

The Strategy of Studying the Herb-Pair Inter-Reinforcement Mechanism Mapping on Chemical Space Between Sparganii Rhizoma and Curcumae Rhizoma

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Research

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Abstract

Background

With the ever-increasing acceptance of combination therapy and the use of traditional Chinese medicine (TCM) has become an emerging trend. The multi-herb formulae therapy is one of the most important characteristics of TCM, but the modernization drive of this conventional wisdom has faced many obstacles due to its unimaginable complexity. Herb pairs, the most fundamental and the simplest form of multi-herb formulae, are a centralized representative of Chinese herbal compatibility. A systematic search of herb pair related research was carried out using multiple online literature databases, books and monographs published in the past 20 years. The herb-pair has an important characteristics—inter-reinforcement (*Xiangxu* in TCM terms) in TCM basic theory. The inter-reinforcement mechanisms of herb pairs are extremely complicated and their exact molecular mechanisms are also still not well elucidated.

Methods

This research integrated the cheminformatics' and network pharmacology approach to elucidate the inter-reinforcement mechanisms in treating cardiovascular diseases (CVDs) with the herb-pair (*Sparganii Rhizoma* and *Curcumae Rhizoma*) at the scale of chemical space. Chemical space is a term often used for 'multi-dimensional descriptor space', geographical map to illustrate the distribution of molecules and their properties. It also encompasses all possible small organic molecules in Chinese herbal medicine (CHM). The concept of chemical space was proposed for the first time to study the herb-pair inter-reinforcement mechanism.

Results

Those compounds from *Sparganii Rhizoma* and *Curcumae Rhizoma* with the HIA \leq 2 were as candidate compounds. The herb-pair shared 2 common compounds with 6 common targets and they have 24 targets from four public databases. The compound-target-pathway-disease networks were constructed to obtain a global perspective of the interactions between *Sparganii Rhizoma* and *Curcumae Rhizoma* in chemical space. On this basis, a series of pathway guided compound combinations were found. Screen with HIA $<$ 2 (Human intestinal Absorption), which were derived from *Sparganii Rhizoma* and *Curcumae Rhizoma* (Chinese herb pair). The CTPAs network embodied 327 nodes (100 compounds, 104 targets, and 128 pathways). After setting compounds similarity to 0.7 in each compound combinations, 497 pairs of compound combinations associated 128 pathways were obtained in Target-Pathway Association Interactions (TPAs). Five, three and thirty-six compound combinations were screened which their targets were in platelet activation pathway, vascular smooth muscle contraction and calcium signaling pathway.

Conclusions

Mapping the chemical compounds of Chinese herbal medicine into chemical space is helpful to explain herb-pair inter-reinforcement mechanism at molecular level. Several combination compounds were mapped into cardiovascular diseases pathways, and the results showed that the strategy of studying the

herb-pair inter-reinforcement mechanism mapping on chemical space is a certain scientific and rational method to illustrate material basis of Chinese herbal medicine at multi-dimension.

Background

Chinese herbal medicine (CHM) is not only medical treasure trove of traditional Chinese medicine (TCM), but also the vastness of natural and biologically relevant chemical space[1,2]. Chemical space is a term often used for 'multi-dimensional descriptor space'[1], geographical map to illustrate the distribution of molecules and their properties[3-5]. Additionally, traditional Chinese medicine (TCM) has been practiced to treat disease from ancient time and it has been recognized as an interesting alternative to conventional medicine[6,7]. Long-term applications and their experiences have demonstrated that herb compatibility is not only a better choice, but also a necessary choice[8]. Herb-pair is the most basic form of herb compatibility in traditional Chinese medicine and is the ancient medical experts of long-term medical practice summary. Herb-pairs are a combination of two relatively fixed herbs, which have been proven effective in clinical practice. The herb-pair has their inherent prescriptions and it is a bridge between single herb and prescriptions. Traditional Chinese medicine (TCM)—herbal mixtures developed through observation and experience accumulated over thousands of years, but with unknown mechanisms of action[9].

In this context, we take herb-pair Sparganii Rhizoma (tuber of *Sparganium stoloniferum* Buch.-Ham.) and Curcumas Rhizoma (rhizome of *Curcuma phaeocaulis* Val.) (SR-CR) as our research object to study the herb-pair inter-reinforcement (Xiang Xu in Chinese ancient medical term) mechanism from holistic perspective in chemical space. The rein-reinforcement is the combination of herbs with similar property and efficacy which can significantly reinforce some kind or several kinds of therapeutic effects[10]. The herb-pair Sparganii Rhizoma and Curcuma Rhizoma (SR-CR) has been increasingly recognized to possess multiple therapeutic activities for treatment of tumor[11-13], thrombosis[14], inflammation[15], blood stasis[16,17], amenorrhea, functional dyspepsia[16]. The details of SR-CR main chemical ingredients see the **supplementary Table S1**.

In a Chinese herbal mixture, the paired-herb is referring to a combined usage of two herbs as to optimize the pharmacological property[18]. Mutual enhancement of herb-pair can be achieved by using pair of herbs with ingredients of similar therapeutic actions, or by using a principal herb with main therapeutic actions and another one acting to enhance or assist the effects of the principle one[19]. Many ingredients of herbs are inactive individually but become active in combination, called coalistic combinations, which are quite common in herb pairs[20,21]. However, the exact molecular mechanisms of these TCM herb-pairs are yet unclear[19]. The herb-pair contains many combinations among the chemical space. Though there are many reports on pharmaceutics, pharmacokinetics, and pharmacodynamics of TCM, there remains a serious lack of summarization and systemic analyses of these reported data to help uncover the compatibility rationale of TCM herb pairs based on their chemical spaces[22]. Chemical space — which encompasses all possible small organic molecules, including those present in biological systems— is vast[1,23]. The vastness of chemical space will guide us to discover biologically active compounds[2],

natural products[24], and the synthesis of new ligands[25,26]. Although herb-pair inter-reinforcement is not entirely clear, the explorations of herb-pair chemical space will greatly enhance our understanding of herb-pair inter-reinforcement mechanism in biology, and lead to new strategies to treat disease[27,28].

Methods

Date collection and visualization Date collection and digitalization of compounds

We collected compounds of *Sparganii Rhizoma* and *Curcumae Rhizoma* from four typical TCM databases, namely TCMID[53], TCMSP[36], TCM Database@Taiwan

[54], BANMAN-TCM[55]. All of these compounds collected were normalized to the canonical SMILES format[56]. Duplicates and compounds without structures were excluded. Data set **S** was collected from *Sparganii Rhizoma* chemical space and contained 47 molecules. Similarly, data set **E** was from *Curcumae Rhizoma* chemical space and contained 91 molecules. Chemical space was used to discuss the similarities and differences between the molecule sets of *Sparganii Rhizoma* (data set **S**) and *Curcumae Rhizoma* (data set **E**). The data set **S** and **E** can be mapped to two-dimensional space while maintaining a lot of geometric structure. A total of 47 chemical components of *Sparganii Rhizoma* were obtained from the databases and were numbered from S001 to S047 (**Supplementary Table S1**). The 91 chemical components of *Curcumae Rhizoma* were numbered from E001 to E093, and we excluded the no clear structure chemical E071, E075 in this research (**Supplementary Table S1**).

All of the related targets were normalized to the official gene symbols using the UniProt database (<http://www.uniprot.org/uploadlists/>). Duplicates from different sources were excluded. Fingerprints for each of the data sets. The MDL 166-key fingerprint is also known as the “Molecular Access System (MACCS)” key in the literature and is a no hashed fingerprint consisting of 166 bits. The MDL 166-key fingerprint is one of the very few available that offers a 1-1 mapping[57]. These fingerprints were assembled into the fingerprint array.

The data sets used to evaluate the models’ ability to cluster compounds by molecular structure were given in **Table 1**. The PaDEL-Descriptor[58] was used to generate a set of MDL 166-key fingerprints for each of the data sets. The MDL 166-key fingerprint is also known as the “Molecular Access System (MACCS)” key in the literature and is a no hashed fingerprint consisting of 166 bits. The MDL 166-key fingerprint is one of the very few available that offers a 1-1 mapping[57]. These fingerprints were assembled into the fingerprint array.

Table 1 Data sets on *Sparganii Rhizoma* and *Curcumae Rhizoma* chemical space to Evaluate Structure Clustering^a

Data set	color	diversity	No. entries	descriptor
S	red	0.6258	47	TCMID,TCMSP,BANMAN-TCM,
E	blue	0.5036	91	TCM Database@Taiwan

a: The colors are those used in the visualization plots.

The diversities were computed using Tanimoto coefficient[57,59], which is a **statistic** used for comparing the **similarity** and **diversity** of **sample** sets. Tanimoto coefficient is also known as the Jaccard coefficient, and, when used to measure dissimilarity rather than similarity[60]. Turning then to the similarity coefficient that is used for comparing fingerprints, the most popular is the Tanimoto coefficient (Equation 1). If two molecules have a and b bits set in their fragment bit-strings, with c of these bits being set in both of the fingerprints, then the Tanimoto coefficient is defined to be:

$$\frac{c}{a+b-c} \quad (1)$$

The Tanimoto coefficient gives values in the range of zero (no bits in common) to unity (all bits the same); and is usually used for Boolean vector, that is, when the components are only 0 or 1. In our research, the compounds of herb pair were normalized to MDL 166-key fingerprints.

Visualization for herb pair's chemical space

Generative Topographic Mapping (GTM) is nonlinear and works by training an Radial Basis Function (RBF) neural network to produce a mapping from two-dimensional latent (i.e., visualization) space to n-dimensional data (i.e. fingerprint) space[57]. Essentially, GTM models was used to reduce the dimensionality of the fingerprint array from 166-dimensional space to two-dimensional space where it could be plotted and visualized (**Fig. 2**). It has reported that greater the structural similarity, the greater the biological similarity[61]. As can be seen from **Fig. 2**, the chemical compounds in the chemical space of SR-RC could be divided into two groups. Nevertheless, there are many compounds with similar structure (**Fig. 2**).

The workflow of studying the herb-pair inter-reinforcement mechanism

We calculated the human intestinal absorption (HIA) properties of the compounds from Sparganii Rhizoma and Curcumae Rhizoma using a model integrated within Pipeline Pilot (version 7.5.2, BIOVIA, San Diego, CA, USA) [62] to rank the molecules into the following four classes: good (0), moderate (1), poor (2) and very poor (3). The very poor class of 10 compounds was discarded. The remaining compounds were further analyzed (for further details of four classifications for these compounds, see **Supplementary Table S1**).

Subsequently, we collected experimentally determined compound-target pairs from four databases, namely STITCH (version 5.0) [63], BindingDB[64], PubChem[65] and DrugBank[66,67]. No target records

among these four typical TCM databases of these compounds were predicted using BATMAN-TCM bioinformatics tool[55] and chemical-protein interactions (CPI) predictor[68]. For each compound, the score above 80 predicted targets were stored as putative targets when using the bioinformatics tool—BATMAN-TCM; the probability above 0.6 predicted targets

were stored as putative targets when using CPI predictor[68]. The calculating score method of the BATMAN-TCM is to rank potential drug-target interactions based on their similarity to the known drug-target interactions[69]. On this basis, the Compound-Target Network were constructed, indicating the interaction between compounds and targets.

We will explain the inter-reinforcement mechanism for herb pair of from the pathway level and the disease level. On this basis, the target-pathway network and target-disease network on the basis of Compound-Target network were constructed. In order to study the contribution of common compounds and non-common compounds for the herb-pair inter-reinforcement in the herb pair, we examined compound-target-pathway and compound-target-disease with common compounds and the non-common compounds. Finally, the CTDIs and TPAs were constructed with the common/non-common compounds to comparative analysis the inter-reinforcement mechanism of the herb pair (**Fig. 3**).

Results

Networks can be used to view global drug-targets-pathway-disease relationships between nodes[29,30]. Network pharmacology approach have been applied in drug discovery[31-35]. In this research, we utilized the network pharmacology method to illustrate SR-CR inter-reinforcement mechanism by mapping compound combinations into SR-CR chemical space.

Target-Pathway association profile of Common compounds for the herb pair

The herb-pair shared 2 common compounds and they have 24 targets from four public databases. We obtained 24 targets associated 15 KEGG pathways and 22 REACTOME pathways in total and the inter-reinforcement mechanisms of herb-pair were mapped to the target-pathway network according to their targets information (**Fig. 4**, see **Supplementary Table S2** for further details of these pathways). The degree of PIK3CG among the 24 targets is the largest one. We also obtained 27 targets associated 87 diseases in total and an average degree of 14 targets per compound (**Fig. 5a**, see **Supplementary Table S3** for further details of these 87 diseases).

Table 2 Physical and chemical properties of the common compounds

Common compound	LogP	OB(%) [36]	pKa
alexandrin	8.615	20.63	12.91±0.70
hederagenin	7.065	36.91	4.63±0.70

The two common compounds were connected to multiple targets and they have 6 common targets, namely PTGS2, PGR, NCOA2, ADH1C, PTGS1, LYZ. The 6 targets associated 35 diseases and the maximum degree of 32 diseases is PTGS2 in **Fig. 5b**. (See **SupplementaryTable S4** for further details of these 35 diseases)

Compound-Target-Pathway Associations (CTPAs) network

To understand the complex interaction of herb-pair's targets at system level, we constructed a Compound-Target-Pathway Associations (CTPAs) network. The CTPAs network embodied 327 nodes (100 compounds, 104 targets, and 128 pathways) (**Fig. 6**, see **Supplementary Table S5** for further details of these pathways associated targets). The mean degree value (the number of associated common targets) of candidate compounds was 1.08, and 24 compounds had a degree value >10, indicating that most of the compounds regulated multiple targets to exert various therapeutic effects in the herb-pair's chemical space. The top 5 degree of Compound-Target-Pathway network were Neuroactive ligand-receptor interaction, Morphine addiction, Retrograde endocannabinoid signaling, Calcium signaling pathway, Nicotine addiction.

Compound-Target-Disease interactions (CTDIs) network

The Compound-Target-Disease interactions (CTDIs) network was built by targets and diseases [37-41]. Node size was proportional to its degree. The light green (green) ellipses indicated *Sparganii Rhizoma* (*Curcumae Rhizoma*) compounds, the pink ellipses indicates the targets of herb-pair's compounds, and the cyan ellipses indicated the disease types (**Fig. 7**, see **SupplementaryTable S6** for further details of these diseases). The involved diseases were type 2 diabetes, hypercholesterolemia, hypertension, cardiovascular diseases, etc.

All of these targets were shown by the gene name. All of the targets and diseases were selected to construct the Compound-Target-Disease interactions (CTDIs) network by linking the targets to the corresponding diseases. All visualized network graphs were constructed by Cytoscape 3.2.1 (<http://www.cytoscape.org/>), an open software package project for visualizing, integrating, modeling and analyzing the interaction networks[42,43].

Target-Pathway Association Interactions (TPAs)

We analyzed Target-Pathway Association Interactions (TPAs) and screened the combinatorial compounds which their targets were in the same pathway[21]. Then we combined the two compounds which one from *Sparganii Rhizoma* and the other from *Curcumae Rhizoma*. Finally, 14370 pairs of pathway-guided combination compounds, including 1267 pairs of compound combinations (**Supplementary Table S7**) were obtained. After setting compounds similarity to 0.7 in each compound combinations (**Fig. 8**), 497 pairs of compound combinations associated 128 pathways (**SupplementaryTable S8**) were obtained.

Pathway guided compound combinations

The pathway of platelet activation is one of 128 pathways, and platelets play a key role in preventing blood loss in response to injury, thrombosis, atherosclerosis, inflammation and metastasis, but they are also responsible for the formation of pathogenic thrombi that cause acute manifestations of vascular atherothrombotic disease[44-46]. In this research, four compound combinations and their associated targets in platelet activation pathway (**Fig 9.**) were obtained. The compound combination (S039, E063) acted on Akt pathway, and the compound combinations ((S039, E085), (S039, E074), (S039, E005)) targeted on the PTGS1 (the numbered chemical ingredients see the **supplementary Table S1**). The mechanism of compound combinations is alike drug combinations. As we knew, drug combinations can improve over single therapeutic agents in two ways[47]. In additionally, drug combinations have been used for treating diseases and reducing suffering in Chinese herbal medicine[48].

Synergy between two drugs may result in a better response than the two drugs independently[47]. In this research, we picked out another two pathways (Calcium signaling pathway, vascular smooth muscle contraction) closely related to cardiovascular diseases (CVDs) and the 3 pathways were consolidated into a “CVDs pathway” under the pathological and clinical data[49]. In order to illustrate the material basis of herb-pair inter-reinforcement mechanism, we mapped compound combinations to pathways associated compounds’ targets (**supplementary Table S9**).

Across all kingdoms in the tree of life, Calcium (Ca^{2+}) is an essential element used by cells to respond and adapt to constantly changing environments[50]. Signaling occurs when the cell is stimulated to release calcium ions (Ca^{2+}) from intracellular stores, and/or when calcium enters the cell through plasma could induce the development of atherosclerosis[49].

This strategy of studying herb-pair inter-reinforcement membrane ion channels[51]. Many diseases, such as cardiovascular diseases, type 2 diabetes, malignant hyperthermia, central core disease, associated with calcium signaling pathway[50,52]. Vascular smooth muscle contraction also plays an important role in the regulation of CVDs, there is due mechanism mapping on chemical space will lead to the development of many today’s drug discovery from Chinese herb-pairs. Future research in our group will focus on investigating compound combinations’ pathological effect on specific diseases, such as, CVDs.

Discussion

This research proposed to explore the inter-reinforcement mechanism of *Sparganii Rhizoma* and *Curcumae Rhizoma* based on the chemical space for the first time. We adopted the chemoinformatics methods to screen the chemical compounds that can be absorbed and utilized by the human body from the chemical space of *Sparganii Rhizoma* and *Curcumae Rhizoma*. And then, the network of Compound-Target-Disease interactions (CTDIs) network, Compound-Target-Disease interactions (CTDIs) network, Target-Pathway Association Interactions (TPAs), Pathway guided compound combinations were constructed with network pharmacology method.

It explains inter-reinforcement mechanism of *Sparganii Rhizoma* and *Curcumae Rhizoma* with the combination compounds in cardiovascular disease-related pathways. Each group of combination compounds was derived from *Sparganii Rhizoma* and *Curcumae Rhizoma*. This strategy could provide research strategies for further research on the mechanism of interaction of TCM pairs. However, this approach to screen out combination compounds on specific disease-related pathways required further animal or cell experiments to confirm its combined effect.

Conclusions

This strategy will provide methodology to study the herb-pairs inter-reinforcement mechanism on compound combinations level. In this context, several compound combinations with potential efficacy to treat diseases were discovered, especially cardiovascular disease.

The compound combinations with the common compounds (hederagenin and alexandrin) plays an important role in the cardiovascular diseases (CVDs) pathways from **Supplementary Table S9**. The common compounds appeared in the CVDs pathways (platelet activation, vascular smooth muscle contraction, calcium signaling pathway), and it indicated that the compound combinations with common compounds through the accumulation of quantity to play a synergistic role. However, the synergistic effects with no-common compounds by accumulating target effects in the same or different pathways. Betulinic acid in *Sparganii Rhizoma* combined with many other compounds played import role in CVDs pathway. Betulinic acid with other compounds may be a potential drug for treating of cardiovascular diseases.

Abbreviations

CHMs: Chinese herbal medicines; TCM: Traditional Chinese medicine; *SR-CR*: *Sparganii Rhizoma* and *Curcumae Rhizoma*; CVDs: cardiovascular diseases; HIA: Human intestinal Absorption; RBF: Radial Basis Function; GTM: Generative Topographic Mapping; CPIs: Chemical-protein interactions; CTDIs: Compound-Target-Disease interactions; TPAs: Target-Pathway Association Interactions

Declarations

Authors' contributions

ZC substantial contributions to the conception or design of the work. ZC and SS drafting the work and revising it critically for important intellectual content. ZC and XR final approval of the version to be published; XR and YC conceived and designed the experiments; SS and XR collected the data; YC contributed reagents/materials/analysis tools; ZC analyzed the data and wrote the paper, YC revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

All of authors consent to publication of this study in Journal of Chinese Medicine.

Availability of data and materials

The readers can use data and materials in this manuscript by quotation of author names and Journal of Chinese Medicine. Raw data in Supplementary Table S1-9 and were summarized as Additional file 1. Availability of raw data for Fig. 1-10 were summarized as Additional file 2.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

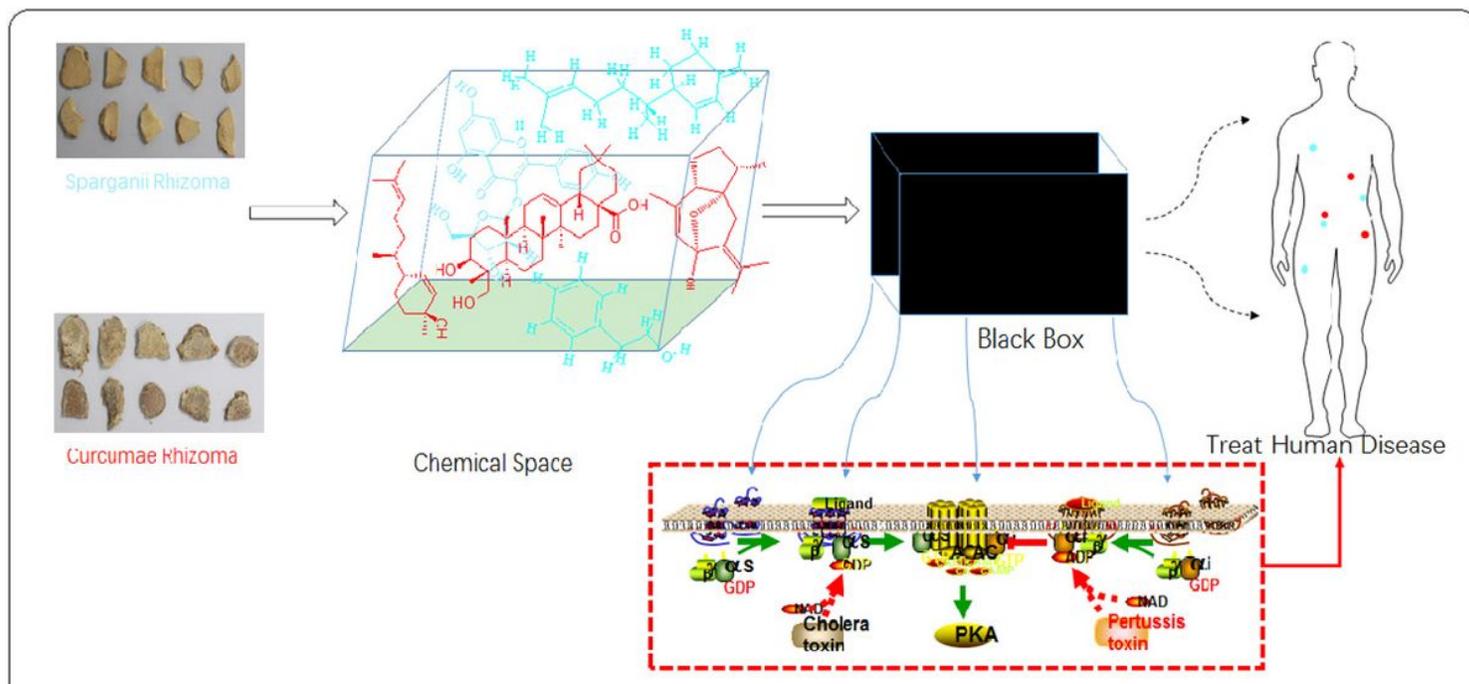


Figure 1

Herb-pair mapping into chemical space to explain the inter - reinforcement mechanism at molecular level.

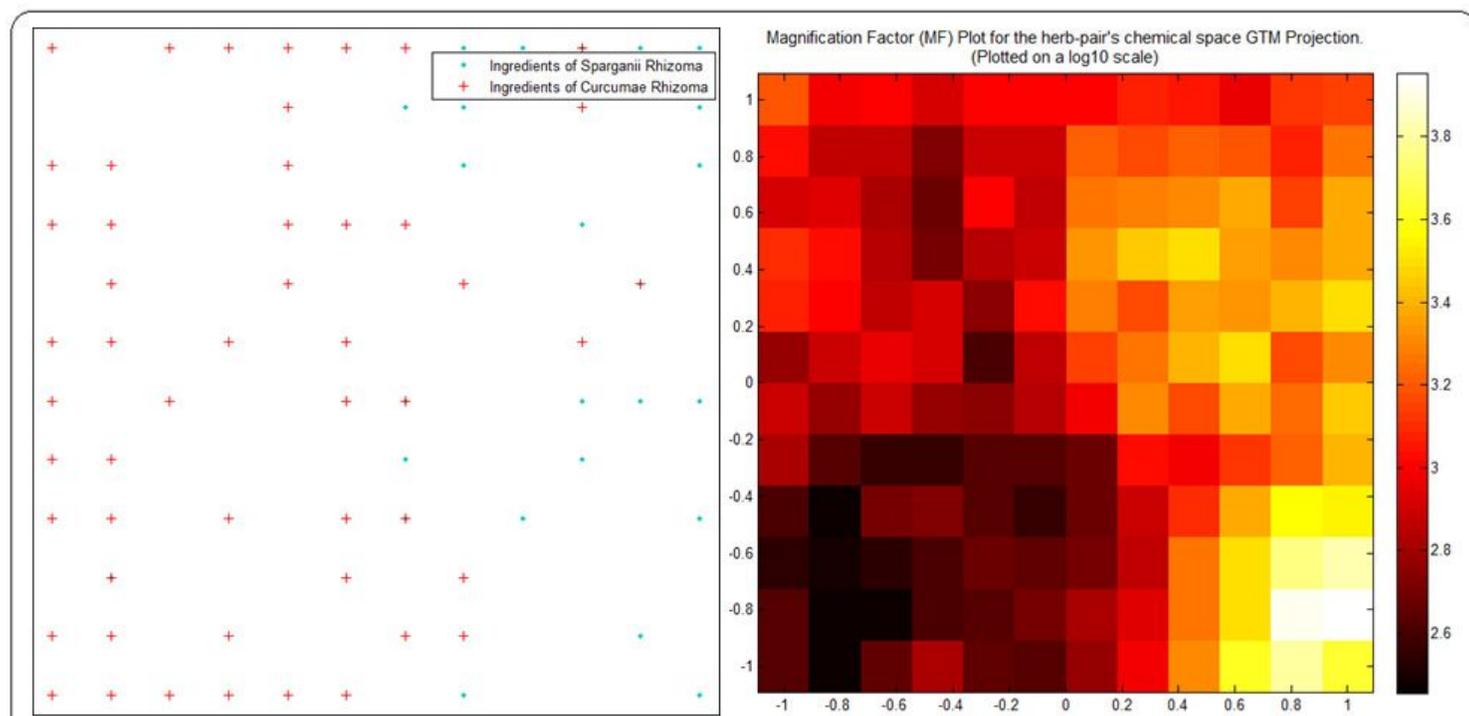


Figure 2

Generative Topographic Mapping (GTM) visualization (a) and Magnification Factor (MF) Plot for herb-pair's (Spargani Rhizoma and Curcumae Rhizoma) chemical space (b)

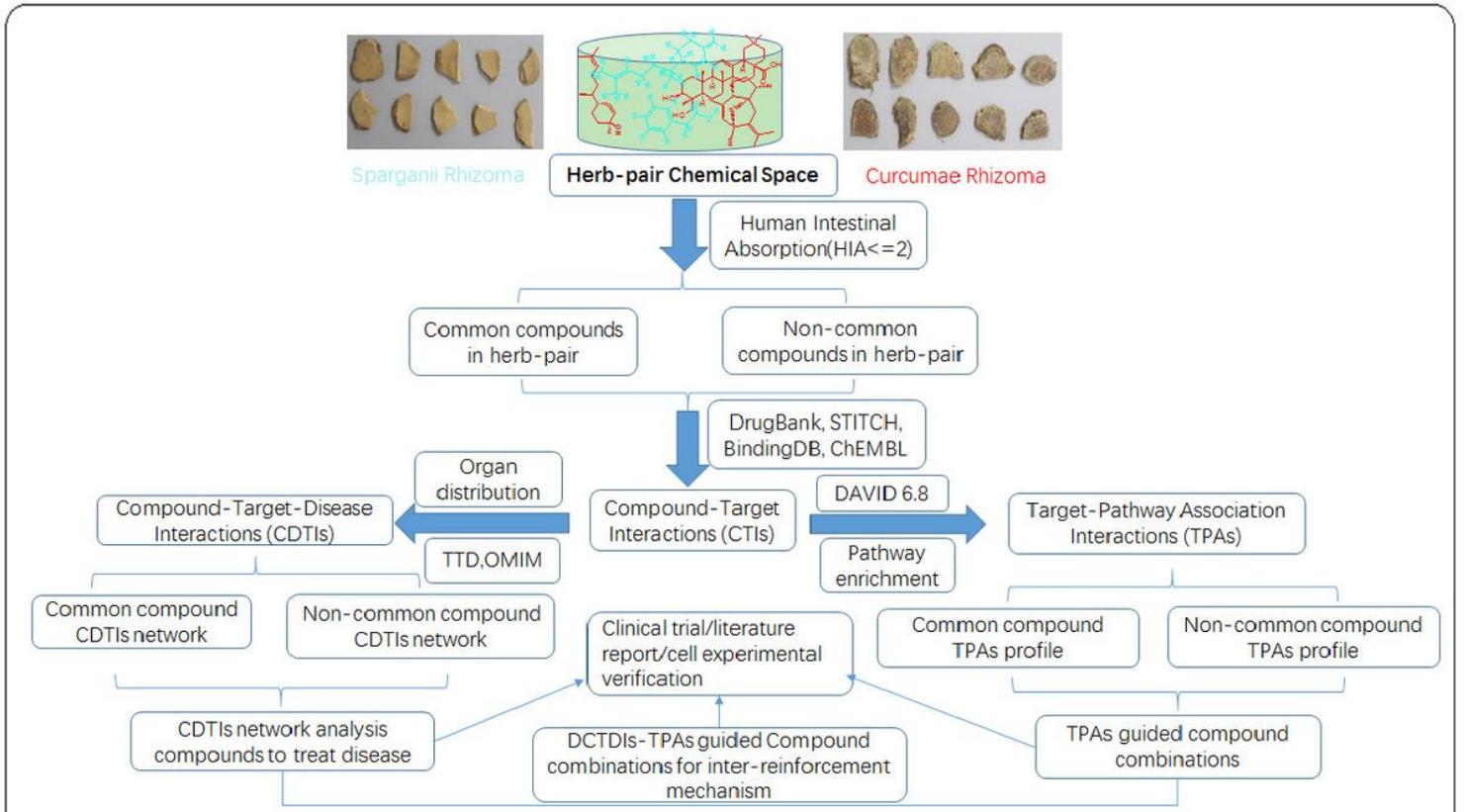


Figure 3

The workflow of studying the herb-pair inter-reinforcement mechanism mapping on chemical space to guide compound combination.

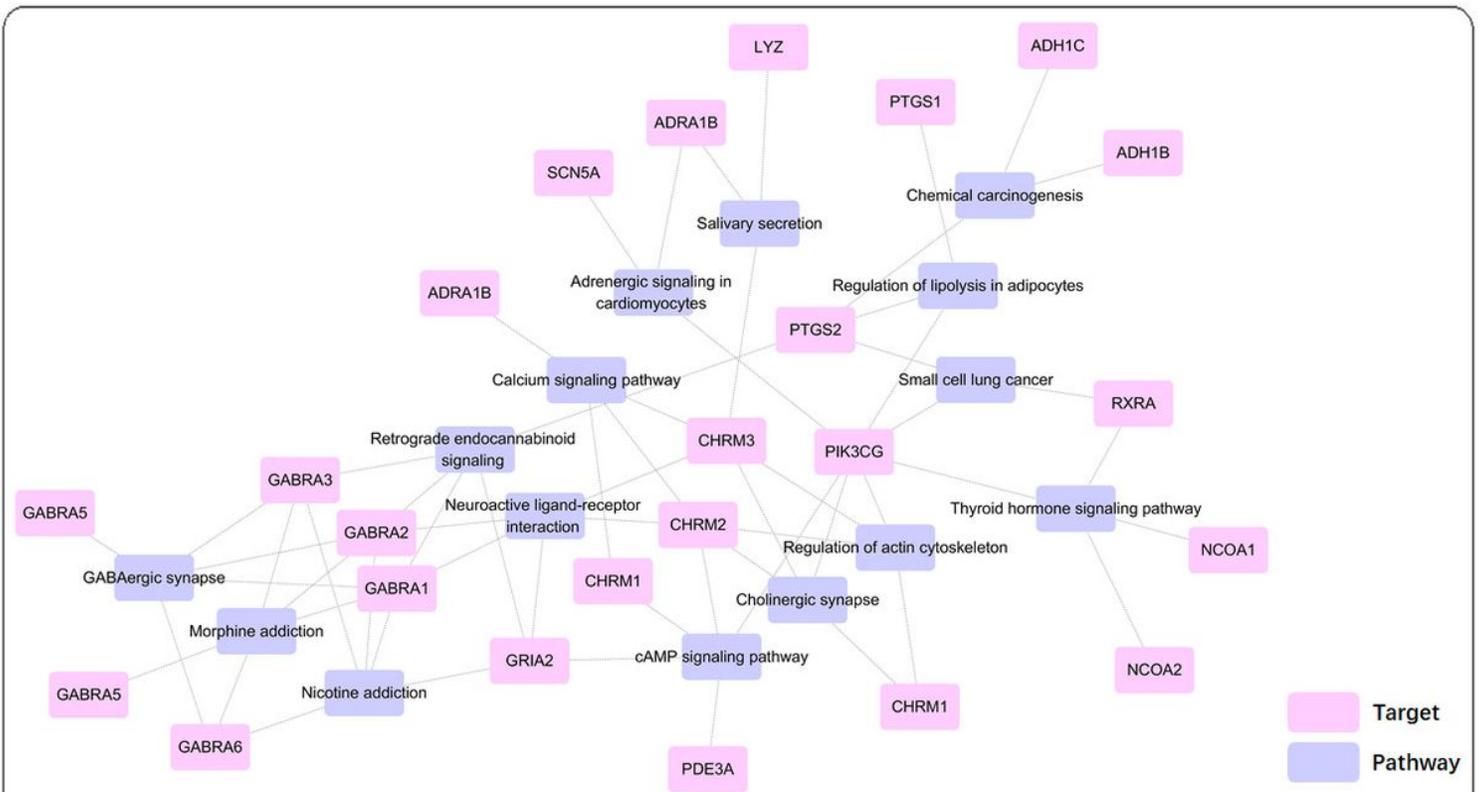


Figure 4

The two common compound (alexandrive and hederagenin) of target-pathway association profile from KEGG database. A target node and a pathway node were linked if the target was associated with the pathway.

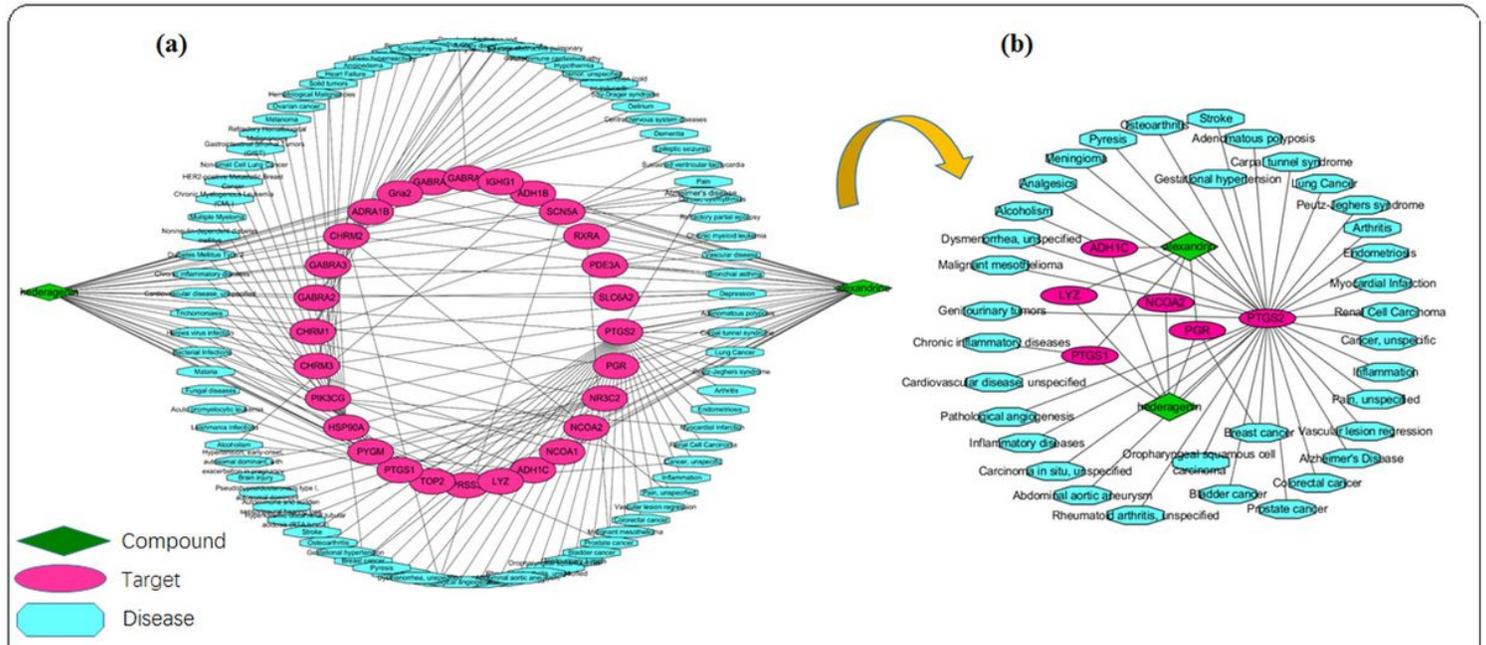


Figure 5

The two common compound (alexandrive and hederagenin) of Compound-Target-Disease Interaction (CTDIs) network. (a) contains 28 targets of the two compound, (b) exacting the 6 common target from the 28 target to construct target node and a disease node were linked if the target was associated with the disease.

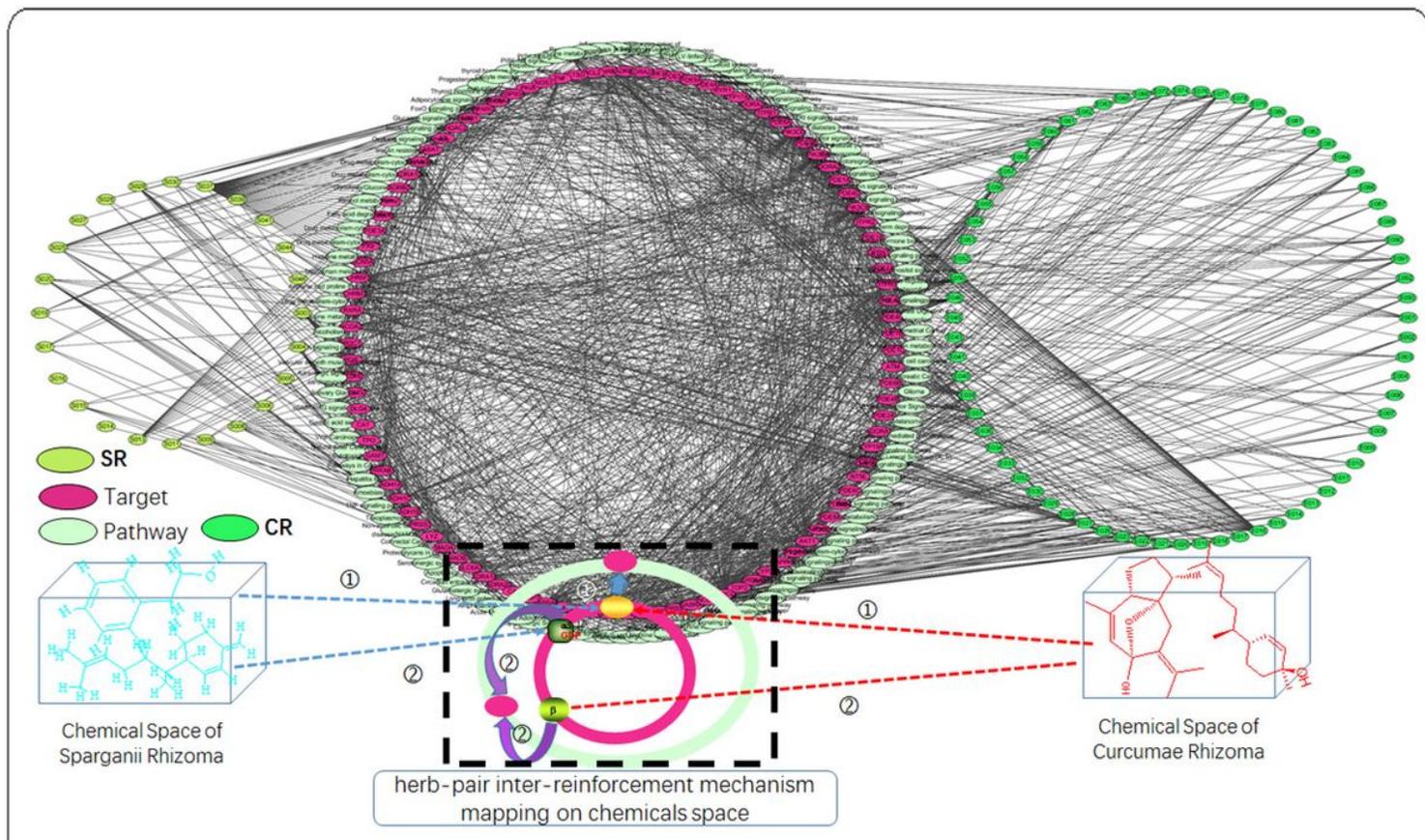


Figure 6

The non-common compounds of target-pathways association profiles. A target node and a pathway node were linked if the target was associated with the disease. The compound combinations were from SR-CR chemical space and each of them may act on the same target as route (1). Otherwise, each of them may act on the different targets in the same pathway as route (2).

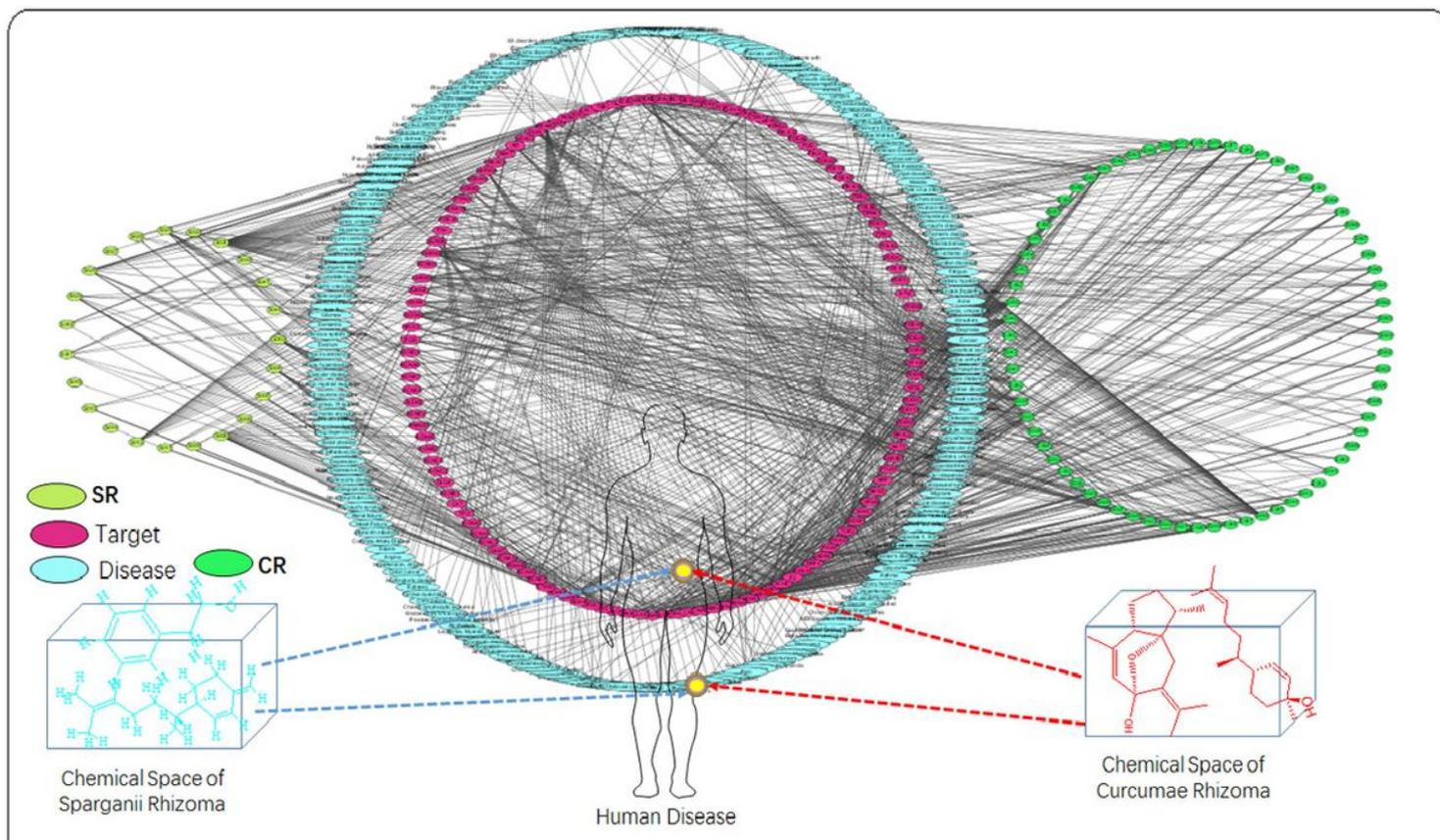


Figure 7

The non-common compound of target-disease association profiles. A targets node and a node were linked if the target was associated with the disease.

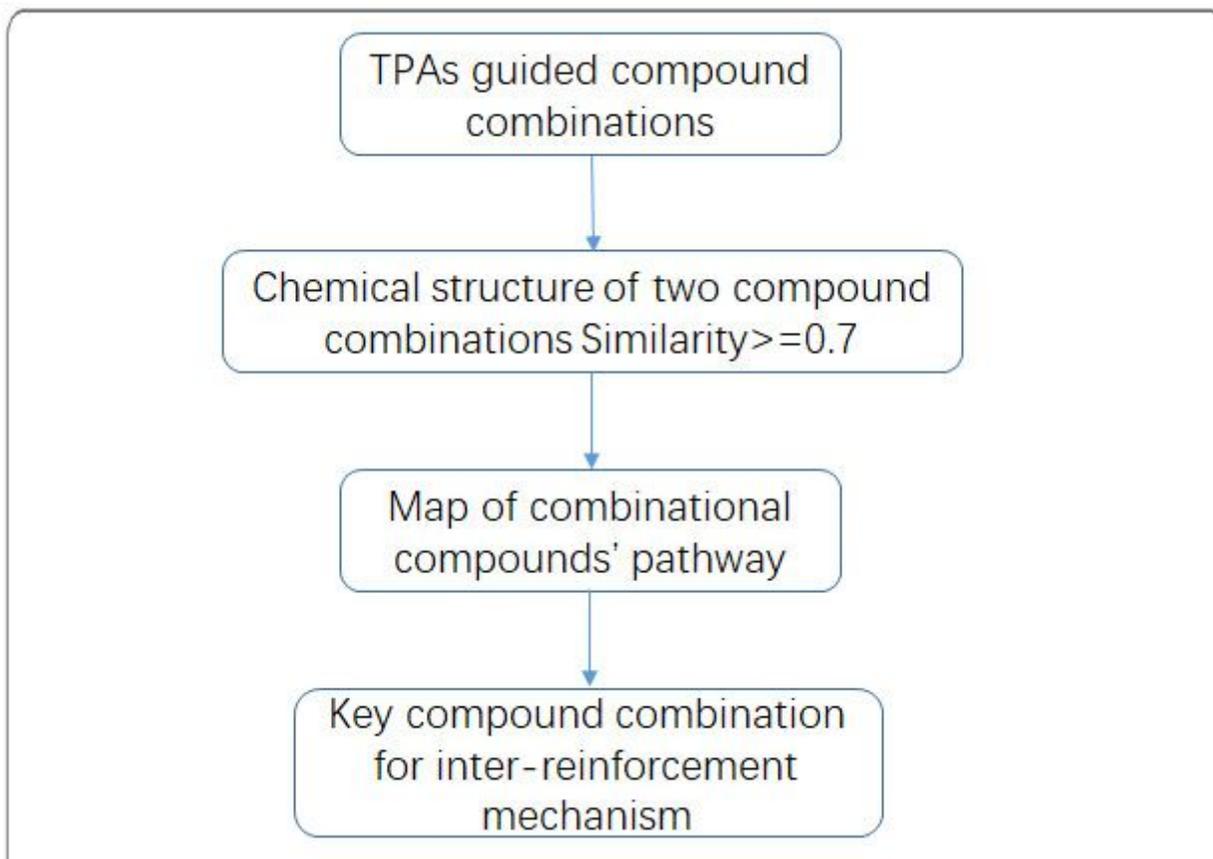


Figure 8

The workflow to discovery the key compound combinations for herb-pair inter-reinforcement mechanism.

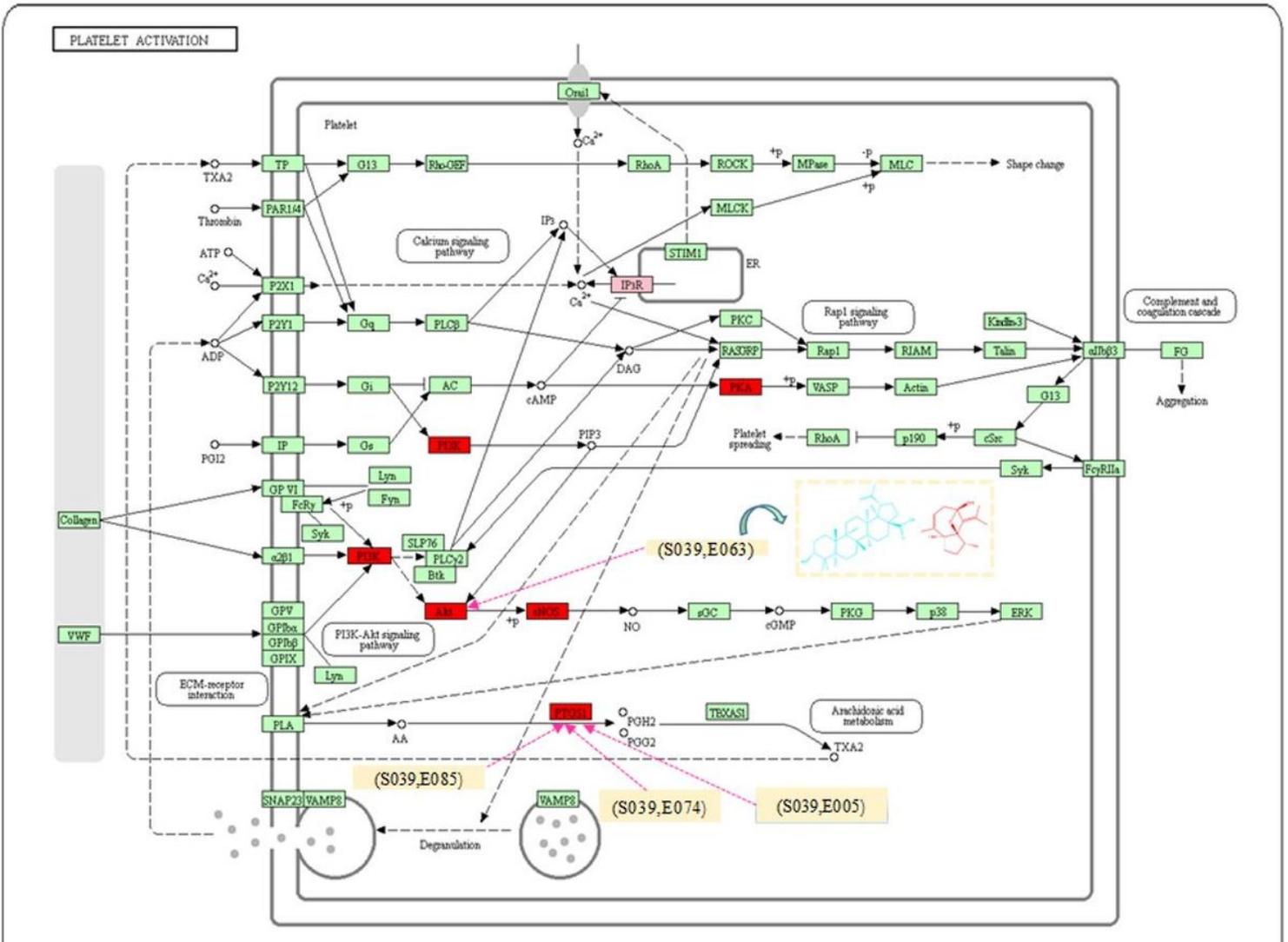


Figure 9

Platelet activation pathway and the compound combinations' associated targets in the pathways.

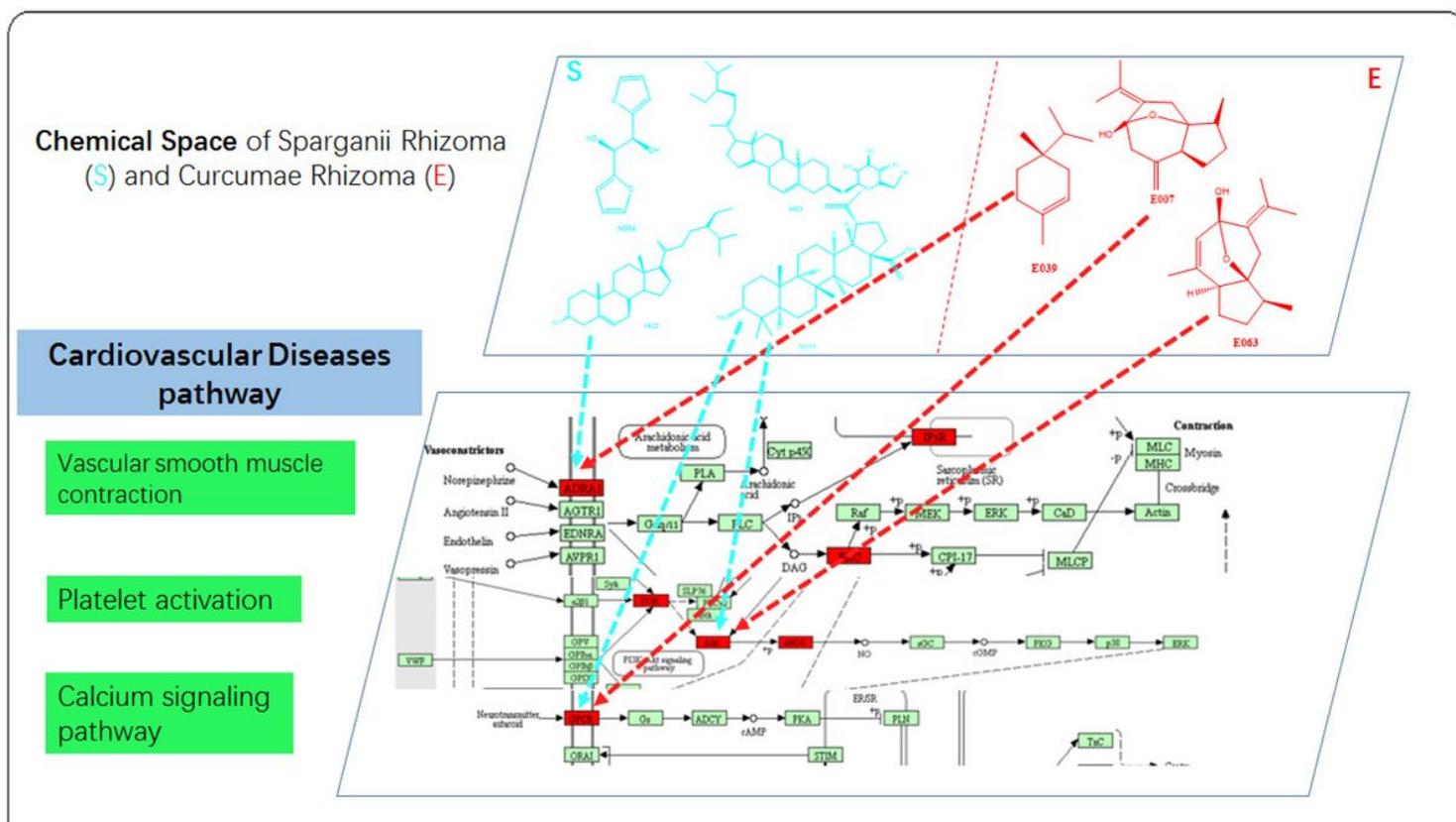


Figure 10

CTDIs-TPAs guide compound combinations for inter-reinforcement mechanism in cardiovascular disease (CVDs) associated pathway and the compound combinations' targets in the pathways.

Supplementary Files

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- [SupplementaryTableS1.xlsx](#)
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