Dissection of effect mechanisms of Kudiezi injection against myocardial ischemia based on virtual screening and systems pharmacology approach

Rongfang Xie (1757640325@qq.com)
Fujian Medical University
Zhenzhen Liu
Fujian Medical University
Peiying Shi
Fujian Agriculture and Forestry University
Zuan Lin
Fujian Medical University
Bing Chen
Fujian Medical University
Shaoguang Li
Fujian Medical University
Guangwen Li
Fujian Medical University
Liying Huang
Fujian Medical University
Xinhua Lin
Fujian Medical University
Hong Yao
Fujian Medical University

Research

Keywords: Kudiezi injection, effect mechanisms, myocardial ischemia, virtual screening, systems pharmacology

Posted Date: March 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-19389/v1

Abstract

Background

Kudiezi (KDZ) injection, a Chinese medicine preparation, has been widely used for the treatment of myocardial injury in recent years. However, the knowledge of the molecular mechanisms is limited to support its clinical application. It is of great importance to explore the underlying mechanisms of this preparation.

Methods

In this research, the 10 main ingredients in this injection were identified firstly by HPLC-Q-TOF-MS. Afterwards, virtual screening and an integrated systems pharmacology approach combined with molecular docking (molecular docking software: Sybyl-X 1.3 and Autodock vina) were adopted to predict its therapeutic mechanisms.

Results

7 proteins targets and 9 KEGG pathways, possessing highly relevance to the therapeutic effects of KDZ against MI, were predicted reasonably. The systems analysis suggested that KDZ injection could exert its therapeutic effects against myocardial ischemia possibly through multi-targets including EGFR, MAPK10, SRC, et al, and multi-pathways referring to MAPK, Focal adhesion, Complement and coagulation cascades, Fluid shear stress and atherosclerosis, etc.

Conclusion

This research provided a scientific basis to clarify the comprehensive pharmacological mechanism of KDZ injection acting on MI, and could provide valuable clues on considering the active ingredient KDZ-6 as a multi-targets leading compound.

Background

Myocardial ischemia (MI), a higher incidence cardiovascular affair, is known as one of the main high mortality diseases globally [1, 2]. Previous studies have demonstrated that MI can lead to acute myocardial infarction (AMI), atherosclerosis, hypertension, cardiac hypertrophy, as well as diabetic associated complications as diabetic atherosclerosis/cardiomyopathy/hypertension [2]. What's more, tissue damage due to the persistent ischemia is irreversible [3]. And the intraluminal coronary thrombosis/coronary artery obstruction and ruptured atherosclerotic plaque impeding blood flow to approach the myocardium are the main causes of it [4].

Herbal medicines have been gradually recognized as safe and effective therapeutics with fewer side effects worldwide [5–11]. Some herbal medicine injections produced by extracting and purifying active ingredients from herbs or decoctions according to modern scientific and technological theories have often been used to treat some emergency and severe diseases in clinics in China. Kudiezi (KDZ) injection, a typical representative of herbal medicine injections, is extracted from the herb Ixeris denticulate and extensively applied to treat ischemic stroke and myocardial infarction [12]. Studies have shown that KDZ injection mainly contains

flavonoids, adenosine, sesquiterpene lactones and triterpene saponins [13]. However, the therapeutic effect of KDZ injection against MI remain unclear due to its complex components.

In the recent years, owing to the convenient and powerful properties on exploring the inner relationship among multiple ingredients, multiple targets and multiple pathways for a complex drug system, systems pharmacology study has become a popular trend on dissecting the action mechanism of compound formula, e.g. traditional Chinese medicines, herbal medicines or their preparation products [14–19]. Also, systems pharmacology was considered to be able to provide valuable clues on identifying the active ingredients from complex herbal formula [17–19]. However, up to nowadays, it is still lack of the comprehensive study on dissecting the effect mechanisms of Kudiezi injection based on systems pharmacology methods.

In this study, an integrated approach was established to unveil the pharmacological mechanisms of the main constitutions in KDZ injection from the whole perspective of system biology. Firstly, the main constitutions in KDZ injection were identified by HPLC-Q-TOF-MS. Then molecular docking simulations, KEGG (Kyoto Encyclopedia of Genes and Genomes) enriched pathway analysis, GO (Gene Ontology) analysis and network analysis were further employed to explore the anti-MI mechanism of KDZ injection.

Methods

HPLC-Q-TOF-MS Analysis

For HPLC-Q TOF-MS analysis, Agilent 1260 HPLC instrument and Agilent 6520 quadrupole time-of-flight tandem mass spectrometer were used in this study. The separation was finally performed on an Agilent ZORBAX SB-C18 column (4.6 mm × 150 mm, 3.5 μm) at 30 °C with a flow rate of 0.5 mL min^{-1}. The Mobile phase consisted of 0.5% glacial acetic acid in water (A) and acetonitrile (B) with the following gradient elution: 0–15 min, 4–11% B; 15–20 min, 11–14% B; 20–40 min, 14–19% B; 40–41 min, 19–95% B. The injection volume was 5 μL.

The acquisition parameters for Q-TOF mass spectra were as follows:drying gas (N_2) flow rate; 10 L min^{-1}, drying gas temperature, 350 °C; atomizing gas flow, 40 psi; electricity Spray voltage 3500 V, fragmentor voltage 145 V. The sample was analyzed in both positive and negative ion modes for full scan (ESI), and the scan mass range was recorded from m/z 100 to 1000.

Target predicting

Firstly, three dimensional molecule structures of the identified compounds were constructed using a molecular docking software Sybyl-X 1.3. Subsequently, an online target fishing sever, PharmMapper (lilab.ecust.edu.cn/pharmmapper), which uses a pharmacophore mapping approach probing potential drug target, was used to predict the target proteins of these compounds [20–23]. The predicted results with fit score, related gene ID, and the correlated diseases, etc. were documented at a Excel table. Next, the results were filtered by a comprehensive consideration of Norm Fit (≥ 0.7) and the definite relationship of the target proteins with cardiovascular diseases (searching PubMed, Web of science and Genecards databases).

Molecular docking

Molecular docking, an important means of modern drug screening, was used to analyze the bind ability of compounds to target proteins [24]. First, the crystal structures of the targets proteins were downloaded from the PDB database (https://www.rcsb.org/) and embellished through the Sybyl-X, including removing the waters and ligands, adding H atoms, optimizing and patching amino acids. The docking scores, including total scores (preferable ≥ 6) and CScores (preferable ≧ 4) were the important evaluation index for predicting the affinity of a target protein to an ingredient. Besides, Autodock vina [25], an open-source program for molecular docking and virtual screening, was also used in this study to access the binding affinity of each compounds/protein complex. It was reported that this program had a high prediction performance when compared to experimental data [26]. With the application of the two molecule docking methods, the total-score and affinity values (kcal/mol) were used to evaluated the interaction between ingredients and targets respectively. And the better docked model was analyzed using PyMOL 2.3.1 and Ligplus [27].

GO and KEGG pathway enrichment analysis

The 39 potential targets were adopted for GO and KEGG analysis were performed by the clusterProfiler package in R (version 3.5.1) to address the functional annotation ($p < 0.01$) [28].

Network construction

The 39 potential targets were inputted into String database (https://string-db.org/cgi/input.pl) to build a PPI network and illustrated the inter-protein interaction (Confidence scores > 0.4). Besides, compounds, candidated targets and pathways for KDZ injection were used to construct the compound-target-pathway network visualized by Cytoscape 3.7.1 software [29].

Results

Identification of the main chemical constituents in KDZ injection

HPLC-Q-TOF-MS analysis of the KDZ injection was performed in both positive and negative ionization modes. The HPLC chromatogram was shown in Fig. 2, and the MS data was listed in Table 1. According to Fig. 2, 10 main compounds were identified, including flavonoids, esters, and organic acids with their standard references. The chemical structures were listed in Table 2.

Table 1

Characterization of compounds in KDZ injection by HPLC-Q TOF-MS.

No.	t_R (min)	Observed mass	Calculated mass	Error (ppm)	Foumula	Identification
1	7.376	311.0397	311.0409	3.83	$C_{13}H_{12}O_9$	Caftaric acid (KDZ-1)
2	8.518	353.0855	353.0878	6.62	$C_{16}H_{18}O_9$	5-CQA (KDZ-2)
3	13.342	353.0866	353.0878	3.52	$C_{16}H_{18}O_9$	3-CQA (KDZ-3)
4	14.870	353.0869	353.0878	2.62	$C_{16}H_{18}O_9$	4-CQA (KDZ-4)
5	15.384	179.0347	179.035	1.67	$C_9H_8O_4$	Caffeic acid (KDZ-5)
6	23.586	473.0692	473.0692	7.2	$C_{22}H_{18}O_{12}$	Chicoric acid (KDZ-6)
7	25.580	609.1432	609.1461	4.90	$C_{27}H_{30}O_{16}$	Luteolin-7-O-β-D-gentiobioside (KDZ-7)
8	29.986	461.0726	461.0725	-0.02	$C_{21}H_{18}O_{12}$	Luteolin-7-O-β-D-glucuronide (KDZ-8)
9	30.742	447.0918	447.0933	3.37	$C_{21}H_{20}O_{11}$	Luteolin-7-O-β-D-glucoside (KDZ-9)
10	37.223	445.0761	445.0776	3.55	$C_{21}H_{18}O_{11}$	Apigenin-7-O-β-D-glucuronide (KDZ-10)

Due to technical limitations, Table 2 is provided in the Supplementary Files section.

Targets Fishing for KDZ injection

In the present work, 39 targets related to MI were predicted by using the online server PharmMapper and its detailed information was described in supplementary Table S1.

Molecular docking

KDZ-6 was found possessing the largest docking scores for many target proteins among the ten ingredients. Thus, taking KDZ-6 as a typical objective, the docking results were introduced next. The three-dimensional structure and the schematic 2D dimensional of KDZ-6 with its best docking state in the active site of the proteins MAOB (PDB ID: 1OJ9), MAPK8 (PDB ID: 4UX9), GSTP1 (PDB ID: 1KBN) and AKR1C1 (PDB ID: 6IJX) displayed in Fig. 3. And the binding energy was − 11.2, -9.3, -6.7 and − 10.6 kcal/mol, respectively. By the way, a lower binding energy predicts a strong capacity between the ingredients and the target proteins. Additionally, the comparison of the results of mentioned above methods for the top 10 docking also was exhibited in Table S2. For the docking of MAOB, Fig. 3A and Fig. 3a showed the KDZ-6 stabilized by the nine hydrogen bonds involving the amino acid residues Tyr398, Gly434, Arg42, Arg36 and Val235 in MAOB, respectively. And it also interacted with sixteen amino acid residues of MAOB via hydrophobic forces. Similarly, from Fig. 3B and Fig. 3b, it can be seen that KDZ-6 is embedded to the active site of MAPK8. Nine hydrogen bonds were established between the ingredient and the protein referring to the six amino acid residues, Asp151, Arg69, Asp169, Gly38, Glu109 and Met111. It was also adjacent to twelve amino acid residues via hydrophobic interactions. Moreover, KDZ-6 possessed a good fit for the active sites of GSTP1 as depicted in Fig. 3C and Fig. 3c. Two hydrogen bond was found in the docking pose referring to the amino acid residues Tyr7 and Tyr108 in GSTP1. At the same time, there are hydrophobic interactions between the ligand and the amino acid residues Val10, Gly205, Trp38, Arg13, Ile104 or Phe8. The representation of the binding interaction of KDZ-6 with the active site of AKR1C1 was exhibited in Fig. 3D and Fig. 3d. KDZ-6 was bound to the amino acid residues Tyr24, Asp50, Thr23, His222, Tyr55, Gln190, Asn167, His117 and Tyr216 though eleven hydrogen bonds, and the hydrophobic interactions involving eleven others amino acid residues in AKR1C1. The docking

results not only showed the active sites in target proteins and its interactive force, but also suggested that KDZ-6 might be of great significance for the development of new drugs as a multi-target ingredient in the future.

Annotation analysis for biological function of potential targets

To study the biological functions of the potential targets, GO enrichment analysis and KEGG analysis were performed in this research. Figure 4 and Fig. 5 shows the top 20 crucially significant GO molecular function annotations and pathways (p value < 0.01) respectively. The results of GO enrichment mainly included endopeptidase activity, serine-type endopeptidase activity, serine-type peptidase activity, serine hydrolase activity, transmembrane receptor protein kinase activity, carboxylic acid binding and organic acid binding, etc.. As shown in Fig. 5, KEGG pathway analysis showed that the potential targets were intensively associated to the pathways as follows: Colorectal cancer, Complement and coagulation cascades, Fluid shear stress and atherosclerosis, Focal adhesion, Prostate cancer, Glycolysis / Gluconeogenesis, Epithelial cell signaling in Helicobacter pylori infection, Adherens junction, Pancreatic cancer, etc..

PPI and Compound-Target-Pathway network construction

The potential protein interaction relationship among the targets was constructed (Fig. 6A). There were 36 nodes and 202 edges after hiding disconnected nodes. Among them the targets CASP3 (degree: 14; Betweenness: 0.14713729), PLG (degree: 12; Betweenness: 0.13976426), MAPK8 (degree: 14; Betweenness: 0.1048131), EGFR (degree: 16; Betweenness: 0.20036317), SRC (degree: 14; Betweenness: 0.12330768) were probably the most important roles in this protein interaction networks.

The Compound-Target-Pathway network was constructed by the connection of potential pathways and corresponding targets (Fig. 6B), which resulted in 69 nodes (10 compounds, 39 targets, 20 signal pathways) and 554 edges. The possible interactions between compounds and target proteins were also evaluated with connection degree and the Betweenness. The targets EGFR (degree: 24; Betweenness: 0.15889369), MAPK10 (degree: 20; Betweenness: 0.09662042), SRC (degree: 19; Betweenness: 0.07250202), MAPK8 (degree: 15; Betweenness: 0.0466211), GSTP1 (degree: 12; Betweenness: 0.02678676), IGF1R (degree: 12; Betweenness: 0.03521009) and MIF (degree: 11; Betweenness: 0.02760243) have higher connection degrees and Betweenness in the network. What's more, the interactions between the identified compounds (KDZ-1 to KDZ-10) and the key targets (EGFR, MAPK10, SRC, MAPK8, GSTP1, IGF1R and MIF) were also modeled by both docking softwares, Sybyl-X and Autodock vina. The docking scores were exhibited in Table 3. Comparatively, the bonds between KDZ-6 and EGFR, KDZ-8 and MAPK10, KDZ-6 and SRC, KDZ-6 and MAPK8, KDZ-6 and GSTP1, KDZ-6 and IGF1R, and KDZ-6 and MIF have better performance with higher the total Score and lower binding energy. Most of these hub target proteins interacted with KDZ-6 compound well, which also provided evidence to support that KDZ-6 might be potential multi-targets active compounds and deserve to be further investigated and developed as a drug candidate.

Table 3

Docking scores of the hub targets with the main active ingredients of KDZ injection.

	Sybyl-X/vina	KDZ-1	KDZ-2	KDZ-3	KDZ-4	KDZ-5	KDZ-6	KDZ-7	KDZ-8	KDZ-9	KDZ-10
EGFR	Total score	7.7	5.65	5.59	5.6	5.37	8.12	5.18	7.86	7.36	7.86
	Binding energy (kcal/mol)	-7.5	-7.1	-7.4	-8.1	-6	-7.8	-6.8	-8.4	-8.6	-8.4
MAPK10	Total score	6.8	6.11	5.4	5.8	4.18	8.29	5.53	9.19	7.78	5.68
	Binding energy (kcal/mol)	-8.4	-8.4	-8.4	-9.7	-6.3	-9	-7.4	-9.8	-9.7	-9.9
SRC	Total score	7.36	6.44	7.58	6.31	5.51	9.8	9.95	1.81	9.12	8.8
	Binding energy (kcal/mol)	-8.2	-7.9	-8.9	-9.5	-6.7	-8.7	-7.5	-9.8	-10.1	-9.7
MAPK8	Total score	6.89	5.93	5.72	5.47	5.35	10.32	5.09	7.09	8.64	7.03
	Binding energy (kcal/mol)	-8.7	-8.7	-9	-9.1	-6.5	-9.3	-7.8	-10.5	-10	-9.3
GSTP1	Total score	3.81	2.92	5.36	6.16	5.58	9.53	6.63	4.55	7.42	6.18
	Binding energy (kcal/mol)	-6.5	-6.4	-6.8	-8	-5.7	-6.7	-5.8	-7.4	-7.6	-7.1
IGF1R	Total score	4.25	4.04	4.22	5.08	4.66	8.7	3.8	5.61	4.97	3.8
	Binding energy (kcal/mol)	-7.2	-6.9	-7.2	-7.6	-5.8	-8	-6.3	-8	-8.3	-8.2
MIF	Total score	6.47	4.22	8.01	1.17	6.01	11.39	8.93	3.05	3.69	0.6
	Binding energy (kcal/mol)	-5.8	-5.8	-6	-6.5	-5.5	-5.8	-5.3	-5.8	-6.7	-6.7

Discussion

In recent years, computational and virtual screening have attracted considerable attention. In the present study, molecular docking and network pharmacology were carried out to elucidate the interactions between the 10 compounds in KDZ injection (KDZ-1 to KDZ-10) and the 39 potential targets.

According the Compound-Target-Pathway network, the target EGFR and MAPK pathway had the highest network connection degree, which could suggest the important significance of EGFR protein and MAPK pathway for the KDZ injection to exert its anti-MI therapeutic effects. Studies have shown that myocardial injuries caused by ischemia is associated with hostile factors like reactive oxygen species (ROS), inadequate angiogenesis and inflammation [30]. ROS as one of the largest hostile factors generated in ischemic surroundings could directly injure cardiomyocytes and vascular cells and further impair myocardial function [31–34]. High concentrations of ROS promotes cell apoptosis by inducing the activation of JNKs and members of MAPK family [35]. In other words, the inhibit of JNKs would attenuate myocardial ischemic apoptosis [36]. Epidermal growth factor (EGF) is also known to activate Src and MAPK pathways [37]. Therefore, down-regulation of epidermal growth factor receptor (EGFR) and MAPK family might be beneficial in preventing and treating myocardial ischemia. According to these results, it can be deduced that the active ingredients of KDZ injection could exert their anti-MI effects via binding the EGFR and inhibit the MAPK pathway.

Cellular apotosis is another critical event mediating the impairment of cardiovascular function [38–40]. In the focal adhesion pathway, the expression of adhesion molecule is a prerequisite for neutrophil-endothelium adherence, which in turn contributes to myocardial ischemia-reperfusion injury [41]. Focal adhesion kinase (FAK), a key enzyme in the integrin-mediated signal transduction process of adhesion molecules, promotes cell survival. Src kinase plays an upstream role in mediating the activation of known adhesion, survival, and stress-activated signaling pathways in response to oxidative stress. Both the FAK and Src are of great significance to mediating cell-matrix adhesion [42]. In the present study, the target Src and the focal adhesion pathway also showed high connection degree, suggesting their considerable roles on anti-MI for the intervening of the multiple active ingredients of KDZ injection.

In addition, MIF elevation could respond to myocardial ischemia, which might be a useful early and specific diagnostic biomarker for myocardial ischemia [43]. GSTP decreases the sensitivity of the heart caused by ischemic injury and IGF-1R takes precautions against the detrimental effects of myocardial infarction. Both of them have a protective effect on the myocardium [44–46]. In general, KDZ injection might correlate with these targets as shown in figure and their related pathways in the treatment of MI, which requires to further experimental verification.

Conclusions

As a traditional Chinese medicine injection, KDZ injection was extensively applied in clinical medicine for invigorating blood circulation for many years. Virtual screening and an integrated system pharmacology approach in this study was used to evaluate the relationship between identified compounds and predicted targets against MI. GO molecular function and KEGG pathway enrichment analysis, constructions of Compound-Target-Pathway network and molecular docking were further performed. A total of 39 MI-related targets of KDZ injection were predicted. The targets of EGFR, MAPK10, SRC, MAPK8, GSTP1, IGF1R and MIF and the signal pathways MAPK, Focal adhesion, Complement and coagulation cascades, Fluid shear stress

and atherosclerosis, Prostate cancer, Glycolysis / Gluconeogenesis, Epithelial cell signaling in Helicobacter pylori infection, Adhesion junction, and Pancreatic cancer could involve in the exerting of anti-MI therapeutic effect of KDZ injection. In conclusion, this research provides a scientific basis to clarify the comprehensive pharmacological mechanism of KDZ injection acting on MI, and it could provide valuable clues on considering the active ingredient KDZ-6 as a multi-targets leading compound.

Abbreviations

KDZ: Kudiezi; HPLC: High-performance liquid chromatography; MI: Myocardial Ischemia; AMI: Acute Myocardial Infarction; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology; PPI: Protein-protein interaction

Declarations

Acknowledgments

Not applicable.

Authors' contributions

HY and GL formulated the idea of the paper and supervised the research. RX and ZZL performed the research, analyzed the data and wrote the manuscript. ZL and BC molecular docking. SL and LH made contribution to the part of network pharmacology. XL participated in revising the data and improving manuscript writing. All authors reviewed the manuscript. And all authors read and approved the final version of the manuscript.

Funding

The study was supported by the National Nature Science Foundation of China (No.81973558), the Fujian Provincial Natural Science Foundation (No.2018J01596 and No.2016J01371), Joint Funds for the innovation of science and Technology, Fujian province (No.2017Y9123), Program for New Century Excellent Talents in Fujian Province University (2018), and Training program for excellent scientific research talents of young teachers in Fujian Province University (2017).

Availability of data and materials

We have presented all our main data in additional file. The datasets supporting the conclusions of this article are available in public database from Pharmapper, PDB database, PDB database.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Department of Pharmaceutical Analysis, School of Pharmacy, Fujian Medical University, Fuzhou 350122, China. [2] Department of Traditional Chinese Medicine Resource, Fujian Agriculture and Forestry University, Fuzhou, 350002, China. [3] Experimental Center of Chemistry, School of Pharmacy, Fujian Medical University, Fuzhou 350122, China.

References

1. Dagres N, Hindricks G. Sudden cardiac death in acute coronary syndromes. Card. Electrophysiol. Clin. 2017;9(4):725–730.
2. Ahmed S, Khan H, Mirzaei H. Mechanics insights of curcumin in myocardial ischemia: Where are we standing?. Eur. J. Med. Chem. 2019;183(1):111658.
3. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Kardiol. Pol. 2018;76(2):229–313.
4. Eltzschig HK, Bratton DL, Colgan SP. Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. Nat. Rev. Drug Discov. 2014;13(11):852–869.
5. Amin S, Khan H. Revival of natural products:utilization of modern technologies. Curr. Bioact. Comp. 2016;12:103–106.
6. Amin S, Ullah B, Ali M, et al. Potent in vitro α-glucosidase inhibition of secondary metabolites derived from dryopteris cycadina. Molecules. 2019;24(3):427.
7. Khan H, Amin S, Patel S. Targeting BDNF modulation by plant glycosides as a novel therapeutic strategy in the treatment of depression. Life Sci. 2018;196:18–27.
8. Khan H, Nabavi SM, Sureda A, et al. Therapeutic potential of songorine, a diterpenoid alkaloid of the genus Aconitum. Eur. J. Med. Chem. 2018;153:29–33.
9. Khan H, Sureda A, Belwal T, et al. Polyphenols in the treatment of autoimmune diseases. Autoimmun. Rev. 2019;18(7):647–657.
10. Sharifi-Rad M, Ozcelik B, Altın G, et al. Salvia spp. plants-from farm to food applications and phytopharmacotherapy. Trends Food Sci. Technol. 2018;80;242–263.
11. Mishra AP, Saklani S, Salehi B, et al, Satyrium nepalense, a high altitude medicinal orchid of Indian Himalayan region: chemical profile and biological activities of tuber extracts. Cell. Mol. Biol. 2018;64(8): 35–43.
12. Liu X, Tao Y, Wang F, et al. Kudiezi injection mitigates myocardial injury induced by acute cerebral ischemia in rats. BMC Complement. Altern. Med. 2017;17(1): 8.
13. Yuan L, Zhang Z, Hou Z, et al. Rapid classification and identification of complex chemical compositions in traditional Chinese medicine based on UPLC-Q-TOF/MS coupled with data processing technique using KuDieZi injection as an example. Anal. Methods. 2015;7:5210–

14. Zhang WJ, Huai Y, Miao ZP, et al. Wang, Systems pharmacology for investigation of the mechanisms of action of traditional Chinese medicine in drug discovery. Front. Pharmacol. 2019;10:743.
15. Liu W, Zhang XW, Mao B, et al. Systems pharmacology-based study of Tanreqing injection in airway mucus hypersecretion. J.Ethnopharmacol. 2020;249:112425.
16. Yao H, Huang XM, Xie YJ, et al. Identification of pharmacokinetic markers for Guanxin Danshen drop pills in rats by combination of pharmacokinetics, systems pharmacology, and pharmacodynamic assays. Front. Pharmacol. 2018;9:1493.
17. Hua Q, Liu Y, Liu C, et al. Revealing synergistic mechanism of multiple components in *Stauntonia brachyanthera*-Mazz. for gout by virtual screening and system pharmacological approach. Bioorg. Chem. 2019;91:103118.
18. Wang Y, Sun Y, Wang Y, et al. Virtual screening of active compounds from *Artemisia argyi* and potential targets against gastric ulcer based on Network pharmacology. Bioorg. Chem. 2019;88:102924.
19. Yu GH, Luo ZQ, Zhou YT, et al. Uncovering the pharmacological mechanism of *Carthamus tinctorius* on cardiovascular disease by a systems pharmacology approach. Biomed. Pharmacother. 2019;117:109094.
20. Liu XF, Ouyang SS, Yu B, et al. PharmMapper Server: a web server for potential drug target identification via pharmacophore mapping approach. Nucleic Acids Res. 2010;38:W609–W614.
21. Wang X, Pan CX, Gong JY, et al. Enhancing the enrichment of pharmacophore-based target prediction for the polypharmacological profiles of drugs. J. Chem Inf. Model. 2016;56(6):1175–1183.
22. Wang X, Shen YH, Wang SW, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. Nucleic Acids Res. 2017;45(W1):W356–W360.
23. Lu JK, Hu YC, Wang LC, et al. Understanding the Multitarget Pharmacological Mechanism of the Traditional Mongolian Common Herb Pair GuangZao-RouDouKou Acting on Coronary Heart Disease Based on a Bioinformatics Approach. Evid Based
24. Liu Y, Hua Q, Ju Y, et al. Chemical constituents and synergistic anti-gout studies on Eurycoma longifolia and potential mechanisms evaluation based on systemic analysis approach. Bioorg. Chem. 2019;92:103302.
25. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010;31(2):455–461.
26. Kolšek K, Mavri J, Sollner Dolenc M, et al. Endocrine disruptome — an open source prediction tool for assessing endocrine disruption potential through nuclear receptor binding. J. Chem. Inf. Model. 2014;54(4)1254–1267.
27. Laskowski RA, Swindells MB. LigPlot+: Multiple ligand–protein interaction diagrams for drug discovery. J. Chem. Inf. Model. 2011;51(10):2778–
28. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters.OMICS. 2012;16(5):284–7.
29. Smoot ME, Ono K, Ruscheinski J, et al. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27(3):431–432.

30. Song H, Cha ME, Song BW, et al. Hwang, Reactive oxygen species inhibit adhesion of mesenchymal stem cells implanted into ischemic myocardium via interference of focal adhesion complex. Stem Cells. 2010;28(3):555–563.
31. Dhalla NS, Elmoselhi AB, Hata T, et al. Status of myocardial antioxidantsin ischemia-reperfusion injury. Cardiovasc. Res. 2000;47(3):446–456.
32. Fan H, Sun B, Gu Q, et al. Oxygen radicals trigger activation of NF-κB and AP-1 and upregulation of ICAM-1 in reperfused canine heart. Am. J. Physiol.-Heart Circul. Physiol. 2002;282(5):H1778–H1786.
33. Kloner RA, Jennings RB. Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2. Circulation.. 2001;104(24):3158–3167.
34. Ren G, Dewald O, Frangogiannis NG. Inflammatory mechanisms in myocardial infarction. Curr. Drug Targets Inflamm. Allergy. 2003;2(3):242–256.
35. Lee HS, Hwang CY, Shin SY, et al. MLK3 is part of a feedback mechanism that regulates different cellular responses to reactive oxygen species. Sci. Signal. 2014;7(382): ra52.
36. Inagaki K, Hahn HS, Dorn GW, et al. Additive protection of the ischemic heart ex vivo by combined treatment with δ-protein kinase C inhibitor and ε-protein kinase C activator. Circulation. 2003;108(7):869–75.
37. Clemente L, Boeldt DS, Grummer MA, et al. Adenoviral transduction of EGFR into pregnancy-adapted uterine artery endothelial cells remaps growth factor induction of endothelial dysfunction. Mol. Cell. Endocrinol. 2020;499:110590.
38. Akki A, Zhang M, Murdoch C, et al. NADPH oxidase signaling and cardiac myocyte function. J. Mol. Cell. Cardiol. 2009;47(1):15–22.
39. Brown DI, Griendling KK. Nox proteins in signal transduction. Free Radic. Biol. Med. 2009;47(9):1239–1253.
40. Doerries C, Grote K, Hilfiker-Kleiner D, et al. Critical role of the NAD(P)H oxidase subunit p47 phox for left ventricular remodeling/dysfunction and survival after myocardial infarction. Circ. Res. 2007;100(6):894–903.
41. Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. Cardiovasc. Res. 2004;61(3):481–497.
42. Hui AY, Meen JA, Schick C, et al. Src and FAK mediate cell–matrix adhesion-dependent activation of met during transformation of breast epithelial cells. J. Cell. Biochem. 2009;107(6):1168–81.
43. Fan F, Dart AM. GW26-e1326 Exercise induced myocardial but not peripheral muscle ischemia is associated with rise in plasma macrophage migration inhibitory factor (MIF). J. Am. Coll. Cardiol. 2015;66(16):C131–C132.
44. Conklin DJ, Guo Y, Jagatheesan G, et al. Genetic deficiency of glutathione s-transferase p increases myocardial sensitivity to ischemia–reperfusion injury. Circ. Res. 2015;117(5):437-49.
45. Enoki C, Otani H, Sato D, et al. Enhanced mesenchymal cell engraftment by IGF-1 improves left ventricular function in rats undergoing myocardial infarction. Int. J. Cardiol. 2010:138(1):9–18.
46. Huynh K, McMullen JR, Julius TL, et al. Cardiac-specific IGF-1 receptor transgenic expression protects against cardiac fibrosis and diastolic dysfunction in a mouse model of diabetic cardiomyopathy. Diabetes.

2010;59(6):1512–1520.

Figures

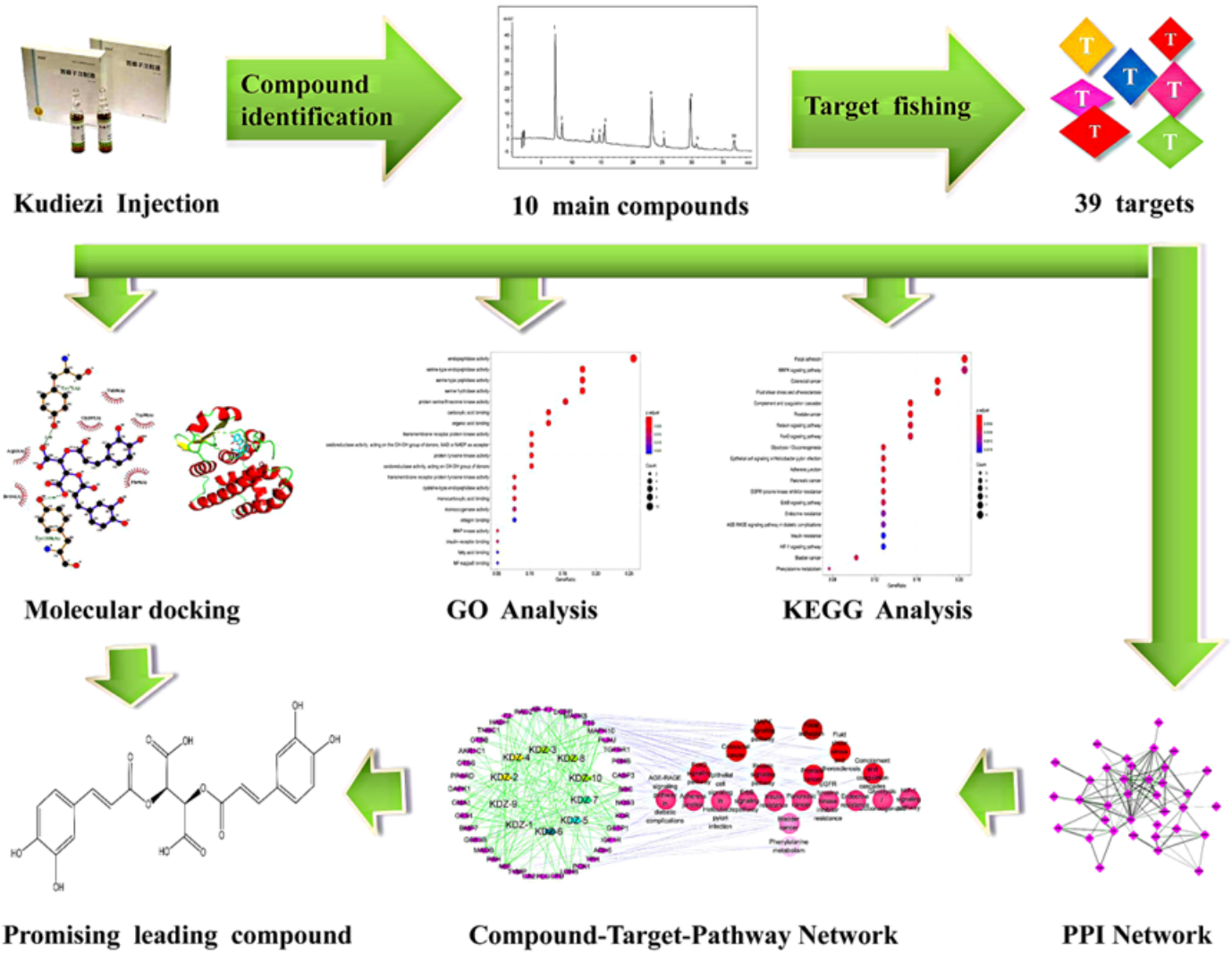

Figure 1

The whole framework of visual screening.

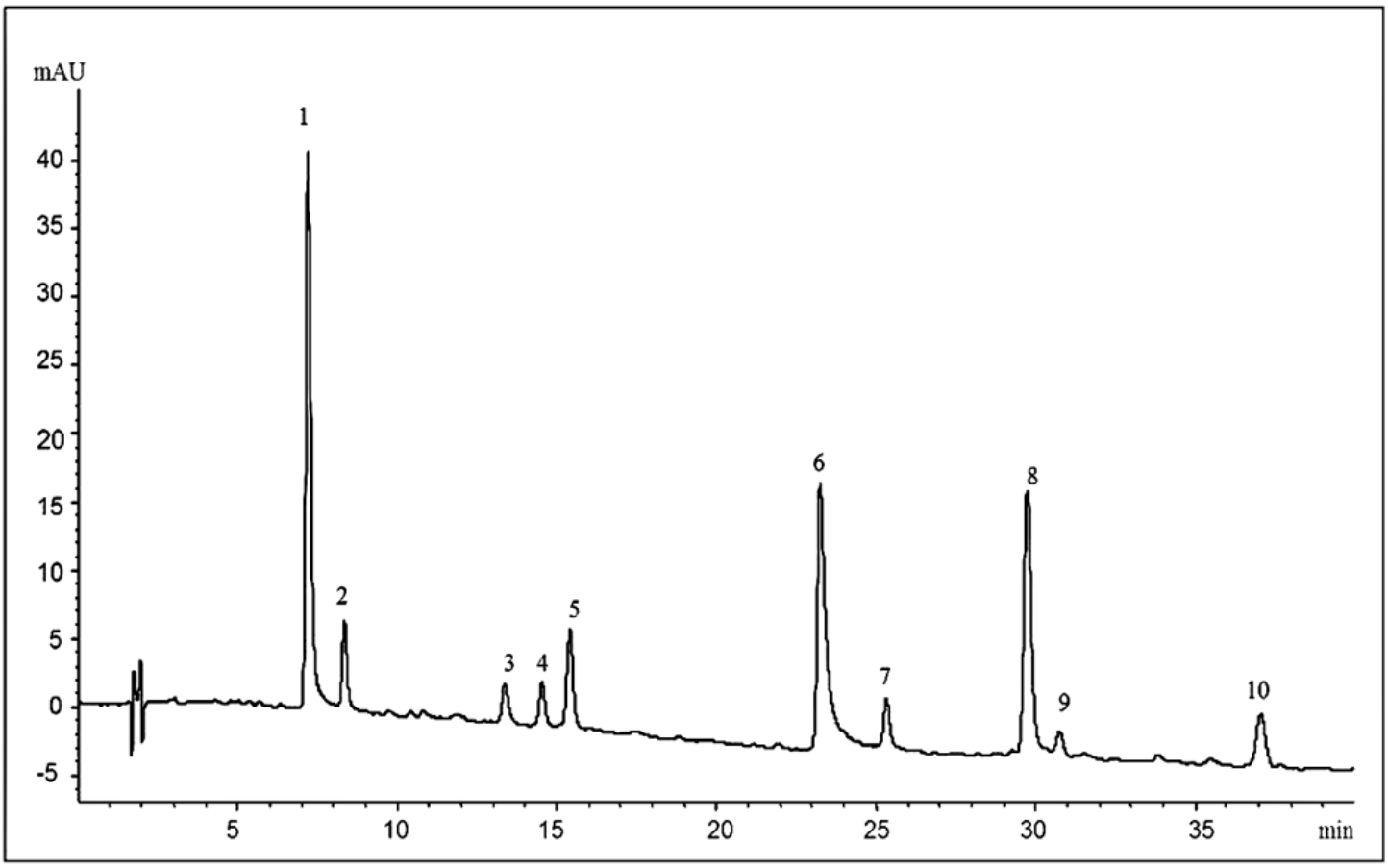

Figure 2

HPLC chromatogram of KDZ injection.

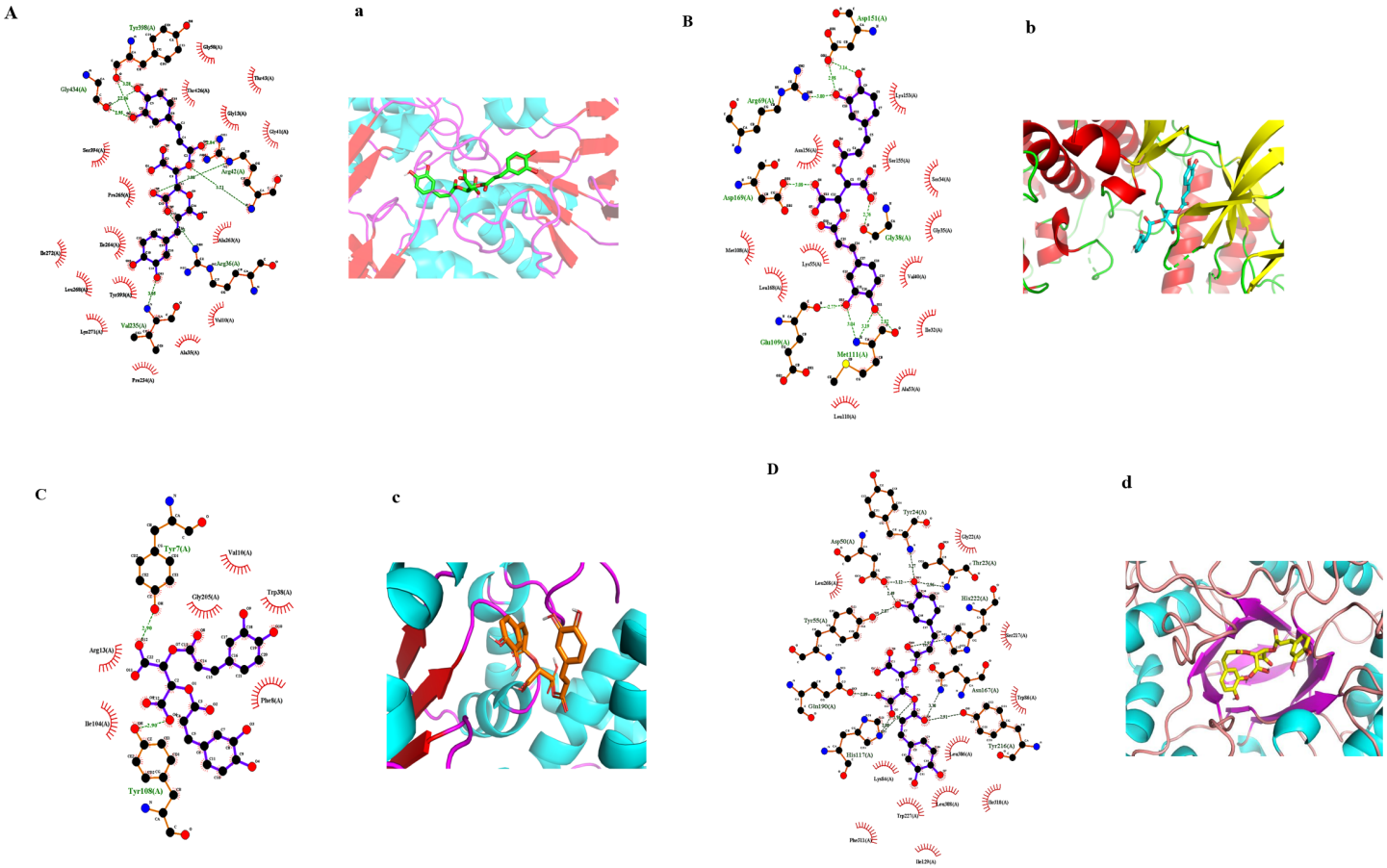

Figure 3

Hydrogen bonds and hydrophobic interactions (A-D). Best-docked conformations (a-d).

Figure 4

GO enrichment entries in the top 20 ($p < 0.01$).

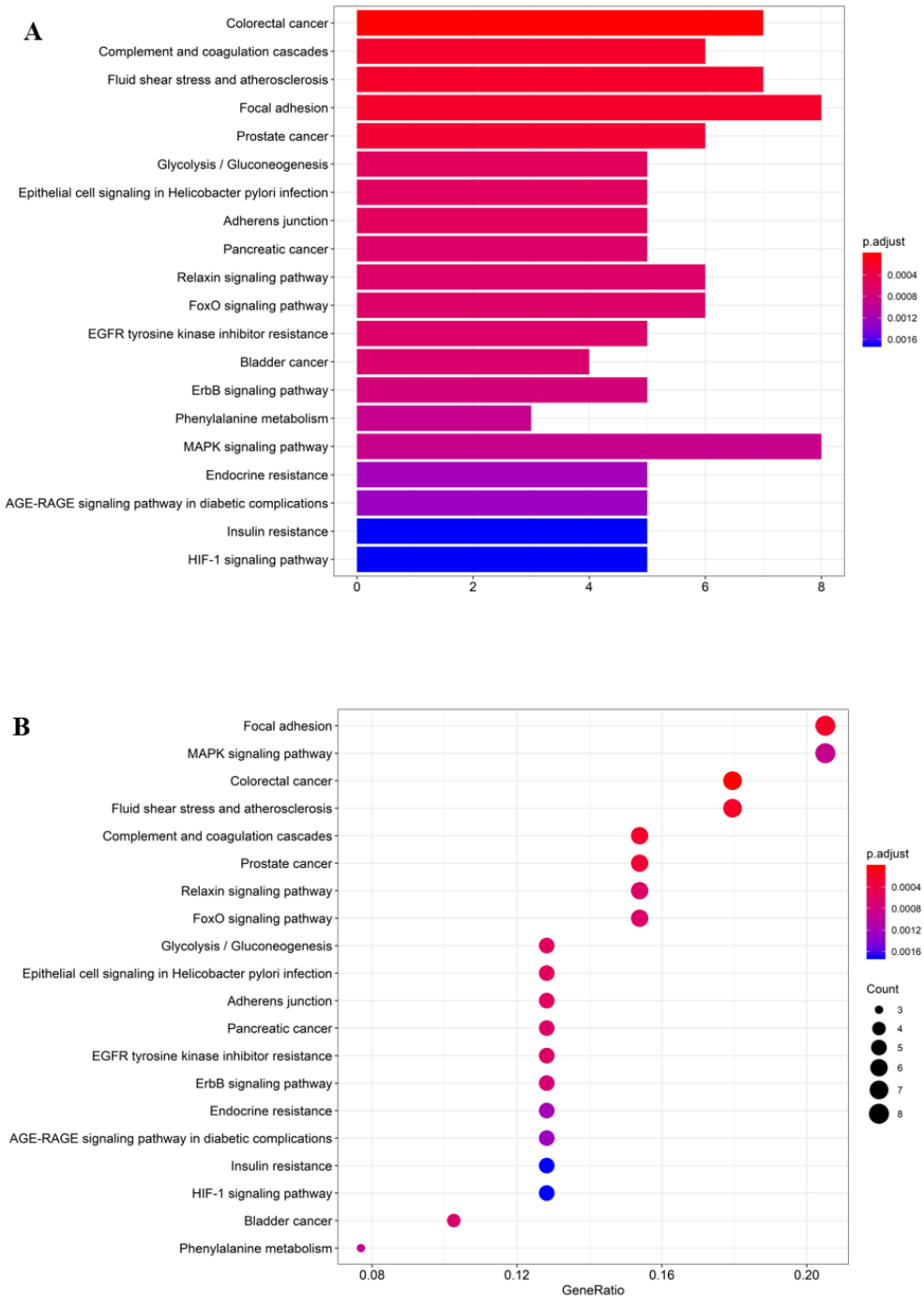

Figure 5

KEGG pathway enrichment entries in the top 20 ($p < 0.01$).

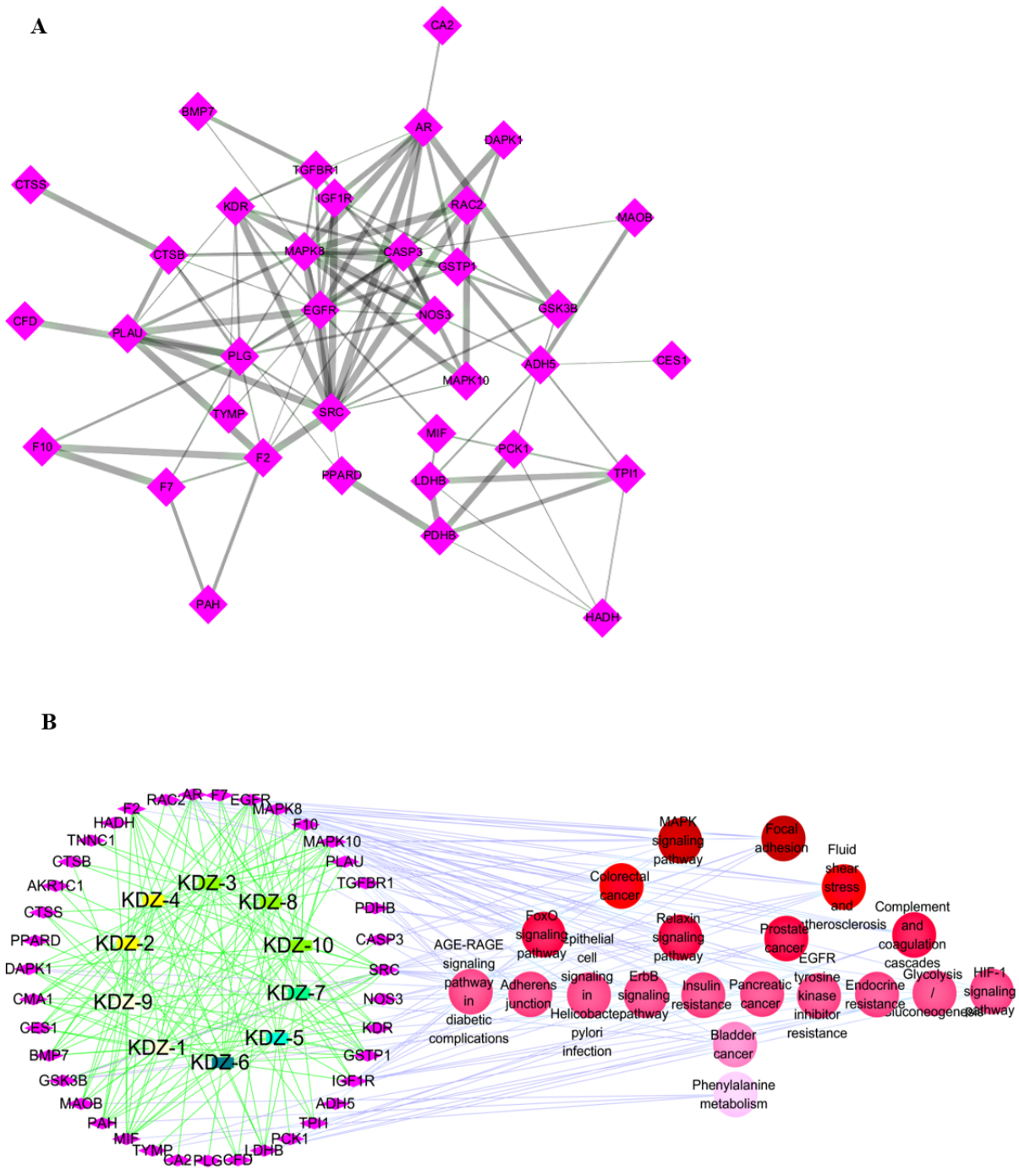

Figure 6

PPI and Compound-Target-Pathway (C-T-P) network. Rectangle nodes represented compounds, which goes better in a clock wise according to color; diamond nodes represented targets; circle nodes represented pathways.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementary.doc
- Table2MainingredientsofKDZinjection.docx