Anticancer Effects of Herbal Medicines in Pancreatic Cancer Through Modulation of Steroid Hormone Response Proteins

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Abstract

Many individual herbs and herbal formulae have been demonstrated to provide safe and effective treatment for Pancreatic cancer (PC); however, the therapeutic mechanisms underlying their effects have not been fully elucidated. A total of 114 herbal formulae comprising 216 single herbal medicines used to treat PC were identified. Cluster analysis revealed a core prescription including four herbs [*Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and *Pinelliae Rhizoma* (Ban Xia)] in combination to treat PC, and 295, 256, 141, and 365 potential targets were screened for each of these four herbs, respectively. PC-related proteins (n = 2940) were identified from the DisGeNET database. Finally, 44 overlapping targets of herbs and PC were obtained, representing potential targets of the herbal medicines for PC treatment. GO enrichment analysis indicated that targets common to herbs and PC primarily functioned in response to steroid hormones. KEGG pathway enrichment analysis indicated that the herbs may prevent PC by influencing apoptotic, p53, and PI3K/Akt signaling pathways. Further, molecular docking analysis indicated that of identified bioactive compounds, stigmasterol, phaseol, perlolyrine, shinpterocarpin, and licopyranocoumarin have good binding ability with proteins involved in responses to steroid hormones, while stigmasterol, phaseol, perlolyrine, and DIOP have good binding ability with PTGS2(also known as COX-2), ESR1, ESR2, AR, and PGR.

Conclusion: The anti-PC activity of herbal medicines may be mediated via regulation of proteins with roles in responses to steroid hormones. This study provides further evidence supporting the potential for use of herbal medicines to treat PC.

1 Introduction

Despite decades of research, the outcomes of patients with pancreatic cancer (PC) remain poor. Compared with favorable prognosis tumors, PC is characterized by its high degree of malignancy, insidious onset, no typical symptoms, many anatomical sites, low resection rate, high recurrence rate and poor prognosis. PC patients have an average 5-year survival rate of less than 10%, and less than 3% for patients with advanced or metastatic disease(Chen et al. 2021). The incidence of PC continues to increase and there is an urgent need to improve survival rates.

Complementary and alternative medicines that are effective for PC patients include gene therapy, immunotherapy, targeted therapy, neoadjuvant therapy and natural medicine/herbal medicine (Mohammed et al. 2015). Among complementary and alternative medicines, natural products/herbal medicines, such as Chinese herbal medicines, have become the choice of more advanced cancer patients due to their good therapeutic effects and fewer side effects(Mohammed, et al. 2015). In the United States, about 50%-60% of cancer patients use agents from plants entirely or in combination with traditional treatment regimens such as chemotherapy and radiation therapy(Gutheil et al. 2012). The combined use of herbal medicines with conventional chemotherapy and radiotherapy can improve the anti-cancer

efficacy and reduce the side effects. Therefore, the development of new anti-PC drugs based on herbal medicines has a good prospect of application (Yue et al. 2017).

Many individual herbs and herb formulae can provide safe and effective treatment for PC (Kuo et al. 2018a; Kuo et al. 2018b; Yang et al. 2015), and recent studies support a better prognosis for patients with PC who receive Chinese medicine treatment compared with those undergoing conventional treatment alone (Wong et al. 2019). Herbal formulations, including Chinese herbs, are derived from thousands of years of human experience and practical application; however, which specific herbs are beneficial for patients with PC requires further investigation. To release the full potential of herbal medicine for cancer therapy and broaden its application, the molecular mechanisms underlying the therapeutic activity of herbal medicine formulae in PC must be further explored.

The current study was designed to explore the herb combinations and underlying molecular mechanisms of traditional Chinese medicine prescriptions used to treat PC. In total, 114 herbal formulae tested for the treatment of PC in randomized controlled experiments were identified. The most frequently used herbs and the associations between use of different herbs were determined. The molecular mechanism underlying the treatment of PC with four core herbs frequently used in combination [*Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and *Pinelliae Rhizoma* (Ban Xia)] was further investigated. Enrichment analysis of pathways and gene ontologies were applied to 44 protein targets common to the core herbs and PC. The results suggested that the four core herbs may function in PC treatment by influencing responses to steroid hormones, as well as apoptotic, p53, and PI3K/Akt signaling pathways. Molecular auto-dock analysis was used to validate predicted herb compound-target interactions.

2 Materials And Methods

2.1 Data Sources and Selection Criteria

PC treatment-related classical prescriptions were retrieved from the published literature by screening the CNKI (https://www.cnki.net/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases.

Only publications meeting the following criteria were selected: randomized controlled or clinical controlled experiments to investigate the treatment of PC; diagnostic criteria meeting the “Guidelines for the Diagnosis and Treatment of Pancreatic Cancer in China”, formulated by the Chinese Medical Association; the treatment evaluation standard adopted was an internationally recognized universal standard; symptoms or indicators of PC were improved or cured following the application of traditional Chinese medicine prescriptions.

A dataset of standardized names was then compiled, by substituting all of the polysemes, synonyms, and acronyms of the herbs, according to Chinese Pharmacopoeia (2020 edition) and Chinese Materia Medica. The frequencies of occurrence of single herbs were calculated.

2.2 Association Rule and Cluster Analysis

Apriori algorithm-based association rule analysis was conducted and the results plotted using SPSS Modeler software. The support degree indicates the probability of simultaneous occurrence of two drugs, and the confidence degree (A→B) represents the probability of occurrence of medicine B under the condition of occurrence of medicine A. An association network diagram of 24 drugs was constructed.

Hierarchical clustering of the 24 herbs used most frequently was conducted using R.

2.3 Establishment of a Database of Core Herb Target Genes and PC-Related Genes

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; https://tcmsp-e.com/) was searched and the chemical components and target genes of herbs retrieved.

PC-related genes were collected from the DisGeNET database (https://www.disgenet.org/home/) using the keywords “Pancreatic carcinoma” or “Pancreatic Ductal Adenocarcinoma”.

A Protein-Protein Interaction (PPI) Network Map was conducted by entering targets common to retrieved herbs and PC into the STRING online database (https:// string-db.org/); species, “*Homo sapiens*”. The resulting “tsv” file was imported into Cytoscape 3.9.1 software for further analysis of the core network.

2.4 Gene Ontology and KEGG Pathway Enrichment Analysis

The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed with R. The results are displayed in bubble charts with homemade R scripts. Network construction was generated using the network visualization software, Cytoscape (ver. 3.5.0).

2.5 Molecular Docking

Download the two-dimensional (2D) structure diagram of the compound from the PubChem database (Kim, Chen, & Cheng, 2019), import it into Chem3D software, draw the three-dimensional structure diagram of the compound, optimize the energy, and save the structure in mol2 format. Next, import the files into AutoDockTools-1.5.6 software, add charging information and display rotatable keys, and save them in pdbqt format. Download the protein crystal structures corresponding to the core target genes from the PDB database (Goodsell, Zardecki, & Di Costanzo, 2020), import PyMOL software to remove water molecules and heteromolecules, import Auto-DockTools-1.5.6 software to add hydrogen atoms, save them in pdbqt format. These compounds were used as ligands, and the proteins were used as receptors for molecular docking analysis. Autodock vina-1.1.2 (http://vina.scripps.edu/,Trott & Olson, 2010) was used to estimate the binding ability of the molecules and targets. Results were analyzed and interpreted using PyMOL software and Discovery Studio 3.5 Client.

3 Results

3.1 High Frequency Herbal Medicines and Association Rule Analysis for Herb Combinations

Of the 216 herbal medicines included in the 114 prescriptions identified by literature screening, the total frequency of drug use was 1613 times (Supplementary Table 1). *Atractylodis macrocephalae Rhizoma* (Bai Zhu), *Poria* (Fu Ling), *Hedyotis diffusa Willd*. (Bai Hua She She Cao), *Astragali Radix* (Huang Qi), and *Glycyrrhizae Radix Et Rhizoma* (Gan Cao) were the herbal medicines most frequently used in the clinic (Fig. 1). Twenty-four Chinese medicines used more than 15 times were identified (Fig. 2A). These results suggest that those herbs were preferred for treatment of PC.

Chinese herbal medicine compatibility refers to the purposeful combination of two or more herbs, according to clinical requirements and pharmacodynamic effects, and is the main method used for clinical drug application and the basis for the composition of Chinese herbal medicine prescriptions.

Association rule analysis was carried out for 24 medicines used at high frequency using an apriori algorithm. We focused on two parameters: support and confidence level, where support was set as ≥ 20% and confidence level as ≥ 80% to obtain the top 10 herb pairs and suitable association rules (Table 1). The association between *Atractylodis macrocephalae rhizoma* (Bai Zhu) and *Poria* (Fu Ling) had the highest degree of support (52.63%), while those of *Glycyrrhizae radix et rhizom*a (Gan Cao) and *Atractylodis macrocephalae rhizoma* (Bai Zhu) with *Poria* (Fu Ling) had the highest confidence level (92%). The resulting association network diagram is presented in Fig. 2B.

Table 1
Apriori algorithm-based association rules for herbs used to treat pancreatic cancer

No.	Association rules	Support(%)	Confidence (%)
1	*Atractylodis macrocephalae rhizoma* (Bai Zhu) => *Poria* (Fu Ling)	52.63	81.67
2	*Poria* (Fu Ling) = > *Atractylodis macrocephalae rhizoma* (Bai Zhu)	51.75	83.05
3	*Scutellariae barbatae herba* (Ban Zhi Lian) = > *Hedyotisdiffusa Willd* (Bai Hua She She Cao)	28.07	81.25
4	*Citri reticulatae pericarpium* (Chen Pi) = > *Poria* (Fu Ling)	26.32	80.00
5	*Astragali radix* (Huang Qi) and *atractylodis macrocephalae rhizoma* (Bai Zhu) => *Poria* (Fu Ling)	26.32	80.00
6	*Astragali radix* (Huang Qi) and *Poria* (Fu Ling) = > *Atractylodis macrocephalae rhizome* (Bai Zhu)	25.44	82.76
7	*Codonopsis radix* (Dang Shen) and *atractylodis macrocephalae rhizoma* (Bai Zhu) => *Poria* (Fu Ling)	22.81	88.46
8	*Glycyrrhizae radix et rhizoma* (Gan Cao) and *atractylodis macrocephalae rhizoma* (Bai Zhu) => *Poria* (Fu Ling)	21.93	92.00
9	*Coicis semen* (Yi Yi Ren) and *atractylodis macrocephalae rhizoma* (Bai Zhu) => *Poria* (Fu Ling)	21.93	82.00
10	*Hedyotisdiffusa Willd* (Bai Hua She She Cao) and *Poria* (Fu Ling) = > *Atractylodis macrocephalae rhizoma* (Bai Zhu)	21.05	91.67

3.2 Rules for Combination of Herbal Medicines Based on Cluster Analysis

Clustering classification is widely used to determine the compatibility of herbs and the rules for combination of different Chinese medicines. Here, we applied hierarchical cluster analysis to identify the core herbs that are used in combination for treatment of PC. The 24 drugs mentioned in the literature at the highest frequency were classified into five categories according to traditional Chinese medicine theory (Fig.3). Based on compatibility rules and clinical experience, the core prescription used for PC treatment included four herbs:*Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and*Pinelliae Rhizoma* (Ban Xia). Our results showed that these fourherbs, which are frequently used in the clinic, areoften used in combination to treat PC.

3.3 Identification of Potential Targets of Core Prescription for PC Treatment

To investigate the possible mechanism underlying the core prescriptions used to treat PC, the targets of the four selected herbs were obtained from the TCMSP database. In total, 295, 256, 141, and 365 potential targets were identified for *Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang

Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and *Pinelliae Rhizoma* (Ban Xia), respectively. Importantly, 84 targets were shared by the four herbs, and these were defined as the core prescription targets. Target proteins were associated with tumors and apoptosis (e.g., TP53, TNF, BAX, BCL2, CASP3, and CASP9, among others).

In addition, 2940 PC-related proteins were identified from the DisGeNET database. Among the 84 core prescription targets, 44 overlapped with proteins in the 2940 PC-related group (hypergeometric p-value < 9.04e-23; Fig. 4A). The 44 common proteins identified as both targets of the herbs and related to PC were considered to represent likely targets of the herbal medicines during PC treatment.

These 44 shared proteins were imported into STRING, and an Herb–PC target PPI network constructed using Cytoscape (Fig.4B).From this PPI network, several nodes (TNF, AKT1, TP53, HSP90AA1, MMP9, JUN, CASP3, and IL6) had high degree values. **Fig.4.** Targets of core prescriptions used for PC treatment. **(A)** Molecules in common between herb targets and PC-associated proteins. **(B)** PPI network diagram of the common targets of the four core herbs used to treat pancreatic cancer. The PPI network contains 44 nodes and 297 edges. Circles represent protein targets; orange circles indicate higher degree values. The node size of gene targets is proportional to the number of degrees.

3.4 GO and KEGG Enrichment Analysis

To elucidate the potential molecular mechanisms by which core prescriptions act on PC, GO biological process and KEGG pathway enrichment analyses were performed using the 44 identified core proteins.

The top 20 enriched GO biological process terms were determined (Fig. 5A), and analysis showed that the targets were closely related to processes involved in responses to steroid hormones and apoptotic signaling pathways. The most significantly enriched KEGG pathways included those involved in cancer, hepatitis B, apoptosis, p53 signaling, and PI3K/Akt signaling (Fig. 5B).

Target–GO term andTarget–KEGG pathway networks were then constructed, basedon the targets involved in each GO term or KEGG pathway. The Target–GO network comprised 46 nodes and 138 edges (Fig.5C). The majority oftargets were primarily implicated in responses to steroid hormones and apoptotic signaling pathways. In addition, the targets participating in the largest number of terms were PTGS2 (also known as COX-2), AKT1, and TNF, which were involved in 10, 9, and 8 GO terms,respectively. The Target–KEGG network included 36 nodes and 126 edges (Fig.5D).The results of both GO and KEGG analysis suggest that the mechanism of action of core prescriptions for treatment of PC involves stimulation of responses to steroid hormones and apoptotic signaling pathways.**Fig.5.** Functional analysis of common targets. **(A)** GO enrichment analysis of putative targets. **(B)** Target–GO network terms. **(C)** KEGG pathway enrichment analysis of putative targets. **(D)** Target–Signaling pathway network. Pink diamond nodes represent main signaling pathways and blue circle nodes refer to putative common targets of the four core herbs used for treatment of pancreatic cancer. Node size is proportional to the number of degrees.

3.5 Molecular Docking

To evaluate whether active compounds from core prescription components that possess good pharmacokinetic properties could bind directly to proteins involved in responses to steroid hormone, we applied molecular docking analysis to explore potential binding modes. The top 10 compounds with highest oral bioavailability and drug-likeness values for each herb were identified as active compounds, and included flavonoids, alkaloids, amino acids, steroids, and volatile oils, among other substances (Supplementary Table 2).

As shown in Fig. 6A, stigmasterol could bind to PTGS2 with the lowest binding energy (− 10.2 kcal/mol). The binding site of stigmasterol in PTGS2 was GLY-225. Further, the binding sites for phaseol in PTGS2 were TYR-130 and VAL-47 and the binding energy for phaseol with PTGS2 was − 10.1 kcal/mol (Fig. 6B). These results suggest that stigmasterol and phaseol could directly bind to PTGS2.

Furthermore, perlolyrine could bind to ESR1 with a binding energy of − 8.8 kcal/mol and DIOP bind to ESR2 with the same binding energy (Fig. 6C,D). Notably, phaseol and AR were able to bind with a free binding energy of − 8.6 kcal/mol, while the free binding energy of licopyranocoumarin with PGR was − 9.7 kcal/mol (Fig. 6E,F).

These results indicate that several active compounds from the four identified medicines could bind to proteins that function in responses to steroid hormones.**Fig.6.** Schematic 3D representation of molecular docking models, active sites, and binding distances. Binding modes of: stigmasterol to PTGS2 **(A)**, phaseol to PTGS2 **(B)**, perlolyrine to ESR1 **(C)**, DIOP to ESR2 **(D)**, phaseol to AR **(E)**, and licopyranocoumarin to PGR **(F)**.

4 Discussion

Plant-based medicines and plant-derived products remain the main source of therapeutics for much of the world's population. Traditional Chinese medicine involves combinations of numerous co-occurring biologically active compounds, and is a valuable source of therapeutic drugs that have been used for a relatively long period of history (T.J 2000). The medicinal compatibility model can reduce the number of targets used to identify active ingredients, which makes the arbitrariness of the drug discovery process more efficient and effective (Ding et al. 2014). Indeed, more than 60% of current anticancer chemotherapeutic drugs used in the clinic were initially developed from natural products/herbal medicines (Li and Leung 2014).

The current study determined effective herbal prescriptions for PC treatment by conducting a comprehensive analysis, based on clinical cases, integration of association rules, cluster analysis, network pharmacology, bioinformatic methods, and molecular auto-dock analysis.The results indicate that *Atractylodis macrocephalae Rhizoma* (Bai Zhu), *Poria* (Fu Ling), *Hedyotis diffusa Willd.* (Bai Hua She She Cao), *Astragali Radix* (Huang Qi), and *Glycyrrhizae Radix Et Rhizoma* (Gan Cao) are the herbs most frequently used for PC treatment. Importantly, several recent studies have suggested that herb combinations including *Atractylodis macrocephalae Rhizoma* (Bai Zhu), *Poria* (Fu Ling), *Astragali Radix* (Huang Qi), and *Glycyrrhizae Radix Et Rhizoma* (Gan Cao) can significantly improve symptoms in

patients with cancer and attenuate metastatic potential (Park et al. 2021; Zhu et al. 2018). The effectiveness of evidence-based strategies in selecting herbs for further treatment can be determined by their efficacy. To our best knowledge, this is the first report of a potential core combination of herbs used in prescriptions for treatment of PC.

Data accumulated from several studies have demonstrated that the use of herbs in combination may have greater pharmacological efficacy than the use of herbs singly (Lu et al. 2020; Qi et al. 2020; Wei et al. 2014). The current research reveals that combinations including four herbs used in Chinese medicine formulae, including *Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and *Pinelliae Rhizoma* (Ban Xia), are potentially useful for the treatment of PC.

To further investigate the molecular mechanisms underlying the effects of combinations use of these four core herbs for treatment of PC, we selected 44 common targets shared by the four core herbs and PC, that were identified via network pharmacology analyses. GO enrichment analysis indicated that the 44 drug and disease targets in common were involved in modulation of cellular processes involved in responses to steroid hormones and apoptotic signaling pathways. Based on KEGG pathway enrichment analysis, our data further reveal that various oncogenic pathways with significant roles in the pathology of PC are significantly enriched for the selected common targets, including cancer, hepatitis B, apoptosis, p53 signaling, and PI3K/Akt signaling pathways.

The sex steroid hormones, estrogen, androgen, and progestin, all have functional roles in the healthy pancreas. PC, which is a highly lethal disease, has a higher prevalence in men than women. Together, these factors may suggest a potential role for sex hormone regulation or dysregulation in pancreatic carcinogenesis.

The results of molecular docking showed that stigmasterol and phaseol had high affinity for PTGS2. PTGS2 is an inducible enzyme with vital roles in the pathophysiological processes of PC (Wang et al. 2014). Importantly, there is evidence that PTGS2 is an attractive target in PC because it is highly upregulated and participates in anti-apoptotic mechanisms (Ding, Tong, and Adrian 2001). Our data indicate that perlolyrine can bind to ESR1 and DIOP to ESR2 with binding energies of − 8.8 kcal/mol. A recently published study revealed that the G protein-coupled estrogen receptor (GPER) inhibits pancreatic ductal adenocarcinoma (Natale et al. 2020). It was demonstrated that GPER acts as a tumor suppressor in cancers that are not classically considered hormone responsive, suggesting that GPER activity may contribute to biological differences between the sexes that influence cancer progression and response to modern therapies (Natale, et al. 2020). An additional study showed that there is an association between thyroid hormone supplementation and PC invasion (Sarosiek et al. 2016).

In summary, the results of the current study suggest that four core herbs may play important roles in treating PC by regulating proteins that function in response to steroid hormones. Hence, bioactive compounds, such as stigmasterol, phaseol, perlolyrine, and DIOP, may exert anti-cancer effects against PC.

5 Conclusion

This study reveals the core prescription used for PC treatment included four herbs: *Glycyrrhizae Radix et Rhizome* (Gan Cao), *Codonopsis Radix* (Dang Shen), *Citri Reticulatae Pericarpium* (Chen Pi), and *Pinelliae Rhizoma* (Ban Xia). The anti-PC activity of core prescription may be mediated via regulation of proteins with roles in responses to steroid hormones. This study provides further evidence supporting the potential for use of herbal medicines to treat PC.

Declarations

Conflict of Interest

All authors declare no conflict of interests.

Author Contributions

CH responsible for concept and design. ZZ, JW and BL are responsible for data analysis and interpretation. CH drafted the paper; CH and JX supervised the study; all authors participated in the analysis and interpretation of data and approved the final paper.

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Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

References

1. Chen, H., Q. Zhuo, Z. Ye, X. Xu, and S. Ji. 2021. "Organoid Model: A New Hope for Pancreatic Cancer Treatment?" Biochim Biophys Acta Rev Cancer 1875, no. 1 (Jan): 188466. https://dx.doi.org/10.1016/j.bbcan.2020.188466.
2. Ding, W., J. Gu, L. Cao, N. Li, G. Ding, Z. Wang, L. Chen, X. Xu, and W. Xiao. 2014. "Traditional Chinese Herbs as Chemical Resource Library for Drug Discovery of Anti-Infective and Anti-Inflammatory." *J Ethnopharmacol* 155, no. 1 (Aug 8): 589 – 98. https://dx.doi.org/10.1016/j.jep.2014.05.066.
3. Ding, X. Z., W. G. Tong, and T. E. Adrian. 2001. "Cyclooxygenases and Lipoxygenases as Potential Targets for Treatment of Pancreatic Cancer." *Pancreatology* 1, no. 4: 291-9. https://dx.doi.org/10.1159/000055827.
4. Goodsell, D. S., Zardecki, C., Di Costanzo, L., et al. (2020). RCSB Protein Data Bank: Enabling biomedical research and drug discovery. Protein Science, 29(1), 52–

65. https://k.sina.com.cn/article_2687386043_a02e41bb02000rw26.html?from=news&su bch = onews.

5. Gutheil, W. G., G. Reed, A. Ray, S. Anant, and A. Dhar. 2012. "Crocetin: An Agent Derived from Saffron for Prevention and Therapy for Cancer." Curr Pharm Biotechnol 13, no. 1 (Jan): 173–9. https://dx.doi.org/10.2174/138920112798868566.
6. Kim, S., Chen, J., Cheng, T., et al. (2019). PubChem 2019 update: Improved access to chemical data. Nucleic Acids Research, 47(D1), D1102–D1109.
7. Kuo, Y. T., T. T. Chang, C. H. Muo, M. Y. Wu, M. F. Sun, C. C. Yeh, and H. R. Yen. 2018a. "Use of Complementary Traditional Chinese Medicines by Adult Cancer Patients in Taiwan: A Nationwide Population-Based Study." Integr Cancer Ther 17, no. 2 (Jun): 531–541. https://dx.doi.org/10.1177/1534735417716302.
8. Kuo, Y. T., H. H. Liao, J. H. Chiang, M. Y. Wu, B. C. Chen, C. M. Chang, M. H. Yeh, T. T. Chang, M. F. Sun, C. C. Yeh, and H. R. Yen. 2018b. "Complementary Chinese Herbal Medicine Therapy Improves Survival of Patients with Pancreatic Cancer in Taiwan: A Nationwide Population-Based Cohort Study." Integr Cancer Ther 17, no. 2 (Jun): 411–422. https://dx.doi.org/10.1177/1534735417722224.
9. Li, L., and P. S. Leung. 2014. "Use of Herbal Medicines and Natural Products: An Alternative Approach to Overcoming the Apoptotic Resistance of Pancreatic Cancer." Int J Biochem Cell Biol 53 (Aug): 224–36. https://dx.doi.org/10.1016/j.biocel.2014.05.021.
10. Lu, P. H., J. L. Keng, K. L. Kuo, Y. F. Wang, Y. C. Tai, and C. Y. Kuo. 2020. "An Apriori Algorithm-Based Association Rule Analysis to Identify Herb Combinations for Treating Uremic Pruritus Using Chinese Herbal Bath Therapy." *Evid Based Complement Alternat Med* 2020: 8854772. https://dx.doi.org/10.1155/2020/8854772.
11. Mohammed, A., N. B. Janakiram, S. Pant, and C. V. Rao. 2015. "Molecular Targeted Intervention for Pancreatic Cancer." *Cancers (Basel)* 7, no. 3 (Aug 10): 1499 – 542. https://dx.doi.org/10.3390/cancers7030850.
12. Natale, C. A., J. Li, J. R. Pitarresi, R. J. Norgard, T. Dentchev, B. C. Capell, J. T. Seykora, B. Z. Stanger, and T. W. Ridky. 2020. "Pharmacologic Activation of the G Protein-Coupled Estrogen Receptor Inhibits Pancreatic Ductal Adenocarcinoma." Cell Mol Gastroenterol Hepatol 10, no. 4: 868–880 e1. https://dx.doi.org/10.1016/j.jcmgh.2020.04.016.
13. Park, J., D. Jeong, M. Song, and B. Kim. 2021. "Recent Advances in Anti-Metastatic Approaches of Herbal Medicines in 5 Major Cancers: From Traditional Medicine to Modern Drug Discovery." *Antioxidants (Basel)* 10, no. 4 (Mar 27). https://dx.doi.org/10.3390/antiox10040527.
14. Qi, Y., X. Cheng, H. Jing, T. Yan, F. Xiao, B. Wu, K. Bi, and Y. Jia. 2020. "Comparative Pharmacokinetic Study of the Components in Alpinia Oxyphylla Miq.-Schisandra Chinensis (Turcz.) Baill. Herb Pair and Its Single Herb between Normal and Alzheimer's Disease Rats by Uplc-Ms/Ms." J Pharm Biomed Anal 177 (Jan 5): 112874. https://dx.doi.org/10.1016/j.jpba.2019.112874.
15. Sarosiek, K., A. V. Gandhi, S. Saxena, C. Y. Kang, G. I. Chipitsyna, C. J. Yeo, and H. A. Arafat. 2016. "Hypothyroidism in Pancreatic Cancer: Role of Exogenous Thyroid Hormone in Tumor Invasion-

Preliminary Observations." *J Thyroid Res* 2016: 2454989. https://dx.doi.org/10.1155/2016/2454989.

16. T.J, Kaptchuk. 2000. "The Web That Has No Weaver—Understanding Chinese Medicine. [Chapter 1]: Medicine East and West: Two Ways of Seeing, Two Ways of Thinking." In *Mcgraw-Hill Education*.
17. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461.
18. Wang, D., X. Z. Guo, H. Y. Li, J. J. Zhao, X. D. Shao, and C. Y. Wu. 2014. "Prognostic Significance of Cyclooxygenase-2 Protein in Pancreatic Cancer: A Meta-Analysis." Tumour Biol 35, no. 10 (Oct): 10301–7. https://dx.doi.org/10.1007/s13277-014-2260-y.
19. Wei, P., H. L. Huo, Q. H. Ma, H. C. Li, X. F. Xing, X. M. Tan, and J. B. Luo. 2014. "Pharmacokinetic Comparisons of Five Ephedrine Alkaloids Following Oral Administration of Four Different Mahuang-Guizhi Herb-Pair Aqueous Extracts Ratios in Rats." J Ethnopharmacol 155, no. 1 (Aug 8): 642–8. https://dx.doi.org/10.1016/j.jep.2014.05.065.
20. Wong, W., B. Z. Chen, A. K. Y. Lee, A. H. C. Chan, J. C. Y. Wu, and Z. Lin. 2019. "Chinese Herbal Medicine Effectively Prolongs the Overall Survival of Pancreatic Cancer Patients: A Case Series." Integr Cancer Ther 18 (Jan-Dec): 1534735419828836. https://dx.doi.org/10.1177/1534735419828836.
21. Yang, X., J. Hao, C. H. Zhu, Y. Y. Niu, X. L. Ding, C. Liu, and X. Z. Wu. 2015. "Survival Benefits of Western and Traditional Chinese Medicine Treatment for Patients with Pancreatic Cancer." Medicine (Baltimore) 94, no. 26 (Jul): e1008. https://dx.doi.org/10.1097/MD.0000000000001008.
22. Yue, Q., G. Gao, G. Zou, H. Yu, and X. Zheng. 2017. "Natural Products as Adjunctive Treatment for Pancreatic Cancer: Recent Trends and Advancements." *Biomed Res Int* 2017: 8412508. https://dx.doi.org/10.1155/2017/8412508.
23. Zhu, B., Q. L. Zhang, J. W. Hua, W. L. Cheng, and L. P. Qin. 2018. "The Traditional Uses, Phytochemistry, and Pharmacology of Atractylodes Macrocephala Koidz.: A Review." J Ethnopharmacol 226 (Nov 15): 143–167. https://dx.doi.org/10.1016/j.jep.2018.08.023.

Figures

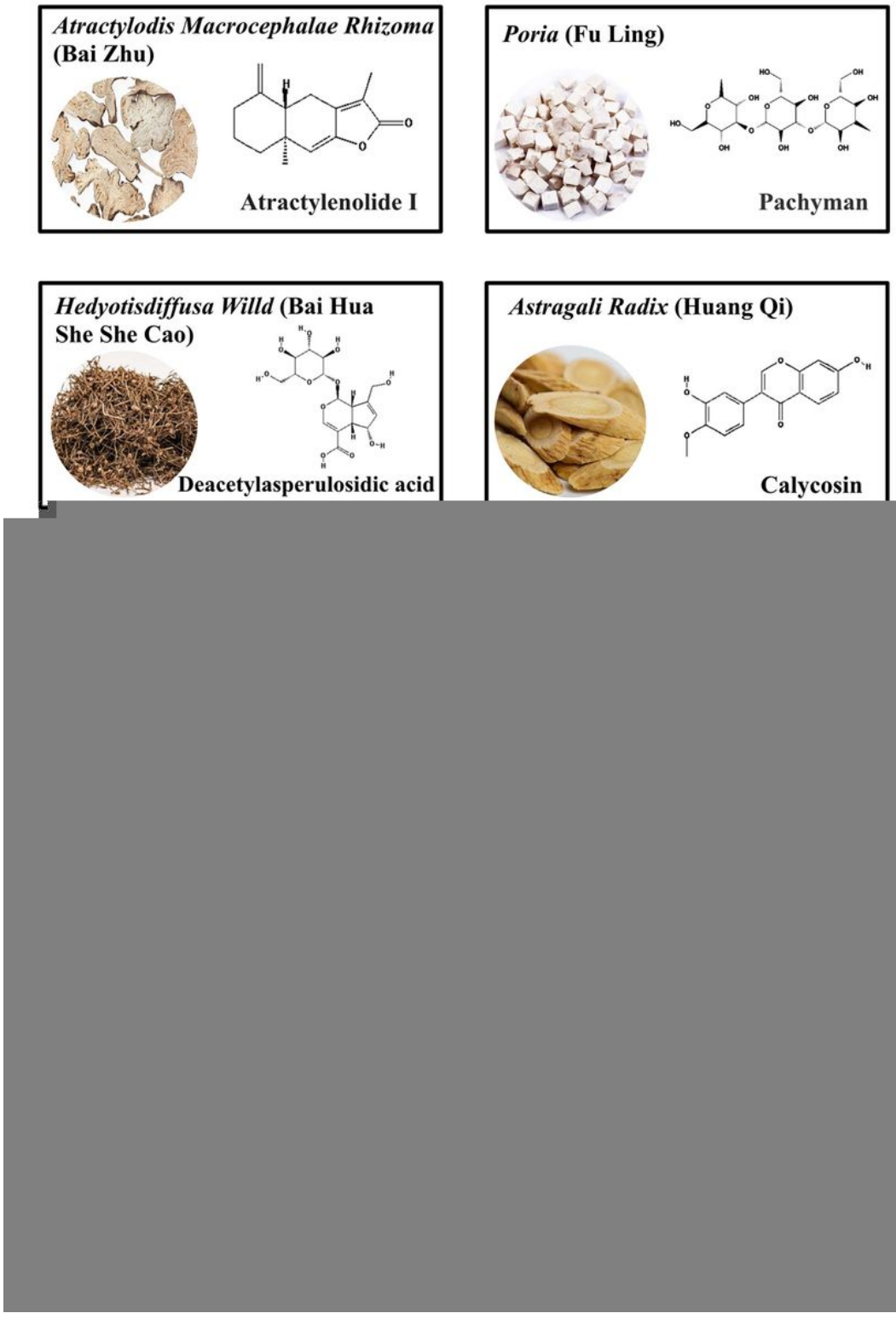

Figure 1

The top 10 herbs most frequently applied in treatment of pancreatic cancer and their chief chemical components with well-defined pharmacological activities. Chemical structures were downloaded from the Traditional Chinese Medicine Systems Pharmacology database.

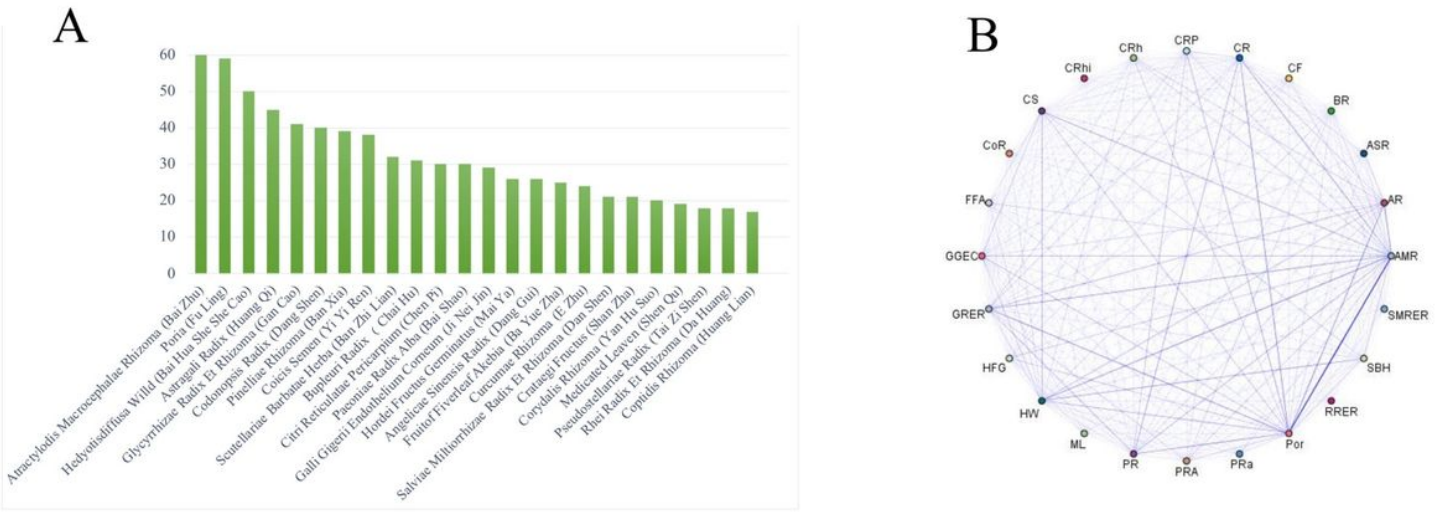

Figure 2

Frequently used herbal medicines and association rule analysis of herb combinations. **(A)** Frequencies of individual herbs. **(B)** Network diagram of association rules among the pharmacological mechanisms of herbs of interest.

Figure 3

Unsupervised hierarchical cluster analysis of the 24 most frequently used herbs.

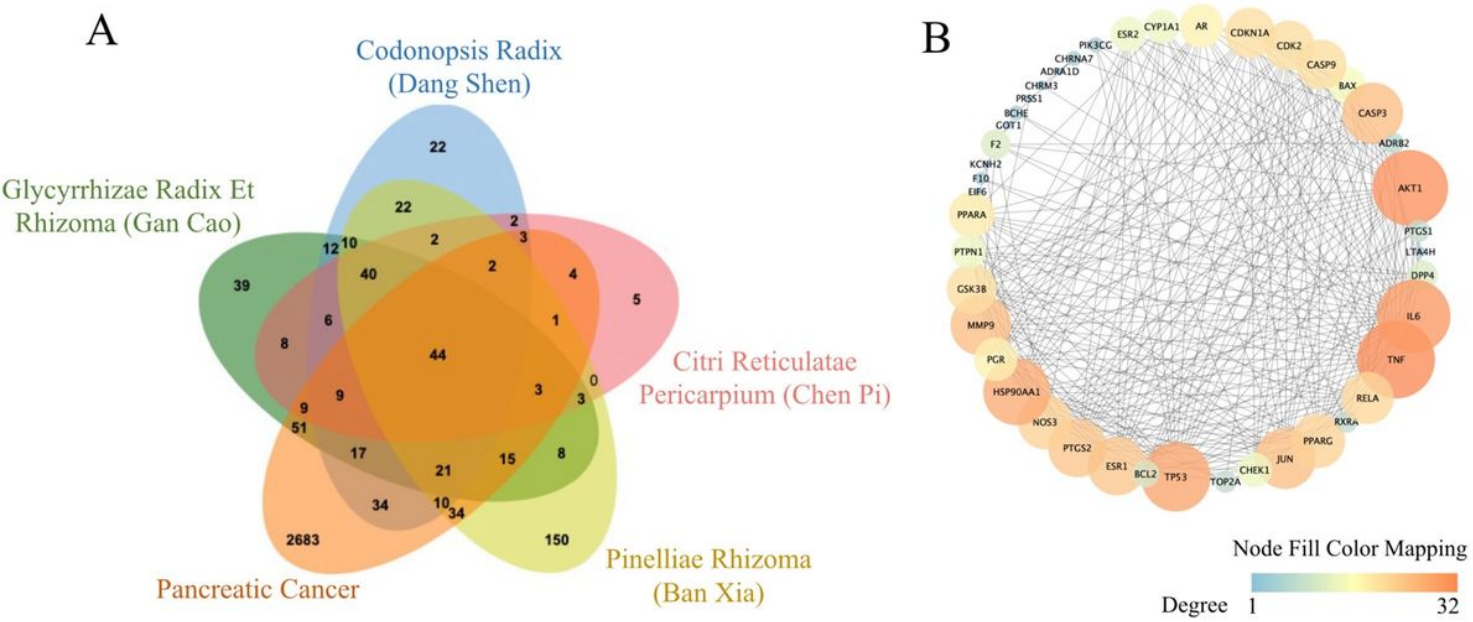

Figure 4

Targets of core prescriptions used for PC treatment. **(A)** Molecules in common between herb targets and PC-associated proteins. **(B)** PPI network diagram of the common targets of the four core herbs used to treat pancreatic cancer. The PPI network contains 44 nodes and 297 edges. Circles represent protein targets; orange circles indicate higher degree values. The node size of gene targets is proportional to the number of degrees.

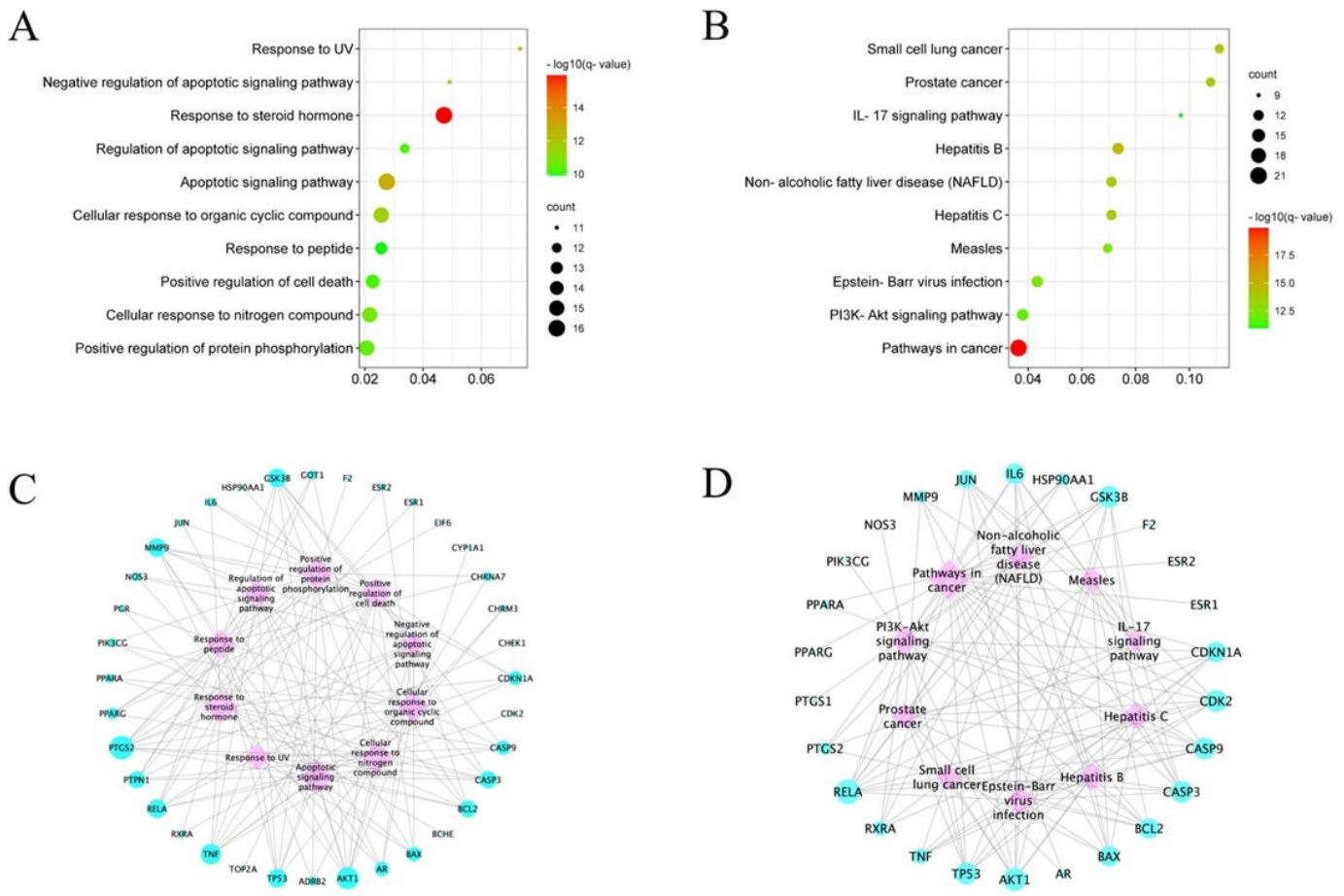

Figure 5

Functional analysis of common targets. **(A)** GO enrichment analysis of putative targets. **(B)** Target–GO network terms. **(C)** KEGG pathway enrichment analysis of putative targets. **(D)** Target–Signaling pathway network. Pink diamond nodes represent main signaling pathways and blue circle nodes refer to putative common targets of the four core herbs used for treatment of pancreatic cancer. Node size is proportional to the number of degrees.

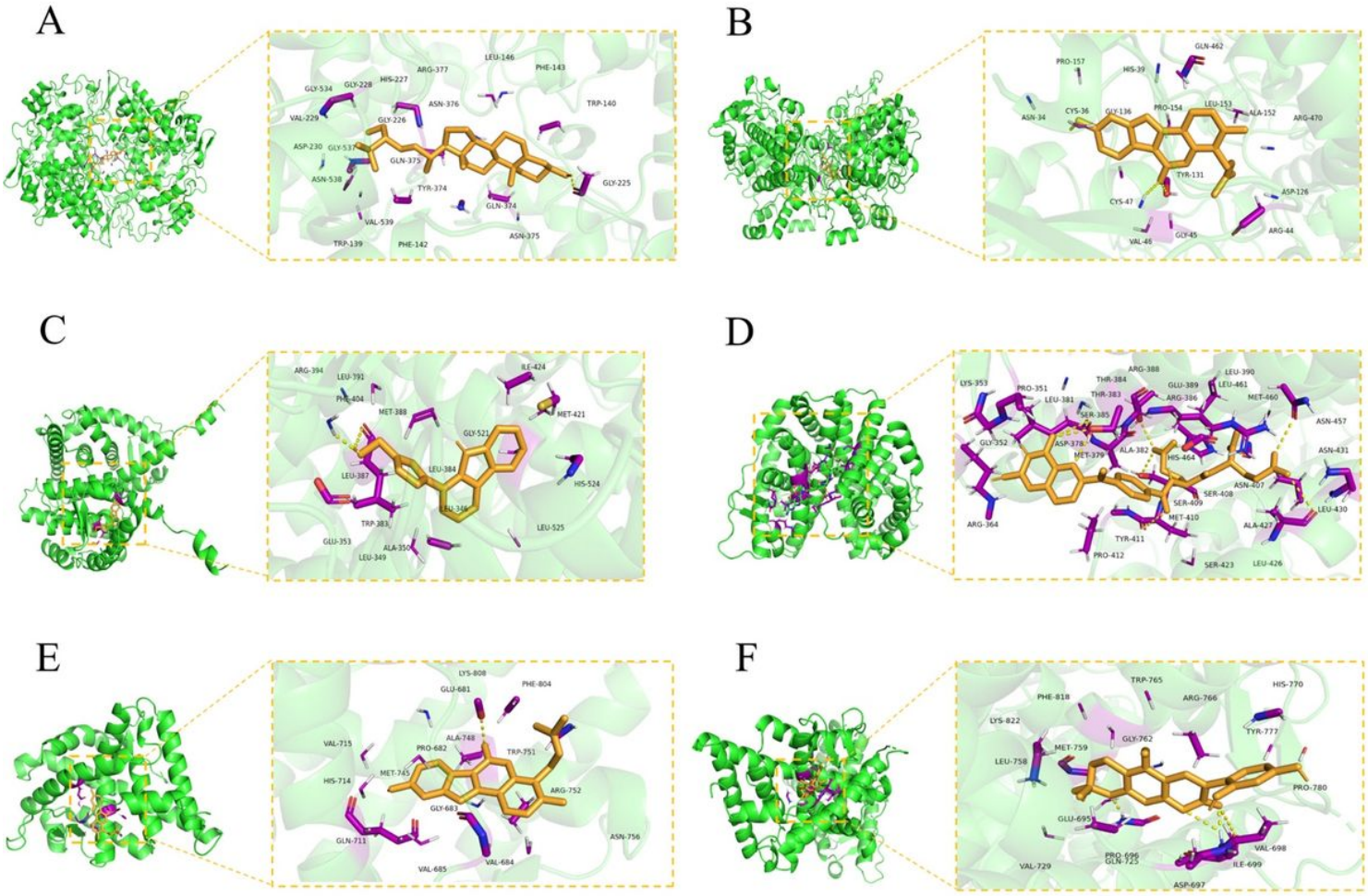

Figure 6

Schematic 3D representation of molecular docking models, active sites, and binding distances. Binding modes of: stigmasterol to PTGS2 **(A)**, phaseol to PTGS2 **(B)**, perlolyrine to ESR1 **(C)**, DIOP to ESR2 **(D)**, phaseol to AR **(E)**, and licopyranocoumarin to PGR **(F)**.

Supplementary Files

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

- 864142Table1.xlsx
- 864142Table2.xlsx