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*Research Article*

# Exploring the Molecular Mechanism of the Antioxidant Activity of Medicine and Food Homology Licorice Flavonoids Based on Pharmacophore Theory and Quantum Calculations

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Licorice flavonoids are a kind of flavonoids extracted from the root of licorice, which have good antioxidant activity and are widely used in the food industry. In the early research of the research group, it has been found that the antioxidant activity of licorice flavonoids can be altered by solvent mediation. However, the molecular mechanism underlying the antioxidant properties of licorice flavonoids is still not fully understood. Therefore, this study aims to explore the molecular mechanism underlying the antioxidant activity of licorice flavonoids based on the principles of pharmacophore theory and quantum computing. Firstly, network pharmacology will be employed to study the antioxidant properties of licorice flavonoids. The major active components and key structural features responsible for the antioxidant activity in licorice flavonoids will be screened using pharmacophore theory and further validated through molecular docking. Lastly, quantum computing will be utilized to uncover the potential mechanism underlying their antioxidant activity. Pharmacophore studies have shown that licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin exhibit low predicted IC50 values, indicating that these compounds are key components of licorice flavonoids in the antioxidant process. Molecular electrostatic potential (MEP) and frontier molecular orbital studies have shown that licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin all exhibit certain chemical reactivity. Quantum computing studies have found that the para-phenol hydroxyl group on the core structure of licochalcone B, isoliquiritigenin, retrochalcone, and isoliquiritin is a crucial functional group for the antioxidant activity of flavonoid compounds. In general, this study successfully elucidated the mode of action of licorice flavonoids in the antioxidant process, providing some guidance for the synthesis of new antioxidant compounds.

## 1. Introduction

Oxygen free radicals (also known as reactive oxygen species) are the primary substances that cause oxidative stress and cellular damage [1]. Under normal circumstances, the body produces free radicals while also generating substances that counteract them, maintaining a dynamic balance between free radicals and antioxidant agents [2]. However, when free radicals accumulate excessively and cannot be effectively cleared, the surplus free radicals can attack the body's tissues, leading to oxidative stress and cellular damage and ultimately contributing to aging and other diseases [3]. The role of antioxidants is to protect cells from oxidative damage by inhibiting the generation of free radicals or clearing formed free radicals [4]. Antioxidants can stabilize free radicals, thereby reducing their damage to biological molecules such as cells, DNA, lipids, and proteins [5]. Common antioxidants include vitamin C, vitamin E, glutathione, and flavonoids. They can exert antioxidant effects through various mechanisms, such as neutralizing free radicals, activating antioxidant enzyme systems, and repairing oxidative damage [6]. Therefore, studying the molecular mechanisms of oxidants is crucial for developing effective antioxidant treatment strategies.

Licorice flavonoids are a class of flavonoid compounds extracted from licorice roots [7]. They are widely used in traditional Chinese medical and other herbal formulations, exhibiting various biological activities, with antioxidant activity being one of the most prominent and important features [8]. Studies have found that licorice flavonoids exert antioxidant effects by clearing oxygen free radicals, inhibiting the generation of peroxides, and enhancing the activity of antioxidant enzymes [9]. These antioxidant mechanisms help protect cells from oxidative stress and maintain the oxidative-antioxidative balance within cells [10]. As a natural antioxidant, licorice flavonoids have broad prospects for applications. They are extensively used in the pharmaceutical and health product industries for the development of antioxidant products, aiming to prevent and treat diseases associated with oxidative damage, such as cardiovascular diseases, cancer, inflammatory diseases, and more [11]. Moreover, licorice flavonoids can also be utilized in the food industry as natural preservatives to extend the shelf life of food [12]. Although previous research has found that the antioxidant activity of licorice flavonoids can be altered under solvent mediation [13], the molecular mechanism of how the structure of licorice flavonoids influences their antioxidant activity remains not fully understood.

Pharmacophore theory and network pharmacology have been widely used to explore the molecular mechanism of antioxidant activity [14]. Pharmacophore theory is a method based on the relationship between specific structural fragments in compounds and their biological activities, which helps reveal the molecular mechanisms of drugs [15]. By identifying and analyzing the key structural features (pharmacophores) of biological activity, we can understand the structure-activity relationships of compounds [16]. In antioxidant research, pharmacophore theory is used to identify and optimize molecules with antioxidant activity. Analyzing these key structural features can reveal the interactions and structure-activity relationships between antioxidant molecules and target molecules [17]. Network pharmacology is an approach that integrates methods from systems biology, computational biology, and other disciplines to study the overall interactions and molecular networks between drugs and targets [18]. In antioxidant research, network pharmacology methods are used to unravel the interactions and signal transduction between antioxidant compounds and multiple molecular targets within cells. By constructing drug-target interaction networks, we can understand the mode of action of antioxidant molecules within cells, the mechanisms of regulating cellular signaling pathways, and their interactions with other molecules [19]. Therefore, the application of pharmacophore theory and network pharmacology will contribute to further revealing the molecular mechanisms of antioxidant activity in licorice flavonoids.

In this study, we will first use network pharmacology to screen for the main active components and key targets of antioxidant activity in licorice flavonoids. Then, we will employ pharmacophore theory to identify potential key structural features that may contribute to the antioxidant activity of licorice flavonoids. Furthermore, utilizing quantum computing methods, we will calculate the electronic structure, orbital distribution, and relevant physicochemical properties of licorice flavonoid molecules to reveal their potential mechanisms of antioxidant activity. Through these analyses, we aim to elucidate the possible mechanisms of licorice flavonoids in antioxidant processes and provide some guidance for the design and development of more potent antioxidant compounds.

## 2. Materials and Methods

*2.1. Research on Network Pharmacology.* To screen for the active components and key targets of the antioxidant activity of licorice flavonoids, we conducted an analysis using network pharmacology methods. Based on the previous analysis of licorice flavonoids using UHPLC-Orbitrap-MS, the composition of licorice flavonoids was collected [20], and further screening was conducted based on the structural characteristics of flavonoid components to identify 34 compounds (Table 1). Identify and download the structures of compounds from the PubChem database, and save them in SDF format. Then, use the Swiss database to predict the targets for each compound. Search for "antioxidant" in the GeneCards database to collect relevant targets with a relevance score greater than 4. Generate a Venn diagram by comparing the predicted targets of the active components with the predicted targets of licorice flavonoids. Take the intersection of the targets (Figure 1(a)) to obtain the potential targets of licorice flavonoids for antioxidant activity. Enter the potential targets of licorice flavonoids for antioxidant activity in the STRING database, set the organism as "Homo" to filter for human-related proteins, and construct a protein-protein interaction (PPI) network diagram of the potential targets of licorice flavonoids for antioxidant activity (Figure 1(b)). Use the DAVID 6.8 database to generate metabolic pathways related to the potential antioxidant targets (Figures 1(c)–1(d)). Finally, use Cytoscape v3.8.2 software to construct a disease network of active components, potential targets, and related pathways of licorice flavonoids. Then, screen and identify the main active components and key targets associated with the antioxidant activity of licorice flavonoids.

*2.2. Building a Pharmacophore Model.* A dataset of IC50 values for 50 experimentally identified tyrosinase inhibitors was obtained from the published literature [21–24]. The chemical structures of these compounds were sketched using the ChemDraw module in ChemOffice, and energy optimization was performed using Discovery Studio 2019 software (BIOVIA; San Diego, USA). A training set for constructing a QSAR pharmacophore was created using compounds from 32 tyrosinase inhibitors (Figure 2). The names of these molecules (Name), their activity (Activ) represented by IC50 values ($\mu$M), and an uncertainty in activity (Uncert) were selected as 1.5. According to the pharmacophore construction algorithm, the top two compounds in terms of ranking are defined as active compounds.

Table 1: Pubchem ID of 34 screened compounds with flavonoid structure.

| No. | Compounds name | Pubchem ID | No. | Compounds name | Pubchem ID |
|---|---|---|---|---|---|
| **1** | DL-liquiritigenin | 1889 | **2** | Licochalcone A | 5318998 |
| **3** | Glycitein | 5317750 | **4** | Neobavaisoflavone | 5320053 |
| **5** | Daidzein | 5281708 | **6** | Retrochalcone | 6442675 |
| **7** | Isoliquiritin | 5318591 | **8** | Osajin | 95168 |
| **9** | 4′-methoxyflavone | 77793 | **10** | Liguiritigenin-7-O-$\beta$-D-apiosyl-4′-O-$\beta$-D-glucoside | 458804988 |
| **11** | 7-hydroxyflavone | 5281894 | **12** | Licoflavone A | 5319000 |
| **13** | Liquiritin | 503737 | **14** | Liquiritigenin | 114829 |
| **15** | Isoliquiritigenin | 638278 | **16** | Glabrone | 5317652 |
| **17** | Hispaglabridin B | 15228661 | **18** | Licochalcone C | 9840805 |
| **19** | Calycosin-7-O-$\beta$-D-glucoside | 440016554 | **20** | Mosloflavone | 471722 |
| **21** | Licochalcone B | 5318999 | **22** | Mulberrin | 5481958 |
| **23** | Kuwanon G | 5281667 | **24** | Formononetin | 5280378 |
| **25** | Naringenin | 439246 | **26** | 5-O-methylgenistein | 5748551 |
| **27** | Sakuranetin | 73571 | **28** | Daidzin | 107971 |
| **29** | Psoralidin | 5281806 | **30** | Nobiletin | 72344 |
| **31** | 8-prenylnaringenin | 480764 | **32** | Kanzonol C | 5316802 |
| **33** | Corylin | 5316097 | **34** | Erysubin F | 12051847 |

$$\mathrm{MA} \times \mathrm{Unc}_{\mathrm{MA}} - \mathrm{UnAc}_{A} > 0.0, \tag{1}$$

where "MA" stands for the Molecular Activity Score, which signifies the pharmacophore activity rating attributed to a compound. A higher MA value suggests that the compound is assessed to possess a higher potential for activity. "$\mathrm{Unc}_{\mathrm{MA}}$" represents the uncertainty of the MA value for a compound. It indicates the level of uncertainty associated with the estimation of the compound's activity. A higher $\mathrm{Unc}_{\mathrm{MA}}$ value suggests that there is a certain degree of uncertainty in the assessment of the compound's activity. "$\mathrm{UnAc}_{A}$" represents the uncertainty of activity for a compound. This value is specific to the measurement method used to evaluate the compound's activity. When the value of "$\mathrm{UnAc}_{A}$" is lower, it indicates a more certain determination of the compound's activity. The 10 compounds with the lowest ranking are defined as nonactive compounds.

$$\log(\mathrm{A}) - \log(\mathrm{MA}) > 3.5, \tag{2}$$

"log(A)" represents the logarithm of the activity value. "log(MA)" indicates the logarithm of the predicted activity value.

The training set molecules were subjected to pharmacophore generation using the 3D QSAR Pharmacophore Generation feature of Discovery Studio 2019 software. A maximum of 255 conformations were generated for each small molecule to characterize its conformational space. Only the conformations whose energy values are within the energy threshold of 10 kcal/mol are preserved [23]. Eighteen tyrosinase inhibitors were selected to construct a test set to verify QSAR pharmacophore (Figure 3). In order to guarantee the accuracy of the model, the selection of tyrosinase inhibitor activity values across four magnitude sets.

*2.3. Molecular Docking.* Selecting TYR as the receptor protein, four licorice flavonoid compounds with good activity identified through pharmacophore screening are chosen as ligand molecules. Molecular docking was performed using Discovery Studio 2019 (BIOVIA; San Diego, USA). Obtain the three-dimensional structure of the target protein from the RCSB-PDB website (https://www.rcsb.org/). Acquire the three-dimensional structure of the ligand molecules from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Make minor modifications to the method based on the previous reports from the research team [13]. In brief, the approach involves first conducting structure optimization for small molecules. Then, the receptor protein is subjected to structural processing. Finally, molecular docking is performed using the LibDock module. Among them, where Libdockscore is greater than or equal to 90, it indicates a good affinity between the receptor and ligand. The formula for calculating the docking score is as follows:

$$\Delta G_{\mathrm{Binding}} = E_{\mathrm{Complex}} - \left(E_{\mathrm{Protein}} + E_{\mathrm{Ligand}}\right). \tag{3}$$

*2.4. DFT Calculation.* The three-dimensional structures of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin were obtained using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Figure S1). To perform theoretical calculations, the DMOL3 module of Materials Studio 2019 software (Accelrys Software Inc., US) was utilized [25, 26]. The calculations were performed using a combination of the generalized gradient approximation (GGA) and the BLYP functional with gradient correction functions [14]. In addition, no constraints were applied during the geometry optimization calculations. The molecular structure and radicals of licorice flavonoids were optimized using the DND3.5 version. Thermodynamic calculations were conducted to analyze the relationship between the antioxidant activity of licorice flavonoids and reaction-free energies.

## 3. Results and Discussion

*3.1. Network Pharmacology Analysis.* Use Cytoscape_v3.8.2 software to generate a network diagram for the antioxidant activity of licorice flavonoids, involving 166 nodes and 958

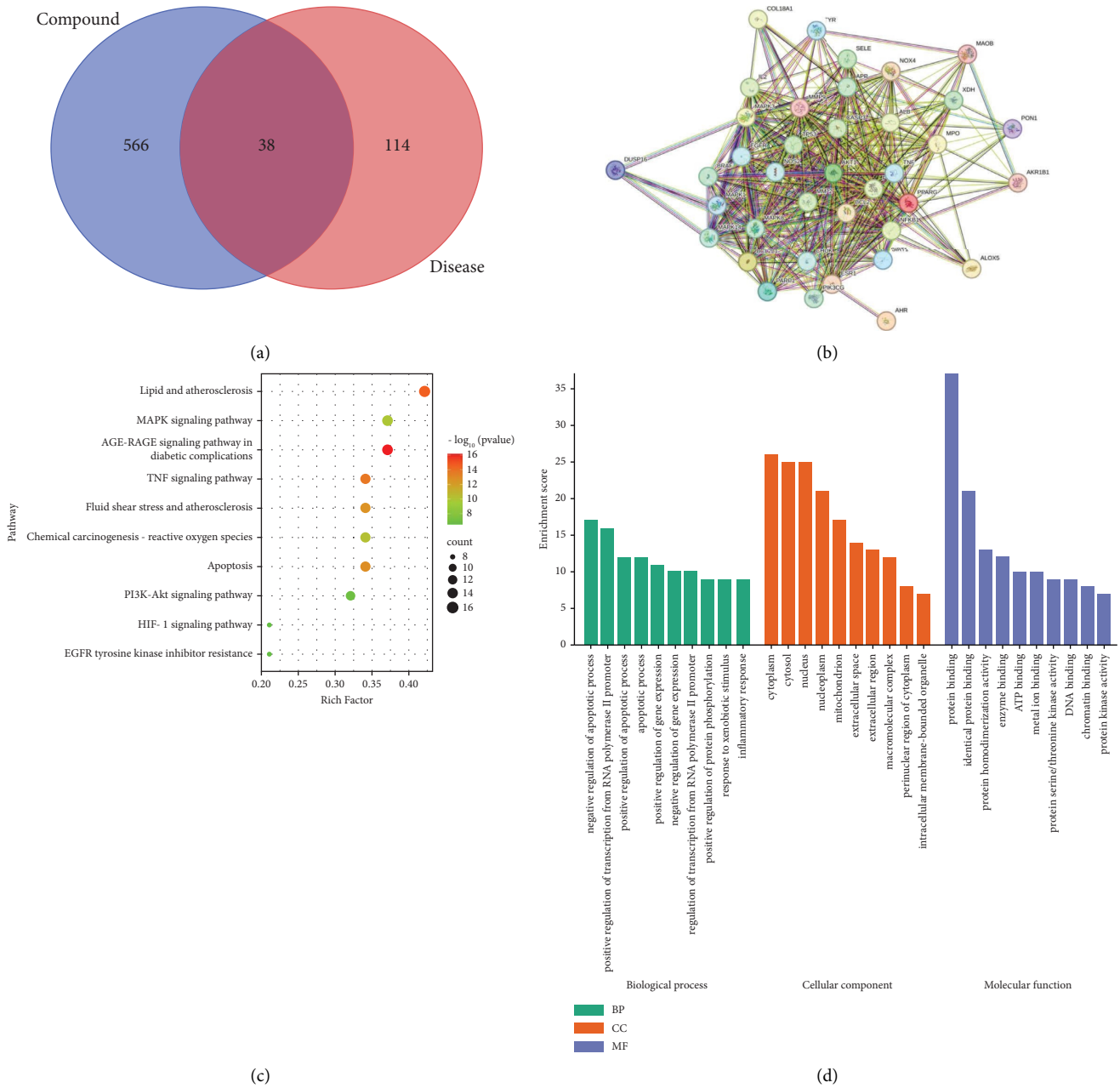
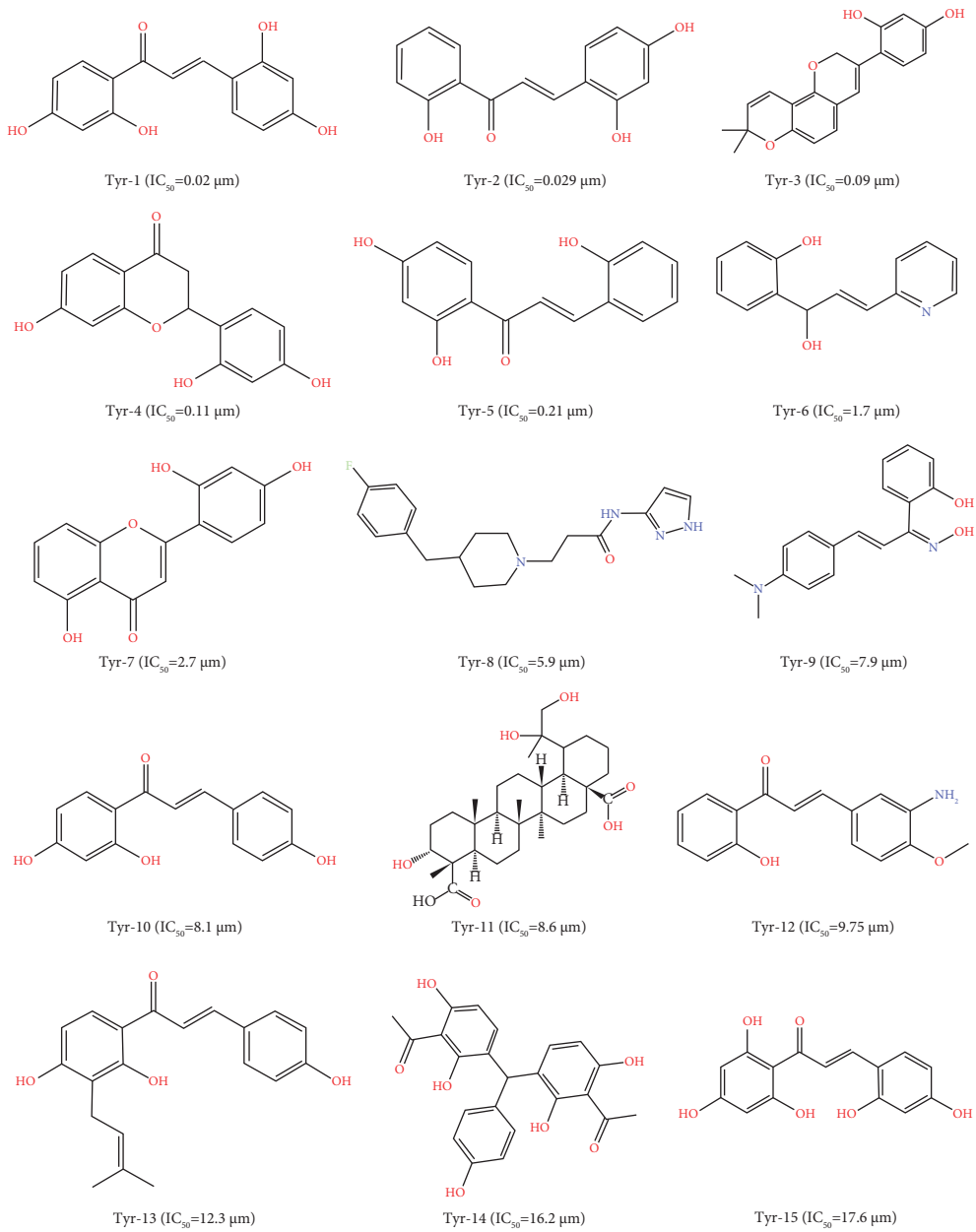



Figure 1: Licorice flavonoids antioxidant network pharmacology analysis.

edges (Figure 4). In the diagram, red circles represent the active components of licorice flavonoids, green diamonds represent the potential targets associated with the antioxidant activity, yellow triangles represent signaling pathways, and blue squares represent licorice flavonoids. The active components ranked in descending order by degree value and identified as the top 10 compounds are isoliquiritigenin, 4′-Methoxyflavone, mosloflavone, isoliquiritin, 7-Hydroxyflavone, retrochalcone, nobiletin, corylin, licochalcone B, and licoflavone A. These compounds are speculated to be the main active components involved in the antioxidant activity of licorice flavonoids (Table 2). In addition, both the PPI network graph and the H-T-P-C network graph indicate that TYR is one of the core targets for the antioxidant activity of licorice flavonoids. Therefore, considering these findings collectively, it is advisable to select TYR as a key target for conducting a structure-activity relationship (SAR) model study.

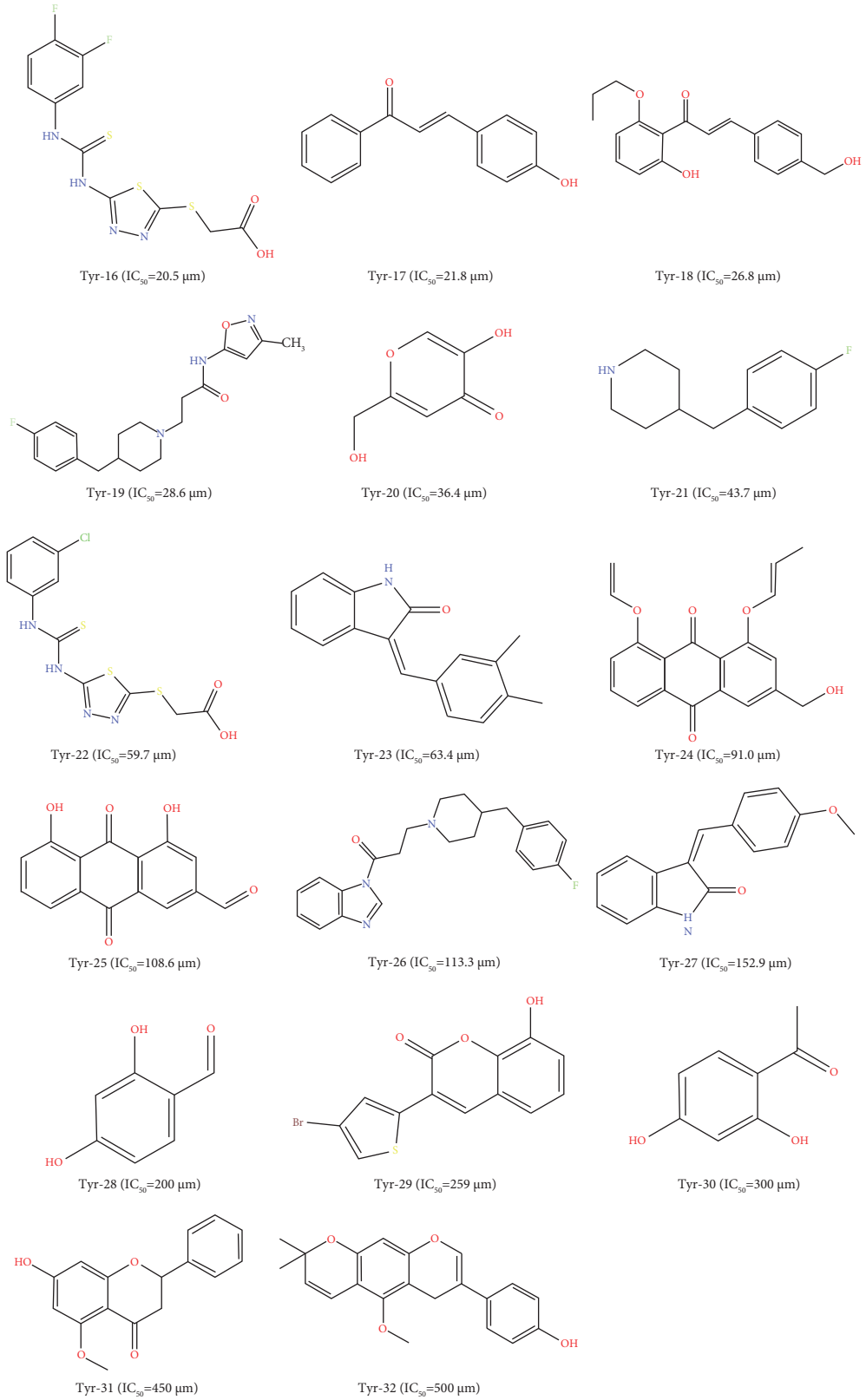
### 3.2. Pharmacophore Model Analysis

*3.2.1. Building a 3D-QSAR Pharmacophore Model with Predictive Capability.* A 3D-QSAR pharmacophore model with predictive capability is constructed using a training set consisting of known active compounds. Figure S2 illustrates the alignment of the compound with the highest predicted activity with the pharmacophore model. It can be observed




(a)

Figure 2: Continued.




(b)

Figure 2: Molecular structure of the training set composed of 32 tyrosinase inhibitors.

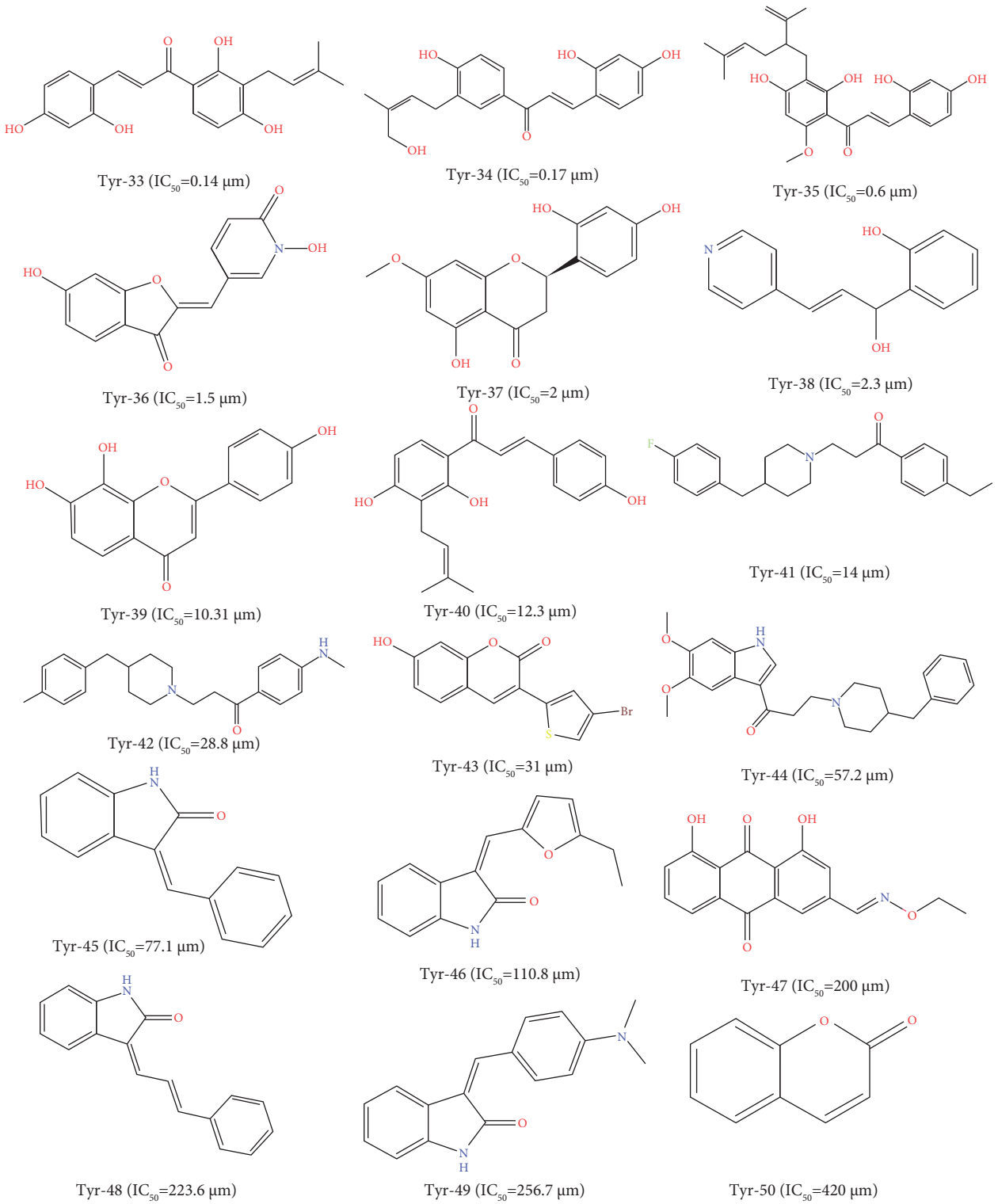



Figure 3: Molecular structure of the test set composed of 18 tyrosinase inhibitors.

that the experimental activity value of this compound is 0.02 $\mu$M, while the predicted activity value is 0.05 $\mu$M. The compound exhibits a good match to all the feature elements of the pharmacophore model. Figure S3 depicts the alignment of the compound with the lowest predicted activity, which represents a nonactive molecule, to the pharmacophore model. It can be observed that the compound has an experimental activity value of 500 $\mu$M, while the predicted activity value is 141 $\mu$M. It can be observed that this nonactive molecule lacks the green feature, which represents a hydrogen bond acceptor. Furthermore, other nonactive molecules also exhibit incomplete alignment with

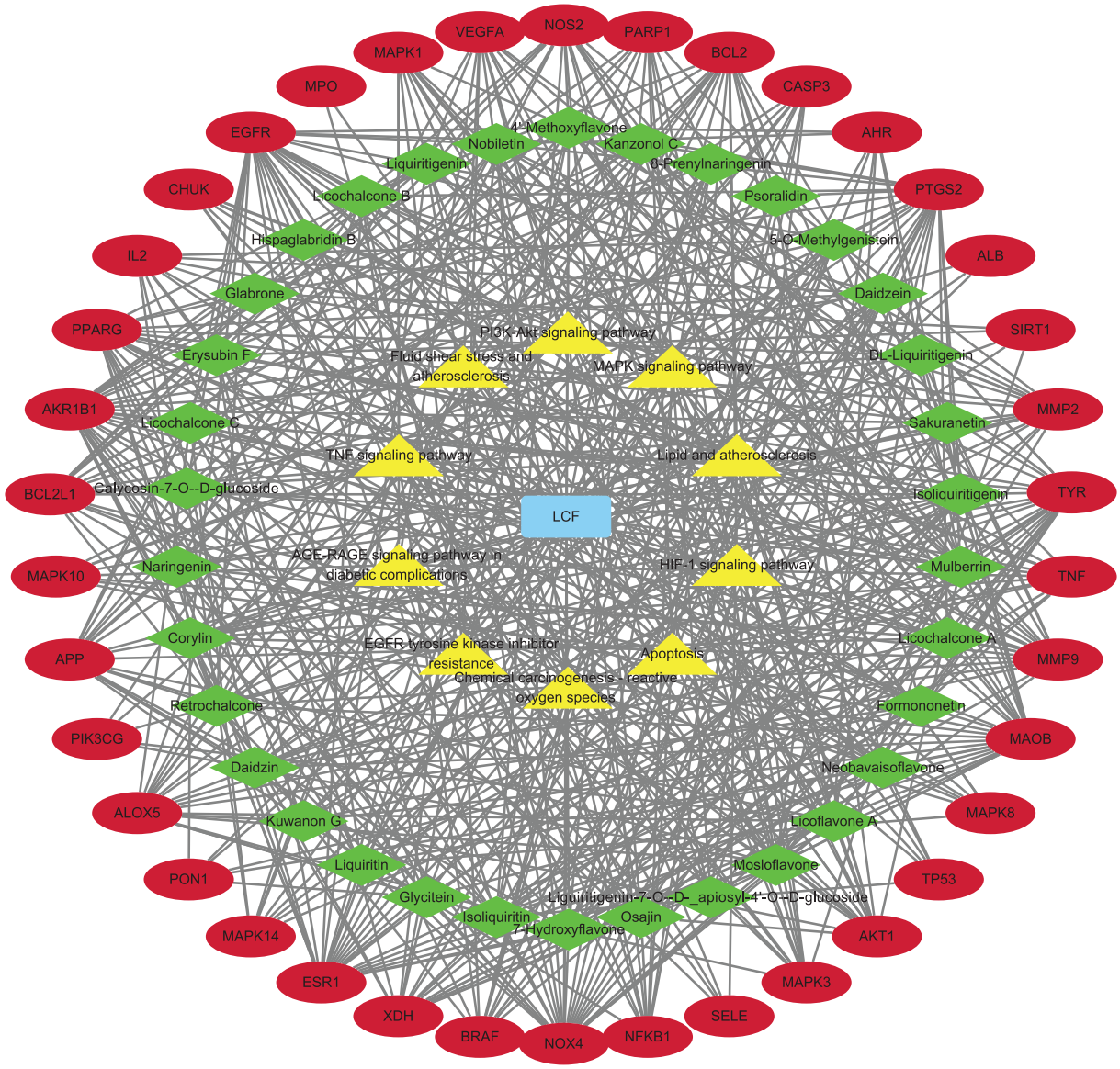



Figure 4: "H-C-T-P" network.

Table 2: Top 10 antioxidant compounds in licorice flavonoids.

| PubChem ID | Compound | Degree |
|---|---|---|
| 638278 | Isoliquiritigenin | 19 |
| 77793 | 4′-methoxyflavone | 18 |
| 471722 | Mosloflavone | 17 |
| 5318591 | Isoliquiritin | 17 |
| 5281894 | 7-hydroxyflavone | 16 |
| 6442675 | Retrochalcone | 16 |
| 72344 | Nobiletin | 16 |
| 5316097 | Corylin | 16 |
| 5318999 | Licochalcone B | 15 |
| 5319000 | Licoflavone A | 15 |

the pharmacophore model to varying degrees. Therefore, this phenomenon indicates that the pharmacophore model has a good discriminatory ability between active and non-active molecules. Figure S4 displays the Δcost values of these pharmacophore models, all of which are greater than 400. This indicates that these models have a high level of confidence. The Δcost (null cost-total cost) is an important indicator for evaluating a pharmacophore model. When this value is less than 40, the confidence interval of the model falls below 50%. A value between 40 and 60 indicates a confidence interval of 75%–90%, while a value greater than 60 indicates a confidence interval of over 90% for the model. Figure S5 demonstrates the predictive ability of the pharmacophore models for the compounds in the training set. It can be observed that these 10 pharmacophore models exhibit correlation coefficients ($r^2$) for predicting activity in the training set as follows: 0.922, 0.896, 0.880, 0.876, 0.874, 0.866, 0.860, 0.857, 0.859, and 0.853. This phenomenon indicates that all of these pharmacophore models have good predictive capabilities.

Figure S6 displays the 10 pharmacophore models constructed using the training set. It includes the statistical data

for validation with the test set and the linear regression curve of LogEstimate vs LogActiv in the test set. It can be observed that these 10 pharmacophore models exhibit correlation coefficients ($r^2$) for predicting activity in the test set as follows: 0.784, 0.776, 0.791, 0.786, 0.674, 0.807, 0.780, 0.755, 0.671, and 0.839. This phenomenon indicates that all of these pharmacophore models have good predictive capabilities. In addition, compared to other pharmacophore models, pharmacophore 10 exhibits the highest correlation coefficient for activity prediction. This indicates that pharmacophore 10 has a higher level of accuracy in predicting activity. Figure 5 displays the matching of each molecule from the test set with pharmacophore models 01-10. Based on the validation results from the test set, pharmacophore model 10 shows a better match with the test set molecules.

*3.3. Activity Prediction of Licorice Flavonoids Based on the Pharmacophore Model.* Based on the pharmacophore models constructed from the training set, activity prediction was performed on the top 10 licorice flavonoid compounds selected through network pharmacology screening. The goal was to identify individual licorice flavonoid monomers with good activity. Figure 6 shows the conformations of these licorice flavonoid compounds that have the highest FitValue (lowest Estimate) when matched with the 10 models. Table 3 presents the activity prediction results of the 10 pharmacophore models for licorice flavonoids. Among them, pharmacophore model 10 predicts an activity value of 1.77 $\mu$M for licochalcone B, and this compound exhibits good matching with all the feature elements of the model (Figure S7). Pharmacophore model 10 predicts an activity value of 786.13 $\mu$M for nobiletin, and this compound only partially matches the feature elements of the model (Figure S8). Additionally, retrochalcone, isoliquiritin, and isoliquiritigenin exhibit low predicted activity values, indicating that these compounds are key components of licorice flavonoids in the antioxidant process.

*3.4. Molecular Docking Verification.* To validate the predicted activity of the licorice flavonoid compounds in antioxidant activity, molecular docking is used for confirmation. Table 4 displays the docking scores and docking energies of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin with the TYR (PDB: 7RK7) target protein. The results demonstrate that these compounds exhibit docking scores above 90 when docked with the TYR target protein, indicating a strong binding affinity between these compounds and the target protein. Figure 7 displays the visualized molecular docking results of these active compounds with the target protein. It can be observed that the binding of these compounds with the target protein primarily occurs through hydrogen bonds, van der Waals forces, and $\pi$-$\pi$ interactions, and the interaction groups are similar to the pharmacophore model. For example, the OH group on licochalcone B forms hydrogen bond interactions with the GLU amino acid residue on the TYR (PDB: 7RK7) target protein at distances of 2.19 Å and 3.02 Å (Figure 7(a)). The benzene ring of retrochalcone forms $\pi$-$\pi$ interactions with the VAL and ARG amino acid residues on the TYR (PDB: 7RK7) target protein at distances of 4.81 Å and 3.80 Å, respectively (Figure 7(b)). The benzene ring of isoliquiritin forms $\pi$-$\pi$ interactions with the TRP amino acid residue on the TYR (PDB: 7RK7) target protein at distances of 4.45 Å and 5.08 Å, respectively (Figure 7(c)). The functional groups on isoliquiritigenin interact with the VAL, CYS, and HIS amino acid residues on the TYR (PDB: 7RK7) target protein through van der Waals forces (Figure 7(d)). In addition, the pharmacophore model's active functional groups, such as benzene ring, OH, and C=O, derived from the training set, form hydrogen bond interactions with the amino acid residues of the TYR target protein. This phenomenon validates the rationality of the 3D QSAR pharmacophore model in predicting the antioxidant activity of licorice flavonoids.

*3.5. Molecular Electrostatic Potential (MEP) Analysis.* Molecular electrostatic potential (MEP) is an effective tool for predicting molecular interactions and reaction behavior between molecules. In this section, the MEP of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin will be used to predict the potential reactive functional groups of these compounds with free radicals. The molecular structures of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin all contain common functional groups such as benzene rings, carbonyl groups, and hydroxyl groups. Among them, the hydroxyl group is a functional group composed of oxygen and hydrogen atoms, which appears in the molecule as an oxygen atom connected to a hydrogen atom and possesses certain oxidative and reductive abilities. Figure 8 shows the MEP distribution maps of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin. It can be observed that the MEP mapping range of charge density goes from the highest electron density (shown as the deepest red) to the lowest electron density (shown as the deepest blue). The carbonyl groups on licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin are regions with higher electron density and can act as electron-donating functional groups, making them prone to undergoing electrophilic reactions with free radicals. The hydroxyl groups on licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin are regions with the lowest electron density and can act as electron-accepting groups, making them prone to undergoing nucleophilic substitution reactions with free radicals. In addition, these functional groups are also the main active moieties in 3D QSAR and molecular docking.

*3.6. Molecular Frontier Orbital Analysis.* HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) are the most important orbitals in molecular frontier orbital analysis. These orbitals determine the way molecules interact with other substances and play a crucial role in chemical reactivity. The HOMO and LUMO orbitals of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin are shown in Figure 9. It can be observed that the -OH and C=O of the flavone skeleton structure of these molecules exhibit higher activity in the HOMO and LUMO orbitals. For example, the -OH (5) on licochalcone B

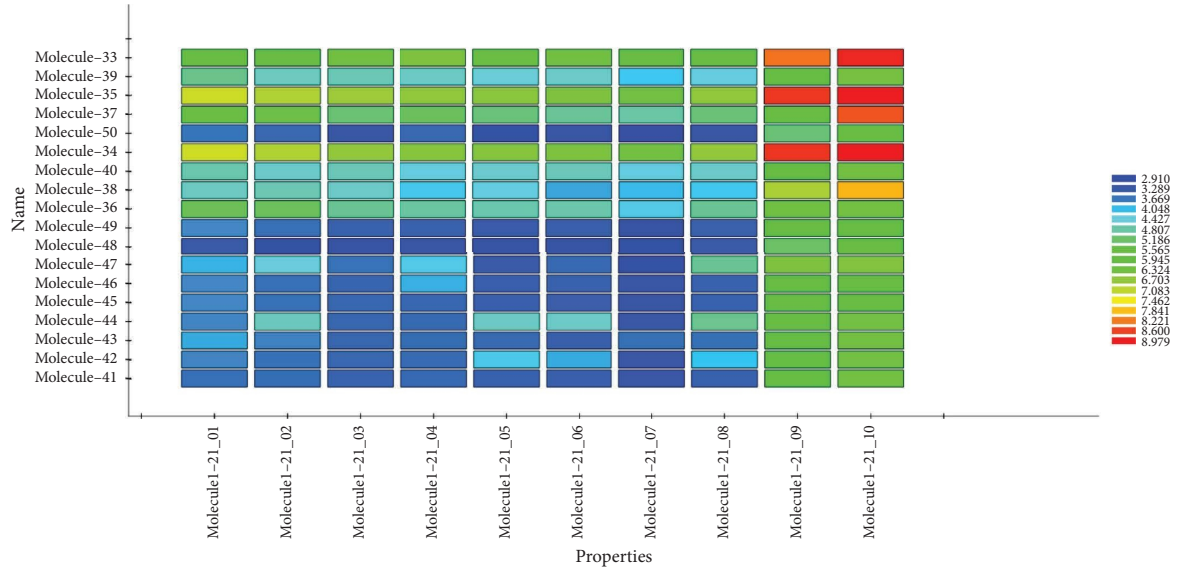



Figure 5: Matched heatmaps of the 10 pharmacophore models of the training set for each of the 18 test set molecules.

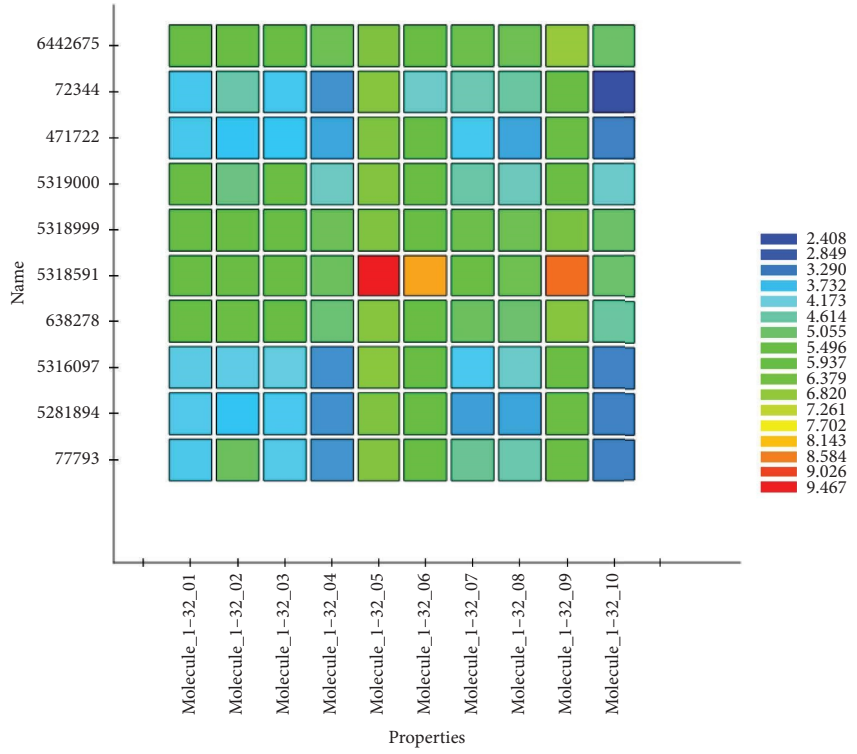



Figure 6: Matching of 10 pharmacophore models in the training set to each molecule of 10 licorice flavonoids small molecules.

is distributed on the LUMO (Figure 9(a)). The -OH (4) on retrochalcone, -OH (8, 9) on isoliquiritin, and -OH (2) on isoliquiritigenin are distributed in both the HOMO and LUMO orbitals. This phenomenon may be due to the conjugation and orbital overlap between the -OH, and the surrounding system [27] (Figures 9(b)–9(d)). Similarly, the C=O (4) on licochalcone B, C=O (3) on retrochalcone, and the C=O (7) on isoliquiritin are also distributed in both the HOMO and LUMO orbitals. This phenomenon may be attributed to the conjugation between the C=O on the flavone skeleton structure and the neighboring phenyl ring, resulting in the rearrangement of electronic levels and contributing to both the HOMO and LUMO orbitals [28] (Figure 9(a)–9(c)). This phenomenon indicates that licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin molecules may exert their antioxidant effects by undergoing

Table 3: 10 Prediction of activity of 10 licorice flavonoids by pharmacophore model.

| PubChem ID | Compound | Predicted IC50 10 |
|---|---|---|
| 5318999 | Licochalcone B | 1.77 |
| 6442675 | Retrochalcone | 1.78 |
| 5318591 | Isoliquiritin | 1.84 |
| 638278 | Isoliquiritigenin | 4.28 |
| 5319000 | Licoflavone A | 9.83 |
| 5316097 | Corylin | 81.76 |
| 5281894 | 7-hydroxyflavone | 82.10 |
| 77793 | 4′-methoxyflavone | 83.91 |
| 471722 | Mosloflavone | 84.98 |
| 72344 | Nobiletin | 786.13 |

Table 4: Molecular docking.

| Compound | Protein | Libdock score | Binding energy (kcal/mol) | Ligand energy (kcal/mol) | Protein energy (kcal/mol) | Complex energy (kcal/mol) |
|---|---|---|---|---|---|---|
| Licochalcone B | TYR (PDB: 7RK7) | 103.365 | 685.2052 | 105946.2090 | −30790.3612 | 75841.0531 |
| Retrochalcone | TYR (PDB: 7RK7) | 96.883 | 310.2326 | 32.4111 | −30790.3612 | −30447.7174 |
| Isoliquiritin | TYR (PDB: 7RK7) | 109.651 | 141.9548 | 227.0134 | −30790.3612 | −30421.3930 |
| Isoliquiritigenin | TYR (PDB: 7RK7) | 108.897 | −25.7910 | 48.6576 | −3.790.3612 | −30767.4946 |

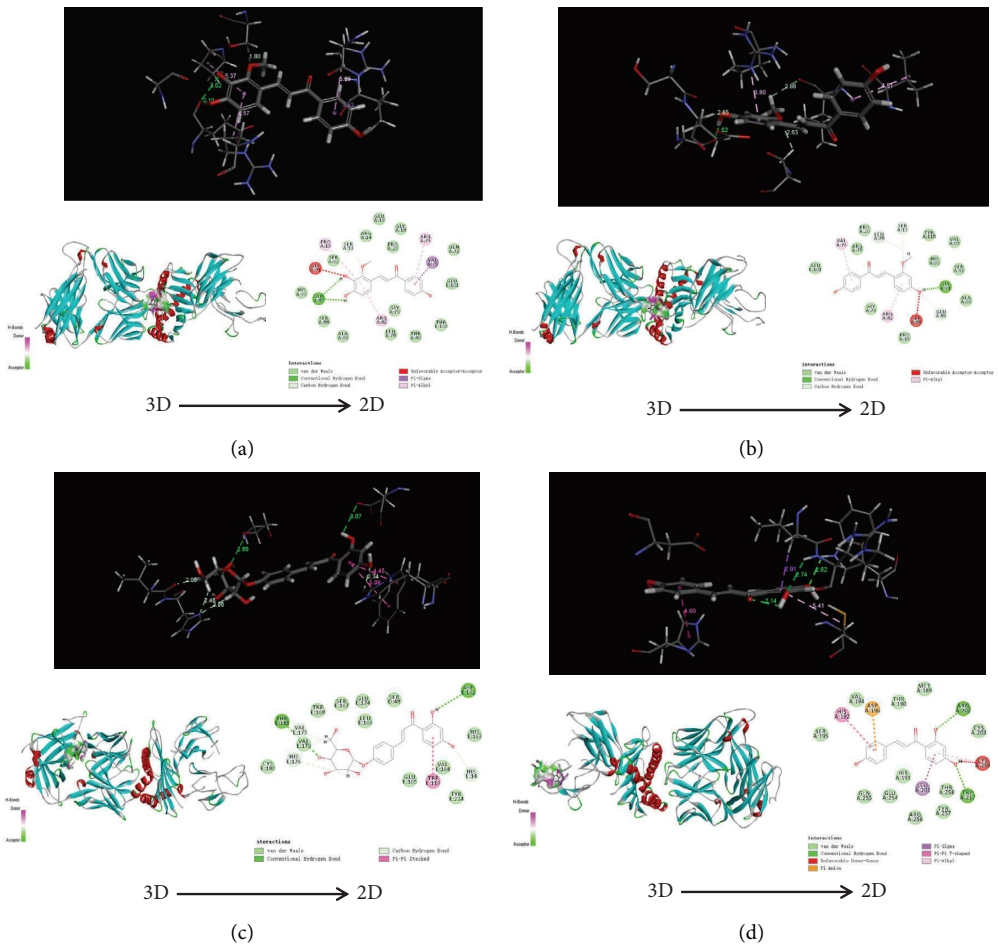



Figure 7: Molecular docking of TYR proteins. Licochalcone B-TYR (PDB: 7RK7) (a), retrochalcone-TYR (PDB: 7RK7) (b), isoliquiritin-TYR (PDB: 7RK7) (c), isoliquiritigenin-TYR (PDB: 7RK7) (d).

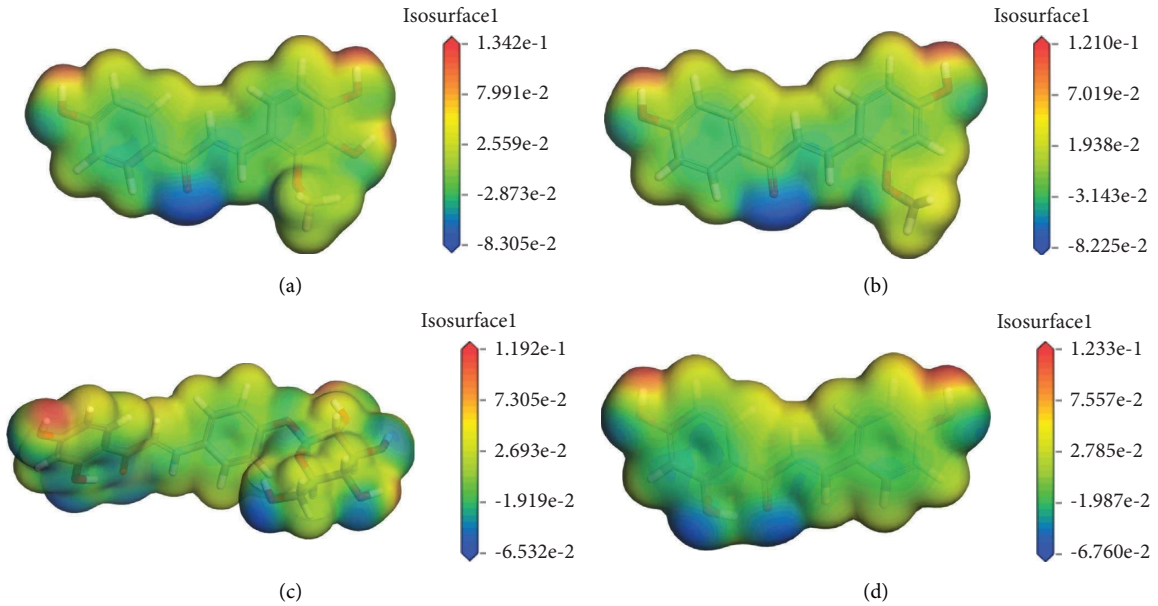



Figure 8: MEP distribution of licochalcone B (a), retrochalcone (b), isoliquiritin (c), and isoliquiritigenin (d).

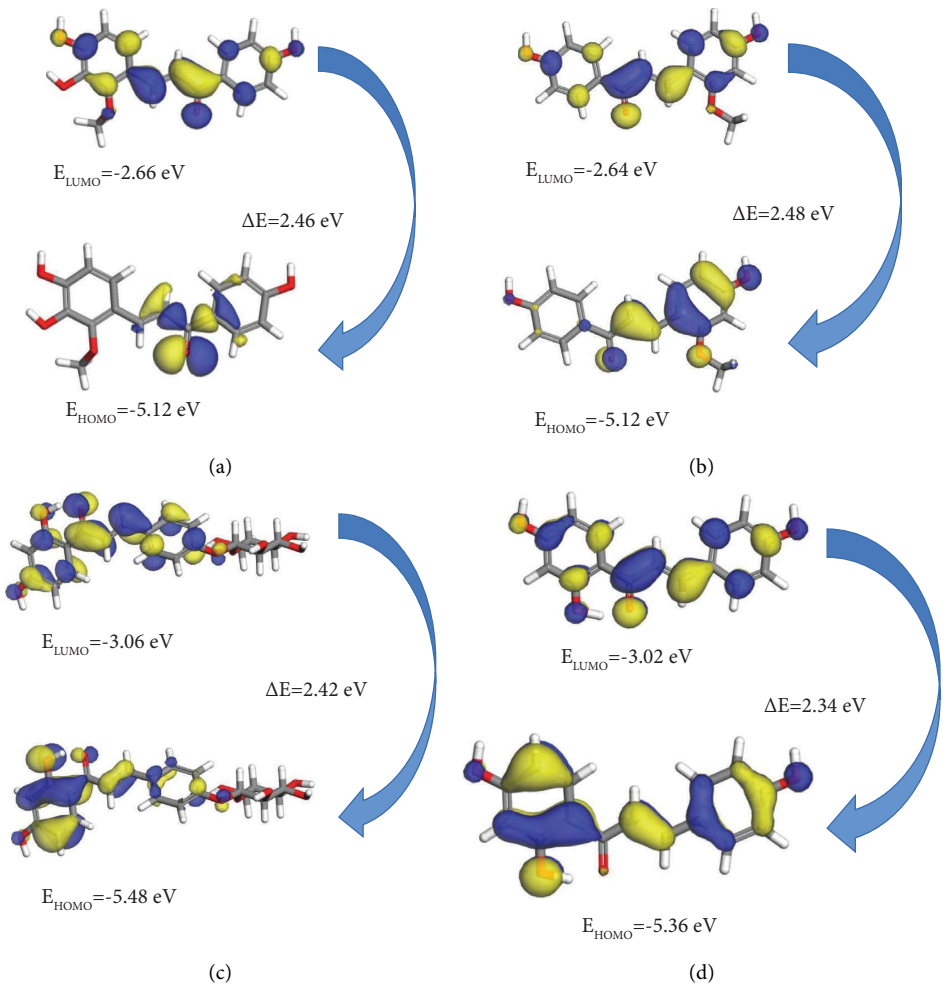



Figure 9: HOMO and LUMO orbital distributions of licochalcone B (a), retrochalcone (b), isoliquiritin (c), and isoliquiritigenin (d).

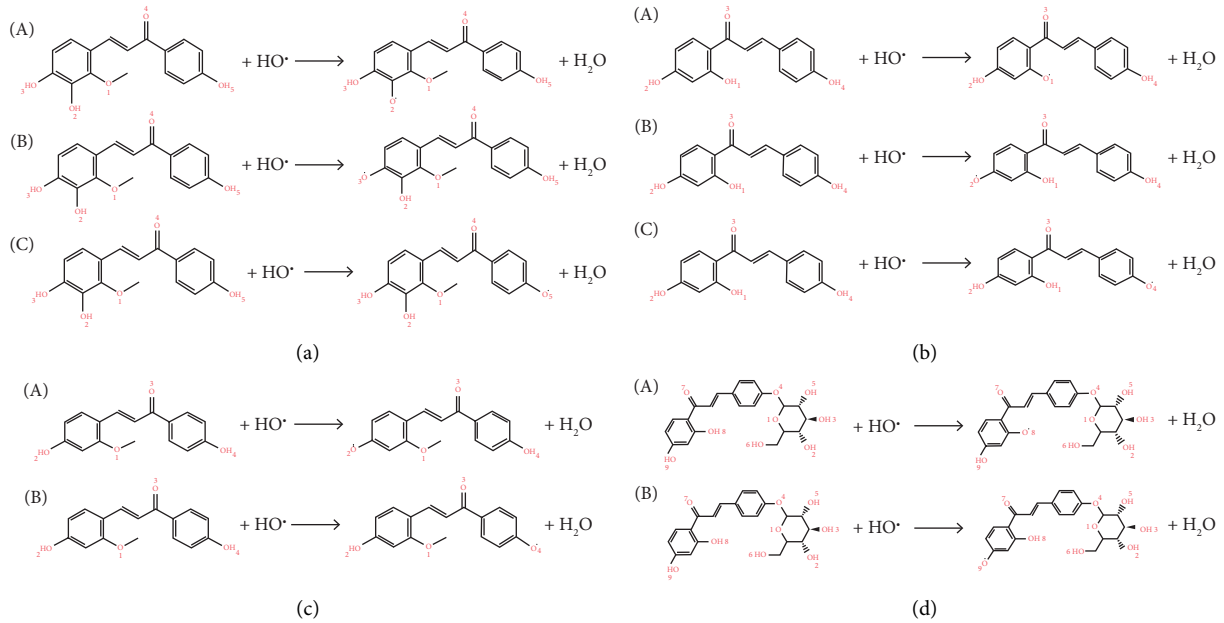



FIGURE 10: Reaction equations of the hydroxyl group with hydroxyl radical of licochalcone B (a), isoliquiritigenin (b), retrochalcone (c), and isoliquiritin (d).

chemical reactions with free radicals through the C=O and OH functional groups on the flavone skeleton structure. In addition, the energy gaps of licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin are 2.46, 2.48, 2.42, and 2.34 eV, respectively (Figure 9). This result indicates that these compounds all possess certain chemical reactivity. Among them, isoliquiritigenin has the smallest energy gap, indicating that it may possess higher biological activity and play a crucial role in the antioxidant process. Therefore, further calculations will be conducted to explore the relationship between different licorice flavonoid compounds and their antioxidant activities.

*3.7. Analysis of Reaction-Free Energy of Licorice Flavonoids.* Although the above results demonstrate that licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin have good antioxidant activity, the previous research conducted by the research team has revealed the structure-activity and dose-response relationships between licorice flavonoids and their antioxidant activity. However, the molecular mechanism regarding the impact of active groups at different positions on their antioxidant activity is still unclear. Therefore, this section will utilize DFT calculations to investigate the molecular mechanisms of antioxidant activity in licorice flavonoids. Figure 10 illustrates that licorice flavonoids eliminate free radicals through chemical reactions with hydroxyl free radicals in the hydrogen atom transfer (HAT) reaction path. Among them, hydroxyl free radicals are hydrogen-oxygen groups (OH) that contain an unpaired electron. They are highly reactive intermediates that can easily react with other molecules and participate in various biochemical processes and cellular signaling pathways. When there is an excessive amount of hydroxyl free radicals or when oxidative stress persists, it can lead to oxidative stress, resulting in cell damage and the occurrence of diseases. Table 5 displays the reaction-free energies between licochalcone B, isoliquiritigenin, retrochalcone, and isoliquiritin with hydroxyl free radicals under the HAT reaction path as determined by quantum calculations. It can be found that the reaction-free energy between the para-phenol hydroxyl group and hydroxyl radical on the left benzene ring structure of licochalcone B, isoliquiritigenin, retrochalcone, and isoliquiritin is lower than that of the ortho and meta-phenol hydroxyl groups. Moreover, the reaction-free energy between the para-phenol hydroxyl group and hydroxyl radical on the left benzene ring structure is less than 0. Specifically, the reaction-free energy of the para-phenol hydroxyl group (R-OH (3)) on the left side of licochalcone B's benzene ring is −2.53 kcal/mol, while the reaction-free energy of the meta-phenol hydroxyl group (R-OH (2)) on the same ring is 11.27 kcal/mol. The reaction-free energy of the para-phenol hydroxyl group (R-OH (2)) on the left side of isoliquiritigenin's benzene ring is −1.29 kcal/mol, while the reaction-free energy of the ortho-phenol hydroxyl group (R-OH (1)) on the same ring is 27.31 kcal/mol. The reaction-free energy of the para-phenol hydroxyl group (R-OH (2)) on the left side of retrochalcone's benzene ring is −6.30 kcal/mol. The reaction-free energy of the para-phenol hydroxyl group (R-OH (9)) on the left side of isoliquiritin's benzene ring is −11.49 kcal/mol, while the reaction-free energy of the ortho-phenol hydroxyl group (R-OH (8)) on the same ring is −0.87 kcal/mol. This phenomenon indicates that the reaction-free energies in the HAT reaction path of the para-phenol hydroxyl groups on the same benzene ring structure of these licorice flavonoid compounds are lower than those of the ortho and meta-phenol hydroxyl groups. This could be due to the following two

Table 5: Free energy of reaction under gas phase.

| Compound (298.15K) | Reaction path | Reactive group | Free radicals | Reactant energy (kcal/mol) | Product energy (kcal/mol) | Free energy of reaction (kcal/mol) |
|---|---|---|---|---|---|---|
| Licochalcone B | HAT | R-OH (2) | (i) OH | −671402.58 | −671391.31 | 11.27 |
| | | R-OH (3) | (ii) OH | −671402.58 | −671405.11 | −2.53 |
| | | R-OH (5) | (iii) OH | −671402.58 | −671388.80 | 13.79 |
| Isoliquiritigenin | HAT | R-OH (1) | (i) OH | −599558.57 | −599531.26 | 27.31 |
| | | R-OH (2) | (ii) OH | −599558.57 | −599559.86 | −1.29 |
| | | R-OH (4) | (iii) OH | −599558.57 | −599541.28 | 17.29 |
| Retrochalcone | HAT | R-OH (2) | (i) OH | −624183.9521 | −624190.26 | −6.30 |
| | | R-OH (4) | (ii) OH | −624183.9521 | −624181.48 | 2.47 |
| Isoliquiritin | HAT | R-OH (8) | (i) OH | −982714.79 | −982715.67 | −0.87 |
| | | R-OH (9) | (ii) OH | −982714.79 | −982726.09 | −11.49 |

reasons: (1) The presence of the para-phenol hydroxyl group can increase the electron density of flavonoid compounds and enhance their electron affinity, resulting in a stronger ability to scavenge free radicals. (2) The para-phenol hydroxyl group is directly attached to the aromatic ring, which makes them more prone to interact with surrounding molecules, thereby increasing their efficiency in capturing free radicals and transferring electrons [14, 29]. Therefore, this study suggests that the para-phenol hydroxyl groups on the same benzene ring structure of licorice flavonoid compounds may play an important role in antioxidant activity. This indicates that the para-phenol hydroxyl group could be one of the key functional groups contributing to the antioxidant activity of flavonoid compounds.

## 4. Conclusion

This study focuses on investigating the molecular mechanisms of antioxidant activity in licorice flavonoids using pharmacophore theory and quantum computing. First, we used network pharmacology methods to screen for the main active components and key targets with antioxidant activity in licorice flavonoids. Next, we employed pharmacophore theory to identify potential key structural features in licorice flavonoids that may contribute to their antioxidant activity and validate them using molecular docking. Finally, we utilized quantum computing methods to calculate the electronic structure, orbital distribution, and related physicochemical properties of licorice flavonoid molecules. Network pharmacology studies have shown that isoliquiritigenin, 4′-methoxyflavone, mosloflavone, isoliquiritin, 7-hydroxyflavone, retrochalcone, nobiletin, corylin, licochalcone B, and licoflavone A are the main active components responsible for the antioxidant activity of licorice flavonoids. TYR has been identified as the primary target of antioxidant activity for licorice flavonoids. The pharmacophore studies have shown that, compared to other pharmacophore models, pharmacophore 10 exhibits the highest correlation coefficient in predicting activity. This indicates that pharmacophore 10 has a high accuracy in predicting activity. In addition, licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin have low IC50 predictive activity values, suggesting that these compounds are crucial components of licorice flavonoids in antioxidant processes. Molecular electrostatic potential (MEP) studies have shown that hydroxyl groups on licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin are the regions with the lowest electron density. These hydroxyl groups can act as electron acceptor groups and are prone to nucleophilic substitution reactions with free radicals. Molecular frontier orbital studies have shown that licochalcone B, retrochalcone, isoliquiritin, and isoliquiritigenin have energy gaps of 2.46, 2.48, 2.42, and 2.34 eV, respectively. This indicates that these compounds possess certain chemical reactivity. Quantum computing research has found that the reaction-free energy between the para-phenol hydroxyl group and hydroxyl radical on the core structure of licochalcone B, isoliquiritigenin, retrochalcone, and isoliquiritin is lower than that of the ortho and meta-phenol hydroxyl groups in the HAT reaction path. The reaction-free energy between the para-phenol hydroxyl group and hydroxyl radical on the core structure is less than 0. This indicates that the para-phenol hydroxyl group is a crucial functional group for the antioxidant activity of flavonoid compounds. In conclusion, this study successfully revealed the possible mechanism of action of licorice flavonoids in the antioxidant process, providing some guidance for the synthesis of new antioxidant compounds.

## Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

Yi Hu conceptualized the study, wrote the original draft, and wrote, reviewed, and edited the manuscript. Peiyi Liang and Zhuxian Wang conceptualized the study and wrote, reviewed, and edited the study. CuiPing Jiang conceptualized the study, wrote, reviewed, edited the study and performed an investigation. Yinglin Guo, Hongkai Chen, and

Chunyan Shen proposed a methodology and curated the data. Yufan Wu proposed a methodology and performed validation. Li Liu proposed a methodology and investigated the data. Yankui Yi was responsible for the Software and validation. Qiang Liu conceptualized the study, administered the project, and wrote, reviewed, and edited the manuscript. Hongxia Zhu was responsible for funding acquisition, project administration, writing of the original draft, and writing, review, and editing.

## Supplementary Materials

Supplementary material related to this article can be found. Figure S1. Molecular structure of licochalcone B (a), retrochalcone (b), isoliquiritin (c), and isoliquiritigenin (d). Figure S2. The pharmacophore with predictive power and its matching with the most active small molecule. Figure S3. The pharmacophore with predictive power and its matching with the lowest active small molecule. Figure S4. △cost analysis of pharmacophore model. Figure S5. Prediction ability of 10 pharmacophore models for training set compounds. Figure S6. Test set validation statistics of the pharmacophore model and correlation curve of the test set LogEstimate vs LogActiv. Figure S7. The pharmacophore model number 10 with predictive ability and its matching with licochalcone B, the most active small molecule of licorice flavonoids. Figure S8. The pharmacophore model number 10 with predictive ability and its matches with nobiletin, a small molecule of licorice flavonoids with the lowest activity. (*Supplementary Materials*)

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