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# A Systems Biology-Based Approach to Uncovering Molecular Mechanisms Underlying Effects of Traditional Chinese Medicine Qingdai in Chronic Myelogenous Leukemia, Involving Integration of Network Pharmacology and Molecular Docking Technology

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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**Background:** The method of multiple targets overall control is increasingly used to predict the main active ingredient and potential target group of Chinese traditional medicines and to determine the mechanisms involved in their curative effects. Qingdai is the main traditional Chinese medicine used in the treatment of chronic myelogenous leukemia (CML), but the complex active ingredients and antitumor targets in treatment of CML have not been clearly defined in previous studies.

**Material/Methods:** We constructed a protein-protein interaction network diagram of CML with 638 nodes (proteins) and 1830 edges, based on the biological function of chronic myelocytic leukemia by use of Cytoscape, and we determined 19 key gene nodes in the CML molecule by network topological properties analysis in a data bank. Then, we used the Surflex-dock plugin in SYBYL7.3 docking and acquired the protein crystal structures of key genes involved in CML from the chemical composition of the traditional Chinese medicine Qingdai with key proteins in CML networks.

**Results:** According to the score and the spatial structure, the pharmacodynamically active ingredients of Qingdai are Isdirubin, Isoindigo, N-phenyl-2-naphthylamine, and Isatin, among which Isdirubin is the most important. We further screened the most effective activity key protein structures of CML to find the best pharmacodynamically active ingredients of Qingdai, according to the binding interactions of the inhibitors at the catalytic site performed in best docking combinations.

**Conclusions:** The results suggest that Isdirubin plays a role in resistance to CML by altering the expressions of PIK3CA, MYC, JAK2, and TP53 target proteins. Network pharmacology and molecular docking technology can be used to search for possible reactive molecules in traditional chinese medicines (TCM) and to elucidate their molecular mechanisms.

**MeSH Keywords:** Leukemia, Myelogenous, Chronic, BCR-ABL Positive • Medicine, Chinese Traditional • Molecular Docking Simulation

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## Background

In recent years, the method of integrated pharmacology and biological networks has been applied to fields in the life sciences [1], such as drug-target identification, the discovery of lead compounds, mechanism of action research, and preclinical drug efficacy and safety evaluation. Among them, based on molecular docking, drug-target network construction, and analysis of network characteristics, the methods of multiple targets overall control are gradually being used to predict the main active ingredients and potential target groups of Chinese traditional medicines, to define the mechanisms by which traditional Chinese medicines (TCM) exert curative effects.

CML is a clonal disorder that is usually easily diagnosed because the leukemic cells of more than 95% of patients have a distinctive cytogenetic abnormality called the Philadelphia chromosome (Ph1) [2,3]. The Ph1 results from a reciprocal translocation between the long arms of chromosomes 9 and 22, and is demonstrable in all hematopoietic precursors [4]. This translocation results in the transfer of the Abelson (Abl) on chromosome 9 oncogene to an area of chromosome 22, termed the breakpoint cluster region (BCR) [4]. This, in turn, results in a fused BCR/Abl gene and in the production of an abnormal tyrosine kinase protein that causes the disordered myelopoiesis found in CML, and chemotherapy drug resistance [5,6].

Imatinib (STI571, Gleevec), a Bcr-Abl tyrosine kinase inhibitor, is highly effective and is currently the first-line therapy for CML [7]. In addition, several first-line drugs are available for therapeutic use in CML, including nilotinib and dasatinib [8–10]. Although imatinib has improved clinical outcomes in the chronic phase of CML, drug resistance emerged in some patients, especially in the accelerated phase and blast crisis. Second- and third-generation inhibitors are effective against most imatinib-resistant (IMR) CML, but some patients become resistant to these drugs [11]. Hence, there is still an urgent need to develop novel agents that can be used to overcome Bcr-Abl inhibitor resistance.

According to reports in the literature, Qingdai is the main traditional Chinese medicine used in the treatment of CML. It was previously believed that the active components of Qingdai is indirubin and its action mechanism is tumor cell DNA synthesis, but use of indirubin alone cannot control or delay the occurrence of acute lesions [13,14].

To discover the Qingdai complex active ingredients and study antitumor targeted treatment in CML, we screened the known genetic genes of chronic myelogenous leukemia (CML) in the OMIM (Online Mendelian Inheritance in Man) database [15] and performed literature mining for the genes using the Agilent Literature Search plugin. We established a protein-protein interaction network based on the biological function of chronic myelocytic leukemia by use of Cytoscape, and determined the key gene nodes in the CML molecule with network topological properties analysis. Then, we used the Surflex-Dock plugin in sybylX1.1 dock analysis between the 9 known chemical compositions of Qingdai with 19 key proteins in CML gene networks to further explore the antitumor molecular mechanism of Qingdai.

## Material and Methods

### Construction of CML protein interaction network

Through OMIM database retrieval, we found that 79 genes were identified (Table 1). Then, the genes were submitted to the Cytoscape 2.8.2 plugin AgilenLiterature Search 2.7.7 (USA Agilent Technologies company) and PubMed, and we found a protein-protein interaction network diagram of CML with 638 nodes (proteins) and 1830 edges. As shown in Figure 1, the triangles represent OMIM genetic disease-related proteins, while the diamonds represent the proteins obtained from text mining.

### The determination of key proteins

Network topology attribute analysis shows the connectivity of nodes in the network greater than or equal to 20, corresponding to a sharp reduction in the number of nodes (Figure 2). Therefore, we regarded the 19 nodes as the key nodes (hubs). Then, we retrieved the protein crystal structures of key genes in CML from the protein data bank (Table 2).

### Database establishment of 3D structure ligands

We retrieved data on the traditional Chinese medicine Qingdai from the chemical and molecular engineering traditional Chinese medicines information database CHDD [16] created by Beijing University of Traditional Chinese Medicines and the Beilstein database, and found 9 compounds with different structures: *Isdirubin, Indigo, Isoindigo, N-phenyl-2-naphthylamine,* β-*sitosterol, tryptan-thren, Qigdainone, Isatin,* and *n-nonacosane.* The 9 chemical components of the Chinese medicine Qingdai acted as ligands. For the ligands preparation, the two-dimensional structures of the compounds were constructed using ChemDraw Ultra 8.0 (Figure 3) and were saved as mol2 format files that could be identified by SYBYL 7.3. Then, mo12 format files were processed with energy minimum after being imported, which gave each compound the most stable three-dimensional conformation. Finally, three-dimensional conformations were joined to the database.





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**Table 1.** 79 genes that were reported to be genes were identified.

| ID | Gene | ID | Gene | ID | Gene | ID | Gene | ID | Gene | ID | Gene | ID | Gene |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ABL1 | 13 | ABL2 | 25 | AKT1 | 36 | ANPEP | 47 | APAF1 | 58 | AQP5 | 69 | ASXL1 |
| 2 | AURKA | 14 | BAX | 26 | BCL11b | 37 | Bcl2 | 48 | BCL2L1 | 59 | BCR | 70 | CBL |
| 3 | CBLB | 15 | CD27 | 27 | CDH13 | 38 | CDKN1A | 49 | DOK2 | 60 | EBAG9 | 71 | EXOSC5 |
| 4 | Fbxw7 | 16 | FLT3 | 28 | FN1 | 39 | FUT4 | 50 | FYN | 61 | H2afx | 72 | HMGA2 |
| 5 | HOXA10 | 17 | HOXA11 | 29 | HRAS | 40 | IDH1 | 51 | IDH2 | 62 | IFNA1 | 73 | Il6 |
| 6 | Irf4 | 18 | Irf8 | 30 | JAK2 | 41 | JUNB | 52 | KBTBD11 | 63 | KIT | 74 | Lcn2 |
| 7 | LOC425684 | 19 | LOC514358 | 31 | LOC556890 | 42 | LOC560982 | 53 | LYN | 64 | MAP2K7 | 75 | Map3k1 |
| 8 | MCL1 | 20 | MIR21 | 32 | MIR328 | 43 | MME | 54 | MPL | 65 | MTHFR | 76 | MUC1 |
| 9 | Myc | 21 | NEWENTRY | 33 | NFKB1 | 44 | NTRK1 | 55 | NUDCD1 | 66 | PCBP2 | 77 | PDCD5 |
| 10 | PTPN6 | 22 | RALA | 34 | RBM15 | 45 | RPS6KB1 | 56 | SIGLEC8 | 67 | SIRT1 | 78 | SOCS3 |
| 11 | SOCS6 | 23 | SPHK1 | 35 | STAT1 | 46 | STAT3 | 57 | STAT5A | 68 | TET2 | 79 | TP53 |
| 12 | USP9X | 24 | Zfp423 | | | | | | | | | | |

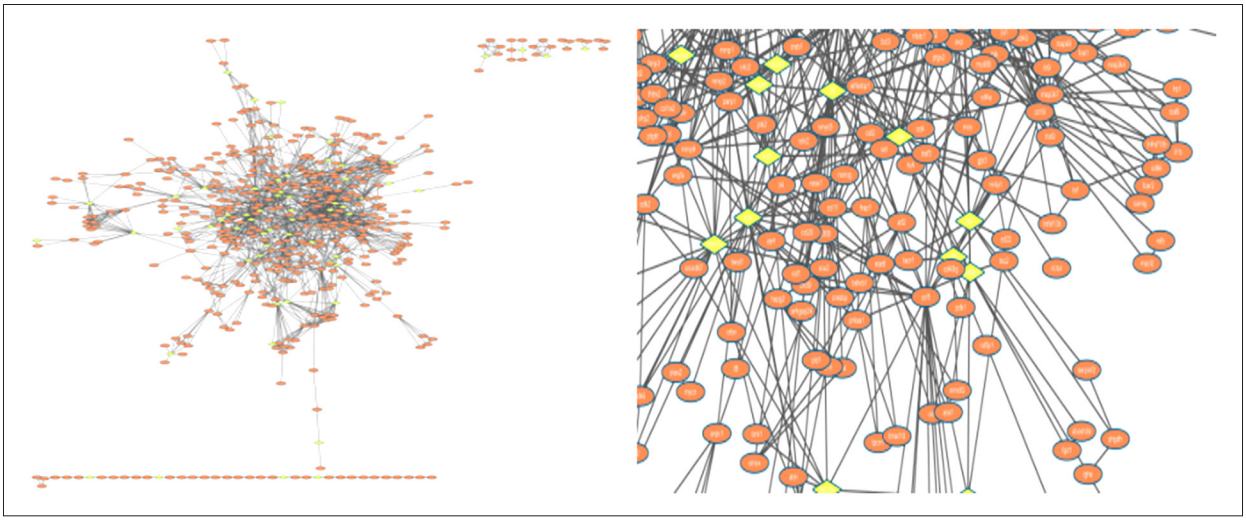

**Figure 1.** Network Map of Chronic Myeloid Leukemia Protein Interaction (Overall + partial).

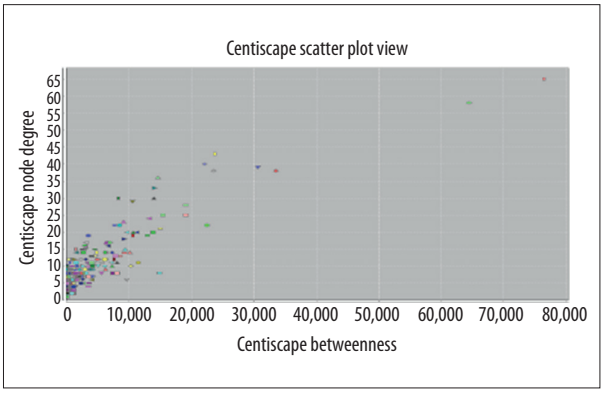


**Figure 2.** Connectivity degree of each node degree and betweenness comparison. The horizontal axis represents betweenness, and the ordinate represents the connectivity degree. The graphic in the table represents each node in the network.





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**Table 2.** Target protein associated with the pathogenesis of chronic myelogenous leukemia.

| Gene | PDB ID | Gene | PDB ID | Gene | PDB ID |
|---|---|---|---|---|---|
| ABL1 | 4WA9 | JAK2 | 5AEP | PARP1 | PARP1 |
| BCL2L1 | 3ZLN | JUN | 2G01 | PIK3CA | 4WAF |
| BCR | 3IK3 | MAPK3 | 2ZOQ | SRC | SRC |
| CD4 | 4KA2 | MAPK8 | 3PZE | STAT3 | 4ZIA |
| CDKN1A | 4NJD | MAPK14 | 2YIX | TNFSF13 | 1U5Y |
| EPHB2 | 1JPA | MYC | 4Y7R | TP53 | 3DCY |
| FRAP1 | 3OAW | | | | |

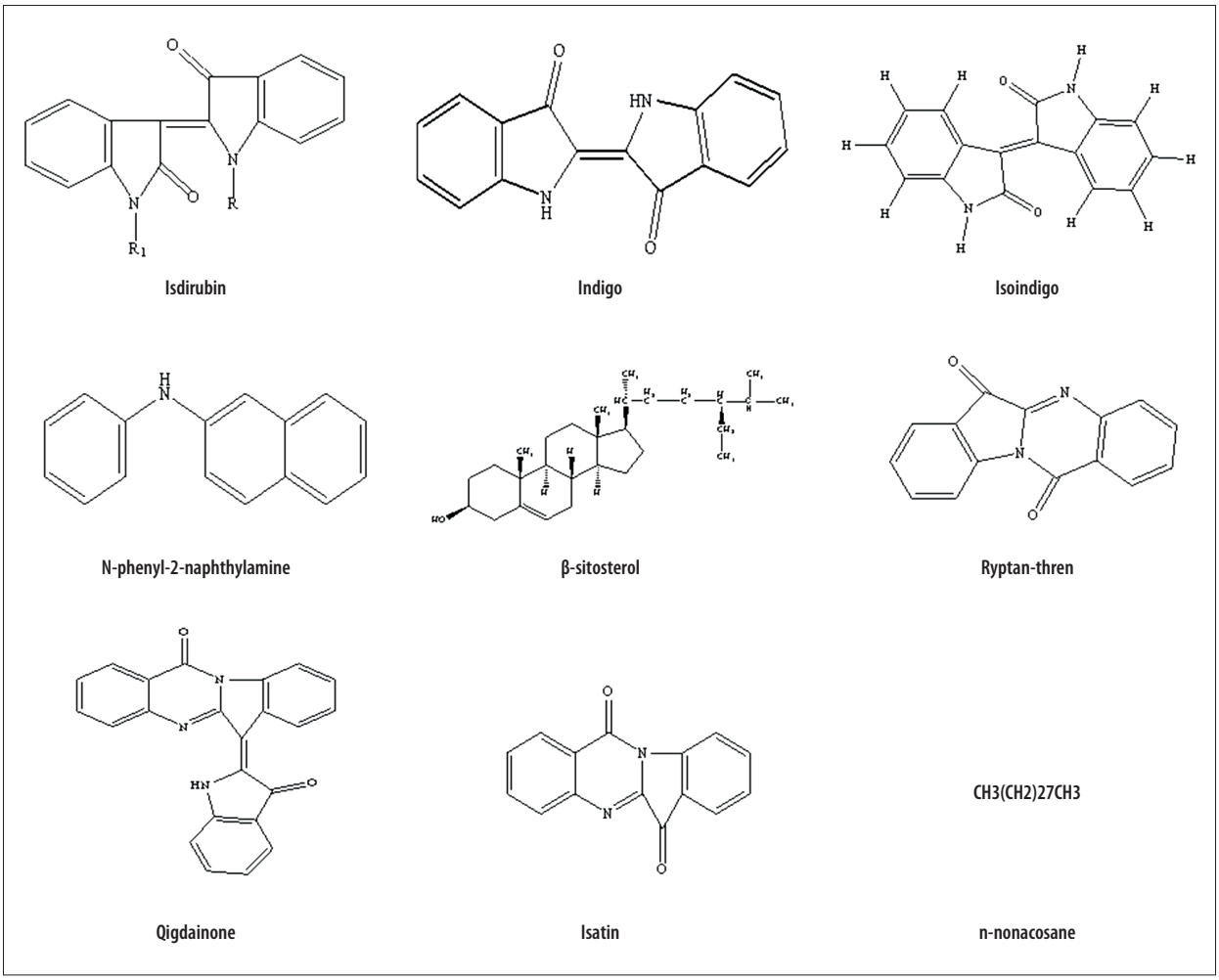


**Figure 3.** The two-dimensional structures of the compounds in Qingdai.

### Preparation of target protein activity pockets

The Surflex-docking module in SYBYL was first proposed and applied by Jain et al. at the University of California, San Francisco [17]; it has good characteristics of high-accuracy docking, high true-positive rate, and fast speed. Its prototype molecule, ProtoMol, is a representative protein activity docking pocket, and it uses 3 probes (NH, CH4, C=0) to populate the protein docking pockets. These 3 probes represent the hydrogen bond donor, receptor, and drain water points to fill the docking pockets, and were converted to protein docking pocket negative images.





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**Table 3.** The results of docking nine compounds of Qingdai (ABL1, BCL2L1, BCR ,CD4) with 19 key proteins of CML.

| Compound | ABL1 | | BCL2L1 | | BCR | | CD4 | |
|---|---|---|---|---|---|---|---|---|
| | T-Score | HB* | T-Score | HB* | T-Score | HB* | T-Score | HB* |
| 1 (Isdirubin) | 3.1568 | 1 | 4.1757 | 1 | 3.3698 | 1 | 2.8296 | 1 |
| 2 (Indigo) | 3.4641 | 1 | 5.4038 | 0 | 3.2092 | 0 | 2.4649 | 1 |
| 3 (Isoindigo) | 3.6801 | 2 | 4.4773 | 0 | 3.0018 | 0 | 3.0925 | 0 |
| 4 (N-phenyl-2-naphthylamine) | 4.2026 | 1 | 5.8536 | 1 | 4.7941 | 1 | 3.9843 | 1 |
| 5 (β-sitosterol) | 0.7455 | 0 | 2.0367 | 0 | 4.5977 | 1 | 5.2632 | 0 |
| 6 (Tryptan-thren) | 3.8393 | 0 | 4.7965 | 0 | 3.5763 | 1 | 2.0821 | 1 |
| 7 (Qigdainone) | 4.0561 | 1 | 5.1180 | 2 | 3.4835 | 0 | 2.0461 | 2 |
| 8 (Isatin) | 4.4990 | 1 | 5.5062 | 0 | 2.7179 | 0 | 2.6113 | 0 |
| 9 (N-nonacosane) | 10.3393 | 0 | 11.2345 | 0 | 11.8919 | 0 | 10.1846 | 0 |

* The number of hydrogen bonds between ligands and protein.

**Table 4.** The results of docking nine compounds of Qingdai (CDKN1A, EPHB2, FRAP1, JAK2) with 19 key proteins of CML.

| Compound | CDKN1A | | EPHB2 | | FRAP1 | | JAK2 | |
|---|---|---|---|---|---|---|---|---|
| | T-Score | HB* | T-Score | HB* | T-Score | HB* | T-Score | HB* |
| 1 (Isdirubin) | 3.6374 | 2 | 3.7304 | 2 | 5.8379 | 1 | 5.8357 | 2 |
| 2 (Indigo) | 3.3901 | 1 | 2.3765 | 0 | 7.4130 | 0 | 3.8144 | 1 |
| 3 (Isoindigo) | 3.1349 | 2 | 3.4825 | 2 | 5.9490 | 1 | 3.9821 | 2 |
| 4 (N-phenyl-2-naphthylamine) | 3.8790 | 1 | 2.9925 | 1 | 5.0802 | 0 | 4.5074 | 0 |
| 5 (β-sitosterol) | 5.8061 | 0 | 1.8533 | 0 | 5.4994 | 1 | 4.7564 | 1 |
| 6 (Tryptan-thren) | 2.3183 | 1 | 2.9325 | 1 | 4.9159 | 1 | 3.2431 | 0 |
| 7 (Qigdainone) | 2.4190 | 0 | 3.9367 | 0 | 7.4917 | 1 | 2.5039 | 0 |
| 8 (Isatin) | 2.3684 | 1 | 2.6611 | 1 | 5.0279 | 1 | 4.1612 | 1 |
| 9 (N-nonacosane) | 9.0341 | 0 | 7.4195 | 0 | 8.4098 | 0 | 9.8562 | 0 |

* The number of hydrogen bonds between ligands and protein.

Before initiating the docking simulations, the co-crystallized ligand and structural water molecules were removed from the crystal structure and the polar hydrogen atoms were added in SYBYL software. Meanwhile, the Kollman-all atom charges were assigned to protein atoms. The small molecular ligands are matched and complemented by the structures of the shape similarity with these probes when docking. Hydrogen bonding receptors and hydrogen bond donor together, and a few water points also unify them. The scored, ignored charge of small molecules and macromolecules in the whole process of docking is entirely based on structural similarity and shape similarity to match.

### Molecular docking and comprehensive score

The binding site was generated by the ligand-based mode. Using the Surflex-Dock program in SYBYL 7.3 software, these 9 compounds were docked into the ligands binding sites of these proteins. In this process, the ProtoMol bloat and ProtoMol threshold parameters, which determine the volume and extent of the ProtoMol, were specified as default values of 0 and 0.50 Å, respectively. Then, a binding pocket was set up and all the compounds were docked into the prepared protein.





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**Table 5.** The results of docking nine compounds of Qingdai (MYC, PARP1, PICK3A, SRC) with 19 key proteins of CML.

| Compound | MYC | | PARP1 | | PICK3A | | SRC | |
|---|---|---|---|---|---|---|---|---|
| | T-Score | HB* | T-Score | HB* | T-Score | HB* | T-Score | HB* |
| 1 (Isdirubin) | 2.4197 | 1 | 4.9958 | 1 | 5.2687 | 3 | 2.8296 | 1 |
| 2 (Indigo) | 3.1056 | 1 | 4.7382 | 0 | 2.7554 | 2 | 2.4649 | 1 |
| 3 (Isoindigo) | 3.0384 | 0 | 4.7824 | 1 | 5.2607 | 3 | 3.0925 | 2 |
| 4 (N-phenyl-2-naphthylamine) | 6.3210 | 1 | 5.5893 | 0 | 3.1753 | 0 | 5.2632 | 0 |
| 5 (β-sitosterol) | 5.5842 | 0 | 5.7791 | 0 | 5.2930 | 0 | 5.2632 | 0 |
| 6 (Tryptan-thren) | 3.2758 | 1 | 3.8816 | 0 | 2.7340 | 0 | 2.0821 | 2 |
| 7 (Qigdainone) | 3.0311 | 1 | 5.1770 | 0 | 1.2001 | 0 | 2.0461 | 0 |
| 8 (Isatin) | 2.8794 | 1 | 5.4552 | 1 | 4.0078 | 1 | 2.6113 | 1 |
| 9 (N-nonacosane) | 6.0826 | 0 | 11.1019 | 0 | 8.9632 | 3 | 10.1846 | 0 |

* The number of hydrogen bonds between ligands and protein.

**Table 6.** The results of docking nine compounds of Qingdai (JUN, MAPK3, MAPK8, MAPK14) with 19 key proteins of CML.

| Compound | JUN | | MAPK3 | | MAPK8 | | MAPK14 | |
|---|---|---|---|---|---|---|---|---|
| | T-Score | HB* | T-Score | HB* | T-Score | HB* | T-Score | HB* |
| 1 (Isdirubin) | 3.5401 | 2 | 4.1810 | 2 | 3.5849 | 0 | 4.4044 | 2 |
| 2 (Indigo) | 2.5436 | 0 | 4.6699 | 0 | 4.2631 | 1 | 4.0518 | 0 |
| 3 (Isoindigo) | 2.9709 | 1 | 4.5529 | 3 | 3.1983 | 0 | 4.8476 | 1 |
| 4 (N-phenyl-2-naphthylamine) | 3.6190 | 1 | 4.6036 | 1 | 3.8663 | 0 | 5.2216 | 0 |
| 5 (β-sitosterol) | 6.0947 | 1 | 2.6685 | 2 | 5.5876 | 1 | 6.4003 | 0 |
| 6 (Tryptan-thren) | 4.4408 | 1 | 4.6631 | 1 | 3.9435 | 0 | 3.9188 | 0 |
| 7 (Qigdainone) | 2.1274 | 0 | 5.0603 | 1 | 4.3059 | 2 | 2.1203 | 2 |
| 8 (Isatin) | 2.5041 | 0 | 4.2444 | 1 | 4.2433 | 0 | 3.7115 | 1 |
| 9 (N-nonacosane) | 9.3536 | 0 | 9.6257 | 0 | 9.2888 | 0 | 9.5436 | 0 |

* The number of hydrogen bonds between ligands and protein.

After molecular docking according to the default parameters, score values were generated for each compound docking with CML key targets:

1. Score value is showed as -log (KD), because RT (KD)=G (in the combination of ligand and receptor binding affinity). With a smaller binding affinity, there is a more stable ligand and receptor. So, the greater the score value, the smaller the G value is, and the greater the stability of the ligand and receptor.
2. The binding modes can observe small-molecule drugs in the combination of the receptor activity sites observed to be combined with domain structure similarity and difference, and greater similarity is associated with greater stability.
3. The stronger the hydrogen bond, the greater the stability of the ligand and receptor. A score was generated after each compound docked with the target value, the binding modes, and the number of hydrogen bonding joint evaluations. The combination evaluation method can improve the docking accuracy.





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**Table 7.** The results of docking nine compounds of Qingdai (STAT3, TNFSF13, TP53) with 19 key proteins of CML.

| Compound | STAT3 | | TNFSF13 | | TP53 | |
|---|---|---|---|---|---|---|
| | T-Score | HB* | T-Score | HB* | T-Score | HB* |
| 1 (Isdirubin) | 2.1319 | 1 | –6.0081 | 1 | 3.7566 | 4 |
| 2 (Indigo) | 1.9192 | 1 | –4.2468 | 2 | 3.2636 | 2 |
| 3 (Isoindigo) | 2.2789 | 2 | –10.8893 | 2 | 3.4965 | 2 |
| 4 (N-phenyl-2-naphthylamine) | 2.5406 | 0 | –1.5608 | 1 | 2.7542 | 0 |
| 5 (β-sitosterol) | 4.1544 | 2 | –58.1653 | 1 | 3.8284 | 1 |
| 6 (Tryptan-thren) | 1.9757 | 2 | –12.2350 | 1 | 4.3113 | 2 |
| 7 (Qigdainone) | 2.0377 | 0 | –27.1805 | 0 | 4.2020 | 1 |
| 8 (Isatin) | 1.9388 | 2 | –12.9568 | 0 | 3.3673 | 2 |
| 9 (N-nonacosane) | 6.3491 | 0 | 0.4307 | 0 | 4.7143 | 0 |

* The number of hydrogen bonds between ligands and protein.

## Results

### Screening results

The Surflex-docking total score is based on the number of hydrogen bonds between ligands and protein, and the intermolecular interactions between ligands and protein, after docking of the 9 compounds of Qingdai with 19 key proteins of CML. The screening results are shown in Tables 3–7.

### Screening of important medicinal ingredients of Qingdai

For further screening for important medicinal ingredients in Qingdai after comprehensive comparison, we took the top 3 traditional Chinese medicine components in each protein group, as shown in Table 8. The results of frequency measurement statistics are isdirubin-16, Isoindigo-11, N-phenyl-2-naphthylamine-8, Isatin-6, Qigdainone-4, β-sitosterol-4, Tryptanthren-4, and Iindigo-3. The results show that isdirubin is the ligand with the best activity, and it can dock well with the 16 key proteins of chronic myelogenous leukemia.

### Best docking combination to Isdirubin

To provide a deeper insight into the binding interactions of the inhibitors at the catalytic site, and to further screen for the most effective and active key protein structures for isdirubin, the docking process was performed. As shown in these docking Figure 4, the structures with the greatest potential were compounds PIK3CA, MYC, JAK2, and TP53, which were selected for more detailed analysis to illustrate the interaction mechanism because these combinations have better spatial structure, number of hydrogen bonds, and scores.

It can be seen that some key amino acids interacted with the inhibitor by hydrogen bond at the binding site. In Figure 4-TP53, there are 4 hydrogen bonds: 1 between the N-H of isdirubin and the oxygen of GLU-89, 1 between the oxygen of carbonyl in isdirubin and the hydrogen amino of ARG-203, and 2 between the oxygen of carbonyl in isdirubin and the hydrogen amino of different positions in ARG-203. In Figure 4-JAK2, there is a pair of hydrogen bonding interactions between isdirubin and the protein backbone: one between the hydrogen amino and the oxygen of carbonyl in GLU-930, and the other between the oxygen of carbonyl and the N-H of LEU-932. Figure 4-MYC shows there is a hydrogen bonding interaction between the hydrogen amino of isdirubin and the oxygen of hydroxyl in SER-91. In Figure 4-PIK3CA, there are 3 hydrogen bonds between isdirubin and the protein backbone: 1 between the oxygen of carbonyl in isdirubin and the N-H of VAL-851, and 2 between the hydrogen amino and oxygen of hydroxyl in SER-854 and oxygen of carbonyl in VAL-851.

## Discussion

Although a great deal of effort has gone into unveiling the mechanisms behind TCM formulas, most remain unknown [18]. Thus, a systematic analysis of the complex mechanism behind TCM formulas is required. The rapid development of computational analyses and systems modeling approaches provides rich and substantial content of “biological networks”,





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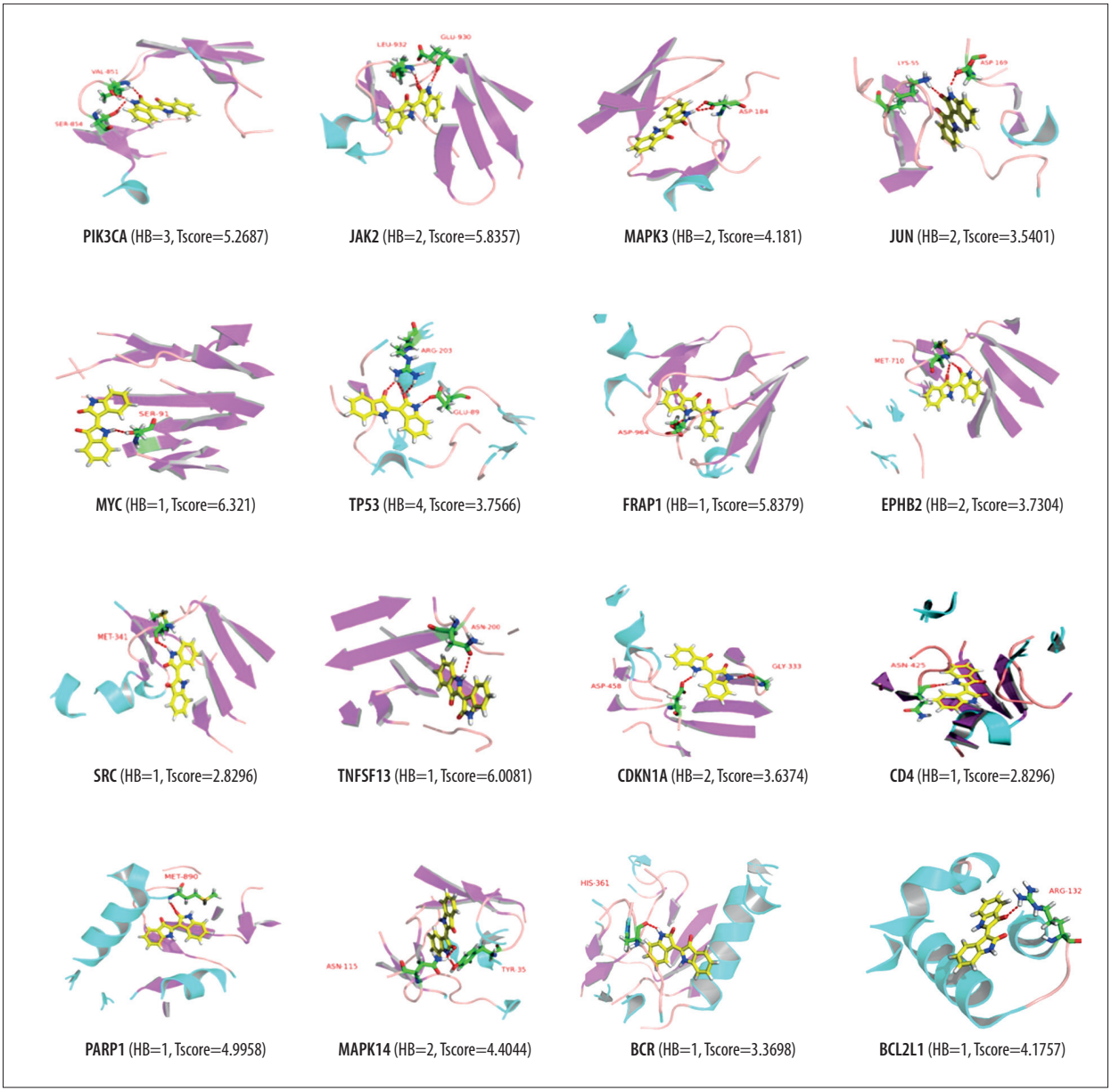


**Figure 4.** Isdirubin key proteins docking combination.

and generates a new view of the life sciences and medical research [19]. Recently, the robustness of multiple systems biology platforms has enabled the discovery of underlying molecular mechanisms and connections between drugs and their targets (e.g., proteomics studies on activated blood circulation of Chinese medicinal herbs) [20]. Newly-developed algorithms and network-based computational models can integrate multilevel omics data and can optimize combinational regimens of drug development.

Computer-Aided Drug Design (CADD) is a new and effective method to design lead compounds of drugs using basic computational chemistry principles, by simulating interaction of a biological macromolecular receptor and drug, and analyzing the structure-activity relationship within known drugs [21]. Molecular docking has a very important role in drug design, and its scope of application is extremely broad. Through molecular docking, it can directly reveal the interaction between drug molecules and targets; molecular docking provides the most reasonable and effective mass model for active confirmation in structure-activity relationship research. Virtual screening via molecular docking can be used for the discovery of lead compounds. After years of development, the molecular docking method has gradually moved from the theoretical research phase into the practical phase. In recent years, the virtual screening method based on molecular docking in drug





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**Table 8.** The ranking top three traditional chinese medicine components in each protein group.

| Key proteins | Compounds of Qingdai | HB* | T-Score | Ranking |
|---|---|---|---|---|
| ABL1 | 8 (Isatin) | 1 | 4.499 | 1 |
| | 4 (N-phenyl-2-naphthylamine) | 1 | 4.2026 | 2 |
| | 3 (Isoindigo) | 2 | 3.6801 | 3 |
| BCL2L1 | 7 (Qigdainone) | 2 | 5.118 | 1 |
| | 4 (N-phenyl-2-naphthylamine) | 1 | 5.8536 | 2 |
| | 1 (Isdirubin) | 1 | 4.1757 | 3 |
| BCR | 4 (N-phenyl-2-naphthylamine) | 1 | 4.7941 | 1 |
| | 5 (β-sitosterol) | 1 | 4.5977 | 2 |
| | 1 (Isdirubin) | 1 | 3.3698 | 3 |
| CD4 | 4 (N-phenyl-2-naphthylamine) | 1 | 3.9843 | 1 |
| | 7 (Qigdainone) | 2 | 2.0461 | 2 |
| | 1 (Isdirubin) | 1 | 2.8296 | 3 |
| CDKN1A | 1 (Isdirubin) | 2 | 3.6374 | 1 |
| | 3 (Isoindigo) | 2 | 3.1349 | 2 |
| | 2 (Indigo) | 1 | 3.3901 | 3 |
| EPHB2 | 1 (Isdirubin) | 2 | 3.7304 | 1 |
| | 6 (Tryptan-thren) | 1 | 2.9325 | 2 |
| | 4 (N-phenyl-2-naphthylamine) | 1 | 2.9925 | 3 |
| FRAP1 | 7 (Qigdainone) | 1 | 7.4917 | 1 |
| | 3 (Isoindigo) | 1 | 5.949 | 2 |
| | 1 (Isdirubin) | 1 | 5.8379 | 3 |
| JAK2 | 1 (Isdirubin) | 2 | 5.8357 | 1 |
| | 3 (Isoindigo) | 2 | 3.9821 | 2 |
| | 8 (Isatin) | 1 | 4.1612 | 3 |
| JUN | 5 (β-sitosterol) | 1 | 6.0947 | 1 |
| | 1 (Isdirubin) | 2 | 3.5401 | 2 |
| | 4 (N-phenyl-2-naphthylamine) | 1 | 3.619 | 3 |
| MAPK3 | 3 (Isoindigo) | 3 | 4.5529 | 1 |
| | 1 (Isdirubin) | 2 | 4.181 | 2 |
| | 6 (Tryptan-thren) | 1 | 4.6631 | 3 |





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**Table 8.** The ranking top three traditional chinese medicine components in each protein group.

| Key proteins | Compounds of Qingdai | HB* | T-Score | Ranking |
|---|---|---|---|---|
| MAPK8 | 5 (β-sitosterol) | 1 | 5.5876 | 1 |
| | 7 (Qigdainone) | 2 | 4.3059 | 2 |
| | 2 (Indigo) | 1 | 4.2631 | 3 |
| MAPK14 | 1 (Isdirubin) | 2 | 4.4044 | 1 |
| | 3 (Isoindigo) | 1 | 4.8476 | 2 |
| | 8 (Isatin) | 1 | 3.7115 | 3 |
| MYC* | 4 (N-phenyl-2-naphthylamine) | 1 | 6.321 | 1 |
| | 1 (Isdirubin) | 1 | 2.4197 | 2 |
| PARP1 | 8 (Isatin) | 1 | 5.4552 | 1 |
| | 1 (Isdirubin) | 1 | 4.9958 | 2 |
| | 3 (Isoindigo) | 1 | 4.7824 | 3 |
| PIK3CA | 1 (Isdirubin) | 3 | 5.2687 | 1 |
| | 3 (Isoindigo) | 3 | 5.2607 | 2 |
| | 8 (Isatin) | 1 | 4.0078 | 3 |
| SRC | 3 (Isoindigo) | 2 | 3.0925 | 1 |
| | 1 (Isdirubin) | 1 | 2.8296 | 2 |
| | 8 (Isatin) | 1 | 2.6113 | 3 |
| STAT3 | 5 (β-sitosterol) | 2 | 4.1544 | 1 |
| | 3 (Isoindigo) | 2 | 2.2789 | 2 |
| | 6 (Tryptan-thren) | 2 | 1.9757 | 3 |
| TNFSF13 | 2 (Indigo) | 2 | –4.2468 | 1 |
| | 4 (N-phenyl-2-naphthylamine) | 1 | –1.5608 | 2 |
| | 1 (Isdirubin) | 1 | –6.0081 | 3 |
| TP53 | 6 (Tryptan-thren) | 2 | 4.3113 | 1 |
| | 1 (Isdirubin) | 4 | 3.7566 | 2 |
| | 3 (Isoindigo) | 2 | 3.4965 | 3 |

* The rest molecules docked with MYC protein did not completely enter into the protein structure, were not valid, so only two groups were here.

design has achieved great success [22], and this advanced method is effective in data mining to discover the effective components in traditional Chinese medicines.

We established a protein-protein interaction network based on the biological function of chronic myelocytic leukemia by using Cytoscape, and we determined 19 key genes in CML with network topological properties analysis. The existing literature on the genes-protein interaction network shows that it can be used to describe the interaction of chronic myeloid leukemia in molecules [23–28].

Molecular docking is the most widely used method for calculating protein-ligand interactions, and we used the Surflex-Dock program in SYBYL 7.3 software to investigate the probable binding modes. The results show that isdirubin has the best activity; it can dock well with 16 key targets of chronic myelogenous leukemia, which is consistent with the results of





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a long-term study showing that isdirubin is an effective drug for the treatment of chronic myelogenous leukemia, and it has beneficial hematologic effects, with a total hematologic remission rate of 61.5% [29]. Isdirubin is used in CML patients in low doses, and long-term administration can have a lasting inhibitory effect on bone marrow activity, with only mild adverse reactions [30,31].

In addition, Isoindigo, N-phenyl-2-naphthylamine, and Isatin also show good activity on docking frequency. Data in the literature show that Meisoindigo (isoindigo isomer) induced apoptotic cell death and disrupted the progression of K562 cells from the G(1) to G(2) phase [32]. The anticancer activity of the substituted pyridone-annelated isoindigo (5'-Cl) involves G0/G1 cell cycle arrest and inactivation of CDKs in the promyelocytic leukemia cell line HL-60 [33], and Isatin and its derivatives have a very good inhibitory effect in leukemia cells and many kinds of tumor cells [34–37]. However, thus far there have been no published reports showing that N-phenyl-2-naphthylamine is an effective treatment for leukemia and tumors. Therefore, our study is the first to show that using the network pharmacology and molecular docking technology to screen the effective active ingredients of Chinese traditional medicines is feasible. This method for screening of effective components of Chinese medicines has important research significance and value.

It can be seen that some key amino acids interacted with the inhibitor by hydrogen bonding at the binding site. Figure 4-TP53 shows that there are 4 hydrogen bonds: 1 between the N-H of isdirubin and the oxygen of GLU-89, 1 between the oxygen of carbonyl in isdirubin and hydrogen amino of ARG-203, and 2 between the oxygen of carbonyl in isdirubin and the hydrogen amino of different positions in ARG-203. In Figure 4-JAK2, there is a pair of hydrogen bonding interactions between the isdirubin and the protein backbone: one between the hydrogen amino and the oxygen of carbonyl in GLU-930, and the other between the oxygen of carbonyl and the N-H of LEU-932. Figure 4-MYC shows that there is a hydrogen bonding interaction between the hydrogen amino of isdirubin and oxygen of hydroxyl in SER-91. In Figure 4-PIK3CA, there are 3 hydrogen bonds between the isdirubin and the protein backbone: 1 between the oxygen of carbonyl in isdirubin and the N-H of VAL-851, and 2 between the hydrogen amino and oxygen of hydroxyl in SER-854 and oxygen of carbonyl in VAL-851.

To further screen the most effective activity key protein structures for isdirubin, the docking process was performed. By comparing the spatial structure, the number of hydrogen bonds, and score, the best potential structures – compounds PIK3CA, MYC, JAK2, and TP53 – were selected for more detailed analysis to illustrate the interaction mechanism. By observing molecular docking (Figure 4) between isdirubin and PIK3CA, MYC, JAK2, and TP53, it can be seen that isdirubin can dock VAL-851, SER-854, and VAL-854 amino 1 acid residues with PIK3CA; it can dock GLU-930, andEU-932 amino acid residues with JAK2; it can dock SER-91 amino acid residues with MYC; and it can dock GLU-89 and ARG-203 amino acid residues with TP53.

The present study confirmed that the traditional Chinese medicine Qingdai has the characteristics of multiple targets and pathways. On the one hand, the drug component can reflect its multicomponent action; and on the other hand, a single drug component also reflects multiple targets. Because of these components of multiple targets, multiple ways and curative effect as well as the "shotgun" principle show the integrity features of treatment and toning. At the same time, according to the SYBYL docking results and evaluation, this study provides the foundation for related research on the chemical composition effects and filter chemical composition, which has strong effects. On the other hand, high-score medicinal ingredients obtained from screening could be used as a potential key quantitative composition of optional fingerprinting. It can provide a scientific and practical basis for establishing the multi-target quantitative fingerprint and perfecting the quality evaluation of compound ingredients.

## Conclusions

The composition of traditional Chinese medicine is complicated, and they act on a variety of complex targets and pathways, which makes research on the active ingredients of Chinese traditional medicines difficult. Network pharmacology and molecular docking research methods, although they have many problems that need to be solved, provide a technology that can predict signaling pathway mechanisms and provide directions for future experimental work on TCM, so as to greatly inform experimental work and provide a new way of thinking about research on the material basis and mechanisms of traditional Chinese medicines [39–41].

## References:

1. Ye H, Liu Q, Wei J: Construction of drug network based on side effects and its application for drug repositioning. PLoS One, 2014; 9(2): e87864
2. Kurzrock R, Kantarjian HM, Druker BJ et al: Philadelphia chromosome-positive leukemias: From basic mechanisms to molecular therapeutics. Ann Intern Med, 2003; 138(10): 819–30
3. Goldman JM, Melo JV: Chronic myeloid leukemia – advances in biology and new approaches to treatment. N Engl J Med, 2003; 349(15): 1451–64
4. Deininger MW, Goldman JM, Melo JV: The molecular biology of chronic myeloid leukemia. Blood, 2000; 96(10): 3343–56
5. Wei G, Rafiyath S, Liu D: First-line treatment for chronic myeloid leukemia: Dasatinib, nilotinib, or imatinib. J Hematol Oncol, 2010; 3: 47





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6. Dai HP, Shen Q, Zhou JW et al: [Influence of Qingdai compound on expression of bcr/abl and JWA in K562 cells.] Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2005; 13(5): 809–11 [in Chinese]
7. Quintas-Cardama A, Kantarjian H, Comes J: Flying under the radar: The new wave of BCR-ABL inhibi-tors. Nat Rev Drug Discov, 2007; 6: 834–48
8. SaglioG, Kim DW, Issaragrisil S et al: Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med, 2010; 362(5): 2251–59
9. Fava C, Rege-Cambrin G, Saglio G: The choice of first-line chronic myelogenous leukemia treatment. Ann Hematol, 2015; 94(Suppl. 2): S123–31
10. Baccarani M, Castagnetti F, Gugliotta G, Rosti G: A review of the European LeukemiaNet recommendations for the management of CML. Ann Hematol, 2015; 94(Suppl. 2): S141–47
11. Sun X, Cai X, Yang J et al: Cantharidin overcomes imatinib resistance by depleting BCR-ABL in chronic myeloid leukemia. Mol Cells, 2016; 39(12): 869–76
12. Kimura S, Ando T, Kojima K: Ever-advancing chronic myeloid leukemia treatment. Int J Clin Oncol, 2014: 19: 3–9
13. Indirubin Clinical Collaboration: [The clinical study of indirubin treatment of 314 cases of chronic myelogenous leukemia.] Chinese Journal of Hematology, 1980; 1(13): 2–135 [in Chinese]
14. Fang F, Guanyun W: [The research progress of Indirubin anticancer mechanism]. Journal of Traditional Chinese Medical, 1982; 21: 312–16 [in Chinese]
15. Hu Z, Tu Y, Xia Y et al: Rapid identification and verification of indirubin-containing medicinal plants. Evid Based Complement Alternat Med, 2015; 5(18): 484670
16. Qiao X, Hou T, Zhang W et al: A 3D structure database of components from Chinese traditional medicinal herbs. J Chem Inf Comput Sci, 2002; 42: 481–89
17. Boström J: Reproducing the conformations of protein-bound ligands: A critical evaluation of several popular conformational searching tools. J Comput Aided Mol Des, 2001; 15(12): 1137–52
18. Li J, Yang L, Li Y et al: Metabolomics study on model rats of chronic obstructive pulmonary disease treated with BuFei JianPi. Mol Med Rep, 2015; 11: 1324–33
19. Li S: Frameworkj and practice of network-based studies for Chinese herbal formula. Chin J Integr Med, 2007; 5: 489–92
20. Liu Y, Yin H, Chen K: Platelet proteomics and its advanced application for research of blood stasis syndrome and activated blood circulation herbs of Chinese medicine. Sci China Life Sci, 2013; 56: 1000–6
21. Xu XJ, Hou TJ, Qiao XB et al: Computer aided drug molecular design. Beijing, chemical industry press, 2004
22. Gao Li, Liu AL, Du GH: [The progress in the application of computer aided drug design in new drug research and development.] Journal of Chinese Pharmacy, 2005; 46(9): 641–45 [in Chinese]
23. Margaret CF: Src in cancer: Deregulation and consequences for cell behaviour. Biochim Biophys Acta, 2002; 1602(2): 114–30
24. Biethahn S, Alves F, Wilde S et al: Expression of granulocyte colony-stimulated factor and granulocyte macrophage colony-stimulating factor associated signal transduction proteins of the JAK/Stat pathway in normal granulopoiesis and in blast cells of acute myelogenous leukemia. Exp Hematol, 1999; 27: 885–94
25. Reddy EP, Korapati A, Chaturvedi P et al: IL-3 signaling and the role of Src kinases, JAKs and STATs: A covert liaison unveiled. Oncogene, 2000; 19(21): 2532–47
26. Reilly JT: Chronic neutrophilic leukaemia: A distinct clinical Entity. Br J Haematol, 2002; 11(6): 10–18
27. *http://www.hgsi.com/products/LR131.html*
28. Donald P, Mc Lornan, Melanie J: Chronic neutrophilic leukemia with an associated V617F JAK2 tyrosine kinase mutation. Haematologica, 2005; 90: 1696–97
29. Gao SX: [26 cases clinical analysis on the curative effect of Isdirubin to chronic myelogenous leukemia.] The Community Physicians Medical Professional, 2006; 13(270): 49 [in Chinese[
30. O'Brien S, Kantarjin H, Keating M et al: Homoharringtonine therapy induces responses in patients with chronic myelogenous leukemia in late chronic phase. Blood, 2006; 86(3): 322–26
31. Gaboriaud-Kolar N, Myrianthopoulos V, Vougogiannopoulou K et al: Natural-based indirubins display potent cytotoxicity toward wild-type and T315I-resistant leukemia cell lines. J Nat Prod, 2016; 79(10): 2464–71
32. Wee XK, Yang T, Go ML: Exploring the anticancer activity of functionalized isoindigos: Synthesis, drug-like potential, mode of action and effect on tumor-induced xenografts. ChemMedChem, 2012; 7(5): 777–91
33. Saleh AM, Aljada A, El-Abadelah MM et al: The anticancer activity of the substituted pyridone-annelated isoindigo (5'-Cl) involves G0/G1 cell cycle arrest and inactivation of CDKs in the promyelocytic leukemia cell line HL-60.Cell Physiol Biochem, 2015; 35(5): 1943–57
34. Pajouhesh H, Parson R, Popp FD: Potential anticonvulsants VI: Condensation of isatin with cyclohexanone and other cyclic ketones. J Pharm Sci, 1983; 72: 318–21
35. Eshbha NH, Salama HM: 5-(2-oxo-3-indolinylidene) thiazolidine -2,4-di-one-1, 3-di-mannich base derivetesaynthesis and evaluation for antileukemic activity. Pharmazine, 1985; 40: 320–22
36. Hung CY, Hsu MH, Huang LJ et al: Synthesisofl-substituted-3-pyridinyl-methylidenylindolin-2-ones and 1- substituted-3-quinolinylmethylidenyl-indolin-2-ones as the rnhancers of ATRA-induced differentiation in HL-60 cells. Bioorg Med Chem, 2008; 16(8): 4222–32
37. Matesic L, Locke JM, Bremner JB et al: N-pnenethyl and N-naphthylmethyl isatin and analogues as *in vitro* cytotoxic agents. Bioorg Med Chem, 2008; 16, 3118–24
38. Yu F, Chen P, Xiang J: [A case of chronic arsenic poisoning was treated by compound indigo tablet in treatment of chronic myeloid leukemia.] Chinese Journal of Hematology, 1999; 20: 7 [in Chinese]
39. Fleischer T, Chang TT, Chiang JH et al: Adjunctive Chinese Herbal Medicine therapy improves survival of patients with chronic myeloid leukemia: A nationwide population-based cohort study. Cancer Med, 2016; 5(4): 640–48
40. Nam S, Scuto A, Yang F et al: Indirubin derivatives induce apoptosis of chronic myelogenous leukemia cells involving inhibition of Stat5 signaling. Mol Oncol, 2012; 6(3): 276–83
41. Cao C, Liu B, Zeng C et al: A polymethoxyflavone from Laggera pterodonta induces apoptosis in imatinib-resistant K562R cells via activation of the intrinsic apoptosis pathway. Cancer Cell Int, 2014; 14(1): 137





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