Research on Active Constituents from Chinese Medicine against Leukemia Targeting ENL based on Molecular Docking

Hao Sun[a] and Jian Tang[b]
School of Chinese Medicine, Bozhou University, Bozhou, Anhui, China

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Abstract: Leukemia is a class of malignant clonal disease of hematopoietic stem cells. It poses a huge threat to human health. Over the years, progress has been made in using traditional Chinese medicine (TCM) in China to treat and relieve various leukemia symptoms. Erb and he Wan et al noted that ENL protein is a key factor affecting the viability of MLL-r cells in leukemia. Molecular adding or removing molecular group modification on the histone can regulate gene expression A prominent structural feature of the ENL protein is a YEATS domain that identifies a specific acetyl (Ac) on the histone H3. Computer-aided Drug Design (CADD) method has gradually mature and plays an increasingly important role in drug development. Molecular docking is the most widely used and successful method in structure-based drug design. Molecular docking generally refers to the process in which two or more molecules identify each other through geometric and energy matching. Two major topics of molecular docking methods are spatial identification and energy identification between molecules.

1 INTRODUCTION

Leukemia is a class of malignant clonal disease of hematopoietic stem cells. It poses a huge threat to human health. Scientists have found that the regulatory protein ENL promotes leukemogenesis. Over the years, progress has been made in using traditional Chinese medicine (TCM) in China to treat and relieve various leukemia symptoms.

Mixed lineage leukemia (MLL) is named for the chromosome translocation of the MLL fusion protein at 11q23.This type of leukemia has attracted wide attention for its unique clinical and biological characteristics.

The cause of leukemia is usually chromosomal translocation, producing fusion proteins formed by fragment junctions of two proteins that ultimately cause disease. Fusion proteins in MLL are typical so commonly present in aggressive childhood leukemia and are associated with poor prognosis. Therefore, it is very necessary to develop leukemia treatment strategies based on MLL rearrangement (MLL-rearranged, MLL-r) fusion proteins. Erb and he Wan et al noted that ENL protein is a key factor affecting the viability of MLL-r cells in leukemia (Wan 2017).

The histones containing DNA in the cells are structural and signaling factors. Molecular adding or removing molecular group modification on the histone can regulate gene expression A prominent structural feature of the ENL protein is a YEATS domain that identifies a specific acetyl (Ac) on the histone H3. This suggests that this "read" ability of ENL to acetylated histone is essential for the induction of MLL-r leukemia.

Another complementary mechanism for SEC and DotCom stability was identified by Erb and he Wan et al. They found that inactivation of ENL impaired the SEC and DotCom function in MLL-r cells. The ability of ENL to bind to SEC, DotCom drops a hint that a model-ENL, through the YEATS domain, recognizes that acetylated H3, enhances the stability of binding of SEC and DotCom complexes to DNA and regulates the activity of aberrant regions of the genome.

Protein ENL is essential for MLL-r leukemia. Some leukemias exist resulting from a mixture of some MLL protein and part of another. The second protein is typically part of the SEC (super elongation complex) protein complex or the DotCom (DOT1L-containing complex). Both protein complexes

[a] https://orcid.org/0000-0001-5748-7516
[b] https://orcid.org/0000-0003-3289-3352

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regulate gene transcriptional programs in MLL-r leukemia (the intact and partially fused SEC/ DotCom complex is highlighted in red in Fig). The ENL protein binds to both complexes while the complex is fused to MLL in cells, and ENL interacts both with unfused SEC/ DotCom and with fused SEC/ DotCom. ENL contains a YEATS domain that can recognize a specific acetyl group (Ac) on the histone H3. Erb and Wan et al confirmed that the YEATS domain of ENL protein helps stabilize the binding of SEC and DotCom to DNA, promoting gene expression in the driving leukemic manner.

This pathway of action presents a way: using a small molecule inhibitor targeting the ENL protein YEATS domain as a drug molecule can selectively kill leukemia MLL-r cells and then treat leukemia. Other cell types seem to largely tolerate ENL loss, but both SEC, DotCom and ENL are expressed in multiple cells, so it is important that when developing such drugs is to understand this difference in tolerance.

In MLL-r leukemia, the importance of ENL is consistent with multiple studies. These studies show that poor regulation of DOT1L viability is required for the survival and proliferation of MLL-r cells. H3K79 methylation has long been associated with gene transcriptional viability and is a regulatory mechanism controlling DOT1L activity. A key unanswered question is how these histone modifications can directly affect transcription.

Methyl-lysine signaling is often coupled to downstream processes by a similar mechanism to the methylation modification of the YEATS domain read. All the reader domains of the major histone methylation sites have been identified except for H3K79. Furthermore, methylated lysine can be dynamically regulated by demethylases, but demethylases that remove methyl groups at H3K79 remain to be characterized. The study by Erb and he Wan et al further encouraged researchers to identify and identify "readers" and demethylases for H3K79 methylation because these enzymes cross with ENL-mediated signaling pathways and may also become therapeutic targets for MLL-r leukemia.

There is currently a new theory that epigenetic regulators can play an important role in disease. Driven by this awareness, a growing number of academics and businesses are working to develop inhibitors of these mechanisms to treat cancer. Clinical trials of MLL-r leukemia have evaluated DOT1L inhibitors, and Erb et al. found that in cellular models, using DOT1L inhibitors, plus knockin of ENL mutant genes that do not recognize acetylated lysine, was more effective than inhibiting gene expression programs that drive leukemenesis in both methods alone. This suggests that there is a synergy between the two therapies.

Wan et al. also investigated the potential of combinatorial therapies for MLL-r leukemia by targeting the ENL YEATS domain with the bromine domain of another reader of lysine acetylation, BET family proteins. BET proteins typically interact with SEC. And also promotes transcriptional elongation. BET inhibitors disrupt the binding of the BET protein to the acetyl-lysine fraction, and there are about 20 clinical trials testing the efficacy of these drugs in cancer treatment. Moreover, the combination of the YEATS domain inhibitor of ENL with the BET inhibitor JQ1 is highly toxic to MLL-r leukemia cells.

The effects of these combinatorial therapies suggest that multiple histone modification signals act together to form a characteristic epigenetic state of MLL-r leukemia. Therefore, multiple targeted therapies are more effective and can reduce the emergence of-with resistance, which is one of the risks of monotherapy.

Drug developers have long focused mainly on targeting enzymatic activity, rather than protein – protein interactions. However, the development of "reader" therapies targeting multiple apparent modification groups has become increasingly popular, thanks in part to the success of BET inhibitors. The binding site of the YEATS domain is also a very attractive target for drug development.

TCM treatment has made progress in the treatment of leukemia for many years, and has developed continuously, while the treatment of leukemia is not limited to western medicine technology. At present, the application of TCM has become an important treatment means of leukemia.

According to researchers, there are six TCM that have a good anti-leukemia effect, and specifically list the relevant active ingredients.

Computer-aided Drug Design (CADD), is a computer chemistry based approach to predicting testing and computing the relationship between ligand and receptor biological macromolecules through computer simulations, enabling optimization and design of lead compounds (Xie 2019). At present, CADD method has gradually mature and plays an increasingly important role in drug development, which can greatly shorten the development cycle of new drugs and reduce development costs.

Molecular docking is the most widely used and successful method in structure-based drug design. Molecular docking generally refers to the process in

which two or more molecules identify each other through geometric and energy matching. Two major topics of molecular docking methods are spatial identification and energy identification between molecules. Spatial matching is the basis of intermolecular interactions, and energy matching is the basis for maintaining a stable binding between molecules. For geometric matching calculation, lattice calculation, fragment growth are usually used, while energy calculation uses simulated annealing and genetic algorithm.

When the binding site of the ligand to the receptor protein is unknown, its site can be predicted by CADD methods and can guide mutation experiments and drug design. It is therefore important to identify the sites where the receptor protein surface interacts with the ligands for drug design.

The molecular docking technology has been used to virtually screen the bioactive constituents from TCM and determine the targets in recent years. Xu Cao and Singh have made outstanding contributions in the drug field using molecular docking (Cao 2021, Singh 2012). Which is efficient in 'narrowing' the chemical database before pharmacological assays in vivo or in vitro.

2 METHODS AND MATERIALS

2.1 Software

ChemOffice Professional (PerkinElmer Inc., USA), AutoDock Tools1.5.6 and AutoDock Vina (The Scripps Research Institute, USA), PyMOL (TM) 1.7.4.5 Edu (Schrodinger, LLC Inc., USA) and Discovery Studio 2016 client (BIOVIA Co., USA).

2.2 Establish A Ligand Database

The Chinese medicinal materials with the effect of treating leukemia contain multiple active ingredients, and the ligand database required for this test can be summarized.

Seven Chinese medicine materials, including Paridis Rhizoma (Guan 2007), Sophorae Flavescentis Radix (Yan 2014), Scutellariae Barbatae Herba (Niu 2021), Herba Hedyotidis (Qin 2008), Paeoniae Radix Rubra (Lu 2015), Salviae Miltiorrhiza Radix et Rhizoma (Xu 2021), Glycyrrhizae Radix et Rhizoma (Huang 2017), were chosen for the remarkable effects in the treatment of leukemia. The total 50 main constituents from these herbs were collected via reviewing the related articles. The structural files of these materials were all downloaded from the PUBCHEM (PubChem (nih.gov) and processed with MM2 in Chem3D, stand as mol2 documents. Followed by Autodock, all the mol2 documents saved as pdbqt documents.

2.3 Locking the ENL Protein Active Site

We used Autodock tools to process ENL with dehydration molecules, deligand, etc, and exported them as pdbqt files. The ENL protein has a YEATS domain (gully) that recognizes the acetylation modification. It can serve as an active site for molecular docking. By operating on the software, the active site of the ENL was determined as center_x =0.954 nm, center_y = -0.12 nm, center_z = 10.368 nm.

2.4 Molecular Docking of ENL with Small Moleculares

The PDBQT format file of the ENL protein as the ligand of the ligand and the ligand library was imported into the Autodock. vina software, and the computer then scored each group. The acceptable ligand GKN displayed by the ENL protein in the RCSB PDB database was exported from GKT to the PDBQT format and used as a ligand as a positive control. The two groups of scores were finally compared, and ligand scores near or beyond the GKT, score were selected as the final screening results, and could be used as a valuable reference for the development of ENL inhibitor drugs for leukemia.

3 RESULTS

The original ligand-GKT in the ENL complex (PDB ID: 6HPW) showed an affinity of -7.2 kcal/mol.

Among the 50 constituents, 26 compounds had higher binding affinity than GKT, respectively listed in the table 1 below.

Table 1: Affinity scores of molecular docking of ENL with 26 compounds.

NO.	Compounds	Binding Affinity (kcal/mol)
1	Cryptotanshinone	-8.5
2	Glabrene	-8.4
3	Tanshinone IIA	-8.3
4	Trifolirhizin	-8.2
5	Paeoniflorigenone	-8.2
6	Polyphyllin I	-8.1
7	Oleanolic acid	-8.1
8	Norkurarinone	-7.9
9	Baicalin	-7.9
10	Ursolic acid	-7.9
11	Benzoylpaeoniflorin	-7.8
12	Pseudoproto-Pb	-7.7
13	Enoxolone	-7.7
14	Glabridin	-7.7
15	Paeoniflorin	-7.6
16	Polyphyllin II	-7.4
17	Quercetin	-7.4
18	Albiflorin	-7.4
19	Benzoyloxypaeoniflorin	-7.4
20	Stigmasterol	-7.4
21	Baicalein	-7.3
22	Naringenin	-7.3
23	Oroxylin A	-7.3
24	Wogonin	-7.3
25	Kuraridin	-7.2
26	Oxypaeoniflorin	-7.2

Cryptotanshinone has a strong binding affinity -8.5 (kcal/mol) with 6HPW, Above the threshold value, The molecular docking plan (Figure 1b) shows the Van Der Waals ineraction between it and the amino acids such as CYS42 in the 6HPW protein; The quinone ring has a Pi-Alkyl interaction between cryptotanshinone and ARG37; The benzene ring between cryptotanshinone has a Pi-Alkyl interaction and ARG37; Alkyl functions between the fat loop of cryptotanshinone and ARG37, PRO70, LYS72, Pi-Alkyl interaction between it and PHE35; Conventional Hydrogen Bond interaction between 2 of cryptotanshinone-O and TYR71, a week hydrogen Bond interaction between it and PRO70; Pi-Sigma interaction between 11-C of cryptotanshinone and PHE35.

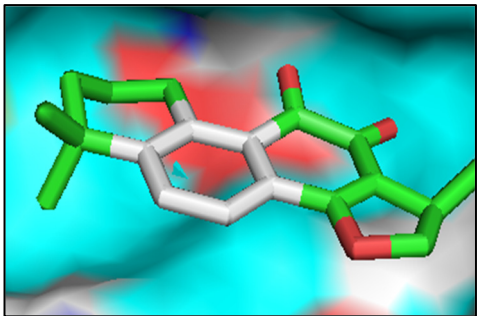


Figure 1a: 3D model.

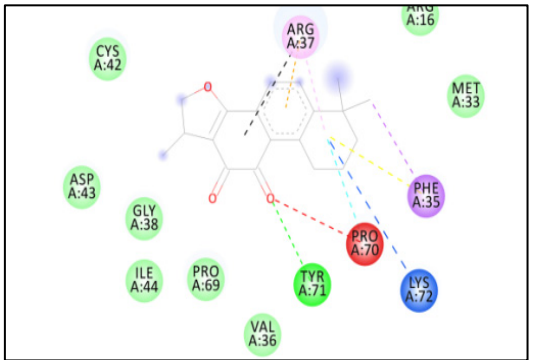


Figure 1b: 2D modle

Figure 1: Molecular docking model for Cryptotanshinone and ENL.

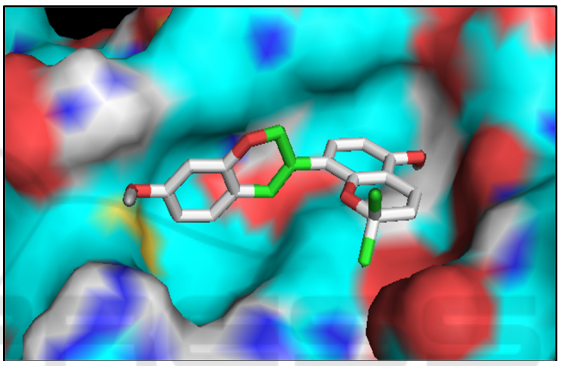


Figure 2a: 3D model

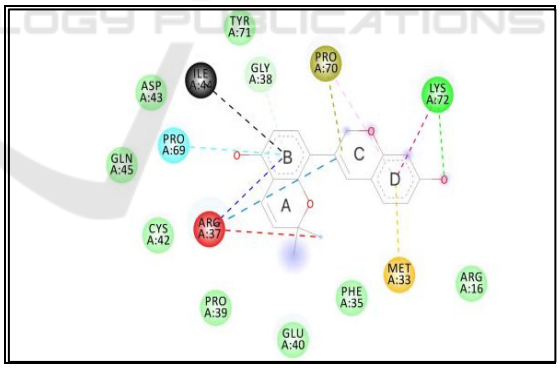


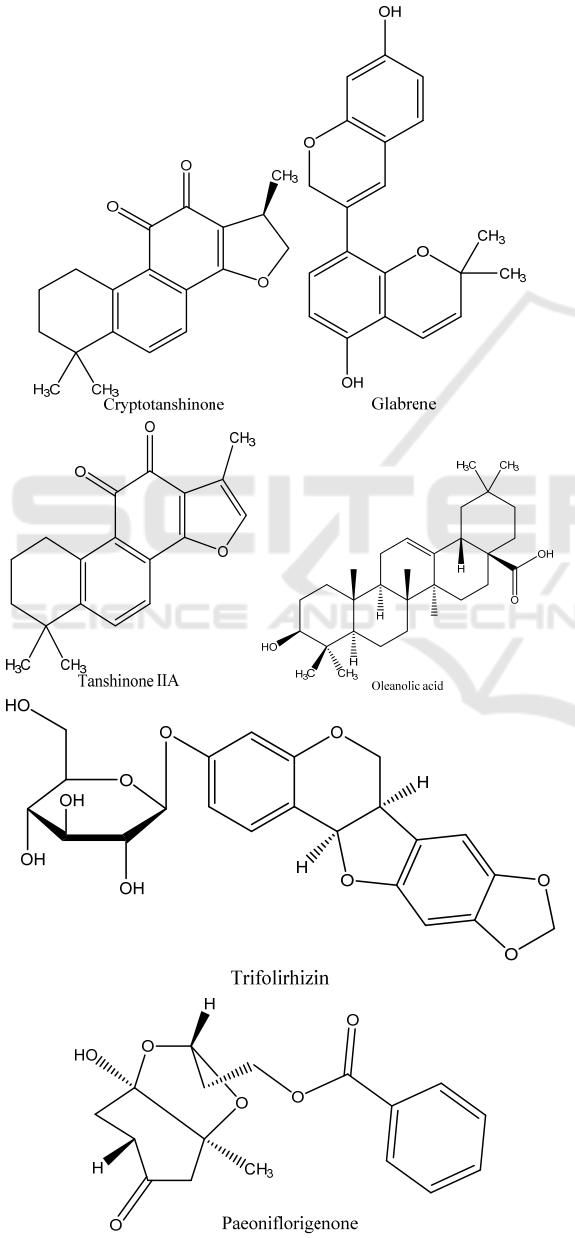
Figure 2b: 2D modle

Figure 2: Molecular docking model for Glabrene and ENL.

Glabrene has a strong binding affinity energy with 6HPW, Above the threshold value, Molecular docking plan (Figure 2b) shows a van der Waals interaction between it and TYR71 et al. in the 6HPW protein; The B loop of Glabrene and GLY38 have a Pi-Donor hydrogen Bond interaction, Pi-Sigma interaction between both and ILE44, PRO69, ARG37; The carbon-carbon double bond on the C

loop of Glabrene and PRO70, ARG37 both have Alkyl interactions; Alkyl interaction between the D loop of ligand and PRO70, Pi-Sigma interaction between it and LYS72, Pi-Sulfur interaction with MET33zhijian; The carbon on the A loop CH3 of Glabrene and ARG37 have Alkyl interaction; The Conventional Hydrogen Bond interaction occurs between O on the D loop of Glabrene and LYS72.

The structures of compounds in the top 8 affinity scores of molecular docking are listed in Figure 3.



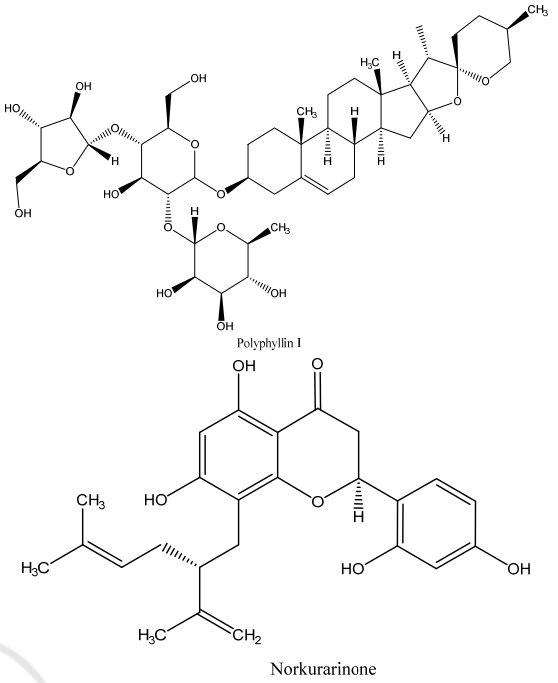


Figure 3: Chemical structures of compounds with top 8 affinity scores.

4 CONCLUSIONS

The results showed that the active TCM components represented by Cryptotanshinone, Glabrene were well and stably bound with the ENL associated with leukemia. The results show that the TCM active Chinese ingredients represented by cryptodenshinone and photoglycyrrhizin bind well and stably to the ENL associated with blood carcinogenesis.This series of molecules represented by cryptodanshinone and photoglycyrrhizin can efficiently bind to ENL proteins and potentially affect the function of ENL proteins. In addition the effect of Cryptotanshinone and Glabrene on cancer have been confirmed to some extent by Yuqing Ge and Yuting Huang (Ge 2015, Huang 2017).

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