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Research Article

Studies of Promising Compounds Aimed to Design Effective Drugs against Leukemia

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Abstract

Leukemia is a serious cancer threatening all ages of people globally. Thousands of scientists are working hard in order to find effective drugs to treat leukemia. In previous studies, we discussed the current obstacles, lead discoveries and drug development strategies [1]. Moreover, we discovered thirteen potential compounds as candidates for further drug development. Following up on previous research, we now studied four promising compounds out of the thirteen candidates. To provide a reference guideline, we have proposed the Sun's Flow Chart for Drug Discovery, as part of efforts to standardize the diverse multi-layers of researches in drug discovery. While "all roads lead to Rome", the Sun's Flow Chart is one of most simple, rational and straightforward. This research is belong to the Step 5 in Sun's Flow Chart, which is focusing on the interactions between the drug candidates and receptors in silico (e.g. docking and ligand-receptor binding tests).

Keywords: Leukemia, Rational Drug Design; Computational Modelling; Ligand-Receptor Binding; Drug Development; Structure-based Drug Design (SBDD); Sun's Flow Chart in Drug Development; Drug Discovery; Topoisomerase; Cancer Therapeutics

Introduction

Leukemia is a serious cancer threatening all ages of people globally. It counts as the 11th most cancer-related mortality worldwide in 2020, accounting for 311,594 deaths [2]. Moreover, leukemia is the most common cancer in children younger than five years of age and accounts for the highest percentage of deaths [3]. In North America, there are more than 200,000 patients suffering from leukemia. Annually, around 34,000 and 4,000 new cases for leukemia happen in USA and Canada respectively. In developed countries, around 1 % populations die from leukemia.

As our previously research [1], the structures of thirteen compounds have been proposed as drug candidates, which have potential anti-leukemia actions by inhibiting human topoisomerase II. They are all derived from WIN 58161, which has specific target selectivity on topoisomerase II, good anti-leukemia activity, and lack of drug resistance [4]. All of these compounds have both the essential tricyclic structure that contains the combined structure of a benzene ring and a pyridine ring, and the optimized thiazine structure. These structures have effective anti-leukemia function [4,5].

Results and Discussions

Furthermore, four out of the aforementioned thirteen compounds are chosen as the ligands for the receptor (topoisomerase II) docking studies. They are named as Compound 1, Compound 2, Compound 3, and Compound 4. Their structures are shown in Figure 1. It is noteworthy that the names could be same as the previous studies [1], but they could refer to different compounds — this is a common trick to protect our intellectual properties. Their logP values, which are the important indicator of lipophility or hydrophility, are shown in Table 1.

Table 1: LogP values of four compounds used for docking studies.

Compound	LogP
1	4.35
2	1.11
3	0.84
4	2.04

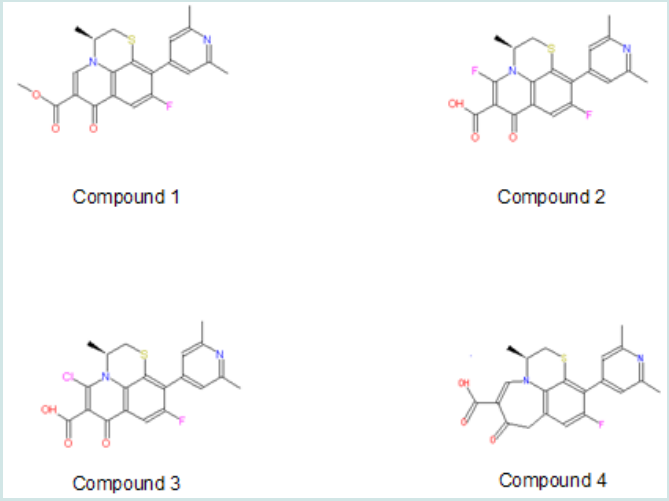

Figure 1: Structures of four compounds that will be used for docking studies.

Compound 1 is an ester with high lipid solubility as indicated by its logP value. Moreover Compound 1 may have longer action time inside body, if it can be used clinically in future, since ester modification avoids the possibility of amino acid conjugation in carboxylic group during drug metabolism. In Compounds 2 and 3, electron withdrawing groups (EWGs) are added to the pyridinyl ring. EWGs decrease pKa and increase acidity of carboxylic acid by stabilizing its negative state, and they have inductive effects to make carboxylic group more electronegative and therefore better for electrostatic interaction. Also, the log P values for Compounds 2 and 3 are low, which indicates better absorption in gastrointestinal tract. Also, chloride and fluoride have different electric and steric effects which may affect binding. Therefore, chloride and fluoride substitutions are studied together for comparative purpose. Compound 4 has increased pyridinyl ring size in quinolone. Expanding ring size is a good way to optimize the position of its substituent(s) for binding, as in the case of developing Cilazaprilat.

Human topoisomerase II is an important enzyme regulating gene expression. It binds to DNA and unwinds the supercoiled DNA by cutting both strands of DNA and hydrolyzing ATP to provide energy. Electromicroscopy and crystallography, on human and yeast topoisomerase IIs respectively, demonstrates that this enzyme contains three domains, ATPase domain, A' domain and B' domain [6,7]. A' domain is responsible for DNA interaction and B' domain has an unclear catalytic role [8]. In yeast topoisomerase II, ATP domain contains 1-409 amino acid residues; A' domain consists of 682-1178 residues, among which tyrosine residue 783 is involved in DNA cleavage; and B' domain contains 420-633 residues [6]. A 92 KD fragment of yeast topoisomerase II without the ATPase domain has been crystallized and its structure has been solved in high resolution [6]. It is the structural-solved protein (PDB ID: 1BGW) that has the highest sequence similarity with the human topoisomerase II (AAA61210 from NCBI protein database; [5], as revealed by protein basic local alignment search (BLAST; NCBI). All these make it possible to build a homology model for human topoisomerase II to explore the binding/active site and study the drug-receptor interactions.

Experimental Protocols

ClustalW (http://www.ch.embnet.org/software/ClustalW.html) is applied to align the sequences of human and yeast topoisomerase IIs which have been described in the introduction section. Both A' and B' domain have good alignment between human and yeast topoisomerase II. Manual adjustment is applied after automatic alignment to enable domain regions to better align together. During manual adjustment, a few amino acid residues are deleted from the template in the connective regions between domains and in the C'-terminal.

created by AutoDockTools. Next, AutoDock 3 in a UNIX operation system is performed for docking studies. Ten pdb files are acquired for every ligand docking onto the receptor. Since the first pdb file, in each set of data, possesses favorable characteristics for docking, the first pdb file is chosen to study the docking of each ligand.

Two kinds of modeling methods are applied to build the comparative model of human topoisomerase II. MODELLER (http://salilab.org/modeller) and 3D-Jigsaw (http://bmm.cancerresearchuk.org/~3djigsaw/) run separately in the default parameters of each program. The MODELLER utilizes the known structure of yeast topoisomerase II and the above alignment to build ten similar models of human topoisomerase II. Manual validation is performed first. A long abnormal N'-terminal loop containing the first 298 amino acid residues is cut off manually. This long loop is due to lack of a piece of amino acid sequence in the N'-terminus of the template (PDB ID: 1BGW). In line with this result, another program, 3D-Jigsaw, automatically selects another template (PDB ID: 1LWZ) for modeling and achieves a similar model. Interestingly, this 3D-Jigsaw model also has its N'-terminus containing the first 303 residues cut off automatically. PROCHECK and WHAT IF (http://biotech.ebi.ac.uk:8400/cgi-bin/sendquery) is performed to evaluate the validity of modeling. The first model built by MODELLER has been chosen for further studies not only because its template has better scores and E-value than the template selected by 3D-Jigsaw but also because this model shows good characteristics. For example, in Ramachandran plot, the target model contains almost same percentage of residues in most favorable regions as the template (82-83%), while it also contains as little percentage of residues in disallowed residue as the template (1.7-1.9%) (Figure 2).

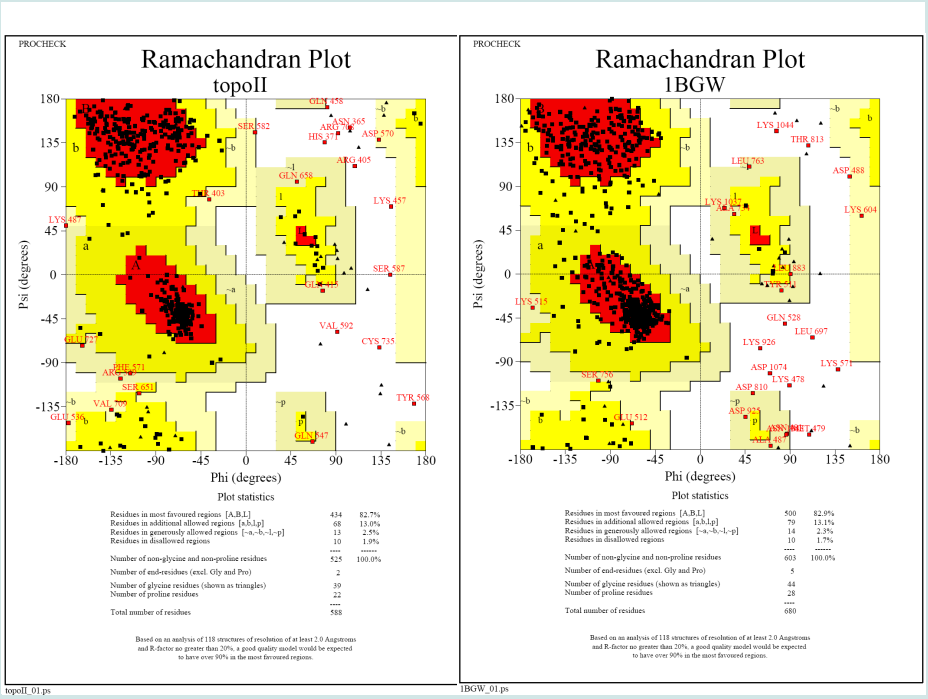

Figure 2: Ramachandran plot to evaluate the backbone conformation of target model (left) and compare with the template (right).

After the comparative model of human topoisomerase II has been established, docking studies are performed by AutoDock (http://autodock.scripps.edu/) to study the binding site and ligand-receptor interaction. First, ligands' pdb files are acquired by CORINA (http://www.molecular-networks.com). Then using AutoDockTools in a Windows operation system, the rigid bonds of ligands are defined automatically and two rotatable bonds in each ligand are defined; hydrogens are added and charges are assigned by the Computer-Gasteiger method. The ligand files are saved to pdbq files. Also, the grid parameter file and docking parameter file of the human topoisomerase II are created by AutoDockTools. Next, AutoDock 3 in a UNIX operation system is performed for docking studies. Ten pdb files are acquired for every ligand docking onto the receptor. Since the first pdb file, in each set of data, possesses favorable characteristics for docking, the first pdb file is chosen to study the docking of each ligand.

Results

Homology Modeling

The refined alignment between human topoisomerase II and yeast topoisomerase II (1BGW) is shown in Figure 3. The regions for domain A' and B' are align well. This provides the base for comparative modeling. During the refinement, deletions on the template are made on some unconserved amino acid sequences in front of the sequence of domain B', between domain B' and A', and at the end of 1BGW.

Figure 3: Sequence alignment between human topoisomerase II (lower line) and template (1bgw; upper line). Identity, strong similarity, and week similarity are denoted as "*", ":", and "." respectively.

To verify the modeling, manual examination is performed as mentioned above. An abnormally long loop in the N'-terminus is deleted. Then PROCHECK is performed to examine the Ramachandran plot, residue Ramachandran plot, chi1-chi2 plot, main-chain parameters, side-chain parameters, residue properties plot, main-chain bond lengths, main-chain bond angles, RMS distances from planarity and distorted geometry. The Ramachandran plot has previously been shown in Figure 2. WHAT IF is also performed on bone length and bone angel for validation purpose. Basically, the model is fairly reasonable. It has good

backbone structure with most residues in favorable regions and as few residue in disallowed region as the template. In WHAT IF test, its Z-score for bond angles is 1.487, close to the score for X-ray high-resolution structures. It also shows normal bone length variability. Therefore, this model is used in further docking studies.

Binding pocket and docking interactions

The docking is viewed by the program of PyMol. All of the four ligands dock into the same binding pocket. The binding pocket is located between A' and B' domains and is formed by more than 30 amino acids in both loops and helices. These residues are Pro 659, Asp 396, 397 and 672, Ala 636, 673, and 674, Gly 399, 441, 633, 661, 664, and 670, Lys 439 and 671, His 440 and 668, Met 638 and 639, Ser 675, Glu 74, 399, 515 and 634, Gln 635, 646 and 662, Phe 663, Asn 643, Val 642, Leu 437, 438, and 644, Ile 641 and 679, Thr 640, and Tyr 395 and 678. The binding cavity is so fit that no manual adjustment is required.

Ligands 1-4 are docked, one by one, onto the receptor, human topoisomerase II in simulation. All ligands docks perfectly onto the binding pocket (Figure 4). Nonetheless, since the flexibility of the protein is not examined due to limited computer power, the induced-fit change in the receptor is not addressed in this study. Ligand 1 is the ester derived from WIN 58161. The chirally extended methyl group from the thiazine ring fits into the extended pocket consisting of Gln 635, Glu 399, and Asp 397. Especially the oxygens in the side chain of Gln 635 and Asp 397 interact with the hydrogens in the methyl group. Interestingly, there seems to be more room available to dock extended chain from the thiazine ring. Therefore, chain extension modifications can be applied to design better drug candidates in further research. Also, the ester modification fits better into the space between Glu 646, Phe 663, and Asn 643. Especially Phe 633 is very close to the methyl group which suggests the presence of hydrophobic interaction. Moreover, the phenyl ring in the tricylic structure interacts with the non-polar residues, Met 639 and Ala 673. Also, the extended phenyl ring may hydrophobically interact with Met 638 and Pro 676. Alkyl extension should be made from the extended phenyl ring since there is extra room for this extension and may form further hydrophobic interaction with Phe 511. Furthermore, the electrostatic map of the receptor is assigned by PyMol (Figure 5). However, no significant electrostatic interaction is perceived for ligand 1 yet. It is noteworthy that the PyMol suggests skepticism for its electrostatic generation function.

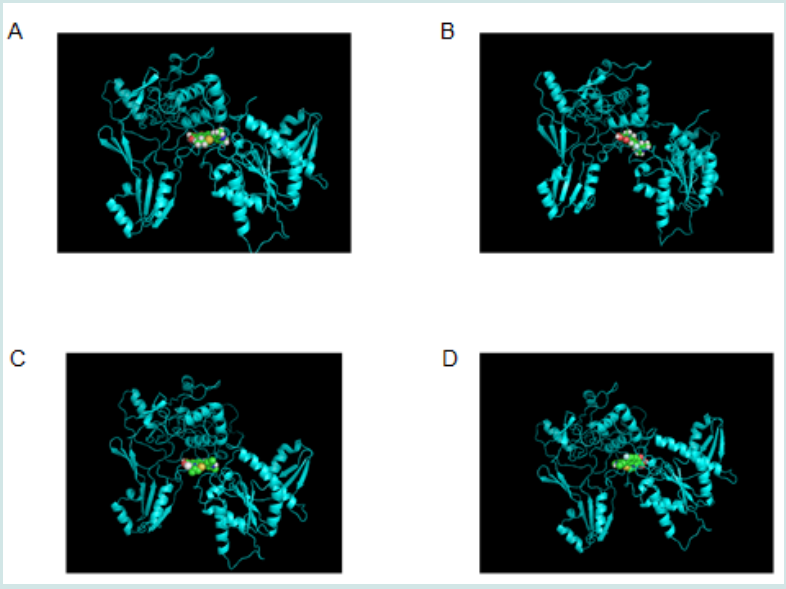

Figure 4: Docking model of four ligands on human topoisomerase II. The bindings for ligands 1-4 are shown in A, B, C, and D respectively.

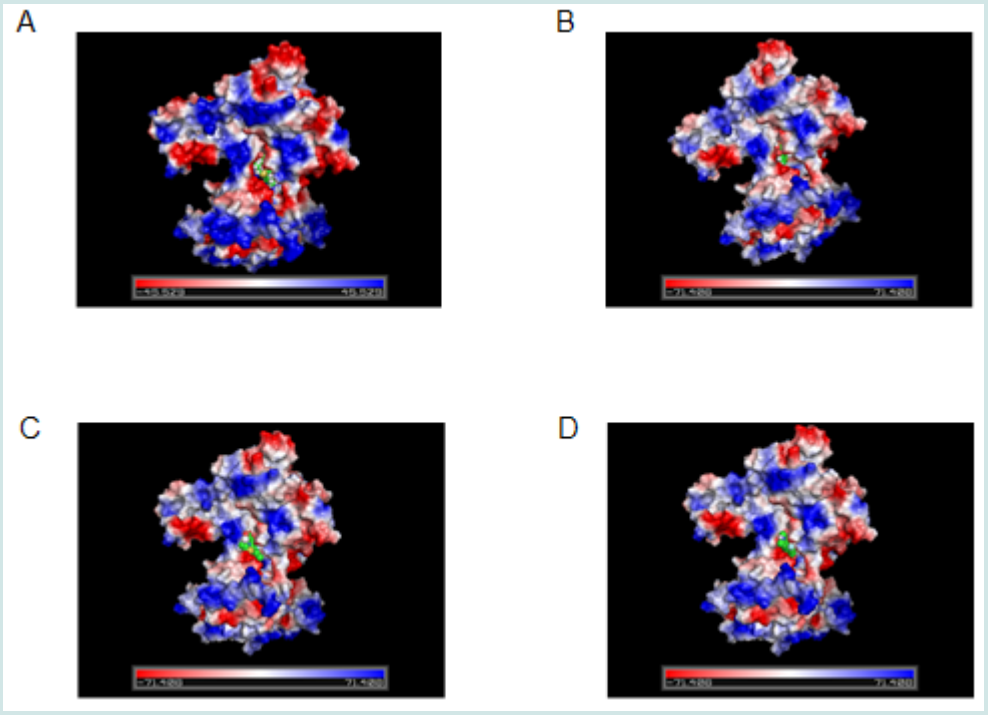

Figure 5: The docking models are shown in the electrostatic map for surface charge of the receptor. Ligands 1-4 are shown in A, B, C, and D respectively.

Ligand 2 is the fluoride substitution of WIN 58161. Its carboxyl acid group may interact with the basic residue Lys 671. Also its hydroxyl group closes to the oxygen in Gly 670, and perhaps there is hydrogen bond interaction between them. The chirally extended methyl group from the thiazine ring has hydrophobic interaction with Ala 636, Met 639, and Phe 663 which form a lipophilic cavity. Moreover, the substituent F atom seems to interact with the hydroxyl group of Thr 640. Interestingly, in the electrostatic map, the oxygen in the hydroxyl group of the ligand, which has negative charge in physical pH (around 7.4), is close to a positively charged region in the receptor. This suggests better-fit of this docking model.

Ligand 3 is the chloride substitution of WIN 58161. Its hydroxyl group may have hydrogen bond interaction with Gly 670. The nitrogen atom in the extended ring may have hydrogen bond interaction with both Asp 397 and Glu 399. The hydrophobic force may be present between the extended phenyl ring and the non-polar Ala 674 and Phe 663. The sulfate in the thiazine ring probably has hydrogen bond interaction with the NH in Lys 439. Nevertheless, no significant interaction is observed for the subsituent Cl atom, which turns to point toward the surface of the receptor. In the electrostatic map, the oxygen in the carboxyl group closes to a positive-charge region, and the chloride atom is also near a positive region, which, nonetheless, is a little far from this atom. All of these suggest good fit.

Ligand 4 is the ring-expansion of WIN 58161. The expanded ring places the attached carboxyl group and oxygen to much better conformation in the binding pocket. Its carboxyl acid group probably interacts with the basic His 440 residue. The carbonyl oxygen may form hydrogen bond with the –NH2 of Lys 439. Also the oxygen attached directly to the ring may form hydrogen bond either with the –NH2 in the side chain of Gln 662 or with the hydroxyl group of Ser 675. Perhaps, the nitrogen in its extended ring could form hydrogen bond with –NH- in the side chain of His 668. Moreover, the F extended from the tricylic ring is placed close to the H2N- in the side chain of Gln 635. The extended methyl group from the thiazine ring is place to a spacious cavity containing non-polar Leu 437, 438 and 644, Ala 673, Val 642, and Ile 641 which can have hydrophobic interaction with the alkyl group. The cavity is spacious and contains more non-polar residues, such as Leu 657 and Ala 674, at the bottom. Therefore, in future research, longer alkyl chain can be designed to fit into the hydrophobic portion of this cavity. Further, in the electrostatic map, all of three oxygens are close to regions with positive charges. Taken together, this ligand well fits into the docking site. This also suggests the ring expansion is a good trial to facility binding. On the other aspect, all of these well-fit docking models are the good indication for the validity of current simulation methods applied in this project.

Discussion

This research successfully reveals the reasonable models for human topoisomerase II, and architecture of its binding pocket and the binding interaction between designed compounds with their target, topoisomerase II. The docking study facilitates the

structure-based drug design on anti-leukemia therapy. Combining the traditional pharmacophore-based drug design in part 1 of this project, this research demonstrates a new line of drug candidates to benefit anti-leukemia research.

Since the Compounds 1-4 contain the key structures for anti-leukemia and bind well in simulation, research can go further on vitro bioassays. For example, to verify the anti-leukemia effect and evaluate the side-effect preliminarily, they can be tested on cultured leukemia cell line (e.g. WEH1-3B/S and WEHI-3B/NOVO; [9] and normal cells, such as normal white cells in blood. The best candidates with less side effects and strong anti-leukemia effect can be applied on animal model test. During designing these ligands, metabolism blocks have been considered. For example, the chloride and fluoride substitutions help increase the action time in body; the ester design helps to pass cellular membranes. If everything goes well, it can apply for clinic trial. Since each level of tests will reduce the number of drug candidates, a large quantity of compounds should be designed and their docking studies should be performed earlier before bioassay in real drug-developing situation. In future, AI could be applied to facilitate this process and overcome obstacles that will be discussed below.

This research is carefully performed, though there are a few drawbacks present. One of problem in current structure-based drug research is the flexibility of macromolecules. For docking study in current available software and computer power, the protein is assumed to be rigid. In real physiological situation, induced-fit is the way of receptor binding ligand. Those hydrophobic residues, Phe 511, Leu 657 and Ala 674, which seem a little far from the alkyl group as mentioned in the result section, can actually get close to bind the alky group after the conformation change of the protein during the process of induced-fit. Also, the homology models have some parameters that seem not perfect. For example, seventeen bones are found to have unusual bond lengths as revealed by WHAT IF. Although homology model is a good way to solve structure, this method itself has unavoidable shortcomings. As matter of fact, even when two proteins have very similar sequences and secondary structures, they do not have exactly same backbone structures. One in ten comparative models has abnormal large root mean square deviation, which is the indication of the difference between model and real structure. In addition, the method of generating electric potential by PyMol is not guaranteed. Although it provides good indication on ligand-receptor interaction, skepticism has to be applied as suggested by the software developer. The above-mentioned shortcoming can be fixed either by future AI or the classic protein crystallography, which is still a non-replaceable method to acquire real high-resolution structures of large proteins.

All in all, this research provides reasonable models for human topoisomerase II and reveals its binding pocket for a certain type of inhibitors. All ligands are successfully docked into this pocket with the ligand-receptor interations of hydrophobic force, electrostatic force, and hydrogen bonds. All of these provides cues for develop a new line of anti-leukemia drugs.

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