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In Silico Design of New Drugs for Myeloid Leukemia Treatment

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Abstract: In this work we use *in silico* tools like *de novo* drug design, molecular docking and absorption, distribution, metabolism and excretion (ADME) studies in order to develop new inhibitors for tyrosine-kinase protein (including its mutate forms) involved in myeloid leukemia disease. This disease is the first cancer directly associated with a genetic abnormality and is associated with hematopoietic stem cells that are manifested primarily with expansion myelopoiesis. Starting from a family of fragment and seeds from known reference drugs, a set of more than 6k molecules were generated. This first set was filtered using the Tanimoto similarity coefficient as criterion. The second set of more dissimilar molecules were then used in the docking and ADME studies. As a result, we obtain a group of molecule that inhibit the tyrosine-kinase family and have ADME properties better than the reference drugs used in the treatment of myeloid leukemia.

Keywords: tyrosine-kinase; de novo design; fragment docking; myeloid leukemia

1. Introduction

The Chronic Myeloid Leukemia (CML) was initially reported in 1825 in France. French literature describes an autopsy done on a 63 years old woman where a large increase in the spleen and liver size was found. The spleen, due to the disease, had been increased 20 times compared to the healthy spleen [1,2].

Another important step on the understanding of CML was given in 1960, with the identification of a chromosomal abnormality as the source of the disease. This anomaly is the subtle exchange of the ends of the chromosomes 9 and 22, respectively (referred to as ABL and BCR gene), thereby creating a new hybrid chromosome which has been assigned the name Philadelphia chromosome. This hybrid chromosome has the ability to start the disease by encouraging disordered cell division of some blood cells [3-7]. The tyrosine-kinase mechanism of BCR-ABL works by binding ATP (adenosine triphosphate)

and transferring a phosphate group from ATP to tyrosine residues on various substrates [8,9]. Activation of these pathways can lead to lack of control of cell proliferation and apoptosis. The major drugs used to fight the disease use these mechanisms to inhibit tyrosine-kinase, choosing tyrosine-kinase as a perfect target for the study and design of new drugs [10].

2. Materials and Methods

The structure of the tyrosine-kinase in its wild form (without any mutation) was downloaded from the Protein Data Bank (PDB) with code 1OPJ and resolution equal to 1.75 Å [11]. To obtain the mutate forms, we apply the mutations following the codes used in the literature for the protein with code 3QRI [12].

Prior to docking studies, the proteins structure was prepared using the Protein Preparation Wizard protocol as implemented in the Schrödinger Suite [13] using the Maestro interface [14]. This protocol adds hydrogen atoms, corrects bonds, complete chains, etc.

The computational modelling of new inhibitors for the tyrosine-kinase was divided into three steps. In the first step, the *de novo* design was carried out using the LigBuilder software [15]. In this work we used the growing/linking modes and the explore mode. In the second step, the generated molecules were used as ligands in the docking studies with all the protein family. The docking studies were carried out using the Glide software [16] from the Schrödinger Suite [13]. To evaluate each pose, it uses an scoring function called GlideScore that consider the van der Waals energy, the Coulomb energy, a lipophilic contact term, an hydrogen-bonding term among other terms [17, 18]. The GlideScore (or GScore) has units of kcal/mol and the lower it is, the better the interaction is. All the docking simulations were performed considering the protein as a rigid structure and the ligand as flexible. In the last step, some physicochemical descriptors related to the Absorption, Distribution, Metabolism and Excretion (ADME) properties were calculated. In this work, we used the QikProp software [19].

3. Results and Discussion

Using the *de novo* design technic, a universe of more than 6000 molecules was obtained. To validate the structural diversity of the generated library we calculated a 2D linear hashed fingerprint with a 64-bit address space. Then, we used the Tanimoto metric to compute the similarity among all the molecules (if the Tanimoto coefficient of two structures is greater than 0.85, the structures are considered similar) [20-22]. In the second step of our methodology, we dock all the molecules into the active site of all the protein family (with and without mutations). To provide a comparative of the potential of the generated molecules, we did the molecular docking of the 4 reference drugs must used as tyrosine-kinase inhibitor: imatinib, dasatinib, nilotinib and ponatinib. Comparing the score of the reference drugs with the generated molecules presented in table 1, we can see that in all the cases (including the mutated proteins) there is more than one molecule with better scores, suggesting potentials tyrosine-kinase inhibitor stronger than the reference drugs.

From the results in table 1, we can see that among the whole population of molecules, the compounds **680**, **781** and **723** repeatedly appear as well ranked ligands. Especial attention for the structure **781** that have a higher score than the reference drugs in all cases.

A comparison of the docking poses of **781** and imatinib in the binding site of the T315I protein is shown in figure 1. The imatinib is interacting with T315I through 4 hydrogen bonds with amino acids Asp400, Ile379 and Met337 and Glu305, a π–cation interaction with His380 and 2 π–π interactions between Phe401 and Tyr272 residues

and the imidazole ring of imatinib. These interactions are dispersed over the whole imatinib molecule. In the case of **781**, it mades 3 hydrogen bonds with residues Glu305, Tyr272 and Asn341 and a π−π interaction between the Tyr272 and the benzofuran ring. In this case the interactions are distributed over the whole molecule structure also.

A widely used descriptor to study the drugability of molecules is the Lipinski's rule of five [24]. It predicts that a molecule will have poor absorption if its molecular weight (MW) is greater than 500Da, the average estimated number of hydrogen bonds that would be accepted by the solute from water molecules (HBAcceptor) is greater than 10, the average estimated number of hydrogen bonds that would be donated by the solute to water molecules (HBDonor) is greater than 5 and its octanol/water partition coefficient (QPlogPo/w) is greater than 5 [24]. Another descriptor that it is important is the QPlogHERG that simulate the blockage of human ether-a-go-go hERG K+ channels.

From table 2 we can see that the reference drugs nilotinib and ponatinib violate the Lipinski' s rule of five. Both have the molecular weight over 500Da and the ponatinib also have a partition coefficient out of the recommended values. On the other hand, our best molecules do not have any violations.

A special attention is needed for the predicted QPlogHERG descriptor. Recently, it has been found that several non-cardiac drugs inhibit the hERG K+ channel causing cardiac side effects. Among them we can mention sudden cardiac death, significant QT prolongation (period between the start of ventricular depolarization and repolarization) and life-threatening ventricular arrhythmia. These undesirable drug interactions make the drugs withdrawn from the market owing to cardiovascular toxicity associated to them [25]. The values of QPlogHERG are concerning when bellow −5 [19]. From our simulations, all the molecules have values lower than −5 but the reference grugs imatinib, nilotinib, dasatinib and ponatinib have the more high-risk values. As it can be found elsewhere, heart problems is a recurrent side-effect of these drugs.

The other compounds (**680**, **723** and **781**) also have the QPlogHERG bellow the recommended value but in a lower extend. In the case of compound **781**, it have the higher value (less risk) among all the studied molecules.

Table 1. Docking score (GScore) for the best molecules and for the references drugs.

Protein Mutation	Molecule: GScore	Imatinib	Dasatinib	Nilotinib	Ponatinib
1OPJ	**680**: -15.34	-13.96	-9.08	-13.63	-12.96
T315I	**781**: -13.57	-13.31	-7.22	-4.89	-11.92
T315A	**781**: -14.16	-13.05	-9.90	-13.49	-13.09
M244V	**723**: -14.95	-13.16	-10.40	-13.51	-13.19
E355G	**781**: -16.13	-10.22	-11,01	-13.58	-12.98
H396A	**781**: -15.82	-13.02	-9.69	-14.12	-13.68

Table 2. ADME properties (quantities out of recommendation values are underlined).

Compound	MW	QPlogPo/w	HBDonor[1]	HBAcceptor[1]	QPlogHERG
Imatinib	493.610	3.476	2	10.00	-9.280
Dasatinib	488.006	2.509	3	10.00	-6.672
Nilotinib	529.523	5.870	2	8.00	-8.246
Ponatinib	532.567	4.602	1	9.50	-9.243

680	487.511	1.856	5	10.00	-6.307
723	430.502	4.471	3	6.25	-8.392
781	459.498	4.96	3	6.75	-5.837

[1] As they are average values, they can be non-integers.

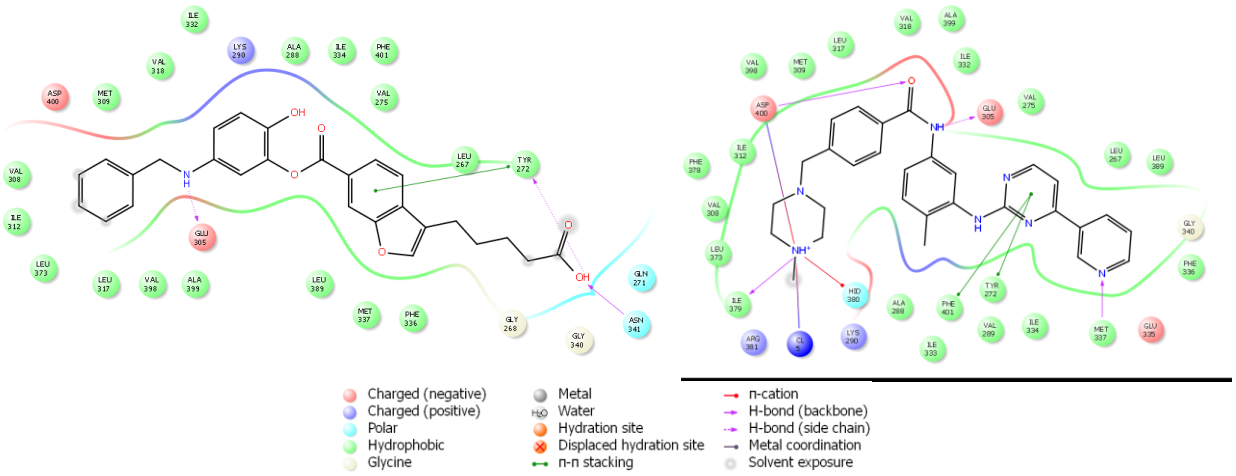

Figure 1. 2D interaction diagram between **781** (left) and imatinib (right) with mutation T315I.

4. Conclusions

The myeloid leukemia is a fatal disease, so it is of great importance to keep the patients in chronic phase where they stay asymptomatic. The fragment based drug design method used in this work turns to be a good alternative to create drugs that can control this neoplasm. Based on the calculated GScore, the de novo designed molecules have better inhibitor capacity than the tyrosine-kinase inhibitors most used in the market. These molecules shown strong potential to become drugs capable to inhibit all mutations, mainly the T315I mutation, now the leading cause of deaths due to the difficulty of inhibitors to control it.

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Author Contributions

IC idealize the experiments and prepare the images and the final manuscript. WP obtain the protein wild structure, produce the mutations and run the simulations.

Conflicts of Interest

The authors declare no conflict of interest.

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