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Virtual screening for novel 1-deoxy-D-xylulose-5-phosphate reductoisomerase inhibitors: A shape-based search approach

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ABSTRACT

A virtual screening study using the ArgusDock docking engine, implemented in ArgusLab 4.0.1 was performed on 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), the second enzyme in the methylerythritol phosphate pathway, in isoprenoid production, which is present in the malarial parasite *Plasmodium falciparum*. This enzyme has been proven to be the molecular target for fosmidomycin, a promising antimalarial drug. However, fosmidomycin exhibits poor pharmacokinetic properties and has low intestinal absorption. In this study, a subset of the ZINC12 database composed of 7,478 molecules was docked on DXR (PDB ID: 5JO0) using the ArgusDock (a shape-based search algorithm), to find a hit that correlated with the fosmidomycin analogs. This shape-based search algorithm was chosen, to virtually screen a large database on a computer, due to its fast docking capability. The screened compounds' outputs were ZINC00310125, ZINC17111658, and ZINC59231267. These three compounds possessed the required ligand-enzyme interactions, as shown in fosmidomycin analog 1. In conclusion, the shape-based search algorithm implemented in ArgusLab 4.0.1 was found to be a relatively easy alternate method of performing virtual screening using a 3D database. The method could be used to develop novel antimalarial drugs.

INTRODUCTION

Malaria is one of the leading causes of death worldwide. It has been indicated that 212 million cases occurred globally in 2015, leading to 429,000 deaths, most of which were in children aged under 5 years in Africa [1]. Therefore, there is an urgent need for novel antimalarial drugs to help eradicate this disease.

The causative *Plasmodium falciparum* species has more than 5,000 genes; however, the number of druggable targets is unknown. To combat the deleterious consequences of resistance spreading, research should focus on drug targets, which are not subject to cross-resistance with currently used antimalarial drugs [2]. 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR; EC 1.1.1.267) is the second enzyme in the methylerythritol phosphate pathway to produce isoprenoids, an alternate biosynthetic route present in many bacteria [3], algae [4], plants [5], and the malarial parasite *P.falciparum* [6]. This enzyme has converted 1-deoxy-D-xylulose 5-phosphate (DXP) into the branched compound 2-C-methyl-D-erythritol-4-phosphate (MEP). The transformation of DXP into MEP requires an isomerization, followed by a NADPH-dependent reduction [7]. DXR is an attractive target for the development of new antimicrobial and herbicides [3,4]. In addition, DXR has been used as a molecular target for new antimalarial and antibacterial compounds. Fosmidomycin, a naturally derived product and promising inhibitor of DXR, is an attractive target for the development of new antimalarial compounds. Due to its hydrophilic properties, fosmidomycin has been shown to exhibit poor pharmacokinetic properties and low intestinal absorption [8]. Many fosmidomycin analogs have been synthesized [9,10]. Recently, Sooriyaarachchi *et al*. modified the fosmidomycin acetyl homolog FR900098 by attaching hydrophobic constituents to improve the lipophilicity. The most improved analog, sodium hydrogen (2-(2-(hydroxyl(methyl)amino)-2-oxoethyl)-5-(m-tolyl) pentyl) phosphate, a fosmidomycin analog 1, exhibited equivalent potency to fosmidomycin with IC_{50} 0.05 μM [11].

During the drug development process, a variety of methods has been applied to discovering new compounds such as isolating active compounds from medicinal plants, De Novo drug design, or combinatorial chemistry with high throughput screening [12]. Virtual screening of chemical structures reported in a known database is an efficient alternative method that can be applied to drug development. This process involves searching for information about similarly structured compounds stored in databases, rather than trying to extract specific data from different journals [13].

The ZINC database is a collection of commercially available chemical compounds prepared, especially for virtual screening. ZINC is used by researchers in pharmaceutical companies and research universities; it is different from other chemical databases because it represents biological activities and three dimensional structures of molecules. The database is updated regularly and can be downloaded and used free of charge [14]. In 2015, ZINC held 12,000,000 purchasable molecules on its database [15].

Normally, there are two main approaches to solving the docking problem. The first is the geometric-based or shape-based approach, and the second focuses on the energy minimization approach. While energy-based docking schemes are based on having knowledge of the approximate positioning of the ligand in the receptor active site; the shape-based method is based on the assumption that the molecular surfaces of the receptor and the ligand need to match, if the molecules are to bind to each other with high affinity [16]. Comparing protein-ligand docking programs is not straightforward. Each program has both advantages and disadvantages in relation to docking accuracy, ranking accuracy, and computational time consumed. It has been difficult to draw general conclusions since both of these programs are based on different docking approaches and use different scoring functions [17]. Kellenberger *et al*. compared the docking accuracy of the shape-based commercial software, DOCK, and FRED, to the genetic algorithm (GA) energy-based software GOLD. As far as docking accuracy was concerned, GOLD mostly compared well with other programs. When computational times consumed to virtually screen were compared, DOCK and FRED were found to execute somewhat faster, with average docking times of 46 and 18 seconds (average time over a set of 100 PDB entries), respectively, whereas GOLD had an average docking time of 137 seconds. The research also revealed that the most accurate docking tools were also the slowest [18].

ArgusLab is freely licensed molecular docking software, which features a graphical user interface. This program runs both the shape-based approach and the GA energy-based approach on the Windows (Microsoft Corp.) operating system. Beginners can learn molecular docking using ArgusLab [19,20]. Oda and Takahashi used the capabilities of ArgusLab to calculate the binding free energy between proteins and ligands. The results showed that ArgusDock was effective for not only pose construction and pose selection but also for virtual screening [19]. Commercial programs such as Surflex [20] or GOLD outperformed the freely available ArgusLab docking engine in almost all the parameters tested. However, their study revealed that although lagging behind in accuracy, results from ArgusLab were biologically meaningful [21]. Tangyuenyongwatana and Jongkon demonstrated that the non-commercial docking programs such as ArgusLab, AutoDock, and AutoDock Vina, had close docking potential when the GA approach was applied [22].

The objective of this research was to demonstrate, through virtual screening, that ArgusLab could be easily applied to finding new enzyme inhibitors. This was achieved by attempting to find a new skeleton of the DXR inhibitor using fosmidomycin analog 1 as a template, and performing virtual screening using ArgusLab 4.0.1, with the ZINC 12 database. Only one cluster of the lead database composed of 7,478 molecules was used in the experiment.

MATERIAL AND METHODS

For the enzyme target, (DXR; EC 1.1.1.267), a crystal structure of DXR from *P. falciparum* (PDB ID: 5JO0) was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/). All the water molecules were removed, missing hydrogens were added, and chain A was selected to perform the experiment.

Software and Database File

To virtually screen the substrates, data were obtained from either ZINC 12 (zinc.docking.org) or ZINC 15 (zinc15.docking.org). For ZINC 12, the data were downloaded in the single structure data file (SDF) format. ZINC 15 is an improvement over ZINC 12 because it offers a more unique set of compounds that can be selected in "Tranches" mode, which shows the scale of Log P and a range of the molecular weights of the compounds. ZINC 15 provides smaller sets of desired compound properties; however, the downloading process is complex as it contains multiple small files. The Linux operating system and the selection of WGET or CURL methods to obtain the database are recommended. For this study, the 3D structures were selected from the leads now (a specific database where all compounds are commercially available) subset of ZINC 12 and downloaded in SDF format. ArgusLab 4.0.1 (www.arguslab.com) was used to perform the database molecular docking. The hit compounds were exported from the SDF database as pdb files using the VEGA ZZ program (nova.disfarm.unimi.it/cms/index). Discovery Studio Visualizer 4.0 (accelrys.com) was used to view the ligand-enzyme interaction and to produce images.

Virtual Screening Studies

The DXR crystal structure (PDB ID: 5JO0) was downloaded to ArgusLab, and the ligand fosmidomycin analog 1 (Figure 1) was chosen, "centered," and "hydrogen atoms added." Then, the ligand was assigned as a group to "make the binding site

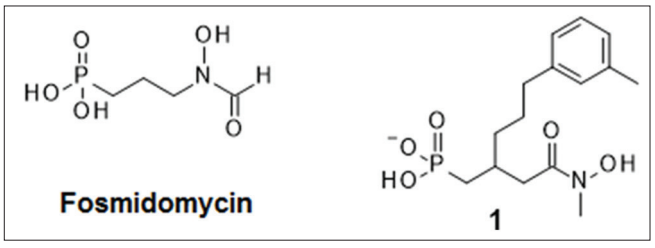

Figure 1: Structure of fosmidomycin and analog 1

for the group." Database docking on the user interface was selected, and the SDF database was chosen. For the binding site option, the calculated size was selected to include the binding site for docking. ArgusDock ran with the AScore scoring function enabled.

Before screening ZINC 12 from the leads now subset database, the docking protocol was validated. 5JO0.pdb, protein-bound ligand fosmidomycin analog 1, was docked into the binding pocket to obtain the dock poses and produce cocrystal bound to the DXR enzyme, which was checked for a root mean square deviation (RMSD) value. The docking times for a small set of 261 compounds in one SDF database using ArgusDock or GA dock were also tested. For the ArgusDock parameters, the docking engine used the exhaustive search method. The flexible ligand was described as a torsion tree, and grids were constructed to overlay the binding site. A group of bonded atoms, which did not have rotatable bonds or ligand root nodes were placed on a search point in the enzyme pocket, and a set of diverse and energetically favorable rotations was created. For each search, torsions in breadth-first order were constructed, and those poses that survived the torsion search were scored. The N-lowest energy poses were reserved, and the final set of poses was submitted to coarse minimization, re-clustering, and ranking [23]. Grid resolution was 0.4, and the binding site box size was 18.570 × 20.259 × 19.580 Å. The docking calculation type was set to "Dock" and "Flexible" ligand docking mode. At the end of the docking, the virtual screening output file was obtained. The free energy of the binding values was ranked from the best binding energy to the poorest binding energy. The hits were chosen using VEGA ZZ software to export the structure from the SDF database. Discovery Studio Visualizer 4.0 was used to show the ligand-binding site interaction in comparison to the fosmidomycin analog 1 orientation.

RESULTS AND DISCUSSION

ArgusLab 4.0.1 features two options for docking algorithms: ArgusDock (shape-based search algorithm) and GA dock. Initially, both docking modes were compared to find a docking time that was suitable for the virtual screening system. For the 261 compounds data set, it was found that using ArgusDock was around 2.24 times faster than using the GA docking algorithm, and the time consumed for 7,478 molecules using ArgusDock docking was about 1.94 times faster than the GA docking algorithm, in terms of producing a screening result (Table 1). The docking time ratios of these two data sets were close to each other. The difference may stem from the different structures of the compounds held in the database, which causes some compounds to spend more or less time in docking. From the results, ArgusDock was a more suitable algorithm for docking a large database because it took less time to produce screening results with a low to medium performance computer. Before starting the screening, the docking protocol was validated by docking 5JO0.pdb protein-bound ligand fosmidomycin analog 1 into the binding site to obtain docking conformation. Then, the RMSD of all atoms between the docking conformation and X-ray crystal structure was calculated and found to be 2.15 Å. This value is slightly higher than 2 Å, which may be due to the number of rotatable bonds of fosmidomycin analog 1 in the structure. It is well known that as the number of the rotatable bonds of the ligand increases, the docking accuracy decreases because a much larger conformational space has to be tested [24].

The amount of compounds that had to be screened the ZINC database (7,478 compounds) was not pre-screened for compounds with similar properties to fosmidomycin analog 1. Random virtual screening was conducted with the intention of obtaining new entities that had different skeletons but retained the active moiety of the DXR inhibitor. From the screening results, hit compounds with a binding energy from −11.926 to −5.153 kcal/mol were obtained. Next, the cutoff was processed as follows: First, the compounds from the virtual screening that had a binding energy in the range of −10.0 to −11.926 kcal/mol were selected, and 225 compounds were passed to next step. Then, Log P of a compound that was equal to 1.75 or higher was considered. After that, 160 compounds were manually docked using ArgusDock to look for the binding interaction of the required amino acids and ligand, which should be the same as the fosmidomycin analog, irrelevant compounds were rejected. This step reduced the amount of compounds to five. Finally, the binding energy and conformation orientation of the filtered compounds were compared to obtain three candidates from 7478 compounds. The three candidates, which could act as an inhibitor against DXR, were ZINC00310125, ZINC17111658, and ZINC59231267 (Figure 2).

Figure 3 shows the topology of fosmidomycin analog 1 bound at the active site of 5JO0.pdb. It can be seen that the phosphonate moiety is coordinated by hydrogen bonding with nitrogen from the enzyme and the Ser270 hydroxyl group. Furthermore, the terminal carboxylate groups of Asp231, Ser232, and Glu233 form hydrogen bonds with hydroxamate. The amide group of Asn311 interacts with oxygen from the hydroxamate moiety and phosphonate. These ligand-enzyme interactions concur with the results reported by Lienau *et al*. [2]. Log P of this compound is 1.75 (calculated from http://www.molinspiration.com), and it has a binding energy of −10.23 kcal/mol.

Many compounds are within the range of −11.926 to −10.00 kcal/mol. First, to narrow down the search and find

Table 1: Time consumed to dock with ArgusDock and the GA docking algorithm

Algorithm	Time for 261 molecules	Time for 7,478 molecules
ArgusDock	18 min 51 s	40 h 41 min 36 s
GA docking	42 min 17 s	78 h 44 min 24 s

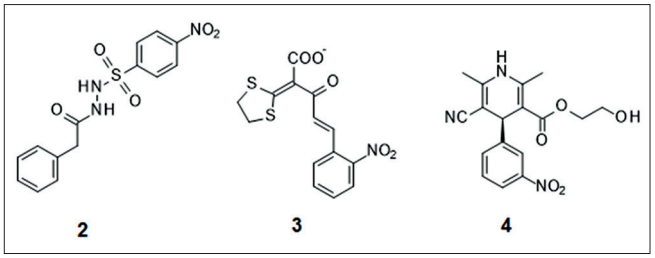

Figure 2: Structures of ZINC00310125 (2), ZINC17111658 (3), and ZINC59231267 (4)

the target compound, the binding conformation that interacts with the active site of DXR was investigated. Then, the ZINC website was searched for compounds that had a similar or higher Log P to fosmidomycin analog 1. Next, manual docking through the shape-based search algorithm was performed and the 3 best hits, ZINC00310125, ZINC17111658, and ZINC059231267, were obtained (Figures 4-6). The binding energies for ZINC00310125, ZINC17111658, and ZINC59231267 were −9.56, −9.56, and -9.03 kcal/mol, respectively (Table 2), which is <10.0 kcal/mol. These conformers were derived, for each docking, by appropriately selecting the binding conformer, which did not always have the highest binding energy.

ZINC17111658 or 2-(1,3-dithiolan-2-ylidene)-5-(2-nitrophenyl)-3-oxo-4-pemtenoic has a binding energy of −9.56 kcal/mol, and the lipophilicity of its structure (Log P = 1.90) is higher than that of fosmidomycin analog 1. This compound contains a hydrogen bond between oxygen of carboxylic moiety with the Ser270 hydroxyl group. Hydrogen bonds also form between the hydrogen of amine terminal groups of Ser232 and the oxygen of nitro group on the benzyl of ligand. The Asn311 amide group interacts with oxygen of α, β-unsaturated carbonyl of ligand (Figure 4).

For ZINC00310125 or N-nosyl-2-phenyl-acetohydrazide, the binding energy is −9.56 kcal/mol and the structure of lipophilicity (Log P) is equal to 1.85, which is higher than that of fosmidomycin analog 1. This compound contains a hydrogen bond between oxygen of nitro moiety with the Ser270 hydroxyl group, between the carboxyl terminal groups of Asp231 and the hydrogen of the amine of ligand, and between the hydroxyl group of Ser232 and the oxygen of the sulfonyl group of ligand (Figure 5).

ZINC59231267 or 2-hydroxyethyl-5-cyano-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate has a binding energy of −9.03 kcal/mol and the lipophilicity (Log P = 2.77) is higher than that of fosmidomycin analog 1. This compound contains a hydrogen bond between oxygen from the hydroxyl group with hydrogen of Ser270. Moreover, the Asp231 and Ser232 carboxyl terminal groups form hydrogen bonds with hydrogen of dihydropyridine (Figure 6).

From the results, all three candidates possessed functional groups that could be substituted for the phosphonate motif and the hydroxamate moiety of fosmidomycin analog 1. These compounds have lipophilicity higher than that of fosmidomycin 1. Table 1 shows that both ZINC00310125 and ZINC59231267 maintained the number of rotatable bonds, H-bond acceptors, and H-bond donors to be close to that of fosmidomycin analog 1. All compounds had approximately the same molecular weight. One interesting thing about the

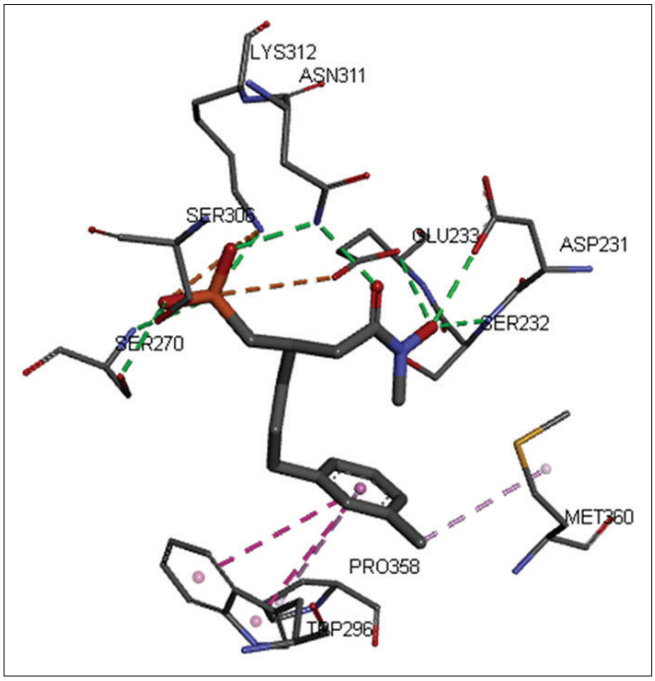

Figure 3: Fosmidomycin analog 1 interactions at the active site of 5JO0.pdb

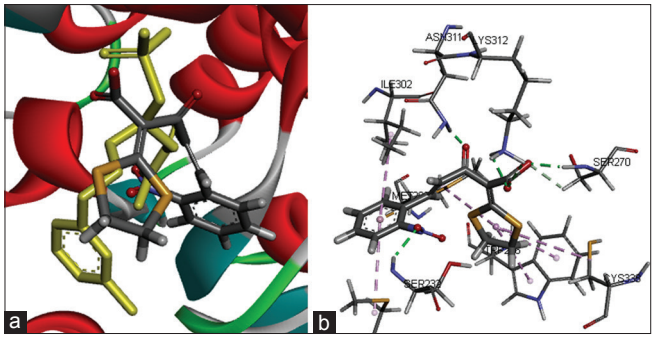

Figure 4: (a) ZINC17111658 overlay on fosmidomycin analog 1 (yellow), (b) ZINC17111658 interactions at the active site of 5JO0.pdb

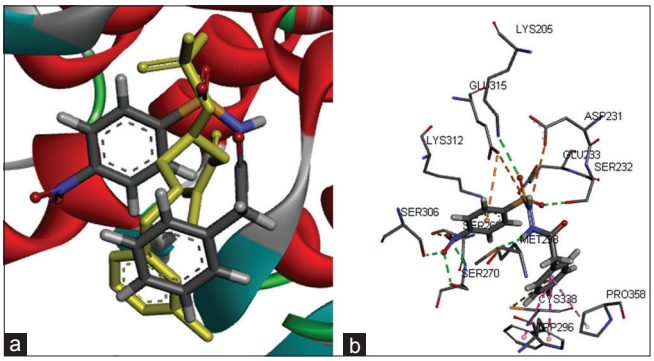

Figure 5: (a) ZINC00310125 overlay on fosmidomycin 1 (yellow), (b) ZINC00310125 interactions at the active site of 5JO0.pdb

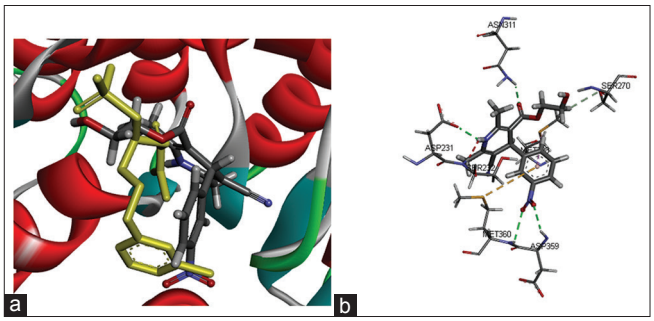

Figure 6: (a) ZINC59231267 overlay on fosmidomycin analog 1 (yellow), (b) ZINC59231267 interactions at the active site of 5JO0.pdb

Table 2: Physical properties of inhibitors

Compounds	Log *P*	Binding energy (kcal/mol)	Rotatable bond	H-bond acceptors	H-bond donors	MW
Fosmidomycin analog 1	1.75[a]	−10.23	8	6	2	328.13
ZINC00310125	1.85[b]	−9.56	6	8	1	335.34
ZINC17111658	1.90[b]	−9.56	5	6	0	336.37
ZINC59231267	2.77[b]	−9.03	6	8	2	343.34

[a]Log *P* was calculated from the ZINC 12 program (http://www.molinspiration.com), [b]Log *P* was obtained from ZINC 12 (ZINC.docking.org)

hits compounds is that all compounds have nitro group in the structure. Nitro group in ZINC00310125 and ZINC17111658 has interaction with the required amino acids in the active site.

CONCLUSIONS

Shape-based search algorithm or shape complementary docking takes into consideration an entire molecular surface rather than purely an active site; consequently, it was found to be a practical alternative approach to docking. The method was fast and efficient. ArgusLab has built-in functions, which dock databases in the SDF format, and it could be installed on a low to medium performance computer. The ZINC database proved to be an invaluable resource, which was used to find new hits for three potential DXR inhibitors: ZINC00310125, ZINC17111658, and ZINC59231267. These compounds can be purchased, synthesized, or further modified to test for their effectiveness as antimalarial drugs.

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