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Original Article

LIGAND-BASED VIRTUAL SCREENING OF FDA-APPROVED DRUGS TO IDENTIFY NEW INHIBITORS AGAINST LACTATE DEHYDROGENASE ENZYME OF MALARIA PARASITES

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ABSTRACT

Objective: The aim of this study is to computationally repurpose FDA-approved drugs as potential inhibitors of the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) by competing with the cofactor NADH.

Methods: In this *in silico* study, we have virtually screened a library of FDA-approved drugs for structural similarity to the dihydro nicotinamide adenine dinucleotide (NADH). Then, the top hits were further assessed for clinical safety and by application of molecular docking and dynamics simulation.

Results: Ligand-based virtual screening reports that the antibiotic Novobiocin has a good similarity to the cofactor NADH with a score of 0.7. Also, molecular docking study indicates that Novobiocin may has the ability to interact with *Pf*LDH enzyme with a docking energy of-8.8 Kcal/mol. However, during molecular dynamics (MD) simulation, the mean ligand proximity root mean square deviation (RMSD) and binding energy for Novobiocin were 4.3 Angstrom and-37.45 Kcal/mol, respectively. These MD simulation parameters are inferior to those recorded for NADH molecule during 50 nanosecond intervals.

Conclusion: The antibiotic Novobiocin may serve as a potential lead candidate toward the design of novel antimalarial agents. However, further evaluation of Novobiocin may be recommended to affirm its capacity against *Pf*LDH enzyme.

Keywords: Ligand-based virtual screening, Repurpose, Docking, Dynamics simulation, Novobiocin, *Plasmodium falciparum* lactate dehydrogenase

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INTRODUCTION

Malaria is a life-threatening neglected disease caused by parasites of the genus *Plasmodium*. The global incidence of this infectious disease is estimated to be 247 million cases in 2021 across 84 endemic regions (https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022) [1, 2]. In human, malaria disease is caused by five species of the *Plasmodium* parasites but *Plasmodium falciparum* is considered the most pathogenic one with a high possibility of drug resistance [3]. The *Plasmodium* parasites is usually transmitted to human beings through bites of the infected female Anopheles mosquitoes [4]. When biting a human, these infected mosquitoes will inject the sporozoites form of the parasite into the host blood. Then, the sporozoites will travel into the host liver and start the asymptomatic asexual reproduction inside hepatocytes. Eventually, the malaria parasites will emerge into the merozoites form, which is then discharged into blood stream to infect red blood cells (RBCs). Once the malaria parasites invade RBCs, it will multiply, destroy these cells and generate more merozoites that infect other RBCs [5]. It is believed that the rupture of these infected RBCs will release malaria toxins into blood stream of the host. These released toxins will activate the liberation of pro-inflammatory cytokines, resulting in the appearance of disease symptoms like fever, anemia and thrombocytopenia [6]. The signs and symptoms of malaria are generally non-specific and thus can complicate the clinical diagnosis of this disease. As such, different laboratory techniques are used to help in the diagnosis of suspected cases like microscopic examination of blood smear, serological tests and polymerase chain reaction (PCR) [7]. To successfully eradicate malaria in endemic regions, different tools must be employed like control of mosquito vectors, use of rapid diagnostic testing, deployment of effective drugs and vaccines. In this direction, the recent introduction of antimalarial vaccines like RTS, S/AS01 and R21/Matrix-M may boosts hopes towards elimination of this disease in endemic countries [8, 9]. Due to the frequent resistance to old antimalarial drugs like Chloroquine, it is now recommended in most regions to use artemisinin-based combination therapy (ACT) for the management of uncomplicated cases of *Plasmodium falciparum* malaria [10]. Unfortunately, there have been several reports of *Plasmodium falciparum* resistance to artemisinin in southeast Asia and other endemic regions [11, 12]. Therefore, it is of interest to design and develop new antimalarial drugs to combat the threat imposed by multidrug-resistant malaria [13]. One of the potential molecular targets to develop new antimalarial drugs is the lactate dehydrogenase (LDH) enzyme of the *Plasmodium* parasites. This enzyme is the last enzyme in the glycolysis pathway and it is essential for the production of energy in the erythrocytic stage of *Plasmodium* parasites life cycle. During this anaerobic stage, the *Plasmodium* LDH enzyme catalyzes the conversion of pyruvate into lactate and regenerates nicotinamide adenine dinucleotide (NAD^+). The LDH enzyme is found plentiful in all species of malaria parasites and it is considered to be distinct from its counterparts in bacteria and mammals [14, 15]. Interestingly, it has been found that chloroquine can act as a competitive inhibitor of the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme. Crystallization studies refer to the ability of chloroquine to interact with *Pf*LDH in the binding pocket of the dihydronicotinamide adenine dinucleotide (NADH) [16]. A comparative illustration for the interaction between either the cofactor NADH or chloroquine with the PfLDH enzyme can be observed in (fig. 1). As such, analogs of the cofactor NADH may have the potential capacity to inhibit *Pf*LDH. In this direction, a previous study had virtually screened DrugBank library of compounds for analogs of NADH and the most similar compounds to NADH were then docked into *Pf*LDH crystal. Based on the findings of this *in silico* study, the top three drugs with best binding energy (itraconazole, atorvastatin, posaconazole) were found to be effective both *in vitro* and *in vivo* against *Plasmodium falciparum* chloroquine resistant parasites [17]. According to the aforementioned facts, we have used the structure of NADH as a template to carry out a ligand-based virtual screening of FDA-approved drugs library in order to identify potential inhibitors against *Pf*LDH enzyme. Then, the best identical drugs to NADH were further assessed by both molecular docking and dynamics simulation to virtually validate its capacity as competitive inhibitors against *Pf*LDH enzyme. Our aim in this study is to repurpose FDA-approved drugs as new potential inhibitors of *Pf*LDH enzyme by applying multiple computational tools.

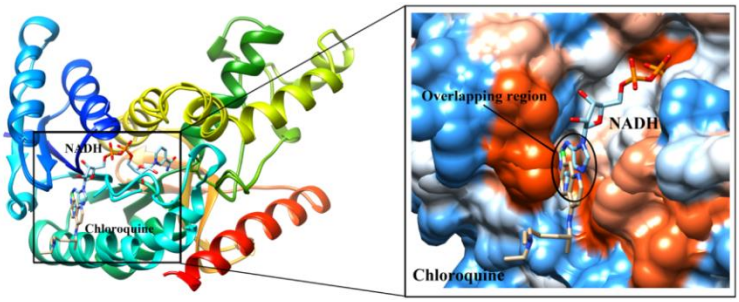

Fig. 1: A comparative representation for the binding between either NADH or chloroquine and the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme (1LDG versus 1CET crystals)

MATERIALS AND METHODS

Setting up a methodological plan for the *in silico* study

A summarized representation for the main steps of this computational study can be viewed in (fig. 2). As can be pointed out from this figure, the first step in this study was ligand-based virtual screening. In this step, a library of FDA-approved drugs was assessed for structural similarity to the cofactor NADH. Then, the clinical uses and safety were evaluated for those drugs with high similarity to NADH. In the next step, only those drugs with acceptable relative safety have been subjected to docking against *Pf*LDH crystal. Finally, the drugs with the best docking energy (least energy of binding) were then submitted to molecular dynamics (MD) simulation for 20 and 50 nanoseconds. In this final stage, both ligand movement and binding energy were calculated throughout the simulation interval.

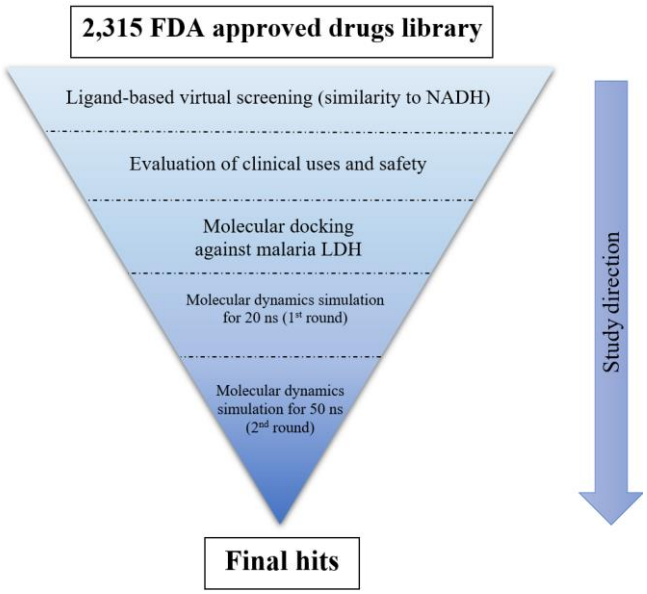

Fig. 2: A schematic overview for the major steps of this virtual screening study

Ligand-based virtual screening

In this initial stage, we have employed FitDock-align tool accessible through DrugRep virtual screening server to carry out ligand-based similarity analysis. The FitDock is a hierarchical alignment tool with multiple features that can detect equivalent atom pairs between two compounds. The result of this screening process is usually ordered based on similarity score that can vary between 0 and 1, where 0 means not identical and 1 means totally identical [18]. For this screening step, a library of 2,315 FDA-approved drugs was screened for similarity to the structure of NADH.

Evaluation of clinical uses and safety

Only those approved drugs with high similarity score were then submitted to an evaluation for clinical uses and safety profile. For this purpose, we have used the Medscape reference website to explore the uses and safety for each drug (https://reference.medscape.com/). Based on this online reference, only these drugs with acceptable safety profile were then submitted to the next step of molecular docking.

Molecular docking

For the docking step, we have used the 1-click docking tool available through the Mcule. com, an online drug discovery website (https://mcule.com/). The 1-click docking tool utilize both AutoDockTools and AutoDock Vina to perform docking process [19, 20]. In this step, each selected drug was docked against the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme crystal with PDB code 1LDG [21]. The employed docking coordinates were X: 33, Y: 25, Z: 35 and the grid box dimensions were 22*22*22 Angstrom. Additionally, we have validated the accuracy of the applied docking procedure by redocking the co-crystalized NADH into *Pf*LDH crystal. After that, the root mean square deviation (RMSD) was computed by using PacDOCK web server to compare the conformations of both the co-crystalized and the docked NADH [22]. For the output of this step, we have selected only those compounds with the best docking energy (least energy of binding) to undergo a molecular dynamics (MD) simulation study. Also, we have used UCSF chimera 1.15, PyMOL 2.4.1 (https://pymol.org/2/) and Discovery studio visualizer 21.1.0 (https://discover.3ds.com/

discovery-studio-visualizer-download) to examine the docking orientation of the minimum binding energy pose for each drug-target complex [23].

Molecular dynamics (MD) simulation

In this *in silico* study, two rounds of MD simulation were executed for 20 and 50 nanoseconds by using the YASARA Dynamics 20.12.24 [24]. At first, The MD simulation was carried out for 20 nanoseconds for each ligand-enzyme complex with the least energy of binding. Then, the second round of MD simulation was performed for only those drugs with average proximity RMSD to enzyme binding pocket of no more than 4 Angstrom. Simulation results of NADH-*Pf*LDH docking complex was used as a positive control for these two rounds of MD study. The detailed steps and options used for MD simulation in this study is similar to what we previously applied in our published articles [25–27]. Briefly, a concentration of 0.9% sodium chloride was used in this MD study and an additional concentration of either sodium or chloride ions was applied to guarantee neutralization of the drug-enzyme complex during simulation. Also, minimizations of steepest descent and simulated annealing were used to avoid any clashes in the simulation process. During this process, the following force fields were utilized: AM1BCC and GAFF2 for ligand, AMBER14 for solute, TIP3P for water [28–30]. The objective of this MD study was to compute ligand proximity RMSD to the designated binding pocket of *Pf*LDH enzyme during the simulation interval. Moreover, the binding energy of molecular mechanics Poisson Boltzmann surface area (MM-PBSA) was also calculated for each drug in this MD study.

RESULTS AND DISCUSSION

For the output of virtual screening based on similarity to NADH, we have only presented the top ten hits as seen in (table 1). In this table, the FitDock similarity score is arranged in a descending order that range between 0.735 and 0.693. This range of values indicates that these top hits have a good molecular similarity to the cofactor NADH. Therefore, these listed drugs should be considered for more assessment.

The clinical uses of these ten drugs are also listed as a column in (table 1). And based on this column, we have decided to exclude those drugs used for hypertension, leukemia, acromegaly or muscle relaxation from further evaluation due to the possibility of serious adverse effects (https://reference.medscape.com/). As such, only three antimicrobials, one anti-migraine drug and one anti-asthma agent, were considered for the next step of molecular docking.

For the molecular docking, initially, the accuracy of 1-click docking tool was evaluated by using the redocking (pose selection) method. In this method, the co-crystalized ligand is taken out of enzyme crystal and then docked into the same binding site. After that, the RMSD is calculated for any variation between native conformation and docked conformation of that ligand. It is a known fact that any RMSD value for conformations variation that falls between 1.5 and 2.0 Angstrom refers to good accuracy of the docking method. In other words, a precise docking method usually coincides with lower conformations difference RMSD [26, 31]. The redocking results of NADH molecule into PfLDH crystal can be seen in (fig. 3). Interestingly, the docking energy for NADH was-11.6 Kcal/mol while the computed RMSD value for the conformations change between docked and co-crystalized NADH was 1.422 Angstrom. This value of conformations change RMSD is less than 1.5 Angstrom and indicates a very good precision of the applied docking tool. Then, the docking method was applied to only those drugs that are similar to NADH and have an acceptable safety profile. The results of docking energy were listed in the last column of (table 1) where the lower energy of binding means better docking behavior. According to (table 1), the reported binding energy values were ranging between-7.4 and-10.8 Kcal/mol and this may indicate good binding affinity against PfLDH. However, the best docking energy-10.8 Kcal/mol was reported to the anti-migraine agent ergotamine, which is inferior to that calculated for NADH of-11.6 Kcal/mol.

Table 1: A tabular list for the ligand-based virtual screening of FDA-approved drugs; these drugs were listed based on their similarity score to the dihydro nicotinamide adenine dinucleotide (NADH) in descending order. This table also presents the clinical use for each of these listed drugs. For only those drugs with relative safety, the docking energy against the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme was calculated.

No.	Generic name	Similarity score	Clinical use	Binding energy (Kcal/mol)
1	Ponatinib	0.735	Leukemia	-
2	Atracurium	0.731	Skeletal muscles relaxation in surgery	-
3	Lanreotide	0.725	Acromegaly	-
4	Cefiderocol	0.717	Bacterial infection	-7.4
5	Rescinnamine	0.716	Hypertension	-
6	Novobiocin	0.701	Bacterial infection	-8.8
7	Ergotamine	0.699	Migraine	-10.8
8	Isavuconazonium	0.699	Fungal infection	-7.8
9	Zafirlukast	0.697	Asthma	-9.1
10	Candesartan	0.693	Hypertension	-

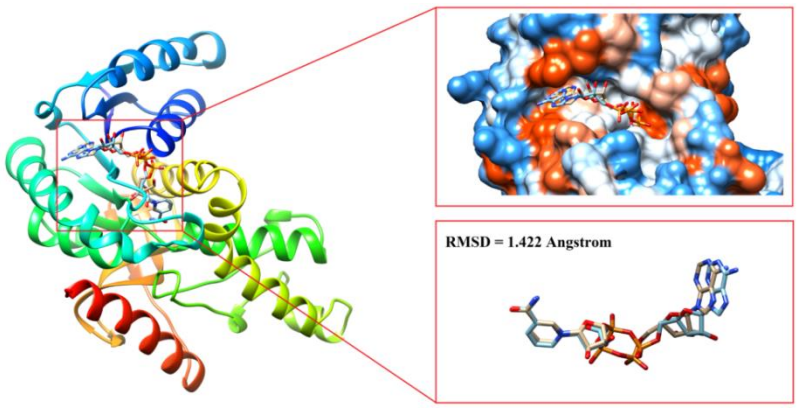

Fig. 3: Redocking of dihydro nicotinamide adenine dinucleotide (NADH) into the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme

Regarding the molecular dynamics (MD) simulation study, the results of the first run of 20 nanoseconds MD simulation are summarized in (table 2). As observed in this table, the term ligand movement RMSD is usually used in MD studies to estimate the proximity of drug molecule to target binding site. In other words, a low ligand movement RMSD value can refer to stronger binding of drug to target site. Such a measurement can be generated through superposing the drug-enzyme complex on its reference structure during MD simulation [24, 32]. Based on the findings in (table 2), the least mean ligand movement RMSD was reported for the cofactor NADH followed by the antibiotic Novobiocin. Both NADH and Novobiocin were able to maintain a proximity to *Pf*LDH binding site with mean RMSD value of less than 4 Angstrom, therefore, only these two compounds were subjected to the second run of MD simulation for 50 nanoseconds. Additionally, the least average MM-PBSA binding energy was calculated to NADH followed by Novobiocin as can be noted in (table 2).

Table 2: A summary of molecular dynamics simulation results for 20 nanoseconds interval

No.	Drug name	Average MM-PBSA binding energy (Kcal/mol)	Ligand movement RMSD (Å)		
			Mean	Minimum	Maximum
1	NADH (Control)	-127.95	2.24	0.71	2.60
2	Cefiderocol	-25.67	7.51	1.13	12.35
3	Novobiocin	-35.53	3.88	1.17	5.46
4	Ergotamine	-19.26	4.63	0.79	8.69
5	Isavuconazonium	-19.43	6.54	0.90	13.89
6	Zafirlukast	-2.24	5.88	1.07	7.60

MM-PBSA: molecular mechanics Poisson Boltzmann surface area; RMSD: Root mean square deviation; Å: Angstrom; NADH: Dihydronicotinamide adenine dinucleotide.

For the extended 50 nanoseconds MD study, a detailed plot of ligand movement RMSD against simulation time can be seen in (fig. 4) for both NADH and Novobiocin. Again, the cofactor NADH was in a position to keep a more stable and closer proximity to *Pf*LDH binding pocket as compared to the antibiotic Novobiocin. In this simulation run, the mean ligand movement RMSD values were 2.29 and 4.30 Å for NADH and Novobiocin, respectively. Also, the average MM-PBSA binding energy were-125.00 and-37.45 Kcal/mol for NADH and Novobiocin, respectively. It is worth mentioning that the comparison of docking results for Novobiocin and NADH against *Pf*LDH refers to the possibility of overlapping in the interactions region of these two compounds with target enzyme as can be seen in (fig. 5). Taken into account these results, it is predicted that the antimicrobial agent Novobiocin may have the potential to bind *Pf*LDH enzyme. However, this binding capacity seems to be inferior to that reported for the cofactor NADH when comparing ligand proximity and MM-PBSA binding energy for these two compounds. Thus, it may be of interest to consider the antibiotic Novobiocin for more *in vitro* and *in vivo* assessments toward the development of new antimalarial agent.

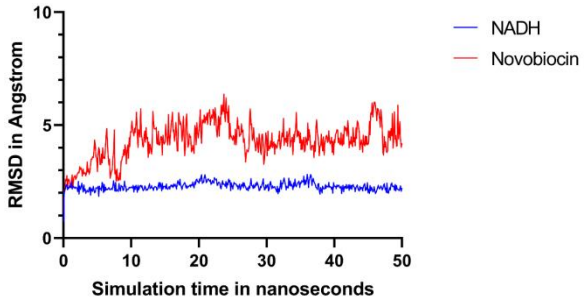

Fig. 4: A plot of ligand movement RMSD against the simulation interval of molecular dynamics study

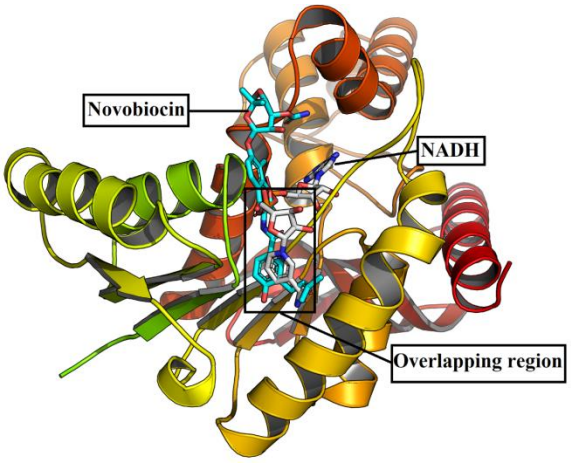

Fig. 5: A comparative illustration for the docking of either NADH or novobiocin against the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme

CONCLUSION

In this *in silico* study, we report that the antibiotic Novobiocin may have the ability to interact with the *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) enzyme. According to ligand-based virtual screening, this antibiotic has a good structural similarity to the cofactor NADH. Also, it displays an acceptable safety profile as mentioned in clinical references. Both molecular docking and dynamics simulation predict that Novobiocin may have a close proximity to *Pf*LDH binding site with good binding energy. However, these calculated parameters of Novobiocin proximity and binding energy were lower than that computed for the cofactor NADH against *Pf*LDH enzyme. As a result, it is recommended to consider Novobiocin for further *in vitro* and *in vivo* evaluations to develop a new drug against malaria parasites.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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