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Machine Learning Predicts 5-Chloro-1-(2-Phenylethyl)-1h-Indole-2,3-Dione as a Drug Target for Fructose Bisphosphate Aldolase in Plasmodium Falciparum

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Abstract:- Malaria is a deadly disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. Of the five species of Plasmodium, P. falciparum is the deadliest, and findings have shown to have growing evidence of drug-resistance mechanisms in malaria treatments. Therefore, the identification of new drug targets is an urgent need for the clinical management of the disease. In this study, we employ an approach of identifying drug leads against fructose bisphosphate aldolase, a potent drug target in P. falciparum.Molecular docking was carried out using PyRx and CBDock to determine the binding affinities of protein-ligand complexes. Two drug leads were generated using machine learning. These drug leads were selected based on Lipinski's drug-likeness criteria. The ligand 5-Chloro-1-(2-phenylethyl)-1H-indole-2,3-dione exerted the highest binding effect on the aldolase as compared to 1-(7,8 Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione using molecular docking. The 5-Chloro-1-(2-phenyl ethyl)-1H-indole-2,3-dione superior binding affinity with bisphosphate aldolase compared to 1-(7,8-Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione imply that it can inhibit the bisphosphate aldolase activity in the plasmodium falciparum.

Keywords:- *BLAST; Molecular Docking; Machine Learning; QSAR; Drug Lead; Drug Target; Aldolase.*

I. INTRODUCTION

Malaria, transmitted by mosquitoes, primarily affects humans and female Anopheles mosquitoes. Typical symptoms encompass fever, tiredness, vomiting, and headaches. Left untreated, recurring episodes of the disease can arise months later. Caused by single-celled microorganisms from the genus Plasmodium, malaria is primarily transmitted by infected female Anopheles mosquitoes, notably prevalent in Africa. Five Plasmodium species can infect and spread via humans. P. falciparum causes most fatalities, while *P. vivax*, *P. ovale*, and *P. malariae* generally result in milder forms of malaria. *P. knowlesi* infrequently causes disease in humans. When an infected mosquito bites, parasites from the liver are introduced into the bloodstream, traveling to the liver to mature and reproduce(Benson & O'Reilly, 2009).

The life cycle of malaria involves a cyclical infection of humans and female adult *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the hepatocytes and then in the erythrocytes of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites that continue the cycle by invading other red cells. The blood-stage parasites are those that cause the symptoms of malaria.

Rapid and effective malaria diagnosis not only combats the disease but also decreases the transmission of the disease. In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears (Tangpukdee et al., 2009), other concentration techniques, e.g. quantitative buffy coat (QBC) method (Bhandari, Raghuveer, Rajeev, & Bhandari, 2008), rapid diagnostic tests, and molecular diagnostic methods, such as polymerase chain reaction (PCR) (Persing, 1991). Malaria is treated with antimalarial medications; the ones used depend on the type and severity of the disease. Currently, available antimalarial drugs are broadly categorized into three types.

Generally speaking, In the realm of malaria control, antimalarial drug resistance has emerged as a significant challenge. This resistance often arises from spontaneous mutations that decrease the effectiveness of a particular drug or drug category. In certain cases, just one genetic mutation can render a drug ineffective against the malaria parasite, while with other drugs, several mutations seem necessary for resistance to manifest. Malaria isolates often consist of diverse parasite populations within a single sample, displaying a spectrum of drug response characteristics ranging from high resistance to complete sensitivity.(Thaithong, 1983).

Fructose bisphosphate aldolase is an enzyme catalyzing a reversible reaction that splits the fructose 1,6 bisphosphate generated by the activity of phosphofructokinase into the triose phosphates dihydroxyacetone phosphate(DHAP), and glyceraldehyde 3- phosphate (G3P) in the glycolytic pathway which is the only source of energy for the *Plasmodium falciparum*. Besides its housekeeping role in glycolysis, fructose bisphosphate aldolase has also been involved in additional functions and is considered a potential drug target against Plasmodium falciparum. *P. falciparum* lacks a functional

citric acid cycle. Unlike most tissues of the mammalian host, it is totally dependent on glycolysis for energy generation. A compound that selectively inhibits the parasite's ATP-generating machine is therefore a potential antimalarial drug target.

Machine learning as a field of study provide a set of algorithms that can help to analyze, predict and make decision based on the analysis of the data given. Machine learning can be used in almost all fields which also cut across the field of drug design and discovery and can be applied in all stages of drug design and discovery. Examples include target validation, identification of prognostic biomarkers, ligand identification, and analysis of pathological data in clinical trials. The application of machine learning can promote valuable decision-making from the analysis of data available and has the potential to speed up the process and reduce failure rates in the case of drug discovery and development.

II. METHODS

A. *Identification of Target with Data Availability On Pub Chem*

In the identification of the target, a Target2Scan tool was used. Target2scan is a programmatic tool that incorporates many features of the bioinformatics, computational biology, and AI-driven drug discovery revolutions into a single workflow assembly. It helps to determine the ligands of a new drug target, predict protein-ligand interactions, discover drug effectiveness, ensure safety biomarkers, and optimize the bioactivity of the ligands.

To identify drug ligands against the target, the target signature (accession number of the target P04075) was provided as an input to the tool. The tool then carried out a BLAST (basic local alignment search tool) protocol with the new target signature provided, and identified known protein drug targets that are similar to the new target submitted to the tool; with data availability, targets were identified on PubChem by the tool.

B. *Retrieval of Ligands for Target and Their Properties*

The first set of compounds was retrieved by this tool on PubChem, which were ligands for the target. The molecular descriptors of these compounds were retrieved. Molecular descriptors such as; ba-id, activity, aid (assay id), cid (compound ID), sid (substance ID), gene-id, pm-id, aid type, hasdrc,rnai, protacxn, ac-name, acqualified, acvalue (activity value) aidsrc name, aid name, compound name, target name, target url, ecs(enzyme classification system), repacxn ,tax-id.

C. *Retrieval of Compounds with Similar Structures to Known Ligands for The Target*

Compounds that have similar structures as the known ligands for the target and their molecular target were retrieved by this tool. The retrieved molecular descriptors of these compounds were CID(compound ID), molecular weight, heavy atom, XlogP, complexity, hydrogen bond acceptor, monoisotope, rotable, TPSA(topological polar surface area) were all retrieved.

D. *Generation of Drug Leads by Machine Learning*

AutoQSAR used machine- learning protocols (training dataset and test dataset) to build, validate and deploy QSAR (Quantitative Structure-Activity Relationships) models. It screened huge databases (PubChem) of compounds in order to determine the biological properties of chemical molecules based on their chemical structure.

All retrieved compounds with similar structures as the known ligands had their features divided into the training set and test set. The training set was now implemented to build up the model, while the test set evaluates/validates the performance of the mode i.e. accuracy of the model.

The AutoQSAR constructed both linear and non-linear regression-based QSAR models by considering all possible combinations of molecular descriptors. Among these models, those exhibiting the highest R2 values or values closest to 1 were preferred, and the one with the closest R2 value to 1 was employed for predictions. These predictions were conducted using PubChem's extensive chemical library. To filter out undesired compounds, Lipinski's drug-likeness criteria, which correlates molecular descriptors with known drug activity, were applied. Subsequently, the top 50 compounds meeting these criteria from the prediction were identified as potential drug leads against the target.

E. *Preparation of Ligands and Receptor (Target Model) For Docking*

AutoDock-Vina tool version 1.5.6 was used to carry out the preparation of the ligand and receptor. The receptor model was downloaded in the PDB format from the Protein Data Bank in Europe (PDBe); https://www.ebi.ac.uk/pdbe-kb/proteins/P0405 which is a database for structural data of biological macromolecules. The receptor model was then taken to AutoDock-Vina for the conversion to the PDQBT format. To generate this format, water molecules were removed; polar hydrogen(s) and Kollman charges were added. Autodock-Vina tool was also used to convert the ligands to PDBQT format also.

F. *Molecular Docking*

Autodock- Vina tool version 1.5.6 was also used in predicting the intermolecular framework formed between the receptor (target model) and the ligands also used in suggesting the binding modes responsible for the inhibition of the target. This docking method was used to fit in the ligands into the binding site of the target in order to predict how strong the binding the ligand and the target is it was also used to generate possible poses which are ranked by scoring functions.

The scoring functions are mathematical functions used to approximately predict the binding affinity between the ligands and the target, the strength of intermolecular interactions between the ligand and the target

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G. Visualization of Protein-Ligand Interaction Complex and Determination of Interactions Between Protein and Ligand

PyMOL, an open source model visualization tool was used in visualizing the protein-ligand interaction complex. It produces high-quality 3D images of small molecules and biological macromolecules.

Information on chemical group that participated in specific interactions (e.g. hydrogen bond donor) and interaction geometry (distance and angle threshold) contributed to the binding affinity of the ligand the ligand for which the target gave the highest binding affinity minimum binding energy) was selected for for visualization.

The bonds that existed in the protein-ligand complex were determined manually using a distance tool in PyMOL. The criteria for identifying the bonds are; distance limit (hydrophobic bond distance = 4.0A, hydrogen bond distance= 4.1A, salt bridge distance= 5.5A), the angle and the atoms (carbon atoms for hydrophobic interactions) Involved in the interactions

III. RESULTS

A. Selection of Compound for Machine Learning

Target 2 scan retrieved 49 compounds from PubChem as ligand that can compelled the target from 98791 compounds as compounds that have similar structures as the known ligands for the target alongside the values of their molecular descriptors and their molecular descriptor was used as features in the downstream machine learning procedure.

B. Generation of Drug Lead by Machine Learning

5-Chloro-1-(2-phenyl ethyl)-1H-indole-2,3-dione and 1-(7,8-Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione were the drug lead generated by machine learning algorithm. These drug leads were selected based on the Lipinski's drug likeness criteria (relates molecular descriptors of the retrieved compounds/ ligands with known drug activity) and the top 50 compounds of the prediction that satisfied the criteria were printed out as drug leads against the target. The CID of these ligands are 2930041 and 152934187.

Table 1: ligands and their molecular descriptors

PARAMETERS	LIGANG 1	LIGAND 2
CID	2930041	1529334187
IUPAC NAME	5-Chloro-1-(2-phenyl ethyl)-1H-indole-2,3-dione	1-(7,8-Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione
Chemical Formula	$C_{16}H_{12}ClNO_2$	$C_{25}H_{24}N_2O_3$
Molecular Weight(G/Ml)	285.32	400.5
Heavy Atom Count	20	30
Xlogp	3.5	3.5
Complexity	3.29	3.29
Hydrogen Bond Acceptor Counts	2	3
Monoisotopic Mass (Da)	285.0556563	400.17869263
Rotatable Bond Counts	3	3
TA /SA (A^2)	37.4	57.7

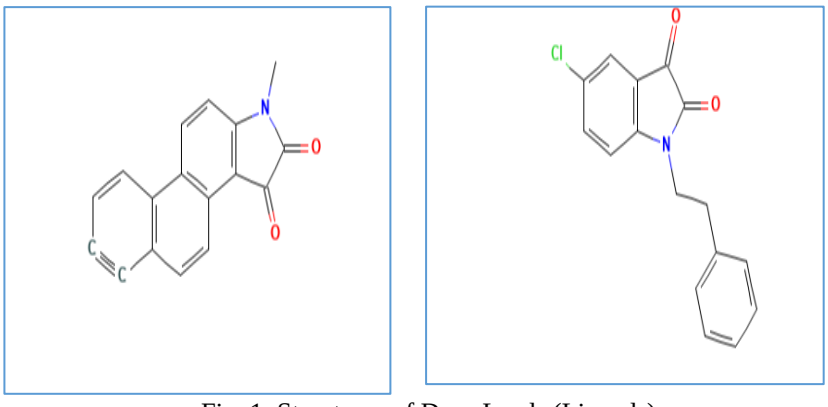

Fig. 1: Structures of Drug Leads (Ligands)

C. Molecular Docking

Molecular docking gave scoring functions which are used to predict the binding affinity between the ligand and target where X Centre= -9.498, Y Centre=- 1.025, Z Centre=- 26.027, size x,y and z= 40, energy,range=4, exhaustiveness=8. The ligand for which the target gave the highest binding affinity (minimum binding energy) was selected for visualization.

Table 2: ligands and their binding affinity

S/N	IUPAC NAME	Binding Affinity
1	5-Chloro-1-(2-phenylethyl)-1H-indole-2,3-dione	-9.1
2	1-(7,8-Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione	-7.8

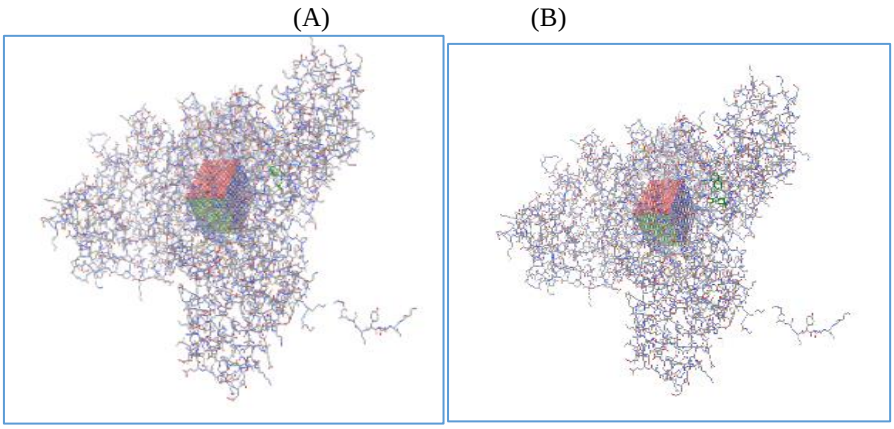

Fig. 2: Grid Boxes of Ligands

D. Fructose Bisphosphate Aldolase- 5-Chloro-1-(2-Phenylethyl)-1h-Indole-2,3-Dione Interaction

The 5-Chloro-1-(2-phenylethyl)-1H-indole-2,3-dione gave the highest binding affinity out of the two ligands and binds and was visualized using PyMOL. The bonds that existed in the protein-ligand complex were determined manually using a distance tool in PyMOL, the parameter are;

Hydrogen bond interaction - 4.6
Hydrophobic bond interaction - 4.8
Salt bridge – 6.0

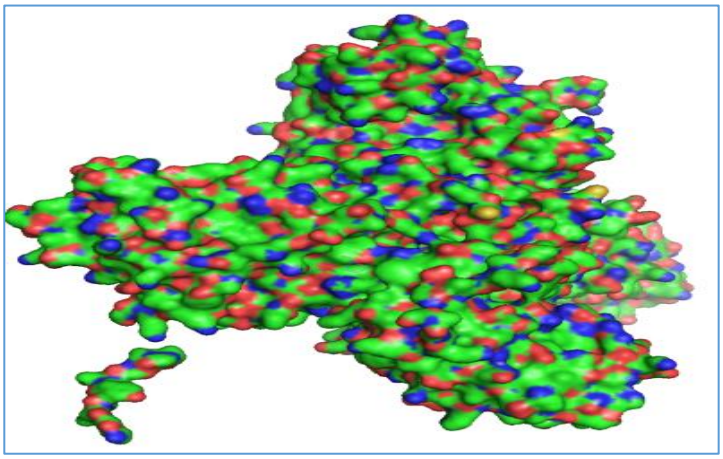

Fig. 3: Surface representation of protein-ligand interaction

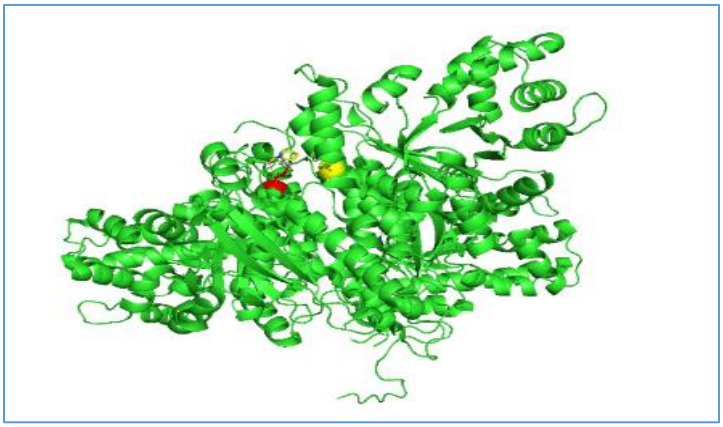

Fig. 4: Cartoon representation of protein-ligand interaction.

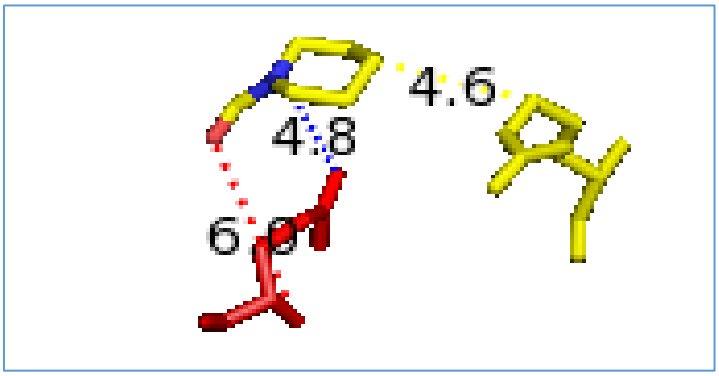

Fig. 5: Interaction between the ligand.

IV. DISCUSSION

Malaria remains a significant public health concern in regions with high transmission rates and even in areas where transmission has been largely controlled or eliminated. Its complexity varies across the globe in terms of epidemiology and clinical presentation.This variability is influenced by factors such as prevalent malaria parasite species, their resistance to antimalarial drugs, climate, environmental conditions, and the acquired immunity levels and behaviors of humans in those environments.

The unique reliance of the Plasmodium parasite on glycolysis for its ATP (adenosine triphosphate) needs sheds light on its resistance to antimalarial drugs. Though targeting the active sites of malarial glycolytic enzymes is hindered by their similarity to human enzymes, exploiting the distinctive structural and functional properties of these enzymes holds promise for antimalarial drug development.

Fructose bisphosphate aldolase, an enzyme crucial in the glycolytic pathway by converting fructose 1,6 bisphosphate into triose phosphates DHAP and G3P, stands out as a potential target for antimalarial drug design. Given the complete reliance of the intra-erythrocytic merozoite life stage of P. falciparum on glycolysis for ATP production, inhibiting glycolytic enzymes in P. falciparum could effectively eliminate the parasite.

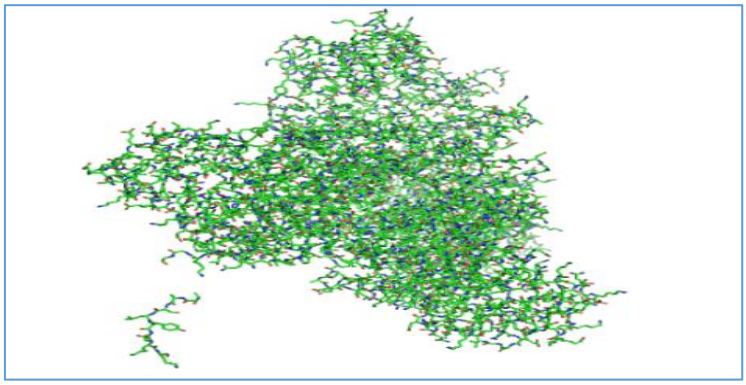

Fig. 6: Structure of fructose- bisphosphate Aldolase

5-Chloro-1-(2-phenylethyl)-1H-indole-2,3-dione which was one of the drug lead generated using machine learning algorithm has been found to have higher binding affinity compared to 1-(7,8-Dihydronaphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione as shown from the score function in molecular docking. These drug leads were selected based on the Lipinski's drug likeness criteria (relates molecular descriptors of the retrieved compounds/ ligands with known drug activity).

V. CONCLUSION

Drug design and development represent critical research realms for pharmaceutical companies and chemical scientists. Yet, challenges like low efficacy, off-target delivery, time-intensive processes, and high costs pose significant obstacles that affect the landscape of drug design and discovery. The integration of machine learning algorithms, a subset of artificial intelligence, has been widely adopted across various drug discovery phases. These algorithms have been applied in peptide synthesis, structure-based virtual screening, ligand-based virtual screening, toxicity prediction, drug monitoring, release mechanisms, pharmacophore modeling, ligand prediction, drug repositioning, and the assessment of physiochemical activity. This study emphasizes the pivotal role of machine learning in leveraging physiochemical activity as a crucial factor in drug discovery.

5-Chloro-1-(2-phenylethyl)-1H-indole-2,3-dione superior binding affinity with bisphosphate aldolase compared to 1-(7,8-Dihydro naphthalen-2-ylmethyl)-5-(piperidine-1-carbonyl)indole-2,3-dione imply that it can inhibit the bisphosphate aldolase activity in the plasmodium falciparum as predicted by machine learning algorithm from this study.

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