Computational Identification of Dihydroorotate Dehydrogenase Interactive Ligands, Therapeutic Aid for Malaria Parasite

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Abstract: This study aims to identify novel inhibitors of dihydroorotate dehydrogenase (DDI) as potential therapeutic agents for malaria. DDIs are crucial in disrupting the Plasmodium life cycle within the human body, and targeting these inhibitors could prevent the proliferation of malaria. We used computational methods to identify the active sites of dihydroorotate dehydrogenase for inhibition. Four bio/organic synthetic molecules were evaluated: comalic acid, 4,4',4"-triaminotriphenylamine, and 6-chloroquinoline-3. Molecular docking studies showed that comalic acid and 4,4',4"-triaminotriphenylamine effectively bind to the enzyme's active sites, indicating they could be strong DDI inhibitors. In contrast, 6-chloroquinoline-3-carbaldehyde demonstrated lower inhibitory potential, and 2-(3-aminoanilino) phenol exhibited no significant activity. Additionally, the study includes a tabulation of oral toxicity results and chemical properties of the ligands, which provides knowledge about their potential therapeutic safety and efficacy. This information is crucial for guiding future research and development of these compounds as viable therapeutic agents. Further investigations into their mechanisms of action and potential side effects will be essential for optimizing their use in clinical applications.

Keywords: Virtual Screening, Plasmodium, Oxidoreductase, Vector-Borne Disease.
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1. Introduction

Malaria is spread by Plasmodium, which is a parasitic disease [1], after asexual reproduction inside the host erythrocyte and produces the crystalline pigment hemozoin as a byproduct [2], which digests host hemoglobin. Due to evolution, the ability to survive and the capability to develop protection against past medication have motivated Plasmodium to develop two hundred species. The world became a global village not just for us; it also provided an edge to other animals. This also spread from malaria-endemic areas to other parts of the world. According to the World Malaria Report 2018 published by WHO, inform us that there is no significant progress in reducing global malaria cases. According to an estimated 219 million cases and 435,000 deaths in 2017 due to malaria. If malaria is not treated, it causes serious complications [3] and ultimately becomes life-threatening in healthy adults [4]. Synthetic drugs are widely used

for the prevention of malaria [5]. The global strategies for fighting malaria have been changed, but the ultimate goal of eradication remains unchanged [6]. Synthetic drugs interrupt the life cycle of Plasmodium inside the human body; this strategy is used to prevent further amplification of malaria [7].

Dihydroorotate dehydrogenase [8] is a flavin-dependent oxidation enzyme of the de novo pyrimidine biosynthetic pathway [9]. This enzyme plays a vital role in the growth and life cycle of Plasmodium in the host. So the ligand, which blocks the further development of Plasmodium and attaches to the target protein considered a suitable candidate against malaria [10]. The crystal structure and nomenclature of DD are already defined. In the protein data base, there are more than 150 structures of DD deposited.

Molecular interaction studies are pivotal in validating virtual model predictions, showcasing the advancements in computational medicinal prediction. These digital methodologies offer a detailed pre-evaluation of potential issues, significantly reducing the reliance on animal testing, which is increasingly restricted or banned in many countries. With the advancement in technology, the practice of using chemicals on animals and harming animals is no longer required, and the sale of products tested on animals is also banned in developing countries. There are many alternatives to animal testing methods for the evaluation of chemical toxicity. Computer-aided design can shorten the cycle of drug development [10-11], and molecular docking technologies have been widely used for the aforementioned purpose. An in silico drug-target interactions study becomes easy using a computational docking mechanism. It makes the process easy to find multiple inhibitors [12]. Alternative approaches, such as computer-aided design, are now crucial for assessing chemical toxicity and accelerating drug development. Molecular docking technologies enable in silico studies of drug-target interactions, facilitating the identification of multiple inhibitors. This study focuses on leveraging digital molecular prediction to develop new antimalarial agents, specifically targeting Dihydroorotate dehydrogenase (DD), a well-established target for malaria. Four candidate molecules have been selected for this purpose.

Figure 1: Graphical representation of Ligands

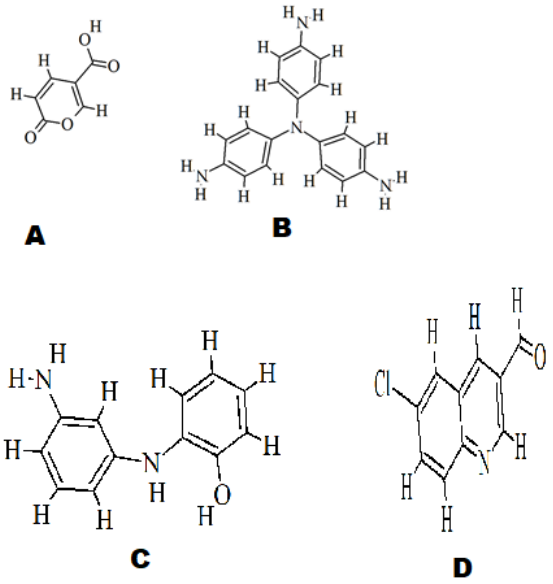

Figure.1. A = Comalic acid, B = 4,4', 4"-triaminotriphenylamine C = 2-(3-aminoanilino)phenol, D = 6-chloroquinoline-3-carbaldehyde.

1.1 Ligand Chemistry

Generally, molecules comprising functional groups have certain properties that are similar to known drugs. Therefore, calculating the molecular property is a significant parameter for oral drugs, and it is shown to be an important feature in drug discovery and development.

The Coumalic acid contains a total of 14 atoms. There are 10 non-H bond(s), four multiple bonds, one rotatable bond, four double bonds, one aromatic ring, four hydrogen atoms, six carbon atoms, and four oxygen atoms, and at the tip there is a hydroxyl group (Fig. **1, A**). Molecular formula is $C_6H_4O_4$. The molecular weight is 140 g/mol. Smiles OC (=O) C1=COC (=O)C=C1, 2-oxo-2H-pyran-5-carboxylic acid is the scientific name.

B= 4,4',4"-triaminotriphenylamine having molecular formula $C_{18}H_{18}N_4$. The molecule contains 40 atoms; there are three aromatic rings with the attachment of a nitrogen atom to each benzene ring. Each aromatic ring contains six hydrogen atoms and six carbon atoms; in this way, there are 18 hydrogen atoms and 18 carbon atoms. Each benzene ring contains a central nitrogen atom and terminal nitrogen atoms. Nine double bonds, no triple bond, are present; there are three rotatable bonds, as shown (Fig. 1, B). The molecular weight is 200.4 g/mol, Smiles Nc1ccc (cc1) N(c1ccc(N)cc1)c1ccc(N)cc1

C = as shown (Fig. 1, C) is a chemical compound where a phenol molecule has a substituent group attached to it. The substituent is a 3-aminoanilino group, which includes a benzene ring with an amino group at the third position. The overall structure consists of a phenol with this specific aromatic amine group attached at the second position of the phenol ring. A benzene ring with a hydroxyl group (-OH) attached at one position. A 3-aminoaniline group is attached to the second position of the phenol ring. This group itself consists of a benzene ring (aniline) with an amino group ($-NH_2$) on the third position of the ring.Smiles Oc1ccccc1Nc1cccc(N)c1

D as shown (Fig. 1, D). 6-Chloroquinoline-3-carbaldehyde is a chemical compound consisting of a quinoline ring system with two specific substituents. A chlorine atom is at the sixth position on the quinoline ring. An aldehyde group (-CHO) at the third position on the quinoline ring. This compound has both a halogen (chlorine) and an aldehyde group on the quinoline structure, which may influence its reactivity and interaction with biological targets. A fused ring system with a benzene ring and a pyridine ring, where the nitrogen is part of the six-membered ring. Chloro Group: A chlorine atom is attached to the sixth position of the quinoline ring. Aldehyde Group: An aldehyde group is attached to the third position of the quinoline ring, Smile Clc1ccc2ncc(cc2c1)C=O

2. Materials &Method

Structures of ligand were drawn with Avogadro 1.2[13]**.** For molecular docking [14] Swiss docking online web system was used. Figure **2** was drawn with the help of Chimera 1.13.1[8]. To minimize the calculation time, Dihydroorotate dehydrogenase chain a (1D3G) is obtained from the RCSB online web database for the protein database. For oral toxicity and surface area, the pro-tox-II server was used 15.

2.1 Target preparation

After obtaining the three-dimensional structure from the Protein Data Bank (www.rcsb.org) in the form of a PDB file. The chain A was used for the docking study.

2.2 Ligand preparation

The three-dimensional structure of concern molecules is drawn with the help of Avogadro. After these UFF force fields were applied to optimize the geometry.

3. Results and Discussion

Table 1. Interaction of amino acid with Dihydroorotate dehydrogenase.

Name of Amino Acid^{+} which interacts with **A**= comalic acid.
Asp Lys Hsd Gly
Position of an Amino acid in Dihydroorotate dehydrogenase chain
106 100 101 107
Name of Amino Acid^{+} which interacts with **B** = 4,4',4"-triaminotriphenylamine.
Ile Thr Ala Gln
Position of an Amino acid in the Dihydroorotate dehydrogenase chain
96 5 91 92
Name of Amino Acid^{+} which interact with **C** =2-(3-aminoanilino)phenol
No interaction found
Position of an Amino acid in Dihydroorotate dehydrogenase chain
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Name of Amino Acid^{+} which interacts with **D** = 6-chloroquinoline-3-carbaldehyde.
Lys Gly
Position of an Amino acid in the Dihydroorotate dehydrogenase chain
100 107

[+] Where name of the amino acid abbreviation?

Fig.**2**. Interaction of ligands with targets.

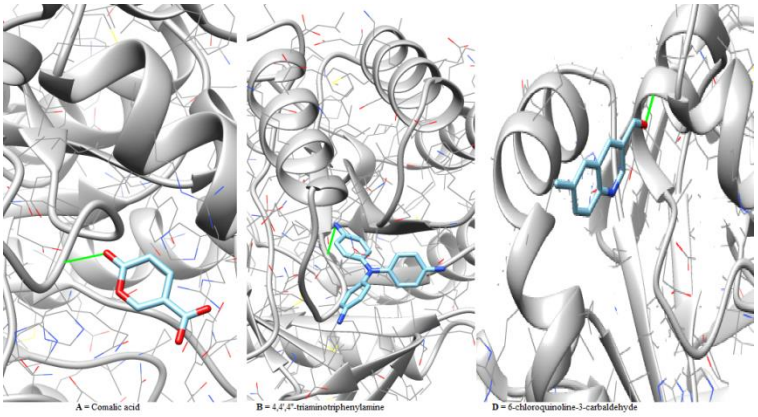
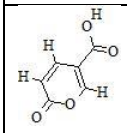
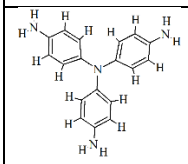
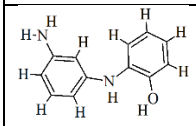
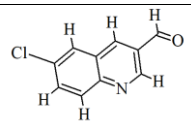


Figure **2**. Show the ligand –Dihydroorotate dehydrogenase interaction. Near the "O" ligand is binding to DD, whereas the ribbon-like structure is human DD.

Table 2. Molecular Structures with LD_{50} Values with Molecular Surface Area.

Molecular structure with name	**Oral toxicity predication LD_{50}**	**Molecular Polar Surface Area**
 comalic acid	200mg/kg	67.51
 4,4',4"-triaminotriphenylamine	244mg/kg	81.3
 2-(3-aminoanilino)phenol	1600mg/kg	58.28
 6-chloroquinoline-3-carbaldehyde	2190mg/kg	29.96

The above-mentioned oxidative enzyme DD is not only a therapeutic aid for malaria but also plays a significant role in rheumatoid arthritis, psoriasis, autoimmune disease, and bacterial and

fungal infections [16]. Based on computational predictions, the three mentioned ligands are likely to serve as promising lead molecules for developing effective drugs against malaria, as shown in the table. 1. Oral toxicity is a non-negligible parameter for any drug design; as mentioned above, all molecules are acceptable. As mentioned earlier, it also provided help with autoimmune disease, so there is also a need to test allergy responses to ensure the safety and efficacy of these potential treatments. Further research is essential to evaluate their interactions with existing therapies and to assess their overall therapeutic profiles in various disease contexts. From this glimpse study, it was clearly observed that a large surface area of the molecule did not play an essential role in DDI selection. The development of feasible inhibitors for the treatment of malaria is still currently under way. There is a need to test DDI results through an assay for further confirmation.

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