Identification of natural inhibitors targeting trehalase of *Anopheles funestus* in the management of malaria: A Biocomputational assessment

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ABSTRACT

Background & objectives: Anopheles funestus is playing an increasingly important role in malaria transmission in sub-Saharan Africa. Trehalase, an enzyme required for trehalose breakdown, is important for mosquito flight and stress adaptation. Hence, its inhibition has emerged as a promising malaria management strategy.

Methods: A collection of 1900 natural compounds from the ZINC database were screened against the 3D modeled structure of *An. funestus* trehalase protein using *in silico* tools. ADMET-AI, a web-based platform, was used to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the selected compounds.

Results: We report 5 natural compounds namely, ZINC00488388, ZINC00488525, ZINC00488566, ZINC00488304, and ZINC00488456 that demonstrated strong binding affinity to the trehalase protein. These compounds interacted with critical residues of the trehalase protein and exhibited good drug-like characteristics.

Interpretation & conclusion: These compounds show promise as trehalase protein inhibitors for malaria management. Nonetheless, additional experimental studies are required to optimize these compounds as potential trehalase inhibitors.

Key words *Anopheles funestus*; malaria; trehalase; natural compounds; drug-likeness

INTRODUCTION

Malaria eradication efforts have included a variety of strategies, including malaria prophylaxis, the use of mosquito nets, development of vaccines, and use of insecticides, among others[1]. The widespread implementation of primary vector control interventions, particularly the use of mosquito nets and indoor residual spraying, has significantly reduced the prevalence of malaria in sub-Saharan Africa[2]. Notably, 68% of the 1.5 billion malaria cases avoided between 2000 and 2015 can be attributed solely to the use of nets. Nonetheless, recent assessments show a discernible slowing in the rates of decline[2].

The continued reliance on insecticide-based malaria control tools has been linked to changes in *Anopheles* mosquito feeding and resting behaviors, as well as shifts in species composition[3–6]. *An. funestus* has historically had a significant impact on malaria transmission[7–9], owing to its anthropophilic and endophilic tendencies[10], pronounced pyrethroid resistance[11–13], and increased daily survival probabilities[14–15]. As resistance to established treatments grows, the need for novel compounds with distinct modes of action against mosquito vectors becomes more apparent. In search for mosquito vector control, various target sites such as the GABA (γ-aminobutyric acid) receptor, acetylcholinesterase, and mitochondrial electron transport have been investigated[16].

Trehalose, a non-reducing disaccharide, has a broad biological distribution. In insects, it is the primary hemolymph sugar, playing an important role as an immediate energy source and in mitigating abiotic stresses. Trehalase directs the enzymatic hydrolysis of trehalose. Trehalase, an enzyme of growing interest in insect physiology, governs energy metabolism and facilitates glucose generation via trehalose catabolism. Tre-1 and Tre-2, the two distinct isoforms of insect trehalase, play critical roles in energy supply, growth, metamorphosis, stress recovery, chitin synthesis, and insect flight facilitation[17], making them a potential target for malaria vector control.

Computer-aided drug design (CADD) is critical for identifying potential targets and compounds for novel drug development. It also has a significant impact on determining biological efficacy and improving drug performance[18]. One significant problem in drug discovery is accounting target flexibility. Most molecular docking methods allow for substantial ligand flexibility while limiting protein

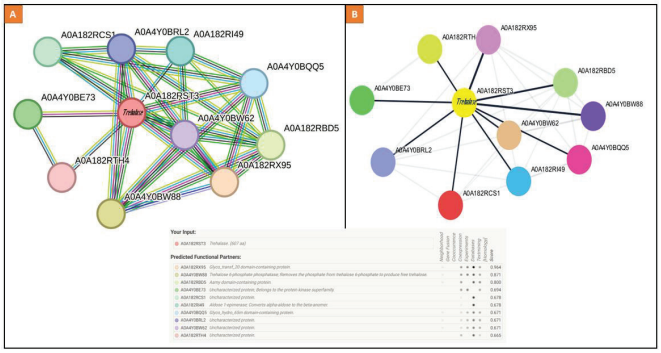

Fig. 1: A. Protein-protein interaction of trehalase with its 10 predicted functional partners, B. network determined by STRING[20], and network analyzed by Cytoscape[21].

flexibility of residues in or near the active site. Fully flexible proteins are computationally difficult, which requires more space and time[19]. However, the three-dimensional (3D) structure of the trehalase enzyme, which is critical for drug discovery, is currently unavailable in the Protein Data Bank (www.pdb.org). This enzyme has not been thoroughly investigated, as protein-protein interaction studies reveal that its interacting partners are largely uncharacterized (Fig. 1).

Consequently, the goal of this research is to create a 3D model of the trehalase enzyme, validate it, identify active sites, and screen for natural compounds that could act as trehalase inhibitors. These steps are carried out using *in silico* tools, to develop future insecticidal measures against malaria transmitting mosquitos.

MATERIAL & METHODS

3D protein structure prediction and preparation

Because the target protein trehalase did not have a 3D structure determined experimentally in the Protein Data Bank, a 3D was generated utilizing the Swiss-Model tool. The generated 3D structure was built using the trehalase amino acid sequence, which was obtained from UniProtKB (Accession number: A0A182RST3). To ensure the modeled structure's reliability, it was validated using PDBSum web tool[22], which provided data on its Ramachandran plot and secondary structure analysis. Subsequently, hydrogen atoms were added and energy minimization was done to prepare the protein model. First, 100 steps of steepest descent minimization were completed, and then, a 10-step conjugate gradient minimization was conducted using Chimera software suite. With this all-encompassing method, the protein model was refined to a degree suitable for further virtual screening processes.

Library preparation

A natural compound library has been downloaded in the SDF file format from the ZINC database. It contains 1900 unique molecules with molecular weights ranging from 200 to 500 Da. These compounds were subjected to an energy minimization using the Universal Force Field (UFF). The compounds were then minimized and converted into .pdbqt format, which was required to make them compatible for further docking analysis. The PyRx0.8 tool was used to carry out these preparation steps[23].

Virtual screening

Using virtual screening methodologies on chemical databases is a quick and precise method for discovering promising new leads with potential for further development[24]. PyRx is a computer tool for virtual screening that aids drug development by screening compound libraries to possible therapeutic targets. PyRx includes a user-friendly docking wizard, making it an indispensable tool for CADD[25]. In this study, PyRx0.8 tool was used to conduct virtual screenings of the curated compound library against the trehalase.

ADMET profiling using ADMET-AI

The pharmacokinetics and toxicity profiles of new therapeutics must be assessed during development. We used ADMET-AI, a web-based tool that employs machine learning to predict ADMET properties efficiently and precisely. This tool enabled us to determine the performance of our top five compounds in biological systems[26].

Ethical statement: Not Applicable

RESULTS

In this study, the target protein, trehalase, was first structurally modeled with Swiss Model tool, using the AlphaFold DB model (A0A4Y0B4A1.1.A Trehalase) as a template. The PDBSum web tool was used to validate the modeled structure by generating a Ramachandran plot and conducting secondary structure analysis. The Ramachandran plot revealed that the most favored regions contained 458 residues, accounting for 93.5% of the structure. This distribution demonstrates that the modeled protein structure is of high quality (Fig. 2).

The validated model was then prepared by adding hydrogen atoms, followed by energy minimization using 100 steps of the steepest descent algorithm and 100 steps of the conjugate gradient algorithm in Chimera[27]. The prepared 3D structure was then analyzed in the Discovery Studio Visualizer to determine the active pockets, yielding 17 active pockets, 5 of which were relatively larger in

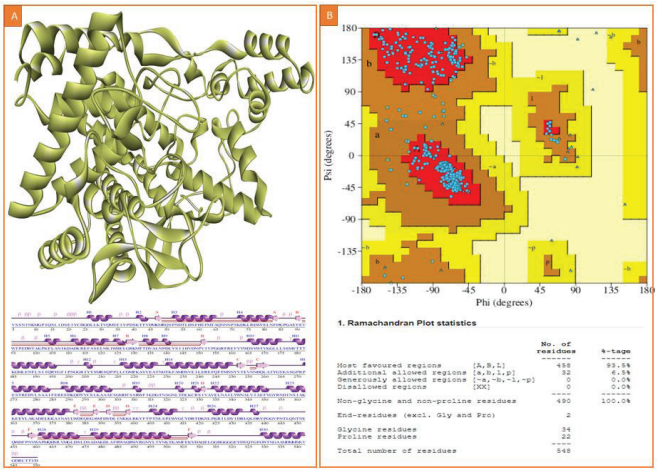

Fig. 2: A. Modelled 3D and 2D structure of trehalase, and B. Ramachandran plot of the model.

size due to their coverage of many residues. Pocket 1 was chosen for further docking purposes (Fig. 3).

To find a possible natural inhibitor of trehalase, a library of 1900 natural compounds obtained from the ZINC database was subjected to a screening process aimed at pocket 1 of the modelled structure. The XYZ coordinates, -6.900000, -3.922000, and 8.721000, were determined by Discovery studio. Validamycin, a well-known trehalase inhibitor, was used as a positive control in this study because of its specific and potent inhibition of the enzyme. Table 1 shows the top ten compounds that demonstrated better binding efficacy and interaction (2D and 3D) with the active site residues of trehalase.

Following a visual inspection of the top ten compounds, a detailed 2D and 3D interaction analysis of the best five compounds was performed to discuss the interacting residues and their binding details (Fig. 4).

Table 1. Top 10 potential inhibitors of trehalase and their binding affinity

Natural compounds	Binding affinity (kcal/mol)
ZINC00488388	-9.1
ZINC00488525	-8.8
ZINC00488566	-8.7
ZINC00488304	-8.5
ZINC00488456	-8.5
ZINC00488339	-7.9
ZINC00488340	-7.9
ZINC00488532	-7.4
ZINC00488282	-7.4
ZINC00487982	-7.3
Validamycin (control)	-7.2

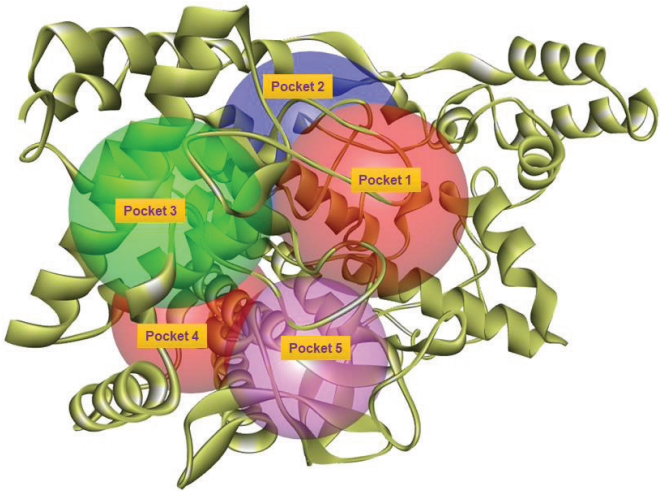

Fig. 3: Top best 5 active pockets of the modelled protein.

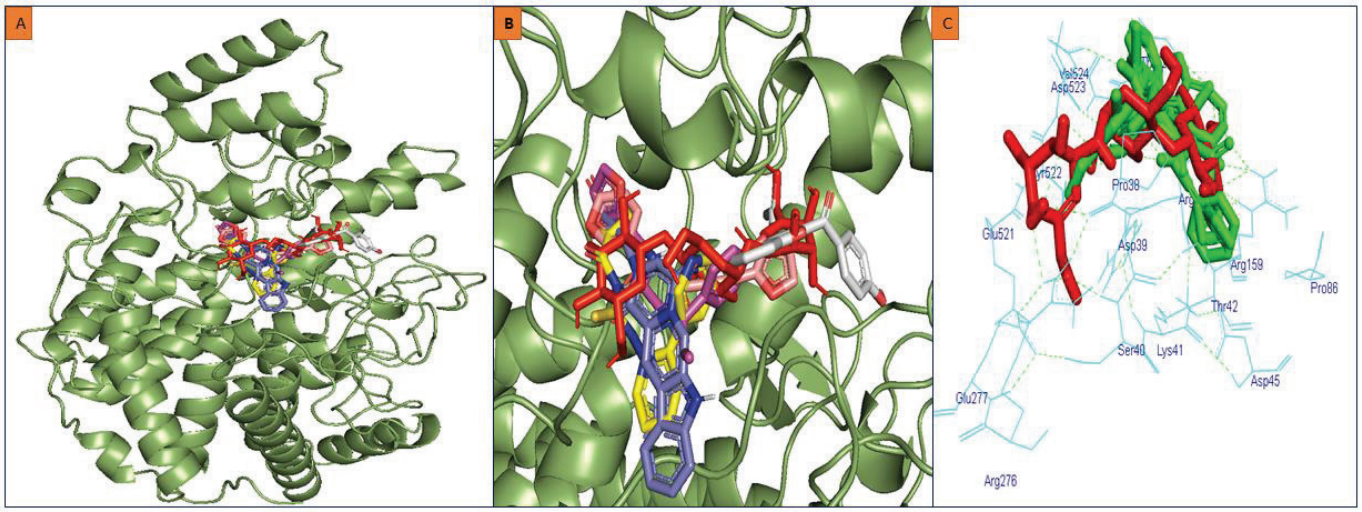

Fig. 4: Visualization of interaction of the top 5 compound and control (red) compound in the active pocket of trehalase. A. Trehalase structure and binding poses of all the selected compounds, B. close-up view of interaction in the active pocket, and C. active site residues involved in binding with control and selected compounds.

ZINC00488566 was found to interact with Asp523, Pro38, Glu521, Arg157, Tyr522, Pro86, Thr42, Asp45, Arg159, Asp39, Val524, and Gln525 residues of trehalase protein (Fig. 5A); while ZINC00488525 was found to interact with Arg157, Asp45, Thr42, Lys41, Arg159, Val524, Asp523, Pro38, Tyr522, Gly156, Gln525, and Asp39 residues of trehalase protein (Fig. 5B). ZINC00488456 interacted with Arg157, Lys41, Thr42, Asp45, Pro86, Arg159, Asp39, Asp523, Tyr522, Glu521, Pro38, and Gln525 residues of trehalase protein (Fig. 5C). In addition, ZINC00488388 was found to interact with Asp523, Pro38, Gly520, Glu277, Glu521, Tyr522, Arg276, Arg157, Ser274, Asp39, and Ser40 residues of trehalase protein (Fig. 5D); while Arg157, Asp45, Thr42, Asp39, Pro38, Gln525, Asp523, Phe505, Thr526, Val524, Lys41, Arg159, and Gly156 residues of trehalase protein were interacted with ZINC00488304 (Fig. 5E). Furthermore, the control compound Validamycin was found to interact with Thr42, Tyr522, Pro38, Glu521, Arg276, Glu277, Ser274, Ser40, Lys41, Asp523, Gln525, Gly156, Arg157, Arg159, and Asp39 residues of trehalase protein (Fig. 5F).

All five compounds had been evaluated for pharmacokinetic properties using ADMET-AI, and the results demonstrated that each compound fell within acceptable ranges for essential ADMET variables. The radial plot in Figure 6 shows that the compounds have profiles that are consistent with favorable Blood-Brain Barrier permeability, hERG safety, bioavailability, solubility, and non-toxicity, implying that they could be safe and effective drugs. Addtional File 1 contains detailed ADMET predictions for each of the five compounds. This includes a variety of ADMET parameters as well as physicochemical property parameters.

DISCUSSION

Anopheles funestus Giles is a significant malaria vector in sub-Saharan Africa. This species, in particular, plays an important role as a bridge vector for malaria transmission during the dry season because its larvae thrive in permanent swampy environments that remain conducive to breeding even as habitats suitable for *Anopheles gambiae* Giles diminish[28]. This study involves screening a library of 1900 natural compounds to identify potential natural inhibitors of the insect trehalase protein.

Validamycin, known for its potent inhibition of several trehalases, has emerged as a promising therapeutic agent for insect and fungal diseases[29]. In molecular interactions, Validamycin was observed to engage with specific amino acid residues including Thr42, Tyr522, Pro38, Glu521, Arg276, Glu277, Ser274, Ser40, Lys41, Asp523, Gln525, Gly156, Arg157, Arg159, and Asp39 with the

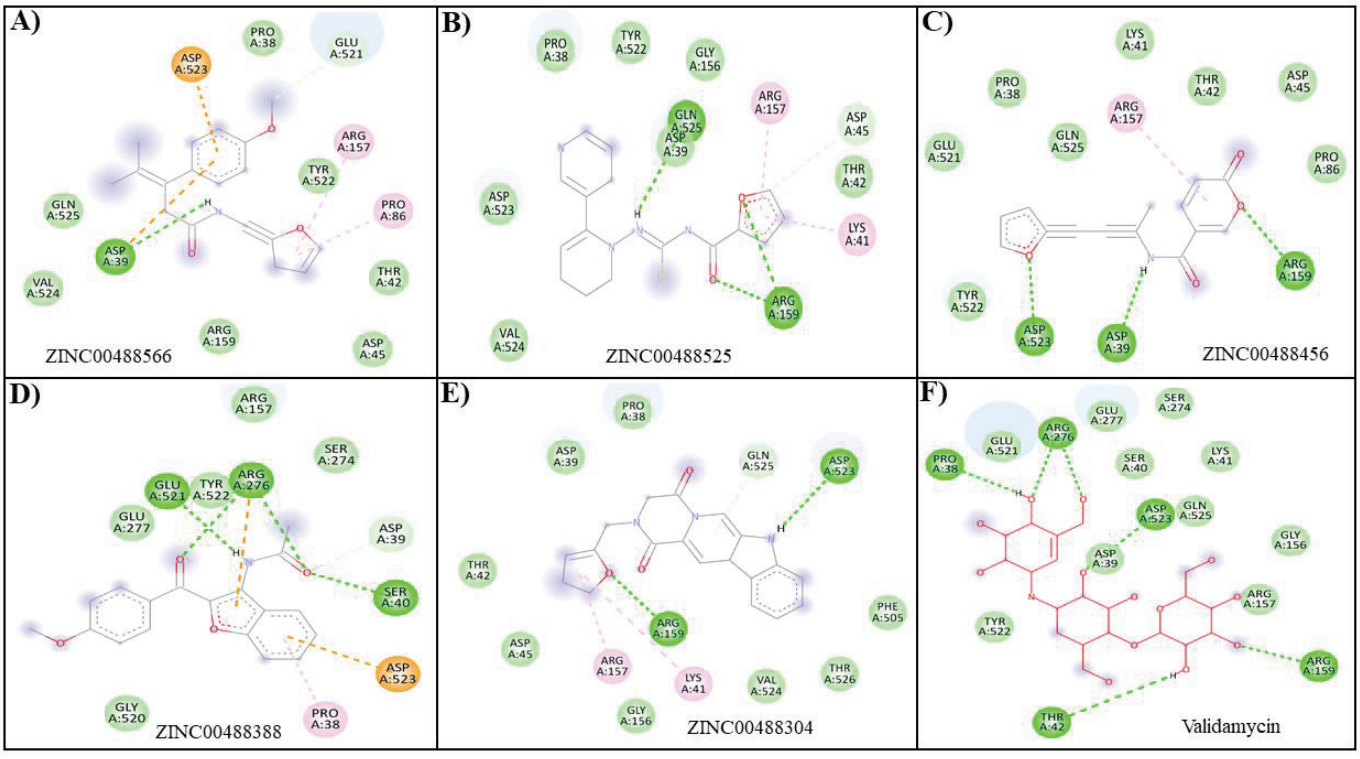

Fig. 5: A. Interacting residues of trehalase protein with compounds ZINC00488566, B. ZINC00488525, C. ZINC00488456, D. ZINC00488388, E. ZINC00488304, F. Validamycin with trehalase protein.

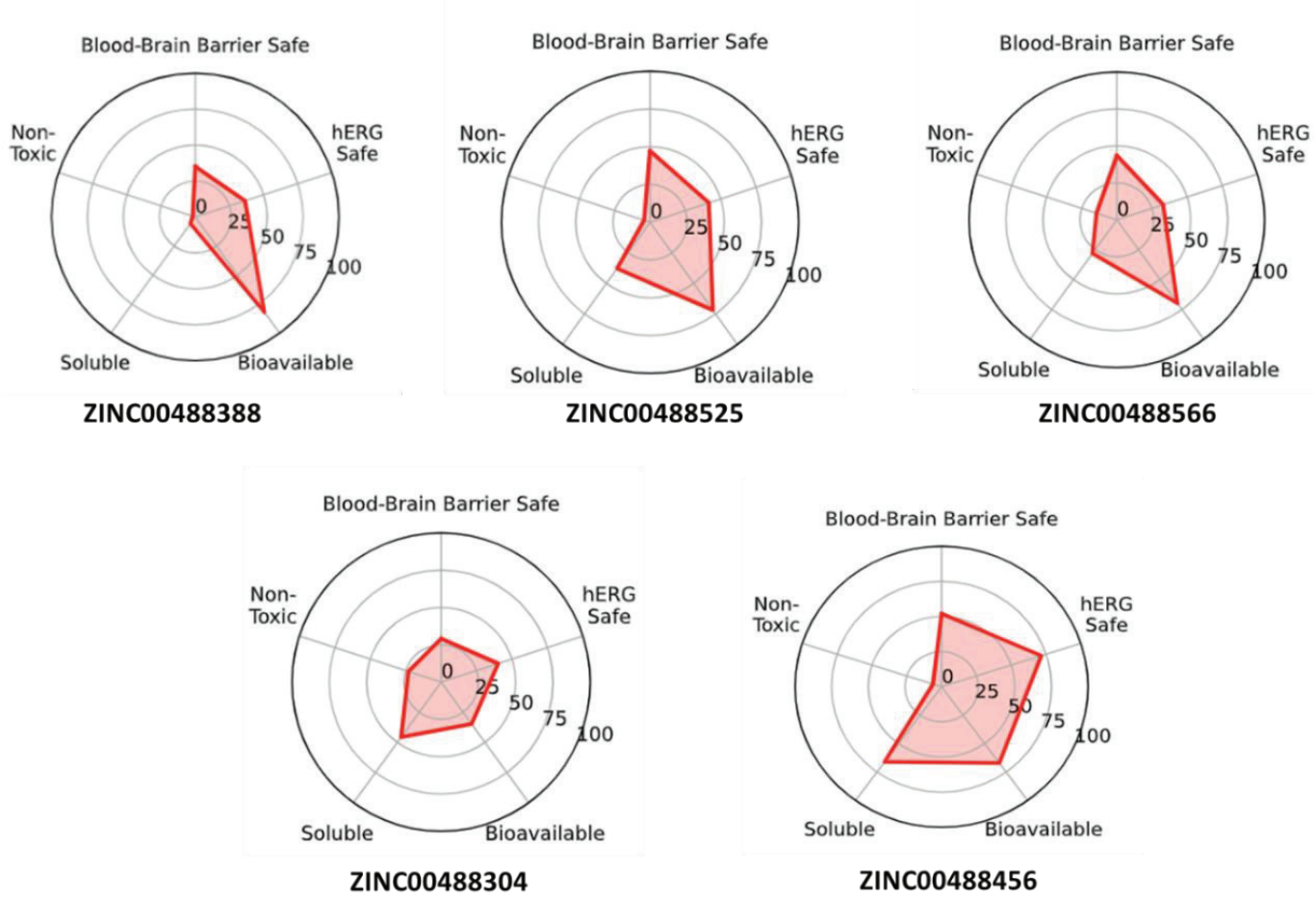

Fig. 6: Radial plot of selected five compounds showing five key ADMET properties.

trehalase protein. Notably, the identified compounds (ZINC00488388, ZINC00488525, ZINC00488566, ZINC00488304, and ZINC00488456) have binding affinities for these residues, implying that they interact within the same binding pocket of the trehalase protein as Validamycin.

Hydrogen bonding is critical for stabilizing the ligand-protein interaction complex[30–31]. Notably, the identified compounds (ZINC00488388, ZINC00488525, ZINC00488566, ZINC00488304, and ZINC00488456) form hydrogen bonds with specific trehalase protein residues. ZINC00488566 was H-bonded with trehalase's Asp39 residue, whereas Gln525 and Arg159 residues interact with ZINC00488525 via H-bonding. ZINC00488456 forms H-bonds with the Asp523, Asp39, and Arg159 residues of the trehalase protein. In addition, ZINC00488388 forms H-bonds with trehalase protein residues Glu521, Arg276, and Ser40. Furthermore, ZINC00488304 was H-bonded with trehalase protein residues Arg159 and Asp523.

During the docking analysis, binding affinity is used to quantify the interaction strength of the ligand-protein complex, with a higher negative value indicating a stronger interaction and a lower ligand dissociation rate[32–35]. Notably, the identified compounds (ZINC00488388, ZINC00488525, ZINC00488566, ZINC00488304, and ZINC00488456) had higher negative binding affinity values for the trehalase protein than the positive control, Validamycin. This suggests that these compounds have strong interactions with the trehalase protein.

Malaria, an infectious disease prevalent in tropical regions where a significant portion of the population relies on plant-derived remedies for treatment, was traditionally treated with quinine until the 1930s[36]. Subsequently, synthetic drugs largely replaced quinine[37]. The initial optimism for global malaria eradication in the mid-1950s faded by the mid-1960s due to emerging drug resistance issues[38], particularly vector mosquito resistance to potent insecticides such as DDT and *Plasmodium falciparum* chloroquine-resistant strains.

The persistence of chemotherapeutic demand highlights the critical need for novel antimalarial compounds, given the escalating resistance problem. Exploration of traditional medicinal plants represents a promising av-

enue for discovering such compounds, drawing on centuries of accumulated knowledge from multiple countries[37]. The compounds identified in this study demonstrate potent binding affinity with the trehalase protein, indicating their potential utility in malaria treatment.

CONCLUSION

In this study, a set of natural compounds was screened against the insect trehalase protein using computational methods. The identified hits, ZINC00488388, ZINC00488525, ZINC00488566, ZINC00488304, and ZINC00488456, showed strong binding affinity to the trehalase protein, interactions with critical trehalase residues, and favorable drug-like properties. Further experimental studies are needed to optimized these compounds as potential trehalase inhibitors.

Conflict of interest: None

ACKNOWLEDGEMENTS

The authors are thankful to the Deanship of Graduate Studies and Scientific Research at the University of Bisha, Bisha, Saudi Arabia for supporting this work through the Fast-Track Research Support Program.

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Received: 2 May 2024 *Accepted in revised form:* 3 July 2024