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Possible inhibitory effects of hoslundal, hoslundin and hoslunddiol on human lactate dehydrogenases: a bioinformatics proof

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Abstract: The development of anti-malarial drugs is of great importance due to the detrimental effects of this disease all around the world. In recent years, bioinformatics tools provide considerable contributions to develop new small molecules which have important bioactivities against many bio-targets. However, missing points in the methodologies or aims of the studies in which in silico tools are used may reveal problematic cases. Hoslundal, hoslundin, and hoslunddiol were proposed by Shadrack et al. (2016) to inhibit *Plasmodium falciparum* lactate dehydrogenase (*Pf*-LDH) to fight malaria. But these molecules may have potential to inhibit mammalian LDHs. To investigate whether these molecules have inhibitions on mammalian LDHs or not, we studied a comprehensive and comparative molecular docking studies as described in the present paper. According to the results, the vina scores of hoslundal without NADH for *Pf*-LDH, Human Muscle-LDH (HM-LDH), Human Heart-LDH (HH-LDH) were found as -7.5, -7.6 and -8.2 kJ/mol, respectively. Moreover, multiple sequence alignment analysis reveals high similarities among sequences. In the light of molecular studies, hoslundal was found to be connected to *Pf*-LDH, HM-LDH, HH-LDH (31, 26, 34), (2, -7, 154), (11, 41, 54), respectively. In conclusion, novel small molecules which are developed via in silico tools could show excellent activities against bio-targets of the pathogenic microorganisms. However, it should not be forgotten that active site of the enzymes may have been conserved, therefore, after a possible proposal of small molecule, its molecular docking and also Swiss-ADME studies for human should be necessarily carried out.

Keywords: anti-malarial drugs, docking, human lactate dehydrogenase, *Plasmodium falciparum*, malaria.

1. Introduction

In anaerobic conditions, the energy as ATP is obtained through the glycolysis pathway in which glucose is converted into pyruvate via ten steps reaction series and this process produces net two moles of ATP. Lactate dehydrogenase (LDH) is a tetrameric enzyme that plays a special role for the continuation of the glycolysis process (Iacovino et al., 2022). It produces nicotinamide adenine dinucleotide (NAD^+) to continue glycolysis, and LDH is classified as the eleventh reaction of glycolysis. LDH produces lactate from pyruvate to provide energy in anaerobic conditions. The result of this process, NAD^+ is produced for use in the sixth reaction of glycolysis in which glyceraldehyde-3-phosphate is transformed into 1,3-bisphosphoglycerate. For mammalians, there are two different types of LDH which can be defined as M and H types. Known isoenzymes of LDH based on their subunits are reported as M_4, M_3H, M_2H_2, MH_3 and H_4 tetrameric structures. H- and M-types are heart and muscle subunits, respectively. M-type is also found in liver (Voet and Voet, 1995). Shadrack et al. (2016) highlighted the danger of the pathogenic microorganism *Plasmodium falciparum* which is the main vector for malaria. It is still a big problem in many countries. According to WHO, estimated cases in 2020 are given 241 million with 627,000 deaths. Most of the cases are reported from Africa (WHO, 2022). Shadrack et al. (2016) proposed hoslundal, hoslundin, and hoslunddiol, the compounds known as secondary metabolites of the plant *Hoslundia opposita*, to inhibit LDH in *P. falciparum* (*Pf*-LDH) for eradication of the disease. Hoslundal, hoslundin, and hoslunddiol (Figure 1) are docked with *Pf*-LDH as a receptor. Since LDH is a haloenzyme, the docking was realized with and without NADH by Shadrack et al. (2016) to estimate the best binding energies, H-bond forming residues and bond lengths. However, there is no information in the paper of Shadrack et al. (2016) related to environmental application of these molecules. One of the application methods of these molecules could be spraying via compressors. If this method is applied, these molecules will be widely spread and they may contaminate water resources. Therefore, there is a very big risk that all animals in the region may negatively be affected by these molecules due to LDH inhibition.

In this investigation, the following research questions will be answered:

1) What are the similarities among *Pf*-LDH, HM-LDH and HH-LDH?
2) What are the docking scores of these compounds against these human LDH forms?
3) If these molecules contaminate water resources, how the animals (mammalians) could be affected?

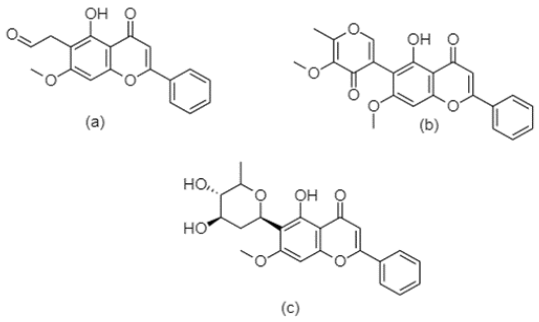

Figure 1. Chemical structures of (a) hoslundal, (b) hoslundin and (c) hoslunddiol

2. Materials and Method

The FASTA sequences of enzymes of *Plasmodium falciparum* lactate dehydrogenase (*Pf*-LDH), Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH) and Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) were retrieved via uniprot.org and the active site information was also accessed (Uniprot, 2021). Each of their FASTA formats was selected for multiple sequence analysis. The aim of this comparison is to determine similarities and conserved regions of the enzymes studied. Multiple sequence alignments of the FASTA sequences were carried out by Clustal Omega (Sievers et al., 2018). The default settings were used in the analysis. This database is widely used in many bioinformatics studies, such as determining genetic or evolutionary relationships, detecting structural similarities, and performing evolutionary analyses. Ligands structures of the compounds were retrieved from PubChem and their sdf formats were downloaded (Kim et al., 2021). Swiss-Similarity is the in silico tool that is used for determination of similarity between small molecules according to their chemical structures (Bragina et al., 2022). Cannonical structure of hoslundal, hoslundin and hoslunddiol were obtained from PubChem (Kim et al., 2021). Molecular docking analysis was used to estimate the vina scores to compare the affinity and also determination of the contact residues of the enzymes by using CB-Dock (Liu et al., 2020). Vina scores were compared with and without NADH. CB-Dock and CB-Dock2 are molecular docking tools used in the field of structural bioinformatics. CB-Dock uses a number of computational algorithms to identify chemical bonds and perform energy minimization (Liu et al., 2020). YASARA was used to model the visualization of each enzyme and ligand. YASARA provides an advanced set of tools for analyzing the structure and interactions of proteins, nucleic acids, and other biomolecules. This tool is widely used in many research fields such as molecular modeling, protein design, drug discovery, molecular dynamics simulations, and molecular interaction analysis (Land and Humble, 2018). Protparam is a bioinformatics tool used for protein sequence analysis. This program is used to predict the basic physical and chemical properties of a protein. It calculates a number of important parameters such as the amino acid composition of the protein, its isoelectric point, molecular weight, theoretical pI value, hydrophobicity and hydrophilia scores, alpha helix and beta sheet formation potential (Gasteiger, 2005). Physicochemical properties such as molar reactivity, rotatable bonds, and the pharmacokinetics of the molecules were estimated. Factors such as cytochromes P450 (CYP), Log Kp (skin permeation), GI absorption, and BBB permeation were evaluated. The BOILED EGG model was utilized to represent blood-brain barrier permeation (BBB permeation), and gastrointestinal absorption (GI absorption) was determined using the Swiss-ADME approach (Daina et al., 2017). Flowchart of the methodology was shown in Figure 2.

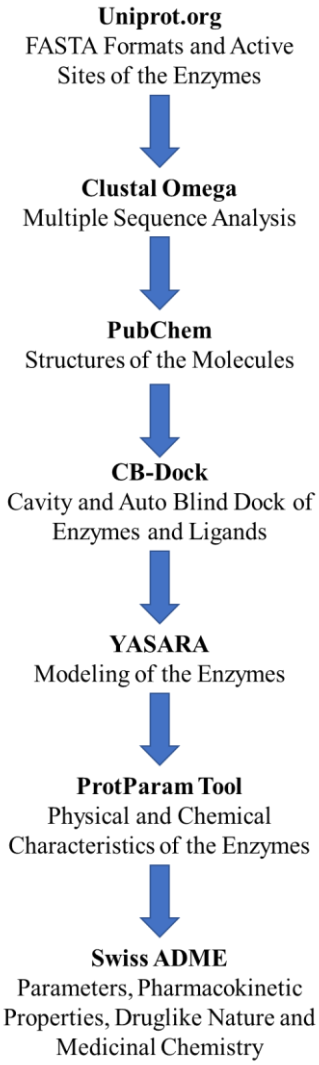

Figure 2. Flowchart of the methodology

3. Results

In this paper, HM-LDH, HM-LDH and *Pf*-LDH were compared by using bioinformatics tools. Clustal Omega was used to determine the similarities between *Pf*-LDH, HM-LDH and HH-LDH (Sievers and Higgins, 2018). According to Figure 3, amino acid lengths of *Pf*-LDH (Uniprot code: A0A024W2N3), HM-LDH (UniProt ID: P00338) and HH-LDH (UniProt ID: P07195) were found as 316, 332 and 334, respectively. As it can be seen from Figure 3, there is a high similarity between HM-LDH and HH-LDH. However, the similarity score was low when *Pf*-LDH was compared with human LDHs.

Swiss-Similarity was used to compare the similarity of hoslundal, holsundin, and hoslunddiol with other small molecules (Bragina et al., 2022). According to Table 1, the three most similar molecules for each hoslundal, hoslundin, and hoslinddiol are identified. Hoslundal shows high similarity with hipidulin (DB14008) with a score of 0.950. The similarity score of hoslundin is very low compared to hetrombogap. Finally, the similarity score of hoslunddiol is determined as 0.832 against puerarin.

Figure 3. Multiple sequence analysis of *Plasmodium falciparum* lactate dehydrogenase (UniProt ID: A0A024W2N3), Human Muscle L-Lactate Dehydrogenase M Chain (UniProt ID: P00338), and Human Heart L-Lactate Dehydrogenase H Chain (UniProt ID: P07195).

Table 1. Swiss-Similarity results of hoslundal, hoslundin and hoslunddiol.

Molecule	Score	Similar Molecule	PubChem ID
Hoslundal	0.950	Hispidulin	DB14008
	0.947	Baicalein	DB16101
	0.925	5,7,2’-trihydoxy-6,8-dimethoxyflavone	DB13983
Hoslundin	0.048	Hetrombopag	DB16184
	0.023	Xanthohumol	DB15359
	0.020	Methyl n-[[2’,4’-difluoro-4-hydroxy-5-iodobiphenyl-3-yl)carbonyl]-beta-alaninate	DB07962
Hoslunddiol	0.832	Puerarin	DB12290
	0.672	Hispidulin	DB14008
	0.647	5,7,2’-trihydroxy-6,8-dimethoxyflavone	DB13983

By using CB-Dock, cavity detection was studied for each enzyme by using hoslundal (Compound CID: 15726097) (Liu et al., 2020). In Figure 4, view of the cavities of each enzyme is shown.

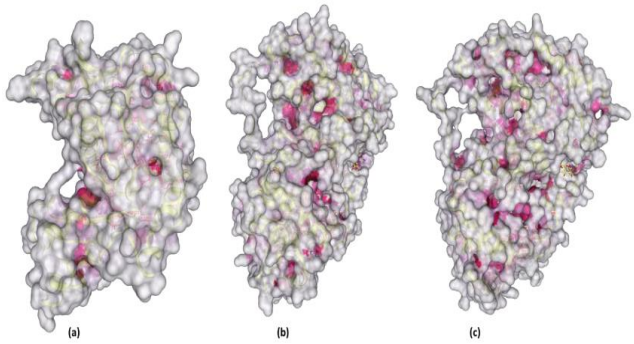

Figure 4. View of the cavities from (a) *Plasmodium falciparum* Lactate Dehydrogenase (UniProt ID: A0A024W2N3), (b) Human Muscle L-Lactate Dehydrogenase M Chain (UniProt ID: P00338), (c) Human Heart L-Lactate Dehydrogenase H Chain (UniProt ID: P07195).

According to Table 2, for each three enzymes, there were five cavities. The highest cavity volume (Å^3) was found for HM-LDH in C1 as 2532 Å^3 and its cavity size coordinates were found as 23, 23, 14. Also, the highest volume values were found at C1 for HH-LDH and *Pf*-LDH as 2003 Å^3 and 809 Å^3, respectively.

The cavities were chosen according to their active sites in Table 3. For this docking, all heteroatoms were removed. Amino acids and their positions were found from uniprot.org (Uniprot, 2021). According to Voet and Voet (1995), Michael Rossman has determined the X-Ray structure of the LDH and NADH complex. As a result of his study, ARG109, ARG171, and HIS195 were determined as active sites of the human LDH. The contact residues were compared and both HM-LDH and HH-LDH had these amino acids at C1. The contact residues of HM-LDH are ARG98, ARG168 and HIS192. HH-LDH contact residues are also found as ARG106, ARG169, and HIS193. When the Table 3 is examined, it is clear that hoslundal is not around the active sites of *Pf*-LDH. HH-LDH has the highest vina score as -8.2 kJ/mol.

The auto blind docking results of *Pf*-LDH, HM-LDH and HH-LDH with coenzyme NADH are shown in Table 4. The ligand was selected as hoslundal. When the contact residues of HH-LDH and HM-LDH are examined, it is clear that the ligand is around the active sites of the enzymes. The contact residues of HM-LDH were found as GLY28, ALA29, VAL30, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ILE241, TYR246, THR247, ILE251. The contact residues in HH-LDH were found to be GLY29, GLN30, VAL31, THR95, GLY97, ARG99, GLN100, ARG106, VAL136, ASN138, SER161, LEU165, ARG169, HIS193, ALA238, ILE242, GLY246, TYR247, THR248, ILE252. *Pf*-LDH contact residues were found as VAL26, GLY27, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, ILE119, ILE123. The highest cavity volume was found as 2532 Å^3 on HM-LDH and the highest vina score was found as -8.2 kJ/mol for HH-LDH.

Table 2. Cavity detection results in the Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) with coenzyme as NADH and ligand as hoslundal by using CB-Dock.

Enzymes	CurPocket ID	Cavity Volume (Å^3)	Center (x, y, z)	Cavity Size (x, y, z)
HM-LDH	C1	2532	2, -7, 154	23, 23, 14
	C2	711	11, -36, 141	16, 10, 17
	C3	393	13, -17, 137	10, 10, 13
	C4	145	17, -2, 151	5, 12, 6
	C5	131	-8, -20, 136	5, 9, 6
HH-LDH	C1	2003	11, 41, 57	22, 18, 11
	C2	861	10, 43, 34	21, 15, 8
	C3	319	24, 14, 51	12, 11, 13
	C4	292	0, 27, 56	9, 10, 12
	C5	259	37, 20, 48	7, 14, 14
Pf-LDH	C1	809	31, 26, 34	15, 13, 22
	C2	738	24, 7, 44	13, 14, 13
	C3	381	8, 17, 43	9, 13, 10
	C4	159	28, 34, 44	7, 9, 6
	C5	154	16, 28, 41	8, 8, 10

Table 3. The molecular docking results of hoslundal for Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) without NADH.

Enzymes	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-7.6	2532	2, -7, 154	28, 28, 21	**Chain A:** GLY28, ALA29, VAL30, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ILE241, TYR246, THR247, ILE251
HH-LDH	C1	-8.2	2003	11, 41, 54	27, 21, 21	**Chain A:** GLY29, GLN30, VAL31, THR95, GLY97, ARG99, GLN100, ARG106, VAL136, ASN138, SER161, LEU165, ARG169, HIS193, ALA238, ILE242, GLY246, TYR247, THR248, ILE252
***Pf*-LDH**	C1	-7.5	809	31, 26, 34	21, 21, 17	**Chain A:** VAL26, GLY27, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, ILE119, ILE123

Table 4. The molecular docking results of hoslundal for Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) with NADH.

Enzymes	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-7.6	2532	2, -7, 154	28, 28, 21	**Chain A:** GLY28, ALA29, VAL30, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ILE241, TYR246, THR247, ILE251
HH-LDH	C1	-8.2	2003	11, 41, 57	27, 21, 21	**Chain A:** GLY29, GLN30, VAL31, THR95, GLY97, ARG99, GLN100, ARG106, VAL136, ASN138, SER161, LEU165, ARG169, HIS193, ALA238, ILE242, GLY246, TYR247, THR248, ILE252
***Pf*-LDH**	C1	-7.5	809	31, 26, 34	21, 21, 27	**Chain A:** VAL26, GLY27, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, ILE119, ILE123

In Table 5, hoslundin was selected as the ligand and it was docked with each enzyme (without NADH). All heteroatoms were released for docking. CurPocket ID of each enzyme was selected as C1. HM-LDH contact residues were found as GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, LYS56, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, ASN112, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, SER248, ILE251. Active sites were found to be ARG105, ARG168 and HIS192. HH-LDH contact residues such as GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, ARG106, LEU109, ASN113, VAL136, SER137, ASN138, LEU165, HIS193, ALA238, ILE242, TYR247, THR248, ILE252. Active sites were determined to be ARG106 and HIS193. *Pf*-LDH contact residues found as VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, PHE100, THR101, LYS102, ILE119, ILE123. The highest cavity volume was found as 2532 on HM-LDH and the highest vina score was found as 9.2 kJ/mol on HH-LDH.

In Table 6, hoslundin was docked to HM-LDH, HH-LDH and *Pf*-LDH with coenzyme NADH. Contact residues of HM-LDH were found as GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, LYS56, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, ASN112, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, ILE251. Contact residues of HH-LDH were found to be GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, ARG106, LEU109, ASN113, VAL136, SER137, ASN138, LEU165, HIS193, ALA238, ILE242, TYR247, THR248, ILE252. *Pf*-LDH contact residues were VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, PHE100, THR101, LYS102, ILE119, ILE123. Active sites were found to be same as without NADH.

Table 5. The molecular docking results of hoslundin for Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) without coenzyme NADH.

Enzymes	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-9.0	2532	2, -7, 154	24, 24, 24	**Chain A:** GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, LYS56, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, ASN112, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, SER248, ILE251
HH-LDH	C1	-9.2	2003	11, 41, 57	24, 24, 24	**Chain A:** GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, ARG106, LEU109, ASN113, VAL136, SER137, ASN138, LEU165, HIS193, ALA238, ILE242, TYR247, THR248, ILE252
***Pf*-LDH**	C1	-8.8	809	31, 26, 34	24, 24, 24	**Chain A:** VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, PHE100, THR101, LYS102, ILE119, ILE123

Table 6. The molecular docking results of hoslundin for Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) with coenzyme NADH.

Enzymes	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-8.9	2532	2, -7, 154	24, 24, 24	**Chain A:** GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, LYS56, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, ASN112, VAL135, SER136, ASN137, LEU164, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, ILE251
HH-LDH	C1	-9.2	2003	11, 41, 57	24, 24, 24	**Chain A:** GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, ARG106, LEU109, ASN113, VAL136, SER137, ASN138, LEU165, HIS193, ALA238, ILE242, TYR247, THR248, ILE252
***Pf*-LDH**	C1	-8.8	809	31, 26, 34	24, 24, 24	**Chain A:** VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, PHE100, THR101, LYS102, ILE119, ILE123

Table 7. The docking results of hoslunddiol for Human L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) without NADH.

Enzyme	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-8.9	2532	2, -7, 154	23, 23, 23	**Chain A:** GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, VAL52, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, VAL135, SER136, ASN137, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, ILE251
HH-LDH	C1	-9.0	2003	11, 41, 57	23, 23, 23	**Chain A:** GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, VAL53, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, LEU109, VAL136, SER137, ASN138, LEU165, ARG169, HIS193, ALA238, ILE242, TYR247, THR248, ASN249, ILE252
***Pf*-LDH**	C1	-8.2	809	31, 26, 34	23, 23, 23	**Chain A:** VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, LYS102, ILE119, ILE123

In Table 7, hoslunddiol was selected as ligand and *Pf*-LDH, HM-LDH and HH-LDH were selected as LDHs without heteroatoms and NADH. The contact residues of HM-LDH were found as GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, VAL52, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, VAL135, SER136, ASN137, ARG168, HIS192, ALA237, ILE241, TYR246, THR247, ILE251. Active sites were found ARG105, ARG168 and HIS192. HH-LDH contact residues were found as GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, VAL53, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, LEU109, VAL136, SER137, ASN138, LEU165, ARG169, HIS193, ALA238, ILE242, TYR247, THR248, ASN249, ILE252. Active sites were found as ARG169 and HIS193. *Pf*-LDH contact residues were found as VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, LYS102, ILE119, ILE123. The highest cavity volume was found to be 2532 on HM-LDH and the highest vina score was found to be -9.0 kJ/mol.

In Table 8, hoslunddiol was selected as ligand and the enzymes were selected with coenzyme NADH. HM-LDH contact residues were found to be GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, VAL52, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, VAL135, SER136, ASN137, HIS192, ALA237, ILE241, TYR246, THR247, ILE251. Active sites of the enzyme were found as ARG105 and HIS192. HH-LDH contact residues were found as GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, VAL53, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, LEU109, VAL136, SER137, ASN138, ARG169, ALA238, ILE242, TYR247, THR248, ASN249, ILE252 and the active site was found to be ARG169. *Pf*-LDH contact residues were found to be VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, LYS102, ILE119, ILE123. The highest cavity volume was found as 2532.

In Figure 5, active sites of the HM-LDH, HH-LDH and *Pf*-LDH without NADH were modeled by YASARA and active sites of the HM-LDH, HH-LDH and *Pf*-LDH were shown. When hoslundal, hoslundin and hoslunddiol are docked to these enzymes, they interact with the active sites. They have methoxy, carbonyl and hydroxyl groups in their structures and these molecules form hydrogen bonds with enzymes. ARG is one of the positively charged R-group amino acids and it is a non-essential amino acid. Also, HIS is a member of the same amino acid group, and it also has an imidazolium ring on its functional group. When hoslundal, hoslindin, and hoslinddiol dock with the enzyme, they are interact with these amino acids and the binding energy must be high.

According to Table 9, most abundance amino acids were found as valine, leucine, alanine, and isoleucine to be 10.1, 9.8 and 8.5% for *Pf*-LDH. Leucine, valine, and lysine were the most abundance amino acids to be 11.4, 10.2 and 8.4% for the HM-LDH and valine, leucine, lysine, and serine to be 11.4, 10.8, and 7.8% for HH-LDH, respectively. Theoretical pI values were found nearly between HM-LDH and *Pf*-LDH to be 8.44 and 7.12. Net charges were found for HM-LDH, HH-LDH, and *Pf*-LDH to be 3, -6, and 0, respectively. The highest instability index was found as 29.73 for *Pf*-LDH and the other values were close (Table 10). Swiss-ADME properties such as molar reactivity, pharmacokinetics, rotatable bonds of hoslundal, hoslundin and hoslunddiol were investigated (Table 11).

Table 8. The molecular docking results of hoslunddiol Human Muscle L-Lactate Dehydrogenase M Chain (HM-LDH), Human Heart L-Lactate Dehydrogenase H Chain (HH-LDH) and *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*-LDH) with coenzyme as NADH.

Enzyme	CurPocket ID	Vina score (kJ/mol)	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
HM-LDH	C1	-8.9	2532	2, -7, 154	23, 23, 23	**Chain A:** GLY26, VAL27, GLY28, ALA29, VAL30, ASP51, VAL52, THR94, ALA95, GLY96, ALA97, ARG98, GLN99, ARG105, LEU108, VAL135, SER136, ASN137, HIS192, ALA237, ILE241, TYR246, THR247, ILE251
HH-LDH	C1	-9.0	2003	11, 41, 57	23, 23, 23	**Chain A:** GLY27, VAL28, GLY29, GLN30, VAL31, ASP52, VAL53, LEU54, THR95, ALA96, GLY97, VAL98, ARG99, GLN100, LEU109, VAL136, SER137, ASN138, ARG169, ALA238, ILE242, TYR247, THR248, ASN249, ILE252
***Pf*-LDH**	C1	-8.2	809	31, 26, 34	23, 23, 23	**Chain A:** VAL26, GLY27, SER28, GLY29, MET30, ILE31, GLY32, PHE52, ASP53, ILE54, VAL55, TYR85, THR97, ALA98, GLY99, THR101, LYS102, ILE119, ILE123

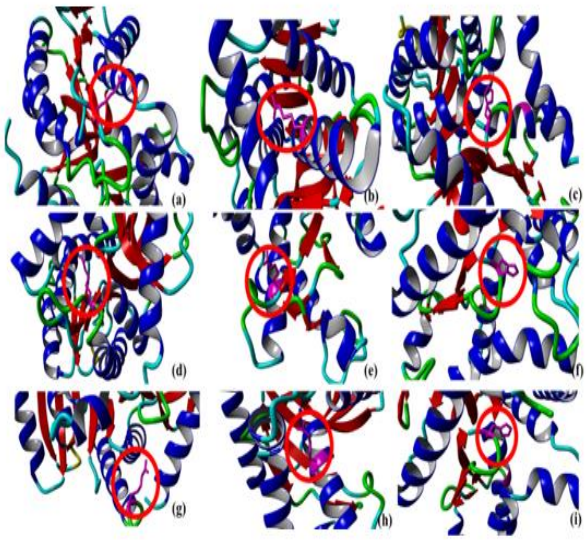

Figure 5. YASARA visualisation of HM-LDH, HH-LDH and *Pf*-LDH without NADH, with active site amino acids (a) ARG98, (b) ARG168, and (c) HIS192, (d) ARG106, (e) ARG171, (f) HIS193, (g) ARG109, (h) ARG171 and (i) HIS195 colored in pink.

Table 9. Number, percentages and abbreviations of the amino acids in *Pf*-LDH, HM-LDH, HH-LDH

Amino acids	*Pf*-LDH		HM-LDH		HH-LDH	
	#	%	#	%	#	%
Ala (A)	27	8.5	18	5.4	21	6.3
Arg (R)	6	1.9	11	3.3	8	2.4
Asn (N)	21	6.6	15	4.5	17	5.1
Asp (D)	17	5.4	18	5.4	19	5.7
Cys (C)	4	1.3	5	1.5	5	1.5
Gln (Q)	8	2.5	12	3.6	11	3.3
Glu (E)	15	4.7	18	5.4	21	6.3
Gly (G)	26	8.2	26	7.8	23	6.9
His (H)	9	2.8	7	2.1	7	2.1
Ile (I)	27	8.5	23	6.9	24	7.8
Leu (L)	31	9.8	38	11.4	36	10.8
Lys (K)	26	8.2	28	8.4	26	7.8
Met (M)	10	3.2	9	2.7	10	3.0
Phe (F)	7	2.2	7	2.1	5	1.5
Pro (P)	12	3.8	11	3.3	11	3.3
Ser (S)	15	4.7	24	7.2	26	7.8
Thr (T)	14	4.4	14	4.2	13	3.9
Trp (W)	1	0.3	6	1.8	6	1.8
Tyr (Y)	8	2.5	8	2.4	7	2.1
Val (V)	32	10.1	34	10.2	38	11.4

Table 10. Protein parameters of HM-LDH, HH-LDH and *Pf*-LDH (aa: amino acids, Mw: molecular weight, pI: isoelectric point).

Enzymes	#aa	Mw	Theoretical pI	Negatively Charged Residues (Asp+Glu)	Positively Charged Residues (Arg+Lys)	Net Charge	Instability Index
HM-LDH	332	36688.72	8.44	36	39	3	24.79
HH-LDH	334	36638.49	5.71	40	34	-6	26.87
***Pf*-LDH**	316	34107.75	7.12	32	32	0	29.73

Also, the pharmacokinetics of the molecules such as cytochromes P450 (CYP), Log Kp (skin permeation), GI absorption, BBB permeant were estimated and the results were given in Table 11. BOILED EGG model represents the blood-brain barrier permeant (BBB permeant) and gastrointestinal absorption (GI absorption). If the molecule is in the yellow part of the BOILED EGG model, BBB permeant and at the white part, GI absorption is observed. The blue dot also indicates that the molecule has a high affinity for P glycoprotein and could be easily released into the system. The red dot shows the affinity of the molecule is low, and it stays in the system for a long time (Daina and Zoete, 2016; Shaaban et al., 2022). In Table 11, Hoslundal is at the intersection of the white and yellow parts, which have both GI absorption and BBB permeant and it has a red dot, which means the molecule stays in the system for a long time. Hoslundin is at the white part with the red dot; it has GI absorption. Hoslunddiol is at the white part with the blue dot.

4. Discussion

About 409,000 people have been dying each year because of malaria. *P. falciparum* has a resistance to anti-malarial drugs and this resistance mechanism has so far been unresolved. Up to date, no validated vaccine has been reported in the scientific literature (Kayamba et al., 2021). Shadrack et al. (2016) selected three molecules that have inhibitory properties on the *Pf*-LDH and they used in silico methods to understand these interactions between enzymes and ligands. However, they did not mention the application method of these three compounds in the real enviromental conditions. This paper was aimed at understanding the inhibitory effects of these molecules on human-LDHs by using in silico methodology as well.

Table 11. Swiss-ADME results of hoslundal, hoslundin and hoslunddiol.

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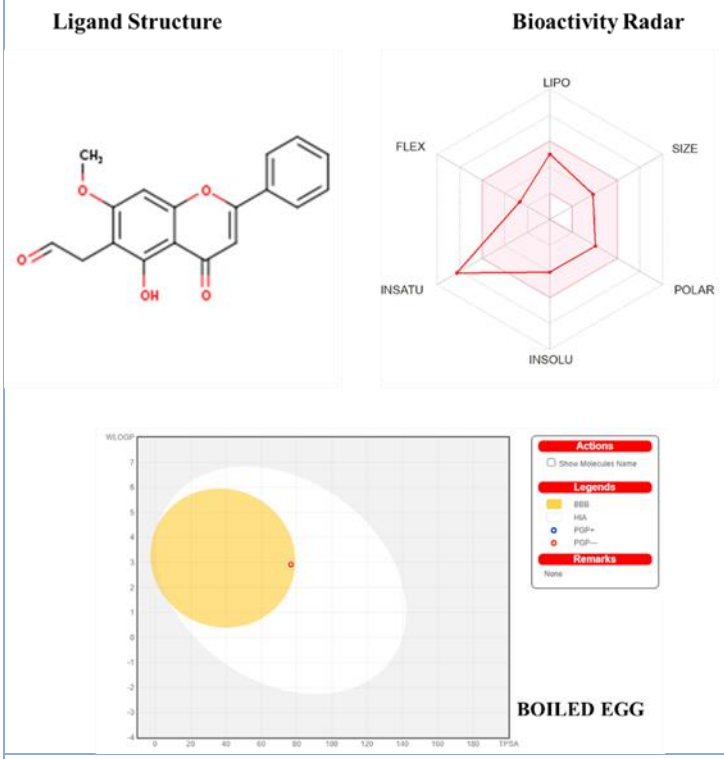
a. Hoslundal



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Pharmacokinetics and drug-likeness

- Lipophilicity: XLOGP3= 3.25
- Molar Refractivity= 86.41
- Polarity: TPSA= 76.74 Å^2
- Saturation: Fraction Csp3= 0.11
- Num. of rotatable bonds= 4
- BBB permeant= Yes
- Log K_P (skin permeation) = -5.89 cm/s
- P-gp substrate= No
- Consensus Log $P_{o/w}$= 2.77
- Bioavailability Score: 0.55
- CYP1A2 inhibitor= Yes
- CYP2C19 inhibitor= Yes
- CYP2C9 inhibitor= Yes
- CYP2D6 inhibitor= No
- CYP3A4 inhibitor= Yes
- GI absorption= High
- Lead likeness= Yes

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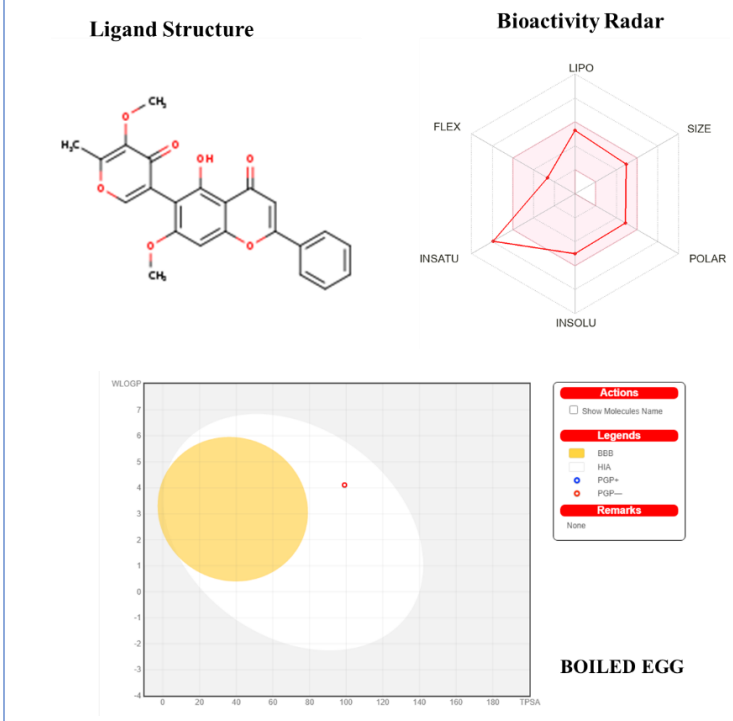
b. Hoslundin



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Pharmacokinetics and drug-likeness

- Lipophilicity: XLOGP3= 3.74
- Molar Refractivity= 111.86
- Polarity: TPSA= 99.11 Å^2
- Saturation: Fraction Csp3= 0.13
- Num. of rotatable bonds= 4
- BBB permeant= No
- Log K_P (skin permeation) = -6.12 cm/s
- P-gp substrate= No
- Consensus Log $P_{o/w}$= 3.42
- Bioavailability Score: 0.55
- CYP1A2 inhibitor= No
- CYP2C19 inhibitor= No
- CYP2C9 inhibitor= Yes
- CYP2D6 inhibitor= No
- CYP3A4 inhibitor= Yes
- GI absorption= High
- Lead likeness= No; 2 violations: MW>350, XLOGP3>3.5

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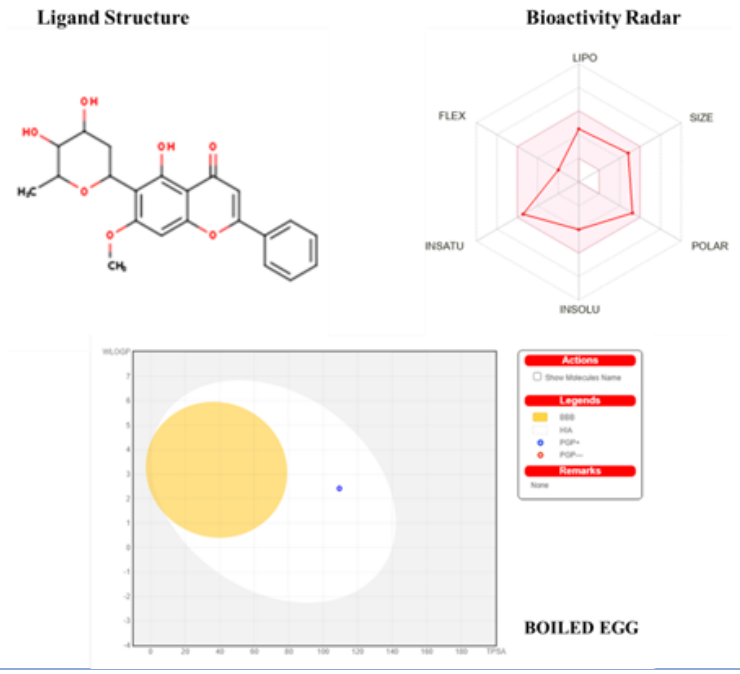
c. Hoslunddiol



Pharmacokinetics and drug-likeness

- Lipophilicity: XLOGP3= 2.36
- Molar Refractivity= 106.73
- Polarity: TPSA= 109.36 Å^2
- Saturation: Fraction Csp3= 0.32
- Num. of rotatable bonds= 3
- BBB permeant= No
- Log K_P (skin permeation) = -7.05 cm/s
- P-gp substrate= Yes
- Consensus Log $P_{o/w}$= 2.24
- Bioavailability Score: 0.55
- CYP1A2 inhibitor= No
- CYP2C19 inhibitor= No
- CYP2C9 inhibitor= No
- CYP2D6 inhibitor= No
- CYP3A4 inhibitor= Yes
- GI absorption= High
- Lead likeness= No; 1 violation: MW>350

A comprehensive review on the LDH inhibitors can be found from Granchi et al (2010). More and very recent information on not only for human LDH inhibitors but also other species can be accessed via brenda-enzymes.org. CB-Dock was used and the results were estimated with and without NADH. The main idea was to elucidate whether these molecules interact with active sites of the enzymes. When the vina scores were compared, the results showed that they bonded with active site amino acids (ARG109, ARG171 and HIS195). As a result of the study, it was understood that these three molecules may also have inhibitory effects on the human-LDH. Ren et al. (2022) studied Methicillin-resistant *Staphylococcus aureus* USA300, which causes pneumonia. They utilized hispidulin as an inhibitor against *S. aureus* LDH. They tested the compound on the mice lungs. According to Swiss-Similarity results, hoslundal and hispidulin are highly similar, suggesting that hoslundal can also be employed for inhibiting *S. aureus* LDH. Singh et al. (2019) mentioned on *Pf*-LDH that there is a high similarity between *Pf*-LDH and human-LDH. Due to this similarity, usage of anti-pLDH drugs could be risky for all aerobic and anaerobic organisms where these anti-pLDH drugs are applied. The researchers have so far investigated the 3D structures of human-LDH and *Pf*-LDHs. Any anti-malarial inhibition focused on LDHs may be effective on human LDH. Some of the previous studies suggested new compounds as anti-pLDH drugs to inhibit pLDH. Vivas et al. (2005) mentioned that 4-hydroxy-1,2,5-oxidiazole-3-carboxylic acid (OXD1) as an inhibitor of *Pf*-LDH. Heterocyclic azole-based inhibitors bind with OXD1 as an inhibitor of *Pf*-LDH. Heterocyclic azole-based inhibitors bind to *Pf*-LDH, but they do not bind the active site of human LDH. In the Vivas et al. (2005) paper, it was shown that OXD1 has anti-malarial activity *in vitro* and *in vivo*. According to this study, Vivas et al. (2005) suggested and proved that while discovering new anti-malarial drugs, azole derivative compounds have more optimization. According to Shadrack et al. (2016), when the compounds were examined, it was clear that there was no azole-based compound. Thus, three of these molecules are alternatives to anti-malarial drugs. According to the findings, these compounds have *Pf*-LDH inhibition properties. The docking results also prove their inhibitory properties. Kayamba et al. (2021) study is about to emphasize the significance of parasitic LDH and MDH as therapeutic treatment targets for a few specific obligate apicoplast parasites. In the light of this study, their potential as therapeutic targets for both aerobic and anaerobic glycolytic pathways are highlighted. Azole derivatives have been extensively studied in the literature for their ability to inhibit *Pf*-LDH and MDH. As a result of this study, inhibition of MDH and LDH is the best strategy for the anti-malaria drugs. In the present study, three molecules were docked to HH-LDH and HM-LDH that are responsible for the continuation of glycolysis. Vina scores of HH-LDH and HM-LDH are higher than *Pf*-LDH when hoslundal, hoslundin and hoslunddiol were docked. According to these data sets, if these molecules are used, they may also inhibit HH-LDH and HM-LDH. Penna-Coutinho et al. (2011) studied NADH analogs to combat with malaria. They investigated 50 different NADH compounds. Itraconazole, atorvastatin and posaconazole were selected to be the best compound to inhibit pLDHs by using Molegro Virtual Docker software. In contrary to Penna-Coutinho et al. (2011), NADH was removed from the LDHs studied in this study and molecular docking was realized. Therefore, the result may exhibit different binding energies. In silico technologies provide a big contribution to the development of anti-pLDH drugs. However, a common and a validated methodology should be developed before obtaining docking scores with respect to candidate drugs. The main idea is to calculate vina scores while the molecules bind to the active sites. Penna-Coutinho et al. (2011) docked the NADH analogs without coenzyme but lactate dehydrogenase is a

haloenzyme that needs a coenzyme in order to show activity (Berg et al., 2007). We propose that itraconazole, atorvastatin and posaconazole should be docked with NADH and the MolDock score should be evaluated. Also, Penna-Coutinho et al (2011) mentioned that their selected compounds strongly bind to active site at and they called this interaction as "competitive inhibition". However, competitive inhibitors are listed under "reversible" inhibition types and strong interaction are not expected in molecular docking studies such hydrogen bonds, hydrophobic interactions electrostatic interactions, etc. According to Singh et al. (2019), chloroquine (CQ) is used in anti-malarial drugs. As a result of this usage, it caused resistance in *P. falciparum* and *P. vivax*. Both of these parasites are responsible for malaria. Long-term treatment led to retinal toxicity and a decrease in vision deficiency, diplopia, and associated vision loss. In the light of this information, new anti-malarial drugs should be developed.

5. Conclusion

The number of deaths due to malaria has been increasing especially in the Africa. In that case, it is very important to produce new anti-*Pf*-LDH drugs to prevent malaria. The development of bioinformatics tools helps produce new drugs. Also, they prevent time and cost losses. As it is known that anti-malarial drugs have inhibitory effects on *P. falciparum*, it should be noted that both mammalians and parasites have lactate dehydrogenases. As a result of this information, new molecules could inhibit mammalian LDH. According to the present paper, it is clear that three molecules exhibit higher binding energies for both HM-LDH and HH-LDH than those of *Pf*-LDH. Application methods play a crucial role in potantial use of these three small molecules in various inhibition studies. For the further studies, effects of specific application methods on the inhibition process of enzymes should be taken into consideration. Wet-lab studies are strongly recommended for specific inhibition.

Authors' contributions: YB: Collected and analyzed the data, wrote first draft and final version of the manuscript. YY: Collected and analyzed the data, draw the figures, wrote the first draft. LC: Designed the project, collected and analyzed the data, revised the first draft and final version of the manuscript.

Conflict of interest disclosure: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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