Chapter 31
Parthenin and Its Similar Structure as Potential Lead Inhibitors of *Plasmodium vivax* and *Plasmodium falciparum* Lactate Dehydrogenase

Pushpendra Singh, Prem P. Kushwaha, and Shashank Kumar

31.1 Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites conveyed to people through malaria vectors. About three million people die and five million have been reported to be infected with malaria annually worldwide (WHO 2011). The dearth of effectual anti-malarial vaccines, chemotherapy assumes a critical part in control of the illness, but unfortunately, drug-resistant strains of *Plasmodium* (*P. falciparum* and *P. vivax*) have shown up against a large portion of antimalarials present till date. Thus, expanded endeavors are instantly desired for antimalarial drug discovery. The objective must be advanced for safe and inexpensive new medications to slug the spread of malaria parasites that are impervious to existing medications. The malarial parasite lives mainly in the host erythrocytes, where they use cell component as a nourishment for their life-cycle growth. These parasites destroyed the hemoglobin fractions of the infected erythrocyte, which leads to severe ailments such as anemia exclusively in both pregnant women and children (Qidwai et al. 2014; Chen 2014). Malarial parasite present in the erythrocyte fundamentally depends on the glycolysis pathway for their energy production. Cytoplasmic pyruvate fermentation and/or mitochondrial electron transport chain are the mechanism by which the consumed NAD^+ during glycolysis is regenerated. In Plasmodia, lactate is the end-product of the glycolytic pathway because pyruvate does not enter the citric acid cycle. In the presence of NADH, reduction reaction converts pyruvate into lactate in the presence of lactate dehydrogenase (LDH) enzyme. Here, *Plasmodium* pyruvate is not able to work as substrate inhibitor, which allows fast energy production, mandatory in the fast-growing malarial parasite (Ramya et al.

P. Singh
Tumor Biology Laboratory, National Institute of Pathology, New Delhi, India

P. P. Kushwaha · S. Kumar (✉)
Department of Biochemistry and Microbial Sciences, Central University of Punjab, Bathinda, Punjab, India

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2002). Plasmodial LDH differs from its human counterpart by the presence of a five amino acid insertion at the pyruvate binding site. Thus LDH has been used for testing the novel *Plasmodium* LDH inhibitors versus human LDH. The malarial LDH also has a vast cleft at its active site which can accommodate consilient colossal inhibitors like gossypol derivatives. Based on the above argument LDH may act as a latent target for malaria. *Parthenium hysterophorus* is a proverbial weed found throughout the world. *P. hysterophorus* have numerous medical advantages such as medication for rheumatic aching, urinary tract infections, neuralgia, diarrhea, dysentery and skin inflammation. Parthenin is an important ingredient of *P. hysterophorus* stem, blossom, leaf, and root (Kumar et al. 2013). Because of this, we used different parthenin like compounds to target *P. falciparum* and *P. vivax* LDH enzyme in the present study.

In-silico screening approach is the foremost strategy for introductory identification of new inhibitors for target proteins and evaluating their interactive mode. The larger parts of known anti-malarial drugs are small molecules intended to interact and temper the biological mode of action of the distinctive pathogen proteins. Molecular docking comprises three distinctive objectives sequentially posture prediction, virtual screening, and binding affinity calculation. Computer-aided drug designing are able to discriminate hits, pick leads and rationalize leads to convert naturally active compounds into decent medications by upgrading their physicochemical, pharmaceutical and ADME\T (absorption, distribution, metabolism, excretion, and toxicity) properties. Thus, in-silico approach is utilized impressively to reduce the risk, time and asset necessities for both in-vitro and in-vivo chemical synthesis and biological testing (Ekins et al. 2007). In the present study, we employed Maestro 9.6 software for docking studies of 85 parthenin like compounds. Furthermore, Maestro 9.6 QikProp module was used to evaluate the ADME/T properties of the best-docked parthenin like compounds.

31.2 Materials and Methods

31.2.1 In-silico Methodology

31.2.1.1 Selection of Protein and Ligand Molecules

Protocol for the GLIDE based molecular docking took from our earlier published article with a few modifications (Singh et al. 2018) (Fig. 31.1). The present work is the extension of previously published in-silico antimalarial drug discovery from our laboratory (Singh et al. 2018). Same ligands reported in the published article were used to check their multi-targeted antimalarial potential. List of ligands and their structural and physiochemical parameters are reported in previously published article (Singh et al. 2018).

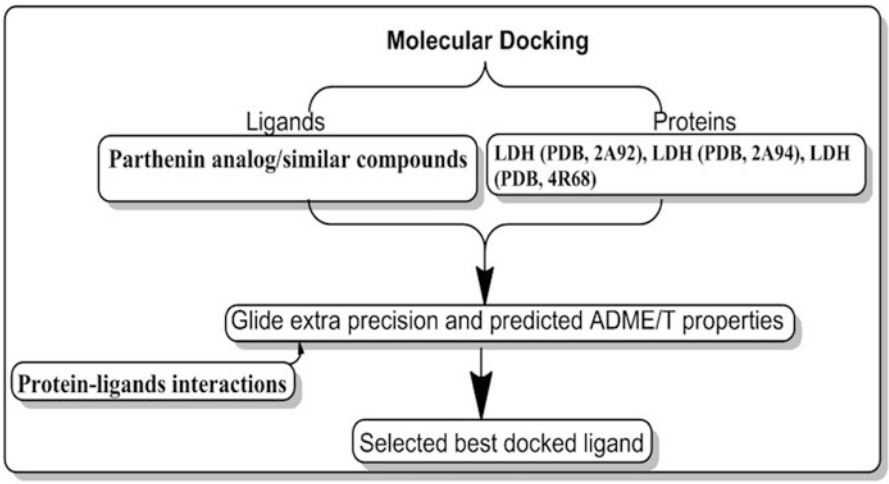

Fig. 31.1 Workflow of study design

Ligprep wizard module of Maestro 9.6 Schrodinger Inc. was used for the ligand preparation. The module accomplishes several modifications in ligands such as hydrogen atom addition, two dimensional to three-dimensional conversion, bond angles and their length corrections, ring conformation, stereochemistry and low energy structure. Ionization was not changed and tautomers were not generated for the ligands. The X-ray crystal structure of test proteins (LDH, PDB: 2A92, LDH PDB: 2A94 and LDH PDB: 4R68) were retrieved from protein data bank (Chaikuad et al. 2005; Labadie et al. 2015). PDB raw structure was modified by using protein preparation wizard module.

31.2.1.2 Molecular Docking

Maestro 9.6 Schrodinger Inc. software package was used for molecular docking studies of selected ligand molecules (Friesner et al. 2004). Ligands were docked against target protein molecules. Ligand dataset and target proteins were prepared by using appropriate modules available in Maestro 9.6 package. The optimized potential for liquid simulations (OPLS_2005) force field was applied for energy minimization and geometry optimization (Shivakumar et al. 2010; Jorgensen and Tirado-Rives 1988; Jorgensen et al. 1996). The one ligand-one conformation was opted for molecular docking protocol. The receptor-grid file was generated to implement the partial atomic charge of 0.25 and 1 Å Van der Waal radii for the receptor atom. After that, ligand-receptor molecular docking was performed. Based on the minimum glide score, ten compounds were selected for the prediction of type of interactions, optimal energy value, bonding potential, and conformations.

31.2.1.3 ADME/T Properties Studies

The ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of lead ligands were evaluated by using the Maestro 9.6 QikProp module. Different pharmacological parameters such as overall CNS activity, log BB, MDCK and Caco-2 cell permeability and logKhsa (human serum albumin binding), etc. of the lead ligand molecules were assessed by QikProp module (Jorgensen and Duffy 2002; Lu et al. 2004).

31.3 Results and Discussion

31.3.1 Analysis of Docking Results of Promising Compounds for Plasmodium vivax *LDH*

Crystal structure of *P. vivax* LDH in complex with inhibitor deliver information about position and conformation of enzyme binding site (Kongsaeree et al. 2005). X-ray structure of *P. vivax* LDH (PDB:2A92) was used for docking study. Antimalarial combinatorial possessions of artemisinin derivatives (ACT) with quinoline compounds (amodiaquine and mefloquine) are known for their widespread high resistance. Our selected lactate dehydrogenase of *Plasmodium vivax* structural insights suggest a general approach for developing new generations of antimalarial LDH inhibitors that accommodate the only substrate for their active site, would retain a binding affinity with the mutant enzymes (Kongsaeree et al. 2005). Molecular docking was achieved by using XP mode of GLIDE. Our result highlighted that; CID72786361, 78178433, and 11552273 yielded a pre-eminent dock score for with proteins *Plasmodium vivax* LDH −8.6, −7.73, −7.55, Kcal/mol respectively (Table 31.1). Most of the interactions made by compounds with residues in the active site of LDH (*P. vivax*) seem to be hydrophobic in nature. Protein-ligand interactions of 2A92 with compounds showed that amino acids Met30, Ile31, Leu112, Val54, Phe100, Ala98, Val138, Pro250, Leu163, Leu167 and Pro246 appeared in the hydrophobic interactions. Furthermore, amino acid amino acids Asn140, Ser245, Ile31 and Gly99 involved in back-bone hydrogen bonding of protein-ligand interactions (Fig. 31.2).

31.3.2 Interaction Modes Between the Parthenin Like Compounds and Human LDH

Molecular docking of *Plasmodium vivax* LDH, *Plasmodium falciparum* LDH and human LDH against natural compounds has been carried out. In the present investigation, our result highlighted that; CID 3482907, 70498184, 73199557, 77977597

Table 31.1 Glide binding score for the ligand-LDH (PDB, 2A92) protein interaction

Ligand type	Compounds ID	GScore	Lipophilic Evdw	HBond	Electro	Protein-ligands interactions
LDH inhibitors (control)	89602938	−7.38	−3.7	−2.88	−0.77	Met30, Ile31, Ieu163, and Leu167
	59637586	−7.32	−3.53	−2.81	−0.81	Met30, Val138, Ieu163, and Leu167
	89602952	−7.3	−3.35	−3.15	−0.72	Met30, Ile31, Ala98, and Phe100
	3503	−6.83	−3.61	−2.31	−0.8	Met30, Ile31, Ala98, and Leu167
	86599376	−6.8	−3.56	−2.4	−0.76	Met30, Ile31, Ala98, and Leu167
Anticancer natural compounds	72786361	−8.6	−2.47	−2.77	−1.15	Met30, Ile31, Asn140, Ieu163, and Ser240
	3482907	−8	−2.76	−2.09	−0.77	Met30, Ile31, Val138, and Ieu163
	78178433	−7.73	−2.38	−2.07	−0.83	Met30, Ile31, Val138, Ieu163, and Ser240
	78178433-2	−7.68	−1.39	−2.74	−1.09	Met30, Ile31, Val138, Ieu163, and Gly99
	11552273	−7.55	−2.72	−2.78	−0.99	Met30, Ile31, Val138, and Ieu163
	44583940	−7.55	−2.72	−2.78	−0.99	Met30, Ile31, Val138, and Ieu163
	3482907-2	−7.49	−2.38	−1.98	−0.76	Met30, Ile31, Val138, and Ieu163
	72791246	−7.47	−2.99	−1.87	−0.75	Met30, Ile31, Val138, and Ieu163
	76391862	−7.46	−3.6	−1.04	−0.53	Met30, Ile31, Val138, and Ieu163
	296217	−7.41	−3.03	−1.37	−0.57	Met30, Ile31, Val138, and Ieu163

yielded a pre-eminent dock score for with proteins human LDH −5.72, −5.58, −6.59, −7.64 Kcal/mol respectively (Table 31.2).

Protein-ligand interactions outline emphasized that the hydrogen bonding, lipophilic, and π-π stacking interactions at the active site plays a vital role in protein-ligand interactions. Molecular docking steps recognize the docking free energy value (Gscore) against the target protein biomolecules. Protein-ligands interactions tinted the electrostatic, lipophilic, and hydrogen bond interactions are a key contributor in protein-ligand interactions. The binding modes of parthenin like compounds in the interactive site of human LDH were identified using intermolecular docking simulations by means of Maestro 9.6 program. All the selected compounds were docked

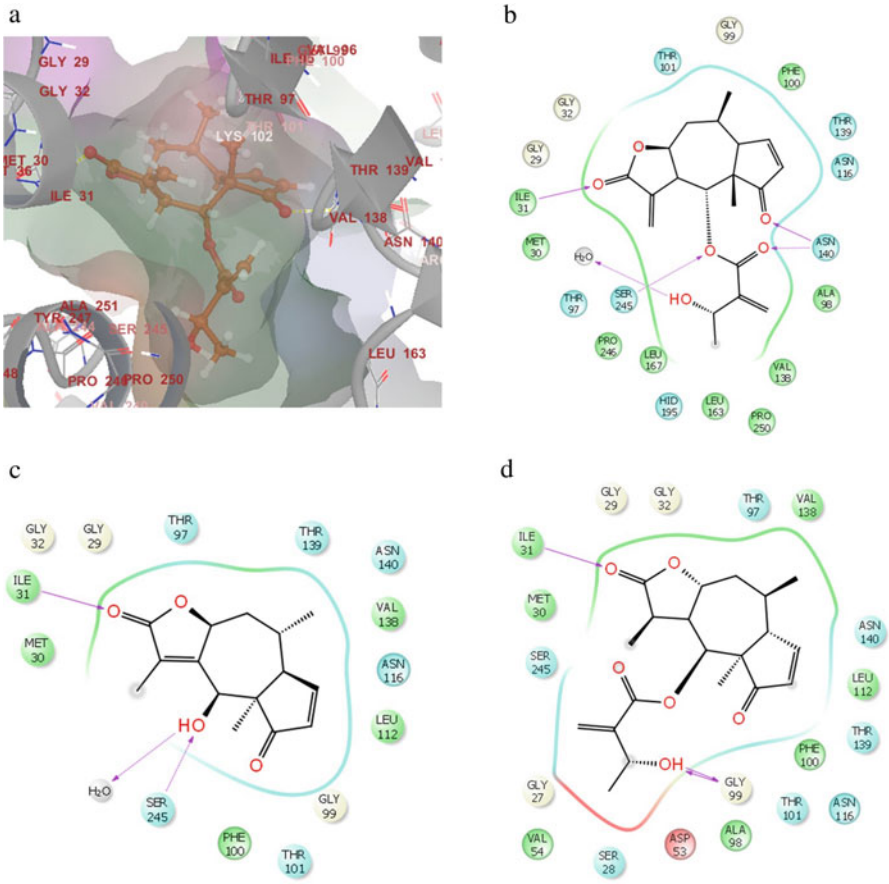

Fig. 31.2 (**a**) Ribbon presentation of LDH (PDB, 2A92) protein molecule with CID 72786361 (**b**) Protein-ligand interactions profile of 2A92 with CID72786361 (**c**) Protein-ligand interactions profile of 2A92 with CID78178433 (**d**) Protein-ligand interactions profile of 2A92 with CID11552273

into the human LDH active site, using the same procedure. Figure 31.3 represents the binding pattern of the parthenin like compounds in the LDH binding pocket. Human LDH active site comprises of mostly hydrophobic amino acids as Pro246, Pro250, Leu167, Ile254, Val138, Leu163, Ile31, Tyr247, Met30, Phe100, and Ala98. These amino acid residues are involved in strong hydrophobic interactions with the parthenin like compounds. As anticipated, inhibitors used in this study interact with the same site like the earlier bounded ligand in the crystallographic complex.

Table 31.2 Glide binding score for the ligand-LDH (PDB, 4R68) protein interaction

Ligand type	Compounds ID	GScore	Lipophilic Evdw	HBond	Electro	Protein-ligands interactions
Compounds	77977597	−7.64	−0.91	−4.24	−2.29	Asn137, Leu164, Hid192, Tyr238, and Ile241
	77977597-2	−5.77	−1.2	−2.69	−1.46	Asn137, Leu164, Hid192, Tyr238, and Ile241
	3482907	−5.72	−1.8	−2.33	−0.98	Leu164, Arg168, Hid192, Tyr238, and Ile241
	73199557	−6.59	−1.5	−2.07	−2.43	Leu164, Arg168, Tyr238, and Ile241
	3482907-2	−5.6	−1.45	−3	−0.64	Leu164, Arg168, Hid192, Tyr238, and Ile241
	70498184	−5.58	−1.13	−2.88	−1.26	Leu164, Tyr238, and Ile241
	73004448	−5.55	−1.85	−2.52	−0.95	Leu164, Tyr238, and Ile241
	77977597-3	−5.54	−1.47	−2.49	−1.21	Asn137, Leu164, Hid192, Tyr238, and Ile241
	56671343	−5.51	−1.73	−2.41	−0.96	Leu164, Tyr238, and Ile241
	3482907-3	−5.49	−1.45	−2.49	−0.87	Leu164, Tyr238, and Ile241

Ligands protein interaction; π-π stacking, π –cat interaction and a hydrogen bond between the ligands and protein

GScore Glide extra precision scores (kcal/mol), *Lipophilic E Vdw* Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy, *HBond* Hydrogen-bonding, *Electro* Electrostatic bounty

31.3.3 Interaction Modes Between the Parthenin Like Compounds and Plasmodium falciparum *LDH*

We used X-ray structure of *Plasmodium falciparum* LDH in complex inhibitor (PDB code 2A94) for the molecular docking study using GLIDE XP module. Our result highlighted that; CID296217, 3482907, 77977597, and 78178433 yielded a pre-eminent dock score for with proteins *Plasmodium falciparum* LDH −6.59, −7.84, −6.73, −6.76 Kcal/mol respectively (Table 31.3). Most of the interactions

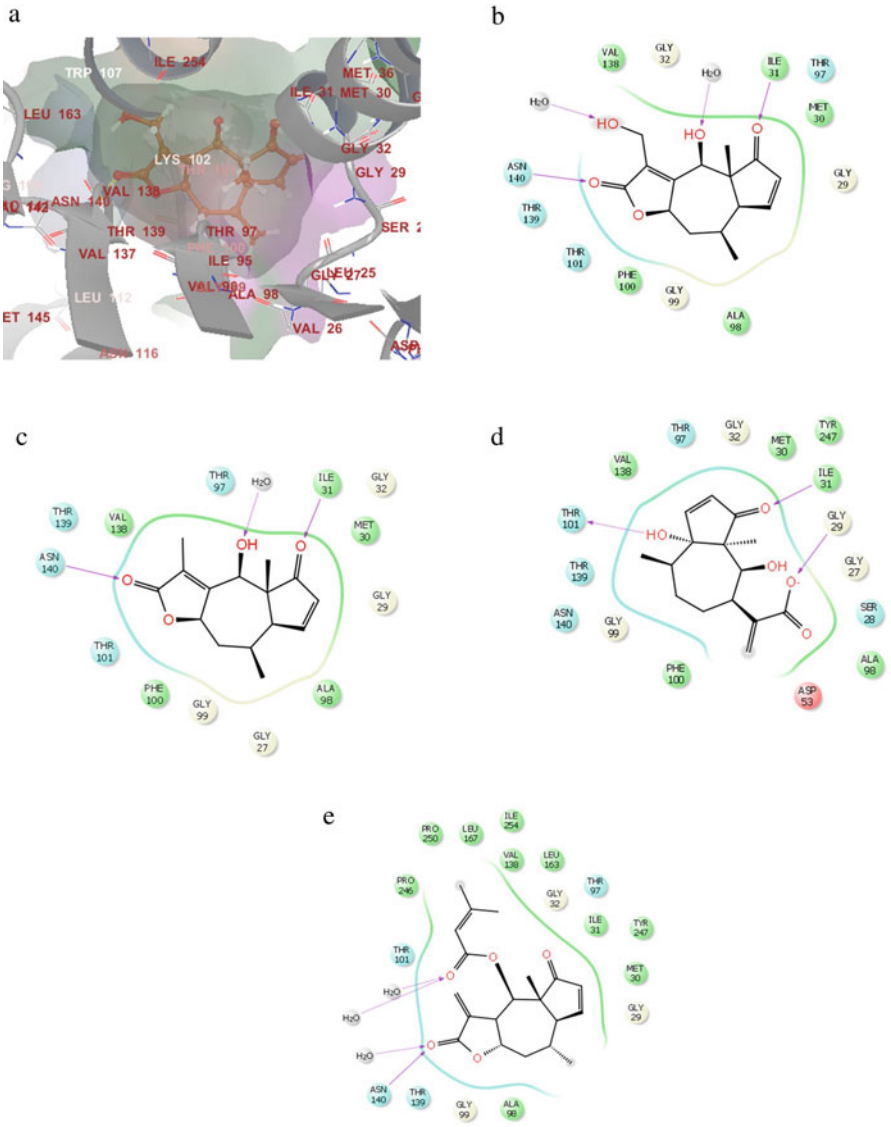

Fig. 31.3 (**a**) Ribbon presentation of LDH (PDB, 2A94) protein molecule with CID 3482907 (**b**) Protein- ligand interactions profile of 2A94 with CID 3482907 (**c**) Protein- ligand interactions profile of 2A94 with CID78178433 (**d**) Protein- ligand interactions profile of 2A94 with CID 77977597 (**e**) Protein- ligand interactions profile of 2A94 with CID296217

made by compounds with residues in the active site of *Plasmodium falciparum* LDH seem to be hydrophobic in nature. Protein-ligand interactions of 2A94 with compounds showed that amino acids Leu164, Ile241, Tyr238, Ala237, Val234, and Pro138 appeared in the hydrophobic interactions. Furthermore, amino acid amino

Table 31.3 Glide binding score for the ligand-LDH (PDB, 2A94) protein interaction

Ligand type	Compounds ID	GScore	Lipophilic Evdw	HBond	Electro	Protein-ligands interactions
Compounds	3482907	−7.84	−1.7	−2.37	−1	Met30, Ile31, Ala98, Phe100, and Asn140
	3482907-2	−7.66	−1.23	−2.68	−0.99	Met30, Ile31, Ala98, Phe100, and Asn140
	78178433	−6.76	−1.47	−1.58	−0.86	Met30, Ile31, Ala98, Phe100, and Thr101
	77977597	−6.73	−1.46	−1.9	−1.07	Met30, Ile31, Ala98, Phe100, and Asn140
	296217	−6.59	−1.91	−2.09	−0.94	Met30, Ile31, Ala98, Phe100, and Val138
	73004448	−6.52	−1.6	−2.3	−1.38	Met30, Ile31, Ala98, Phe100, and Val138
	10265551	−6.32	−2.34	−1.84	−0.6	Met30, Ile31, Ala98, Phe100, and Val138
	13918467	−6.32	−2.34	−1.84	−0.6	Met30, Ile31, Ala98, Phe100, and Val138
	10333765	−6.26	−2.66	−2.02	−0.99	Met30, Ile31, Ala98, Phe00, and Val138
	13918467-2	−6.26	−2.66	−2.02	−0.99	Met30, Ile31, Ala98, Phe100, and Val138

acids Arg168, Asn137 and Hid192 involved in back-bone hydrogen bonding of protein-ligand interactions (Fig. 31.4).

31.3.4 ADME/T Properties of Leads Molecules

ADME/T properties of lead compounds were reviewed by Qikprop module of Maestro 9.6 (Kerns and Di 2010). Compound EGCG was found to be promising based on their docking free energy score and binding mode. A most fascinating aspect of CID73199557, 3482907, 73199557, 72786361, 77977597 are their admirable, Qplogpo/w, QplogHERG, QplogBB, QPP MDCK, Qplogkhsa, and proportion of human oral value which justify the Lipinski's rule of five (Table 31.4). Moreover, high oral bioavailability, polar surface area, H-bond acceptors and donors being imperious criteria for the therapeutic agent's development. It has been suggested that compounds having a polar surface area equal to or less than 140 angstrom and 10 or less rotatable bonds (or 12 or fewer H-bond donors and acceptors) may have a high possibility for best oral bioavailability in-vivo (Veber et al. 2002). Furthermore, it is also reported that the polar surface area is inversely proportional to permeation rate (Becker et al. 1998). These compounds have batter SASA values that are claimed to be suitable for therapeutic agents. These results designate that these compounds will have a better penetration rate.

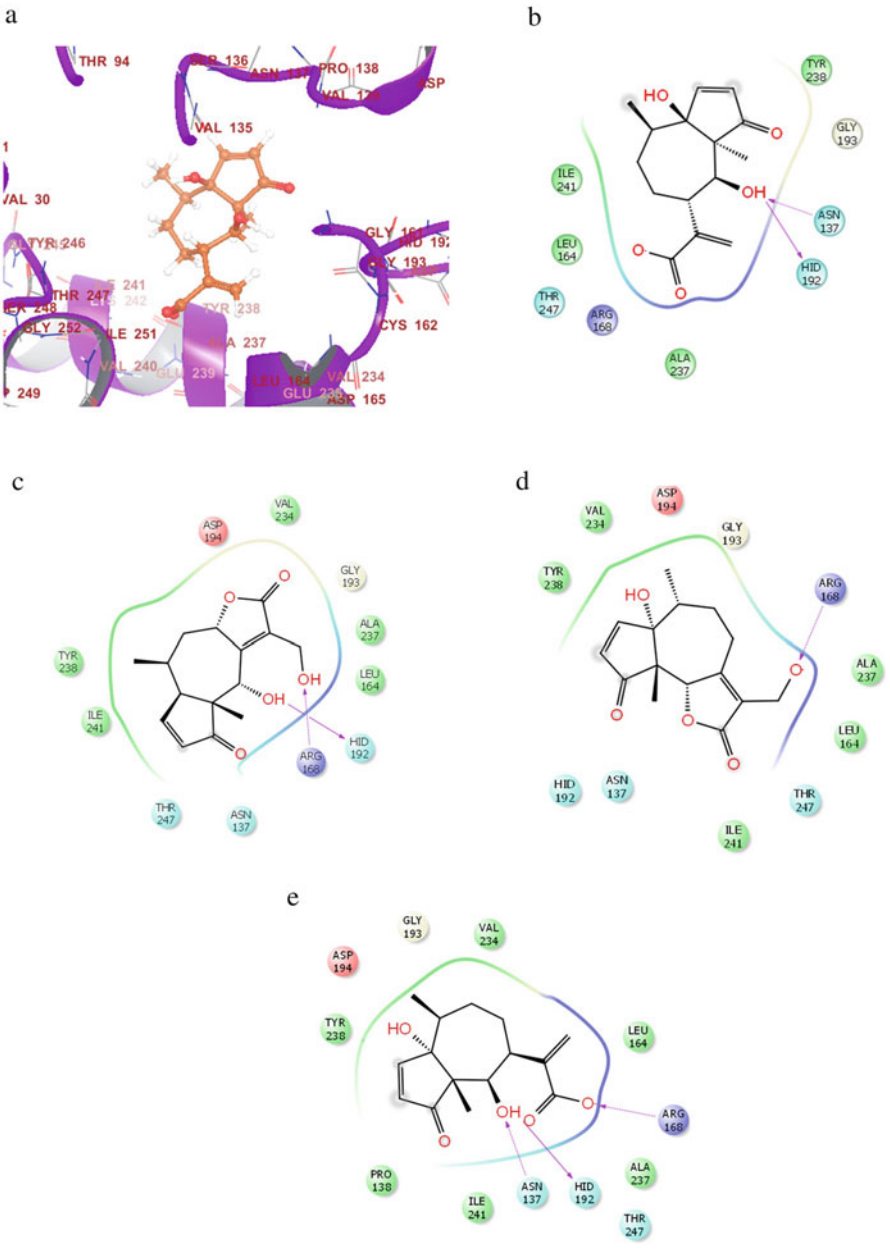

Fig. 31.4 (**a**) Ribbon presentation of LDH (PDB, 4R68) protein molecule with CID77977597 (**b**) Protein-ligand interactions profile of 4R68 with CID77977597 (**c**) Protein-ligand interactions profile of 4R68 with CID3482907 (**d**) Protein-ligand interactions profile of 4R68 with CID73199557 (**e**) Protein-ligand interactions profile of 4R68 with CID70498184

Table 31.4 ADME/T properties. Structural, physicochemical, biochemical, pharmacokinetics and toxicity properties of the compound

S. N	Molecule	QP log $P_{o/w}$ (−2.0 to 6.5)	QPlog HERG (acceptable range: above −5.0)	QPP Caco (nm/s) <25-poor >500- great	QP log BB (−3 to 1.2)	QPP MDCK (nm/s) <25-poor >500- great	QPlog Khsa (acceptable range: −1.5 to 1.5)	Percentage of human oral absorption; (<25% is poor and >80% is high)
1	73199557	0.861	−3.318	259.85	−0.963	115.275	−0.39	75.209
2	155585	0.47	−3.011	92.799	−1.008	83.49	−0.794	70.597
3	3482907	0.47	−3.011	192.799	−1.008	83.49	−0.794	70.597
4	73199557	0.865	−3.253	244.135	−0.972	107.758	−0.38	74.744
5	3482907	0.633	−3.366	256.749	−0.974	113.789	−0.76	73.775
6	72786361	1.522	−3.444	592.017	−0.767	280.718	−0.654	85.479
7	3482907	0.552	−3.063	310.41	−0.845	139.699	−0.826	74.78
8	73199557	0.87	−3.222	271.872	−0.928	121.05	−0.391	75.608
9	3482907	0.52	−2.993	228.077	−0.951	100.118	−0.792	72.196
10	77977597	1.758	−1.654	43.355	−1.185	21.164	−0.325	66.539

QPlogPo/w (−2.0 to 6.5) Predicted octanol/water partition coefficient
QPlogHerg (acceptable range: above −5.0) IC50 for HERG K^+ channels inhibition
QPPCaco (nm/s) <25-poor; >500-great. Apparent Caco-2 cell permeability (nm/s)
QPlogBB (−3 to −1.2) Predicted brain/blood partition coefficient
QPPMDCK (nm/s) <25-poor; >500- great. Apparent MDCK cell permeability (nm/s)
QPlog Khsa- Binding to human serum albumin prediction; (acceptable range: −1.5 to 1.5)
Percentage of human oral absorption; (<25% is poor and >80% is high)

31.4 Conclusion

The present study utilized in-silico approach to search novel *Plasmodium spp.* lactate dehydrogenase inhibitors using parthenin like compounds as a scaffold. Pharmacological and drug-likeness properties of the selected test compounds showed anti-LDH potential. Beside various lead compounds, CID 78178433 showed potentially against both *P. vivax* and *P. falciparum* LDH enzyme. Strong hydrophobic and H-bonding interaction between phytochemicals and pathogen protein was found as revealed by high Dock score. We hereby suggest the in-vitro and in-vivo validation of these lead antimalarial compounds which might provide cost-effective and safer natural antimalarial drug.

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