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In Silico Evaluation of Anti-Malarial Agents from *Hoslundia opposita* as Inhibitors of *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*LDH) Enzyme

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Abstract

Malaria has continued to be a health and economic problem in Africa and the world at large. Many anti-malarial drugs have been rendered ineffective due to the emergence of resistant strains of *Plamodium falciparum.* A key malaria parasite enzyme in glycolytic pathway, *P. falciparum* lactate dehydrogenase (*Pf*LDH) is specially targeted for anti-malarial drugs development. Thus, the aim of this investigation was to determine the *in silico* inhibition effects of antimalarial compounds from *Hoslundia opposita* Vahl. namely hoslundin, hoslundal and hoslunddiol on *Pf*LDH enzyme. The compounds were docked to the three-dimensional structure of *Pf*LDH as enzyme using AutoDock Vina in PyRx virtual screening software. Binding affinity and position of the inhibitors were evaluated using PyMol software. The *Pf*LDH enzyme showed two binding sites: the cofactors binding site (Site A) and secondary binding site (Site B). In the absence of the cofactor all ligands showed higher affinity than NADH, and were bound to the cofactors binding site (Site A). When docked in the presence of the cofactor, site B was the preferred binding site. Binding to cofactor site with higher binding energy than NADH suggests that these ligands could act as preferential competitive inhibitors of *Pf*LDH. However, the binding to site B also suggests that they may be non-competitive allosteric inhibitors. Amino acid residues Gly99, Asn140, Phe100 and Thr97 were indicated to form hydrogen bonds with Hoslundin. Hoslunddiol showed hydrogen bonding with Thr97 and Met30, while Hoslundal formed hydrogen bond with Thr101 and Asn140.

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Keywords

***Pf*LDH, Hoslundin, Hoslundal, Hoslunddiol, Anti-Malarial, Docking**

1. Introduction

Malaria remains to be a health and economic problem in the tropical Africa and the world at large. In Africa malaria is responsible for over 430,000 children deaths every year [1]. In the year 2013, the World Health Organization (WHO) estimated 198 million cases of malaria leading to 584,000 deaths worldwide [1]. The disease mostly prevails in the poor countries of tropical and sub-tropical regions of Sub-Saharan Africa, America and Asia [2]-[7]. The *Plamodium* species namely *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, and the lately reported species *P. knowlesi* are known to cause malaria [4] [5] [8] [9]. Of the five species *P. falciparum* is the most responsible for causing the disease [2] [3] [5] [7].

Over the recent past years, chloroquine was used to treat malaria [4] [8]. Artemisinin and its derivatives were later discovered and took over to treat the disease [3]. However, the occurrence of resistant strains of *Plasmodium* to chloroquine and artemisinin based drugs [3] [4] [8], has necessitated the search and development of new drug targets to combat the disease. Development of resistance to antimalarial drugs is associated to mutations in the parasite active site for the drug target [3] [4] [8]. Thus, identifying new drug targets with new mechanisms of action of the drug may help in fighting the disease [4] [8]. Today, many other natural products and synthetic anti-malaria agents have been designed to target different enzymes involved in parasitic life cycle [6] [10]-[15].

Enzymes of the glycolytic pathways are thought to be an important drug target due to parasitic dependence on glycolysis for energy production [3] [4]. *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*LDH) enzyme is involved in the final step of glycolysis and catalyzes the interconversion of pyruvate to lactate [3] [4]. The enzyme LDH is further involved in the formation of NAD^+ which is required for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase [4]. *P. falciparum* parasites depend on this enzyme for energy production needed for biochemical process, growth and development. Thus, this enzyme is thought to be an important drug target in malaria treatment. Inhibition of this enzyme activities results in death of the parasite. Thus, some anti-malarial drugs have been designed to target this enzyme. Thus, efforts to search for new potent anti-malarial drugs have been a constant ambition to scientists in this field of research.

In a search for potent anti-malarial compounds, Ngadjui and co-workers isolated anti-malarial compounds namely hoslundin, hoslundal and hoslunddiol from *Hoslundia opposita* (**Figure 1**) [16]. The compounds showed anti-malarial activity against multi-drug resistant K1 strain of *P. falciparum*, hoslundal being the most active [16]. An interesting anti-malarial activity of hoslundal suggested it to have a different mode of action from chloroquine and other anti-malaria drugs [16]. *H. opposita* is traditionally used in the treatment of malaria and other diseases in East and West Africa [16] [17]. Several research groups have also reported the anti-malarial activities of the compounds from *H. opposita* [17]-[21]. However, the *in silico* assessment of molecular interaction of these compounds with *Pf*LDH enzyme remains unreported. In this work, a molecular docking study was carried on hoslundin, hoslundal and hoslunddiol to evaluate their potential inhibitory activity against *Pf*LDH enzyme. The binding interactions were investigated and are reported herein.

2. *In Silico* Experimental Procedures

The three dimensional protein crystal structures of *Pf*LDH (PDB ID: IT2C) with resolution of 2.01 Å, (**Figure 2(a)**), [22] was obtained from the RCSB Protein Data Bank and used for docking studies. Docking experiments

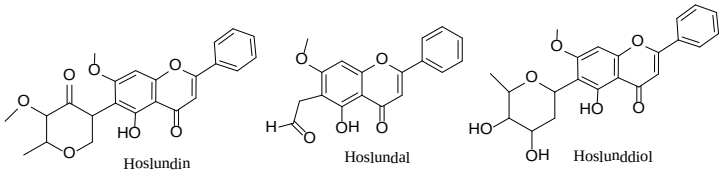


Figure 1. Structures of anti-malaria compounds from *Hoslundia opposita* [16].

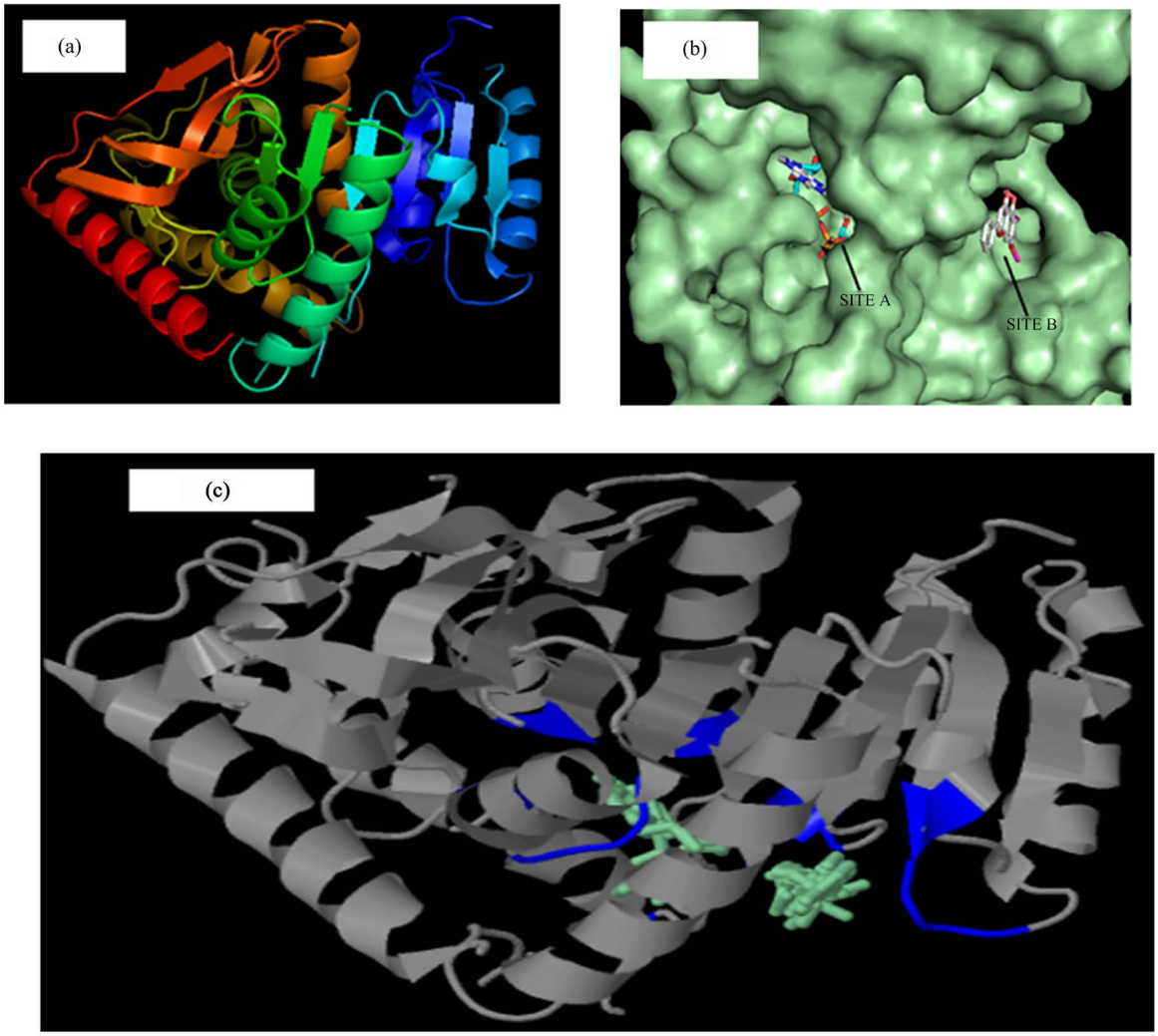


Figure 2. Structure of *Pf*LDH enzyme (PDB ID: IT2C) retrieved from the RCSB Protein Data bank as shown in (a); (b) shows the binding surface pockets present in *Pf*LDH enzyme. Site A is the cofactor binding pocket as viewed in PyMol software; (c) shows the amino acids binding pocket in blue colour near the surface of the enzyme.

were performed following the procedure reported in the literature by [23] [24]. Briefly, Protein binding pockets sites were analysed by using of 3D Ligand Binding Site Predication Server. Possible proteins clashes and amino acid from the active sites were checked by PyMol software v 0.9 (DeLano Scientific LLC). The protein crystal structure was cleaned by using Arguslab software by deleting all heteroatoms, cofactor and water in the protein active sites. Hydrogen atom was added to the geometry. The crystal structure was converted to PDBQT, then refined and geometries optimized using AutoDock Vina in PyRx tools to generate atomic coordinates. Ligands used in this study are chemical structures of hoslundin, hoslundal and hoslunddiol which are anti-plasmodial compounds isolated *Hoslundia opposita* [16]. A list of ligands was generated and optimized using ChemDraw Ultra 12.0 and ChemDraw 3D Pro. The optimized geometry structures were served in .pdb file format. Ligands were then converted to PDBQT in PyRx. Finally, ligands were automatically docked to *Pf*LDH enzymes using AutoDock Vina in PyRx (v 0.8) virtual screening tool with 8 ligand exhaustiveness. PyRx employs Lamarckian Geometric Algorithm (LGA) in docking processes; ligands were docked using flexible conformation. Ligands were re-docked three times in the active sites. Docking grid from autogrid with dimensions 25 × 25 × 25 Å size was used. A default vina search space grid dimensions of 26.09, 26.99 and 9.106 for x, y, z, coordinates was used, respectively. PyMol v 0.9 software (DeLano Scientific LLC,) was used to visualize the binding sites and orientations of the complex.

3. Results and Discussion

3.1. Binding Pockets Analysis

The *Pf*LDH enzyme possesses two important binding pockets, the cofactors binding pocket (Site A) and Site B (**Figure 2(b)**) [4]. Site A, identified as NADH binding pocket comprised of amino acid residues: Gly29, Met30, Ile31, Phe52, Asp53, Ile54, Val55, Tyr85, Thr97, Ala98, Thr101, Val138, Thr139, Asn140, Val142, Leu163, Leu167 and Pro250 (**Appendix**). Site B comprised of amino acid residues: Asp230, Lys198, Val233, Lys314, Glu317, Asp230, Leu201, Glu226, Phe229, Val200, Leu237, and Asn241. These amino acid residues were near the surface end of enzyme (**Figure 2(c)**) [4]. The presence of NADH cofactor affected the distribution and conformation of the docked ligands. It was observed that, in the presence of the cofactors all ligands bound to site B, while in the absence of the cofactor all docked ligands showed a stable conformation at site A.

3.2. Molecular Docking

All ligands were successfully docked to binding sites of *Pf*LDH enzyme. All ligands showed better docking score with stable conformation than the crystalized NADH cofactor when docked in the absence of the cofactor (in the cofactor binding site). In the absence of cofactor, all ligands bound to site A which is the binding site of the cofactor. When ligands were docked in the presence of cofactor, site B was the preferred binding site for all ligands and possessed lower docking scores (**Table 1**).

In the present study, the anti-malarial compound hoslunddiol from *Hoslundia opposita* had a good docking score (−8.0 kcal/mol) higher than all the other ligands to *Pf*LDH enzyme. The best docking score was obtained in the absence of the cofactor (**Table 1**). When re-docked in the absence of the cofactor, similar binding affinities were obtained (**Table 2**). The interaction of hoslunddiol with amino acid residues of *Pf*LDH involved four hydrogen bonds which were formed as follows: The first hydrogen bond was formed by the oxygen atom of the ligand (**Figure 3(d)**). The second hydrogen bond was formed by the hydroxyl group bonding with -NH_2 of Met30 (HO----Met30, 2.38 Å). The third and fourth hydrogen bonds involved oxygen of methoxy group (MeO ----Thr97, 3.22 Å), carbonyl group (C=O----Thr97, 3.54 Å) and oxygen atom of the pyran ring (-O----Thr97, 3.30 Å). **Figure 3(e)** shows the binding of hoslunddiol in the *Pf*LDH active site A. The docking results showed hoslundin to have the second docking score rank with −7.8 kcal/mol. Docking of hoslundin involved four hydrogen bonds between *Pf*LDH and oxygen of the ligand (**Figure 3(b)** and **Figure 3(c)**). The first hydrogen bond formed between C=O of Gly99 (C=O----Gly99, 3.06 Å) while the second hydrogen bond was formed between -NH_2 of Asn140 (C=O----Asn140, 3.05 Å). The hydroxyl (OH) group in the ligand formed the third hydrogen bond with -NH_2 group of Phe100 (HO----Phe100, 3.02 Å) whereas the fourth bond was formed between amine group of Thr97 with the carbonyl group in the ligand (C=O----Thr97, 2.35 Å). Molecular docking studies of hoslundal showed only two hydrogen bonds, which involved Thr101 (3.08 Å) and Asn140 (3.26 Å) (**Figure 3(a)**). Other amino acid residues which interacted with hoslundal in the *Pf*LDH binding pocket were; Gly99, Thr97, Ala98, Gly27, Lys102, Ala103, Phe101, Met30, Ile31, Gy29, Gly32, Pro246, Asn116, Thr139, Ser28, Met36, Tyr247, Gly33, Ala251, Asp110, Leu112, Ser245 and Val248.

Docked ligands showed higher binding affinity than NADH in absence of the cofactor, implying a possible competitive inhibition. However, on closer examination, the interaction of NADH and *Pf*LDH indicated many more hydrogen bonds than the ligands (**Figure 4**). Concomitantly, other studies have reported many hydrogen bonds formed between NADH and *Pf*LDH, regardless of the lower binding energy obtained for NADH in the

Table 1. Best binding energy (kcal/mol) of the favourable conformation based on PyRx

Compounds	Best binding energy (kcal/mol)	
	Absence of cofactor (NADH)	Presence of cofactor (NADH)
Hoslundal	−7.3	−5.9
Hoslundin	−7.8	−6.1
Hoslunddiol	−8.0	−6.4
NADH	−6.8	

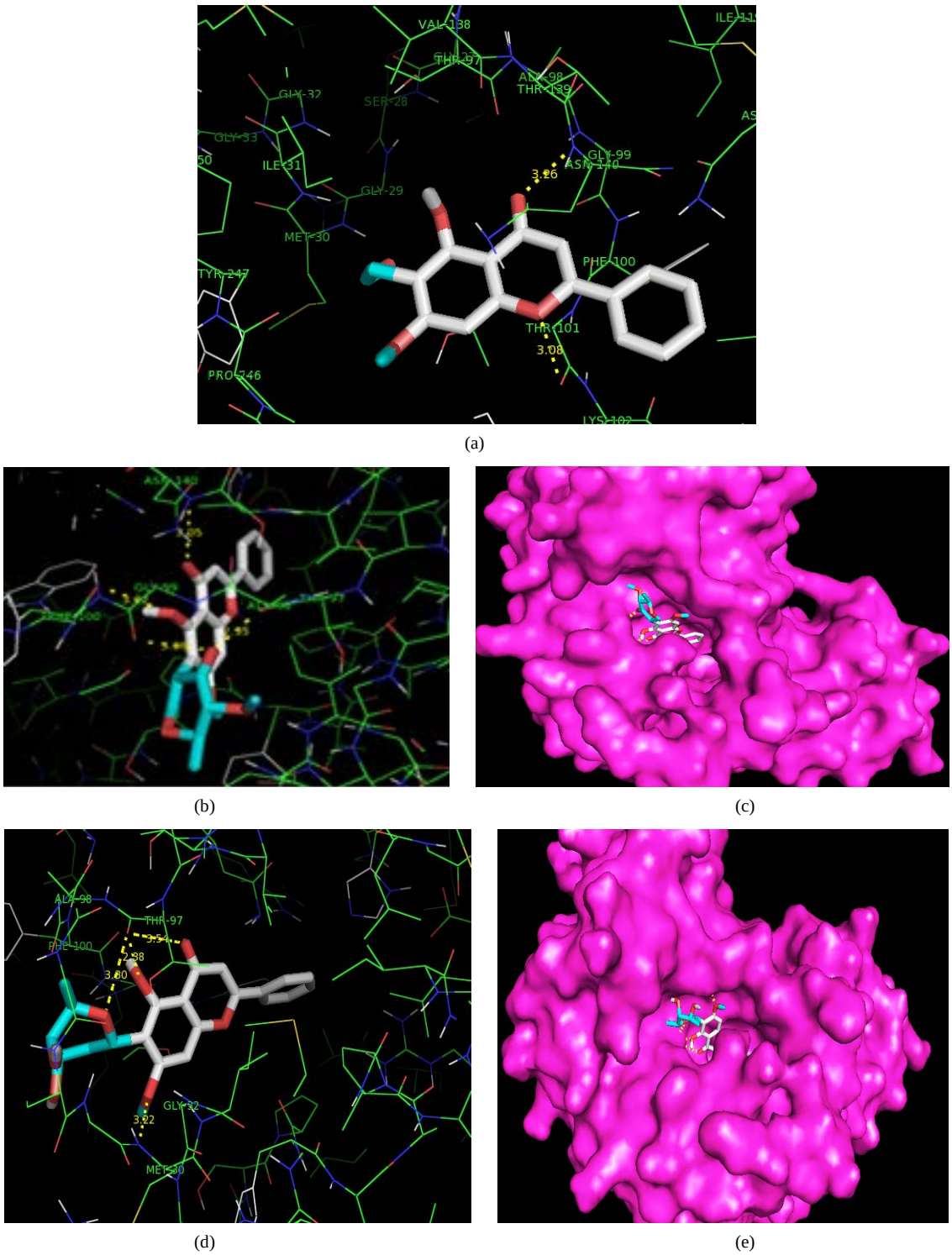


Figure 3. (a) shows the interactions of hoslundal with *Pf*LDH enzyme, two hydrogen bonds were involved, the H-bond formed involved Thr101 (3.08 Å) and Asn140 (3.26 Å); (b) shows the interaction of hoslundin with *Pf*LDH enzyme, four hydrogen bonds were involved (indicated by yellow dotted lines). The amino residues forming hydrogen bond with hoslundin were: Thr97 (2.35 Å), Asn140 (3.05 Å), Phe100 (3.02 Å) and Gly100 (3.06 Å); (c) shows the binding position of hoslundin in the cavity of *Pf*LDH in the absence of cofactor; (d) indicates the *Pf*LDH-hoslunddiol complex. The yellow dotted lines indicates the hydrogen bonds formed between Met30 (2.38 Å), Thr97 (3.22 Å), Thr97 (3.54 Å) and Thr97 (3.30 Å); (e) shows the binding conformation of hoslunddiol in the cavity of *Pf*LDH at site A.

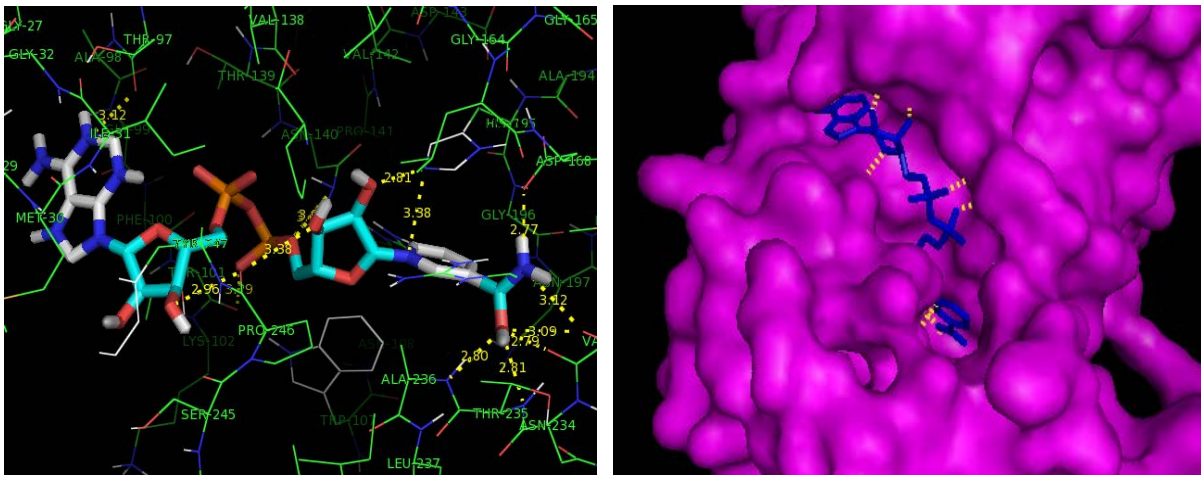


Figure 4. Interaction of NADH to PfLDH (left) and binding position in the pocket (right).

Table 2. Best binding affinity on re-docking experiments

Compounds	Docking run (kcal/mol)			
	1	**2**	**3**	**Mean ± SE**
Hoslunddiol	−8.0	−8.1	−8.1	−8.06 ± 0.03
Hoslundin	−7.8	−7.8	−7.8	−7.8 ± 0.0
Hoslundal	−7.3	−7.0	−7.0	−7.1 ± 0.1

*Pf*LDH enzyme active site [3]. The presence of a high number of hydrogen bonds may reduce the binding affinity of other ligands in the cofactor binding sites. Hydrogen bonds stabilize the ligand interaction in the active site [4] [25]. Docking of ligands in the presence of NADH reduced the affinity and frequency of binding to the cofactor active site. Such phenomenon has also been reported when ligands were docked in the presence of NADH [4], the frequency and affinity of binding to the cofactors binding site was found to be low. In the present study, all ligands showed lower binding affinity than NADH when they were docked in the presence of the NADH (**Table 1**). However, in the absence of NADH all ligands showed higher docking score than NADH. This suggests competitive inhibition in the NADH cofactor binding site, thus, inhibiting *Pf*LDH enzyme from producing energy [3]. Binding of ligands to site B may suggest allosteric inhibition [4]. The amino acid residues forming H-bonds and the bond length in the active site with hoslundal, hoslundin, hoslunddiol and NADH are presented in **Table 3**.

4. Conclusion

Plasmodium falciparum is an important malaria parasite in Africa and other parts of the world. The glycolytic *Pf*LDH enzyme is thought to be one of the important anti-malarial drug targets due to the parasite dependence on glycolysis for energy production. The present study investigated the *in silico* inhibition effects of the three *Hoslundia opposita* compounds (hoslunddiol, hoslundin and hoslundal) to *Pf*LDH enzyme. Results indicated that hoslunddiol (−8.0 kcal/mol) and hoslundin (−7.8 kcal/mol) had better interaction with the target. Hoslundal showed less binding energy (−7.3 kcal/mol) and had few interactions. The former two compounds showed four hydrogen bonds while the latter showed only two hydrogen bonds. Since hoslundal was reported to be the most active anti-malarial compound against the multi-drug resistant K1 strain yet weakly active against the chloroquine sensitive strain NF54 *in vitro*, the present findings further affirm that hoslundal possesses antimalarial activities with probable different modes of action. Within the limitations of the docking procedure, these findings suggest that the studied compounds could act as competitive inhibitors as they had higher binding energy than the cofactor. The pharmacokinetics and *in vivo* studies of these promising anti-malarial compounds are recommended. Furthermore, studies on the interaction and mode of action with other *Plasmodium falciparum* enzymes

Table 3. Amino acid residues forming H-bonds with their bond length in *Pf*LDH active site

	H-bond forming residues	Bond length (Å)
Hoslundal	Thr101	3.08
	Asn140	3.26
Hoslundin	Thr97	2.35
	Phe100	3.02
	Asn140	3.05
Hoslunddiol	Gly99	3.06
	Met30	2.38
	Thr97	3.22
	Thr97	3.54
	Thr97	3.3
	Thr101	3.29
	Gly164	2.99
NADH	(2) Asn140	3.03 and 3.18
	(2) His195	2.81 and 3.38
	Ile31	3.12
	Ala236	2.8
	Val233	2.27
	Asn234	2.81

and any other life cycle target points are recommended for the studied and other anti-plasmodial compounds from *H. opposita*.

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References

[1] World Health Organization (2014) World Malaria Report. World Health Organization, Geneva.

[2] Hasan, A., Mazumder, H.H., Chowdhury, A.S., Datta, A. and Khan A. (2015) Molecular-Docking Study of Malaria Drug Target Enzyme Transketolase in *Plasmodium falciparum* 3D7 Portends the Novel Approach to Its Treatment. *Source Code for Biology and Medicine*, **10**, 1-14. http://dx.doi.org/10.1186/s13029-015-0037-3

[3] Penna-Coutinho, J., Cortopassi, W.A., Oliveira, A.A., Franca, T.C.C. and Krettli, A.U. (2011) Antimalarial Activity of Potential Inhibitors of *Plasmodium falciparum* Lactate Dehydrogenase Enzyme Selected by Docking Studies. *PLoS ONE*, **6**, e21237. http://dx.doi.org/10.1371/journal.pone.0021237

[4] Waingeh, V.F., Groves, A.T. and Eberle, J.A. (2013) Binding of Quinoline-Based Inhibitors to *Plasmodium falciparum* Lactate Dehydrogenase: A Molecular Docking Study. *Open Journal of Biophysics*, **3**, 285-290. http://dx.doi.org/10.4236/ojbiphy.2013.34034

[5] Chinsembu, K.C. (2015) Plants as Antimalarial Agents in Sub-Saharan Africa. *Acta Tropica*, **152**, 32-48. http://dx.doi.org/10.1016/j.actatropica.2015.08.009

[6] Navarro, M., Castro, W., Madamet, M., Amalvict, R., Benoit, N. and Pradines B. (2014) Metal-Chloroquine Derivatives as Possible Anti-Malarial Drugs: Evaluation of Anti-Malarial Activity and Mode of Action. *Malaria Journal*, **13**,

1-8. http://dx.doi.org/10.1186/1475-2875-13-471

[7] Silva, N.S.R., Gonçalves, L.K.S., Duarte, J.L., Silva, J.S, Santos, C.F., Branga, F.S., Silva, R.C., Costa, J.S., Hage-Melim, L.I.S. and dos Santos C, B.R. (2014) Computational Analysis of Physicochemical, Pharmacokinetic and Toxicological Properties of Deoxyhypusine Synthase Inhibitors with Antimalarial Activity. *Computational Molecular Bioscience*, **4**, 47-57. http://dx.doi.org/10.4236/cmb.2014.44006

[8] de Souza, N.B., Carmo, A.M.L., da Silva, A.D., França, T.C.C. and Krettli, A.U. (2014) Antiplasmodial Activity of Chloroquine Analogs against Chloroquine-Resistant Parasites, Docking Studies and Mechanisms of Drug Action. *Malaria Journal*, **13**, 1-12. http://dx.doi.org/10.1186/1475-2875-13-469

[9] White, N.J. (2008) *Plasmodium knowlesi*: The Fifth Human Malaria Parasite. *Clinical Infectious Diseases*, **46**, 172-173. http://dx.doi.org/10.1086/524889

[10] Srivastava, M., Singh, H. and Naik, P.K. (2010) Molecular Modeling Evaluation of the Antimalarial Activity of Artemisinin Analogues: Molecular Docking Rescoring using Prime/MM-GBSA Approach. *Current Research Journal of Biological Science*, **2**, 83-102.

[11] Sessions, R.B., Dewar, V., Clarke, A.R. and Holbrook, J.J. (1997) A mode of *Plasmodium falciparum* Lactate Dehydrogenase and Its Implications for the Design of Improved Antimalarials and the Enhanced Detection of Parasitaemia. *Protein Engineering*, **4**, 301-306. http://dx.doi.org/10.1093/protein/10.4.301

[12] Prakash, N., Patel, S., Faldu, N.J., Ranjan, R. and Sudheer, D.V.N. (2010) Molecular Docking Studies of Antimalarial Drugs for Malaria. *Journal of Computer Science & Systems Biology*, **3**, 070-073. http://dx.doi.org/10.4172/jcsb.1000059

[13] Bhat, H.R., Ghosh, S.K., Prakash, A., Gogoi, K. and Singh, U.P. (2012) *In Vitro* Antimalarial Activity and Molecular Docking Analysis of 4-Aminoquinoline-clubbed 1,3,5-Triazine Derivatives. *Letters in Applied Microbiology*, **54**, 483-486. http://dx.doi.org/10.1111/j.1472-765X.2012.03234.x

[14] Boechat, N., Pinheiro, L.C.S., Santos-Filho, O.A. and Silva, I.C. (2011) Design and Synthesis of New *N*-(5-Trifluoromethyl)-1*H*-1,2,4-triazol-3-yl Benzenesulfonamides as Possible Antimalarial Prototypes. *Molecules*, **16**, 8083-8097. http://dx.doi.org/10.3390/molecules16098083

[15] Sharma, R.B. and Chetia, D. (2013) Docking Studies on Quinine Analogs for Plasmepsin-II of Malaria Parasir Using Bioinformatics Tools. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 681-685.

[16] Ngadjui, B.T., Ayafor, J.F., Sondengam, B.L., Connolly, J.D. and Rycroft, D.S. (1991) Hoslundin, Hoslundal and Hoslunddiol: Three New Flavonoids from the Twigs of *Hoslundia opposita* (Lamiaceae). *Tetrahedron*, **47**, 3555-3564. http://dx.doi.org/10.1016/S0040-4020(01)80869-3

[17] Nkunya, M.H.H. (2002) Natural Chemicals for Disease and Insect Management. Professorial Inaugural Lecture, University of Dar es Salaam, Dar es Salaam.

[18] Salame, R., Cheikh-Ali, Z., Bories, C., Adiko, M., Poapon, E. and Champy, P. (2012) Pyrone and Unusually Furanone-Substituted Flavones from the Leaves of *Hoslundia opposite*. *Planta Medica*, **78**, 1777-1779. http://dx.doi.org/10.1055/s-0032-1315256

[19] Sarker, S.D., Batholomew, B., Nash, R.J. and Robinson, N. (2000) 5-*O*-Methylhoslunding: An Unusual Flavonoid from *Bidens pilosa* (Asteraceae). *Biochemical Systematics and Ecology*, **28**, 591-593. http://dx.doi.org/10.1016/S0305-1978(99)00091-5

[20] Ngadjui, B.T., Ayafor, J.F., Sondengam, B.L., Connolly, J.D., Rycroft, D.S. and Tillequin, F. (1993) Oppositin and 5-*O*-Methylhoslundin, Pyrone-Substituted Flavonoids of *Hoslundia opposite*. *Phytochemistry*, **32**, 1313-1315. http://dx.doi.org/10.1016/S0031-9422(00)95112-4

[21] Murithi, C.K., Nguta, J.M. and Lukhoba, C.W. (2014) Anti-Malaria Activity and *in Vivo* Toxicity of Selected Medicinal Plants Naturalised in Kenya. *International Journal of Education and Research*, **2**, 395-406.

[22] Cameron, A., Ready, J., Tranter, R., Winter, V.J., Sessions, R.B., Brady, R.L., Vivas, L., Easton, A., Kendrick, H., Croft, S.L., Barros, D., Lavandera, J.L., Martin, J.J., Risco, F., Garcia-Ochoa, S., Gamo, F.J., Sanz, L., Leon, L., Ruiz, J.R., Gabarro, R., Mallo, A. and De Las Heras, F.G. (2004) Identification and Activity of a Series of Azole-Based Compounds with Lactate Dehydrogenase-Directed Anti-Malarial Activity. *Journal of Biological Chemistry*, **279**, 31429-31439. http://dx.doi.org/10.1074/jbc.M402433200

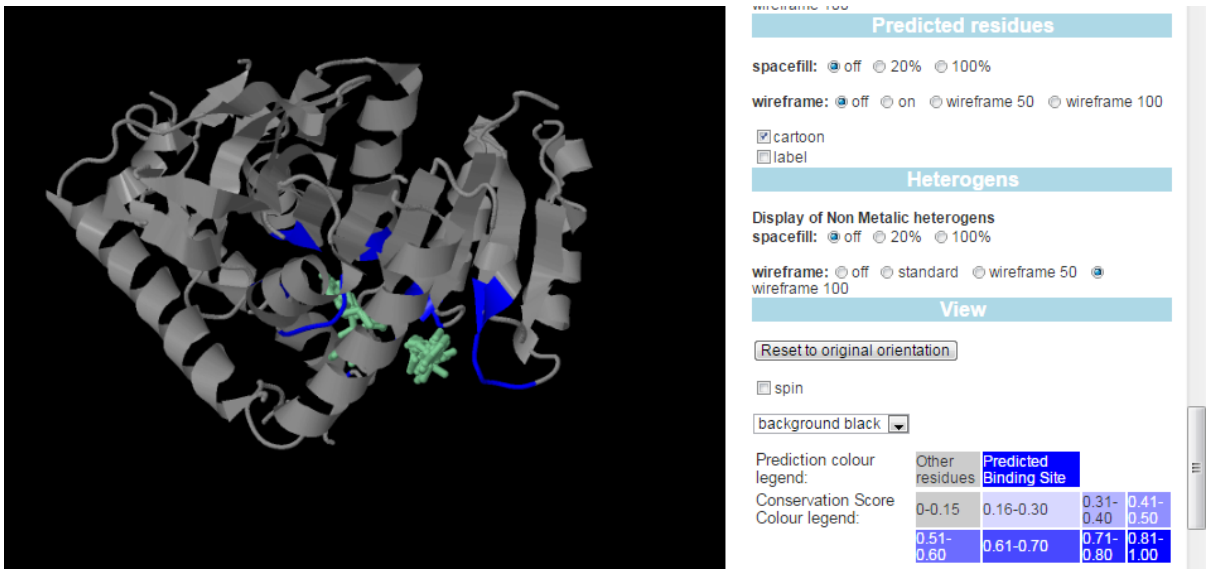
[23] Muhammad, S.S. and Fatima, N. (2015) *In Silico* Analysis and Molecular Docking Studies of Potential Angiotensin-Converting Enzyme Inhibitor Using Quercetin Glycosides. *Pharmacognosy Magazine*, **11**, 123-126. http://dx.doi.org/10.4103/0973-1296.157712

[24] Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S. and Olson, A.J. (2009) AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexiblity. *Journal of Computational Chemistry*, **30**, 2785-2791. http://dx.doi.org/10.1002/jcc.21256

[25] Navya, A., Rayalu, J.D. and Devi, U.M.P. (2011) Docking Studies on Xanthones of Mangosteen as COX-2 Inhibitors. *International Journal of Applied Biology and Pharmaceutical Technology*, **2**, 263-268.

Appendix

Predicated binding active site in the *Pf*LDH enzyme by 3D ligand site prediction server.



Predicted Binding Site

Residue	Amino acid	contact	av distance	JS divergence
29	GLY	20	0.57	
30	MET	25	0.18	
31	ILE	24	0.14	
52	PHE	10	0.62	
53	ASP	16	0.56	
54	ILE	13	0.41	
55	VAL	14	0.44	
85	TYR	25	0.06	
97	THR	23	0.23	
98	ALA	25	0.06	
101	THR	25	0.01	
138	VAL	19	0.59	
139	THR	21	0.59	
140	ASN	24	0.16	
142	VAL	17	0.15	
163	LEU	23	0.13	
167	LEU	13	0.64	
250	PRO	24	0.24	

Selected letter amino acid code sequence of P*f*LDH in docking server.

Single letter amino acid code sequence

Selected amino acids:

18	A	P	K	A	K	I	V	L	V	G	S	G	M	**I**	G	G	V	M	A	T	-38
39-	L	I	V	Q	K	N	L	G	D	V	V	L	F	D	I	V	K	N	M	P	-59
60-	H	G	K	A	L	D	T	S	H	T	N	V	M	A	S	N	C	K	V	S	-79
80-	G	S	N	T	Y	D	D	L	A	G	A	D	**V**	V	**I**	**V**	**T**	**A**	G	F	-100
101-	T	K	A	D	E	W	N	R	D	D	L	L	P	L	N	N	K	I	M	I	-121
122-	E	I	G	G	H	I	K	K	N	C	P	A	**F**	I	**I**	**V**	**V**	**T**	**N**	P	-141
142-	**V**	**D**	**V**	**M**	**V**	Q	L	L	H	Q	H	S	G	V	P	K	N	K	I	**I**	-161
162-	**G**	**L**	**G**	**G**	**V**	**L**	**D**	**T**	S	R	L	K	Y	Y	I	S	Q	K	L	N	-181
182-	V	C	P	R	D	V	N	**A**	H	I	V	G	A	**H**	**G**	N	K	M	V	L	-201
202-	L	K	R	Y	I	T	V	G	L	E	F	I	N	N	K	L	I	S	D	A	-222
223-	E	L	E	A	I	F	D	R	T	V	N	T	A	L	E	I	V	N	L	H	-243
244-	A	S	**P**	**Y**	V	**A**	**P**	**A**	**A**	**A**	**I**	**I**	**E**	**M**	**A**	E	S	Y	L	K	-263
264-	D	L	K	K	V	**L**	**I**	**C**	**S**	**T**	L	L	E	G	Q	Y	G	H	S	D	-283
285-	I	**F**	G	G	T	P	**V**	V	L	G	A	N	G	**V**	E	Q	V	I	E	L	-305
306-	Q	L	N	S	E	E	K	A	K	F	D	E	A	I	A	E	T	K	R	M	-325
326-	K	A	L	A																	