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Research Article

POTENTIAL DRUG TARGETS FOR ALOIN AND MICRODONTIN: AN *IN-SILICO* ANALYSIS

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ABSTRACT

Objective: The aim of this study was to study the interactions of Aloin and microdontin with antimalarial drug targets.

Methods: The ADMET properties of Aloin and microdontin were analyzed using LigandScout and Osiris Molecular Property Predictor tools. The protein-ligand docking was performed in AutoDock software. AutoDock result was analyzed using PyMol and LigPlot+ software.

Results: ADMET analysis suggests no major side effects for both Aloin and microdontin. Docking results show that Aloin had the highest significance with *Plasmodium falciparum* calcium-dependent protein kinase (PfCDPK2) with a free binding energy of –8.01 Kcal/Mol, Ki value of 1.35 μM and 6 hydrogen bonds. Microdontin had the highest significance toward Glutaredoxin-1 with –8.04 Kcal/Mol, Ki value of 1.28 μM and 3 hydrogen bonds.

Conclusion: Based on the observed results for the studied drug targets, the proposed mechanism of action of Aloin is suggestively concluded as PfCDPK2 and for microdontin as glutaredoxin-1.

Keywords: Anti-malaria, Aloin, Microdontin, Autodock, Ligandscout.

INTRODUCTION

Aloe vera predominantly distributed in the tropical and subtropical regions throughout the world is a succulent xerophyte belonging to the family Liliaceae. Though *A. vera* plants are indigenous to Africa now, their existence could be found all over the world [1]. This genus Aloe contains more than 600 species [2]. Their water storage tissue is large, and so they are capable of adapting to areas with low water availability [1]. *A. vera* consists of many biologically important compounds. *Aloe percrassa* is, traditionally, used in Ethiopia to treat Malaria; it is also used to treat wounds and gastrointestinal problems; it is locally known as Ere-Senay in Ethiopia. Recently, Gereziher *et al*. evaluated the antimalarial potency of anthrone derivatives and leaf latex extracted from *Aloe percrassa*. Chloroquine-resistant *Plasmodium berghei* was used to infect the test mice. It was reported that anti-malarial activity of Aloin and microdontin were lower than that of the leaf latex and concluded that the components of leaf latex had synergistic activity [2]. Several anti-malarial studies are being carried out all over the globe using *Aloe* and other medicinal, to compensate the current demand for the effective anti-malarial drug. The mortality rates of malaria have decreased by 47% in the global level and by 54% in the WHO African region; yet, 5,84,000 people died due to malaria in 2013 out of which 78% were children under the age of 5 [3].

Here, in this study, Aloin and microdontin were docked with some of the known *Plasmodium* proteins to find the potential drug targets using AutoDock. The studied proteins were; *P. falciparum* lactate dehydrogenase (PfLDH) with PDB ID: 2 × 8L. It plays a key catalytic role in the reduction of pyruvate [4]. Plasmepsin-2 with PDB ID: 1LF4. It is an asparatic protease, with an important role in hemoglobin degradation pathway [5]. Thioredoxin-2 with PDB ID: 3UL3, it is a redox protein that guards the plasmodium cells against high fluxes of reactive oxygen species [6]. Glutaredoxin-1 with PDB ID: 4MZC, it is also a redox protein, working against reactive oxygen species [7]. *P. falciparum* protein kinase-5 with PDB ID: 1OB3. Is an important protein kinase in *P. falciparum* and is believed to be essential for regulation of nuclear division [8]. Falcipain-2 with PDB ID: 2GHU. It is a cysteine protease, with key function in degradation of hemoglobin [9]. *P. falciparum* phosphoethanolanime methyltransferase with PDB ID: 3UJ9. It is a methyltransferase enzyme, with a key function in membrane biogenesis in *Plasmodium*. This protein is unique for *Plasmodium* and hence is an ideal drug target [10]. *P. falciparum* ADP-Ribosylation factor-1 with PDB ID: 3LRP. It is an important vesicular trafficking protein [11]. *P. falciparum* gamete antigen-27 with PDB ID: 1N81. It is a crucial protein involved in the development of the gamete in *P. falciparum* [12].

METHODS

All protein molecules were downloaded from RCSB Protein Data Bank website. Structure with the following PDB ID were used in this study; 3UL3, 1LF4, 3UJ9, 1OB3, 2X8L, 1N81, 4MVF, 3LRP, 4MZC, 2GHU, 3K7Y. The structure of the Aloin was downloaded from PubChem website, Aloin 313325. The structure of microdontin was sketched using ChemSketch. Properties of the ligand molecules were analyzed using LigandScout.

Protein-ligand docking was performed using AutoDock. 4.2.1 [13-15], using Generic Algorithm parameters. Binding sites of each protein molecule was predicted using MetaPocket online tool [16,17]. The docking results from AutoDock were saved in pdb file format and were analyzed using PyMol [18,19] and LigPlus software [20,21] to view the docking positions and bonding.

Osiris property explorer an online molecular properties prediction tool was used to analyze the biological properties of Aloin and micrdontin. LigandScout [22,23] was used to analyze the chemical nature and structural arrangement of the ligand molecules.

RESULTS

LigandScout analysis of properties of Aloin and microdontin are listed in Table 1. The pictorial representation of H-bond acceptors and H-bond donors are given in Fig. 1. Osiris toxicity analysis suggested no major side effects, signifying their drug ability. A drug score of with yellow color code is considerable as an acceptable drug molecule as given in Table 2.

Auto dock results of Ligand-Protein docking for Aloin and 10 chosen drug target proteins are listed in Table 3, in the order of their binding

energy requirement. Similarly, Auto dock results of Ligand-Protein docking for Aloin and 10 chosen drug target proteins are listed in Table 4, in the order of their binding energy requirement.

Aloin showed the highest affinity toward *P. falciparum* calcium-dependent protein kinase (PfCDPK-2) with binding energy of –8.01 Kcal/Mol and with IC_{50} value of 1.35 uM. The PyMol analysis of Aloin and PfCDPK-2 complex is given in Fig. 2. Aloin formed 6 hydrogen bonds with 4 amino acid residues, Ile-212 (2.0Å), Cys-149 (1.9Å, 2.5Å and 2.1Å), Gly-151 (2.2Å) and Glu-153 (2.1Å).

Microdontin showed the highest affinity toward Glutaredoxin-1 with a binding energy of –8.04 Kcal/Mol and IC_{50} value of 1.28 uM. LigPlot + analysis of microdontin and glutaredoxin-1 is given in Fig. 3. microdontin formed 3 hydrogen with two amino acid residues, Lys-82 (2.96Å) and Leu-69 (2.90Å and 2.76Å).

Table 1: Ligand scout analysis of Aloin and microdontin

Parameters	Aloin	Microdontin
Formula	$C_{21}H_{24}O_9$	$C_{30}H_{32}O_{11}$
Mol. weight	420.41 Da	568.57 Da
cLogP	–1.536	0.354
TPSA	171.07	197.37
HBA	9	10
HBD	8	8

HBA: Hydrogen bond acceptor, HBD: Hydrogen bond donor, AR: Aromatic ring, TPSA: Topological polar surface area

Table 2: Osiris molecular property analyzer drug score for Aloin and microdontin

Parameters	Aloin	Microdontin
Score for cLogP	0.997	0.983
Score for LogS	0.89	0.608
Score for Mol.Wt	0.727	0.316
Score for drug likeness	0.089	0.338
No risk for mutagenicity	1	1
No risk for tumorigenicity	1	1
No risk of irritating effects	1	1
No risk reproductive effects	1	1
Total drug score	0.444 (yellow)	0.351 (yellow)

Table 3: AutoDock results of Aloin with chosen target proteins

Target protein	Binding energy	Inhibition constant	Number of H-bonds
PfCDPK2	–8.01	1.35µM	6
Thioredoxin-2	–7.49	3.22 µM	7
Glutaredoxin-1	–7.11	6.19 µM	6
PfPMT	–7.00	7.35 µM	6
PfPK5	–6.76	11.09 µM	6
Plasmepsin-2	–6.18	29.36 µM	8
PfLDH	–5.97	42.01 µM	4
Falcipain-2	–5.91	46.50 µM	6
PfARF1	–5.58	81.87 µM	6
Pfg27	–5.23	146.48 µM	5

PfCDPK-2: *Plasmodium falciparum* calcium-dependent protein kinases, PfPMT: *Plasmodium falciparum* phosphoethanolamine, PfLDH: *Plasmodium falciparum* lactate dehydrogenase, PfPK-5: *Plasmodium falciparum* protein kinase 5

Table 4: AutoDock results of microdontin with chosen target proteins

Target protein	Binding energy	Inhibition constant	Number of H-bonds
Glutaredoxin-1	–8.04	1.28 µM	3
Plasmepsin-2	–7.92	1.57 µM	4
Thioredoxin-2	–7.70	2.25 µM	5
PfPK5	–7.50	3.17 µM	5
PfLDH	–7.27	4.69 µM	7
PfPMT	–6.47	18.13 µM	1
PfCDPK2	–6.42	19.61 µM	4
Falcipain-2	–6.00	39.92 µM	5
PfARF1	–4.78	312.08 µM	2
Pfg27	–3.32	3.7 mM	3

PfCDPK-2: *Plasmodium falciparum* calcium-dependent protein kinases, PfPMT: *Plasmodium falciparum* phosphoethanolamine, PfLDH: *Plasmodium falciparum* lactate dehydrogenase, PfPK-5: *Plasmodium falciparum* protein kinase 5

DISCUSSION

Ligand scout and osiris toxicity analysis revealed the physiochemical properties of the ligand molecules. Both Aloin and microdontin

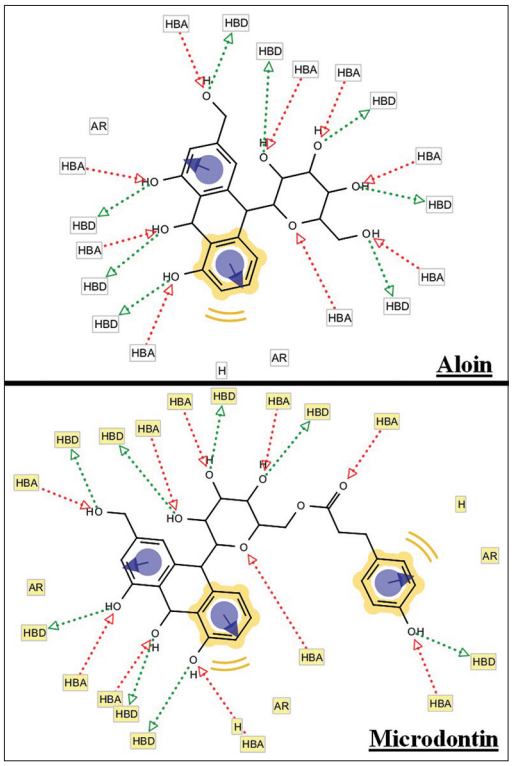

Fig. 1: H-bond acceptor and donor sites in Aloin and microdontin, (HBA: Hydrogen bond acceptor, HBD: Hydrogen bond donor, AR: Aromatic ring)

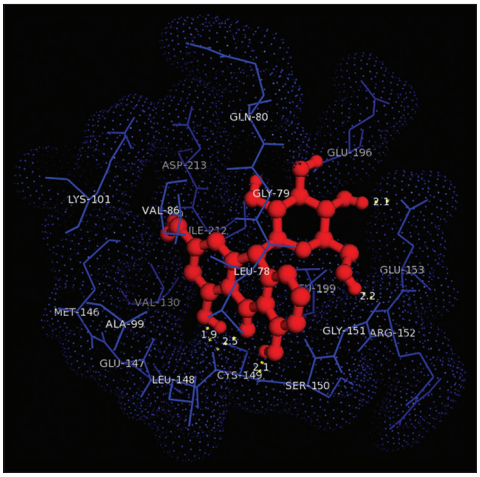

Fig. 2: PyMol view of Aloin and *Plasmodium falciparum* calcium-dependent protein kinases-2 complex

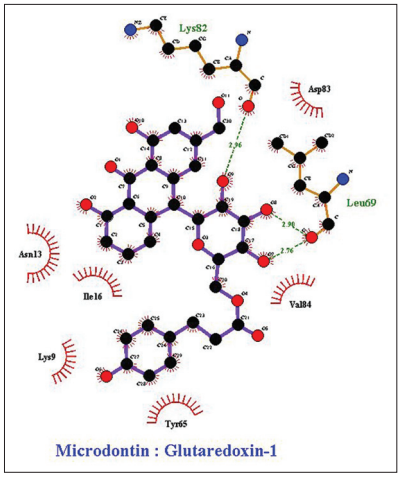

Fig. 3: LigPlot + analysis of microdontin and glutaredoxin-1 complex

contains a high number of H-Bond Acceptors and H-Bond Donors. Osiris toxicity analysis displayed that, both Aloin and microdontin possess no major side effects and has significant properties for being used as drug. The report by Gerziher *et al*., 2014, demonstrated that Aloin and microdontin did not display any adverse side effects and toxicity, with a maximum of 2 g/kg body weight of pure compound for up to 14 days in mice models. Though the anti-malarial activity of Aloin and microdontin have been proven *in-vitro* and *in-vivo* (Gerziher *et al.,* 2014), the mechanism by which these compounds exerts its action is still unclear. It was also reported by Gerziher *et al*., 2014, that Aloin and microdontin displayed reduced activity individually when compared to crude latex.

Auto dock results along with visual confirmation have led to the suggestion of possible target proteins of the test molecules. Aloin showed the highest significance of inhibition toward PfCDPK-2 (RCSB-PDB ID: 4MVF). Aloin demonstrated 1.35 µM inhibition constant with -8.01 Kcal/Mol binding energy. Since Aloin is rich in hydroxyl group, it has formed 6 hydrogen bonds with PfCDPK-2. This suggests PfCDPK-2 could be the potential target protein for Aloin to exert its antimalarial activity. CDPKs are one of the most common drug targets used for malaria and other protozoan diseases since CDPKs does not exist in mammals. Microdontin on the other hand demonstrated highest significant inhibition toward glutaredoxin-1 (RCSB-PDB ID: 4MZC). It is a well-known drug target andGrx-1 is a key redox protein present in *Plasmodium*. Microdontin showed 1.28 µM inhibition constant with –8.04 Kcal/Mol free binding energy and formed 3 hydrogen bonds.

CONCLUSION

The objective of this study was achieved by suggesting the potential target protein for Aloin and microdontin for its anti-malarial activity. PfCDPK-2 has been suggestively concluded as the possible drug target for Aloin. CDPK family proteins are a novel drug targets for plasmodium diseases since CDPKs does not exist in mammals.

Glutaredoxin-1 has been suggestively concluded as the potential drug target for microdontin. Since Grx-1 plays key role in the protection of plasmodium cells against Reactive-Oxygen-Species, inhibiting these proteins would lead to the destruction of plasmodium cells by reactive oxygen species. Since Gerziher *et al*., 2014 has reported that Aloin and microdontin showed reduced anti-malarial activity when administered individually, it could be suggested that, these two molecules work synergistically together by inhibiting PfCDPK-2 and Grx-1, respectively.

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