An In Silico Approach for Targeting Plasmodium Phosphatidylinositol 4-Kinase to Eradicate Malaria

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Abstract Accomplishing the destination of malaria evacuation will depend upon directing Plasmodium pathways necessity throughout all life stages. Here, we selected a lipid kinase, phosphatidylinositol 4-kinase (PI4K), as the potential drug target, In order to achieve a novel antimalarial compound that inhibits the intracellular development of multiple Plasmodium species at each stage of infection in the vertebrate host. Virtual screening was performed against more than thousands of compounds from ZINC database to get some potent natural compounds which are able to inhibit PI4K. Binding affinity of screened compounds was compared with well-known inhibitor like Primaquine as a reference molecule, by analyzing their docking score and binding efficiency with the receptor. ADMET properties of the obtained screened compounds were analyzed to check drug like property. Based on the aforementioned analysis, it has been suggested that these screened potent compounds are capable to inhibit PI4K for the prevention, treatment and elimination of malaria.

Keywords Phosphatidylinositol 4-kinase (PI4K) · Virtual screening · ADME/T

1 Introduction

To eradicate malaria, broadly acting medicines must be formulated that therapeutic the diagnostic asexual blood stage, clear the coming before liver phase transmission that can induce relapses and block parasite infection to mosquitoes [1]. Relapse prevention is particularly significant for *P. falciparum* and *P. vivax*, which makes

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intra-hepatic hypnozoites that, can remain for year earlier reinitiating growth and activating blood stage infection. Primaquine is the drug of choice for treating malaria and only accredited marketed antimalarial drug capable of eradicating the hypnozoite reservoir and perform a radicalcure. Nevertheless, side-effects and weak activity against blood stages prevent widespread usage of primaquine [2]. Subsequently primaquine's target and mechanism of action are not well known, the explore for new revolutionary therapeutic drugs has been limited to concerned analogues, such as Tafenoquine [3]. There is a clear demand for druggable and chemically related validated targets that are necessity in all lifecycle stages of the malaria parasite. Here we describe that a parasite phosphatidylinositol 4-kinase, anomni present eukaryotic enzyme that phosphorylates lipids to govern intracellular signaling and trafficking, is inhibited by screened molecules. In blood stages, inhibitors block a late step in parasite growth by interrupting plasma membrane ingression throughout growing daughter merozoites. This probably stems from varied phosphatidylinositol 4-phosphate (PI4P) pools and disrupted Rab11A-mediated membrane trafficking. Determinations corroborate PI4K as the first known drug target required across all Plasmodium lifecycle stages [4].

2 Methodology

All molecular source and computational analysis was performed by using Schrodinger Maestro version 2014-1, 9.7 build panel. Ligands were prepared using LigPrep application and condition by means of the Optimized Potentials for the Liquid Simulations of electrostatic force field. The protein was modeled before virtual screening and molecular docking the ligands into the active site of the protein molecule. Further the modeled structure of the protein molecule was used to predict the active site of the protein molecule which was then introduced for generation of grid. The energy minimized inhibitor were docked into the generated grid using Glide (Glide, version 6.2, Schrödinger, Inc.) on a Windows 7& Linux based (CentOS release 6.5 Linux-86x-64 platform in Lenovo Intel(R) core i3-3220 (TM) CPU @ 3.30 GHz processor 6 GB RAM workstation. The QikProp program (QikProp, v3.9, Schrödinger software) was used to receive the ADME/T characteristics of the screened compounds along with reference compound Primaquine.

2.1 Protein Preparation

The X-ray crystal structure of PI4K protein was not available in the Protein Data Bank (PDB) therefore we modeled the structure with the help of SWISS-MODEL

(http://swissmodel.expasy.org) [5] followed by NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [6]. The structural features, catalytic residues and active site residues of PI4K were analyzed. For further studies, preparation of protein structure was processed through "Protein Preparation Wizard" [7] of Maestro v9.7 interface of Schrodinger [8]. Protein preparation process involved assigning bond orders, addition of hydrogen bonds, creation of disulphide bonds, conversion of selenomethionine to methionine, filling of missing residues using Prime, capping of termini, deletion of waters and optimization. Energy was minimized using the OPLS_2005 molecular mechanics force field with default value of cut off RMSD (Root Mean Square Deviation).

2.2 Ligand Preparation

Approximately two thousands compounds were extracted from the ZINC databases (http://zinc.docking.org/browse/catalogs/natural-products/) [9] and then processed with Phase v3.8 module of Schrodinger suite [10] for creating a Phase database format (.phdb) of the Schrödinger software for virtual screening followed by LigPrep v2.9 wizard of Maestro v9.7 interface of Schrodinger [11]. It involved generation of maximum possible isomeric and ionization variants. Applying Lipinski's filter, the ligands having poor pharmacological properties were discarded to prepare a virtual library having pharmacologically preferred ligands [12].

2.3 Grid Generation

There may not be any information about the potential ligand binding site for PI4K protein, while in few literatures a putative binding site has been identified by experimental means, but the druggability of the target is not known [4]. Hence it is predicted by SiteMap, which is used for identifying and analyzing binding sites and for predicting target druggability [13]. A receptor grid was generated in the region of these residues of PI4K using Glide v6.2 of Maestro v9.7 interface of Schrodinger with default parameters. Grid point scale for X-axis, Y-axis and Z-axis (−5.86, 364.88 and 90.24) at 10 Å respectively within the grid parameters and grid generation was performed using OPLS 2005 [14].

2.4 Virtual Screening and Molecular Docking Studies

A lead molecule with best docking score was retained through implementation of three subsequent docking operations such as HTVS, SP and XP were applied using

Glide v6.2 of Maestro v9.7 interface of Schrodinger. Based on XP GScore, favorably docked ligands were ranked [15].

2.5 ADME/T Prediction Analysis

To evaluate the ADMET properties QikProp v3.9 module of Maestro v9.7 interface of Schrodinger was used [16]. Various physio-chemical descriptors were calculated to further account for the potential of the lead molecule to act as efficient drug candidate. Violation of Lipinski's rule, if any, was assessed using obtained values for these physio-chemical descriptors. With reference to these values for proposed lead molecule PI4K inhibitors, a comparative study was performed.

3 Result and Discussion

Computational Simulation and Virtual screening was carried out for finding screening potent inhibitors of PI4K including library thousand chemical molecules from generated library. Top eight compounds are presented here along with their glide score energy in drug molecule of hydrogen bond, Van Der Waals and electrostatic energy.

3.1 Validation of Receptor (Target) Protein

The PI4K protein was validated by Ramachandran plot through RAMPAGE by Paul de Bakker and Simon Lovell (http://mordred.bioc.cam.ac.uk/). It showed that 95 % residues were in the most common favorable zone, 2.7 % residue in the generously allowed region, 2.3 % residues in the outlier regions. Hence the protein structure has been selected from the stable structure for the study [17].

3.2 Virtual Screening and Molecular Docking Studies

The prepared protein structure of PI4K was virtually screened against a library of approximately two thousands compounds extracted from ZINC database

Table 1 Molecular docking energy calculations of top eight screened compounds along with primaquine as a reference compound

S. No.	Compound ID	Popular name	Docking score	Glide g score	Glide E-model
1	ZINC00056474	Nordihydroguaiaretic acid	−9.986	−9.986	−66.327
2	ZINC04098166	Picrotoxinin	−9.255	−9.255	−38.587
3	ZINC03881558	Morin	−9.010	−9.015	−55.349
4	ZINC00389747	Naloxone hydrochloride	−8.588	−8.602	−46.325
5	ZINC05037532	Cryptomeridiol	−8.445	−8.445	−37.798
6	ZINC96221670	2-[(4S)-1-(1,3-benzodioxol-5-ylmethyl)-2,5-dioxo-imidazolidin-4-yl]-N-(2-thiazol-2-ylethyl)acetamide	−8.426	−8.426	−62.239
7	ZINC13481200	Xanthopurpurin	−8.221	−8.253	−51.606
8	ZINC04098133	Lactucin	−7.681	−7.705	−39.714
9	PRIMAQUINE	8-(4-Amino-1-methylbutylamino)-6-methoxyquinoline	−4.376	−4.376	−41.636

(http://zinc.docking.org). Three subsequent docking procedures such as HTVS, SP and XP were implemented using Glide v6.2 of Maestro v9.7 interface of Schrodinger. Based on XP G Score, favorably docked ligands were ranked. To find the top poses of the ligands, Glide E-model was used. The lead compound ID ZINC00056474 had a Glide Score of −9.986 and had better binding affinity against PI4K receptor. Similar docking parameters against PI4K are also reported in Table 1 for the comparative purpose. The docking studies indicated that the proposed lead compound ZINC00056474 showed strong hydrogen bond and hydrophobic interactions with the important binding residues of PI4K. The lead compound ZINC00056474 occupies the better binding efficiency against PI4K with lowest docking score and strong interaction in comparison of some other screened compounds against PI4K along with Primaquine as a reference compound (Fig. 1).

3.3 ADME/T Analysis

ADMET properties such as Molecular weight, Hydrogen bond donors, Hydrogen bond acceptors, log P (Octanol/water partition coefficient), percentage of human oral absorption, CNS activity and BBB (blood brain barrier) partition coefficient are

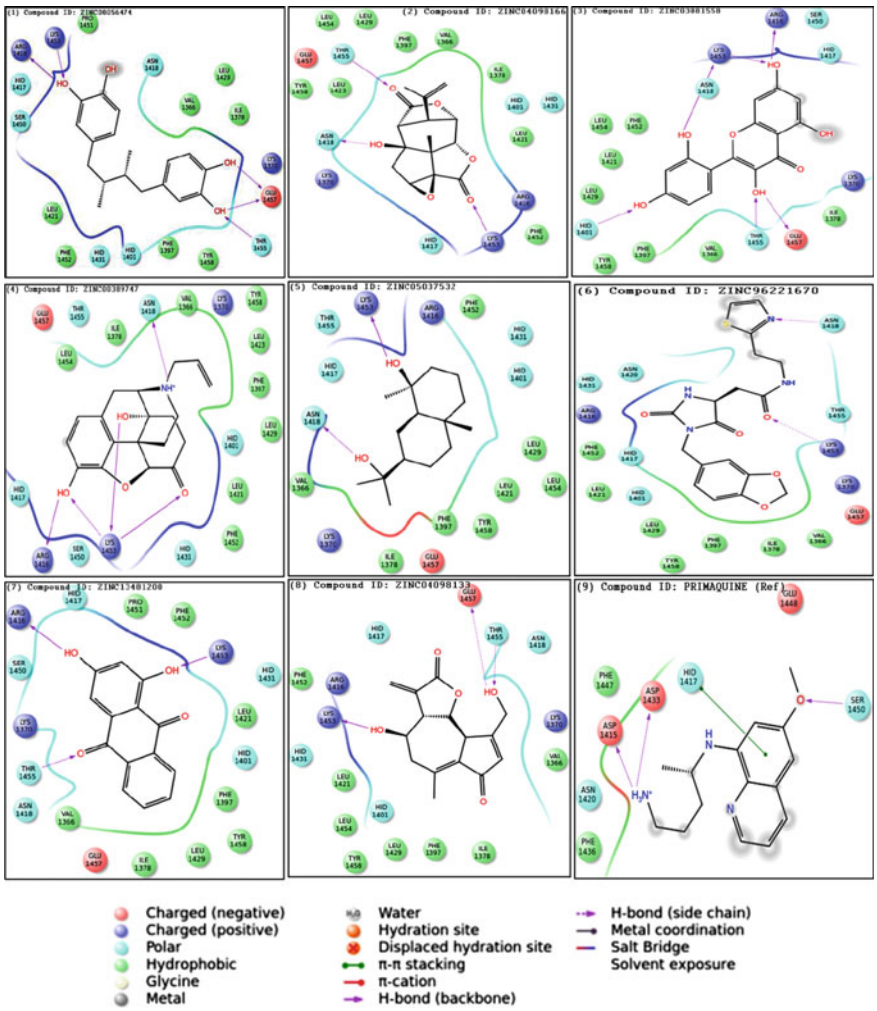

Fig. 1 This shows a figure consisting of different types Protein Ligand Interaction *1* ZINC00056474, *2* ZINC04098166, *3* ZINC03881558, *4* ZINC00389747, *5* ZINC05037532, *6* ZINC96221670, *7* ZINC13481200, *8* ZINC04098133 and *9* PRIMAQUINE

important for ADME estimation. All these values for Screened molecule are following the recommended ranges determined below in Table 2 for a drug with acceptable pharmacological properties. This depicts the excellent potential of ZINC00056474 as prospective lead PI4K inhibitor.

Table 2 ADMET Properties analysisof top eight screened compounds along with primaquine as a reference compound

ADMET	ZINC00056474	ZINC04098166	ZINC03881558	ZINC00389747	ZINC05037532	ZINC96221670	ZINC13481200	ZINC04098133	Primaquine (ref)
Mol. Wt.	302.3	292.2	302.2	327.3	240.3	402.4	240.2	276.2	259.347
Dipole	5.457	9.505	2.712	7.840	2.833	5.239	1.269	4.304	1.218
H-bond donor	4.000	0.000	4.000	2.000	2.000	1.000	1.000	2.000	3.000
H-bond acceptors	3.000	7.750	5.250	6.250	1.500	8.000	4.500	8.400	3.750
Potential energy	62.689	320.739	70.638	352.178	128.586	97.683	86.611	127.512	82.393
QPPcaco	118.645	712.910	23.713	159.174	2673.3	169.838	182.498	158.885	435.057
QPlogPw	10.487	8.816	14.329	10.848	5.036	13.705	9.198	12.976	9.415
QPlogPo/w	2.414	−0.313	0.414	1.441	3.398	1.949	1.040	0.039	2.161
QPlogS	−3.264	0.656	−2.749	−1.699	−3.741	−3.577	−2.442	−2.088	−2.415
QPlogHERG	−4.635	−1.318	−4.922	−4.135	−3.003	−4.264	−4.432	−3.663	−5.553
QPlogBB	−1.802	−0.331	−2.236	−0.267	−0.101	−1.409	−1.077	−1.276	−0.188
QPlogKP	−3.762	−3.481	−5.315	−5.078	−2.337	−3.100	−3.804	−4.382	−3.695
QPPMDK	49.399	343.161	8.668	75.084	1432.0	220.103	78.679	67.735	222.608
CNS	−2	−1	−2	1	0	−2	−2	−2	0
% HOA	78.204	76.175	53.978	74.793	100.00	78.272	73.505	66.568	86.825

Recommended ranges:

Mol. Wt.: <500; Hydrogen Bond Donor: <5; Hydrogen Bond Acceptor: <10; Log P (Octanol/water partition coefficient): <5; % Oral Absorption: >80 % High, <25 % Poor; BBB Partition Coefficient: −3; CNS Activity: −2 Inactive, 2 Active

4 Conclusion

Virtual screening method is widely used for reducing cost and time of drug discovery process. It has been clearly shown that the Structure based virtual screening approach utilized in this study successfully find eight potentially active compound on the basis of virtual screening against PI4K from the ZINC database which may be potential inhibitors of PI4K. The docked poses of these compounds resembles similar orientation as observed ligand. Therefore this kind of study shows the importance of large libraries of molecule and their use to intensify drug development process former synthesis. PI4K plays an important role in intracellular development of multiple Plasmodium species at each stage of infection in the vertebrate host. Thus, it can act as a therapeutic target for the treatment of malaria. Various safety, tolerability and immunological concerns related with already documented PIK4, making them unfit for clinical use motivated us to discover safe compound with acceptable pharmacological properties. We have proposed top eight inhibitors against PI4K, based on rational drug design. Docking studies revealed that the better binding interaction of ZINC00056474 against PI4K with reference to Primaquine. ZINC00056474 is having acceptable pharmacological properties thus it could be a futuristic perspective chemical compound for the treatment of malaria.

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