Success Case of the Application of Virtual High Throughput Screening Against Molecular Antimalarial Targets

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INTRODUCTION

Malaria is an infectious disease caused by parasites belonging to the genus Plasmodium, having five species which infect humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi.*[1]

The main symptoms presented by the disease are fever, chills, headache, vomiting, anemia, diarrhea, anorexia, fatigue. The untreated malaria can evolve to pulmonary edema, renal complications, obstructive jaundice, causing the death of infected individual.[2]

According to the World Health Organization (WHO), about three billion people are exposed to the risk of having malaria, of these, 216 million are affected by the disease and 655,000 die from it. In Brazil, the most important species are *P. vivax*, responsible for 90% of the cases, and *P. falciparum*, responsible for the most serious cases and for the mortality increase. A major factor contributing to the spread of the disease is the resistance of parasites to current antimalarial drugs used in therapy.[3]

In this context, new groups started to develop strategies to search for new antimalarial drugs. Based on molecular biology techniques and high throughtput screening. [4]

Although many studies have focused on the screening of new molecules with *in vitro* and *in vivo* antimalarial activity, few compounds reached the stage of clinical trials. Therefore, it is of fundamental importance to focus efforts to find new molecules with potential antimalarial activity to create a therapeutic arsenal.[3]

A major factor contributing to the spread of the disease is the resistance of parasites to current antimalarial drugs used in therapy.[3] In addition, the plasmodium resistance to commercially available drugs for antimalarial therapy is a threatening factor in controlling the disease worldwide.[5]

One strategy used for the development of new drugs is the use of *in silico* techniques due to high experimental costs such as X-ray crystallography and *in vivo* biological assay for few molecular targets. Hence, molecular modeling techniques, such as comparative modeling[6], docking[7], molecular dynamics[8] and virtual screening[9], have been used as a tool to development of new drugs. Such techniques allow researchers to build molecular target scaffolds to simulate and predict toxicity, activity, bioavailability and effectiveness. Therefore this rational drug design project, reduces the time and the costs to develop a new drug, wherein, virtual screening approaches, consists in the identification of novel molecules against specific molecular targets.[10] It has been largely used to obtain the pharmacoforic conformation and the binding energy, of a set of compounds against a biological receptor.[11]

In this context, our research group has studied specific targets building a database denoted by Our Own Molecular Targets (OOMT)[12] with 40 structures from Protein Data Bank (PDB)[13] and built by comparative homology modeling.[6]

In a previous study, we performed virtual screening process on 10 compounds using OOMT database. Following, the compound I showed specificity for the malaria targets. Hence, this compound was addressed for antimalarial assay. As a result of experimental work, the compound I had a satisfactory antimalarial activity. In this study, we described a success case of a hit compound obtained from virtual High Throughput Screening (vHTS). This process consisted the use of docking approach between the compound I and specific *P. falciparum* molecular targets, such as plasmepsin IV, plasmepsin II, falcipaina II[14] and PfATP6. [15]

All these proteins are present in the digestive vacuole of *P. falciparum*, except PfATP6 present in membrane. Additionally, the digestive vacuole enzymes work with optimum pH of 4-5.[16]

METHODS

The previous results of virtual screening using OOMT database motivated us to study new molecular targets against selected *P. falciparum* targets. The molecular targets plasmepsin IV plasmepsin II, falcipain II, which were obtained from PDB under codes 2ANL, 1LF3, 3BPF, respectively;[13] while PfATP6 was obtained by previous comparative modeling methodology.[15]

The promising compound was designed in MarvinSketch program where its protonation was adjusted to pH 4.5. Next, it was refined in the MOPAC[17] program using the semi-empirical parametric method 7 (PM7).[18]

The compound was oriented toward the binding site through a grid box with dimensions of 20 Å covering all binding site. The coordinates X, Y and Z were defined according to table 1 with spaced points of 1 Å centered in the ligand. Following, crystallographic ligands were re-docked into the targets to evaluate the docking methodology, obtaining the root mean square deviation (RMSD) values for heavy atoms. Two distinct approaches were used, rigid and flexible docking using programa AutoDock Vina[7]. After the rigid docking, the binding site amino acid residues were chosen for flexible docking (table 2).

	Coordenates (Å)		
	x	y	z
2ANL	54.924	13.448	25.686
1LF3	16.215	6.850	27.605
3BPF	-36.87	31.066	-47.069
PfATP6	-5.142	-48.212	8.979

Table1. Grid box size and position for all molecular targets.

In addition, the targets state of protonation was adjusted to acid pH using PROPKA from Maestro software[19].

All docking simulations were carried using AutoDock vina[7], DockThor[20] and SwissDock[21] softwares. The exhaustiveness was set to 8 to improve the docking search.

2ANL	1LF3	3BPF	PfATP6
ASP34	ILE14	GLN36	ILE251
GLY36	MET15	CYS42	LEU253
ILE75	ILE32	TRP43	PHE254
TYR77	ASP34	LEU72	GLN257
GLY78	GLY36	ASN81	LEU258
LEU131	TYR77	GLY82	ILE261
ASP214	VAL78	GLY83	ILE748
THR217	SER79	LEU84	ILE752
VAL292	ILE123	HIS174	ASN 755
ILE300	TYR192		ILE756
	ASP214		VAL759
	SER218		PHE763
			LEU815
			ILE816
			LEU821
			TYR824
			ILE825

Table 2. Flexible residues in the binding sites of molecular targets

Finally, DS Visualizer v.4.0 (Accelrys Software Inc, USA) was used to show the docking results of the binding conformations; thereby establishing the best molecular target for the compound. Moreover, logP, Molecular Weight (MW), number of hydrogen atoms acceptors and donor, Log S, Druglikeness, properties were calculated using DataWarrior program.

RESULTS AND DISCUSSION

Initially, the re-docking using AutoDock Vina process showed that the crystallographic and docked ligand shared the same conformation into the binding site. The RMSD values are represented in table 3.

Software	Molecular targets - RMSD values (Å)			
	2ANL	1LF3	3BPF	PfTP6
AutoDockVina	0.25	0.40	1.51	1.12
DockThor	7.09	2.37	1.59	3.46
SwissDock	9.16	2.94	1.89	3.04

Table 3. Root mean square deviation (RMSD)

values found after crystallographic ligands were re-docked into the targets to evaluate the docking methodology

These results evaluated the docking methodology considering the RMSD value less 2.0 Å. In this context, the AutoDock Vina program showed the best RMSD results compared with SwissDock and DockThor softwares[7].

The AutoDock Vina[7], DockThor[20] and SwissDock[21] software were used to generate the binding energy of compound for four enzymes. Table 4 shows the binding energies between the promise ligand and all targets for rigid and flexible approach.

A)

Flexible Dock using AutoDock Vina				
	2ANL	1LF3	3BPF	PfATP
Compound	-10.1	-10.4	-8.0	-12.2
Crystal	-12.7	-12.1	-8.1	-6.8

B)

Rigid Dock using AutoDock Vina				
	2ANL	1LF3	3BPF	PfATP
Compound	-8.1	-8.6	-6.7	-8.6
Crystal	-12.5	-17.9	-6.8	-7.7

C)

Using Dock DockThor				
	2ANL	1LF3	3BPF	PfATP
Compound	-30.96	-47.08	-23.38	-16.39
Crystal	-31.22	-37.96	-31.79	-20.84

D)

Using Dock SwissDock				
	2ANL	1LF3	3BPF	PfATP
Compound	-8.18	-8.36	7.50	-6.62
Crystal	-9.16	-11.33	-7.25	-6.98

Table 4. Binding energy (Kcal/mol) between the compound and binder crystallographic against Plasmepsin IV, Plasmepsin II, Falcipain II and PfATP6, using the software AutoDock Vina (Flexible and rigid), DockThor and SwissDock. Table A, B, C and D, respectively

As can be seen, the Autodock Vina program obtained more accuracy results than other softwares. In addition, both rigid and flexible approaches through AutoDock Vina suggested the 3BPF and PfATP6 as molecular targets for compound I.

The compound could perform electrostatic, van der Waals and Pi interaction. Fig. 1 shows the interaction of compound with 3BPF3 and PfATP6 into the binding site. The crystallographic ligand and compound shared the same amino acids in the binding site Fig.2.

In addition, the compound was evaluated against Lipinski rule (logP < 5, number of hydrogen bond groups acceptors (HBA) < 10, number of hydrogen bonds groups donors (HBD) (<5 and MW < 500)[22] using Data Warrior software. As a result, the compound respects the Lipinski rule having MW, HBA, HBD, and logP of 291.377 g/mol, 4, 2 and 3.33, respectively. Moreover, this molecule has aqueous solubility and druglikeness of -3.205 mol/liter and 2.76, respectively. These results are close with antimalarial drugs, like chloroquine. Furthermore, Datawarrior could not estimate any mutagenic, tumorgenic, irritant activity.

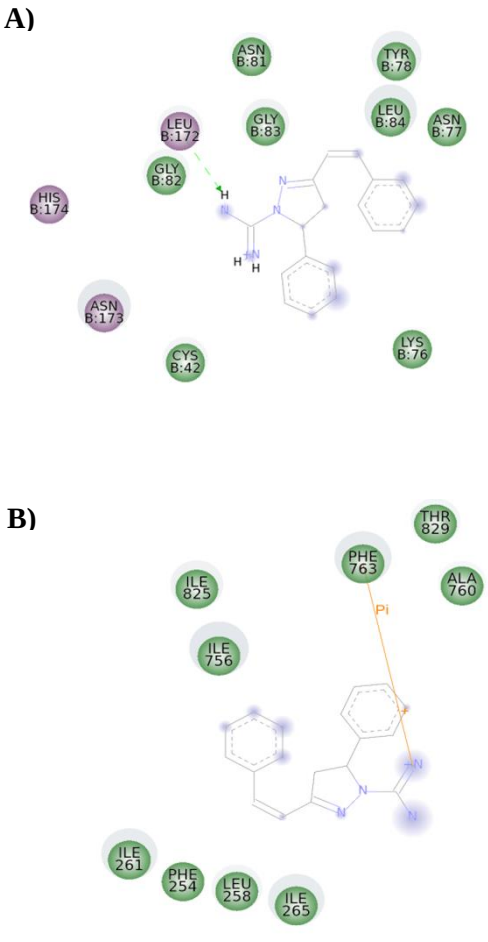

Figure 1. Electrostatic interactions, van der Waals and Pi interaction between target and compound. a) falcipaina II. b) PfTPA6. Green and pink show van der Waals interactions and electrostatic, respectively.

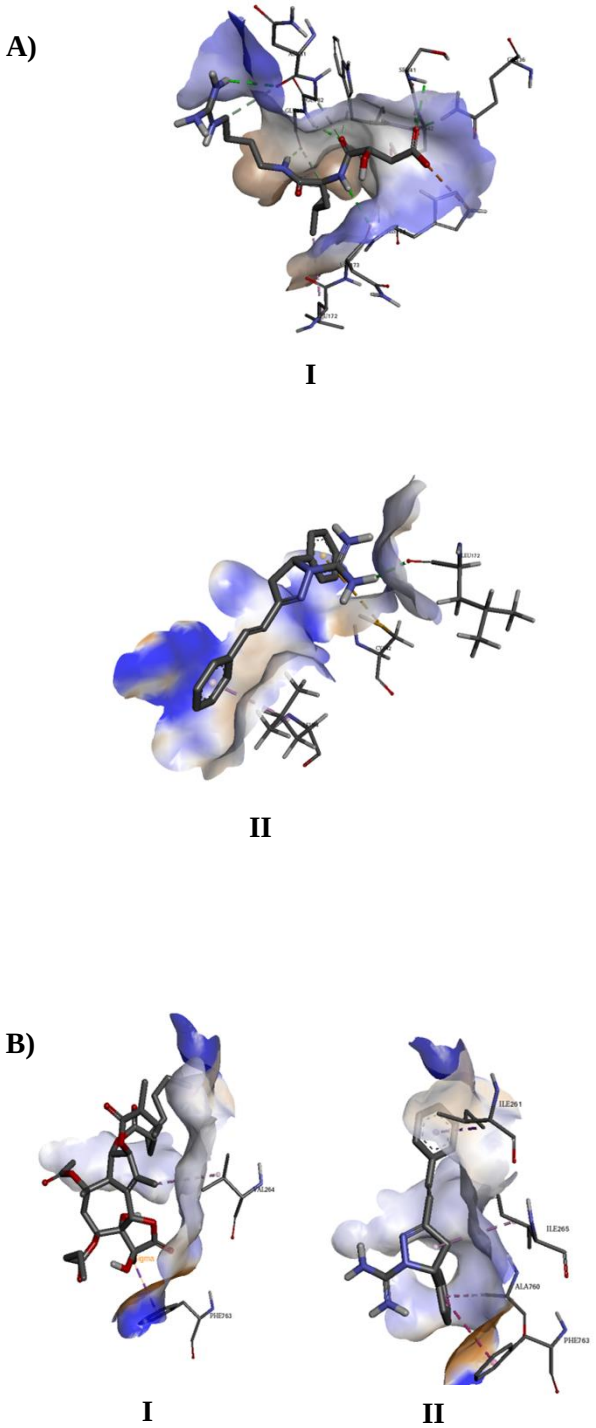

Figure 2. Binding site kept a) falcipain II; I – Crystallographic ligand and II - compound b) PfATP6; I - crystallographic ligand and II - compound.

CONCLUSIONS

This work evaluated the accuracy among three different docking methodologies, which AutoDock Vina showed more suitable results for our system.

The data addressed PfATP6 and falcipain II as a potential molecular target for this synthetic compound. In addition, this compound fits into Lipinski rule with acceptable values of Log S and druglikeness, suggestion it as a potentianl new antimalarial drug. Noteworthy, after docking studies, this compound was addressed to antimalarial assay. As a result, this compound was able to kill 78% of parasites *in vitro* test. Further ligand optimization cycle had begun generating new hit for docking and biological assay.

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