International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 190-202 www.ijprajournal.com ISSN: 2249-7781

Identification of Potential Natural Products as Novel Anti-malarials via Comparative Docking Study, Network Pharmacology and DFT Analysis

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Date of Submission: 10-1-2021 Date of Acceptance: 22-01-2021

ABSTRACT: Network Pharmacology based virtual screening was performed in order to identify potential inhibitor of Plasmodium with the polypharmacologic nature of action. A Natural Products (NPs) Library of 582 compounds with the evidence of anti-malarial activity was retrieved from extensive literature study. The Library was further compared with a set of known anti-malarials using the 3D Space analysis via Principle Component Analysis (PCA). NPs were used to dock with 15 potential drug targets of Plasmodium falciaparum and NPs-PfTarget Network was developed based on the docking score-weighted prediction. NPs-PfTarget network study revealed five molecules as selective natural products viz. Cryptopleurine, Gallic acid, Mucobromic acids, Xanthohumol and Mucochloric acid with polypharmacologic nature of action against Plasmodium drug targets. Mucochloric acid was found to form maximum interactions (relationship) with five important drug target namely, Enoyl-ACP Reductase; Triosphosphate Isomerase; Dihydroorotate Dehydrogenase; Triosphosphate Isomerase and Subtilisin-like Protease 1 of Plasmodium falciparum, indicating its multi therapeutics potential as Plasmodium inhibitor. Xanthohumol was found to be minimum ΔE (energy difference between LUMO-HOMO frontier orbital) of 0.092 kcal/mol indicating its strong reactivity as selective Plasmodium inhibitor. This investigation has successfully hypothesized the synergistic principle of the identified NPs towards the inhibition of Plasmodium.

Key Words: Network Pharmacology, Plasmodium, Natural Product, LUMO-HOMO, Malaria

I. INTRODUCTION

Malaria is an infectious disease caused by the protozoan parasites of Plasmodium genus. Plasmodium parasites are transmitted during the bite of an infected female Anopheles mosquito. Malaria became of the major cause of mortality, with an estimated report of more than 200 million cases and 600,000 deaths till 2012. There were more than 80% of all cases, of which 90% of all deaths reported form the African Sub-continent, whereas sub-Saharan Africa is facing the major malaria burden. There are five species of Plasmodium, which are pathogenic for humans, Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi, where P. falciparum accounting more than 90% cases of malaria with high mortality rate. P. vivax is accounted for more than 50% of the worldwide malaria cases, whereas the other species only shows limited impact over global malaria burden. Presently, artemisinin-based combination therapies (ACTs) were generally recommended as the first line treatment of Malaria. ACTs consisting of an artemisinin derivative along with a long-acting partner drugs. Now a day's 79 of 88 malaria-endemic countries prescribed ACTs to control uncomplicated P. falciparum malaria [1]. Artemisinins are class of fast-acting drugs and effectively targeted on the erythrocytic life cycle stages of the parasite including young gametocytes and transmission stage. However, the artemisinin activity in Falciparum malaria has been found apparently inactive, emphasizing that treatment should not depend on single substance class [2]. Chemoprophylactic strategies for malaria control were employed in most of malaria-endemic countries, targeting pregnant women and small children. Sulfadoxine-pyrimethamine combinations are the recommended drug by WHO for intermittent preventive treatment (IPT) in both populations [3]. However, the resistance of this combination are again a problematic issue in order to combat malaria. Infections with P. vivax are usually treated with Chloroquine, whereas ACTs applied mostly in areas where ACTs are the first-line treatment of P. falciparum and in areas of chloroquine-resistance of P. vivax.

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Volume 6, Issue 1 Jan-Feb 2021, pp: 190-202 www.ijprajournal.com ISSN: 2249-7781

Chemoprophylaxis is suggested for short-term stays and mostly relies on atovaquone in combination with Proguanil, Mefloquine and Doxycycline. In vitro studies and some in vivo investigation suggested a high rate of to Atovaquone and Proguanil. Hence, a more widespread use in endemic study should not be implemented [4, 5].

Increase the rate of resistance against mainstay drugs, shortcomings of existing drugs in certain populations and the scarcity on the treatment options for hypnozoites of P. vivax malaria has demanding the newer therapeutics of malaria [6].

Application and exploration of natural products (NPs) as anti-malarial agent, is gaining a tremendous attention for modern biologist to develop novel and selective inhibitor of Plasmodium [7]. Selection of NPs for drug development is adventitious over the synthetic drugs, due to lower side effects in order to establish as candidate inhibitor. Till date, extensive work on the exploration of new molecular entities as anti-malarial agents from natural sources has been conducted across the Globe. However, more extensive study on these NPs is necessary to reveal their anti-malarial potency.

Computational methodologies in the field of drug discovery were occupying a pivotal role in drug screening yardsticks. Network Pharmacology is a novel method applied to study the systems-level polypharmacology of natural products with desired therapeutics indices. Network Pharmacology study could help us to understand the mechanism of multiple action inhibitors across multiple scales from the molecular to organism level by analyzing the important features of biological networks [8]. Network pharmacology approach is extensively using in the scientific understanding the molecular mechanisms in Traditional Chinese Medicine [9, 10]. A interactive work of TCM network pharmacology and its application on a herbal formula was studied by Liang et al [11]. Li et al. also reported the action mechanisms of Ge-Gen-Qin-Lian decoction as for its efficacy against type 2 diabetes by network pharmacology Method [12]. Jiangyong Gu et al, has demonstrated the application of Natural Products as Chemical Library for Drug Discovery and Network Pharmacology based on the docking score-weighted prediction model [13].

In this study, a network pharmacology study on a large set of Natural Product (NPs) was established through molecular docking against 15 important drug targets of Plasmodium. This study provides a powerful tool for explaining the anti-malarial mechanism of NPs with multiple therapeutics potential.

II. MATERIALS AND METHODS

A systems-level polypharmacology approach was employed in order to reveal the potential natural products (NPs) as a selective and effective anti-malarial agent. A natural products (NPs) library was developed and molecular docking was performed against potential Plasmodium drug targets. A NPs-PfTargets network was developed from the Ligand-protein docking data and Density Function Theory was further used to compare the reactivity of top hits as a novel inhibitor of Plasmodium falciparum of natural origin.

Compound Library Development

Natural products (NPs) plays an important role in the treatment of Malaria. A library of 582 NPs with evidence of antimalarial/antiplasmodial activity were retrieved from extensive literature survey [14-20]. Compounds were sketched using MarvinSketch v15.7.27. Three dimensional structure of all the compounds were generated by using BIOVIA Discovery Studio v4.5 (DS v4.5) and their drug-like descriptors were predicted. Structural optimization was performed by using CHARMM based force field. Conformers of all the chemical entities were computed and further Library was in cooperated with the DS by using 3D database development module of DS. Meanwhile, a set 34 anti-malarial drug molecules from PubChem database were retrieved. The Drugs library consisting of 26 approved anti-malarial drugs and eight molecules in different clinical stages as candidate inhibitor of Plasmodium. Dataset compounds were further checked and hydrogen were added using CHARMm based smart minimizer which performs 1500 steps of Steepest Descent followed by Conjugate Gradient algorithms with a convergence gradient of 0.001 kcal mol^{-1} [21]. Diverse conformation option was applied and 250 conformations were generated using BEST conformation generation module of DS using Poling Algorithm at an energy threshold of 15 kcal mol^{-1}. The principle of rigorous energy minimization in both Torsional and Cartesian space that is employed in this option ensures the best coverage of conformational space by application of the poling algorithm. [22, 23]. Further, NPs library was used to study the ADMET properties such as

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Volume 6, Issue 1 Jan-Feb 2021, pp: 190-202 www.ijprajournal.com ISSN: 2249-7781

Blood-brain barrier (BBB) permeability, Solubility, Human intestinal absorption (HIA), Oral Bioavailability and Hepatotoxicity [24-26]. Density Functional Theory based descriptor such as HOMO (Highest Occupied Molecular Orbital Energy) and Lowest Unoccupied Molecular Orbital's (LUMO) are also annotated in order to reveal their reactivity to the target protein.

Collection of the Plasmodium Drug Targets

Potential Plasmodium drug targets were collected from Protein Data Bank (PBD) after extensive literature Survey. We have selected 15 important drug targets with their 3D structures from PDB as presented in the Table 1. All the structures were cleaned and optimized using Steepest Descent Algorithm (200 steps) at Protein Preparation module of DS v4.5. Potential Ligand binding site of all the structures were further computed using the Edit and Built binding site tool of DS v4.5. Out of 15 protein 3D structures, majority of structure were belongs to Oxidoreductase protein class and their X-ray resolution were ranging from 1.7 Å to 3.0 Å.

Table 1. Plasmodium drug targets selected for docking analysis.

Sl. No.	Protein	Short Name	PDB ID	Resolution (Å)	Classification	Reference
1	Pf Dihydropteroate Synthetase	PfDHPS	3O8A	2.3	Oxidoreductase	[27]
2	Pf Glutathione S-transferase	PfGSTs	1OKT	1.9	Transferase	[28]
3	Pf Plasmepsin-2	Pf Plas2	2IGY	2.6	Hydrolase	[29]
4	Pf Glutathione Reductase	PfGR	1ONF	2.6	Oxidoreductase	[30]
5	Pf Dihydroorotate Dehydrogenase (quinone) mitochondrial	PfDHODH	1TV5	2.4	Oxidoreductase	[31]
6	Pf Dihydrofolate reductase	PfDHFR	1J3J	2.3	Oxidoreductase	[32]
7	Pf Cytochrome b	PfCyt-b	3CX5	1.9	Oxidoreductase	[33]
8	Pf Subtilisin-like Protease 1	PfSUB1	4LVN	2.25	Hydrolase	[34]
9	**Pf** Prolyl-tRNA Synthetase	PfPRS	4TWA	3.0	Ligase	[35]
10	**Pf** Lactate Dehydrogenase	PfLDH	3ZH2	2.1	Oxidoreductase	[36]
11	Pf DOXP Reductoisomerase	PfDXP	1ONP	2.5	Oxidoreductase	[37]
12	**Pf** Enoyl-ACP Reductase	PfENR	3LSY	2.85	Oxidoreductase	[38]
13	**Pf** Triosphosphate Isomerase	PfTPI	2VFF	1.7	Isomerase	[39]
14	**Pf** Enoyl acyl carrier protein Reductase	PfENR	2O2Y	2.2	Oxidoreductase	[40]
15	**Pf** Triosephosphate Isomerase	PfTPI	4YWI	1.85	Isomerase	[41]

Chemical Space Analysis of NPs

Principal component analysis (PCA) is an orthogonal linear statistical transformation technique which can transform the data into a new coordinate system in a three dimensional system. Principal component analysis (PCA) was conducted on the NPs and the Drugs library by using the Library Analysis module of DS v4.5. In a PCA model, variance of the data which was maximized on the first coordinate was called first

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Volume 6, Issue 1 Jan-Feb 2021, pp: 190-202 www.ijprajournal.com ISSN: 2249-7781

principal component and rest of variance maximized on the second coordinate, and so on [13, 42]. Herein, we have performed the PCA analysis using 15 important drug-like descriptors as shown in the Table 2.

Table 2. Statistics of molecular descriptors of molecules in NPs and Drugs Library.

Descriptor Variable	NPs (582 Molecules)				Drugs (34 Molecules)			
	Min	Max	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.
nHBD	0.0	17	1.984	1.901	0	6	1.764	1.373
nHBA	0.0	30	5.573	3.347	2	9	4.735	1.754
MW	84.159	1169.3	418.7	139.03	248.3	503.67	383.93	68.163
MFPSA	0.0	0.622	0.194	0.084	0.072	0.425	0.205	0.095
LUMO	-0.253	0.059	-0.074	0.036	-0.200	-0.030	-0.082	0.031
HOMO	-0.324	-0.116	-0.179	0.025	-0.214	-0.153	-0.182	0.016
ALogP	-1.797	12.863	4.217	2.120	0.895	7.533	3.248	1.790
Initial RMS Gradient	5.042	1.508	1.387	1.396	13.204	2.41	7.144	4.07
Dihedral Energy	0.0054	163.01	44.238	29.994	.8996	58.168	22.464	18.834
Bond Energy	0.0	268.78	3.367	11.342	0.418	3.822	1.715	0.851
Angle Energy	0.0	222.44	15.995	15.222	3.130	185.93	18.109	30.381
Electrostatic Energy	-414.14	58.609	-18.67	39.602	-74.024	31.867	-8.174	22.353
Potential Energy	-174.91	778.43	44.756	50.446	-56.543	171.37	35.899	40.343
RMS Gradient	-4.408	4.6669	0.363	0.445	0.046	0.648	0.215	0.134
Van der Waals Energy	-29.474	151.26	-1.576	10.77	-14.438	14.003	1.550	6.876

NB: The descriptor abbreviations used in this table include: MW, molecular weight; nHBAs, number of hydrogen acceptors; nHBDs, MPSA, molecular polar surface area; HOMO, highest occupied molecular orbital energy; LUMO, lowest unoccupied molecular orbital energy.

Docking Computation

Molecular Docking techniques are widely applied molecular methods used to evaluate the binding orientation of an inhibitor to its target receptor. Ligand-protein docking allows us to understand the molecular events happening at the binding interface of ligand-protein interaction site. Docking utilities is paramount in complementing and supplementing the experimentally determined data. Herein, we have employed the CHARMm based docking software (CDOCKER) of the BIOVIA Discovery Studio v4.5 for docking simulation. CDOCKER is based on the grid-based molecular dynamics simulated annealing method by using CHARMm force field. In the CDOCKER algorithm, ligands were remain kept flexible, whereas non-bonded interactions are softened during the docking simulation. In the entire docking process the protein structures were held rigid[43].

CDOCKER algorithm is based on the following equation:

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$$V = \sum_{bonds} k_b\,(b - b_o)^2 + \sum_{angles} k_\theta(\theta - \theta_o)^2 + \sum_{dihedrals} k_\phi[1 + \cos(n\phi - \delta)$$
$$+ \sum_{impropers} k_\omega(\omega - \omega_o)^2 + \sum_{Urey-Bradley} k_u\,(u - u_o)^2$$
$$+ \sum_{nonbonded} \epsilon\left[(R_{\min_{i,j}}/r_{i,j})^{12} - (R_{\min_{i,j}}/r_{i,j})^{6}\right] + \frac{q_i q_j}{\epsilon r_{ij}}$$

where kb is the bond force constant and b − b0 is the distance from equilibrium that the atom has moved. The second term in the equation accounts for the bond angles where kθ is the angle force constant and θ − θ0 is the angle from equilibrium between 3 bonded atoms. The third term is for the dihedrals where k_Φ is the dihedral force constant, n is the multiplicity of the function, Φ is the dihedral angle and δ is the phase shift. The fourth term accounts for the impropers, that is out of plane bending, where kω is the force constant and ω − ω0 is the out of plane angle. The Urey-Bradley component (cross-term accounting for angle bending using 1,3 nonbonded interactions) comprises the fifth term, where ku is the respective force constant and u is the distance between the 1,3 atoms in the harmonic potential. Nonbonded interactions between pairs of atoms (i, j) are represented by the last two terms. By definition, the nonbonded forces are only applied to atom pairs separated by at least three bonds. The van Der Waals (VDW) energy is calculated with a standard 12-6 Lennard-Jones potential and the electrostatic energy with a Coulombic potential. In the Lennard-Jones potential above, the Rmini,j term is not the minimum of the potential, but rather where the Lennard-Jones potential crosses the x-axis [44, 45].

Network Pharmacology

Network pharmacology is a new-fangled method used to understand the polypharmacologic features of novel lead molecules in the drug discovery research. The Network pharmacology was first proposed by Hopkins [46]in 2007, where network analysis methods are used to explore the pharmaceutical action of molecules in the context of biological networks. It help us to study the action mechanisms to assess the drug efficiency by analyzing the network properties or exploring the action of compounds to the biological networks[47]. In this method relationship between drug-targets were established to figure out the multi targeted nature of small molecular inhibitor. Network pharmacology is now comprehensively used to identify the possible targets of natural products [13, 42]. The drug–target network (DTN) study is an apt method for the mapping of polypharmacologic features of natural products. It is also helpful to understand the action mechanism of NPs in order to exploring new clinical applications [9].

NPs and experimental drug targets were connected in Cytoscape v3.0.1 software [48]. The interactions between molecules and target proteins (CDOCKER docking scores lower than -10 kcal/mol) were chosen to generate a Drug Target Network (NPs-PfTarget) in which nodes represent molecules or Plasmodium target proteins. The network properties and node centralities were calculated by Network Analyzer Plugin and CentiBin in the Cytoscape workspace [49] .

Density Functional Theory analysis

Density Functional Theory analysis on the best hits were computed in order to understand the reactivity of potential NPs identified through Network Pharmacology Study. Herein, DFT analysis was carried out using the Becke's three-parameter formulation B3LYP complete geometry optimization method using basis set 6-31G* level [50]. B3LYP is the best known hybrid functional use for DFT computation with greater precision than the Hartree–Fock theory [51]. Orbital energy parameters such as LUMO (lowest unoccupied molecular orbital), and HOMO (highest occupied molecular orbital) energy values of the selected molecules were computed and compared. Further, NPs with least energy gap (LUMO-HOMO) was considered to be the most selective natural product in the NPs Library.

III. RESULTS AND DISCUSSION

Compounds Library Development

A compound's library with anti-malarial activity was developed from extensive literature. Compounds Library consisted of 582 Natural

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Products (NPs). Physiochemical Properties such as Molecular Weight, Number of Hydrogen Bond Acceptor, Number Hydrogen Bond Donor, Molecular Polar Surface Area, LUMO (Lowest Unoccupied Molecular Orbital) and HOMO (Highest Occupied Molecular Weight) etc were. Further, a Library of known drug of Malaria were also compiled and subjected for 3D space comparison with the developed NPs Library. Statistics of molecular descriptors used for the 3D space analysis using Principle component analysis were given in the Table 2. In the 3D Space plot, NPs and Drugs were found to large overlap in the chemical surface, indicating the potency of these NPs as drug-like molecules with possible anti-malarial activity. The variances of PCA1, PCA2 and PCA3 as given the Figure 1 are 0.451, 0.201 and 0.170, respectively.

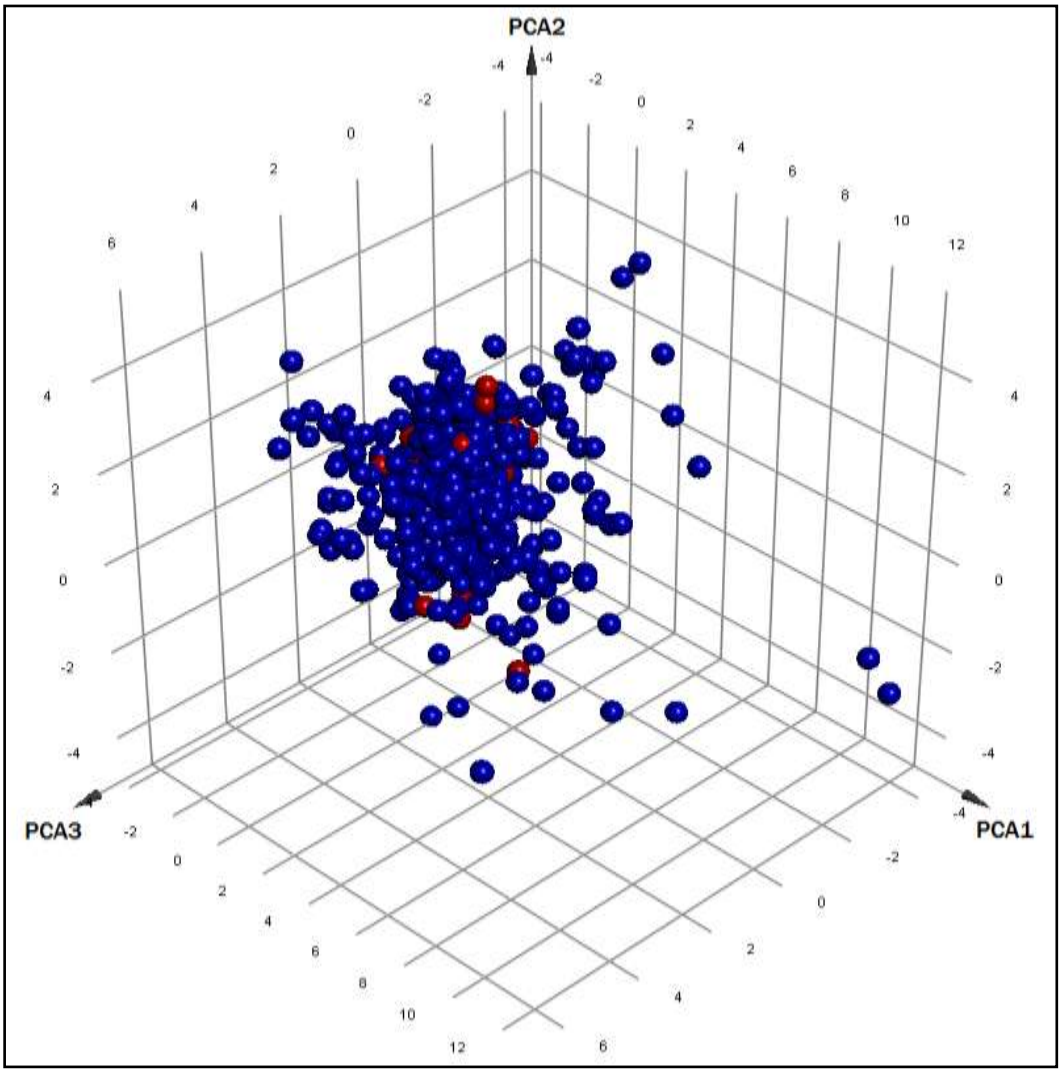

Figure 1. The distributions in the chemical space between molecules in NPs Library and Drug Library according to PCA. Blue Circles and Red circles represent NPs and Drugs, respectively.

Important ADME (Absorption, Distribution, Metabolism and Excretion) properties of the NPs Library was also computed and a larger numbers of the compounds were found to be ADME positive as presented in the Figure 2.

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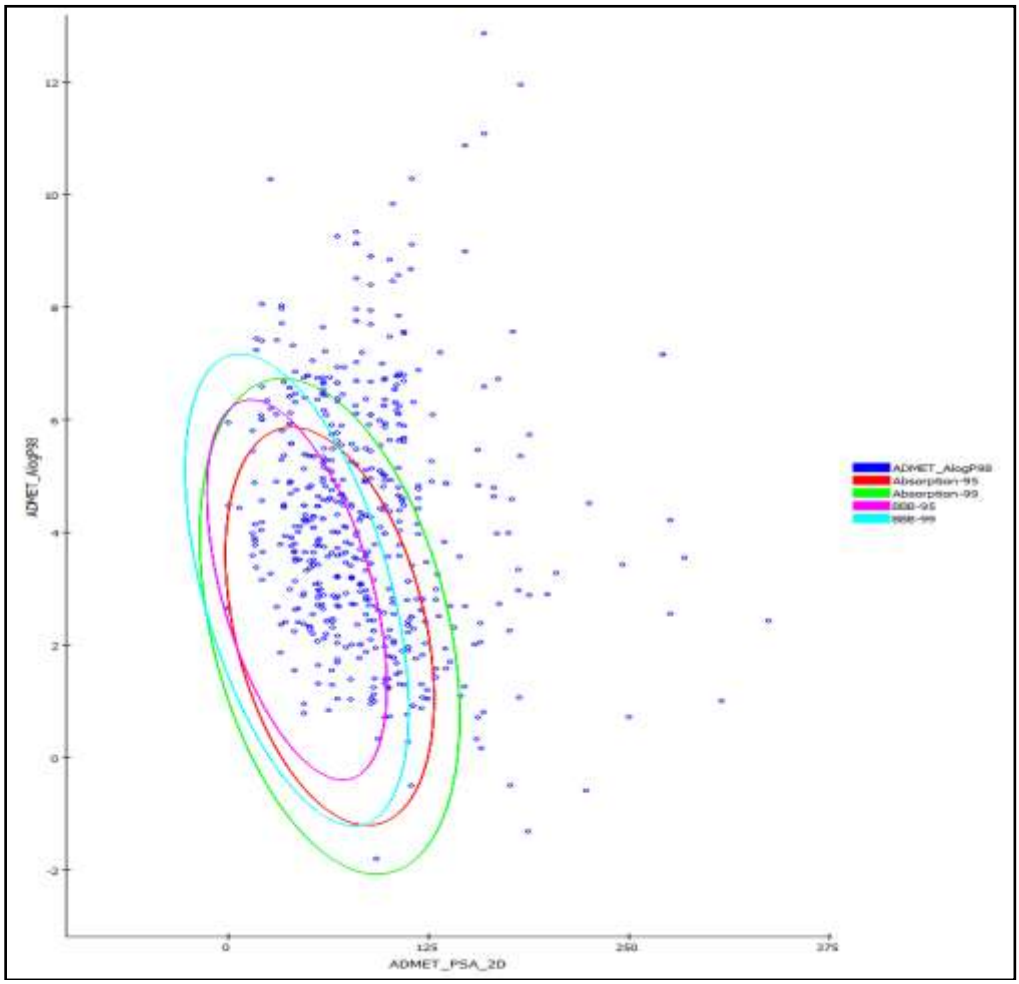

Figure 2 ADME profiling of NPs.

Docking Computation of NPs

Molecular Docking is one of the important methodology in the in silico drug designing process used to reveal the binding orientation of small molecular entities with potential drug targets. Protein-ligand binding interaction study is a pivotal, to understand the molecular mechanisms of small molecules as candidate inhibitor of a specific target. Herein, we have employed the CDOCKER algorithm of BIOVIA Discovery Studio v4.5 for the docking computation of NPs with potential Plasmodium drug targets. Docking result is analyzed based on the negative score of CDOCKER energy. NPs found to dock with only 10 potential Plasmodium drug targets, out of 15 with negative CDOCKER energy (lower than -10 kcal/mol) as presented in the Figure 3. Further, the docking results were subjected for the NPs-PfTarget network construction in order to understand the polypharmacological nature of selected NPs as Plasmodium inhibitors.

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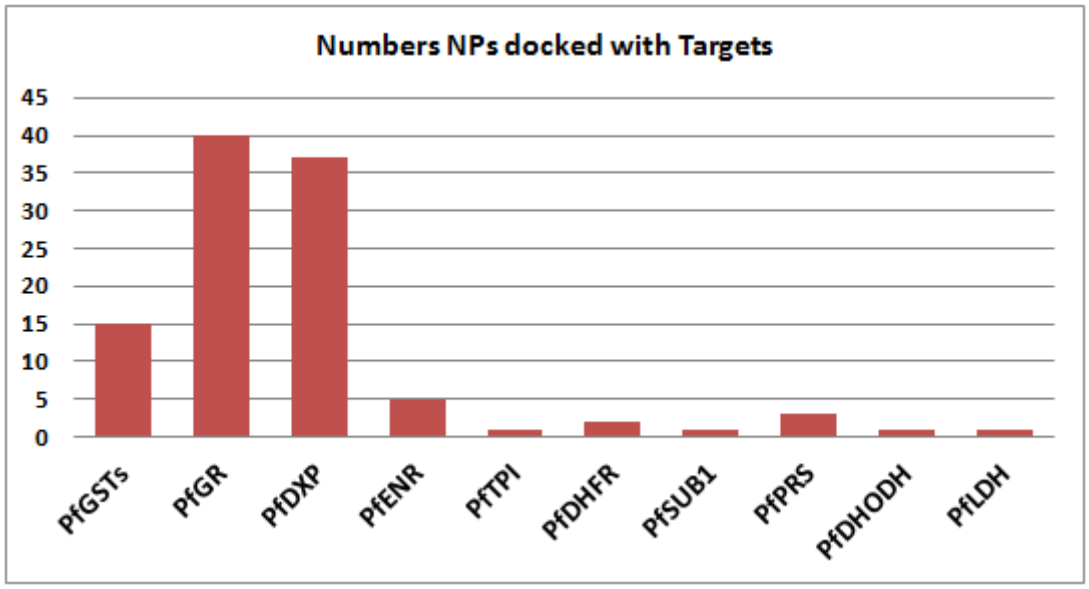

Figure 3 Plasmodium drug targets found to interact with NPs.

Network Pharmacology

Network pharmacology is a novel systems biology-based methodology used to understand the mechanism of multiple action of to drugs against multiple biological targets by analyzing the features of biological network [8]. In this investigation, we have endeavoured to reveal the polypharmacological potency of NPs to identify potential anti-malarials using Network Pharmacology approach. NPs-Targets docking result (-CDOCKER Energy) were used to develop the NPs-PfTarget Network. NPs-PfTarget Network consisting of 104 nodes (94 molecules and 10 proteins) and 107 edges as in shown in **Figure 4.4.** In the NPs-PfTarget network, the close association between NPs and Pf targets were observed (9.4 molecules per target), indicating their probable synergism as selective Plasmodium inhibitor.

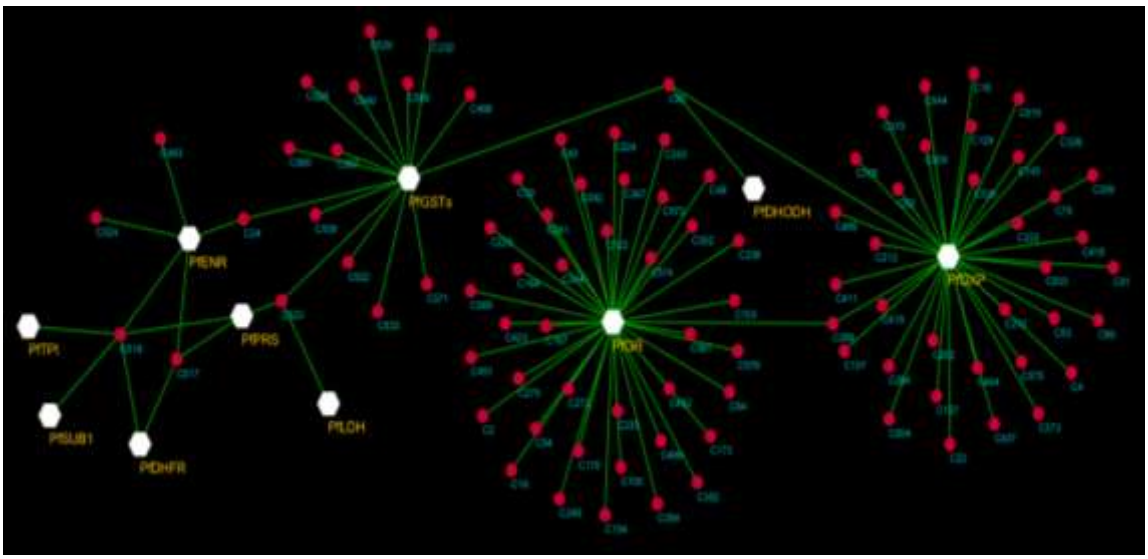

Figure 4 NPs-Pf Target Network, Red Sphere representing the NPS and while hexagon representing the Pf drug targets.

The possible interaction between NPs with Pf Targets were revealed based on the two important topological parameter namely, Degree and Betweenness Centrality [52]. These parameters were used to quantify the node (NPs or Pf Target proteins) and the extent of the influence

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of the node on the spread of information through the network. The most selected NPs with binding to multiple proteins were selected based on the Degree of the NPs- PfTarget network. The Degree and Betweenness of each node (molecules or proteins) were analyzed in the network. Among 582 compounds, only 104 were found to dock with the ten Pf targets. Finally five compounds were selected based on the maximum Degree score as presented in the Table 4.3. Important topological parameters such as Betweenness Centrality, Stress, Toplogical Coefficient were also taken in to consideration to select the top hits. Mucochloric acid was found to form maximum interactions (relationship) with five important drug target namely, Enoyl-ACP Reductase, Triosphosphate Isomerase, Dihydroorotate Dehydrogenase, Triosphosphate Isomerase and Subtilisin-like Protease 1 of Plasmodium falciparum. Finally five NPs viz. Cryptopleurine, Gallic acid, Mucobromic acids, Xanthohumol and Mucochloric acid were identified as selective NPs with polypharmacological nature of action against Plasmodium falciparum drug targets. (Figure 5). NPs were identified based on the maximum Degree score (≥ 2) in the NPs-PfTarget Network as shown in the Table 4. Further, these selected NPs may be recommended as combinatorial therapeutics for Malaria related syndromes. On, the other hand the plants sps. namely Vernonia staehelinoides Harv., Boehmeria cylindrica (L.), Terminalia bellerica (Gaertn.) Roxb, and Humulus lupulus (L.) can be also proposed as combination herbal therapy of Malaria. DFT study was further proceeded in order to reveal the reactivity of proposed NPs.

Table 3. Topological parameters of the selected NPs in NPs- PfTarget network.

Compound	Compound Name	Closeness Centrality	Stress	Degree	Betweenness Centrality	Radiality	Topological Coefficient
C518	Mucochloric acid	0.166	1184	5	0.048	0.444	0.28
C90	Cryptopleurine	0.341	5146	3	0.385	0.785	0.333
C523	Gallic acid	0.231	1508	3	0.08	0.632	0.333
C517	Mucobromic acids	0.165	402	3	0.01	0.44	0.466
C266	Xanthohumol	0.375	5600	2	0.479	0.815	0.5

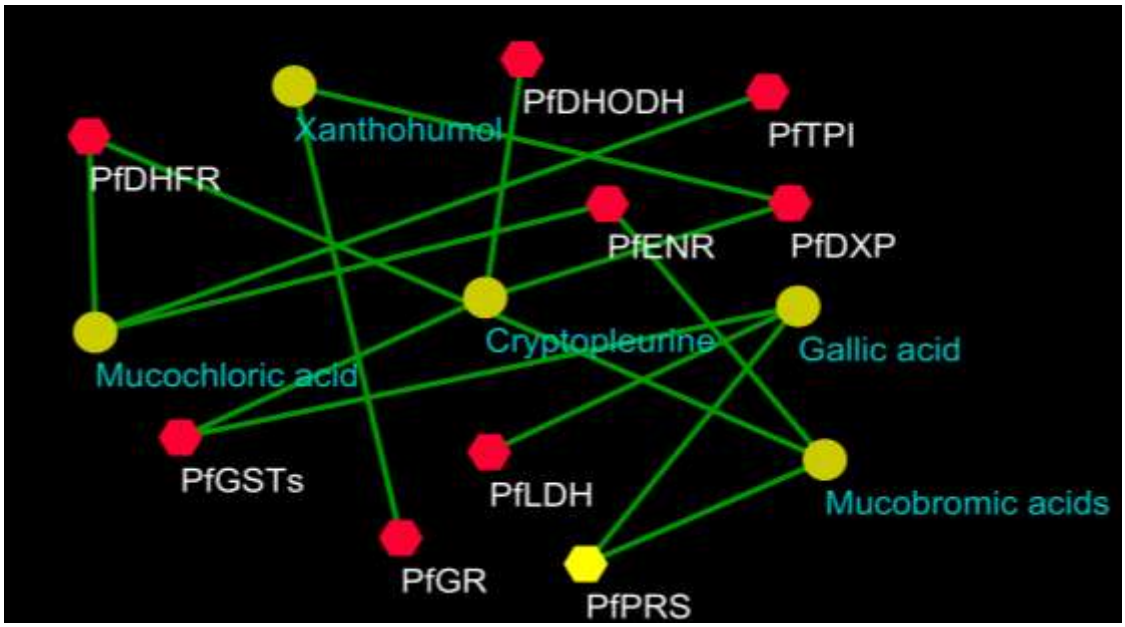

Figure 5. Compounds in the NPs-Pf Target Network with highest degree with Targets.

Table 4. Selected NPs as proposed combination therapy for Malaria Treatment.

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Compound Name	Scientific Name	Family
Mucochloric acid	Vernonia staehelinoides Harv.	Compositae
Cryptopleurine	Boehmeria cylindrica (L.)	Urticaceae
Gallic acid	Terminalia bellerica (Gaertn.) Roxb	Combretaceae
Mucobromic acids	Vernonia staehelinoides Harv. and	Compositae
Xanthohumol	Humulus lupulus (L.)	Cannabaceae

DFT Study

DFT is today one of the suitable method to study medium size and larger molecular systems [53]. Frontier molecular orbital energies-HOMO and LUMO are crucial in predicting the reactivity of molecule in the binding site of protein receptor [54]. Higher HOMO value indicates that, the molecule has good electron donating ability and as lower value implies weak electron donating ability. A smaller energy gap (between the LUMO and HOMO) of a molecules demonstrated more reactive in nature [55, 56]**.** LUMO and HOMO energy value for all the potential hits were computed as presented in the Table 4.4. Further, energy gap (LUMO-HOMO, ΔE) for all the hits and Xanthohumol was found to be minimum ΔE of 0.092 indicating its strong reactivity as a selective Plasmodium inhibitor.

Table 4 Density Functional Theory based descriptors and other 2D descriptors of identified NPs.

Formula	MW	Alogp	MPSA	LUMO (kcal/mol)	HOMO (kcal/mol)	Band Gap Energy (LUMO-HOMO) (kcal/mol)
Mucochloric acid	168.963	0.786	0.337	-0.128	-0.261	0.132
Cryptopleurine	377.476	4.835	0.127	-0.052	-0.155	0.102
Gallic acid	170.12	0.733	0.606	-0.065	-0.181	0.115
Mucobromic acids	257.865	0.954	0.316	-0.121	-0.250	0.1286
Xanthohumol	434.438	4.421	0.279	-0.0735	-0.165	0.092

NB: MW, Molecular Weight, Alogp, Water/Octanol partition correlation coefficient, MPSA, Molecular Polar Surface Area.

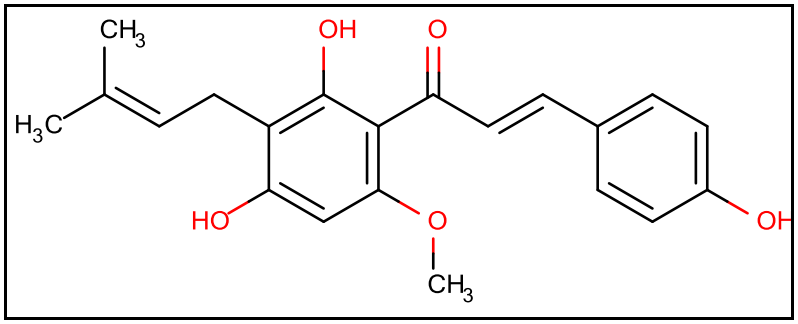

Figure 6: 2D representation of Xanthohumol

IV. CONCLUSION

Systems-level polypharmacology approach was employed to identify potential and selective Plasmodium inhibitors from a NPs Library of 582 compounds. NPs-PfTarget was developed based on the docking of NPs against important Plasmodium drug targets. From, the network analysis Pf Glutathione Reductase was found to form network with 41 NPs (Figure 4). On the other hand Mucochloric acid was found to form maximum relationship with five important drug target targets namely, Enoyl-ACP Reductase,

Triosphosphate Isomerase, Dihydroorotate Dehydrogenase, Triosphosphate Isomerase and Subtilisin-like Protease 1 of Plasmodium falciparum. In summary, five top compounds with maximum Degree values viz. Cryptopleurine, Gallic acid, Mucobromic acids, Xanthohumol and Mucochloric acid subjected for DFT analysis. DFT study has reveled Xanthohumol (Figure 4.6) as most potential inhibitor of Plasmodium with minimum ΔE (difference between LUMO and HOMO energy) of 0.092 kcal/mol. The result of current investigation is clearly indicating the novelty of these NPs as a synergistic potential as Plasmodium inhibitor.

REFERENCES

[1]. Held, J., S. Jeyaraj, and A. Kreidenweiss, Antimalarial compounds in Phase II clinical development. Expert Opin Investig Drugs, 2015. **24**(3): p. 363-82.
[2]. Noedl, H., et al., Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med, 2008. **359**(24): p. 2619-20.
[3]. von Seidlein, L., et al., Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: a workshop report. Malar J, 2013. **12**: p. 112.
[4]. Looareesuwan, S., et al., Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. Am J Trop Med Hyg, 1996. **54**(1): p. 62-6.
[5]. Korsinczky, M., et al., Mutations in Plasmodium falciparum cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob Agents Chemother, 2000. **44**(8): p. 2100-8.
[6]. Burrows, J.N., et al., Designing the next generation of medicines for malaria control and eradication. Malar J, 2013. **12**: p. 187.
[7]. Taiwo, B.J., E.O. Akinkunmi, and N. Omisore, Antimicrobial and antiplasmodial activities of a quaternary compound from Ritchiea capparoides var. longipedicellata. Afr J Tradit Complement Altern Med, 2013. **10**(6): p. 528-31.
[8]. Berger, S.I. and R. Iyengar, Network analyses in systems pharmacology. Bioinformatics, 2009. **25**(19): p. 2466-72.
[9]. Gu, J., et al., Drug-target network and polypharmacology studies of a Traditional Chinese Medicine for type II diabetes mellitus. Comput Biol Chem, 2011. **35**(5): p. 293-7.
[10]. Tao, W., et al., Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease. J Ethnopharmacol, 2013. **145**(1): p. 1-10.
[11]. Liang, X., H. Li, and S. Li, A novel network pharmacology approach to analyse traditional herbal formulae: the Liu-Wei-Di-Huang pill as a case study. Mol Biosyst, 2014. **10**(5): p. 1014-22.
[12]. Li, H., et al., A network pharmacology approach to determine active compounds and action mechanisms of ge-gen-qin-lian decoction for treatment of type 2 diabetes. Evid Based Complement Alternat Med, 2014. **2014**: p. 495840.
[13]. Gu, J., et al., Use of natural products as chemical library for drug discovery and network pharmacology. PLoS One, 2013. **8**(4): p. e62839.
[14]. Bero, J., M. Frederich, and J. Quetin-Leclercq, Antimalarial compounds isolated from plants used in traditional medicine. J Pharm Pharmacol, 2009. **61**(11): p. 1401-33.
[15]. Shankar, R., S. Deb, and B.K. Sharma, Antimalarial plants of northeast India: An overview. J Ayurveda Integr Med, 2012. **3**(1): p. 10-6.
[16]. Kaur, K., et al., Antimalarials from nature. Bioorg Med Chem, 2009. **17**(9): p. 3229-56.
[17]. Adebayo, J.O. and A.U. Krettli, Potential antimalarials from Nigerian plants: a review. J Ethnopharmacol, 2011. **133**(2): p. 289-302.
[18]. Amoa Onguene, P., et al., The potential of anti-malarial compounds derived from African medicinal plants, part I: a pharmacological evaluation of alkaloids and terpenoids. Malar J, 2013. **12**: p. 449.
[19]. Ntie-Kang, F., et al., The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malar J, 2014. **13**: p. 81.
[20]. Silva, J.R., et al., A review of antimalarial plants used in traditional medicine in communities in Portuguese-speaking countries: Brazil, Mozambique, Cape Verde, Guinea-Bissau, Sao Tome and Principe and Angola. Mem Inst Oswaldo Cruz, 2011. **106 Suppl 1**: p. 142-58.

[21]. John, S., et al., Development, evaluation and application of 3D QSAR Pharmacophore model in the discovery of potential human renin inhibitors. BMC Bioinformatics, 2011. **12 Suppl 14**: p. S4.
[22]. Arooj, M., et al., 3D QSAR pharmacophore modeling, in silico screening, and density functional theory (DFT) approaches for identification of human chymase inhibitors. Int J Mol Sci, 2011. **12**(12): p. 9236-64.
[23]. Niu, M., et al., Pharmacophore modeling and virtual screening for the discovery of new type 4 cAMP phosphodiesterase (PDE4) inhibitors. PLoS One, 2013. **8**(12): p. e82360.
[24]. Xia, X., et al., Classification of kinase inhibitors using a Bayesian model. J Med Chem, 2004. **47**(18): p. 4463-70.
[25]. Cheng, A. and K.M. Merz, Jr., Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships. J Med Chem, 2003. **46**(17): p. 3572-80.
[26]. Egan, W.J. and G. Lauri, Prediction of intestinal permeability. Adv Drug Deliv Rev, 2002. **54**(3): p. 273-89.
[27]. Booker, M.L., et al., Novel inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase with anti-malarial activity in the mouse model. J Biol Chem, 2010. **285**(43): p. 33054-64.
[28]. Fritz-Wolf, K., et al., X-ray structure of glutathione S-transferase from the malarial parasite Plasmodium falciparum. Proc Natl Acad Sci U S A, 2003. **100**(24): p. 13821-6.
[29]. Boss, C., et al., Achiral, cheap, and potent inhibitors of Plasmepsins I, II, and IV. ChemMedChem, 2006. **1**(12): p. 1341-5.
[30]. Sarma, G.N., et al., Glutathione reductase of the malarial parasite Plasmodium falciparum: crystal structure and inhibitor development. J Mol Biol, 2003. **328**(4): p. 893-907.
[31]. Hurt, D.E., J. Widom, and J. Clardy, Structure of Plasmodium falciparum dihydroorotate dehydrogenase with a bound inhibitor. Acta Crystallogr D Biol Crystallogr, 2006. **62**(Pt 3): p. 312-23.
[32]. Yuvaniyama, J., et al., Insights into antifolate resistance from malarial DHFR-TS structures. Nat Struct Biol, 2003. **10**(5): p. 357-65.
[33]. Solmaz, S.R. and C. Hunte, Structure of complex III with bound cytochrome c in reduced state and definition of a minimal core interface for electron transfer. J Biol Chem, 2008. **283**(25): p. 17542-9.
[34]. Withers-Martinez, C., et al., The malaria parasite egress protease SUB1 is a calcium-dependent redox switch subtilisin. Nat Commun, 2014. **5**: p. 3726.
[35]. Jain, V., et al., Structural and functional analysis of the anti-malarial drug target prolyl-tRNA synthetase. J Struct Funct Genomics, 2014. **15**(4): p. 181-90.
[36]. Cheung, Y.W., et al., Structural basis for discriminatory recognition of Plasmodium lactate dehydrogenase by a DNA aptamer. Proc Natl Acad Sci U S A, 2013. **110**(40): p. 15967-72.
[37]. Steinbacher, S., et al., Structural basis of fosmidomycin action revealed by the complex with 2-C-methyl-D-erythritol 4-phosphate synthase (IspC). Implications for the catalytic mechanism and anti-malaria drug development. J Biol Chem, 2003. **278**(20): p. 18401-7.
[38]. Maity, K., et al., X-ray crystallographic analysis of the complexes of enoyl acyl carrier protein reductase of Plasmodium falciparum with triclosan variants to elucidate the importance of different functional groups in enzyme inhibition. IUBMB Life, 2010. **62**(6): p. 467-76.
[39]. Gayathri, P., et al., Biochemical and structural characterization of residue 96 mutants of Plasmodium falciparum triosephosphate isomerase: active-site loop conformation, hydration and identification of a dimer-interface ligand-binding site. Acta Crystallogr D Biol Crystallogr, 2009. **65**(Pt 8): p. 847-57.
[40]. Muench, S.P., et al., Studies of Toxoplasma gondii and Plasmodium falciparum enoyl acyl carrier protein reductase and implications for the development of antiparasitic agents. Acta Crystallogr D Biol Crystallogr, 2007. **63**(Pt 3): p. 328-38.
[41]. Pareek, V., et al., Connecting active site loop conformations and catalysis in triosephosphate isomerase: insights from a rare variation at residue 96 in the plasmodial enzyme. Chembiochem, 2016.
[42]. Zhang, X., et al., Network pharmacology study on the mechanism of traditional Chinese medicine for upper respiratory tract infection. Mol Biosyst, 2014. **10**(10): p. 2517-25.

[43]. Wu, G.S., et al., Detailed analysis of grid-based molecular docking: A case study of CDOCKER - A CHARMm-based MD docking algorithm. Journal of Computational Chemistry, 2003. **24**(13): p. 1549-1562.
[44]. MacKerell, A.D., et al., All-atom empirical potential for molecular modeling and dynamics studies of proteins. Journal of Physical Chemistry B, 1998. **102**(18): p. 3586-3616.
[45]. Abdel-Hamid, M.K. and A. McCluskey, In Silico Docking, Molecular Dynamics and Binding Energy Insights into the Bolinaquinone-Clathrin Terminal Domain Binding Site. Molecules, 2014. **19**(5): p. 6609-6622.
[46]. Hopkins, A.L., Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol, 2008. **4**(11): p. 682-90.
[47]. Hoeng, J., et al., A network-based approach to quantifying the impact of biologically active substances. Drug Discov Today, 2012. **17**(9-10): p. 413-8.
[48]. Smoot, M.E., et al., Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics, 2011. **27**(3): p. 431-432.
[49]. Junker, B.H., D. Koschutzki, and F. Schreiber, Exploration of biological network centralities with CentiBiN. Bmc Bioinformatics, 2006. **7**.
[50]. Lee, C., W. Yang, and R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B Condens Matter, 1988. **37**(2): p. 785-789.
[51]. Panchmatia, P.M., et al., Halide ligated iron porphines: a DFT+U and UB3LYP study. J Phys Chem A, 2010. **114**(51): p. 13381-7.
[52]. Newman, M.E.J., A measure of betweenness centrality based on random walks. Social Networks, 2005. **27**(1): p. 39-54.
[53]. Karunagaran, S., et al., Investigation on the isoform selectivity of novel kinesin-like protein 1 (KIF11) inhibitor using chemical feature based pharmacophore, molecular docking, and quantum mechanical studies. Comput Biol Chem, 2016. **61**: p. 47-61.
[54]. Aihara, J., Reduced HOMO-LUMO gap as an index of kinetic stability for polycyclic aromatic hydrocarbons. Journal of Physical Chemistry A, 1999. **103**(37): p. 7487-7495.
[55]. John, S., et al., Potent BACE-1 inhibitor design using pharmacophore modeling, in silico screening and molecular docking studies. BMC Bioinformatics, 2011. **12 Suppl 1**: p. S28.
[56]. Perepichka, D.F. and M.R. Bryce, Molecules with exceptionally small HOMO-LUMO gaps. Angew Chem Int Ed Engl, 2005. **44**(34): p. 5370-3.