In silico identification of promiscuous scaffolds as potential inhibitors of 1-deoxy-D-xylulose 5-phosphate reductoisomerase for treatment of Falciparum malaria

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\textbf{ABSTRACT}

\textbf{Context:} Malaria remains one of the prevalent infectious diseases worldwide. \textit{Plasmodium falciparum} 1-deoxy-D-xylulose-5-phosphate reductoisomerase (\textit{PfDXR}) plays a role in isoprenoid biosynthesis in the malaria parasite, making this parasite enzyme an attractive target for antimalarial drug design. Fosmidomycin is a promising \textit{DXR} inhibitor, which showed safety as well as efficacy against \textit{Plasmodium falciparum} malaria in clinical trials. However, due to its poor oral bioavailability and non-drug-like properties, the focus of medicinal chemists is to develop inhibitors with improved pharmacological properties.

\textbf{Objective:} This study described the computational design of new and potent inhibitors for deoxy-D-xylulose 5-phosphate reductoisomerase and the prediction of their pharmacokinetic and pharmacodynamic properties.

\textbf{Material and methods:} A complex-based pharmacophore model was generated from the complex X-ray crystallographic structure of \textit{PfDXR} using MOE (Molecular Operating Environment). Furthermore, MOE-Dock was used as docking software to predict the binding modes of hits and target enzyme.

\textbf{Results:} Finally, 14 compounds were selected as new and potent inhibitors of \textit{PfDXR} on the basis of pharmacophore mapping, docking score, binding energy and binding interactions with the active site residues of the target protein. The predicted pharmacokinetic properties showed improved permeability by efficiently crossing blood–brain barrier. While, in \textit{silico} promiscuity binding data revealed that these hits also have the ability to bind with other \textit{P. falciparum} drug targets.

\textbf{Discussion and conclusion:} In conclusion, innovative scaffolds with novel modes of action, improved efficacy and acceptable physiochemical/pharmacokinetic properties were computationally identified.

\section*{Introduction}

In 2014, the World Health Organization (WHO) reported an estimated 198 million cases of malaria worldwide, and 584,000 subsequent deaths from the disease, mostly among children below five years of age (Zhang et al. 2015; Bertin et al. 2016). Anopheles mosquitoes cause malaria in human and cause infection by intracellular parasites, i.e., \textit{Plasmodium} (Winzeler 2008). There are four species of the \textit{Plasmodium}, and \textit{Plasmodium falciparum} is the most dangerous one that causes infection in human beings. \textit{P. falciparum} is highly resistant to various antimalarial drugs, for example amodiaquine, sulphadoxine-pyrimethamine and chloroquine. \textit{P. falciparum} is a multidrug resistant species and cause malaria, so for its treatment new antimalarial drugs with novel modes of action are urgently needed.

Emergence of resistance in \textit{P. falciparum} strains has hampered the treatment of malaria and has been one of the major challenges for medicinal/drug discovery chemists. There are several malarial enzymes and pathways, such as nucleic acid metabolism, oxidative stress that can serve as a potential validated drug targets for antimalarial drug design. 2-Methyl-D-erythritol-4-phosphate (MEP) pathway (present in many bacteria and \textit{P. falciparum}) to isoprenoid biosynthesis is an attractive target for developing novel antimalarial compounds. In isoprenoid biosynthesis pathway, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR, EC 1.1.1.267) is an essential enzyme that converts DXP (deoxy-D-xylulose 5-phosphate) to MEP (methylerythritol 5-phosphate) (Proteau 2004; Alam et al. 2009).

Fosmidomycin, a phosphonic acid derivative, is a potent inhibitor of \textit{PfDXR} and showed safety as well as efficacy against \textit{P. falciparum} malaria in clinical trials. However, due to its poor oral availability and non-drug-like properties, medicinal chemists focused to develop more lipophilic inhibitors (Murakawa et al. 1982; Kuemmerle et al. 1985). Fosmidomycin derivative is the diminutive molecule, which has the ability to inhibit the recombinant \textit{P. falciparum} DXR enzyme in \textit{in vitro} and \textit{in vivo} studies. It has an anti-malarial action and well tolerated in the human beings. Modification in the simple structure of the fosmidomycin derivative to improve its drug characteristics has been challenging (Kurz et al. 2007; Ortmann et al. 2007; Tahar & Basco 2007; Wiesner et al. 2007; Haemers et al. 2008). In the current era of drug design and discovery, bridging the gap between the computational and a medicinal chemist is indispensable for synthesizing...
a blockbuster drug molecule. According to Langer, pharmacophore modelling technique is one of the successful approaches that could open the road-blocks faced due to the gap between computational and medicinal chemistry scientists (Langer 2011). Pharmacophore modelling has emerged as a powerful and fast tool for identifying and optimizing the drug candidates. A pharmacophore is an ensemble of essential features (hydrogen bonds, hydrophobic, metallic or ionic contacts, etc.) responsible for optimal interaction between a small active molecule and a target protein to trigger its biological response (Langer & Hoffmann 2006; Langer 2011). This 3D feature-based pharmacophoric models have been successfully applied in virtual screening (VS) to select other molecules from virtual databases to match the specified structural libraries. VS is an alternative approach to high-throughput screening (HTS) and is an efficient tool for early identification of potential hit scaffolds from the databases of millions of compounds. VS process, either ligand or receptor based, include successive steps: target selection, database preparation, pharmacophore model generation, 3D screening and prioritization of compounds for final confirmation of biological activity (Lavecchia & Giovanni 2013).

In our group, a major part of our research is focused on computer-aided drug design with subsequent synthesis and testing of new chemical entities as putative drugs for the treatment of various diseases (Hassan et al. 2013; Rashid et al. 2013; Rehman et al. 2014). In continuation of our endeavor and, considering the importance of pharmacophore modelling (Wadood et al. 2012, 2014; Wadood & Ul-Haq 2013), it was planned to explore novel and promiscuous scaffolds for the treatment of Falciparum malaria with improved efficacy and acceptable ADMET properties. Herein, we describe a model for the design of complex-based pharmacophore to facilitate the discovery of PfDXR inhibitors.

Materials and methods

Preparation of receptor protein

The three dimensional (3D) crystal structure of PfDXR, was retrieved from the Protein Data Bank (PDB) [PDB Code 4GAE]. The 3D protonation and energy minimization of the retrieved protein were carried out by using Molecular Operating Environment (MOE) software with the default parameters of energy minimization algorithm [gradient: 0.05, Force Field: MMFF94X] (www.chemcomp.com).

Pharmacophore model generation and validation

The crystal structure of PfDXR in complex with a fosmidomycin derivative was used for the generation of the complex-based pharmacophore model (Umeda et al. 2011) using MOE (2014-15). The generated pharmacophore model was validated by a test database of 50 known inhibitors (Altincicek et al. 2006; Singh et al. 2007; Deng et al. 2011; Jansson et al. 2013) of PfDXR enzyme.

Pharmacophore-based virtual screening

Virtual screening of chemical databases could aid in finding novel lead compounds. Virtual screening has the advantage over other de novo design methods because hit compounds can be easily obtained from commercial sources for biological activity assay (Thangapandian et al. 2011). The validated pharmacophore model was used as a 3D query to screen Chembridge database (www.chembridge.com) using the software MOE. Then the screened results were further filtered by following the Lipinski’s ‘Rule of five’, which describes the drug-like properties of compounds. Drug likeness is a property that is most often used to characterize novel lead compounds (Muiegge 2003) by screening of structural libraries. Lipinski’s rule of five states that a molecule having druggable properties must have molecular weight less than 500 Da, the hydrogen bond acceptor atoms (HBA) less than 10, hydrogen bond donor atoms (HBD) less than five and the value of log P should be less than five (Lipinski et al. 1997).

Molecular docking

All the retrieved hits were docked into the binding pocket of PfDXR to further reduce the number of hits by using MOE-Dock implemented in MOE using the default parameters of MOE, i.e., Placement: Triangle Matcher, Rescoring 1: London dG, Refinement: Forcefield, Rescoring 2: London dG for each ligand 20 conformations were generated and saved. Then the top ranked conformation for each hit was saved in a separate database. On the basis of docking score, the top poses were selected for further evaluation. The resulted binding interactions between these hits and protein were visually observed using LigPlot implemented in MOE.

Calculations of the binding affinity and binding energy

To find the most potential ligands, the binding affinities of the hits-PfDXR complexes were estimated with GB/VI (generalized Born/volume integral) contained solvent scheme executed in MOE (Labute 2008). The non-bonded interaction energies are the Generalized Born interaction energy between the ligand and receptor molecule which comprises Van der Waals, Coulomb electrostatic interactions and implied solvent interaction energies. The receptor molecule and ligands have strain energies, which were not taken into account. The solvent molecules were ignored during the calculation. The receptor atoms in the vicinity of the ligand are retained flexible during calculation while the receptor atoms which are away from the ligand were retained rigid, but were focused to tether restraints that discourage gross movement. In the binding pocket, the atoms of the ligand were set free to move. Before an estimation of the binding affinity, an energy minimization of the binding pocket in the PfDXR-ligand complex was done in each case. After energy minimization, the binding affinity for each hit was calculated and reported in units of Kcal/mol.

In silico prediction of pharmacokinetic and pharmacodynamic properties

Various descriptors like logP, solubility (Sw, calculated as logS by MOE software and converted to mg/ml units), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), number of rotatable bonds (NOR) and molecular weight (MW) were calculated by MOE software. While, logD7.4 and total polar surface area (TPSA) was calculated via Marvin 6.0.0 software of Chemaxon (Marvin 2013). Renal clearance (Cl), plasma protein binding (PPB), percentage unbound in plasma (% unbound) and Log Permeability were calculated theoretically. Log of distribution (S + logD) was calculated by MedChemDesigner™ Studio of Simulation Plus (2014) (www.simulations-plus.com).

Results and discussion

The aim of this study was to design a virtual screening approach useful to predict new structural scaffolds for the inhibition of
PfDXR enzyme. Pharmacophore modelling has been widely used in drug discovery and is considered an important technique for its use in virtual screening. The current study describes the use of a complex-based pharmacophore model, determines interaction points from structural data of receptor-ligand complex to lead the improvement of binding affinity and increasing selectivity. The in silico ADME and Toxicity predictions were carried out of the predicted hit compounds. Additional investigations on their in silico promiscuity binding of the retrieved hit compounds with different P. falciparum targets was also carried out. An overview of the study design is shown in Figure 1.

**Generation and validation of pharmacophore model**

Crystal structure of PfDXR in complex with a fosmidomycin derivative (Figure 2(a)) was used for the generation of the complex-based pharmacophore model. In the first step, chemical features were visualized by analyzing 2D structure of the co-crystallized ligand. Standard pharmacophore features such as HBA, HBD, ionizable or charge transfer (positive or negative), aromatic ring and hydrophobic (HYD) were considered. Subsequently, a detailed analysis of binding site was carried out to investigate favourable ligand-receptor contact regions. Interactions correspond to HBD and HBA were retained with a distance of 4.0 Å and hydrophobic interactions were retained if they were within 4.5 Å from hydrophobic residues such as the Met, Phe or Cys. In the building of a pharmacophore model, the essential chemical features for the generation of pharmacophore were recognized by visualizing 2D functionalities of ligand (Figure 2(b)). A detailed structural analysis of PfDXR binding site and protein-ligand complex interactions were investigated via ligand interaction tool in MOE, showing three regions; (1) binding region for phosphonate moiety containing polar environment of Ser269, Ser270, Ser306, Asn311, (2) a hydrophobic region of pyridine group with His293, Trp296, Met298, Cys338 and Pro358 residues and, (3) a binding pocket for hydroxymate group with Mn$^{2+}$ coordinated with carboxylate groups of Asp231, Glu233 and Glu315 residues. In addition, carbonyl oxygen of acetyl group pointed towards Ser232 and formed a hydrogen bond with its side chain hydroxyl group (Xue et al. 2013). The schematic 2D view of the interactions between fosmidomycin derivative and binding pocket residues is shown in
These Ligand-complex interaction points were used to generate pharmacophoric features. The analysis of the phosphonate binding region revealed that the ligand should exhibit both hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) features presumably due to the pH in which PfDXR-complex was crystallized. Hence, the first two pharmacophoric features (F1 and F2, red colour) were suggested on the hydroxyl group of phosphoric acid moiety of ligand in the region favourable for HBD (Don) interactions. The third pharmacophoric feature (F3, Acc[HydA], brown colour) was developed on a hydrophobic region of pyridine group. The fourth feature F4 was Atom Q, developed on the nitrogen of the hydroxylamine moiety. This feature was generated in the pharmacophore model to enhance its specificity. There were two HBA sites on the ligand corresponds to the carbonyl oxygen of the acetyl group and oxygen atom bonded with phosphorus. These two favourable acceptor features corresponded to the HBA interaction in the regions of Ser232 and Asn311 respectively. Therefore, the fifth and sixth (F5 and F6, cyan colour) pharmacophoric points were defined by hydrogen bond acceptor interactions. The final six-point pharmacophore model (Figure 2(d)) consists of, two hydrogen bond donors (Don), a hydrophobic point (Acc[HydA]), one Atom Q (heavy atom feature), and two hydrogen bond acceptors (Acc) points.

From previous literature, a test database of fifty known inhibitors (Altincicek et al. 2000; Singh et al. 2007; Deng et al. 2011; Jansson et al. 2013), having 37 active and 13 inactive compounds, was used for the validation of the pharmacophore model. The pharmacophoric mapping of three hits is shown in Figure 3 which are correctly mapped by the developed pharmacophore model. In order to check the drug-like properties of the retrieved hits, the properties of each hit were studied for Lipinski’s rule of five. Strictly, following the Lipinski’s rule of five, finally, 700 hits were selected for further assessment using molecular docking.

Pharmacophore-based database screening

The validated pharmacophore model was used for the screening of ChemBridge database. From this screening 1238 hits were identified that mapped on the developed pharmacophore model. The pharmacophoric mapping of three hits is shown in Figure 3 which are correctly mapped by the developed pharmacophore model. In order to check the drug-like properties of the retrieved hits, the properties of each hit were studied for Lipinski’s rule of five. Strictly, following the Lipinski’s rule of five, finally, 700 hits were selected for further assessment using molecular docking.

Molecular docking

To further refine the hits, all the retrieved hits were docked into the active site of PfDXR enzyme. In order to check the reliability of the docking method, co-crystallized ligand was re-docked into the active binding site of PfDXR. The root mean square deviation (RMSD) between co-crystallized and re-docked conformation was found 1.089 Å, suggesting high docking reliability. On the basis of docking scores, top ranked 200 compounds were selected for visual inspection. The criteria for the selection of the potential compounds included, appropriate filling of the binding site, overall matching of interactions correspond to HBD, HBA and hydrophobic interactions in the range of 4.0–5.5 Å, and metal ligation with divalent manganese (Mn²⁺) in the ligand–enzyme complex. After the critical assessment of the binding modes, 60 hits revealed the important interactions with the active site residues of the target protein. These 60 hits were further ranked on the basis of their fit value, i.e., binding affinity and binding energy.
Binding energy and binding affinity calculations

The binding affinities of these 60 compounds comprising the ligand of the complex structure were calculated with GB/VI (generalized Born/volume integral) through MOE and identify the most promising ligands. After the energy minimization, for each hit, binding affinity was measured in units of Kcal/mol. Finally, on the basis of pharmacophore mapping, molecular docking, binding energy and binding affinity calculations, 14 compounds were selected as most promising inhibitors for PfDXR enzyme (Table 1).

Binding interactions of finally selected hits

The docking conformation of the top-ranked compound 1 (one of the finally selected hit) showed interactions with the important active site residues Lys205, Asp 231, Ser269, Ser306 and Asn311. Carbonyl oxygen of the compound 1 formed hydrogen bonds with active site residues Asn311 and Ser269 at the distance of 3.24 and 3.22 Å, respectively (Figure 4(a)). The oxygen atom of the hydroxyl moiety of the compound showed interactions with active site residues Asn311 and Lys205. The nitrogen atom of the compound showed a polar contact with active site Ser 306 of the enzyme. The coordination pattern of compound 1, divalent manganese (Mn\(^{2+}\)) and carboxylate groups of Asp231, Glu233 and Glu315 residues was similar to that of the native ligand. Taking into the consideration of the fitness value, compound 1 have a high docking score (−16.6152), strong binding affinity (−11.254 Kcal/mol) and lower binding energy (−45.698 Kcal/mol) (Table 1). This is the indication of full occupancy in the binding site and thus showed strong binding.

Top-ranked compound 2 also showed favourable interactions and with a good docking score −16.1193, binding affinity −9.806 Kcal/mol and binding energy −42.262 Kcal/mol. This compound showed coordination with Mn\(^{2+}\) via oxygen atoms of hydroxyl and carbonyl moieties of the compound. Asn311 and Lys205 formed hydrogen bonds with the oxygen atom of carbonyl moiety of the compound whereas Asp232 and Glu233 showed hydrogen bonds with oxygen atom of hydroxyl moiety of the compound (Figure 4(b)). Furthermore, one hydrogen bond between the nitrogen atom of the compound 2 and the active residue Ser269 was also observed.

Similar results were observed for other finally selected hit compounds, for example the docking conformations of compounds 12–14, showed that these compounds also showed interactions with the important active site residues of the enzyme. In case of compound 13, the docking conformation showed that Ser306 and Ser270 formed hydrogen bonds with nitrogen and carbonyl oxygen atoms of the compound respectively. Lys205, Asp231, Ser232 and Asn311 were found to show polar interactions with the oxygen atoms of hydroxyl moieties of the compound 13 (Figure 4(c)). In case of compound 14, it was observed that this compound showed polar interactions with important active site residues Lys205, Asp231, Ser269, Ser270 and Asn311 of the enzyme (Figure 4(d)).

To the best of our knowledge, no other group has done any pharmacological study on the above predicted compounds. However, these compounds were found to be derivatives of piperidine, piperidine-4-carboxamide and imidazolidine and from the literature it was confirmed that these derivatives have many pharmacological functions. For example, compounds 1, 2, 4, 5 and 6 were found to be piperidine derivatives while compound 3 was found to be imidazolidine derivative. Piperidines are used as local anesthetics, such as mepivacaine, ropivacaine, and bupivacaine are extensively used in clinical practice (Brau et al. 2000). Selective inhibition of a number of enzymes has rendered piperidine alkaloids as important paraphernalia in the study of biochemical pathways (Gulluoglu et al. 2007; Khalid et al. 2013). 4-Piperidone derivatives were found to be superior

![Figure 3. (a–c) Pharmacophore mapping of most active Hits 1–3 respectively.](image-url)
| S.NO | Cambridge ID | Docking score (S) | Binding affinity Kcal/mol | Binding energy Kcal/mol |
|------|--------------|-------------------|---------------------------|-------------------------|
| 1    | 19754435     | -16.6152          | -11.254                   | -45.698                 |
| 2    | 54282478     | -16.1193          | -9.806                    | -42.262                 |
| 3    | 13118929     | -15.8232          | -8.215                    | -41.122                 |
| 4    | 31049317     | -13.1053          | -5.518                    | -39.471                 |
| 5    | 70996071     | -13.9176          | -7.605                    | -39.178                 |
| 6    | 11967450     | -12.8502          | -6.920                    | -39.765                 |
| 7    | 11996146     | -12.9556          | -6.589                    | -39.853                 |
| 8    | 26587127     | -12.8264          | -7.841                    | -25.880                 |
| 9    | 57569821     | -12.5040          | -7.833                    | -25.135                 |

(continued)
Like piperidines, imidazolidines are also very important class of compounds. Many imidazolidine derivatives have psychopharmacological properties, such as phenytoin, famous for its anticonvulsant efficacy, but also effective in the treatment of neuropathic pain (Queiroz et al. 2015). Pharmacological functions of imidazolidine or hydantoin derivatives are mainly shown in antibacterial, diminishing inflammation, relieving cough and asthma, lowering blood sugar (Xue et al. 2013). Compounds 4–5 are piperidine-4-carboxamide derivatives act as novel antihypertensive agents (Watanuki et al. 2011). These findings further support our prediction about compound 1–14 to be act as potent chemotypes against malaria.

### Computational pharmacokinetic studies

Setbacks have greatly outnumbered success in drug design and development process. During the past decade, there was a long series of unsuccessful drug candidates from clinical trials. Currently, computational predictions of the physiochemical properties of drugs at design stage have enabled the medicinal chemists to select the suitable drug candidates for lead optimization and potential drug development. The eventual objective of the computational ADMET property modelling is the prediction of the *in vivo* pharmacokinetics of a new potential drug molecule in man, whilst it exists as only a virtual structure. Fosmidomycin is a promising DXR inhibitor showed safety as well as efficacy against *P. falciparum* malaria in clinical trials. However, due to poor oral bioavailability and non-drug-like properties of fosmidomycin, medicinal chemists focused to develop more lipophilic inhibitors with an aim to get new chemical entities with improved effect on their biological activity and pharmacokinetic properties.

Molecular descriptors like *n*-octanol-water partition co-efficient (*Log P*), *Log D* at various physiological pH, molecular weight of the compound and permeability of cell membrane (*LogPer*) were computed to determine the absorption and compliance with Lipinski’s rule of five of compounds. The results
suggest that all the compounds followed the Lipinski’s rule of five. The enzyme DXR is located inside the malarial parasite and also in various species of *E. coli*. For this, the logPer of all the compounds were computed and compared with fosmidomycin (van de Waterbeemd & Gifford 2003). A high logPer value of all compounds demonstrate that they can be efficiently absorbed (Table 2). The lead compound 1 and 13 exhibited high logP and logPer and thus can be efficiently absorbed through the bacterial cell membrane. Likewise, compounds 6, 8, 12 and 13 can be absorbed through the bacterial cell membrane due to their improved logP and logPer values. The predicted low logPer value for fosmidomycin (−7.9172) could be the reason of its high dosage (1200 mg t.i.d) selection for patients (could be explained). The logD value at various physiological pH of the gut was computed and is shown in Table 2. All the compounds were found highly polar at pH of 1.5 and 5 in the stomach. However, improved logD values of compounds 1, 5, 7 and 13 in the intestinal environment (pH 6.5) predicted the potential of the compounds to be absorbed from the intestinal compartment more efficiently than in the stomach.

The tendency of compounds to remain in the blood stream for an extended span of time is vital for their efficient delivery to already invaded liver sporozoites and blood merozoites or gametocytes. The computed parameters used to assess the propensity of compounds to sustain in the blood stream are the predicted plasma protein binding (PPB, represented as Log K) (Figure 5) (Yang et al. 2012). Lead compound 1 showed efficient PPB with reduced plasma unbound percentage (29.14%) and Log K of −0.38 as compared to fosmidomycin (Log K = 1.99) and this was further supported by the experimental PPB of 1% as obvious from literature (Na-Bangchang et al. 2007). Compounds with logD >3 and molecular weights in the range of 500–700 Da was associated with high PPB (90–98%) (van de Waterbeemd et al. 2001, Dalvie et al. 2010). Therefore, lead compound 1 was

![Figure 4. The three dimensional binding mode analysis of most active and least active compounds into the active binding site of PfDXR (a) Hit compound 1; (b) Hit compound 2; (c) Hit compound 13; (d) Hit compound 14.](image)

Table 2. Pharmacokinetic and pharmacodynamic descriptors of HITS.

| Comp | S + logD | MW | HBD | Acc | NOR | TPSA | RO5 | % unbound | LogK | LogPermeability | pKa | logD1.5 | logD3 | logD6.5 | logP |
|------|----------|----|-----|-----|-----|------|------|-----------|------|----------------|-----|---------|------|---------|------|
| 1    | 2.505    | 322.472 | 2    | 5   | 5   | 7    | 91.9 | 0.14      | −0.38| −5.2606       | 7.46| 12.68   | −0.47| 0.43    | 1.82 |
| 2    | 0.476    | 344.413 | 3    | 6   | 6   | 5    | 68.9 | 8.4       | −0.24| −2.16         | 0.99| 1.08    | −0.54| 0.49    | 2.22 |
| 3    | 0.576    | 375.471 | 4    | 7   | 7   | 7    | 162.0| 0.97      | −2.24| −2.24         | −0.99| 0.91    | −0.54| 0.49    | 2.22 |
| 4    | −0.423   | 469.604 | 2    | 4   | 9   | 120.6| 347.6| 1.33      | −0.97| −2.16         | 0.99| 1.08    | −0.54| 0.49    | 2.22 |
| 5    | 0.745    | 373.517 | 2    | 4   | 7   | 95.66| 78.92| 0.57      | −6.2902| 5.61        | −2.56| 0.14    | 0.79 | 0.85    | 1.27 |
| 6    | 1.776    | 396.917 | 2    | 4   | 6   | 73.24| 49.76| −0.004    | −5.1389| 8.13        | −2.23| 1.7      | −0.36| 1.27    | 1.27 |
| 7    | 1.146    | 342.345 | 3    | 6   | 6   | 78.87| 67.59| 0.32      | −5.8177| 5.54        | −2.62| 0.09    | 0.52 | 0.56    | 1.51 |
| 8    | 1.888    | 344.457 | 2    | 4   | 6   | 76.56| 46.59| −0.05     | −5.7014| 8.35        | −1.99| 1.6      | −0.33| 1.51    | 1.51 |
| 9    | −0.404   | 311.424 | 2    | 5   | 5   | 73.16| 98.45| 1.8       | −5.8195| 8.62        | −3.59| −3.35   | −2.2 | −0.09   | 1.27 |
| 10   | 0.835    | 309.414 | 2    | 5   | 7   | 94.14| 76.38| 0.51      | −6.7377| 2.14        | −4.57| 2.4      | −1.09| 1.07    | 0.67 |
| 11   | 1.032    | 362.472 | 3    | 6   | 6   | 73.24| 70.81| 0.38      | −5.4145| 8.18        | −2.84| 2.34    | −1.01| 1.06    | 0.67 |
| 12   | 0.827    | 351.446 | 3    | 5   | 6   | 79.23| 76.61| 0.52      | −5.7603| 8           | −2.16| 1.54    | −0.16| 1.34    | 1.34 |
| 13   | 1.718    | 389.418 | 2    | 5   | 5   | 70   | 51.41| 0.02      | −5.0597| 8.01        | −3.05| 1.78    | −0.78| 0.45    | 0.45 |
| 14   | 0.11     | 309.408 | 3    | 5   | 5   | 73.16| 96.89| 1.49      | −5.8356| 8.75        | −3.05| 2.86    | −1.78| 1.8    | 2.46 |
| 15a  | −2.244   | 188.472 | 3    | 5   | 6   | 98.07| 99    | 1.99      | −7.9172| 1.85        | −2.38| 4.46    | −4.53| 2.21    | 1.27 |

*Fosmidomycin.
predicted to have a potential of sustained duration of action as compared to all the other compounds, including fosmidomycin and a correlation of logD with Log K ($R^2 = 0.8817$) is shown in Figure 5.

Cerebral malaria is one of the most devastating etiologies and its aggravated symptoms are due to blocking of cerebral capillaries. Therefore, the central nervous system (CNS) penetration criteria of compounds was also computed and encouragingly, most of the compounds were found to possess improved CNS penetration due to their MW < 400 Da and TPSA < 90Å² (Figure 6). Furthermore, it was found that with the increase in the number of rotatable bonds (NOR) and decrease in permeability (negative LogPer), the compounds are less likely to enter the CNS. Likewise, compounds with decreased logP values may not exhibit low CNS penetration (Table 2). The proneness of compounds towards multidrug resistance transporters such as P-glycoprotein (P-gp) was also computed and used as a criterion to predict CNS penetration. The results suggest that all the compounds, except compound 4, were found to be less substrate at P-gp efflux transporter due to their reduced MW < 400 Da, logP < 5, HBD count, TPSA < 90Å² and increased basic pKa values (Table 2) (Hitchcock 2012). Negative logD values may reduce the blood brain barrier (BBB) penetration of compounds. Compounds 4, 9 and 15 (fosmidomycin) exhibited low BBB penetration due to their negative logD values ($-0.423$, $-0.404$ and $-2.244$, respectively). However, there are some reports of headache and vertigo from Fosmidomycin mono-therapy to patients and these adverse effects are considered due to its MW (<300 Da) and some amount of fosmidomycin molecule squeezing along the gap junctions of BBB (Na-Bangchang et al. 2007; Meyers 2008).

The malarial attack in pregnant women has severe symptoms due to a depressed immune system. This is a particular health concern in areas with characteristically large family sizes, like The Gambia. Moreover, distribution and entrance of to the placenta of pregnant women is a critical scenario and may aggravate foetus deformities. For this, the compounds should not cross the placental barrier of pregnant women. The increased pKa value of
all the compounds (>6) further negate the entry of compounds to foetal environment and ameliorates the safety criteria for our hit compounds (Pacifici & Nottoli 1995). The drug of choice in the early stage of pregnant women is chloroquine, as it does not enter the foetal environment through placenta due to its pKa = 7.29 and is therefore prescribed as a first line treatment for the initial therapy of malaria to pregnant women. Encouragingly, all the compounds, except 5, 7 and 10 can be administered to pregnant women due to increased basic pKa of compounds (Table 2).

Number of HBDS, molecular weight and logD values are the important factors in predicting the metabolic fate of the compound. Renal clearance is related to the molecular weight distribution and HBD capacity of compounds. Small molecules with MW below 350 Da will eliminate via renal and higher molecular weight compounds via partially faecal route. In addition, the increase in logD values of compound is responsible for increased metabolic clearance over renal clearance with lipophilicity. Similarly, compounds with HBD > 4 will have a greater tendency to eliminate via phase-II metabolism (glucoronidation) (Proudfoot 2005). Glucuronide conjugations for compound 3 is predicted to be high due to the number of HBD = 4. Likewise, compounds 3, 4, 5, 6, 11 and 13 will be eliminated majorly via the faecal route as have molecular weight greater than 350 Da. All the other compounds, including lead compound 1 will eliminate partially via renal and the faecal route due to molecular weight range from 250–350 Da (Ghibellini et al. 2006). Similarly, the highest log D value possessed by the lead compound 1 > 8 > 6 > 13 also conferred them to be released metabolically via bile canaliculi and faecal route (Smith et al. 1996). Fosmidomycin will be released solely by the renal clearance due to MW less than 250 Da (Na-Bangchang et al. 2007).

G-protein coupled receptors, also known as GPCRs, and various protein kinases are among the two largest target families that are linked frequently with promiscuity. They are also highly safety-relevant. Further the compounds being prone towards serotonergic receptor type 5-HT2B may elevate the heart valve damage (Rothman et al. 2000; Hutcheson et al. 2011). These seven transmembrane helical proteins preferably bind to the basic compounds; therefore, liabilities of synthesized compounds towards these GPCRs were assessed (Figure 7) (Andrews & Lloyd 1982; LaBella 1991). It is shown in the Figure 7 that compounds pertaining pKa> 7 are more substrate at GPCR ligands.

The importance of ion channels in medicinal chemistry point view relates to the inhibition of human Ether-à-go-go Related Gene (hERG) potassium channel. hERG potassium channel is associated with electrical activity of the heart that coordinates with heart’s beating and its inhibition leads to major consequences of prolonged QT-syndrome provoking cardiac arrhythmia and sudden death (Pearlstein et al. 2003). While highlighting the importance of human immune-deficiency virus (HIV), the protease inhibition potential was additionally computed by utilizing the free online programing of molinspiration (www.molinspiration.com). The more positive values direct the proneness of compound to be liable to the specific receptor as shown in Table 3. Negative E-values ensured the safety prediction towards specific receptor family as a toxicological point of view. Compound 9 (E = 0.51) was estimated to possess minor cytochrome enzyme...
High proneness of compound 9 (indicated in italic font) to act as enzyme inhibitor was estimated, however, rest of the compounds were predicted non liable to other off-targets, except being prone towards GPCRs are protease inhibitors. *Indicates the reference compound (Fosmidomycin).

**In silico promiscuity binding**

Resistance to current antimalarial drugs has certainly increased the global health concern. Emergence of resistance in different *P. falciparum* strains due to mutation has rendered all current treatments ineffective, which create problems for medicinal chemists to develop new antimalarial agents. In recent years, the main focus of drug design and discovery has shifted away from one target-one disease paradigm and most of the strategies are now aiming to develop drugs that can modulate multiple targets in parallel fashion. These multi-targeted treatments, also referred as pharmacological promiscuity, preclude rapid drug resistance against the infectious diseases and inflammation. The term ‘pharmacological promiscuity’ describes the activity of a single compound against multiple targets. In an attempt to gain insights and to explore the probable binding modes of hit compounds, molecular docking of these compounds was performed into the active site of *Pf*LDH via MOE docking program. Crystal structure of *Pf*LDH (PDB ID 1LDG) with NADH as the co-crystallized ligand and substrate oxamate was selected for these studies. The binding model of compound 4 is shown in Figure 7(a). Key interactions stabilized compounds and the important contact residues for the docked ligands were Met 30, Ile 31, Gly 99, Asn 116, Arg 171, His 195, Ser 245, Pro 246 and Pro 240. All these compounds were fitted well in the NADH pocket. The MOE docking score of NADH/ligand-protein complex was also compared. The best five compounds showed docking score values closest to NADH (16.0480 Kcal/mol). Compound 4 has the docking score -15.8243 Kcal/mol and showed hydrogen bond interactions with Thr 139, Asn 116 and Arg 171 (Figure 7(a)). On the other hand, fitness values of interactions for compound 3, 5 and 2 were observed, −12.6623, −11.3723and −10.5436 Kcal/mol, respectively (Table 5).

**Plasmodium falciparum dihydroorotate dehydrogenase**

Dihydroorotate dehydrogenase (DHODH) is a flavin mononucleotide (FMN)-dependent present in mitochondria that catalyzes the oxidation of dihydroorotate (DHO) to produce orotate, the fourth step in the de novo biosynthesis of pyrimidine. The human DHODH (*Hs*DHODH) and *P. falciparum* DHODH (*Pf*DHODH) belong to class 2 DHODHs (Phillips & Rathod 2010). Molecular docking of these compounds was performed into the active site of *Pf*DHODH via MOE docking program. Crystal structure of *Pf*DHODH (PDB ID 3OA8) was selected for these studies (Booker et al. 2010). Hit compound 4, with high a MOE score (−14.8259 Kcal/mol), established HBD interactions with Gly 181 at the distance of 1.65 Å. Sulfonyl oxygen anchored itself in such a way that enables a strong hydrogen bond with Tyr 528. Similarly, the oxygen atom of the hydroxyl group stabilized through an H-bond with Arg 265 (Figure 7(b,c)). MOE docking scores of all retrieved hits is shown in Table 4.
The observed negative fitness values of binding interactions (i.e., docking score) revealed that the active site residues of the top scoring poses were examined. Key interactions stabilized hit compounds and the importance of a functional/lipophilic group to the lead compound in order to develop more lipophilic inhibitors and also to probe for extra binding interactions with the target; (2) on in vivo models. The efforts to design new and promiscuous chemical scaffolds with improved effect on their biological activity and pharmacokinetic properties are the future perspectives for the treatment of Plasmodium falciparum malaria.

Table 5. MOE docking scores (in Kcal/mol) of retrieved hits showing promiscuity binding with different P. falciparum targets.

| Hit compound no. | MOE Docking Scores |
|------------------|---------------------|
|                  | 1LDG | 308A | 1J3K (qM) | 1J3J (WT) | 1J3J (dM) |
| 1                | 9.2675 | 12.5662 | 11.8362 | 10.9831 | 11.0900 |
| 2                | 9.2677 | 14.3089 | 12.4862 | 12.6259 | 11.9734 |
| 3                | 12.6663 | 14.5123 | 12.7736 | 12.2308 | 12.9205 |
| 4                | 12.8263 | 14.8259 | 12.8598 | 13.3367 | 13.7884 |
| 5                | 12.6723 | 12.1097 | 12.2094 | 12.3811 | 11.4676 |
| 6                | 10.3436 | 11.3174 | 12.3297 | 11.6160 | 11.3610 |
| 7                | 8.7451 | 13.1541 | 10.9049 | 11.2097 | 12.1894 |
| 8                | 8.8891 | 13.0939 | 13.4182 | 11.9330 | 12.2181 |
| 9                | 9.6732 | 10.6460 | 9.8219 | 10.7437 | 11.0437 |
| 10               | 9.7762 | 9.3029 | 9.5540 | 10.1136 | 11.2766 |
| 11               | 9.6596 | 10.5546 | 9.9551 | 10.9400 | 10.8403 |
| 12               | 9.3396 | 10.5081 | 9.4946 | 10.3982 | 10.8906 |
| 13               | 9.3454 | 9.5650 | 9.2500 | 9.7040 | 10.0582 |
| 14               | 9.4954 | 10.7130 | 10.2995 | 11.0763 | 11.0158 |

For better visualization, a color map is used; for compounds having docking scores smaller than 10 Kcal/mol are indicated with italic font. Value between 10 and –12 Kcal/mol are indicated with bold font and the values higher than –12 Kcal/mol are indicated with bold-italic font.

Table 4. Different targets of human malaria parasite P. falciparum and their PDB IDs.

| S. no. | Target | Abbreviation | Organism | PDB ID |
|--------|--------|--------------|----------|--------|
| 1      | Dihydroorotate dehydrogenase | PDOXDH | P. falciparum | 308A   |
| 2      | Lactate dehydrogenase | pLDH | P. falciparum | 1LDG   |
| 3      | Dihydroorotate reductase-Thymidylate synthase (double mutant) | pDHFR-TS | P. falciparum | 1J3I   |
| 4      | Dihydroorotate reductase-Thymidylate synthase (quadruple mutant) | pDHFR-TS | P. falciparum | 1J3K   |
| 5      | Dihydroorotate reductase-Thymidylate synthase (wild type) | pDHFR-TS | P. falciparum | 1J3J   |

Plasmodium falciparum dihydroorotate reductase-thymidylate synthase

Dihydroorotate reductase-thymidylate synthase from P. falciparum (P/DHFR-TS) is a well-defined and extensively investigated target for malarial as well as other protozoal drug design. It catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) in the biosynthesis of deoxythymidylate (dTMP) (Vaitchankel et al. 2012). P. falciparum has acquired resistance to the antifolate marketed drugs such as pyrimethamine and cycloguanil. Resistance is caused by mutations in the active site residues of P/DHFR-TS. In this preliminary study, we performed molecular docking of hit compounds into the active site of the wild type P/DHFR-TS complexed with a triazine inhibitor (PDB ID 1J3I), a double mutant P/DHFR-TS (C59R and S108N) complexed with pyrimethamine (PDB ID 1J3I) and a quadruple mutant P/DHFR-TS (N51I, C59R, S108N, I164L) complexed with WR99210 (PDB ID 1J3K) (Yuvaniyama et al. 2003). Key interactions stabilized hit compounds and the important contact residues for the top scoring poses were examined. The binding models of top scoring compounds were illustrated in Table 5 and Figure 7(d,e,f). The observed negative fitness values of binding interactions (i.e., docking score) revealed that these hits were tightly fitted into the active sites (Table 5). Hit compound 4 exhibited the highest docking score in all cases.

Conclusion

In this work, we described the development of pharmacophore-based virtual screening approach for DXR inhibitors. Virtual screening of complex-based pharmacophore has led to the identification of 14 hits scaffold from the ChemBridge database. These fourteen compounds selected from the final hits were subjected to in silico ADME studies. Compound 1 is predicted to have acceptable pharmacokinetics/drug-like properties and could be used as a novel scaffold for further development of novel multi-functional inhibitors for Falciparum malaria. Based on our findings in this study, we are convinced that our understanding of the ADME/Toxicity predictions and binding orientations of these promiscuous hit compounds against PDXR and other targets could help the medicinal chemist to design future chemotherapeutic strategy to avoid rapid drug resistance against Falciparum malaria. These predictions are promising and hence deserve to be extended to more comprehensive study including; (1) further investigation on structural optimization of these hits focusing on the medicinal chemistry strategy of addition of another functional/lipophilic group to the lead compound in order to develop more lipophilic inhibitors and also to probe for extra binding interactions with the target; (2) on in vivo models. The efforts to design new and promiscuous chemical scaffolds with improved effect on their biological activity and pharmacokinetic properties are the future perspectives for the treatment of Falciparum malaria.
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