Molecular docking studies of the selected derivatives of Nitroketene dithiolate, Decalin-β-keto ester enolate and Furo pyrazole - Better drug candidate screening for the treatment of Malaria

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ABSTRACT

The Malaria virus is a serious threat to human Beings. The present investigation is about the in-silico drug screening of 75 (Nitrogen, sulphur and oxygen containing) derivatives of Nitroketene dithiolate, decalin-β-keto ester enolate and Furo pyrazole to control the viral growth by blocking the active sites of Malaria protein dihydrofolate reductase enzyme. The molecular docking tools used were PyRx 0.8, PyMol 2.3.2, and ligplus 1.4. The presented study identified two derivatives of Decalin-β-keto ester enolate, as a potential better alternative to the commercial drugs proguanil which are being used for the treatment of Malaria. As the two derivatives, predicted to have better binding and drug properties with simple structure and positive Blood Brain Barrier, it is of interest to consider these 2 compounds for further in vitro and in vivo evaluation to declare them as better drug candidates for Malarial infection.

KEYWORDS

Malaria, dihydrofolate reductase, Nitroketene dithiolate, Decalin-β-keto ester enolate, Furo pyrazole derivatives, PyRx, PyMol, Ligplus, SWISS ADME, Blood Brain Barrier.

INTRODUCTION

Malaria is the most chief parasitic disease in humans, over 100 countries, three million people were affected by transmission. One to two million deaths are caused by this disease which is equal to 150 to 300 deaths per hour [1-2]. The parasites protozoan of genus plasmodium is responsible for this disease. The deadly life cycle of this malaria parasites is carried out depends on both humans and mosquitoes. The female anopheles mosquito transmits this parasite from one person to another. Plasmodium develops in the abdomen of the mosquito and is transmitted in the saliva of an infected insect each time it takes a new blood meal. When the human is bitten by this infected mosquito the parasites reaches the liver in 30 minutes. Here the reproduction of the parasite starts swiftly. The parasites that enter into the red blood cells got reproduced after bursting, which spreads in to the host’s blood. It is spread by another mosquito and the life cycle continues in this manner [3]. Inspite of its complexity the disease can be cured and prevented. Human lives can be saved if the disease is detected in the earlier stage and treated proportionately. To prevent and to avoid or
contain epidemics and other unfavourable situations a known action is necessary for this disease. The Hi-tech to prevent, monitor, diagnose and treat malaria exists. dihydrofolate reductase is a small enzyme that plays an assisting role, but an important role, in the building of DNA and other processes. It manages the state of folic acid (vitamin C), a sly organic molecule that commutes carbon atoms to enzymes that need them in their reactions of particular importance. An essential component of DNA thymine bases is built by the enzyme thymidylate synthase uses these carbon atoms. It has to be recycled once folate has released its carbon atoms. This process is done by dihydrofolate reductase. Malaria is treated by using many antimalarial drugs. Patients suffering from malaria are treated with different combination of drugs. Malaria parasite is stopped by the Proguanil is a prophylactic antimalarial drug, Plasmodium falciparum and Plasmodium vivax, from reproducing once it is in the red blood cells. It is done by inhibiting the enzyme, dihydrofolate reductase, which is responsible in the reproduction of the parasite [4]. Attempts also have been made to mimic human transketolase to block Plasmodium falciparum transketolase.[5] The aim of our present investigation is to perform in silico drug screening of 75 (Nitrogen, sulphur and oxygen containing) reported derivatives [6-14] of Nitroketene dithiolate, decalin-β-keto ester enolate and Furo pyrazole to control the viral growth by blocking the active sites of Malaria protein dihydrofolate reductase. An attempt was made to identify the derivatives with more binding energies and drug property than the commercial drug Proguanil.

MATERIALS AND METHODS

In our study we used biological databases like PDB (Protein Data Bank) and free software’s like PyMol 2.3.2, PyRx 0.8 and ligplus 1.4. The PDB (Protein Data Bank) is the common worldwide accounts of Structural data of Biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971 (The Protein Data Bank, 2000)[15]. It uses X-ray crystallographic and NMR methods to determine the structural data of Biological macromolecules. Ramachandran plot [16] of dihydrofolate reductase protein, depicted in Figure 1, is generated using Pro Check 2.3 to find stable conformations. The red, brown, yellow regions represent the favoured, allowed and generously allowed regions respectively, where the circles and squares represent amino acid of protein [17].

![Ramachandran plot of dihydrofolate reductase protein](image)

**Fig. 1.** Ramachandran plot of dihydrofolate reductase protein generated using Pro Check 2.3
Synthesis of Lead Compounds

Of the 75 derivatives [6-14] docked for in silico screening, the Decalin-β- keto ester enolate derivatives were found to be the best drug candidates. The general procedure [13-14] for the synthesis of the derivatives of the Decalin-β- keto ester enolate is described below as presented in Scheme 1. The β-ketoester I (0.5 mmol) in dry acetone (10ml) was added to potassium carbonate (2.5 mmol), which was dispersed in dry acetone (15 ml) under magnetic stirring and nitrogen atmosphere. After stirring at room temperature for about 20 min alkylation agent (benzyl bromide II or Methyl Iodide IIa) (2.5 mmol) in dry acetone (15 ml) was added over a period of 10 min the reaction mixture was stirred for 12 h by which time reaction was complete (TLC), the crude reaction mixture filtered through a pad of Celite and the solvent was removed.

The 1H NMR spectra of crude diastereomeric benzylated products (III & IV / IIa & IVa) were recorded to determine the ratio of E/Z benzylated products. The individual isomers were separated by column chromatography on silica gel 200 mesh with 5-10% EtOAc-hexanes. Analytical samples were obtained by repeated column chromatography. The alkylated products obtained were either colourless solids or light-yellow oils.

Scheme 1. Synthesis of derivatives of decalin-β-keto ester enolate

Preparation of ligand structure

The chemical structure of derivatives was prepared by ChemDraw Ultra 12.0. A total 45 compounds of derivative of nitroketene dithiolates, 18 compounds of derivative of decalin-β-keto ester enolate and 12 compounds of Furo Pyrazole was minimize the optimization energy of the molecule using Gaussian 3 and Gauss view 5.0. The standard control compound structures (Proguanil) and these derivative of molecules was minimize in the MOL SDF format were converted to PDBQT file using PyRx tool to generate atomic coordinates while 3D structure of glycoprotein of neuraminidase was accessed from Protein Data Bank. Decalin-β- keto ester enolate SMILES was obtained from SDF files. The structure of drug was visualized through Ligplot. Different analogues of Decalin-β- keto ester enolate derivatives drug were generated by using the SMILES of the original drug and then modifying it. The 3D structures of the analogues were obtained by using the PyMol 2.3.2.
The docking analysis of Decalin-β-keto ester enolate derivatives with dihydrofolate reductase inhibitor was performed by using PyRx 0.8 docking software.

**Preparation of Protein structure**

Protein target were downloaded from database Protein Data Bank (PDB). 1MVT is the PDB ID of the target protein (PubMed: 12657784). The resolution of the protein is 1.80 Å. The target protein was visualized through PyMol. The following Figure 2 shows the 3-D structure of the receptor.

![Figure 2. Crystal Structure of dihydrofolate reductase (PDB:1MVT)](image)

X-ray crystal of protein structure from protein Data Bank are downloaded based on literature survey. PDB ID for protein structure and The active site is situated in the N-terminal half of the sequence, which includes a conserved Pro-Trp dipeptide; the tryptophan has been shown to be involved in the binding of substrate by the enzyme [18].The protein had co-X-ray ligand (co-crystallized ligand) in the binding site. All heteroatoms were removed from PDB ID:1MVT, which make the complex receptor free of any ligand before docking. The Graphical User Interface Program “PyRx” was used to run and analyse the docking simulations.

2.4. Protein-ligand docking

(i) **Grid Box Generation**

The grid parameter of protein was generated using AutoDock Tool. The grid-box was created that was large enough to cover the entire protein binding site and accommodate the ligand to move freely in it. The grid center of x, y, z-axes are 0.5662, 35.598, 5.2785 and number of grid points in x, y, and z-axes were set to 50×50×50. The distance between two connecting grid points was 0.375 Å. The center of the ligand in the X-ray crystal structure was used as the center of the grid-box. To the protein structures that do not have ligands in the binding site, the center of the active binding site was estimated from the structure and taken as the center of the grid-box.

(ii) **Ligand Docking**

The docking of ligands to the protein was performed by using AutoDock Vina Software. Docking was performed to obtain the possible conformations and orientations for
the ligand at the binding site. Non-polar hydrogen atoms were merged by using the software. All bonds of ligands were set to be rotatable. The best conformation was chosen with the lowest docked energy. An illustration of the screen shot of the docked structure of the ligand 53 with the virus protein 1MVT if given in the Figure 3.

![Figure 3](image)

**Fig. 3.** Pyrx view for Ligand 53 -1MVT complex

PyRx explores the path to dock the two molecules, namely drugs and an enzyme Dihydrofolate reductase receptor fit together. The molecules binding to a receptor, inhibit its function, and thus act as drug. The collection Decalin-β- keto ester enolate, its derivatives and receptor complexes were identified via docking and their relative stabilities.

### RESULTS AND DISCUSSION

In our study of the virtual screening of the drug candidates for the treatment of Swine Flu by using the free molecular docking software PyRx 0.8, we screened 75 organic molecules (Ligands / Analogs) whose structures are presented in Table 1 and 2. They are, 45 derivatives of Nitroketene Dithiolate, 18 derivatives of Decalin-β- keto ester enolate & 12 derivatives of Furopyrazole. They were subjected to molecular docking with the selected proteins of the pathogens causing, Malaria (protein-1MVT). The structure and nature of the peptide chains of the protein is verified by ‘Ramachandran plots’

The ligand scoring parameters such as binding energies with the proteins, [19] Lipinski parameters, [20] ADME / BBB parameters and drug likeness scores [21] are presented in the Table 3 and Figure 4. All the 75 Ligand molecules pass the 4 rules of the Lipinski and 75% of them, pass all the 5 rules. 25% of the ligands log P values ranges from 5 to 8 as presented in the Table 3. Most of the ligands (80%) pass the ADME test by having good GI absorption and 65% of the ligands have positive Blood Brain barrier (BBB) diffusion, as given in the Table 3. As BBB values for most of the ligand are positive, they can also be considered for potential CNS drugs.
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<td>-5.7</td>
<td>-1.31</td>
<td>50*</td>
<td>-9</td>
<td>0.16</td>
<td>75</td>
<td>-8.3</td>
<td>-0.42</td>
</tr>
</tbody>
</table>

*The best Binding affinity and drug score of Decalin-β- keto ester enolate derivatives.
Fig. 4. Graphical representation of (a) Binding energy Vs Derivatives (Analogs) (b) Drug-likeness score (Positive scores are more drug likeness)
The results of protein–ligand energy of binding affinities is presented in the Table 3 and Figure 3, are encouraging. For the 1MVT protein of the Malaria, the binding energies of the derivatives range from -9.8 to -3.9 kcal/mol for the 75 ligands and the binding energy of Decalin-β- keto ester enolate derivatives ranges from -6.6 to -9.4 kcal/mol. In Molecular docking studies, generally, the best drug candidates are decided based on the drug scores of the ligands. The drug scores for the 75 ligands are arrived by considering the 4 criteria: (i) The binding energies of the 75 ligands, (ii) Their Lipinski parameters, (iii) Their ADME parameters and (iv) Their Drug likeliness score. The drug scores of the best 2 ligands and structural features of the Protein – ligand complexes for 1MVT type of protein under each criterion, are discussed as follows:

**Analog 53:** From the Lig-Plot Figure 5(b), it evident that the hydrogen bonding interaction of the compound 53 with Serine 59 (A) of the protein is detected. The hydrogen of the hydroxyl group of Serine 59 (A) makes a hydrogen (2.85Å) bond with the carbonyl oxygen atom of the methoxy ester group present in compound 53. Apart from hydrogen bonding interaction there are strong hydrophobic interactions of 11 amino acids, lining the protein cavity, with the compound 53. The amino acids involved in hydrophobic interaction with the compound 53 are Alanine 9 (A); Asparagine 64(A); Glutamic Acid 30 (A); Isoleucine 7(A)/60 (A); Leucine 22 (A); Phenylalanine 31 (A)/34 (A); Proline 61 (A); Threonine 56 (A); Tyrosine 121 (A) and Valine 18 (A)/115 (A).

**Analog 50:** From the Lig-Plot Figure 7(b), it evident that the hydrogen bonding interaction of the compound 50 with Serine 59 (A) of the protein is detected. The hydrogen of the hydroxyl group of Serine 59 (A) makes a hydrogen (3.01Å) bond with one of the oxygen atoms of methoxy group present in compound 50. Apart from hydrogen bonding interaction there are strong hydrophobic interactions of 8 amino acids, lining the protein cavity, with the compound 50.

The amino acids involved in hydrophobic interaction with the compound 50 are Alanine 9 (A); Asparagine 64 (A); Glutamic Acid 30 (A); Isoleucine 60 (A); Leucine 67 (A)/22 (A); Phenylalanine 31 (A)/34 (A); Threonine 56 (A) and Valine 8 (A).

Docking results of the drug derivatives via PyRx software reveals that the e-value of Analog 53 is -9.4 Kcal/mol is better Binding affinity compared to other derivatives of Decalin-β- keto ester enolate derivatives. The important inference of our study is that the 2 derivatives of Decalin-β- keto ester enolate (53 and 50), have better binding energies than the reference compound Proguanil as presented in Table 4. Compared to the recently reported variants of Pleconaril, the compounds 53 and 50 have simple structure and positive BBB. The drug properties of our reported best drug candidates (53 & 50) are compared with the commercial drug proguanil in Table 4. The structures of the Ligands (53 & 50) & commercial drug proguanil are compared in Figure 5. The protein –ligand complexes for the Analogs 53 & 50 with the protein 1MVT are presented in the Figure 6 -9.

**Table No: 4.** The Best two drug candidates based on criteria (i), (ii), (iii) & (iv) for the treatment of Malaria.
<table>
<thead>
<tr>
<th>Lig. No / Name</th>
<th>Mol. Formula</th>
<th>Mass</th>
<th>H-Bond Donor</th>
<th>H-Bond acceptor</th>
<th>B.E. (k.cal/mol)</th>
<th>Log P</th>
<th>GI Abs</th>
<th>BBB</th>
<th>Drug score</th>
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<tr>
<td>53</td>
<td>C_{21}H_{25}ClO_{5}</td>
<td>392</td>
<td>0</td>
<td>5</td>
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<td>+</td>
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<tr>
<td>50</td>
<td>C_{20}H_{26}O_{4}</td>
<td>330</td>
<td>0</td>
<td>4</td>
<td>-9</td>
<td>3.66</td>
<td>+</td>
<td>+</td>
<td>0.16</td>
</tr>
<tr>
<td>Proguanil</td>
<td>C_{11}H_{16}ClN_{5}</td>
<td>253</td>
<td>3</td>
<td>1</td>
<td>-6.5</td>
<td>-0.32</td>
<td>+</td>
<td>-</td>
<td>1.15</td>
</tr>
</tbody>
</table>

BBB - Blood Brain Barrier Penetration; GI Abs - Gastrointestinal Absorption
B.E – Binding Energy in Kcal/mol

![Chemical structures](image)

**Fig. 5.** Structural comparison of the (a) Ligand 50, (b) Ligand 53, (c) Commercial drug Proguanil.

![PyMol view](image)

**Fig. 6.** PyMol view of the (a) Ligand 53 (b) Protein – Ligand 53 complex the protein Malaria of 1MVT
Fig. 7. (a) The Egg plot of the Ligand 53  (b) LigPlot view of the Protein – Ligand 53 Complex of the protein 1MVT of Malaria

Fig. 8. PyMol view of the (a) Ligand 50  (b) Protein – Ligand 50 complex the protein 1MVT of Malaria
Fig. 9. (a) The Eggplot of the Ligand 50 (b) LigPlot view of the Protein – Ligand 50 Complex of the protein 1MVT of H1N1

CONCLUSION

Molecular docking of (2R,4aR,8aS)-8a-ethyl 2-methyl 2-(4-chlorobenzyl)-3-oxodecahydronaphthalene-2,8a-dicarboxylate 53 and (2S,4aR,8aR)-methyl 2-(4-methoxybenzyl)-8a-methyl-3-oxodecahydronaphthalene-2-carboxylate 50 with Malaria protein of 1MVT shows stronger binding energy, good ADME properties and Drug Score compared to reference compound Proguanil. It is of interest to find that our drug with the best binding energy - 9.4 k.cal /mol structurally resemble the commercial drug in having the aromatic ring and chlorine substitution. This fact is very useful for further SAR studies in this field.

Compared to the commercially reported variants of Proguanil for the treatment of Malaria, the compounds 53 and 50 involve simple method of preparation as we have synthesised and reported the compounds [13-14]. They have simple structure and positive BBB. The positive BBB drugs are very rare, as they can penetrate the blood barrier of the brain, mix with blood of the brain and neutralise the virus even if it has reached the brain.

Based on our ‘in-silico’ prediction, the two reported derivatives of Decalin-β-Keto ester enolate (53 and 50) can act as better (‘potential’) drugs to treat malaria as their binding energy with the virus protein is 50% more than that of the commercially available drug, further in vitro and in vivo studies can be made to declare them as the best drugs for treating Malaria.
ACKNOWLEDGEMENT

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