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Study on the Antimalarial Activity of Actinonin Derivatives by Molecular Modeling

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ABSTRACT
The drug resistance is a serious problem in malaria. So, the present strategy for new drug development is directed towards identifying essential enzyme systems in the parasite and developing potent molecules to inhibit them. A peptide deformylase (PDF) gene was identified in the Plasmodium falciparum genome and was suggested as a new target for antimalarial therapy. The aim of this study was to analyze the interactions between the PDF of Plasmodium falciparum (pPDF) and the actinonin, naturally occurring PDF inhibitors to explore their binding modes and to make tests of modelling with a view to identify novel and more efficient antimalarial drugs. The binding modes have been studied using molecular docking software FlexX. The study of the modelling realized on the actinonin shows that the binding energy can be decreased in a significant way by a judicious choice of fragments to be substituted. Replacement of the hydroxymethyl group of the pPDF inhibitor with an hydroxyl and the Pentyl group by a cyclopentyl-ethyl enhances the binding energy from -24.73 to -35.11 kJ mol\(^{-1}\). The biological potentialities of these proposed compounds were checked by their pharmacokinetic properties and they showed no toxicity.

Key words: Peptide deformylase, antimalarial drug, actinonin, molecular docking

INTRODUCTION
Malaria is a serious health problem and according to a world malaria report of World Health Organization in 2010 (http://www.who.int/malaria/world_malaria_report_2010/en/index.html) there are one million deaths and 250 million clinical malaria cases due to malaria annually, predominantly attributed to Plasmodium falciparum. The treatment of malaria is becoming extremely difficult due to the emergence of drug-resistant parasites (Wellens, 2002). Therefore, development of new drugs and a better understanding of the targets of antimalarial drugs and drug resistance are urgently needed (Alam et al., 2009).

The present strategy for new drug development is directed towards identifying essential enzyme systems in the parasite and developing potent molecules to inhibit them.

The peptide deformylase (PDF) is an essential enzyme in a wide variety of pathogenic microorganisms. It catalyzes the removal of a formyl group from newly synthesized proteins. (Giglione and Meinmel, 2001; Giglione et al., 2004). Its activity was not believed to be important in eukaryotic cells until recently (Serero et al., 2003).
In an article in Parasitology Today by Meinnel (2000), a putative PDF gene was identified in the *Plasmodium falciparum* genome and was suggested as a new target for antiparasitic therapy.

Actinonin, a naturally occurring PDF inhibitor, is a potent competitive inhibitor of bacterial PDF (Chen et al., 2000; Chikhi et al., 2006). This compound is an apparent competitive inhibitor of *Plasmodium falciparum* PDF (PfPDF) (Nguyen, 2005).

Wiesner et al. (2001) and Nguyen (2005) have reported that actinonin inhibits the growth of *Plasmodium falciparum* with an half maximal inhibitory concentration value of 3.0 and 2.5 μM, respectively.

Following the same study, the interactions between the PfPDF and the Actinonin have been analyzed by molecular docking method using the FlexX 1.3.0, 2010 (http://www.biosolveit.de/FlexX) to explore their binding modes and to modeling, with a view to identify new and more efficient PfPDF inhibitors.

**MATERIALS AND METHODS**

**Method:** Molecular docking is a widely-used computational tool for the study of molecular recognition, which aims to predict the binding mode and binding affinity of a complex protein-ligand (Wodak and Janin, 1978). It plays an important role in the rational design of drugs (Kitchen et al., 2004). Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

**Molecular docking software:** FlexX is one of the most established protein-ligand docking tools in the literature. It has proved to be highly successful in numerous drug discovery applications (Forino et al., 2005). Several subnanomolar inhibitors have been discovered with FlexX and are on the market after having proved their potential as a drug (Kubinyi, 2006; Stoermer, 2006). The technology behind FlexX is based on a robust incremental construction algorithm. In this algorithm rigid base fragments are identified first. At the next step, the selected fragment is placed into the active site of the receptor using a hashing technique. The complete ligand is constructed by adding the remaining components step by step. At each step of reconstruction a specified number of optimal partial solutions are selected for the next extension step. The scoring is done using a modified Böhm scoring function:

\[
\Delta G = \Delta G_0 + \Delta G_{rot} \times N_{rot} 
\]

\[
+ \Delta G_{hb} \sum_{\text{non-H-bonds}} f(\Delta R, \Delta \alpha)
\]

\[
+ \Delta G_{ip} \sum_{\text{internal}} f(\Delta R, \Delta \alpha)
\]

\[
+ \Delta G_{as} \sum_{\text{asym.}} f(\Delta R, \Delta \alpha)
\]

\[
+ \Delta G_{lip} \sum_{\text{lipid}} f^*(\Delta R)
\]

The first two terms (Eq. 1) of the function are a fixed ground term (DG0 = 5.4 kJ mol⁻¹) and a term taking into account the loss of entropy during ligand binding due to the hindrance of rotatable
bonds ($DG_{rot} = 1.4 \text{ kJ mol}^{-1}$). The following terms (Eq. 2-4) are sums over all interactions (hydrogen bonding, ionic and aromatic interactions). The last part (Eq. 5) of the scoring function rates the atom-atom contacts between protein and ligand, which are hydrophobic contacts. The functions $f$, $f^*$ are heuristic distance and angle dependent penalties (Rarey et al., 1996; Kramer et al., 1999).

**Docking strategy:** In this study, *Pf* PDF is taken as a drug target, *Plasmodium falciparum* causes the most deadly form of malaria. The structure was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb). The PDB ID of this enzyme is 1RL4, it is a cobalt metalloenzyme composed of 232 amino acids. For Docking study the actinonin was selected because it is a natural inhibitor of *Pf*PDF, it can inhibit the growth of *Plasmodium falciparum* (Nguyen, 2005). Its structure was obtained from PDB and represented in the Fig. 1.

**RESULTS AND DISCUSSION**

**Interaction between the *Pf*PDF and the actinonin:** Interesting interactions were detected between *Pf*PDF and actinonin with high number of matches (11 matches) and high interaction energies values (-22.1532 kJ mol$^{-1}$ of the matched interacting groups, -10.6627 kJ mol$^{-1}$ of the lipophilic contact area and -9.7587 kJ mol$^{-1}$ of the lipophilic-hydrophilic contact area), but the effect of rotatable bonds has been carried out, they decreases the total score of the docking solution to -24.7270 kJ mol$^{-1}$ (Table 1). The interactions are represented in the Fig. 2.

The hydrophobic and hydrogen bonds play very important roles in the interactions between actinonin and *Pf*PDF, which were confirmed sufficiently by molecular docking.

Actinonin makes several hydrogen bonds with amino acid residues of the binding pocket. In particular, the metal binding group is involved in five hydrogen bonds (Fig. 2), two by its carbonyl group with NH of Leu157 and metal ion with interaction energies of -4.21 and -0.47 kJ mol$^{-1}$ respectively, one by its NH with carboxyl of Glu199 (-4.45 kJ mol$^{-1}$) and two by its hydroxyl with lateral amine of Cln112 (-1.01 kJ mol$^{-1}$) and the imidazole ring of His198 (-4.70 kJ mol$^{-1}$). A hydrogen bond is formed between the carbonyl in position 13 and the NH of Ile106 residue (-3.88 kJ mol$^{-1}$). The NH in position 14 is hydrogen bonded to the carbonyl of Gly155 (-4.34 kJ mol$^{-1}$) and the bonding distance between them is 2.60 Å. An additional hydrogen bond of the hydroxyl hydrogen in position 27 with the carbonyl of Ile158 (2.74 Å) further stabilizes the inhibitor in the binding pocket. The actinonin is also stabilized by hydrophobic interactions with the region S$_1$' residues which is the binding site for the methionine side chain of the substrate: His198, Ile195, Gly155 and Leu157 (Fig. 2).
Table 1: Interaction energy (kJ mol⁻¹) calculated between PfPDF residues and the actinin

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Score</th>
<th>Match</th>
<th>Lipo</th>
<th>Ambig</th>
<th>Clash</th>
<th>Rot</th>
<th>Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinin</td>
<td>-24.7270</td>
<td>-32.1532</td>
<td>-10.6927</td>
<td>-0.7578</td>
<td>4.2468</td>
<td>18.3000</td>
<td>11</td>
</tr>
</tbody>
</table>

Score: Total score of the docking solution, Match Score: Contribution of the matched interacting groups, Lipo Score: Contribution of the lipophilic contact area, Ambig Score: Contribution of the lipophilic-hydrophilic contact area, Clash Score: Contribution of the clash penalty, Rot Score: Ligand conformational entropy score, Match: Number of matches

Fig. 2: Docking results for actinin. Hydrogen bonding is represented by dotted lines with interaction energies also shown. Hydrophobic interactions are shown as green lines

These results confirm those obtained by Guilloteau et al. (2002) where the interactions between the actinin and four PDFs were studied to determine their binding mode. Indeed, the actinin was involved in hydrophobic interactions with the residues of the region S₁ and in hydrogen interactions with the others amino acid residues of the binding pocket.

**Designing of new inhibitors:** The study of the PfPDF-actinin interaction offers certain guidelines for the design of high-affinity PfPDF inhibitors.

The second part of this study was devoted to modeling, with a view to identify novel and more efficient PDF inhibitors. The actinin was taken and several types of substitution were carried out: two mono-substitutions (compound 1 and 2) and four bi-substitutions (compound 3-6) represented in the Table 2.

Binding of Actinin derivatives and the active site of the PfPDF enzyme was further studied with molecular modeling docking experiments using FlexX. The result is shown in the Table 2.

Docking calculations revealed that the binding energy increased with all docked ligands. The best docking results were obtained using the bi-substituted compounds, particularly compound 4 (ΔG = -35.1089 KJ mol⁻¹) followed by compound 6 (ΔG = -31.1076 KJ mol⁻¹). The increase might be attributed to the formation of new interactions with amino acid residues of the binding pocket.
Table 2: Interaction energies (kJ mol\(^{-1}\)) calculated between p/PDF and the actinomycin derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>R2</th>
<th>Binding energy (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin</td>
<td>Hydroxymethyl</td>
<td>Pentyl</td>
<td>-34.7270</td>
</tr>
<tr>
<td>1</td>
<td>Hydroxyl</td>
<td>Pentyl</td>
<td>-30.9562</td>
</tr>
<tr>
<td>2</td>
<td>Carboxyl</td>
<td>Pentyl</td>
<td>-27.6227</td>
</tr>
<tr>
<td>3</td>
<td>Hydroxyl</td>
<td>Butyl</td>
<td>-29.0928</td>
</tr>
<tr>
<td>4</td>
<td>Hydroxyl</td>
<td>Cyclopentyl ethyl</td>
<td>-35.1089</td>
</tr>
<tr>
<td>5</td>
<td>Carboxyl</td>
<td>Butyl</td>
<td>-27.7047</td>
</tr>
<tr>
<td>6</td>
<td>Carboxyl</td>
<td>Cyclopentyl ethyl</td>
<td>-31.1076</td>
</tr>
</tbody>
</table>

Fig. 3: Binding modes of compound 4 with the active site of p/PDF

A representative binding mode of the compound 4 with the active site of p/PDF is given in the Fig. 3.

Compound 4 binds in the binding pocket of p/PDF through the formation of various distinct hydrogen bonds and hydrophobic interactions with selected amino acid residues within the binding pocket.

The results show that substituent like cyclopentyl-ethyl is preferred for the R\(_1\)’ region. The cyclopentyl-ethyl has strong hydrophobic interaction with the residues Glu154, Ile106 and Arg194, which can be seen in the Fig. 3.
The metal binding group, hydroxamate, forms an additional hydrogen bonds with the Co\(^{2+}\) by its hydroxyl group. Moreover, the first hydrogen bonds becomes more strongly (from -0.47 to -4.70 kJ mol\(^{-1}\)).

This study confirms the results reported by Lee et al. (2010) where a judicious choice of fragments to be substituted led to the discovery of new actinonin derivatives, which exhibit more potent enzyme inhibition and antibacterial activity against *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*.

**Pharmacokinetic study:** Lipinski’s Rule of Five is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Lipinski et al. (2001). The rule describes molecular properties important for a drug’s pharmacokinetics in the human body, including their absorption, distribution, metabolism, excretion and toxicity (ADME/Tox).

The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski’s rule.

Lipinski’s Rule of Five states that, in general, an orally active drug has:

- Not more than 5 hydrogen bond donors
- Not more than 10 hydrogen bond acceptors
- Not more than 15 rotatable bonds
- A molecular weight under 500 g mol\(^{-1}\)
- A partition coefficient log P less than 5

Molinspiration cheminformatics package (http://www.molinspiration.com/) was used for the determination of the inhibitor molecular properties. The results are described in the Table 3.

The ADME/Tox screening of the proposed compounds has not shown any negative result; it could be seen that the molecular weights of all those compounds are less than 500 g mol\(^{-1}\) which fulfill the criteria of Lipinski’s rule, the values of the hydrogen bond donors, acceptors and flexible bonds were also less than the maximum extent permitted by Lipinski’s rule, Log P Values also met the standards of Lipinski’s rule, which indicates the potentiality of those molecules to become drug. They are accepted to be orally bioavailable.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight (g mol(^{-1}))</th>
<th>Log P</th>
<th>H Donors</th>
<th>H Acceptors</th>
<th>Flexible bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinonin</td>
<td>385.505</td>
<td>1.615</td>
<td>4</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>371.478</td>
<td>1.502</td>
<td>4</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>383.489</td>
<td>1.967</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>357.451</td>
<td>0.997</td>
<td>4</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>397.516</td>
<td>1.378</td>
<td>4</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>369.462</td>
<td>1.462</td>
<td>3</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>409.527</td>
<td>1.843</td>
<td>3</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Log P: Partition coefficient
CONCLUSION

The inhibitory activity against PfPDF of actinonin and its derivatives was examined and docking studies on the active site of the enzyme were performed. Among the compounds used in this study, compound 4 constitutes the best inhibitor of PfPDF.

Replacement of the hydroxymethyl group of the PfPDF inhibitor with an hydroxyl and the pentyl group by a cyclopentyl-ethyl (compound 4) enhances the binding energy to -35.1089 kJ mol⁻¹. Docking results show that the compounds 1-6 may aid the development of more potent PfPDF inhibitors.

Also all the actinonin derivatives have passed successfully the ADME/Tox filter and all are found to be non-toxic.

REFERENCES


