Indian Journal of Pure & Applied Physics
Vol. 61, September 2023, pp. 803-806
DOI: 10.56042/ijpap.v61i9.3064

Identification of Potential Dipeptide Inhibitors for *Pf*ENR Enzyme in Fatty Acid Biosynthesis Pathway II: A Computational Study for Developing Novel Antimalarials

Kanika Devi, Jyoti Varsha Yadav, Rajat Kumar Gupta, Anshuman Chandra & Vijay Kumar Goel*

School of Physical Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Received 24 June 2023; accepted 2 August 2023

Malaria is a life-threatening disease caused by parasites of the genus Plasmodium that are transmitted through the bite of infected female Anopheles mosquitoes. The essential role of fatty acids in the malarial parasite's liver and blood stages makes it a promising target for combating *P. falciparum*. However, the emergence of strains of the malarial parasite has limited the efficacy of currently available drugs against malaria. Therefore, there is an urgent need to develop new drugs that can target the parasite and overcome drug resistance. This study aimed to identify potential dipeptide inhibitors for the *Pf*ENR enzyme using in-silico methods. Virtual screening was performed using thelibrary of 400 dipeptides to identify lead dipeptides with an affinity towards *Pf*ENR. We observed dipeptides Trp-Trp, Trp-Phe, Trp-Tyr, Tyr-Phe are showing the best affinity against *Pf*ENR. Density Functional Theory (DFT) analysis was used to reveal the electronic structure and reactivity of the top dipeptides by calculating the HOMO-LUMO gap. Additionally, we assessed the pharmacokinetic and other relevant properties of the lead dipeptides. All the lead dipeptides followed Lipinski's rule of five (Ro5). Our findings suggest that the identified dipeptides have significant potential as inhibitors of *Pf*ENR and could lead to the development of a novel class of antimalarial drugs. This research provides valuable insights into developing effective drugs to combat malaria.

Keywords: *Pf*ENR; P. Falciparum; Dipeptide inhibitors; DFT

1 Introduction

Malaria is responsible for devastating effects on vulnerable populations around the world. Development of antimalarials may improve overall health and wellbeing of affected people. Plasmodium falciparum enoyl-acyl carrier protein reductase (*Pf*ENR), a crucial enzyme involved in fatty acid biosynthesis and presents a good chance to interfere with the parasite's critical metabolic pathways. Fatty acids serve an essential role in supplying metabolic precursors of biological membranes[1–3]. They are essential source of metabolic energy, making their biosynthetic pathway an ideal target for antimicrobial agents[4–7].

*Pf*ENR functions in the last step of fatty acid elongation, reducing enoyl-ACP (acyl carrier protein) intermediates to acyl-ACP[2,8–10]. This enzymatic activity is essential for synthesizing fatty acids, which are crucial for various biological processes and membrane formation in the parasite[11–13].

The catalytic residues of *Pf*ENR facilitate the transfer of hydride ions from NADPH to the enoyl-ACP substrate, reducing the double bond and forming the acyl-ACP product[14].

Targeting *Pf*ENR with specific inhibitors has the potential to hinder the growth and progression of the malaria parasite, making it an attractive candidate for the development of antimalarial drugs. Triclosan is the available inhibitor of the FAS-II pathway and interacts with *Pf*ENR at its active site. Triclosan interacts with the Try277 residue and forms a hydrogen bond[7,15,16].

Using *in-silico* approaches, we identified lead dipeptide inhibitors of the *Pf*ENR enzyme. For this virtual screening is done utilizing the complete dipeptide library of natural dipeptides. Further, for the lead dipeptides found from virtual screening Density Functional Theory (DFT) was performed to reveal the electronic structure and activity of the molecules. We also looked at the pharmacokinetics and other features of the top dipeptides. Our results indicate that discovered dipeptides have significant promise as *Pf*ENR inhibitors, potentially leading to a new class of antimalarial medicines.

*Corresponding author: (E-mail: vijaykgoel@mail.jnu.ac.in)

2 Material and methods

2.1 Virtual Screening

Virtual screening (VS) is the method used in drug discovery[17–19]. It is utilized to screen a large library of molecules, for finding the best molecules against the protein target. Using VS, a large collection of molecules can be screened in a short time against a target enzyme. We have done a virtual screening of 400 dipeptides using Auto Dock Vina[20]. The best dipeptides are shortlisted from the library of dipeptides after screening. Further, these top hits are analysed for their ADME properties[16,21].

2.2 Density functional theory

Density functional theory (DFT) is an in-silico quantum mechanical methodology used to analyse electronic properties and activity of the molecule. Based on DFT analyses E_{HOMO} (highest occupied molecular orbital), E_{LUMO} (lowest unoccupied molecular orbital) and energy gap (ΔE) of respective HOMO and LUMO are calculated for the best tripeptides using Argus Lab[22]. We performed single point energy calculation using Parametric Model number 3(PM3) Semi empirical Method with max SCF of 200. A lower energy gap indicates that molecule is more active since it requires less energy to remove electrons.

3 Results and Discussion

3.1 Virtual Screening

For determining the most suitable dipeptide as *Pf*ENR inhibitor, a dipeptide library is screened against *Pf*ENR (Table 1). Dipeptides having the highest binding score are selected and analysed. The binding score of the top four dipeptidesis illustrated in the table1 ranging from -12.8kcal/mol to -10.9kcal/mol. Docking results show that these dipeptides form stable complexes with *Pf*ENR, compared with the control Triclosan with a score of -9.2kcal/mol. Trp-Trp, Trp-Phe, Trp-Tyr and Tyr-Phe are found to be the best dipeptide having affinity towards *Pf*ENR.

3.2 Molecular Docking

The molecular docking simulations revealed intriguing interactions between the dipeptides and PFENR, providing valuable insights into their potential as inhibitors. Notably, the dipeptides exhibited enhanced interactions compared to Triclosan, particularly with Tyr277, a crucial residue in the active site of *Pf*ENR.

All four dipeptides (Trp-Trp, Trp-Phe, Trp-Tyr, Tyr-Phe) formed a strong pi-pi interaction with Tyr277 and Tyr267 (Fig. 1). This interaction is highly significant as it promotes stability and contributes to the overall binding affinity of the test dipeptides. Pi-pi interactions involve stacking aromatic rings, allowing for effective electron delocalization and attractive

Table 1 — Binding affinity of ligands against PfENR with binding energy.

Sr No	Ligands	Binding energy (kcal/mol)
1	Trp-Trp	-12.8
2	Trp-Phe	-12.2
3	Trp-Tyr	-12.0
4	Tyr-Phe	-10.9
5	Triclosan	-9.2

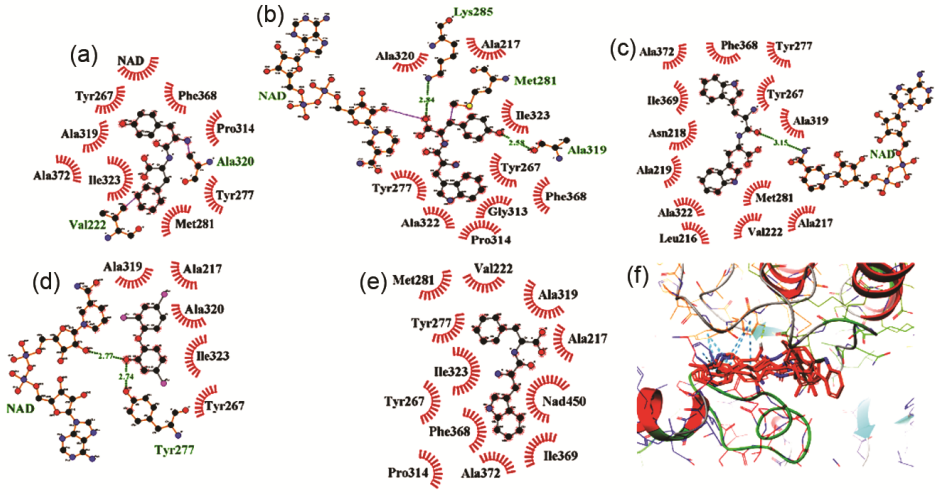

Fig. 1 — PfENR-ligand interaction plot. (a) Trp-Phe, (b) Trp-Trp, (c) Trp-Tyr, (d) Tyr-Phe, (e) Triclosan and (f) Binding site of PfENR. Images are drawn using Maestro[23] and Ligplot+.

forces. Pi-pi interactions between the dipeptides and Tyr277 suggest a favourable binding orientation and improved inhibition potential. Furthermore, Trp-Tyr, demonstrated a unique interaction pattern. In addition to the pi-pi interaction with Tyr277, Trp-Tyr formed a stable salt bridge with a nearby Lys285 residue of *Pf*ENR. The salt bridge between Trp-Tyr and lysine adds an extra layer of stability. It likely contributes to the enhanced binding affinity and potential inhibitory activity of Trp-Tyr compared to Triclosan. (Fig. 1(c))

The results suggest that the test dipeptides exhibit superior interactions with *Pf*ENR compared to Triclosan. These findings hold significant promise for developing novel and more effective inhibitors targeting *Pf*ENR, paving the way for advancements in antimicrobial research.

3.3 Pharmacokinetic Study

The four dipeptides, Trp-Phe, Trp-Trp, Trp-Tyr and Tyr-Phe exhibit distinct ADME properties and are found favourable.

Trp-Phe, with a molecular formula of $C_{20}H_{21}N_3O_3$ and a molecular weight of 351.4, has moderate solubility. It is very soluble according to the ESOL and Ali solubility prediction models. Trp-Trp, with a molecular formula of $C_{22}H_{22}N_4O_3$ and a molecular weight of 390.44, has varying solubility properties. It is soluble according to the ESOL solubility prediction model but poorly soluble according to the Silicos-IT solubility prediction model. Trp-Trp has high GI absorption, is not BBB permeant, and is a substrate for Pgp. Trp-Tyr, with a molecular formula of $C_{20}H_{21}N_3O_4$ and a molecular weight of 367.4, has good solubility. It is very soluble according to both the ESOL and Ali solubility prediction models. Trp-Tyr has high GI absorption, is not BBB permeant, and is a substrate for Pgp. Tyr-Phe, with a molecular formula of $C_{18}H_{20}N_2O_4$ and a molecular weight of 328.36, has moderate solubility.

3.4 Electronic properties

DFT analysis provides information about the overlap between the molecular orbitals of the ligand, indicating regions of interaction and the possibility of electron transfer. The HOMO-LUMO energy gap of the ligand can affect the orbital overlap and thus influence the strength and nature of the ligand's reactivity. HOMO-LUMO energy gap of top dipeptides Trp-Trp, Trp-Phe, Trp-Tyr, Tyr-Phe and Triclosan (control) are 8.4157eV, 8.5193eV, 8.5197eV, 9.1982eV and 8.8552eV respectively.

4 Conclusion

The compressive study aimed to identify potential dipeptide inhibitors for the *Pf*ENR enzyme, which is involved in the fatty acid biosynthesis pathway (FAS-II) of the malaria parasite. Our findings unveiled promising candidates dipeptides which form stable complexes with *Pf*ENR and exhibited enhanced interactions compared to the control inhibitor, Triclosan. Notably, the presence of pi-pi interactions and a unique salt bridge in Tyr-Phe dipeptide suggested favourable binding orientations and improved inhibition potential. Our investigation also delved with the pharmacokinetic properties of the identified dipeptides. To gain a deeper understanding of the electronic properties of these inhibitors, DFT analysis was conducted, unravelling key insights into their reactivity and overall efficacy. Overall, this research provides valuable insights and significant advancement into the development of potential antimalarial drugs. The identified dipeptides (Trp-Trp, Trp-Phe, Trp-Tyr, Tyr-Phe) show promise as inhibitors of the *Pf*ENR enzyme, and further experimental validation and optimization can pave the way for the creation of novel and more effective antimalarial medications.

Acknowledgement

KD thanks CSIR, New Delhi for junior research fellowship. AC thanks DHR, New Delhi for young scientist fellowship (R.12014/30/2022). VKG thanks Sant Sanganeria Foundation for financial support.

References

1 Manhas A, Patel A, Lone M Y, Jha P K & Jha P C, *J Cell Biochem*, 119 (2018) 8490.
2 Neves B J, Bueno R V, Braga R C & Andrade C H, *Bioorganic Med Chem Lett*, 23 (2013) 2436.
3 Lindert S, Tallorin L, Nguyen Q G, Burkart M D & McCammon J A, *J Comput Aided Mol Des*, 29 (2015) 79.
4 Bieri C, *et al.*, *Int J Mol Sci*, 24 (2023).
5 Frecer V, Megnassan E & Miertus S, *Eur J Med Chem*, 44 (2009) 3009.
6 Kapoor M, Mukhi P L S, Surolia N, Suguna K & Surolia, *Biochem J*, 381 (2004) 725.
7 Perozzo R, *et al.*, *J Biol Chem*, 277 (2002) 13106.
8 Maity K, *et al. IUBMB Life*, 63 (2011) 30.
9 Makam P, Thakur P K & Kannan, *Eur J Pharm Sci*, 52 (2014) 138.
10 Costa D B, *et al.*, *J Biomol Struct Dyn*, 40 (2022) 6295.
11 Pandey A, Shyamal S S, Shrivastava R, Ekka S & Mali S N, *Chem Africa*, 5 (2022) 1469.
12 Tasdemir D, *Phytochem Rev*, 5 (2006) 99.
13 Perozzo R, *et al.* , *J Biol Chem*, 277 (2002) 13106.
14 Banerjee T, Sharma S K, Surolia N & Surolia A, *Biochem Biophys Res Commun*, 377 (2008) 1238.

15 da Silva M A, *et al. Parasitol Res*, 119 (2020) 1879.
16 Ibrahim Z Y, Uzairu A, Shallangwa G A, Abechi S E & Isyaku S, *Malaysian J Pharm Sci*, 20 (2022) 51.
17 Shukla R & Singh T R, *J Genet Eng Biotechnol*, 19 (2021) 1.
18 Shukla R & Singh T R, *J Biomol Struct Dyn*, 38 (2019) 248.
19 Shukla R & Singh T R, *J Biomol Struct Dyn*, 40 (2020) 2815.
20 Trott O & Olson A J, *J Comput Chem*, (2009).
21 Daina A, Michielin O & Zoete V, *Sci Rep*, **7** (2017).
22 Bitencourt-Ferreira G & de Azevedo, *Methods Mol Biol*, 2053 (2019) 203.
23 Schrödinger. Maestro | Schrödinger, *Schrödinger Release*, 2019-1 at (2019).