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Research

Exploring Aminohydantoin derivatives as promising antimalarial agents through *In-silico* Studies

Khushboo Gupta*, Shourya Pratap, Amresh Gupta
Institute of Pharmaceutical Sciences and Research, Unnao (U. P)

Corresponding Author:
Khushboo Gupta

Email: kg123vns@gmail.com

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Abstract:
Background: Our research highlights the *In-silico* of newer antimalarial compounds using molecular docking studies. **Objective:** The study investigates a series of aminohydantoin derivatives from previous literature, focusing on their biological activities as antimalarial agents. **Method:** Computational methods such as molecular docking employed to gain insights into the interaction between the synthesized compounds and the target enzyme *PfDHFR-TS.* **Result:** The compounds were showed good docking score like moldock score and re-rank score. The finding of docking studies shows a typical molecular interaction pattern with lactate dehydrogenises. The binding interaction information derived from these molecules will be useful in future antimalarial agent design. **Conclusion:** From the docking study, it was observed that ligands bind to the electrostatic, hydrophobic clamp formed by the residues Asp 76(B), Tyr 190(B), Tyr 80(B) and Lys 72(B) which play an important role for *Plasmodium falciparum* inhibition. The binding affinity, grid calculation and RMSD percentage lower and upper parameters were calculated. Hence, the observable data indicated that, above compounds can serve as good leads for further modification and optimization in the of treatment malaria.

Keywords: Aminohydantoin, docking studies, *Plasmodium falciparum*, moldock score.

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INTRODUCTION

Malaria is a well-documented and widely recognized tropical disease that can have severe consequences, including occasional fatalities. A parasite causes it, primarily transmitting it through the bites of female mosquitoes in the Anopheles genus, known to carry the disease [1, 2, 3]. The annual death toll from this cause exceeds 445,000 individuals, with a significant proportion of fatalities occurring among the younger population in Africa [4, 5]. Five types of protozoa, *Plasmodium falciparum, P. vivax, P. malariae, P. ovale*, and the recently identified *P. knowlesi*, cause malaria, a disease that mosquitoes transmit [6, 7]. The World Health Organization (WHO) World Malaria Report of 2019 estimates that there were 228 million malaria cases globally in 2018. These cases resulted in approximately 405,000 deaths, with a significant number of fatalities occurring among children under the age of 5 [8, 9]. Data from available sources indicates that malaria is prevalent in over 90 countries. This infectious disease has a significant impact on the global population, affecting approximately 40% of people worldwide [10, 11]. Countries with limited healthcare infrastructure, inadequate funding, and insufficient resources for prevention and treatment exhibit a significantly higher fatality rate of malaria another contributing factor could be the prevalence of high levels of poverty [12, 13]. The Chinese botanical specimen Artemisia annua, also known as sweet wormwood or Qinghao, initially yielded artemisinin, a crucial compound in the therapeutic intervention of malaria

[14, 15, 16]. People typically administer Artemether, a commonly employed antimalarial agent, in conjunction with another antimalarial drug, lumefantrine, resulting in a therapeutic combination known as artemether-lumefantrine [17, 18, 19]. Furthermore, the bark of the cinchona tree yields quinine, a medication and natural compound commonly used to treat malaria [20, 21, 22]. Regrettably, subsequent to an initial triumph, the malaria parasite, particularly Plasmodium falciparum, developed resistance to chloroquine [23, 24]. Despite the existence of several efficacious antimalarial drugs and treatments, the issue of drug resistance persists due to the malaria parasite's ability to rapidly evolve and adapt [25, 26]. *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (P*f*DHFR-TS) was selected as the biological target in this study due to its essential role in the parasite's folate biosynthesis pathway, which is critical for DNA synthesis and cellular replication. P*f*DHFR-TS catalyze the conversion of dihydrofolate to tetrahydrofolate, a vital step for the synthesis of purines and thymidylate, both of which are necessary for DNA replication [27]. This enzyme has long been a prime target for antimalarial drugs, including pyrimethamine and cycloguanil, due to its central role in the parasite's survival [28]. However, the widespread emergence of drug-resistant *P. falciparum* strains, caused by mutations in the P*f*DHFR-TS gene, especially in malaria-endemic regions, has significantly reduced the efficacy of current antifolates, necessitating the discovery of novel inhibitors [29]. Recent research has focused on new classes of P*f*DHFR-TS inhibitors, such as propargyl-linked antifolates and triazine-based molecules, which have demonstrated potential against resistant strains of *P. falciparum* [30, 31]. In addition, hybrid molecules combining various pharmacophores have been explored to improve efficacy against resistant strains [32]. The selection of **Aminohydantoin** derivatives for antimalarial research is grounded in their structural similarity to both indole and quinoline scaffolds, which are known for their antimalarial potency [33].

MATERIAL AND METHODS:

A dataset of 28 Aminohydantoin analogues from a single series is acquired. The biological activities of these analogues were obtained from previous literature Marvin J. Meyers *et al.* 2013 [34]. This study rendered the structures in a two-dimensional format using the software Chemdraw Ultra 2D Version 8.0. Chemdraw Ultra 3D then transformed these two-dimensional representations into three-dimensional models. The MOPAC algorithm was utilized to perform the energy minimization process [35].

The molecular docking studies were conducted using the Surflex Dock module in Molegro virtual docker 6.0 software. The protein structures of the specified PDB entries, 4RAO, and their corresponding inhibitors were obtained from the RCSB Protein Data Bank [36, 37]. The PDB structures were selected for docking studies due to their high-resolution crystallographic data (better than 2.5 Å) and validated R-free values below 0.25, ensuring structural reliability. The selection of 4RAO was also based on their inclusion of biologically relevant cofactors and inhibitors, which are essential for maintaining the proper binding environment [38, 39]. NADPH was retained in the structure during the docking process because it plays a crucial role in maintaining the active site conformation and can directly or indirectly interact with the ligand. Removing NADPH could lead to an inaccurate representation of the binding pocket and affect the docking results [40, 41]. Additionally, the pKa values of ionizable residues in the protein were computed using the PROPKA method at pH 7.4, a pH that is representative of physiological conditions. This step ensures that the protonation states of amino acid residues in the binding site are accurate, which is critical for predicting correct interactions during docking simulations [42]. The PROPKA method allows the prediction of pKa values based on the protein structure, taking into account factors such as residue environment and hydrogen bonding [43].The protein structures were prepared for docking by performing energy minimization and charge calculations using the AMBER7FF99 force field. All ligands and water molecules, except NADPH, were removed to avoid interference during docking. Bloat values were set to 1.0, and threshold values to 0.5 to generate the docking data [44, 45, 46]. To validate the docking protocol, re-docking of the co-crystallized ligands from the PDB entries 4RAO was performed. The root-mean-square deviation (RMSD) between the re-docked and experimental ligand poses was calculated, and an RMSD value below 2 Å was

considered acceptable, which is shown in the figure, this step ensured that the docking protocol accurately predicted ligand binding poses [47, 48]. The final docking results were further analyzed through visual inspection of the key interactions between the docked ligands and the active site residues, confirming the preservation of critical hydrogen bonds, hydrophobic contacts, and interactions with NADPH.

EXPERIMENTAL:

Each ligand was chosen for molecular docking analyses to assess its efficacy against malaria. The inhibitor protein structure and PDB name were acquired from the RCSB protein data bank under the PDB identifiers 4RAO, respectively. Molecular docking investigations were performed utilizing the Surflex Dock module integrated within the Molegro virtual docker 6.0 software. MolDock Score and MolDock Rerank Score of most active compound to that of reference ligand reveals the efficient docking interactions of the compound. Asp-76(B), Try-190(B), Leu-77(B), Thr-45(B), His-38(B), Gly-39(B), Asp-43(A), Tyr-80(B) and Met-42(B) are the major amino acid bindings responsible for biological activity. Among the reference ligand and compounds 1,9,18,19,26,27 and 28 of these amino acids bindings are in common which proves that the binding of the compounds takes place with the desired amino acids in the protein. Molecular docking scores and data also correlate and reconfirm the assumption regarding the design and development of new molecules.

Table 1: Docking Score of Pdb-4RAO reported compounds

Comp No.	MolDock Score	Re-rank Score	H-Bond
01	-131.531	-78.5204	-3.10212
02	-103.249	-75.8402	-0.384916
03	-101.301	-68.5639	-0.752536
04	-113.662	-87.5361	-2.37363
05	-113.777	-90.9875	-1.40004
06	-115.143	-94.3147	-
07	-110.555	-85.7039	-0.00511253
08	-102.064	-65.8804	-1.97476
09	-219.323	-161.412	-0.159418
10	-100.391	-58.364	0.199877
11	-97.2375	-57.0105	-0.383658
12	-102.768	-81.1365	-
13	-100.765	-58.0613	-0.516466
14	-98.8683	-72.2189	-
15	-100.875	-61.7457	0.351308
16	-100.477	-59.5785	-1.33243
17	-97.9958	-74.6467	-1.65333
18	-133.322	-60.3441	-0.914538
19	-131.747	-96.0003	-0.258545
20	-105.738	-25.2223	-0.0212229
21	-116.014	-80.3198	-0.244676
22	-140.886	-106.217	-1.56805
23	-118.095	-80.9378	-0.452932
24	-119.617	-78.4913	-2.5
25	-175.065	-121.149	-1.37088
26	-178.584	-121.801	-2.41728
27	-169.936	-133.218	-
28	-152.656	-94.752	-4.26752

Pose analysis of docking of standard drug chloroquine and co-crystallized ligand (3L7) explore the common amino acid interaction like: Met-42(B), Leu-77(B), Thr-45(B), Asp-76(B), Tyr-190(B), His-38(B), Gly-39(B), Asp-43(A) and Tyr-80(B) which are considered to be the active amino acids important for binding of ligand on active site. Compound-1 exhibit common binding interaction like: Met-42(B), Asp-76(B), Try-190(B), Leu-77(B), Thr-45(B), Gly-39(B), His-38(B), Asp-43(A) and Tyr-80(B) to that of standard active amino acids.Compound-9 exhibit common binding interaction Met-42(B), Asp-76(B), His-38(B), Try-190(B), Leu-77(B), Thr-45(B) and Tyr-80(B) with amino acids responsible for activity. Compound-18 has common binding interactions Met-42(B), Asp-76(B), Try-190(B), Asp-79(D), His-38(B) and Asp-43(A) with amino acids. Compound-19 exhibit binding with essential amino acids like: Met-42(B), Asp-76(B), Try-190(B), Thr-45(B), Asp-76(B), Gly-39(B), His-38(B) and Asp-43(A).Compound-26 exhibit binding with Asp-76(B), Try-190(B), Leu-77(B), Thr-45(B), Gly-39(B), His-38(B), Tyr-80(B) and Asp-43(A).Compound-27 exhibit common amino acid bindings with Asp-76(B), Try-190(B), Leu-77(B), Thr-45(B), Gly-39(B), His-38(B) and Asp-43(A). Compound-28 exhibit common amino acid bindings with Asp-76(B), Try-190(B), Leu-77(B), Thr-45(B), Gly-39(B), His-38(B) and Asp-43(A).

RESULT AND DISCUSSION:

Computer aided drug designing (CADD) helps the researcher to decrease the time and money for drug designing projects Molecular docking is very helpful in studying the interactions of ligand molecules with the target protein before its in vitro synthesis. Docking is performed through computer programs like Autodock , arguslab and discovery studio 3.1. All these molecules were taken from ligand database or draw with help of chemical organizer (draw) software like chemdraw ultra 2d & 3d in mol or pdb format and were stored in a database of MOE in mdb format or Pubchem database. All these molecules were docked against the same pocket where reference drug bound. Molecules were selected from a library of molecules and were further assessed by the interaction analysis. Finalized molecules showed the interactions with the active residue and with other residues, docking studies helps to predict the binding orientations and interactions of the ligands with the target protein 4RAO. These docking experiments provided key structural insights into the ligand-receptor interactions, particularly revealing the importance of hydrogen bonding, hydrophobic interactions, and electrostatic interactions in the binding pocket. For instance, the hydroxyl and methoxy substituent were shown to form hydrogen bonds with key residues in the binding site, which correlated with enhanced activity.

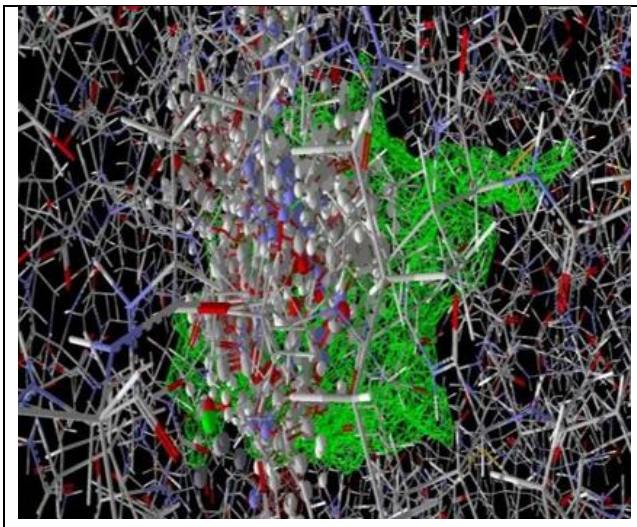

Fig 1: Docking of all compounds

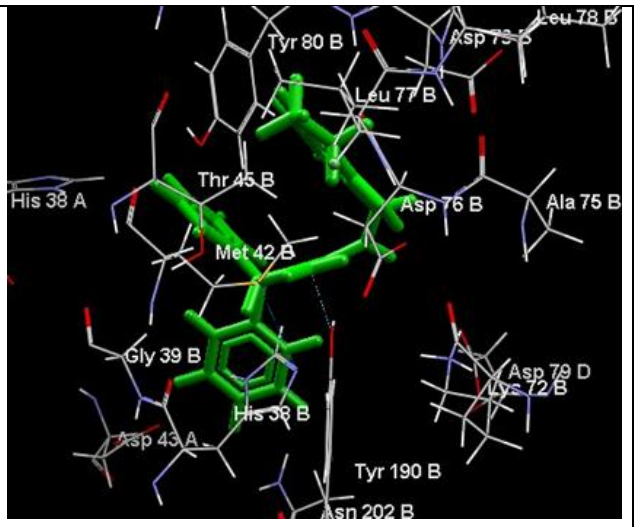

Fig 2: Docking pose of compound

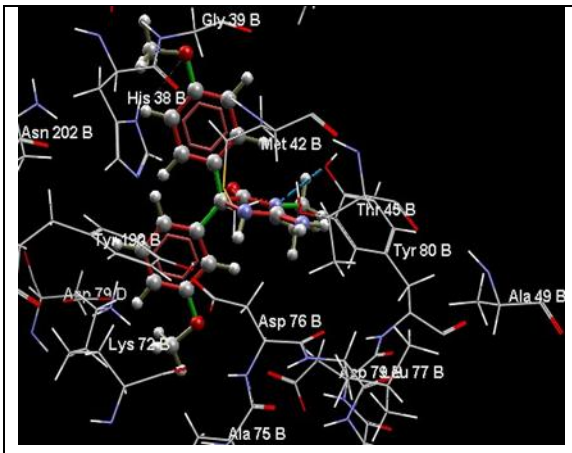

Fig 3: Docking pose of compound 9

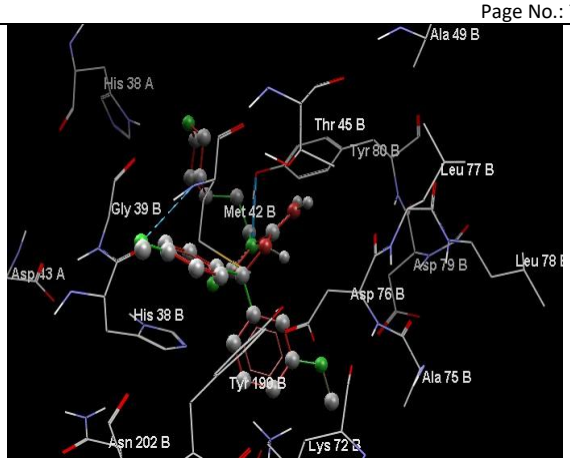

Fig 4: Docking pose of compound 18

CONCLUSION:

The current study demonstrates the potential of Aminohydantoin derivatives as antimalarial agents using molecular docking, study concluded that the synthesize derivatives of aminohydantoin, showed the good docking score and more stable bonding with the *Plasmodium falciparum* so it can be said that, these derivatives may be significant against *Plasmodium falciparum* inhibitor. Further study will need to be conducted for other properties of drug like, absorption metabolism and excretion in human body. On the basis of comparison with standard drugs like chloroquine and other derivatives selected in this study, which have less docking score as compared to the standard. It can be concluded that these seven derivatives have good RMSD limit so it was made virtual derivatives in these molecules could be used as promising inhibitor of *Plasmodium falciparum* to therapeutic value for various types of malaria in future.

Credit authorship contribution statement:

Khusbu Gupta: Conceptualization, Methodology, Investigation, Data curation, Writing- Original draft, Supervision; **Shourya Pratap:** Writing-reviewing and editing; **Amresh Gupta:** Writing-reviewing and editing.

Declaration of competing interest:

The authors state that there are no competing interests. The study generated all the data internally, without any involvement from outside sources or paper mills. All the authors in this study have agreed to take responsibility for every aspect of the work, making sure that the findings are accurate and trust worthy.

Conflict of Interest:

The authors declare no competing financial interest.

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REFERENCES

[1] Talapko, J.; Skrlec, I.; Alebic, T.; Jukic, M.; Vcev, A. Malaria: The Past and the Present. Microorganisms, 2019, 7(6), 179. https://doi.org/10.3390/microorganisms7060179

[2] Cox, F.E. History of the discovery of the malaria parasites and their vectors. Parasites Vectors., 2010, 3, 5. https://doi.org/10.1186/1756-3305-3-5

[3] Mace, K.E.; Arguin, P.M.; Tan, K.R. Malaria Surveillance - United States, 2015. MMWR. Morb. Mortal Wkly. Rep., 2018, 67(7), 1–28. https://doi.org/10.15585/mmwr.ss6707a1

[4] Islam, M.R.; Dhar, P.S.; Rahman, M.M. Recently outbreak of malaria in current world: species, etiology, life cycle, transmission, symptoms, vaccination, diagnostic tests, treatment, and complications. Int. J. Surg., 2023, 109(2), 175-177. https://doi:10.1097/JS9.0000000000000165

[5] Sato, S. *Plasmodium* a brief introduction to the parasites causing human malaria and their basic biology. J. Physiol. Anthropol., 2021, 40, 1. https://doi.org/10.1186/s40101-020-00251-9

[6] Lee, W.C.; Cheong, F.W.; Amir, A.; Lai, M.Y.; Tan, J.H.; Phang, W.K.; Shahari, S.; Lau, Y.L. Plasmodium knowlesi: the game changer for malaria eradication. Malar. J., 2022, 21(1), 140. https://doi.org/10.1186/s12936-022-04131-8

[7] Phillips, M.A.; Burrows, J.N.; Manyando, C.; Van

Huijsduijnen, R.H.; Van Voorhis, W.C.; Wells, T.N. Malaria. Nat. Rev. Dis. Primers., 2017, 3(1), 1-24. https://doi.org/10.1038/nrdp.2017.50

[8] Wambani, J.; Okoth, P. Impact of Malaria Diagnostic Technologies on the Disease Burden in the Sub-Saharan Africa. J. Trop. Med., 2022, 7324281. https://doi.org/10.1155/2022/7324281

[9] Varo, R.; Chaccour, C.; Bassat, Q. Update on malaria. Med. Clin., 2020; 155(9), 395-402. https://doi.org/10.1016/j.medcle.2020.05.024

[10] Zekar, L.; Sharman, T. Plasmodium falciparum Malaria. Treasure Island (FL): StatPearls Publishing; 2024 https://www.ncbi.nlm.nih.gov/books/NBK555962/

[11] Schantz-Dunn, J.; Nour, N.M. Malaria and Pregnancy: A Global Health Perspective. Obstet. Gynecol., 2009, 2(3), 186-192. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2760896/

[12] Andrade, M.V.; Noronha, K.; Diniz, B.P.C.; Guedes, G.; Carvalho, L.R.; Silva, V.A.; Calazans, J.A.; Santos, A.S.; Silva, D.N.; Castro, M.C. The economic burden of malaria: a systematic review. Malar. J., 2022, 21(1), 283. https://doi.org/10.1186/s12936-022-04303-6

[13] Oladipo, H.J.; Tajudeen, Y.A.; Oladunjoye, I.O.; Yusuff, S.I.; Yusuf, R.O.; Oluwaseyi, E.M.; AbdulBasit, M.O.; Adebisi, Y.A.; El-Sherbini, M.S. Increasing challenges of malaria control in sub-Saharan Africa: Priorities for public health research and policymakers. Ann. med. Surg., 2022, 81, 104366. https://doi.org/10.1016/j.amsu.2022.104366

[14] Miller, L.H.; Su. X. Artemisinin: discovery from the Chinese herbal garden. Cell., 2011, 146 (6), 855-858. https://doi.org/10.1016/j.cell.2011.08.024

[15] Graziose, R.; Lila, M.A.; Raskin, I. Merging traditional Chinese medicine with modern drug discovery technologies to find novel drugs and functional foods. Curr. Drug. Discov. Technol., 2010, 7(1), 2-12. https://doi.org/10.2174/157016310791162767

[16] Jigang, W.; Xu, C.; Wong, Y.K.; Li, Y.; Liao, F.; Jiang, T.; Tu. Y. "Artemisinin, the Magic Drug Discovered from Traditional Chinese Medicine." Eng., 2019, 5(1), 32-39. https://doi.org/10.1016/j.eng.2018.11.011

[17] Omari, A.A.; Gamble, C.; Garner, P. Artemether-lumefantrine (four-dose regimen) for treating uncomplicated falciparum malaria. Cochrane Database Syst. Rev., 2006, (2), CD005965. https://doi.org/10.1002/14651858.CD005965

[18] Peto, T.J.; Tripura, R.; Callery, J.J.; Lek, D.; Nghia, H.D.T.; Nguon, C.; Thuong, N.T.H.; Van. der. Pluijm, R.W.; Dung, N.T.P.; Sokha, M.; Van, L.V.; Long, L.T.; Sovann, Y.; Duanguppama, J.; Waithira, N.; Hoglund, R.M.; Chotsiri, P.; Chau, N.H.; Ruecker, A.; Dondorp, A.M. Triple therapy with artemether–lumefantrine plus amodiaquine versus artemether–lumefantrine alone for artemisinin-resistant, uncomplicated falciparum malaria: An open-label, randomised, multicentre trial. Lancet. Infect. Dis., 2022, 22(6), 867-878. https://doi.org/10.1016/S1473-3099(21)00692-7

[19] Faurant, C. From bark to weed: the history of artemisinin. Parasite., 2011, 18(3), 215–218. https://doi.org/10.1051/parasite/2011183215

[20] Wells, T.N. Natural products as starting points for future anti-malarial therapies: going back to our roots? Malar. J., 2011, 10 (1), S3. https://doi.org/10.1186/1475-2875-10-S1-S3

[21] Permin, H.; Norn, S.; Kruse, E.; Kruse, P.R. On the history of Cinchona bark in the treatment of Malaria. Dan. Medicinhist. Arbog., 2016, 44, 9-30. PMID: 29737660.

[22] Gachelin, G.; Garner, P.; Ferroni, E.; Trohler, U.; Chalmers, I. Evaluating Cinchona bark and quinine for treating and preventing malaria. J. R. Soc. Med., 2017, 110(1), 31-40. https://doi:10.1177/0141076816681421

[23] Sa, J.M.; Chong, J.L.; Wellems, T.E. Malaria drug resistance: new observations and developments. Essays. Biochem., 2011, 51, 137-160. https://doi:10.1042/bse0510137

[24] Ecker, A.; Lehane, A.M.; Clain, J.; Fidock, D.A. PfCRT and its role in antimalarial drug resistance. Trends. Parasitol., 2012, 28(11), 504-514. https://doi:10.1016/j.pt.2012.08.002

[25] Sinha, S.; Medhi, B.; Sehgal, R. Challenges of drug-resistant malaria. Parasite., 2014, 21, 61. https://doi:10.1051/parasite/2014059

[26] Plowe, C.V. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. Malar. J., 2022, 21, 104. https://doi.org/10.1186/s12936-022-04115-8

[27] Hyde, J.E. Exploring the folate pathway in Plasmodium falciparum. Acta Trop. 2005, 94(3), 191-206. https://doi:10.1016/j.actatropica.2005.04.002

[28] Wang, P.; Lee, C.S.; Bayoumi, R.; Djimde, A.; Doumbo, O.; Swedberg, G.; Dao, L.D.; Mshinda, H.; Tanner, M.; Watkins, W.M.; Sims, P.F.; Hyde, J.E. Resistance to antifolates in Plasmodium falciparum monitored by sequence analysis of dihydropteroate synthetase and dihydrofolate reductase alleles in a large number of field samples of diverse origins. Mol Biochem Parasitol., 1997, *89*(2), 161–177. https://doi.org/10.1016/s0166-6851(97)00114-x

[29] Nzila, A. The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. J. Antimicrob. Chemother., 2006, 57(6), 1043–1054. https://doi.org/10.1093/jac/dkl104

[30] Nduati, E.; Diriye, A.; Ommeh, S.; Mwai, L.; Kiara, S.; Masseno, V.; Kokwaro, G.; Nzila, A. Effect of folate derivatives on the activity of antifolate drugs used against malaria and cancer. Parasitol Res., 2008, 102(6), 1227–1234. https://doi.org/10.1007/s00436-008-0897-4

[31] Pathak, M.; Ojha, H.; Tiwari, A.K.; Sharma, D.; Saini, M.; Kakkar, R. Design, synthesis and biological evaluation of antimalarial activity of new derivatives of 2,4,6-*s*-triazine. Chem. Cent. J., 2017, 11, 132. https://doi.org/10.1186/s13065-017-0362-5

[32] Yuvaniyama, J.; Chitnumsub, P.; Kamchonwongpaisan, S.; Vanichtanankul, J.; Sirawaraporn, W.; Taylor, P.; Walkinshaw, M.D.; Yuthavong, Y. Insights into antifolate resistance from malarial DHFR-TS structures. Nat. Struct. Biol., 2003, 10(5), 357–365. https://doi.org/10.1038/nsb921

[33] Wang, J.; Zhang, C.J.; Chia, W.N.; Loh, C.C.; Li, Z.; Lee, Y.M.; He, Y.; Yuan, L.X.; Lim, T.K..; Liu, M.; Lin, Q. Haem-activated Promiscuous Targeting of Artemisinin in Plasmodium Falciparum. Nat. Commun., 2015, 6 (1), 1-11. https://doi.org/10.1038/ncomms10111

[34] Meyers, M. J.; Tortorella, M. D.; Xu, J.; Qin, L.; He, Z.; Lang, X.; Zeng, W.; Xu, W.; Qin, L.; Prinsen, M. J.; Sverdrup, F. M.; Eickhoff, C. S.; Griggs, D. W.; Oliva, J.; Ruminski, P. G.; Jacobsen, E. J.; Campbell, M. A.; Wood, D. C.; Goldberg, D. E.; Liu, X.; Chen, X. Evaluation of aminohydantoins as a novel class of antimalarial agents. *ACS medicinal chemistry letters*, 2013, *5*(1), 89–93. https://doi.org/10.1021/ml400412x

[35] Bahareh H.; Thavendran G.; Glenn, E.M.M.; Mahmoud, E.S.S.; Hendrik G.K. Integrated Approach to Structure-Based Enzymatic Drug Design: Molecular Modeling, Spectroscopy, and Experimental Bioactivity. Chem. Rev., 2014, 114 (1), 493-537. https://doi.org/10.1021/cr300314q

[36] Ren, Y.; Long, S.; Cao, S. Molecular Docking and Virtual Screening of an Influenza Virus Inhibitor That Disrupts Protein–Protein Interactions. Viruses., 2021, 13(11), 2229. https://doi.org/10.3390/v13112229

[37] Li, X.; Chu, Z.; Du, X.; Qiu, Y.; Li, Y. Combined molecular docking, homology modelling and density functional theory studies to modify dioxygenase to efficiently degrade aromatic hydrocarbons. RSC adv., 2019, 9(20), 11465–11475. https://doi.org/10.1039/c8ra10663k

[38] Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P. E. The Protein Data Bank. Nucleic acids research, 2000, 28(1), 235–242. https://doi.org/10.1093/nar/28.1.235

[39] Read, R.J.; Adams, P.D.; Arendall, W.B.; Brunger, A.T.; Emsley, P.; Joosten, R.P.; Kleywegt, G.J.; Krissinel, E.B.; Lütteke, T.; Otwinowski, Z.; Perrakis, A.; Richardson, J.S.; Sheffler, W.H.; Smith, J.L.; Tickle, I.J.; Vriend, G.; Zwart, P.H. A new generation of crystallographic validation tools for the protein data bank. Structure., 2011, 19(10), 1395–1412. https://doi.org/10.1016/j.str.2011.08.006

[40] Singh, R.; Joseph, L.; Ryan J.M.; Vasu D.A. A Novel Strategy Involved Anti-Oxidative Defense: The Conversion of NADH into NADPH by a Metabolic Network. PLoS ONE., 2008, 3(7), e2682. https://doi.org/10.1371/journal.pone.0002682

[41] Meng, X.Y.; Zhang, H.X.; Mezei, M.; Cui, M. Molecular docking: a powerful approach for structure-based drug discovery. Curr. Comput.-Aided Drug Des., 2011, 7(2), 146–157. https://doi.org/10.2174/157340911795677602

[42] Li, H.; Robertson, A.D.; Jensen, J.H. Very fast empirical prediction and rationalization of protein pKa values. Proteins, 2005, 61(4), 704–721. https://doi.org/10.1002/prot.20660

[43] Olsson, M.H.; Søndergaard, C.R.; Rostkowski, M.; Jensen, J.H. PROPKA3: Consistent Treatment of Internal and Surface Residues in Empirical pKa Predictions. J. Chem. Theory Comput., 2011, 7(2), 525–537. https://doi.org/10.1021/ct100578z

[44] Gao, X.; Han, L.; Ren, Y. In Silico Exploration of 1,7-Diazacarbazole Analogs as Checkpoint Kinase 1 Inhibitors by Using 3D QSAR, Molecular Docking

Study, and Molecular Dynamics Simulations. Molecules, 2016, 21(5), 591. https://doi.org/10.3390/molecules21050591

[45] Baammi, S.; Daoud, R.; El Allali, A. In silico protein engineering shows that novel mutations affecting NAD^+ binding sites may improve phosphite dehydrogenase stability and activity. Sci. Rep., 2023, 13(1), 1878. https://doi.org/10.1038/s41598-023-28246-3

[46] Xue, Q.; Liu, X.; Russell, P.; Li, J.; Pan, W.; Fu, J.; Zhang, A. Evaluation of the binding performance of flavonoids to estrogen receptor alpha by Autodock, Vina and Surflex-Dock. Ecotoxicol. Environ. Saf., 2022, 233, 113323. https://doi.org/10.1016/j.ecoenv.2022.113323

[47] Meng, X.Y.; Zhang, H.X.; Mezei, M.; Cui, M. Molecular docking: a powerful approach for structure-based drug discovery. Curr. Comput. Aided Drug Des., 2011, 7(2), 146–157. https://doi.org/10.2174/157340911795677602

[48] Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K..; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem., 2009, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

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