Original Article

A RATIONAL APPROACH FOR DESIGNING THE MULTITARGETING ANTIMALARIAL COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM* KINASES TO EFFECTIVELY COMBAT THE RAPIDLY EMERGING DRUG RESISTANCE

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ABSTRACT

The development of parasite resistance to primary antimalarial drugs has caused a significant number of fatalities in malaria-affected countries, with drug target mutations being the sole reason. As a potential solution, the development of multitargeting drugs has gained attention. Studies have shown that the probability of multiple target mutations is low because it would significantly impact the parasite's fitness. Furthermore,

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multitargeting drugs have the potential to be effective with reduced dosages, increased efficiency, and improved safety profiles. Among the viable targets for malaria drug development, kinases, a class of enzymes that play a critical role in various stages of the parasite's life cycle, are highly promising. Therefore an integrative approach combining computational methods, biochemical target-specific inhibition assays, and phenotypic screening may be an effective strategy for developing antimalarial compounds targeting multiple kinases.

Keywords: drug discovery, high-throughput virtual screening, molecular dynamics, drug resistance, kinase, multitargeting

Malaria is a life-threatening infectious disease caused by the *Plasmodium* parasite and transmitted through the bites of infected *Anopheles* mosquitoes. *P. falciparum* is responsible for the maximum number of death cases. The disease is a significant public health concern, particularly in tropical and subtropical regions of the world, where the majority of cases and deaths occur. According to the latest malaria report (World Malaria Report, 2022), there were nearly 247 million cases, and 619,000 reported deaths in 2021. This represents a 2 million increase in cases compared to the previous year. The group most susceptible were young children aged five years or below, who accounted for 80% of all malaria-related deaths https://www.who.int/health-topics/malaria. Despite the significant progress in reducing the malaria burden over the last two decades, the disease continues to pose a major global health challenge[1]. The parasite has developed resistance to most of the frontline antimalarial medications, making it increasingly challenging to treat and control the disease[2].

The statistics mentioned above pose the considerable public health challenge of malaria, emphasizing the urgent need for effective strategies to combat the disease.

THE QUEST TO DISCOVER THE MULTITARGETING DRUGS FOR MALARIA THERAPY

Compared to single-target and combination therapy, the multitarget strategy stands out as a novel and promising approach in terms of combatting drug resistance[3].

Multitargeting drugs exhibit polypharmacology by targeting multiple molecular targets[4]. It is commonly observed that biological network systems have robust homeostasis and redundant mechanisms. This suggests that relying on single-target drugs may not be sufficient and may lead to adverse side effects. On the other hand, a coordinated pharmacological approach that simultaneously modulates multiple targets is necessary to achieve the desired therapeutic outcome[5]. There is less potential for drug resistance with multitargeting medications compared to single or combination therapy, and additionally, the drugs are associated with a more effective, safer profile, comparatively less expensive, and require fewer daily doses[6,7].

The statistical and network analysis of the new molecular entities (NMEs) approved by the U.S. FDA between 2000-15 showed that from among the 361 total NMEs, 146 were single-target ones, 66 with two targets, 25 with three, and 16 with four targets, respectively. The remaining 95 NMEs had higher target numbers between 5-31[8]. Among the 101 FDA-approved NMEs from 2015-17, the contribution of multitargeting drugs was nearly 21%[4]. In January 2019, a search of the Journal of Medicinal Chemistry website for papers using the term "multitarget" yielded 265 results. Surprisingly, nearly 88% of them were published in the recent decade, indicating a growing interest in multitarget drug discovery and development in recent years. This trend highlights the increasing recognition among researchers of the potential benefits of developing drugs that can simultaneously target multiple pathways or proteins, rather than just a single target, in treating complex diseases[5].

1. *Plasmodium falciparum* kinases are exploitable drug targets

Kinases are a group of enzymes that catalyze the transfer of γ-phosphate from ATP to target proteins, thereby modulating their activity and function. Malaria kinases exhibit only 35-60% sequence similarity with the corresponding human orthologues[9]. In *P. falciparum*, kinases are involved in critical cellular processes such as cell division, differentiation, invasion, and egress from host cells. The parasite employ diverse range of kinases to phosphorylate proteins, lipids, and carbohydrates for its survival and proliferation. Some of the kinases extensively studied as potential targets for antimalarial drug development include Calcium-Dependent Protein Kinase 4 (*Pf*CDPK4), Calcium-Dependent Protein Kinase

1(*Pf*CDPK1), Cyclin-Dependent-Like Kinase 3 (*Pf*CLK3), Thymidylate Kinase (*Pf*TMK), Mitogen-Activated Protein Kinase 2 (*Pf*MAP2), Phosphatidylinositol 4-Kinase (*Pf*PI4K), cGMP-Dependent Protein Kinase (*Pf*PKG) and Protein Kinase 5 (*Pf*PK5), etc. Inhibiting these kinases can disrupt vital cellular pathways and interfere with the parasite's ability to infect and replicate within the host. The versatile roles of these kinases in *Plasmodium falciparum* make them attractive targets for drug development[10].

Additionally, the availability of crystal structures of the kinases provides valuable insights into their functional mechanisms and enables the rational design of small molecule inhibitors that selectively bind and inhibit their activity. Kinases also represent a highly druggable class of molecules extensively studied in cancer therapeutics[11]. Furthermore, *Pf*PI4K, an exploitable drug target, has gained significant attention, with one of its inhibitors (MMV390048) currently undergoing Phase II clinical trials[12].

2. High Throughput virtual screening is the preferred initial approach for multitarget drug discovery

High Throughput virtual screening (HTVS) and molecular docking-based studies have emerged as effective strategies in identifying potential lead molecules for drug development. This approach encompasses several stages: target identification, homology modeling, ligand preparation, protein preparation, grid generation, molecular docking, MMGBSA calculation, and molecular dynamics simulations (Fig.1). These methods have become a valuable primary step in drug discovery, enabling the identification of novel compounds to treat various diseases. In particular, virtual screening and molecular docking have proven to be powerful tools in the development of antiviral and antibacterial drugs, as well as antiprotozoal agents[13-16]. This approach can efficiently predict the binding affinity of small molecules to target proteins, aiding in identifying potential drug candidates. Several kinase inhibitor libraries accessible in various databases can be used for HTVS against the *P. falciparum* kinase targets.

Overall, this approach holds great promise in the identification of new leads for the development of therapeutic interventions[17-19]. This approach has proven to be an effective strategy for developing multitargeting drugs, extensively utilized against SARS-CoV-2[20,21]. The effectiveness of the structures predicted by HTVS against

Leishmania was demonstrated through *in vitro* testing[22], which validated the efficacy of this approach.

3. Experimental Design for Testing Hypothesis: Key Considerations and Approaches

The multitargeting hits may be further validated through the kinase inhibition assay to check for their multikinase inhibition nature. The kinase assay may be carried out using NADH/ATPase coupled assay, HPLC assay, ELISA-based protein kinase assay, etc.[23-25]. Parasite growth inhibition assay may be carried out to check the antimalarial effect of the hit compounds. Additionally, the standard *in vitro* resistance mutant generation experiment may be conducted using single-targeting drugs as a baseline to assess the likelihood of resistance to the multitargeting hit compounds[26]. The *in vivo* studies may be carried out to check the safety and efficacy of the best multitargeting inhibitors in animal models (Fig.1)[27].

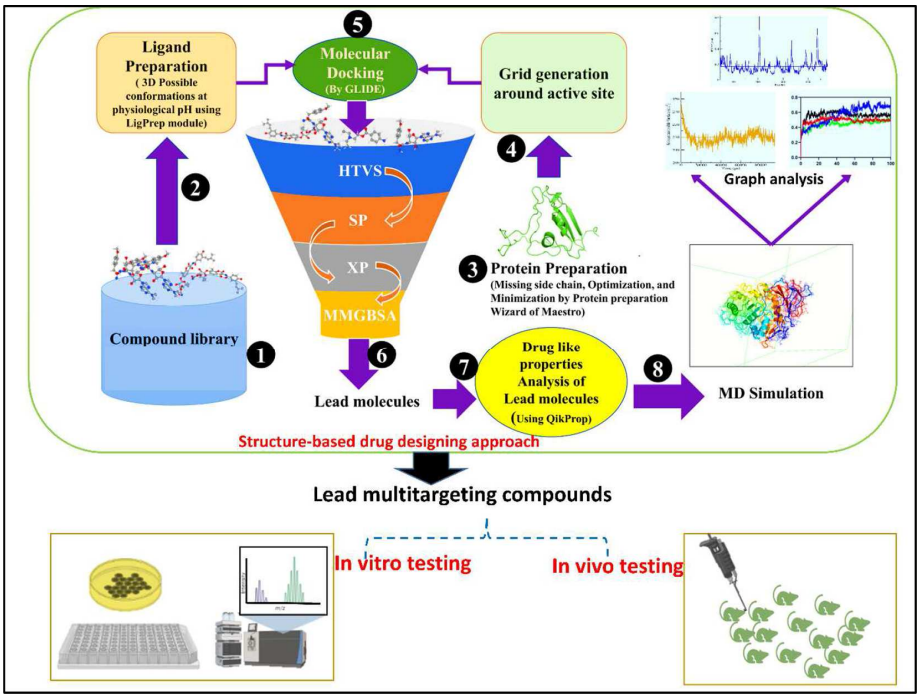

Fig 1. The schematic representation of the *in silico*, *in vitro,* and *in vivo* approaches for multitarget drug discovery against *Plasmodium falciparum* kinases.

CONCLUSION

In conclusion, the above discussed integrative approach combining computational methods, biochemical target-specific studies, and inhibition phenotypic screening has the potential to pave the way for the discovery of antimalarial compounds that target multiple *P. falciparum* kinases and, in doing so, may help address the issue of drug resistance that is becoming increasingly prevalent.

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Conflict of Interest Statement:

The authors declare that there are no conflicts of interest.

Author contributions:

PG carried out work and writing original manuscript, and DP reviewed and edited.

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