Design, Synthesis and Biological Evaluation of Some Novel Anti-malarial Agents

A THESIS SUBMITTED TO

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
PHARMACY

BY
RASANA YADAV

UNDER THE SUPERVISION OF

DR. PRASHANT R MURUMKAR

Faculty of Pharmacy
The Maharaja Sayajirao University of Baroda
Vadodara-390001

May 2025

Table of Content

1. Introduction

Malaria remains one of the most devastating parasitic diseases affecting humanity, with historical roots tracing back to ancient Indian, Chinese, and Egyptian civilizations[1]. Despite global health advancements, malaria continues to pose a severe threat, particularly in tropical and subtropical regions, where it has re-emerged as a leading cause of morbidity and mortality[2].

The disease is caused by protozoan parasites of the genus *Plasmodium*, of which over 150 species exist. However, only five species primarily infect humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*[3-4]. Among these, *P. falciparum* and *P. vivax* account for the vast majority of malaria cases and deaths worldwide. *P. falciparum*, the most virulent species, is prevalent in Africa, South America, and Southeast Asia, and is known for causing severe and often fatal infections. *P. vivax*, while widespread globally, is particularly dominant in India and contributes significantly to malaria-related illness[5].The other three species—*P. ovale*, *P. malariae*, and *P. knowlesi*—have more localized distributions and comparatively lower virulence[6]. However, infections from these parasites can still lead to serious complications if untreated. In particular, *P. malariae* can cause chronic conditions such as nephrotic syndrome, while *P. knowlesi*, a zoonotic parasite found in Southeast Asia, has emerged as a public health concern due to its capacity to infect humans from macaque hosts[7].

2. Aim and Objective

Malaria continues to be a major global health threat, particularly in tropical and subtropical regions, due in part to the rapid emergence of drug-resistant *Plasmodium* strains. Traditional antimalarial monotherapies targeting single biological pathways are increasingly compromised by

non-target site resistance mechanisms[8]. These mechanisms allow the parasite to evade the effects of treatment without modifying the direct drug target, posing a significant challenge to effective disease control.

To overcome this, the present research aims to design novel hybrid antimalarial agents that incorporate dual pharmacophores into a single molecular framework. This dual-targeting approach is intended to disrupt multiple parasite survival pathways simultaneously, thereby reducing the probability of resistance development and enhancing therapeutic efficacy.

The study strategically integrates various potent scaffolds known to target key biochemical processes within the parasite:

- Vicinal diaryl derivatives are potent inhibitors of *Plasmodium falciparum* dihydrofolate reductase (DHFR), a key enzyme in folate biosynthesis. These compounds have demonstrated IC_{50} values as low as 8.4 nM and show efficacy against both chloroquine-sensitive and resistant strains[9]. Notably, vicinal diaryl compounds have not been previously explored as antimalarial agents, presenting a novel direction for drug design.

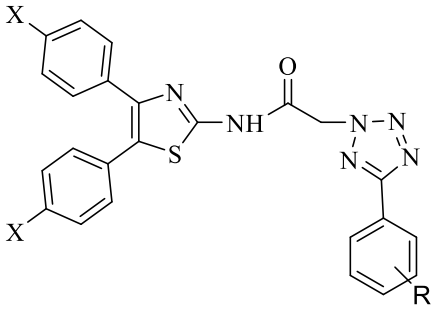
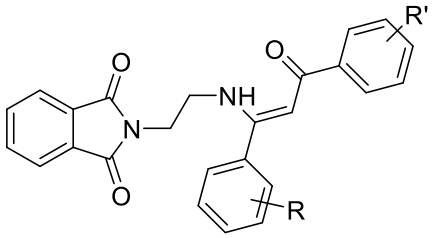
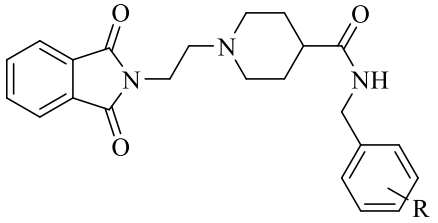
- Thiazole-containing compounds possess dual-action potential by inhibiting both DHFR and the heme detoxification pathway. A thiazole-based lead compound exhibited remarkable potency with an IC_{50} of 0.5 nM[10], reinforcing the scaffold's value in multi-targeted antimalarial strategies.

- Phthalimide derivatives also act as DHFR inhibitors. The imide moiety interacts with the enzyme's catalytic site, halting DNA synthesis. A representative compound showed an IC_{50} of 11 nM[11]. Continued optimization aims to improve selectivity and bioavailability.

- Chalcone derivatives, with their α,β-unsaturated carbonyl system, disrupt essential parasite enzymes through covalent interaction. A chalcone-based analog showed antimalarial activity with an IC_{50} of 200 nM[12], with further structural modification expected to enhance its potency.

- Piperidine derivatives target the parasite's heme detoxification process by preventing the formation of inert hemozoin, leading to toxic heme buildup. A piperidine compound demonstrated excellent activity with an IC_{50} of 4.19 nM[13].

Collectively, these scaffolds offer diverse mechanisms of action, including inhibition of folate metabolism, disruption of heme detoxification, and enzyme inactivation. By integrating these features into hybrid molecules, this research aims to develop resistance-resilient, next-generation antimalarial agents capable of maintaining high efficacy in the face of evolving drug resistance.

Derivatives	Structure	Remark
Vicinal diaryl thiazole fused tetrazole derivatives		R = H, Br, Cl, F, NO_2, Me, OMe, 4-*i*pr, 3,4 Di-OMe. X = H, Cl, OMe
Phthalimide fused chalcone derivatives		R = H, Br, Cl, F, NO_2, Me, OMe, 4-*i*pr, 3,4-Di-Ome R' = H, Br, Cl, Me, OMe, 3,4-Di-OMe
Phthalimide fused piperidine derivatives		R = H, 3-Cl, 4-Cl, F, NO_2, Me, OMe, 2,5-DiCF_3, 3,4-Di-OMe

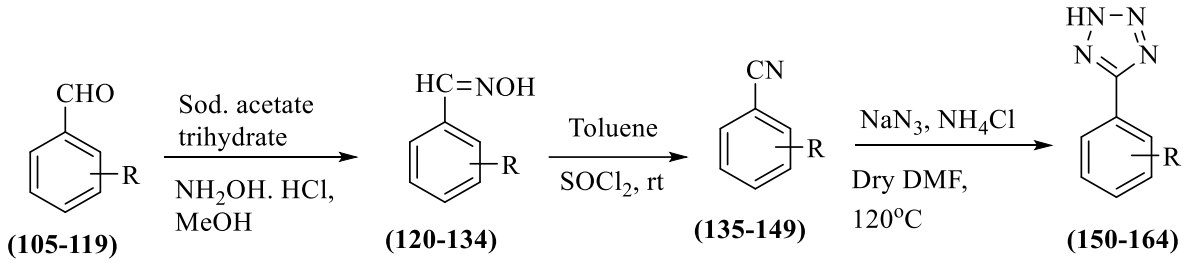
3. Research Methodology

In order to synthesis the compounds of our interest two different schemes were followed (General scheme 1-2).

Under General scheme 1 vicinal diaryl substituted tetrazole derivatives (186-224) were synthesized.

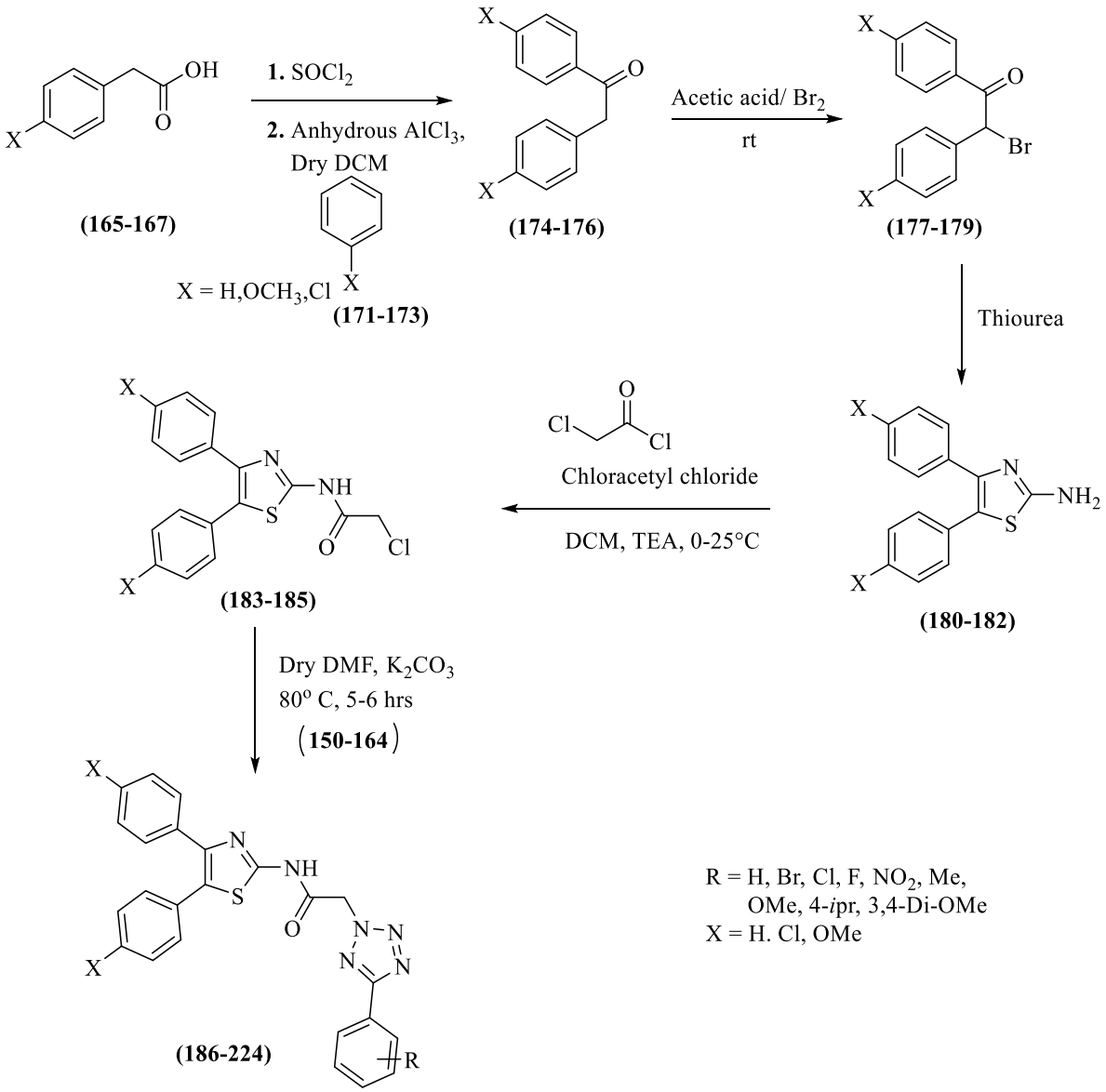
Synthesis of aryl-substituted tetrazoles (**150-164**) begins with the conversion of substituted benzaldehydes (**105-119**) to benzaldoximes, followed by their dehydration to benzonitriles. These benzonitriles undergo a 1,3-dipolar cycloaddition to yield 5-substituted-2*H*-tetrazoles (**150-165**).

For the preparation of the proposed compounds, vicinal diaryls (H, Cl, OCH_3 substituted) were synthesized using the Friedel-Crafts acylation reaction. The obtained ethanones were then brominated and cyclized using thiourea to form thiazole derivatives. These thiazole-2-amines were further modified through nucleophilic substitution and coupled with tetrazoles in the presence of potassium carbonate to obtain the final compounds, *N*-(4,5-di-(4-substitutedphenyl)thiazol-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl) acetamide (186-224).



(105-119) **(120-134)** **(135-149)** **(150-164)**

R = H, Br, Cl, F, NO_2, Me, OMe, 4-*i*pr, 3,4-di-OMe

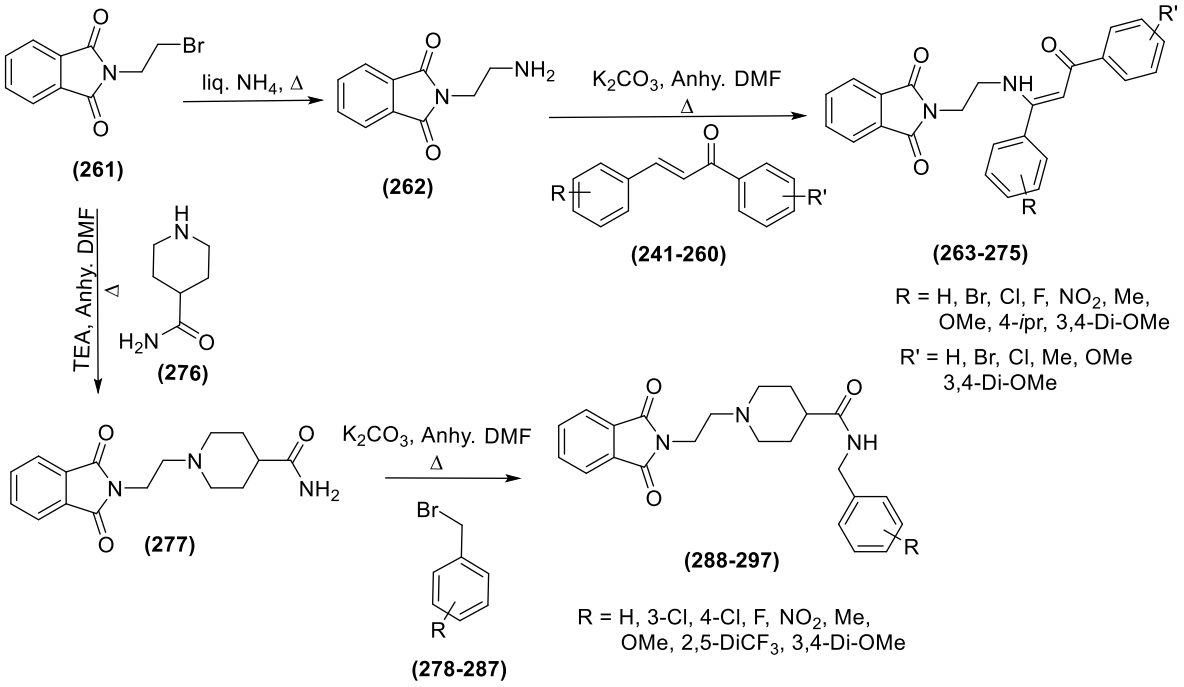


General Scheme 1: Synthesis of *N*-(4,5-di-(4-substitutedphenyl)thaizol-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl) acetamide (171-209)

Phthalimide moiety substituted various chalcone and benzyl halides derivatives i.e. (**261-273**) and (**285-294**) were synthesized using various strategies as discussed in General scheme 2.

Under the discussed scheme 2-(2-bromoethyl)isoindoline-1,3-dione was heated with liquid ammonia at 40°C under solvent free conditions to offer 2-(2-aminoethyl)isoindoline-1,3-dione. The resulted compound was further condensed with different substituted chalcones in the presence of base and anhy. DMF to offer desired compounds (**263-275**).

1-(2-(1,3-dioxoisoindolin-2-yl)ethyl)piperidine-4-carboxamide was synthesized on condensation of 2-(2-bromoethyl)isoindoline-1,3-dione with isonipecotamide which was further reacted with substituted benzyl halides to yield final desired compounds (**288-297**).



General scheme 2: Synthesis of 2-(2-substititedethyl)isoindoline-1,3-dione (**263-275**) and (**288-297**)

4. Key Findings

All the proposed compounds from general schemes 1 to 3 were successfully synthesized and structurally confirmed through IR, ^{1}H & ^{13}C NMR, HRMS, and mass spectrometry. The derivatives synthesized from general scheme 1, incorporating vicinal diaryl-tetrazole and phthalimide scaffolds, were evaluated for their in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7 strain. The biological evaluation revealed a concentration-dependent inhibitory profile, with several compounds displaying >70% inhibition at 10 µM.

Among the tested compounds, (**198, 219, 290, 293**, and **294**) exhibited consistent and potent activity across all concentrations, including at 50 nM, indicating their potential as strong lead antimalarial agents. Compound (**293)** demonstrated the most promising profile, showing 84.1%, 82.1%, and 76.6% inhibition at 10 µM, 500 nM, and 50 nM, respectively. Compounds (**189, 190,**

209, 273, and **280**) also showed measurable inhibition at the lowest concentration, suggesting favorable pharmacological properties.

Conversely, compounds (**188, 265, 266, 268**, and **269**) were found to be inactive across all concentrations, likely due to unfavorable structural or physicochemical characteristics. Computational docking studies revealed that active compounds formed stable complexes with *P. falciparum* target enzymes involved in heme detoxification, through hydrogen bonding, π–π stacking, and hydrophobic interactions, supporting their proposed mechanism of action.

Structure–activity relationship (SAR) analysis indicated that the presence of halogens, nitro, methoxy, and alkyl groups at specific positions enhanced activity by improving lipophilicity and binding affinity. Compounds bearing electron-withdrawing substituents displayed superior inhibition profiles. ADME predictions further confirmed the drug-like properties of the most potent derivatives. These findings support the hybrid design approach and provide a strong foundation for further optimization and in vivo validation of these novel tetrazolyl-thiazole and phthalimide-based scaffolds as antimalarial agents.

5. Conclusion

With an aim to design and develop novel therapeutics for the treatment of malaria, three general synthetic schemes were adopted to prepare proposed compounds. Based on the literature survey conducted, a combination of diverse bioactive scaffolds—namely vicinal diaryl, tetrazole, thiazole, and phthalimide—was selected and strategically integrated to enhance pharmacological profiles. All the proposed compounds were confirmed for their structures by IR, ^{1}H & ^{13}C-NMR, HRMS, and mass spectrometry.

Using general scheme 1, vicinal diaryl-based tetrazole derivatives (**186–224**) were synthesized and evaluated for their in vitro antimalarial activity against the *Plasmodium falciparum* 3D7 strain. These compounds exhibited a concentration-dependent inhibition pattern, with several derivatives, including (**198, 219, 290, 293**, and **294)**, demonstrating consistent and potent inhibition across all tested concentrations (10 µM, 500 nM, and 50 nM). Compound (**293)** showed particularly promising activity (84.1%, 82.1%, and 76.6%), indicating strong target engagement and pharmacological potential. Other compounds such as (**189, 190, 209, 273**, and **280)** also exhibited measurable inhibition at nanomolar levels. In contrast, compounds like (**188, 265, 266, 268**, and

269) were found to be inactive, suggesting unfavorable structural features.

Molecular docking studies confirmed stable binding interactions of active compounds with *P. falciparum* target enzymes involved in heme detoxification, involving hydrogen bonding, π–π stacking, and hydrophobic contacts. ADME predictions further supported the drug-likeness and pharmacokinetic favorability of the active compounds. The incorporation of nitrogen-containing heterocycles such as tetrazole and thiazole is believed to enhance binding interactions and influence lipophilicity, potentially aiding membrane permeability.

Using general schemes 2 and 3, novel phthalimide-based derivatives (**263–275** and **288–297**) were synthesized, incorporating various electron-donating and electron-withdrawing groups. SAR analysis revealed that halogens, nitro, methoxy, and alkyl substituents at specific aryl positions significantly influenced antimalarial potency. Notably, compound (**290)** showed strong and stable inhibition (97.1%, 42.9%, and 42.9%) across all concentrations, while compound 294, though not highly potent at 10 µM, demonstrated consistent activity across the dose range. A subset of derivatives, however, lacked activity due to excessive polarity or steric hindrance.

6. Recommendation

The compounds synthesized from general scheme-1, comprising vicinal diaryl-based tetrazole-thiazole derivatives, were found to exhibit potent in vitro antimalarial activity against *Plasmodium falciparum*. Among them, compound (**293)**, which maintained >75% inhibition across all tested concentrations (10 µM, 500 nM, and 50 nM), is recommended for further in vivo evaluation to determine its therapeutic efficacy, toxicity profile, and pharmacokinetic/pharmacodynamic behavior in animal models. Similarly, compounds (**198, 219**, and **290**), with consistent nanomolar-level inhibition, can be advanced to animal studies to confirm their biological relevance and metabolic stability.

On the other hand, several derivatives from general scheme-2 and 3, including compounds (**188, 265, 266, 268,** and **269**), showed no significant in vitro activity, indicating the need to reassess their physicochemical characteristics. These molecules may provide insights into undesirable steric and electronic configurations, and further exploration could involve structural simplification or scaffold hopping strategies to restore activity.

Molecular docking analysis and ADME predictions supported the high binding affinity and drug-

likeness of active compounds, especially for those engaging in hydrogen bonding and π-π stacking interactions at key sites of *P. falciparum* enzymes. Based on these computational findings, structure-based optimization is recommended to improve target selectivity and binding strength, potentially via fragment-based lead design or bioisosteric replacement strategies.

The hybrid scaffold design integrating phthalimide, tetrazole, and thiazole moieties proved valuable. However, more extensive mechanistic studies, including biochemical pathway assays and heme detoxification interference experiments, should be performed to confirm the exact mode of action of the lead compounds. Additionally, kinetic inhibition studies could help determine whether these compounds act via competitive, non-competitive, or allosteric mechanisms.

In conclusion, this work lays a solid foundation for the preclinical advancement of selected hybrid molecules, while also highlighting the importance of scaffold-specific tuning in overcoming antimalarial drug resistance. Further interdisciplinary studies are recommended to fully exploit the therapeutic potential of these promising chemical entities.

7. References

(1) Finnigan., E. B. N. A. Malaria - StatPearls - NCBI Bookshelf. 2020.

(2) Perkins, S. L. Malaria's Many Mates: Past, Present, and Future of the Systematics of the Order Haemosporida. *The Journal Of Parasitology* **2014**, *100* (1), 11–25.

(3) Phillips, M. A.; Burrows, J. N.; Manyando, C.; van Huijsduijnen, R. H.; Van Voorhis, W. C.; Wells, T. N. C. Malaria. *Nature Reviews Disease Primers* **2017**, *3* (1), 17050.

(4) Cox, F. E. G. History of the Discovery of the Malaria Parasites and Their Vectors. *Parasites & Vectors* **2010**, *3* (1), 5.

(5) Carlton, J. M. Malaria Parasite Evolution in a Test Tube. *Science (New York, N.Y.)* **2018**, *359* (6372), 159–160.

(6) Theunissen, C.; Janssens, P.; Demulder, A.; Nouboussié, D.; Van-Esbroeck, M.; Van-Gompel, A.; Van-Denende, J. Falciparum Malaria in Patient 9 Years after Leaving Malaria-Endemic Area. *Emerging Infectious Diseases*. United States January 2009, pp 115–116.

(7) Ortiz-Ruiz, A.; Postigo, M.; Gil-Casanova, S.; Cuadrado, D.; Bautista, J. M.; Rubio, J. M.; Luengo-Oroz, M.; Linares, M. Plasmodium Species Differentiation by Non-Expert on-Line Volunteers for Remote Malaria Field Diagnosis. *Malaria Journal* **2018**, *17* (1), 54.

(8) Feng, L. S.; Xu, Z.; Chang, L.; Li, C.; Yan, X. F.; Gao, C.; Ding, C.; Zhao, F.; Shi, F.; Wu, X. Hybrid Molecules with Potential in Vitro Antiplasmodial and in Vivo Antimalarial Activity against Drug-Resistant Plasmodium Falciparum. *Medicinal Research Reviews* **2020**, *40* (3), 931–971

(9) Wright, A. D.; Wang, H.; Gurrath, M.; König, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. Inhibition of Heme Detoxification Processes Underlies the Antimalarial Activity of Terpene Isonitrile Compounds from Marine Sponges. *Journal of Medicinal Chemistry* **2001**, *44* (6), 873–885

(10) Murumkar, P. R.; Chikhale, R. V. Chapter 6 - Vicinal Diaryl Triazoles and Tetrazoles. In *Vicinal Diaryl Substituted Heterocycles*; Yadav, M. R., Murumkar, P. R., Ghuge, R. B., Eds.; *Elsevier,* 2018; pp 191–219

(11) Bueno, J. M.; Carda, M.; Crespo, B.; Cuñat, A. C.; de Cozar, C.; León, M. L.; Marco, J. A.; Roda, N.; Sanz-Cervera, J. F. Design, Synthesis and Antimalarial Evaluation of Novel Thiazole Derivatives. *Bioorganic & Medicinal Chemistry Letters* **2016**, *26* (16), 3938–3944.

(12) Bansal, M.; Upadhyay, C.; Poonam; Kumar, S.; Rathi, B. Phthalimide Analogs for Antimalarial Drug Discovery. *RSC Medicinal Chemistry* **2021**, *12* (11), 1854–1867.

(13) Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O. In Vitro Antimalarial Activity of Chalcones and Their Derivatives. *Journal Of Medicinal Chemistry* **1995**, *38* (26), 5031–5037.