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Design, synthesis and biological evaluation of functionalized phthalimides: A new class of antimalarials and inhibitors of falcipain-2, a major hemoglobinase of malaria parasite

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ABSTRACT

Phthalimides functionalized with cyclic amines were synthesized, characterized and screened for their in vitro antimalarial efficacy against *Plasmodium falciparum* (*Pf*3D7). Of all the listed phthalimides evaluated, **14** and **24** were identified as potent antimalarial agents as advocated by assessment of their ability to inhibit [^{3}H] hypoxanthine incorporation in the nucleic acid of parasites. In addition, phthalimides **14** and **24** were incubated for 60 and 90 h and an enhanced antimalarial effect was noticed with increase in time to great extent. A reduction in IC_{50} values was observed with increase in exposure time of the parasite to the compounds. A symmetric phthalimide, **24** possessing piperazine as linker unit was identified as the most potent antimalarial agent with IC_{50} values of 5.97 ± 0.78, 2.0 ± 1.09 and 1.1 ± 0.75 μM on incubation period of 42, 60 and 90 h, respectively. The abnormal morphologies such as delay in developmental stages, growth arrest and condensed nuclei of parasite were observed with the aid of microscopic studies upon exposure with **14** and **24**. The evaluation of **14** and **24** against chloroquine resistant strain, (*Pf*7GB) of *P. falciparum* afforded IC_{50} values, 13.29 ± 1.20 and 7.21 ± 0.98 μM, respectively. The combination of **24** with artemisinin (ART) showed enhanced killing of parasite against *Pf*3D7. Further, all phthalimides were evaluated for their activity against falcipain-2 (FP2), a major hemoglobinase of malarial parasite. The enzymatic assay afforded **6** as most active member against FP2. To the best of our knowledge this is the initial study represents phthalimide protected amino acids functionalized with cyclic amines as potent antimalarial agents.

1. Introduction

Malaria is one of the most deadly protozoan parasitic infectious diseases caused by single celled *plasmodium* sp. According to WHO reports, nearly 1.2 million deaths worldwide occurred in 2010 alone.[1] The seriousness of the disease may be attributed to the increased drug resistance of *Plasmodium* strains, and the unavailability of an effective vaccine against these strains or insecticide against the mosquito which causes more than 95% of malaria related mortality.[2,3] Among various human parasites, *Plasmodium falciparum* causes cerebral malaria by exhibiting severe neurological symptoms and death.

During asexual blood stages of *P. falciparum* ~60–80% of hemoglobin (Hb) degradation takes place inside the acidic digestive vacuole (DV), and is pivotal for the survival of parasite inside the host cells.[4] The mechanistic action of Hb degradation that leads to hemozoin formation is poorly understood. However, recent report strongly suggests that this process is catalyzed by a complex of degrading enzymes like aspartic proteases, cysteine proteases and histo-aspartic proteases (HAP).[5] Cysteine proteases such as falcipain 2 (FP2), which is an enzyme responsible for the majority of Hb hemoglobin degradation, are considered potential drug targets.[6,7] Therefore, the malarial infections are being treated with drugs usually targeting the parasite at erythrocyte stages.[8,9]

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At present, clinically symptomatic infections are causing menace in the treatment regimen due to development of multi-drug resistant strains of *P. falciparum*[10–12] and the adverse effects of currently available antimalarial drugs.[13,14] This problem possesses vulnerable threat and increase in prevalence in the developing nations. For past few decades, commonly used drugs in malaria chemotherapy like chloroquine (CQ), amodiaquine and mefloquine fail to treat endemic malaria due to the increased resistance of *P. falciparum*.[15] In order to overcome the problem of drug resistance, artemisinin (ART) based therapy is suggested as first-line treatment for *P. falciparum* infections in malaria prevalent countries. However, it is really unfortunate to notice that indiscriminate use of ART as monotherapy led to emergence of drug resistance[16–18] and triggers us to look into new alternatives, including the development of new chemical libraries which is one of the most successful strategies to reduce the spread of drug resistance and to decrease the sensitivity of antimalarial agents.

Further, design and synthesis of new molecules representing the structural diversity and intricacy are considered as one of the potential pathways to enable the discovery of potent bioactive agents.[19–21] Thus, due to the urgent need for effective and economically viable as antimalarial agents, we herein present design, synthesis, characterization and antimalarial activity of chiral phthalimides functionalized with cyclic amines. Functionalities like pyrrolidines, piperidines, piperazines and 4-benzylpiperidine have been proven to be important scaffolds in antimalarial agents.[22–24] Phthalimide, **14** and **24** possessing piperazine and 4-benzylpiperidine, respectively showed a strong antiplasmodial activity against *Pf*3D7. The antimalarial effect of phthalimide **14** and **24** was also studied on the incubation period of 42, 60 and 90 h. The abnormal morphologies viz. delay in developmental stages, growth arrest and condensed form of nuclei of parasites upon the treatment of **14** and **24** were also shown with the aid of microscopic examinations. The antimalarial evaluation of **14** and **24** was further tested against CQ resistant strain, *Pf*7GB that exhibited significant inhibition as revealed by IC_{50} values 13.29 ± 1.20 and 7.21 ± 0.98 μM, respectively. Further, in vitro combination of most potent phthalimide **24** with ART was evaluated against *Pf*3D7. In order to find out the reasoning for their antimalarial activity, all the listed phthalimides were screened for their actions against falcipain 2 (FP2). The enzymatic assay afforded **6** as potent inhibitor of FP2. To the best of our knowledge, this exploratory study presents compounds with cyclic amine based phthalimide-protected amino acid linkers as strong leads for the development of novel antimalarial agents. For the first time, we hope that these results will influence promising therapeutic strategy in the treatment of malarial infection.

2. Results and discussion

2.1. Chemistry

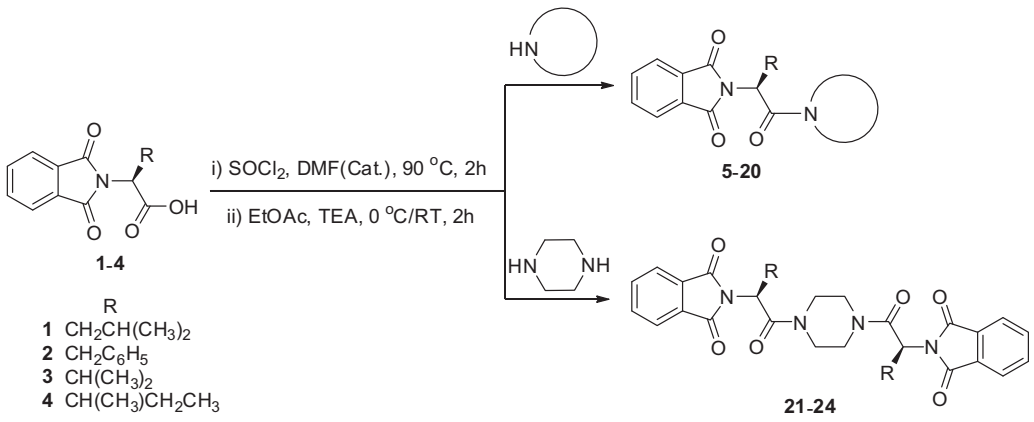
In the present investigation phthalic anhydride, amino acid and cyclic amines were simply employed to prepare 20 chiral phthalimides (**5**–**24**). In solution phase, standard reactions can be used to easily accomplish the formation of amide bonds by the protection or deprotection of amine and carboxylic group of amino acid is a usual process. Initially, the amine group of amino acid was protected by fusion with phthalic anhydride following the reported procedure[25] and the formation of *N*-phthaloyl-L-amino acids was confirmed by their melting points and NMR (^1H & ^{13}C) spectroscopy. The conversion of carboxylic group of *N*-phthaloyl-L-amino acids to their more reactive acid chloride derivatives was considered as a suitable path for the coupling reaction. In order to make the overall reaction economic thionyl chloride was used as an activating reagent rather any coupling reagents. Acid chlorides of corresponding *N*-phthaloyl-L-amino acids so obtained were used for the next step without any prior purification. Subsequently, cyclic amines such as pyrrolidine, piperidine, piperazine and 4-benzylpiperidine coupled with acid chloride in the presence of triethylamine. Triethylamine used in the reaction enhances the nucleophilicity of cyclic amines and also neutralizes the by-product of reaction.

The details of synthesis are depicted in Scheme 1. The composition of newly prepared molecules was confirmed by NMR (^1H & ^{13}C), FT-IR and HR-MS techniques. In NMR spectra, protons and carbons were assigned with the aid of COSY, DEPT and HETCOR experiments (see Supporting information). All the data were in good agreement with our proposed structure.

2.2. In vitro antimalarial assay

We first evaluated the antimalarial activity of all the listed phthalimides (**5**–**24**) on *P. falciparum* (3D7) in asynchronous culture. *P. falciparum* infected erythrocytes (4% final hematocrit and 2% parasitemia) were incubated for 24 h at 37 °C in the presence of various concentrations of test compounds. The known drugs, CQ and ART were used as positive control. The IC_{50} values were assessed by measuring the level of [^3H] hypoxanthine incorporation into nucleic acids for additional 18 h as described in Section 4. The mean value ± standard error is indicated for each compound and values are representative of two separate experiments. The results are summarized in Table 1.

By determining the antimalarial activity of phthalimides, it is clearly evident that **14** and **24** are the most effective



Scheme 1. Synthesis of functionalized phthalimides (**5**–**24**).