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Topic of Research: **Structure-based design, synthesis and biological evaluation of Antiparasitic agents**

Findings

Malaria is one of the most devastating parasitic diseases with severe global social and economic impact. The most virulent form of malaria is caused by *plasmodium falciparum* species and is accountable for more than one million of deaths each year. Due to the limited number of antimalarial medications available, acquired resistance poses a hazard to both individual patients' health and the disease's eradication strategies. Thus, there is an urgent need to develop novel antimalarial drugs with high efficacy, cost-effective and different mode of action.

In this thesis, we have chiefly focussed on the development of new hybrid antimalarials. We successfully designed and synthesised different core moiety-based series of compounds and screened them against both sensitive and resistance strain of *plasmodium falciparum.* The thesis comprises of five chapters.

Chapter 1: This chapter deals with the general introduction about about parasitic disease and malaria, its present status, life cycle of *plasmodium falciparum*, symptoms, causes, various drug targets of the malaria, available FDA approved antimalarial drugs, various drugs in pipeline and up-to-date literature review about the development of various anti-parasitic agents.

Chapter 2: we report a series of sixteen compounds of 2-((substituted)(4-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)piperazin-1-yl)methyl)-substituted phenol derivatives **(F1- F16).** The development of these compounds (F1‑F16) was justified through the study of H^1 NMR, C^{13} NMR, mass spectra. Compound F1 and F2 were also structurally validated by single crystal X-ray diffraction analysis. All the compounds were evaluated for their *in vitro* anti-plasmodial assessment against the W2 strain (chloroquine-resistant) of *P. falciparum.* Two compounds, **F4** and **F16** exhibited significant activity against W2 strain of *P. falciparum* with 0.75 and 0.74 μM. The compounds were also evaluated for *in vitro* cytotoxicity against two cancer cell lines, human lung (A549) and cervical (HeLa) cells, which demonstrated non-cytotoxicity with significant selectivity indices. Thus, all these results indicate that tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine scaffolds may serve as models for the development of antimalarial agents.

Chapter 3: we report a series of novel molecular hybrids based on tetrahydrobenzothieno[2,3-d]pyrimidine-acetamide (**GA1-GA8** and **A1-A8**) were synthesized and examined for their antimalarial activity. Compound **A8**, **A5** and **A4** are the most potent showed excellent anti-

plasmodial activity against CQ-resistant strain in the low nanomolar range with IC_{50} values 55.7 nM, 60.8 nM and 68.0 nM respectively. To assess the parasite selectivity, the in vitro cytotoxicity of selected compounds (A3-A6, A8) was tested against HPL1D cells, demonstrating low cytotoxicity and good selectivity indices. Haemolytic assay also showed non-toxicity of these compound on normal uninfected human RBCs. Docking studies of compounds were also performed to perceive the compounds' possible mode of action with both wild type and quadruple mutant *Pf*-DHFR-TS, exhibited good binding interactions in the active site.

Chapter 4: we report sixteen new 2-(4-cinnamoylpiperazin-1-yl)-2-(3,4-dichlorophenyl)-N-(subsituited)acetamide and (E)-1-(4-(1-(3,4-dichlorophenyl)-2-(subsituited)-2-oxoethyl)piperazin-1-yl)-3-phenylprop-2-en-1-one derivatives were designed, synthesised and biologically evaluated against malaria. We integrated molecular hybridization strategy with *in silico* drug design to develop Falcipain-2 (FP2) inhibitors. Docking results proposed that compounds M4 and M9 could efficiently bind to residue of amino acid of the active site of falcipain-2. All the synthesised compounds were also evaluated against CQ-sensitive 3D7 strains *of P. falciparum.* **M4**, **M9**, and **M1** were the most active compound having IC_{50} 1.18, 1.30, and 1.72 μM respectively. All the compounds were also screened for haemolysis assay for evaluating toxicity against uninfected RBCs.

Chapter 5: A library of series of 2-(4-subsituitedpiperazin-1-yl)-N-(5-((naphthalen-2-yloxy)methyl)-1,3,4-thiadiazol-2-yl)acetamide conjugates was designed, synthesized and structurally characterized by using different spectroscopic techniques. All compounds were also evaluated for their antimalarial activity and cytotoxicity evaluation. They exhibited good to excellent inhibitory activity against the growth of CQ-sensitive *Plasmodium falciparum* Nf-54 strain with IC_{50} values ranging from 0.940 to 106.90 μM and CQ-resistant Plasmodium falciparum W2 strain with IC_{50} values ranging from 10.00 μM to 3.919 μM. All the tested compounds were non-toxic against normal mammalian cell lines (Hek293) with good selectivity indices. Hemolysis assay also showed non-toxicity of these compound on normal uninfected human RBCs. The interaction of these hybrids was also investigated by the molecular docking studies in the binding site of both wild type and quadruple mutant *Pf*-DHFR enzyme. The physiochemical and pharmacokinetic properties analysis of best active compounds (**T5**, **T6**, & **T8**) was also studied using ADMET prediction. These data suggest that such molecular frameworks can behave as therapeutic templates for the design and synthesis of new antimalarials.

In summary, the present thesis addresses the synthesis of designed compounds that hit *in vitro* both *Plasmodium falciparum* CQ-resistant and CQ-sensitive strain by targeting *Pf*FP2 or *Pf*DHFR enzyme. Altogether, the thesis provides preclinical evidence that the lead molecules are therapeutic agents of high potency with multiple functions against malaria diseases.