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DOCKING STUDY, SYNTHESIS AND EVALUATION OF AN ANTI-MALARIAL AGENT

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ABSTRACT: Cysteine protease Falcipain-2 (FP2), the major cysteine protease of the human malaria parasite Plasmodium falciparum, is a hemoglobinase and promising drug target.*de novo* designed analogue of Parasitic Cysteine Protease Inhibitors, considering chemical stock feasibility of Analogue - 2-hydroxy-5-(1-hydroxy-2-((4-phenylbutan-2-yl)amino)ethyl)benzamide.) was synthesized and evaluated for its inhibition against Plasmodium falciparum cysteine protease falcipain-2. Compound was synthesized in good yield over 95% and characterized using 1H NMR, LC and Mass spectroscopy. The molecular properties of designed compound were also studied in silico for drug-likeness assessment based on Lipinski's rule of five. Antimalarial activity showed that, compound was showing activity against the sensitive and resistant malarial strain .Compound showing better results as per the data obtained from IC^{5o} values. The outcome of drug-likeness experiment showed that all newly designed analogues of Cysteine protease inhibitors possess good drug-like properties, indicating their drug likeness behavior is favorable for optimal ant malarial action. Assessment of drug-likeness score further implies the suitability of hybrid derivatives as drug-like molecules.

KEYWORDS: Analogue Design, Docking, NMR, Mass, Antimalarial.

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1. INTRODUCTION

Over the last few decades and more, the burden of malaria has increasingly become a serious health concern around the globe. The disease remains one of the most lethal infectious diseases of human beings affecting around 300-600 million people with about three million deaths per year globally.[1]Malaria persists to be one of the critical public health problems in India. Around 1.13 million confirmed cases and 287 deaths were reported in 2015 by the National Vector Borne Disease Control Programme (NVBDCP), out of which 67.1% was due to *Plasmodium falciparum.*[1]To get over this crucial disease burden and treat, a number of drugs candidates are available in the market. But due to the several drawbacks in curing phase such as toxicity, high cost, and diminishing efficacy of drug molecules, sometime treatments become very complicated and less effective. In recent times, the artemisinin-based combination therapies (ACTs) are reported as highly effective against P. falciparum in Africa region and most other malaria endemic countries.[1-5]Hence, globally research on malaria is a vital and important aspect to be recognized .There is an extensive need of new and improved drug candidates ideally directed against new protein target or receptor targets to control the malaria globally. Among the several newly acknowledged or identified parasitic targets, cysteine proteases constitute is an important antimalarial chemotherapeutic target.[6]. Proteases of various classes appear to be involved in critical processes during parasite development [7-10].One of these processes is hemoglobin degradation. Malaria parasites hydrolyze hemoglobin in an acidic food vacuole to obtain free amino acids for protein synthesis and possibly to maintain osmotic stability.[11-13]Plasmodia aspartic, cysteine, and metallo proteases all appear to participate in hemoglobin hydrolysis and co-operative functions of different classes of proteases in this process have been proposed.[11,14-18]Cysteine proteases are divided into clans and are dissimilar in terms of sequence or structural individuality and possibly arose separately.[19]Cys, His and Asn residues are worn by Clan CA proteases and can be found always in the same arrangement in the primary sequence of the cysteine protease. Four genes encode cysteine proteases of *P. falciparum*, recognized as falcipains. Falcipain-1, a fairly outlying relative of the other falcipains, is encoded on chromosome 14 and has been implicated in erythrocyte invasion by merozoites.[20-21] Remaining three falcipains, two closed identical copies of falcipain-2 [22] and a single falcipain-3,[23]are encoded within a 12.5-kb stretch of chromosome 11 (http://PlasmoDB.org) and play a key role in hemoglobin hydrolysis by parasites. First, cysteine protease inhibitors block the hydrolysis of hemoglobin by cultured parasites, causing the food vacuole to swell and fill with un-degraded hemoglobin.[24-26]Secondly, cysteine protease inhibitors block the growth of cultured *P. falciparum* parasites.[27]and cure mice infected with murine malaria parasites.[28]Third, falcipain-2 and falcipain-3 localize to the food vacuole, are active at the acidic pH of the food vacuole, and readily hydrolyze native and denatured hemoglobin[22,23]have been proposed. Cysteine proteases are divided into clans and are dissimilar in terms of sequence or structural individuality and possibly

arose separately.[19] Cys, His and Asn residues are worn by Clan CA proteases and can be found always in the same arrangement in the primary sequence of the cysteine protease.

2. MATERIALS AND METHODS

2.1 Analogue Design

All chemicals obtained for the experiment are from Sigma Aldrich and TCI, and were used without further purification. The solvents and reagents used in the antimalarial experiment were of analytical grade. The development of reactions was observed by the silica gel-G thin layer chromatography (TLC) and the spots/dots were visualized by iodine vapors. Compounds purity check was done by HPLC. With D2 O (deuterated water) as a solvent.1 H (NMR) spectra were recorded on Bruker AD II 400 FT-NMR spectrometer at 400 and 100 MHz, respectively using tetramethylsilane (TMS) as an internal standard (δ 0.00 ppm) Chemical shift (δ) values were expressed in parts per million (ppm) relative to TMS (δ 0.00 ppm).In this paper, an effort has made to synthesize novel analogue designed analogues (Cysteine protease inhibitor) and validate the synthesized compounds by NMR, Mass spectroscopy with 93% yield for compound. Synthesis process for compound became bit complicated as compare to compound.Further biological evaluation has been carried out to validate the inhibitory action of novel synthesized analogues or cysteine protease inhibitors and for the consideration of possible ant malarial agents.

Table 1:*de novo* designed analogue with cysteine protease falcipain-2 protein of *Plasmodium falciparum* (PDB ID: 3BPF)

De novo designed compound	SMILE structure format	Interacting residues in H-bond interactions	Number of H-bond interaction
Analogue	OCCclccc(ccl)CC[C@H](NCC[C@H(CCN(C)C)O)C	Gln 36, Gly 83 (TwoH-bonds), Trp 206	4

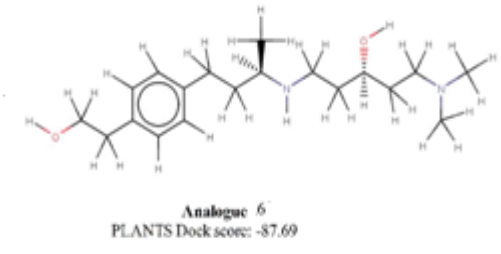


Fig.1 Molecular structure for Analogue

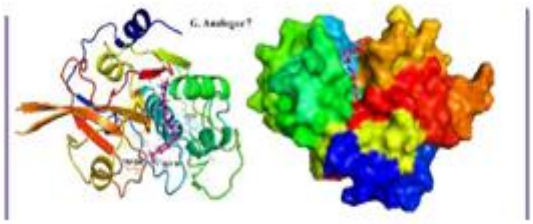
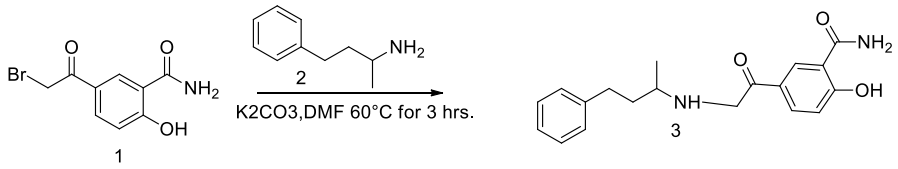


Fig.2 3D Structure for Analogue

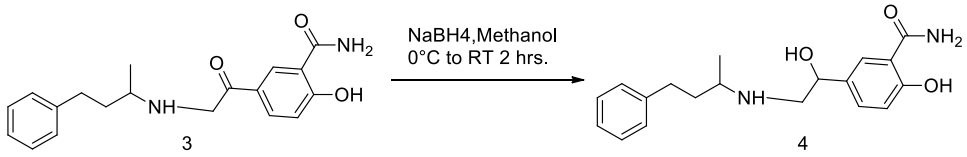
2.2 Synthesis:

Step-1: Synthesis of 2-hydroxy-5-(2-((4-phenylbutan-2-yl) amino) acetyl) benzamide.



To a solution of Int-1 (1 g, 1.0 eq.) in DMF (15 ml) was added K2CO3 (1.0 g, 2 eq.) followed by drop wise addition of Int-2 (0.8 ml, 1.5 eq.) and reaction mixture stirred at 60°C for 3 Hrs. After completion of reaction, Reaction mixture was diluted with water (100 ml) and extracted with DCM (3 X 30 ml).combined organic layer dried over anhydrous sodium sulphate and concentrate under reduced pressure to give pure Product Int-3.

Step 2: Synthesis of 2-hydroxy-5-(1-hydroxy-2-((4-phenylbutan-2yl) amino) ethyl) benzamide.



To a solution of Int-3 (0.8 g, 1.0 eq.) in Methanol (10 ml) was cooled to 0°C and NaBH4 (100 mg, 1.2 eq.) and reaction mixture was stirred at 0°C for 2 Hrs. After z of reaction, reaction mixture was concentrated and diluted with water and extracted with DCM (3 X 30 ml).combined organic layer dried over anhydrous sodium sulphate and concentrate under reduced pressure to give pure Product Int-3. Yield: 0.3 g.

3. RESULTS AND DISCUSSION

De novo compound was designed as the antimalarial potential cysteine protease protein 3BQA (pfEMP1) and found good molecular weights for the compounds and shown good binding energy which is compatible for H bond interaction. Analogue was synthesized and characterized for its purification through NMR, Mass and HPLC evaluation. Also biological evaluation was performed for antimalarial activity against P. falciparum strain 3D7 culture and shown good IC50 value (12 nM). This study found a potent antimalarial agent.

3.1 NMR Data

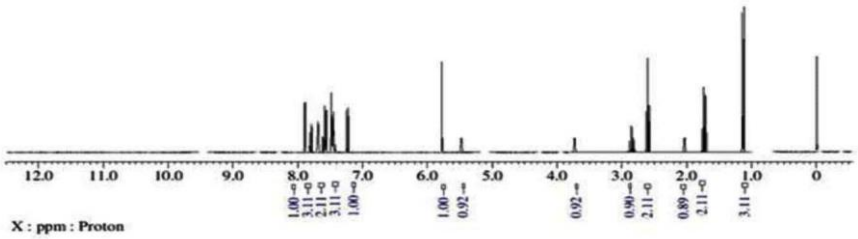


Fig.3 1H NMR (400 MHz, DMSO) δ 8.2 (d, J = 8.8 Hz,2H) , δ 8.1 (d, J = 8.8 Hz,1H), δ 7.3 (d, J = 8.8 Hz,5H), 4.65 (bs, 1H),3.650 (m, 1H), 2.950 (m, 2H), 2.66 (m, 4H), 2.1 (t, 2H), 2.3 (t, 1H), 1.12 (s, 3H)

3.2 Mass Spectra

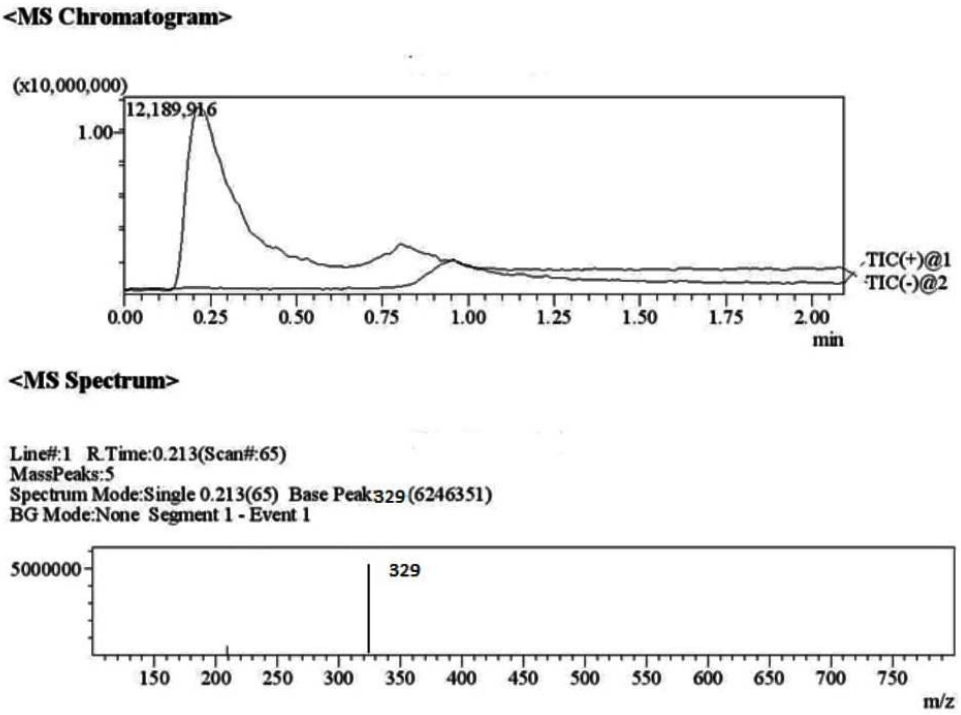


Fig.4 LC/MS (m/z) 329 (M + 1). Purification: Column chromatography in 0.5% MeOH; DCM to afford vi (0.75 g)

3.3 HPLC Method

HPLC System : Waters

Column :C18 (50*3.0mm), 2.6µ+ Guard Column

Mobile Phase : A- 5M Ammonium acetate in water.
B- Acetonitrile

Flow Rate : 1.2mL/Min

Injection Volume : 10.0 µL

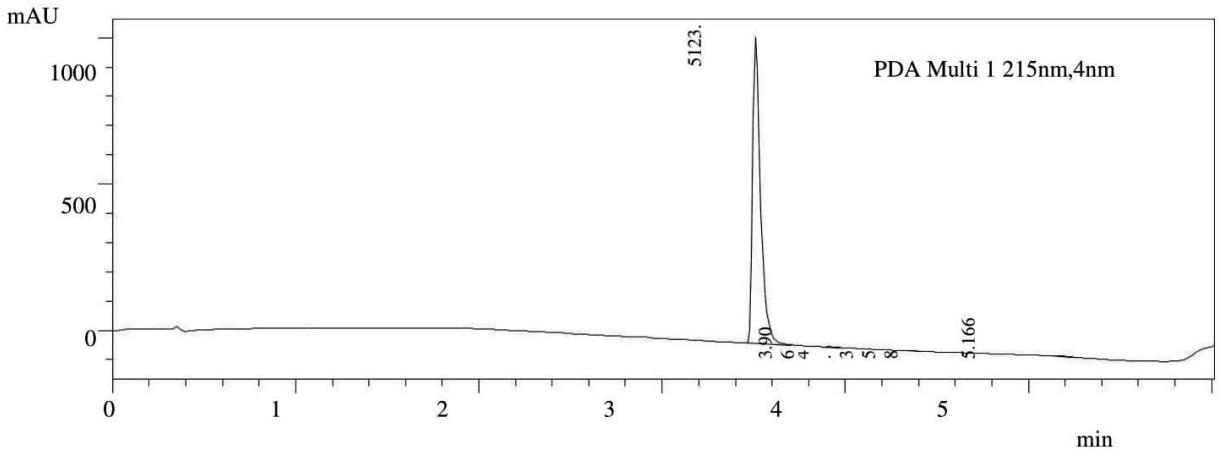


Fig.5. Chromatogram of the analogue 2-hydroxy-5-(1-hydroxy-2-((4-phenylbutan-2-yl)amino)ethyl)benzamide.)

Table 2: Retention time and % area of the analogue 2-hydroxy-5-(1-hydroxy-2-((4-phenylbutan-2-yl) amino)ethyl)benzamide)

Peak	RT	Area	Area%
1	3.512	3160403	99.550
2	3.906	4311	0.136
3	4.358	1940	0.061
4	5.166	8033	0.253
Total	4.233	3174687	100.000

3.4 Biological Evaluation

Assessment of inhibition of growth of P. falciparum and determination of IC50

P. falciparum strain 3D7 culture were used for the in vitro screening of the compounds (Radfar et al., 2009). Briefly, parasites are cultured at 10% parasitemiain RPMI1640 medium (Pan-Biotech, Germany) supplemented with 25 mM HEPES, 0.5% AlbuMAX I, 1.77 mM sodium bicarbonate, 100 µM hypoxanthine and 12.5 µg ml-1 gentamycin sulphate at 37°C. Red Blood Cells (RBCs) are prepared from fresh whole blood obtained from healthy donors and stored at 4°C for at least 1 day. A parasite culture in early ring stage is synchronized with 5% sorbitol treatment twice with a gap of 8 hours for the compound screening. Typically uninfected or infected (1–2% parasitemia, ring stage) red blood cells (2% heamatocrit) were added to the culture medium in the 6-well plate (Nunc, Roskilde, Denmark) along with different concentrations of the compound. Growth inhibition observed first with a broad range of concentrations of the compound of interest. Subsequently, growth inhibition was performed for a narrow range of the compound in triplicates. A positive control (artemisinin) was used. DMSO (0.05%) will be used as the solvent control. SYBR-green

staining was used for the determination of parasitemia after drug-treatment on day-4. The IC50 calculated from a plot of relative percent parasitemia versus log concentration of the inhibitor.

Table 3: Antimalarial activities (IC 50) againts *P. falciparum*

Compound	RSA values (% survival) at different concentrations		
	175 nM	350 nM	700 nM
Analogue	62	45	12

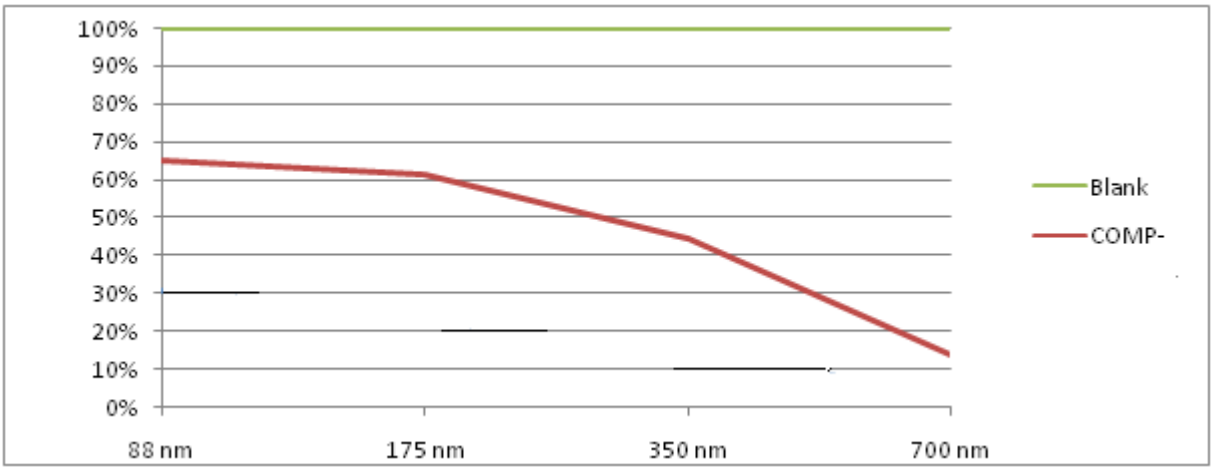


Fig.6.Mean % survival +/- SE of Plasmodium falciparum isolate Parasites after an 6 hr. exposure to a range of concentrations of compound

4. CONCLUSION

Synthesis of Analogue 2-hydroxy-5-(1-hydroxy-2-((4-phenylbutan-2-yl) amino) ethyl) benzamide wascarried out successfully with 93% yield with high purity, as checked by thin layer chromatography (TLC). The compounds was analyzed satisfactorily, both by the spectral and analytical data,Mass and 1H-NMR data have been comprehensively discussed and complement each other for compound. The Cyeteine Protease inhibitors with desired structural features to trigger anti malarial properties were presented to display desirable anti malarial activity. To confirm the inhibitors properties insillico investigation also done in background .Therefore, in conclusion synthesized compound is acknowledged as promising antimalarial agents with a scope to analyze more with remaining derivatives.

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CONFLICT OF INTEREST

Author has no any conflict of Interest.

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