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Designing novel inhibitors against falcipain-2 of *Plasmodium falciparum*

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ABSTRACT

Coumarin containing pyrazoline derivatives have been synthesized and tested as inhibitors of in vitro development of a chloroquine-sensitive (MRC-02) and chloroquine-resistant (RKL-2) strain of *Plasmodium falciparum* and in vivo *Plasmodium berghei* malaria. Docking study was also done on cysteine protease falcipain-2 which showed that the binding pose of **C-14** molecule and epoxysuccinate, inhibitor of falcipain-2, binds in the similar pattern. The most active antimalarial compound was 3-(1-benzoyl-5-(4-flurophenyl)-4,5-dihydro-1H-pyrazol-3yl)-7-(diethyamino)-2H-chromen-2-one **C-14**, with an IC_{50} of 4.21 μg/ml provided complete protection to the infected mice at 24 mg/kg X 4 days respectively.

Malaria is one of the major causes of morbidity and mortality in population of endemic countries. Annually, 500 million cases of malaria are registered and 3 million deaths due to malaria are reported in these countries. As per WHO report, one African child succumbs to malaria in every 30 s.[1–3] The gravity of the situation is increased due to development of resistance against drug in Plasmodium strain, unavailability of vaccine for human and non- availability of insecticide for malaria causing mosquitoes.[4]

During the intra-erythrocytic stage of *P. falciparum*, degradation of haemoglobin into hemozoin inside the acidic food vacuole is essential for the survival of parasite into the host cell.[5] This process is catalysed by the Cysteine proteases enzyme such as falcipain-2.[6] In the absence of cysteine protease, swelling occurs due to osmosis which renders the food vacuoles impaired and consequently, the parasite dies because of starvation.

Chemical literature reveals that the coumarins derivatives are having diverse biological and pharmacological properties.[7–14] Coumarins also displayed potential in vitro antiplasmodial and in vivo antimalarial activities.[15–17] Therefore, coumarin derivatives play a pivotal role in medicinal chemistry, also making them promising candidates for treatment of malaria. Pyrazolines derivatives have attractive intense interest in recent years as antimalarial activities.[18] Thus in the search for novel antimalarial drugs having synergistic effect of both moeity against the specific parasitic targets; we herein present design, synthesis, characterization and antimalarial activity of pyrazolines functionalized with coumarin. 15 compounds were synthesized which showed moderate in vitro antimalarial activity against chloroquine-sensitive and chloroquine resistant *P. falciparum*. Afterwards, three selected compounds C-8, C-14 and C-15 with better activity were also evaluated for their *in vivo* activity and showed moderate activity in a mouse model of *P. berghei*. The interaction of these conjugates in the binding site of *P. falciparum* falcipain 2 protein structures using molecular docking studies was also investigated.

Computer aided drug design play an imperative role in designing selective and potent inhibitors as well as vaccines. Among others, molecular docking approach is one of the most rational and authentic approaches in the drug design and discovery for studying the molecular interaction of small molecules.[19–21] The binding pattern of the synthesized most active compound was analysed using docking study in the active site of *P. falciparum* falcipain 2 protein. Our study presents compounds with coumarin based pyrazolines as strong leads for the development of novel antimalarial agents.

The target compounds were synthesized as shown in Scheme 1. 4-(diethylamino)salicylaldehyde on condensation with ethyl acetoacetate gave 3-acetyl-7-(diethylamino) coumarin **A**, which on Claisen-Schimdt condensation with different aromatic aldehydes in piperidine afforded compounds **B(i-v)** which on further treatment with hydrazine hydrate in ethanol at reflux temperature gave the target compounds **C(1–15)**. All compounds were obtained in good yields depicted in Table 1. All the synthesized compounds were characterized by their physical and spectral data (IR, ^{1}H NMR and Mass spectroscopy). The IR spectra of the compounds **C (1–15)** showed absorption due to lactone of coumarin at ~1725 cm^{-1} whereas, the —CH stretching band was seen around 2950–

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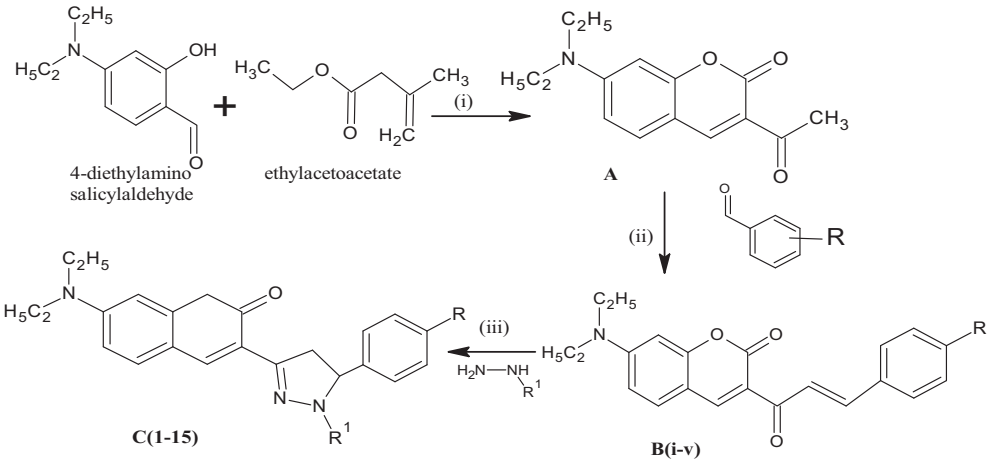
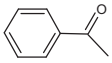
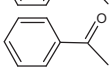
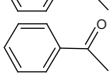
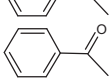
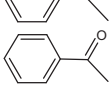


Scheme 1. Synthesis of 3-acetyl-7-(diethylamino)coumarin **A** then its chalcones **B(i–v)** and then pyrazolines **C(1–15)**. Reagents and condition: (i) piperidine, ethanol, rt, stirring; (ii) piperidine, *n*-butanol, reflux 6hr; (iii) glacial acetic acid, reflux 3hr.

Table 1
Pyrazoline derivatives.

Compound No.	R	R1	% yield
C-1	H	H	61
C-2	*p*-OH	H	59
C-3	*p*-Cl	H	83
C-4	*p*-F	H	68
C-5	*p*-NO_2	H	55
C-6	H		53
C-7	*p*-OH		48
C-8	*p*-Cl		54
C-9	*p*-F		55
C-10	*p*- NO_2		63
C-11	H		52
C-12	*p*-OH		60
C-13	*p*-Cl		62
C-14	*p*-F		72
C-15	*p*- NO_2		57

2850 cm^{-1}. The three proton of pyrazoline ring produced well defined resonances at d 5.28–5.12 (dd), 4.11–3.67 (dd) and 3.32–3.17 (dd), respectively.

The antimalarial activity of all the compounds was determined by in vitro against *P. falciparum* (MRC-02 and RKL9 strains). The results are shown in Table 2 indicate that nine of the 15 compounds evaluated, showed significant antimalarial activity against the chloroquine sensitive (MRC-02) strain. Interestingly, three of these nine compounds showed good activity (IC_{50} < 10 μM) also against RKL9, the chloroquine-resistant field isolate of *P. falciparum*. It is noteworthy that the resistance index of **C-8**, **C-14** and **C-15** compounds is higher than 1 (for **C-14**, its 2.2). This is due to their surprisingly higher potencies against chloroquine-resistant strain than that against chloroquine sensitive one (Table 2).

The results have indicated that these compounds have resistance index >1 which is significant due to the fact that the more the resistance index of a compound, it will be more effective in inhibiting the growth of the resistant strain at lower doses than that is required for inhibiting the growth of non resistant strain. The compound **C-14** (IC_{50}^{MRC-02} = 4.2 μM; IC_{50}^{RKL9} = 1.9 μM) and **C-15** (IC_{50}^{MRC-02} = 3.3 μM; IC_{50}^{RKL9} = 2.1 μM) were the most potent analogs of the series.

The most active compounds (IC_{50}^{RKL9} < 10 μM, i.e., **C-8**, **C-14** and **C-15**) were further evaluated for in vivo antimalarial activity against P. *berghei* at 25 mg/kg/day dose. The in vivo study was performed using the Peter's four day suppressive test. The chloroquine was used as treated control of the experiments at 20 mg/kg/day dose. The percentage inhibition of parasite multiplication was calculated comparing the treated group with the untreated group, by means of the following formula: [(A − B)/A] * 100, where A = parasitaemia in the untreated group and B = parasitaemia in the test group. Compound **C-14** provided 100% protection at this dose and all the rodents were alive after four weeks as indicated in Table 2. Whereas **C-15** and **C-8** showed 62% and 34% suppression of parasitaemia on day 4 but none of the treated mice survived beyond day 28.

To understand the binding mechanism of newly designed pyrazoline derivatives, docking study was performed for most active compound C-14 in the active site of falcipain-2. The docking experiment was carried out on Fujitsu linux workstation (Xeon quad-core E3-1220 processor) using LigPrep 3.0, Impact 6.3, Glide 6.3 modules of Maestro 9.8 (Schrödinger, LLC, New York, NY, 2014. The coordinates of falcipain-2 enzyme (PDB ID: 3BPF) were retrieved from RSCB protein data bank. Among the existing four crystal structure of falcipain-2 in the RCSB protein data bank, 3BPF is crystallised with the inhibitor (epoxysuccinate- crystal ligand) which help to understand the binding mechanism. This protein structure was prepared using Protein Preparation Wizard (Impact 6.3, Schrodinger)[22] as previously described.[23,24] In brief hydrogen atoms were added, treated with formal charges and correct bond orders were assigned. Molecules were sketched and prepared using LigPrep 3.0 with Epik 2.8 and tautomeric state and protonation states were expanded at 7.0 ± 2.0 pH units. The OPLS 2005 force field was used for both molecules and protein minimization. The grid was generated defining the centroid of crystal ligand. The docking was performed on extra precision mode and the docked complex was analysed for the H-bonds.

The Fig. 1a showed the binding pose of the most active compound C-14 in the active site of falcipain-2. The compound C-14