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Review Article

SYNTHESIS AND ANTIPLASMODIAL ACTIVITY OF SOME NOVEL CHALCONE DERIVATIVES

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ABSTRACT

Increased drug resistance in malaria toward many of the existing antimalarials make the condition worse. Hence, indicates the necessity of the novel molecules to overcome the problem. A series of chalcone derivatives (3a-4e) were primed via Claisen-Schmidt condensation of substituted aldehydes with substituted methyl ketones. These derivatives were tested against *Plasmodium falciparum* clinical isolate for their antiplasmodial activity. Furthermore, *in-vitro* β-hematin formation assay has been conducted in order to gain insight into the possible mechanism of action. Out of the 10 synthesized compounds, two compounds 4a and 4d exhibited promising antiplasmodial activities (50% inhibitory concentration [IC_{50}] values 7.45±0.65 and 6.01±0.29 µg/ml, respectively). Other compounds (3a, 4b, 4e and 3d) showed moderate inhibition against *P. falciparum.* Among all the compounds, 4a showed good hemozoin inhibitory activity (IC_{50} - 19.75 µg/ml) while 3a and 4d showed moderate type inhibition. These molecules may act as templates for medicinal chemistry to discover novel and hybrid molecules with improved characteristics, which may become future candidates for the treatment of malaria.

Keywords: Chalcone, Malaria, *Plasmodium falciparum*, β-hematin.

INTRODUCTION

Malaria is a one of the most devastating disease widely distributed and endemic in near about 106 countries of the world map. Malaria continues to be an enormous global health issue with approximately 250-500 million clinical episodes and nearly one million deaths annually [1]. Among the five human malaria species, *Plasmodium falciparum* is the most severe form, causing malignant malaria globally, while *Plasmodium vivax* is the most widespread species outside Africa, with enormous morbidity and can be severe and fatal [2]. This malaria parasite develops a very strong and selective resistance due to the widespread and indiscriminate use of some potent antimalarials. Artemisinin-based combination therapies are now recommended as first-line treatment of uncomplicated falciparum malaria in all areas in which malaria is endemic [3]. Recently, there have been signs that the efficacy of artemisinin - based combination therapy has declined in western Cambodia [4]. Artemisinin resistance would be disastrous for global malaria control. In the absence of a vaccine, chemotherapy plus vector control remain the main tools to reduce malaria related morbidity and mortality [5]. So, there is an urgent need of new bioactive compounds from natural sources and synthetic approaches against multi-resistant *Plasmodium* strains through the identification of new targets with antimalarial activity.

Chalcones exposed their importance in the field of antimalarial drug discovery when licochalcone A, a natural product isolated from Chinese liquorice roots, was reported to exhibit potent antimalarial activity [6]. Afterwards, a synthetic analogue 4-hydroxy-2-methoxy-4′-butoxy chalcone was reported to have outstanding antimalarial activity [7]. Ever since then, a succession of natural and synthesized alkoxylated, hydroxylated, prenylated, oxygenated, quinolylated chalcones have been examined as antiplasmodial agents [8-12]. On the basis of previous reports, here in the present study a series of chalcone derivatives have been synthesized and evaluated for their antiplasmodial activities with mechanism based study to explore their possible mode of action.

CHEMICAL SYNTHESIS OF DERIVATIVES

A series of chalcone derivatives were synthesized by Claisen-Schmidt condensation method, which is contributed by two step protocols (Fig. 1). Briefly, the first step involved the treatment of 4-chloro acetophenone with various cyclic amines in dimethylformamide (DMF) and potassium carbonate (K_2CO_3) at 110°C for 18 hrs to yield substituted acetophenone. After this cycle (as checked by thin-layer chromatography [TLC]), DMF was evaporated, and the contents were dissolved in water followed by extraction with chloroform. Then chloroform was removed by evaporation and substituted acetophenones were purified by flash column chromatography. In the next step, the substituted acetophenones were treated with suitably substituted aldehyde using sodium hydroxide as a catalyst in methanol at room temperature; the precipitate was collected by filtration, washed with water and recrystallized. The purity of the compounds was checked by TLC and elemental analysis while the homogeneity of the final compounds was also ensured by column chromatography. The compounds were characterized by both analytical and spectral data (^{1}H nuclear magnetic resonance (NMR),^{13}CNMR, infrared [IR] and mass spectroscopy [MS]). All the synthesized compounds were in full agreement with the proposed structures. Melting points were determined by open tube capillary method and were uncorrected. IR spectra were recorded on a Perkin-Elmer Fourier transform IR spectrometer (spectrum 2000) in KBr pellets.^{1}HNMR spectra were recorded on brucker (AMX-300) using $CDCl_3$ as solvent. Tetra methyl silane (TMS) was used as internal reference for^{1}HNMR.^{13}CNMR spectra were recorded on brucker top-stin-300 MHz using $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ ppm). Mass spectra were recorded on a Macromass G spectrophotometer.

General procedure for the synthesis of various substituted acetophenone

To a solution of 4-chloroacetophenone (6.0 ml, 40 mmo1) in 20 ml anhydrous DMF, imidazole (2.72 g, 40 mmol) and K_2CO_3 (11.1 g, 50 mmol) were added. The reaction mixture was refluxed for 18 hrs at 110°C. On completion of the reaction, as checked by TLC, the DMF was

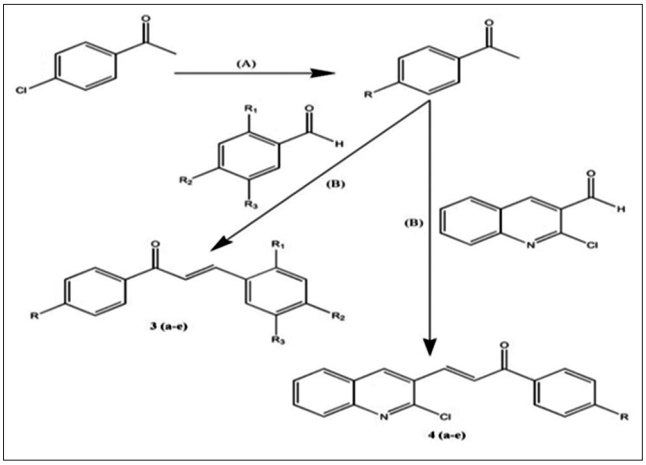

Fig. 1: General procedure for the synthesis of chalcone derivatives, Scheme for the synthesis of derivatives and reagents, (a) Cyclic amines, K_2CO_3, dimethylformamide, 18 hrs, 110°C, (b) 10% NaOH, MeOH, 18-20 hrs, rt, R=Cyclic amines (imidazole, triazole, pyrazole, benzimidazole and benzotriazole), R_1=Cl, R_2=Cl, R_3=H

evaporated in vacuum and the mixture was dissolved in water (50 ml) followed by extraction with chloroform (3 ml×50 ml). The combined organic solution was then dried over anhydrous sodium sulfate and evaporated in vacuum to yield 4-imidazole acetophenone. The formed intermediate was further purified by flash column chromatography and characterized using mass and NMR spectral analysis prior to use in the next step. The remaining intermediates were prepared by adopting the similar method.

General procedure for the synthesis of substituted chalcone derivatives

To a stirred solution of substituted acetophenone (6 mmol) and substituted aldehyde (6 mmol) in a minimum amount of methanol (normally 10 ml), NaOH pellets (600 mg) were added at 0°C. The reaction mixture was allowed to draw closes to room temperature and stirred for 18-20 hrs. The appearance of off-white to yellow solids in solution within a few minutes to several hours indicated successful synthesis of chalcone. The product was filtered and washed with ice cold water (3 ml×10 ml). The compound was purified by column chromatography using chloroform and methanol as eluent. The remaining substituted chalcones derivatives were prepared by a similar method.

1-(4-(1H-imidazol-1-yl) phenyl)-3-(2,4- dichlorophenyl) prop-2-en-1-one (3a)

Yield 69%, yellow crystals, m.p 160-162°C, MS m/z: 343 (M+1), IR (KBr)/cm^{-1}: 1659 (C=O), 1604 (C=C, Ar), 1524 (C=C, COC=C), 835, 806/cm (C-C1),^{1}HNMR ($CDC1_3$, 300 MHz): δ 7.90 (s, 1H, imidazolyl), 7.50 (d, 2H, imidazolyl), 7.31-8.09 (m, 9H, Ar),^{13}CNMR($CDC1_3$, 300MHz): δ117.75, 120.86, 124.34, 127.66, 128.55, 130.26, 130.67, 131.24, 131.60, 135.41, 136.22, 136.41, 136.81, 140.06, 140.72, 188.53.

1-(4-(1H-1, 2, 4 triazol-1-yl) phenyl)-3-(2, 4- dichlorophenyl) prop-2-en-1-one (3b)

Yield 79%, yellow crystals, m.p 160-161°C, MS m/z: 344 (M+1), IR (KBr)/cm^{-1}: 1670 (C=O), 1593 (C=C, Ar), 1464 (C=C, COC=C), 852/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHZ): δ 7.25 (s, 2H, triazolyl), 6.71-7.25 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ 117.45, 118.50, 123.80, 124.78, 127.82, 129.65, 129.81, 130.30, 131.59, 131.49, 132.51, 136.20, 138.28, 148.51, 185.65.

1-(4-(1H- pyrazol-1-yl) phenyl)-3-(2, 4-dichlorophenyl) prop-2-ene-1-one (3c)

Yield 74%, yellow crystals, m.p 153-155°C, MS m/z: 381 (M+K), IR (KBr)/cm^{-1}: 1660 (C=O), 1594 (C=C, Ar), 1389 (C=C, COC=C), 859/cm (C-C1),^{1}HNMR ($CDC1_3$,300 MHz): δ 6.52 (t, 1H, pyrazolyl- CH-), 7.25 (d, 2H, pyrazolyl-CH-N-N-CH-), 6.91-7.61 (m,9H,Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ117.87, 118.91, 120.60, 121.81, 122.72, 123.82, 124.86, 127.89, 129.65, 129.86, 129.75, 131.45, 131.56, 132.62, 148.71, 186.71.

1-(4-(1H-benzimidazol-l-yl) phenyl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (3d).

Yield 62%, yellow crystals, m.p 158-159°C, MS m/z: 393 (M+), IR (KBr)/cm^{-1}: 1670 (C=O), 1604 (C=C, Ar), 1487 (C=C, COC=C), 836/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHz): δ7.38 to 8.27 (m, 14H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ110.43, 120.87, 122.92, 123.31, 123.39, 124.18, 124.23, 127.60, 128.49, 130.19, 130.63, 131.48, 132.97, 136.16, 136.73, 136.77, 140.08, 140.12, 141.78, 144.20, 188.56.

1-(4-(1H-benzo [d] [1, 2, 3] triazol-1-yl) phenyl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (3e)

Yield 60%, light yellow crystals, m.p 154-155°C, MS m/z: 394 (M+1), IR (KBr)/cm^{-1}: 1660 (C=O), 1589 (C=C, Ar), 1383 (C=C, COC=C), 836/cm (C-C1),^{1}HNMR ($CDC1_3$, 300 MHz): δ7.27 to 8.44 (m, 13H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ117.87, 118.91, 123.82, 124.86, 127.71, 127.89, 128.22, 128.40, 128.72, 129.12, 129.65, 129.75, 129.86, 130.12, 131.45, 131.56, 132.62, 148.71, 186.71.

1-(4-(1H-imidazol-1-yl) phenyl)-3-(2-chloro-quinolin-3-yl) prop-2-en-1-one (4a)

Yield 69%, yellow brown crystals, m.p 94-95°C, MS m/z: 360 (M+1), IR (KBr)/cm^{-1}: 1686 (C=O), 1598 (C=C, Ar), 1475 (C=C, COC=C), 823/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHz): δ 7.76 (s, 1H, imidazolyl), 7.32 (merged, 2H, -N-CH=CH-N-), 7.29-8.79 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ 110.83, 117.80, 119.99, 120.84, 124.36, 124.52, 125.06, 125.32, 125.86, 126.96, 127.26, 127.92, 129.77, 130.43, 130.63, 132.62, 135.42, 136.95, 138.31, 148.95, 189.44.

1-(4-(1H-1, 2, 4-triazol-1-yl) phenyl)-3-(2-chloro-quinolin-3-yl) prop-2-en-1-one (4b)

Yield 85%, yellow crystals, m.p 150-152°C, MS m/z: 383 (M+Na), IR (KBr)/cm^{-1}: 1650 (C=O), 1594 (C=C, Ar), 1398 (C=C, COC=C), 810/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MH_z): δ 7.90 (s, 2H, triazolyl), 7.19-8.00 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ 111.41, 116.76, 118.12, 119.60, 123.82, 124.56, 127.71, 127.78, 127.80, 129.64, 130.31, 130.82, 131.30, 131.81, 136.72, 138.22, 148.62, 186.65.

1-(4-(1H- pyrazol-1-yl) phenyl) -3- (2-Chloro-quinolin-3-yl) prop-2-en-1-one (4c)

Yield 45%, yellow crystals, m.p 90-92°C, MS m/z: 360 (M+1), IR (KBr)/cm^{-1}: 1686 (C=O), 596 (C=C, Ar), 1497 (C=C, COC=C), 831/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHz): δ 8.09 (m, 3H, pyrazolyl), 7.45-8.01 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ119.98, 123.71, 124.22, 124.35, 125.05, 126.89, 127.26, 127.42, 128.16, 128.57, 128.95, 129.20, 129.77, 130.93, 132.61, 133.66, 135.50, 148.94, 189.42.

1-(4-(1H- benzimidazol-1-yl) phenyl)-3- (2-chloro-quinolin-3-yl) prop-2-en-1-one (4d)

Yield 54%, yellow crystals, m.p 160-162°C, MS m/z: 410 (M+1), IR (KBr)/cm^{-1}: 1660 (C=O), 1597 (C=C, Ar), 1450 (C=C, COC=C), 848/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHz): δ 7.82 (s, 1H, benzimidazolyl), 7.92 (m, 4H, benzimidazolyl), 7.40-8.50 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ, 112.20, 117.45, 118.50, 119.20, 123.38, 124.48, 127.71, 127.72, 127.82, 127.91, 128.12, 128.12, 128.71, 129.11, 130.11, 130.30, 130.65, 130.77, 131.32, 131.36, 147.50, 148.51, 185.65.

1-(4-(1H-benzo [d] [1, 2, 3] triazol-1-yl) phenyl) -3- (2-chloro - quinolin-3-yl) prop-2-en-1-one (4e)

Yield 56%, yellowish white crystals, m.p 170-172°C, MS m/z: 433 (M+Na), IR (KBr)/cm^{-1}: 1650 (C=O), 1590 (C=C, Ar), 1394 (C=C, COC=C), 809/cm (C-Cl),^{1}HNMR ($CDCl_3$, 300 MHz): δ 7.25-7.36 (m, 4H, benzotriazolyl), 7.30-7.87 (m, 11H, Ar),^{13}CNMR ($CDC1_3$, 300 MHz): δ 111.41, 116.76, 118.12, 119.60, 123.82, 124.56, 127.71, 12.71, 127.78, 127.80, 128.22, 128.40, 128.72, 129.12, 129.64, 130.12, 130.31, 130.82, 131.30, 131.80, 148.62, 186.65.

BIOLOGICAL ACTIVITY

Antiplasmodial activity

The synthesized derivatives were dissolved in dimethyl sulfoxide (10 mg/ml) and further diluted in RPMI at a suitable concentration. The compounds were screened for antiplasmodial activity against *P. falciparum* clinical isolate provided by National Institute of Malaria Research (NIMR), New Delhi. *In-vitro* drug sensitivity of derivatives was assessed using the standard procedure described by Trager and Jensen [13] by using candle jar method. Briefly, culture was maintained in A positive erythrocytes using RPMI 1640 medium supplemented with AB Rh positive human serum (10%), sodium bicarbonate (0.2%), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (25 mM) and gentamycin (50 µg/ml). The culture was treated with selected concentrations (1-100 µg/ml) of extracts. After 24-72 hrs of incubation, blood smears were prepared and stained with giemsa stain. Percentage maturation of schizonts against control was recorded. Chloroquine was used as a standard reference. The inhibitory concentration value that killed 50% of the parasites (IC_{50}) was obtained by plotting a linear curve dose - response of extract used and percentage inhibition obtained.

In-vitro β-hematin inhibitory assay (BHIA)

The *in-vitro* BHIA was carried out as reported earlier [14]. Male Swiss mice, weighing 15-20 g were inoculated with 1×10^5 *Plasmodium yoelii* infected red blood cells. Blood of the infected animal at 50% parasitemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 2500 rpm for 15 minutes at 4°C. The plasma was used for β-hematin formation inhibitory assay. The assay mixture contained 100 mM sodium acetate buffer pH (5.1), 50 µL plasma, 100 µM hemin as the substrate and 1-50 µg compound/drug in a total volume of 1.0 mL. The control tube contained all reagents except compound. The reaction mixture in triplicate was incubated at 37°C for 16 hrs in a rotary shaker. The reaction was stopped by centrifugation at 10,000 rpm for 10 minutes at 30°C. The pellet was suspended in 100 mM tris-HCl buffer pH (7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free hemin attached to polymerized β-hematin. The pellet was solubilized in 50 µL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm. The IC_{50} was determined using non-linear regression analysis dose response curves.

RESULTS AND DISCUSSION

The chalcone derivatives 3(a-e) and 4(a-e) were synthesized using base catalyzed clasien-schmidt condensation of the substituted methyl ketone with appropriate 2-chloro-3-formyl quinoline and 2, 4-di-chlorobenzaldehyde derivatives as depicted in Fig. 1. In the synthesized chalcone derivatives the aldehyde rings were 2, 4-dichlorobenzaldehyde and 2-chloro-quinolaldehyde, while 4-chloro functional group in acetophenone ring was replaced by N-containing heterocyclic ring such as imidazol, pyrazol, triazol, benzimidazol, benzotriazol to generate new compounds. The structures of all synthesized compounds were confirmed by IR,^{1}HNMR,^{13}CNMR and Mass spectral analysis. The IR band at 1660-70/cm suggesting the presence of (C=O) group, 1593/cm indicates the presence of (C=C) group and 852/cm indicates the presence of (C-Cl) group. The^{1}HNMR spectra of these compounds gave singlet for heterocylic ring protons at δ 7.25-7.91 and a multiplet for aromatic protons at δ 6.71-8.79.^{13}CNMR spectra of the compounds showed absorptions at 188, 129, 136 ppm due to C=O, C-Cl, N-C=N groups respectively, indicating the formation of synthesized compounds. The mass spectra of these compounds provide molecular ion peaks corresponding to their molecular masses.

A series of chalcone derivatives were synthesized and identified as novel antimalarial agents using *in-vitro* testing against the intact parasite. Previously, it was found that chalcone derivatives (2,4-dimethoxy-4'-butoxychalcone) have good antimalarial activity [6]. Therefore, the present study was carried out to synthesize a series of chalcone analogues and their evaluation against chloroquine sensitive *P. falciparum* strain. The IC_{50} values ≤16 µg/ml were considered to be of interest and are compiled in Table 1. Previously, Liu *et al*. [12] classified synthesized antimalarial chalcones with IC_{50} values ranging from 10 to 20 µm, as good antiplasmodial compounds. Results exhibited that derivatives 4a and 4d having quinoline ring showed good antiplasmodial activity with their IC_{50} values 7.45±0.65 and 6.01±0.29 µg/ml respectively while compounds 3a and 3d (having dicholoroaryledene moiety) and compounds 4b and 4e (having quinoline ring) showed moderate inhibition against *P. falciparum* (Table 1). Whereas the rest of the compounds showed, IC_{50} >16 µg/ml were considered to be moderate active or inactive.

Enormous amount of free heme is released by hemoglobin degradation in the food vacuole of the parasite. This free heme is highly toxic for parasite, causing extensive damage to the biomembranes and inhibits a variety of metabolic enzymes, resulting in the death of the parasite [15]. Polymerization of toxic free heme into non-toxic crystalline hemozoin is one of the prominent pathway followed by the parasite for its safety [16]. Hence, hemozoin pathway inhibitors become ideal in malaria drug discovery program as evident by various existing antimalarial such as chloroquine, which is known to be a potent inhibitor of hemozoin (β-hematin; *in-vitro* analog of hemozoin) formation [17]. Among all the compounds, 4a showed good hemozoin inhibitory activity (IC_{50} - 19.75 µg/ml) while 3a and 4d showed moderate type inhibition (Table 2). The difference in the antiplasmodial activity and hemozoin inhibition of compound 4d (*in-vitro* lead) may due to factors like the degree of accumulation of compound in parasite food vacuole. These results from *in-vitro* β-hematin formation revealed that the active molecules showing the promising antimalarial activity may inhibit heme polymerization process.

Table 1: Antimalarial activity chalcones derivatives against *P. falciparum* isolate

S.No.	Compound	IC_{50} (µg/ml)
1.	3a	9.75±0.85
2.	3b	33.82±3.91
3.	3c	31.62±4.65
4.	3d	15.82±3.24
5.	3e	20.00±3.45
6.	4a	7.45±0.65
7.	4b	10.10±1.52
8	4c	18.88±3.52
9	4d	6.01±0.29
10	4e	12.59±2.11
	Chloroquine	0.06±0.001

IC_{50}: Concentration corresponding to 50% growth inhibition of the parasite. Data are the mean±SD of three different experiments. SD: Standard deviation, *P. falciparum: Plasmodium falciparum*

Table 2: Effect of synthesized derivatives on β-hematin inhibition assay

S.No.	Compound	Inhibition of β-hematin formation IC_{50} (µg/ml)
1.	3a	24.66
2.	3b	ND
3.	3c	ND
4.	3d	>50
5.	3e	ND
6.	4a	19.75
7.	4b	>50
8	4c	ND
9	4d	37.38
10	4e	>50
	Chloroquine	5.72±0.81

IC_{50} represents the concentration of compound that inhibits β-hematin formation by 50%. ND: Not done

CONCLUSIONS

In conclusion, the present study demonstrates synthesis of chalcone derivatives and their *in-vitro* antiplasmodial activity against *P. falciparum* isolate. Two of the chalcones *viz.*, 4a and 4d showed appreciable antiplasmodial activity while compounds 3a, 4b, 4e and 3d displayed moderate antiplasmodial activity. Future aspects of this study will be a determination of the cytotoxity as well as *in-vivo* antimalarial activity of these synthesized chalcones. Thus, these compounds may act as templates for medicinal chemistry to discover novel molecules with improved characteristics, which can become preclinical candidates for the treatment of malaria.

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