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Synthesis and biological screening of some thienyl and phenyl pyrazoline derivatives as antimalarial agent

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Abstract

Malaria is a common parasitic disease of the developing world and drug resistance has hindered their efficient control. Pyrazole derivatives were synthesized using aldol condensation and subsequent cyclization reactions. The compounds were synthesized in a good yield (71.39 %-95.00 %). The compounds were purified by recrystallization and their chemical structure was characterized by elemental microanalysis, IR, and ^{1}H NMR spectroscopy. *In vivo* antimalarial activity was conducted using four-day suppression test method. The results for antimalarial activity conducted using *P. berghei* infected mice at a dose level of 48.46 µmol/kg/day showed that all the synthesized compounds had lower activity than the standard drug chloroquine phosphate. Compound IIc,1-phenyl-4-(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)-3-p-tolyl-1*H*-pyrazole, showed relatively the highest % suppression, 63.40 %.

Keywords: Antimalarial agent, Pyrazole derivative, Phenyl pyrazoline, Biological screening

Introduction

Malaria is one of the most prevalent parasitic infections in the world and certainly the most harmful [1] and still remains one of the most important parasitic diseases of the developing world [2]. Despite substantial advances in treatment and prevention over the past decade, malaria still threatens the lives of millions in tropical countries [2]. The risk of death from malaria is considerably higher in Africa than other parts of the world [3]. In Ethiopia, malaria is endemic in three quarters of the national territory, with *P. falciparum* predominating over *P. vivax* [4]. An estimated 51 million people (68 % of the population) live in areas at risk of malaria. Malaria is still the leading cause of health problem in the country [5].

Malaria contributes to health problems and deaths on many ways, especially in younger adults: (1) frequent acute infections, (2) anemia as a result of repeated or chronic malaria infection, (3) malaria in pregnant resulting in low birth weight in the new born and (4) increase susceptibility to other diseases such as respiratory infection, diarrhea, etc [6]. Malaria is a

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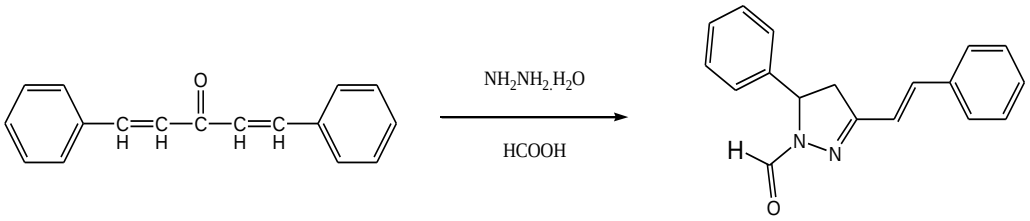

Figure 1 Synthesis of pyrazolines through reaction of dibenzalacetone with hydrazine hydrate and formic acid

major obstacle to socioeconomic development in Africa and the disease also contributes to poverty [7]. Malaria constitutes a major public health problem and impediment to socioeconomic development in Ethiopia [8].

Parasite resistance to anti-malarial drugs and mosquito resistance to insecticides are major threats to achieving global malaria control [2]. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. The economics of developing new pharmaceuticals for tropical diseases, including malaria, are such that there is a great gap between the public health importance of the disease and the amount of resources invested in developing new cures [9-11]. Most of the antimalarial drugs currently available have been in use for decades, but their use is now severely limited by the emergence and spread of drug resistance [12]. This disparity comes at a time when malaria parasites have demonstrated some levels of resistance to almost every anti-malarial drug currently available, significantly increasing the cost and complexity of achieving parasitological cure.

Pyrazolines can be synthesized by reaction of alkyl dihalides and hydrazines under microwave irradiation via a simple and efficient cyclocondensation in an alkaline aqueous medium [13]. The other synthetic method to produce pyrazolines is through the reaction of α, β unsaturated ketones with diazomethanes or hydrazine derivatives. It was reported that the reaction of dibenzalacetone and hydrazine hydrate with formic acid by heating under reflux for 24 hr under constant stirring gave 5-phenyl-3-(2-phenylvinyl]-4,5-dihydro-1*H*-pyrazole-1- carbaldehyde [14] (Figure 1).

It was reported that the diazomethane leads to the formation of pyrazoline type compound on reaction with dimethyl fumarate. The primary product of such cycloaddition is 1-pyrazoline but it spontaneously isomerizes into the more thermodynamically stable compound, 2-pyrazoline by 1, 3-hydride shift [15]. When arylhydrazines regioselectively react with 4-butynol in the presence of a catalytic amount of zinc triflate, aryl-substituted pyrazolines are obtained [16].

Different pyrazole derivatives were found to possess various important biological activities such as; antibacterial [17, 18], anti-inflammatory [19, 20], antioxidant [21], ACE inhibitory [22], anti-cancer [23], MAO-B inhibitory [24], antidepressant [25], antiviral [26], anti-mycobacterial [27, 28], anti-leishmanial [29, 30] and anti-malarial [31, 32] activities. These promising findings and continuation of the efforts to find a new class of antimalarial agents initiated research on such hetrocyclic compounds. Pyrazoline derivatives were reported to possess significant anti-malarial activity [33]. Based on these promising findings, as a continuation to this ongoing program, the aim of this project has been designed to synthesize and investigate safe, effective and cheap anti-malarial agent of pyrazoline derivatives containing phenyl or thiophenyl moiety.

Materials

Instruments and apparatus. Silica gel TLC plates (Merck, Germany) with UV light and iodine vapor as a detection system, Bruker Avance DMX400 FT-NMR spectrometer to collect ^{1}H NMR spectral data, IR spectra (Shimadzu 8400SP Spectrophotometer in the range of 4000-500 cm^{-1}), Eelectro thermal IA9100 hot storage melting point apparatus using an open melting tube, Perkin Elmer 2400 elemental analyzer and BIO-PLUS microscope to count Malaria parasites. In addition micropipettes (pipetman ultra), oven (Gallenkamp), eppendroff tube, heating mantle, autoclave (Pristage), thermoshake (Gerhardt), Vortex (model- whirl VIB2), pipette tips (Oxford), slides, aluminum foil, suction filter, class II biosafety cabinet (Labconco) have been used throughout the experiment for anti-malarial activity test.

Chemicals and reagents. Acetophenone, 2-acetylthiophene and hydrazine hydrate (Sigma Aldrich), ethanol, glacial acetic acid, propanoic acid, hydrochloric acid, KOH, absolute methanol, acetonitrile, chloroform, ethyl acetate, benzene, Tween 80, normal saline, sodium citrate, distilled H_2O, dimethyl sulfoxide (BDH, England), Giemsa stain, RPMI 1640 medium have been used throughout the experiments. 1-phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde was donated by Drug Discovery Centre, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Egypt.

Experimental animals and parasite strain. Swiss albino mice of both sex, weighing 24-38 g and of age 4-6 weeks obtained from Ethiopian Health and Nutrition Institute were used in testing the anti-leishmanial activity. The animals were acclimatized for a period of 7 days at room temperature (23-25 °C) and relative humidity of 60-65% before starting the assay. The animals were housed in standard cages and maintained on standard pelleted diet and water.

Standard drugs. Chloroquine phosphate (EPHARM) was used as a reference drug in determination of the antimalarial activity of the synthesized compounds.

Methods

Synthesis of target compounds. The intermediate α, β unsaturated ketones, (II and III) were synthesized by aldol condensation of 1-phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde I with 2-acetylthiophene and acetophenone respectively in alcoholic KOH. The target thienyl and phenyl pyrazolines were synthesized by cyclization of the intermediate α, β unsaturated ketones (II and III) with hydrazine hydrate in ethanol or the appropriate aliphatic acid.

(E)-3-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-1-(thiophen-2-yl)-prop-2-en-1-one (II). One of the intermediate compounds, which is an *α, β* unsaturated ketone II, was synthesized by condensation of 1-phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde I (2 g, 7.63 mmol) with 2-acetylthiophene (0.96 g, 7.63 mmol) in 20% ethanolic solution of KOH (20 ml). The yellow precipitate formed was filtered, washed with ethanol, dried and then recrystallized from chloroform/ethanol mixture (4:1). (Figure 2)

1-(5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-ethanone (IIa). A mixture of α,β-unsaturated ketone II (0.37 g, 1 mmol) and equimolar amount of hydrazine hydrate (0.048 g, 1 mmol) in glacial acetic acid (5 ml) was heated under reflux for 4 hr. The formed white solid product was filtered, washed with ethanol, dried and recrystallized from ethanol. (Figure 2)

1-(5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)propan-1-one (IIb). A mixture of α,β-unsaturated ketone II (0.37 g, 1 mmol) and equimolar amount of hydrazine hydrate (0.048 g, 1 mmol) in propionic acid (5 ml) was heated under reflux for 15 min. The formed white solid product was filtered, washed with ethanol, dried and recrystallized from ethanol. (Figure 2)

1-phenyl-4-(3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-3-p-tolyl-1H-pyrazole (IIc). A mixture of α,β-unsaturated ketone II (0.37 g, 1 mmol) and equimolar amount of hydrazine hydrate (0.048 g, 1 mmol) in ethanol (10 ml) was heated under reflux for 15 min. The separated white solid product was filtered, washed successively with water, dried and recrystallized from ethanol. (Figure 2)

(E)-1-phenyl-3-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-prop-2-en-1-one (III). The other intermediate compound, which is also an α, β unsaturated ketone III was synthesized by condensation of 1-phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde I (1 g, 3.81 mmol) with acetophenone (0.457 g, 3.81 mmol) in 20% ethanolic solution of KOH (20 ml). The yellow precipitate was filtered, washed with ethanol, dried and then recrystallized from chloroform/ethanol mixture (4:1). (Figure 3)

1-(3-phenyl-5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-4,5-dihydropyrazol-1-yl)ethanone (IIIa). A mixture of α,β-unsaturated ketone III (0.364 g, 1 mmol) and equimolar amount of hydrazine hydrate (0.048 g, 1 mmol) in glacial acetic acid (5 ml) was heated under reflux for 4 hr. The formed white solid product was filtered, washed with ethanol, dried and recrystallized from ethanol/chloroform mixture (1:1). (Figure 3)

1-(3-phenyl-5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-4,5-dihydropyrazol-1-yl)propan-1-one (IIIb). A mixture of α,β-unsaturated ketone III (0.364 g, 1 mmol) and equimolar amount of hydrazine hydrate (0.048 g, 1 mmol) in propionic acid (5 ml) was heated under reflux for 15 min. The obtained white solid product was filtered, washed with ethanol, dried and recrystallized from ethanol. (Figure 3)

Determination of physical constants for the synthesized compounds. Physical constants such as percentage yield and melting point were determined. R_f values were determined on precoated silica gel of 0.25 mm thickness plates using ethyl acetate:n-hexane (3:7) as a mobile phase and UV-light and iodine vapor as visual detection system.

Spectroscopic analysis of the synthesized compounds

IR analysis. IR spectra of the synthesized compounds were recorded in the range of 4000-500 cm^{-1} in nujol.

^{1}H NMR analysis. ^{1}H NMR spectra were recorded using Bruker Avance DMX400 FT-NMR spectrometer operating at 400 MHz. All the compounds were dissolved in $CDCl_3$ for NMR analysis. Chemical shift values are reported in δ (ppm) using tetramethylsilane (TMS) as an internal standard.

Condition of test protocol. The synthesized compounds II, III, IIa, IIIa, IIb, IIIb and IIc were evaluated for their *in vivo* antimalarial activity using *P. berghei* infected mice at a dose level of 48.46 µmol/kg/day. Chloroquine phosphate and the solvent were used as positive and negative control, respectively.

Biological activity test: In vivo antimalarial activity test. The *in vivo* antimalarial activity of the synthesized compounds was determined by standard 4 day

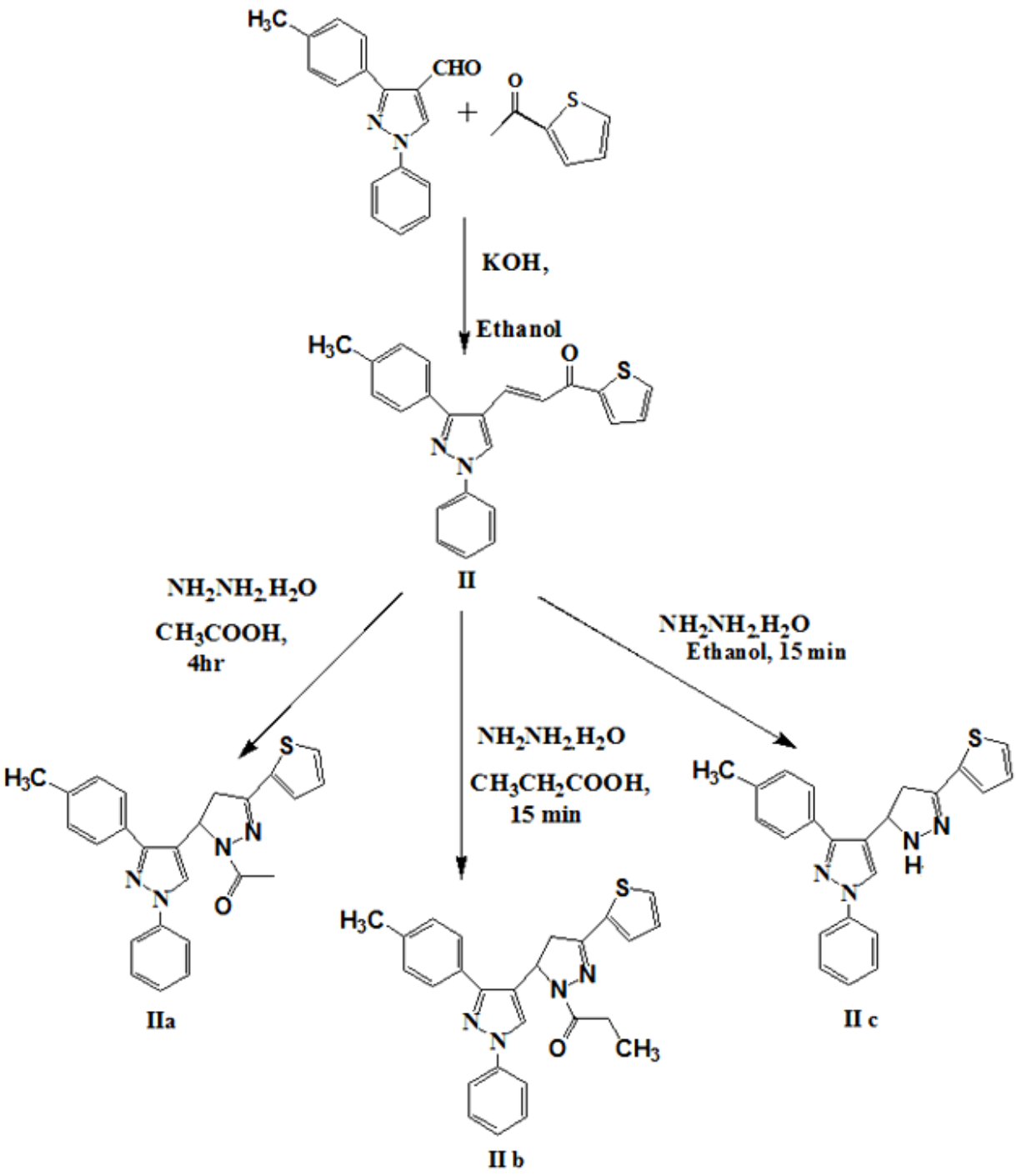

Figure 2 Synthesis of intermediate α,ß unsaturated ketone (III) and thienyl pyrazoline derivatives.

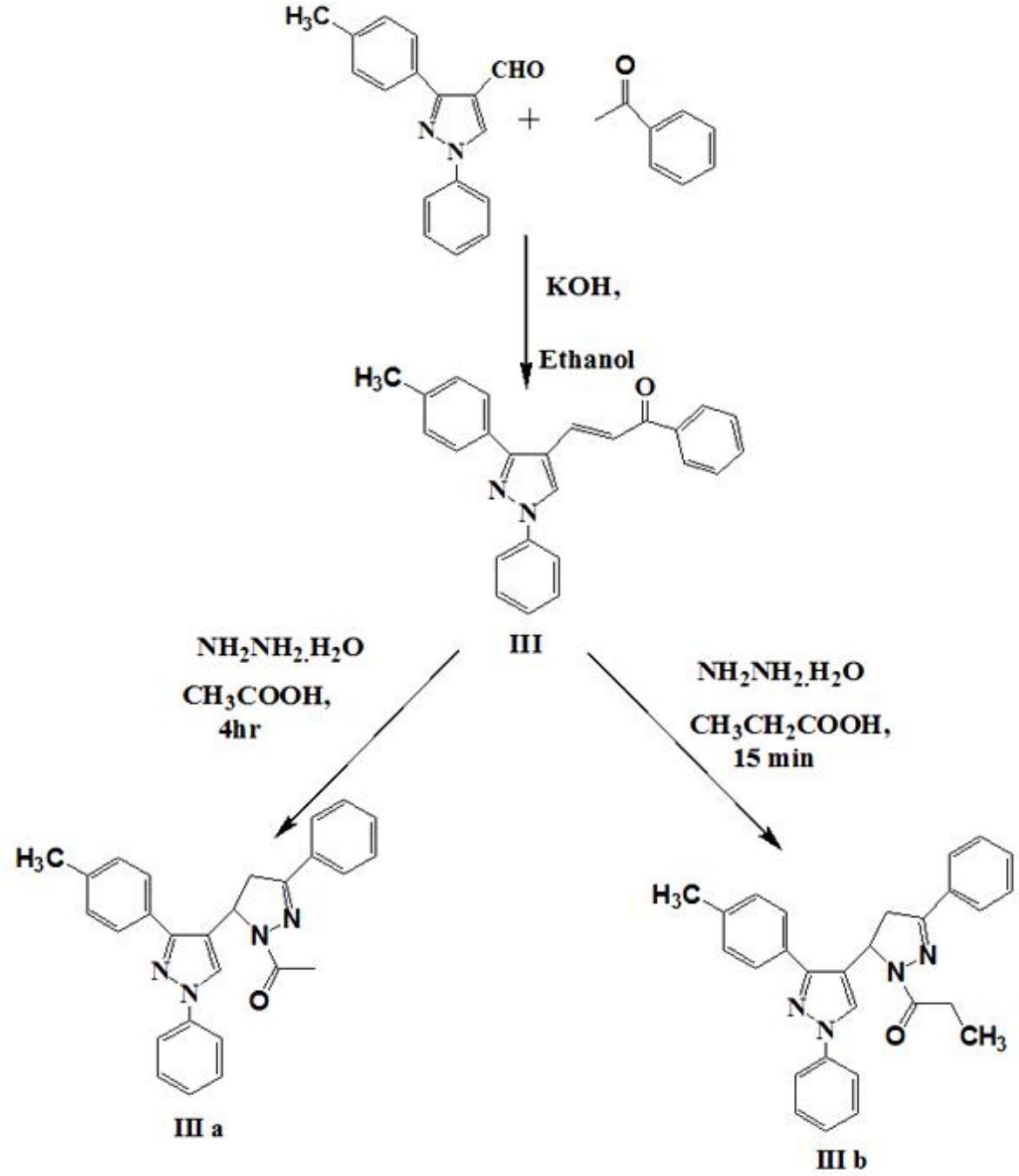

Figure 3 Synthesis of intermediate α,ß unsaturated ketone (III) and phenyl pyrazoline derivatives.

suppressive test using *P. berghei* ANKA strain infected mice as described by David, et al. (2004) [34]. This test is the most widely used preliminary test by which activity of various compounds is assessed by comparison of blood parasitemia and mouse survival time in treated and untreated mice [35]. Accordingly, test mice were infected with 0.2 ml of 2X107 parasitized erythrocytes (*P. berghei* ANKA strain) intraperitonialy on day 0. These parasitized erythrocytes were obtained from the blood of a donor mouse with 27 % parasitemia which was then diluted with normal saline (1:4). Mice were then weighed and randomly divided into nine groups of five mice per cage 2 hr after infection. The first seven groups received the synthesized compounds suspended in a vehicle containing 7 % Tween and 3 % ethanol in water orally at 48.46 µmol/kg dose level. Group eight received the vehicle only and acted as a negative control. The standard drug, chloroquine phosphate dissolved in the same solvent, was administered orally at a dose of 48.4 µmol/kg to mice in group nine and served as positive control [36, 37].

On day 1 to 3 (24 hr interval on successive dosing), animals in treatment groups were treated again with the same dose of the synthesized compounds through the same route as on day 0. On day 4 (24 hr after the last dose or 96 hr post-infection), the mice were weighed and blood smear was prepared on slides. The blood was then fixed with absolute methanol and stained with Giemsa. Level of parasitemia was determined microscopically by counting 4 fields of approximately 100 erythrocytes per field. The difference between the mean parasitemia level of the negative control group (taken as 100 %) and that of test compound treated group was calculated and expressed as percent suppression. The survival time for each test mouse was recorded except for chloroquine treated ones which were completely cured of the parasite [25]. Percent parasitemia and percent suppression was calculated using the following two formulas

$$\% \text{ parasitemia} = \frac{\text{Number of infected RBC}}{\text{Number of total RBC}} \times 100$$

$$\% \text{ Suppression} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in study group}}{\text{Parasitemia in negative control}} \times 100$$

Data analysis. Results of the antimalarial activity test were expressed as mean ± standard deviation. Statistical significance for suppressive test was determined by one-way ANOVA. All data were analyzed at 95 % confidence limits (p = 0.05).

Results and Discussion

Synthesis of the intermediate compound. The intermediate α,β-unsaturated ketone compounds II and III were synthesized by applying aldol condensation. Aldol condensation reaction involves nucleophilic addition of a ketone enolate to an aldehyde. The aldol product, when loses a molecule of water, the corresponding α, β-unsaturated ketone is formed. A strong base, potassium hydroxide, was used as a catalyst to produce ketone enolate.

Synthesis of thienyl and phenyl pyrazoline derivatives. The pyrazoline derivatives were obtained via Michael type addition. The compounds were synthesized by nucleophilic attack of hydrazine hydrate on the α,β-unsaturated ketone followed by cyclisation.

Physical properties, percentage yield and elemental microanalysis. Thin layer chromatography (TLC) was used to monitor the progress of chemical reactions and confirm their completion. The purity of the synthesized compounds was also inferred on TLC from one spot for each target compound in three different developing solvents. Percentage yield, R_f values, melting point and elemental microanalyses of the synthesized compounds were determined. Compound IIIa was produced in the highest yield (95.23%) while the least percentage yield was observed for compound IIc (71.39%). All the synthesized compounds were completely soluble in chloroform. Elemental microanalyses were also performed to find out their C, H, N, and S percentage composition. The results obtained were found to be within ± 0.4% of the theoretical values. This was done to assure the formation and purity of the proposed compound.

Spectral analysis of synthesized compounds. Spectral analysis such as IR and ^{1}H NMR were done to confirm the presumed structures of the synthesized compounds. All the functional groups and protons were observed at the expected values.

(E)-3-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-1-(thiophen-2-yl)-prop-2-en-1-one (II). Percentage yield: 90.0%, Melting point: 203-204 ^{o}C, retardation factor (Rf): 0.62, IR (nujol) cm^{-1}: 1636 (C=O); 1563 (C=N). ^{1}H NMR ($CDCl_3$/ CCl_4): δ (ppm) ; 2.45 (s, 3H, phenyl-CH_3), 7.17-7.40 (m, 5H, C<u>H</u>=CH-CO, phenyl-$C_{3,4,5}$H, thiophen- C_4 H), 7.45-7.53 (m, 2H, phenyl-$C_{2,6}$H), 7.60 (d, 2H, J= 8.27 Hz, *p*- tolyl-$C_{3,5}$ H), 7.65 (d, 1H, J= 5.96 Hz, thiophen-C_3 H), 7.75 (d, 1H, J= 4.85 Hz, thiophen-C_5 H), 7.80 (d, 2H, J= 8.27 Hz, *p*-tolyl-$C_{2,6}$ H), 7.92 (d, 1H, J= 15.6 Hz, CH=C<u>H</u>-CO), 8.36 (s, 1H,

pyrazole-C_5 H). Anal. Calcd for $C_{23}H_{18}N_2OS$: C, 74.53; H, 4.90; N, 7.56. Found C, 74.58; H, 5.21; N, 7.70.

1-(5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-ethanone (IIa). Percentage yield: 85.3%, Melting point: 193-194 oC, retardation factor (Rf): 0.67, IR (nujol) cm-1: 1658 (C=O); 1600 (C=N). NMR (CDCl3/ CCl4): δ (ppm) ; 2.40 (s, 3H, phenyl-CH3), 2.45 (s, 3H, COCH3), 3.65 (dd, 1H, J= 11.5Hz, pyrazoline-C4H), 3.09 (dd, 1H, J=4.4Hz, pyrazoline-C4H), 5.91 (dd, 1H, J=4.36Hz, pyrazoline-C5 H), 7.05 (dd, 1H, thiophene-C4), 7.13 (d, 1H, J=2.8Hz, thiophene-C3H), 7.26-7.3 (m, 3H, N1-phenyl-C3,4,5H), 7.4-7.5 (m, 3H, thiophene-C5H, N1-Phenyl-C2,6H), 7.66 (d, 2H, J=7.83Hz, p- tolyl-C3,5 H), 7.71 (d, 2H, J=7.83Hz, p-tolyl-C2,6H), 7.8 (s, 1H, pyrazole-C5H). Anal. Calcd for C25H22N4OS: C, 70.40; H, 5.20; N, 13.14. Found C, 69.95; H, 5.35; N, 13.27.

1-(5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl) propan-1-one (IIb). Percentage yield: 75.0%, Melting point: 204-205 ^oC, retardation factor (Rf): 0.48, IR (nujol) cm^{-1}: 1640 (C=O); 1600 (C=N). ^1H NMR ($CDCl_3$/ CCl_4): δ (ppm) ; 1.23 (t, 3H, J= 7.5Hz, $C\underline{H}_3$-CH_2), 2.38 (s, 3H, tolyl-CH_3), 2.82 (q, 2H, J= 7.5Hz, $CH_3C\underline{H}_2$), 3.60 (dd, 1H, J= 11.6Hz, pyrazoline-C_4 H), 3.05 (dd, 1H, J= 4.3Hz, pyrazoline-C_4 H), 5.88 (dd, 1H, J= 4.3, 11.6Hz, pyrazoline-C_5 H), 7.02 (t, 1H, thiophen-C_4H), 7.10 (d, 1H, J=3.59Hz, thiophen-C_5H), 7.23 (m, 3H, thiophen-C_3H, p-tolyl-$C_{3,5}$H), 7.38-7.43 (m, 3H, N_1-phenyl-$C_{3,4,5}$H), 7.63 (d, 2H, J=7.69Hz, N_1-phenyl-$C_{2,6}$H), 7.69 (d, 2H, p-tolyl-$C_{2,6}$H), 7.8 (s, 1H, pyrazole-C_5H). Anal. Calcd for $C_{26}H_{24}N_4OS$: C, 70.88; H, 5.49; N, 12.72. Found C, 71.11; H, 5.72; N, 12.92.

In vivo antimalarial activity. The synthesized compounds II, IIa, IIb, IIc, III, IIIa, and IIIb were evaluated for their *in vivo* antimalarial activity using *P. berghei* infected mice at a dose level of 48.46 µmol/kg/day chloroquine phosphate and the solvent were used as positive and negative control, respectively (Table 1). The antimalarial activity results revealed that all the synthesized compounds had lower activity than the standard drug chloroquine sulphate at the dose level of evaluation. The study revealed that the antimalarial activity of thienyl and phenyl pyrazoline ring, compound II and III, without substitution of the second pyrazole ring had lower activity with a percent suppression of 6.78 % and 36.99 %, respectively. However, the phenyl pyrazole ring, compound III, had a better antimalarial activity than the thienyl pyrazole ring, compound II.

Table 1 Antimalarial activities for the synthesized compounds at a dose of 48.4 µmol/kg.

Test compound	Dose (µmol/kg)	%Parasitemia*	%Suppression	Mean survival time (Days)*
II	48.46	46.71 ± 0.25	6.78	5.9 ± 0.21
IIa	48.46	40.16 ± 0.18	19.86	6.7 ± 0.85
IIb	48.46	33.82 ± 2.9	32.51	7.4 ± 0.38
IIc	48.46	18.34 ± 0.56	63.40	10.2 ± 0.46
III	48.46	31.57 ± 0.49	36.99	7.6 ± 0.13
IIIa	48.46	29.96 ± 0.73	40.21	6.9 ± 0.54
IIIb	48.46	27.30 ± 0.35	45.52	9.2 ± 0.50
Chloroquine phosphate	48.46	0	100	ND**
NC**		50.11 ± 0.13	0	5.3 ± 0.53

*Values are mean ± SD, P<0.05, **NC: Negative control, ND: No death recorded over the experimental period.

On top of this, the study showed that further substitution of the compound II and III with the second pyrazole moiety brought a better antimalarial activity than unsubstituted compound. Therefore, to a compound II and III, substitution of the pyrazole ring by nucleophylic attack of hydrazine hydrate on α, β unsaturated ketone followed by cyclization enhanced the antimalarial activity. Beside this, the phenyl derivatives, i.e. compound III, IIIa, and IIIb showed better antimalarial activity than their respective thienyl derivatives, like compound II, IIa, and IIIb. From the entire synthesized compound, the thienyl prazole derivative compound IIc and phenyl pyrazole derivative compound IIIb had the highest antimalarial activity with a percent suppression of 63.40 % and 45.52 %, respectively. Hence, the thienyl pyrazoline derivative, compound IIc had the highest antimalarial activity with a percent suppression of 63.40 % from whole synthesized compound, i.e. compound II, IIa, IIIb, III, IIIa and IIIb. (Table 1)

Conclusion

Seven pyrazole derivatives, four thienylpyrazoles and three phenylpyrazoles, were synthesized using aldol condensation and subsequent cyclization reactions. The compounds were produced in a good yield (71.39%-95.20%). The compounds were purified with recrystallization method and their structure was elucidated by elemental microanalysis, IR, and ^{1}H NMR spectroscopy. *In vivo* antimalarial activity was conducted using four day suppression test. The resultsfor antimalarial activity conducted using *P. berghei* infected mice showed that all the synthesized compounds displayed lower activity than the standard drug chloroquine sulphate with compound IIc showing the highest % suppression, 63.40%.

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