Evaluation of some 1*H*-pyrazole derivatives as a dual acting antimalarial and anti-leishmanial agents

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Abstract: The synthesis of a novel series of 1*H*-pyrazole derivatives was achieved by condensation of pyrazole aldehyde 1 with hydrazine hydrate to give hydrazone 7. On the other hand, cyclization of α,β-unsaturated ketone counterpart 2 using hydrazine hydrate in liquid aliphatic acids rendered compounds 4-6 and hydrazine hydrate in ethanol afforded compound 3. The later was allowed to react with aroyl chloride giving rise to compounds 8, 9. All compounds were tested for their *in vivo* anti-malarial and *in vitro* antileishmanial activities. The anti-malarial activity was performed using *Plasmodium berghei* infected mice, while the anti-leishmanial activity of the compounds was determined against *Leishmania aethiopica* promastigotes using alamar blue reduction assay. Compound 3, 1-(4-methylphenyl)-3-phenyl-4-[3-(2-thienyl)-2-pyrazolin-5-yl]-1*H*-pyrazole, possessed the highest anti-malarial activity with suppression of 70.26%. The highest anti-leishmanial activity was exhibited by compound 2, 1-(4-methylphenyl)-3-phenyl-4-[1-(2-thienyl)-prop-2-en-1-one]-1*H*-pyrazole, with an IC_{50} of 0.079µg/ml. Hydrazone 7 showed appreciable dual anti-malarial (suppression = 62.30%) and anti-leishmanial activity (IC_{50} = 1.823µg/ml).

Keywords: *1H*-pyrazole, *plasmodium berghei*, anti-malarial activity, alamar blue, *leishmania aethiopica*, anti-leishmanial activity, acute toxicity

INTRODUCTION

Malaria is a parasitic infection that has drastic effects on human worldwide. According to WHO malaria report, 243 million cases of malaria were estimated worldwide. The preponderance of infection cases (85%) are found in Africa. This disease resulted in about 863, 000 death cases in 2008 with approximately 89% were in African countries (WHO, 2009). Malaria is a protozoan disease, which is caused by parasites of the genus *Plasmodium* and transmitted to man by the sting of certain species of infected female anopheline mosquito. Four types of *Plasmodium* species commonly encountered in human infection: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Among these, *P. falciparum* and *P. vivax* are frequently found to cause the illness. However, the majority of severe disease and death has been recorded due to *P. falciparum* (Kalra *et al*., 2006). The condition is getting worse primarily due to the absence of effective drugs and development of drug resistance. Quinoline derivatives, anti-folates and artemisinins have been the mainstays for treatment of malaria (Valderramos and Fidock, 2006).

Leishmaniasis is worldwide distributed with major infection regions being Southern Europe, North and East Africa, Central and South America, the Middle East, and the Indian subcontinent (Habtemariam, 2003). It is caused by obligate intracellular vector-borne parasites, which belong to genus *Leishmania* and family Trypanosomatidae. The parasites are transferred to humans by an insect vector, phlebotomine sand flies, through bite attacks. There are three clinical types of leishmaniasis, visceral leishmaniasis, cutaneous leishmaniasis and mucocutaneous leishmaniasis. Sodium stibogluconate, amphotericin B, miltefosine, pentamidine and paramomycin are drugs used for treatment of leishmaniasis (Croft *et al.,* 2006) Resistance was reported to all currently existing anti-malarial and anti-leishmanial drugs, in addition to their toxicity. To tackle this problem new agents are urgently needed.

It has been reported that several pyrazole derivatives showed anti-malarial (Acharya *et al.*, 2010) and anti-leishmanial activity (Santos *et al.*, 2011). We have already reported the anti-malarial activity of some pyrazole derivatives (Bekhit *et al.*, 2012). This work presents our attempt to design and synthesize novel pyrazole derivatives with selected substitution pattern to study the effect of such molecular variation on the anti-malarial and anti-leishmanial activities.

MATERIALS AND METHODS

Chemistry

The compounds were synthesized according to the scheme shown in fig. 1. The determinations of melting points were performed in open glass capillaries using electro thermal BUCHI (B-540) hot storage melting-point apparatus and are uncorrected. Infra-red (IR) spectra was recorded on Shimadzu 8400SP infrared spectro-photometer using nujol and KBr. ^{1}H NMR spectra was recorded on Bruker Avance DMX400 400MHz FT-NMR

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spectrometer using $CDCl_3$ and the chemical shifts are given in δ (ppm) using tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; dd: doublet of doublet and m: multiplet. Perkin Elmer 2400 elemental analyzer was used for the determination of the elemental analyses and values obtained were within the theoretical values (±0.4%). Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60 GF_{254}, Merck) and the spots were detected by the aid of iodine vapour and by exposure to UV lamp at λ_{254} nm for few seconds (table 1).

1-(4-Methylphenyl)-3-phenyl-4-[1-(2-thienyl)-prop-2-en-1-one]-1H-pyrazole 2

An equimolar mixture of 1-(4-methylphenyl)-3-phenyl-1*H*-pyrazole-4-carboxaldehyde 1 (2.62g, 10 mmol) and 2-acetylthiophene (1.26g, 10mmol) in 3% alcoholic KOH (20ml) was stirred for 6 hrs under room temperature. The yellow precipitate formed was then filtered, washed with ethanol, dried and recrystalized from chloroform/ ethanol (3:1). Yield: 91.2%. mp 211-12°C.

1-(4-Methylphenyl)-3-phenyl 4-[3-(2-thienyl)-2-pyrazolin-5-yl]-1H-pyrazole 3

A mixture of the intermediate α,β-unsaturated ketone **2** (0.56 g, 1.5 mmol) and equimolar amount of hydrazine hydrate (0.072 g, 1.5 mmol) in ethanol (20 ml) was heated under reflux for 30 min. The white precipitate obtained after cooling was filtered, washed with ethanol, dried and recrystalized from ethanol. Yield (73.3%). mp 156-57°C.

4-[1-Acetyl-3-(2-thienyl)-2-pyrazolin-5-yl]-1-(4-methylphenyl)-3-phenyl-1H-pyrazole 4

A mixture of the intermediate α,β-unsaturated ketone 2 (0.56g, 1.5mmol) and equimolar amount of hydrazine hydrate (0.072g, 1.5mmol) in glacial acetic acid (20ml) was heated under reflux for 8 hr. The white solid product formed was filtered, washed with ethanol, dried and recrystalized from ethanol. Yield: 78.7%. mp 170-1°C.

1-(4-Methylphenyl)-3-phenyl-4-[1-propyl-3-(2-thienyl)-2-pyrazolin-5-yl]-1H-pyrazole 5

An equimolar mixture of α,β-unsaturated ketone 2 (0.56g, 1.5 mmol) and hydrazine hydrate (0.072g, 1.5mmol) in propanoic acid (20ml) was heated under reflux for 6 hr. The reaction mixture was concentrated, cooled and poured into crushed ice (30g). The grey solid product separated was filtered, washed with ethanol, dried and recrystalized from ethanol. Yield: 72.7%. mp 204-6°C.

4-[1-Butyl-3-(2-thienyl)-2-pyrazoline-5-yl]-1-p-methylphenyl-3-phenyl-1H-pyrazole 6

A mixture of α, β-unsaturated ketone 2 (0.56gm, 1.5 mmol) and equimolar amount of hydrazine hydrate (0.072 gm, 1.5mmol) in butyric acid (20ml) was heated under reflux for 6 hr. The reaction mixture was concentrated, cooled and poured into crushed ice (30g). The white solid product formed was filtered, washed with ethanol, dried and recrystalized from ethanol. Yield: 68.6%. mp 211-3°C.

1-(4-Methylphenyl)-3-(phenyl)-1H-pyrazole-4-carboxaldehyde hydrazone 7

A mixture of aldehyde 1 (0.3g, 1.08mmol) and excess hydrazine hydrate (0.25g, 5 mmol) in ethanol (20ml) and one drop of HCl was heated under reflux for 15 minutes. The yellow solid product formed after cooling was filtered, washed with ethanol, dried and recrystallized from acetonitrile. Yield: 60.1%. mp 265-6°C.

4-[1-Aroyl-3-(2-thienyl)-2-pyrazolin-5-yl]-1-(4-methylphenyl)-3-phenyl-1H-pyrazole 8 & 9

To a solution of 3 (0.38 gm, 1mmol) in dry pyridine (5ml) an equivalent amount of the selected aroyl chloride was added. The reaction mixture was then heated under reflux on a boiling water bath for 20min, cooled and poured onto crushed ice (30g). The white precipitate formed was separated by filtration, washed with water, dried and recrystalized from ethanol. Compound 8**:** Yield: 83.6%. mp 274-5°C. Compound 9: Yield: Yield: 81.2%. mp 268-9°C.

Biology

Male Swiss albino mice were obtained from the Ethiopian Health and Nutrition Research Institute. The protocols used in the present study followed the guidelines set in "The Guide for the Care and Use of Laboratory Animals". The anti-malarial activity was determined by counting the parasites using BIO-PLUS microscope.

In vivo anti-malarial activity

The *in vivo* anti-malarial activity of the synthesized compounds was determined by a standard 4 day suppressive test using *Plasmodium berghei* ANKA strain infected mice. Male Swiss albino mice (weight 20-25 gm) were used in this study. Before starting the assay, the animals were acclimatized for one week to room temperature (23-25°C) at relative humidity of 60-65%. The animals were maintained on standard pelleted diet, housed in standard cages and had free access to water. This test is the most widely used preliminary test to assess the anti-malarial activity of compounds by examining blood parasitaemia and mouse survival time in treated and control-untreated mice. The assay was performed as according to Dominguez *et al* (2009). The test mice were infected with parasitized (*Plasmodium berghei* ANKA strain) erythrocytes obtained from the blood of a mouse with approximately 20-30% parasitaemia. The parasitized erythrocytes was diluted with normal saline (1:4) and given intravenously (0.2ml of 2×10^7) on day 0 of the trial.

Table 1: Elemental analyses and spectral data of compounds 2-9

Cpd. No.	Elemental analyses	IR	^{1}H NMR ($CDCl_3$)
2	For $C_{23}H_{18}N_2OS$: C,74.57; H, 4.90; N, 7.56; S, 8.66. Found: C, 74.28; H, 5.15; N, 7.35; S, 8.80.	(Nujol, cm^{-1}) 1632 (C=O); 1594 (C=N)	2.50 (s, 3H, phenyl-CH_3), 7.15-7.38 (m, 5H, phenyl-H), 7.22 (d, 1H, J= 15.5 Hz, CH=C<u>H</u>-CO), 7.51 (t, 1H, thioph-C_4 H), 7.61 (d, 2H, J= 8.0 Hz, *p*- tolyl-$C_{3,5}$ H), 7.66 (d, 1H, J= 4.7 Hz, thioph-C_3 H), 7.76 (d, 1H, J= 4.7 Hz, thioph-C_5 H), 7.82 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{2,6}$ H), 7.93 (d, 1H, J= 15.5 Hz, C<u>H</u>=CH-CO), 8.36 (s, 1H, pyrazole-C_5 H).
3	For $C_{23}H_{20}N_4S$: C, 71.85; H, 5.24; N, 14.57; S, 8.34. Found : C, 72.06; H, 5.48; N, 14.32; S, 8.16.	(KBr, cm^{-1}) 3300 (N-H); 1600 (C=N)	2.50 (s, 3H, phenyl-CH_3), 3.05 (dd, 1H, J=4.4 Hz, pyrazoline-C_4 H), 3.60-3.70 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.90-6.00 (dd, 1H, J= 4.4 Hz, pyrazoline-C_5 H), 7.10-7.44 (m, 9H, thioph-H, phenyl-H, pyrazoline- NH), 7.64 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{3,5}$ H), 7.72 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{2,6}$ H), 7.79 (s, 1H, pyrazol-C_5 H)
4	For $C_{25}H_{22}N_4OS$: C, 70.4; H, 5.20; N,13.14; S, 7.52. Found: C, 70.62; H, 4.94; N, 12.89; S, 7.82.	(KBr, cm^{-1}) 1660(C=O); 1601.95 (C=N)	2.40 (s, 3H, phenyl-CH_3), 2.50 (s, 3H, <u>CH_3</u>CO), 3.00-3.10 (dd, 1H, J= 4.3 Hz, pyrazoline-C_4 H), 3.60-3.70 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.90- 6.00 (dd, 1H, J= 4.3 Hz, pyrazoline-C_5 H), 7.00-7.45 (m, 8H, phenyl-H, thioph-H), 7.64 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{3,5}$ H), 7.71(d, 2H, J= 8.0 Hz, *p*-tolyl- $C_{2,6}$ H), 7.78 (s, 1H, pyrazol-C_5 H).
5	For $C_{26}H_{24}N_4S$: C, 71.21; H, 5.06; N, 12.78; S, 7.52. Found: C, 70.94; H, 4.82; N,13.04; S, 7.14.	(KBr, cm^{-1}) 1653 (C=O); 1600 (C=N)	1.25 (t, 3H, C<u>H</u>$_3$-CH_2), 2.40 (s, 3H, phenyl-CH_3), 2.80-2.90 (q, 2H, CH_3C<u>H</u>$_2$), 3.00-3.10 (dd, 1H, J= 4.4 Hz, pyrazoline-C_4 H), 3.55-3.65 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.80- 5.90 (dd, 1H, J= 4.4 Hz, pyrazoline-C_5 H), 7.00-7.45 (m, 8H, phenyl-H, thioph-H), 7.63 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{3,5}$ H), 7.71 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{2,6}$H), 7.78 (s, 1H, pyrazol-C_5 H).
6	For $C_{27}H_{26}N_4OS$: C, 71.65; H, 5.35; N, 12.38; S, 7.09. Found: C, 71.86; H, 5.21; N, 12.58; S, 6.87.	(KBr, cm^{-1}) 1651(C=O); 1601 (C=N)	1.05 (t, 3H, C<u>H</u>$_3$$CH_2$$CH_2$), 1.70-1.90 (m, 2H, CH_3C<u>H</u>$_2$$CH_2$), 2.40 (s, 3H, pheyl-$CH_3$), 2.72-2.88 (m, 2H, $CH_3$$CH_2$C<u>H</u>$_2$), 3.00-3.10 (dd, 1H, J= 4.4 Hz, pyrazoline-C_4 H), 3.56-3.66 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.85- 5.95 (dd, 1H, J= 4.4 Hz, pyrazoline-C_5 H), 7.00-7.45 (m, 8H, phenyl-H, thioph- H), 7.63 (d, 2H, J=8.0 Hz, *p*-tolyl-$C_{3,5}$ H), 7.71 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{2,6}$ H), 7.77 (s, 1H, pyrazole-C_5 H).
7	For $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.27. Found: C, 74.08; H, 6.02; N, 19.86.	(KBr, cm^{-1}) 3420, 3370 (NH_2); 1620(C=N)	2.50 (s, 3H, *p*-tolyl-CH_3),7.20-7.52 (m, 7H, phenyl-H & NH_2), 7.65 (d, 2H, J= 8.0 Hz, phenyl-$C_{3,5}$ H), 7.85 (d, 2H, J= 8.0 Hz, *p*-tolyl-$C_{2,6}$ H), 8.65 (s, 1H, pyrazole-C_5 H), 8.75 (s, 1H, C<u>H</u>=N)
8	For $C_{30}H_{24}N_4OS$: C, 73.75; H, 4.95; N, 11.47. Found: C, 73.66; H, 5.22; N, 11.36.	(KBr, cm^{-1}) 1668 (C=O); 1625 (C=N)	2.51 (s, 3H, phenyl-CH_3), 3.07-3.18 (dd, 1H, J=4.4 Hz, pyrazoline-C_4 H), 3.62-3.71 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.93-6.02 (dd, 1H, J= 4.4 Hz, pyrazoline-C_5 H), 7.11-7.54 (m, 11H, thioph-H, phenyl-H, benzoyl-$C_{3,4,5}$ H), 7.64 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{3,5}$ H), 7.73 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{2,6}$ H), 7.96 (d, 2H, J= 8.05 Hz, benzoyl-$C_{2,6}$ H), 8.61 (s, 1H, pyrazol-C_5 H).
9	For $C_{31}H_{26}N_4OS$: C, 74.08; H, 5.21; N, 11.15. Found: C, 74.26; H, 5.32; N, 15.36.	(KBr, cm^{-1}) 1670 (C=O); 1625 (C=N)	2.51, 253 (2s, 6H, 2 CH_3), 3.06-3.17 (dd, 1H, J=4.4 Hz, pyrazoline-C_4 H), 3.63-3.72 (dd, 1H, J= 11.4 Hz, pyrazoline-C_4 H), 5.92-6.04 (dd, 1H, J= 4.4 Hz, pyrazoline-C_5 H), 7.13-7.58 (m, 13H, thioph-H, phenyl-H, benzoyl-C_4 H), 7.65 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{3,5}$ H), 7.68 (d, 2H, J = 8.08, , benzoyl-$C_{3,5}$ H), 7.72 (d, 2H, J= 8 Hz, *p*-tolyl-$C_{2,6}$ H), 7.82 (d, 2H, J= 8.05 Hz, benzoyl-$C_{2,6}$ H), 8.59 (s, 1H, pyrazol-C_5 H).

Table 2: Antimalarial activity of the synthesized compounds at a dose of 48.4 µmol/kg

Test Substance	Dose (µmol/kg)	% Parasitaemia*	% Suppression	Mean survival time (Days)*
2	48.4	79.82± 1.41	-55.9	7.5± 0.2
3	48.4	15.22± 0.22	70.26	11± 0.9
4	48.4	33.99± 2.63	33.6	9.1± 1.4
5	48.4	36.9± 0.58	27.9	8.7±1.0
6	48.4	58.93± 0.94	-15.1	8.0± 0.3
7	48.4	19.3± 1.86	62.3	9.4± 1.1
8	48.4	64.18±0.26	-25.35	7.8± 0.3
9	48.4	73.28± 1.42	-43.12	7.4± 1.0
CQ**	48.4	0.0	100	> 14
NC***	1 ml/100 g	51.2± 1.66	0	7.3± 0.8

* Values are M ± SD, P<0.05, ****** Chloroquine phosphate, ******* Negative control

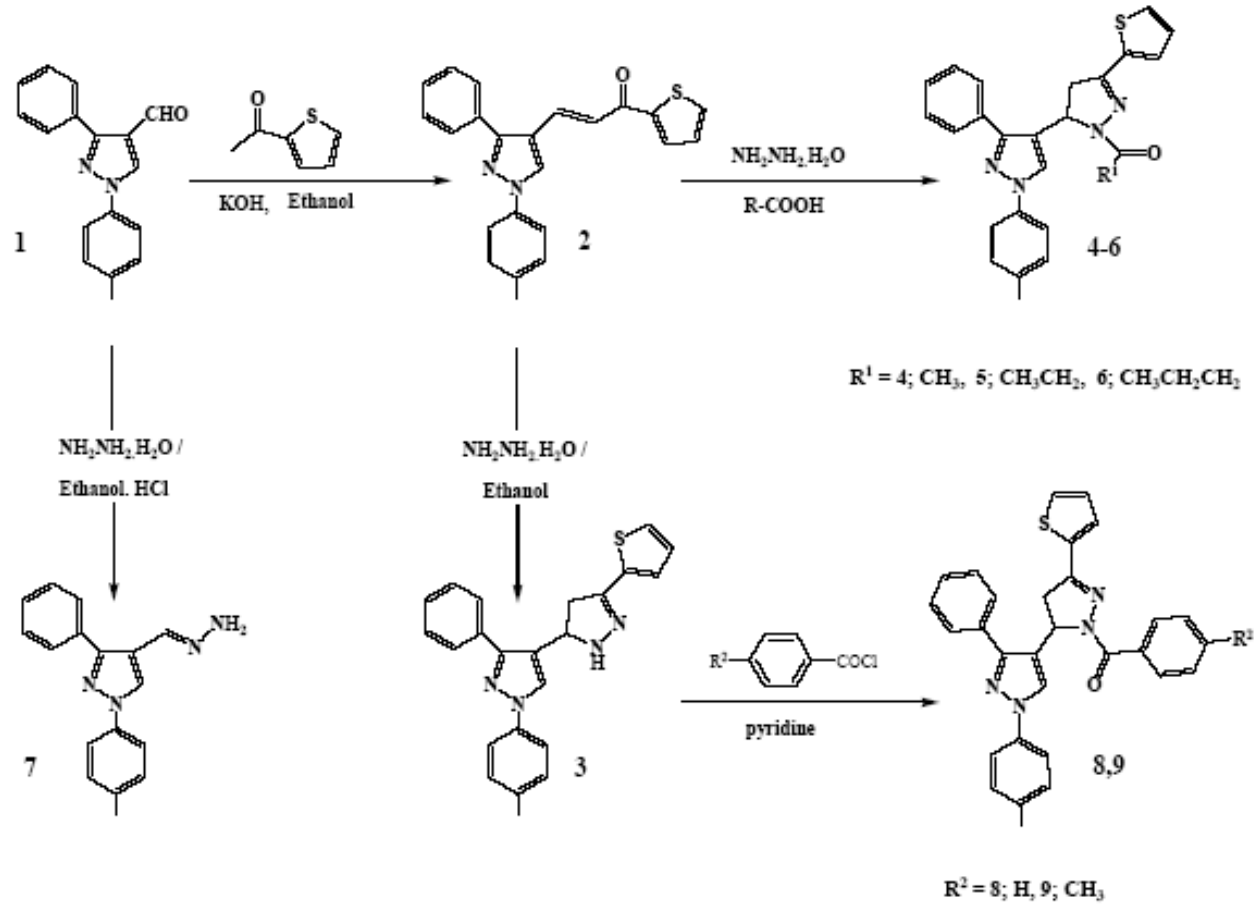

Fig. 1: synthesis of intermediate and target compounds

Two hour after infection, mice were weighed and randomly divided into ten groups of five mice each. The first eight groups received each of the synthesized compounds orally which was suspended in a vehicle containing 7% Tween 80 and 3% ethanol in water, at 48.4 µmol/kg dose level. Group nine received the vehicle only and acted as a negative control. Mice in-group ten were administered 48.4µmol/kg (25mg/kg) of the standard drug chloroquine phosphate orally and served as positive control.

On days 1 to 3 with a 24 hr interval, animals in the test groups were treated again with the same dose of the synthesized compounds using the same route as in day 0. On day 4, that is 24 hr after the last dose or 96 hr post-infection, the mice were weighed and smear was prepared on slides by taking blood from each mouse. The blood was then fixed with absolute methanol and stained with Giemsa stain. Level of parasitaemia was determined microscopically by counting 4 fields of approximately 100 erythrocytes per field. The difference between the

mean parasitaemia 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 of the mice was recorded with the exception of chloroquine-treated mice, which were completely cured from the infection (Dominguez *et al.,* 2009).

In vitro antipromastigote assay

Promastigote forms of *L. aethiopica* and standard drugs of amphotericin B deoxycholate and miltefosine were used for the assay. 3x 10^6 promastigotes of *L. aethiopica* in 100 µl were seeded in RPMI 1640 media to each well in a 96 well flat bottom plate. A volume of 100 µl of each of the concentrations of test compounds (10, 3.33, 1.11, 0.37, 0.12, 0.04 µg/ml) were added to the parasites in duplicates (Yang *et al.,* 2010). The media (RPMI 1640) and DMSO alone were used as a negative control. After 24hr, 20µl of alamar blue (12.5mg resazurin dissolved in 100ml of distilled water) was added to each of the wells. The plates were kept at room temperature. Absorbance of the resulting mixture was measured after 48 hr at a wavelength of 540 and 630 nm using ELISA plate reader. The test was done in triplicates (Al-Nasiry *et al.*, 2007; Foroumadi *et al.,* 2005; Bekhit *et al.,* 2005).

Acute toxicity

The acute toxicity of the most active compounds 2, 4 (anti-leishmanial) 3 (anti-malarial) and 7 (dual acting) was investigated using male mice (20-25g each, from Ethiopian Health and Nutrition Research Institute) according to previously reported methods. The mice were divided into groups of six animals each. The compounds were suspended in 1% gum acacia and orally administrated to the mice, in doses of 10, 50, 100, 200 and 300mg/kg. The percentage mortality in each test group was recorded after 24 hr. Additionally, the parenteral acute toxicity of the test compounds was investigated in groups of mice of six animals each. The compounds or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 20, 40, 80, 120 and 140mg/kg. The percentage survival was followed up to 7 days (Bekhit *et al.,* 2004).

RESULTS

Chemistry

The synthesis of *1H*-pyrazole derivatives is outlined in scheme 1, the key intermediate α,β-unsaturated ketone 2 was obtained through condensation of 1-(4-methylphenyl)-3-phenyl-1*H*-pyrazole-4-carboxaldehyde 1 and 2-acetylthiophene in alcoholic KOH. Compound 2 was cyclized by hydrazine hydrate in the presence of different liquid aliphatic acid or hydrazine hydrate in ethanol to afford the corresponding 1-acylpyrazoline 4-6 derivatives or *1H*-pyrazoline derivative 3. The latter was allowed to react with aroyl chloride in pyridine to render 1-aroylpyrazoline derivatives 8, 9. ^{1}HNMR spectra of pyrazoline derivatives showed the characteristic three double doublet peaks for pyrazoline-$C_{4\&5}$ protons. On the other hand, aldehyde 1 was condensed with hydrazine hydrate to give hydrazone 7. The results from IR, ^{1}H NMR and elemental microanalyses confirmed the structures of the synthesized compounds.

Table 3: Results of *In vitro* antipromastigote assay of compounds 2-9

Test compound	IC_{50}
2	0.079
3	11.76
4	0.2465
5	0.9788
6	1.006
7	1.823
8	5.438
9	7.536
Miltef*	3.18
Amphot**	0.047

* Miltefosine, ** Amphotericin B deoxycholate

The results of antimalarial testing and the anti-promastigote assay are listed in tables 2& 3.

DISCUSSION

In vivo anti-malarial activity test

The synthesized compounds were tested for their *in vivo* anti-malarial activity using *P. berghei* infected mice at a dose level of 48.4µmol/kg (table 2). Compounds 2, 6, 8 and 9 resulted in higher parasite load compared to the negative control, indicating these compounds may have a negative effect on the animal biochemical system. On the other hand, compounds 3, 4, 5 and 7 showed lower parasitaemia level and were considered to have significant suppressive effect against *P. berghei*. Compound 3 showed the highest inhibition of 70.26% followed by compound 7 with 62.3%. The presence of NH group in the latter compound may play a role in the interaction with vital important biochemical process. The anti-malarial activity is decreased as the carbon chain of the acyl group is increased, compounds 3 (70.26%), 4 (33.6), 5 (27.9) and 6 (-15.1%). From this result, it may be concluded that substitution of NH of pyrazoline ring contributed for the decrease in anti-malarial activity.

In vitro antipromastigote assay

The *in vitro* anti-leishmanial activity of synthesized compounds showed encouraging results against promastigotes of *L. aethiopica* (table 3). From the result obtained, the α, β-unsaturated ketone (2) is the most active (IC_{50}= 0.079), may be due to the formation of hydrogen bonding between its carbonyl group and backbone of certain receptor active site. Compound 2 was found to be more active than the standard drug,

Miltefosine. A linear decrease was seen in anti-leishmanial activity from compound 4 to 6 as the acyl chain increased. Similar behavior was observed for compounds 8 and 9. From this observation, it could be concluded that substitution of NH of the pyrazoline derivatives by small acyl moiety appeared to be necessary. On the other hand compound 7 with free NH_2 group could be considered the most promising within this work to be a dual acting anti-malarial anti-leishmanial agent that deserves more derivatization and investigation.

In vivo acute toxicity test

Compounds 2, 3, 4 and 7 were evaluated for their oral acute toxicity using a standard method. The results indicated that the test compounds were not toxic and well tolerated by experimental animals up to 300mg/kg. In addition, evaluation of the compounds' toxicity via parenteral route showed that all test compounds were well tolerated up to 140mg/kg.

CONCLUSION

Among all the synthesized compounds, compound 3, 1-(4-methylphenyl)-3-phenyl-4-[3-(2-thienyl)-2-pyrazolin-5-yl]-1*H*-pyrazole, possessed the highest anti-malarial activity with suppression of 70.26%. On the other hand, the highest anti-leshmanial activity was shown by compound 2, 1-(4-methylphenyl)-3-phenyl-4-[1-(2-thienyl)-prop-2-en-1-one]-1*H*-pyrazole, with an IC_{50} of 0.079 µg/ml. compound 7 possessing free NH_2 has good anti-malarial and anti-leishmanial activities. This compound could represent a fruitful matrix for the development of dual acting anti-malarial and anti-leishmanial agents that would deserve further derivatization and investigation.

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