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SYNTHESIS OF SOME SUBSTITUTED PYRAZOLE DERIVATIVES AND THEIR EVALUATION AS ANTIPROTOZOAL AGENTS

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ABSTRACT

Compounds incorporating a pyrazole moiety have attracted a great deal of research owing to its therapeutic utility of the templates as useful drug molecule scaffolding. The synthesis of pyrazoles moiety substituted with anilines at the fifth position of the ring as anti-protozoal lead moiety have been reported. All the compounds were screened for the anti-protozoal activity. The compounds **(3a)**, 3-amino-5-phenylamino-4*H*-pyrazole-4-carboxylic acid ethyl ester and **(3c)** 3-amino-5-(-4-methoxy-phenylamino)-4*H*-pyrazole-4-carboxylic acid ethyl ester showed better antimalarial as well as anti-leishmanial activity with IC_{50} value of 0.132 µmol/L and 0.150 µmol/L against *P. falciparum* and IC_{50} value of 0.132 µmol/L and 0.168 µmol/L against *Leishmania donovani.* These results open up new avenues in designing novel anti-protozoal drugs as dual inhibitor with utilization of pyrazole template as part of the pharmacophore.

Key words: Pyrazoles, Antiprotozoal agent.

INTRODUCTION

Protozoal parasitic diseases continue to pose serious health problem in third world countries. In the search for orally active drug, priority is given considering the increased prevalence of drug resistant *Plasmodium falciparum* parasites and *Leishmania donovani* parasites[1]. Malaria remains the world's most devastating parasitic disease. According to the world health organization, at least one million deaths and over 300 million acute illness can be attributed to malaria[2]. Leshmaniasis affects people in 88 countries with 350 million at risk of contracting the disease and 59,000 death each year is triggered by another unrelated protozon parasite. In recent decades parasite resistance to standard drug therapies has been developed. Chloroquine resistant *Plasmodium falciparum* has now spread to the

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most malarial areas and is resistant to other antimalarial drugs, including meloquine and sulfadoxin – pyrimithamine, which has become significant problem worldwide. Some problem arises in cases of *Leishmania donovani,* when resistance developed against sodium antimony gluconate (SAG) and pentamidine[3]. A series of pyrozoles are described as part of efforts directed towards the synthesis of some potent antiprotozoal agents against *Plasmodium falciparum* and *Leishmania donovani* parasites[4]. Pyrazole compounds have attracted a great deal of attention of chemists and pharmacologists. Some pyrazole compounds have been reported in literature out of which, some are potent nonsteroidal analgesic and anti-inflammatory drugs[5] as well as immunosuppressive compounds[6].

Authors were prompted to consider the synthesis of pyrazole derivatives with ketene dithioacetals to improve their activity in both the causes, which are posing serious health problems in the third world countries. Ketene dithioacetals are important and versatile reagents specially, when they are used for the synthesis of poly-functionalized heterocyclics[7-9].

EXPERIMENTAL

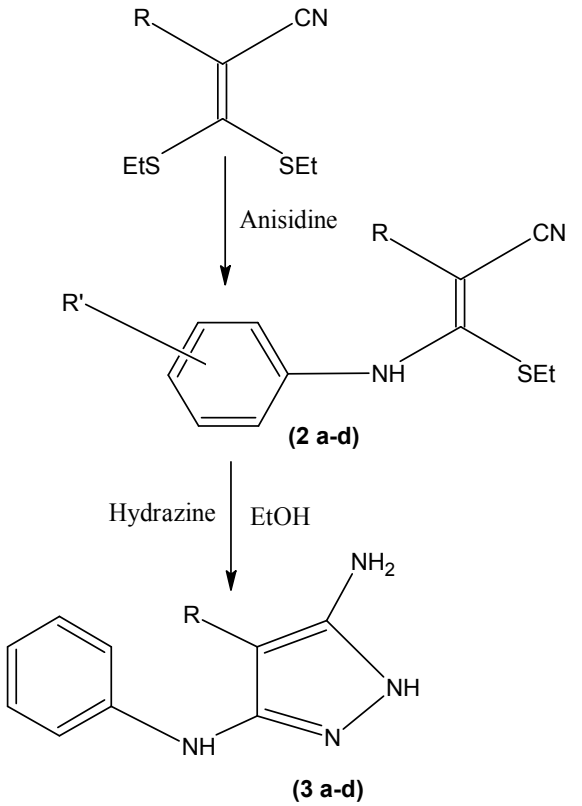
Substituted ketene dithioacetals **(1)** were synthesized using the method reported by Jesnsen et al.[10]. Ketene dithioacetals, anisidine and hydrazine hydrate were purchased from SD fine chemicals.

IR Spectra were recorded on a Perkin Elmer Model – 881. NMR spectra were obtained in $CDCI_3$ (with tetramethyl silane used as internal standard, Aldrich) and reported in ppm. Electron impact (EI) mass spectra were recorded on a JEOl-D-300 spectrometer with the ionization potential of 70 eV.

Synthesis of 2-cyano-3-ethylsulfanyl-(substituted methoxy-phenylamino)-acrylic acid ethyl derivatives

The synthesis sequence **(Scheme 1)**, which was followed for preparing thioacrylate derivatives was analogous to the method reported in literature[10,11]. The reaction was brought about by reacting equimolar amount of acrylates and anisidine at 110^{o}C for 4 h. After cooling, the product was crystallized out from reaction mixture and it was recrystellized by methanol to give the title compounds **(2a-d)**.

(2a) Yield - 76%, m.p. 92^{o}; IR (KBr) cm^{-1} : 2210 (CN str.), 1665 (CO str. ester); ^{1}H NMR ($CDCl_3$, 300 MHz) : 1.32 (3H, s, -CH_3), 2.27 (2H, m, -CH_2), 3.65 (3H, s, -OMe), 7.23 -7.31 (5H, m, Ar), 11.5 (1H, br, NH); Mass : m/z-247 (M^+)



R: COOEt, R': H, 4-OMe, 3 -OMe, 2 -OMe

Scheme 1 : Synthesis of substituted pyrazoles

(2b) Yield - 89%, m.p.- 90°; IR (KBr) cm^{-1} : 2195 (CN str.), 1665 (CO str. ester); ^1H NMR ($CDCl_3$, 300 MHz) : 1.35 (3H, s, $-CH_3$), 2.28 (2H, m, $-CH_2$), 3.81 (3H, s, -OMe), 3.91 (3H, s, -OMe), 6.65 -7.02 (5H, m, H $_{3',4'}$ Ar), 7.26 (1H, m, $H_{6'}$), 7.47 (1H, d, $H_{5'}$), 11.5 (1H, br, NH); Mass : m/z-277 (M^+)

(2c) Yield - 75%, m. p. 74°; IR (KBr) cm^{-1}: 2190 (CN str.), 1650 (CO str. ester); ^1H NMR ($CDCl_3$, 300 MHz) : 1.37 (3H, s, $-CH_3$), 2.3 (2H, m, $-CH_2$), 3.85 (3H, s, -OMe), 3.93 (3H, s, -OMe), 6.82 -6.95 (5H, m, H $_{2',3',6'}$), 7.3 (1H, m, $H_{5'}$), 11.5 (1H, br, NH); Mass : m/z-277 (M^+)

(2d) Yield - 86%, m. p. 88°; IR (KBr) (cm^{-1}): 2200 (CN str.), 1665 (CO str. ester); ^1H NMR ($CDCl_3$, 300 MHz) : 1.32 (3H, s, $-CH_3$), 2.27 (2H, m, $-CH_2$), 3.8 (3H, s, -OMe), 3.82 (3H, s, -OMe), 6.92 (2H, m, H $_{2',6'}$), 7.21 (1H, m, $H_{5'}$), 11.4 (1H, br, NH); Mass :

m/z-277 (M^+).

Synthesis of 3-amino-5-phenylamino-4*H*-pyrazole-4-carboxylic acid ethyl ester derivatives

Equimolar amount of **(2a-d)** and hydrazine hydrate in ethanol was refluxed at 110° for 6 h. The reaction mixture was allowed to cool at room temperature, the precipitate was filtered off and recrystallized from methanol to give the title compounds **(3a-d)**.

(3a) Yield - 84%, m. p. 178°; IR (KBr) (cm^{-1}) : 3447 (NH str.), 1643 (CO str. ester); ^{1}H NMR (DMSO-d_6, 300 MHz) : 3.5 (3H, s, -OMe), 5.18 (2H, br, -NH_2), 6.49 (1H, t, $H_{4'}$), 6.89 (2H, t, H $_{3', 5'}$), 7.06 (2H, d, H $_{2', 6'}$), 7.68 (1H, br, NH), 10.14 (1H, NH); Mass : m/z-276 (M^+)

(3b) Yield - 65%, m. p. 200°; IR (KBr) (cm^{-1}) : 3465 (NH str.), 1682 (CO str. ester); ^{1}H NMR (DMSO-d_6, 300 MHz) : 3.72 (3H, s, -OMe), 3.78 (3H, s, -OMe), 5.23 (2H, br, -NH_2), 6.74 (4H, m, Ar, NH), 8.09 (1H, d, H_3), 8.4 (1H, br, NH); Mass : m/z-306 (M^+)

(3c) Yield - 70%, m. p. 168°; IR (KBr) (cm^{-1}): 3468 (NH str.), 1684 (CO str. ester); ^{1}H NMR (DMSO-d_6, 300 MHz) : 3.77 (3H, s, -OMe), 3.83 (3H, s, -OMe), 5.26 (2H, br, -NH_2), 6.42 (1H, d, $H_{4'}$), 7.0 (1H, d, $H_{5'}$), 7.13 (3H, m, H $_{2', 6'}$), 8.4 (1H, br, NH); Mass : m/z-306 (M^+)

(3d) Yield - 60%, m. p. 200°; IR (KBr) (cm^{-1}) : 3468 (NH str.), 1684 (CO str. ester); ^{1}H NMR (DMSO-d_6, 300 MHz) : 3.75 (3H, s, -OMe), 3.8 (3H, s, -OMe), 5.26 (2H, br, -NH_2), 6.42 (1H, d, $H_{3',5'}$), 6.62 (1H, s, NH), 6.9 (2H, d, H $_{2', 6'}$), 8.23 (1H, br, NH); Mass : m/z-306 (M^+)

Assessment of antiprotozoal activity

Synthesized pyrazole **(3a-d)** were tested for their antimalarial and antileishmanial activity *in vitro* against *Plasmodium falciparum* and *Leishmania donovani,* respectively. The 50% inhibitory concentration (IC_{50}) values of the above synthetic compounds were determined by series of dilutions at given concentrations, which were determined using method described by Cherris et al.[12], against *Plasmodium falciparum.* A chloroquin resistant strain (FCBI) was propagated in human erthtrocytes at a 2% haematrocrit in RPMI 1640 containing 25 mmol/L Hepes, 50 µg/mL hypoxanthin, 0.25% sodium bicarbonate and 10% human plasma and cultured at 37°C in jar. Ciprofloxacin drug was used as a control. Antileishmanic activity[13] of the final compounds against stage of

Leishmania donovani parasite measured in 3 d non-radioactive cell proliferation assay using tetrazolium dye based cell filter 96 aque. The known antileishmanial drug pentamidine was used as a control.

RESULTS AND DISCUSSION

The NMR, IR, and Mass data confirm the formation of intermediates as well as the final compounds. The compounds **(3a)**, **(3c)** showed highest activity against *Plasmodium falciparum* with IC_{50} value of 0.149 µ mol/L and IC_{50} 0.150 µ mol/L, respectively and highest antileishmanial activity against *Leishmania donovani* with IC_{50} value of 0.132 µ mol/L and IC_{50} 0.168 µ mol/L, respectively. Compounds **(3b)** and **(3d)** have their activity at concentration higher than 100-µ mol/L. (Table 1)

Table 1. Inhibitory effects of pyrazole derivatives

Compounds	IC_{50} for malaria	IC_{50} for Leishmaniasis
	(µ M)*	(µ M)*
(3a)	0.149	0.132
(3b)	>100	NT
(3c)	0.15	0.168
(3d)	>100	>100
Ciproloxacin	39.8	-
Pentamidine	-	46.8

* IC_{50} (µ M) = micro molecular dose (µ moles / liter) required to produce 50 % inhibition of *Plasmodium falciparum* and *Leishmania donovani.*

> greater than

NT – Not tested

The unsubstituted phenyl analogue **(3a)** was found to be most potent as anti-protozoal agent. Interestingly, while a 3-OMe substituted in the phenyl ring of compound **(3c)**, it showed same activity. It is most likely due to the existence of same molecular geometry where the molecules display an intramolecular hydrogen bond cycle. This requirement for two hydrogen bonded cycles **(3a)**, **(3c)** is consistent with their activity.

Factor, which we could consider is the electronic effect caused by the substituents in the phenyl ring, meta position would have benefited for the activity as antiprotozoal agent, due to activation of the ortho- and para- position by moderate electron releasing groups. In summary, we found that the pyrazoles had a good activity against *Plasmodium falciparum* parasites and *Leishmania donovani* parasites. However, further studies are required to improve their activity.

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