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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL PYRAZOLE DERIVATIVES

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ABSTRACT

Objectives: To synthesize novel pyrazole derivatives and their evaluation for anti-inflammatory activity.

Methods: The synthesis of chalcone (1) was carried out by using Claisen-Schmidt condensation. which on further cyclization with thiosemicarbazide gives the substituted 3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothoic acid amide (2), further reaction with different aldehydes yield title compounds (3). Using this scheme 8 compounds were synthesized which further have been evaluated for anti-inflammatory activity by egg-albumin induced paw edema.

Results: All the synthesized compounds have been supported by spectral analysis. The anti-inflammatory activity of synthesized compounds was compared with standard anti-inflammatory agent Diclofenac sodium.

Conclusion: Compound-8, compound-2 and compound-3 showed greater anti-inflammatory activity due to the presence of alkene and electron withdrawing groups (Cl and NO_2).

Keywords: Chalcone, Thiosemicarbazide, Pyrazole derivatives, Anti-inflammatory activity.

INTRODUCTION

Pyrazole chemically known as 1, 2-diazole has become a popular topic due to its manifold uses. The chemistry of pyrazole and its derivatives are particularly interesting because of their potential application in medicinal chemistry as antitumor [1], antibacterial [2], antifungal [3], antiviral [4], antiparasitic [5], anti-tubercular [6], and insecticidal agents [7]. Some of these compounds have also anti-inflammatory [8], anti-diabetic [9], anesthetic [10], and analgesic [11] properties. Moreover, chalcones have played a crucial part in the development of theory of heterocyclic compounds, and also they used extensively in organic synthesis. A classical synthesis of these compounds involves the base-catalyzed claisen-schmidt reaction of ketones and aldehydes to give α, β-unsaturated ketones (chalcones), which undergo a subsequent reaction with thiosemicarbazide affording pyrazoles. Inflammation (Latin, *inflammo*, "I ignite, set alight") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.

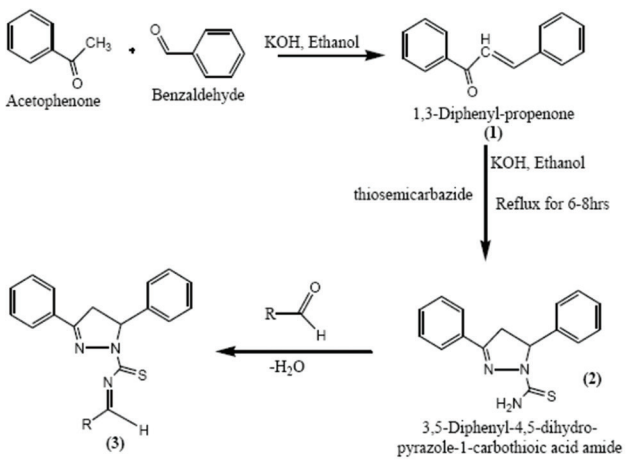
METHODS

General

All the chemicals were obtained from SD Fine chem. Limited (Mumbai). All the glassware is of borosilicate grade. Melting points were determined in open capillaries and were uncorrected. The melting point of an organic compound was determined by Thiel's melting point apparatus. The purity of the compounds was ascertained by thin-layer chromatographic (TLC) on silica gel-G plate. TLC is an important method for synthetic chemistry to infer the formation of the compound based on the R_f value since different compound will have different R_f values. It also helps in the confirming the reaction.

Fourier transform infrared (FT-IR) spectra were taken in KBr on a thermo nicolet nexus 670 spectrophotometer. ^{1}HNMR spectra were recorded on BRUKER AVANCE 300MHz spectrophotometer in $CDCl_3$ with TMS as an internal standard. The chemical shift values are in delta (ppm). Mass spectra were recorded on Polaris Q apparatus (thermo electron) and the fragmentations were obtained by electronic impact (EI). The data are given as mass to charge ratio (m/z) and nominal masses were used for the calculation of molecular weights of the synthesized products.

Scheme



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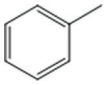
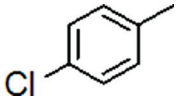
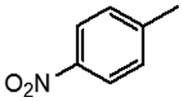
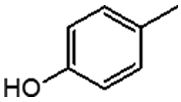
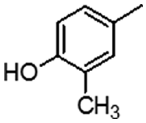
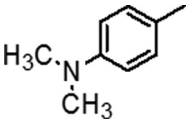
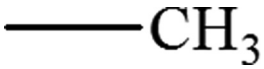
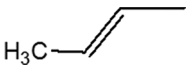
CP-1	
CP-2	
CP-3	
CP-4	
CP-5	
CP-6	
CP-7	
CP-8	

Synthetic procedure

Step-1: Synthesis of chalcone (1)

Equimolar quantity of benzaldehyde (0.01 mol) (1.06 ml) and acetophenone (0.01 mol) (1.2 ml) were placed in 30 mL of ethanol. The mixture was allowed to stir for 15 minutes. A 10 mL 40% aqueous potassium hydroxide solution was slowly added dropwise to the reaction flask via a dropping funnel. The reaction solution was allowed to stir at room temperature for 1.5 hrs. A precipitate formed was then collected by filtration.

Step-2: Synthesis of 3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothoic acid amide (2)

A mixture of chalcones (1) (0.01 mol) (2.08 g), thiosemicarbazide (0.01 mol) (0.91 g), and KOH (0.0025 mol) (1.4 g) was refluxed in ethanol (30 mL) for 6 hrs. The completion of is monitored by TLC. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol.

Step-3: Synthesis of Substituted 3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid benzylideneamide (3)

A mixture of different aldehyde (0.05 mol) and glacial acetic acid (18 ml) is refluxed in RBF (100 ml) capacity, 0.05 moles of 3, 5-Diphenyl-4, 5-dihydro-pyrazole-1-carbothoic acid amide (2) is added through the neck and mixture is refluxed for 30 minutes, the completion of reaction is known by TLC. Then, the solution is cooled in the ice-salt bath with stirring. The separated product is filtered, washed with water and recrystallized with ethanol.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid benzylideneamide (CP-1)

Steps 1 and 2 products were dissolved in glacial acetic acid and following the above general procedure desired compound was obtained in 62.33% yield, m.p. > 300°C, IR (KBr): 1509 cm^{-1} (-C=N), 1444 cm^{-1} (C=S), and 3160 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 2.2 s (-CH_2), 8.2 s (-CH=N), 3.8 s (-CH) and 6.8-7.8 m (Ar-H), EI-MS *m/e:* M^+ ion peak 368.

Table 1: Group of animals, drugs and their dosage forms

Groups	Sample	Dose
Group-1	Control (5% gum acacia suspension)	10 ml/kg
Group-2	Standard (diclofenac sodium)	5 mg/kg
Group-3	Compound-1	100 mg/kg
Group-4	Compound-2	100 mg/kg
Group-5	Compound-3	100 mg/kg
Group-6	Compound-4	100 mg/kg
Group-7	Compound-5	100 mg/kg
Group-8	Compound-6	100 mg/kg
Group-9	Compound-7	100 mg/kg
Group-10	Compound-8	100 mg/kg

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-chloro-benzylideneamide (CP-2)

73.69% yield, m.p. >300°C, IR(KBr): 1593 cm^{-1} (-C=N), 1444 cm^{-1} (C=S), 837cm^{-1} (C-Cl) and 3163 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 1.9 s (-CH_2), 8.0s (-CH=N), 3.8 s (-CH) and 7.2-7.9 m (Ar-H), EI-MS *m/e:* M^+ ion peak 402.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-nitro-benzylideneamide (CP-3)

61.04% yield, m.p. >300°C, IR (KBr): 1561 cm^{-1}(-C=N), 1469 cm^{-1} (C=S), 1469 cm^{-1} (C-NO_2) and 3059 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 2.0 s (-CH_2), 8.1 s (-CH=N), 3.8 s (-CH) and 7.2-7.7 m (Ar-H), EI-MS *m/e:* M^+ ion peak 413.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-hydroxy-benzylideneamide (CP-4)

77.41% yield, m.p. >300°C, IR (KBr): 1568 cm^{-1} (-C=N), 1465 cm^{-1} (C=S), 3483 cm^{-1} (C-OH, phenolic) and 3049 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 2.1s (-CH_2), 8.0s (-CH=N), '3.9s(-CH) and 6.79-7.9 m (Ar-H), EI-MS *m/e:* M^+ ion peak 384.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-hydroxy-3-methoxy-benzylideneamide (CP-5)

70% yield, m.p. > 300°C, IR (KBr): 1599 cm^{-1} (-C=N), 1447 cm^{-1} (C=S), 3483 cm^{-1} (C-OH, phenolic), 2931 cm^{-1} (-OCH_3) and 3026 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 2.0 s (-CH_2), 8.2 s (-CH=N), 3.9 s (-CH) and 6.8-7.9 m (Ar-H), EI-MS *m/e:* M^+ ion peak 414.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-dimethylamino-benzylideneamide (CP-6)

63.02% yield, m.p. >300°C, IR (KBr): 1568 cm^{-1} (-C=N), 1468 cm^{-1} (C=S), 1164 cm^{-1} (-$N(CH_3)_2$) and 3049 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 2.1s (-CH_2), 8.0s (-CH=N), 3.9s (-CH) and 6.9-7.9 m (Ar-H), EI-MS *m/e:* M^+ ion peak 411.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid ethylideneamide (CP-7)

65.36% yield, m.p. > 300°C, IR(KBr): 1599 cm^{-1} (-C=N), 1448 cm^{-1} (C=S), 1493 cm^{-1} (-CH_3) and 2940 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 1.9s (-CH_2), 8.0s (-CH=N), 3.9s (-CH) and 6.8-7.8m (Ar-H), EI-MS *m/e:* M+ ion peak 306.

3, 5-diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid but-2-enylideneamide (CP-8)

54.27% yield, m.p. > 300°C, IR (KBr): 1590 cm^{-1} (-C=N), 1385 cm^{-1} (C=S), 1455 cm^{-1} (-CH_3) and 2950 cm^{-1} (-CH-Ar), ^1H NMR ($CDCl_3$): 1.9s (-CH_2), 8.1s (-CH=N), 3.8s (-CH) and 6.9-7.8 m (Ar-H), EI-MS *m/e:* M+ ion peak 332.

Biological evaluation

Animals

Male or female Wistar-albino rats with a body weight between 100 and 150 g are used. They were acclimated to laboratory conditions for 7 days before commencement of experiments and were allowed free access to standard drug pellet diet and water ad libitum. The animals are starved overnight.

The grouping of animals according to the type of compound and its dose are represented in Table 1.

Experimental procedure for fresh egg white induced paw edema method [12]

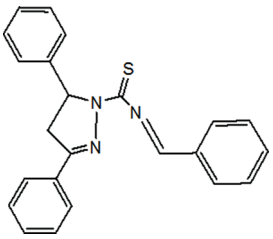
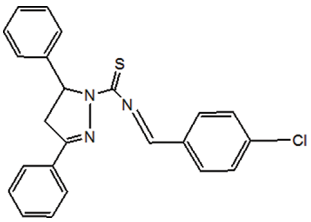
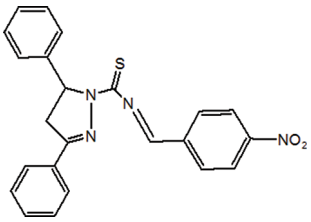
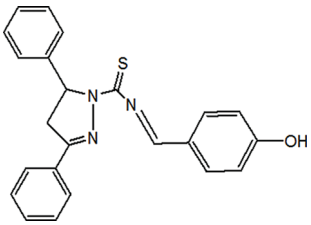
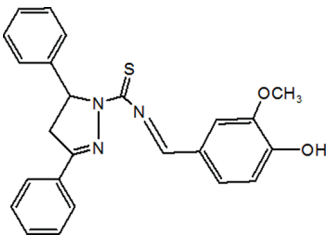
To ensure uniform hydration, the rats receive the test drug at a dose level of 100 mg/kg body weight suspended in 5% acacia solution. 30 minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of egg albumin into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured with plethysmometer immediately after injection, again after 1 hr, 2 hrs, and 3 h and 5 hrs % inhibition was calculated by following a formula.

$$\%\text{Inhibition} = ([\text{control}-\text{test}]/\text{control}) \times 100$$

The values are calculated by Dunnett method by comparing all the compounds with control in One-way Analysis of Variance (ANOVA) and are expressed in Mean ± SEM.

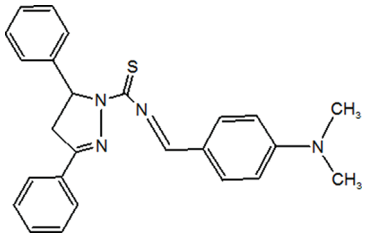
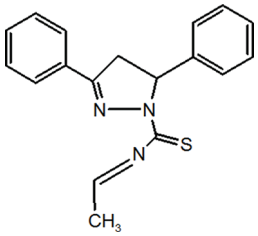
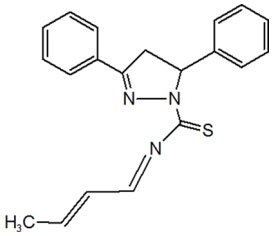
The physical data of synthesized compounds are given in table 2

Table 2: Physical data of synthesized compounds

Compounds	Structure	Molecular formula	Molecular weight	% yield
CP-1	 3, 5-Diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid benzylideneamide	$C_{23}H_{19}N_3S$	369.4821	62.33
CP-2	 3, 5-Diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-chloro-benzylideneamide	$C_{23}H_{18}C_1N_3S$	403.9271	73.69
CP-3	 3,5-Diphenyl-4,5-dihydro-pyrazole-1-carbothioic acid 4-nitro-benzylideneamide	$C_{23}H_{18}N_4O_2S$	414.4796	61.04
CP-4	 3, 5-Diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-hydroxy-benzylideneamide	$C_{23}H_{18}N_3OS$	385.1249	77.41
CP-5	 3, 5-Diphenyl-4, 5-dihydro-pyrazole-1-carbothioic acid 4-hydroxy-3-methoxy-benzylideneamide	$C_{24}H_{21}N_3O_2S$	415.5074	70

(*Contd*...)

Table 1: (*Continued*)

Compounds	Structure	Molecular formula	Molecular weight	% yield
CP-6	 3,5-Diphenyl-4,5-dihydro-pyrazole-1-carbothioic acid 4-dimethylamino-benzylideneamid	$C_{25}H_{24}H_4S$	412.5499	63.02
CP-7	 3,5-Diphenyl-4,5-dihydro-pyrazole-1-carbothioic acid ethylideneamide	$C_{18}H_{17}N_3S$	307.412	65.36
CP-8	 3,5-Diphenyl-4,5-dihydro-pyrazole-1-carbothioic acid but-2-enylideneamide	$C_{20}H_{19}N_3S$	333.450	54.27

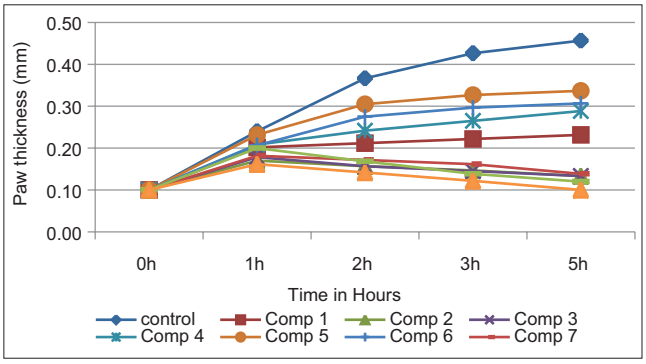


Fig. 1: Effect of diclofenac sodium and test compounds on paw thickness

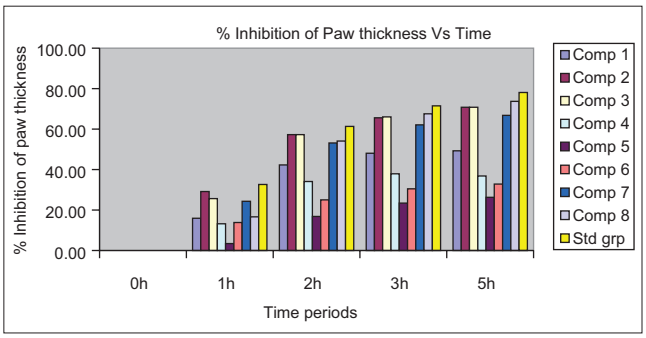


Fig. 2: % inhibition of paw thickness

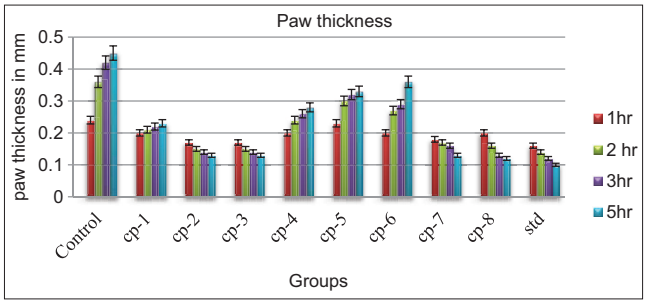


Fig. 3: Bar diagram with mean and standard error of mean at 1 hr-5 hrs

RESULTS

The physical data of synthesized compounds are given in Table 2. The results obtained from the synthesized compounds with a dose of 100 mg/kg confirmed that maximum activity was obtained when R was substituted by alkene (compound-8) with 73.72% inhibition, when R was substituted by a chlorine group (compound-2) with 70.80% inhibition; R was substituted by $-NO_2$ group (compound-3) with 70.80% inhibition, R was substituted by alkane group (compound-7) with 66.83% inhibition, R was substituted by benzaldehyde (compound-1) with 49.27% inhibition, R was substituted by –OH group (compound-4) with 36.86% inhibition, R was substituted by $-N(CH_3)_2$ group (compound-6) with 32.85% inhibition and R was substituted by vanillin group (compound-5) with 26.28% inhibition. Based on the "p" value, compound-8, 2 and 3 showed higher significance from 1 hr to 5 hrs when compared to control. It was found that the electron

Table 3: Comparison of significance of synthesized compounds with control

Groups	1 hr	2 hr	3 hr	5 hr
Control	0.24±0.00*	0.3667±0.006*	0.4267±0.008	0.4567±0.009
CP-1	0.2017±0.001*	0.2117±0.001	0.2217±0.001*	0.2317±0.001*
CP-2	0.17±0.00**	0.1567±0.002**	0.1467±0.002**	0.1333±0.004**
CP-3	0.1783±0.001**	0.1567±0.002**	0.1450±0.002**	0.1333±0.002**
CP-4	0.2083±0.001*	0.2417±0.001	0.2650±0.002*	0.2883±0.003
CP-5	0.2317±0.001	0.3050±0.002	0.3267±0.002	0.3367±0.002
CP-6	0.2067±0.002	0.2750±0.002	0.2967±0.002	0.3667±0.002
CP-7	0.1817±0.001	0.1717±0.002	0.1617±0.001	0.1383±0.011
CP-8	0.2±0.00**	0.1683±0.001**	0.1383±0.001**	0.1200±0.002**
Standard	0.1617±0.001**	0.1417±0.001**	0.1217±0.001**	0.100±0.002**

Value are mean±SEM (n=6), *significant at p<0.05, **significant p<0.01 significantly different compare to control

withdrawing groups and alkene containing synthesized compounds enhanced the anti-inflammatory activity. The effect of Diclofenac sodium and test compounds on paw thickness shown in the Fig. 1 and percentage inhibition of paw thickness shown in Fig. 2 and comparison data for significance of synthesized compounds versus control given in Table 3 and bar diagram shown in Fig. 3.

CONCLUSION

The synthesized novel pyrazole derivatives were subjected to *in vivo* anti-inflammatory evaluation. Anti-inflammatory activity of the synthesized compounds was evaluated by egg-albumin induced rat paw edema method. The activity was studied at the dose levels of 100 mg/kg body weight, and their effects were measured at 1, 2, 3, and 5 hrs.

The paw volume of the rat in inhibiting inflammation by the synthesized compounds at different time intervals is measured by mercury displacement method. The anti-inflammatory studies revealed that all the synthesized novel pyrazole derivatives showed significant anti-inflammatory activity when compared with that of standard drug diclofenac sodium. CP-8, CP-2 and CP-3 showed greater Pharmacological activity due to the presence of alkene and electron withdrawing groups Cl, NO_2 respectively. Whereas, CP-5, CP-6, CP-4, CP-1, and CP-7 showed mild to moderate activity. Therefore, further studies required for pharmacologically more potent compounds in these series.

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