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SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF PYRAZOLE DERIVATIVES

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ABSTRACT

Several pharmacological activities like antitubercular, analgesic, anti-cancer, anti-inflammatory, antiasthmatic, antioxidant and antibacterial activities have been attributed to pyrazoles. The above observations prompted us to synthesize some novel pyrazole derivatives as possible antimicrobial agents. A series of novel 1, 3, 5-trisubstituted pyrazole derivatives have been synthesized by the reaction of substituted chalconeswith hydrazine hydrate. The starting material, chalcones were prepared by Claisen Schmidt condensation of acetophenone with aldehydes in the presence of sodium hydroxide in ethanol. All thesynthesized compounds were characterized by IR, ^{1}H-NMR, and Elemental Analysis.

Keywords: Chalcones, Pyrazole, Antimicrobial, Ant-inflammatory, Analgesic,^{1}H-NMR.

INTRODUCTION

Clinically biological activity is the result of a chemical compound's interaction with a human organism. The biological activity is dependent upon the compound's structure and its physical-chemical characteristics, as well as the biological entity and its mode of therapeutic treatment. Pyrazoles are unique in their chemical behavior not only among heterocyclic compounds in general, but also among related azoles. This is because pyrazole possesses the typical properties of the aromatic system, which are in fact rather pronounced in these derivatives, together with the high liability of the ring under certain condition. Pyrazole un substituted in 1-position show NH- acidity. The pKa value of Pyrazole is 14.21 and equal to that of imidazole. Now a day's vast numbers of compounds with pyrazole nucleus have been reported to show a broad spectrum of biological activity includingantimicrobial[1], anti-cyclooxygenase[2], anticonvulsant[3], antitubercular[4], antitumor[5], antiinflammatory[6], analgesic[7], antidiabetic[8], antipshycotic[9-11] etc.

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Since most of thepyrazole derivatives show anti-microbial activity, the synthesized compounds are also expected to show anti-microbial activity. Hence, our plan is to synthesize some substituted pyrazole derivatives and subsequently screen for their antimicrobial activity.

METHODS AND METERIALS

Melting points were recorded on an X4-Data microscopic melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded on SHIMADZU FT-IR8400S with KBr Pellets. ^{1}H-NMR spectra were recorded on JNM-ECS400, 400 MHz.The chemical shifts are reported as parts per million down fields from tetramethylsilane (TMS). Mass spectra were recorded on LC–MS 2010A SHIMADZU instrument. The elemental analyses were performed on a EuroERElemental Analyser.The purity of the compounds was checked by TLC on precoatedsilica gel G (E Merck).

General procedure for synthesis of 3,5-diphenyl-4, 5-dihydro-1*H*-pyrazole[12](I):

A mixture of chalcone (0.01mol), hydrazine hydrate (0.01mol) and acetic acid (5ml) in ethanol was refluxed for 8 hrs. The reaction mixture was cooled and poured over ice water. The solid separated was filtered, washed with water, dried and recrystallized from ethanol gave pale yellowcrystals, Yield:86.55%. The completion

of reaction was monitored by TLCusing chloroform: petroleum ether (8:2) as mobile phase.

2-chloro-N-substituted-phenyl-acetamide[15] (II):

Aromatic amines (0.05mol) were dissolved in glacial acetic acid (25 ml) containing (25 ml) of saturated solution of sodium acetate.In case if the substance did not dissolve completely,the mixture was warmed and then the solution was cooled in ice bath with stirring .To this chloro- acetyl chloride(0.06 mol) was added drop wise avoid the vigorous reaction.After half an hour a white coloured product was separated and filtered.The product was washed with 50% aqueous acetic acid and finally with water.It was recrystallized from aqueous alcohol,m.p125-127^{0}C, and yield 82%.

***N*-(substituted phenyl)-2-(3, 5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)acetamide[13]:III(A_1-A_6):**

3,5-diphenyl-4, 5-dihydro-1*H*-pyrazole **(1)** (0.05mol) and different 2-chloro-N-substituted-phenyl acetamide (0.05mol) were mixed in 15 ml of 1,4-dioxane.To this (0.005 ml) of triethylamine(TEA) solution was added and the reaction mixture was refluxed for 2-3 h.It was then cooled and poured into crushed ice.The solid filtered was washed with 10% K_2CO_3 and finally water.

SCHEME

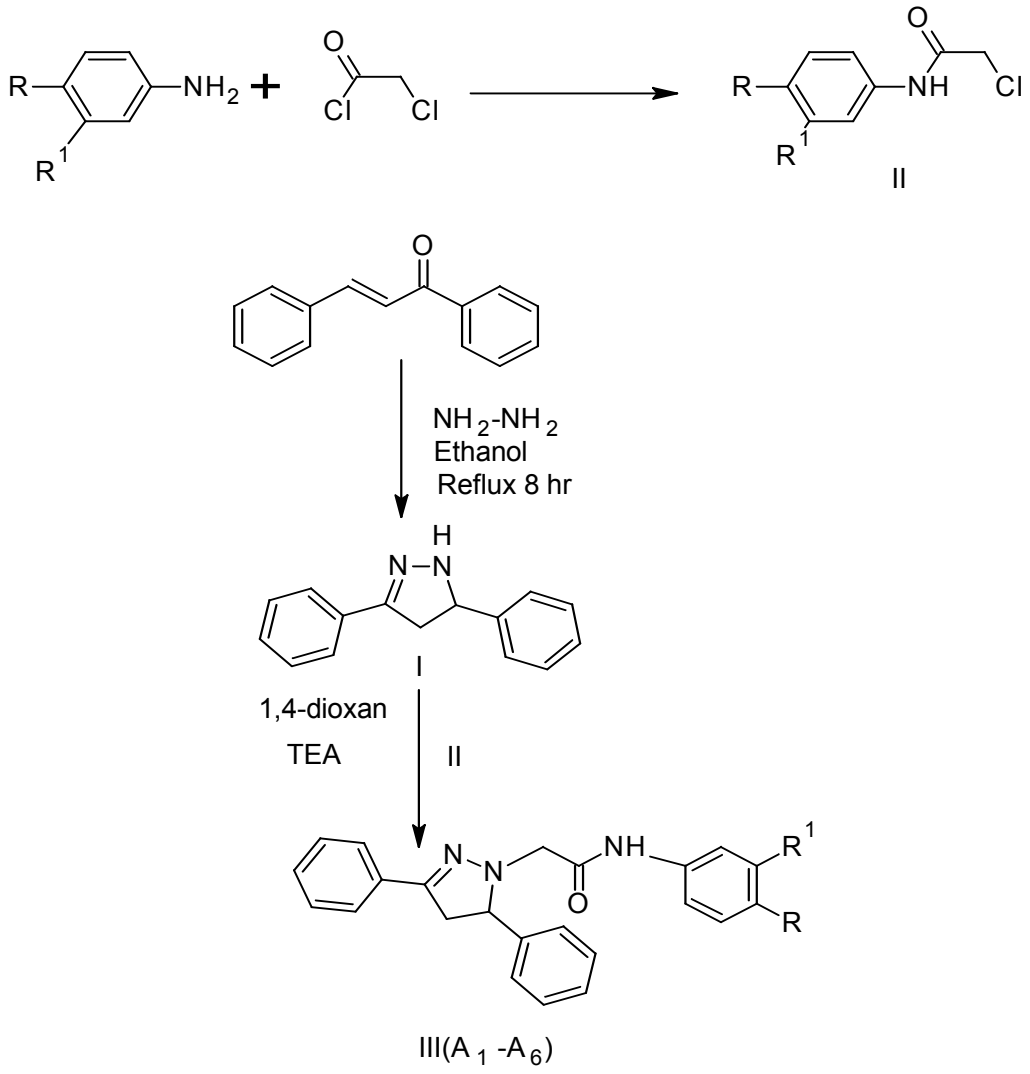

Table 1: Physical data of the synthesized compounds:

Compound No.	Mol. Formula	R	R^1	Mol. Wt.	M.P ^{0}C	R_f Value	Yield %
A1	$C_{23}H_{21}N_3O$	H	H	355.4	153-155	0.75	67
A2	$C_{23}H_{19}ClFN_3O$	Cl	F	407.8	178-180	0.78	64
A3	$C_{23}H_{20}N_4O_3$	NO_2	H	400.4	210-212	0.68	59
A4	$C_{23}H_{20}N_4O_3$	H	NO_2	400.4	198-200	0.64	70
A5	$C_{23}H_{20}ClN_3O$	H	Cl	389.8	163-165	0.72	74
A6	$C_{23}H_{20}ClN_3O$	Cl	H	389.8	192-194	0.67	53

Spectral data of the synthesized compounds:

2-(3,5-diphenyl-4, 5-dihydropyrazol-1-yl)-N-phenylacetamide, (A1):

IR(KBr): 3102 (C-H Ar); 2938 (CH_2alkane); 1647 (C=O), 1315 (C-N), 1626-1633(C=N),1455 (C=C,Ar) (cm^{-1}); ^{1}H NMR(400 MHz ,$CDCl_3$) 7.01-7.64 (m,15H,Ar-H), 5.55(s,1H,Ar-C-NH),3.55 (s,2H,CH_2), 3.49 (d,2H,CH_2); MS : m/z 355 $(M)^+$; Anal. Calcd.for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82.Found: C, 77.63;H,5.90;N,11.09.

N-(3-chloro-4-fluorophenyl)-2-(3, 5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl) acetamide, (A2):

IR(KBr): 3134 (C-H Ar),3015(C=C Ar), 2822 (CH_2 alkane) , 3410 (NH) ,1733 (C=O), 1190 (C-F),622 (C-Cl), 1134 (C-N), 1631 (C=N), 1491 (C=C, Ar) (cm^{-1}); ^{1}H NMR(400 MHz ,$CDCl_3$): 7.24-7.68 (m,13H,Ar-H),4.51 (s,1H,Ar-C-NH),3.82 (s,2H, CH_2), 3.40(d, 2H, CH_2); MS : m/z 407$(M)^+$, 408(M+1) $^+$; Anal. Calcd. for $C_{23}H_{19}ClFN_3O$: C, 67.73;H, 4.70; N, 4.70. Found: C, 68.89; H, 4.99; N, 13.93.

2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(4-nitrophenyl)acetamide,(A3):

IR(KBr): 3210 (C-H Ar), 2959 (C=C Ar) , 2943(CH_2 alkane), 3483 (NH), 1678 (C=O), 1363 (C-N),1618 (C=N), 1437 (C=C, Ar),1356(NO) (cm^{-1}); ^{1}H NMR (400 MHz ,$CDCl_3$): 7.34-8.17 (m, 14H, Ar-H), 4.51 (s, 1H, Ar-C-NH), 3.82 (s, 2H, CH_2), 3.45 (d, 2H, CH_2); MS : m/z:-400$(M)^+$, 401(M+1) $^+$; Anal. Calcd. for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.93. Found: C, 68.89; H, 5.05; N, 13.90.

2-(3, 5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(3-nitrophenyl)acetamide ,(A4):

IR(KBr): 3105 (C-H Ar) , 3018 (C=C Ar) , 2959 (CH_2 alkane) , 3430 (NH) , 1651 (C=O) , 1387 (C-N), 1580 (C=N) , 1445 (C=C, Ar) , 1376 (*p*-NO) (cm^{-1}) ; ^{1}H NMR (400 MHz ,$CDCl_3$) : 7.41-8.01 (m,14H,Ar-H), 4.13 (s,1H,Ar-C-NH),4.02 (s,2H,CH_2), 3.48 (d, 2H, CH_2); MS : m/z: 400$(M)^+$; Anal. Calcd. for $C_{23}H_{20}N_4O_3$: C, 68.99 ; H, 5.03 ; N, 13.93. Found: C, 68.92; H, 4.98; N, 13.95.

N-(3-chlorophenyl)-2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)acetamide, (A5):

IR(KBr): 3060 (C-H ,Ar), 2995(C=C Ar) , 3056 (CH_2 alkane), 3421(NH), 1726 (C=O), 1360 (C-N), 1568 (C=N) , 1447 (C=C, Ar) , 686 (C-Cl)(cm^{-1}); MS : m/z: 389$(M)^+$,390(M+1) $^+$; Anal. Calcd. for $C_{23}H_{20}ClN_3O$: C, 70.85; H, 5.17; N, 10.78. Found: C, 70.92; H, 5.18; N, 10.95.

N-(4-chlorophenyl)-2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)acetamide(A6):

IR(KBr): 3145(C-H, Ar), 3085(C=C Ar), 3062 (CH_2 alkane), 3483(NH), 1652(C=O), 1457 (C=C,Ar), 617(C-Cl) (cm^{-1}); ^{1}H NMR (400 MHz ,$CDCl_3$) : 7.24-8.05(m,14H, Ar-H), 4.51(s,1H,Ar-C-NH), 3.82 (s,2H,CH_2), 3.52 (d, 2H, CH_2); MS : m/z: 390 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{20}ClN_3O$: C, 70.85; H, 5.17; N, 10.78. Found: C, 70.80; H, 5.15; N, 10.72.

***In-vitro* antimicrobial activity[14]:**

Evaluation of antibacterial and antifungal activity is done by the agar dilution method. All bacteria were grown on Mueller–Hinton agar (Hi-media) plates (37^0C, 24 h) and fungi were grown on Sabourand dextrose agar (Hi-media) plates (26^0C, 48–72 h). The synthesized compounds were subjected to antimicrobial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various gram positive and gram negative bacteria and antifungal activity against various fungal stains compared with standard drug (Amoxycilline and Ketoconazole) using solvent control. The microorganisms selected for antimicrobial activity were *Staphylococcus aureus* (NTCC-6571), *Bacillus subtilis*(ATCC11774), *Echerichia coli*(TG1) 4, *Aspergillusniger, Candida albicans*. Results have been given in Table 2.

Experimental animals:

Adult swiss albino mice (20+2 g) and albino rats weighing (150–200 g) of either sex were used as experimental animals. All the animals were housed in groups of 4–8 per cage at a temperature of 25 $\pm1^0$C and a relative humidity of 45–55%. A 12 h dark and 12 h light cycle was followed during the experiments. Animals were allowed free access to food and water. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals.

Acute toxicity studies[16-22]:

The acute toxicity studies were carried out in groups of six Swiss albino mice, weighing 20±2 g which were fasted overnight and treated orally with the test compounds. The dosage was varied from 100-1000 mg/kg body weight orally. All the animal experiments were performed with the approval of Institutional Animal Ethics Committee (Reg.No. 1028/T/07/CPCSEA), Himalayan Pharmacy Institute,Majhitar, Rangpoo,East Sikkim-737136.

Analgesic activity by Tail-flick method in mice[23]:

The analgesic activity was carried out by Tail-flick method using Swiss albino mice. In this method, heat is used as a source of pain. Overnight fasted healthy and adult male Swiss albino mice weighing 20±2 g, in a group of six each were taken for the investigation. The animals were kept into a small cage with an opening for the tail at the rear wall. The tail was held gently and a light beam exerting radiant heat was directed to the proximal third of the tail. The tip of the tail of the mice was individually placed on the radiant heat source at constant temperature 55^0C. The cut-off reaction time was fixed at 15 second to avoid tissue damage. The tail flick response was measured at 0 h, 1 h, 2 h, 3 h and 4 h after treatment of test compounds by digital analgesiometer (INCO, Ambala, India). The drug pentazocine (3.9 mg/kg,i.p.) was used as standard drug for comparison and test groups received synthesized pyrazoline derivatives at 100 mg/kg p.o. The results were described in the Table no.3.

Anti-inflammatory activity by Carrageenan-induced rat paws edema method[24]:

The anti-inflammatory activity of the test compounds was evaluated by carrageenan induced rat paw edema model of Winter et al. (1962). Rats of either sex were treated with pyrazoline derivatives (100 mg/kg p.o.) and standard drug diclofenac sodium (100 mg/kg p.o.), one hour prior to the 1% w/v solution injection of 0.1 ml carrageenan into the plantar region of left hind paw. The marking was just made beyond the tibia-tarsal junction of (knee joint) left hind paw in each animal of all groups. Paw volume was measured by Plethysmometer (Model 520, IITC, Life sciences, USA) at 0 h, 1 h, 2 h, 3 h and 4 h after carrageenan injection. The difference between the paw volume at 4th and 0 h measurement was calculated and taken as edema volume. Percentage inhibition in the paw was calculated by using the formula, percentage inhibition= 100 (1-Vt/Vc), where Vt= mean increase in paw volume of test, and Vc =mean increase in paw volume with the control. The results were described in the Table no.4.

Table 2.Antibacterial activity of Synthesized Compounds:

Compd. code	*Zone of inhibition in (mm)					
	Bacteria				Fungi	
	S. aureus	*E. coli*	*S. typhi*	*B.subtilis*	*A. niger*	*C.albicans*.
A1	20	15	17	18	10	10
A2	30	32	35	10	14	13
A3	25	31	33	37	13	15
A4	18	24	13	23	-----	12
A5	14	13	20	16	11	15
A6	22	28	29	13	12	14
Amoxycilline	31	36	39	38	-----	-----
Ketoconazole	-----	-----	-----	-----	15	18
DMSO	-----	-----	-----	-----	-----	-----

'–' indicates no sensitivity or mean inhibition zone diameter lower than 10 mm. *Average three reading

Table 3. Analgesic activity of synthesized compounds on mice by using tail-flick method.

Comp. code	Tail withdrawing time in second (Mean±SEM)				
	0 h	1 h	2 h	3 h	4h
Control	1.60±0.16	2.21±0.16	2.36±0.21	2.58± 0.21	2.98± 0.76
Std.	2.36±0.16	7.95±0.34	10.98±0.21	10.56±0.30	10.87±0.28
A1	2.0±0.25	6.76±0.21	7.85±0.33	7.53± 0.21	6.76± 0.41
A2	2.16±0.16	5.66±0.21	8.12±0.22	8.45± 0.22	8.65± 0.38
A3	2.10±0.25	5.65±0.10	4.48±0.21	6.33± 0.21	7.96± 0.29
A4	2.09±0.25	2.17±0.25	7.62±0.33	7.65± 0.22	7.83±0.34
A5	1.61±0.30	2.08±0.25	3.45±0.09	5.63± 0.21	6.36± 0.31
A6	2.16±0.30	1.61±0.30	5.74±0.42	4.88± 0.33	6.56± 0.37
n=6 animals in each group. All synthesized compounds tested at a dose of 100 mg/kg p.o. body weight, Std. -pentazocine (3.9 mg/kg i.p), Control-vehicle (0.5% CMC).					

Table 4. Anti-inflammatory activity of synthesized compounds on carrageenan-induced acute paws edema in rats

Comp. code	Tail withdrawing time in second (Mean± SEM)					% Inhibition
	0 h	1 h	2 h	3 h	4h	4h
Control	0.16± 0.01	0.21 ±0.01	0.24±0.02	0.26± 0.01	0.26±0.01	------
Std	0.13± 0.01	0.10 ±0.01	0.09±0.01	0.06± 0.01	0.06±0.01	76
A1	0.10± 0.01	0.13± 0.01	0.09± 0.01	0.11± 0.01	0.11±0.01	57
A2	0.10± 0.01	0.11± 0.01	0.10± 0.01	0.09± 0.01	0.08±0.01	69
A3	0.12± 0.01	0.14± 0.01	0.12± 0.01	0.10± 0.01	0.09±0.01	65
A4	0.12± 0.01	0.11± 0.01	0.12± 0.01	0.11± 0.01	0.12±0.01	53
A5	0.14± 0.01	0.15± 0.01	0.10± 0.01	0.12± 0.01	0.13±0.01	50
A6	0.15± 0.01	0.14± 0.01	0.11± 0.01	0.10± 0.01	0.10±0.01	61
n=6 animals in each group. All synthesized compounds tested at a dose of 100 mg/kg p.o. body weight, Std-pentazocine (3.9 mg/kg i.p), Control-vehicle (0.5% CMC).						

RESULTSAND DISCUSSION

Pyrazolederivatives were synthesized and the structures of the compounds were established by means of IR,^{1}H-NMR and elemental analysis. All the compounds were evaluated for their *in-vivo* antimicrobial activity (Table 2),*in-vivo* analgesic activity (Table 3), anti-inflammatory activity (Table 4).

Acute toxicity studies:

Acute toxicity and gross behavior studies revealed that the tested compounds in the present investigation were found to be nontoxic up to 1000 mg/kg p.o.

Analgesic activity:

Table 3, revealed that almost all the compounds showed very potent analgesic activity when compared with standard pentazocine. Among the tested compounds **A2** and **A3**showed profound analgesic activity. The rest of the compounds**A1**,**A4, A5**and **A6**showed moderate activitywhen compared with the control.

Anti-inflammatory activity:

From the **Table 4**, it was found that most of the tested compounds showed significant results in comparison with standard diclofenac sodium. Amongst all the compounds **A2, A3**and **A6**showed potent anti-inflammatory activity and the rest of the compounds showed moderate activity.

Antimicrobial Evaluation:

The *in vitro* antimicrobial activity was performed using the Cup plate method with different strains of bacteria and fungi. Amoxicillin and Ketoconazole were used as positive control for bacteria and fungi.Theresults revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms.The chloro group at 5, and nitro at 5position of phenyl ring in the molecule of pyrazole has the best overallantibacterial profile as the 3-chloro,3-nitro and without substituentson phenyl ring at pyrazole nucleus displayed moderate activity. The compounds showed activity which is comparable with control against bacterial strains in increasing order of 5-Cl,3-F > 5-NO_2>3-Cl> H.

CONCLUSION

All derivatives of pyrazole have been successfully synthesized and screened forantimicrobial activity by cup plate method. The antibacterial activity, of the synthesized *N*-(substituted phenyl)-2-(3, 5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)acetamidederivatives revealed that the compounds A_2,A_3 and A_6 were effective against gram Positive and gram negative organisms respectively. The antifungal activity, of the synthesized *N*-(substituted phenyl)-2-(3, 5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)acetamidederivatives revealed that the compound **A3**,**A5** and **A6** showed good activity against tested fungi.

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