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Synthesis, characterization and biological evaluation pyrazole derivatives containing indole ring as a potent analgesic and anti-inflammatory agents

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ABSTRACT

A novel series of pyrazole derivatives containing indole ring were synthesized from 1-Propyl-1H-indole-2-carbohydrazide and substituted chalcones were refluxed on an oil bath. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Ampicillin and Greseofulvin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis, anti-inflammatory, analgesic and antimicrobial screening of the new pyrazole derivatives containing indole ring are reported.

Keywords: Thiophene, Antibacterial activity, Antifungal activity.

INTRODUCTION

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole alkaloids have been proved to be medicinally important natural compounds. Indole compounds include the plant hormone Auxin, the anti-inflammatory drug indomethacin, the β-blocker pindolol, and the naturally occurring hallucinogen dimethyltryptamine. The indole skeleton is one of the most attractive frameworks with a wide range of biological and pharmacological activities. Many researchers have described synthesis of indole and its derivatives along with its applications in literature. A large number of heterocyclic compounds containing the indole ring are associated with diverse pharmacological properties such as Analgesic [1], Antiallergic [2], Antibacterial [3], Anticonvulsant [4], Antifungal [5], Antihistaminic 6], Anti-inflammatory [7], Anticancer [8], Antiviral [9], Anthelminthic [10] and Anti-hypertensive [11].

Derivatives of pyrazole have played a crucial role in the history of heterocyclic chemistry and been used extensively important pharmacophores and synthons in the field of organic chemistry. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei. Pyrazole derivatives exhibit various biological activities such as, antibacterial [12], anticonvulsant [13], anticancer [14], anthelmintics [15], anti-inflammatory [16], herbicidal [17] and hypoglycemic [18] activities.

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active heterocycles. The novel series of pyrazole containing indole ring derivatives anticipated to have potential biological activity. Thus the efficient synthesis novel series of pyrazole derivatives containing indole ring still represent highly pursued target.

MATERIAL AND METHODS

All the chemicals used were of laboratory grade and procured from E.Merck and S.D. Fine Chemicals (NSP, Guntur). Diclofenac sodium is a gift sample from Biophore Pharmaceuticals Pvt, Ltd, Hyderabad. Melting points were determined in digital melting point apparatus and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. All chemicals were reagent grade and used without further purification, and all solvents were freshly distilled before use.

Synthesis of 1*H*-Indole-2-carbohydrazide:
Methyl 1*H*-indole-2-carboxylate (1.75 g, 0.01 mol) in absolute ethanol (25 ml) was refluxed with 1.0 ml of hydrazine hydrate for 2 hour. After the completion of the reaction checked by TLC, the reaction mixture was cooled to room temperature. The separated solid was filtered, washed with cold ethanol and recrystallized from ethanol. Yield 98%, M.P 247-248^{0}C.

Preparation of 1-Phenyl-3-(thiophen-2-yl)prop-2-en-1-ones: These were prepared by condensation of thiophen-2-carbaldehyde and substituted acetophenones in the presence of sodium hydroxide.

General procedure for the preparation of 1H-Indol-2-yl[5-Aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]methanones:
1*H*-Indole-2-carbohydrazide (1.75 g, 0.01 mol) was charged into 250 ml round bottom flask. 10 ml of glacial acetic acid was added to dissolve it. Then add substituted chalcones (0.01 mol). The reaction mixture was refluxed on an oil bath for 12 hour. The progress and the completion of the reaction were monitoring by TLC. After the completion of reaction the mixture was poured onto crushed ice to give solid product. Finally, it was purified by column chromatography. (Eluent3: 7 = E.A: Hexane) Similarly other compounds were prepared. The physical constants of the product are recorded in Table-1.

[5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl)methanone (TD-1): Purity by HPLC: 94 %; IR (KBr): 3365 (N-H str), 3090 (Ar, C-H str), 2955 (C-H str), 2908 (C-H str), 1637 (amide, C=O str), 1610 (C=N str), 1556 (Ar, C=C str), 1475 (Ar, C=C str), 1184 (N-N str), 1014 (C-N str), 796 (C-Cl) cm^{-1}; 1H NMR (400 MHz, $CDCl_3$): δ ppm 3.20-3.26 (dd, *J*=3.92&17.60 Hz, 1H, CH), 3.70-3.76 (dd, *J*=8.64&16.60 Hz, 1H, CH), 5.82-5.86 (dd, *J*=3.80&11.40 Hz, 1H, CH), 6.87-6.98 (m, 2H, ArH), 7.02-7.16 (m, 2H, ArH), 7.19-7.25 (m, 1H, ArH), 7.27-7.44 (m, 4H, ArH), 7.45-7.57 (m, 2H, ArH), 7.59-7.68 (m, 1H, ArH), 9.61 (s, 1H, NH). 13C NMR (100MHz, $CDCl_3$): δ ppm 41.97, 55.43, 115.84, 124.70, 124.83, 125.08, 126.87, 127.86, 127.95, 128.15, 128.40, 129.07, 129.31, 129.74, 130.37, 130.45, 131.10, 137.01, 159.23, 164.58; MS: *m/z* = 405 $[M]^+$; Anal. Calcd for $C_{22}H_{16}ClN_3OS$: C, 65.10; H, 3.97; N, 10.35. Found: C, 65.03; H, 3.85; N, 10.21%.

1*H*-Indol-2-yl[5-(4-methylphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl] methanone (TD-2): Purity by HPLC: 97 %; IR (KBr): 3580, 3072, 2956, 2842, 1682, 1600, 1582, 1438, 1146, 1021, 840 cm^{-1}; 1H NMR (400 MHz, CDCl3): δ ppm 2.44 (s, 3H, CH3), 3.36-3.42 (dd, *J*=4&17.6 Hz, 1H, CH), 3.73-3.80 (dd, *J*=11.36&17.6 Hz, 1H, CH), 6.12-6.16 (dd, *J*=3.96&11.32 Hz, 1H, CH), 7.08-7.19 (m, 4H, ArH), 7.27-7.33 (m, 4H, ArH), 7.39-7.46 (m, 1H, ArH), 7.63-7.68 (m, 1H, ArH), 7.74-7.76 (d, *J*=8.16 Hz, 2H, ArH), 9.79 (s, 1H, NH). 13C NMR (100 MHz, CDCl3): δ ppm 21.64, 21.72, 41.13, 56.65, 110.44, 111.85, 120.39, 120.82, 122.63, 124.84, 124.94, 126.87, 126.91, 128.26, 128.35, 128.58, 128.65, 129.36, 129.47, 129.72, 131.97, 136.87, 141.54, 143.95, 156.00, 158.42; MS: *m/z* = 387 $[M+2]^+$; Anal. Calcd for $C_{23}H_{19}N_3OS$: C, 71.66; H, 4.97; N, 10.90. Found: C, 71.49; H, 4.82; N, 10.86%.

1*H*-Indol-2-yl[5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]methanone (TD-3): IR (KBr): 3561, 3071, 2941, 2862, 1683, 1621, 1558, 1423, 1175, 1012 cm^{-1}; MS: *m/z* = 371 $[M]^+$; Anal. Calcd for $C_{22}H_{17}N_3OS$: C, 71.14; H, 4.61; N, 11.31. Found: C, 71.02; H, 4.55; N, 11.27%.

[5-(4-Fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl)methanone (TD-4): IR (KBr): 3568, 3086, 2955, 2872, 1681, 1593, 1588, 1568, 1422, 1156, 1113, 820 cm^{-1}; MS: *m/z* = 390 $[M+1]^+$; Anal. Calcd for $C_{22}H_{16}FN_3OS$: C, 67.85; H, 4.14; N, 10.79. Found: C, 67.76; H, 4.10; N, 10.69%.

[5-(2,4-Dichlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl)methanone (TD-5): IR (KBr): 3572, 3071, 2936, 2858, 1686, 1598, 1570, 1554, 1429, 1188, 1050, 720 cm-1; MS: *m/z* = 441 $[M+1]^+$; Anal. Calcd for $C_{22}H_{15}Cl_2N_3OS$: C, 60.01; H, 3.43; N, 9.54. Found: C, 59.87; H, 3.31; N, 9.42%.

[5-(3-Aminophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl)methanone (TD-6): IR (KBr): 3564, 3068, 2976, 2828, 1684, 1613, 1579, 1546, 1438, 1164, 1049, 780 cm^{-1}; MS: *m/z* = 386 $[M]^+$; Anal. Calcd for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 67.35; H, 4.47; N, 14.37%.

[5-(4-Aminophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (TD-7): IR (KBr): 3579, 3079, 2982, 2837, 1690, 1609, 1559, 1558, 1429, 1186, 1020, 740 cm^{-1}; MS: *m/z* = 386 $[M]^+$; Anal. Calcd for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 67.89; H, 4.52; N, 14.35%.

1*H*-Indol-2-yl[5-(3-nitrophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl] methanone (TD-8): IR (KBr): 3548, 3062, 2979, 2842, 1689, 1606, 1576, 1568, 1434, 1164, 1024, 780 cm^{-1}; MS: *m/z* = 417 $[M+1]^+$; Anal. Calcd for $C_{22}H_{16}N_4O_3S$: C, 63.45; H, 3.87; N, 13.45. Found: C, 62.35; H, 3.75; N, 13.38%.

[5-(4-Bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (TD-9): IR (KBr): 3589, 3079, 2961, 2853, 1682, 1600, 1575, 1562, 1451, 1174, 1029, 855 cm^{-1}; MS: *m/z* = 450 $[M]^+$; Anal. Calcd for $C_{22}H_{16}BrN_3OS$: C, 58.67; H, 3.58; N, 9.33. Found: C, 57.82; H, 3.22; N, 9.27%.

[5-(4-Hydroxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (TD-10): IR (KBr): 3588, 3086, 2946, 2866, 1686, 1611, 1588, 1573, 1466, 1166, 1010, 842 cm^{-1}; MS: *m/z* = 388 $[M]^+$; Anal. Calcd for $C_{22}H_{17}N_3O_2S$: C, 68.20; H, 4.42; N, 10.85. Found: C, 67.88; H, 4.29; N, 10.68%.

Scheme-1: Pyrazole derivatives containing indole ring (IP-1 to IP-10)

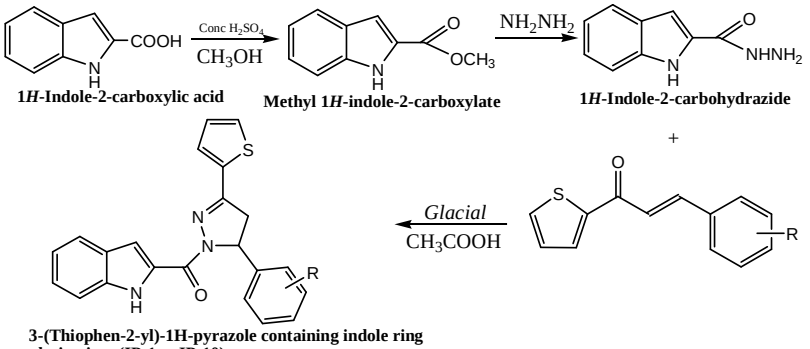
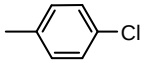
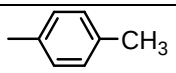
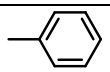
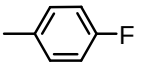
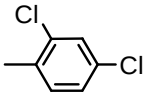
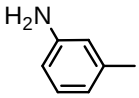
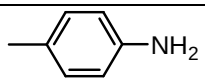
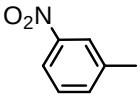
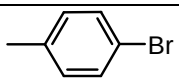
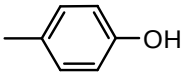


Table-1: Physical constant of synthesized pyrazole derivatives containing indole ring

Compound	Substitution (R)	M.F	M.W	M.P (^{0}C)
IP-1		$C_{22}H_{16}ClN_3OS$	405.89	145-147
IP-2		$C_{23}H_{19}N_3OS$	385.48	231-232
IP-3		$C_{22}H_{17}N_3OS$	371.45	169-171
IP-4		$C_{22}H_{16}FN_3OS$	389.44	133-135
IP-5		$C_{22}H_{15}Cl_2N_3OS$	440.34	128-130
IP-6		$C_{22}H_{18}N_4OS$	386.46	205-207
IP-7		$C_{22}H_{18}N_4OS$	386.46	117-119
IP-8		$C_{22}H_{16}N_4O_3S$	416.45	123-124
IP-9		$C_{22}H_{16}BrN_3OS$	450.35	165-167
IP-10		$C_{22}H_{17}N_3O_2S$	387.45	159-161

BIOLOGICAL EVALUATION:

Preparation of Culture Media: Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Sabouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture: Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^0C.

Standardization of Stock Culture: Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15×10^8cfu/ml.

Preparation of Working Stock Culture: Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions: Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening: Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus*(MTCC 96), *Staphylococcus pyogenus*, *Pseudomonas aeruginosa*(MTCC 1688),*Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans*(MTCC 227) and *Aspergilla niger* (MTCC 282).

Determination of MIC: The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8 ml of seeded broth and 0.2 ml of title compound (1 mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37 ^{0}C and 25 ^{0}C respectively for 24 hours for bacteriae and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).The MIC values of the test compounds are recorded in Table-2.

Table-2: Antimicrobial activity of pyrazole derivatives containing indole ring

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	***S.pyogenus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
IP-1	250	200	100	62.5	200	200
IP-2	500	100	100	200	250	500
IP-3	200	500	200	250	500	250
IP-4	100	200	100	200	1000	1000
IP-5	200	250	62.5	100	200	1000
IP-6	200	125	250	500	1000	500
IP-7	500	100	250	200	500	1000
IP-8	125	250	500	250	200	200
IP-9	100	200	200	100	250	200
IP-10	50	100	125	200	500	500
Ampicillin	250	100	100	100	NT	NT
Greseofulvin	NT	NT	NT	NT	500	100

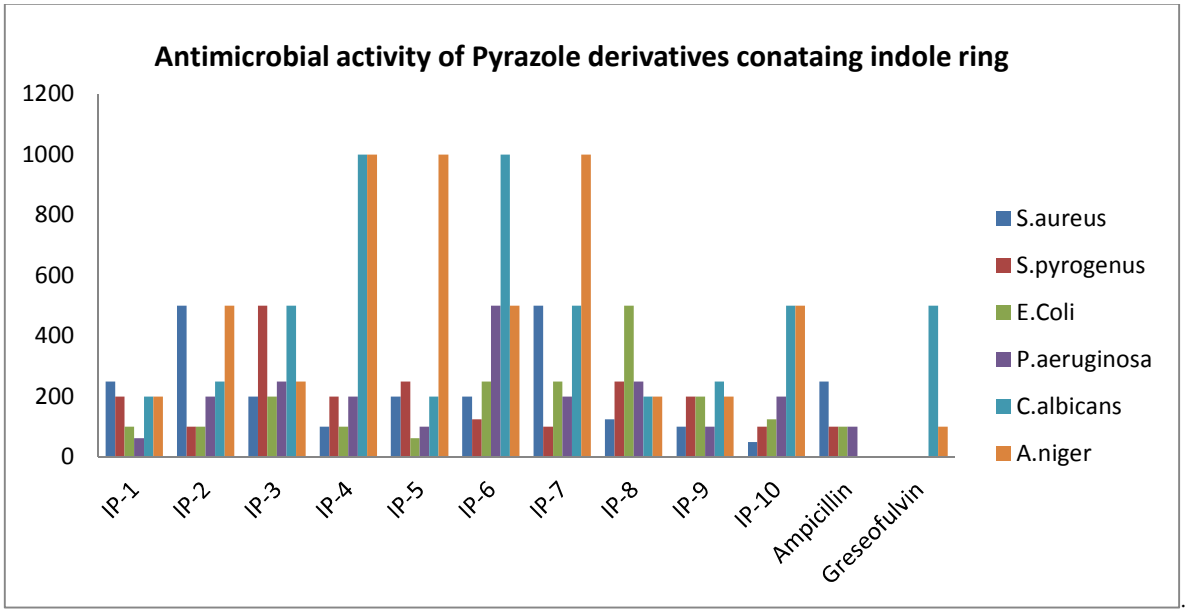


Figure 1: Antimicrobial activity of pyrazole derivatives containing indole ring

ANTI-INFLAMMATORY ACTIVITY: Carrageenan-induced rat paw edema method employing Zeitlin's apparatus was used to determine the anti-inflammatory activity of the newly synthesized pyrazole derivatives containing indole ring (IP-1 to IP-10).

Materials: Carrageenan from Sigma-Aldrich Chemicals, USA, whereas sodium CMC was of Merck grade and the required saline (Core Health Care) was purchased from a local supplier (National Scientific Pharmaceuticals, Guntur). Indomethacin used as a standard drug purchased form Research Lab fine Chem Industries.

Preparation of sodium CMC suspension: 1gm of sodium CMC was triturated in 100 ml of distilled water to give the required stock suspension of sodium CMC. This stock suspension was used for suspending all the test compounds as well as the standard drug.

Experimental procedure: Albino rats of either sex, weighing between 150-200 gm, supplied by Chalapathi Institute of Pharmaceutical Sciences, Guntur were divided into twenty seven groups of six animals each. All these groups were kept for fasting overnight and only allowed water adlibitum.

0.05 ml of 1% carrageenan suspension was slowly injected subcutaneously into the subplantar region of the left hind paw to produce inflammation in all the groups. Groups III to XXVII were treated with pyrazole derivatives containing indole ring (IP-1 to IP-10) (10 mg/kg) after carrageenan administration and the time gap is at an interval of 0.5, 1, 2, 3, 4 and 6 h. Group I used as carrageenan treated control was given only 1% sodium CMC suspension (1 ml/kg) whereas group II received indomethacin (2 mg/kg). All these doses were administered orally and the induced paw edema in each group was measured to assess the anti-inflammatory activity.

Measurement of paw thickness: Before carrageenan injection, the thickness of both the paws of each rat was measured using Zeitlin's constant load lever method. The paws thickness was also measured in a similar way after carrageenan injection at time intervals 0, 0.5, 1, 2, 3, 4 and 6 h. The dose selection for the compound in the preliminary screening is usually 5 times the dose of the standard drug indomethacin, which was used at a dose of 2 mg/kg.

The percent increase at each time interval was determined by using the formula: Yt-Yo / Yo×100 Yt = Paw thickness at time t hours (after injection), Yo = Paw thickness at time 0 hours (before injection)

The percent inhibition of paw oedema thickness was calculated by using the formula: Percentage inhibition = [1-Yt/Yc] × 100

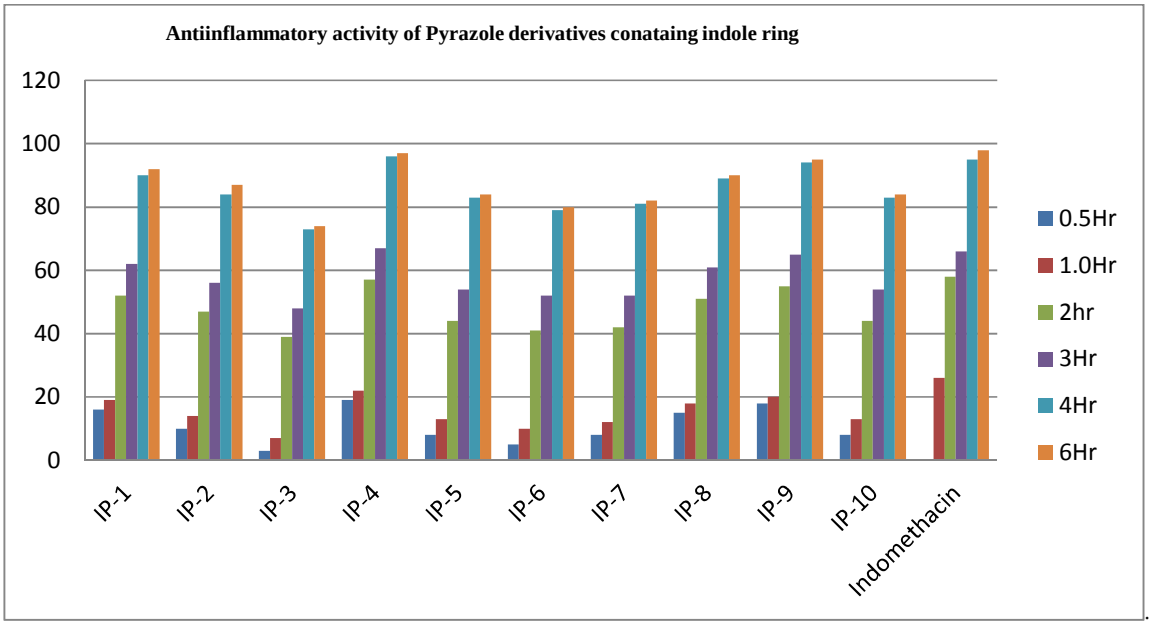
Where Yt= Average increase in paw thickness in groups tested with pyrazole derivatives containing indole ring (IP-1 to IP-10) and the standard.
Yc= Average increase in paw thickness in control

The results of anti-inflammatory activity of indomethacin and the pyrazole derivatives containing indole ring (IP-1 to IP-10) tested are shown in Table 3.

Table 3: Percentage inhibition in paw thickness at various time intervals

Compd code	% inhibition in paw thickness at various time intervals					
	0.5hr	1hr	2hr	3hr	4hr	6hr
IP-1	16 ± 1*	19 ± 2	52 ± 1	62 ± 1**	90 ± 1	92 ± 1
IP-2	10 ± 1*	14 ± 2	47 ± 1	56 ± 2	84 ± 2	87 ± 1
IP-3	03 ± 1	07 ± 1**	39 ± 2	48 ± 1	73 ± 1	74 ± 2
IP-4	19 ± 1	22 ± 1	57 ± 1**	67 ± 1	96 ± 1	97 ± 2
IP-5	08 ± 1**	13 ± 1*	44 ± 1	54 ± 1	83 ± 1	84 ± 1
IP-6	05 ± 1	10 ± 2*	41 ± 2	52 ± 2*	79 ± 1	80 ± 1*
IP-7	08 ± 2**	12 ± 1*	42 ± 1	52 ±2	81 ± 1	82 ± 2
IP-8	15 ± 1	18 ± 2**	51 ± 1	61 ± 2	89 ± 2	90 ± 2*
IP-9	18 ± 1	20 ± 1	55 ± 1	65 ± 1*	94 ± 1	95 ± 2
IP-10	08 ± 1**	13 ± 1*	44 ± 1	54 ± 1	83 ± 1	84 ± 1
Indomethacin	21± 1	26 ± 1	58 ± 1	66 ± 1	95 ± 2	98 ± 1

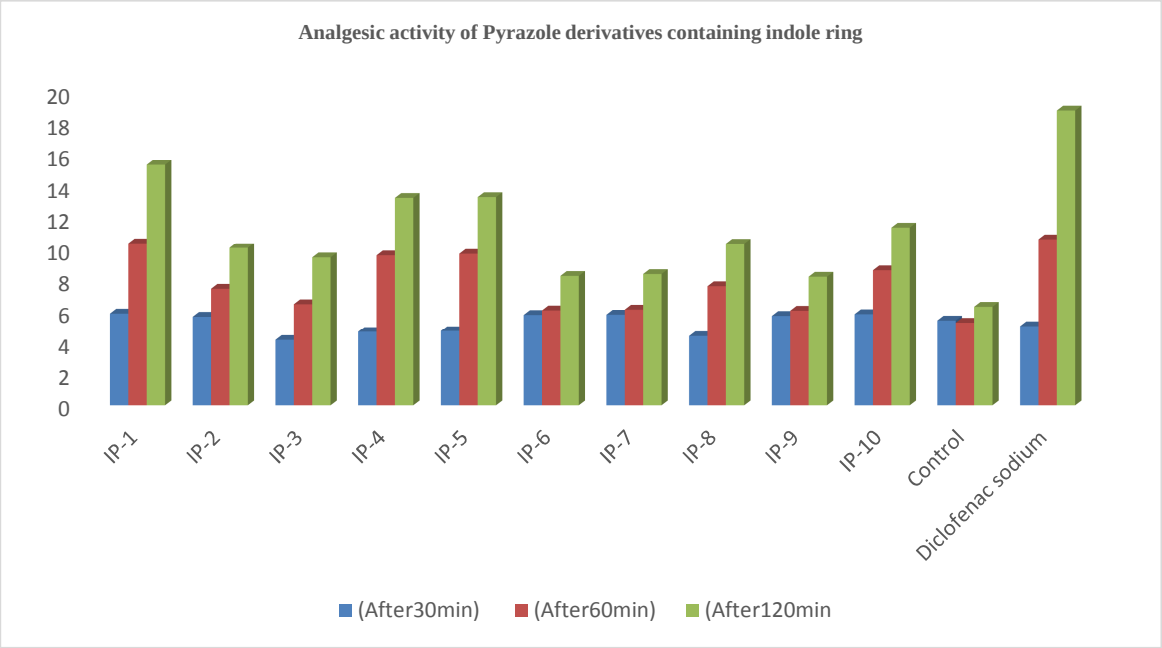
Values are expressed as mean ±(n=6)
P<0.05, P**<0.01 compared to control,Student t-test (Unpaired), Value for the control group in all the cases is zero*



ANALGESIC ACTIVITY: Tail immersion method is based on the observation that morphine-like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. Albino rats were divided in twenty-six groups each containing six animals. The tail of mice was immersed (12cm) in warm water kept constant at 55 ^{0}C.Their action time was recorded by stopwatch (the reaction time is the time taken by the rats to flick their tails).The latent period of the tail flick response will be determined before and 15, 30, 60, and120 min after drug administration. Diclofenac sodium is used as standard in analgesic activity. The results were expressed as mean ±SEM for six animals in each group analgesic activity.

Compd code	Reaction time(sec.)		
	(After30min)	(After60min)	(After120min
IP-1	5.87 ± 1.1	10.34 ± 0.09∗	15.37 ± 2.0∗
IP-2	5.67 ± 0.8	7.44 ± 0.05	10.03 ± 2.5
IP-3	4.17 ± 0.9	6.46 ± 0.02	9.42 ± 1.9
IP-4	4.70 ± 1.0	9.55 ± 0.01∗	13.25 ± 1.6∗
IP-5	4.75 ± 1.1	9.65 ± 0.02∗	13.30 ± 1.7∗
IP-6	5.78 ± 1.3	6.07 ± 0.02	8.25 ± 2.4
IP-7	5.80 ± 1.4	6.12 ± 0.02	8.37 ± 2.4
IP-8	4.42 ± 0.7	7.60 ± 0.06	10.32 ± 2.0
IP-9	5.72 ± 1.2	6.05 ± 0.02	8.20 ± 2.4
IP-10	5.83 ± 1.6	8.61 ± 0.08	11.35 ± 1.6
Control	5.43 ± 1.7	5.29 ± 0.9	6.30 ± 1.2
Diclofenac sodium	5.07 ± 1.6	10.60 ± 2.0	18.83 ± 2.4

All values are expressed as means ±SEM (n=6), ∗P< 0.05 versus control.



RESULTS AND DISCUSSION

1*H*-Indole-2-carbohydrazide was dissolved in glacial acetic acid, added substituted chalcones and refluxed on an oil bath for 12 hours to prepare pyrazole derivatives containing indole ring (**IP-1 to IP-10**).All the synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique.

The data recorded in Table 2 indicated that compound IP-4, IP-8, IP-9 and IP-10 are more potent towards *Staphylococcus aureus.* The compounds IP-1, IP-5 and IP-6 are moderately potent towards the *Staphylococcus aureus.* Compounds IP-2, IP-7 and IP-10 are moderately potent towards the *Streptococcus pyogenes.* Compound IP-5 is more potent towards the *Escherichia coli* and compounds IP-1, IP-2 and IP-4 were moderately potent towards the *Escherichia coli.*CompoundIP-1 is more potent towards the *Pseudomonas aeruginosa.* Compounds IP-5 and IP-9 are moderately potent towards the *Pseudomonas aeruginosa.*All these compounds are compared with the standard reference (Ampicillin) for their antibacterial activities. The compounds IP-1, IP-2, IP-5, IP-8 and IP-9 are more potent towards the *Candida albicans.* All these compounds are compared with the standard reference (Greseofulvin) for their antifungal activities.

The anti-inflammatory activity of the newly synthesized pyrazole derivatives containing indole ring (IP-1 to IP-10) has been evaluated by using carrageenan-induced rat paw edema method. The results of the evaluation have been viewed by taking indomethacin as the standard drug. From all the compounds, IP-1, IP-4, IP-5 and IP-9 having a chloro fluoro, and bromo group at para and ortho positions were found to be potent in anti-inflammatory activity which is comparable to standard. Also the presence of para and ortho substituted halogens (–Cl) in the compound results in enhanced biological activities. This significant increase in biological activities is attributed due to the electron withdrawing nature of halogens, which ultimately results in enhancement in lipophilicity. This enhanced lipophilicity could facilitate the penetration or passage of these compounds across the biological membrane easily.

The analgesic activity of the newly synthesized pyrazole derivatives containing indole ring (IP-1 to IP-10) has been evaluated by using Tail immersion method. The results of the evaluation have been viewed by taking Diclofenac sodium as the standard drug. From all the compounds, IP-1, IP-4, IP-5 and IP-9 having electron withdrawing groups were found to be potent in analgesic activity which is comparable to standard.

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CONCLUSION

In this study, certain pyrazole derivatives containing indole ring were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* activity. Compound IP-1, IP-2, IP-5, and IP-10 are more potent. Remaining compounds also showed moderate to weak antimicrobial activities.

The results of anti-inflammatory and analgesic activity revealed that the compounds IP-1 to IP-10exhibited moderate to considerable activity when compared with reference standard indomethacin and Diclofenac sodium. CompoundsIP-1, IP-4, IP-5, and IP-9having the electron withdrawing groups like the halogens showed maximum activity and this is consistent with the literature reports that such groups enhance the lipophilic properties of the molecule.

The study would be a fruitful matrix for the development of pyrazole derivatives containing indole ring for further bio-evaluation. But it should be suggested that further exact mechanism of action is necessary.

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