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Synthesis of some novel pyrazoline derivatives and evaluation of their biological activity

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Abstract
Medicinal Chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. Some other survey of literature reveals that no sufficient work regarding synthesis and biological evaluation of some novel pyrazoline derivatives have been reported. So the present study was designed to synthesis and evaluates the biological potential of some novel pyrazoline derivatives. Pyrazoline derivatives were chemically synthesized, crystallized and identified and characterized by melting point determination, Thin Layer Chromatography (TLC), Infrared (IR) and Nuclear Magnetic Resonance (NMR) techniques. The albino rats of either sex weighing 180-250 g were used for the study. Animals were procured from the animal housing facility of SPS, IFTM University Moradabad (Reg. no. 837/ac/04/CPCSEA). In screening of anti-inflammatory effect, Indomethacin was used as standard drug against carrageenan induced rat paw edema by mercury displacement method (Plethysmometer). Zone of inhibition technique was used for evaluation of anti-bacterial potential against Gram +ve= Bacillus subtilis, Staphylococcus aureus and Gram –ve= E.coli, Pseudomonas Aeruginosa; ciprofloxacin was kept as standard. In results, both tests exhibited significant level of anti-inflammatory and antibacterial activity comparable to standard. In conclusion, the present study reveals that some pyrazoline derivatives could be used as a template for the future development through modification or derivatization to design more potent therapeutic agents.

Keywords: NMR, E. coli, anti-inflammatory and anti-bacterial activity

1. Introduction
Medicinal Chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level [1]. Antimicrobial agents are the chemical compounds biosynthetically or synthetically produced which either destroy or usefully suppress the growth of metabolism of a variety of microscopic or submicroscopic forms of life. On the basis of their primary activity, they are more specifically called antibacterial, antifungal, antiprotozoal, anti-parasitic, or antiviral agents. Of the thousands of antimicrobial agents, only a small number are safe chemotherapeutic agents, effective in controlling infectious diseases in plants, animals, and humans [2-4].
Modern therapy by chemicals, chemotherapy, is attributed to Paul Ehrlich (Germany, Nobel Prize in 1908) who synthesized the first antibiotic, an arsenic compound patterned after an azo dye that he found to stain a microorganism selectively. The compound first called compound 606 was introduced commercially in 1910 as a treatment of syphilis under the name of Sylvarasan [5]. Pyrazolines- Among a wide variety of heterocyclic compounds that have been explored for developing pharmaceutically important molecules, pyrazolines have emerged as an interesting group of compounds possessing a broad spectrum of useful medicinal properties. The di-hydro pyrazoles are called pyrazolines. Pyrazoline are prominent class of nitrogen containing five membered heterocyclic compounds play importantrole in medicinal chemistry. Considerable attention has been focused on pyrazoline derivatives due to their interesting biological activities [6, 7]. Bhaskar *et al*. (2011) synthesized eight different derivatives of substituted 5-phenyl-1-(5-substituted phenyl) -4,5-dihydro-1H-pyrazol-3-yl)-1H-tetrazole (4a-h) by reacting the chalcones with hydrazine hydrate in presence of glacial acetic. The compounds were screened for analgesic activity by acetic acid induced writhing method and hot plate method, anti-inflamatory and antimicrobial activities [8]
Some other survey of literature reveals that no sufficient work regarding synthesis and biological evaluation of some novel pyrazoline derivatives has been reported. So the present study was designed to synthesis and evaluates the biological potential of some novel pyrazoline derivatives.

Materials and Methodology

A. Chemical Synthesis

Reagents and Solvents: Most of the solvents used were of L.R. grade and purified before the use in different reactions. Chemicals used were obtained from Central Drug House Pvt. Ltd. (CDH), Merck and Hi-media.

Purification of organic solvents: Commercially available grade of organic solvents of adequate purity were used in the reactions. The commercially available grade for general use is often accompanied by specifications indicating the around and nature of any impurity present. When however, the level of impurities, including moisture, is unacceptable for particular reaction and when large volume of such solvents is likely to be required, it is frequently more economical to purify the commercial grade.

Ethanol: Ethanol of high degree purity is frequently required in preparative organic chemistry. For some purpose ethanol of 99.5% purity is required. Rectified spirit is the constant boiling mixture, which ethanol forms with water and usually contains 95.5% of ethanol by weight.

Pyrazoline derivatives were synthesized by the following step of synthesis-

STEP-1: In first step 1H-indole-3-carbaldehyde will undergo nucleophilic addition reaction with substituted aromatic ethanone in presence of sodium hydroxide and alcohol to give corresponding aromatic substituted chalcones.

STEP-2: In second step the above mentioned substituted aromatic chalcone derivatives will undergo cyclization reaction with isoniazide and dimethyl formamide to give (4-5-dihydro-5-(1H-inden-3-yl)-3-(substitutedaromatic) pyrazole-1-yl)(pyridine-4 yl) methanone derivatives.

General procedure for the Synthesis of Chalcones [Intermediates][9]

To a stirred mixture of 1H-indole-3-carbaldehyde (0.05 mole) and substituted aromatic ethanones (0.05 mole) in 15ml ethanol (95%) at room temperature, 40% sodium hydroxide aqueous solution was added portion wise after which stirring was continued for further 8-10 hrs and kept a in refrigerator overnight, On the next day, the product was collected by suction filtration on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then with 3% aqueous hydrochloric acid. The crude chalcone, after drying in the air was recrystallised from ethanol (95%).

Table 1: Physical constants of synthesized Chalcones [Intermediates]

S. N.	Compound Code	M.P. (˚C)	Yield (%)	Mol. Formula	Rf Value	Mol. Wt.
1.	CH-1	94-95 ˚C	81%	C17H12ONBr	0.85	325
2.	CH-2	98-99 ˚C	83%	C17H12ONCl	0.87	281.5
3.	CH-3	96-97 ˚C	82%	C18H15ON	0.86	261
4.	CH-4	100-101 ˚C	85%	C18H15O2N	0.88	277
5.	CH-5	102-103 ˚C	84%	C15H11OSN	0.89	253
6.	CH-6	108-109 ˚C	86%	C17H14O2N	0.90	262
7.	CH-7	104-105 ˚C	88%	C17H13ON	0.92	263
8.	CH-8	106-107 ˚C	87%	C17H13ON	0.94	247
9.	CH-9	103-104 ˚C	89%	C15H11O2N	0.93	235
10.	CH-10	101-102 ˚C	90%	C23H17ON	0.91	310

General procedure for the synthesis of Pyrazoline Derivatives [10]

In a 250 ml of round bottom flask equimolar quantity of isoniazid (0.01 mole) and individual chalcone (0.01) were added in 40 ml of dimethyl-formamide. The mixture was refluxed at 120° to 140° for a period of 14-16 hours. After cooling, the reaction mixture was poured into a beaker containing ice-cold water and the product which precipitated out was collected by suction filtration on a Buchner funnel, washed with sufficient amount of cold water dried over a clean filter paper. The pyrazoline obtained was recrystallized using benzene-petroleum ether.

Table 2: Physical constants of different Pyrazoline Derivatives

S.N.	Compound Code	M.P. (˚C)	Yield (%)	Mol. Formula	Rf Value	Mol. Wt.
1.	PY-1	136-137 ˚C	74%	C23H17ON4Br	0.76	450
2.	PY-2	134-135 ˚C	76.8%	C23H17ON4Cl	0.79	406
3.	PY-3	162-163 ˚C	75%	C24H20ON4	0.75	380
4.	PY-4	140-141 ˚C	75.4%	C24H20O4N4	0.78	428
5.	PY-5	127-128 ˚C	77%	C21H16ON4S	0.72	372
6.	PY-6	141-142 ˚C	73.5%	C23H19ON5	0.73	381
7.	PY-7	120-121 ˚C	76.9%	C23H18O2N4	0.72	366
8.	PY-8	128-129 ˚C	74.3%	C23H17ON4	0.77	365
9.	PY-9	134-135 ˚C	77.8%	C21H16O2N4	0.71	356
10	PY-10	124-125 ˚C	75.2%	C29H22ON4	0.79	442

Physicochemical properties

Identification and characterization

The compounds synthesized were identified and characterized by following methods-

- Melting point determination
- Thin layer chromatography
- Infra-red spectroscopy
- Nuclear magnetic resonance spectroscopy

Melting point determination: The melting point of an organic compound was determined by Thiel's melting point tube (capillary tube method). The determination of melting point is the most important and easy way of differentiating this physical constant of one compound from other.

Thin layer chromatography (TLC): TLC is an important method for synthetic chemistry to infer the formation of compound based on the Rf value since different compound will have different Rf values. It also helps in confirming the progress of the reaction.

Infrared spectroscopy (IR): IR is the most important tools for determining the various functional groups and the possible chemical structure. The important advantage of IR over other technique is that it gives fingerprints (1300-650 cm-1) information about the structure (functional group, bonding with each other) of molecules easily. No two compounds have identical fingerprint region. This technique is based upon the molecular vibration of the compound such that each and every bond will vibrate at the different frequency and this vibration frequency corresponds to the IR frequency. Thus IR spectra of each and every bond will be formed. FTIR spectra were recorded in KBr powder on a Jasco V410 FTIR spectrometer by diffuse reflectance technique.

Nuclear magnetic resonance spectroscopy: The introduction between matter and electromagnetic forces can be observed by subjecting a substance simultaneously to two magnetic forces, one stationary and other varying at some radio frequency. At a particular combination of fields, energy is observed by the sample and absorption can be observed as a change in single developed by a radio frequency detector and amplifier. This energy of absorption can be related to a magnetic dipolar nature of a spinning nucleus. This technique is useful in assuming the structure of the molecule. 1H- NMR spectra were measured in CDCl3 and d6-DMSO on a Bruker Ultra-space 500MHz/ AMX400MHz spectrometer.

B. Pharmacological Activity

I. Evaluation of anti-inflammatory activity [11-14]

Animals

Albino rats of either sex, weighing 180-250 g, were used for the study. Animals were procured from the animal housing facility of SPS, IFTM University Moradabad (Reg. no. 837/ac/04/CPCSEA). The animals were fed with standard chow diet, water *ad libitium*. They were housed in poly propylene cages maintained under standard condition (12 hour light / 12 hour dark cycle). The animals were deprived of food for 24 hours before experiment but allowed free access to drinking water throughout. The experimental protocol was subjected to the scrutiny of the institutional Animal Ethical Committee and was cleared by the same before starting.

The standard and the test compounds were administered in the form of a suspension [5% w/v carboxy methyl cellulose (CMC) as vehicle].

Standard drug solution (Indomethacin, dose- 10 mg/kg)

The Indomethacin was taken as standard drug. Indomethacin 75 mg tablet were procured for the preparation of standard drug solution Indomethacin (75 mg) was transferred to 10 ml measuring cylinder and dissolved in 7.5 ml of 5% acacia solution. 1 ml of resulting solution (10 mg/ml) was transferred to 10 ml measuring cylinder and dissolved in 5 ml of 5% acacia solution. This final solution (2mg/ml) was standard drug solution.

Control vehicle (5% w/v CMC solution): Accurately weighed 5 gm of CMC was transferred to 100 ml volumetric flask. It was dissolved in 10 ml of distilled water and the volume was made-up to 100 ml by distilled water.

Preparation of 1% Carrageenan solution

Accurately weighed 1 gm of carrageenan was transferred to 100 ml of volumetric flask. This was dissolved in 10 ml of normal saline solution (0.9% w/v) and volume was made up to 100 ml with normal saline solution. Dosing solution of synthesized compounds (dose-100 mg/kg): 100 mg of synthesized compounds were weighed and transferred to 50 ml beaker. This was suspended in 4 ml of 5% CMC solution.

Experimental design

Six animals were kept in every groups enlisted below-

Control group: 5% w/v CMC at a dose level of 10 ml/kg body wt. was given to control group *p. o.*

Standard group: Indomethacin at a dose level of 10 mg/kg body wt. was given *subcutaneously* as standard drug for comparison.

Test group: The test drugs (PY -1, PY -2, PY -3, PY -4, PY -5, PY -6 and PY -7, PY -8, PY -9, PY -10) were given *p. o.* at a dose level of 100 mg/kg body wt. to test groups.

Oedematogenic agent

0.1 ml of a 1% w/v freshly prepared suspension of carrageenan in normal saline solution (0.9% w/v) was used to induce swelling or inflammation in paw of rats.

Protocol

The albino rats (180-250 g) were divided into twelve groups of six animals each as discussed above. A mark was made on left hind paw of each rat just beyond tibio-tarsal junction, so that every time the paw could be dipped in the column up to the fixed mark to ensure constant paw volume. The initial paw volume of each rat was noted by mercury displacement method with the help of a plethysmometer. To Group-I, serving as control group, 1% w/v CMC at a dose level of 10 ml/kg body wt was given orally. To Group-II, serving as standard, Diclofenac at a dose level of 10 mg/kg body wt. was given orally. To Test groups, test drugs at a dose level of 100 mg/kg body wt. were given orally. One hour after the oral administration of control, standard and test drugs, 0.1, l of 1% carrageenan in normal saline was injected into the plantar aponeurosis of the left hind paw of each rat. The paw volumes were measured using the mercury displacement technique with the help of a plethysmometer immediately 30 min, 1, 2 and 3 h after carrageenan injection. The percentage increase in paw volume in animals treated with standard, synthesized compound (test drugs) were compared with the increase paw volume of animals of control group after 30 min, 1, 2 and 3 hour.

The percent inhibition was calculate by following formula-

$\% \text{ Inhibition} = (1 - Vt/Vc) \times 100$

Where, Vt and Vc are the mean change in paw volume of treated and control rats respectively.

I. Evaluation of antibacterial activity [15-20]

In vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically.

There are two official methods for determining antimicrobial activity-:

1. Paper-disk-plate technique (cylinder plate method)
2. Tube-dilution technique (broth micro dilution technique)

Paper-disk-plate technique (cylinder plate method)

Sensitivity testing is done to determine the range of microorganisms that are susceptible to the compound under specified conditions. It can be done by disk diffusion method. This method is suitable for the organisms that grow well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi. This method is solely depends upon the diffusion of the drug substance from a disk to an extent such than the observed growth of the microorganism is prevented totally in a zone just around the disk impregnated in a solution of drug substances. A zone of inhibition (a clear area) around the disk indicates that the organism was inhibited by the drug, which diffused into the agar from the disk.

Tube-dilution technique (broth micro dilution technique)

Dilution susceptibility testing methods are used to determine the minimal concentration, usually expressed in units or microorganisms/ml, of an agent required to inhibit or kill a microorganism.

Procedures for determining antimicrobial activity are carried out by either agar or broth based methods. By this method, one can determine the smallest amount of drug substances required to inhibit the growth of the organism *in vitro*, this method is referred to as the MIC (Minimum inhibitory concentration). MIC can be determined by tube dilution technique. Antimicrobial agents are usually tested at 10 g sub (2) (two fold) serial dilutions, & the lowest concentration that inhibits visible growth of an organism recorded as the MIC.

Materials

1. Gram +ve = Bacillus subtilis, Staphylococcus aureus
2. Gram –ve = E.coli, Pseudomonas Aeruginosa

Composition of media

Nutrient Broth was used for the preparing stock cultures of bacteria.

Table 3: Composition of Nutrient Broth

Ingredients	Quantity
Peptic digest of animal Tissue	10 g
Meat Extract	10 g
NaCl	5 g
Distilled water	up to 1000 ml
pH	7.5 ± 0.2

Nutrient agar was prepared for the growth of bacterias in petri-dish which is to be inhibited by drugs.

Table 4: Composition of Nutrient Agar Medium: (2%, pH±6.8 0.2)

Ingredients	Quantity
Peptone	10 g
Beef Extract	10 g
NaCl	5 g
Agar	20 g
Distilled water	up to 1000 ml

Procedure

Prepration of Nutrient Broth

Peptone (5 gms.) beef extract (5 gms.) and sodium chloride (2.5 gms.) (All of biological grades) were weighed and dissolved in 400 ml of distilled water in a 500 ml volumetric flask and warmed. This nutrient broth was sterilized in an autoclave at 15 lb/inch2 pressure (1210 C) for 30 min.

Prepration of Nutrient Agar

Peptone (5 gms.) beef extract (5 gms.) and sodium chloride (2.5 gms.) (All of biological grades) were weighed and dissolved in 400 ml of distilled water in a 500 ml volumetric flask and warmed. 10 g agar was dissolved in 50 ml of warm distilled water. The two solutions were mixed and the volume in volumetric flask was made up to 500 ml with warm distilled water. This nutrient agar media was sterilized in an autoclave at 15 lb/inch2 pressure (1210 C) for 30 min.

Standard Drug and Test Compounds

Preparation of solution of synthesized compound [21]

Accurately weighed 25 mg and 50 mg of each synthesized compound were transferred to different 50 ml Beakers. These compounds were then dissolved in 5 ml of DMSO to form clear solution and volumes in each Beaker were made up to 10 ml with sterile distilled water. These solutions (each having conc. 50 and 100 µg/disc) were used as drug derivative solutions. 6 mm dics of whatman paper were prepared, sterilized and dipped in the drug solutions so that the drug absorbs itself on the disc. 6 mm of disc was dipped in the test solutions made. A disc absorbs about 0.02 ml of the solution which shows that if 25 mg was made into 10 ml of solution, it will contain 0.25 mg/.01 ml = 0.50 mg/0.02 ml = 50 µg/ disc Similarly for 50 mg drug dissolved in 10 ml of solution 0.50 mg/.01 ml = 1 mg/0.02 ml = 100 µg/ disc.

Preparation of standard drug: (Ciprofloxacin)

Disc of standard drug 10 µg/disc was used as standard for comparison.

Preparation of Inoculum:

Preparation of Seeded Broth

Vial containing Lactose dilution (dehydrated powder) of E. coli was broken using sterile scalpel knife in aseptic condition in a conical flask containing 100 ml of Nutrient Broth. This flask was incubated for 24 hrs at 37 degree Celsius in BOD incubator. After 24 hrs a turbid solution was obtained.

Same procedure was followed for the preparation of the cultures of Pseudomonas aeruginosa, Bacillus subtilis and Staphyloccocus aureus.

Standarization of Seeded Broth

1 ml of seeded broth was diluted in 99 ml of sterile water containing 0.05 % of Tween 80 (8 drops of Tween 80 in 100 ml of Normal Saline). From this 1 ml was diluted to 10 ml of sterile water to yield 10-1 dilution. Similarly different dilutions in the range of 10-2 to 10-10 dilutions were made.

Inoculation of Nutrient Agar Petri-dish

The dilutions were studied by inoculating 0.2 ml of each dilution on the dried Nutrient Agar medium by spreading method. After incubation at 37 ± 10C for 24 hrs the no. of well-formed colonies on the petridishes were counted. The seeded broth was suitably diluted to contain 106 to 107 m/o of CFU (Colony forming Units). This working stock was used for anti-bacterial studies.

Inoculation of drug on the petri-dish

Laminar airflow bench was swapped with 70% alcohol and UV lamp was switched on. After 30 min, the UV lamp was switched off.

All the reagents, media, inoculum and glassware were placed in laminal airflow bench observing all aseptic conditions. The sterilized solution Nutrient Agar was evenly spread in all pertidishes. It was allowed to stand for some time so that agar solidifies. After that 0.2 ml of bacteria stock solution was poured on the petridish and was evenly spread with the help of L- shaped rod. The discs of the drug of different conc. were placed on the pertidish with the help of forcep at four sides of petridish. All this was done in laminar flow. Another petridishes containing standard drug was also prepared.

The petridishes were then placed in an incubator for 24 h at 37 degree Celsius.

Negative controlled plate- In this plate, only nutrient agar medium was poured i.e. it did not contain drug dilution and inoculum.

Positive controlled plate- In this plate, nutrient agar medium was poured and after its solidification, inoculum was spread over the surface. But this petriplate did not contain drug solution.

DMSO (10%) was used as solvent control.

After 24 h the zone of inhibition of different drugs was measured by mm scale and it was compared with standard.

Results and Discussion

Anti-inflammatory effect

The following table 5 depicts the anti-inflammatory effect of the synthesized compound on carrageenan induced rat paw edema.

Table 5: The anti-inflammatory effect of synthesized compounds on carrageenan induced rat paw edema

Treatment	Dose	Mean Increase in Paw Volume (ml) ± SE			
		Time in Minutes			
		30	60	120	180
Control	10 ml/Kg	0.66±0.03	0.80±0.02	0.88±0.04	0.71±0.03
Indomethacin (standard)	10 mg/Kg	0.40±0.05 (37.87)	0.34±0.03* (62.52)	0.25±0.5* (70.45)	0.30±0.04* (56.33)
PY -1	100 mg/kg	0.49±0.06 (25.71)	0.46±0.02* (42.50)	0.44±0.04* (50.00)	0.42±0.07 (40.84)
PY -2	100 mg/kg	0.53±0.05 (19.69)	0.48±0.6* (40.00)	0.47±0.07 (46.59)	0.44±0.03 (38.02)
PY -3	100 mg/kg	0.50±0.03 (24.24)	0.45±0.2* (43.75)	0.40±0.4* (54.54)	0.39±0.06 (45.07)
PY -4	100 mg/kg	0.55±0.02 (16.66)	0.51±0.04 (36.25)	0.48±0.06 (45.45)	0.45±0.05 (36.61)
PY-5	100 mg/kg	0.51±0.03 (22.7)	0.46±0.05 (42.5)	0.41±0.04* (53.4)	0.39±0.03* (45.07)
PY -6	100 mg/kg	0.48±0.03 (27.27)	0.42±0.02* (47.50)	0.39±0.07 (55.68)	0.38±0.06 (46.47)
PY -7	100 mg/kg	0.51±0.07 (22.72)	0.48±0.04 (40.00)	0.46±0.02 (47.72)	0.41±0.03* (42.25)
PY -8	100 mg/kg	0.56±0.03 (15.15)	0.52±0.06* (35.00)	0.50±0.05* (43.18)	0.48±0.04 (32.39)
PY -9	100 mg/kg	0.54±0.06 (18.18)	0.53±0.04 (33.75)	0.53±0.05* (39.77)	0.49±0.03 (30.98)
PY -10	100mg/kg	0.53±0.05 (19.69)	0.48±0.06 (45.45)	0.42±0.02* (47.50)	0.39±0.07 (55.68)

All values are expressed as mean ± SEM: n=6,

($P < 0.05$) control vs. treated group.

The anti-inflammatory potential of prepared drug was observed that showed decreased level of increased paw edema in rats. The effect was screen-out at different time intervals including 30, 60, 120 and 180 minutes after administration to drug. The effect was observed in time dependent manner. The effect was found significant when compared with the standard group. All the groups including PY-1, PY-2, PY-3, PY-4, PY-5, PY-6, PY-7, PY-8, PY-9 and PY-10 were demonstrated a healthy and effective pharmacological effect. The maximum effect of indomethacin was noted 0.25±0.5* (70.45) and PY-5 was noted 0.39±0.03* (45.07) that make it valuable. Indomethacin was kept as standard anti-inflammatory drug. Carrageenan was used to induce inflammation among rats.

The anti-inflammatory effect proves about its actual chemically synthesized compound and being biologically active.

Anti-bacterial effect

Table 6: Antibacterial activity data of synthesized compounds

S.N.	Compound Code	Zone of Inhibition (mm)							
		B. subtilis (ATCC-441)		S. aureus (209p)		E. coli (ATCC) 11775)		P. aeruginosa	
		50µg/disc	100µg/disc	50µg/Disc	100µ g/disc	50µg/disc	100µ g/disc c	50µg/disc	100µ g/disc
1	PY -1	5	4	7	5	9	9	9	12
2	PY -2	6	7	6	6	8	8	7	9
3	PY -3	8	4	8	8	11	9	8	16
4	PY -4	5	5	4	7	12	10	9	15
5	PY -5	9	4	6	3	9	12	11	12
6	PY -6	4	6	9	6	8	10	9	8
7	PY -7	4	9	9	8	12	9	8	9
8	PY -8	6	7	3	4	14	14	12	9
9	PY -9	7	8	4	6	15	12	11	12
10	PY -10	9	10	7	5	11	11	10	9
11	Ciprofloxacin	25	25	25	25	25	25	25	25
12	Solvent control (DMSO)	-	-	-	-	-	-	-	-

From the antibacterial activity data, it can be inferred that the various formed derivatives showed significant activity against Gram –ve microbes i.e., E. coli, Pseudomonas aeruginosa. Compound PY -3 showed good activity against Gram –ve m/o, Compound PY -4, P Y -5, Y -9, PY -10, PY -8 showed moderate activity at 50 micro gm and 100 micro gm concentration, and others showed less activity. All compounds showed less to moderate activity against Gram+ve microbes.

The anti-bacterial effect was observed significantly against all species. The method was incorporated Zone Inhibition (mm). It demonstrated a potent biological effect as compared to control. The standard group was administered Ciprofloxacin.

Conclusion

A series of pyrazoline derivatives (PY-1, PY-2, PY-3, PY-4, PY-5, PY-6, PY-7, PY-8, PY-9, PY-10) were synthesized by refluxing different chalcones with isoniazid in presence of N, N-dimethylformamide. The different chalcones were obtained by reaction of 1H-indole-3-carbaldehyde with different substituted aromatic ethanone in the presence of sodium hydroxide and ethanol.

The identification of the compounds was confirmed on the basis of IR, NMR spectroscopy and by determining the Melting point and T L C.

All the synthesized compounds were tested for anti-inflammatory (carrageenan induced rat paw edema method) and anti-microbial (disc-diffusion method).

All the compounds showed good to moderate activity as anti-inflammatory agents. The synthesized compounds were found to be active against Gram –ve micro-organisms and for Gram +ve all the compounds showed less to moderate activity.

The present study reveals that some pyrazoline derivatives could be used as a template for the future development through modification or derivatization to design more potent therapeutic agents.

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