International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 4; July 2017; Page No. 03-06

Synthesis, characterization and biological screening of novel pyrazolone derivatives for certain pharmacological activities

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Abstract
Pyrazolone derivatives are prepared by condensation reaction of ethyl etoacetate, hydrazine hydrate and substituted aromatic aldehyde in alcohol. All synthesized compounds were characterized by physicochemical properties, IR, NMR etc. The compounds are screened for different biological activities like antimicrobial, anti-inflammatory, analgesic, anticonvulsant, and anti-diabetic activities.

Keywords: Pyrazolone, antimicrobial, anti-inflammatory, analgesic, anti-diabtic, anticonvulsant activities

Introduction
Medicinal chemistry is a multidisciplinary subject involving organic chemistry, pharmacology, biochemistry, physiology, microbiology, toxicology, genetics and computer modeling. It is concerned with the design, development, identification, synthesis of compounds that can be used for prevention, treatment and the interpretation of their mechanism of action at molecular level.

Pyrazolones
In the last few decades, the chemistry of six membered heterocyclic moieties has received considerable attention owing to their synthetic and effective biological importance. Heterocyclic compounds containing nitrogen and oxygen have been attracting increasing interest over the past decades owing to their utility in various applications. Intensive research in diverse heterocyclic derivatives continues to yield new medicinal agents. One such compound is pyrazolone. Pyrazolone is a widely used precursor to a variety of compounds, documented well for their numerous applications such as products and intermediates in analytical, agricultural, biological, and pharmaceutical chemistry. The pyrazolone derivatives have still attracted medicinal chemist's interest. Medicinal chemistry and pharmacology together aim for the discovery and development of new therapeutic agents. Pyrazolones are important class of heterocyclic compounds that occur in many drugs and is a non-steroidal anti-inflammatory agent used in the treatment of arthritis and other musculo skeletal and joint disorders.

Materials and Methods
Methodology for synthesis
Step-1: Synthesis of 3-methyl pyrazol-5-one
Ethyl acetoacetate (0.5 mol) was taken in a 250 mL conical flask and stirred magnetically during slow drop wise of a solution of (0.5 mol) hydrazine hydrate in 40 mL absolute ethanol. The temperature of the reaction mixture was increased during reaction and regulated around 60ºC. The crystalline deposit was separated after stirring for 1h at 60ºC and cooled in an ice bath to complete the crystallization. After standing for completion of crystallization, it was filtered and the residue was recrystallized from cold alcohol.
Step- II: Synthesis of 3-methyl pyrazol-5-one derivatives
A mixture of 3-methyl-pyrazolone (0.01 mol, 1.74 g) and substituted aromatic aldehydes (0.012 mol) was heated at 150-160ºC for 2 hrs. The progress of the reaction was monitored by TLC. The mixture was cooled, triturated with ether (20 ml) and filtered off. The coloured residue was recrystallised from ethanol to give the corresponding 3-methyl-pyrazolone derivatives.

Biological Evaluation
Experimental protocol was approved by Institutional Animal Ethical Committee, Pushpagiri college of pharmacy, Thiruvalla. All compounds were tested for oral acute toxicity study as per OECD guideline before evaluation of pharmacological activities.

Antimicrobial activity: Done by agar disc diffusion method against both gram positive and negative organisms. In disc diffusion method bacterial inoculum is prepared and inoculated into the entire surface of solid agar plate with a sterile cotton-tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution(100µg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps. The forceps were sterilized using flame. The plates were incubated for 2-3 days at 20 -25 ^{0}C and observed without opening them and the zone of inhibition was measured. The antibacterial screening was carried out in a laminar air flow unit and all types of precautions were strictly maintained to avoid any type of contamination during the test.

Anti-inflammatory activity-in vitro protein denaturation method: A solution of 0.2 % w/v of Bovine Serum Albumin(BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid [5]. Test drug of

100µg/ml concentration were prepared using ethanol as solvent. 50µl of each test drug was transferred to test tubes using micropipette. 5ml of 0.2% w/v BSA was added to the test tubes. The control consists of 5 ml of 0.2%w/v BSA solution and 50µl of alcohol. Diclofenac sodium 100µg/ml is used as standard. The test tubes were heated at 72^0C for 5 minutes and then cooled for 10 minutes. The absorbances of these solutions were determined using UV-VIS spectrophotometer at a wavelength of 660nm. Percentage inhibition = $(A_c - A_t)/A_c * 100$
A_c : absorbance of control
A_t : absorbance of test

***Invivo* studies; Carrageenan induced paw edema in rats**
The animals were divided into group of three animals in each group(one control, one standard and five test group).Acute inflammation was induced by sub-planar injection of 0.1% w/v freshly prepared carrageenan suspension into the right hind paw of the rats. The product were suspended in vehicle and administered orally (60mg/kg). 1 hour before carrageenan injection indomethacin (10mg/kg) was given to standard group. The control group animals received vehicle. The paw volume was measured with mercury plethysmometer at 0,1,2,3 and 4 hours after carrageen injection. The percentage inhibition of edema was calculated for each group with respect to the control group% inhibition of edema = $(V_c - V_t)/ V_c \times 100$
Where V_c = paw volume in test group
V_t = paw volume in control group animal

Analgesic activity: Eddy's hot plate method : The mice were divided into 3 groups each containing six animals. Control group is treated with CMC. Standard group is treated with diclofenac (10mg/kg) i.p and test(60mg/kg) is administered to third group. Maintained the hot plate temperature at 55ºC and noted the reaction time at 0,15,30,60 minutes. Calculate the increase in the reaction time(as index of analgesia).

Anticonvulsant activity: Maximal electro shock induced convulsion method: Rats were divided into 3 groups each containing six animals. Control group is treated with CMC orally. Standard group is treated with phenytoin(25mg/kg) i.p and test(60mg/kg) is administered to third group. Applied a current of 150 mA for 0.2 seconds and noted the abolition of extensor phase

Anti diabetic activity: *In vitro* by α amylase inhibitory method
Porcine pancreatic α-amylase (PPA) was used for the preliminary screening of α amylase inhibitors from the compounds. The inhibition assay was performed using the chromogenic dinitro salicylic acid (DNSA) method. A mixture of 500µl of test (100µg/ml)[44] and 500µl of α amylase solution prepared in 0.02M sodium phosphate buffer (pH 6.9 with 0.0006M NaCl) was incubated at 25^0C in a BOD incubator for 10 minutes. 500µl of preincubated 1% starch solution in 0.02M phosphate buffer of pH 6.9 was added to the above mixture. The reaction mixture was then incubated at 25^0C for 10 minutes. The reaction was stopped by adding 1.0ml dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and then cooled to room temperature. The reaction mixture was then diluted by adding 10ml distilled water and the absorbance was measured at 540nm. The control was taken 100% enzyme activity not containing any compound.

Results and Discussion

Table 1: Physiochemical properties

Sample Code	State	Colour	Molecular Formula	Molecular Weight	M.P (^{0}C)	Yield %w/w	R_F value	Perentage purity (in%)
A1	Solid powder	Light yellow	$C_{13}H_{14}N_2O_2$	230.2619	148	44.84	0.72	88
A 2	Solid powder	Orange yellow	$C_{11}H_{10}N_2O_2$	202.2088	160	60.4	0.63	92
A 3	Solid powder	Orange	$C_{11}H_9N_2O_3Cl$	220.6534	125	57.2	0.38	84
A 4	Solid powder	Orange	$C_{11}H_9N_3O_3$	231.2070	129	51.3	0.42	86
A 5	Solid powder	Orange	$C_{14}H_{16}N_2O_4$	276.2873	116	64	0.48	90

Spectral Analysis

4-[(2-ethoxyphenyl)methylidene]-3methyl-2,4-dihydro-3*H*-pyrazol-3-one(A1)
IR-(cm^{-1})2800-CH stretching, 1700-C=0 streching, 1500-C=N streching,1451-Aromatic C=C stretching.
H^1NMR -δ in ppm **:** 1.277-4.210 (8H, R-CH_3), 6.802-7.284(5H,Ar-H)

4-[(4-hydroxyphenyl)methylidene]-3-methyl-2,4-dihydro-3*H*-pyrazol-3-one(A2)IR-(cm^{-1}) 3350-NH stretching, 2870-C=H streching, 1500-C=Ostreching, 1453-Aromatic C=Cstretching.H^1NMR -δ in ppm **:** 1.216-1.601 (3H, R-CH_3), 6.962-7.847(5H,Ar-H),9.902(1 H,CH_2=NH

4-[(4-chlorophenyl)methylidene]-3-methyl-2,4-dihydro-3*H*-pyrazol-3-one(A3)
IR-(cm^{-1}) 3302-NH stretching, 1710-CH streching, 1500-C=N streching, 1430-Aromatic C=Cstretching., 750-C-Cl bond
H^1NMR δ in ppm **:** 2.415-2.549 (3H, R-CH_3), 7.284-7.967(4H,Ar-H),10.519(1 H,CH_2=NH)

4-**[(4-nitrophenyl)methylidene]- 3-methyl 2,4-dihydro-3*H*-pyrazol-3-one (A4)**
IR-(cm^{-1}) 3400-NH stretching, 2980-C=H streching, 1700-C=Ostreching, 1645-Aromatic C=Cstretching
H^1NMR δ- in ppm **:** 2.317-2.502 (3H, R-CH_3), 6.528-7.502(5H,Ar-H),9.021(1 H,CH_2=NH)

4-[(3,4,5-trimethoxyphenyl)methylidene]-3-methyl-2,4-dihydro-3*H*-pyrazolone(A5)
IR-(cm^{-1}) 2850-CH stretching, 1900-C=O streching, 1550-C=Nstreching, 1400c C-Cstretching
H^1NMR=δ in ppm : 3.953 (3H, -3.995 (12H,R-CH_3), 7.151-7.284(3H,Ar-H),9.889(1 H,CH_2=NH)

Activity Studies

Table 2: Antibacterial activity of A1-A 5

S. No	Sample	Zone of inhibition in cm			
		Bacillus subtilis	***Styphylococus aureus***	***Escherchia coli***	***Pseudomonas aeruginosa***
1	Standard 1(ciprofloxacin, 10mcg)	2.7±0.03**	2.5±0.01**	2.8±0.03**	3±0.01**
2	A1	0.5±0.02	-	0.2±0.01	-
3	A 2	-	1.7±0.03**	-	-
4	A 3	-	1.6±0.02**	-	-
5	A 4	-	1.3±0.04**	-	0.5±0.04
6	A 5	-	-	-	-

Each value represent Mean ± SEM, n = 3, **=p < 0.01 vs. control (One way ANOVA followed by Dunnett's test

In vitro Anti-Inflammatory Activity

Table 3: Percentage inhibition of protein denaturation by A1-A 5

Sl.no.	SAMPLE	Absorbance at 660nm	Percentage..of inhibition
1	A1	1.371±0.003	21.5%
2	A2	0.920±0.002**	47.33%
3	A3	1.021±0.003**	41.55%
4	A4	1.489±0.004	14.76%
5	A5	1.546±0.001	11.50%
6	Control	1.747±0.001	-
7	Standard (diclofenac 100µg/ml)	0.689±0.002**	60.23%

Each value represent Mean ± SEM, n = 3, **=p < 0.01 vs. control (One way ANOVA followed by Dunnett's test

In vivo studies; Carrageenan induced paw edema in rats

Table 4: anti-inflammatory activity of A1-A 5 using Digital Plethysmometer (ml)

S. No.	Sample	Mean paw volume measured by digital plethysmometer (in ml)									
		Zeroth hour		After 1 hour		After 2 hour		After 3 hour		After 4 hours	
		R	L	R	L	R	L	R	L	R	L
1.	A1	0.48 ± 0.006	0.48 ± 0.007	0.46 ± 0.009	0.48 ± 0.003	0.45 ± 0.006	0.48 ± 0.005	0.45 ± 0.006	0.48 ± 0.005	0.43 ± 0.009	0.48 ± 0.004
2.	A2	0.51 ± 0.009**	0.53 ± 0.007**	0.48 ± 0.001**	0.53 ± 0.006**	0.42 ± 0.007**	0.53 ± 0.001**	0.35 ± 0.006**	0.53 ± 0.001**	0.29 ± 0.003**	0.53 ± 0.001**
3.	A3	0.51 ± 0.004**	0.51 ± 0.006**	0.46 ± 0.009**	0.51 ± 0.008**	0.40 ± 0.003**	0.51 ± 0.009**	0.33 ± 0.002**	0.51 ± 0.003**	0.26 ± 0.001**	0.51 ± 0.009**
4.	A4	0.52 ± 0.003	0.53 ± 0.009	0.5 ± 0.004	0.53 ± 0.005	0.46 ± 0.006	0.53 ± 0.009	0.44 ± 0.007	0.53 ± 0.007	0.43 ± 0.004	0.53 ± 0.007
5.	A5	0.53 ± 0.009	0.50 ± 0.003	0.48 ± 0.009	0.50 ± 0.004	0.47 ± 0.006	0.50 ± 0.001	0.44 ± 0.008	0.50 ± 0.007	0.41 ± 0.005	0.50 ± 0.008
6.	Standard (Indomethacin 10mg/kg)	0.50 ± 0.007**	0.53 ± 0.006**	0.40 ± 0.005**	0.53 ± 0.006**	0.38 ± 0.008**	0.53 ± 0.006**	0.31 ± 0.008**	0.53 ± 0.006**	0.19 ± 0.008**	0.53 ± 0.006**
7.	Control (CMC)	0.51 ± 0.008	0.52 ± 0.01	0.56 ± 0.005	0.52 ± 0.01	0.59 ± 0.009	0.52 ± 0.01	0.63 ± 0.005	0.50 ± 0.01	0.65 ± 0.005	0.50 ± 0.01

Each value represent Mean ± SEM, n = 6, **=p < 0.01 vs. control (One way ANOVA followed by Dunnett's test)

Analgesic Property

Table 5: Analgesic property of A1-A 5 using Hot plate apparatus

S. No	Sample	Basal. reaction before. drug admn. (sec)	Pain perception After drug administration (sec) Mean±SEM		
			After 15minute	After 30 minute	After 60 minute
1	A1(60mg/kg)	2.3±0.2108**	3.8±0.2230**	7.33±0.2106**	8.8±0.223**
2	A2(60mg/kg)	2.33±0.2108	3.5±0.2236	4.16±0.2106	4.25±0.3073
3	A3(60mg/kg)	2.33±0.2108**	3.8±0.2236**	7.33±0.2136**	8.5±0.210**
4	A4(60mg/kg)	2.4±0.2108	3.1±0.2263	4±0.3651	5±0.2582
5	A5(60mg/kg)	2.4±0.3333**	4.2±0.4014**	6.4±0.4773**	8.8±0.2108**
6	Control	2.2±0.2108	2.3±0.2108	2.3±0.2108	2.6±0.108
7	Standard Tramadol (10mg/kg)	3.6±0.2108**	6.5±0.2236**	12.3±0.226**	12.5±0.2582**

Each value represent Mean ± SEM, n = 6, ** = p < 0.01 (one way ANOVA followed by Dunnett's 't' test

Anti Convulsant Property

Table 6: Anticonvulsant activity of A1-A5 BY MES method

Group	Time (seconds)				
	Tonic flexion	Tonic extensor	Clonic convulsion	stupor	Recovery/ death
A1(60mg/kg)	4.6±0.223	4±0223	4±0.223	75±1.04	Recovery
A2(60mg/kg)	5.5±0.223	4.6±0.2108	4.3±0.21	86±0.577	Recovery
A3(60mg/kg)	7.5±0.202	6.3±0.2189	5.5±0.213	97.3±0.71	Death
A4(60mg/kg)	6.5±0.236	8.1±0.3073	5.5±0.3073	89.16±0.347	Recovery
A5(60mg/kg)	4.5±0.2108**	2.5±0.3651**	2.5±0.3651**	65.8±0.3651**	Recovery
Control	4.5±0.223	10.5±0.232	3.5±0.235	0	Death
standard	3.3±0.210**	0.33±0.210**	2.3±0.210**	10.5±0.210 **	Recovery

Each value represent Mean ± SEM, n = 6, ** = $p < 0.01$ (one way ANOVA followed by Dunnett's 't' test)

In vitro Anti Diabetic Activity

Table 7: Percentage inhibition of α amylase inhibitory activity byA1-A5

Test drug (100µg/ml)	Percentage of inhibition
A1	10.1%
A2	15.8%
A3	8.9%
A4	62.7%
A5	56.6%

Conclusion

The objective of the study was to synthesize Pyrazolone derivatives (A1-A5). All the synthesized compounds were characterized by their physicochemical properties like melting point, R_f value, solubility IR spectrum and NMR spectrum. All these confirmed the structure of the synthesized compounds. Compound A1 showed significant activity and significant analgesic activity. Compound A2 showed significant anti inflammatory activity and anti-bacterial activity against gram positive bacteria. Compound A3 showed anti-bacterial activity against gram positive bacteria. Compound A4 showed anti-bacterial activity against gram positive bacteria. Compound A5 derivatives showed significant anti convulsant activity. In this study it can be concluded that pyrazolone ring possess antibacterial activity against gram positive and gram negative bacteria, anti-inflammatory, analgesic and anticonvulsant activities, The activities varied according to the substituents attached to the ring, so it is concluded that presence of substitution at 4 th position of pyrazolone ring increases the activity.

Acknowledgement

The authors are very thankful to all teaching and non-teaching staffs of Pushpagiri College of pharmacy, Thiruvalla, kerala for providing all facilities to carry out this work. We also thank IIRBS, MG University Kottayam, for providing spectral data.

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