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Research Article

SYNTHESIS AND STUDY OF ANALGESIC, ANTI-INFLAMMATORY ACTIVITIES OF 3-METHYL-5-PYRAZOLONE DERIVATIVES

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ABSTRACT

Considerable amount of research has been carried out in the field of pyrazolone derivatives due to their diverse biological, pharmacological and chemical properties. The need was felt to synthesize certain 3-methyl-5-pyrazolone derivatives with potent analgesic and anti-inflammatory activity and with lesser side effects as it had been proved of late that the beneficial effects of pyrazolones outweigh its adverse effects.

The present work involved the synthesis of some aryl 3-methyl-5-pyrazolone derivatives by Knorr-Pyrazolone condensation followed by subsequent one step Knovenagel-Michael condensation and the evaluation of the analgesic and anti-inflammatory activities of the synthesized compounds using Acetic acid writhing method in Swiss Albino mice and Carageenan induced rat paw edema in Wistar rat respectively.

Keywords: Pyrazolone, Knorr, Konvenagel, Analgesic, Anti-inflammatory, Anti-thrombic.

INTRODUCTION

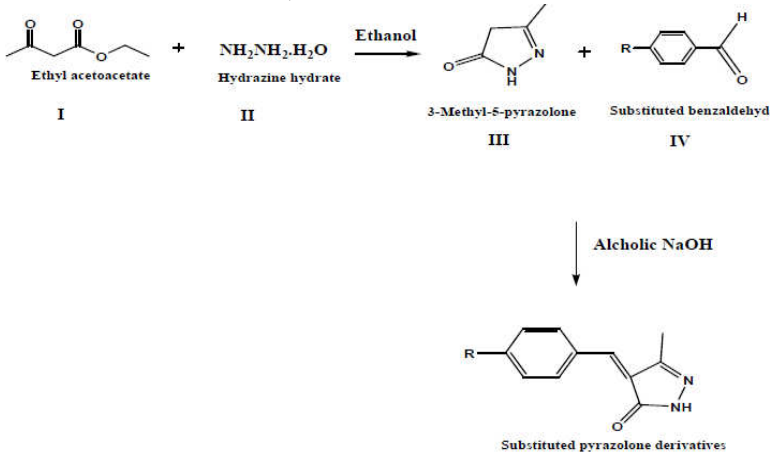
Inflammation is part of the body's natural defense system. It is a process whereby the body's cells & natural chemicals protect us from physical damage & infection from foreign substances such as bacteria & viruses. White blood cells or leukocytes are the body's major infection fighting cells. The primary objective of inflammation is to isolate, localize & eradicate foreign substances and repair damaged tissues. [1] Over the last few years despite intensive global research, cures for pain and inflammation with no toxicity have still not been found. [2] Keeping in view the potential for potent & superior anti-inflammatory agents & in continuation of our efforts in search of bioactive molecules, it was thought of interest to design the novel new chemical entities containing heterocycle like substituted pyrazolone derivatives with different moieties. [3] From the literature survey, in recent years pyrazolone derivatives have attracted considerable interest because of their therapeutic and pharmacological properties. Several of them have been found to exhibit a wide spectrum of biological actions like anti-inflammatory, ulcerogenic, antibacterial, diuretic, analgesic, antiviral, antifungal, antimycobacterial activity etc. So it has been planned to synthesize a novel series of some pyrazolone derivatives with different moiety and to check their analgesic anti-inflammatory activity. [4]

The Pyrazolone moiety is a five-membered lactam ring containing two nitrogen atoms and a ketone group. Pyrazolone and its derivatives have been the focus of medicinal chemists since the last century because of the outstanding pharmacological properties shown by several of its derivativese.g. ampyrone, metamizole etc. It is a constituent structural feature of many NSAIDS clinically useful in the treatment of arthritis and other musculoskeletal and joint disorders. [5] They act mainly by inhibiting the cyclooxygenase enzymes and thereby inhibiting the production of prostaglandins which are the mediators of pain and inflammation. Recent advances in this field have led to the development of Pyrazolone derivatives which are being used extensively in the treatment of cerebral ischaemia [5] and cardiovascular diseases. Some of the earlier developed pyrazolone based drugs like antipyrine and amidopyine have been banned as they are found to have serious side effects like bone marrow depression, agranulocytosis and blood dyscriasis.

Currently, a lot of research has been carried out in the field of pyrazolones as it has been justified that the occurrence of agranulocytosis in patients administered with a pyrazolone drug is infinitesimally small.

MATERIALS AND METHODS

All chemicals used were of laboratory grade and were procured from Merck specialities Pvt. Ltd. and Sisco Research Laboratories (SRL). Melting points were determined by Melting Point apparatus (*Veego, Model No. MP I*). The TLC plates were prepared by using Silica gel-G. The spots were visualized by exposure to iodine vapor and UV light. The UV-Spectra (λ_{max}) were recorded on *Shimadzu*, UV-1800, UV-VIS spectrophotometer. The FTIR spectra of the synthesized compounds were recorded on *Bruker FTIR* at the Lab. of Dept.of Pharm. Sciences, Dibrugarh University. The ^{1}H-NMR spectra were recorded in DMSO 400.40 MHz by Bruker Advance-II 400 NMR spectrometer and the [13]C-NMR spectra were recorded in $CDCl_3$ at 100 MHz by Bruker Advance–II 100 NMR spectrometer. The *in vivo* experiments were performed after the approval of the protocol by the CPCSEA Rgtn. No.1576/GO/a/11/CPCSEA dated 17-02-12.



Synthesis of 3-methyl-5-pyrazolone (Fig.1) [6]

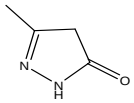
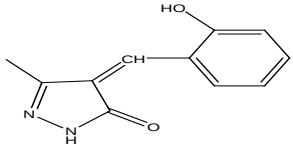
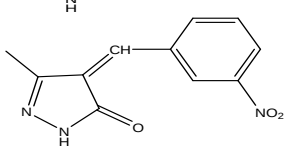
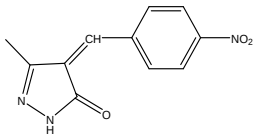
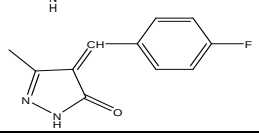
Ethyl acetoacetate was taken in a beaker and stirred magnetically and to it a solution of hydrazine hydrate and absolute alcohol were added drop wise. [7] The temperature of the reaction mixture was maintained at 60^0c and the crystalline deposit was separated after stirring for 1 hr at 60^0c. The reaction mixture was cooled in ice bath and kept to stand for some time to complete the crystallization. The crystalline solid was then washed with cold alcohol and dried. The 3-methyl-5-pyrazolone so obtained was kept for next step of the synthetic scheme. [8]

Synthesis of 3-methyl-5-pyrazolone derivatives [9-11]

The synthesized pyrazolone was taken and to it freshly prepared 20% NaOH (alcoholic solution) was poured into it and stirred magnetically for 30 min. Substituted aromatic aldehyde was added to the reaction mixture and kept under constant stirring for 8 hrs. Reaction mixture was transferred to crushed ice and neutralized with dil. HCl to precipitate the product.

Fig.1 Synthesis of 3-methyl-5-pyrazolone (intermediate) via Knorr pyrazolone reaction and further reaction with substituted aryl aldehydes to obtain 3-methyl-5-pyrazolone derivatives.[12]

Table 1: Compounds Synthesized

S. No.	Compound Code	Structure	Molecular weight	% Yield
1)	PYZ-1		98.1	81%
2)	PYZ-2		202.21	69%
3)	PYZ-3		231.06	71%
4)	PYZ-4		231.21	54%
5)	PYZ-5		204.2	55%

Biological Evaluation studies

Animals

Male Swiss albino mice (20-50 g) and male Wistar rats (120-160 g) were used to carry out the analgesic and anti-inflammatory studies of the synthesized pyrazolone derivatives respectively.

Animals were acclimatized in the animal house of the pharmacology Dept., College of Veterinary sciences, Khanapara for at least one week prior to experimentation. Animals were kept at 22 ± 3^0C and 55 ± 5% relative humidity during the whole experiment. Standard food pellets and water were supplied *ad libitum*. All tested compounds were dispensed in 10% Tween-80 solution in distilled water. Animals' treatment protocol was approved by the CPCSEA Rgtn. No.1576/GO/a/11/CPCSEA dated 17-02-12.

Acute Toxicity Studies: Toxicological studies of the test compounds as a suspension in 1% Tween 80 were carried out by administering high dose of 1000mg/kg and low dose 100mg/kg body weight in Swiss albino mice. The control group received 1% Tween 80 suspension. Animals were kept in fasting condition prior to dosing. Following the period of fasting, the animals were weighed, properly marked and the test substance administered. After administration of test compounds, food was withheld for a further 1-2 hours. OECD guideline No. 420; (Annexure -2d) method of CPCSEA was adopted for toxicity studies. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. After carrying out the acute toxicity study of all the test compounds, it was found that the entire compounds in the present investigation were non-toxic up to 1000mg/kg body weight.

Analgesic Activity: Analgesic activity of the synthesized compounds was carried out using the Acetic acid writhing method in Swiss Albino mice by *Seigmund et al* [13]. 19 groups of mice, male and female weighing 25-40g were taken with 5 in each group. Acetic acid (0.6 %) was used as an irritant and administered intraperitoneally for the control group and the number of writhing movements were noted and recorded for a time span of 15 minutes. The test compounds and the standard drug (Aspirin) were given in doses of 100mg, 250 mg and 500 mg per kg body weight orally half an hour prior to the administration 1ml (0.6 %) acetic acid injection. The number of writhings which the animal demonstrated within a span of 15 minutes were noted and reported.

Anti-inflammatory Activity: For Anti-inflammatory studies the method described by Winter *et al* [14] was followed. Acute inflammation was produced by injecting 0.1ml of carrageenan 1% suspension in 1% Tween 80 under the planter aponeurosis of the left hind paw of Wistar rats. 7 groups of rats were used each group containing 5 animals. The control group received vehicle (1%Tween 80). The standard drug Diclofenac and the test compounds were administered orally one hour prior to the carrageenan injection. Hind paw volume was recorded for each group after intervals of ½, 1, 2 and 3 hours. Initial as well as the oedema volume was measured by Plethysmograph.

RESULTS AND DISCUSSION

A new series 3-methyl-5-pyrazolone derivative have been synthesized and screened for their analgesic and anti-inflammatory activities. Various physico-chemical data of the synthesized compounds obtained are recorded in the Table 2.

Table 2: Physicochemical properties of the synthesized compounds

Compound Code	State	Colour	M. Point 0⁰C	Solubility	R_f (TLC)*
PYZ1	Solid	Yellow	207-210⁰C	DMSO, Ethanol	0.32.
PYZ2	Solid	White	371-380⁰c	DMSO, Methanol	0.37
PYZ3	Solid	Pale brown	159-167⁰c	DMSO, Ethanol	0.83
PYZ-4	Solid	Pale brown	161-167⁰c	Methanol	0.44
PYZ-5	Solid	Yellow	299-305⁰c	Ethanol	0.36

*Solvent system of TLC-Ethyl acetate:hexane for PYZ1, PYZ2, PYZ4, PYZ5 is 9:1,[15]Methanol:Hexane::7:3 for PYZ 3[16]

The UV-Spectra (λ_{max}) were recorded on *Shimadzu*, UV-1800, UV-VIS spectrophotometer. The FTIR spectra of the synthesized compounds were recorded on *Bruker FTIR* at the Lab. of Dept.of Pharm. Sciences, Dibrugarh University. The ^{1}H-NMR spectra were recorded in DMSO 400.40 MHz by Bruker Advance-II 400 NMR spectrometer and the [13]C-NMR spectra were recorded in $CDCl_3$ at 100 MHz by Bruker Advance–II 100 NMR spectrometer.[17]

Analgesic Activity: Synthesized compounds were evaluated for analgesic activity by acetic acid induced writhing method. The percentages of analgesic activity of the synthesized compounds at three different doses calculated are listed in the Table 4.

Anti-inflammatory Activity: Anti-inflammatory activity of the synthesized compounds were determined by carrageenan induced rat paw edema method and listed in the Table 5.

Table 3: Spectroscopic data of the synthesized compounds

Compd. Code	FTIR Spectrum	UV λ_{max} (nm) Ethanol	^{1}H- NMR -and [13] C-NMR Spectra
PYZ-1	2566.47 cm^{-1}(CH_3 group), 3283.05 cm^{-1}(N-H Stretch),1609.91 cm^{-1} (CH=CH stretch), 1735.77 cm^{-1},1676.28 cm^{-1}(ketone group), 1342-1266 cm^{-1}(C-N stretch), 3000-2840 cm^{-1}(C-H stretch)	237	**^{1}H NMR (400 MHz, DMSO):** δ, ppm: 7.265 (s, 1H, NH, Pyrazolone nucleus), δ,ppm: 1.652(s, 2H, CH_2), δ, ppm: 0.8263(s, 3H, CH_3) **[13]C NMR (100 MHz, DMSO):** δ,ppm: 169.267(C=O), δ,ppm: 21.37(CH_3), δ,ppm: 53.327(C-NH), δ,ppm: 31.9314(-CH_2-)
PYZ-2	3669.54 cm^{-1}(OH group), 2981.14 cm^{-1}(CH_3 group), 1587.70 cm^{-1}(CH=CH stretch), 1710.21 cm^{-1}(Ketone group),3609.39 cm^{-1}(N-H stretch), 1274.55 cm^{-1}(C-N stretch), 1452.50 cm^{-1}(N-N stretch)	355	**^{1}H NMR (400 MHz, DMSO):** δ,ppm: 7.2261(s, 1H, NH, Pyrazolone nucleus), δ, pm: 11.4152 (s, 1H, Ar-OH), δ, ppm: 4.8812 (s, 1H, Ar-CH=CH), δ, ppm: 1.62 (s, 3H,CH_3). **[13]C NMR (100 MHz, DMSO):** δ, ppm: 169.2676 (C=O), δ, pm: 21.8439 (CH_3), δ, pm: 151.3277 (C=N), δ, pm: 53.3170 (C-NH), δ, pm: 141.6841 (-CH=CH-), δ, pm: 158.8177(C-O),δ, pm: 115.8077-127.6778 (Aromatic C), δ, pm: 154.8704 (Phenolic group) δ,ppm: 31.9314(-CH_2-)
PYZ-3	3394.77 cm^{-1}(N-H stretch), 2938.76 cm^{-1}(CH_3 group), 1526.68 cm^{-1}(C-NO_2), 1727.32 cm^{-1}(Ketone group), 1584.70 cm^{-1}(CH=CH stretch), 1265.69 cm^{-1}(C-N stretch)	298.50	**^{1}H NMR (400 MHz, DMSO):** δ, ppm: 7.7884 (s, 1H, NH Pyrazolone nucleus), δ, ppm: 4.8403(s, 1H, Ar-CH=CH), δ, ppm: 1.1735 (s, 3H,CH_3), δ,ppm:1.3858 (s, 2H, CH_2), δ, ppm: 6.5707-7.5461(m, 4H, Ar-H) **[13]C NMR (100 MHz, DMSO):** δ, ppm: 170.1824 (C=O), δ, pm: 21.4143 (CH_3), δ, pm: 155.3127 (C=N), δ, pm: 53.3179 (C-NH), δ, pm: 141.6841 (-CH=CH-), δ, pm: 156.8816 (C-O), δ, pm: 119.3240-130.9677 (Aromatic C), δ, pm: 146.4 (C-NO_2) δ,ppm: 31.9314(-CH_2-)(M-Nitro)
PYZ-4	1550.98 cm^{-1}(C-NO_2), 2799.73 cm^{-1}(CH_3 group), 1785.88 cm^{-1}(Ketone group), 1524.94 cm^{-1}(CH=CH stretch), 1454.99 cm^{-1}(N-N stretch), 1287.24 cm^{-1}(C-N stretch), 3523.58 cm^{-1}(N-H stretch)	300	**^{1}H NMR (400 MHz, DMSO):** δ, ppm: 7.8755(s, 1H, NH, Pyrazolone nucleus), δ, ppm: 4.9107 (s, 1H, HC=CH-Ar), δ, ppm: 1.6394 (s, 2H, CH_2), δ, ppm: 0.8781 (s, 3H, CH_3), δ, ppm: 6.6749-7.8755 (m, 4H, Ar-H) **[13]C NMR (100 MHz, DMSO):** δ, ppm: 170.1824 (C=O), δ, pm: 21.4143 (CH_3), δ, pm: 155.3127 (C=N), δ, pm: 53.3179 (C-NH), δ, pm: 141.6841 (-CH=CH-), δ, pm: 156.8816 (C-O), δ, pm: 119.3240-130.9677 (Aromatic C), δ, pm: 146.4 (C-NO_2) δ,ppm: 31.9314(-CH_2-)(P-Nitro)
PYZ-5	1225.73 cm^{-1}(C-F), 1453.39 cm^{-1}(N-N stretch), 1337.82 cm^{-1}(C-N stretch), 3394.35 cm^{-1}(N-H stretch), 1728.27 cm^{-1}(Ketone group),1585.50 cm^{-1}(CH=CH stretch), 2969.89 cm^{-1}(CH_3 group)	305	**^{1}H NMR (400 MHz, DMSO):** δ, ppm: 7.9459, (s, 1H, NH, Pyrazolone), δ, ppm: 4.6805 (s, 1H, HC=CH-Ar), δ, ppm: 1.1714 (s, 3H, CH_3), δ, ppm: 1.300 (s, 2H, CH_2), δ, ppm: 7.047-7.34 (m, 4H, Ar-H) **[13]C NMR (100 MHz, DMSO):** δ, ppm: 169.2676 (C=O), δ, pm: 21.73 (CH_3),δ, pm: 151.3277 (C=N),δ, pm: 53.3270 (C-NH), δ, pm: 141.6841 (-CH=CH-),δ, pm: 156.8816 (C-O),δ, pm: 115.8077-128.4214 (Aromatic C),δ, pm:158.8177 (C-F) δ,

Table 4: Analgesic activity of 3-methyl-5-pyrazolone derivatives

Group	No. of writhings noted for 15 minutes			Percentage of analgesic activity		
	100 mg/ kg Dose	250 mg/ kg Dose	500 mg/ kg Dose	100 mg/ kg Dose	250 mg/ kg Dose	500 mg/ kg Dose
Control	103.67±1.02			-		
Aspirin	52±1.24**	41±3.26**	29±0.83**	49.84	60.45	72.02
PYZ 1	68.2±0.83*	53.4±2.07*	48.2±2.16*	34.21	48.49	53.50
PYZ 2	78.4±1.14*	70.8±1.48*	61.8±1.92*	24.37	31.70	40.38
PYZ 3	81.8±1.92*	77.4±1.51*	67±3.08*	21.09	25.34	35.37
PYZ 4	65±2.12*	57.2±1.78*	54.6±2.79*	37.30	44.82	47.33
PYZ 5	74.6±1.51*	67±1.87*	60.8±1.92*	28.04	35.37	41.35

No of writhings are expressed in mean ± SE (n=5). Statistical analysis is carried out by using one way ANOVA (F-test) followed by Dunnett's t test. **Significantly different from the control value at P<0.001 and *significantly different from control value at P<0.01.

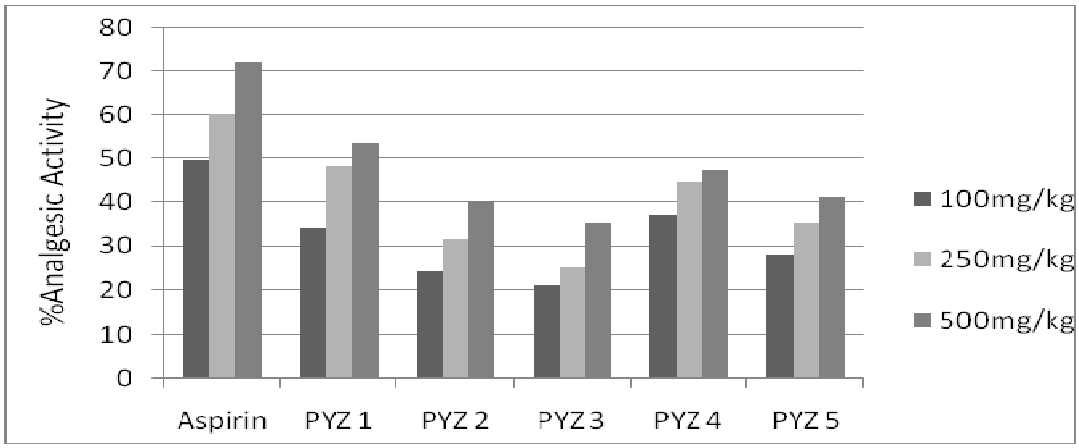


Fig. 2: Histogram of synthesized compounds showing analgesic activity.

Table 5: Anti-inflammatory activity 3-methyl-5-pyrazolones

Group	Increase in paw volume (ml)				% of anti-inflammatory activity			
	½ Hour	1 Hour	2 hour	3 hour	½ Hour	1 Hour	2 hour	3 hour
Control	0.25±0.02	0.35±0.03	0.55±0.02	0.77±0.01	-			
Diclofenac	0.126±0.008	0.174±0.005	0.22±0.007	0.256±0.005	49.6	51.4	60	67.53
PYZ 1	0.19±0.007	0.216±0.008	0.232±0.008	0.26±0.011	24	38.28	58.18	66.23
PYZ 2	0.218±0.008	0.234±0.005	0.276±0.001	0.32±0.007	12	33.14	49.81	58.44
PYZ 3	0.162±0.008	0.22±0.007	0.24±0.05	0.26±0.008	36	37.14	56.36	66.23
PYZ 4	0.23±0.01	0.21±0.005	0.23±0.008	0.27±0.008	8	40	58.18	64.93
PYZ 5	0.206±0.005	0.228±0.008	0.278±0.008	0.32±0.01	17.6	34.85	50.90	58.44

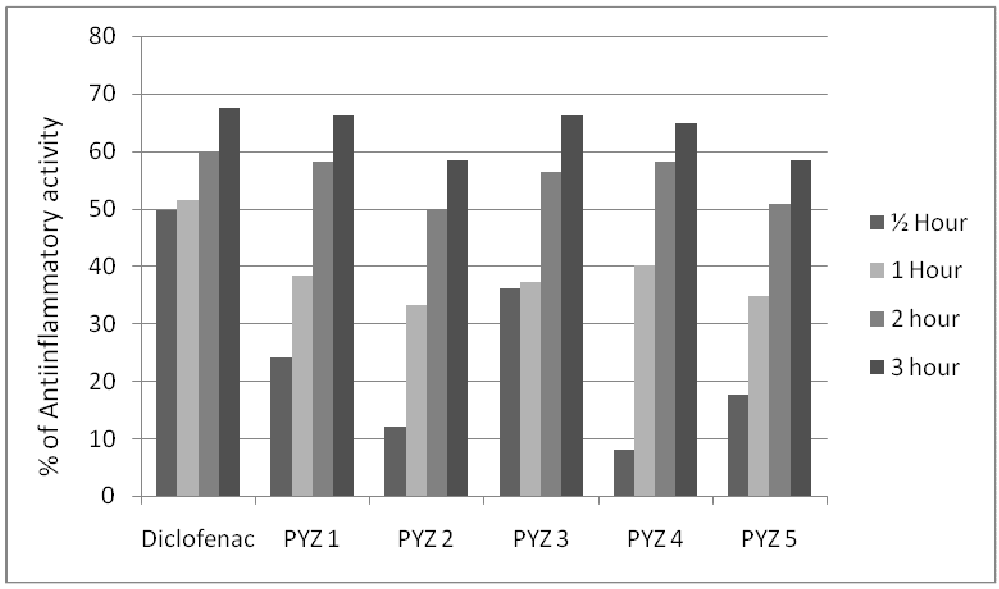


Fig. 3: Histogram of synthesized compounds showing anti-inflammatory activity

CONCLUSION

Synthesis and study of acute toxicity, analgesic and anti-inflammatory activity of certain 3-methyl-5-pyrazolone derivatives in mice and rats have been carried out and described here. In acute toxicity study no mortality was observed in the test compounds. The compounds were found safe in between the dose of 100 mg/kg to 1000mg/kg. However, further detailed toxicological investigations are required particularly to elucidate their chronic toxicity. The results obtained clearly indicate that the synthesized compounds possess significant analgesic as well as anti-inflammatory activity at a dose of 500 mg/kg. Among the five synthesized compounds, the compounds **PYZ 1** (53.50%), **PYZ 2** (40.38%), **PYZ 4** (47.33%) and **PYZ 5** (41.35%) showed significant analgesic activity as compared to the standard Aspirin, whereas compound **PYZ 3**(35.37%) have shown moderate activity. The compound **PYZ 1** is found to possess most effective analgesic activity among the all synthesized compounds. In case of anti-inflammatory study, all the compounds have shown significant activity as compared to the standard drug Diclofenac. The compounds **PYZ 1** (67.53%), **PYZ 2** (66.23%), **PYZ 3** (58.44%) **PYZ 4** (64.93%) and **PYZ 5** (58.44%) have shown most effective anti-inflammatory activities.

Hence, it is concluded that **PYZ 1** and **PYZ 4** might be attractive candidates for further development as they are the most active compounds among the compounds that have both analgesic and

anti-inflammatory activities. The experimental details of the scheme of synthesis of the compounds reported here have been carefully documented, so that it may pave the way for researchers in their effort to make new analgesic and anti-inflammatory compounds in future.

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