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Research Article

Synthesis and Evaluation of (1-Phenyl) – (5- Pyrrol)-Pyrazole Derivatives as Non-Acidic Anti-Inflammatory and Analgesic Agent

Elessy Abraham^{1*}, Anil V Chandewar², Nithin Kocher²¹ Prist University, Vallam, Thanjavur – 613403 (T.N.), India² P. Wadhvani College of Pharmacy, (Amravati University), Moha Fata, Yavatmal – 445001 (M.S.), India

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Abstract

A series of 1-Phenyl, 5-pyrrol- pyrazole derivatives (**7a-7f**) have been synthesized. The structures of these compounds were established by IR, ¹H NMR, Mass spectral data and elemental analysis. Compounds were evaluated for their in-vivo analgesic and anti inflammatory activity. Derivatives **7b** and **7c** exhibited good anti inflammatory activity in carrageenan- induced rat paw edema compared with the standard drug diclofenac sodium.

1. INTRODUCTION

The treatment of pain and inflammation are done widely with Non-steroidal anti-inflammatory drugs (NSAIDs). Most currently used NSAIDs have limitations for therapeutic uses, since they cause gastrointestinal and renal side effects which are inseparable from their pharmacological activities. These compounds act via inhibition of the enzyme cyclooxygenase, thus preventing prostaglandin synthesis. Cyclooxygenase (COXs) are main enzymes in the synthesis of prostaglandin H₂ which is a precursor for the biosynthesis of prostaglandins, thromboxanes, and prostacyclins. It was discovered that this enzyme exists as two isomers, one constitutive (COX-1) and the other inducible (COX-2)¹. COX-1 is an enzyme is constitutively expressed and provides cell protection in the gastrointestinal tract (GIT); whereas the inducible COX-2 mediates inflammation, pain and oncogenesis². The conventional NSAIDs causes inhibition of both enzymes. In fact, most of them show greater selectivity for COX-1 than COX-2³. Consequently long term therapy with nonselective NSAIDs may cause gastrointestinal problems ranging from stomach irritation to GI ulceration and bleeding⁴. Most of the clinical NSAIDs possess acidic carboxyl (COOH) group, which further causes GI irritation by direct contact of –COOH group in GIT. Also selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs. But coxibs with thiazole ring has revealed unexpected cardiovascular adverse effects⁵. These serious side effects limit the use of NSAIDs as a safer drug for the treatment of inflammation and pain. Non acidic⁶ and acid bioester of the type of 1, 3, 4-thiadiazole and 1, 3, 4-oxadiazole derivatives⁷ were reported for anti-inflammatory activity. Several studies have described the derivatization of the carboxylate function of representative NSAID with less acidic azoles: thiazole⁸, oxadiazole⁹, thiadiazole¹⁰ etc. which resulted in an increased anti inflammatory activity with reduced ulcerogenicity. Here an attempt to synthesize new, safer and potent agents for treatment of inflammatory and pain diseases, we used made derivatives of pyrazole moiety.

2. MATERIALS AND METHODS

Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 157 spectrometer using KBr pellets. The ¹H NMR spectra was taken on a Bucker WM-400 (400 MHz FT NMR) spectrophotometer using CDCl₃ and DMSO-d₆ as solvents with TMS as an internal reference. Chemical shifts (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. The chemicals were purchased from Aldrich and Merck. Reagents and solvents were of analytical grade. Diclofenac sodium was procured from Zydus Cadila, Ahmadabad. Ethical Approval- The proposed protocols were approved before the experimentation by the CPCSEA approved IAEC of P. Wadhvani College of Pharmacy, Yavatmal.

2.1 Chemistry

2.1.1 Synthesis of intermediated 2 to 6 is as follows:

Potassium 1-cyano-3-ethoxy-3-oxoprop-1-en-2-olate (**2**) was obtained by treatment of diethyl oxalate (**1**) with acetonitrile in the presence of 18-crown-6 and potassium tert-butoxide. Reaction of (**2**) with phenylhydrazine hydrochloride (**3**) in refluxing ethanol furnished ethyl 1-phenyl-5-amino-1H-pyrazole-3-carboxylate (**4**). Pyrrole derivative (**5**) were prepared by heating the ester compound (**4**) with 2, 5-dimethoxytetrahydrofuran in glacial acetic acid according to the Clauson-Kaas procedure. Lithium hydroxide hydrolysis of esters (**5**) at room temperature afforded the acid (**6**). Finally, carboxamides or carboxyhydrazide (**7a-f**) were obtained through parallel solution-phase synthesis by coupling acids (**6**) with selected amine or hydrazide in the presence of EDC/HOBt, using ethyl diisopropyl amine as scavengers for acid (**Scheme 1**).

2.1.2 General method for synthesis of amide and hyrazide (**7a-f**)

To a solution of the appropriate acid **6** (1.0 mmol) in dichloromethane, kept at 0 °C, EDC hydrochloride (1.2 mmol), HOBt (1.0 mmol) and triethylamine (2.0 mmol) were added followed by the appropriate amine or hydrazide (1.5 mmol). The solution was allowed to warm up at room temperature and stirred overnight. The residue is diluted with water. Solid precipitates were filtered under suction on Buchner funnel and then washed with water (20 ml).

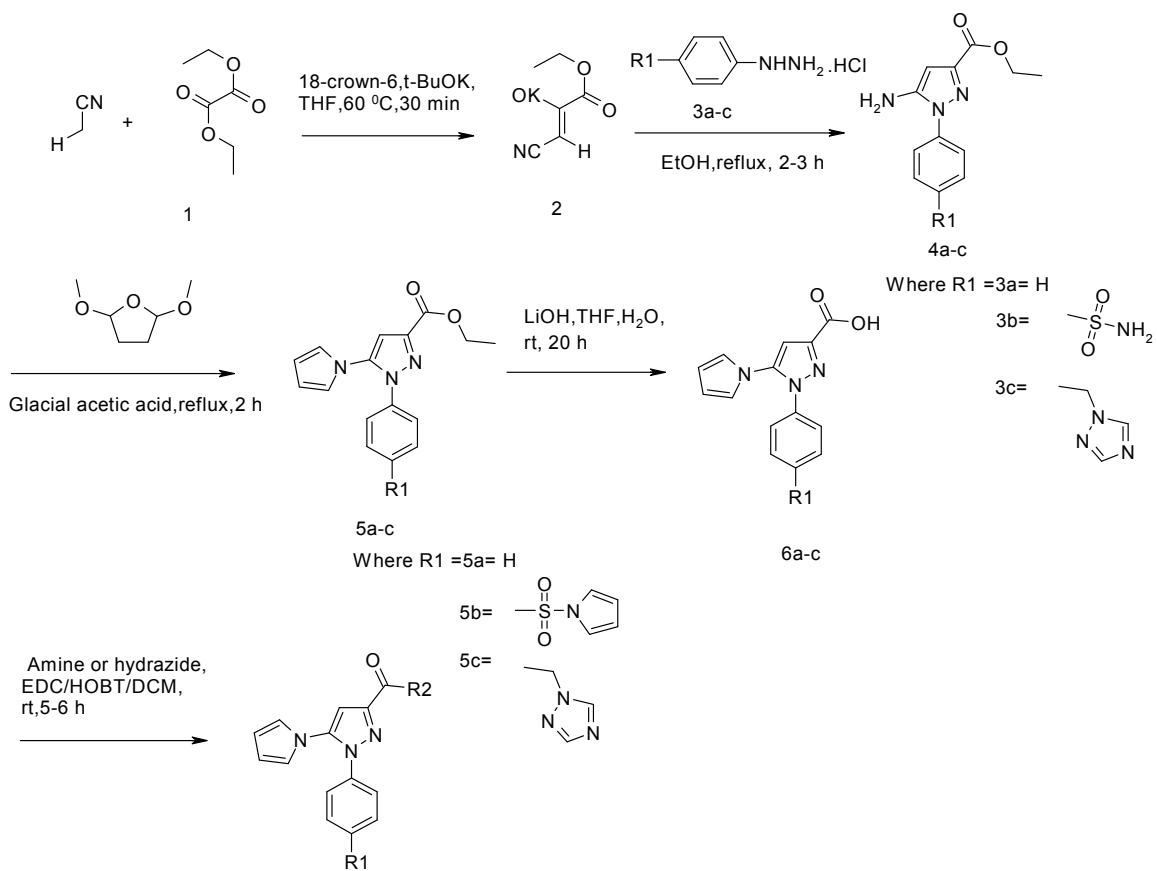
*Corresponding Author:

Elessy Abraham

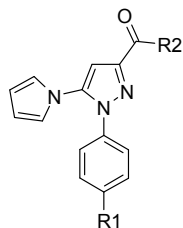
Prist University, Vallam, Thanjavur – 613403 (T.N.)

Email: rejelessy@yahoo.com

Contact no.: +91-9495204940



Scheme I: Synthesis of derivatives 7a -7f



Comp	R1	R2
7a	H	
7b	H	
7c	H	
7d	H	
7e	H	
7f	H	

N-acetyl-1-phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxhydrazide (7a)

Yield: 0.2 g (67%); **mp.:** 138-141 °C; **IR** (KBr, cm^{-1}): 3310 (NH), 3022 (Ar-CH), 1645 (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.33 (bs, 1H, NH), 8.01 (bs, 1H, NH), 7.33–7.36 (m, 3H, Ar-H), 7.11–7.16 (m, 2H, Ar-H), 7.05 (s, 1H, pyrazole-H), 6.58 (t, 2H, pyrrol-H), 6.23 (t, 2H, pyrrol-H), 2.15 (s, 3H, CH_3); **ESI-MS** (m/z): 310 [M+1]; **Molecular weight:**- 309; **Molecular Formula:**- $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_2$.

N-trifluoroacetyl-1-phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxhydrazide (7b)

Yield: 0.15 g (43%); **mp.:** 111-114 °C; **IR** (KBr, cm^{-1}): 3315 (NH), 3021 (Ar-CH), 1648 (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.41 (bs, 1H, NH), 8.05 (bs, 1H, NH), 7.42–7.48 (m, 3H, Ar-H), 7.15–7.19 (m, 2H, Ar-H), 7.10 (s, 1H, pyrazole-H), 6.61 (t, 2H, pyrrol-H), 6.25 (t, 2H, pyrrol-H); **ESI-MS** (m/z): 364 [M+1]; **Molecular weight:**- 363; **Molecular Formula:**- $\text{C}_{16}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$.

[(1-Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carbonyl)-amino]acetic acid ethyl ester (7c)

Yield: 0.18 g (55%); **mp.:** 141-144 °C; **IR** (KBr, cm^{-1}): 3325(NH), 3020 (Ar-CH), 1750 (C=O of ester), 1645 (C=O of amide); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.50 (bs, 1H, NH), 7.34–7.39 (m, 3H, Ar-H), 7.13–7.17 (m, 2H, Ar-H), 6.98 (s, 1H, pyrazole-H), 6.65 (t, 2H, pyrrol-H), 6.29 (t, 2H, pyrrol-H), 5.29 (s, 2H, CH_2), 4.24 (q, 2H, CH_2), 1.26 (t, 3H, CH_3); **ESI-MS** (m/z): 339 [M+1]; **Molecular weight:**- 338; **Molecular Formula:**- $\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_3$.

(1-Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-yl)-(4-pyrimidin-2-yl-piperazin-1-yl)-methanone (7d)

Yield: 0.22 g (56%); **mp.:** 246-249 °C; **IR** (KBr, cm^{-1}): 3012 (Ar-CH), 1648 (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.34 (d, 2H, Ar-H), 7.37–7.40 (m, 3H, Ar-H), 7.15–7.18 (m, 2H, Ar-H), 6.84 (s, 1H, pyrazole-H), 6.60 (t, 2H, pyrrol-H), 6.52 (t, 1H, Ar-H), 6.25 (t, 2H, pyrrol-H), 4.29 (m, 2H, CH_2 of piperazinyl), 3.94 (m, 2H, CH_2 of piperazinyl), 3.63 (m, 4H, CH_2 of piperazinyl); **ESI-MS** (m/z): 400 [M+1]; **Molecular weight:**- 399; **Molecular Formula:**- $\text{C}_{22}\text{H}_{21}\text{N}_7\text{O}$.

(1Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-yl)-(4-pyridin-2-yl-piperazin-1-yl)-methanone (7e)

Yield: 0.25 g (64%); **mp.:** 232-235 °C; **IR** (KBr, cm^{-1}): 3014 (Ar-CH), 1645 (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.22 (d, 1H, Ar-H), 7.49–7.53 (m, 1H, Ar-H), 7.35–7.38 (m, 3H, Ar-H), 7.14–7.16 (m, 2H, Ar-H), 6.85 (s, 1H, pyrazole-H), 6.67 (m, 2H, Ar-H), 6.69 (t, 2H, pyrrol-H), 6.28 (t, 2H, pyrrol-H), 4.29 (m, 2H, CH_2 of piperazinyl), 3.95 (m, 2H, CH_2 of piperazinyl), 3.62 (m, 4H, CH_2 of piperazinyl); **ESI-MS** (m/z): 399 [M+1]; **Molecular weight:**- 398; **Molecular Formula:**- $\text{C}_{23}\text{H}_{22}\text{N}_6\text{O}$.

N-Diethylaminoethyl-1-phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxamide (7f)

Yield: 0.21 g (62%); **mp.:** 122-125 °C; **IR** (KBr, cm^{-1}): 3320 (NH), 3011 (Ar-CH), 1647 (C=O); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , δ ppm): 8.45 (bs, 1H, NH), 7.37–7.40 (m, 3H, Ar-H), 7.15–7.18 (m, 2H, Ar-H), 6.82 (s, 1H, pyrazole-H), 6.66 (t, 2H, pyrrol-H), 6.27 (t, 2H, pyrrol-H), 3.65 (t, 2H, CH_2), 2.84 (t, 2H, CH_2), 2.81 (q, 4H, 2CH_2), 1.17 (t, 6H, 2CH_3); **ESI-MS** (m/z): 352 [M+1]; **Molecular weight:**- 351; **Molecular Formula:**- $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}$.

2.2 Biological evaluation

Diclofenac sodium was used as a reference standard at a dose 25mg/kg for anti-inflammatory and analgesic studies. The experiments were performed on Albino rats of Wister strain of either sex, weighing 180-200 g for anti-inflammatory activity and Albino mice of either sex weighing 25-30 g for analgesic activity. The animals were divided into groups (control, standard and test groups) of 6 animals each. The tested compounds and the standard drugs were administered in the form of a suspension (using 1%w/v carboxy methyl cellulose) in distilled water by oral route of administration for analgesic and anti-inflammatory studies. The animals were maintained in colony cages at $25\pm 2^\circ\text{C}$, relative humidity 45–55%, under a 12 h light–dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use.

2.2.1 Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method [11]. A freshly prepared aqueous

suspension of carrageenan (0.1% w/v in 0.9%NaCl, 0.1 ml) was injected in the sub planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs and standard drug 1 h before the carrageenan treatment. The paw volume of the all groups of rats were measured before injection of carrageenan for 0 minute and measured again after 1, 3 and 5 h after carrageenan injection with the help of digital plethysmometer. The oedema was expressed as a mean reduction in paw volume (ml) after treatment with test compounds and the percent of oedema inhibition was obtained as follows:

$$\text{Percent inhibition} = \frac{[V_t - V_o]_{\text{control}} - [V_t - V_o]_{\text{treated}}}{[V_t - V_o]_{\text{control}}} \times 100$$

Where V_t = Paw volume at time t (i.e at 1, 3 and 5h) after carrageenan injection

V_o = Paw volume before carrageenan injection (i.e at 0h).

Control= Group treated with saline

Treated= Group treated with standard drug or test compounds

The results are summarized in Table 1.

2.2.2 Analgesic activity

Analgesic activity was evaluated using acetic acid induced writhing method¹². After 50 mins of the oral administration of test compound and standard drug, each animal was injected with 1ml/100g, i.p of 0.6% v/v acetic acid solution intraperitoneally. After 5 min of acetic acid injection, the numbers of muscular contractions (writhing) in mice were counted for a period of 15 min. A significant reduction in the number of writhing by any treatment as compared to control animals was considered as a positive analgesic response. The average number of writhes in each group of treated mice was compared with that of the control. The % analgesic activity was expressed according to the formula:

$$\% \text{ Inhibition} = \left[\frac{n - n'}{n} \times 100 \right]$$

Where; n is the number of writhes in control group of mice and n' is the number of writhes in test and standard group of mice. The results are summarized in Table 2.

2.2.3 Acute Ulcerogenicity

Acute ulcerogenesis test was done according to literature method¹³. Albino rats were divided into groups consisting of 6 animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard at the dose of 80mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% CMC). Food but not water was removed 24hr before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage is examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system : 0.0 score is given to normal stomach (no injury, bleeding and latent injury), 0.5 score is to latent injury or widespread bleeding(>2mm), 1.0 score to slight injury (2-3 dotted lines), 2.0 score for severe injury (continuous lined injury or 5-6 dotted injuries), 3.0 score to very severe injury (several continuous lined injuries) and 4.0 for wide spread injury or widened injury. The mean score of each treated group minus the mean score of control was regarded as severity index of gastric mucosal damage. The observations are tabulated as Table 3.

2.3 Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds was evaluated using a one-way analysis of variance (ANOVA). In all cases, post hoc comparisons of the means of individual groups were performed using Dunnett's test. A significance level of $P < 0.05$ denoted significance in all cases. All values are expressed as mean \pm SEM. Statistical analysis was carried out using Graph Pad Prism (Graph Pad Prism 3.0 version).

3. RESULTS**3.1 Anti-inflammatory activity**

The title compounds (7a-f) were screened for their anti-inflammatory activity using carrageenan induced rat paw edema method. The observations are tabulated as Table 1.

Table 1: Results of anti-inflammatory activity of title compounds (7a-f) against carrageenan induced rat paw oedema model in rats.

Compound	Mean change in paw volume (ml) after treatment (\pm SEM)			% Inhibition		
	1h	2h	3h	1h	2h	3h
Control	0.81 \pm 0.04	1.24 \pm 0.06	1.66 \pm 0.08	-----	-----	-----
Diclofenac	0.49 \pm 0.05*	0.55 \pm 0.06*	0.59 \pm 0.02*	39.50	55.64	64.45
7a	0.59 \pm 0.07*	0.82 \pm 0.05*	1.06 \pm 0.08*	27.16	33.87	36.14
7b	0.56 \pm 0.04*	0.68 \pm 0.03*	0.83 \pm 0.17*	31.86	45.16	50.01
7c	0.53 \pm 0.04*	0.65 \pm 0.02*	0.74 \pm 0.15*	34.56	47.58	55.42
7d	0.63 \pm 0.03*	0.94 \pm 0.03*	1.25 \pm 0.11*	22.23	24.19	24.69
7e	0.64 \pm 0.06	0.92 \pm 0.04*	1.26 \pm 0.13*	20.98	25.80	24.09
7f	0.55 \pm 0.04*	0.75 \pm 0.05*	0.86 \pm 0.15*	32.09	39.51	48.19

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6), *P<0.05 significant from control.
Dose levels: Test compounds and diclofenac sodium (25 mg/kg, p. o.).

3.2 Analgesic activity

The title compounds (7a-f) were screened for their analgesic activity using acetic acid induced writhing method by using the

same procedure as mentioned above. The observations are tabulated as Table 2.

Table 2: Analgesic activity of title compounds (7a-f) against acetic acid induced writhing tests in mice.

Compounds	No. of writhes in 15min after treatment (mean \pm SEM)	% Inhibition
Control	22.6 \pm 1.25	-----
Diclofenac sodium	8.7 \pm 0.88*	61.23
7a	13.5 \pm 0.99*	40.15
7b	12.5 \pm 1.17*	44.05
7c	13.0 \pm 1.07*	42.05
7d	15.53 \pm 1.23*	31.29
7e	16.0 \pm 1.18*	29.17
7f	13.1 \pm 0.88*	41.93

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6),
*P<0.05 significant from control. Dose levels: Test compounds and diclofenac sodium (25 mg/kg, p. o.).

3.3 Acute Ulcerogenicity Activity

The most active anti-inflammatory compound 7c was evaluated for ulcerogenicity and the data is presented in Table 3.

Table 3: Evaluation of ulcerogenicity of compound 7c

Compound	Dose mg/kg, p.o.	Ulcer score	Ulcer index
Control	CMC 1% w/v	0.16 \pm 0.10	---
Diclofenac	80	2.20 \pm 0.21*	2.04
7c	80	0.95 \pm 0.08* ^a	0.79

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6),
* P < 0.05 significant from control, ^aP < 0.05 significant from diclofenac sodium

4. DISCUSSION AND CONCLUSION

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats. The activity of the newly synthesized compounds was measured before and at 1 h, 2 h and 3 h after carrageenan injection. The anti-inflammatory activity data (Table 1) indicated that all the test compounds protected rats from carrageenan-induced inflammation reached to peak level at 3 h. All the test compounds showed a reasonable inhibition of edema size ranging 24.09% to 55.42% whereas diclofenac sodium displayed anti-inflammatory activity 64.45% after 3h carrageenan injection. A comparative study of the anti-inflammatory activity of test compounds relative to the reference drug at different time intervals indicated the following: after 1 h compound 7c was nearly as effective in inhibiting the paw edema with percentage activity 34.56% when compared with that of diclofenac sodium (39.50%). Taking the anti-inflammatory activity after 3 h time interval as a criterion for comparison, it was observed that compounds 7c showed potent anti-inflammatory activity 55.42% when compared with diclofenac sodium (64.45%). Compounds 7b displayed a good anti-inflammatory activity (50.01%). From the results of acetic acid induced writhing test as given in Table 2, which showed that all compounds exhibited analgesic activity in the range of 29.17% to 44.05%. It was noticed that the compounds 7a, 7b, 7c and 7f showed 40.15%, 44.05%, 42.05% and 41.93% reduction of writhing respectively, where as standard showed 61.23%.

The most active anti-inflammatory compound 7c was subjected to ulcerogenicity activity and was found to be less ulcerogenic than the standard diclofenac.

The structure activity relationship study showed that phenyl at first position and glycine ethylester group (7c) at three position of pyrazole exhibited good anti-inflammatory activity, whereas phenyl group at first position with trifluorocarboxyhydrazide group at three position of pyrazole (7b) and N-diethylaminoethylamine group at three position of pyrazole (7f) exhibited moderate anti-inflammatory activity.

REFERENCES

- Parsit P, Reindeau D, "Selective Cyclooxygenase -2 Inhibitors" Annual Reports in Medicinal Chemistry, 1997, 32: 211-220.
- Almansa C, Alfon J, et al, "Synthesis and Structure-Activity Relationship of a New Series of COX-2 Selective Inhibitors: 1,5-Diarylimidazoles" Journal Medicinal Chemistry, 2003, 46: 3463-3475.
- Jackson LM, Hawkey CJ, "Gastrointestinal effects of COX-2 inhibitors" Expert Opinion on Investigational Drugs, 1999, 8: 963-71.
- Allison MC, Howatson AG, et al, "Gastrointestinal damage associated with the use of non steroidal anti-inflammatory drugs" New England Journal of Medicine, 1992, 327: 749-754.
- Dongre JM, Pratico D, "Adverse cardiovascular effects of coxibs, Journal of Medicinal Chemistry" 2005, 48(7): 2251-2257.
- Shasikant VB, Kailash GB, et al, "Design, synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenic studies of Novel S- substituted phenacyl- 1,3,4 oxadiazole-2- thiol and Schiff bases of Diclofenac acid as non ulcerogenic derivatives" Bioorg Medicinal Chemistry, 2008, 16(4): 1822-1831.
- Manjunatha K, Boja P, et al, "Synthesis and biological evaluation of some 1,3,4-oxadiazole derivatives" European Journal of Medicinal Chemistry, 2010, 45: 5225-5233.
- Giri RS, Thaker HM, et al, "Design, synthesis and characterization of novel 2-(2,4-disubstituted- thiazole- 5-yl)-3-aryl-3H-quinazoline-4-one derivatives as inhibitors of NF-kB and AP-1 mediated transcription activation and as potential anti-inflammatory agents" European Journal of Medicinal Chemistry, 2009, 44(5): 2184-2189.
- Akhter M, Husain A, et al, "Aroyl propionic acid based 2,5, disubstituted 1,3,4-oxadiazoles: Synthesis and their anti-

- inflammatory and analgesic activities" *European Journal of Medicinal Chemistry*, 2009, 44(6): 2372-2378.
10. Diane HB, David T, "1,3,4-Oxadiazole, 1,3,4-Thiadiazole and 1,2,4-Triazole Analogues of the fenamates: In vitro inhibition of cyclooxygenase and 5-lipoxygenase activities. *Journal of Medicinal Chemistry*, 1993, 36(13): 1802-1810.
 11. Winter CA, Risley EA, et al, "Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs" *Proceedings of Society for Experimental Biology and medicine*, 1962, 111: 544-547.
 12. Koster R, Anderson M, et al, "Acetic acid for analgesic screening" *Federation Proceedings*, 1959, 18: 412-418.
 13. Cioli V, Putzolu S, et al, "The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats" *Toxicology and Applied Pharmacology*, 1979, 50(2): 283-289..