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Synthesis and Biological Evaluation of pyrazole derivatives containing ethanone skeleton as Anti-inflammatory agents

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ABSTRACT

Current research had focused on the synthesis of the novel pyrazole ethanone linked compounds with improved biological activity. In this synthetic process 1st step was to synthesize the intermediate chalcone and 2nd step was the synthesis of final compound pyrazole derivative containing ethanone moiety. This procedure is the type of cyclization reaction using proton transfer mechanism. By using this method 8 derivatives synthesized. After synthesis these were subjected to identification tests by using various methods like melting point study, thin layer chromatography, solubility study and characterization by using UV, IR and NMR Spectroscopy.

Keywords: Selective COX-2 inhibitor, anti-inflammatory, pyrazole ethanone linked compounds, pyrazole derivatives, inflammation

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1. INTRODUCTION

Inflammation is the part of natural defense system of the body. It is a process where the cells & natural chemicals of the body protect us from physical damage & infection from foreign substances such as bacteria & viruses. WBCs or leukocytes are the major infection fighting cells of the body. The principal objective of inflammation is to isolate, localized & eradicate foreign substances & repair damaged tissues. In recent years pyrazole derivatives have been a topic of interest among the researchers because of their pharmacological properties like anti-inflammatory, ulcerogenic, antibacterial, diuretic, analgesic, antiviral, antifungal, anti-mycobacterial activity etc[1]. (*Rainsford et al., 2001)*.

The inflammatory process includes a series of events that can be initiated by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interaction and thermal or other physical injuries). Almost two decades ago, steroids namely prednisolone, dexamethasone, betamethasone, etc. were considered to be the choicest anti-inflammatory drugs. Owing to several adverse effects caused by either short-term or long-term steroid therapy, these have been more or less replaced by much safer and better-tolerated non-steroidal anti-inflammatory drugs (NSAID)*[2] (Sujatha et al., 2009).*

Cycloxiginase (COX) enzyme exists in two isomeric forms, namely COX-1 and COX-2, while the existence of a third isoforom (COX-3) is still into debate. However, recent studies have shown that COX-2 inhibitors are associated with increased thromboembolic phenomena in specific patient populations such as cardiovascular disease patients challenging the benefits of selective COX-2 inhibition. COX-1 and COX -2 are very similar in structure and are almost identical in length varying from 599 (human) to 602 (mice) amino acid in COX -1 and 603 (mice) to 604 (human) for COX-2. COX-2 contains an 18 amino acid insert near the C-terminal end of the enzyme that is not present in COX-1, but all other residues that have been previously identified as being essential to the catalytic activity of COX-1 are present in COX-2.

A major difference between COX-1 and COX-2 is that COX-2 lacks a sequence of 17 amino acid from the N-terminus but contains a sequence of 18 amino acid at the C-terminus compared to COX-1. From a therapeutic viewpoint, the major difference between COX-1 and COX-2 lies in physiological function rather than structure. The main mediators of

inflammation are derivatives of arachidonic acid (AA) which is an a unsaturated fatty acid containing 20 carbon chain, produced from membrane phospholipids.

The principal pathways of Arachidonic acid metabolism are:

- The 5-Lipoxygenase pathway, which produces a collection of leukotrienes (LT)
- The Cyclooxygenase (COX) pathway, which produces prostaglandin H_2 (PGH_2).

PGH_2 is the substrate for two enzymatic pathways, one leading to the production of several Prostaglandins (PG); the other leading to the production of thromboxanes (Tx).[3] (*Dhondge R. et.al, 2009)*

The Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used medications in the world (Zarghi et al., 2009a). Their anti-inflammatory activity is due to inhibition of cyclooxygenases (COXs), which catalyze the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs) *(Zarghi et al., 2009b)*. Prostaglandins such as PGE2 are produced in the cyclooxygenase pathway of the arachidonic acid cascade by the action of the isoenzymes COX-1 and COX-2 *(Schuhly et al., 2009)* [4].

Prostaglandins are among the most important mediators of inflammation. They promote blood vessel dilation and vascular permeability, causing the typical redness, heat and swelling phenomena involved in inflammation. Moreover, they promote pain transmission from nociceptors to the brain by increasing the sensitivity of the nerve endings. However, prostaglandins also play a cytoprotective role in the gastrointestinal tract and they are necessary for normal platelet aggregation and renal function *(Girgis et al., 2009)*[5].

The success of NSAIDs is in the treatment of various inflammatory disorders such as inhibition of COX enzyme as a target of anti-inflammatory therapies.

However, the GI toxicities associated with the use of NSAIDs proved to be a major problem during long term therapy (Zebardast et al., 2009)[6]. Side effects such as gastrointestinal pain have been associated with NSAID use due to the inhibition of COX-1 *(Kouatly et al., 2009)*[7].

The identification of COX-2 and the subsequent introduction of the COX-2 selective inhibitor NSAIDs were thought to be a major breakthrough, with the expectation of a significant reduction in GI side effects *(Sondhi et al., 2008)*[8]. The differential tissue distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) provides a rationale for the development of selective COX-2 inhibitors as anti-inflammatory-analgesic agents that lack the GI side effects exhibited by traditional NSAIDs *(Navidpour et al., 2007)*[9].

In recent years active research has been initiated on halogen containing heterocycles, particularly fluorine and chlorine. 2, 4-Dichloro-5-fluoroacetophen is used in the synthesis of drugs like Ciprofloxacin and their analogs. Incorporation of fluorine or CF_3 group into an organic molecule largely enhances the pharmacological properties and also could lead to increased lipid solubility, enhancing the rates of absorption and transport of drugs in vivo. The replacement of hydrogen or hydroxyl group by fluorine. The preliminary study towards new potential COX-2 selective compounds as novel drug candidates for inflammatory and related diseases, new introduced systematic modifications to the 4,5diphenyloxazolone core structure. It is well established that 3,4diaryloxazolones having a sulfone or sulfonamide on the 4-phenyl is a good template for selective COX-2 inhibition. Recently evaluating ester and amide derivatives of indomethacin and meclofenamic acid resulted in highly potent and selective compounds and it was suggested that derivatization of the carboxylate moiety in moderately selective COX-1 inhibitors can be used as a novel strategy for generation of potent and selective COX2 inhibitors.

COX-2 selective inhibitors play a role in reducing the incidence of GI side effects among NSAIDs are the reports of other potential therapeutic uses for this new class of drugs including potential use in the treatment of Alzheimer's disease and various carcinomas.

Epidemiological studies suggest a significant reduction in the risk for colon cancer in patients regularly taking aspirin. NSAIDs have also been reported to reduce the growth rate of polyps in the colon in human as well as the incidence of tumors of the colon in animals. The effectiveness of NSAIDs in the prevention and treatment of other cancers such as prostate cancer and mammary carcinoma has also been reported.

Pyrazole represents one of the most active classes of compounds possessing wide spectrum of biological activities. (*Karthikeyan MS et.al, 2009)*[10]

Current research has focused on developing safer NSAIDs-selective COX-2 inhibitors

(Lv *et al.*, 2010)[11] by synthesis of new compound that is absolutely required for the inhibition or antagonism of the abnormalities due to inflammatory response

1.2 Future prospects

COX-2 selective inhibitors play a role in reducing the incidence of GI side effects among NSAIDs are the reports of other potential therapeutic uses for this new class of drugs including potential use in the treatment of Alzheimer's disease and various carcinomas.

Epidemiological studies suggest a significant reduction in the risk for colon cancer in patients regularly taking aspirin. NSAIDs have also been reported to reduce the growth rate of polyps in the colon in human as well as the incidence of tumors of the colon in animals. The effectiveness of NSAIDs in the prevention and treatment of other cancers such as prostate cancer and mammary carcinoma has also been reported.

Pyrazole represents one of the most active classes of compounds possessing wide spectrum of biological activities. Many of the therapeutically useful compounds such as Phenylbutazone, Oxyphenbutazone and Antipyrine, belong to Pyrazoles.

2. MATERIALS AND METHODS

2.1 Synthesis Of Compounds:

Step 1 General Synthetic Procedure for Chalcones:

Equimolar quantities (10mmol, 1equiv) of the aromatic aldehydes and substituted acetophenones were dissolved in approximately 15 ml of ethanol. The mixture was allowed to stir for several minutes at 5-10°C. A 10 ml portion of a 40% aqueous potassium hydroxide solution was then slowly added drop wise to the reaction flask. The reaction solution was allowed to stir at room temperature for approximately 4 hours. Most commonly, a precipitate formed was then collected by suction filtration. (*Figure 1*)

Figures:

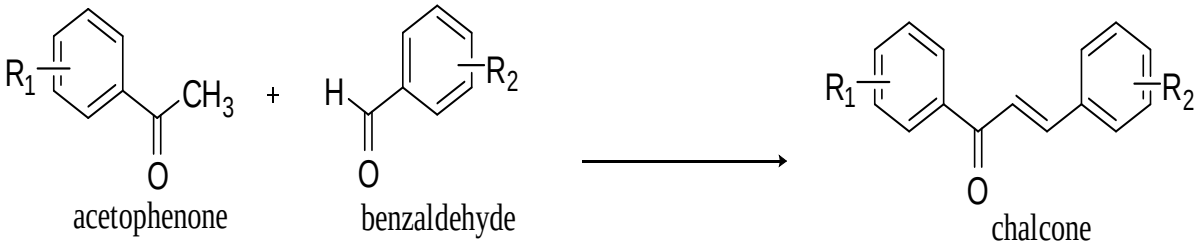


Figure 1: Step 1 General Synthetic Procedure for Chalcones

Step 2 Synthesis of pyrazole Derivative

A solution of chalcone (5mmol) in 30 ml of acetic acid was added drop wise to 0.6ml of hydrazine Hydrate (12.5mmol) and kept under stirring at 120^{0}C for 24hrs. The mixture was then poured into ice-water, obtaining the crude pyrazole Derivatives which were recrystallised twice from ethanol. (*Figure 2*)

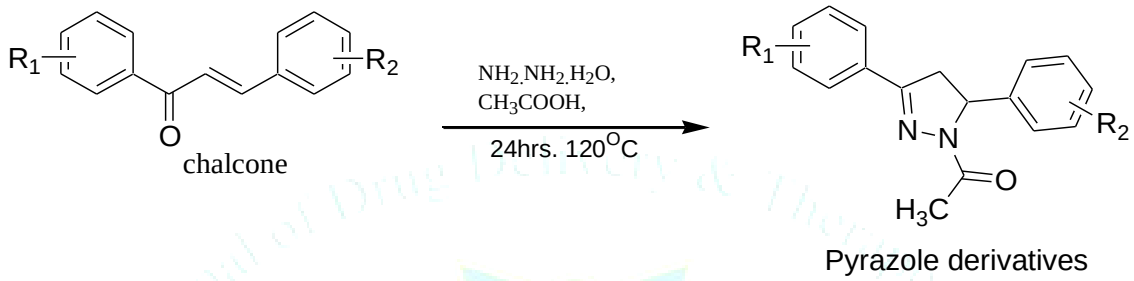


Figure 2: Step 2 Synthesis of pyrazole Derivative

2.2 Synthesis Of Pyrazole Derivatives:

2.2.1 1-[3-(3, 4-bromophenyl)-5-(4-methoxyphenyl)-4, 5-dihydro-1h-pyrazole-1-yl] ethanone [KM-1]:

Anisaldehyde (1.2ml, 10mmol) and 4-bromoacetophenone (1.94g, 10mmol) were dissolved in approximately 15 ml of ethanol. The mixture was allowed to stir for several minutes at 5-10^{0}C. A 10 ml portion of a 40% aqueous potassium hydroxide solution was then slowly added drop wise to the reaction flask. The reaction solution was allowed to stir at room temperature for approximately 4 hrs. Then obtained precipitate (Chalcone) was collected by suction filtration.

A solution of Chalcone (5mmol) in 30 ml of acetic acid was added drop wise to 0.6 ml of hydrazine hydrate (12.5mmol) and kept under stirring at 120°C for 24 hrs. The mixture was then poured into ice-water, obtaining the crude pyrazole derivatives which were recrystallized twice from ethanol.

2.2.2 1-[3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1h-pyrazole-1-yl] ethanone[KM-2]

4-Chlorobenzaldehyde (1.40g, 10mmol) and 4-bromoacetophenone (1.99g, 1-mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound KM-1.

2.2.3 1-[3-(3,4-dimethoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1h-pyrazole-1-yl] ethanone [KM-3]

4-Chlorobenzaldehyde (1.40g, 10mmol) and 3,4-dimethoxyacetophenone (1.80g, 10mmol) were dissolved in approximately 15 ml of ethanol the remaining procedure was same as used for the compound KM-1.

2.2.4 1-[3-(4-ethylphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1h-pyrazole-1-yl]ethanone [KM-4]

4-Chlorobenzaldehyde (1.40g, 10mmol) and 4-ethylacetophenone (1.34g, 10mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound **KM-1.**

2.2.5 1-[3-(3,4-dimethoxyphenyl)-5-(4-fluororophenyl)-4,5-dihydro-1h-pyrazole-1-yl] ethanone [KM-5]:

4-fluorobenzaldehyde (1.24g, 10mmol) and 3,4-dimethoxyacetophenone (1.80g, 10mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound **KM-1**.

2.2.6 1-[3-(2-bromo-4-methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1h-pyrazole-1-yl]ethanone [KM-6]:

4-Chlorobenzaldehyde (1.40g, 10mmol) and 2-bromo-4-methoxyacetophenone (2.29g, 10mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound KM-1.

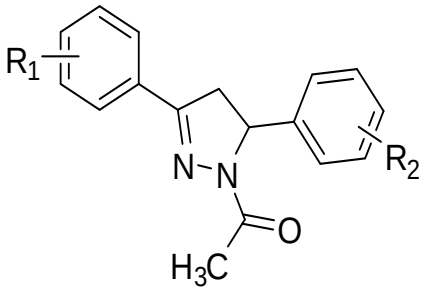
2.2.7 1-[3-(4-bromophenyl)-5-(4-fluororophenyl)-4,5-dihydro-1h-pyrazole-1-yl] ethanone [KM-7]:

4-fluorobenzaldehyde (1.24g, 10mmol) and 4-bromoacetophenone (1.99g, 10mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound KM-1.

2.2.8 1-[3-(4-ethylphenyl)-5-(4-fluororophenyl)-4,5-dihydro-1h-pyrazole-1-yl] ethanone [KM-8]:

4-fluorobenzaldehyde (1.24g, 10mmol) and 4-ethylacetophenone (1.34g, 10mmol) were dissolved in approximately 15 ml of ethanol and the remaining procedure was same as used for the compound KM-1.

List of synthesized compounds given in table 1



S. no.	Compound code	R_1	R_2
1	KM-1	4-Br	4-OCH_3
2	KM-2	4-Br	4-Cl
3	KM-3	3,4-OCH_3	4-Cl
4	KM-4	4-C_2H_5	4-Cl
5	KM-5	3,4-OCH_3	4-F
6	KM-6	2-Br, 4-OCH_3	4-Cl
7	KM-7	4-Br	4-F
8	KM-8	4- C_2H_5	4-F

2.3 Physiochemical characterization:

The following tests were performed to check the quality of the synthesized compounds. Physicochemical characterization of synthesized compounds was done by using following various parameters.

Melting point of synthesized compounds was determined in open capillary tube by VEEGO apparatus. Practically obtained melting point indicates the purity of synthesized compounds. The yield values and melting points of synthesized compounds are reported in table 2

The purity of synthesized compounds was verified by thin layar chromatography using silica gel-G as adsorbent and Benzene: Petroleum ether: Chloroform (6:2:2) and spots were observed when exposed to iodine vapors under iodine chamber. The R_f of synthesized compounds are reported in table 2

Table 2: Physical constant data of synthesized compounds:

S. No.	Compound	% Yield	Melting Point (°C)	R_f Value
1	KM-1	75.12%	105-110°C	0.45
2	KM-2	55.24%	115°C	0.60
3	KM-3	59.09%	110-115°C	0.47
4	KM-4	50.15%	80-85°C	0.53
5	KM-5	70.13%	115-120°C	0.24
6	KM-6	46.74%	100-105°C	0.58
7	KM-7	65.80%	110-115°C	0.61
8	KM-8	50.65%	110°C	0.50

2.3.1 Solubility study:

Each of the synthesized compounds (2 mg) was transferred in 5 ml of different pure solvent and results are reported in table 3

After completion of the synthesis, physicochemical characterizations of synthesized compounds have been performed. Practically obtained melting points were sharp which indicates the purity of the synthesized compounds. The result of solubility study shows that all the synthesized compounds gives maximum solubility in ethanol, chloroform, benzene etc. which confirm that the synthesized compounds are semi polar in nature.

Table 3: Solubility studies of the synthesized compounds in different solvents

S. No.	Compound	Water	Alcohol	Acetone	Chloroform	Benzene	Hexane
1	KM-1	--	++	+++	++	++	+
2	KM-2	--	++	+++	++	++	+
3	KM-3	--	++	+++	++	++	+
4	KM-4	--	++	+++	++	++	+
5	KM-5	--	++	+++	++	++	+
6	KM-6	--	++	+++	++	++	+
7	KM-7	--	++	+++	++	++	+
8	KM-8	--	++	+++	++	++	+

+++ Freely soluble + sparingly soluble ++ Soluble - - practically insoluble

3 RESULTS AND DISCUSSION

3.1 Spectroscopy:

The structural determination of the compounds had been done by using UV, IR and NMR spectroscopic methods. For determination of λ_{max} of synthesized compounds, ethanol was used as solvent. For UV- spectroscopy, UV SHIMADZU 1700s Spectrophotometer was used from the sophisticated instrument lab of the institution. The obtained UV spectra of the compounds KM-1 to KM-8 and their λ_{max} values were given in the table 4

The IR spectra of synthesized compounds were obtained from SAIF, Punjab University, using KBR pellets. IR spectra of synthesized compounds were shown characteristic absorption for the functional groups present in compounds. The Interpretation of IR spectra of synthesized compounds given in the table 4. The NMR spectra (Nuclear Magnetic Resonance ^{1}H-NMR Spectra) of synthesized compounds were obtained from Sophisticated Analytical Instrumentation Facility (SAIF), central instrument laboratory, Punjab University, Chandigarh on a Burker Avance II- 400 MHz NMR spectrometer, Deuteriated Chloroform ($CDCl_3$) as a solvent and TMS (Tetra Methyl Silane) as internal standard. NMR spectra of synthesized compounds were shown splitting patterns which is described as singlet (s), doublet (d), and multiplet (m). NMR values are given in chemical shift in ppm. Interpretation of NMR spectra of the synthesized

The NMR was also performed at Punjab University, Chandigarh and recorded spectrums were interpreted. Spectroscopic data to support the laboratory synthesis of compounds KM-1 to KM-8 given in table 4

Table 4: λ_{max} (nm) and NMR data of synthesized compounds

S No.	Compounds	UV analysis λ_{max}	IR interpretation	NMR Interpretation
1.	KM-1	295.0	1658- C=O stretch (s), 3020- C-H stretch (m), 1418- C-C stretch (aromatic), 548- C-Br stretch (m), 1252- C-N stretch (m), 1514- C-H bend (m)	**^{1}H NMR ($CDCl_3$): δ values** 2.36-2.39 (s, 3H, CH_3-CO), 3.14 (m, 3H, O-CH_3), 3.66-3.84 (m, 1H, CH-Br), 5.53-5.57 (m, 2H, CH_2), 6.82 -7.61 (m, 8H, Ar)
2.	KM-2	294.5	1658-C=O stretch (s), 3060-C-H stretch (m), 1436-C-C stretch(aromatic), 543-C-Br stretch (m), 1323-C-N stretch (m), 823-C-Cl stretch (m), 1483-C-H bend	**^{1}H NMR ($CDCl_3$): δ values** 2.40(s, 3H, CH_3-CO), 3.06- 3.10 (d, 1H, CH-Br), 3.69-3.75 (m, 1H, CH-Cl), 5.54 (s, 2H, CH_2), 7.15-7.58 (m, 8H, Ar)
3.	KM-3	312.5	1649- C=O stretch (s), 2958- C-H stretch (m), 1467- C-C stretch (aromatic), 1323- C-N stretch (m), 823- C-Cl stretch (m), 1517- C-H bend	**^{1}H NMR ($CDCl_3$): δ values** 2.42 (s, 3H, CH_3-CO), 3.07-3.77 (m, 6H, O-CH_3), 3.92-3.96 (m, 1H, CH-Cl), 5.52-5.56 (m, 2H, CH_2), 6.86-7.43 (m, 8H, Ar)
4.	KM-4	358.0	1659- C=O stretch (s), 2960- C-H stretch (m), 1595- C-C stretch (aromatic), 1247- C-N stretch (m), 836- C-Cl stretch (m), 1445- C-H bend (m)	**^{1}H NMR ($CDCl_3$): δ values** 1.25 (m, 3H, CH_3), 2.66-2.71 (m, 2H, Ar-CH_2), 2.41(s, 3H, CH_3-CO), 3.69-3.77 (m, 1H, CH-Cl), 5.51-5.56 (m, 2H, CH_2), 7.15-7.66 (m, 8H, Ar)
5.	KM-5	313.5	1664- C=O stretch (s), 3002- C-H stretch (m), 1516- C-C stretch(aromatic), 1246- C-N stretch (m), 1401- C-F stretch, (m), 1445- C-H bend	**^{1}H NMR ($CDCl_3$): δ values** 2.42 (s, 3H, CH_3-CO), 3.08-3.76 (6H or CH_3-O) (m), 3.92-3.96 (d, 1H, CH-F), 5.54-5.58 (m, 2H, CH_2), 6.8-7.4 (m, 8H, Ar)
6.	KM-6	264.5	1664- C=O stretch (s), 2960- C-H stretch (m), 1504- C-C stretch(aromatic), 1252- C-N stretch (m), 833- C-Cl stretch (m), 1445- C-H bend, 530- C-Br stretch (m)	**^{1}H NMR ($CDCl_3$): δ values** 2.1-2.58 (m, 1H, CH-Br), 2.69-3.73 (m, 3H or CH_3-O), 3.82 (s, 1H, CH-Cl) (s) , 5.52 (s, 2H, CH_2), 6.41-8.09 (m, 8H, Ar)
7.	KM-7	293.0	1661- C=O stretch (s), 2960- C-H stretch (m), 1588- C-C stretch(aromatic), 1218- C-N stretch (m), 549- C-Br stretch (m), 1445- C-H bend, 1394- C-F stretch (m)	**^{1}H NMR ($CDCl_3$): δ values** 2.40(CH_3-C-O) (s), 3.07-3.12 (CH-Br) (m), 3.68-3-3.76 (CH-F) (m), 5.55-5.59 (methene) (m), 6.97-7.61 (Ar-8H) (m)
8.	KM-8	290.0	1661- C=O stretch (s), 2927- C-H stretch (m), 1507- C-C stretch(aromatic), 1218- C-N stretch (m), 1394- C-F stretch (m), 1427- C-H bend	**^{1}H NMR ($CDCl_3$): δ values** 2.41(CH_3-C-O) (s), 6.96-7.67 (Ar-8H), 2.58-2.71(m, 2H, Ar-CH_2), 1.20-1.28 (m, 3H, CH_3), 5.53-5.57 (m, 2H, -CH_2), 6.96-7.87 (m, 8H, Ar)

4. PHARMACOLOGICAL SCREENING

The anti-Inflammatory activity of newly synthesized pyrazole derivatives was carried out using Formaldehyde induced rat hind paw edema method. In this method, rat hind paw edema produced by sub planter injection of 2% formaldehyde, and paw volume measured by vernier calipers.

4.1 Method:

Inhibition of formaldehyde induced inflammation in rat paw

Animals used: Swiss Albino Rats

No. of groups: 10 groups

No. of animals used: 6 animals in each group

Dose of compound: 50mg/kg

Dose of standard drug: 10mg/kg (Indomethacin)

Route of administration: Oral (Suspended in 2% Acacia solution)

Mice were assigned into 10 groups of 6 animals each. They were marked for individual animal identification. The animals were deprived of food overnight (allowed free access to water ad libitium) and synthetic compounds were administered once before 30 minutes the injection of 2% formaldehyde. Dose volume not exceeding 0.5ml/100gm orally was administered. After 30 minutes of test compound administration, 0.1ml of 2%w/v of formaldehyde in normal saline was injected in to the sub-planter region of the left hind paw of mice. Immediately after the formaldehyde injection, the volume of its displacement was measured using Vernier Callipers. The reading was recorded at 0, ½ & 3 hours. The % inhibition of edema was calculated at the end of 3 hrs by using the following formula (Winter et al., 1962).

%inhibition = $(1-V_t/V_c)*100$

Vt and Vc is a edema volume in the mice treated with the test drug and control respectively

4.2 Statistical analysis:

The results are presented as Mean ± SEM (Standard Error Mean) of Six Rats. Statistical analysis were performed using one way analysis of variance (ANOVA) followed by Dunnett's t-Test for multiple comparisons, Using Graph Pad Software. P Values <0.05-0.01 were Considered to be significant, *given in table 5 figure 3*

Table 5: Anti-inflammatory activity (formaldehyde induced rat hind paw edema method)

Groups	Dose (mg/kg)	Mean paw volume(mm) ± SEM and % Inhibition (P.I.)		% Inhibition $(1-V_t/V_c)*100$
		Time after formaldehyde injection		
		Before Formaldehyde (V_0)	3hrs. (V_t)	
I (Control)	----	5.11±0.39	6.85±0.35	----
II (Std.)	10	5.265±0.085	5.40±0.10	88.500
III (KM-1)	50	5.00±0.60	6.395±0.845	19.820
IV (KM-2)	50	5.195±0.675	6.35±0.65	33.620
V (KM-3)	50	5.05±0.05	5.75±0.05	59.770
VI (KM-4)	50	4.75±0.35	6.125±0.125	20.977
VII (KM-5)	50	5.23±0.31	5.5±0.30	84.850
VIII (KM-6)	50	5±0.70	5.75±0.55	56.890
IX (KM-7)	50	5.505±0.205	6.12±0.62	64.655
X (KM-8)	50	5.65±0.25	6.10±0.25	74.130

(n= 6 albino rats per groups; values are represent mean ± SEM; probability value with respect to control group *P<0.01 considered to be significant)

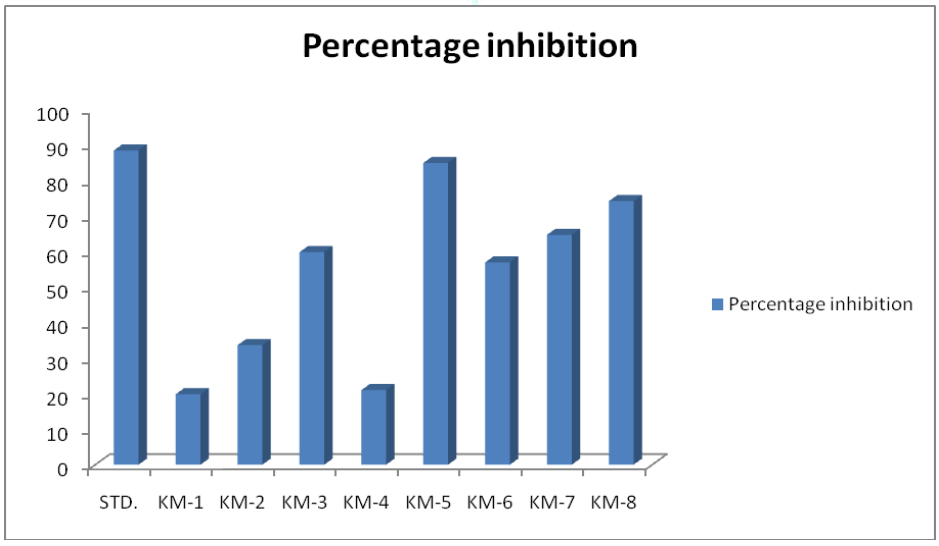


Figure 3: Graph representing % inhibitions of the compounds

Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin. Indomethacin gave more or less uniform inhibition of edema in early intermediate and later phases. Compound KM-5 showed less significant inhibition of formaldehyde induced edema in early phases while significant inhibition at later phases. Out of other remaining compounds Compound KM-5 was more significant than the other compound in percentage inhibition of paw edema, as some of the above compounds significantly inhibited this model of inflammation.

5. CONCLUSION

The initial postulate that a selective COX-2 inhibitor would reduce inflammation without causing gastric irritation was validated following the introduction of selective COX-2 inhibitors such as celecoxib and rofecoxib. However, it was subsequently observed that selective COX-2 inhibitors may alter the balance in the Cyclooxygenase pathway resulting in a decrease in the level of the vasodilatory and anti-aggregatory prostacyclin (PGI2), relative to an increase in the level of the prothrombotic thromboxane A2 (TxA2), leading to increased incidences of an adverse cardiovascular thrombotic event. COX-2 selective inhibitors play a role in reducing the incidence of GI side effects among NSAIDs are the reports of other potential therapeutic uses for this new class of drugs including potential use in the treatment of Alzheimer's disease and various carcinomas.

Pyrazole represents one of the most active classes of compounds possessing wide spectrum of biological activities. Many of the therapeutically useful compounds such as phenylbutazone, Oxyphenbutazone and antipyrine, belong to pyrazoles.

My current research work had focused on the synthesis of the novel pyrazole ethanone linked compounds with improved biological activity. The 2 step synthesis of pyrazole derivatives was done into the pharmaceutical laboratory facility provided by the institute under the guidance of my supervisor. In this synthetic process 1st step was to synthesize the intermediate chalcone and 2nd step was the synthesis of final compound pyrazole derivative containing ethanone moiety. This procedure is the type of cyclization reaction using proton transfer mechanism. By using this method I synthesized 8 derivatives as given below:

1. 1-[3-(3,4-Bromophenyl)-5-(4-Methoxyphenyl)-4,5-dihydro-1H-Pyrazole-1-yl]Ethanone **[KM-1]**
2. 1-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazole-1-yl] Ethanone**[KM-2]**
3. 1-[3-(3,4-Dimethoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazole-1-yl] Ethanone **[KM-3]**
4. 1-[3-(4-Ethylphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazole-1-yl]Ethanone**[KM-4]**
5. 1-[3-(3,4-Dimethoxyphenyl)-5-(4-fluororophenyl)-4,5-dihydro-1H-Pyrazole-1-yl] Ethanone **[KM-5]**
6. 1-[3-(2-Bromo-4-methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazole-1-yl Ethanone **[KM-6]**
7. 1-[3-(4-Bromophenyl)-5-(4-Fluororophenyl)-4,5-dihydro-1H-Pyrazole-1-yl]Ethanone **[KM-7]**
8. 1-[3-(4-Ethylphenyl)-5-(4-Fluororophenyl)-4,5-dihydro-1H-Pyrazole-1-yl]Ethanone **[KM-8]**

All these compounds were synthesized and after synthesis these were subjected to identification tests by using various methods like melting point study, thin layer chromatography, solubility study and characterization by using UV, IR and NMR Spectroscopy. All the data of compounds confirms the synthesis of the above mentioned derivatives. Like melting point of all the pyrazole derivatives ranges between 80-120**°**C, the thin layer chromatography by using silica gel-G as adsorbent and benzene: petroleum ether: chloroform (6:2:2) as a solvent system, solubility data represents that the synthesized compounds were semi polar in nature like it is insoluble in water soluble in ethanol, acetone, chloroform and sparingly soluble in hexane.

The UV spectroscopic data represents the λ_{max} (nm) values of the compounds. IR spectroscopy helps to identify the chemical structure of the compounds, like in the given data all the compounds show the peak values of the representing group which is present in the compounds so here all these pyrazole derivatives show C=O stretch, C-H stretch, C-C stretch (aromatic), C-Br stretch, C-N stretch, C-H bend, C-Cl stretch, C-F stretch peaks that confirms the structure of the pyrazole derivatives.

NMR spectroscopic data also helps to confirm the presence of hydrogen bonds in the compound so here the NMR spectroscopy was done by using $CDCl_3$ as the solvent and tetra methyl silane as the internal standard and the chemical shift values (ppm) gave the much more conformation about the structural determination of the compound that it contains pyrazole ring with ethanone skeleton and 2 neighboring substituted benzene rings given in figure.

After structural determination the in-vivo testing of the synthesized compounds also had been done by using Formaldehyde-induced rat hind paw edema method in which swiss albino rats were used for animal model, 2% formaldehyde solution (for induction of inflammation in animals), indomethacin (NSAID) in a dose of 10mg/kg body weight, and for test compounds 50mg/kg body weight, method includes 10 groups on which this study was taken place as control, standard, and 8 test compound groups. % inhibition values were represented as mean ± SEM.

All the above given data summarizes that among all the synthesized compounds KM-5 gave 84% inhibition of inflammation, while the standard drug (indomethacin) shows 88.5% inhibition of the inflammation. Thus it can be concluded that the compound KM-5 is active and belongs to the class of COX-2 inhibitory anti-inflammatory agents. *(Figures: 4,5,6)*

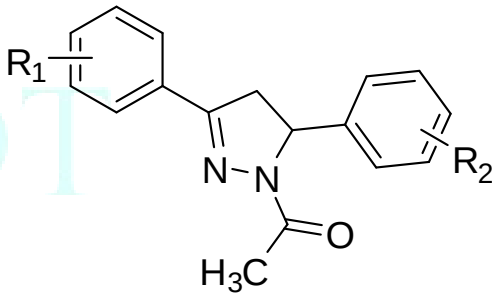


Figure 4: representing the synthesized basic synthesized pyrazole nucleus

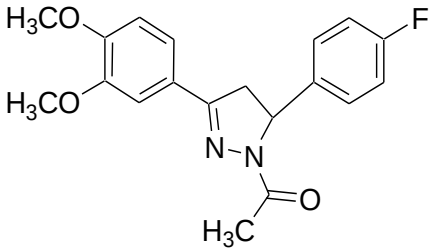


Figure 5: Chemical structure of the synthesized compound KM-5

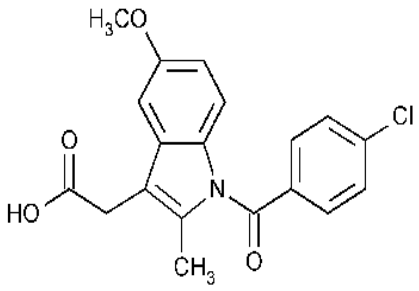


Figure 6: Chemical structure of the synthesized compound Indomethacin.

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