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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF P38 KINASE INHIBITOR AS ANTIINFLAMMATORY AGENT

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ABSTRACT

Inflammation occurs as a defensive process by the organism followed by redness, heat, swelling and pain due to damage to the tissues or the organs. With the help of this mechanism our body fight with the pathogens such as parasites, bacteria, virus and other harmful microorganisms. There are various disease caused by chronic inflammation including gastritis, colitis, dermatitis, rheumatoid arthritis, pulmonary diseases, and type II diabetes, which causes damage to millions of people's health every year. There are so many growing evidences that show that inflammation is a critical initiation factor which induces a variety of other major diseases such as cancer, atherosclerosis, Alzheimer's disease, cardiovascular disease, neurological disorders, and pulmonary diseases. Therefore knowing deeply about inflammation is basically required for better treatment strategies. P38 mitogen-activated protein kinases are a class of mitogen-activated protein kinases which are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation and apoptosis. P38 MAP Kinase (MAPK), also called RK or CSBP, is the mammalian orthologue of the yeast HOG kinase which participates in a signalling cascade controlling cellular responses to cytokines and stress. Numerous p38 kinase inhibitors have been reported and some of them are various stage of clinical evaluation. New analogue containing α-ketoamides were synthesized and evaluate for p38 kinase inhibitory activity and reported to other then ATP binding site.

KEYWORDS: MAPK, P38 KINASE Inhibitors, ATP Binding Site, α-ketoamides.

1. INTRODUCTION

Inflammation occurs as a defensive process by the organism followed by redness, heat, swelling and pain due to damage to the tissues or the organ. With the help of this mechanism

our body fight with the pathogens such as parasites, bacteria, virus and other harmful microorganisms. There are various disease caused by chronic inflammation including gastritis, colitis, dermatitis, rheumatoid arthritis, pulmonary diseases, and type II diabetes, which causes damage to millions of people's health every year. There are so many growing evidences that show that inflammation is a critical initiation factor which induces a variety of other major diseases such as cancer, atherosclerosis, Alzheimer's disease, cardiovascular disease, neurological disorders, and pulmonary diseases. Therefore knowing deeply about inflammation is basically required for better treatment strategies.

Macrophages within tissues are very important in playing an essential role in the initiation, development, and resolution of inflammation. Macrophages are white blood cells that are differentiated from monocytes. Their roles are to clean up damaged cells and pathogens by phagocytosis and to activate immune cells, such as neutrophils, dendritic cells, macrophages, and monocytes, in response to pathogens and diseases. They can be activated or deactivated during inflammatory processes depending on the signaling molecules produced. Stimulation signals include lipopolysaccharide (LPS), cytokines (interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)), other chemical mediators, and extracellular matrix proteins. A variety of membrane receptors are expressed on the surfaces of macrophages, including pattern recognition receptors (PRRs) such as dectin-1 and Toll-like receptors (TLRs). These receptors recognize activation signals and subsequently activate downstream protein kinases, eventually resulting in the stimulation of transcription factors including activator protein-1 (AP-1), nuclear factor-kappa B (NF-κB), and cAMP response element-binding protein (CREB).

Various intracellular proteins can initiate inflammation. p38 proteins are a class of mitogen-activated protein kinases (MAPKs) that are major players during inflammatory responses, especially in macrophages. p38, also called RK or cytokinin-specific binding protein (CSBP), was identified in 1994 and is the mammalian ortholog of the yeast Hog1p MAP kinase. p38 was isolated as a 38 kDa protein that is rapidly phosphorylated at a tyrosine residue in response to LPS stimulation, and the p38 gene was cloned through binding of the p38 protein with pyridinyl imidazole derivatives. p38 expression is upregulated in response to inflammatory and stress stimuli, such as cytokines, ultraviolet irradiation, osmotic shock, and heat shock, and is involved in autophagy, apoptosis, and cell differentiation. Accumulating evidence suggests that p38 plays an important role in arthritis and inflammation of the liver,

kidney, brain, and lung and that it acts as a critical player in inflammatory diseases mediated by macrophages.

P38 mitogen-activated protein kinases are a class of mitogen-activated protein kinases which are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation and apoptosis. P38 MAP Kinase (MAPK), also called RK or CSBP, is the mammalian orthologue of the yeast HOG kinase which participates in a signalling cascade controlling cellular responses to cytokines and stress. Four isoforms of p38 MAP kinase, p38-α (MAPK14), -β (MAPK11), -γ (MAPK12 or ERK6) and -δ (MAPK13 or SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), Ultraviolet light and growth factors. MKK3 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182, Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 and to phosphorylate the transcription factors ATF-2, Mac and MEF2.

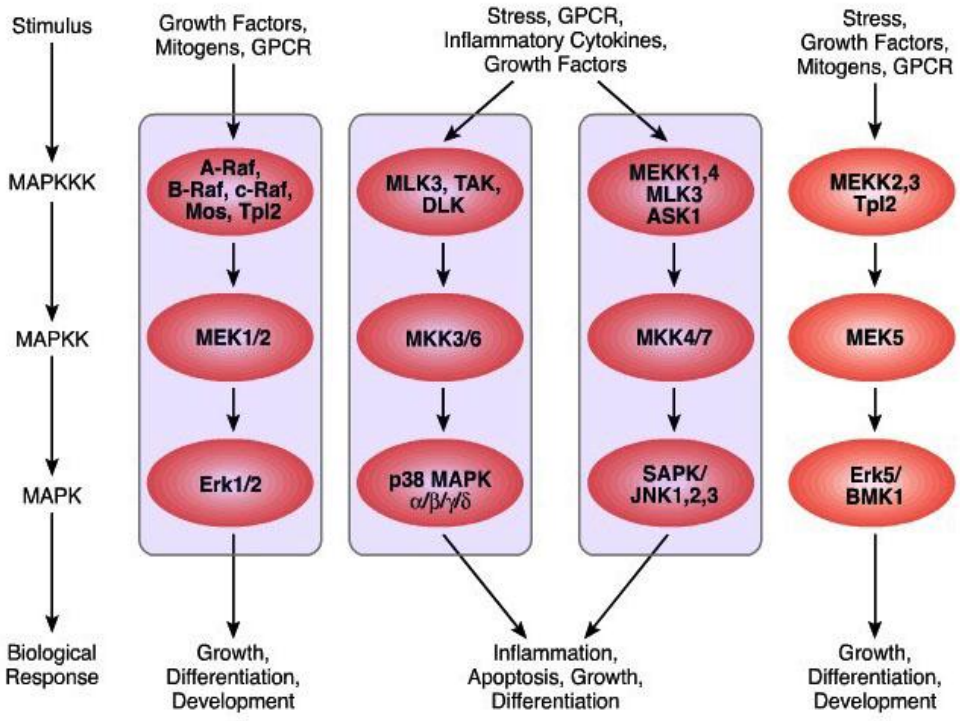


Figure 1.1: P38 Kinase signal transduction pathway

The mitogen-activated protein (MAP) kinase p38 has been recognized as a highly attractive target for therapeutic intervention due to its role in the stress-activated signal transduction

pathway leading to the release of pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-α) and interleukin-1b (IL-1βb). It is well established that these cytokines play an important role in the pathogenesis of various inflammatory diseases. The signal transduction pathway leading to the production of TNF-α from stimulated inflammatory cells, regulated by p38 MAP kinase. P38 kinase belongs to a group of serine/threonine kinase that includes c-Jun NH2 terminal kinase (JNK) and extracellular regulated protein kinase (ERK). Upon extracellular stimulation by a variety of condition and agents, p38 is activated through bis-phosphorylation on a Thr-Gly-Tyr motif located in the activation loop. Activation is achieved by dual-specificity serine/threonine MAPK kinase, MKK3 and MKK6. Once activated, p38 can phosphorylate and activate other kinases or transcription factors leading to stabilized mRNA and increase or decrease in the expression of certain target genes.

1.1 p38 Kinase Inhibitors

P38 Kinase inhibitors are the drugs, which block the MAP Kinase cascade, it plays a crucial role in regulating the production of pro inflammatory cytokines such as TNFα and IL-1β. Blocking this kinase may offer an effective therapy for treating many inflammatory diseases. P38 MAP Kinase inhibitors are classified into two classes: Drugs which compete with ATP, Drugs which bind to allosteric site.

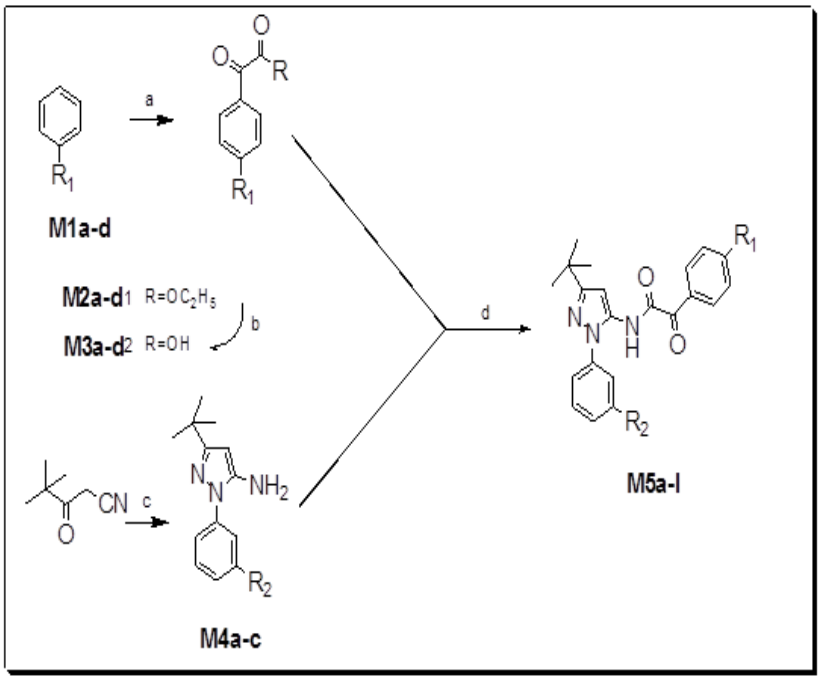
Numerous p38 kinase inhibitors have been reported and some of them are various stage of clinical evaluation. In the current study new compounds have been synthesized and their pharmacological activity has been found out using various models.

2. MATERIALS AND METHODS

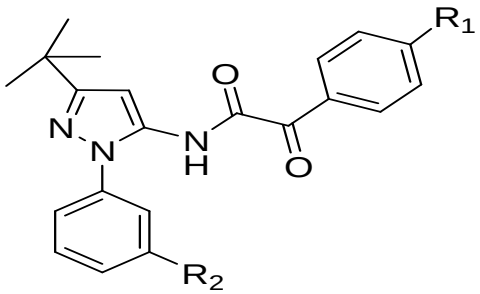
2.1 Synthesis

The materials required are benzene, chloro-benzene, anisol, toluene, $AlCl_3$ chloroform, 10% sod.bicarbonate, $MgSO_{4,}$, methanol, 1N HCl, ethoxyl chloride, phenyl hydrazine, 3-nitro phenyl hydrazine, 3-methyl phenyl hydrazine, 4,4,dimethyl phentane -nitrile, ethanol, acetic acid, EDCI, HOBt, dry dichloromethane.

Scheme



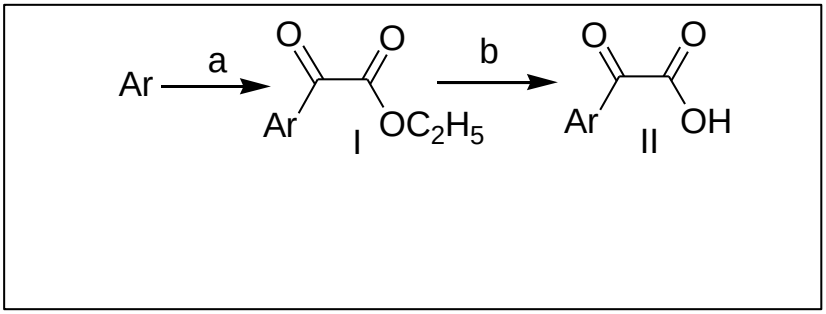
Scheme-:Reagents and conditions (a) $CHCl_3$, ethoxyl chloride, 0-5°C, 40 min, then, $AlCl_3$, 80 min (b) MeOH, 4N NaOH, 40 min, 1N HCl (c) $PhNHNH_2$, ethanol, reflux ,16 h (d) EDCI , HOBt, dry CH_2Cl_2, 0 ^{0}C ,r.t., 12 h.



M5a-l

compound	R_2	R_1
M5a	H	H
M5b	H	CH_3
M5c	H	Cl
M5d	NO_2	H
M5e	NO_2	CH_3
M5f	NO_2	Cl
M5g	CH_3	H
M5h	CH_3	CH_3
M5i	CH_3	Cl
M5j	NO_2	OCH_3
M5k	H	OCH_3
M5l	CH_3	OCH_3

Synthesis of aryl α-keto acid



Reagents and conditions: (a) $CHCl_3$, ethoxyl chloride, 0-5 ^{0}C,40 min, then $AlCl_3$, 0-5 ^{0}C, 80 min (b) MeOH, 4N NaOH, 40 min, 1N HCl.

2.2 General procedure

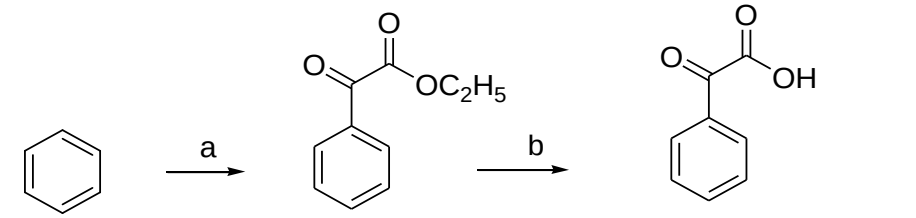
Step 1: synthesis of aryl α-keto ester

The substituted benzene (0.05 mmol) and monoethyloxalyl chloride (0.05 mmol) were stirred in 100 mL of $CHCl_3$ and kept at 0-5ºC on the ice bath. Powdered $AlCl_3$ (0.1 mmol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then $CHCl_3$ was added, this reaction mixture was extracted two times and the $CHCl_3$ solution was washed with a 10% solution of sodium hydrogen carbonate and then water and dried with $MgSO_4$. The crude product thus obtained was purified over silica and characterized by NMR.

Step 2: synthesis of aryl α-keto acid

Acid was dissolved in MeOH at 0°C (cooling) and 4 N NaOH was added and allowed to stir for 30 min with TLC monitored. After completion of reaction MeOH was removed. Reaction mixture was neutralized with 1N HCl. Compound was extracted into EtOAc, dried over Na_2SO_4.

2.2.1 2-Oxo-2-phenylacetic acid (M3a)



Benzene — Ethyl 2-oxo-2-phenylacetate — 2-Oxo-2-phenylacetic acid

M1a **M2a** **M3a**

Reagents and conditions: (a) $CHCl_3$, ethoxyl chloride, 0-5 ^{0}C, 40 min, then $AlCl_3$, 0-5^0 C, 80 min (b) MeOH, 4N NaOH, 40 min, 1N HCl

Procedure

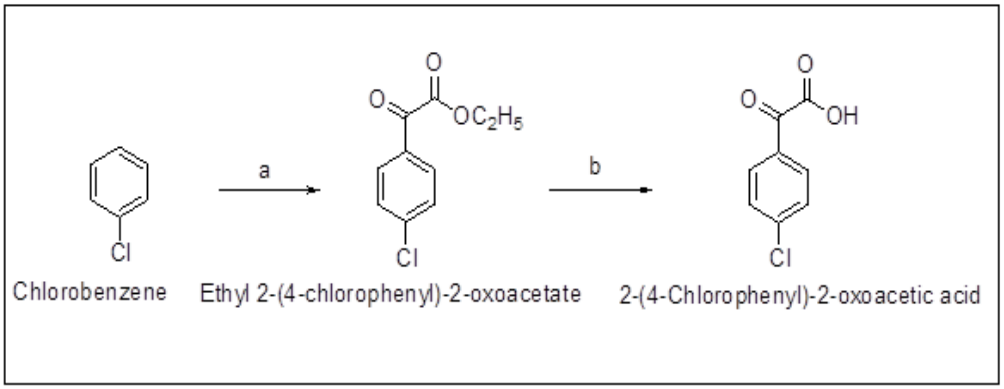
Step1: Ethyl 2- oxo-2-phenylacetate

To 4.5mL benzene (0.05mol) and 5.6 mL monoethyloxalyl chloride (0.05 mol) were stirred in 100 mL of $CHCl_3$ and kept at 0-5 ºC on the ice bath. 12.35gm Powdered $AlCl_3$ (0.1 mol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then $CHCl_3$ was added, this mixture was extracted two times and the $CHCl_3$ solution was washed with a 10% solution of sodium hydrogen carbonate and then with water and dried with $MgSO_4$. The crude product thus obtained was purified over silica gel and characterized by NMR.

Step 2: 2- Oxo-2-phenylacetic acid

Ethyl 2-oxo-2-phenylacetae was dissolved in MeOH at 0°C (cooling) and 4 N NaOH was added and allowed to stir for 30 min with TLC monitored. After completion of reaction MeOH was removed. Reaction mixture was neutralized with 1N HCl. Compound was extracted with EtOAc, dried over Na_2SO_4.

2.2.2 2-(4-Chlorophenyl)-2-oxoaceticacid: (M3b)



M1b **M2b** **M3b**

Reagents and conditions: (a) $CHCl_3$, ethoxyl chloride, 0-5 ^{0}C, 40 min, then $AlCl_3$, 0-5^0 C, 80 min
(b) MeOH, 4N NaOH, 40 min, 1N HCl

Step 1: Ethyl 2 – (4-chlorophenyl)-2-oxoacetate

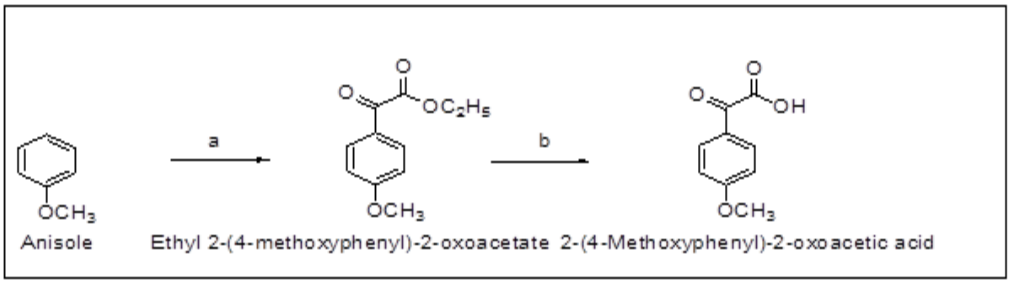
To 5.08 mL chlorobenzene (0.05 mol) and 5.56 mL monoethyloxalyl chloride (0.05 mol) were stirred in 100 mL of $CHCl_3$ and kept at 0-5 ºC on the ice bath.12.35 gm Powdered $AlCl_3$ (0.1 mol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then $CHCl_3$ was added, this mixture was extracted two times and the $CHCl_3$ solution was washed with a 10% solution of sodium hydrogen carbonate

and then with water and dried with $MgSO_4$. The crude product thus obtained was purified over silica and characterized by NMR.

Step 2: 2 – (4-Chlorophenyl)-2-oxoacetic acid

Ethyl 2 – (4-chlorophenyl)-2-oxoacetate was dissolved in125 mL MeOH (4 mL/mmol) at 0°C (cooling) and 4 N 125mL NaOH (4 mL/mmol) was added and allowed to stir for 30 min .Reaction was monitored by TLC. After completion of reaction MeOH was removed. Reaction mixture was neutralized with 1N HCl. Compound was extracted with EtOAc, dried over Na_2SO_4.

2.2.3 2-(4-Metoxyphenyl)-2-oxoacetic acid (M3c)



M1c **M2c** **M3c**

Reagents and conditions: (a) $CHCl_3$, ethoxyl chloride, 0-5 ^{0}C, 40 min, then $AlCl_3$, 0-5^0 C, 80 min
(b) MeOH, 4N NaOH, 40 min, 1N HCl

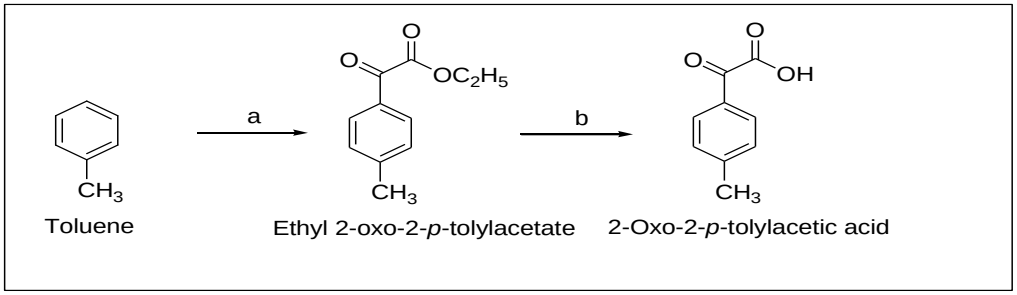
Step 1: Ethyl 2 – (4-metoxyphenyl)-2-oxoacetate

To 5.5 mL anisole (0.05 mol) and 5.56 mL monoethyloxalyl chloride (0.05 mol) were stirred in 100 mL of $CHCl_3$ and kept at 0-5 ºC on the ice bath.12.35 gm. Powdered $AlCl_3$ (0.1 mol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then $CHCl_3$ was added, this mixture was extracted two times and the $CHCl_3$ solution was washed with a 10% solution of sodium hydrogen carbonate and then with water and dried with $MgSO_4$. The crude product thus obtained was purified over silica gel and characterized by NMR.

2 – (4-Metoxyphenyl)-2-oxoacetic acid

Ethyl 2 – (4-metoxyphenyl)-2-oxoacetate was dissolved in125 mL MeOH (4 mL/mmol) at 0 °C (cooling) and 4N 125 mL NaOH (4 mL/mmol) was added and allowed to stir for 30 min.Reaction mixture was monitored by TLC. After completion of reaction MeOH was removed. Reaction mixture was neutralized with 1N HCl. Compound was extracted with EtOAc, dried over Na_2SO_4.

2.2.4 2-Oxo-2-p-tolylacetic acid (M3d)



M1d **M2d** **M3d**

Reagents and conditions: (a) $CHCl_3$, ethoxyl chloride, 0-5 ^{0}C, 40 min, then $AlCl_3$, 0-5^0 C, 80 min
(b) MeOH, 4N NaOH, 40 min, 1N HCl

Step 1: Ethyl 2- oxo-2-p tolylacetate

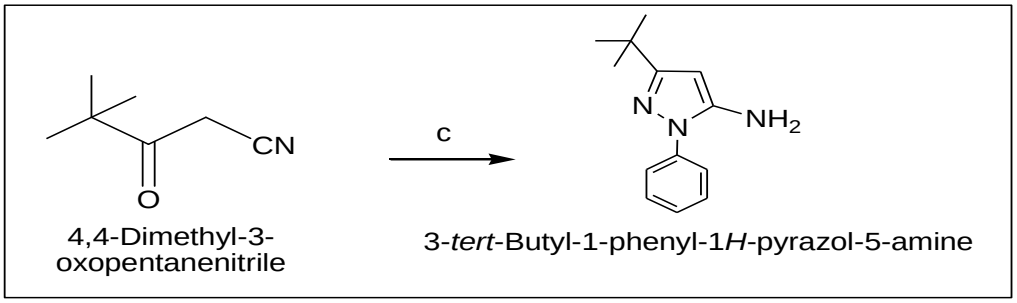
To 5.3 mL toluene (0.05 mol) and 5.56 mL monoethyloxalyl chloride (0.05 mol) were stirred in 100 mL of $CHCl_3$ and kept at 0-5 ºC on the ice bath.12.35 gm. Powdered $AlCl_3$ (0.1 mol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then $CHCl_3$ was added, this mixture was extracted two times and the $CHCl_3$ solution was washed with a 10% solution of sodium hydrogen carbonate and then with water and dried with $MgSO_4$. The crude product thus obtained was purified over silica gel and characterized by NMR.

Step 2: 2 – (4-Metoxyphenyl)-2-oxoacetic acid

Ethyl 2 – oxo 2-p-tolylacetate was dissolved in125 mL MeOH (4 mL/mmol) at 0 °C (cooling) and 4N 125 mL NaOH (4 mL/mmol) was added and allowed to stir for 30 min. Reaction was monitored by TLC. After completion of reaction MeOH was removed. Reaction mixture was neutralized with 1N HCl. Compound was extracted with EtOAc, dried over Na_2SO_4.

2.3 Synthesis of pyarazolamine

2.3.1 3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-amine (M4a)

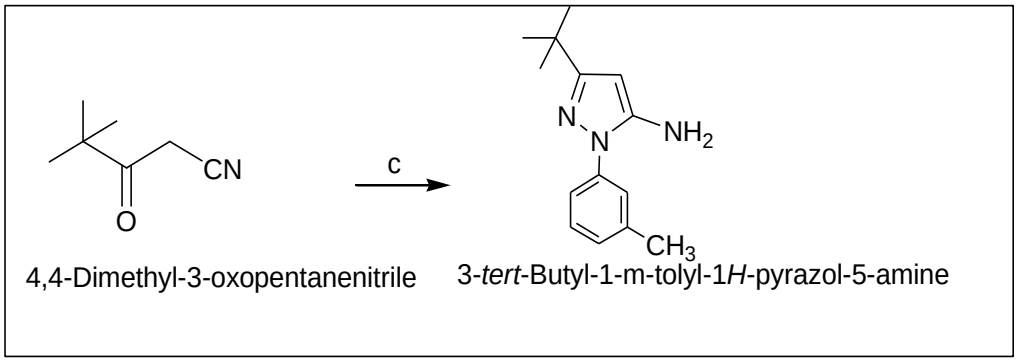


M 4a

Reagents: (c) phenylhydazine, toluene, reflux or aqueous HCl, ethanol, reflux, 16h

A solution of phenyl hydrazine (0.83 mL, 8.39 mmol) and 4, 4-dimethyl-3-oxo-pentanenitrile (1.0 g, 8.0 mmol) in toluene (3 mL) was heated to reflux overnight. Removal of the volatiles in vacuo provided a residue, which was purified by silica gel chromatography using 50% ethyl acetate in hexanes as the eluent. Concentration in vacuo of the product-rich fractions provided 3-amino-5-*tert*-butyl-2- phenyl-*2H*-pyrazole as a light orange solid (1.53 g, 89%).

2.3.2 3-*tert*-Butyl-1-m-tolyl-1*H*-pyrazol-5-amine (M4b)

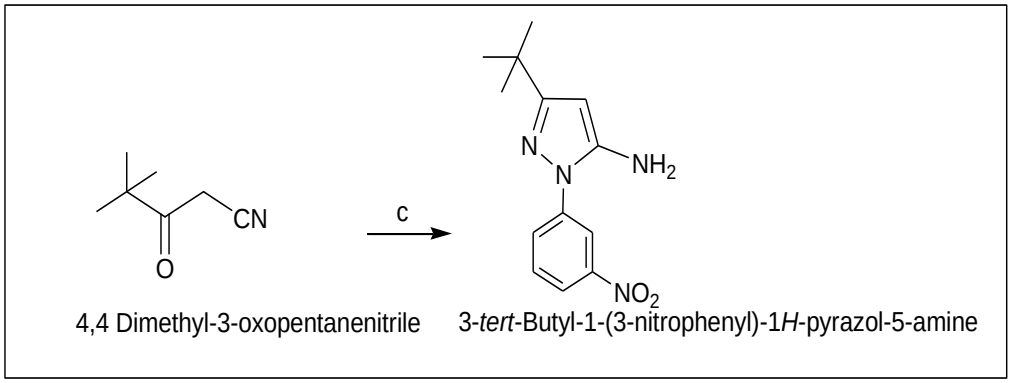


4b

Reagents: (c) *m*-tolylhydazine, toluene, reflux or aqueous HCl, ethanol, reflux, 16h

A solution of *m*-tolylhydrazine hydrochloride (3 g, 18.9 mmol), 4,4-dimethyl-3 oxopentanenitrile (2.6 g, 20.8 mmol), and concentrated HCl (2 mL) in ethanol (100 mL) was heated to reflux for 12 h, cooled to room temperature, basified with 20% aqueous NaOH to pH 12 (litmus), and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried (MgSO4). Removal of the volatiles in vacuo afforded 3-*tert*-butyl-1-*m*-tolyl-1*H*-pyrazol-5-amine as a yellow solid (3.7 g, 85%).[26]

2.3.2 3-*tert*-Butyl -1-(3-nitrophenyl)-1*H*-pyrazole-5-amine (4c)



M4c

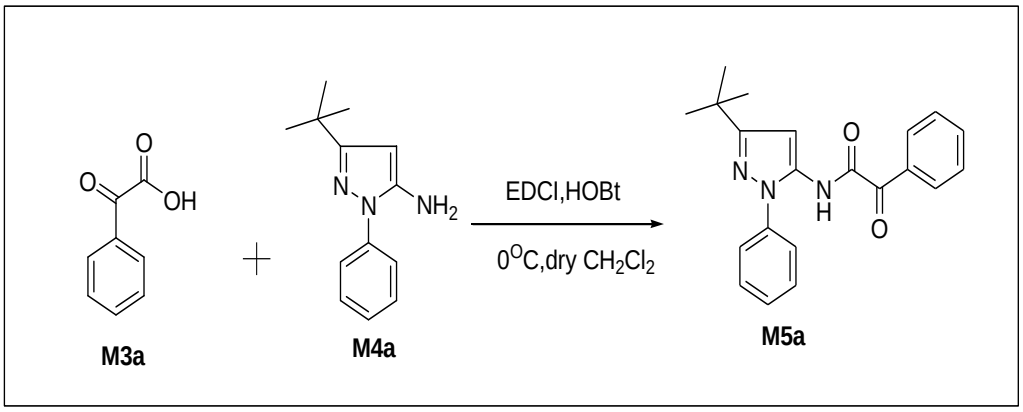
Reagents: (c) 3-nitrophenyllhydazine, toluene, reflux or aqueous HCl, ethanol, reflux, 16h

A solution of 3-nitro-phenylhydrazine hydrochloride (2.0 g, 10.5 mmol), 4,4-dimethyl-3-oxopentanenitrile (1.45 g, 11.6 mmol), and concentrated HCl (2 mL) in ethanol (100 mL) was heated to reflux for 12 h, cooled to room temperature, basified with 20% aqueous NaOH to pH 12 (litmus), and extracted with ethyl acetate (3 X 20 mL). The combined extracts were dried ($MgSO_4$). Removal of the volatiles in vacuo afforded compound as a yellow solid (1.9 g, 70%).[26]

2.4 Coupling of amines and acids

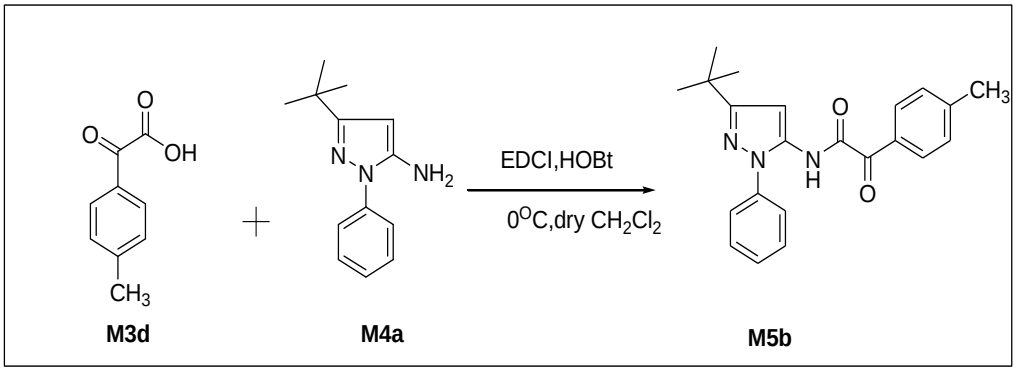
2.4.1. [N-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazole-5-yl)-2-oxo-2-phenylacetamide] (M5a)

Procedure



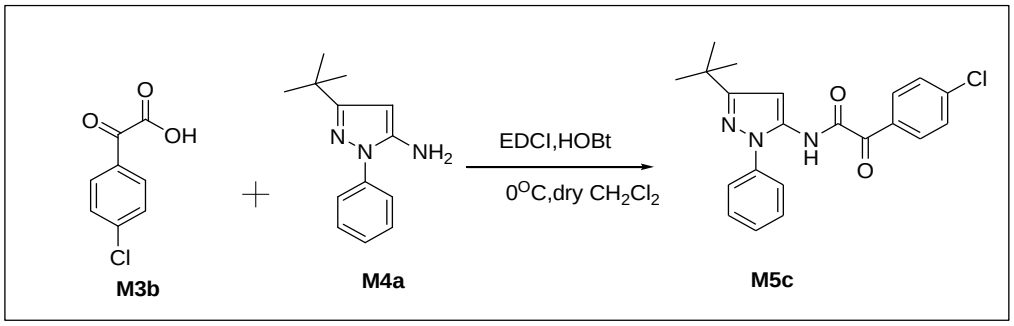
To a well stirred and ice cooled mixture of 3a (1.1 eq), EDCI (1.2 eq), and HOBt (1.2 eq) in dry DCM was added a solution of free amine **(4a)** (1 eq) in dry DCM. After 5 Hr stirring at r.t. the reaction mixture was washed with 1N H_2SO_4, (20 mL), H_2O (20 mL) and sat. $NaHCO_3$ (20 mL).The organic layer was dried ($MgSO_4$) and solvent evaporate in vacuum. The residue on chromatograph on silica-gel and elution with Hexane: EtOAc.[27]

2.4.2. [N-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazole-5-yl)-2-oxo-2-p-tolylacetamide] (M5b)



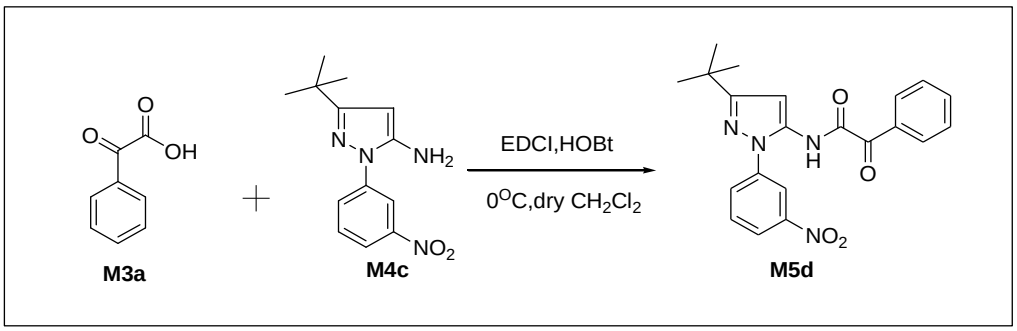
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.3.[N-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazole-5-yl)-2-(4-chlorophenyl)-2-oxo-acetamide] (M5c)



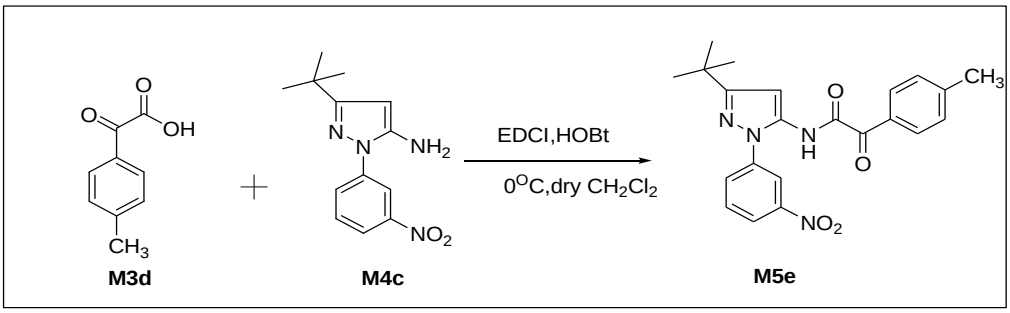
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.4.[N-(3-*tert*-Butyl-1-(3-nitrophenyl)-1*H*-pyrazole-5-yl)-2-oxo-2-phenylacetamide] (M5d)



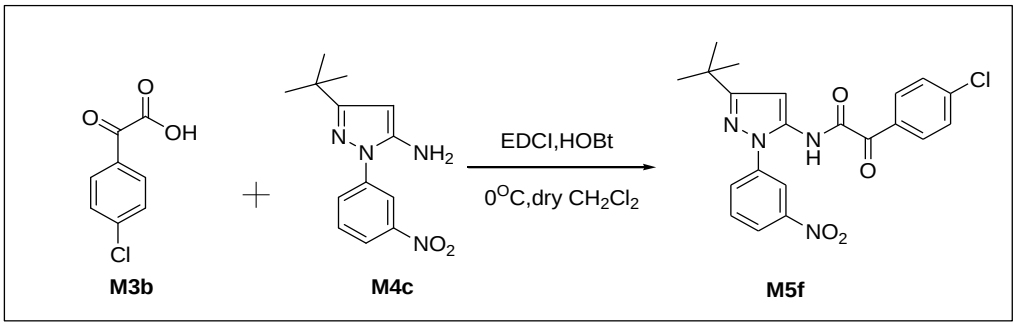
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.5.[N-(3-*tert*-Butyl-1-(3-nitrophenyl)-1*H*-pyrazole-5-yl)-2-oxo-2-*p*-tolylacetamide] (M5e)



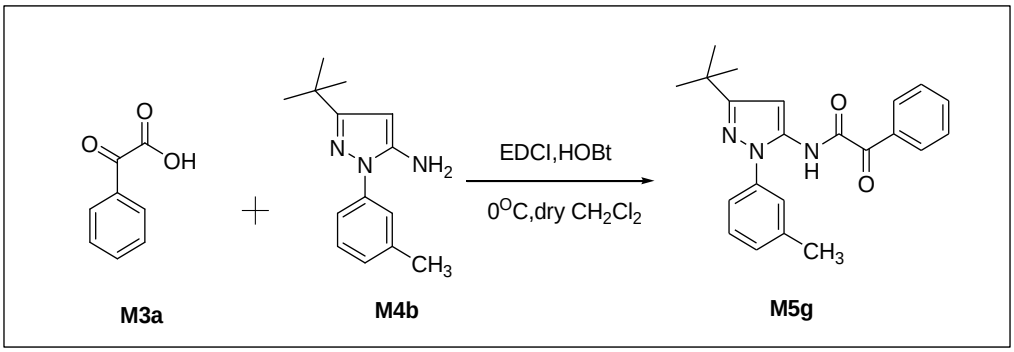
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.6.[N-(3-*tert*-Butyl-1-(3-nitrophenyl)-1*H*-pyrazole-5-yl)-2-(4-cholorophenyl)-2-oxoacetamide (M5f)



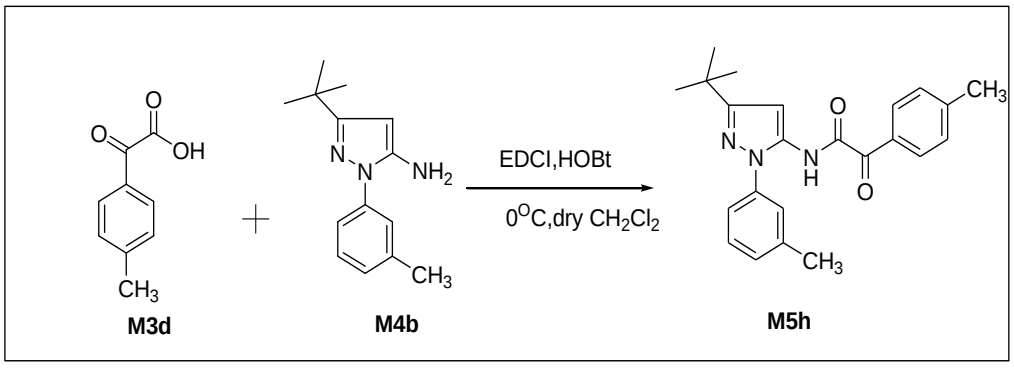
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.7. [N-(3-*tert*-Butyl-1-m-tolyl-1*H*-pyrazole-5-yl)-2-oxo-2-phenlylacetamide] (M5g)



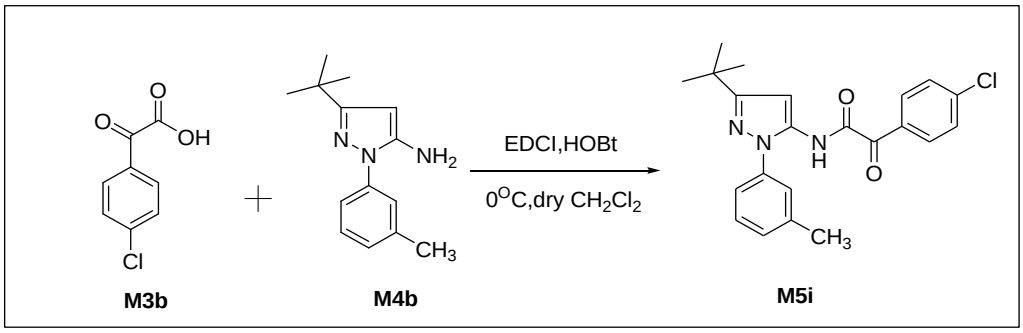
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.8. [N-(3-*tert*-Butyl-1-m-tolyl-1*H*-pyrazole-5-yl)-2-oxo-2-p-tollylacetamide] (M5h)



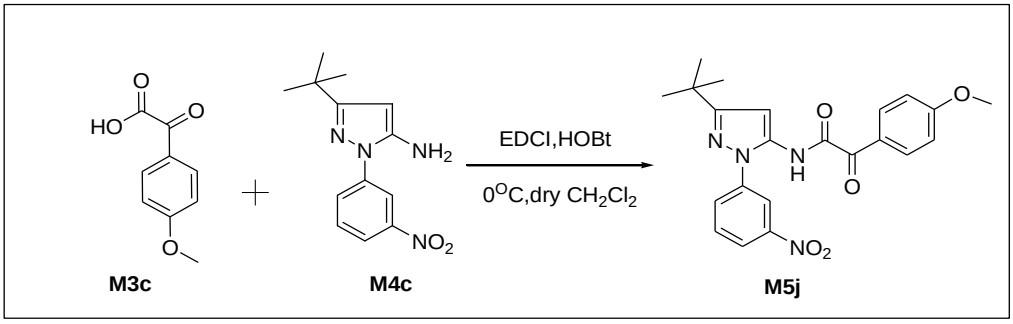
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.9.[N-(3-*tert*-Butyl-1-m-tolyl-1*H*-pyrazole-5-yl)-2-(4-chlorophenyl)-2-oxoacetamide] (M5i)



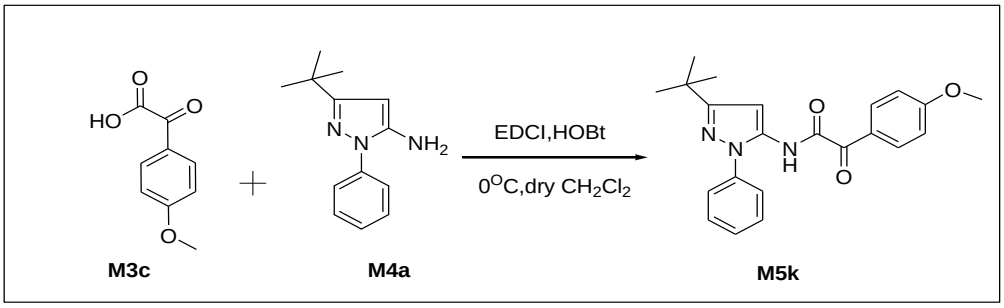
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.10.[N-(3-*tert*-Butyl-1-(3-nitrophenyl)-1*H*-pyrazole-5-yl)-2-(4-methoxyphenyl)-2-oxaacetamide] (M5j).



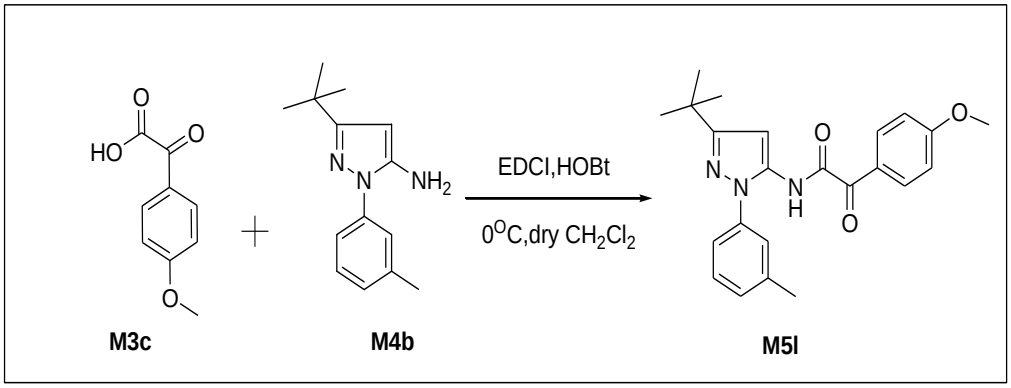
Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.11. [N-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazole-5-yl)-2-(4-methoxyphenyl)-2-oxaacetamide] (M5k).



Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.4.12. **[N-(3-*tert*-Butyl-1-m-tolyl-1*H*-pyrazole-5-yl)-2-(4-methoxyphenyl)-2-oxaacetamide](M5l).**



Procedure: followed the similar reaction procedure as described in the earlier for compound (M5a).

2.5. PHARMACOLOGICAL EVALUATION

2.5.1 Acute toxicity study

Acute toxicity of a drug can be determined by the calculation of LD50, i.e., the dose that will kill 50% of animals of a particular species.

This study is needful before pharmacological screening on animals .The acute toxicity study was carried out according to OECD (Organization for Economic and Cultural Development-2006) 423 guidelines which are based on a stepwise procedure with the use of a minimum number of animals per step.

The acute toxicity for the synthesized compounds was determined by the Miller and Tainter method administering the compounds orally. LD_{50} of the test compounds calculated by Miller and Tainter (1944) method, initially least tolerated dose (100% mortality) and most tolerated dose (0% mortality) were determined by hit and trial method. If mortality observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality not observed, the procedure was repeated for further higher doses such as100, 500 and 2000 mg / kg body weight. After determination of two doses we have selected five doses in between the least tolerated and most tolerated doses were given orally to 6 groups of rats, 06 animals in each group. The animals were observed for first 2 hours and then at 6^{th} and 24^{th} hour for any toxic symptoms. After 24 hours, the number of deceased animals was counted in each group

and percentage of mortality calculated. The percentage of animals that had died at each dose level is then transformed to probit. From the obtained data determined the LD_{50} of the test compounds by using probit value transformations.

Ethical clearance

Ethical Clearance has been obtained from Institutional Animal Ethics Committee (IACE). Protocol used in this study for use of mice as animal model for cancer research was approved by Institutional Animal Ethical committee, **NIMS University, Jaipur-Rajasthan (NIMSUR/IAEC/CERT/2014/09/03)**. The copy of the ethical clearance certificate obtained from Institutional Animal Ethical Committee (IAEC) is attached.

Animals for experiment

Healthy Mature wistar albino rats of either sex weighing 200–350g were taken for the study. All the animals were procured from the Central Animal House of the NIMS University. The animals were housed in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (25±2^{o}c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. The animals are randomized into experimental and control groups. The animals were provided with Standard pellets diet (Hindustan Lever, Kolkata, India) and tap water *ad libitum* and maintained at natural day night cycle. The animals were acclimatized to laboratory condition for one week before commencement of experiment.

Housing Conditions

All pharmacological activities were conducted in 2-3 months old either sex wistar albino rats. The animals were housed under specific conditions in polypropylene cages for group rearing. The cages simply contained husk bedding material. The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25°C (± 30°C) and 45-55% respectively with a light-dark cycle of 12 h (light from 06:00 AM to 06:00 PM). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow (Hindustan Lever, Kolkata, India) and water *ad libitum*. All experiments were approved by Institutional Animal Ethics Committee.

Determination of Median Lethal Dose (LD50)

The synthesized compounds (10mg-100mg/kg) produced physical signs such as gasping for air, palpitation, depression, decreased respiratory rate, loss of appetite, feeling sleepy, and death depending on the dose. The LD50 values of the synthesized compounds in Sprague-Dawley rats were calculated as following-

Procedure

Healthy, young, adult wistar albino rats of either sex (150-200gm.) were used for this study. Animals were fasted prior to dosing. On next day, the fasted body weight of each animal was determined and the dose was calculated according to the body weight.

36 animals were divided into six groups (n=06) for giving each synthesized compounds dose 10, 20, 50, and 100mg/kg respectively.

The synthesized compound was dissolved in distilled water and injected intraperitoneally to six groups of rats (each containing 6 rats) at different doses (10, 20, 50, 100, 150 and 200 mg/kg). LD50 was evaluated by recording mortality after 24 hours.

Group1: 10 mg/kg, the dose of **compound M5a** given to animals. At this dose animal showed normal behavior.
Group2: 20 mg/kg, the dose of **compound M5a** given to animals. At this dose initially animals were uncomfortable but after sometimes they became normal.
Group3: 50 mg/kg, the dose of **compound M5a** given to animals. At this dose initially animals were uncomfortable but after sometimes they became normal.
Group4: 100 mg/kg, the dose of **compound M5a** given to animals. At this dose 30 to 50% animals died.
Group5: 150 mg/kg, the dose of **compound M5a** given to animals. At this dose 60 to 80% animals died.
Group6: 200 mg/kg, the dose of **compound M5a** given to animals. At this dose 90 to 100% animals died.

Animals were observed individually 30 minutes after dosing, periodically during the first 24 hours. Following changes were examined in the treated animals.

1) *Behavioral profile*

Awareness**:** Alertness, Visual placing, Stereotypy, passivity.

Mood**:** Grooming, restlessness, irritability, fearfulness.

2) *Neurological profile*

Motor activity: Spontaneous Activity, reactivity touches response, pain response startle response tremor gait grip strength pinna reflex, and corneal reflex.

3) *Autonomic profile*: Writhing, Defecation, Urination, Pile erection, heart rate, respiratory rate.

The percentage dead for 0 and 100 are corrected before the determination of probits as under:

Corrected % Formula for 0 and 100% mortality:

For 0% dead: 100(0.25/n)

For 100% dead: 100(n-0.25/n)

The probit values are plotted against log-doses and then the dose corresponding to probit 5, i.e., 50%, is found out.

No mortality was noticed up to 20 mg/kg body weight (i.p.), whereas, 100% mortality was noticed at the dose of 150-200 mg/kg (i.p.). **Same procedure was followed for all the synthesized compounds to obtain the LD50.** The LD50 of the synthesized **compounds** was found to be 100 mg/kg for **M5a- M5l.**

Selection of dose: 1/10 and 1/5 of the maximum tolerated dose was selected as treatment dose for further any other pharmacological activity. (Ghosh MN, 1984).

Statistical analysis

The data are expressed as mean ± SD. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnet and Tukey's test. *P*-value <0.05 was regarded as statistically significant.

2.5.2 ANTI-INFLAMMATORY SCREENING

2.5.2.1 Carrageenan induced paw edema

Materials and Method

Preparation of drug solutions and reagents

- Diclofenac Sodium.
- Normal saline solution (9% NaCl).
- Vehicle (1% Carboxymethylcellulose, CMC, 10 ml/kg) were prepared and used.

Animals

Wistar albino rats (150-180 g) of both sexes were selected for the anti-inflammatory study. The rats were given food and water ad libitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage.

Experimental Procedure

Carrageenan induced paw oedema model was used to determine the anti-inflammatory activity of the synthesized compounds by the method of Winter et al. (1962). Paw oedema was induced injecting 0.1 ml of 1% carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat (Winter et al., 1962). 84 rats were allowed to fast for 18 h and divided into 14 groups of 6 animals each.

Group I served as Control received the vehicle 0.1 ml of 1% carrageenan in 1% Carboxymethylcellulose, CMC, 10 ml/kg).

Group II served as Standard, received Diclofenac Sodium (10 mg/kg).

Group III served as test, received compound M5a at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group IV served as test, received compound M5b at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group V served as test, received compound M5c at doses of 10 mg/kg + 0.1 ml of 1% carrageenan

Group VI served as test, received compound M5d at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group VII served as test, received compound M5e at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group VIII served as test, received compound M5f at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group IX served as test, received compound M5g at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group X served as test, received compound M5h at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group XI served as test, received compound M5i at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group XII served as test, received compound M5j at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group XIII served as test, received compound M5k at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

Group XVI served as test, received compound M5l at doses of 10 mg/kg + 0.1 ml of 1% carrageenan.

The animals pretreated with synthesized compounds (M5a-M5l) or diclofenac sodium one hour before injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after carrageenan application at 1 and 5 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. The results were expressed as a percentage of inhibition of edema.

$$\text{Percentage inhibition of oedema} = \frac{Vc - Vt}{Vc} * 100$$

Where, Vc is the inflammatory increase in paw volume in control group of animals and Vt is the inflammatory increase in paw volume in drug-treated animals.

OBSERVATIONS AND RESULTS

The results were expressed as a percentage of inhibition of inflammation.

2.5.2.2 PGE2-induced hind paw edema model

Materials and Method

Preparation of drug solutions and reagents

- Indomethacin.
- 5 µl PGE2 (1 mg/ml) in Tyrode's solution.
- Vehicle (Tyrode's solution 5µl) were prepared and used.

Animals

Wistar albino rats (150-180 g) of both sexes were selected for the anti-inflammatory study. The rats were given food and water ad libitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage.

Experimental Procedure

PGE2-induced hind paw edema model is also used for the determination of anti-inflammatory activity. Six animals per group will be given either sample, control or reference drug (indomethacin, 10mg/kg). The sample dose will be 10 mg/kg for each of synthesized compound. One hour after oral administration of the sample, drug or vehicle (control), each mouse received 5 µl of freshly prepared suspension of PGE2 (1 mg/ml) in Tyrode's solution by injection into the subplantar tissue of the right hind paw except that for control, 5 µl of Tyrode's solution was injected into the left hind paw. Thereafter, paw edema will be measured at 15 min interval for 75 min. The difference in the thickness of the footpad will be measured as earlier described.

Group I served as Control received the vehicle 5 µl Tyrode's solution.

Group II served as Standard, received indomethacin (10 mg/kg).

Group III served as test, received compound M5a at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group IV served as test, received compound M5b at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group V served as test, received compound M5c at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group VI served as test, received compound M5d at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group VII served as test, received compound M5e at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group VIII served as test, received compound M5f at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group IX served as test, received compound M5g at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group X served as test, received compound M5h at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group XI served as test, received compound M5i at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group XII served as test, received compound M5j at doses of 10 mg/kg + 5 µl of Tyrode's solution.

Group XIII served as test, received compound M5k at doses of 10 mg/kg + 5 µl PGE2 suspension.

Group XIV served as test, received compound M5l at doses of 10 mg/kg + 5 µl PGE2 suspension.

The animals pretreated with the synthesized compound or indomethicin one hour before were injected with 5µl of freshly prepared suspension of PGE2 (1 mg/ml) in Tyrode's solution by injection into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after PGE2 application at 15 and 75 min after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. The results were expressed as a percentage of inhibition of edema.

$$\text{Percentage inhibition of oedema} = \frac{Vc - Vt}{Vc} * 100$$

Where, Vc is the inflammatory increase in paw volume in control group of animals and Vt is the inflammatory increase in paw volume in drug-treated animals.

Observations and Results

The results were expressed as a percentage of inhibition of inflammation.

2.5.2.3 Ethyl phenylpropiolate (EPP) induced ear edema in rats

Materials and Method

Preparation of drug solutions and reagents

- Indomethacin.
- EPP (1 mg/20 µl/ear) dissolved in acetone.
- Vehicle (acetone) were prepared and used.

ANIMALS

Wistar albino male rats (100-150 g) were selected for the anti-inflammatory study. The rats were given food and water ad libitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage.

Procedure

Wistar albino male rats of 100–160 g were used. The inflammogen EPP was dissolved in acetone and ear edema was induced by topical application of EPP (1 mg/20µl/ear) to the inner and outer surfaces of both ears using an automatic microliter pipette. Synthesized compounds dissolved in dimethylsulfoxide (DMSO) and acetone (1:1) was administered topically (20 µl/ear) just before the inflammogen. The thickness of each ear was measured with vernier calipers before and at 15, 30, 60 and 120 min after EPP induction. The inhibitory effect on the edema formation was compared with that of the vehicle-control group and the percent inhibition will be calculated.

Group I served as **Control** received the vehicle as acetone solution.
Group II served as **Standard** received phenylbutazone (1 mg/ear).
Group III served as **test**, received **compound M5a** at doses of 1 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.
Group IV served as **test**, received **compound M5b** at doses of 1 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.
Group V served as **test**, received **compound M5c** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.
Group VI served as **test**, received **compound M5d** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group VII served as **test**, received **compound M5e** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group VIII served as **test**, received **compound M5f** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group IX served as **test**, received **compound M5g** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group X served as **test**, received **compound M5h** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group XI served as **test**, received **compound M5i** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group XII served as **test**, received **compound M5j** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group XIII served as **test**, received **compound M5k** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

Group XIV served as **test**, received **compound M5l** at doses of 10 mg/kg + EPP (1 mg/20 µl acetone/ear) solution.

The animals pretreated with the synthesized compound or phenylbutazone one hour before were injected with 20 µl EPP (1 mg/20 µl acetone/ear) solution by topical application of EPP (1 mg/20 µl/ear) to the inner and outer surfaces of both ears using an automatic microliter pipette.

The thickness of each ear was measured with vernier calipers before and at 15, 30, 60 and 120 min after EPP induction. The inhibitory effect on the edema formation was compared with that of the vehicle-control group and the percent inhibition was calculated.\

The results were expressed as a percentage of inhibition of edema.

$$\text{Percentage inhibition of oedema} = \frac{Vc - Vt}{Vc} * 100$$

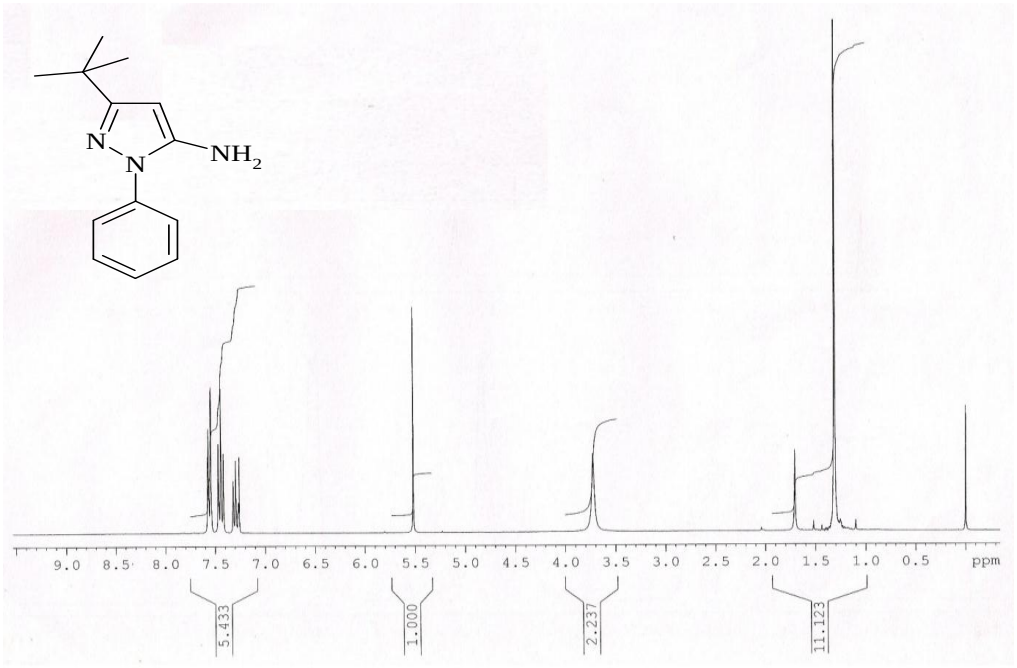
Observations and Results

The results were expressed as a percentage of inhibition of inflammation.

3. RESULT AND DISCUSSION

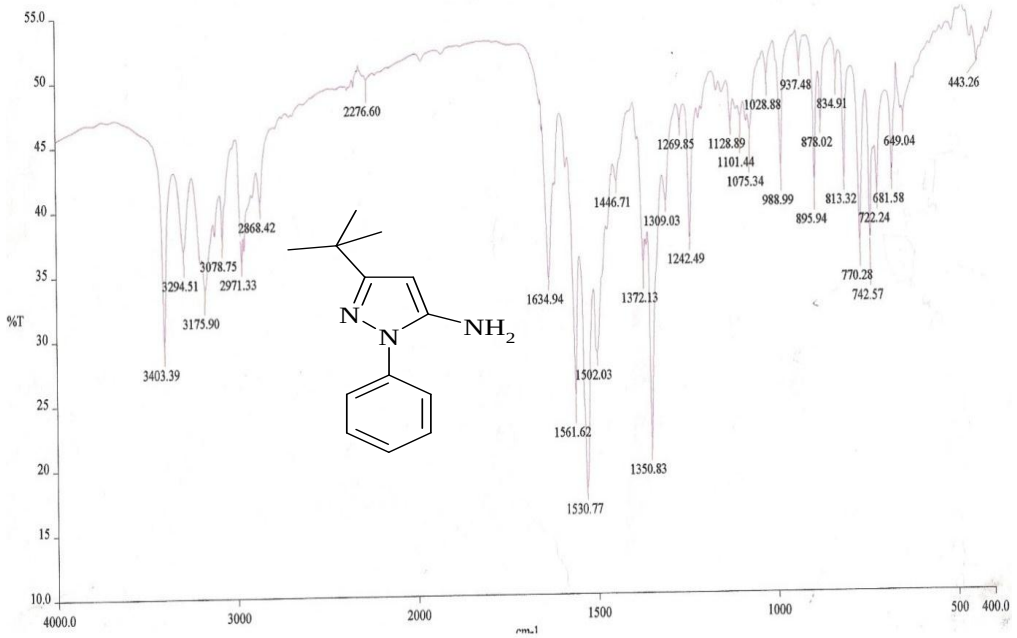
3.1 Supporting Information or Spectral Data

^{1}H NMR Spectrum of 5-amino, 1-phenyl-3-*t*-butylpyrazole



Spectra No. 1: NMR Spectrum of 5-amino, 1-phenyl-3-*t*-butylpyrazole

IR Spectrum of 5-amino, 1-phenyl-3-*t*-butylpyrazole



Spectra No. 2: IR Spectrum of 5-amino, 1-phenyl-3-*t*-butylpyrazole

3.2. Table 1: Effect of the synthesized compounds against carrageenan induced paw edema in rats

Group	Paw edema volume (ml)				
	1h	2h	3h	4h	5h
Control (Inhibition %)	0.27±0.03 ----	0.43±0.04 ---	0.58±0.02 ---	0.72±0.03 ---	0.86±0.01 ---
Standard (Inhibition %)	0.23±0.02 14.8%***	0.21±0.03 51.1%**	0.19±0.01 67.2%*	0.25±0.01 65.2%*	0.34±0.02 60.4%**
Compound M5a (Inhibition %)	0.22±0.02 18.5%***	0.25±0.02 41.8%**	0.28±0.03 51.7%**	0.21±0.04 70.8%*	0.28±0.05 67.4%*
Compound M5b (Inhibition %)	0.23±0.02 14.8%***	0.18±0.03 58.1%*	0.23±0.03 60.3%*	0.26±0.02 63.8%*	0.35±0.04 59.3%*
Compound M5c (Inhibition %)	0.19±0.02 29.6%***	0.21±0.02 51.1%**	0.25±0.03 56.8%**	0.22±0.04 69.4%*	0.29±0.05 66.2%*
Compound M5d (Inhibition %)	0.21±0.02 22.2%***	0.25±0.02 41.8%**	0.22±0.03 62.0%*	0.23±0.04 68.0%*	0.29±0.05 66.2%*
Compound M5e (Inhibition %)	0.19±0.02 29.6%***	0.25±0.02 41.8%**	0.23±0.03 60.3%*	0.24±0.04 66.6%*	0.31±0.05 63.9%*
Compound M5f (Inhibition %)	0.21±0.02 22.2%***	0.23±0.02 46.5%**	0.25±0.03 56.8%**	0.21±0.04 70.8%*	0.27±0.05 68.6%*
Compound M5g (Inhibition %)	0.20±0.02 25.9%***	0.26±0.02 39.5%***	0.28±0.03 51.7%**	0.23±0.04 68.0%*	0.30±0.05 65.1%*
Compound M5h (Inhibition %)	0.23±0.02 14.8%***	0.29±0.02 32.5%***	0.25±0.03 56.8%**	0.26±0.04 63.8%*	0.33±0.05 61.6%*
Compound M5i (Inhibition %)	0.20±0.02 25.9%***	0.24±0.02 44.1%**	0.22±0.03 62.0%*	0.26±0.04 63.8%*	0.35±0.05 59.3%**
Compound M5j (Inhibition %)	0.19±0.02 29.6%***	0.26±0.02 39.5%***	0.20±0.03 65.5%*	0.22±0.04 69.4%*	0.32±0.05 62.7%*
Compound M5k (Inhibition %)	0.24±0.02 %11.1***	0.26±0.02 39.5%***	0.23±0.03 60.3%*	0.27±0.04 62.5%*	0.38±0.05 55.8%**
Compound M5l (Inhibition %)	0.22±0.02 18.5%***	0.28±0.02 34.8%***	0.32±0.03 44.8%**	0.35±0.04 51.3%**	0.45±0.05 47.6%**

* Extremely significant (P<0.01), **Significant (p< 0.05), ***- Not significant (P> .05)

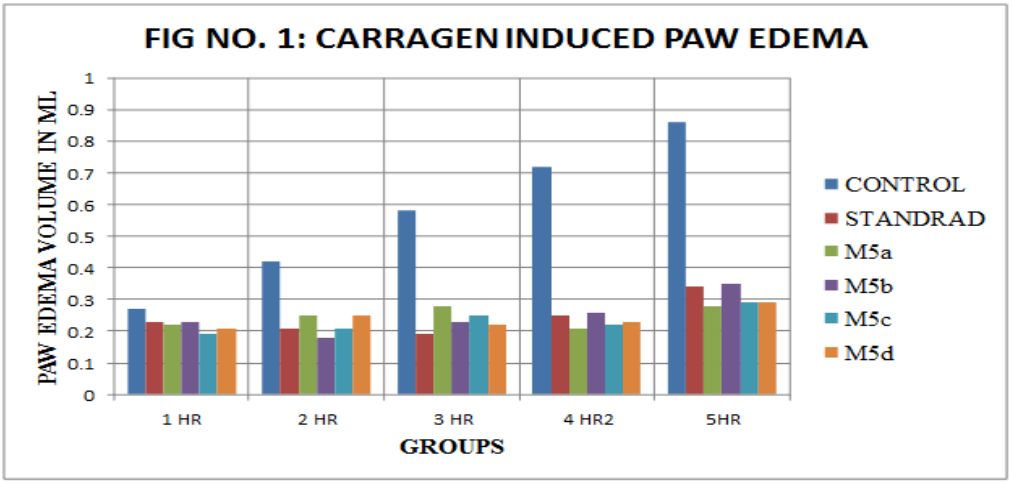


Fig no 1: Comparative Representation of Carragen Induced Paw Edema of M5a-M5d.

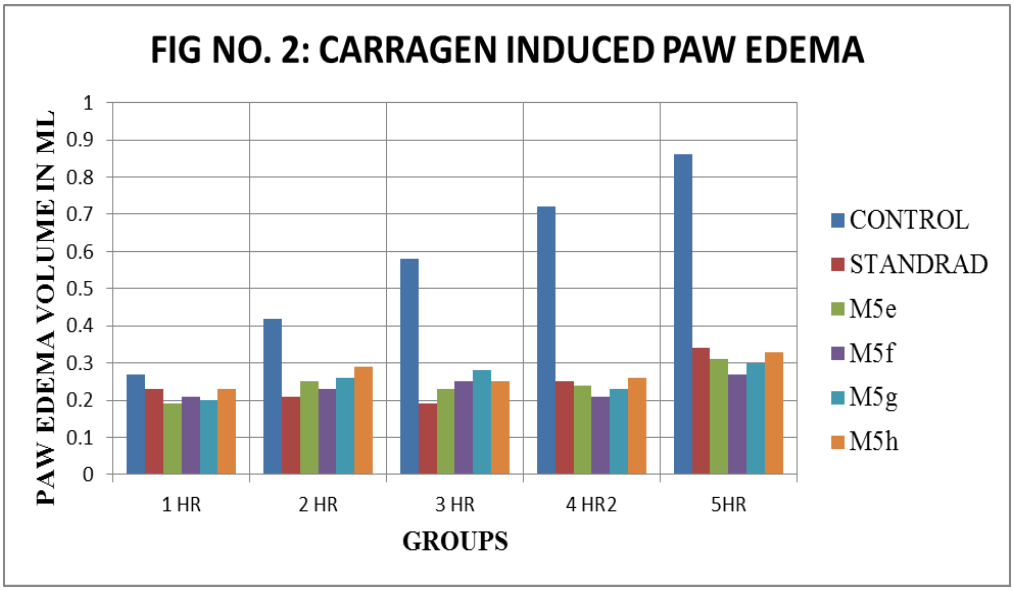


Fig No. 2: Comparative Representation of Carragen Induced Paw Edema of M5e-M5h.

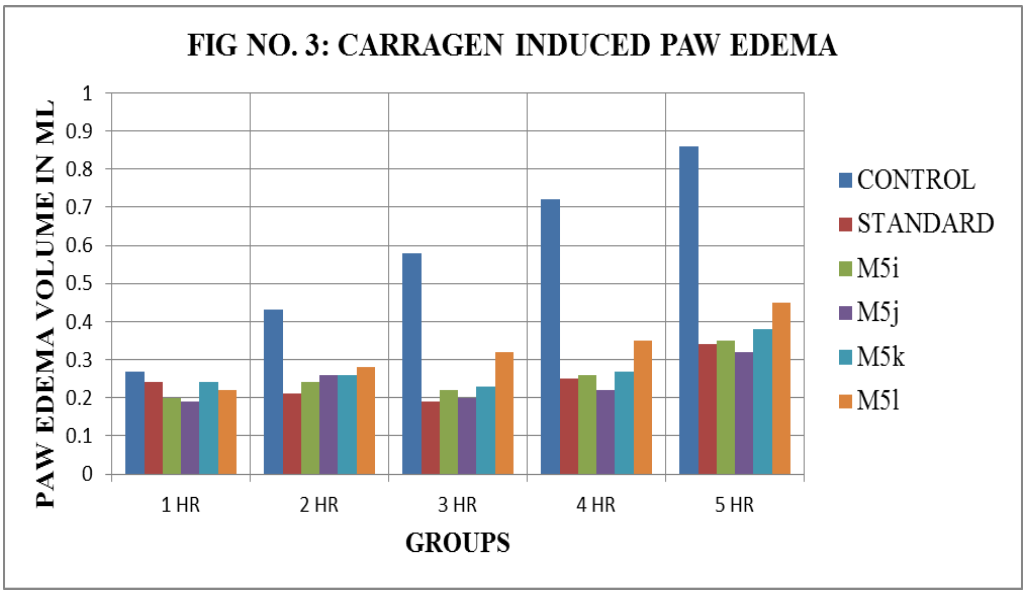


Fig no 3: Comparative Representation of Carragen Induced Paw Edema of M5i- M5l.

Discussion

All the synthesized compounds have given the satisfactory result for anti inflammatory action. All have given the best inhibition at 4th hour with maximum inhibition of 70.8% by compound M5a and M5f.

3.3 Table 2: Effect of the synthesized compounds against PGE2 induced paw edema in rats

Group	Paw edema volume (ml)					
	0 min	15min	30min	45min	60min	75min
Control	0.017±0.01	0.14±0.02	0.25±0.03	0.28±0.01	0.31±0.02	0.35±0.01
Standard (Inhibition %)	0.015±0.01 11.7%***	0.11±0.03 21.4%***	0.17±0.02 32%***	0.11±0.02 60.7%*	0.18±0.01 41.9%**	0.26±0.03 25.7%***
Compound M5a (Inhibition %)	0.014±0.03 17.6%***	0.10±0.02 28.5%***	0.16±0.01 36.0%***	0.14±0.02 50.0%**	0.19±0.04 38.7%***	0.24±0.05 31.4%***
Compound M5b (Inhibition %)	0.014±0.01 17.6%***	0.09±0.01 35.7%***	0.16±0.03 36.0%***	0.17±0.03 39.2%**	0.20±0.02 35.4%***	0.25±0.04 19.3%***
Compound M5c (Inhibition %)	0.012±0.01 29.4%***	0.09±0.02 35.7%***	0.13±0.02 48.0%**	0.12±0.02 57.1%*	0.22±0.04 29.0%***	0.27±0.05 22.8%***
Compound M5d (Inhibition %)	0.015±0.02 11.7%***	0.10±0.02 28.5%***	0.15±0.01 40.0%**	0.10±0.02 64.2%*	0.18±0.04 41.9%**	0.29±0.04 17.1%***
Compound M5e (Inhibition %)	0.013±0.02 23.5%***	0.09±0.02 35.7%***	0.13±0.03 48.0%**	0.10±0.03 64.2%*	0.18±0.04 41.9%**	0.28±0.03 20.0%***
Compound M5f (Inhibition %)	0.015±0.04 11.7%***	0.09±0.02 35.7%***	0.11±0.02 56.0%*	0.15±0.01 46.4%**	0.21±0.04 32.2%***	0.26±0.05 25.7%***
Compound M5g (Inhibition %)	0.015±0.03 11.7%***	0.10±0.03 28.5%***	0.13±0.02 48.0%**	0.11±0.04 60.7%*	0.21±0.04 32.2%***	0.24±0.02 31.4%***
Compound M5h (Inhibition %)	0.012±0.01 29.4%***	0.09±0.02 35.7%***	0.15±0.01 40.0%**	0.14±0.03 50.0%**	0.19±0.04 38.7%***	0.25±0.04 28.5%***
Compound M5i (Inhibition %)	0.014±0.02 17.6%***	0.10±0.02 28.5%***	0.16±0.03 36.0%***	0.12±0.02 57.1%*	0.21±0.04 32.2%***	0.26±0.05 25.7%***
Compound M5j (Inhibition %)	0.016±0.03 5.8%***	0.10±0.02 28.5%	0.15±0.01 40.0%**	0.10±0.03 64.2%*	0.22±0.04 29.0%***	0.26±0.03 25.7%***
Compound M5k (Inhibition %)	0.014±0.01 17.6%***	0.11±0.02 21.4%***	0.19±0.04 24.0%***	0.15±0.01 46.4%**	0.21±0.04 32.2%***	0.24±0.03 31.4%***
Compound M5l (Inhibition %)	0.017±0.02 -----	0.12±0.02 14.2%***	0.18±0.03 28.0%***	0.20±0.03 28.5%***	0.25±0.04 19.3%***	0.29±0.05 17.1%***

* Extremely significant (P<0.01), * *Significant (p< 0.05), ***- Not significant (P> .05)

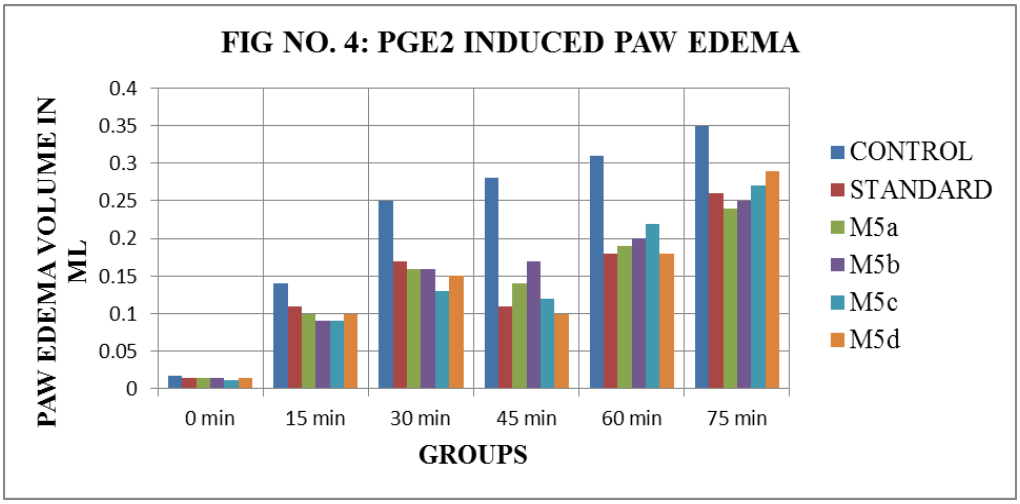


Fig no. 4: Comparative Representation of Pge2 Induced Paw Edema of m5a- m5d.

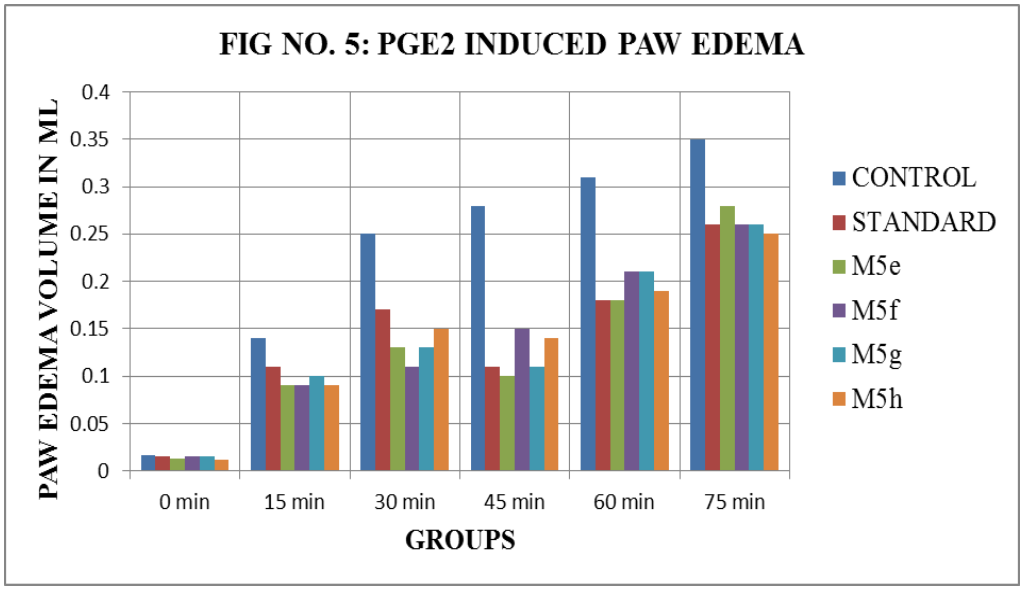


Fig No. 5: Comparative Representation of pge2 induced paw edema of m5e- m5h.

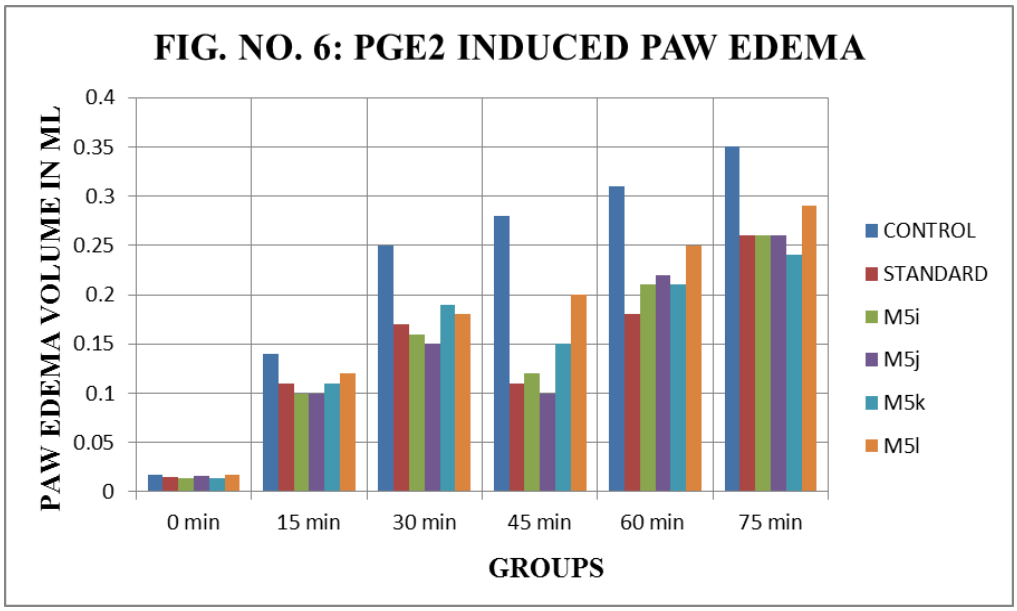


Fig no 6: Comparative Representation of pge2 induced paw edema of m5i- m5l.

Discussion

All the synthesized compounds have given the satisfactory result for anti inflammatory action. All have given the best inhibition at 45 min with maximum inhibition of 64.2% by compound M5d and M5e.

3.4 Table 3: Effect of the synthesized compounds against EPP induced ear edema in rats

Group	ear edema thickness (mm)			
	15 min	30 min	60min	120min
Control	0.583±0.04	0.597±0.01	0.575±0.04	0.589±0.05
Standard (Inhibition %)	0.477±0.03 18.1%***	0.487±0.01 18.4%**	0.437±0.03 24.0%*	0.429±0.04 27.1%*
Compound M5a (Inhibition %)	0.522±0.01 10.4%***	0.515±0.04 13.7%***	0.483±0.03 16.0%**	0.445±0.01 24.4%*
Compound M5b (Inhibition %)	0.524±0.04 10.1%***	0.511±0.03 14.4%***	0.484±0.01 15.8%**	0.447±0.04 24.1%*
Compound M5c (Inhibition %)	0.512±0.02 12.1%***	0.509±0.03 14.7%**	0.483±0.02 16.0%**	0.452±0.05 23.2s%*
Compound M5d (Inhibition %)	0.515±0.01 11.6%***	0.510±0.02 14.5%**	0.480±0.03 16.5%**	0.450±0.04 23.5%*
Compound M5e (Inhibition %)	0.523±0.02 10.2%***	0.515±0.02 13.7%**	0.470±0.03 18.2%**	0.447±0.03 24.1%*
Compound M5f (Inhibition %)	0.525±0.01 09.9%***	0.518±0.03 13.2%**	0.484±0.01 15.8%**	0.445±0.01 24.4%*
Compound M5g (Inhibition %)	0.522±0.01 10.4%***	0.530±0.02 11.2%***	0.491±0.01 14.6%**	0.441±0.03 25.1%*
Compound M5h (Inhibition %)	0.522±0.01 10.4%***	0.512±0.01 14.2%**	0.482±0.02 16.1%**	0.454±0.03 22.9%*
Compound M5i (Inhibition %)	0.524±0.03 10.1%***	0.517±0.01 13.4%**	0.488±0.02 15.1%**	0.452±0.03 23.2%*
Compound M5j (Inhibition %)	0.516±0.01 11.5%***	0.510±0.02 14.5%**	0.485±0.02 15.6%**	0.446±0.04 24.2%*
Compound M5k (Inhibition %)	0.524±0.02 10.1%***	0.511±0.03 14.4%**	0.482±0.02 16.2%**	0.445±0.2 24.4%*
Compound M5l (Inhibition %)	0.527±0.04 9.6%***	0.522±0.03 12.5%***	0.498±0.02 13.3%**	0.490±0.04 16.8%**

* Extremely significant (P<0.01), * *Significant (p< 0.05), ***- Not significant (P> .05)

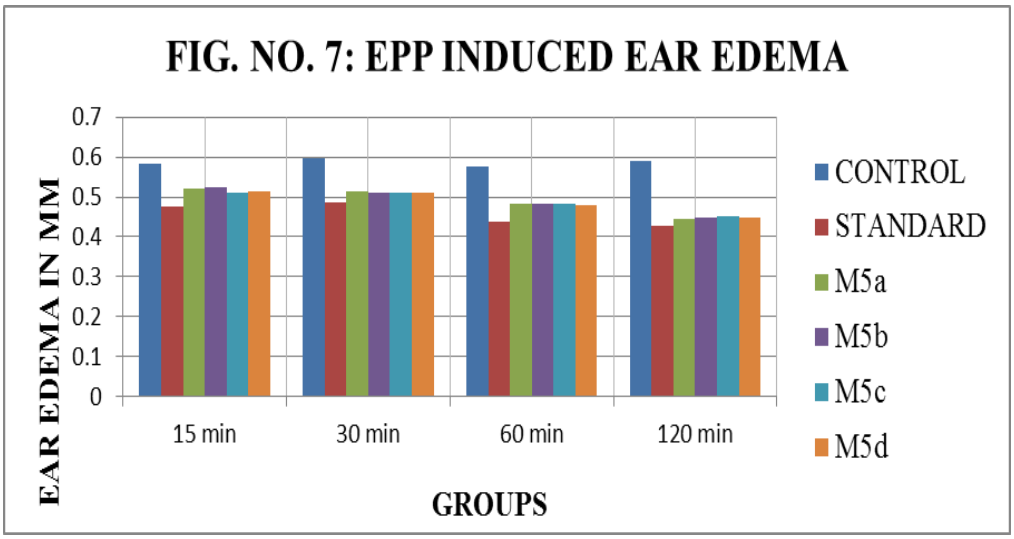


Fig. No. 7: Comparative Result of epp induced ear edema of m5a- m5b.

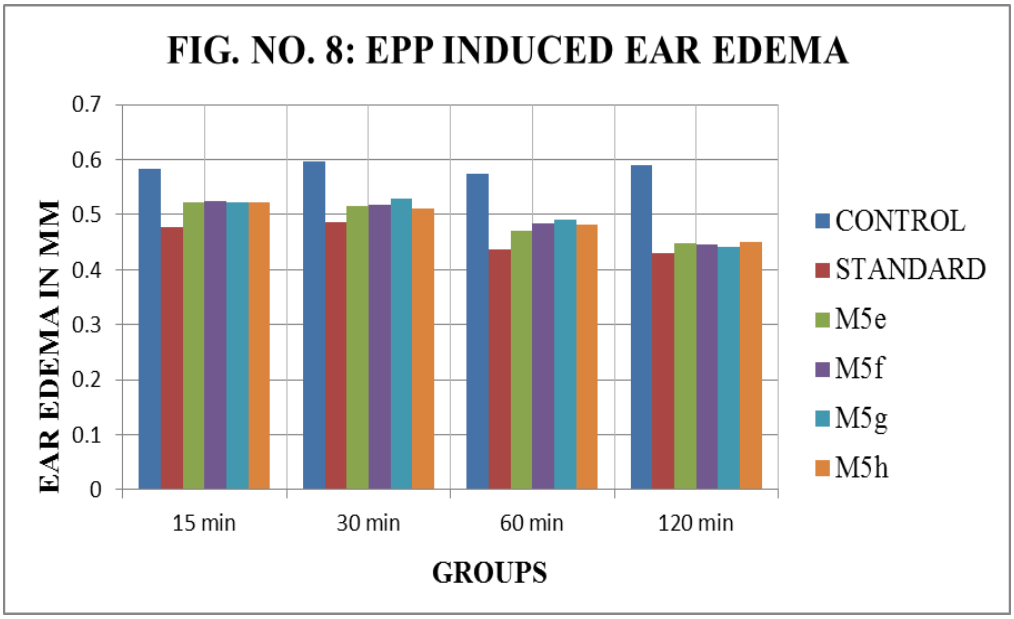


Fig. No. 8: Comparative Result of epp induced ear edema of m5e- m5h.

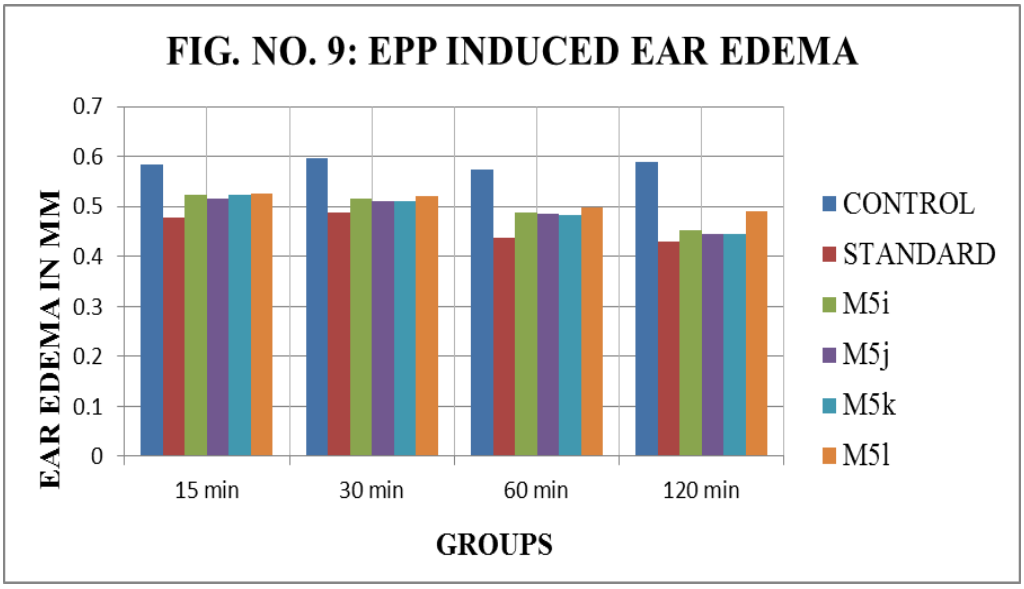


Fig. No. 9: Comparative Result of Epp Induced Ear Edema of M5i- M5l.

DISCUSSION

Inhibition of acute inflammation by synthesized compounds (M5a- M5l) was clearly seen in Table 3. The compounds significantly inhibited ear edema when compared to a control group. During the 1st hour of assessment time the percentage inhibition of ear edema in rats was lower than that of rats treated with phenylbutazone (1 mg/ear). Nevertheless, the compounds displayed a comparable percent of inhibition to the anti-inflammatory drug at 2 h after topical application.

4. CONCLUSION

This study was performed to synthesize P38 Kinase inhibitors and to evaluate their anti-inflammatory activity using various models to clarify the pain and inflammation relieving effects. The various results show that the synthesized compounds posses the anti-inflammatory effects.

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