

## SYNTHESIS OF NOVEL DRUGS OF P38 KINASE INHIBITOR

**Mithilesh Singh\*, Sonu Sharma and Dr. Ashish K. Sharma**

Nims Institute of Pharmacy, Nims University, Shobha Nagar, Jaipur.

**\*Corresponding Author: Prof. Mithilesh Singh**

Nims Institute of Pharmacy, Nims University, Shobha Nagar, Jaipur.

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### ABSTRACT

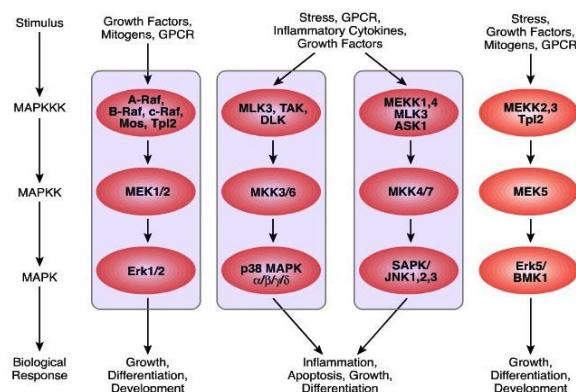
The MAP kinase p38 has been implicated in cytokine signaling, and its inhibitors are potentially useful for the treatment of arthritis and osteoporosis. Novel small-molecule inhibitors of p38 kinase were derived from a combinatorial chemistry effort. The p38 protein kinase is a serine-threonine mitogen activated protein kinase, which plays an important role in inflammation and arthritis. Many p38 $\alpha$  inhibitors with diverse chemical structures and modes of protein interaction have been designed on the basis of their ability to compete with ATP site or Allosteric site for binding to p38 $\alpha$ . In the late 1970's and early 1980's the initial p38 chemo type, triaryl imidazole was discovered. During the last ten years a number of novel p38 chemotypes were discovered via high through put screening. A step has been taken forward to give a little contribution in the synthesis of p38 kinase inhibitors to make it more effective.

**KEYWORDS:** MAPK, p38 kinase, allosteric site.

## 1. INTRODUCTION

### 1.1. P<sup>38</sup> mitogen-activated protein kinases

P<sup>38</sup> 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. P<sup>38</sup> 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 P<sup>38</sup> MAP kinase, p<sup>38</sup>- $\alpha$  (MAPK14), - $\beta$  (MAPK11), - $\gamma$  (MAPK12 or ERK6) and - $\delta$  (MAPK13 or SAPK4) have been identified. Similar to the SAPK/JNK pathway, <sup>2</sup> P<sup>38</sup> 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 p<sup>38</sup> MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 and to phosphorylate the transcription factors ATF-2, Mac and MEF2.



**Figure 1.4.1: 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- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ). 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- $\alpha$  from stimulated inflammatory cells, regulated by p38 MAP kinase. P38 kinase belongs to a group of serine/threonine kinase that includes c-Jun NH<sub>2</sub> 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 Inflammation

Inflammation is defined as local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues.

Inflammation is distinct from infection – the former being a protective response by the body the later is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basis processes with some overlapping early *inflammatory response* and later followed by *healing*.

#### 1.1.1. Causes of inflammation

- *Physical agents* like heat, cold, radiation, mechanical trauma.
- *Chemical agents* like organic and inorganic poisons.
- *Infective agent* like bacteria, viruses and their toxins.
- *Immunological agents* like cell mediated and antigen antibody reactions.

#### 1.1.2. Signs of inflammation.

The roman writer Celsus in 1<sup>st</sup> century A.D. name the famous four *cardinal signs of inflammation* as:

- **Rubor** (redness),
- **Calor** (increased heat),
- **Tumor** (swelling),
- **Dolor** (pain).

To these, fifth sign *functio laesa* (loss of the function) was later added by Virchow.

#### 1.1.3. Types of inflammation

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as:

1. *Acute inflammation* is a short duration and represents early body reaction and is usually followed by repair.
2. *Chronic inflammation* is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it includes chronic inflammation from the beginning.

#### 1.1.4. Chemical mediators of acute inflammation

##### 1. Cell derived mediators

1. Vasoactive amines (Histamine, 5-hydroxytryptamine)
2. Arachidonic acid metabolites (Eicosanoids)
  - a. Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A<sub>2</sub>, prostacyclin)

- b. Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes)
3. Lysosomal components
4. Platelets activating factors
5. Cytokines (IL-1, TNF- $\alpha$ , TNF- $\beta$ , IF- $\gamma$ , chemokines)
6. Nitric oxides and oxygen metabolite.

##### 2. Plasma derived mediators (Plasma Proteases)

These are the product of:

1. The kinin system
2. The clotting system
3. The fibrinolytic system
4. The complement system

##### Cell derived mediators

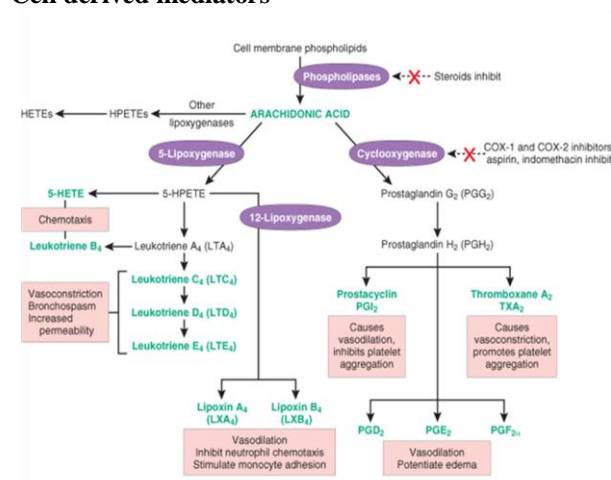


Figure No1.1.4.1: Generation of arachidonic acid metabolites and their roles in inflammation. The molecular targets of some anti-inflammatory drugs are indicated by a red X.

##### Plasma derived mediators

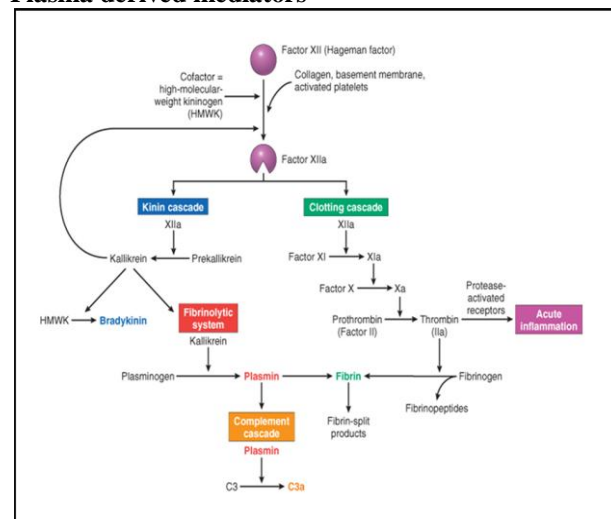
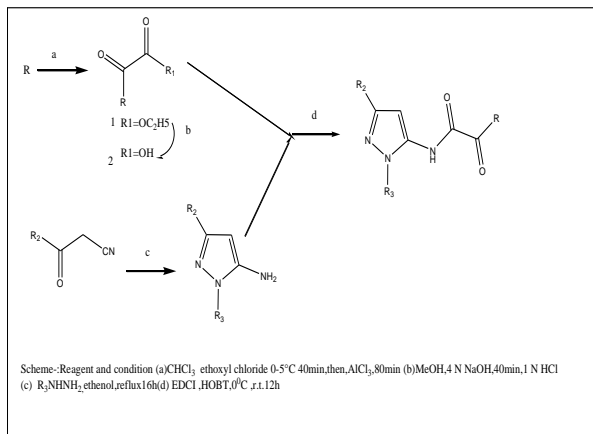


Figure No1.1.4.2: Interrelationships between the four plasma mediator systems triggered by activation of factor XII (Hageman factor). Note that thrombin induces inflammation by binding to protease-activated receptors (principally PAR-1) on platelets, endothelium, smooth muscle cells and other cells.

## 2. MATERIAL AND METHODS

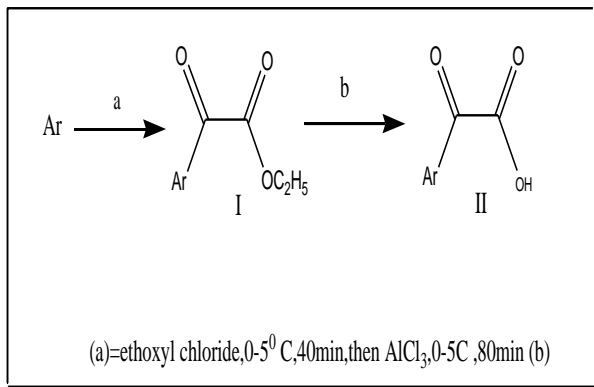
### 2.1. Scheme

The scheme was planned accordingly to synthesize the  $\alpha$ -keto amides, the key intermediates, substituted phenyl glyoxalic acid have been synthesized by acylation of substituted benzene and characterized. The acid further will be coupled with several aromatic and heterocyclic amines in presence of coupling agent to afford desired  $\alpha$ -keto amides



## EXPERIMENTAL SECTION

### Aryl $\alpha$ -ketoester (I) and $\alpha$ -keto acid (II)



(b)=MeOH, 4N NaOH, r.t., 40min, (2)=1N HCl

### 2.2. Procedure (1)

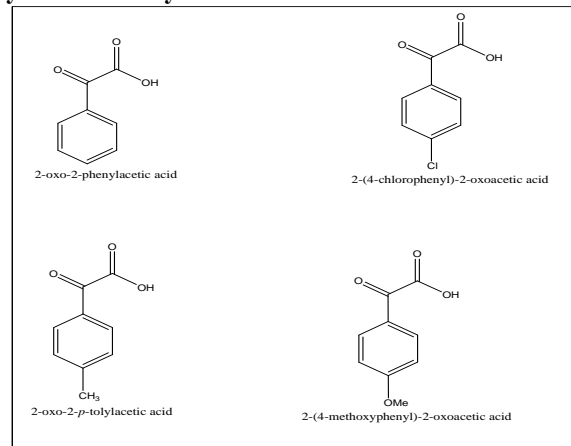
The substitute benzene (0.05 mol) and monoethyloxalyl chloride (0.05 mol) were stirred in 100 ml of  $\text{CHCl}_3$  and kept at  $0-5^\circ\text{C}$  on the ice bath. Powdered  $\text{AlCl}_3$  (0.1 mol) was added into well-stirred reaction mixture for 20-40 min. The reaction mixture was poured into ice water and then  $\text{CHCl}_3$  was added; this mixture was extracted two times and the  $\text{CHCl}_3$  solution was washed with a 10% solution of sodium hydrogen carbonate and then with water and dried with  $\text{MgSO}_4$ . The crude product thus obtained was purified over silica and characterised by NMR.

### Aryl $\alpha$ -keto Acid (II)

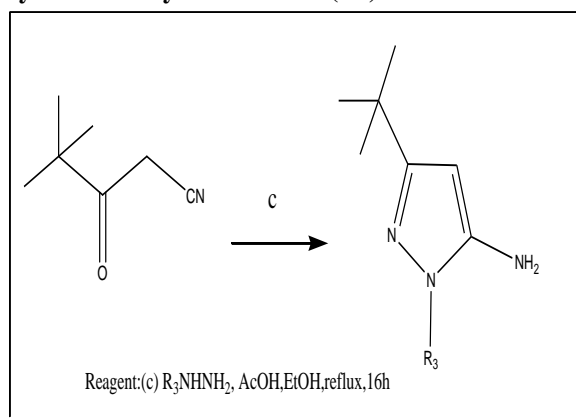
Acid was dissolved in MeOH at  $0^\circ\text{C}$  (cooling) 4 N NaOH was added and allowed to stir for 30 min with TLC monitored. After completion of reaction MeOH was

removed. Reaction mixture was neutralized with 1N HCl, compound extracted into EtOAc dried over  $\text{Na}_2\text{SO}_4$ .

### Synthesized Aryl $\alpha$ -keto Acid



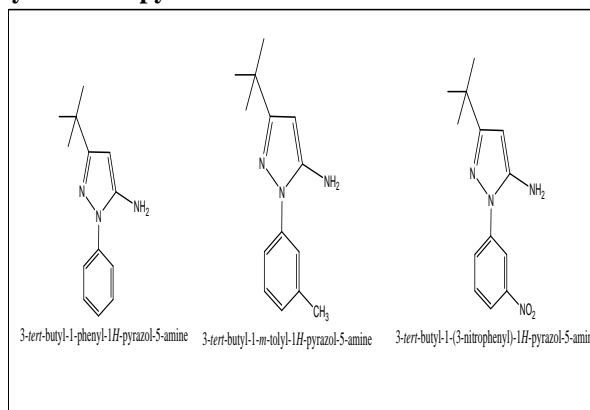
### Synthesis of Pyrazole amine (III)

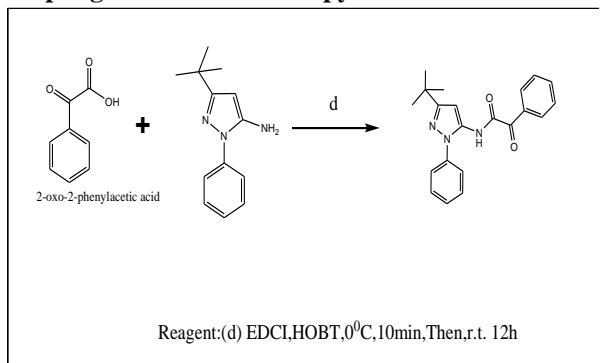
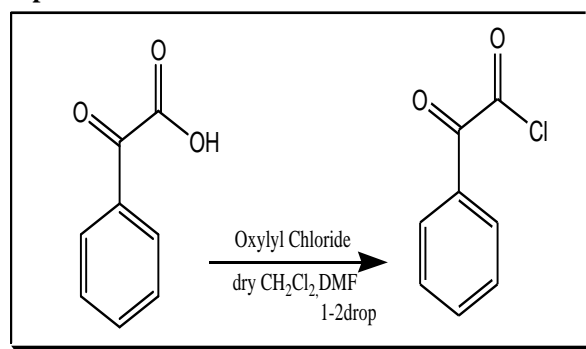
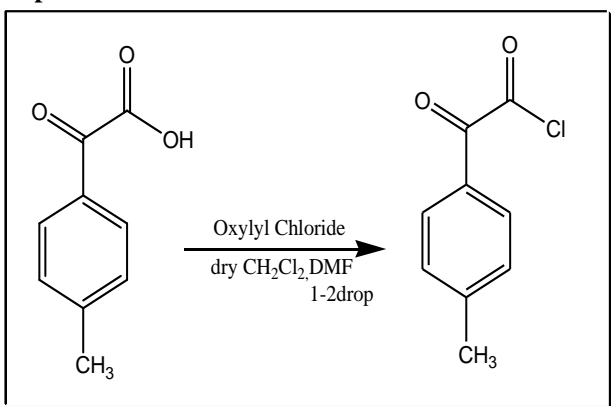
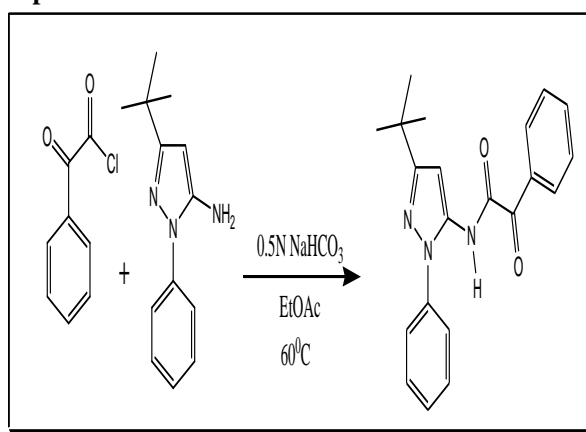
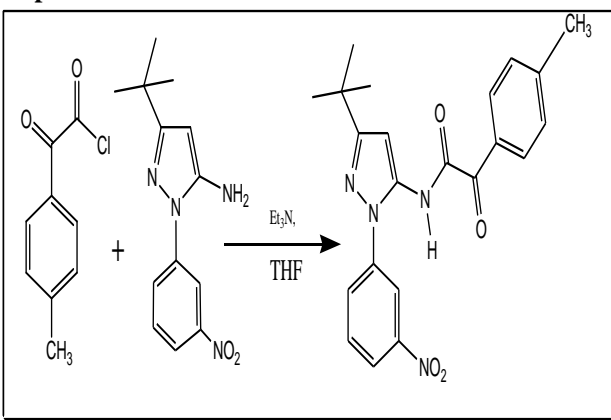
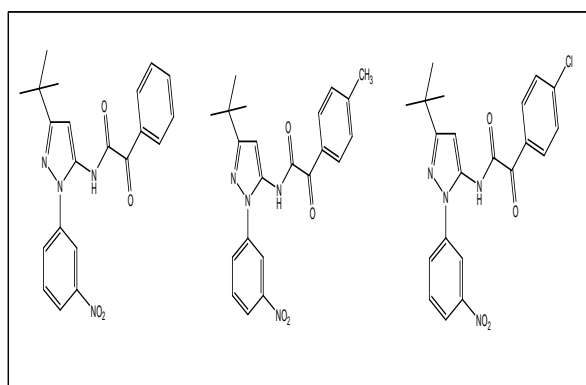
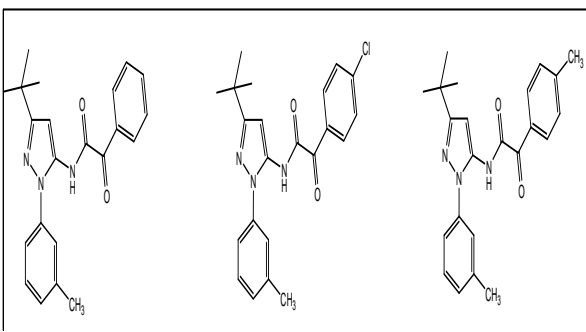
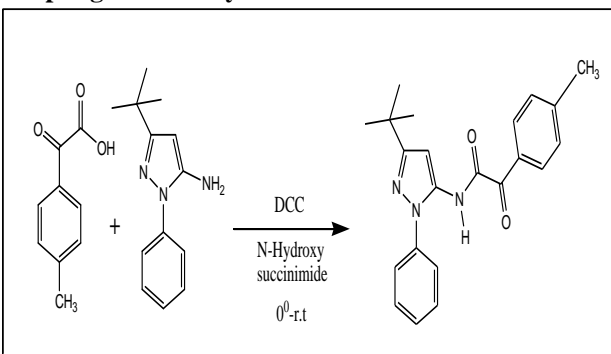


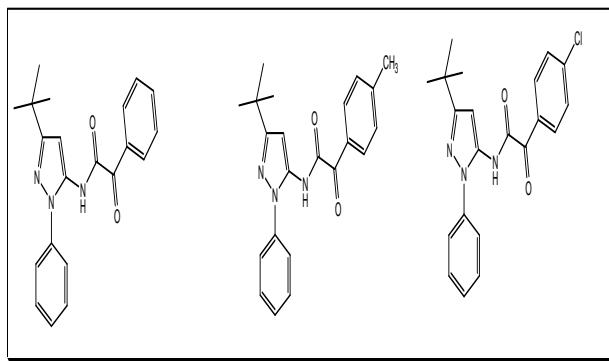
### Procedure

A mixture of 3-oxo- alkane nitrile (6.53mmol), aromatic hydrazine (6.53mmol) and AcOH (0.3ml) in EtOH (10-12ml) was heated at the reflux temperature over night. Then cooled down to rt. and concentrated under reduced pressure. The solid residue was washed with  $\text{Et}_2\text{O}$ , suspended in EtOAc and treated with 300ml of 1 M  $\text{NaHCO}_3$ . The organic layer was separated, washed with brine, dried with  $\text{Na}_2\text{SO}_4$ .

### Synthesized pyrazole amine



**Coupling of  $\alpha$ -keto acid and pyrazole amine****Step-1****Other coupling reactions****Step-1****Step-2****Step-2****SYNTHESIZED ANALOGUES****1. NPPB****2. NPPT****3. NPPC****Coupling reaction by DCC****4. MPPB****5. MPPC****6. MPPT**



7. PPAB

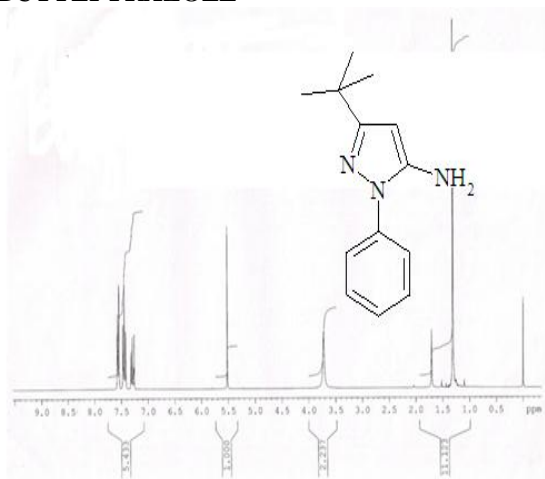
8. PPAT

9. PPAC

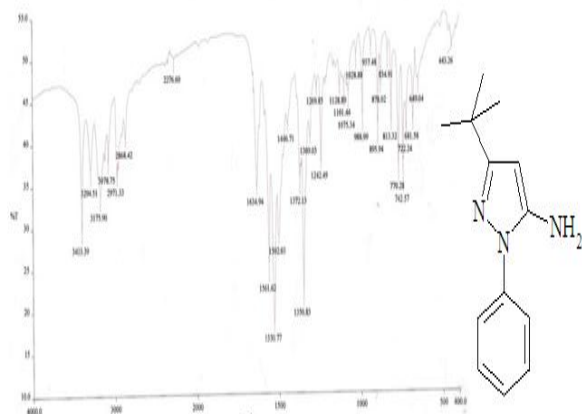
### 3. RESULT

After the synthesis of the various compounds, 5-amino, 1-phenyl-3-t-butylpyrazole (main compound) was studied under NMR spectrophotometer and IR spectrophotometer. Following results were obtained which shows that the synthesis was correct.

#### <sup>1</sup>H NMR SPECTRUM OF 5-AMINO, 1-PHENYL-3-T-BUTYLPYRAZOLE



#### IR SPRCTEUM OF 5-AMINO, 1-PHENYL-3-T-BUTYLPYRAZOLE



### CONCLUSION

Increased understanding of signal transduction mechanisms and gene regulation involved in immune responses has created opportunities for the discovery of novel therapeutic compounds useful in treating inflammatory disorders. Drugs which are binding to allosteric binding site of p38 $\alpha$  are selective and potent inhibitors of p38 kinase as compared to drugs which bind to ATP binding site of p38 $\alpha$ . Pyrazolyl ureas and its derivatives were identified as selective and potent p38 kinase inhibitors as they selectively binding to allosteric binding site of p38 kinase.

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