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Review Article

A REVIEW OF p38 KINASE INHIBITORS AS ANTI-INFLAMMATORY DRUG TARGETS

D.Swapna*, V.Prathyusha Pooja, D.Sucharitha, K.Sunitha, M.Swetha.
Email: swapna_pharmacy@yahoo.co.in

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ABSTRACT:

The p38 protein kinase is a serine-threonine mitogen activated protein kinase, which plays an important role in inflammation and arthritis. p38 subgroup of the mitogen activated protein kinase super family has four isoforms: p38α, p38β, p38δ, p38γ. p38α is involved in inflammation, proliferation, differentiation and apoptosis. The biological functions of p38β, p38δ, p38γ are not understood completely. Many p38α inhibitors with diverse chemical structures and modes of protein interaction have been designed on the basis of their ability to compete with ATPsite or Allostericsite for binding to p38α. 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. Among several novel p38 chemotypes developed, pyrazolyl ureas and its derivatives were identified as potent and selective p38 kinase inhibitors.

Key Words: MAPK, p38 kinase, pyrazolylurea.

INTRODUCTION:

Inflammation (Latin, inflammatio, to set on fire) is the complex biological response of vascular tissue to harmful stimuli, such as pathogens, damaged cells or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. The inflammatory process is regulated by the actions of various inflammatory mediators. It normally leads to repair of structure and function and thus is essential for the survival of an organism.

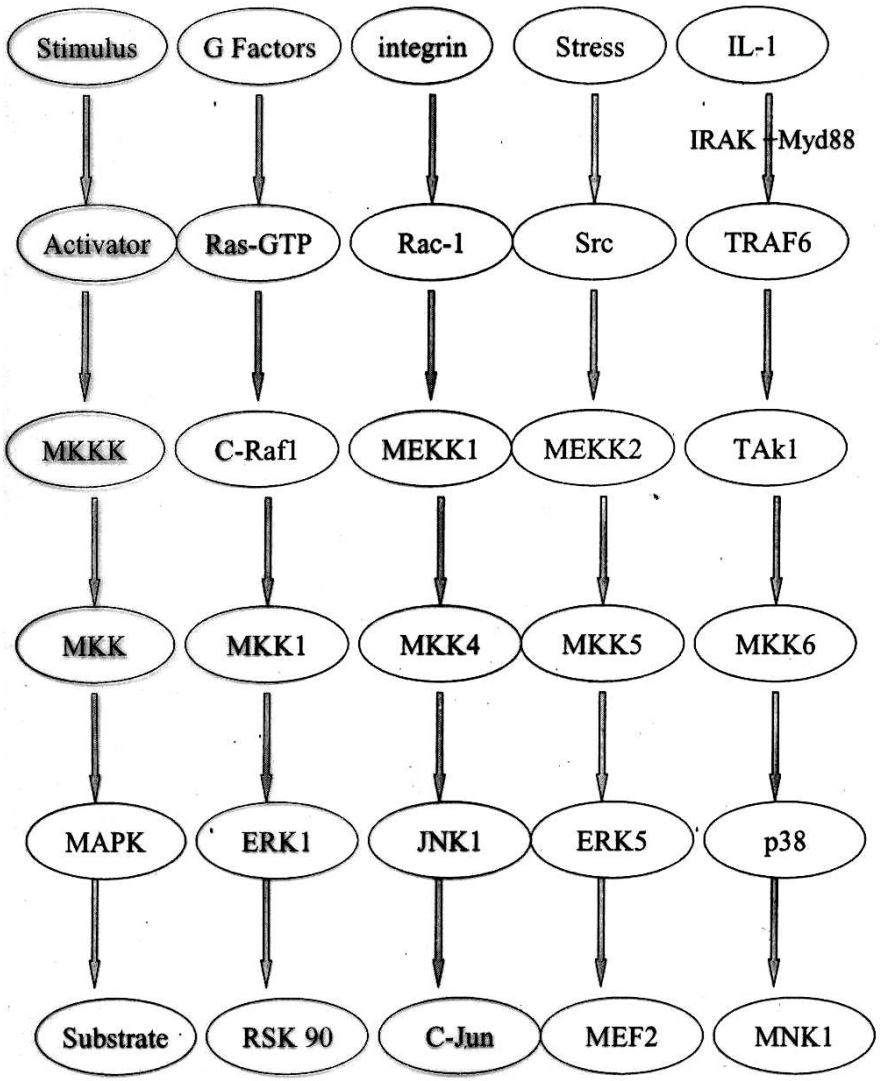
Mitogen activated protein kinases (MAP kinase) play crucial roles in the signal transduction pathways and are activated by various extracellular signals triggered by growth factors, physicochemical stress and cytokines. Four major cascades of MAP kinases have been reported namely, extracellular signal regulated kinases [ERK], c-Jun N-terminal kinase or stress activated protein kinase (JNK/SAPK), ERK5 or big MAP kinase (BMK1) and p38 group of MAP kinases.

The activation of MAP kinases involves dual phosphorylation of thereonine and tyrosine residues in TXY motif that are located in the regulatory loop.

TARGETING MAP KINASE PATHWAYS FOR ANTI-INFLAMMATORY DRUG DEVELOPMENT

The MAP kinase signaling pathways are new hunting grounds for pharmaceutical companies. Developing the drugs that interfere with signalling routes and gene expression, aiming at attenuating the proinflammatory response.

MAP kinase cascade



p38 Kinase and its Inhibitors:

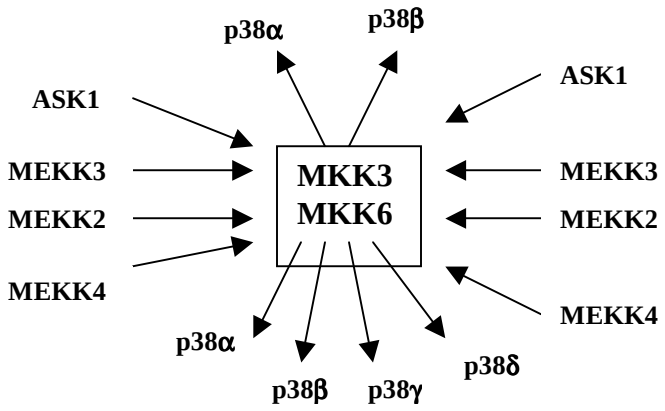
The p38 protein kinase is a serine-threonine mitogen activated protein kinase, which plays a central role in numerous pro-inflammatory responses. There are four isoforms of the p38 kinase (p38α,p38β,p38γ,p38δ)

p38 Isoform	Cellular Expression
p38α	All cell types
p38β	Endothelial cells, T cells
p38δ	Macrophages, Neutrophils, T cells, Monocytes
p38γ	Little or no expression in immune system

Structure of p38 Kinase:

p38 kinase has two domains i.e N-terminal domain and C-terminal domain. These two domains are separated by a channel, where substrates are likely to bind. The N-terminal domain creates a biding pocket for the adenine ring of ATP and the C- terminal domain contains catalytic base, magnesium bindingsite and phoshorylationloop. Eg: The pyridinyl imidazole inhibitors bind in the ATP pocket and is therefore competitive against ATP. Diaryl urea series compounds binds to an allosteric pocket(that is, a site other than the protein's active site) in p38.

p38 Kinase cascade:



Phosphorylated p38 kinase activates:

i) Different transcription factors including activating transcription factor-2 (ATF-2), cAMP response element binding protein (CREB), Elk-1, CHOP (growth arrest and DNA damage inducible gene 153 or GADD 153) and myocyte enhance factor 2c (MEF 2C).

ii) Protein kinases Mnk1 and Mnk2, which regulate the activity of the translational intiation factor elF4E.

iii) Additionally MAPkinase activated protein kinase-2 (MAPKAPK-2) and MAPKAPK-3.Activated MAPKAPK-2 and MAPKAPK-3 kinases inturn phosphorylate the small heat shock protein HSP 25/27 play a role in regulating actin dynamics and provides an actin based cytoprotective response of cells to new environment conditions.

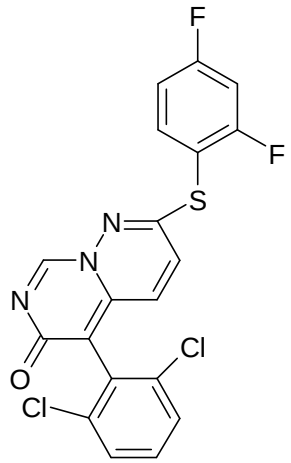
p38 Kinase Inhibitors:

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

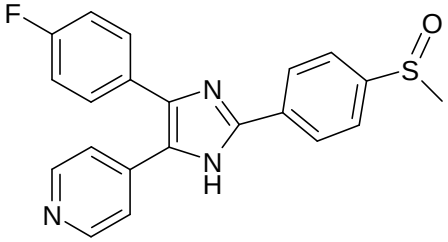
Drugs which compete with ATP:

Drugs which compete with ATP binding site of p38 kinase result in inhibition of MAP kinase cascade i.e inhibit the production of proinflammatory cytokines.

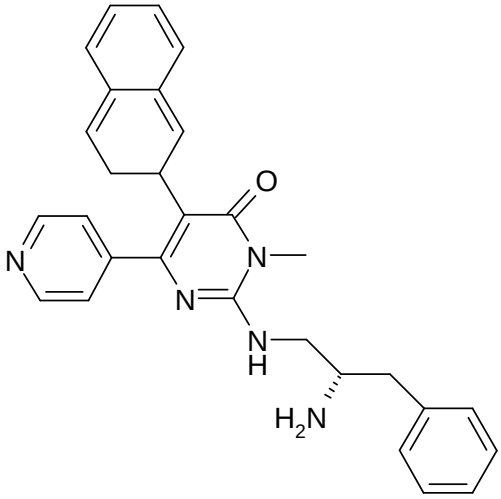
Inhibitor name	Structural class	Mode of interaction with p38α	IC_{50} (nM)	Pharmaco-kinetics	Adverse effects
SB203580	Pyridinyl imidazole	An H-bond between pyrimidine nitrogen and Met109 and m-CF_3 substituted phenyl ring occupies the hydrophobic pocket.	20	-	NA
VX745	Pyrimidopyr-idazinone (Bicyclic 6,6-heterocycle)	H-bonds between the heterocycle carbonyl and Met109 and the heterocycle carbonyl and Gly110 and the occupation of diflurophenyl and dichlorophenyl groups in the deep and lower hydrophobic pockets.	10	-	Neurological effects in dogs.
Bristo-Myer Squibb compound	Substituted Benzamide	H-bond between amide NHandGlu71carboxylate and amide carbonyl and NH of ASP168 and triazine ring nitrogen interact with Met109 and N-methylhomopiperizine nitrogen interact with ASP 168.	44	-	-
RO3201195	Pyrazole ketone (Diaryl ketone)	H-bond between benzoyl oxygen and Met109, two H-bonds between amine and His107and between amine and Thr106 and occupation of N-phenyl ring in deep hydrophobic pocket.	700	Rat t1/2= 1.64 h	-
SCIO469	Indole amide	H-bond between carbonylamide and Met109 and occupation of benzyl group and indole ring in lipophilic pockets.	9	Rat t1/2=30 min	Light headedness, dizziness.
AMG548	Pyrimidinone	H-bond between pyridine nitrogen and Met109 and carbonyl of pyrimidinone and Lys53 and occupation of naphthyl and benzyl groups in deep hydrophobic pocket.	0.5 (Ki)	Rat t1/2=4.6h	Liver enzyme elevations.



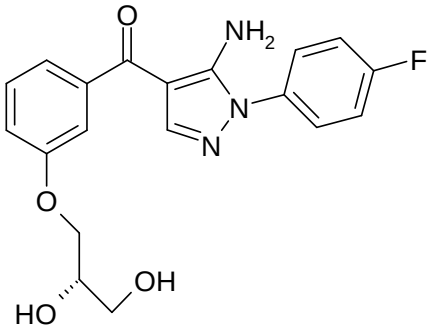
Pyrimidopyridazinone
VX745



Pyridinyl imidazole
SB203580



Pyrimidinone
AMG548



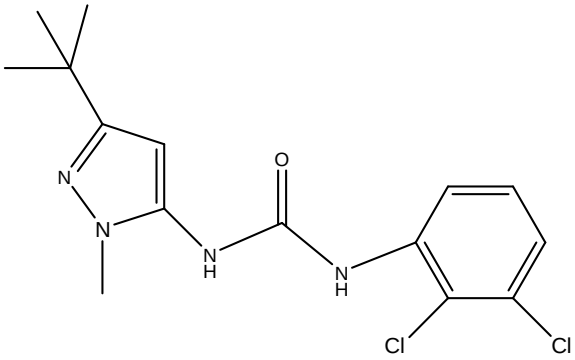
Pyrazole ketone
RO3201195

Drugs which bind to allosteric site:

Allosteric binding site is a site other than the proteins active site, when drug binding to allosteric binding site causes a large conformational change in Asp-phe-Gly (DFG) motif.

Pyrazolyl ureas

Eg: 1-Phenyl-5-pyrazolyl Ureas

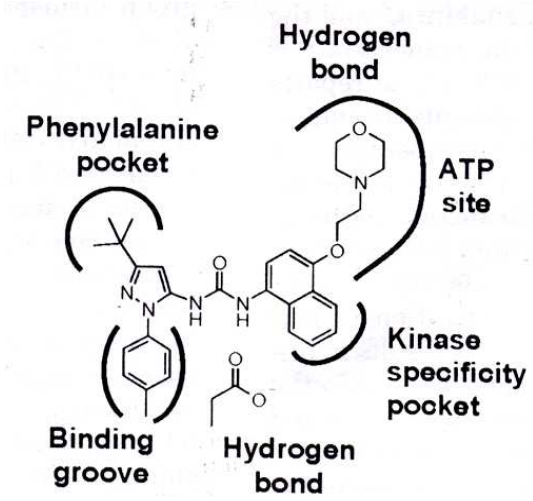


Side effects that are caused by lack of target selectivity is found to be a major problem for many drugs, particularly those that target the protein kinases. In this regard advantage of diaryl urea inhibitors is that they target the unique allosteric site, as a result of which BIRB 796 has shown higher selectivity for p38 kinase over 11 other protein kinases.

PYRAZOLYL UREAS:

Discovery of p38 kinase inhibitor through high through put screening is the discovery of class Pyrazolyl urea p38 inhibitors, discovered by Bayer and elucidation of its binding mode by Boehringer Ingelheim, disclose the unique binding mode of this class of inhibitors as illustrated for BIRB 796. BIRB 796 is 1-(5-tert-Butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalene-1-yl]urea

MOLECULAR MECHANISM:



New allosteric binding site for pyrazolyl ureas are highly potent and selective inhibitors against human p38 MAP Kinase. Molecular mechanism for their inhibitory activity was determined by the crystal structure of p38 MAP Kinase in complex with BIRB 796, binding to allosteric binding site causes a large conformational change in Asp-Phe-Gly(DFG) motif, it is within the active site of the kinase.

DFG-IN Conformation:

In DFG motif Phe 169 residue buried in a hydrophobic pocket in the groove between two lobes of the kinase.

DFG-OUT Conformation:

When Diaryl ureas bind to allosteric site Phe side chain has moved by 10A° to a new position

Additional interactions that are important in the binding of BIRB 796 to p38 include[22]:

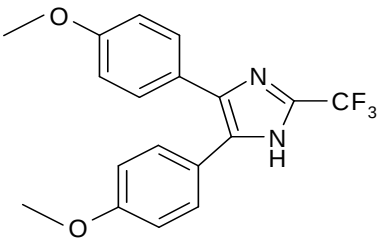
1) The lipophilic interaction of the pyrazole tolyl group with the alkyl side portion of Glu 71.
2) The hydrogen bonding of one of the urea nitrogens with Glu 71 carboxylate moiety.
3) The hydrogen bonding of one of the urea carbonyl with the Asp 168 NH.
4) The lipophilic interaction of the naphthyl ring system in the deep hydrophobic pocket.
5) The hydrogen bonding of the morpholine ring oxygen with the Met 109 residue. This bonding is potent for p38 activity.

Of these interactions the urea hydrogen bonding interactions appears to be the most crucial for this class of compounds, since replacement of either NH of the urea by a methylene or an N-methyl group has been reported to result in a complete loss of p38 activity. Removal of the t-butyl group or replacement of the toluyl group with a methyl or the complete removal of the morpholino ethoxy side chain results in decreased p38 activity relative to BIRB 796 by 20,000 fold (K_d=200 nM), 230 fold (K_d=23nM) and 11 fold (K_d=1.1 nM)

The p38 MAP kinase plays a central role in the regulation of a wide range of immunological responses. The main biological response of p38 activation involves the production and activation of inflammatory mediators, which initiate leukocyte recruitment and activation. But there is a considerable overlap with other signaling routes such as ERK and JNK.

The p38 discovery:

The discovery of the Smithkline series of triaryl p38 inhibitors in part were critical to the discovery of p38 itself. SKF 86002 was first reported as a dual cyclooxygenase / 5-lipooxygenase inhibitor.



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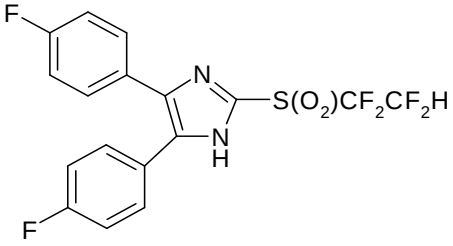
Flumizole



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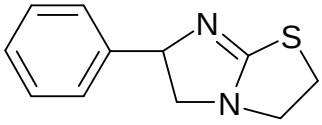
Tiflamizole



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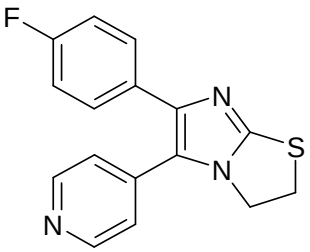
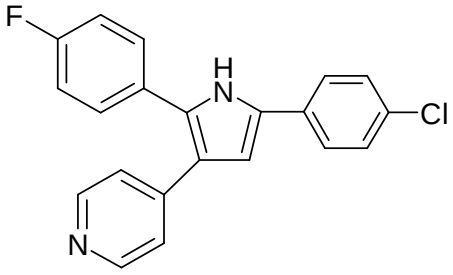
Levamisole



SKF 86002



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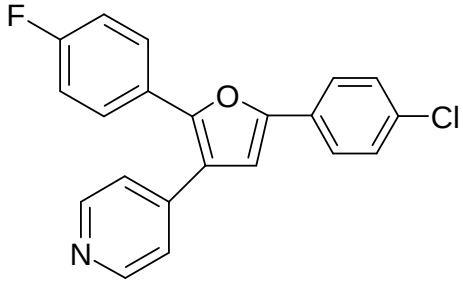

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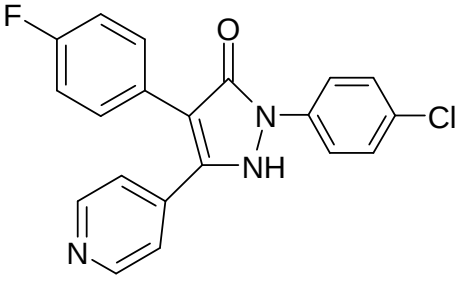

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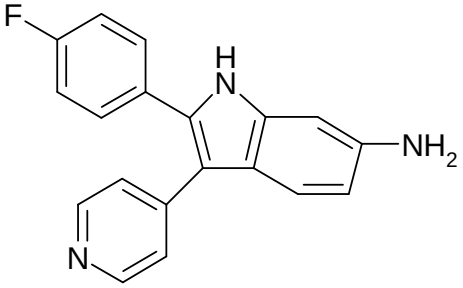
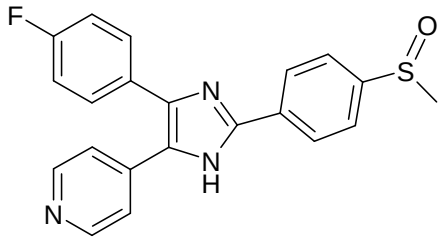


10

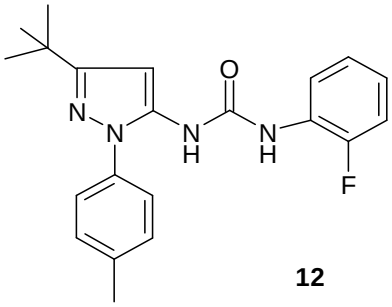
In 1972, researchers at Novartis claimed compound **5** as anti-inflammatory agent, more than two decades prior to the discovery of p38 itself. Numerous novel chemotypes were developed by several groups using the SKF 86002 series as starting point, in addition to the research work examining the effects of substitution around the imidazole core.

A number of novel p38 chemotypes have been discovered in the last few years using a variety of high through put screening approaches. These chemotypes differ significantly not only from the original SKF triaryl imidazoles but also were quite different from any inhibitors for kinases in general.



Pyridinyl imidazole
SB203580

Warrior and co workers at Abbot labs described the process for finding p38 inhibitors via high through put screening, which was based on competitive displacement of tritium labeled SB 20190 (a p38 inhibitor related to SKF 86002). Almholt and co-workers at BioImage carried out a high through put screening in search of p38 inhibitors by redistribution assay which can detect inhibitors that act through non-ATP competitive mechanisms such as compounds that bind at substrate binding site or an allosteric site.



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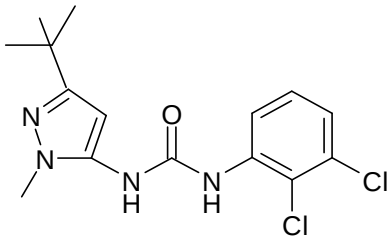
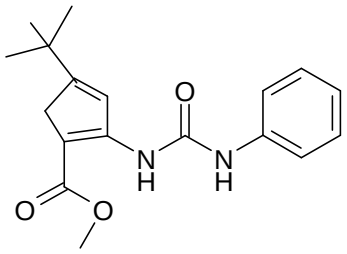


13

p38 IC_{50} = 53 nM



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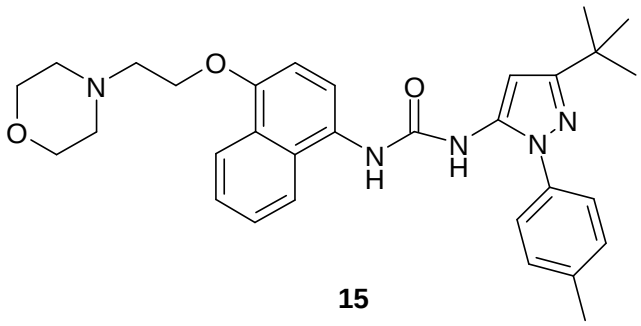
p38 IC_{50} = 290 nM



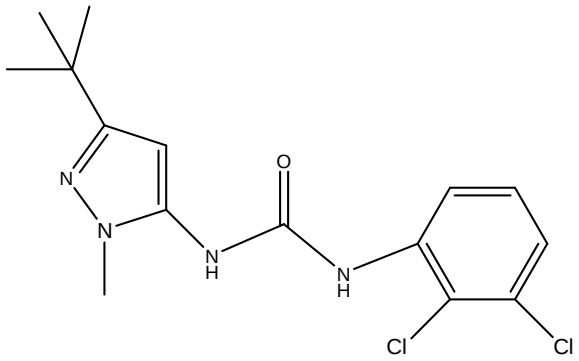
15

BIRB796

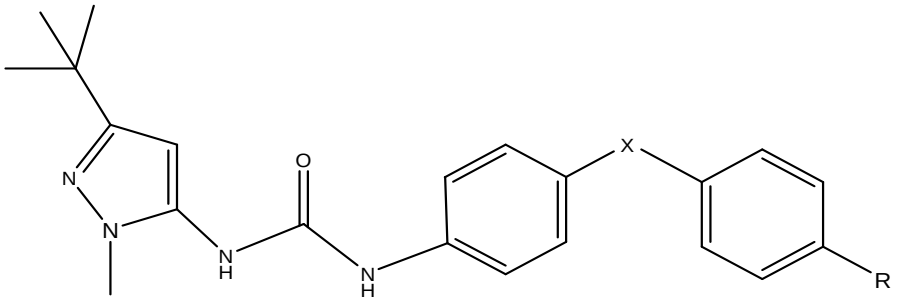
The authors reported compound **12** as one of p38 inhibitor that they discovered from this screen.

The most well known discovery of p38 inhibitors via high through put screening is the discovery of the diaryl ureas, examples of which are compounds **13** is referred to as a screening hit from a combinatorial chemistry effort. These compounds not only structurally distinct from triaryl imidazoles, but ultimately shown to have a different mechanism of p38 inhibition. Researchers at Boehringer Ingelheim showed using protein crystallography that this series inhibits p38 allosterically by displacing the flexible activation loop and causing it to fold into the ATP binding site. Hence they selected this series for an optimization program that ultimately led to the development of their clinical candidate BIRB 796 **(15)**.

Dumas et al. reported a new class of highly potent and selective p38 kinase inhibitors 1-phenyl-5-pyrazolyl ureas **(13)** and evaluated for anti-inflammatory activity.



Dumas et al. optimized the 1-phenoxy phenyl-5-pyrazolyl ureas **(16)** through combined combinatorial and medicinal chemistry effort resulted in improvements of both potency and drug like properties.



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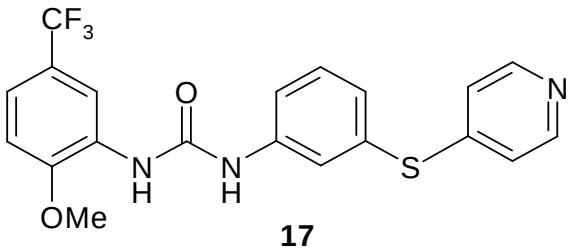

17

Recently compound of structure **17** has been patented, which was synthesized as potent p38 kinase inhibitor.

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 bindingsite of p38α are selective and potent inhibitors of p38 kinase as compared to drugs which bind to ATP binding site of p38α. Pyrazolyl ureas and its derivatives were identified as selective and potent p38 kinase inhibitors as they selectively binding to allosteric bindingsite of p38 kinase.

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ABBREVIATIONS:

MAPK - Mitogen-activated Protein kinase

MKK - Mitogen active Protein kinase kinase

MEKK (MAPKKK, MKKK) - MAP Kinase kinase kinases

IL - Interleukin

TNFα - Tumor necrotic factor α

MAPKAPK - MAPK Activating Protein Kinase

HSP - Heat Shock Protein

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Address for Correspondence:

D. Swapna*
Department of Pharmaceutical Chemistry,
Maheshwara College of Pharmacy,
Patancheru, Hyderabad –502307.
Email: swapna_pharmacy@gmail.com