"Design and Synthesis of Novel Pyridine Containing Tetrazole Derivatives and Evaluation of Anti-inflammatory, Analgesic, Ulceroganic Potential, Antioxidant and Antibacterial Activities"

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in

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BY

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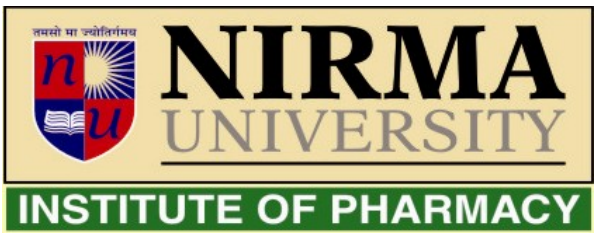

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APRIL 2010

CERTIFICATE

This is to certify that Mr. Keshav Kant Kshatri (08MPH403) has prepared his thesis entitled ***"Design and Synthesis of Novel Pyridine Containing Tetrazole Derivatives and Evaluation of Anti-inflammatory, Analgesic, Ulceroganic Potential, Antioxidant and Antibacterial Activities"****, in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University.*

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DECLARATION

I declare that the thesis entitled ***"Design and Synthesis of Novel Pyridine Containing Tetrazole Derivatives and Evaluation of Anti-inflammatory, Analgesic, Ulceroganic Potential, Antioxidant and Antibacterial Activities"****, under the guidance of Prof. Manjunath Ghate (Guide), Professor* and *Mr. Kuntal Manna (Co-guide), Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously in our institute and elsewhere.*

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No research is ever the outcome of single individual's efforts. This work is also no exception. Some of the personalities teaching and non-teaching staff, friends and well wisher have contributed to and made it possible for me to present in this shape.

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Date:

Place: Ahmedabad **(Keshav Kant Kshatri)**

DEDICATED TO:

MY MUMMY, MY PAPA,

MY BROTHER AND MY SISTER

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A series of novel 2-(5’-(2’’,4’’ –disubstituted phenyl)-1*H*-tetrazol-1-yl)pyridine **(67)** , and 2-(5’-(furan-2’’-yl)-1*H*-tetrazol-1’-yl)pyridine **(73)** were synthesized from 2,4-disubstituted-N-(pyridin-2’-yl)benzimidoyl chloride **66** and N-(pyridin-2’-yl)furan-2-carbimidoyl chloride **(72)** excess amount of sodium azide and sodium acetate at - 2^{o}C. 6-(5’-(2’’,4’’–disubstituted phenyl)-1*H*-tetrazol-1’-yl)pyridine-3-sulfonamide (**69)** was prepared from 6-(5’-(2’’,4’’-disubstituted-phenyl)-1*H*-tetrazol-1’-yl)pyridine-3-sulfonyl chlorides **(68)** in presence of liquor ammonia., Compound **(68)** was prepared from compound **(67)** with chlorosulfonic acid. 2,4-disubstituted-N-(pyridin-2’-yl)benzamides **65** and N-(pyridin-2’-yl)furan-2-carboxamide **(71)** were prepared by reacting with 2-amino pyridine **(63)** and 2.4-disubstituted benzoyl chloride **(64)** and corresponding furan-2-carbonyl chloride **(70)** in presence of TEA and DCM, which was further treated with PCl_5 to obtained 2,4-disubstituted-N-(pyridin-2’-yl)benzimidoyl chloride **(66)** and N-(pyridin-2’-yl)furan-2-carbimidoyl chloride **(72)**. All the synthesized compounds were evaluated for anti-inflammatory, Analgesic, ulceroganic potential, antioxidant and antibacterial activities. Most of the compounds showed significant *in-vivo* anti-inflammatory and analgesic activities. Compounds KPT-Cl_2 **(75)** and KPST-Cl_2 **(76)** were found to be the most potent among all the synthesized compounds. Low ulceroganic potential activity was done in rats and found reduced and equal activity against standard drugs diclofenac sodium and nimesulide by compounds KPT-Cl_2 **(75)** and KPST-Cl_2 **(76)**. KPT-Cl_2 **(75)**, KPST-Cl_{24} **(80)** and KST-Me_4 **(81)** were produced good in-vitro antioxidant activity by DPPH method. In antibacterial screening was done by ager diffusion method against *E. coli* and *S. aureus.* Compounds KPT-Cl_2 **(75)**, KPST-Cl_{24} **(80)** and KPST-Me_4 **(82)** were found good potential against *S. aureus.*

1.1 INTRODUCTION

Most of the active drug molecules consist of five-member and six member heterocyclic rings are widely distributed in nature and often play an important role in various biochemical processes. As a result they are incorporated into new chemical entities by medicinal chemists[1]. Tetrazole and pyridine rings directly fused or coupled through carbon and nitrogen bridges may be produce potent biological activities. Several researcher and biologist interest to explore and synthesised such similar molecules for better activity and lesser toxicity during last two decades.

Tetrazole nucleus has attracted the attention of many medicinal chemists due to its interesting and wide range of biological activities like anti-inflammatory activity [2,] analgesic activity [3], antibacterial activity [4], antifungal activity [5], antiviral activity [6], anticholinergic activity[7], antiasthmatic[8] and antihypertensive activity[9], antiemetic activity[10], estrogen agonist and antagonist activities[11,] anticonvulsant activity[12]. Last few decades tetrazole moiety is the centre of research interest due to its stable molecular structure, greatest number of nitrogen atoms, synthetic methods, chemical and physicochemical properties. Thus, tetrazole exhibit the extreme values of acidity, basicity, zwitterions, complex formation and specific thermo chemical properties [13]. Pyridine is extensively studied due to their occurrence in living systems. Pyridine containing compounds have been reported as antibacterial[14], antifungal agents[14], herbicidal[14], bacteriostatic[14], antiviral[14], and antitumor[14] and anti-HIV activity[15], anti-inflammatory activity[16] and analgesic[17], antiparkisonian[18], anticonvulsant activity[18] and antihypertensive activity[19].

Tetrazole and pyridine ring alone having good and versatile biological activities already reported and fused heterocyclic ring also reported, but combined rings through carbon-carbon bond and small linkages not yet studied for anti-inflammatory, analgesic, antibacterial, antioxidant activities. Total eleven tetrazole and pyridine containing novel were synthesized and evaluated for anti-inflammatory. Recently most of the anti-inflammatory agents (COX-I & II) are produce severe side effect, for that, they are force to withdrawn from potential anti-inflammatory market. Hence, lack of low cast anti-inflammatory agents in the market for third world country. Therefore we plan to design tetrazole containing pyridine moiety which may be solve the recent burden of anti-inflammatory tragedy and fulfill the crises.

Inflammation is a complex phenomenon involving interrelationships of humoral and cellular reactions through a number of inflammatory mediators. It is a usual symptom covering different pathologies, and there are still many questions to be answered in order to understand the inflammatory process as well as a need for better-tolerated and more efficient non-steroidal anti-inflammatory drugs[19]. All classical NSAIDs such as aspirin, ibuprofen, and indomethacin, can inhibit both COX-1 and COX-2, but bind more tightly to COX-1[19]. Selective COX-2 inhibitors are proving to have the same anti-inflammatory, anti-pyretic, and analgesic activities as do nonselective NSAID inhibitors, but its having few or none of their gastrointestinal side-effects. Research on the nonsteroidal anti-inflammatory and analgesic drugs is receiving continuous interest in industrial and academic laboratories. Due to frequent presence with this class of drugs of undesirable side effect, for the gastrointestinal (GI) (i.e., dyspepsia, ulcer, perforation, occlusion, and bleeding) and cardiovascular (CV) system (myocardial infarction, stroke, hypertension, sodium retention with edema and heart failure), which plausibly involve the inhibition of COX-1 and COX-2, respectively[20]. Due to the high toxicity potential of NSAIDs recently many selective COX-2 inhibitor derivatives (celcoxib, rofecoxib and valdecoxib) and nonselective COX inhibitor are now withdrawn in the market.

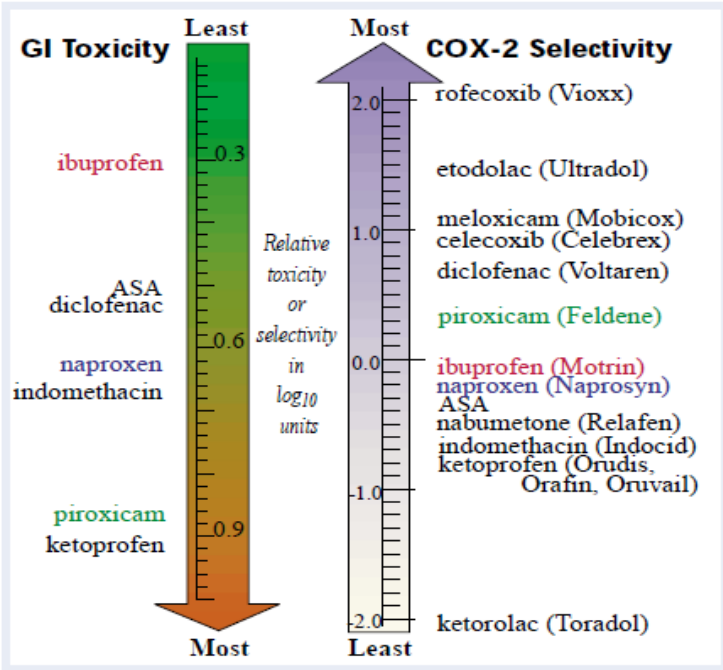
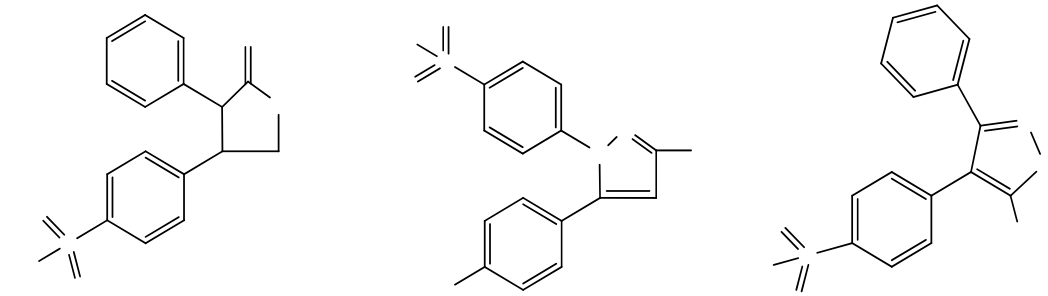


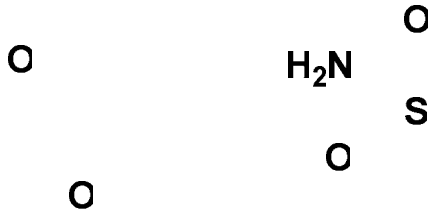
Fig. 1: COX-2 selectivity explain GI toxicity

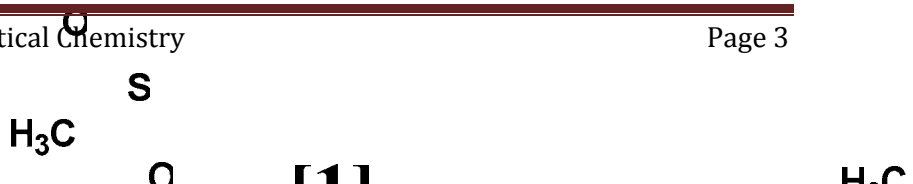
1.2 Aim and scope of study:

Literature survey reveals that pyridine and tetrazole ring is important for anti-inflammatory activity. Tetrazole containing pyridine moiety to possess an interesting biological activities because they are form a zwitterions like phenomena. And may be toxicity is low as compared to other anti-inflammatory agents.

Novel pyridine containing tetrazole derivatives are based on the modification of the structures of the known potent non-selective NSAID inhibitors. . The strategy is intended to obtain potent anti-inflammatory activity without ulcerogenic effects using traditional medicinal chemistry techniques motivated by the comparative pharmacophore modelling of COX-I & II.

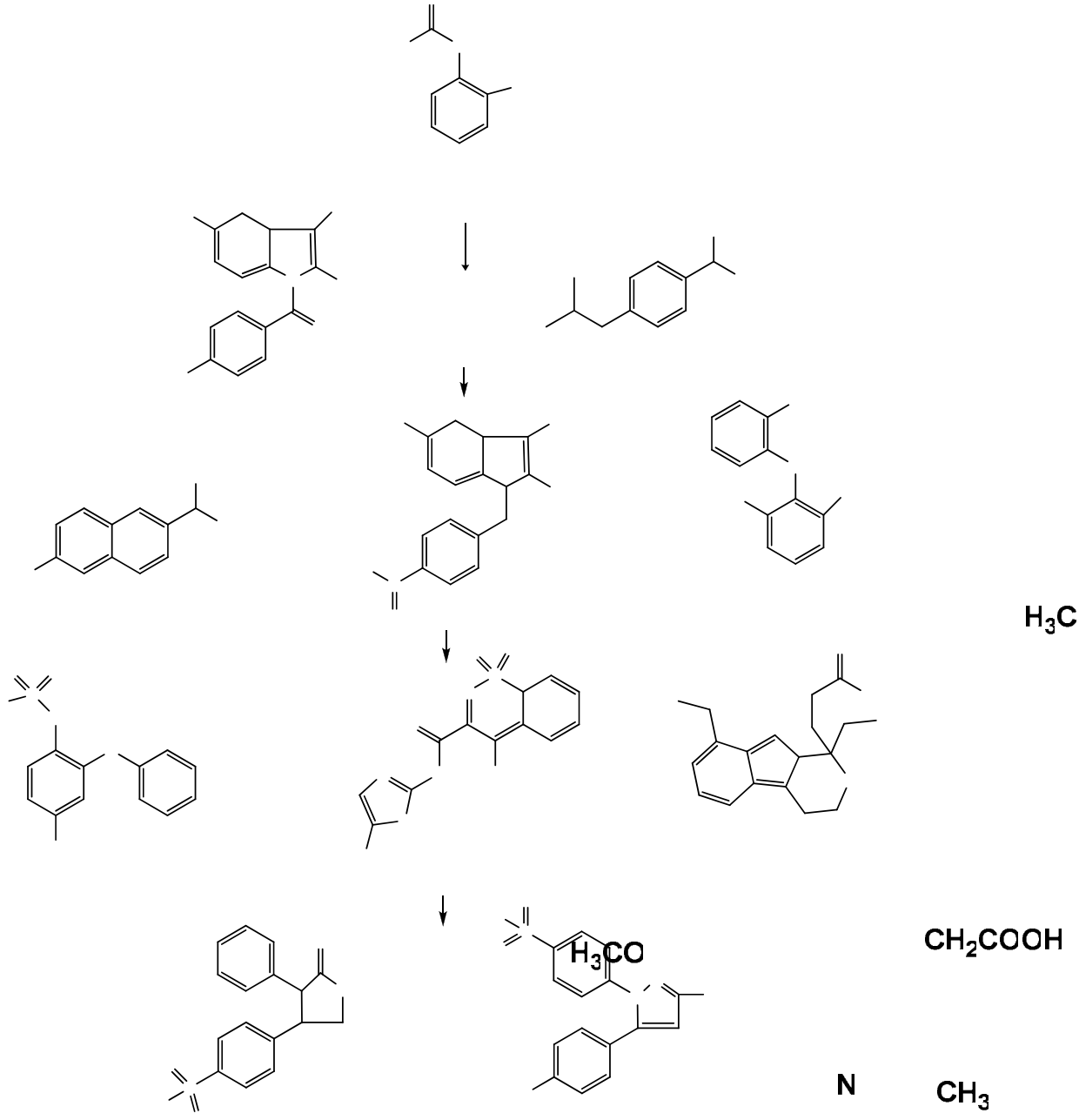






2.1 Introduction:

History of NSAIDs beginning with aspirin (top), the sequesntial development of nonselective competitively acting NSAIDs (middle) followed by preferential COX-2 inhibitors which possess comparatively less selectivity for COX-1 or an intermediate group between selective and non-selective inhibition and finally COX-2 selective inhibitor (bottom).

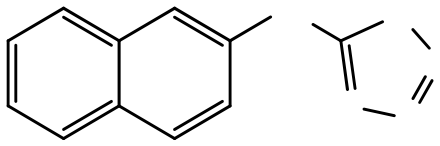


[8]

Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation. However, their therapeutic use is often limited by the common side effects, such as gastrointestinal (GI) haemorrhage and ulceration and also affects the functioning of platelets [21]. NSAIDs work by inhibiting the cyclooxygenase (COX), a key enzyme so preventing the formation of inflammatory prostaglandins from metabolism of arachidonic acid. Since prostaglandins have dual functions, mediation of inflammation and cytoprotection in the stomach and intestine, and possible dissociation of anti-inflammatory effects from GI toxicity is suggested by recent discovery that COX exists in two isoforms, COX-1 and COX-2, which are encoded by two distinct genes. COX- 1 is thought to provide cytoprotection; COX-2 inhibitor might have selective anti-inflammatory properties and lack of GI side effects[22].

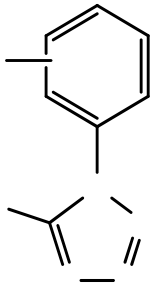
2.2 Review of Tetrazoles:

Disli A. and Salman M., et.al., (2009) Synthesised of Some New 5-Substituted 1*H*-Tetrazoles , A series of 4-Substituted 1-naphthyl selenocyanates **(18)** and thiocyanates, as well as 2-naphthyl selenocyanate synthesised and screened anti-inflammatory activity. It possess both acidic and basic properties, therefore, they can be easily bound to various structures to produce tetrazole-containing drugs or complex molecules stable in acidic and basic media and resistant to oxidants and reducing agents.[23]



[18]

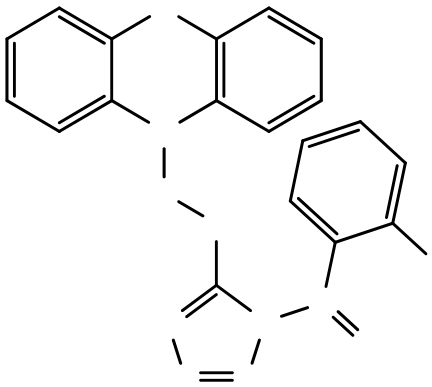
Katritzky A.R., Jain R., Petrukhin, Denisenko S. and Schelenz T., *et.al.,* (2001) Were synthesised a series of 5-Amino-1-Aryl-1*H*-tetrazole **(19)** and evaluate QSAR correlation of the algistatic activity of these derivatives and it possess anti-inflammatory, muscle relaxant and algistatic activity [24]



[19]

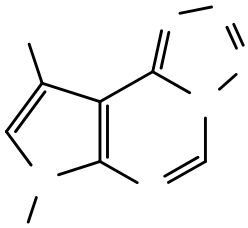
R = H, 3-F, 3-Cl, 3-Me, 3-NO_2, 3-OMe, 4-F, 4-Me, 4-NO_2

Rajasekaran A. and Thampi P.P *et.al.* (2003) were synthesized a series of novel 10-[(-1*H*-tetrazole-5'-yl)ethyl]-10*H*-phenothiazine **(20)** and screened for its anti-inflammatory activity. Compound was found to potent anti-inflammatory activity similar to Diclofenac[2].



[20]

Chaitanya G. Dave and Shah R. D., *et.al.* (2002) were synthesised A series of novel 7,9-disubstituted-7*H*-tetrazolo[1,5-*c*]pyrrolo[3,2-*e*]pyrimidines **(21)** derivatives and screened for antibacterial activity. Compound are found to antibacterial Agents.[25]

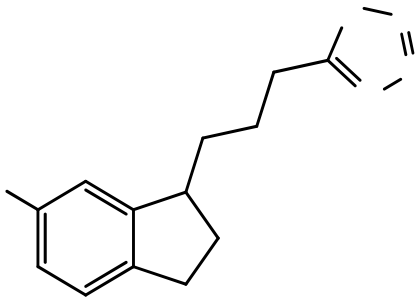


[21]

R = C_6H_5,4-$OCH_3C_6H_4$, , 4-ClC_6H_4,

R_1 = 4-ClC_6H_4, C_6H_5, 4-$OCH_3C_6H_4$

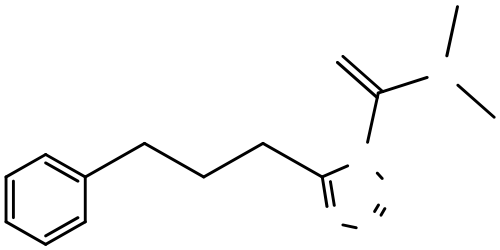
Bepary S., Das B K, Bachar S C., Kundu J K., Rouf A. S. S. and Datta B K., *et. al.* (2008), were synthesised A number of indanyl tetrazole derivatives namely 5-(6′-chloroindan-1′-yl)tetrazole (CIT), 5-(6′-bromoindan-1′-yl)tetrazole (BIT), 5-(6′-chloroindan-1′-yl) methyltetrazole (CIMT), 5-(6′-bromoindan-1′-yl)methyltetrazole (BIMT) **(22)** and evaluated for the anti-inflammatory activity in carragennan induced rat paw oedema[26] .



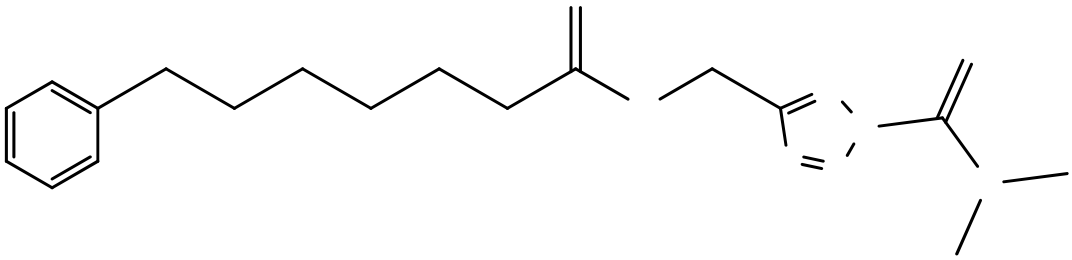
[22]

5-(6′-chloroindan-1′-yl)tetrazole (CIT)	X=Cl, n=0
5-(6′-bromoindan-1′-yl)tetrazole (BIT)	X=Br, n=0
5-(6′-chloroindan-1′-yl)methyltetrazole (CIMT)	X=Cl, n=1
5-(6′-bromoindan-1′-yl)methyltetrazole (BIMT)	X=Br, n=1

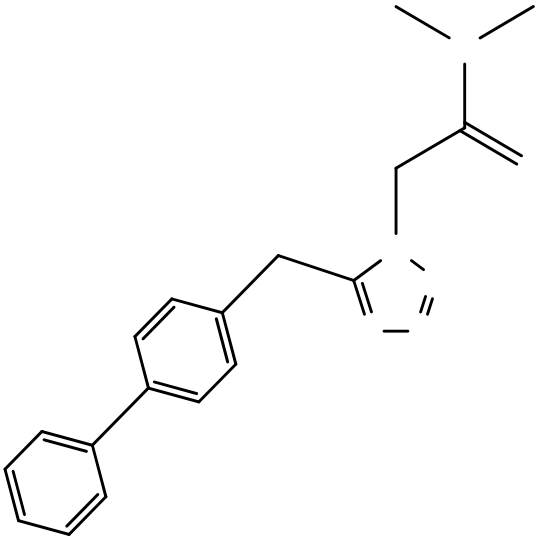
Maione S., Morera E., Marabese I, Ligresti A., Luongo L., Ortar G., Marzo V. D., *et al.* (2008), The series of tetrazole derivative are synthesised and screened the antinociceptive effect by cendocannabinoid inactivation**. (23), (24), (25), (26), (27)** [27].



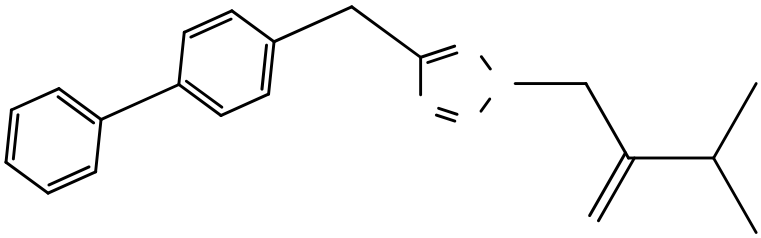
[23]



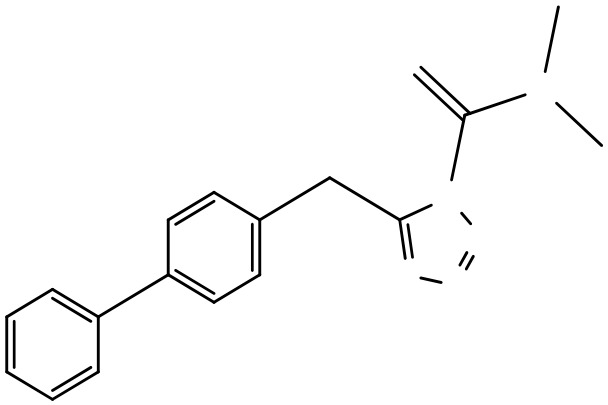
[24]



[25]

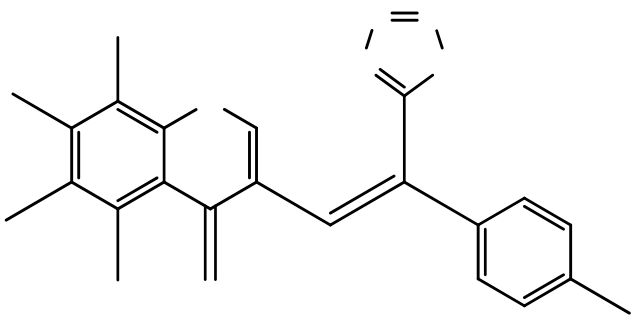


[26]



[27]

Diwakar S. D., Bhagwat S S., Shingare M. S., Gill C. H., *et.al.*(2008), were synthesised a series of Substituted 3-((*Z*)-2'-(4''-nitrophenyl)-2'-(1*H*-tetrazol-5''-yl)vinyl)-*4H*-chromen-4-ones (**28).** All the synthesized compounds were assayed for their in-vitro antibacterial activities against gram-negative and gram-positive bacteria and these four derivatives having well anti bacterial activity[28].

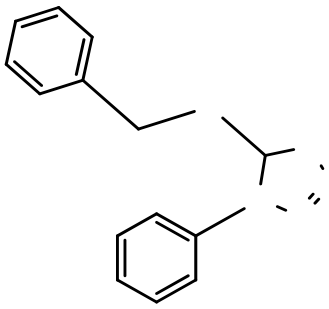


[28]

	R_1	R_2	R_3	R_4
1	H	F	H	H
2	H	Cl	H	Cl
3	H	Cl	CH_3	H
4	H	CH_3	H	CH_3

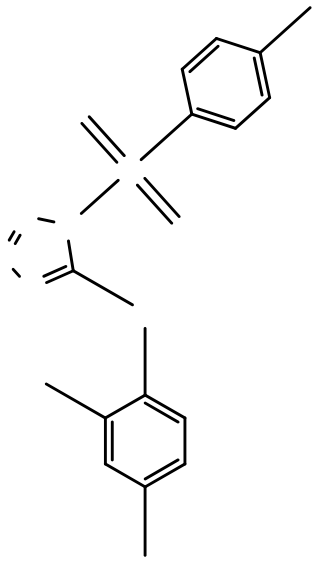
Adamec J., Beckert R., Weib D., ova V. K., Waisser K., llmann U. M., Kaustova J., and Buchta V., *et.al*., (2008), Synthesised a series of 5-benzylsulfanyl-1-phenyltetrazoles (**29)** derivative and screened antibacterial, antimycobacterial, antifungal, and antiproliferative activities.[6]

R_4



[29]

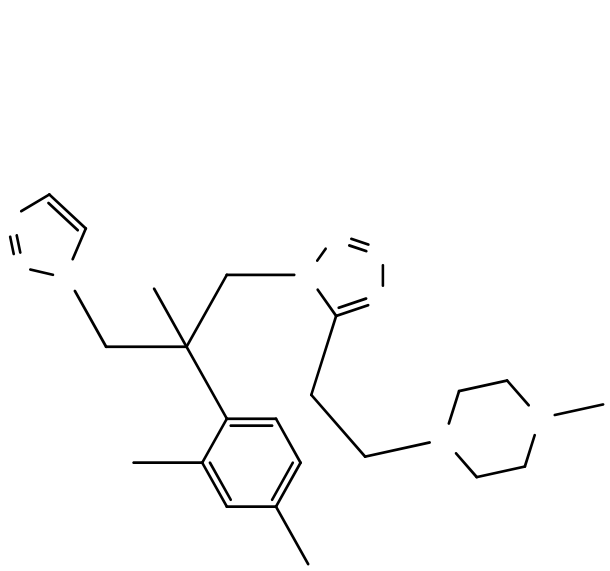
Yıldırır Y., Us M. F., Colak N., Ozkan H., Yavuz S., *et.al.*, (2009), were synthesised some new phenylselanyl-1-(toluene-4'-sulfonyl)-1*H*-tetrazole derivatives (**30),** and evaluated its antimicrobial activity.[5]



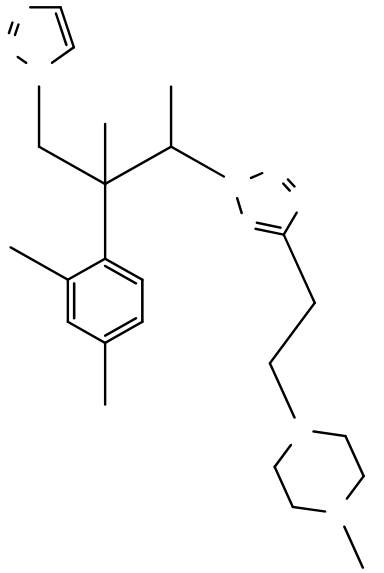
[30]

	R_1	R_2
1	H	Cl
2	Cl	H

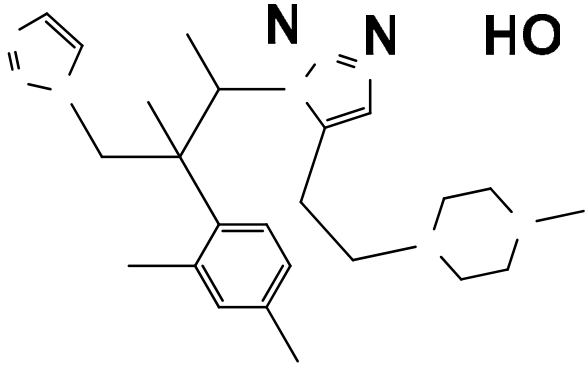
Upadhayaya R. S., Jain S., Sinha N., Kishore N., Chandra R., Arora S. K., *et.al.* (2004), were synthesised of novel 5-substituted tetrazole **(31), (32), (33)** and evaluated antifungal activity against *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp.[29]



[31]

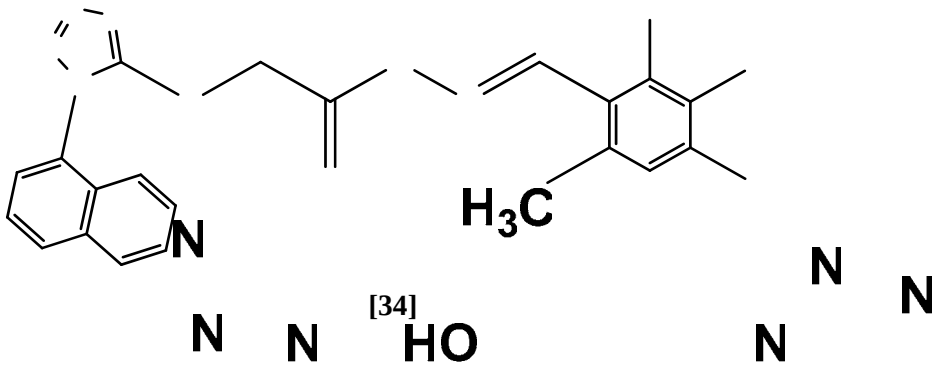


[32]



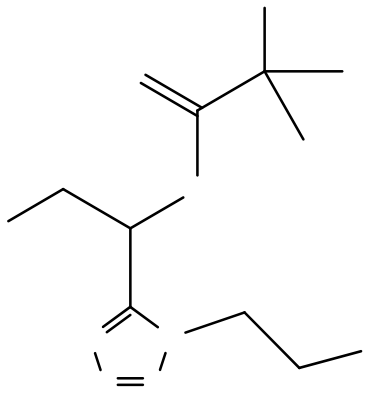
[33]

Zhan P., Liu H., Liu X., Wang Y., Pannecouque C.,*et. al.,* (2008), Synthesised a series of novel N'-arylidene-2-[1'-(naphthalen-1''-yl)-1'*H*-tetrazol-5-yl-thio]acetohydrazides, **(34)** evaluated as nonnucleoside reverse transcriptase inhibitors (NNRTIs), for their in vitro HIV-1 and HIV-2 activity using the IIIB strain and ROD strain, respectively[8].



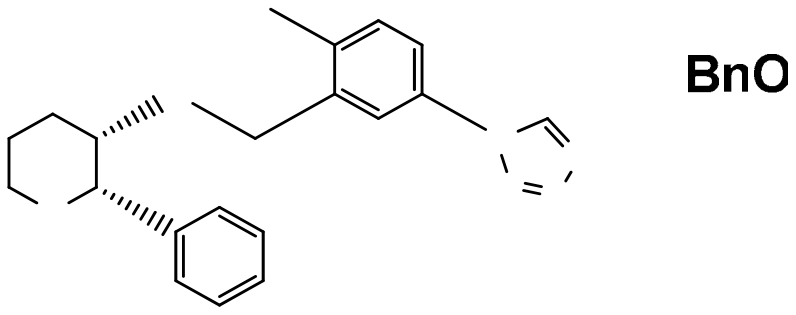
[34]

Hernandez A. S., Cheng P.T. W., Musial C. M., Swartz S. G., George R. J., *et al*., (2009), has been discovered A novel class of Growth Hormone Secretagogues (GHS), based on a tetrazole template, and synthesised tetrazole derivates **(35)** and evaluated good oral bioavailability in rats and dogs as well as efficacy following an oral 10 mg/kg dose in dogs[10].



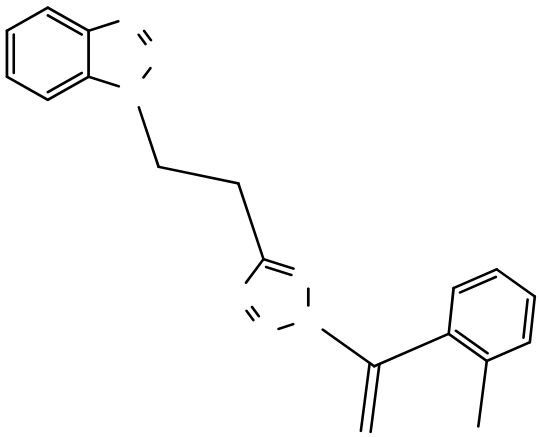
[35]

Armour D.R., Chung K.M.L., Congreve M., Evans B., Hubbard T., *et al.,* (1996), were synthesised a novel N-(2'-methoxy-5'-(1''*H*-tetrazol-1''-yl)benzyl)-2-phenylpiperidin-3-amine **(36)** an orally active non-peptide neurokinin-1 receptor antagonist that is the most potent broad-spectrum antiemetic agents[10].



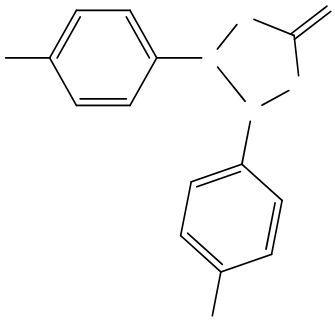
[36]

Rajsekaran A., Murugesan S., Anadarajgopal K.,*et.al.,* (2009), were synthesized novel newly 1-[2'-(1''*H*-tetrazole-5''-yl)ethyl]-*1H*-benzo[d][1,2,3]triazoles **(37)**, and screened Antibacterial, antifungal, and anticonvulsant activities compound elicited excellent anticonvulsant activity[12].



[37]

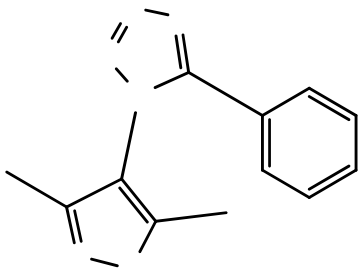
Zhao P.S., Jian F.F., Xiao H. L., and Hou Y. X., *et.al*., (2004), were Synthesised a novel Crystal Structure of 2,3-Substituted-1,4-2*H*-tetrazolthione (**38)**, and evaluated antibacterial and anti-inflammatoryactivity activities.[30]



[38]

R= H. CH_3

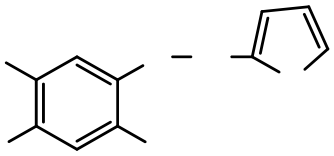
Rajanarendar E., Rao E.K. and Reddy A.S. R., *et.al.,* (2008) were synthesized 3,5-dimethyl-4-(5'-phenyl-*1'H*-tetrazol-1'-yl)isoxazole derivatives **(39)** and synthesized structure of these tetrazole derivative have been established by their elemental analysis and spectral data analysis and it was found good anti-inflammatory activity[31].



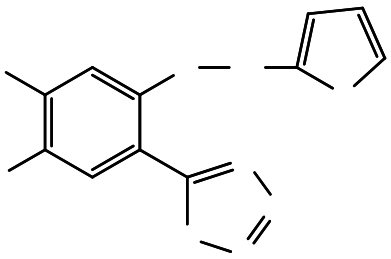
[39]

Block H.J *et.al.*, (2004), "Wilson and Gisvold,s textbook of organic medicinal and pharmaceutical chemistry" in there reported Both compounds **(40), (41)** having diuretic activity 1st one is Furosemide and 2nd one is Azosimide.[32]

The –COOH group is replaced in 2nd structure but it's having low toxicity as compare to 1st one.

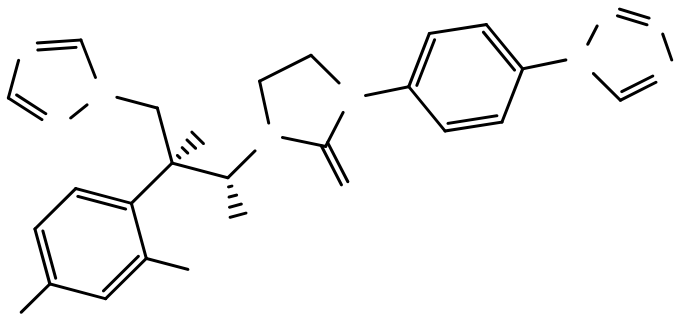


[40]



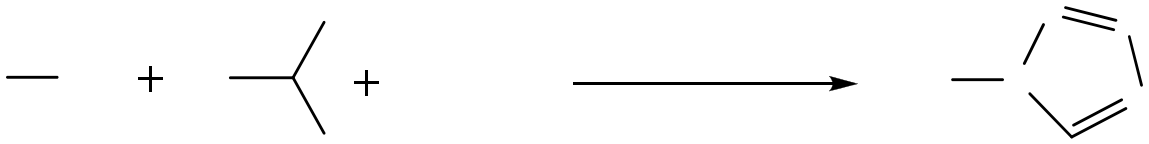
[41]

Myznikov L. V., Hrabalek A., and Koldobskii *et.al.*, (2007) were found 1, 1-Substituted tetrazoles have not yet been widely used for the creation of pharmaceutical products. The best known are certain derivatives of β-lactam antibiotics and optically active tetrazole-containing antifungal preparations of the azole type, such as TAK-456 **(42)**.[33]



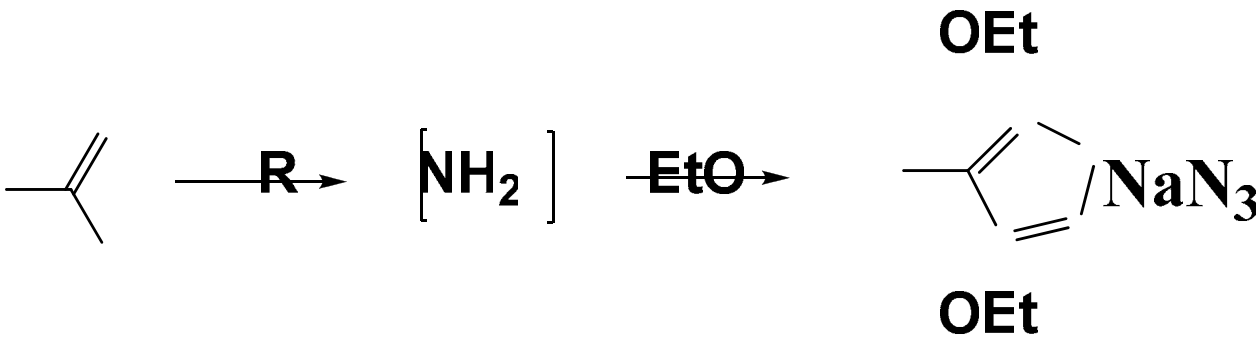
[42]

Su W.K., Hong Z., Shan W.G., Zhang X.X., *et.al.,* (2006), were a series of 1-substituted 1*H*-1,2,3,4-tetrazole **(43)** compounds have been synthesized in good yields from amines, triethyl orthoformate, and sodium azide through the catalyzed reaction with $Yb(OTf)_3$. Some of the 1-susbstituted 1*H*-1,2,3,4-tetrazole compounds showed strong phytocidal activity.[34]



[43]

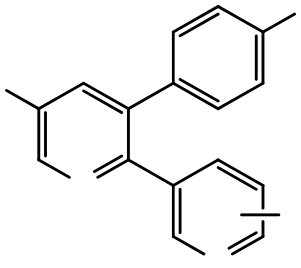
Shie J.J. and Fang J.M., *et.al.,* (2007), Microwave-Assisted One-Pot Tandem Reactions for Direct Conversion of Primary Alcohols and aldehydes to Triazines and Tetrazoles **(44)** in Aqueous Media.[35]



[44]

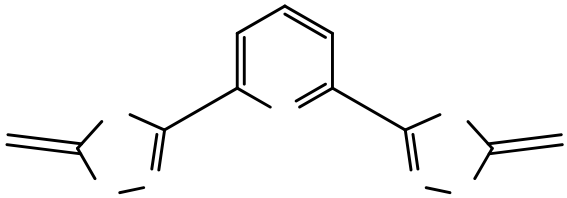
2.3 Review of Pyridine:

Dube D., Lazare S., Friesen R., Ormeaux D., Fortin R., *et al*., (1999), Synthesised a series of substituted pyridines **(45)** and evaluate its anti-inflammatory activity as selective cyclooxygenase-2 inhibitor.[16]



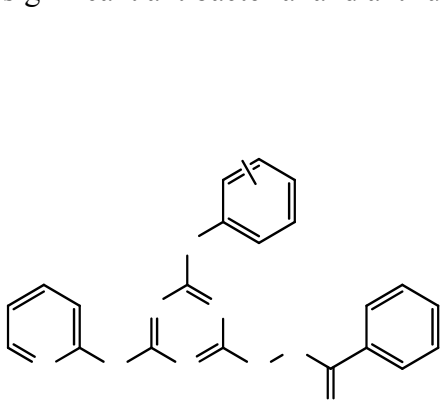
[45]

Ghozlan S.A.S., Mohamad S. F., Amr A.E.E., Mustafa E.S.E., Wahab A.E., *et al*., (2003) synthesised a series of 2,6-Bis-substituted pyridine derivatives 3,3'-(pyridine-2',6'-diyl)-bis-(1*H*-1',2',4'-triazole-5'(4*H*)-thione) hydrosulfide **(46)** and evaluated its antimicrobial activity[17].

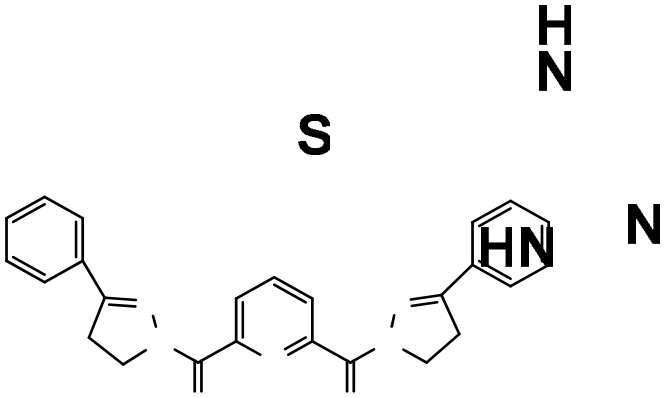


[46]

Raval J. P., Rai A. R., Patel N. H., Patel H. V. , Patel P. S.,*et.al*., (2009), Synthesised a variety of N'-(4''-(arylamino)-6''-(pyrazin-2''-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazide **(47)**, and compounds were evaluated for antimicrobial activity against variety of bacterial strains and some of these **(48)** compounds have shown significant antibacterial and antifungal activities.[36]

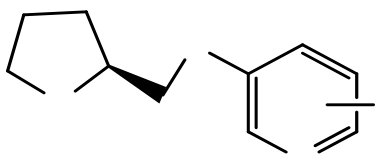


[47]



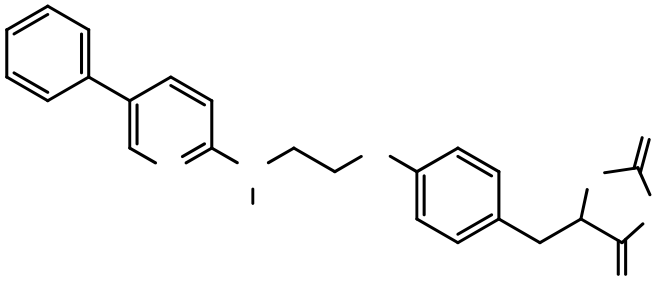
[48]

Lin N.H., Gunn D.E., Li Y., He Y., Bay H., *et.al.,* (1998), Were synthesised analogy of 3-[2'-((s)-pyrrolidinyl)methoxy]pyridine **(49)** with 1,4,5,6 substituted on the pyridine and tested in-vitro for neuronal nicotinic acetylcholine receptor binding activity.[37]



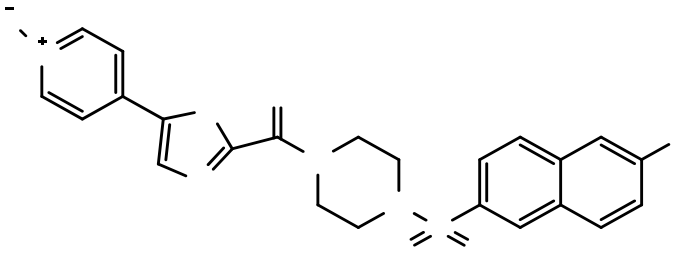
[49]

Kim B.Y., Ahn J. B., Lee H.W., Kang S.K., Lee J. H. *et al.*,(2004), were designed and synthesized a series of substituted pyridines and purines containing 2,4-thiazolidinedione from their corresponding pyridines and purines. On the basis of their biological activities, 5-(4'-{2''-[*N*-methyl-(5'-phenyl-pyridin-2-yl)amino]ethoxy}benzyl)thiazolidine-2,4-dione **(50)** was selected as a candidate for further pharmacological studies.[38]



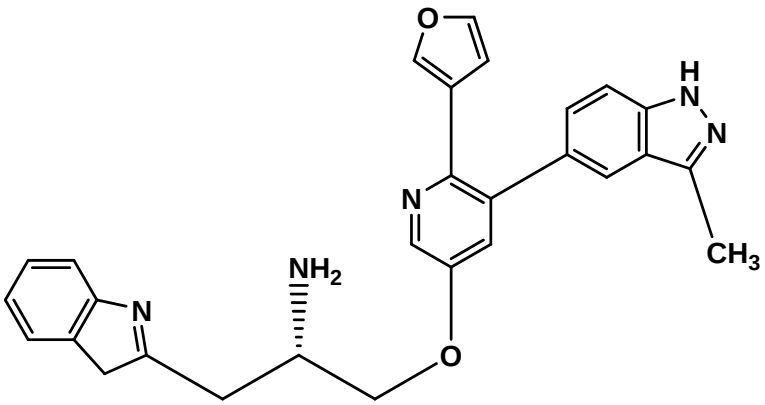
[50]

Haginoya N., Kobayashi S., Komoriya S., Yoshino T., Nagata T., *et..al*., (2004), Designed and synthesised a series of thiazol-5-ylpyridine derivatives **(51)** containing pyridine N-oxide and 2-carbamoylthiazole units to optimize the S4 binding element and biological activity of non-amidine factor Xa inhibitors containing pyridine N-oxide and 2-carbamoylthiazole units. N-Oxidation of thiazol-5-ylpyridine increased the anti-fXa activity more than 10-fold independent on the position of N-oxide.[39]



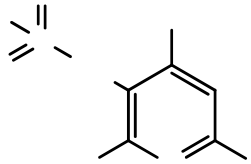
[51]

Lin H., Yamashita D.S., Zeng J., Xie R., Verma S., *et al*., (2010), were synthesised a novel series of AKT inhibitors containing 2,3,5-trisubstituted pyridines (**52)** with novel azaindazoles as hinge binding elements are described and displays greater than 80% inhibition of GSK3b phosphorylation in a BT474 tumor xenograft model in mice.[40]



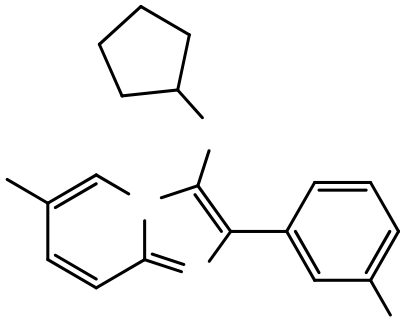
[52]

Chand P., Kotian P. L., Morris P. E., Bantia S., Walshb D.A.,*et.al.,* (2005), were Synthesised benzoic acid and pyridine derivatives **(53)** and evaluated its pharmacological activity on influenza neuraminidase as anti-vital.[41]

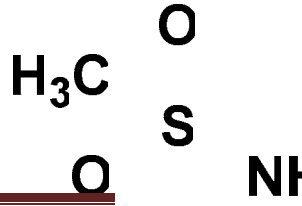


[53]

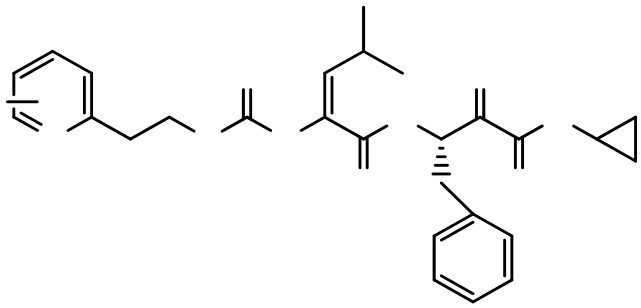
Luke R., Odell a, Mikael T. Nilsson B, Johan Gising A., *et.al*., (2009), were synthesised a series of Functionalized 3-amino-imidazo[1,2-a]pyridines **(54)** and evaluated A novel class of drug-like Mycobacterium tuberculosis glutamine synthetase.[42]



[54]

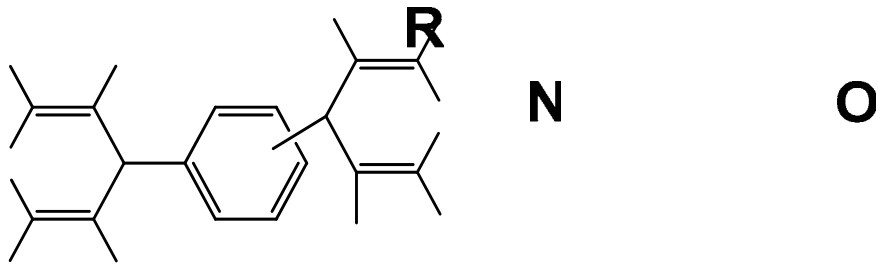


Shirasaki Y., Miyashita H. and Yamaguchi M., *et.al.,* (2006), were designed and synthesized a series of water-soluble dipeptidyl a-ketoamides containing a pyridine moiety, and evaluated for their oral bioavailability and retinal penetration. Introduction of a pyridine ethanol moiety provided the potent a-ketoamide inhibitor **(55)** with good oral bioavailability.[43]



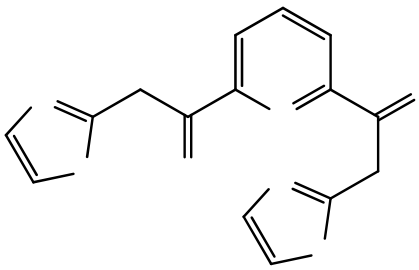
[55]

Tu S., Miao C., Fang F., F. Youjian F., Li T., *et al*., (2004), designed and synthesised a series of compounds containing two pyridine **(56)**, pyrimidine, pyridone, quinoline and acridine units under microwave irradiation, and screened pharmacological activity as New potential calcium channel modulators.[44]



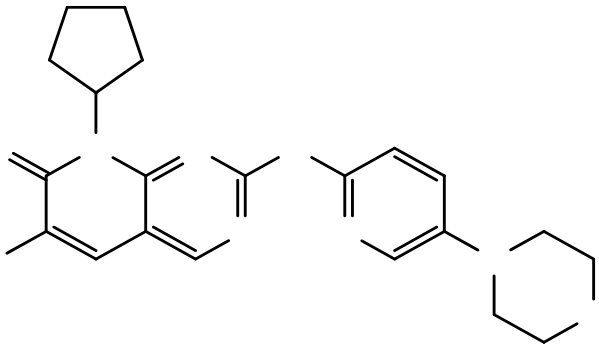
[56]

Luo Q.L., Li J.Y., Liu Z.Y., Chen L.L., Li J., *et al*., (2005), Synthesised a series of pyridine-2-carboxylic acid thiazol-2-ylamide **(57)** and SAR studies on the determination of the key scaffold, as Inhibitors of type I.[45]



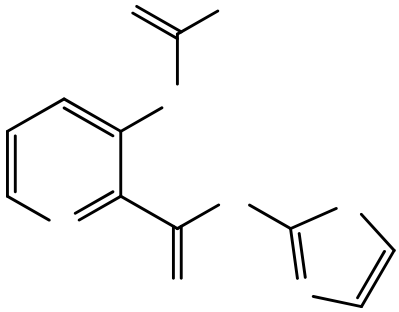
[57]

Mascarenhas N.M., Bhattacharyya D. and Ghoshal N., *et.al.,* (2010), were designed and synthesised pyridine containing pyrido[2,3-d]pyrimidin-7-ones **(58)** and evaluated it was selectively inhibit CDK4 than CDK2: Insights from molecular dynamics simulation.[46]



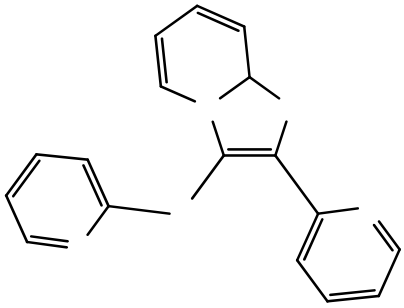
[58]

Luo Q.L., Li J.Y., Chen L.L., Li J., Yea Q.Z.,*et al.,* (2005), designed and SAR study of pyridine-2-carboxylic acid thiazol-2-ylamide **(59)** as Inhibitors of type I MetAPs.[47]



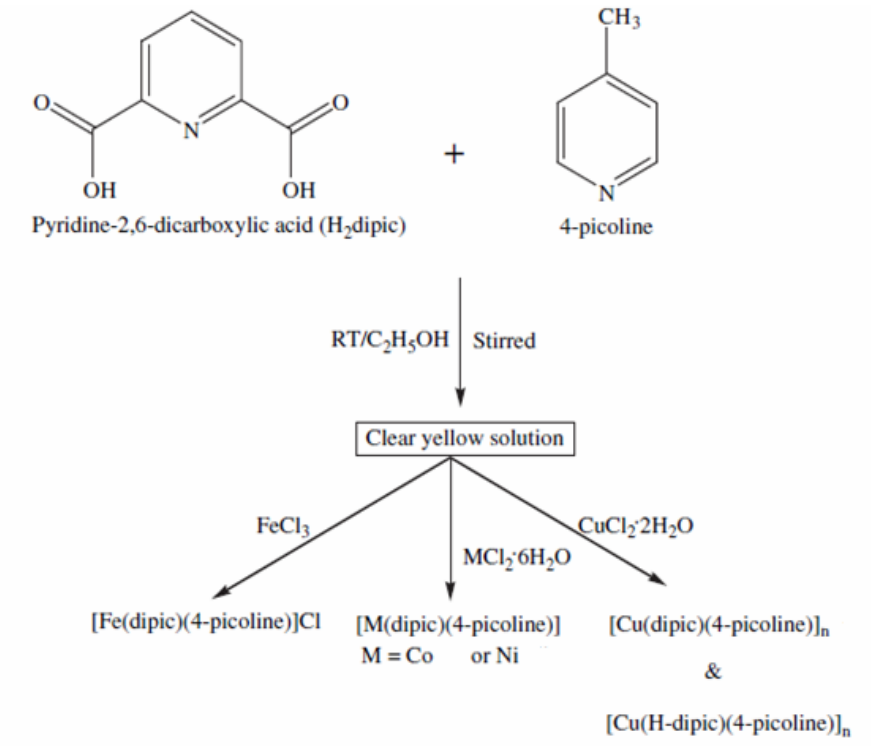
[59]

Lacerda R.B., Lima C.K.F., Silva L. L., Romeiro N.C., Miranda A. L. P.,*et al.*, (2009), Discovery and synthesised of novel analgesic and anti-inflammatory 3-arylamine-imidazo[1,2-a]pyridine **(60)** symbiotic prototypes.[48]



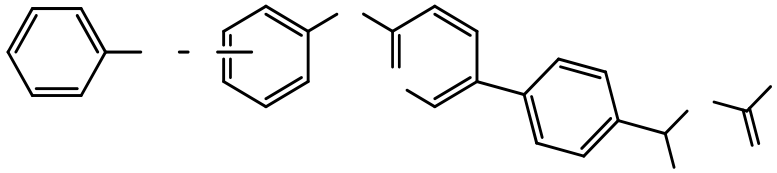
[60]

Siddiqi Z.A., Khalid M., Kumar S., Shahid M., Noor S., *et al.*, (2010), were synthesised novel transition metal complexes of pyridine-2,6-dicarboxylic acid **(61)** containing 4-picoline as auxiliary ligand and evaluated its antimicrobial activity and SOD activity.[49]



[61]

Haque T.S., Liang N., Golla R., Seethala R., Mac Z., *et al.*, (2009), were synthesised biphenyl- and 3-phenyl pyridine-based moieties **(62)** and evaluated its pharmacology and found potent inhibitors of acetyl-CoA carboxylase.[50]



[62]

O

H_2
C O

3.1 Table 3.1 Materials:

List of reagents used for synthetic work are stated as below:

Sr. No.	Reagents	Supplier company
1	2-aminopyridine	S d Fine Chem Limited, Mumbai
2	Dichloromethane	CDH
3	Triethyl amine	CDH
4	Methanol	Merck Pvt. Ltd., Mumbai
5	Ethanol	Baroda channel ind. Limited
6	Benzoylchloride	S d Fine Chem Limited, Mumbai
7	2- Chloro benzoyl chloride	S d Fine Chem Limited, Mumbai
8	4- Chloro benzoyl chloride	S d Fine Chem Limited, Mumbai
9	2,4- Dichloro benzoyl chloride	Merck Pvt. Ltd., Mumbai
10	4-Methoxy benzoyl chloride	S d Fine Chem Limited, Mumbai
11	2-Bromo benzoyl chloride	Spectrochem Pvt. Ltd., Mumbai
12	2-furoyl chloride	S d Fine Chem Limited, Mumbai
13	Phosphorous pentachloride	CDH
14	Sodium acetate	CDH
15	Sodium azide	Thomas baker chem Ltd., Mumbai.
16	Chlorosulphonic acid	S d Fine Chem Limited, Mumbai
17	Ammonia	S d Fine Chem Limited, Mumbai
18	Ethyl acetae	Merck Pvt. Ltd., Mumbai
19	Carbon tetrachloride	CDH
20	Toluene	Merck Pvt. Ltd., Mumbai
21	Acetone	CDH, S d Fine Chem Limited, Mumbai
22	Hexane	CDH
21	Chloroform	Merck Pvt. Ltd., Mumbai
22	Calcium-oxide	CDH
23	Hydrochloric acid	Merck Pvt. Ltd., Mumbai
24	Carragenann salt	S d Fine Chem Limited, Mumbai
25	Carboxy methy cellulose	CDH
26	DPPH(2,2-Diphenyl-1-	HI-media laboratory Pvt. Ltd.

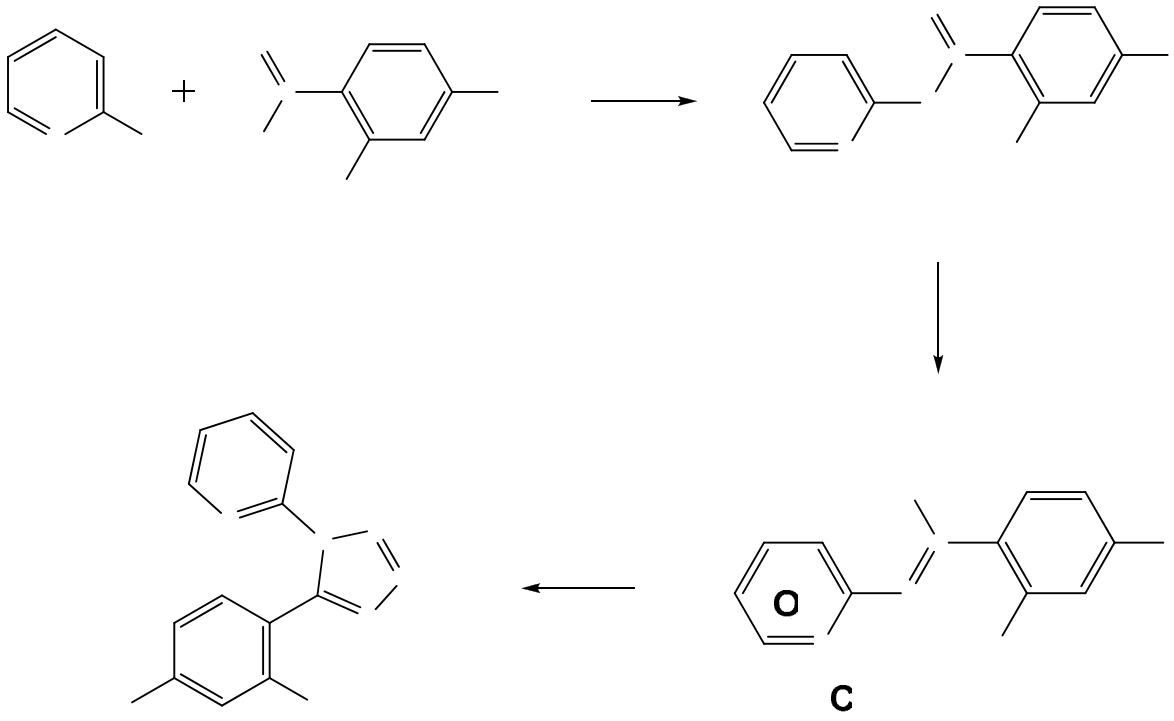
	Picrylhydrazyl)	
27	Sodium chloride	CDH
28	Diethyl ether	CDH
29	Dimethylsulfoxide	CDH
30	Actonitrile	CDH
31	Agar	CDH
32	Hydrogen peroxide	CDH
33	Activated chalcoal	CDH
34	Ascorbic acid	CDH
35	Pet ether	CDH

3.2 Instruments:

List of instruments used during experimental work are stated below:

1. Hot air oven (EIE Ltd, 230V)
2. U. V. Chamber (EIE Ltd)
3. Soxhlet apparatus (Durga Scientific Equipment, 500ml)
4. Rotary evaporator (BUCHI Type)
5. Plethysmometer (PTH 707)
6. Hot plate (EIE Ltd)
7. U.V and visible spectrometer(SHIMADZU)
8. Autoclave (Indfos)
9. Incubator (EIE Instruments Pvt. Ltd).
10. IR spectrometer. (JASCO- FTIR)

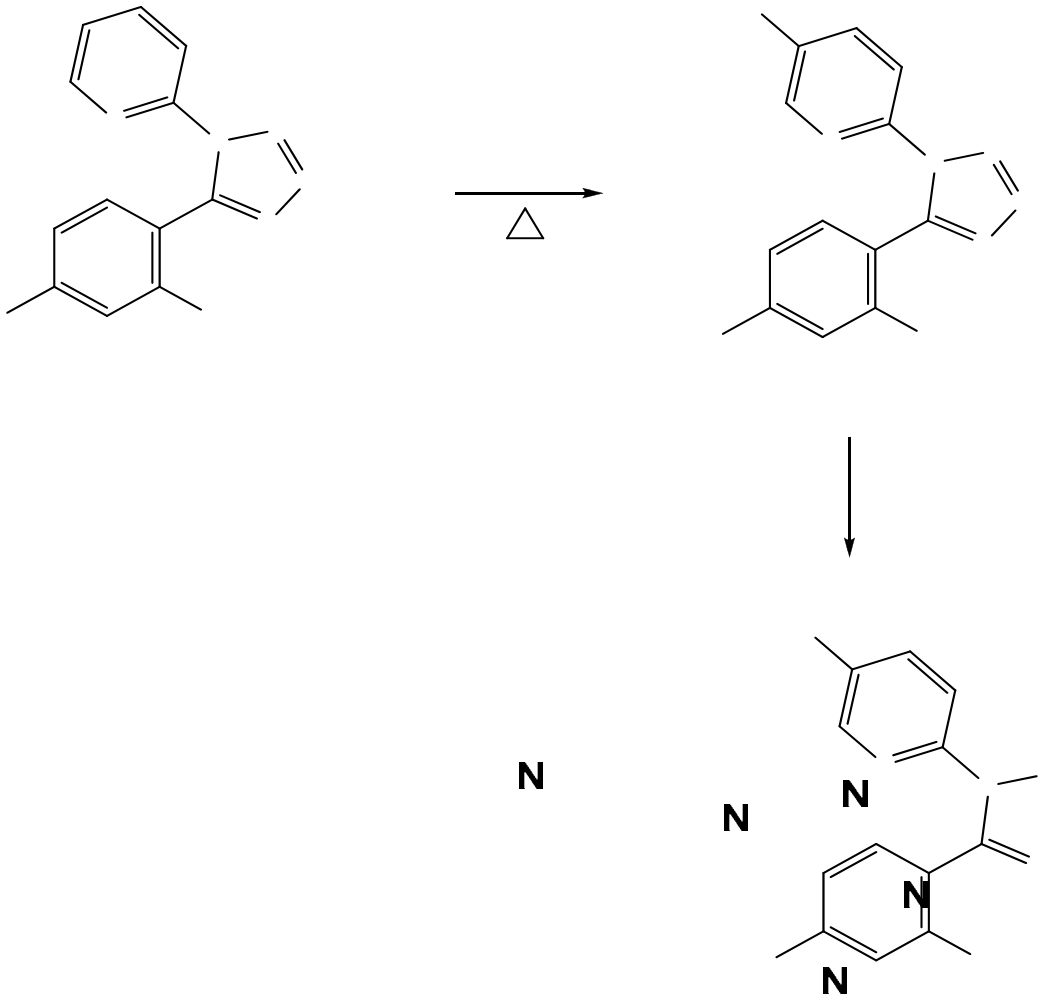
4. 1 Scheme – I: Synthesis of 2-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine (67).



63 **64**

R_1, R_2 = H, Cl, Br, OCH_3,

4. 2 Scheme – II: Synthesis of 6-(5'-(2'',4''-disubstitutedphenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonamide (69).

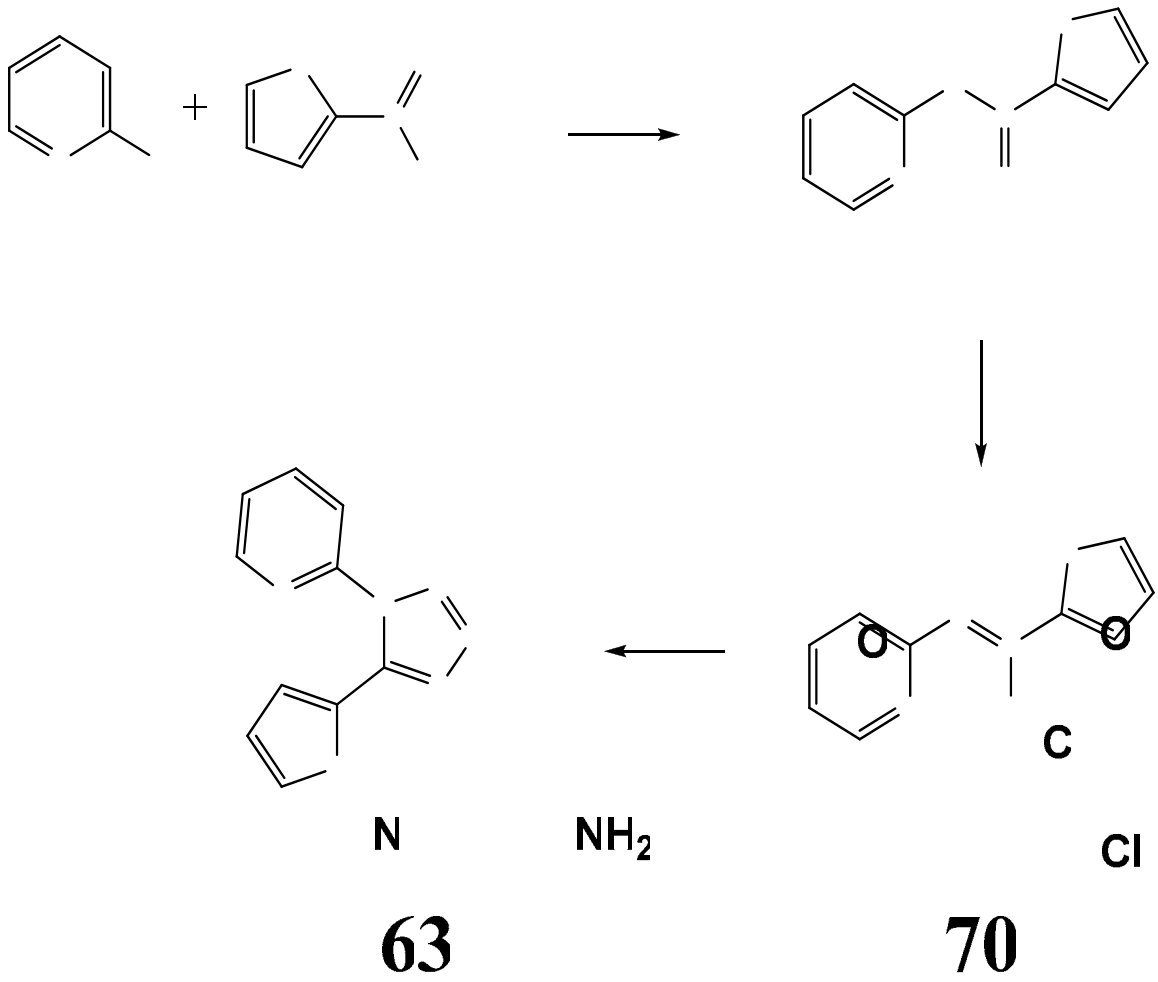


R_1, R_2 = H, Cl, OCH_3,

R_2 R_1

67

4. 3 Scheme – III: Synthesis of 2-(5'-(furan-2''-yl)-1'*H*-tetrazol-1'-yl) pyridine (73).



4. 4 Synthesis of 2,4-disubstituted-N-(pyridin-2'-yl)benzamide (65) .

Procedure[51, 52, 53 54]**:**

2-animo pyridine **63** (5gm, 0.053 mole) was taken to a in 250 ml round bottom flask. To that 60 ml of dichloromethane (DCM) was added and stirred for 15 minutes. Than triethylamine (1.03 ml, 0.079 mole) was added and stirred for another 5 minutes. Substituted-Benzoylchloride **64** (5.74 ml, 0.053 mole) was added drop wise and reaction mixture was stirred for overnight at room temperature. Reaction mixture was extracted with water and ethyl acetate by separating funnel. Organic layer was separated and moisture was removed by magnesium sulphate. DCM and ethyl acetate were evaporated using vacuum rotary evaporator. Product was collected and recrystallized using methanol and dried under vacuum desiccator. Percentage of yield was found 53.2%, Melting point was found 196-199ºC and TLC R_f value 0.71 was found. Reaction was monitored by TLC.

Solvent system used for TLC:

Toluene : Ethyl acetate (6 : 4).

4. 5 Synthesis of 2,4-disubstituted-N-(pyridin-2'-yl)benzimidoyl chloride (66) from of 2,4-disubstituted-N-(pyridin-2'-yl)benzamide (65)

Procedure[55, 56, 57]**:**

2,4-disubstituted-N-(pyridin-2'-yl)benzamide **65** (2 gm, 0.01 mole) was taken into a two naked 100 ml round bottom flask and added 2.3 gm, 0.02 mole of PCl_5 and mixed with glass rod. The reaction mixture was than refluxed at 120ºC on sand bath for 2 hours, attached with moisture trapped guard tube. Excess chlorine gas was removed under vacuum condition. Solid was washed with cold carbon tetrachloride to removed excess PCl_5. Percentage of yield was found 46.3%, Melting point was found 60-63ºC and TLC R_f value 0.67 was found. Reaction was monitored by TLC.

Solvent system used for TLC:

Toluene : Ethyl acetate (6 : 4).

4. 6 Synthesis of 2-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine (67) from 2,4-disubstituted-N-(pyridin-2'-yl)benzimidoyl chloride (66).

Procedure[58, 59, 31]**:**

2. 4-disubstituted-N-(pyridin-2'-yl)benzimidoyl chloride **66** (3gm, 0.013 mole) was taken into a two naked 100ml round bottom flask and maintained the temperature at 0-5ºC. Sodium azide and excess solution of sodium acetate was made in aqueous acetone at cold condition, NaN3 solution was added in to the flask with cold condition stirring for overnight. The organic layer was separated by separating funnel and excess solvent was evaporated at room temperature. The crude product was washed with methanol and recrystallized with 95% ethanol. Percentage of yield was found 28%, Melting point was found 230ºC and TLC R_f value 0.60 was found. Reaction was monitored by TLC.

Solvent system used for TLC:

Toluene : Ethyl acetate (6 : 4)

4. 7 Synthesis of 6-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonyl chloride(68) from 2-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine(67).

Procedure[61, 62, 63]**:**

2-(5-(2'',4''-disubstituted-phenyl)-*1'H*-tetrazol-1'-yl)pyridine **67** (400 mg, 0.00074 mole) was taken in to a 100 ml of round bottom flask and maintained the temperature at bellow 15°C. Chlorosulphonic acid (1.4 ml, 0.002) was added drop wise into the reaction mixture with constant stirring for 20 minutes. Resulting mixture was stirred and refluxed for 2 hours at 60°C. Cool the reaction mixture and poured in to the crushed ice and white product was obtained under vacuum, washed the crude with cold water. Percentage of yield was found 41%, Melting point was found 231ºC and TLC R_f value 0.79 was found. Reaction was monitored by TLC.

Solvent system used for TLC:

1) Toluene : Ethyl acetate (5 : 5)**.**

2) Chloroform : methanol (9.5:0.5)

4. 8 Synthesis of 6-(5'-(2'',4''-disubstitutedphenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonamide (69) from 6-(5'-(2'',4''-disubstituted-phenyl)-1*H*-tetrazol-1-yl)pyridine-3-sulfonyl chloride (68).

Procedure [62, 77, 78]:

6-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonyl chloride **68** (20mg, 0.0005 mole) was taken into a 100 ml round bottom flask and added excess amount of ammonia solution and refluxed for 2 hours . The reaction mixtures was cool and acidified with conc. HCl. Solid product was obtained and separated by vacuum filtration and washed with cold water. Crude product was recystallized with 95% ethanol. Percentage of yield was found 20%, Melting point was found 340ºC and TLC, R_f value (0.22) was found. Reaction was monitored by TLC.

Solvent system used for TLC:

Toluene : Ethyl acetate (5 : 5)**.**

Chloroform: methanol (9.5:0.5)

5.1: Inflammation:

Inflammatory diseases are a major cause of morbidity of the working force throughout the world. This has been called the king of human miseries[60].

Inflammation is defined as the local response of living mammalian vascularised connective tissue to injury due to various exogenous and endogenous stimuli. It is a body defence reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues[61, 62.]

An initial inflammatory stimulus triggers the release of chemical mediators from plasma or cells, which then regulate the subsequent vascular and cellular responses[61].

The agents causing inflammation may be grouped as:

1. Physical agents- like heat, cold, radiation and mechanical trauma.

2. Chemical agents- like organic and inorganic poisons.

3. Infective agents- like bacteria, virus and their toxins.

4. Immunological agents- like cell-mediated and antigen-antibody reactions.

Inflammation involves two basic processes with some overlapping, viz. early inflammatory response and later followed by healing. Though these both processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to body as well, example anaphylaxis to bites by insects or reptiles, drugs and toxins leading to atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesion in intestinal obstruction[63].

5. 2: Signs of inflammation:

The 4 cardinal signs of inflammation are given by roman writer Celsus in 1st century A.D. as: rubor (redness), tumor (swelling), calor (heat), dolor (pain), fifth sign of inflammation function laesa (loss of function) was later added by Virchow. The word inflammation means burning but burning is not only one of the signs of inflammation[63, 64.]

5.3: Pathway and Mechanism of Inflammation

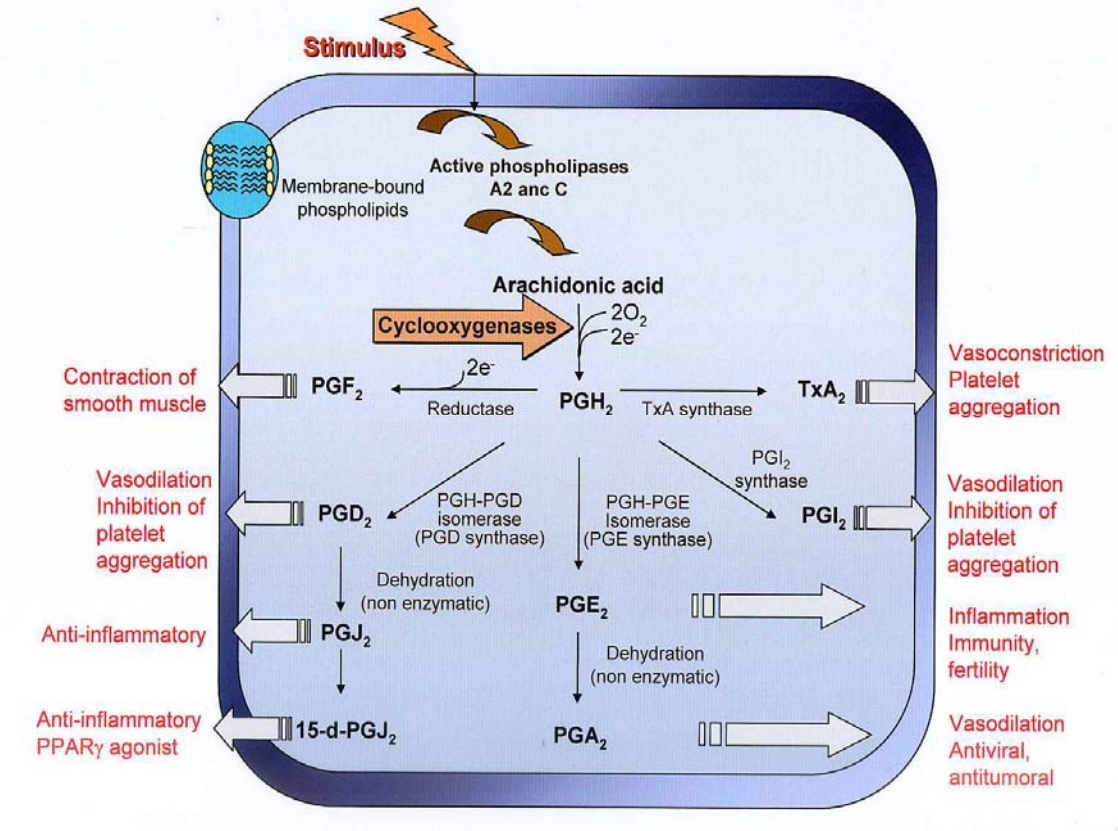


Fig.-10: pathway and mechanism of inflammation

- **Redness (Rubor):** Due to gross and persistent dilatation of arterioles, capillaries and venules in the injured area.
- Swelling (Tumour): Due to increased permeability of small blood vessels which allows the exudates to escape into the tissues of the damaged area.
- **Heat (Calor):** Due to considerable increase in blood flow.
- **Pain (Dolar):** Due to the release of certain endogenous chemical substances such as bradykinin, 5HT and certain prostaglandins.

5. 4: Animal models to screen anti-inflammatory activity:

Inflammation has different phases: In first phase vascular permeability is increased, resulting in exudation of fluid from blood into interstital fluid. In second phase infiltration of leukocytes from blood into tissue and in third phase the granuloma is formed. Accordingly, the anti-inflammatory tests have to be divided into those

measuring acute inflammation, subacute inflammation and chronic inflammation. In some cases, the screening is directed to test compounds for local application[63, 64].

5. 4.1: *In-vitro* models:

1. 3H-Bradykinin receptor binding
2. Constitute and inducible cellular arachidonic acid metabolism in vitro
3. COX-1 and COX-2 inhibition
4. Screening for interleukin-1 antagonists

5. 4. 2 *In-vivo* models:

1. Ultraviolet erythema in guinea pigs
2. Vascular permeability
3. Inhibition of leukocyte adhesion on rat mesenteric venules
4. Oxazolone and croton oil induced ear edema in mice and rats
5. Rat paw edema: Carrageenan, formalin, prostaglandin etc. induced paw edema.
6. Granuloma pouch technique
7. Cotton wool granuloma
8. Sponge implantation technique

5.5: Experimental protocol

Animals

Wistar rat of either sex were procured from Zydus Research Centre, ahmedabad which were used in the present study and were maintained in colony cages at 25±2°C and relative humidity of 45-55% under a 12 hrs light and dark cycle. They were fed standard animal food. The institutional animal ethics committee approved the protocol adopted for the experimentation of animals **(IPS/PCHEM/MPH10/001)**

Test compounds

The test compound and standard drugs were administered in the form of suspension 0.5% W/V sodium carboxy methyl cellulose (CMC) as vehicle by the same route of administration. Diclofenac sodium as dose of 13.5 mg/kg of body weight and nimesulide as dose 20 mg/kg of body weight were administered orally as reference of drug comparison. The test compounds as dose of 20 mg/kg of body weight were also administered orally.

5. 6: Screening Method

For this activity overnight starved wistar rats of 250-350 g were divided into 8 groups of six animals in each group:

All the animals were injected subcuteneus with 0.1 ml. of 1% freshly prepared Carrageenan suspension in normal saline, into subplantar region of right hind paw to induce inflammation.

Procedure:

The acute inflammation was produced according to the method of Winter *et al.* (1962) in all the test animals. The animals were received vehicle/test drug, Diclofenac sodium and Nimesulide orally and sixty minute later all the animals were challenged by injecting of 0.1 ml of 1% freshly prepared carrageenan suspension into the sub plantar region of the right hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in water up to this mark. The paw volume was measured plethysmographically before injection, immediately after injection and again at 1, 2, and 4 hours after challenge with carrageenan.[65, 66]. The percentage inhibition of oedema was calculated for each group on each h recording as follows:

Percentage inhibition = $[(Vo - Vt) / Vo] \times 100$

Where, Vo = Volume of the paw of control at time‘t’.

Vt = Volume of the paw of drug treated at time‘t’.

5. 7 Table -10: Anti-inflammatory activity:

Values are mean ± SEM, (n = 6); * $p < 0.05$, student t’ test as compared to control. (* = significant differences between to control and other)

5. 8 Fig.-11: Graphical representation of Anti-inflammatory activity:

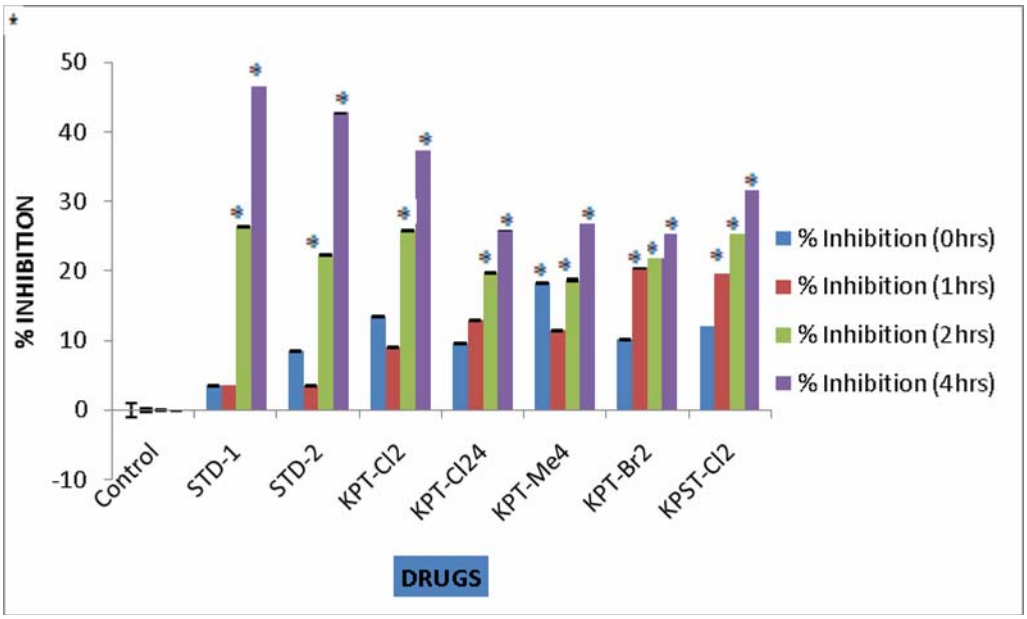


Fig.-11 % inhibition of paw volume of rat (anti-inflammatory activity) (* $p < 0.05$, student t' test as compared to control.)

STD-1- Diclofenac sodium

STD -2- Nimesulide

5. 9: PAIN

The International association for the study of pain defines it as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"[67, 68]

Pain is typically classified as either **acute or chronic**.

Acute pain is usually short-term, lasting anything from a few seconds to a few hours or a few days to a few weeks. Acute pain acts as a warning signal that alerts you to possible injury. Some types of acute pain e.g. back strain or a headache may be relieved without any medical treatment as the pain will ease off by itself. Other types of acute pain will be more serious e.g. appendicitis and will require swift medical attention to correct the problem and relieve the pain. Acute pain is perceived in the peripheral tissues by pain receptors nociceptors that come in separate types responding to stimulation by heat, cold or pressure and mechanical stress. This type of pain

generally comes on suddenly for example after trauma or surgery and may be accompanied by anxiety or emotional distress[69, 70.]

Chronic pain was originally defined as pain that has lasted 6 months or longer. Chronic pain is widely believed to represent disease itself. It can be made much worse by environmental and psychological factors. Chronic pain persists over a longer period of time than acute pain and is resistant to most medical treatments. It can cause severe problems for patients. Pain management is an integral part of treating chronic pain. Chronic pain is pain that does not go away for extended periods of time. Chronic pain can be caused by depression, and depression can be caused by chronic pain. Chronic pain can be accompanied by swelling, stiffness, discoloration, changes in temperature, skin sensitivity, tremors, sweating and changes in skin texture or hair growth. Chronic pain can be caused by any number of factors. It is not uncommon for an injury or surgical site to heal only to have continued chronic pain. Pain relief is vital to overall health and mobility, as well as improving an individual's ability to fight diseases[70].

5. 10: In-Vivo Method for Evaluation of Analgesic Activity

A great verity of nociceptive tests is currently used differing from each other byte nature of stimuli, parameter, and site of application, nature of response, quantisation and apparatus. Depending upon the nature of stimulus, they can be classified in to chemical, electrical, mechanical and thermal methods.

5. 10. 1: Electrical stimulation methods

1. Electrical stimulation of the tail.

Electrical stimulation of the tail through intracutaneous needles in animals produces consistent responses. This method is sensitive to opioid agonists, antagonists and NSAIDs.

2. Flinch-jump test in mice

In the flint jump technique, a constant-current shock is applied to the grid floor of the cage and the behaviour of the animal is observed. The current level is either increased

or decreased after each presentation and the ascending and descending series determined the order of the shock intensity presentation. The method is useful for the screening of opioid and non-opioid analgesic.

3. Tooth pulp stimulation in rabbits

Electrical stimulation of the tooth pulp has been used in antiniciceptive screening and is a useful model for studying facial pulp. Stimulation of the tooth pulp produced characteristic painful reaction such as licking, biting, chewing, and head flick, which can be observed easily.

5. 10. 2: Mechanical method

Selective stimulation of the mechanoreceptors can be achieved by the application of the high pressure. When applying with high pressure, it is not possible to avoid stimulating low threshold mechanoreceptors. The main disadvantage of this method is that the stimuli may produce receptor damage and therefore repeated stimulation may not elicit reproducible results. Haffiner tail-clip test in mice is based on this method. NSAIDs are not detectable by this method.

5. 10. 3: Thermal stimulation method

1. Radiant heat method (Tail-flick method)

The tail flick produce of amour and smith (1941) has become a standard screening method for evaluation of analgesic in rats and mice. It's effective for estimating the efficiency and potency of centrally analgesics.

2. Tail immersion method.

The rodent tail withdrawal reflex can be elicited by immersion of the tail in hot water at 55°C. This test is specific for opioids like central analgesics and a use to differentiate them from peripheral analgesics.

3. Hot plate method

The paw of rodents is highly sensitive to heat at temperature, which do not damage their skin. They usually respond by jumping, withdrawing of paws and licking them. The time required for the onset of the response in animal is prolonged by centrally acting analgesic whereas peripheral analgesics and NSAIDs do not affect this response.

5.11: Experimental protocol

Animals

Wistar rat of either sex were procured from Zydus Research Center, ahmedabad which were used in the present study and were maintained in colony cages at 25±2°C and relative humidity of 45-55% under a 12 hrs light and dark cycle. They were fed standard animal food. The institutional animal ethics committee approved the protocol adopted for the experimentation of animals**. (IPS/PCHEM/MPH10/001)**

Test compounds

The test compound and standard drugs were administered in the form of suspension 0.5% W/V sodium carboxy methyl cellulose (CMC) as vehicle by the same route of administration. Diclofenac sodium as dose of 13.5 mg/kg of body weight and nimesulide as dose 20 mg/kg of body weight were administered orally as reference of drug comparison. The test compounds as dose of 20 mg/kg of body weight were also administered orally.

5.12: Screening Method

For this activity overnight starved wistar rats of 250-350 g were divided into 8 groups of six animals in each group:

Procedure:

The hot plate test was carried out according to the method described by Eddy and Leimbach (1953). The animals were received vehicle/test drug, Diclofenac sodium and Nimesulide orally and thirty minute later wistar rats were placed on the hot plate maintained at 55±0.5^{o}C and the time between placement of animal on the hot plate and the occurrence of either licking of the paws, shaking, or jumping off from the plate was recorded as response latency. Rats with basal latency of more than 10 sec were not included in the study. The response latencies were measured before distraction (basal) and after drug treatment at 30, 60 and 120 min. The cut off time for hot plate latency was set at 30 sec[72, 73].

Effects of various drugs administered orally on the latency of rat exposed to the hot plate, Analgesic activity of test compounds was recorded in terms of mean latency time of each compound

5. 13 Table-11: Analgesic activity

Values are mean ± SEM, (n = 6); * $p < 0.05$, student t' test as compared to control.

5.14 Fig.-12: Graphical representation of Analgesic activity.

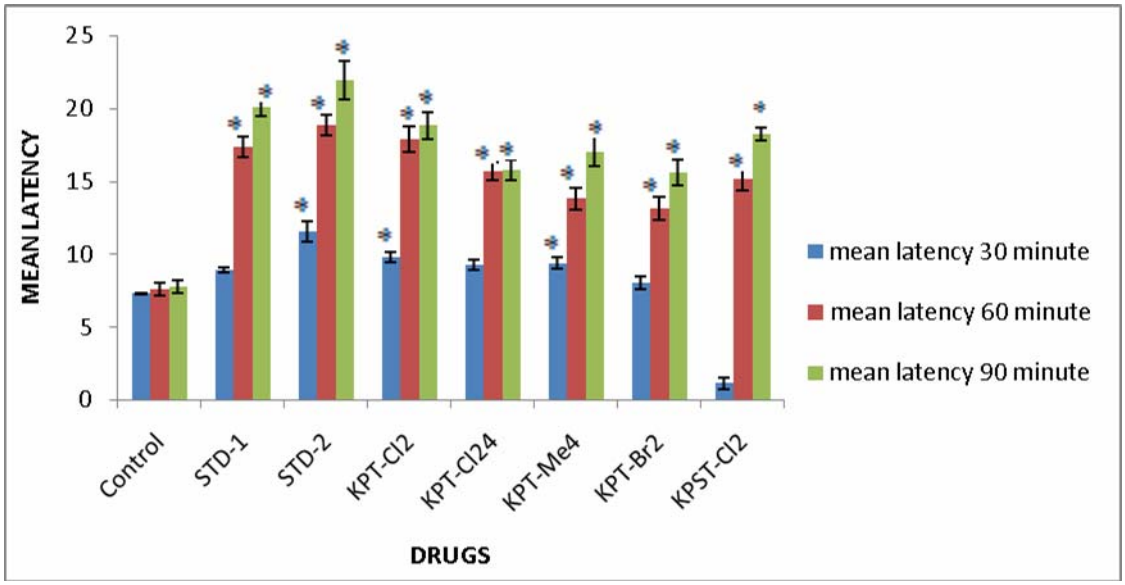


Fig.-12: Latency Time (Second) of Hot Plate in Rat versus compound (* $p < 0.05$, student t' test as compared to control.)

STD-1- Diclofenac sodium,

STD -2- Nimesulide

5.15: Ulceroganic Potential

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammation, pain and arthritis. The beneficial effect is associated with inhibition of cyclooxygenases (COX) that convert arachidonic acid into prostaglandins in inflammatory processes[88]. The major limitation of long-term therapeutic use of NSAIDs (COX-1 inhibitors) is their gastrotoxicity. This side effect produced by NSAIDs are believed to involved two different mechanism: inhibition of prostaglandin synthesis in the stomach, responsible for inducing mucus production and a local action exerted by direct contact of the drugs with the gastric mucosa due the acidic nature of the NSAIDs.[73]

5.16: Experimental protocol

Animals

Wistar rat of either sex were procured from Zydus Research Centre, ahmedabad which were used in the present study and were maintained in colony cages at 25±2°C and relative humidity of 45-55% under a 12 hrs light and dark cycle. They were fed standard animal food. The institutional animal ethics committee approved the protocol adopted for the experimentation of animals**. (IPS/PCHEM/MPH10/001)**

Test compounds

The test compound and standard drugs were administered in the form of suspension 0.5% W/V sodium carboxy methyl cellulose (CMC) as vehicle by the same route of administration. Diclofenac sodium as dose of 20 mg/kg of body weight and nimesulide as dose 20 mg/kg of body weight were administered orally as reference of drug comparison. The test compounds as dose of 20 mg/kg of body weight were also administered orally.

5.17: SCREENING METHOD

For this activity 48 hrs starved wistar rats of 250-350 g were divided into 5 groups of six animals in each group:

Procedure:

The animals were received vehicle/test drug, Diclofenac sodium and Nimesulide orally and 7 hrs later animals were sacrificed. Control animals are sacrifice after 7 hrs. Stomach was removed and placed on saline-soak filter paper until inspection. A longitudinal incision along the greater curvature is made with fine scissors. The stomach is inverted over the index finger and the presence or absence of gastric irritation was determined. The presence of a single or of multiple lesions (erosion, pataches, ulcer or perforation) was considered to be positive[74].

Ulceroganic potential of the compounds was recorded in terms of area of ulceration in mm^2

5.18 Table-12: Ulceroganic potential

Values are mean ± SEM, (n = 6); * $p < 0.05$, student t' test as compared to control.

5.19 Fig.-13: Graphical representation of Ulceroganic area mm^2 versus compounds.

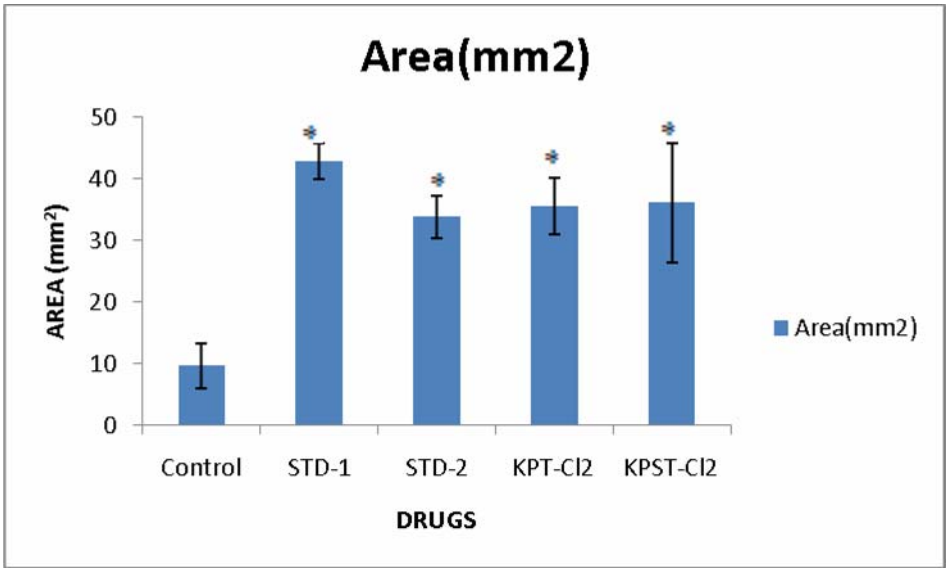
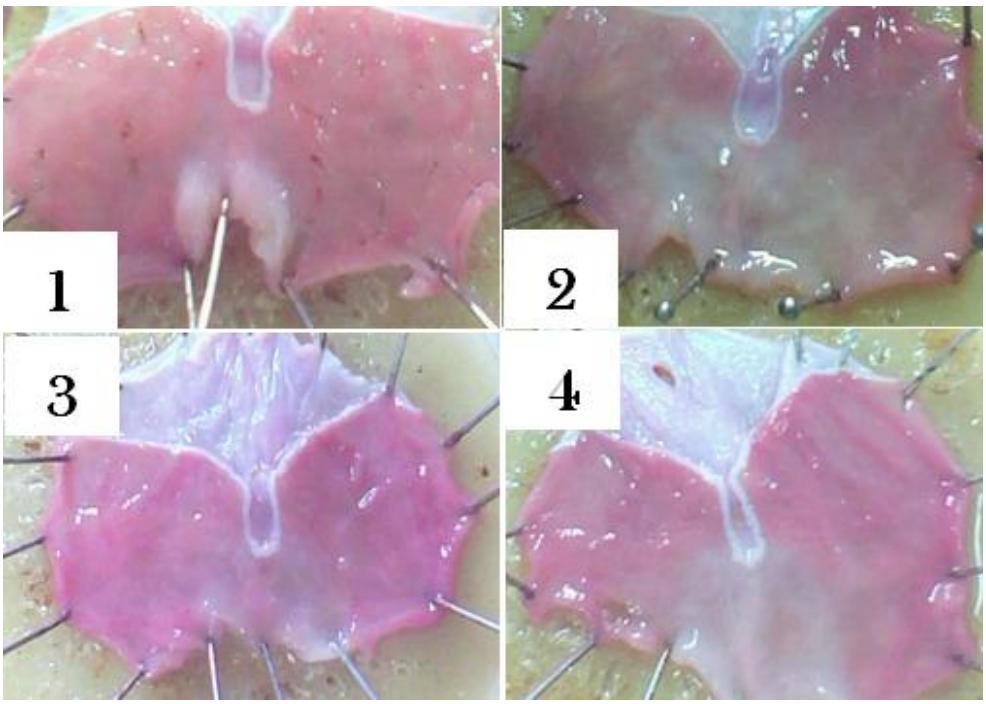


Fig.-13: Graph of ulceroganic area mm^2 versus compound (* $p < 0.05$, student t' test as compared to control.)

5.20 Fig.-20: Photographs of ulcer produced in rat stomach



1- Diclofenace treated, 2- Nimesulide treated,

3- KPT-Cl_2 treated, 4- KPST-Cl_2 treated stomach

5.21 ANTIOXIDANT

An antioxidant is a molecule capable of showing or preventing the oxidation of other molecules. Oxidation reaction can produce free radicals, which start chain reaction that damage cells. Antioxidant terminates these chain reactions by removing free radical intermediate and inhibited oxidation reaction by being oxidised themselves**.**

Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by haemolytic cleavage of covalent bond of a molecule, by the loss of a single electron from a normal molecule. Most of the molecular oxygen consumed by aerobic cell during metabolism is reduced to water by using cytochrome oxidase in mitochondria. However, when oxygen is partially reduced it become 'activated' and reacts readily with a variety of bio-molecules. This partial electron occur in one

electron steps, by addition of one, two, and four electron to O_2 which lead to successive formation of reactive oxygen metabolites[75].

Different types of free radicals in the body are produced at different sites and causes diseases. The over activity and generation of this free radicals are inhibited by natural or synthetic antioxidants. The evaluation of the antioxidant activity can be done by both in-vivo and in-vitro activity can be evaluated without sacrifice the animals. In-vitro activity can be determined by the following method.

1. DPPH (2, 2 diphenyl-2-picryl-hydrazyl) radical scavenging activity

2. Superoxide radical scavenging activity

3. Nitric oxide radical scavenging activity

4. Hydrogen peroxide radical scavenging activity

5. Reductive power assay

6. Hydroxyl radical scavenging activity

7. ABTS (2, 2-azino bis(3-ethyl-benzo-thiazoline-6-sulfonate)) radical scavenging activity

8. Lipid peroxidation assay

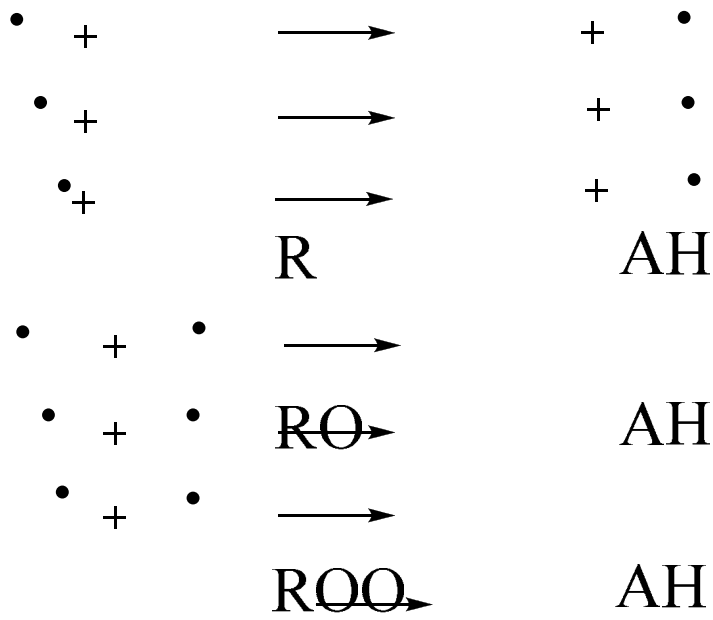
5.22 Mechanism of antioxidant

The possible mechanisms of action of antioxidant were first explored when it was recognised that a substance with antioxidant activity is likely to be one that itself readily oxidised. Research into how vitamin E prevents the process of lipid peroxidation led to identification of as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells.

Mechanism by

1) Hydrogen donation to free radicals by antioxidants.

2) Formation of a complex between the lipid radical and the antioxidant radical (free radical acceptor).



5.23 DPPH (2, 2 diphenyl-2-picryl-hydrazyl) radical scavenging activity

DPPH is characterised as a stable free radical by virtue of delocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise. The delocalization also gives rise to the deep violat color. The effect of antioxidants on DPPH scavenging is due to their hydrogen donating ability. DPPH is stable free radical & accepts an electron or hydrogen redical to become 2, 2 diphenyl-2-picryl-hydrazyl stable dimagnatic molecule. The reduction capability of DPPH redical is determined by decrease in its absorbance at 517 nm induce by antioxidant. The absorption maximum of a stable DPPH radical in methanol is at λ-max of 517nm. The decreased in the absorption of DPPH radical caused by antioxidants due to the reaction between antioxidants molecule and the radical, result in the scavenging of radical by hydrogen donation. The change in the color fron violet to yellow is visually noticeable. Tribromodihydroxybenzyl methyl ether has higher DPPH redical scavenging as compared to L-ascorbic acid[76].

Diphenypicrylhydrazyl (free radical) Diphenypicrylhydrazyl (nonradical)

Procedure:

1ml of 0.1mM of DPPH in methanol was added in 1ml of references standard (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml)/ test solution of different concentration (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml). It was kept in dark for 30 minute to protect from light and the absorbance was measured at λ-max517nm in UV Visible spectrophotometer (SHIMADZU). The assay was performed in triplicate.

Reference standard: Ascorbic acid

All solution was freshly prepared.

Test solution was prepared by dilution with methanol form stock solution of 10mg/ml

The DPPH scavenging was measured by following equation

% inhibition = A_0-A_t/A_0

A0 = absorbance of control (containing all measurement except test)

At = absorbance of test solution

5.24 Table-13: Antioxidant activities of test compounds were recorded in terms of % scavenging shown by each compound.

Values are mean ± SEM, (n = 3); * $p < 0.05$, student t' test as compared to control.

5.25 Fig.-14: Graphical presentation of antioxidant activities.

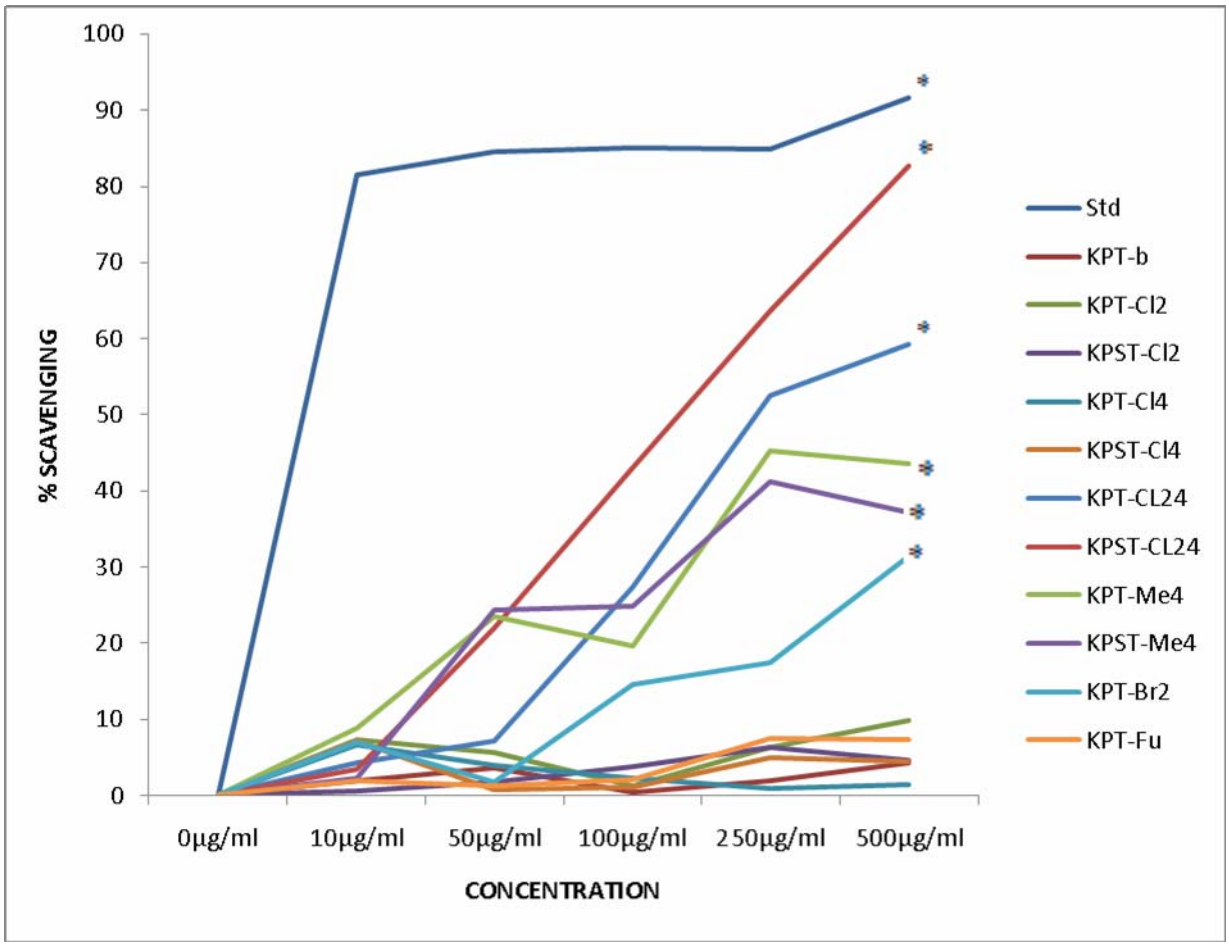


Fig.14: Graph between % DPPH scavenging radical versus concentration of each drugs (* $p < 0.05$, student t' test as compared to control.)

5.26 Introduction:

The Bacteria (*singular*: bacterium) are a large group of unicellular microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial. However, a few species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people a year, mostly in sub-Saharan Africa. In developed countries, antibiotics are used to treat bacterial infections and in agriculture, so antibiotic resistance is becoming common. In industry, bacteria are important in sewage treatment, the production of cheese and yoghurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

In this era, the prevalence of infectious diseases has increased to a great extent. Antimicrobial agents are among the most commonly used to treat the variety of infectious diseases. Literature review revealed that tetrazole and pyridine containing compounds show different biological activities. These compounds are also evaluated for their antibacterial activities.

There are various *in vivo* and *in vitro* methods are available for evaluation of antibacterial activity. Synthesized compounds were evaluated for their antibacterial activity against 1 gram-positive bacteria and 1 gram-negative bacteria.

5.27 Methods of Antimicrobial Susceptibility Testing

Several evaluation methods are available for the determination of bacterial sensitivity to the antibacterial agents. The most commonly used methods include

A. Diffusion test

- Agar disc-diffusion method
- Agar well diffusion method

B. Dilution test

- Agar dilution method
- Broth dilution method

C. Diffusion and Dilution

- E-Test method

A. Agar Disc-diffusion method

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition"[1, 2].

Many conditions can affect a disc diffusion susceptibility test. When performing these tests certain things are held constant so only the size of the zone of inhibition is variable. Conditions that must be constant from test to test include the agar used, the amount of organism used, the concentration of chemical used, and incubation conditions (time, temperature, and atmosphere).

The disc diffusion method for antibiotic susceptibility testing is the Kirby- Bauer method. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method.

B. Agar Well-diffusion method

This method is similar to agar disk diffusion method. The only difference is that well is produced on the surface of agar in place of filter paper disk that kept on the agar. In that well antimicrobial agent is added and around these well bacteria will not grow.

5.28 Experimental Protocol

In the present study, the *in vitro* antibacterial activity of the synthesized compounds was assessed against a panel of one gram-positive bacterial species and one gram-negative bacterial species by the Agar well-diffusion method for the preliminary *in vitro* antibacterial using nutrient broth culture media (CDH).

Bacterial strain used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and that are

1. Gram-positive bacteria
    - *Staphylococcus aureus* (MTCC 737)
2. Gram-negative bacteria
    - *Escherichia coli* (MTCC 1687)

Preparation of nutrient broth Agar media

Agar was prepared according to directions provided by manufacturer which is 45.00 g of media was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. The melted agar medium was filled in 20 ml test-tubes. The media was then sterilized by autoclaving (Indfos) at 15 lbs pressure at 121 ^{o}C for 15 minutes. All media containing test-tubes were preserved at -20 ^{o}C in the deep freezer and used as required.

Preparation of Test and Standard Compounds

All the test compounds were freely soluble in dimethyl sulfoxide (DMSO). The solutions of required concentrations- 50 µg, 100 µg, and 250 µg were prepared by dissolving in DMSO. All the test compounds were prepared from the stock solution of 10mg/ml. Ofloxacine (Zydus Cadila Pvt. Ltd) 50µg/1ml. was taken as a reference standard drug.

Preparation of Bacterial Culture

From the stock solution of bacterial culture 0.1 ml was taken and diluted to 10 ml with Water

Or Injection (WFI) (*Nirlife* Healthcare), so 100 times dilution was done.

From this diluted cultures 0.1 ml was taken into the petri dish containing solid agar and spread properly by spreader. After 24 hours of incubation at 37^{o}C Colony Forming Unit (CFU)/ml was checked. (CFU/ml was observed to be 10^{-5} to 10^{-6}). All colonies were counted by Digital Colony Counter (Toshiba, EIE-1901).

Determination of Zone of Inhibition

Agar well diffusion method (Cup plate method) nutrient broth Agar was employed to study the preliminary *in vitro* antibacterial activity of the synthesized compounds against one gram-positive and one gram-negative bacterial species.

All petri dishes were sterilized by Hot air oven at 180^{o}C for 30 minutes. nutrient broth Agar was sterilized and about 20 ml of the agar media was poured in to the sterilized petri-dish. Petri dishes were allowed to cool and solidify, and then diluted bacterial cultures were applied on the plates by micropipette. After spreading by spreader wells were prepared on agar surface by sterile cork borer of 6 mm diameter. The reference standard (50 µg/1 ml) and test compounds (50 µg/1 ml, 100 µg/1 ml, and 250 µg/1 ml) were loaded into the well with the help of micropipette and kept aside to allow the solution to diffuse totally in the medium. The plates were incubated at 37^{o}C for 24 hours in Biological Oxygen Demand (BOD) incubator (EIE Instruments Pvt. Ltd). The zone of inhibition was measured in millimetres on antibiotic zone reader (Hally Instruments) in all the incubated plates. DMSO was taken as a control. Same procedure was applied for the Standard drug, ofloxacin. All the experiments were performed in triplicates.

5.29 Table -14: Zone of Inhibition (mm) in *S.aureus*

E1*, E2*, E3* represent data of 1, 2, 3 experiment respectively.

5.30 Fig.-15: graphical representation of Zone of Inhibition (mm) in *S.aureus*

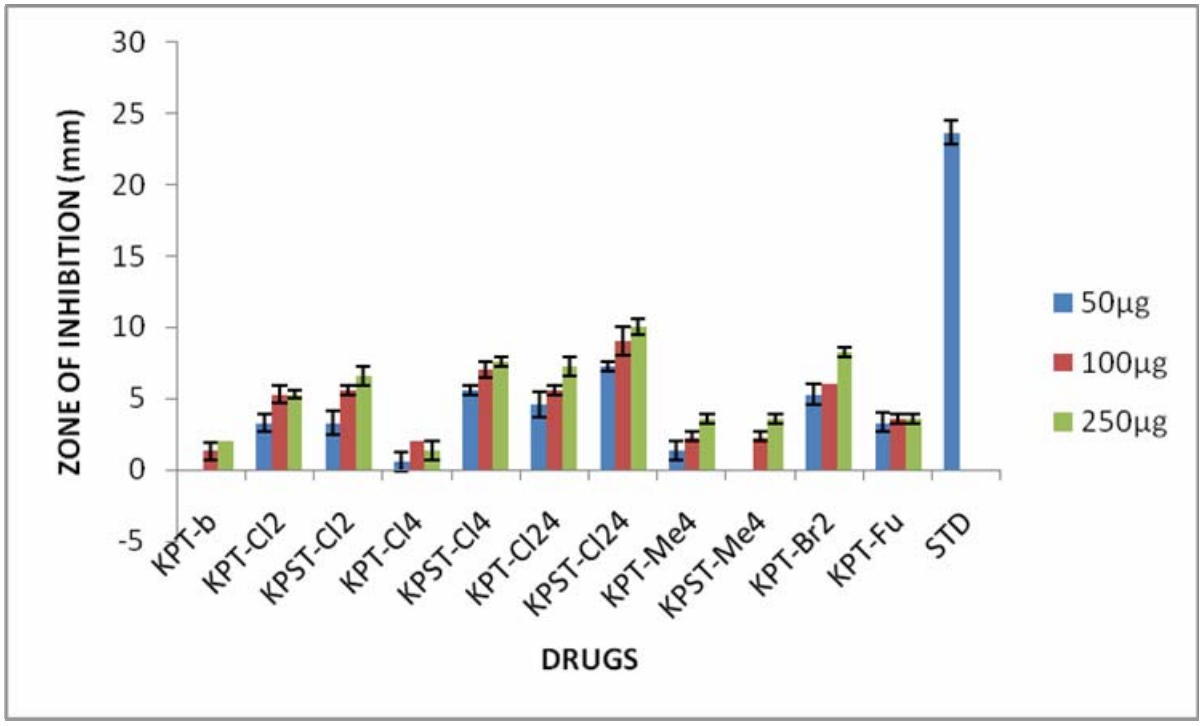


Fig.-15: Graph of Zone of inhibition in *S.aureus*

5.31 Table-15: Zone of Inhibition (mm) in *E.coli*

Antibacterial activity of test compounds was recorded in terms of zone of inhibition (in millimeters) shown by each compound against various bacteria.

E1*, E2*, E3* represent data of 1, 2, 3 experiment respectively.

5.32 Fig.-16: Graphical representation of Zone of Inhibition (mm) in *E.coli*

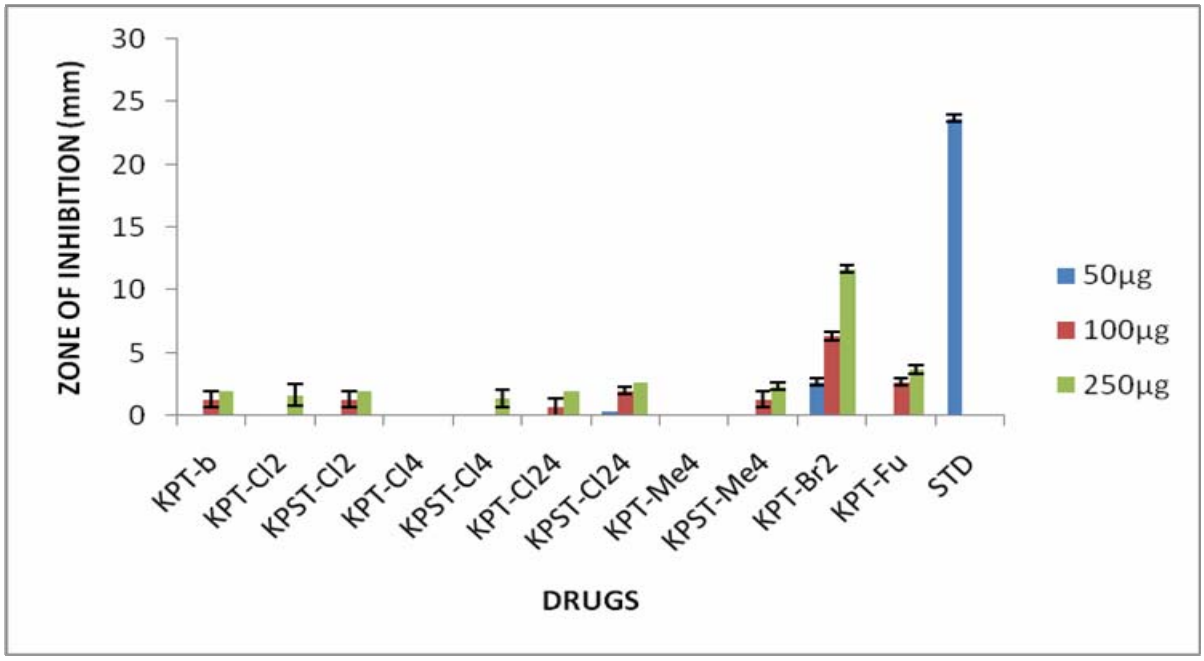


Fig.-16: Graph of zone of Inhibition (mm) in *E.coli*

6.1 Result and Discussion

Final structures of synthesized compounds were elucidated by FTIR, ^{1}H NMR, Mass spectra. Compound, **(75)** was found light yellowish colour solid, melting point was recorded at 240^{o}C. The purity of the compound was established by TLC All the spectral data and TLC results were found satisfactory. The structure of compound **(75)** was elucidated and found pure.

All the Final structures of synthesized compounds were elucidated by the same way and all the spectral data and TLC assure that, compounds were pure and formed.

The synthesized compounds KPT-Cl_2, KPST- Cl_2, KPT- Cl_{24}, KPT- Me_4 and KPT- Br_2 were screened in-vivo anti-inflammatory activity by using right hind paw edema method, diclofenace sodium and nimesulide was as used as the standard drug in the form of oral suspension in 0.5%CMC standard as well as tests. All the biological results of the compounds are given in chapter 6 in Table-10. Anti-inflammatory activity is recorded in term of % inhibition of the paw volume of rat. According to our synthesised compound, KPT-Cl_2 (**75)**, KPT- Cl_{24} **(79)** and KPST- Cl_2 (**76)** derivatives showed significant anti-inflammatory activity. Statistical analyses of all data were represented as mean ± S.E.M and as percentage. Results were statistically evaluated using student t- test. $P<0.05$ was considered significant. So we conclude that alternate of the substituent's attached at 2 and 4-substituted phenyl ring in tetrazole and 3-sulfonamide substituted pyridine in tetrazole made important difference for anti-inflammatory activity.

The synthesized compounds KPT-Cl_2, KPST- Cl_2, KPT- Cl_{24}, KPT- Me_4 and KPT- Br_2 were screened in-vivo analgesic activity by using hot plat method, diclofenace sodium and nimesulide were as used as the standard drug in the form of oral suspension in 0.5% CMC standard as well as tests. All the biological results of the compounds are given in chapter 6 in Table-11. The compounds increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Analgesic activity of test compounds was recorded in terms of mean latency time of each compound. Statistical analyses of all data were represented as mean ± S.E.M. Results were statistically evaluated using student t- test. $P<0.05$ was considered significant. According to our synthesised compound, KPT-Cl_2 (**75)**, KPT- Cl_{24} (**79)**, KPT-Me_4 (**81)**

and KPST- Cl_2 **(76)** derivatives showed significant analgesic activity among all the derivatives. Substituent's attached at 2 and 4-substituted phenyl ring in tetrazole and 3-sulfonamide substituted pyridine in tetrazole are to be in more responsible for the activity.

The five synthesised compounds were screened anti-inflammatory and analgesic activity and two good active compounds KPT-Cl_2 (**75)** and KPST- Cl_2 (**76)** derivatives were screened ulceroganic potential and its shows less ulceroganic effect as compare to diclofenace and similar to nimesulide . All the biological results of the compounds are given in chapter 6 in Table-12. Statistical analyses of all data were represented as mean±S.E.M. Results were statistically evaluated using student t- test. $P<0.05$ was considered significant. So we can say that these compound having good anti-inflammatory activity as well as low ulceroganic potential.

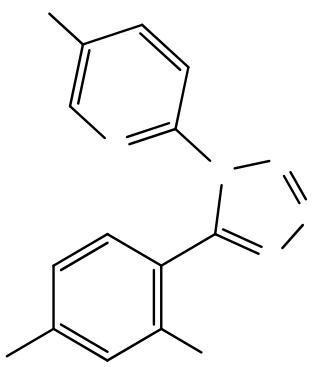
The synthesized compounds KPT-b, KPT-Cl_2, KPST- Cl_2, KPT- Cl_4, KPST- Cl_4, KPT-Cl_{24}, KPST- Cl_{24}, KPT- Me_4, KPST- Me_4, KPT- Br_2 and KPT-Fu were tested in vitro antioxidant activity by DPPH scavenging method. And compared to their anti-oxidant activity with ascorbic acid was used as the standard. All the biological results of the compounds are given in chapter 6 in. Antioxidant activities of test compounds were recorded in terms of % scavenging shown by each compound. All the biological results of the compounds are given in chapter 6 in Table-13.Statistical analyses of all data were represented as mean ± S.E.M. Results were statistically evaluated using student t-test. $P<0.05$ was considered significant. According to our synthesized compounds KPT- Cl_{24} (**79)**, KPST- Cl_{24} (**80)**, KPT- Me_4 (**81)**, KPST- Me_4 (**82)** and KPT- Br_2 (**83)** derivatives showed significant activities. Even if we concluded that the alternates of the substituent's attached at 2^{nd} and 4^{th} position of the phenyl ring and sulfonyl moiety in pyridine made important difference for the anti-oxidant activity. Therefore, we put different substituents such as 2-Cl, 4-Cl, 2, 4-di Cl, 4-OCH_3 and 2-Br in phenyl ring, and 2-furoyl ring.

The synthesized compounds KPT-b, KPT-Cl_2, KPST- Cl_2, KPT- Cl_4, KPST- Cl_4, KPT-Cl_{24}, KPST- Cl_{24}, KPT- Me_4, KPST- Me_4, KPT- Br_2 and KPT-Fu were tested in vitro anti-bacterial activity against two antibacterial species by using the cup-plate agar diffusion method against *S. aureus and E. Coli* compared to their anti-bacterial activity

with ofloxacine was used as the standard drug in the form of injection (Forcan,Cipla). All the biological results of the compounds are given in chapter 6 in Table-14, 15. Antibacterial activity of test compounds was recorded in terms of zone of inhibition (in millimetres) shown by each compound against various bacterial species. According to our synthesized compound, we pointed that the KPST- Cl_2 (**76)**, KPST- Cl_4 (**78)**, KPT- Cl_{24} (**79)**, KPST- Cl_{24} (**80)** and KPT- Br_2 (**83)** showed significant activities against *S. aureus*, and *E. coli.* Even if we concluded that the alternates of the substituent's attached at sulfonyl group in the pyridine made important difference for the anti-bacterial activity, our goal here was to keep on investigating the role of the 2^{nd} position of the phenyl ring and sulfonyl group of pyridine ring for anti-bacterial activity. Therefore, we put different substituents such as 2-Cl, 4-Cl, 2, 4-di Cl, 4-OCH_3 and 2-Br in phenyl ring, and 2-furoyl ring. The synthesized compounds show good anti-bacterial activity against certain strains of bacteria. Synthesized compounds have shown good anti-bacterial activity against *S.aureus, and E. coli* still they are somewhat less potent than the standard. Subsequent purification yielded final compounds in moderate to higher yields.

Compounds (**75), (76)** and (**79)** proved to be the most active anti-inflammatory, analgesic activity it's having low ulceroganic potential. Structure activity relationship studies revealed that the type of substituent at 2-Cl and 4-Cl of phenyl and 5-SO_2NH_2 of pyridine manipulate the activity. Compound (**75)** showed very good activity as comparable to known anti-inflammatory agents' diclofenac and nimesulide. Compounds (**79), (80), (81), (82)** and (**83)** exert their antioxidant activity through DPPH method. Compounds **(80)** and **(81)** showed very good activity then other. So concluded that 5-SO_2NH_2 substituted of pyridine is most active antioxidants. Compounds (**76), (78),** (**79), (80)** and (**83)** proved to be moderate active antibacterial activity against *S. aureus* and *E. coli*. So finally concluded that the 5-SO_2NH_2 substituted of pyridine containing compounds are showed more active as compared to other derivatives because this is look like sulphonamide.

6.2 Structure Activity Relationship



1. The rings system and the skeleton are responsible for biological activity, may be pharmacophore of the compound is suggested as per literature review to inhibiting both COX-I & II receptors.

2. Tetrazole rings having extreme value of acidity so it is responsible for the anti-inflammatory activity.

3. Pyridine ring having basic in nature so overall the acidic nature of ring was decreased and decreased its GIT toxicity.

4. Substitution of R_1 in phenyl ring electron withdrawing group shows good anti-inflammatory and analgesic activities.

5. Substitution of R_2 position in phenyl ring electron donating group – OCH_3 is shown good antioxidant activity. Electron withdrawing group at R_1 and R_2 position shows good antioxidant activity as well as anti-bacterial activity against *S. aureus*.

6. Substitution of SO_2NH_2 at R_3 position and electron withdrawing substitution at R_1 and R_2 having good anti-inflammatory, analgesic, antioxidant and antibacterial activity, as well as very low ulcerogannic potential.

7. Substitution of furan ring at the replacement of phenyl ring shown moderate antibacterial activity.

7.1 Summery and Conclusion

Research on the nonsteroidal anti-inflammatory and analgesic drugs is receiving continuous interest in industrial and academic laboratories. Due to frequent presence with this class of drugs of undesirable side effect, for the gastrointestinal and cardiovascular which plausibly involve the inhibition of COX-1 and COX-2.

Most of the active drug molecules consist of five-member and six member heterocyclic rings are widely distributed in nature and often play an important role in various biochemical processes. As a result they are incorporated into new chemical entities by medicinal chemists[1]. Tetrazole and pyridine rings directly fused or coupled through carbon and nitrogen bridges may be produce potent biological activities. Several researcher and biologist interest to explore and synthesised such similar molecules for better activity and lesser toxicity during last two decades.

Tetrazole nucleus has attracted the attention of many medicinal chemists due to its interesting and wide range of biological activities. The stable structures and hetero-aromatic system contain the greatest number of nitrogen atoms. That is why tetrazole exhibit the extreme values of acidity, basicity, and complex formation constants. Pyridine is the parent hetero-ring of a very important group of compounds that are extensively studied due to their occurrence in living systems. Compounds containing a pyridine ring have been reported as many activities. The pyridine having basic in nature so it was decrease the acidic nature of tetrazole and overall decreased the toxicity of drugs.

Tetrazole and pyridine both rings having good and versatile activity so now I choose both rings in my project work. So now we make a new molecule having both rings for anti-inflammatory, analgesic, antibacterial, antioxidant activities.

Prompted by these observations, as a part of present study aimed at developing new biologically active substituted a series of novel 2-(5’-(2’’,4’’–disubstituted phenyl)-1’*H*-tetrazol-1’-yl)pyridine (**67)** , and 2-(5’-(furan-2’’-yl)-1’*H*-tetrazol-1’-yl)pyridine (**73)** were synthesized from 2,4-disubstituted-N-(pyridin-2’-yl)benzimidoyl chloride (**66)** and N-(pyridin-2’-yl)furan-2-carbimidoyl chloride (**72)** excess amount of sodium

azide and sodium acetate at - 2^{o}C. 6-(5'-(2'',4'' –disubstituted phenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonamide (**69)** was prepared from 6-(5'-(2'',4''-disubstituted-phenyl)-1'*H*-tetrazol-1'-yl)pyridine-3-sulfonyl chlorides **(68)** in presence of liquor ammonia., Compound (**68)** was prepared from compound (**67)** with chlorosulfonic acid. 2,4-disubstituted-N-(pyridin-2'-yl)benzamides (**65)** and N-(pyridin-2'-yl)furan-2-carboxamide (**71)** were prepared by reacting with 2-amino pyridine (**63)** and 2.4-disubstituted benzoyl chloride (**64)** and corresponding furan-2-carbonyl chloride (**70)** in presence of TEA and DCM, which was further treated with PCl_5 to obtained 2,4-disubstituted-N-(pyridin-2'-yl)benzimidoyl chloride (**66)** and N-(pyridin-2'-yl)furan-2-carbimidoyl chloride (**72)**. The structures of the synthesized compounds were established by MASS 1H NMR and IR spectroscopic techniques. All the synthesized compounds were evaluated for anti-inflammatory, Analgesic, ulceroganic potential, antioxidant and antibacterial activities. Most of the compounds showed significant *in-vivo* anti-inflammatory and analgesic activities. Compounds KPT-Cl_2 **(75)** and KPST-Cl_2 **(76)** were found to be the most potent among all the synthesized compounds. Low ulceroganic potential activity was done in rats and found reduced and equal activity against standard drugs diclofenac sodium and nimesulide by compounds KPT-Cl_2 **(75)** and KPST-Cl_2 **(76)**. KPT-Cl_2 **(75)**, KPST-Cl_{24} **(80)** and KST-Me_4 **(81)** were produced good in-vitro antioxidant activity by DPPH method. In antibacterial screening was done by ager diffusion method against *E. coli* and *S. aureus.* Compounds KPT-Cl_2 **(75)**, KPST-Cl_{24} **(80)** and KPST-Me_4 **(82)** were found good potential against *S. aureus.*

Compounds (**75), (76)** and (**79)** proved to be the most active anti-inflammatory, analgesic activity it's having low ulceroganic potential. Structure activity relationship studies revealed that the type of substituent at 2-Cl and 4-Cl of phenyl and 5-SO_2NH_2 of pyridine manipulate the activity. Compound (**75)** showed very good activity as comparable to known anti-inflammatory agents' diclofenac and nimesulide. Compounds (**79), (80), (81), (82)** and (**83)** exert their antioxidant activity through DPPH method. Compounds **(80)** and **(81)** showed very good activity then other. So concluded that 5-SO_2NH_2 substituted of pyridine is most active antioxidants. Compounds (**76), (78),** (**79), (80)** and (**83)** proved to be moderate active antibacterial activity against *S. aureus* and *E. coli.* So finally concluded that the 5-SO_2NH_2 substituted of pyridine containing compounds are showed more active as compared to other derivatives because this is look like sulphonamide.

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Project Number:IPS/PCHEM/MPH10/001

CERTIFICATE

This is to certify that the project title **Evaluation of anti-inflammatory, ulcerogenic effect and analgesic activities of novel pyridine containing tetrazole derivatives** has been approved by the IAEC on dated 23-01-2010 in 6th IAEC meeting.

Name of Chairperson/Member Secretary IAEC:
Dr. Avani F. Amin
Chairperson

Signature with date

Name of CPCSEA nominee:
Dr. P. Y. Guru
CPCSEA nominee

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)