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Synthesis Of Pyrazolopyrimidine Derivatives Along With Its Biological Activity Including Toxicity Studies

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Abstract: Pyrazolopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. The heterocyclic fusion of pyrimidine ring and pyrazole ring resulted in formation of pyrazolopyrimidines, the structural analogues of biogenic purine class, undoubtedly, has high significance in the field of pharmaceutical and biotechnological sciences with wide spectrum of biological activities and its several derivatives. Toxicity may be due to the accumulation in a specific organ/ tissue (e.g. bosentan), the co - administration of other drugs affecting ADMET (absorption, distribution, metabolism, elimination and toxicity) Cmax reaching off target IC_{50}, or the high Cmax required for therapeutic effects. Assessing the relative drug efficacy and toxicity is important for medicinal chemists, pharmacologists, pharmacists, physicians. As multiple treatment options are available for many diseases, relative toxicity assessment is necessary. Difficulty in direct clinical trial comparisons forces network meta-analyses for estimating the relative toxicity. Therapeutic index (TI) assumes simplified linear relationships between receptor affinity, maximum unbound plasma drug concentration (Cmax) and toxicity. But high TI does not guarantee safety. For drugs metabolized by cytochrome P450 (CYP450),estimating TI based on target potencies alone is insufficient.

Keywords: Anti-inflammatory activity, Analgesic activity, In Silico toxicity

1. INTRODUCTION:

The field of medicinal chemistry has evolved from an emphasis on synthesis, isolation, and classification of drugs to an increased awareness of the biochemistry of disease states and designing drugs for the prevention of diseases. An important aspect of medicinal chemistry has been establishing a relationship between chemical structure and biological activity [1].This involves the identification, synthesis, and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological

properties, and their quantitative structure-activity relationships (QSAR). Pharmaceutical chemistry is focused on the quality of medicines and aims to ensure fitness for the purpose of medicinal products [2].

Medicinal chemistry is a chemistry-based discipline involving features of biological, medical and pharmaceutical sciences. It is concerned with the invention, discovery, design, and identification of biologically active compounds.It is also concerned with the study of their metabolism, the interpretation of their mode of action at the molecular level, and the construction of structure activity relationships (SARs), which is the relationship between chemical structure and pharmacological aspects [3]. Although there has been a great deal of success in understanding the relationship between the chemical structure and biological activities in numerous areas, especially for antibacterial drugs, there are still many human afflictions that requires new and improved drugs [4].

When a new pharmaceutical lead compound is discovered, extensive and costly efforts are usually made to prepare a series of analogues so that better activity can be found. The metabolism of the drug is an important object of study in medicinal chemistry and considerable efforts are to spent on detailed analysis of the bioconversion that a new drug series undergoes. Modern analytical methods such as mass spectrophotometery permit the identification of minute amounts of metabolites. The intellectual goal of a medicinal chemistry is to determine the mode of action of drugs at the molecular level. The objective of medicinal chemistry is the design and production of compounds that can be used in medicine for the prevention, treatment and cure of humans or animal diseases [5].

Five and six-membered heterocyclic nitrogen containing systems such as pyrazole, imidazole, triazoles, thiozolidine, pyrazolidine, piperidine, oxane pyrimidine, pyridine, thiane, and pyran far the most important in the ongoing search for more efficacious drugs in the fields of antibacterials, antifungal,antitubercular, anti-inflammatory, diuretics, antirheumatics, and antihistaminics. Nitrogen-containing heterocyclic compounds have received considerable attention due to their wide range of pharmacological activity. Pyrimidine and their derivatives are considered to be important for medicinal drugs as well. Because pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities. Pyridine, a heterocyclic nucleus, has played a pivotal role in the development of different medicinal agents. Current studies have demosnstrated that pyridine congeners are associated with different biological activities, such as pesticidal, fungicidal and antibacterial activity. Pyrimidines and pyridines have contributed to the diverse library of compounds demonstrating selective affinity to the 5-HT7 receptor. Pyrimidines are six-member heterocyclic rings, containing two nitrogen atoms on the 1, 3 positions, as depicted in Fig.1.1

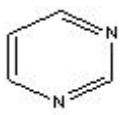


Fig.1.1

Pyrimidines are present among the three isomeric diazines. Several pyrimidines mainly cytosine (I), uracil (II) and thymine (III) have been isolated from the nucleic acid hydrolysis

as shown in Fig 1.2. The nucleic acid are essential constituent of all cell and thus of all living matter cytosine is found to be present in both types of nucleic acid i.e. ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) [6].

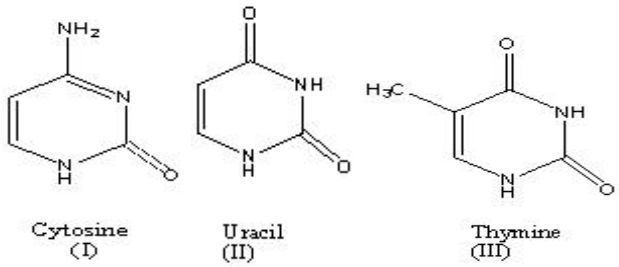


Fig.1.2

In addition to this, Pyrimidines ring is also found in Vitamin B_1, Barbituric acid (IV) and its several derivatives e.g. Veranal (V) which are used as hypnotics (fig.1.3) [7].

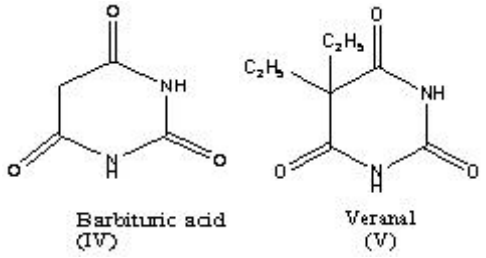


Fig.1.3

Numerous reports have appeared in the literature that highlights chemistry and uses of pyrimidines, and their derivatives like , Sulfamerazine, and Sulfamethazine. These agents are inhibitors of folic acid biosynthesis in microorganism. Pyridine is a ubiquitous chemical compound. The aromatic, monocyclic azine is utilized as a reagent or as a polar aprotic solvent. It is salient in a number of biological systems and industrial applications. Naturally occurring pyridines include the nicotinamides, a component of the vitamin B group. Pyridines are precursors to various pharmaceuticals, adhesives, agrichemicals, and synthetic pigments.A pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier [8]. **Synthesis of pyrimidine:** Several approaches are available for synthesis of pyrimidine as follows:

1.1.1 Synthesis from enamines, triethyl orthoformate:

A $ZnCl_2$-catalyzed three-component coupling reaction allows the synthesis of various 4,5-disubstituted pyrimidine derivatives in a single step from functionalized enamines, triethyl orthoformate, and ammonium acetate.

The procedure can be successfully applied to the efficient synthesis of mono- and disubstituted pyrimidine derivatives, using methyl ketone derivatives instead of enamines (as shown in figure 4) [9].

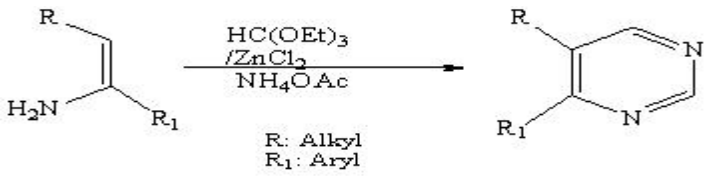


Fig.1.4

1.1.2. Synthesis from N-vinyl/aryl amides:

The direct condensation of cyanic acid derivatives with N-vinyl/aryl amides affords the corresponding C4-heteroatom substituted pyrimidines (as shown in fig 1.5)[10].

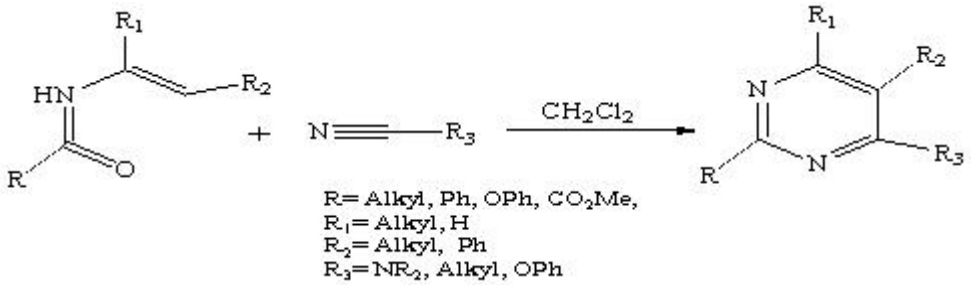


Fig.1.5

1.1.3. Synthesis of pyrimidine from β-formyl enamides:

A novel and efficient synthesis of pyrimidine from β-formyl enamide involves samarium chlori1.de catalyzed cyclisation of β-formyl enamides using urea as source of ammonia under microwave irradiation (as shown in fig.1.6) [11].

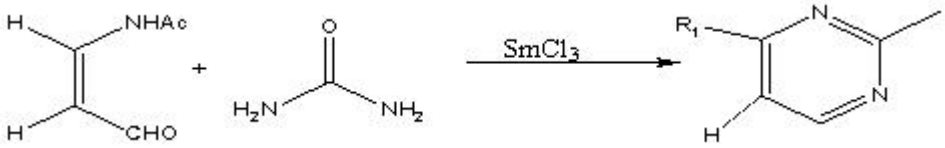


Fig.1. 6

1.1.4. Synthesis from activation of amide with 2-chloropyridine

A single-step conversion of various N-vinyl and N-aryl amides to the corresponding pyrimidine and quinazoline derivatives involves amide activation with 2-chloropyridine and trifluoromethanesulfonic anhydride followed by nitrile addition into the reactive intermediate and cycloisomerization (as shown in fig1.7)[12].

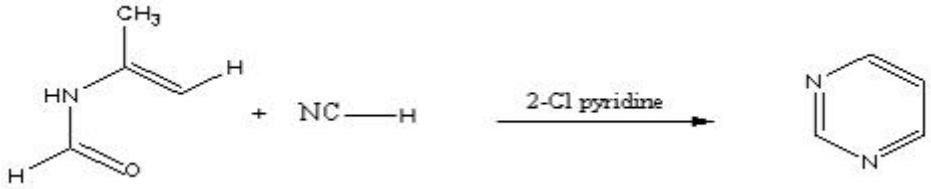
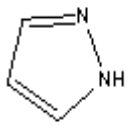


Fig.1.7

Pyrazole: The simple doubly unsaturated compound containing two nitrogen and three carbon atoms in the ring and with the nitrogen containing neighboring, is known pyrazole

(fig. 1). Pyrazole derivatives are well-known in the literature as significant biologically active heterocyclic compounds. These derivatives are the subject of many research studies due to their extensive potential biological activities such as anti-inflammatory , antipyretic , antimicrobial , antiviral , antitumour , anticonvulsant , antihistaminic , antidepressant , insecticides and fungicides.



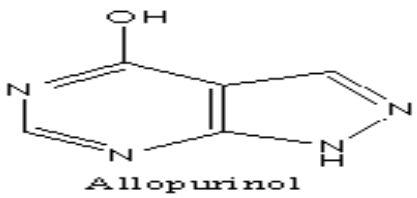
Pyrazole

Fig. 1.8

The physical properties of the pyrazole can be usefully compared and contrasted with those of their 1,3-isomeric counter parts. Echoing the higher boiling point of pyrazole which is only one solid at room temperature, also has much higher B.P. (187 °C) than isoxazole (95°C) and again reflecting the intermolecular hydrogen bonding available only to pyrazole. This association probably takes the form of dimers, trimers and oligomers. The dihydro and tetrahydro heterocycles are named as pyrazoline/ pyrazolidine.Rapid tautomerism, involving switching of the hydrogen from one nitrogen to the other, as in imidazoles, means that substituted pyrazoles are inevitably mixtures. Example: 3(5)-methyl pyrazole.

Fig. 1.9

Pyrazolopyrimidine: Pyrazolopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. The heterocyclic fusion of pyrimidine ring and pyrazole ring resulted in formation of pyrazolopyrimidines, the structural analogues of biogenic purine class, undoubtedly, has high significance in the field of pharmaceutical and biotechnological sciences with wide spectrum of biological activities and its several derivatives. e.g. (fig.1.10).



Allopurinol

Fig. 1.10

2. RESEARCH METHODOLOGY:

Synthesis and purification of Pyrazolopyrimidine derivatives.

1. Characterization of synthesized compounds: Synthesized Pyrazolopyrimidine derivatives will characterized by using-

1. Infrared spectroscopy

2. NMR spectroscopy

3. MASS spectroscopy

2. Biological Evaluation: Synthesized Pyrazolopyrimidine derivatives will screened for following biological activities:

1. Analgesic activity

2. Anti-inflammatory activity

3. Prediction of Toxicity of Synthesized Pyrazolopyrimidine derivatives

4. SAR of synthesized Pyrazolopyrimidine derivatives

3. METHODOLOGY:

3.1. Material and Methods:

The purified pyrimidine derivatives were obtained in yields of 45-95%. The synthetic route is illustrated in scheme 1. Thin layer chromatography was used to reach completion of reaction and purity of compounds synthesized, using silica gel as stationary phase and Toulene:ethyl acetate:formic acid as solvent system (4:2:1) and visualized by U.V. visualizing cabinet.

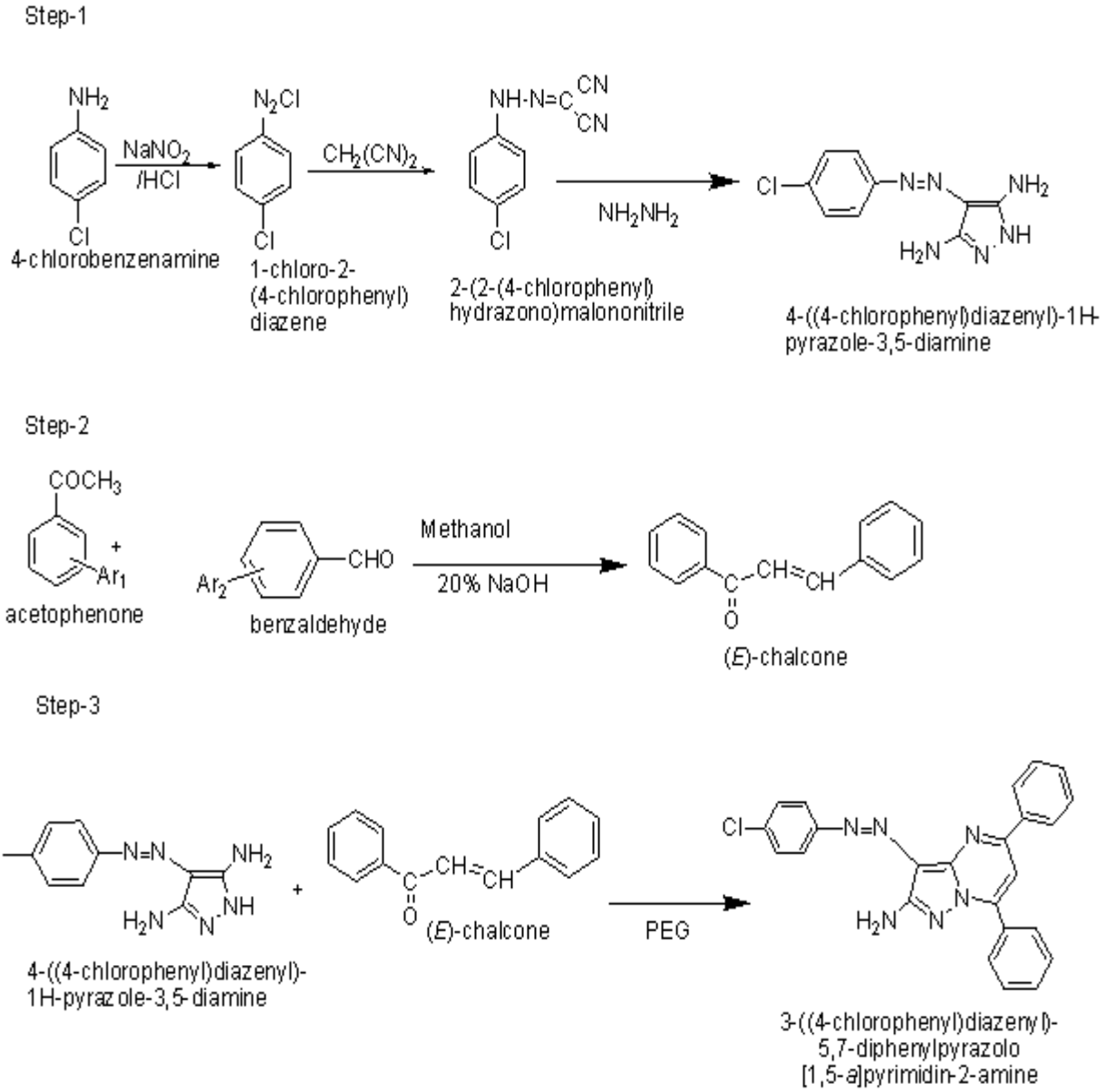
All solvents used were analytical grade. The chemicals used were obtained from sigma – Aldrich (St. Louis Mossuri, USA).

The structures of compounds were identified using infrared spectroscopy, Mass spectroscopy and proton nuclear magnetic resonance studies. IR Spectra were recorded by KBR pellet technique using FTIR-84005 Shimadzu spectrophotometer. 1HNMR Spectra were obtained on Bruker model DRX (300MHz NMR) Spectrometer in DMSO-d6/$CDCl_3$ as solvent and using tetramethylsilane as internal standard. Mass were recorded on API 2000 triple quadrapole mass spectrophotometer.

Procedure: A Mixture of α, β- unsaturated carbonyl compounds (chalcone) (1mmol), substituted pyrazole (1mmol) and 1-2 pellets of NaOH in polyethylene glycol (PEG-400) (20ml). The reaction mixture was heated for the period. The progress of the reaction was monitered by TLC.

After completion, the reaction mixture was extracted with diethyl ether (2×20mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was recrystallized from proper solvent to give the product (**PP_1- PP_5)**.

3.2. REACTION SCHEME:



Physicochemical properties of Pyrazolopyrimidine derivatives:

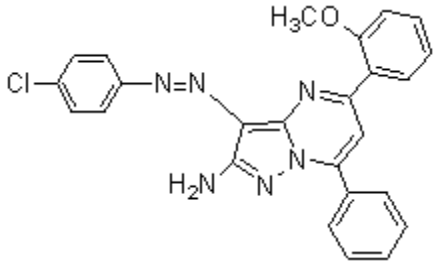
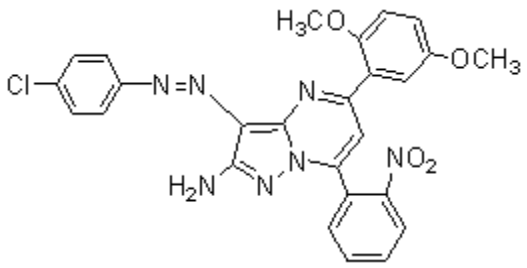
[a]Compounds	Ar_1	Ar_2	Mol. Formula (Mol.Wt.)	[b]R_f value	Yield (%)	m.p. (ºC)
PP_1	$-C_6H_5$	$2\text{-}OCH_3C_6H_4$	$C_{25}H_{19}ClN_6O$ (454)	0.90	58	72-75

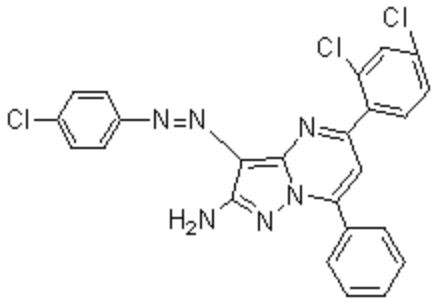
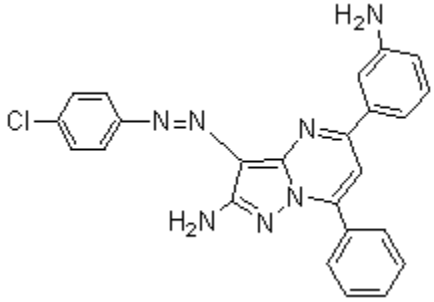
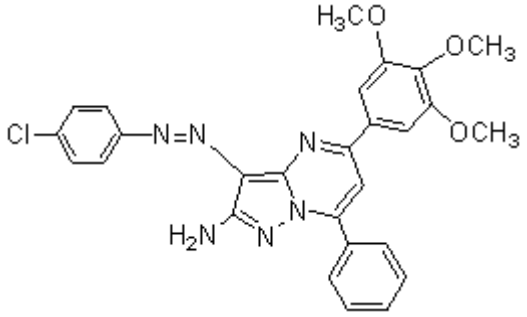
PP_2	-2,5-$(OCH_3)_2C_6H_3$	2-$NO_2C_6H_4$	$C_{26}H_{20}ClN_7O_4$(529)	0.83[1]	84.15	113-115
PP_3	-2,4-$(Cl)_2C_6H_3$	-C_6H_5	$C_{24}H_{15}Cl_3N_9$ (492)	0.88[1]	41.66	146-148
PP_4	-3-$NH_2C_6H_5$	-C_6H_5	$C_{24}H_{18}ClN_7$ (439)	0.84[1]	47.34	132-134
PP_5	-C_6H_5	-3,4,5,$(OCH_3)_3$ C_6H_2	$C_{27}H_{23}ClN_6O_3$ (514)	0.78[1]	81.25	92-94

[a] Products were characterized by IR, NMR, MS.

[b] 1; Toulene : EthylAcetate : Formic Acid (4:2:1), 2; EthylAcetate : n-Hexane (3:7), 3; Pet. Ether : Ethyl Acetate (2:1)

3.3. Spectral characterizations of synthesized compounds:

1.	**3-(4-chlorophenyl)diazenyl)-5-(2-methoxyphenyl)-7-phenylpyrazolo [1,5-a]pyrimidin-2-amine** 	**IR (KBr, cm^{-1}):** 3240 (NH_2), 3316 (C-H Ar str), 1548 (C=C str),1576 (C=N str), 765(C-Cl str), 1178(OCH_3) **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.8 (s, 1H, CH of pyrimidine), 3.98 (d, 2H, NH_2), 7.7 (m, 14H, Ar-H), 2.5 (s. 3H, OCH_3), , **MS (m/z)** : **(**M^+= 455); 390, 380, 375.
2.	**3-(4-chlorophenyl)diazenyl)-5-(2,5-dimethoxyphenyl)-7-(2 nitrophenyl)pyrazolo[1,5-a]pyrimidin-2-amine** 	**IR (KBr, cm^{-1})**: 3572(NH_2 str), 3157 (C-H Ar str), 1565 (C=N str), 1548.02 (C=C Ar str), 755 (C-Cl str.), 1189 (OCH_3 str). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.72 (s, 1H, CH of pyrimidine), 7.5-8.2 (m, 12H, Ar-H), 8.8 (s, 1H, NH), 3.26-3.3 (s, 6H, OCH_3), **MS (m/z)** : **(**M^+= 529), 527, 522.

3.	**3-(4-chlorophenyl)diazenyl)-5-(2,4-dichlorophenyl)-7phenylpyrazolo[1,5-a]pyrimidin-2-amine** 	**IR (KBr, cm^{-1})**: 3561 (NH_2 str), 3132 (C-H str), 1610 (C=O str), 1545 (C=N str), 1178.02 (C=C Ar str),765 (C-Cl str.), **^{1}H NMR: ($CDCl_3$, δ, ppm):** 7.1(s, 1H, CH of pyrimidine), 7.8-8.6 (m, 13H, Ar-H), 8.9 (s, 1H, NH_2), **MS (m/z)** : **(**M^+= 492), 440, 429.
4.	**5-(3-aminophenyl)-3-((4chlorophenyl)diazenyl)-7-phenylpyrazolo[1,5-a]pyrimidin-2-amine** 	**IR (KBr,cm^{-1})**: 1668 (C=N str), 1595 (Ar C=C) , 3570 (NH_2 str), 3152 (C-H str), 1695 (C=O str), 1545 (C=N str), 751 (C-Cl str). **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.72 (s, 1H, CH of pyrimidine), 7.9-8.5 (m, 14H, Ar-H), 8.8 (d, 4H, NH_2). **MS (m/z)** : **(**M^+= 439), 320, 329.
5.	**4-(4-chlorophenyl)diazenyl)-7-phenyl-5-(3,4,5-trimethoxyphenyl)pyrazolo[1,5-a]pyrimidin-2-amine** 	**IR (KBr, cm^{-1})**: 3752 (NH_2 str), 3437 (C-H str), 1706 (C=O str), 1668 (C=N str), 1608 (Ar C=C) , (-OCH_3 str), 752 (C-Cl str.) **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 7.4 (s, 1H, CH of pyrimidine), 7.9-8.7 (m, 7H, Ar-H), 8.8 (s, 12H, NH_2), 7.36 (s, 1H, CH of pyrimidine), 3.26-3.3 (s, 9H, OCH_3), **MS (m/z)** : **(**M^+= 514), 500, 498.

4. BIOLOGICAL EVALUATION:

4.1. Anti-inflammatory activity:

Material and methods:

Animals Rats (100-180gm) six in each group

Standard drug Diclofenac Sodium

Control 0.1% CarboxyMethyl Cellulose (CMC) solution

Experimental procedure

The study was carried out on healthy rats, divided in different groups of six animals each and housed in cages. Rats were weighed and marked/numbered.

1. A mark was made on the left hind paw near tibio-tarsus junction so that every time the paw was dipped in mercury column up to fixed mark to ensure constant paw volume.

2. The drug used as standard was Diclofenac sodium in dose of 50-mg/kg-body weight. The doses of test compounds were 50-mg/kg-body weight. The standard and test compound were administered through oral route in the form of (0.1% CMC) suspension. The control group was administered normal saline orally. Carrageenan was injected subcutaneously, 0.1 ml of a 1% w/v carrageenan suspension (in 0.5% CMC) to hind paw of each of the rats.

3. Initial paw volume of all the animals was recorded.

4. After 30 min, carrageenan was injected subcutaneously into subplantar region of left hind paw of all animals.
5. The paw volume was measured by digital plethysmograph at 0, 0.5, 1 and 2 hours after carrageenan injection. Thus the oedema volume in control group (Vc) and oedema volume in groups treated with test compounds (Vt) was measured and percentage inhibition of oedema was calculated using the formula

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

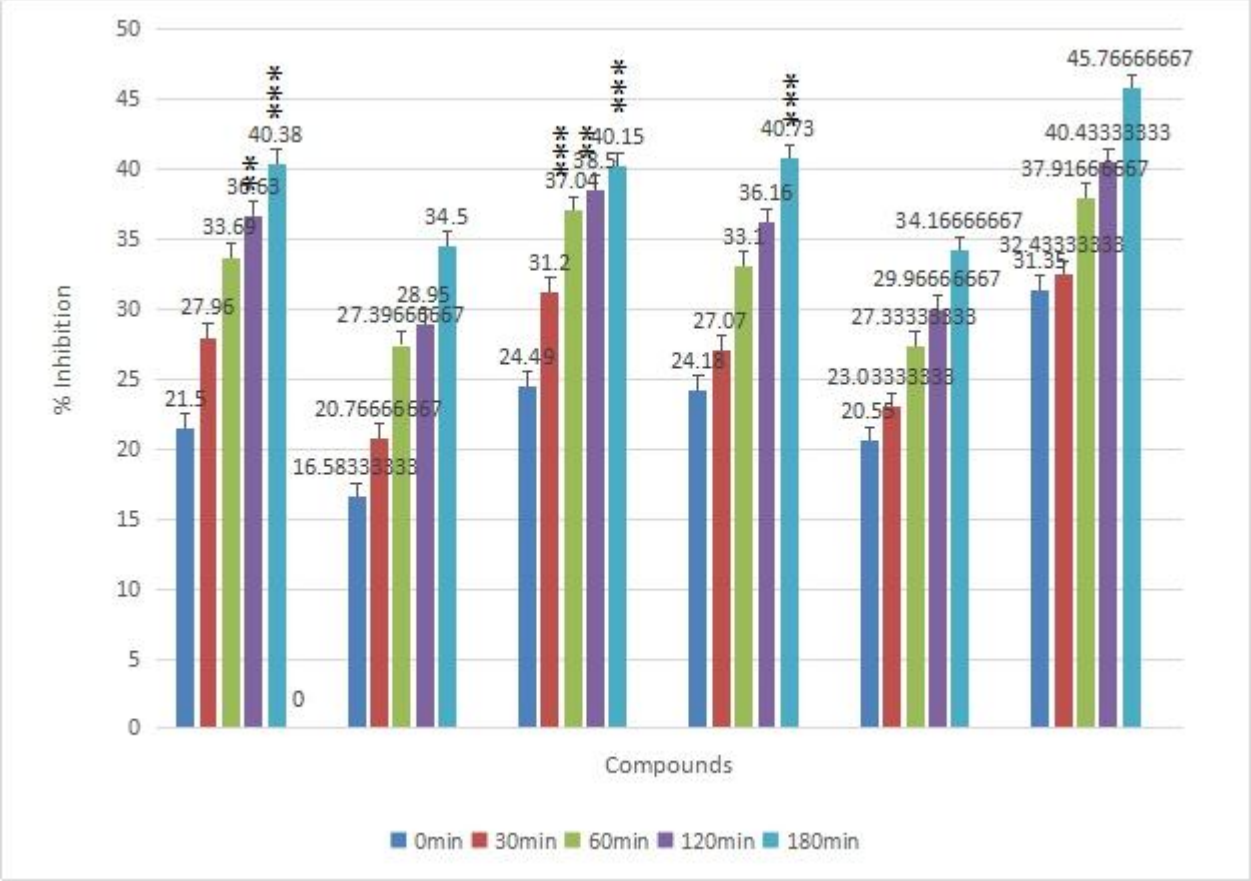
Anti-inflammatory activity of synthesized compounds

Group	Dose(mg/kg)	After Carrageenan injection at various time intervals				
		Mean ± SEM				
		0 min	30 min	60 min	120 min	180 min
		EI (%)	EI (%)	EI (%)	EI (%)	EI (%)
Std.(Diclofenac sodium)	50	31.35± 0.1	32.43± 0.3	37.91±0.3	40.43± 0.1	45.76± 0.7
PP_1	50	16.58± 0.2	20.76± 0.04	27.38± 0.2	28.95± 0.2	34.5± .08
PP_2	50	21.50±.09	27.96± 0.09	33.69± 0.22	36.63± 0.21**	40.38± 0.09***

PP_3	50	24.49± 0.08	31.20± 0.08**	37.04± 0.09***	38.5± 0.08**	40.15± 0.90***
PP_4	50	24.18± 0.03	27.07± 0.08	33.1± 0.13	36.16±0.11	40.73± 0.50***
PP_5	50	20.55± 0.11	23.03± 0.26	27.33± 0.06	29.96±0.06	34.16±0.08

EV: Oedema Vol.

EI: Oedema Inhibition



Statistical analysis Data were analyzed by One-Way ANOVA followed by Tukey's t-test using computerized Graph Pad Instat version 5.04 (Graph Pad software)

4.2. ANALGESIC ACTIVITY:

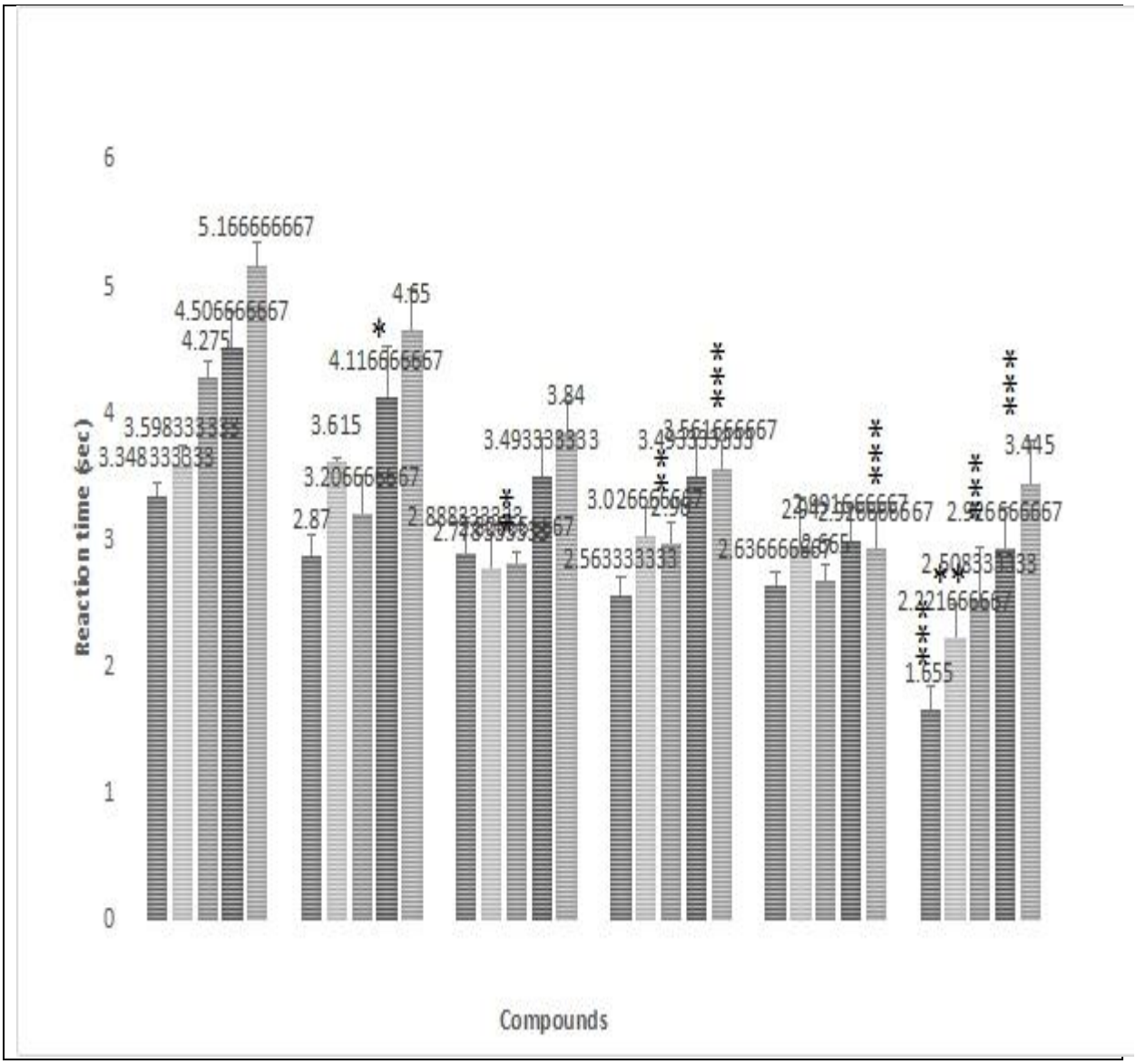
Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. It is the feeling common to such experiences as stubbing a toe, burning a finger, putting iodine on a cut, and bumping the. Pain motivates us to withdraw from damaging or potentially damaging situations, protect the damaged body part while it heals, and avoid those situations in the future. It is initiated by stimulation of nociceptors in the peripheral nervous system, or by damage to or malfunction of the peripheral or central nervous systems.

Experimental procedure:

- Tail immersion method was used to determine the analgesic activity.
- Rats of wistar strain were randomly divided into a six groups having six animals in each.
- They were fasted overnight but during the experiment had free access to water. All the extracts were administered orally (100mg/kg) 60 minutes priorto the commencement of the estimation of reaction time.
- The temperature of the water in the organ bath was set at 55 ± 0.5 °Ċ with the help of thermostat.
- The reaction time was determined by immersing the tail in hot water and the time taken by the rat to withdraw its tail clearly out of water was noted. Observations were repeated at an interval of 30 minutes up to 120 minutes.

Analgesic activity of synthesized compound:

Compounds	**Reaction time (sec)** **Mean ± SEM**				
	0 min	**30 min**	**60 min**	**120 min**	**180 min**
Std.	3.34 ± 0.10	3.59 ± 0.14	4.27 ± 0.12	4.50 ± 0.28	5.16 ± 0.18
PP_1	2.63 ± 0.10	2.94 ± 0.39	2.66 ±0.12	2.99 ± 0.25*	2.92 ± 0.28***
PP_2	2.87 ± 0.15	3.61 ± 0.03	3.20 ± 0.29	4.11 ± 0.39*	4.65 ± 0.31**
PP_3	1.65 ±0.19***	2.22±0.26**	2.50±0.43***	2.92±0.32*	3.44 ± 0.33***
PP_4	2.56 ± 0.14	3.02 ± 0.21*	2.96 ±0.17**	3.23 ± 0.31	3.56 ± 0.31***
PP_5	2.88 ± 0.28*	2.77 ± 0.25*	2.80 ± 0.094**	3.49 ± 0.31	3.84 ± 0.25

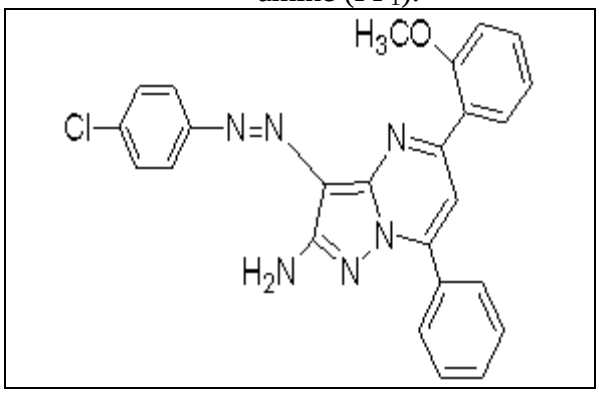


Method: Tail immersion method; Albino rats; number of animals per group: 6; route of administration: oral; standard: Diclofenac sodium (50mg/kg.); test compound 50 mg/kg. ***$p \leq 0.001$ statistically significant; Statistical analysis was performed by one way─ANOVA followed by Tukey's 't' test. All the values were expressed as means sem and $p \leq 0.001$ indicates the level of statistical significance compared with standard Diclofenac sodium.

4.3.TOXICITY:

Toxicity screening was performed for: Drug Induced Toxicity, Genomic Toxicity, Aquatic & Terrestrial Toxicity, Reproductive Toxicity, Environmental Factor. These toxicity values were adapted from literature support.

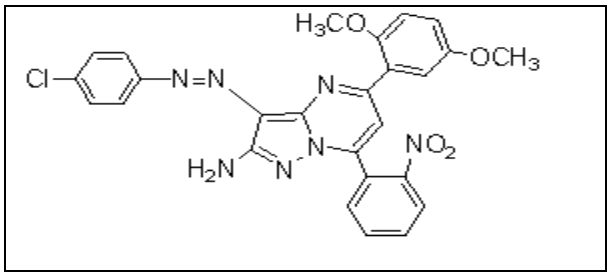
3-(4-chlorophenyl)diazenyl)-5-(2-methoxyphenyl)-7-phenylpyrazolo[1,5-a]pyrimidin-2-amine (PP_1):



PP_1: Toxicity (Qualitative Prediction & Probability)		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8709
	Non-inhibitor	0.7389
AMES Toxicity	Non AMES toxic	0.6022
Carcinogens	Non-carcinogens	0.7681
Fish Toxicity	High FHMT	0.6507
Tetrahymena Pyriformis Toxicity	High TPT	0.8762
Honey Bee Toxicity	Low HBT	0.8932
Biodegradation	Not ready biodegradable	1.0000
Acute Oral Toxicity	III	0.7106
Carcinogenicity (Three-class)	Non-required	0.5377

Toxicity (Predicted Activity through model)		
Rat Acute Toxicity	1.9116	LD_{50}, mol/kg
Fish Toxicity	1.3171	pLC_{50}, mg/L
Tetrahymena Pyriformis Toxicity	1.1173	$pIGC_{50}$, ug/L

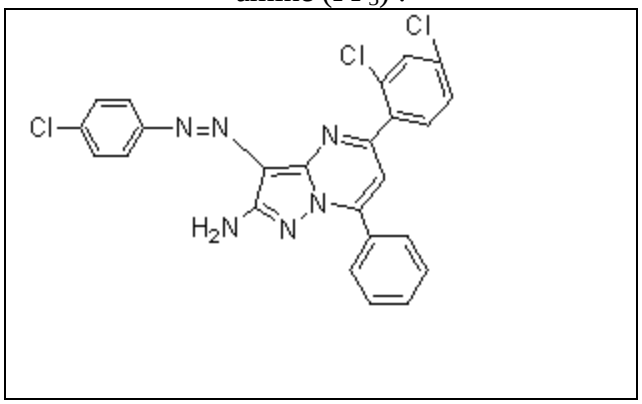
3-(4-chlorophenyl)diazenyl)-5-(2,5-dimethoxyphenyl)-7-(2 nitrophenyl)pyrazolo[1,5-a]pyrimidin-2-amine PP_2



PP_2: Toxicity (Qualitative Prediction & Probability)		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8391
	Non-inhibitor	0.6654
AMES Toxicity	Non AMES toxic	0.4148
Carcinogens	Non-carcinogens	0.9057
Fish Toxicity	High FHMT	0.7745
Tetrahymena Pyriformis Toxicity	High TPT	0.9734
Honey Bee Toxicity	Low HBT	0.8402
Biodegradation	Not ready biodegradable	0.7969
Acute Oral Toxicity	III	0.5895
Carcinogenicity (Three-class)	Non-required	0.3786

Toxicity (Predicted Activity through model)		
Rat Acute Toxicity	2.0823	LD_{50}, mol/kg
Fish Toxicity	1.2008	pLC_{50}, mg/L
Tetrahymena Pyriformis Toxicity	0.8920	$pIGC_{50}$, ug/L

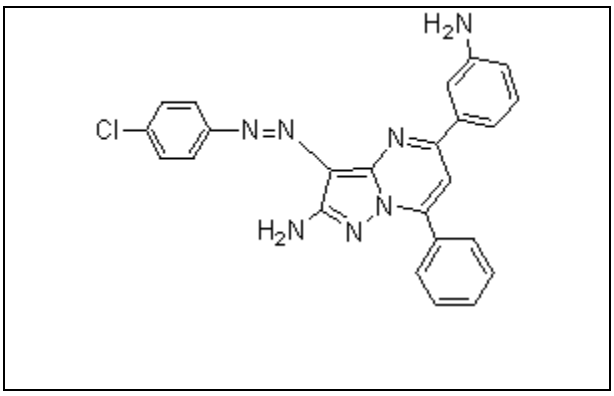
5- (4-chlorophenyl)diazenyl)-5-(2,4-dichlorophenyl)-7phenylpyrazolo[1,5-a]pyrimidin-2-amine (PP_3) :



PP_3: Toxicity (Qualitative Prediction & Probability)		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9384
	Non-inhibitor	0.8159
AMES Toxicity	Non AMES toxic	0.5437
Carcinogens	Non-carcinogens	0.8996
Fish Toxicity	High FHMT	0.6163
Tetrahymena Pyriformis Toxicity	High TPT	0.9903
Honey Bee Toxicity	Low HBT	0.7857
Biodegradation	Not ready biodegradable	0.9974
Acute Oral Toxicity	III	0.7109
Carcinogenicity (Three-class)	Non-required	0.4549

Toxicity (Predicted Activity through model)		
Rat Acute Toxicity	2.1544	LD50, mol/kg
Fish Toxicity	1.3695	pLC50, mg/L
Tetrahymena Pyriformis Toxicity	0.8863	pIGC50, ug/L

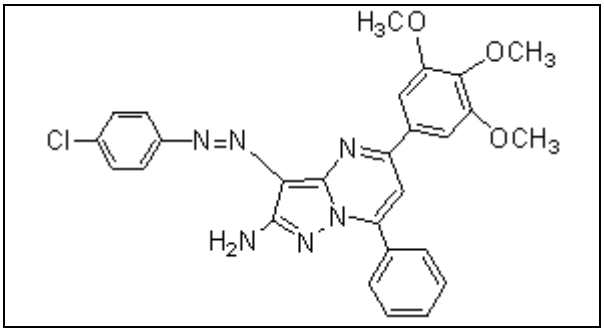
5-(3-aminophenyl)-3-((4chlorophenyl)diazenyl)-7-phenylpyrazolo[1,5-a]pyrimidin-2-amine(PP_4):



PP_4: Toxicity (Qualitative Prediction & Probability)		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9693
	Non-inhibitor	0.8329
AMES Toxicity	Non AMES toxic	0.6366
Carcinogens	Non-carcinogens	0.8483
Fish Toxicity	High FHMT	0.7275
Tetrahymena Pyriformis Toxicity	High TPT	0.8801
Honey Bee Toxicity	Low HBT	0.6181
Biodegradation	Not ready biodegradable	1.0000
Acute Oral Toxicity	III	0.6184
Carcinogenicity (Three-class)	Non-required	0.4708

Toxicity (Predicted Activity through model)		
Rat Acute Toxicity	0.0891	LD_{50}, mol/kg
Fish Toxicity	0.5441	pLC_{50}, mg/L
Tetrahymena Pyriformis Toxicity	0.9762	$pIGC_{50}$, ug/L

3-(4-chlorophenyl)diazenyl)-7-phenyl-5-(3,4,5-trimethoxyphenyl)pyrazolo[1,5-a]pyrimidin-2-amine (PP_5):



PP_5: Toxicity (Qualitative Prediction & Probability)		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9851
	Non-inhibitor	0.8265
AMES Toxicity	Non AMES toxic	0.6427
Carcinogens	Non-carcinogens	0.8404
Fish Toxicity	High FHMT	0.8580
Tetrahymena Pyriformis Toxicity	High TPT	0.9860
Honey Bee Toxicity	Low HBT	0.8064
Biodegradation	Not ready biodegradable	1.0000
Acute Oral Toxicity	III	0.5548
Carcinogenicity (Three-class)	Non-required	0.3980

Toxicity (Predicted Activity through model)		
Rat Acute Toxicity	2.2961	LD_{50}, mol/kg
Fish Toxicity	0.9373	pLC_{50}, mg/L
Tetrahymena Pyriformis Toxicity	1.0086	$pIGC_{50}$, ug/L

Interpretation of result:

Compounds	**Probability (Acute Oral Toxicity)**	**Low (<0.6); Mild (>=0.6 to <0.7); High (>=0.70)**
PP_1	0.7106	High
PP_2	0.6895	**Mild**
PP_3	0.6034	Mild
PP_4	0.4674	**LOW**
PP_5	0.6548	**mild**

5. RESULT AND DISCUSSION:

Anti-inflammatory activity was performed by carrageenan induced rat paw oedema method, it was observed that, compounds **PP_3 , PP_1**, and **PP_4** exhibited significant activity after 2 and 3 hr, comparable to standard drug Diclofenac sodium which was administered at 50 mg/kg/p.o.

The analgesic activity was performed by tail immersion method. Compounds **PP_3** , **PP_5** **PP_4** **(**(having of nitro group at 4^{th} position) showing good analgesic activity. The maximum

reaction time was observed in the case of the standard was 5.16 sec and among the synthesized compound **PP_3**, **PP_4** and **PP_5**, showed reaction time of 3.56, 3.44 and 2.92.

Among the synthesized compounds **PP_4** showed good activity and low toxicity due to low LD_{50} Value.

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