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VIRTUAL SCREEENING AND PHARMACOLOGICAL EVALUATION OF NEWER BTK INHIBITIORS AS POTENT ANTI-RHEUMATOID ARTHRITIC AGENTS

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Keywords:

Complete Freund's Adjuvant, Rheumatoid Arthritis, Bone erosion, Histopathology, Protein denaturation, Rheumatoid factor

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ABSTRACT: Rheumatoid arthritis is a chronic autoimmune disease affecting 1% of the world wide population. Recent researches suggested that the need for discovery of new therapeutic disease modifying anti-rheumatoid arthritis drugs (DMARD's). BTK (Bruton's tyrosine kinase) is cytoplasmic, non receptor tyrosine kinase and it is efficient target for the treatment of rheumatoid arthritis. This encouraged us to design a novel series of potent BTK inhibitor. The BTK inhibitor containing five features of Pharma-cophore, One Hydrogen bond donor (HBD), one hydrogen bond acceptor lipid (HBAL) and three hydrophobic features (HYP). Five different ligands containing 4, 5-di-substituted imidazole as heterocyclic nucleus have been designed and optimized by using Molecular docking, Lipinski rule of five and toxicity prediction (ADMET). The optimized ligands were synthesized based on the synthetic feasibility and characterized by using different spectral studies such as IR, 1H NMR, 13C NMR and GC-MS. The synthesized compounds were evaluated for their *in vitro* Anti-arthritic activity using protein denaturation method and all were found to exhibit an effective inhibition against the protein. Out of the five synthesized compounds IPABA was found to be a most effective with percentage inhibition of protein denaturation at 1000µg/ml when compared with the other three synthesized compounds and it will take for further *in-vivo* anti-rheumatoid arthritis activity by using adjuvant induced arthritic model. The effect of synthesized compound IPABA decreased the bone erosion, spleen enlargement and rheumatoid factor at the dose of 100mg/kg and 200mg/kg compared to the disease control group but significantly less compared to standard drug dexamethasone 5mg/kg.

INTRODUCTION: Rheumatoid arthritis (RA) is a progressive, disabling, chronic multisystem disease characterized by pain, swelling and stiffness of synovial joints. An inflammatory reaction, increased cellularity of synovial tissue and joint damage are the pathological hallmarks of RA [1].

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Approximately 1% of the general population is afflicted by RA, in which the occurrence is two to three times more predominant in women than men [2]. The treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, Celecoxib disease modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunomide, hydroxychloroquine, and corticosteroids like prednisolone, methyl-prednisolone, Dexamethasone have all been associated with adverse effects [3].

Bruton's tyrosine kinase (BTK) is a member of the Tec family of non-receptor tyrosine kinases, which is expressed in all cells of hematopoietic lineage exclude plasma cells, natural killer cells, and T lymphocytes [4]. Selective inhibition of BTK may be a promising therapeutic target for B cell inhibition in RA as well as for B cell lymphoma, designing potent and specific BTK inhibitors becomes vital [5]. Here we are using computer-aided drug design approaches to identify potent and novel BTK inhibitors which can cause inhibition of BTK. By reviewing the literature, the best model, Hypo 1(HBD, HBAL, HYP), was used for data base screening. Based on the 3D Pharmacophore model Hypo 1, about five ligands were designed and docked against BTK using Glide 10.2 and the potential ligands were checked their drug like properties such as ADMET properties, Lipinski rule of five and Novelty prediction. The designed ligands were synthesized based on the synthetic feasibility and characterized by Chromatographic and different Spectral studies.

In-vitro Anti-arthritic activity was screened for all the five synthesized compounds IPABA, ISA, IHA, IPAA, IAA by using protein denaturation method and all were found to exhibit an effective inhibition against the protein. Out of the five synthesized compounds IPABA was found to be a most effective with percentage inhibition of protein denaturation at 1000µg/ml. Then the *In-vivo* investigation of anti-arthritic activity of compound IPABA in treating rheumatoid arthritis using Adjuvant induced arthritis model. Overall experimental chart were depicted in **Fig. 1**. Overall experimental flow chart is depicted below,

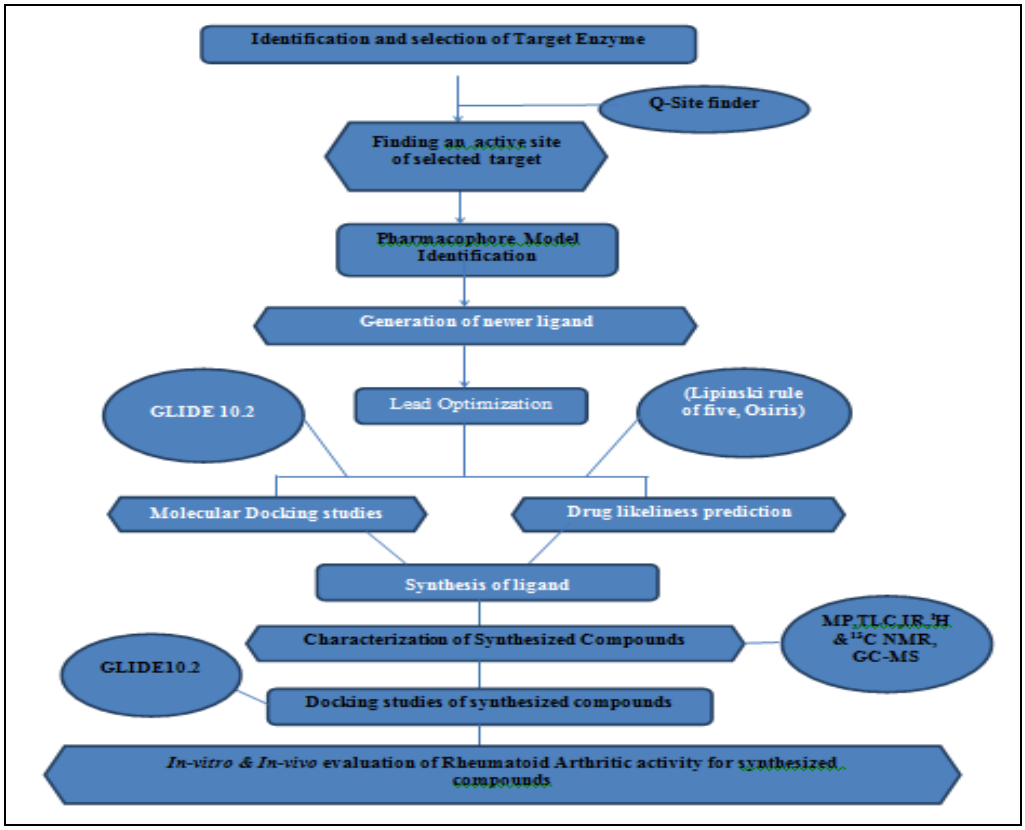


FIG. 1: FLOW CHART

MATERIALS AND METHODS:

Selection of Target Enzyme: The targets creating the greatest enthusiasm at this time for the treatment of Rheumatoid Arthritis and Inflammatory diseases. Bruton's tyrosine kinase (BTK) is a cytoplasmic, non-receptor, tyrosine kinase which is expressed in most of the hematopoitic cells and plays an important role in the development, differentiation and proliferation of B-lineage cells, thus making BTK an efficient therapeutic target for the treatment of rheumatoid arthritis. Recent researchers suggested BTK as a therapeutic potential target to treat Rheumatoid Arthritis and Cancers which prompted us to select the BTK as the protein target in this study [6].

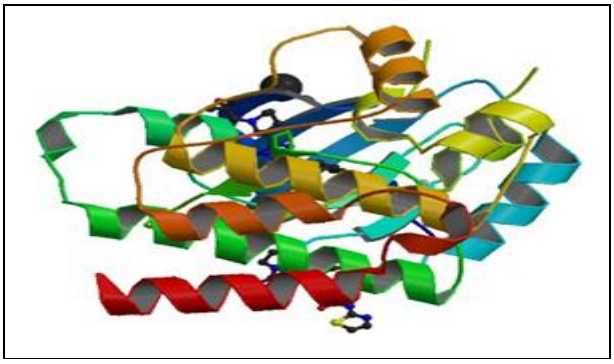


FIG. 2: 3D STRUCTURE OF BTK

Crystal Structure:		BTK
PDB code	:	5FBN
Method	:	X-RAY DIFFRACTION
Resolution	:	1.8 Å
R-Value Free	:	0.226
R-Value Work	:	0.179

Active Site of Selected PDB: The active site of selected protein was identified by using Q-site finder software in which ligand mapping is used to show the active site of the existed co-crystal ligand.

Pharmacophore Identification: When reviewing the efficient journals and research articles, the best pharmacophore model was identified. For designing BTK Inhibitors (HBD, HBAL, HYP). The BTK inhibitors also found to contain acidic head attached to an aromatic scaffold, a linker and a hydrophobic tail. Based on these features, five ligands were generated as potent BTK inhibitors [7]. Common structural features of BTK Inhibitor were depicted below,

Examples of HBAL and HBD Molecular Fragments:

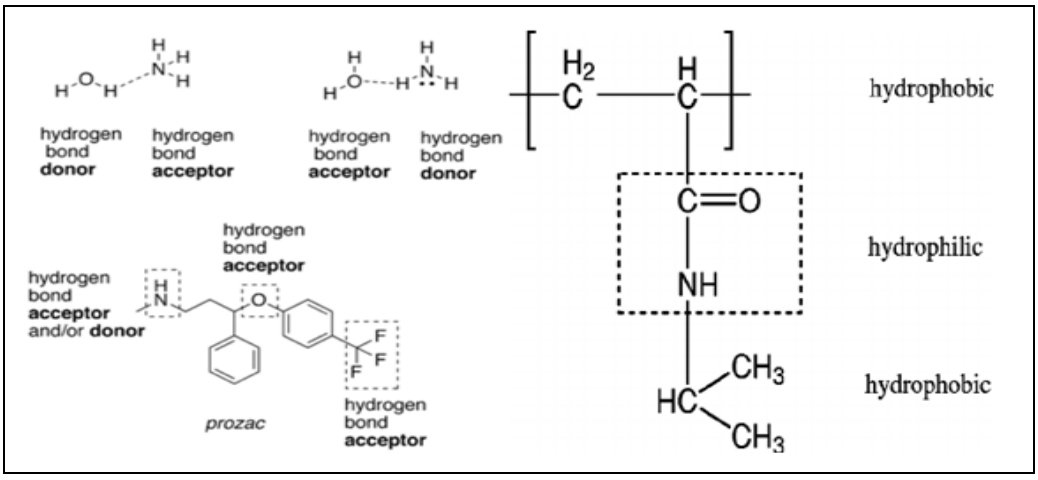


FIG. 3: HBAL AND HBD MOLECULAR FRAGMENTS

Construction of Ligands: About five ligands as potent BTK inhibitors were generated based on the knowledge of binding interaction of Ligand with the protein and also the common features necessary for the biological activity of molecule. The structures of some of the molecular fragments, substituents used for the incremental construction of molecules have been shown in following **Fig. 4**.

TABLE 1: MOLECULAR FRAGMENTS USED IN CONSTRUCTION OF LIBRARY

HBD	HBA	Linker	Hydrophobic tail
Imidazole, Benzimidazole, Aminothiazole, Phenolic-OH, Aniline, Alkyl amines, Hydrazines, Morpholine	C=O of aliphatic and aromatic amides, C=O of aromatic ketones	Phenyl, Methyl, Ethyl, Phenoxy,	Phenyl, Diazole, Pyridine Triazole, Benzthiazole, Quinaxoline, dimethyl benzene,

Virtual Screening: The newly generated ligands were subjected into Molecular docking, ADME properties, Lipinski's rule of five, Novelty prediction and Toxicity prediction. From the molecular docking studies, the ligands were docked against the active site of BTK using GLIDE 10.2 all the five ligands has a best docking score. During ADME investigation the compounds were checked for low blood brain barrier (BBB), optimal solubility, good absorption, Non-Hepatotoxicity.

Lipinski's rule of five estimates the absorption and intestinal permeability of the compound. Lipinski's rule of five states that, the compounds that are well absorbed have a LogP value less than 5, Hydrogen bond donor less than 5, Hydrogen bond acceptor less than 10, Molecular weight of less than 500 Da and fewer than ten rotatable bonds. Then the designed ligands were subjected into toxicity prediction with the "OSIRIS" online software. That shows toxicological properties of the newly designed ligands. The properties like Terato-genicity, Mutagenicity, Irritant and Reproductive effect. Then the novelty of the compounds was checked by using online software "Zink Data Base".

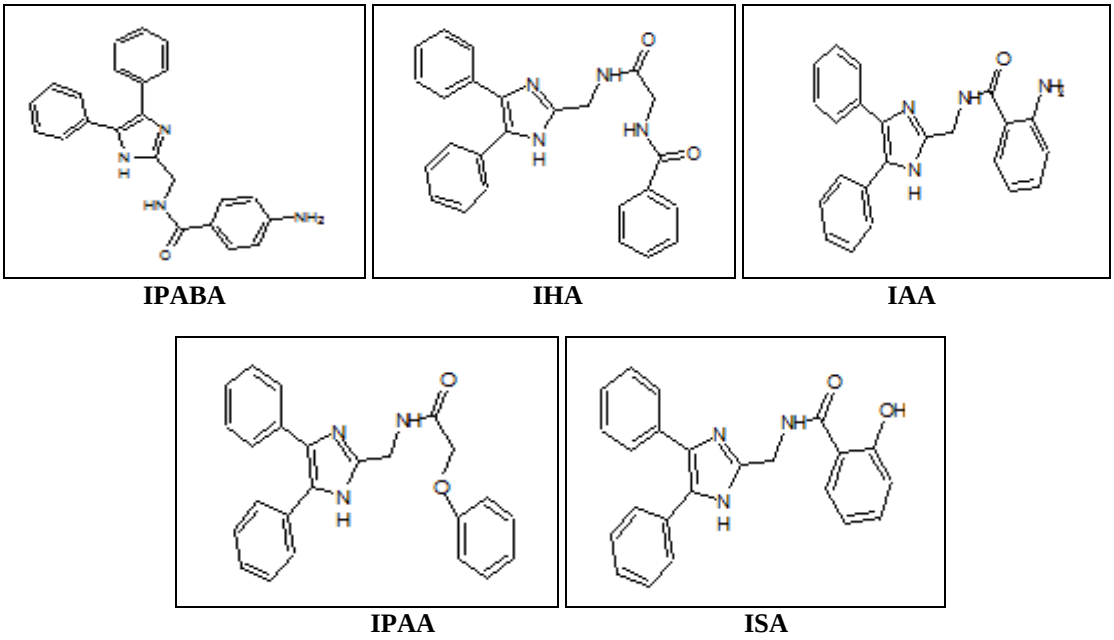
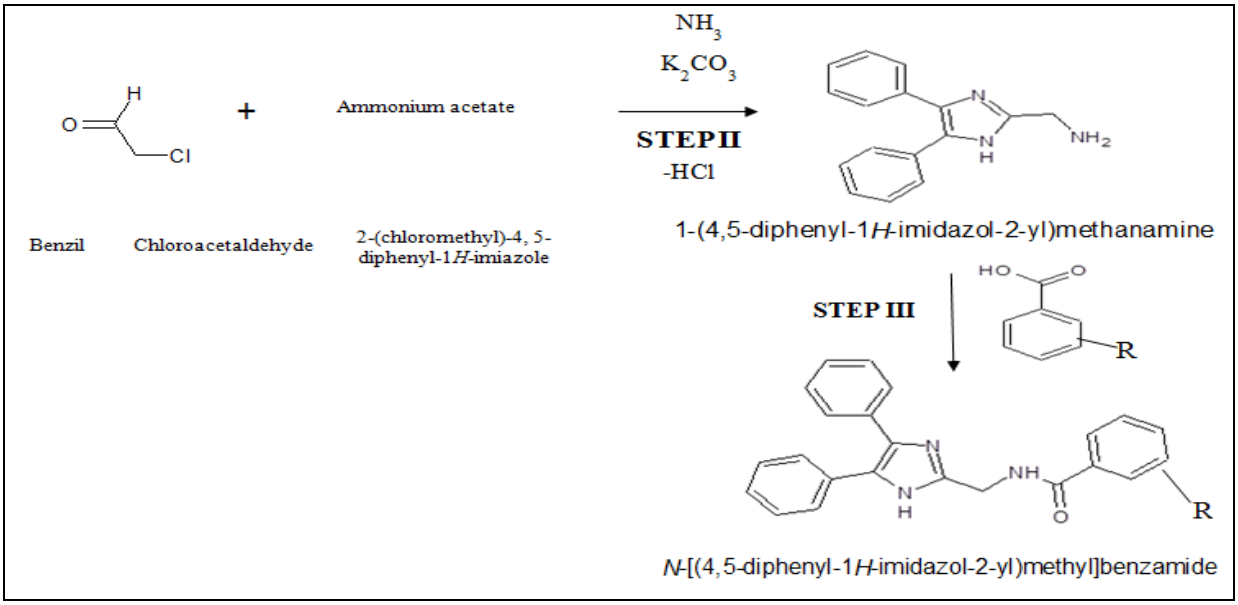


FIG. 4: NEWLY DESIGNED LIGANDS AS BTK INHIBITORS

Chemical Synthesis: All the five ligands exhibiting the drug likeliness properties were synthesized based on the synthetic feasibility and characterized by using different spectral studies such as IR, ^{1}HNMR, ^{13}CNMR, GC-MS.



SCHEME: 1

R = 2-NH_2, 4-NH_2, 2-OH

Evaluation Studies:

***In vitro* Anti-arthritic Activity Using Protein Denaturation Assay:** The reaction mixture (5ml) consisted of 0.2ml of bovine serum albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of synthesized compounds (100 and 200µg/ml). Similar Volume of double-distilled water served as control. Then the mixtures were incubated at 37 ± 2 °C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium was used as reference drug and treated similarly for determination of absorbance [8]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ of inhibition} = \frac{100 - [\text{OD of test solution} - \text{OD of product control}]}{\text{OD of test control}} \times 100$$

***In-vivo* Evaluation Studies:**

Acute Toxicity Test: Acute toxicity tests were performed according to OECD-423 guidelines (Acute toxic class method). Wistar rats (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 12 hrs with free access to water only. The newly synthesized compounds IPABA, ISA, IPAA, IHA and IAA were suspended in CMC 0.5% w/v was administered orally at a dose 2000mg/kg (limit dose) initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four rats, out of 6 rats then the same dose was repeated again to confirm the toxic effect. The animals were observed for toxic symptoms like behavioral changes, locomotion, convulsions and mortality for 72 hrs [9]. The Animal Ethical Committee meeting held at Madras Medical College, Chennai - 600 003 on 21/11/2016.

Induction of CFA and Drug Treatment: Adult wistar rats with an initial body weight of 100-200g were taken, and divided into five groups each containing 6 animals. On day zero, all rats were injected into the sub plantar region of left hind paw with 0.1ml of Complete Freund's Adjuvant. This consist of *Mycobacterium butyricum* suspended in heavy paraffin oil by through grinding with mortar and pestle to give a concentration of 6mg/ml. Dosing with test and standard compound was started on zero day and continued for 12 days according to the following schedule: group I: Normal control (Distilled water), Group II: Disease control (suspension of 1% CMC), Group III: Dexamethazone (5mg/kg, IP. Standard), Group IV: Test compound (High Dose) Group V: Test compound (Low dose). From day 13th to 21st, the animals were not dosed with the test compound or the standard [10]. Then the following parameter were measured,

Evaluation of Development of Arthritis: Rats were inspected daily for the onset of arthritis characterized by edema in the paws. The incidence and the severity of arthritis were evaluated using a system of arthritic scoring, and measurement of bi-hind paw volumes every 3 days beginning on the when arthritic signs were first visible. Animals were observed for presence or absence of nodules in different organs like ear, fore paw, hind paw, nose and tail. Animal were score 0 for absence and 1 for presence of nodules. 5 was the potential maximum of combined arthritic score per animal. Hind paw volume was measured using plethys-mometer. Paw volumes of both hind limbs were recorded from day of Treatment started to 21st day at three day interval using mercury column Plethysmometer [11].

Rheumatoid Factor: The latex turbidimetry method was used in the present study using RF turbi-latex kit of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml. The reading of RF factor of all the groups obtained was compared with the control animals and was expressed as IU/ml Rf [12].

Radiography: Wistar rats were sacrificed on 21st day of CFA administration and legs were removed and placed on formalin containing plastic bags. This plastic bag was kept at a distance of 90 cm from the X-ray source and Radiographic analysis of arthritic and treated animal hind paw were performed by X-ray machine with a 300-mA exposition for 0.01 s. An investigator blinded for the treatment regimen performed radiograph score. The following radiograph criteria were considered: These scores (destroyed or intact joint) were used

as a quantal test for bone necrosis. Radiographs were carefully examined using a stereo microscope and abnormalities were graded as follows:

- Periosteaic reaction, 0 - 3 (None, Slight, Moderate, Marked);
- Erosions, 0 - 3 (None, Few, Many Small, Many Large);
- Joint space narrowing, 0 - 3 (None, Minimal, Moderate, Marked);
- Joint space destruction, 0 - 3 (None, Minimal, Extensive, Ankylosis).
- Bone destruction was scored on the patella as described previously [13].

Effect on Spleen-Index: At the end of the experiment, after sampled for serum, all mice were sacrificed by ether anaesthesia. All the spleens of mice were weighed immediately after dissection [14]. The spleen indexes were calculated by using the following formula:

$$\text{Spleen Index} = \frac{\text{Spleen weight of CFA rat / Body weight of CFA rat}}{\text{Spleen weight of normal rat / Body weight of normal rat}}$$

Total Leukocyte Count and Neutrophil Count: Blood samples were collected by puncturing the retro-orbital plexus into heparanized vials and analysed for total leucocyte counts (TLC) and differential leucocyte counts (DLC).

Histological Processing and Assessment of Arthritis Damage: Rats were sacrificed by ether anaesthesia. Knee joints were removed and fixed for 4 days in 4% formaldehyde. After decalcification in 5 % formic acid, the specimens were processed for paraffin embedding tissue sections (7µm thick) and were stained with haematoxylin and eosin, or safranin. An experienced pathologist, unaware of the different drug treatments scored the condition of tibiotarsal joints. Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale from 0 to 3, depending on the amount of inflammatory cells in the synovial tissues. Inflammatory cells in the joint cavity were graded on a scale from 0 to 3 and expressed as exudate. A characteristic parameter in arthritis is the progressive loss of articular cartilage.

This destruction was separately graded on a scale from 0 to 3, ranging from the appearance of dead chondrocytes (empty lacunae) to complete loss of the articular cartilage. Bone erosion was scored on a scale ranging from 0 to 3, ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage / subchondral bone); 3, severe change (pannus invasion into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels). Histopathological changes in the knee joints were scored in the femur region on 5 semi-serial sections of the joint, spaced 70µm apart. Scoring was performed on decoded slides by two observers, as described earlier [15-17].

Statistical Analysis: Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by student t test. The values $P < 0.05$ were regarded as significant and the values $P < 0.01$ were considered as highly significant.

RESULTS AND DISCUSSION:

Virtual Screening: All the ligands IPABA, ISA, IHA, IPAA, IAA physiochemical properties and ADMET properties results were tabulated below:

TABLE 2: ADMET PROPERTIES OF NEWLY SYNTHESIZED LIGANDS

Code	MUT	TUM	Irritant	REP	Clog P	Sol	TPSA	Drug Likeness	Drug Score
IPABA	-	-	-	-	3.44	-4.28	79.19	2.97	0.68
ISA	-	-	-	-	3.77	-3.91	73.39	2.41	0.68
IHA	-	-	-	-	3.2	-3.97	82.26	2.58	0.68
IPAA	-	-	-	-	3.69	-3.99	62.39	3.22	0.68
IAA	-	-	-	-	3.44	-4.29	79.18	-0.1	0.51

MUT: Mutagenicity; TUM: Tumarogenicity; REP: Reproductive effect; Sol: Solubility; TPSA: Topological Polar Surface Area. (-): Negative sign of Toxicity.

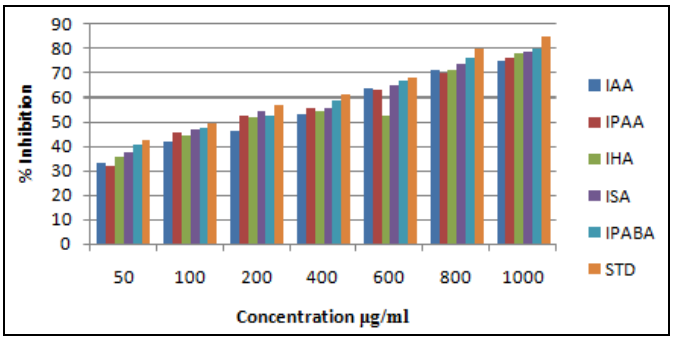
TABLE 3: PHYSIOCHEMICAL PROPERTIES OF NEWLY SYNTHESIZED LIGANDS

Code	Nrotb	MR	Mol Wt	HBD	HBA	Violation
IPABA	5	108.616570	364.0000	3	4	0
ISA	5	105.868965	365.0000	2	4	0
IHA	7	117.703156	404.0000	1	5	0
IPAA	7	110.375664	375.0000	1	4	0
IAA	5	108.616570	364.0000	3	4	0

Nrotb: Number of rotatable bonds; MR: Molar refractivity; Mol Wt: Molecular weight; HBD: Hydrogen Bond Donor; HBA: Hydrogen bond Acceptor.

Evaluation Studies:

***In vitro* Anti-arthritic Activity Using Protein Denaturation Assay:** The *In-vitro* Anti-arthritic activity has been carried out using most popular inhibition of protein denaturation method. The synthesized compounds IPABA, ISA, IPAA, IHA, IAA showed significant activity at various concentration ranging from 50-1000µg/ml. Both the minimum inhibitory (50µg/ml) and maximum inhibitory (1000 µg/ml) concentration of synthesized compounds, IPABA has effectively inhibited the protein denaturation when compared with the standard drug (Diclofenac sodium). So all the synthesized compound may be responsible for potent Anti-arthritic activity.



GRAPH 1: PERCENTAGE INHIBITION VS CONCENTRATION

Oral Acute Toxicity Study OECD-423: Oral acute toxicity of newly synthesized imidazole-yl Heterocycles such as IPAA, ISA, IPABA, IHA, IAA were studied and the results were tabulated.

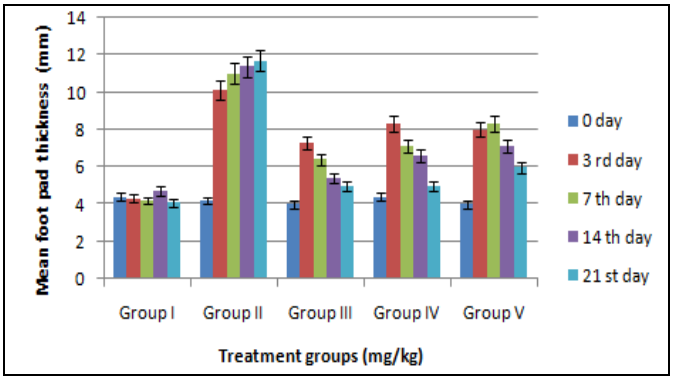
TABLE 4: OBSERVATION IN ORAL ACUTE TOXICITY STUDY (OECD-423)

S. No	Observation	For 30 min	4 hrs	24 hrs	48 hrs	7 day	14 day
1	Sedation	Absent	Absent	Absent	Absent	Absent	Absent
2	Excitation	Absent	Absent	Absent	Absent	Absent	Absent
3	Jumping	Normal	Normal	Normal	Normal	Normal	Normal
4	Writhing	Absent	Absent	Absent	Absent	Absent	Absent
5	Scratching	Absent	Absent	Absent	Absent	Absent	Absent
6	Grooming	Absent	Absent	Absent	Absent	Absent	Absent
7	Aggression	Absent	Absent	Absent	Absent	Absent	Absent
8	Ptosis	Absent	Absent	Absent	Absent	Absent	Absent
9	Loss of writhing reflux	Absent	Absent	Absent	Absent	Absent	Absent
10	Lose of pinel reflux	Absent	Absent	Absent	Absent	Absent	Absent
11	Lose of coreneal reflux	Absent	Absent	Absent	Absent	Absent	Absent
12	Salivation	Absent	Absent	Absent	Absent	Absent	Absent
13	Lacrimation	Absent	Absent	Absent	Absent	Absent	Absent
14	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
15	Color of eye	Normal	Normal	Normal	Normal	Normal	Normal
16	Tremors	Absent	Absent	Absent	Absent	Absent	Absent
17	Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent
18	Coma	Absent	Absent	Absent	Absent	Absent	Absent
19	Inflammation	Absent	Absent	Absent	Absent	Absent	Absent
20	Urination	Absent	Absent	Absent	Absent	Absent	Absent

In acute toxicity study test, No mortality and the sign of toxicity was observed for all the selected doses during the study. The acute toxicity studies showed that the non-toxic nature of the newly synthesized Imidazol-yl Heterocycles such as IPAA, ISA, IPABA, IHA, IAA up to the level of 2000mg/kg body weight selected doses.

***In-vivo* Anti- Arthritic activity:** The results were obtained after daily administration of the test doses 100 mg/kg, 200 mg/kg and standard drug 5 mg/kg in this experimental protocol arthritis revealed that the test compounds exerted the Anti-arthritic activity.

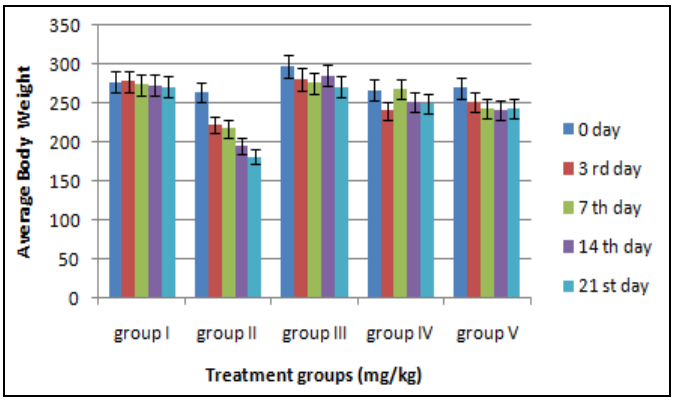
Food Pad Thickness / Paw Volume: Rats treated with Dexamethasone (5mg/kg) shows significantly decreased in paw volume ($P < 0.001$) when compared with disease control. Test compound IPABA (100 mg/kg) and (200 mg/kg) also shows significant changes in paw volume with $P < 0.001$.



GRAPH 2: FOOT PAD THICKNESS

Group I- Normal control. Group; II- Disease control;
Group III- Standard group; Group IV and V- Test group.

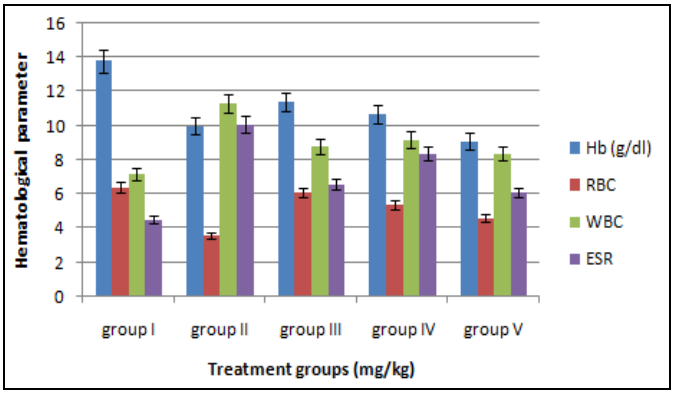
Effect of Average Body Weight: Rats treated with Dexamethasone (5mg/kg) shows significantly increased in body weight ($P < 0.001$) when compared with disease control. Test compound IPABA (100mg/kg) and (200mg/kg) also shows significant changes in body weight with $P < 0.001$.



GRAPH 3: AVERAGE BODY WEIGHT

Group I- Normal control. Group; II- Disease control;
Group III- Standard group; Group IV and V- Test group.

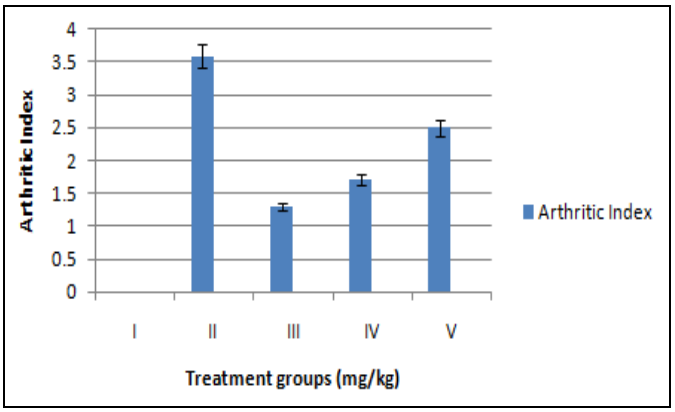
Effect of Hematological Parameter: There was a significant ($p < 0.001$) decrease in RBC count and hemoglobin and increase in WBC count and ESR of arthritic rats as compared to control rats. Rats treated with Dexamethasone (5mg/kg) shows significantly brought back the altered hemato-logical changes when compared with disease control. Test compound IPABA (100mg/kg) and (200mg/kg) also shows significant changes in hematological parameter with $P < 0.001$.



GRAPH 4: HEMATOLOGICAL PARAMETER

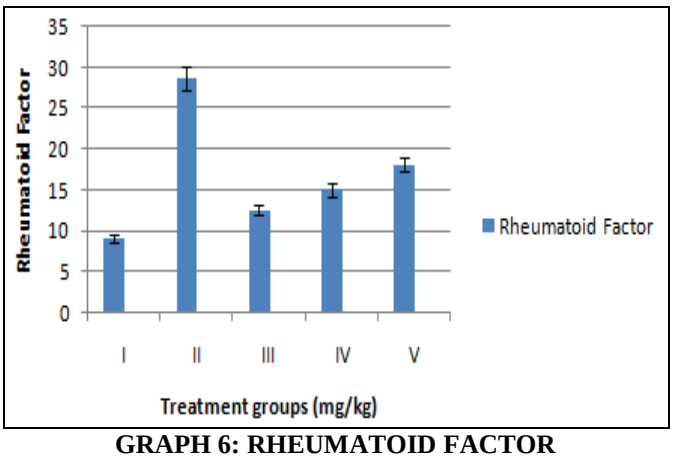
Group I- Normal control. Group; II- Disease control;
Group III- Standard group; Group IV and V- Test group.

Arthritic Index and Rheumatoid Factor: Sub plantar administration of CFA results in significant increased ($P<0.001$) in arthritic score in all arthritic treated rats as compared to control rats. Albino rats treated with Test compounds IPABA showed significant and dose dependent decreased in arthritic score ($P<0.001$) as compared to arthritic rats.



GRAPH 5: ARTHRITIC INDEX

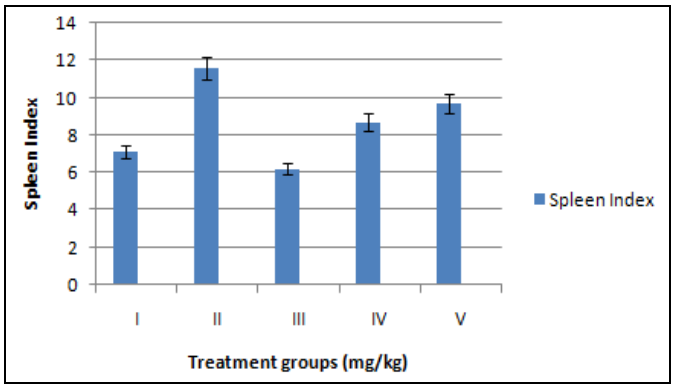
Group I- Normal control. Group; II- Disease control;
Group III- Standard group; Group IV and V- Test group.



GRAPH 6: RHEUMATOID FACTOR

Group I- Normal control. Group; II- Disease control;
Group III- Standard group; Group IV and V- Test group.

Spleen Index: Spleen Index was significantly increased ($P < 0.001$) in all arthritic treated rats as compared to control rats. The Spleen Index reduction in test compound IPABA (100 and 200 mg/kg) and Dexamethasone (5mg/kg) treated rats were significantly ($P < 0.001$) lesser than that of arthritic treated rats.

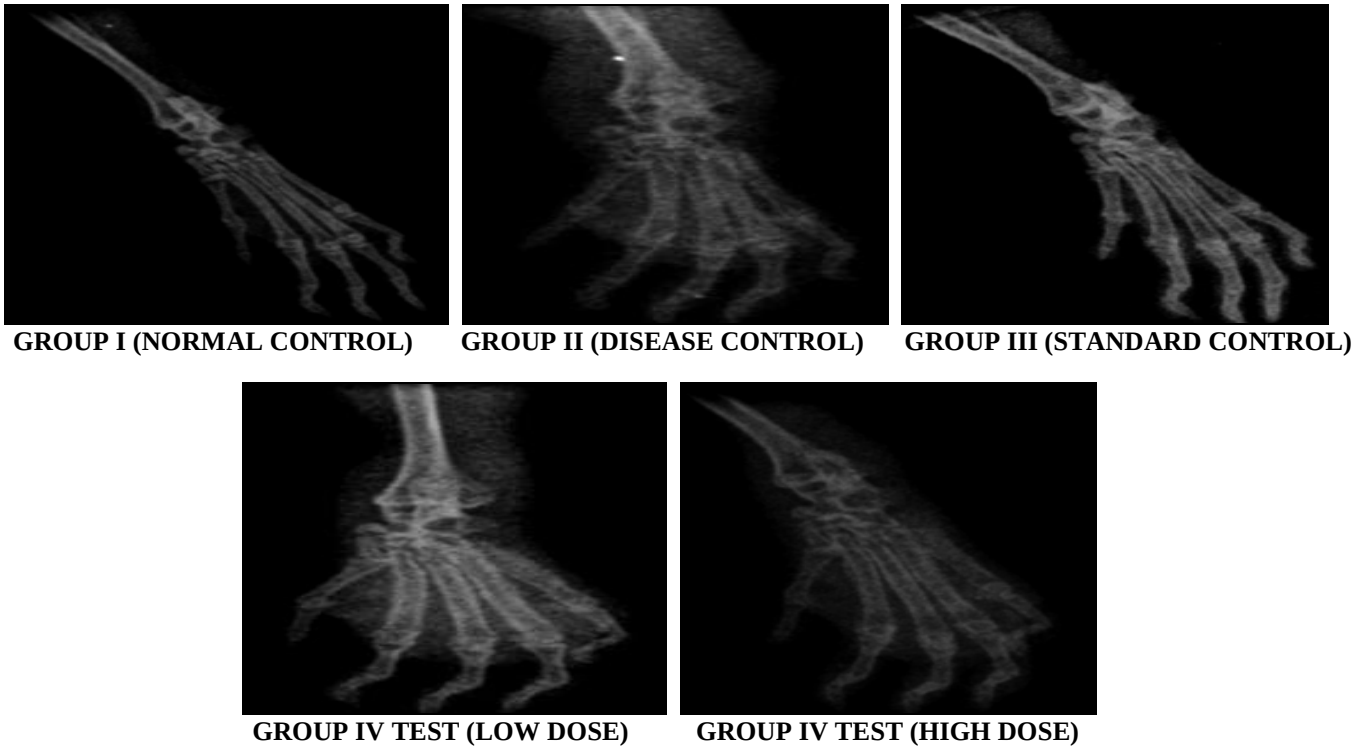


GRAPH 7: SPLEEN INDEX

Group I- Normal control. Group; II- Disease control; Group III- Standard group; Group IV and V- Test group.

Radiography: Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis treated rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissues swelling that includes the digits, diffuse demineralization of bone, marked periosteal thickening and cystic enlargement of bone and extensive erosions produced narrowing or pseudo widening of all joints space. In contrast, in rats treated with test compound IPABA attenuate abnormalities consisted of asymmetric soft tissue swelling and small erosions, periosteal thickening and minimal joint space narrowing areas of the paws and the result were shown in **Fig. 5.**

Despite a similar chemical course of arthritis, disease control rats suffered from more pronounced bone destruction than test compounds treated animals as soon on radiograph taken on 21st day in CFA induced arthritis.



GROUP I (NORMAL CONTROL) **GROUP II (DISEASE CONTROL)** **GROUP III (STANDARD CONTROL)**

GROUP IV TEST (LOW DOSE) **GROUP IV TEST (HIGH DOSE)**

FIG. 5: EFFECT OF TEST COMPOUND IPABA ON TIBIOTARSAL JOINTS

Histopathology: Test compound IPABA treated animals had a more pronounced decrease in bone density, destruction of bony structure, as compare to disease control as shown in **Fig. 6**. Abrogation of disease progression by test compound was further supported by the Histopathology analysis of the joints from these animals. Rats that had been treated with test compounds at the time CFA immunization showed no histological abnormalities with no evidence of cartilage erosion in their joints in contrast to the disease control rats that displayed completely destroyed joint architecture as shown in following **Fig. 6**.

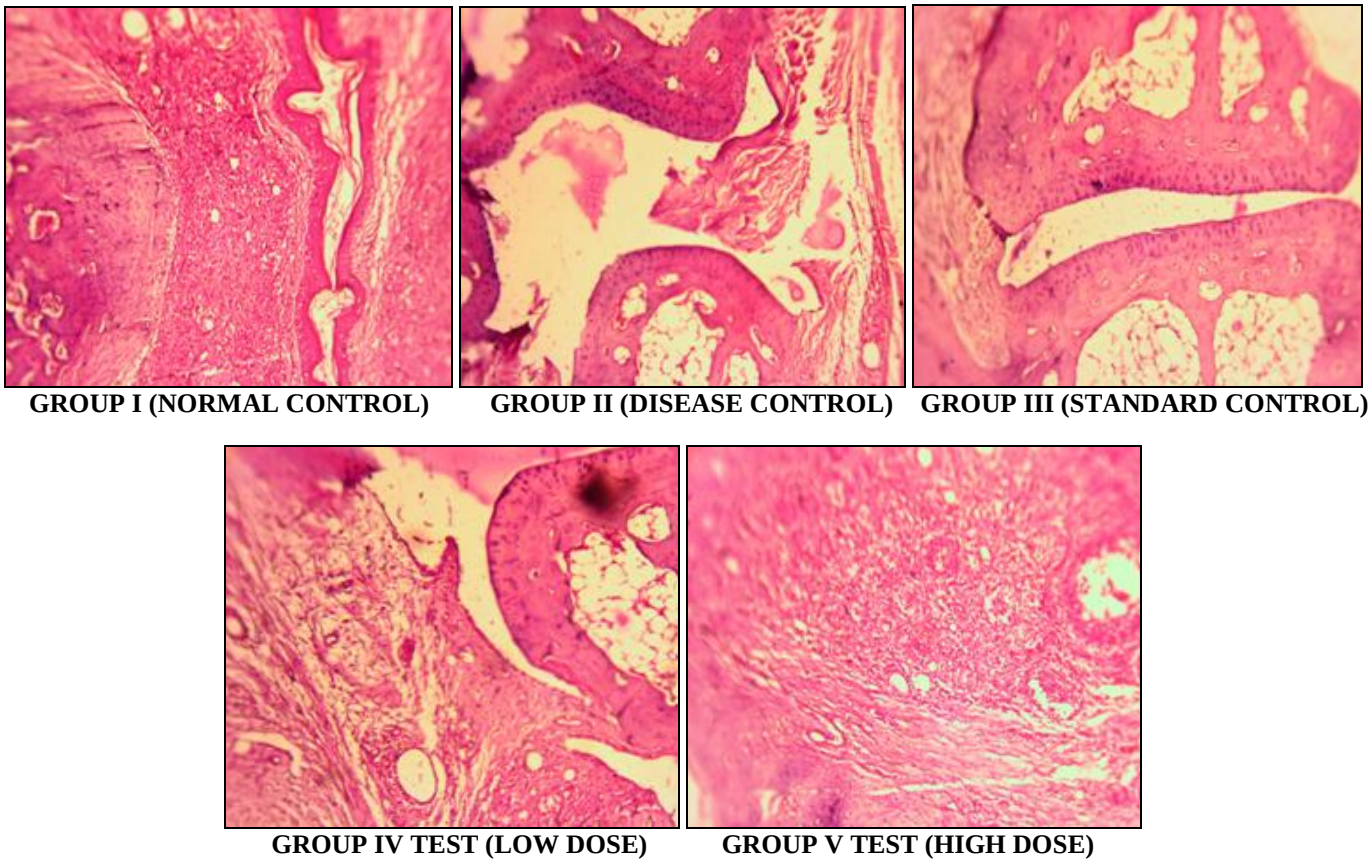


FIG. 6: HISTOPATHOLOGY OF JOINTS INDICATES TREATMENT WITH TEST COMPOUND IPABA PREVENT BONE EROSION

Our data suggested that test compound possesses significant Anti-Arthritic activity of best compound appears to be possessing Anti-Inflammatory activity showed in arthritic parameters like paw edema, Arthritic Index, Rheumatoid arthritis, improving bone erosion. All these results thus predict that the drug provide pharmacological rationale for the uses of the drug against inflammatory disease such as Rheumatoid Arthritis.

CONCLUSION: Inhibition of BTK as developed as a new promising target in the field of Rheumatoid Arthritis and Malignancy, allergy or hypersensitivity as it is involved in several signalling pathways. Thus as an attempt to ligand based pharmacophore modelling was done to find the important chemical features which can inhibit the BTK activity. The five feature pharmacophore models, Hypo1, were developed consisting of 1 HBAL, 1 HBD and 3HYP features. The best hypothesis Hypo1was used as a 3D structural query to screen the chemical database for retrieving new potent inhibitors of BTK. Lipinski's rule of five, ADMET properties screening assisted us to discard the non-drug-like leads. Furthermore, the screened drug-like ligands was subjected to molecular docking study against BTK as a target using Glide 10.2. Ligands were synthesized with different aromatic acids and also characterized by different spectral studies. The purity was also checked by TLC chromatographic technique. The potency of the synthesized compounds to inhibit BTK will be evaluated by *in vitro* anti-arthritic activity using Protein denaturation assay and *in vivo* Arthritis activity will also be carried out to prove the efficacy of the newly synthesized compound IPABA as potent anti-rheumatoid arthritic agents showed in arthritic parameter like Arthritic index, Rheumatoid factor, improving bone erosion. All these results thus predict that the drug provide pharmacological rationale for the uses of the drug against inflammatory disease such as Rheumatoid Arthritis.

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