DESIGN, MOLECULAR DOCKING, SYNTHESIS AND EVALUATION OF SOME NOVEL HETEROCYCLIC ANALOGUES OF 2-SUBSTITUTED BENZIMIDAZOLE AS POTENT ANTIMICROBIAL, ANALGESIC, ANTI-INFLAMMATORY AGENTS

Dissertation

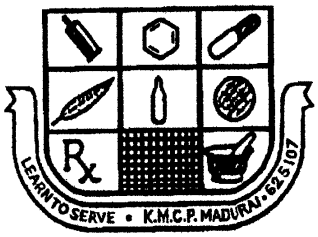
Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

OF

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

K.M.COLLEGE OF PHARMACY

UTHANGUDI, MADURAI - 625 107

APRIL – 2012

CERTIFICATE

This is to certify that the dissertation entitled "**DESIGN, MOLECULAR DOCKING, SYNTHESIS AND EVALUATION OF SOME NOVEL HETEROCYCLICANALOGUES OF 2-SUBSTITUTED BENZIMIDAZOLE AS POTENT ANTIMICROBIAL, ANALGESIC AND ANTI-INFLAMMATORY AGENTS**" submitted by **Mr**. **ANOOP.P** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment for the award of Master of Pharmacy in Pharmaceutical chemistry at K.M. College of Pharmacy, Madurai, is a bonafide work carried out by him under my guidance and supervision during the academic year 2011-2012. This Dissertation partially or fully has not been submitted for any other degree or diploma of this University or any other Universities.

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Anoop.P

INTRODUCTION

Medicinal Chemistry or Pharmaceutical chemistry is a discipline with a traditional focus on organic synthetic chemistry with the broad goals of drug discovery and optimization of pharmaceuticals. Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. The process of establishing new pharmaceutical is exceedingly complex and involves the talents of people from variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics, and medicine. Thus medicinal chemistry occupies a strategic position at the interface of chemistry and biology. [1]

During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are equally concerned with the creation of new synthetic drug compounds, possibly based on newly discovered mechanisms. Now medicinal chemist have efficient methods for optimizing the potency and the profile of a given active substances. These methods may consist of more or less intense approaches such as the synthesis of analogues and isomers and isosters, or the modification of ring system. They may also rest on computer assisted design, such as identifying pharmacophore by molecular modelling or optimizing activity by means of quantitative structure-activity relationships.

Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the interest of medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds. [2]

Medicinal chemistry covers the following steps,

1. In the first stage new active substance or drugs are identified and prepared from natural sources, organic chemical reactions or biotechnological processes. They are known as lead molecules.
2. The second stage is optimization of lead molecules to improve the potency, selectivity and to reduce toxicity.

3. The next stage involves optimization of synthetic route for bulk production and modification of pharmacokinetic properties of active substance to render it clinically useful.[3]

Medicinal chemistry is almost always geared toward drug discovery and development. Development is an intense, lengthy and an interdisciplinary endeavour. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. [4]

IN-SILICO DRUG DESIGN

Traditionally, scientists identify new drugs either by fiddling with existing drugs or by testing thousands of compounds in a laboratory. Such a development processes has resulted in high attrition rates with failures attributed to poor pharmacokinetics (40%), lack of efficacy (31%), animal toxicity (10%), adverse effect in humans (9%), and various commercial and miscellaneous factors. Using a structure-based strategy, researchers have an initial advantage. They start with a computerized model of the detailed, three-dimensional structure of the lock and of its key (the natural molecule called a substrate, which fits into the lock, triggering viral replication). Then scientists try to design a molecule that will plug up the lock to keep out the substrate key. Knowing the exact three-dimensional shape of the lock, scientists can discard any of the small molecules that are not the right size or shape to fit the lock. They might even be able to design a small molecule to fit the lock precisely. Such a molecule may be a starting point for pharmaceutical researchers who are designing a drug.

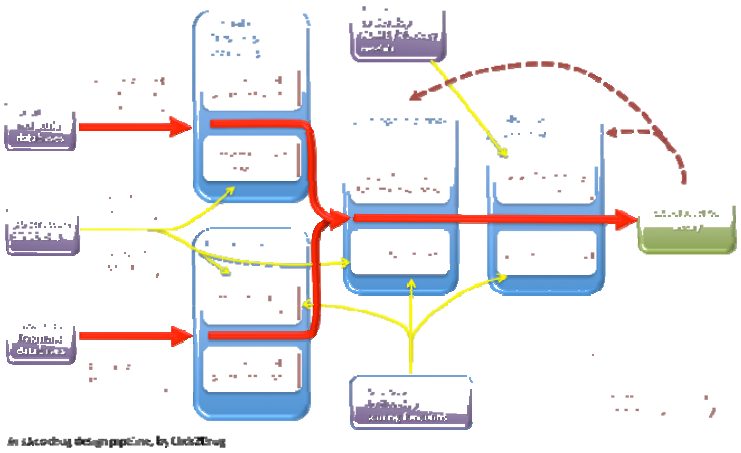


Fig 1 – *In silico* drug designing

In silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of structure-based drug design. High-performance computing, data management software and internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into workable knowledge in modern day drug discovery process. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. Major roles of computation in drug discovery are;

(1) Virtual screening & de novo design.

(2) In- silico ADMET prediction and

(3) Advanced methods for determining protein-ligand binding. [5]

RATIONAL DRUG DESIGN

Rational drug design has begun to replace the old methods; rational drug design is a more streamlined process that requires careful consideration of the target of the drug as well as the drug itself. This method of drug design uses special equipment to examine the three-dimensional structure of a drug target and then find a compound that can interact with the target. Rational drug design therefore requires significant knowledge of chemistry as well as biology, because chemical interactions between drugs and their targets are what determine whether a drug is biologically active. [6]

Rational drug design often involves the use of molecular design software, which researchers use to create three-dimensional models of drugs and their biological targets. For this reason, the process is also known as computer-aided drug design. It saves a great amount of time and money in R&D projects. [7]

A drug target, or biological target, is usually one of two types. The first type is a molecule in the human body that causes disease when it is defective in some way. The second is a molecule from a disease-causing microorganism. Drug development involves discovering or designing new chemical compounds that interact with these targets in a beneficial way, such as by interacting with cholesterol to remove it from the body or by interacting with a virus to cause its death.

Techniques

- **Molecular Docking** and **Virtual Screening:** Molecular docking is a computer simulation procedure to predict the conformation of receptor-ligand complex, where the receptor is usually a protein or a nucleic acid molecule (DNA or RNA) and the ligand is either a small molecule or another protein. It can also be defined as a simulation process where a ligand position is estimated in a predicted or predefined binding site. Docking is one of main tools for virtual screening procedures, where a library of several compounds is "docked" against one drug target and returns the best hit. Structure databases, such as Protein Data Bank (PDB) and Worldwide Protein Data Bank (wwPDB) have over 50,000 protein structures, many of these structures may be considered as potential drug targets.
- **Molecular Dynamics:** The prediction of the evolution of molecular systems over time, the study of protein conformation, protein-protein interactions, the simulation of biological membranes.
- **Quantum Mechanics:** The study of chemical reactions, the effects of substitutions on electronic properties and reactivity of molecules.
- **QSAR** (Quantitative structure-activity relationship): The ability of predicting biological properties of molecules without even the need of knowing their target
- **Homology Modelling:** predicting the structures of proteins that has not been yet crystallised. [8]

CYCLO-OXYGENASE

Cyclooxygenase (COX) - An enzyme that is responsible for the formation of prostanoids (prostaglandins, prostacyclins, and thromboxanes) each involved in the inflammatory response.

The COX enzyme contains two active sites:

- A Cyclooxygenase site- where arachidonic acid is converted into PGG_2 (hydroperoxy endoperoxide prostaglandin G_2). The reaction proceeds through H atom abstraction from arachidonic acid by a tyrosine radical generated by the peroxidase active site. Two O_2 molecules then react with the arachidonic acid radical, yielding PGG_2.
- A heme with peroxidase activity- responsible for the reduction of PGG_2 to PGH_2,

Two Forms of Cyclooxygenase (COX)

Two different COX enzymes existed, known as COX-1 and COX-2. Cyclooxygenase-1 (COX-1) is known to be present in most tissues, in the GIT, COX-1 maintains the normal lining of the stomach. Cyclooxygenase-2 (COX-2) is primarily present at sites of inflammation. Selective COX 2 inhibitors may give good anti-inflammatory activity. [9]

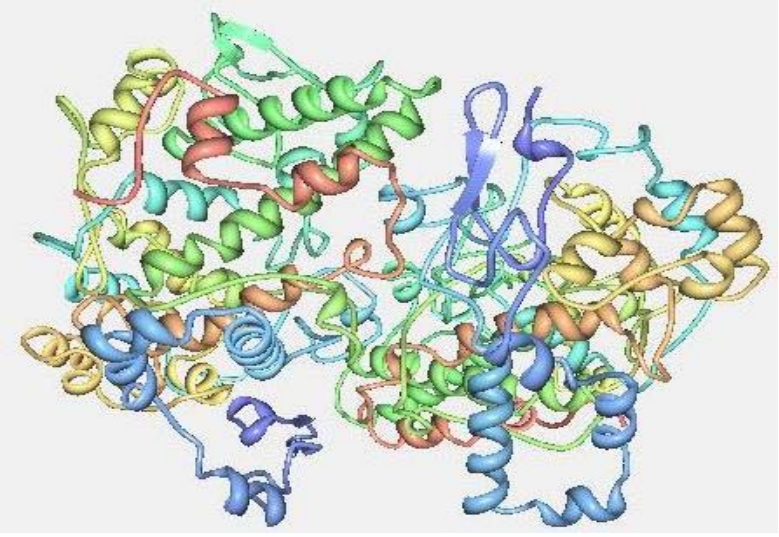


Fig 2- Crystallographic model of Cyclooxygenase-2

14 ALPHA DEMETHYLASE

14 alpha demethylase is a cytochrome P450 enzyme that involved in the steroid production. It is the main target for antifungal drugs, inhibiting the production of ergosterol. The inhibition of 14 alpha demethylase leads to the depletion of ergosterol and accumulation of steroid precursors, including 14 alpha methylated steroids (lanosterol, 14, 14- dimethyl zymosterol) resulting in the formation of plasma membrane with altered structure and function. [9]

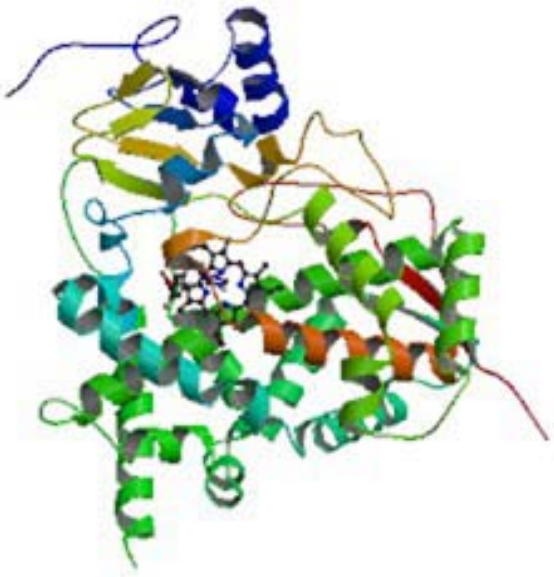


Fig 3- Crystallographic model of 14 α- demethylase

ANALGESICS

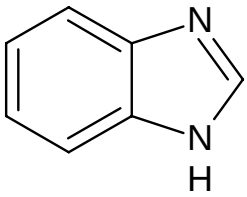
An analgesic may be defined as a drug bringing about insensitivity to pain without loss of consciousness. The pain has been classified in to the following types: physiological, inflammatory, and neuropathic. The first is the most common, for ex. touching a hot object or getting a cut. Inflammatory pain can be initiated in a wide variety of ways, such as infection and tissue injury. The last type is due to injury to the peripheral of central nervous system (CNS). [10]

Several classes of pain relieving drugs are there, the three major classes of drugs used to manage pain are opioids, NSAIDs, and acetaminophen. The consideration of naturally occurring and synthetic analgesic is facilitated greatly by dividing them into 2 groups; (a) morphine and related compounds and (b) the antipyretics and anti-inflammatory analgesics.

Prostaglandin plays a major role in inflammatory processes. The NSAIDs inhibit PGs synthesis in several tissues. NSAIDs strongly inhibit the conversion of arachidonic acid into prostaglandin E_2 (PGE_2). This occurs at the stage of conversion of arachidonic acid, released by the action of phospholipase A_2 on damaged tissues, by prostaglandin H_2 synthase called Cyclooxygenase to the cyclic to the endoperoxides PGG_2 and PGH_2. These are known to cause vasoconstriction and pain. They in turn, are converted in part to PGE_2 and $PGF_{2\alpha}$, which can cause pain and vasoconstriction. [11]

SUBJECT INTRODUCTION

Benzimidazole



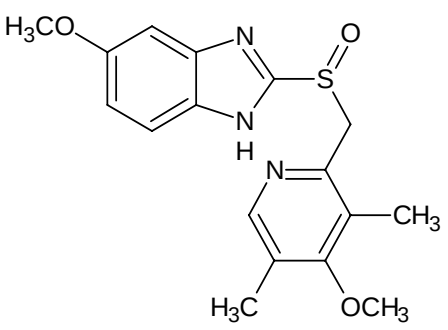
All the heterocyclic compounds have a great interest in pharmaceutical chemistry. Out of these heterocyclic compounds the benzfused heterocyclic compound that is benzimidazole and its derivatives have wide varieties of biological activity. In addition to that the benzimidazole have played a very important role in the development of theory in heterocyclic chemistry and also extensively in organic synthesis. [12]

Benzimidazole is an important pharmacophore and privileged structure in medicinal chemistry. Literatures surveys show that among the benzimidazole derivative 2-subsituted ones are found to be pharmacologically more potent and hence the design and synthesis of 2-susituted benzimidazole are the potential area of the research.

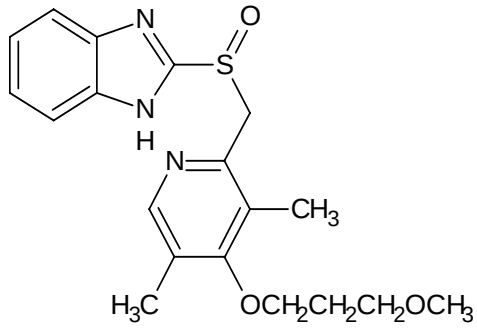
Extensive biochemical and pharmacological studies have confirmed that their derivatives are active against various strains of microorganism. The reason for a special interest of researchers towards benzimidazole derivatives has been 5,6-dimethyl benzimidazole which is a constituent of naturally occur vitamin-B_{12}. Vit B_{12} capable of inducing the growth of bacteria, benzimidazole component and some of its derivatives depress the bacterial growth. Due to the structural similarity to purine, antibacterial ability of benzimidazole is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acid and proteins. [13, 14]

Compounds containing Benzimidazole nucleus [6]

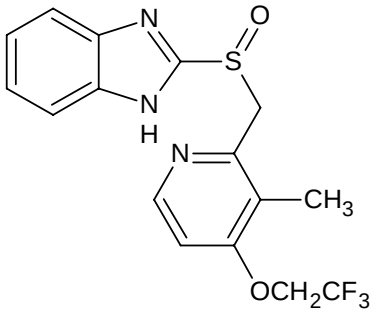
As antiulcer agents



Omeprazole

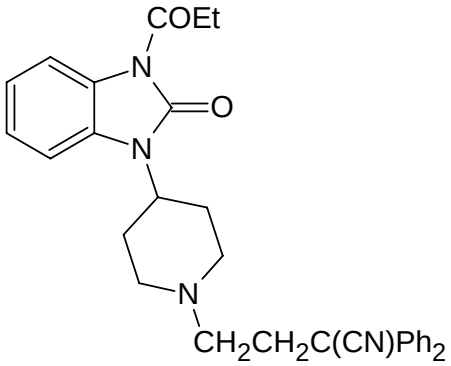


Rabeprazole



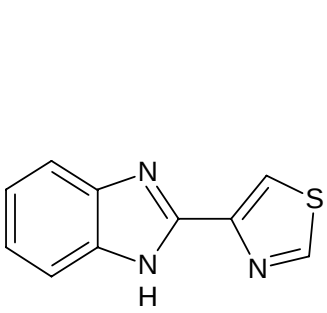
Lansoprazole

Analgesic agent

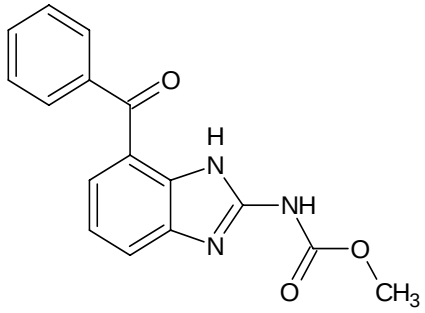


Benzitramide

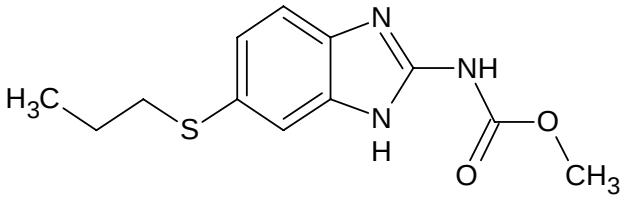
As anthelmintic agents



Thiabendazole



Mebendazole

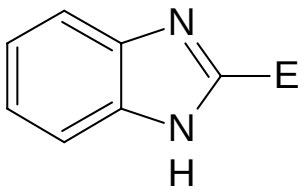


Albendazole

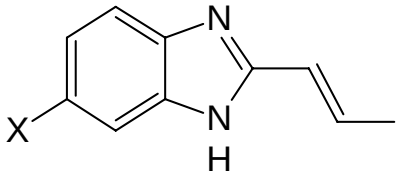
Fig 4- Compounds containing Benzimidazole nucleus.

REVIEW OF LITERATURE

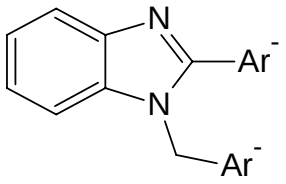
1. **Alan R.Kartritzky et al.,** [15] (1988) done the lithiation of N- N-(Dialkyl amino) methyl (aminal) derivatives of imidazole, benzimidazole and pyrazole which occurs smoothly at the 2-, 2- and 5-positions, respectively, on treatment with n-butyl lithium in ether or tetrahydrofuran. Reaction with electrophiles and subsequent facile acid-catalyzed hydrolysis of the protecting group provides 2-substituted imidazoles, 2-substituted benzimidazoles and 3(5)-substituted pyrazoles in good overall yields.



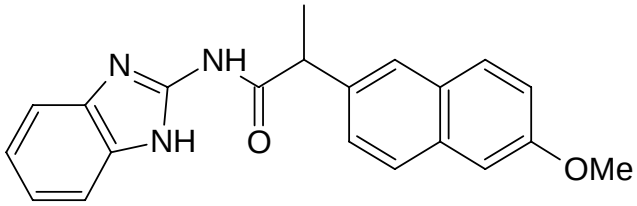
2. **M.Hasan et al.,** [16] (1989) concluded that when β Unsaturated carboxylic acids reacted with unsubstituted *o*-phenylenediamine under Philip's reaction gave benzodiazapinone as expected and crotonic acid reacts with 4-nitro-o-phenylenedimine to form benzimidazole. The products were identified and their structure was confirmed with the help of elemental analysis. These results indicate that not only the structure of unsaturated carboxylic acid, but also the substituent present in the ring of o-phenylenediamine influence the mechanism of Philip's reaction.



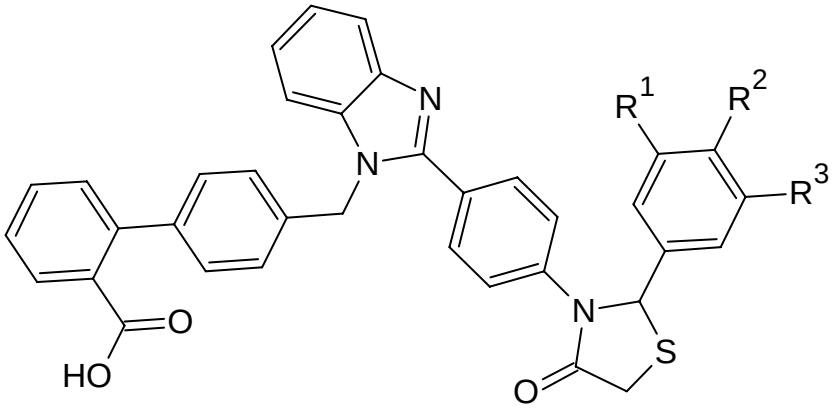
3. **Mohammad R.Mohammadizadeh et al.,**[17] (2010) had introduced trifluro acetic acid as commercially available, inexpensive and effective catalyst for the selective and eco-compatible synthesis of 2-aryl-1-arylmethyl-1H,3-benzimidazoles via condensation reaction of o-phenylenediamine and aromatic aldehydes in ethanol/water at room temperature.



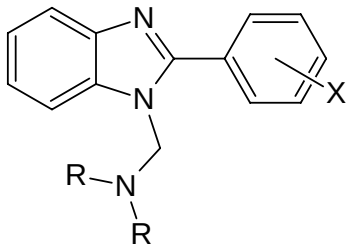
4. **Abdullah GM Al-Sehemi et al., [18]** (2005) had synthesized the novel isothiocyanate and azide derivatives by the reaction between naproxenoyl chloride 2 and nucleophilic reagent ammonium thiocyanate. Interaction of isothiocyanate with 1,2-phenylenediamine and anthranilic acid has produced the corresponding benzimidazole and 3,1-benzoxacine derivatives respectively. Treatment of acid azide with p-toluidine afforded urea derivative. The novel quinazolinone was synthesized by acylation of methyl anthranilate with acid chloride followed by treatment with hydrazine hydrate.



5. **M.C Sharma, et al., [19]**(2010) had reacted prepared schiff bases like 4'-{2-[4-[2-(substituted-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl}benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acids with mercapto acetic acid and 4'-bromo methyl biphenyl-2 carboxylic acid and all synthesized compounds are screened for angiotensin(A II). Receptor antagonist antihypertensive activity with biphenyl carboxylic acid Schiff's basesthiazolidine-4-one shows goods activity compared with losartan and telmisartan.



6. **Mohamed Al Messmary et al.,**[20] (2010) prepared 2-substituted benzimidazoles by reaction of substituted benzoic acid with o-phenylenediamine and the products were treated with secondary amines in presence of formaldehyde in order to synthesize Mannich bases. The final products were characterized by physical and spectral analysis.

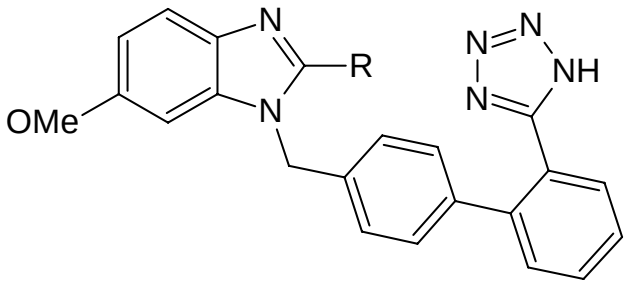


R=CH3

R=CH2CH3

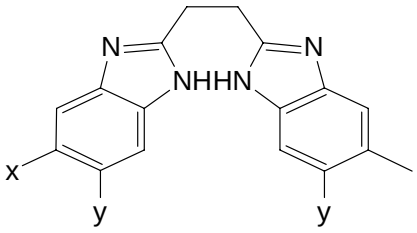
R=Ph

7. **M.C Sharma et al.,** [21] (2010) had synthesized a series of substituted benzimidazoles [2-(substituted-phenyl)-6-methoxy-1-[2'-(1H-tetrazole-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazole] from 4-methoxy-1,2-phenylenediamine and different substituted carboxylic acids in the presence of $BF_3.OEt_2$ as a catalyst with biphenyl tetrazole. Synthesized compounds have been evaluated for antihypertensive activity direct and indirect methods and confirmed by IR, NMR, MS and elemental analysis.



8. **Kiumars Bahrami et al., [22]** (2008) synthesized 2-substituted benzimidazoles and benzothiazoles and this offers short reaction times, large scale synthesis, easy and quick isolation of products, excellent chemo selectivity, and excellent yields as main advantages.

9. **Pinar Alcil et al., [23]** (2009) had studied synthesis and illumination of 8 compounds that were expected to display antifungal and antibacterial activity in addition to interaction with DNA. It was performed by heating o-phenylenediamine and derivatives with succinic acid and malonic acid in 4N HCl. Reactions with succinic acid yields bis-benzimidazole derivative. Reactions with malonic acid yield benzimidazole derivatives. During analysis it was found that compound 1 requires higher concentration and longer time to be able to interact with DNA than comparing with compound 5. All compounds were examined for antibacterial activity. Compound 7 was effective against E.coli and compound 5 against Enterococcus faecalis.7 and 8 were effective against Candidia albicans.



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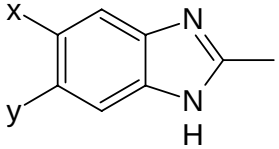
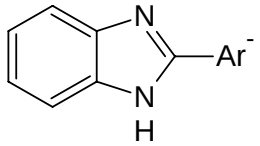
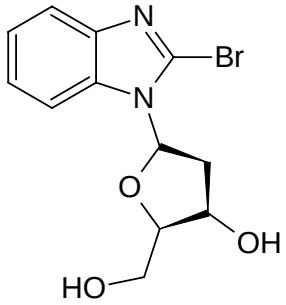


(5)

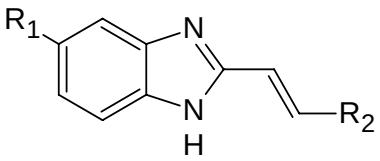
10. **Sachin V.et al., [24]** (2011) developed a novel approach for the synthesis of 2-substituted benzimidazole via a tandem reaction following sp^3 C-H functionalization. Here they reported a simple, efficient and tandem oxidative dehydrative coupling reaction of N-benzylbenzene-1,2-diamine in the presence of oxidant tert-butyl hydro peroxide(TBHP) in solvent acetonitrile to give substituted benzimidazole.



11. **Simone Budow et al., [25]** (2009) evaluated the anti viral activity of a series of benzimidazole and substituted benzimidazole β-L-and β-D-2'deoxyribonucleosides against RNA and DNA viruses including HIV-1, BVDV, YFV, DENV-2, WNV etc. Several other syntheses of compounds, stereo selective glycosilation of anions and certain crystalline structures of compounds were also reported.



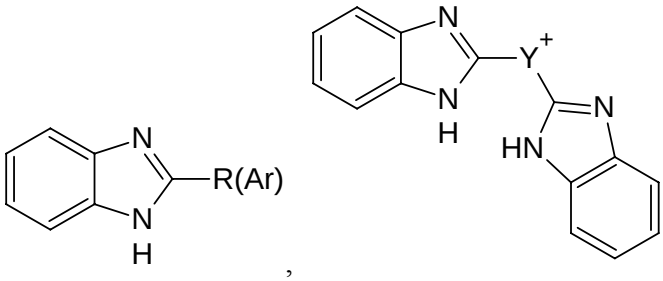
12. **Santhosh.S.Chhajed et al.,[26]** (2011) had synthesized substituted benzimidazole by the reaction between 2-methyl 1-H benzimidazole, with different (un)/ substituted aldehydes to form 2-(un)/substituted styryl/vinyl-1H-benzimidazole. Structures and purity were determined by elemental analysis and TLC respectively. Synthesized compounds were screened for their antioxidant properties. Results shown that two of them active same as standard ascorbic acid while other three are more active than ascorbic acid.



R_1= -H, -NO_2

R_2= -H, -CH_3, -C_6H_5, -C_6H_4Cl (o,m,p), -C_6H_4OH (o,p), -$C_6H_4NO_2$

13. **K.Niknam et al.,** [27] (2007) reported a microwave assisted method for the synthesis of 2-substituted benzimidazole in presence of alumina-methanesulphonic acid (AMA). In addition, by the direct reaction of phenylenediamine and dicarboxylic acid, new bis-benzimidazoles were formed in excellent yields.



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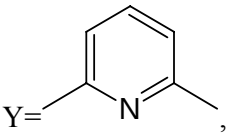
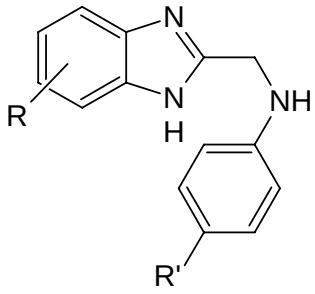


Y= , CH_2-CH_2, CH_2-S-CH_2, CH_2-O-CH_2

14. **Mashoda Hasan et al.,** [28] (1997) had condensed methyl crotonic acid with 4-chloro-o-phenylenediamine in 4N HCl yielded 5-chloro-2(1-isobutenyl)benzimidazole and 7-chloro-4,4-dimethyl-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one.condensation of methacrylic acid with 4-chloro-o-phenylenediamine in 4N HCl yielded 5-chlooro-2(2-propenyl)benzimidazole. The structures where confirmed with the help of mass and ^{1}HNMR spectral analysis.

15. **Kavitha C.S.Achar et al.,** [29] (2010) had synthesized 2-methyl-aminobenzimidazole derivative (1-11) by the reaction of 2-(chloro methyl)-1H-benzimidazole derivatives with primary aromatic amines. The compounds

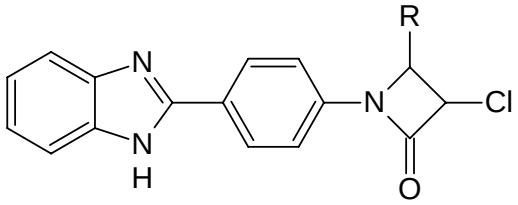
were characterized by IR, ^{1}H NMR, ^{13}C NMR, GC-MS and elemental analysis and were screened for analgesic and anti-inflammatory activities on acetic acid induced writhing in mice and carrageenan induced paw oedema in rats. Compounds(7) and (2) showed a potent analgesic and anti-inflammatory activities compared with standard drug nimesulide respectively. The other compounds showed good analgesic and anti-inflammatory activities.

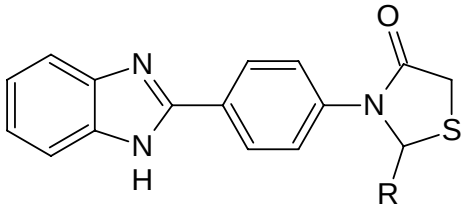


R=H, Br, NO_2

R'=H, Cl, Br, CH_3, OCH_3

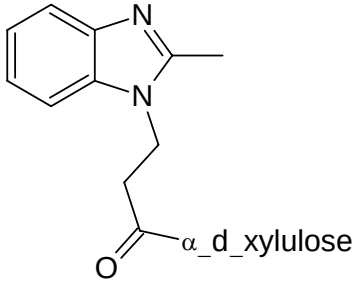
16. **Panneer Selvam et al.,** [30] (2011) had synthesized and characterized 2-substituted benzimidazole derivatives by IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. Compounds were screened for anti bacterial and anti fungal activities. The minimum inhibitory concentration (MIC) was determined by agar streak dilution method. 1-(4-(1H-benzo[d]imidazol-2yl)phenyl)-3-chloro-4-(4-nitrophenyl)acetidin-2-one was found to exhibit potent and some others exhibit moderate activity against the bacterial and fungal organism tested.



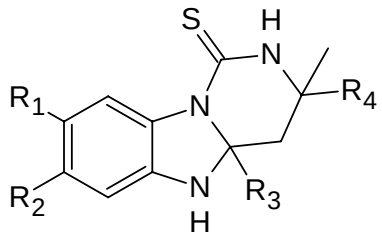


R=$C_6H_4NO_2$, C_6H_4Cl, $C_6H_4OCH_3$, $C_6H_4CH_3$

17. **Ahmed O.H.El-Nezhawy et al.,** [31] (2009) had synthesized a series of 2-methyl-N-substituted-benzimidazoles, bearing hydroxypyrrolidinone-5-yl or hydroxylpyrrolidine-2-yl, 2,3:5,6-di-O-isopropylidine-α-D-mannofuranoside, 2,3,5,6-tetrahydroxy-α-D-xylo-heptofuranos-5-ulose, 3-O-benzyl-6,7-dideoxy-1,2-dihydroxy-α-D-xylo-heptofuranose-5-ulose, 1,2,5,6-tetrahydroxy-α-D-glucofuranose sugar moieties. These compounds were obtained in good yields from 2-methyl N-(trichloroacetamidomethyl) benzimidazole as a donor and carbohydrate residues as acceptor precursors in the presence catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as Lewis acid. Out of 16 synthesized compounds 6 showed significant anti-inflammatory and analgesic activities.



18. **Shefali Arora et al.,** [32] (2011) found out that the side effects of some analgesic agents were overcome by achieving an effect at a lower dose. Modification of the structure of a known drug had optimized the pyrimidobenzimidazole derivatives using molecular modeling studies, using the physicochemical parameter and molecular descriptors. The data base was subjected to QSAR studies using Chemsketch software version 10.0e and all the parameter and descriptors were calculated using TSAR 3D version 3.3 for windows. The results of QSAR study suggested that molecule must contain at least substituent on R_1 and R_2 should be NO_2 for good analgesic activity.



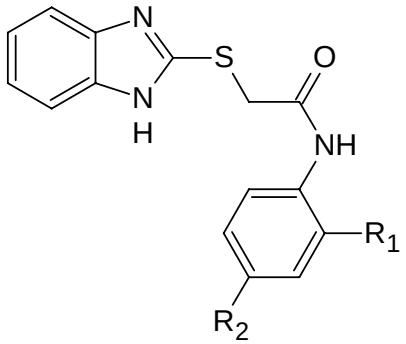
R_1= -H, -NO_2, -COOH, -C_6H_5CO, -CH_3

R_2= -H, -CH_3, -Cl

R_3= -H, -CH_3

R_4= -H, -CH_3

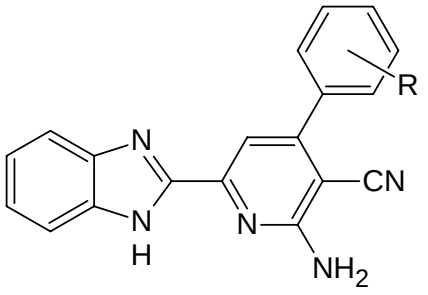
19. **V.Mohan Goud et al.,** [33] (2011) had synthesized benzimidazole derivatives like 2-(1H-benzimidazole-2-yl-sulfanyl)-N-(2-hydroxy-5-methylphenyl) acetamide, and the structures were confirmed by IR, HNMR and MS. The compounds were screened for analgesic and anti-inflammatory activities by biological evaluation method and antibacterial activity. Some synthesized compounds have shown significant analgesic anti-inflammatory activity and moderate antibacterial activity.



R_1= -CH_3, -H, -NO_2, -Cl

R_2= -OH, -NH_2, -$COCF_3$

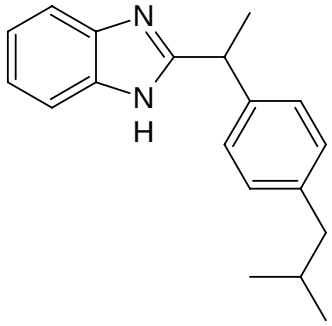
20. **Janardan.S.Yadav et al.,** [34] (2011) had synthesized 2-amino-6-(1H-benzimidazolle-2-yl)-4-phenyl pyridine-3-carbonitriles by reacting 2-acetyl benzimidazoles(1) with substituted benzaldehydes(2) in presence of ethanol to furnish substituted chalcones (3a-f).These chalcones were further treated with Malononitrile and Ammonium Acetate to afford substituted 2-Amino-6-(1H benzimidazol-2-yl)-4-phenyl pyridine-3-carbonitriles(4a-f). The structures were confirmed by spectral data and elemental analysis. The compounds were screened for antibacterial activity. The results exhibited good antibacterial and moderate antifungal activities.



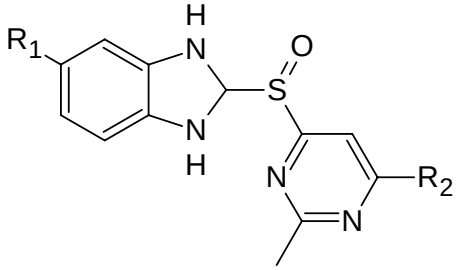
R= -H, -OCH_3, -Cl, -$N(CH_3)_2$

21. **Avinash Patil et al.,** [35] (2008) proven the antiulcer activity as potent inhibitor of H^+/K^+-ATPase of a series of benzimidazole derivatives. The therapeutic significance of these drugs for the treatment of peptic ulcer and GIT diseases encouraged the development of potent and significant compounds. The pathophysiology of disease, structural and molecular chemistry of benzimidazole derivatives was also discussed. This study summarizes the different derivatives of substituted benzimidazole along with biological evaluation and factors that make the drug more specific for proton pump inhibitors.

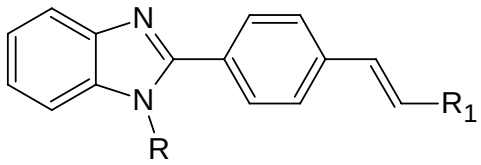
22. **A.Anton Smith et al.,** [36] (2008) had synthesized novel benzimidazole derivatives from Ibuprofen with 0-phenylenediamine and was subjected to antibacterial activity against Staphylococcus aureus ATCC 250, Pseudomonas aeruginosa ATCC 25619, etc and antifungal efficacy against Candida albicans RSKK 628. It was shown that benzimidazole derivatives was more effective and found more effective and showed antibacterial and antifungal activity against the entire organism tested. The MIC was found in the range 25-100mg/ml on all the microorganisms.



23. **Thakare P.B et al.,** [37] (2011) had synthesized new substituted 2-(pyrimidinylsulphenyl)benzimidazole derivatives from different starting material and evaluated against antiulcer anti-secretory activity as a inhibition of gastric H^+/K^+-ATPase by induction of gastric ulcerations experimentally in male WISTER rats according to the method. The compound was characterized by elemental analysis and IR. The compounds were tested to antiulcer and anti-secretory activities. The antiulcer activities were assessed by acetyl salicylic acid (ASA) method-induced gastric ulcer.



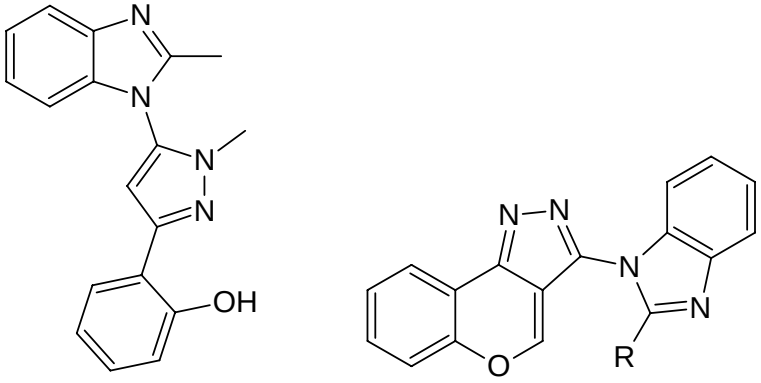
24. **Raju B Chaudhari et al.,** [38] (2011) prepared the derivatives by the condensation of O-phenylenediamine with 4-bromobenzoic acid to give 2-(4-bromophenyl)-1H-benzimidazole which undergoes Heck reaction with alkyl acrylates to give the above benzimidazole derivatives. These compounds react with electrophiles to give 1N-substituted alkyl/acyl/aroyl/sulphonyl derivatives. The antibacterial activities were also checked but the results are found to be disappointing.



R= Methyl, Ethyl, Butyl, Benzyl, Methoxycarbonyl, Phenylsulfonyl

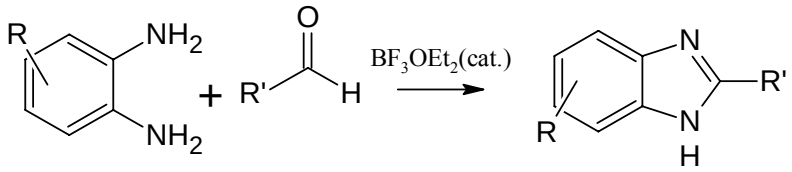
R_1= Methoxycarbonyl, Ethoxycarbonyl,

25. **Saoussen Hammami et al., [39]** (2010) had synthesized new N-pyrazoyl-benzimidazoles through a two-step reaction using 5,2-aminophenylamino-3-(2-hydroxy phenyl)-1H-pyrazole as a useful precursor. The compounds were characterized by M.S, ^{1}H, ^{13}C and 2D NMR techniques.



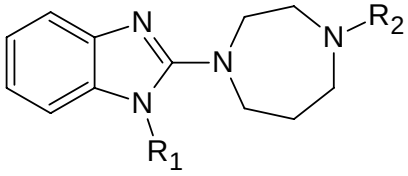
R= $-CH_3$, C_2H_5

26. **Rahul R Nagawade et al., [40]** (2006) had synthesized substituted benzimidazoles by the reaction with 0-phenylenediamine and aldehydes in the presence BF_3OEt_2 as a catalyst in solvent free conditions. They found that the method is applicable to aromatic, unsaturated and aliphatic aldehydes and to substituted O-phenylenediamine without significant differences.



27. **Hiroyuki Nakano et al., [41]** (1999) had synthesized novel benzimidazole derivatives that suppress histamine release from mast cells, inhibit 5-lippooxygenase and possess anti-oxidative action. Among the synthesized compounds 1-[2-[2(4-hyydroxy-2,3,5-trimethylphenoxy)ethoxy]ethyl]-2-(4-methyl-1-homopiperazino)benzimidazole potently suppressed histamine release from rat peritoneal mast cells triggered by the antigen-antibody

reaction, inhibited 5-lipoxygenase in rat basophilic leukemia-1(RBL-1) cells and prevented the NADPH-dependent lipid peroxidation induced by Fe^{3+}-ADP in rat liver microsomes, in addition to an antagonizing the contraction of guinea pig ileum caused by histamine.

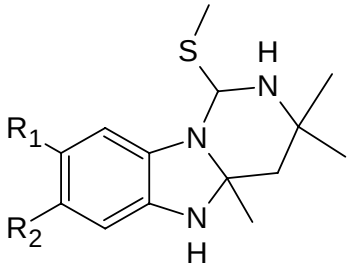


28. **Anshul Chawla** [42] (2011) discussed about the benzimidazole prepared from amino acids associated with diverse pharmacological activities such as antimicrobial, antiviral, anti-diabetic and anticancer activity. This review is deals with the "green" synthesis of benzimidazole derivatives by microwave induced reactions.

29. **Devender Pathak et al.,** [43] (2010) suggested in this review article that benzimidazole derivatives posses biological actions such as antimicrobial, anti-inflammatory, anticancer, anticonvulsant, antidepressant, antioxidant, radio protective and anti-leishmanial activities. In this review article the SAR of benzimidazole derivatives where also discussed to develop newer compounds possessing benzimidazole moiety that could be better agents in terms of efficacy and safety.

30. **Shefali Arora** [44] (2011) found out that compounds of pyrimidine, pyridine, and benzimidazole nucleus have wide range of therapeutic uses such as anti tubercular, anti cancer, anti-helmintic, anti oxidant and anti microbial activities. It was also believed that the presence of >N-C=S linkage is responsible for the amoebicidal, anti-convulsant, fungicidal and antiviral activities. Various benzimidazole derivatives of o-phenylenediamine, 4,5-dimethyl-1,2-phenylenediamine, 4-chloro-1,2-phenylenediamine, S-methylated o-phenylenediamine, S-mehylated-4,5-dimethyl-1,2-phenylenediamine, S-methylated-4-chloro-1,2-phenylenediamine have been synthesized. The derivatives were screened with various bacterial and fungal

strains. After the study it was showed that very good activity against the fungal strains of Aspergillus niger and Aspergillus japanicus.



R_1= -H, -CH_3

R_2= -H, -CH_3, Cl

31. **Davood azarifar et al.,** [45] (2010) developed a simple procedure for the green synthesis of various 2-aryl-1(arylmethyl)-1*H*-benzimidazoles in high yields by acetic acid-promoted condensation of *o*-phenylenediamine with aldehydes in air under microwave irradiation and transition metal catalyst-free conditions.

32. **S. O. Podunavac kuzmanovi et al.,** [46] (2007) had reacted Zinc (II) chloride with some 1-benzylbenzimidazole derivatives (L) to give complexes of the formula ZnL_2Cl_2. All the ligands and their zinc (II) complexes were evaluated for their *in vitro* antibacterial activity against *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Sarcina lutea*. The majority of the investigated compounds displayed *in vitro* antimicrobial activity against very persistent microorganisms. It was found that all the tested compounds were more active against gram-positive than gram-negative bacteria. The minimum inhibitory concentration (MIC) was determined for all ligands and their complexes. The effect of the structure of the ligands and complexes on the antimicrobial activity is discussed. The complexes were found to be more toxic than the ligands.

33. **P.C.Santosh et al.,** [47] (2011) published a review article suggest that benzimidazole is an important nucleus and a large variety of 2-substituted

benzimidazoles have been to possess antiulcer, antihelminthic, analgesic, anti inflammatory, antispasmodic, antihistaminic, antimicrobial, anticancer, Cyclooxygenase inhibitor and HIV-1 reverse transcriptase inhibitor activities.

34. **Raamanpreet Walia et al., [48]** (2011) had reviewed that benzimidazole derivatives play an important role in medical field with so many pharmacological activities such as antimicrobial, antiviral, antidiabetic, and anticancer activity. The potency of these clinically useful drugs in treatment encouraged the development of some more potent and significant compounds. Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective. They summarized about the chemistry of different derivatives of substituted benzimidazoles along with their pharmacological activities.

35. **Punit P. Seth et al., [49]** (2003) had done the preparation and evaluation of different 2-amino benzimidazole dimmers as antibacterial agents. The biological evaluation indicated that compounds with multiple chloro substituent possessed optimal antibacterial activity**.**

36. **M.Shahar Yar et al., [50]** (2009) had synthesized a series of pyrazoline and phenyl pyrazoline derivatives of benzimidazoles by reacting 1-(1H-benzimidazole-2yl)-3-(substituted phenyl)-2-propane-1-one with hydrazine hydrate and phenyl hydrazine in submitted reactions. All the compounds entered for screening at the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF) for their in vitro antibacterial activity against Mycobacterium Tuberculosis using Microplate Alamar Blue Assay (MABA) susceptibility test. The result expressed as MIC in µg/ml. Among the fifteen compounds, eight compounds were found to have MIC values less than 10 µg/ml. These were subjected for cytotoxicity assay in VERO cells to determine CC50 values and finally the SI (selectivity index) were calculated. 2-[5-(4fluorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole was considered the best candidate of the series that could be a

good starting point to develop new lead compounds in the fight against tuberculosis.

37. **Paris.M.Allen et al., [51]** (1970) reported that thiabendazole inhibit the growth of Penicillium atrovenetum at 8-10µg/ml. Thiabendazole did not inhibit peptide synthesis in cell-free protein synthesizing system. Probably the primary site of inhibition is the terminal electron transport system and other effects are secondary.

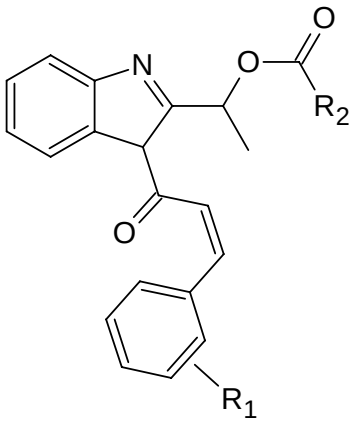
38. **Rajesh kumar et al., [52]** (2007) had synthesized a series of imidazolines and benzimidazoles from various aldehydes and 1,2-diamines in the presence of Ceric(IV) ammonium nitrate (CAN). They prepared via one step synthesis method. This method is more attractive for organic chemists because of the simplicity of the reaction conditions with shorter reaction time and without the use of column chromatography to get the pure products in high yield.

39. **Raquel Dias et al., [53]** (2008) reviewed several molecular docking search algorithms and the programs which apply such methodologies and also discussed how virtual screening can be optimized, describing methods that may increase accuracy of the simulation process, with relatively fast docking algorithms. Molecular docking is a computer simulation procedure to predict the conformation of a receptor-ligand complex. Each docking programme makes use of one or more specific search algorithms, which are the methods used to predict the possible conformation of a binary complex.

40. **Sabiha Alper et al., [54]** (2002) done a detailed investigation on bi- and ter-benzimidazole derivative revealed that these compounds are new classes of topoisomerase I inhibitors that poisons mammalian topoisomerase I. Some recent result about new derivatives, some SAR and comparison of activity of various functional groups are discussed. In general 5-position of terbenzimidazole is an important position for their activity. The presence of phenyl, naphthyl or pyridyl groups at this position influence cytotoxicity. In

addition to this para substituents at 5-position needed for the activity as topoisomerase I poisoning.

41. **Chhajed.S.S et al.,** [55] (2010) had designed and novel substituted benzimidazoles and docked into active site of Cyclooxygenase II. The ligands are designed based on the structure of the receptor, COX II and well known NSAID celecoxib. Further in-silico docking analysis of designed ligands was performed to predict binding mode, orientation and affinity. Ligands having less binding energy are said to possess more affinity for receptor than other molecules.



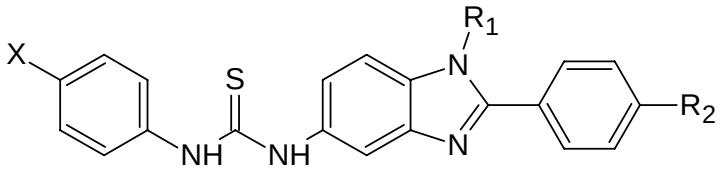
R_1= -H, -ClC_6H_4

R_2= -C_6H_5, -ClC_6H_4, -$NO_2C_6H_4$, -CH_3, -OHC_6H_4, -$NH_2C_6H_4$

42. **Sreena.K et al.,** [56] (2009) had synthesized some benzimidazole derivatives and screened for their anthelmintic activity. O-phenylenediamine was condensed with acids in presence of polyphosphoric acid and solvents like water and HCl and the synthesized compounds were characterized by IR, NMR and MS. All the synthesized compounds showed significant anthelmintic activity.

43. **Gulgun Ayhan Kilcigil et al.,** [57] (2006) had synthesized some benzimidazole derivatives and tested their in-vitro antifungal activities against Candida albicans, Candida glabrata, Candida krusei. Among the synthesized

compounds two of them possessed activity compatible to fluconazole against Candida albicans with a MIC of 12.5µg/ml.

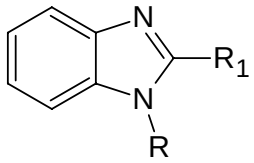


R_1= -C_3H_7, -C_5H_4

R_2= -H, -F

R_3= -H, -Cl

44. **Ashish Kumar Tewari et al.,** [58] (2006) had synthesized two series of N-substituted-2-substituted benzimidazole derivatives. 1-benzyl-2-substituted benzimidazole and 1-(p-chlorophenyl)-2-substituted benzimidazole had been synthesized and tested for their antiviral activities. The compounds had been screened for Tobacco mosaic viruses and Sunhemp rosette viruses and show significant activities.



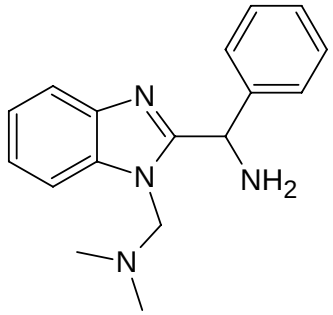
R= -H, -$CH_2C_6H_5$, C_6H_5Cl

R_1= -CH_2CH_2COOH, -C_6H_5OH, - $(CH_2)_4COOH$, -C_6H_4COOH

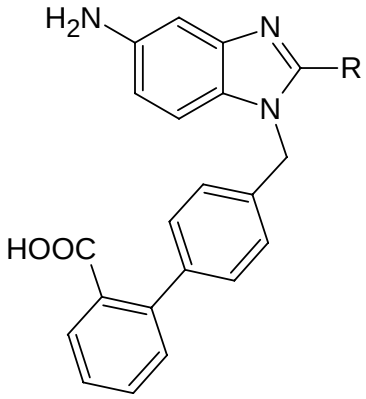
45. **K.Vijayakumar et al.,** [59] (2010) had synthesized a series of novel benzimidazole derivatives by the condensation of different diamines with anthranilic acids. The subsequent reaction of benzimidazole derivatives had been also carried out with different aromatic acid chlorides to get tetrazole moieties. These compounds were screened for their potential anticancer, anti-diabetic, antitumor and anti-asthmatic properties, which exhibited some authentic results towards testing organism in-vitro and in-vivo studies.

46. **B.Anil Reddy** [60] (2009) had synthesized various 2-substituted benzimidazole derivatives and subsequent reaction to get the corresponding Mannich bases. The formations of the products were confirmed by the analytical and spectral

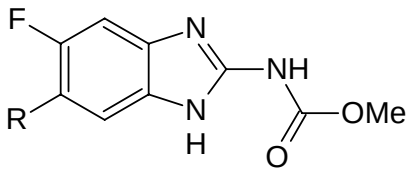
data. The biological evaluation showed that the prepared compounds possess anti-inflammatory activity.



47. **Jat rakesh kumar et al.,** [61] (2006) had synthesized a new seris no of non peptide angiotensin(A-II) receptor antagonist. This 5-substituted (amino)-2-phenyl-1-(2'carboxy biphenyl-4-yl) benzimidazoles produce potent anti-hypertensive effect upon oral administration. It had been found out that 2'-position of biphenyl is essential. Only ortho substituted acids possess both high affinity for the AII receptor and oral anti-hypertensive potency.

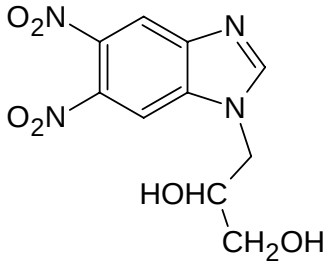


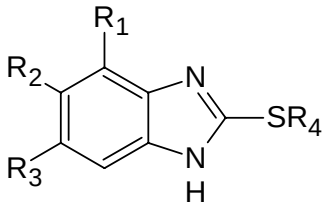
48. **Canan Kus et al.,** [62] (2003) had synthesized methyl-5(6)-fluro-6(5)-substituted-1H-benzimidazole carbamate derivatives and their antifungal activities were evaluated against Candida albicans.



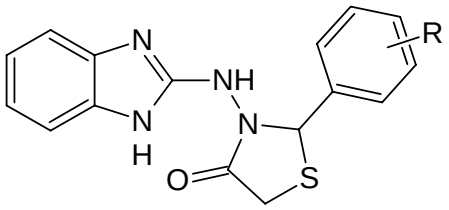
R= -Cl, -4-morpholinyl, 1-pyrrolidinyl, 3-methylpiperidin-1-yl

49. **Zygmunt Kazimierczuk et al.,** [63] (2002) synthesized two series of benzimidazole derivatives. The first one was based on 5,6-dinitrobenzimidazole, second one comprises 2-thioalkyl- and thioaryl-substituted modified benzimidazoles. Antibacterial and antiprotozoal activities of the newly synthesized compounds were studied. Some thioalkyl derivatives showed remarkable activity against Stenotrophonomas malthophilia and an activity comparable to that of metronidazole against gram positive and gram negative bacteria. Out of the tested compounds, 4,6-dichloro-2-(4-nitrobenzylthio)-benzimidazole showed the most distinct antiprotozoal activity.



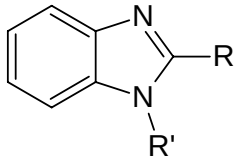


50. **Srinivas Bethi et al.,** [64] (2011) had fused the moieties of benzimidazole & thiazolidinones and synthesized novel 3-(1H-benzimidazole-2-yl-amino)-2-phenyl-1,3-thiazolidin-4-one, such 5 compounds are characterized by its physical datas and screened for possible CNS activity like gross behavioral studies and locomotion activity. Among all the test compounds, compound with 4-Chloro substitution on phenyl ring showed more promising depressant activity.



R= -H, -Cl, -$N(CH_3)_2$, -OCH_3, -CH_3, -OH

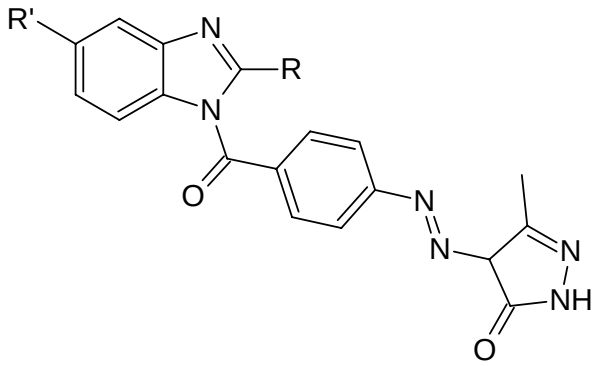
51. **Ashish Kumar Tewari et al.,** [65] (2006) had synthesized two series of N-substituted benzimidazole derivatives viz 1-benzyl-2-substituted benzimidazole and 1-(P-Chlorophenyl)-2-substituted benzimidazole and tested for Antiviral activities by Tobacco mosaic viruses and Sunhemp rosette viruses and show significant activities.



R= -CH_2CH_2COOH, -C_6H_4OH, -C_6H_4COOH. …

R'= -H, -$CH_2C_6H_5$, C_6H_5Cl

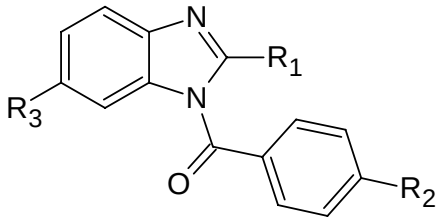
52. **Ramaraj sivakumar et al.,** [66] (2010) done molecular docking of benzimidazole containing pyrazoline-5-one derivatives as inhibitor of 14-α-demethylase which is the enzyme targeted in treatment of fungal infection in organisms ranging from humans to plants. The inhibitory activities against 14-α-demethylase were investigated by molecular docking using the HEX docking software. These compounds docked into the active site of receptor(PDB code, IE9X) using HEX docking tools and compounds show good affinity for the enzyme when compared with the binding energies of standard drugs such as clotrimazole and griseofulvin. Among all the designed compounds, the compound 7 show more binding energy values.



R= -H, -CH_3, -$CH_2C_6H_5$, -CH_2CH_2COOH, -C_6H_4COOH, -C_6H_4OH

R'= -H, -NO_2

53. Parmender Sing Rathee et al., [67] (2011) had synthesized two series of novel benzimidazole derivatives. The first one comprises of 2-methyl, second one comprises of 2-phenyl substitution on benzimidazole moiety. Seven novel benzimidazole derivatives were synthesized and characterized physicochemically. The synthesized compounds were screened for antimicrobial activity by tube dilution method. Among the synthesized compounds four of them showed appreciable antifungal activity.



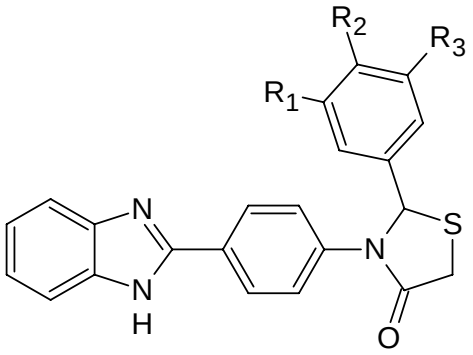
R1= -CH_3, -$CH_2C_6H_5NO_2$, -$CH_2C_6H_5$

R_2= -NO_2, -H

R3= -H, -OH

54. P. Shanmugapandian et al., [68] (2010) synthesized a new series of 2-[4-(azetidin-2-one)-3-Chloro-4-phenyl]-1H-Phenylbenzimidazole and 2-[(thiazolidin-4-one)-phenyl]-1H-Phenylbenzimidazoles. The synthesized compounds were screened for antibacterial, antifungal, analgesic and anti-inflammatory activity. All the compounds shows significant antimicrobial activity compared to the standard, and two of the thiazolidinone derivative

shows prominent analgesic anti-inflammatory activity equivalent to diclofenac.



R_1= -H, -OCH_3

R_2= -H, -Cl, -OH, -CH_3, -$N(CH_3)_2$, -OCH_3

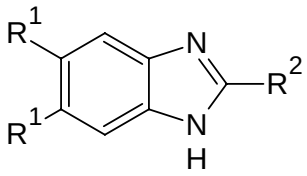
R_3= -H, -OCH_3

55. **S.L.Khokra et al.,** [69] (2011) published an article which reveals the different actions of benzimidazole; suggesting that benzimidazole is a versatile heterocycle possessing a wide spectrum of biological activities like antihelminthic, antifungal, anti-allergic, antimicrobial, antiviral, and antineoplastic activities and highlights the importance of benzimidazole in medicinal world.

56. **Ganesh Akula et al.,** [70] (2011) had synthesized a new series of 3-[1H-benzimidazole-2-yl-amino]-2-phenyl-1,3-thiazolidin-4-one and screened for depressant activity by the gross behavioral studies and the loco motor activity method. Among all the five synthesized compounds two of them showed excellent depressant activity.

57. **Harjyoti Thakuria et al.,** [71] (2008) developed a simple, highly efficient, convenient, and one pot solvent free green synthetic method for the synthesis of biologically important benzimidazole derivative. They explore the utility of simple one pot, solvent free, solid phase grinding method for efficient synthesis of biologically active benzimidazole derivatives.

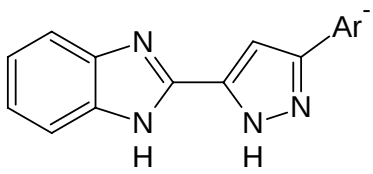
58. Peyman Salehi et al., [72] (2006) had developed a highly selective synthesis of 2-aryl-1-aryl methyl-1H-1,3-benzimidazole from O-phenyl diamine and aromatic aldehydes in presence of silica sulfuric acid in ethanol or water.



R_1= -H, $-CH_3$

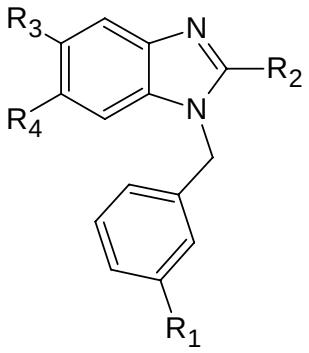
R_2= $-C_6H_5$, $-MeOC_6H_4$, $-MeC_6H_4$, $-ClC_6H_4$, $Me_2NC_6H_4$

59. R. Kalirajan et al., [73] (2010) had synthesized a series of pyrazole derivatives of benzimidazole; microwave irradiation and further reaction results in the final products. Among the synthesized compounds two of them had shown significant anti-cancer activity and all compounds had showed the antibacterial activity.



Ar= $-C_6H_5$, $-C_6H_4Cl$, $-C_6H_3Cl_2$, $-C_6H_4NO_2$, $-C_6H_4OCH_3$

60. Dijana J.Barna et al., [74] (2007) investigated the antifungal activity of some 1-benzylbenzimidazole derivatives against yeast Saccharomyces cerevisiae. Quantitative structure-activity relationship (QSAR) had been used to study the relationship between the antifungal activity and lipophilicity parameter, log P, calculated by using CS Chem-Office Software version 7.0 and three high-quality mathematical models relating the inhibitory activity, log $1/C_{MIC}$, and log P were defined. The developed QSAR mathematical model is used to predict inhibitory activity of the benzimidazole.



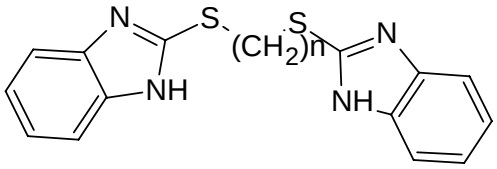
R_1= -CH_3, -Cl, -F, -OCH_3, -CH_3

R_2= -H, -NH_2

R_3= -CH_3, -H

R_4= -CH_3, -H

61. Srikanth Gurrala et al., [75] (2011) had adopted a convenient methodology for the symmetrical coupling of 2-mercapto benzimidazole derivatives by suitable linking agents. The synthesized compounds were screened for antifungal activity using Candida albicans by disc diffusion method on nutrient agar media and antibacterial activity using DMF as a solvent against the organisms, S.aureus and E.coli using Ketoconazole and Ampicillin as standard drug.



R= -OCH_3, -$OCHF_2$

62. Rakesh R. Somani et al., [76](2009) summarized in the review about oxadiazole, a heterocyclic nucleus; suggest that out of its four possible isomers, 1,3,4-oxadiazole is widely exploited for various applications. They explained about the various routes of synthesis and reactions of 1,3,4-oxadiazole and its derivatives and focus on their biological potential.

AIM OF THE WORK

In the present study, the main motto was to develop new chemical entities as potential anti-inflammatory, analgesic and antimicrobial agents by chemical modification of the 2nd position of prepared benzimidazole by different heterocyclic rings.

In our attempt, firstly 3-(1*H*-benzimidazol-2-yl) propanoic acid was prepared. Suitable heterocyclics which give potent ligands were identified by molecular docking then synthetic approaches based upon the chemical modification of the carboxylic group present in the prepared 3-(1*H*-benzimidazol-2-yl)propanoic acid with the heterocyclics like pyridazine-3,4-dione, 2,3-dihydrophthalazine-1,4-dione, 1,3,4-oxadiazole. Furthermore, it has been reported in literature that certain compounds bearing these nucleus possesses a wide spectrum of activities like analgesic, anti-inflammatory, anticonvulsant, antipyretic, antihypertensive, antiviral, antituberculosis, antimicrobial, anticancer activities etc. then identification and characterization of synthesized compounds by different methods like physical properties (M.P, Solubility, Thin layer chromatography) and spectral analysis (IR, NMR).

Finally selective pharmacological activities like Antimicrobial, Analgesic and Anti-inflammatory activities need to examine.

PLAN OF THE WORK

Step 1:

Designing various ligands by substituting different heterocyclic rings like phthalazine, triazine, 1, 3, 4- oxadiazole, and pyridazine on benzimidazole in order to identify the most potent compounds with the help of molecular docking studies.

Step 2:

The most potent compounds which have been identified by molecular docking studies were planned to synthesis.

Step 3:

The synthesized compounds were planned to characterized by physical data and spectral analysis (IR, HNMR)

Step 4:

Finally all the synthesized compounds were planned to evaluate for Antimicrobial, Analgesic and Anti-inflammatory activities.

MOLECULAR DOCKING

EXPERIMENT

In the docking studies, the protein sequences for Cyclooxygenase-2 and 14 α demethylase were taken from NCBI (National Center for Biotechnology Information). The sequences were converted into FASTA format. The FASTA format sequences were allowed to BLAST (Basic Local Alignment Search Tool) data base to identify the PDB code of required protein. The protein structure files of Cyclooxygenase-2 and 14α demethylase (PDB code: 6COX, 3KHM) were taken from Protein Data Bank (www.rcsb.org/pdb) and edited by removing the hetero atom. CAST P (Computed Atlas of Surface Toporaphy of Protein) server was used to cross check the active pockets on target protein molecules. All the ligand molecules were designed and the structure was analyzed using Chemoffice2004 software. ACD/ChemSketch software was used to draw the chemical structures. Argus lab.exe was used to perform molecular docking. Pymolsoftware was used to view the structure and calculating the length of the hydrogen bond.

LIGANDS DESIGNING

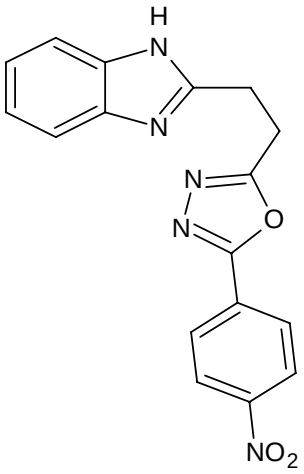
Various compounds were designed by replacing the carboxylic acid group present in the prepared 3-(1*H*-benzimidazol-2-yl)propanoic acid with the heterocyclics like pyridazine, phthalazine, 1,3,4-oxadiazole, pyrazole, imidazole, thiazole etc. in order to identify the new ligands. Docking simulation was carried out to all the designed compounds against 6COX (COX-2 enzyme) and 3KHM (14α demethylase enzyme) by using Argus Lab Program and the docking scores of each compound were analyzed.

FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY- Out of all the designed compounds, eight compounds showed good interaction energies against 6COX (see table 1). Among them structure containing 1,3,4-oxadiazole (Ligand1, Ligand 2), pyridazine (Ligand 6, Ligand 7), and phthalazine (Ligand 8) showed highly negative interaction energy. The compounds which have highly negative energy were considered as potent compounds.

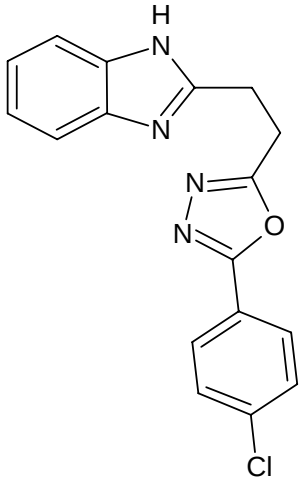
FOR ANTIMICROBIAL ACTIVITY- Out of all the designed compounds, eight compounds showed good interaction energies against 3KHM (see table 2). Among them structure containing 1,3,4-oxadiazole (Ligand 2, Ligand 3, Ligand 4), pyridazine (Ligand 6), and phthalazine (Ligand 8) showed highly negative interaction energy and these compounds were considered as potent compounds.

STRUCTURE OF LIGANDS DESIGNED

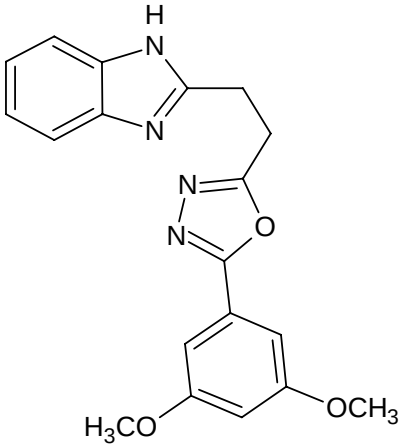
1,3,4-OXADIAZOLE DERIVATIVES:



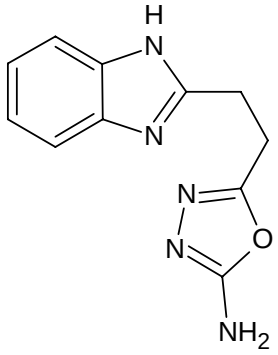
Ligand 1



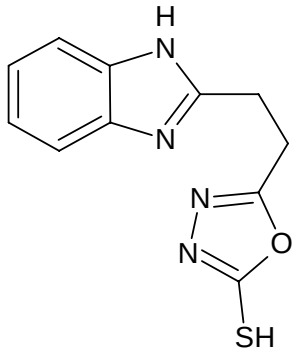
Ligand 2



Ligand 3

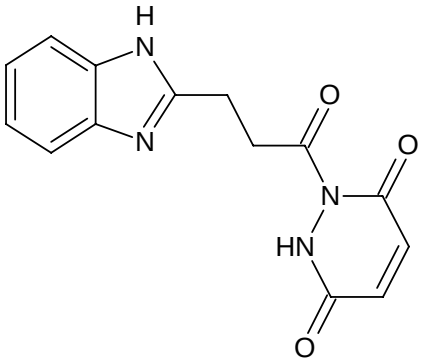


Ligand 4

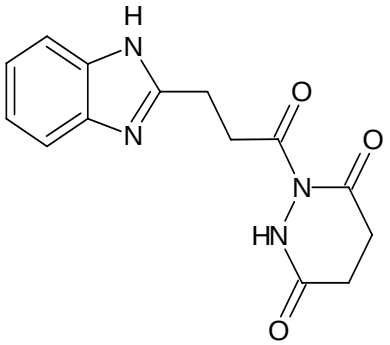


Ligand 5

PYRIDAZINE DERIVATIVES:

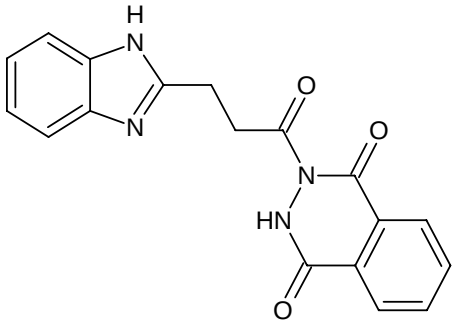


Ligand 6



Ligand 7

PHTHALAZINE DERIVATIVE:



Ligand 8

Table No 1. DOCKING SCORES (kcal/mol) OF VARIOUS NEWLY DESIGNED LIGANDS (1-8) WITH CYCLOOXYGENASE-2 (6COX)

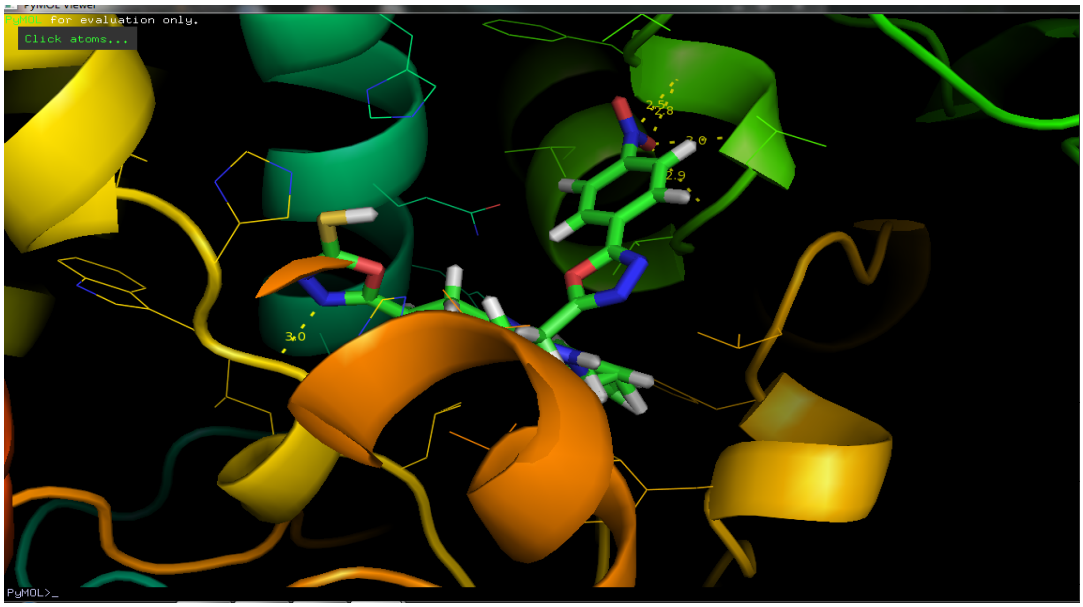
Sl.no	Compound code	Energy level (kcal/mol)	No. of hydrogen bond	Length of hydrogen bond	Interacting residue
1	Ligand 1	-10.5347	5	2.5 2.8 3.0 2.9 3.0	VAL291 VAL291 LEU294 VAL295 TRP387
2	Ligand 2	-12.8915	2	1.9 3.0	GLU203 TRP387
3	Ligand 3	-10.3525	1	3.0	TRP387
4	Ligand 4	-9.41254	NIL	NIL	NIL
5	Ligand 5	-9.37935	1	3.0	TRP387
6	Ligand 6	-10.6027			
7	Ligand 7	-11.2328			
8	Ligand 8	-11.8177			

Table No 2. DOCKING SCORES (kcal/mol) OF VARIOUS NEWLY DESIGNED LIGANDS (1-8) WITH 14α-demethylase (3KHM)

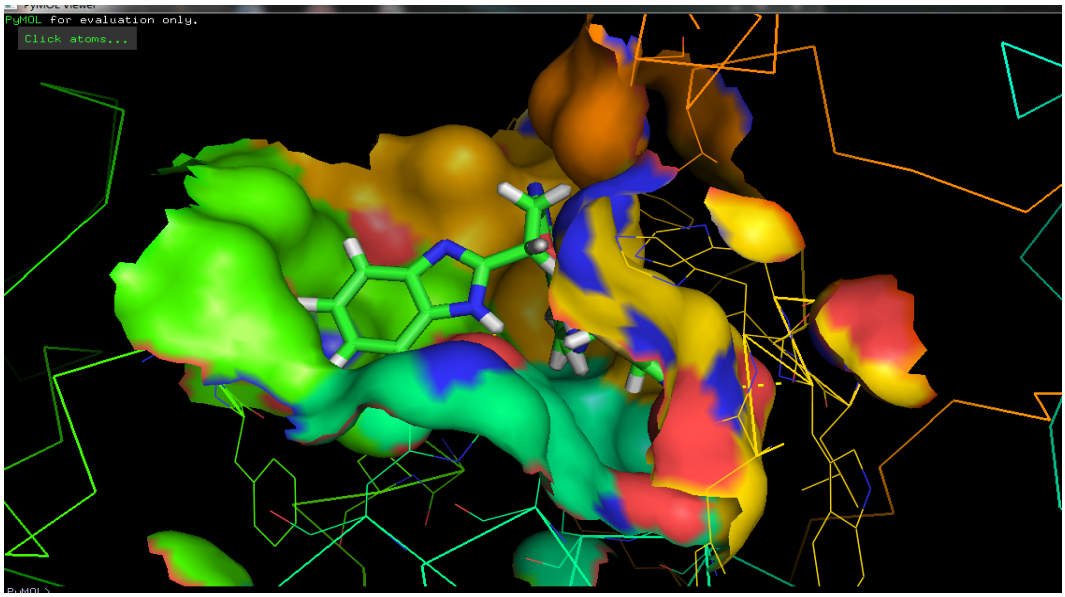
Sl.no	Compound code	Energy level (kcal/mol)	No. of hydrogen bond	Length of hydrogen bond	Interacting residue
1	Ligand 1	-7.16042	2	3.4 2.5	GLN294 LYS248
2	Ligand 2	-8.91847	1	2.5	ASN213
3	Ligand 3	-7.90829	2	2.6 3.0	LEU214 ASN213
4	Ligand 4	-7.49149	2	1.9 2.6	GLU204 ASN213
5	Ligand 5	-7.45405	2	2.0 2.4	GLU204 ASN213
6	Ligand 6	-7.63357	3	2.7 3.0 1.6	LEU214 ASN213 GLU204
7	Ligand 7	-6.81676	1	3.0	LEU214
8	Ligand 8	-7.6645	1	2.1	ASN213

BINDING MODE OF VARIOUS LIGANDS WITH COX-2 ENZYME (6COX)

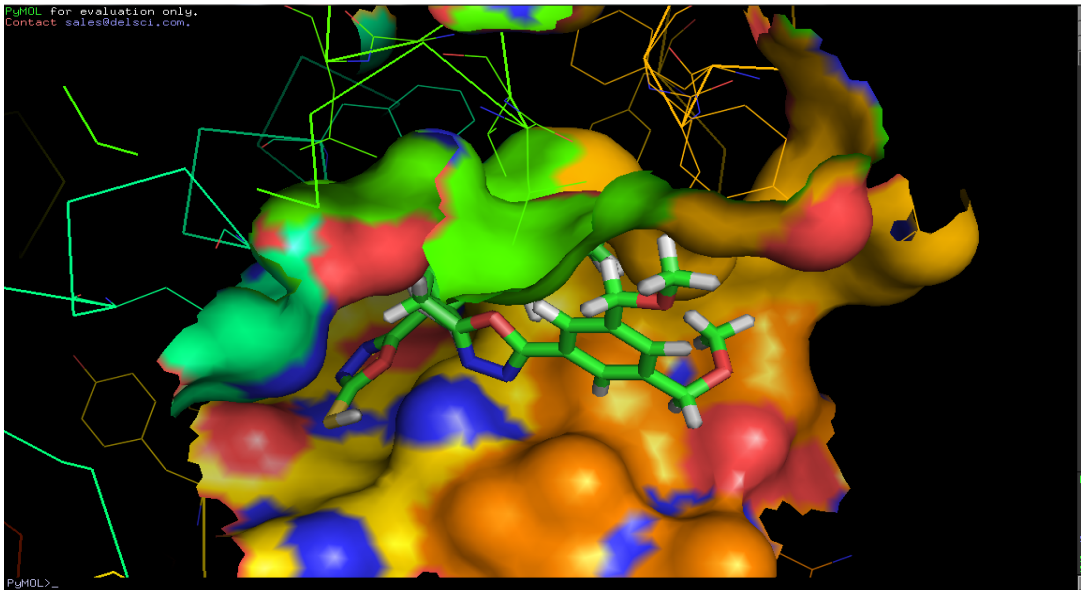
Fig 5



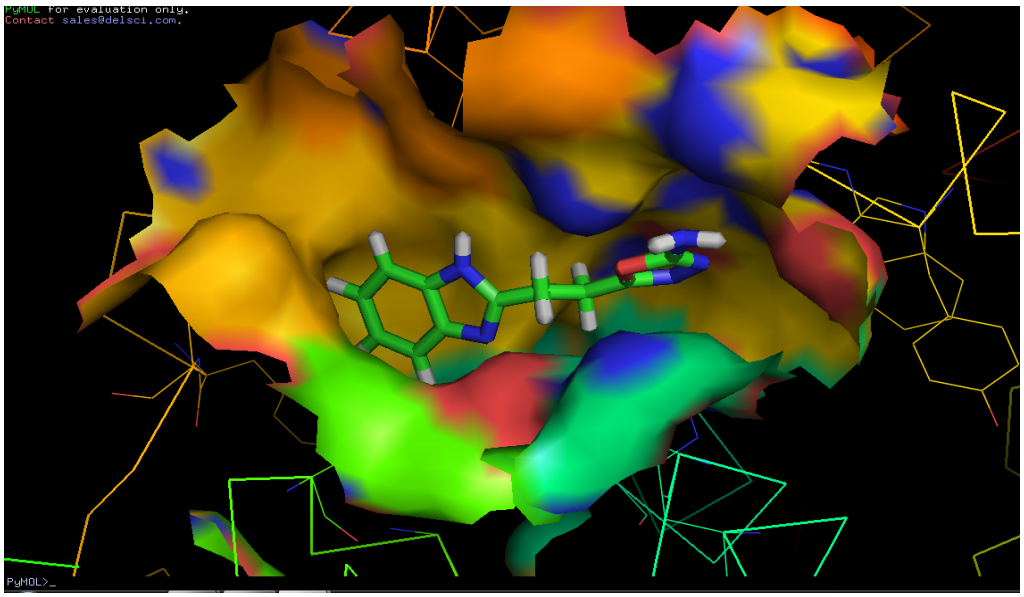
Ligand 1



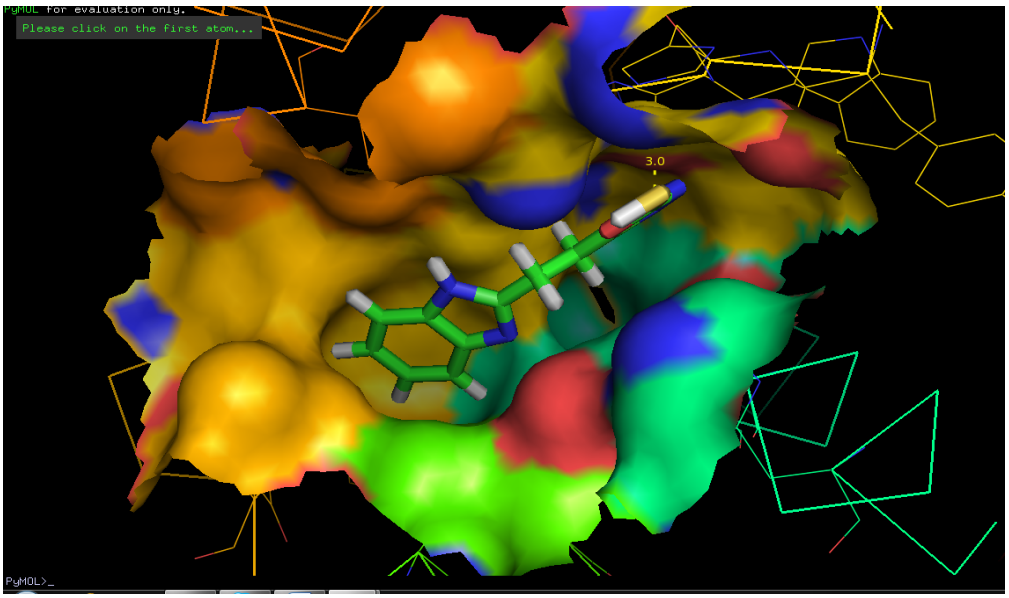
Ligand 2



Ligand 3



Ligand 4

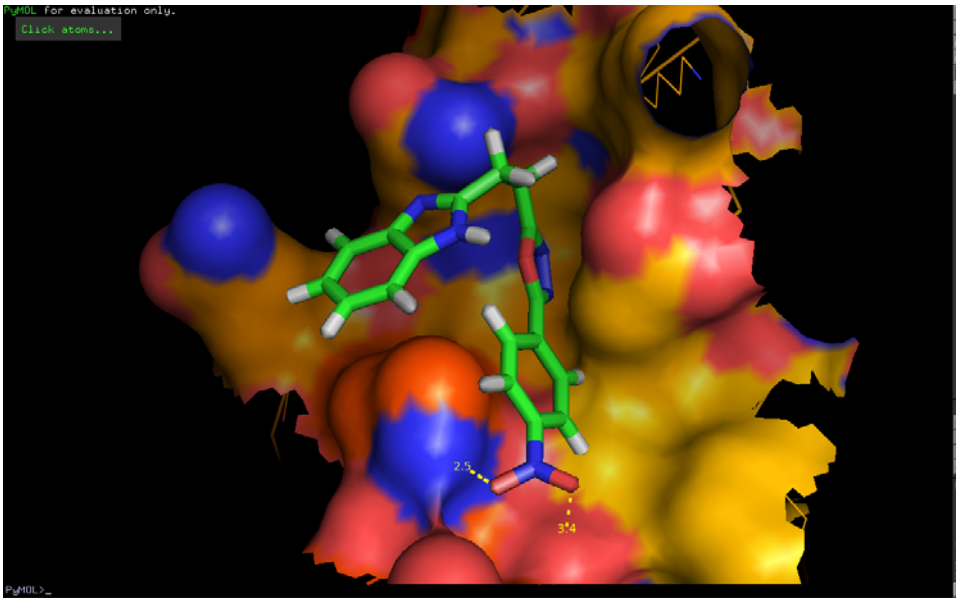

PyMOL for evaluation only.
Please click on the first atom...
3.0
PyMOL>_

Ligand 5

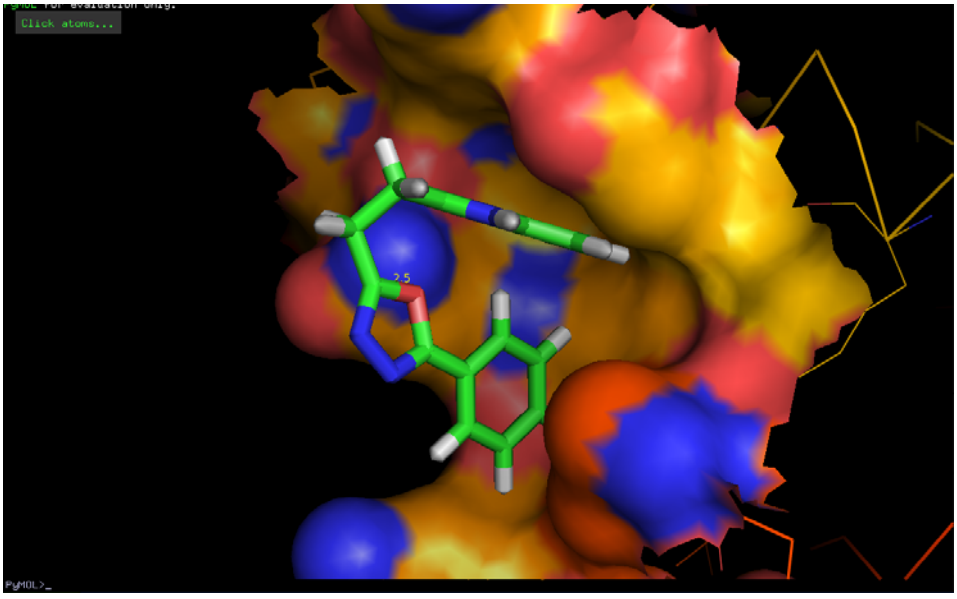
BINDING MODES OF VARIOUS LIGANDS WITH

14 Alpha Demethylase ENZYME (3KHM)

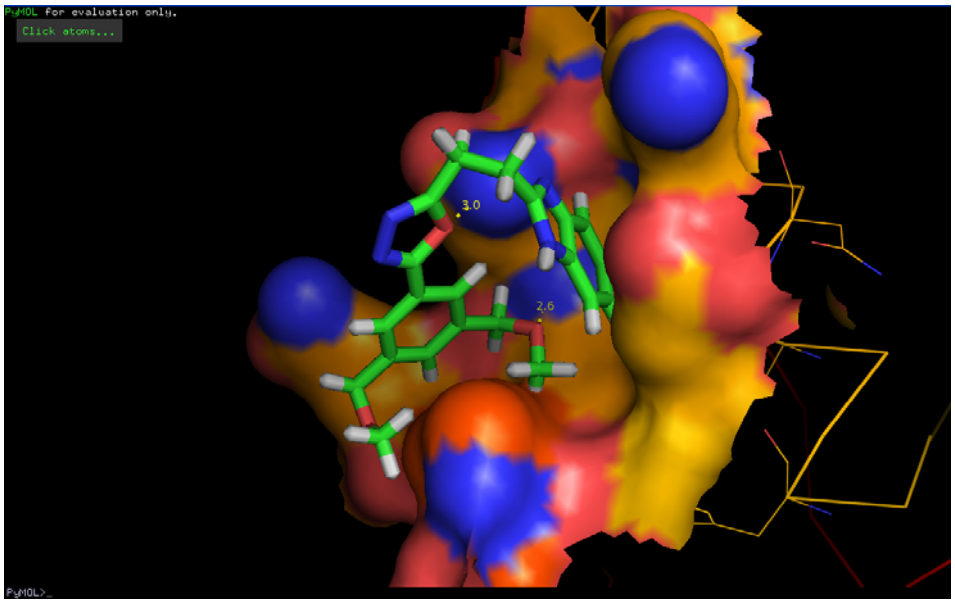
Fig 6



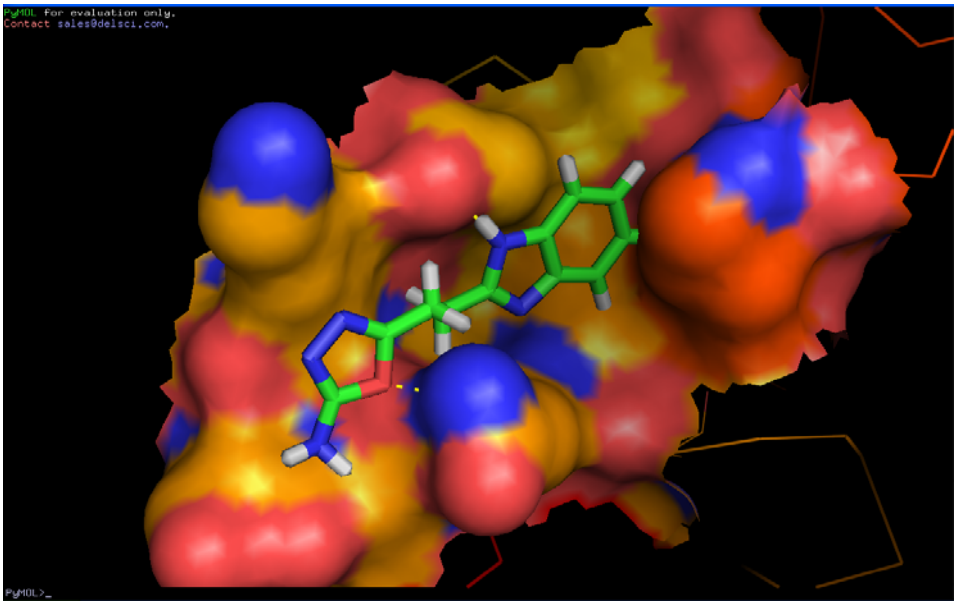
Ligand 1



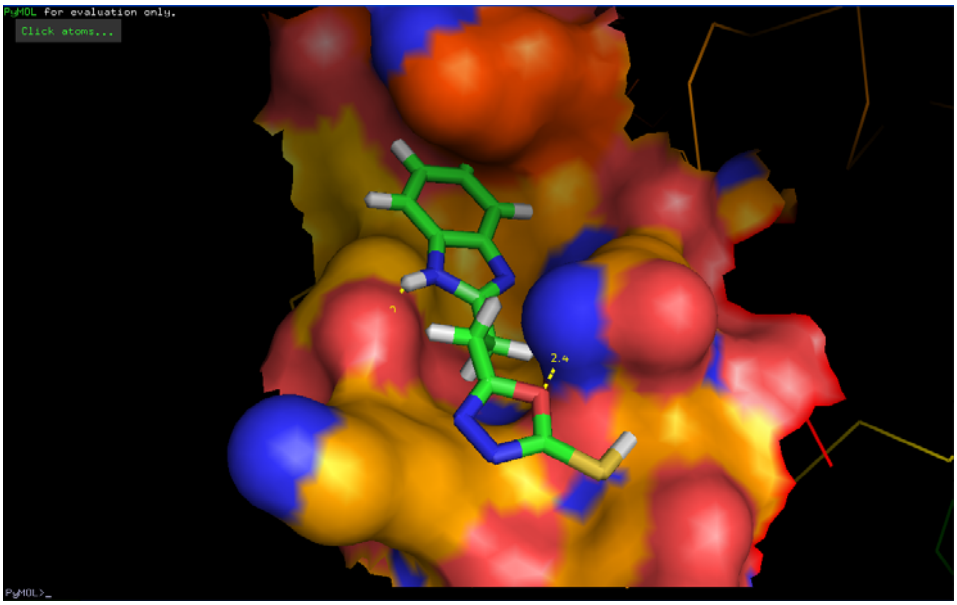
Ligand 2



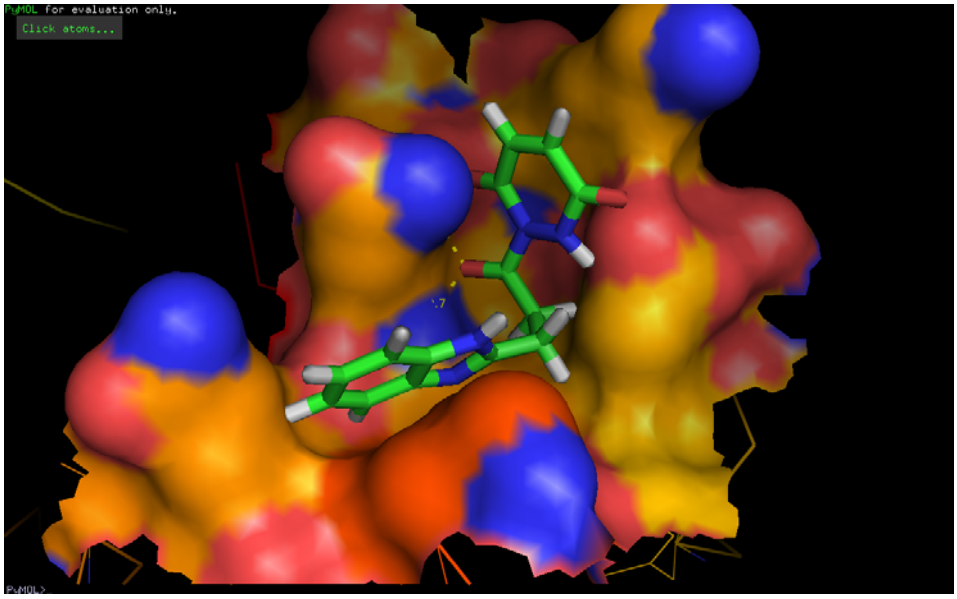
Ligand 3



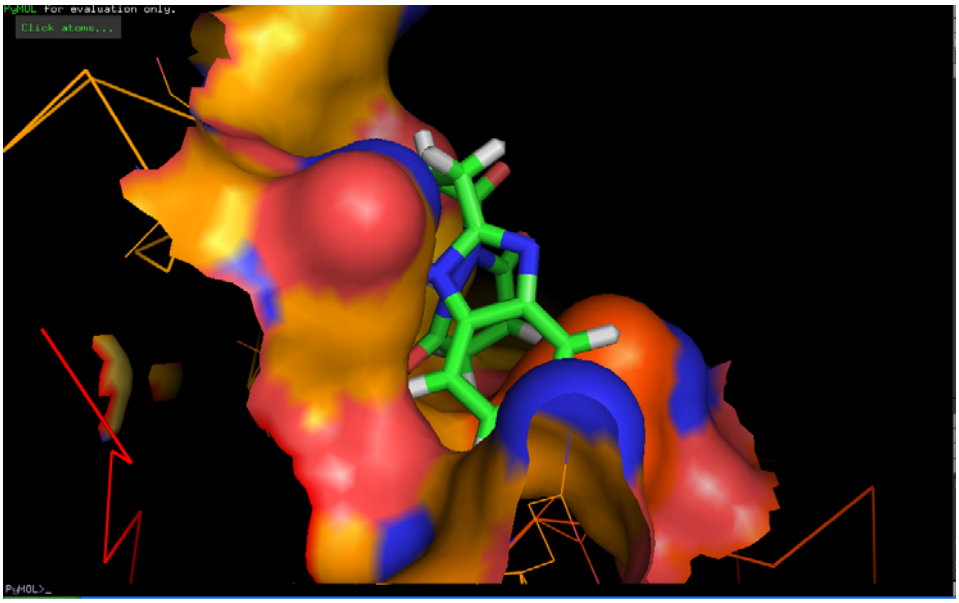
Ligand 4



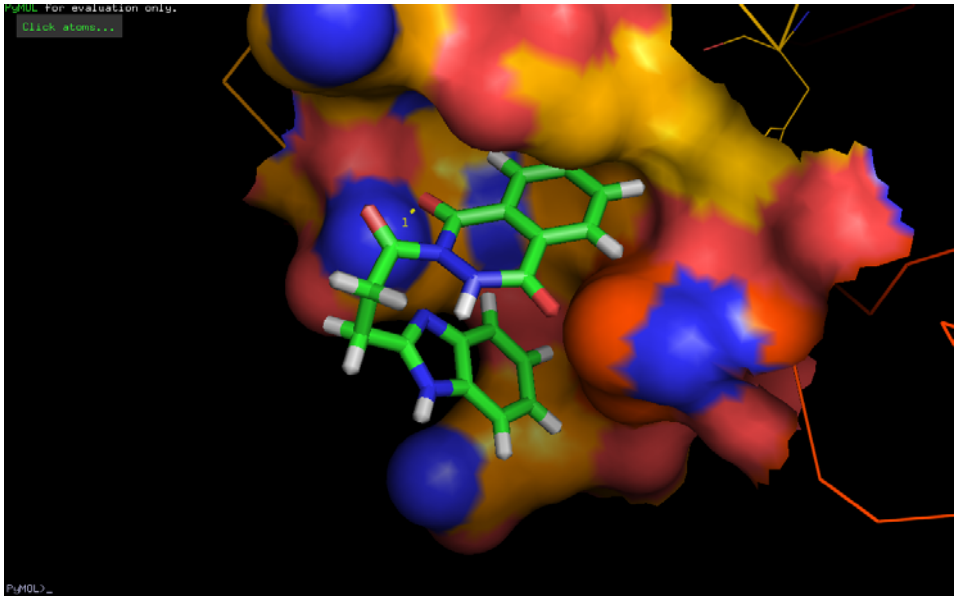
Ligand 5



Ligand 6

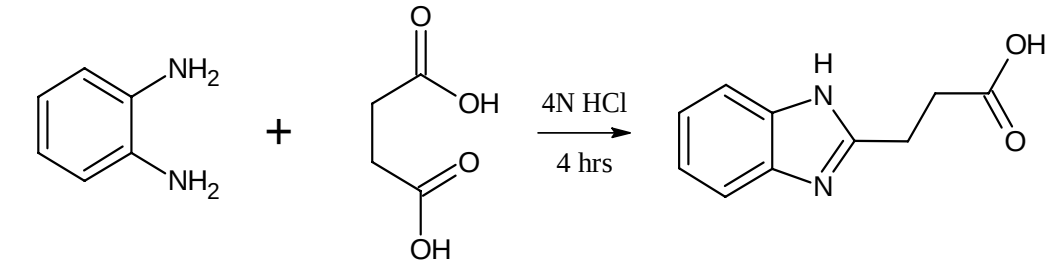


Ligand 7

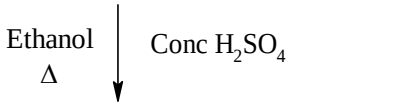


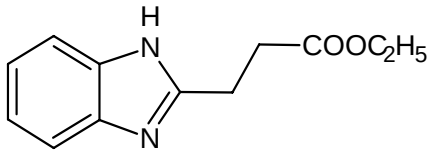
Ligand 8

SCHEME OF PRESENT WORK

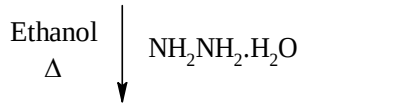


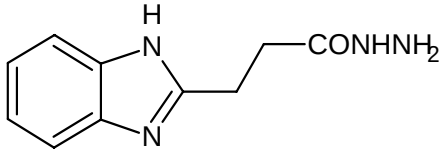
benzene-1,2-diamine butanedioic acid 3-(1*H*-benzimidazol-2-yl)propanoic acid





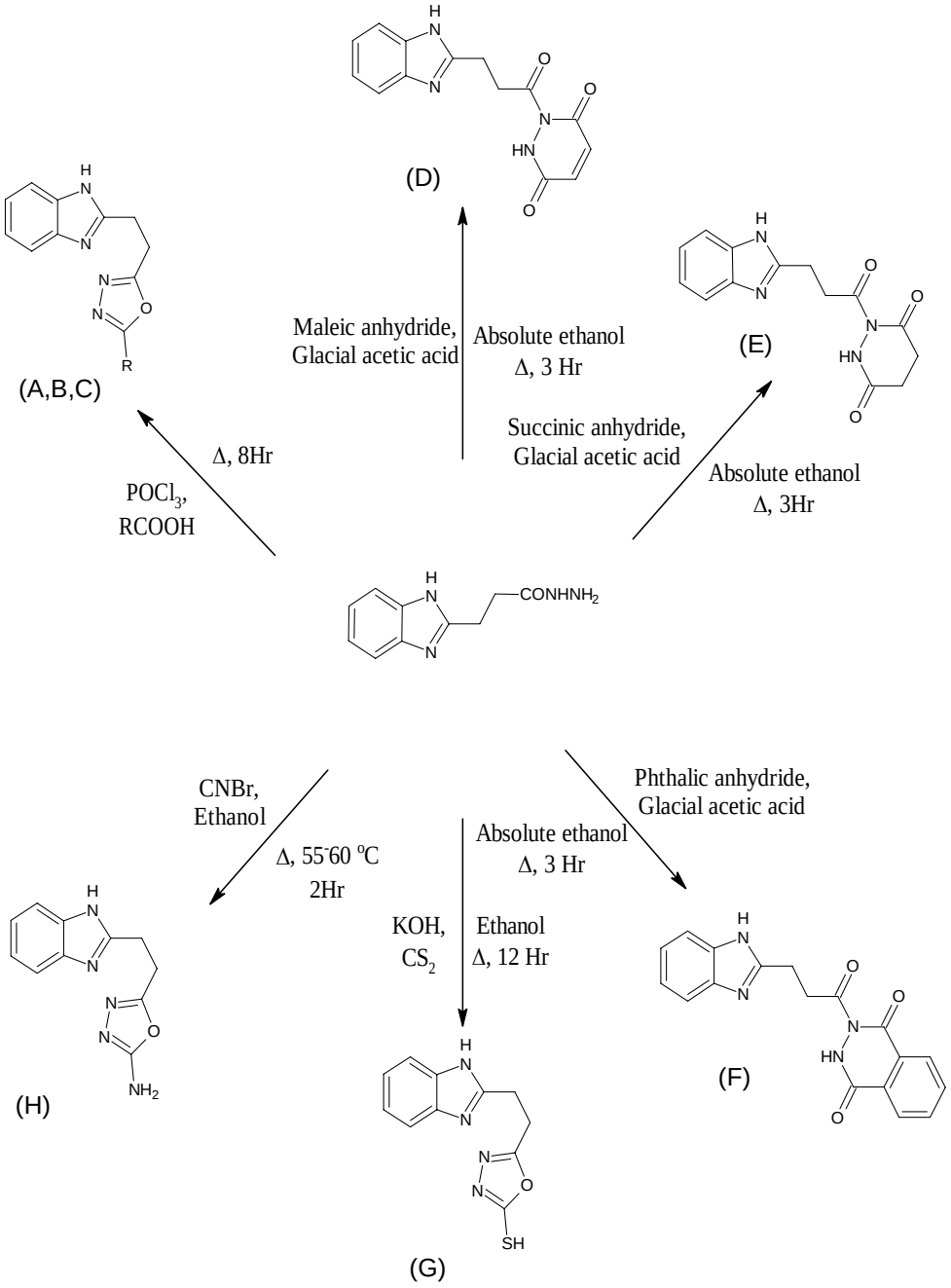
ethyl 3-(1*H*-benzimidazol-2-yl)propanoate





3-(1*H*-benzimidazol-2-yl)propanehydrazide

Cont…..



R=P-Nitro phenyl (A), P-Chloro phenyl(B), 3,5-Dimethoxy phenyl (C)

EXPERIMENTAL WORK

Chemicals and instruments

1. *o*-Phenylene diamine
2. Succininc acid
3. HCl
4. Absolute ethanol
5. Conc.H_2SO_4
6. Hydrazine hydrate
7. Conc.Ammonia solution
8. Sodium bicarbonate
9. Methanol
10. Succinic anhydride
11. Maleic anhydride
12. Glacial acetic acid
13. Phthalic anhydride
14. Phosphorus oxychloride
15. 4-Nitro benzoicacid
16. 4-Chloro benzoicacid
17. 3,5-Dimethoxy benzoicacid
18. Potassium hydroxide
19. Carbon disulphide
20. Cyanogen bromide
21. Chloroform
22. Benzene
23. Silica gel G
24. DMF
25. DMSO

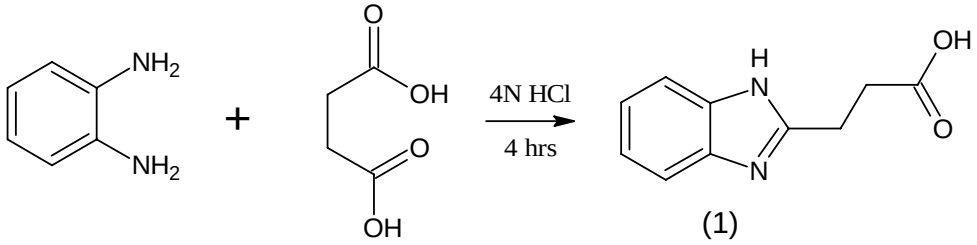
All the above chemicals and reagents used were analytical grade.

Instruments	**Model**
Melting point apparatus	VEEGO-VMPT (Silicon oil bath)
FT-IR spectrometer	Perkin elmer
NMR spectrometer	Bruker-Ultra shield (300MHz)

SYNTHESIS OF VARIOUS PYRIDAZINE, PHTHALAZINE, 1,3,4-OXADIAZOLE DERIVATIVES OF BENZIMIDAZOLE

Synthesis of 3-(1*H*-benzimidazol-2-yl)propanoic acid (1)

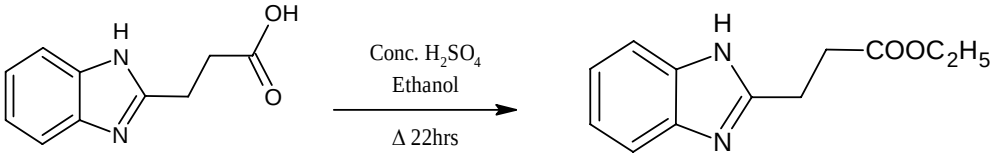
o-Phenylenediamine (0.03 mol) and Succinic acid (0.09 mol) dissolved in 30 ml of HCl (4N) and the reaction mixture was refluxed for 4 hrs. Cooled the mixture and add Ammonia solution (Conc.) to increase the basicity. The solid thus precipitated out was filtered washed with cold water and recrystallized from absolute ethanol.



(1)

Synthesis of ethyl 3-(1*H*-benzimidazol-2-yl) propanoate (2)

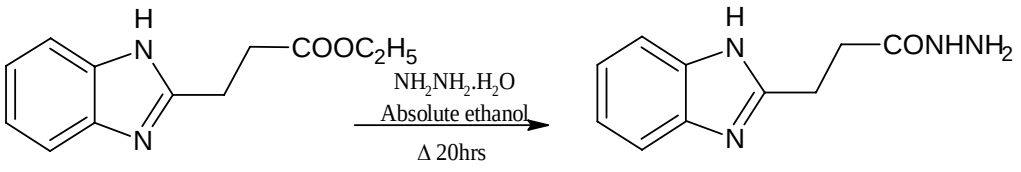
0.05 mol of 3-(1*H*-benzimidazol-2-yl) propanoic acid (1) in absolute ethanol (10ml), and Conc H_2SO_4 (1ml) was added. The reaction mixture was refluxed for 22hrs. Reaction mixture gave on processing ethyl ester (2). The solid obtained was washed with 50 ml of Sodium bicarbonate solution (10%) and recrystallized from methanol.



(2)

Synthesis of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3)

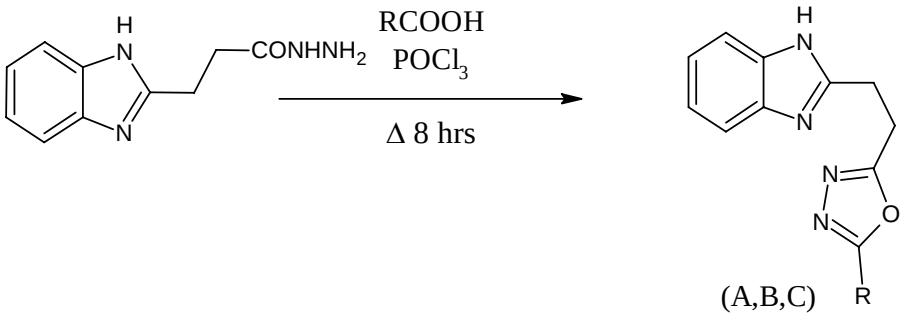
Compound 2 (0.01 mol) and Hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (50 ml) for 20 hrs. The mixture was concentrated, cooled and poured in ice cooled water. The solid thus precipitated out was filtered, dried and recrystallized from ethanol.



(3)

Synthesis of 2-[2-(5-substituted aryl-1,3,4-oxadiazol-2-yl)ethyl]-1*H*-benzimidazole (A, B, C)

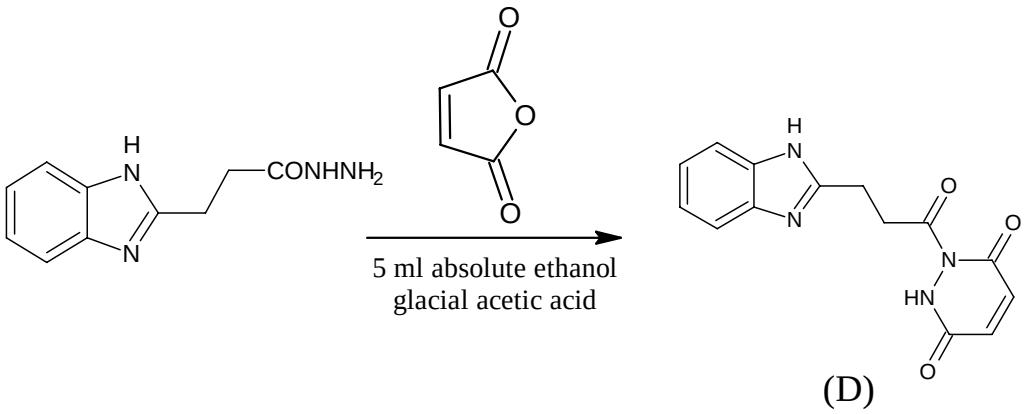
0.001 mol of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3) and appropriate aromatic acid (0.001 mol) was dissolved in phosphorus oxychloride and refluxed for 8 hrs. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol.



(A,B,C)

Synthesis of 1-[3-(1*H*-benzimidazol-2-yl) propanoyl]-1,2-dihydropyridazine-3,6-dione (D)

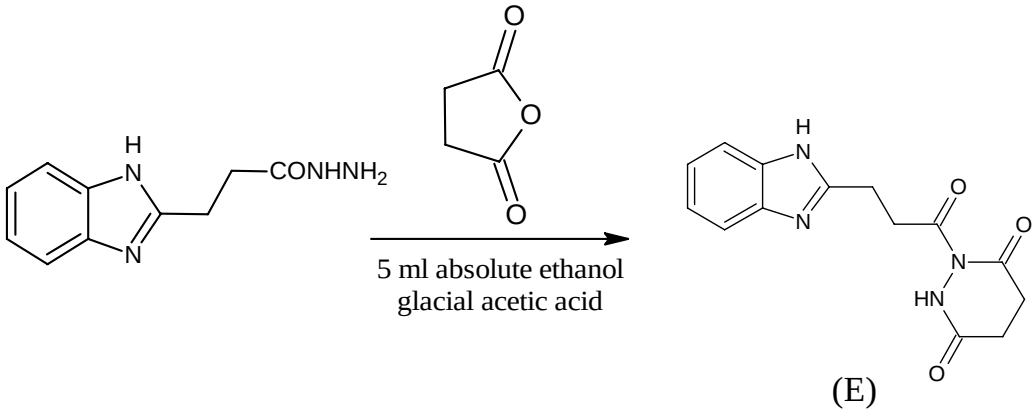
A mixture of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3) (0.001 mol) and maleic anhydride (0.001 mol) in 5 ml absolute ethanol, add glacial acetic acid (0.005 mol). The reaction mixture was refluxed for 3 hrs. It was then cooled and poured into crushed ice. The solid obtained was filtered, washed with 50 ml of sodium bicarbonate solution (50 ml) and recrystallized from ethanol.



(D)

Synthesis of 1-[3-(1*H*-benzimidazol-2-yl)propanoyl]tetrahydropyridazine-3,6-dione (E)

A mixture of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3) (0.001 mol), succinic anhydride (0.001 mol) in 5 ml absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hrs. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, washed with 50 ml of sodium bicarbonate solution (50 ml) and recrystallized from ethanol.

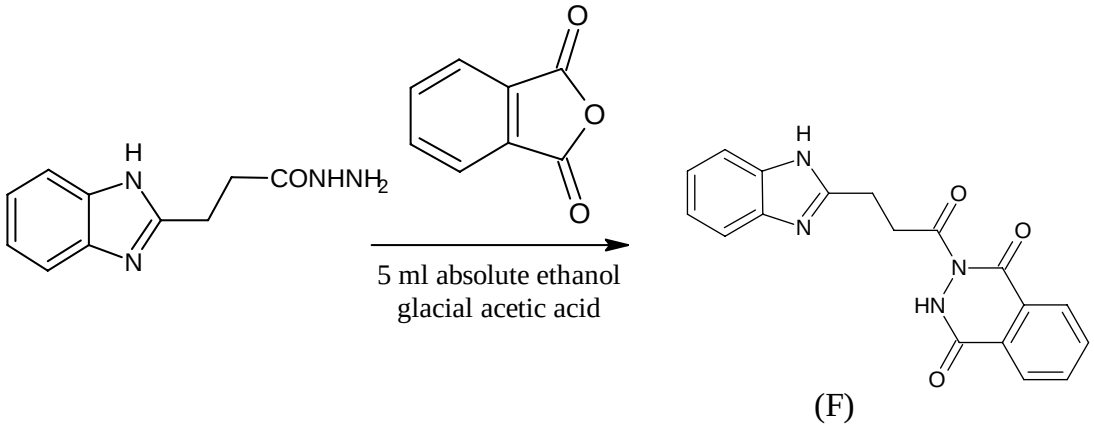


(E)

Synthesis of 2-[3-(1*H*-benzimidazol-2-yl)propanoyl]-2,3-dihydrophthalazine-1,4-dione (F)

A mixture of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3) (0.001 mol), phthalic anhydride (0.001 mol) in 5 ml absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hrs. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, washed

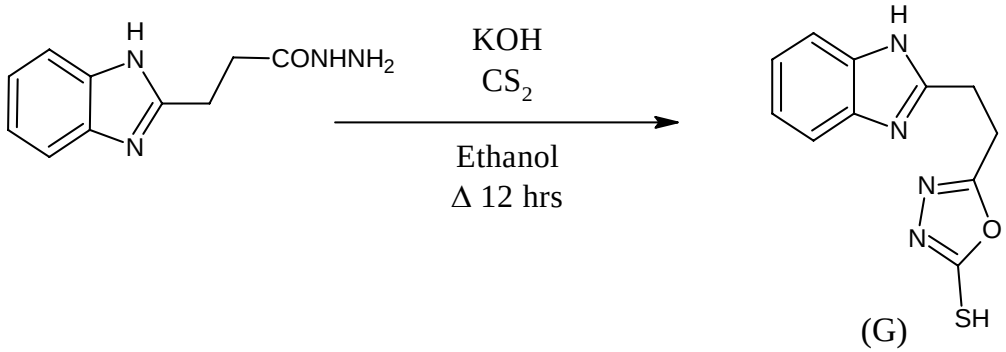
with 50 ml of sodium bicarbonate solution (50 ml) and recrystallized from ethanol.



(F)

Synthesis of 5-[2-(1*H*-benzimidazol-2-yl)ethyl]-1,3,4-oxadiazole-2-thiol (G)

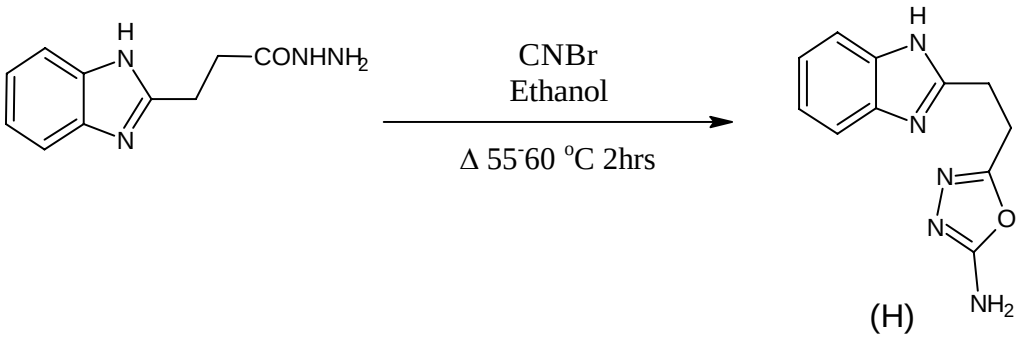
A mixture of 0.001 mol of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3), KOH (0.001 mol) and Carbon disulphide (5 ml) in ethanol (50 ml) was refluxed for 12 hrs. The solution was then concentrated, cooled and acidified with dilute HCl. The solid thus precipitated was filtered, washed with ethanol, dried and recrystallized from ethanol.



(G)

Synthesis of 5-[2-(1*H*-benzimidazol-2-yl)ethyl]-1,3,4-oxadiazol-2-amine (H)

To an ethanolic solution of 3-(1*H*-benzimidazol-2-yl)propanehydrazide (3) (0.001 mol), cyanogens bromide (0.001 mol) was added. The reaction mixture was stirred with heating at 55-60 °C for 2 hrs. The resulting solution was cooled and it was neutralized with sodium bicarbonate (10%) solution. The solid thus precipitated was filtered, washed with water, dried and recrystallized from methanol.



(H)

PHYSICAL CHARACTERISTICS

The synthesized compounds were characterized by the following physical properties.

THIN LAYER CHROMATOGRAPHY

The purity of the synthesized compounds was ascertained by TLC.

Absorbent used : Silica Gel G

Developing solvent : Benzene:Chloroform(9:1)

Detecting agent : Iodine vapour.

Colour of the spot : Brown (iodine vapour)

R_f values of synthesized compounds were calculated by the following formula,

R_f= (Distance travelled by the solute) / (Distance travelled by the solvent)

R_f values of the synthesized compounds were shown in the table No.4.

MELTING POINT

Melting point of the synthesized compounds was determined by open capillary tube method. The melting points of the synthesized compounds were shown in the table No.4.

SOLUBILITY

At room temperature solubility of the synthesized compounds were determined. The solubility of the synthesized compounds was shown in table No.4.

TABLE NO.3

PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

Compound code	**Molecular formula**	**Molecular weight**	**Colour**	**Nature**	**Percentage yield**
A	$C_{17}H_{13}N_5O_3$	335.32	Cream	Powder	95.34
B	$C_{17}H_{13}ClN_4O_3$	324.76	Pale white	Powder	94.13
C	$C_{19}H_{18}N_4O_3$	350.37	Grey	Crystalline	85.47
D	$C_{14}H_{12}N_4O_3$	284.27	Creamy white	Powder	80.21
E	$C_{14}H_{14}N_4O_3$	286.29	Pale white	Powder	69.52
F	$C_{18}H_{14}N_4O_3$	334.33	Pale white	Powder	86.43
G	$C_{11}H_{10}N_4OS$	246.29	Ash	Crystalline	78.53
H	$C_{11}H_{11}N_5O$	229.24	White	Powder	80.44

TABLE NO.4

PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

Compound code	**Solubility**					**Melting point °C**	**Rf value**
	$CHCl_3$	**DMF**	**DMSO**	**CH_3OH**	**C_2H_5OH**		
A	+	+	+	-+	-+	238-240	0.62
B	+	+	+	-+	-+	248-250	0.57
C	+	+	+	-+	-+	190-195	0.72
D	+	+	+	+	+	260-265	0.51
E	+	+	+	+	+	265-270	0.54
F	+	+	+	+	+	273-278	0.65
G	+	+	+	+	+	250-254	0.70
H	+	+	+	+	+	255-260	0.53

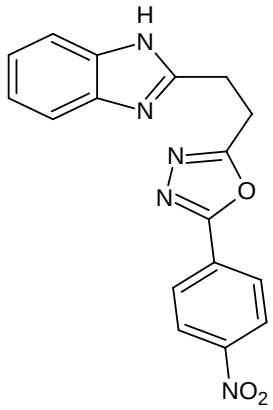
Where + Soluble

- Insoluble

-+ slightly soluble

INTERPRETATION OF SPECTRA

COMPOUND A



2-{2-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]ethyl}-1*H*-benzimidazole

IR Spectral data [14, 77]

KBr pellet

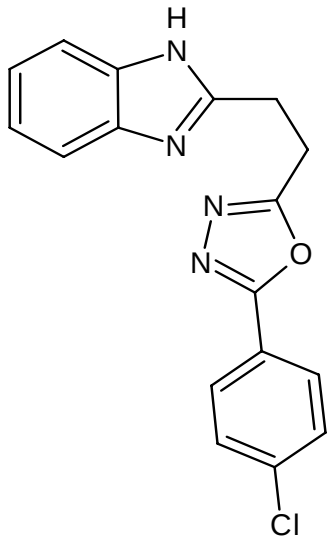
IR value (cm^{-1})	**Groups assigned**
2850.68, 2713.95	Alkyl C-H stretching
1700.67	Aromatic C=C stretching
717.05, 747.74, 857.17	Aromatic C-H bending
1522.57	N-H bending
1295.86, 1312.96, 1338.14	C-N stretching
1030.03	C-O-C stretching
1421.03, 1350.45	N=O stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
8.167-8.226	4H, 3,4,5,6Aryl protons
7.699-7.721	4H, 2,3,5,6Aryl protons
3.427	1H, NH protons
8.0167-1.234	4H, CH_2 protons

COMPOUND B



2-{2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]ethyl}-1*H*-benzimidazole

IR Spectral data [14, 77]

KBr Pellet

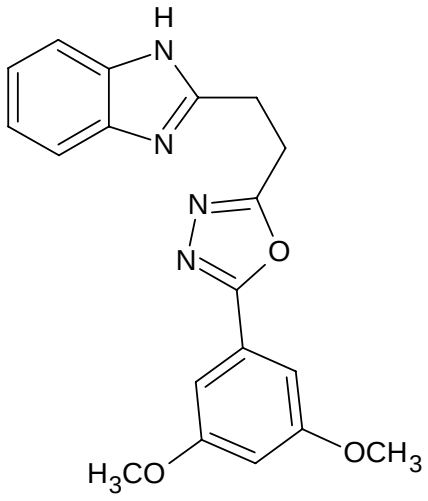
IR Value (cm^{-1})	**Groups assigned**
2842.48	Alkyl C-H Stretching
1592.68, 1425.96	Aromatic C=C Stretching
762.30, 853.03	Aromatic C-H Bending
1684.30	N-H Bending
1332.30	Aromatic C-N Stretching
1092.73	C-O-C Stretching
549.03	C-Cl Stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.934-7.955	4H, 3,4,5,6 Aryl protons
7.564-7.585	4H, 2,3,5,6 Aryl protons
2.513	1H, NH protons
0.828-1.236	4H, CH_2 protons

COMPOUND C



2-{2-[5-(3,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]ethyl}-1*H*-benzimidazole

IR Spectral data [14, 77]

KBr Pellet

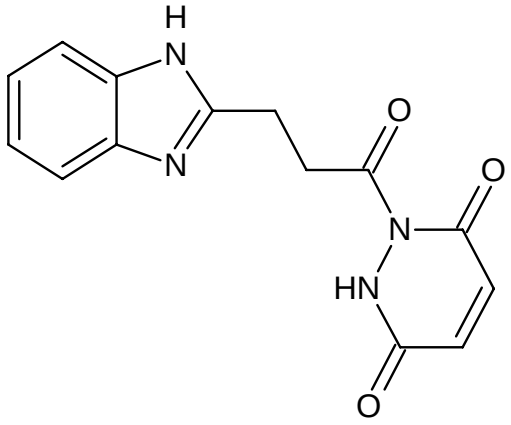
IR value (cm^{-1})	**Groups assigned**
2959.26	Alkyl C-H Stretching
1459.22	Aromatic C=C Stretching
768.01, 726.99	Aromatic C-H Bending
1351.99, 1308.93	C-N Stretching
1686.31	N-H bending
1048.67	C-O-C stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.056-7.061	4H, 3,4,5,6 Aryl protons
6.735-6.747	3H, Dimethoxy aryl protons
3.785	3H, OCH_3 protons
2.509	1H, NH protons
1.151-1.332	4H, CH_2 protons

COMPOUND D



1-[3-(1*H*-benzimidazol-2-yl)propanoyl]-1,2-dihydropyridazine-3,6-dione

IR Spectral data [14, 77]

KBr Pellet

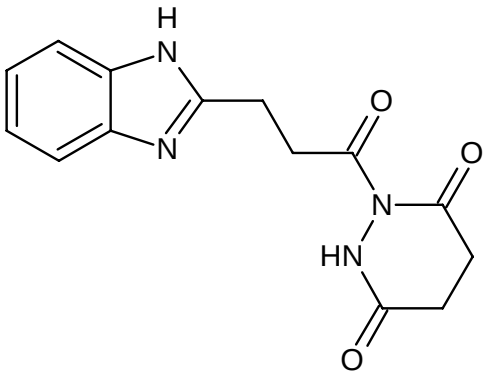
IR Value (cm^{-1})	**Group assigned**
2928.13	Alkyl C-H Stretching
1431.98	Aromatic C=C Stretching
748.43	Aromatic C-H Bending
1599.50	C=O Stretching
1222.37	Alkyl C-N stretching
1275.66	Aromatic C-N Stretching
3781.31	N-H Stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.108-7.122	4H, 3,4,5,6 Aryl protons
7.463	1H, CONH protons
3.412	1H, NH protons
1.237-2.509	2H, CH_2CO protons
0.828-0.856	4H, CH_2 protons

COMPOUND E



1-[3-(1*H*-benzimidazol-2-yl)propanoyl]tetrahydropyridazine-3,6-dione

IR Spectral data [14, 77]

KBr pellet

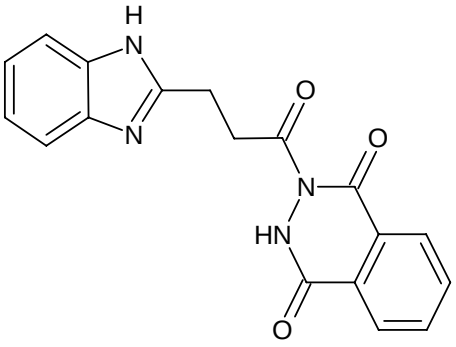
IR value (cm-1)	Groups assigned
2671.73	Alkyl C-H Stretching
1437.72	Aromatic C=C Stretching
749.55	Aromatic C-H Bending
1609.74	C=O Stretching
1222.85, 1152.40	Alkyl C-N Stretching
1276.29, 1320.13	Aromatic C-N Stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.103-7.125	4H, 3,4,5,6 Aryl protons
7.469	1H, CONH protons
3.412	1H, NH protons
2.509	2H, $COCH_2$ protons
0.742-1.236	2H, CH_2 protons

COMPOUND F



2-[3-(1*H*-benzimidazol-2-yl)propanoyl]-2,3-dihydrophthalazine-1,4-dione

IR Spectral data [14, 77]

KBr pellet

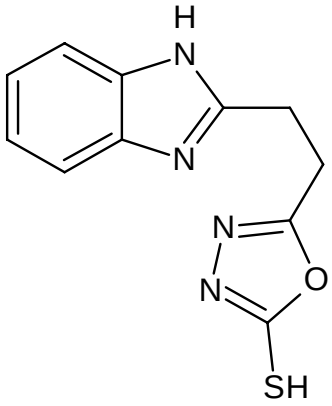
IR value (cm-1)	Group assigned
2851.96, 2921.13	Alkyl C-H Stretching
3052.00	Aromatic C-H Stretching
740.36, 749.81	Aromatic C-H Bending
1450.38, 1436.07	Aromatic C=C Stretching
1668.52	C=O Stretching
1154.42, 1222.74	Alkyl C-N Stretching
1319.75	Aromatic C-N Stretching
3229.21	N-H Stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.461-7.484	4H, Phthalazine protons
7.451	1H, CONH protons
7.098-7.126	4H, 3,4,5,6 Aryl protons
3.424	1H, NH proton
2.509	2H, $COCH_2$ protons
1.322	2H, CH_2 protons

COMPOUND G



5-[2-(1*H*-benzimidazol-2-yl)ethyl]-1,3,4-oxadiazole-2-thiol

IR Spectral data [14, 77]

KBr pellet

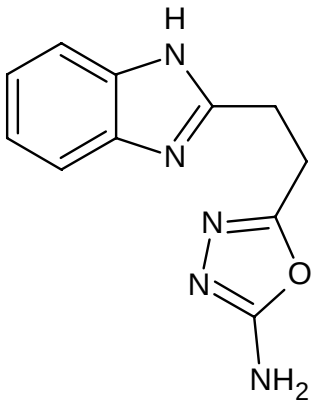
IR value (cm^{-1})	**Groups assigned**
2921.33, 2854.88	Alkyl C-H Stretching
1570.23, 1497.07	Aromatic C=C Stretching
775.09, 759.09	Aromatic C-H Stretching
1277.78, 1301.02, 1326.99	C-N Stretching
3435.65	N-H Stretching
1627.53	N-H Bending
1069.54	C-O-C Stretching
2732.37	S-H

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.484-7.507	4H, 3,4,5,6 Aryl protons
3.444	1H, NH proton
2.509	1H, SH proton
0.834-1.040	2H, CH_2 protons

COMPOUND H



5-[2-(1*H*-benzimidazol-2-yl)ethyl]-1,3,4-oxadiazol-2-amine

IR Spectral data [14, 77]

KBr pellet

IR value (cm^{-1})	**Group assigned**
1451.38, 1435.74	Aromatic C=C Stretching
739.35, 848.52	Aromatic C-H bending
1275.62, 1223.11	C-N Stretching
3274.08,	N-H Stretching
1543.28, 1654.64	N-H bending
1031.67	C-O-C Stretching

NMR Data [14, 77]

DMSO

δ Value (ppm)	**Groups assigned**
7.087-7.108	4H, 3,4,5,6 Aryl protons
3,434	1H, NH proton
2.509	2H, CH_2 protons
1.637	2H, NH_2 protons

ANALGESIC ACTIVITY

Analgesic activity of various synthetic drugs was evaluated by acetic acid induced writhing reflux in mice. Painful reaction in animals may be produced by the chemicals such as phenylquinone, bradykinin etc. Like that, acetic acid pain reaction which is characterized as a writhing response. Construction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to chemically induced pain. Analgesics (both narcotic and non-narcotic) inhibit writhing response.

REQUIREMENTS:

Animal : Swiss albino mice (20-25g) either sex

Drugs and chemicals : Diclofenac sodium (standard),

Acetic acid (1%v/v), various synthetic compounds such as A to H

METHOD:

TREATMENT PROTOCOL

Group-1 Treated as normal control received 10ml/kg of normal saline through orally.

Group-2 Treated as Standard control received 10mg/kg of diclofenac sodium through Intraperitonially.

Group-3 Treated as treatment control received 10mg/kg of synthetic drug comp-A dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-4 Treated as treatment control received 10mg/kg of synthetic drug comp-B dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-5 Treated as treatment control received 10mg/kg of synthetic drug comp-C dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-6 Treated as treatment control received 10mg/kg of synthetic drug comp-D dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-7 Treated as treatment control received 10mg/kg of synthetic drug comp-E dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-8 Treated as treatment control received 10mg/kg of synthetic drug comp-F dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-9 Treated as treatment control received 10mg/kg of synthetic drug comp-G dissolved with 0.5ml of DMSO administered through intraperitonially.

Group-10 Treated as treatment control received 10mg/kg of synthetic drug comp-H dissolved with 0.5ml of DMSO administered through intraperitonially.

All the synthetic drugs were administered half hour prior to the acetic acid administration. Note the onset on writhing. Record the numbers of abdominal contractions, trunk twist and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes were noted.

STATISTICS:

Data are expressed as mean ± SEM; data analyzed by one way ANOVA followed by Newman's keul's multiple range tests to determine the significance of the difference between the control group and rats treated with the extracts.

* Values were considered significant at $P< 0.01$.

TABLE No.5

ANALGESIC ACTIVITY OF VARIOUS SYNTHETIC DRUGS

Treatment	**Dose (mg/kg)**	**No. of writhing**	**% reduction in reaction time**
Group I Normal control	Inject 1%v/v acetic acid 1ml/100g of body weight	37.0±3.2	-
Group II Std control	10mg/kg I.P.Diclofenac sodium	6.0±0.8	83.78%*
Group III Treatment control	10mg/kg comp-A through I.P.	13.2±1.68	64.32%*
Group IV Treatment control	10mg/kg comp-B through I.P.	12.6±1.24	65.94%*
Group V Treatment control	10mg/kg comp-C through I.P.	13.2±1.68	64.32%*
Group VI Treatment control	10mg/kg comp-D through I.P.	13.4±1.98	63.78%*
Group VII Treatment control	10mg/kg comp-E through I.P.	12.9±1.62	65.13%*
Group VIII Treatment control	10mg/kg comp-F through I.P.	12.9±1.62	65.13%*
Group IX Treatment control	10mg/kg comp-G through I.P.	14.0±2.06	62.16%*
Group X Treatment control	10mg/kg comp-H through I.P.	14.05±2.26	62.02%*

Values are expressed as mean±SEM

Values were find out by using one-way ANOVA followed by Newman's keuls multiple range tests.

* Values were considered significant at P< 0.01.

RESULTS

The table values show that analgesic activity of various synthetic compound such as comp A – H by acetic acid induced writhing reflex. The results reveals that all synthetic drugs possess significant analgesic activity at p<0.01.

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activities of synthetic drugs at a dose of 10 mg/kg doses were evaluated using carrageenan-induced paw edema method. The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and edema.

REQUIREMENTS:

Animal : Albino rat (180-200 g)

Drugs and chemicals : Carrageenan (1%w/v), Diclofenac sodium (standard), DMSO

Digital plethysmo meter. U G O Basile (Italy)

Test compounds : Synthetic drugs such as A to H.

METHOD:

The animals were divided into 10 groups each having six animals. A freshly prepared suspension of carrageenan (1% w/v, 0.1 ml) was injected to the planter region of left hind paw of each rat. One group was kept as control and the animals of the other groups were pretreated with Synthetic drugs such as A to H test Compounds dissolved with 0.5 ml DMSO administered through intraperitonially 30 min before the carrageenan treatment. The paw volumes of the test compounds, standard and control groups were measured at 60,240,360 minutes of carrageenan treatment with the help of Digital plethysmometer (Ugo basile, Italy). Mean increase in paw volume was measured and the percentage of inhibition was calculated.

% Anti-inflammatory activity = (Vc-Vt / Vc) x 100

Where, ***Vt***- means increase in paw volume in rats treated with test compounds,

Vc- means increase in paw volume in control group of rats.

TABLE No.6

ANTI-INFLAMMATORY ACTIVITY OF SYNTHETIC DRUGS

Treatment	**Dose (mg/kg)**	**Paw volume(ml) as measured by mercury displacement at 6 hour**	**Percentage inhibition of paw edema**
Group I Normal saline	10ml/kg orally	5.90±0.90	-
Group II Std	10mg/kg I.P.Diclofenac sodium	1.72±0.43	70.84%*a
Group III (A)	10mg/kg.i.p.	2.50±0.42	57.62%*a
Group IV (B)	10mg/kg.i.p.	2.08±0.28	64.74%*a
Group V (C)	10mg/kg.i.p.	2.10±0.33	64.40%*a
Group VI (D)	10mg/kg.i.p.	2.42±0.38	58.98%*a
Group VII (E)	10mg/kg.i.p.	2.10±0.33	64.40%*a
Group VIII (F)	10mg/kg.i.p.	2.04±0.30	65.42%*a
Group IX (G)	10mg/kg.i.p.	2.60±0.48	55.93%*a
Group X (H)	10mg/kg.i.p.	2.64±0.50	55.25%*a

* Data are expressed as Mean ± S.E.M.

*Data were analyzed by one way ANOVA followed by Newman's keul's multiple range tests, to determine the significance of the difference between the control group and rats treated with the test compounds.

*a Values were significantly different from normal control at P< 0.01.

RESULTS

Various synthetic drugs such as A to H at a dose of 10mg/kg were tested for their Anti- inflammatory activity by using carrageenan Induced rat paw edema method and the results are tabulated in table no 1. The results reveals that synthetic drugs A to H at 10mg/kg doses possesses significant Anti- inflammatory activity when compared to control group at p<0.01.

ANTIMICROBIAL STUDIES

Organism used

Gram +ve microorganism : Staphylococcus aureus

Gram –ve microorganism : Pseudomonas aurogenosa

Fungi microbial : Candida albicans

Media used

Muller-Hinton agar media

Saboured Dextrose Agar media

Standard used

Amikacin disc

Ketoconazole

Method

Filter paper disc method

Principle

Gel diffusion

ANTIBACTERIAL ACTIVITY

They have relatively broad spectrum of action and are effective against Gram positive and Gram negative organism.

Bacteria

Bacteria are unicellular microscopic prokaryotic organism lacking chlorophyll. They are found in animals, plant, soil, water, atmosphere and dead organic matters. Bacteria generally vary in size from 0.5µ to 3µ.

Infection

Infection can involve in any organs or system in body. The present trend is to refer to all disease caused by living micro organism as infectious diseases.

- Bacteristatic if it inhibits the growth of bacteria
- Bactericidal if it destroys and kills the bacteria

Treatment

Chemotherapy is defined as the treatment of specific infections with chemical agents so as to destroy offending micro organism or parasites without damaging the caused tissues.

Mechanism of action of antibacterial agents

- Interference with cell wall synthesis
- Damage to the cytoplasmic membrane
- Inhibition of protein synthesis and impairment of function of the ribosome
- Interference with translation of genetic information
- Inhibition of viral enzymes

Experimental

Purpose and rationale

The in vitro antibacterial activities of the various synthesized benzimidazole derivatives were evaluated against staphylococcus aureus and pseudomonas aurogenosa.

Requirements

- Petri dish (sterilized)
- Muller-Hinton agar media (sterilized)
- Amikacin disc (standard)
- Dimethyl sulphoxide (control)
- Synthesized compound 1-8 (A-H)

Organism used

Gram +ve microorganism

Staphylococcus aureus

Gram –ve microorganism

Pseudomonas aurogenosa

Method: Filter paper disc method

Principle: Gel diffusion

Composition of media: Muller-Hinton agar media

Beef extract	30gm
Peptone	17.5gm
Starch	1.5gm
Agar	17gm
Sodium hydroxide	5gm
Distilled water	1000ml
Final pH at 25°C	7.4±0.2

The beef extract, peptone, starch and agar were taken in the above proportions and dissolved up to 100ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of the medium was adjusted to 7.4 using sodium hydroxide. The pH tested using a universal

indicator paper, which showed green colour at pH 7.4, and then it was transferred to boiling tubes in hot conditions and sealed with non absorbent cotton and sterilized by autoclaving at 121°C (15lbs pressure) for 15 min, poured aseptically in to sterile Petri dishes.

Conditions of the work

The entire work was done using horizontal laminar flow cabinet so as to provide aseptic condition. Before commencement of the work, air sampling was carried using a sterile Mull- Hinton agar plate and exposing in to the environment inside the cabinet. On incubation it was checked for the growth of micro organism and absence of growth confirmed the aseptic working conditions.

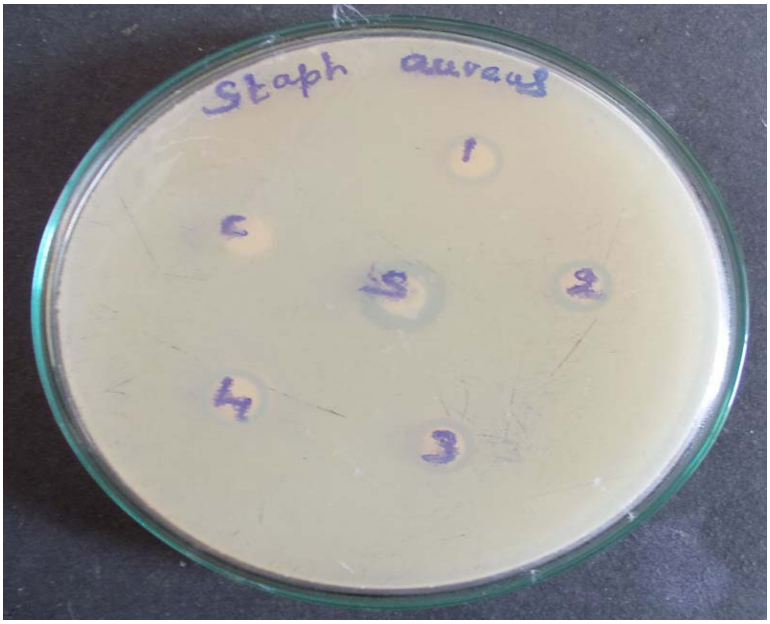
Inoculation of microorganism

The sterilized Muller-Hinton agar media was heated on a water bath to melt the media. When the media was luke warm, the organism was inoculated separately and poured aseptically into sterile petridishes and allowed to solidify. The standard drug Amikacin disc was placed on the media and the Whatmann No.2 filter disc (5mm diameter) were cut and filled into vials plugged with cotton. These vials were kept in hot air oven at 160°C for 30 min for sterilization. Then it was soaked in synthesized compounds separately and evaporated to dryness and kept on the media (5mm height). One more disc immersed in dimethyl sulphoxide and kept on the media as control. It was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug and later kept in the incubator for a period of 24 hours at 37°C. Observations were made for the zone of inhibition around the synthesized compounds with that of standard.

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST STAPHYLOCOCCUS AUREUS

Standard- Amikacin Control- DMSO

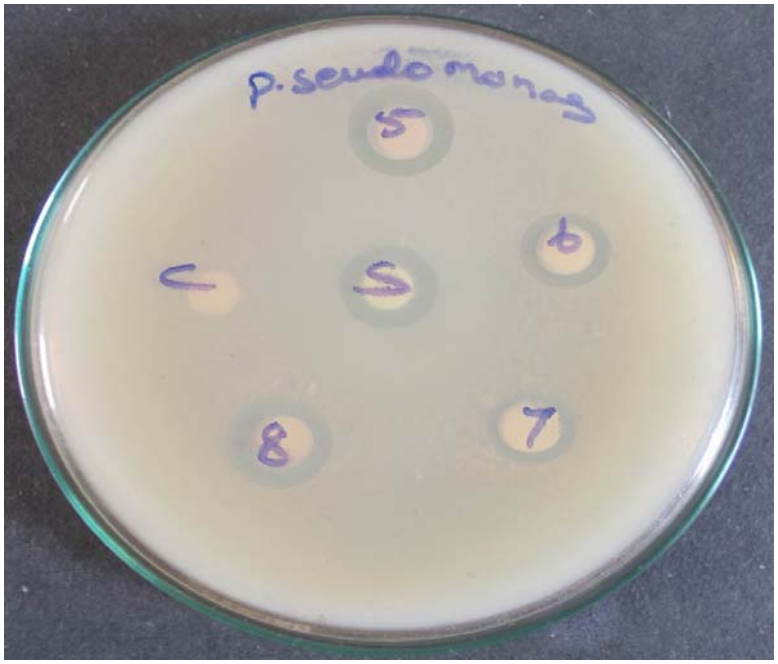
Fig 7



ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST PSEUDOMONAS AUROGENOSA

Standard- Amikacin Control- DMSO

Fig 8



ANTIBACTERIAL ACTIVITY OF VARIOUS SYNTHESIZED COMPOUNDS

Table No 7.

Activities against G +ve and G –ve organisms

Compounds	**Zone of inhibition**	
Code	**G +ve S.aureus in mm (%)**	**G –ve P.aurogenosa in mm (%)**
1 (A)	**10**	**15**
2 (B)	**12**	**10**
3 (C)	**11**	**10**
4 (D)	**11**	**13**
5 (E)	**14**	**16**
6 (F)	**18**	**12**
7 (G)	**15**	**11**
8 (H)	**17**	**17**
Control	**Resistant**	**Resistant**
Standard	**17**	**16**

Standard : Amikacin

Control : DMSO

ANTIFUNGAL ACTIVITY

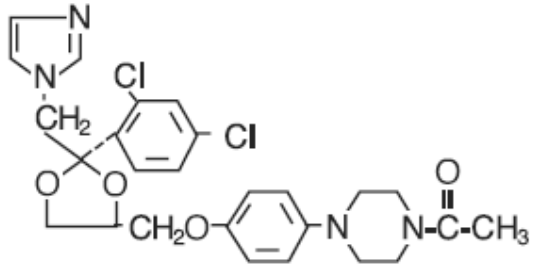
INTRODUCTION

Fungi are plant like non photosynthetic eukaryotes growing either in colony of single cell (yeast) or in filamentous multi-cellular aggregates (molds). Most fungi live as Saprophytes in soil or dead plant materials and they are very important in the mineralization of organic matter. Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells.

The azole antifungal drugs inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth.

Treatment

The standard drug used is Ketoconazole.



Ketoconazole is usually prescribed for topical infections such as athlete's foot, ringworm, candidiasis (yeast infection or thrush), and jock itch.

Experiment

Purpose and rationale

The in-vitro antifungal activity of the various synthesized benzimidazole derivatives were evaluated on Candida albicans.

Requirement

- Petri-dishes (sterilized)
- Sabouraud Dextrose Agar media (sterilized)
- Ketoconazole (standard)
- DMSO (control)
- Synthesized compounds

Organisms used

Candida albicans

Method

Filter paper disc method

Principle

Gel diffusion

Composition of media

Saboured Dextrose Agar media

Glucose	-40gm
Peptone	-10gm
Agar	-15gm
Distilled water up to	-1000ml
Final pH	-5.4

Procedure

Glucose, peptone and agar were taken in the above proportions and dissolved up to 1000ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of the media was adjusted to 5.4. Then it was transformed to boiling tube in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes then poured aseptically in to sterile Petri-dishes.

Microorganisms- Candida albicans

Candida albicans is seldom isolated outside the bodies of animals and is known from 58 species including wild and domestic mammals and birds. Candiasis, the disease caused by C.albicans. The cell wall polysaccharides mannan and mannan protein complexes are involved in several different interaction and mannan structure types of Candida albicans.

Working conditions

The entire work was done using horizontal laminar flow cabinet so as to provide aseptic condition before commencement of work, a sampling was carried out using a sterile Muller-Hinton agar plate and exposing it to the environment inside the cabinet. On incubation it was checked for the growth of microorganism and absence of growth confirmed the aseptic working condition

Inoculation of microorganism

For the screening of antifungal activity disc diffusion method was used. Sabouraud dextrose agar plate were prepared aseptically to get a thickness of 5-6mm. the plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°C just before inoculation.

From the solid culture the clinical sample of Candida albicans were inoculated in to Sabouraud dextrose agar plates by using sterile inoculation loop and agar plates were incubated for about 24 hours, at 37°C, which show significant growth of fungi.

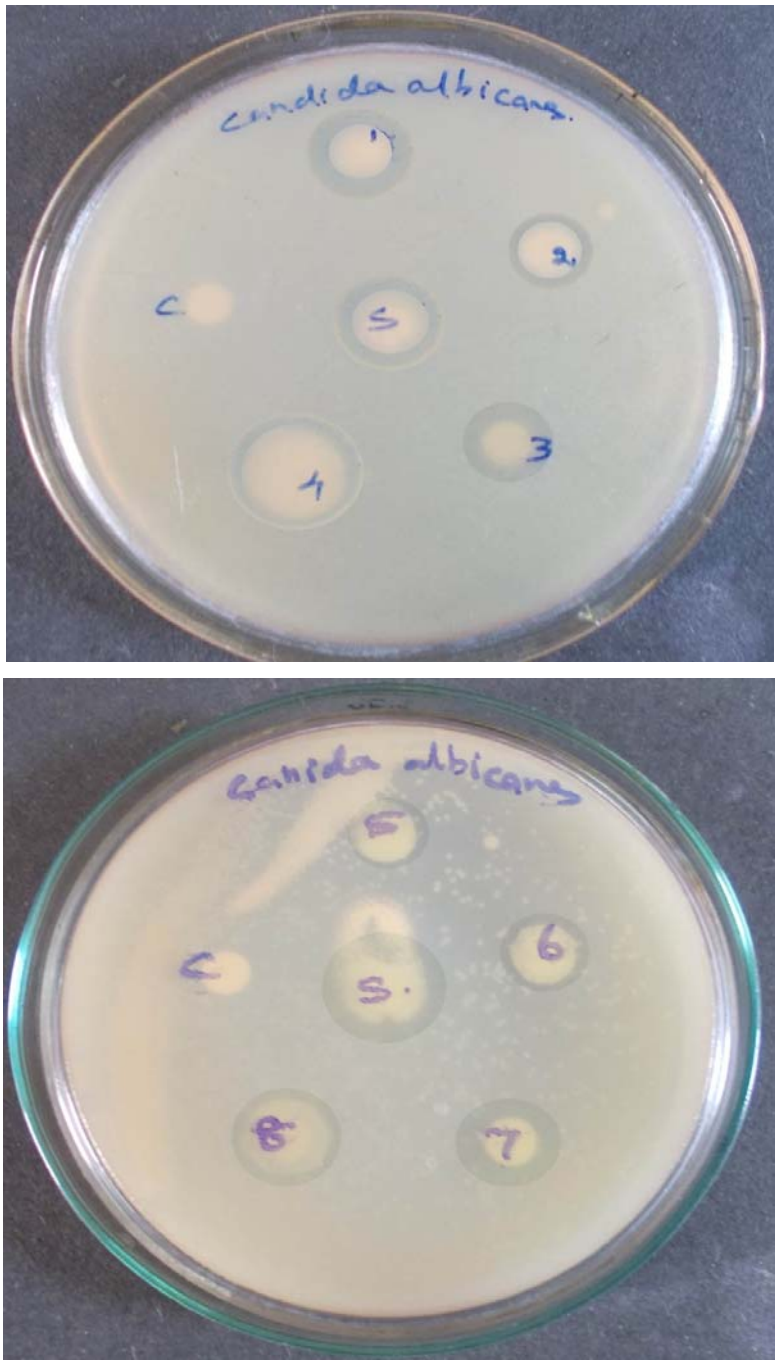
The temperature of the medium should not exceed about 50°C when the organisms were inoculated. The standard drug Ketoconazole (10µgm/disc) was placed on the media. The sterile Whatmann No.2 filter paper disc (5mm diameter) was soaked in synthesized compound (20µgm/disc) separately and evaporated to dryness and then kept on the media. One more disc immersed in dimethyl sulphoxide and kept on the media as control. The Petri dishes were incubated at 37°C for 24 hours, after placing them in refrigerator for 1 hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the synthesized compound and with that of standard.

ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS

AGAINST CANDIDA ALBICANS

Standard- Ketoconazole Control- DMSO

Fig 9



ANTIFUNGAL ACTIVITY OF VARIOUS SYNTHESIZED COMPOUNDS

Table No8

Microorganism used –Candida albicans

Compounds code	Zone of inhibition in mm
1 (A)	14
2 (B)	12
3 (C)	15
4 (D)	11
5 (E)	10
6 (F)	12
7 (G)	16
8 (H)	12
Control	Resistant
Standard	15 mm

Standard –Ketoconazole

Control – DMSO

RESULTS AND DISCUSSION

i. Molecular docking studies

Various ligands were designed by replacing the carboxylic acid group present in the prepared 3-(1H-benzimidazole-2-yl) propeonic acid and its binding affinity in COX-2 and 14 α-demethylase were determined with the help of molecular docking studies using Argus Lab program. The binding scores of designed ligands 1-8 with COX-2 and 14 α-demethylase enzymes ranging from -12.8915 to -9.37935 Kcal/Mol and -8.91847 to -6.81676 Kcal/Mol respectively (see table no) and the binding modes of these ligands with COX-2 and 14 α-demethylase enzymes are given in Fig . These data clearly indicates their potency as Cyclooxygenase inhibitor and 14 α-demethylase inhibitor. Almost all the designed ligands showed good interaction energy.

ii. Characterization of synthesized compounds

The selected ligands 1-8 which showed good interaction energy were designed and then characterized by various methods such as Infra red Spectrometry (IR), and Nuclear Magnetic Resonance (NMR) and its reports were in complete arrangement with their chemical structure. The purity of the synthesized compounds was further established by chromatographic methods (TLC).

iii. Pharmacological studies

The pharmacological screening of these derivatives were carried out according to standard procedure and compared with standard.

a) Analgesic activity

All the synthesized compounds were tested for their analgesic activity by using acetic acid induced writhing reflux in mice and the results are tabulated in table no.5. The results reveals that all the compounds possess significant analgesic activity when compared to control group at $p<0.01$.

b) Anti-inflammatory activity

All the synthesized compounds were also tested for their anti-inflammatory activity by using carrageenan induced rat paw edema method and the results are tabulated in table no.6. The results reveals that the synthetic drugs A to H at 10mg/kg doses possesses significant Anti- inflammatory activity when compared to control group at $p<0.01$.

c) Antimicrobial activity

All the synthesized compounds were tested for antimicrobial activity like antibacterial and antifungal activity. Antibacterial activity was evaluated against Streptococcus aureus (G +ve) and Pseudomonas aerogenosa (G –ve). Antifungal activity was evaluated against Candida albicans. The zone of inhibition was measured as the parameter of activity.

Staphylococcus aureus (G+ve) - Antibacterial activity was evaluated against Staphylococcus aureus and the zone of inhibition was measured. Amikacin, the standard showed a zone of inhibition of 17 mm. Out of 8 synthesized compounds three (F and H) showed high degree of activity and two (E and G) showed considerable activity and the remaining four compounds showed moderate activity when compared with standard. The results are tabulated in table no.7.

Pseudomonas aerogenosa- Antibacterial activity was evaluated against Pseudomonas aerogenosa and the zone of inhibition was measured. Amikacin, the standard showed a zone of inhibition of 16 mm. Out of 8 synthesized compounds three (A, E, H) showed high degree of activity and two (D and F) showed considerable activity and the remaining compounds showed moderate activity when compared with standard. The results are tabulated in table no 7.

Candida albicans- Antifungal activity was evaluated against Candida albicans and the zone of inhibition was measured. Ketoconazole, the standard showed a zone of inhibition of 15 mm. Out of 8 synthesized compounds three (A, C, G) showed high degree of activity and

remaining five showed moderate activity when compared with the standard. The results are tabulated in table no 8.

CONCLUSION

Various pyridazine-3,6-dione, 2,3-dihydrophthalazine-1,4-dione, 1,3,4-oxadiazole derivatives of 3-(1*H*-benzimidazol-2-yl)propanoic acid were designed and synthesized with the aim of developing better analgesic anti-inflammatory molecules with antimicrobial activity.

The results generated in this study leads to the following conclusions.

- Molecular docking studies reveal that all the designed ligands 1-8 possess very good binding ability in COX-2 and 14 α-demethylase enzymes.
- All the test compounds exhibit significant analgesic activities against acetic acid induced writhing reflux.
- All the test compounds exhibit significant anti-inflammatory activity in carrageenan induced rat paw edema method.
- The test compounds A, F, E, H showed high degree of antibacterial activity when compared with standard Amikacin and test compounds A, C, G showed high degree of antifungal activity against when compared with standard Ketoconazole.

The result obtained support the statement that newly synthesized heterocyclic analogues of benzimidazole can be considered as good analgesic anti-inflammatory agent with antimicrobial activity, however further structural modification are planned to increase the activity of this series to obtain a clinically useful antimicrobial, analgesic and anti-inflammatory agent.