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Research Article

DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF CERTAIN NOVEL IMIDAZOLE DERIVATIVES

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ABSTRACT

Imidazole and its derivatives occupied a unique position in the field of medicinal chemistry and pharmacology, thus the synthesis of novel imidazole derivatives plays an important role in the synthetic strategy of drug design and discovery. Imidazole derivatives shows various biological activities such as anti-microbial, anti-inflammatory activity, analgesic activity, anti-tubercular activity, anti-depressant activity, anti-cancer activity, anti-viral activity, and antileishmanial activity etc,. Debus radziswiski imidazole synthesis is the synthesis of an imidazole derivative by the condensation of an 1,2-dicarbonyl compound, an aldehyde, and two equivalents of dry ammonia in alcohol. The main objective of this study, derivatives of 2,3,4-triphenyl-3,4-dihydroimidazo[4,5-*b*]indole were designed, synthesized and their biological activities such as anti microbial and anti diabetic activity. The structure elucidation of the compounds was performed by IR, 1 H-NMR and mass spectroscopic data.

KEYWORDS: Debus radziswki reaction, imidazole, 1,2 dicarbonyl compound, condensation

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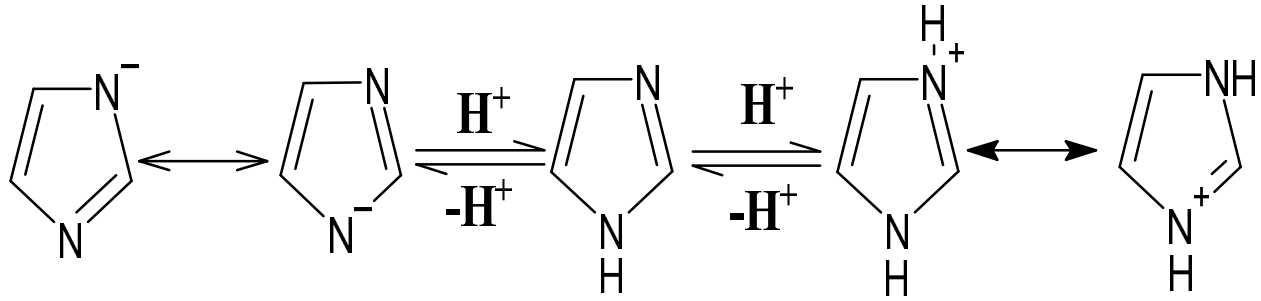
INTRODUCTION

Imidazole is a 5-membered aromatic ring with two nitrogen atoms at 1st and 3rd positions. The solubility of Imidazole is found to be good in polar solvents and water. Due to the presence of hydrogen atoms on either of the two nitrogen atoms in the imidazole ring, it exists in two equivalent tautomeric forms i.e., 1*H*-imidazole and 3*H*-imidazole[1]. Imidazole is an organic aromatic compound with the molecular formula $C_3N_2H_4$ and resonance value of 14.2 Kcal/mol. Imidazole's show pKa value of 14.5 and dipole moment of 3.61D. N-3 of the Imidazole ring is the basic site, protonation gives the imidazolium cation. Imidazole is a weak base and tautomeric substance since positions 4 and 5 are equivalent.The physical characteristics of imidazoles occur in colourless solid having a melting point of 89□-91□ and boiling point 256□[2].



This reaction was first reported by Debus in 1858 fully developed by Radziszewski beginning in 1882, and further modified by Weidenhagen in 1935. This reaction is the main synthetic reaction of industrial importance for the synthesis of imidazole derivatives but the problem with debus radziswiski imidazole synthesis is it gives poor yields of imidazoles and side reactions[3]. Imidazole derivatives has been proven for various biological activities based on the research and review articles available such as anti-microbial, anti-viral activity[4], anti-inflammatory activity[5], analgesic activity[6], anti-tubercular activity, anti-cancer activity[7], anti-depressant activity, and antileishmanial activity[8]. The synthesis of imidazole derivatives can be done using Debus Radziswski imidazole synthesis. It is the synthesis of an imidazole derivative by the condensation of an 1,2-dicarbonyl compound, an aromatic aldehyde and two equivalents of dry ammonia in alcohol[9]. It is the main structure present in natural products such as purine, histamine, histidine, nucleic acids, etc. In the field of medicinal chemistry and pharmacology, Imidazole and its derivatives occupied a unique position, thus the synthesis of novel imidazole derivatives plays a vital role in the synthetic strategy of drug discovery[10].

OBJECTIVE OF THE STUDY

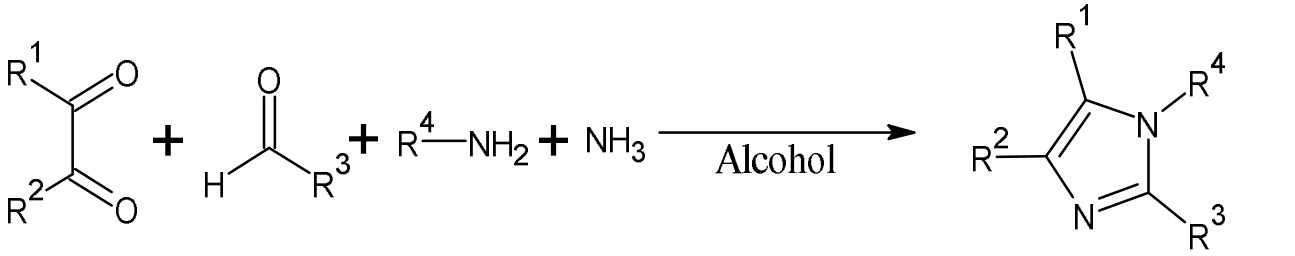
The present study is aimed in designing and synthesis of the Imidazole analogues for the anti microbial and antidiabetic lead molecule.

The main objective of the study is to find out the molecule which promote anti bacterial activity.

1. Molecular docking study for imidazole analogues
2. Synthesis of newer derivative of imidazole
3. Characterization of the new molecule
4. To screen the synthesized compounds for their antimicrobial and antidiabetic activity.

Debus Radziswski imidazole synthesis general procedure

Imidazole derivatives can be synthesized by condensation of an alpha-dicarbonyl compound for example glyoxal, a substituted aldehyde and two equivalents of dry ammonia in alcohol. Probable mechanism is that ammonia or primary amine reacts with a α-dicarbonyl compound to form α-diimine, which then condenses with an aldehyde to form Imidazole derivatives (Reaction-1), but the problem with the Debus Radziswiski imidazole synthesis is, it gives poor yields of imidazoles and side reactions[3]. N-phenyl isatin is synthesised and used as dicarbolyl compound in the synthesis of imidazole derivaties.



Reaction-1 (R_1~R_4 = H, alkyl, aryl)

Review of literature

- Deepika Sharma *et,al.*, have synthesized 2-(substituted phenyl)-1H-imidazole (1–12) and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanone (13–26) analogues and screened them for their antimicrobial activity against Gram positive, Gram negative and fungal species. The results of antibacterial study indicated that compounds showed appreciable antibacterial activity and antifungal activity[31].

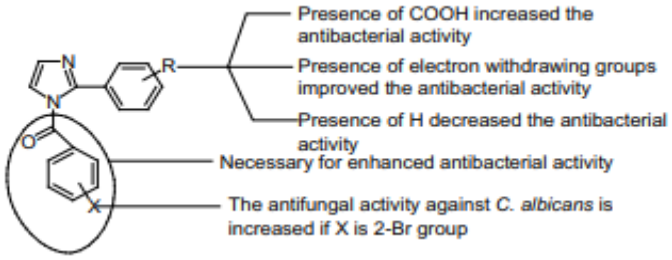
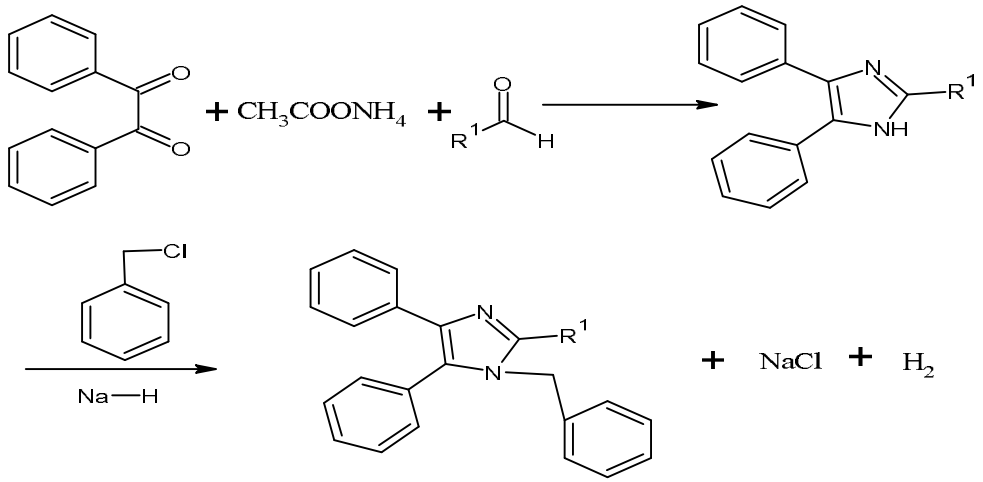


Figure no.8- SAR for antibacterial activity

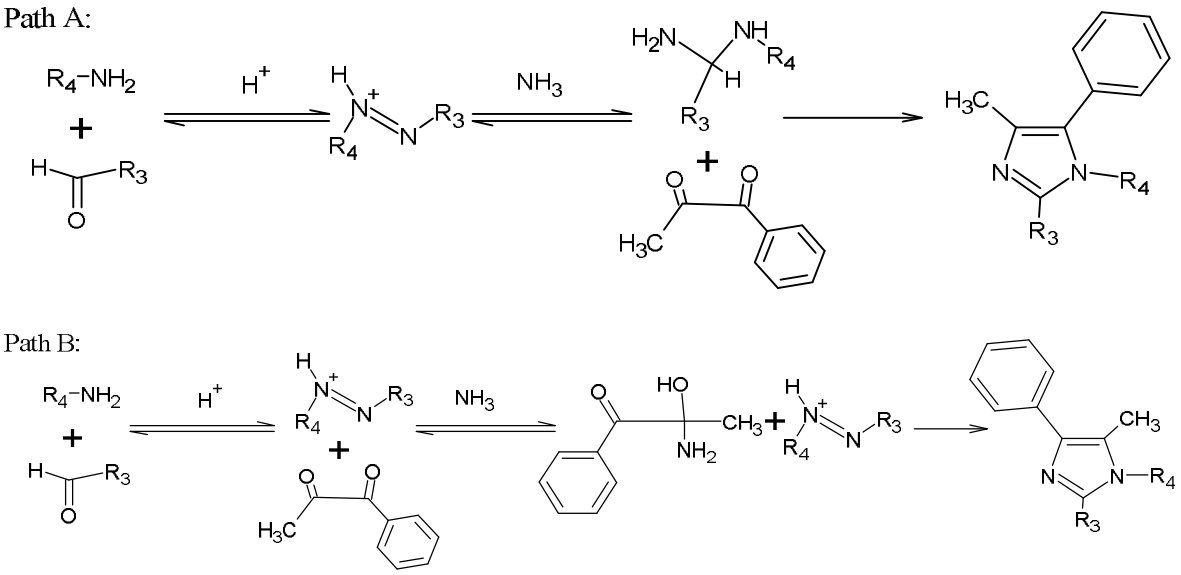
- Derivatives of 1-benzyl-2-substituted-4,5-diphenyl-1H-imidazole were synthesized by Umit ucucu et,al., and their analgesic activity assayed. 1,2,4,5-Tetrasubstituted imidazole compounds were obtained by the treatment of purified imidazole compounds with benzyl chloride in the presence of sodium hydride. Generally the prepared compound exhibited only moderate analgesic activity in mice at the dose of 100 mg/kg i.p. however, a few of them exhibited good activity, almost equivalent to that of morphine at 1 mg/kg i.p. At the above dosage, no toxicity was observed for all compounds[32].



Reaction 2- Synthesis of substituted imidazole

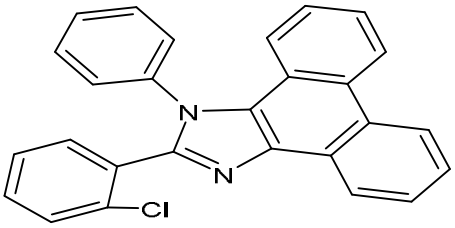
- E. Gelens *et al*,. proposed reaction mechanism of Debus-Radziszewski imidazole synthesis. The data suggest that the reaction may proceed via two pathways. The aldehyde and the amine first

react to give protonated aldimine in the presence of acetic acid, then the two pathways differentiate. In pathway A, ammonia, present in the reaction as ammonium acetate, reacts with aldamine to give a diamino intermediate. This diamino intermediate reacts with the diketone and in the following steps, the five membered ring system is formed and water is eliminated. In pathway B, ammonia reacts with the diketone to give an aminoalcohol intermediate. Then, protonated aldimine and aminoalcohol intermediate react, after which the five-membered ring system, is formed and water is eliminated[33].



Reaction 3

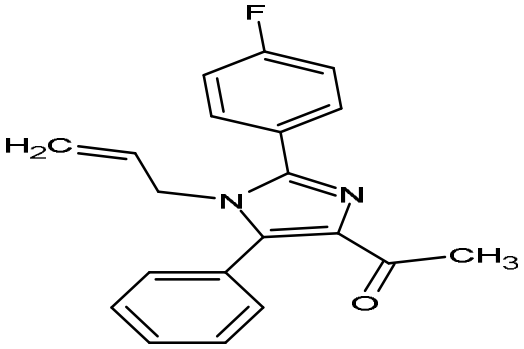
- Jawaharmal ***et.al.,*****Synthesized Tetra substituted imidazole derivatives and** screenedthe compounds for *in vitro* antibacterial activity at 100 and200 ppm concentration.**2-(2-chlorophenyl)-1-phenyl-1H-phenanthro [9, 10-d] imidazole were found to have good antimicrobial activity.** In this investigation, it was of interest to synthesize imidazole by refluxing 9, 10-phenenthraquinone with aryl aldehyde, primary amines and ammonium acetate in the presence of glacial acetic acid and a novel series of imidazole derivatives[34]**.**



2-(2-chlorophenyl)-1-phenyl-1H-phenanthro [9, 10-d] imidazole

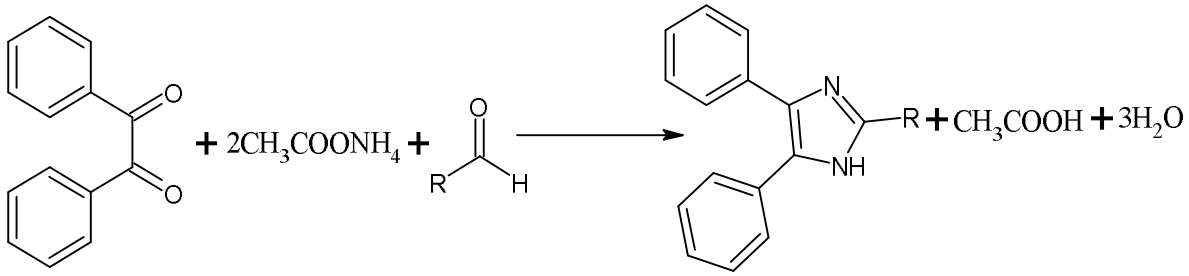
- Marcus Vinicius P. S. Nascimento *et al*., synthesized novel tetrasubstituted imidazolfor the Development of Anti-inflammatory activity. In that methyl 1-allyl-2-(4-fluorophenyl)-5-phenyl-1Himidazole-4-acetate shows a distinctive anti-inflammatory profile both in vitro and in vivo. The tetrasubstituted imidazoles were synthetized through a multicomponent reaction involving azirines, amines, and aldehydes. The compounds were dissolved in a solution of sterile saline (0.9% NaCl) and dimethyl sulfoxide 1% (DMSO) to a final concentration of 2.5 mg/mL as a stock solution. These solutions were aliquoted and stored at − 20 °C until the

moment of the experiments, when it was properly dissolved in cell medium for in vitro experiments, or sterile saline to the in vivo experiments[35].



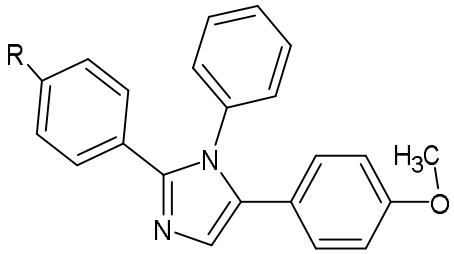
1-allyl-2-(4-fluorophenyl)-5-phenyl-1Himidazole-4-acetate

- Satyajit dutta synthesized 2-substituted-4,5-diphenyl imidazole's refluxing benzil with different substituted aldehydes in the presence of ammonium acetate and glacial acetic acid and were screened for anthelmintic activity. 2-substituted-4,5-diphenyl imidazole derivatives were found to have improved activity compared to albendazole and piperazine citrate. 2-Hydroxyphenyl, 3- -methoxyphenyl, 2-phenylethenyl, 4-fluorophenyl and 3-nitrophenyl groups at the 2nd position in imidazole derivatives seem to be resposible for high activity. Test results revealed that compounds shows that the paralysis timewas 0.24 to 1.54 min and death time of 0.39 to 4.40 min while the standard drugs albendazole and piperazine citrate showed paralysis time of 0.54 and 0.58 min and death time of 2.16 and 2.47 min[36].



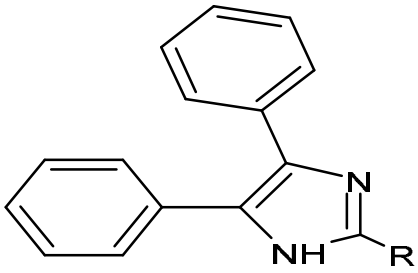
Reaction 4- Synthesis of imidazole derivatives

- Asif Husain *et al*,. Synthesized di- and tri-substituted imidazoles as safer anti-inflammatory-antifungal agents. In that 2-(4-nitrophenyl)-4-(4-methoxyphenyl)1-phenyl-1 H-imidazole and 2,4-di-(4-methoxyphenyl)1-phenyl-1 H-imidazole have good anti-inflammatory-analgesic activity and also showed less GI irritation. 1,2,4-trisubstituted-1 H-imidazoles were synthesized by refluxing 2,4-disubstituted-1 H-imidazoles with chlorobenzene in tetrahydrofuran (THF) using triethylamine as catalyst. 2,4-Disubstituted-1 H-imidazoles were synthesized by refluxing 4-methoxyphenyl glyoxal (1) with aryl aldehyde in glacial acetic acid using ammonium acetate as catalyst[37].



1,2,4-trisubstituted-1 H-imidazoles

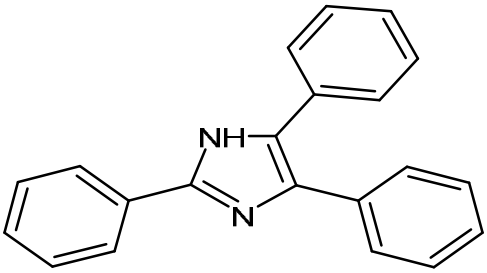
- Puratchikody *et al*,. Synthesized 2-substituted-4,5-diphenyl-1H-imidazoles by condensation of benzil with ammonium acetate and appropriate aldehydes for Anti-nociceptive and anti-inflammatory activities. It is proven that the compounds with phenyl substitution with –F, –Cl, –NH_2, –$N(CH3)_2$, –OH and –OCH_3 at p-position showed higher activity than all other substitutions. In this paper, the determination of antinociceptive and antiinflammatory activities of 2-substituted-4,5- diphenyl-1H-imidazoles is reported. The antinociceptive activity is based on hot plate and tail flick methods and the antiinflammatory studies are based on carageenan-induced paw oedema[38].



Tri-substituted imidazole

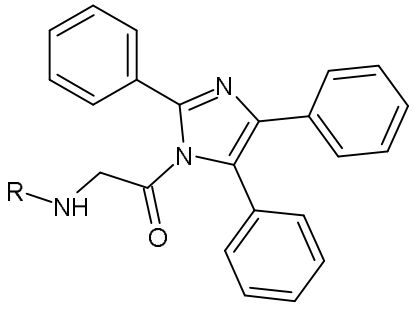
- Rambabu *et al*., Synthesized 2, 4, 5- trisubstituted imidazole's taking different aldehydes as substitutions. The compounds were tested *in-vitro* for their antibacterial and anti-fungal activity and also screened for anti-depressant activity using forced swimming test in mice. Some of the compounds show comparable activity with that of the standard. The present work has

given out many active antibacterial, antifungal and antidepressant agents. Some of the compounds have showed moderate activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future. The promising biological activities may consider these compounds as a lead for drug development and drug discovery [39].



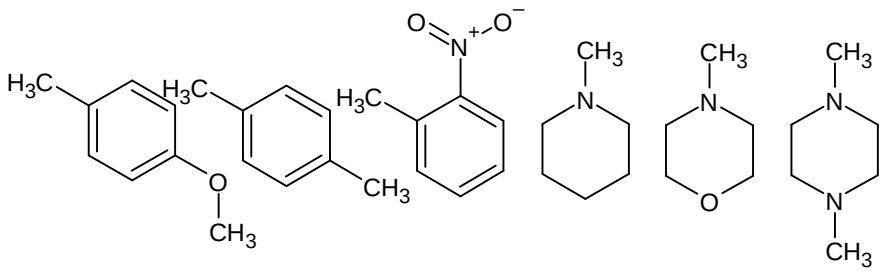
Triphenyl imidazole

- 2,4,5-triphenyl-1H-imidazole-1-yl derivatives were synthesized and tested for their antiinflammatory activity in-vitro using Phenylbutazone as a reference drug and antimicrobial activity using clotrimazole and ciprofloxacin as a standard drug. Shailesh P. Zala et.al., stated that when increases in aromaticity while substitution showed decrease in activity. Results of substituted compounds containing electron donating groups like methyl and methoxy functional group are showed more active than electron withdrawing nitro functional group. The pharmacological screening of the synthesized compounds showed anti-inflammatory activity ranging from 10.44 to 76.11% inhibition of rat paw oedema volume after 4hr, whereas the standard drug phenylbutazone showed 85.07% inhibition of rat paw oedema volume after 4hr[40].

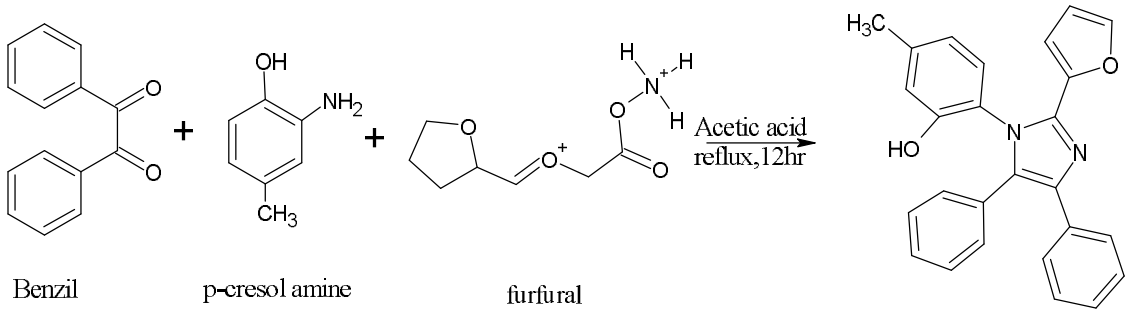


Substituted 2-chloro-1-(2,4,5-triphenyl-1H-imidazole-1-yl)ethanone

Where R=



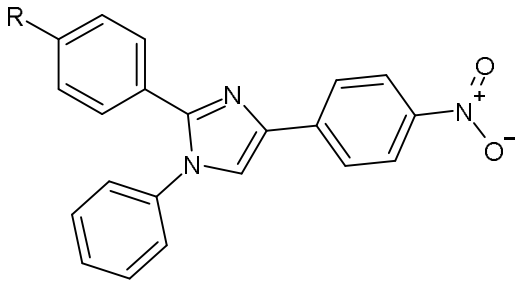
- Khurram Shahzad *et.al.*, synthesized a new class of tetrasubsituted imidazole compounds using multicomponent one pot synthesis scheme through cyclocondensation reaction of benzil, aromatic primary amines, aldehydes and ammonium acetate in glacial acetic acid. The synthesized compounds have been examined against various bacterial strains. The antibacterial study of these compounds demonstrated that the imidazole compounds shown considerable to significant activity against the strains[41].



Benzil p-cresol amine furfural

Reaction 5-Synthesis of 2-[2-(furan-2-yl)-4,5-diphenyl-1H-imidazol-1-yl]-5- methylphenol

- 1, 2, 4-Trisubstituted-1H-imidazoles were synthesized by Kundlik S. Khandare *et.al.*, using nitrophenyl glyoxal with various substituted aryl aldehyde and evaluated them for In vitro and In vivo anti-inflammatory and antibacterial activity. The molecular docking study and 3D-QSAR study were performed. They were docked to COX-2 (PDB code: 4COX) and Glucosamine-6-phosphate synthase (PDB code: 1MOQ). The 3D-QSAR study was performed on MLR, PLSR, PCR and kNN-MFA models of which MLR model was found to most suitable. In vitro anti-inflammatory activity of newly synthesized imidazole derivatives was carried out by protein denaturation method and In vivo anti-inflammatory activity was carried out by carrageenan induced rat paw edema method. Antibacterial activity was carried out by the cylinder (cup) plate[42].



4-(4-Nitrophenyl)-2- (substituted phenyl)-1 H-imidazole

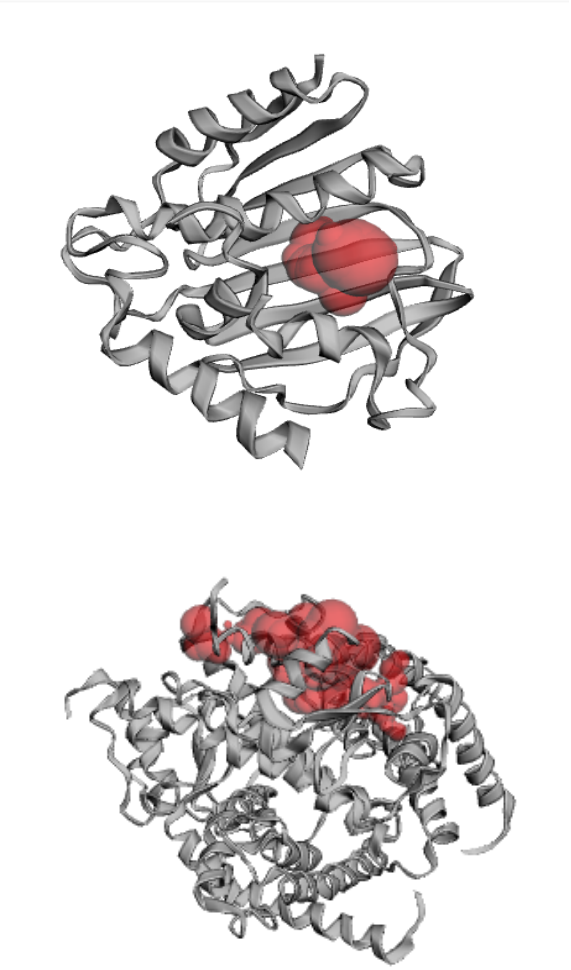
METHODALOGY

Ligand preparation

The novel imidazole analogues that is 4-(4-phenyl-2-(3-nitrophenyl)imidazo(4,5-b)indol-3(4H)yl)benzoicacid, 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole, 4-(4-phenyl-2-(4-methoxy phenyl)-imidazo(4,5-b)indol-3(4H)phenyl)acetamide, 4-(4-phenyl-2-(4-nitrophenyl)imidazo(4,5-b)indol-3(4H)-yl)benzoic acid and 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole were retrieved from PubChem database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi). The ligand optimisation was performed by Hyperchem Professional 7.0.

ACTIVE SITE PREDICTION

The active site of the model which was revealed by means of CASTp web server used as a binding site for molecular docking and ligand binding site. The active site was validated and cross checked with the information present in the NCBI database VAL43, ASN46, ALA47, GLU50, VAL71, ASP73, ARG76, ILE78, ALA91, ILE94, MET95, VAL120, LEU132, ILE134,THR165 and VAL167 of 7C7N Protein are found to be the best binding site. The resultant protein structure was used to predict the active site amino acids and to calculate the electrostatic and Vander Waals interactions between residues of complex proteins. The active site of amino acid was predicted based on the RMSD values and the resulting amino acid further used as active site of aminoacid. Same procedure is followed to determine the active binding sites of 6FZY protein using CASTp web server.



Active site of 7C7N and 6FZY protein predicted by CASTp server

Molecular Docking

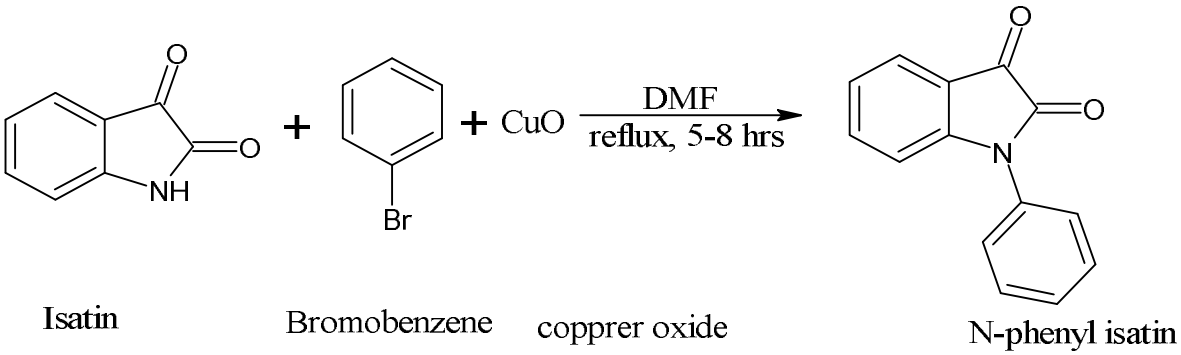
The docking evaluations of five main ligands (Imidazole derivatives) were docked with 7C7N and 6FZY using Auto dock tools. Molecular docking studies were carried out using Auto dock 4.2 and Auto dock tools 1.5.4. The Lamarckian genetic algorithm was used for ligand conformational searching. The local search algorithm, which builds a population of individuals (genes), each being a different random conformation of the docked molecule. The grid was generated around the active site at 80 × 80 × 80 to calculate molecular simulation using AMBER tools, showed autogrid of active site residues around the complex structure. There were 150 populations with a mutation rate of 0.02, crossover rate of 0.8 and default grid spacing 0.375Å were used as parameters settings for docking. Consequently, these simulations were performed using up to 2.5 million energy evaluations with a maximum of 27,000 generations and each simulation was performed by 10 times that yielded 10

docked conformations. At last, the lowest energy conformations were regarded as the binding conformations between ligands and the protein.

Synthesis of Imidazole derivatives

Step 1- Synthesis of N-phenyl isatin

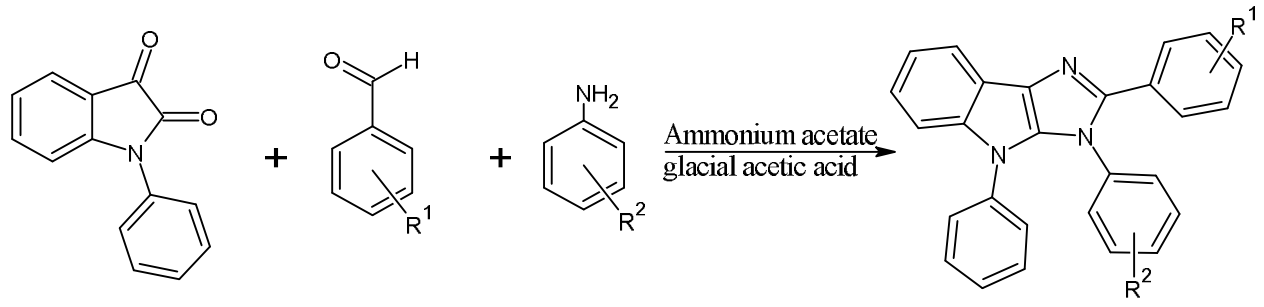
- A mixture of 0.04 mole of isatin, 0.05 mole of bromobenzene and 0.08mole of copper oxide was refluxed (Reaction-2) for 5-8 hours in a round bottomed flask using 100ml of dimethyl formamide (DMF). Filter the solution in hot condition and the filtrate was poured into cold water. Filter the mixture and the residue was collected and recrystillized using ethonl.



Reaction-2

Step 2- Synthesis of imidazole derivative

- A mixture of 0.01 mole of n-phenyl isatin, 0.01 mole of substituted benzaldehydes, 0.01 mole of substituted anilines and 0.01 mole of ammonium acetate are refluxed (Reaction-3) in round bottomed flask using glacial acetic acid for 3 hours. Cool the solution and add 200ml of ice water and neutralize using ammonium hydroxide. Filter the precipitate and residue is collected and recrystillized using ethanol.

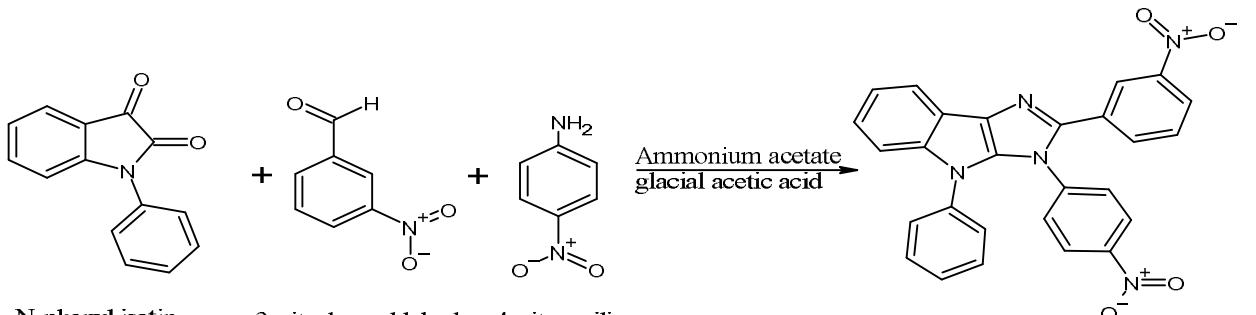


N-phenyl isatin Substituted aldehyde Substituted aniline Substituted imidazole

Reaction-3

Synthesis of 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole

- A mixture of o.o1 mole of n-phenyl isatin, 0.01 mole 3-nitro benzaldehyde, 0.01 mole of 4-nitro aniline and 0.01 mole of ammonium acetate are refluxed (Reaction-4) in round bottom flask using glacial acetic acid for 3 hours. Cool the solution and add 200ml of ice water and neutralize using ammonium hydroxide. Filter the precipitate and residue is collected and recrystalized using ethanol.

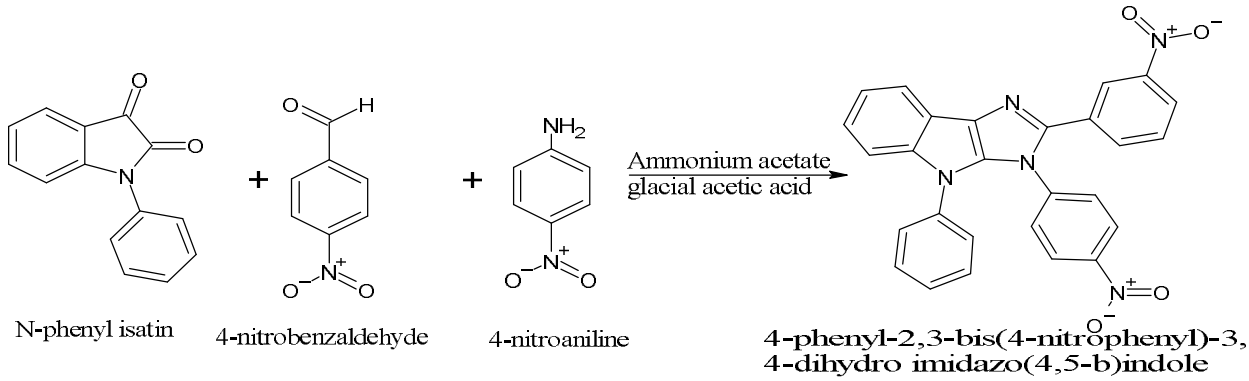


N-phenyl isatin 3-nitrobenzaldehyde 4-nitroaniline 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole

Reaction-4

Synthesis of 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole

- A mixture of o.o1 mole of n-phenyl isatin, 0.01 mole 4-nitro benzaldehyde, 0.01 mole of 4-nitro aniline and 0.01 mole of ammonium acetate are refluxed (reaction-5) in round bottom flask using glacial acetic acid for 3 hours. Cool the solution and add 200ml of ice water and neutralize using ammonium hydroxide. Filter the precipitate and residue is collected and recrystalized using ethanol.



Reaction-5

INVITRO ANTI MICROBIAL STUDY

Broth dilution method

- The broth dilution method depends upon microbial inoculation at a specific inoculum density of broth media (in tubes or microtiter plates) containing antibiotics at varying levels—usually doubling dilutions are used (e.g., 1, 2, 4, 8, and 16 μg/mL).
- The standardized bacterial suspension is typically 1–5 × 10^5 CFU/mL. Following incubation at 35 °C, turbidity is recorded either visually or with an automated reader, and the breakpoint concentration established.
- The lowest concentration of antibiotic that prevented growth represents the MIC.
- The precision of this method is to be ± 1 twofold concentration

RESULTS AND DISCUSSION

Ligand Preparation

Ligand were prepared using chemsketch software and the structure were built using Chemsketch to prevent any unsolicited mistake in the structure. The ligands can be retrieved from the PubChem databases and these compounds are analyzed by Hyperchem Professional 7.0 based on biological properties. The prevailed ligand molecules have done by molecular docking and the active site receptor proteins were performed using auto dock program.

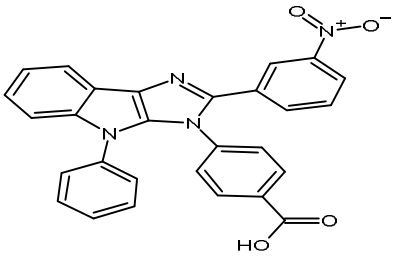
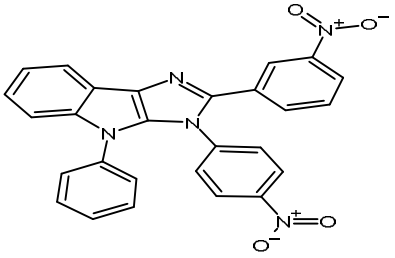
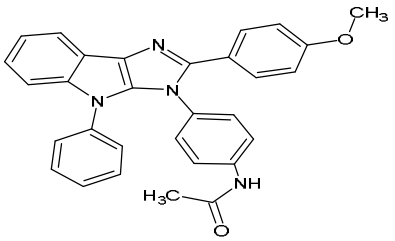
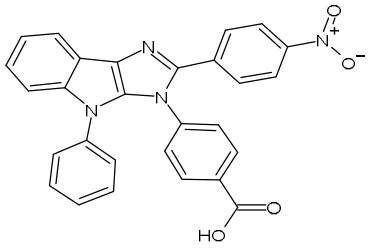
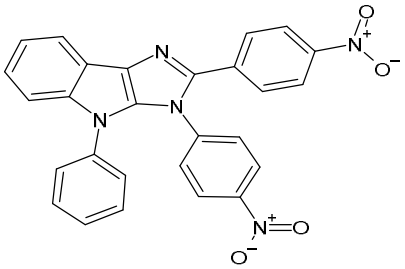
Compound Name	Molecular Formula	Mass	Structure
Compound 1A 4-(4-phenyl-2-(3-nitrophenyl)imidazo(4,5-b)indol-3(4H)yl)benzoicacid	$C_{28}H_{18}N_4O_4$	474.47g/mole	
Compound 1B 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole	$C_{27}H_{17}N_5O_4$	475.45g/mole	
Compound 1C 4-(4-phenyl-2-(4-methoxy phenyl)-imidazo(4,5-b)indol-3(4H)phenyl)acetamide	$C_{30}H_{24}N_4O_2$	472.54g/mole	
Compound 1D 4-(4-phenyl-2-(4-nitrophenyl)imidazo(4,5-b)indol-3(4H)-yl)benzoic acid	$C_{28}H_{18}N_4O_4$	474.47g/mole	
Compound 1E 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole	$C_{27}H_{17}N_5O_4$	475.45g/mole	

Table no. (1) Properties of ligand molecules

Pharmacophore and ADMET prediction

The pharmacophore properties of the ligand were calculated using molinspiration online database which is reported in the table. Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecular properties needed in QSAR, molecular modelling and drug design. The pharmacokinetics of the compounds was calculated using admetSAR web server. The results are reported in the table which was in the range.

Lipinski rule:

Ligand	**Molecular weight**	**Hydrogen Bond Acceptor**	**Hydrogen Bond Doner**	**LogP**	**MR**
1A	474.47	5	1	3.64	139.12
1B	475.45	5	0	3.89	140.98
1C	472.54	3	1	4.73	144.14
1D	474.47	5	1	3.64	139.12
1E	475.45	5	0	3.89	140.98

Table no. (2) Quality parameters

The pharmacophore prediction reported explains the properties such as partition coefficient, topological molecular surface area, Molecular weight, number of rotatable bonds, number of hydrogen bond donors and number of hydrogen bond acceptors.

The components of the Lipinski's rule states that no more than one violation of the following criteria.

- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors
- A molecular mass less than 500 Daltons
- An octanol- water partition coefficient that does not exceed 5.

ADMET prediction of Ligand molecules

Ligand	Carcinogenicity	Oral acute toxicity	LogS (water solubility)
1A	0.4366	0.6650	-3.495
1B	0.3973	0.6766	-3.644
1C	0.4942	0.4898	-3.754
1D	0.4356	0.6650	-3.495
1E	0.3973	0.6766	-3.644

All the compounds in the table passes the Lipinski rule of five and the compounds show druglikeliness property.

ADMET stands for absorption, Distribution, Metabolism, Excretion and Toxicity. The prediction of the ADMET properties plays an important role in the drug design process because these properties account for the failure of about 60% of all drugs in the clinical phases.

According to the results shown in the table, the compounds showed goodcell permeability and good permeability on lipid absorption and metabolism. All the compounds are in the acceptable range.

Molecular docking study

The molecular docking results detected the position and orientation of the inhibitor or substrate within protein structure. The docking of bioactive molecules 4-(4-phenyl-2-(3-nitrophenyl)imidazo(4,5-b)indol-3(4H)yl)benzoicacid, 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole, 4-(4-phenyl-2-(4-methoxy phenyl)-imidazo(4,5-b)indol-3(4H)phenyl)acetamide, 4-(4-phenyl-2-(4-nitrophenyl)imidazo(4,5-b)indol-3(4H)-yl)benzoic acid, 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole onto the conserved domain region of UniProtKB Knowledge base were performed using Autodock software. The homology model UniProtKB protein knowledge base was added polar hydrogen atoms and its non-polar hydrogen atoms were merged. For the ligands, nonpolar hydrogen atoms were merged with Gustier charges assigned. All rotatable bonds of ligands were set to be rotatable. Docking was performed using genetic algorithm and local search methods. A population size of 150 and 10 million energy evaluations was used for 100 times searches, with 40 x 40 x 40 dimension of grid box size and 0.375 Å grid spacing around the domain. Clustering histogram analyses were performed after the docking searches. The best conformations were chosen from the lowest docked energy that populated in the highest number of molecules in a particular cluster with not more than 1.5Å root-mean-square deviations (RMSD). After analyzing the data and the result obtained, 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole for antibacterial (7C7N) 4-phenyl-2,3-bis(4-

nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole for anti-diabetic (6FZY) activity was showing the least binding energy and least inhibition constant (Ki) and higher no. of hydrogen bonds which can consider these two compound as a best molecule for binding to the particular proteins. Further investigation has to be done for conformation and evaluation of these compounds.

Ligand	Binding free energy (Kcal/mol)	RMSD (A)	Inhibition constant KI (µm)	No. of Hydrogen Bonds
1A	-5.43	7.938	104.26	1
1B	-5.25	8.947	142.84	4
1C	-6.26	8.036	25.64	1
1D	-4.31	8.627	688.50	2
1E	-4.69	7.627	363.17	3
STD	-4.51	19.70	496.23	4

Table no. (3) Molecular docking study with ligand molecules of protein 7C7N

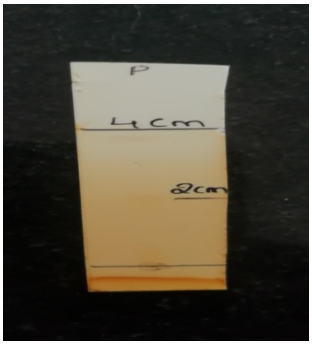
Ligand	Binding free energy (Kcal/mol)	RMSD (A)	Inhibition constant KI (µm)	No. of Hydrogen Bonds
1A	-6.51	25.204	16.81	1
1B	-6.63	25.022	13.81	1
1C	-9.22	24.20	174.93	2
1D	-6.99	25.236	7.56	4
1E	-7.51	24.705	3.13	4
STD	-5.49	25.236	7.56	3

Table no. (4) Molecular docking study with ligand molecules of protein 6FZY

The conclusion of this study was suggesting that 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole (7C7N) and 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole (6FZY) has good binding energy with PfGST Protein which can be considered as antibmicrobial and antidiabetic lead molecule.

CHARACTERIZATION

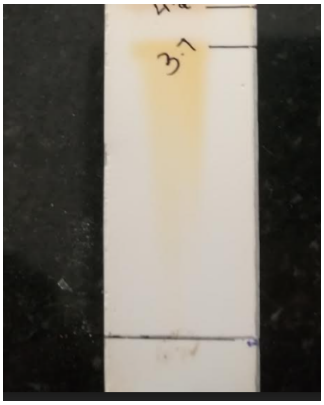
N-phenyl isatin can be synthesized by reacting the isatin and bromobenzene in presence of DMF ansd Copper oxide. Melting point of the synthesized n-phenyl isatin was found to be 142□ (138□ to 140□[59]). The synthesized 1-phenyl isatin then tested for its purity by performing the TLC method and observed single spot in TLC plate.



TLC: Mobile phase preparation: n-Hexane : Ethyl acetate (8:2)

Calculation of RF Value =Distance travelled by the solute / Distance travelled by the solvent

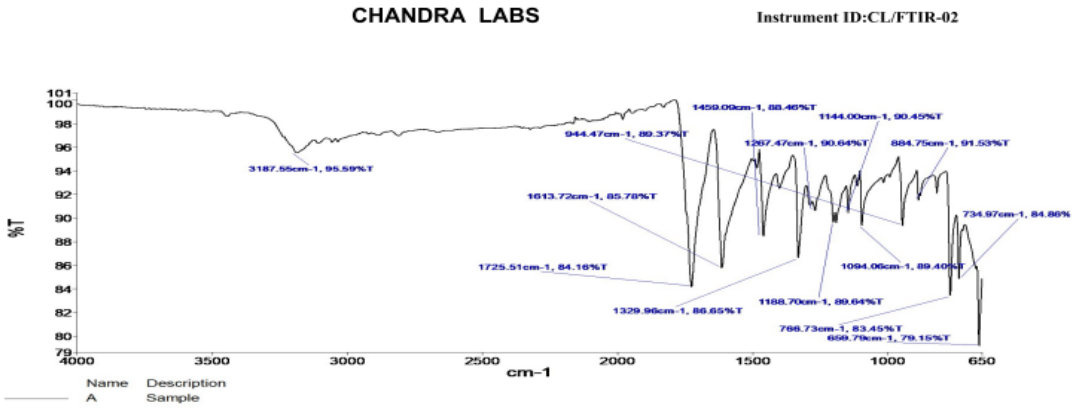
RF of isatin = 2/4 = 0.5



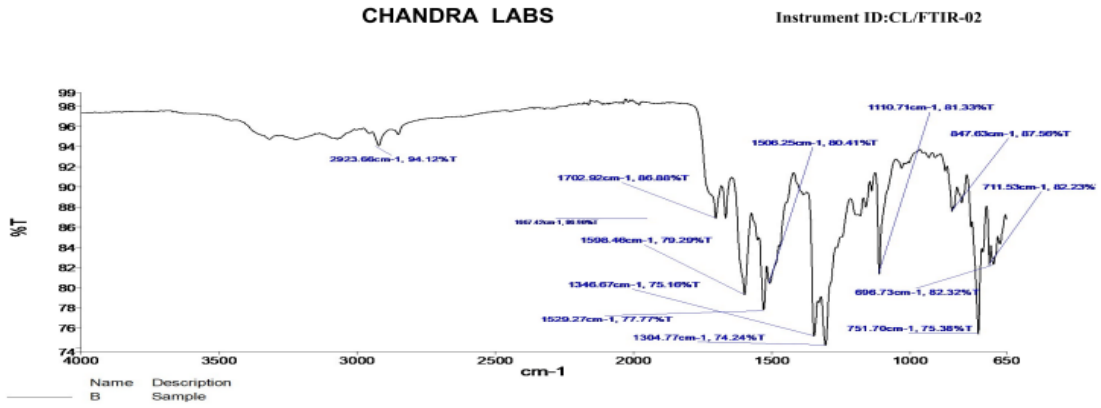
Rf value compound 1E = 0.76 **Rf value of compound 1E = 0.88**

INTERPRETATION OF THE LEAD COMPOUND

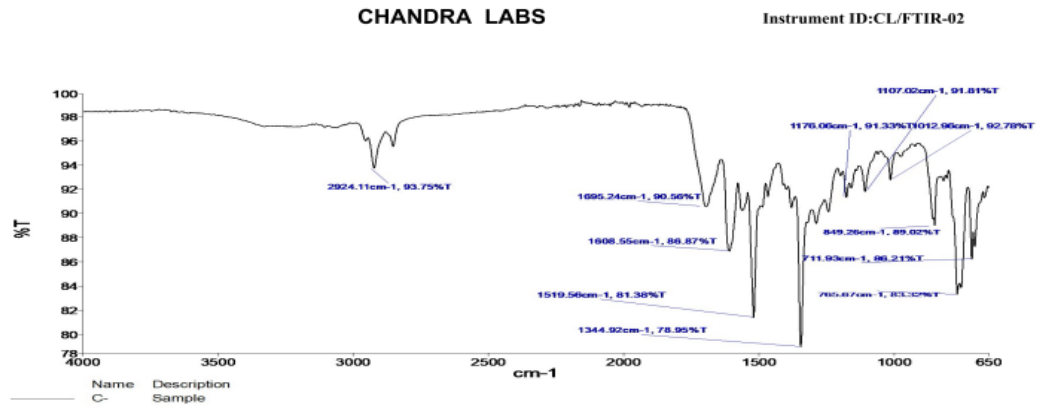
IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substance.



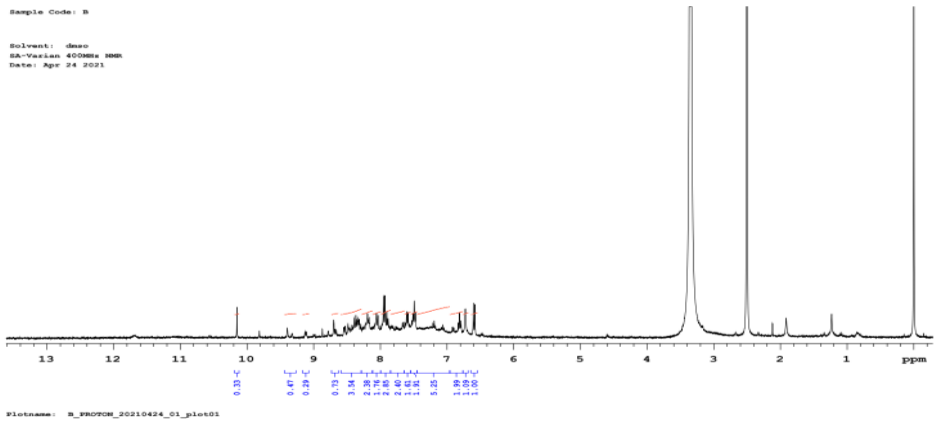
IR Spectra of n-phenyl isatin



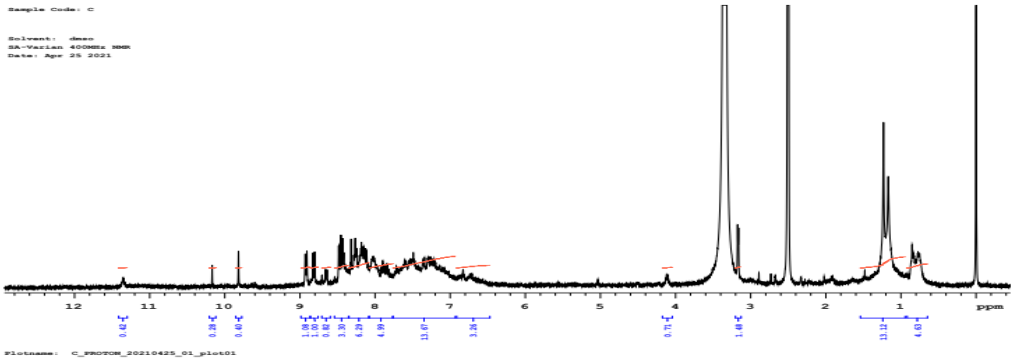
IR Spectra of 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole



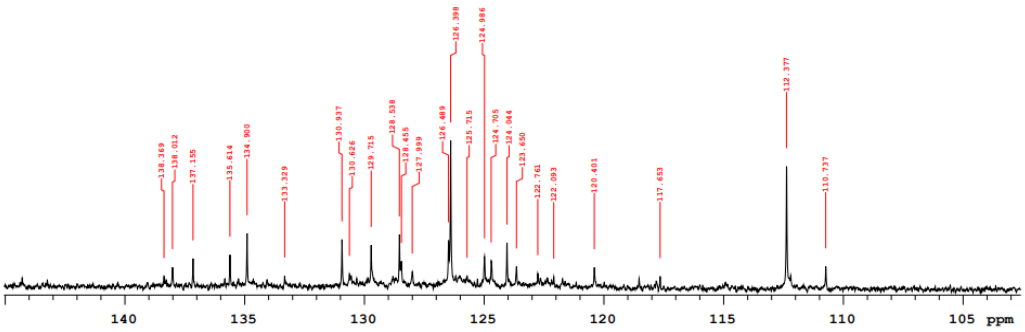
IR Spectra of 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydroimidazo(4,5-b)indole



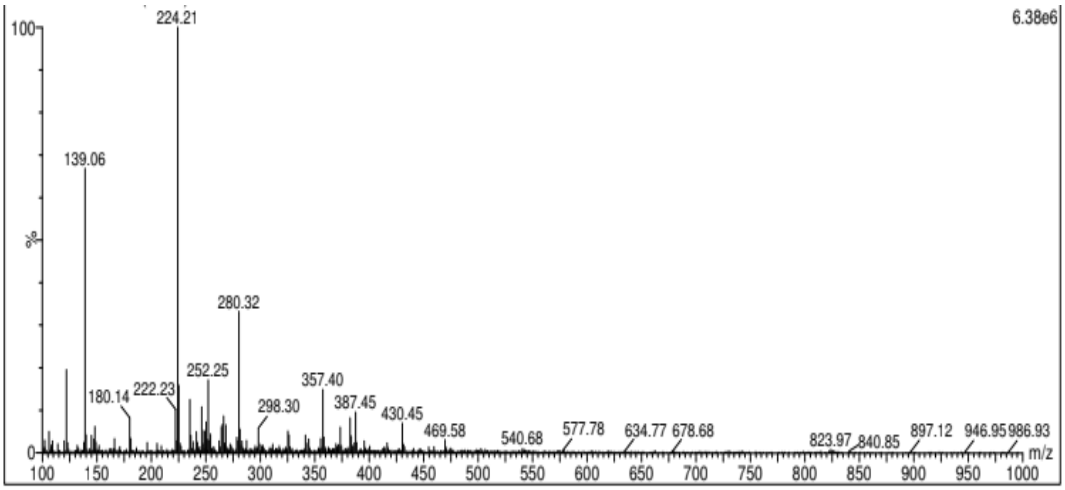
H-NMR spectra of 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole



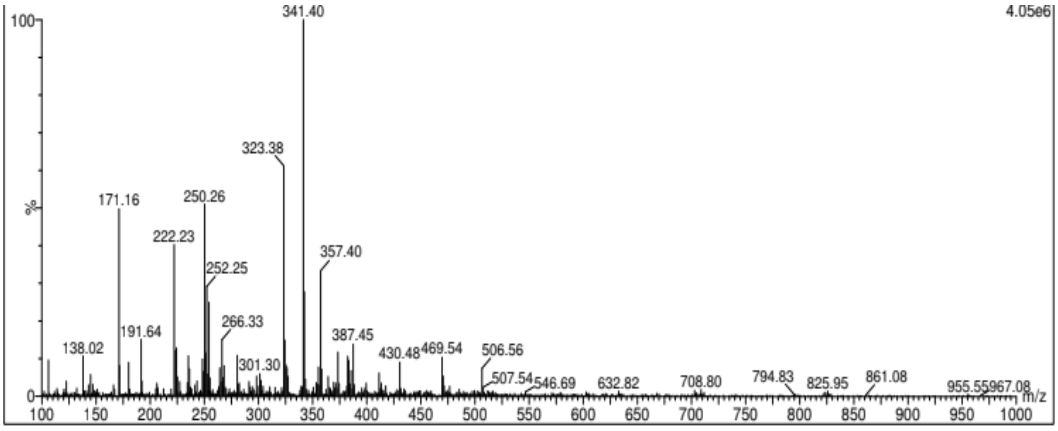
H-NMR spectra of 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydroimidazo(4,5-b)indole



C-NMR spectra of 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole



Mass spectra of 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro imidazo(4,5-b)indole



Mass spectra of 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydroimidazo(4,5-b)indole

CONCLUSION

Molecular docking was taken as first step in this research as it is a powerful tool for ligand based drug discovery due to its ability to predict the binding conformation of small molecule ligands to appropriate binding site. The investigation infer that the ligands were docked with the target P-glycoprotein. The pharmacophore properties of the ligands obeyed the Lipinski rule of five parameters Also, the ADMET prediction using admetSAR revealed that the docked compounds were in the acceptable range.

The compounds showed good permeability on ligand absorption and metabolism but slightly aqueous solubility. After analysing the data and the result obtained, 4-phenyl-2-(3-nitrophenyl)-3-(4-nitrophenyl)-3,4-dihydro-imidazo(4,5-b)indole and 4-phenyl-2,3-bis(4-nitrophenyl)-3,4-dihydro

imidazo(4,5-b)indole was showing the least binding energy and least inhibition constant which considered this compound as a best molecule binding to the 7C7N and 6FZY respectively.

The approach for molecular docking has been advantageous because it was helpful to study the size, shape, charge distribution, polarity, hydrogen bonding and hydrophobic interactions of both ligand and receptor.

Bioinformatic modelling overcomes the time consuming process in synthesis of all compound and cost effective as only the lead compound need to be synthesized.

Further investigation has done to confer the synthesis and evaluation of the lead compound.

New imidazole derivatives were synthesized from multistep approach involving a debus radziswski imidazole synthesis reaction from a reactin of n-phenyl isatin, substituted benzaldehydes and substituted anilines.

Formation of the compound was confirmed with TLC and IR spectral analysis. The target molecules can be considered for biological screening in order to develop active pharmaceutical compound.

The compound was screened for antimicrobial and antidiabetic activity. It was found that the lead compound showed significant activity. The chemical structure of the synthesized compound revealed that the compound with electron withdrawing group shows better activity than electron donating groups.

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