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Molecular docking, synthesis, characterization toxicity studies and in-vitro anti-bacterial activity of some novel benzimidazole derivatives

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ABSTRACT : Benzimidazole is a fused heterocyclic compound, it contain phenyl ring fused with imidazole nucleus. Benzimidazole derivatives have been found in various therapeutic applications such as anti-viral, anti-fungal, anti-cancer etc. Due to wide variety of bacterial infection in worldwide, we have more concentrate on bacterial resistance and toxicity to design new molecules having better anti-bacterial activity with low toxicity. An importance to design novel N-Substituted benzimidazole derivatives five compounds were selected and subjected to molecular docking studies by using argus lab 4.0 software. The docked molecules were synthesized by condensation of aromatic primary amine and benzimidazole in the presence of formaldehyde, this reaction is based on mannich base reaction. The structure of synthesized molecule was elucidated by spectral and analytical studies like UV, IR and NMR spectroscopy. In-silico toxicity studies were carried out by using Osiris molecular property explorer and evaluation of in-vitro antibacterial activity against gram-positive organism staphylococcus aureus and gram-negative organism E.coli by cup plate method. The docked molecules (C_1 C_2 C_3 C_4 and C_5) having moderate to better activity (-7.4163 to-10.5517). The synthesized compound C_1 which exhibit the zone of inhibition of in highest concentration, i.e., 100 µg/ml was found to be >31 mm against two the strains of bacteria.

KEY WORDS: Mannich reaction; Benzimidazole ; Antibacterial activity; Docking study;

I. INTRODUCTION

A bacterial infection is a proliferation of a harmful strain of bacteria on or inside the body. Bacteria can infect any area of the body. Pneumonia, meningitis, TB, Gastritis and food poisoning are just a few illnesses that may be caused by harmful bacteria. Examples of bacteria that cause infections include Streptococcus, Staphylococcus and Escherichia coli.

Salmonella is a type of infection (salmonellosis) associated with food poisoning caused primarily by Non-typhoid salmonellae bacteria found in intestinal tract of humans & other animals.

An antibiotic is the most important type of antibacterial agent for fighting against bacterial infections. It is capable of killing or inhibiting the growth of other micro-organisms. Amoxicillin, Ciprofloxacin, Clindamycin, Metronidazole and Levofloxacin are some of the antibiotics which are generally used.

Whenever, an antibiotic is taken to treat bacterial infection, it will increases the chances that the bacteria in the body will learn to resist them causing antibiotic resistance. Later, the infected person could get or spread an infection that those antibiotics cannot cure.

In the present work, the main goal was to discover the novel compounds of N- substituted Benzimidazole derivatives with potential anti-bacterial activity and to prevent the antibiotic resistance. The designed compounds were docked against a specific crucial target protein (PDB id: 207T) tetra family transcriptional regulator from Corynebacterium glutamicum and the compounds were synthesized and they are expected to act against G(+ve) and G(-ve) organism by in-vitro antibacterial studies.

The target Corynebacterium glutamicum [1] is a Gram-positive, rod-shaped bacterium in the family corynebacteriaceae and the order corynebacteriales. It is obtained from protein data bank and used for further docking studies.

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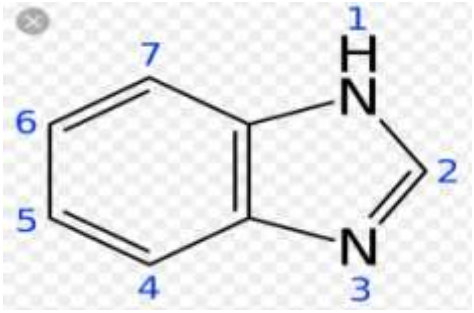
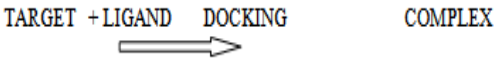


Benaimidazole

The benzimidazole contains a phenyl ring fused to an imidazole ring. Imidazole nucleus was first discovered by Debus1 in the year 1859 by reacting glyoxal and ammonia to indicate its source, he proposed the name glyoxaline. The term imidazole which is due to Hantzsch implies a five membered heterocyclic ring system containing imino group in addition to a tertiary nitrogen atom, that are located at the positions 1 and 3 respectively. Thus, the ring system in which benzene ring is fused to the 4, 5-positions of imidazole ring is designated as benzimidazole**.** Benzimidazole derivatives are being explored in pharmaceutical industries and substituted benzimidazoles have also been found in the various therapeutic applications such as Anti-ulcers, Anti-hypertensives, Anti-virals, Anti-fungals, Anti-cancer and Anti-histaminic activity.

Drug design, often referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the bio-molecular target with which they interact and therefore will bind to it. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex .It may also be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.[27-28]



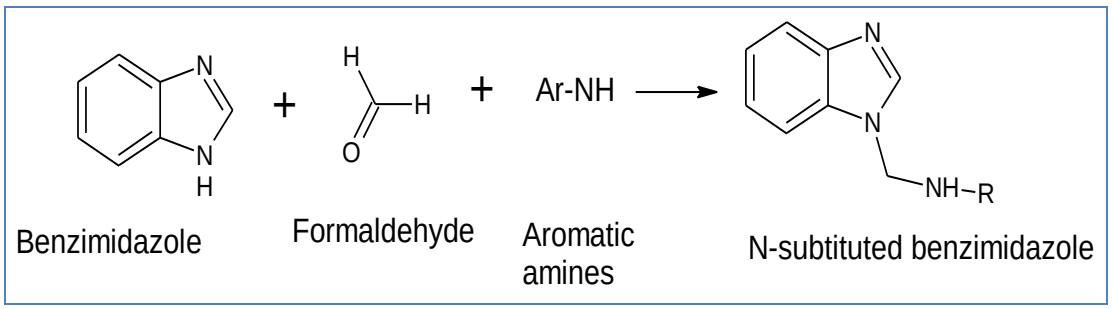
II. MATERIALS AND METHODS (ALL UPPERCASE, 12 PT., BOLD)

Insilico Studies: Docking and toxicity studies are carried out by Software.

- Chemsketch -Draw the structure and find the property of drugs.
- Argus lab -Docking
- Molegro molecular viewer-Viewed the docked molecule and interaction.
- Osiris property explorer -Toxicity studies.

SYNTHETIC SCHEME

The designed molecues are docked and screened for their better score and best interactions and based on the docking score and feasibility, one compound (C1) was synthesized by the condensation of (1 mole) Benzimidazole, (1 mole) different aromatic primary amines in the presence of formaldehyde, this reaction is based on Mannich base reaction.



R-Aromatic amine groups

Ar-amines: P-chloro aniline; Dimethoxy anthranilic acid; 6-Aminopyrimidine; 4-Aminopyrimidine; Benzylaniline.

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III. CHARACTERIZATION:

The purification of the synthesized molecules were carried out by Thin layer chromatography technique and are identified by identification test and characterized by using chemical methods such as UV, IR, and NMR which is Shown in FIG:4,5 & 6.

IV. IN-VITRO ANTI-BACTERIAL STUDIES:

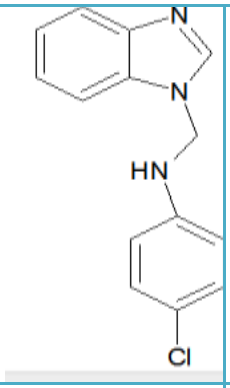
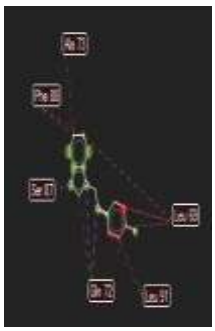
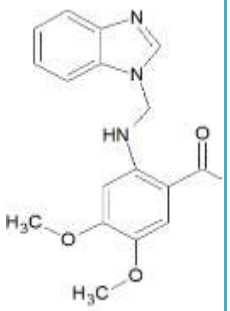
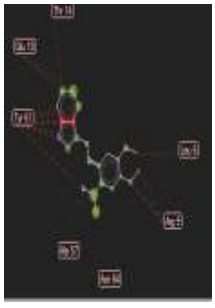
The synthesized compound was subjected to in-vitro anti-bacterial activity by cup plate method using nutrient agar medium, against both G(+ve) and G(-ve) organism, and 100 µg/ml concentration of sample and Amoxicillin as a standard & ethanol as the vehicle.

METHOD:

It is done by using disc diffusion method shown in FIG:3. Inoculate the test organism into nutrient agar plate with a depth of 4-5 mm and allow it to solidify. Divide the nutrient agar plate into four equal portions and cavities were formed with the help of sterile borer. Then, fill the cavities with antibiotic disc and slowly incubate the plates at 37°C for 24 hours. After incubation, measure the zone of inhibition. The results of in-vitro antibacterial activity obtained is given in FIG:12 to 15.

V. RESULTS AND DISCUSSION

The designed molecules were docked against the target protein (2O7T) Crystal structure of a tetra family transcriptional regulator, from Corynebacterium glutamicum and the results are shown in the given table.1.

CODE. NO	STRUCTURE	DOCKING	INTERACTION	ENERGY(K cal mole)
GIJ1				-8.51885
GIJ2				-7.86194

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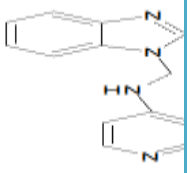
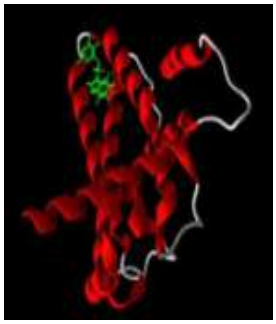
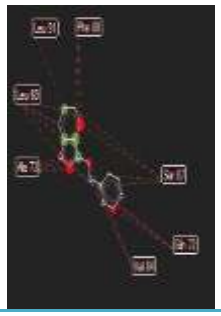
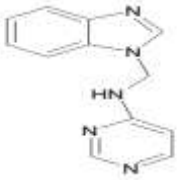
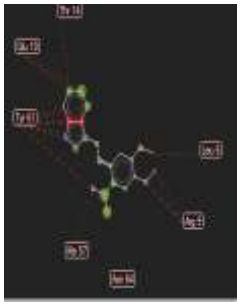
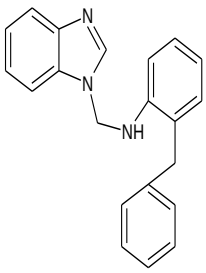
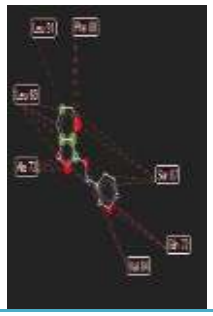
GIJ3				**-7.03069**
GIJ4				**-7.41635**
GIJ5				**-10.5517**

Molecular docking studies were performed to describe the interaction of the title compound with bacterial protein **(Pdb id: 2o7t)**.The docked compounds (C_1 to C_5) having good docking energy (-7.03069 to -10.5517) and the compounds bind to the various target amino acids in bacterial protein 2o7t are (Ala 73,Phe 88,Ser 87,Leu 69,Gln 72,Leu 91,Ala 57,Tyr 61,Glu 10,Thr 14).

From the above selected molecules, one compound was undergo toxicity by software studies (Osiris property explore) and the selected compound was synthesized(C_1) and characterized by spectral analytical techniques.

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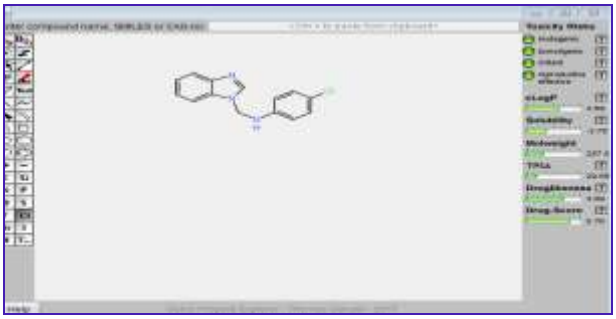
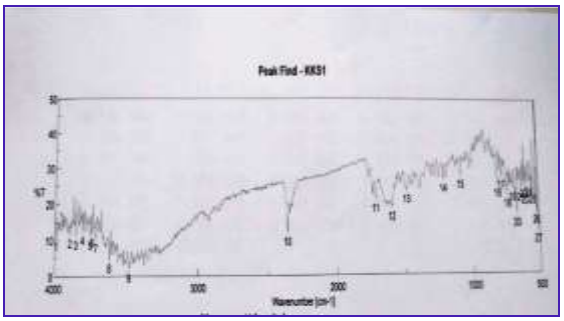


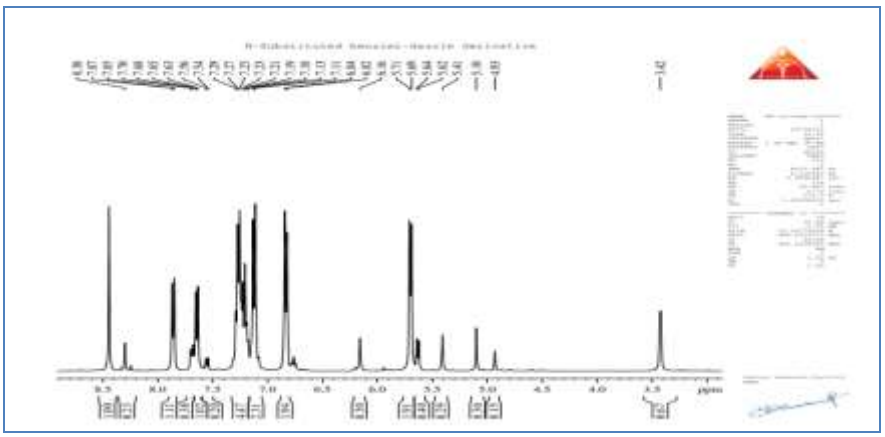
Toxicity report



IR spectrum of compound C1

IR spectrum of C1 shows respective bands for functional group present and the NMR spectrum indicates presents of corresponding Protons in the synthesized compound

S.NO	FUNCTIONAL GROUPS	WAVE NUMBER(cm^{-1})
1.	C-C	1216.66
2.	C=C	1601.59
3.	C-H	3481.85
4.	C-H	2361.41
5.	C=N	1719
6.	C-N	1088.62
7.	C-Cl	809.95
8.	N-H	3618.77



NMR Spectrum of C1

The synthesized compound C_1, which exhibit the zone of inhibition at highest concentration, i.e.,100 µg/ml was found to be >31 mm against the two strains of bacteria and hence, it possesses good antibacterial activity against G(+) ve and G(-) ve organism.

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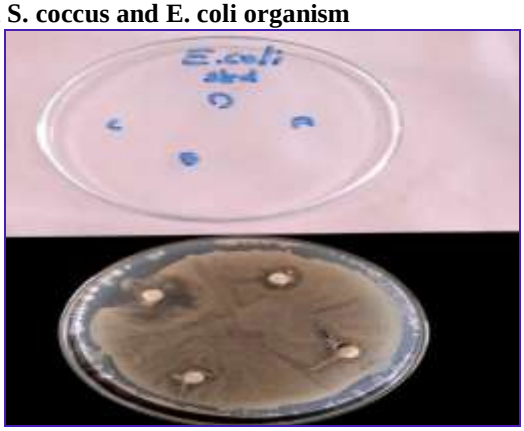
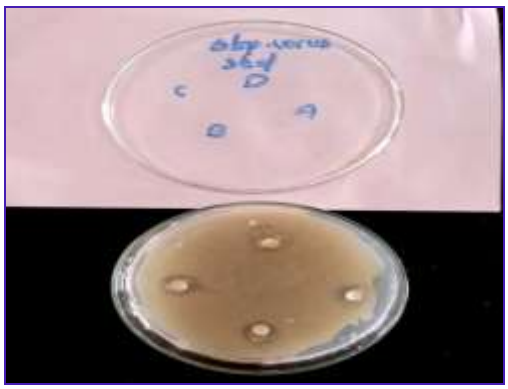
FIG 1-4 : Antibacterial activity against S. coccus and E. coli organism

Zone of inhibition of C1 in **E. coli**



Zone of inhibition of Standard



Zone of inhibition of C1 in **S. coccus**

Zone of inhibition of Standard

VI. CONCLUSIONS

The present study shows that N-substituted benzimidazole derivative compounds (C1 C2 C3 C4 & C5) possess moderate to better activity (-7.4163 to-10.5517) and having low toxicity. The synthesized compound also shows good anti-bacterial activity. Hence, we conclude that the further refinement to the structure of the synthesized compounds is expected to yield new outlook to the development of promising molecules against the bacterial infections.

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