Evaluation and Molecular Docking of Benzimidazole and its Derivatives as a Potent Antibacterial Agent

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The study was performed to identify a potent antibacterial benzimidazole derivative using in vitro and in silico techniques. Benzimidazole and its derivatives were synthesized by reflux process. The derivatives were screened for antibiotic susceptibility test (AST) and minimum inhibitory concentration (MIC) against Gram-negative and Gram-positive clinical isolates and compared with the positive control Norfloxacin. Insilico molecular docking was performed to screen the binding potential of the derivatives with target enzymes topoisomerase II /DNA gyrase of Escherichia coli (E.coli) and Staphylococcus aureus (S.aureus) along with the control Norfloxacin. Totally fifty-four isolates were screened for antimicrobial susceptibility test (AST) and minimum inhibitory concentration (MIC) and 35 clinical isolates of Gram-negative showed 86% resistance to Norfloxacin and 19 isolates of Gram-positive showed 90% resistance to Norfloxacin. However, these isolates were found to be sensitive to 1-((4-((1H–benzimidazol-1-yl)methylamino) phenyl) ethanone (3) (C2), and 2-methyl-1H-benzimidazole (C4) compounds, with MIC ranges from 6.25- 12.5 µg/ml. Molecular docking analysis revealed that the compound C2 exhibited better binding affinity towards topoisomerase II / DNA gyrase of E.coli and S.aureus when compared with C4 and control Norfloxacin. The antibacterial activity of these may due to the inactivation of these enzymes which is supported by the MIC results. The obtained in vitro and in silico results suggested that C2 showed better antimicrobial activity.

Keywords: Synthesis; Benzimidazole; 1-((4-((1H–benzimidazol-1-yl)methylamino)phenyl) ethanone (3); Antibacterial activity; Minimum inhibitory concentration; Molecular docking.

In recent years, the increased multidrug-resistant (MDR) bacteria have raised an alarm throughout the world.1,2 Around 50% of the antimicrobial drugs prescribed for human diseases, found to be unnecessary3. This use, misuse, overuse or over the counter has driven the major source towards the antimicrobial resistance4. Thus usage of antibiotics has become the major cause for the emergence and spreading of multi-drug resistant isolates of a group of microorganisms.5

Gram-negative pathogens are predominantly troublesome since it has
become resistant to all antibiotics that are in current use. The emergence of MDR (PAN resistance) in Gram-negative infectious occur in health care center commonly caused by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species. Among Gram-positive pathogens, *S.aureus* and *Enterococcus* species are the biggest threats. Furthermore, the antimicrobial drugs are expensive and cause undesirable side effects. Therefore, in light of the evidence of rapid global spread of resistant clinical isolates, we are in need to find out the new antimicrobial agents for future use.

Even though the newer use of antibiotics possesses a different mode of action against emerging bacterial resistance strains, we are still in need of more effective and potent antimicrobials. In recent years, benzimidazole has gained more attention, as the ring is an important pharmacophore in modern drug discovery. Benzimidazole derivatives have been reported to possess various biological activities such as anti-cancer, anti-viral, anti-bacterial, anti-fungal, anti-helmintic, anti-inflammatory, proton pump inhibitor and anti-coagulant property. Also, benzimidazole moiety has attracted attention in recent times because of its presence in Vitamin B12 as 5, 6-dimethylbenzimidazole which suppresses the growth of bacterial cells.

Therefore, we attempted to investigate benzimidazole and its derivatives against Gram-positive and Gram-negative bacteria through in vitro and in silico studies.

**MATERIALS AND METHODS**

**Scheme 1**

**General preparation of Benzimidazole**

In the scheme 1, benzimidazole was prepared by the mixture of O-phenylenediamine 1% and 90% formic acid through refluxed process at 150°C-160°C for 1h as show in figure 1. The product obtained was filtered, washed with water and dried at 100°C and chosen as first compound, benzimidazole.

**Synthesis of 1-(4-((1H-benzimidazol-1-yl)methylamino) phenyl) ethanone (3)**

As the continuation of scheme 1, second derivative was synthesized by adding benzimidazole (1.18 g, 0.01 mol) and p-aminoacetophenone (1.35 g, 0.01 mol) which was dissolved in 40 mL of ethanol, to this formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in ice bath as shown in figure 2. The product was separated by filtered, dried and crystallized from ethanol.

**Scheme 2**

**General Method for the Synthesis of 2-substituted benzimidazole derivatives**

In the scheme 2, carboxylic groups were selected for the study. These carboxylic groups are stearic and tartaric acid, which are replaced in the place of R position shown in figure 3. O-phenylenediamine (0.25 mol) and appropriate carboxylic acid (0.34 mol) was heated on a water bath at 100°C for 6-8 h. The completion of reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using 10% sodium hydroxide solution. The crude benzimidazole was filtered and washed with ice-cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added and digested for 15 min. The hot solution was filtered and the filtrate was cooled to temperature of 10°C. The pure product was filtered, washed with 25 ml of cold water and dried at 100°C.

Finally, Benzimidazole and its derivatives, 1-(4-((1H-benzimidazol-1-yl)methylamino) phenyl) ethanone; 2-(1H-benzimidazol-2-yl) butanedioic acid; 2-methyl-1H-benzimidazole were suspended in DMSO. The antibiotics used are listed in Table 1. As control, MTCC *K. pneumoniae* 7407, MTCC *S.aureus* 3160 and ATCC *E.coli* J53 were used.

**Antimicrobial disc diffusion susceptibility**

AST was performed by disc-diffusion method using Muller Hilton agar (MHA) for all the derivatives at the concentration of 1 mg/ml to find the zone of inhibition. Different concentrations of derivatives were added to the 9 mm sterilized filter paper disc and placed on the culture swabbed MHA plates. The zone of inhibition was measured in millimeters.

**Minimal inhibitory concentration**

MIC was performed based on the standard procedure. Ampicillin (Hi-Media) was used as a control, at the concentration of 1 mg/ml.
Benzimidazole and its derivatives were prepared at the concentration of 1 mg/ml using dimethyl sulphoxide (DMSO). Finally, the MIC value was converted to mL.

**Gas chromatography and Mass spectrometry (GC-MS)**

The JEOL GCMATE II GC-MS with data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI).

**In silico molecular docking studies**

The binding activity of the derivatives against *Escherichia coli* and *Staphylococcus aureus* was analysed using docking software Schrodinger Maestro Version 10.2. The compounds were sketched using ChemSketch version 12.01, developed by ACD labs and subjected to minimization using macromodel module in maestro 10.2. Ligand minimization deals with conversion of 2D structures to 3D, assigning proper bond orders, bond lengths, torsion angles, correct chirality, generation of ionization states, stereochemistry and ring conformation. Further, the protein targets from *E.coli* (4KFG) and *S.aureus* (4URM) were downloaded from RCSB-Protein Data Bank (PDB) and subjected to energy minimization to assign bond orders, bond length, charge fixing using protein preparation module in Schrodinger maestro 10.2. Induced fit docking (IFD) was carried out by specifying the active site region of the protein, which was identified using PDBsum. The best docked conformation was chosen based on glide energy, docking score and hydrogen bonded interactions. Ligand interaction map from Schrodinger suite was used for visualization of the interactions between the residues and the compound.

**RESULTS AND DISCUSSION**

The yield of synthetic compound benzimidazole derivatives is shown in table 2. Totally, 54 bacterial strains (Gram-negative - *E.coli*, *K.pneumoniae* and Gram-positive – *S.aureus*) were collected from different sources in Chennai (ACS Medical College, Chennai) during October 2016 – May 2017. ATCC *E.coli*353, MTCC *K.pneumoniae* 7407 and MTCC *S.aureus* 3160 strains were used as a control. Gram-negative isolates showed 80% resistance to amikacin, 86% resistance to norfloxacin and chloramphenicol, and 80% resistance to cephoxitin. Gram-positive isolates showed 82% resistance to amikacin and vancomycin, 90% resistance to norfloxacin and cephoxitin and 74% resistance to chloramphenicol. Among these derivatives, C2 and C4 showed better results compared to other compounds. Subsequently, these two derivatives exhibited better MIC ranges between 6.25-12.5 µg/ml for *E.coli*, *K.pneumoniae* and *S.aureus* compared with other derivatives and norfloxacin (Table. 3). Figure 4 and 5 shows the GC MS analysis of the C2 and C4 compounds.

As *in vitro* analysis showed better antibacterial activity with C2 compound, since NMR simulated structure was performed using ChemDraw, shown in figure 6, 7.

**In silico molecular docking study** was performed to understand the interaction of the derivatives C2 and C4 with topoisomerase II DNA gyrase enzyme of *E.coli* and *S.aureus*. The binding affinity of the synthetic compounds was compared with the control norfloxacin and the results were analysed based on docking score, glide energy and hydrogen bond interactions. The compound C2 showed docking score and glide energy of -5.366, -41.777 and -4.139, -36.748 Kcal/mol with topoisomerase II DNA gyrase of *E.coli* and *S.aureus* respectively. The compound C2 also had hydrogen bonded interactions with active site residue (Arg 76) of topoisomerase II DNA gyrase of *E.coli* and *S.aureus* respectively. The compound C2 also had hydrogen bonded interactions with active site residue (Arg 76) of topoisomerase II DNA gyrase of *E.coli* and *S.aureus*. The compound formed hydrogen bond interaction with Arg 144 and hydrophobic interactions with residues such as Arg 84, Pro 87, Glu 58, Ile 86, Gly 85, Thr 173, Asn 54, Asp 81, Ile 102, and Ser 128.

Similarly, synthesized compound C4 showed docking score and glide energy of -4.537, 31.046 and -4.69, -29.474 Kcal/mol against topoisomerase II DNA gyrase of *E.coli* and *S.aureus*. The compound formed hydrogen bond interaction with Asp 73 and hydrophobic interactions with residues such as Arg 47, Thr 165, Gln 72, Met 166, Val 71, Val 43, Val167, Asn 46, Val 120, Met 91, Ile 90, and Ile 78 with active site residues.
of topoisomerase II DNA gyrase of *E. coli*. For *S. aureus*, the compound showed hydrogen bond interactions with Asn 54 and hydrophobic interactions with Glu 58, Ile 86, Ile 175, Ile 51, Val 79, Val 174, Asp 81, Thr 80, Thr 173, and Ser 55. The docking results of synthesized compounds were compared with the commercially available drug norfloxacin in order to study the binding affinity. The drug norfloxacin showed docking score and glide energy of -5.708, -38.096 and -7.126, -31.484 against topoisomerase II DNA gyrase of *E. coli* and *S. aureus* enzyme respectively. The drug formed hydrogen and hydrophobic interactions with active site residues such as, Asp 73, Val 71 and Val 43, Val 44, Arg 136, Leu 132, Met 166, Val 120, Asp 49, Gly 50, Ile 78, Ala 47, Asn 46, Val 167, Gly 77, Asp 73, Thr 165 and Val 43 with active site residues of of topoisomerase II DNA gyrase *E. coli*. In case of *S. aureus*, the drug had hydrogen bond interactions with active site residues Arg 144, Asp 81 and hydrophobic interactions with Pro 87, Ile 102, Glu 58, Thr 173, Ser 55, Asn 54, Ile 175, Gly 85, Ile 86, Gly 83, and Arg 54 (Figure 9). The molecular docking studies revealed that the compound C2 showed better binding affinity towards topoisomerase II DNA gyrase of *E. coli* and *S. aureus* in terms of glide energy than C4 and norfloxacin drug as shown in table 4, which supports the above in vitro studies.

Structural activity relationship studies disclose that C2 compound possess an electron withdrawing group at the phenyl ring attached to benzimidazole, which displayed better antimicrobial activity. Thus SAR revealed that the electron with drawing group possesses high efficiency anagistic microbial activity than the electron donating groups. Structural activity relationship of C2, supports *in vitro* and *in silico* docking studies.

**Fig. 1.** Schematic representation of synthesis of benzimidazole (scheme 1)

**Fig. 2.** Schematic representation of synthesis of 1-(4-(1H-benzimidazol-1-yl) methylamino) phenyl) ethanone
**DISCUSSION**

Benzimidazole, a bioactive heterocyclic compound that contain nitrogen and benzene ring fused at 4,5-positions of imidazole. Benzimidazole and its derivatives are used as bioactive molecules in drug and pharmaceutical research sectors. The present work deals with the identification of potent benzimidazole derivative using antimicrobial studies. AST was performed with different mode of antibiotics and synthetic compounds to screen the resistant pattern of bacterial isolates. A total of 54 isolates were screened and found to be resistant to aminoglycosides, fluoroquinolones, tetracyclines and macrolides class of antibiotics but chloramphenicol sensitive as it prevents protein chain elongation by inhibiting the peptidyltransferase activity of the bacterial ribosome. However, chloramphenicol has got its own side effects as it causes mitochondrial stress and decreased ATP biosynthesis as stated. In our present investigation, all the 54 isolates showed above 80% resistance to the above mentioned

| S. No. | Antibiotic disc used | Antibiotic group         | Mode of action                      |
|-------|----------------------|--------------------------|-------------------------------------|
| 1.    | Amikacin AK 30 mcg   | Aminoglycoside Antibiotics | Inhibits RNA synthesis (30S)        |
| 2.    | Norfloxacin NX 10 mcg | 2nd generation Fluoroquinolones | Inhibits DNA synthesis             |
| 3.    | Cephoxitin CX 30 mcg | 2nd generation Cephalosporins | Inhibits Cell wall Synthesis        |

Table 1. Antibiotics and its mode of action

| Synthetic compound | Yield (g) |
|--------------------|-----------|
| Benzimidazole      | 7.8       |
| 1-(4-((1H–benzimidazol-1-yl)methylamino)phenyl) ethanone (3) | 6.0       |
| 2-(1H-benzimidazol-2-yl) butanedioic acid | 5.0       |
| 2-methyl-1H-benzimidazole | 5.0       |

Table 2. The yield of benzimidazole and its derivatives

![Fig. 3. Schematic representation of synthesis of 2-substituted benzimidazoles, r group replaced with stearic and tartaric acid](image)
Fig. 4. GC-MS analysis of (1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl) ethanone (3)
classes of antibiotics except chloromphenicol but found to be sensitive to all the benzimidazole derivatives. Among them C2 and C4 showed better results compared to other two compounds. It was reported that, fluoroquinolone derivatives, showed potent activity against S.aureus, Streptococcus pneumoniae, E.coli and P.aeruginosa as compared to norfloxacin.22 Another reported on the substituted molecule of benzimidazole, namely N-(4-(3-(5-bromo-1H-indol-3-yl)acryloyl)phenyl)-2-((5-methoxy-1H-benzo[d]imidazol-2-yl)thio)acetamide (7d) and 2-(benzo[d]thiazol-2-ylthio)-N-(4-(3-(5-bromo-1H-indol-3-yl)acryloyl)phenyl)acetamide (8d) have exhibited good activity against E. coli (MTCC-723).23

Following antimicrobial susceptibility test, the minimum inhibitory concentrations of these compounds were analyzed. In comparison with the AST, the MIC was also found to be low for C2 and C4 compounds for both Gram-positive and Gram-negative isolates when compared with the control norfloxacin. Among C2 and C4 compounds, C2 showed better MIC. The parent compound, benimidazole inhibits DNA synthesis of bacteria by inactivating the topoisomerase II/DNA gyrase enzyme.22 In this investigation, we made an attempt to screen the antibacterial activity of its substituted derivatives namely benzimidazole, 1-(4-(1H-benzimidazol-1-yl)methylamino)phenyl)ethanone (3), 2-(1H-benzimidazol-2-yl) butanedioic acid and 2-methyl-1H-benzimidazole and we found 1-(4-((1H-benzimidazol-1-yl)methylamino)phenyl)ethanone (3) and 2-methyl-1H-benzimidazole possess better antibacterial activity among the four compounds by inhibiting, topoisomerase class of enzymes especially type II enzymes, essential for introducing negative supercoiling into bacterial DNA, which is important for initiation and propagation of DNA and transcription of mRNA from many genes.

To evaluate the binding mechanism of the compounds, in silico molecular docking was performed against bacterial topoisomerase II/DNA gyrase enzyme of E.coli and S.aureus. In vitro analysis revealed that the compounds C2 and C4 exhibits antibacterial activity where as in silico docking studies of C2 compound showed better binding affinity against the target protein of E.coli and S.aureus. Supporting our results, we have reported that, 2-(2-Chlorophenyl)-1H-benzo[d]imidazoloderivatives showed microbial activity against Gram-positive and Gram-negative bacterial strains using norfloxacin as standard.24 As the lack of newer antibiotics and increased in the resistance pattern for different modes of antibiotics, we are in need of new and active compound to control the increased mortality, morbidity. Therefore in recent studies, benzimidazoles showed better activity along with various substitutions in different active positions. Benzimidazoles strive with purines due to their structural resemblance, thus it results in inhibition of synthesis of bacterial nucleic acids and proteins.25

It is generally accepted that any compound containing electron withdrawing group possess antimicrobial activity than the electron donating groups as reported.26 Structural activity relationship between the topoisomerase II/DNA gyrase enzyme

### Table 3. MIC of synthetic compounds

| Cultures       | C1  | C2  | C3  | C4  | NX |
|----------------|-----|-----|-----|-----|----|
| ATCC S.aureus  | 6.25| 6.25| <6.25| <6.25| 25 |
| MTCC S.aureus  | 6.25| 6.25| <6.25| <6.25| 25 |
| MTCC K.pneumoniae | 6.25| 6.25| <6.25| <6.25| 25 |
| MRSA S.aureus  | 100-50| 6.25-12.5| >100| 6.25-12.5| 50 |
| Sensitive S.aureus | 6.25| >6.25| 12.5 | >6.25| 25 |
| ESBL K.pneumoniae | 50 | 6.25-12.5| >100| 6.25-12.5| 50 |
| Sensitive K.pneumoniae | 6.25| >6.25| 25 | >6.25| 25 |
| ESBL E.coli     | 25 | 6.25-12.5| >100| 6.25-12.5| 25-50 |
| Sensitive E.coli| 6.25| >6.25| 12.5 | >6.25| 25 |

C1- Benzimidazole, C2- 1-(4-((1H-benzimidazol-1-yl)methylamino)phenyl)ethanone, C3- 2-(1H-benzimidazol-2-yl) butanedioic acid, C4- 2-methyl-1H-benzimidazole NX- Norfloxacin.
Fig. 5. GC-MS analysis of 2-methyl-1H-benzimidazole
Fig. 6. Carbon NMR simulated using Chemdraw
Fig. 7. Proton NMR simulated using Chemdraw
and the synthesized derivatives showed that the C2 compound possessed electron withdrawing group when compared with C4 compound, which may account for the antibacterial activity of the derivative.

**CONCLUSION**

The protein – ligand studies is vital for identifying drug likeness or inhibitors in the structure based drug designing. In this screening method, induced fit docking was used to acquire the reliable energy evaluation and binding affinity details of the synthetic compounds. The docking studies with norfloxacin, benzimidazole and its derivatives performed for the first time against topoisomerase gyrase II of *E.coli* and *S.aureus*. Our aim was to identify the potent benzimidazole derivative against the target topoisomerase II / DNAgyrase enzyme. The derivative, 1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl) ethanone (3) exhibited better glide energy and hydrogen bond interactions with the active site residues of the target when compared with other derivatives and control norfloxacin.

![Fig. 8. Interactions of norfloxacin and derivative C2 : 1-(4-((1H –benzimidazol -1-yl) methylamino) phenyl) ethanone (3) , C4 : 2-methyl-1H-benzimidazole in the active site region of *E.coli* DNA gyrase B](image-url)
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