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# *In silico* Studies and Assessment of Antimicrobial Activities for Synthesised Nitrobenzimidazole Derivetives

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**ABSTRACT**

Nitro substituted benzimidazole derivatives were synthesized using o-phenylenediamine and different aromatic acid. The reaction is carried out in ethanol as solvent at room temperature by using mechanical stirrer. Nitration of the synthesized benzimidazole derivative was carried out using mixed acid as a nitrating agent. The synthesized product characterized by using FT-IR, and $^1$H NMR. The synthesized benzimidazole derivative were studied for antimicrobial activity using *Gram-ve* and *Gram+ve* micro-organism. Maximum zone of inhibition is 18mm for *B. cereus Gram+ve* microorganism and 17 mm for *E. coli Gram-ve* microorganism in comparison with streptomycin as control drug. In silico studies were adopted for synthetic derivatives by Chem bioDraw, and online software tool and the synthesized compounds XY-1, XY-2 and XY-3 showed good binding affinity than streptomycin. The results suffice *In vitro* studies.

**Keyword:** Benzimidazole, Nitration, FTIR, Antimicrobial activity, In silco.

## INTRODUCTION

Benzimidazole has been observed to show the biological application, make the benzimidazole derivative interesting for the research in medicinal chemistry. The biological activity of the derivatives are attributed to its structure which comprises that benzene ring is fused with a five member ring system having hetero atom at 1 and 3 position. Due to the structural similarity with nucleotides, its interaction with the biopolymers of living system is synergistic and exhibit number of biological activities that include anthelmintic[1], antifungal[2], anti-allergic,

antimicrobial[3-5] etc. Benzimidazole derivatives are reported as it effectively seizes the growth of microbes[6]. Which manifest against the combat with various pathogens. Since then, numerous classes of antimicrobial agents have been discovered. Antimicrobials are among the most commonly used of all drugs[7]. Benzimidazole dragged the attention of researchers as the hetero cyclic ring is an important pharmacophore for current drug discovery.[8] Some known drugs e.g., Amoxicillin, streptomycin, norfloxacin, ciprofloxacin etc., are coined with some serious side effects[9]. Some novel compounds were synthesized.

In the present study the antimicrobial activities are evaluated by synthesizing some 2-substituted benzimidazole derivatives, prepared by reacting o-phenylenediamine with different aromatic acids followed by the nitration at room temperature to get 5-nitro 2-substituted benzimidazole derivatives. In silico" antibacterial modeling was performed by using online available computational software to anticipate the biological and physiological parameters. The software Py-Rx tool, Py-Mol and Discovery Studio Visualize, ACD lab Chemsketch were used for in silico studies.[10,11]

## EXPERIMENTAL

### Materials and Techniques

o-phenylenediamine, aromatic acids like salicylic acid; p-hydroxy benzoic acid and benzoic acid and solvents were procured from Loba Chemicals Ltd. Baroda (Gujarat) and they are of LR grade. *B. cereus*, *(Gram +ve)* and *E. coli (Gram -ve)* were procured from the Lab. N-agar was purchased from Loba Chemicals Ltd. Baroda (Gujarat).

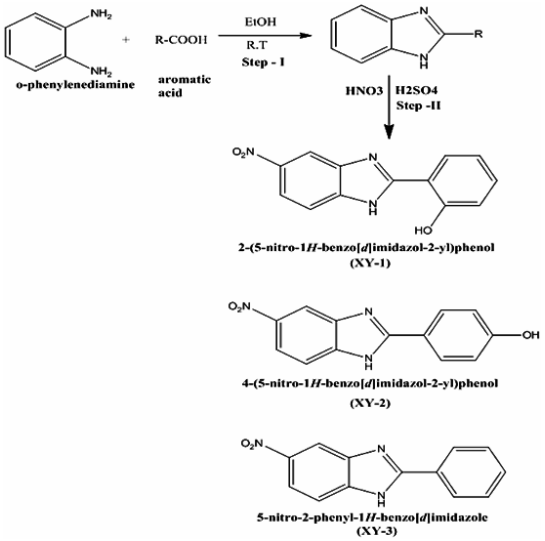
### Scheme for the synthesis of 2-(5-nitro1H-benzo imidazole -2yl) phenol (XY-1)

### Benzimidazole Synthesis

Equimolar o-phenylenediamine and salicylic acid were taken in methanol as solvent. The reaction mixture was mixed at room temperature for 2 h on magnetic stirrer. Afterward the reaction mixture was transferred in the ice cold water. The granular mass was obtained, further the mass is filtered and oven dried the product to recrystallize from the alcohol. Thin layer chromatography was performed to assess the progression of the reaction.

### Nitration of benzimidazole Compound

Take above synthesized compound (1 g) and mixture of $H_2SO_4$ (5 mL) and $HNO_3$ (5 mL) were taken in a beaker and stirred for 3 h at room temperature. Yellow color solid was filtered and recrystallized by ethanol with the yield of 85.00%. All the compounds (XY-1, XY-2 & XY-3) were synthesized by following the said method by using different substituted aromatic acids (XY-(a-c) respectively as given in Table 1. Thin layer chromatography was performed to assess the progression of the reaction.




### Reaction Scheme

The proposed reaction scheme for preparation of benzimidazole derivatives.

### Characterization

For the progress of reactions, Aluminium TLC plate silica gel with fluorescent indicator F254 were taken for the Thin layer chromatography and By using 1:1 methanol: hexane solvent system were used.

FT-IR Spectrum GX spectrophotometer (Parkin Elmer, USA), used to record IR spectra and the characteristic peaks were recorded in $cm^{-1}$.

$^1H$ NMR spectra were DMSO-d6 solvent using TMS as an internal standard at 400 MHz in Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland)

### Biological activity

*In vitro* growth inhibitory action of

synthesized compounds was assessed against selected bacteria, *Gram-positive Bacillus cereus* ATCC 11778 and *Gram-negative* bacteria: *E. coli* ATCC 25922. The inhibition zones of both standard drug and synthesized compounds were compared. For inhibition zone measurement the nutrient agar plates medium of 7.1±0.2 pH was prepared Whatman paper (grade No-1) was taken for the disc's preparation of 6mm diameter and 2mm thickness subsequently sterilize by dry heat for 1 hours. For discs impregnation, samples and standard (Streptomycin) concentration were made 25 µg/mL in DMF (dimethyl formamide) and a negative control impregnated with DMF was also taken. Standard inoculums were delivered into the surface of surface of sterile agar plate by adjusting the 105 CFU/mL. The agar plates were inverted and incubated for 24 h at 37±2°C. All the Petri dishes were then inverted and kept in an incubator for a period of 24 h at 37±2°C. The size of the zone of inhibition were measured to identify the potency of the compounds against the selected microbes[12] Result of antimicrobial activity shown in Table 1.

**In silico Molecular Docking study[13]**

For the study, synthesized compounds,. pdb format structure were obtained by drawing the structure in ACDlabs Chemsketch freeware followed by converting through Chem Bio 3D Ultra 6.0. 3D. Structure of streptomycin was taken from online source using chemspider. The scaffold proteins of bacteria were downloaded from the Protein Data Bank encoded with PDB ID: 2VH1 and 5H67 for *E coli* and *Bacillus cereus* respectively. The downloaded protein structures were cleaned and removed water molecules from the proteins and polar hydrogen atom were added further preparation for molecular docking by using discovery studio visualize was performed. For imaging the docked conformation, PyMOL software package and discovery studio visualize were used. PyRx tool 4.2 were used for molecular docking studies. Online availablepre-ADMET was assessing in silico ADMET parameter.

## RESULTS AND DISCUSSION

**Benzimidazole synthesis**

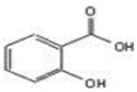
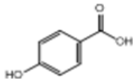
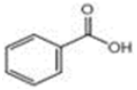
In order to synthesize substituted benzimidazole derivatives (XY-1 to XY-3), a comparatively useful and simple process were chosen. Our objective was direct condensation of o-phenylenediamine and aromatic acid at room temperature to obtain substituted benzimidazole without catalyst.[14] The adopted scheme was working as per the expectation in obtaining the desired product in appreciable yields. The Compound XY-1 gave more yield compare to other compounds. It may be presence of OH- group on ortho position in starting material. These reactions were observed relatively faster in comparison to the conventional solution phase synthesis. The conversion into product was found satisfactory and recoveries of the reactants were also observed.

**Nitration of benzimidazole Compound**

Nitration of the benzimidazole compound using this techniques nitro group attached on the 5th position of the benzimidazole compounds so we state that using these methods the nitration may be possible of the benzimidazole compound.

**Characterization**

**Table 1: Elemental analysis of synthesized compound**

| Sr. No | Name | -R XY-(a-c) | Yield (%) | Melting Point °C | Molecular Formula | Elemental analysis (Cal.) (%) C | H | N |
|---|---|---|---|---|---|---|---|---|
| 1 | XY-1 2-(5-nitro-1-H-benzo[d] imidazole-2-yl)phenol |  Salicylic acid | 88.78 | 185 | $C_{13}H_9N_3O_3$ | 60.12 (61.18) | 3.20 (3.55) | 16.00 (16.46) |
| 2 | XY-2, 4(-5-nitro-1H-benzo[d] imidazole-2yl)phenol |  P-hydroxy benzoic acid | 80.00 | 192 | $C_{13}H_9N_3O_3$ | 60.23 (61.18) | 3.01 (3.55) | 15.55 (16.46) |
| 3 | XY-3 (5-nitro-2 -phenyl-1H-benzoimidazole) |  Benzoic acid | 78.08 | 70 | $C_{13}H_9N_3O_2$ | 64.56 (65.27) | 3.21 (3.79) | 17.00 (17.56) |

**IR and NMR Spectral data of the synthesized derivative compounds**

| | |
|---|---|
| Compound:(XY-1)2-(5-nitro-1-H-benzo[d] imidazole-2-yl)phenol | IR : 3288 $cm^{-1}$ (N-H Str), 3111 $cm^{-1}$ (C-H Str), 1608 $cm^{-1}$ (C=N Str), 1446 $cm^{-1}$ (C-N Str), 1502 $cm^{-1}$ (N-O Str)<br>$^1$H NMR: 7.1-7.8 $cm^{-1}$ (9 H, Ar - CH); 8.2 $cm^{-1}$ (1H, NH) |
| Compound:(XY-2)4(-5-nitro-1H-benzo[d] imidazole-2yl) phenol | IR : 3285 $cm^{-1}$ (N-H Str), 3118 $cm^{-1}$ (C-H Str), 1650 $cm^{-1}$ (C=N Str), 1440 $cm^{-1}$ (C-N Str), 1504 $cm^{-1}$ (N-O Str)<br>$^1$H NMR: 7.0-7.8 $cm^{-1}$ (9 H, Ar- CH); 8.4 $cm^{-1}$ (1H, NH) |
| Compound:(XY-3) (5-nitro-2-phenyl-1H-benzoimidazole) | IR : 3268 $cm^{-1}$ (N-H Str), 3120 $cm^{-1}$ (C-H Str), 1628 $cm^{-1}$ (C=N Str), 14330 $cm^{-1}$ (C-N Str), 1570 $cm^{-1}$ (N-O Str)<br>$^1$HNMR: 7.1-7.6 $cm^{-1}$ (9H, Ar-CH); 8.3 $cm^{-1}$ (1H, NH) |

**Biological activity**

Synthesized compounds were screened for antimicrobial activities given in Table 2. *B. cereus (Gram+ve)*, *Escherichia coli (Gram-ve)* strains were used. The antibacterial activity of synthesized benzimidazole derivatives were compared with Streptomycin as a standard.

In case of *B. cereus,* compounds (5-nitro-2 -phenyl-1H-benzoimidazole) (XY-3) showed equipotent antibacterial activity. Compounds (XY-2) and (XY-1) showed moderate activity compared with standard drug.

In case of *E. coli* compound 2-(5-nitro-1-H-benzo[d]imidazole-2-yl) phenol (XY-1) showed good antibacterial activity. Compounds (XY-2) and (XY-3) show moderate activity compared with standard drug.

***In silico* antibacterial studies**

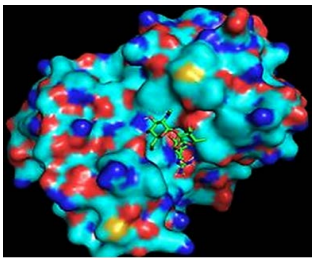
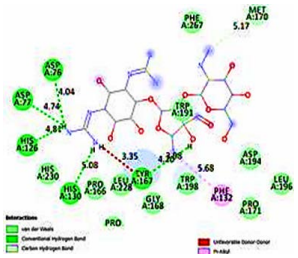
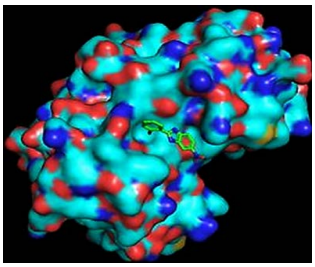
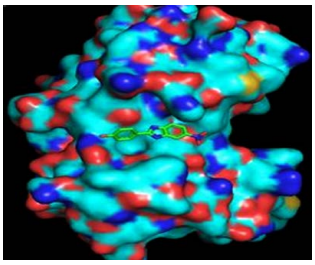
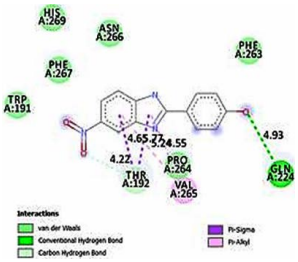
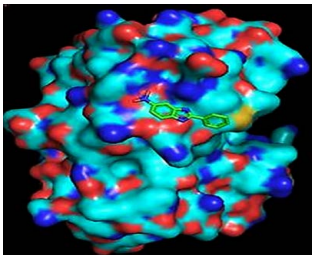
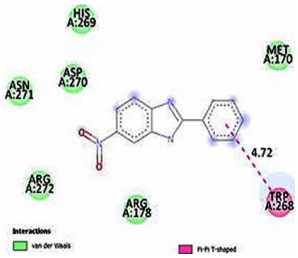
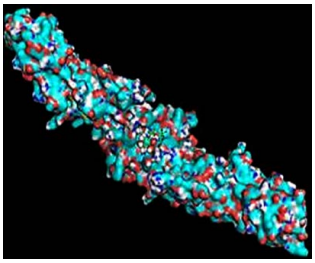
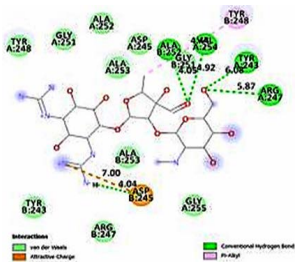
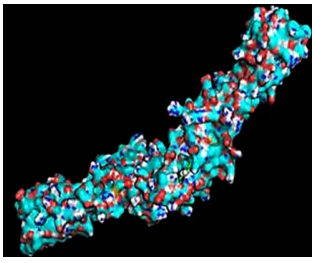
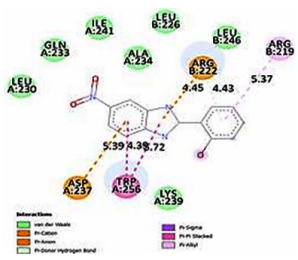
In the study, protein-ligand docking technique applied using online tool e.g. Py-Rx tool, Py-Mol and discovery studio visualize. Antibacterial studies by in-silico method were carried out on bacterial proteins of *Escherichia coli* (2VH1) and *Bacillus cereus* (5N1P) to observe detailed insight among the binding sites of receptor and the type of interactions. This study is in accordance to the in vitro antibacterial studies. The docking results were taken in the form of Binding affinity in (kcal/mol) for bacterial protein and applied compound. The results were compared with standard and tabulated in Table 2. From the table it has been observed that binding affinity of the synthesized compounds XY-1, XY-2 and XY-3 in comparison to standard streptomycin with the protein receptor of *E coli* were appreciable however binding affinity with the protein of *B. cereus* were observed moderate. The interactions of molecule with the proteins of microbe's 3D and 2D view are represented in Table 3. The obtained results are in accordance to the results of *In vitro* studies.

**Table 2: Antimicrobial activity of synthesized compound (zone of inhibition)**

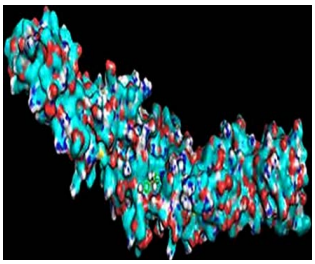
| Compound | Zone of inhibition (in mm) | | | | | |
|---|---|---|---|---|---|---|
| | *B .cereus* | | | *E. coli* | | |
| | 25 μg/mL | 50 μg/mL | 75 μg/mL | 25 μg/mL | 50 μg/mL | 75 μg/mL |
| XY-1 | 14 | 15 | 15 | 15 | 16 | 17 |
| XY-2 | 13 | 13 | 15 | 13 | 15 | 16 |
| XY-3 | 12 | 14 | 18 | 14 | 15 | 16 |
| Streptomycin | 17 | 19 | 20 | 16 | 17 | 19 |

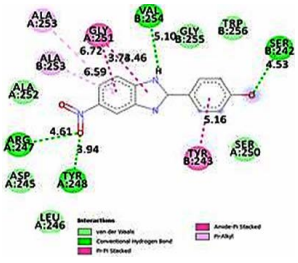
**Table 3: In silico molecular docking studies of ligand and its metal complexes with bacterial proteins in terms of binding affinity**

| Compound | Binding affinity for different proteins (kcal/mol) | |
|---|---|---|
| | *B. cereus* (PDB.ID: 5N1P) | *E. coli* (PDB.ID: 2VH1) |
| XY-1 | -5.9 | -7.6 |
| XY-2 | -5.7 | -7.1 |
| XY-3 | -4.3 | -7.4 |
| Streptomycin | -6.5 | -6.5 |

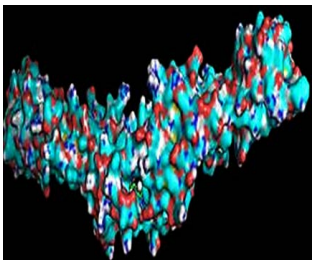
| Name | 3D Interaction View | 2D Interaction View |
|---|---|---|
| Streptomycine-5N1P |  |  |
| XY1-5N1P |  |  |
| XY2-5N1P |  |  |
| XY3-5N1P |  |  |
| Streptomycine-2VH1 |  |  |
| XY1-2VH1 |  |  |

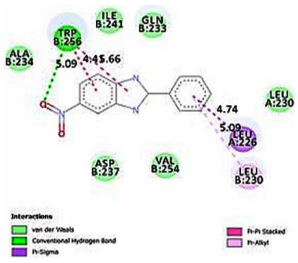
XY2-2VH1






XY3-2VH1






## CONCLUSION

For the synthesis of all substituted benzimidazole derivatives a simplified procedure were followed. Condensation of o-phenylenediamine organic compound and aromatic acid at room temperature is facilitated to produce substituted benzimidazole derivative without catalyst. The nitration on the 5$^{th}$ position of the benzimidazole compound was carried out by using this method. The compounds are produced in respectable yield of 88.78 for XY1, 80.00% for XY-2 and 78.08% for XY-1 (Table 1). A little quantity of starting materials (reactant) was recovered after completion of the reaction. The synthesized and purified compounds were characterized by spectral analyses using IR and NMR. They were assessed for antimicrobial activity by using cup-plate method and all the synthesized compounds were exhibiting appreciable antimicrobial activity against *Gram-positive* and *Gram-negative* microorganism.

In-silico studies of the synthesized compound using online software tools and the synthesized compounds XY-1, XY-2 and XY-3 showed good binding affinity than streptomycin (Table 3). The results suffice *In vitro* studies.

## ACKNOWLEDGMENT

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### Conflict of interest

The authors have no conflicts of interest.

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