In silico and In vitro screening of novel 2-substituted benzimidazole against Escherichia coli

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Abstract

Benzimidazole has been considered as an important scaffold because of its wide range of pharmacological activities. The current research work was aimed to synthesis some novel benzimidazole derivatives and evaluate them by in silico and in vitro screening. A series of newer 2-substituted benzimidazole derivatives were synthesized by reacting o-phenylenediamine with various carboxylic acids in the presence of ammonium chloride as catalyst at 80-90^{0}C. All the synthesized compounds BI01, BI02, BI03, BI04 and BI05 were characterized by TLC and melting point analysis. The compounds were confirmed by IR, NMR and Mass spectroscopy. The compounds were tested in silico and in vitro methods to determine the antimicrobial activity. The results showed that the compounds, BI01 and BI04 had good docking results also they were showing good zone of inhibition against Escherichia coli.

Keywords: *Benzimidazole, In silico screening, In vitro antibacterial activity, Escherichia coli.*

1. Introduction

Benzimidazole is an aromatic bicyclic heterocyclic scaffold. It is considered as an important lead compound in medicinal chemistry and modern drug discovery. Several 2-Substituted benzimidazoles have been known to poses antibacterial **[1]** , antifungal [2], Anti-malarial [3], antimicrobial [1] and anti-protozoal, antihypertensive [4] activity against cancer [5], Antiparasitic, Antiulcer activity, anti-inflammatory [6], antioxidant property [7], anti HIV, anthelmintic [8], antidiabetic [9] and antiviral hepatitis [10, 11, 12 13] activities. Literature survey showed that among the benzimidazoles (BZD) reported, 2-substituted derivatives are found to be pharmacologically more potent and hence the design and synthesis of 2-substituted benzimidazoles are the potential area of research. In recent times, BZD is more emphasized on complementary and alternative medicines to manage and treat various cancer diseases. It is achieved very easily through the development of computer aided drug design and developmental studies.

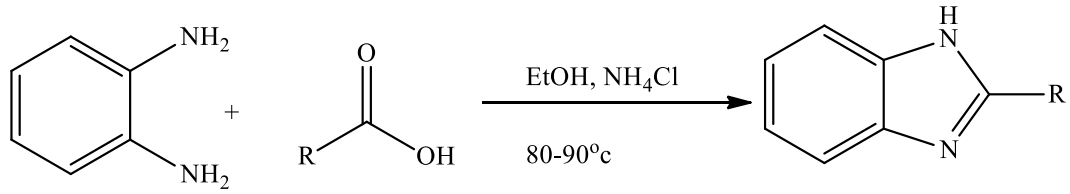
In most of the research studies it was reported that the heterocyclic possess broad range of therapeutic values, one such important heterocycle is benzimidazole. Its Derivatives are widely listed in United States Food and Drug administration. They are the nitrogen containing heterocyclic compounds and widely known for various biological and pharmacological activities like angiotensin II receptor blockers (azilsartan), anthelmintic agents (albendazole, ciclobenazole), antihistamines (astemizole, bilastine), fungicides (benomyl, carbendazim), opioids (bezitramide, brophine), Proton-Pump Inhibitors (dexlansoprazole, esomeprazole), antipsychotics (benperidol, clopimozide) etc., Further, the associations of computer in the drug design and discovery is found to be more essential in the drug research, The docking study done previously revealed that the benzimidazole scaffold forms good interactions with the amino acid residues of the target proteins or receptors through hydrogen bond or π-π conjugation and hydrophobic interactions.

As a part of an ongoing study of biologically active benzimidazoles, the present work was directed to synthesize certain novel 2-substituted benzimidazoles that comprise the aforementioned moieties in their framework in order to investigate the *in silico* and *in vitro* antimicrobial activities.

2. Materials and methods

2.1.Chemistry: General procedure for Synthesis of 2-substituted benzimidazole

Equimolar mixture of o-phenylenediamine (0.01M) and carboxylic acids (0.01M) in the presence of ammonium chloride and ethanol was refluxed in a reflux condenser for 2 hours at 80^0C. The products were then cooled and made alkaline using 10% NaOH until the red litmus turned blue. Then, Thin Layer Chromatography was performed to check out the purity of the products. Then the products were recrystallized and the melting points were recorded [14].



o-phenylenediamine carboxylic acid 2-substituted benzimidazole derivative BI 01-10

Figure 1. Synthesis of 2-substituted benzimidazole derivatives

All the chemicals used were of AR grade. The reaction was monitored using TLC method. The melting point of all synthesized compound were recorded and are uncorrected. IR Spectra were recorded on Perkin-Elmer-1800 FTIR Spectrophotometer. ^{1}H NMR spectra ($CDC1_3$) were recorded on Bruker Advance 400 NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded on LC-MS Q-T of Micro Mass analyzer (shimadzu).

2.2.In Silico Screening of 2-substituted Benzimidazole Derivatives on 3ERT

Molecular docking is a tool of computational investigation of ligand binding to a receptor. This *in silico* approach reduces the laboratory works as well as justifies chemical/physical explanations. In addition, it helps to predict the biological activity of a given ligand and for lead optimization. The X-ray crystal structure of the protein, 3ERT was downloaded from the RSCB Protein Data Bank (RCSB PDB) for the docking studies.

2.2.1. Steps Involved in Docking Studies:

To get the docking score value, at first ligand structure were drawn on the chemdraw software in mdl (.sdf) file format. And next, PDB format of protein structure were downloaded from the RCSB website i.e., 3ERT. Then pre-processed protein in Swiss PDB Viewer software and make a new folder in the desktop with attaching both the ligand along with the pre-processed macromolecule in the same. Open PyRx software and click the following (Edit-preferences-workspace-browse-ok) and close the software. Again, open PyRx software and click the following (file-import-chemical table file-next) and then choose the ligand which will display on the screen. At the bottom row, the selected option will be seen just right click and choose to minimize all and again do right click and choose to convert all to auto dock option. Then choose vina wizard option and press start, add macromolecule. Afterwards, select the ligand and macromolecule shown onto the screen, choose forward option, the docking process will start and shows the highest score value at the first and can be saved in .csv format. [15], *in silico* molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents.

2.2.3. In Vitro antimicrobial activity on E.coli

In this study, all the synthesized compounds were screened for antimicrobial activity by the disk diffusion method. The antibacterial activity of the compounds was evaluated against Gram-negative bacteria: *Escherichia coli.* In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

Nutrient agar plates were prepared by mixing agar, peptone, NaCl and beef extract in a conical flask and gently heating the mixture. The mixture was then poured onto the petri dishes which hardens on cooling. Then, the agar plates were inoculated with *Escherichia coli*. Antimicrobial activity for the five compounds BI01, BI02, BI03, BI04, and BI05 were performed using ciprofloxacin as standard. 6mm discs were prepared using Whatman filter paper. The discs used for standard were loaded with 1% concentration of ciprofloxacin. The discs used for test were loaded with 200µg, 100µg and 50µg of the benzimidazole compounds. One disc was used as solvent control. The discs were then, gently placed on the bacteria containing agar plates. The agar plates were then placed in the incubator for 24 hours to measure the zone of inhibition [16].

3. Results and discussion

3.1.Chemistry

In the present study, 2-substituted benzimidazole derivatives were synthesized from o-phenylenediamine with different carboxylic acids in the presence of ethanol and 10% NaOH. under reflux condensation in a water bath at 100^0 Celsius for 2 hours. Then the crude products were cooled and NaOH solution was mixed slowly with continuous stirring to make the products alkaline until the red litmus is turned blue. Then the recrystallization of the crude products were carried out by dissolving the crude products in the boiling water. Then the solutions were cooled on an ice bath. The filtrates were then cooled and the crystals of benzimidazole were separated, washed with cold water and dried. The dried crystals were used to determine the melting point.

Table 1. Compound code, their structure and IUPAC name.

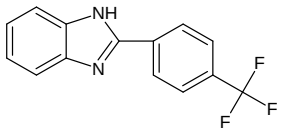
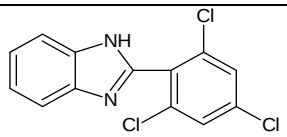
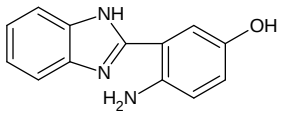
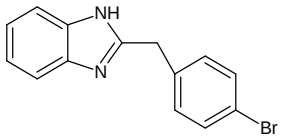
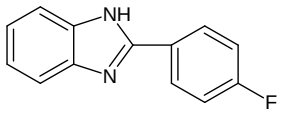
S. NO.	Compound Code	Structure	IUPAC Name	Mol. Formula	Mol. Wt. (g/mol)
1	BI01		2-[4-(trifluoromethyl) phenyl]-1*H*-1,3-benzimidazole	$C_{14}H_9F_3N_2$	262.23
2	BI02		2-(2,4,6-trichlorophenyl)-1*H*-1,3-benzimidazole	$C_7H_3Cl_3N_2$	221.5
3	BI03		4-amino-3-(1*H*-1,3-benzimidazol-2-yl) phenol	$C_{13}H_{11}N_3O$	225.25
4	BI04		2-[(4-bromophenyl) methyl]-1*H*-1,3-benzimidazole	$C_{14}H_{11}BrN_2$	287.15
5	BI05		2-(4-fluorophenyl)-1*H*-1,3-benzimidazole	$C_{13}H_9FN_2$	212.22

Table 2. Physicochemical properties of the synthesized compounds.

S. NO.	Compound code	Yield (%)	Melting Point (^{0}C)	RF value
1	BI01	81.20	120	0.83
2	BI02	75.67	172	0.82
3	BI03	61.30	194	0.91
4	BI04	66.70	80	0.79
5	BI05	85.89	200	0.67

3.1.1.Characterization of the Compound BI02:

IR Spectroscopy

The major use of infrared spectroscopy is to determine the functional group of molecules, relevant to both organic and inorganic chemistry. The compound BI02 showed the presence of the following functional groups:

Aromatic -CH: 3076cm^{-1}
-NH: 3439cm^{-1}
Aromatic C=C: 1575cm^{-1}
Alkene C=C:1643cm^{-1}

NMR Spectroscopy

NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The synthesized compound, BI02 was soluble in DMSO.

Analysis of the NMR Spectra:
Aromatic protons: 6.5 – 6.6, doublet
CH=CH: 6.1, multiplet
CH=CH: 6.46 – 6.48, multiplet
Hetero-atomic NH: 7.67 - 7.68, doublet

Mass Spectroscopy

A mass spectrum is a type of plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.

Formula: $C_7H_3Cl_3N_2$

Molecular Weight: 221.5g/mol

m/z = 221
M+ = 222
M+Z = 223
Base line correction peak was observed at 109M/Z

3.2. *In Silico* Screening of 2-substituted Benzimidazole Derivatives on 3ERT

The docking studies was carried out by using PyRx software along with different other software were used for screening. The software includes BIOVIA discovery studio, Swiss PDB viewer, Chemdraw software.

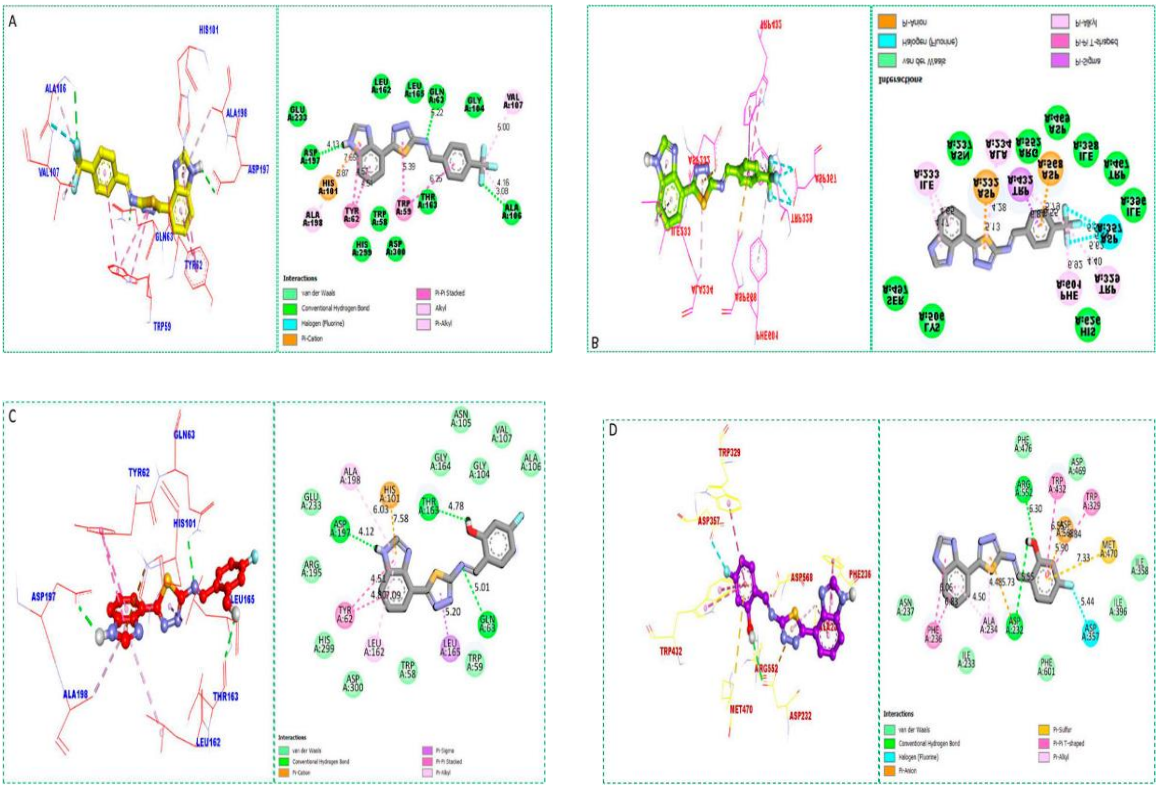


Figure 2. Docking Studies showed the interactions of the compound with the receptor.

Table 3. *In silico* screening of the compounds on 3ERT

S. No.	Compound Code	Score Value
1	BI01	-6.9
2	BI02	-6.4
3	BI03	-6.1
4	BI04	-6.8
5	BI05	-6.4

The synthesized compounds with significant potential α-amylase and α-glucosidase showed better interactions for the superimposed complex. Bonded functional groups at various positions on the aromatic ring provided analogs with a strong affinity. Trifluoro, nitro and hydroxyl-containing molecules showed stronger hydrogen bonding. The attached substituents have contributed to the good-to-poor interactions observed in most of the analogs.

Table 4. ADME properties of the compound 2-[4-(trifluoromethyl)phenyl]-1H-1,3-benzimidazole

Physicochemical Properties	
Formula	C14H9F3N2
Molecular weight	262.23 g/mol
Num. heavy atoms	19
Num. arom. heavy atoms	15
Fraction Csp3	0.07
Num. rotatable bonds	2
Num. H-bond acceptors	4
Num. H-bond donors	1
Molar Refractivity	66.53
TPSA	28.68 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.10
Log $P_{o/w}$ (XLOGP3)	4.12
Log $P_{o/w}$ (WLOGP)	5.40
Log $P_{o/w}$ (MLOGP)	3.72
Log $P_{o/w}$ (SILICOS-IT)	4.52
Consensus Log $P_{o/w}$	3.97
Water Solubility	
Log *S* (ESOL)	-4.51
Solubility	8.04e-03 mg/ml ; 3.06e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.43
Solubility	9.77e-03 mg/ml ; 3.73e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-6.32
Solubility	1.26e-04 mg/ml ; 4.82e-07 mol/l
Class	Poorly soluble
Pharmacokinetics	

GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.97 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	1.81

Table 5. ADME properties of the compound, 2-(4-Bromobenzyl)-1H-benzimidazole

Physicochemical Properties	
Formula	C14H11BrN2
Molecular weight	287.15 g/mol
Num. heavy atoms	17
Num. arom. heavy atoms	15
Fraction Csp3	0.07
Num. rotatable bonds	2
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar Refractivity	73.25
TPSA	28.68 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.22
Log $P_{o/w}$ (XLOGP3)	4.23
Log $P_{o/w}$ (WLOGP)	3.92
Log $P_{o/w}$ (MLOGP)	3.46
Log $P_{o/w}$ (SILICOS-IT)	4.57
Consensus Log $P_{o/w}$	3.68
Water Solubility	
Log *S* (ESOL)	-4.81
Solubility	4.49e-03 mg/ml ; 1.56e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.54
Solubility	8.23e-03 mg/ml ; 2.86e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-6.68
Solubility	6.01e-05 mg/ml ; 2.09e-07 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.05 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	1.81

Table 6. ADME properties of the compound, 2-(2,4,6-trichlorophenyl)-1H-benzimidazole

Physicochemical Properties	
Formula	C13H7Cl3N2
Molecular weight	297.57 g/mol
Num. heavy atoms	18
Num. arom. heavy atoms	15
Fraction Csp3	0.00
Num. rotatable bonds	1
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar Refractivity	76.56
TPSA	28.68 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.32
Log $P_{o/w}$ (XLOGP3)	5.12
Log $P_{o/w}$ (WLOGP)	5.19
Log $P_{o/w}$ (MLOGP)	4.38
Log $P_{o/w}$ (SILICOS-IT)	5.42
Consensus Log $P_{o/w}$	4.49
Water Solubility	
Log *S* (ESOL)	-5.46
Solubility	1.03e-03 mg/ml ; 3.46e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-5.47
Solubility	1.02e-03 mg/ml ; 3.42e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-7.27
Solubility	1.61e-05 mg/ml ; 5.42e-08 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.48 cm/s
Druglikeness	
Lipinski	Yes; 1 violation: MLOGP>4.15
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	1.87

Table 7. ADME properties of the compound, 2-(4-Fluorophenyl)-1H-benzimidazole

Physicochemical Properties	
Formula	C13H9FN2
Molecular weight	212.22 g/mol
Num. heavy atoms	16
Num. arom. heavy atoms	15
Fraction Csp3	0.00
Num. rotatable bonds	1
Num. H-bond acceptors	2
Num. H-bond donors	1
Molar Refractivity	61.49
TPSA	28.68 Å^2
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.93
Log $P_{o/w}$ (XLOGP3)	3.34
Log $P_{o/w}$ (WLOGP)	3.79
Log $P_{o/w}$ (MLOGP)	3.21
Log $P_{o/w}$ (SILICOS-IT)	3.98
Consensus Log $P_{o/w}$	3.25
Water Solubility	
Log *S* (ESOL)	-3.89
Solubility	2.75e-02 mg/ml ; 1.30e-04 mol/l
Class	Soluble
Log *S* (Ali)	-3.62
Solubility	5.10e-02 mg/ml ; 2.40e-04 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-5.71
Solubility	4.10e-04 mg/ml ; 1.93e-06 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes

CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.22 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.65

3.3.*In vitro* antibacterial activity of the synthesized compounds on *E.coli*

The antimicrobial activity of the compounds BI01, BI02, BI03, BI04 and BI05 were carried out by disc diffusion method. In the disc diffusion method, the zones of inhibition of the compounds were studied. The zone of inhibition shows the resistance against the bacteria. The bacterium Escherichia coli was used for the antimicrobial study. Ciprofloxacin was used as standard. 1% concentration of ciprofloxacin was used on one disc.

Table 8. Antibacterial activity of the synthesized compounds

S. No.	Compound Code	Zone of Inhibition(mm)		
		200µg	100µg	50µg
1	BI01	12	7	4
2	BI02	15	9	6
3	BI03	10	5	3
4	BI04	11	6	3
5	BI05	14	7	5

The concentrations of 200µg, 100µg and 50µg of each of the compounds BI01, BI02, BI03, BI04 and BI05 were used on separate discs. A control was placed. After incubation of about 24hours, all the five compounds showed maximum zone of inhibition for the concentration of 200µg and the least zone of inhibition for the concentration of 50µg. The disc for the standard showed zone of inhibition of 18mm. The control showed no zone of inhibition. The compounds BIO2 and BI05 showed the best resistance against *Escherichia coli.* Compound BI02 and BI05 showed good zone of inhibition of about 15mm and 14mm respectively for the concentration of 200µg.

4. Conclusion

An attempt was made to synthesize some novel 2-substitued benzimidazole derivatives through the condensation reaction of *o*-phenylenediamine and various carboxylic acids. The compounds were characterized by Thin Layer Chromatography, Melting Point, IR, NMR and Mass Spectral Analysis. The spectral study reports were satisfactory, and they confirm the structure of the compounds. The *in Silico* and *in vitro* Antimicrobial Studies revealed that the compounds BI01, BI02 and BI04 showed good docking score values and were found to be most active on E.coli. The results revealed that the compounds containing fluoro, chloro and bromo substitutions played a crucial role in determining the antibacterial activity of the benzimidazole scaffold.

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