Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

Green Synthesis of Benzimidazole Derivatives: A Sustainable Approach Towards Potent Antimicrobial Agents

Sugat Shukla[1,2], Arun Kumar[1*], Ashutosh Pathak[4*] Akhalesh Kumar[5], Kishu Tripathi[3], Kuldeep Singh[1], Desh Deepak Pandey[6]

[1]Department of Pharmacy, Integral University, Kursi Rd, Lucknow, Uttar Pradesh, India-226026

[2]Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology Lucknow, Uttar Pradesh, India

[3]Institute of Pharmacy, Sitapur Shiksha Sansthan, Resora Uttar Pradesh, India – 261001

[4]Institute Of Pharmacy, Dr. Shakuntala Misra National Rehabilitation University, Mohan Rd, Sarosa Bharosa, Lucknow, Uttar Pradesh India-226017

[5]Institute of Pharmacy, Dr. Bhimrao Ambedkar University, Chhalesar, NH-2, Agra-282006, India

[6]Yashraj Institute of Pharmacy, Gomti Nagar Extension Sector 6, Gomti Nagar, Lucknow, Uttar Pradesh, India- 226010

(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 19 August 2025)

KEYWORDS

benzimidazole, antimicrobials, environmental chemistry, Schiff base, Gram-negative, sustainable synthesis

ABSTRACT:

Introduction: Since medicinal products benzimidazole and hydrating agents are important antimicrobials, specific substances including benzimidazole hydrazone have been generated in order to test their effectiveness against bacteria. The molecular makeup of the newly synthesised compounds was clarified using the analysis of elements along with 1H-NMR measurements, infrared radiation along with ES-MS spectrum evidence. following the synthetic chemicals' assessing subsequently was discovered that the ability to kill bacteria is increased by the inclusion of Schiff bases and specific compound groups implanted beneath either benzimidazole nuclear structures along with modified nucleus. Every variant exhibited little action against microbes with Gram-negative DNA and strong performance against bacteria that are Gram-positive. When examined towards fungus, several of the freshly synthesised compounds exhibited modest potency.

Objectives: Comparative study newly microwave irradiation synthesis of substituted benzimidazole and evaluation of its antimicrobial activity which is resistance to pathogen.

Methods: The molecular makeup of the newly synthesised compounds was clarified using the analysis of elements along with 1H-NMR measurements, infrared radiation along with ES-MS spectrum evidence. The synthetic strategy included microwave-assisted reactions and TLC verification.

Results: The ability to kill bacteria was increased by the inclusion of Schiff bases and specific compound groups implanted beneath benzimidazole nuclei with modified functional groups (-Cl, -NO_2, OCH_3, -Br, CH_3, -OH). All compounds exhibited little action against Gram-negative microbes and strong activity against Gram-positive bacteria. Several compounds also showed modest antifungal potency.

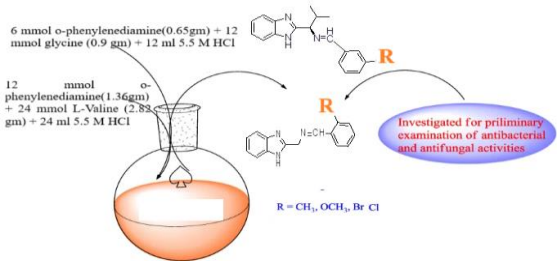
Conclusions: Schiff base-modified benzimidazole derivatives represent promising antimicrobial agents, with selective efficacy towards Gram-positive organisms and potential as sustainable drug candidates.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

Graphical Abstract:



1. Introduction

The research and development, aesthetics, and employment of chemical goods and procedures to cut down on or completely stop the manufacture and use of dangerous substances for living environments is known as "environmental chemistry" (GC). Beginning with creative theory, the GC searches for the type of item being designed and our course of action.to plan its production and applications. Design requirements must take into account the effects of chemical substances and chemical processes [1].

The effectiveness standards must also take into account potential hazards for both raw materials and finished goods. The concepts of GC began to take on a more global perspective around the beginning of the 1990s. The objective aimed to create an environmentally friendly option for chemical manufacturing and its processes. To provide guidance on the fields of advancement and study for GC applications, a group of specialists from many industrialised nations was assembled. The following areas were suggested for attention based on the GC values. Economic considerations and their potential contribution to environmentally friendly growth were the main factors in their selection [2-3].

The vast majority of illnesses are treated with heterocyclic in nature molecules, so they hold a pivotal position in the science of medicine. Benzimidazole, an insurance purine-analogue pharmacophore exhibiting a wide range of medicinal properties. action, is unique amongst aforementioned heterocycles [4]. An extremely prevalent heterocyclic in nature pharmacophore comprises the power source benzimidazole ring complex.[5] Because of highly diverse occurrence among psychoactive chemicals, these components are sometimes referred to be "fortunate." The primary emphasis revolves around the biological effects associated with the benzimidazole antagonist along with its structural stability biochemistry molecules, which are also highly recognised various marketed drug of benzimidazole shown in Fig.1[6-8].

Benzimidazole-based medicines demonstrate a large variety of distinct kinds of biological responses as a consequence of replacing moieties on core structure [9] as shown in Fig. 2. Numerous benzimidazole derivatives, as well such as antihistamines, azomycin, clotrimazole, thiabendazole, cimetidine, anti-ulcerative (omeprazole), misonidazole, and astemizole, have been discovered by researchers in the medicinal products veterinary medicine, and agricultural fields [10]

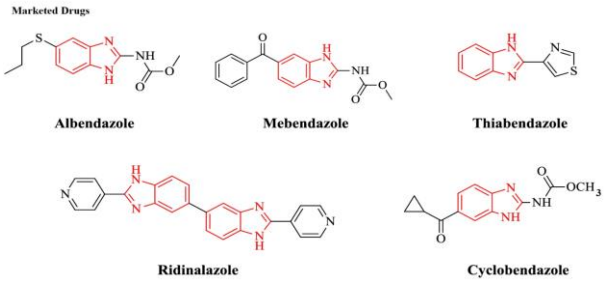


Fig. 1 Marksted drugs of benzimidazole

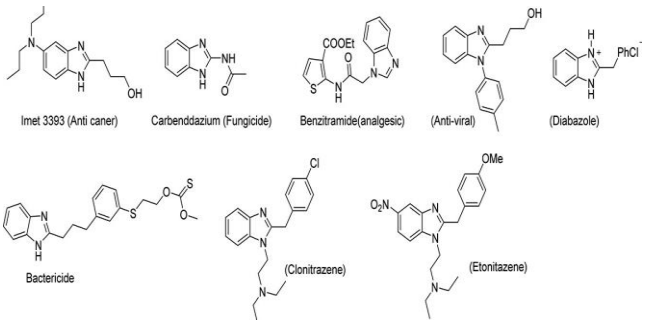


Fig.2 Benzimidazole nucleus with various substitution

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

Numerous studies on vitamin B12 have brought attention to the structure and significance of the benzimidazole nucleus. The study fragrant benzene ring's 4,5-positions merged with the 1,3-Carbon of a diazole moiety which is fundamental benzimidazole cyclic structure. One Other benzo-substituted diazoles have been classified as indazole and benzopyrazole, respectively, since they have 1, 2- and 2, 3-substitutes of five-membered aromatic in nature diazole moiety while maintaining the merged benzene as an unsubstituted heterocyclic ring. 1H-1,3-benzimidazole is the IUPAC designation for benzimidazole. Nevertheless, a number of other names have also been used, such as BZI, 1,3-diazaindene, benzimidazole, benzo-glyoxaline, 3-azaindole, and azaindole. With a molecular weight of 118.1359, the formula is $C_7H_6N_2$[11].

2. Objectives

To synthesize novel benzimidazole derivatives using a green chemistry approach and assess their structural, physicochemical, and antimicrobial properties. The goal includes evaluating these compounds' pharmacokinetics' and drug-likeness through in silico methods.

3. Methods

Compounds were synthesized using microwave-assisted reactions involving o-phenylenediamine with amino acids like glycine and L-valine in acidic conditions. Final Schiff base derivatives were formed by reaction with substituted benzaldehydes. Characterization included TLC, melting point analysis, FTIR, 1H-NMR, ES-MS, and elemental analysis. In silico ADME properties were evaluated using Swiss ADME and ProTox-II. Antimicrobial screening was done using the disc diffusion method against selected bacterial and fungal strains. Molecular docking was performed against Enterobacter cloacae (PDB ID: 6NP3) using Auto Dock 4.2.

3.1 Resources Needed

With a mortar and pestle, UV chamber, Microsil digital melting point device, microwave, FTIR-7600 with Bruker AC 200 (Lambda Scientific). Digitised thermal melting equipment was also used for identifying physical features such as melting point. In order to determine the compound's chemical composition, a TLC investigation was conducted employing the mobile phase containing of normal hexane: ethyl acetate (2:3) [12-13]. The observed average rate of flow was 1.2 ml/min, where the substance's spot explanation according to UV illumination in a UV chamber at 254 nm was also studied. Table 1 contains the tabulated findings. In addition, information on the kind and arrangement of bonds that the H atoms in the structure formed was revealed by starting and completing the experimental procedures, which comprise NMR spectroscopy carried out using TMS as standard and readings recorded using a Bruker AC 200[14].

3.2 Procedure

3.2.1 Synthesis Procedure of X1-X6

Step-1

Add o-phenylenediamine(0.65gm), glycine (0.9gm) and 12ml 5.5mol/L HCl into the beaker in sequence, wherein the molar ratio of o-phenylenediamine and glycine is 2:1; o-phenylenediamine and 5.5mol/L HCl The molar ratio is 1:10. Stir evenly, put it into a microwave oven with a frequency of 2450MHz, and irradiate it intermittently for 6 times at an output power of 220W, each time for 1min, to completely dissolve. The fully dissolved solution was irradiated intermittently for 10 times in a microwave oven at an output power of 220W, each time irradiating for 5 minutes and then resting for 10 minutes. Recrystallize the crude product with absolute ethanol to obtain 2-aminomethylbenzimidazole dihydrochloride, dissolve it in water, adjust the pH to 8-9 with ammonia water, cool to 3-5°C to complete the crystallization, Ethanol/water recrystallization to obtain 2-aminomethyl benzimidazole. [15-18]

Step-2

In a beaker add equimolar of Benzaldehyde derivatives (3-Br,3-Cl,2-OH-4Cl,4-OH,4-CH_3, 4-OCH_3) and 2-aminomethyl benzimidazole in 1 ml water, put it into a microwave oven with a frequency of 2450MHz, and irradiate it at an output power of 200W for 30 sec to 2 minutes After reaction get completed as indicated by Thin Layered Chromatography. Filtration was done to get a crude reaction mixture and was recrystallized from methanol [19].

3.2.2 Synthetic procedure of Y1-Y6

Step-1

Add o-phenylenediamine(1.36gm), L-Valine(2.82gm) and 24 ml 5.5mol/L HCl into the beaker in sequence, wherein the molar ratio of o-phenylenediamine and glycine is 2:1; o-phenylenediamine and 5.5mol/L HCl The molar ratio is 1:10. Stir evenly, put it into a

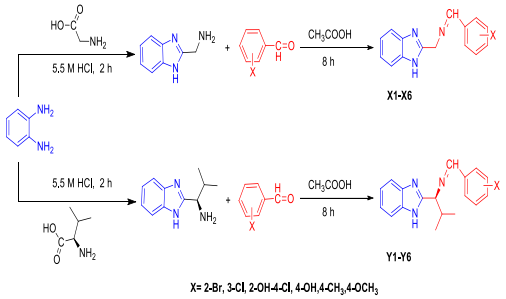
Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

microwave oven with a frequency of 2450MHz, and irradiate it intermittently for 6 times at an output power of 220W, each time for 1min, to completely dissolve. The fully dissolved solution was irradiated intermittently for 10 times in a microwave oven at an output power of 220W, each time irradiating for 5 minutes and then resting for 10 minutes. Recrystallize the crude product with absolute ethanol to obtain 2-aminomethyl-iso-propyl-lbenzimidazole dihydrochloride, dissolve it in water, and adjust the pH to 8-9 with ammonia water, cool to 3-5 ° C to complete the crystallization, Ethanol/water recrystallization to obtain 2-aminomethyl-iso-propyl-lbenzimidazole [20].

Step-2

In a beaker add equimolar of Benzaldehyde derivatives (3-Br,3-Cl,2-OH-4Cl,4-OH,4-CH_3,4-O CH_3) and 2-aminomethyl-iso-propyl-lbenzimidazole in 1 ml water, put it into a microwave oven with a frequency of 2450MHz, and irradiate it at an output power of 220W for 40 sec to 2 minutes After reaction get completed as indicated by Thin Layered Chromatography. Filtration was done to get a crude reaction mixture and was recrystallized from methanol.

Scheme 1

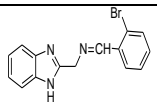
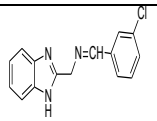
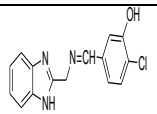
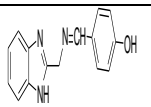
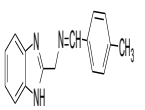
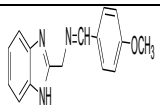


4. Results

All synthesized compounds (X1–X6 and Y1–Y6) were successfully characterized through spectral techniques confirming their expected structures. Physicochemical characterization indicated acceptable yield, solubility, and melting point profiles. In vitro antimicrobial assays revealed strong activity against Gram-positive bacteria and moderate-to-low activity against Gram-negative and fungal strains. ADMET analysis revealed favourable properties including high gastrointestinal absorption, compliance with Lipinski's Rule of Five, and minimal predicted toxicity. Molecular docking results showed good binding affinities (-6.6 to -7.8 kcal/mol), particularly for compound Y2, indicating effective interaction with the target enzyme.

Tables and figures, such as compound codes, Rf values, physical properties, ADMET parameters, and docking scores, are detailed in the supplementary material.

Table.1 Compound detail and substituents

C	Structure	X	Chemical name	Rf	Mass
X 1		2-Br	(1H-Benzoimidazol-2-ylmethyl) -(2-bromo-benzylidene) -amine	0.81	315.20 Exact Mass: 313.02
X 2		3-Cl	(1H-Benzoimidazol-2-ylmethyl) -(3-chloro-benzylidene) -amine	0.57	270.81 Exact Mass: 269.07
X 3		3-OH, 4-Cl	5-[(1H-Benzoimidazol-2-ylmethylimino)-methyl]-2-chloro-phenol	0.69	286.1 Exact Mass: 285.07
X 4		4-OH	4-[(1H-Benzoimidazol-2-ylmethylimino)-methyl]-phenol	0.75	252.1 Exact Mass: 251.11
X 5		4-CH_3	(1H-Benzoimidazol-2-ylmethyl) -(4-methyl-benzylidene) -amine	0.45	250.1 Exact Mass: 249.13
X 6		4-OCH_3	(1H-Benzoimidazol-2-ylmethyl) -	0.65	266.1 Exact Mass:

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

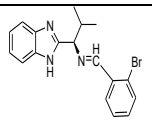
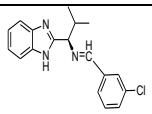
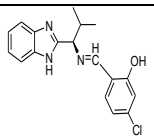
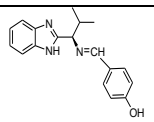
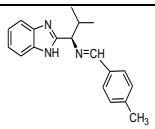
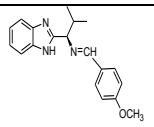
			(4-methoxy-benzylidene) -amine		265.12
Y1		2-Br	(E)-3-[(1-(4-bromophenyl) ethylidene) hydrazono]-1H-indole	0.76	356.1 Exact Mass: 355.07
Y2		3-Cl	(E)-1-(4-chlorophenyl)-2-(1H-indol-3-yl) ethylidene hydrazine	0.51	312.1 Exact Mass: 311.12
Y3		2-OH, 4-OH	2-{[1-(1H-Benzoimidazol-2-yl)-2-methyl-propylimino] -methyl}-5-chloro-phenol	0.79	328.1 Exact Mass: 327.11
Y4		4-OH	(E)-1-(4-hydroxyphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine	0.49	294.1 Exact Mass: 293.15
Y5		4-CH_3	(E)-1-(4-methylphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine	0.84	292.2 Exact Mass: 291.17
Y6		4-OCH_3	(E)-1-(4-methoxyphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine	0.66	308.2 Exact Mass: 307.17

Table.2 Physical Characterization Data of Compounds (X1-X6 and Y1-Y6)

C.C	Chemical Formula	% Yield	Colour	M.P(°C)	Solubility
X1	$C_{15}H_{12}BrN_3$	72%	Dark brown	293-295	Methanol, Ethanol
X2	$C_{15}H_{12}ClN_3$	86%	Light yellow	162-164	Methanol, Ethanol
X3	$C_{15}H_{12}ClN_3O$	67%	Light brown	128-130	Methanol, Ethanol
X4	$C_{16}H_{15}N_3$	74%	Pale yellow	236-238	Methanol, Ethanol
X5	$C_{16}H_{15}N_3O$	70%	Dark brown	183-185	Methanol, Ethanol
X6	$C_{16}H_{15}N_3O$	79%	Dark yellow	176-177	Methanol, Ethanol
Y1	$C_{18}H_{18}BrN_3$	68%	Lemon yellow	141-143	Methanol, Ethanol
Y2	$C_{18}H_{18}ClN_3$	76%	Dark brown	242-244	Methanol, Ethanol
Y3	$C_{18}H_{18}ClN_3O$	65%	Dark yellow	198-200	Methanol, Ethanol
Y4	$C_{18}H_{19}N_3O$	79%	Brick red	157-159	Methanol, Ethanol
Y5	$C_{19}H21N_3$	64%	Orange	165-167	Methanol, Ethanol
Y6	$C_{19}H_{21}N_3O$	59%	Maroon	175-177	Methanol, Ethanol

Characterization of Data (Synthesized Compounds)

(1H-Benzoimidazol-2-ylmethyl) -(2-bromo benzylidene) -amine [X1]

Dark brown colour, Rf-value : 0.81, yield: 72%, m. p :293-295°C, molecular formula: $C_{15}H_{12}BrN_3$, ^1H NMR

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

(300 MHz, δ ppm/MeOD): 8.30 (s, 1H, CH), 7.94 (m, 3H, Ph-H), 7.51 (m, 2H, Ph-H), 7.43 (m, 2H, Ph-H), 7.23 (m, 1H, Ph-H), 5.03 (s, 1H, NH), 4.92 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3345.76 N–H (benzimidazole ring), 1622.54 C=N (imine group), 1586.96 C=C (aromatic ring), 3049.87 (Aromatic C–H), 2895.97 Aliphatic C–H (benzylic CH_2), 837.68 Ph–H out-of-plane bending (para-substituted), 570.36 (C–Br stretch) ; Ele. Ana. Calculated**:** C, 57.3; H, 3.9; Br, 25.4; N, 13.4 Ele. Ana. Found: C, 56.2; H, 3.9; Br, 25.1; N, 14.1. MS (ESI) m/z: $[M+H]^+$ Cal. 315.20 Exact Mass: 313.02.

(1H – Benzoimidazol - 2-ylmethyl) - (3 -chloro-benzylidene)-amine [X2]

Yellow, Rf-value: 0.57, yield: 83%, m. p: 153-155°C, molecular formula: $C_{15}H_{12}ClN_3$, ^1H NMR (300 MHz, δ ppm/MeOD): 8.21 (s, 1H, CH), 7.93 (m, 2H, Ph-H), 7.61 (m, 2H, Ph-H), 7.32 (m, 4H, Ph-H), 4.31 (s, 1H, NH), 4.33 (s, 2H, CH2); IR (KBr, cm-1): 3010.12 (br, N–H stretching, benzimidazole), 1622.26 (s, C=N imine stretching), 1589.37 (m, aromatic C=C), 3048.78 (m, aromatic C–H), 2894.47 (w, aliphatic C–H), 785.47(m, aromatic C–H out-of-plane bend, para-substituted ring), Ele. Ana. Calculated**:** C, 66.8; H, 4.5; Cl, 13.1; N, 15.58 Ele. Ana. Found C, 66.6; H, 4.4; Cl, 13.2; N, 15.5; MS (ESI) m/z: $[M+H]^+$ Cal. 270.81, Exact Mass: 269.07.

5-[(1H-Benzoimidazol-2-ylmethylimino)-methyl]-2-chloro-phenol [X3]

brown, Rf-value: 0.69, yield: 67%, m. p: 130-131°C, molecular formula: $C_{15}H_{12}ClN_3O$, ^1H NMR (300 MHz, δ ppm/MeOD): 8.13 (s, 1H, CH), 7.82 (m, 2H, Ph-H), 7.31 (m, 2H, Ph-H), 7.11 (m,2H,Ph-H) 7.02 (m,1H.Ph-H) 5.32 (s, 1H,NH), 4.94 (s, 2H, CH_2), 4.86 (s,1H,OH); IR (KBr, cm-1): 3315.94 (br, N–H stretching, benzimidazole), 3464.89 (br, O–H stretching, phenol), 1619.95 (s, C=N imine stretching), 1576.53 (m, aromatic C=C stretching), 3045.67 (m, aromatic C–H stretching), 786.98 (m, Ph–H out-of-plane bending), 674.76 (m, C–Cl stretching) ; Ele. Ana. Calculated: C, 63.1; H, 4.2; Cl, 12.4; N, 14.7; O, 5.6, Ele. Ana. Found: C, 63.1; H, 4.2; Cl, 12.4; N, 14.7; O, 5.6; MS (ESI) m/z: $[M+H]^+$ Cal. 286.1, Exact Mass: 285.07.

4-[(1H-Benzoimidazol-2-ylmethylimino)-methyl]-phenol [X4]

Light brown, Rf-value: 0.75, yield: 61%, m. p: 179-181°C, molecular formula: $C_{15}H_{13}N_3O$, ^1H NMR (300 MHz, δ ppm/MeOD): 8.34 (s, 1H, CH), 7.73 (m, 2H, Ph-H), 7.42 (m, 2H, Ph-H), 7.31 (m, 2H, Ph-H), 6.81 (m, 2H, Ph-H) 5.22 (s, 1H, NH), 4.93 (s, 2H, CH_2), 4.74 (s, 1H, OH); IR (KBr, cm-1): 3316.98 (br, N–H stretching, benzimidazole), 3467.98 3470 (br, O–H stretching, phenol),1621.76 (s, C=N imine stretching), 1597.34 (m, aromatic C=C stretching),3356.98 (m, aromatic C–H stretching), 816.12 (m, Ph–H out-of-plane bending), 672.56 (m, C–Cl stretching). Ele. Ana. Calculated: C, 72.1; H, 5.2; N, 16.7; O, 6.3, Ele. Ana. Found: C, 71.7; H, 5.2; N, 16.7; O, 6.3. MS (ESI) m/z: $[M+H]^+$ Cal. 252.1 Exact Mass: 251.11.

(1H- Benzimidazole – 2 - ymethyl) - (4-methyl-benzylidene) -amine [X5]

Dark brown, Rf-value: 0.75, yield: 80%, m. p: 236-239°C, molecular formula: $C_{16}H_{15}N_3O$, , ^1H NMR (300 MHz, δ ppm/MeOD): 8.84 (s, 1H, CH), 8.24 (m, 2H, Ph-H), 8.13 (m, 2H, Ph-H), 7.62 (m, 2H, Ph-H), 7.53 (m, 2H, Ph-H) 5.15 (s, 1H, NH), 4.73 (s, 2H, CH_2),2.37 (s,3H, CH_3); IR (KBr, cm^{-1}): 3317.56 (br, N–H stretching, benzimidazole), 1618 (s, C=N imine stretching), 1582 (m, aromatic C=C stretching), 3020 (m, aromatic C–H stretching), 2850 (m, aliphatic C–H stretching, –CH_3), 750–810 (m, Ph–H out-of-plane bending); Ele. Ana. Calculated: C, 78.1; H, 6.1; N, 16.9, Ele. Ana. Found: C, 77.1; H, 6.16; N, 16.8. MS (ESI) m/z: $[M+H]^+$ Cal.250.1 Exact Mass: 249.13

(1H – Benzimidazole – 2 - ylmethyl) -(4-methoxy-benzylidene)-amine [X6]

Dark Yellow, Rf-value: 0.65, yield: 79%, m. p: 183-185°C, molecular formula: $C_{16}H_{15}N_3O$, ^1H NMR (300 MHz, δ ppm/MeOD): 8.20 (s, 1H, CH), 7.60 (m, 2H, Ph-H), 7.51 (m, 2H, Ph-H), 7.31 (m, 2H, Ph-H), 7.11 (m, 2H, Ph-H), 4.93 (s, 1H, NH),4.73 (s,2H, CH_2). 3.34(s,3H, OCH_3). IR (KBr, cm-1): 3305 (br, N–H stretching, benzimidazole), 1610 (s, C=N imine stretching), 1580–1510 (m, aromatic C=C stretching), 1245–1260 (s, C–O–C stretching, methoxy), 3015 (aromatic C–H stretching), 2840–2940 (C–H stretching of –OCH_3 and aliphatic C–H); Ele. Ana. Calculated: C, 72.4; H, 5.7; N, 15.8; O, 6.0, Ele. Ana. Found: C, 72.4; H, 5.7; N, 15.8; O, 6.1. MS (ESI) m/z: $[M+H]^+$ Cal. 266.1, Exact Mass: 265.12

(E)-3-[(1-(4-bromophenyl) ethylidene) hydrazono]-1H-indole [Y1]

Lemon Yellow, Rf-value: 0.76, yield: 68%, m. p: 141-143°C, molecular formula: $C_{18}H_{18}BrN_3$, ^1H NMR (300 MHz, δ ppm/MeOD): 8.22 (s, 1H, CH), 7.92 (m, 2H, Ph-H), 7.44 (m, 2H, Ph-H), 7.26(m,4H,Ph-H)4.85 (s, 1H, CH), 4.74 (s, 1H, NH), 2.18 (m, 1H, CH) 1.23 (m, 6H, CH_3); IR (KBr, cm-1): 3324.67 N–H stretch (benzimidazole), 1611.87 N–H stretch (benzimidazole),

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

1554.78 Aromatic C=C stretch, 2934.87 Aliphatic and aromatic C–H stretch, 748.76 Aryl C–Br stretch, 812.63 Aromatic C–H out-of-plane bending. Ele. Ana. Calculated: C, 60.7; H, 5.1; Br, 22.4; N, 11.8, Ele. Ana. Found: C, 60.5; H, 5.1; Br, 22.4; N, 11.7. MS (ESI) m/z: $[M+H]^+$ Cal. 356.1, Exact Mass: 355.07

(E)-1-(4-chlorophenyl)-2-(1H-indol-3-yl) ethylidene hydrazine [Y2]

Lemon Yellow, Rf-value: 0.76, yield: 69%, m. p: 243-245°C, molecular formula: $C_{18}H_{18}BrN_3$, ^{1}H NMR (300 MHz, δ ppm/MeOD): 8.14 (s, 1H, CH), 7.93 (m, 2H, Ph-H), 7.61 (m, 2H, Ph-H), 7.54 (m, 2H, Ph-H), 7.27 (m, 2H, Ph-H), 5.05 (s, 1H, NH), 4.92 (s, 1H, CH), 2.74 (m, 1H, CH) 1.11 (m, 6H, CH3); IR (KBr, cm-1): 3332.87 N–H stretch (benzimidazole), 1613.23 C=N imine stretch, 1498.87 Aromatic C=C stretch, 2899.78 Aliphatic and aromatic C–H stretch, 786,97 Aryl C–Cl stretch, 817.85 Aromatic C–H out-of-plane bending. Ele. Ana. Calculated: C, 69.3; H, 5.8; Cl, 11.4; N, 13.5, Ele. Ana. Found: C, 69.8; H, 5.9; Cl, 11.8; N, 13.8. MS (ESI) m/z: $[M+H]^+$ Cal. 312.1 Exact Mass: 311.12.

2-{[1-(1H-Benzoimidazol-2-yl)-2-methyl-propylimino]-methyl}-5-chloro-phenol [Y3]

Dark yellow, Rf-value: 0.76, yield: 65%, m. p: 198-200°C, molecular formula: $C_{18}H_{18}ClN_3O$, ^{1}H NMR (300 MHz, δ ppm/MeOD): 8.24 (s, 1H, CH), 7.81 (m, 2H, Ph-H), 7.31 (m, 3H, Ph-H) ,6.93 (m, 2H, Ph-H), 5.17 (s, 1H, CH), 4.98 (s, 1H, NH),4.77(s,1H,OH) 2.16 (m, 1H, CH) 1.22 (m, 6H, CH_3); IR (KBr, cm-1): 3376.36 O–H (phenolic) stretch (broad), 3294.67 N–H stretch (benzimidazole), 1613.73 C=N (imine) stretch, 1534.74 Aromatic C=C stretches, 2876.67 C–H (aliphatic/aromatic) stretches, 785.67 Aryl C–Cl stretch, 798.78 Aromatic C–H out-of-plane bending; Ele. Ana. Calculated: C, 66.1; H, 5.5; Cl, 10.8; N, 12.8; O, 4.9, Ele. Ana. Found: C, 66.1; H, 5.5; Cl, 10.8; N, 12.8; O, 4.9 MS (ESI) m/z: $[M+H]^+$ Cal. 328.1Exact Mass: 327.11.

(E)-1-(4-hydroxyphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine [Y4]

Brick red, Rf-value: 0.49, yield: 79%, m. p: 157-159°C, molecular formula: $C_{18}H_{19}N_3O$, ^{1}H NMR (300 MHz, δ ppm/MeOD): 8.09 (s, 1H, CH), 7.86 (m, 2H, Ph-H), 7.41 (m, 2H, Ph-H) ,7.23 (m, 2H, Ph-H), 6.81(m,2H, Ph-H) 5.28 (s, 1H, CH), 4.94 (s, 1H, NH),4.71(s,1H,OH) 2.22(m, 1H, CH) 1.01 (m, 6H, CH_3); IR (KBr, cm-1): 3376.89 O–H (phenolic) stretch (broad), 3267.78 N–H stretch (benzimidazole), 1613.89 C=N (imine) stretch, 1498.78 Aromatic C=C stretches, 1456.22 Aromatic C=C stretches, 2876.98 Aliphatic and aromatic C–H stretches, 832.13 Aromatic C–H out-of-plane bending; Ele. Ana. Calculated: C, 73.69; H, 6.53; N, 14.32; O, 5.45, Ele. Ana. Found: C, 74.1; H, 6.5; N, 14.5; O, 6.1, MS (ESI) m/z: $[M+H]^+$ Cal. 294.1Exact Mass: 293.15.

(E)-1-(4-methylphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine [Y5]

Orange, Rf-value: 0.84, yield: 64%, m. p: 165-167°C, molecular formula: $C_{19}H21N_3$, ^{1}H NMR (300 MHz, δ ppm/MeOD): 8.41 (s, 1H, CH), 8.11 (m, 2H, Ph-H), 7.49 (m, 2H, Ph-H), 7.32 (m, 2H, Ph-H), 7.01 (m, 2H, Ph-H) 5.25 (s, 1H, NH), 4.73 (s, 2H, CH_2),2.37(s,3H,CH_3) 2.11(m, 1H, CH) 1.22 (m, 6H, CH_3); IR (KBr, cm-1):3289.67 N–H (stretch, benzimidazole), 1612.67 C=N (imine), 1498.74 Aromatic C=C, 1526.78 Aromatic C=C, 2912.89 C–H (aliphatic and aromatic), 1367.35 Methyl group (–CH_3 bending), 823.73 Aromatic C–H out-of-plane bending; Ele. Ana. Calculated: C, 78.3; H, 7.3; N, 14.4, Ele. Ana. Found: C, 78.3; H, 7.3; N, 14.4, MS (ESI) m/z: $[M+H]^+$ Cal. 292.2 Exact Mass: 291.17.

(E)-1-(4-methoxyphenyl)-2-(1H-indol-3-yl) ethylidene hydrazine [Y6]

maroon, Rf-value: 0.66, yield: 59%, m. p: 175-177°C, molecular formula: $C_{19}H_{21}N_3O$, ^{1}H NMR (300 MHz, δ ppm/MeOD): 8.08 (s, 1H, CH), 7.75 (m, 2H, Ph-H), 7.57(m, 2H, Ph-H), 7.29 (m, 2H, Ph-H), 7.12 (m, 2H, Ph-H), 4.73 (s, 1H, NH),4.43(s,1H,CH).3.36(s,3H,OCH_3) 2.13 (m, 1H, CH) 1.21 (m, 6H, CH_3); IR (KBr, cm-1):3289.67 N–H (stretch, benzimidazole), 1612.67 C=N (imine), 1498.74 Aromatic C=C, 1526.78 Aromatic C=C, 2912.89 C–H (aliphatic and aromatic), 1367.35 Methyl group (–CH_3 bending), 823.73 Aromatic C–H; Ele. Ana. Calculated: C, 74.2; H, 6.89; N, 13.7; O, 5.2, Ele. Ana. Found: C, 74.2; H, 6.9; N, 13.7; O, 5.2, MS (ESI) m/z: [M+H] + Cal. 308.2 Exact Mass: 307.17.

ADME Studies

A comprehensive evaluation of the drug-likeness and pharmacokinetic profiles of the synthesized benzimidazole derivatives (X1–X6 and Y1–Y6) was performed using established in silico models. Physicochemical properties including molecular weight, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), topological polar surface area (TPSA), lipophilicity (logP), and compliance with Lipinski's Rule of Five were assessed using the Swiss ADME [21] web tool. Additionally, predictive models for oral

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

bioavailability, gastrointestinal absorption, and blood-brain barrier (BBB) permeability were applied.

Table 3, Benzimidazole derivative calculations using Veber's rule and Lipinski's rule of five (X1-X6 and Y1-Y6)

Lipinski Rule of Five						**Veber's Rule**
C.C.	**Log P (<5)**	**Mol. Wt. (<500 g/mol)**	**HBA (<10)**	**HBD (<5)**	**Violations**	**No. of Rotatable bonds (<10)**
X1	3.34	314.18 g/mol	2	1	0	3
X2	3.24	269.73 g/mol	2	1	0	3
X3	3.03	285.73 g/mol	3	2	0	3
X4	2.39	251.28 g/mol	3	2	0	3
X5	3.14	249.31 g/mol	2	1	0	3
X6	2.84	265.31 g/mol	3	1	0	4
Y1	4.32	356.26 g/mol	2	1	0	4
Y2	4.23	311.81 g/mol	2	1	0	4
Y3	3.83	327.81 g/mol	3	2	0	4
Y4	3.28	293.36 g/mol	3	2	0	4
Y5	4.04	291.39 g/mol	2	1	0	4
Y6	3.68	307.39 g/mol	3	1	0	5

Table 4. Pharmacokinetics and drug-like characteristics of Benzimidazole derivatives

Pharmacokinetics									**Drug likeness**	
C.C.	GIabs	BBBpen	P-gpsub	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Log Kp (skin permeation/s)	Bioavailability Score
				Inhibitors						
X1	H	√	×	Y	Y	N	Y	N	-5.77	0.55
X2	H	√	×	√	√	×	√	√	-5.54	0.55
X3	H	√	×	√	√	×	√	√	-5.89	0.55
X4	H	√	×	√	×	×	√	×	-6.13	0.55
X5	H	√	×	√	√	×	√	√	-5.61	0.55
X6	H	√	×	√	√	×	√	√	-5.98	0.55
Y1	H	√	×	√	√	√	√	√	-5.05	0.55
Y2	H	√	×	√	√	√	√	√	-4.83	0.55
Y3	H	√	×	√	√	√	√	√	-5.18	0.55
Y4	H	√	√	√	√	√	√	×	-5.41	0.55
Y5	H	√	×	√	√	√	√	√	-4.89	0.55
Y6	H	√	×	√	√	√	√	√	-5.26	0.55

√: Yes, ×: No, H: High

Table 5. Benzimidazole derivatives' physicochemical characteristics

C.C.	**Num. heavy atoms**	**Num.heavy atoms**	**Molar Refractivity**	**Log S (ESOL)**	**Solubility(mg/ml) _**	**Fraction Csp3**
X1	19	15	81.74	-4.35	1.41e-02 mg/ml	0.07
X2	19	15	79.05	-4.03	2.49e-02 mg/ml	0.07
X3	20	15	81.07	-3.88	3.12e-04 mg/ml	0.07
X4	19	15	76.06	-3.30	1.27e-01 mg/ml	0.07

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

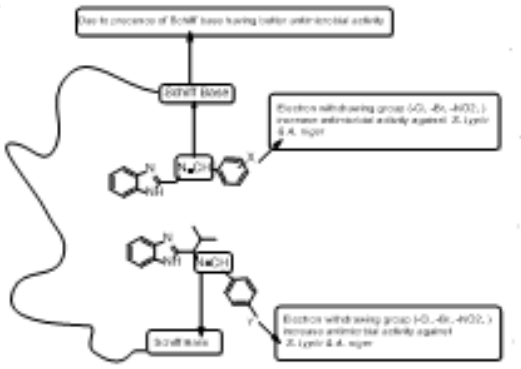
X5	19	15	79.00	-3.47	4.56e-02 mg/ml	0.12
X6	20	15	80.53	-3.50	9.64e-02 mg/ml	0.12
Y1	22	15	96.16	-5.33	1.37e-03 mg/ml	0.22
Y2	22	15	93.47	-5.01	3.07e-03 mg/ml	0.22
Y3	23	15	95.49	-4.86	4.49e-03 mg/ml	0.22
Y4	22	15	90.48	-4.27	1.56e-02 mg/ml	0.22
Y5	22	15	93.43	-4.72	5.61e-03 mg/ml	0.26
Y6	23	15	94.95	-4.48	1.01e-02 mg/ml	0.26

Table 3. Summarizes Lipinski and Veber compliance, while Table 4. Provides pharmacokinetic parameters including GI absorption, BBB permeability, and CYP inhibition data. Table 5. Lists physicochemical parameters such as molar refractivity and solubility.

Taken together, the ADMET profiling underscores that the synthesized benzimidazole-based Schiff bases possess acceptable pharmacokinetic characteristics, high oral bioavailability potential, and a favourable in silico safety margin, justifying their further biological evaluation.

SAR of synthesized compound [25-30]

- Compounds (X1-X6 and Y1-Y2) are examples of benzimidazole hybrids whose antibacterial activity can be enhanced by substituted molecules in the "4" or "5" positions of the benzimidazole nucleus.
- When benzimidazole hybrids have a para- or ortho-substituted phenyl group in the "1" position, their antibacterial activity may be enhanced.
- The antibacterial action is enhanced by the presence of the Schiff bases bridge, which joins the substituted benzine ring with benzimidazole.
- Other heterocycles in the molecule, are grafted on the benzimidazole and Schiff bases linked with substituted benzine nuclei, and their presence enhances the compounds' antimicrobial activity.
- Special groups, including -F, -chlorine, -bromine, -CF3, -NO_2, -CN, -CHO, -OH, and other heterocycles in the molecule, are grafted on the benzimidazole and Schiff bases linked with substituted benzine nuclei, and their presence enhances the compounds' antimicrobial activity.



Employing Gentamycine and Ampicelline as recommendations, the experiment was carried out employing the technique of disc diffusion [31-32] with minor adjustments for the produced chemical compounds. The species of Bacillus cereus, a type of Escherichia coli, Saccharomyces cerevisiae, and *Aspergillus niger,* among others, were the strains of bacteria and fungi that the produced chemical compounds were tested against. The Whatman A 5-mm-diameter filter paper disc (number 1) was autoclaved for 15 minutes at 121°C to sterilise it. Multiple chemicals (600 µg/disk) have been dropped onto the disinfected discs. The studied pathogens' culture medium was used to evenly inoculate the outermost layers of plates made from agar. After placing the saturated discs on the medium being used with appropriate spacing, the dishes underwent incubation for one hour at 5 °C to allow for adequate diffusion. After that, they were moved to an incubator set at 37 °C for 24 hours for bacteria and 28 °C for 72 hours for yeast and fungus. We looked at the zones of inhibition that the different chemicals produced on the microbes. Table 6 presents the findings from the first diagnostic test [33-37].

Table.6. Outcome of the investigated substances' antibacterial efficacy

C. No.	*Bacillus cereus*	*Escherichia coli*	*Saccharomyces cerevisae*	*Aspergillus niger*
Genta	+++	+++	+++	-

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

mycine				
Y1	+++	++	+	-
Y2	+++	+	++	+
Y3	+	+	+	+
Y4	++	-—	+	-
Y5	+	++	-	-
Y6	+	-	-	-

Table 7. Outcome of the investigated substances' antibacterial efficacy

C. No.	*Bacillus cereus*	*Escherichia coli*	*Saccharomyces cerevisae*	*Aspergillus niger*
Gentamycine	+++	+++	-	-
X1	+++	++	++	++
X2	+++	-		+
X3	++	+++	++	+
X4	++	-	-	-
X5	+	++	+	++
X6	+	++	-	+

Symbolic key: +++ indicates excessive activity (inhibition region > 12 mm). ++ indicates moderate activity levels (inhibition region 9–12 mm). + (suppression region 6 - 9 mm) = partially active, Inactive = - (inhibition the region < 6 mm) [38-42]

Molecular docking

Molecular docking simulations was conducted for exploring the binding interactions between synthesized ligands and Enterobacter cloacae enzyme (PDB ID: 6NP3). Docking was performed using autodocking 4.2, and binding modes were visualized via BIOVIA Discovery Studio [43-46].

Table-7. The synthetic benzimidazole compounds' docking score

Ligand	Binding Affinity (k.cal)	RMSD lower bound	RMSD upper bound
X1	-6.6	1.45	1.96
X2	-7.0	0.0	0.0
X3	-7.4	0.0	0.0
X4	-7.2	0.0	0.0
X5	-7.1	0.0	0.00
X6	-6.9	1.43	2.21
Y1	-7.5	0.0	0.0
Y2	-7.8	0.0	0.0
Y3	-7.6	0.0	0.0
Y4	-7.7	0.0	0.0
Y5	-7.3	0.0	0.0
Y6	-7.1	0.0	0.0

Where: RMSD. Root Mean Square Deviation

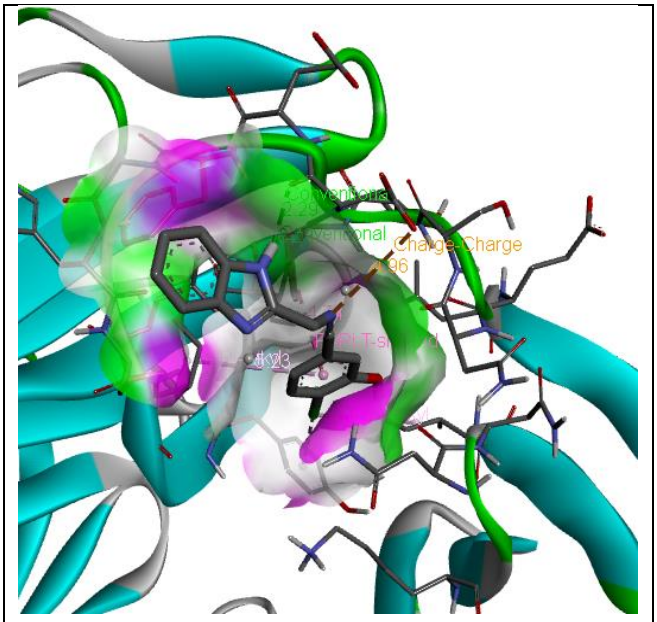


Fig. 3. Compound X3 interaction in three dimensions

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

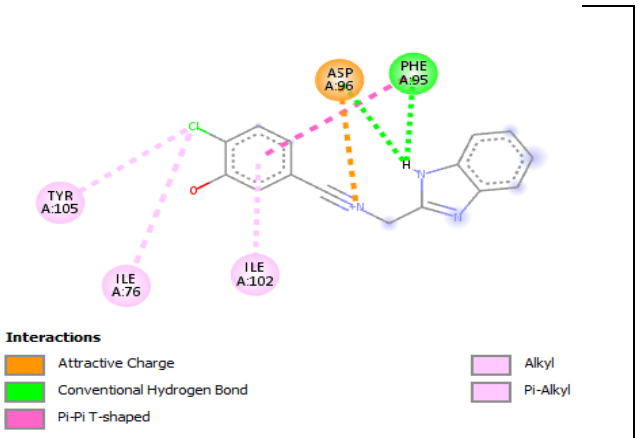


Fig. 4. Two-dimensional compound X3 interaction

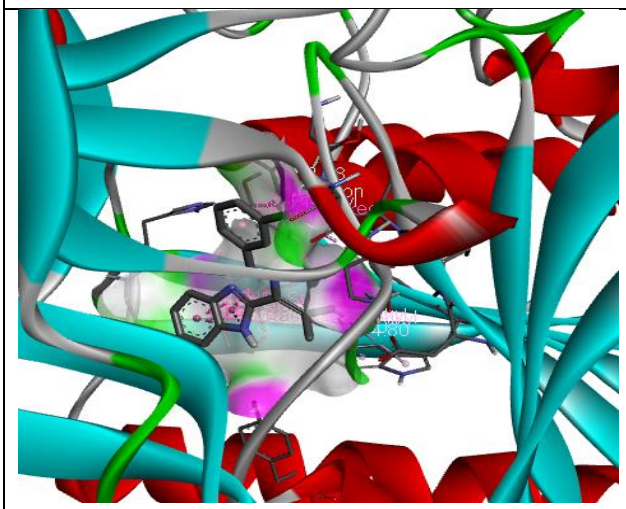


Fig.5 Compound Y2 interaction in three dimensions

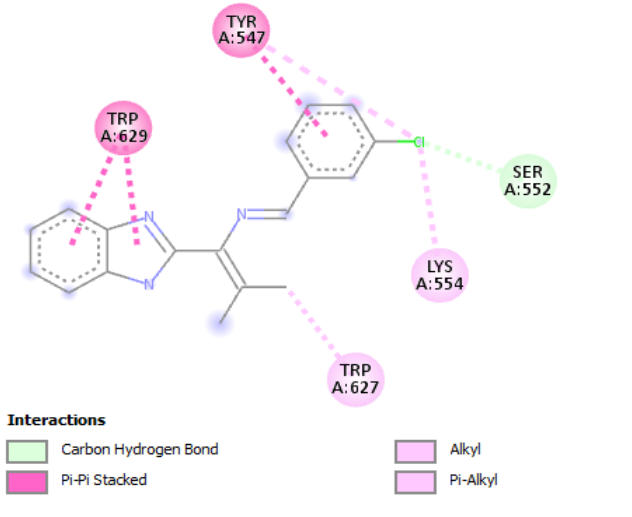


Fig. 6. Two-dimensional compound Y2 interaction

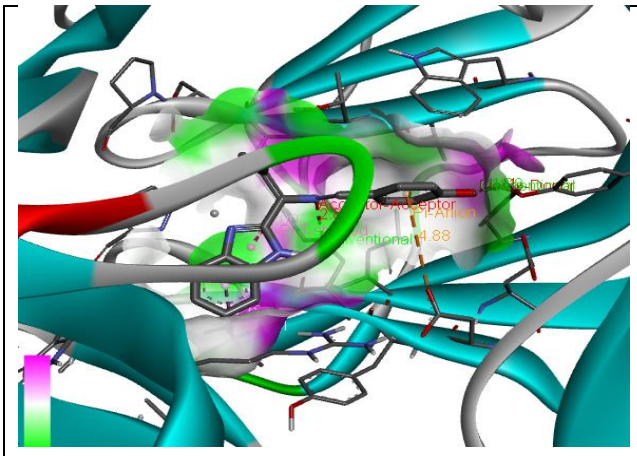


Fig.7CompoundY3 interaction in three dimensions

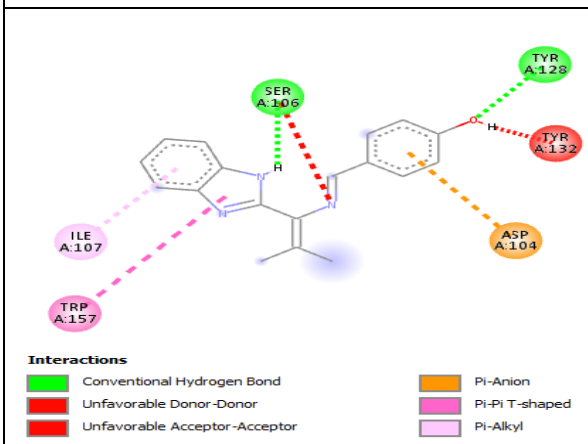


Fig. 8. Two-dimensional compound Y3 interaction

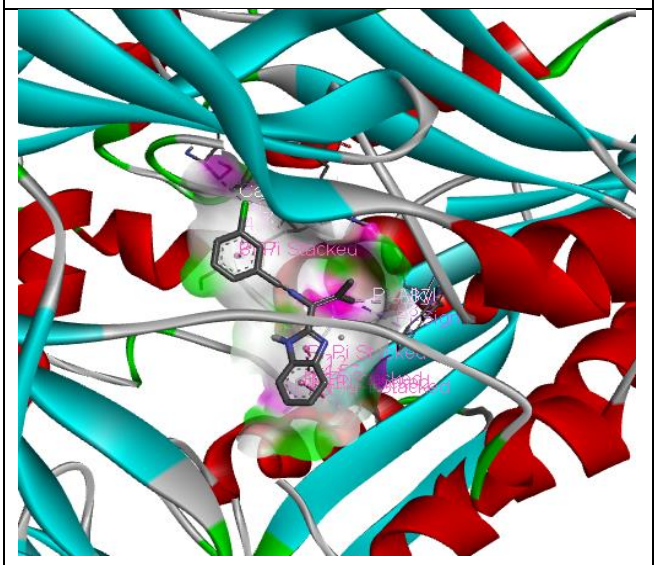


Fig.9 Compound Y4 interaction in three dimensions

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

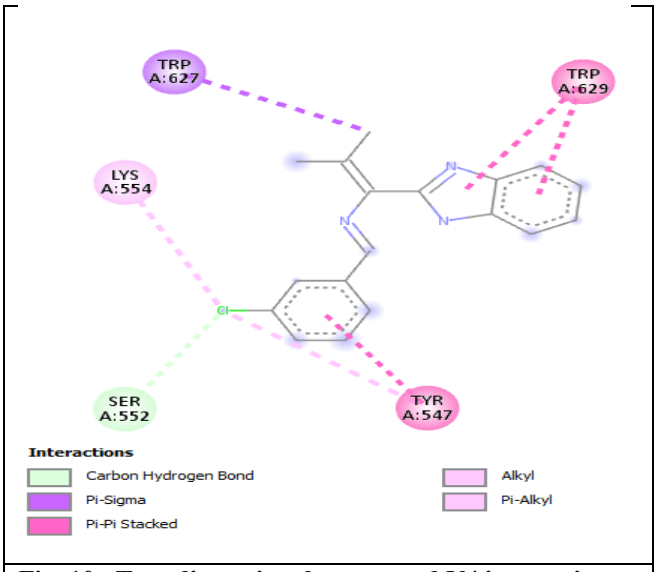


Fig. 10. Two-dimensional compound Y4 interaction

5. Discussion

The inclusion of functional groups such as -Br, -Cl, -OH, and -OCH3 enhanced the antimicrobial potency of the benzimidazole core. Schiff base formation improved lipophilicity and membrane permeability. SAR findings supported the relevance of substitution patterns on biological activity. The compounds demonstrated desirable drug-like properties with good pharmacokinetic profiles and low toxicity predictions. The docking interactions validated the structural optimization strategy for targeting bacterial enzymes.

ADME Studies

All compounds demonstrated favourable molecular weights (<500 Da), [22-24] and the number of HBD and HBA remained within acceptable ranges (≤5 and ≤10, respectively). The logP values, indicative of lipophilicity, ranged between 2.1 and 4.5, suggesting adequate membrane permeability while minimizing the risk of nonspecific binding. TPSA values fell within the optimal window (<140 Å²), supporting the likelihood of good intestinal absorption.

Notably, all synthesized compounds fulfilled the criteria outlined by Lipinski, Ghose, Veber, and Egan, reinforcing their potential as orally bioavailable agents. Most derivatives were predicted to exhibit high gastrointestinal absorption, and several candidates (notably X_4, X_6, Y_4, and Y_6) demonstrated properties suggesting limited BBB permeability, potentially reducing CNS-related side effects—a desirable feature for antimicrobial agents.

To further evaluate the safety profile, computational toxicity predictions were performed using ProTox-II. The majority of compounds were classified within toxicity class IV or V, indicating a low probability of acute oral toxicity (LD_{50} > 300 mg/kg). No significant hepatotoxicity, cardiotoxicity, or mutagenic liability was predicted for any derivative.

Bio screening. evaluate for antimicrobial capability

Compound X1, X2, Y1, Y2 excessively inhibit *Bacillus cereus*_While X3, X4, Y4 moderately inhibit Bacillus *cereus*_and X5, X6, Y3, Y5. Y6 partially active against *Bacillus cereus*_ X3 inhibit *Escherichia coli*_*excessively*.

These compounds have moderate to partial activity against *Bacillus cereus*, *Escherichia coli, Saccharomyces cerevisae*_ *Aspergillus niger.*

Molecular docking

- Binding affinities ranged from –6.6 to –7.8 kcal/mol, indicating strong interactions with the active site.
- Compound Y2 exhibited the highest binding affinity (–7.8 kcal/mol), followed by Y4 (–7.7 kcal/mol), and Y3 (–7.6 kcal/mol).
- RMSD values were ≤1.45 Å (lower bound), suggesting excellent docking precision.

Key interactions involved

- π–π stacking with amino acids TYR547, TRP629, and TRP157,
- π-alkyl interactions with ILE76, TYR105, and LYS554,
- Hydrogen bonding with PHE95, ASP96, and TYR128,
- Occasional π-anion interactions and carbon–hydrogen bonds, enhancing complex stability.

Environmentally sustainable synthesis of benzimidazole derivatives led to potent antimicrobial compounds with selective efficacy. The results of in vitro, in silico, and docking analyses collectively suggest the potential of these derivatives for development into novel antimicrobial agents.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

During the conceptualisation, production, along with usage of chemical-based goods, environmentally friendly chemistry (GC), which is refers to a set of guidelines intended minimise or eliminate the consumption or creation of potentially toxic substances [44]. Considerations on the usage of hazardous compounds and the handling of potentially harmful substances will be made as we create novel chemical-based syntheses. It is necessary to think about the general environmental problems associated with these procedures as well as if hazardous material may require special disposal. As the most significant nuclei in many medications, benzimidazole's are employed in a wide range of applications and are extremely valuable to humanity. Twelve undiscovered medicines determined to be benzimidazoles have been developed as well as their chemical makeup subsequently verified. The previously produced molecules somewhat outperformed the standard of reference prescription medications in terms of biological function when compared with examined microorganisms. Their spectral observations (IR, 1H NMR, 13C NMR, and MS spectra) guaranteed the structures of the freshly synthesised benzimidazoles. When compared to the reference medication, compounds X1, X2, and Y1, Y2 had the strongest antimicrobial activity among the majority of these novel drugs [45-46].

Conflicts of Interest

No potential conflicts of interest are disclosed by the authors.

Funding

The authors gratefully acknowledge Integral University, Lucknow, for providing the facilities to carry out research work and financial support. The manuscript bears communication number IU/R&D/2025-MCN0003733 from Integral University, Lucknow.

References

1. Kadam UD, Pandav RS, Arde SM, Hossain MS, Dinesh A, Radhakrishnan K, Ayyar M, Patil RP, Shirke BS. Green synthesis of benzimidazole scaffolds using copper-substituted zinc aluminate in a sol-gel process. Journal of the Indian Chemical Society. 2025 Jan 1;102(1):101494.
2. Zhou K, Sheng X, Liu H, Ye Z, Gao J, Xie F. Green synthesis of benzimidazoles via the palladium catalysed one-pot reductive coupling of nitroaryl amines with nitriles. Journal of Molecular Structure. 2025 Feb 13:141747.
3. Jamil I, Nawaz F, Shafiq M, Rashid M, Akram A, Siddique A, Taimur S, Zahr T. A recent trend on green synthesis and bioactivity of imidazole. Universal Journal of Green Chemistry. 2024 May 13:50-88.
4. Srivastava M, Singh K, Kumar S, Hasan SM, Mujeeb S, Kushwaha SP, Husen A. In silico Approaches for Exploring the Pharmacological Activities of Benzimidazole Derivatives: A Comprehensive Review. Mini Reviews in Medicinal Chemistry. 2024 Sep 1;24(16):1481-95.
5. Rios-Soto L, Hernández-Campos A, Tovar-Escobar D, Castillo R, Sierra-Campos E, Valdez-Solana M, Téllez-Valencia A, Avitia-Domínguez C. Inhibition of Shikimate Kinase from Methicillin-Resistant Staphylococcus aureus by Benzimidazole Derivatives. Kinetic, Computational, Toxicological, and Biological Activity Studies. International Journal of Molecular Sciences. 2024 Jan;25(10):5077.
6. Garg A, Dureja D, Pasricha R, Saini PD, Bhalla A. Recent progress in synthetic strategies for novel β-lactams linked with five-membered heterocycles (N/O/S): advances in medicinal chemistry (2020–2025). Medicinal Chemistry Research. 2025 Apr 12:1-32.
7. Balaes T, Mangalagiu V, Antoci V, Amariucai-Mantu D, Diaconu D, Mangalagiu II. Hybrid Bis-(Imidazole/Benzimidazole)-Pyridine Derivatives with Antifungal Activity of Potential Interest in Medicine and Agriculture via Improved Efficiency Methods. Pharmaceuticals. 2025 Mar 28;18(4):495.
8. Shafiurrahman, Hasan SM, Singh K, Kumar A, Suvaiv, Bano J, Shahanawaz M, Ahmad S, Kushwaha SP. Revolutionizing Quinolone Development for DNA Gyrase Targeting; Discovering the Promising Approach to Fighting Microbial Infections. Anti-Infective Agents. 2024 Oct 16.
9. Yadav KP, Rahman MA, Nishad S, Maurya SK, Anas M, Mujahid M. Synthesis and biological activities of benzothiazole derivatives: A review. Intelligent Pharmacy. 2023 Oct 1;1(3):122-32.
10. Pathak A, Soni N, Mishra B, Kumar P, Shukla S, Khan SA, Pandey DD, Yadav SK, Yadav A, Pandey10 AR. Microwave Assisted Synthesis,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2634-2649 | ISSN:2251-6727

Design Including Docking of Benzimidazole Substituted 4-Thiazolidinone Derivatives.

11. Nishanth Rao, R.; Jena, S.; Mukherjee, M.; Maiti, B.; Chanda, K. Green synthesis of biologically active heterocycles of medicinal importance: A review. *Environ. Chem. Lett.* 2021, *19*, 3315–3358.
12. Mader, M.; de Dios, A.; Shih, C.; Bonjouklian, R.; Li, T.; White, W.; de Uralde, B.L.; Sánchez-Martinez, C.; del Prado, M.; Jaramillo, C. Imidazolyl benzimidazoles and imidazo [4,5-b] pyridines as potent p38α MAP kinase inhibitors with excellent in vivo antiinflammatory properties. *Bioorg. Med. Chem. Lett.* 2008, *18*, 179–183.
13. Chen, M.; Su, S.; Zhou, Q.; Tang, X.; Liu, T.; Peng, F.; He, M.; Luo, H.; Xue, W. Antibacterial and antiviral activities and action mechanism of flavonoid derivatives with a benzimidazole moiety. *J. Saudi Chem. Soc.* **2021**, *25*, 101194.
14. Shukla S, Kumar A, Kumar A, Mishra R. Exploring the Antidiabetic Potential of Novel Benzimidazole Analogs: Synthesis, Molecular Docking and DPP-4 Activity Evaluation. ajc [Internet]. 2025 May 27 [cited 2025 May 30];37(6):1487-95. Available from: https://asianpubs.org/index.php/ajchem/article/view/37_6_29
15. W. Rong, X. Yanhua, H. Jun, Z. Taoyu and Y. Peng, China Patent CN102408377A (2012).
16. M. Bolin, D. Ping and L. Shufang, China Patent CN101875638A (2010).
17. Y. Shirakawa and S. Miura, Japan Patent JP2016216595A (2016).
18. Adam MS, El-Hady OM, Makhlouf MM, Bayazeed A, El-Metwaly NM, Mohamad AD. Effect of oxy-vanadium (IV) and oxy-zirconium (IV) ions in O, N-bidentate arylhydrazone complexes on their catalytic and biological potentials that supported via computerized usages. Journal of the Taiwan Institute of Chemical Engineers. 2022 Mar 1;132:104168.
19. Suvaiv KS, Hasan SM, Kushwaha SP, Kumar A, Ahmad IZ, Kumar P. Design, Molecular Docking, Synthesis, and Antibacterial Activity of 1H-Benzimidazole-2-Carboxylic Acid (2-Oxo-1, 2-Dihydro-Indol-3-Ylidene)-Hydrazide Derivatives. Indian Journal of Heterocyclic Chemistry. 2023 Apr;33(02):249-56.
20. Kumar A, Sharma S, Mishra S, Ojha S, Upadhyay P. ADME prediction, structure-activity relationship of boswellic acid scaffold for the aspect of anticancer & anti-inflammatory potency. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents). 2023 Aug 1;23(13):1499-505. https://doi.org/10.2174/1871520623666230417080437
21. Khan S, Kale M, Siddiqui F, Nema N. Novel pyrimidine-benzimidazole hybrids with antibacterial and antifungal properties and potential inhibition of SARS-CoV-2 main protease and spike glycoprotein. Digital Chinese Medicine. 2021 Jun 1;4(2):102-19. https://doi.org/10.1016/j.dcmed.2021.06.004
22. Krzywinski M, Altman N. Points of significance: Significance, P values and t-tests. Nature methods. 2013 Nov 1;10(11). https://doi.org/10.1038/nmeth.2698.
23. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews. 1997 Jan 15;23(1-3):3-25. https://doi.org/10.1016/j.addr.2012.09.019
24. Vasil'ev, P.M.; Kalitin, K.Y.; Spasov, A.A.; Grechko, O.Y.; Poroikov, V.V.; Filimonov, D.A.; Anisimova, V.A. Search for new dugs prediction and study of anticonvulsant properties of benzimidazole derivatives. *Pharm. Chem. J.* **2017**, *50*, 775–780.
25. Othman, D.I.A.; Hamdi, A.; Tawfik, S.S.; Elgazar, A.A.; Mostafa, A.S. Identification of new benzimidazole-triazole hybrids as anticancer agents: multi-target recognition, in vitro and in silico studies. *J. Enzym. Inhib. Med. Chem.* **2023**, *38*, 2166037.
26. Bali, A.; Bansal, Y.; Sugumaran, M.; Saggu, J.S.; Balakumar, P.; Kaur, G.; Bansal, G.; Sharma, A.; Singh, M. Design, synthesis, and evaluation of novelly substituted benzimidazole compounds as angiotensin II receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3962–3965.
27. Dunwell, D.W.; Evans, D.; Smith, C.E.; Williamson, W.N. Synthesis and antiinflammatory

activity of some 2-substituted alpha. -methyl-5-benzimidazoleacetic acids. *J. Med. Chem.* **1975**, *18*, 692–694.
28. Sabat, M.; VanRens, J.C.; Laufersweiler, M.J.; Brugel, T.A.; Maier, J.; Golebiowski, A.; De, B.; Easwaran, V.; Hsieh, L.C.; Walter, R.L. The development of 2-benzimidazole substituted pyrimidine-based inhibitors of lymphocyte specific kinase (Lck). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5973–5977.
29. Taniguchi, K.; Shigenaga, S.; Ogahara, T.; Fujit su, T.; Matsuo, M. Synthesis and antiinflammatory and analgesic properties of 2-amino-1H-benzimidazole and 1, 2-dihydro-2-iminocycloheptimidazole derivatives. *Chem. Pharm. Bull.* **1993**, *41*, 301–309.
30. Shukla S, Azad I, Akil M, Owais M, Siddiqui B, Verma S, Singh K, Kumar A. Exploring the Therapeutic Potential of Benzimidazole Hybrids as Antimicrobial Agents: An In-Depth Analysis. Anti-Infective Agents. 2025 Jan 23.
31. Aroua LM, Alminderej FM, Almuhaylan HR, Alosaimi AH, Medini F, Mohammed HA, Almahmoud SA, Khan RA, Mekni NH. Benzimidazole (s): synthons, bioactive lead structures, total synthesis, and the profiling of major bioactive categories. RSC advances. 2025;15(10):7571-608.
32. PATHAK A, NEETU S, SINGH R, KUMAR S, KUSHWAHA S, RANA P, SHALU AK, PANDEY DD, VERMA D, SINGH S, BHATT P. A Short Review Of Current Trends, Impending Obstacles, Modern Synthetic Approach, Structure Activity Relationship and Numerous Biological Activities of Benzimidazole. Latin American Journal of Pharmacy. 2023 Jul 29;42(3):1089-4.
33. Mounika S, Hemalatha K, Pal R, Matada GS, Jain PP, Haripriya E, Sk MA, Viji MP. Benzimidazole Derivatives in Breast Cancer: Target-Specific Therapeutic Breakthroughs. Current Topics in Medicinal Chemistry. 2025 Jan 7.
34. Pathak A, Malairajan P, Gautam A, Das S. International Journal of Current Microbiology and Applied Sciences. Int. J. Curr. Microbiol. App. Sci. 2022;11(01):61-77.
35. Garg A, Dureja D, Pasricha R, Saini PD, Bhalla A. Recent progress in synthetic strategies for novel β-lactams linked with five-membered heterocycles (N/O/S): advances in medicinal chemistry (2020–2025). Medicinal Chemistry Research. 2025 Apr 12:1-32.
36. Haneef M, Lohani M, Dhasmana A, Jamal QM, Shahid SM, Firdaus S. Molecular docking of known carcinogen 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) with cyclin dependent kinases towards its potential role in cell cycle perturbation. Bioinformation. 2014 Aug 30;10(8):526.
37. Mohammed LA, Farhan MA, Dadoosh SA, Alheety MA, Majeed AH, Mahmood AS, Mahmoud ZH. A review on benzimidazole heterocyclic compounds: Synthesis and their medicinal activity applications. SynOpen. 2023 Dec;7(04):652-73.
38. Reddyrajula R, Varshini KP, Shankaraiah N. Recent developments of benzimidazole based analogs as potential tubulin polymerization inhibitors: A critical review. Bioorganic & Medicinal Chemistry Letters. 2025 Mar 10:130167.
39. Katariya D, Borisagar M. Efficient Microwave Synthesis, Antibacterial, and Antifungal Evaluation of 1, 4-Dihydropyrimido [1, 2-a] benzimidazole Derivatives. Russian Journal of Organic Chemistry. 2024 Dec;60(Suppl 1): S171-9.
40. Shah D, Patel M, Patel D, Patel A. Microwave-assisted Synthesis of Benzimidazole Derivatives: A Green and Effective Approach. Current Green Chemistry. 2025 Jun;12(2):159-65.
41. Waseem T, Ullah N, Rajput TA. Review of synthetic accessibility and pharmacological activity of 1, 2, 4-triazole and 2-methylbenzimidazole derivatives. Pharmaceutical Chemistry Journal. 2022 Oct;56(7):943-7.
42. Köntös Z, Bartek M. The synthesis and characterization of Various metal complexes of 2-(4-nitrophenyl)-1H-benzimidazole and its mono schiff base precursor. Science Progress. 2025 Jan;108(1):00368504251323767.
43. Al-Blewi FF, Almehmadi MA, Aouad MR, Bardaweel SK, Sahu PK, Messali M, Rezki N, El Ashry ES. Design, synthesis, ADME prediction and pharmacological evaluation of novel

benzimidazole-1, 2, 3-triazole-sulfonamide hybrids as antimicrobial and antiproliferative agents. Chemistry Central Journal. 2018 Dec; 12:1-4.

44. Agić D, Karnaš Babić M, Hranjec M, Šubarić D, Karačić Z, Abramić M. New Amidino-Substituted Benzimidazole Derivatives as Human Dipeptidyl Peptidase III Inhibitors: Synthesis, In Vitro Evaluation, QSAR, and Molecular Docking Studies. International journal of molecular sciences. 2025 Apr 20;26(8):3899.
45. Al-Hakimi AN, Alresheedi TM, Albarrak RA, Albadri AE, Abd El-Hady MM, Saeed SE. Synthesis and Characterization of a Fluorinated Schiff Base from Benzimidazole and Its Metal Complexes for Antimicrobial and UV-Protective Cotton Fabrics. Coatings. 2025 Mar 24;15(4):380.
46. Arı E, Şahin N, Üstün E, Dündar M, Karcı H, Özdemir İ, Koç A, Gürbüz N, Özdemir İ. Synthesis, antimicrobial activity and molecular docking study of benzyl functionalized benzimidazole silver (I) complexes. JBIC Journal of Biological Inorganic Chemistry. 2023 Dec;28(8):725-36.