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Original Paper

Benzimidazole derivatives as β-lactamase inhibitors with antibiofilm activity against *Acinetobacter baumannii*: in vitro and in silico evaluation

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ABSTRACT

This study reports the synthesis and characterization of three benzimidazole derivatives, N1, N2, and N 3 , and their evaluation as potential antibacterial agents against multidrug-resistant (MDR) *Acinetobacter baumannii*. The synthetic pathway began with 2-mercapto-5-nitrobenzimidazole, leading to the intermediate N1, which was subsequently converted into compounds N2 and N3. The structures of all synthesized compounds were confirmed using FTIR and ^{1}H NMR spectroscopy. The in vitro antibacterial activity was evaluated using agar diffusion, minimum inhibitory concentration (MIC) determination, growth-curve analysis, and biofilm inhibition assays. The results showed that all compounds exhibited antibacterial activity, with compound N3 producing the largest inhibition zone, outperforming amikacin and vitamin C, and demonstrating strong antibiofilm activity. Furthermore, molecular docking studies against β-lactamase enzyme (PDB ID: 5L2F) revealed favorable binding affinities for all ligands, with N3 showing the best docking score, -8.239kcal/mol, surpassing that of the reference antibiotic amikacin. These findings suggest that the synthesized benzimidazole derivatives, particularly N3, may represent promising lead candidates for the development of antibacterial and antibiofilm agents against MDR infections.

Keywords: *Antibacterial drug design, Benzimidazole derivatives, β-lactamase inhibition, Molecular docking, Multidrug-resistant (MDR) Acinetobacter baumannii*

1 INTRODUCTION

Antibiotic resistance is a global issue that can cross international borders and spread rapidly across continents. Global health experts have described antibiotic-resistant organisms as "nightmare bacteria" that pose a "catastrophic threat" to individuals in every country worldwide. The excessive and inappropriate use of antibiotics is one of the main factors contributing to the global emergence of antibiotic resistance [1]. In recent years, the prevalence of multidrug-resistant pathogens has increased substantially worldwide. Currently, antimicrobial resistance (AMR) represents a major challenge to patient care, leading to increased morbidity and mortality, prolonged hospitalization, and substantial economic losses for both patients and healthcare systems [2].

Clinical isolates, including Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus (MRSA), enterococci, especially vancomycin-resistant enterococci (VRE), and members of the Enterobacteriaceae family, such as Klebsiella pneumoniae, Escherichia coli, and Proteus species, can rapidly develop antibiotic resistance and persist in hospital environments [3].

Heterocyclic compounds are among the most important chemical classes attracting the interest of scientists

and researchers in chemistry, biology, and medicine. These compounds are characterized by rings containing heteroatoms, such as oxygen, nitrogen, or sulfur, in addition to carbon atoms. This diversity in chemical composition gives them unique properties and supports a wide range of applications [4, 5].

Benzimidazole is an important heterocyclic compound in organic chemistry and plays a vital role in many industrial and medical applications. Its molecular structure consists of two fused rings, which provide unique properties suitable for different applications [6–8]. Benzimidazole derivatives are important compounds in modern medicine and exhibit several pharmacological activities [9, 10], including anticancer properties, as some derivatives have shown activity against different cancer types [11, 12]. Benzimidazole derivatives have also been used in skin-care products because of their antibacterial properties [13]. In addition, some studies have shown that benzimidazole and its derivatives may contribute to environmental applications because some compounds have biodegradable properties and may be useful in pollution-related studies [14].

In recent decades, advances in biochemistry and pharmacology have led to major progress in the use of computational techniques to analyze and predict the chemical and biological properties of organic compounds. Among these modern computational techniques, molecular docking has gained considerable attention [15, 16].

Due to the importance of benzimidazole derivatives and their broad applications in pharmacy, biochemistry, and related fields, this study synthesized three benzimidazole derivatives and evaluated their in vitro antibacterial and antibiofilm activities, together with in silico molecular docking, against multidrug-resistant *Acinetobacter baumannii*. A. baumannii was selected as the target organism because it is listed among WHO priority pathogens and represents a serious clinical challenge because of its multidrug resistance and high prevalence in hospital-associated infections.

2 MATERIALS AND METHODS

Figure 1 presents the overall framework of the current investigation

2.1 Chemicals

All chemicals used in this study were obtained from Riedel-de Haën, SDH, and Sigma-Aldrich, as shown in Figure 2.

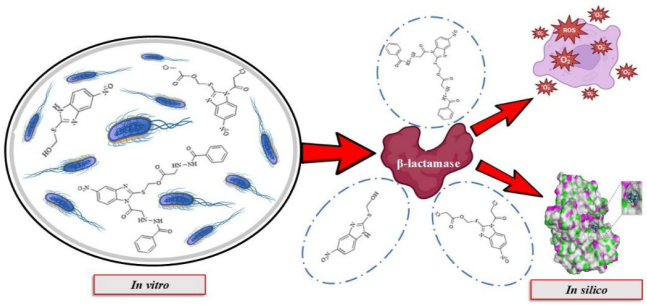

Fig. 1 Graphical representation of this study

2.2 Instrumentation

FTIR spectra were recorded using a Shimadzu FTIR spectrophotometer at the BPC Analysis Center, Baghdad, using the KBr pellet method. ^{1}H NMR spectra were recorded using a Bruker spectrometer at 400 MHz, with chemical shifts reported in ppm and DMSO- d_6 used as the solvent. The purity of the synthesized compounds was confirmed to be >95% by repeated thin-layer chromatography (TLC).

2.3 Methods

2.3.1 Synthesis of N1: 2-(Hydroxymethylthio)-5-nitrobenzimidazole

A mixture of 2-mercapto-5-nitrobenzimidazole (1.952 g, 0.01 mol, ≥ 98%) and formaldehyde solution (5.0 mL, 37% w/w) was dissolved in 30 mL of absolute ethanol (≥ 99.8%) in a 100 mL round-bottom flask. The reaction mixture was stirred under reflux at 80°C for 5 h . After completion of the reaction, as monitored by TLC, the mixture was cooled to room temperature. The resulting solid was collected by filtration, washed with cold ethanol (2 × 10 mL), and dried under vacuum to obtain a white solid. Yield: 82%; Rf = 0.68(TLC, EtOAc = 2 : 1); m.p. = 80 – 82 °C [17].

2.3.2 Synthesis of N2: (2-Chloroacetoxy)methyl-2-(2-chloroacetyl)-5-nitrobenzimidazole-1-thioether

Compound N1 (1.952 g, 0.01 mol) was dissolved in 30 mL of 1.0 M ethanolic KOH, prepared by dissolving KOH pellets in ethanol. The solution was stirred at room temperature for 10 min . Chloroacetyl chloride (1.129 g, 0.01 mol, approximately 0.80 mL, density 1.418 g/mL) was added dropwise under stirring, and the reaction mixture was refluxed at 85 °C for 6 h. Reaction progress was monitored by TLC. After cooling, the mixture was poured onto crushed ice and carefully acidified with 1

M HCl to pH6 – 7. The precipitated solid was filtered, washed with water and cold ethanol, and dried. Yield: 78%; Rf = 0.54(EtOAc = 1 : 1); decomposition m.p. = 243°C.

2.3.3 Synthesis of amide derivative N3

Compound N2 (3.50 g, 0.01 mol) was dissolved in 15 mL of absolute ethanol in a 100 mL roundbottom flask. Hydrazine hydrate (80% w/w, 15.0 mL) was added dropwise to the stirred solution at room temperature. The reaction mixture was then heated under reflux at 90 °C for 4 h. After cooling to room temperature, the formed hydrazide intermediate was collected by filtration, washed with cold ethanol (2 × 10 mL), and dried under vacuum. The isolated hydrazide was dissolved in 15 mL of absolute ethanol, and benzoyl chloride (0.012 mol, 1.69 g, approximately 1.39 mL) was added dropwise at room temperature. The mixture was stirred for 30 min. The precipitated amide derivative N3 was filtered, washed with cold ethanol, and dried under vacuum to obtain the final product. Yield: 87%; Rf = 0.61 ($CHCl_3$ = 3 : 1); m.p. = 195 – 197°C.

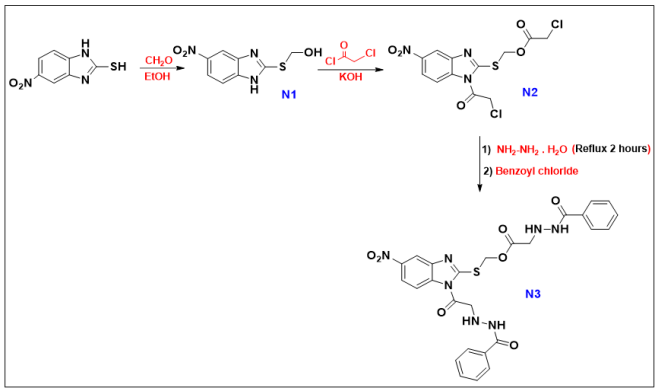

Fig. 2 Preparation of compounds N1-N3

2.4 In vitro antibacterial study

2.4.1 Collection of samples

All clinical isolates, including *Acinetobacter baumannii*, were obtained from Ramadi Teaching Hospital under the Al-Anbar Health Directorate, Iraq, in accordance with ethical guidelines approved by the hospital and the University Ethics Committee. Informed consent was obtained where applicable, and all samples were handled according to biosafety regulations. The *A*. baumannii isolate was previously identified using the VITEK system. According to antibiotic susceptibility testing reports, the isolate was classified as multidrug-resistant (MDR). The *A*. baumannii isolate was further cultured on nutrient agar and in nutrient broth and designated as strain code Ab-RAM-01 as an internal laboratory code. Its identity was confirmed against the reference strain *A. baumannii* ATCC 19606 [18].

2.4.2 Antimicrobial screening

The synthesized benzimidazole derivatives, N1, N2, and N3, were evaluated for their in vitro antibacterial activity against *A. baumannii* using the agar diffusion method [19]. Mueller-Hinton agar was used as the culture medium. Wells were prepared in agar plates inoculated with bacterial suspension adjusted to 1.5×10^8CFU/mL. Each well was treated with the synthesized compounds N1, N2, and N3 at a concentration of 200μ g/mL. The diameters of the growth inhibition zones were measured after incubation at 37 °C for 24 h. Amikacin and vitamin C were used as positive controls [20]. Vitamin C was included because of its reported antibiofilm properties against *A. baumannii*, which may be mediated through oxidative stress modulation [21].

2.4.3 Assessment of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) of all prepared compounds against *A*. baumannii were determined according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method [22]. Amikacin and vitamin C were used as positive controls. The tested compounds were diluted in tryptic soy broth (TSB) to obtain the required final concentrations. Two-fold serial dilutions were prepared in 96-well plates to obtain final concentrations ranging from 0.2 to 200μ g/mL for N1, N2, and N3. Amikacin and vitamin C were also tested at concentrations ranging from 0.2 to 200μ g/mL. Each well was inoculated with a microbial suspension adjusted to 0.125 absorbance, equivalent to 0.5 McFarland, and then incubated at 37 °C. The MIC was defined as the lowest concentration that inhibited visible bacterial growth.

2.4.4 Analysis of growth patterns

Growth-curve analysis was performed to evaluate the effects of sub-inhibitory concentrations of the synthesized benzimidazole compounds N1, N2, and N3 on bacterial proliferation. Overnight cultures were inoculated into 100 mL of TSB medium containing half the MIC of each synthesized compound, with untreated cultures used as

negative controls. The samples were incubated at 37 °C for 24 h, and optical density was measured at 600 nm (OD600) at 1 h intervals over a 24 h period [23].

2.4.5 The inhibitory impact of the investigated substances on biofilm formation

The effects of the synthesized benzimidazole compounds on biofilm formation were evaluated in 96-well polystyrene plates. The experiment was conducted according to the method previously described by Elkhatib et al. [24], with minor modifications. To achieve a final concentration of 1.5×10^8CFU/mL, equivalent to 0.5 McFarland, the *A. baumannii* isolate was inoculated into TSB medium, vortexed, and diluted 1:100 in fresh TSB medium for biofilm formation. The cultures were then incubated at 37 °C for 24 h with or without the tested compounds.

After incubation, non-adherent cells were removed by washing with sterile phosphate-buffered saline (PBS). Adherent cells were stained with 1% crystal violet solution for 15 min. The wells were washed with water to remove excess stain and then air-dried. Crystal violet bound to the biofilm was dissolved using 95% ethanol. The absorbance of the crystal violet solution was measured spectrophotometrically at OD570. Wells containing medium supplemented with phosphate buffer served as negative controls. Amikacin and vitamin C were used as positive controls. The biofilm inhibition percentage was calculated using the following formula [25]:
Biofilm inhibition (%) = [(Control OD - Test OD) / Control OD] × 100.

2.5 In silico molecular docking strategy

Molecular docking calculations and scoring were performed using Molecular Operating Environment software, MOE 2015 [26]. The crystal structure of β-lactamase from *A*. baumannii (PDB ID: 5L2F; resolution: 1.77) was retrieved from the Protein Data Bank [27]. Before docking, solvation water molecules and heteroatoms were removed, except for a conserved water molecule located in the active site, which was retained to facilitate hydrogenbond formation between the ligands and the target enzyme [28–30]. Docking parameters were optimized using genetic algorithms, generating 30 conformations or poses for each ligand. The most favorable conformation was selected based on the lowest binding free energy, reflecting stable non-covalent interactions between the ligand and receptor. Hydrophobic interactions between the docked compounds and active-site residues were also analyzed to support binding stability.

2.6 Statistical analysis

Biofilm inhibition assays and all other experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Data were statistically analyzed using one-way ANOVA after confirming normality, followed by Tukey's post hoc test to assess group differences. A p value <0.05 was considered statistically significant. Error bars shown in the figures represent SD values from three independent replicates. Statistical analyses and graphing were performed using Microsoft Excel 365, GraphPad Prism version 9.0, and OriginLab 2023 version 10.0.

3 RESULTS

3.1 Synthesis and characterization of benzimidazole derivatives (N1-N3)

Three benzimidazole derivatives, N1 – N3, were successfully synthesized, and their structures were confirmed using FTIR and ^{1}H NMR spectroscopic analyses (Figure 3).

The FTIR spectra of compounds N1 – N3 are summarized in Table 1, showing the characteristic absorption bands and their corresponding functional group assignments [31–33]. Similarly, the ^{1}H NMR data are compiled in Table 2 and presented in Figure 3, showing the δ values, multiplicities, and proton assignments [34]. This tabular arrangement provides a clearer and more systematic comparison among the three derivatives.

Table 1 FT-IR spectral data of synthesized benzimidazole derivatives (N1–N3)

Compound	Main absorption bands ($\mathbf{cm^{-1}}$)	Assignment
N1	3392, 2843, 2960, 1620, 1519, 1460, 802	O-H stretch, aliphatic C-H stretch, C=N stretch, C=C aromatic vibration, C-S stretch
N2	1716, 2879, 2974, 1543-1460, 1014, 731-615	C=O stretch, aliphatic C-H stretch, C=C aromatic vibration, C-O stretch, C-Cl stretch
N3	3199, 3049, 3003, 2833, 1666, 1629, 1575, 1531, 1485, 1286, 1155, 1072 , 869	N-H stretch, aromatic C-H stretch, aliphatic C-H stretch, ester/amide C=O stretch, aromatic C=C vibration, C-N stretch, C-O-C stretch, C-S stretch

Table 2 ^{1}H-NMR spectral data of synthesized benzimidazole derivatives (N1-N3), (400MHz, DMSO − d_6)

Compound	δ(ppm)	Multiplicity (J, Hz)	Assignment
N1	4.92	d, J = 5.2	-S-CH_2- (coupled to OH)
	6.01	br s	-OH
	7.35-8.67	m	Aromatic protons
	11.23	s	-NH (imidazole)
N2	8.97	d, J = 7.98	Aromatic proton
	8.49	d, J = 7.98	Aromatic proton
	7.78	s	Aromatic proton
	5.42	s	$-S-CH_2-O-$
	4.66	s	$-N-CO-CH_2-Cl$
	4.22	s	$-O-CO-CH_2-Cl$
N3	8.27	s	-NH-CO- (amide)
	8.19	s	-NH-CO- (amide)
	7.95	t, J = 7.6	Aromatic proton
	7.70	d, J = 7.9	Aromatic proton
	7.58	d, J = 2.1	Aromatic proton
	7.46	t, J = 7.6	Aromatic proton
	7.38	d, J = 8.1	Aromatic proton
	5.53	s	$-S-CH_2-$
	4.00	s	$-CH_2-$ (near hydrazide)
	3.83	s	$-CH_2-$ (near hydrazide)
	6.24	s	-NH-CO-
	5.30	s	-NH-CO-

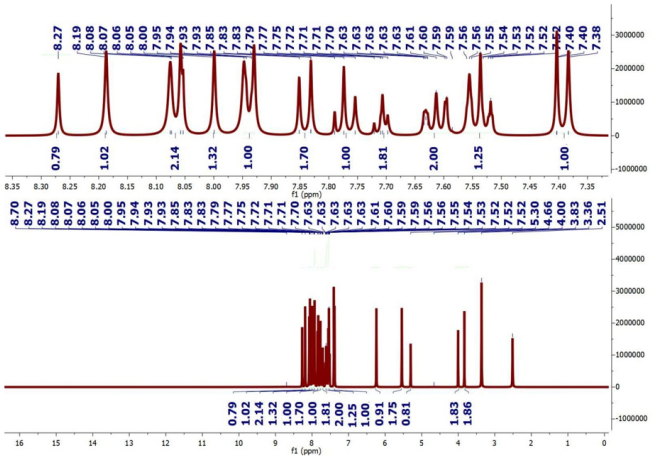

Fig. 3 ^{1} H-NMR spectrum of N3

3.2 In vitro antibacterial assessment

3.2.1 Initial assessment of antibacterial efficacy

The antibacterial activity of the synthesized benzimidazole derivatives N1, N2, and N3 was evaluated against multidrug-resistant (MDR) *Acinetobacter baumannii* using the well-diffusion method. Vitamin C and amikacin were used as positive controls. As shown in Figure 4, all three compounds showed inhibitory effects, with inhibition zones increasing in the order N1 < N2 < N3.

At 100μ g/mL, compound N3 produced the largest inhibition zone, measuring 15 ± 0.02 mm, compared with amikacin, 12 ± 0.02 mm, and vitamin C, 10 ± 0.02 mm. Compounds N2 and N1 produced smaller inhibition zones of 2 mm and 8 mm, respectively, compared with the control groups. Overall, the benzimidazole derivatives demonstrated antibacterial activity, which may be attributed to their functional groups and their possible interactions with bacterial surfaces in a concentration-dependent manner [35–38].

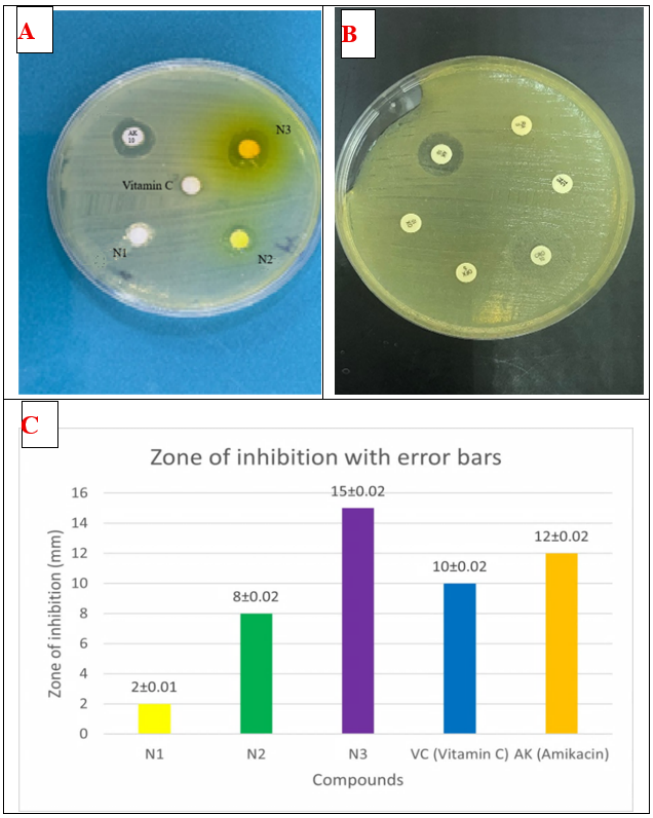

Fig. 4 Zone of inhibition of benzimidazole compounds (N1, N2, and N3) against *Acinetobacter baumannii*. (A) Inhibition zones with Vitamin C (VC) and Amikacin (AK) as reference controls. (B) Inhibition zones against the multidrug-resistant (MDR) isolate of *A. baumannii*. (C) Bar chart summarizing inhibition zone diameters with error bars representing standard deviations from triplicate experiments

Table 3 Zone of inhibition of benzimidazole derivatives (N1–N3) against MDR *A. baumannii*, compared with positive controls

Compounds	Zone of inhibition (mm) ± SD
N1	2 ± 0.01
N2	8 ± 0.02
N3	15 ± 0.02
Vitamin C (VC)	10 ± 0.02
Amikacin (AK)	12 ± 0.02

Note. Values are mean ± SD (n = 3). One-way ANOVA with Tukey's test was applied. Significance is denoted as ns ($p > 0.05$), * ($p < 0.05$), * * ($p < 0.01$), and *** ($p < 0.001$).

3.2.2 Minimum inhibitory concentrations (mic) and growth curve analysis

The minimum inhibitory concentrations (MICs) of the benzimidazole derivatives N1, N2, and N3 were determined against *A. baumannii* using the standard broth microdilution method according to CLSI guidelines. Growth-curve analysis was conducted to assess the effects of sub-MIC concentrations, equivalent to 1/2 MIC, on bacterial proliferation.

As shown in Table 4, the MIC values of the compounds ranged from 19.25 to 38.5 μg/mL. Sub-MIC concentrations did not significantly affect bacterial growth compared with untreated controls (ns, $p > 0.05$). These findings suggest that the observed antibacterial activity occurred at MIC levels, whereas the tested sub-MIC concentrations did not significantly impair planktonic bacterial growth.

Table 4 MIC and sub-MIC values of benzimidazole derivatives (N1–N3) and controls against MDR *A. baumannii*

Compounds	MIC (μg/mL)	Sub-MIC (1/2 MIC, μg/mL)
N1	19.25	9.62
N2	38.5	19.25
N3	38.5	19.25
Vitamin C (VC)	38.5	19.25
Amikacin (AK)	39.63	19.81

Note. Values are mean ± SD (n = 3). One-way ANOVA with Tukey's test was applied. ns indicates $p > 0.05$ compared with control.

3.2.3 inhibitory impact on biofilm development

The ability of the benzimidazole derivatives to inhibit biofilm formation by *A.* baumannii was evaluated using the crystal violet assay in 96-well plates. Sub-MIC concentrations of N1-N3 were compared with amikacin and vitamin C as positive controls.

The results showed that all compounds reduced biofilm formation compared with the untreated control (Table 5). Among the derivatives, N1 exhibited the strongest biofilm inhibition, at 94%, followed by N3, at 78%, and N2, at 61%. These inhibition rates were higher than those recorded for amikacin, 41.3%, and vitamin C, 39.1%. The graphical presentation of these findings is provided in Figure 5, which illustrates the comparative inhibition rates and standard deviations across all treatments.

Table 5 Biofilm inhibition of benzimidazole derivatives (N1–N3) and controls against MDR *A. baumannii* at sub-MIC concentrations

Compound	Absorbance at 570 nm ± SD	% Biofilm inhibition
Control	0.552 ± 0.01	-
N1	0.123 ± 0.07	78
N2	0.217 ± 0.02	61
N3	0.031 ± 0.02	94
Vitamin C (VC)	0.101 ± 0.01	81
Amikacin (AK)	0.120 ± 0.06	78

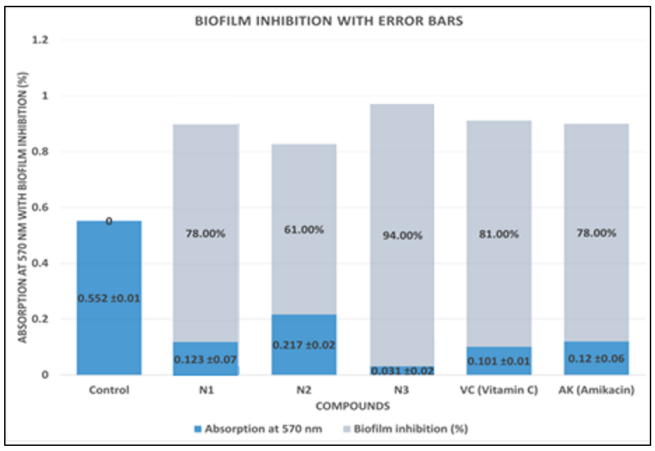

Fig. 5 Biofilm inhibition of *A. baumannii* at sub-MIC concentrations of benzimidazole derivatives (N1-N3), compared with Vitamin C (VC) and Amikacin (AK). Blue bars represent absorbance at 570 nm (OD570 ± SD) and grey bars represent percentage inhibition of biofilm formation. Error bars indicate standard deviation from triplicate experiments (n = 3, one-way ANOVA with Tukey's test).

3.3 In silico investigation

3.3.1 Molecules library preparation

The chemical structures of the synthesized benzimidazole derivatives N1, N2, and N 3 , as well as vitamin C and amikacin, were generated and converted into three-dimensional formats using MarvinSketch. These ligands were prepared for docking analysis against the target

protein β -lactamase (PDB ID: 5L2F) [39]. The structures were pre-optimized using the semi-empirical AM1 method [40] with HyperChem 8.08 software [41]. The optimized structures were then integrated into a unified ligand database using Molecular Operating Environment software, MOE 2015, to assess ligand binding affinity, as shown in Figure 6 and Table 6.

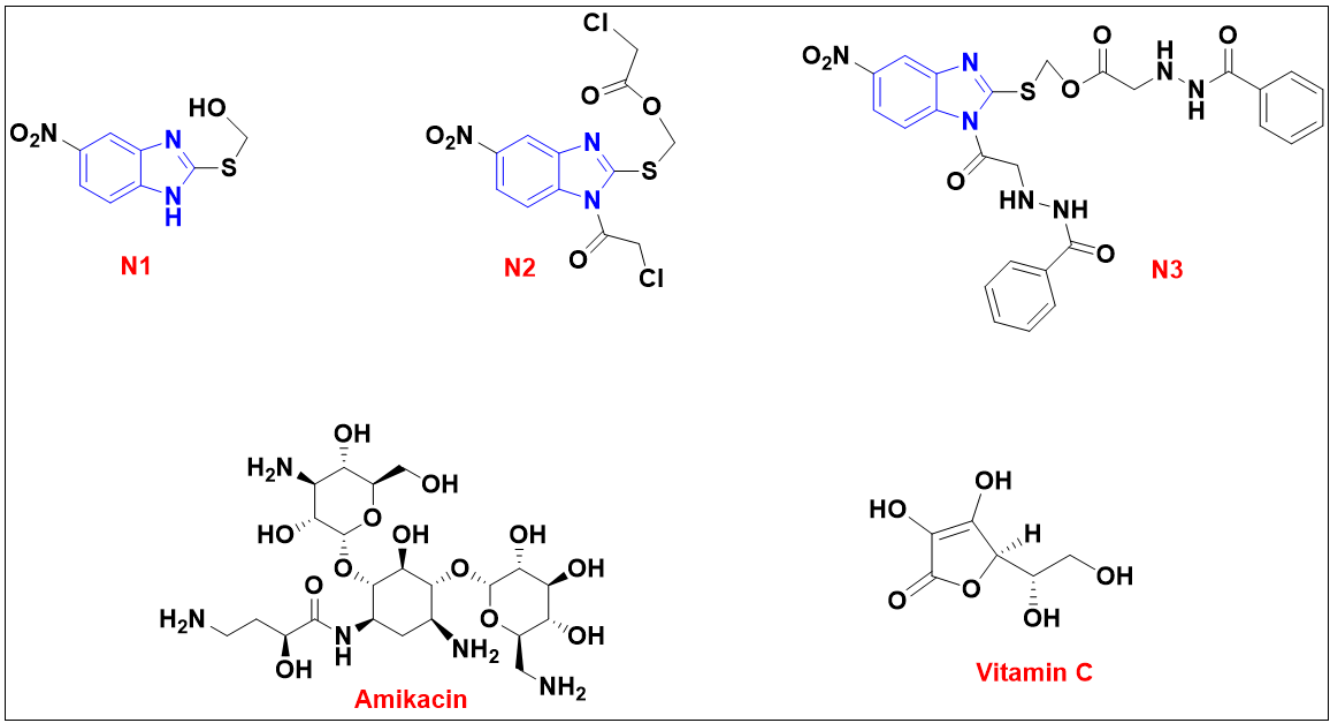

Fig. 6 The chemical structures of selected benzimidazole derivatives (N1, N2, N3) and the chemical structure (Vitamin C and Amikacin), as compared standard inhibitors.

Table 6 The SMILES representations of chosen benzimidazole compounds (N1, N2, and N3) with standard controls (Vitamin C and Amikacin)

Compounds	SMILES
N1	OCSC1=NC2=CC([N+]([O−]) = O) = CC = C2 N1
N2	ClCCC (N1C2 = CC = C([N+]([O−]) = 0)C = C2 N = C1SCOC(CCCl) = O) = 0
N3	O = C(CNNC(C1 = CC = CC = C1) = O)N2C3 = CC = C([N+]([O]) = O) C = C3 N = C2SCOC(CNNNC(C4 = CC = CC = C4) = O) = 0
Vitamin C	[H][C@@]1(OC(=O)C(O)=C10)[C@@H](O)CO
Amikacin	NCC[C@H](O)C(=O)N[C@@H]1C[C@H](N)[C@@H](O[C@H]2O[C@H] (CN)[C@@H](O)[C@H] (O)[C@H]2O)[C@H](O)[C@H]10[C@H]10[C@H](CO)[C@@H](O) [C@H] (N)[C@H]10

3.3.2 Receptor preparation

The crystal structure of β-lactamase from *A. baumannii* (PDB ID: 5L2F) was obtained from the Protein Data Bank (PDB). Before docking, non-essential water molecules were removed using the sequence editor. However, a conserved water molecule located in the active site was deliberately retained because of its potential role in facilitating hydrogen-bond formation between the ligand and the target enzyme [28–30].

Structural irregularities in the receptor were corrected, and the finalized protein structure was saved in PDB format [42]. The three-dimensional conformation of the protein and its active-site pocket prepared for docking are illustrated in Figure 7. Furthermore, the structure was refined by restoring chemical bonds that may have been affected during structural preparation. The key active-site residues selected for docking are summarized in Table 7.

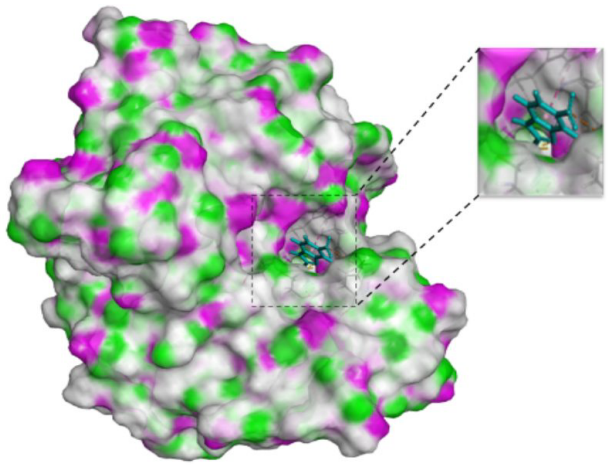

Fig. 7 3D structure of protein and active site of β-lactamase from *A. baumannii* (PDB ID: 5L2F) was performed using Discovery Studio Visualizer [43], version 2024)

3.3.3 Molecular docking analysis of compounds N1, N2, and N3 against β-lactamase *A. baumannii*

β-Lactams represent a major class of bactericidal drugs used to treat infections caused by both Gram-positive and Gram-negative microorganisms. Their bactericidal activity is mediated through inhibition of penicillin-binding proteins (PBPs), which are essential for bacterial cell wall synthesis [44]. In this study, compounds N1, N2, and N3 were docked against OXA-51 class D β-lactamase from *A. baumannii* (PDB ID: 5L2F), using amikacin and vitamin C as reference controls. The compounds formed at least one hydrogen bond with key amino acid residues in the active site of β-lactamase from *A. baumannii*. Figure 8 and Table 8 summarize the binding affinities, hydrogen bonding, hydrophobic interactions, and van der Waals interactions of the tested compounds.

Table 7 Induced fit Docking takes binding site residues as input to generate the receptor grid

Receptor	Sites	Residues
5L2F	Site 1	1:(Ala79, Ser80, Leu110, Phe111, Glu113, Trp114, Lys125, Ala126, Ser127, Leu129, Leu167, Ile206, Ala216, Lys217, Ser218, Gly219, Trp220, Trp222, Gln227, Leu231, Ser257, Ser258, Val259, Arg260, Lys261)

As shown in Figure 8, the benzimidazole compounds, amikacin, and vitamin C interacted with β -lactamase from *A. baumannii*, as illustrated by both three-dimensional and two-dimensional docking representations. The results showed that compound N3 exhibited the strongest docking affinity within the β-lactamase binding pocket, with a docking score of -8.239kcal/mol, followed by amikacin, -7.234kcal/mol, N2, -6.158kcal/mol, and vitamin C, -5.596kcal/mol, as shown in Table 9. The ribbon model presents the binding pocket of β-lactamase from *A.* baumannii in complex with N1, N2, N3, amikacin, and vitamin C. Hydrogen bonds between the compounds and amino acid residues are represented by green dashed lines, whereas hydrophobic interactions are shown by pink lines.

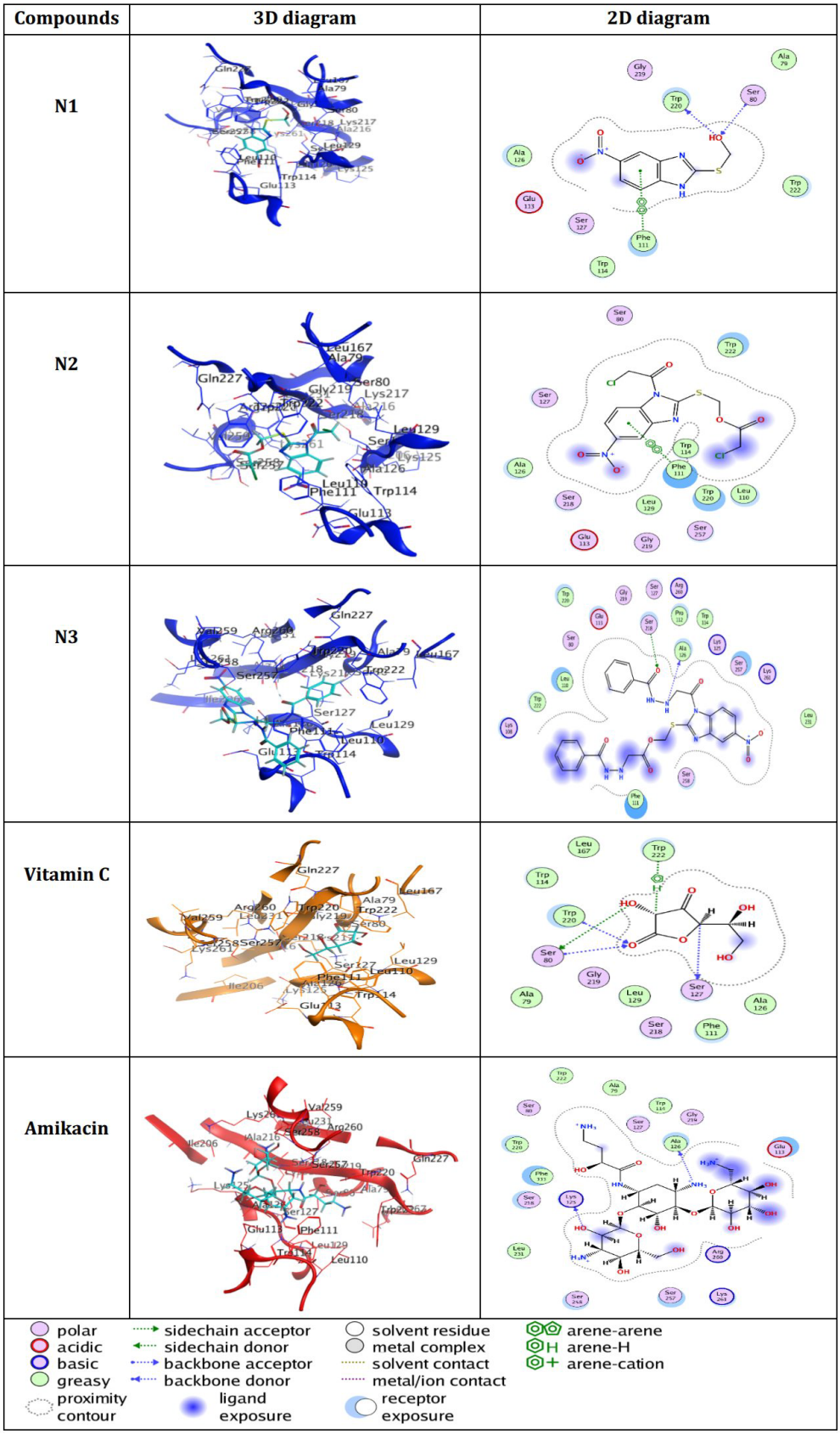

Fig. 8 The 3D and 2D diagrams binding interactions of compounds N1, N2, N3, Vitamin C, and Amikacin against β-lactamase

Table 8 Ligand interactions of β-lactamase in the *A. baumannii* (PDB: 5L2F)

Compounds	Ligand	Receptor	Interaction	Distance / Å	E / (kcal/mol)
N1	0115	O TRP 220 (A)	H-donor	3.09	-1.6
	0115	N SER 80 (A)	H-acceptor	3.43	-0.6
	6-ring	6-ring PHE111 (A)	pi-pi	3.70	-0.0
N2	6-ring	6-ring PHE111 (A)	pi-pi	3.64	-0.0
N3	N3 44	0 ALA 126 (A)	H-donor	2.84	-1.3
	050	OG SER 218 (A)	H-acceptor	3.13	-1.6
Vitamin C	019	O VAL 143 (A)	H-donor	3.21	-1.3
	08	NH2 ARG 30 (A)	H-acceptor	3.04	-2.7
	014	N PHE 105 (A)	H-acceptor	3.46	-0.6
	019	N ARG 104 (A)	H-acceptor	3.04	-2.0
Amikacin	N 26	0 ALA 126 (A)	H-donor	3.46	-1.0
	086	O LYS 125 (A)	H-donor	2.68	-3.2

Table 9 Binding energy values S (in units of kcal/mol) and RMSD (mean square deviation between the pre- and post-refinement laying positions; in units of 10^{-10} m) of the investigated compounds with β-lactamase in the *A. baumannii* (PDB: 5L2F)

Compounds	S (kcal/mol)	RMSD (10^{-10} m)
N1	-5.725	1.540
N2	-6.158	1.475
N3	-8.239	2.913
Vitamin C	-5.596	0.919
Amikacin	-7.234	3.963

4 DISCUSSION

The antibacterial activity demonstrated by the benzimidazole derivatives supports their potential as agents against multidrug-resistant (MDR) *A. baumannii*. The inhibition zones showed a clear activity pattern among the tested compounds, with N3 displaying the strongest antibacterial effect and showing activity comparable to that of amikacin. This activity may be attributed to the presence of functional groups that enhance interactions with the bacterial surface [35, 36].

MIC testing further supported these findings, indicating that inhibitory activity was observed at MIC concentrations, whereas sub-MIC concentrations did not significantly affect bacterial growth. This suggests that the observed antibacterial activity was mainly associated with inhibitory concentrations rather than growth impairment at sub-MIC levels. The biofilm inhibition results further demonstrated the antibiofilm potential of the synthesized derivatives, with N3 showing strong inhibitory activity and outperforming vitamin C and amikacin. These findings are consistent with previous reports on benzimidazole derivatives, in which heteroaromatic modifications improved antimicrobial efficacy [37, 38].

Docking studies provided molecular insight into the possible interaction of the synthesized compounds with β-lactamase. Compound N3 showed the highest predicted binding affinity, with a docking score of -8.239 kcal/mol, through hydrogen bonding and $\pi - \pi$ stacking interactions with β-lactamase residues. The RMSD values, although indicating some flexibility, remained within acceptable ranges, supporting the reliability of the docking poses. Although amikacin is not a β-lactam antibiotic, its inclusion as a clinical comparator provided a reference point for evaluating the relative performance of N3.

Together, the experimental and in silico findings indicate that N3 is the most promising candidate among the synthesized derivatives for further investigation. However, the absence of molecular dynamics simulations is a limitation of this study. Future studies should include molecular dynamics analysis to further validate the stability of ligand-receptor interactions.

5 CONCLUSION

In this study, three benzimidazole derivatives, N1-N3, were synthesized and characterized using FTIR and ^{1}H NMR spectroscopy. Biological evaluation showed that these compounds possess promising antibacterial and antibiofilm activities against multidrug-resistant *Acinetobacter baumannii*. Among the synthesized derivatives, compound N3 showed the strongest overall antibacterial activity and the most favorable predicted binding interaction with β-lactamase, with the highest docking affinity of -8.239 kcal/mol.

These findings suggest that benzimidazole scaffolds, particularly N3, may serve as promising candidates for the development of antibacterial and antibiofilm agents. However, this study was limited

to *A. baumannii*, and other bacterial species were not included. In addition, mammalian cytotoxicity assays and in vivo validation were not performed. Future research should prioritize molecular dynamics simulations, cytotoxicity evaluation, broader antibacterial testing, and in vivo studies to further support the therapeutic potential of these compounds.

Authors' contribution

Nuaman F. Alheety: Conceptualization; Writing – Original Draft; Review and Editing. Noureddine Raouafi: supervision; project administration; methodology; investigation. Mustafa A. Alheety: formal analysis; investigation; methodology. Rafaa Besbes: supervision; project administration; methodology; investigation

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Data availability

N/A

DECLARATIONS

Conflict of interest

The authors declare no competing interests.

Consent to publish

N/A

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N/A

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