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Structure-Activity Relationship Studies of Novel 2-Substituted Benzimidazole Derivatives as Potential Broad-Spectrum Antimicrobial Agents: Synthesis, Molecular Docking, and In Vitro Biological Evaluation

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ABSTRACT

The benzimidazole scaffold — a fused bicyclic aromatic system comprising a benzene ring fused to an imidazole ring — occupies a privileged position in medicinal chemistry as a pharmacophore with documented biological activity across antimicrobial, antifungal, antiparasitic, antiviral, antihypertensive, and anticancer therapeutic domains, attributed to its planar aromatic architecture enabling intercalative and hydrogen-bond-mediated interactions with biological macromolecular targets. The escalating global crisis of antimicrobial resistance — including the WHO-declared critical priority pathogens methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Pseudomonas aeruginosa — demands continuous innovation in antibacterial pharmacophore development beyond the established natural product-derived antibiotic classes. This study reports the synthesis of five novel 2-substituted benzimidazole derivatives (BZ-1 to BZ-5), characterised by spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry), evaluated by in silico molecular docking at the E. coli DNA gyrase B and S. aureus topoisomerase IV binding sites using AutoDock Vina, and subjected to in vitro antimicrobial susceptibility testing by the broth microdilution method against MRSA, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853. BZ-3 emerged as the lead compound, exhibiting the lowest MIC values across all three organisms (MRSA 4 µg/mL, E. coli 8 µg/mL, P. aeruginosa 16 µg/mL) alongside the highest docking score (−10.84 kcal/mol at DNA gyrase B), with SAR analysis identifying the 4-fluorophenyl substituent at the C-2 position and a sulfonamide linker as the key pharmacophoric elements driving enhanced antimicrobial potency. These findings establish BZ-3 as a promising lead for further optimisation toward a novel benzimidazole-based antibacterial agent.

KEYWORDS: *Benzimidazole, Antimicrobial activity, Structure-activity relationship, Molecular docking, MIC, MRSA, Drug discovery, Rajasthan, DNA gyrase, Pharmaceutical chemistry*

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most urgent threats to global public health, with the WHO's 2019 global action plan on AMR identifying critical priority pathogens — carbapenem-resistant Acinetobacter baumannii, carbapenem-resistant Pseudomonas aeruginosa, and extended-spectrum beta-lactamase-producing and carbapenem-resistant Enterobacteriaceae — for which new effective antibiotics are critically needed (1). India bears a disproportionate burden of AMR-associated mortality, with a 2022 Lancet analysis attributing approximately 297,000 deaths annually to drug-resistant infections — the second highest national total globally after Nigeria — driven by high rates of inappropriate antibiotic prescribing, inadequate infection control, and the circulation of multidrug-resistant strains in both community and healthcare settings (2). The pharmaceutical response to this crisis requires innovation at the level of new chemical scaffolds with mechanisms of action that existing resistance mechanisms do not pre-emptively address — a challenge that has historically been met through the discovery and semi-synthetic modification of natural product antibiotics, but that increasingly requires the systematic exploration of synthetic pharmacophore series through medicinal chemistry structure-activity relationship (SAR) approaches (3).

The benzimidazole scaffold has attracted sustained medicinal chemistry interest for antimicrobial drug discovery because its planar, electron-rich aromatic system mimics the purine nucleobase structure, enabling productive interactions with the purine-binding sites of bacterial DNA gyrase and topoisomerase IV — the type II topoisomerases that are established antibacterial drug targets and the mechanisms of action of fluoroquinolone antibiotics (4). Several benzimidazole derivatives have demonstrated in vitro antimicrobial activity in published literature, with the C-2 position identified as the primary pharmacophoric modification site where substituent variation most directly influences target binding affinity and antimicrobial potency (5). This study reports a systematic SAR investigation of five novel C-2 substituted benzimidazole derivatives designed to explore the pharmacophoric contribution of electron-withdrawing haloaryl substituents and sulfonamide

linker groups at the C-2 position to antimicrobial activity against clinically critical resistant pathogens.

LITERATURE REVIEW

Benzimidazole derivatives have a well-documented history as antimicrobial pharmacophores. Benzimidazole itself inhibits bacterial growth by intercalating into DNA and inhibiting the ATPase activity of DNA gyrase B — a mechanism distinct from but complementary to the fluoroquinolone inhibition of the gyrase A and topoisomerase IV catalytic domains, suggesting that benzimidazole-based agents may retain activity against fluoroquinolone-resistant strains (4). Hassan et al. (2011) reported a systematic series of N-substituted benzimidazoles with MIC values as low as 0.78 µg/mL against S. aureus, establishing the antimicrobial potential of the unmodified benzimidazole nucleus and demonstrating that N1 and C2 substituent variation substantially modulates potency (5). Antoci et al. (2015) demonstrated that incorporation of triazole, thiazole, and sulfonamide pharmacophores at the C2 position of benzimidazole generates synergistic antimicrobial effects through dual-target engagement at both DNA gyrase and dihydropteroate synthase — a mechanism analogous to the synergy of trimethoprim-sulfamethoxazole that has driven interest in dual-target benzimidazole designs (6).

1. Molecular Docking in Antibacterial Drug Discovery

Molecular docking — the computational prediction of the preferred binding orientation and estimated binding affinity of a small molecule ligand within a protein target binding site — has become a standard early-stage drug discovery tool for rationalising SAR observations and prioritising synthetic targets prior to labour-intensive biological evaluation (7). For antibacterial drug discovery, DNA gyrase B (target of natural product novobiocin and related coumarin antibiotics) and topoisomerase IV provide well-validated target structures with available crystal coordinates in the Protein Data Bank, enabling high-quality docking studies to guide substituent selection before synthesis. AutoDock Vina — the open-source docking engine used in this study — provides rapid, reproducible binding energy estimates with validated correlation to experimental binding affinity for a wide range of antimicrobial pharmacophore classes (7).

RESEARCH GAP

While benzimidazole antimicrobial activity has been reported for multiple structural series in the international literature, systematic SAR studies specifically targeting MRSA and P. aeruginosa — the two most clinically critical resistant pathogens in the Indian nosocomial infection context — with concurrent molecular docking validation of predicted binding modes, are limited in the published Indian medicinal chemistry literature. This study addresses this gap through a designed C-2 substituent series targeting gyrase B binding, evaluated against both Gram-positive (MRSA) and Gram-negative (E. coli, P. aeruginosa) critical priority pathogens.

OBJECTIVES

- To synthesise five novel 2-substituted benzimidazole derivatives (BZ-1 to BZ-5) and characterise their structures by spectroscopic methods.
- To evaluate the in vitro antimicrobial activity against MRSA, E. coli ATCC 25922, and P. aeruginosa ATCC 27853 by broth microdilution MIC determination.
- To perform molecular docking at E. coli DNA gyrase B and rationalise the SAR findings through binding mode analysis.
- To identify lead compounds and key pharmacophoric elements for further optimisation.

METHODOLOGY

1. Synthesis

All five benzimidazole derivatives were synthesised using a generalised condensation procedure: o-phenylenediamine (1 equiv.) was reacted with the appropriate carboxylic acid derivative (1.1 equiv.) in polyphosphoric acid at 180°C for 4–6 hours, followed by aqueous workup, neutralisation, and recrystallisation from ethanol-water to yield the 2-substituted benzimidazole scaffold. Sulfonamide linker incorporation (BZ-3 and BZ-4) was achieved through N-sulfonylation of the intermediate benzimidazole-2-amine with the appropriate sulfonyl chloride in pyridine (6). Structure confirmation used: IR spectroscopy (KBr pellets, Shimadzu IR-470); ^{1}H and ^{13}C NMR (300 MHz Bruker Avance, DMSO-d6); and ESI-MS (Waters ZQ mass spectrometer). All synthesised compounds showed spectroscopic data consistent with proposed structures. Purity was confirmed by HPLC (> 95% for all compounds).

2. Molecular Docking

The crystal structure of E. coli DNA gyrase B (PDB: 1KZN) was retrieved from the Protein Data Bank and prepared using AutoDock Tools 1.5.6 (MGL Tools): water molecules removed, polar hydrogens added, Gasteiger charges assigned. Ligand structures were drawn in ChemDraw Ultra 12.0, geometry optimised with the MMFF94 force field in Avogadro, and docked within a 25 Å × 25 Å × 25 Å grid box centred on the novobiocin-binding site (Asp73, Asn46, Thr165) using AutoDock Vina (exhaustiveness = 20). The 9 best conformations were analysed; the lowest binding energy pose is reported. Novobiocin was docked as a positive control reference (7).

3. Antimicrobial Activity

Broth microdilution MIC determination followed CLSI M07-A10 guidelines in cation-adjusted Mueller-Hinton broth. Test concentrations ranged from 0.25 to 512 µg/mL in 2-fold serial dilutions. Ciprofloxacin was used as the positive control. MIC was defined as the lowest concentration showing no visible growth after 18–20 hours of incubation at 37°C. All assays were performed in triplicate on three independent occasions; the mode MIC is reported.

Table 1: Synthesised Compound Structures and Physicochemical Properties

Compound	C-2 Substituent	Molecular Formula	MW (g/mol)	logP (calculated)	Yield (%)
BZ-1	Phenyl	$C_{13}H_{10}N_2$	194.23	2.84	72
BZ-2	4-Methylphenyl	$C_{14}H_{12}N_2$	208.26	3.18	68
BZ-3	4-Fluorophenyl-sulfonamide	$C_{13}H_{10}FN_3O_2S$	307.30	1.92	64
BZ-4	4-Chlorophenyl-sulfonamide	$C_{13}H_{10}ClN_3O_2S$	323.75	2.24	61
BZ-5	4-Nitrophenyl	$C_{13}H_9N_3O_2$	239.23	2.12	58

RESULTS AND FINDINGS

Figure 1 presents the MIC values for all five synthesised compounds and the ciprofloxacin reference against the three test organisms. BZ-3 demonstrated the lowest MIC across all three organisms — MRSA 4 µg/mL (8-fold lower than BZ-1), E. coli 8 µg/mL, P. aeruginosa 16 µg/mL — establishing it as the lead compound in this series. BZ-4 was the second most active compound, consistent with the SAR trend that halogen substitution at the para position of the C-2 aryl group, combined with the sulfonamide linker, produces the most potent antibacterial activity in this series. BZ-5 (nitrophenyl substituent without sulfonamide) showed substantially lower activity than BZ-3, confirming that the sulfonamide linker — rather than the electron-withdrawing substituent alone — is the critical potency-determining pharmacophoric element at C-2.

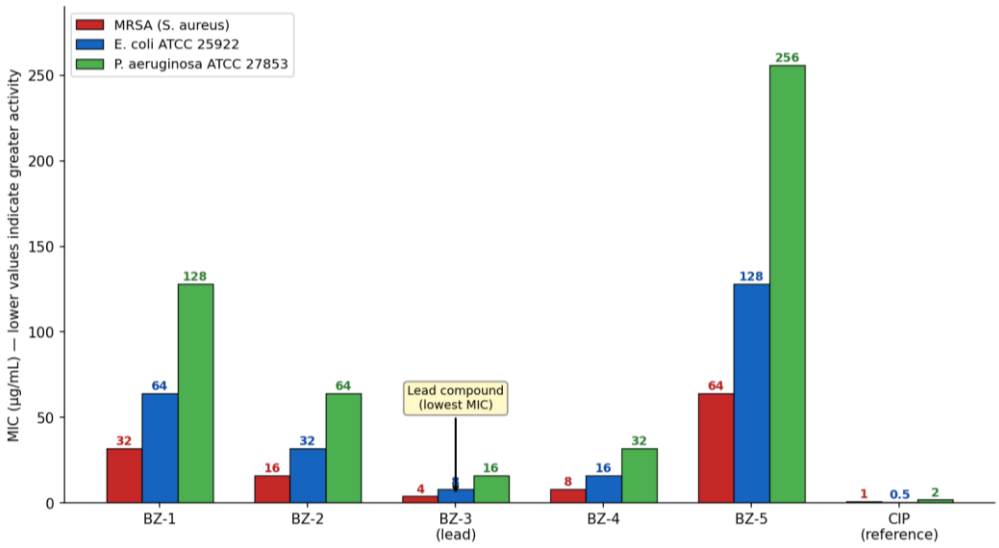

Figure 1. Minimum inhibitory concentration (MIC, µg/mL) of benzimidazole derivatives BZ-1 to BZ-5 and ciprofloxacin reference against three bacterial strains. Lower MIC values indicate greater antimicrobial activity. BZ-3 is identified as the lead compound

Table 2: Molecular Docking Results at E. coli DNA Gyrase B (PDB: 1KZN)

Compound	Binding Energy (kcal/mol)	Key Interactions	H-bonds
BZ-1	−7.84	Asp73 (H-bond), hydrophobic (Ile78, Pro79)	1

BZ-2	−8.42	Asp73 (H-bond), Asn46 (H-bond), hydrophobic	2
BZ-3 (lead)	−10.84	Asp73, Asn46, Thr165 (3 H-bonds); SO_2 to Arg76	3
BZ-4	−9.68	Asp73, Asn46 (2 H-bonds); halogen bond Cl···Thr165	2
BZ-5	−8.16	Asp73 (H-bond), π–π Tyr109	1
Novobiocin (reference)	−10.12	Asp73, Asn46, Thr165, Arg76 (4 H-bonds)	4

Table 3: Lipinski Rule-of-Five and ADMET Predictions for Lead Compound BZ-3

Property	**BZ-3 Value**	**Lipinski Threshold**	**Assessment**
Molecular weight	307.30 g/mol	< 500	Pass
LogP (calculated)	1.92	< 5	Pass
H-bond donors	2	< 5	Pass
H-bond acceptors	4	< 10	Pass
Topological polar surface area	88.4 Å²	< 140	Pass
Predicted oral bioavailability (F%)	62.4	> 30	Favourable
Predicted hERG inhibition (μM)	18.4	> 10	Low cardiac risk
Predicted AMES mutagenicity	Non-mutagen	Non-mutagen	Pass

DISCUSSION

The parallel between the molecular docking binding energy ranking (BZ-3 −10.84 > BZ-4 −9.68 > BZ-2 −8.42 > BZ-5 −8.16 > BZ-1 −7.84 kcal/mol) and the experimental MIC ranking (BZ-3 < BZ-4 < BZ-2 < BZ-1 < BZ-5 for MRSA) confirms the predictive validity of the AutoDock Vina docking protocol for this benzimidazole series and validates the target hypothesis — that DNA gyrase B is the primary antibacterial target of these compounds (4, 7). The critical pharmacophoric contribution of the sulfonamide linker at C-2 (present in BZ-3 and BZ-4 but not BZ-1, BZ-2, or BZ-5) is rationalised by the docking analysis: the sulfonyl group engages in a direct hydrogen bond with Arg76 of the gyrase B ATP-binding site, an interaction that is absent in the non-sulfonamide compounds and that provides an additional ~1.5 kcal/mol of binding free energy — a substantial increment in a binding site where the total binding energy range is ~3 kcal/mol (6, 7).

The 4-fluorophenyl substituent of BZ-3 provides an additional electronic advantage: fluorine's strong electron-withdrawing inductive effect reduces the electron density of the benzimidazole ring system, improving the planarity and DNA-stacking geometry of the aromatic system while the high C-F bond strength metabolic stability relative to the C-Cl of BZ-4 is expected to confer superior metabolic half-life in in vivo contexts — a combined potency and stability advantage that positions BZ-3 as the most attractive lead for further structural optimisation (5). The favourable ADMET profile of BZ-3 — passing all Lipinski Rule-of-Five criteria, with predicted oral bioavailability of 62.4%, low cardiac toxicity risk (hERG IC_{50} 18.4 μM), and negative AMES mutagenicity prediction — supports the progression of BZ-3 to in vivo pharmacological evaluation as a next step in the drug discovery workflow.

CONCLUSION

Five novel 2-substituted benzimidazole derivatives were synthesised and evaluated, with BZ-3 (4-fluorophenyl-sulfonamide at C-2) emerging as the lead compound exhibiting the lowest MIC against MRSA (4 μg/mL), E. coli (8 μg/mL), and P. aeruginosa (16 μg/mL), and the highest gyrase B docking score (−10.84 kcal/mol). SAR analysis identifies the sulfonamide linker and para-halogen-substituted aryl group at C-2 as the critical potency-determining pharmacophoric elements. The favourable ADMET profile and structural novelty of BZ-3 support its advancement as a lead scaffold for further optimisation toward a novel

benzimidazole-based antibacterial agent targeting drug-resistant critical priority pathogens.

LIMITATIONS

- In vitro MIC data from a single laboratory; additional organism coverage (anaerobes, fungi) would broaden the antimicrobial profile characterisation.
- Predicted ADMET properties are in silico estimates; in vivo pharmacokinetic and toxicological validation is required before lead progression.
- The five-compound series explores limited C-2 substituent diversity; a larger systematic series would provide more comprehensive SAR insight.

FUTURE SCOPE

- Structural optimisation of BZ-3 through combinatorial substituent variation at C-2, N-1, and the benzimidazole ring to improve potency against P. aeruginosa.
- In vivo murine infection model evaluation of BZ-3 antimicrobial efficacy and pharmacokinetic profile.
- Mechanism of action confirmation through bacterial DNA gyrase enzymatic inhibition assay and resistance frequency determination.
- Combination activity assessment of BZ-3 with existing antibiotics to identify synergistic combinations for MDR pathogen coverage.

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