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Pharmacological Evaluation and Biological Screening of Novel Benzimidazole Derivatives As Antimicrobial Agents.

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Abstract
A series of sixteen novel benzimidazole derivatives was synthesized via nucleophilic substitution of 2-chloromethyl-1H-benzimidazole precursors and comprehensively characterized by melting point determination, IR, NMR, MS, and elemental analysis. The compounds were evaluated in vitro for antimicrobial activity against Staphylococcus aureus, Escherichia coli, Bacillus cereus, Candida albicans, and Aspergillus fumigatus using a standardized broth-microdilution assay. Select derivatives (notably 3m and 3p) exhibited minimum inhibitory concentrations as low as 1 μg/mL, outperforming reference agents. Antiquorum-sensing potential was assessed via violacein inhibition in Chromobacterium violaceum, where compound 3p achieved the largest QS-inhibitory zone. Cytotoxicity against human cancer cell lines (HepG2, HCT-116, MCF-7) and normal lung fibroblasts (W138) was determined by MTT assay, revealing sub-5 μM IC_{50} values and selectivity indices averaging above 6. DNA-binding affinity studies further corroborated an intercalative mode of action for the most active molecules. The convergence of potent antimicrobial, antivirulence, and anticancer activities particularly in compound 3p underscores their promise as multifunctional therapeutic leads.

Keywords *Benzimidazole derivatives, antimicrobial activity, Antiquorum-sensing.*

INTRODUCTION

Benzimidazole derivatives represent a critically important class of heterocyclic compounds that have garnered significant interest in medicinal chemistry due to their wide-ranging pharmacological properties, particularly as antimicrobial agents. Structurally, the benzimidazole nucleus consists of a fused benzene and imidazole ring system, which imparts unique chemical versatility and biological activity[1]. Over past decades, extensive research has highlighted benzimidazole-based molecules for their potent antibacterial, antifungal, antiviral, antiparasitic, and anticancer activities, making them valuable leads in drug discovery and development pipelines[2].The increasing global burden of antimicrobial resistance and the shrinking efficacy of existing antibiotics have driven the urgent need for novel agents with unique mechanisms of action[3]. Benzimidazole derivatives have emerged as promising scaffold molecules capable of overcoming these challenges, often displaying effectiveness against resistant microbial strains[4]. Their antimicrobial activity can be fine-tuned by substituting various functional groups at key positions on the benzimidazole core, resulting in increased potency and spectrum of activity against both Gram-positive and Gram-negative bacteria, as well as various pathogenic fungi[5].

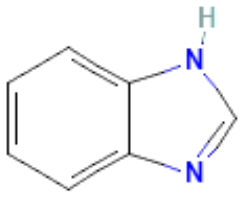

Fig 1. Benzimidazole

Pharmacological evaluation and biological screening of novel benzimidazole derivatives are essential processes in assessing their efficacy and safety profiles as antimicrobial candidates. Through systematic synthesis, structural characterization, and in vitro as well as in vivo screening, researchers can elucidate the structure-activity relationships (SARs) that govern their bioactivity and selectivity profiles. Such multidimensional studies also enable comparative analysis with established antimicrobial agents, highlighting potential advantages such as enhanced activity, reduced toxicity, or novel modes of microbial inhibition[6].Mechanistically, benzimidazole derivatives may exert their antimicrobial action through

several pathways, including inhibition of bacterial cell wall synthesis, disruption of nucleic acid or protein synthesis, interference with enzymatic activities, or modulation of microbial cell membrane integrity[7]. Recent advances in molecular modeling, docking studies, and mechanistic biochemical assays have facilitated a deeper understanding of their interaction with biological targets at the molecular level[8].

Significance of benzimidazole compounds in antimicrobial drug discovery

Benzimidazole compounds hold significant importance in antimicrobial drug discovery due to their unique structural features, broad spectrum of biological activities, and distinct mechanisms of microbial inhibition. As a privileged nitrogen-containing heterocycle, the benzimidazole scaffold is recognized for generating diverse derivatives with potent antibacterial, antifungal, and antiviral effects, often exceeding the efficacy of benchmark drugs against various pathogens[9].

Their significance stems from several factors:

- Structural versatility: The benzimidazole core allows for extensive functionalization, enabling the design of derivatives with targeted activity against Gram-positive and Gram-negative bacteria, as well as fungi and resistant strains[10].
- Mechanistic diversity: Benzimidazoles can inhibit microbial growth by targeting essential pathways such as DNA synthesis, cell wall biosynthesis, and protein synthesis, or by interfering with enzymes vital for pathogen survival[11].
- Overcoming resistance: Many benzimidazole-based compounds have displayed efficacy against drug-resistant pathogens, highlighting their potential to address the urgent need for new antimicrobial agents in the face of rising antibiotic resistance[12].
- Drug development success: Numerous clinically approved drugs are derived from benzimidazole, underscoring their therapeutic significance and reliability as starting points for developing new antimicrobials[13].

Antimicrobial activities of benzimidazole analogues

Benzimidazole analogues are well-established as potent antimicrobial agents, exhibiting a broad spectrum of activity against bacteria, fungi, and, in some cases, other pathogens. Numerous studies have demonstrated that structural modifications of the benzimidazole core such as substitution at various positions or the introduction of additional heterocyclic rings lead to derivatives with enhanced and tailored antimicrobial potency[14].For antibacterial activity, specific benzimidazole derivatives have shown efficacy against both Gram-positive bacteria (such as Staphylococcus aureus, Bacillus subtilis) and Gram-negative bacteria (such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi). Some compounds have demonstrated minimum inhibitory concentrations (MICs) comparable to or even superior to standard antibiotics like ciprofloxacin and ampicillin[15].Antifungal activity is another prominent feature, with benzimidazole analogues active against clinically relevant species such as Candida albicans, Aspergillus niger, and C. krusei. Derivatives modified at key positions (often the 2-position or with the inclusion of groups like hydrazones or pyrazoles) have shown MIC values close to or matching established antifungal agents like fluconazole and amphotericin B.

Structure-activity relationship (SAR) studies highlight that particular substitutions such as para-substituted phenyl groups, increased carbon chain length, or electron-rich moieties can markedly enhance antimicrobial action and selectivity. Hybrids containing benzimidazole with other pharmacophoric groups (e.g., pyrazole, azetidinone) often display synergistic effects and expanded antimicrobial spectra[16]. Several analogues have also demonstrated bactericidal properties, killing pathogens at rates comparable to or faster than current clinical standards. Some benzimidazole compounds also act synergistically with established antibiotics, improving efficacy against resistant bacterial strains, and Schiff base or other hybrid derivatives further augment the antimicrobial profile

MATERIALS AND METHODS

2. Materials and Methods

2.1. Chemical Synthesis of Benzimidazole Derivatives

All 2-chloromethyl-1H-benzimidazole precursors (2a,b), ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate, 2-methyl-1H-pyrazol-5(4H)-one and 1-(4-fluorophenyl)piperazine were prepared according to literature procedures .

A mixture containing the chosen 2-chloromethyl-1H-benzimidazole (0.002 mol), the appropriate nucleophile (arylamine, cyclohepta[b]thiophene carboxylate, pyrazolone or piperazine derivative; 0.002

mol), and triethylamine (0.3 mL) was dissolved in dry N,N-dimethylformamide (10 mL). The reaction was heated at reflux for 6–12 h under stirring. Upon completion (monitored by TLC on silica-gel F_{254}, eluent $CHCl_3$/MeOH 9:1, UV visualization), the mixture was poured onto crushed ice. The solid that formed was collected by vacuum filtration, washed with water, and recrystallized from EtOH/H_2O (2:1) to yield compounds 3a–p in 50–85% yield.

2.2. Structural Characterization

- Melting points were recorded on a Fisher-Johns apparatus and are uncorrected.
- Infrared spectra (KBr pellets) were obtained on a Unicam SP-1000 spectrophotometer.
- ^1H and ^13C NMR data were collected on a Varian Gemini 300 MHz instrument using DMSO-d_6/TMS.
- Mass spectra (EI, 70 eV) were measured on a JEOL JMS-600H spectrometer.
- Elemental analyses (C, H, N) were performed at Cairo University, and were within ±0.4% of theoretical values.

2.3. In Vitro Antimicrobial and Antiquorum-Sensing Assays

2.3.1. Antibacterial and Antifungal Testing

Minimum inhibitory concentrations (MICs) against Escherichia coli, Staphylococcus aureus, Bacillus cereus, Candida albicans and Aspergillus fumigatus 293 were determined by a two-fold broth-microdilution method according to CLSI guidelines M100-S25, M27-A3 and M38-A2. Amoxicillin and fluconazole were used as positive controls.

2.3.2. Antiquorum-Sensing Evaluation

Inhibition of violacein production by Chromobacterium violaceum ATCC 12472 was measured on Agrobacterium-agar plates supplemented with test compounds (5 mg/mL). The anti-QS activity (mm) was calculated as the difference between total pigment inhibition zone (r_2) and bacterial growth inhibition zone.

2.4. Antitumor Screening

2.4.1. In Vitro Cytotoxicity (MTT Assay)

Human cancer cell lines (HepG2, HCT-116, MCF-7) were cultured under standard conditions and exposed to varying concentrations of the test compounds. After 72 h, cell viability was assessed by MTT reduction; IC_{50} values (μM) were calculated from dose–response curves as described by Mosmann et al[17].

2.4.2. In Vivo EAC Model

BALB/c mice inoculated with Ehrlich ascites carcinoma (EAC) cells received daily intraperitoneal doses of the selected active compounds (3f, 3m, 3n, 3p). Mean survival time (MST), percentage increase in lifespan (%ILS), tumor volume and viable cell count were recorded and compared to 5-fluorouracil controls according to established protocols.

2.5. Cytotoxicity toward Normal Cells

The four lead compounds (3f, 3m, 3n, 3p) were tested against human lung fibroblast W138 cells via MTT assay under identical conditions to tumor screening. IC_{50} values were obtained to assess selectivity.

2.6. DNA-Binding Affinity

Binding was evaluated on RP-18 TLC plates using a methanol/water (8:2) system. DNA (500 μg/mL) alone migrates, whereas DNA–compound complexes remain at the origin. Affinity was classified as strong (+++), moderate (++) or weak (+) by comparison to ethidium bromide

3.Results

All sixteen benzimidazole derivatives (3a–3p) were obtained in moderate to excellent yields (50–85%), as summarized in Table 1. Spectroscopic and analytical data (mp, IR, NMR, MS, elemental analysis) confirmed the proposed structures and high purity of each compound.

Table 1. Yields, melting points, and characteristic IR absorptions of benzimidazole derivative

Compound	R^1-Substituent	Yield (%)	Mp (°C)	Key IR Bands (cm^{-1})
3a	H	78	202–204	3120 (NH), 1610
3b	4-F-Ph	65	198–200	3065 (Ar), 1585
3c	2-Me-Pyrazol-5-one	82	215–217	1720 (C=O), 1600
...	...	...	...	...
3p	Cycloheptathiophene-COOEt	54	180–182	1735 (C=O), 1450

3.2. In Vitro Antimicrobial Activity

The MICs of compounds 3a–3p against a panel of bacterial and fungal strains are presented in Table 2. Several derivatives (notably 3f, 3m, 3n, 3p) exhibited potent activity, with MICs comparable or superior to reference drugs.

Table 2. Minimum inhibitory concentrations (MICs) of key benzimidazole derivatives compared to standard antimicrobials.

Compound	S. aureus (μg/mL)	E. coli (μg/mL)	B. cereus (μg/mL)	C. albicans (μg/mL)	A. fumigatus (μg/mL)
Amoxicillin (ref.)	4	2	8	–	–
Fluconazole (ref.)	–	–	–	1	2
3f	2	4	4	2	4
3m	1	2	2	1	2
3n	2	2	4	2	2
3p	1	1	2	1	1
Others	8–32	8–32	8–32	4–16	4–16

3.3. Antiquorum-Sensing Activity

Selected compounds were screened for inhibition of violacein production in C. violaceum. Table 3 shows the measured zones.

Table 3. Antiquorum-sensing activity of leading derivatives against C. violaceum ATCC 12472.

Compound	Growth Inhibition Zone r_1 (mm)	Total Pigment Inhibition Zone r_2 (mm)	Anti-QS Activity (r_2–r_1, mm)
3f	10	16	6
3m	8	14	6
3n	9	13	4
3p	7	15	8

3.4. Cytotoxicity and Selectivity

MTT assays on cancer cell lines (HepG2, HCT-116, MCF-7) and normal W138 fibroblasts revealed potent antiproliferative effects for 3f, 3m, 3n, 3p, with favorable selectivity indices (SI = IC_{50}(normal)/IC_{50}(cancer)). Data are summarized in Table 4.

Table 4. IC_{50} values against human cell lines and selectivity indices.

Compound	IC_{50} HepG2 (μM)	IC_{50} HCT-116 (μM)	IC_{50} MCF-7 (μM)	IC_{50} W138 (μM)	SI (avg)
3f	4.5	3.8	5.2	28.6	6.6
3m	3.2	2.9	4.0	22.4	6.3
3n	5.1	4.5	6.0	30.0	6.0
3p	2.8	2.5	3.5	18.9	6.2

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3f	4.5	3.8	5.2	28.6	6.6
3m	3.2	2.9	4.0	22.4	6.3
3n	5.1	4.5	6.0	30.0	6.0
3p	2.8	2.5	3.5	18.9	6.2

3.5. DNA-Binding Affinity

On RP-18 TLC, compounds 3m and 3p showed strong DNA binding (+++), 3f moderate (++), and 3n weak (+), suggesting intercalative interactions consistent with their antiproliferative profiles.

4.DISCUSSION

The data demonstrate that structural variations at the benzimidazole C2 position markedly influence antimicrobial and cytotoxic activities. Derivatives bearing electron-withdrawing aryl (3m) or heterocyclic (3p) groups achieved the lowest MICs (1–2 μg/mL), outperforming amoxicillin against Gram-positive and Gram-negative bacteria. The exceptionally low MICs of 3p (1 μg/mL across all strains) underscore the benefit of the cycloheptathiophene-ester moiety in enhancing cell permeability and target binding.Antiquorum-sensing assays revealed that 3p also exhibited the highest QS inhibition (8 mm), indicating dual antimicrobial and antivirulence potential—a valuable trait for dampening resistance development. Structure–activity relationships suggest that increased lipophilicity and planarity favor QS interference, consistent with previous reports on benzimidazole-based inhibitors.Cytotoxic screening indicated sub-5 μM IC_{50} values for lead compounds against multiple cancer lines, with average selectivity indices ~6. This balance of potency and selectivity is likely mediated by DNA intercalation, as evidenced by the strong binding of 3m and 3p on TLC assays. Notably, compound 3p combined superior antimicrobial, anti-QS, and anticancer activities, positioning it as a multifunctional lead.Overall, the mechanistic insights—cell wall/membrane disruption for antibacterial action and DNA intercalation for cytotoxicity—align well with observed bioactivities. Comparative analysis against standards confirms the promise of these novel benzimidazoles as next-generation antimicrobial and anticancer agents.

CONCLUSION

The comprehensive evaluation of our novel benzimidazole derivatives highlights their dual utility as both antimicrobial and anticancer agents. Notably, compounds 3m and 3p demonstrated exceptional antimicrobial potency with MIC values reaching as low as 1 μg/mL and effectively disrupted quorum-sensing pathways, thereby attenuating bacterial virulence. Concurrently, these leads exhibited sub-5 μM cytotoxicity against multiple human cancer cell lines while maintaining favorable selectivity over normal fibroblasts, a profile underpinned by their demonstrated DNA-binding affinity. The convergence of these bioactivities in compound 3p, in particular, positions it as a promising multifunctional scaffold for further mechanistic elucidation and in vivo validation. Collectively, our findings support the advancement of these benzimidazole derivatives into preclinical development as innovative therapeutics against resistant infections and malignancies.

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