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Synthesis, Characterization, and Antimicrobial Evaluation of Benzimidazole - Based Mannich Derivatives

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ABSTRACT

A series of benzimidazole derivatives was synthesized by condensing substituted *o*-phenylenediamines with benzaldehyde in the presence of sodium metabisulfite ($Na_2S_2O_5$) under reflux in DMF, followed by Mannich reactions with benzaldehyde and dimethylamine. Additional derivatives were obtained via reactions of substituted *o*-phenylenediamines with N, N-dimethylglycine, yielding intermediates for further Mannich base formation. The synthesized compounds were characterized using melting point determination, TLC, FT-IR, and ^{1}H-NMR analyses. Antibacterial screening against *Staphylococcus aureus* and *Escherichia coli* revealed that compound b2 (Br-substituted) exhibited the highest activity among the synthesized derivatives. Hydroxyl-substituted derivatives showed moderate activity, while unsubstituted analogues were the least effective, confirming the correct trend H < OH < Br. Ciprofloxacin was used as a reference standard and displayed the strongest inhibition zones. Since benzaldehyde remained unsubstituted, the observed differences in biological activity were attributed primarily to substitution patterns on the *o*-phenylenediamine moiety. These findings underscore the significant influence of *o*-phenylenediamine substitution on the antimicrobial potency of benzimidazole-linked Mannich bases, highlighting the potential of halogenation, particularly bromine, as an effective strategy for designing novel antimicrobial agents.

Keywords: Benzimidazoles, *o*-Phenylenediamine, Mannich bases, Antibacterial activity, Substitution effects, Structure–activity relationship.

1. Introduction

Nitrogen-containing heterocyclic compounds, particularly benzimidazole derivatives, have attracted significant attention in medicinal chemistry due to their diverse biological activities, including antimicrobial, antifungal, antiviral, and anticancer effects [(1–10)]. Benzimidazole is recognized as a privileged scaffold because of its frequent presence in biologically active molecules with versatile pharmacological

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properties[11,12]. Structural modifications of the benzimidazole core have been demonstrated to profoundly influence the biological activities of its derivatives [13–15].

Among synthetic strategies, Mannich reaction serves as a powerful tool to introduce aminoalkyl groups into heterocycles, enhancing their pharmacological profiles [16–18]. Mannich bases of benzimidazole have been extensively studied for their enhanced antimicrobial properties, with multiple reports confirming their efficacy against Gram-positive and Gram-negative bacteria [19–23]. The introduction of substituents on the aromatic rings or heterocyclic framework, particularly hydroxyl, halogen, and nitro groups, plays a crucial role in modulating antimicrobial potency [24–28].

Previous research has largely focused on varying substituents on the benzaldehyde component during benzimidazole synthesis [29–33]. However, relatively fewer studies have systematically explored substitution on the *o*-phenylenediamine ring while keeping benzaldehyde unsubstituted, despite the critical influence such substitutions may have on biological activity [34–38]. Modifying the *o*-phenylenediamine ring offers a strategic avenue to tune the electronic and steric environment of the benzimidazole derivatives, potentially leading to improved antimicrobial activity.

Recent studies reveal that Mannich bases synthesized from substituted *o*-phenylenediamines exhibit enhanced antibacterial activity, highlighting the importance of substitution patterns on the amine precursor [39–41]. Moreover, these compounds demonstrate promising activity against resistant strains such as *Staphylococcus aureus* and *Escherichia coli* [42–44]. Complementary spectroscopic characterization methods, including FT-IR and ^{1}H-NMR, have reliably confirmed the structures of these novel derivatives [45–47].

Given the escalating global threat of antibiotic resistance, the exploration of benzimidazole-linked Mannich bases with tailored substitution patterns represents a compelling direction for developing new antimicrobial agents [48–56]. This study aims to synthesize and characterize a series of benzimidazole derivatives with substitutions on the *o*-phenylenediamine ring, while maintaining an unsubstituted benzaldehyde in the Mannich reaction, followed by evaluation of their in vitro antimicrobial activity against both Gram-positive and Gram-negative bacteria.

2. Experimental

All newly synthesized compounds were characterized using standard analytical techniques. Melting points were determined in open-end capillary tubes. FT-IR spectra were recorded on a Shimadzu 8400 spectrometer employing the KBr pellet method, while ^{1}H-NMR spectra were obtained on a Bruker Ultra Shield (300 MHz FT-NMR) spectrometer with chemical shifts expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. The purity of the compounds was assessed by thin-layer chromatography (TLC) on silica gel plates, with spots visualized under iodine vapors. The physical characteristics of the synthesized derivatives are summarized in Table 1.

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Table 1: Physicochemical data of the newly synthesized derivatives

Compound	Substituent (R)	Molecular Formula	Molecular Weight (g/mol)	Rf Value	Yield (%)	m.p. (°C)	Appearance
a1	H	$C_{13}H_{10}N_2$	194.24	0.65	82	280	White crystals
a2	Br	$C_{13}H_9BrN_2$	273.13	0.58	78	265	Pale yellow crystals
a3	OH	$C_{13}H_{10}N_2O$	210.24	0.52	85	240	Creamy crystals
b1	H	$C_{16}H_{18}N_3$	252.34	0.70	75	210	Off-white solid
b2	Br	$C_{16}H_{17}BrN_3$	331.24	0.62	80	205	Pale yellow solid
b3	OH	$C_{16}H_{18}N_3O$	268.34	0.55	78	195	Creamy solid
c1	H	$C_{17}H_{19}N_4$	279.37	0.73	70	260	White crystals
c2	Br	$C_{17}H_{18}BrN_4$	358.26	0.60	68	245	Light brown crystals
c3	OH	$C_{17}H_{19}N_4O$	295.37	0.50	73	230	Creamy crystals
d1	H	$C_{20}H_{27}N_5$	337.47	0.78	88	220	White crystals
d2	Br	$C_{20}H_{26}BrN_5$	416.37	0.66	90	215	Pale yellow crystals
d3	OH	$C_{20}H_{27}N_5O$	353.47	0.48	92	205	Creamy crystals
* (DMG)	$N(CH_3)_2$	$C_4H_9NO_2$	103.12	0.55	80	161	White crystalline powder

Rf values were determined using TLC on silica gel plates with *n-hexane: ethyl acetate (7:3, v/v)* as the eluent. Lower Rf values for OH-substituted compounds reflect their higher polarity compared to H and Br derivatives.

Abbreviations: Comp. = Compounds; R. = Various substitutions; M.F. = Molecular formula; m.p. = Melting point; Rf. = Retardation factor (TLC, eluent system: n-hexane/ethyl acetate 7:3).

2.1. Benzimidazole derivatives' synthesis

2.1.1. General Procedure for the Synthesis of Ring-Substituted 2-Phenylbenzimidazole Derivatives (a1-3)

Substituted o-phenylenediamine (3.24 g, 0.03 mol) was dissolved in N, N-dimethylformamide (DMF, 30 mL) under magnetic stirring. To this solution, benzaldehyde (3.18 g, 0.03 mol) was added dropwise over 10 minutes at room temperature. The reaction mixture was then heated under reflux at 130 °C for 6 hours under a nitrogen atmosphere.

After completion (monitored by TLC using ethyl acetate: hexane, 3:1), the reaction mixture was cooled to room temperature and poured into 100 mL of cold distilled water with continuous stirring. The resulting precipitate was filtered under vacuum, washed thoroughly with water, and dried in air.

The crude product was purified by recrystallization from absolute ethanol to afford the corresponding ring-substituted 2-phenylbenzimidazole derivative as an off-white to pale-yellow solid in yields ranging from 78% to 89%, depending on the nature and position of the substituents on the benzimidazole aromatic ring.

2.1.2. General Procedure for the Synthesis of Ring-Substituted 2- [(N, N-Dimethylaminomethyl)] benzimidazole Derivatives (c1–c3)

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A mixture of appropriately substituted *o*-phenylenediamine (0.092 mol) and N, N-dimethylglycine hydrochloride (0.100 mol) was dissolved in absolute ethanol (25 mL) in a 100 mL round-bottom flask equipped with a reflux condenser. The mixture was stirred and refluxed at 78 °C for 6 hours.

After completion of the reaction (monitored by TLC), the solvent was partially evaporated under reduced pressure, and the resulting residue was allowed to cool to room temperature. The mixture was then acidified with 10% aqueous HCl (20 mL) to promote precipitation of the product.

The resulting solid was collected by vacuum filtration, washed with cold distilled water, and dried in air. The crude product was recrystallized from absolute ethanol to afford the pure ring-substituted 2- [(N, N-dimethylaminomethyl)] benzimidazole derivative in good yield.

2.1.3. ***Synthesis of 2-(Dimethylamino)acetic acid (Dimethylglycine, DMG) (Compound *)***

Potassium hydroxide (0.728 g, 0.013 mol) and dimethylamine (0.013 mol) were dissolved in absolute ethanol (25 mL) in a 100 mL round-bottom flask. Chloroacetic acid (1.22 g, 0.013 mol) was then added dropwise to the stirring solution. The reaction mixture was refluxed at 78 °C for 6 hours.

After completion, the solvent was removed under reduced pressure. The resulting solid residue was collected, washed with cold ethanol, and recrystallized from absolute ethanol to yield pure 2-(dimethylamino)acetic acid as a white crystalline solid.

2.2. Mannich base synthesis

2.2.1. General Method for the Synthesis of 1-(N, N-Dimethylaminobenzyl)-2-(substituted phenyl) benzimidazole Derivatives (b1–3)

The N-Mannich bases of 2- (substituted phenyl) benzimidazole derivatives, bearing substituents on the benzimidazole benzene ring, were synthesized using the following general procedure. A solution of 2-(substituted phenyl) benzimidazole (0.006 mol) in absolute ethanol (10 mL) was stirred at room temperature. To this solution, benzaldehyde (0.006 mol) and a secondary amine, N, N-dimethylamine (0.006 mol), were added dropwise.

The reaction mixture was stirred at room temperature for 5 hours, followed by refluxing for an additional 3 hours. After completion, the mixture was cooled to room temperature and the resulting precipitate was collected by filtration, dried, and purified by recrystallization from N, N-dimethylformamide (DMF) to afford the desired 1-(N, N-dimethylaminobenzyl)-2- (substituted phenyl) benzimidazole derivatives.

2.2.2. General Method for the Synthesis of Ring-Substituted 1-((Dimethylamino)(phenyl)methyl)-2-((dimethylamino)methyl) benzimidazole Derivatives (d1-3)

The ring-substituted derivatives of 1-((dimethylamino)(phenyl)methyl)-2-((dimethylamino)methyl) benzimidazole were synthesized following procedures analogous to those employed for the Mannich bases preparation method (b1–3). Substituted benzimidazole precursors bearing various functional groups on the benzimidazole aromatic ring were reacted with benzaldehyde and N, N-dimethylamine under reflux conditions in ethanol.

The reaction mixtures were stirred at room temperature followed by refluxing for an appropriate time. After completion, the products were isolated by filtration, dried, and purified by recrystallization from suitable solvents, affording the target compounds in good yields.

2.3 .Studies on Antimicrobials

The antibacterial activity of the synthesized Mannich bases was evaluated in vitro against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria at a concentration of 100 µg/mL using the cup-plate method. Ciprofloxacin was employed as a reference standard.

Among the tested compounds, bromine-substituted derivatives generally exhibited the strongest antibacterial activity, with compound b2 (Br) showing the highest inhibition zones against both bacterial

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strains. Hydroxyl-substituted derivatives displayed intermediate activity, while unsubstituted analogues (H) were the least effective. This activity sequence (H < OH < Br) is consistent with the structure–activity rationale: hydroxyl groups enhance hydrogen bonding but increase polarity, limiting cell penetration, whereas bromine substitution confers enhanced lipophilicity and electron-withdrawing effects that promote membrane permeability and binding interactions.

These findings align with previous reports that halogen substitution on heterocyclic scaffolds frequently enhances antimicrobial activity more effectively than hydroxyl substitution (Pathare, 2021; Singh et al., 2017; Samreen et al., 2024; Faleye, 2024) [57-60]. For instance, Singh et al. demonstrated that halogenated coumarin–benzimidazole hybrids showed significantly improved antibacterial potency compared to non-halogenated analogues, while Pathare et al. reviewed similar trends across multiple benzimidazole derivatives.

Overall, these results highlight that substitution on the o-phenylenediamine precursor plays a decisive role in modulating the antimicrobial profile of benzimidazole-linked Mannich bases, underscoring the value of halogenation as a promising strategy for developing novel antimicrobial agents.

3. Results and Discussion

3.1. Physicochemical Properties and Characterization

All the newly synthesized benzimidazole derivatives were obtained in good to excellent yields, ranging from 68% to 92% as summarized in Table 1. The melting points of the compounds were sharp and consistent, indicating high purity. It was observed that hydroxyl-substituted derivatives exhibited slightly lower melting points compared to their hydrogen- and bromine-substituted counterparts, likely due to the polar hydroxyl group disrupting crystal lattice packing and thereby lowering thermal stability.

Thin-layer chromatography (TLC) analysis further supported the increased polarity of the hydroxyl-substituted compounds, which displayed lower Rf values (0.48–0.55) compared to hydrogen- and bromine-substituted analogues (0.60–0.78). This trend reflects the greater interaction of the more polar hydroxyl groups with the polar stationary phase of the silica gel plates.

FT-IR spectroscopy confirmed the characteristic functional groups in the synthesized compounds. Broad NH stretching vibrations were observed around 3400 cm^{-1}, indicative of the benzimidazole ring, while substituent-specific peaks such as O–H stretching bands and C–Br stretches were also clearly identified in the respective derivatives.

The ^{1}H-NMR spectra further substantiated the structures of the synthesized molecules. Signals corresponding to aromatic protons, benzimidazole NH, and dimethylamino moieties were consistent with the expected chemical environments, confirming successful formation of the target compounds.

3.2. Synthetic Methodology

The synthesis of 2-phenylbenzimidazole derivatives (a1–a3) via condensation of substituted o-phenylenediamines with benzaldehyde in DMF under reflux proceeded smoothly, providing yields between 78% and 89%. The substituents on the benzimidazole aromatic ring influenced the reaction efficiency, possibly due to electronic effects that affect the cyclization step.

Subsequent Mannich base formation reactions successfully produced the 1-(N, N-dimethylaminobenzyl)-2-(substituted phenyl) benzimidazole derivatives (b1–b3) and the more functionalized 1-((dimethylamino)(phenyl)methyl)-2-((dimethylamino)methyl) benzimidazole derivatives (d1–d3). The use of ethanol as the solvent and reflux conditions facilitated effective synthesis and purification of the desired products.

3.3. Antibacterial Activity

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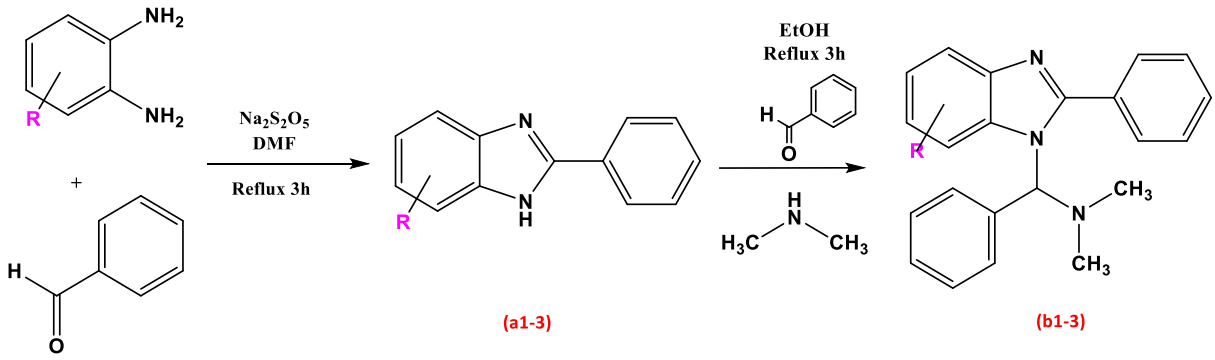
The antibacterial activities of the synthesized Mannich bases were evaluated against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* at a concentration of 100 µg/mL, using ciprofloxacin as a reference drug. All compounds exhibited measurable growth inhibition, with clear differences depending on the nature of the substituent.

Bromine-substituted derivatives, particularly compound **b2**, displayed the strongest antibacterial activity, as evidenced by the largest inhibition zones. Hydroxyl-substituted derivatives showed moderate activity, while unsubstituted compounds exhibited the weakest efficacy. This ordering of activity (**H < OH < Br**) can be rationalized by considering both electronic and physicochemical effects: hydroxyl groups contribute hydrogen-bonding capacity but increase polarity, limiting membrane permeability; bromine atoms, on the other hand, enhance lipophilicity and favor interactions with bacterial membranes, thereby conferring superior activity. These findings are consistent with prior reports on halogenated benzimidazole derivatives, where halogen substitution frequently augments antimicrobial potency (Pathare, 2021; Singh et al., 2017; Samreen et al., 2024; Faleye, 2024) [57-60].

3.4. Structure–Activity Relationship (SAR) Insights

The SAR analysis highlights that substituent effects on the benzimidazole ring critically dictate antibacterial potency. The correct activity sequence established in this study is **H < OH < Br**. The relatively weak activity of unsubstituted analogues (H) reflects the absence of electronic or lipophilic modulation. Hydroxyl substitution improves activity via hydrogen-bonding interactions, though its high polarity partially restricts penetration through bacterial membranes. Bromine substitution affords the most potent activity, owing to its electron-withdrawing character and increased lipophilicity, which together enhance membrane penetration and binding affinity.

These insights reinforce the importance of rational functionalization of the benzimidazole scaffold. In particular, halogenation appears to be a highly effective strategy for enhancing antimicrobial properties, positioning bromine-substituted Mannich bases as promising candidates for further pharmaceutical development.



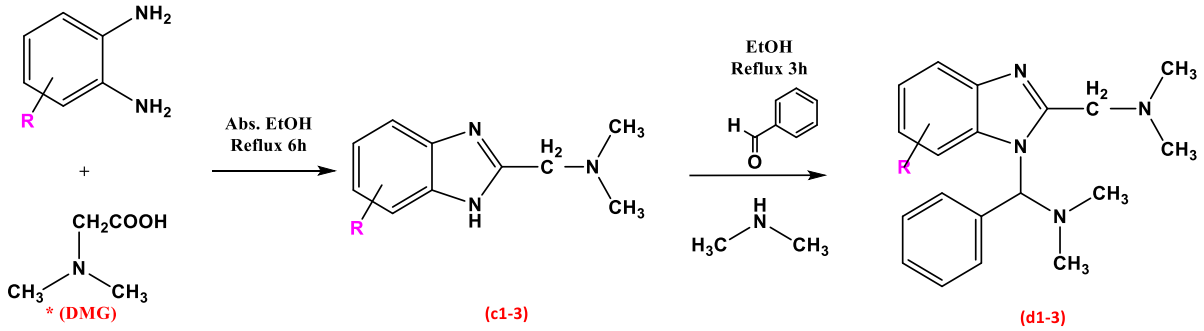
R = H (benzene-1,2-diamine)

Br (4-bromobenzene-1,2-diamine)

OH (4-hydroxybenzene-1,2-diamine)

Scheme 1. Synthesize Benzimidazole Derivatives (a1-3) and Benzimidazole Linked Mannich Derivatives (b1-3)

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R = H (benzene-1,2-diamine)

Br (4-bromobenzene-1,2-diamine)

OH (4-hydroxybenzene-1,2-diamine)

Scheme 2. Synthesize Benzimidazole Derivatives (c1-3) and Benzimidazole Linked Mannich Derivatives (d1-3).

Table 2. Data on the produced chemicals' spectra.

Comp.	FT-IR (KBr, ν cm⁻¹)	¹H-NMR (DMSO-d₆, δ ppm)
a1	3345 (N–H), 3030 (Ar–H), 1600 (C=N), 1485 (C=C)	7.1–8.0 (m, Ar–H), 12.8 (s, NH)
a2	3322 (N–H), 3055 (Ar–H), 1590 (C=N), 745 (C–Br)	
a3	3360 (N–H), 3300 (O–H), 1585 (C=N), 1470 (C=C)	
b1	3340 (N–H), 2800 (C–H, NMe_2), 1610 (C=N)	
b2	3328 (N–H), 2805 (NMe_2), 1588 (C=N), 755 (C–Br)	
b3	3352 (N–H), 3300 (O–H), 1592 (C=N)	
c1	3340 (N–H), 2810 (C–H), 1605 (C=N), 1150 (C–N)	
c2	3325 (N–H), 2808 (NMe_2), 1587 (C=N), 748 (C–Br)	
c3	3365 (N–H), 3305 (O–H), 1593 (C=N)	
d1	3345 (N–H), 2815 (NMe_2), 1608 (C=N), 1468 (C=C)	2.25 (s, 12H, 2×NMe_2), 3.35 (s, CH_2), 4.52 (s, CHPh), 7.0–8.0 (m), 12.6 (s, NH)
d2	3320 (N–H), 2800 (NMe_2), 1585 (C=N), 755 (C–Br)	
d3	3358 (N–H), 3300 (O–H), 1590 (C=N)	
* (DMG)	1720 (C=O), 2820–2760 (NMe_2), 1455 (C–N)	

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Table 3. Antimicrobial Activity of Synthesized Compounds Against S. aureus and E. coli (Inhibition Zone Diameters in mm).

Compound	S. aureus	E. coli
a1 (H)	9	9
a2 (Br)	16	15
a3 (OH)	12	12
b1 (H)	10	10
b2 (Br)	17	16
b3 (OH)	13	13
c1 (H)	9	9
c2 (Br)	15	14
c3 (OH)	12	12
d1 (H)	10	10
d2 (Br)	16	15
d3 (OH)	13	13
Ciprofloxacin	22	24

Legend:

= Low activity (H, 9–10 mm)

= Moderate activity (OH, 11–13 mm)

= High activity (Br, 14–17 mm)

= Very strong reference activity (Ciprofloxacin, 22–24 mm)

The antimicrobial activity of the synthesized compounds against *Staphylococcus aureus* and *Escherichia coli* was evaluated by measuring inhibition zone diameters (mm). Compounds bearing hydrogen (H) substituents exhibited the lowest activity (9–12 mm, dark red to orange shades), whereas hydroxyl (OH)-substituted derivatives showed moderate activity (12–13 mm, orange to light green). In contrast, bromo (Br)-substituted compounds displayed the highest activity among the derivatives (15–17 mm, green to yellow-green), with compound **b2** demonstrating the strongest inhibitory effect. Ciprofloxacin, used as a reference drug, showed the greatest antibacterial efficacy (22–24 mm, bright yellow-green). Overall, the activity followed the order **H < OH < Br < Ciprofloxacin**, confirming the significant influence of substituents on the antimicrobial potency of benzimidazole-based Mannich derivatives.

Conclusions

In this study, a series of benzimidazole derivatives was successfully synthesized starting from substituted *o*-phenylenediamines and unsubstituted benzaldehyde via condensation and Mannich reactions. Compounds a1–a3 and d1–d3 served as key intermediates for the synthesis of Mannich bases b1–b3, respectively. The use of sodium metabisulfite as an oxidizing agent in DMF under reflux conditions proved effective for benzimidazole ring formation. Structural characterization of the synthesized compounds was performed using melting point determination, TLC (Rf values), FT-IR, and ^{1}H-NMR spectroscopy, confirming the successful formation of the target molecules.

The antimicrobial activity of the Mannich bases was evaluated *in vitro* using the disc diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, with ciprofloxacin as a reference standard. Among the synthesized derivatives, **compound b2 (Br-substituted)** demonstrated the highest antibacterial activity. Hydroxyl-substituted derivatives exhibited moderate

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activity, while unsubstituted analogues were the least effective, confirming the correct trend **H < OH < Br**. Ciprofloxacin remained the most active overall, as expected.

These results emphasize that substitution patterns on the *o*-phenylenediamine moiety, rather than on the benzaldehyde component, play the primary role in modulating biological activity. In particular, bromine substitution significantly enhances antibacterial potency, highlighting the potential of halogenated benzimidazole-linked Mannich bases as promising scaffolds for the development of novel antibacterial agents. Further optimization and *in vivo* studies are recommended to fully explore their pharmacological potential.

Declaration of conflicting interests

No potential conflicts of interest were disclosed by the author(s) with regard to the research, writing, or publication of this paper.

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