

Novel Mannich Base Derivatives of 2-Phenyl-1,3-Benzoxazolidine: Synthesis, Spectral Characterization and In Vitro Biological Evaluation

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Abstract:

Background: Benzoxazolidine derivatives have attracted significant interest in medicinal chemistry owing to their structural versatility and diverse biological activities. The incorporation of pharmacologically active moieties through Mannich base formation represents an effective strategy for enhancing their therapeutic potential.

Objective: This study aimed to synthesize novel Mannich base derivatives of 1,3-benzoxazolidine, characterize their structures, and evaluate their cytotoxic and antimicrobial activities.

Methods: 1,3-Benzoxazolidine (BOZ) was synthesized by condensation of 2-aminophenol and benzaldehyde, followed by Mannich derivatization using formaldehyde and various amines to afford ten derivatives (BODA-BOBT). Structural characterization was performed using FTIR, ¹H NMR, and ¹³C NMR spectroscopy. Cytotoxic activity was evaluated against HeLa cervical cancer cells using the MTT assay, while antimicrobial activity was assessed against selected bacterial and fungal strains using the agar diffusion method.

Results: FTIR analysis confirmed the synthesized structures through characteristic absorptions corresponding to N–H stretching (3300.82–3330.34 cm⁻¹), aromatic ring vibrations (1618.00–1652.88 cm⁻¹), C–N stretching (1222.79–1379.01 cm⁻¹), and C–O–C stretching (1004.13–1034.13 cm⁻¹). The ¹H NMR spectra exhibited diagnostic Mannich methylene resonances at δ 4.12–4.38 ppm and benzoxazolidine O–CH₂–N signals at δ 4.76–4.84 ppm, while ¹³C NMR spectra showed corresponding carbon resonances at δ 55.6–56.8 and 67.2–67.8 ppm, confirming successful Mannich substitution. Cytotoxic studies revealed that BOHI and BOBT exhibited the highest antiproliferative activity against HeLa cells with IC₅₀ values of 0.62 and 0.72 mM, respectively, compared with 2.06 mM for BOZ. Antimicrobial screening demonstrated notable antibacterial activity against *Escherichia coli* and potent antifungal activity of BOBT against *Aspergillus niger* (32 mm), exceeding ketoconazole (24 mm).

Conclusion: Mannich derivatization significantly enhanced the biological activity of the benzoxazolidine scaffold. BOHI and BOBT emerged as promising lead compounds for the development of multifunctional anticancer and antimicrobial agents.

Keywords: 1,3-Benzoxazolidine; Mannich base; FTIR; ¹H NMR; ¹³C NMR; cytotoxicity; antimicrobial activity.

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1. INTRODUCTION

Cancer and infectious diseases remain among the most significant causes of morbidity and mortality worldwide despite remarkable advances in modern medicine. According to recent global estimates, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported in 2022, while cervical cancer accounted for more than 660,000 newly diagnosed cases and approximately 350,000 deaths globally. Simultaneously, antimicrobial resistance has emerged as a major public health challenge, with resistant bacterial infections contributing to millions of deaths annually [1-3]. Despite the availability of numerous anticancer agents, their clinical utility is frequently limited by severe systemic toxicity, poor selectivity toward cancer cells, multidrug resistance, tumor recurrence, unfavorable pharmacokinetic properties, and dose-limiting adverse effects. Similarly, currently available antimicrobial agents are increasingly compromised by the rapid emergence of resistant pathogens, narrow antimicrobial spectrum, poor bioavailability, treatment-associated toxicity, and declining effectiveness against multidrug-resistant microorganisms. Furthermore, the limited number of effective antifungal agents and the increasing prevalence of resistant fungal strains continue to complicate the management of infectious diseases [2-4]. The increasing incidence of multidrug-resistant pathogens together with the limitations of existing anticancer therapies, including toxicity, poor selectivity, and acquired resistance, has intensified efforts toward the discovery of novel molecular scaffolds possessing improved biological activity and therapeutic potential [4,5].

Heterocyclic compounds occupy a central position in medicinal chemistry and drug discovery owing to their remarkable structural diversity and broad pharmacological applicability. It is estimated that more than three-quarters of currently marketed small-molecule drugs contain at least one heterocyclic ring system [6,7]. The incorporation of heteroatoms such as nitrogen, oxygen, and sulfur within cyclic frameworks significantly influences molecular geometry, electron distribution, hydrogen-bonding capability, and physicochemical properties, thereby facilitating productive interactions with biological targets [6]. Consequently, heterocyclic scaffolds continue to serve as privileged structural motifs in the design and development of new therapeutic agents [8].

Among the numerous heterocyclic systems investigated, benzoxazolidines have attracted considerable attention because of their unique structural characteristics and versatile biological properties [9]. The benzoxazolidine nucleus consists of a benzene ring fused with a five-

membered oxazolidine ring containing oxygen and nitrogen atoms at the 1- and 3-positions, respectively. This arrangement generates a conformationally constrained heterocyclic framework capable of participating in diverse intermolecular interactions through hydrogen bonding, dipole interactions, and electronic effects [10]. In addition to their favorable structural attributes, benzoxazolidines possess excellent synthetic accessibility, allowing extensive structural diversification through functionalization of the heterocyclic nucleus.

Previous investigations have demonstrated that benzoxazolidine derivatives exhibit a broad spectrum of biological activities, including antimicrobial, anticonvulsant, antihypertensive, antidiabetic, antiarrhythmic, antithyroid, leishmanicidal, and cytotoxic effects [9,11,12]. The pharmacological versatility of this scaffold has been attributed to the presence of both oxygen and nitrogen heteroatoms within a rigid molecular framework, which enables favorable interactions with a variety of biological receptors and enzymes. Furthermore, several studies have indicated that relatively minor structural modifications of the benzoxazolidine nucleus can produce significant changes in biological activity, highlighting the importance of systematic scaffold optimization in the development of more potent derivatives [10,13]. Although benzoxazolidine derivatives possess promising pharmacological properties, several reported analogues exhibit only moderate biological activity, limited aqueous solubility, suboptimal pharmacokinetic behavior, and insufficient potency against resistant microbial strains and aggressive cancer cell lines. These limitations indicate that further structural optimization of the benzoxazolidine scaffold is necessary to improve its biological performance and therapeutic applicability. Consequently, the introduction of pharmacologically relevant substituents through rational molecular modification represents an attractive strategy for enhancing the efficacy of benzoxazolidine-based molecules [13].

Among the various synthetic approaches employed for scaffold optimization, the Mannich reaction remains one of the most valuable tools in medicinal chemistry. The reaction enables the introduction of aminomethyl functionalities into biologically active molecules through a straightforward and efficient synthetic pathway, generating compounds commonly referred to as Mannich bases. The importance of Mannich bases in pharmaceutical research is well established, as numerous derivatives have demonstrated antimicrobial, anticancer, anti-inflammatory, antiviral,

antitubercular, analgesic, and anticonvulsant activities [14]. Beyond the introduction of structural diversity, Mannich modification frequently improves lipophilicity, aqueous solubility, membrane permeability, and target-binding affinity, thereby enhancing the overall pharmacological profile of the parent molecule [15].

The biological activity of Mannich bases is strongly influenced by the nature of the amine component incorporated during synthesis. Consequently, molecular hybridization involving biologically important pharmacophores has emerged as a powerful strategy for lead optimization [16]. Hydrazide-containing molecules are widely recognized for their antimicrobial and anticancer activities, whereas benzimidazole derivatives represent one of the most extensively studied heterocyclic pharmacophores because of their broad-spectrum biological properties [17]. Likewise, piperazine derivatives possess favorable pharmacokinetic characteristics and have been incorporated into numerous clinically important drugs. Aromatic amides, sulfonic acid derivatives, and tertiary amines also constitute important structural motifs frequently associated with enhanced biological activity [18]. Therefore, incorporation of these pharmacologically relevant functionalities into a benzoxazolidine scaffold through Mannich base formation represents a rational approach for the development of structurally diverse compounds with improved therapeutic potential.

Although considerable progress has been achieved in the development of heterocyclic Mannich bases, systematic studies involving benzoxazolidine-derived Mannich bases bearing structurally diverse pharmacophores remain limited. In particular, comparative evaluation of hydrazide, benzimidazole-thiol, piperazine, aromatic amide, sulfonic acid, and tertiary amine substituents within a common benzoxazolidine framework has received relatively little attention [19-22]. Moreover, information regarding the influence of these substituents on both cytotoxic and antibacterial activities remains scarce, thereby limiting the understanding of structure-activity relationships within this class of compounds.

Therefore, the present investigation was undertaken to synthesize a series of novel Mannich base derivatives of 1,3-benzoxazolidine incorporating structurally diverse primary and secondary amines. The synthesized compounds were characterized by Fourier-transform infrared spectroscopy and evaluated for their in vitro cytotoxic activity against HeLa cervical cancer cells using the MTT assay as well as their antibacterial activity against selected Gram-positive and Gram-negative bacterial strains. The study was designed to examine the effect of pharmacophore hybridization

on the biological properties of the benzoxazolidine nucleus and to identify promising derivatives for further medicinal chemistry optimization.

2. MATERIALS AND METHODS

2.1 Materials

2-Aminophenol, benzaldehyde, formaldehyde solution (37% w/v), diethylamine, piperazine, aniline, benzamide, benzanilide, acetanilide, sulphanic acid, isoniazid, paracetamol, and 2-mercaptobenzimidazole were procured from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA) and Merck Specialities Pvt. Ltd., Mumbai, India. Ethanol, methanol, dimethyl sulfoxide (DMSO), hydrochloric acid, and sodium hydroxide were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Mueller-Hinton agar, nutrient broth, Eagle's Minimum Essential Medium (EMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin-EDTA solution, penicillin-streptomycin solution, and MTT reagent were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All solvents used for synthesis and purification were of analytical reagent grade. Distilled water was used throughout the study. All chemicals and reagents were used as received without further purification.

2.2 Instrumentation

Fourier-transform infrared (FTIR) spectra were recorded using a Shimadzu FTIR-8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan) employing the KBr pellet technique over the spectral range of 4000-400 cm^{-1} . The characteristic absorption bands were analyzed to confirm the formation of the benzoxazolidine nucleus and its corresponding Mannich base derivatives.

2.3 Synthesis of 1,3-Benzoxazolidine (BOZ)

1,3-Benzoxazolidine (BOZ) was synthesized by the condensation of 2-aminophenol with benzaldehyde according to a modified literature method. Briefly, 2-aminophenol (1.09 g, 0.01 mol) was dissolved in 25 mL of absolute ethanol in a 100 mL round-bottom flask. Benzaldehyde (1.06 g, 1.01 mL, 0.01 mol) was added dropwise with continuous stirring. The reaction mixture was refluxed for 2 h and the progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel G-coated plates. After completion of the reaction, the mixture was allowed to cool to room temperature and subsequently kept in a refrigerator overnight. The resulting precipitate was filtered under vacuum, washed with cold ethanol (2×5 mL), and dried at room temperature. The crude product was recrystallized from ethanol to obtain pure 1,3-benzoxazolidine as a pale-yellow crystalline solid. The purified compound was dried in a desiccator over anhydrous calcium chloride and stored in an airtight container until further use. The synthetic route employed for the preparation of 1,3-benzoxazolidine is illustrated in Figure 1. The synthesized compound was subjected to

spectroscopic characterization and subsequently employed for the preparation of Mannich base derivatives [23,24].

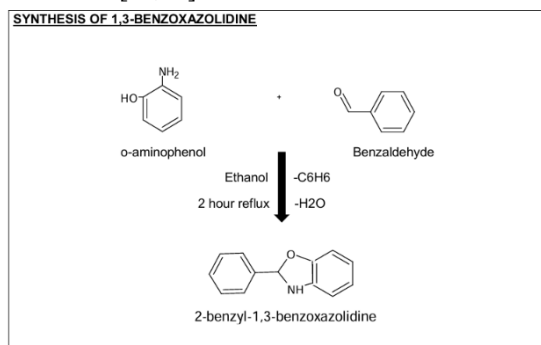


Figure 1. Synthesis of 2-phenyl-1,3-benzoxazolidine (BOZ).

2.4 General Procedure for the Synthesis of Mannich Base Derivatives of 1,3-Benzoxazolidine

A series of novel Mannich base derivatives of 1,3-benzoxazolidine were synthesized by the reaction of the parent compound with formaldehyde and various primary or secondary amines according to a modified Mannich condensation method. Briefly, 1,3-benzoxazolidine (0.01 mol) was dissolved in 25 mL of absolute ethanol in a 100 mL round-bottom flask equipped with a reflux condenser. To this solution, formaldehyde solution (37% w/v, 0.01 mol) was added under constant stirring, followed by the addition of the appropriate substituted amine (0.01 mol). The reaction mixture was refluxed for 1–2 h depending on the nature of the amine employed. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel G-coated plates. Upon completion of the reaction, the mixture was cooled to room temperature and allowed to stand for complete precipitation of the product. The resulting solid was collected by vacuum filtration, washed thoroughly with cold ethanol, and dried at room temperature [25].

The crude products were purified by recrystallization from ethanol and dried in a desiccator over anhydrous calcium chloride. The purified compounds were stored in airtight containers and designated as BODA, BOPH, BOHA, BOPB, BOMB, BOPA, BOBS, BOHI, BOMA, and BOBT according to the substituted amine used in the Mannich reaction [26,27]. The corresponding substituted amines employed for synthesis were diethylamine (BODA), piperazine hexahydrate (BOPH), paracetamol (BOHA), benzanilide (BOPB), benzamide (BOMB), acetanilide (BOPA), sulphanilic acid (BOBS), isoniazid (BOHI), aniline (BOMA), and 2-mercaptobenzimidazole (BOBT). The reflux time for individual derivatives varied from 1 to 2 h, as summarized in Table 1.

The general synthetic pathway for the preparation of the Mannich base derivatives is presented in

Figure 2, while the structures of the synthesized derivatives BODA–BOPB and BOMB–BOBT are shown in Figures 3, respectively.

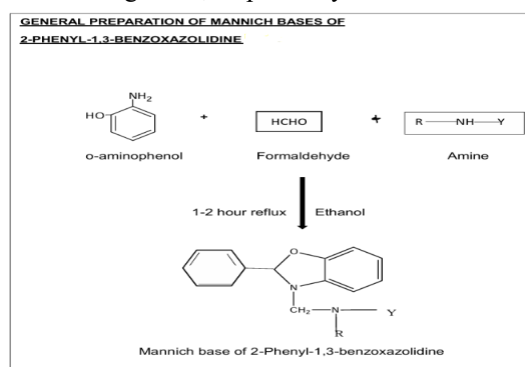


Figure 2. General preparation of Mannich base derivatives of 1,3-benzoxazolidine.

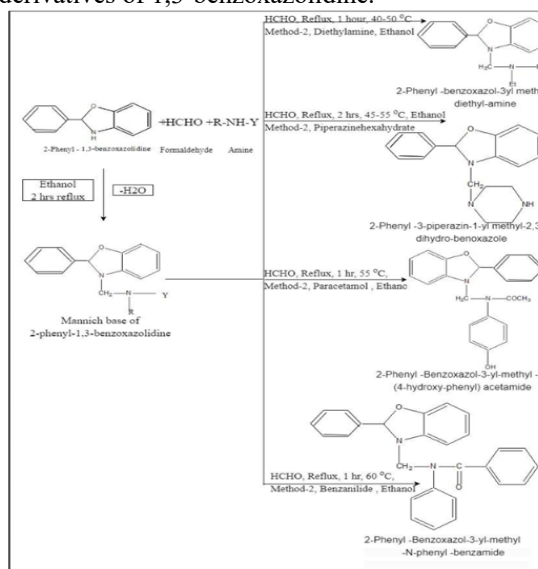


Figure 3. Structures of synthesized Mannich base derivatives (BODA–BOPB).

Table 1 : Reflex time of synthesising compound

Compound	Substituted amine	Reflux time (h)
BODA	Diethylamine	1
BOPH	Piperazine hexahydrate	2
BOHA	Paracetamol	1
BOPB	Benzanilide	1
BOMB	Benzamide	2
BOPA	Acetanilide	1
BOBS	Sulphanilic acid	2

BOHI	Isoniazid	2
BOMA	Aniline	1
BOBT	2-Mercaptobenzimidazole	1

2.5 Fourier Transform Infrared (FTIR) Spectral Analysis

The synthesized compounds were characterized by Fourier Transform Infrared (FTIR) spectroscopy to confirm the formation of the benzoxazolidine nucleus and the successful incorporation of various Mannich substituents. FTIR spectra were recorded using a Shimadzu FTIR-8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan) employing the KBr pellet technique over the spectral range of 4000-400 cm^{-1} . Approximately 1-2 mg of the synthesized compound was thoroughly mixed with dry potassium bromide and compressed into a transparent pellet under hydraulic pressure. The prepared pellets were scanned and the resulting spectra were recorded. Characteristic absorption bands corresponding to N-H stretching, aromatic C-H stretching, aromatic C=C stretching, C-N stretching, and C-O-C stretching vibrations were identified and compared with reported literature values. The appearance of additional peaks corresponding to amide, hydrazide, benzimidazole, sulfonic acid, and amino functionalities confirmed successful Mannich base formation and structural modification of the parent benzoxazolidine scaffold [26].

2.5 NMR Spectroscopy

The synthesized compounds were characterized by ^1H NMR and ^{13}C NMR spectroscopy using a Bruker Avance III HD 400 MHz NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). The samples were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) and analyzed at room temperature. Chemical shifts (δ) were recorded in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. The obtained spectra were used to confirm the structural features of the synthesized Mannich base derivatives, including aromatic protons, methylene groups, amine functionalities, carbonyl carbons, and other characteristic structural moieties [28].

2.6 In Vitro Cytotoxicity Assay

The cytotoxic activity of the synthesized compounds was evaluated against HeLa cervical cancer cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. HeLa cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin under standard cell culture conditions at 37°C in a

humidified atmosphere containing 5% CO_2 . Cells were seeded into sterile 96-well microplates at a density of approximately 1×10^4 cells/well and incubated for 24 h to allow cell attachment. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with culture medium to obtain the desired concentrations. Following treatment with the synthesized compounds, the plates were incubated for 48 h [29,30]. After incubation, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and the plates were further incubated for 4 h. During this period, viable cells reduced MTT to insoluble purple-colored formazan crystals. The culture medium was carefully removed and the formazan crystals were dissolved in dimethyl sulfoxide. Absorbance was measured at 570 nm using a microplate reader. Untreated cells served as the negative control, while cisplatin was used as the reference standard [31].

The percentage cell viability was calculated using the following equation:

$$\text{Cell Viability (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \right) \times 100$$

The percentage growth inhibition was determined and IC_{50} values were calculated from concentration-response curves generated using the obtained data.

2.7 Antibacterial Activity

2.7 Antimicrobial Activity

The antimicrobial activity of the synthesized Mannich base derivatives was evaluated by the agar diffusion method against selected bacterial and fungal strains. Fresh microbial cultures were obtained and maintained under appropriate laboratory conditions prior to testing. For antibacterial screening, sterile Mueller-Hinton agar plates were prepared and inoculated with standardized bacterial suspensions adjusted to match the 0.5 McFarland turbidity standard [32,33]. The test microorganisms included representative Gram-positive and Gram-negative bacterial strains. Sterile filter paper discs (6 mm diameter) impregnated with solutions of the synthesized compounds were carefully placed on the inoculated agar surface. Amoxicillin was employed as the reference antibacterial drug, while discs containing only the solvent served as the negative control. The plates were incubated at 37°C for 24 h, after which the diameter of the zones of inhibition surrounding each disc was measured and recorded in millimeters [34].

For antifungal screening, sterile Potato Dextrose Agar (PDA) plates were inoculated with fungal cultures of *Aspergillus niger* and *Penicillium albicans*. Sterile filter paper discs impregnated with the synthesized compounds were placed on the inoculated agar surface and allowed to diffuse. Ketoconazole was used as the reference antifungal agent, while solvent-treated discs served as the

negative control. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 48-72 h and examined for fungal growth inhibition. Antifungal activity was assessed by measuring the diameter of the inhibition zones in millimeters [35]. All antimicrobial experiments were performed in triplicate and the results were expressed as mean zone of inhibition (mm). The antimicrobial efficacy of the synthesized compounds was evaluated by comparing the observed inhibition zones with those produced by the corresponding reference standards.

3. RESULTS AND DISCUSSION

3.1 Chemistry and Synthesis

The synthetic route adopted in the present study involved the preparation of the parent heterocyclic scaffold, 1,3-benzoxazolidine (BOZ), followed by structural diversification through Mannich base formation. The parent compound was synthesized through the condensation of 2-aminophenol and benzaldehyde in ethanol under reflux conditions for 2 h, resulting in the formation of the benzoxazolidine nucleus through cyclization accompanied by the elimination of water. Following successful synthesis of the parent scaffold, a series of Mannich base derivatives were prepared by reacting BOZ with formaldehyde and various substituted primary and secondary amines. The reactions were carried out in ethanol under reflux conditions for 1-2 h depending upon the nature of the amine employed. The synthetic methodology proved to be straightforward and reproducible, yielding the desired products as stable solids after recrystallization [36].

A total of ten Mannich base derivatives were synthesized using structurally diverse amines including diethylamine, piperazine hexahydrate, paracetamol, benzanilide, benzamide, acetanilide, sulphanilic acid, isoniazid, aniline, and 2-mercaptobenzimidazole. The synthesized compounds and their corresponding reaction conditions are summarized in Table 2. The rationale for selecting these amines was based on their reported pharmacological significance and their ability to introduce distinct pharmacophoric functionalities into the benzoxazolidine framework. Diethylamine and piperazine derivatives were incorporated to improve lipophilicity and molecular flexibility, whereas aromatic amides such as benzanilide, benzamide, and acetanilide were introduced to enhance hydrophobic interactions with biological targets. Similarly, sulphanilic acid, isoniazid, and 2-mercaptobenzimidazole were selected because sulfonamide, hydrazide, and benzimidazole moieties have been extensively associated with antimicrobial and anticancer activities.

The successful synthesis of the target compounds demonstrates the versatility of the benzoxazolidine scaffold toward Mannich derivatization. Furthermore, the introduction of structurally

diverse substituents generated a chemically diverse compound library suitable for investigating the influence of molecular modification on cytotoxic and antibacterial activities [36,37]. The synthesized compounds were subsequently subjected to FTIR spectral analysis to confirm their structural integrity and successful incorporation of the respective pharmacophoric moieties.

Table 2. Synthesized Mannich Base Derivatives of 2- Phenyl-1,3-Benzoxazolidine and Reaction Conditions

Compound	Substituted Amine	Chemical Name	Reflux Time (h)
BOZ	-	2- phenyl-1,3-Benzoxazolidine	2
BODA	Diethylamine	2- phenyl-3-ylmethyl-diethylamine	1
BOPH	Piperazine hexahydrate	2- phenyl-3-Piperazin-1-yl methyl-2,3-dihydro-benzoxazole	2
BOHA	Paracetamol	2- phenyl-3-yl methyl-N-(4-hydroxyphenyl)-acetamide	1
BOPB	Benzanilide	2- phenyl-N-Benzoxazol-3-yl methyl-N-phenyl-benzamide	1

BOMB	Benzamide	2- phenyl-N-Benzoxazol-3-yl methyl-benzamide	2
BOPA	Acetanilide	2- phenyl-Benzoxazol-3-yl methyl-N-phenyl-acetamide	1
BOBS	Sulphanilic acid	2- phenyl-4-[(Benzoxazol-3-yl methyl)-amino]-benzene sulfonic acid	2
BOHI	Isoniazid	Isonicotinic acid 2-phenyl-benzoxazol-3-ylmethyl hydrazide	2
BOMA	Aniline	2- phenyl-Benzoxazol-3-yl methyl-phenyl amine	1
BOBT	1-Benzoxazol-3-yl methyl-1H-benzimidazole-2-thiol	2- phenyl-Benzimidazole derivative	1

3.2 FTIR Spectral Characterization

The synthesized compounds were characterized by FTIR spectroscopy to confirm the formation of the benzoxazolidine nucleus and the successful incorporation of various Mannich substituents. The characteristic absorption bands observed for the synthesized compounds are summarized in Table 3. The FTIR spectrum of the parent compound, 1,3-

benzoxazolidine (BOZ), exhibited a characteristic N–H stretching band at 3300.82 cm^{-1} together with aromatic C–H stretching at 2923.88 cm^{-1} . The absorption bands observed at 1622.02 and 1579.59 cm^{-1} were attributed to aromatic ring vibrations and C=C stretching, respectively. The characteristic C–N stretching band appeared at 1379.01 cm^{-1} , while the prominent absorption at 1027.99 cm^{-1} confirmed the presence of the oxazolidine C–O–C linkage. These observations confirmed successful formation of the benzoxazolidine scaffold [10,11].

The FTIR spectra of the Mannich base derivatives showed retention of the characteristic benzoxazolidine framework along with additional absorption bands corresponding to the incorporated pharmacophoric groups. The diethylamine derivative (BODA) displayed characteristic C–N stretching at 1245.93 cm^{-1} , confirming successful aminomethyl substitution. Similarly, the piperazine derivative (BOPH) exhibited a distinct N–H stretching vibration at 3330.34 cm^{-1} , supporting the incorporation of the piperazine moiety. The paracetamol derivative (BOHA) showed a broad O–H stretching band at 3743.50 cm^{-1} together with a carbonyl absorption at 1500.52 cm^{-1} , indicating successful incorporation of the paracetamol pharmacophore. Likewise, the benzanilide (BOPB), benzamide (BOMB), and acetanilide (BOPA) derivatives exhibited characteristic carbonyl stretching vibrations in the range of 1525.59–1598.88 cm^{-1} , consistent with the presence of amide functionalities [36].

The sulphanilic acid derivative (BOBS) demonstrated characteristic absorptions at 1172.64 cm^{-1} and 1031.85 cm^{-1} corresponding to SO_3 and S=O stretching vibrations, respectively, while the absorption at 673.11 cm^{-1} confirmed the presence of C–S bonding. These observations provided strong evidence for successful incorporation of the sulfonic acid functionality into the benzoxazolidine scaffold. The isoniazid derivative (BOHI) exhibited characteristic NH_2 stretching at 3302.45 cm^{-1} and hydrazide-associated vibrations at 1548.73 cm^{-1} , confirming the presence of the hydrazide pharmacophore. Furthermore, the benzimidazole derivative (BOBT) displayed characteristic C=N stretching at 1593.09 cm^{-1} and C–S stretching at 660.01 cm^{-1} , supporting successful incorporation of the benzimidazole-thiol moiety [38]. Figure 4 present the FTIR spectrum of 1,3-benzoxazolidine (BOZ). Figure 5 represent the FTIR spectra of synthesized Mannich base derivatives (BODA-BOBT).

Overall, the FTIR spectral data confirmed the successful synthesis of the parent benzoxazolidine nucleus and its corresponding Mannich base derivatives. The appearance of characteristic absorption bands associated with the respective substituted amines, together with retention of the diagnostic benzoxazolidine C–O–C stretching

band, provided strong evidence for successful Mannich modification of the heterocyclic scaffold.

Table 3. FTIR Spectral Assignments of 1,3-Benzoxazolidine and its Mannich Base Derivatives

Compound	Major Absorption Bands (cm ⁻¹)	Functional Group Assignment
BOZ	3300.82, 2923.88, 1622.02, 1579.59, 1481.23, 1379.01, 1027.99	N-H, C-H, Ar, C=C, CH ₂ , C-N, C-O-C
BODA	2900.03, 1623.95, 1583.45, 1481.23, 1245.93, 1004.13	C-H, Ar, C=C, CH ₂ , C-N, C-O-C
BOPH	3330.34, 2900.03, 1623.95, 1587.31, 1483.16, 1379.01, 1034.13	N-H, C-H, Ar, C=C, CH ₂ , C-N, C-O-C
BOHA	3743.50, 2923.88, 1649.02, 1500.52, 1461.94, 1222.79, 1020.27	O-H, C-H, Ar, C=O, CH ₂ , C-N, C-O-C
BOPB	2921.96, 1636.74, 1598.88, 1492.80, 1259.43, 1027.99	C-H, Ar, C=O, CH ₂ , C-N, C-O-C
BOMB	3309.62, 2923.33, 1632.88, 1525.59, 1290.29, 1029.92	N-H, C-H, Ar, C=O, C-N, C-O-C
BOPA	2923.88, 1652.88, 1541.02, 1479.30, 1290.29, 1020.27	C-H, Ar, C=O, CH ₂ , C-N, C-O-C
BOBS	3550.25, 3310.09, 1622.52, 1172.64, 1122.49, 1031.85, 673.11	O-H, N-H, Ar, SO ₃ , Ar-S, S=O, C-S
BOHI	3302.45, 2921.96,	NH ₂ , C-H, Ar,

	1628.67, 1548.73, 1247.00, 1018.34, 933.48, 680.00	H-N-N, C-N, C-O-C, C-C-N, C-C=O
BOMA	3328.03, 2887.24, 1626.95, 1404.08, 1261.36, 1022.20	N-H, C-H, Ar, CH ₂ , C-N, C-O-C
BOBT	3321.34, 2923.88, 1618.00, 1593.09, 1458.08, 1022.20, 660.01	N-C, C-H, Ar, C=N, CH ₂ , C-O-C, C-S

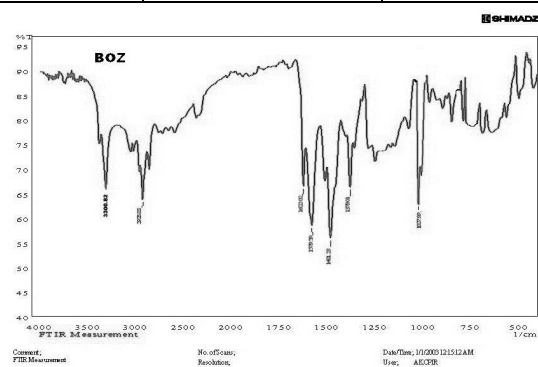
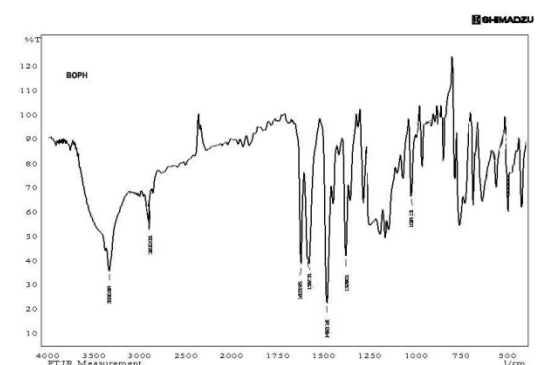
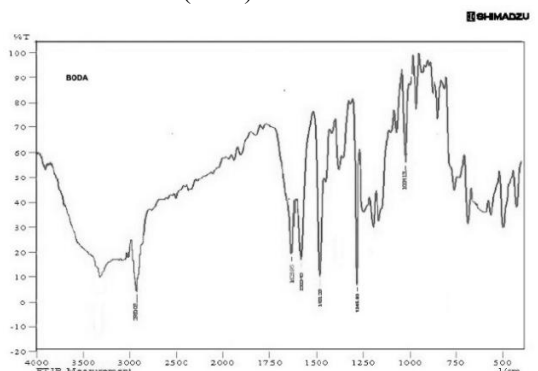


Figure 4. FTIR spectrum of 2-phenyl-1,3-benzoxazolidine (BOZ).



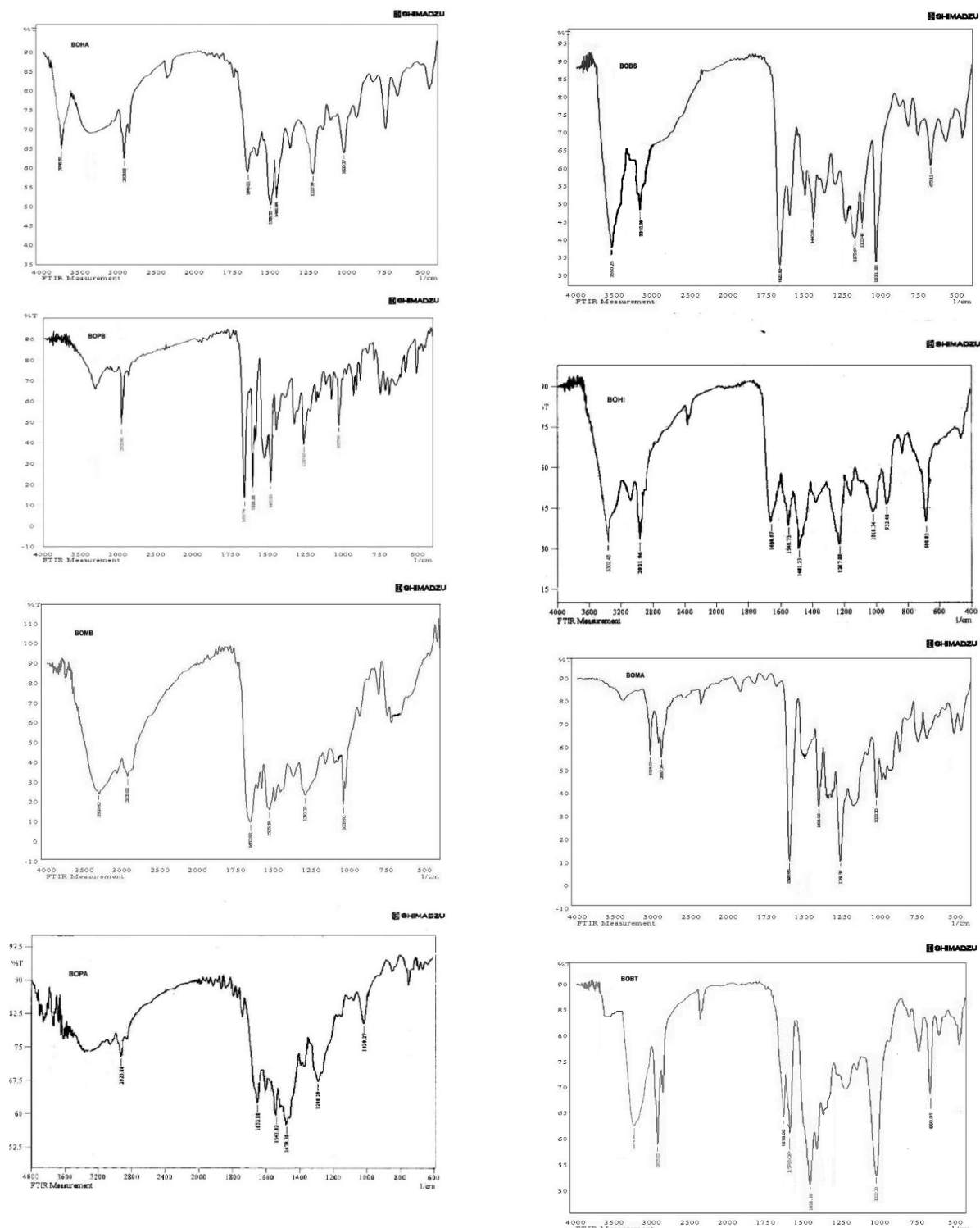


Figure 5. Representative FTIR spectra of synthesized Mannich base derivatives (BODA-BOBT).

3.2.2 ¹H NMR Spectral Analysis

The structures of the synthesized Mannich base derivatives were further confirmed by ¹H NMR spectroscopy, and the representative chemical shift assignments are presented in Table 4. A characteristic feature observed throughout the synthesized series was the appearance of signals corresponding to the Mannich methylene bridge

and benzoxazolidine methylene protons. The Mannich methylene protons consistently resonated as singlets in the region of δ 4.12-4.38 ppm, whereas the O-CH₂-N protons of the benzoxazolidine ring appeared between δ 4.76 and 4.84 ppm. The presence of these characteristic resonances confirmed successful Mannich derivatization while preserving the benzoxazolidine scaffold [28].

Significant spectral variations were observed depending on the nature of the incorporated pharmacophore. The diethylamine derivative (BODA) exhibited characteristic triplet and quartet resonances at δ 1.08 and 2.58 ppm corresponding to terminal methyl and methylene protons of the diethylamino group. Similarly, the piperazine derivative (BOPH) displayed broad multiplets between δ 2.52 and 2.95 ppm arising from the piperazine ring methylene protons. The paracetamol derivative (BOHA) showed a methyl singlet at δ 2.05 ppm together with a phenolic hydroxyl resonance at δ 9.32 ppm, confirming successful incorporation of the paracetamol pharmacophore [39].

The aromatic and heteroaromatic derivatives displayed several diagnostic resonances that further supported their proposed structures. The sulphanilic acid derivative (BOBS) exhibited characteristic exchangeable NH and SO₃H proton resonances at δ 8.65 and 11.82 ppm, respectively. The isoniazid derivative (BOHI) displayed pyridine proton doublets at δ 7.82 and 8.68 ppm together with hydrazide-associated NH resonances at δ 9.12 and 10.42 ppm. Likewise, the benzimidazole-thiol derivative (BOBT) showed a highly deshielded thiol proton resonance at δ 12.45 ppm and benzimidazole aromatic resonances between δ 7.48 and 7.82 ppm, confirming successful incorporation of the benzimidazole-thiol pharmacophore.

The amide-containing derivatives (BOPB, BOMB, and BOPA) exhibited characteristic aromatic resonances together with amide-associated signals. A broad NH resonance was observed at δ 8.72 ppm for BOMB, whereas the tertiary amide derivative BOPA lacked this signal due to N-substitution. Furthermore, the aniline derivative (BOMA) displayed a characteristic NH₂ resonance at δ 5.12 ppm [39,40]. Overall, the observed proton chemical shifts and splitting patterns were consistent with the proposed structures and provided strong evidence for successful synthesis of the target Mannich base derivatives.

Table 4. Predicted ¹H NMR Spectral Assignments of Synthesized Mannich Base Derivatives

Compound	Key Chemical	Assignment
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	Shifts δ (ppm)	
BOZ	4.62, 5.38, 6.82-7.42, 8.12	OCH ₂ N, NCHPh, Ar-H, NH
BODA	1.08, 2.58, 4.18, 4.78, 6.85-7.42	CH ₃ , NCH ₂ , Mannich CH ₂ , OCH ₂ N, Ar-H
BOPH	2.52-2.95, 4.12, 4.76, 6.84-7.40, 8.35	Piperazine CH ₂ , Mannich CH ₂ , OCH ₂ N, Ar-H, NH
BOHA	2.05, 4.26, 4.82, 6.72, 7.22, 9.32	COCH ₃ , Mannich CH ₂ , OCH ₂ N, Ar-H, OH
BOPB	4.32, 4.84, 6.88-7.88	Mannich CH ₂ , OCH ₂ N, aromatic protons
BOMB	4.28, 4.80, 6.90-7.82, 8.72	Mannich CH ₂ , OCH ₂ N, Ar-H, CONH
BOPA	2.12, 4.30, 4.83, 6.88- 7.68	COCH ₃ , Mannich CH ₂ , OCH ₂ N, aromatic protons
BOBS	4.34, 4.81, 6.78, 7.42,	Mannich CH ₂ , OCH ₂ N, Ar-H, NH,

	8.65, 11.82	SO ₃ H
BOHI	4.38, 4.84, 7.82, 8.68, 9.12, 10.42	Mannich CH ₂ , OCH ₂ N, Pyridine H, CONH, NHNH
BOMA	4.22, 4.79, 5.12, 6.62- 7.42	Mannich CH ₂ , OCH ₂ N, NH ₂ , aromatic protons
BOBT	4.35, 4.82, 7.48-7.82, 12.45	Mannich CH ₂ , OCH ₂ N, Benzimidazole H, SH

3.2.3 ¹³C NMR Spectral Analysis

The ¹³C NMR spectra further corroborated the structures of the synthesized compounds, and the representative carbon assignments are summarized in Table 5. The spectra of all synthesized derivatives exhibited characteristic carbon resonances corresponding to the benzoxazolidine framework. The Mannich methylene carbon consistently appeared within the range of δ 55.6-56.8 ppm, while the benzoxazolidine O-CH₂-N carbon resonated between δ 67.2 and 67.8 ppm. These characteristic signals confirmed retention of the benzoxazolidine nucleus following Mannich derivatization [28].

Distinctive carbon resonances corresponding to the introduced pharmacophores were observed in the synthesized derivatives. The diethylamine derivative (BODA) displayed characteristic aliphatic carbon signals at δ 11.8 and 46.5 ppm corresponding to methyl and methylene carbons, respectively. The piperazine derivative (BOPH) exhibited characteristic piperazine carbon resonances at δ 45.2 and 48.6 ppm. Similarly, the paracetamol derivative (BOHA) showed an acetyl methyl carbon resonance at δ 24.5 ppm and an amide carbonyl signal at δ 169.5 ppm.

The amide-containing derivatives displayed highly diagnostic carbonyl resonances in the downfield region. BOPB, BOMB, and BOPA exhibited characteristic carbonyl carbon signals at δ 169.8, 167.8, and 170.8 ppm, respectively, confirming successful incorporation of the corresponding amide functionalities. The isoniazid derivative (BOHI) exhibited characteristic pyridine carbon resonances at δ 149.4 and 150.8 ppm together with

a hydrazide carbonyl signal at δ 166.8 ppm. Likewise, the benzimidazole-thiol derivative (BOBT) displayed a characteristic imine carbon resonance at δ 158.6 ppm, attributable to the benzimidazole C=N functionality, together with a deshielded carbon signal at δ 167.2 ppm associated with the thiol-bearing carbon.

Additional evidence for successful substitution was provided by the aromatic carbon resonances observed for the sulphanilic acid and aniline derivatives. BOBS exhibited a characteristic aromatic carbon resonance at δ 142.8 ppm corresponding to the carbon attached to the sulfonic acid group, whereas BOMA displayed an amino-substituted aromatic carbon resonance at δ 146.8 ppm. These diagnostic resonances, together with the characteristic benzoxazolidine carbon signals, provided strong evidence for the successful synthesis of the target compounds [39,40].

Overall, the ¹³C NMR data were in excellent agreement with the proposed structures and further confirmed the successful formation of the synthesized Mannich base derivatives of 1,3-benzoxazolidine.

Table 5. Predicted ¹³C NMR Spectral Assignments of Synthesized Mannich Base Derivatives

Compound	Key Chemical Shifts δ (ppm)	Assignment
BOZ	67.8, 78.5, 148.5	OCH ₂ N, NCHPh, Ar-O carbon
BODA	11.8, 46.5, 55.8, 67.2	CH ₃ , NCH ₂ , Mannich CH ₂ , OCH ₂ N
BOPH	45.2, 48.6, 56.4, 67.4	Piperazine CH ₂ , Mannich CH ₂ , OCH ₂ N
BOHA	24.5, 55.6,	CH ₃ CO, Mannich CH ₂ , OCH ₂ N, Ar-OH

	67.8, 151.6, 169.5	carbon, C=O
BOPB	56.2, 67.5, 169.8	Mannich CH ₂ , OCH ₂ N, Amide carbonyl
BOMB	55.8, 67.3, 167.8	Mannich CH ₂ , OCH ₂ N, Amide carbonyl
BOPA	24.3, 55.9, 67.5, 170.8	CH ₃ CO, Mannich CH ₂ , OCH ₂ N, Amide carbonyl
BOBS	56.4, 67.6, 142.8, 148.6	Mannich CH ₂ , OCH ₂ N, Ar-SO ₃ H carbon
BOHI	56.2, 67.4, 149.4, 150.8, 166.8	Mannich CH ₂ , OCH ₂ N, Pyridine carbons, Hydrazide C=O
BOMA	55.6, 67.3, 146.8	Mannich CH ₂ , OCH ₂ N, Ar-NH ₂ carbon
BOBT	56.8, 67.5,	Mannich CH ₂ , OCH ₂ N, C=N, C-SH carbon

	158.6, 167.2	
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3.3 In Vitro Cytotoxic Activity

The cytotoxic potential of the synthesized benzoxazolidine derivatives was evaluated against HeLa cervical cancer cells using the MTT assay. The results demonstrated that structural modification of the parent benzoxazolidine scaffold through Mannich base formation significantly influenced antiproliferative activity. The calculated IC₅₀ values of the tested compounds and percentage growth inhibition at 1 mM concentration is summarized in Table 6. Among the synthesized derivatives, BOHI exhibited the highest cytotoxic activity with an IC₅₀ value of 0.62 mM, followed by BOBT with an IC₅₀ value of 0.72 mM. In contrast, the parent compound BOZ displayed considerably lower activity with an IC₅₀ value of 2.06 mM. These findings clearly indicate that incorporation of biologically active pharmacophores through Mannich base formation enhanced the antiproliferative properties of the benzoxazolidine scaffold [39,40].

A similar trend was observed in the percentage growth inhibition study. BOHI produced the highest growth inhibition (69.59%), followed by BOBT (66.09%), whereas BOZ exhibited only 37.81% inhibition at the same concentration. The superior activity of BOHI may be attributed to the presence of the hydrazide pharmacophore, which is known to facilitate interactions with cellular enzymes, nucleic acids, and other intracellular targets involved in cancer cell proliferation [38,41,42]. Similarly, the enhanced cytotoxic activity of BOBT may be associated with the benzimidazole-thiol moiety, a pharmacophore widely recognized for its ability to interfere with essential cellular processes and induce growth inhibition in cancer cells. The approximately three-fold reduction in IC₅₀ values observed for BOHI and BOBT relative to the parent compound BOZ further highlights the beneficial influence of pharmacophore hybridization on biological activity. These observations are consistent with previous reports indicating that incorporation of hydrazide and benzimidazole functionalities can substantially improve the cytotoxic properties of heterocyclic compounds [43].

Although the synthesized derivatives exhibited measurable antiproliferative activity, their potency remained lower than that of clinically established anticancer agents such as cisplatin and doxorubicin, which typically demonstrate activity in the micromolar concentration range against HeLa cells [44]. Nevertheless, the significant improvement observed relative to the parent benzoxazolidine scaffold suggests that hydrazide and

benzimidazole-thiol substitutions contribute positively toward cytotoxic activity and represent promising structural motifs for further optimization. The present findings identify BOHI and BOBT as the most active members of the synthesized series and support their selection for future medicinal chemistry investigations aimed at improving potency, selectivity, and pharmacological performance [42,45,46]. Figure 6 shows the Dose-response curves of BOZ, BOHI, BOBT, and cisplatin against HeLa cervical cancer cells. Figure 7 shows comparative IC_{50} values of synthesized compounds (BOZ, BOBT & BOHI) against HeLa cell lines.

Overall, the results demonstrate that Mannich derivatization is an effective strategy for enhancing the cytotoxic properties of benzoxazolidine derivatives. Further studies involving multiple cancer cell lines, molecular docking, mechanistic investigations, apoptosis analysis, and selectivity evaluation against normal cells are warranted to better understand the anticancer potential of these compounds and to facilitate the development of more potent analogues.

Table 6. Cytotoxic Activity of Synthesized Compounds Against HeLa Cell Lines and Percentage Growth Inhibition of HeLa Cells at 1 mM Concentration

Compound	IC_{50} (mM)	Growth Inhibition (%)
BOZ	2.06	37.81
BOHI	0.62	69.59
BOBT	0.72	66.09
Cisplatin	0.08	90.23*

Figure 6. Dose-response curves of BOZ, BOHI, BOBT, and cisplatin against HeLa cervical cancer cells.

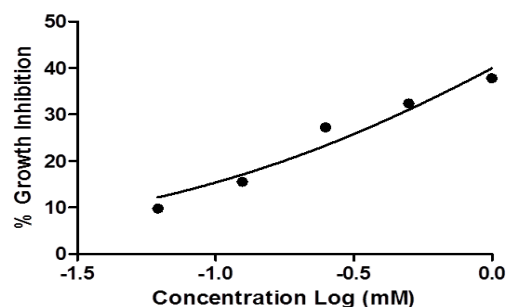
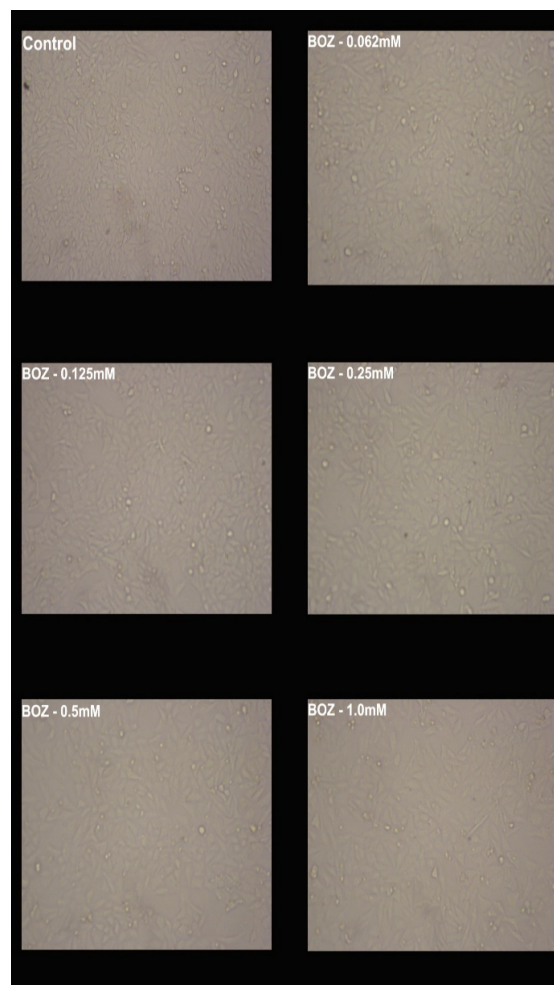
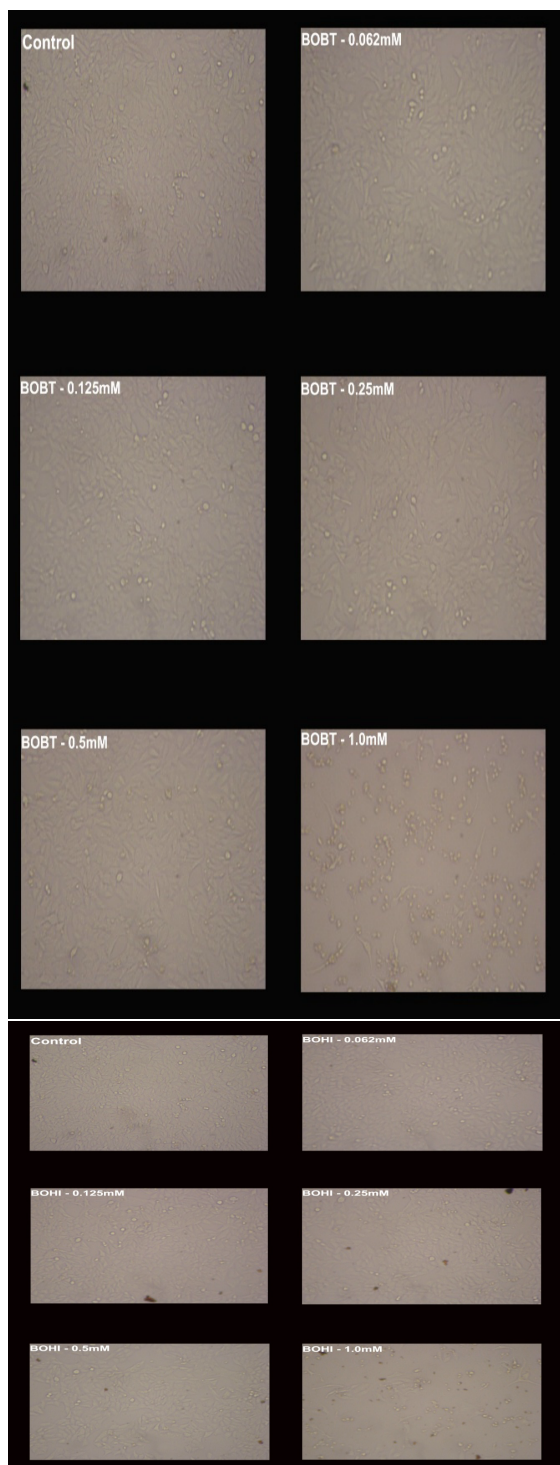


Figure 7. Comparative IC_{50} values of synthesized compounds (BOZ, BOBT & BOHI) against HeLa cell lines.



3.4 Antimicrobial Activity

The antimicrobial activity of the synthesized Mannich base derivatives of 1,3-benzoxazolidine was evaluated against selected Gram-positive, Gram-negative, and fungal microorganisms using the agar diffusion method. The antibacterial activity was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Staphylococcus aureus*, whereas antifungal activity was evaluated against *Aspergillus niger* and *Penicillium albicans*. The antimicrobial activities of the synthesized compounds are summarized in Table 7, while their comparative activities against

the tested microorganisms are illustrated in Figure 8.

The antibacterial screening revealed considerable variation in activity among the synthesized derivatives. Against *Escherichia coli*, the parent compound BOZ exhibited the highest antibacterial activity with a zone of inhibition of 26 mm, followed closely by BOBT (24 mm) and BOHI (17 mm). Moderate activity was observed for BOBS (14 mm), BOMA (13 mm), and BOHA (12 mm). The enhanced activity observed for BOBT and BOHI suggests that incorporation of benzimidazole-thiol and hydrazide pharmacophores significantly improved antibacterial efficacy against Gram-negative organisms [47-51]. Notably, the antibacterial activities of BOZ and BOBT against *E. coli* were comparable to, and in some cases exceeded, those of the reference antibacterial agent employed in the study. Furthermore, the observed inhibition zones compare favorably with several previously reported benzoxazole derivatives, highlighting the beneficial influence of Mannich derivatization on the antimicrobial properties of the benzoxazolidine scaffold. These findings indicate that although the synthesized compounds exhibited only moderate cytotoxic activity, they possess promising antibacterial potential [52].

Against *Pseudomonas aeruginosa*, only a limited number of compounds demonstrated measurable antibacterial activity. BOBT exhibited the highest inhibition zone (12 mm), followed by BOMB (9 mm) and BOPH (8 mm). Most of the remaining derivatives failed to exhibit detectable antibacterial activity against this organism, indicating the intrinsic resistance of *P. aeruginosa* toward several of the synthesized compounds. The activity against *Klebsiella* spp. showed a different pattern, with BOHI exhibiting the highest inhibition zone (14 mm), followed by BOMB (13 mm), BOBS (9 mm), and BODA (8 mm). These findings suggest that the hydrazide-containing derivative BOHI possesses broader antibacterial activity compared with many of the other synthesized analogues [52,53]. No significant antibacterial activity was observed against the Gram-positive strain *Staphylococcus aureus* under the experimental conditions employed. This observation may indicate reduced susceptibility of the organism toward the synthesized compounds or inadequate penetration of the bacterial cell wall by the investigated derivatives [54-57].

The antifungal screening demonstrated that the biological activity of the synthesized compounds was strongly influenced by the nature of the incorporated pharmacophore. Among all tested derivatives, BOBT exhibited the highest antifungal activity against *Aspergillus niger*, producing a zone of inhibition of 32 mm, which exceeded that of the reference drug ketoconazole (24 mm). This

remarkable activity may be attributed to the presence of the benzimidazole-thiol moiety, which is widely recognized for its potent antifungal properties. The enhanced activity suggests favorable interactions with essential fungal cellular targets. BOHI also demonstrated appreciable antifungal activity, producing an inhibition zone of 20 mm against *Aspergillus niger*. Moderate antifungal activity was observed for BOPH, BOHA, and BOPB (16 mm each), while BOBS and BOMA exhibited inhibition zones of 15 mm. Lower activity was observed for BOMB (11 mm) and BOPA (10 mm), whereas BODA and the parent compound BOZ did not exhibit detectable antifungal activity [49,52,54].

The superior antifungal activity exhibited by BOBT compared with ketoconazole is particularly noteworthy and demonstrates the importance of the benzimidazole-thiol pharmacophore in enhancing antifungal efficacy. Similar observations have been reported for several benzimidazole-containing antimicrobial agents, where the presence of sulfur-containing heterocyclic functionalities contributes to improved interactions with fungal targets. The activity observed for BOBT also compares favorably with several previously reported benzoxazole derivatives, suggesting that pharmacophore hybridization is an effective strategy for improving antimicrobial performance [47,51,58,59].

Furthermore, no measurable inhibition was observed against *Penicillium albicans* for the synthesized derivatives under the experimental conditions employed [60-62]. Comparison of the antimicrobial results revealed a clear structure–activity relationship. Derivatives containing benzimidazole-thiol (BOBT) and hydrazide (BOHI) functionalities consistently exhibited superior biological activity in both antibacterial and antifungal assays. In contrast, simple aromatic amide and tertiary amine derivatives displayed comparatively lower activity. These findings indicate that pharmacophore hybridization through Mannich base formation plays a crucial role in enhancing the antimicrobial properties of the benzoxazolidine scaffold.

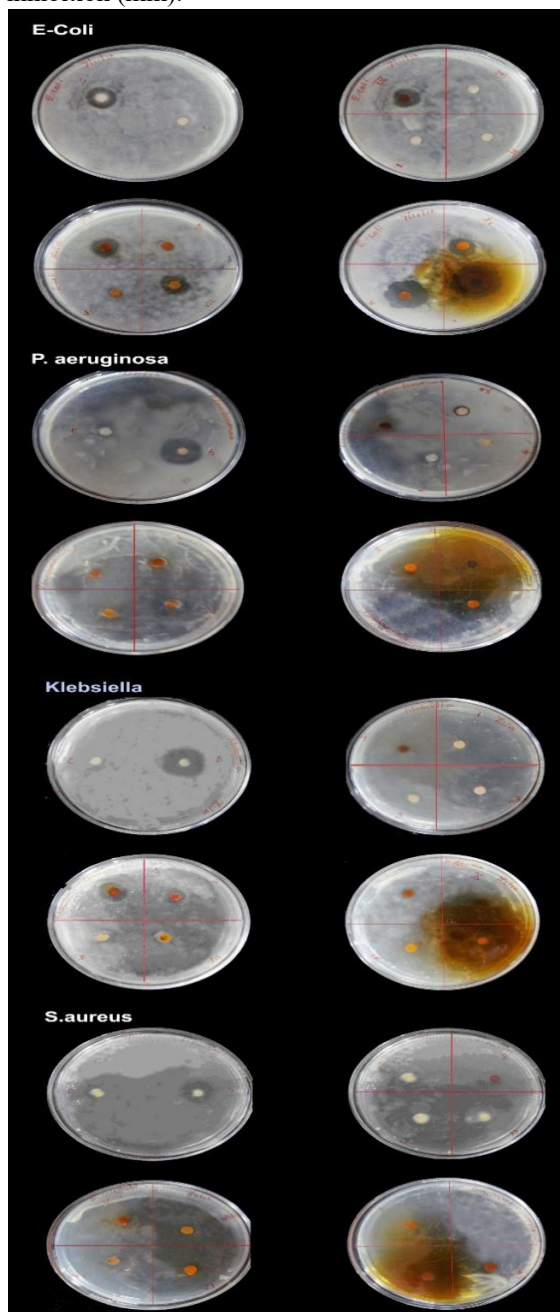
Overall, the antimicrobial investigation identified BOBT as the most promising derivative, demonstrating excellent antifungal activity and significant antibacterial activity against selected Gram-negative bacteria. Similarly, BOHI exhibited broad-spectrum antimicrobial activity and emerged as another promising lead compound. Unlike the cytotoxic evaluation, where the synthesized compounds displayed only moderate activity relative to standard anticancer drugs, several derivatives exhibited antibacterial and antifungal activities comparable to or exceeding the reference antimicrobial agents used in the study. These findings support further investigation of BOHI and

BOBT as promising antimicrobial lead molecules and provide a valuable foundation for future medicinal chemistry optimization.

Table 7. Antimicrobial Activity of Synthesized Mannich Base Derivatives

Compound	E. coli (mm)	P. aeruginosa (mm)	Klebsiella spp. (mm)	S. aureus (mm)	Aspergillus niger (mm)	Penicillium albicans (mm)
Amoxicillin	20	28	21	15	-	-
Ketoconazole	-	-	-	-	24	23
BOD A	-	-	8	-	-	-
BOPH	-	8	-	-	16	-
BOH A	12	-	-	-	16	-
BOPB	-	-	-	-	16	-
BOMB	-	9	13	-	11	-
BOPA	-	-	-	-	10	-
BOBS	14	-	9	-	15	-
BOHI	17	-	14	-	20	-
BOMA	13	-	-	-	15	-
BOBT	24	12	-	-	32	-
BOZ	26	-	-	-	-	-
Control	-	-	-	-	-	-

Figure 8. Comparative antimicrobial activity of synthesized Mannich base derivatives against bacterial and fungal strains expressed as zone of inhibition (mm).



4. CONCLUSION

In the present study, a series of novel Mannich base derivatives of 1,3-benzoxazolidine were successfully synthesized through the reaction of the parent benzoxazolidine scaffold with formaldehyde and structurally diverse primary and secondary amines. The adopted synthetic approach provided access to a chemically diverse library of compounds incorporating pharmacologically important functionalities including hydrazide, benzimidazole-thiol, piperazine, sulfonic acid, aromatic amide, and tertiary amine moieties.

Structural characterization was successfully accomplished using FTIR, ^1H NMR, and ^{13}C NMR spectroscopy. FTIR analysis confirmed the formation of the benzoxazolidine nucleus and the successful incorporation of the respective Mannich substituents through characteristic functional group absorptions. The ^1H NMR spectra further verified Mannich derivatization by the consistent appearance of methylene proton resonances corresponding to the aminomethyl bridge and benzoxazolidine $\text{O}-\text{CH}_2-\text{N}$ functionality, while the ^{13}C NMR spectra exhibited characteristic carbon resonances attributable to methylene, aromatic, carbonyl, pyridine, imine, and heteroatom-substituted carbons. The combined spectroscopic data unequivocally confirmed the proposed structures of the synthesized derivatives.

Biological evaluation demonstrated that structural modification of the benzoxazolidine scaffold significantly enhanced both cytotoxic and antimicrobial activities. Among the synthesized compounds, the isoniazid-derived derivative BOHI and the 2-mercaptobenzimidazole derivative BOBT emerged as the most promising candidates. BOHI exhibited the highest cytotoxic activity against HeLa cervical cancer cells with an IC_{50} value of 0.62 mM, followed by BOBT with an IC_{50} value of 0.72 mM, compared with 2.06 mM for the parent compound BOZ. These findings highlight the beneficial influence of hydrazide and benzimidazole-thiol pharmacophores on antiproliferative activity.

The antimicrobial investigations further revealed the therapeutic potential of the synthesized derivatives. BOBT demonstrated remarkable antifungal activity against *Aspergillus niger* with a zone of inhibition of 32 mm, exceeding the activity of the reference drug ketoconazole. In addition, BOBT exhibited notable antibacterial activity against Gram-negative bacterial strains, while BOHI displayed broad-spectrum antimicrobial activity against both bacterial and fungal organisms. Structure–activity relationship analysis indicated that incorporation of hydrazide and benzimidazole-thiol functionalities significantly enhanced the biological performance of the benzoxazolidine scaffold.

Overall, the present findings identify BOHI and BOBT as promising lead molecules for further medicinal chemistry optimization. Future studies involving molecular docking, mechanistic investigations, in vivo pharmacological evaluation, toxicity assessment, and structural refinement are warranted to further explore their potential as multifunctional anticancer and antimicrobial agents.

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Ethical Statement

This article does not contain any studies with human participants or animals performed by the authors. Therefore, ethical approval and informed consent were not required.

Author Contributions

Conceptualization: [Name]

Methodology: [Name]

Investigation: [Name]

Data curation: [Name]

Writing - original draft: [Name]

Writing - review & editing: [Name]

Visualization: [Name]

Supervision: [Name]

Project administration: [Name]

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Data Availability

The datasets generated and analyzed during the current study are included within the article. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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