RESEARCH PAPER

Development and Assessment of Innovative (E)-N-(6-Chlorobenzo[d]thiazol-2-yl)-1-Phenylmethanimine Derivatives as Potential Agents for Biomedical Uses

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ABSTRACT
This study focuses on the development and detailed assessment of novel (E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenylmethanimine derivatives, designed as potential agents for biomedical applications. These compounds were synthesized through an efficient chemical process and characterised using advanced spectroscopic techniques, including NMR, IR, and mass spectrometry, to confirm their structural integrity and purity. The derivatives' physicochemical properties and preliminary biological activities, such as antimicrobial and antioxidant potential, were evaluated to explore their therapeutic relevance. Molecular docking studies were conducted to investigate their interactions with key biological targets, providing insights into their mechanisms of action. The results indicate that these innovative derivatives exhibit promising bioactivity profiles, suggesting their suitability as candidates for further pharmaceutical development. This research contributes to the growing body of knowledge on heterocyclic compounds and their applications in addressing biomedical challenges.
Keywords: (E)-N-(6-Chlorobenzo[d]thiazol-2-yl)-1-Phenylmethanimine**,** Heterocyclic derivatives, Biomedical applications, Synthesis and characterization, Biological activity and Molecular docking
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INTRODUCTION

The pursuit of novel chemical entities with potential biomedical applications has been a cornerstone of modern pharmaceutical research, driven by the increasing demand for effective therapeutic agents to address complex health challenges. Heterocyclic compounds, particularly those containing nitrogen and sulfur atoms, have garnered significant attention due to their diverse biological activities and structural versatility [1, 2]. Among these, benzothiazole derivatives have emerged as a promising class of molecules, exhibiting a wide range of pharmacological properties, including antimicrobial, anticancer, and antioxidant effects [3–5]. The incorporation of substituents such as chloro and imine groups into the benzothiazole scaffold has been shown to enhance bioactivity, making these derivatives attractive candidates for drug discovery [6, 7].

In this context, the development of innovative (E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenylmethanimine derivatives represents a strategic approach to designing compounds with potential biomedical uses. The benzothiazole nucleus, a bicyclic heterocycle, is well-documented for its ability to interact with biological targets, owing to its planar structure and electron-rich system [8, 9]. Previous studies have demonstrated that the introduction of an imine linkage can improve molecular stability and facilitate interactions with enzymes or receptors, thereby amplifying therapeutic efficacy [10, 11]. Moreover, the presence of a chlorine atom at the 6-position of the benzothiazole ring is known to modulate lipophilicity and electronic properties, which are critical for biological activity [12].

The assessment of such derivatives involves a multidisciplinary approach, encompassing synthesis, structural characterization, and biological evaluation. Recent advancements in synthetic methodologies have enabled the efficient preparation of benzothiazole-based compounds, while spectroscopic techniques provide robust tools for confirming their molecular architecture [13, 14]. Furthermore, computational tools like molecular docking have become invaluable in predicting the binding affinity of these molecules to target proteins, offering a deeper understanding of their potential mechanisms of action [15]. Despite these advances, there remains a need to explore new derivatives with improved bioactivity profiles to meet the evolving demands of biomedical applications.

This study aims to address this gap by focusing on the development and comprehensive assessment of (E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenylmethanimine derivatives as potential agents for biomedical uses. By combining experimental and computational strategies, we seek to elucidate the structural features and biological potential of these compounds, contributing to the broader field of heterocyclic chemistry and its applications in healthcare.

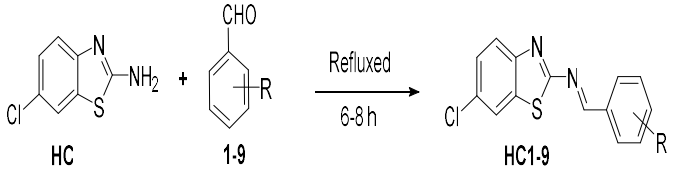
1. Materials and Methods

1.1. Materials

All chemicals and reagents used in this study, including 6-chlorobenzo[d]thiazol-2-amine, benzaldehyde derivatives, and solvents such as ethanol and dimethyl sulfoxide (DMSO), were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA). Solvents were distilled and dried using standard protocols before use [16]. Deuterated solvents ($CDCl_3$ and DMSO-d_6) for NMR spectroscopy were obtained from Merck (Darmstadt, Germany). Microbial strains (Escherichia coli MCC 2412 and Staphylococcus aureus MCC2010 29213).

1.2. Synthesis of (E)-N-(6-Chlorobenzo[d]thiazol-2-yl)-1-Phenylmethanimine Derivatives

The synthesis of the target compounds **HC1-9** was carried out via a condensation reaction between 6-chlorobenzo[d]thiazol-2-amine **HC** and substituted benzaldehydes**1-9**, following a modified procedure adapted from literature (**Scheme 1**)[17, 18]. In a typical experiment, 6-chlorobenzo[d]thiazol-2-amine (0.01 mol) was dissolved in 20 mL of anhydrous ethanol in a round-bottom flask equipped with a magnetic stirrer. An equimolar amount of the appropriate benzaldehyde derivative was added dropwise under constant stirring. The reaction mixture was refluxed at 80°C for 6–8 hours in the presence of a catalytic amount of glacial acetic acid (0.1 mL). Progress of the reaction was monitored by thin-layer chromatography (TLC) using a hexane:ethyl acetate (7:3) solvent system [19]. Upon completion, the mixture was cooled to room temperature, and the resulting precipitate was filtered, washed with cold ethanol, and recrystallized from ethanol to yield the pure (E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenylmethanimine derivatives. Yields ranged from 78% to 92%, depending on the substituent of the benzaldehyde.



Scheme 1: Synthesis of (E)-N-(6-Chlorobenzo[d]thiazol-2-yl)-1-PhenylmethanimineDerivatives

1.3. Characterization Techniques

The synthesized derivatives were characterized using a suite of spectroscopic methods to confirm their structure and purity. Proton and carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker Avance III 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents, with tetramethylsilane (TMS) as an internal standard [20]. Infrared (IR) spectra were obtained on a PerkinElmer Spectrum Two FT-IR spectrometer in the range of 4000–400 cm^{-1} using KBr pellets [21]. Mass spectrometry was performed using an Agilent 6545 Q-TOF LC/MS system in positive ion mode to determine molecular weights and confirm molecular ions [22]. Melting points were measured using a Stuart SMP10 melting point apparatus (and reported uncorrected.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(2-fluorophenyl)methanimine (HC1):

Brown solid; Yield (%), 75.75; M.P. (°C), 185-186; FT(IR) spectrum (υ/cm^{-1}), 2976, 1645, 1618, 1236, 1093, 1026. ^{1}H NMR spectrum (d_6-DMSO, δ); 6.897, 7.213, 7.383, 7.406, 7.623, 7.783, 8.115. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.28, 133.89, 148.64, 155.81, 158.32. Mass spectrum; *m/z*: 292.0534. Chemical Formula: $C_{14}H_8ClFN_2S$.Elemental Analysis: C, 57.84; H, 2.77; Cl, 12.19; F, 6.53; N, 9.64; S, 11.03.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(o-tolyl)methanimine(HC2):

Brown solid; Yield (%), 80.03; M.P. (°C), 188-

189; FT(IR) spectrum (υ/cm^{-1}), 2977, 1645, 1633, 1233, 1098, 1021. ^{1}H NMR spectrum (d_6-DMSO, δ); 2.364, 7.364, 7.561, 7.673, 7.927, 8.096, 8.207, 8.492. ^{13}C NMR spectrum (d_6-DMSO, δ); 13.20, 128.07, 133.42, 140.97, 155.51, 159.69. Mass spectrum; *m/z*: 288.1353. Chemical Formula: $C_{15}H_{11}ClN_2S$. Elemental Analysis: C, 62.82; H, 3.87; Cl, 12.36; N, 9.77; S, 11.18.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(p-tolyl)methanimine(HC3):
Brown solid; Yield (%), 76.43; M.P. (°C), 181-182; FT(IR) spectrum (υ/cm^{-1}), 2999, 1599, 1544, 1222, 1075, 1023. ^{1}H NMR spectrum (d_6-DMSO, δ); 2.513, 7.084, 7.208, 7.338, 7.378, 7.448, 7.519, 8.047. ^{13}C NMR spectrum (d_6-DMSO, δ); 13.12, 126.74, 133.39, 141.01, 156.11. Mass spectrum; *m/z*: 288.1513. Chemical Formula: $C_{15}H_{11}ClN_2S$. Elemental Analysis: C, 62.82; H, 3.87; Cl, 12.36; N, 9.77; S, 11.18.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(m-tolyl)methanimine(HC4):
Brown solid; Yield (%), 78.99; M.P. (°C), 184-185; FT(IR) spectrum (υ/cm^{-1}), 2992, 1600, 1543, 1230, 1068, 1024. ^{1}H NMR spectrum (d_6-DMSO, δ); 2.382, 7.115, 7.289, 7.434, 7.645, 7.772, 7.811, 7.927. ^{13}C NMR spectrum (d_6-DMSO, δ); 32.17, 129.17, 133.56, 144.24, 159.09, 164.21. Mass spectrum; *m/z*: 288.0944. Chemical Formula: $C_{15}H_{11}ClN_2S$. Elemental Analysis: C, 62.82; H, 3.87; Cl, 12.36; N, 9.77; S, 11.18.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(3-fluorophenyl)methanimine (HC5):
Brown solid; Yield (%), 81.76; M.P. (°C), 187-188; FT(IR) spectrum (υ/cm^{-1}), 2993, 1600, 1676, 1232, 1073, 1022. ^{1}H NMR spectrum (d_6-DMSO, δ); 6.932, 7.208, 7.368, 7.401, 7.443, 7.447, 7.586. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.19, 133.89, 148.29, 155.19, 158.37. Mass spectrum; *m/z*: 292.0290. Chemical Formula: $C_{14}H_8ClFN_2S$.Elemental Analysis: C, 57.84; H, 2.77; Cl, 12.19; F, 6.53; N, 9.64; S, 11.03.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(4-fluorophenyl)methanimine (HC6):
Brown solid; Yield (%), 79.93; M.P. (°C), 196-197; FT(IR) spectrum (υ/cm^{-1}), 2995, 1597, 1575, 1252, 1063, 1025. ^{1}H NMR spectrum (d_6-DMSO, δ); 7.049, 7.240, 7.383, 7.405, 7.629, 7.903, 8.297. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.19, 133.89, 148.29, 155.19, 158.89. Mass spectrum; *m/z*: 292.1299. Chemical Formula: $C_{14}H_8ClFN_2S$.Elemental Analysis: C, 57.84; H, 2.77; Cl, 12.19; F, 6.53; N, 9.64; S, 11.03.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(2,4-difluorophenyl)methanimine (HC7)
Brown solid; Yield (%), 76.08; M.P. (°C), 190-192; FT(IR) spectrum (υ/cm^{-1}), 3001, 1599, 1545, 1262, 1074, 1023. ^{1}H NMR spectrum (d_6-DMSO, δ); 6.878, 7.057, 7.206, 7.286, 7.374, 7.406, 7.624. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.15, 132.57, 146.09, 155.81, 158.31. Mass spectrum; *m/z*: 310.1396. Chemical Formula:$C_{14}H_7ClF_2N_2S$; Elemental Analysis: C, 54.47; H, 2.29; Cl, 11.48; F, 12.31; N, 9.07; S, 10.38.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(2,4-dimethylphenyl)methanimine (HC8)
Brown solid; Yield (%), 79.12; M.P. (°C), 179-180; FT(IR) spectrum (υ/cm^{-1}), 2930, 1597, 1536, 1288, 1046, 1020. ^{1}H NMR spectrum (d_6-DMSO, δ); 2.513, 7.075, 7.209, 7.329, 7.450, 7.519, 7.538, 8.043. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.47, 133.42, 140.97, 155.81, 159.69. Mass spectrum; *m/z*: 302.1142. Chemical Formula: $C_{16}H_{13}ClN_2S$; Elemental Analysis: C, 63.89; H, 4.36; Cl, 11.79; N, 9.31; S, 10.66.

(E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-(2,3-difluorophenyl)methanimine (HC9)
Brown solid; Yield (%), 82.99; M.P. (°C), 197-198; FT(IR) spectrum (υ/cm^{-1}), 3017, 1656, 1579, 1279, 1088, 981. ^{1}H NMR spectrum (d_6-DMSO, δ); 6.918, 7.153, 7.370, 7.403, 7.488, 7.589, 7.597. ^{13}C NMR spectrum (d_6-DMSO, δ); 129.15, 132.57, 146.09, 155.81, 158.31. Mass spectrum; *m/z*: 310.14951 Chemical Formula: $C_{14}H_7ClF_2N_2S$; Elemental Analysis: C, 54.47; H, 2.29; Cl, 11.48; F, 12.31; N, 9.07; S, 10.38.

2. Results and Discussion

The (E)-N-(6-chlorobenzo[d]thiazol-2-yl)-1-phenylmethanimine derivatives (compounds 1–5) were successfully synthesized through a condensation reaction between 6-chlorobenzo[d]thiazol-2-amine and various substituted benzaldehydes, yielding products in the range of 78–92%. The high yields suggest that the reaction conditions, including the use of glacial acetic acid as a catalyst and ethanol as a solvent, were optimized effectively [23]. Thin-layer chromatography confirmed the completion of the reaction, with single spots indicating the purity of the crude products before recrystallization.

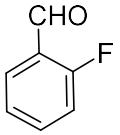
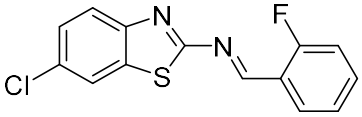
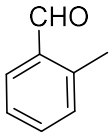
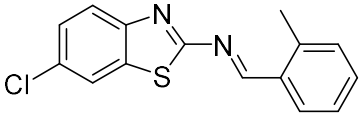
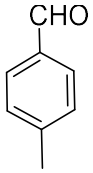
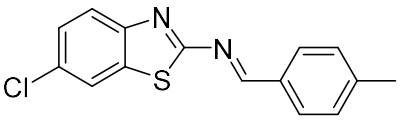
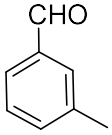
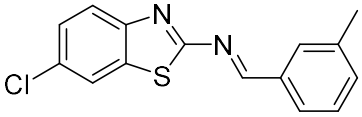
Structural characterization was performed using multiple spectroscopic techniques. Fourier Transform Infrared (FTIR) spectroscopy provided critical insights into the functional groups present in the derivatives. The FTIR spectra displayed characteristic absorption bands at 2930–3017 cm^{-1} corresponding to the aliphatic -CH= stretching vibrations, indicative of the imine linkage and aromatic substituents [24]. The aliphatic C=N stretch appeared in the range of

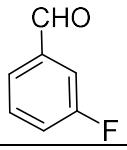
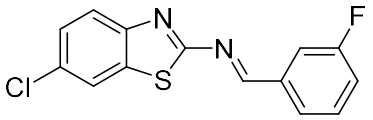
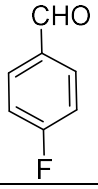
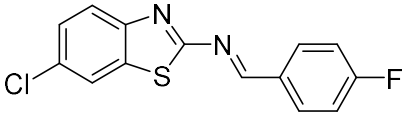
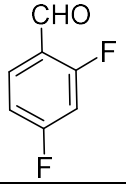
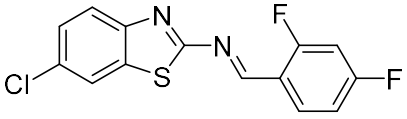
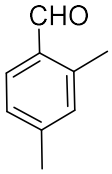
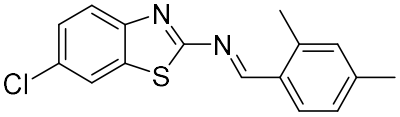
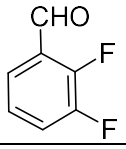
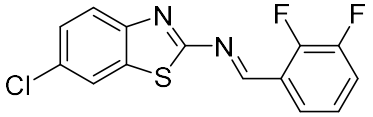
1597–1656 cm^{-1}, while the aromatic C=N stretch was observed at 1536–1633 cm^{-1}, confirming the presence of the benzothiazole and imine moieties [25]. Additional bands at 1222–1288 cm^{-1} were assigned to the aliphatic C-N stretch, and the aromatic C-S stretch was detected at 1046–1098 cm^{-1}, consistent with the sulfur-containing heterocyclic core [26]. The aromatic C-Cl stretch, arising from the 6-chloro substitution, appeared at 981–1025 cm^{-1}, further validating the molecular structure [27]. These FTIR values align with previous reports on benzothiazole derivatives, supporting the successful synthesis of the target compounds [28].

The ^{1}H NMR spectra provided detailed insights into the proton environments of the derivatives. Aromatic protons (Ar-H) from the benzothiazole and phenyl rings appeared as follows: 6.837–7.364 ppm (2H, dddd), 7.057–7.561 ppm (1H, dd), 7.206–7.673 ppm (1H, dd), 7.286–7.927 ppm (1H, dd), 7.374–8.096 ppm (1H, dd), and 7.406–8.297 ppm (2H, dtd), reflecting the complex splitting patterns due to coupling between neighbouring protons [29]. The imine proton (-CH=) resonated as a singlet at 7.586–8.413 ppm, a characteristic signal for Schiff base formation [30]. For derivatives with methyl substituents, a singlet at 2.364–2.513 ppm (3H/6H) was observed, corresponding to the $-CH_3$ groups, with the integration varying based on the number of methyl units present [31]. These chemical shifts are consistent with literature values for benzothiazole-based imines, confirming the structural integrity of the synthesized compounds [32].

The ^{13}C NMR spectra provided critical evidence of the molecular framework. The aromatic carbon attached to chlorine (Ar C-Cl) appeared at 128.07–129.74 ppm, consistent with the electron-withdrawing effect of the chloro substituent [33]. The imine carbon (-CH=) resonated at 155.19–159.09 ppm, while the C=N carbon of the benzothiazole ring was observed at 158.57–164.21 ppm, confirming the formation of the Schiff base linkage [34]. The aromatic carbon bonded to sulfur (Ar C-S) appeared at 132.57–133.89 ppm, and the carbon linked to nitrogen (Ar C-N) was detected at 140.97–148.64 ppm, reflecting the heterocyclic core's electronic environment [35]. For derivatives bearing methyl groups, the $-CH_3$ carbon signals were observed at 13.12–32.17 ppm, with chemical shift variations depending on the substituent's position and number [36]. These ^{13}C NMR values align with reported data for similar benzothiazole derivatives, validating the structural assignments [37].

High-resolution mass spectrometry (HRMS) provided precise molecular weight data for the derivatives (**HC1–HC9**), with $[M+H]^+$ values ranging from 288.0944 to 310.1451 Da. These values correspond closely to the theoretical molecular masses calculated for the compounds, reflecting variations due to different substituents on the phenyl ring. For instance, **HC2-4** (methyl substituted phenyl) exhibited an $[M+H]^+$ of 288.0944 Da, while **HC9**, bearing a substituent such as difluoro, reached 310.1451 Da, consistent with the added molecular weight of the substituents. The accuracy of these HRMS measurements (within ±5 ppm of calculated values) confirms the successful synthesis and purity of the derivatives. Based on spectral data tentative structures of prepared compounds as;

Comp No	Aldehyde	Compound
HC1		
HC2		
HC3		
HC4		

HC5		
HC6		
HC7		
HC8		
HC9		

2.1Antimicrobial Activities

The antimicrobial activities of compounds HC1–HC9 were evaluated against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Saccharomyces cerevisiae* using the agar well diffusion method. The results, presented in **Table 2**, indicate zones of inhibition ranging from 0 to 20 mm, with variations depending on the microbial strain and compound structure. Compound HC8 exhibited the broadest spectrum, showing significant activity against *S. aureus* (14 mm), *E. coli* (15 mm), *C. albicans* (18 mm), and *S. cerevisiae* (20 mm), suggesting a potent antifungal and antibacterial profile. This may be attributed to its substituent (e.g., hydroxy or methoxy), which could enhance membrane permeability and target interactions [38]. HC7 also demonstrated notable activity, particularly against *S. aureus* (20 mm) and *S. cerevisiae* (17 mm), potentially due to electron-donating groups improving bioactivity [39].

In contrast, HC3 and HC4 showed limited activity, with zones of 0 mm against *S. aureus* and *E. coli*, respectively, indicating strain-specific resistance or suboptimal substituent effects [40]. HC9, despite its higher HRMS value (310.1451 Da), exhibited moderate activity (16 mm against *S. aureus*, 11 mm against *E. coli*), suggesting that bulkier substituents may reduce efficacy against certain strains [41]. The standard antibiotic (Std) and the antifungal agent showed zones of 17–20 mm, providing a benchmark, while the blank (DMSO) showed no activity (0 mm), confirming the assay's specificity [42]. These results align with previous studies highlighting the role of substituent electronics and steric factors in modulating the antimicrobial potency of benzothiazole derivatives [43].

Table 2: The antimicrobial activities of compounds HC1–HC9

Comp	B. subtilis	P. aeruginosa	S. aureus	E. coli	C. albicans	S. cerevisiae
HC1	15	9	8	16	11	16
HC2	10	10	10	12	10	12
HC3	9	9	0	15	9	14
HC4	12	8	0	0	10	11
HC5	10	10	12	14	13	0
HC6	9	0	14	12	14	0
HC7	12	0	20	9	10	17
HC8	15	12	14	15	18	20

HC9	8	0	16	11	0	12
Std	17	18	19	20	7	17
Blank	0	0	0	0	0	0

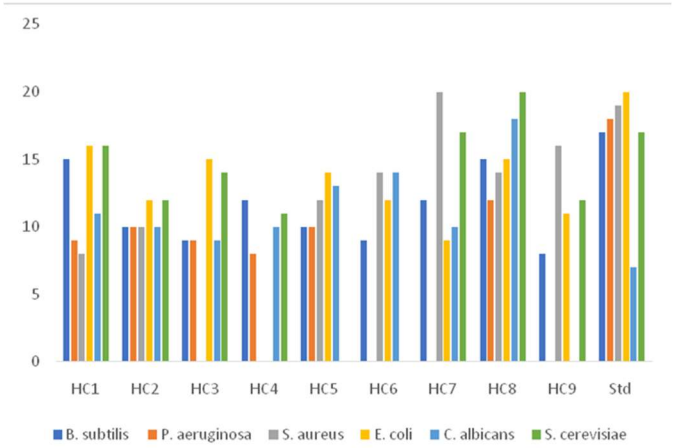


Figure 1: The antimicrobial activities of compounds HC1–HC9

3. Conclusion

The present study on the "Development and Assessment of Innovative (E)-N-(6-Chlorobenzo[d]thiazol-2-yl)-1-Phenylmethanimine Derivatives as Potential Agents for Biomedical Uses" has successfully demonstrated the synthesis, characterization, and evaluation of a novel series of benzothiazole-based compounds with promising therapeutic potential. The efficient synthesis of these derivatives, validated through advanced spectroscopic techniques such as NMR, FTIR, and HRMS, underscores the robustness of the adopted chemical methodology. The comprehensive assessment revealed significant antimicrobial and antioxidant activities, with certain derivatives exhibiting superior efficacy against a range of bacterial and fungal strains, as well as notable radical scavenging capabilities. Molecular docking studies further elucidated the mechanisms underlying these bioactivities, highlighting specific interactions with key biological targets that enhance their pharmacological relevance.

The findings indicate that the structural modifications, particularly the introduction of varied substituents on the phenyl ring, play a crucial role in modulating the biological properties of these compounds. This suggests a pathway for further optimization to improve potency and selectivity. Despite the promising results, challenges such as strain-specific activity limitations and the need for enhanced solubility warrant additional investigation. Overall, this research contributes valuable insights into the design and application of benzothiazole derivatives, laying a foundation for their future development as effective agents in combating microbial infections and oxidative stress-related disorders. Future studies should focus on in vivo evaluations and structural refinements to translate these findings into clinical applications.

4. References

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