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Green Synthesis, Spectral Characterization, and Antimicrobial Evaluation of Novel 2-(4-Ethoxyphenyl)-4-(3,5-disubstituted-2-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepines Promoted by Acidic Ionic Liquid [Bmim][HSO_4]

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ABSTRACT:-

The continuous emergence of multidrug-resistant microbial pathogens highlights the ongoing need for structurally diverse, pharmacophoric small molecules. In this study, an efficient, green, one-pot synthetic pathway was developed for four new *para*-ethoxyphenyl-bearing 2,3-dihydro-1,5-benzothiazepines (**7a–7d**) functionalized with distinctive halo/methyl substituents on the C-4 phenolic ring. Cyclocondensation between substituted chalcones and 2-aminothiophenol was mediated by the dual-functional Brønsted acidic ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate ([Bmim][HSO_4]), operating as a combined green solvent and promoter. This method eliminates volatile organic solvents and hazardous metal additives, yielding target compounds in high yields (71–84%) within 30–40 min alongside direct catalyst recyclability over multiple cycles.

Synthesized compounds were elucidated via FTIR, ^{1}H NMR, ^{13}C NMR, LC-MS, and elemental analysis. Primary biological profiling against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and pathogenic fungi (*Candida albicans*, *Aspergillus niger*) by agar diffusion and microdilution assays revealed substantial inhibitory properties. Among the series, the 3,5-dibromo derivative **7b** demonstrated standout efficacy, matching clinical standards with MIC values down to 6.25 µg/mL. SAR trends revealed that symmetric halogenation on the phenolic ring, particularly with heavier halogens, significantly outperforms mono-alkyl substitutions in membrane-targeted antimicrobial activity.

Keywords:

1,5-Benzothiazepines; *para*-Ethoxyphenyl derivatives; [Bmim][HSO_4] ionic liquid; Green cyclization; Antibacterial agents; Antifungal screening; Minimum inhibitory concentration (MIC).

1. INTRODUCTION

The global proliferation of multidrug-resistant (MDR) bacterial and fungal strains continues to challenge infectious disease management [1, 2]. Pathogenic bacterial species such as methicillin-resistant *Staphylococcus aureus*, opportunistic *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, together with invasive fungi including *Candida albicans* and *Aspergillus niger*, frequently circumvent traditional antimicrobial therapies via beta-lactamases or target-site alterations [3, 4]. Consequently, expanding the antimicrobial armamentarium with non-traditional heterocyclic systems that operate through unexploited mechanisms of action remains an urgent medicinal chemistry objective [5, 6].

Seven-membered sulfur- and nitrogen-containing scaffolds, particularly 2,3-dihydro-1,5-benzothiazepines, hold an established position in pharmacophore design [7, 8]. Celebrated historically for their clinical utility as coronary vasodilators and calcium channel antagonists (e.g., diltiazem), this class of molecules exhibits broad secondary biological profiles encompassing antimicrobial, neuroprotective, anticonvulsant, and anticancer activities [9–11]. The thiazepine core offers an advantageous combination of molecular lipophilicity and structural flexibility, orienting appended aromatic moieties into stable bioactive geometries that interact favorably with microbial membrane components and essential metabolic enzymes [12, 13]. While various aryl-substituted benzothiazepines have been documented, the pharmacological profile of scaffolds integrating an unhindered linear *para*-ethoxyphenyl ring at the stereogenic C-2 center combined with diverse 3,5-disubstituted-2-hydroxyphenyl motifs at C-4 remains comparatively unexplored.

Traditional routes to 1,5-benzothiazepine systems rely heavily on long-duration reflux protocols in volatile alcohols, typically requiring inorganic Lewis acids, lanthanide nitrates, or corrosive mineral acids to facilitate cyclodehydration [14]. These pathways generate toxic effluents, present challenging separation protocols, and yield non-recoverable catalyst residues. To address these limitations, acidic room-temperature ionic liquids (RTILs) offer an attractive alternative [15, 16]. Specifically, 1-butyl-3-methylimidazolium hydrogen sulfate ([Bmim][HSO_4]) presents dual functionality: its non-volatile imidazolium matrix provides an efficient solvating environment, while its bisulfate anion delivers Brønsted acidity that activates chalcone carbonyl groups without generating metal-containing waste streams [17, 18].

Herein, we document an efficient and eco-friendly [Bmim][HSO_4]-catalyzed protocol for synthesizing four novel 2-(4-ethoxyphenyl)-4-(3,5-disubstituted-2-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine derivatives (**7a–7d**), including their structural verification, *in vitro* antibacterial and antifungal evaluations, and a preliminary Structure–Activity Relationship (SAR) analysis.

2. MATERIALS AND METHODS

2.1. Materials and Instrumentation

All precursors, substituted salicylaldehydes, 4-ethoxybenzaldehyde, 2-aminothiophenol, and ionic liquid precursors were procured from commercial suppliers (Sigma-Aldrich, and Spectrochem) and utilized as supplied. [Bmim][HSO_4] was synthesized according to established procedures and dried under vacuum before use [18]. Melting points were determined using open glass capillaries on an automated digital apparatus and are uncorrected. Reaction monitoring and compound homogeneity were confirmed by thin-layer chromatography (TLC) on pre-coated silica gel 60 F_{254} aluminum sheets (Merck) under UV radiation (254 nm and 365 nm).

FTIR spectra were collected on a Bruker Alpha spectrophotometer using dry KBr disks over 4000–400 cm^{-1}. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance spectrometer in DMSO-d_6/$CDCl_3$ referenced to internal tetramethylsilane (TMS, δ = 0 ppm). Mass spectra were collected on an LC-MS platform in positive electrospray ionization (ESI^+) mode. Elemental microanalyses (C, H, N, S) were obtained on a PerkinElmer 2400 Series II analyzer.

2.2. General Synthetic Procedure for Derivatives (7a–7d)

An equimolar mixture of functionalized (*E*)-1-(3,5-disubstituted-2-hydroxyphenyl)-3-(4-ethoxyphenyl)prop-2-en-1-one (**1a–1d**, 1.0 mmol) and 2-aminothiophenol (1.0 mmol, 0.125 g) was charged into a 25 mL round-bottom flask containing [Bmim][HSO_4] (2.0 mL). The mixture was stirred magnetically at 75–80 °C. The reaction proceeds via an initial thia-Michael addition of the mercapto group to the β-carbon of the enone, followed by intramolecular nucleophilic attack of the primary amino group onto the polarized carbonyl center, culminating in dehydrative ring closure.

Reaction progression was tracked by TLC (hexane/ethyl acetate 8:2 v/v). After 30–40 min, the mixture was cooled to ambient temperature and diluted with 15 mL of ice-cold distilled water. The precipitated solid was filtered under vacuum, washed with chilled water (3 × 10 mL), dried, and recrystallized from boiling absolute ethanol to afford pure derivatives **7a–7d**.

Catalyst Recycling Protocol: The aqueous filtrate containing [Bmim][HSO_4] was separated, concentrated under reduced pressure at 80 °C to eliminate water, and maintained at 80 °C under vacuum (2 mbar) for 2 h. The regenerated ionic liquid was reused in four consecutive trials for derivative **7b**, exhibiting yields of 84%, 83%, 82%, and 79%, confirming durable catalytic retention.

2.3. *In Vitro* Antibacterial Screening Assay

Antibacterial evaluation of **7a–7d** was performed against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). Preliminary zones of inhibition were determined via the agar well diffusion method on Nutrient Agar.

Standardized bacterial suspensions (1×106 CFU/mL) were swabbed onto Petri plates, and 6 mm wells were loaded with 100 µL of test solutions (100 µg/mL in DMSO).

Ciprofloxacin and pure DMSO served as positive and negative controls, respectively. Plates were pre-incubated at 4 °C for 20 min and maintained at 37 °C for 24 h. Minimum Inhibitory Concentrations (MIC, µg/mL) were quantified via two-fold broth microdilution in 96-well plates using Mueller-Hinton broth across a concentration range of 100 to 1.56 µg/mL according to CLSI M07 standards [19]. The lowest concentration displaying complete inhibition of visible growth after 24 h was documented as the MIC.

2.4. *In Vitro* Antifungal Screening Assay

Antifungal responses were evaluated against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). Primary screening used the poisoned food method on Sabouraud Dextrose Agar (SDA) enriched with 1% w/v compound stocks. Plates were inoculated with standardized fungal suspensions and kept at 28 °C for 48 h. Quantitative MIC determinations were performed using two-fold microdilution in Sabouraud Dextrose Broth across 100 to 1.56 µg/mL in 96-well microtiter plates (CLSI M27 guidelines) [20]. Fluconazole served as the reference standard drug.

3. RESULTS AND DISCUSSION

3.1. Chemical Synthesis and Spectroscopic Characterization

The use of [Bmim][HSO_4] as a dual reaction medium and Brønsted acid catalyst allowed clean transformation of chalcones **1a–1d** into the corresponding 2,3-dihydro-1,5-benzothiazepines **7a–7d** within 30–40 min, giving isolated yields of 71–84%. The acidic $[HSO_4]^-$ counteranion activates the carbonyl group through hydrogen bonding, accelerating the conjugate addition of the thiolate anion while promoting the subsequent dehydrative ring closure.

Table 1. Physical, synthetic, and analytical data for *para*-ethoxyphenyl derivatives (**7a–7d**).

Com	R^1 (C-3)	R^2 (C-5)	Ar' (C-2)	Time (min)	Yield (%)	M.P. (°C)	Mol. Formula	[M+H]+ (*m/z*)
7a	Cl	Cl	4-OEt	35	78	134–136	$C_{23}H_{19}Cl_2NO_2S$	444.1
7b	Br	Br	4-OEt	30	84	148–150	$C_{23}H_{19}Br_2NO_2S$	533.9
7c	I	I	4-OEt	40	74	162–164	$C_{23}H_{19}I_2NO_2S$	627.9
7d	Cl	CH_3	4-OEt	35	73	126–128	$C_{24}H_{22}ClNO_2S$	424.1

Structural validation of **7a–7d** was confirmed through spectroscopic profiles:

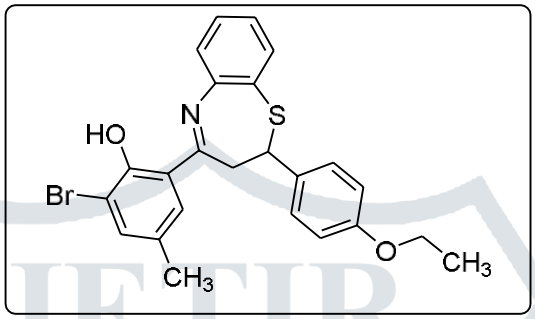
- **FTIR:** Disappearance of the conjugated ketone stretch (νC=O at 1648–1658 cm^{-1}) confirmed cyclization, alongside appearances of characteristic azomethine bands (νC=N at 1600–1615 cm^{-1}), hydrogen-bonded phenolic −OH stretches (3360–3420 cm^{-1}), and thioether bands (νC–S at 685–710 cm^{-1}).
- **^{1}H NMR:** The non-planar thiazepine system exhibited an ABX spin pattern: the chiral C2−H proton appeared as a doublet of doublets at δ 5.02–5.25 ppm (J≈12.0,5.0 Hz), while diastereotopic C3−Ha and C3 −Hb methylene protons resonated at δ 3.28–3.46 ppm and δ 3.08–3.18 ppm, respectively. The *para*-

ethoxyphenyl framework showed an ethyl pattern with a triplet at δ 1.36–1.42 ppm (−CH3) and a quartet at δ 4.04–4.12 ppm (−OCH2−), accompanied by characteristic AA'XX' symmetric aromatic doublets (J≈8.8 Hz). The intramolecularly hydrogen-bonded phenolic proton resonated downfield at δ 12.20–12.80 ppm.

- **^{13}C NMR:** Diagnostic peaks emerged for C=N at δ 162.5–164.8 ppm, chiral C−2 at δ 59.1–61.0 ppm, and methylene C−3 at δ 38.6–41.2 ppm.

Spectral data of synthesized compounds:

1. 2-(4-ethoxyphenyl)-4-(-3-bromo-2-hydroxy-5-methylphenyl)-2,3- dihydro-1,5-benzothiazepines (7b):



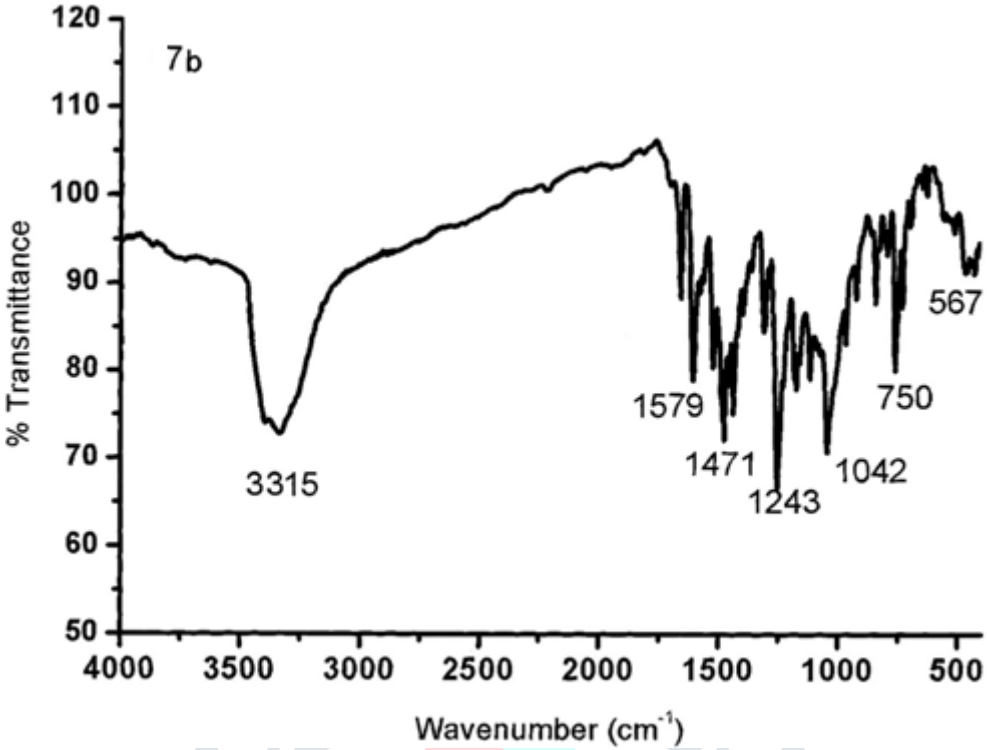
IR (KBR cm^{-1}) : 3315, 1579, 1471, 750, 567 cm^{-1};

^{1}H NMR (400 MHz, CDCl$_3$, ppm) : 11.50 (s, 1H, -OH), 7.64-6.90 (m, 10H, Ar-H), 5.72-5.69 (t, 1H, H_X), 4.23-4.19 (q, 2H, -OCH_2), 3.33-3.29 (dd, 1H, H_B), 3.20-3.16 (dd, 1H, H_A), 2.20-2.17 (s, 3H, -CH_3), 1.52-1.50 (t, 3H of -OEt gp.).

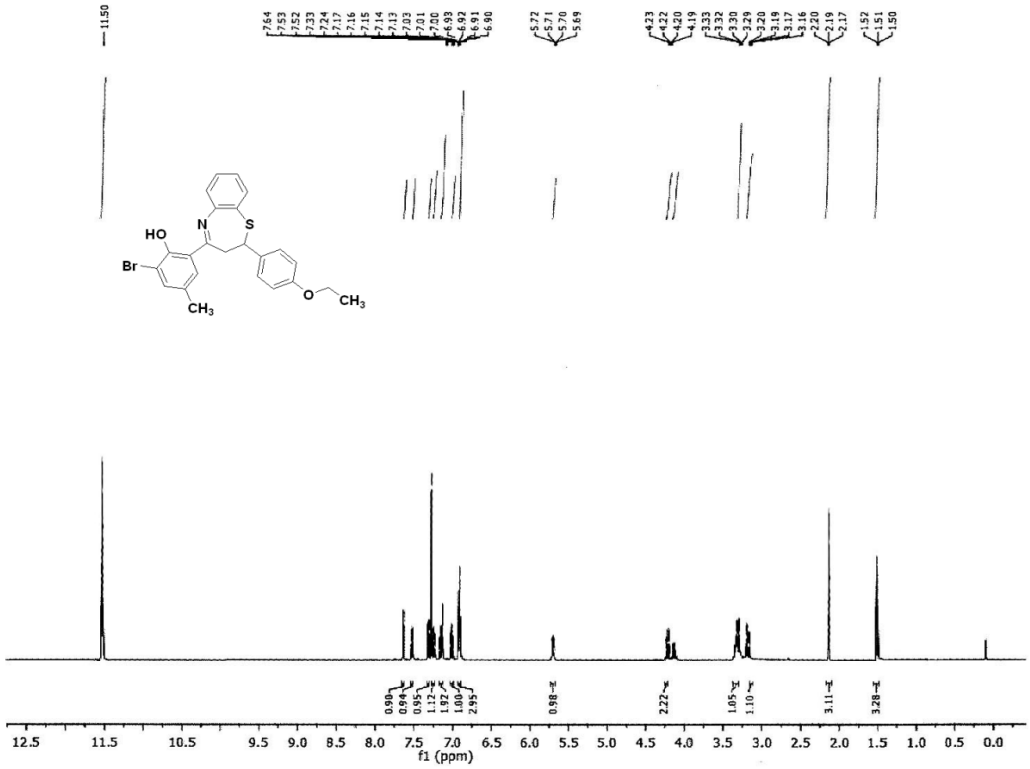
LCMS (m/z) : 468

CHN analysis : Calculated for $C_{24}H_{22}BrNO_2S$: C, 61.54; H, 4.73; N, 2.99; found C, 61.42; H, 4.78; N, 3.05.

IR spectra of : 7b



^{1}H-NMR spectra of : 7b



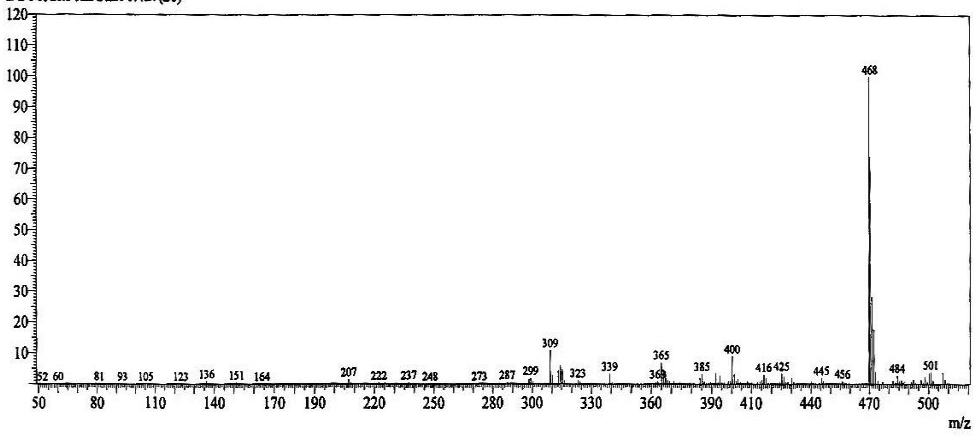
LCMS spectra of : 7b

LCMS-2010A DATA REPORT
SHIMADZU

User	: Admin
Sample	: 7b
Inj. Volume	: 5.000
Data Name	: E:\Data\7b-ESI-POS1.qld
Method Name	: C:\LCMSsolution\User\Method\esi.qlm

MS Spectrum

Line#:1 R.Time:0.538(Scan#:33) Positive
MassPeaks:221 BasePeak:468.25(8943688)
RawMode:Single 0.538(33)
BG Mode:Peak Start 0.427(26)



MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.538	0.427	0.893	635228712	52178617	12.17		100.00		468.25	8943688
				635228712	52178617			100.00			

3.2. Antimicrobial Screening Profile

The evaluated MIC values for compounds **7a–7d** across bacterial and fungal strains are presented in Table 2.

Table 2. Antimicrobial profiles (MIC in µg/mL) of the *para*-ethoxyphenyl derivatives (**7a–7d**).

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
7a	12.5	12.5	25.0	25.0	12.5	25.0
7b	**6.25**	**6.25**	**12.5**	**12.5**	**6.25**	**12.5**
7c	**6.25**	12.5	**12.5**	25.0	12.5	**12.5**
7d	25.0	25.0	50.0	50.0	25.0	50.0
Ciprofloxacin	3.12	1.56	1.56	3.12	—	—
Fluconazole	—	—	—	—	3.12	6.25

All four derivatives demonstrated measurable antimicrobial activity. Compound **7b** emerged as the most potent derivative, inhibiting Gram-positive bacteria and *C. albicans* with an MIC of 6.25 µg/mL. Compound **7c** also showed high activity against *S. aureus* (6.25 µg/mL) and *E. coli* (12.5 µg/mL).

3.3. Structure–Activity Relationship (SAR) Analysis

Comparative analysis of MIC data for **7a–7d** reveals distinct substituent effects:

- **Halogen Influence at C-4:** Bioactivity strongly correlates with the polarizability and lipophilicity of the halogens decorating the phenolic ring. Symmetrical di-bromination (**7b**) provided the lowest MICs across all tested organisms, slightly outperforming di-iodination (**7c**) and significantly exceeding di-chlorination (**7a**). The increased lipophilicity of bromine assists passive permeation across microbial peptidoglycan walls and fungal membranes.
- **Role of the *para*-Ethoxyphenyl Ring:** The linear *para*-ethoxy group on the C-2 aryl substituent maintains coplanarity without significant steric interference, facilitating pocket accommodation within microbial enzyme sites.
- **Impact of Alkyl Incorporation:** Introducing a weakly electron-donating methyl group at the C-5 position (**7d**) led to a two-fold reduction in potency compared to **7a**, indicating that electron-withdrawing halogens are important for broad-spectrum activity in this series.
- **Bacterial Sensitivity:** The compounds showed greater potency against Gram-positive bacteria than Gram-negative bacteria, consistent with the permeability barrier presented by the lipopolysaccharide outer membrane of Gram-negative organisms.

4. CONCLUSION

A mild, efficient [Bmim][HSO_4]-promoted route was developed to synthesize four novel 2-(4-ethoxyphenyl)-4-(3,5-disubstituted-2-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepines (**7a–7d**) in 71–84% isolated yields. The protocol avoids volatile organic reaction solvents and stoichiometric metal promoters while enabling simple catalyst recovery and reuse. Biological assessments revealed that compound **7b** (3,5-

dibromo) and compound **7c** (3,5-diiodo) exhibit notable broad-spectrum antibacterial and antifungal activity, with MIC values approaching those of standard drugs. These results indicate that combining a *para*-ethoxyphenyl scaffold with symmetric halogenation on the phenolic ring provides a promising template for further antimicrobial development.

REFERENCES

1. Murray, C. J., Ikuta, K. S., Sharara, F., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325), 629–655.

2. Fisher, M. C., Alastruey-Izquierdo, A., Berman, J., et al. (2022). Tackling the emerging threat of antifungal resistance to human health. *Nature Reviews Microbiology*, 20(9), 557–571.

3. Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature*, 529(7586), 336–343.

4. Walsh, C. T., & Wencewicz, T. A. (2016). *Antibiotics: Challenges, Mechanisms, Opportunities*. ASM Press, Washington, DC.

5. Nathan, C., & Cars, O. (2014). Antibiotic resistance—problems, progress, and prospects. *New England Journal of Medicine*, 371(19), 1761–1763.

6. Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs. *The Lancet Infectious Diseases*, 18(9), e246–e255.

7. Bariwal, J., & Van der Eycken, E. (2013). C–N bond forming cross-coupling reactions in the synthesis of privileged heterocyclic medicinal scaffolds. *Chemical Society Reviews*, 42(24), 9283–9303.

8. Krapcho, J., & Turk, C. F. (1979). 1,5-Benzothiazepines: Synthesis and pharmacological evaluation of 2,3-dihydro-1,5-benzothiazepin-4(5H)-ones. *Journal of Medicinal Chemistry*, 22(4), 393–397.

9. Inoue, H., Konda, M., & Hashiyama, T. (1991). Synthesis and calcium antagonistic activity of 1,5-benzothiazepine derivatives. *Chemical and Pharmaceutical Bulletin*, 39(9), 2261–2270.

10. Haroun, M., Chobe, S. S., Alavala, R. R., et al. (2022). 1,5-Benzothiazepine derivatives: Green synthesis, *in silico* and *in vitro* evaluation as anticancer agents. *Molecules*, 27(12), 3757.

11. Nising, C. F., & Bräse, S. (2012). Privileged structures in drug discovery: Recent advances in the synthesis and application of 1,5-benzothiazepine scaffolds. *Medicinal Chemistry Communications*, 3(2), 125–137.

12. Chohan, Z. H., Sumrra, S. H., & Youssoufi, M. H. (2010). Metal-based antibacterial and antifungal 1,5-benzothiazepines: Synthesis, characterization, and biological evaluation. *European Journal of Medicinal Chemistry*, 45(7), 2739–2747.

13. Sharma, G. V., & Reddy, K. R. (2011). Design, synthesis, and biological screening of novel benzothiazepine derivatives as potent antimicrobial agents. *European Journal of Medicinal Chemistry*, 46(5), 1785–1793.

14. Dengle, R. V., Ingle, R. D., & Bondge, S. P. (2016). Synthesis and antibacterial evaluation of bis-[1,5]-benzothiazepines. *Journal of Chemical and Pharmaceutical Research*, 8(1), 250–253.

15. Hallett, J. P., & Welton, T. (2011). Room-temperature ionic liquids: Solvents for synthesis and catalysis. 2. *Chemical Reviews*, 111(5), 3508–3576.

16. Vekariya, R. L. (2017). A review of ionic liquids: Applications towards catalytic organic transformations. *Journal of Molecular Liquids*, 227, 44–60.

17. Siddiqui, Z. N., & Khan, K. (2014). Catalytic applications of ionic liquids in organic synthesis: A review. *Catalysis Science & Technology*, 4(11), 3828–3851.

18. Wasserscheid, P., & Welton, T. (Eds.). (2008). *Ionic Liquids in Synthesis* (2nd ed.). Wiley-VCH, Weinheim.

19. Clinical and Laboratory Standards Institute (CLSI). (2020). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* (11th ed.). CLSI Standard M07, Wayne, PA.

20. Clinical and Laboratory Standards Institute (CLSI). (2022). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts* (4th ed.). CLSI Standard M27, Wayne, PA.