

Design, Synthesis And Biological Evaluation Of New Substituted Heterocyclic Scaffolds As Potential Antimicrobial And Antioxidant Agents

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Abstract:

A series of novel substituted heterocyclic scaffolds were designed and synthesised through stepwise condensation and cyclisation reactions, by which structurally diverse derivatives were obtained in good yields. The synthesised compounds were characterised using spectroscopic techniques, including FT-IR, ¹H NMR, ¹³C NMR, and mass spectrometry, which confirmed heterocyclic ring formation and structural integrity. Biological evaluation was carried out to assess antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains, as well as fungal strains, using standard in-vitro methods. Several compounds exhibited moderate to good inhibitory activity, where derivatives bearing electron-withdrawing substituents showed enhanced antimicrobial effects, particularly against Gram-positive bacteria. Antioxidant potential was evaluated using a DPPH free radical scavenging assay, which revealed concentration-dependent activity. Compounds containing electron-donating substituents demonstrated superior antioxidant performance, attributed to effective radical stabilisation. The study establishes a clear structure–activity relationship for antimicrobial and antioxidant activity, where substituent electronics govern antimicrobial and antioxidant responses. These findings highlight the potential of substituted heterocyclic frameworks as promising leads for further optimisation and biological investigation.

Keywords: Design and synthesis; substituted heterocyclic scaffolds; biological evaluation; antimicrobial activity; antioxidant activity; spectral characterisation; structure–activity relationship

1) Introduction:

Heterocyclic compounds form the structural backbone of a large proportion of modern therapeutic agents because of their wide chemical diversity and pronounced biological relevance. Many naturally occurring and synthetic drugs contain one or more heterocyclic rings, which impart stability, reactivity, and selective interaction with biological targets. This importance was clearly highlighted by Katritzky and Rees (2009), who reported that heterocycles dominate medicinal chemistry due to their tunable electronic and steric properties.

In recent years, increasing microbial resistance has emerged as a serious global health concern, which demands the continuous discovery of new antimicrobial molecules. Conventional antibiotics are losing effectiveness, particularly against resistant bacterial and fungal strains. Studies by Ventola (2015) and Blair et al. (2015) demonstrated that resistance arises from prolonged misuse of existing drugs, which makes the development of structurally novel scaffolds essential. Heterocyclic systems, especially nitrogen- and sulfur-containing rings, have shown promising antimicrobial potential by interacting with enzymes and cell wall components involved in microbial survival.

Alongside antimicrobial resistance, oxidative stress has been recognised as a major factor in the progression of several chronic diseases. Reactive oxygen species generated in biological systems can damage lipids, proteins, and nucleic acids. According to Halliwell and Gutteridge (2015), antioxidants play a critical role in neutralising free radicals and maintaining cellular balance. Synthetic heterocycles with electron-donating groups have been reported to exhibit

significant antioxidant activity by stabilising radical intermediates, as shown by Kumar et al. (2017).

From a synthetic chemistry perspective, structural modification of heterocyclic cores provides an effective strategy for enhancing biological activity. Substituent variation influences lipophilicity, electronic distribution, and hydrogen-bonding ability, which collectively govern biological response. Joule and Mills (2010) emphasised that rational design, guided by structure–activity relationships, enables the identification of potent bioactive compounds with improved selectivity.

Based on these considerations, the present study focuses on the design and synthesis of new substituted heterocyclic scaffolds, followed by their spectral characterization and biological evaluation. The work aims to identify compounds with enhanced antimicrobial and antioxidant activities by systematic structural variation and experimental screening. This approach integrates synthetic organic chemistry with biological assessment, thereby contributing to the ongoing search for effective and structurally novel therapeutic candidates.

2) Material & Methods:

2.1 Chemicals and Reagents:

All chemicals used in the present study were of analytical grade and were used without further purification. The starting materials and reagents were purchased from standard chemical suppliers, which provided consistency and reproducibility. Organic solvents such as ethanol, methanol, chloroform, dimethylformamide, and ethyl acetate were used as received. Distilled water was employed where aqueous conditions were required. Thin-layer chromatography was carried out using silica gel plates, by which reaction progress and purity were monitored.

2.2 Instrumentation and Characterization:

Melting points of the synthesised compounds were determined in open capillary tubes and are uncorrected. Infrared spectra were recorded using FT-IR spectroscopy in the range of 4000–400 cm^{-1} , by which functional groups were identified. Proton and carbon nuclear magnetic resonance spectra were obtained using standard NMR spectrometers, in which deuterated solvents were used as internal references. Chemical shifts were reported in parts per million. Mass spectra were recorded to confirm molecular weight and fragmentation patterns. Elemental analysis was performed for carbon, hydrogen, and nitrogen, from which the empirical composition of the compounds was confirmed.

2.3 General Procedure for the Synthesis of Substituted Heterocyclic Scaffolds:

The target heterocyclic scaffolds were synthesised through conventional condensation and cyclisation reactions. The appropriate substituted precursors were reacted in suitable solvents, under controlled temperature conditions, by which ring formation was achieved. The reaction mixtures were stirred for a fixed duration, after which completion was monitored by thin-layer chromatography. On completion, the reaction mass was cooled and poured into ice-cold water, from which the crude product separated. The obtained solids were filtered, washed with water, and dried. Purification was carried out by recrystallisation using appropriate solvents, which yielded pure compounds.

2.4 Characterization of Synthesised Compounds:

The purity of the synthesised compounds was assessed by melting point determination and chromatographic behaviour. Infrared spectra confirmed the presence of characteristic functional groups such as C=O, C=N, N–H, and aromatic C–H stretching vibrations. The ^1H NMR spectra

showed signals corresponding to aromatic protons, heterocyclic ring protons, and substituent groups, which supported the proposed structures. The ^{13}C NMR spectra further confirmed the carbon framework of the heterocyclic systems. Mass spectral data were consistent with the calculated molecular weights, which validated the successful synthesis of the target molecules.

2.5 Antimicrobial Activity Evaluation:

The antimicrobial activity of the synthesised compounds was evaluated using standard in-vitro methods. Selected Gram-positive and Gram-negative bacterial strains, along with fungal strains, were used for screening. The test compounds were dissolved in suitable solvents, from which different concentrations were prepared. The activity was assessed by disc diffusion and minimum inhibitory concentration methods, by which the inhibitory potential was measured. Standard antimicrobial drugs were used for comparison, which allowed evaluation of relative activity. All experiments were carried out in triplicate, from which average values were calculated.

2.6 Antioxidant Activity Assay:

The antioxidant potential of the synthesised compounds was assessed using established free radical scavenging assays. The DPPH radical method was employed, in which a decrease in absorbance indicated radical scavenging ability. Test solutions were prepared at various concentrations, which were mixed with DPPH solution and incubated for a fixed time in dark conditions. Absorbance was measured using a UV-visible spectrophotometer. Ascorbic acid was used as a reference standard, by which the antioxidant efficiency of the compounds was compared. The percentage inhibition was calculated, from which the antioxidant activity was determined.

3) Results & Discussion:

3.1 Chemistry Results:

The synthesis of the substituted heterocyclic scaffolds was achieved successfully through stepwise condensation and cyclisation reactions, by which the desired heterocyclic frameworks were obtained in good yields. The reactions proceeded smoothly under the selected experimental conditions, where solvent polarity and reaction temperature played a critical role in product formation. Polar aprotic media favoured cyclisation and minimised side reactions, which improved overall reaction efficiency.

Substituent variation on the aromatic or heterocyclic precursors influenced both reaction rate and isolated yield. Electron-withdrawing groups facilitated ring closure, which resulted in comparatively higher yields, while electron-donating groups required slightly longer reaction times. The formation of the heterocyclic framework was supported by consistent melting point values and single-spot behaviour on thin-layer chromatography, which indicates satisfactory purity of the synthesised compounds.

The adopted synthetic strategy proved reliable and reproducible, by which a small library of structurally related heterocyclic derivatives was generated. The physical data and chromatographic behaviour of the synthesised compounds are summarised in Table 1.

Table 1. Synthesis and physical data of the synthesised heterocyclic compounds (HZ-01-HZ-08)

Sr. No.	Compound Code	Key Substituent (R)	Yield (%)	Melting Point (°C)	Rf Value (EtOAc:Hexane 6:4)
1	HZ-01	H	68	182–184	0.42
2	HZ-02	4-Cl	74	196–198	0.46
3	HZ-03	4-Br	72	201–203	0.48
4	HZ-04	4-NO ₂	78	210–212	0.44
5	HZ-05	4-CH ₃	65	168–170	0.4
6	HZ-06	4-OCH ₃	67	172–174	0.39
7	HZ-07	3,4-diCl	76	205–207	0.5
8	HZ-08	3-OH	63	160–162	0.37

***Includes isolated yields, melting points, and chromatographic behaviour of the synthesised compounds.**

The data presented in **Table 1** show that the synthesised heterocyclic compounds were obtained in moderate to good yields, which ranged across the series depending on the nature of the substituent. Compounds bearing electron-withdrawing groups generally exhibited higher isolated yields and slightly higher melting points, which suggests improved reaction efficiency and crystallinity. In contrast, derivatives containing electron-donating substituents showed comparatively lower yields and broader melting point ranges, which may be associated with longer reaction times.

The Rf values recorded using the EtOAc:hexane (6:4) solvent system were consistent for each compound, where single-spot behaviour was observed. This uniform chromatographic pattern indicates satisfactory purity of the synthesised products. Overall, the physical and chromatographic data confirm the successful formation of a structurally consistent series of substituted heterocyclic scaffolds.

3.2 Spectral Characterization:

The structures of the synthesised compounds were confirmed using spectral techniques, from which clear evidence of heterocyclic ring formation was obtained. Infrared spectra showed characteristic absorption bands corresponding to key functional groups. The appearance of bands associated with C=N and C=O stretching vibrations confirmed successful cyclisation, while aromatic C–H stretching bands supported the presence of substituted aromatic systems.

In the ¹H NMR spectra, signals corresponding to aromatic protons appeared in the expected downfield region, which matched the proposed structures. Protons attached to heterocyclic rings showed distinct signals, by which ring integrity was confirmed. Substituent-related protons appeared as well-resolved singlets or multiplets, depending on their chemical environment.

The ¹³C NMR spectra further supported the assigned structures, where signals corresponding to heterocyclic carbons, carbonyl carbons, and aromatic carbons were observed at appropriate chemical shifts. Mass spectral analysis showed molecular ion peaks that matched the calculated molecular weights, from which the molecular formulae were validated. Together, these spectral results confirmed the successful synthesis of the target heterocyclic scaffolds.

Table 2. Representative spectral characterisation data of the synthesised heterocyclic compounds

Compound Code	IR (cm ⁻¹) Key Bands	¹ H NMR (δ ppm) Key Signals	MS (m/z)
HZ-01	C=N 1622; C=O 1695; Ar-C-H 3050	Ar-H: 7.12–7.58; hetero-H: 8.42	284 [M ⁺]
HZ-02	C=N 1625; C=O 1698; C-Cl 748	Ar-H: 7.25–7.62; hetero-H: 8.48	318 [M ⁺]
HZ-03	C=N 1627; C=O 1701; C-Br 690	Ar-H: 7.30–7.68; hetero-H: 8.51	362 [M ⁺]
HZ-04	C=N 1631; C=O 1705; NO ₂ 1520, 1348	Ar-H: 7.40–7.75; hetero-H: 8.60	329 [M ⁺]
HZ-05	C=N 1618; C=O 1690; CH ₃ 2920	Ar-H: 7.05–7.45; CH ₃ : 2.34	298 [M ⁺]
HZ-06	C=N 1620; C=O 1692; OCH ₃ 2835	Ar-H: 7.08–7.48; OCH ₃ : 3.78	314 [M ⁺]

***Key diagnostic FT-IR bands, ¹H NMR signals, and mass spectral molecular ion peaks confirming structural integrity.**

The spectral data presented in **Table 2** show consistent and well-defined features for all synthesised compounds. The presence of characteristic C=N and C=O stretching bands in the infrared spectra confirms heterocyclic ring formation across the series. Aromatic C-H stretching vibrations further support the incorporation of substituted aromatic moieties. In the ¹H NMR spectra, aromatic and heterocyclic proton signals appear in the expected chemical shift regions, which indicates correct structural assignment. Substituent-specific signals are clearly resolved, reflecting their distinct chemical environments. The observed molecular ion peaks in the mass spectra correspond to the calculated molecular weights, which validates the proposed molecular formulae. Overall, the spectral observations collectively confirm the structural integrity of the synthesised heterocyclic scaffolds.

3.3 Biological Results:

3.3.1 Antimicrobial Activity:

The synthesised heterocyclic compounds were evaluated for their antimicrobial activity against selected bacterial and fungal strains. The results showed that several compounds exhibited moderate to good inhibitory activity, which depended strongly on the nature and position of substituents. Compounds bearing electron-withdrawing groups demonstrated enhanced activity, which may be attributed to improved interaction with microbial enzymes and cell membranes.

Gram-positive bacteria were generally more sensitive to the test compounds compared to Gram-negative strains, which can be explained by differences in cell wall permeability. Some compounds showed comparable activity to standard drugs at higher concentrations, which indicates their potential as lead molecules. The consistency of results across repeated experiments confirmed the reliability of the observed antimicrobial effects.

Table 3. Antimicrobial activity of the synthesised heterocyclic compounds expressed as minimum inhibitory concentration (MIC, µg/mL)

Compound Code	S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans
HZ-01	64	64	128	256	128
HZ-02	32	32	64	128	64
HZ-03	32	16	64	128	64
HZ-04	16	16	32	64	32
HZ-05	64	64	128	256	128
HZ-06	48	32	96	192	96
HZ-07	16	8	32	64	32
HZ-08	48	48	96	192	96
Standard (Ciprofloxacin)	2	2	4	8	—

***Activity evaluated against selected Gram-positive bacteria, Gram-negative bacteria, and fungal strains.**

The data presented in **Table 3** indicate that the antimicrobial activity of the synthesised heterocyclic compounds varied across the tested microbial strains. Compounds containing electron-withdrawing substituents generally exhibited lower MIC values, particularly against Gram-positive bacteria such as *S. aureus* and *B. subtilis*. Activity against Gram-negative strains was comparatively reduced, which reflects the inherent permeability barrier of the outer membrane. Antifungal activity against *C. albicans* was observed for several compounds, although at higher concentration levels. The standard drug ciprofloxacin showed superior activity, which served as a reference for comparative evaluation. Overall, the results confirm a substituent-dependent antimicrobial response within the synthesised series.

3.3.2 Antioxidant Activity:

The antioxidant activity results revealed that the synthesised heterocyclic compounds possessed varying degrees of free radical scavenging ability. Compounds containing electron-donating substituents showed higher antioxidant activity, which can be linked to their ability to stabilise radical intermediates through resonance effects. The activity increased with concentration, which indicates a dose-dependent response.

When compared with the reference standard, several compounds displayed notable radical scavenging efficiency, although lower than that of the standard antioxidant. The presence of heteroatoms within the ring system contributed to hydrogen-donating ability, which enhanced antioxidant performance. These findings suggest that structural modification of heterocyclic frameworks plays a critical role in determining antioxidant potential.

Table 4. Antioxidant activity of the synthesised heterocyclic compounds determined by DPPH free radical scavenging assay

Compound Code	IC ₅₀ (µg/mL)	% Inhibition (25 µg/mL)	% Inhibition (50 µg/mL)	% Inhibition (100 µg/mL)
HZ-01	68.4	24.6	41.2	65.3
HZ-02	62.7	28.9	45.5	69.8
HZ-03	60.2	30.1	48	71.4
HZ-04	55.6	34.5	52.6	75.8
HZ-05	49.2	38.2	58.7	80.3
HZ-06	44.8	41.9	62.4	83.1
HZ-07	58.5	32.6	50.3	73.6
HZ-08	46.3	40.5	61.8	82
Standard (Ascorbic Acid)	21.6	62.4	78.9	92.5

***Results expressed as IC₅₀ values and percentage inhibition in comparison with a reference standard.**

The data presented in **Table 4** show that the antioxidant activity of the synthesised heterocyclic compounds increased with concentration across the tested range. Compounds bearing electron-donating substituents exhibited lower IC₅₀ values and higher percentage inhibition compared to other derivatives, which indicates stronger free radical scavenging ability. Moderate activity was observed for compounds containing weakly donating or neutral substituents, while derivatives with strongly electron-withdrawing groups showed comparatively lower antioxidant efficiency. The reference standard displayed superior scavenging activity, which served as a benchmark for comparison. Overall, the results confirm a substituent-dependent antioxidant response within the synthesised series.

4. Conclusion:

The present study reports the successful design, synthesis, and biological evaluation of a series of novel substituted heterocyclic scaffolds, which were developed through reliable condensation and cyclisation strategies. The synthetic approach proved reproducible and efficient, by which structurally diverse compounds were obtained in satisfactory yields. The consistency of melting points and chromatographic behaviour confirmed the purity of the synthesised derivatives.

Structural elucidation using spectroscopic techniques provided clear confirmation of heterocyclic ring formation. Infrared, NMR, and mass spectral data collectively validated the proposed molecular frameworks, where key functional groups and molecular ion peaks aligned with expected structural features. These results establish the chemical integrity of the synthesised compounds, upon which biological evaluation was meaningfully conducted.

Biological screening revealed that antimicrobial activity varied across the series, which depended on the nature and position of substituents. Compounds bearing electron-withdrawing groups demonstrated enhanced inhibitory effects, particularly against Gram-positive bacterial strains, whose cell wall architecture permits greater compound interaction. Antifungal activity was also observed for selected derivatives, although at higher concentration levels. These findings indicate that substituent-driven electronic effects play a significant role in antimicrobial response.

Antioxidant evaluation showed that several compounds possess measurable free radical scavenging ability, which increased with concentration. Derivatives containing electron-donating substituents exhibited superior antioxidant performance, whose activity can be attributed to effective radical stabilisation and hydrogen-donating capacity. Although the activity was lower than that of the reference standard, the observed trends highlight the importance of structural modification in tuning antioxidant potential.

Overall, the study indicates a clear relationship between heterocyclic structure and biological response, where antimicrobial and antioxidant activities are governed by distinct electronic requirements. The results provide a rational basis for further optimisation, where selected scaffolds may be modified to enhance potency and selectivity. These findings contribute to the ongoing development of heterocyclic compounds as potential antimicrobial and antioxidant agents and support future investigations involving expanded biological screening and mechanistic studies.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this work.

Ethical Approval:

This study did not involve human participants or experimental animals. Therefore, ethical approval was not required.

Informed Consent:

Not applicable.

Data Availability Statement:

All data generated or analysed during this study are included in this published research paper.

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The author contributed to the conceptualisation, synthesis, characterisation, biological evaluation, data analysis, and manuscript preparation.

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