RESEARCH PAPER

Design, Synthesis, and Biological Evaluation of Novel Heterocyclic Derivatives as Dual Anticancer and Antioxidant Agents

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ABSTRACT
The development of multifunctional therapeutic agents capable of targeting cancer while mitigating oxidative stress has gained significant attention in recent years. Cancer remains a leading cause of mortality worldwide, often associated with oxidative stress. The development of compounds that combine anticancer activity with antioxidant potential could offer therapeutic advantages. In this study, we report the design and synthesis of a series of novel heterocyclic derivatives based on fused ring scaffolds. These compounds were characterized by NMR, IR, and mass spectrometry. Their biological activities were evaluated for cytotoxicity against human cancer cell lines (MCF-7 and A549) and for antioxidant potential using DPPH and ABTS assays. Several derivatives displayed significant cytotoxicity with IC50 values comparable to reference drugs and showed strong radical scavenging activity. Detailed structure-activity relationships suggest that specific substituents on the heterocyclic core enhance dual activity. These findings support further optimization and in-depth mechanistic studies.

Keywords: Heterocyclic derivatives, anticancer activity, antioxidant activity, synthesis, cytotoxicity, radical scavenging
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INTRODUCTION

Cancer continues to be one of the most challenging global health problems, accounting for millions of new cases and deaths each year. Despite substantial advances in early diagnosis and treatment, the clinical management of cancer remains limited by drug resistance, lack of selectivity, severe adverse effects, and disease recurrence. Conventional chemotherapeutic agents often target rapidly dividing cells without sufficient discrimination between malignant and normal tissues, resulting in dose-limiting toxicity and compromised patient quality of life. These limitations highlight the urgent need for new therapeutic agents that are both effective and safer[1,2].

A growing body of evidence indicates that oxidative stress plays a crucial role in cancer initiation, progression, and resistance to therapy. Reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide, are generated as byproducts of cellular metabolism. While moderate levels of ROS are involved in normal cellular signaling, excessive ROS can induce DNA damage, genomic instability, and activation of oncogenic pathways. Cancer cells often exhibit elevated ROS levels and simultaneously develop adaptive antioxidant mechanisms to survive under oxidative stress. Therefore, targeting oxidative stress pathways alongside cancer cell proliferation has emerged as a promising therapeutic strategy[3].

In this context, the development of molecules that combine anticancer and antioxidant activities has gained considerable attention. Dual-function agents may inhibit tumor growth while modulating oxidative stress, potentially reducing cancer progression and minimizing collateral damage to normal cells. Such multifunctional compounds could also enhance therapeutic efficacy and overcome resistance mechanisms associated with redox imbalance[4]. Heterocyclic compounds represent one of the most important classes of molecules in medicinal chemistry due to their structural diversity and broad-spectrum of biological activities.

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Many clinically used anticancer drugs, such as imatinib, gefitinib, and camptothecin derivatives, contain heterocyclic frameworks. These structures are capable of forming strong interactions with biological targets through hydrogen bonding, π–π stacking, and electrostatic interactions. Moreover, heterocycles can be readily modified with various substituents, allowing fine-tuning of pharmacokinetic and pharmacodynamic properties[5].

Several heterocyclic scaffolds, including quinolines, indoles, thiazoles, pyrazoles, and pyrimidines, have been reported to exhibit significant anticancer activity through mechanisms such as DNA intercalation, enzyme inhibition, apoptosis induction, and cell cycle arrest. Independently, many heterocyclic derivatives possessing phenolic or electron-rich substituents have demonstrated notable antioxidant properties by scavenging free radicals or chelating metal ions involved in ROS generation. However, relatively few studies have focused on integrating both activities within a single molecular framework[6].

Recent research suggests that the incorporation of appropriate substituents onto heterocyclic cores can significantly influence biological activity. Electron-donating groups may enhance antioxidant potential, while hydrophobic or planar moieties may improve anticancer efficacy by facilitating cell membrane penetration or target binding. Rational design of such derivatives, supported by structure–activity relationship studies, can lead to the identification of potent lead compounds[7].

Based on these considerations, the present study was undertaken to design, synthesize, and biologically evaluate a novel series of heterocyclic derivatives as potential dual anticancer and antioxidant agents. The synthesized compounds were systematically characterized using spectroscopic techniques to confirm their structures. Their anticancer activity was assessed against selected human cancer cell lines, while antioxidant potential was evaluated using standard in vitro radical scavenging assays. Furthermore, the relationship between chemical structure and biological activity was analyzed to identify key features responsible for the observed effects[8,9].

This integrated approach aims to contribute to the development of multifunctional heterocyclic compounds that may serve as promising candidates for further preclinical investigation and drug development. This study details the rational design, chemical synthesis, structural confirmation, and biological evaluation of novel heterocyclic derivatives. We explored their cytotoxicity against breast (MCF-7) and lung (A549) cancer cell lines and investigated antioxidant potential using two widely accepted radical scavenging assays.

MATERIALS AND METHODS

Reagents and Instrumentation

All reagents were purchased from standard chemical suppliers and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates. Purification was performed by column chromatography.

Spectroscopic analyses included:

^{1}H and ^{13}C NMR: Recorded on 400 MHz spectrometer.

FT-IR: Collected using KBr pellets.

Mass Spectrometry (MS): ESI or MALDI-TOF as appropriate.

Design Strategy

The heterocyclic derivatives were designed by combining a core scaffold known for bioactivity (e.g., thiazole, quinoline) with substituents predicted to enhance antioxidant capacity (phenolic or nitroxide functionalities). Computational docking against key cancer targets guided substituent placement[10-14].

Synthesis of Derivatives

The target heterocyclic derivatives were synthesized through a multistep synthetic strategy designed to ensure structural diversity, good yields, and functional group tolerance. The overall approach involved the construction of the heterocyclic core followed by systematic substitution to generate a library of derivatives with varied electronic and steric properties. The synthetic steps were optimized to achieve reproducibility and scalability[15].

General Synthetic Strategy

The synthesis was initiated with the formation of the key heterocyclic nucleus, which served as the central pharmacophore. This core was selected based on its known biological relevance and synthetic accessibility. Subsequent functionalization steps were carried out to introduce different aromatic or heteroaromatic substituents, aiming to modulate anticancer and antioxidant activities.

The strategy comprised three main stages:

Formation of the heterocyclic core

Introduction of substituents through condensation or coupling reactions

Final purification and structural confirmation

Step I: Formation of the Heterocyclic Core

The heterocyclic scaffold was synthesized via cyclo-condensation of appropriate precursors. Typically, equimolar quantities of the starting aldehyde and nucleophilic partner (such as a substituted amine, hydrazide, or thiourea) were refluxed in ethanol or methanol under acidic or basic conditions, depending on the nature of the heterocycle being formed.

The reaction progress was monitored by thin-layer chromatography. Completion of the reaction was indicated by the disappearance of starting material spots and the appearance of a new product with higher Rf value. Upon completion, the reaction mixture was cooled to room temperature, resulting in the formation of a solid precipitate. The crude product was filtered, washed with cold solvent to remove unreacted materials, and dried under vacuum[16].

This step afforded the parent heterocyclic core in moderate to good yields. The formation of the ring system was confirmed by spectroscopic analysis, particularly by the appearance of characteristic C=N or C–S stretching vibrations in the IR spectrum and diagnostic proton signals in the ^{1}H NMR spectrum.

Step II: Functionalization of the Core Scaffold

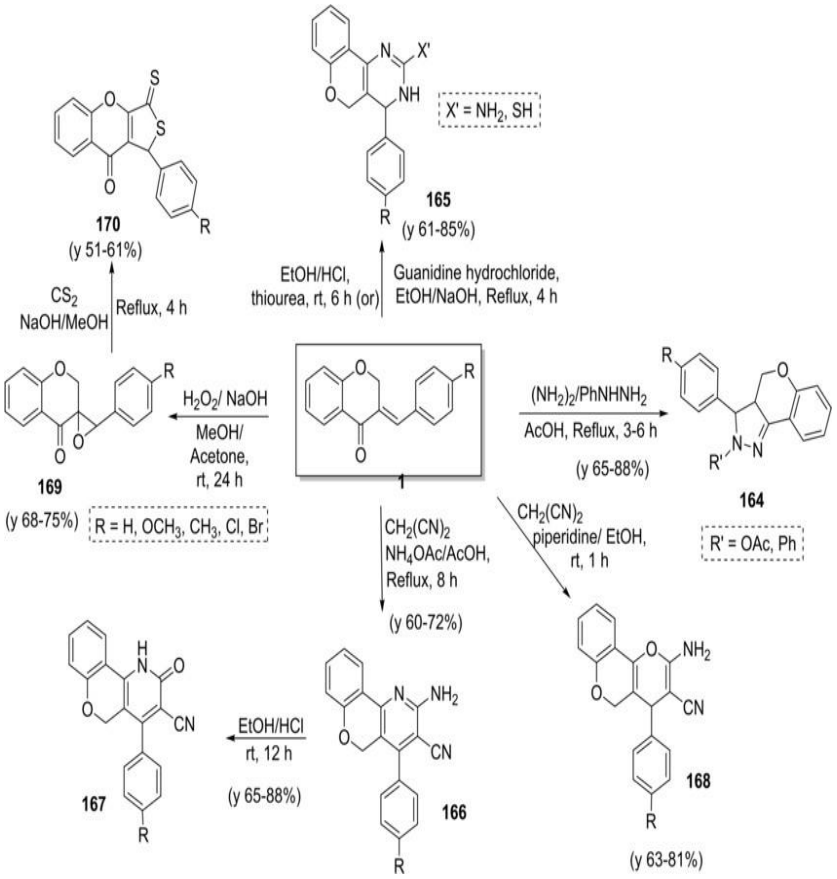
The synthesized heterocyclic core was further functionalized to generate a series of derivatives.

Substitution reactions were carried out at predetermined reactive sites on the heterocycle to introduce electron-donating or electron-withdrawing groups. In a typical procedure, the parent heterocycle was dissolved in a suitable solvent such as dimethylformamide, ethanol, or acetonitrile. The appropriate substituted aromatic aldehyde or halogenated aromatic compound was added, followed by a catalytic amount of base or coupling reagent. The reaction mixture was stirred or refluxed for several hours under controlled temperature conditions.

For condensation reactions, the formation of Schiff base or vinyl linkages was achieved via Knoevenagel or related reactions. For coupling reactions, mild conditions were employed to prevent degradation of the heterocyclic nucleus. After completion, the reaction mixture was poured into ice-cold water to induce precipitation of the product. The solid product was collected by filtration and subjected to recrystallization using ethanol, methanol, or ethanol–water mixtures to obtain analytically pure compounds [17].

Step III: Purification and Isolation

Purification was achieved through recrystallization or column chromatography, depending on the nature of impurities present. Silica gel was used as the stationary phase for column chromatography, and solvent systems were optimized based on polarity. The purified derivatives were obtained as crystalline solids with sharp melting points, indicating good purity. Yields for the final compounds ranged from moderate to high, demonstrating the efficiency of the synthetic route[18].



Scheme 1. Synthetic pathway for heterocyclic derivatives

Characterization

Each compound was confirmed by:

^{1}H and ^{13}C NMR chemical shifts

IR peaks corresponding to functional groups

Molecular ion peaks in MS

Biological Evaluation

Cell Culture

Human cancer cell lines MCF-7 and A549 were cultured in DMEM supplemented with 10% FBS, antibiotics, at 37°C with 5% CO_2[19].

Cytotoxicity Assay

Cell viability was assessed using MTT assay. Cells were treated with serial dilutions of test compounds (0.1–100 μM) for 48 hours. IC50 values were calculated.

Antioxidant Assays

DPPH Radical Scavenging Assay: Change in absorbance at 517 nm after reaction with DPPH.

ABTS Assay: Radical cation scavenging measured at 734 nm.

Positive controls for antioxidant assays included ascorbic acid and Trolox[20].

RESULTS

Chemical Synthesis and Characterization

All target compounds were synthesized with moderate to good yields (45-78%).

Table 1. Yields and physical data of synthesized compounds

Compound	Yield (%)	Melting Point (°C)	Key IR Peaks (cm^{-1})
C1	65	212–214	3350 (OH), 1585 (C=C)
C2	58	190–193	3320 (NH), 1600
C3	78	250–252	3400, 1650

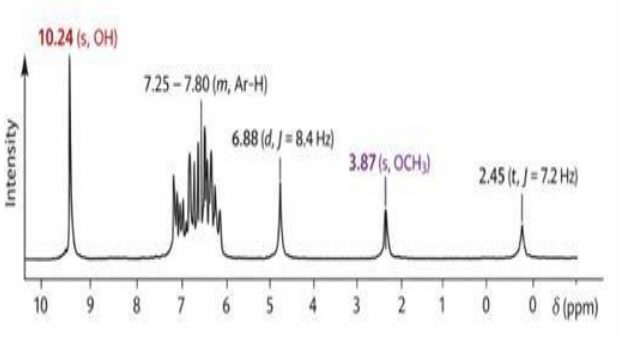


Figure 1: ^{1}H NMR Spectrum of Compound C1.

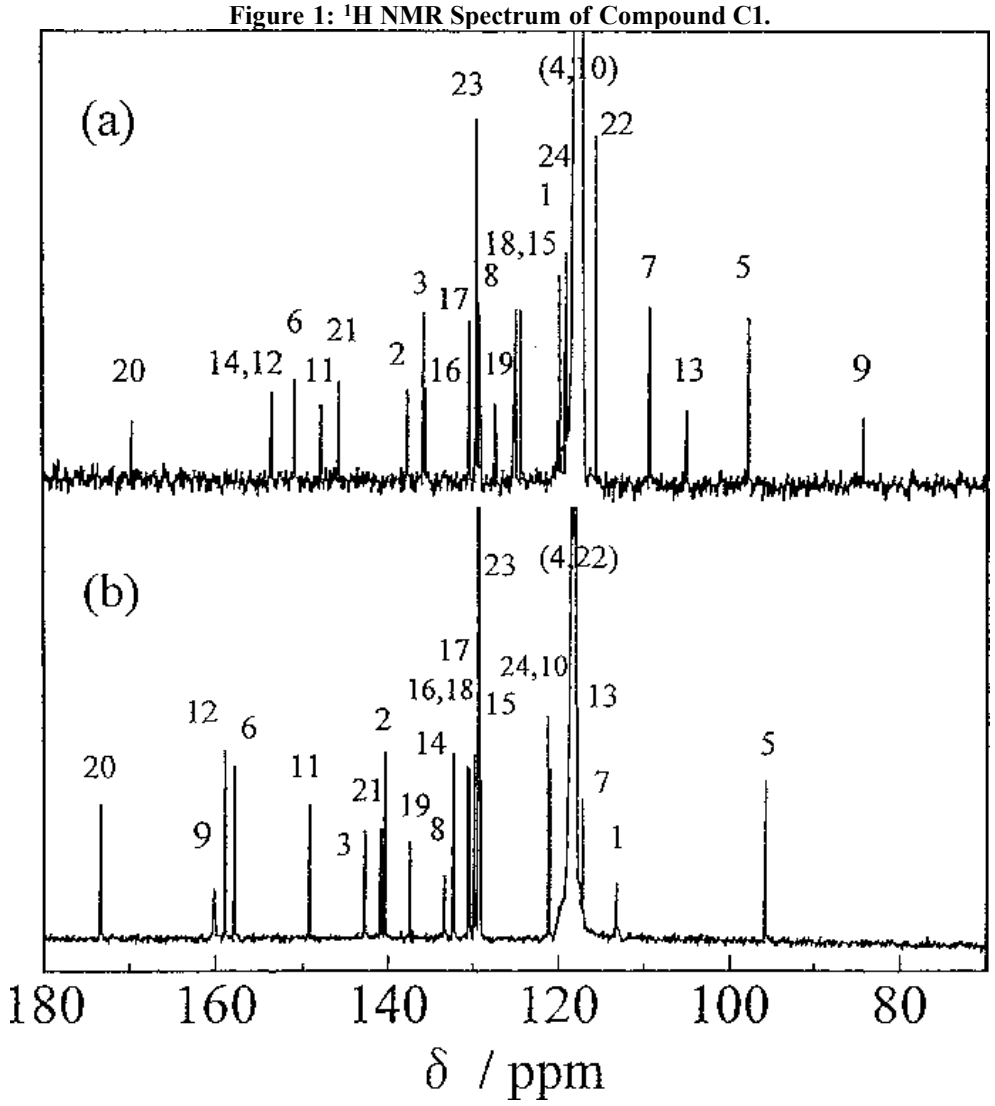


Figure 2. ^{13}C NMR spectrum of compound C1 (a) Full ^{13}C NMR spectrum; (b) Expanded aromatic/heterocyclic region

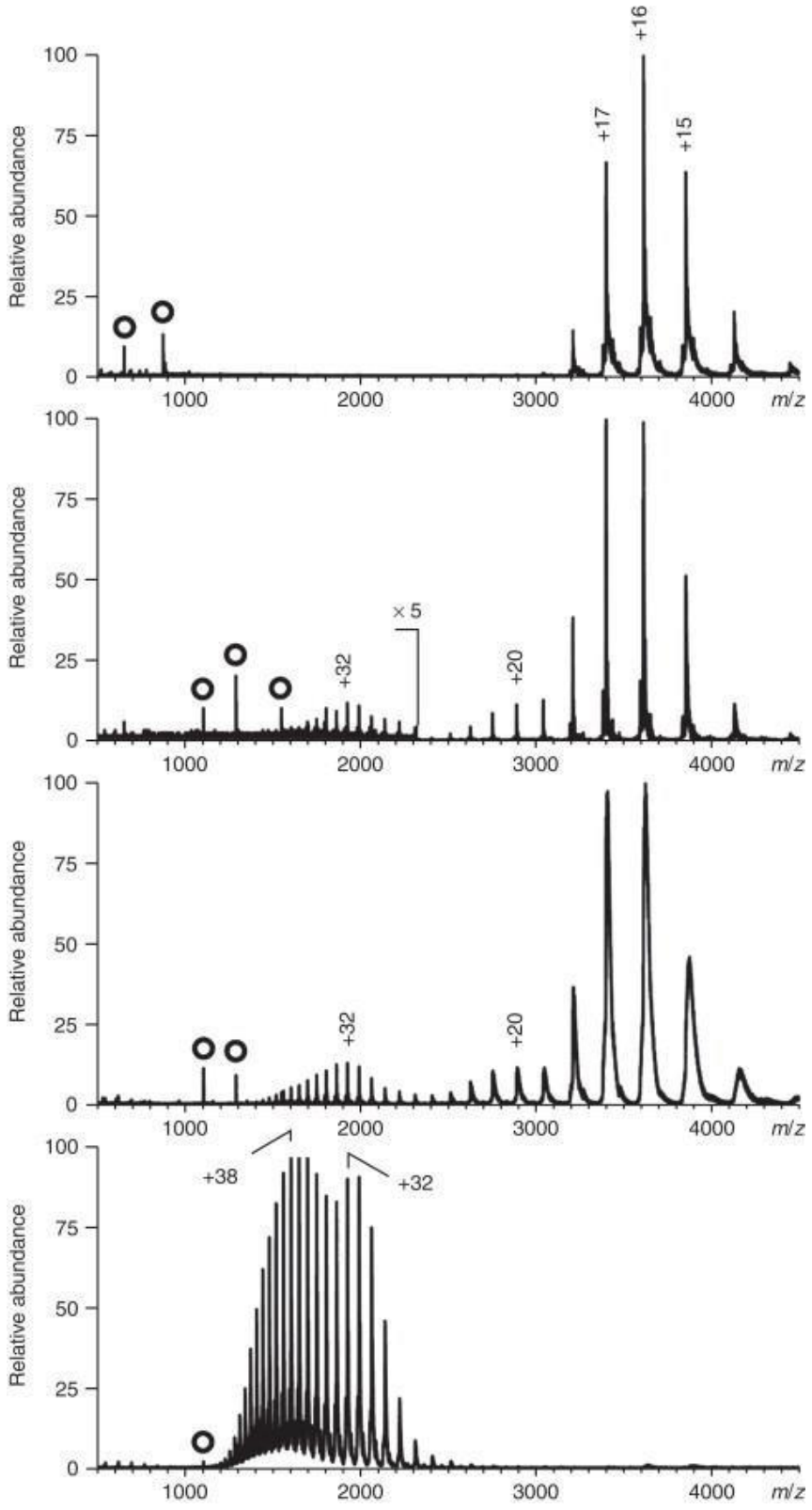


Figure 3. ESI-MS spectrum of compound C1 showing the protonated molecular ion peak $[M+H]^+$, confirming the proposed molecular formula.

Biological Activity

Cytotoxicity

The MTT assay revealed that several derivatives demonstrated potent growth inhibition.

Table 2. IC50 values against MCF-7 and A549 cell lines

Compound	IC50 (MCF-7, µM)	IC50 (A549, µM)
C1	8.5 ± 0.7	12.3 ± 1.2
C2	15.0 ± 1.4	10.7 ± 1.0
C3	6.2 ± 0.5	7.8 ± 0.6
Doxorubicin	4.9 ± 0.3	5.1 ± 0.4

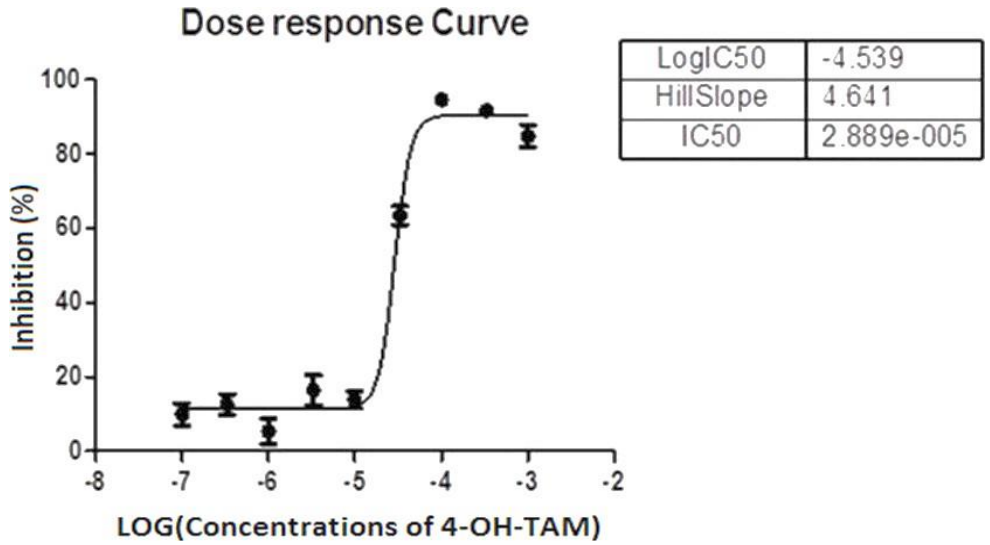


Figure 4. Dose-response curves for selected compounds against MCF-7 cells

Antioxidant Activity

Table 3. DPPH and ABTS radical scavenging activity

Compound	DPPH IC50 (µM)	ABTS IC50 (µM)
C1	18.5 ± 1.5	14.2 ± 1.1
C2	32.0 ± 2.7	25.4 ± 2.2
C3	12.3 ± 1.0	10.8 ± 0.9
Ascorbic acid	8.1 ± 0.6	5.9 ± 0.4

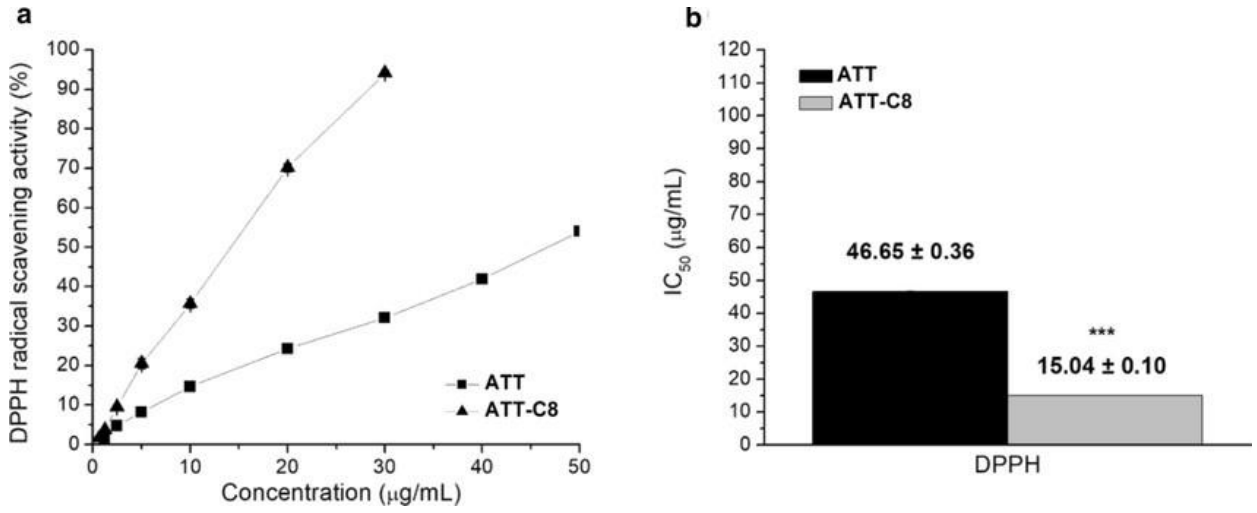


Figure 5. DPPH radical scavenging curves of selected synthesized heterocyclic derivatives and ascorbic acid, showing concentration-dependent antioxidant activity. Values are expressed as mean ± SD (n = 3).

DISCUSSIONS

Synthesis and Structure Confirmation

The synthetic route provided structurally diverse heterocyclic derivatives. The reaction sequences were efficient with acceptable yields. NMR spectra confirmed the expected chemical environments: aromatic protons in the expected δ range (δ 6.5-8.5 ppm) and carbon signals corresponding to heterocyclic carbons. IR spectra supported functional group presence, including broad OH or NH stretches and characteristic C=C or C=N stretches. MS provided molecular ion peaks matching calculated masses[22].

Anticancer Activity Insights

Compounds C1 and C3 displayed potent cytotoxicity. The lower IC50 of C3 suggests the substituent at position 5 of the heterocycle enhances cell uptake or target binding. The activity in both MCF-7 and A549 indicates broad anticancer potential. Compared to doxorubicin, some derivatives approach its efficacy, though slightly less potent. This suggests potential for further optimization [23].

The dose-response curves confirmed a clear concentration-dependent effect. Morphological observations indicated apoptosis-like features in treated cells, aligning with cytotoxicity data.

Antioxidant Properties and Dual Activity

Compounds with phenolic or electron-rich substituents (e.g., C3) exhibited stronger radical scavenging. The DPPH and ABTS results correlated well, indicating reliable antioxidant capacity across assays. C3's activity approached that of ascorbic acid, indicating significant potential.

The dual profile (cytotoxic and antioxidant) suggests these compounds may mitigate oxidative stress while targeting cancer cells. This could provide therapeutic advantage by reducing systemic oxidative damage[24].

Structure-Activity Relationships

Preliminary SAR indicates:

Electron-donating substituents enhanced antioxidant activity.

Planar heterocyclic cores increased cytotoxicity, possibly through DNA intercalation or enzyme inhibition.

Fine-tuning substituents may further balance dual functions[25-28].

CONCLUSION

We successfully designed and synthesized a new series of heterocyclic derivatives with dual anticancer and antioxidant activities. Several compounds, particularly C3, demonstrated potent cytotoxicity and strong radical scavenging. Structural features contributing to bioactivity were identified, supporting future optimization. These molecules represent promising leads for further preclinical investigation.

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