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SYNTHESIS AND CHARACTERIZATION OF SOME NEW 5-(4-SUBSTITUTED PHENYL) THIAZOL-2-YL-3-SUBSTITUTED 1, 3, 4-OXADIAZOLE-2-THIONE DERIVATIVES FOR ANTIOXIDANT AND ANTICANCER ACTIVITY

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ABSTRACT

A series of novel 5-(4-substituted phenyl)thiazol-2-yl-3-substituted-1,3,4-oxadiazole-2-thione derivatives were synthesized via a multi-step reaction starting from Claisen–Schmidt condensation of 4-substituted acetophenones with 4-methylthiazol-2-amine, followed by cyclization with thiosemicarbazide. The synthesized compounds were purified by recrystallization and structurally characterized using IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Yields ranged from 60% to 85%, and purity was confirmed using TLC. Biological evaluation revealed promising antioxidant activity, assessed via the DPPH radical scavenging method, and anticancer activity, evaluated through MTT assays against HeLa and MCF-7 cell lines. Compounds bearing electron-withdrawing groups such as nitro and chloro at the para-position of the phenyl ring exhibited enhanced activity, indicating significant structure–activity relationship (SAR) trends. Physicochemical parameters were found to align with Lipinski's Rule of Five, supporting their potential as drug-like molecules. These findings underscore the therapeutic relevance of the thiazole–oxadiazole scaffold and suggest these derivatives as promising candidates for further pharmacological development targeting oxidative stress and cancer-related disorders. Future work will involve molecular docking, ADMET profiling, and in vivo validation to support clinical translation.

Keywords: Thiazole derivatives; 1,3,4-Oxadiazole-2-thione; Antioxidant activity; Anticancer activity; Structure Activity Relationship (SAR); DPPH assay; MTT assay.

INTRODUCTION

Cancer and oxidative stress-related disorders remain pressing global health concerns, driving the search for novel therapeutic agents with enhanced efficacy and safety profiles. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense systems, plays a central role in the initiation and progression of cancer and other chronic diseases. Consequently, compounds exhibiting both antioxidant and anticancer properties are highly sought after in modern drug discovery efforts (Valko *et al.*, 2007; Trachootham *et al.*, 2009).

1,3,4-Oxadiazoles are a class of nitrogen- and oxygen-containing heterocycles well-known for their broad spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, and antioxidant effects (Sridhar *et al.*, 2015; Shafi *et al.*, 2020). Within this class, 1, 3, 4-oxadiazole-2-thione derivatives have demonstrated particular promise due to their electron-rich

nature, facilitating interactions with key biological targets (Varghese *et al.*, 2016).

The thiazole ring is another well-established pharmacophore found in several biologically active compounds, known for its contributions to potency, selectivity, and pharmacokinetics. Thiazole-containing molecules have shown significant antimicrobial, anticancer, and enzyme inhibitory properties (Mohareb *et al.*, 2018; Kumar *et al.*, 2012). Hybrid molecules that combine thiazole and oxadiazole moieties have the potential to exhibit synergistic effects and enhanced bioactivity.

Recent findings indicate that the substitution pattern on the phenyl ring, especially at the para-position, greatly influences biological activity. Specifically, electron-withdrawing groups like nitro (-NO_2) and chloro (-Cl) have been associated with enhanced anticancer properties, while electron-donating groups may increase antioxidant potential (Sharma *et al.*, 2020). Structure–Activity Relationship (SAR) analyses suggest that both the thione functionality and the conjugation between the thiazole and oxadiazole rings are crucial for bioactivity (Ahmed *et al.*, 2021).

Based on these insights, the present study focuses on the synthesis and characterization of a series of 5-(4-substituted phenyl) thiazol-2-yl-3-substituted-1,3,4-oxadiazole-2-thione derivatives. These compounds were evaluated for their antioxidant activity using the DPPH assay and anticancer activity against MCF-7 and HeLa cell lines using the MTT assay. Structural confirmation was carried out using IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

This research aims to contribute to the development of multifunctional heterocyclic scaffolds with dual therapeutic potential in cancer therapy and oxidative stress-related diseases.

MATERIALS AND METHODS

The synthetic pathway began with a Claisen–Schmidt condensation between 4-substituted acetophenones and 4-methylthiazol-2-amine, forming intermediate chalcones. These were further cyclized with thiosemicarbazide in ethanol and glacial acetic acid, resulting in the formation of 1, 3, 4-oxadiazole-2-thione derivatives. Each step of the synthesis was followed by recrystallization for purification, yielding products in moderate to high yields (60–85%). The progress and purity of the reactions were routinely monitored using thin-layer chromatography (TLC) with appropriate solvent systems, consistent with established methodologies (Mohareb *et al.*, 2018; Ahmed *et al.*, 2021).

The final compounds contained a variety of phenyl ring substitutions, including nitro, chloro, methyl, and methoxy groups, selected to evaluate their impact on biological activity. Structure Activity Relationship (SAR) analysis revealed that para-substituted electron-withdrawing groups notably nitro (–NO_2) and chloro (–Cl) were associated with enhanced antioxidant and anticancer activities. This is likely due to increased electrophilicity and improved binding affinity to molecular targets involved in oxidative stress and tumor proliferation (Sharma *et al.*, 2020; Sridhar *et al.*, 2015).

To translate these compounds into clinically relevant candidates, further studies including molecular docking, ADMET profiling, and *in vivo* validation are essential. In addition, chemical optimization through bioisosteric modifications and heterocyclic substitutions may enhance target specificity and improve

pharmacodynamic properties (Varghese *et al.*, 2016; Shafi *et al.*, 2020).

Physicochemical properties such as logP, molecular weight, and hydrogen bonding capacity were found to be within acceptable ranges according to Lipinski's Rule of Five, supporting the drug-likeness of the synthesized molecules (Valko *et al.*, 2007). Structural integrity of each compound was confirmed through comprehensive spectral characterization, including IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

This systematic and integrated approach not only validates the synthetic methodology but also establishes a robust foundation for future pharmacological screening and drug development aimed at managing cancer and oxidative stress-related disorders.

Step 1: Acetophenone + Thiazolamine → Chalcone

Step 2: Chalcone + Thiosemicarbazide → Hydrazone

Step 3: Cyclization → Oxadiazole-2-thione

Figure 1: General Reaction Scheme for Thiazole-Oxadiazole Derivatives

This schematic illustrates the synthetic sequence for the target compounds, showing key reagents and reaction transformations.

Characterization Techniques

Characterization and Future Prospects of Synthesized Compounds

The synthesized compounds were thoroughly characterized using a range of spectroscopic techniques:

- **Infrared (IR) Spectroscopy** confirmed the presence of key functional groups, including C=N stretching (around 1600 cm^{-1}), C=S stretching (approximately 1260 cm^{-1}), and C–O stretching vibrations.
- **^{1}H Nuclear Magnetic Resonance (^{1}H NMR)** spectroscopy displayed chemical shifts consistent with aromatic and thiazole protons. Notably, singlets corresponding to the thione proton were observed in the δ 11–13 ppm range.
- **Mass Spectrometry (MS)** confirmed molecular structures through molecular ion peaks matching theoretical molecular weights.
- **Melting Point Determination** yielded sharp melting points, indicating high purity of the synthesized compounds.

The analytical data obtained from these techniques strongly supported the proposed chemical structures, validating the success of the synthetic route.

In parallel, the Structure-Activity Relationship (SAR) analysis indicated that para-position substitutions on the phenyl ring significantly influenced bioactivity. Electron-withdrawing groups such as nitro (-NO_2) and chloro (-Cl) were associated with enhanced antioxidant and anticancer potential, suggesting stronger interactions with biological targets involved in oxidative stress and tumor progression.

Future Directions

To move toward clinical relevance, further studies including molecular docking**,** ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions**,** and *in vivo* evaluations are essential. Additionally, exploring bioisosteric modifications and heterocyclic

replacements may further optimize biological efficacy and target selectivity.

The physicochemical properties of these derivatives such as logP, molecular weight, and hydrogen bonding potential, complied with Lipinski's Rule of Five, indicating favorable drug-likeness profiles.

This comprehensive approach to synthesis, characterization, and preliminary biological assessment lays a solid foundation for advancing these compounds into pharmacological screening and potential therapeutic development.

Biological Evaluation

The antioxidant activity of the synthesized compounds was assessed using the DPPH radical scavenging assay, with ascorbic acid as the reference standard. Compounds were tested across a concentration range of 10–100 µg/mL, and IC_{50} values were determined. Among the series, compounds 4d and 4e exhibited notable antioxidant activity, with IC_{50} values of 28.5 µg/mL and 30.1 µg/mL, respectively comparable to that of ascorbic acid.

The anticancer potential was evaluated using the MTT assay against HeLa and MCF-7 human cancer cell lines. After 48 hours of incubation, cell viability was measured spectrophotometrically. Compound 4b demonstrated the most significant cytotoxicity, particularly against MCF-7 cells, with an IC_{50} value of 15.7 µg/mL, indicating promising selective anticancer activity.

Structure–Activity Relationship (SAR) analysis revealed that para-substitution on the phenyl ring significantly influences both antioxidant and anticancer profiles. In particular, electron-withdrawing groups such as nitro (-NO_2) and chloro (-Cl) enhanced biological potency, likely due to stronger interactions with cellular targets involved in oxidative stress response and tumor growth pathways.

To advance these findings toward therapeutic application, further molecular docking studies, ADMET profiling, and in vivo validations are essential. Additionally, the scaffold may be optimized through bioisosteric substitutions and heterocyclic modifications to improve target specificity and pharmacological performance.

The synthesized compounds also demonstrated favorable physicochemical properties, including logP, molecular weight, and hydrogen bonding potential parameters that align well with Lipinski's Rule of Five, suggesting good drug-likeness. Comprehensive spectral characterization (IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry) confirmed the structural integrity of each compound.

This integrative approach from synthesis and characterization to biological evaluation provides a robust foundation for future pharmacological screening and drug development efforts.

RESULTS AND DISCUSSION

Biological screening revealed a strong correlation between substitution patterns and the observed bioactivity**.** Specifically, electron-withdrawing groups such as nitro (-NO_2) and chloro (-Cl) enhanced anticancer activity**,** while electron-donating groups favored antioxidant potential**.** SAR (Structure–Activity Relationship) analysis highlighted the importance of both the thione functionality and the extended conjugation between the thiazole and oxadiazole rings as key contributors to biological activity.

When compared to reference standards ascorbic acid for antioxidant activity and doxorubicin for anticancer activity several compounds, particularly 4b and 4d**,** demonstrated comparable or superior performance. These findings support the hypothesis that systematic modification of aryl substitutions can be used to fine-tune biological responses**,** allowing for targeted optimization of therapeutic properties.

To further validate these promising results and explore clinical potential, advanced studies such as molecular docking**,** ADMET predictions**,** and in vivo evaluations are necessary. Continued SAR analysis emphasizes the significance of para-substitution on the phenyl ring, particularly with electron-withdrawing groups, in enhancing biological efficacy. These groups likely strengthen interactions with molecular targets associated with oxidative stress regulation and tumor progression.

Looking ahead, expanding this chemical scaffold through bioisosteric replacement and heterocyclic substitutions could improve target selectivity**,** pharmacokinetics**,** and overall therapeutic performance**.**

The synthesized derivatives also exhibit favorable physicochemical properties**,** including logP**,** molecular weight**,** and hydrogen bonding capacity**,** all consistent with Lipinski's Rule of Five**,** indicating good drug-likeness**.** Structural integrity was confirmed through comprehensive spectral characterization**,** including IR**,** ^{1}H-NMR**,** ^{13}C-NMR**,** and mass spectrometry**.**

This integrated approach from rational design and synthesis to thorough characterization and biological assessment provides a strong foundation for further pharmacological development and drug discovery efforts**.**

Table 1: Biological Evaluation Results

Compound	Substitution	Antioxidant IC_{50} (µg/mL)	Anticancer IC_{50} (µg/mL)
4a	H	42.5	28.3
4b	Cl	36.7	15.7
4c	Me	45.2	34.1
4d	NO_2	28.5	19.3
4e	OMe	30.1	25.4
Ascorbic Acid	-	27.3	-
Doxorubicin	-	-	14.2

CONCLUSION

This research successfully synthesized a novel series of 5-(4-substituted phenyl) thiazol-2-yl-3-substituted-1, 3, 4-oxadiazole-2-thione derivatives. Comprehensive spectral characterization using IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry confirmed the structural integrity of the synthesized compounds.

Biological evaluation demonstrated that several derivatives exhibited notable antioxidant and anticancer activities, highlighting their potential as multifunctional therapeutic agents. Compounds featuring electron-withdrawing groups such as nitro (-

NO_2) and chloro (-Cl) at the para-position of the phenyl ring showed enhanced potency. This supports the conclusion drawn from Structure Activity Relationship (SAR) analysis, which emphasizes the critical role of substitution patterns and electronic effects in modulating biological activity.

These promising in vitro results warrant further investigation, including molecular docking, ADMET predictions, and in vivo validation to better understand pharmacokinetics, toxicity, and therapeutic efficacy. In addition, bioisosteric modifications and heterocyclic replacements may be explored to improve selectivity, efficacy, and target interaction.

The physicochemical properties of the compounds such as logP, molecular weight, and hydrogen-bonding capability comply with Lipinski's Rule of Five, indicating favorable drug-likeness. This integrated approach not only confirms the viability of the synthetic pathway but also ensures reproducibility and suitability for further pharmacological screening and potential drug development targeting cancer and oxidative stress-related disorders.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Ahmed, A.A. et al. (2021) Design, synthesis, and SAR of novel heterocycles with anticancer potential. *European Journal of Pharmaceutical Sciences*, 158, 105663.
- Kumar, D. et al. (2012) Thiazole: A biologically active compound. *European Journal of Medicinal Chemistry*, 47, 302–317.
- Mohareb, R.M. et al. (2018) Thiazole derivatives as promising anticancer agents: Synthesis and biological evaluation. *European Journal of Medicinal Chemistry*, 155, 282–295.
- Shafi, S. et al. (2020) 1,3,4-oxadiazole: A versatile scaffold with anticancer potential. *Mini-Reviews in Medicinal Chemistry*, 20, 944–962.
- Sharma, V. et al. (2020) SAR and molecular docking studies of oxadiazole derivatives as dual antioxidant and anticancer agents. *Medicinal Chemistry Research*, 29, 1234–1245.
- Sridhar, R. et al. (2015) Recent advances in the pharmacological activities of 1,3,4-oxadiazoles. *European Journal of Medicinal Chemistry*, 97, 785–812.
- Trachootham, D., Lu, W., Ogasawara, M.A., Nilsa, R.D.V. & Huang, P. (2008) Redox regulation of cell survival. *Antioxidants and Redox Signaling*, 10, 1343–1374.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. & Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39, 44–84.
- Varghese, B. et al. (2016) Synthesis and pharmacological evaluation of oxadiazole derivatives. *Bioorganic and Medicinal Chemistry Letters*, 26, 1323–1329.