SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING ANALYSIS, AND EVALUATION OF ANTIOXIDANT PROPERTIES OF NOVEL 1-(2-CHLOROQUINOLIN-3-YL)N-(4-SUBSTITUTED PHENYLTHIAZOL-2-YL)METHANIMINE DERIVATIVES

Shweta Keshari[1,*], Uma Shanker Maurya[2]

[1]*Department of Pharmaceutical Chemistry, Goel Institute of Pharmacy & Sciences, Lucknow, Uttar Pradesh-226010, India*
[2]*Department of Pharmaceutical Chemistry, Goel Institute of Pharmacy & Sciences, Lucknow, Uttar Pradesh-226010, India*
[*]*Corresponding Author: shwetakeshari1996@gmail.com*

ABSTRACT

Quinoline, a heterocyclic aromatic compound containing nitrogen, is widely distributed in various plants and holds significant value in medicinal chemistry. Meanwhile, benzimidazole derivatives, another class of heterocyclic molecules, have garnered substantial interest in pharmaceutical research. In this study, a novel series of 1(2chloroquinolin-3yl)-N-(4phenyl thiazol-2yl)methanimine derivatives (4a-e) was designed and synthesized to explore their potential as antioxidant agents. The investigation focused on five distinct quinoline-thiazole derivatives for their antioxidant using computational tools. AutoDock tools were employed to analyze the binding site, binding energy, and receptor-ligand interactions of each compound with human peroxiredoxin-5. Additionally, the synthesized derivatives underwent evaluation for antioxidant activity using the DPPH (2,2-diphenyl1picrylhydrazyl) method. Among these, derivatives bearing bromo and methyl substitutions exhibited superior free radical scavenging potency compared to other compounds, with ascorbic acid serving as the standard.

The structures of the final derivatives were confirmed through comprehensive spectroscopic analyses including FT-IR, ^{1}H NMR, and Mass spectrometry, which yielded distinct and characteristic spectral features. These findings validate the synthesis and structural integrity of the designed quinoline-thiazole derivatives, highlighting their potential for further exploration as therapeutic agents with antioxidant and anti-inflammatory properties. Future studies could delve deeper into elucidating their mechanisms of action and optimizing their efficacy for clinical applications in oxidative stress-related disorders.

Keywords: Quinoline, Vilsmayer Haack reaction, Thiazole, Hantzsch reaction, Schiff base, Antioxidant activity.

INTRODUCTION

Oxidation in the human body, commonly known as O.S, is a fundamental concept interlinked with cellular homeostasis and the regulation of numerous physiological processes. "Central to oxidative stress isthe balance between reactive oxygen species and the body's antioxidant defenses. ROS, including superoxide radicals ($O_2^{\bullet -}$ hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and singlet oxygen ($1O_2$), are by-products of normal metabolic processes in biological systems. These reactive species play critical roles in various cellular functions, such as protein phosphorylation, activation of transcription factors, apoptosis, immune responses, and cellular differentiation." While low levels of ROS are necessary for these cellular functions, excessive ROS cause significant damage to vital cellular structures like proteins, lipids, and nucleic acids.**[1]**

QUINOLINE

Quinoline is a heterocyclic aromatic organic compound, characterized by a fused benzene and pyridine ring structure. Its molecular formula is C_9H_7N, and it is known for its distinct nitrogen atom within the ring system, which significantly influences its chemical behavior and reactivity. This arrangement confers quinoline with aromaticity, contributing to its stability and reactivity. The nitrogen atom in the pyridine ring introduces basicity to the molecule, allowing it to participate in various chemical reactions such as nucleophilic substitutions and electrophilic aromatic substitutions. Quinoline and its derivatives are of great interest in various fields, including medicinal chemistry, organic synthesis, and materials science, due to their versatile applications and unique properties.**[2]**

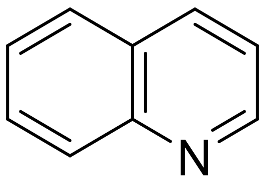


Figure 1. Quinoline ring.

THIAZOLE

"Thiazole is a five-membered heterocyclic compound containing both sulfur and nitrogen atoms in its ring structure with molecular formula C_3H_3NS. This intriguing molecule serves as a fundamental building block inorganic chemistry and plays a critical role in various biological and industrial applications." The unique electronic and structural properties of thiazole and its derivatives contribute to their wide range of chemical reactivity and biological activities, making them invaluable in the development of pharmaceuticals, agrochemicals, dyes, and more.

Thiazole and its derivatives are of profound biological significance, playing essential roles in several biological processes. Notably, thiazole is a key component of thiamine (vitamin B_1), which is crucial for carbohydrate metabolism and neural function. Thiamine pyrophosphate, the active form of vitamin B1, serves as a coenzyme in several enzymatic reactions, underscoring the importance of the thiazole ring in biochemistry.**[3]**

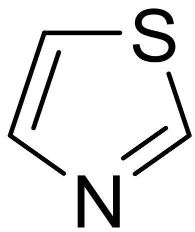


Figure 2. Thiazole ring.

THIAZOLE AS AN ANTIOXIDANT AGENT

"Thiazole, a five-membered heterocyclic compound containing both sulfur and nitrogen, has gained significant attention in the field of medicinal chemistry due to its diverse biological activities. One of the promising roles of thiazole derivatives is their function as antioxidant agents. Antioxidants are crucial in protecting the body against O.S., which involves the damaging effects of reactive oxygen species such as superoxide radicals ($O_2^{\bullet -}$), H_2O_2, -OH radicals (•OH), and singlet oxygen ."

Thiazole derivatives exhibit antioxidant properties through several mechanisms:

Free Radical Scavenging: Thiazole derivatives can directly scavenge free radicals. Their structure allows them to donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative damage to vital bio-molecules such as lipids, proteins, and DNA.

Metal Chelation: Transition metals like Fe and Cu can increase the development of free radicals. Thiazole compounds can chelate these metals, reducing their availability to participate in redox reactions that generate ROS. This chelation process is crucial in minimizing metal-induced oxidative damage.

Inhibition of Oxidative Enzymes: Certain enzymes, like NADPHoxidase and xanthine oxidase, leads to ROS development. Thiazole derivatives can inhibit these enzymes, thereby reducing the overall oxidative burden in cells.**[4]**

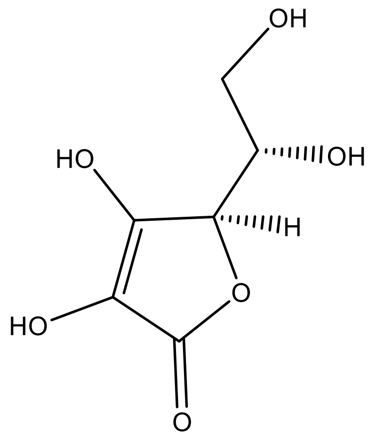
Table 1. Thiazole Derivatives with Antioxidant Activity

Compound/Derivative	Activity	References
Thiosemicarbazones/ Thiosemicarbazide	Significant free radical scavenging activity	**[5]**
Benzothiazoles	Exhibit potent antioxidant activity	**[6]**
Thiazolidinediones	Known for their antidiabetic properties, also exhibit antioxidant effects	**[7]**
Thiazole-containing Peptides and Proteins	Antioxidant potential	**[8]**

EXPERIMENTAL
MATERIAL AND METHODS
Molecular docking

Various Quinoline-thiazole derivatives have been designed by various substituted phenacyl bromide and docked by the help of AUTODOCK software and their interactions have been visualized by PyMOL tool.

Table 2. Target protein and standard used.

Target Protein	PDB ID	Structure	Standard used
Human peroxiredoxin 5	1HD2		 Ascorbic acid

Molecular Docking Analysis

Molecular docking assesses the binding affinity between a target protein and a specific ligand, quantified by a docking score in kcal/mol.
Detailed protein-ligand docking studies were meticulously performed using AutoDock Vina to ensure precision and accuracy.

The default parameters of AutoDock Vina included a comprehensive grid box with dimensions of 40x40x40 Å and a grid spacing of 0.375 Å, defined by the Auto Grid module, which ensured thorough coverage of the protein's active site region.

To further enhance the analysis, BIOVIA DSV 19 Client software was employed for sophisticated visualization, producing intricate 3D and 2D interaction maps of each docking complex. These visualizations provided deeper insights into the molecular interactions and binding conformations.

This robust approach not only facilitated a better understanding of the binding mechanisms but also aided in the identification of potential therapeutic candidates by highlighting key interactions and conformational details.

GENERAL SYNTHESIS PROCEDURE

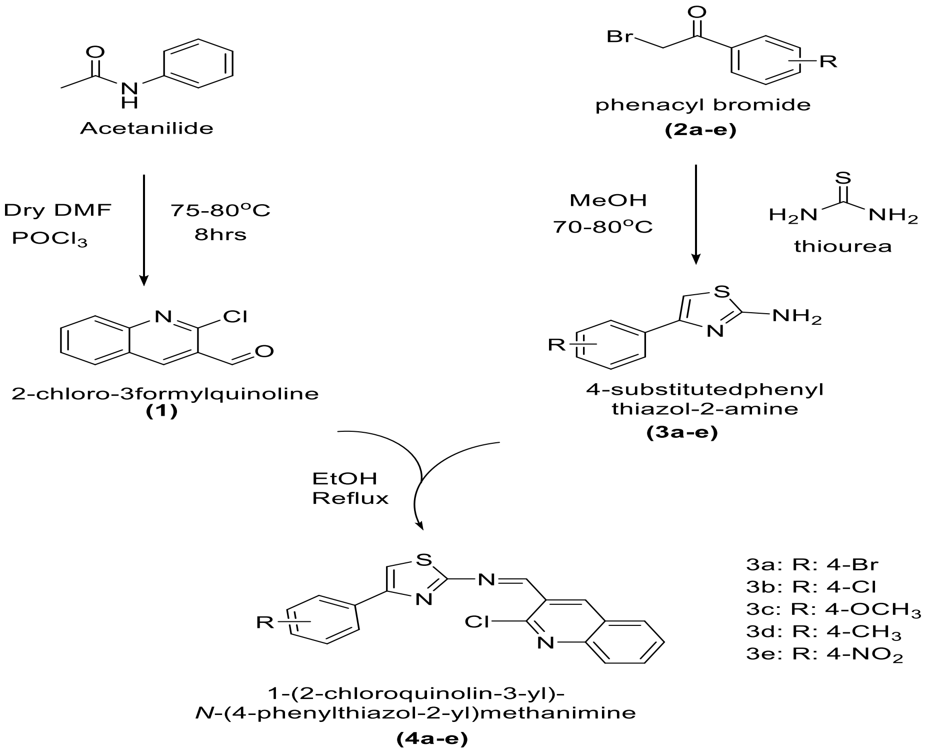
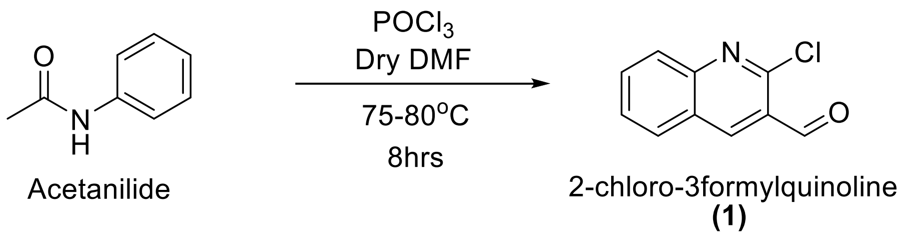


Figure 2. Synthesis scheme.

Method for synthesis of 2-chloro-3formylquinoline (1)

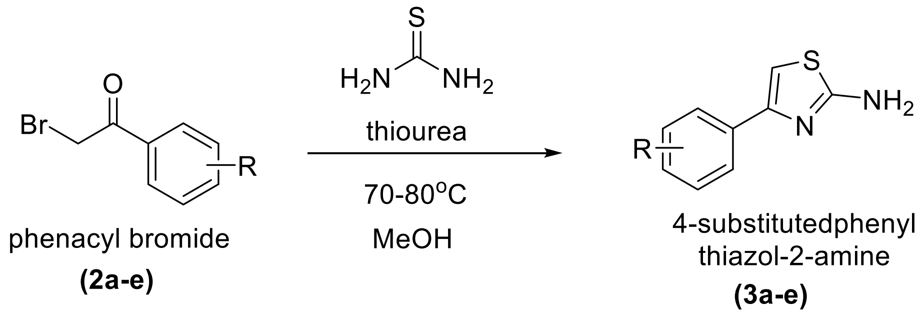
Vilsmeier haack reaction

"$POCl_3$ (13.77g, 0.09 mol) will be slowly added to anhydrous DMF (2.19g, 0.03 mol) while maintaining the temp. between 0-5°C, and the mixture will be stirred for 5 minutes. Following this, acetanilide (1.35g, 0.01 mol) (1) will be added to the mixture, and the solution will be heated under reflux at 75-80°C for 8 hours. After the reaction is complete, the mixture will be cooled and poured into crushed ice with stirring, leading to the formation of a yellowish precipitate of 2-chloro-3formyl quinoline (2)."



Method for synthesis of 4-substitutedphenyl thiazol-2-amine (3a-e)

"In a round-bottom flask, a solution of thiourea (0.76 g, 0.01 mol) and 4-bromophenacyl bromide (1.38 g, 0.005 mol) will be dissolved in 100 ml of absolute methanol and then refluxed for 3-4 hours. The completion of the reaction is checked by TLC. After the reaction is complete, the mixture will be allowed to cool to 25°C (R.T) and then added into cold water. The resulting solid will be collected by filtration, dried, and then recrystallized from absolute ethanol". **[19]**



Method for synthesis of 1-(2chloroquinolin-3-yl)-N-(4-phenylthiazol-2-ylmethanimine (4a-e)

Equimolar quantities of 2-chloro-3-formylquinoline (1) (0.01 mol) and 4-substituted phenyl thiazol-2-amine (2a-e) "will be added in 40 milliliter of ethanol. Then, 2 mL of glacial-acetic acid will be added to the solution, and the mixture will be refluxed for 8-12 hours. Once the reaction is complete, the mixture will be poured over crushed ice." The resulting crystalline product will be filtered, dried, and then recrystallized. **[20]**

ANTIOXIDANT ACTIVITY

DPPH method

To determine the antioxidant activity of synthesized derivatives their ability to scavenge the free radical 2,2-diphenyl1picrylhydrazyl (DPPH) was measured, using following standard protocol. The DPPH assay is a common method used in antioxidant research to analyze the free radical scavenging ability of substances.

Procedure

0.004% w/v solution of DPPH

" DPPH (4mg) was dissolved in 40 milliliter of methanol, and then the mixture was diluted Upto 100 mL in a calibrated flask. The sample was incubated in the dark for 30 minutes to prepare a 0.004% DPPH solution."

Standard ascorbic acid solution

10 mg of A.A was mixed in 5 ml methanol and then volume make up to 10 ml.

Determination of percentage inhibition

5 test tubes were prepared with aliquots of ascorbic acid at concentrations of 20, 40, 100, 200, and 400 µg/mL. Each test tube then received 3 mL of the DPPH solution. The same procedure was followed for the synthesized derivatives (4a-e), with each test tube also receiving 3 mL of the DPPH solution." The samples were incubated in the dark for 30 min. After incubation, the Abs was measured at 517 nm using a Spectrophotometer. The absorbance values of both the standard and test solutions were observed and recorded."

DPPH scavenging activity (% inhibition) = $\frac{(Ab\ sample - Ab\ blank)}{Ab\ blank} \times 100$

Where, Ab = Absorbance.

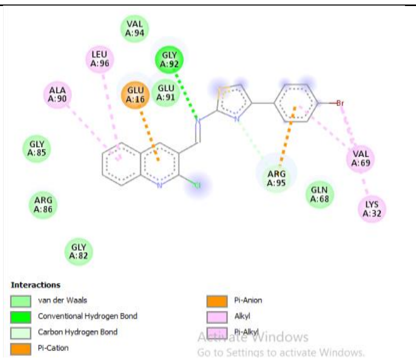
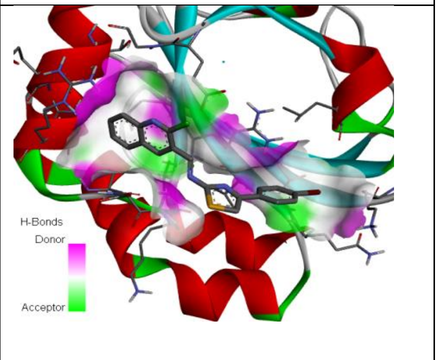
RESULTS AND DISCUSSION

MOLECULAR DOCKING STUDIES

Molecular-docking was conducted to assess the interaction profiles of novel quinoline-thiazole analogues within the active site of Human peroxiredoxin 5 (PDB code: 1HD2). This enzyme was selected based on its relevance to the potential therapeutic activity of the analogues. Docking simulations revealed detailed interaction patterns for the analogues. For instance, compound 4d exhibited conventional hydrogen bonding interactions with Arg-124 and Asn-76 residues, along with π-π stacking interactions involving Phe-43 within the binding pocket of Human peroxiredoxin 5. The calculated interaction affinity for this interaction was determined to be -6.9 kcal/mol. On the other hand, compound 4a demonstrated conventional hydrogen bonding interactions with Gly-92, as well as pi-alkyl interactions with Val-69 and Ala-90 with binding affinity of -6.9 kcal/mol.

Overall, the synthesized derivatives demonstrated favorable binding affinities comparable to the standard drug ascorbic acid. These findings underscore the potential of the quinoline-thiazole analogues as promising candidates for further exploration in therapeutic development against targets associated with Human peroxiredoxin 5.

Table 3. Receptor-Ligand Interaction (2D & 3D) and Binding affinity.

<table>
<tr><th>Ligand</th><th>Docking Score</th><th>Receptor-Ligand Interaction (2D)</th><th>Receptor-Ligand Interaction (3D)</th></tr>
<tr><td>4a</td><td>-6.9</td><td>
</td><td>
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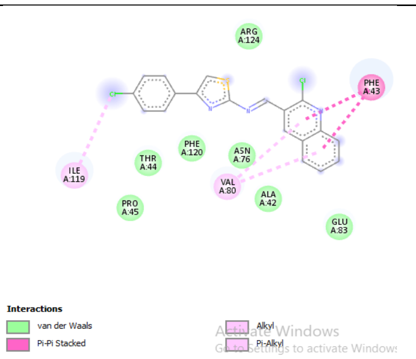
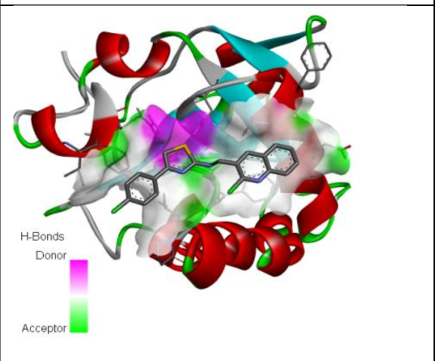
<tr><td>4b</td><td>-6.9</td><td>
</td><td>
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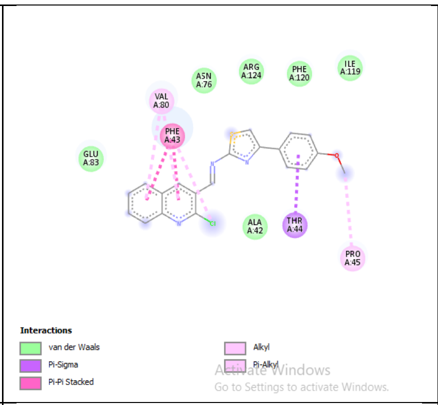
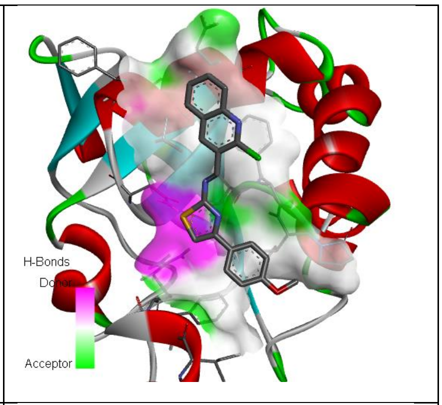
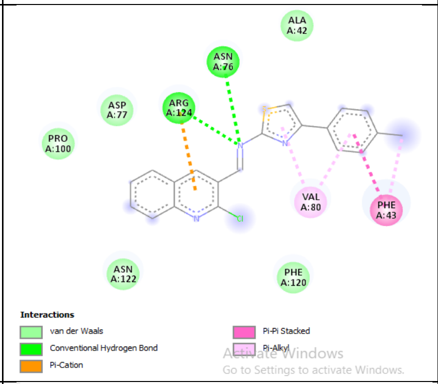
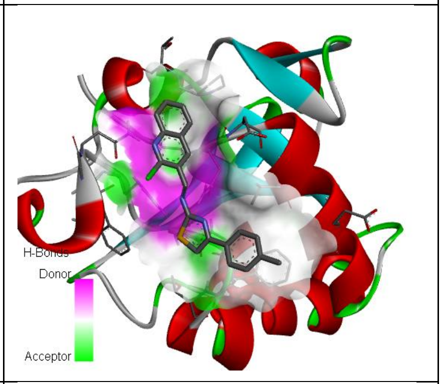
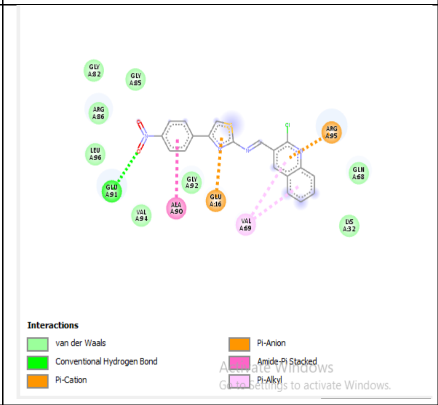
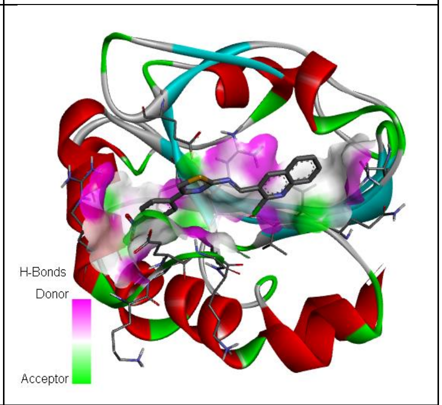
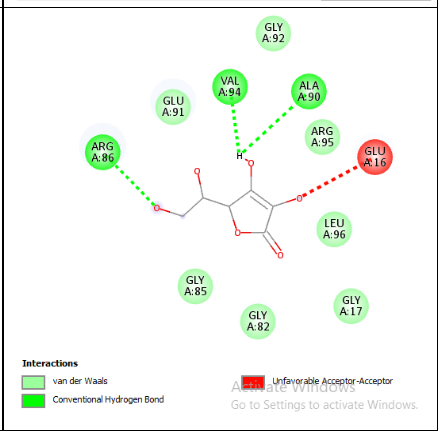
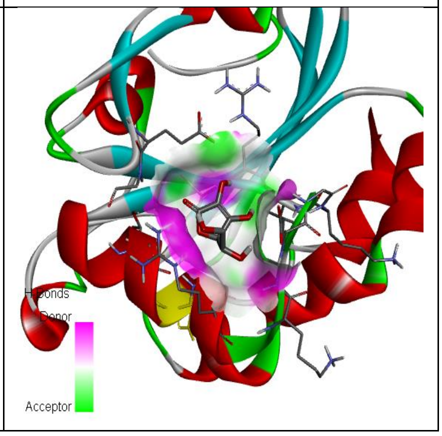
4c	-6.4		
4d	-6.9		
4e	-6.8		
Standard (Ascorbic Acid)	-5.5		

SYNTHESIS OF DESIGNED DERIVATIVES

Novel quinoline-thiazole scaffolds were synthesized through a meticulously designed multistep process. Initially, the synthesis of 2-chloro-3formylquinoline (1) was achieved via the Vilsmeier Haack reaction. In this step, acetanilide was added to a solution of the Vilsmeier reagent, which comprises dimethylformamide (DMF) and phosphorus oxychloride ($POCl_3$), and the resulting mixture was refluxed for duration of 7-8 hrs at a temp. range of 75-80°C.

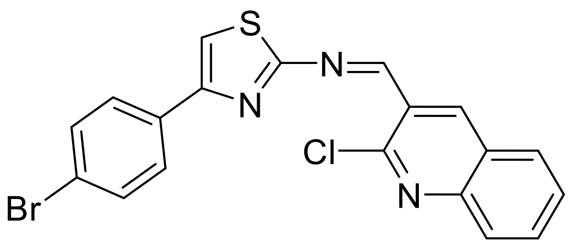
Subsequently, the synthesis of thiazole derivatives (3a-e) was carried out using the Hantzsch reaction. This involved heating a series of substituted phenacyl bromides (2a-e) in a solution of thiourea and methanol. The reaction was carefully controlled to ensure the efficient formation of the desired thiazole derivatives.

In the final step of the synthetic pathway, both the 2-chloro-3-formylquinoline (1) and the thiazole derivatives (3a-e) were combined and heated in methanol for 7-8 hours. This crucial step facilitated the formation of the quinoline-thiazole scaffolds (4a-e). The entire process was carefully monitored and optimized to ensure high yields and purity of the final products.

CHARACTERIZATION OF COMPOUNDS

The synthesized derivatives were characterized Physicochemically as well as Spectroscopically to determine the Physicochemical and Spectral features of the synthesized derivatives. Various parameters such as Colour, Yield, Solubility, Melting point, Rf value, FT-IR, ^{1}H-NMR and ESI-MS were analysed to determine structural and physicochemical features of the synthesized motifs.

Compound 4a

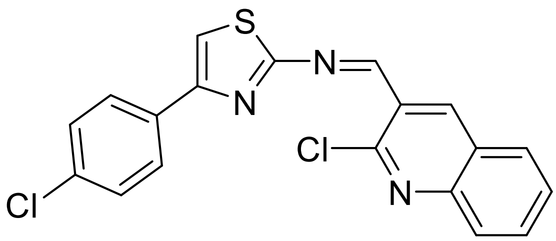


Molar Mass: $C_{19}H_{11}BrClN_3S$.
Mol. weight. : 428.73 g/mol.
Yield : 58%.
Melting point : 338-340°C.

Solubility data
Freely soluble : Ethanol, methanol.
Sparingly soluble : ethyl acetate, acetone
Insoluble : benzene, toluene
TLC Analysis
Solvent system: Chloroform and Methanol ratio (70:30, v/v); **R_f :** 0.62

IR spectra [KBr; cm^{-1}]: 641.92 (C-S Thia), 718.98 (C-Cl), 1211.08 (C-N Thia), 1591.39 (Ar C=C), 1663.73 (HC=N), 3064.01 (Ar C-H).
^{1}H NMR spectra]: 5.66 (s, 2H, Ar-H), 6.70-7.62 (m,8H, Ar-H), 9.98 (s, 1H, CH=N)

Compound 4b



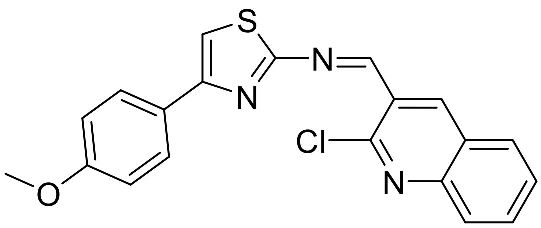
Molar Mass: $C_{19}H_{11}Cl_2N_3S$
Mol. weight. : 384.28 g/mol
Yield : 62%
Melting point : 324-326°C

Solubility data
Freely soluble : Ethanol, methanol
Sparingly soluble : ethyl acetate, acetone
Insoluble : benzene, toluene

TLC Analysis
Solvent system: Chloroform and Methanol ratio (70:30, v/v); **R_f :** 0.73

IR spectra [KBr; cm^{-1}]: 688.41 (C-S Thia), 794.75 (C-Cl), 1291.50 (C-N Thia), 1591.07 (Ar C=C), 1671.47 (HC=N), 3063.94 (Ar C-H).
^{1}H NMR spectra]: 5.80-5.84 (m, 2H, Ar-H), 7.16-8.46 (m, 8H, Ar-H), 9.00 (s, 1H, CH=N)

Compound 4c



Molar Mass: $C_{20}H_{14}ClN_3OS$
Mol. weight. : 379.86 g/mol
Yield : 70%
Melting point : 319-321°C

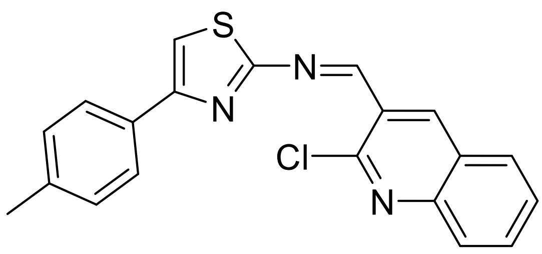
Solubility data

Freely soluble : Ethanol, methanol
Sparingly soluble : ethyl acetate, acetone
Insoluble : benzene, toluene

TLC Analysis
Solvent system: Chloroform and Methanol ratio: (70:30, v/v); **R_f :** 0.74

IR spectra [KBr; cm^{-1}]: 631.62 (C-S Thia), 775.91 (C-Cl), 1196.28 (C-O methoxy), 1267.93 (C-N Thia), 1510.01 (Ar C=C), 1665.29 (HC=N), 2852.06 (C-H methoxy), 3062.60 (Ar C-H).
^{1}H NMR spectra]: 3.30-3.34 (s, 3H, OCH_3), 8.66 (s, 1H, CH=N), 6.97-8.65 (m, 10H, Ar-H)

Compound 4d



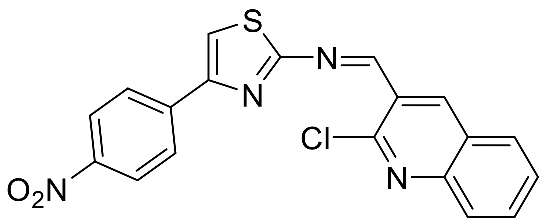
Molar Mass: $C_{20}H_{14}ClN_3S$
Mol. weight. : 363.86 g/mol
Yield : 64%
Melting point : 307-309°C

Solubility data
Freely soluble : Ethanol, methanol
Sparingly soluble : ethyl acetate, acetone
Insoluble : benzene, toluene

TLC Analysis
Solvent system: Chloroform and Methanol ratio: (70:30, v/v); **R_f :** 0.82

IR spectra [KBr; cm^{-1}]: 645.31 (C-S Thia), 753.32 (C-Cl), 1211.52 (C-N Thia), 1448.60 (HC=N), 2851.92 (C-H str.), 2919.23 (Ar C-H).
^{1}H NMR spectra]: 3.35 (s, 3H, CH_3), 6.50-8.54 (m, 10H, Ar-H), 8.93 (s, 1H, HC=N)

Compound 4e



Molar Mass: $C_{19}H_{11}ClN_4O_2S$
Mol. weight. : 394.03 g/mol
Yield : 59%
Melting point : 312-314°C

Solubility data
Freely soluble : Ethanol, methanol
Sparingly soluble : ethyl acetate, acetone
Insoluble : benzene, toluene

TLC Analysis
Solvent system: Chloroform and Methanol ratio:(70:30, v/v); **R_f :** 0.76

IR spectra [KBr; cm^{-1}]: 771.19 (C-Cl), 1217.86 (C-N Thia), 1383.58 (N-O Sym. Str.), 1623.27 (HC=N), 2919.22 (Ar C-H).
^{1}H NMR spectra 7.16-8.46 (m, 10H, Ar-H), 9.00 (s, 1H, HC=N)

ANTIOXIDANT ACTIVITY
Biological screening experiments demonstrated that quinoline-thiazole derivatives (4a-e) exhibit significant antioxidant activity. Among these, derivative 4d exhibited the highest percentage inhibition, achieving an impressive 82.34% inhibition rate compared to the other derivatives. Th remaining derivatives displayed moderate antioxidant activity relative to ascorbic acid used as the standard. These findings underscore the potential of derivative 4d and its counterparts as promising candidates for further exploration in antioxidant-related therapeutic applications. The results highlight their ability to effectively inhibit oxidative processes, which are implicated in various diseases and aging processes.

Table 1: Percentage inhibition with respect to concentrations

S.No.	**µg/ml**	**Ascorbic acid (Std)**	**4a**	**4b**	**4c**	**4d**	**4e**
1.	20	95.31	28.31	23.98	26.53	30.51	22.46
2.	40	97.46	39.56	31.26	37.41	35.64	31.22
3.	100	97.53	46.57	36.57	40.48	42.13	46.28

4.	200	97.61	61.33	67.72	60.37	59.57	60.54
5.	400	98.03	81.09	79.76	75.82	82.34	71.33

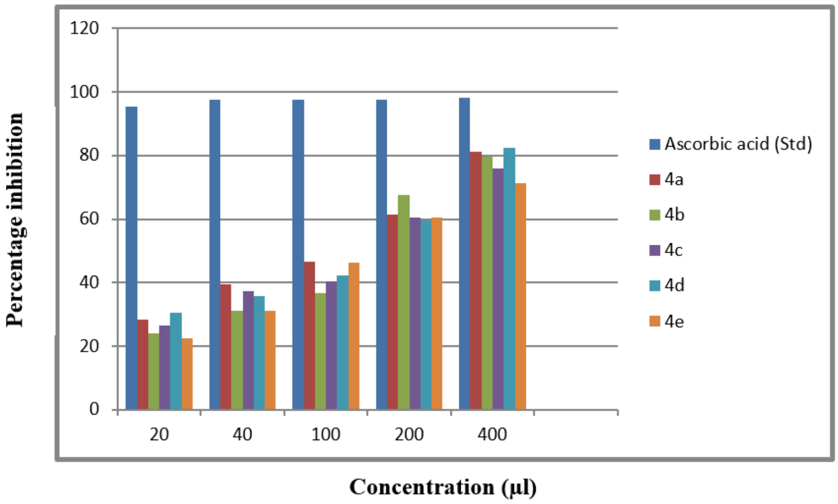


Figure 4. Graphical representation of Antioxidant potential (4a-4e)

CONCLUSION

The dissertation focuses on synthesizing and evaluating various quinoline-thiazole analogues as potential compounds possessing antioxidant properties. These derivatives were synthesized through a multi-step process. Initially, quinoline was synthesized using the Vilsmeyer-Hack reaction, followed by its conjugation with substituted thiazole derivatives..

Antioxidant activity ofthe derivatives was assessed using the DPPH method, evaluating their ability to scavenge free radicals at different concentrations. Results indicated that the incorporation of both quinoline and thiazole rings could serve as a promising strategy for identifying potent antioxidant agents. Among the synthesized derivatives, compounds 4a and 4d demonstrated enhanced therapeutic efficacy as antioxidant agents.

In conclusion, further exploration of quinoline-thiazole hybrids is warranted to uncover novel and potent antioxidant agents. This study underscores the potential of these hybrid molecules in the development of effective antioxidant therapies. Future investigations could delve into the mechanistic insights and optimization of these compounds for broader therapeutic applications in oxidative stress-related disorders.

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Conflict of Interest
Authors declare no conflict of interest

Ethical approval
Not applicable

Informed consent
Not applicable

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