

Antioxidant potential of Schiff base: A short Review

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Abstract:

Schiff bases are a significant class of organic compounds characterized by an imine ($-\text{CH}=\text{N}-$) functional group, attracting extensive attention due to their diverse chemical, biological, and medical applications. Their biological properties are increasingly recognized for antioxidant activity, particularly in combating oxidative stress and reactive oxygen species (ROS) implicated in various pathological illnesses. This review emphasizes current research on the synthesis, characterization, and antioxidant activity of Schiff bases, especially those with phenolic and heterocyclic analogs.

Common characterization tools include FT-IR, UV-Vis, NMR, mass spectrometry, and X-ray diffraction, while synthetic methods range from traditional condensation to microwave-assisted and environmentally friendly techniques. Antioxidant activity is evaluated using standard in vitro methods like DPPH and ABTS radical-scavenging assays, alongside reducing-power and metal-chelation assays. Structural elements significantly influence antioxidant activity, including the quantity and placement of hydroxyl groups, electron-donating and electron-withdrawing substituents, conjugation, heterocyclic rings, and metal coordination.

Electron-rich substances with hydroxyl groups exhibit enhanced radical-scavenging activity through sequential proton-loss electron-transfer (SPLET), single-electron transfer (SET), and hydrogen atom transfer (HAT) pathways. Many Schiff base derivatives and transition metal complexes demonstrate antioxidant qualities comparable to or better than conventional standards like quercetin, Trolox, BHT, BHA, and ascorbic acid. Solvent effects considerably impact antioxidant effectiveness, especially in DPPH and ABTS assays. Research indicates that metal complexation and strategic structural modifications can significantly enhance antioxidant characteristics, establishing Schiff bases as viable platforms for developing new antioxidant and potentially therapeutic drugs.

Key words: Schiff bases, Antioxidant activity, Radical-scavenging assays, DPPH assay, ABTS assay, Oxidative stress
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Introduction

Schiff bases are organic compounds formed when a primary amine reacts with a carbonyl compound, creating a characteristic imine bond ($-\text{CH}=\text{N}-$). First discovered by Hugo Schiff in 1864, these compounds have attracted significant attention due to their diverse applications in medicine, chemistry and biology. When heterocyclic rings, such as thiazole, pyridine, or thiophene, are incorporated into Schiff bases, they become heterocyclic Schiff bases, which often exhibit enhanced biological properties due to their electron-rich structures.

Oxidative stress, defined as an imbalance between the formation of reactive oxygen species (ROS) and the biological system's capacity to detoxify these harmful substances, is implicated in a multitude of diseases, including cancer, neurological disorders, and cardiovascular diseases.[1,2]. To combat the negative consequences of oxidative stress, scientists are looking into new antioxidant molecules that may protect cells and tissues from oxidative damage. Schiff bases, with their variable chemical structures and potential for diverse biological functions, have emerged as interesting candidates for therapeutic development[3,4]. Schiff bases are becoming more popular, but we still don't fully understand the protective qualities of the compounds that are made from meta-substituted diethylenetriamine and 2-

hydroxybenzaldehyde. Unique antioxidant capabilities may be demonstrated by these specific compounds, which could have therapeutic implications as medicinal chemistry. Oxidative stress is an imbalance of free radicals and antioxidants in the body that can lead to cell and tissue damage. During regular metabolic processes, cells in the body make free radicals. Cells, on the other hand, make antioxidants that fight them. Free radicals and antioxidants are usually able to continue functioning in balance in the body. Oxidative stress can also be caused briefly by the body's immune system. This type of oxidative stress causes mild inflammation. Inflammation is caused by this type of oxidative stress. It goes away after the immune system fights off an illness or heals an injury. Oxidative stress that isn't under control can speed up the aging process and may play a role in the development of a number of diseases. Antioxidants donate electrons to neutralize or eliminate free radicals. A substance called an antioxidant can neutralize reactive stress, which helps protect the body from damage. Antioxidants include vitamins A, C, and E. Chronic inflammation and resulting cell damage are one way that oxidative stress can impair the body.[5,6] During oxidative stress, excess free radicals can damage brain structures and change critical proteins such as amyloid-beta peptides. According to Cheignon et al. (2018), oxidative stress

may alter these peptides, contributing to the formation of amyloid plaques in the brain. This is a key indicator of Alzheimer's disease.[7]

Oxidative stress may play a role in the development of a range of conditions, including:

- cancer
- Alzheimer's disease
- Parkinson's disease
- diabetes
- cardiovascular conditions such as high blood pressure, atherosclerosis, and stroke
- inflammatory disorders
- chronic fatigue syndrome
- asthma
- male infertility

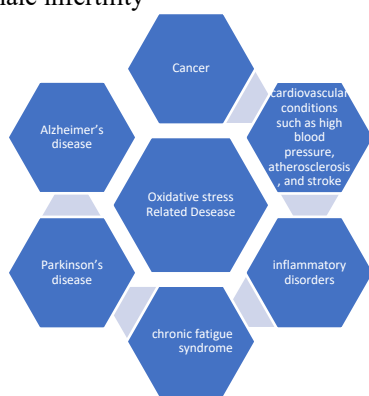


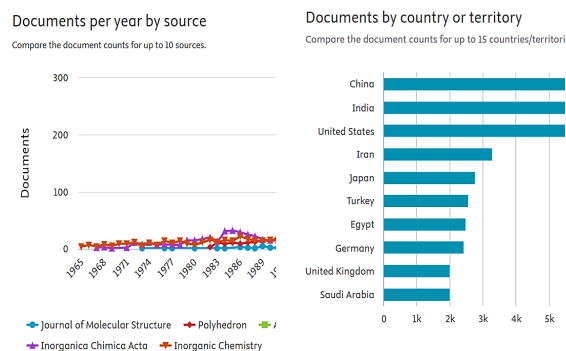
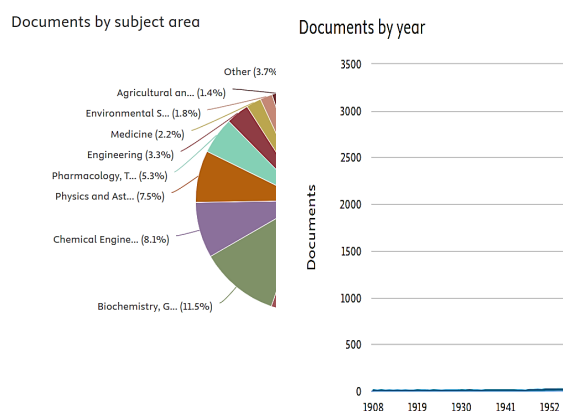
Fig.1

Molecules with unpaired electrons are reactive and unstable known as free radicals. The endogenous and external sources are reason to develop of these reactive oxygen species (ROS) and reactive nitrogen species (RNS) [8] Free radicals are developed via organic waste products of metabolic activities such as immunological responses, enzymatic reactions and mitochondrial electron transport. [9] Some of the factor are enhancing ROS and RNS such as pollution, tobacco smoke, industrial chemicals, and ultraviolet (UV) radiation.[10]. This oxidative stress causes an imbalance between the body's capacity to detoxify free radicals or repair the harm. Increment the amount of free radicals can weak and imbalance antioxidant defences. [3] Free radicals react with biological components such as lipids, proteins, and DNA. [11] DNA damage results mutations, genomic instability and protein oxidation, which causes loss of function, structural integrity, and lipid peroxidation damages to cell membranes [12]. Scopus analysis with 240 papers indicates that the network map of antioxidant work is published. Furthermore, the provided data was analysed in VOSviewer to check the connection of each other. Each analysed item within the visualization.[13] The inclusion of this number of papers suggests a substantial body of literature which can be sufficient to reveal meaningful patterns, clusters, and

relationships.[14,15]

Fig.2

The collection of charts visualizes global research trends in chemistry and related fields through document analytics. China, India, and the United States lead in research output, with chemistry and materials science dominating subject areas. The yearly distribution reveals exponential growth in research publications, particularly after the year 2000, while a minor decline is noted after 2026. Most documents are research articles (94.3%), highlighting the focus on original research. Leading journals, such as the Journal of Molecular Structure, are significant contributors, and notable authors like S. Chattopadhyay and R.J. Butcher stand out for their prolific publication records. These insights collectively underscore the expanding landscape and collaborative nature of scientific research worldwide, especially in the chemical sciences.



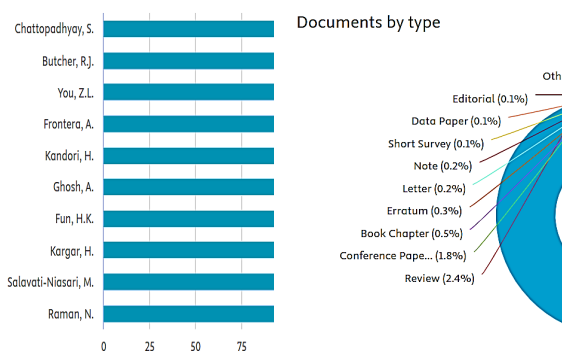


Fig.3

Table 1

Metric	Key Figures & Trends
Top Countries	China, India, USA, Iran, Japan, Europe
Dominant Subject	Chemistry (39.6%), Materials Science (15.6%)
Main Document Type	Research Articles (94.3%)
Leading Journals	J. Mol. Structure, Polyhedron, Inorganica Chimica Acta
Author Leaders	S. Chattopadhyay, R.J. Butcher, Z.L. You
Growth Trend	Fast rise post-2000, peak before 2027
Industrial Focus (2025+)	Sustainability, AI, digitalization, circular economy
Innovation Metrics	>2.9 million patents, global startup ecosystem

This review focuses on heterocyclic Schiff bases, exploring their synthesis, characterization and antioxidant potential, primarily

through assays like DPPH and ABTS. It examines how structural features, such as electron-donating groups and heterocyclic rings, influence their activity. Additionally, the review compares their performance to standard antioxidants like Trolox and discusses their potential in pharmaceutical applications. By analyzing recent studies, this paper aims to highlight the importance of heterocyclic Schiff bases as antioxidants and identify future research directions to enhance their therapeutic use.

Synthesis of Heterocyclic Schiff Bases

Heterocyclic Schiff bases are typically synthesized via condensation reactions. A common method involves mixing equimolar amounts of amine with substituted aldehydes or ketones in ethanol/methanol, with or without catalytic acetic acid, under reflux for 4-6 hours. Yields range from 76-88% and products are purified by recrystallization.[16]

Microwave-assisted synthesis offers faster reaction times and higher yields.[17] For instance, chitosan-based heterocyclic Schiff bases are prepared by reacting chitosan with quinolinone derivatives.[18] Other approaches include using S-methyl dithiocarbamate with aromatic aldehydes to form thiocarbamate Schiff bases.[19] Green solvents and catalysts are increasingly used to make synthesis eco-friendly.[20]

2.7 Characterization Techniques

Characterization ensures the structure and purity of synthesized compounds. Fourier Transform Infrared (FT-IR) spectroscopy identifies the imine stretch at $1610-1640\text{ cm}^{-1}$ and phenolic O-H at $\sim 3400\text{ cm}^{-1}$. UV-Vis spectroscopy shows $\pi \rightarrow \pi^*$ transitions at 280-300 nm and $n \rightarrow \pi^*$ at 320-360 nm.[21]

^1H NMR reveals imine protons at δ 8.3-8.8 ppm and phenolic OH at δ 9.5-10.2 ppm. X-ray diffraction provides crystal structures, confirming tautomers like keto-amine forms. Mass spectrometry and elemental analysis further validate molecular formulas.[22-24]

2.8 Antioxidant Assays

Antioxidant activity is essential because it helps your body stay healthy by protecting its cells from harmful molecules called free radicals. These unstable particles can build up and cause oxidative stress, which contributes to aging, inflammation, and diseases such as heart problems, cancer, and diabetes.

Table 2:

Main Assays	Types Of Assays
	DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay[25]

[25]Free Radical Scavenging Assay	ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Assay[25]
	Superoxide Radical Scavenging Assay[26,27]
	Hydroxyl Radical Scavenging Assay[28]
	Nitric Oxide Scavenging Assay[29]
Reducing Power Assay	Ferric Reducing Antioxidant Power (FRAP) Assay[30][29]
	CUPRAC (Cupric Ion Reducing Antioxidant Capacity Assay[31,32]
	Total reducing power assay[32]
Metal Chelating Assay	Ferrous Ion Chelating Activity[33]
	Metal Ion Chelating Activity[32]
Lipid Peroxidation Inhibition Assay	Thiobarbituric Acid Reactive Substances (TBARS) Assay[34]
	Lipid peroxidation Assay[35]
	Conjugated Diene Assay[36]
Cellular Antioxidant Activity (CAA) Assay	Cellular Antioxidant Capacity (CAA) Assay[37]
Total Antioxidant Capacity (TAC) Assay	Phosphomolybdenum Method[30]
	Oxygen Radical Absorbance Capacity (ORAC) Assay[30]
Enzyme-based Antioxidant Assay	Superoxide Dismutase (SOD) Activity[38]
	Catalase (CAT) Activity[38]

	Glutathione Peroxidase (GPx) Activity[39]
Other Assay	Total Phenolic Content (TPC)[29,32]
	Total flavonoid content (TFC)[29]
	Electrochemical assays (e.g. Cyclic Voltammetry)[40]

Antioxidant potential is commonly assessed using in vitro assays. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay measures the ability to reduce purple DPPH to yellow diphenyl picrylhydrazine at 517 nm. IC₅₀ values indicate concentration for 50% inhibition; lower values mean higher activity.[22]

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay involves blue-green ABTS radicals, decolorized by antioxidants. Other methods include ferric reducing antioxidant power (FRAP) and superoxide scavenging. Assays are performed in triplicate, with statistical analysis like ANOVA.[24]

2.9 Structure-Activity Relationships (SAR)

SAR studies reveal how structural features affect antioxidant activity. Electron-donating groups (e.g., methoxy, hydroxy) on the aromatic ring enhance activity by stabilizing radicals via resonance.[41–44] Ortho-hydroxy positions enable intramolecular hydrogen bonding with imine nitrogen, boosting efficacy.[24,45]

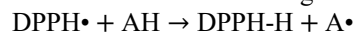
Heterocyclic rings like thiazole provide better activity than pyridine due to sulfur's electron delocalization.[46] Electron-withdrawing groups (e.g., nitro, halogens) reduce activity. Solvent effects matter; polar solvents favor proton transfer mechanisms like HAT or SPLET. Metal complexes often show superior activity due to redox cycling.

2.10 The Antioxidant Assay: Mechanism and Principles

Antioxidant assay mechanisms involve measuring the ability of compounds to neutralize free radicals or reduce oxidants, typically using hydrogen atom transfer (HAT) and single-electron transfer (SET) principles.[47,48]

2.10.1 Hydrogen Atom Transfer (HAT) Mechanism

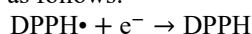
In the HAT mechanism, antioxidants neutralize free radicals by donating a hydrogen atom. This mechanism is most relevant for compounds like phenolics and flavonoids that possess a hydrogen atom in their structure. The reaction can be generalized as:



Where AH is an antioxidant, and A• is the resulting antioxidant radical.[24,49–53]

2.10.2 Single Electron Transfer (SET) Mechanism

The SET mechanism involves the transfer of a single electron from the antioxidant to the DPPH radical, leading to the formation of a neutral molecule. Unlike HAT, SET does not involve proton transfer. This mechanism is prominent in antioxidants like ascorbic acid and other electron-donating compounds. The reaction is as follows:



Here, e^- represents the electron provided by the antioxidant.[53–58]

2.10.3 Sequential Proton Electron Transfer (SPLET) Mechanism

The SPLET mechanism combines both SET and HAT. Initially, the antioxidant donates a proton to the DPPH radical, followed by electron transfer. This dual donation makes SPLET more efficient in neutralizing free radicals than either HAT or SET alone. SPLET is typically observed in antioxidants like polyphenols, which can donate both protons and electrons.[50,51,59]

The DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely accepted, simple, and fast spectrophotometric method used to evaluate the free radical scavenging ability and antioxidant potential of synthesized compounds or natural extracts.[24,46,60–72]

Principle and Mechanism of the DPPH Assay

The DPPH assay is based on the reduction of the stable DPPH free radical.[46,62,67,70] DPPH contains an unpaired electron[46,66,70] that gives it a deep violet color[46,61–63,70] when dissolved in ethanol or methanol. When an antioxidant is added to this solution, it donates a hydrogen atom or electron to the DPPH radical,[24,46,63,64,67,70] converting it into its non-radical form, diphenylpicrylhydrazine.[46,70]

As this reaction occurs, the solution's color gradually changes from deep violet to yellow[46,61–63,70] due to the neutralization of the radical. The change in color intensity is measured spectrophotometrically at the maximum absorption wavelength (λ_{max}) of 517 nm.[24,46,61–63,66,69,70] A lower absorbance indicates stronger radical scavenging activity.[24,60]

Methodology and Calculation

The assay is carried out by monitoring the change in absorbance of the DPPH solution after mixing with the test sample.[61,66]

General Procedure:

- **Preparation:** A DPPH stock solution is prepared in ethanol[60,61,70] or methanol,[46,62–64,69,72] usually at 0.1[62,63]–0.4[61] mM concentration. Test

samples are prepared at various concentrations (for example, 1.25–10 $\mu\text{g/mL}$).[72]

- **Reaction:** A measured volume of DPPH solution is mixed with the test sample, shaken well, and incubated in the dark at room temperature or 37 °C for about 30 minutes.[61,62,64,66,69,70,72]
- **Measurement:** After incubation, the absorbance is recorded at 517 nm using a UV–Vis spectrophotometer. Tests are performed in triplicate for accuracy.[60,61,66,70]

Calculation:

The percentage of DPPH radical scavenging activity is calculated as:

$$\% \text{ Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control (DPPH only) and A_1 is the absorbance of the sample.[24,60,61,64–66]

IC₅₀/EC₅₀

Determination:

The IC₅₀ (half-maximal inhibitory concentration) or EC₅₀ (effective concentration) is determined by plotting % inhibition against concentration.[61,70,71] IC₅₀ represents the concentration required to achieve 50% inhibition of the DPPH radical.[70,71]

Reference Standards

Common standards used for comparison include:

- Ascorbic acid (Vitamin C)[24,46,60–62,64–66,68,69,70,72]
- Butylated hydroxytoluene (BHT)[63,67,70]
- Butylated hydroxyanisole (BHA)[67,71]
- Quercetin[62]
- α -Tocopherol (Vitamin E)[63,67]

Mechanistic Insights and Structure–Activity Relationship (SAR)

The DPPH assay helps in understanding how structural features influence antioxidant efficiency.

- **Hydroxyl groups:** These are key contributors to antioxidant activity since they can donate hydrogen atoms to neutralize radicals.[64,70,71] Molecules with multiple hydroxyl groups often show higher scavenging capacity.[70]
- **Substituents:**
 - Electron-donating groups (e.g., methyl, $-\text{OCH}_3$) improve activity by stabilizing the formed phenoxyl radical.[65,68,69,71]
 - Electron-withdrawing groups (e.g., $-\text{NO}_2$) generally reduce activity by destabilizing the radical.[65,71]
- **Conjugation:** The presence of imine ($\text{C}=\text{N}$) groups or conjugated systems allows for delocalization of electrons, enhancing radical stabilization.[62,71]
- **Chelation Effect:** Complex formation with metal ions can increase antioxidant activity due to enhanced charge delocalization and lipophilicity.[65,68]

Influence of Solvent and Reaction Pathways

The DPPH mechanism may follow two main pathways:

- **Sequential Proton-Loss Electron Transfer (SPLET):** Favored in polar protic solvents such as methanol.[71] The phenolic compound first loses a proton, forming an anion that then reacts with the DPPH radical.[71]
- **Hydrogen Atom Transfer (HAT):** Favored in non-polar solvents such as chloroform, where the antioxidant directly donates a hydrogen atom.[71]

Comparison with Other Antioxidant Assays

The DPPH assay specifically evaluates radical scavenging activity, but results can differ from other methods due to the varying nature of radicals and reaction media.[62]

- **ABTS assay:** Involves the ABTS⁺ cation radical in aqueous medium; compounds often show stronger activity in this system than in DPPH.[62,70–72]
- **CUPRAC assay:** Measures the reduction of Cu(II) to Cu(I) under near physiological pH (around 7). Generally, DPPH radicals are less reactive than ABTS⁺ radicals, emphasizing the need to perform multiple assays for a comprehensive antioxidant evaluation.[62,71]

Table 1: Results reported in Micromolar (M) Concentration of Standards

Compound/Ligand	IC50 (μ M) $\pm$ SD/SEM	Key Finding/Activity Note
Ascorbic acid (AA)	23.80 $\pm$ 1.21	Reference standard [61]
Quercetin	8.69 $\pm$ 0.24	Reference standard [62]
TA (Schiff base)	0.028 $\pm$ 0.003	Exhibited IC50 = EC50, indicating rapid scavenging [71]
PMA (Schiff base)	0.023 $\pm$ 0.003	Best activity in its series (IC50 = EC50) [71]

Conclusion of table: TA and PMA, are reported numerically equivalent for IC₅₀ and EC₅₀ in methanol, confirming rapid radical scavenging activity for those compounds. The highest reported IC₅₀ among the spiro-isatin Schiff bases was 204.90 $\pm$ 3.52 M for (5q).[7,12]

Table 2: Results of Standards reported in Mass Concentration (g/mL or mg/mL)

Compound/ Ligand	Unit	IC50 / EC50 $\pm$ SD/S EM	Standar d Used for Compar ison	Activit y Ranki ng
Ascorbic acid	μ g/m L	3.98	N/A	Refere nce standa rd [72]
Ascorbic acid	mg/ mL	49.68 $\pm$ 3.68	N/A	Refere nce standa rd [69]

In the DPPH assay using different solvents, phenolic Schiff bases were found to exhibit the general trend in antioxidant activity: PMA > TMA > PA > TA > PNA > TNA [7]

Binesh Kumar et. al.[72] investigates the antioxidant properties of transition metal complexes derived from heterocyclic Schiff base ligands, including 1,4-bromo-2-

(((3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl)imino)methyl)phenol **(1)**, 2,4-dichloro-6-(((3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl)imino)methyl)-phenol **(2)**, 2-6-methoxy-4-nitrophenol **(3)**, and 1-1-((3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl)imino)methyl)naphthalen-2-ol **(4)**. The structure of these complexes and ligands is also discussed.

The synthesized compounds were prepared in DMSO and dissolved in DPPH solution. The absorbance was assessed at 517 nm in triplicates, and the % radical scavenging values. The results showed the concentration and scavenging activity of the compounds, calculating % radical scavenging and IC50 values, and comparing them with the standard drug.

The antioxidant activity of synthesized compounds and standard drugs is dose-dependent, with increased concentration and decreasing IC50 value. Ligand antioxidant activity is influenced by polarity, metallic effect, hydrophobicity, lipophilicity, and spreading of negative charge. Metal complexes have good antioxidant behavior, with copper(II) complex **(5)** being highly active and promising for treating oxidative stress and pathogenic illnesses.

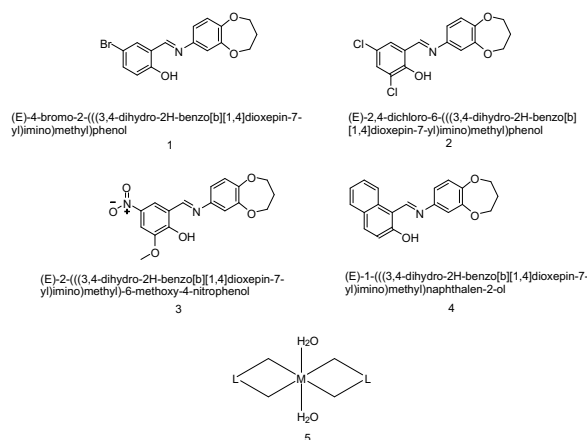


Figure 4 Schiff base Ligands and General Formula of Transition metal ligand complexes

Anthony C. Ekenia, [70] and his team develop a some heterocyclic ligands like 2-[(5-methoxy-1,3-benzothiazol-2-yl)amino]naphthalene-1,4-dione (**6**), 3-[(5-methoxy-benzothiazol-2-ylimino)-methyl]-naphthalen-2-ol (**7**), 2-methyl-6-[(5-methoxybenzothiazol-2-ylimino)-methyl]-2-methoxycyclohexa-1,5-dienol (**8**) and that ligand metal (Co and Pd Metal) complexes.

They studied antioxidant study of heterocyclic base structure shown in figure 5.

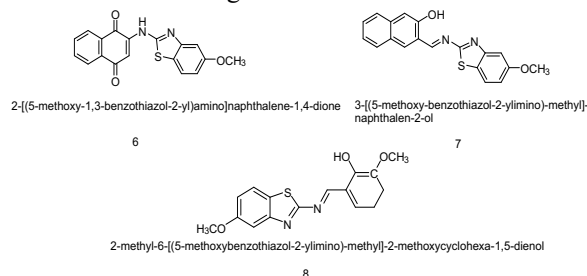


Figure 5 Heterocyclic Schiff Base Used for Antioxidant Activity

The antioxidant capacity of ligands was investigated using a DPPH radical scavenging assay. The ligands were added to a pre-dissolved solution of 2,2-diphenyl-1-picrylhydrazyl, and the mixture was incubated for 30 minutes. The absorbance was measured against a blank solution of DMSO. The % DPPH radical scavenging ability was determined in triplicate, using ascorbic acid as a standard. the % DPPH radical scavenging ability was determined following the expression below:

$$\text{DPPH Scavenging Effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c represents the absorbance of the control mixture, while A_s represents the absorbance of the sample mixture or the absorbance of the standard.

The study analyzed the antioxidant activity of ligands using the DPPH Assay, finding that HL1 ligand had the highest DPPH radical scavenging ability at 35 $\mu\text{g/mL}$, while (**8**) had the highest at 96 $\mu\text{g/mL}$, showing a concentration-dependent effect.

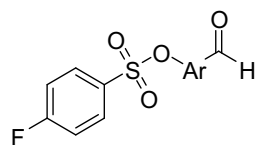
Table 1 DPPH Radical Scavenging Activity for the HL¹-HL³ ligands

Name	Concentration ($\mu\text{g/mL}$)	% DPPH radical scavenging ability	IC50
6	50	50.12($\pm$ 1.132)	35
	100	61.89($\pm$ 0.331)	
	150	69.08($\pm$ 0.137)	
	200	72.17($\pm$ 0.117)	
	250	87.67($\pm$ 0.052)	
7	50	47.75($\pm$ 1.203)	56
	100	53.89($\pm$ 1.032)	
	150	63.15($\pm$ 0.115)	
	200	65.89($\pm$ 0.133)	
	250	71.08($\pm$ 0.122)	
8	50	39.12($\pm$ 0.137)	96
	100	41.71($\pm$ 0.215)	
	150	55.23($\pm$ 0.168)	
	200	59.65($\pm$ 0.124)	
	250	63.91($\pm$ 0.110)	

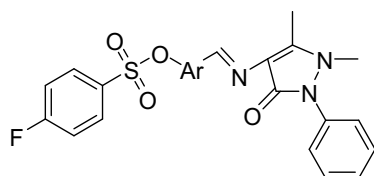
Ayfer Kamah et. al. [66] synthesized Schiff base compounds and compared their antioxidant properties using the DPPH Assay. The compounds 15, 16, and 17 showed higher antioxidant activity than BHT but lower than BHA and α -TOC. None of the remaining Schiff base derivatives or aryl sulfonates showed higher antioxidant activity than standard compounds.

and aryl sulfonates showed higher antioxidant activity than standard compounds.

Ar	
9, 18	2-OHC ₆ H ₄
10, 19	3-OHC ₆ H ₄
11, 20	4-OHC ₆ H ₄
12, 21	2-OH-3-OCH ₃ -C ₆ H ₃
13, 22	2-OH-4-OCH ₃ -C ₆ H ₃
14, 23	3-OH-4-OCH ₃ -C ₆ H ₃
15, 24	4-OH-3-OCH ₃ -C ₆ H ₃
16, 25	4-(N(CH ₂ CH ₃) ₂)-2-OH-C ₆ H ₃
17, 26	2-OH-C ₁₀ H ₆



Aryl Sulfonates (9-17)



The Target Molecules (18-26)

Figure 6 Compounds which Worked by Author
The DPPH assay compared the antioxidant activity of all tested molecules and standard compounds. The newly synthesized Schiff base derivatives generally had better antioxidant activities than aryl sulfonates. Compounds 23 (IC₅₀ = 161.93 μM), 24 (IC₅₀ = 191.76 μM), and 25 (IC₅₀ = 105.55 μM) had higher antioxidant activity than BHT but lower than BHA and α-TOC. None of the remaining Schiff base derivatives

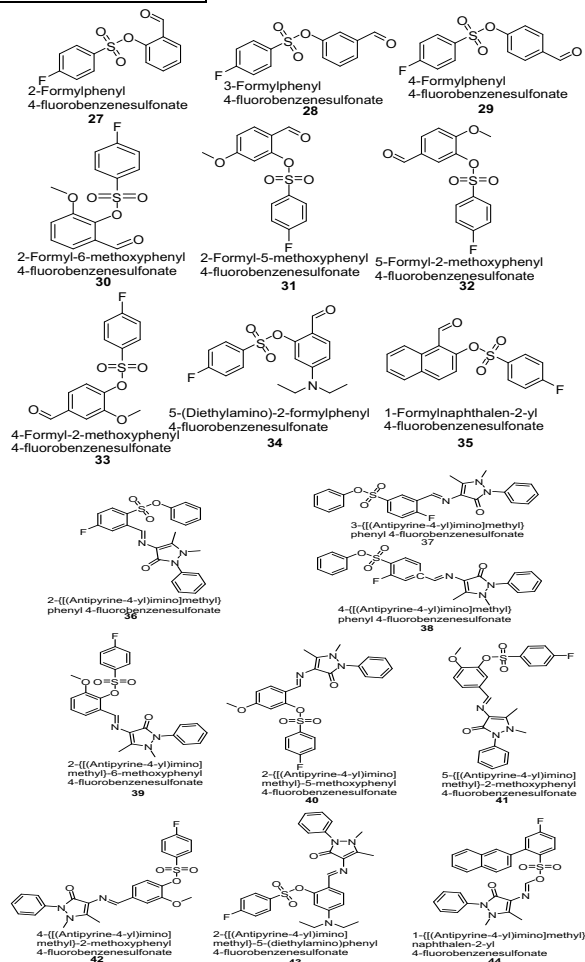


Table 2 IC₅₀ value of DPPH Assay of newly synthesized compound

Compounds	IC ₅₀ Values (μM) DPPH
27	>1,000
28	274.59 ± 3.07
29	744.59 ± 4.07
30	346.77 ± 2.74
31	386.12 ± 3.06
32	665.74 ± 4.84
33	362.19 ± 3.13
34	746.36 ± 2.53
35	542.70 ± 2.94
36	>1,000
37	255.57 ± 1.69
38	205.17 ± 0.83
39	253.72 ± 0.87
40	228.36 ± 1.16
41	161.93 ± 2.29
42	191.76 ± 0.82
43	107.55 ± 0.88
44	288.33 ± 3.20
BHA	50.58 ± 0.77
α-TOC	16.30 ± 0.79
BHT	203.50 0.66

Manju Rani et. Al. [65] Synthesized Benzo Derivative Schiff Base Ligand and after ligand formation they have develop some metal ligand complexes. In this complexes author used Cobalt, nickel, copper, and zinc metal is used. Then after author confirmed these ligand and metal complexes using some techniques like FT-IR, UV-Vis, NMR, Mass Spectra etc. that synthesized ligand and metal complexes general structure is shown in figure 5. After that author studied some biological activity like ADMET, antimalarial, antioxidant, anti-inflammatory etc.

The study examines the antioxidant potential of compounds (45-54) in vitro using DPPH, a potent component in radical scavenging activity. Test samples were prepared at concentrations ranging from 200 to 6.25 μg/mL in DMSO, using vitamin C as a reference drug. A DMSO-based DPPH solution was used as a control. The samples were blended with a DPPH

solution, and the absorbance and color changes indicated the compounds' antioxidant capability. The percentage of radical scavenging power of each compound was also examined.

$$\% \text{ radical scavenging ability of sample} = (X_c - X_s / X_c) \times 100$$

X_c = Control's Absorbance

X_s = Sample's Absorbance

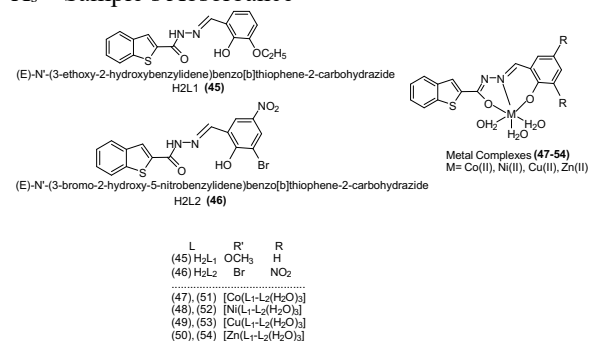


Figure 1 Schiff Base Ligand Structure and Their Metal Complex General Structure

The study examines antioxidant properties in a dose-dependent manner, focusing on Schiff base ligand H2L1's highest inhibition ability and H2L2's less inhibition ability. The antioxidant potential of ligand H2L2 is less than H2L1. The order of transition metal chelates for antioxidant effectiveness is Ni(II), Co(II), Zn(II), Cu(II), and Ascorbic Acid. The Copper Complex and Zinc Complex have the strongest potency, providing a protective layer against free radical attacks and suppressing oxidative effects.

Sumita Saznin Marufa et.al[61] Synthesized eighteen thiazole derivatives and characterize it. After synthesis and characterize authors check antioxidant activity, antimicrobial activity and molecular docking properties of these compounds. These synthesized compound structure shown in figure 6 shown below.

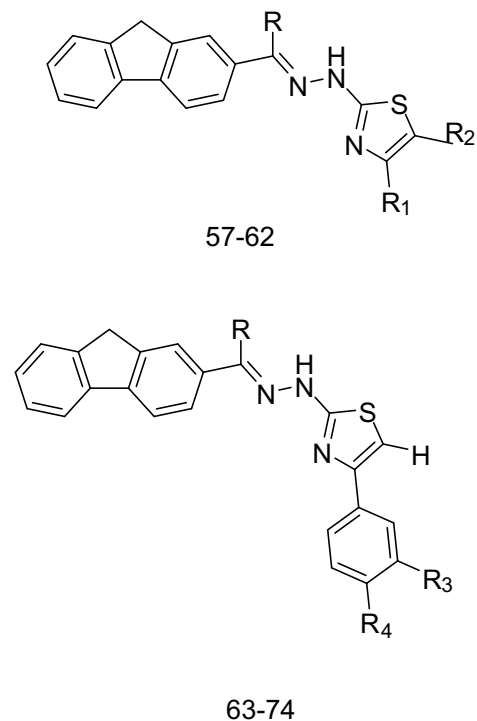
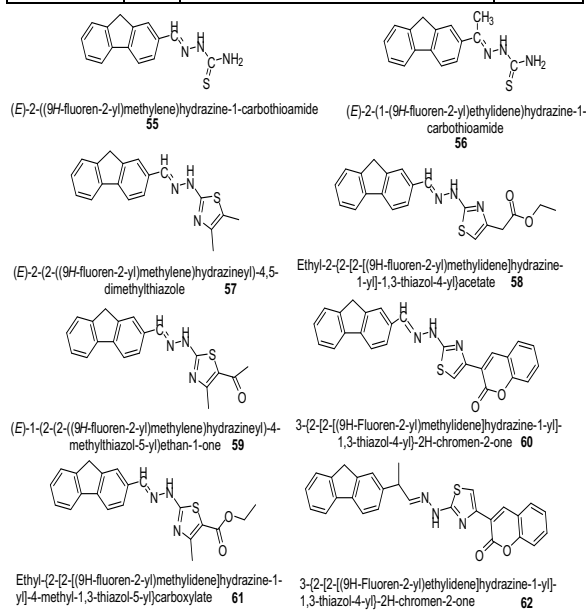


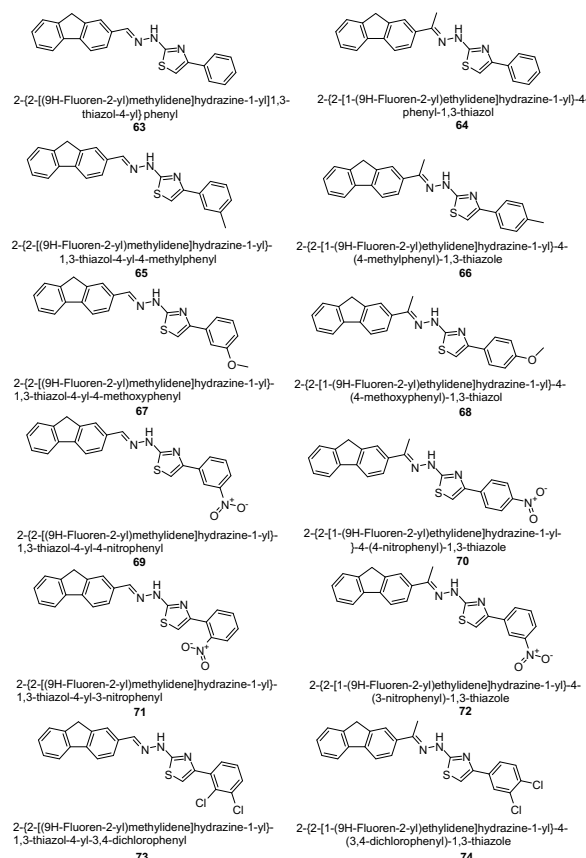
Figure 2 Thiazole Derivatives

Table 3 Thiazole Derivative Compounds R,R₁,R₂,R₃ and R₄

Compound	R	R ₁ /R ₃	R ₂ /R ₄
57	H	Me	Me
58	H	Me	CO Me
59	H	Me	COO Et
60	H	CH ₂ COOEt	H
61	H	3-methyl-2H-chromen	H
62	Me	3-methyl-2H-chromen	H
63	H	H	H
64	H	H	Me

65	H	H	OMe
66	H	H	NO ₂
67	H	NO ₂	H
68	H	Cl	Cl
69	Me	H	H
70	Me	H	Me
71	Me	H	OMe
72	Me	H	NO ₂
73	Me	NO ₂	H
74	Me	Cl	Cl





The antioxidant activity of tested compounds varied from 15 to 250 microlitres, with absorbance fluctuating with concentration. 2m showed highest activity at 125 mg/mL-1, while coumarin and methyl groups had the highest IC₅₀ values. Compound 2j had the lowest IC₅₀ due to a thiazole substituted phenyl ring. The IC₅₀ value of DPPH Assay of all compounds showed in table 3.

Table 4 Antioxidant Properties (IC₅₀ values) of compounds 2a-2r and ascorbic acid

Compound	IC ₅₀ (ug mL ⁻¹)	Compound	IC ₅₀ (ug mL ⁻¹)
57	117.08 ± 1.43	66	171.73 ± 2.80
58	48.62 ± 2.41	67	134.8 ± 1.42
59	57.39 ± 2.47	68	133.62 ± 0.72
60	99.86 ± 0.38	69	48.99 ± 2.11
61	140.87 ± 1.76	70	38.35 ± 1.69

62	11.73 ± 1.22	71	118.15 ± 1.43
63	111.57 ± 1.22	72	132.48 ± 3.11
64	101.72 ± 0.69	73	68.95 ± 0.56
65	120.68 ± 0.80	74	118.44 ± 1.19
Ascorbic Acid		49.68 ± 3.68	

Abdelmadjid madi et. Al. [60] synthesized a novel Schiff Base 2,2'-(1E,1'E)-((4-methyl-1,3-phenylene)bis(benzylidene))bis(methylethylidene)bis(4-methoxyphenol) (L) from 2,4- diaminotolunene and -hydroxy—methoxy benzaldehyde. After that author characterized synthesized compound. Then after author check antioxidant activity of new synthesized Schiff base. The structure of newly synthesize Schiff base is shown in figure.

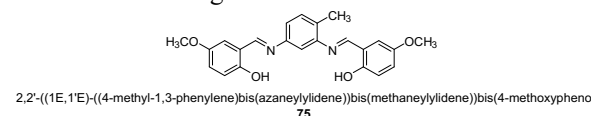


Figure 3 Structure of novel Schiff base

The DPPH Assay is used to evaluate the antioxidant properties of a novel Schiff base ligand. DPPH is a stable free radical with an odd electron structure, used for detecting radical scavenging activity. The test measures the antioxidant's ability to sweep free radicals. The Results of the DPPH test show that the effective concentration (ug/ml) of the compound L has poor antioxidant activity compared to the effective concentration of the BHT standard. This large difference between the two can be explained by the high ability of BHT standard to reduce the DPPH radical. It is well known that the BHT is one of the best antioxidants. However, the percent of scavenging effect (%) increases with the increase of the concentration of the compound L.

Table 5 DPPH Assay of newly synthesized compound

Samples	DPPH EC ₅₀
75	210.477 ± 31.607
BHT	57.310 ± 5.361

Table 6 Evolution of the percent inhibition (%) for the compound L and BHT

Compound (75)		BHT	
Concentration (µg/mL)	(%) DPPH	Concentration (µg/mL)	(%) DPPH
7	39.448	20	37.026

20	42.306	27	39.130
60	43.469	33	44.133
200	48.620	67	79.710
400	58.828	133	79.310
470	63.310	270	93.314
530	67.035	333	93.548
700	70.422	500	93.454
870	74.875	600	93.127
		700	93.595

Antony Shanty et. Al.[24] synthesized some thiophene and pyrrole-based Schiff base derivatives and that structure are shown below figure 9. After synthesis author describe antioxidant properties of synthesized Schiff base in different solvents. That antioxidant activity table also shown below table 6.

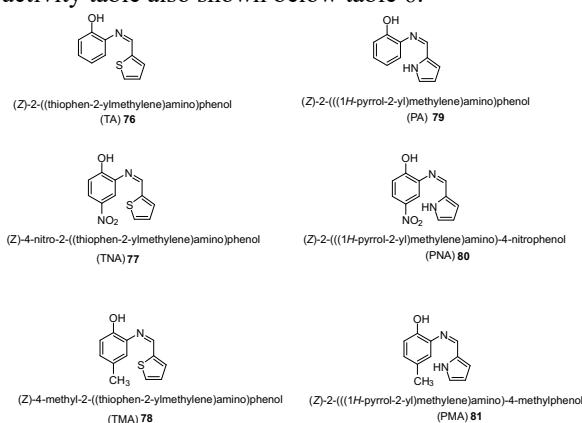


Figure 4 Some Synthesized Heterocyclic Schiff bases

The method determines the decrease in absorbance from one minute to one hour, depending on the antioxidant. Kinetics are plotted for each antioxidant, and the percentage of DPPH radical remaining is determined. EC₅₀ values are obtained, similar to IC₅₀, which is the efficient concentration required to decrease DPPH concentration by 50%.

The synthesized Schiff base derivatives insoluble in water and soluble in ethanol, acetone, DMF, and DMSO. The Invitro antioxidant properties of all developed compounds were evaluated by DPPH Assay. The IC₅₀ Values in different solvents were shown in table 6. A lower IC₅₀ value indicated greater antioxidant activity. The compound showed very good DPPH activity. All the compound except TNA showed higher antioxidant activity that that of standard synthetic antioxidant BHA. General trend for antioxidant activity of synthesized compounds was TNA<PNA<TA<PA<TMA<PMA.

Table 7 DPPH Scavenging activity of synthesized compound in different solvents

Comp ounds	Meth anol	chloro form	Aceto nitrile	Acet one	Eth yl acet ate
TA (76)	1.85	2.94	23.2	33.3	150
TNA (77)	4.08	6.01	43.2	90.3	206
TMA (78)	1.60	2.77	18.5	26.0	134
PA (79)	1.66	2.80	26.0	32.0	123
PNA (80)	2.25	4.15	42.1	52.9	150
PMA (81)	1.50	2.31	22.0	26.1	120
BHA	3.46	5.34	41.9	56.0	170

Fitsum Lemilemu et. Al.[46] The team synthesized five heterocyclic thiazole-based Schiff bases from different compounds; the structures of these synthesized compounds are given in Figure 10. After synthesis, the authors assess the compound's antibacterial and antioxidant activities and perform a computational study. This antioxidant study summary is given below. First shown in Figure 10 is below.

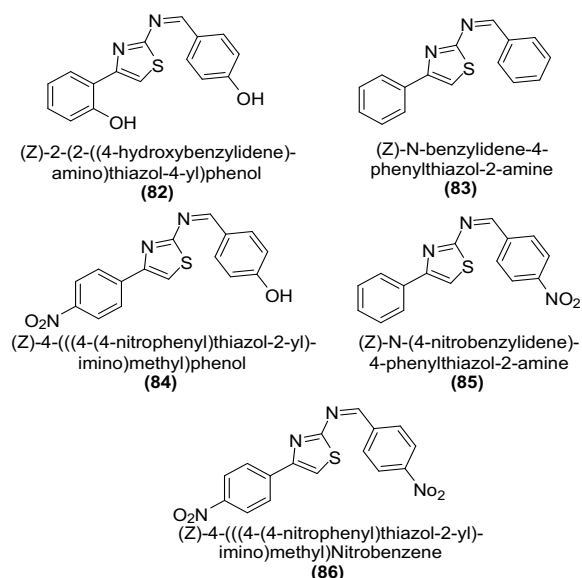


Figure 5 Thiazole based synthesized Schiff bases and their green synthesis of Schiff bases

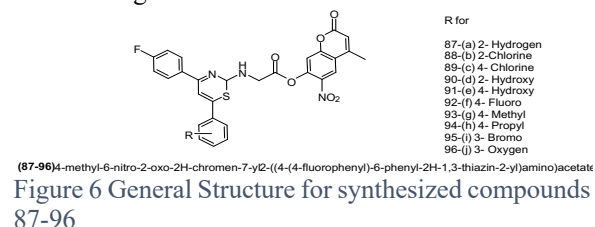
Oxidative stress causes cancer, neurological, and cardiovascular diseases. Synthesized compounds **82** and **84** showed promising antioxidant activity, with A's high activity likely due to electron-donating -OH substituents, compared to ascorbic acid's 91.2%.

Table 8 DPPH Assay For the synthesized compound A-E

Concentration Ug/mL	Ascorbic Acid	82	83	84	85	86
1.25	22.6	15.	17.	17.	21.	15.
	9%	73	13	13	60	40
		%	%	%	%	%
2.50	35.0	58.	42.	64.	27.	22.
	0%	15	70	50	20	00
		%	%	%	%	%
5.00	73.1	71.	43.	66.	59.	58.
	0%	35	80	00	83	15
		%	%	%	%	%
10.00	91.2	80.	75.	70.	74.	88.
	0%	89	80	00	50	80
		%	%	%	%	%
IC ₅₀	3.98	3.6	5.5	3.6	5.3	5.1
		0	6	5	6	4

Nilesh Chauhan et al.[69] Synthesized a new series of 4-methyl-6-nitro-2-oxo-2H-chroman-7-yl-2-(4-

fluorophenyl)-6-phenyl-2H-1,3-thiazin-2-yl-amino)acetates a-j from 6-nitro-4-methyl coumarinyl chloroacetate and 2-amino thiazines. these synthesized compound structure confirmed by IR, ¹H NMR, ¹³C NMR and Mass. After characterization check antimicrobial, antitubercular, and antioxidant activities. These synthesized compound structures are shown in figure 11.



The DPPH antioxidant bioassay is a widely used method for evaluating the free radical scavenging action of tested compounds. It is used to screen berberine-based compounds for their in vitro antioxidant profiles. The study used the DPPH bioassay to analyze the percentage of radical scavenging antioxidant activity (RSA %) of each substance. The reaction of title compounds with DPPH resulted in a change in color from deep violet to light yellow at 517 nm. Ascorbic acid served as a control drug in the assay. The results of this bioassay, RSA % was determined according to (Mensor et al. 2001) as described in the below equation.

% Scavenging = Absorbance Blank – Absorbance of Test / Absorbance of Blank × 100

A plot of concentration (fig.) of test compounds and % Scavenging introduced IC₅₀ in the presence of an ascorbic acid as standard.

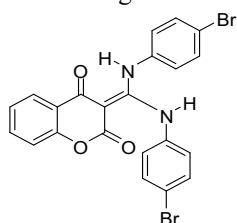
From the antioxidant Assay of compound, a-j compound h and b were appeared to have high radical scavenging efficacies showed by IC₅₀ Values in DPPH Assay and can be comparable to that of control ascorbic acid while other compounds have moderate poor antioxidant power. The results were shown in following table 8.

Table 9 Screening results of DPPH radical scavenging activity of 87-96

Compound no.	DPPH IC ₅₀ ug/mL ± SD
87	40.02 ± 0.199
88	35.35 ± 0.470
89	37.80 ± 0.246
90	64.21 ± 0.895
91	69.17 ± 0.866
92	58.90 ± 1.069

93	51.97 ± 1.098
94	33.99 ± 0.301
95	69.58 ± 0.330
96	49.15 ± 0.140
Ascorbic Acid	36.22 ± 0.469

Drifa Belkhir-Talbi et. Al.[68] synthesized N,N'-di(4-bromophenyl)-4-hydroxycoumarin-3-carboximidamide for their antimicrobial and antioxidant studies. The Schiff Base Ligand Structure is Given in the figure 12.



97 N,N'-di(4-bromophenyl)-4-hydroxycoumarin-3-carboximidamide

Figure 7 Ligand L₁ Structure

The antioxidant activity of synthesized L₁ and its complexes was evaluated using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) scavenging assay. The DPPH solution was prepared by dissolving it in ethanol, and the compounds were dissolved in DMSO. The concentrations of the compounds were diluted with DMSO, and the DPPH solution was added to the mixtures. The absorbances were measured at 517 nm, and the DPPH scavenging effect was calculated. The ligand L₁ exhibited higher scavenging activity than its corresponding complexes, possibly due to the presence of the OH group. The decrease in antioxidant activity of the complexes was due to the low oxidation potential of the metal ions. The minimum inhibitory concentration IC₅₀ of the tested compounds is illustrated in Fig.

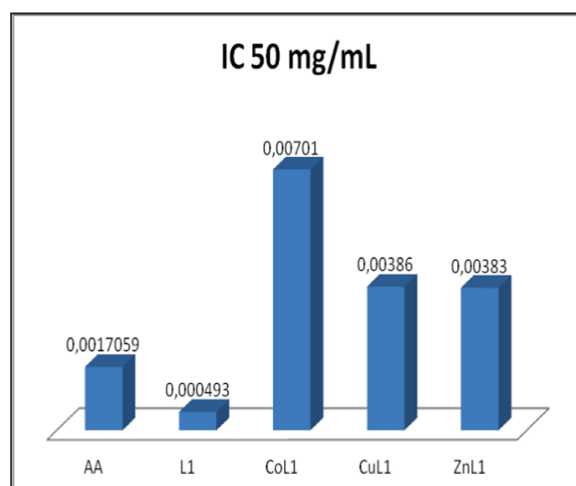
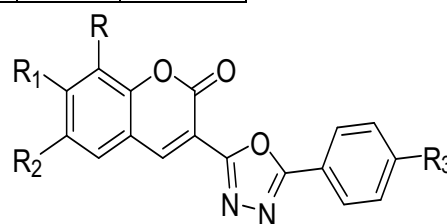


Figure 8 IC₅₀ (mg/mL) values of the antioxidant compounds tested[68]

Vagish Channa Basappa et. Al. [67] synthesized a series of substituted 3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-ones (**98-107**) using substituted aldehydes with analogues of hydrazine hydrates by grinding technique in the presence of iodine which helps in the cyclization process. The structures of the synthesized compounds were elucidated by spectroscopic techniques such as FT-IR, NMR, and LCMS. The antioxidant activity also checks for the synthesized compound (**98-107**) and standards. The general structure of the synthesized compounds are given in the figure 14.

98-107 Series			
(98) R=H	R ₁ =H	R ₂ =Br	R ₃ =H
(99) R=H	R ₁ =H	R ₂ =Cl	R ₃ =H
(100) R=OC ₂ H ₅	R ₁ =H	R ₂ =H	R ₃ =H
(101) R=OCH ₃	R ₁ =H	R ₂ =H	R ₃ =Cl
(102) R=H	R ₁ =OCH ₃	R ₂ =H	R ₃ =Cl
(103) R=OC ₂ H ₅	R ₁ =H	R ₂ =H	R ₃ =Cl
(104) R=H	R ₁ =H	R ₂ =Br	R ₃ =CH ₃
(105) R=H	R ₁ =H	R ₂ =Cl	R ₃ =CH ₃
(106) R=H	R ₁ =OCH ₃	R ₂ =H	R ₃ =CH ₃
(107) R=OC ₂ H ₅	R ₁ =H	R ₂ =H	R ₃ =CH ₃



98-107

The

Figure 9 General structure of Synthesized Coumarin Based Series 7(98-107) antioxidant properties of synthesized oxadiazole compounds were assessed through free radical scavenging assay. The DPPH radical solution, produced in ethanol, changes color to yellow/pale yellow in the presence of antioxidants. The experiment involved mixing a known volume of DPPH radical solution with test samples of varying concentrations, measuring absorbance using UV-Vis spectrophotometer, and calculating the percentage DPPH radical scavenging activity. The experiments were done in triplicate. The percentage DPPH radical scavenging activity was calculated using the equation: % DPPH radical scavenging activity = $[(A_0 - A_1) / A_0] \times 100$

where A_0 is the absorbance of the standard, and A_1 is the absorbance of the test samples at different concentrations. The % inhibition was plotted against concentration, and from the graph, IC_{50} was calculated.

The synthesized compounds 7 (**98-107**) showed good to moderate antioxidant activity compared to standard drugs. Some compounds displayed promising radical scavenging activities, comparable with ascorbic acid, suggesting they might act as lead molecules for DPPH radical scavenging activity. Compound 7c showed the lowest activity, while compounds 7d and 7i showed better DPPH radical scavenging activity.

Table 10 DPPH Scavenging activity of compounds (98-107)

Compound	DPPH radical Scavenging IC_{50} (uM)
98	25.08 ± 0.87
99	26.14 ± 0.93
100	29.33 ± 1.02
101	19.47 ± 1.28
102	18.62 ± 1.46
103	27.51 ± 1.07
104	23.28 ± 0.88
105	22.78 ± 0.98
106	17.19 ± 0.81
107	25.81 ± 1.41
Ascorbic acid	23.80 ± 1.21

Faith Sonmez et. Al.[64] synthesized a novel series of Spiro-isatin based Schiff base compounds and investigate their Antioxidant activity. The synthesized compound general structural formula shown in given figure 15.

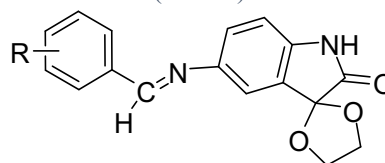


Figure 10 General Structure of Spiro-isatin based Schiff base series

Here R is the different different functional group for series is shown in the table 10. The DPPH method measures antioxidants' ability to sweep free radicals, focusing on compounds with strong hydrogen donor groups. Synthesized compounds showed IC_{50} values ranging from 4.49 μ M to 204.90 μ M, with (**108**) showing stronger antioxidant activity than quercetin. Other compounds showed lower DPPH activity. The antioxidant activity result IC_{50} values given in the table.

Table 11 IC_{50} Values of DPPH Assay of a series compound

Compounds	R	DPPH IC_{50} (uM)
108	H	171.74 ± 1.24
109	3-OH	94.81 ± 0.83
110	2,3-di-OH	4.49 ± 0.45
111	2,5-di-OH	18.65 ± 0.03
112	2,4,6-tri-OH	64.03 ± 0.39
113	4-OCH ₃	145.00 ± 0.25
114	3,4-di-OCH ₃	130.00 ± 0.20
115	2,5-di-OCH ₃	81.78 ± 0.38
116	4-N(CH ₃) ₂	112.33 ± 2.49
117	2-F	125.4 ± 0.63
118	3-F	137.75 ± 2.67
119	4-F	90.15 ± 0.59
120	2-Cl	129.40 ± 0.46
121	3-Cl	178.91 ± 0.37
122	4-Cl	110.95 ± 0.72
123	2-Br	143.90 ± 1.85
124	3-Br	204.90 ± 3.52
125	4-Br	127.16 ± 0.97
126	2-NO ₂	162.91 ± 0.98
127	3-NO ₂	126.62 ± 0.21

128	4-NO ₂	118.86 ± 0.68
Quercetin		8.69 ± 0.24

The DPPH Method is a widely used method for removing free radicals, with antioxidant properties based on the ability to degrade DPPH radicals. Synthesized compounds, with IC₅₀ values ranging from 4.49 uM to 204.90 uM, show higher antioxidant activity.

K. Karrouchi, L. Chemlal et.al.[71] synthesized a novel series of 4-amino-1,2,4-triazole derivatives containing pyrazole (**129-136**). The structure of this series was elucidated by FT-IR, NMR, and mass spectroscopy. All the synthesized compounds were screened for their in vivo analgesic and in vitro antioxidant activities revealing analgesic and antioxidant properties. The general structure of the series compound is given in figure 16.

Figure 11 General structure of a novel series 129-136

The DPPH Activity of synthesized compounds is shown in the table 11. The synthesized compounds 127-134 were tested for their antioxidant activity using the free radical DPPH. This assay was used to test the capacity of the antioxidant compounds as proton radical scavengers or hydrogen donors. The DPPH radical reacts with suitable reducing agents, then electrons become paired off and solution loses colour and the results compared with standard ascorbic acid. All compounds showed excellent antioxidant activity when compared with DPPH Assay of ascorbic acid. The DPPH Assay values are shown in table 11 given below. And here, 133 shows the best antioxidant activity.

Table 12 Antioxidant activity of the synthesized compounds (129-136)

Compoun ds	DPPH Radical Scavenging activity %			
	60 (ug/m L)	125 (ug/m L)	250 (ug/m L)	500 (ug/m L)
129	55.98 ± 0.34	63.52 ± 0.17	71.20 ± 0.34	77.76 ± 0.98
130	49.95 ± 0.22	64.06 ± 1.13	79.87 ± 0.15	86.19 ± 0.40
131	45.03 ± 0.43	61.13 ± 2.19	75.26 ± 0.57	79.56 ± 1.59
132	60.69 ± 0.88	71.47 ± 2.09	78.49 ± 0.58	82.53 ± 0.01

133	37.06 ± 0.10	68.87 ± 0.14	77.28 ± 0.14	81.27 ± 0.71
134	49.31 ± 0.07	78.81 ± 1.43	84.73 ± 0.02	80.03 ± 0.61
135	83.88 ± 0.74	86.20 ± 0.79	86.91 ± 0.20	88.51 ± 0.33
136	56.41 ± 0.80	74.14 ± 0.81	85.71 ± 0.49	87.88 ± 1.58
Ascorbic Acid	95.69 ± 0.10	96.30 ± 0.08	96.34 ± 0.03	96.37 ± 0.12

Hakan Sahal et. Al.[62] synthesized a new four schiff base ligands from aldehyde derivatives and Cu(II) Salts. That ligand structure is confirmed by FT-IR, NMR, TGA etc. The antioxidant activity of the schiff base ligands and their complexes was determined by DPPH Assay. The obtained IC₅₀ value of the DPPH activity for the L¹-Cu complex and L³H ligand were higher antioxidant activity shown compared to other synthesized compounds. The structure of ligand is shown in figure 17 and ligand and their metal complexes antioxidant value is given in the table 12.

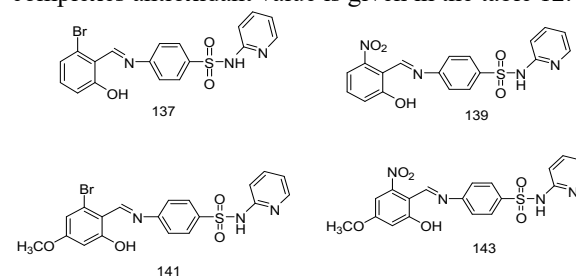


Figure 12 New Synthesized Schiff Base Ligand Structure

The antioxidant activity of extracts was measured using a method involving DPPH free radical scavenging. The color change was measured at 517 nm using a UV visible light spectrophotometer. Standard antioxidants were α-tocopherol and BHT. The inhibition activity was calculated using an equation after 30 minutes of shaking.

$$\text{Inhibition activity, \%} = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100$$

Table 13 Ligand And metal complexes in DPPH Activity

Compounds	DPPH IC ₅₀ uM
(137). L ¹ H	1.264 ± 0.069
(138). L ¹ -Cu	1.469 ± 0.024
(139). L ² H	1.401 ± 0.031
(140). L ² -Cu	1.271 ± 0.023

(141). L ³ H	1.285 ± 0.049
(142). L ³ -Cu	1.237 ± 0.078
(143). L ⁴ H	1.292 ± 0.051
(144). L ⁴ -Cu	1.326 ± 0.044
α -tocophenol	0.113 ± 0.019
^b BHT	0.052 ± 0.090

From The table authors conclude that L¹-Cu Complex showed higher scavenging activity compared to another compound. and L³-Cu exhibited lower value than all the ligand and their complexes. Therefore, said than all compound gives antioxidant activity but ligand 1 with Cu (**138**) gives better antioxidant activity and ligand 3 cu (**142**) complex gives very lower antioxidant activity.

Siham Slassi et. Al. [63] synthesize Four Different Ligands Which associate with an azo group and an imidazole unit and a Schiff base fragment. Then after the author identified the structure of ligand using NMR and mass spectroscopy after that author check antifungal activity and antioxidant activity. Structure of the synthesized ligand is given in the figure 16.

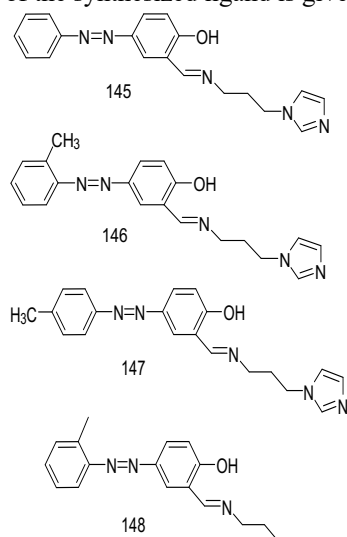


Figure 13 Newly synthesized New Schiff base ligands
The study used DPPH free radical scavenging assays, prepared by dissolving 2 mg DPPH in methanol and mixing with different concentrations of compounds 145-148. The percentage inhibition of DPPH was calculated using the absorbance of the control and the tested sample. IC₅₀ values were calculated by plotting inhibition percentages against sample concentration, with ascorbic acid used as a positive control.

Antioxidants are crucial bioactive species that capture free radicals, causing diseases like cancer. UV-Visible spectroscopy evaluated the antioxidant activity of Schiff bases 145-148 compared to ascorbic acid. Results showed promising activity due to the hydroxyl

group's DPPH scavenging activity. 145-147 ligands, with a methyl group, showed similar inhibitory concentrations and more activity than 148 ligands, suggesting the presence of an imidazole ring significantly contributes to their bioactivity.

Table 14 The antioxidant activity of L1-L4 in DPPH

Compound	Concentration (ug/ml)						
	10	50	100	200	400	600	IC ₅₀
145	39, 12	44, 48	51, 62	64, 28	76, 29	88, 63	95, 98
146	41, 07	43, 83	51, 13	63, 14	77, 75	91, 07	94, 48
147	35, 87	46, 1	51, 13	64, 61	83, 44	91, 55	96, 01
148	33, 60	37, 01	49, 83	71, 42	83, 27	88, 31	116, 39
Ascorbic acid	49, 18	52, 07	55, 31	65, 04	82, 34	98, 73	25, 88

The ABTS Assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay is a popular method for determining the radical scavenging activity of substances.[73–88] The assay's results, which are usually given as the half maximum effective concentration (IC₅₀ or EC₅₀), show how much of the compound is needed to lower the amount of ABTS•⁺ radical by 50%. [76,88] It is often performed as part of the Trolox equivalent antioxidant capability (TEAC) test.[85]

I. ABTS Assay Methodology :

The ABTS assay analyzes an antioxidant's ability to scavenge the free ABTS•⁺ radical. [76,88]

Radical Formation:

The ABTS•⁺ radical is made by mixing ABTS salt or solution (usually 2 mM, 4 mM, or 7.4 mM) with a strong oxidizing agent, which is usually potassium persulfate (K₂S₂O₈) (1.41 mM to 2.6 mM).[73,75,77,82,84] This combination is normally left to react in the dark for a long time, such 6, 12, 16, or 24 hours. [73,77,82,84,87]

Measurement:

The blue-green ABTS•⁺ solution[76,88] that comes out is diluted to a certain absorbance level (for example, 0.706 ± 0.001 units).[74,84] The

sample/standard and diluted ABTS•+ solution are mixed and allowed to react for a certain amount of time (usually 5, 10, 30, or 2 hours).[75,77,81,82,86,87] The change in absorbance is measured at 734 nm. [75,77,79,86,88]

II. Results of Comparative and Structural Activity

Numerous investigations have revealed that antioxidant properties differ according to molecule structure, namely the presence and positioning of hydroxyl groups and their complexation with metal ions. [75]

Effect of Hydroxyl Groups (Coumarins):

In a study of coumarin-hydroxytyrosol conjugates (14a–t), the addition of the hydroxyl group enhanced ABTS•+ scavenging activity.[75] Compound 14q (7,8-OH substitution) exhibited exceptional activity with an IC₅₀ value of $30.31 \pm 0.97 \mu\text{M}$. [75]

For traditional coumarins, HMD (EC₅₀ of $39.98 \mu\text{M}$) and DHC (EC₅₀ of $30.83 \mu\text{M}$) exhibited superior radical scavenging activity compared to the Trolox standard. In a study that looked at hydroxylated coumarin-based thiosemicarbazones, compounds T5 and T6, both of which have a catechol-like structure, showed strong antioxidant activity in the ABTS test. [85]

Schiff Base Derivatives vs. Intermediates/Standards:

Schiff base derivatives frequently exhibit greater antioxidant activity compared to their respective ester derivatives or precursors.[73,83] For instance, in a particular investigation, aryl sulfonate derivative Schiff bases exhibited superior ABTS antioxidant activity compared to aryl sulfonates.[83] In the examination of fluorinated sulfonate derivatives, all evaluated compounds (1–28) exhibited extremely low antioxidant activity ($>1000 \mu\text{M}$) in the ABTS assay. [82]

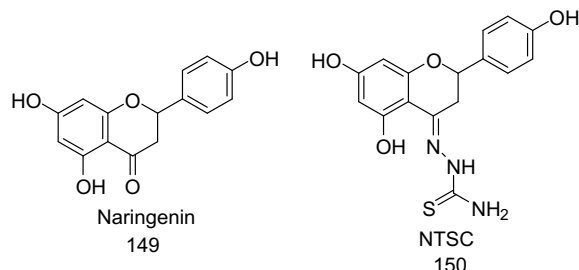
Effect of Solvents:

The solvent used can have a big impact on how well ABTS radicals are scavenged. For naringenin compounds, DMSO exhibited significantly superior antioxidant properties compared to methanol. [81]

TABLE. ABTS IC₅₀/SC₅₀ Values The most potent substances exhibited action that was comparable to or surpassed established benchmarks (Trolox, BHA, Ascorbic acid).

Compound Type (Reference)	Compound	IC ₅₀ / EC ₅₀ Value	Comparison/Notes
Standards	Trolox	$4.13 \pm 0.18 \text{ g/mL}$ (SC ₅₀)	Most active standard in one study [87][76]
	BHA	$7.14 \pm 0.09 \text{ g/mL}$	Standard activity [88]
	Ascorbic acid (AA)	$1.22 \pm 0.07 \text{ g/mL}$	Standard activity [88]

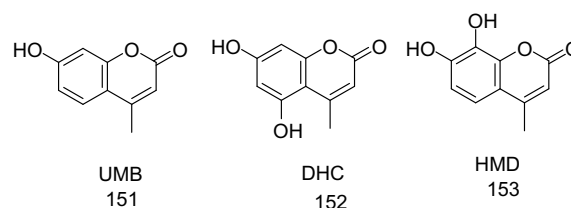
Katarzyna Brodowska et. Al.[75] synthesized and characterized Schiff base derived from naringenin (NTSC) and its complex with Cu(II) ([Cu(H₃L)(OAc)]H₂O, Cu(II) NTSC). Antioxidant studies confirmed better radical scavenging activity of both NTSC and its Cu(II) complex compared to naringenin. The TEAC values from the ABTS assay demonstrate that the highest antioxidant (radical scavenging) capacities are observed at the highest concentration tested ($100 \mu\text{M}$) for all compounds. Free naringenin in DMSO had a TEAC(ABTS) value of $71.25 \pm 12.03 \mu\text{mol/L}$, which is higher than naringenin in methanol ($24.29 \pm 3.36 \mu\text{mol/L}$). Adding excess copper(II) to naringenin increased TEAC values in DMSO ($40.18 \pm 2.56 \mu\text{mol/L}$) but decreased them in methanol ($15.96 \pm 2.26 \mu\text{mol/L}$). Cu(II)–NTSC complex exhibited the highest antioxidant capacity in the ABTS assay ($99.43 \pm 0.45 \mu\text{mol/L}$ at $100 \mu\text{M}$ concentration). IC₅₀ values were also calculated: the best ABTS radical cation inhibitors (smallest IC₅₀) were the synthesized copper complex Cu(II)–NTSC (0.17 mM), NTSC/Cu = 1/1 (0.19 mM), NTSC/Cu = 1/2 (0.20 mM), and NTSC (0.21 mM). The weakest antioxidant capacity in the ABTS assay was found for naringenin complexed with copper(II) in methanol (1/2, v/v ratio; IC₅₀ = $6.33 \pm 0.01 \text{ mM}$). The solvent had a significant effect: DMSO generally resulted in higher antioxidant capacities compared to methanol. The authors attribute the strong radical scavenging activity, especially for the metal complexes, to the presence of thiazole and phenolic moieties.

**Table: ABTS (TEAC) and IC50 Values (Key Highlights)**

Compound	TEAC(ABTS) at 100 μ M (μ mol/L)	IC50 (mM)
(149) Naringenin in DMSO	71.25 $\pm$ 12.03	0.63 $\pm$ 0.02
(149) Naringenin in MeOH	24.29 $\pm$ 3.36	2.59 $\pm$ 0.01
(149-a) Nar/Cu = 1/1 in DMSO	29.84 $\pm$ 1.79	1.83 $\pm$ 0.05
(149-a) Nar/Cu = 1/1 in MeOH	15.52 $\pm$ 1.54	3.54 $\pm$ 0.10
(149-a) Nar/Cu = 1/2 in DMSO	40.18 $\pm$ 2.56	1.29 $\pm$ 0.02
(149-a) Nar/Cu = 1/2 in MeOH	15.96 $\pm$ 2.26	6.33 $\pm$ 0.01
(150) NTSC	98.08 $\pm$ 1.40	0.21 $\pm$ 0.01
(150-a) Cu(II)-NTSC complex	99.43 $\pm$ 0.45	0.17 $\pm$ 0.00
(150-a) NTSC/Cu = 1/1	98.52 $\pm$ 0.30	0.19 $\pm$ 0.00
(150-a) NTSC/Cu = 1/2	97.30 $\pm$ 1.20	0.20 $\pm$ 0.00

Alan Couttolenc [79] synthesized and studied the primary and secondary antioxidant activities of three hydroxy-4-methylcoumarins. Cu(II)-NTSC complex (IC₅₀ = 0.17 mM, TEAC = 99.43 μ mol/L at 100 μ M). Antioxidant capacity was greater in DMSO than methanol. Schiff base formation and Cu(II) complexation significantly enhance naringenin's ABTS radical scavenging ability. Both the structure of the compound (presence of thiosemicarbazone and phenolic groups) and the solvent (DMSO over MeOH)

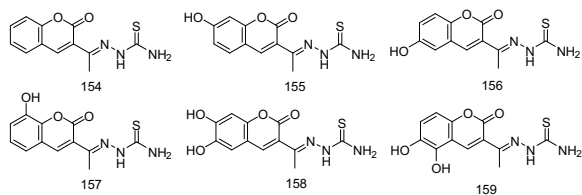
played significant roles in antioxidant efficacy. The study concludes that both NTSC and its copper complexes provide **better ABTS radical scavenging than naringenin alone**, demonstrating their potent antioxidant potential. The number and position of hydroxyl groups in the coumarin structure influence the ABTS radical scavenging activity strongly. DHC's higher activity over HMD and Trolox is attributed to its structure, with catechol groups suggested as the key feature. The study highlights the superior radical scavenging potential of HMD and especially DHC compared to standard antioxidants like Trolox in the ABTS assay. Experimental findings are supported by theoretical calculations on free radical scavenging mechanisms and kinetics.



Compound	ABTS (EC50, μ M)	Relative Activity (Lower is better)
UMB (151)	1,150 $\pm$ 60	Low
DHC (152)	30.83 $\pm$ 5.06	Highest
HMD (153)	39.98 $\pm$ 4.14	High
Trolox	83.50 $\pm$ 3.07	Moderate (reference)

Sebastiano Masuri et. Al. [84] synthesised o new bishydroxylated and two new monohydroxylated derivatives of (1E)-2-(1-(2-oxo-2H-chromen-3-yl)ethylidene)hydrazine-1-carbothioamide and evaluated the antioxidant and tyrosinase inhibition abilitythe antioxidant activity appears to be influenced according to the number and substitution pattern of the hydroxyl groups. These results indicate that (1E)-2-(1-(2-oxo-2H-chromen-3-yl)ethylidene)hydrazine-1-carbothioamide and its hydroxylated derivatives are promising molecules for further drug development studies

Dihydroxylated coumarin thiosemicarbazones (158 and 159) are superior to Trolox in ABTS radical scavenging activity. Antioxidant potential is highly structure-dependent, with the catechol motif being the most advantageous for free radical scavenging by this method.



• **Table 1 summarizes key ABTS EC₅₀ (μM) values:**

Compound	ABTS EC ₅₀ (μM)	Interpretation
154	72 ± 6	Weak activity
155	25.7 ± 0.4	Moderate activity
156	36 ± 1	Moderate activity
157	21.8 ± 0.8	Moderate activity
158	9 ± 1	High (strongest, dihydroxy motif)
159	8.8 ± 0.4	High (strongest, dihydroxy motif)
Trolox	13 ± 1	Reference standard

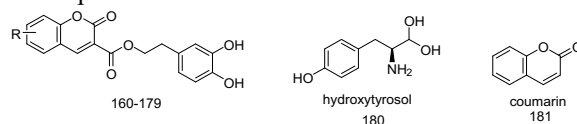
Wen-Bo Li et. Al.[83] synthesized hydroxytyrosol and coumarin based compounds and evaluated for their free radical scavenging. **Most compounds (160-179) showed superior ABTS+• scavenging activity** (IC₅₀: 30.31–164.22μM) versus hydroxytyrosol (170.47μM) and coumarin (>10,000μM).

• IC₅₀ values of select examples (lower = stronger)

- 176- (7,8-dihydroxy): **30.31±0.97μM**
- 163- (6-hydroxy): 78.88±4.23μM
- 172- (8-hydroxy): 105.40±8.24μM
- 160- (H): 108.26±3.25μM
- BHT (standard): 127.07±0.40μM
- Hydroxytyrosol: 170.47±5.28μM

Substitution of a hydroxyl group on the coumarin ring improved ABTS scavenging (163, 168, 172). Most electron-donating groups (Me, OMe) decreased activity. Compounds with multiple hydroxyl substituents, especially **compound 176 (7,8-dihydroxy substitution)**, were by far most active. 6Fold improvement (relative to BHT) for 176 in ABTS+• scavenging: **4.19× stronger**. Hydroxyl group presence/number is crucial: more and ortho-positioned hydroxyls (e.g., 7,8-dihydroxy) dramatically boost ABTS+• scavenging. Electron-withdrawing groups (OH, Cl, NO₂) increased antioxidant activity; electron-donating groups (Me, OMe) diminished it. Compound **176 (7,8-dihydroxy)** stood out for potent ABTS radical scavenging, low toxicity, enhanced cell

viability, and increased cellular antioxidant enzyme (SOD, GSH) levels. Coumarin–hydroxytyrosol conjugates—especially with strategic hydroxylation—exhibited much stronger ABTS+• scavenging than standard antioxidants. The **ABTS assay confirmed compound 176 as a particularly potent and promising antioxidant candidate** for further development.



1	R=R	1	R=R ₁	1	R=R ₁	1	R=6-
6	₁ =H	6	=6-	6	=6-Cl	6	OH
0		1	CH ₃	2		3	R ₁ =6-
							OBn
1	R=R	1	R=R ₁	1	R=R ₁	1	R=R ₁
6	₁ =6-	6	=6-	6	=7-	6	=7-Cl
4	OC	5	NO ₂	6	CH ₃	7	
	H ₃						
1	R=7	1	R=R ₁	1	R=R ₁	1	R=R ₁
6	-OH	6	=7-	7	=8-	7	=8-Cl
8	R ₁ =	9	OCH ₃	0	CH ₃	1	
	7-						
	OBn						
1	R=8	1	R=R ₁	1	R=R ₁	1	R=R ₁
7	-OH	7	=8-	7	=5,7-	7	=6,8-
2	R ₁ =	3	OCH ₃	4	OCH ₃	5	C(CH
	8-						₃) ₃
	OBn						
1	R=7,	1	R=R ₁	1	R=R ₁	1	R=R ₁
7	8-	7	=7,8-	7	=6,8-	7	=H
6	OH	7	OCH ₃	8	Cl	9	
	R ₁ =						
	7,8-						
	OBn						

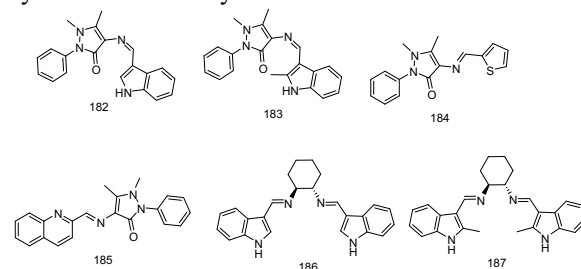
Summary Table: ABTS+• Scavenging Activity (Selected Compounds)

Compound	Substituent(s)	ABTS IC ₅₀ (μM)	Relative to BHT
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176	7,8-dihydroxy	30.31±0.9 7	4.19×
163	6-hydroxy	78.88±4.2 3	1.61×
172	8-hydroxy	105.40±8. 24	1.21×
160	H	108.26±3. 25	1.17×
161	6-methyl	137.46±4. 60	0.92×
177	7,8-dimethoxy	194.61±1. 81	0.65×
180	Hydroxytyrosol	170.47±5. 28	0.75×
181	Coumarin	>10,000	—
BHT	—	127.07±0. 40	1.00×

Hakan Kizilkaya et Al.[82] synthesized schiff base by treatment of 4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one. **Compound 152** showed the best ABTS scavenging among the new Schiff bases, with an IC₅₀ only about 1.5× weaker than BHT, and notably more potent than most synthesized analogs. **Compound 183** (a new analog) also revealed good ABTS scavenging effect, close to compound 182. **Compounds 184 (thiophenyl analog) and 185 (quinolyl analog)** demonstrated moderate ABTS scavenging. **Compounds 186 and 187** (cyclohexane diamine bis-indolyl derivatives) had weaker ABTS activity (higher IC₅₀s). **Standards:** BHT, BHA, and Trolox had lower IC₅₀s (stronger activities) than all synthesized compounds, but compound 182's value was fairly close to BHT. The indole-based Schiff bases, especially compound 3, exhibit notably higher activity in the ABTS assay. The results suggest that substituents on the indole or heteroaromatic significantly influence radical scavenging. Although none of the synthesized Schiff bases surpassed the standard antioxidants in potency, their relatively low IC₅₀s indicate promising activity and potential for development as synthetic antioxidants. The study highlighted compounds 182, 183, 184 as promising antioxidants based on their performance in the ABTS assay, supporting their potential as synthetic antioxidant agents. The ABTS assay was central to determining the free radical scavenging abilities and

structure–activity relationships among these newly synthesized heterocyclic Schiff bases.

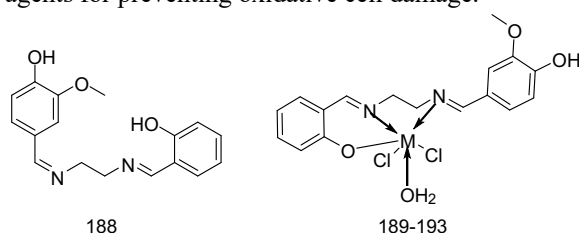


Compound	ABTS IC ₅₀ (μg/ml)	Interpretation
182	15.09 ± 0.15	Outstanding ABTS scavenging
183	17.89 ± 0.10	Good ABTS scavenging
184	32.03 ± 0.16	Moderate ABTS scavenging
185	27.97 ± 0.15	Moderate ABTS scavenging
186	45.53 ± 0.17	Weaker ABTS scavenging
187	44.23 ± 0.28	Weaker ABTS scavenging
BHT	10.34 ± 0.07	Standard Antioxidant
BHA	7.14 ± 0.09	Strong Standard Antioxidant
Trolox	6.92 ± 0.04	Reference Standard

Ikechukwu P. Ejidike et. Al.[80] synthesized 4-((Z)-((2-((E)-((2-hydroxyphenyl)methylidene)amino)ethyl)imino)methyl)-2-methoxyphenol based Schiff base. Trivalent metal complexes of [M(L)(H₂O)_nCl_y] obtained from the interaction of metal³⁺ ion salts with organic N, N, O-Schiff base (HL). All metal complexes (189-193) showed improved (lower) IC₅₀ values compared to the free ligand 188, indicating enhanced ABTS radical scavenging activity due to metal coordination. Among the synthesized complexes, **193 (Ti(III))** exhibited the best ABTS scavenging activity (lowest IC₅₀: 8.70 ± 2.78 μM), closely followed by **190 (Ru(III))** (8.81 ±

3.43 μ M). However, the standards Gallic acid and Rutin showed much lower IC₅₀ values, indicating higher scavenging activity than the synthesized compounds. The ABTS radical scavenging activity ranking: **Gallic acid > Rutin > 193 > 190 > 189 > 191 > 192 > 188**.

- The results indicate that the metal complexes, especially 193 and 190, have potential as therapeutic agents for preventing oxidative cell damage.

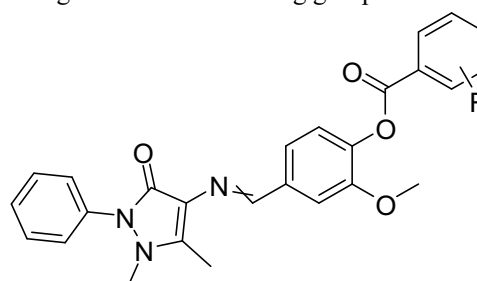


Data Table (As Reported in the Paper)

Compound	ABTS IC ₅₀ ± SEM (μ M)	Correlation Coefficient (R ²)
188	15.32 ± 5.34	0.972
(189) C1 (Cr)	10.49 ± 3.24	0.939
(190) C2 (Ru)	8.81 ± 3.43	0.805
(191) C3 (Fe)	12.02 ± 4.60	0.977
(192) C4 (Al)	15.01 ± 4.98	0.864
(193) C5 (Ti)	8.70 ± 2.78	0.976
Gallic Acid	1.22 ± 1.08	0.948
Rutin	2.86 ± 0.92	0.962

Ercan Çınar et. Al. [73] synthesized pyrazolone-based Schiff base derivatives. **Schiff base derivatives 194-198** Generally showed improved ABTS activity **195**, Displayed the best activity among synthesized compounds (IC₅₀ = 17.95±0.47 μ M), comparable to the standard BHA (IC₅₀ = 17.59±0.10 μ M). **Order of activity among studied Schiff bases:** α -TOC > BHT > BHA > 195 > 194 > 196 > 198 > 197. 195's activity overlapped BHA and was only slightly less potent than BHT and α -TOC; others (194, 196, 198) were distinctly weaker. Introduction of electron-withdrawing nitro groups (as in 195) greatly enhanced ABTS scavenging activity. **195** was the most promising candidate for further antioxidant studies but did not outperform standard antioxidants. These

results suggest the pyrazolone-containing Schiff bases are worth exploring as moderate ABTS-type antioxidants, especially when functionalized with strong electron-withdrawing groups.



194-198

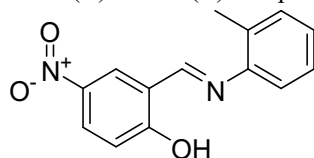
Entry	R ₂
194	H
195	2-NO ₂
196	3-NO ₂
197	4-NO ₂
198	3,5-NO ₂

Tabulated ABTS Radical Scavenging Activity

Compound	ABTS IC ₅₀ (μ M) ± SD	Note
194	22.49 ± 0.59	Moderate
195	17.95 ± 0.47	Highest among compounds
196	28.98 ± 0.52	Moderate
197	>1,000	No activity
198	87.16 ± 0.75	Weak
BHA	17.59 ± 0.10	Standard
BHT	13.25 ± 0.27	Standard
α -TOC	9.74 ± 0.42	Standard

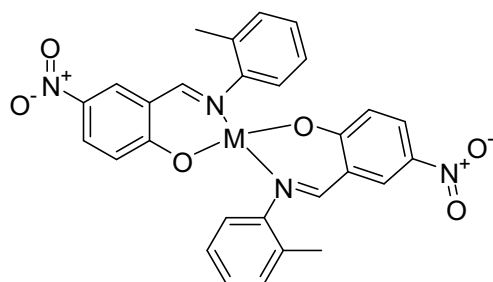
Halil Gökce et. Al [81] synthesized Schiff base, (E)-4-nitro-2-[(o-tolylimino)methyl]phenol and its Co(II) and Pd(II) complexes were evaluated using the ABTS assay alongside other methods. The Pd(II) and Co(II) complexes both showed strong ABTS radical scavenging activities, with IC₅₀ values very close to the standard antioxidants BHA and ascorbic acid. The text states: "The compounds demonstrated comparable activity to standard antioxidants, particularly in the CUPRAC and ABTS tests. ... The ABTS+ test results mirrored these findings. Notably, metal complexes demonstrated stronger radical scavenging abilities compared to ligands and showed activities similar to BHA and AA antioxidants. **Pd(II) and Co(II)**

complexes exhibited significant ABTS radical scavenging activity, approaching the efficacy of standard antioxidants. Metal complexation enhanced the ABTS activity compared to the ligand alone, but all compounds (L, Co(II), Pd(II)) had IC₅₀s in the 1.22–1.28 µg/mL range. The ABTS assay confirms the strong antioxidant potential of the synthesized Pd(II) and Co(II) complexes.



(E)-4-nitro-2-((o-tolylimino)methyl)phenol

199

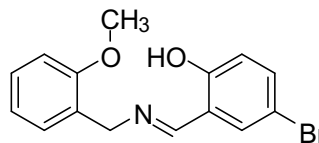


200-201

Sample	ABTS activity (IC ₅₀)
199	1.28 ± 0.01
200 (Co(II) complex)	1.25 ± 0.05
201 (Pd(II) complex)	1.25 ± 0.05
BHA	1.23 ± 0.02
AA (ascorbic acid)	1.22 ± 0.07

(All IC₅₀ values are presumably in µg/mL.) Nada Hiba Boukoucha et al.[74] synthesized ligand using methoxy substituted amine and 5-bromo salicylaldehydic by condensation reaction in methanolic medium. The ABTS radical scavenging activities were compared with standard antioxidants: BHA, BHT, ascorbic acid, quercetin, and Trolox. The synthesized Schiff base 202 showed **moderate ABTS radical scavenging activity** compared to the standards. Lower IC₅₀ values reflect higher antioxidant activity; thus, the standards showed more potent ABTS scavenging than compound. The ABTS scavenging ability ranked: **BHA > Trolox > BHT > Ascorbic acid > Quercetin > 202**. The paper also correlates the experimental ABTS scavenging capacity with theoretical studies (DFT parameters), noting moderate efficacy of the ligand compared to antioxidant standards. The **ligand (1) effectively scavenged ABTS radicals**, though less efficiently than the tested reference antioxidants, with an IC₅₀ of

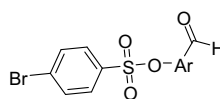
17.22 µM versus ≤5 µM for standards. **ABTS scavenging was the most sensitive among the three in vitro antioxidant assays** performed (ABTS, FRAP, SNP), as the ligand demonstrated its lowest IC₅₀ in the ABTS test. Experimental results were consistent with computed antioxidant descriptors supporting the observed activity efficacy.



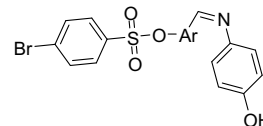
202

Compound	ABTS IC ₅₀ ± SD (µM)
202	17.22 ± 0.03
BHA	2.26 ± 0.01
BHT	2.98 ± 0.01
Ascorbic Acid	3.37 ± 0.02
Quercetin	4.86 ± 0.27
Trolox	8.01

- (n=3, mean ± SD)1.



203-209



210-216

Entry	Ar
203 210	2-OH-C ₆ H ₄ -
204 211	4-OH-C ₆ H ₄ -
205 212	4-OH-3-OCH ₃ -C ₆ H ₃ -
206 213	2-OH-4-N(C ₂ H ₅) ₂ -C ₆ H ₃ -
207 214	3-OH-C ₆ H ₄ -
208 215	2-OH-3-OCH ₃ -C ₆ H ₃ -
209 216	3-OH-3-OCH ₃ -C ₆ H ₃ -

Results: ABTS Scavenging Activity (IC₅₀ values, µM)

From Table 2 in the article:

Compound	ABTS IC ₅₀ ± SD (µM)
203	10.16 ± 0.20
204	9.65 ± 0.14
205	9.10 ± 0.21
206	9.55 ± 0.16
207	8.32 ± 0.35
208	12.14 ± 0.29
209	9.54 ± 0.14

210-216 (precursors)	450.73 $\pm$ 3.45 to 584.36 $\pm$ 3.29
Standard Antioxidants:	
BHA	8.53 $\pm$ 0.24
α-TOC	15.41 $\pm$ 0.27
BHT	11.69 $\pm$ 0.17

- All values are means of three parallel measurements $\pm$ SD.

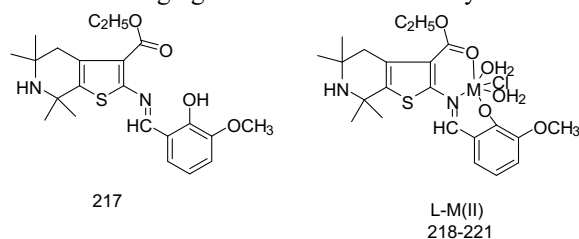
Ercan Çınar[77] synthesized Schiff base derivatives (203-209) demonstrated much stronger ABTS radical scavenging activity than their aryl sulfonate precursors (210-216). The paper concludes that these new Schiff base derivatives demonstrate **strong antioxidant potential in the ABTS assay**, further supported by their performance in DPPH and CUPRAC antioxidant assays. In the ABTS method, the molecule 3-((Z)-(4-hydroxyphenylimino)methyl)phenyl-4-bromobenzenesulfonate (207) showed good activity with 8.32 $\pm$ 0.35 μ M compared to the standard antioxidant BHA (8.53 $\pm$ 0.24 μ M). The tested Schiff base-derived molecules showed higher antioxidant activities than aryl sulphonates. All target molecules showed high antioxidant activities compared to α -TOC. The ABTS assay confirmed **high antioxidant capacity** for the newly synthesized Schiff base derivatives, especially for 207, whose activity is on par with standard antioxidants. Schiff bases containing aryl sulfonate groups may serve as promising antioxidant agents for further biological study and development. The **most active compound in the ABTS assay was 207 (8.32 $\pm$ 0.35 μ M)**, which outperformed or was comparable to the reference antioxidant BHA (8.53 $\pm$ 0.24 μ M). Other Schiff base derivatives showed comparable activities, most notably 205, 204, 206, and 209 (all around 9-10 μ M). The aryl sulfonate precursors (210-216) displayed much weaker activity, with IC₅₀ values in the hundreds of μ M range. All target molecules (except 208) were more active than α -tocopherol (α -TOC, 15.41 $\pm$ 0.27 μ M) and BHT (11.69 $\pm$ 0.17 μ M). **Schiff base derivatives (203-209)** are significantly more potent ABTS radical scavengers than their starting aryl sulfonates. **207** demonstrated the highest activity (lowest IC₅₀) in the ABTS assay, indicating it is the most efficient radical scavenger among the test set. All target molecules (except 208) showed ABTS scavenging comparable to or better than typical antioxidants like BHT and α -TOC, and similar to BHA.

Table for ABTS Activity

Compound/Class	ABTS IC ₅₀ (μ M)	Notes
207 (Schiff base derivative)	8.32 $\pm$ 0.35	Most active in ABTS assay
203, 204, 205, 206, 209	9.10-10.16	Comparable to BHA, BHT
208	12.14 $\pm$ 0.29	Lower activity than other A-series
210-216 (precursors)	450.73-584.36	Much weaker than A1-A7
BHA (reference)	8.53 $\pm$ 0.24	Standard antioxidant
BHT (reference)	11.69 $\pm$ 0.17	Standard antioxidant
α -TOC (reference)	15.41 $\pm$ 0.27	Standard antioxidant

Nevin Turan et al. [88]. Synthesized Schiff base and among the metal complexes, 218 (**L-Ru(II)**) showed the strongest ABTS radical scavenging activity (lowest IC₅₀), even more potent than the standards. 220 (L-Ni(II)) (74.1 μ M) and 221 (L-Co(II)) (102.3 μ M) showed moderate activity, close to ascorbic acid and α -tocopherol. The Schiff base ligand and 219 (L-Pd(II)) exhibited poor activity (high IC₅₀ values above 250 μ M). The 218 (**L-Ru(II)**) complex displayed the most effective ABTS radical scavenging (lowest IC₅₀) among all tested compounds and standards. 221 L-Co(II) and 220 (L-Ni(II)) complexes demonstrated moderate ABTS activity (IC₅₀: 74-102 μ M), somewhat close to that of BHA, BHT, ascorbic acid, and α -tocopherol. Schiff base ligand and 219 (L-Pd(II)) complex were the least effective in the ABTS assay. In both ABTS and DPPH radical scavenging methods, the data indicated that the Ru(II) complex had strong antioxidant properties, while the ligand and other complexes were much less efficient. According to the ABTS cation radical scavenging activity method, all of the complexes except the 219 (L-Pd(II)) complex were detected to have effective radical scavenging antioxidant activities. The lowest IC₅₀ value of the 218 (**L-Ru(II)**) complex (16.9 $\pm$ 2.4 μ M) was obtained among the complexes and standards. Also, the 220 (L-Ni(II)) complex (74.1 $\pm$ 6.4 μ M) and the 221 L-Co(II) complex (102.3 $\pm$ 4.0 μ M) were detected to have good ABTS cation radical scavenging activities which were

close to the IC₅₀ values of the standards. However, the 219 (L-Pd(II)) complex ($260.9 \pm 12.8 \mu\text{M}$) and the Schiff base ($252.1 \pm 20.2 \mu\text{M}$) were found to have poor radical scavenging effect in the ABTS assay.



Results: ABTS Scavenging Activity (IC₅₀ values, μM)

(Table 2, n=3, mean $\pm$ SD)

Compound/Standard	ABTS IC ₅₀ (μM)
BHA	55.5 ± 11.1
BHT	25.8 ± 2.7
Ascorbic acid	75.4 ± 5.1
α -Tocopherol	67.3 ± 10.3
Schiff base (217)	252.1 ± 20.2
218 (L-Ru(II))	16.9 ± 2.4
219 (L-Pd(II))	260.9 ± 12.8
220 (L-Ni(II))	74.1 ± 6.4
221 (L-Co(II))	102.3 ± 4.0

Ahmet Savcı et. AL.[87] obtained schiff base from the condensation reaction of benzene-1,2-diamine and 5-fluoropyrimidine 2,4(1H,3H)-dione (5-FU) and Metal(II) complexes were synthesized with Fe(II), Co(II) and Ni(II) chloride salts. Lower IC₅₀ values indicate stronger ABTS radical scavenging. Among the new compounds, the 225: **Ni(II) complex showed the strongest activity (lowest IC₅₀)**, nearly matching the standard antioxidants BHA and BHT. All three metal complexes performed better than the parent ligand 222 or 5-FU. **5-FU** (the chemotherapy drug) exhibited the weakest ABTS scavenging in this set. The % scavenging of ABTS radicals at 20 $\mu\text{g/mL}$ for each compound was recorded as:

- BHT (85.16%) > BHA (84.70%) > 225: Ni(II) complex (81.85%) > 223: Fe(II) complex (70.43%) > 224: Co(II) complex (62.78%) > 222 (60.50%) > 5-FU (34.82%).

Metal complexation notably enhanced the ABTS scavenging activity over the ligand alone, with Ni(II) complex especially effective, approaching the potency of standard antioxidants. **The synthesized metal complexes (Ni, Fe, Co) all outperformed the clinical drug 5-FU** in the ABTS assay. The ABTS assay

results indicate that these new metal-Schiff base compounds, particularly the Ni(II) derivative, are promising candidates for antioxidant applications due to their high radical scavenging capacity.

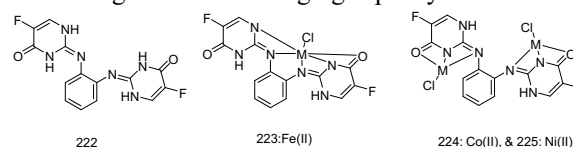


Table 1 in the paper reports ABTS radical scavenging IC₅₀ values ($\mu\text{g/mL}$) for each compound:

Compound	ABTS IC ₅₀ ($\mu\text{g/mL}$)
222	84.5 ± 11.82
223: Fe(II) complex	50.97 ± 1.13
224: Co(II) complex	54.76 ± 2.50
225: Ni(II) complex	54.53 ± 2.49
5-FU (the chemotherapy drug)	116.28 ± 0.16
BHA (standard)	43.5 ± 0.65
BHT (standard)	43.68 ± 0.64

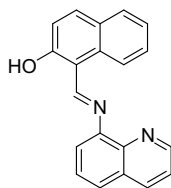
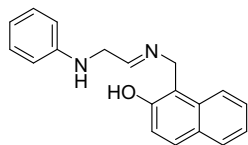
Ianka J. Nunes et AL.[77] synthesized Naphthol-imine compounds containing N-phenylethylenediamine (1) and quinoline amine (2) moieties for scavenging free radicals. Compound 226 demonstrated ABTS⁺ radical scavenging with an IC₅₀ of $17.4 \pm 0.9 \mu\text{M}$.

- **Trolox (positive control)** had an IC₅₀ of $10.8 \pm 0.2 \mu\text{M}$ (max inhibition 100%).
- **Compound 227** showed limited or no significant ABTS⁺ scavenging activity; at 200 μM , I_{max} was only $31.0 \pm 1.3\%$.
- Table: % of control absorbance at 10 and 20 μM (from Table 6)

At 20 μM : 226 inhibited 43% of the ABTS⁺ radical vs. 77% for Trolox, while 227's inhibition was only 9%. At the highest tested dose (100 μM), 226 nearly maximized scavenging (~98%), similar to Trolox. 227 consistently showed much lower inhibition, signifying weaker activity.

226 is a moderately effective ABTS scavenger, with potency similar to Trolox but requiring higher concentration. 227 was essentially inactive in the ABTS⁺ assay at all tested doses. The difference in activity is attributed to structural features (substantially discussed in the SAR section). ABTS scavenging by 1a (reference compound) was even stronger (IC₅₀ = 7.5 μM). **The ABTS assay was more sensitive than the DPPH test for these Schiff bases—only 226 showed radical scavenging at all.** The paper confirmed ABTS radical scavenging for

226 ($IC_{50} = 17.4\mu M$), with **Trolox** at **10.8 μM** as reference. **227** did not show substantial ABTS^{•+} scavenging. **ABTS was a more effective test than DPPH** for distinguishing the antioxidant activity of these naphthol-imine Schiff bases.



226						227	
Compound	10 μM	20 μM	50 μM	100 μM	200 μM	IC ₅₀ (μM)	IC ₅₀ (μM)
Trolox	55.8%	23.1%	0.0%	0.0%	0.0%	10.0	10.8
226	77.6%	43.0%	11.9%	2.1%	2.1%	97.9	17.4
227	91.1%	90.7%	83.2%	81.2%	69.0%	31.0	- (inactive)

CONCLUSION

Both DPPH and ABTS radical-scavenging assays are well-established, reproducible methods for evaluating the antioxidant activity of Schiff base ligands and their metal complexes. DPPH protocols typically involve measuring absorbance at 517 nm after 30 min incubation in methanol or ethanol, while ABTS assays generate the ABTS^{•+} radical cation via persulfate oxidation and measure absorbance at 734 nm.

Reporting IC_{50} values in both assays enables direct comparison across diverse structural classes and reference antioxidants (ascorbic acid, BHT, BHA, Trolox, quercetin).

A consistent finding across both assays is that coordination of Schiff base ligands with transition metals (Ru(II), Ni(II), Co(II), Zn(II), Cu(II)) significantly improves radical-scavenging activity relative to free ligands or precursors. Several metal complexes achieved IC_{50} values lower than or comparable to standard antioxidants in both DPPH and ABTS assays, indicating that metal coordination enhances electron donation, charge delocalization, and radical stabilization.

Ligand architecture critically influences antioxidant potency in both assays. Electron-donating substituents ($-OH$, $-OCH_3$), ortho-hydroxy arrangements, conjugated imine ($-CH=N-$) systems, and heterocyclic rings (thiazole, pyridine, oxadiazole) consistently lower IC_{50} values. Phenolic Schiff bases with multiple hydroxyl groups exhibit enhanced activity through HAT, SET, and SPLET mechanisms. Aryl sulfonate-derived and methoxy-substituted derivatives often outperformed their starting materials and, in some cases, surpassed conventional standards. DPPH and ABTS assays provide complementary information. ABTS frequently demonstrates greater sensitivity, particularly for compounds with specific radical-scavenging mechanisms; several derivatives inactive or weak in DPPH showed moderate-to-strong activity in ABTS. Solvent effects are more pronounced in DPPH (favoring HAT in non-polar media and SPLET in polar protic solvents), whereas ABTS operates primarily in aqueous or hydroalcoholic media, better mimicking physiological conditions. Employing both assays yields a more comprehensive antioxidant profile.

Together, these findings validate DPPH and ABTS assays as rigorous, complementary tools for antioxidant discovery and structure–activity relationship (SAR) studies. Metal complexation, rational ligand design, and systematic comparison to established standards have advanced the development of highly efficient synthetic antioxidants. Nevertheless, further mechanistic investigations, standardized assay conditions, toxicity profiling, cellular assays, and in vivo studies are essential to confirm the biomedical potential of Schiff base derivatives and support their translation into therapeutic agents.

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