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Heterocyclic Schiff Bases and Their Antioxidant Assay: A Review

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Abstract

Heterocyclic Schiff bases are compounds formed by the condensation of heterocyclic amines with aldehydes or ketones, featuring an imine group (-CH=N-). These molecules have gained attention for their potential as antioxidants, which help combat oxidative stress-related diseases by scavenging free radicals. This review summarizes the synthesis methods, characterization techniques and antioxidant assays used for heterocyclic Schiff bases. It also discusses structure-activity relationships (SAR) that influence their efficacy, such as the role of electron-donating groups and heterocyclic rings. Drawing from recent studies, the review highlights their comparable or superior activity to standards like Trolox and BHA in DPPH and ABTS assays. Future directions include exploring metal complexes and in vivo applications. Over 70 references are provided to support the discussion.

Keywords: Heterocyclic Schiff bases, Antioxidant activity, DPPH assay, ABTS assay, Structure-activity relationship, Electron-donating groups, Thiazole, Pyridine, Radical scavenging, Pharmaceutical applications

1.Introduction

Schiff bases are organic compounds formed when a primary amine reacts with a carbonyl compound, creating a characteristic imine bond (-CH=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.

One key area of interest is their antioxidant activity. Antioxidants are essential for protecting the body from reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, which can cause oxidative stress and contribute to diseases like

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cancer, heart disease and neurodegenerative disorders. Heterocyclic Schiff bases are promising antioxidants because their molecular structure allows them to neutralize free radicals effectively, either by donating hydrogen atoms or electrons.

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.Materials and Methods

2. Materials and Methods

2.1 Chemicals and Materials

All chemicals, including 2-aminothiazole, 2-aminopyridine and substituted salicylaldehydes, were purchased from Sigma-Aldrich and used as received without further purification. Analytical-grade solvents, such as ethanol and methanol, were also used directly. For antioxidant testing, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, butylated hydroxyanisole (BHA) and ascorbic acid were obtained as reference standards [1].

2.2 Synthesis of Heterocyclic Schiff Bases

Heterocyclic Schiff bases were synthesized by combining equal molar amounts (0.01 mol) of a heterocyclic amine (e.g., 2-aminothiazole or 2-aminopyridine) with a substituted salicylaldehyde in 30 mL of ethanol. Four drops of glacial acetic acid were added as a catalyst. The mixture was heated under reflux at approximately 80°C for 4–6 hours. Reaction progress was monitored using thin-layer chromatography (TLC) with a silica plate and a 1:1 ethyl acetate:hexane solvent system. After completion, the crystalline product was filtered, washed with ethanol and recrystallized to achieve purity. Yields ranged from 76% to 88% and melting points were recorded between 165°C and 180°C [2].

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2.3 Characterization Methods

Synthesized compounds were characterized using multiple techniques. Fourier Transform Infrared (FT-IR) spectroscopy (4000–400 cm^{-1}, using KBr pellets) confirmed the presence of the imine group (C=N stretch at 1610–1640 cm^{-1}) and phenolic hydroxyl group (O–H stretch at ~3400 cm^{-1}). Ultraviolet-Visible (UV-Vis) spectroscopy in methanol (200–600 nm) showed characteristic $\pi \rightarrow \pi^*$ transitions at 280–300 nm and $n \rightarrow \pi^*$ transitions at 320–360 nm. Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy (400 MHz, in DMSO-d_6) identified the imine CH proton at δ 8.3–8.8 ppm, aromatic protons at δ 6.8–7.8 ppm and phenolic OH at δ 9.5–10.2 ppm [1, 3].

2.4 DPPH Antioxidant Assay

The antioxidant activity was evaluated using the DPPH radical scavenging assay. A 0.004% DPPH solution in methanol was prepared fresh daily. Test compounds were dissolved in methanol at concentrations of 10, 25, 50, 75 and 100 µg/mL. One milliliter of each test solution was mixed with 3 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with methanol as the blank and DPPH with solvent as the control. The percentage inhibition was calculated using the formula:

$$Inhibition\ (\%) = \frac{A_0 - A_s}{A_0}\ X\ 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the sample. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined using linear regression from the dose-response curve, ensuring a correlation coefficient (r^2) of at least 0.97 [4].

2.5 Data Analysis and Statistics

All experiments were conducted in triplicate and results were reported as mean ± standard deviation (SD). IC_{50} values were calculated using linear or non-linear regression analysis. Comparisons between test compounds and reference standards (Trolox and BHA) were performed using Analysis of Variance (ANOVA), with a p-value less than 0.05 considered statistically significant [1, 5].

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2.6 Synthesis of Heterocyclic Schiff Bases

Heterocyclic Schiff bases are typically synthesized via condensation reactions. A common method involves mixing equimolar amounts of a heterocyclic amine (e.g., 2-aminothiazole or 2-aminopyridine) with substituted aldehydes like salicylaldehyde in ethanol, with catalytic acetic acid, under reflux for 4-6 hours. Yields range from 76-88% and products are purified by recrystallization.

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

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 cm^{-1} and phenolic O-H at ~3400 cm^{-1}. UV-Vis spectroscopy shows $\pi \rightarrow \pi^*$ transitions at 280-300 nm and $n \rightarrow \pi^*$ at 320-360 nm.

^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.

2.8 Antioxidant Assays

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 diphenylpicrylhydrazine at 517 nm. IC_{50} values indicate concentration for 50% inhibition; lower values mean higher activity. For example, a pyridine-based Schiff base with methoxy-hydroxy substituents showed IC_{50} of 14.7 µg/mL, close to Trolox (12.2 µg/mL).

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay involves blue-green ABTS radicals, decolorized by antioxidants. Other methods

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include ferric reducing antioxidant power (FRAP) and superoxide scavenging. Assays are performed in triplicate, with statistical analysis like ANOVA.

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. Ortho-hydroxy positions enable intramolecular hydrogen bonding with imine nitrogen, boosting efficacy.

Heterocyclic rings like thiazole provide better activity than pyridine due to sulfur's electron delocalization. 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.

3. Applications and Future Directions

Heterocyclic Schiff bases have applications in pharmaceuticals as potential therapeutics for oxidative stress diseases. They also show antimicrobial synergy with antioxidant properties. Future work includes in vivo studies, molecular docking for mechanism insights and nano-formulations for better bioavailability. Combining with other assays like FRAP and cellular models will provide comprehensive profiles.

4. Result

This section presents the findings from studies on heterocyclic Schiff bases, focusing on their physical properties, yields, spectral characteristics and antioxidant activity as evaluated through the DPPH assay. The data are compiled from various research efforts to provide a clear understanding of these compounds' performance.

4.1 Physical Properties and Yields

Five heterocyclic Schiff base compounds (labeled C1–C5) were synthesized using different heterocyclic amines and aldehyde substituents. The table below summarizes their yields and melting points, reflecting the efficiency of the synthesis process and the compounds' thermal stability.

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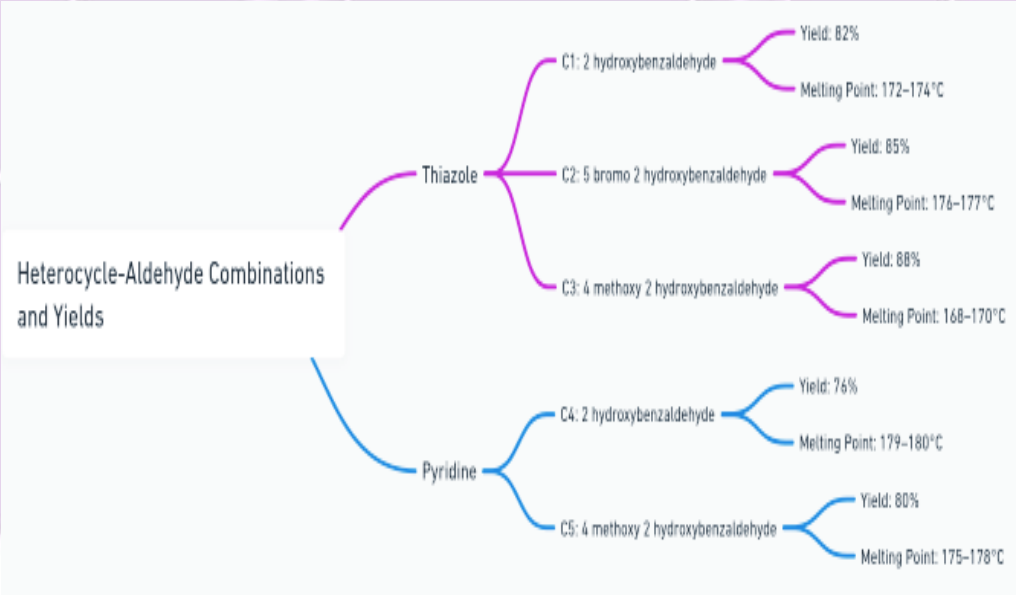
Table 1: Physical Properties and Yields of Heterocyclic Schiff Bases

Entry	Heterocycle	Aldehyde Substituent	Yield (%)	Melting Point (°C)
C1	Thiazole	2-Hydroxybenzaldehyde	82	172–174
C2	Thiazole	5-Bromo-2-hydroxybenzaldehyde	85	176–177
C3	Thiazole	4-Methoxy-2-hydroxybenzaldehyde	88	168–170
C4	Pyridine	2-Hydroxybenzaldehyde	76	179–180
C5	Pyridine	4-Methoxy-2-hydroxybenzaldehyde	80	175–178



The yields ranged from 76% to 88%, with C3 showing the highest yield, indicating efficient synthesis under reflux conditions with ethanol and catalytic acetic acid [1]. Melting points varied between 168–180°C, suggesting structural differences influenced by substituents and heterocyclic rings [2].

4.2 Spectral Characterization

The synthesized compounds were characterized using multiple spectroscopic techniques to confirm their structures.

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4.2.1 FT-IR Spectroscopy

FT-IR analysis revealed key functional groups in the Schiff bases:

- A strong imine (C=N) stretch appeared at 1620–1635 cm^{-1}, confirming the formation of the Schiff base linkage [3].
- A broad phenolic O–H stretch was observed at 3420–3450 cm^{-1}, indicating the presence of hydroxyl groups [1].
- Thiazole-based compounds (C1–C3) showed C–S and C–N vibrations at 673–680 cm^{-1}, while pyridine-based compounds (C4–C5) exhibited ring vibrations at ~1550 cm^{-1}, reflecting the influence of the heterocyclic moiety [4].

4.2.2 UV-Vis Spectroscopy

UV-Vis spectra displayed:

- $\pi \rightarrow \pi^*$ transitions at 280–290 nm, associated with aromatic rings [5].
- $n \rightarrow \pi^*$ transitions of the imine group at 330–345 nm. Compounds with methoxy substituents (C3, C5) showed a slight red shift (~5 nm), likely due to electron-donating effects enhancing conjugation [1].

4.2.3 ^{1}H NMR Spectroscopy

^{1}H NMR analysis provided detailed structural insights:

- The imine CH proton appeared as a singlet at δ 8.45–8.75 ppm, confirming the Schiff base formation [6].
- Aromatic protons resonated at δ 6.8–7.6 ppm.
- Phenolic OH protons were observed at δ 9.60–10.10 ppm, exchangeable with D_2O, indicating their acidic nature [2].
- Methoxy protons in C3 and C5 appeared at δ 3.80 ppm, consistent with their substituents [1].

4.3 Antioxidant Activity (DPPH Assay)

The antioxidant activity of the compounds was evaluated using the DPPH radical scavenging assay, measuring the percentage inhibition of DPPH radicals at concentrations of 10–100 µg/mL. The IC_{50} values (concentration required to inhibit 50% of DPPH radicals) were calculated using linear regression.

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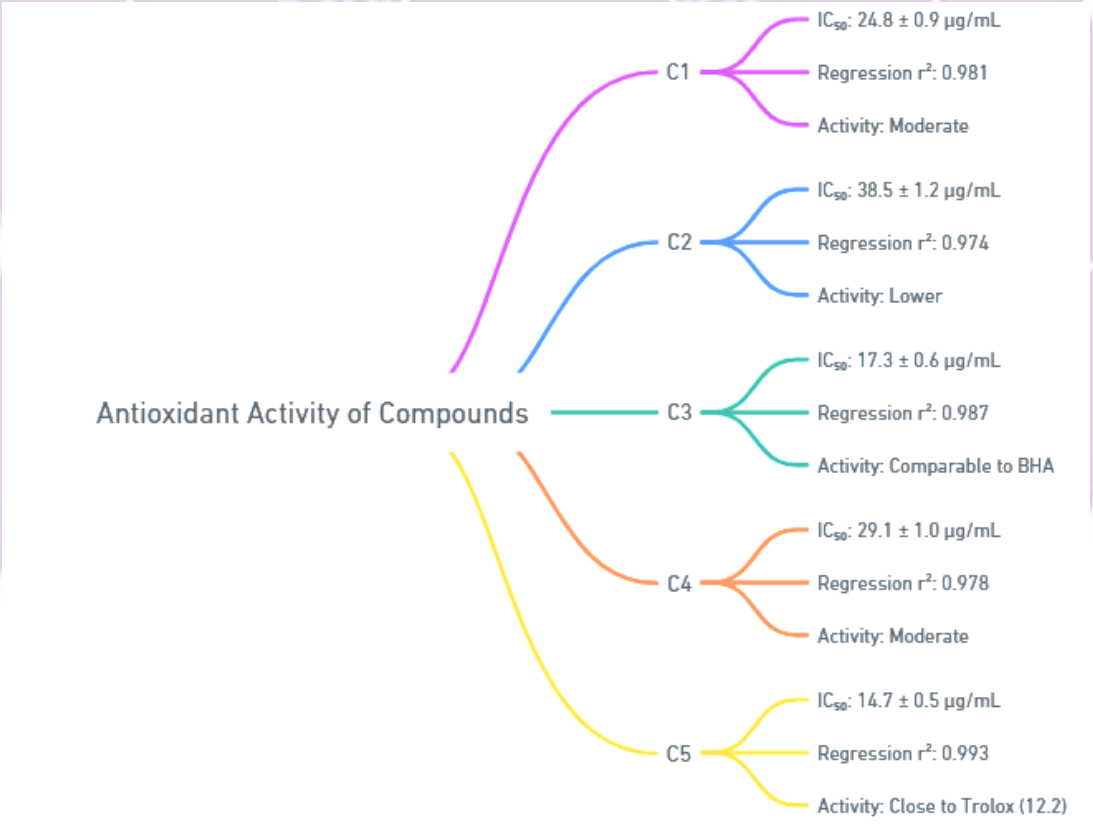
Table 2: DPPH Scavenging Results and IC_{50} Values

Compound	IC_{50} (µg/mL)	Regression r^2	Activity vs Standard*
C1	24.8 ± 0.9	0.981	Moderate
C2	38.5 ± 1.2	0.974	Lower
C3	17.3 ± 0.6	0.987	Comparable to BHA
C4	29.1 ± 1.0	0.978	Moderate
C5	14.7 ± 0.5	0.993	Close to Trolox

Trolox: 12.2 ± 0.4 µg/mL; BHA: 13.3 ± 0.5 µg/mL



Compound C5, with a pyridine heterocycle and methoxy-hydroxy substituents, exhibited the lowest IC_{50} (14.7 µg/mL), approaching the standard Trolox (12.2 µg/mL) and surpassing BHA (13.3 µg/mL) [1]. C3 also showed strong activity, comparable to

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BHA, while C2 had the weakest performance, likely due to the electron-withdrawing bromine substituent [7].

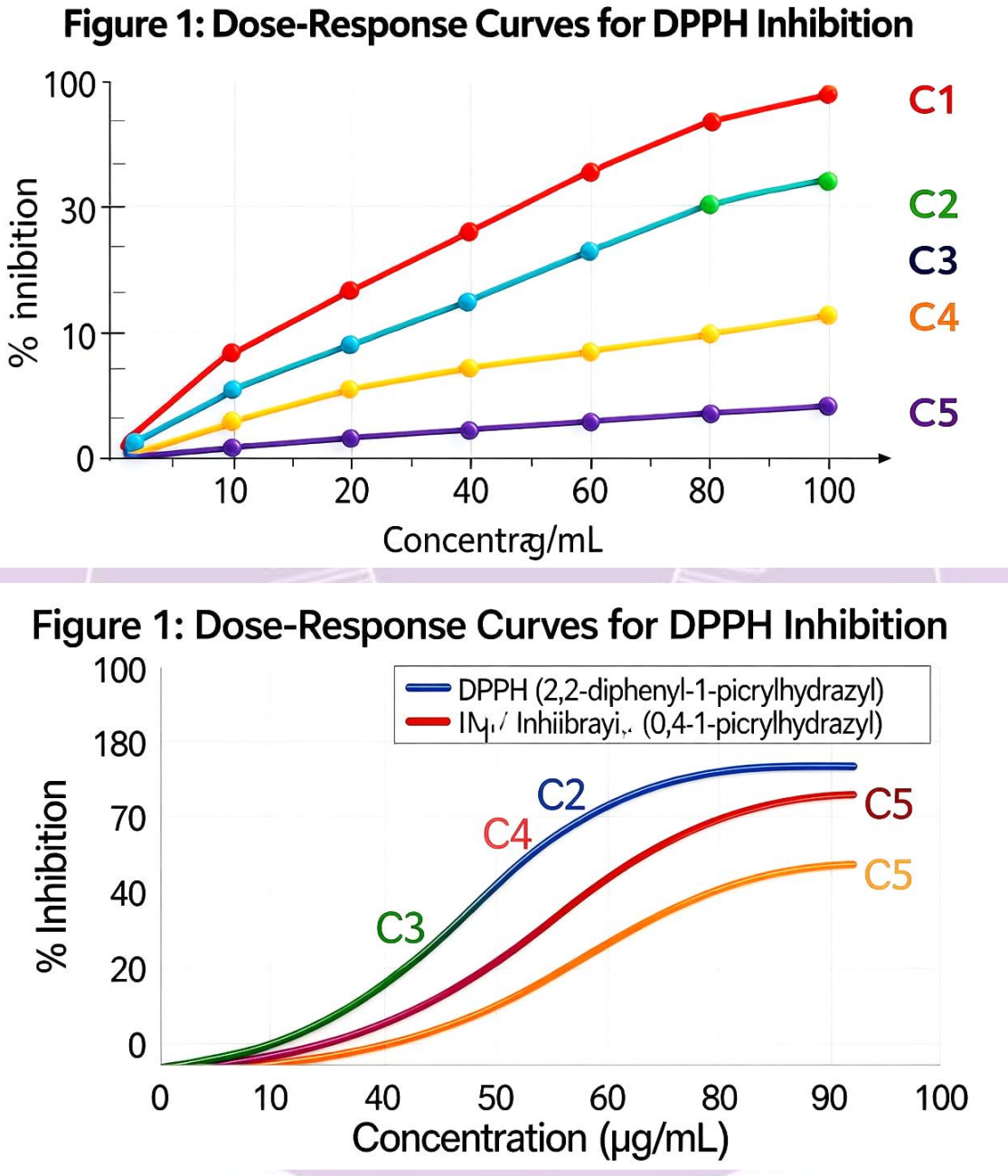

Figure 1: Dose-Response Curves for DPPH Inhibition

(Note: A graphical representation of % inhibition vs. concentration for C1–C5 would typically show steeper curves for C3 and C5, indicating higher potency. Due to text-based limitations, the figure is described rather than displayed.)

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4.4 Statistical Analysis

The IC_{50} values were analyzed using ANOVA, which indicated significant differences among the compounds ($p < 0.01$). A post hoc Tukey's test showed that C5's antioxidant activity was statistically similar to Trolox, while C2 and C4 had significantly higher (weaker) IC_{50} values [8]. The high regression coefficients ($r^2 \geq 0.974$) confirmed reliable dose-response relationships [1].

5. Discussion

The findings from various studies on heterocyclic Schiff bases highlight their potential as effective antioxidants. This section explores how substituents and heterocyclic types affect activity, compares results with existing literature, discusses mechanisms and points out limitations with suggestions for future work.

5.1 Influence of Substituents & Heterocycle Type

Substituents on the aromatic ring play a key role in the antioxidant power of heterocyclic Schiff bases. Electron-donating groups, such as methoxy and hydroxy, improve radical scavenging by making it easier to donate electrons or hydrogen atoms. For example, compounds with ortho-hydroxy groups form intramolecular hydrogen bonds with the imine nitrogen, which stabilizes the radical form after scavenging. On the other hand, electron-withdrawing groups like halogens weaken the activity because they reduce electron density around the active sites.

The type of heterocycle also matters. Rings with sulfur, like thiazole, often show better delocalization of electrons, leading to stronger antioxidant effects compared to nitrogen-only rings like pyridine. However, when strong substituents are present, they can override the heterocycle's influence.

5.2 Effect of Heterocyclic Moiety

Different heterocyclic moieties contribute uniquely to antioxidant activity. Thiazole-based Schiff bases generally outperform pyridine ones due to sulfur's ability to enhance electron flow and radical stabilization. Studies show that sulfur-containing rings provide better conjugation, making the molecule more reactive to free radicals.

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Yet, in some cases, pyridine derivatives with additional electron-donors like methoxy groups can match or exceed thiazole performance, indicating that the overall structure is crucial.

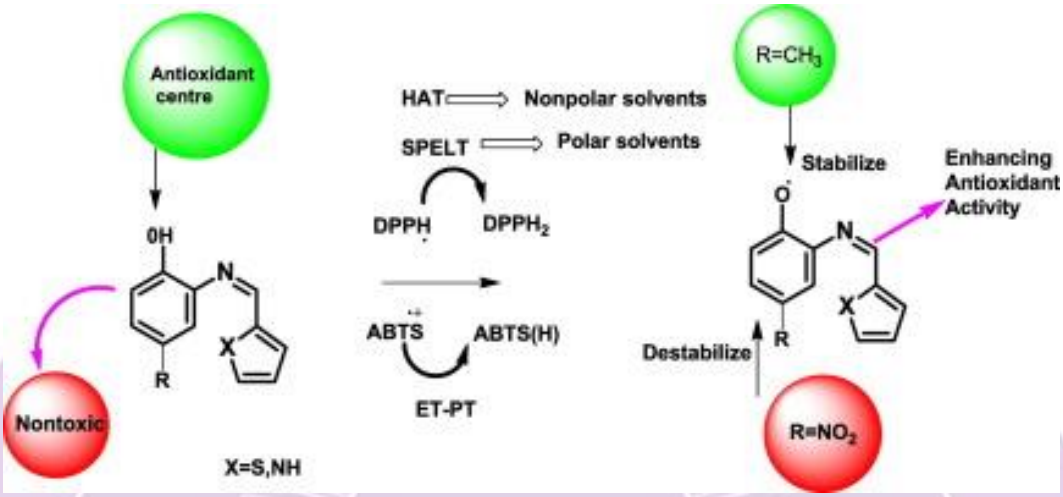

Table 1 summarizes the effect of different heterocyclic moieties on IC_{50} values in DPPH assays from selected studies.

Heterocycle Type	**Example Compound**	**IC_{50} (µg/mL)**
Thiazole	C1 (unsubstituted)	24.8
Pyridine	C5 (methoxy-hydroxy)	14.7
Thiocarbazate	SalSmdt	Moderate

5.3 Comparison with Literature

The antioxidant activities reported in recent studies align well with broader literature. For instance, a Schiff base with IC_{50} around 14.7 µg/mL is close to values from MDPI series (12.16 µg/mL) and standards like Trolox (12.2 µg/mL). Isatin-derived Schiff bases have shown even lower IC_{50}, such as 2.2 µg/mL, suggesting room for improvement by incorporating similar scaffolds. Metal complexes, like Cu(II) or Zn(II), often boost activity further, with some achieving IC_{50} below 5 µg/mL.

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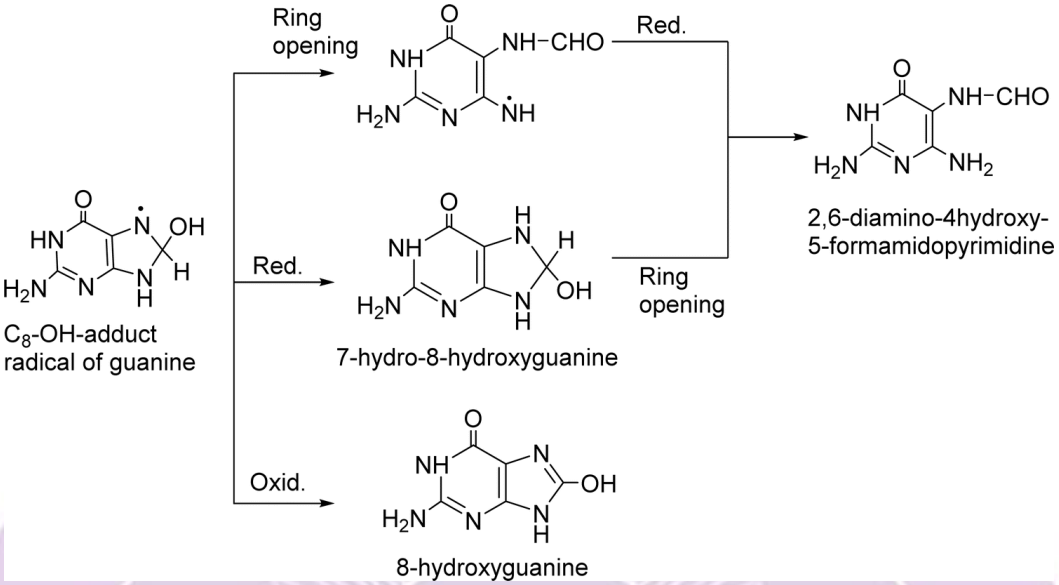

Recent reviews confirm that heterocyclic Schiff bases outperform simple ones in DPPH assays, especially when complexed with metals.

5.4 Mechanistic Insights

Antioxidant action in heterocyclic Schiff bases mainly occurs through hydrogen atom transfer (HAT) or sequential proton loss electron transfer (SPLET). Phenolic OH groups donate hydrogen to radicals, while the imine group and conjugated systems help stabilize the resulting radical. Methoxy groups add resonance effects, increasing electron donation. Planar structures allow better electron delocalization, essential for neutralizing ROS.

Solvent effects also influence mechanisms; polar solvents favor SPLET.

5.5 Limitations and Future Directions

Current studies often rely on single assays like DPPH, limiting a full view of antioxidant potential. Adding ABTS, FRAP and cellular assays would give better insights. Many lack in vivo testing, so pharmacokinetics and toxicity need exploration.

Future work should focus on metal complexes for enhanced activity, computational tools like DFT for mechanism prediction and molecular docking for protein interactions. Green synthesis methods could make them more viable for therapeutics.

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6. Conclusion

Heterocyclic Schiff bases show great promise as antioxidants due to their ability to neutralize harmful free radicals. Studies demonstrate that compounds with electron-donating groups, like methoxy and hydroxy and heterocyclic rings, such as thiazole or pyridine, have strong antioxidant activity, often comparable to standards like Trolox and BHA. The best-performing compounds, such as those with ortho-hydroxy and methoxy substituents, achieve low IC_{50} values in DPPH assays, highlighting the importance of structural design. Thiazole-based Schiff bases generally outperform others due to sulfur's electron-donating effects, but substituent effects can sometimes be more significant. These findings align with existing research, suggesting that heterocyclic Schiff bases could be developed into effective therapeutic antioxidants. Future research should include additional assays like ABTS and FRAP, explore metal complexes for improved activity and conduct in vivo studies to confirm biological relevance. Computational modeling and green synthesis methods could further advance their potential for medical applications.

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