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COPPER CHELATION BY SYRINGIC HYDRAZONES: A PROMISING STRATEGY FOR COMBATING OXIDATIVE STRESS-RELATED DISEASES

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ABSTRACT. Oxidative stress is a critical factor in vascular damage and the development of diseases such as atherosclerosis. This study investigates the complexation properties of two syringic hydrazones, (E)-4-Hydroxy-3,5-dimethoxybenzaldehyde -1,3-benzothiazol-2-ylhydrazone **1** and (E)-4-Hydroxy-3,5-dimethoxybenzaldehyde phthalazin-1-ylhydrazone hydrochloride **2**, with copper ions ($CuSO_4 \cdot 5H_2O$ and $CuCl_2 \cdot 2H_2O$). Using UV spectrophotometry, we determined stability constants and stoichiometries of the complexes formed. Compound **1** forms three distinct types of complexes (ML, ML_2, and M_2L) with higher stability constants compared to those formed by compound **2**, particularly when using $CuCl_2 \cdot 2H_2O$. These findings extend our previous work, where syringic hydrazones demonstrated potent antioxidant properties through scavenging $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, inhibiting superoxide anion generation, and reducing TBARS formation in human cell-mediated LDL oxidation. These results highlight the dual potential of syringic hydrazones as radical scavengers and metal chelators.

Keywords: *Antioxidant activity, ROS scavenging, metal chelation, UV spectrophotometry, Cu(II) complexes, stability constants*

INTRODUCTION

Oxidative stress underpins vascular damage and cardiovascular diseases, arising from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses [1]. Cellular processes like mitochondrial

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respiration and NADPH oxidase activity generate ROS, leading to lipid peroxidation, protein modifications, and cellular dysfunction [2, 3]. Transition metals, notably copper and iron, catalyze Fenton and Haber-Weiss reactions, producing hydroxyl radicals that exacerbate oxidative stress [4, 5]. Antioxidants mitigate this stress by scavenging free radicals or forming stable complexes with transition metals [6, 7].

Hydrazones, characterized by a C=N-NH bond, exhibit dual functionality as antioxidants and metal chelators [8, 9]. Our earlier work [11] demonstrated the potent antioxidant properties of syringic hydrazones through their scavenging effects on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, inhibition of superoxide anion generation, and reduction of TBARS formation during human cell-mediated LDL oxidation. Furthermore, these compounds effectively decreased protein carbonyl content in cells exposed to oxidized LDL, showcasing their carbonyl scavenger efficacy. However, their potential as metal chelators remained unexplored.

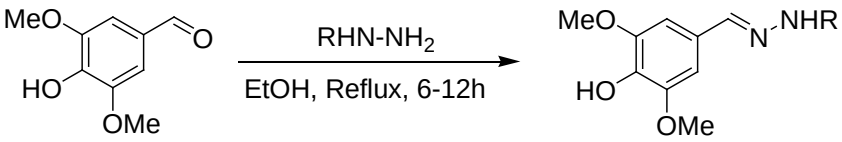
This study investigates the complexation behavior of two syringic hydrazones (E)-4-Hydroxy-3,5-dimethoxybenzaldehyde -1,3-benzothiazol-2-ylhydrazone **1** and (E)-4-Hydroxy-3,5-dimethoxybenzaldehyde phthalazin-1-ylhydrazone hydrochloride **2** with copper ions. Using UV spectrophotometry, we determined the stability constants and stoichiometries of the resulting complexes, providing insights into their therapeutic potential for oxidative stress-related diseases.

In our publication [11], we conducted a comprehensive physicochemical evaluation of syringic hydrazones, focusing on their antioxidant properties. The study revealed their potent scavenging effects on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, expressed as Trolox equivalent antioxidant capacity (TEAC). Additionally, these compounds inhibited superoxide anion ($O_2^{\bullet-}$) generation and reduced TBARS formation during human cell-mediated low-density lipoprotein (LDL) oxidation, highlighting their ability to protect against lipid peroxidation. Furthermore, their carbonyl scavenger efficacy was assessed by measuring the reduction of protein carbonyl content in cells challenged with oxidized LDL, demonstrating their protective role against protein oxidation. Despite these promising findings, the potential of syringic hydrazones as metal chelators was not explored, leaving a gap in understanding their full therapeutic potential.

This current study addresses this gap by investigating the complexation behavior of syringic hydrazones with copper ions, thereby expanding their application as dual-action agents capable of mitigating oxidative stress through both radical scavenging and metal sequestration.

RESULTS AND DISCUSSION

In this study, after synthesizing compounds **1** and **2**, we investigate the complexation behavior of two syringic hydrazones, designated as compounds **1** and **2**, with copper sulfate and copper chloride.



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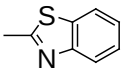
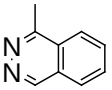
1		89%
2		97%

Scheme 1. General synthesis of ligands **1** and **2**

Synthesis

The following **Table 1** present the spectroscopic analyses of compounds **1** and **2**, including RMN, IR, and UV techniques.

Table 1. Characterization of Compounds **1** and **2**

Compound	(E)-4-Hydroxy-3,5-dimethoxybenzaldehyde -1,3-benzothiazol-2-ylhydrazone (1)	(E)-4-Hydroxy-3,5-dimethoxybenzaldehyde phthalazin-1-ylhydrazone hydrochloride (2)
Yield (%)	97	75
Melting Point (°C)	219-221	154-156
IR Spectrum (KBr) v cm^{-1}	Peaks at 3543 (O-H); 3480 (N-H); 3185 (=C-H ethyl.); 3068 (C-H arom.); 2846 (C-H, O-CH_3); 1672 (C=N ethyl.); 1608 (C=C arom.); 1510 (C=C arom.), 1249 (O-C arom); 1219 (O-C), 1110 (O-C)	Peaks at 3415 (O-H); 1630 (C=N); 1619 (C=C); 1592 (C=C arom.); 1516 (C=C arom.); 1464 (C=C arom.); 1222 (C-O); 1122 (C-O)
^{1}H NMR (CD_3OD, 300 MHz)	3.97 (s, 6H, OCH_3); 6.94 (s, 2H, $H_{2,6}$);	3.84 (s, 6H, OCH_3); 7.38 (s, 2H, $H_{2,6}$); 8.15 (td, J = 8.2 Hz, J = 1.5, 1H, H_{11});

Compound	(E)-4-Hydroxy-3,5-dimethoxybenzaldehyde -1,3-benzothiazol-2-ylhydrazone (1)	(E)-4-Hydroxy-3,5-dimethoxybenzaldehyde phthalazin-1-ylhydrazone hydrochloride (2)
δ ppm	7.18 (t, 1H, J = 7.8 Hz, $H_{5'}$); 7.36 (t, 1H, J = 8.1 Hz, H_{11}); 7.56 (d, 1H, J = 8.1 Hz, H_{10}); 7.68 (d, 1H, J = 7.8 Hz, H_{13}); 7.90 (s, 1H, H_7).	8.21 (t, J = 8.2 Hz, 1H, H_{12}); 8.25 (d, J = 8.0 Hz, 1H, H_{13}); 8.98 (s, 1H, H_7); 9.04 (s, 1H, H_{15}); 9.23 (d, J = 7.6 Hz, 1H, H_{10})
UV Spectrum ((EtOH, 50 µM, 25°C)	λ = 342 nm, ε = 34,920 L mol^{-1} cm^{-1}	λ = 300 nm, ε = 13,220 L mol^{-1} cm^{-1}, λ = 372 nm, ε = 22,180 L mol^{-1} cm^{-1}

Complexation Studies by UV-Vis Spectrophotometry

The complexation behavior of syringic hydrazones **1** and **2** with copper(II) ions was investigated using UV-Vis spectrophotometric titrations. For each ligand, a solution in absolute ethanol (10^{-5} M) was titrated with incremental additions of an aqueous solution of the metal salt ($CuSO_4 \cdot 5H_2O$ or $CuCl_2 \cdot 2H_2O$, 10^{-3} M in deionized water). For compound **2**, which was isolated as a hydrochloride salt, the ethanolic medium was first neutralized by adding a few microliters of triethylamine (Et_3N) prior to titration. This ensured that complexation studies were performed under comparable, near-neutral pH conditions for both ligands.

The spectral evolution during the titration of **1** with both copper salts is shown in **Figure 1**. The addition of metal ions resulted in a significant bathochromic shift and changes in absorbance, indicating the formation of copper-ligand complexes. The presence of clear isosbestic points in the spectra (e.g., at ~370 nm and ~420 nm for **1** with $CuCl_2 \cdot 2H_2O$) confirms the existence of well-defined equilibria between distinct absorbing species.

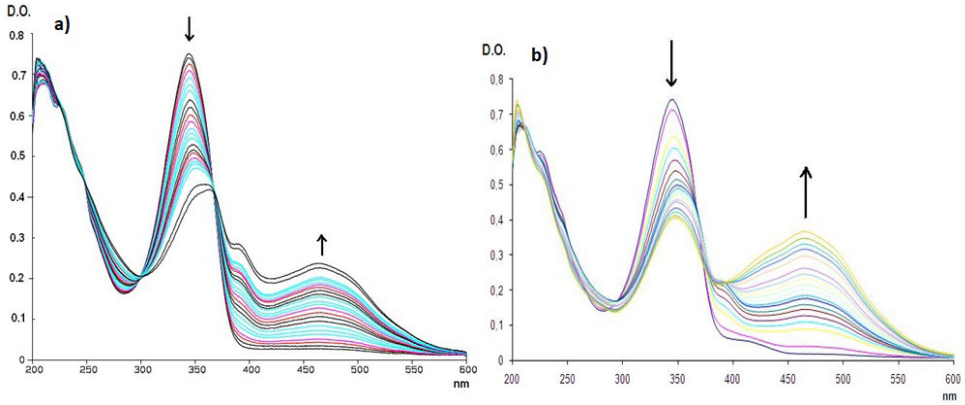


Figure 1. UV-Vis spectral changes during the titration of compound **1** (10^{-5} M in ethanol) with incremental additions of aqueous solutions of (a) $CuSO_4 \cdot 5H_2O$ and (b) $CuCl_2 \cdot 2H_2O$ (10^{-3} M in water). The arrows indicate the direction of spectral changes upon increasing metal concentration

Figure 2 displays the analogous spectral changes observed during the titration of compound **2** with $CuCl_2 \cdot 2H_2O$. A qualitatively similar, though less pronounced, variation in the absorbance spectra was observed upon complexation, suggesting the formation of weaker or fewer complexes compared to **1**.

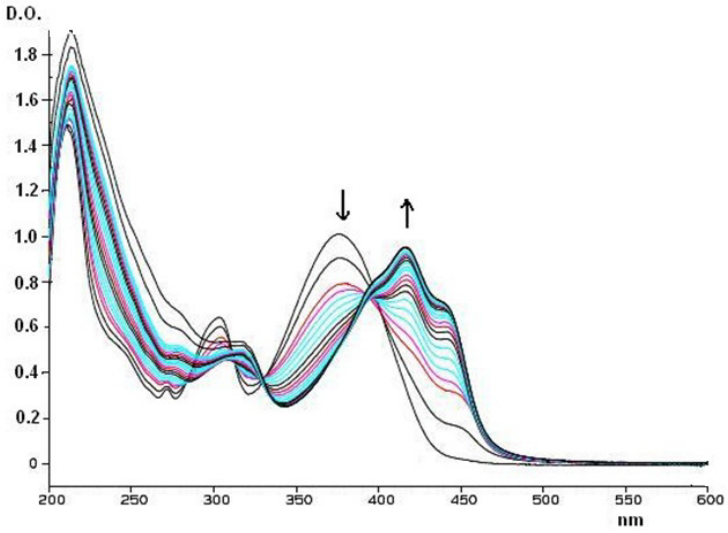


Figure 2. UV-Vis spectral changes during the titration of compound **2** (10^{-5} M in ethanol, neutralized with Et_3N) with incremental additions of an aqueous solution of $CuCl_2 \cdot 2H_2O$ (10^{-3} M in water)

Determination of Stability Constants

Stability constants and stoichiometries were determined using the STAR program [13], which performs global multi-wavelength fitting. Analysis was carried out over the wavelength ranges listed in Table 2. Various binding models (ML, ML_2, M_2L) were tested, and the best-fit model was selected based on minimal residual variance (S(A) < 0.005), satisfactory statistical parameters (χ^2 < 12.6, R(%) < 1%, kurtosis ≈ 3), and physical consistency.

The resulting formation constants are summarized in Table 2. Notably, **1** forms three complex types (ML, ML_2, M_2L) with significantly higher stability constants than **2**, especially when $CuCl_2 \cdot 2H_2O$ is used as the metal source. The exceptionally high value for the ML_2 species of **1** with $CuCl_2 \cdot 2H_2O$ (log β = 16.56 ± 0.01) indicates very strong chelation.

It should be noted that the reported stability constants are conditional values, valid under the specific experimental conditions (mixed ethanol–water medium, near-neutral pH after neutralization where applicable, and ambient temperature)

Table 2. Formation constants and statistical tests of complexes of **1** and **2** with $CuSO_4.5H_2O$ and $CuCl_2.2H_2O$

Compound	**1**	**1**	**2**	**2**
Metal source	$CuSO_4 \cdot 5H_2O$	$CuCl_2 \cdot 2H_2O$	$CuSO_4 \cdot 5H_2O$	$CuCl_2 \cdot 2H_2O$
λmax(nm)	340-487	330-540	370-455	395-450
Logβ ML	5.84±0.02	7.97±0.02	4.3±0.2	4.2±0.04
Logβ ML_2	10.76±0.08	16.56±0.01	-	8.4±0.1
Logβ M_2L	11.04±0.03	13.10±0.01	8.7±0.3	-
S(A) < 0.005	0.000577	0.0012	0.000991	0.000963
k2 < 12.6	7.4	11.3	8.96	6.9
R(%) < 1	0.46	0.85	0.64	0.34
Kurtosis ≈ 3	3.3	2.8	2.25	2.77

Complexation Behavior of Compound 1 vs. Compound 2

Compound **1** [(E)-4-Hydroxy-3,5-dimethoxybenzaldehyde -1,3-benzothiazol-2-ylhydrazone] exhibited markedly superior Cu^{2+}-binding ability compared to **2**, forming ML, ML_2, and M_2L species. This enhanced metal-chelating property may contribute to its observed antioxidant effects previously reported for **1** [11], as effective sequestration of redox-active copper reduces its participation in ROS-generating reactions.

By contrast, **2** formed fewer complexes and displayed lower stability constants, particularly with $CuSO_4 \cdot 5H_2O$. Interestingly, our earlier biological assays showed **2** to be more cytoprotective in certain in vitro models (TBARS and MTT) [11]. This apparent discrepancy may arise from structural differences: the benzothiazole moiety in **1** enhances electron donation and metal coordination, whereas the phthalazinyl group in **2** may favor alternative protective mechanisms, such as direct carbonyl scavenging or modulation of cellular pathways [12].

Interpretation of Stability Constants and Biological Implications

The stability constants (logβ) presented in Table 2 provide a quantitative measure of the affinity between the syringic hydrazones and copper ions. A higher logβ value indicates a more stable complex and a greater thermodynamic driving force for complex formation. The exceptionally high value for the **1**–$CuCl_2$ ML_2 complex (logβ = 16.56) signifies an extremely stable chelate, suggesting that compound **1** can effectively sequester Cu^{2+} ions even at low concentrations. In contrast, the significantly lower constants for compound **2** (e.g., logβ ML ≈ 4.2–4.3) reflect a weaker and more labile interaction. Qualitatively, this places the chelating strength of compound **1** on par with some known high-affinity copper chelators, while compound **2** functions as a much weaker binder. This distinction has direct implications for their potential biological activity: a chelator with relatively high affinity such as compound 1 has greater potential to sequester redox-active copper ions in a cellular environment, thereby inhibiting metal-catalyzed radical generation a key mechanism in mitigating oxidative stress-related damage.

Effect of Metal Source on Complexation Efficiency

The choice of copper salt significantly influenced complex stability. $CuCl_2 \cdot 2H_2O$ consistently yielded higher log β values than $CuSO_4 \cdot 5H_2O$, particularly for the ML_2 species of **1**. This may stem from chloride ions stabilizing specific coordination geometries or promoting mixed-ligand complexes [14,15]. The presence of two clear isosbestic points during titration with $CuCl_2 \cdot 2H_2O$ further supports stepwise, well-defined binding equilibria.

Implications for Antioxidant Activity

The dual role of syringic hydrazones as radical scavengers and metal chelators positions them as promising multifunctional agents against oxidative stress. They directly neutralize ROS (e.g., $O_2^{-\bullet}$, $ROO^{\bullet}$) while

simultaneously reducing metal-catalyzed hydroxyl radical formation [6,7]. The present physicochemical data complement our earlier findings [11] by providing a mechanistic basis for the contribution of copper chelation to overall antioxidant efficacy.

Structure-Activity Relationships

Spectroscopic and stability data highlight key structural features governing chelation. The hydroxyl and methoxy groups increase electron density at the binding site, while the planar benzothiazole ring in **1** likely facilitates favorable π-interactions [8,9]. In **2**, the bulkier phthalazinyl moiety may introduce steric hindrance, reducing metal accessibility. These insights underscore the value of targeted structural modification in future hydrazone design.

Clinical Relevance and Future Directions

The physicochemical insights gained from this study provide a foundation for future research into therapeutic strategies targeting oxidative stress-related diseases. Compounds with the ability to chelate transition metals, such as the syringic hydrazones studied here, represent an important class of molecules for further investigation in pathological contexts where metal ion dysregulation and ROS production are implicated, including atherosclerosis, neurodegenerative disorders, and cancer [5]. The dual functionality of syringic hydrazones, combining radical scavenging and metal chelation, makes them particularly interesting candidates for further structure-activity relationship studies and mechanistic exploration.

Future research should focus on evaluating the in vivo efficacy of these compounds in preclinical models of oxidative stress-related diseases. Investigations into their pharmacokinetic and pharmacodynamic profiles will provide critical insights into their suitability for clinical translation. Additionally, studies examining the synergistic effects of combining syringic hydrazones with existing antioxidant therapies may uncover new avenues for improving treatment outcomes [6].

In conclusion, this study provides a comprehensive analysis of the complexation behavior of syringic hydrazones with copper ions, highlighting their potential as therapeutic agents for combating oxidative stress. The superior metal-chelating ability of compound **1**, combined with its potent antioxidant properties, positions it as a lead candidate for further physicochemical and biological evaluation. Continued research in this area holds great promise for advancing our understanding of the role of metal ions in oxidative stress and developing innovative solutions to address this pressing health challenge.

CONCLUSIONS

This study demonstrates the ability of syringic hydrazones to form stable complexes with copper(II) ions. Compound **1** exhibited superior complexation, forming ML, ML_2, and M_2L species with higher stability constants than compound **2**, particularly when using $CuCl_2 \cdot 2H_2O$. These findings provide a physicochemical basis for understanding the metal-chelating capacity of these compounds, complementing their previously established radical-scavenging antioxidant activity.

The results highlight the dual functionality of syringic hydrazones and underscore the importance of structural features—such as the benzothiazole moiety in **1** in determining metal-binding affinity. Future work should focus on elucidating the molecular mechanisms underlying these differences and evaluating the efficacy of these chelators in biological models of oxidative stress.

EXPERIMENTAL SECTION

Synthesis of Compounds 1 and 2

Compounds **1** and **2** were synthesized according to the procedures outlined in **Scheme 1**. Commercially available syringaldehyde was refluxed in absolute ethanol with different hydrazines for 6-12 hours. The reaction was monitored by thin-layer chromatography (TLC) until completion. The solution was then cooled to room temperature, and the resulting precipitate was collected by filtration to provide the corresponding hydrazones as hydrochloride salts or non-salt forms, depending on the nature of the commercially available hydrazines [11].

The synthesis and full spectroscopic characterization (including ^{1}H NMR, ^{13}C NMR, IR, UV-Vis, and elemental analysis) of compounds **1** and **2** have been reported previously [11]. The ^{1}H and ^{13}C NMR spectra for both compounds are provided in the Supplementary Material.

Complexation

Compounds **1** and **2**, previously synthesized and fully characterized [11], were used without further purification and were of sufficient purity (>95% as assessed by NMR) for the spectrophotometric complexation studies

UV spectrophotometry was employed to study the complexation of **1** and **2** with Cu^{2+}. Solutions of the ligands (10^{-5} M) were prepared in ethanol,

and incremental volumes of $CuSO_4 \cdot 5H_2O$ or $CuCl_2 \cdot 2H_2O$ (10^{-3} M) were added. Spectra were recorded over the range of 200-600 nm after each addition. The presence of isobestic points confirmed the formation of specific equilibria between the ligand and metal complexes.

The stability constants were calculated using the STAR program [13], which evaluates the best fit between experimental and calculated spectra. Statistical tests were applied to validate the models:

- S(A): Must be < 0.005.
- R (%): Must be < 1%.
- Kurtosis: Should be around 3.
- χ^2 (Chi-Square): Must be < 12.6.

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