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# THE EFFECT OF S-ALKYLATION OF THE THIOSEMICARBAZONE FRAGMENT ON THE ANTIRADICAL ACTIVITY OF SOME 3*d* METAL COMPLEXES

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**Abstract**

This study examines how *S*-alkylation of thiosemicarbazones affects the antiradical activity of 3*d*-metal complexes. Comparative analysis of free salicylaldehyde 4-allyl-*S*-alkylisothiosemicarbazones and their coordination compounds revealed a dual effect: *S*-alkylation diminishes the activity of free ligands by blocking proton-donor capacity but markedly enhances it in metal complexes via efficient redox-pair formation. Nitrate complexes of Fe(III), Co(III), and Cr(III) exhibited the highest potency, with $IC_{50}$ values (0.50 μM), surpassing Trolox by 66-fold. Structure–activity relationships indicated that compact *S*-substituents and outer-sphere nitrate anions boost activity, whereas bulky benzyl groups impose steric hindrance. Further optimization was achieved by introducing electron-donating methoxy groups or extending conjugation in the aldehyde fragment. These findings establish a rational design strategy for highly efficient isothiosemicarbazone-based antioxidants.

***Keywords:*** coordination compounds; isothiosemicarbazone; 3*d* metal complexes; antiradical activity; antioxidant activity; S-alkylation; structure-activity relationship; $ABTS^{\cdot+}$ radical scavenging.

**Rezumatul**

Acest studiu examinează modul în care *S*-alchilarea tiosemicarbazonei afectează activitatea antiradicalică a complecșilor metalelor 3*d*. Analiza comparativă a 4-alil-*S*-alchilizotiosemicarbazonei aldehidei salicilice libere și a compușilor lor coordinativi a evidențiat un efect dual: *S*-alchilarea reduce activitatea liganzilor liberi prin blocarea capacității de donare a protonului, dar o sporește semnificativ în complecșii metalici prin formarea unor perechi redox eficiente. Complecșii nitrați ai Fe(III), Co(III) și Cr(III) au demonstrat cea mai mare potență, cu valori $IC_{50}$ (0,50 μM), depășind activitatea Trolox de 66 de ori. Relațiile structură-activitate au indicat faptul că substituenții *S*- compacți și anionii nitrat din sfera exterioară stimulează activitatea, în timp ce grupările benzil voluminoase induc impedimente sterice. O optimizare suplimentară a fost obținută prin introducerea de grupări metoxi donatoare de electroni sau prin extinderea conjugării în fragmentul aldehidic. Aceste rezultate stabilesc o strategie de proiectare rațională pentru antioxidanți altamente eficienți bazați pe izotiosemicarbazone.

***Cuvinte-cheie:*** compuși coordinativi; izotiosemicarbazone; complecși ai metalelor 3d; activitate antiradicalică; activitate antioxidantă; S-alchilare; relație structură-activitate; captarea radicalului $ABTS^{\cdot+}$.

## Introduction

Thiosemicarbazones and their derivatives have held the status of one of the most promising classes of compounds in bioinorganic chemistry for several decades [1-9]. The high biological potential of these compounds, particularly their antioxidant and radical-scavenging properties, provides broad opportunities for the development of new therapeutic agents aimed at combating oxidative stress. To optimize pharmacological properties, researchers actively employ chemical modification of the ligand environment. In particular, alkylation of heteroatoms allows fine-tuning of molecular lipophilicity and alters the nature of interactions with transition metal ions [10-11].

A crucial factor in shaping antiradical activity is the state of the thione group and the system's ability to undergo tautomeric transformations. Converting a classical thiosemicarbazone into an isothiosemicarbazone by introducing an alkyl substituent on the sulfur atom significantly modifies the donor–acceptor characteristics of the ligand. However, the effect

of this structural modification on antioxidant potential is ambiguous: it can manifest in diametrically opposite ways for free organic molecules versus their coordination complexes.

The coordination chemistry of isothiosemicarbazones has been extensively documented in the literature, with numerous complexes of these ligands with transition metal ions having been synthesized and structurally characterized [12-19]. Nevertheless, a systematic study of how *S*-alkylation specifically affects antiradical activity in free ligands and their metal complexes remains limited. Most previous work has focused on synthesis and structural features, while the relationship between sulfur-center modification and radical-scavenging ability requires further analysis [20].

In this context, the present study provides a comparative analysis of the activity of parent thiosemicarbazones and their *S*-alkylated analogues, both in the free state and as coordination complexes with 3*d*-metal ions. Special attention is given to identifying synergistic effects arising from the combination of different metal centers (Fe, Co, Cr, Cu, Ni) with modified ligands, as well as to examining the influence of the outer-sphere environment and the nature of the aldehyde fragment on overall radical-inhibiting efficiency. The results offer a new perspective on the principles of designing highly active antioxidants.

This work presents a systematic study focused on the investigation of 3*d* metal complexes with 4-allyl-*S*-alkylisothiosemicarbazones of salicylaldehyde and its substituted derivatives (**Figure 1, Table 1**).

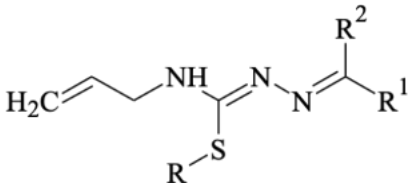
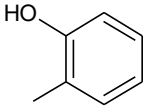
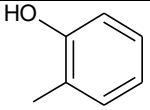
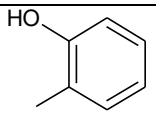
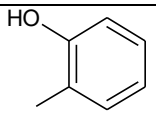
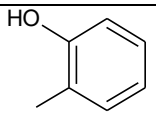
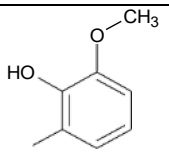
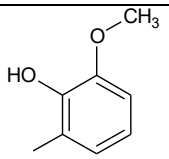
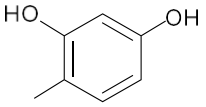
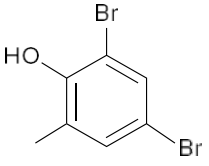
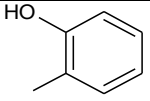
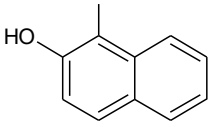
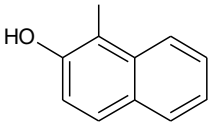



**Figure 1.** The structural formula of isothiosemicarbazones.

**Table 1.** Chemical structures and substituents of the studied 4-allyl-*S*-alkylisothiosemicarbazones ($HL^3$ – $HL^{14}$)

| Isothiosemicarbazones | R | $R^1$ | $R^2$ |
|---|---|---|---|
| $HL^3$ | $CH_3$ | H |  |
| $HL^4$ | $C_2H_5$ | H |  |
| $HL^5$ | Allyl | H |  |
| $HL^6$ | Bn | H |  |

| | | | |
|---|---|---|---|
| $HL^7$ | 4-$NO_2$Bn | H |  |
| $HL^8$ | $CH_3$ | H |  |
| $HL^9$ | $C_2H_5$ | H |  |
| $HL^{10}$ | $CH_3$ | H |  |
| $HL^{11}$ | $CH_3$ | H |  |
| $HL^{12}$ | $CH_3$ | $CH_3$ |  |
| $HL^{13}$ | $CH_3$ | H |  |
| $HL^{14}$ | $C_2H_5$ | H |  |

**Materials and methods**

**Assessment of Antiradical Activity: $ABTS^{\bullet+}$ Assay**

The ability of the studied organic precursors and coordination compounds (1–85) to scavenge free radicals was evaluated using the spectrophotometric $ABTS^{+\bullet}$ decolorization assay [21].

**Preparation of the radical cation**

The working solution was generated by mixing a 7 mM solution of ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) with a 140 mM solution of potassium persulfate. The reaction mixture was incubated in the dark at 25 °C for 12–16 h until complete oxidation

occurred. The resulting radical cation solution was diluted with 0.02 M acetate buffer (pH 6.5) to an optical density of 0.70 ± 0.01 at 734 nm.

**Assay procedure**

Test samples were pre-dissolved in dimethyl sulfoxide (DMSO). In a 96-well microplate, 20 μL of the sample solution was added to 180 μL of the $ABTS^{+\bullet}$ working solution. Trolox, prepared in the same way, was used as a reference standard. The mixture was automatically shaken for 15 s and then incubated at 25 °C for 30 min.

Data acquisition. Optical density was measured at 734 nm using a BioTek microplate reader. Control wells containing only buffer and DMSO were used to correct for background absorbance and solvent effects. Antiradical activity was expressed as the half-maximal inhibitory concentration ($IC_{50}$), calculated from calibration curves. All measurements were performed in triplicate to ensure reproducibility.

**Results and discussions**

**Structural Transition from Thiosemicarbazones to *S*-Alkylisothiosemicarbazones: Impact on Antiradical Potential**

Thiosemicarbazones and their derivatives remain one of the most promising classes of compounds in modern coordination chemistry. Their high biological activity, including antioxidant properties, makes them attractive candidates for the development of new therapeutic agents. In an effort to optimize the pharmacological profile of these substances, researchers often resort to chemical modification of the ligand environment. One of the key approaches is the alkylation of heteroatoms, which allows for fine-tuning of molecular lipophilicity and modulation of their interactions with both biological targets and transition metal ions.

The state of the thione group and the possibility of tautomeric transformations play a particularly important role in shaping antiradical properties. The conversion of a classical thiosemicarbazone into an isothiosemicarbazone via the introduction of an alkyl substituent at the sulfur atom significantly alters the donor–acceptor characteristics of the ligand. However, the effect of this structural modification on antioxidant potential is not straightforward: it may manifest differently in free organic molecules and in their coordination compounds. To elucidate these patterns, a comparative analysis of the activity of the parent thiosemicarbazones and their *S*-alkylated analogues, both in the free state and as part of complexes with 3*d* metals, is required.

A transition from classical thiosemicarbazones to their *S*-alkylated derivatives in the coordination chemistry of 3*d* metals reveals significant differences in biochemical properties. Among free ligands, the non-alkylated thiosemicarbazone $\mathbf{H_2L^2}$ exhibits high antiradical activity ($IC_{50}$ = 9.00 μM). However, the introduction of a methyl group at the sulfur atom in compound $\mathbf{HL^3}$ leads to a decrease in activity to 31.0 μM, which is comparable to that of the reference compound Trolox (33.0 μM). From a chemical standpoint, this phenomenon is explained by the blocking of the thiol group: in classical thiosemicarbazones, the mobile proton of the sulfur atom or adjacent nitrogen acts as a donor, effectively neutralizing free radicals. Replacement of this hydrogen atom with an alkyl group eliminates the proton-transfer mechanism, shifting the reaction toward a less efficient electron-transfer pathway **(Table 2)**.

The situation changes fundamentally upon coordination of these ligands with metal ions. Within complexes, *S*-alkylation promotes a significant increase in antioxidant efficiency. While the iron complex with a classical thiosemicarbazone **(8)** exhibits an $IC_{50}$ value of 2.41 μM, its isothiosemicarbazone analogue **(17)** shows substantially higher activity ($IC_{50}$ = 0.60 μM). A similar trend is observed in the cobalt series: methylation of the ligand reduces the half-maximal inhibitory concentration from 8.73 μM **(6)** to 0.50 μM **(15)**.

The obtained data indicate that a more efficient redox system is formed in isothiosemicarbazone complexes. Whereas the free alkylated ligand shows only moderate

activity, its coordination with a 3*d* element leads to polarization of electron density, facilitating electron transfer to the $ABTS^{\cdot+}$ radical more effectively than in non-alkylated precursors. Copper complexes constitute an exception: here, *S*-alkylation—especially with bulky substituents (e.g., a benzyl group)—creates steric hindrance, as evidenced by the reduced activity in complexes 32–35 ($IC_{50}$ > 100 μM). Thus, the transition to *S*-alkylated structures represents an effective modification strategy which, with an optimal choice of the central metal atom, can enhance the antioxidant response of the coordination system by an order of magnitude.

Of particular interest in the context of structural evolution is the comparison of modified systems with the parent "building block," *N4*-allylthiosemicarbazide **($HL^1$)**. This unsubstituted reagent itself exhibits high antiradical activity ($IC_{50}$ = 6.38 μM), surpassing even the parent thiosemicarbazone **$H_2L^2$**. Upon coordination with nickel(II) ions, a significant enhancement of properties is observed: complexes **1** (1.99 μM) and 2 (2.57 μM) demonstrate a threefold increase in efficiency compared to the free ligand. This confirms a general trend: the presence of an accessible thiol (or thione) fragment, combined with metal coordination, provides a strong foundation for radical scavenging.

However, whereas in the case of $HL^1$ and $H_2L^2$ the activity is primarily governed by mobile protons of the sulfur-containing fragment, in the studied *S*-alkylated systems this mechanism is completely replaced by metal–ligand synergy. The fact that *S*-methylated iron and cobalt complexes **(15–17)** ultimately outperform even the nickel complexes of the unsubstituted thiosemicarbazide highlights that the transition to isothiosemicarbazones represents a more advanced antioxidant design strategy, enabling the achievement of submicromolar activity levels.

In summary, *S*-alkylation of thiosemicarbazone ligands exerts a dual effect on their antioxidant potential depending on the state of the system. For free organic molecules, this modification leads to decreased activity due to the loss of the proton-transfer mechanism, whereas in coordination compounds it promotes the formation of more efficient redox pairs through synergy with the central metal ion.

**Table 2.** $ABTS^{\bullet+}$ scavenging activity of the studied substances

| Compound | Name / Coordination formula | $IC_{50}$, μM |
|---|---|---|
| Trolox | 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid | 33.0 |
| $HL^1$ | $N^4$-allylthiosemicarbazide | 6.38 |
| 1 | $[Ni(HL^1)_3](NO_3)_2 \cdot 2H_2O$ | 1.99 |
| 2 | $[Ni(HL^1)_3]Cl_2 \cdot 2H_2O$ | 2.57 |
| $H_2L^2$ | 2-hydroxybenzaldehyde $N^4$-allylthiosemicarbazone | 9.00 |
| 3 | $[Cu_2(H_2O)_2(HL^2)_2](NO_3)_2$ | 39.7 |
| 4 | $[Cu(HL^2)Cl] \cdot H_2O$ | 51.8 |
| 5 | $[Cu(HL^2)Br]$ | 52.7 |
| 6 | $[Co(HL^2)_2]NO_3$ | 8.73 |
| 7 | $[Co(HL^2)_2]Cl \cdot 2H_2O$ | 21.4 |
| 8 | $[Fe(HL^2)_2]NO_3 \cdot 2H_2O$ | 2.41 |
| $HL^3$ | 2-hydroxybenzaldehyde $N^4$-allyl-*S*-methylisothiosemicarbazone | 31.0 |
| 9 | $[Cu(H_2O)_2(L^3)]Cl$ | 43.0 |
| 10 | $[Cu(H_2O)_2(L^3)]Br$ | 18.1 |
| 11 | $[Cu(L^3)CH_3COO]$ | 17.5 |
| 12 | $[Co(L^3)_2]Cl$ | 23.2 |
| 13 | $[Co(L^3)_2]Br$ | 24.4 |
| 14 | $[Co(L^3)_2]I$ | 21.7 |
| 15 | $[Co(L^3)_2]NO_3$ | 0.50 |

| | | |
|---|---|---|
| 16 | $[Fe(L^3)_2]Cl$ | 0.70 |
| 17 | $[Fe(L^3)_2]NO_3$ | 0.60 |
| 18 | $[Cr(L^3)_2]NO_3$ | 0.50 |
| $HL^4$ | 2-hydroxybenzaldehyde *N*4-allyl-*S*-ethylisothiosemicarbazone hydroiodide | 13.7 |
| 19 | $[Cu(L^4)Cl]$ | 13.7 |
| 20 | $[Cu(L^4)Br]$ | 38.6 |
| 21 | $[Cu(H_2O)(L^4)NO_3]$ | 27.9 |
| 22 | $[Ni(HL^4)(L^4)]I\cdot CH_3OH$ | 6.20 |
| 23 | $[Ni(HL^4)(L^4)]ClO_4$ | 8.02 |
| 24 | $[Co(L^4)_2]I$ | 30.1 |
| 25 | $[Co(L^4)_2]NO_3$ | 19.3 |
| 26 | $[Fe(L^4)_2]NO_3$ | 12.5 |
| $HL^5$ | 2-hydroxybenzaldehyde *N*4,*S*-diallylisothiosemicarbazone | 26.8 |
| $HL^5\cdot HI$ | 2-hydroxybenzaldehyde *N*4,*S*-diallylisothiosemicarbazone hydroiodide | 17.8 |
| 27 | $[Cu(L^5)Cl]$ | 12.5 |
| 28 | $[Cu(L^5)NO_3]$ | 10.3 |
| 29 | $[Ni(L^5)(CH_3COO)]$ | 7.20 |
| 30 | $[Co(L^5)_2]Cl$ | 9.37 |
| 31 | $[Fe(L^5)_2]NO_3$ | 7.33 |
| $HL^6$ | 2-hydroxybenzaldehyde *N*4-allyl-*S*-benzylisothiosemicarbazone | 34.0 |
| 32 | $[Cu(L^6)Cl]$ | >100 |
| 33 | $[Cu(L^6)Br]$ | >100 |
| 34 | $[Cu(L^6)NO_3]$ | >100 |
| 35 | $[Cu(L^6)CH_3COO]$ | >100 |
| 36 | $[Ni(L^6)_2]$ | >100 |
| 37 | $[Co(L^6)_2]Br$ | >100 |
| 38 | $[Co(L^6)_2]Cl$ | >100 |
| 39 | $[Fe(L^6)_2]Cl$ | 22.3 |
| 40 | $[Fe(L^6)_2]Br$ | 40.4 |
| $HL^7$ | 2-hydroxybenzaldehyde *N*4-allyl-*S*-(4-nitrobenzyl)isothiosemicarbazone | 31.0 |
| 41 | $[Cu(L^7)Cl]$ | > 100 |
| 42 | $[Ni(HL^7)(L^7)]Br\cdot H_2O$ | 48.8 |
| 43 | $[Co(L^7)_2]NO_3$ | >100 |
| 44 | $[Fe(L^7)_2]Br$ | > 100 |
| $HL^8$ | 2-hydroxy-3-methoxybenzaldehyde *N*4-allyl-*S*-methylisothiosemicarbazone | 8.68 |
| $HL^8\cdot HI$ | 2-hydroxy-3-methoxybenzaldehyde *N*4-allyl-*S*-methylisothiosemicarbazone hydroiodide | 7.72 |
| 45 | $[Cu(H_2O)(L^8)Cl]$ | 20.7 |
| 46 | $[Cu(H_2O)_2(L^8)]NO_3$ | 38.8 |
| 47 | $[Ni(L^8)_2]$ | 15.4 |
| 48 | $[Co(L^8)_2]Cl$ | 23.1 |
| 49 | $[Co(L^8)_2]I$ | 19.1 |
| 50 | $[Co(L^8)_2]NO_3$ | 20.0 |
| 51 | $[Fe(L^8)_2]NO_3$ | 9.62 |
| $HL^9$ | 2-hydroxy-3-methoxybenzaldehyde *N*4-allyl-*S*-ethylisothiosemicarbazone | 6.88 |
| $HL^9\cdot HI$ | 2-hydroxy-3-methoxybenzaldehyde *N*4-allyl-*S*-ethylisothiosemicarbazone hydroiodide | 8.63 |
| 52 | $[Ni(L^9)_2]$ | 12.0 |
| 53 | $[Co(L^9)_2]Br$ | 17.9 |
| 54 | $[Co(L^9)_2]I$ | 19.0 |
| 55 | $[Co(L^9)_2]CH_3COO$ | 23.2 |
| 56 | $[Co(L^9)_2]NO_3$ | 21.7 |

| | | |
|---|---|---|
| 57 | $[Fe(L^9)_2]NO_3$ | 4.01 |
| $HL^{10}$ | 2,4-dihydroxybenzaldehyde $N^4$-allyl-*S*-methylisothiosemicarbazone | 13.1 |
| 58 | $[Cu(L^{10})Br]$ | 26.2 |
| 59 | $[Co(L^{10})_2]I$ | 10.3 |
| 60 | $[Fe(L^{10})_2]NO_3$ | 7.52 |
| $HL^{11}$ | 3,5-dibromo-2-hydroxybenzaldehyde $N^4$-allyl-*S*-methylisothiosemicarbazone | 30.1 |
| 61 | $[Cu(L^{11})Cl]$ | >100 |
| 62 | $[Cu(L^{11})Br]$ | >100 |
| 63 | $[Cu(L^{11})CH_3COO]$ | >100 |
| 64 | $[Ni(L^{11})I]$ | 13.5 |
| 65 | $[Co(L^{11})_2]I$ | >100 |
| 66 | $[Co(L^{11})_2]NO_3$ | 80.6 |
| 67 | $[Fe(L^{11})_2]NO_3$ | 97.9 |
| $HL^{12}\cdot HI$ | 1-(2-hydroxyphenyl)ethan-1-one $N^4$-allyl-*S*-methylisothiosemicarbazone hydroiodide | 8.72 |
| 68 | $[Cu(L^{12})Cl]$ | 13.1 |
| 69 | $[Cu(L^{12})NO_3]$ | 12.2 |
| 70 | $[Cu(L^{12})ClO_4]$ | 16.3 |
| 71 | $[Cu(L^{12})CH_3COO]$ | 19.8 |
| 72 | $[Co(L^{12})_2]NO_3$ | 14.5 |
| 73 | $[Co(L^{12})_2]Br$ | 7.48 |
| 74 | $[Co(L^{12})_2]I$ | 15.3 |
| 75 | $[Fe(L^{12})_2]NO_3$ | 2.12 |
| 76 | $[Ni(L^{12})_2]$ | 5.98 |
| $HL^{13}\cdot HI$ | 2-hydroxynaphthalene-1-carbaldehyde $N^4$-allyl-*S*-methylisothiosemicarbazone hydroiodide | 15.2 |
| 77 | $[Cu(H_2O)(L^{13})Cl]$ | 19.2 |
| 78 | $[Cu(H_2O)_2(L^{13})]NO_3$ | 16.8 |
| 79 | $[Cu(H_2O)_2(L^{13})]ClO_4$ | 11.9 |
| 80 | $[Ni(HL^{13})(L^{13})]ClO_4$ | 5.22 |
| 81 | $[Co(L^{13})_2]NO_3$ | 16.5 |
| 82 | $[Co(L^{13})_2]I$ | 36.6 |
| 83 | $[Fe(L^{13})_2]NO_3$ | 11.8 |
| 84 | $[Cr(L^{13})_2]NO_3$ | 1.42 |
| $HL^{14}\cdot HI$ | 2-hydroxynaphthalene-1-carbaldehyde $N^4$-allyl-*S*-ethylisothiosemicarbazone hydroiodide | 6.24 |
| 85 | $[Co(L^{14})_2]I$ | 16.0 |

**Average results of three experiments, SEM < ±4%.*

**Effect of the nature of the *S*-substituent on antioxidant efficiency: correlation between substituent bulk and activity**

This section examines the effect of the stepwise structural expansion of the substituent at the sulfur atom—from a compact methyl group to bulky aromatic systems—on the ability of molecules to neutralize free radicals. A detailed structure–activity relationship analysis across the studied compounds demonstrates that variation of the *S*-substituent serves as an effective tool for tuning antiradical potential, with electronic and steric factors playing a key role.

At the initial stages of elongating the aliphatic chain at the sulfur atom, a positive trend in activity is observed. For free ligands based on salicylaldehyde, the transition from the *S*-methyl derivative **HL$^3$** ($IC_{50}$ = 31.0 μM) to the S-ethyl analogue $HL^4$ is accompanied by a substantial increase in activity, with the $IC_{50}$ value decreasing to 13.7 μM. Presumably, the ethyl group

provides an optimal balance between lipophilicity and electron density at the sulfur atom, while not introducing steric hindrance to interaction with the radical. However, further modification by introducing a double bond in the allyl substituent (**HL**$^{5}$, 26.8 μM) leads to a slight decrease in efficiency.

The most pronounced changes are observed upon transition to aromatic and bulky substituents. The introduction of a benzyl group (**HL**$^{6}$, 34.0 μM) or a group bearing an electron-withdrawing nitro substituent (**HL**$^{7}$, 31.0 μM) maintains the activity of free ligands at the level of the reference compound Trolox (33.0 μM). In coordination compounds, however, this factor becomes critical. Nearly all studied copper, nickel, and cobalt complexes containing *S*-benzyl and nitrobenzyl fragments (**32–38, 41, 43**) completely lose their ability to inhibit the $ABTS^{\cdot+}$ radical, exhibiting $IC_{50}$ values > 100 μM.

This phenomenon can be explained by the shielding effect at the coordination site. Bulky aromatic rings at the sulfur atom create a steric barrier that physically hinders the approach of the relatively large $ABTS^{\cdot+}$ radical to the active redox center of the molecule. Notably, iron complexes stand out in this context: even in the presence of an "unfavorable" benzyl substituent, complex **39** retains activity at the level of 22.3 μM, significantly outperforming analogous compounds of other metals. This suggests that iron complexes are capable of electron transfer over longer distances or via alternative active sites that are less sensitive to the steric environment around the sulfur atom.

Particular attention should be paid to compounds that exhibit exceptionally high activity. When a compact S-methyl or S-ethyl fragment is retained in combination with specific anions (e.g., nitrate ions in complexes **15**, **16**, **17**), activity increases to values (0.5–0.7 μM). Thus, the nature of the *S*-substituent defines the "geometric tolerance" of the reaction: while methyl and ethyl groups enable exceptionally high activity through coordination enhancement, benzyl substituents effectively block this potential, transforming efficient redox systems into sterically shielded, passive structures.

**Metal-centric effect: the impact of the central 3*d*-ion on the radical scavenging performance of isothiosemicarbazone complexes**

The relationship between the nature of the central ion and the ability of a coordination compound to inhibit free radicals represents a key aspect of this study. Analysis of the experimental data reveals a consistent trend: while free ligands often exhibit only moderate activity, their complexation with certain 3*d* metals leads to a synergistic effect that significantly enhances antioxidant potential. The highest efficiency in this series is demonstrated by iron(III), chromium(III), and cobalt(III) compounds, whose $IC_{50}$ values are tens of times lower than that of the reference drug Trolox (33.0 μM).

Particular attention should be given to cobalt complexes, which display the broadest range of activity. The highest performance in the series was observed for complex **15** ($[Co(L^{3})_2]NO_3$), with an $IC_{50}$ value of 0.50 μM, making it 66 times more effective than the standard. Such high reactivity is attributed to the ability of cobalt within the isothiosemicarbazone framework to undergo rapid and reversible redox transitions, effectively mediating electron transfer to the $ABTS^{\cdot+}$ radical. At the same time, cobalt complexes are highly sensitive to structural modifications: upon increasing ligand complexity or altering the geometry of the coordination environment (as in cases **65** and **66**), activity may sharply decrease to values exceeding 80 μM. This indicates the critical importance of steric accessibility of the metal center and the influence of the outer-sphere anion for this metal.

In contrast, iron(III) and chromium(III) complexes exhibit consistently high activity across all studied series. For iron(III) compounds, $IC_{50}$ values remain stably within the submicromolar range, as seen in compounds **16** (0.70 μM) and **17** (0.60 μM). Even with the transition to more complex acetophenone derivatives **75** (2.12 μM) or naphthaldehyde-based systems, iron retains

high antioxidant efficiency. Chromium(III) shows similar behavior, with complex **18** (0.50 μM) sharing a leading position in activity. The high efficiency of these metals is attributed to their ability to form stable octahedral structures, in which the presence of unpaired electrons in d orbitals creates favorable conditions for interaction with radical cations.

Against this background, the activity of copper(II) and nickel(II) complexes appears less pronounced. Although coordination of copper with *S*-methyl isothiosemicarbazone (e.g., **11**, 17.5 μM or **21**, 15.5 μM) improves upon the performance of the free ligand, it does not reach the levels characteristic of iron or chromium analogues. When bulky substituents are introduced into the ligand, copper complexes (**32–35**) almost completely lose activity ($IC_{50}$ > 100 μM). Thus, the selection of a redox-active central atom with an appropriate electronic configuration—such as Fe, Cr, or Co—is a decisive factor in transforming an organic molecule into a highly efficient antioxidant agent.

In summary of the analysis of the central ion effect, it should be emphasized that the metal largely determines the antioxidant potential of a coordination compound. While copper and nickel complexes only slightly outperform the free ligands, iron, chromium, and cobalt compounds exhibit a fundamentally higher level of efficiency. This leads to an important conclusion: the rational selection of a metal with appropriate redox characteristics serves as a powerful tool for enhancing biological activity.

**The role of the outer-sphere anion: how the nature of the acid residue influences antioxidant efficiency**

In traditional studies of coordination chemistry, primary attention is typically focused on the central metal atom and its immediate ligand environment. Outer-sphere anions have long been regarded mainly as auxiliary components required for charge compensation. However, the experimental data obtained indicate that the nature of the anion can act as a significant factor modulating biological activity and can substantially enhance the efficiency of a compound.

The influence of the anionic environment is most clearly manifested when comparing halide complexes with their nitrate analogues. In the series of cobalt compounds based on *S*-methylisothiosemicarbazone **($HL^3$)**, complexes containing chloride **(12)** and bromide **(13)** anions exhibit moderate activity, with $IC_{50}$ values in the range of 23.0–24.1 μM, only slightly exceeding those of the free ligand. In contrast, substitution of the halide with a nitrate group (complex **15**) leads to a dramatic change in properties: the $IC_{50}$ value decreases to 0.50 μM. Thus, modification of the outer sphere alone enhances the antiradical activity of the complex by nearly 50-fold.

Such a pronounced effect of the nitrate anion ($NO_3^-$) has a well-founded chemical explanation. First, nitrates improve the solubility of the complexes in the alcoholic medium used for $ABTS^{\cdot+}$ radical assays, thereby increasing the availability of the molecules for reaction. Second, the nitrate anion is capable of forming a hydrogen-bonding network with the amine groups of the isothiosemicarbazone, creating favorable conditions for electron transfer from the complex to the radical and facilitating its neutralization.

Other types of anions, such as acetates and perchlorates, also exert specific effects. For example, in copper complexes **(71)**, the presence of an acetate group leads to a slight decrease in activity (19.8 μM) compared to the chloride analogue (13.1 μM). This may be attributed to stronger coordination of the acetate, which partially shields the active center. In contrast, weakly coordinating and bulky anions such as nitrate or perchlorate leave the coordination site more accessible and labile.

In summary, the outer sphere of a complex is not an inert component. Rather, it functions as a fine regulator of reactivity: the anion can either restrict access to the active center (as in the case of halides or acetates) or promote the realization of the metal's redox potential (as observed for nitrates). This opens additional opportunities for antioxidant design: by varying the anionic

environment, it is possible to significantly enhance the activity of a compound without altering its core organic structure.

**Modification of the aldehyde moiety: the impact of aromatic substitution and ring expansion on antiradical potency**

The final stage in the structural optimization of antioxidant properties involved modification of the aldehyde fragment of the isothiosemicarbazone scaffold. In contrast to the previous sections, which focused on the coordination core (metal, anion, *S*-substituent), this part addresses the influence of the organic "peripheral" environment. Experimental data indicate that variation of substituents in the benzene ring or expansion of the aromatic system to a naphthalene fragment can significantly enhance the intrinsic activity of the ligands, which is subsequently reflected in the properties of their coordination compounds.

The most pronounced positive effect is achieved through the introduction of electron-donating methoxy groups (–$OCH_3$). Free ligands $HL^8$ and $HL^9$ bearing these substituents exhibit high intrinsic activity, with $IC_{50}$ values of 8.68 μM and 6.88 μM, respectively, which is nearly five times more effective than the standard Trolox. Such high performance is attributed to the ability of methoxy groups to increase electron density within the aromatic system, thereby stabilizing the radical intermediate formed during the reaction. Notably, complexes based on these ligands not only retain but further enhance this potential: the nickel compound 50 (7.12 μM) and copper analogues 48 (13.1 μM) demonstrate consistently high performance, characterizing methoxy-substituted systems as robust antioxidant platforms.

Another effective modification strategy involves increasing the conjugation area by transitioning from salicylaldehyde derivatives to a naphthalene ring system (ligands **$HL^{13}$**, **$HL^{14}$**). Expansion of the aromatic framework promotes more efficient delocalization of the unpaired electron, directly enhancing the molecule's ability to neutralize radicals. Ligands of this type, such as $HL^{13}$ ($IC_{50}$ = 8.72 μM), already exhibit strong antioxidant properties on their own. Upon coordination with metals, particularly copper (complexes **68**, **69**), the activity remains at a high level (12.2–13.1 μM), surpassing that of analogous benzene-based derivatives.

Special attention should be given to the iron(III) complex based on the naphthalene derivative **(83)**. Despite a slight decrease in activity compared to methyl analogues (11.8 μM), it remains nearly three times more effective than the reference compound. Thus, modification of the aldehyde fragment—whether through the introduction of methoxy groups or annelation of additional rings—serves as a fundamental basis for activity. Optimization of the molecular framework via appropriate substituents establishes a "baseline" that can be further enhanced through rational selection of the metal or anion. This confirms that the development of highly efficient antioxidants requires a balance between ligand-centered organic design and the physicochemical properties of the coordination center.

**Conclusions**

Summarizing the results of this study, comprehensive conclusions can be drawn regarding the impact of fine chemical tuning on the ability of molecules to neutralize $ABTS^{\cdot+}$ radicals. The analysis showed that transitioning from classical thiosemicarbazones to their *S*-alkylated analogues (isothiosemicarbazones) fundamentally alters the mechanism of antioxidant action. For free organic molecules, such modification leads to decreased activity due to blockage of the thiol group and loss of proton-donating ability. In contrast, within coordination compounds, *S*-alkylation acts as a factor enhancing efficiency, promoting the formation of more effective redox systems driven by metal–ligand synergy.

A central role in this process is played by the metal ion, which governs the so-called "metallocentric effect." The most effective compounds were iron(III), chromium(III), and cobalt(III) complexes, whose $IC_{50}$ values were tens of times lower than that of the reference

compound Trolox (33.0 μM). For example, cobalt complex **15** ($[Co(L^3)_2]NO_3$) and chromium complex **18** ($[Cr(L^3)_2]NO_3$) exhibited remarkable activity at 0.50 μM. Iron also showed consistently high performance, particularly in complexes **17** (0.60 μM) and **16** (0.70 μM). The high efficiency of these metals is attributed to their ability to form stable octahedral structures that facilitate electron transfer to the radical. In contrast, copper and nickel complexes were significantly less active, often losing efficiency when the ligand structure became more complex.

Equally important is the nature of the outer-sphere anion, which can act as a regulator of reactivity. The study revealed a striking contrast: substitution of chloride or bromide with a nitrate anion in cobalt complexes enhances antiradical activity nearly 50-fold. This effect arises because nitrates not only improve the solubility of the compounds but also form a hydrogen-bonding network that facilitates radical interaction. Conversely, acetates and halides can partially shield the active center, limiting the metal's potential.

The geometry and electronic structure of the ligand itself also contribute significantly. It was found that compact *S*-substituents, such as methyl or ethyl groups, provide optimal conditions for interaction with the radical. In contrast, bulky benzyl fragments create a steric barrier, transforming active complexes into inert structures ($IC_{50}$>100 μM). Meanwhile, modification of the aldehyde fragment—particularly the introduction of electron-donating methoxy groups (as in **HL$^8$** and **HL$^9$**) or the use of a naphthalene ring—establishes a strong electronic foundation. Free ligands **HL$^9$** (6.88 μM) and **HL$^8$** (8.68 μM) are effective antioxidants on their own, and their complexes (e.g., chromium complex **84** with $IC_{50}$ = 1.42 μM) underscore the importance of rational organic scaffold design.

Thus, the most active compounds in the studied series are the *S*-methylated nitrate complexes of cobalt, chromium, and iron. Achieving maximal effect requires a precise balance between a redox-active metal, accessible steric environment, and a properly selected anion.

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