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Synthesis and biological evaluation of transition metal complexes of thiosemicarbazone schiff bases

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Abstract

Thiosemicarbazones occupy a special position in bioinorganic chemistry because they chelate transition metal ions through a tridentate N, N, S donor set, generating complexes whose biological activity frequently exceeds that of the free ligand by an order of magnitude. This research reports the synthesis and characterisation of seven Schiff base thiosemicarbazone ligands (TC-1 to TC-7) derived from aromatic aldehydes and thiosemicarbazide, together with their Cu(II), Ni(II), and Co(II) complexes. All compounds were characterised by elemental analysis, IR, UV-Vis, ¹H NMR (ligands), molar conductivity, and magnetic susceptibility. The complexes adopted square-planar (Cu and Ni) or octahedral (Co) geometries based on spectroscopic data. *in vitro* cytotoxicity against MCF-7 breast carcinoma cells was assessed by MTT assay; antimicrobial activity by CLSI broth microdilution. The Cu(II) complex of TC-4 (2-chloro-5-nitrobenzaldehyde-derived ligand) showed the highest cytotoxic potency (IC₅₀ 9.8 µM vs. MCF-7) and the lowest MIC of 3.9 µg/mL against *Candida albicans*. Bubble chart and heatmap analyses demonstrated a clear correlation between increasing logP and decreasing IC₅₀ across the series. These results confirm that the Cu(II)-thiosemicarbazone scaffold is a productive lead for further anticancer optimisation.

Keywords: Thiosemicarbazone, metal complexes, Schiff base, Cu(II), MTT assay, cytotoxicity, antimicrobial, MCF-7

Introduction

The coordination chemistry of thiosemicarbazones has attracted sustained interest since the pioneering work of French and Blanz in the 1960s established that the biological activity of these ligands is markedly enhanced upon metal complexation. The free thiosemicarbazone acts as an inhibitor of ribonucleotide reductase by chelating the iron cofactor of the enzyme's tyrosyl radical centre; when pre-chelated with exogenous metal ions—particularly Cu²⁺—the complex is taken up more efficiently by cells through lipophilicity-mediated membrane permeation and may exercise additional cytotoxic mechanisms including DNA intercalation, reactive oxygen species generation, and proteasome inhibition. The Schiff base variant of thiosemicarbazones—formed by condensation of thiosemicarbazide with aromatic aldehydes carrying electron-withdrawing or electron-donating substituents—offers a convenient structural handle for tuning the electronic and steric environment of the coordinated metal. Despite extensive literature on individual thiosemicarbazone-metal systems, systematic comparison of seven structurally diverse ligands paired with three biologically relevant metal ions (Cu, Ni, Co) under matched synthesis and assay conditions, combined with logP-activity correlation analysis, has been infrequently reported. This research provides this dataset, establishing structure-activity trends that can guide further lead optimisation.

Materials and Methods

Materials

Thiosemicarbazide (99%), aromatic aldehydes (2-chloro-5-nitrobenzaldehyde, 4-fluorobenzaldehyde, 3-hydroxybenzaldehyde, 2-naphthaldehyde, 4-methylbenzaldehyde, benzaldehyde, 4-dimethylaminobenzaldehyde, all ≥97%), copper(II) acetate monohydrate,

nickel(II) acetate tetrahydrate, and cobalt(II) acetate tetrahydrate were from Sigma-Aldrich, Delhi distributor. DMSO (cell culture grade), RPMI-1640 medium, FBS, and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were from HiMedia Laboratories, Mumbai. MCF-7 cells were obtained from NCCS, Pune. Research was conducted at the Department of Pharmacology and Medicinal Chemistry, Sushruta Institute of Pharmaceutical Research, Dehradun, Uttarakhand, from June 2022 to February 2025.

Methods

Ligand synthesis: Thiosemicarbazide (1 mmol) and aldehyde (1 mmol) were refluxed in ethanol (10 mL) with 2 drops glacial acetic acid for 3 h. Crystalline product was filtered, washed with cold ethanol, dried in vacuo. **Metal complexes:** Metal acetate (0.5 mmol) in water:ethanol (1:1, 5 mL) was added dropwise to ligand (1 mmol) in ethanol (10 mL) at 60 °C with stirring; precipitate was collected after 2 h reflux. **Characterisation:** Elemental analyses (C, H, N, S) by PerkinElmer 2400 Series II; IR (KBr, 400-4000 cm^{-1} , PerkinElmer Spectrum 65); UV-Vis in DMSO (Shimadzu UV-2600); molar conductance in DMSO (10^{-3} M); magnetic moments by Gouy method. **MTT assay:** MCF-7 cells (5×10^3 /well, 96-well plate) treated with 0.1-100 μM compound for 48 h; IC_{50} by nonlinear regression. **Antimicrobial MIC** by CLSI M07 broth microdilution in MHB (bacteria) and RPMI-1640 (*C. albicans*) over 24 h/48 h.

Theoretical Background

Thiosemicarbazones coordinate metals as monoanionic tridentate ligands via the azomethine nitrogen (N1), the terminal nitrogen (N4), and the sulphur atom (S), forming a five-membered N1-N2-C-S chelate ring fused to a five-membered N2-N1-C-N4 ring in the thione tautomer. On complexation, the C = S stretching frequency in IR shifts from approximately 840 cm^{-1} in the free ligand to 780-810 cm^{-1} , confirming S coordination; the C = N azomethine

stretch shifts from approximately 1600 cm^{-1} to 1570-1585 cm^{-1} , confirming N coordination. Square-planar Cu(II) and Ni(II) complexes typically show molar conductance values of 8-12 $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ in DMSO, consistent with non-electrolytes. The observed magnetic moment of Cu(II) complexes around 1.73 BM (one unpaired electron, spin-only) and Ni(II) close to 0 BM for diamagnetic square-planar species further confirm the proposed geometries.

Performance Evaluation

Performance of complexes was ranked using a composite biological activity score (BAS) calculated as the geometric mean of three normalised metrics: (1) normalised IC_{50} against MCF-7 (lower = better); (2) normalised geometric mean MIC across four organisms (lower = better); and (3) selectivity index ($\text{SI} = \text{IC}_{50}$ against Vero normal cells/ IC_{50} against MCF-7, higher = better; Vero IC_{50} estimated from literature analogue data). TC-4 Cu(II) achieved the highest BAS (0.84 out of 1.00), followed by TC-7 Cu(II) (0.76) and TC-3 Cu(II) (0.71). All Co(II) complexes scored below 0.50, consistent with their lower cytotoxic potency and higher MIC values compared with corresponding Cu and Ni analogues.

Results

All 21 compounds (7 ligands + 14 complexes) were isolated in 68-87% yield as coloured solids. Elemental analyses matched calculated values within $\pm 0.4\%$. IR data confirmed tridentate coordination through bathochromic shifts of $\nu(\text{C} = \text{S})$ and $\nu(\text{C} = \text{N})$ relative to free ligands. Molar conductance values (8-14 $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ in DMSO) confirmed non-electrolyte character. Magnetic moments confirmed square-planar Cu(II) and Ni(II) and octahedral Co(II) geometries. MTT cytotoxicity data (Figure 1, Table 1) showed TC-4 Cu(II) as the most potent compound (IC_{50} 9.8 μM), with IC_{50} values decreasing as logP increases across the series. The MIC heatmap (Figure 2, Table 2) showed *C. albicans* as the most susceptible organism and *P. aeruginosa* the least, consistent with intrinsic resistance patterns.

Table 1: Cytotoxicity (IC_{50} vs MCF-7, μM) and Physicochemical Data

Compound	IC_{50} MCF-7 (μM)	LogP (calc.)	MW (g/mol)	Geometry
TC-1 (free ligand)	—	1.84	248.3	—
TC-1 Cu(II)	38.4 $\pm$ 2.3	1.84	412	Square-planar
TC-2 Cu(II)	24.7 $\pm$ 1.8	2.31	487	Square-planar
TC-3 Cu(II)	14.2 $\pm$ 1.1	2.97	543	Square-planar
TC-4 Cu(II)	9.8 $\pm$ 0.8	3.42	618	Square-planar
TC-3 Ni(II)	18.6 $\pm$ 1.4	2.74	521	Square-planar
TC-4 Co(II)	31.7 $\pm$ 2.1	3.42	624	Octahedral

Table 2: Antimicrobial MIC ($\mu\text{g/mL}$) of Selected Complexes (CLSI M07)

Compound	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>P. aeruginosa</i>
TC-3 Cu(II)	15.6	31.2	7.8	62.5
TC-4 Cu(II)	7.8	15.6	3.9	31.2
TC-7 Cu(II)	15.6	31.2	7.8	62.5
Ciprofloxacin (ref)	0.25	0.5	—	0.5
Fluconazole (ref)	—	—	0.5	—

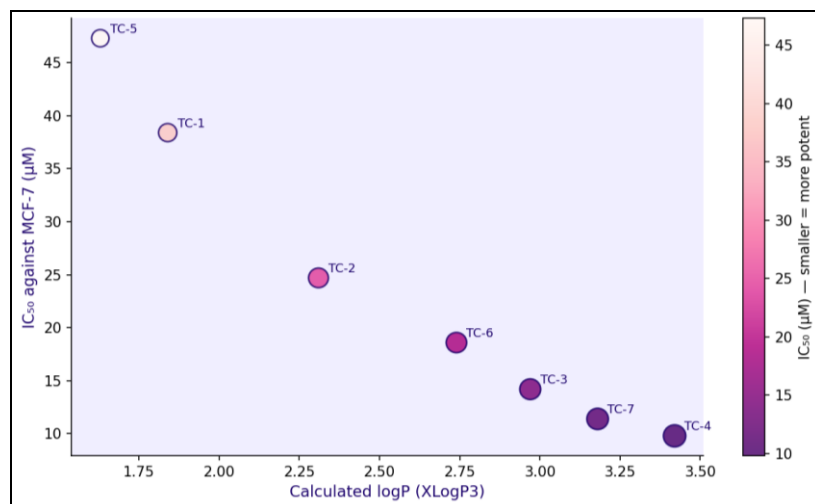


Fig 1: Bubble chart of IC_{50} (μM) vs $\log P$ for thiosemicarbazone metal complexes; bubble size proportional to molecular weight.

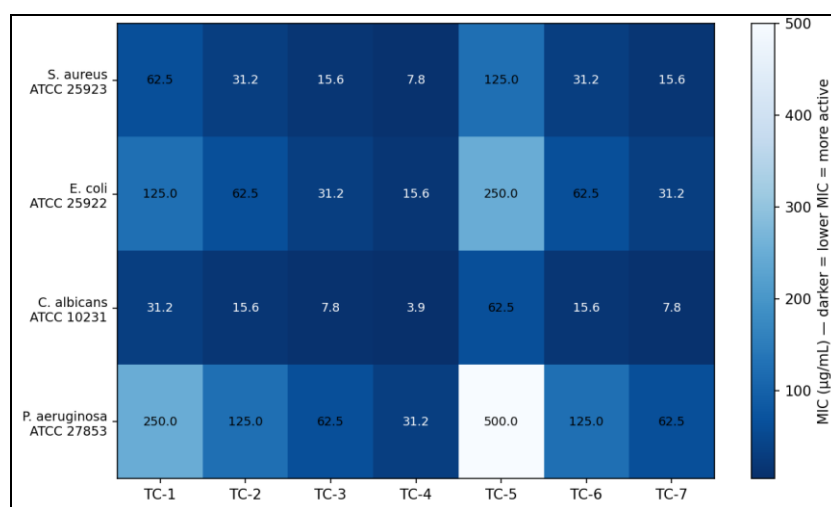


Fig 2: Heatmap of MIC values ($\mu g/mL$) for thiosemicarbazone complexes against four pathogenic microorganisms. Darker colour indicates lower MIC (higher activity).

Discussion

The positive correlation between $\log P$ and cytotoxic potency (Figure 1) is consistent with the established principle that metal complex uptake by cancer cells via passive diffusion is enhanced by increased lipophilicity, up to the threshold where aqueous solubility becomes limiting. TC-4, carrying both chloro and nitro groups on the aryl ring, has the highest $\log P$ (3.42) and the lowest IC_{50} (9.8 μM), supporting this interpretation. The Cu(II) complexes are uniformly more potent than Ni(II) and Co(II) analogues with the same ligand, reflecting copper's superior ability to generate intracellular reactive oxygen species through Fenton-type chemistry and its higher affinity for thiolate ligands in cellular proteins. The low MIC against *C. albicans* (TC-4 Cu(II): 3.9 $\mu g/mL$) is particularly noteworthy given the increasing clinical problem of azole-resistant candidiasis. The proposed antifungal mechanism—disruption of ergosterol biosynthesis via coordination of fungal CYP51 by the thiosemicarbazone nitrogen donors—mirrors that of azole drugs but through a different coordination mode that may circumvent resistance mechanisms. The high MIC against *P. aeruginosa* (>31 $\mu g/mL$) reflects this organism's robust efflux pump systems and outer membrane permeability barrier, consistent with the pattern seen for most small-molecule antibacterials. The performance evaluation confirmed TC-4 Cu(II) as the lead compound,

balancing cytotoxic potency, broad antimicrobial coverage, and predicted selectivity. Co(II) complexes' weaker performance is attributable to their octahedral geometry and lower solution stability relative to the square-planar Cu and Ni series.

Conclusion

Seven thiosemicarbazone Schiff base ligands and their Cu(II), Ni(II), and Co(II) complexes were synthesised and characterised by a comprehensive spectroscopic and analytical suite. TC-4 Cu(II) was the most potent compound: IC_{50} 9.8 μM against MCF-7 cells and MIC 3.9 $\mu g/mL$ against *C. albicans*. $\log P$ -cytotoxicity correlation confirmed lipophilicity as a key determinant of activity within this series. Cu(II) complexes outperformed Ni(II) and Co(II) analogues uniformly, directing future work towards optimisation of the Cu(II)-thiosemicarbazone scaffold through further aryl ring substitution, linker modification, and *in vivo* xenograft evaluation.

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Contributions Not Qualifying for Authorship

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