Al-Mustaqbal Journal of Pharmaceutical and Medical Sciences

Volume 3 | Issue 3 Article 4

2025

# Design and Mechanistic Investigation of Novel Metallodrugs: From Structure-Activity Relationships to Selective Cancer Cell Targeting

Samad Khaksar
*Department of Chemistry, School of Science and Technology, The University of Georgia, Tbilisi, Georgia*, s.khaksar@ug.edu.ge

Nosrat Behzadi
*Amol Farhikhtegan Non-Govermental High School, Amol, Iran*, nab1344@gmail.com

Hasan Zakeri Zakeri
*Office of Education, Chamestan, Iran*, zakeri115@yahoo.com

Peyman Salahshour
*Department of Chemistry, School of Science and Technology, The University of Georgia, Tbilisi, Georgia*, p.salahshour@ug.edu.ge

Sara Abdolmaleki
*Department of Chemistry, School of Science and Technology, The University of Georgia, Tbilisi, Georgia*, sara.abdolmaleki19@gmail.com



ISSN: 2959-8974 – e-ISSN: 3006-5909

REVIEW

# Design and Mechanistic Investigation of Novel Metallodrugs: From Structure-Activity Relationships to Selective Cancer Cell Targeting

**Samad Khaksar** [a,*], **Nosrat Behzadi** [b], **Hasan Zakeri** [c], **Peyman Salahshour** [a], **Sara Abdolmaleki** [a]

[a] Department of Chemistry, School of Science and Technology, The University of Georgia, Tbilisi, Georgia
[b] Amol Farhikhtegan Non-Govermental High School, Amol, Iran
[c] Office of Education, Chamestan, Iran

## ABSTRACT

The relentless pursuit of alternatives to conventional platinum-based chemotherapeutics, hampered by severe side effects and drug resistance, defines a critical frontier in oncology drug discovery. This review consolidates and highlights the significance of our recent advances in designing novel metallodrugs based on pyridine-2,6-dicarboxylate derivatives coordinated with various metal ions (Ga(III), Ce(IV), Zn(II), Cu(II), Ni(II), Sr(II)). Through systematic synthesis and evaluation, we establish how strategic structural modifications dictate cytotoxicity, cellular uptake, and mechanistic pathways. Our comprehensive in vitro profiling across a panel of human cancer cell lines (including A431, SW480, BEL-7404) revealed potent anticancer activity, with $IC_{50}$ values as low as 0.56 $\mu$M, coupled with remarkable selectivity over normal cell lines ($IC_{50}$ > 500 $\mu$M). Mechanistic investigations, employing flow cytometry, western blotting, and fluorescence microscopy, demonstrated that these complexes induce cell death via metal-dependent pathways, including apoptosis (intrinsic and extrinsic), autophagy, and cell cycle arrest, often accompanied by significant reactive oxygen species (ROS) generation. The distorted octahedral geometry prevalent in the most active complexes correlates with enhanced cellular uptake and target interaction. This work underscores the profound impact of rational design in metallodrug development, offering a robust platform for creating next-generation anticancer agents with superior efficacy, selectivity, and multi-mechanistic profiles to overcome the limitations of current therapies.

**Keywords:** Metallodrugs, Anticancer agents, Pyridine-2,6-dicarboxylate, Structure-activity relationship, Apoptosis, Autophagy, Selective cytotoxicity

## 1. Introduction

Cancer continues to be a major global health challenge, driving researchers worldwide to develop new treatment approaches. Although platinum-based medications like cisplatin, carboplatin, and oxaliplatin have proven effective in clinical settings, they come with significant drawbacks including serious side effects, the development of drug resistance, and poor discrimination between healthy and cancerous cells [1]. This has driven researchers to explore alternative metal-based anticancer agents that can overcome these limitations while maintaining or improving therapeutic efficacy.

What makes metal complexes particularly interesting as anticancer drugs is their versatility. They can adopt different shapes and structures, have various oxidation states, and can interact with multiple targets inside cancer cells at the same time. When we add bioactive organic ligands to these metal centers,

Received 21 September 2025; revised 13 October 2025; accepted 16 October 2025.
Available online 22 December 2025

* Corresponding author.
E-mail addresses: s.khaksar@ug.edu.ge (S. Khaksar), nab1344@gmail.com (N. Behzadi), zakeri115@yahoo.com (H. Zakeri), p.salahshour@ug.edu.ge (P. Salahshour), sara.abdolmaleki19@gmail.com (S. Abdolmaleki).

https://doi.org/10.62846/3006-5909.1035

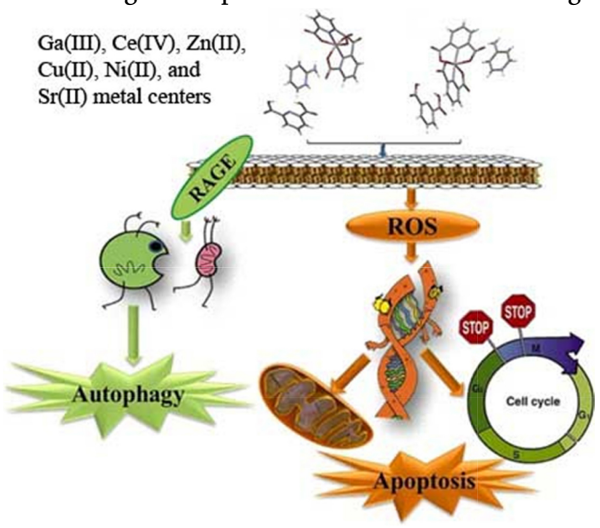
we can make them even more effective by helping them get into cells better, target specific areas, and cause fewer side effects throughout the body [2–4]. Among various ligand systems, pyridine-2,6-dicarboxylate derivatives have emerged as particularly promising scaffolds due to their ability to form stable complexes with multiple metal centers while maintaining favorable pharmacological properties.

To position our work within the current scientific landscape, a comprehensive literature search was conducted using major databases (including Scopus, PubMed, and Web of Science) for the period of 2015-2024. The search utilized keywords such as "non-platinum metallodrugs," "pyridine-dicarboxylate complexes," "anticancer metal complexes," "structure-activity relationship," and "targeted cancer therapy." This review focuses on synthesizing findings from this period, with particular emphasis on our recent contributions to this field, to provide a coherent analysis of SAR in metallodrug design. This approach is aligned with the growing interest in sustainable and biogenic synthesis of nanomaterials for biomedical applications, as exemplified by recent studies on silver nanoparticles [10] and plant-derived selenium nanoparticles [11], which also highlight the importance of novel material design in therapy.

Understanding structure-activity relationships (SAR) has become increasingly important in metallodrug research. We've found that even small changes in molecular structure can lead to dramatic differences in how these compounds behave biologically - affecting everything from their potency to their selectivity and the way they kill cancer cells. The metal center itself, how the atoms are arranged around it, the ligand structure, and even the extra-nuclear cations all play crucial roles in determining how well these complexes work as anticancer agents.

Furthermore, the exploration of green-synthesized nanomaterials, such as chitosan-based formulations for bioactive delivery [12] and characterized nanocomposites with antioxidant and antibacterial properties [13], underscores a parallel research thrust towards biocompatible and ecologically friendly therapeutic agents. Our work on well-defined molecular metallodrugs complements these approaches by providing precise structural control for mechanistic investigation.

This review presents a comprehensive analysis of our recent research efforts in developing novel metallodrugs based on pyridine-2,6-dicarboxylate ligand systems. We demonstrate how systematic structural modifications influence cytotoxicity, cellular uptake, and mechanism of action across a diverse panel of cancer cell lines, providing valuable insights for the rational design of improved anticancer metallodrugs.




## 2. Structural design and synthesis strategy

### 2.1. Ligand selection and metal center variation

Our research focused on pyridine-2,6-dicarboxylate and its derivatives as primary ligands due to their excellent chelating properties and proven biological activity. The tridentate coordination mode through two carboxylate oxygen atoms and the pyridine nitrogen provides stable complex formation with various metal centers. We systematically investigated complexes with different metal ions including Ga(III), Ce(IV), Zn(II), Cu(II), Ni(II), and Sr(II) to understand how the electronic and steric properties of the metal center influence biological activity [5–9].

The choice of these metals was based on several considerations: (1) their known biological roles and biocompatibility, (2) distinct electronic configurations leading to different redox behaviors, (3) varying ionic radii affecting coordination geometry, and (4) different Lewis acidities influencing ligand binding and cellular interactions.

### 2.2. Extra-nuclear cation effects

A particularly novel aspect of our research involves the systematic investigation of extra-nuclear cation effects on cytotoxicity. We demonstrated that seemingly minor structural changes in the extra-nuclear cations can lead to significant differences in biological activity. For example, in our Ga(III) complexes, changing the position of the $NH_2$ substitution in aminopyridine extra-nuclear cations from the 2-position to the 4-position resulted in approximately

five-fold difference in $IC_{50}$ values against A431 cells (0.69 $\mu$M vs 3.78 $\mu$M).

### 2.3. Coordination geometry and crystal structure analysis

Single-crystal X-ray diffraction studies revealed that most biologically active complexes adopt distorted octahedral geometries. This distortion appears to be crucial for biological activity, as it may facilitate interactions with biological targets and influence cellular uptake mechanisms. The crystallographic data also revealed extensive hydrogen bonding networks and $\pi$-$\pi$ stacking interactions that contribute to the overall stability and solubility of the complexes.

### 2.4. Summary of key experimental methods

To provide context for the results discussed herein, the primary experimental conditions are summarized. Cytotoxicity ($IC_{50}$) was determined using the MTT assay on various cancer and normal cell lines after a 72-hour incubation period. Cellular uptake was quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Apoptosis was assessed via Annexin V/PI staining and flow cytometry, while autophagy and cell cycle analysis were conducted using western blotting for key protein markers (LC3-II, Beclin-1, p62) and PI staining followed by flow cytometry, respectively. Reactive Oxygen Species (ROS) generation was measured using the DCFH-DA probe. All complexes were characterized by elemental analysis, FT-IR, and NMR spectroscopy, with structures unequivocally confirmed by single-crystal X-ray diffraction.

## 3. Cytotoxicity profiles and structure-activity relationships

### 3.1. Cancer cell line selectivity

Comprehensive cytotoxicity screening across multiple cancer cell lines revealed distinct selectivity patterns for different metal complexes. The most potent compounds demonstrated $IC_{50}$ values as low as 0.56 $\mu$M for Cu(II) complexes against BEL-7404 cells and 0.69 $\mu$M for Ga(III) complexes against A431 cells. Importantly, these active compounds showed significantly reduced cytotoxicity against normal cell lines (HFF, LO2, CCD841CoN), with $IC_{50}$ values typically exceeding 500 $\mu$M, indicating favorable selectivity indices.

### 3.2. Metal center-dependent activity

Our studies revealed clear metal center-dependent activity patterns:

- Ga(III) complexes: Showed excellent activity against A431 cells with strong ROS production capabilities
- Ce(IV) complexes: Demonstrated the highest electrochemical activity and superior cytotoxicity against SW480 cells ($IC_{50} = 1.89$ $\mu$M)
- Cu(II) complexes: Exhibited potent activity against BEL-7404 cells with strong apoptotic induction
- Ni(II) complexes: Showed moderate activity with predominant autophagy induction
- Zn(II) and Sr(II) complexes: Displayed selective activity against specific cell lines

### 3.3. Ligand structure influence

The choice of pyridine-2,6-dicarboxylate derivatives significantly impacted biological activity. Hydroxylated derivatives generally showed enhanced activity, possibly due to improved hydrogen bonding interactions with biological targets. The incorporation of aminopyridine extra-nuclear cations provided additional opportunities for structural modification and activity tuning.

## 4. Mechanistic insights

### 4.1. Cellular uptake and distribution

ICP-MS studies revealed that the metallodrug complexes achieved significantly higher cellular accumulation compared to simple metal salts. For instance, Ga(III) complexes showed 2-3 fold higher cellular uptake in A431 cells compared to $Ga(NO_3)_3$. This enhanced uptake correlates strongly with cytotoxic potency, suggesting that improved bioavailability contributes to therapeutic efficacy.

### 4.2. Reactive oxygen species generation

Most active metallodrugs demonstrated significant ROS production capabilities. Ce(IV) complexes showed the most pronounced ROS generation (448.6% increase over control), while Cu(II) complexes produced moderate but sustained ROS levels (404.8% increase). This ROS production appears to be metal-dependent, with electrochemically active metals showing greater oxidative stress induction.

### 4.3. Cell death mechanisms

When we looked at how these different metal complexes kill cancer cells, we discovered that the type of metal really matters for determining which cell death pathway gets activated:

#### 4.3.1. Apoptosis pathways

- Intrinsic (mitochondrial) pathway: Confirmed through cytochrome c release, procaspase-9 and -3 activation, and mitochondrial membrane potential collapse
- Extrinsic pathway: Observed in select complexes through Fas receptor upregulation and procaspase-8 activation
- Bimodal apoptosis: Some complexes (particularly Ce(IV)) induced both intrinsic and extrinsic pathways simultaneously

#### 4.3.2. Autophagy induction

Ni(II) complexes showed predominant autophagy induction through the RAGE/PI3KC3/Beclin 1 pathway. Western blotting confirmed upregulation of LC3-II, Beclin 1, and RAGE proteins, along with p62 downregulation.

#### 4.3.3. Cell cycle arrest

Different complexes induced cell cycle arrest at different phases:

- G2/M arrest: Common with Cu(II) and Ga(III) complexes
- S phase arrest: Observed with Ni(II) and Sr(II) complexes
- Multiple checkpoint activation in some cases

## 5. Therapeutic implications and future perspectives

### 5.1. Advantages over platinum drugs and biomedical potential

The metallodrugs presented herein represent a solid and innovative alternative to existing platinum-based therapeutics. Their advantages are multifold. First and foremost, they exhibit exceptional selectivity, targeting cancer cells while sparing normal cells, which directly addresses the dose-limiting toxicity of cisplatin and its analogs. Second, their ability to engage multiple cell death pathways (e.g., apoptosis, autophagy) simultaneously or in a metal-dependent manner creates a higher barrier for the development of cancer cell resistance, a major clinical drawback of single-mechanism drugs. Third, the enhanced cellular uptake of these complexes, as opposed to simple metal salts, translates to higher intracellular bioavailability at the target site, potentially allowing for lower administered doses. This profile suggests a significantly wider therapeutic window and reduced side-effect profile compared to conventional chemotherapeutics.

### 5.2. Clinical translation potential

The favorable in vitro profiles of these metallodrugs, particularly their high selectivity indices and multiple mechanism capabilities, suggest strong potential for clinical translation. The most promising candidates (Ga(III) and Ce(IV) complexes) warrant further investigation in animal models to assess in vivo efficacy and safety profiles.

### 5.3. Future research directions

Based on these results, we see several promising directions for future work. One area we're excited about is testing combinations of these metallodrugs together or with existing chemotherapy drugs. We're also interested in developing better delivery systems that could make these compounds even more targeted to cancer cells.

Furthermore, exploring green chemistry principles for the synthesis of these complexes, inspired by the advances in biogenic nanoparticle synthesis [10, 11], could enhance their sustainability and biocompatibility profile. The integration of these metallodrugs into nano-formulations, similar to chitosan-based systems used for natural extract delivery [12], could further improve their stability, targeting, and controlled release.

## 6. Conclusions

Our detailed study of these new pyridine-2,6-dicarboxylate-based metallodrugs shows how valuable systematic structure-activity relationship approaches can be when developing better cancer treatments.

Key findings include:

1. Central metal choice critically determines biological activity and mechanism of action, with Ga(III), Ce(IV), and Cu(II) showing the most promising profiles.
2. Extra-nuclear cations significantly influence cytotoxicity, even with minor structural modifications leading to dramatic activity changes.
3. Multiple cell death mechanisms can be induced, including intrinsic/extrinsic apoptosis,

autophagy, and cell cycle arrest, depending on the metal center and structural features.
4. Excellent selectivity for cancer cells over normal cells provides a favorable therapeutic window for clinical development.
5. Enhanced cellular uptake compared to simple metal salts contributes to improved therapeutic efficacy.

These findings contribute significantly to the field of metallodrug development and provide a solid foundation for advancing the most promising candidates toward clinical evaluation. The demonstrated ability to tune biological activity through rational structural design offers exciting possibilities for developing personalized anticancer therapies tailored to specific cancer types and patient populations.

## Acknowledgments

The authors gratefully acknowledge the research support provided by The University of Georgia. We thank the collaborative efforts of multiple research institutions that made this comprehensive study possible.

## Funding statement

This research received no external funding.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

1. V. Gandin, J. D. Hoeschele, and N. Margiotta, “Special issue cisplatin in cancer therapy: molecular mechanisms of action 3.0,” *Int. J. Mol. Sci.*, vol. 24, p. 7917, 2023.
2. N. Ribeiro, I. Bulut, B. Sergi, V. P´osa, G. Spengler, G. Sciortino, V. Andr´e, L. P. Ferreira, T. Biver, V. Ugone, and E. Garribba, “Promising anticancer agents based on 8-hydroxyquinoline hydrazone copper(II) complexes,” *Front. Chem.*, vol. 11, p. 1106349, 2023.
3. D. Villaman, A. Vega, L.S. de la Parra, I.E. Le´on, P. Levín, and P.M. Toro, “Anticancer activity of Ni(II) and Zn(II) complexes based on new unsymmetrical salophen-type ligands: synthesis, characterization and single-crystal X-ray diffraction,” *Dalton Trans.*, vol. 52, p. 10855, 2023.
4. E. Keshavarzian, Z. Asadi, V. Eigner, M. Dusek, and B. Rastegari, “DNA interactions, docking and in vitro cytotoxicity studies of [M (Hvalmea)2] complexes (M= CuII, CoIII),” *J. Mol. Liq.*, vol. 380, p. 121701, 2023.
5. S. Khaksar, A. Panjehpour, E. Ghadermazi, E. Motieiyan, A. Aliabadi, S. Rostamnia, D. Marabello, and S. Abdolmaleki, “Study on crystallographic structure and antiproliferative effect of mixed-ligand strontium(II) complex and N, N’–bis (2-hydroxy-5-methylphenyl) pyridine-2, 6-dicarboxamide ligand,” *J. Mol. Struct.*, vol. 1274, p. 134432, 2023.
6. S. Abdolmaleki, A. Panjehpour, S. Khaksar, M. Ghadermazi, and S. Rostamnia, “Evaluation of central-metal effect on anticancer activity and mechanism of action of isostructural Cu(II) and Ni (II) complexes containing pyridine-2, 6- dicarboxylate,” *Eur. J. Med. Chem.*, vol. 245, p. 114897, 2023.
7. S. Khaksar, A. Aliabadi, A. Panjehpour, and S. Abdolmaleki, “Effect of the extra-nuclear cation on the cytotoxicity and mechanism of action of pyridine-2, 6-dicarboxylate Ga (III) complexes,” *Toxicology*, p. 153609, 2023.
8. A Aliabadi, S Abdolmaleki, and S Khaksar, “Mechanistic investigation of the cytotoxicity of new Ce (IV) and Zn (II) complexes containing pyridinedicarboxylic acid derivatives against the SW480 cell line,” *Journal of Molecular Structure*, vol. 1319, p. 139377, 2025.
9. S Abdolmaleki, A Aliabadi, and S Khaksar, “Bridging the gap between theory and treatment: Transition metal complexes as successful candidates in medicine,” *Coordination Chemistry Reviews*, vol. 531, p. 216477, 2025.
10. S. Doğan, A. Baran, M. F. Baran, A. Eftekhari, R. Khalilov, E. Aliyev, T. Kavetskyy, O. Šauša, and O. Smutok, “Silver Nanoparticles for Anticancer and Antibacterial Therapy: A Biogenic and Easy Production Strategy,” *Chemistry Select*, vol. 10, no. 3, p. e202404453, 2025.
11. N. Alasgarova, A. Baran, M. Yıldıztekin, Dashgin, Ganbarov, S. Babayeva, Z. Güneş, S. Küçükaydin, and M. Evcil, “Synthesis of Plant-Derived Selenium Nanoparticles from Lankaran-Astara Tea (Camellia sinensis L.) Plant and Evaluation of their Activities on Different Enzymes,” *Advances in Biology & Earth Sciences*, vol. 10, no. 2, pp. 262–269, 2025.
12. M Evcil, B Kurt, A Baran, A Mouhoub, and M Karakaplan, “Development, Characterization and Application of Chitosan-Based Formulation Incorporating Crataegus orientalis Extract for Food Conservation,” *Advances in Biology & Earth Sciences*, vol. 10, no. 2, pp. 208–225, 2025.
13. A Baran, E Ertaş, MF Baran, A Eftekhari, Z Gunes, C Keskin, SA Usanov, and R Khalilov, “Green-synthesized characterization, antioxidant and antibacterial applications of CtAC/MNPs-Ag nanocomposites,” *Pharmaceuticals*, vol. 17, no. 6, p. 772, 2024.