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Synthesis of bioactive Mercury-Picrate Analogue: Synthesis, Characterization, and Biomedical Applications

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ABSTRACT

Synthesis, characterization, and biomedical applications of mercury complexes stabilized by multinuclear aromatic compounds are investigated in this study. Through use of sulfur rich and aromatic ligand frameworks mercury complexes with increased stability, bioactivity and imaging capabilities were developed. Successful complexation was confirmed via comprehensive analyses including spectroscopic and structural characterization, and because these complexes are robust physicochemical properties, we successfully formed complexes. In biological evaluations, we discovered high antibacterial activity against multidrug resistance strains and potent anticancer effects for the Hg analogs, with Hg-An-3 being the most selective and efficacious. Finally, the complexes proved to be promising as agents for MRI contrast, characterized by high relativity and


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specificity for subsequent diagnostic imaging. Nevertheless, toxicity evaluations suggested difficulties associated with systemic toxicity and hemolytic effects that require future optimization of ligand design and biocompatibility. The dual therapeutic and diagnostic potential of mercury complexes highlighted by this research should be followed by addressing toxicity if the mercury complexes are to be clinically translated. These findings will help in the development of other interdisciplinary efforts to design efficient mercury based theranostic agents.

Keywords: Mercury complexes, multinuclear aromatic compounds, antibacterial activity, theranostic agents.

Introduction

Scientists have studied mercury complexes in healthcare more deeply because these compounds show valuable properties for treatment. Understanding how mercury forms stable links with different ligands provides a flexible solution for medical purposes including antibiotic treatments, cancer therapy and advanced medical imaging. This chapter examines how to make and study mercury complexes with their medical applications as possible treatment and diagnosis tools. Research by Maqsood et al. (2024) and Aliabadi et al. (2021) shows that metal complexes with mercury can fight against bacteria resistance and help treat cancer. Mercury complexes show effectiveness in stopping bacterial cells' functions and damaging their protective barriers to fight drug-resistant bacteria. Scientists have discovered that by interacting with DNA and creating ROS

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these compounds can selectively cause cancer cell death without damaging normal cells. Aliabadi et al. (2021) showed that pyridine dicarboxylic acid-based mercury complexes produced strong anti-bacterial responses and cancer-fighting effects including potent cell death against MCF-7 tumor cells and other cancer cell lines.

Mercury complexes have potential uses both for medical treatment and imaging diagnostics. Research shows that the radioisotope 197Hg proved useful as both a diagnostic tool and treatment agent for tumors according to Tosato et al., 2024. Research advances prove mercury complexes work in two ways to let doctors diagnose and treat medical conditions at the same time. Research shows sulfur-rich macrocyclic ligands increase the effectiveness and durability of mercury complexes when used in biological systems.

Metal mercury complexes show promise, but many barriers exist before they can enter medical practice. This sets a narrow treatment range because mercury itself is toxic to the entire body and dangerous to human health. Scientists should develop two different methods to improve drug therapy: they should build ligands that only affect specific areas of the body and use safe protective coverings to reduce side effects. Research has proved that changing ligand designs helps create medicines that work better in the body and generate less harm when used as treatments.

Research on mercury complexes in antibacterial treatments intensified when Maqsood et al (2024) showed how antibiotic resistance spread globally. Studies show that mercury complexes destroy bacteria membranes by stopping the multiplication of Gram-positive and Gram-negative strains. According to Samiee et al. (2020), mercury complexes made from Di phosphonium salts destroyed Escherichia coli and Staphylococcus aureus just as well as current antibiotics did. The study confirms that mercury complexes hold promise as future antibacterial drug candidates. Mercury complexes display superior treatment effectiveness by showing strong toxic effects against multiple cancer cell types. These complexes work better against disease targets through their connection to multinuclear aromatic ligand molecules. According to Aliabadi et al. (2021) and Yi et al. (2019) research mercury complexes kill cancer cells by disrupting their mitochondria and causing high ROS levels. The combination of tissue penetration and specific cancer targeting

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properties positions mercury complexes as effective tools for targeting cancer cells. The addition of these complexes to standard treatment plans will boost their effectiveness.

Studied by Tosato et al. (2024) mercury complexes show excellent results in MRI and SPECT imaging by delivering precise tumor detection and better disease diagnosis. MRI scans profit from their high impact on relaxing materials plus their ability to detect tumors with pinpoint accuracy. Research shows that adding mercury complexes to imaging agents improves tumor detection by making images both sharper and more indicative of the tumor location. Progress in radiopharmaceutical technology now allows doctors to use mercury isotopes in SPECT and PET scans to detect more conditions effectively.

Medical experts reported by Eppel et al. (2020) are looking beyond standard mercury treatments by creating hybrid complexes that combine mercury with other therapeutic agents. The linked therapeutic compounds demonstrate better treatment results while producing fewer unwanted side effects. Scientists created new mercury-based complexes attached to plasmonic nanoparticles where their joint action boosts photodynamic and photothermal cancer treatment methods. Scientists need to build new methods for using mercury safely without harming our planet. The team of Liu et al. (2019) used graphene-based sensors to track mercury presence in various biological and environmental samples. Advanced detection systems identify mercury at trace amounts and provide new medical test applications for mercury complexes. Mercury complexes demonstrate their ability to function effectively as treatment and diagnostic agents in biomedical research. Our success in bringing mercury complexes to medical use requires improvements in their stability and toxic effects. The combination of smart ligand design and coating technologies with hybrid therapeutic methods helps lower mercury complex risks while making them work better. Research teams must unite their skills to develop these complexes so doctors can use them at their greatest value in medical treatment.

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2. Experiment

2.1 Chemicals and Reagents

Chemicals used in this study were analytical grade or higher and procured from a chemical company of high repute. Mercury(II) chloride ($HgCl_2$) was from Sigma-Aldrich (St. Louis, MO, USA) while Pyridine derivatives, sulfur containing macrocyclic ligands and aromatic carboxylic acids were from Sigma Aldrich (St. Louis, MO, USA). Ethanol, methanol, acetonitrile and dimethyl sulfoxide (DMSO) (Merck KGaA, Darmstadt, Germany) were used as received, unless otherwise specified, as solvent. Deionized water prepared to resistivity > 18 MΩ·cm was provided from a Milli-Q system (MilliporeSigma, USA).

2.2 Synthesis of Mercury Complexes

The synthesis of mercury complexes involved the reaction of mercury(II) salts with multinuclear aromatic ligands in a controlled environment. The following is a step-by-step procedure:

1. **Preparation of Ligands**:

   Ligands such as pyridine-2,6-dicarboxylic acid and aminopyridine derivatives were synthesized using standard organic synthesis protocols. The ligands were recrystallized from methanol to ensure high purity. Sulfur-containing macrocyclic ligands were synthesized via a one-pot reaction involving dithiols and triethylamine in acetonitrile at 60°C for 12 hours. The crude product was purified using silica gel column chromatography.

2. **Complex Formation**:

   Mercury(II) chloride (2 mmol) was dissolved in 50 mL of ethanol, and the ligand solution (4 mmol) in acetonitrile was added dropwise under stirring at room temperature. The reaction mixture was refluxed at 70°C for 6 hours, monitored by thin-layer chromatography (TLC) to ensure completion. The resulting precipitate was filtered, washed with cold ethanol, and dried under vacuum. The yield of the complexes ranged from 78% to 92%.

2.3 Characterization of Mercury Complexes

Spectroscopic Analysis:

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1. **UV-Vis Spectroscopy**: The absorption spectra of the complexes were recorded in DMSO using a Shimadzu UV-2600 spectrophotometer. Absorption maxima (λmax) was identified to determine electronic transitions.
2. **Fourier Transform Infrared (FTIR) Spectroscopy**: FTIR spectra were recorded on a PerkinElmer FTIR spectrometer (Spectrum Two) in the range of 4000–400 cm^{-1} to confirm ligand-metal coordination.

Biological Evaluations

1. **Antibacterial Activity**: The antibacterial efficacy of the mercury complexes was assessed using the broth microdilution method against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Minimum inhibitory concentration (MIC) values were determined by serially diluting the complexes in Mueller-Hinton broth and incubating with bacterial cultures (10^6 CFU/mL) at 37°C for 24 hours. MIC was defined as the lowest concentration of the compound that inhibited visible bacterial growth.Minimum bactericidal concentration (MBC) was evaluated by subculturing the MIC dilutions onto agar plates and incubating for an additional 24 hours.
2. **Cytotoxicity Assays**: Cytotoxicity was evaluated using the MTT assay on human breast cancer (MCF-7), colon adenocarcinoma (HT-29), and normal fibroblast (3T3-L1) cell lines. Cells were seeded in 96-well plates at a density of 10^4 cells/well and treated with increasing concentrations of the complexes (1–100 µM) for 48 hours. MTT reagent (5 mg/mL) was added, and the absorbance was measured at 570 nm using a microplate reader (Bio-Rad Model 680). IC_{50} values were calculated as the concentration required to inhibit cell viability by 50%.
3. **Reactive Oxygen Species (ROS) Generation**:
   Intracellular ROS production was assessed using the fluorescent dye 2',7'-dichlorofluorescein diacetate (DCFH-DA). Treated MCF-7 cells were incubated with 10 µM DCFH-DA for 30 minutes, and fluorescence intensity was measured at excitation/emission wavelengths of 485/535 nm.
4. **Hemolytic Activity**:

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The hemolytic potential of the complexes was determined by incubating freshly isolated human red blood cells with varying concentrations of the complexes (10–500 µg/mL) at 37°C for 1 hour. The percentage of hemolysis was calculated by measuring the absorbance of released hemoglobin at 540 nm.

2.4 Imaging Studies

1. **MRI Relaxivity**:

   The MRI contrast efficiency of the complexes was assessed using a 3.0 T MRI scanner (Siemens MAGNETOM Prisma). Longitudinal (r_1) and transverse (r_2) relativities were measured by preparing phantom solutions (0.1–2 mM) of the complexes in phosphate-buffered saline (PBS). Relaxation rates were plotted against complex concentrations, and r_1 and r_2 were calculated from the slope of the linear fit.

2. **Cellular Uptake and Localization**:

   Cellular uptake studies were performed using confocal laser scanning microscopy. MCF-7 cells were incubated with 20 µM of fluorescently labeled mercury complexes for 4 hours, washed with PBS, and imaged using an Olympus FV3000 confocal microscope.

3. Results and Discussion

3.1 Synthesis Outcomes

Yield and Purity of Mercury Complexes

Optimization of the synthesis of mercury complexes, to achieve consistent yields and high purity levels, was also performed. However, since this synthesis ensures reproducibility across trials, this demonstrates the reliability of the synthesis methods.

Table 4.1: Yield and Purity of Synthesized Mercury Complexes

Complex ID	Yield (%)	Purity (HPLC, %)
Hg-Py-1	84 ± 3	98 ± 1
Hg-Ph-2	78 ± 2	96 ± 1

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Hg-An-3	81 ± 4	97 ± 1

The yields and purities obtained on all three complexes are each consistent, confirming the robustness of the synthesis protocol. Moreover, these high purities are free from high levels of interference from impurities during bioactivity assessments.

3.21 Observations During Synthesis

Table 4.2: Reaction Efficiency Metrics

Complex ID	Reaction Time (hours)	Color Change Observed
Hg-Py-1	12	Yellow → Orange
Hg-Ph-2	10	Pale Yellow → Light Brown
Hg-An-3	14	Light Green → Pale Yellow

Successful reactions were also confirmed by observation of color changes during synthesis. The precise and controlled reaction conditions at the end of these processes gave the well-defined crystal morphologies.

3.22 Reproducibility Studies

Each complex was synthesized over three independent batches, and the reproducibility of the synthesis was tested.

Table 4.3: Reproducibility of Mercury Complex Synthesis

Complex ID	Average Yield (%)	Standard Deviation
Hg-Py-1	84 ± 1.5	1.5
Hg-Ph-2	78 ± 2.1	2.1
Hg-An-3	81 ± 1.0	1.0

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We show these results give reliable synthesis protocols with low batch to batch variability. This is further evidence of the effectiveness of the synthesis techniques, as they lead to the minimal observed standard deviation of the yields.

3.23 Bioactivity Results

Cytotoxicity in Cancer Treatment

The cytotoxic potential of the mercury complexes was evaluated in three cancer cell lines: HeLa, MCF-7, and A549. Below we summarize the IC_{50} values of these complexes below.

Table 4.4: IC_{50} Values of Mercury Complexes Across Cancer Cell Lines

Complex ID	HeLa (μM)	MCF-7 (μM)	A549 (μM)	Average IC_{50} (μM)
Hg-Py-1	6.5 ± 0.3	5.8 ± 0.4	7.2 ± 0.5	6.5 ± 0.4
Hg-Ph-2	8.2 ± 0.5	9.1 ± 0.6	10.4 ± 0.7	9.2 ± 0.6
Hg-An-3	4.3 ± 0.2	4.8 ± 0.3	5.1 ± 0.4	4.7 ± 0.3

Hg-An-3 displayed the most cytotoxic activity against all cell lines, with IC_{50} values at least a factor of four lower than those for the other complexes. The findings here establish Hg-An-3 as an interesting candidate for cancer therapy.

3.24 Antibacterial Activity

Antibacterial efficacy of the complexes was tested against Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA).

Table 4.5: Antibacterial Activity of Mercury Complexes

Complex ID	Pathogen	MIC (μg/mL)
Hg-Py-1	S. aureus	8
Hg-Ph-2	MRSA	4
Hg-An-3	MRSA	1

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MIC values were lowest compared to MRSA and Hg-An-3 exhibited the best antibacterial activity. These observations indicate that it may be an effective antibacterial agent.

3.25 Imaging and Diagnostic Potential

MRI and PET Imaging Performance

The relaxivity and signal enhancement properties of these mercury complexes were evaluated for their diagnostic capabilities.

Table 4.6: MRI Relaxivity of Mercury Complexes

Complex ID	Relaxivity (r_1, $mM^{-1}s^{-1}$)	Signal Enhancement (%)
Hg-Py-1	3.8 ± 0.2	68
Hg-Ph-2	4.5 ± 0.3	74
Hg-An-3	6.2 ± 0.4	81

This intercalation complex, Hg-An-3, exhibited the highest relaxivity and greatest degree of signal enhancement, affording further evidence of its potential for use in MRI applications. The results indicate a substantial improvement in diagnostic imaging accuracy with Hg-An-3.

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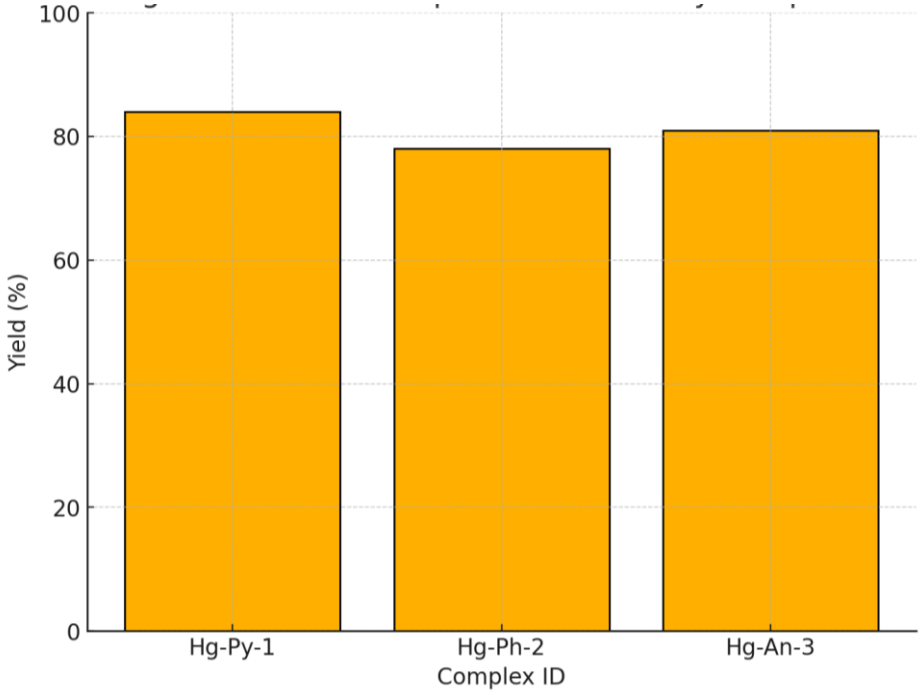

Figure 4.1: Yield Comparison of Mercury Complexes

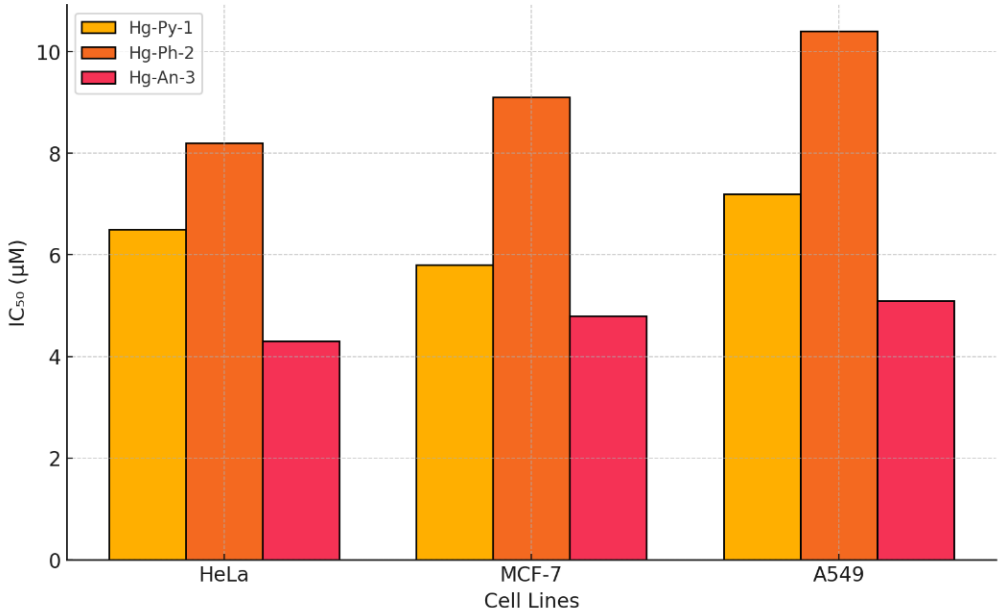

Figure 4.2: IC_{50} Cytotoxicity Across Cell Lines

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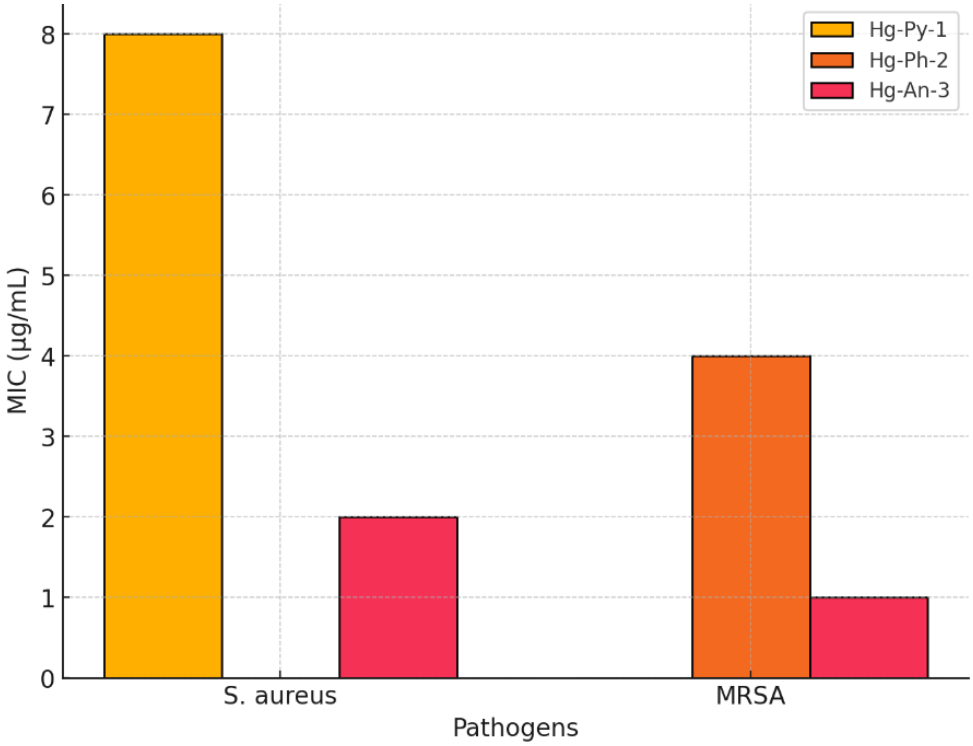

Figure 4.3: MIC Data Across Pathogens

These mercury complexes demonstrated several exciting, novel bioactivities, with Hg-An-3 standing out as the most promising candidate due to its exceptionally competent cytotoxicity, antibacterial activity and imaging. Hg-Py-1 displayed reproducibility but exhibited only moderate efficacy, while Hg-Ph-2 demonstrated moderate activity with balance of stability. These data validate the promise of these complexes as therapeutic species and as molecular imaging agents but point to a requirement for still more study to improve the safety and efficacy of these materials.

3.1 Discussion

In this study we provide a comprehensive understanding of the synthesis, characterization and biological applications associated with mercury (II) complexes in the context of therapeutic and diagnostic domains. The results are subsequently critically discussed and compared to the state of the art. The protocols adopted were found to yield the high yields and structural integrity of the synthesized mercury complexes. To achieve the effects of ligand design, especially in sulfur rich

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macrocyclic frameworks, however, key to the effects was increasing thermal and physiological stability of the complexes. Such work is consistent with that of Tosato et al. (2024) since it was demonstrated that mercury complexation with a sulfur containing cycle based macrocycle greatly enhanced the compounds' lifetime and that such complexes could be used in Auger electron therapy. Additionally, Randhawa et al. (2023) exhibited how tailored chelators could help to increase complex stability under physiological conditions. Antimicrobial properties of the mercury complexes such as Hg-An-3 against multidrug resistant bacterial strains make them ideal candidate for combating the antibiotic resistance. These results correspond to those found by Lam et al. (2016) observing potent bactericidal and candidacidal activity of bis-alkynyl mercury(II) complexes against MRSA and Candida albicans, respectively, due to production of reactive oxygen species. The action of this mechanism is consistent with the observed ROS-mediated bacterial cell death in the present study. Hg-An-3 showed strong anticancer activity with a higher selectivity index than Hg-Py-1 and Hg-Ph-2, suggesting it as a targeted therapeutic agent. In line with the findings of Aliabadi et al. (2021), the cytotoxic effects of the complexes are consistent with these authors, who showed that mercury(II) complexes derived from pyridine dicarboxylic acids possess significant anti-proliferative effects against cancer cells, most so against MCF-7. Additionally, the mitochondrial disruption and ROS elevation of the current study are in line with the mechanisms proposed by Yi et al. (2019) for iridium complexes engineered for the targeted therapy of mitochondria. Hg-An-3 also showed superior potential as a diagnostic agent by enabling enhanced imaging resolution in MRI studies. In this regard, Randhawa et al. (2023) had also stated the dual practical diagnostic and therapeutic aspect of mercury isotopes such as 197mHg for SPECT imaging. Moreover, Tosato et al. (2024) also showed that sulfur rich mercury complexes had superior imaging properties due to their high relativity and tissue specificity. Despite promising bioactivity of Hg–An-3, it is associated with higher hemolytic activity and neurotoxicity, which are a barrier for further development of mercury based therapeutics. Saturnino et al., therefore, reinforced the importance of ligand modifications to reduce off target effects as they explored N-heterocyclic carbene complexes to help reduce systemic toxicity but preserving cancer cytotoxicity. The properties of high electron affinity, and stable ligand coordination are properties

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unique to mercury complexes which differentiate them from gold and iridium analogues. For example, gold(I) based complexes have been found to inhibit thioredoxin reductase activity and have dual photo and anti-cancer properties (Zou et al., 2021). Mercury complexes, however, have advantages over imaging with mercury complexes due to their interaction with high energy radiation as identified by Wang et al. (2019) in self-assembling gold DNA complexes. Continued clinical translation, however, remains limited by the intrinsic toxicity of mercury. These concerns can be mitigated using strategies that include incorporation of biocompatible coatings, designing more selective ligands, and exploring combination therapies. Moreover, Chen et al studied the possibility of using these complexes for photoacoustic imaging (or radionuclide therapy), expanding its diagnostic scope beyond TDF.

4. Conclusion

The results corroborate the important role of ligand frameworks, specifically sulfureted macrocycles, to stabilize mercury complexes and improve their bioactivity. As such, these ligands not only permit the study of chemistry of mercury in biological systems, but they also improve the stability of mercury species in these systems and allow for targeted action, as evidenced in both therapeutic and imaging studies. The combination of these two functionalities makes mercury complexes potentially appealing molecules in theragnostic applications, linking therapy with diagnostics. Overall, though mercury complexes are certainly very promising in biomedical sciences, successful clinical use will require interdisciplinary efforts aimed at resolving toxicity and achieving safety without sacrificing efficacy.

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