ChemMedChem

Review
doi.org/10.1002/cmdc.202300645

Chemistry Europe
European Chemical Societies Publishing

www.chemmedchem.org

The Therapeutic Potential in Cancer of Terpyridine-Based Metal Complexes Featuring Group 11 Elements

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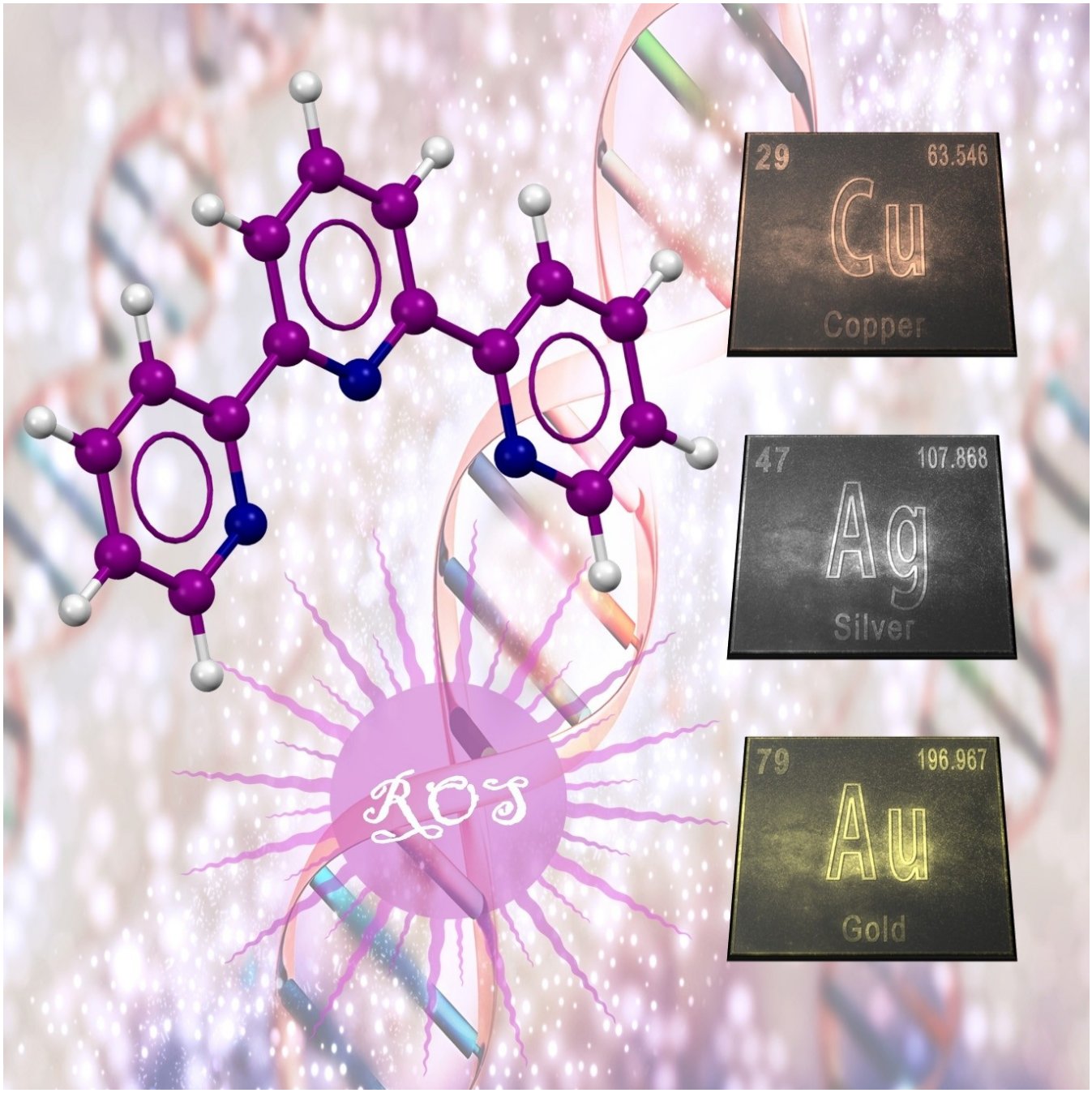

Terpyridine-based complexes with group 11 metals emerge as potent metallodrugs in cancer therapy. This comprehensive review focuses on the current landscape of anticancer examples, particularly highlighting the mechanisms of action. While Cu(II) complexes, featuring diverse ancillary ligands, dominate the field, exploration of silver and gold species remains limited. These complexes exhibit significant cytotoxicity against various cancer cell lines with a commendable selectivity for non-tumorigenic cells. DNA interactions, employing intercalation and groove binding, are pivotal and finely tuned through terpyridine ligand functionalization. In addition, copper complexes showcase nuclease activity, triggering apoptosis through ROS generation. Despite silver's high affinity for nitrogen donor atoms, its exploration is relatively sparse, with indications of acting as intercalating agents causing DNA hydrolytic cleavage. Gold(III) compounds, overshadowing gold(I) due to stability concerns, not only intercalate but also induce apoptosis and disrupt the mitochondrial membrane. Further investigations are needed to fully understand the mechanism of action of these compounds, highlighting the necessity of exploring additional biological targets for these promising metallodrugs.

1. Introduction

The era of metallodrugs in medicine was initiated with the introduction of platinum-based anticancer agents, with cisplatin leading the way as one of the earliest anticancer drugs.[1] Despite its known side effects and resistance issues in various cancers, cisplatin continues to be used, even in the presence of newer alternatives. The initial success of cisplatin paved the way for extensive research into other platinum-containing compounds.[2] Additionally, metals, especially ruthenium[3] and gold,[4] have demonstrated effectiveness in similar applications, with several of these metallodrugs entering clinical trials for the treatment of various types of cancer. Research carried out has provided valuable insights into the mechanisms of action and the kinetics of ligand exchange in these compounds.[5]

The success of metal-based drugs is contingent on several factors, including not only their specific structure but also the structure and properties of the ligands bonded to the metal center, and consequently, they often differ from typical purely organic molecules. Combining various organic ligands with metals can yield a wide range of properties and biological activities. Numerous organic scaffolds suitable for designing different complexes have been described to date, with nitrogen-containing heterocycles being the most commonly employed.[6]

Terpyridine-based ligands have gained significant attention across various disciplines, including supramolecular chemistry, materials science, and coordination chemistry, due to their distinctive characteristics. In this study, we will focus on exploring the intriguing anticancer properties exhibited by terpyridine complex compounds.[7]

It is noteworthy that while terpyridine-based metal complexes exhibit excellent biological properties, the free ligands themselves also display inherent anticancer activity. Information about the biological properties of these free ligands is limited in the literature. However, Musiol *et al.* have conducted a comprehensive review on this subject.[8] In Figure 1, selected structures of free terpyridine-based ligands with cytotoxicity are presented. It is important to highlight that when terpyridines are substituted with aromatic moieties, they exhibit enhanced antiproliferative activity, as well as improved intercalating potency and DNA degradation. This improvement can be attributed to their higher lipophilicity and enhanced permeability through biological membranes.[9]

This review focuses on terpyridine-based group 11 metal complexes and their biological properties. These metals are well-known for their intriguing properties in the design of potential metallodrugs.

Copper is a redox-active metal, readily transitioning between the reduced Cu(I) and oxidized Cu(II) states, whether in standard chemical reactions or under physiological conditions. Copper forms a diverse range of coordination complexes in both Cu(II) and Cu(I) oxidation states, although Cu(I) complexes are generally less stable and tend to oxidize to Cu(II). Consequently, fewer studies have explored the potential of Cu(I) complexes as metallodrugs. Copper is an essential micronutrient for all living organisms, and its intracellular concentration is tightly regulated. Therefore, researchers have investigated copper-based complexes under the assumption that endogenous metals may be less toxic to normal cells. The altered metabolism of cancer cells and the differing responses of normal and tumor cells to copper provide a basis for the

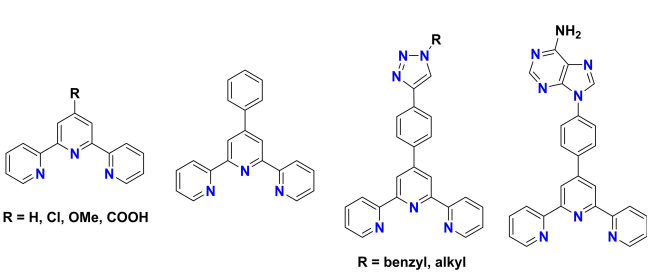

Figure 1. Terpyridine-based ligands with biological activity.

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development of copper complexes as potential anticancer agents.[10]

Silver, known for its excellent biocompatibility and easy elimination, has long been recognized for its antimicrobial properties. These properties have led to the incorporation of silver compounds into medicinal drugs due to their potent antimicrobial activity. However, it has become increasingly evident in recent years that silver complexes also exhibit significant cytotoxicity against numerous types of cancer cells. Additionally, the synthesis of silver(I) compounds has garnered increased attention owing to their reduced toxicity to healthy cells, sparking optimism about their potential as anticancer agents.[11]

The biological properties of gold have been recognized since ancient times. Extensive research involving gold complexes as potential anticancer agents has revealed their ability to interact with various biological targets. Notably, gold complexes exhibit great affinity for sulfur and selenium atoms, making thiol-containing enzymes potential targets for gold complexes. However, thioredoxin reductase (TrxR), a critical enzyme involved in numerous redox processes and essential for cell growth, stands from all the others. The capacity of gold complexes to target TrxR has generated significant interest in their potential as effective anticancer agents. This underscores the complex and multifaceted role of gold in the field of medical research and its growing importance in the development of innovative cancer therapies.[12]

2. Terpyridine-Based Copper Complexes

Copper complexes with terpyridine ligands as anticancer agents have been extensively studied. The aim of this review is to emphasize key examples, evaluate common mechanisms exhibited by these derivatives, and contribute to the scientific community's understanding for the rational design of enhanced anticancer properties. It is worth noting that existing literature already contains reviews addressing this topic, underscoring the ongoing interest in these types of complexes.[13] Additionally, it is noteworthy that the majority of studies focus on Cu(II) complexes.

Considering the ease of functionalizing terpyridine-based ligands and copper's versatility in varying its coordination environment, it is not surprising that numerous studies have explored the anticancer properties of Cu-Terpy complexes. These investigations encompass various ancillary ligands, including N-donor, inorganic donor ligands, O-donor and polynuclear complexes.

2.1. Complexes with nitrogen donors as ancillary ligands

Kumar and co-workers studied the anticancer activity of various copper(II) bis(terpyridine) derivatives substituted at the 4′ position with different aromatic moieties (**Cu1**). The anthracenyl-terpy complex, **Cu1a** (Figure 2), exhibited significant toxicity against a broad range of cancerous cell lines. Regarding the mechanism, **Cu1a** binds to DNA both through intercalation and groove binding. Additionally, the authors were able to demonstrate that copper played a redox role in DNA-plasmid cleavage, exhibiting nuclease activity. Furthermore, **Cu1a** induces apoptosis.[14] The compounds **Cu1b** to **Cu1e** also shows cytotoxicity against different tumorigenic cell lines and great selectivity index against non-tumorigenic cells. In these cases, all show DNA binding through intercalation mode and DNA-cleavage in presence of H_2O_2. These compounds also bind BSA.[15,16] Further studies with **Cu1b** shows that the complex can go inside the cytoplasm and accumulate in the cell nuclei. In addition, the mechanism of cell dead was via apoptosis through a ROS-mediated mitochondrial pathway. it is important to highlight that the *in vivo* assays also gave good results and administration of **Cu1b** significantly inhibits tumour growth in EAC cells in female Swiss albino mice.[17]

Karges *et al.* evaluated the influence of different substituents at the 4′ position of the terpy ligand in this case they used substituents such as H (**Cu2a**), Cl (**Cu2b**), Br (**Cu2c**), COOH (**Cu2d**), OMe (**Cu2e**) and NMe_2 (**Cu2 f**). All compounds exhibited excellent activity in the low micromolar range, except for **Cu2d** which was significantly less toxic. They also synthesized monoterpy derivatives with general formula [R-Terpy-Cu-Cl_2]. However, these compounds showed solubility problems and could not be studied.[18]

Maria Gil-Moles received her PhD in 2019 from University of La Rioja (Spain). After completed the PhD she obtained a postdoctoral contract at Technical University of Braunschweig (Germany) which started on 2020 until 2022. Additionally, she has recently been a beneficiary of a fellowship for the re-qualification of the Spanish University system through the Margarita Salas modality, this fellowship entered into force on April 2022 until March 2024. This fellowship is a collaborative project between Technical University of Braunschweig and University of Zaragoza. María Gil-Moles is currently in the period of her fellowship at the University of Zaragoza.

M. Concepción Gimeno received her PhD from the University of Zaragoza. After completing her postdoctoral work, she joined the Institute of Chemical Synthesis and Homogeneous Catalysis (CSIC-University of Zaragoza), where she has been a professor since 2008. Her research centers on designing novel group 11 metal compounds with catalytic, luminescent, and biological applications. She has authored more than 300 scientific publications and has received several awards, including the IUPAC 2017 Distinguished Women in Chemistry or Chemical Engineering, the GEQO-Excellence in Organometallic Chemistry Research Award in 2017, the RSEQ-Excellence Research Award in 2018, and the Rafael Usón Medal in 2022.

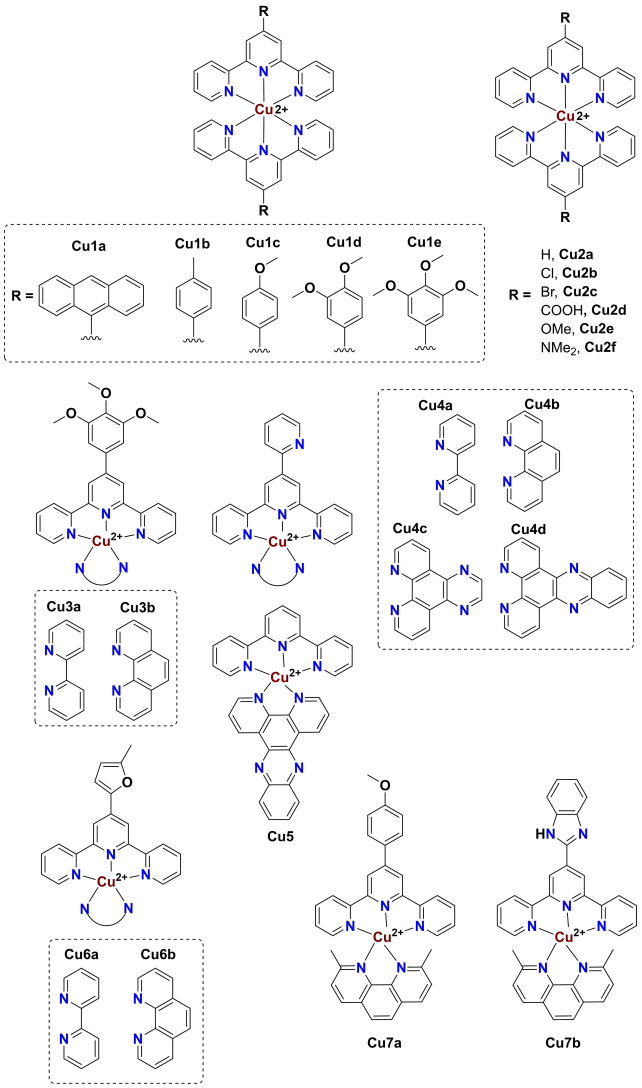

Figure 2. Selected Terpy-Cu N-donor complexes.

There are many examples in the literature of copper(II) based terpyridine compounds and bipyridine or phenanthroline type ligands. In this context, Kumar and co-workers evaluated analogous complexes of **Cu1e** with phenanthroline **Cu3a** and with bipyridine **Cu3b**. These complexes were also found to interact with DNA by intercalation and to exhibit DNA nuclease activity. However, the observed toxicity could not be solely correlated with DNA binding/cleavage, as **Cu3a** was found to be the most cytotoxic and showed the worst interaction with DNA. Therefore, these derivatives demonstrate a multi-target mechanism and require further studies.[16] In another work, the effect of using different bidentate ligands such as bipyridine (**Cu4a**), phenanthroline (**Cu4b**), dipyridoquinoxaline (**Cu4c**) and dipyridophenazine (**Cu4d**) was also evaluated. In this case, the compounds showed moderate cytotoxicity, and as in the previous cases, they were found to be DNA intercalators. A clear trend was observed: increasing the number of aromatic rings in the N–N ligand increased the interaction. Thus **Cu4d** proved to be the best intercalating agent and the one with the best toxicity.[19]

Cu5 also proved to be an excellent intercalating agent and showed high cytotoxicity. It seems that the high planarity of dipyridophenazine greatly supports the intercalation processes.[20] Compounds **Cu6a** and **Cu6b** show the same tendency as in the previous cases, both are intercalating agents but **Cu6b** was found to be the strongest binding agent than **Cu6a**. In this study, the authors also examined the free ligand and observed that it displayed very similar characteristics to the studied complexes.[21] Other authors evaluated how the substituent in the 4′ position of the terpy ligand affected (**Cu7a** and **Cu7b**). Both complexes bound to DNA through intercalation. **Cu7b** shows better binding due to the presence of its N and NH groups, that can form hydrogen bonds with DNA bases. **Cu7a** and **Cu7b** also induced hydrolytic DNA cleavage and exhibited great cytotoxicity against cancer cells and good selectivity index against non-tumorigenic cells. Notably, **Cu7b**, which had stronger DNA binding, showed higher cytotoxicity and was able to induce mitochondrial-mediated and caspase-dependent apoptosis.[22] On the basis of these results, it can be concluded that DNA is a clear and key target for Terpy-Cu-N-N complexes. In addition, it seems that as far as N–N ligands are concerned, phenanthroline derivatives show better properties than bipyridine derivatives. Furthermore, is also important to note the relevance of the substituents present on the terpyridine ligand. However, further studies are needed to try to elucidate the full mechanism of Terpy-Cu complexes with N-donor ligands.

2.2. Complexes with halogen or pseudo-halogens as ancillary ligands

Li and *et al.* analyzed several complexes wit general formula [R-phenylterpyCuCl$_2$] (**Cu8a**–**Cu8h**). All derivatives exhibited significant cytotoxicity in the low micromolar range. However, as there were no significant differences, a structure-property relationship could not be established. These compounds were also demonstrated to be DNA intercalating agents. Molecular docking studies revealed that binding interactions primarily depended on π-π stacking and hydrogen bonding between the compounds and DNA. Complexes containing hydroxyl, methoxy, and methylsulfonyl groups predominantly relied on hydrogen bonding.[23] Other authors have evaluated how the coordinated anions and the presence of an aromatic substituent in the terpyridine can affect biological properties. Compounds **Cu9a** to **Cu9c** (see Figure 3) displayed moderate cytotoxicity and exhibited interactions with DNA. Significant differences were observed among them: **Cu9a** and **Cu9b** interacted with the minor groove, while **Cu9c** acted as an intercalating agent, possibly due to its higher number of aromatic rings. The three complexes displayed nuclease activity in the presence of H_2O_2, which was attributed to the hydroxyl radicals generated by them. In addition, these complexes induce apoptosis, although some differences can be observed. **Cu9a** induces early apoptosis, **Cu9b** induces both early apoptosis at low dosage and late apoptosis at high dosage, whereas **Cu9c** induces both

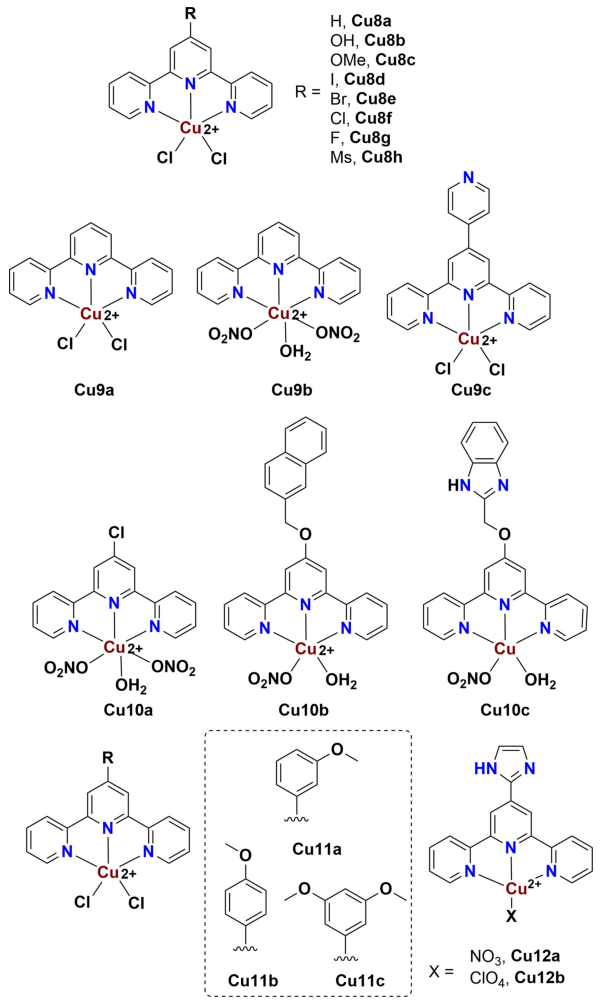

Figure 3. Selected Terpy-Cu complexes with halogen or pseudo-halogen as ancillary ligands.

regardless of concentration. These results suggest that the change in the coordinated anion does not significantly affect. However, the increase in aromatic rings changes the mechanism by which they interact with DNA.[24]

Grau and *et al.* assessed the impact of the substituent in the 4′ position of the terpyridine (R = Cl **Cu10a**, naphthyl **Cu10b** or benzimidazole **Cu10c**). These ligands were chosen to investigate the potential role of supramolecular interactions in the DNA-interacting and cytotoxic properties of the corresponding copper complexes. The observed cytotoxicity was moderate, including free ligands, with **Cu-10b** yielding the best results. This compound also proved to be the most effective DNA intercalating agent but did not induce DNA cleavage, unlike **Cu10a** and **Cu10c**, both of which exhibited activity. Surprisingly, this lack of DNA cleavage did not translate to cytotoxicity.[25] Other authors have also studied the influence of the substituent in the 4′ position. Thus, Liang *et al.* evaluated the influence of increasing the number of methoxy groups on the phenyl ring (**Cu11a**, **Cu11b**, **Cu11c**). The three complexes showed high antitumor activity and good DNA binding following the order **Cu11b** > **Cu11a** > **Cu11c**. However, **Cu11b** has the highest binding capacity but the lowest antitumour activity. The reason is related to the amount of intracellular ROS generated by the complexes, being **Cu11b** the one that generates the least amount of ROS. Therefore, this study concludes that not only does DNA binding benefit antitumor activity, but ROS generation also plays a crucial role.[26] It seems that the determining factor is the substituents present on the terpyridine ligand rather than the anions coordinated to the copper atom. For instance, **Cu12a** (NO_3) and **Cu-12b** (ClO_4) complexes, which differ only in the coordinated anion, both act as intercalating agents and induce DNA cleavage in the presence of H_2O_2. Additionally, both exhibit similar toxicity in the low micromolar range.

2.3. Complexes with oxygen donors as ancillary ligands

The use of different ancillary ligands can modify the anticancer activity of Terpy-Cu derivatives. Hence Guedes da Silva and co-workers explored the anticancer activity of five complexes in which different oxygen donors were used as ancillary ligands (Figure 4), such as *p*-toluenesulfonate (**Cu13a**), benzoate (**Cu13b**) and *o*- (**Cu13c**), *m*- (**Cu13d**) or *p*-hydroxybenzoate (**Cu13e**). All compounds showed excellent antiproliferative properties with **Cu13a** (the only cationic one) being the most active of all in the nano molar range. In addition, all complexes showed better activity against tumor cell lines than against non-tumorigenic ones, and all were able to induce apoptosis. These compounds proved to be good intercalating agents, with **Cu13c** and **Cu13d** being the best ones, exhibiting binding constants in the range of 10^6 and **Cu13a**, **Cu13b**, **Cu13c** showed an order of magnitude lower binding constants. All five compounds are able to induce DNA cleavage in the absence of H_2O_2. These results cannot be correlated with the antiproliferative activity of the compounds.[27]

Deka *et al.* studied the influence of different O-donor ligands and the influence on the substituent in the terpyridine ligand. They employ 4′-phenyl-terpyridine (**Cu14a**–**Cu14c**) and 4′-ferrocenyl-terpyridine (**Cu14d**–**Cu14f**) and the O-donor ligands plumbagin, chrysin, and curcumin. All compounds showed antiproliferative activity, but ferrocene derivatives were found to be much more active against different cell lines than phenyl derivatives. **Cu14f**, displayed the higher cytotoxicity demonstrated lower toxicity towards normal cells. In addition, **Cu14f** showing an apoptotic pathway and it is accumulated in the mitochondria, suggesting a potential involvement of mitochondrial apoptosis, possibly through the generation of ROS.[28] Rahiman and co-workers also evaluated the influence of the substituent in the 4′ position of the terpyridine ligand. They studied several complexes with different terpyridines and naproxen as ancillary ligand (**Cu15a**–**Cu15c**). These compounds were groove DNA binders, and **Cu15b** was found to be the best binding agent, probably due to the ability of the substituent to form hydrogen bonds. All the complexes displayed significant hydrolytic DNA cleavage activity. Additionally, these compounds showed moderate antiproliferative activity against various cancer cells, with **Cu15b** being the most active.

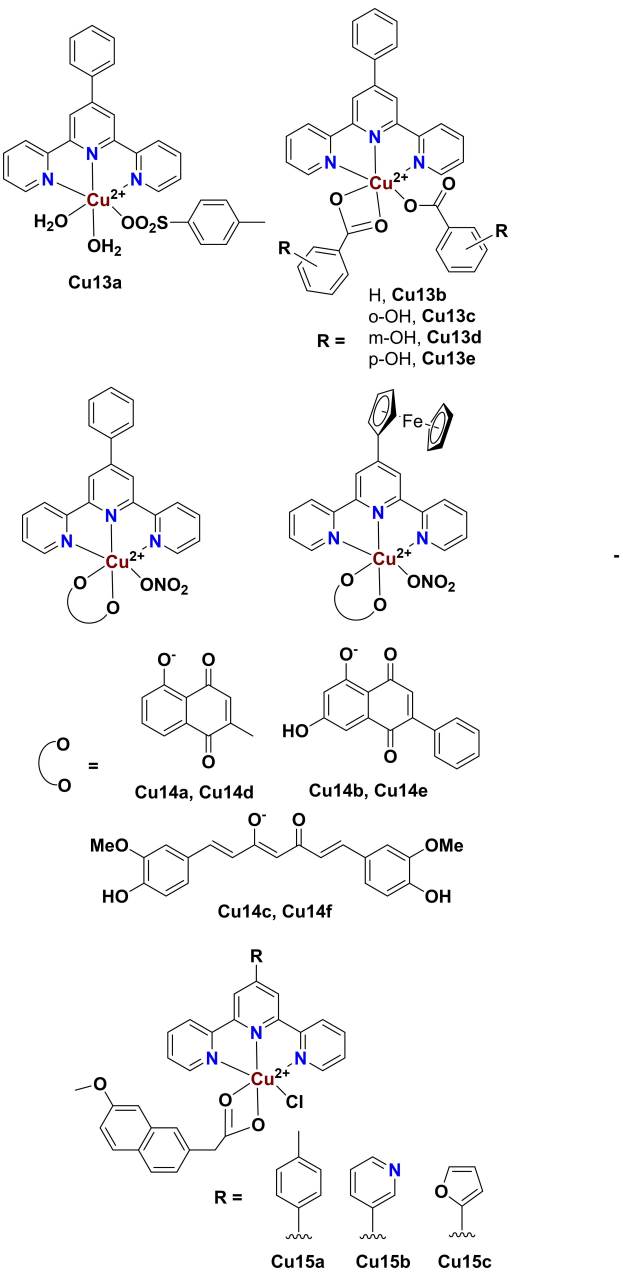

Figure 4. Selected Terpy-Cu complexes with O-donors as ancillary ligands.

Regarding the cell death mechanism, the complexes induce mitochondrial-mediated and caspase-dependent apoptosis.[29]

2.4. Polynuclear Complexes

Some authors have explored the anticancer properties of polynuclear Cu-terpy complexes based on the idea that the redox properties of copper are responsible for DNA cleavage. Vilar and co-workers studied a family of mono (**Cu16a**), di (**Cu16b**) and tri-copper (**Cu16c**) complexes (Figure 5). The complexes showed moderate binding affinity with DNA and the binding mode depend on the number of arms. **Cu16a** was an

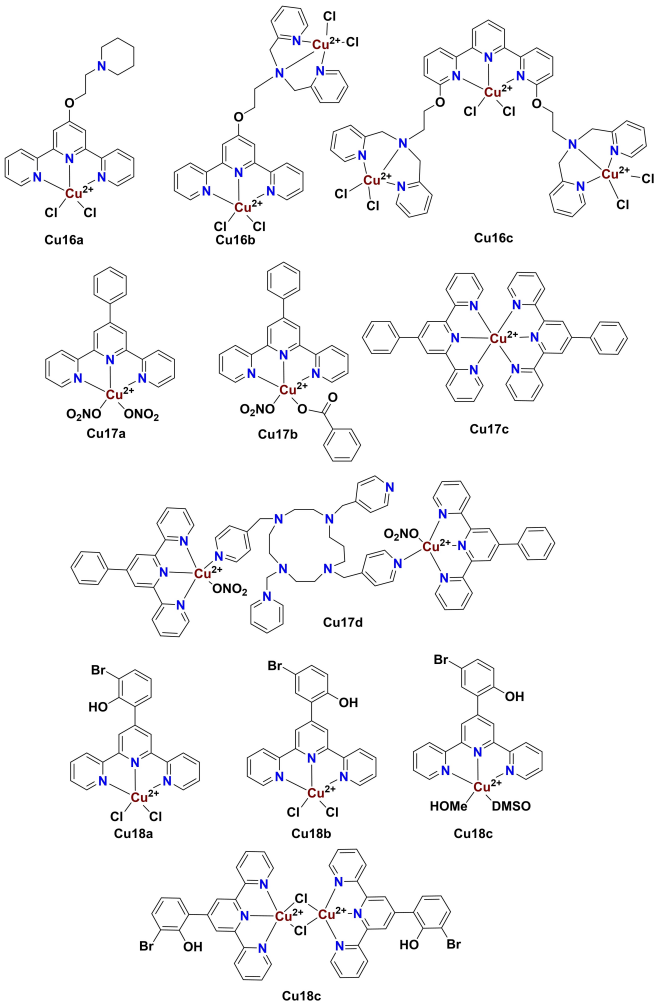

Figure 5. Selected polynuclear complexes.

intercalator; however, when the number of Cu ions increases, and the side arms are modified accordingly, groove binding is preferred over intercalation. The nuclease activity also depends on the number of Cu centers, with **Cu16c** (tri-copper) being the most effective. In addition, the tri-copper complex could cleave DNA without requiring external co-reductants. **Cu16c** shows a moderate toxicity against different cell lines. Cellular uptake studies have shown that a significant portion of **Cu16c** is accumulated in the cytoplasm. However, a portion of the complex localizes in the nucleus, allowing it to damage DNA.[30]

Ma and co-workers analyse the antiproliferative activity of some mono-copper complexes (**Cu17a**–**Cu17c**) and one di-copper complex (**Cu17d**). All were active in the low micromolar range against different cancer cell lines with **Cu17d** being more active, but the difference was not very significant. Molecular docking simulations were conducted to examine the interaction of the compounds with DNA sequences and proteins. These simulations revealed robust binding affinities between the complexes and macromolecules, indicating their potential for therapeutic applications.[31] Chen and co-workers also studied different mononuclear derivatives (**Cu18a**–**Cu18c**) and a dinuclear one (**Cu18d**). In this case the dinuclear complex did not

stand out from the mononuclear complexes either, and **Cu18a**–**Cu18d** exhibited greater cytotoxic activity against multiple tested cancer cell lines. Moreover, the authors evaluated the free ligands and observed that they exhibit slightly lower cytotoxicity. Additionally, they demonstrated low toxicity towards normal cells. ICP-MS detection indicated the accumulation of copper complexes in mitochondria. Mechanistic studies revealed that these copper complexes induced G0/G1 cell cycle arrest and altered the expression of related cell cycle proteins. They also led to a reduction in mitochondrial membrane potential, increased intracellular ROS levels, Ca^{2+} release, up-regulation of Bax, down-regulation of Bcl-2 expression levels, cytochrome c release, caspase cascade activation, and induced mitochondrion-mediated apoptosis. *In vivo* studies showed that **Cu18a** effectively inhibited tumor growth in a mouse xenograft model with BEL-7402 tumor cells.[32]

3. Terpyridine-Based Silver Complexes

Given the high affinity of silver for nitrogen donor ligands, it is surprising that the number of silver complexes with terpyridine-based ligands is very scarce, despite the interesting biological activity they exhibit.

The combination of Ag(I) ions and the ligand 6,6′-dimethyl-2,2′:6′,2″-terpyridine resulted in the formation of dinuclear double helicates **Ag1a-d** (Figure 6). The structure and complexity of these helicates varied depending on the counterions and the solvent used during synthesis. Extensive investigations were carried out to assess the biological activity of these complexes, showing antiproliferative effects on several human cancer cell lines, although the ligand itself shows antiproliferative activity. A noteworthy discovery was that the Ag(I) complexes displayed a specific affinity for the DNA double helix, interacting with it through intercalation, a vital mechanism. Confocal microscopy was employed to observe how these complexes bound to the nuclei of cancer cells, revealing that they aggregated in the nucleoli region, indicating a potential mechanism for DNA interference leading to apoptosis. Moreover, the newly synthesized silver helicates displayed remarkable antibacterial efficacy against both Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus bacterial strains.[33]

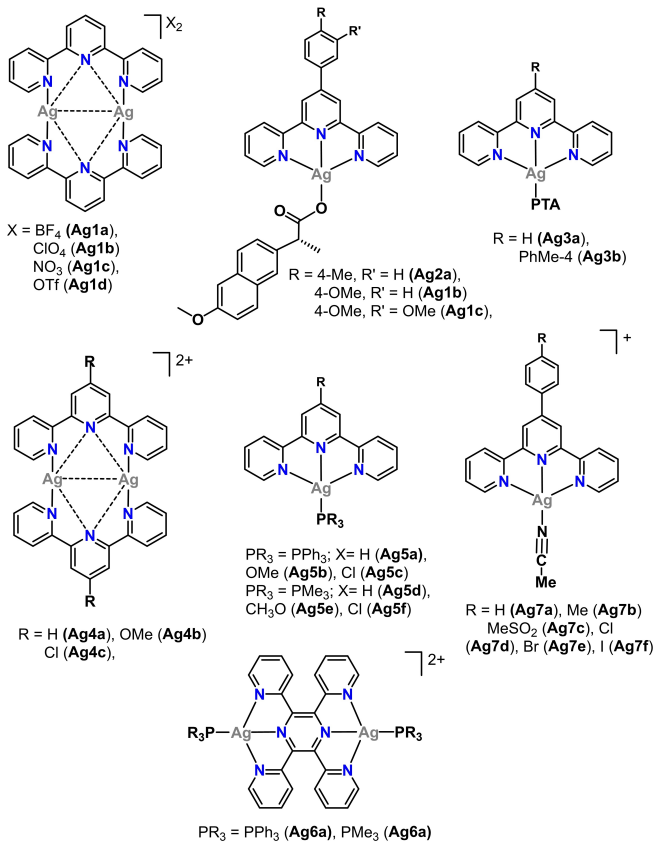

Figure 6. Silver(I) complexes with terpyridine-based ligands.

A series of heteroleptic silver(I) complexes, **Ag2a-c**, bearing 4′-substituted 2,2′:6′,2″-terpyridine and naproxen ligands, were prepared and their cytotoxicity was tested in several tumor cell lines, exhibiting high antiproliferative activity. Interestingly, they exhibited low toxicity towards healthy fibroblasts. Examination of their binding ability with CT-DNA revealed an intercalative mode of DNA binding, with complex **Ag2c** exhibiting the highest affinity. Molecular docking studies showcased strong binding with epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2). All the complexes have been found to promote DNA cleavage through a hydrolytic pathway. Morphological studies using Hoechst 33258 staining revealed that complex **Ag2c** induces apoptosis much more effectively than the other complexes.[34] A series of silver(I) compounds has been developed by incorporating 2,2′:6′,2″-terpyridine and 4′-(4-methylphenyl)-2,2′:6′:2″-terpyridine derivatives with PTA (1,3,5-triaza-7-phosphaadamantane). The free ligands did not exhibit any activity but compounds, **Ag3a-b**, showed significant antiproliferative activity in several cancer cell lines, whereas the with 10 to 40 times higher activity compared to cisplatin, a commonly used chemotherapy drug. Importantly, these compounds had up to 30 times lower toxicity to normal cells.

The study also investigated the interaction of these compounds with human serum albumin (HSA) using various spectroscopic methods, such as UV-Vis, fluorescence, and circular dichroism spectroscopy (CD), sowing a moderate affinity for HSA, suggesting that serum albumin could serve as an effective carrier for potential anticancer drugs based on silver complexes, or the adduct formed can act as a reservoir for therapeutic purposes.[35]

Silver complexes featuring 4′-substituted terpyridine, either as homoleptic dinuclear species **Ag4a-c**, or with phosphines as ancillary ligands **Ag5a-f**, or tetra-2-pyridinylpyrazine ligands with phosphines as ancillary ligands **Ag6a-b**, were synthesized and evaluated for their cytotoxic properties. These compounds demonstrated significant cytotoxicity against various tumor cells and, importantly, displayed a substantial selectivity index between tumor cells and healthy Lymphocytes T for certain derivatives. The study of their interaction with CT-DNA revealed that these compounds can moderately intercalate with DNA. Furthermore, cell death studies suggest that these derivatives

induce apoptosis through the mitochondrial pathway. The authors aimed to establish a structure-activity relationship, observing that the presence of different substituents on the terpyridine ligand provided virtually no change. On the other hand, the use or non-use of PPh_3 or PMe_3 appears to be a key factor, as the presence of PPh_3 promotes greater cytotoxicity but no selectivity, while the use of the PMe_3 ligand provides some selectivity against healthy Lymphocytes T.[36]

Cationic silver complexes bearing 4′-(4′-substituted-phenyl)-2,2′:6′,2″-terpyridine and acetonitrile ligands, **Ag7a-f**, have been developed. These compounds exhibited intriguing photoluminescence properties in both solid and solution states. In vitro experiments revealed that they displayed higher antiproliferative activities compared to cisplatin and the free ligands when tested against three human carcinoma cell lines: A549, Eca-109, and MCF-7.

The exploration of the interaction of these compounds with DNA was conducted through fluorescence titration, circular dichroism spectroscopy, and molecular modeling techniques. Spectrophotometric findings showed that the compounds exhibited a strong affinity for binding with DNA, acting as intercalators, while molecular docking studies suggested that the binding was facilitated by π-π stacking and hydrogen bonds.[37]

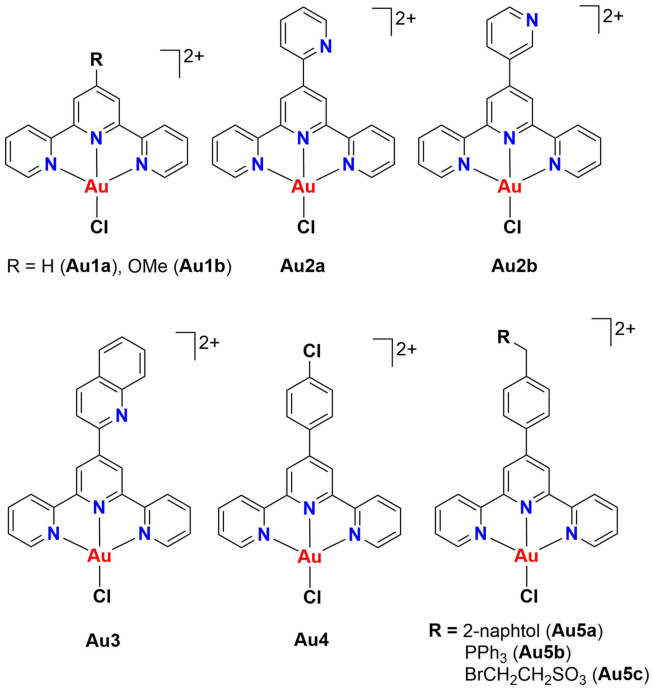

Figure 7. Gold(III) complexes with terpyridine-based ligands.

4. Terpyridine-Based Gold Complexes

The striking similarity between gold(III) complexes and platinum(II) compounds has positioned them as promising candidates for the development of a highly effective class of potential anticancer agents. In comparison to cisplatin, gold(III) complexes possess distinctive features, including high kinetic lability and substantial positive redox potentials. However, under physiological conditions, gold(III) complexes tend to exhibit a relatively low stability, which has posed a challenge for their advancement as cytotoxic and antitumor agents.

Nonetheless, the quest for greater stability in gold(III) complexes has led researchers to explore the use of multidentate N-donor ligands, such as derivatives of 2,2′:6′,2″-terpyridine. The simple cationic gold complexes with the terpyridine ligand $[Au(terpy)Cl]Cl_2$ **Au1a** and **Au1b** (Figure 7) exhibited significant cytotoxic activity in A2780 human ovarian cancer cells. Preliminary interaction studies with calf thymus DNA revealed a weak and reversible interaction, which is proposed to be electrostatic in nature or by interaction.[38]

4′-Substituted terpyridine gold(III) derivatives have also been described to have biological properties. 4-methoxyphenyl substituted species were reported to be DNA intercalators in a seminal work by Che and coworkers, who did a comparison with the NNC cyclometallated complex observing a higher DNA-binding constant for the latter.[39] Subsequent studies have shown that structural modifications of terpyridine ligands, especially in the 4′ position, allow for the tuning of the antitumor properties of the complexes. Significant cytotoxic activities were confirmed for two gold(III) complexes incorporating 2-pyridyl (**Au2a**) and 3-pyridyl (**Au2b**), which are higher in relation to those of the free ligands, $[AuCl(terpy)](PF_6)_2$ and the standard anticancer drug cisplatin in HCT116 human cancer cells. Pro-apoptotic cell death was found for the 2-pyridyl complex, while pro-necrotic activity was confirmed for the 3-pyridyl complex.[40] A structurally similar 4′-quinoline-substituted terpyridine gold(III) (**Au3**) derivative exhibited potent antiproliferative effects in ovarian cancer cells (A2780) and colorectal carcinoma cells (HCT116). Although it didn't match the efficacy of its copper counterpart, it was notably less toxic to healthy dermal fibroblasts than cisplatin. This complex showed diverse interactions with calf-thymus DNA (CT-DNA), favoring intercalation at higher DNA concentrations. The gold(III) species demonstrated efficient passive cellular uptake and induced the generation of reactive oxygen species (ROS) as well as alterations in the mitochondrial membrane, triggering apoptosis and autophagy. Interestingly, these complexes also exhibited a compelling anti-angiogenic effect by inhibiting the formation of new blood vessels without causing harm.[41]

The 4′-chlorophenyl terpyridine ligand builded a gold(III) (**Au4**) derivative showed a significantly enhanced antitumor effect against human oral squamous carcinoma cells (CAL-27) when compared to cisplatin. The studies carried out with this complex revealed a decreasing reactivity trend among selected nucleophiles, with 5′-GMP exhibiting the highest reactivity, followed by L–Met and GSH, implying that the guanine derivative is a more effective entering nucleophile than the studied S-donor ligands. The complex displayed moderate binding affinities for both CT DNA and BSA, whereas molecular docking confirmed intercalation as the primary binding mode with DNA and identified the binding pocket at site I of BSA.[42]

A distinct family of terpyridine-gold(III) complexes (**Au5a-Au5c**), featuring various 4′-phenyl substituents, exhibited

remarkable cytotoxicity, surpassing cisplatin in multiple cancer cell lines. Notably, the corresponding ligands also demonstrated significant cytotoxicity against the tested cell lines. This study underscores the stability of gold(III)-terpyridine complexes in the presence of glutathione (GSH) in aqueous solutions. Of particular significance, this research provides the first instance where gold(III) complexes are shown to target intracellular DNA *in vitro*. The extent of DNA metallation observed in gold(III) complexes parallels that of platinum(II) complexes, with both steric and electrostatic factors significantly influencing their interactions with DNA. Reduced steric hindrance and increased positive charge appear to enhance the DNA binding affinity of gold(III) complexes, which intriguingly correlates with their antitumor activity. This implies that the cytotoxicity and DNA binding capacity of gold(III)-terpyridine complexes can be finely tuned by adjusting the steric and electrostatic properties of the ligand substituents.[43]

5. Summary and Outlook

Terpyridine-based complexes with group 11 metals present promising potential as candidates for the development of new metallodrugs against cancer. In this study, we systematically evaluated and summarized the anticancer properties of such complexes, emphasizing their mechanisms of action. These derivatives exhibit notable cytotoxicity against various cancer cell lines, and several compounds demonstrate a favorable selectivity index, sparing non-tumorigenic or healthy cells.

The interaction of copper, silver, or gold complexes with terpyridine-based ligands with DNA is a key aspect of their mechanism of action. Generally, these interactions involve intercalation and, in some instances, groove binding. The versatility of the terpyridine ligand allows for modulation of the DNA interaction through functionalization, particularly at the 4′ position, with aromatic rings that enhance planarity and the incorporation of heteroatoms facilitating hydrogen bond formation.

Copper compounds, in addition to their DNA interactions, display nuclease activity in the presence of H_2O_2, primarily attributable to the redox properties of copper. This redox activity also promotes the generation of reactive oxygen species (ROS), contributing to the apoptotic mechanism via the mitochondrial pathway observed in Cu-terpy derivatives.

Research on silver and terpyridine remains somewhat limited, but existing studies consistently identify DNA as a primary biological target. These investigations underscore the role of these compounds as intercalating agents, especially with silver compounds demonstrating DNA hydrolytic cleavage and the induction of apoptosis. Furthermore, these substances display noteworthy antiproliferative activity in cancer cells, along with notable selectivity against healthy cells.

Gold compounds, besides acting as intercalators, demonstrate the ability to generate ROS and induce alterations in the mitochondrial membrane. Apoptosis is the predominant observed mechanism of cell death, although some derivatives induce necrosis. Notably, there is a lack of studies on the inhibition of thioredoxin reductase, a known target for gold compounds, emphasizing the need for further investigations.

However, a comprehensive understanding of the full mechanism of terpyridine-based complexes with group 11 metals requires additional studies. While it is established that DNA is a crucial target and ROS generation is significant, not all instances of cytotoxicity can be exclusively attributed to DNA interactions. Nevertheless, a comprehensive understanding of these metallodrugs demands further investigations, particularly in untangling the intricate mechanisms, emphasizing the urgency of exploring additional biological targets. This review underscores the promising trajectory of terpyridine-based complexes, urging intensified efforts towards unlocking their full therapeutic potential.

Acknowledgements

The authors thank Agencia Estatal de Investigación (PID2022-136861NB–I00/AEI/10.13039/501100011033) and Gobierno de Aragón (Research Group E07_23R) for financial support of our research. M. G.-M. acknowledges support by a Margarita-Salas Fellowship (European Union, NextGenerationEU).

Conflict of Interests

The authors declare no conflict of interest.

Keywords: Terpyridine · Group 11 · Biological activity · DNA-binding · Cancer

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Manuscript received: November 19, 2023
Revised manuscript received: January 22, 2024
Accepted manuscript online: February 8, 2024
Version of record online: February 28, 2024