

Research Article

Development of Cu(II) complexes of substituted polypyridyl and salicylaldehyde ligands as anticancer agents

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ABSTRACT

A comprehensive panel of mixed ligand Cu(II)-complexes with general formula $\text{Cu}(\text{X-dppz})(\text{X}_2\text{-sal})(\text{NO}_3)$ was synthesized and properly characterized through elemental analysis, FT-IR, mass spectrometry, UV-Vis and EPR spectroscopy. Theoretical calculations using DFT and TD-DFT methods were carried out to support the interpretation of the absorption spectra, revealing that most of the observed absorptions exhibit either Ligand Centered (LC) or Ligand-to-Ligand Charge Transfer (LLCT) character. The study was aimed to investigate them as anticancer agents against three cancer cell lines selected based on the incidence (MDA-MB 231) and aggressiveness (U87 and MG 63) of the related tumor type, and to investigate the effect of substitutions on both ligands (i.e., the polypyridyl diimine ligand, dppz, and the auxiliary ligand, sal) on antiproliferative activities of the complexes. The results showed that complexes bearing the $-\text{NO}_2$ group on the dppz ligand exhibited significantly higher cytotoxicity, particularly against MDA-MB 231 cells, compared to their Cl-substituted counterparts, with the exception of the complex with the 3-bromo-5-chloro-salicylaldehyde as auxiliary ligand. The latter also turned out to be essential for the cytotoxicity (comparative analysis with sal-free Cu(II)-complexes) as well as the complete structure of the complexes (comparative analysis with pure polypyridyl ligands). Mechanistic studies (*in vitro* and *in silico*) revealed that these complexes induce cytotoxicity through Reactive Oxygen Species (ROS) generation and intercalation into the DNA double helix, as well as through G-quadruplex stabilization. Among the synthesized compounds, complexes Cl_2CuBrCl (4) and NO_2CuBrCl (14) demonstrated the most promising biological activity, making them compelling candidates for further investigation as anticancer agents.

1. Introduction

According to the World Health Organization (WHO), cancer, which is the result of uncontrolled cell division that can eventually spread to other organs or tissues, is the second leading cause of death worldwide [1,2]. Unfortunately, if the incidence rate remains stable and in line with the current upward trend, the number of new cancer cases is set to increase, reaching 28.4 million per year by 2040 [3]. Therefore, further effort in the synthesis of new effective and less toxic anticancer drugs appears necessary.

Since Rosenberg's discovery of cisplatin cytotoxicity in 1965, chemotherapy with coordination compounds has received increasing attention. However, despite cisplatin's well-known efficacy in killing cancer cells and the discovery other, better-tolerated platinum-based drugs (e.g., carboplatin and oxaliplatin) [4–6], some of their drawbacks – such as significant side effects and inherited or acquired resistance – have limited their clinical use and encouraged scientists to investigate non-platinum-based metal complexes as an alternative to overcome these major issues [7,8], as well as the development of metal complexes which can interact noncovalently with DNA [9,10].

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From this perspective, metal complexes with polypyridyl ligands have been considered an attractive option due to the unique physicochemical, photochemical and biological properties of these ligands, which can be modulated and enhanced by varying the metal core [11]. The resulting metal complexes, in fact, are known for their inertness, being coordinatively saturated and exhibiting peculiar redox properties [12]. Recent studies have shown that they can act as effective DNA intercalators and/or groove binders, forming noncovalent adducts and providing compounds with moderate-to-strong cytotoxic activity against cancer cells [13]. Examples include platinum(II)-based **Pt-dppz-Py₂** (also known as **LDP-17**, which we tested on the same three tumor cell lines used in this study and whose analogues have previously been prepared and tested for telomerase inhibition and G-quadruplex binding activity) [10,14], and ruthenium(II)-based **Ru-dppz₃** (topoisomerase inhibition and DNA binding activity) [15], Fig. 1.

Applications of polypyridyl complexes in other fields include their use as catalysts [16], molecular devices [17], dye sensitizers for solar cells [18], fluorescent probes [19,20], and agents for photodynamic chemotherapy [21]. Copper(II)-based complexes are capable of generating reactive oxygen species (ROS) and inducing oxidative DNA cleavage at multiple sites [22], resulting in antineoplastic potential, as in the case of Casiopeinas® (specifically **CasIII-ia**; Fig. 2A) [23]. The extraordinary therapeutic potential of copper(II)-based compounds can also be envisaged by considering Cu(II)-ATSM, a thiosemicarbazone complex that is permeable to the blood-brain barrier, currently under development for the treatment of neurodegenerative diseases such as Parkinson's disease and amyotrophic lateral sclerosis (phase III clinical trials) and also proposed as a promising PET agent for hypoxia imaging [24,25]. Other relevant examples of Cu(II)-polypyridyl complexes with activity on tumor cells and nuclease activity related to ROS production were provided by Salimi et al. (i.e., **[Cu(tpy)(dppz)]²⁺** and **[Cu(tptz)₂]²⁺** (Fig. 2A); MCF-7 cells) [26], Molphy et al. (i.e., **Cu-dpq-Phen**, **Cu-dppz-Phen** and **Cu-dppn-Phen** (Fig. 2A); Cu(II)-complexes with DNA binding constant of the order $\sim 10^7$ M(bp)⁻¹) [27], and Eremina et al. (Hep2, HepG2 and MCF-7 cells) that developed three mixed-ligand copper-based complexes with general formulas **[Cu(dmbipy)(L)₂]**, **[Cu₂(dmphen)₂(L)₄]** and **[Cu(phendione)(L)₂]** [28].

Copper(II) has received considerable attention in medicinal inorganic chemistry also for other reasons: first, Cu(II) salts are inexpensive compared to salts of noble metals such as Pt, Au and Ru used to obtain complexes of therapeutic interest. Secondly, being an essential endogenous metal, Cu²⁺ is less toxic to normal cells than to tumor cells, in which it can induce cell death by affecting different pathways (oxidative stress, cell cycle, angiogenesis) [29]. On the other hand, tumor cells

require more Cu²⁺ than normal cells for their metabolism, which makes them more vulnerable to imbalanced Cu²⁺ levels [30,31]. Indeed, providing extra amounts of Cu²⁺ brings about a unique non-apoptotic cell death pathway defined as cuproptosis which consists of the direct binding of Cu²⁺ to lipoylated components of the tricarboxylic acid cycle [32–34].

Despite the promising results obtained with Cu(II)-complexes in medicine and the importance of this trace element in regulating numerous biological functions, Cu(II)-complexes with polypyridyl ligands have received generally limited attention to date. Therefore, our research group has recently focused on developing these types of compounds as potential anticancer agents. In this regard we have previously synthesized a series of mixed-ligand Cu(II)-complexes based on a diimine ligand and an auxiliary halogenated ligand, namely Cu(diimine)(X-sal)(NO₃), where diimine is bipy or phen, sal is salicylaldehyde, and X is Cl, Br, I, or H (Fig. 2B) [35]. The evaluation of the antiproliferative activity of these complexes revealed that it depends largely on the nature of the diimine ligand and the substitution pattern present on salicylaldehydes. Interestingly, the complex lacking halogens on the salicylaldehyde auxiliary ligand showed the least cytotoxicity, highlighting the importance of these substituents in the biological activity profile [35,36]. Also recently, Selaković et al. [37] reported two novel Cu(II)-complexes, namely **[Cu(bipy)(L')(BF₄)]** and **[Cu(phen)(L')(H₂O)](BF₄)·H₂O**, bearing α-diimine ligands, which exhibit strong anticancer activity (Fig. 2B). In particular, the phenanthroline-based complex showed potent cytotoxic effects against several cancer cell lines at micromolar concentrations, mainly through induction of apoptosis and cell cycle disruption. Additionally, strong DNA binding and antiangiogenic properties were observed, while no genotoxic effects were detected, highlighting their potential as promising anticancer agents.

The result of these works motivated us to pursue further investigations. Consequently, in 2021, we synthesized a series of Cu(II)-complexes and chiral Pd(II)-complexes containing halogen-substituted tridentate Schiff base ligands along with 2-picolyamine. We concluded that, in both cases, the presence of halogen atoms on the ligand positively influenced the antiproliferative activity profile on cancer cells [36,38]. In 2022, we conducted a similar study by preparing additional Cu(II)-complexes and analogous Pt(II)-complexes for comparative analysis [39]. This study essentially highlighted the validity of Cu(II)-complexes as an alternative to Pt(II)-ones. We further highlighted the influence of chirality and presence of halogen atoms on the Schiff base ligands by developing also enantiopure Pd(II)-complexes [40,41]. Moreover, we confirmed that halogenation of ligands plays a

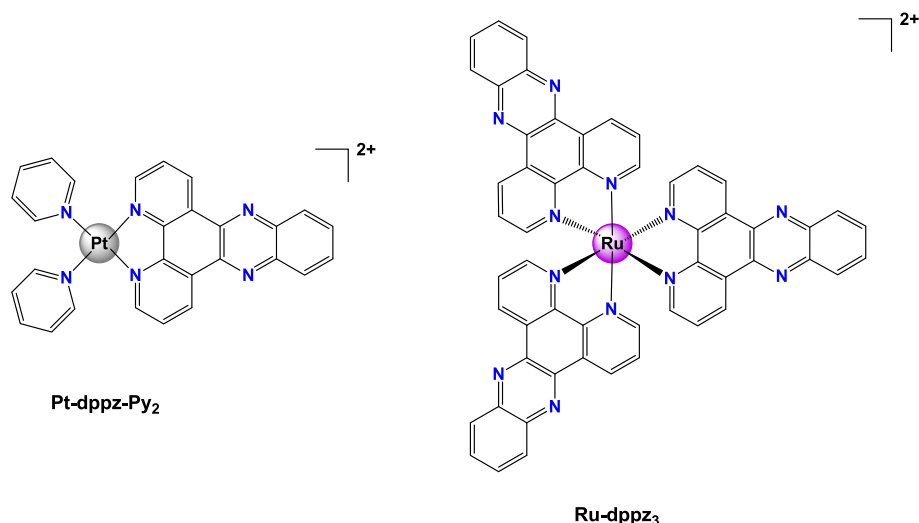
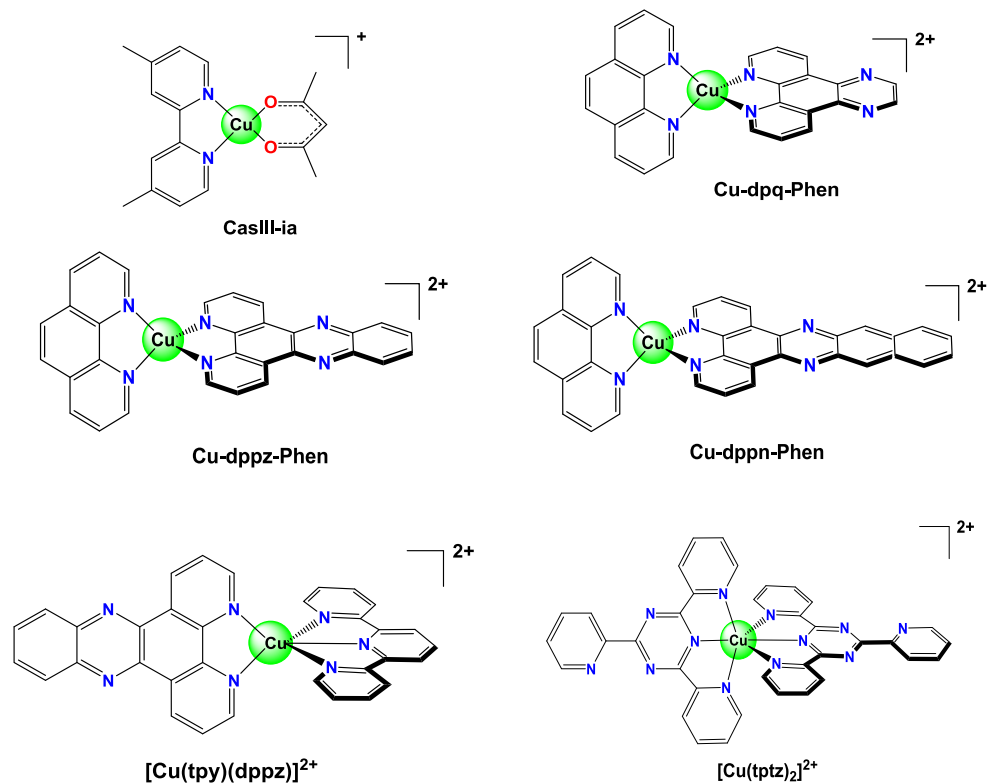


Fig. 1. Representative examples of Pt(II)- and Ru(II)-polypyridyl complexes endowed with anticancer activity developed so far.

A



B

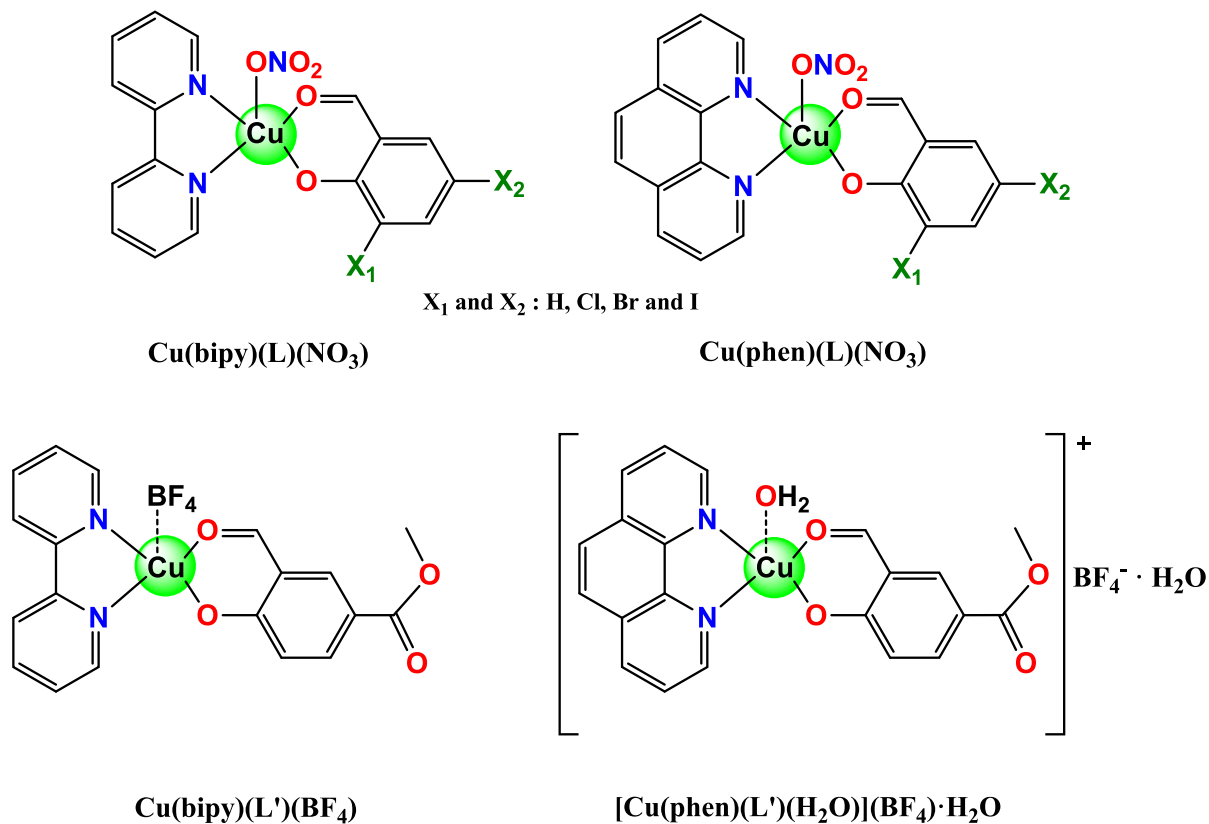
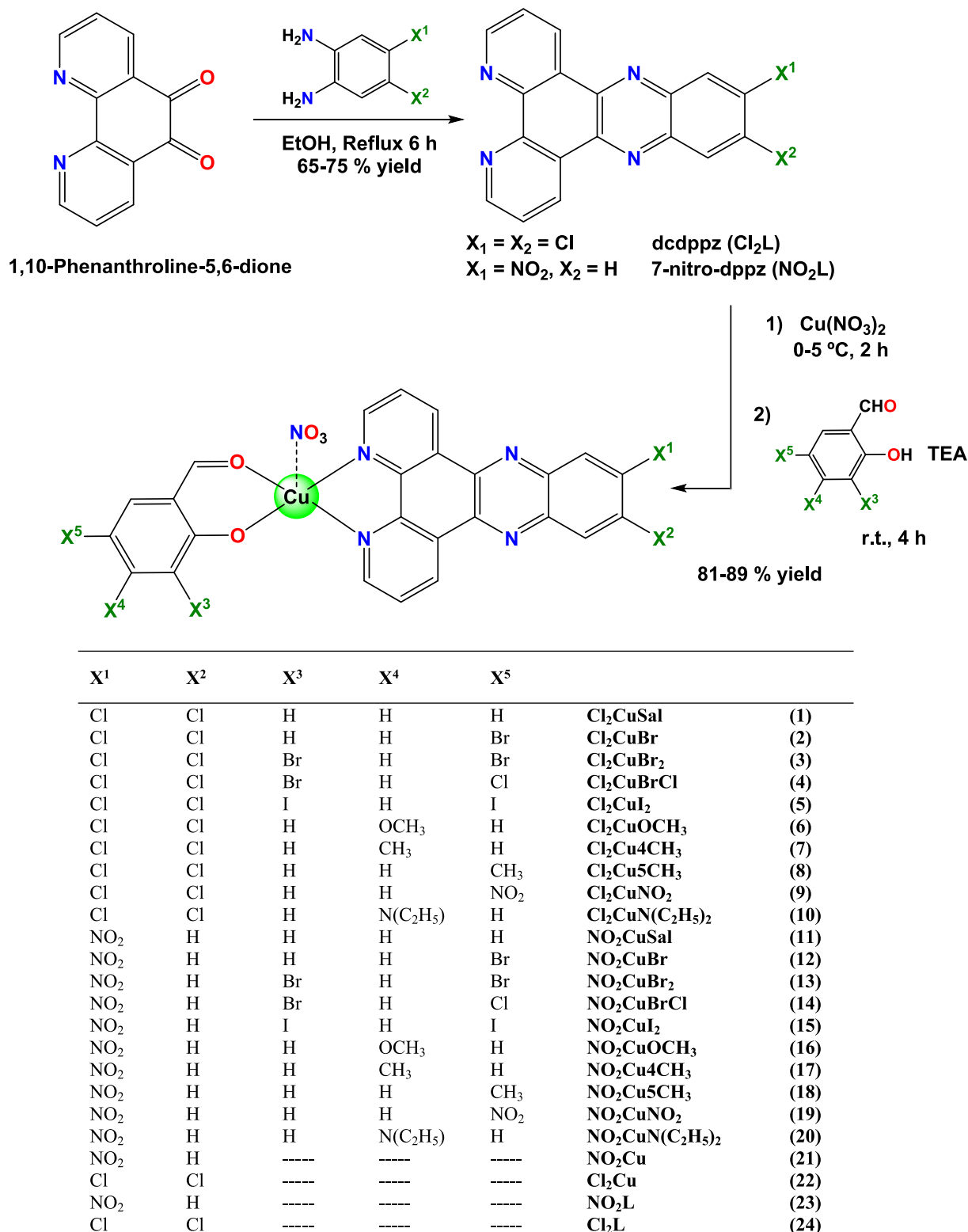


Fig. 2. A) Some representative examples of Cu(II)-polypyridyl complexes endowed with anticancer activity developed so far. B) Examples of Cu(II)-diimine-complexes with X-sal-derivative ligands endowed with anticancer activity.

crucial role not only in their cytotoxicity but also in their mechanism of action [42]. More recently, we moved to a series of Ru(II)-polypyridyl complexes, namely $[\text{Ru}(\text{phen})_2(\text{X-sal})]\text{BF}_4$, in order to investigate the impact of the chemical/electronic characteristics and position of the substituents in the structure of the salicylaldehyde ligand on the cytotoxic behavior of these complexes. The results showed that 4-position electron-donating group (4-EDG) and 3,5-dihalogen substitution

provide the highest cytotoxicity [43].

In this study, we aimed to investigate a comprehensive panel of Cu(II)-complexes and examine the effect of substitutions on both the salicylaldehyde ligand (*sal*) and diimine ligand (specifically the polypyridyl derivative dppz (dipyrido[3,2-*a*:2',3'-*c*]phenazine)), on the anti-proliferative activity against three tumor cell lines (i.e., MDA-MB 231, U87, and MG 63).



Scheme 1. Synthetic route and chemical structure of the new complexes with the general formula $[\text{Cu}(\text{X-dppz})(\text{X}_2\text{-sal})(\text{NO}_3)]$.

There is some ambiguity regarding the influence of the electronic structure of metal complexes on their biological activities. For instance, studies by Notaro et al. [44] and Lu et al. [45] suggest that complexes containing EDGs exhibit higher cytotoxicity than those with electron-withdrawing groups (EWGs). In contrast, other researchers have reported that the presence of EWGs is crucial for biological activity [46,47]. Therefore, we decided to conduct a detailed study on the effect of different substituents on the anticancer activity of metal complexes.

2. Results and discussion

2.1. Synthesis and characterization

The synthesis of the twenty Cu(II)-complexes named [Cu(X-dppz)(X₂-sal)(NO₃)] was carried out with good yields by reacting copper(II) nitrate with the corresponding ligands in methanol (Scheme 1). Despite extensive efforts to obtain suitable single crystals for X-ray diffraction analysis of the copper complexes, including the use of various crystallization techniques such as slow evaporation, vapor diffusion, and solvent-solvent layering with different solvent systems, all attempts were unsuccessful. Therefore, structural characterization was performed using alternative analytical techniques, including elemental analysis, mass spectrometry and FT-IR, UV-Vis and EPR spectroscopies. According to the results obtained from the IR-spectra of the complexes, the oxygen atoms of the salicylaldehyde ligand and nitrogen atoms of the diimine ligand are coordinated to the metal ions. The presence of a very strong band at 1600–1630 cm⁻¹ is indicative of the presence of $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{C})$ belonging to the heterocyclic rings. Moreover, several medium intensity bands were observed in the range of 2910–3100 cm⁻¹, confirming the existence of the C–H of aliphatic and aromatic protons (Figs. S1–S20).

The ESI-MS spectra of the obtained compounds are displayed in Figs. S21–S40. For the most representative complexes, the experimental mass spectra were validated by comparison with simulated isotopic abundance profiles calculated at both high and instrumental-like mass resolutions.

Notably, in the ESI-MS spectra, the systems investigated were observed as cationic species (molecular species minus the NO₃⁻ anion) because of the loss of the NO₃⁻ anion during the desorption/ionization process.

The experimental data support the proposed complexes structures. Given the nitrate anion's poor coordination ability and on the basis of the X-ray crystal structure of similar Cu(II)-complexes which exhibit a square-pyramidal geometry with the apical position occupied by the nitrate oxygen atom with the Cu–O bond longer than the equatorial ones [35], in solution the Cu(II) nitrate bond is broken, resulting in the formation of the complex [Cu(X-dppz)(X₂-sal)]⁺ as confirmed by DFT calculations and EPR (see hereinafter). It should be noted that such a case has been observed previously in similar complexes with tetrafluoroborate ion (BF₄⁻) [37]. Moreover, conductivity measurements carried out on complexes NO₂CuBrCl (14) and Cl₂CuBrCl (4), in a 10 μM aqueous solution, show the behavior of a 1:1 electrolyte (at 25 °C, $\Lambda_{\text{M}} = 149.2$ and 156.3 S cm² mol⁻¹, respectively) consistent with the assumption that the complexes, once in solution, undergo the loss of the coordinated NO₃⁻ group.

The UV-Vis characterizations of the samples Cl₂CuSal (1), Cl₂CuBr (2) and Cl₂CuBr₂ (3) are reported in Fig. 3. In the magnification of the spectra are evident the lower energy absorptions (inset of Fig. 3). The UV-Vis spectra of all complexes are reported in Fig. S41.

To obtain some hint as to the coordination geometry of Cu(II) in the investigated compounds we recorded Electron Paramagnetic Resonance (EPR) spectra on selected samples, both in solid state and frozen solution. The powder spectra (Fig. 4a) of Cl₂CuBrCl (4) and NO₂CuBrCl (14) are typical for a tetragonally axial Cu(II), characterized by a d_{x₂-y₂} magnetic orbital; in particular, the value of $g_{\parallel} = 2.23$ and $g_{\perp} = 2.08$ are consistent with a square pyramidal coordination geometry. On the

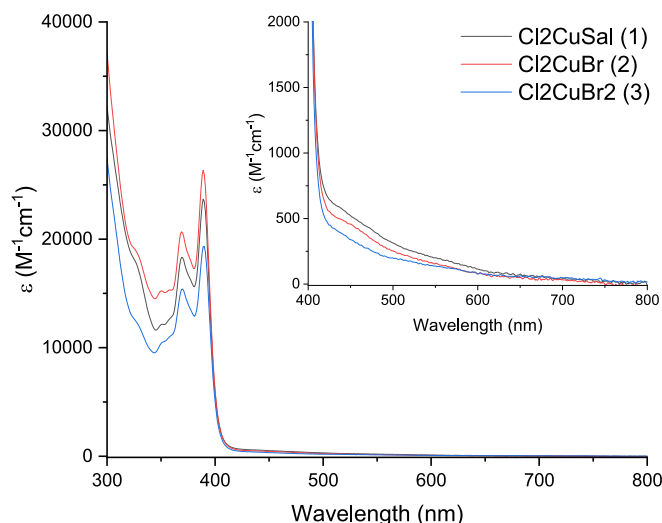


Fig. 3. UV-Vis spectra of the samples Cl₂CuSal (1), Cl₂CuBr (2) and Cl₂CuBr₂ (3) in DMSO. In the inset, the spectra magnification in the range 400–800 nm.

contrary, Cl₂Cu (21) and NO₂Cu (22) clearly show an “inverted pattern” with $g_{\perp} = 2.14 > g_{\parallel} = 2.02$, which indicates a strong contribution of d_{z²} to the ground state. This suggests that in these two samples Cu(II) are penta-coordinated with a geometry close to trigonal bipyramidal. In addition to this, one can notice in Cl₂CuBrCl (4) and NO₂CuBrCl (14) the presence of an half field transition (Fig. S42), occurring at an effective $g = 4.17$ which is consistent with twice the g value of the main transition. This is a clear indication of the presence of magnetic interactions among Cu(II) centers in the solid state in these two samples. To check whether such interactions are intermolecular or intramolecular, and the stability of the coordination geometry in solution, we further measured the frozen solution spectra in DMSO of the four samples.

The resulting spectra (Fig. 4b) evidence a few relevant points: i) the spectral pattern of Cl₂Cu (21) and NO₂Cu (22) in frozen solution is typical for a d_{x₂-y₂} magnetic orbital, with $g_{\parallel} = 2.32$ $g_{\perp} = 2.08$, indicating a major structural rearrangement toward a tetragonal geometry upon dissolution; we tentatively attribute this phenomenon to dissociation of a weakly bound methanol molecule (reaction solvent) and consequent rearrangement; ii) for samples Cl₂CuBrCl (4) and NO₂CuBrCl (14), the parallel series of signals due to hyperfine coupling with Cu nuclei is apparently doubled, suggesting the presence of two different isomers in solution; iii) also for these two samples a different coordination environment compared to the solid state is hypothesized, considering the different g values observed compared to solid state samples ($g_{\parallel} = 2.32$ $g_{\perp} = 2.08$ for the frozen solution); iv) the very similar g values observed for the four samples indicate that in solution their coordination geometry is essentially the same; v) interestingly, despite being weak, the half-field transition which is an indication of the presence of magnetic interactions is still present in the frozen solution spectra of Cl₂CuBrCl (4) and NO₂CuBrCl (14) (Fig. S42). This suggests that these two complexes might actually tend to establish intermolecular interacting structures even in solution.

2.2. Biological evaluation

All metal complexes were tested *in vitro* to evaluate their anti-proliferative activity. For this purpose, three cancer cell lines, namely MG 63 (osteosarcoma), breast adenocarcinoma (MDA-MB 231) and glioblastoma (U87) were selected based on the incidence (MDA-MB 231) and aggressiveness (U87 and MG 63) of the related tumor type. Breast cancer remains the leading cause of death among women, with 2.4 million new cases reported globally in 2024 [48]. It frequently

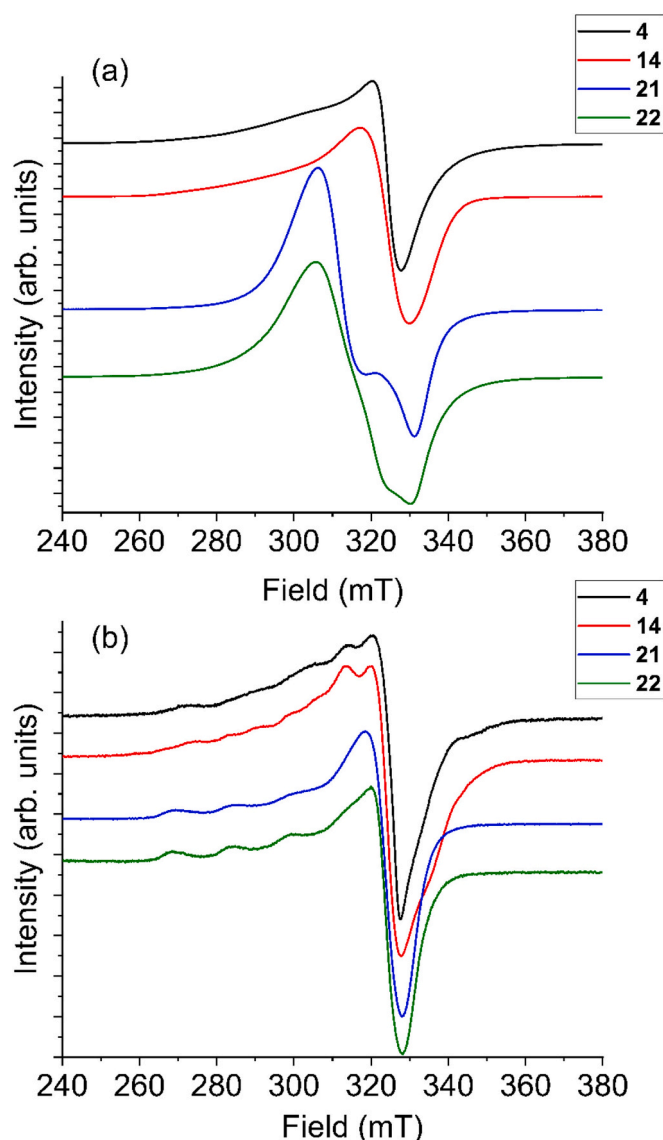


Fig. 4. Low temperature (60 K) X-band ($\nu = 9.390$ GHz) EPR spectra of (a) microcrystalline powder samples and (b) DMSO frozen solution spectra.

metastasizes to the liver, lungs, brain, and bones, with bone metastases occurring in 70% of cases [49]. Osteosarcoma, the most prevalent bone cancer in young patients, responds poorly to chemotherapy, significantly reducing life expectancy [50]. Glioblastoma, an aggressive central nervous system cancer originating from glial cells, also has a poor prognosis. The 5-year survival rate stands at 5%, with no notable advancements in recent decades [51].

A first biological screening was carried out to evaluate the cytotoxicity of all the complexes at 10 μM , testing cell viability after an overnight incubation using the MTT assay, in which MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was applied to measure metabolic activity as an indicator of viable cells [52,53]. The two polypyridyl ligands, i.e., NO_2L (23) and Cl_2L (24), plus the two polypyridyl Cu(II)-complexes devoid of the salicylaldehyde ligand, i.e., NO_2Cu (21) and Cl_2Cu (22), were considered for comparative SAR analysis. MDA-MB 231 cells displayed a marked reduction in cell viability for all complexes bearing the $-\text{NO}_2$ group at the polypyridyl ligand, i.e., NO_2CuSal (11)– $\text{NO}_2\text{CuN}(\text{C}_2\text{H}_5)$ (20) (Fig. 5 D). Complexes bearing two $-\text{Cl}$ groups at the polypyridyl ligand instead, i.e., Cl_2CuSal (1)– $\text{Cl}_2\text{CuN}(\text{C}_2\text{H}_5)$ (10), did not show a remarkable inhibition of cell viability except for complex Cl_2CuBrCl (4) (Fig. 5 A). Pure ligands

(NO_2L (23) and Cl_2L (24)) were poorly active, as well as the *sal*-free complex NO_2Cu (21), whereas *sal*-free complex Cl_2Cu (22) showed $\sim 70\%$ reduction of cell viability (Figs. 5 A and D). The screening on U87 cells highlighted much less structure-related selectivity, as almost all complexes showed ~ 40 – 60% reduction of cell viability. The only complexes with superior antiproliferative activity were Cl_2CuBrCl (4), NO_2CuBrCl (14), NO_2CuI_2 (15) and NO_2CuNO_2 (19). Again, the ligands were found to be less active than the complexes, although with a difference not as marked as in the case of MDA-MB 231 (Figs. 5 B and E). MG 63 cells proved to be much more sensitive than U87 cells, still following the same trend of reduction of cell viability (Figs. 5 C and F). Regardless of cellular variability in the cytotoxic response, this 10 μM screening revealed that Cu(II)-complexes with the $-\text{NO}_2$ group at the polypyridyl ligand are, in general, more cytotoxic than those with two chlorine atoms at the same ligand (with the exception of compound Cl_2CuBrCl (4)) and that the presence of halogens (specifically, double substitution at positions 3 and 5) or an $-\text{NO}_2$ group (at position 5) on the *sal* ligand is decisive for the activity.

In view of these preliminary results, the complexes Cl_2CuBrCl (4), NO_2CuBrCl (14), NO_2CuI_2 (15) and NO_2CuNO_2 (19) were selected for further biological assessments, including continuous assays for IC_{50} values determination on the same cancer cell lines, cell morphology evaluation and ROS detection. The two Cu(II)-complexes lacking the auxiliary *sal* ligand NO_2Cu (21) and Cl_2Cu (22) were also selected for these detailed assessments for comparative SAR analysis.

The IC_{50} determinations were carried out using serial dilutions of the samples (0.065, 0.125, 0.5, 1, 5, 10, and 20 μM) after 24 h of incubation. As illustrated in Fig. 6, the IC_{50} values and corresponding dose-response curves demonstrated that the tested compounds (Cl_2CuBrCl (4), NO_2CuBrCl (14), NO_2CuI_2 (15), NO_2CuNO_2 (19)) were more effective than their respective *sal*-free Cu(II) metal counterparts (NO_2Cu (21), Cl_2Cu (22)) in all the cell lines. Notably, the complexes exhibited greater cytotoxicity against the breast cancer cell line MDA-MB 231, with complex NO_2CuBrCl (14) showing the lowest IC_{50} concentration (i.e., 3.55 μM) among the compounds bearing the $-\text{NO}_2$ group on the polypyridyl ligand. This range of IC_{50} values obtained against MDA-MB 231 cells (3.55–10.22 μM) roughly aligns with other Cu(II)-complexes tested in the same conditions such as two acylhydrazone derivatives with heterocyclic bases as coligands, namely $[\text{Cu}(\text{HL})(\text{bipy})](\text{NO}_3)$ ($\text{IC}_{50} = 11.4$ μM) and $[\text{Cu}(\text{L})(o\text{-phen})]$ ($\text{IC}_{50} = 5.3$ μM), recently developed by Parajón-Costa and co-workers [54], which also obtained nonlinear findings using poly-functional ligands derived from *o*-vanillin and thiophene (i.e., $\text{IC}_{50} = 1.6$ μM for the acylhydrazone derivative $[\text{Cu}(\text{HL})(\text{H}_2\text{O})](\text{NO}_3) \cdot \text{H}_2\text{O}$ and $\text{IC}_{50} = 23.0$ μM for the Schiff base derivative $\text{Cu}(\text{oVATPNH}_2)_2$, although the latter evaluated at 48 h) [55,56]. The best compound against MG 63 cells turned out to be Cl_2CuBrCl (4), whose IC_{50} value (4.70 μM) perfectly aligns with those of the above-mentioned acylhydrazone derivatives ($[\text{Cu}(\text{HL})(\text{bipy})](\text{NO}_3) \rightarrow \text{IC}_{50} = 5.6$ μM ; $[\text{Cu}(\text{L})(o\text{-phen})] \rightarrow \text{IC}_{50} = 3.5$ μM) [54], and is slightly higher compared to a new acylhydrazone derivative with promising dual anticancer and antimetastatic action developed by the same research group ($[\text{Cu}(\text{N-N-Fur}(\text{NO}_3)(\text{H}_2\text{O}))]$ [57]. No significant findings regarding cytotoxicity of Cu(II)-complexes against the U87 cell line have yet been reported in the literature that are comparable to those we obtained in this study.

Based on these findings, a deeper characterization of the interaction between the MDA-MB 231 cell line and complexes Cl_2CuBrCl (4) (the most effective among the complexes containing two $-\text{Cl}$ groups on the polypyridyl ligand), NO_2CuBrCl (14) (the most effective among the complexes featuring the $-\text{NO}_2$ group on the polypyridyl ligand), as well as that of their *sal*-free Cu(II) counterparts (Cl_2Cu (22) and NO_2Cu (21), respectively), was investigated. Specifically, cells were treated with the IC_{50} concentration of each compound for 6 and 24 h, followed by morphological analysis to assess cellular state. Since cytoskeletal integrity and nuclear shape are critical indicators of cell health, actin/DAPI staining was conducted. The observed cell density aligned with cell viability data, and in most cases, cytoskeletal integrity was highly

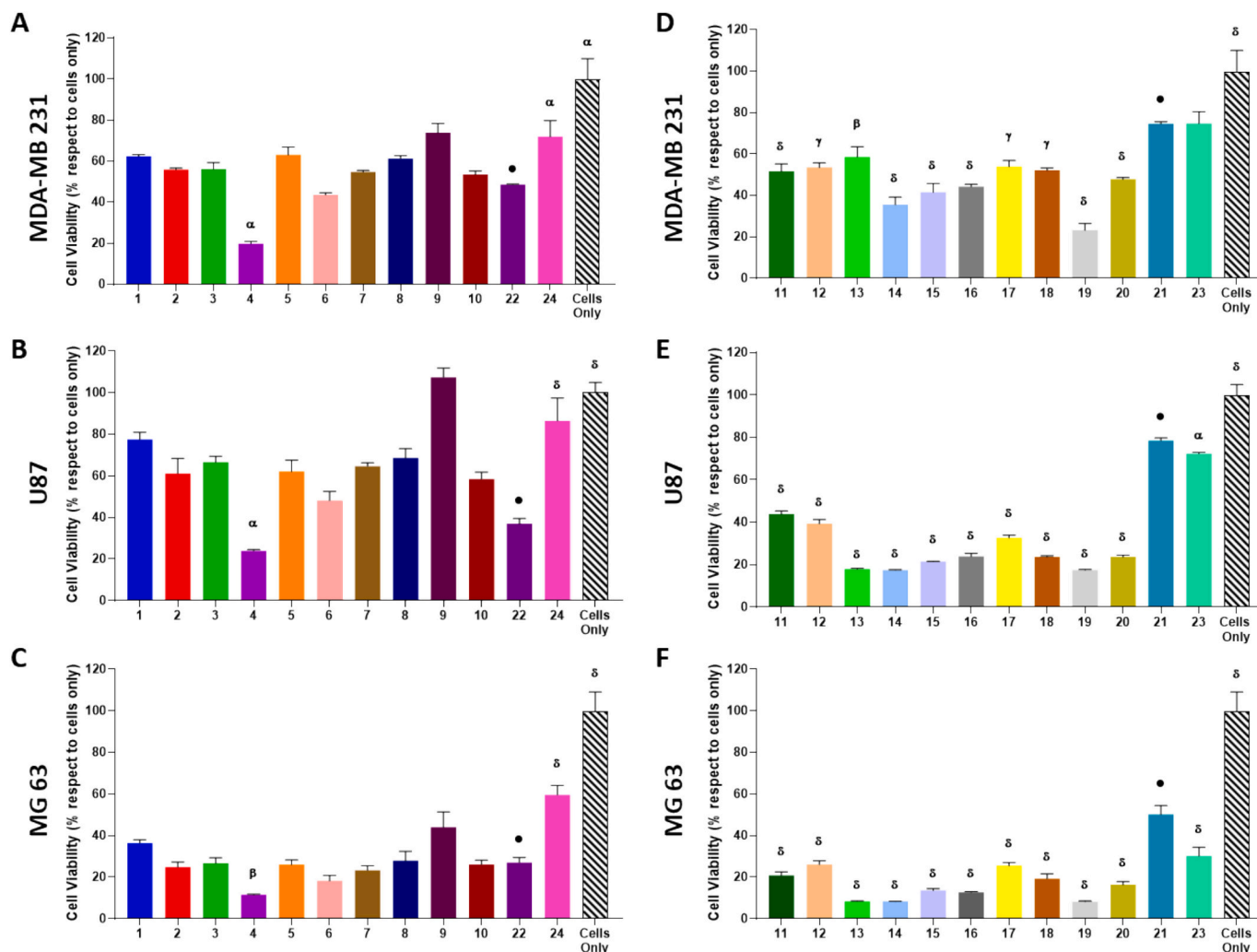


Fig. 5. Antiproliferative effect of the synthesized compounds against MDA-MB 231, U87 and MG 63 cancer cell lines after 24 h treatment at 10 μ M. (A–C) Complexes bearing two $-\text{Cl}$ groups at the polypyridyl ligand. (D–F) Complexes bearing the $-\text{NO}_2$ group at the polypyridyl ligand. Data are shown as mean $\pm$ SD. Statistical analyses with respect to *sal*-free Cu(II) metal counterparts (NO_2Cu (21), Cl_2Cu (22); shown as ●) are reported in the graph, p value is $\alpha \leq 0.05$, $\beta \leq 0.01$, $\gamma \leq 0.001$ and $\delta \leq 0.0001$. Cells Only = negative control.

compromised, further corroborating the previously obtained viability results (Fig. 7).

To further investigate the mechanisms underlying cell cytotoxicity, ROS production was evaluated (Figs. 8 and S43). Elevated ROS levels can lead to oxidative stress, damaging vital cellular components such as lipids, proteins, and DNA, ultimately causing oxidative cell death [58]. After treating cells with the IC_{50} concentration of each compound for 2 and 24 h, the assay revealed that complex Cl_2CuBrCl (4) induced significantly higher ROS production compared to its *sal*-free counterpart (Cl_2Cu (22)), likely contributing to its greater activity. Conversely, compound NO_2CuBrCl (14) showed lower ROS production than its *sal*-free counterpart (NO_2Cu (21)). It should be noted that the CellROX Green assay provides an indication of increased intracellular ROS levels, but does not allow the identification of the specific ROS species involved or the precise mechanism underlying their generation. This apparent discrepancy indicates that the extent of ROS generation does not directly correlate with the cytotoxic potency of these complexes and suggests that ROS generation alone is unlikely to account for their biological activity. This discrepancy between the cytotoxicity of compound NO_2CuBrCl (14) and the higher ROS production of compound NO_2Cu (21) may therefore reflect differences in their overall mechanisms of action, including the relative contribution of ROS-dependent and ROS-independent pathways. In particular, the cytotoxic activity of

NO_2CuBrCl (14) may involve mechanisms that are not directly reflected by the intracellular ROS levels measured by the CellROX assay, such as DNA interactions and G-quadruplex stabilization. Although Cu(II)/Cu(I)-mediated redox processes represent a plausible source of oxidative stress for copper complexes, the present experiments do not allow us to establish this mechanism or to distinguish it from other possible ROS-generating pathways, including photophysical processes [59].

Overall, these findings indicate that complexes Cl_2CuBrCl (4) and NO_2CuBrCl (14) are promising candidates for further investigation and hold potential as novel anticancer agents.

The biological evaluation in the present study focused on tumor cell models, allowing us to identify the most promising structural features within this new family of Cu(II) complexes and to define the corresponding structure–activity relationships through comparative screening across representative tumor cell lines. Future investigations may extend this analysis to appropriate non-tumor cell models to further characterize the selectivity profile of the lead compounds and assess their potential for preclinical development.

2.3. Theoretical calculations

As already mentioned, based on our previous study [35], the coordination geometry around copper(II) in all synthesized complexes with

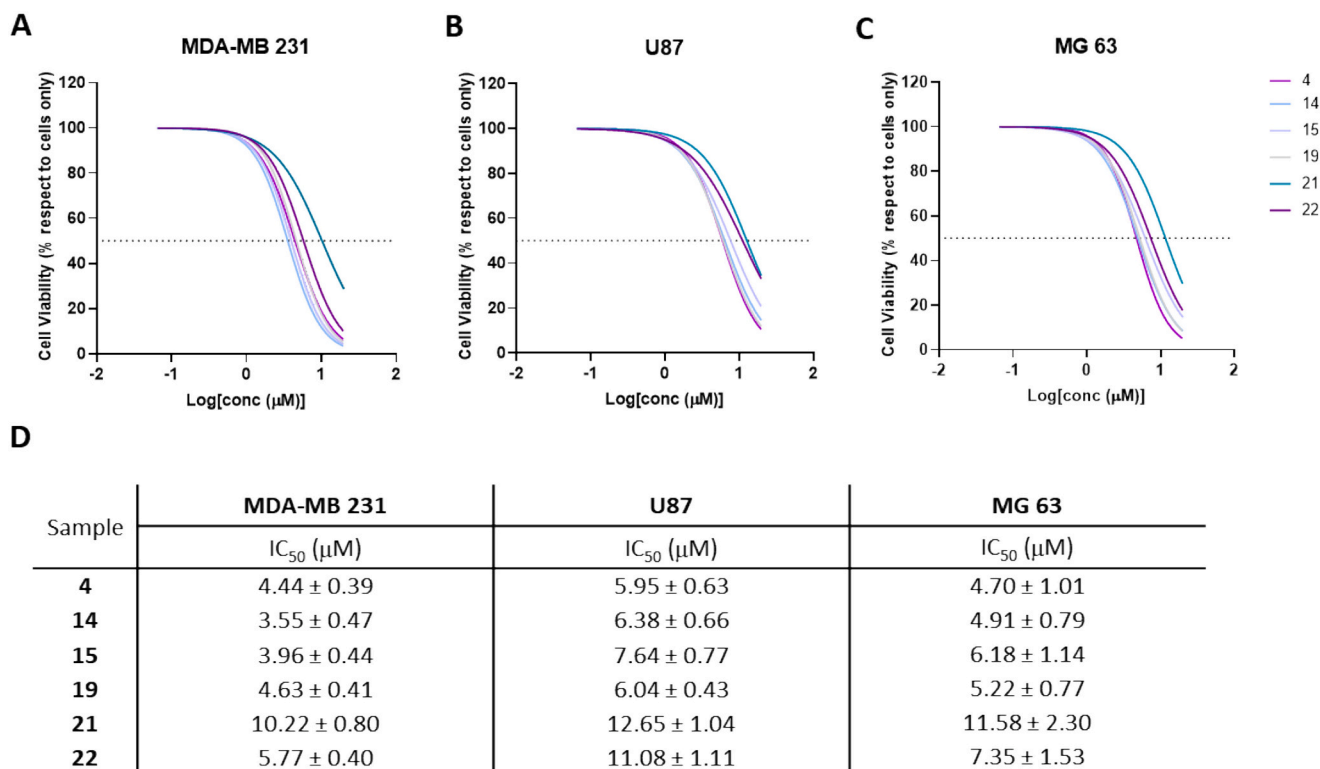


Fig. 6. IC₅₀ (μM) values of the compounds Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂CuI₂ (15), NO₂CuNO₂ (19), NO₂Cu (21) and Cl₂Cu (22) against MDA-MB 231 (A), U87 (B) and MG 63 (C) cells. (D) Data are reported as mean ± standard error.

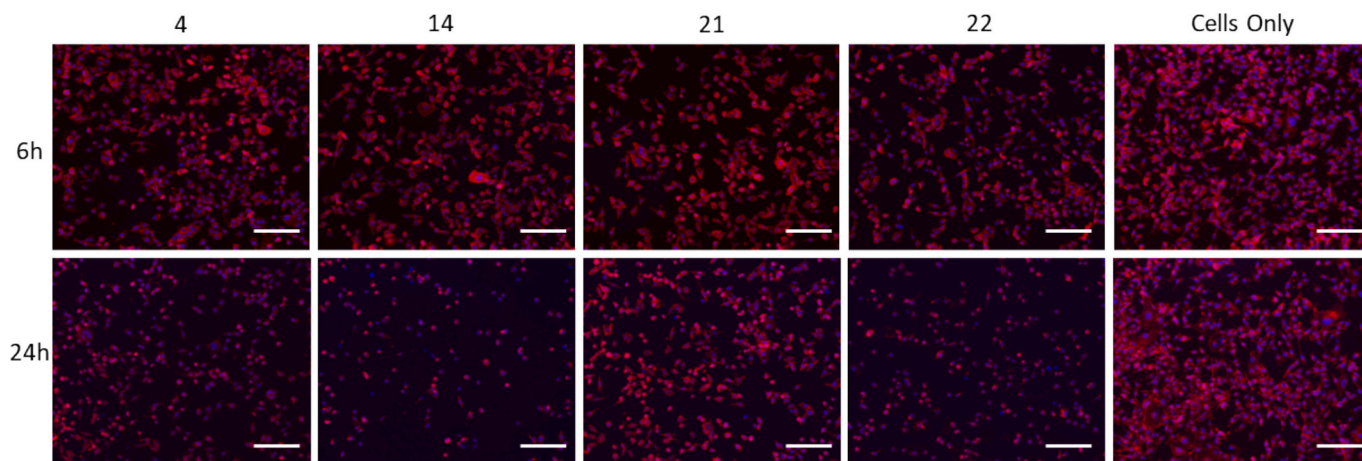


Fig. 7. Morphological evaluation after 6 h and 24 h incubation complexes Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂Cu (21) and Cl₂Cu (22) at IC₅₀ concentration on MDA-MB 231 cells. F-actin filaments in red; cell nuclei in blue. Scale bars: 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

general formula Cu(X-dppz)(X₂-sal) is square-pyramidal in solid state, with the CuN₂O₂ square plane occupied by nitrogen atoms of diimine ligands and oxygen atoms of coligand. The apical position was also occupied by the oxygen atom of a nitrate anion. However, the Cu(II)-complexes are square-planar in solution, due to breaking of the weak Cu-nitrate bond. Other Cu(II)-complexes with similar structures to our compounds have been reported [37]. The optimization of the structure of these complexes has been carried out considering a coordination number of 4, because the calculations are focused on explaining the UV-Vis spectra in solution, where a square-planar coordination is expected [37]. The calculated structural parameters around the copper center are in good agreement with the values of the solid-state structure

(see Table S1). The structure of complexes Cl₂CuSal (1), Cl₂CuBr (2), Cl₂CuBr₂ (3), NO₂CuSal (11), NO₂CuBr (12) and NO₂CuBr₂ (13) was optimized by DFT calculations using the B3LYP hybrid functional and def2-TZVP basis describing the atoms. Table S1 gathers some structural parameters compared with the related experimental values from close compounds. In order to help the understanding of the electronic structure and the excited states, TD-DFT calculations were also performed (see also the “Additional data on theoretical calculations” section in ESI).

2.3.1. Docking and molecular dynamic simulations

The G-quadruplex (G4) is a unique DNA secondary structure formed

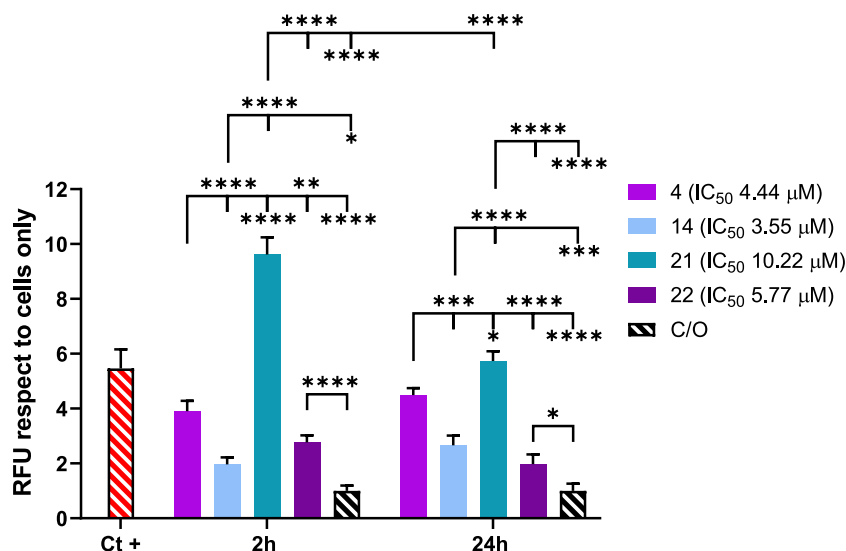


Fig. 8. Reactive oxygen species (ROS) induced after 2 h and 24 h of exposure of MDA-MB 231 cells to IC₅₀ concentrations of complexes Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂Cu (21) and Cl₂Cu (22). TBHP (200 μM) was used as a positive control (Ct +). Data are shown as mean ± SD. Statistical analyses comparing the groups to cells only are reported in the graph; *p* value is * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001 and **** ≤ 0.0001. C/O (Cells Only) = negative control.

by guanine-rich sequences and has recently garnered significant attention in oncology research [60]. Guanine bases can fold into stacked tetrads (G-quartets), adopting various topologies that influence biological processes, particularly during the cellular replication phase. The formation and stabilization of these structures can lead to gene silencing, making them promising therapeutic targets. Current research is focused on identifying selective G4-binding compounds that specifically recognize individual quadruplex topologies, with the goal of developing targeted anticancer therapies for specific tumor types [61–63].

In our study, we evaluated the cytotoxic activity of copper complexes

on three cell lines known for their ability to form G-quadruplexes: MDA-MB 231 (breast adenocarcinoma), U87 (glioblastoma), and MG 63 (osteosarcoma). To investigate whether the activity of our complexes could be potentially ascribed to their G4-stabilizing properties, we selected four complexes, i.e. Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂Cu (21), and Cl₂Cu (22), as representatives of –Cl (4, 22) and –NO₂ (14, 21) derivatives, and with (4, 14) and without (21,22) auxiliary salicylaldehyde ligand, for molecular docking and subsequent molecular dynamics (MD) simulations. Simulations were performed using four target G4 models: KRAS (breast cancer – PDB ID: 5I2V) [64]; hTERT (glioblastoma – PDB IDs: 2ZKE, 2ZKD) [65]; and wtTel23 (osteosarcoma – PDB ID:

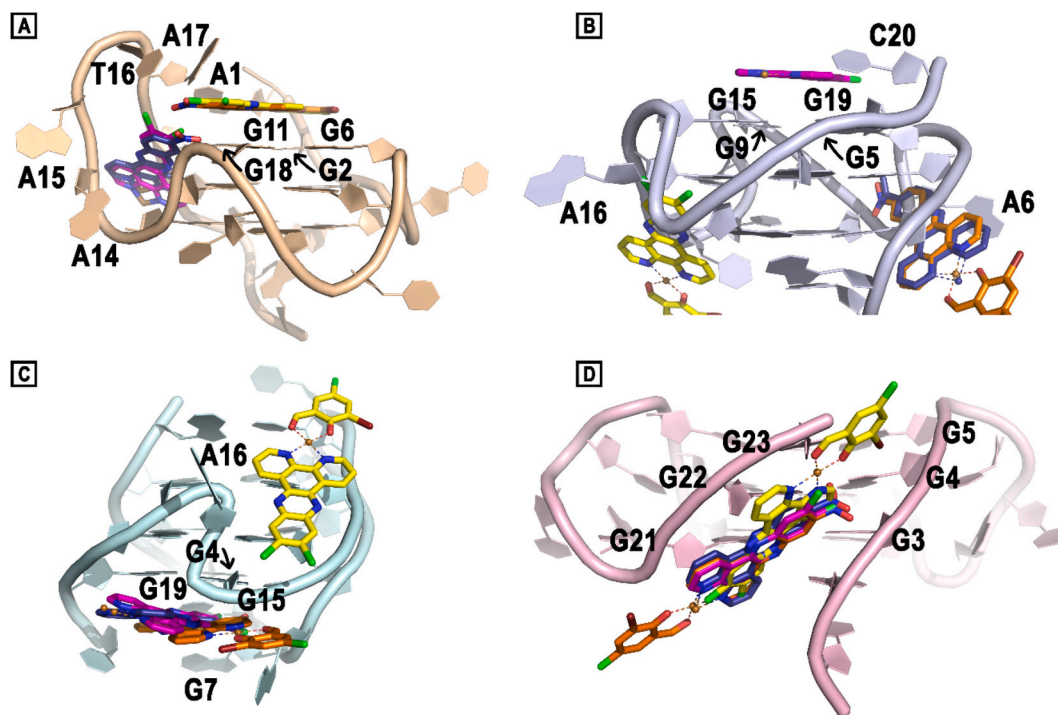


Fig. 9. Docking-predicted bound conformations of complexes Cl₂CuBrCl (4) (yellow sticks), NO₂CuBrCl (14) (orange sticks), NO₂Cu (21) (blue sticks) and Cl₂Cu (22) (magenta sticks) on target G4 models 5I2V (panel A), 2ZKE (panel B), 2ZKD (panel C), and 2LD8 (panel D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2LD8) [66].

Ten NMR-resolved conformations from each of the aforementioned G4 PDB models were used as targets for ensemble docking simulations (see experimental section), that were used to predict the G4-bound conformation of **Cl₂CuBrCl** (4), **NO₂CuBrCl** (14), **NO₂Cu** (21), and **Cl₂Cu** (22). The complexes, in their top scoring docking poses, were found to interact with G4 by π -stacking onto bases layers, by groove binding, or by interaction within loop structures lined by the phosphodiester backbone.

On 5I2V, complexes **Cl₂CuBrCl** (4) and **NO₂CuBrCl** (14) are predicted to stack on a layer composed of bases G2, G6, G11 and G18, with A1 and A17 stacking with the compounds from above. Complexes **NO₂Cu** (21) and **Cl₂Cu** (22) instead bind into a phosphodiester backbone loop created by the extrusion of the A14, T15 and T16 bases (Fig. 9, panel A). It is worth noting that the Lowest Binding Energies (LBE) for compounds **Cl₂CuBrCl** (4) (LBE = −12.91) and **NO₂CuBrCl** (14) (LBE = −13.46) (Table 1) are the most favorable among the simulated compounds, in agreement with the biological data (Fig. 5). On 2ZKE, the best scoring clusters of complexes **NO₂CuBrCl** (14) and **NO₂Cu** (21), mainly interact within a phosphodiester backbone-lined loop created by the extrusion of the unpaired basis A6, while complex **Cl₂CuBrCl** (4) binds into a loop created by the extrusion of A16. Complex **Cl₂Cu** (22) is predicted to interact by π -stacking onto the bases layer formed by G5, G9, G15 and G19, with C20 stacking on top of the complex (Fig. 9, panel B). On 2ZKD, complexes **NO₂CuBrCl** (14), **NO₂Cu** (21) and **Cl₂Cu** (22) are predicted to stack onto the layer composed of bases G4, G8, G15 and G19, with G7 stacking on top of the compounds. Complex **NO₂CuBrCl** (14) is found rotated on its plane of 90° with respect to complexes **NO₂Cu** (21) and **Cl₂Cu** (22). Complex **Cl₂CuBrCl** (4) is instead predicted to bind into a phosphodiester backbone loop created by extrusion of A16 (Fig. 9, panel C). Finally, on 2LD8 all the best scoring bound conformation bind to the groove lined by residues G3, G4, G5, G21, G22 and G23 (Fig. 9, panel D).

To date, no experimentally determined structures of the G-quadruplex topologies investigated in this study in complex with stabilizers have been reported, although similar topologies complexed with stabilizers have been described [67,68]. In these structures, the ligands feature large aromatic moieties that promote π - π stacking interactions with the terminal G-tetrads, a binding mode also predicted for our organometallic complexes. Additionally, these experimental structures highlight secondary interactions occurring within the loop regions and along the grooves of the quadruplex scaffold. Such interaction patterns closely resemble the alternative binding poses observed in our docking simulations (Fig. 10).

Overall, comparison with these experimentally characterized G4–ligand complexes reinforces the structural plausibility of the binding modes proposed herein and supports the hypothesis that the aromatic character of the investigated organometallic compounds plays a central role in mediating G-quadruplex stabilization through stacking-driven molecular recognition mechanisms.

To investigate the potential of the complexes in stabilizing the G4 topology, we carried out MD simulations (see experimental section) using the docking-predicted bound conformations described above. The DNA RMSD values of each MD trajectory are plotted in Fig. 11 as a function of the simulation time. To get insight into the potential G4 stabilizing properties of the complexes, the RMSD values of the four

unbound G4 models are plotted as reference. Overall, G4-stabilizing potential of the complexes is evident in 5I2V and 2LD8 models, while no clear clue of stabilizing properties is evident for 2ZKE and 2ZKD models, in line with the biological data that show, for U87 cells, the lowest complexes induced cytotoxicity (see Fig. 6). The MD trajectories of the complex-bound G4 models 5I2V and 2LD8 clearly agree on the G4 stabilizing properties of the chloro-derivatives, both in the presence (complex **Cl₂CuBrCl** (4)) or in the absence (complex **Cl₂Cu** (22)) of the salicylaldehyde ligand. On the other hand, MD simulations of the nitro-derivatives bound conformations do not show agreement on the effect on G4 stabilizing properties of the salicylaldehyde ligand. Indeed, the presence of this latter auxiliary ligand (complex **NO₂CuBrCl** (14)) appears detrimental in the case of 5I2V, beneficial in the case of 2LD8.

Fig. 12 depicts the last MD trajectory frames of unbound and **Cl₂CuBrCl** (4)-bound 2LD8 G4 models, where it is evident that, in the absence of a stabilizing complex, the G4 loses its folding trying to match the bases and recover the duplex conformation (Fig. 12, panel A). On the other hand, in the presence of **Cl₂CuBrCl** (4), the G4 conformation is preserved. Overall, simulation studies suggest that G4 stabilization may be involved in the cytotoxicity observed in the MDA-MB231 and MG 63 cell models (Fig. 5). However, in contrast to the observations made for the two aforementioned cellular models, the *in silico* studies do not support G4 stabilization as mechanism of action in the case of the cytotoxicity observed on the U87 cell model. In the latter case, the cytotoxicity activity of Cu(II)-complexes may be mainly due to different and concomitant mechanisms, such as ROS production and consequent DNA cleavage, as supported by the ROS production evaluation.

2.4. Complexes–DNA interaction

Based on the promising cytotoxic activity of the new copper-based compounds and the results of the docking studies with G-quadruplexes compatible with a noncovalent binding process, we decided to investigate the interaction of these complexes with calf thymus DNA (ct-DNA). This direction was further motivated by the well-documented intercalative properties of the dppz ligand which is known to intercalate effectively into DNA base pairs in complexes with octahedral geometry, such as those of Ru(II) and Os(II) [69–71], and even more in square-planar complexes, like those of Pt(II) [72–74], in which reduced steric bulk with the DNA backbone facilitates the interaction.

Among the Cu(II)-complexes with superior antiproliferative activity against the selected cell lines, although biological studies identified **Cl₂CuBrCl** (4) and **NO₂CuBrCl** (14) as the most promising anticancer agents, we chose to investigate the interaction of **NO₂CuNO₂** (19) with ct-DNA. The synthesized Cu(II) complexes, in fact, are highly soluble in DMSO, but have low solubility in aqueous solutions and complex **NO₂CuNO₂** (19) exhibited the highest molar absorbance in DMSO compared to the others, specifically in the region of the spectrum above 300 nm where DNA does not absorb. Therefore, **NO₂CuNO₂** (19) was first dissolved in DMSO and then diluted with 1 mM phosphate buffer (pH 7) containing enough NaCl to give the desired ionic strength, resulting in a final DMSO concentration of 5% v/v to help maintain the complex in solution. Nevertheless, we preferred not to exceed a total complex concentration of 11 μ M.

The interaction of complex **NO₂CuNO₂** (19) with ct-DNA was studied using various experimental techniques. Circular dichroism and absorption studies confirmed a reversible interaction between the copper complex and DNA. As shown in Fig. 13, spectropolarimetric titration of a DNA solution (30 μ M) with increasing concentrations of **NO₂CuNO₂** (19) revealed the emergence of two CD bands due to the rigid orientation of the complex upon binding to the DNA helix: a positive band around 265 nm and a smaller negative one around 305 nm, both increasing with the amount of added complex. Since this spectral region is characterized by the DNA signals, the spectra shown in Fig. 13 were obtained by subtracting the DNA signals at the concentration used. Remarkably, as expected for a reversible interaction of species with the

Table 1

Lowest binding energies (kcal/mol) of docking poses for complexes **Cl₂CuBrCl** (4), **NO₂CuBrCl** (14), **NO₂Cu** (21) and **Cl₂Cu** (22).

Compound/Target	5I2V	2KZE	2ZKD	2LD8
Cl₂CuBrCl (4)	−12.91	−10.36	−9.52	−10.87
NO₂CuBrCl (14)	−13.46	−11.76	−10.96	−10.76
NO₂Cu (21)	−12.39	−10.74	−10.10	−11.76
Cl₂Cu (22)	−10.29	−11.45	−8.57	−9.49

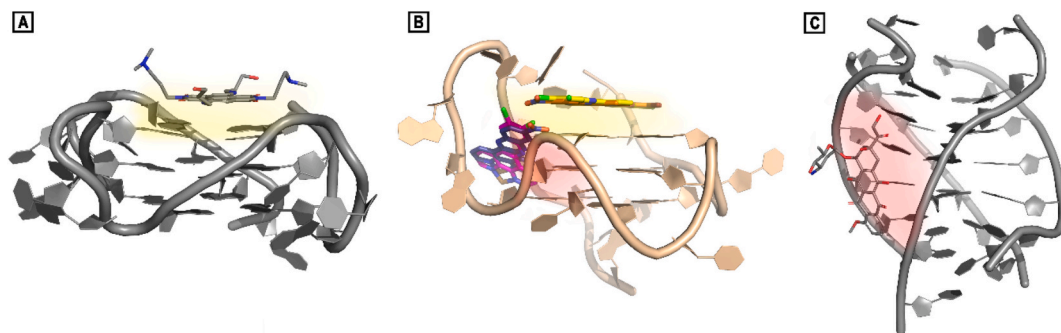


Fig. 10. Crystallographic structures of two G-quadruplex (G4) DNA topologies in complex with aromatic stabilizers: the intramolecular G4 structure (PDB ID: 3CDM, panel A) and the intermolecular G4 structure (PDB ID: 7CPS, panel C), compared with our representative docking model (panel B). Two analogous binding modes can be observed across the experimental and computational models, highlighted by colour-coded regions: i) stacking on the G-tetrad surface (yellow area); ii) ligand insertion within the loop region (red area). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

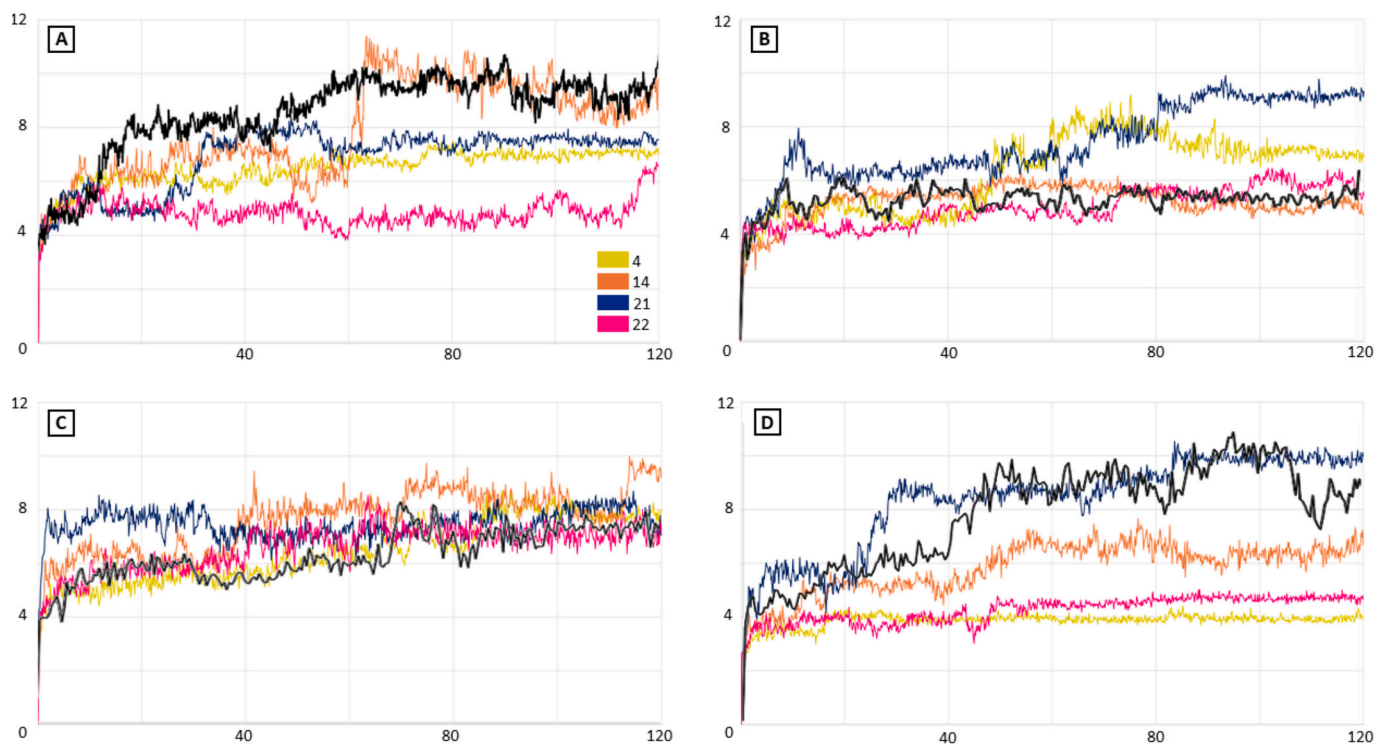


Fig. 11. RMSD values, as a function of MD simulation time, of the selected G-quadruplex structures in apo form (black line), or bound to complexes Cl_2CuBrCl (4) (yellow line), NO_2CuBrCl (14) (orange line), NO_2Cu (21) (blue line), and Cl_2Cu (22) (magenta line). Panel A: 5I2V; panel B: 2ZKE; panel C: 2ZKD; panel D: 2LD8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 12. Final frames of the MD trajectory of apo (A) and Cl_2CuBrCl (4)-bound (B) 2LD8 G-quadruplex.

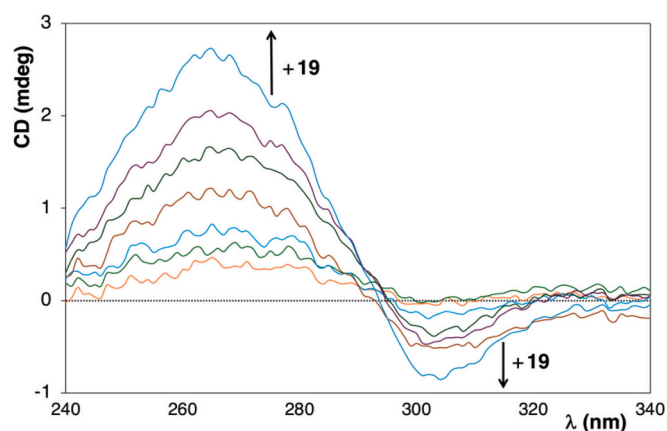


Fig. 13. Spectropolarimetric titration of DNA (30 μ M) with increasing amounts of complex NO_2CuNO_2 (19) at $T = 25^\circ\text{C}$ and $\text{pH} = 7$ (1 mM phosphate buffer, 10 mM NaCl and 5% v/v DMSO). Spectra reported were obtained by subtracting the DNA signals at the concentration used.

DNA, the CD signals shown in Fig. 13 are reversible upon the addition of NaCl.

UV–Vis spectroscopy also confirms the presence of a noncovalent interaction between compound NO_2CuNO_2 (19) and DNA. Indeed, the addition of the biopolymer to the solution of complex NO_2CuNO_2 (19) causes hypochromism of the absorption maximum of the complex at 370 nm, due to the stacking of the aromatic rings of the phenazine moiety with the DNA nucleobases (Fig. 14), accompanied by a slight bathochromic shift. This interaction is also reversible upon addition of NaCl.

Intercalation of a species into DNA leads to stabilization of the double helix, which can be revealed by measuring the melting point of a DNA solution and observing how it changes in the presence of the interacting species. If the species destabilizes the DNA double helix, a decrease in the thermal denaturation temperature (ΔT_m) will be observed; conversely, if the compound stabilizes the helix as is expected for an intercalating agent, an increase in ΔT_m will be measured. In the case of the complex NO_2CuNO_2 (19) an increase of $8.4(\pm 0.5)^\circ\text{C}$ was found, comparable to that of the well-known intercalator $[\text{Pt}(\text{bipy})(\text{py})_2]^{2+}$ [75], $\Delta T_m = 11.2(\pm 0.5)^\circ\text{C}$ under the same experimental conditions (1 mM buffer phosphate at pH 7, containing 2 mM NaCl and 5% v/v DMSO). To further confirm the intercalative ability of NO_2CuNO_2 (19), the effect of $[\text{Pt}(\text{en})(\text{py})_2]^{2+}$, a complex structurally incapable of intercalating into DNA due to the absence of a sufficiently large aromatic system, was also evaluated. The small increase of ΔT_m

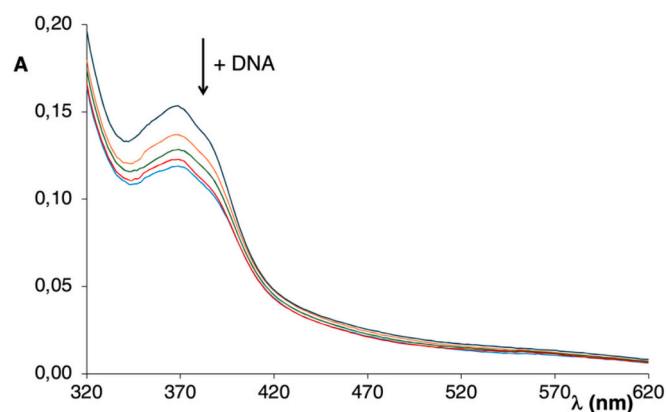


Fig. 14. Spectrophotometric titration of complex NO_2CuNO_2 (19) (11 μ M) with increasing amount of DNA at $T = 25^\circ\text{C}$ and $\text{pH} = 7$ (1 mM phosphate buffer, 21 mM NaCl and 5% v/v DMSO).

determined in this case, $2.2(\pm 0.3)^\circ\text{C}$, due only to the stabilization of the double helix resulting from the positive charge of the complex, confirms the intercalative capability of NO_2CuNO_2 (19).

Moreover, the spectrophotometric titration data were used to estimate the binding constant value of the NO_2CuNO_2 (19)–DNA interaction ($K_B > 1 \times 10^6 \text{ M}^{-1}$ in 1 mM phosphate buffer, at pH 7, containing 21 mM NaCl and 5% v/v DMSO) using the McGhee–von Hippel Eq. [76]. It should be noted that this is only a rough estimated value, given the very small spectral variations recorded at 370 nm and the poor solubility of the complex in aqueous solution, which prevents larger and more accurate variations from being recorded.

3. Conclusion

In this study, a series of twenty polypyridyl Cu(II)-complexes substituted at their ligand with $-\text{Cl}_2$ or a $-\text{NO}_2$ group and containing a coligand (*sal*-derivative), in turn variously substituted with different halogens or EDG/EWG have been synthesized and exhaustively characterized by elemental analysis and analytical methods (MS, FT-IR, UV–Vis and EPR). DFT calculations confirmed the square-planar geometry of these complexes in solution. The electronic structure analysis revealed minimal participation of the atomic orbitals of the metal in the frontier molecular orbitals. TD-DFT calculations were used to assign the absorption bands observed in the UV–Vis spectra, which were predominantly attributed to Ligand-Centered (LC) and/or Ligand-to-Ligand Charge Transfer (LLCT) transitions. The limited contribution of the metal orbitals to the frontier molecular orbitals supports the LC and LLCT character of these transitions. All Cu(II)-complexes underwent a preliminary screening at 10 μ M against three tumor cell lines, chosen based on the incidence and aggressiveness (MDA-MB 231, U87 and MG 63), to assess their anticancer potential. From this preliminary assay emerged that the Cu(II)-complexes are by far more cytotoxic than both the pure polypyridyl ligands and the Cu(II)-complexes devoid of the *sal* auxiliary ligand (though with some exceptions, such as the *sal*-free complex Cl_2Cu (22) which showed $\sim 70\%$ reduction of cell viability). The antiproliferative assay also showed that Cu(II)-complexes with the $-\text{NO}_2$ group at the polypyridyl ligand are, in general, more cytotoxic than those with two chlorine atoms at the same ligand. Regarding the *sal* ligand, the presence of halogens (specifically, the double substitution at position 3,5) or a $-\text{NO}_2$ group is also determinant for the activity. Accordingly, some compounds, namely Cl_2CuBrCl (4), NO_2CuBrCl (14), NO_2CuI_2 (15), NO_2CuNO_2 (19), were selected for further investigations such as IC_{50} determination against the three tumor cell lines, ROS production, morphological analysis, *in vitro* DNA-binding and computational studies (docking and MD simulations). The ROS production assay indicated that the presence of the auxiliary ligand is essential in the dichloro-polypyridyl Cu(II)-complexes $[\text{Cl}_2\text{CuBrCl}$ (4) $>>$ Cl_2Cu (22)], whereas it is not in the nitro-polypyridyl Cu(II)-complexes $[\text{NO}_2\text{CuBrCl}$ (14) $<$ NO_2Cu]. These results indicate that the auxiliary ligand can modulate the intracellular oxidative stress induced by the Cu(II) complexes. However, the CellROX Green assay provides an indication of increased intracellular ROS levels but does not allow the identification of the specific ROS species involved or the precise mechanism underlying their generation. A Cu(II)/Cu(I)-mediated redox mechanism may represent a plausible contribution to the observed oxidative stress, as reported for related copper-based anticancer complexes, although copper redox cycling, intracellular copper accumulation, and copper influx/efflux were not directly investigated in the present study. Importantly, the different ROS responses observed for the investigated complexes also suggest that ROS generation alone cannot account for their cytotoxic activity. On the other hand, the *in vitro* DNA-binding assay (performed on NO_2CuNO_2 (19) given its highest molar absorbance compared to the other Cu(II)-complexes) showed an intercalative mechanism of action for this class of derivatives, suggesting multiple possibilities for the mechanism of interaction with the predicted biological targets. Cell morphology analysis (performed on MDA-

MB 231 cells) indicated that these compounds also compromise the integrity of the cell's cytoskeleton. The possibility that these compounds have multiple activities has been corroborated by docking and MD simulation studies that clearly suggest that the cytotoxicity observed in MDA-MB 231 and MG 63 cell models could be, at least in part, due to G4 stabilizing properties of the complexes, in particular for Cl_2CuBrCl (**4**) and Cl_2Cu (**22**). Taken together, these results indicate that these Cu(II)-complexes are worthy candidates for further in-depth studies as anti-cancer agents.

4. Experimental section

4.1. Chemicals and instrumentation

Copper(II) nitrate hydrate (99,999%), ethanol ($\geq 99.5\%$, ACS reagent), methanol ($\geq 99.9\%$, ACS reagent), 3,5-dibromosalicylaldehyde, 3,5-diiodosalicylaldehyde, 3-bromo-5-chlorosalicylaldehyde, 5-bromosalicylaldehyde, 5-methoxysalicylaldehyde, 4-methylsalicylaldehyde, 5-methylsalicylaldehyde, 5-nitrosalicylaldehyde, 5-(diethylamino)-salicylaldehyde, and salicylaldehyde were purchased from Sigma-Aldrich Co. (Milan, Italy) and used without further purification. Calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and purified as previously described [77]. Its concentration, expressed in base pairs, was determined spectrophotometrically using the molar absorptivity $1.31 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (260 nm) [78]. The FT-IR spectra were recorded on a Perkin-Elmer (Bridgeport Ave Shelton, CT) Spectrum-One spectrometer ($4000\text{--}400 \text{ cm}^{-1}$) in KBr pellets. Elemental analysis was performed on Leco, CHNS-932 and PerkinElmer 7300 DV elemental analyzers. Absorption spectra were recorded using a Cintra 3030 GBC spectrophotometer. Conductivity measurements were carried out using a XS Cond 50 Violab benchtop conductivity meter. Positive ESI mass spectra were acquired on an API 2000 mass spectrometer (ABSciex—Milano, Italy) equipped with an API ion source. The DMSO solutions of the investigated samples were introduced into the source at a flow rate of $5 \mu\text{L}/\text{min}$. The mass spectra were elaborated with the “Analyst” software (from AB Sciex). X-band ($\nu \text{ ca. } 9.4 \text{ GHz}$) EPR spectra were acquired using an Elexsys E500 (Bruker) spectrometer equipped with a SHQ cavity. Low temperatures were achieved by using an ESR900 (Oxford Instruments) continuous flow cryostat.

4.2. Synthesis of the ligands

The synthesis of the ligand precursor 1,10-phenanthroline-5,6-dione, and the ligands 7,8-dichlorodipyrido[3,2-a:2',3'-c]-phenazine (dcdppz)

and 7-nitro dipyrido[3,2-a:2'-3'-c] phenazine (7-nitro-dppz) ligands were synthesized as previously reported [79–81].

4.3. Synthesis of complexes

The complexes of general formula $[\text{Cu}(\text{X-dppz})(\text{X}_2\text{-sal})(\text{NO}_3)]$ were synthesized by the reaction of the copper(II) nitrate salt and the ligand dcdppz or 7-nitro-dppz ligand in methanol in a 1:1 M ratio at room temperature. After adding the reagents (1 mmol) in an ice bath, the reaction was stirred 2 h. A solution of salicylaldehyde or substituted salicylaldehyde (1 mmol) in methanol and in presence of trimethylamine (1.2 mmol) was added dropwise to the above reported mixture, and the reaction was stirred for an additional 4 h at room temperature (see Scheme 1). The final complexes were obtained as precipitates directly from the reaction, filtered through a sintered glass funnel, washed with cold methanol and vacuum-dried. All complexes are stable in the solid state (for several months) and exhibited good stability in dimethyl sulfoxide (DMSO) (see Fig. S41). The stability of this type of complexes in phosphate buffer (pH 7) was verified using complex NO_2CuNO_2 (**19**), the same used for the DNA-binding assay (Fig. 15).

$\text{Cu}(\text{dcdppz})(\text{salicylaldehyde})\text{NO}_3$ (Cl_2CuSal ; **1**): The complex **1** (532 mg, 89% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{13}\text{CuCl}_2\text{N}_5\text{O}_5$ ($M_r = 597.85$): C, 50.23; H, 2.19; N, 11.71. Found: C, 50.20; H, 2.19; N, 11.70. IR (KBr, cm^{-1}): 1624.73 (s, C=O). Molecular mass: 597.85 Da; ESI-MS: m/z 535.8 Da (detected as $[\text{Cu}(\text{dcdppz})(\text{salicylaldehyde})]^+$ ion, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(5\text{-bromosalicylaldehyde})\text{NO}_3$ (Cl_2CuBr ; **2**): The complex **2** (541 mg, 80% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{12}\text{CuBrCl}_2\text{N}_5\text{O}_5$ ($M_r = 676.75$): C, 44.37; H, 1.79; N, 10.35. Found: C, 44.39; H, 1.80; N, 10.34. IR (KBr, cm^{-1}): 1620.88 (s, C=O). Molecular mass: 676.75 Da; ESI-MS: m/z 614.7 Da (detected as $[\text{Cu}(\text{dcdppz})(5\text{-bromosalicylaldehyde})]^+$ ion, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(3,5\text{-dibromosalicylaldehyde})\text{NO}_3$ (Cl_2CuBr_2 ; **3**): The complex **3** (642 mg, 85% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{11}\text{CuBr}_2\text{Cl}_2\text{N}_5\text{O}_5$ ($M_r = 755.64$): C, 39.74; H, 1.47; N, 9.27. Found: C, 39.75; H, 1.48; N, 9.28. IR (KBr, cm^{-1}): 1612.2 (s, C=O).

Molecular mass: 755.64 Da; ESI-MS: m/z 693.4 Da (detected as $[\text{Cu}(\text{dcdppz})(3,5\text{-dibromosalicylaldehyde})]^+$ ion, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(3\text{-bromo-5-chlorosalicylaldehyde})\text{NO}_3$ (Cl_2CuBrCl ; **4**): The complex **4** (633 mg, 89% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{11}\text{CuBrCl}_3\text{N}_5\text{O}_5$ ($M_r = 711.19$): C, 42.22; H, 1.56; N, 9.85. Found: C, 42.23; H, 1.55; N, 9.86. IR (KBr, cm^{-1}): 1614.13 (s, C=O). Molecular mass: 711.19 Da; ESI-MS: m/z 647.5 Da (detected as $[\text{Cu}(\text{dcdppz})(3\text{-bromo-5-chlorosalicylaldehyde})]^+$ ion, due to the loss of

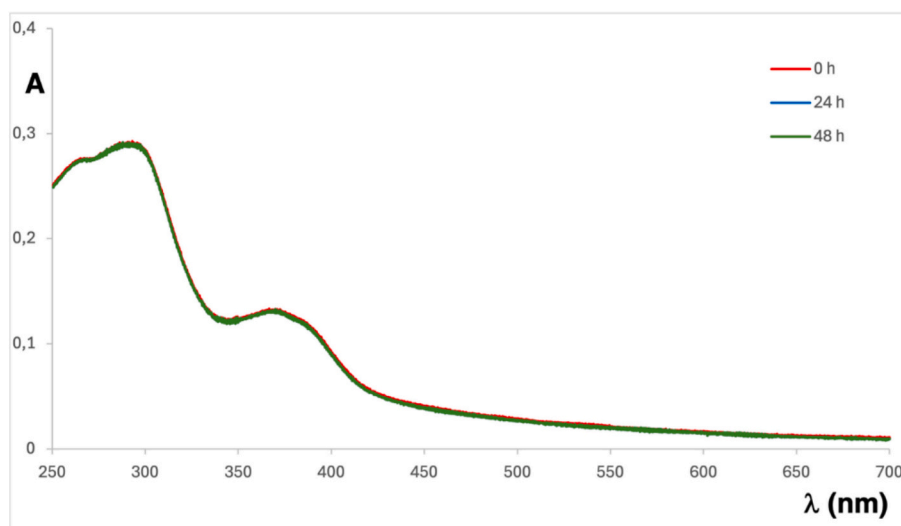


Fig. 15. UV-Vis spectra of complex NO_2CuNO_2 (**19**) in phosphate buffer 1 mM (pH 7), NaCl 21 mM and 5% v/v DMSO at 25 °C recorded at 0 h, 24 h, and 48 h.

NO_3^- ion).

$\text{Cu}(\text{dcdppz})(3,5\text{-diiodosalicylaldehyde})\text{NO}_3$ (Cl_2CuI_2 ; **5**): The complex **5** (714 mg, 84% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{11}\text{Cl}_2\text{I}_2\text{CuN}_5\text{O}_5$ ($M_r = 849.65$): C, 35.34; H, 1.31; N, 8.24. Found: C, 35.32; H, 1.33; N, 8.24. IR (KBr, cm^{-1}): 1619.91 (s, C=O). Molecular mass: 849.65 Da; ESI-MS: m/z 786.9 Da (detected as $[\text{Cu}(\text{dcdppz})(3,5\text{-diiodosalicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(2\text{-hydroxy-4-methoxybenzaldehyde})\text{NO}_3$ ($\text{Cl}_2\text{CuOCH}_3$; **6**): The complex **6** (521 mg, 83% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{26}\text{H}_{15}\text{Cl}_2\text{CuN}_5\text{O}_6$ ($M_r = 627.88$): C, 49.74; H, 2.41; N, 11.15. Found: C, 49.75; H, 2.40; N, 11.14. IR (KBr, cm^{-1}): 1600.63 (s, C=O). Molecular mass: 627.88 Da; ESI-MS: 565.6 m/z Da (detected as $[\text{Cu}(\text{dcdppz})(2\text{-hydroxy-4-methoxybenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(2\text{-hydroxy-4-methylbenzaldehyde})\text{NO}_3$ (Cl_2CuCH_3 ; **7**): The complex **7** (526 mg, 86% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{26}\text{H}_{15}\text{Cl}_2\text{CuN}_5\text{O}_5$ ($M_r = 611.88$): C, 51.04; H, 2.47; N, 11.45. Found: C, 51.05; H, 2.48; N, 11.44. IR (KBr, cm^{-1}): 1604.48 (s, C=O). Molecular mass: 611.88 Da; ESI-MS: 549.6 m/z Da (detected as $[\text{Cu}(\text{dcdppz})(2\text{-hydroxy-4-methylbenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(2\text{-hydroxy-5-methylbenzaldehyde})\text{NO}_3$ (Cl_2CuCH_3 ; **8**): The complex **8** (490 mg, 80% yield) was obtained as a green solid. Anal. Calc. for $\text{C}_{26}\text{H}_{15}\text{Cl}_2\text{CuN}_5\text{O}_5$ ($M_r = 611.88$): C, 51.04; H, 2.47; N, 11.45. Found: C, 51.02; H, 2.47; N, 11.46. IR (KBr, cm^{-1}): 1601.59, 1626.66 (d, C=O). Molecular mass: 611.88 Da; ESI-MS: 549.7 m/z Da (detected as $[\text{Cu}(\text{dcdppz})(2\text{-hydroxy-5-methylbenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(2\text{-hydroxy-5-nitrobenzaldehyde})\text{NO}_3$ (Cl_2CuNO_2 ; **9**): The complex **9** (527 mg, 82% yield) was obtained as a dark green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{12}\text{Cl}_2\text{CuN}_6\text{O}_7$ ($M_r = 642.85$): C, 46.71; H, 1.88; N, 13.07. Found: C, 46.70; H, 1.89; N, 13.06. IR (KBr, cm^{-1}): 1626.66 (s, C=O). Molecular mass: 642.85 Da; ESI-MS: 581.0 m/z Da (detected as $[\text{Cu}(\text{dcdppz})(2\text{-hydroxy-5-nitrobenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{dcdppz})(4\text{-(diethylamino)salicylaldehyde})\text{NO}_3$ ($\text{Cl}_2\text{CuN}(\text{C}_2\text{H}_5)_2$; **10**): The complex **10** (569 mg, 85% yield) was obtained as a dark brown solid. Anal. Calc. for $\text{C}_{29}\text{H}_{22}\text{Cl}_2\text{CuN}_6\text{O}_5$ ($M_r = 668.98$): C, 52.07; H, 3.31; N, 12.56. Found: C, 52.08; H, 3.33; N, 12.54. IR (KBr, cm^{-1}): 1575.56 (s, C=O). Molecular mass: 668.98 Da; ESI-MS: 606.5 m/z Da (detected as $[\text{Cu}(\text{dcdppz})(4\text{-(diethylamino)salicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(\text{salicylaldehyde})\text{NO}_3$ (NO_2CuSal ; **11**): The complex **11** (482 mg, 84% yield) was obtained as a yellow solid. Anal. Calc. for $\text{C}_{25}\text{H}_{14}\text{CuN}_6\text{O}_7$ ($M_r = 573.97$): C, 52.32; H, 2.46; N, 14.64. Found: C, 52.34; H, 2.45; N, 14.65. IR (KBr, cm^{-1}): 1614.13 (s, C=O). Molecular mass: 573.97 Da; ESI-MS: 510.5 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(\text{salicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(5\text{-bromosalicylaldehyde})\text{NO}_3$ (NO_2CuBr ; **12**): The complex **12** (529 mg, 81% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{13}\text{BrCuN}_6\text{O}_7$ ($M_r = 652.86$): C, 45.99; H, 2.01; N, 12.87. Found: C, 45.97; H, 2.00; N, 12.88. IR (KBr, cm^{-1}): 1615.09 (s, C=O). Molecular mass: 652.86 Da; ESI-MS: 590.3 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(5\text{-bromosalicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(3,5\text{-dibromosalicylaldehyde})\text{NO}_3$ (NO_2CuBr_2 ; **13**): The complex **13** (593 mg, 81% yield) was obtained as a brown solid. Anal. Calc. for $\text{C}_{25}\text{H}_{12}\text{Br}_2\text{CuN}_6\text{O}_7$ ($M_r = 731.76$): C, 41.03; H, 1.65; N, 11.48. Found: C, 41.01; H, 1.66; N, 11.49. IR (KBr, cm^{-1}): 1617.98 (s, C=O). Molecular mass: 731.76 Da; ESI-MS: 668.9 m/z Da (detected as

$[\text{Cu}(\text{7-nitro-dppz})(3,5\text{-dibromosalicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(3\text{-bromo-5-chlorosalicylaldehyde})\text{NO}_3$ (NO_2CuBrCl ; **14**): The complex **14** (570 mg, 83% yield) was obtained as a bright green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{12}\text{CuBrClN}_6\text{O}_7$ ($M_r = 687.31$): C, 43.69; H, 1.76; N, 12.23. Found: C, 43.70; H, 1.75; N, 12.22. IR (KBr, cm^{-1}): 1619.91 (s, C=O). Molecular mass: 687.31 Da; ESI-MS: 624.0 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(3\text{-bromo-5-chlorosalicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(3,5\text{-diiodosalicylaldehyde})\text{NO}_3$ (NO_2CuI_2 ; **15**): The complex **15** (694 mg, 84% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{12}\text{I}_2\text{CuN}_6\text{O}_7$ ($M_r = 825.76$): C, 36.36; H, 1.46; N, 10.18. Found: C, 36.35; H, 1.47; N, 10.17. IR (KBr, cm^{-1}): 1612.2 (s, C=O). Molecular mass: 825.76 Da; ESI-MS: 762.7 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(3,5\text{-diiodosalicylaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-4-methoxybenzaldehyde})\text{NO}_3$ ($\text{NO}_2\text{CuOCH}_3$; **16**): The complex **16** (501 mg, 83% yield) was obtained as a bright green solid. Anal. Calc. for $\text{C}_{26}\text{H}_{16}\text{CuN}_6\text{O}_8$ ($M_r = 603.99$): C, 51.70; H, 2.67; N, 13.91. Found: C, 51.69; H, 2.68; N, 13.92. IR (KBr, cm^{-1}): 1605.46 (s, C=O). Molecular mass: 602.99 Da; ESI-MS: 541.0 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-4-methoxybenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-4-methylbenzaldehyde})\text{NO}_3$ (NO_2CuCH_3 ; **17**): The complex **17** (506 mg, 86% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{26}\text{H}_{16}\text{CuN}_6\text{O}_7$ ($M_r = 587.99$): C, 53.11; H, 2.74; N, 14.29. Found: C, 53.12; H, 2.73; N, 14.30. IR (KBr, cm^{-1}): 1598.7 (s, C=O). Molecular mass: 587.99 Da; ESI-MS: 525.0 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-4-methylbenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-5-methylbenzaldehyde})\text{NO}_3$ (NO_2CuCH_3 ; **18**): The complex **18** (482 mg, 82% yield) was obtained as a brown solid. Anal. Calc. for $\text{C}_{26}\text{H}_{16}\text{CuN}_6\text{O}_7$ ($M_r = 587.99$): C, 53.11; H, 2.74; N, 14.29. Found: C, 53.12; H, 2.76; N, 14.29. IR (KBr, cm^{-1}): 1600.63 (s, C=O). Molecular mass: 587.99 Da; ESI-MS: 525.1 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-5-methylbenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-5-nitrobenzaldehyde})\text{NO}_3$ (NO_2CuNO_2 ; **19**): The complex **19** (520 mg, 84% yield) was obtained as a sage green solid. Anal. Calc. for $\text{C}_{25}\text{H}_{13}\text{CuN}_7\text{O}_9$ ($M_r = 618.97$): C, 48.51; H, 2.12; N, 15.84. Found: C, 48.52; H, 2.13; N, 15.85. IR (KBr, cm^{-1}): 1608.34 (s, C=O). Molecular mass: 618.97 Da; ESI-MS: 556.7 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(2\text{-hydroxy-5-nitrobenzaldehyde})]^+$, due to the loss of NO_3^- ion).

$\text{Cu}(\text{7-nitro-dppz})(4\text{-(diethylamino)salicylaldehyde})\text{NO}_3$ ($\text{NO}_2\text{CuN}(\text{C}_2\text{H}_5)_2$; **20**): The complex **20** (542 mg, 84% yield) was obtained as a dark brown solid. Anal. Calc. for $\text{C}_{29}\text{H}_{23}\text{CuN}_7\text{O}_7$ ($M_r = 645.09$): C, 54.00; H, 3.59; N, 15.20. Found: C, 54.01; H, 3.60; N, 15.18. IR (KBr, cm^{-1}): 1580.38 (s, C=O). Molecular mass: 645.09 Da; ESI-MS: 581.9 m/z Da (detected as $[\text{Cu}(\text{7-nitro-dppz})(4\text{-(diethylamino)salicylaldehyde})]^+$, due to the loss of NO_3^- ion from molecular specie).

In the ESI-MS spectra (Figs. S21–40), the systems investigated were observed as cations (i.e., the molecular species minus the NO_3^- anion) because the NO_3^- anion was removed during the acceleration process. In other words, the obtained experimental m/z values are in full accordance with the molecular mass of the related compounds, but as cationic species because the molecular system loses a nitrate anion during the ESI-MS acceleration process by the extraction of the cationic species.

Although a mass spectrometer with a mass resolution of approximately 1000 FWHM was used, it was sufficient to evaluate the isotopic

pattern abundances and compare them with the theoretical ones. To improve clarity, a comparison with the simulated isotopic patterns of compounds containing chlorine, bromine, and iodine species is reported in ESI.

Regarding the FT-IR analyses, the spectra of all samples (1–20) are reported in ESI (Figs. S1–20).

4.4. *In vitro* biological evaluation

4.4.1. Cell culture

Three different cancer cell lines were used: MDA-MB 231 (breast cancer, ATCC HTB26), U87 (glioblastoma, ATCC HTB14) and MG 63 (osteosarcoma, ATCC CRL1427). All of them were purchased from American Type Culture Collection (ATCC). MDA-MB 231 cell line was cultured in RPMI 1640 Medium (Gibco), U87 in alpha-MEM w/o ascorbic acid Medium (Gibco) and MG 63 McCoy's 5 A Medium (Gibco), all supplemented with 10% fetal bovine serum (FSB) (Gibco) and 1% penicillin/streptomycin mixture (pen/strep) (100 U/ml – 100 µg/mL, Gibco). Cells were grown in an incubator at 37 °C under controlled humidity and 5% CO₂ atmosphere conditions, detached by trypsinization and centrifuged. The viability was assessed by Trypan Blue Dye Exclusion test, and all the cell handling procedures were carried out under a laminar flow hood in sterile conditions.

4.4.2. Cell viability assessment

MDA-MB 231 and U87 were seeded at a density of 12×10^3 cells/well in a 96-well plate, whereas MG 63 was 8×10^3 cells/well. After 24 h, the compounds were added at a concentration equal to 10 µM for the first screening, whereas a concentration range (0.065, 0.125, 0.50, 1, 5, 10, 20 µM) was used to determine the IC₅₀; cells only were used as control. An overnight incubation was done and then the MTT assay was used to quantitatively evaluate the cell viability following the manufacturer's instructions. Briefly, MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (5 mg/mL, Sigma) was dissolved in Phosphate Saline Buffer 1× (PBS 1×) (Gibco), added to the cell media 10% v/v and incubated for 2 h at 37 °C, 5% CO₂ and controlled humidity conditions. Lastly, the media was removed and the formazan crystals produced by the metabolically active cells dissolved in dimethyl sulfoxide (DMSO) (Sigma). The formazan absorbance was read at 570 nm by using a Multiskan FC Microplate Photometer (Thermo Scientific). The values were directly proportional to the number of metabolically active cells ($n = 3$).

4.4.3. Cell morphology evaluation

MDA-MB 231 cells were seeded at a density of 12×10^3 cells/well in a 96-well plate. After 24 h, the selected compounds (Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂Cu (21) and Cl₂Cu (22)) were added to IC₅₀ concentration and incubated for 6 and 24 h. Then, the cells were fixed in 4% buffered Paraformaldehyde (PFA) (Sigma) following the manufacturer's instructions. To permeabilize the cells, a 5-min incubation with 0.1% (v/v) Triton X-100 (Sigma) in PBS 1× was performed. F-actin filaments were stained using Actin Red 555 Ready Probes Reagent (Invitrogen) following the manufacturer's instructions. Then the cell nuclei were counterstained with 4',6-diamidino-2-ylindole, dihydrochloride (DAPI) (600 nM, Invitrogen) for 7 min. All the images were acquired by using an Inverted Ti-E Fluorescent Microscope (Nikon).

4.4.4. ROS detection

MDA-MB 231 were seeded at a density of 12×10^3 cells/well in a 96 well plate. After 24 h, the selected compounds (Cl₂CuBrCl (4), NO₂CuBrCl (14), NO₂Cu (21) and Cl₂Cu (22)) were added to IC₅₀ concentration and incubated for 2 and 24 h. Tert-Butyl hydroperoxide (TBHP, 200 µM) was added and incubated for 2 h as positive control (Ct +). After the incubation, the CellROX Green (Invitrogen) ROS detection kit was performed following the manufacturer's instructions. Briefly, the kit reagent was added to each well at the final concentration equal to 5

µM and incubated for 30 min at 37 °C. Then, the cells were washed 3 times with PBS 1× and fixed with 4% PFA as described above. A quantitative analysis was performed by detecting the green fluorescence using the Fluoroskan Microplate Fluorometer (Thermo Scientific), setting the excitation wavelength equal to 485 nm and the kit reagent product emission wavelength being 520 nm. Then, the cell nuclei were stained as described above, and qualitative images were acquired by using an Inverted Ti-E Fluorescent Microscope (Nikon), ($n = 3$).

4.4.5. Statistical analysis

MTT assay results were plotted as the mean percentage with respect to cells only ± standard deviation (SD) and analysed by One-way analysis of variance (One-way ANOVA) and Dunnett's multiple comparisons test (* p value ≤ 0.05, ** p value ≤ 0.01, *** p value ≤ 0.001, **** p value ≤ 0.0001). IC₅₀ values were calculated as Log(inhibitor) versus mean percentage of dead cells with respect to cells only and the obtained values are reported ± SD. Quantitative ROS detection data were plotted as the mean with respect to cells only ± SD and analysed by Two-way analysis of variance (Two-way ANOVA) and Tukey's multiple comparisons test (* p value ≤ 0.05, ** p value ≤ 0.01, *** p value ≤ 0.001, **** p value ≤ 0.0001).

All the calculations and statistical analyses were performed using GraphPad Prism Software (8.0.1 version).

4.5. Theoretical calculations

All calculations have been developed using the Gaussian16 software package [82]. The DFT and TD-DFT calculations were performed using Becke's three-parameter B3LYP exchange-correlation functional [83,84]. The basis set used to define the atoms were def2tzvp for the all the atoms [85,86]. The empirical dispersion correction was taken into account using Grimme's dispersion with Becke-Johnson damping, D3BJ [87,88]. The solvent (DMSO) effects were considered within the self-consistent reaction field (SCRF) theory using the solvation model SMD of Truhlar et al. [89]. Time-dependent DFT (TD-DFT) calculations of the lowest-lying 50 excited states were performed in the presence of the solvent for all the complexes at the minimum-energy geometry optimized for the ground state [90–92].

4.5.1. Docking studies

The NMR conformations of G-quadruplex (PDB IDs: 5I2V, 2ZKE, 2ZKD, 2LD8) were employed as target structures for docking simulations. Structures were retrieved from the protein data bank [93], and prepared using AutoDockTools v1.5.66 [94]. Docking grids were generated for each model and conformation using AutoGrid v4.2.6 [94], with dimensions set to $x = 84$, $y = 104$, $z = 84$ grid points and a spacing of 0.375 Å, covering the entire G-quadruplex structure. The optimized geometries of the metal complexes, obtained through DFT calculations, were subsequently subjected to docking simulations using AutoDock v4.2.6 [94]. Specific parameters for copper were added (atom_par Cu 2.75 0.080 12.000-0.00110 0.0 0.0 0-1 -1 4 # Non H-bonding) were added for Copper. For each ligand, 200 runs of the Lamarckian Genetic Algorithm were performed, with a population size of 150, while all other settings were kept at their default values. From the resulting docking poses, the conformation with the lowest binding energy for each compound was selected as the bound structure and used as the starting point for Molecular Dynamics (MD) simulations. MD simulations were conducted using Desmond [95], employing partial charges obtained from DFT calculations. Solvation was modeled implicitly using the TIP3P water model [96], and 22 Na⁺ ions were added to neutralize the system. The OPLS2005 force field was used throughout. Subsequently, 120 ns MD simulations were run in the NPT ensemble at 310 K, using a Nose-Hoover chain thermostat and a Martyna-Tobias-Klein barostat at 1.01325 bar. Finally, PyMOL v1.8.4.0 (open-source version) was utilized for the visual inspection and molecular representation of the ligand-bound conformations.

4.5.2. In vitro DNA-complexes interaction

Spectrophotometric titration was performed by adding successive aliquots of DNA, also containing the complex, to the complex solution (11 μM) in a 10 mm stoppered quartz cell and recording the spectrum after each addition. The data were analysed by a nonlinear least-squares fitting program, applied to McGhee and von Hippel Eq. [76]. The binding constant, K_B , was estimated by the program, using the extinction coefficient of the compound, the free complex concentration and the ratio of bound complex/mol of DNA. Extinction coefficient for bound compound was determined by Beer's law plots in the presence of a large excess of DNA. The thermal denaturation temperature of compound–DNA mixtures (1:10) was determined in 1 mM phosphate buffer (pH = 7) solutions containing the complex (7.8 μM) and 2 mM NaCl in 5% v/v DMSO. Melting curves were recorded at 260 nm. The temperature has been increased at a rate of 0.5 $^{\circ}\text{C}/\text{min}$ by using a GBC Peltier system. Spectropolarimetric titration was performed by adding increasing amounts of complex NO_2CuNO_2 (19), also containing DNA at the same concentration used for the titration not to dilute, to the DNA solution (30 μM) and recording the spectrum after each addition. Spectra reported were obtained subtracting the DNA signal at the used concentration.

CRediT authorship contribution statement

Hadi Amiri Rudbari: Writing – review & editing, Project administration. **Zahra Kazemi:** Investigation. **Angelo Nicosia:** Visualization, Investigation. **Placido Mineo:** Validation, Investigation. **Chiara Riera:** Investigation. **Arianna Rossi:** Investigation, Formal analysis. **Monica Montesi:** Validation, Resources. **Silvia Panzeri:** Visualization, Resources. **Maria Letizia Di Pietro:** Writing – review & editing, Investigation. **Nunzio Iraci:** Software, Data curation. **Claudio Stagno:** Investigation. **Lorenzo Sorace:** Investigation, Writing – review & editing. **José V. Cuevas-Vicario:** Writing – review & editing, Formal analysis. **Nicola Micale:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hadi Amiri Rudbari reports financial support was provided by Iran National Science Foundation. Jose Vicente Cuevas Vicario reports was provided by Junta de Castilla y León (Consejería de Educación). Claudio Stagno reports a relationship with University of Namur that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.inoche.2026.117734>.

Data availability

Data will be made available on request.

References

- [1] J. Karges, K. Xiong, O. Blacque, H. Chao, G. Gasser, Highly cytotoxic copper (II) terpyridine complexes as anticancer drug candidates, *Inorg. Chim. Acta* 516 (2021) 120137, <https://doi.org/10.1016/j.ica.2020.120137>.
- [2] D.I. Ugwu, J. Conradie, Anticancer properties of complexes derived from bidentate ligands, *J. Inorg. Biochem.* 246 (2023) 112268, <https://doi.org/10.1016/j.jinorgbio.2023.112268>.
- [3] J. Karges, Encapsulation of Ru (II) polypyridine complexes for tumor-targeted anticancer therapy, *BME Front.* 4 (2023) 0024, <https://doi.org/10.34133/bmef.0024>.
- [4] J.J. Rani, S. Roy, Recent development of copper (II) complexes of polypyridyl ligands in chemotherapy and photodynamic therapy, *ChemMedChem* 18 (8) (2023) e202200652, <https://doi.org/10.1002/cmde.202200652>.
- [5] G. Gasser, Metal complexes and medicine: a successful combination, *Chimia* 69 (2015) 442–446, <https://doi.org/10.2533/chimia.2015.442>.
- [6] H. Zhang, L. Tian, R. Xiao, Y. Zhou, Y. Zhang, J. Hao, Y. Liu, J. Wang, Anticancer effect evaluation in vitro and in vivo of iridium (III) polypyridyl complexes targeting DNA and mitochondria, *Bioorg. Chem.* 115 (2021) 105290, <https://doi.org/10.1016/j.bioorg.2021.105290>.
- [7] A. Skoczynska, A. Lewinski, M. Pokora, P. Paneth, E. Budzisz, An overview of the potential medicinal and pharmaceutical properties of Ru (II)/(III) complexes, *Int. J. Mol. Sci.* 24 (2023) 9512, <https://doi.org/10.3390/ijms24119512>.
- [8] G.-B. Jiang, W.-Y. Zhang, M. He, Y.-Y. Gu, L. Bai, Y.-J. Wang, Q.-Y. Yi, F. Du, Development of four ruthenium polypyridyl complexes as antitumor agents: design, biological evaluation and mechanism investigation, *J. Inorg. Biochem.* 208 (2020) 111104, <https://doi.org/10.1016/j.jinorgbio.2020.111104>.
- [9] K.M. Deo, D.L. Ang, B. McGhie, A. Rajamanickam, A. Dhiman, A. Khoury, J. Holland, A. Bjelosevic, B. Pages, C. Gordon, J.R. Aldrich-Wright, Platinum coordination compounds with potent anticancer activity, *Coord. Chem. Rev.* 375 (2018) 148–163.
- [10] A. Rossi, C. Stagno, A. Piperno, N. Iraci, S. Panzeri, M. Montesi, M. Feizi-Dehnbeybi, G. Bassi, M.L. Di Pietro, N. Micale, Anticancer activity and morphological analysis of Pt(II) complexes: their DFT approach, docking simulation, and ADME-Tox profiling, *Appl. Organomet. Chem.* 38 (2024) e7403, <https://doi.org/10.1002/aoc.7403>.
- [11] A.C. Hachey, D. Havrylyuk, E.C. Glazer, Biological activities of polypyridyl-type ligands: implications for bioinorganic chemistry and light-activated metal complexes, *Curr. Opin. Chem. Biol.* 61 (2021) 191–202, <https://doi.org/10.1016/j.cbpa.2021.01.016>.
- [12] L. Salassa, Polypyridyl metal complexes with biological activity, *Eur. J. Inorg. Chem.* 32 (2011) 4931–4947, <https://doi.org/10.1002/ejic.201100376>.
- [13] M. He, Q.-Y. Yi, W.-Y. Zhang, L. Bai, F. Du, Y.-Y. Gu, Y.-J. Liu, P. Wei, Evaluation of anticancer activity in vitro and in vivo of iridium (III) polypyridyl complexes, *New J. Chem.* 43 (2019) 8566–8579, <https://doi.org/10.1039/c9nj01001g>.
- [14] D.-L. Ma, C.-M. Che, S.-C. Yan, Platinum (II) complexes with dipyrrophenazine ligands as human telomerase inhibitors and luminescent probes for G-quadruplex DNA, *J. Am. Chem. Soc.* 131 (2009) 1835–1846, <https://doi.org/10.1021/ja806045x>.
- [15] X. He, L. Jin, L. Tan, DNA-binding, topoisomerases I and II inhibition and in vitro cytotoxicity of ruthenium(II) polypyridyl complexes: $[\text{Ru}(\text{dppz})_2\text{L}]^{2+}$ (L = dppz-11- CO_2Me and dppz), *Spectrochim. Acta Part A* 135 (2015) 101–109, <https://doi.org/10.1016/j.saa.2014.06.147>.
- [16] N. Elgrishi, M.B. Chambers, X. Wang, M. Fontecave, Molecular polypyridine-based metal complexes as catalysts for the reduction of CO_2 , *Chem. Soc. Rev.* 46 (2017) 761–796, <https://doi.org/10.1039/c5cs00391a>.
- [17] K. Kalyanasundaram, M. Grätzel, Applications of functionalized transition metal complexes in photonic and optoelectronic devices, *Coord. Chem. Rev.* 177 (1998) 347–414, [https://doi.org/10.1016/S0010-8545\(98\)00189-1](https://doi.org/10.1016/S0010-8545(98)00189-1).
- [18] J. Conradie, Polypyridyl copper complexes as dye sensitizer and redox mediator for dye-sensitized solar cells, *Electrochem. Commun.* 134 (2022) 107182, <https://doi.org/10.1016/j.elecom.2021.107182>.
- [19] K.K.-W. Lo, Luminescent rhenium (I) and iridium (III) polypyridine complexes as biological probes, imaging reagents, and photocytotoxic agents, *Acc. Chem. Res.* 48 (2015) 2985–2995, <https://doi.org/10.1021/acs.accounts.5b00211>.
- [20] M.L. Di Pietro, G. La Ganga, F. Nastasi, F. Puntoriero, Ru(II)-Dppz derivatives and their interactions with DNA: thirty years and counting, *Appl. Sci.* 11 (2021) 3038, <https://doi.org/10.3390/app11073038>.
- [21] J. Karges, Clinical development of metal complexes as photosensitizers for photodynamic therapy of cancer, *Angew. Chem. Int. Ed.* 61 (2022) e202112236, <https://doi.org/10.1002/anie.202112236>.
- [22] T. McGivern, S. Afsharpour, C. Marmion, Copper complexes as artificial DNA metallo-nucleases: from Sigman's reagent to next generation anti-cancer agent? *Inorg. Chim. Acta* 472 (2018) 12–39, <https://doi.org/10.1016/j.ica.2017.08.043>.
- [23] Y. Figueroa-DePaz, J. Pérez-Villanueva, O. Soria-Arteche, D. Martínez-Otero, V. Gómez-Vidales, L. Ortiz-Frade, L. Ruiz-Azuara, Casiopinas of third generations: synthesis, characterization, cytotoxic activity and structure–activity relationships of mixed chelate compounds with bioactive secondary ligands, *Molecules* 27 (2022) 3504, <https://doi.org/10.3390/molecules27113504>.
- [24] G.R. Walke, S. Meron, Y. Shenberger, L. Gevorkyan-Airapetov, S. Ruthstein, Cellular uptake of the ATSM–Cu (II) complex under hypoxic conditions, *ChemistryOpen* 10 (2021) 486–492, <https://doi.org/10.1002/open.202100044>.
- [25] G.R. Walke, S. Ruthstein, Does the ATSM-Cu (II) biomarker integrate into the human cellular copper cycle? *ACS Omega* 4 (2019) 12278–12285, <https://doi.org/10.1021/acsomega.9b01748>.

- [26] M. Salimi, K. Abdi, H.M. Kandelous, H. Hadadzadeh, K. Azadmanesh, A. Amanzadeh, H. Sanati, Antiproliferative effects of copper (II)-polypyridyl complexes in breast cancer cells through inducing apoptosis, *BioMetals* 28 (2015) 267–278, <https://doi.org/10.1007/s10534-015-9820-5>.
- [27] Z. Molphy, A. Prisecaru, C. Slatore, N. Barron, M. McCann, J. Collieran, D. Chandran, N. Gathergood, A. Kellett, Copper phenanthrene oxidative chemical nucleases, *Inorg. Chem.* 53 (2014) 5392–5404, <https://doi.org/10.1021/ic500914j>.
- [28] J.A. Eremina, E.A. Ermakova, K.S. Smirnova, L.S. Klyushova, A.S. Berezin, T. S. Sukhikh, A.A. Zubenko, L.N. Fetisov, K.N. Kononenko, E.V. Lider, Cu (II), Co (II), Mn (II) complexes with 5-phenyltetrazole and polypyridyl ligands: synthesis, characterization and evaluation of the cytotoxicity and antimicrobial activity, *Polyhedron* 206 (2021) 115352, <https://doi.org/10.1016/j.poly.2021.115352>.
- [29] S.S. Massoud, F.R. Louka, N.M. Salem, R.C. Fischer, A. Torvisco, F.A. Mautner, J. Vančo, J. Belza, Z. Dvořák, Z. Trávníček, Dinuclear doubly bridged phenoxido copper (II) complexes as efficient anticancer agents, *Eur. J. Med. Chem.* 246 (2023) 114992, <https://doi.org/10.1016/j.ejmech.2022.114992>.
- [30] V.C. Shanbhag, N. Gudekar, K. Jasmer, C. Papageorgiou, K. Singh, M.J. Petris, Copper metabolism as a unique vulnerability in cancer, *Biochim. Biophys. Acta, Mol. Cell Res.* 1868 (2021) 118893, <https://doi.org/10.1016/j.bbamcr.2020.118893>.
- [31] X. Tang, Z. Yan, Y. Miao, W. Ha, Z. Li, L. Yang, D. Mi, Copper in cancer: from limiting nutrient to therapeutic target, *Front. Oncol.* 13 (2023) 1209156, <https://doi.org/10.3389/fonc.2023.1209156>.
- [32] C. Wang, X. Yang, C. Dong, K. Chai, J. Ruan, S. Shi, Cu-related agents for cancer therapies, *Coord. Chem. Rev.* 487 (2023) 215156, <https://doi.org/10.1016/j.ccr.2023.215156>.
- [33] D. Tang, X. Chen, G. Kroemer, Cuproptosis: a copper-triggered modality of mitochondrial cell death, *Cell Res.* 32 (2022) 417–418, <https://doi.org/10.1038/s41422-022-00653-7>.
- [34] P. Tsvetkov, S. Coy, B. Petrova, M. Dreishpoon, A. Verma, M. Abdusamad, J. Rossen, L. Joesch-Cohen, R. Humeidi, R.D. Spangler, Copper induces cell death by targeting lipoylated TCA cycle proteins, *Science* 375 (2022) 1254–1261, <https://doi.org/10.1126/science.abf0529>.
- [35] N. Kordestani, H.A. Rudbari, A.R. Fernandes, L.R. Raposo, P.V. Baptista, D. Ferreira, G. Bruno, G. Bella, R. Scopelliti, J.D. Braun, Antiproliferative activities of diimine-based mixed ligand copper (II) complexes, *ACS Comb. Sci.* 22 (2020) 89–99, <https://doi.org/10.1021/acscmbci.9b00202>.
- [36] N. Kordestani, H.A. Rudbari, I. Correia, A. Valente, L. Corte-Real, M.K. Islam, N. Micale, J.D. Braun, D.E. Herbert, N. Tumanov, Heteroleptic enantiopure pd (ii)-complexes derived from halogen-substituted Schiff bases and 2-picolyamine: synthesis, experimental and computational characterization and investigation of the influence of chirality and halogen atoms on the anticancer activity, *New J. Chem.* 45 (2021) 9163–9180, <https://doi.org/10.1039/D1NJ01491A>.
- [37] S. Selaković, M.V. Rodić, I. Novaković, I.Z. Matic, T. Stanjoković, A. Pirković, L. Živković, B. Spremo-Potparević, M. Milčić, V. Medaković, Cu (II) complexes with a salicylaldehyde derivative and α -diimines as co-ligands: synthesis, characterization, biological activity. Experimental and theoretical approach, *Dalton Trans.* 53 (2024) 2770–2788, <https://doi.org/10.1039/D3DT03862A>.
- [38] N. Kordestani, H.A. Rudbari, A.R. Fernandes, L.R. Raposo, A. Luz, P.V. Baptista, G. Bruno, R. Scopelliti, Z. Fatemina, N. Micale, Copper (ii) complexes with tridentate halogen-substituted Schiff base ligands: synthesis, crystal structures and investigating the effect of halogenation, leaving groups and ligand flexibility on antiproliferative activities, *Dalton Trans.* 50 (2021) 3990–4007, <https://doi.org/10.1039/D0DT03962D>.
- [39] H.A. Rudbari, A. Saadati, M. Aryaeifar, O. Blaque, J.V. Cuevas-Vicario, R. Cabral, L.R. Raposo, A.R. Fernandes, Platinum (II) and copper (II) complexes of asymmetric halogen-substituted [NN'O] ligands: synthesis, characterization, structural investigations and antiproliferative activity, *Bioorg. Chem.* 119 (2022) 105556, <https://doi.org/10.1016/j.bioorg.2021.105556>.
- [40] H.A. Rudbari, N. Kordestani, J.V. Cuevas-Vicario, M. Zhou, T. Efferth, I. Correia, T. Schirmeister, F. Barthels, M. Enamullah, A.R. Fernandes, Investigation of the influence of chirality and halogen atoms on the anticancer activity of enantiopure palladium (ii) complexes derived from chiral amino-alcohol Schiff bases and 2-picolyamine, *New J. Chem.* 46 (2022) 6470–6483, <https://doi.org/10.1039/D2NJ00321J>.
- [41] M. Zhou, J.C. Boulos, E.A. Omer, H.A. Rudbari, T. Schirmeister, N. Micale, T. Efferth, Two palladium(II) complexes derived from halogen-substituted Schiff bases and 2-picolyamine induce parthanatos-type cell death in sensitive and multi-drug resistant CCRF-CEM leukemia cells, *Eur. J. Pharmacol.* 956 (2023) 175980, <https://doi.org/10.1016/j.ejphar.2023.175980>.
- [42] M.T. Shool, H.A. Rudbari, T. Gil-Antón, J.V. Cuevas-Vicario, B. García, N. Busto, N. Moini, O. Blaque, The effect of halogenation of salicylaldehyde on the antiproliferative activities of $\{\Delta/\Lambda\text{-[Ru (bpy) 2 (X, Y-sal)] BF 4}\}$ complexes, *Dalton Trans.* 51 (2022) 7658–7672.
- [43] M. Taghizadeh Shool, H.A. Rudbari, J.V. Cuevas-Vicario, A. Rodríguez-Rubio, C. Stagno, N. Iraci, T. Efferth, E.A. Omer, T. Schirmeister, O. Blaque, Investigating the cytotoxicity of Ru (II) polypyridyl complexes by changing the electronic structure of salicylaldehyde ligands, *Inorg. Chem.* 63 (2023) 1083–1101, <https://doi.org/10.1021/acs.inorgchem.3c03414>.
- [44] A. Notaro, M. Jakubaszek, N. Rotthowe, F. Maschietto, R. Vinck, P.S. Felder, B. Goud, M. Tharaud, I. Ciofini, F. Bedioui, Increasing the cytotoxicity of Ru (II) polypyridyl complexes by tuning the electronic structure of dioxo ligands, *J. Am. Chem. Soc.* 142 (2020) 6066–6084, <https://doi.org/10.1021/jacs.9b12464>.
- [45] Y. Lu, Z. Hou, M. Li, N. Wang, J. Wang, F. Ni, Y. Zhao, B. Zhang, N. Xi, Increasing the cytotoxicity of Ru(ii) polypyridyl complexes by tuning the electron-donating ability of 1,10-phenanthroline ligands, *Dalton Trans.* 51 (2022) 16224–16235, <https://doi.org/10.1039/D2DT02332F>.
- [46] Z. Xu, J. Huang, D. Kong, Y. Yang, L. Guo, X. Jia, G. Zhong, Z. Liu, Potent half-sandwich Ru (II) N⁻ N (aryl-BIAN) complexes: lysosome-mediated apoptosis, in vitro and in vivo anticancer activities, *Eur. J. Med. Chem.* 207 (2020) 112763, <https://doi.org/10.1016/j.ejmech.2020.112763>.
- [47] A.A. Adeniyi, P.A. Ajibade, The anticancer activities of some nitrogen donor ligands containing bis-pyrazole, bipyridine, and phenanthroline moiety using docking methods, *Bioinorg. Chem. Appl.* 2018 (2018) 5796287, <https://doi.org/10.1155/2018/5796287>.
- [48] World Health Organization, Breast Cancer (2026). <https://www.who.int/new-s-room/fact-sheets/detail/breast-cancer>.
- [49] M.E. Sowder, R.W. Johnson, Bone as a preferential site for metastasis, *JBM R Plus* 3 (2019) e10126, <https://doi.org/10.1002/jbm4.10126>.
- [50] G. Ottaviani, N. Jaffe, The epidemiology of osteosarcoma, in: N. Jaffe, O. Bruland, S. Bielack (Eds.), *Pediatric and adolescent osteosarcoma. Cancer Treatment and Research*, Springer, Boston, MA, 2009, pp. 3–13, https://doi.org/10.1007/978-1-4419-0284-9_1.
- [51] V. Rajaratnam, M.M. Islam, M. Yang, R. Slaby, H.M. Ramirez, S.P. Mirza, Glioblastoma: pathogenesis and current status of chemotherapy and other novel treatments, *Cancers* 12 (2020) 937, <https://doi.org/10.3390/cancers12040937>.
- [52] J. van Meerloo, G.J.L. Kaspers, J. Cloos, Cell sensitivity assays: The MTT assay, in: I. Cree (Ed.), *Cancer Cell Culture. Methods in Molecular Biology*, Humana Press, 2011, pp. 237–245, https://doi.org/10.1007/978-1-61779-080-5_20.
- [53] Y. Liu, D.A. Peterson, H. Kimura, D. Schubert, Mechanism of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction, *J. Neurochem.* 69 (1997) 581–593, <https://doi.org/10.1046/j.1471-4159.1997.69020581.x>.
- [54] Y. Burgos-López, L.M. Balsa, O.E. Piro, I.E. León, J. García-Tojal, G.A. Echeverría, A.C. González-Baró, B.S. Parajón-Costa, Tridentate acylhydrazone copper(II) complexes with heterocyclic bases as coligands. Synthesis, spectroscopic studies, crystal structure and cytotoxicity assays, *Polyhedron* 213 (2022) 115621, <https://doi.org/10.1016/j.poly.2021.115621>.
- [55] L.M. Balsa, M.R. Rodríguez, B.S. Parajón-Costa, A.C. González-Baró, M. J. Lavecchia, I.E. León, Anticancer activity and mechanism of action evaluation of an acylhydrazone Cu(II) complex toward breast cancer cells, spheroids, and mammospheres, *ChemMedChem* 17 (2022) e202100520, <https://doi.org/10.1002/cmdc.202100520>.
- [56] M.R. Rodríguez, J. Del Plá, L.M. Balsa, I.E. León, O.E. Piro, G.A. Echeverría, J. García-Tojal, R. Pis-Diez, B.S. Parajón-Costa, A.C. González-Baró, Cu(II) and Zn (II) complexes with a poly-functional ligand derived from o-vanillin and thiophene. Crystal structure, physicochemical properties, theoretical studies and cytotoxicity assays against human breast cancer cells, *New J. Chem.* 43 (2019) 7120, <https://doi.org/10.1039/c8nj06274a>.
- [57] L.S.M. de la Parra, A.I.B. Romo, J. Rodríguez-López, O.R. Nascimento, G. A. Echeverría, O.E. Piro, I.E. León, Promising dual anticancer and antimetastatic action by a Cu(II) complex derived from acylhydrazone on human osteosarcoma models, *Inorg. Chem.* 63 (2024) 4925, <https://doi.org/10.1021/acs.inorgchem.3c0408>.
- [58] X. An, W. Yu, J. Liu, D. Tang, L. Yang, X. Chen, Oxidative cell death in cancer: mechanisms and therapeutic opportunities, *Cell Death Dis.* 15 (2024) 556, <https://doi.org/10.1038/s41419-024-06939-5>.
- [59] H. Yang, R.M. Villani, H. Wang, M.J. Simpson, M.S. Roberts, M. Tang, X. Liang, The role of cellular reactive oxygen species in cancer chemotherapy, *J. Exp. Clin. Cancer Res.* 37 (2018) 266, <https://doi.org/10.1186/s13046-018-0909-x>.
- [60] N. Kosiol, S. Juranek, P. Brossart, A. Heine, K. Paeschke, G-quadruplexes: a promising target for cancer therapy, *Mol. Cancer* 20 (2021) 40, <https://doi.org/10.1186/s12943-021-01328-4>.
- [61] J. Qian, R. Liu, N. Liu, C. Yuan, Q. Wu, Y. Chen, W. Tan, W. Mei, Arene Ru(II) complexes acted as potential KRAS G-quadruplex DNA stabilizer induced DNA damage mediated apoptosis to inhibit breast cancer progress, *Molecules* 27 (2022) 3046, <https://doi.org/10.3390/molecules27103046>.
- [62] S. Sharma, A.K. Mukherjee, S.S. Roy, S. Bagri, S. Lier, M. Verma, A. Sengupta, M. Kumar, G. Nesse, D.P. Pandey, S. Chowdhury, Human telomerase is directly regulated by non-telomeric TRF2-G-quadruplex interaction, *Cell Rep.* 35 (2021) 109154, <https://doi.org/10.1016/j.celrep.2021.109154>.
- [63] J. Weynand, A. Diman, M. Abraham, L. Marcélis, H. Jamet, A. Decottignies, J. Dejeu, E. Defrancq, B. Elias, Towards the development of photo-reactive ruthenium(II) complexes targeting telomeric G-quadruplex DNA, *Chem. Eur. J.* 24 (2018) 19216–19227, <https://doi.org/10.1002/chem.201804771>.
- [64] A. Kerkour, J. Marquieville, S. Ivashchenko, L.A. Yatsunyk, J.-L. Mergny, G. F. Salgado, High-resolution three-dimensional NMR structure of the KRAS proto-oncogene promoter reveals key features of a G-quadruplex involved in transcriptional regulation, *J. Biol. Chem.* 292 (2017) 8082–8091, <https://doi.org/10.1074/jbc.M117.781906>.
- [65] K.W. Lim, L. Lacroix, D.J.E. Yue, J.K.C. Lim, J.M.W. Lim, A.T. Phan, Coexistence of two distinct G-quadruplex conformations in the hTERT promoter, *J. Am. Chem. Soc.* 132 (2010) 12331–12342, <https://doi.org/10.1021/ja101252n>.
- [66] B. Heddi, A.T. Phan, Structure of human telomeric DNA in crowded solution, *J. Am. Chem. Soc.* 133 (2011) 9824–9833, <https://doi.org/10.1021/ja200786q>.
- [67] G.N. Parkinson, F. Cuenca, S. Neidle, Topology conservation and loop flexibility in quadruplex–drug recognition: crystal structures of inter- and intramolecular telomeric DNA quadruplex–drug complexes, *J. Mol. Biol.* 381 (2008) 1145–1156, <https://doi.org/10.1016/j.jmb.2008.06.022>.
- [68] <https://www.rcsb.org/structure/7CPS>.

- [69] A.E. Friedman, J.C. Chambron, J.P. Sauvage, N.J. Turro, J.K. Barton, A molecular light switch for DNA: $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, *J. Am. Chem. Soc.* 112 (1990) 4960–4962.
- [70] P. Lincoln, A. Broo, B. Nordén, Diastereomeric DNA-binding geometries of intercalated ruthenium(II) Tris-chelates probed by linear dichroism: $[\text{Ru}(\text{phen})_2\text{DPPZ}]^{2+}$ and $[\text{Ru}(\text{phen})_2\text{BDPPZ}]^{2+}$, *J. Am. Chem. Soc.* 118 (1996) 2644–2653, <https://doi.org/10.1021/ja953363l>.
- [71] E. Trovato, M.L. Di Pietro, F. Puntoriero, Shining a new light on an old game: An OsII-based near-IR light-switch, *Eur. J. Inorg. Chem.* (2012) 3984–3988, <https://doi.org/10.1002/ejic.201200787>.
- [72] C.-M. Che, M. Yang, K.-H. Wong, H.-L. Chan, W. Lam, Platinum(II) complexes of dipyrrophenazine as metallointercalators for DNA and potent cytotoxic agents against carcinoma cell lines, *Chemistry* 5 (1999) 3350–3356, 10.1002/(SICI)1521-3765(19991105)5:11<3350::AID-CHEM3350>3.0.CO;2-J.
- [73] M. Cusumano, M.L. Di Pietro, A. Giannetto, DNA interaction of platinum(II) complexes with 1,10-phenanthroline and extended phenanthrolines, *Inorg. Chem.* 45 (2006) 230–235, <https://doi.org/10.1021/ic050880o>.
- [74] M. Cusumano, M.L. Di Pietro, A. Giannetto, F. Nicolò, B. Nordén, P. Lincoln, Ambivalent intercalators for DNA: L-shaped platinum(II) complexes, *Inorg. Chem.* 43 (2004) 2416–2421, <https://doi.org/10.1021/ic035016f>.
- [75] M. Cusumano, M.L. Di Pietro, A. Giannetto, Stacking surface effect in the DNA intercalation of some polypyridine platinum(II) complexes, *Inorg. Chem.* 38 (1999) 1754–1758, <https://doi.org/10.1021/ic9809759>.
- [76] J.D. McGhee, P.H. von Hippel, Theoretical aspects of DNA-protein interactions: co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice, *J. Mol. Biol.* 86 (1974) 469–489, [https://doi.org/10.1016/0022-2836\(74\)90031-X](https://doi.org/10.1016/0022-2836(74)90031-X).
- [77] R.F. Pasternack, E.J. Gibbs, J.J. Villafranca, Interactions of porphyrins with nucleic acids, *Biochemistry* 22 (1983) 5409–5417.
- [78] R.D. Wells, J.E. Larson, R.C. Grant, B.E. Shortle, C.R. Cantor, Physicochemical studies on polydeoxyribonucleotides containing defined repeating nucleotide sequences, *J. Mol. Biol.* 54 (1970) 465–497, [https://doi.org/10.1016/0022-2836\(70\)90121-X](https://doi.org/10.1016/0022-2836(70)90121-X).
- [79] T. Pivetta, F. Trudu, E. Valletta, F. Isaia, C. Castellano, F. Demartin, R. Tuveri, S. Vascellari, A. Pani, Novel copper (II) complexes as new promising antitumor agents. A crystal structure of $[\text{Cu}(1,10\text{-phenanthroline-5,6-dione})_2(\text{OH}_2)(\text{ClO}_3)](\text{ClO}_4)$, *J. Inorg. Biochem.* 141 (2014) 103–113, <https://doi.org/10.1016/j.jinorgbio.2014.08.011>.
- [80] H.L. Huang, Z.Z. Li, Z.H. Liang, Y.J. Liu, Cell cycle arrest, cytotoxicity, apoptosis, DNA-binding, photocleavage, and antioxidant activity of octahedral ruthenium (II) complexes, *Eur. J. Inorg. Chem.* (2011) 5538–5547, <https://doi.org/10.1002/ejic.201100848>.
- [81] K.L. Reddy, Y.H. Kumar Reddy, K.A. Kumar, S. Vidhisha, S. Satyanarayana, Synthesis, characterization, DNA-binding, and DNA-photocleavage properties of $[\text{Co}(\text{bpy})_2(7\text{-NO}_2\text{-dppz})]^{3+}$, $[\text{Co}(\text{dmb})_2(7\text{-NO}_2\text{-dppz})]^{3+}$, and $[\text{Co}(\text{phen})_2(7\text{-NO}_2\text{-dppz})]^{3+}$ complexes: (7-Nitro-dppz = 7-Nitro dipyrro[3, 2-a: 2'-3'-c] phenazine; bpy = 2, 2'-bipyridine; dmb = 4, 4'-dimethyl-2,2'-bipyridine; phen = 1,10-phenanthroline) and their toxicity on different microorganisms, *Nucleosides Nucleotides Nucleic Acids* 28 (2009) 204–219, <https://doi.org/10.1080/15257770902865399>.
- [82] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, G.A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B.G. Janesko, R. Gomperts, B. Mennucci, H.P. Hratchian, J. V. Ortiz, A.F. Izmaylov, J.L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J.A. Jr. Montgomery, J.E. Peralta, F. Ogliaro, M.J. Bearpark, J.J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, T.A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, S.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, J.M. Millam, M. Klene, C. Adamo, R. Cammi, J.W. Ochterski, R.L. Martin, K. Morokuma, O. Farkas, J.B. Foresman, D.J. Fox, Gaussian 16, Revision C.01, Gaussian, Inc., Wallingford, CT, 2019.
- [83] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, *J. Chem. Phys.* 98 (1993) 5648–5652, <https://doi.org/10.1063/1.464913>.
- [84] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, *Phys. Rev. B* 37 (1988) 785–789, <https://doi.org/10.1103/PhysRevB.37.785>.
- [85] F. Weigend, Accurate coulomb-fitting basis sets for H to Rn, *Phys. Chem. Chem. Phys.* 8 (2006) 1057–1065, <https://doi.org/10.1039/B515623H>.
- [86] F. Weigend, R. Ahlrichs, Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: design and assessment of accuracy, *Phys. Chem. Chem. Phys.* 7 (2005) 3297–3305, <https://doi.org/10.1039/B508541A>.
- [87] S. Grimme, J. Antony, S. Ehrlich, H. Krieg, A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu, *J. Chem. Phys.* 132 (2010) 154104, <https://doi.org/10.1063/1.3382344>.
- [88] S. Grimme, S. Ehrlich, L. Goerigk, Effect of the damping function in dispersion corrected density functional theory, *J. Comput. Chem.* 32 (2011) 1456–1465, <https://doi.org/10.1002/jcc.21759>.
- [89] A.V. Marenich, C.J. Cramer, D.G. Truhlar, Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions, *J. Phys. Chem. B* 113 (2009) 6378–6396, <https://doi.org/10.1021/jp810292n>.
- [90] M. Petersilka, U. Gossmann, E. Gross, Excitation energies from time-dependent density-functional theory, *Phys. Rev. Lett.* 76 (1996) 1212, <https://doi.org/10.1103/PhysRevLett.76.1212>.
- [91] C. Jamorski, M.E. Casida, D.R. Salahub, Dynamic polarizabilities and excitation spectra from a molecular implementation of time-dependent density-functional response theory: N2 as a case study, *J. Chem. Phys.* 104 (1996) 5134–5147, <https://doi.org/10.1063/1.471140>.
- [92] M.E. Casida, C. Jamorski, K.C. Casida, D.R. Salahub, Molecular excitation energies to high-lying bound states from time-dependent density-functional response theory: characterization and correction of the time-dependent local density approximation ionization threshold, *J. Chem. Phys.* 108 (1998) 4439–4449, <https://doi.org/10.1063/1.475855>.
- [93] G.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I. N. Shindyalov, P.E. Bourne, The Protein Data Bank, *Nucleic Acids Res.* 28 (2000) 235–242.
- [94] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, *J. Comput. Chem.* 30 (2009) 2785–2791, <https://doi.org/10.1002/jcc.21256>.
- [95] K.J. Bowers, E. Chow, H. Xu, R.O. Dror, M.P. Eastwood, B.A. Gregersen, J. L. Klepeis, I. Kolossvary, M.A. Moraes, F.D. Sacerdoti, J.K. Salmon, Y. Shan, D. E. Shaw, Scalable algorithms for molecular dynamics simulations on commodity clusters, in: *Proceedings of the ACM/IEEE Conference on Supercomputing (SC06)*, 2006. Tampa, Florida.
- [96] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, Comparison of simple potential functions for simulating liquid water, *J. Chem. Phys.* 79 (1983) 926–935, <https://doi.org/10.1063/1.445869>.



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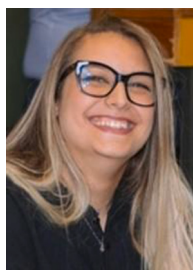
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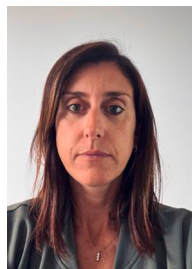
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