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Ruthenium-based complexes as anti-tumor agents

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ABSTRACT

Extensive research into platinum-based chemotherapeutics has been underway for decades with ruthenium-based complexes emerging as interesting and potent candidates. Even still, there is no evidence of a single mechanism of action across all synthesized and tested Ru-based complexes, prompting the continuance of research in this field. In addition, the mechanism of action varies according to cell line and/or animal model and is seemingly highly individualized and personalized. In accordance with this, the ruthenium complexes are able to activate specific molecular pathways and interact with certain targets within the cell, sometimes reported simultaneously. In this review, we attempt to give a new perspective on ruthenium complexes' anti-cancer properties and organize selected results from the past 15 years of research connecting their structure with the reported mechanism of action. These results corroborate the previously reported great potential that ruthenium complexes have on cancer *in vitro*. In addition, the review provides insight into Ru drugs in their clinical trials and their efficacy against cancer including a historical context on metallodrugs, particularly platinum-based complexes, and their antitumor capability.

Keywords: Ruthenium complexes; cancer; metallodrug; mechanism of action; antitumor properties

INTRODUCTION

Cancer treatment, although heavily dependent on cancer type and stage, involves one or a combination of the following approaches: Surgery, chemotherapy, radiotherapy, immunotherapy, or biological therapy. A more modern approach of targeted therapy tailored to every individual patient known as personalized medicine is on the rise, providing hope and betterment for cancer patients worldwide (1). The race for research and development of alternatives to current chemotherapeutics is far from over with the main motivators being overcoming tissue toxicity (mainly neurotoxicity and nephrotoxicity), as well as both intrinsic and acquired drug resistance. While cisplatin and other platinum-based chemotherapeutics remain at the helm of present-day chemotherapy, their use is not without adverse effects. One of the most important issues to note with cisplatin use is drug resistance for many cancer types such as ovarian, breast, lung, head and neck, and many others, severely limiting its effectiveness (2,3). Due to the described need for Pt-based alternatives, there has been a significant increase in published research on the topic of ruthenium-based complexes as potential anti-cancer drugs within the past 40 or so years. The purpose of this review is to summarize the advancements made in this field and give comments on prospects of ruthenium-based compounds aiding or replacing currently in-use drugs.

Ruthenium has proved to be an excellent target for anti-cancer drug research due to its innate biochemical characteristics including (a) mimicking iron-binding properties of serum albumin and transferrin; (b) pro-drug potential, as the inactive Ru^{3+} state in circulation can be reduced to the active Ru^{2+} state when in the target cell; (c) slow ligand exchange kinetics, much like cisplatin; and (d) binding to DNA, although more research is needed to clarify it this mechanism of action is similar or different than cisplatin (4). It is worth noting that despite decades-long research into Ru-based cancer drugs, the mechanism behind its anti-cancer effect remains elusive.

Ruthenium complexes have been proposed as non-toxic alternatives to commonly used chemotherapeutics (5). They are organometallic complexes that have antimicrobial and antitumor activities, where the ruthenium atom is coordinated to an established organic compound.

This review attempts to organize the results of selected published research from the past 15 years and give commentary on how the chemical structure of referenced Ru-complexes impacts its reported mechanism of action. To achieve this, we classified referenced complexes into nine groups based on their chemical structure with ligands and commented on their reported mechanism of action. These mechanisms of action were grouped into cellular effects, cell cycle

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disruptions, DNA interactions, protein interactions, and molecular effects.

CISPLATIN AND OTHER TRANSITION METAL-BASED COMPLEXES

Cisplatin is a chemotherapy drug used for the treatment of patients with bladder, lung, neck, testicular, cervical, esophageal, breast, and brain cancer, among others (6). Cisplatin was first discovered and synthesized by Dr. Rosenberg after the drug showed great potential for the degradation of bacterial cell walls, specifically researched on *Escherichia coli* strains. This discovery propelled the research into cisplatin (7). Cisplatin's mode of action is characterized by covalent binding to the DNA, forming adducts, and induction of apoptosis (8). The mode of activation occurs after the metal enters the cytoplasm coupled with a water molecule and forms the electrophile and activity toward the sulfhydryl group on protein and nitrogen donor on nucleic acid (6,9-13). There are several other anticancer regimens that are used in the treatment of cancer patients such as metal-based drugs (14-16). The mode of action of metal-based drugs is due to the metal's inherent characteristics: Redox reaction, variable coordinate modes, and reactivity toward other substrates (17). While the research into various ruthenium-based metallodrugs has accelerated, providing a general mechanism of action for these compounds has proved to be challenging. This is due to the sheer possibility of combinations giving unique Ru-complex structures, either organometallics or coordinated complexes, of which multiple simultaneous mechanisms of action can be present.

RUTHENIUM-COORDINATION COMPLEXES – STRUCTURE, CLASSIFICATION, AND MECHANISM OF ACTION

Ruthenium complexes have been proposed as non-toxic alternatives to commonly used chemotherapeutics. As reported by Lazarević et al. (2017), ruthenium species are found in two oxidation states of interest to cancer researchers: Ru(II) d^6 which is diamagnetic, and Ru(III) d^5 which is paramagnetic, both having a good affinity for ligands containing N and S donor atoms. Depending on its oxidation state, Ru(II) has a higher affinity for S- and N-donor ligands, while Ru(III) favors O- and N-donors (18). Gianferrara et al. have classified anti-cancer metallodrugs into five classes according to the role of each part of the complex: (class I) the metal center has a functional role where it directly interacts with the target molecule; (class II) the metal center provides a structural role and non-covalently interacts with the target; (class III) the metal center serves as a carrier to deliver and protect the active ligand *in vivo*; (class IV) the metal complex catalyzes reactive oxygen species (ROS) *in vivo* ultimately damaging cancer cells; and (class V) the metal complex is a photosensitizer due to its photoactivity (19).

Ruthenium bipyridine/phenanthroline complexes

The first group of Ru-based complexes is ruthenium bipyridine/phenanthroline complexes which contain at least one bipyridine and/or phenanthroline derivative as their ligand. $[Ru(bpy)_2Ld](OTf)\cdot 2H_2O$ (**1**), where bpy is 2,2'-bipyridine and Ld is a monobasic anion of 3-hydroxyflavone, showed moderately strong DNA intercalating potential and strongly bound to bovine serum albumin (BSA) (20). Its reported chemical structure, alongside all Ru-bipyridine/phenanthroline complexes, is available in Figure 1. The anti-cancer potential of this complex was tested against HeLa, SW620, HepG2, and MCF-7 with all IC_{50} values but for HepG2 being under 1.0 μM (0.78 ± 0.20 μM, 0.75 ± 0.15 μM, 2.51 ± 0.67 μM, and 0.52 ± 0.38 μM, respectively) (20). $[Ru(bpy)_2(L)]CF_3SO_3$ (**2**), where bpy is 2,2′-bipyridine and L is a Schiff base derived from salicylaldehyde and amino acid phenylalanine, was more effective than cisplatin only against MCF-7 in the study (26 ± 1 vs. 41.7 ± 1.5 μM) but not against NCI-H460 or SW620 (>100 μM) (Osmanković et al. 2022). The complex showed weak intercalation with CT DNA as measured by spectroscopic titration (binding constant in $10^3/M^{-1}$ range) but strongly binds to BSA in a 1:1 ratio as investigated by spectrofluorimetry (21). Han et al. tested the anti-cancer potential and mechanism of action of two Ru(II) complexes out of which $[Ru(dmb)_2(dcdppz)](ClO_4)_2$, (**3**) where dmb is 4,4'-dimethyl-2,2'-bipyridine and dcdppz is h,j-dichlorodipyrido[3,2-a:2',3'-c]phenazine, was more potent than $[Ru(bpy)_2(dcdppz)](ClO_4)_2$ but less than cisplatin in all three cancer cell lines used in this study. The complex was able to enter the cytoplasm and accumulate in the nucleus disrupting the G0/G1 checkpoint of the cell cycle (22). $[Ru(dmb)_2(dcdppz)](ClO_4)_2$, (**3**) was also able to induce apoptosis by ROS-mediated mitochondrial dysfunction pathway, intercalate dsDNA, down-regulate the expression of Bcl-2 and Bad, and upregulate the expression of Bax (22). $[Ru(II)(bmbp)(phen)]^{2+}$ (**4**), where bmbp is 2,6-bis(6-methylbenzimidazol-2-yl)pyridine) and phen is 1,10- phenanthroline, IC_{50} values were significantly lower than those of NAMI-A for the following cancer cell lines: A375, HeLa, MCF-7, PC3, and MDA-MB-231 (25.2 μM, 47.9 μM, 45.4 μM, 49.6 μM, and 12.5 μM, respectively). The complex induced apoptosis through both the intrinsic and extrinsic pathways by activating Caspases 3, 8, and 9 in a dose-dependent manner. As there was no blue nucleus fluorescence detected through live-cell fluorescence test in the study, the authors concluded that nucleic acids are not the cellular target of this Ru-bipyridine/phenanthroline complex (23). Another Ru-bypiridine complex with the basic formula $[Ru(bpy)_3]^{2+}$ - SST (**5**), where bpy is 2,2'-bipyridine and SST peptide hormone somatostatin, was tested for its anti-cancer potential against the A549 cancer cell line. The complex exhibited significant toxicity against the chosen cell line after light irradiation but was found to be non-toxic at concentrations over 300 μM without light irradiation (22). $[Ru(bpy)_3]^{2+}$ – SST (**5**) proved to be more potent than its Ru-alkyne counterpart or complex 3 in the study, with IC_{50} values of 13.2 ± 1.1 μM. Lovison et al. described $[Ru(\eta^1\text{-OPiv})(CO)(dppb)(phen)]OPiv$ (**6**), where Piv is pivalate, dppb is 1,4-bis(diphenylphosphino) butane), and phen is 1,10-phenanthroline and tested it for anti-cancer properties against two thyroid adenocarcinoma cell lines. The complex is highly soluble in organic solvents and stable in air at room temperature but the pivalate ligand is not coordinated to Ru in an aqueous state (24). It induced apoptosis as confirmed by Western blot of cleaved-PARP protein levels and possesses strong metastatic activity. The complex was more potent than the

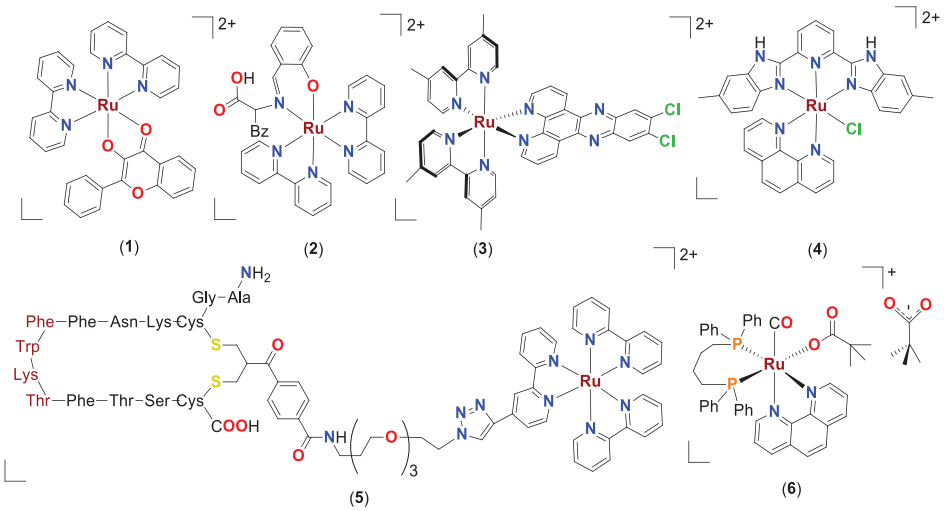

FIGURE 1. Anticancer ruthenium bipyridine/phenanthroline complexes.

reference cisplatin with EC_{50} values <0.20 μM (24). Out of these six Ru-complexes, five of them reported apoptosis as the primary cellular effect (**1-2,4-6**), while complex (**3**) reported ROS accumulation. For complex (**6**), it was additionally reported to have metastatic properties. As for interactions with DNA, complexes (**1-3**) reported DNA intercalation, while complex (**4**) induced apoptosis through both cas3/9 and cas8 activation. Complexes (**1-2**) are able to interact with proteins as well. We would like to infer that Ru-complexes with bipyridine derivatives as ligands show promising intercalating properties worth exploring further; however, they have been reported to be less potent than reference drugs used in the study, while complexes with bipyridine/phenanthroline ligands or phenanthroline ligand alone were reported to be more effective. Due to the small sample size and varying mechanisms of actions reported, it is impossible to give a definitive conclusion. As these complexes were tested against various cancer cell lines and compared to differing drugs (cisplatin, NAMI-A, and other Ru-based drugs), the implications of our conclusion remain to be confirmed in a more robust study.

Ruthenium-terpyridine complexes

Next group of complexes with anti-cancer properties, we chose to analyze are Ru-terpyridine complexes, of which we chose seven reference complexes (Figure 2). In a study by Lazić et al., two Ru(II)-terpyridine complexes were synthesized and tested for possible interactions with L-histidine [Ru(Cl-tpy)(en)Cl][Cl] or compound 1 in the study and [Ru(Cl-tpy)(dach)Cl][Cl] (**7**) or compound 2 in the study (where Cl-tpy is 4′-chloro-2,2′:6′,2″-terpyridine; en is 1,2-diaminoethane; dach is 1,2-diaminocyclohexane). [Ru(Cl-tpy)(dach)Cl][Cl] (**7**), where Cl-tpy is 4′-chloro-2,2′:6′,2″-terpyridine and dach is 1,2-diaminocyclohexane, is one of the water-soluble ruthenium(II) terpyridine complexes Lazić et al. researched. Cytotoxicity was dose-dependent but lower than the control drug under the same conditions (25). A dual mode of action was proposed, where there is a covalent bonding with 5'-guanine N7 in dsDNA forming adducts and a non-covalent intercalation happening simultaneously. This highlights the importance of expanding the scope of research for Ru-complexes' intracellular targets and activity, as it is evident, certain complexes are able to interact with DNA directly, while others target proteins or enzymes involved in cellular processes. The results indicate bond formation with L-histidine and the imidazole group, being the most likely target for coordinated bond formation with Ru(II); however, the interaction was described as having relatively low reactivity when compared to DNA (25).

Both compounds seem to coordinate with the L-histidine firstly N3 atom before binding to N1 for enhanced thermodynamic stability. Compound 1 is slightly less hydrophilic when compared to compound 2, having the advantage when it comes to cell uptake and possibly anti-cancer properties. In addition to that, compound 1 exhibits a combination of MoA where both N7 covalent coordination and intercalation of tpy ligand between adjacent bases of the double helix are recorded. Similar chemical behavior is described for compound 2, although less potent. Both compounds stand in competition with ethidium bromide's intercalating affinity toward DNA, facilitated by the tpy ligand. [Ru(Cl-tpy)(dach)Cl][Cl] (**7**) despite having a dual mechanism of action wherein non-covalent intercalation and covalent bonding with N7 of guanine in dsDNA, subsequently forming DNA adducts, was detected, the anti-cancer effect against A549 and HCT116 cell lines was lesser than cisplatin (IC_{50} values: 58.40 ± 0.10 μM and 66.30 ± 0.20, respectively) (25). [Ru(Cl-tpy)(en)Cl][Cl] (**8**), where en is ethylenediamine and tpy is terpyridine was less effective against HCT116 and SW480 than oxaliplatin (19.1 ± 1 μM and 44.7 ± 4 μM, respectively). However, the complex showed significant cytotoxicity against chosen cell lines with lower nephrotoxicity when compared to the reference drug and exerted this effect faster. In addition to this, the tested complex possibly affects cell membrane integrity as shown by the LDH test. It induced late apoptosis in chosen cell lines and G2/M cell cycle phase arrest, while early apoptosis had no statistical difference between treated and control cells (26). Conjugating the Ru(II)-complex [Ru(terpy)(terpy*)]$(PF_6)_2$ (**9**), where terpy = 2,2′:6′,2″-terpyridine and terpy* = 2,2′:6′,2″-terpyridine]-4′-carboxylic acid, with a cyclic pentapeptide **RGDfK** (Arg-Gly-Asp-D-phen-Lys) did not yield higher cytotoxicity regardless of the motif present. The complex was tested against A549 and SKOV3 cancer cell lines with IC_{50} values for both being

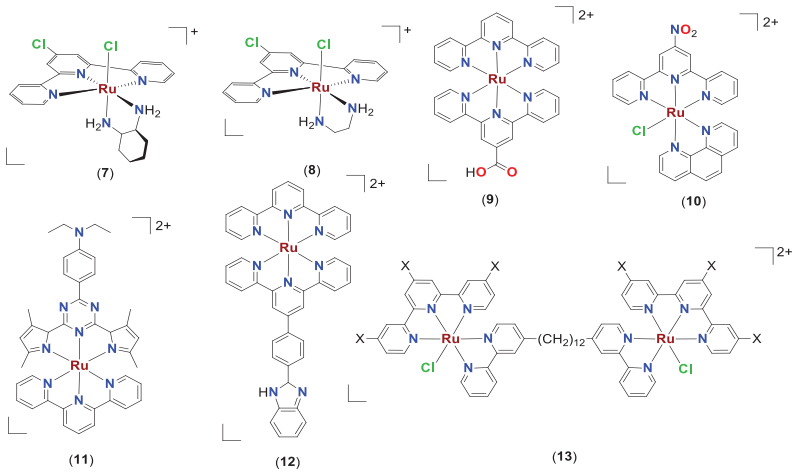

FIGURE 2. Anticancer ruthenium-terpyridine complexes.

above 70 μM. Selectivity toward the $\alpha_v\beta_3$ subclass of integrins expressed on both A549 and SKOV3 cancer cell lines was reasonably high (27).

Deng et al. tested several Ru(II) phenylterpyridine complexes against the following cancer cell lines: A375, HepG2, MCF-7, and A549. Out of the 12 tested complexes in this study, [RuII(4-NO_2-phtpy)(phen)Cl]ClO_4, (**10**) where phtpy is phenylterpyridine and phen is 1,10-phenanthroline, was found to be most potent against chosen cancer cell lines. The introduction of NO_2 group into the phenylterpyridine ligand greatly improved cytotoxicity. After 24-hour incubation, the complex can be found accumulated in the cytoplasm with very little presence in the nucleus and was able to induce apoptosis in a dose-dependent manner. As the apoptosis was mainly triggered by cas8 involvement, a DR pathway is suggested as the main apoptotic pathway, as well as p53-mediated pathway. The complex targets death receptors and its transport is mediated by the transferrin receptor. It is stable in physiological conditions enabling interaction with cellular membrane receptors but does not induce apoptosis through ROS overproduction (28).

Another ruthenium-based complex [Ru(bdpta)(tpy)]$^{2+}$ (**11**), where bdpta ligand is 4-(4,6-bis(3,5-dimethyl-1H-pyrazole-1-yl)-1,3,5,-triazine-2-yl)-N,N-diethylaniline, and tpy stands for 2,2':6',2"-terpyridine, shows promising results against both MCF-7 and CD44$^+$ MCF-7, as well as HCT-116 and CD133$^+$ HCT-116 cancer cell lines. For all 4 tested cell lines, IC_{50} values were higher than for reference drug cisplatin (2.1 ± 0.06 μM, 3.48 ± 0.07 μM, 2.62 ± 0.05 μM, and 3.89 ± 0.09 μM, respectively). The complex is distributed within the cell mostly in the mitochondria and ER as demonstrated by the use of organelle-specific dyes in both cell lines; however, the nuclear distribution was found to be poor. A statistically significant increase in Bax and Bak levels was detected in complex-treated cells but not in control and a suppressed Bcl-2 expression indicated intrinsic pathway of apoptosis (29). Ru-UCN3 or [(tpy)Ru(tpy-ph-bzH)]$(Cl)_2$ (**12**) was able to strongly increase the activity of caspases 3/7 observed after 24 hour of incubation and increase the expression of PUMA and DIABLO affecting downstream signalization of the intrinsic apoptotic pathway as tested against G-415 cancer cell line (30). A multinuclear Ru(II)-based complex referenced as Cl-Rubb12 or [{Ru(tpy)Cl}$_2${m-bbn}]$^{2+}$ (**13**), where tpy is 2,2':6',2"-terpyridine, bbn is bis[4(4'-methyl-2,2'-bipyridyl)]-1,n-alkane (n = 12)} showed higher anti-proliferative potential against two breast adenocarcinoma cell lines when compared both to cisplatin and carboplatin. Cl-Rubb12 IC_{50} values for the MCF-7 cell line were 8 ± 4, cisplatin's 34 ± 2, and carboplatin's 273 ± 7, while the IC_{50} values of the three drugs for MDA-MB-231 cell line were 9 ± 4, 31 ± 3, and 451 ± 8, respectively (31). While complex (**9**) did not have detailed *in vivo* testing, out of the seven described Ru-terpyridine complexes, only complex (**11**) reported ROS accumulation. Similarly, only complex (**8**) had any effects on the cell cycle reported, mainly cell cycle arrest at G2/M. Complex (**7**) was reported to simultaneously cause DNA adducts as well as intercalation, while no such effects were noted for the remaining six complexes of this group. Complexes (**11-12**) induced apoptosis through the intrinsic pathway, while complex (**10**) induced apoptosis through the extrinsic pathway. Complex (**10**) was also the only complex reported to have any interactions with proteins in this group, which could be attributed to the introduction of a nitro-NO_2 group, as most other complexes of the group possess -Cl as the leaving ligand. All Ru-terpyridine complexes for which effectiveness compared to reference drug was reported, those with a -Cl leaving ligand were found to be less potent than cisplatin or oxaliplatin, highlighting the importance of ligand choice.

Prodrug with phenyl-1h-imidazophenanthroline ligand

Zhao et al. designed and researched an RGD-functionalized prodrug Ru-RGD or cis-[Ru(POP)$_2$Cl$_2$] + Arg-Gly-Asp peptide (**14**), where POP is phenanthroline. The $\alpha_v\beta_3$ integrin receptor-mediated mechanism of action most likely contributed to the selective uptake of the described conjugate. The conjugate exhibited higher selectivity and lower toxicity when compared to the parent complex devoid of the peptide sequence but failed to top the effectiveness of cisplatin for all but CaSki cell line (IC_{50} 3.8 μM) (32). The chemical formula for this Ru pro-drug is available at Figure 3.

Heteroleptic ruthenium coordination complexes

This group of Ru-complexes comprises five complexes in which more than one distinct ligand is coordinated to the Ru metal core (Figure 4). As stated previously, the structure

of the complex not only affects efficacy but also influences the mechanism of action against cancer cell lines. [Ru(GA)(dppe)2]PF_6 (**15**) complex, where GA is gallic acid (3,4,5-trihydroxybenzoic acid) as the ligand and dppe is 1,2-bis(diphenylphosphino)ethane, showed moderate interaction with CT DNA when compared to classic intercalators. The complex exhibited metastatic properties and apoptosis was induced in a concentration-dependent manner. At 12.5 μM concentration, the complex selectively caused damage to the cytoskeleton by promoting F-filament condensation and upregulated the expression of Bax, Cas9, and Cas3 but did not affect the expression of Cas-8 and Bcl-2 (33). The $[Ru_2L_2Cl_2(Et_2NH)x2(H_2O)]$ (**16**) complex, where L is N-(2-pyridyl)-5-H-salicylideneimine, was reported to have a high binding affinity to CT DNA (groove binding) comparable to known groove binders; however, its intercalating potential was significantly lower than the control (Kahrović et al., 2017a). Spectroscopic study of this dinuclear Ru(II) complex confirmed the complex moderately binds non-covalently to the DNA groove (34). It had several-fold lower IC_{50} values than cisplatin against HeLa, SW620, A549, and MCF-7 cancer cell lines (1.66 ± 0.48 μM, 1.99 ± 0.56 μM, 0.68 ± 0.88 μM, and 4.09 ± 0.78 μM, respectively) (18). In an earlier study by Kahrović et al. (2014), the binding affinity of a newly synthesized Ru(III) complex of the general formula: **$Na[Ru(N\text{-}R\text{-}5\text{-}X\text{-}salim)_2]\cdot 0.5Et_3N$** (where R = C_6H_4O; X = Cl, Br, NO_2; salim = salicylidene aminato; Et_3N = triethylamine) to calf thymus DNA (CT DNA) was researched. The spectrophotometric titration results, confirmed also by cyclic voltammetry, deemed the complexes to have moderate DNA-intercalating potential. The substituent "X" of the general formula above was found to have a prominent effect on the complex' binding potential with Cl having the highest value binding constant (35).

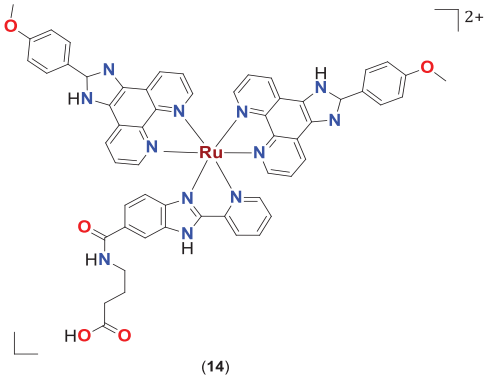

FIGURE 3. A multifunctional Ru prodrug with phenyl-1H-imidazophenanthroline ligand having theranostic activity against cervical carcinoma.

Devagi et al., synthesized four cyclopentadienyl ruthenium(II)-acetophenone-4(N)-substituted thiosemi-carbazone complexes, out of which **$[Ru(\eta^5\text{-}C_5H\text{-})(H\text{-}Ap\text{-}etsc)PPh_3]$**. Cl was the most potent in all performed assays. The complex interacts with CT DNA by means of intercalation as demonstrated by EB displacement assay. The complex is also able to cleave supercoiled plasmid pBR322 DNA and possesses high radical scavenging potential when compared to ascorbic acid (36). A kaempferol-based Ru(II) complex (**17**) synthesized and characterized by Thangavel et al. was able to strongly inhibit the proliferation of A549 cells in a time- and dose-dependent manner while having a minimal effect on non-cancerous fibroblasts. After 6 hour of incubation, the cells began to show signs of apoptosis with membranes and microtubules disintegrating after 12 hour of incubation. The complex induced DNA damage, especially in higher concentrations (37).

Cervinka et al. tested [RuCl(κ3-tpm)$(PPh_3)_2$]Cl (**18**), where tpm is tris(pyrazolyl)methane, against MCF-7, HeLa, and HCT-116 cancer cell lines and discovered the complex localizes in membranes of organelles, such as ER and mitochondria, with up to 8% localizing within the nucleus. TMRE assay results indicate the complex affects mitochondrial membrane potential in a concentration-dependent manner and its IC_{50} values were lower compared to cisplatin (2.4 ± 0.6 μM, 4.0 ± 0.4 μM, 1.5 ± 0.1 μM, respectively). The complex also reduced the flux of Ca^{2+} ions in mitochondria followed by an influx in the cytosol (38).

[Ru([9]aneS_3)(en)Cl][PF_6] (**19**) complex, where (([9]aneS_3) is 1,4,7-trithiacyclononane and en is 1,2-diaminoethane) undergoes rapid Cl- ligand hydrolysis in aqueous solution giving rise to [Ru([9]aneS_3)(en)$(H_2O)]^{2+}$. 5′-GMP binds said compound first to the phosphate group within minutes before switching to its preferred target the N7 G within hours. The Ru center preferably binds to N7 of 9MeG (9-methylguanine) and similarly to free guanosine (39).

Precise location and timing of the metal complex activation is important in regard to its effectiveness and toxicity (19). While seemingly quite different, complexes of this group all induced apoptosis without ROS accumulation; however, complex (**18**) disrupted mitochondrial membrane potential, and complex (**15**) exhibited metastatic properties. All heteroleptic coordination complexes for which there was data on efficiency were more potent against chosen cancer cell lines when compared to reference cisplatin. Thus, the key may lie in coordinating ligands of varying innate chemical properties to the Ru metal core. This may explain the discrepancies between reported MoA within this group

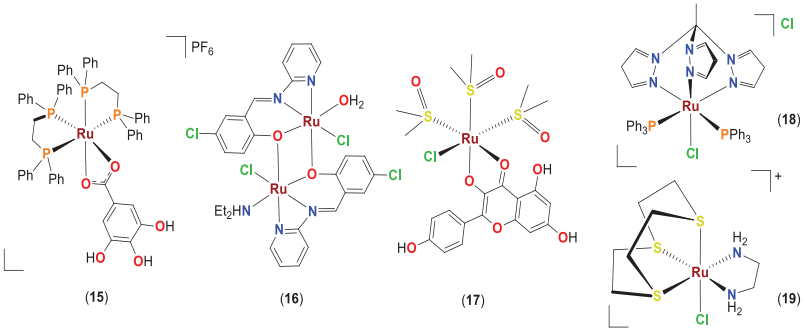

FIGURE 4. Heteroleptic ruthenium coordination complexes with anti-cancer activity.

– complex (**15**) induces apoptosis through the intrinsic pathway involving cas3/9 activation, while complex (**16**) forms DNA adduct.

Promising ruthenium organometallics

The structures of the three most promising ruthenium organometallics **RAED-C**, **RAPTA-C**, and **RM175** are available in Figure 5. Adhireksan et al., in their study from 2014 compared the properties and anti-proliferation capacity of [(η^6-*p*-cymene)Ru(ethylene-diamine)Cl] PF_6 or **RAED-C** and [(η^6-*p*-cymene)Ru(1,3,5-triaza-7-phosphaadamantane)Cl_2] or **RAPTA-C** against A2780 and A2780cis cisplatin-resistant ovarian cancer cell lines. After treatment with **RAED-C**, approximately 8% of the total intracellular ruthenium was associated with chromatin, and of the chromatin-bound adducts 71% associated with DNA, preferring binding to 5'-guanine NT atom of GG dinucleotide sites, likely due to electrostatic attraction. While the anti-proliferative effect of **RAED-C** against A2780 cancer cell line was weaker than the reference drug cisplatin (4.53 ± 0.93 vs. 1.00 ± 0.05), its IC_{50} values were lower in comparison against the cisplatin-resistant A2780cis cell line (6.8 ± 0.3 vs. 14.0 ± 0.3) and induced a faster S-phase cell cycle arrest and apoptosis. **RAPTA-C** tested on the same ovarian cancer cell lines had significantly higher IC_{50} values (247 ± 15 μM and 507 ± 38 μM, respectively) (40). **RM175** or [(η^6-biphenyl)RuII(ethylenediamine)Cl]$^+$ was less potent than its osmium counterpart AFAP51 against MDA-MB-231 and MCF-7 breast cancer cell lines (IC_{50} >60 μM for both cell lines). The complex had no statistically significant effect on the cancer cell line migration ability in a 3D matrix; however, it did exhibit strong inhibition of MMP-2 production and activity (41). While all three described Ru-organometallics induced apoptosis, they were less effective when compared to the reference drugs used in their respective studies, except for **RAED-C** tested against the cisplatin-resistant A2780 cancer cell line. Both **RAED-C** and **RAPTA-C** arrested the cell cycle at the S0/S1 phase; however, only **RAED-C** interacts with DNA, forming adducts as described. On the other hand, both **RAPTA-C** and **RM175** complexes have direct interaction with proteins, mainly histones and MMP-2, respectively.

Heteroleptic ruthenium organometallics with P – donor ligands

Engelbrecht et al. tested several Ru-based complexes against A375 melanoma cell line out of which the complex references as GA105 or [Ru(*p*-cymene)Cl_2]$_2$ (**20**) in dichloromethane with bis-amino-phosphine as the ligand was most potent with an IC_{50} value of 6.72 μM (± 2.02 μM) versus cisplatin with IC_{50} > 20 μM. The complex exhibits dose-dependent induction of apoptosis confirmed through light microscopy and starts exhibiting toxicity on non-cancer cell lines at concentrations >20 μM (42). A Ru(II)-cyclopentadienyl complex $RuCp(PPh_3)_2$(ATZ) BF_4 (**21**), where Cp is cyclopentadienyl and ATZ is anastrozole ligand, was found to be stable at physiological conditions and highly soluble in both water and culture media. The anastrozole ligand alone had IC_{50} values above 100 μM, while the complex had IC_{50} lower than cisplatin for all tested cell lines MCF-7 (0.5 ± 0.09 μM), T47D (0.32 ± 0.03 μM), MDA-MB-231 (0.39 ± 0.09 μM), and H295R (0.63 ± 0.05 μM). It is highly unlikely for the complex to bind to aromatase and it does not inhibit the enzyme activity, however, the free ligand is able to achieve this inhibition. For *in vivo* toxicity, a zebrafish embryo model was also used and showed no significant toxicity for tested concentrations in this study (43). A Ru-complex of the following structure [Ru(CCC-Nap)(Ibu)(PTA)] (**22**), where CCC-Nap is a CCC-pincer containing naproxen moiety and PTA is 1,3,5-triaza-7-phosphaadamantane, was tested for its anti-cancer potential against three cell lines: MCF-7, MDA-MB-231, and HT-29. The complex was deemed potent against chosen cancer cell lines (0.91 ± 0.02 μM and 1.32 ± 0.05 μM, respectively), although less effective than cisplatin for HT-29 cell line (35.82 ± 0.52 μM), while the free ligands were ineffective in all cases (IC_{50} >100 μM). When it comes to selectivity, Ru-complex selectivity was approximately 25 times higher than the reference drug. The complex is determined to be of hydrophobic nature adding to its high cellular uptake and to be a potent inhibitor of the COX2 enzyme isomer, as well as an enhancer of intracellular ROS production (44). The structures of these three heteroleptic organometallics with P-donor ligands are available in Figure 6. All three complexes were able to induce apoptosis and had lower IC_{50} values than reference cisplatin with the exception of complex (**22**) for HT-29 cell line. Unlike the other two, complex (**22**) reported ROS accumulation and COX inhibition, hinting at the possibility its main intracellular target(s) may be proteins instead of nucleic acids.

Ruthenium organometallics with N – donor ligands

Colina-Vegas et al. characterized 10 piano-stool Ru-complexes with chloroquine and chelating ligands and primarily tested their DNA and protein interactions against lung and breast cancer cell lines. The [Ru(η^6-$C_{10}H_{14}$)(dph-phen)Cl]PF_6 (**23**) or complex 2 in their study, where dph-phen is 4,7-diphenyl-1,10-phenanthroline, had a low DNA binding constant and no indication of intercalating ability,

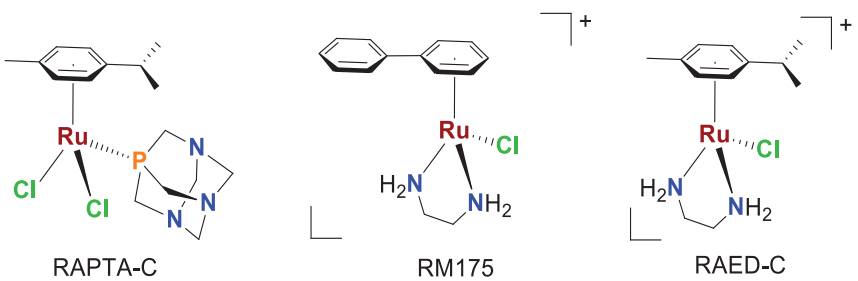

FIGURE 5. The most promising ruthenium organometallics with anti-cancer activity.

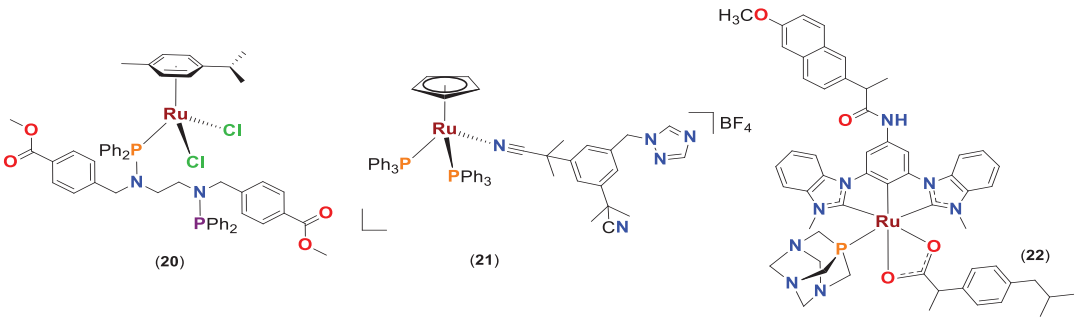

FIGURE 6. Heteroleptic anti-cancer ruthenium organometallics with P-donor ligands.

nor did the complex alter pBR322 plasmid DNA in any significant manner. On the other hand, the complex did exhibit moderate binding affinity toward BSA through static quenching where hydrogen bonding was suggested to play a major role in the process. On the other hand, complex 7 in the study or ($[Ru(\eta^6\text{-}C_{10}H_{14})(bipy)CQ](PF_6)_2$) (**24**) had the lowest IC_{50} values against chosen cancer cell lines compared to cisplatin, doxorubicin, free chloroquine, and other Ru-complexes (A549 0.95 ± 0.10 μM; MDA-MB-231 2.30 ± 1.64 μM; 0.80 ± 0.10 μM). The DNA binding constant for complex (**24**) was comparable to free chloroquine ligand but an increase in viscosity as a measure of intercalating potential indicated the complex's DNA intercalating properties, further attributed to the CQ ligand. It is proposed that the addition of CQ also attributed to an increase in oxidation potential, subsequently affecting hydrophobic association with BSA (45). These results provide insight into how the ligand's innate chemical properties affect anti-cancer potential when complexed with a metal ion core.

The complex OC-6-24-$[RuCl\{(Me_2N)_2CS\}(pp)(cod)](CF_3SO_3)]$ (**25**), where pp is 5,6-Me_2phen, induced apoptosis through a dose-dependent inhibition of mitochondrial respiration in MCF-7 cell line (IC_{50} 0.73 ± 0.34 μM). Concentrations of <5 μM with Annexin V and PI staining revealed an equal ratio of early/late apoptosis after 48 h incubation, while treatment with concentrations >10 μM leaned toward late-stage apoptosis with very low necrosis percentage which was tested on the Jurkat cell line (46).

Fuster et al. tested $[Ru(p\text{-cymene})Cl_2(\mu\text{-}(4ampy)]$, where 4ampy is 4-aminopyridine (**26**) or complex IV in the study against HeLa cell line. While it was the most potent complex in the study, it was three times less potent than cisplatin under the same conditions (IC_{50} 1.60 ± 0.004 mM) (47).

$[(\eta^6\text{-}p\text{-cymene})Ru(L)Cl](BF_4)$ (**27**) where L is 9-ethyl-3-(1-pyridin-2-yl-imidazo-[1,5-a]pyridin-3-yl)-9H-carbazole] strongly binds to CT DNA as shown by the EB displacement assay and a high binding affinity toward BSA determined by tryptophan quenching. The complex also showed a higher percentage of typical apoptotic cells under a fluorescence microscope, especially early apoptosis, and has a significantly lower IC_{50} value than cisplatin against A549 cell line (17.5 ± 0.5 vs. 71.0 ± 2.0 μM) (48).

Pavlović et al. tested $[(\eta^6\text{-}p\text{-cymene})Ru(L1)Cl]PF_6$ (**28**), where L1 is the ligand 2-amino-4-methylbenzamide against HCC1937, MDA-MB-231, and MCF-7 breast cancer cell lines. Concentration-dependent PARP-1 inhibition with IC_{50} 25.5 ± 0.2 μM attributed to its bidentate coordinated ligand and the free ligands L1 and L2 from the study shows low cell growth inhibition with this potential greatly increasing when coordinated to the ruthenium(II)-arene moieties. Varying sensitivity of the complex is attributed to the uniqueness of genotype and phenotype of cancer cell lines used in this study; however, its potency remained significantly lower when compared to cisplatin (IC_{50} values: 428.1 μM, 196.9 μM, 156.6 μM, respectively). Observed cell morphology changes confirmed $[(\eta^6\text{-}p\text{-cymene})Ru(L1)Cl]PF_6$ (**28**) has some level of anti-proliferative effect. Treatment with 200 μM after 72-hour incubation induced G2/M phase cell cycle arrest, while the free ligands had no such effect. When compared to other complexes from the study, this complex had the lowest penetration or intracellular content for the HCC1937 cells but the highest retention in organelle/membrane fraction and in the nucleus. Treatment with 200 μM did not affect the electrophoretic mobility of pHOT-1 plasmid DNA (49). Kacsir et al. (2021) screened 14 newly synthesized ruthenium half-sandwich complexes with bidentate monosaccharide ligands on the A2780 ovarian cancer cell line. Four out of the 14 complexes showed cytostatic properties; however, none were cytotoxic and their IC_{50} values were higher or comparable to conventional chemotherapeutics such as cisplatin, oxaliplatin, and carboplatin. The lowest IC_{50} value of 0.9 μM was for Ru-2a complex $[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2$-L (**29**), where the L ligand is 1-(2',3,4',6'-tetra-O-benzoyl-β-D-glucopyranosyl)-4-(pyridin-2-yl)-1,2,3-triazole. The A2780 cancer cell treated with the IC_{50} concentration of the complex had elevated levels of 4-hydroxy-nonenal, an oxidative stress marker but the mitochondria were not involved in the generation of ROS detected in the study (50). Vyas et al. in their study from 2021 proved a newly synthesized Ru-cymene type organometallic drug $[Ru_2(\eta^6\text{-}p\text{-cymene})_2(\mu\text{-L1})(\mu\text{-Cl})Cl_2]$ (**30**), where L1 is the 5-phenyl-2H-tetrazole ligand, was more effective against MCF-7, HepG2, and HCT116 cancer cell lines when compared to cisplatin. The compound also increased the production of ROS within the tested cell line, upregulating Cas3 and Cas9 while downregulating Bcl2. In addition to this, the treated cells showed signs of mitochondrial membrane disruption consistent with apoptosis and the compound exhibited metastatic activity by decreasing the wound healing ability (51). The chemical structures of the above-described Ru-organometallics featuring N-donor ligands are available in Figure 7. It is evident that various Ru-complexes in terms of structure and chemical properties have been synthesized and characterized in recent years. Depending on said properties, different mechanisms of action have been attributed to the anti-cancer

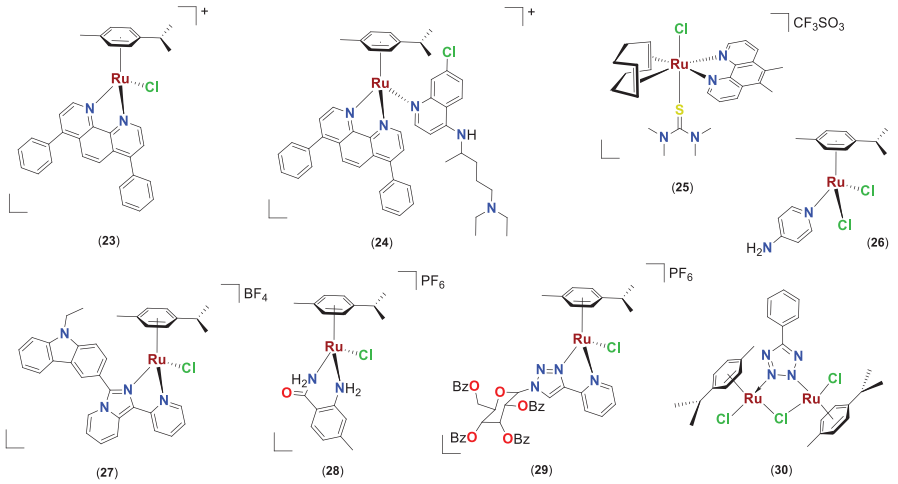

FIGURE 7. Anticancer ruthenium organometallics featuring N-donor ligands.

potential of researched complexes, adding to the elusiveness of any common mechanism of action or intracellular target. Complexes (**29-30**) reported ROS accumulation as the primary cellular effect with complex (**30**) exhibiting metastatic properties as well. The only complex within this group for which DNA interactions were reported was complex (**27**) with DNA intercalation listed as a MoA. Complexes (**28** and **30**) induced apoptosis through G2/M cell cycle arrest and cas3/9 activation, respectively. While overall Ru-organometallics featuring N-donor ligands appear to induce apoptosis intrinsically, the effectiveness of described complexes when compared to study reference drugs was equally distributed between "less potent" and "more potent" categories. Thus, there is no conclusive evidence for N-donor ligands increasing Ru-organometallics potency measured in IC_{50} values *in vitro*.

Ruthenium organometallics with monobasic O,O-donor ligands

Mandal et al. researched the anti-cancer properties of a Ru-cymene metallodrug with ibuprofen as the ligand [Ru(η^6-*p*-cymene)(ibu)Cl] (**31**). After 24 hour of incubation, GI values for all three cancer cell lines used in the experiment (A549, HeLa, MCF-7) were <0.1 nM/mL and were lower when compared to the reference drug adriamycin. [Ru(η^6-*p*-cymene)(ibu)Cl] (**31**) was able to displace EB from CT DNA confirming intercalating properties. In addition to this, the Ru-cymene complex type with ibuprofen significantly inhibited the activity of COX and 15-LOX enzymes and is able to bind to certain amino acid residues in the following preferential order: Histidine>methionine>cysteine which gradually replaces the ibuprofen ligand coordinated to the Ru(II) center (52). The Ru-cymene complex of the following structure: Chlorido{3-(oxo-κO)-2-(4-bromophenyl)-chromen-4-onato-κO}(η^6-*p*-cymene)ruthenium(II) (**32**) was able to inhibit CDK2, although in a minor way and also inhibited the catalytic activity of human topoisomerase Iiα. While there were no IC_{50} values provided for any reference drug used in the study, after 96-hour treatment with complex (**32**), MTT assay confirmed IC_{50} values <10 μM for all three cancer cell lines CH1, SW480, and A549 (1.2 ± 0.2 μM; 3.4 ± 0.1 μM; 8.6 ± 0.7 μM, respectively) (53). [Ru(O_2CNEt_2)Cl(*p*-cymene)] (**33**), one of the

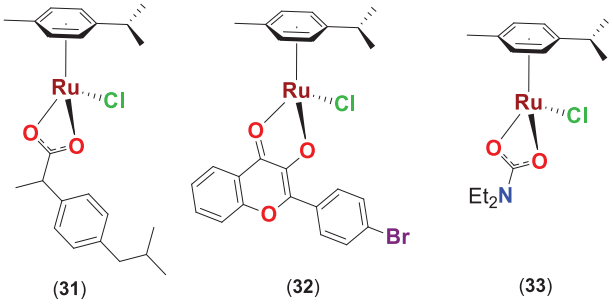

FIGURE 8. Ruthenium organometallics with monobasic O,O-donor ligands.

transitional metal-based complexes researched by Bresciani et al., had significantly higher IC_{50} values (>100 μM) than cisplatin against A549 and A2780 cell lines and thus was not researched further (54). The structure of the three described Ru organometallics with monobasic O,O-donor ligands is found in Figure 8. The MoA of complex (**33**) has not been reported and it was less effective than the reference drug in the study. However, both complexes (**31-32**) were reported to induce apoptosis by inhibiting key proteins in the cell, such as topoisomerase IIα and CDK2 (complex **32**), as well as interacting with important enzymes of metabolic pathways (15-lipoxygenase and cyclooxygenases) in the case of complex (**31**). In addition, complex (**31**) was reported to have DNA intercalating properties. It is worth exploring further if the presence of monobasic O,O-donor ligands in Ru-organometallics affects target choice.

Neutral ruthenium organometallics with bidentate monoanionic ligands

In our final group of Ru-complexes, we have four neutral organometallics with bidentate monoanionic ligands, whose chemical structures are represented in Figure 9. A Ru-organometallic [(η^6 -benzene)Ru(L3)Cl] (**34**), where L3 is (Z)-4-methoxy-N'-(2,3,4,9-tetrahydro-1Hcarbazol-1-ylidene)benzohydrazide, was tested against A549 and A2780 cancer cell lines and had 3-4 times more potency than cisplatin under same conditions (IC_{50} values of both were reported at 3 μM). The complex has high lipophilicity implying its transporting ability across the cell membrane. The fee ligand's IC_{50} was above 25 μM, while its cytotoxic effect on the non-cancerous 16HBE cell line was at 93 ± 0.8 μM. The complex [(η^6 -benzene)Ru(L3)Cl] (**34**)

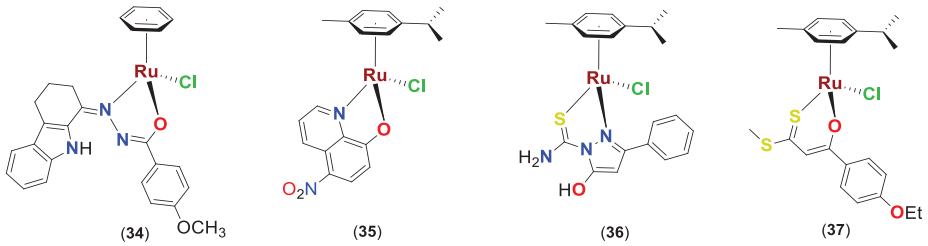

FIGURE 9. Neutral ruthenium organometallics with bidentate monoanionic ligands having anti-cancer activity.

had the highest DNA synthesis inhibition in the study, as confirmed by the EdU assay. Acridine orange-ethidium bromide dual staining analysis confirmed the complex induces apoptosis and the V-FITC/propidium iodide (PI) double staining with flow cytometry confirmed early apoptosis with cell cycle arrest at S phase (55). Kljun et al. synthesized and characterized 10 novel Ru(II)-*p*-cymene complexes with anti-proliferative potential and tested them against aggressive ovarian cancer cell lines COV362 and OVCAR-4. In the study, complex 9 or [(η^6-*p*-cymene)Ru(nitroxolinato)(Cl)]$^{2+}$ (**35**) had IC_{50} values several-fold higher than carboplatin but was deemed less potent than cisplatin against chosen cell lines (21.53 μM and 14.36 μM, respectively). In general, complexes which contained ligands nitroxoline and -Cl exhibited a stronger anti-proliferative effect than other complexes described in the study. Nitroxoline as a free ligand was up to four-fold more potent than when bound in complex (**35**) and two-fold more than cisplatin tested on COV362. The complex was proven to have anti-migratory potential as well, however, exhibited cytotoxicity after 48-hour incubation (56). Many studies report results on the anti-cancer potential measured in IC_{50} values not only for Ru-complexes but also their free ligands as well. It is common to see in published works that free ligands have a higher anti-cancer potential due to their unique innate biochemistry. Even so, most reports show that even if the free ligand is potent on its own, the anti-cancer potential is only amplified when coordinated to a Ru metal core. This indicates the continuance of research into the synthesis and characterization of metallodrugs varying in structure is needed for a better understanding of their anti-cancer properties. Namiecińska et al. 2019) tested [(η^6-p-cymene)Ru(1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1H-pyrazole)Cl_2 (**36**) against HL-60, NALM-6, and WM-115 cancer cell lines. The complex was cytotoxic against all chosen cancer cell lines, although with varying levels (IC_{50} values were 86.5 ± 8.02 μM, 11.71 ± 1.62 μM, 26.66 ± 3.28 μM, respectively), however, not more than reference cisplatin. The cytotoxic potential of the free ligand was researched as well, but it had no such anti-cancer effect. In addition, the complex did not affect the migration of the supercoiled pUC57 plasmid DNA (57).

In a study by Hildebrandt et al., a total of 17 different Ru-based and Os-based organometallic drugs have been tested for their anti-cancer potential. Among those, a complex with cinnamic acid derivatives as O, S bidentate ligands referenced in the study as Ru14 (**37**) proved more potent than the reference drug cisplatin (except for A2780 cell line); however, the osmium counterparts were more effective in general. While the IC_{50} values of Ru14 for SKOV3, SKOV3cis, A2780cis, and A549 were lower than for cisplatin (3.5 ± 2.0 μM, 5.1 ± 2.8 μM, 2.9 ± 0.8, and 2.7 ± 1.2, respectively), the complex had a minuscule effect on disrupting the cell cycle, therefore, a mechanism of action not involving DNA damage was proposed (58).

All four described neutral Ru organometallics with bidentate monoanionic ligands were able to induce apoptosis with complex (**35**) having metastatic potential. In addition, complex (**35**) was the only among groups for which protein interactions were reported. Complex (**34**) induced cell cycle arrest at S0/S1 phase and was more potent than the reference drug, as was complex (**37**) for all but A2780 cancer cell line from its respective study. Research into this group of Ru organometallics is lacking, especially concerning their intracellular target(s) and mechanism of apoptosis induction.

A RETROSPECTIVE ON RU-METALLODRUGS MECHANISMS OF ACTION AGAINST CANCER CELL LINES

While the research into various Ru-based metallodrugs has accelerated, providing a general mechanism of action for these compounds has proved to be challenging. This is due to the sheer possibility of combinations giving unique Ru-complex structures, either organometallics or coordinated complexes, of which multiple simultaneous mechanisms of action can be present. For the purpose of writing this review, we looked further into 40 published studies of novel Ru-based complexes testes against various cancer cell lines. Out of this set of data, approximately about 5% did not report researching the mechanism of action behind their Ru-complexes at all, while over 50% reported apoptosis in general to be the primary effect. Other most commonly reported mechanisms of action included protein binding and DNA adduct formation as the primary effects; activation of cas3/cas9, DNA intercalation, and cell cycle arrest at S0/S1 as the secondary effects; and a significant increase in ROS accumulation as the notable tertiary effect. Out of these 40 complexes referenced throughout this review, Ru-bipyridine/phenanthroline, heteroleptic organometallic with P-donor ligands, Ru-terpyridine complexes, and Ru organometallics with N-donor ligands equally reported ROS accumulation as the primary cellular effect observed. Besides reporting apoptosis, Ru-bipyridine/phenanthroline, neutral organometallics with bidentate monoanionic ligands, heteroleptic coordination complexes, and Ru organometallics with N-donor ligands exhibited anti-migratory properties. As for interactions with the DNA helix, between adduct formation and intercalation, heteroleptic coordination complexes, Ru organometallics, and Ru-terpyridine complexes were more likely to be reported as adduct forming complexes versus Ru-bipyridine/

phenanthroline, Ru organometallics with N-donor ligands, and other Ru-terpyridine complexes which had intercalating properties. Cell cycle arrest at one of the checkpoints is another common effect of Ru-based complexes. Out of all reported S0/S1 cell cycle arrests, complexes belonging to the Ru-organometallics group were responsible, for G2/M, this was equally distributed between Ru organometallics with N-donor ligands and Ru-terpyridine complexes, and for G0/G1 arrest, all belonged to the Ru-bipyridine/phenanthroline complexes group. Close to 2.5× more Ru-complexes reported inducing apoptosis than having any protein interactions in general. While it seems Ru-complexes are more likely to disrupt the cell cycle or interact with nucleic acids, it is possible for the same complex to have dual MoA, as was reported for complex (**7**). Thus, future studies of Ru-based complexes and their anti-cancer potential should, if possible, include assays for both nucleic acid and protein interactions to shine a light on this conundrum. Much like Pt-based compounds, Ru-based ones possess a similar mechanism of action and are considered to be prodrugs targeting guanine (N7) residues in DNA forming Ru-N7 from G coordination bond (39). Intercalating metal-based compounds either possess aromatic ligands attached to coordinatively saturated square-planar Pt(II) and Pd(II) complexes which play an important part in DNA disruption or the ligands are bonded to the complex whose aromatic side arms function as intercalators between the DNA base pair, while the metal coordinates to the DNA base directly. Pt(II) complexed with an intercalating ligand and an ancillary ligand is the typical active complex exhibiting anti-cancer properties. The advantages the positive charge of the complex brings are threefold: Improved solubility, selective uptake, and higher affinity to DNA. For both the DNA binding and cytotoxicity, the role of the ancillary ligand must not be dismissed (59). In general, complexes with a metal center are able to bind to DNA, either covalently or non-covalently. Covalent binding can be intra-strand or inter-strand, often including better-leaving groups in the ligand such as Cl- anion. DNA groove binding and intercalation are examples of non-covalent binding of metal complexes (35). As the number of published studies on Ru-complexes' mechanism of action increases, undoubtedly more information on the diverse effects of these complexes will be available to the scientific community. While there is indication that certain chemical structures influence particular mechanisms of action, overall it is not clear how ruthenium metallodrugs exert their anti-cancer effect. Another Ru(II)-complex property worth noting is stability and bioactivity in the target cell versus in the bloodstream when bound to serum proteins. It is important to design a Ru-based complex that is stable in both environments while being active once it is internalized in the target cells. In addition, targeting of Ru-based complexes is one of the most important properties in design as off-target toxicity, even with high anti-cancer potential, would prove disadvantageous in a clinical setting. The Ru mimicking mechanism for iron-containing compounds such as serum albumin and transferrin suggests that Ru-based complexes do not have just the DNA as their target for exhibiting anti-cancer effects. Drug efficiency *in vivo* is influenced by the degree to which it is able to bind blood plasma proteins (25). Evidence shows that ruthenium complexes could inhibit thioredoxin reductase (TrxR), indicating that the anticancer action of the inert Ru-complexes results from redirecting TrxR (60). A study by Casini et al. on a group of ruthenium (II)-arene complexes (RAPTA) reveals that inhibiting thioredoxin reductases was significantly less potent than a cathepsin B inhibitor. However, specific metallodrugs were far better inhibitors of both enzyme classes compared to other metal-based medications (61). Moreover, another study on Ru (II) salicylate complexes by Zhao et al. suggested that these complexes could progress into effective TrxR targeting agents. Thus, cancer therapy such complexes can have many different applications (32).

Besides classical compounds, other complexes such as cyclometalated compounds (RCD family) and iminophosphorane ruthenium(II) compounds have been developed and characterized (61,62).

CURRENT RESEARCH AND CLINICAL TRIALS WITH RU-COMPLEXES

The following compounds NAMI-A, KP1019, KP1339, and TLD1433 have been tested in clinical trials (chemical structures available in Figure 10). **NAMI-A** is the first ruthenium-based drug that entered clinical trials as a potential chemotherapeutic, but the clinical trial has been terminated. Novel Anti-tumor Metastasis Inhibitor A (NAMI-A) has the following chemical formula ($C_8H_{15}Cl_4N_4ORu(S)$ or imidazolium-trans-tetrachloro(dimethylsulfoxide) imidazole ruthenium(III) was reported to limit actin-dependent cell motility and adhesion *in vitro*, regulate TGFβ1 expression in turn having anti-invasive potential, and having anti-angiogenic potential. While it was in clinical phase I, NAMI-A was administered at 12 different doses for 5 consecutive days every 3 weeks with the advised dose for further testing being 300 mg/m^2/day. Adverse effects from the treatment involved the GI tract and mild hematological toxicity, phlebitis was reported for non-port-a-cath administration, and the dose-limiting factor was blister

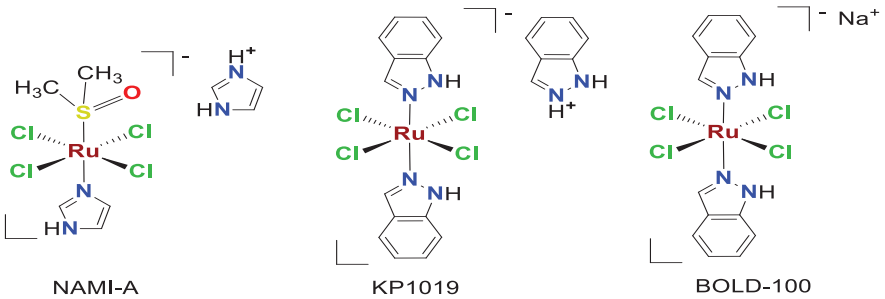

FIGURE 10. The most promising anti-cancer ruthenium coordination complexes.

formation. When it was in Phase I/II, it involved a combination with gemcitabine for 31 NSCLC patients, where Phase I was a non-randomized, dose escalation study of 300 mg/m^2 of NAMI-A at day 1, day 8, and day 15 plus 1000 mg/m^2 of gemcitabine at days 2, 9, and 16. Phase II had the dose escalation to 450 mg/m^2 of NAMI-A with the same dose of gemcitabine in a 21-day schedule for 15 eligible patients. The most commonly noted adverse effects were neutropenia and anemia reported at higher NAMI-A doses, vomiting/nausea, diarrhea/constipation, elevated liver enzymes, transiently creatinine, and fatigue. Overall, anti-tumor activity was noted for 56% of enrolled patients (5). It has been suggested that the prevention of HCT116 cell metastasis by NAMI-A might include the reduction of integrin $\alpha_5\beta_1$. Preclinical testing of Ru-based drugs was performed on mice, where NAMI-A successfully slowed growth and development of metastases in the lungs and brain, increasing the survival of treated mice. Moreover, NAMI-A showed accuracy in targeting metastases without influencing the growth of the primary tumor. Being directly injected into a primary tumor, it resulted in a small growth reduction, when compared to the effect on metastases of the lungs, with no connection to pharmacokinetics. Compared to NAMI-A, **KP1019** showed significant activity, resulting in 95% tumor reduction in platinum-resistant colorectal carcinoma model rats. More than 50 primary human tumors *in vitro* testing showed a positive outcome rate surpassing 70% (63). KP1019 and KP1339 are salt variants of the same ruthenium complex that has an impact on primary tumors due to their inability to spread. In addition, as a sodium salt form, **KP1339** has a higher water solubility than K1019. Clinical trials revealed that KP1339 and KP1019 both have an effect on GRP78 protein, whose function is crucial for protein misfolding and tumor survival. KP1339 exhibits therapeutic efficacy against solid tumors by inhibiting the GRP78 protein, and it is well-tolerated with manageable side effects, while KP1019 reduces levels of the same protein. On the other hand, **TLD1433** has entered Phase I and Phase IIa as a treatment for bladder cancer using photodynamic therapy, including different approaches from combination therapy to monotherapy (5). **BOLD-100** exhibits the affinity for the blood proteins albumins and transferins. The interaction and activity of the BOLD-100 are mediated due to the reduction from Ru (II) to Ru (I). All of these processes and interaction between the BOLD-100 and molecules result in the activation of the apoptosis or inhibition of the DNA machinery and induction of the ROS (in breast cancer). The BOLD-100 showed great results in the first Phase I clinical trial, due to these results, the BOLD-100 is currently in Ib/IIa dose–escalation study with FOLFOX as chemotherapy treatment for solid tumors (64). In the clinical phase Ib, the BOLD-100 is tested against colorectal, pancreatic, and gastric cancers, as well as cholangiocarcinoma (NCT04421820) (65). Besides these cancer types, the BOLD-100 has shown great potential and efficiency in the preclinical models against breast, lung, and liver cancer (66). RAPTA is another Ru(II) complex that causes detachment from the primary tumor cell mass and spreads while activating mitochondrial apoptosis. **RM175 and ONCO4417** both influence apoptosis in the G2/M phase. Cisplatin and ONCO4417 cause similar DNA damage (67). To further improve the

TABLE 1. Clinical trials for ruthenium drugs (information taken from clinicaltrials.gov and trial search.who.int)

Study title	NCT Number	Status	Conditions	Treatments	Enrollment	Study start	Study completion	Study type	Phase
BOLD-100 in combination with FOLFOX for treatment of advanced solid tumors	NCT04421820	ACTIVE/NOT RECRUITING	Colorectal cancer Pancreatic cancer Gastric cancer Cholangiocarcinoma	1. BOLD-100 in combination with FOLFOX Chemotherapy (dose expansion) 2. BOLD-100 in combination with FOLFOX chemotherapy (dose escalation)	117	2020	2024 (Estimated)	Interventional	Phase 1 Phase 2
Dose Escalation Study of NKP-1339 to treat advanced solid tumors	NCT01415297	COMPLETED	Solid tumors	NKP-1339	46	2009	2016	Interventional	Phase 1
Intravesical PDT in BCG refractory high-risk NMIBC patients	NCT03053635	COMPLETED	NMIBC Refractory to BCG	TLD1433 infusion and PDT treatment	6	2016	2018	Interventional	Phase 1I
A Phase I/II study with NAMI-A a novel ruthenium anticancer agent, and gemcitabine combination second-line therapy in NSCLC patients	NL-OMON31433	RECRUITING	Advanced NSCLC	NAMI-A and Gemcitabine	45	2008	/	Interventional	Phase 1 Phase 2
NAMI-A and KP1019/1339, two iconic ruthenium anticancer drug candidates face-to-face: A case story in medicinal inorganic chemistry*	/	COMPLETED	Advanced solid tumors	KP-1019	34	2006	/	/	Phase 1

PDT: Photodynamic therapy, NMIBC: Non-muscle invasive bladder cancer, NAMI-a: Novel anti-tumor metastasis inhibitor A. *Information found in article (62)

effectiveness of ruthenium complexes, research should be focused on understanding the primary molecular mechanisms and the function of metal-drug delivery systems. Thorough analysis is essential to identify specific cells that are targeted by ruthenium metallodrugs and to understand how their interactions with these cells affect their structure, function, and recognition by other components within the cell (68-70). Table 1 offers information on clinical trials of BOLD-100, KP-1333, TLD1433, NAMI-A, and KP1019.

CONCLUSION

In recent years, ruthenium complexes have gained significant interest for their potential as cancer drugs. These complexes exhibit enhanced cancer cell targeting capabilities while reducing toxicity toward healthy cells, making them potentially safer and more effective treatment options. Notably, ruthenium-based complexes such as NAMI-A, KP1019, NKP1339, and TLD1433 underwent clinical trials, showing a considerable inhibitory effect on platinum-resistant tumors, demonstrating more powerful actions than platinum drugs. In this review, we provide an overview of ruthenium-based anti-cancer complexes. The extensive research on these compounds shows great promise as potential anti-cancer agents due to their unique chemical properties and ability to target cancer cells with high precision. In the future, research should focus on minimizing the drug's negative side effects while simultaneously addressing drug resistance, which could significantly enhance the therapeutic advantage of ruthenium complexes. In addition, efforts to enhance the effectiveness of these drugs in the diagnosis and treatment of cancer could lead to their widespread usage in clinical settings, offering new hope for patients with various forms of cancer.

REFERENCES

1. Hoeben A, Joosten EA, Van den Beuken-Van Everdingen MH. Personalized medicine: Recent progress in cancer therapy. Cancers (Basel) 2021;13(2):242.
   https://doi.org/10.3390/cancers13020242
2. Kryczka J, Kryczka J, Czarnecka-Chrebelska KH, Brzeziańska-Lasota E. Molecular mechanisms of chemoresistance induced by Cisplatin in NSCLC cancer therapy. Int J Mol Sci 2021;22(16):8885.
   https://doi.org/10.3390/ijms22168885
3. Lugones Y, Loren P, Salazar LA. Cisplatin resistance: Genetic and epigenetic factors involved. Biomolecules 2022;12(10):1365.
   https://doi.org/10.3390/biom12101365
4. Leijen S, Burgers SA, Baas P, Pluim D, Tibben M, Van Werkhoven E, et al. Phase I/II study with ruthenium compound NAMI-A and gemcitabine in patients with non-small cell lung cancer after first line therapy. Invest New Drugs 2014;33(1):201-14.
   https://doi.org/10.1007/s10637-014-0179-1
5. Lee SY, Kim CY, Nam TG. Ruthenium complexes as anticancer agents: A brief history and perspectives. Drug Des Devel Ther 2020;14:5375-92.
   https://doi.org/10.2147/DDDT.S275007
6. Aldossary SA. Review on pharmacology of cisplatin: Clinical use, toxicity and mechanism of resistance of cisplatin. Biomed Pharmacol J 2019;12:7-15.
7. Minerva N, Bhat A, Verma S, Chander G, Jamwal RS, Sharma B, et al. Cisplatin-based combination therapy for cancer. J Cancer Res Ther 2023;19(3):530-6.
   https://doi.org/10.4103/jcrt.jcrt_792_22
8. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int J Mol Sci 2022;23:1532.
   https://doi.org/10.3390/ijms23031532
9. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol 2021;13:303-28.
   https://doi.org/10.2147/JEP.S267383
10. Basu A, Krishnamurthy S. Cellular responses to cisplatin-induced DNA damage. J Nucleic Acids 2010;2010:201367.
    https://doi.org/10.4061/2010/201367
11. Sharma B, Shukla S, Rattan R, Fatima M, Goel M, Bhat M, et al. Antimicrobial agents based on metal complexes: Present situation and future prospects. Int J Biomater 2022;2022:6819080.
    https://doi.org/10.1155/2022/6819080
12. Boros E, Dyson P, Gasser G. Classification of metal-based drugs according to their mechanisms of action. Chem 2020;6(1):41-60.
    https://doi.org/10.1016/j.chempr.2019.10.013
13. Wang L, Zhao X, Fu J, Xu W, Yuan J. The role of tumour metabolism in cisplatin resistance. Front Mol Biosci 2021;8:691795.
    https://doi.org/10.3389/fmolb.2021.691795
14. Zhang CL, Huang T, Wu BL, He WX, Liu D. Stem cells in cancer therapy: Opportunities and challenges. Oncotarget 2017;8(43):75756-66.
    https://doi.org/10.18632/oncotarget.20798
15. Amjad MT, Chidharla A, Kasi A. Cancer Chemotherapy. StatPearls; 2023. [cited 2024 Apr 3]; Available from: https://www.ncbi.nlm.nih.gov/books/nbk564367
16. Nirmala MJ, Kizhuveetil U, Johnson A, Balaji G, Nagarajan R, Muthuvijayan V. Cancer nanomedicine: A review of nano-therapeutics and challenges ahead. RSC Adv 2023;13(13):8606-29.
    https://doi.org/10.1039/d2ra07863e
17. Lucaciu RL, Hangan AC, Sevastre B, Oprean LS. Metallo-drugs in cancer therapy: Past, present and future. Molecules 2022;27(19):6485.
    https://doi.org/10.3390/molecules27196485
18. Kahrović E, Zahirović A, Pavelić SK, Turkušić E, Harej A. *In vitro* anticancer activity of binuclear Ru(II) complexes with Schiff bases derived from 5-substituted salicylaldehyde and 2-aminopyridine with notably low IC50 values. J Coord Chem 2017;70(10):1683-97.
    https://doi.org/10.1080/00958972.2017.1308503
19. Gianferrara T, Bratsos I, Alessio E. A categorization of metal anticancer compounds based on their mode of action. Dalton Trans 2009;37:7588.
    https://doi.org/10.1039/b905798f
20. Zahirović A, Kahrović E, Cindrić M, Pavelić SK, Hukić M, Harej A, et al. Heteroleptic ruthenium bioflavonoid complexes: From synthesis to *in vitro* biological activity. J Coord Chem 2017;70(24):4030-53.
    https://doi.org/10.1080/00958972.2017.1409893
21. Osmanković I, Turkušić E, Zahirović A, Kralj M, Uzelac L, Kahrović E. CT DNA, BSA and antiproliferative activity of Ru(II) bipyridine complexes containing schiff bases derived from amino acids. Croat Chem Acta 2022;94(3):149-158.
    https://doi.org/10.5562/cca3872
22. Han BJ, Jiang GB, Yao JH, Li W, Wang J, Huang HL, et al. DNA interaction, antioxidant activity, and bioactivity studies of two ruthenium(II) complexes. Spectrochim Acta A Mol Biomol Spectrosc 2015;135:840-9.
    https://doi.org/10.1016/j.saa.2014.07.075
23. Li L, Wong YS, Chen T, Fan C, Zheng W. Ruthenium complexes containing bis-benzimidazole derivatives as a new class of apoptosis inducers. Dalton Trans 2012;41:1138-41.
    https://doi.org/10.1039/c1dt11950h
24. Lovison D, Allegri L, Baldan F, Ballico M, Damante G, Jandl C, et al. Cationic carboxylate and thioacetate ruthenium (ii) complexes: Synthesis and cytotoxic activity against anaplastic thyroid cancer cells. Dalton Trans 2020;49(24):8375-88.
    https://doi.org/10.1039/d0dt01390k
25. Lazić D, Arsenijević A, Puchta R, Bugarčić ŽD, Rilak A. DNA binding properties, histidine interaction and cytotoxicity studies of water soluble ruthenium(ii) terpyridine complexes. Dalton Trans 2016;45(11):4633-46.
    https://doi.org/10.1039/c5dt04132e
26. Savic M, Arsenijevic A, Milovanovic J, Stojanovic B, Stankovic V, Simovic AR, et al. Antitumor activity of ruthenium(II) terpyridine complexes towards colon cancer cells *in vitro* and *in vivo*. Molecules 2020;25(20):4699.
    https://doi.org/10.3390/molecules25204699
27. Hahn EM, Estrada-Ortiz N, Han J, Ferreira VF, Kapp TG, Correia JD, et al. Functionalization of ruthenium(II) terpyridine complexes with cyclic RGD peptides to target integrin receptors in cancer cells. Eur J Inorg Chem 2016;2017(12):1667-72.
    https://doi.org/10.1002/ejic.201601094
28. Deng Z, Gao P, Yu L, Ma B, You Y, Chan L, et al. Ruthenium complexes with phenylterpyridine derivatives target cell membrane and trigger death receptors-mediated apoptosis in cancer cells. Biomaterials 2017;129:111-26.
    https://doi.org/10.1016/j.biomaterials.2017.03.017
29. Purushothaman B, Arumugam P, Ju H, Kulsi G, Samson AA, Song JM. Novel ruthenium(II) triazine complex $[Ru(bdpta)(tpy)]^{2+}$ co-targeting drug resistant GRP78 and subcellular organelles in cancer stem cells. Eur J Med Chem 2018;156:747-59.
    https://doi.org/10.1016/j.ejmech.2018.07.048

30. Villota H, Pizarro S, Gajardo F, Delgadillo Á, Cortés-Mancera F, Bernal G. Ruthenium complex induce cell death in G-415 gallbladder cancer cells. J Gastrointest Cancer 2019;51(2):571-8.

https://doi.org/10.1007/s12029-019-00278-x

31. Gorle AK, Ammit AJ, Wallace L, Keene FR, Collins JG. Multinuclear ruthenium (ii) complexes as anticancer agents. N J Chem 2014;38(9):4049-59.

https://doi.org/10.1039/c4nj00545g

32. Zhao Z, Zhang X, Li CE, Chen T. Designing luminescent ruthenium prodrug for precise cancer therapy and rapid clinical diagnosis. Biomaterials 2019;192:579-89.

https://doi.org/10.1016/j.biomaterials.2018.12.002

33. Naves MA, Graminha AE, Vegas LC, Luna-Dulcey L, Honorato J, Menezes AC, et al. Transport of the ruthenium complex [Ru(GA)(dppe)2]PF6 into triple-negative breast cancer cells is facilitated by transferrin receptors. Mol Pharm 2019;16(3):1167-83.

https://doi.org/10.1021/acs.molpharmaceut.8b01154

34. Kahrović E, Zahirović A, Turkušić E, Bektaš S. A dinuclear ruthenium (II) schiff base complex with dissimilar coordination: Synthesis, characterization, and biological activity. Z Anorg Allg Chem 2016;642(6):480-5.

https://doi.org/10.1002/zaac.201600008

35. Kahrovic NE, Zahirovic NA, Turkusic NE. Calf thymus DNA intercalation by anionic Ru (III) complexes containing tridentate schiff bases derived from 5-x-substituted salicyladehyde and 2-aminophenol. J Chem Chem Eng 2014;8(4):335-43.

https://doi.org/10.17265/1934-7375/2014.04.002

36. Devagi G, Dallemer F, Kalaivani P, Prabhakaran R. Organometallic ruthenium (II) complexes containing NS donor schiff bases: Synthesis, structure, electrochemistry, DNA/BSA binding, DNA cleavage, radical scavenging and antibacterial activities. J Organomet Chem 2018;854:1-14.

https://doi.org/10.1016/j.jorganchem.2017.10.036

37. Thangavel P, Viswanath B, Kim S. Synthesis and characterization of kaempferol-based ruthenium (II) complex: A facile approach for superior anticancer application. Mater Sci Eng C Mater Biol Appl 2018;89:87-94.

https://doi.org/10.1016/j.msec.2018.03.020

38. Cervinka J, Gobbo A, Biancalana L, Markova L, Novohradsky V, Guelfi M, et al. Ruthenium (II)-Tris-pyrazolylmethane complexes inhibit cancer cell growth by disrupting mitochondrial calcium homeostasis. J Med Chem 2022;65(15):10567-87.

https://doi.org/10.1021/acs.jmedchem.2c00722

39. Rilak A, Bratsos I, Zangrando E, Kljun J, Turel I, Bugarčić ŽD, et al. Factors that influence the antiproliferative activity of half sandwich RuII-[9]aneS3 coordination compounds: Activation kinetics and interaction with guanine derivatives. Dalton Trans 2012;41(38):11608-18.

https://doi.org/10.1039/c2dt31225e

40. Adhireksan Z, Davey GE, Campomanes P, Groessl M, Clavel CM, Yu H, et al. Ligand substitutions between ruthenium-cymene compounds can control protein versus DNA targeting and anticancer activity. Nat Commun 2014;5(1):3462.

https://doi.org/10.1038/ncomms4462

41. Bergamo A, Masi A, Peacock AF, Habtemariam A, Sadler PJ, Sava G. *In vivo* tumour and metastasis reduction and *in vitro* effects on invasion assays of the ruthenium RM175 and osmium AFAP51 organometallics in the mammary cancer model. J Inorg Biochem 2010;104(1):79-86.

https://doi.org/10.1016/j.jinorgbio.2009.10.005

42. Engelbrecht Z, Roberts KE, Hussan A, Amenuvor G, Cronjé MJ, Darkwa J, et al. Synthesis and anti-cancer activity of bis-amino-phosphine ligand and its ruthenium (II) complexes. Bioorg Med Chem Lett 2020;30(20):127492.

https://doi.org/10.1016/j.bmcl.2020.127492

43. Golbaghi G, Pitard I, Lucas M, Haghdoost MM, De Los Santos YL, Doucet N, et al. Synthesis and biological assessment of a ruthenium (II) cyclopentadienyl complex in breast cancer cells and on the development of zebrafish embryos. Eur J Med Chem 2020;188:112030.

https://doi.org/10.1016/j.ejmech.2019.112030

44. Tabrizi L, Olasunkanmi LO, Fadare OA. Experimental and theoretical investigations of cyclometalated ruthenium (ii) complex containing CCC-pincer and anti-inflammatory drugs as ligands: Synthesis, characterization, inhibition of cyclooxygenase and *in vitro* cytotoxicity activities in various cancer cell lines. Dalton Trans 2019;48(2):728-40.

https://doi.org/10.1039/c8dt03266a

45. Colina-Vegas L, Villarreal W, Navarro M, De Oliveira CR, Graminha AE, Da S Maia PI, et al. Cytotoxicity of Ru(II) piano-stool complexes with chloroquine and chelating ligands against breast and lung tumor cells: Interactions with DNA and BSA. J Inorg Biochem 2015;153:150-61.

https://doi.org/10.1016/j.jinorgbio.2015.07.016

46. Kasper C, Alborzinia H, Can S, Kitanovic I, Meyer A, Geldmacher Y, et al. Synthesis and cellular impact of diene-ruthenium (II) complexes: A new class of organoruthenium anticancer agents. J Inorg Biochem 2012;106(1):126-33.

https://doi.org/10.1016/j.jinorgbio.2011.08.027

47. Fuster MG, Moulefera I, Montalbán MG, Pérez J, Víllora G, García G. Synthesis and characterization of new ruthenium (II) complexes of stoichiometry [Ru(p-Cymene)Cl_2L] and their cytotoxicity against HeLa-Type cancer cells. Molecules 2022;27(21):7264.

https://doi.org/10.3390/molecules27217264

48. Khamrang T, Kartikeyan R, Velusamy M, Rajendiran V, Dhivya R, Perumalsamy B, et al. Synthesis, structures, and DNA and protein binding of ruthenium(ii)-p-cymene complexes of substituted pyridylimidazo [1,5-a] pyridine: Enhanced cytotoxicity of complexes of ligands appended with a carbazole moiety. RSC Adv 2016;6(115):114143-58.

https://doi.org/10.1039/c6ra23663d

49. Pavlović M, Tadić A, Gligorijević N, Poljarević J, Petrović T, Dojčinović B, et al. Synthesis, chemical characterization, PARP inhibition, DNA binding and cellular uptake of novel ruthenium(II)-arene complexes bearing benzamide derivatives in human breast cancer cells. J Inorg Biochem 2020;210:111155.

https://doi.org/10.1016/j.jinorgbio.2020.111155

50. Kacsir I, Sipos A, Ujlaki G, Buglyó P, Somsák L, Bai P, et al. Ruthenium half-sandwich type complexes with bidentate monosaccharide ligands show antineoplastic activity in ovarian cancer cell models through reactive oxygen species production. Int J Mol Sci 2021;22(19):10454.

https://doi.org/10.3390/ijms221910454

51. Vyas KM, Sharma D, Magani SK, Mobin SM, Mukhopadhyay S. *In vitro* evaluation of cytotoxicity and antimetastatic properties of novel arene ruthenium(II)-tetrazolato compounds on human cancer cell lines. Appl Organomet Chem 2021;35(5):e6187.

https://doi.org/10.1002/aoc.6187

52. Mandal P, Kundu BK, Vyas K, Sabu V, Helen A, Dhankhar SS, et al. Ruthenium (ii) arene NSAID complexes: Inhibition of cyclooxygenase and antiproliferative activity against cancer cell lines. Dalton Trans 2018;47(2):517-27.

https://doi.org/10.1039/c7dt03637j

53. Kurzwernhart A, Kandioller W, Bächler S, Bartel C, Martic S, Buczkowska M, et al. Structure-activity relationships of targeted ruii (η6-p-cymene) anticancer complexes with flavonol-derived ligands. J Med Chem 2012;55(23):10512-22.

https://doi.org/10.1021/jm301376a

54. Bresciani G, Busto N, Ceccherini V, Bortoluzzi M, Pampaloni G, Garcia B, et al. Screening the biological properties of transition metal carbamates reveals gold (I) and silver (I) complexes as potent cytotoxic and antimicrobial agents. J Inorg Biochem 2022;227:111667.

https://doi.org/10.1016/j.jinorgbio.2021.111667

55. Kamatchi TS, Subarkhan MK, Ramesh R, Wang H, Małecki JG. Investigation into antiproliferative activity and apoptosis mechanism of new arene Ru (ii) carbazole-based hydrazone complexes. Dalton Trans 2020;49(32):11385-95.

https://doi.org/10.1039/d0dt01476a

56. Kljun J, Pavlič R, Hafner E, Lipec T, Silva SM, Tič P, et al. Ruthenium complexes show potent inhibition of AKR1C1, AKR1C2, and AKR1C3 enzymes and anti-proliferative action against chemoresistant ovarian cancer cell line. Front Pharmacol 2022;13:920379.

https://doi.org/10.3389/fphar.2022.920379

57. Namiecińska E, Sadowska B, Więckowska-Szakiel M, Dołęga A, Pasternak B, Grazul M, et al. Anticancer and antimicrobial properties of novel η6-p-cymene ruthenium (ii) complexes containing a N,S-type ligand, their structural and theoretical characterization. RSC Adv 2019;9(66):38629-45.

https://doi.org/10.1039/c9ra08736b

58. Hildebrandt J, Häfner N, Kritsch D, Görls H, Dürst M, Runnebaum IB, et al. Highly cytotoxic osmium (II) compounds and their ruthenium (II) analogues targeting ovarian carcinoma cell lines and evading cisplatin resistance mechanisms. Int J Mol Sci 2022;23(9):4976.

https://doi.org/10.3390/ijms23094976

59. Lazarević T, Rilak A, Bugarčić ŽD. Platinum, palladium, gold and ruthenium complexes as anticancer agents: Current clinical uses, cytotoxicity studies and future perspectives. Eur J Med Chem 2017;142:8-31.

https://doi.org/10.1016/j.ejmech.2017.04.007

60. Prast-Nielsen S, Cebula M, Pader I, Arnér ES. Noble metal targeting of thioredoxin reductase--covalent complexes with thioredoxin and thioredoxin-related protein of 14 kDa triggered by cisplatin. Free Radic Biol Med 2010;49(11):1765-78.

https://doi.org/10.1016/j.freeradbiomed.2010.09.008

61. Frik M, Martínez A, Elie BT, Gonzalo O, Ramírez de Mingo D, Sanaú M, et al. *In vitro* and *in vivo* evaluation of water-soluble iminophosphorane ruthenium(II) compounds. A potential chemotherapeutic agent for triple negative breast cancer. J Med Chem 2014;57(23):9995-10012.

https://doi.org/10.1021/jm5012337

62. Meng X, Leyva ML, Jenny M, Gross I, Benosman S, Fricker B, et al. A ruthenium-containing organometallic compound reduces tumor growth through induction of the endoplasmic reticulum stress gene CHOP. Cancer Res 2009;69(13):5458-66.

https://doi.org/10.1158/0008-5472.CAN-08-4408

63. Alessio E, Messori L. NAMI-A and KP1019/1339, two iconic ruthenium anticancer drug candidates face-to-face: A case story in medicinal inorganic chemistry. Molecules 2019;24(10):1995.

https://doi.org/10.3390/molecules24101995

64. Happl B, Brandt M, Balber T, Benčurová K, Talip Z, Voegele A, et al. Synthesis

and Preclinical evaluation of radiolabeled [^{103}RU] BOLD-100. Pharmaceutics 2023;15(11):2626.

https://doi.org/10.3390/pharmaceutics15112626

65. Baier D, Schoenhacker-Alte B, Rusz M, Pirker C, Mohr T, Mendrina T, et al. The anticancer ruthenium compound BOLD-100 targets glycolysis and generates a metabolic vulnerability towards glucose deprivation. Pharmaceutics 2022;14(2):238.

https://doi.org/10.3390/pharmaceutics14020238

66. Park BJ, Raha P, Pankovich J, Bazett M. Utilization of cancer cell line screening to elucidate the anticancer activity and biological pathways related to the ruthenium-based therapeutic BOLD-100. Cancers (Basel) 2022;15(1):28.

https://doi.org/10.3390/cancers15010028

67. Sahu G, Patra SA, Lima S, Das S, Görls H, Plass W, et al. Ruthenium (II)-dithiocarbazates as anticancer agents: Synthesis, solution behavior, and mitochondria-targeted apoptotic cell death. Chemistry 2023;29(18):e202202694.

https://doi.org/10.1002/chem.202202694

68. Singh A, Barman P. Recent advances in schiff base ruthenium metal complexes: Synthesis and applications. Top Curr Chem (Cham) 2021;379(4):29.

https://doi.org/10.1007/s41061-021-00342-w

69. Rodríguez J, Mosquera J, Couceiro JR, Vázquez ME, Mascareñas JL. Ruthenation of non-stacked guanines in DNA G-quadruplex structures: Enhancement of c-MYC expression. Angew Chem Int Ed Engl 2016;55(50):15615-8.

https://doi.org/10.1002/anie.201607965

70. Alcalá S, Villarino L, Ruiz-Cañas L, Couceiro JR, Martínez-Calvo M, Palencia-Campos A, et al. Targeting cancer stem cell OXPHOS with tailored ruthenium complexes as a new anti-cancer strategy. J Exp Clin Cancer Res 2024;43(1):33.

https://doi.org/10.1186/s13046-023-02931-7