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REVIEW

Ruthenium Complexes as Anticancer Agents: A Brief History and Perspectives

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Abstract: Platinum (Pt)-based anticancer drugs such as cisplatin have been used to treat various cancers. However, they have some limitations including poor selectivity and toxicity towards normal cells and increasing chemoresistance. Therefore, there is a need for novel metallo-anticancers, which has not been met for decades. Since the initial introduction of ruthenium (Ru) polypyridyl complex, a number of attempts at structural evolution have been conducted to improve efficacy. Among them, half-sandwich Ru-arene complexes have been the most prominent as an anticancer platform. Such complexes have clearly shown superior anticancer profiles such as increased selectivity toward cancer cells and ameliorating toxicity against normal cells compared to existing Pt-based anticancers. Currently, several Ru complexes are under human clinical trials. For improvement in selectivity and toxicity associated with chemotherapy, Ru complexes as photodynamic therapy (PDT), and photoactivated chemotherapy (PACT), which can selectively activate prodrug moieties in a specific region, have also been investigated. With all these studies on these interesting entities, new metallo-anticancer drugs to at least partially replace existing Pt-based anticancers are anticipated. This review covers a brief description of Ru-based anticancer complexes and perspectives.

Keywords: metallo-anticancer, ruthenium, photodynamic therapy, photoactivated chemotherapy

Introduction

Transition metal complexes consisting of organic ligands bound to the center metal have played an important role in terms of their applications related to human civilization.[1,2] Among them, ruthenium (Ru) complexes have received attention in many aspects.[3,4] Positioned in the center of the second row of the transition metal series, Ru shows both early and late transition metal properties.[5] Due to its Lewis acidic but less oxophilic nature, the element displays a distinct array of properties utilized in many industrial and scientific fields such as solar cells,[6] electronics,[7] alloys,[8] catalysts,[9,10] and diagnostic and therapeutic agents.[11–16] Ru has also been studied[17,18] in the field of medicinal inorganic chemistry, which has constantly grown during the past few decades. Organometallic complexes are generally considered unstable in air or in wet conditions. However, a variety of bioactive Ru complexes, which are stable in aqueous and alcoholic solutions and less sensitive to oxygen and sulfur, have been developed.[19] Researchers from both academia and industry have been focusing on the development of noble Ru complexes with prominent bioactivity and bioavailability.

In physiological conditions, Ru ion is stable in two oxidation states, Ru(II) and Ru(III), and the reduced state is considered to be more reactive.[20] Both oxidation states accommodate a six-coordinated octahedral configuration, but it is possible that

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they could be coordinated by ligands with different geometries and are expected to participate in various biological redox reactions.[21] Proper variation of ancillary ligands can modulate the steric and electronic properties of a complex to enable the construction of a large platform of chiral Ru complexes. Labile axial ligands are expected to coordinate to the disease targets through ligand-exchange reactions with biomolecules. The rate of ligand exchange in Ru(II) complexes (ranging from 10^{-2} to 10^{-3} s^{-1}) is similar to that of platinum (II), which is on the scale of an average cell's lifetime.[22] Thus, Ru is considered to be an alternative to platinum (Pt)-based drugs. In particular, many Ru compounds are considered to be less toxic than Pt-based drugs, and some of them are quite selective for cancerous cells.[23] These phenomena might have arisen from the ability of Ru to mimic iron in binding to biomolecules.[24] Overexpressed transferrin receptors on cancer cells due to their increased demand for iron may efficiently deliver Ru complexes to cancer cells.[25]

Since Dwyer et al first developed a series of bioactive Ru polypyridyl complexes **1**–**3** in 1952 (Figure 1),[26] Ru has been a prominent subject in the search for therapeutic and diagnostic agents, and a number of bioactive Ru complexes have been reported.[27–31] The major research field is the synthesis of new Ru(II) and Ru(III) complexes as potential anticancer agents and the investigation of their mechanism of action.[32] Although the applications of metal drugs are mainly related to the treatment of cancer, a significant amount of research has also been conducted to obtain therapies for other uses such as antivirals,[33] antibiotics,[34,35] and anti-parasitic agents.[36,37] Ru complexes are expected to be effective against infections due to the same mechanisms as those in the treatment of cancer. Thus, most of the Ru compounds tested for their cytotoxicity in different tumor cells have also been assessed in terms of their antimicrobial activity. Another area of growth is the study of the interactions between DNA and Ru complexes owing to the recent expansion of their roles such as chemical and stereoselective probes of nucleic acid structures,[38] molecular light switching and bioimaging,[39] and DNA bioanalysis agents.[40] The structurally complex three-dimensional architectures of metal complexes are ideal templates for constructing DNA interaction systems. As a result, Ru complexes have received attention by virtue of their unique binding ability to DNA, together with their rich photophysical, photochemical, and electrochemical properties.

Carbon Monoxide-Releasing Ru Complexes

An emerging research field for Ru complexes is the preparation of Ru-based carbon monoxide (CO)-releasing scaffolds (CORMs) to provide novel vehicles for intracellular CO delivery.[41] Carbon monoxide (CO), which is produced endogenously from the heme oxygenase (HO)-catalyzed degradation of heme,[42] is an important gas signaling molecule that plays significant anti-inflammatory, anti-apoptotic, anti-proliferative, and cytoprotective roles at low concentration.[43] Thus, controlled intracellular CO delivery in a specific target cell is expected to modulate cellular functions, and the development of biomaterials that can deliver CO into target cells in a dose-dependent manner is an attractive approach to achieve therapeutic values. However, its toxicity at high concentrations and the challenges for specific delivery to target sites are limiting its facile application. To overcome these problems, a wide range of Ru-based CORMs have been prepared for controlled dose-release of CO at the target tissue. The most extensively investigated CORM is $Ru(CO)_3$ Cl(glycinate), termed as CORM-3,[44] which has been reported to show interesting biological properties,

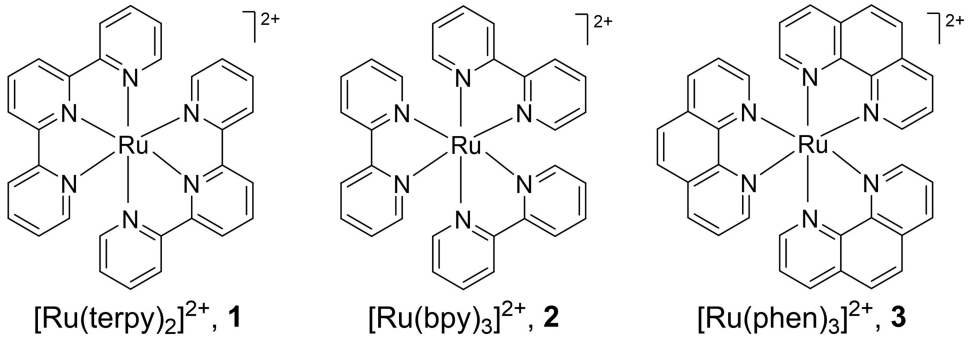

Figure 1 Ru complexes developed by Dwyer.[26]

including vasodilatory, anti-inflammatory, antibacterial, anti-ischemic and anti-apoptotic effects in preclinical studies.[45–47] There have been several recent review papers that have followed the update of CORMs. Therefore, this interesting topic will not be discussed further. Since studies on the preparation and evaluation of bioactive Ru complexes have been reported and have been the subject of many comprehensive reviews,[1–4,48–50] this review will specifically focus on a brief history and future perspectives in anticancer agent research.

Ru-Based Anticancer Agents in Clinical Trials

The clinical success of the platinum (Pt) anticancer drug cisplatin (**4**) is an excellent example of how to advance a serendipitous discovery to a pharmaceutical.[51,52] At present, three Pt-based anticancer compounds, cisplatin (**4**),[53] carboplatin (**5**),[54] and oxaliplatin (**6**),[55] have been approved and are used worldwide in clinical practice (Figure 2).[56,57] However, despite their clinical successes as chemotherapeutics, Pt-based drugs have some limitations: they are not active against many common types of cancer, drug resistance is common, and they cause a deplorable range of side effects such as nerve damage, hair loss, and nausea.[58–60] In search of alternative metal-based anticancer agents, Ru compounds have turned out to be the most promising candidates.[1,61]

A number of Ru-based anticancer agents have been developed to date, yet none of them are in clinical use as anticancer drugs. Successful entries to clinical trials of NAMI-A (**7**),[62] KP1019 (**8**),[63] NKP1339 (**9**),[64] and TLD1443 (**10**)[65,66] together with many reports on the promising in vitro and in vivo activities of other types of Ru complexes have caused Ru-based chemotherapeutics to be seen as a major area in anticancer drug research (Figure 3).[67,68] Despite their structural similarity, NAMI-A and KP1019 have shown quite different in vitro and in vivo activities. NAMI-A showed antiangiogenic and antimetastatic activities in secondary tumors[69,70] whereas KP1019 is active in a broad spectrum of primary tumors.[71,72] NKP1339, a sodium salt version of KP1019, was initially developed as a precursor in the formulation of KP1019 but reevaluated as a clinical candidate owing to its higher aqueous solubility, which allows for the clinical application of large doses to patients.[73] TLD1433 (**10**) entered Phase I and phase 2a clinical trials for bladder cancer treatment with photodynamic therapy (PDT).[66]

Development of Anticancer Half-Sandwich Arene-Ru Complexes

Perhaps the most prosperous structural moiety of Ru-based anticancer agents over the last few decades has been half-sandwich Ru-arene complexes containing 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane (PTA) ligand(s) called RAPTAs. Their pharmacological properties can be easily modulated by ligand modification (**11**–**18** as shown in Figure 4).[67,74–76] Their structures are composed of a "piano stool" geometry where an $\eta^{5 \text{ or } 6}$ π-arene ligand forms the seat, and the combination of mono- and bidentate

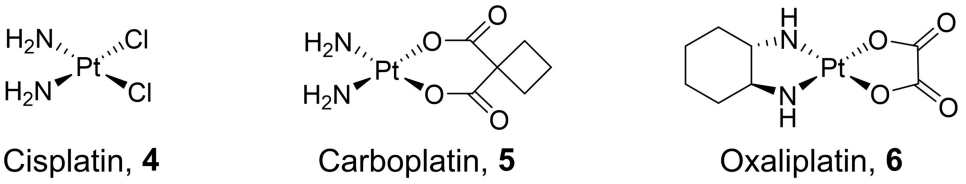

Figure 2 Approved Pt-based anticancer drugs.

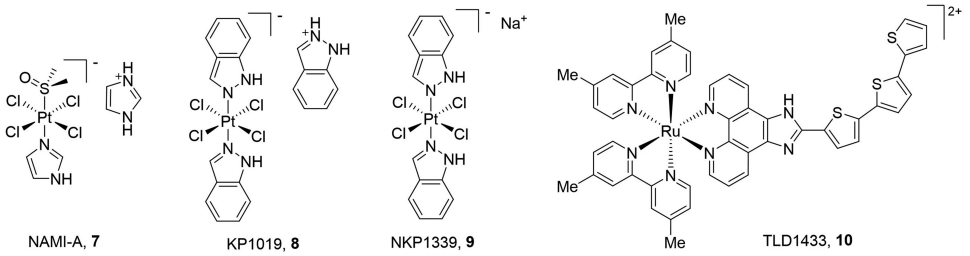

Figure 3 Anticancer Ru complexes in clinical trials.

ligands forms three legs. The chelating nature of the bidentate ligand appears to be beneficial for anticancer activity. Ru-arene complexes can display both hydrophilic and hydrophobic properties, which are expected to exhibit not only additive but also synergistic effects. The robust Ru-arene unit, together with other finely tuned ligands, can create diverse structural deviation and various modes of interaction with biomolecular targets that provide a high potential for the development of anticancer drugs. The first stable monomeric benzene-Ru complexes were reported by Zelonka et al in 1972,[77] and the development of anticancer arene-Ru complexes was initiated by the observation of Tocher et al in 1992 that the cytotoxicity of metronidazole, an antibiotic agent, was increased when coordinating to a benzene-Ru dichloro complex.[78] Since then, the RAPTA family has been a focus of research, and a number of analogues that display in vitro and in vivo anticancer activities have been prepared and evaluated. Interestingly, RAPTA complexes displayed a similar spectrum of activity as NAMI-A in spite of their differences in oxidation state, ligands, charge, and geometry.[79] The target of arene-Ru anticancer compounds may be DNA or RNA, but serum proteins might also become targets.[80] The development of RAPTA analogues along with the evaluation of their bioactivity has been described in detail in previous reviews;[74–76] therefore, only recent advances in the development of RAPTA analogues will be discussed in this review.

Recent Advances in the Development of RAPTA Family Anticancer Agents

Kurzwernhart et al[81] reported on the preparation and evaluation of a series of Ru(cymene) complexes with bioactive flavonol ligands, which are considered as topoisomerase inhibitors for use as anticancer agents. Studies on their mode of action have indicated that they form covalent bonds with DNA showing only a minor impact on the cell cycle but inhibit CDK2 and topoisomerase IIα in vitro. A cytotoxicity study against a panel of human cancer cell lines displayed IC_{50} values in a low μM range, which were lower than those of the parent compounds, flavonols. Complexes with para- and meta-substituted phenyl ligands exhibited lower IC_{50} values than unsubstituted ones. The structure of the most promising compound (**19**) is depicted in Figure 5. Côrte-Real et al[82] prepared three cyclopentadienyl-Ru(II) bipyridyl complexes, including **TM34**. These complexes inhibited lactate production and trans-plasma membrane electron transport activity and showed inhibitory cell growth activity. Their uptake was facilitated without loss of activity when they were conjugated with transferrin. The most active compound, **TM34** (**20**), exhibited cytotoxic activity against human tumor cell lines, A2780 and MDA-MB-231, in low μM IC_{50} values. Pettinari et al[83] prepared other RAPTA complexes consisting of fixed acylpyrazolonato bidentate ligand with varying arenes and monodentate ligands. The antitumor activity of the complexes was shown to be highly dependent on the nature of the arene ligand, and the complexes with the hexamethylbenzene (hmb) group turned out to be the most effective. The antiproliferative activity of such complexes against four human cancer cell lines was determined, and three hmb-Ru complexes displayed dose- and cancer cell line-dependent IC_{50} values in the low μM range. In particular, the most promising compound [(hmb)Ru(Q^{biph})(PTA)][PF_6] (**21**) was active in all tumor cell lines with a potency comparable to the reported values of cisplatin. The hmb-Ru complexes might activate caspase activity, thereby inducing DNA fragmentation, accumulation of proapoptotic proteins, and down-

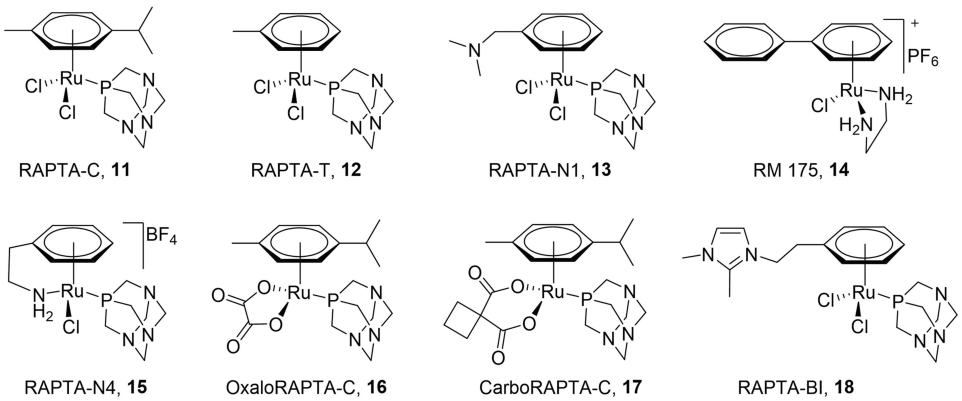

Figure 4 Selected RAPTA complexes from Ang and Dyson.[75]

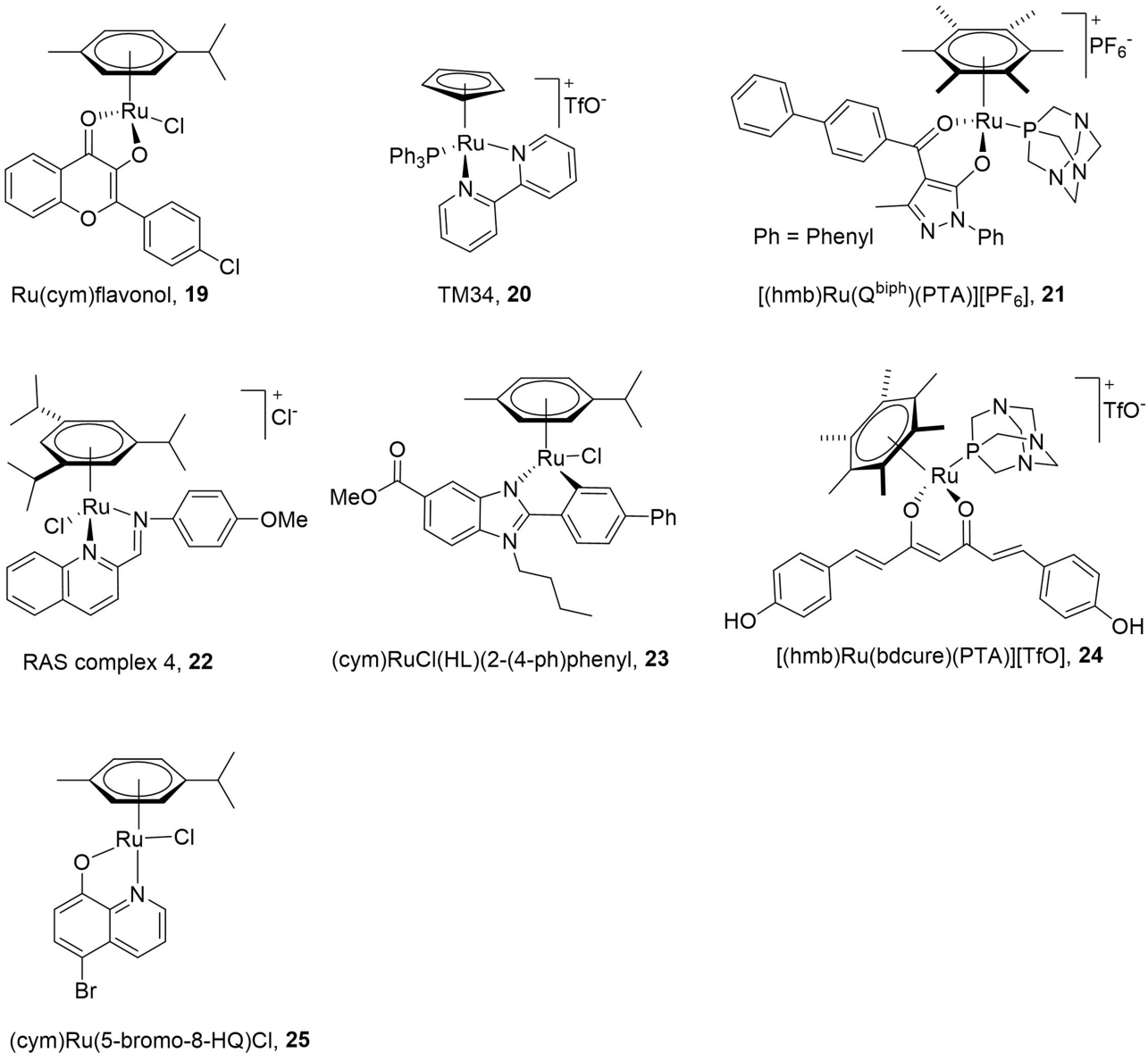

Figure 5 Selected anticancer RAPTA complexes.

regulation of antiapoptotic protein Bcl-2, which results in apoptosis.

A library of water-soluble 442 arene-Ru Schiff base (RAS) complexes was efficiently constructed via a one-pot combinatorial metal three-component reaction by Chow et al.[84] The screening of the library for the anticancer activity showed that several compounds had low μM IC_{50} values in the inhibition of cell viability against a panel of cancer cell lines. The most active compound, the RAS complex 4 (**22**), exhibited cytotoxicity superior to that of the positive control, cisplatin. A mechanistic study suggested compound **22** induced apoptosis via a p53-independent mechanism, suggesting that DNA is not its primary biological target. In addition, Yellol et al[85] synthesized a series of C, *N*−bidentate Ru(II) and Ir (III) complexes, [(cymene)RuCl(HL)] and [(Me_5Cp)IrCl(HL)] having various 2-(4-varied phenyl)benzimidazole ligands. All the complexes displayed high cell growth inhibitory activity in tested cells including cisplatin-resistant A2780cisR. In general, the Ru cytotoxic activity of metal compounds was evaluated against a panel of cancer cell lines, and all compounds displayed high cell growth inhibitory activity in tested cells including cisplatin-resistant A2780cisR. In general, Ru complexes are more active than their corresponding iridium complexes, and substitution on the 4-position of the phenyl ring caused increases in

the potency of both the Ru and Ir complexes. The most potent compound [(cym)RuCl(HL)(2-(4-ph)phenyl] (**23**) showed superior inhibitory activity compared to the positive control, cisplatin. Additionally, six novel RAPTA type complexes containing curcumin-based ligands and 1,3,5-triaza-7-phosphaadamantane (PTA) were synthesized and characterized by Pettinari et al.[86] The antitumor activity of the complexes was evaluated in vitro against human ovarian carcinoma cells, A2780 and A2780cisR, and a non-tumorous human embryonic kidney HEK293 cell. Despite leading to different hydrolysis rates, the presence of a methoxy substituent on the phenyl rings of curcumin did not strongly affect the biological activity. However, the PTA ligand significantly enhanced the activity and selectivity of Ru complexes compared to the previously reported values for the parent compound, curcumin. All the Ru-curcumin complexes showed superior cytotoxicity and a cancer cell selectivity index compared to the positive control, cisplatin. In particular, the most promising compound [(hmb)Ru(bdcure)(PTA)] (**24**) was approximately 70-fold more selective against cancer cells than a noncancerous HEK cell.[86] A (cym)Ru(5-bromo-8-hydroxyquinoline) (**25**) hampered cell proliferation, migration and invasion in a monolayer of cancer cells. In addition, it showed anti-metastatic activity in spheroid model.[87,88]

Recent Advances in the Development of Anticancer Ruthenium Polypyridyl Analogues

In search of Ru-based antitumor compounds with a different spectrum of activity and fewer side effects, scientists have continued to search traditional Ru polypyridyl complexes as potent anticancer agents.[4] Cadoso et al[89] reported the preparation of five water-soluble, near IR luminescent Ru(II) polypyridyl complexes. The complexes were bound to HSA protein via non-covalent interaction and were luminescent in the near IR region. This method allows visualization of cellular localization and the distribution of administrated metal complexes. The high uptake of the complexes into HCT116 cells was detected by luminescent confocal microscopy. The inhibition of the proliferation of the cancer cell lines, A2780, HCT116 $p53^{+/+}$, and HCT116 $p53^{-/-}$ by the complexes was evaluated, and the IC_{50} values for all the complexes were comparable to that of cisplatin. Among them, RuphenImH (**26**) displayed the highest activity, and the hydrogen-bonding ability of the imidazole ligand seemed to play an important role in their cytotoxicity (Figure 6). Similarly, Huang et al[90] synthesized an inert Ru(II) complex $[Ru(bpy)(phpy)(dppz)]^{+}$ (**27**) as an effective anticancer agent. Compound **27** was found to be rapidly taken up by cancer cells so that ~90% of the complex was accumulated in the nuclei of cancer cells 2 h after incubation. The anticancer activity of **27** was screened against a panel of cancer cell lines, and the compound exhibited IC_{50} values with a range of 0.6–4.3 μM, which is an order of magnitude lower than that of cisplatin. The formation of a Ru-carbon covalent bond in **27** enhanced the stability and lipophilicity, which would be beneficial for penetration to the cancer cell nuclei. The high DNA binding affinity of **27** caused a disruption in the binding of the transcription factor NF-κB to DNA, thereby inhibiting cellular transcription and leading to irreversible cancer cell apoptosis. In addition, Zeng et al[91] reported Ru(II) anthraquinone complexes that were highly cytotoxic to both normoxic and hypoxic cancer cells. The complexes exhibit similar or superior cytotoxicity compared to cisplatin in HeLa, A549, and multidrug-resistant A549R tumor cell lines. Their anticancer activities were correlated to their lipophilicity and cellular uptake properties. The most active compound, **28**, exhibited 46-fold and 61-fold higher cytotoxic potency than cisplatin in hypoxic cells and 3D multicellular tumor spheroids, respectively. Compound **28** was preferentially accumulated in the mitochondria of hypoxic HeLa cells and induced apoptosis through multiple synergistic pathways. Similarly, three Ru(II) complexes with a bidentate bisimidazolo ligand were synthesized and characterized by Xia et al.[92] An interaction study between human telomeric G-quadruplex DNA and Ru complexes showed that they tightly bind to the human telomeric DNA. Among them, $[Ru(phen)_2(biim)]^{2+}$ (**29**), was the most effective in the formation of mixed/hybrid type G-quadruplexes. Their antitumor activity was closely related to their ability to interact with G-quadruplex DNA so that **29** showed the highest inhibitory activities comparable to the positive control, cisplatin, against HeLa, A549, and HepG2 cells. Compound **29** can effectively promote the apoptosis of tumor cells by acting on mitochondrial apoptotic pathways. Additionally, Han et al[93] reported the preparation of Ru(II) polypyridyl complexes and the evaluation of their anticancer activities. All the compounds reduced the mitochondrial membrane potential and inhibited cell growth in A549 cells in the G0/G1 phase. The IC_{50} values of complexes against BEL-7402, A549, MG-63, and SK-BR-3 cells were in the low

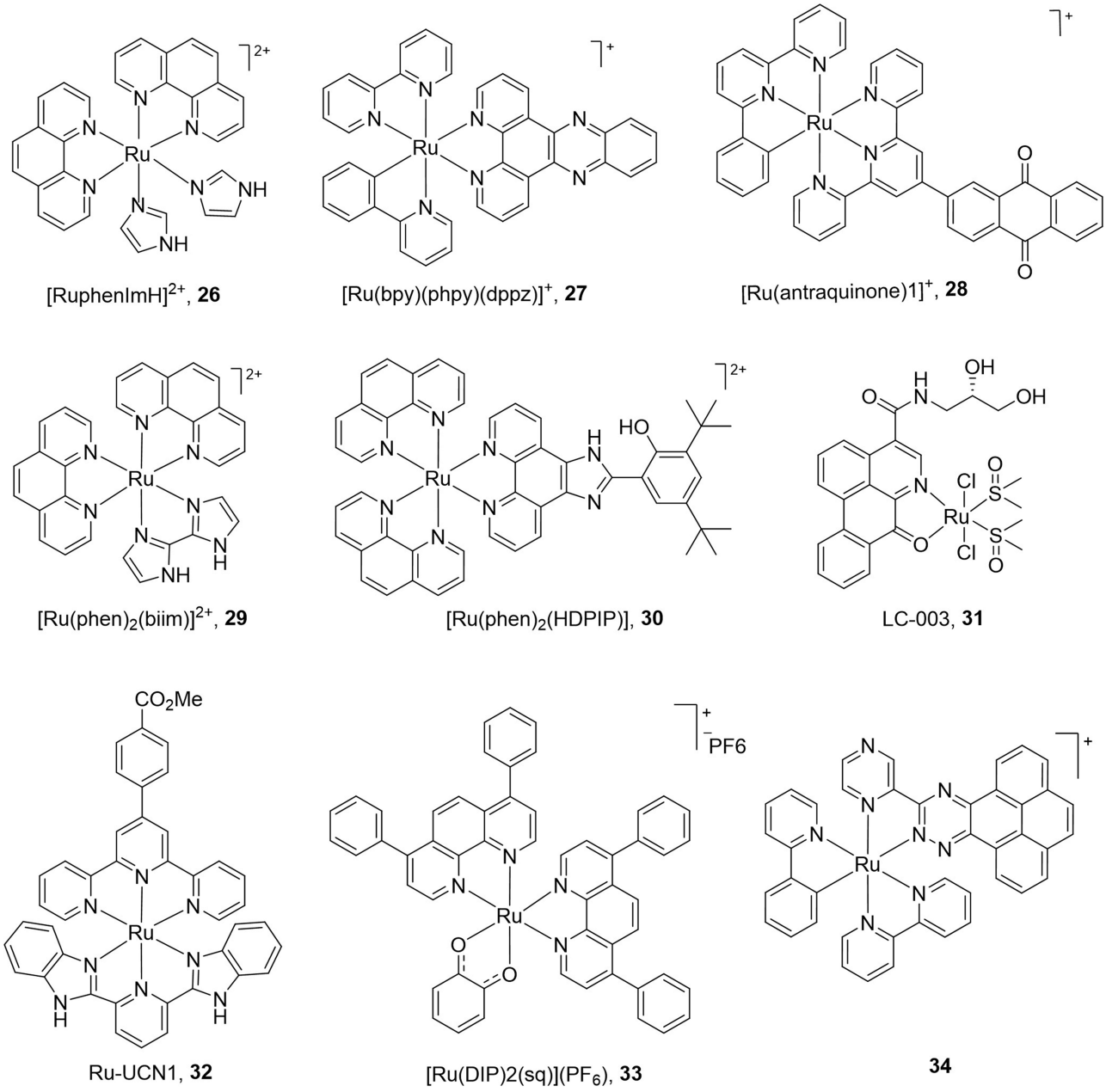

Figure 6 Selected anticancer Ru polypyridyl complexes.

μM range, and the cytotoxicity of the most active compound, [Ru(phen)$_2$(HDPIP)] (**30**), was comparable to that of cisplatin. Among the four cell lines, A549 was found to be the most sensitive, while MG-63 was the least sensitive. Compound **30** enhanced the level of reactive oxygen species (ROS) and decreased the mitochondrial membrane potential, suggesting a compound-induced apoptosis in A549 cells through an ROS-mediated mitochondrial dysfunction pathway. Additionally, Chen et al[94] synthesized three water-soluble Ru(II) complexes with chiral 4-(2,3-dihydroxypropyl)-formamide oxoaporphine (FOA) ligand and evaluated their in vitro and in vivo antitumor activities. The compounds effectively stabilized telomeric and G-quadruplex DNA in the promoter of c-myc, thereby acting as a telomerase inhibitor. In the in vitro cytotoxicity against six human tumor cell lines (BEL-7404, A549, MGC80-3, HeLa, Hep-G2, BEL-7402 and one normal liver cell line HL-7702), compound LC-003 (**31**) displayed the highest inhibitory activity that was comparable to the positive control, cisplatin. Compound **31** was more selective for the BEL-7404 tumor cell than the normal HL-7702 cell. Moreover, **31** exhibited in vivo inhibition efficacy on

tumor growth in the BEL-7402 xenograft mouse model and a higher in vivo safety profile than cisplatin. Another series of polypyridyl compounds (**32**) showed comparable anticancer activity to that of cis-platin, especially for gastric cancer cells.[95,96] Tables 1 and 2 summarize anticancer activities of selected Ru complexes in in vitro and in/ex vivo system, respectively.

Photoactivation of Ru Complexes

Poor selectivity and lack of efficacy have been the main bottlenecks for anticancer chemotherapies. Thus, the selective delivery of a photoactivatable agent to tumorous cells and its activation by irradiation is an attractive approach to enhance the cytotoxicity of tumor cells while minimizing side effects on normal cells.[99] Two types of photoactivation modalities for Ru-based anticancer agents have been developed: photodynamic therapy (PDT) and photoactivated chemotherapy (PACT). In PDT treatment,[100–103] photosensitizer molecules are administered either topically or intravenously, and the target tissue layers are irradiated with light of a specific wavelength after internalization of the agents into tumor cells.[17,104] The excited photosensitizer molecules then activate nearby oxygen and/or biomolecules to generate a reactive oxygen species, mainly singlet oxygen ($^1O_2$), causing the death of cancer cells. Because light has to reach deeper tissue layers, the light of a long wavelength is usually chosen in PDT, and the two-photon excitation (TPE) is preferred as the photoactivation method.[105] The advantages of TPE are reduced scattering of near-infrared (NIR) photons in turbid biological tissues and better definition of the focal spot.[106] However, PDT is still hindered by poor depth efficacy and reduced toxicity against hypoxic cancer cells. Porfimer sodium (Photofrin),[107] aminolevulinic acid (ALA),[108] and methyl ester of ALA (Metvixia)[109] have been approved for clinical practice as photosensitizing agents by the FDA. In contrast, PACT[65,110–112] utilizes light to induce activation of the internalized prodrug moiety independent of the presence of oxygen inside cells. Thus, PACT is an attractive approach to treat tumors in a hypoxic condition, which is the notorious characteristic of solid cancers.[113] The photoactivation might induce DNA cross-linking, release of cytotoxic compounds from molecular carriers, or activation of prodrugs by ligand displacement. Because Ru complexes feature at least one coordination site in them that can be occupied by a labile ligand, construction of photo-dissociable Ru-based drug carriers or prodrugs is feasible.[114] During the last few decades, a number of photoactive Ru compounds that have exhibited multiple types of biologically relevant activity have been reported. There has also been a recent thorough review of Ru-based PDT and PACT agents.[115]

For an update, Liu et al reported Ru(II) polypyridyl complexes as mitochondria that targeted two photon-absorbing PDT photosensitizers.[116] These complexes exhibited efficient singlet oxygen generation in methanol, significant two photon absorption (TPA) cross sections, and a substantial amount of mitochondrial accumulation. They are virtually nontoxic towards 3D HeLa multicellular spheroids (MCSs) in the dark but triggered cell death by a generation of singlet oxygen upon either one- or two-photon irradiation. The most promising complex, **RuL4** (**35**) displayed IC_{50} values of 9.6 μM in one-photon and 1.9 μM in two-photon PDT, respectively, against 3D MCSs, which were lower than those of the positive control, cisplatin (Figure 7). Likewise, Chen et al[117] developed Ru-arene complexes as potential candidates for dual PDT and PACT agents. The most active compound, $[(cymene)Ru(dpb)(py)]^{2+}$ (**36**), absorbs long wavelength light and generated reactive $^1O_2$, leading to photocleavage of DNA. In addition, compound **36** underwent photodissociation of both dpb and py ligands upon irradiation, and the resulting Ru-cymene fragment was bound to the nearby DNA bases. Hence, compound **36** has potential as a new type of antitumor agent with dual PACT and PDT pathways. The dissociated dpb ligand is a fluorescent process that might provide another opportunity to monitor real-time imaging of the photo-activated interaction between the biomolecule and Ru complex. Hence, compound **36** has potential as a new type of anticancer agent with dual PACT and PDT mechanisms. A dissociated dpb ligand can be a fluorescent probe that might provide another opportunity to monitor real-time images of photo-activated interaction between biomolecules and the Ru complex. Compound **36** showed significant light-enhanced cytotoxicity: IC_{50} of 27.6 μM in the dark and 4.0 μM under illumination against human lung carcinoma A549 cells. In addition, Huang et al[118] synthesized several mixed ligand Ru(II) terpyridyl complexes as PDT photosensitizing agents and investigated their photocytotoxicity. These complexes exhibited red luminescence between 670 and 710 nm and functioned as photosensitizers by generating both singlet oxygen and reactive radical species. They were located in the nucleus and exhibited significant photocytotoxicity upon irradiation. The most active compound, **37**, exhibited a remarkable phototoxicity index (PI) together with a lower IC_{50} value than that of the positive control, cisplatin, toward human hepatocellular carcinoma Bel7402 and HepG2 cells. Similarly, Frei et al[119] prepared two Ru(II) polypyridyl complexes, $Ru(DIP)_2(bdt)$ (**39**) and $[Ru(dqpCO_2Me)(ptpy)]^{2+}$

Table 1 In vitro Anticancer Activity of the Ru-Based Compounds Selected in the Literature

No	Compound Number (or Name)	IC_{50} (Assay System)		Ref
		Monolayer Cells	3D or Spheroids	
1	KP1019	39 μM (SW480)		[71]
2	KP1339	123 μM (SW480)		[71]
4	Cmpd 19	0.9–19 μM (CH1, SW480, A549, 5637, LCLC-103H, DAN-G)		[81]
5	Cmpd 20	13–25 μM (A2780, MCF7, MDA-MB-231)		[82]
6	Cmpd 21	14–27 μM (HeLa, MCF-7, HepG2, HCT-116)		[83]
7	Cmpd 22	3 μM (MCF7, A2780, A2780cisR)		[84]
8	Cmpd 23	1–2 μM (A2780, A2780cisR, S637, A427, LCLC, SISO, HT29, EA.hy926)		[85]
9	Cmpd 24	13 μM (HEK293)		[86]
		0.2–0.27 μM (A2780, A2780cisR)		[86]
10	Cmpd 25	19–51 μM (MG-63, A549, MCF7, MDA-MB-231)	104–251 μM (MG-63, A549, MCF7)	[87]
11	Cmpd 26	0.7–1.3 μM (HCT116 ($p53^{+/+}$), HCT116($p53^{-/-}$), A2780)		[89]
12	Cmpd 27	0.6–4 μM (HeLa, A549)	1.5–2.9 μM (MCTSs)	[90]
13	Cmpd 28	0.5–4.5 μM (HeLa, A549, A549R)		[91]
14	Cmpd 29	14–30 μM (HeLa, A549, HepG2)		[92]
15	Cmpd 30	7–15 μM (BEL-7402, A549, MG-63, SK-BR-3)		[93]
16	Cmpd 31	7–16 μM (BEL-7404, A549, MGC80–3, HeLa, HepG2, BEL-7402)		[94]
17	Cmpd 32	29.5 μM (AGS)		[95]
18	Cmpd 35		9.6 μM (1P*, HeLa) 1.9 μM (2P**. HeLa)	[116]
	Cmpd 36	27.6 μM (dark), 4.0 μM (λ>400 nm) (A549)		[117]
	Cmpd 37	92 μM (dark), 1.5 μM (450 nm) (HepG2)		[118]
	Cmpd 38	1–3 μM (light), 300–470 μM (dark) (HeLa)	2–20 μM (light), >500 (dark) (HeLa)	[102]
	Cmpd 39	49.7 μM (dark), 0.62 μM (420nm) (HeLa)		[119]
	Cmpd 40	>1007 μM (dark), 25.3 μM (420nm) (HeLa)		[119]
20	Cmpd 43	17 μM (UV-A), >100 μM (dark) (HeLa, U2OS, MRC-5)		[123]
21	[(p-cymene)Ru(maleonitrile-dithiolate)]	0.32–1.14 μM (HCT116 p53+/+, HCT116 p53-/-, A2780, A2780cisR, H460)		[149]
22	Cmpd 33	0.45–4.13 μM (HeLa, A2780, A2780 ADR, A2780 cis, CT-26, CT-26 Luc, RPE-1, MRC-5)	14.1 μM (HeLa)	[97]
23	Cmpd 41	> 100 μM (dark), 0.7 μM (480 nm), 0.9 μM (540 nm) (CT-26)	> 1.4 μM (800nm, HeLa)	[121]
24	Cmpd 42		2P (900 nm) melanoma spheroid	[124]
25	Cmpd 34	3 μM (A549, A549R, SGC-7091, SGC-7091/DDP)		[98]
26	Dendrimer	1–5.9 μM (HeLa, PC-3)		[150]

Notes: *1P = single photon, **2P=two photon.

Table 2 In/ex vivo Anticancer Activity of the Ru-Based Compounds Selected in the Literature

No	Compound Number (or Name)	Assay System (Cell, Administration)	Results	Ref
1	Dendrimer	Prostate cancer	Inhibited tumor growth 40% compared to control	[150]
2	Cmpd 34	A549R (2 mg/kg)	More effective than cis-platin (4 mg/kg)	[98]
3	Cmpd 41	SW620/AD300 (2 mg/kg, iv)	1P (500 nm) and 2P (800 nm) showed dramatic tumor reduction	[121]
4	Cmpd 33	Ehrlich carcinoma (5 mg/kg, IP)	Insufficient tumor suppression (p=0.108)	[97]
5	[(p-cymene)Ru(maleonitrile-dithiolate)]	H460 (7.5 mg/kg, IP)	Tumor growth delayed on day 3, but relapsed afterwards	[149]

(**40**), as PDT photosensitizers. Their phototoxicity was measured against the human cervical cancer cell line HeLa, and both compounds showed remarkable phototoxicity. Complex **40** displayed low μM range phototoxicity but no significant toxicity in the dark. However, complex **39** displayed μM range toxicity in the dark and nm range phototoxicity upon irradiation with a PI of 80, which is more impressive than that of the clinically approved photosensitizers, ALA and porfimer sodium. Both **39** and **40** displayed lower phototoxicity and PI values in similar experimental settings.[120] Around 67% of complex **39** taken up by the cell was accumulated in mitochondria. Nevertheless, the cellular uptake of **40** was shown to be diffused in all cellular compartments with a slight preference to the nucleus. The phototoxicity of both complexes against the two bacterial strains, S. *aureus* and E. *coli*, was also tested. Compound **39** effectively reduced cell viability in the Gram (+) strain S. *aureus* whereas no toxicity was observed against the Gram (−) strain E. *coli*. On the other hand, **40** effectively reduced the cell viability in both S. *aureus* and E. *coli*. The phototoxic profile of **40** against bacteria is particularly promising as Gram (−) bacterial strains are reported to be less affected by PDT than Gram (+) bacteria. One of the excellent examples would be **41** which showed a promising phototoxic profile.[121] It displayed IC_{50} > 100 μM in dark without irradiation while its IC_{50} is less than 1 μM when irradiated against CT-26 colon carcinoma cells reaching safety index >100.

As for the recent examples of PACT, Karaoun et al[122] constructed Ru(II) complexes with either one or two imidazole-based antifungal agent econazole ligands for dual applications in cell imaging and PACT agents. Both complexes were stable and luminescent in the dark, yet the irradiation of green light induced the release of an econazole ligand and turn-off of the luminescence. Although both complexes showed enhanced cytotoxicity and photocytoxicity against a panel of tumor cell lines compared to the parent drug econazole nitrate, which is known to induce apoptosis, $Ru(phen)_2(Ec)_2Cl_2$ (**42**), which has two econazole ligands, displayed a higher PI value than the Ru(II) complex with one econazole ligand (Figure 8). Compound **42** acts as a prodrug of econazole and offers several advantages, such as improved aqueous solubility and stability, enhanced intracellular accumulation, reduced toxicity, and real-time imaging of drug release by the turn-off luminescence response over the parent drug. Joshi et al[123] prepared a photolabile DMNPB ester capped Ru(II) complex (**43**) as a prodrug of the cytotoxic complex $[Ru(dppz)_2(CppH)]^{2+}$ (44),[124] which is known to disrupt the mitochondrial function. The hydrolytic stability test of **43** in the PBS buffer (pH 7.2) demonstrated that about 7% of **43** was hydrolyzed to be converted to **44** after 24 h in a dark environment. Compound **43** displayed negligible toxicity toward two cancerous (HeLa and U2OS) and non-cancerous (MRC-5) cells after 48 h in the dark. However, the cytotoxic action of the prodrug **43** can be regained in living cells under light illumination (350 nm), reaching a similar level of cytotoxicity as the parent cytotoxic compound **44**, which is comparable to that of cisplatin. In order to photoactive in deep hypoxic legion in the body, compound **45** was developed to be excited by near-infrared light (NIR) via two-photon irradiation to treat melanoma cancer. The compound was readily absorbed by melanoma spheroid and showed rapid cell death in the hypoxic region.[125]

Mode of Actions

Protein Binding

After intravenous administration, NAMI-A has a stronger interaction with human serum albumin (HSA) than KP1019, although both compounds bind to HSA in

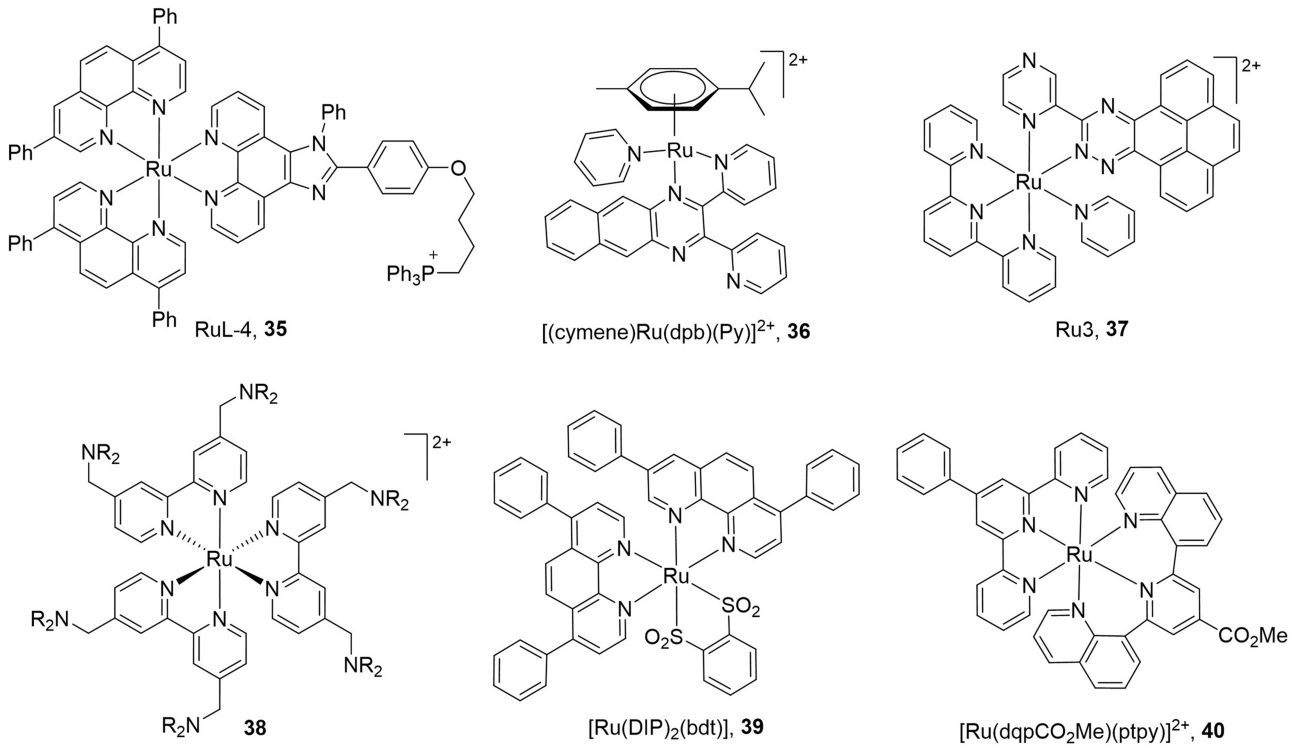

Figure 7 Selected Ru-based PDT agents.

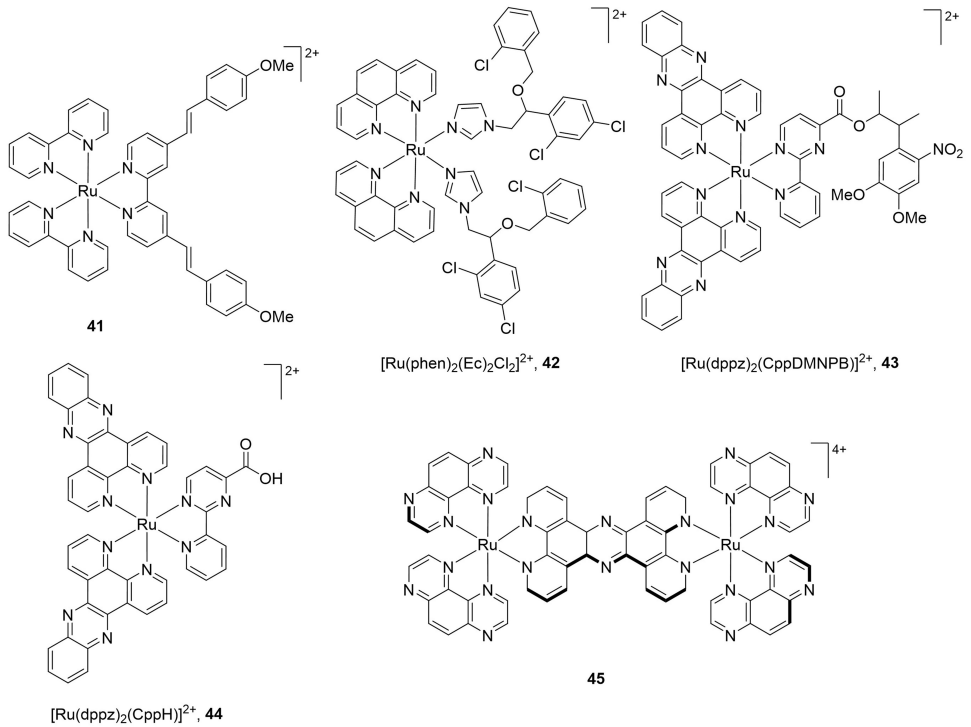

Figure 8 Selected Ru-based PACT agents.

a noncovalent manner.[126–131] The stability of their noncovalent interaction has been shown to correlate with the ability of ligands to interact with the hydrophobic binding domains of HSA, and their different binding modes may play an important role in their distinct pharmacologic properties and efficacies. As shown in Figure 9, which depicts the structure of

the HSA-myristate-KP1019 complex,[132] Ru moieties bind to HAS. Both Ru metal centers are bound to the imidazole nitrogen of histidine 146 and histidine 242, which are located within the well-known drug binding sites, on subdomain IB (Ru binding site 1) and IIA (Ru binding site 2). The indazole ligands of KP1019 are important as binding sites recognizing moieties, which promote metal binding to other proteins in serum, which might lead to a decrease in selectivity and cytotoxicity and suggests an important reason for the pharmacologically different behavior between KP1019 and cisplatin, which was found to bind His residue located at the surface of HSA.[133]

DNA Binding, Cytotoxicity, and Apoptosis

The cytotoxicity of cisplatin against cancer cells is mainly associated with binding to DNA via both interstrand and intrastrand cross-links, whereas the biologic targets of NAMI-A and KP1019 have not yet been totally elucidated.[134,135] Both compounds are able to target DNA and proteins, implying the feasibility of either a different binding mode than that of cisplatin or the existence of multiple pathways. Both compounds are known as prodrugs; they are reduced to more reactive Ru(II) species by reducing molecules such as glutathione, cysteine, and ascorbic acid in a physiological medium.[136–138] There are two major proposed mechanisms by which Ru compounds are less toxic than platinum drugs in general: activation by reduction and the iron mimicking hypotheses. Activation by reduction theory is based on the observation that Ru(III) complexes are more inert than Ru(II), and cancerous cells, especially solid tumor issues, tend to have a greater reducing environment due to their lower oxygen level and pH condition than normal healthy cells.[139] Thus, the administrated Ru(III) compound causes minimal damage to healthy cells but can be activated to an Ru(II) oxidation state under a hypoxic environment inside cancerous cells.[140] The other postulation emerged from the fact that iron and Ru belong to the same group in the periodic table so that Ru is able to mimic iron during its interaction with biomolecules such as serum transferrin and albumin. Since rapidly growing tumor cells have a higher demand for iron uptake and often overexpress transferrin receptors on their cell surfaces, it is thought that they achieve only selective delivery and entry into metal complexes.[25] However, these theories have received considerable criticism. Even the question of how Ru compounds enter cells has been the subject of some debate in the literature.[141]

NKP-1339, which is a leading clinical candidate among Ru-based anticancer compounds, showed very promising anticancer activity. As shown in Figure 10A,[64] it decreased tumor volume and increased Td values (day when tumor volumes reach 300 mm^3) in a xenograft model at a comparable level to sorafenib when administered i.v. once a week for two weeks. However, the tumor volume in

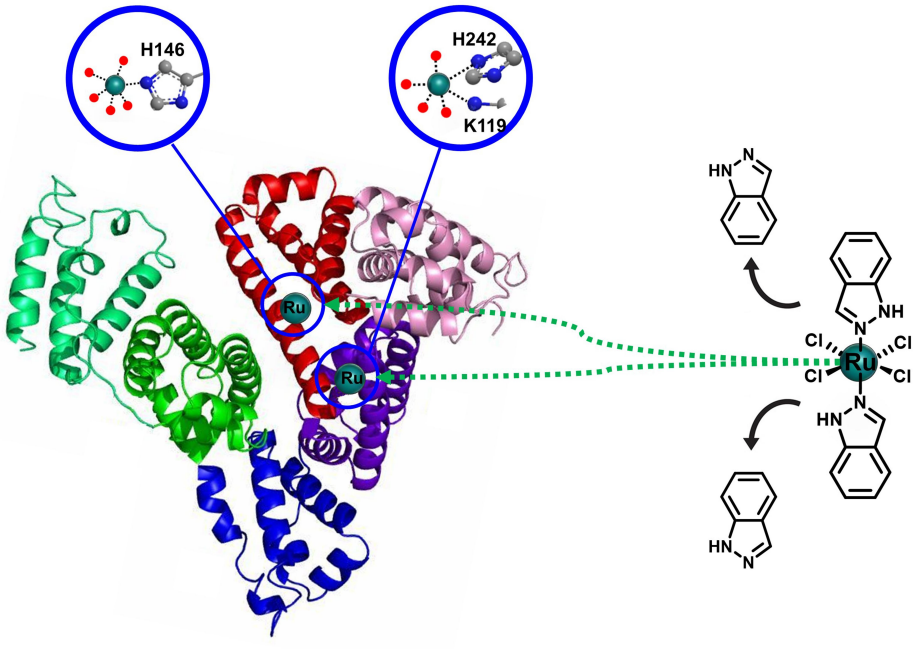

Figure 9 Crystal structure of the HAS-myristate-KP1019 complex. Two molecules of KP1019 bind to HAS at two histidine sites (H146 and H242).

both NKP-1339- and sorafenib-treated groups slowly caught up to that of the vehicle-treated group in the end. Combined with sorafenib, NKP-1339 reduced tumor volume more dramatically than its single treatment and kept it low for a long period of time. As for the mechanism of action, evidence to support other mechanisms than its direct interaction with DNA molecules was reported. It was proposed that if NKP-1339 is reduced to Ru(II) like other Ru-based anticancer compounds, then it could interact with unfolded protein response (UPR) machinery to regulate endoplasmic reticulum (ER) stress in cancer cells, or it could lead either to apoptosis or cell cycle arrest in cancer cells via mitochondrial damage or the p38 MAPK control (Figure 10B).

Conclusion and Perspectives

Over the last decades, Ru complexes have been targets of considerable attention and the fields of their application have rapidly grown. Ru complexes are in six-coordinated octahedral configurations, and proper variations of the ligands can allow the construction of a large platform of Ru compounds. The major research field of Ru complexes is the synthesis of potential anticancer agents among which the most prominent structural moiety of Ru-based anticancer agents has been half-sandwich Ru-arene complexes that display both hydrophilic and hydrophobic properties. A number of Ru complexes have shown superior anticancer profiles such as increased selectivity toward cancer cells and ameliorating toxicity against normal cells compared to existing Pt-based anticancer drugs. As a result, four Ru-based anticancer agents, NAMI-A (**7**), KP1019 (**8**), and NKP1339 (**9**), and TLD1433 (**10**) have entered clinical trials, but only two entities, NKP1339 (**9**) and TLD1433 (**10**) are still under investigation at this point.[142] One of the mainstream trends of oncology drugs is so-called targeted therapy where the agents are developed with a specific molecular target in hand. Ru-complexes have few target molecules known and therefore might be considered as non-specific. However, given that numerous oncology regimens still include non-specific Pt-based anticancer drugs, a new generation of metalloanticancers to overcome the existing drawbacks, such as poor selectivity for cancer cells and high toxicity against normal cells, would be an attractive alternative.

In order to overcome the poor selectivity and lack of toxicity associated with chemotherapy, PDT and PACT, which can selectively activate prodrug moieties in a specific region, have become a promising strategy. Studies in these applications have demonstrated feasibility as non-invasive and organelle-specific therapies such as mitochondrial-[116] and lysosome-targeting.[102] Despite very promising in vitro results of Ru-based PDT and PACT agents, insufficient in vivo studies have hampered full assessment of the feasibility of such compounds in a clinical context, which we believe researchers will have to now focus on.

An interesting approach to overcome the uptake, efficacy and biocompatibility issues related to Ru complexes will be a nanomaterial-conjugated PDT.[142] Another idea worthy of consideration would be a drug combination to synergize the efficacy without increasing the toxicity and drug resistance[143] such as a combination with poly(ADP-ribose)polymerase (PARP) inhibitors[144] to treat BRCA wild-type triple-negative breast cancer (TNBC). Recent studies are shedding new light

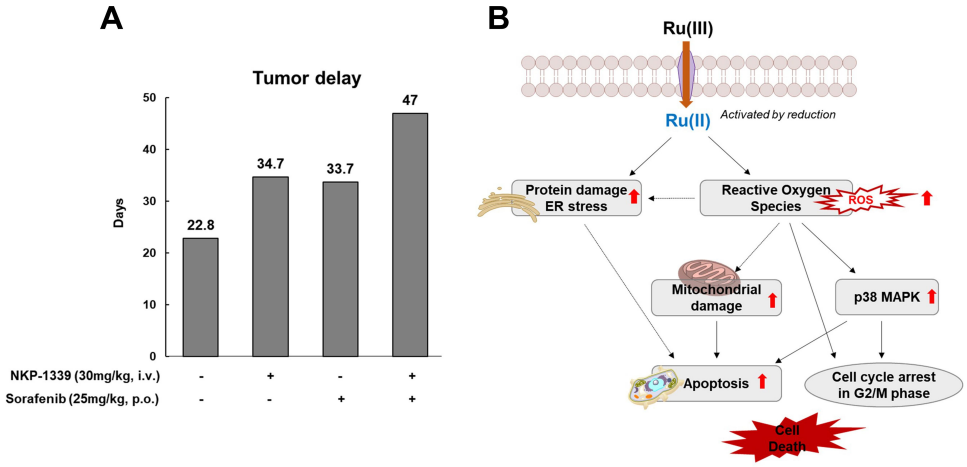

Figure 10 Anticancer activity of NKP-1339. (**A**) Hep3B xenografts in Balb/c SCID mice was treated with NKP-1339 (30 mg/kg, iv, once a week) and/or sorafenib (25 mg/kg, po, five consecutive days per week) for two weeks. (**B**) The mechanisms underlying the anticancer activity of representative Ru complexes: KP1019 and NKP-1339.

on the anticancer potential of Ru complexes. Some have shown a promising characterization as an immune-modulating anticancer agent,[145] excellent redox potential,[97] strong topoisomerase inhibitor,[98] while others have shown encouraging results such as antimetastatic activity, tubulin formation inhibition, and high selectivity against cancer cells over normal cells.[146–148] Recent investigations cover structurally novel scaffolds including electron-deficient ruthenium complexes[149] and macromolecular ligands such as dendrimers.[150]

In this review, we present a brief description of Ru-based anticancer complexes. With all the studies on these interesting entities, it is evident that new metalloanticancer drugs with improved efficacy and selectivity, and less toxicity compared to existing Pt-based anticancers should be seen in clinical use to provide new hope for cancer patients.

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Disclosure

The authors report no conflicts of interest in this work.

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