

## RUTHENIUM(II) COMPLEXES AS POTENTIAL APOPTOSIS INDUCERS IN CANCER THERAPY

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## RUTENIJUM(II) KOMPLEKSI KAO MOGUĆI INDUKTORI APOPTOZE U TERAPIJI MALIGNIH TUMORA

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### ABSTRACT

The compound cis-diamminedichloroplatinum(II) (cisplatin) is the most widely used anticancer drug, but due to its serious side effects (including gastrointestinal symptoms, renal tubular injury, neuromuscular complications, and ototoxicity), clinical applications of cisplatin are limited. Therefore, these limitations have provided an encouragement for further research into other transition metal complexes, with an aim to overcome the disadvantages related with cisplatin therapy. In the search for effective complexes that can be targeted against tumor cells, many research groups synthesized various ruthenium(II) complexes with different ligands. Also, newly synthesized ruthenium(II) complexes showed selective anticancer activity against different types of cancer cells. Activity of ruthenium(II) complexes in some cases was even higher than that of cisplatin against the same cells. Precise mechanism of action of ruthenium(II) complexes is not fully understood. The different examples mentioned in this review showed that ruthenium(II) complexes decreased viability of cancer cells by induction of apoptosis and/or by cell cycle arrest which implies their different mechanism of action against different types of cancer cells.

**Keywords:** ruthenium complexes, apoptosis, cancer therapy.

### SAŽETAK

Jedinjenje cis-diamino-dihloroplatina(II) (cisplatin) je najčešće primenjen citostatik, ali usled ozbiljnih neželjenih efekata koje izaziva (simptomi koji nastaju kao poremećaj funkcionisanja gastrointestinalnog trakta, oštećenje bubrenih tubula, neuromišićne komplikacije i ototoksičnost), mogućnost njegove kliničke primene je ograničena. Zbog ovih ograničenja cisplatine, postoji podsticaj za ispitivanjem drugih kompleksa prelaznih metala. U potrazi za efikasnijim kompleksima koji bi mogli da se koriste u antitumorskoj terapiji, veliki broj istraživača je sintetisao različite komplekse rutenijuma(II). Takođe, novosintetisani rutenijum(II) kompleksi su ispoljili selektivno antitumorsko dejstvo na različite tipove ćelija malignih tumora. Aktivnost rutenijum(II) kompleksa je u pojedinim slučajevima bila čak i veća na određene tipove tumorskih ćelija u poređenju sa cisplatinom. Tačan mehanizam dejstva rutenijum(II) kompleksa još uvek nije precizno objašnjen. U različitim studijama koje su prikazane u ovom preglednom članku, pokazano je da rutenijum(II) kompleksi smanjuju vijabilnost ćelija malignih tumora izazivanjem apoptoze i/ili zaustavljanjem tumorskih ćelija u nekoj fazi ćelijskog ciklusa, što sugeriše da postoje različiti mehanizmi delovanja rutenijum(II) kompleksa na različite vrste ćelija malignih tumora.

**Ključne reči:** kompleksi rutenijuma, apoptoza, terapija tumora.



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## INTRODUCTION

The compound *cis*-diamminedichloroplatinum(II) (cisplatin) is the most widely used anticancer drug, but due to its serious side effects (including gastrointestinal symptoms, renal tubular injury, neuromuscular complications, and ototoxicity), clinical applications of cisplatin are limited (1). Therefore, these limitations have provided an encouragement for further research into other transition metal complexes, with an aim to overcome the disadvantages related with cisplatin therapy (1, 2). Ruthenium compounds proved to be the most promising ones in the search for anticancer agents containing metals other than platinum (3). Reduced toxicity, good selectivity for tumors, inhibition of antimetastatic progression and antiangiogenic properties are advantages of ruthenium based drugs (4). This is believed to be due to the ability of ruthenium to mimic the binding of iron to serum transferrin, which solubilises and transports iron in plasma. Therefore, ruthenium-based drugs may be delivered more efficiently to cancer cells as cancer cells overexpress transferrin receptors, to satisfy their increased demand for iron (1, 5). After binding to the transferrin receptor on cell membranes, the inert complex Ru(III) in tumor cells can be activated and reduced to a more reactive Ru(II) complex (4). The redox potential of the ruthenium complex allows for more effective antitumor therapy. Glutathione, ascorbate and proteins have the ability to reduce, while molecular oxygen and cytochrome oxidize Ru(II). In malignant cells there is a lower concentration of oxygen and an increased glutathione concentration, which results in the reduction of the Ru (III) complex in a more reactive Ru (II) complex (6).

### Potential targets of Ru(II) complexes

Cancer cells divide relentlessly due to a loss of control of normal restraints on cell cycle division. Proliferation of cancer cell is regulated by DNA, and many ruthenium complexes have high selectivity for binding to DNA (7). Ruthenium(II) compounds can be bonded to the DNA covalently and non-covalently (1, 7). For example, ruthenium(II) complexes can bind covalent to the N7 atom in guanosine (8, 9). Ru–DNA covalent binding distorts the DNA backbone, which impairs processes of replication and transcription (7-9). The non-covalent interaction of complexes with DNA includes electrostatic interactions, intercalation and groove binding (7, 10). Intercalation occurs when compounds are added between adjacent base pairs in the DNA double helix. Despite the fact that ruthenium complexes can bind to DNA, recent researches also indicate that certain proteins may be molecular targets for ruthenium compounds (11).

### Ruthenium complexes as protein kinase inhibitors

Ru(II) complexes have been demonstrated to be promising agents as protein kinase inhibitors (12). Protein kinases are known regulators of various aspects of cellular life and moreover, they are one of the main targets for different anticancer drugs (13). They are large enzyme family with homologous active sites and have highly conserved ATP binding sites (13-

15). Platinum complexes are reported as protein kinase inhibitors (14). However, various ruthenium complexes are also reported as protein kinase inhibitors (15-20). In general, inert metal complexes, such as ruthenium complexes, can be promising scaffolds for the design of different enzyme inhibitors (15). Maksimoska et al. designed ruthenium complexes that are selective inhibitors of p21-activated kinase 1 (PAK1). PAK1 have significant roles in metastasis and tumorigenesis and for that reason these compounds are potential candidates for cancer therapy (15). Bregman and Meggers synthesized ruthenium half-sandwich complexes that are inhibitors of glycogen synthase kinase 3 (GSK3) and proto-oncogene serine/threonine-protein kinase Pim-1 (16). Both GSK3 and Pim-1 play important role in the regulation of apoptosis and cell cycle progression, but Pim-1 has also been implicated in numerous cancers including Burkitt's lymphoma and prostate cancer (16-18). Meggers et al also investigated inhibitory activities of the ruthenium compounds against the protein kinases GSK-3, Pim-1, MSK-1 and CDK2/CyclinA and these results were also promising (18). Mitogen- and stress-activated protein kinase-1 (MSK1) and Cyclin-dependent kinase 2 (CDK2) are also involved in cell cycle progression (18, 21, 22).

### Ruthenium(II) complexes and their role in endoplasmic reticulum stress pathway and ROS generation

Another approach for the treatment of cancer involves thioredoxin system. This system consists of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH and plays an important role in regulating the redox balance, redox-regulated signaling cascades, cell function and cell proliferation (23). From the literature reports so far, anticancer actions of ruthenium compounds may be exerted by reactive oxygen species (ROS)-mediated apoptosis (24, 25). Luo et al. reported that ruthenium polypyridyl complexes  $[\text{Ru}(\text{bpy})_3]^{2+}$ ,  $[\text{Ru}(\text{phen})_3]^{2+}$  (2),  $[\text{Ru}(\text{ip})_3]^{2+}$  (3),  $[\text{Ru}(\text{pip})_3]^{2+}$  (4) (bpy = 2,20-bipyridine, phen = 1,10-phenanthroline, ip = imidazole[4,5-f][1,10] phenanthroline, pip = 2-phenylimidazo[4,5-f][1,10] phenanthroline), and demonstrated that these compounds exhibited anti-proliferative activities against A375 human melanoma cells through inhibition of TrxR (23). Costa et al. also demonstrated that piplartine-containing ruthenium complexes  $[\text{Ru}(\text{piplartine})(\text{dppf})(\text{bipy})](\text{PF}_6)_2$  and  $[\text{Ru}(\text{piplartine})(\text{dppb})(\text{bipy})](\text{PF}_6)_2$  were able to induce caspase-dependent and mitochondrial intrinsic apoptosis on human colon carcinoma HCT116 cells by ROS-mediated pathway (26). Complexes of ruthenium have been used in the deprotection of alloc/allyl-protected substrates, and to carry out olefin metathesis in cells (27, 28). Ruthenium complexes have been reported to reduce cofactor nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) to NADH inside cancer cells. This way of toxic activity of ruthenium complexes aims inherent redox vulnerability of cancer cells which is consequence of their dysfunctional mitochondria (28). One emerging tendency is the identification of drug candidates that target the endoplasmic reticulum (ER) with the goal of inducing ER stress and leading to eventual cell death. Ruthenium compounds of various investigators targeted ER (29-35). ROS-mediated ER stress is involved in several novel



strategies for cancer treatment. For example, ROS-mediated ER stress is necessary to activate Type-II immunogenic cell death, a concurrent killing of cancer cells and activation of the immune system, which has been shown to significantly decrease cancer recurrence *in vivo* (29). The endoplasmic reticulum is a key participant in tumor cell apoptosis and drug resistance and, therefore, is one of the main targets in anticancer research. ER stress induced activation of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) leads to the phosphorylation of the eucariotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) that inhibits the translation and subsequently triggers cell cycle arrest (35). Ruthenium complexes are also known to cause induction of oxidative stress or endoplasmic reticulum stress (ERS), and consequent apoptosis of tumor cells (30, 31). Three ruthenium(II) complexes synthesized by Xu et al., namely [Ru(NeN)<sub>2</sub>(HIPMP)](ClO<sub>4</sub>)<sub>2</sub> (N-N = 2,2'-bipyridine (bpy, Ru1), 1,10-phenanthroline (phen, Ru2), and 4,4'-dimethyl-2,2'-bipyridine (dmb, Ru3), were shown to induce apoptosis of human cervical carcinoma cells HeLa through endoplasmic reticulum stress and reactive oxygen species production (29). It is compelling that clinically investigated ruthenium-based metal complex, which showed promising results in solid tumors, sodium trans-[tetrachloridobis(1H-indazole) ruthenate(III)] (NKP-1339), involved both ROS- and ER-related cytotoxic effects in human colon carcinoma cell lines SW480 and HCT116 (33).

Although ruthenium complexes affect DNA and induce ROS-production, it has been discovered that these compounds accumulated in organelles predominantly (31). Mitochondria is a key target of ruthenium complexes, because ruthenium complexes can swiftly reduce mitochondrial membrane potential, leading to dysfunction of mitochondria or mitochondrial apoptosis pathways activation (24, 25, 31).

### Ruthenium(II) complexes, mitochondria and cell death

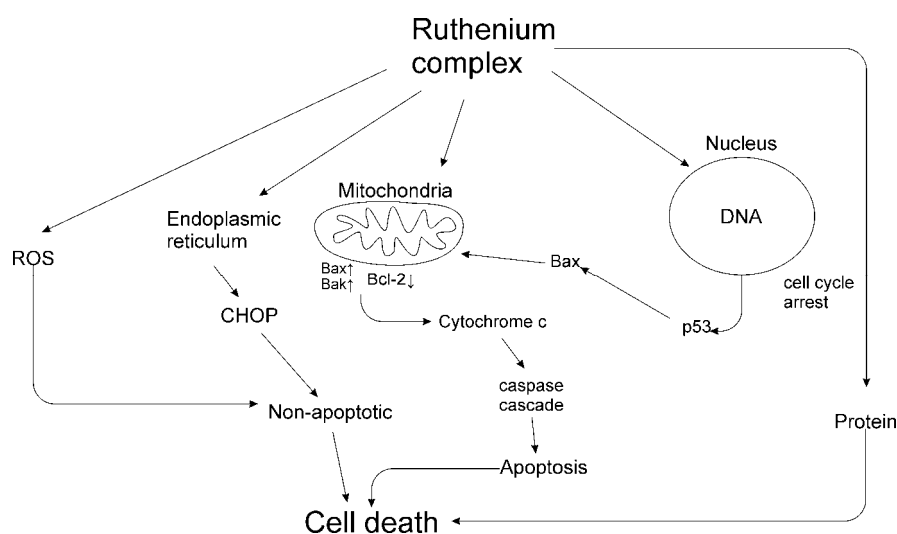
Mitochondria are complex organelles found in almost all eukaryotic cells that play fundamental roles in the regulation of cellular functions. Some nonnuclear targets, and especially mitochondria, have also been reported to be targets for the anticancer activity of Ru(II) compounds (36). Under certain cellular conditions, mitochondria can release molecules that can activate the extrinsic and intrinsic apoptotic pathways (36, 37). As we already stated, ruthenium complexes may affect DNA, mitochondria, endoplasmic reticulum, protein kinases and induce ROS-generation (Figure 1) (24).

Cell death can be result of several mechanisms. Apoptosis is a process of programmed cell death and includes cell membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (38). This process is primarily induced by two basic apoptotic signaling pathways: the extrinsic and the intrinsic pathways (38, 39). Extrinsic apoptosis is activated via specific transmembrane receptors belonging to the tumour necrosis factor (TNF) receptor superfamily (40, 41). The intrinsic pathway, also known as the mitochondria-mediated pathway is activated by various intracellular stimuli, including DNA damage,

growth factor deprivation, oxidative stress and endoplasmic reticulum (ER) stress (42, 43). During mitochondria-mediated pathway, cytochrome c is released from mitochondria into the cytosol of the cell and activates Apaf1 and consequently regulates other proteins involved in apoptosis (42-44). Non-apoptotic cell death includes necrosis and ER stress. Autophagy allows the orderly degradation and recycling of cellular components, and process is regulated by autophagy-related (ATG) proteins (45). The role of autophagy in cancer is complex, and this process can inhibit or promote cell death (45, 46). Necrosis is a form of cell death which results in the premature death of cells (47, 48). Cellular death due to necrosis is different than apoptotic cell death, and result is loss of cell membrane integrity and uncontrolled release of products into the extracellular space (49). Apoptosis and necrosis can be induced by increased production of reactive oxygen species (ROS), including hydrogen peroxide, superoxide anion and nitric oxide, and decreased reduced glutathione levels (50).

### Cytotoxicity of RU(II) complexes

In the research for effective complexes that can be targeted against tumor cells, many groups of researchers synthesized various ruthenium(II) complexes with different ligands (51-53). The first ruthenium(II) arene complexes reported is [Ru( $\eta^6$ -C<sub>6</sub>H<sub>6</sub>)(DMSO)Cl<sub>2</sub>]. Investigations into its antitumor effects showed that the complex strongly inhibited the activity of topoisomerase II which was crucial for structural organization of the mitotic chromosomal scaffold during cell replication process (54). Lazic et al. evaluated cytotoxicity of five Ru(II) terpyridine complexes against human lung carcinoma A549, human colon carcinoma HCT116 and mouse colon carcinoma CT26 cell lines by MTT assay. Complex [Ru(Cl-tpy)(en)Cl][Cl] (**1**), had IC<sub>50</sub> values ranging between 32.80 and 66.30  $\mu$ M and showed the highest anticancer activity compared to the other four ruthenium(II) complexes: [Ru(Cl-tpy)(dach)Cl][Cl] (**2**), [Ru(Cl-tpy)(bpy)Cl][Cl] (**3**), [Ru(tpy)Cl<sub>3</sub>] (**4**) and [Ru(Cl-tpy)(pic)Cl] (**5**) (1). The cytotoxicity of the Ru(II) benzene complexes was screened for a panel of cancer cell lines: human hepatocyte carcinoma HepG2, human lung carcinoma A549, human breast carcinoma MCF7 and human ovary carcinoma cells SKOV3 by Jeyalakshmi et al. Results showed that the complexes [RuCl<sub>2</sub>( $\eta^6$ -benzene) N-(phenylcarbamothioyl)thiophene-2-carboxamide] (**6**), [RuCl<sub>2</sub>( $\eta^6$ -benzene) N-(o-tolylcarbamothioyl)thiophene-2-carboxamide] (**7**) and [RuCl<sub>2</sub>( $\eta^6$ -benzene) N-(o-tolylcarbamothioyl)thiophene-2-carboxamide] (**8**) displayed modest activity against HepG2 cells. Complex **6** showed moderate cytotoxicity against A549 cell lines and had IC<sub>50</sub> value 95,6  $\mu$ M (55). Canovic et al. explored cytotoxic properties of two new monofunctional ruthenium(II) polypyridyl complexes. The cytotoxicity of complexes [Ru(Cl-Ph-tpy)(phen)Cl]Cl (**9**) and [Ru(Cl-Ph-tpy)(o-bqdi)Cl]Cl (**10**) was evaluated against four human cancer cell lines: lung carcinoma A549, breast carcinoma MCF7, cervical carcinoma HeLa and melanoma Hs294T and against one non-cancer, fibroblast cell line MRC-5 by MTT assay. Ruthenium complex **9** displayed high cytotoxic activity against A549 and MCF7 cell lines with IC<sub>50</sub> values of 4.6  $\pm$  2.1 and 13.8  $\pm$  1.8  $\mu$ M.



**Figure 1.** Principal targets and mechanisms of action of ruthenium complexes as oncotherapeutics. Reproduced from reference 24.

The cytotoxicity of complex **9** was about 2 times higher compared to the cytotoxicity of cisplatin under the same conditions. Ruthenium complex **10** significantly decreased viability of A549, MCF7 and HeLa cells, with  $IC_{50}$  values of  $21.7 \pm 4.3$ ,  $4.6 \pm 0.9$  and  $6.4 \pm 1.3$   $\mu\text{M}$ , respectively. Complex **10** showed higher cytotoxic activity against MCF7 and HeLa cells compared to the cytotoxicity of cisplatin under the same conditions. Also, both ruthenium complexes displayed an insignificant effect on viabilities of Hs294T cells and fibroblasts MRC-5 (9). Also, Milutinovic et al. have developed a series of six new monofunctional Ru(II) complexes (**11–16**) of the general formula  $\text{mer-}[\text{Ru}(\text{Cl-Ph-tpy})(\text{N-N})\text{Cl}]\text{Cl}$  (where N-N = en (**11**), dach (**12**) or bpy (**13**)) and  $[\text{Ru}(\text{Cl-tpy})(\text{N-N})\text{Cl}]\text{Cl}$  (where Cl-tpy = 4'-chloro 2,2':6',2''-terpyridine; N-N = en (**14**), dach (**15**) or bpy (**16**)). Only complexes **13** and **14** had  $IC_{50}$  values against non-cancer cells MRC-5 (human fibroblasts) that were less than 100  $\mu\text{M}$  (56). Liao et al. reported three newly synthesized ruthenium(II) polypyridine complexes, which demonstrated potential as anticancer agents:  $[\text{Ru}(\text{bpy})_2(\text{icip})]^{2+}$  (**17**),  $[\text{Ru}(\text{bpy})_2(\text{pdppz})]^{2+}$  (**18**) and  $[\text{Ru}(\text{bpy})_2(\text{tactp})]^{2+}$  (**19**). Cytotoxicity of three newly synthesized polypyridine complexes was tested against human cervical carcinoma HeLa cells and  $IC_{50}$  values for complexes **17**, **18** and **19** were 37.45  $\mu\text{M}$ , 21.37  $\mu\text{M}$  and 23.85  $\mu\text{M}$ , respectively (57).

### Ruthenium compounds and apoptosis

Since all these results have shown that Ru(II) complexes exhibited selective cytotoxic effects against different types of tumor cells, the next step was to determine and explain mechanism of action of Ru(II) complexes. Mazuryk et al. results showed that cellular uptake of Ru(II) complexes occurs through passive diffusion and showed that Ru(II) complexes induced apoptosis of breast cancer (4T1) and human lung adenocarcinoma epithelial cells (A549). Apoptosis was induced

by activation of oxidative stress by both tested Ru(II) complexes (58). Also, Cinara et al. have developed two novel Ru(II) complexes, and they discovered that complexes induced caspase-dependent and mitochondrial activated intrinsic apoptotic pathway in human colon carcinoma HCT116 cells by ROS-mediation (59). Furthermore, Martin et al. designed lipophilic Ru(II) complexes which targeted the lipid-dense endoplasmic reticulum in cells and showed the high anticancer activity against MCF-7 and HeLa cells (60). A series of Ru(II)–polypyridyl complexes synthesized by MacDonnell et al. were proven to induce apoptosis affecting both the intrinsic and extrinsic pathways (61). Recently, Canovic et al. demonstrated that ruthenium complexes decreased Bcl-2/Bax ratio causing cytochrome c mitochondrial release, the activation of caspase-3 and induction of mitochondrial apoptotic pathway (9). Three Ru(II) polypyridyl complexes were synthesized and characterized by Han et al.:  $[\text{Ru}(\text{dmb})_2(\text{HDPIP})](\text{ClO}_4)_2$  (**20**),  $[\text{Ru}(\text{bpy})_2(\text{HDPIP})](\text{ClO}_4)_2$  (**21**) and  $[\text{Ru}(\text{phen})_2(\text{HDPIP})](\text{ClO}_4)_2$  (**22**). Ruthenium complexes **20**, **21** and **22** accumulated in the cell nuclei and increased the ROS levels. Complex **22** showed no impact on the expression of the anti-apoptotic protein Bcl-2, but increased the levels of the pro-apoptotic proteins Bax and Bid (62).

### Ruthenium(II) complexes and cell cycle arrest

Both, induction of apoptosis and/or cell cycle arrest may decrease the viability of cancer cells. Lai et al. demonstrated that four ruthenium(II) polypyridyl complexes inhibited cell growth at the G0/G1 phase in A549 cells, and that the complexes could induce both autophagy and apoptosis (63). However, Mazuryk et al. showed that polypyridyl complexes of ruthenium arrest cell growth in the S-phase and induce apoptosis (58). Furthermore, Canovic et al. showed that ruthenium complex **9** induced G2/M phase arrest in A549 cells and G0/G1 phase arrest in MCF7 and Hs294T cells. Also, complex **10** in-



duced G2/M cell cycle arrest in A549 and HeLa cells, and G0/G1 cell cycle arrest in Hs294T cells (9).

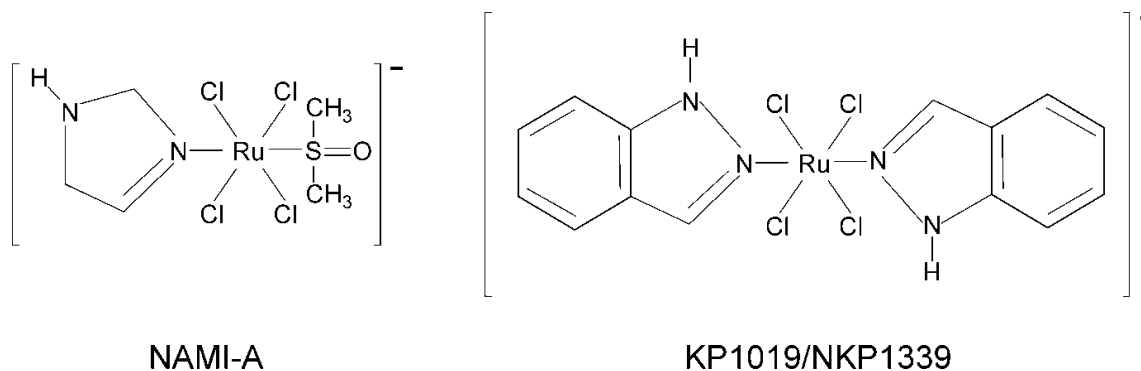
### Ru(II) complexes which entered preclinical and clinical trials

The first step in discovering novel chemotherapeutics is reserved for their evaluation *in vitro*. Afterwards, characterization of the anticancer activity and toxicity *in vivo* of compounds that showed promising results *in vitro*, is fundamental to their further development (64). A number of *in vivo* studies have discovered that Ru(II) complexes exhibited anti-cancer activities comparable to, or even better than cisplatin, but with significantly less toxicity and side effects than cisplatin (65-76). Weiss et al. synthesized ruthenium complex,  $[\text{Ru}(\text{h}^6\text{-p-cymene})\text{Cl}_2(\text{pta})]$ , where pta = 1,3,5-triaza-7-phosphaadamantane (RAPTA-C). This complex decreased the growth of primary tumors in preclinical models for ovarian and colorectal carcinomas via anti-angiogenic mechanism. When the authors applied this complex every day at low doses (0.2 mg/kg), RAPTA-C significantly decreased the growth of the A2780 ovarian carcinoma transplanted onto the chicken chorioallantoic membrane model. Similar effect was noted in LS174T colorectal carcinoma in athymic mice, although at a higher dose (68).

Recent advances in discovering improved selectivity and cytotoxic activity of photocaged complexes led to *in vitro* testing of various ruthenium(II) complexes after their photoactivation by UVA and visible light (77-85). In general, after the excitation of the photosensitizing agent's moiety at suitable wavelength, energy or electron transfer process ultimately lead to the production of ROS which are cytotoxic to living cells and can be used for targeted singlet oxygen chemotherapy (77). Photocaged Ru complexes are usually nontoxic to nonirradiated tissues and can become toxic in tumor cells through photoactivation (78-84). Also, in other cases, light can be used to uncage toxic Ru ligands or species from the complexes for photochemotherapy (85). Therefore, Sun et al. synthesized a novel Ru-containing block copolymer (PolyRu) as a photoactivated polymetallo drug for combined photodynamic therapy and photochemotherapy *in vivo* (69).

They demonstrated *in vivo* that PolyRu accumulated at tumor tissue in mice and reduced the growth of tumors under light irradiation with minimal systemic toxicity (69). Chen et al. reported that complex (LC-003)  $\text{cis-}[\text{RuCl}_2(\text{S-(-)-FOA})(\text{dmsO})_2]$  inhibited tumor growth in BEL-7402 xenograft mouse model via multiple mechanisms that included DNA damage, telomerase dysfunction, inhibition of p53 expression and caspase cascade activation. This complex showed higher *in vivo* safety than cisplatin (70). Other ruthenium complexes also exhibited similar effects *in vivo*; all of them inhibited growth of tumor tissue and produced less systemic toxicity in comparison to cisplatin *in vivo* (71-75). It is worth noting that complexes synthesized by Milutinovic et al., Ru(II)-tpy/ferrocene complexes  $[\text{Ru}(\text{tpy})\text{Cl}_2(\text{mtefc})]$  and  $[\text{Ru}(\text{tpy})\text{Cl}_2(\text{mtpfc})]$  (where tpy = 2,2':6',2''-terpyridine, mtefc = (2-(methylthio)ethyl)ferrocene, and mtpfc = (3-(methylthio)propyl)ferrocene), promoted activation of acquired and innate antitumor immunity, which led to growth reduction of mammary carcinoma *in vivo* (76).

About 50% of patients undergoing chemotherapy receive some type of a platinum medication at this time (24). But, drug resistance to platinum drugs and serious side effects limits its applications (1, 24). Four therapeutics containing ruthenium entered human clinical trials (24, 84). Sadly, the results of phase 1 and phase 2 clinical studies didn't show promising results, which consequently stopped two of these drugs (NAMI-A, and KP1019) from entering to phase III clinical trials (Figure 2) (7, 24). However, remaining two ruthenium compounds are still under consideration in clinical trials: NKP1339 and the theranostic compound TLD1433 (7, 24, 65, 74, 86, 87). We also must mention that 95% of potential oncotherapeutics entering clinical development failed, correlating with an average of 90% for compounds in all therapeutic areas (24). Nevertheless, recent results of *in vitro* and *in vivo* studies mentioned in this review give us hope that designing ruthenium compound that will selectively target tumor cells is a realistic achievement.



**Figure 2.** NAMI-A and KP1019/NKP1339 are ruthenium complexes that entered clinical trials. NAMI-A prevents metastasis and hinder neo-angiogenesis. KP1019 and its sodium salt equivalent, NKP1339, induce G2/M cell cycle arrest by ROS-generation (12).



## CONCLUSIONS

After carefully reviewing the biological activity of ruthenium(II) compounds *in vitro*, it becomes clear that these complexes offer a promising approach to the advancement of new anticancer agents. These compounds exhibit remarkable features, such as low general toxicity against non-tumour cells, the ability to mimic iron binding to transferrin and albumin and exhibition of stronger affinity for tumor tissues over normal tissues. Also, newly synthesized ruthenium(II) complexes showed selective anticancer activity against different types of cancer cells. Activity of ruthenium(II) complexes in some cases is even higher than that of cisplatin against the same type of cancer cells. Precise mechanism of action of ruthenium(II) complexes is still not fully understood. The different examples mentioned in this review showed that ruthenium(II) complexes decrease viability of cancer cells by induction of apoptosis and/or by cell cycle arrest which implies their different mechanism of action against different types of cancer cells.

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