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Organoruthenium Complexes with C^N Ligands are Highly Potent Cytotoxic Agents that Act by a New Mechanism of Action

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Abstract: Our study demonstrates that four novel kinetically inert C,*N*-cyclometalated Ru^{II} complexes of the type $[Ru(C^N)(N^N)_2][PF_6]$ containing a handle for functionalization on the C^N ligand are very potent cytotoxic agents against several different human cancer cell lines and are up to 400-fold more potent than clinically used cisplatin. In addition, the investigated ruthenium complexes are less cytotoxic in noncancerous cells, and exhibit higher selectivity for cancer cells than conventional platinum anticancer drugs. The high potency of the investigated ruthenium compounds can be attributed to several factors, including enhanced internalization and their capability to change mitochondrial transmembrane potential in cells. The new ruthenium complexes also interfere with protein synthesis with a markedly higher potency than conventional inhibitors of DNA translation. Notably, the latter mechanism has not been hitherto described for other cytotoxic Ru compounds and cisplatin.

The clinical success of cisplatin and its derivatives, carboplatin, and oxaliplatin[1] has stimulated the search for alternative transition metal-based drugs that possess anticancer activity.[2] New metal-based anticancer drugs could widen the spectrum of treatable cancers, reduce toxic side effects, and overcome platinum resistance. Several studies focusing on the potential alternatives to platinum-based anticancer drugs have employed ruthenium complexes.[2d,3] Notably, two anionic Ru^{III} coordination compounds, NAMI-A (imidazolium *trans*-[tetrachlorido(1*H*-imidazole)(*S*-dimethyl sulfoxide)ruthenate(III)]) and KP1019 (indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]), have been evaluated in Phase I clinical trials for treating metastatic cancer.

A series of ruthenium(II) complexes that exhibit promising antitumor activity have been prepared and their biological (antitumor) activity evaluated. In general, antitumor Ru^{II} complexes can be divided into two primary families: complexes that adopt the so-called "piano-stool" conformation and heteroleptic cyclometalated/polypyridyl-type complexes.[4,5] Organometallic ruthenium(II) complexes of the type $[(\eta^6\text{-arene})Ru(en)(Cl)]^+$ account for another class of antitumor ruthenium(II) complexes. Their DNA binding mode involves combined coordination to guanine residues and noncovalent, hydrophobic interactions, such as intercalation of the arene ligand into DNA and minor groove binding.[6] Further, a 2-phenylpyridine C^N ligand has been employed to generate heteroleptic ruthenium(II) complexes, so called RDCs (ruthenium-derived compounds), which represent another class of propitious cytotoxic and cytostatic agents.[7] These complexes act by an atypical pathway involving endoplasmatic reticulum stress.

Earlier reports have shown that benzimidazole is a widely used pharmacophore,[8] acting either as *anti*-angiogenic and antitumor agents[9] or amyloid-β aggregation inhibitors.[10] To obtain new biologically active transition-metal complexes with improved ability to kill cancer cells and elucidate the mechanism of action of organometallic complexes with C^N ligands along with the optimization of the choice of the transition-metal moiety and C^N ligands, we designed four kinetically inert ruthenium-based agents of the type $[Ru(C^N)(N^N)_2][PF_6]$ (Figure 1). These complexes contain either (i) an in-house benzimidazole C^N ligand (complexes **1** and **2**) with an ester group for further chemical functionalization and a butyl group for *N*-substitution chosen initially to adjust lipophilic properties of the final complex; or (ii) a modified 2-phenylpyridine/1-phenylpyrazole C^N ligand with an aldehyde group (complexes **3** and **4** respectively, Figure 1). Complexes **3** and **4** were prepared also for comparative purposes and for the possibility to incorporate an additional functionality to further affect their biological potency. It is important to note that, in this context,

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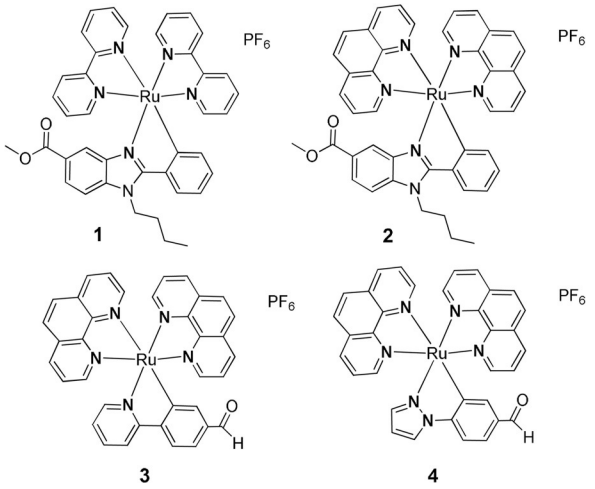

Figure 1. Ruthenium(II) complexes with C^N ligands investigated in this work.

Table 1. Cytotoxicity (IC_{50} mean values, μM) for Ru complexes and cisplatin.[a]

	MRC-5	A2780	A2780cisR[b]	HCT-116	MCF-7
1	5.1 ± 0.5	0.13 ± 0.01	0.067 ± 0.008 (0.50)	0.47 ± 0.03	0.25 ± 0.07
2	2.3 ± 0.4	0.13 ± 0.02	0.045 ± 0.005 (0.35)	0.41 ± 0.01	0.32 ± 0.06
3	8.8 ± 0.2	0.15 ± 0.01	0.14 ± 0.02 (0.94)	1.02 ± 0.04	0.47 ± 0.02
4	8.8 ± 0.1	0.21 ± 0.08	0.12 ± 0.01 (0.61)	0.78 ± 0.09	0.72 ± 0.03
cisplatin	15.2 ± 0.5	3.3 ± 0.8	18 ± 2 (5.28)	5.9 ± 0.9	9 ± 1

[a] The drug-treatment period was 72 h. The results are expressed as mean values ± SD (standard deviation) for two independent samples, each made in quadruplicate. [b] Resistance factor, defined as IC_{50} (resistant, A2780cisR)/IC_{50} (sensitive, A2780), is given in parentheses.

a kinetically inert heteroleptic Rh^{III} complex containing two 2-phenylpyridine C^N ligands with a CHO group that acts as an inhibitor of STAT3 and reduces tumor size and angiogenesis in an in vivo mouse xenograft model was recently reported.[11] Another goal was to study how the nature of the C^N ligand affects the various parameters associated with cytotoxicity (cellular accumulation and localization, mitochondrial dysfunction, cell cycle, protein synthesis, etc.).

Ruthenium complexes were prepared by a synthetic procedure described in the Supporting Information (synthesis and characterization of ruthenium compounds and Figures S1–S4). The final compounds were characterized by elemental analysis, ^{1}H, and ^{13}C NMR spectroscopy, including COSY and HSQC spectroscopy, and UV/Vis absorption spectroscopy; additionally, the structures of **3** and **4** were unambiguously confirmed by the X-ray crystallographic study (for details, see the Supporting Information, synthesis and characterization of ruthenium compounds and Figures S5–S33, Tables S1–S5).

The cytotoxic effects of the investigated Ru compounds were evaluated against four tumor cell lines, ovarian (A2780), cisplatin-resistant ovarian (A2780cisR), colon (HCT-116), and breast (MCF-7) cells. For comparison, the cytotoxic effects of cisplatin were also determined. The toxicities of the complexes were also tested against the noncancerous human lung fibroblasts (MRC-5). All Ru complexes were found to be markedly more cytotoxic than cisplatin, and the IC_{50} values were in the submicromolar range (Table 1).

Interestingly, all investigated Ru complexes were markedly more toxic (125- to 390-fold) than cisplatin in cisplatin resistant A2780cisR cells and (13- to 29-fold) in MCF-7 (inherently resistant to cisplatin). Furthermore, the resistance factor [defined as the ratio of IC_{50} values in resistant (A2780cisR) and cisplatin-sensitive parent cells (A2780)] was 0.35–0.94 for Ru complexes, whereas it was markedly higher for cisplatin (5.28) (Table 1 and Table S6 in the Supporting information). This suggests that the mechanism underlying the biological action of the investigated Ru complexes is different from that of cisplatin and its direct derivatives, allowing the investigated Ru complexes to successfully overcome the resistance mechanisms operating in the case of cisplatin. Importantly, the IC_{50} values found for the investigated Ru complexes in cancer cells tested in our study were 6–76-fold lower than those for non-carcinoma cells MRC-5, whereas the IC_{50} values found for cisplatin under identical conditions in cancer cells were only 0.8–4.6-fold lower than those found for non-carcinoma cells MRC-5 (Table S7 in the Supporting information). Thus, these results confirm a higher selectivity of the investigated Ru compounds for cancer cells compared with cisplatin.

One of the most important factors for the biological activity of metal-based drugs is the intracellular accumulation and the way the complexes are transported into the intracellular space.[12] The key factor relevant for cell uptake and anticancer activity of some metallodrugs is the hydrophobicity of complexes.[13] The total cellular accumulation of the investigated Ru complexes and cisplatin in A2780 and HCT-116 cell lines was studied (see the Supporting Information, biological evaluation–details and Tables S8, S9). The results summarized in Table S8 indicate that the levels of ruthenium associated with the cells after exposure to **1**–**4** of both cell lines were 23- to 66-fold higher than the level of Pt if both cell lines were treated with cisplatin. As also shown in Table S8, the cellular accumulation is a function of hydrophobicity of the metal complex, which suggests that these complexes are mostly dependent on a passive diffusion uptake mechanism. The extent of cellular accumulation also correlates with the cytotoxicity of the complexes. As presented in Table 1 and Table S8 in the Supporting information, **1** and **2** with the highest level of cellular accumulation are the most hydrophobic and also exhibit the best cytotoxic effects. An interesting summary of these results is that the presence of C^N ligands in the Ru complexes ensures hydrophobicity, thereby significantly facilitating the association of these compounds with cells, and enhancing the cytotoxicity of this class of Ru complexes. This is in agreement with a similar observation previously found by Chao et al. for related ruthenium heteroleptic polypyridyl-type complexes.[11]

Cellular accumulation studies were also performed to investigate the localization of the investigated Ru complexes and cisplatin in A2780 cells treated with these complexes (see Supporting Information). The results (Table S9 in the Supporting In-

formation) indicate that the largest amounts of ruthenium from **1**–**4** become trapped in the cell membrane(s), probably due to their lipophilicity. An appreciable amount is also detected in cytosol. Minor amounts of Ru from complexes **1**–**4** were found in the cytoskeleton and nucleus. Thus, the results demonstrating cellular accumulation of ruthenium suggest that nuclear macromolecules including DNA are not a likely target for cytotoxic effects of this class of Ru complexes.

In contrast, it has been proposed that in the case of several metallodrugs, DNA damage plays a key role in the mechanism of their antitumor action. To determine whether DNA is a pharmacological target for the investigated Ru complexes, the sensitivity of DNA repair (NER)-deficient cells towards these complexes and cisplatin was tested and compared to that of isogenic DNA repair-proficient cells.[14] The details of this approach and the detailed results are described in the Supporting Information (biological evaluation–details). The results (Table S10 in the Supporting Information) indicate that in contrast to cisplatin, there is no significant difference in cell-killing of wild type cells and cells deficient in DNA repair by the investigated Ru complexes. These data clearly show that, in contrast to cisplatin, DNA-repair status does not significantly affect the effectiveness of Ru complexes, suggesting that reparable DNA damage is not the major factor contributing to the cytotoxic activity of the Ru complexes.

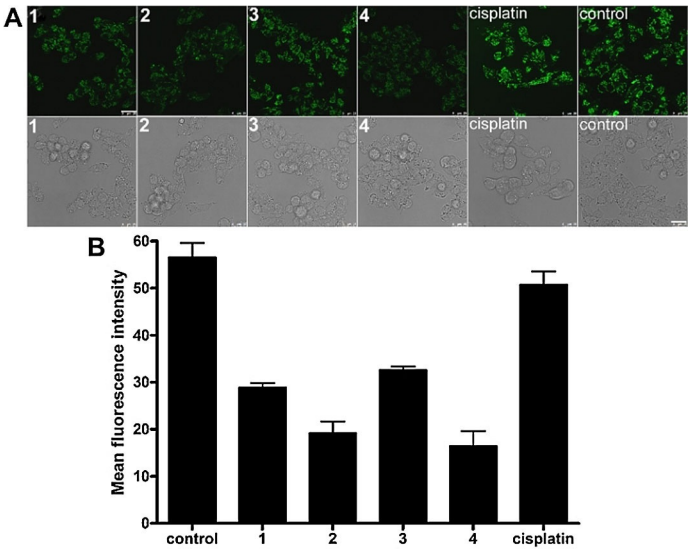

Figure 2. A) Fluorescence images of A2780 cells untreated or treated with equitoxic concentrations (IC_{50}, 72 h) of cisplatin, **1**–**4** for 18 h which were stained with the mitochondria-selective dye TMRE for changes in mitochondrial membrane potential and then analyzed by confocal laser-scanning microscope. Top row: fluorescence channel, bottom row: bright field. Scale bars represent 25 μm. Images are the representatives from three independent experiments. B) Quantification of mitochondrial dysfunction. Single-cell analysis was performed by using ImageJ software and results are expressed as the mean fluorescence intensity per cell; error bars are the standard deviations calculated from at least one hundred single cells analyzed out from three independent experiments.

There is a large body of evidence indicating that antitumor Ru complexes may be effective as mitochondria-targeting anticancer drugs.[15] We determined changes in the mitochondrial transmembrane potential ($\Delta\psi_m$) in A2780 cells induced by the investigated Ru compounds and cisplatin (for comparative purposes) by a TMRE staining assay (tetramethylrhodamide methyl ester). TMRE is a lipophilic but slightly cationic fluorescent dye that is preferentially sequestered by active mitochondria. Inactive mitochondria are characterized by reduced membrane potential and consequently do not sequester TMRE.[16] The depletion of $\Delta\psi_m$ was then monitored by a confocal microscope, as described in the Experimental section in the Supporting Information. The results (Figure 2) showed that in the TMRE assay, the signals observed by confocal microscopy (fluorescence channel) were not significantly changed from the A2780 cells treated for 18 h with cisplatin (Figure 2 A, image 6 and Figure 2 B). In contrast, if the A2780 cells were treated under the same conditions, the signals were strongly attenuated in the case of the investigated Ru complexes (Figure 2 A, images 1–4 and Figure 2 B).

Thus, complexes **1**–**4** caused a significant decrease in the mitochondrial transmembrane potential in comparison with non-treated control cells (Figure 2), which is a hallmark of mitochondria dysfunction. However, it has to be noted that on the basis of these results, it cannot be definitely decided whether the mitochondria are the primary target for these complexes or whether the mitochondrial membrane depolarization represents a downstream effect related to the cell death (apoptosis) (Figure S34 in the Supporting information) primarily induced by the effect on another cellular site(s).

The perturbations in mitochondrial function associated with reduced mitochondrial membrane potential may result in the oxidative stress related to increased generation of reactive oxygen species (ROS). Intracellular ROS were quantified to determine the oxidative stress in A2780 cells after the treatment with **1**, **3**, or cisplatin as described in the Supporting Information. Treatment of A2780 cells with **1** and **3** resulted in a concentration-dependent increase in the production of ROS (Figure S35 in the Supporting Information). In contrast, the effect of cisplatin on ROS production in treated cells was insignificant. This observation suggests that the investigated Ru complexes can cause disruption of mitochondrial function and associate production of reactive oxygen species (ROS).

It has been shown[16,17] that impedance-based monitoring of cellular responses to biologically active small molecule compounds produces TCRPs (time-dependent cell response profiles), which can be predictive of the mechanism of action of small molecules. The TCRPs of cisplatin and Ru complexes **1**–**4** are displayed in Figures 3 A–E.

Significant differences in the effects of the investigated Ru complexes and cisplatin can be observed. The effect of cisplatin (Figure 3 E) is characterized by a slight initial increase in the cell index in comparison with the control, followed by a concentration-dependent decrease in the cell index below control levels, reflecting cytotoxic responses; cisplatin at the concentration corresponding to IC_{90} fails to kill adherent cells completely even at the longest times of their growth. The TCRP of

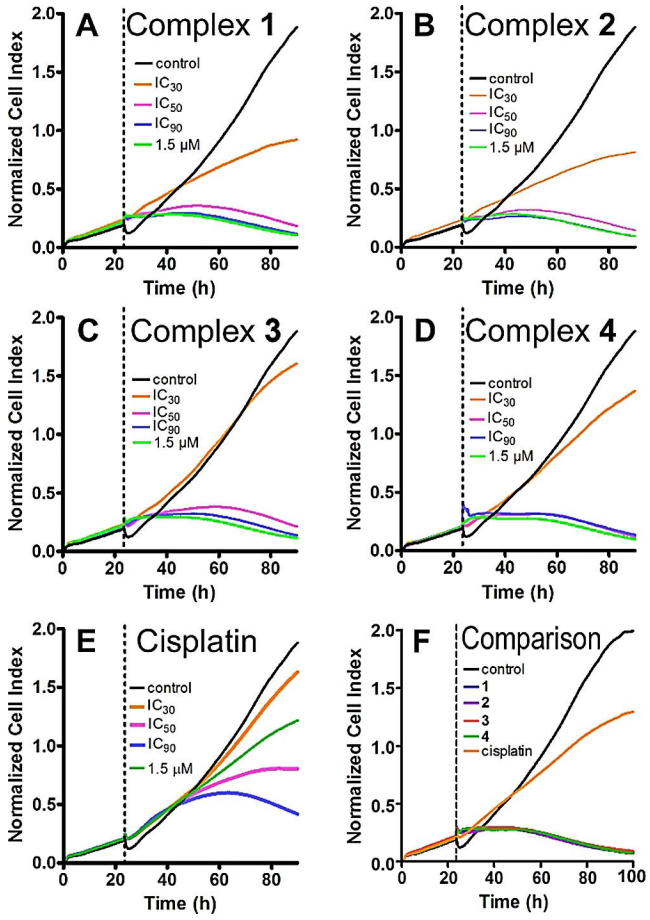

Figure 3. Interaction of **1** (A), **2** (B), **3** (C), **4** (D), and cisplatin (E) with A2780 cells monitored by a real-time cell analyzer (RTCA). For **1**: IC_{30} = 0.08 μM, IC_{50} = 0.13 μM, IC_{90} = 0.4 μM; for **2**: IC_{30} = 0.08 μM, IC_{50} = 0.12 μM, IC_{90} = 0.40 μM; for **3**: IC_{30} = 0.1 μM, IC_{50} = 0.15 μM, IC_{90} = 0.6 μM; for **4**: IC_{30} = 0.1 μM, IC_{50} = 0.20 μM, IC_{90} = 0.60 μM; for cisplatin: IC_{30} = 1.0 μM, IC_{50} = 3.0 μM, IC_{90} = 8.0 μM. F: A comparison of RTCA profiles found for **1**, **2**, **3**, **4**, and cisplatin in their equimolar concentrations (1.5 μM). The vertical lines indicate the start of treatment after allowing the cells to grow and adhere to microelectrodes for 24 h. Cell indices were normalized to account for differences in cell counts that exist across the wells prior to treatment. Incubations were performed in quadruplicate with 10000 cells per well using inhibitory drug concentrations determined for 72 h of incubation in colorimetric cell viability assay.

cisplatin (Figure 3E) coclustered with the TCRPs of DNA-damaging compounds interfering with DNA synthesis and replication and transcription, which are also known to induce cell-cycle arrest in S or G2/M phases followed by the induction of cell death.[16,17] In contrast, treatment with IC_{50}, IC_{90}, or equimolar (1.5 μM) concentrations of the investigated Ru complexes (Figures 3A–D) resulted in an immediate, more pronounced decrease in the cell index and complete killing of adherent cells at the longest times of cell growth. Thorough examination of the profile obtained for the investigated Ru complexes (Figure 3A–D) revealed that their TCRPs can be coclustered with subclusters containing TCRPs for compounds inhibiting protein translation and inducing cell-cycle arrest principally in G1, S, or G1/S phases, followed by initiation of cell death.[17]

This conclusion was further supported by the results of cell cycle analysis by flow cytometry. The results of this analysis (Figures S36, S37 and Table S11 in the Supporting Information) show the cell-cycle arrest primarily in the G1 phase if the A2780 cells were treated with **1** or **3**. For comparative purposes, cell cycle analysis was also performed with the A2780 cells treated with cisplatin. Cisplatin caused cell-cycle arrest mainly in the S and G2 phases, which is consistent with previous reports.[18] The effect on cellular distribution into the particular cell cycle phases was manifested in a concentration-dependent manner and is elevated with increasing concentrations of Ru complexes (Figure S37 in the Supporting Information). Thus, the ability of the investigated Ru complexes to block the cell cycle primarily in the G1 phase is consistent with the conclusion drawn from the analysis of TCRP profiles (Figure 3).

The impedance-based TCRP obtained for the investigated Ru complexes (Figure 3) revealed that these TCRPs can be interpreted to mean that a possible mechanism of action of Ru complexes may involve inhibition of protein translation. In order to verify the inhibition effect of Ru complexes on protein synthesis in living cells, the incorporation of [^{35}S] methionine ([^{35}S]-Met) was detected as a measure of translation activity of the cells in the presence of Ru complexes). In these experiments, we intentionally used low concentrations of **1** or **3** and short incubation times (0.5 and 1 h) to secure cell viability so as to mainly detect the upstream effects of the Ru complexes. A2780 cells were incubated with [^{35}S]-Met for 2 h to allow for their incorporation into newly synthesized proteins. Ruthenium complexes significantly decreased protein synthesis (Figure 4).

To verify that the observed effect of Ru complexes on protein synthesis is specific, we evaluated how they affect both

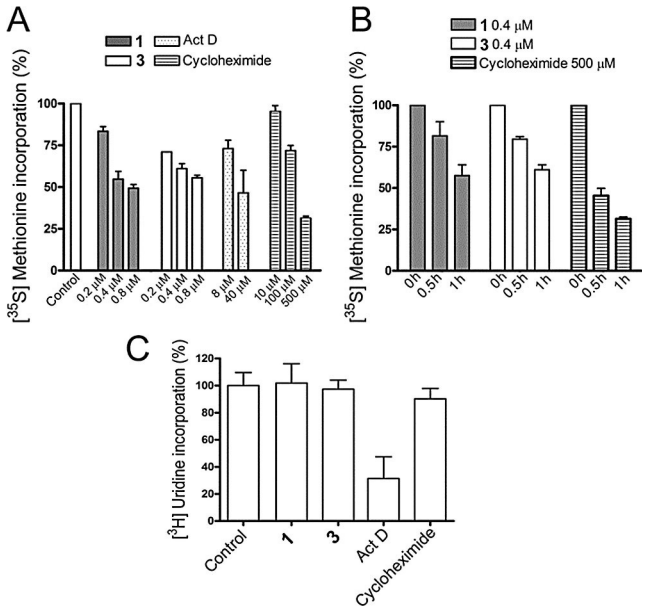

Figure 4. Effect of Ru complexes on translation and transcription in A 2780 cells as measure of incorporation of ^{35}S-methionone or ^{3}H-uridine, respectively. The radioactivities of control, untreated cells were taken as 100%. A) Cells were treated with **1**, **3**, Actinomycin D, and cycloheximide at indicated concentrations for 1 h. B) Cells were treated with **1** (0.4 μM), **3** (0.4 μM), and cycloheximide (500 μM) for indicated time intervals. C) Cells were treated with **1** (0.8 μM), **3** (0.8 μM), Actinomycin D (40 μM), cycloheximide (500 μM) for 1 h.

translation and transcription by metabolic labeling. Transcriptional activity was monitored by incubation with [^{3}H]uridine for 2 h. Actinomycin D (ActD) and cycloheximide served as controls as well-known transcription and translation inhibitors, respectively. As expected, cycloheximide strongly inhibited translation but only affected transcription at high doses, whereas ActD concomitantly blocked both transcription and translation (Figure 4) since protein synthesis requires a supply of mRNA. Similarly to cycloheximide, both **1** and **3** inhibited protein synthesis without a significant impact on transcription. Our results also show that these Ru complexes are capable of inhibiting translation at concentrations approximately three orders of magnitude lower than conventional cycloheximide, a well-known specific inhibitor of protein synthesis in eukaryotic cells.[19]

The results of this experiment show that the inhibition of protein synthesis takes place at a translational but not a transcriptional level (Figure 4). The membrane fraction obtained after cell fractionation (Table S9 in the Supporting Information) contained whole cellular membranes including the cellular organelles membranes with their membrane proteins (but excluding the nuclear membrane and nuclear membrane proteins) [see the protocols for use of FractionPREP cell fractionation kit from BioVision (Mountain View, CA)]. Hence, the prevailing accumulation of Ru from the investigated Ru complexes in the membrane fraction is not in contradistinction with the assumption that the investigated Ru complexes inhibit protein synthesis by interactions with ribosomes or tRNA at endoplasmatic reticulum, where translation takes place.

Collectively, the ruthenium complexes tested in the present work are significantly more potent than clinically used cisplatin and its derivatives in cancer cells, and their therapeutic index is at least 10 times higher than that found for clinically used cisplatin. The data presented in this work also demonstrate that the investigated ruthenium complexes differ significantly from the previously reported cytotoxic ruthenium-based compounds and cisplatin in their mechanism of action. They interfere with protein synthesis, which is a mechanism hitherto not described for other cytotoxic ruthenium-based compounds and cisplatin. On the other hand, it was shown very recently[20] that in contrast to the presumed DNA-damage-response mechanism of antitumor action of clinically used platinum drug oxaliplatin, oxaliplatin in fact kills cells by inducing ribosome biogenesis stress. Thus, oxaliplatin, unlike cisplatin, was shown to markedly perturb the global translation machinery, thereby disturbing the synthesis of the entire set of proteins, similar to that reported in this communication for the investigated Ru complexes.

Further, importantly, the cycloruthenated complexes examined in the present work inhibit protein synthesis with a markedly higher potency than the conventional inhibitors. The unique mechanism of action together with their high selectivity to the cancer over the normal cells predestines these complexes and their derivatives for further biological testing as potent anticancer drugs.

Last but not least here we demonstrate that the investigated Ru complexes cause mitochondrial dysfunction involving ROS production as well. Thus, it cannot be excluded that the investigated Ru complexes exhibit a dual effect in killing cancer cells causing primarily inhibition of proteosynthesis and in a lesser extent disruption of mitochondrial function simultaneously.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: anticancer agents · cell growth · mitochondrial disfunction · protein synthesis · ruthenium

[1] a) L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573 – 584; b) T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, *116*, 3436 – 3486.

[2] a) C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391 – 401; b) A. Casini, C. G. Hartinger, A. A. Nazaroy, P. J. Dyson, in *Med. Organometal. Chem. Vol. 32* (Eds.: G. Jaouen, N. MetzlerNolte), **2010**, pp. 57 – 80; c) E. Alessio, *Bioinorganic Medicinal Chemistry*, Wiley-VCH, **2011**; d) A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen, G. Sava, *J. Inorg. Biochem.* **2012**, *106*, 90 – 99; e) N. P. E. Barry, P. J. Sadler, *Pure Appl. Chem.* **2014**, *86*, 1897 – 1910.

[3] a) N. Muhammad, Z. Guo, *Curr. Opin. Chem. Biol.* **2014**, *19*, 144 – 153; b) B. S. Murray, M. V. Babak, C. G. Hartinger, P. J. Dyson, *Coord. Chem. Rev.* **2016**, *306*, 86 – 114.

[4] a) S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, M. A. Zoroddu, *Coord. Chem. Rev.* **2015**, *284*, 329 – 350; b) H. Y. Huang, P. Y. Zhang, H. M. Chen, L. N. Ji, H. Chao, *Chem. Eur. J.* **2015**, *21*, 715 – 725.

[5] P. Nowak-Sliwinska, J. R. van Beijnum, A. Casini, A. A. Nazarov, G. WagnieĚ€res, H. van den Bergh, P. J. Dyson, A. W. Griffioen, *J. Med. Chem.* **2011**, *54*, 3895 – 3902.

[6] O. Novakova, H. Chen, O. Vrana, A. Rodger, P. J. Sadler, V. Brabec, *Biochemistry* **2003**, *42*, 11544 – 11554.

[7] a) C. Gaiddon, P. Jeannequin, P. Bischoff, M. Pfeffer, C. Sirlin, J. P. Loeffler, *J. Pharmacol. Exp. Ther.* **2005**, *315*, 1403 – 1411; b) X. Meng, M. L. Leyva, M. Jenny, I. Gross, S. Benosman, B. Fricker, S. Harlepp, P. Hebraud, A. Boos, P. Wlosik, P. Bischoff, C. Sirlin, M. Pfeffer, J.-P. Loeffler, C. Gaiddon, *Cancer Res.* **2009**, *69*, 5458 – 5466; c) V. Vidimar, X. Meng, M. Klajner, C. Licona, L. Fetzer, S. Harlepp, P. Hébraud, M. Sidhoum, C. Sirlin, J.-P. Loeffler, G. Mellitzer, G. Sava, M. Pfeffer, C. Gaiddon, *Biochem. Pharmacol.* **2012**, *84*, 1428 – 1436; d) M. Klajner, C. Licona, L. Fetzer, P. Hebraud, G. Mellitzer, M. Pfeffer, S. Harlepp, C. Gaiddon, *Inorg. Chem.* **2014**, *53*, 5150 – 5158; e) C. Licona, M.-E. Spaety, A. Capuozzo, M. Ali, R. Santamaria, O. Armant, F. Delalande, A. Van Dorsselaer, S. Cianferani, J. Spencer, P. Michel, G. Mellitzer, C. Gaiddon, *Oncotarget* **2016**, *8*, 2568 – 2584; f) C. Gaiddon, M. Pfeffer, *Eur. J. Inorg. Chem.* **2017**, *12*, 1639 – 1654.

[8] a) M. Boiani, M. Gonzalez, *Mini-Rev. Med. Chem.* **2005**, *5*, 409 – 424; b) Y. Bansal, O. Silakari, *Bioorg. Med. Chem.* **2012**, *20*, 6208 – 6236.

[9] a) G. S. Yellol, A. Donaire, J. G. Yellol, V. Vasylyeva, C. Janiak, J. Ruiz, *Chem. Commun.* **2013**, *49*, 11533 – 11535; b) J. Yellol, S. A. Perez, A. Buceta, G. Yellol, A. Donaire, P. Szumlas, P. J. Bednarski, G. Makhloufi, C.

Janiak, A. Espinosa, J. Ruiz, *J. Med. Chem.* **2015**, *58*, 7310 – 7327; c) J. Yellol, S. A. Pérez, G. Yellol, J. Zajac, A. Donaire, G. Vigueras, V. Novohradsky, C. Janiak, V. Brabec, J. Ruiz, *Chem. Commun.* **2016**, *52*, 14165 – 14168.

[10] G. S. Yellol, J. G. Yellol, V. B. Kenche, X. M. Liu, K. J. Barnham, A. Donaire, C. Janiak, J. Ruiz, *Inorg. Chem.* **2015**, *54*, 470 – 475.

[11] a) D. L. Ma, L. J. Liu, K. H. Leung, Y. T. Chen, H. J. Zhong, D. S. H. Chan, H. M. D. Wang, C. H. Leung, *Angew. Chem. Int. Ed.* **2014**, *53*, 9178 – 9182; *Angew. Chem.* **2014**, *126*, 9332 – 9336; b) H. J. Zhong, L. H. Lu, K. H. Leung, C. C. L. Wong, C. Peng, S. C. Yan, D. L. Ma, Z. W. Cai, H. M. D. Wang, C. H. Leung, *Chem. Sci.* **2015**, *6*, 5400 – 5408.

[12] D. P. Gately, S. B. Howell, *British J. Cancer* **1993**, *67*, 1171 – 1176.

[13] a) S. Y. Loh, P. Mistry, L. R. Kelland, G. Abel, K. R. Harrap, *Br. J. Cancer* **1992**, *66*, 1109 – 1115; b) S. P. Oldfield, M. D. Hall, J. A. Platts, *J. Med. Chem.* **2007**, *50*, 5227 – 5237; c) M.-G. Mendoza-Ferri, C. G. Hartinger, R. E. Eichinger, N. Stolyarova, K. Severin, M. A. Jakupec, A. A. Nazarov, B. K. Keppler, *Organometallics* **2008**, *27*, 2405 – 2407; d) P. Gramatica, E. Papa, M. Luini, E. Monti, M. Gariboldi, M. Ravera, E. Gabano, L. Gaviglio, D. Osella, *J. Biol. Inorg. Chem.* **2010**, *15*, 1157 – 1169; e) Z. Liu, L. Salassa, A. Habtemariam, A. M. Pizarro, G. J. Clarkson, P. J. Sadler, *Inorg. Chem.* **2011**, *50*, 5777 – 5783.

[14] N. P. Johnson, J.-L. Butour, G. Villani, F. L. Wimmer, M. Defais, V. Pierson, V. Brabec, *Prog. Clin. Biochem. Med.* **1989**, *10*, 1 – 24.

[15] L. L. Zeng, Y. Chen, J. P. Liu, H. Y. Huang, R. L. Guan, L. N. Ji, H. Chao, *Sci. Rep.* **2016**, *6*, 19449.

[16] V. Novohradsky, L. Zerzankova, J. Stepankova, A. Kisova, H. Kostrhunova, Z. Liu, P. J. Sadler, J. Kasparkova, V. Brabec, *Metallomics* **2014**, *6*, 1491 – 1501.

[17] a) Y. A. Abassi, B. Xi, W. F. Zhang, P. F. Ye, S. L. Kirstein, M. R. Gaylord, S. C. Feinstein, X. B. Wang, X. Xu, *Chem. Biol.* **2009**, *16*, 712 – 723; b) B. Liskova, L. Zerzankova, O. Novakova, H. Kostrhunova, Z. Travnicek, V. Brabec, *Chem. Res. Toxicol.* **2012**, *25*, 500 – 509.

[18] a) H. Chen, C. N. Landen, Y. Li, R. D. Alvarez, T. O. Tollefsbol, *J. Oncol. Pharm. Pract.* **2013**, *19*, 111; b) R.-R. Mi, H. Ni, *Gynecol. Oncol.* **2003**, *90*, 238 – 244; c) X. Li, G. Wang, J. Zhao, H. Ding, C. Cunningham, F. Chen, D. C. Flynn, E. Reed, Q. Q. Li, *Cell. Mol. Life Sci.* **2005**, *62*, 894 – 904.

[19] T. Schneider-Poetsch, J. H. Ju, D. E. Eyler, Y. J. Dang, S. Bhat, W. C. Merrick, R. Green, B. Shen, J. O. Liu, *Nat. Chem. Biol.* **2010**, *6*, 209 – 217.

[20] P. M. Bruno, Y. Liu, G. Y. Park, J. Murai, C. E. Koch, T. J. Eisen, J. R. Pritchard, Y. Pommier, S. J. Lippard, M. T. Hemann, *Nat. Med.* **2017**, *23*, 461 – 471.

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