Supplementary Information

Biological effect of nitroimidazole derivative of polypyridyl Ru complex on cancer and endothelial cells

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Scheme S1. Schematic representation of the determination of retention of [Ru(dip)\textsubscript{2}(bpy-NitroIm)]\textsuperscript{2+} in A549 and MLuMEC cell lines.

Fig. S1. Time dependence accumulation of [Ru(dip)\textsubscript{2}(bpy-NitroIm)]\textsuperscript{2+} (4 µM) under normoxic (filled) and hypoxic (dashed) conditions in MLuEC FVB cells in medium with 2% serum.
Fig. S2. Time dependence accumulation of $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$(2 and 4 µM) under normoxic (filled) and hypoxic (dashed) conditions in A549 cells in medium without $(S^-)$ or with $(S^+, 2\%)$ serum.

Fig. S3. Influence of serum addition on accumulation of $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$(2 µM) in A549 cells lines under normoxic conditions for various incubation time. The filled bars denote serum free $(S^-)$ while the dashed bars stand for the presence of serum $(S^+, 2\%)$.

Fig. S4. Accumulation profile of $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$(4µM) in 4T1 cells conducted in the presence of 2% serum under normoxic (red) and hypoxic (blue) conditions after 2 (A), 4 (B) and 24 h (C) incubation time. Black line stands for control.
Fig. S5. Concentration dependence of accumulated [Ru(dip)$_2$(bpy-NitroIm)]$^{2+}$ in MLuEC FVB cells conducted in medium without serum under normoxic (filled bar) and hypoxic (dashed bar) conditions after 2 (blue), 4 (red) and 24 h (black) of incubation time.

Fig. S6. Confocal microscopy of A549 cells showing subcellular localization of [Ru(dip)$_2$(bpy-NitroIm)]$^{2+}$. (A, B, C) CellLight® ER-RFP was used to image endoplasmic reticulum (ER). (D, E, F) CellLight® Golgi-RFP was used to image Golgi. Green color denotes intrinsic emission of Ru complexes (8 µM, 30 min of incubation at 37 °C), whereas
red color arises from organelle-specific dyes. The yellow color occurs due to the overlap of the green luminescence from the $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$ and red emission from dyes, indicating co-localization. Ru complex primary localizes in ER. Scale bar is 20 µm.

Fig. S7. Fluorescence imaging of MLuMEC FVB cells incubated with 6 µM $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$ for 1 h at 37 °C observed in fixed (A) and alive (B) cells.

Fig. S8. Effect of incubation temperature on $[\text{Ru(dip)}_2(\text{bpy})]^2^+$ (A) and $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^2^+$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h (black – white cells, blue – incubation at 4 °C, green – incubation at 20 °C, red – incubation at 37 °C).
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Fig. S9. Effect of membrane fluidity on $[\text{Ru(dip)}_2(\text{bpy})]^{2+}$ (A) and $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h with (red) or without (black) pre-incubation with MβCD.

Fig. S10. Fluorescence imaging of MLuMEC FVB cells incubated with 2 µM $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^{2+}$ for 24 h at 37 °C. The dot-like staining pattern of labelling suggests accumulation of Ru complex in endosomes.

Fig. S11. Effect of depolarization of membrane with gramicidin on $[\text{Ru(dip)}_2(\text{bpy})]^{2+}$ (A) and $[\text{Ru(dip)}_2(\text{bpy-NitroIm})]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h with (red) or without (green) 30 min pre-incubation with 5 µM gramicidin.
Fig. S12. Effect of hyperpolarization of membrane with valinomycin on [Ru(dip)₂(bpy)]^{2+} (A) and [Ru(dip)₂(bpy-NitroIm)]^{2+} (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h with (blue) or without (red) 30 min pre-incubation with 50 µM valinomycin.

Fig. S13. DIC (C) and corresponded fluorescence (D) images of 4T1 cells after treatment with 8 µM [Ru(dip)₂(bpy-NitroIm)]^{2+} for 24 h. (A) and (B) show control cells.

Fig. S14. The effect of [Ru(dip)₂(bpy-NitroIm)]^{2+} (A, C) and [Ru(dip)₂(bpy)]^{2+} (B, D) in the resistance of trypsion test for 4T1 cancer cells (A, B) and MLuMEC cells (C, D).
Fig. S15. The relative mRNA expression level of several genes (fold change) after 24 h incubation with [Ru(dip)$_2$(bpy-NitroIm)]$^{2+}$ (filled bars) and [Ru(dip)$_2$(bpy)]$^{2+}$ (dashed bars) under normoxic (A) and hypoxic (B) conditions in 4T1 cell line. The results presented in graphs are means ± SEM of the experiments performed in three biological replicates. Student’s t-test was used for statistical analyses: *p<0.05 was considered statistically significant.
Fig. S16. The relative mRNA expression level of several genes (fold change) after 24 h incubation with [Ru(dip)$_2$(bpy-NitroIm)]$^{2+}$ (filled bars) and [Ru(dip)$_2$(bpy)]$^{2+}$ (dashed bars) under normoxic (A) and hypoxic (B) conditions in MLuMEC cell line. The results presented in graphs are means ± SEM of the experiments performed in three biological replicates. Student’s t-test was used for statistical analyses: *p<0.05 was considered statistically significant.