Supporting Information

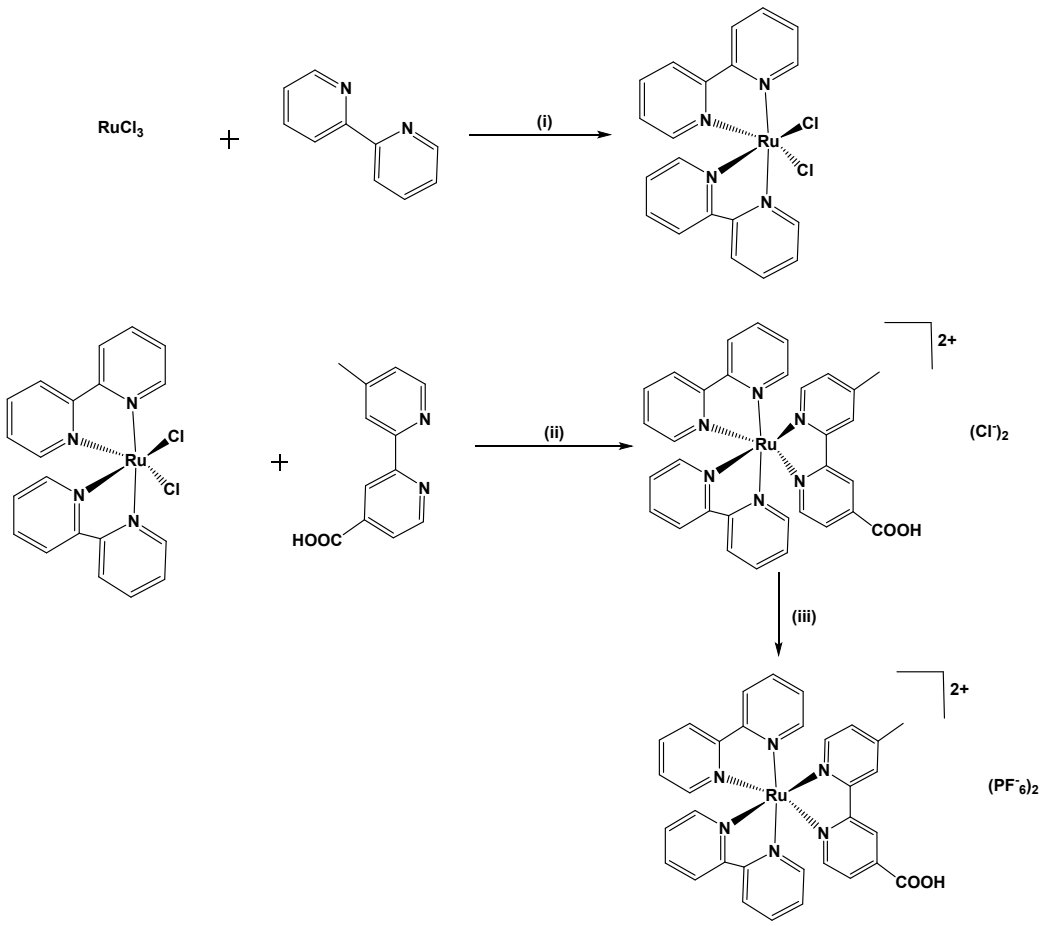
Development of novel GnRH and Tat$^{48-60}$ based luminescent agents with enhanced cellular uptake and bioimaging properties

Anastasia Kougioumtzi[1], Maria V. Chatziathanasiadou[2], Eirinaios I. Vrettos[2], Nisar Sayyad[2], Mariana Sakka[2], Panagiotis Stathopoulos[2], Michalis D. Mantzaris[1], Ab Majeed Ganai[5], Rajshekhar Karpoormath[5], Georgios Vartholomatos[3], Vassilios Tsikaris[2], Theodore Lazarides[4], Carol Murphy[1], Andreas G. Tzakos*[2,6]

[1]Institute of Molecular Biology & Biotechnology, Foundation of Research and Technology-Hellas, Department of Biomedical Research, University Campus, 45110 Ioannina, Greece
[2]University of Ioannina, Department of Chemistry, Section of Organic Chemistry and Biochemistry, 45110, Ioannina, Greece
[3]Hematology Laboratory, Unit of Molecular Biology, University Hospital of Ioannina, Ioannina, 45110 Greece
[4]Laboratory of Inorganic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[5]Department of Pharmaceutical Chemistry, Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal (Westville), Durban 4000, South Africa
[6]University Research Center of Ioannina (URCI), Institute of Materials Science and Computing, Ioannina, Greece

***Corresponding author**

Andreas G. Tzakos, Department of Chemistry, University of Ioannina, 45110, Greece; Tel.: +30 2651008387, email: atzakos@uoi.gr



Scheme S1: Synthesis of the chromophore group $[Ru(bipy)_2(mcb)](PF_6)_2$. **(i)** LiCl in DMF; **(ii)** MeOH; **(iii)** NH_4PF_6.

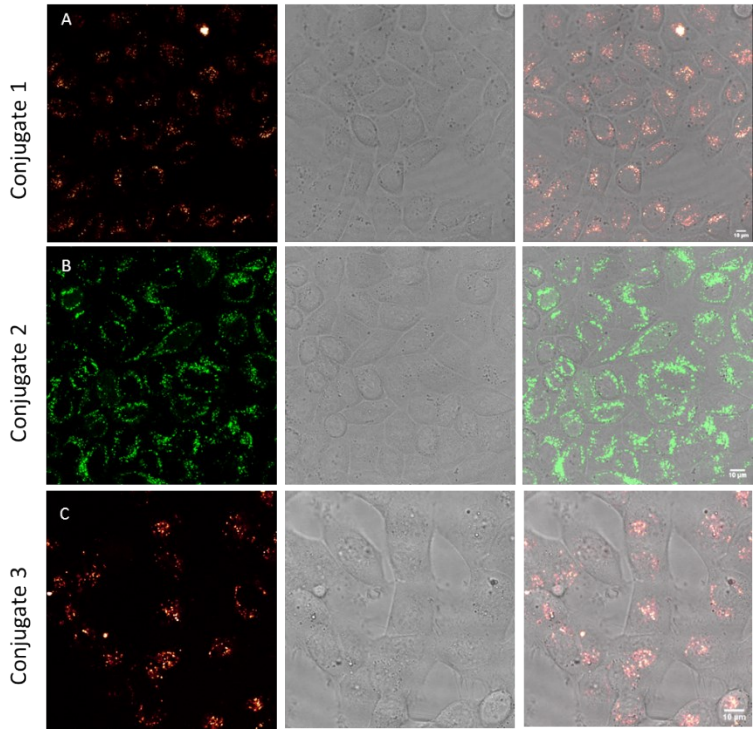


Figure S1. Cellular uptake of the conjugates 1-3 by live cell confocal microscopy after acid stripping. Before live cell microscopy analysis, the cells were washed 3 times with Phosphate Buffered Saline (PBS), acid washes were performed twice with plain medium (pH 2) and 1 mL of fresh medium was added to the dishes. **A.** Fluorescence analysis of HeLa cells incubated at 37 °C for 2 hours with 25 μM of **conjugate 1**. **B.** Fluorescence analysis of HeLa cells incubated at 37 °C for 2 hours with 25 μM of **conjugate 2**. C. Fluorescence analysis of T24 cells incubated at 37 °C for 24 hours with 25 μM of **conjugate 3**. Samples were analyzed with LSCM using a Leica Sp5 confocal microscope. Representative fluorescence and bright-field images are shown. In merge images Scale bar = 10 μM.

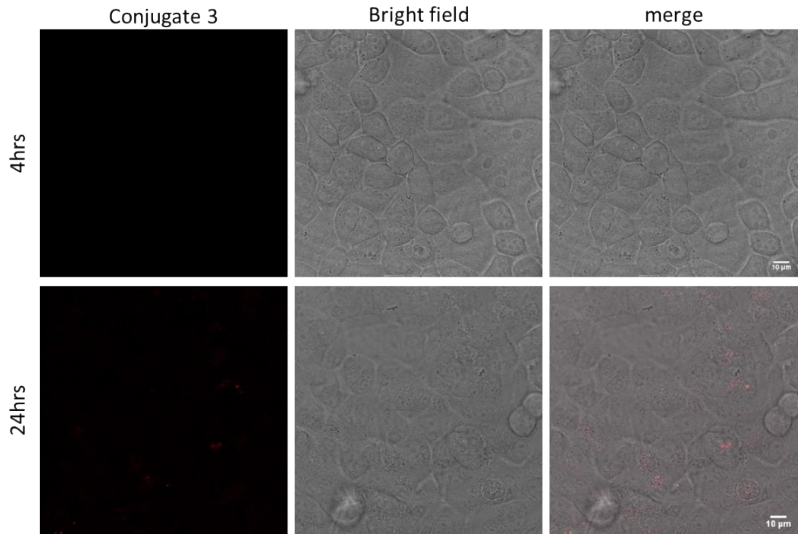


Figure S2. **Cellular uptake of the conjugate 3 in HeLa cells by live cell confocal microscopy.** Live-cell microscopy on HeLa cells incubated at 37 °C for 4 hours and 24 hours with 25 μM of **conjugate 3**. Samples were analyzed with LSCM using a Leica Sp5 confocal microscope. Representative fluorescence and bright-field images are shown. In merge images Scale bar = 10 μM.

Photophysical characterization

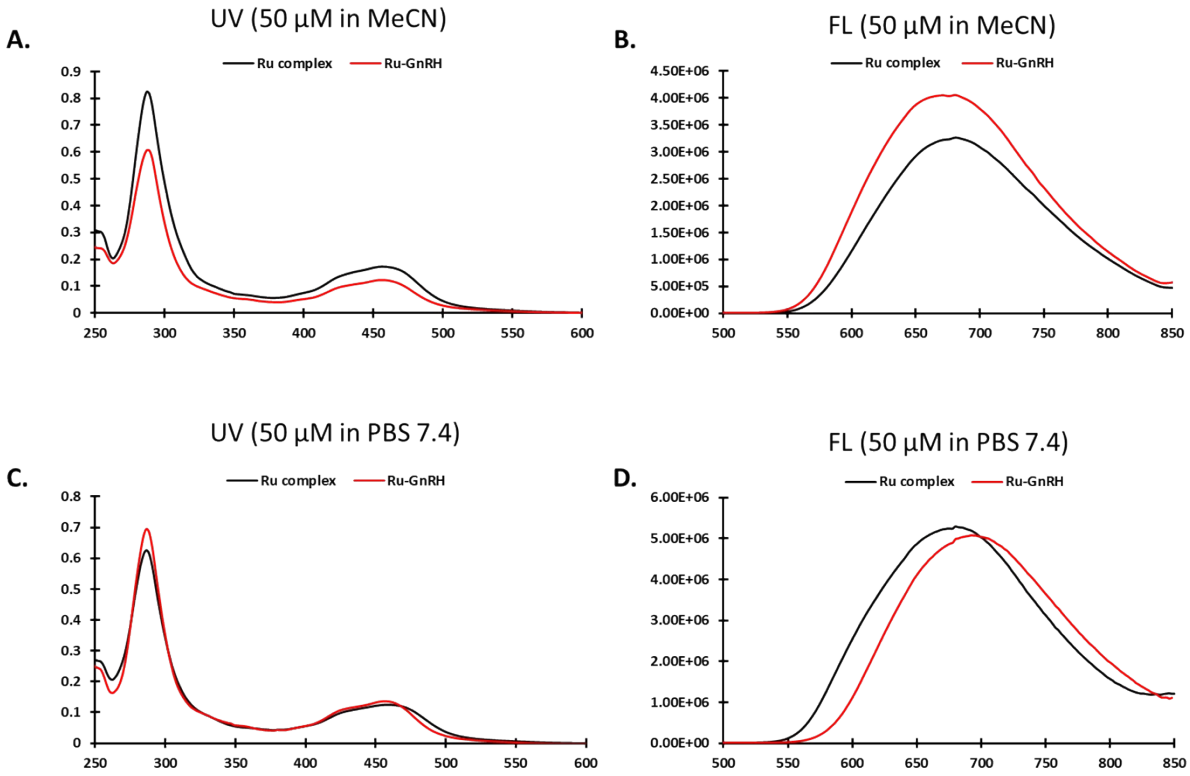


Figure S3. Photophysical characterization of Ru complex $[Ru(bipy)_2(mcb)](PF_6)_2$ and Ru-GnRH (**conjugate 3**) via **A.** UV in MeCN, **B.** Fluorescence in MeCN, **C.** UV in PBS pH 7.4 and **D.** Fluorescence in PBS pH 7.4. The measurements were conducted at 298 K using 50 µM samples.

Stability in physiological conditions

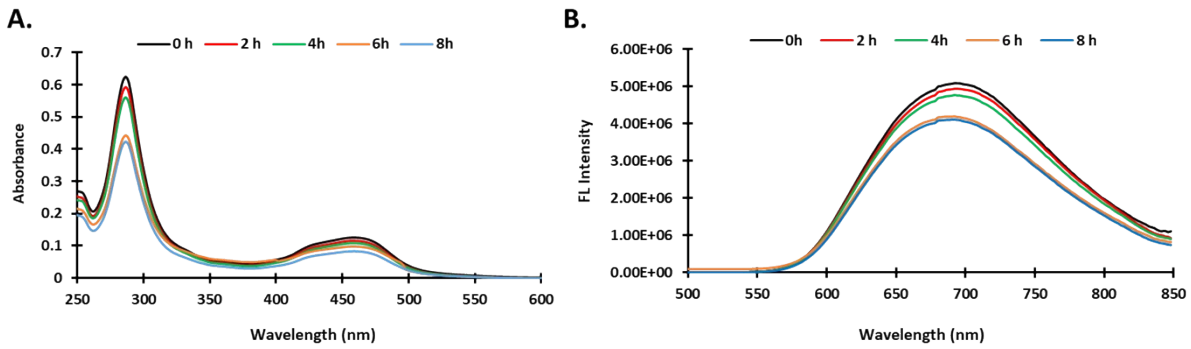


Figure S4. Stability of **conjugate 3** in PBS pH=7.4 over time assessed via **A.** UV and **B.** Fluorescence spectroscopy. The measurements were conducted at 298 K using 50 µM samples.

Videos of photostability of the conjugates after continuous laser scanning

Video 1.avi

Video 1. Conjugate 2: HeLa cells were incubated at 37 °C for 2 hours with 25 µM **conjugate 2** and images were taken every 5 seconds for 5 minutes by live confocal microscopy. Video was generated using ImageJ software, Playback acceleration 50x.

Video 2.avi

Video 2. Conjugate 1: HeLa cells were incubated at 37 °C for 2 hours with 25 µM **conjugate 1** and images were taken every 5 seconds for 5 minutes by live confocal microscopy. Video was generated using ImageJ software, Playback acceleration 50x

Video 3.avi

Video 3. Conjugate 3: T24 cells were incubated at 37 °C for 2 hours with 25 μM of **conjugate 3**, and images were taken every 5 seconds for 9 minutes by live confocal microscopy. Video was generated using ImageJ software, Playback acceleration 90x