# SUPPORTING INFORMATION

## Somatostatin receptor-targeted organometallic iridium(III) complexes as novel theranostic agents

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## 1.- Materials and Methods

Unless otherwise noted, common chemicals and solvents (HPLC-grade or reagent-grade quality) were purchased from commercial sources and used without further purification. Milli-Q water was directly obtained from a Milli-Q system equipped with a 5000 Da ultrafiltration cartridge. Peptide-grade DMF was from Scharlau. Fmoc-protected amino acids, resins, and coupling reagents for solid-phase synthesis were obtained from Novabiochem, Bachem, or Iris Biotech. Fmoc-Hag-OH was purchased from Bachem. Fmoc-*L*-threoninol *p*-carboxyacetal was synthesized following previously reported procedures.[1] Solid-phase syntheses were performed manually in a polypropylene syringe fitted with a polyethylene disk. Second-generation Grubbs catalyst and Wilkinson's catalyst were purchased from Aldrich. Metal complex Ir-COOMe (**1**),[2] the peptides Octreotide (**3**)[3] and dicarba Octreotide (**4**),[3-4] as well as Fluorescein-labeled Octreotide (FITC-OCT)[3] and Fluorescein-labeled dicarba analogue of Octreotide (FITC-dcOCT)[3] were synthesized and characterized as previously reported. All the assayed compounds displayed a purity of ≥95%, determined by HPLC analysis.

Solid-phase peptide synthesis was performed on a Rink amide resin-*p*-MBHA (f = 0.34 mmol/g, 100−200 mesh) using standard Fmoc/$^{t}$Bu chemistry with the following side-chain protecting groups: Boc ($N^{i}$-*tert*-butoxicarbonyl, tryptophan, and $N^{\varepsilon}$-*tert*-butoxycarbonyl, lysine), $^{t}$Bu (*O*-*tert*-butyl, threonine), and Trt (*S*-trityl, cysteine).

Analytical reversed-phase HPLC analyses were carried out on a GraceSmart RP C18 column (250 × 4 mm, 5 μm, flow rate: 1 mL/min), using linear gradients of 0.1% formic acid or 0.045% TFA in $H_2O$ (solvent A) and 0.1% formic acid or 0.036% TFA in ACN (solvent B). Large-scale purification was carried out on a Jupiter Proteo semipreparative column (250 × 10 mm, 10 μm, flow rate: 3−4 mL/min), using linear gradients of 0.1% HCOOH in $H_2O$ (solvent A) and 0.1% HCOOH in ACN (solvent B). After several runs, pure fractions were combined and lyophilized. A [Vydac C18]-filled glass column (22x2 cm, 15-20 mm, 300 Å) was used for medium pressure liquid chromatography (MPLC), using aqueous and ACN solutions containing 0.1% HCOOH (flow rate: 2-3 mL/min). Elution was carried out by connecting a piston pump to the mixing chamber of a gradient-forming device and to the top of the glass column. The mixing chamber of the gradient-forming device was the flask containing solvent A, which was connected through a stopcock to the flask containing solvent B. The bottom of the preparative column was connected to an automatic fraction collector through a UV/Vis detector which was also connected to a chart recorder using the appropriate ports. Equilibration

of the column was carried out with 200 mL of solvent A, and 600 mL of each mobile phase was introduced in the appropriate compartments of the gradient-forming device.

NMR spectra were recorded at 25 °C on a Bruker 400 MHz spectrometer using DMSO-$d_6$. The residual signal of the solvent was used as a reference for $^{1}H$ and $^{13}C$ spectra. High-resolution MALDI-TOF mass spectra were recorded on a 4800 Plus MALDI-TOF/TOF spectrometer (Applied Biosystems) in the positive-ion mode using 2,4-dihidroxybenzoic acid as a matrix. ESI mass spectra (ESI-MS) were recorded on a Micromass ZQ instrument with single quadrupole detector coupled to an HPLC. High-resolution electrospray mass spectra (HR ESI MS) were obtained on an Agilent 1100 LC/MS-TOF instrument

The human lung carcinoma A549 cells, human cervical carcinoma HeLa cells, human mammary gland carcinoma MCF-7 cells and The Chinese hamster ovary CHO-K1 cells (wild type, non-carcinoma cells) were from ECACC. Cells were grown in DMEM medium (high glucose 4.5 g $L^{-1}$, Biosera, Boussens, France) supplemented with gentamycin (50 μg $mL^{-1}$, Serva, Heidelberg, Germany) and 10% heat inactivated fetal bovine serum (FBS, Biosera). The cells were cultured in a humidified incubator at 37 °C in a 5% $CO_2$ atmosphere and subcultured 2-3 times a week with an appropriate plating density.

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## 2.- Synthesis and characterization of the compounds (2, 5-6)

### Ir-COOH complex (2)

To a solution of Ir-COOMe (**1**) (125 mg, 0.134 mmol) in a MeOH/THF mixture (1:5, $V_F$=18 mL) was added dropwise an aqueous solution of LiOH (4 mol equiv., 32 mL $H_2O$), and the resulting solution was stirred at 50ºC for 5 h. After cooling the reaction, the aqueous phase was acidified with HCl 10% until pH≈3 and extracted with DCM (3 × 10 mL). The combined organic phases were partially reduced under vacuum and then 10 mL of $H_2O$ was added. The product was precipitated out by the addition of 1–2 mL of a saturated aqueous $KPF_6$. The $PF_6^-$ salt of the compound was extracted with a mixture of DCM/ACN (3 × 10 mL), the organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum to yield a yellow solid (118 mg, 96%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm): 8.87 (d, 1H, $J_{HH}$ = 2.8 Hz), 8.81 (d, 1H, $J_{HH}$ = 2.4 Hz), 8.60 (d, 1H, $J_{HH}$ = 8.3 Hz), 8.34 (td, 1H, $J_{HH}$ = 8.0 Hz, $J_{HH}$ = 1.6 Hz), 8.13 (dd, 1H, $J_{HH}$ = 5.4 Hz, $J_{HH}$ = 1.1 Hz), 7.97 (dd, 1H, $J_{HH}$ = 8.7 Hz, $J_{HH}$ = 1.3 Hz), 7.91 (br, 1H), 7.70 (m, 2H), 7.65 (d, 1H, $J_{HH}$ = 7.3 Hz), 7.18 (s, 1H), 7.13 (dd, 2H, $J_{HH}$ = 6.7 Hz, $J_{HH}$ = 1.9 Hz), 7.05 (td, 1H, $J_{HH}$ = 7.7 Hz, $J_{HH}$ = 1.3 Hz), 7.00 (td, 1H, $J_{HH}$ = 7.9 Hz, $J_{HH}$ = 1.2 Hz), 6.83 (m, 2H), 6.64 (m, 2H, $J_{HH}$ = 2.4 Hz), 6.62 (m, 2H), 4.91 (m, 2H), 1.91 (m, 2H), 1.41 (m, 2H), 0.91 (t, 3H, $J_{HH}$ = 7.4 Hz). $^{13}$C{$^1$H} NMR (100 MHz, DMSO-$d_6$) δ (ppm): 166.8, 153.3, 151.6, 147.0, 143.2, 143.0, 140.0, 139.3, 139.2, 138.3, 133.6, 133.1, 132.5, 128.7, 128.6, 128.2, 128.1, 126.6, 126.3, 125.9, 125.5, 122.9, 122.8, 118.8, 112.1, 111.9, 108.4, 108.2, 45.4, 31.4, 19.3, 13.6. ESI-MS (pos. ion mode, $H_2O$/MeOH/THF): *m*/*z* 773.71 ($[M–PF_6^-]^+$, calcd. 773.71).

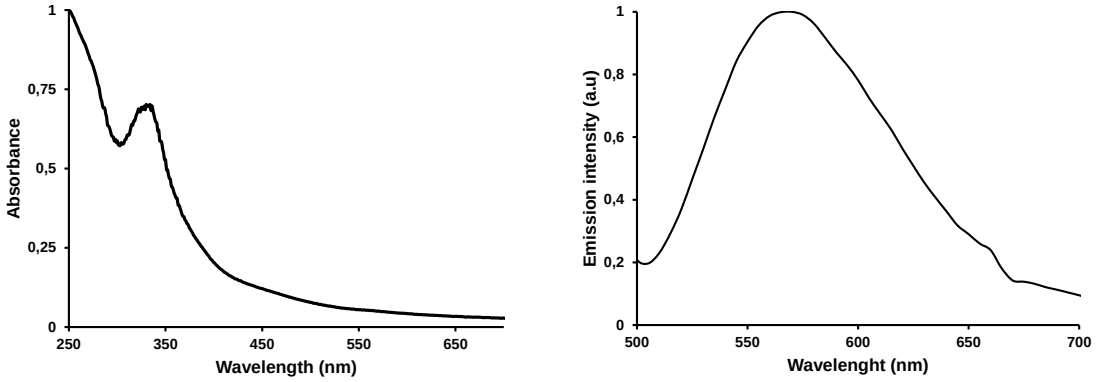


**Figure S1.** UV−vis spectra (left, 30 μM) and luminiscent emission spectra (right, 10 μM, $\lambda_{ex}$ = 480 nm) of complex **1** in PBS.

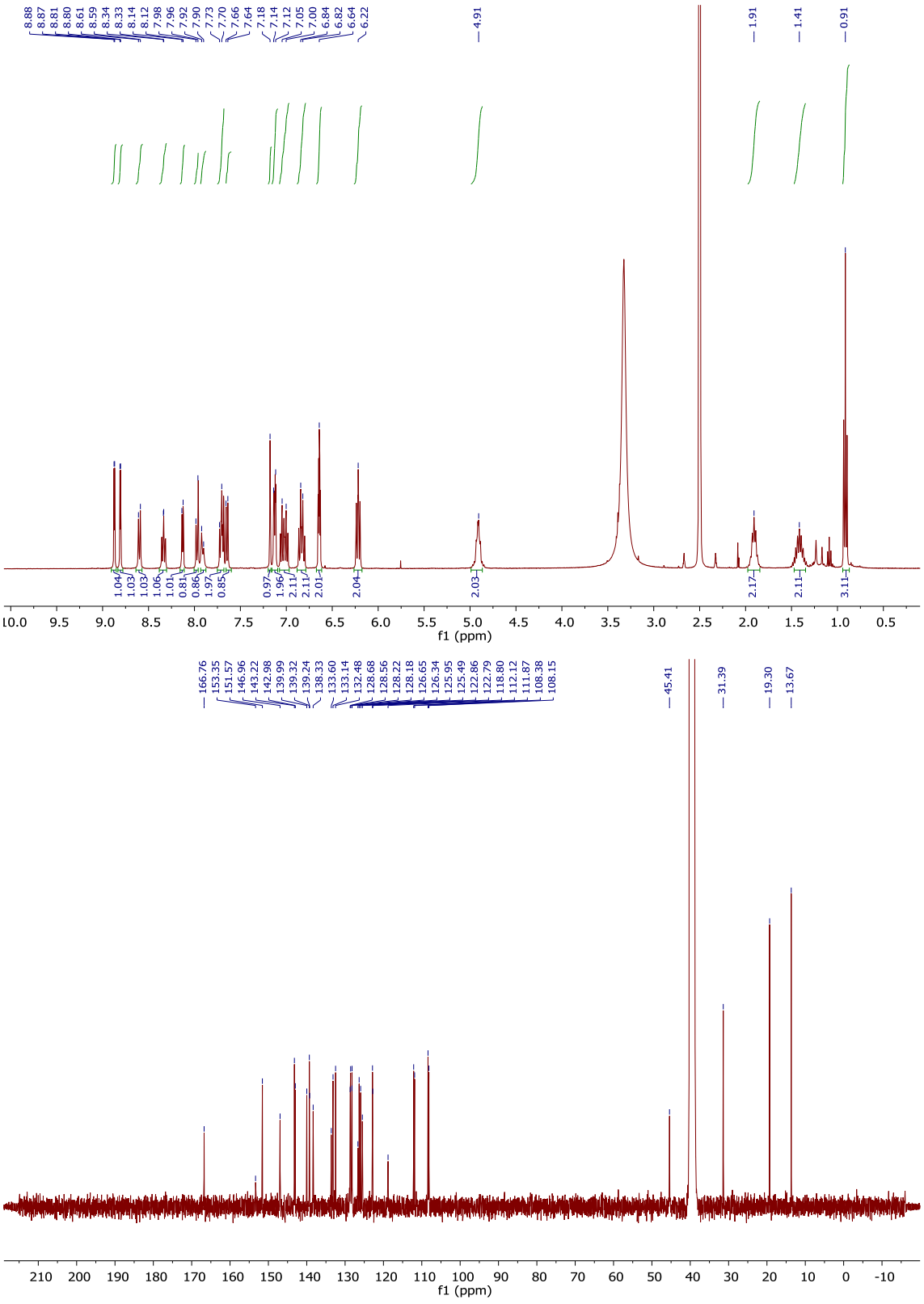


**Figure S2.** $^{1}H$ and $^{13}C$ NMR spectra of complex **2**.

**Ir-OCT conjugate (5)**

Complex **2** (1.3 mol equiv) was coupled onto the linear octreotide-bound resin with HATU (1.2 mol equiv) in anhydrous DMF in the presence of DIPEA (5 mol equiv.) for 3 h at RT. Side-chain deprotection and cleavage were performed simultaneously by treatment with TFA/TIS/$H_2O$/EDT (94:2.5:2.5:1) under an argon atmosphere for 1 h at RT. Most of the TFA was removed by bubbling $N_2$ into the solution, and the resulting residue was poured onto cold ether to precipitate the target compound. The solid was isolated by centrifugation, dissolved in $H_2O$/ACN (9:1) and lyophilized. Cyclisation was accomplished after continuously stirring a solution of the crude material in aqueous $NH_4HCO_3$ (5%) in the presence of $O_2$ overnight at RT. The solution was lyophilized and the conjugate purified by MPLC using a linear gradient from 20 to 100% of 0.1% formic acid in $H_2O$ (solvent A) and 0.1% formic acid in ACN (solvent B). Overall yield (synthesis + purification): 15.9 mg of a yellow solid, 12%. HR ESI MS, positive mode: *m/z* 960.3628 (calcd mass for $C_{90}H_{107}IrN_{18}O_{14}S_2$ $[M+H]^{2+}$: 960.3640), *m/z* 640.5810 (calcd mass for $C_{90}H_{108}IrN_{18}O_{14}S_2$ $[M+2H]^{3+}$: 640.5784). Analytical RP-HPLC (0−100% B in 30 min; A, 0.1% formic acid in $H_2O$; B, 0.1% formic acid in ACN; $t_R$ = 14.2 min).

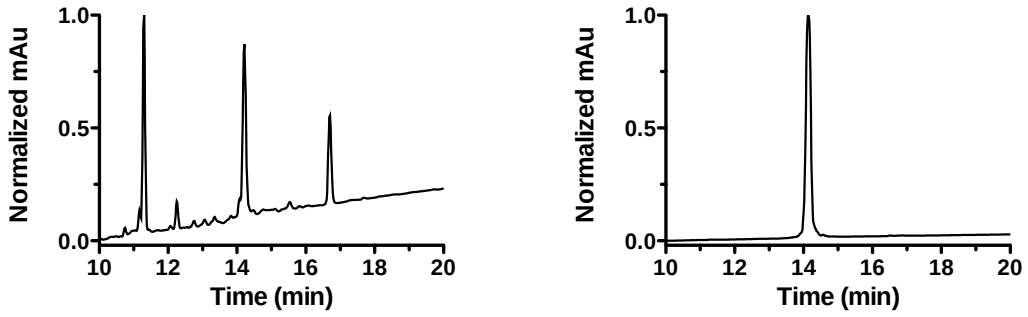


**Figure S3** Reversed-phase HPLC traces for conjugate **5**: reaction crude (left) and purified (right).

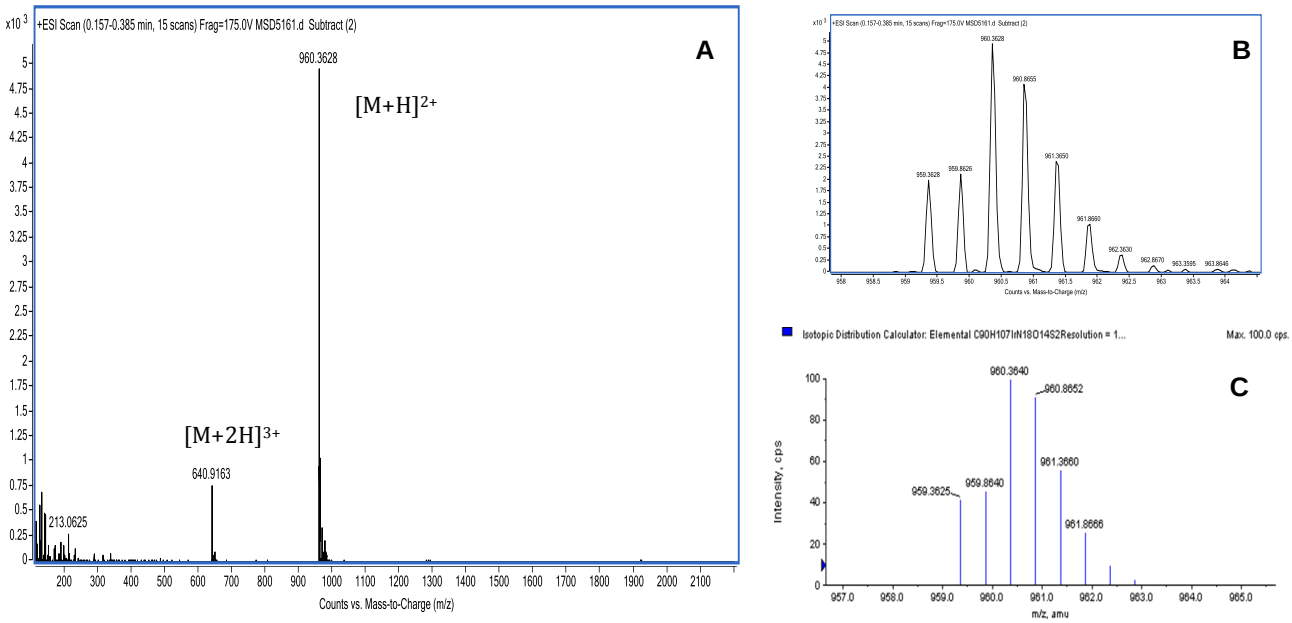


**Figure S4.** High resolution ESI MS of conjugate **5**: experimental (A, B) and calculated (C).

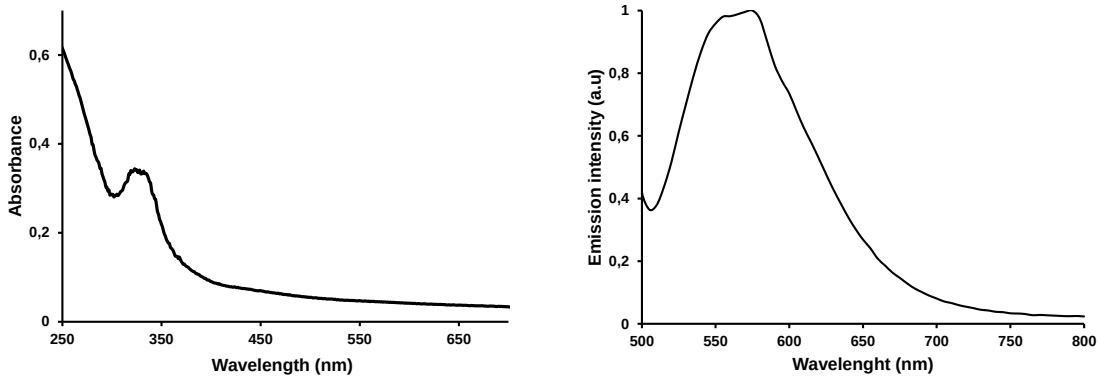


**Figure S5.** UV−vis spectra (left, 30 μM) and luminiscent emission spectra (right, 10 μM, $\lambda_{ex}$ = 480 nm) of Ir-OCT conjugate **5** in PBS.

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**Ir-dcOCT conjugate (6)**

The assembly of the dicarba analogue of octreotide was performed as previously described.[3,5] Briefly, Fmoc-*L*-threoninol *p*-carboxyacetal (1.4 mol equiv.) was first coupled using DIPC (1.4 mol equiv.) and HOBt (1.4 mol equiv.) in anhydrous DMF for 3 h. The following Fmoc-protected amino acids (3 mol equiv.) were incorporated with HOAt (3 mol equiv.) and DIPC (3 mol equiv.) in anhydrous DMF for 2 h. Microwave-assisted ring-closing metathesis using second-generation Grubbs catalyst and hydrogenation reaction with Wilkinson's catalyst were carried out as previously described.[5a] Finally, the linker Fmoc-8-amino-3,6-dioxaoctanoic acid was coupled onto the cyclic dicarba analogue of octreotide bound to resin with DIPC (3 mol equiv.) and HOAt (3 mol equiv.) in anhydrous DMF for 2 h. After removal of the Fmoc group (20% piperidine in DMF) from the Fmoc-protected linker-derivatized dicarba analogue of octreotide bound to resin, coupling of the iridium complex **2** (2 mol equiv.) on the free N-terminal end was carried out with DIPC (2 mol equiv.) and HOAt (2 mol equiv.) in anhydrous DMF for 2 h. Side-chain deprotection and cleavage from the resin was performed simultaneously with TFA/TIS/$H_2O$ (95:2.5:2.5) under an argon atmosphere for 2 h at RT. The product was precipitated and purified by semipreparative RP-HPLC (gradient from 20 to 80% B in 30 min, A: 0.1 % formic acid in $H_2O$, B: 0.1% formic acid in ACN, flow rate: 3 mL/min, $t_R$ = 14.8 min). Overall yield (synthesis + purification): 5.23 mg of a yellow solid, 14%. HR ESI MS, positive mode: *m/z* 942.4068 (calcd mass for $C_{92}H_{111}IrN_{18}O_{14}$ $[M+H]^{2+}$: 942.4078), *m/z* 628.6049 (calcd mass for $C_{92}H_{112}IrN_{18}O_{14}$ $[M+2H]^{3+}$: 628.6076). Analytical RP-HPLC (0 to 100% B in 30 min; A, 0.1% formic acid in $H_2O$; B, 0.1% formic acid in ACN; $t_R$ = 14.1 min).

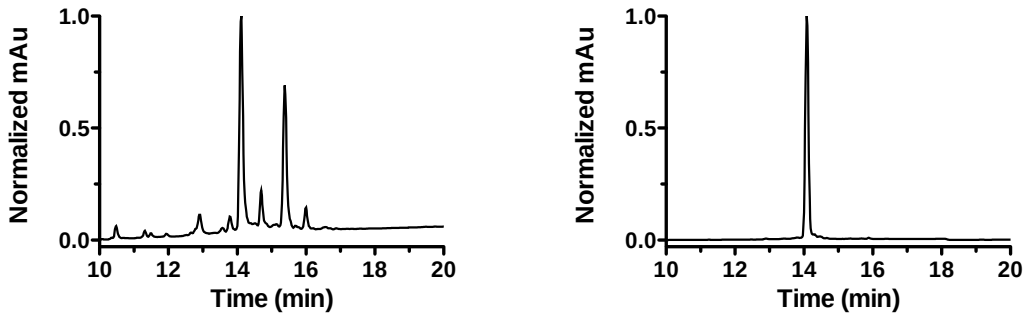


**Figure S6.** Reversed-phase HPLC traces for conjugate **6**: reaction crude (left) and purified (right).

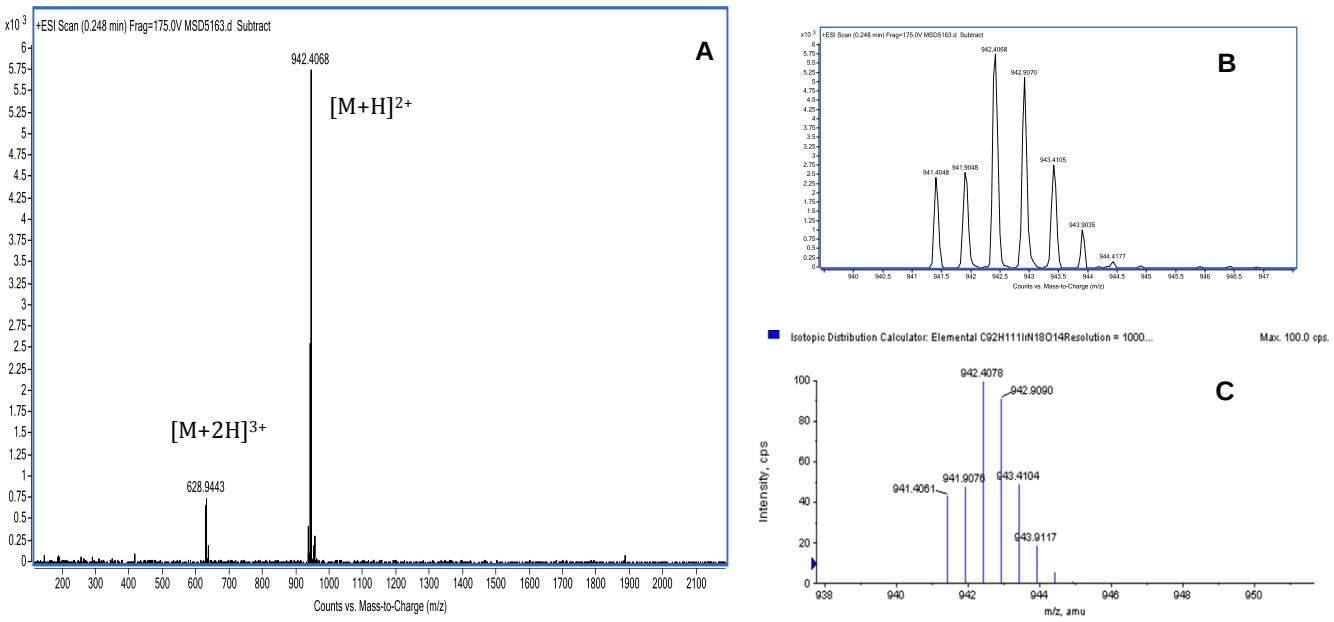


**Figure S7.** High resolution ESI MS of conjugate **6**: experimental (A, B) and calculated (C).

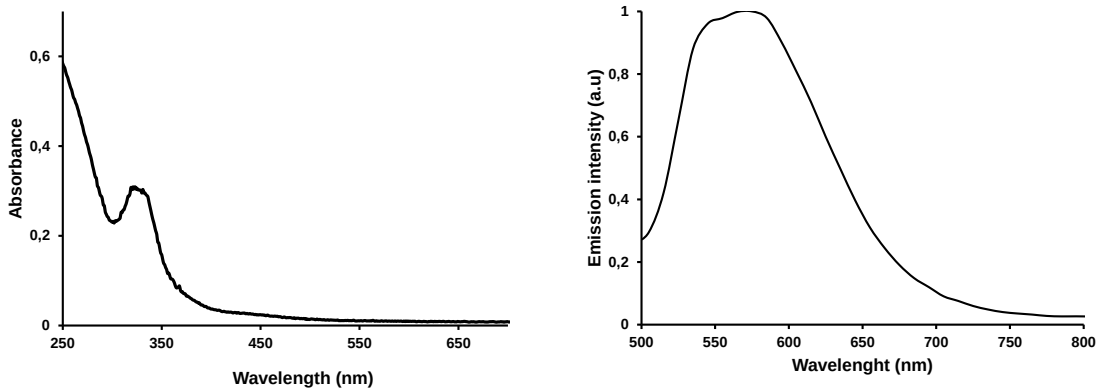


**Figure S8.** UV−vis spectra (left, 30 μM) and luminiscent emission spectra (right, 10 μM, $\lambda_{ex}$ = 480 nm) of Ir-dcOCT conjugate **6** in PBS.

## 3.- hSSTR2 mRNA expression assessed by quantitative real-time PCR

Total RNA was isolated from A549, HeLa, MCF-7 and MDA-MB-231 cells using NucleoSpin® RNA plus (Machery-Nagel, Düren, Germany) and subsequently reverse transcribed into their respective cDNAs with ProtoScript® II First strand cDNA synthesis kit (New England Biolabs, Hitchin, UK). cDNAs (1 ng $\mu L^{-1}$) were submitted to PCR with reaction mix HOT FIREPol® EvaGreen® qPCR mix plus (Solis BioDyne, Tartu, ES). PCR reaction was carried out on Eco real-time PCR system (Illumina, CA, USA). Primers used to quantify mRNA expressions were as following: β-actin (sense 5´-AGCTACGAGCTGCCTGAC-3´, antisense 5´-AAGGTAGTTTCGTGGATGC-3´; amplicon size 122 bp), hSSTR2 (sense 5´-TGGCTATCCATTCCATTTGACC-3´, antisense 5´-AGGACTGCATTGCTTGTCAGG-3´; amplicon size 98bp). β-actin was used as an internal housekeeping control. Primers were selected from PrimerBank (The Massachusetts General hospital), specific single amplicon product was checked using melting curve analysis. Negative controls were prepared without template cDNA. Polymerase was activated at 95 °C for 15 min followed with 40 cycles: 95 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s. Samples were normalized by using the difference in critical thresholds (CT) between hSSTR2 and β-actin. Comparative ΔΔCT method was used, $\Delta\Delta CT_{hSSTR2}=\Delta CT_{SSTR2}-\Delta CT_{\beta\text{-actin}}$, where $\Delta CT_{SSTR2}$ is the $CT_{SSTR2}$ subtracted from negative control and $\Delta CT_{\beta\text{-actin}}$ is the $CT_{\beta\text{-actin}}$ subtracted from negative control. The mRNA levels in each cell line were compared using the relative expression $2^{-\Delta\Delta CTSSTR2}$, the lowest mRNA level of hSSTR2 was arbitrarily normalized to 1.

**Table S1**: mRNA expression of hSSTR2 in A549, HeLa, MCF-7 and MDA-MB-231 cells determined by using qRT-PCR[a]

| **Cell line** | $\Delta\Delta\mathbf{CT_{hSSTR2}=\Delta CT_{SSTR2}-\Delta CT_{\beta\text{-actin}}}$ | Normalized relative expression relative to MCF7 $\mathbf{2^{-\Delta\Delta CTSSTR2}}$ |
|---|---|---|
| **A549** | 8.98 ± 0.37 | 3.08 |
| **HeLa** | 8.45 ± 0.34 | 4.44 |
| **MCF-7** | 10.60 ± 0.14 | 1.00 |
| **MDA-MB-231** | 10.20 ± 0.10 | 1.32 |

[a]PCR was performed with 40 cycles of 95 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s. Gene expression levels of hSSTR2 were compared with the lowest one found for MCF-7 cells which was arbitrarily assigned the value 1. ΔΔCT values are the means ±SDs from four independent experiments.

## 4.- Double-immunofluorescence SSTR2 expression analysis

SSTR2 expression was determined in A549, HeLa, MCF-7 and MDA-MB-231 cells by double immunofluorescence analysis and detected by flow cytometry. Cells were cultured according to a standard procedure, harvested and then fixed with 1.5% formaldehyde in PBS for 1 h at 4 °C. Subsequently the cells were washed with PBS and permeabilized with PBS-Tween 20 (0.2%) for 15 min at 37 °C. Cells were washed with ice cold PBS and incubated with SSTR2 primary mouse $IgG_{2A}$ monoclonal antibody (Clone#402038, 2.5 µg/$10^6$ cells; R&D systems, MN, USA) for 30 min at 4 °C. Cells were washed twice with ice cold PBS and incubated with mouse F(ab)2 IgG (H+L) APC-conjugated mouse secondary antibody (R&D systems, MN, USA). Cells were washed three times with ice cold PBS and immediately analyzed on flow cytometer (FACSVerse, BD, San Jose, CA). 20 000 single cells with normal characteristics (standard relative size and granularity) were analyzed. Background fluorescence signal from mouse $IgG_{2A}$ isotype control (Clone#133304; R&D systems, MN, USA) was subtracted from the mean specific fluorescence intensity of the stained SSTR2 samples. Thus, the levels of expression of hSSTR2 in four human carcinoma cell lines on RNA level by qRT-PCR and on the protein level by double-immunofluorescence staining with flow-cytometric detection have been determined (Fig. S9). The two independent methods were used also to select representative SSTR2 positive (HeLa) and SSTR2 negative (MDA-MB-231) cell lines. The data obtained with the aid of both methods indicate a correlation between the RNA and protein expression levels, although results obtained with the MCF-7 cells showed the lowest RNA expression, but not the lowest protein expression. The cell lines for the subsequent experiments were chosen primarily on the basis of their protein expression levels determined by double-immunofluorescence.

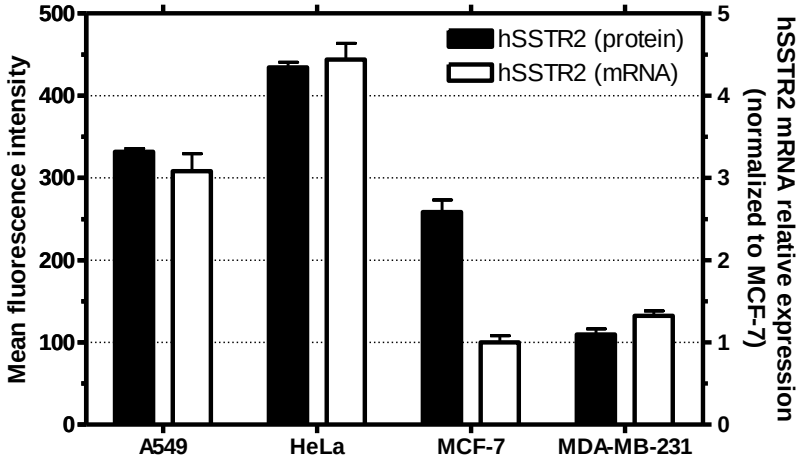


**Figure S9** Expression of hSSTR2 in selected cell lines examined by using double-immunofluorescence staining with flow-cytometric detection correlated with mRNA expression determined by using qRT-PCR. SSTR2 protein expression is depicted as the mean fluorescence intensity coming from APC-conjugated $IgG_{2A}$ secondary antibody (isotype control was subtracted). Cellular levels of hSSTR2 mRNA are expressed as $2^{-\Delta\Delta CTSSTR2}$, mRNA levels in MCF-7 cells were arbitrary assigned to 1. Experiments were done in triplicate (FC analysis) or in quadruplicate (qRT-PCR analysis). Error bars are the standard deviations.

## 5.- Internalization experiments with FITC-OCT and FITC-dcOCT conjugates

The intracellular accumulation efficiency of FITC-OCT and FITC-dcOCT conjugates was analyzed with flow cytometry. SSTR2 positive HeLa cells were seeded on a 100 mm petri dishes at the density of $1.5 \cdot 10^6$ cells/dish and incubated overnight. Cells were treated with 50 μM of FITC-OCT and FITC-dcOCT and incubated for 5 h at 37 °C, 5% $CO_2$. Cells were rinsed with ice cold PBS, harvested, and washed again with ice cold PBS. Fluorescence of the cells corresponding to the accumulation of octreotide conjugates was analyzed on flow cytometer (FACSVerse, BD, San Jose, CA) and plotted on the log scale histogram. Untreated cells were used as the internal control for autofluorescence. 20 000 single cells with standard relative size and granularity were analyzed.

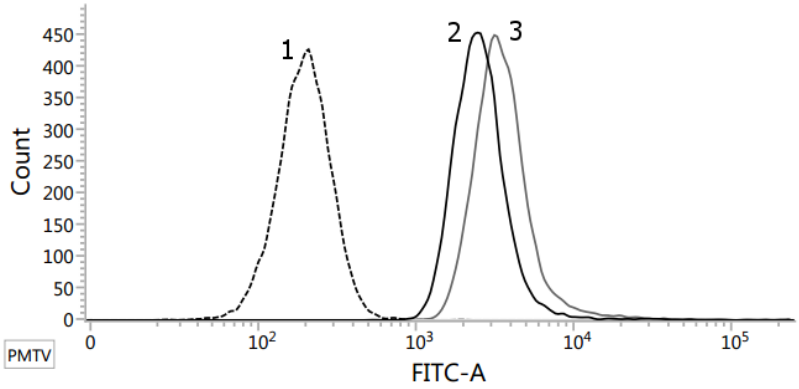


**Figure S10** Internalization of FITC-OCT (3) and FITC-dcOCT (2) in HeLa cells determined by flow cytometry (1= untreated cells). Internalization of fluorescently labelled peptides corresponds to the fluorescence intensity of 20000 cells on a log scale. Experiments were done in quadruplicate.

## 6.- Cellular accumulation studies by ICP-MS

Cellular accumulation of iridium from iridium compounds was measured in HeLa and MDA-MB-231 cells. The cells were seeded in 100 mm petri dishes at a density of $1.5 \cdot 10^6$ cells/dish. After 24 h of incubation, the cells were treated with the compounds (1 μM) for 24 h. For determination of interactions of Ir compounds with SSTR receptors the cells were treated with 5 μM of tested compounds and incubated for 2 h at 4 °C (interaction partially inhibited) or at 37 °C (unobstructed interaction). Kinetics of Ir cellular accumulation was determined in HeLa cells treated for 2, 5 and 24 h with 1 μM of tested compounds at 37 °C. Then the cells were washed with PBS, harvested, counted and pelleted by centrifugation at 300 g, 3 min, 4 °C. Cell pellets were digested by a high pressure microwave digestion system (MARS5, CEM) with HCl (11 M) to give a fully homogenized solution, and final iridium content was determined by ICP-MS (Agilent technologies, CA, USA).

**Table S2**: Accumulation studies of iridium complexes and iridium-peptide conjugates determined with ICP-MS in HeLa and MDA-MB-231 cells[a]

| | | HeLa | | MDA-MB-231 | |
|---|---|---|---|---|---|
| | | ng Ir/$10^6$ cells | pmol Ir/$10^6$ cells | ng Ir/$10^6$ cells | pmol Ir/$10^6$ cells |
| **1** | 4°C | 272.1 ± 10.1 | 1417.2 ± 52.7 | 172.7 ± 13.1 | 899.4 ± 68.3 |
| | 37°C | 592.9 ± 47.0 | 3088.1 ± 244.6 | 448.9 ± 12.4 | 2338.0 ± 64.7 |
| **2** | 4°C | 3.3 ± 0.5 | 17.3 ± 2.6 | 5.4 ± 0.4 | 28.2 ± 2.3 |
| | 37°C | 3.5 ± 0.1 | 18.1 ± 0.5 | 6.1 ± 0.3 | 31.8 ± 1.5 |
| **5** | 4°C | 32.8 ± 3.4 | 171.0 ± 17.5 | 44.2 ± 7.2 | 230.2 ± 37.5 |
| | 37°C | 52.1 ± 3.5 | 271.2 ± 18.4 | 45.9 ± 4.9 | 239.3 ± 25.4 |
| **6** | 4°C | 22.7 ± 1.8 | 118.4 ± 9.2 | 24.7 ± 0.8 | 128.8 ± 4.4 |
| | 37°C | 30.4 ± 1.3 | 158.2 ± 6.9 | 24.8 ± 1.8 | 129.1 ± 9.5 |

[a]Cells were treated with 5 μM of tested compounds and incubated for 2 h at 37°C or 4ºC. Cellular complex accumulation was expressed in ng of iridium per $10^6$ cells, or alternatively in pmol of Ir per $10^6$ cells. Results are the mean ±SDs from three independent experiments.

## 7.- Cellular accumulation studies by ICP-MS with SSTR2 competitive inhibitor somatostatin

Cellular accumulation of iridium from iridium compounds and platinum from cisplatin was measured in HeLa cells. The cells were seeded in 100 mm petri dishes at a density of $1.5x10^6$ cells/dish and incubated overnight. In the experiments aimed at determination of interactions of Ir compounds with SSTR receptors the cells were pretreated with increasing concentrations of somatostatin (SSTR competitive inhibitor) (0, 1, 100 nM and 1 μM) for 1 h before the application of tested compounds (5 μM). Cells were incubated for further 2 h at 37 °C in 5% $CO_2$. Then the cells were washed with PBS, harvested, counted and pelleted by centrifugation at 300 g, 3 min, 4 °C. Cell pellets were digested by a high pressure microwave digestion system (MARS5, CEM) with HCl (11 M) to get a fully homogenized solution, and final iridium content was determined by ICP-MS (Agilent technologies, CA, USA).

**Table S3**: Accumulation studies of iridium complexes and iridium-peptide conjugates determined with ICP-MS in HeLa cells[a]

| Cellular Pt/Ir accumulation ($ng/10^6$ cells) | Concentration of somatostatin | | | |
|---|---|---|---|---|
| | 0 | 1 μM | 100 nM | 1 nM |
| **1** | 592.9 ± 47.0 | 597.4 ± 7.3 | 580.2 ± 5.3 | 587.5 ± 5.0 |
| **2** | 3.5 ± 0.1 | 4.5 ± 0.4 | 4.1 ± 0.3 | 4.6 ± 0.2 |
| **5** | 52.1 ± 3.5 | 39.4 ± 0.7* | 45.6 ± 1.7* | 49.6 ± 3.4 |
| **6** | 30.4 ±1.3 | 22.2 ± 1.4* | 29.6 ± 1.7 | 31.3 ± 2.2 |
| **cisplatin** | 3.8 ± 0.7 | 4.1 ± 1.0 | 4.0 ± 0.9 | 4.0 ± 1.3 |

[a]Cells were treated with Ir compounds or cisplatin (5 μM) for 2 h. Cells were pretreated for 1 h with SSTR2 competitive inhibitor somatostatin (0, 1, 100 nM and 1 μM). Cellular accumulation was expressed in ng of iridium per $10^6$ cells, or alternatively in pmol of Ir per $10^6$ cells. An asterisk denotes a significant difference ($p<0.05$) from the sample not treated with somatostatin. Results are the mean ±SDs from three independent experiments.

## 8.- Cytotoxicity

SSTR2 positive HeLa and SSTR2 negative MDA-MB-231 cells were selected as the representatives for evaluation of the cytotoxicity of tested compounds. Light/dark experimental setting was used to determine possible photo-activation of the compounds. Cells were seeded on the 96-well plates at the density of 5000 cells/well and incubated overnight. Then the medium was removed, cells were washed with HBSS and treated with increasing concentration of tested compounds diluted in HBSS (without antibiotics). Treatment schedule was as following: 30 min of incubation in the dark, followed by 30 min of the incubation under irradiation conditions (irradiated) [using Photoreactor LZC-ICH2 from Luzchem (Canada) fitted with Vis lamps (cool white fluorescent tubes, 400–700 nm with a maximum of ca. 580 nm, 2.8 mW $cm^{-2}$; the temperature in the light chamber during irradiation was 37 °C) or in the dark (non-irradiated) and then incubated again for 1 h in the humidified $CO_2$ incubator. After that the cells were washed again with HBSS and the wells were loaded with 200 μL of culture medium. The MTT assay was performed after 24 or 72 h of recovery time since the end of the incubation with the compounds. 10 μL of a freshly diluted MTT solution (2.5 mg $mL^{-1}$) was added to each well and the plate was incubated at 37 °C in a humidified 5% $CO_2$ atmosphere for 4 h. At the end of the incubation period the medium was removed and the formazan product was dissolved in 100 μL of DMSO. The cell viability was evaluated by measurement of the absorbance of 570 nm, using an Absorbance Reader SUNRISE TECAN SCHOELLER. $IC_{50}$ values were calculated from curves constructed by plotting cell survival (%) versus drug concentration (μM). All experiments were made in triplicate. The reading values were converted to the percentage of control (% cell survival). Cytotoxic effects were expressed as $IC_{50}$.

**Table S4**. Cytotoxicity of iridium compounds tested after 2 h of incubation with the cells followed by 24 h (CHO-K1 cells) or 72 h (HeLa, MDA-MB-231 and CHO-K1 cells) of the recovery time in drug-free media.[a]

| $IC_{50}$ (μM) | HeLa (72 h) | | | MDA-MB-231 (72 h) | | |
|---|---|---|---|---|---|---|
| | Non-irradiated | Irradiated | PI[b] | Non-irradiated | Irradiated | PI[b] |
| **1** | 0.32 ± 0.05 | 0.24 ± 0.03 | 1.3 | 0.75 ± 0.09 | 0.74 ± 0.08 | 1.0 |
| **2** | 154.4 ± 8.4 | 139.1 ± 7.0 | 1.1 | >200 | >200 | nd. |
| **5** | 25.6 ± 3.8 | 13.5 ± 2.2 | 1.9 | 25.8 ± 3.3 | 13.0 ± 1.9 | 2.0 |
| **6** | 29.6 ± 3.1 | 18.4 ± 2.4 | 1.6 | 46.3 ± 4.0 | 39.2 ± 4.1 | 1.2 |
| **OCT** | >200 | >200 | nd. | >200 | >200 | nd. |
| **dcOCT** | >200 | >200 | nd. | >200 | >200 | nd. |
| | CHO-K1 (24 h) | | | CHO-K1 (72 h) | | |
| | Non-irradiated | Irradiated | PI[b] | Non-irradiated | Irradiated | PI[b] |
| **1** | 7.7 ± 0.9 | 3.2 ± 0.7 | 2.4 | 5.3 ± 1.1 | 2.7 ± 0.8 | 2.0 |
| **5** | 38.7 ± 4.1 | 17.0 ± 2.9 | 2.3 | 27.4 ± 4.2 | 23.3 ± 1.6 | 1.2 |

[a]Cells were irradiated with visible light or leaved in the dark when incubated with the complex. Cytotoxicity was determined with MTT method. Results are the means ±SDs from three independent experiments.

[b]PI – Phototoxic Index ($IC_{50}$ of non-irradiated cells / $IC_{50}$ irradiated cells).

## 9.- Determination of reactive oxygen species

Generation of reaction oxygen species (ROS) was determined in HeLa cells by using 2´,7´-dichlorofluorescein diacetate (DCFH-DA) assay. Cells were seeded in 96-well black plates (corning), incubated overnight and then treated with increasing concentration of the compounds or with the concentration corresponding to the $IC_{50}$ at 72 h. Following 5 or 24 h of incubation with the compounds the cells were loaded with 10 μM of and incubated for another 45 min. ROS generated after the irradiation in Photoreactor LZC-ICH2 from Luzchem (Canada) fitted with Vis lamps (cool white fluorescent tubes, 400–700 nm with a maximum of ca. 580 nm, 2.8 mW $cm^{-2}$) were determined 5 and 24 h after the irradiation. The irradiation and treatment schedule was the same as in the cytotoxicity experiments (30 min of treatment in EBSS, 30 min of irradiation, followed by another 1 h of incubation in the dark). Fluorescence signal coming from cleaved DCFH-DA by ROS was determined by Varian cary eclipse fluorescence spectrophotometer. Control samples were loaded with the concentration of DMSO corresponding to the maximal concentration used for dilution of the tested compounds (usually ≤ 0.2%). Fluorescence signal in the control sample was arbitrary assigned to 1.

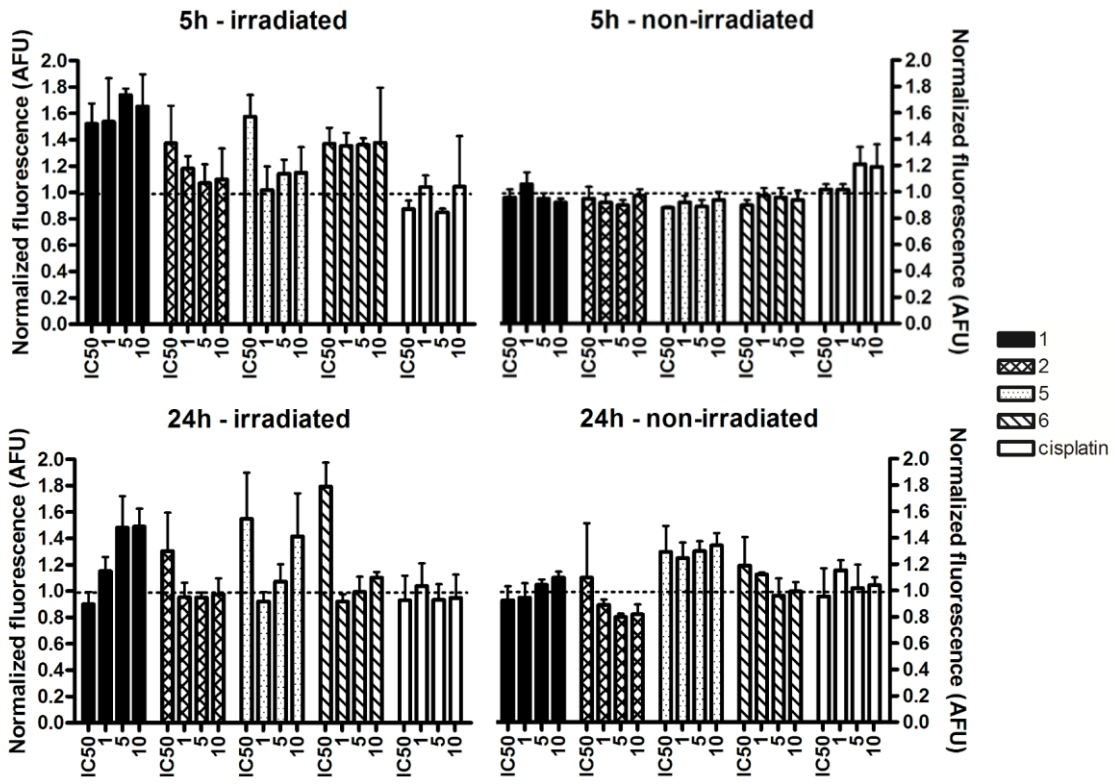


**Figure S11**. Detection of ROS in HeLa cells detected by DCHF-DA assay. Cells were incubated with increasing concentration of tested compounds or with the equitoxic concentration corresponding to the $IC_{50}$ values at 72 h. Cells were incubated with the metal compounds for 2 h (30 min of incubation in the dark, 30 min of irradiation under the visible light followed by further 1 h of incubation in the dark) and then incubated for further 5 or 24 h in the drug free medium. Fluorescence signal in the untreated (control) samples was arbitrarily assigned to 1. Experiment was repeated in triplicate with octuplicate in each run. Results are the means ±SD.

## 10.- Confocal microscopy

Cellular localization of tested iridium compounds in HeLa cells was determined by using confocal microscopy. $0.3x10^6$ cells were seeded on the 35 mm glass bottom dishes (MatTek Co., Ashland, USA). After overnight incubation the cells were treated with 1 μM of iridium compounds and imaged after 24 h of the treatment. Cells were incubated in phenol red-free culture medium at least one passage before the seeding for the confocal microscopy experiment and for all the time needed for subsequent visualization. Cells were visualized under the standard cultivating conditions (5% $CO_2$, humidified atmosphere) with confocal microscope Olympus FV10i. Cells were illuminated with 405 nm diode laser and the fluorescence detection window was set approximately from 500 to 700 nm. Negative control samples were loaded with an appropriate concentration of DMSO used for the dilution of tested compounds.

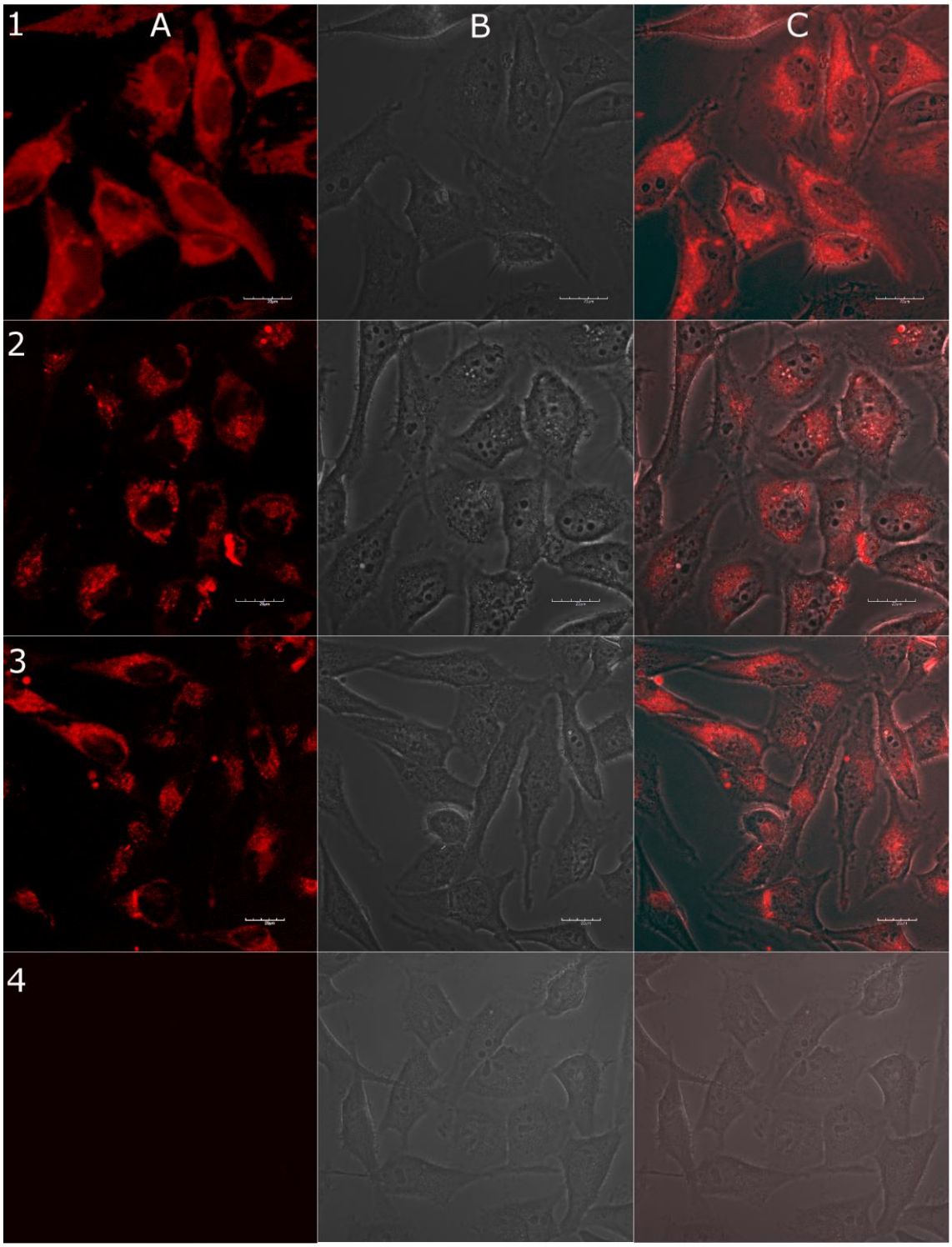


**Figure S12.** Confocal microphotograph of Hela cells treated for 24 h with 1 μM of 1) Ir complex **1**, 2) Ir-OCT **5**, 3) Ir-dcOCT **6** and 4) untreated control. Channels: A) Luminiscent channel, B) bright field, C) merge. Scale bars represent 20 μm. Figures are the representatives of three independent experiments.

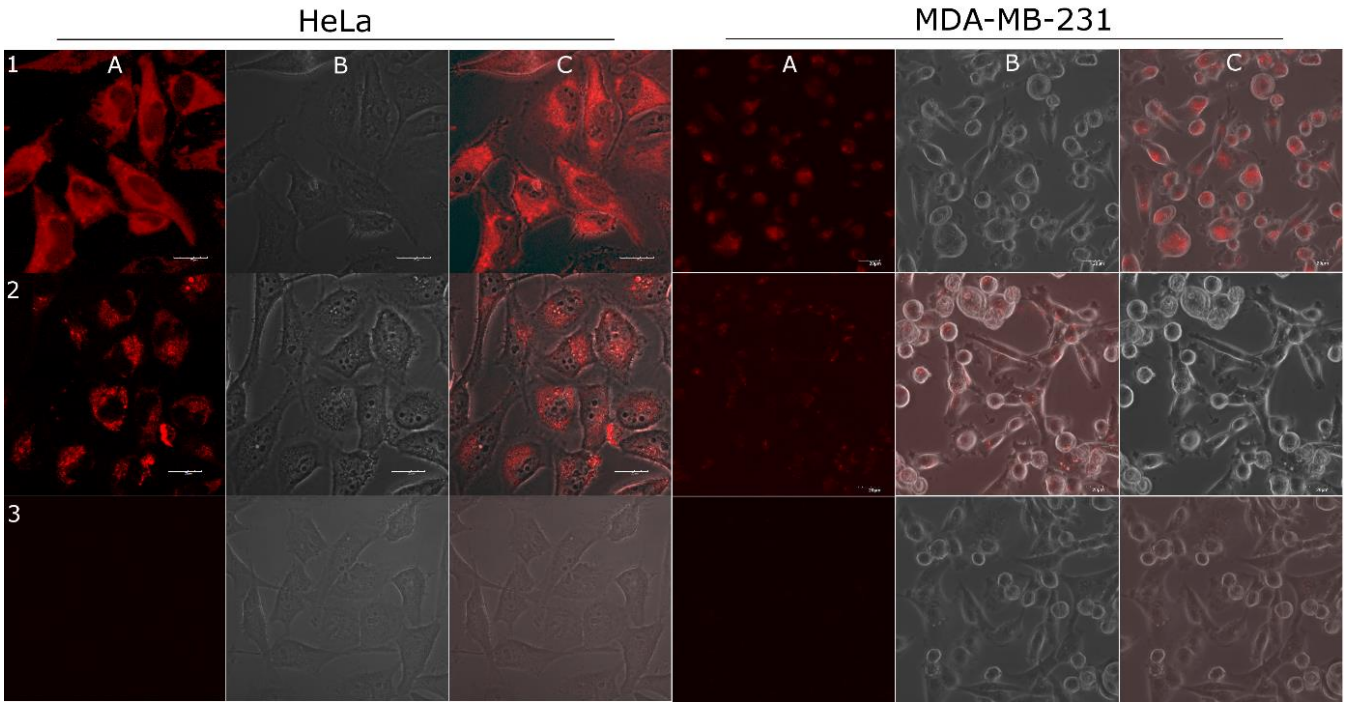


**Figure S13:** Confocal microphotograph of HeLa and MDA-MB-231 cells treated for 24 h with 1 µM of **1** (1), **5** (2), or untreated control cells (3). Luminiscent channel (A), bright field (B) and (C) merged images. Scale bars: 20 µm

## 11.- References

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