

## SUPPLEMENTARY INFORMATION

### Harnessing Mitochondrial Targeting: Biguanide–Iridium(III) Complexes with Enhanced Anticancer Activity in Pancreatic Cells

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**Materials :** All starting materials were purchased from commercial sources (Sigma Aldrich, Fisher Scientific and TCI) and used without further purification. Analytical thin layer chromatography (TLC) was carried out on precoated TLC plates Alugram Sil G/ UV254. Column chromatography purifications were done with silica gel (ultrapure, 60-200  $\mu\text{m}$  (60  $\text{\AA}$ )). Experiments were performed as follows:

**Nuclear Magnetic Resonance (NMR):**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (201 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in deuterated solvents as noted, namely chloroform- $d$  ( $\text{CDCl}_3$ ) and dimethyl sulfoxide- $d_6$  ( $\text{DMSO}-d_6$ ), which were purchased from Fisher Scientific. All chemical shifts are reported in  $\delta$  (ppm) referenced to tetramethylsilane ( $\text{Si}(\text{CH}_3)_4$ ) and the peak multiplicities are referred to as singlet (s), doublet (d), triplet(t), quartet (q), multiplet (m). Spin-spin coupling effect is quantified with the coupling constants, which is abbreviated with the capital letter J.

**Mass Spectrometry:** High-resolution mass spectral data measurements were performed by Xiao Feng at the Mass Spectrometry Laboratory (Dalhousie University, Halifax, Canada). High-resolution mass spectra were recorded on Bruker Daltonics MicrOTOF instrument. The ionization method used for low/high-resolution analysis was positive electrospray ionization (ESI+). The sample was introduced by a syringe pump at a flow rate of 2  $\mu\text{L}/\text{min}$  and the spray voltage applied to the ESI needle was 4.5 kV. The dry gas flow rate was 4 L/min with a pressure of 1 Bar and a temperature of 180°C. For the analysis, samples sizes were between 1-2.5 mg and the experiment was replicated 2-3 times to ensure the viability of the results (sample sizes of 5-7 mg were sent).

**High-Performance Liquid Chromatography:** The samples were analyzed using the Ultivo Triple Quadrupole LC-MS/MS system from Agilent (Santa Clara, CA, USA), equipped with an Avantor® ACE® UltraCore C18 2.5 Super C18 column (50 × 3 mm, particle size 2.5 µm) as well as a photodiode array detector and a mass spectrometer. For analysis, the following solvent systems were used: Solvent A, methanol:acetonitrile:ammonium acetate (1 M):water at 29:71:2:398; solvent B, methanol: acetonitrile:ammonium acetate (1 M):water at 130:320:0.25:49.7. The following linear elution gradient was used: 0–0.5 min 100% A, 0% B; 0.5–5.0 min 1% A, 99% B; 5.8–8.0 min 100% A, 0% B; the flow during the analysis was constant and 0.6 mL/min. The photodiode array detector range was 200–500 nm.

**UV-Vis Spectroscopy:** UV-Vis spectra for the cyclometalated Ir(III) complexes X-Z 5-11 were recorded with a Cary Series UV-Vis Spectrophotometer (Agilent Technologies). Chloroform – HPLC grade (C606-1, LOT 214513) was used as the blank solution and the solvent to prepare the solutions of the several Ir(III) complexes X-Z5-11. Two standard Q macro cuvettes with an optical path length of 10 millimetres which are made of fused quartz glass were used to measure the absorbance of these compounds. UV-Vis spectra were collected with a concentration of the cyclometalated Ir(III) complexes IRB1-IRB3 of  $1.1 \times 10^{-5}$  –  $2.8 \times 10^{-6}$  M

**Fluorescence Microscopy :** Excitation and emission spectra for the cyclometalated Ir(III) complexes **IRB1-IRB3** were recorded with a FS5 Spectrofluorometer (Edinburgh Instruments) and a SC-05 (Standard Cuvette Holder). A Hellma Fluorescence Cuvette (Suprasil 6 Quartz, spectral range 200-2,500 nm, pathlength 10x4 mm and chamber volume 3,500  $\mu$ L) was used to measure the excitation spectra of **IRB1-IRB3**. Chloroform – HPLC grade (C606-1, LOT 214513) was used as a blank solution and the solvent to prepare the solutions of **IRB1-IRB3** (concentration of  $\sim 1 \times 10^{-5}$  M).

Photoluminescence quantum yields were calculated using the relative method. A solution of quinine sulphate in 0.1 M sulfuric acid was used as a reference sample. Absorbance spectra were recorded in triplicate for both the reference and Ir(III) complexes **IRB1-IRB3** at a fixed absorbance value below 0.1, ranging from 0.075 to 0.080. (absorbance value recommended by Edinburgh Instruments). An average of these absorbances was used as the standard value for both the reference and the Ir(III) complexes **IRB1-IRB3**. Both samples were degassed for 20min with Argon and emission spectra were then recorded in triplicate for both the reference and Ir(III) complexes IRB1-IRB3 with all samples being excited at 350 nm. The area under the curve of the emission scan was then calculated. An average of these integration values was used as the standard value for both the reference and the Ir(III) complexes **IRB1-IRB3**. Photoluminescence quantum yield

for each Ir(III) complex **IRB1-IRB3** were then calculated using **Eq. 1**, and the values reported in **Table S1**.

**Equation 1:**

$$QY \text{ of Ir(III) complexes} = QY_{ref} \times \frac{I_{sample}}{I_{ref}} \times \frac{A_{ref}}{A_{sample}} \times \frac{\eta_{sample}^2}{\eta_{ref}^2}$$

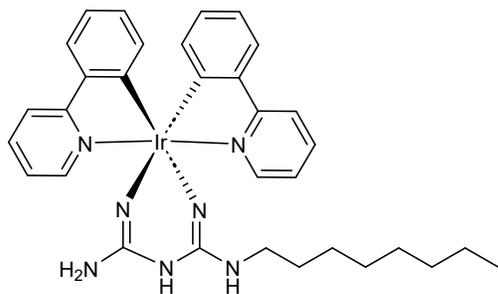
**I** = Integrated area under the emission curve

**A** = Absorbance at the wavelength of excitation maxima

$\eta$  = Refractive index

Citation: Brouwer, A. M. (2011). Standards for photoluminescence quantum yield measurements in solution (IUPAC technical report). Pure and Applied Chemistry, 83(12), 2213–2228

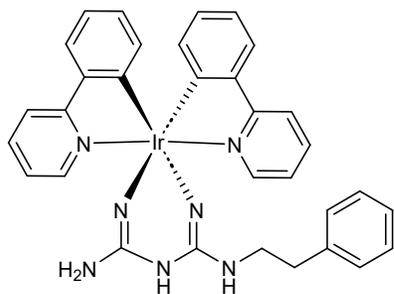
**Octyl biguanide-iridium(III) complex (IRB1)**



Iridium phenylpyridine dimer (0.130 g, 0.121 mmol) and the C8 biguanide compound (0.0517 g, 0.243 mmol) were dissolved in anhydrous ethanol and stirred at room temperature. Triethylamine (0.08 mL, 0.606 mmol) was added and the reaction flask was flushed with nitrogen before it was sealed and placed in the microwaved for 21 minutes at 150°C. The solvent was removed by rotary evaporation and the resulting solid was dissolved in chloroform (25 mL) and extracted with water (3 x 25 mL). The organic phases were combined and dried over MgSO<sub>4</sub>. The solution was filtered and rinsed with chloroform and then the

solvent was removed by rotary evaporation, resulting in a yellow dark yellow solid. The crude product was passed through a column of silica (10:0.2 DCM:MeOH à 10:0.6 DCM:MeOH) and the polarity of the solvent was gradually increased to get good separation of bands. The desired product was a yellow fluorescent band in the column and was collected into an RBF. The solvent was removed by rotary evaporation leaving a pure green yellow fluorescent solid (0.878 g, 50% yield). **HRMS:** Calculated  $m/z = 713.2818$  found  $m/z = 714.2891$  (ESI+). **HPLC:** 96.0%. **<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)**  $\delta$  11.11 (s, 1H), 8.66 – 8.56 (m, 2H), 7.81 (d,  $J = 2.2$  Hz, 2H), 7.74 (t,  $J = 8.3$  Hz, 2H), 7.54 (d,  $J = 9.4$  Hz, 1H), 7.50 (d,  $J = 7.8$  Hz, 1H), 7.21 – 7.13 (m, 2H), 6.87 – 6.81 (m, 1H), 6.77 (t,  $J = 8.2$  Hz, 1H), 6.74 – 6.63 (m, 1H), 6.32 (t,  $J = 5.1$  Hz, 1H), 6.25 (d,  $J = 6.6$  Hz, 2H), 5.16 (s, 2H), 4.90 (s, 1H), 4.61 (s, 1H), 2.94 – 2.69 (m, 2H), 1.41 (q,  $J = 6.5, 5.9$  Hz, 2H), 1.16 (m, 10H), 0.86 (t,  $J = 7.1$  Hz, 3H). **<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)**  $\delta$  168.8, 153.15, 152.7, 151.1, 151.0, 149.0, 148.9, 144.7, 144.68, 137.0, 136.9, 132.7, 129.6, 129.6, 124.1, 124.1, 122.4, 122.3, 121.4, 121.3, 118.9, 118.8, 41.2, 31.87, 29.2, 29.1, 28.5, 26.9, 22.7, 14.2

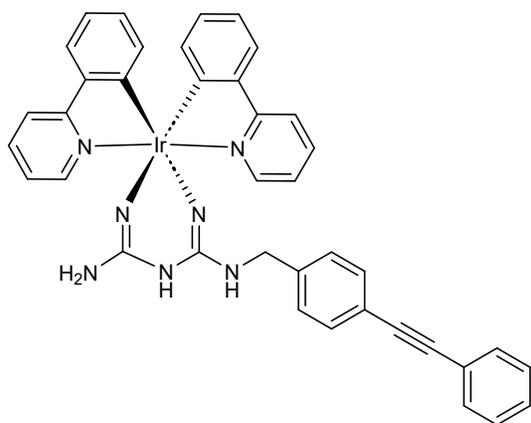
### Phenformin biguanide-iridium(III) complex (IRB2)



The phenformin biguanide (0.020 g, 0.0827 mmol) and iridium phenylpyridine dimer (0.0443 g, 0.0414 mmol) were dissolved in THF (5 mL) and stirred at room temperature. KHMDS (0.020 g, 0.1034 mmol) was added to the reaction flask and flushed with nitrogen before it was sealed. The reaction took place in the microwave for 15 minutes at 180 °C and then solvent was removed by rotary evaporation. The solid was dissolved in chloroform (3 mL) and extracted with water (3 x 5 mL). The combined organic phases were dried over MgSO<sub>4</sub> then filtered and rinsed with chloroform. The solvent was removed by rotary evaporation and left to

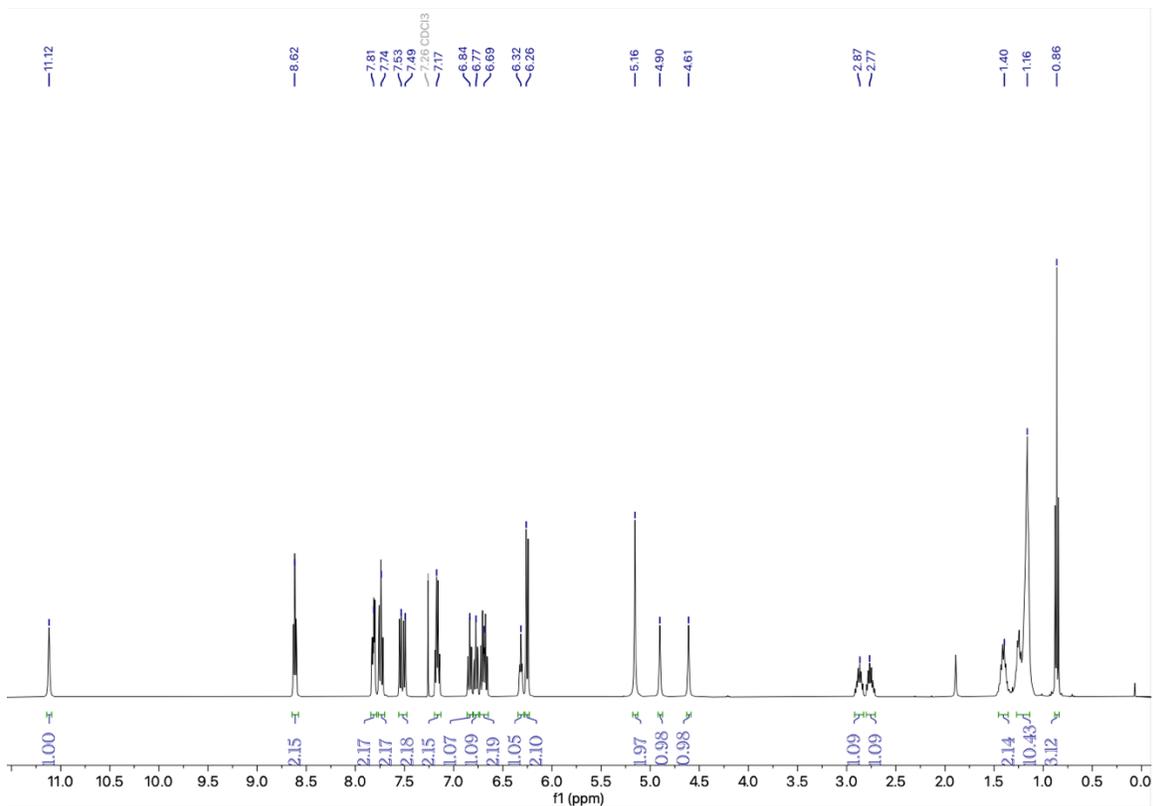
dry on a high vac. The crude product passed through a silica column (10:0.1 à 10:1 DCM:MeOH) and the desired fluorescent green yellow band was collected into a RBF. The solvent was removed by rotary evaporation and the resulting product was a yellow solid with bright green fluorescence (0.012 g, 21% yield). **HRMS:** Calculated  $m/z = 705.2192$  found  $m/z = 706.2259$  (ESI+). **HPLC:** 92.0%. **<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)**  $\delta$  11.41 (s, 1H), 8.67 – 8.62 (m, 1H), 8.46 (ddd,  $J = 5.8, 1.6, 0.8$  Hz, 1H), 7.88 – 7.82 (m, 2H), 7.77 (tt,  $J = 7.2, 6.5, 1.5$  Hz, 2H), 7.60 – 7.52 (m, 2H), 7.23 – 7.13 (m, 5H), 7.02 – 6.98 (m, 2H), 6.92 – 6.81 (m, 2H), 6.73 (dtd,  $J = 11.6, 7.4, 1.3$  Hz, 2H), 6.50 (t,  $J = 5.4$  Hz, 1H), 6.26 (ddd,  $J = 7.4, 3.6, 1.2$  Hz, 2H), 5.17 (s, 2H), 4.87 (s, 1H), 4.63 (s, 1H), 3.20 – 3.01 (m, 2H), 2.79 – 2.59 (m, 2H). **<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)**  $\delta$  168.6, 168.6, 152.7, 152.4, 150.9, 150.8, 148.9, 148.8, 144.6, 144.5, 137.8, 136.8, 132.6, 132.6, 129.5, 129.5, 128.7, 128.6, 126.8, 124.0, 124.0, 122.3, 121.2, 118.7, 118.7, 43.1, 35.0.

### Phenylethynylbenzene biguanide-iridium(III) complex (IRB3)

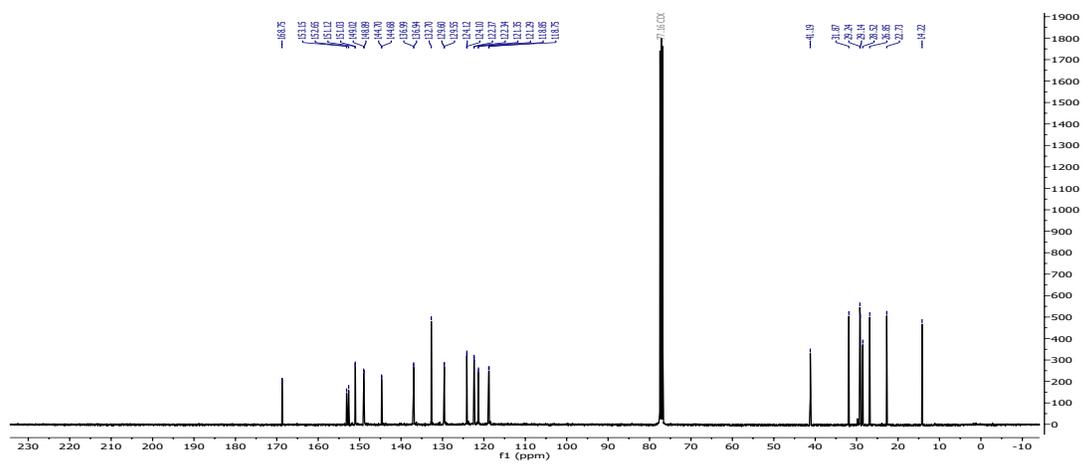


After dissolving PEB-biguanide HCl (0.015 g, 0.0513 mmol) in THF (5 mL), KHMDS (0.026 g, 0.1283 mmol) was added and stirred under nitrogen at room temperature for 5 minutes. Iridium phenylpyridine dimer (0.027 g, 0.0256 mmol) was added to solution and stirred under nitrogen and then sealed in the microwave flask. The flask was placed in the microwave for 15 minutes at 180 °C. The solvent was removed by rotary evaporation leaving an orange solid. The solid was dissolved in chloroform (5 mL) and extracted with water (3 x 10 mL). The organic phases were combined and dried with MgSO<sub>4</sub>. The crude solution was filtered and

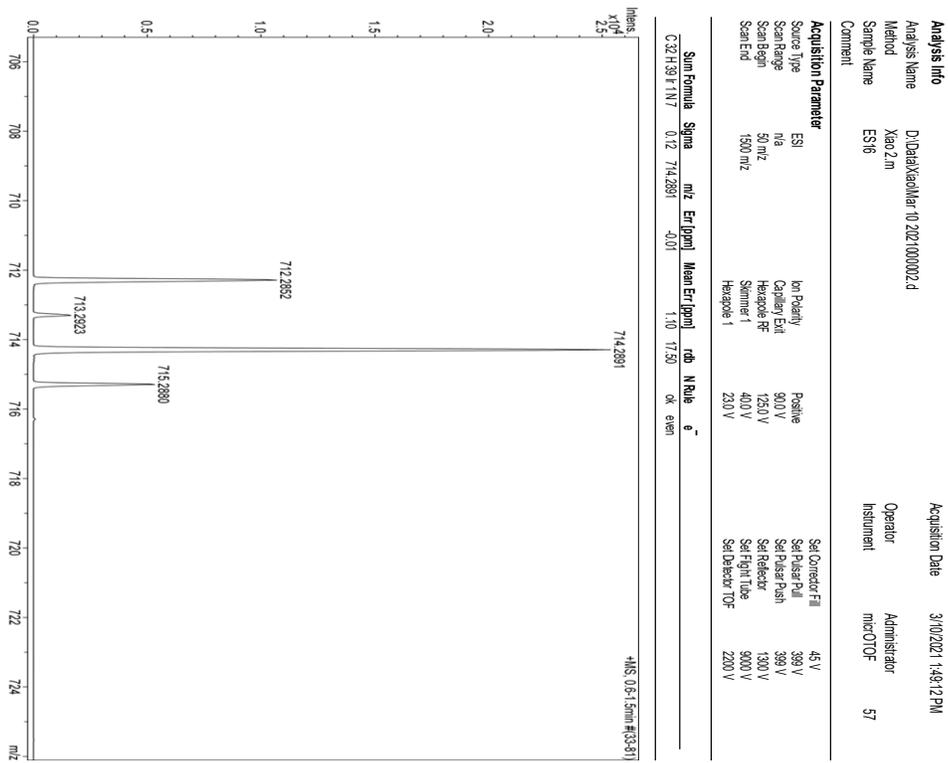
rinsed with chloroform and the solvent was removed by rotary evaporation. The product was purified through a column of silica (DCM à 10:0.6 DCM:MeOH) and the desired band was yellow with a fluorescent blue-green colour. The solvent was removed by rotary evaporation and resulted in an orange solid. **HRMS**: Calculated  $m/z = 791.2348$  found  $m/z = 792.2408$  (ESI+). **HPLC**: 90.1%. **<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)**  $\delta$  11.55 (s, 1H), 8.55 (s, 1H), 8.21 (s, 1H), 7.77 (s, 4H), 7.61 (s, 2H), 7.52 (s, 2H), 7.41 (s, 3H), 7.22 (s, 3H), 7.15 (s, 1H), 7.09 (s, 1H), 6.94 (s, 2H), 6.85 (s, 2H), 6.71 (s, 2H), 6.26 (s, 1H), 6.16 (s, 1H), 5.19 (s, 2H), 4.87 (s, 1H), 4.63 (s, 1H), 4.14 (s, 2H). **<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)**  $\delta$  168.7, 168.4, 152.8, 152.2, 150.9, 150.6, 148.8, 148.5, 144.7, 144.5, 136.9, 136.7, 136.6, 132.8, 132.5, 132.2, 131.8, 129.5, 129.4, 128.6, 127.0, 124.2, 124.1, 123.3, 122.8, 122.3, 122.2, 121.3, 119.1, 118.7, 90.1, 89.2, 45.0, 31.1, 29.8.



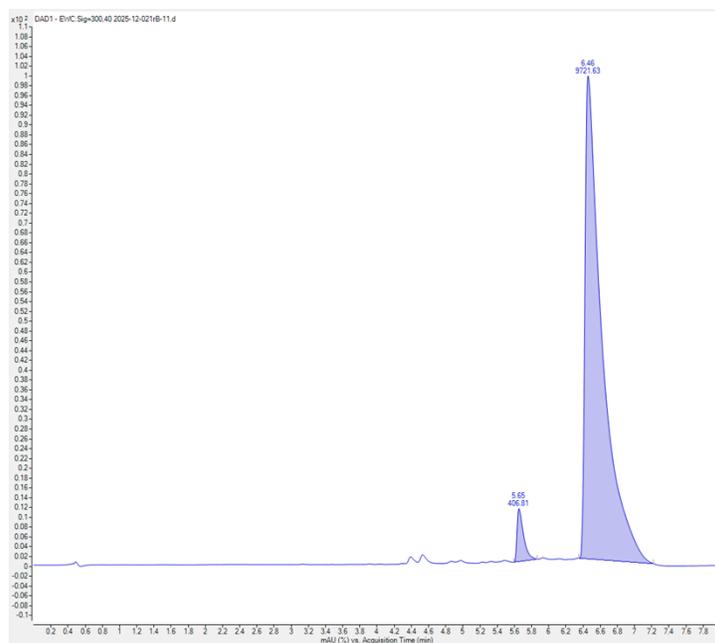
**Figure S1:**  $^1\text{H}$  NMR of compound **IRB1** in  $\text{CDCl}_3$ .



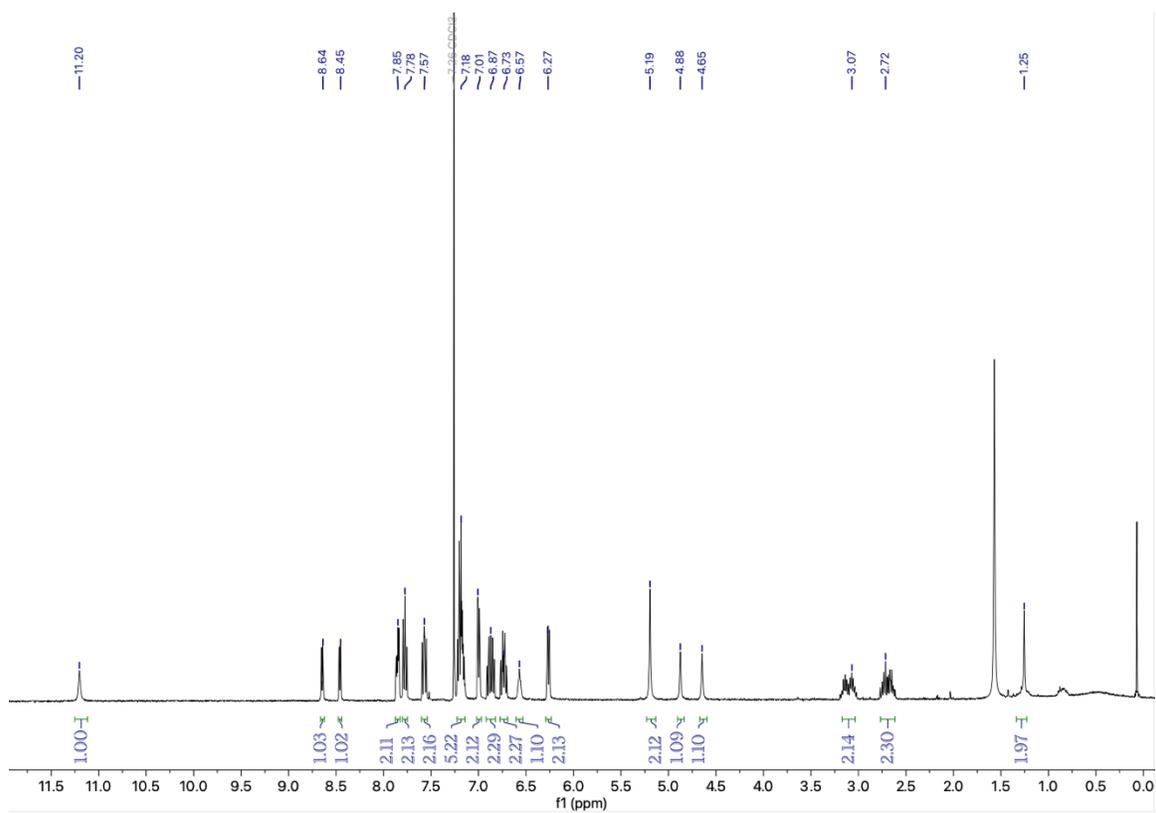
**Figure S2:**  $^{13}\text{C}$  NMR of compound **IRB1** in  $\text{CDCl}_3$ .



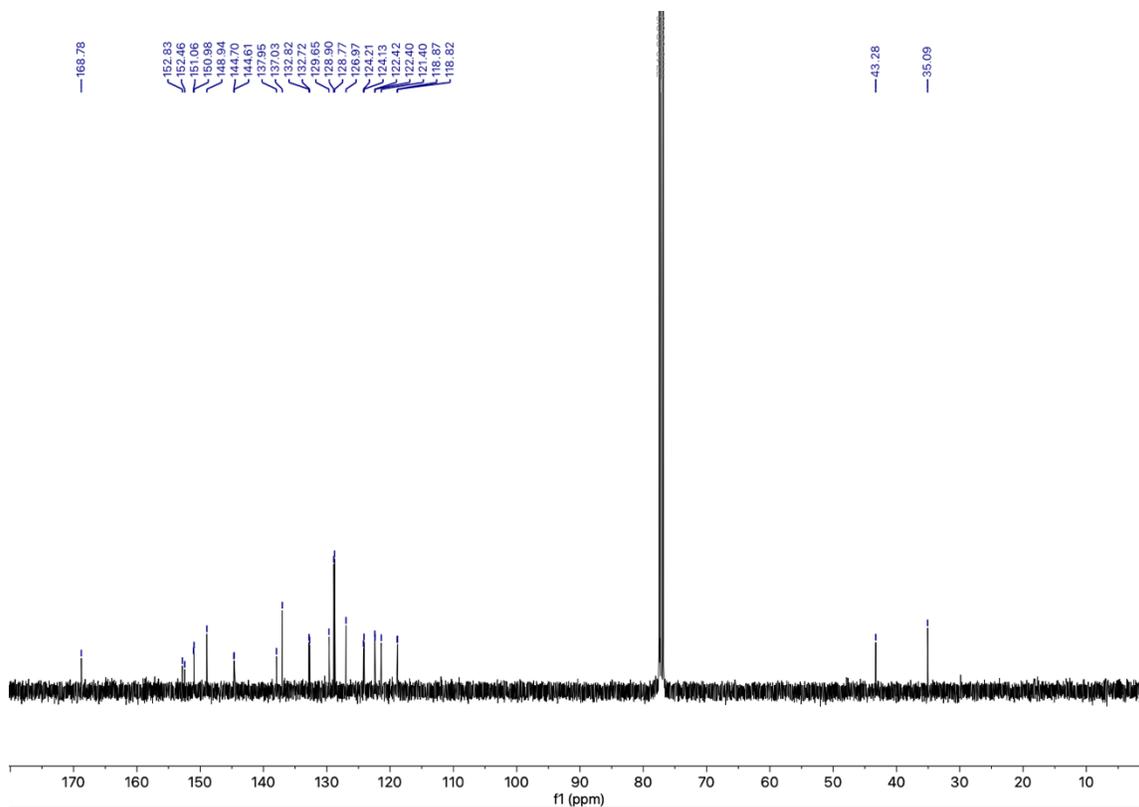
**Figure S3:** HRMS of compound **IRB1** (ESI+).



**Figure S4:** HPLC of compound **IRB1** (96.0% purity by integration).

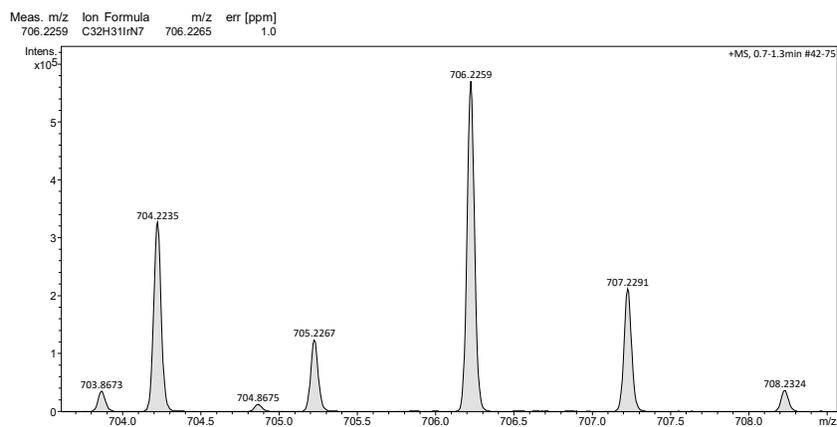


**Figure S5:** <sup>1</sup>H NMR of compound **IRB2** in CDCl<sub>3</sub>.



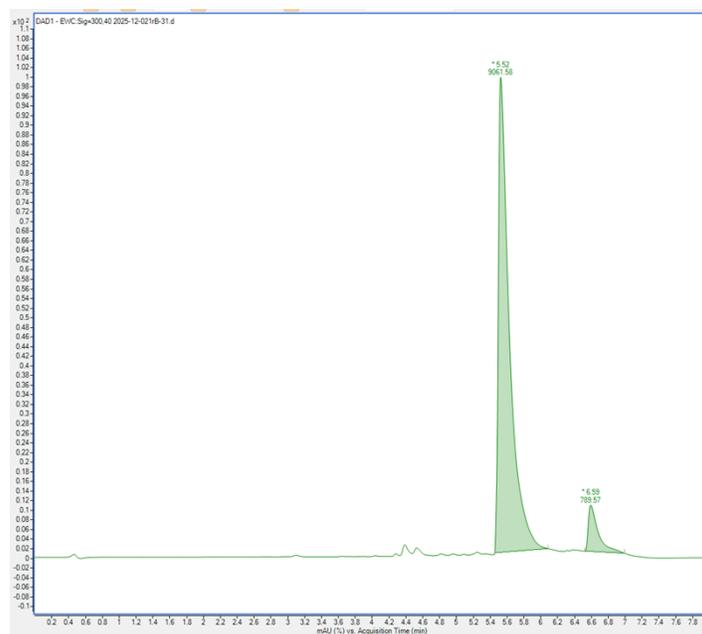
**Figure S6:**  $^{13}\text{C}$  NMR of compound **IRB2** in  $\text{CDCl}_3$ .

Analysis Info		Acquisition Date			
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Method	Xiao all 1.m	Operator	x		
Sample Name	IRB-21	Instrument	compact	8255754.20059	
Comment					
Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

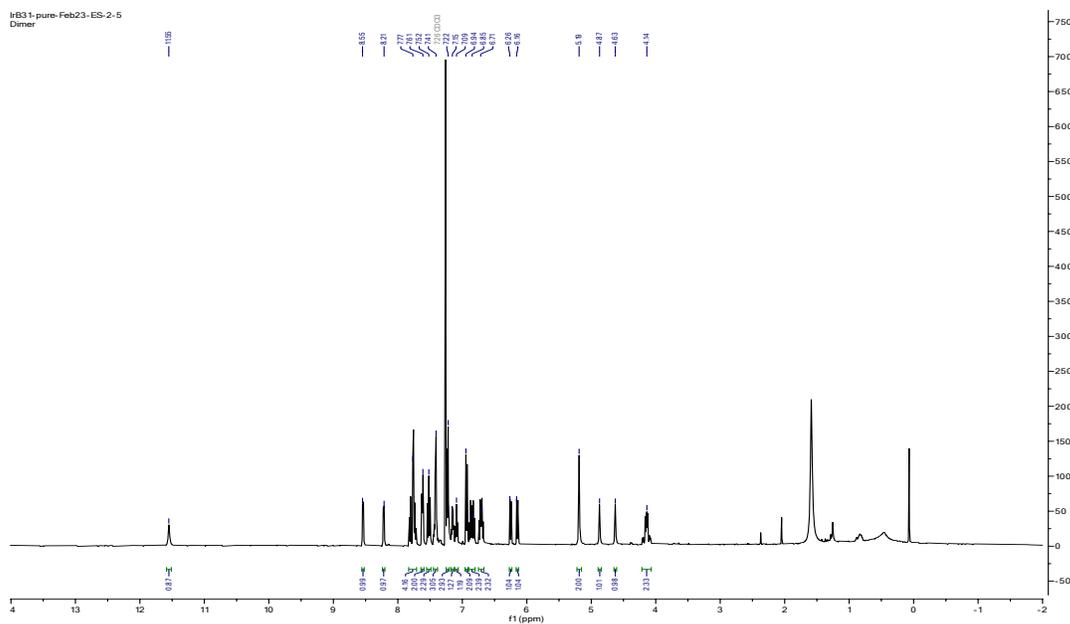


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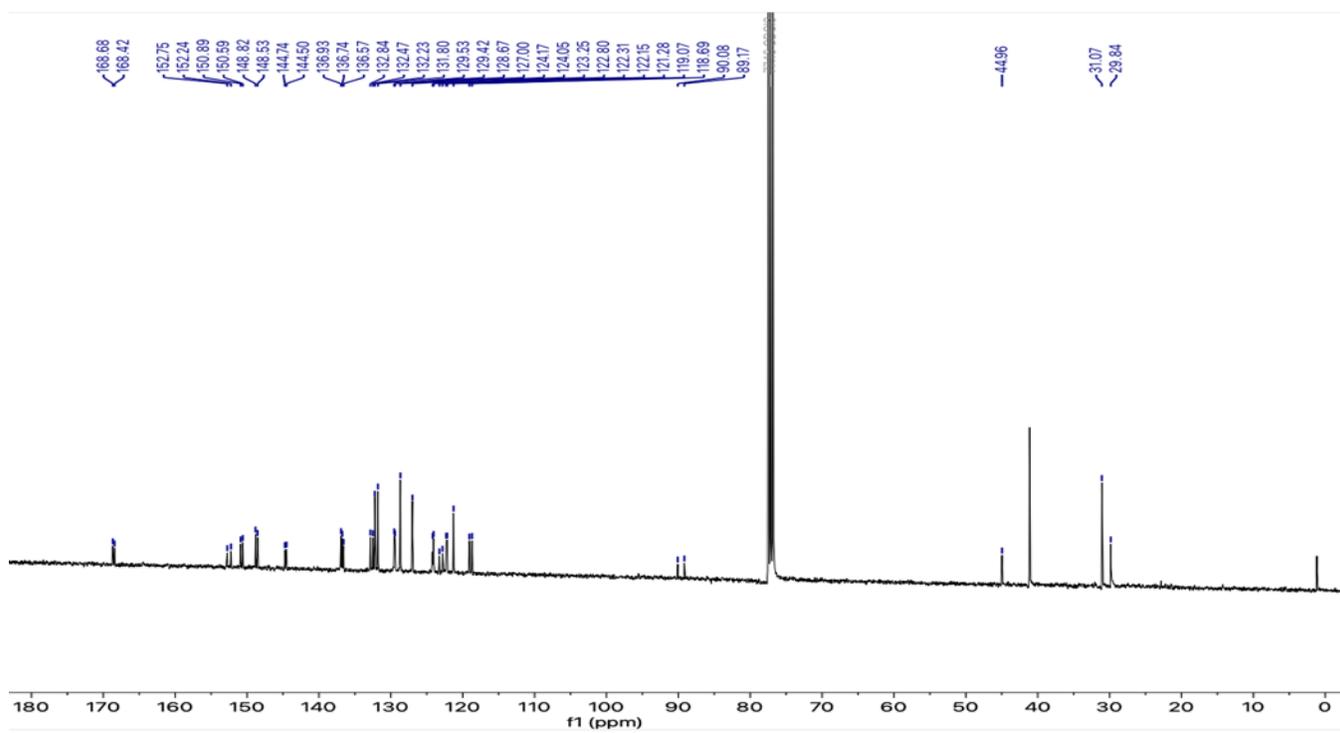
**Figure S7:** HRMS of compound **IRB2** (ESI+).



**Figure S8:** HPLC of compound **IRB2** (92.0% purity by integration).



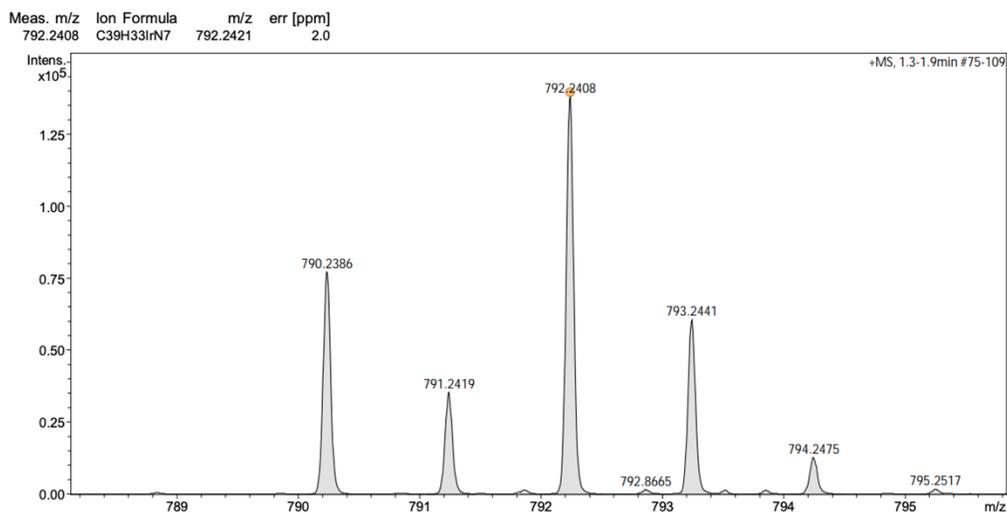
**Figure S9:** <sup>1</sup>H NMR of compound **IRB3** in CDCl<sub>3</sub>.



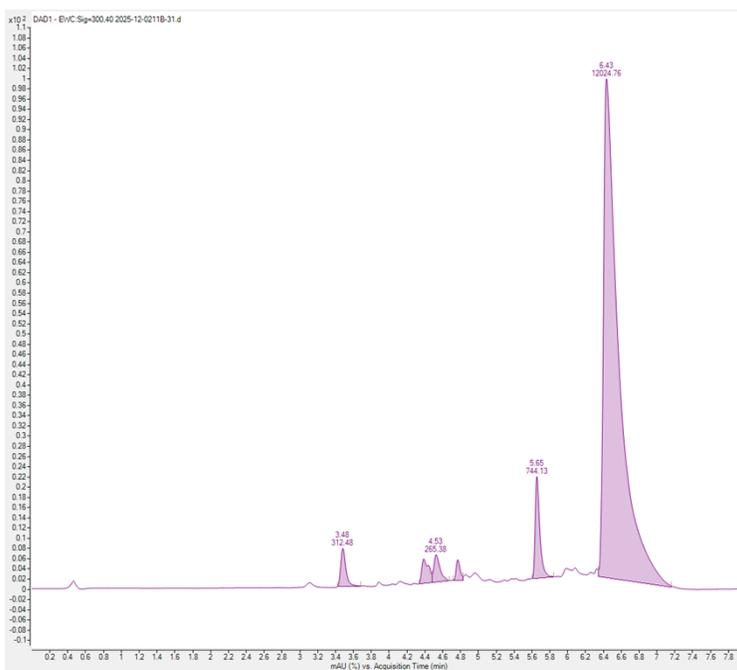
**Figure S10:** <sup>13</sup>C NMR of compound **IRB3** in CDCl<sub>3</sub>.

## Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date		2025-10-07 10:35:41 AM	
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Method	Xiao all 1.m				8255754.20059
Sample Name	IRB-31				
Comment					
Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	181 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



**Figure S11: HRMS of IRB3 (ESI+).**



**Figure S12: HPLC of compound IRB3 (90.1% purity by integration).**

## **Biological assays**

Absorbance measurements for the determination of  $IC_{50}$  and  $CC_{50}$  values were performed using a SpectraMax 190 microplate reader from Molecular Devices. The obtained data were analyzed with PRISM software by fitting a curve with nonlinear regression (log(inhibitor) vs. response -- Variable slope (four parameters)).

*hTERT*-HPNE cells (*hTERT*-immortalized epithelial cells) were obtained from Dr. M. Ouellette (University of Nebraska, Medical Center). The pancreatic cells PANC-1 (epithelial morphology cell line isolated from the pancreatic duct of a 56-year-old white male with an epithelioid carcinoma) and KP-4 (human pancreatic ductal carcinoma cell line derived from human ascites) were obtained from ATCC or the Massachusetts General Hospital Center for Molecular Therapeutics (CMT).

## **Cellular growth, $IC_{50}$ and $CC_{50}$ determination**

KP4, PANC1, IMR90, and *hTERT*-HPNE cells were cultured in DMEM (319-015-CL, Wisent) supplemented with 10% fetal bovine serum (FBS, Wisent) and Penicillin/Streptomycin/L-Glutamine (Wisent) at 37°C with 5%  $CO_2$ . The cells were seeded at a density of  $1 \times 10^3$  cells per well for KP4 and PANC1, and  $4 \times 10^3$  for IMR90 and *hTERT*-HPNE in 96-well plates for 24 hours. Following this, treatments with different concentrations of vehicle (DMSO) and compounds were carried out for 72 hours. The culture medium was then removed. The cells were washed twice with cold PBS to remove any dead cells. A 1% glutaraldehyde solution was added to the wells for 10 minutes to fix the remaining viable cells. The cells were then washed with PBS, stained with 0.2% violet crystal for 30 minutes at room temperature, washed with water, and dried. The adherent violet crystal was solubilized in 10% acetic acid, and absorbance was measured at a wavelength of 590 nm to calculate cell viability.

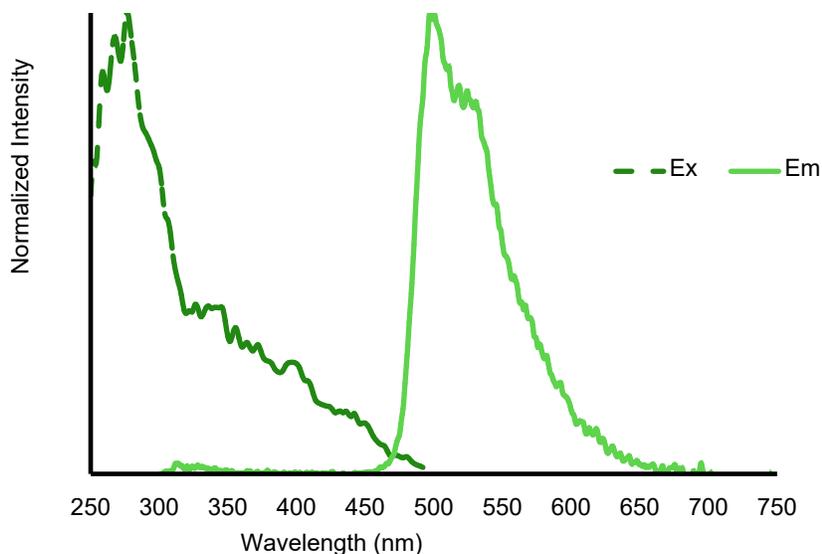
### Stability of Ir(III) complexes in PBS buffer solution

Solutions of Ir(III) complexes were made by taking stock solutions of **IRB1-3** ( $1.0 \times 10^{-4}$  M, DMSO) and diluting in PBS buffer to optically dilute concentration ( $1.0 \times 10^{-5}$  M) in a total volume of 3 mL. UV-Vis spectra were taken of each solution at  $t=0$ . UV-Vis was repeated at  $t= 0.5$  h, 1 h, 3 h, 6 h, 12, and 24 h and compared to the  $t=0$  spectrum.

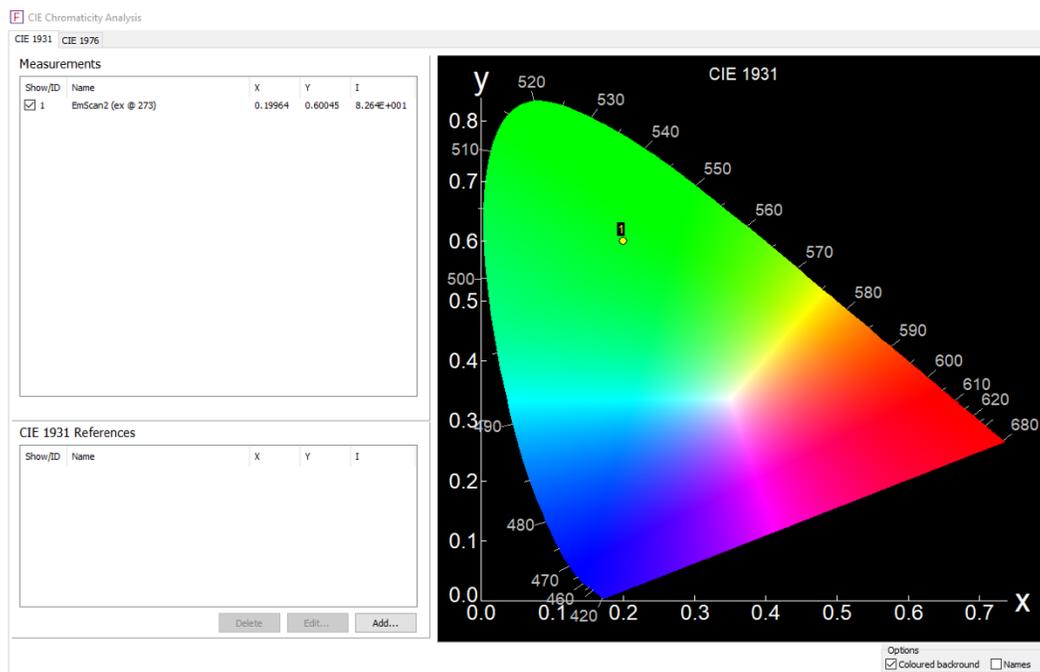
### Fluorescence Spectroscopy

**Table S1:** Fixed excitation wavelengths, emission ranges and average PLQYs for Ir(III) complexes **IRB1-3**; in  $\text{CHCl}_3$  degassed for 20 min (Ar) at 298K.

Ir(III) complex	Excitation maxima (nm)	Emission maxima (nm)	PLQY (%)	CIE Coordinates (x, y)
<b>IRB1</b>	278	501	40	(0.199, 0.600)
<b>IRB2</b>	280	501	45	(0.202, 0.595)
<b>IRB3</b>	286	501	58	(0.240, 0.590)



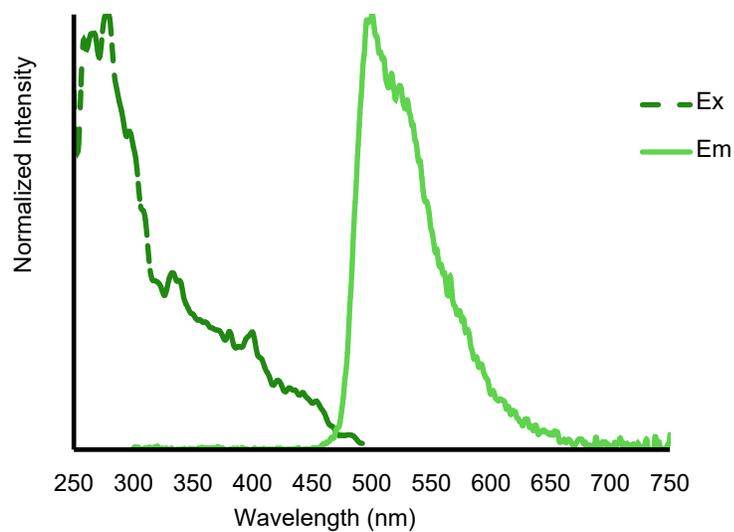
**Figure S13:** Normalized emission and excitation spectra of compound **IRB1** in  $\text{CHCl}_3$  ( $1 \times 10^{-5}$  M) at 298 K ( $\lambda_{\text{Ex}} = 273$  nm).



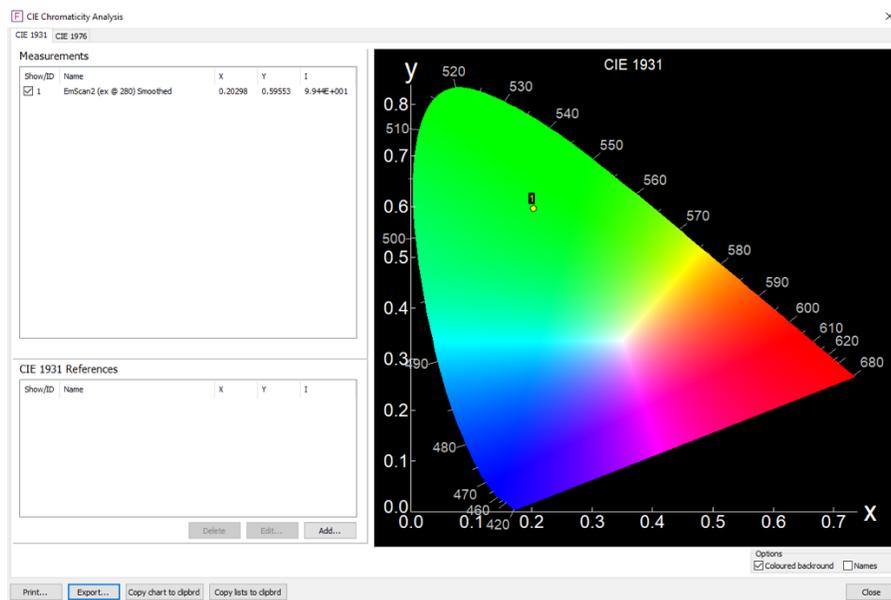
**Figure S14:** CIE chromaticity analysis of compound **IRB1**.

Table 1: Data Concerning the Standard				Table 2: Data Concerning the Sample			
Stock solution reference	Quinine Sulfate			Sample Structure	IrB-11		
Solvent	0.1 M Sulfuric Acid			Solvent	$\text{CHCl}_3$		
Refractive Index	1.33			Refractive Index	1.445		
Wavelength used (nm)	350			Wavelength used (nm)	350		
Run	1	2	3	Run	1	2	3
Volume of Mother solution (mL)	3	3	3	Volume of Mother solution (mL)	3.05	3.05	3.05
Theoretical Abs (optical)	0.065	0.065	0.065	Degassed 1 minute	0.05	0.05	0.05
Theoretical Abs (average)	0.065			Total Volume (mL)	3	3	3
Emission Integral	4.46E+07	4.09E+07	4.23E+07	Theoretical Abs (optical)	0.083	0.084	0.084
Emission Integral (average)	4.26E+07			Theoretical Abs (average)	0.083666667		
Standard Quantum Yield (%)	54.6			Emission Integral	3.56E+07	3.47E+07	3.27E+07
Values needed to be plugged in				Emission Integral (average)	3.43E+07		
				Calculated QY (%)	40.34		

**Figure S15:** Calculation table used for the determination of quantum yield for **IRB1** using the relative method.



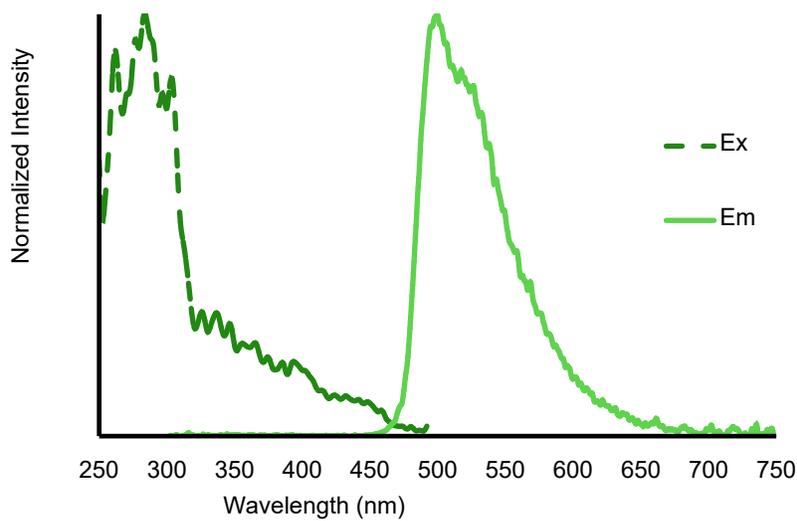
**Figure S16:** Normalized emission and excitation spectra of compound **IRB2** in  $\text{CHCl}_3$  ( $1 \times 10^{-5}$  M) at 298 K ( $\lambda_{\text{Ex}} = 280$  nm).



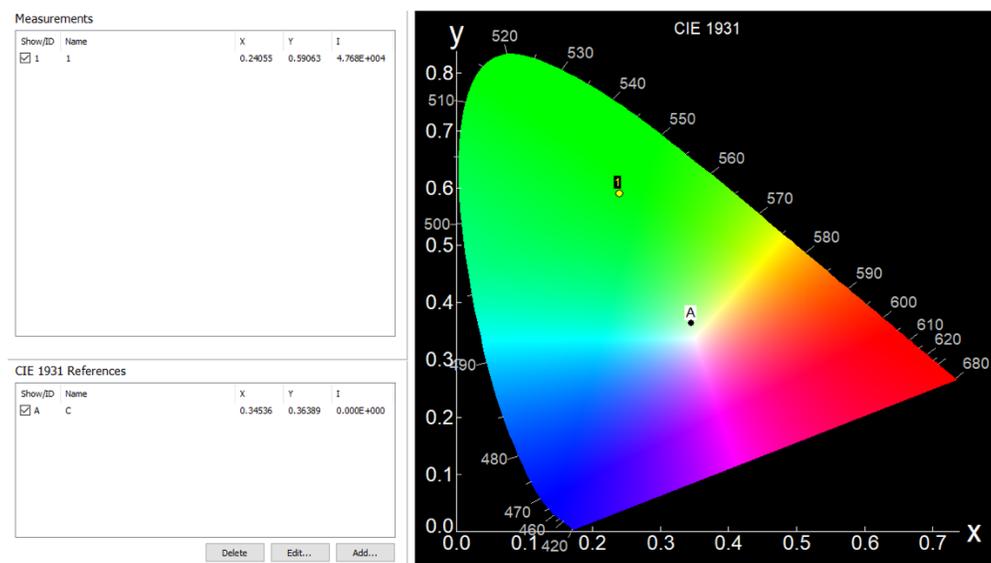
**Figure S17:** CIE chromaticity analysis of compound **IRB2**.

Table 1: Data Concerning the Standard				Table 2: Data Concerning the Sample			
Stock solution reference	Quinine Sulfate			Sample Structure	IrB-21		
Solvent	0.1 M Sulfuric Acid			Solvent	CHCl <sub>3</sub>		
Refractive Index	1.33			Refractive Index	1.445		
Wavelength used (nm)	350			Wavelength used (nm)	350		
Run	1	2	3	Run	1	2	3
Volume of Mother solution (mL)	3	3	3	Volume of Mother solution (mL)	3.05	3.05	3.05
Theoretical Abs (optical)	0.065	0.065	0.065	Degassed 1 minute	0.05	0.05	0.05
Theoretical Abs (average)	0.065			Total Volume (mL)	3	3	3
Emission Integral	4.66E+07	4.49E+07	4.37E+07	Theoretical Abs (optical)	0.071	0.071	0.071
Emission Integral (average)	4.51E+07			Theoretical Abs (average)	0.071		
Standard Quantum Yield (%)	54.6			Emission Integral	3.55E+07	3.23E+07	3.42E+07
Values needed to be plugged in				Emission Integral (average)	3.40E+07		
				Calculated QY (%)	44.54		

**Figure S18:** Calculation table used for the determination of quantum yield for **IRB2** using the relative method.



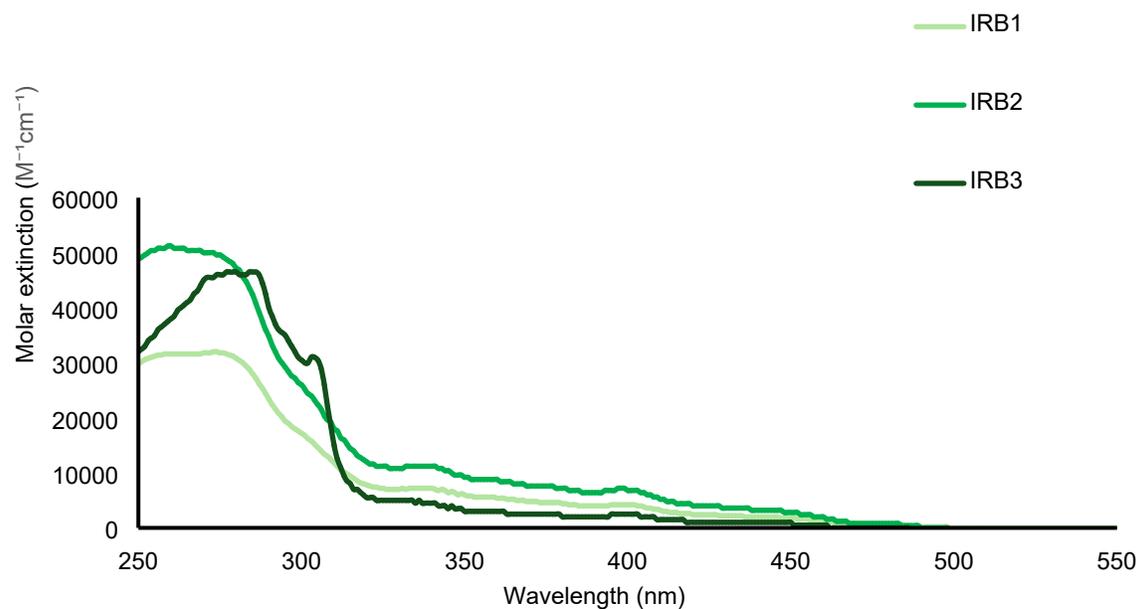
**Figure S19:** Normalized emission and excitation spectra of compound **IRB3** in CHCl<sub>3</sub> ( $1 \times 10^{-5}$  M) at 298 K ( $\lambda_{\text{Ex}} = 285$  nm).



**Figure S20:** CIE chromaticity analysis of compound **IRB3**.

Table 1: Data Concerning the Standard				Table 2: Data Concerning the Sample			
Stock solution reference	Quinine Sulfate			Sample Structure	IrB-31		
Solvent	0.1 M Sulfuric Acid			Solvent	CHCl <sub>3</sub>		
Refractive Index	1.33			Refractive Index	1.445		
Wavelength used (nm)	350			Wavelength used (nm)	350		
Run	1	2	3	Run	1	2	3
Volume of Mother solution (mL)	3	3	3	Volume of Mother solution (mL)	3.05	3.05	3.05
Theoretical Abs (optical)	0.065	0.065	0.065	Degassed 1 minute	0.05	0.05	0.05
Theoretical Abs (average)	0.065			Total Volume (mL)	3	3	3
Emission Integral	4.66E+07	4.49E+07	4.37E+07	Theoretical Abs (optical)	0.067	0.069	0.069
Emission Integral (average)	4.51E+07			Theoretical Abs (average)	0.068333333		
Standard Quantum Yield (%)	54.6			Emission Integral	4.33E+07	4.14E+07	4.22E+07
				Emission Integral (average)	4.23E+07		
Values needed to be plugged in				Calculated QY (%)	57.55		

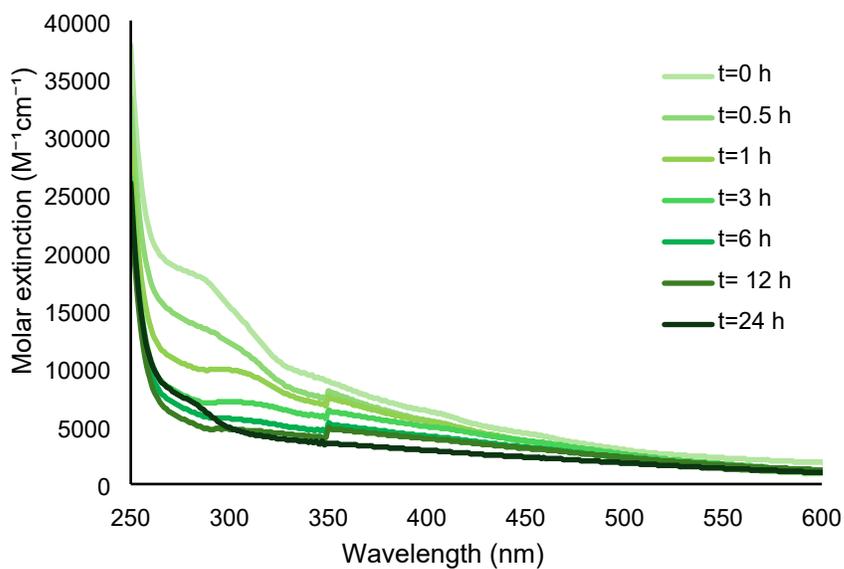
**Figure S21:** Calculation table used for the determination of quantum yield for **IRB3** using the relative method.



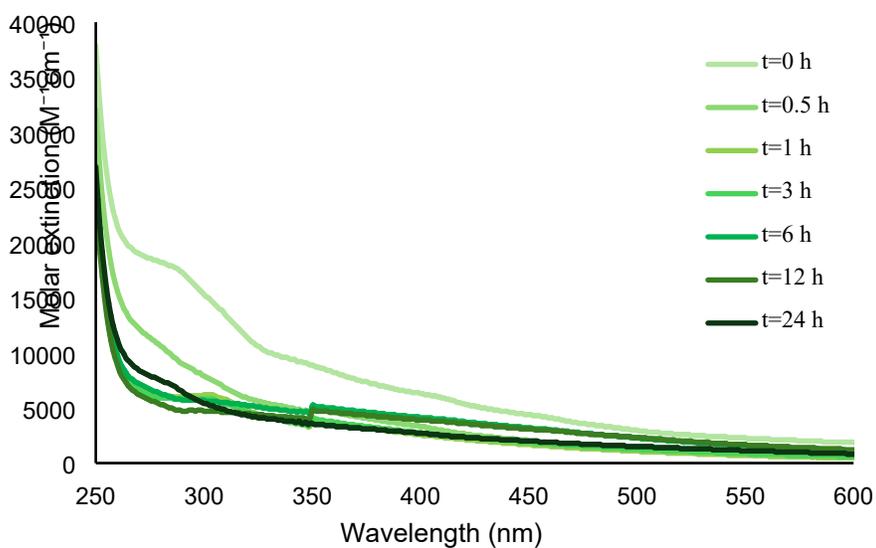
**Figure S22:** UV-Vis spectra of **IRB1**, **IRB2**, and **IRB3** plotted in terms of molar extinction coefficient ( $\epsilon$ ,  $\text{Mol}^{-1}\text{cm}^{-1}$ ).

**Table S2:** Summary of molar extinction coefficients determined for notable transitions from UV-Vis spectra of **IRB1-3** recorded at 298 K.

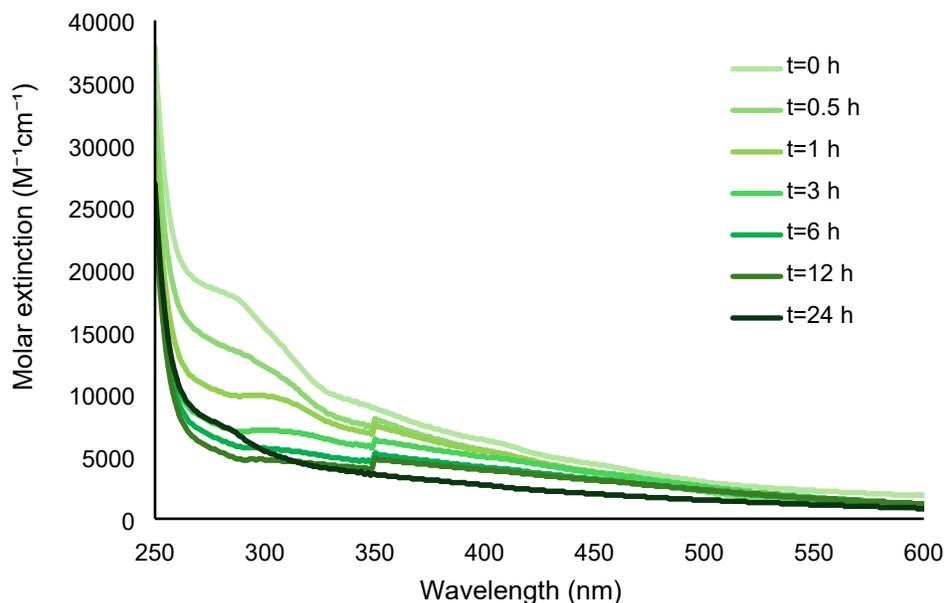
Complex	$\lambda_1$ (nm)	$\epsilon_1$ ( $\text{Mol}^{-1}\text{cm}^{-1}$ )	$\lambda_2$ (nm)	$\epsilon_2$ ( $\text{Mol}^{-1}\text{cm}^{-1}$ )	$\lambda_3$ (nm)	$\epsilon_3$ ( $\text{Mol}^{-1}\text{cm}^{-1}$ )
<b>IRB1</b>	280	38000	340	7200	406	3600
<b>IRB2</b>	265	54000	339	11200	400	6800
<b>IRB3</b>	287	46000	334	5000	398	2500



**Figure S23:** UV-Vis spectra of **IRB1** in PBS buffer plotted in terms of molar extinction coefficient ( $\epsilon$ ,  $\text{Mol}^{-1}\text{cm}^{-1}$ ) over a 24 h period.



**Figure S24:** UV-Vis spectra of **IRB2** in PBS buffer plotted in terms of molar extinction coefficient ( $\epsilon$ ,  $\text{Mol}^{-1}\text{cm}^{-1}$ ) over a 24 h period.



**Figure S25:** UV-Vis spectra of **IRB3** in PBS buffer plotted in terms of molar extinction coefficient ( $\epsilon$ ,  $\text{M}^{-1}\text{cm}^{-1}$ ) over a 24 h period.

### Fluorescence Microscopy

KP4 cancer cells were seeded at a density of  $8 \times 10^4$  cells per well on microscope slides in 6-well plates overnight. They were then treated with either the vehicle or the studied compounds for the desired treatment times (24h, 4h, 2h, 30min). The culture medium was replaced, and a solution of MitoTracker® Deep Red FM (100 nM in DMEM) was added. After 30 minutes at  $37^\circ\text{C}$ , the medium was removed. The cells were washed twice with PBS. A 4% paraformaldehyde solution was added to the wells for 10 minutes to fix the cells onto the microscope slides. The cells were then washed three times with a 0.1 M glycine solution in PBS. 8  $\mu\text{L}$  of mounting medium for fluorescence without DAPI was used to mount each slide onto a microscope slide. The slides were sealed, dried, and observed under a microscope. Microscopy images were acquired using the Zeiss Axio-Imager Z2 Microscope. The fluorescence filter sets used were: GFP HE (Zeiss Set 38): BP 470/40x FT 495 BP 525/50m; Cy5 (Chroma 49009): BP ET640/30x BS TLPXR660 BP ET690/50m, along with the DIC analyser. Pearson coefficients were calculated with Zeiss Zen 3.8 Software.

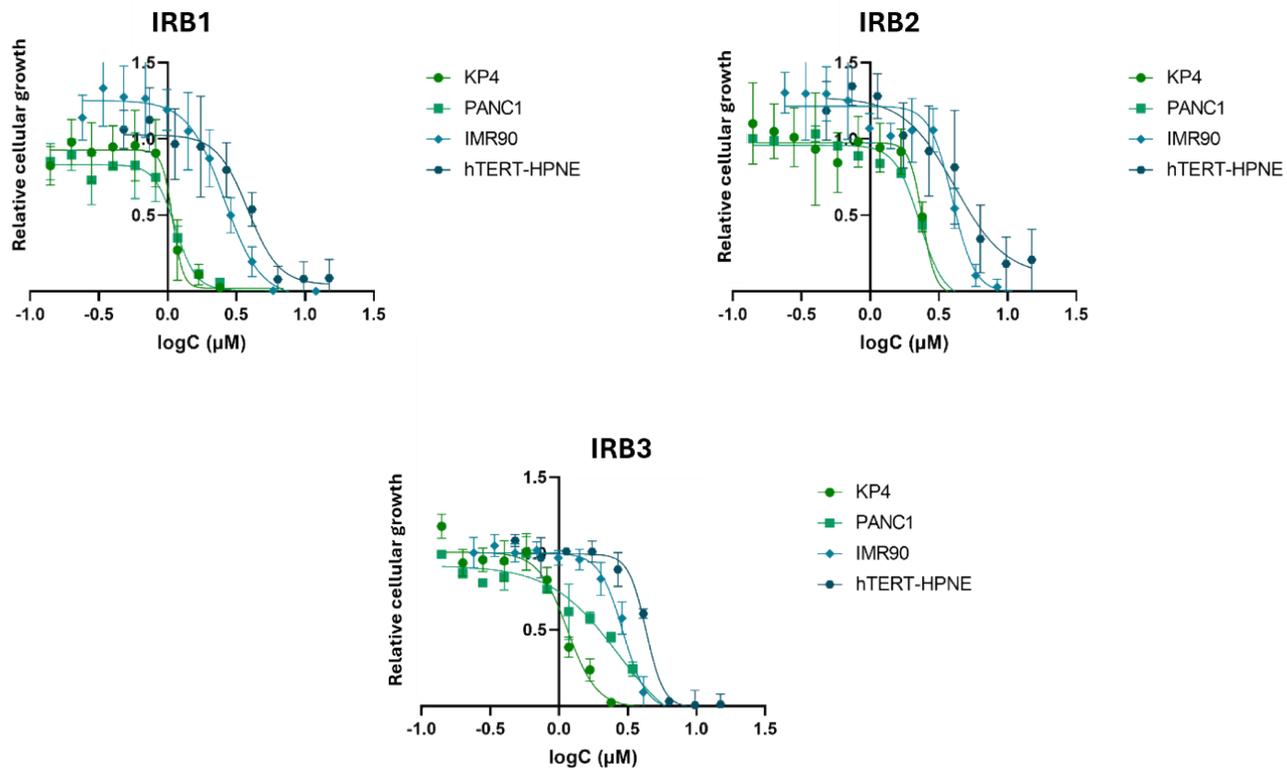


Figure S26:  $IC_{50}$  of IRB1-IRB3 on all the tested cell lines.