Electronic Supplementary Information

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Structure-guided discovery of a luminescent theranostic toolkit for living cancer cells and the imaging behavior effect

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Part A. Chemicals and Materials

Iridium chloride hydrate (IrCl$_3$·xH$_2$O) was purchased from Precious Metals Online (Australia). Phosphate-buffered saline (10×) pH 7.4 (AM9625) was purchased from Life Technologies Ltd. (Hong Kong). Recombinant Human EGFR protein (10001–H08B) was purchased from Sino Biological Inc. (China). Unless specified, all the reagents were purchased from J&K Chemical Ltd. (China) and used as received without further purification, and all aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm$^{-1}$) unless specified. A431 and LO2 cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). EGFR kinase assay kit was purchased from BPS Bioscience, Inc.

Part B. Synthesis experiment

Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. $^1$H and $^{13}$C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz ($^1$H) and 100 MHz ($^{13}$C). $^1$H and $^{13}$C chemical shifts were referenced internally to solvent shift (CDCl$_3$–d: $^1$H, 7.26, $^{13}$C, 77.16; DMSO–d$_6$: $^1$H, 2.50, $^{13}$C, 39.52; acetone–d$_6$: $^1$H, 2.05, $^{13}$C, 29.8). Chemical shifts are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for $^1$H and ±0.05 for $^{13}$C. Coupling constants are typically ±0.1 Hz for $^1$H–$^1$H and ±0.5 Hz for $^1$H–$^{13}$C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data were acquired and processed using standard Bruker software (Topspin).

Synthesis of complexes 2a–2h

Complex 2a was synthesized according to reported literatures.$^{1-3}$ Briefly, 2.1 equivalents of 2-phenylpyridine (ppy) and 1 equivalent of IrCl$_3$·xH$_2$O were mixed together and further heated overnight at 120 °C in 12 mL of 2-methoxyethanol/H$_2$O (v/v, 3/1). Afterwards, the mixture was filtered and washed by excessive deionized
water and then diethyl ether for three times, respectively to generate the dichloro-bridged dimer \([\text{Ir}(ppy)_2\text{Cl}]_2\). 100 mg of compound S2 (1 eq.) was firstly dissolved in 50 mL tetrahydrofuran (THF) with further addition of 2-chloroacetyl chloride (1.2 eq.) and triethylamine (1.5 eq.) for reaction at 0 °C to generate the light yellow compound S3. After purification of compound S3 by silica gel column chromatography, the oven-dried dimer S5 was treated with 2.1 equivalents of compound S3 in DCM (4 mL) and methanol (4 mL) at ambient temperature for 10 h. Then, an excess of solid ammonium hexafluorophosphate (NH₄PF₆) was added and the reaction was stirred for another 20 min. The brown powder S6 thus obtained was isolated and filtrated by removing the solvent under reduced pressure, and the residue was purified by silica gel column chromatography employing dichloromethane (DCM) and methanol as solvents. Afterwards, compound S6 (1 eq.) was added and dissolved in acetonitrile (ACN), followed by compound S1 (2 eq.), cesium carbonate (Cs₂CO₃, 2 eq.), potassium iodide (KI, 2 eq.) for overnight reaction at 80 °C. The solvent was then removed under reduced pressure and the crude mixture was further purified by silica gel column chromatography to obtain compound S7. Finally, the Knoevenagel condensation of propanedinitrile to compound S7 (1 eq.) was then performed by subsequent addition of cyanoacetonitrile (2 eq.) and L-proline (2 eq.) in DCM at ambient temperature. Further purification of the crude mixture was conducted to collect the final compound 2a by DCM-methanol based column chromatography. Compounds 2b–2h were synthesized by the same method as that of compound 2a by the replacement of auxiliary C^N ligands (CN2–CN8) of compound S6.
Scheme S1 Synthesis routes of iridium(III) complexes 2a–2h. Conditions: (A) H₂O, rt; (B) triethylamine, THF; (C) Cs₂CO₃, KI, ACN; (D) DCM:MeOH (1:1, v/v); (E) Cs₂CO₃, KI, ACN; (F) Cs₂CO₃, KI, ACN; (G) Cs₂CO₃, KI, ACN; (H) DCM, L-Proline.

¹H NMR (400 MHz, Acetone) δ 9.96 (s, 1H), 8.91 (d, J = 8.4 Hz, 1H), 8.74 (d, J = 8.4 Hz, 1H), 8.58 (d, J = 5.2 Hz, 1H), 8.35 (d, J = 4.8 Hz, 1H), 8.25 (d, J = 4.8 Hz, 1H), 8.10 (d, J = 8.0 Hz, 2H), 7.97 (dd, J = 8.4, 5.2 Hz, 1H), 7.90 (dd, J = 8.6, 5.0 Hz, 1H), 7.81 – 7.75 (m, 4H), 7.57 (t, J = 6.0 Hz, 2H), 6.95 (t, J = 7.6 Hz, 2H), 6.88 – 6.82 (m, 4H), 6.32 (dd, J = 7.2, 3.6 Hz, 2H), 4.41 (s, 2H). ¹³C NMR (101 MHz, Acetone) δ 167.76, 167.73, 166.19, 151.46, 150.60, 150.04, 149.70, 149.49, 149.46, 147.39, 145.07, 144.27, 144.23, 138.59, 138.36, 133.99, 133.59, 131.78, 131.74, 131.70, 131.20, 130.36, 127.61, 127.24, 126.63, 124.93, 123.49, 123.44, 122.60, 120.85, 119.83, 43.19. HRMS: Caled. for C₃₆H₂₆ClIrN₅OPF₆ [M–PF₆]⁺: 772.1443 Found: 772.1405.

¹H NMR (400 MHz, Acetone) δ 10.26 (s, 1H), 9.80 (s, 1H), 8.96 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 7.6 Hz, 1H), 8.57 (s, 1H), 8.33 (d, J = 4.4 Hz, 1H), 8.23 (d, J = 4.0 Hz, 1H), 8.10 (d, J = 6.4 Hz, 2H), 7.95 – 7.86 (m, 2H), 7.84 – 7.74 (m, 6H), 7.56 (d, J = 5.2 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 6.94 (t, J = 7.2 Hz, 2H), 6.87 – 6.81 (m, 4H), 6.31 (d, J = 7.2 Hz, 2H), 5.02 (s, 2H). ¹³C NMR (101 MHz, Acetone) δ 191.29, 168.66, 163.51, 152.31, 151.42, 150.97, 150.65, 150.35, 148.24, 145.91, 145.17, 145.13, 139.48, 139.20, 135.40, 132.69, 132.66, 132.56, 132.11, 132.02, 131.24, 128.09, 127.41, 125.83, 124.39, 124.36, 123.48, 120.73, 116.20, 68.34. HRMS: Caled. for C₄₃H₃₀IrN₃O₃PF₆ [M–PF₆]⁺: 857.9549 Found: 857.8516.
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**H NMR (400 MHz, Acetone)** \(\delta\) 9.00 (dd, J = 8.8, 1.2 Hz, 1H), 8.72 (dd, J = 8.4, 1.2 Hz, 1H), 8.67 (s, 1H), 8.46 (dd, J = 4.8, 1.2 Hz, 1H), 8.35 (dd, J = 4.8, 1.2 Hz, 1H), 8.21-8.18 (m, 3H), 8.08 (d, J = 8.8 Hz, 2H), 8.03 (dd, J = 8.8, 4.8 Hz, 1H), 7.94 – 7.83 (m, 5H), 7.68 (d, J = 5.6 Hz, 2H), 7.37 (d, J = 9.2 Hz, 2H), 7.06 – 6.89 (m, 7H), 6.50 – 6.42 (m, 2H), 5.17 (s, 2H). \(^{13}C\) NMR (101 MHz, Acetone) \(\delta\) 168.62, 168.60, 168.13, 163.67, 160.62, 152.34, 151.45, 151.01, 150.72, 150.34, 148.26, 145.99, 145.24, 145.21, 139.55, 139.25, 139.24, 135.27, 134.34, 132.74, 132.71, 132.07, 131.30, 131.28, 128.71, 128.08, 127.50, 126.25, 125.88, 124.47, 123.55, 122.12, 120.79, 116.89, 115.32, 114.50, 79.63, 68.33. HRMS: Calcd. for C\(_{46}\)H\(_{31}\)IrN\(_{6}\)O\(_{2}\)PF\(_{6}\) [M–PF\(_{6}\)]\(^+\): 906.0010 Found: 906.0756.

**H NMR (400 MHz, CDCl\(_3\))** \(\delta\) 9.46 (s, 1H), 8.91 – 8.82 (m, 3H), 8.51 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.25 – 8.22 (m, 2H), 7.97 (dd, J = 9.6, 4.8 Hz, 2H), 7.78 – 7.60 (m, 9H), 7.13 – 7.10 (m, 5H), 6.90 – 6.86 (s, 2H), 6.35 – 6.27 (m, 2H), 4.40 (s, 2H). \(^{13}C\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 169.09, 166.96, 152.94, 150.97, 150.09, 146.97, 145.67, 144.88, 140.36, 140.24, 138.31, 137.02, 134.50, 133.45, 132.44, 131.83, 131.00, 130.86, 128.91, 128.10, 127.64, 126.92, 126.59, 126.28, 122.61, 122.19, 121.50, 43.31. HRMS: Calcd. for C\(_{44}\)H\(_{36}\)ClIrN\(_{6}\)OPF\(_{6}\) [M–PF\(_{6}\)]\(^+\): 872.4119 Found: 872.6614.

**H NMR (400 MHz, CDCl\(_3\))** \(\delta\) 10.09 (s, 1H), 9.91 (s, 1H), 9.05 – 8.89 (m, 3H), 8.60 (s, 1H), 8.47 (d, J = 7.2 Hz, 1H), 8.31 (t, J = 7.2 Hz, 2H), 8.07 (dd, J = 5.0, 1.3 Hz, 1H), 8.03 (dd, J = 5.1, 1.4 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.87 – 7.72 (m, 6H), 7.68 (dd, J = 8.4, 5.2 Hz, 4H), 7.30 – 7.27 (m, 2H), 7.19 – 7.14 (m, 4H), 6.98 – 6.93 (m, 2H), 6.39 (dd, J = 13.3, 7.4 Hz, 2H), 5.11 (s, 2H). \(^{13}C\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 189.92, 168.22, 167.80, 167.14, 161.33, 151.87, 151.56, 149.92, 148.80, 145.98, 144.74, 144.49, 144.03, 139.38, 139.04, 137.07, 136.03, 135.93, 133.95, 132.59, 131.45, 131.30, 130.81, 130.68, 130.04, 129.99, 129.79, 129.64, 127.84, 127.69, 127.24, 126.63, 126.47, 125.95, 125.78, 125.49, 125.37, 125.31, 125.21, 121.56, 121.46, 121.20, 120.82, 120.49, 114.34, 66.15. HRMS: Calcd. for C\(_{35}\)H\(_{29}\)IrN\(_{6}\)O\(_{2}\)PF\(_{6}\) [M–PF\(_{6}\)]\(^+\): 958.0723 Found: 958.2371.

**H NMR (400 MHz, CDCl\(_3\))** \(\delta\) 9.90 (s, 1H), 9.05 – 9.01 (m, 3H), 8.70 (s, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.39 (t, J = 6.4 Hz, 2H), 8.16 (d, J = 4.4 Hz, 1H), 8.11 (d, J = 4.8 Hz, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.92 – 7.88 (m, 4H), 7.85 – 7.82 (m, 4H), 7.79 – 7.74 (m, 1H), 7.69 (d, J = 9.2 Hz, 2H), 7.35 – 7.28 (m, 4H), 7.24 (d, J = 6.4 Hz, 2H), 7.03 (t, J = 7.2 Hz, 2H), 6.50 – 6.43 (m, 2H), 5.14 (s, 2H). \(^{13}C\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 169.29, 168.90, 167.73, 162.57, 158.92, 152.85, 152.57, 151.01, 149.87, 147.04, 145.78, 145.54, 145.13, 140.38, 138.12, 137.10, 137.01, 134.88, 133.59, 133.49, 132.37, 131.74, 131.05, 130.85, 130.69, 128.88,
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1H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 9.01 (d, J = 8.8 Hz, 1H), 8.90 (dd, J = 8.4, 1.2 Hz, 1H), 8.64 (s, 1H), 8.32 (dd, J = 5.2, 1.0 Hz, 1H), 8.28 (d, J = 8.8 Hz, 2H), 8.21 (dd, J = 5.2, 1.2 Hz, 1H), 8.08 (dd, J = 8.8, 5.2 Hz, 1H), 8.01 – 7.93 (m, 3H), 7.52 (d, J = 5.6 Hz, 2H), 7.06 (t, J = 6.6 Hz, 2H), 7.00 (t, J = 11.0 Hz, 2H), 5.68 (td, J = 8.7, 2.3 Hz, 2H), 4.55 (s, 2H). 13C NMR (101 MHz, DMSO) δ 166.88, 163.20, 161.94, 159.91, 154.56, 154.51, 154.20, 154.15, 151.89, 150.91, 150.35, 146.70, 144.24, 140.43, 139.41, 135.40, 133.82, 131.23, 128.27, 128.05, 127.62, 127.40, 124.86, 123.89, 123.69, 120.99, 113.99, 99.64, 43.82. HRMS: Calcd. for C₆₅H₅₅IrN₃O₂PF₆ [M – PF₆]⁺: 1006.1184 Found: 1006.2544.

1H NMR (400 MHz, Acetone) δ 12.62 (s, 1H), 10.11 (d, J = 8.4 Hz, 1H), 9.74 (s, 1H), 8.70 (s, 1H), 8.64 (d, J = 8.3 Hz, 1H), 8.40 (dd, J = 5.6 Hz, 1H), 8.27 (d, J = 4.7 Hz, 1H), 8.23 (d, J = 8.1 Hz, 2H), 7.94 – 7.80 (m, 4H), 7.71 (d, J = 7.6 Hz, 3H), 7.62 (d, J = 5.6 Hz, 1H), 7.14 (d, J = 8.0 Hz, 2H), 6.99 – 6.87 (m, 2H), 6.64 (t, J = 11.0 Hz, 2H), 5.77 (d, J = 8.4 Hz, 2H), 5.45 (s, 2H). 13C NMR (101 MHz, Acetone) δ 191.24, 169.29, 164.64 (d, J = 14.5 Hz), 152.33, 151.24, 150.97, 150.87, 147.93, 144.87, 140.48, 139.48, 138.27, 136.17, 132.67, 132.44, 131.41, 129.10, 128.47, 127.96, 127.14, 125.03, 124.96, 124.49, 124.29, 118.90, 116.18, 114.92, 114.70, 99.93, 99.66, 99.33, 68.32. HRMS: Calcd. for C₄₆H₅₂F₆IrN₅O₆PF₆ [M – PF₆]⁺: 930.1676 Found: 930.1714.

1H NMR (400 MHz, CDCl₃) δ 12.65 (s, 1H), 10.04 (d, J = 8.4 Hz, 1H), 8.82 (s, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.37 – 8.33 (m, 2H), 8.29 (d, J = 4.8 Hz, 1H), 8.19 (d, J = 4.8 Hz, 1H), 8.00 (dd, J = 8.4, 4.8 Hz, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.83 – 7.77 (m, 3H), 7.65 (s, 1H), 7.18 – 7.24 (m, 3H), 7.22 (d, J = 5.6 Hz, 1H), 6.94 (t, J = 6.0 Hz, 1H), 6.90 (t, J = 6.4 Hz, 1H), 6.67 – 6.61 (m, 2H), 5.78 (t, J = 9.2 Hz, 2H), 5.56 (s, 2H). 13C NMR (101 MHz, CDCl₃) δ 168.98, 164.51, 163.62, 159.03, 152.93, 150.57, 149.08, 149.08, 148.46, 148.14, 146.70, 144.03, 139.32, 139.24, 138.54, 137.83, 135.50, 133.49, 131.59, 128.36, 127.56, 126.66, 126.48, 124.45, 124.24, 123.60, 123.49, 119.48, 116.18, 114.57, 114.28, 114.10, 113.31, 99.56, 78.60, 67.65. HRMS: Calcd. for C₄₆H₅₂F₆IrN₅O₆PF₆ [M – PF₆]⁺: 978.1792 Found: 978.1740.

1H NMR (400 MHz, Acetone) δ 10.08 (s, 1H), 9.04 (d, J = 8.4 Hz, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.71 (s, 1H), 8.51 (d, J = 5.2 Hz, 1H), 8.40 (d, J = 5.2 Hz, 1H), 8.18 (d, J = 8.0 Hz, 2H), 8.11 (dd, J = 8.4, 4.8 Hz, 1H), 8.07 – 8.00 (m, 1H), 7.89 – 7.83 (m, 4H), 7.66 (t, J = 5.9 Hz, 2H), 6.98 – 6.88 (m, 4H), 6.33 – 6.29 (m, 2H), 4.57
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\( ^{13} \text{C NMR (101 MHz, Acetone)} \) δ 168.73, 168.70, 167.11, 152.32, 151.45, 151.29, 150.97, 150.18, 150.16, 148.28, 145.99, 142.58, 142.54, 141.11, 141.09, 139.30, 139.20, 134.84, 134.49, 133.43, 133.39, 132.05, 128.50, 128.08, 127.50, 125.77, 125.75, 124.52, 124.51, 123.80, 123.77, 121.78, 120.33, 44.08, 21.88. HRMS: Calcd. for C\(_{38}\)H\(_{36}\)ClIrN\(_5\)OPF\(_6\) [M–PF\(_6\)]: 800.3477 Found: 800.8413.

\( ^{1} \text{H NMR (400 MHz, CDCl3)} \) δ 12.57 (s, 1H), 9.88 (s, 1H), 8.74 (s, 1H), 8.41 (d, \( J = 8.4 \text{ Hz}, 1H \)), 8.23 (d, \( J = 4.8 \text{ Hz}, 1H \)), 8.20 – 8.16 (m, 1H), 8.15 (d, \( J = 4.8 \text{ Hz}, 1H \)), 7.91 – 7.79 (m, 6H), 7.71 – 7.66 (m, 3H), 7.64 – 7.60 (m, 2H), 7.31 – 7.28 (m, 2H), 7.21 (d, \( J = 6.4 \text{ Hz}, 1H \)), 6.93 – 6.91 (m, 2H), 6.85 – 6.77 (m, 2H), 6.19 (d, \( J = 8.8 \text{ Hz}, 2H \)), 5.53 (s, 2H), 2.18 (s, 6H). \( ^{13} \text{C NMR (101 MHz, CDCl3)} \) δ 191.04, 169.22, 168.00, 163.40, 150.67, 149.98, 149.30, 148.36, 148.07, 147.13, 144.50, 141.13, 141.11, 137.92, 137.88, 136.90, 135.22, 132.66, 132.03, 126.27, 124.75, 123.97, 122.55, 120.02, 119.70, 119.28, 115.42, 67.53, 21.90. HRMS: Calcd. for C\(_{48}\)H\(_{36}\)IrN\(_7\)O\(_3\)PF\(_6\) [M–PF\(_6\)]: 886.2367 Found: 886.2370.

\( ^{1} \text{H NMR (400 MHz, CDCl3)} \) δ 9.32 (s, 1H), 8.78 (d, \( J = 8.4 \text{ Hz}, 1H \)), 8.54 (s, 1H), 8.42 (d, \( J = 8.0 \text{ Hz}, 1H \)), 8.24 (dd, \( J = 18.3, 5.0 \text{ Hz}, 2H \)), 7.89 – 7.82 (m, 5H), 7.71 – 7.64 (m, 3H), 7.60 (dd, \( J = 8.2, 5.6 \text{ Hz}, 2H \)), 7.47 (s, 1H), 7.31 (d, \( J = 5.6 \text{ Hz}, 1H \)), 7.27 – 7.24 (m, 1H), 7.19 (d, \( J = 8.8 \text{ Hz}, 2H \)), 6.87 – 6.79 (m, 4H), 6.18 (d, \( J = 7.2 \text{ Hz}, 2H \)), 4.93 (s, 2H), 2.13 (s, 6H). \( ^{13} \text{C NMR (101 MHz, Acetone)} \) δ 168.67, 168.21, 163.84, 160.55, 152.22, 151.37, 151.26, 151.03, 150.21, 150.13, 148.22, 145.77, 142.56, 142.52, 141.09, 141.06, 139.29, 139.04, 135.60, 134.58, 134.25, 133.42, 133.40, 132.07, 130.59, 128.47, 128.06, 127.28, 125.94, 124.51, 124.51, 123.80, 123.80, 121.30, 120.32, 116.81, 115.29, 114.46, 79.51, 68.33, 21.91. HRMS: Calcd. for C\(_{48}\)H\(_{36}\)IrN\(_7\)O\(_3\)PF\(_6\) [M–PF\(_6\)]: 934.0542 Found: 934.2518.

\( ^{1} \text{H NMR (400 MHz, Acetone)} \) δ 10.08 (s, 1H), 9.05 (d, \( J = 8.4 \text{ Hz}, 1H \)), 8.87 (d, \( J = 8.0 \text{ Hz}, 1H \)), 8.72 (s, 1H), 8.57 (d, \( J = 4.4 \text{ Hz}, 1H \)), 8.46 (d, \( J = 4.4 \text{ Hz}, 1H \)), 8.14 – 8.10 (m, 3H), 8.05 (dd, \( J = 8.1, 5.1 \text{ Hz}, 1H \)), 7.90 (d, \( J = 8.6 \text{ Hz}, 2H \)), 7.84 (t, \( J = 7.6 \text{ Hz}, 2H \)), 7.62 (t, \( J = 5.3 \text{ Hz}, 2H \)), 6.93 – 6.89 (m, 2H), 6.71 (dd, \( J = 8.7, 2.5 \text{ Hz}, 2H \)), 5.96 (s, 2H), 4.57 (s, 2H), 3.63 (s, 6H). \( ^{13} \text{C NMR (101 MHz, Acetone)} \) δ 167.62, 167.58, 166.20, 161.29, 161.27, 152.33, 152.00, 151.54, 150.66, 149.15, 147.35, 145.05, 138.31, 137.01, 136.98, 133.95, 133.58, 131.14, 128.76, 127.57, 127.23, 126.64, 126.59, 126.57, 122.09, 122.06, 120.84, 119.00, 117.02, 116.98, 107.77, 107.75, 54.14, 43.20. HRMS: Calcd. for C\(_{38}\)H\(_{36}\)ClIrN\(_5\)OPF\(_6\) [M–PF\(_6\)]: 832.1656 Found: 832.5770.

\( ^{1} \text{H NMR (400 MHz, CDCl3)} \) δ 12.56 (s, 1H), 9.96 (t, \( J = 8.8 \text{ Hz}, 1H \)), 9.11 (d, \( J = 8.8 \text{ Hz}, 1H \)), 8.75 (t, \( J = 8.8 \text{ Hz}, 1H \)), 8.66 (d, \( J = 8.8 \text{ Hz}, 2H \)), 8.41 (d, \( J = 3.6 \text{ Hz}, 1H \)), 8.26 (t, \( J = 6.0 \text{ Hz}, 1H \)), 8.23 – 8.16 (m, 2H), 8.07 (d, \( J = 7.6 \text{ Hz}, 1H \)), 8.04 (t, \( J = 7.6 \text{ Hz}, 1H \)).
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H NMR (400 MHz, CDCl3) δ 8.73 (d, J = 8.4 Hz, 1H), 6.83 (t, J = 5.6 Hz, 1H), 6.75 (t, J = 5.2 Hz, 1H), 6.05 (d, J = 8.8 Hz, 1H), 5.16 (d, J = 10.8 Hz, 1H), 4.90 (s, 2H), 3.64 (s, 6H). 13C NMR (101 MHz, Acetone) δ 167.84, 167.69, 167.24, 162.49, 161.39, 161.19, 158.95, 151.90, 151.55, 151.00, 149.81, 148.53, 148.05, 147.03, 145.16, 138.07, 137.97, 133.76, 136.77, 136.38, 135.12, 132.47, 128.25, 126.55, 126.39, 126.30, 124.98, 122.36, 121.84, 121.52, 118.99, 118.73, 117.35, 117.19, 116.11, 114.32, 113.03, 108.19, 107.75, 99.99, 79.35, 67.19, 54.85. HRMS: Calcd. for C48H33IrN3O6PF6 [M–PF6]$: 966.0530 Found: 966.2416.

H NMR (400 MHz, Acetone) δ 9.89 (s, 1H), 8.93 (d, J = 8.8 Hz, 1H), 8.76 (d, J = 8.4 Hz, 1H), 8.56 – 8.54 (m, 2H), 8.44 (d, J = 5.2 Hz, 1H), 8.04 (dd, J = 8.4, 5.2 Hz, 1H), 7.99 – 7.87 (m, 3H), 7.88 (d, J = 7.6 Hz, 2H), 7.19 (td, J = 7.8, 3.1 Hz, 2H), 7.03 (t, J = 7.5 Hz, 2H), 6.87 – 6.74 (m, 4H), 6.42 (d, J = 7.6 Hz, 2H), 5.88 (d, J = 8.5 Hz, 1H), 5.82 (d, J = 8.5 Hz, 1H), 4.38 (s, 2H). 13C NMR (101 MHz, Acetone) δ 182.78, 182.73, 167.03, 152.92, 151.99, 150.96, 150.63, 150.13, 150.10, 149.02, 146.67, 141.54, 141.51, 139.87, 135.58, 134.53, 134.42, 134.37, 132.95, 132.93, 132.45, 132.43, 131.81, 128.82, 128.73, 128.39, 128.15, 127.93, 127.74, 126.93, 126.91, 124.97, 124.96, 124.28, 121.43, 118.19, 118.12, 44.04. HRMS: Calcd. for C46H35ClIrN5O6PF6 [M–PF6]$: 884.4673 Found: 884.4271.

H NMR (400 MHz, Acetone) δ 12.50 (s, 1H), 9.89 (s, 1H), 8.84 (s, 1H), 8.81 (dd, J = 8.3, 1.3 Hz, 1H), 8.61 (d, J = 4.8 Hz, 1H), 8.48 (dd, J = 5.2, 1.2 Hz, 1H), 8.11 – 7.98 (m, 6H), 7.87 (d, J = 8.8 Hz, 2H), 7.34 – 7.26 (m, 4H), 7.20 – 7.12 (m, 2H), 7.02 – 6.91 (m, 5H), 6.55 (d, J = 8.0 Hz, 2H), 6.02 (d, J = 8.0 Hz, 1H), 5.96 (d, J = 8.4 Hz, 1H), 5.54 (s, 2H). 13C NMR (101 MHz, Acetone) δ 190.31, 185.96, 184.89, 182.55, 181.62, 168.81, 167.88, 164.15, 162.94, 151.51, 150.11, 148.03, 145.78, 140.64, 139.12, 138.50, 137.57, 136.95, 135.43, 133.47, 131.96, 131.55, 131.35, 130.57, 130.50, 128.09, 127.92, 127.06, 126.83, 126.00, 125.94, 123.97, 123.96, 123.93, 123.26, 123.23, 123.19, 117.84, 117.40, 117.32, 115.27, 68.69. HRMS: Calcd. for C45H31IrN6O6S2PF6 [M–PF6]$: 970.1492 Found: 970.1486.
Electronic Supplementary Information

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl}_3) & \quad \delta \quad 9.38 \ (s, 1H), 8.96 \ (d, J = 8.4 \ Hz, 1H), 8.66 \ (s, 1H), 8.50 \ (d, J = 8.2 \ Hz, 1H), 8.43 \\
& \quad (d, J = 5.2 \ Hz, 1H), 8.37 \ (d, J = 4.8 \ Hz, 1H), 7.98 \ (dd, J = 8.4, 5.2 \ Hz, 1H), 7.91 \ (d, J = 8.8 \ Hz, 2H), 7.88 - 7.73 \\
& \quad (m, 6H), 7.61 \ (s, 1H), 7.23 \ (t, J = 3.6 \ Hz, 2H), 7.14 \ (dd, J = 12.8, 7.2 \ Hz, 2H), 7.05 \ (t, J = 8.0 \ Hz, 1H), 6.98 - \\
& \quad 6.83 \ (m, 4H), 6.48 \ (d, J = 7.6 \ Hz, 2H), 5.83 \ (d, J = 8.4 \ Hz, 1H), 5.76 \ (d, J = 8.4 \ Hz, 1H), 5.00 \\
& \quad (s, 2H). \quad ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta \quad 181.36, 180.97, 167.42, 162.44, 158.97, 151.23, \\
& \quad 150.04, 149.53, 149.19, 149.15, 149.00, 147.92, 145.93, 140.31, 140.13, 138.08, 135.00, \\
& \quad 133.66, 133.59, 133.51, 133.16, 132.45, 132.20, 131.25, 130.73, 129.91, 129.13, 128.03, \\
& \quad 127.26, 126.76, 126.76, 126.63, 126.39, 126.33, 126.01, 124.97, 123.58, 123.53, 123.43, \\
& \quad 123.17, 120.19, 117.44, 117.11, 116.08, 114.31, 113.19, 79.22, 67.03. \text{HRMS: Calcd. for } \\
& \quad \text{C}_6\text{H}_3\text{IrN}_5\text{O}_2\text{S}_2\text{PF}_6 [\text{M–PF}_6]^{-}: 1018.1738 \text{ Found: 1018.1646.}
\end{align*}
\]

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl}_3) & \quad \delta \quad 9.40 \ (s, 1H), 8.93 \ (d, J = 8.4 \ Hz, 1H), 8.51 \ (d, J = 5.2 \ Hz, 1H), 8.43 \ (d, J = 4.8 \ Hz, 1H), 8.39 \ (s, 1H), 8.27 \ (d, J = 8.4 \ Hz, 1H), 8.23 - 8.12 \ (m, 4H), 8.05 \ (d, J = 8.0 \ Hz, 2H), 7.88 \ (dd, J = 8.5, 5.1 \ Hz, 1H), 7.68 \ (dd, J = 8.2, 5.1 \\
& \quad (d, J = 8.0 \ Hz, 1H), 7.56 \ (d, J = 8.0 \ Hz, 1H), 7.23 - 7.16 \ (m, 5H), 7.06 \ (d, J = 8.8 \ Hz, 1H), 6.87 \ (t, J = 7.5 \ Hz, 2H), 6.82 \ (t, J = 8.0 \ Hz, 1H), \\
& \quad 6.75 \ (t, J = 8.0 \ Hz, 1H), 6.65 \ (t, J = 6.4 \ Hz, 2H), 4.42 \ (s, 2H). \quad ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta \quad 170.17, 167.38, 150.88, 150.48, 148.12, 147.85, 147.65, 147.01, 146.90, 145.75, 145.63, \\
& \quad 144.90, 140.12, 139.80, 137.87, 135.08, 131.62, 131.14, 130.91, 130.86, 130.47, 129.25, \\
& \quad 128.81, 127.58, 127.24, 127.11, 126.87, 126.20, 125.85, 124.00, 123.93, 123.35, 123.25, \\
& \quad 119.52, 117.43, 117.25, 43.37. \text{HRMS: Calcd. for } \text{C}_5\text{H}_3\text{IrN}_5\text{OPF}_6 [\text{M–PF}_6]^{-}: 872.1756 \\
& \quad \text{Found: 872.5274.}
\end{align*}
\]

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl}_3) & \quad \delta \quad 12.19 \ (s, 1H), 9.87 \ (dd, J = 8.4, 1.2 \ Hz, 1H), 9.77 \ (d, J = 5.2 \ Hz, 1H), 8.86 \ (d, J = 8.4 \\
& \quad (1H), 8.49 \ (dd, J = 4.0 \ Hz, 1H), 8.41 \ (d, J = 5.2 \ Hz, 1H), 8.30 \ (d, J = 5.2 \ Hz, 1H), 8.21 \ (d, J = 5.0 \ Hz, 1H), \\
& \quad 8.15 - 8.03 \ (m, 5H), 8.02 - 7.95 \ (m, 2H), 7.80 \ (dd, J = 8.4, 5.2 \ Hz, 1H), 7.70 \ (t, J = 8.4 \ Hz, 2H), 7.60 - 7.47 \ (m, 2H), 7.18 - 7.02 \ (m, 7H), 6.80 \ (t, J = \\
& \quad 7.2 \ Hz, 2H), 6.75 - 6.60 \ (m, 2H), 6.58 \ (dd, J = 12.0, 8.4 \ Hz, 2H), 5.38 \ (s, 2H). \quad ^{13}\text{C NMR (101 MHz, CDCl}_3) \delta \quad 189.98, 189.98, 168.83, 168.09, 162.37, 149.80, 149.67, 146.66, 145.91, \\
& \quad 145.18, 144.66, 144.60, 143.32, 139.06, 138.75, 136.55, 135.65, 134.06, 133.98, 133.53, \\
& \quad 130.97, 130.63, 129.91, 129.77, 129.18, 128.29, 127.82, 126.47, 126.83, 126.32, 126.01, \\
& \quad 125.85, 124.78, 123.03, 122.92, 122.21, 122.08, 118.94, 116.28, 114.31, 66.57. \text{HRMS: Calcd. for } \\
& \quad \text{C}_5\text{H}_3\text{IrN}_5\text{OPF}_6 [\text{M–PF}_6]^{-}: 958.0723 \text{ Found: 958.2046.}
\end{align*}
\]

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl}_3) & \quad \delta \quad 9.20 \ (s, 1H), 8.79 \ (dd, J = 8.4, 1.2 \ Hz, 1H), 8.54 \ (dd, J = 5.2, 1.2 \ Hz, 1H), 8.44 \ (dd, J = 5.2, 1.6 \ Hz, 1H), 8.40 \ (s, 1H), 8.30 - 8.10 \ (m, 5H), \\
& \quad 8.06 \ (dd, J = 7.6, 5.2 \ Hz, 2H), 7.89 \ (dd, J = 8.4, 5.2 \ Hz,
\end{align*}
\]
$^1$H NMR (400 MHz, Acetone) δ 10.06 (s, 1H), 9.72 (s, 1H), 9.12 (t, $J = 7.4$ Hz, 1H), 8.68 (d, $J = 8.4$ Hz, 1H), 8.61 (d, $J = 4.8$ Hz, 1H), 8.37 (d, $J = 8.2$ Hz, 2H), 8.31 (d, $J = 4.1$ Hz, 1H), 8.03 – 8.01 (m, 2H), 7.90 – 7.71 (m, 6H), 7.67 (d, $J = 8.4$ Hz, 1H), 7.46 (d, $J = 7.6$ Hz, 2H), 7.32 – 7.27 (m, 2H), 7.19 (d, $J = 8.2$ Hz, 2H), 7.10 (t, $J = 7.8$ Hz, 2H), 6.89 (d, $J = 8.4$ Hz, 2H), 6.34 (dd, $J = 7.1$, 4.2 Hz, 2H), 5.10 (s, 2H). $^{13}$C NMR (101 MHz, Acetone) δ 191.27, 166.8, 161.7, 160.9, 149.79, 144.2, 139.18, 138.39, 137.36, 137.21, 136.45, 135.37, 135.31, 134.65, 133.97, 133.08, 132.80, 132.80, 132.55, 131.87, 131.65, 131.24, 130.70, 130.06, 129.93, 129.27, 128.77, 128.41, 128.18, 126.96, 125.62, 124.94, 123.25, 121.52, 116.71, 116.20, 68.38. HRMS: Calcd. for C$_{46}$H$_{32}$ClIrN$_5$OPF$_6$ [M–PF$_6$]$^+$: 906.2053 Found: 906.2074.
Part C: Luminescence experiments

Photophysical measurement

Emission spectra for complexes 2a–2h were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 335 nm) fitted with a 395 nm filter. Error limits were estimated: λ (±1 nm); τ (±10 %); φ (±10 %). UV/Vis absorption spectra were recorded on an Agilent Cary 8454 UV–Vis Spectrophotometer. The luminescence lifetime of the complex was measured by time-correlated single-photon counting (TCSPC) on the Horiba fluorescence spectrometer (FL3C–21), following excitation at 340 nm with a NanoLED light source. The lifetime of complexes 2a–2h were calculated according to a reported equation (1):

\[ F(t) = F_0 e^{-t/\tau} \]  

Where the lifetime \( \tau \) is equal to the time after which the intensity \( F \) drops to 1/e of its initial value \( F_0 \). \( \tau_{\text{ave}}, \tau_1 \) and \( \tau_2 \) were determined by the software DAS6 v6.8 on the Horiba fluorescence spectrometer (FL3C-21).

Luminescence quantum yields were determined using the method of Demas and Crosby with [Ru(bpy)_3][PF_6]_2 in degassed acetonitrile (ACN) as a standard reference solution (\( \Phi_r = 0.062 \)) and were calculated according to the following reported equation (2):

\[ \Phi_s = \Phi_r (B_r/B_s)(n_s/n_r)^2(D_s/D_r) \]  

Where the subscripts s and r refer to the sample and reference standard solution, respectively, n is the refractive index of the solvents, D is the integrated intensity, and \( \Phi \) is the luminescence quantum yield. The quantity B was calculated by \( B = 1 - 10^{-AL} \), where A is the absorbance at excitation wavelength and L is the optical path length.

Time-resolved emission spectroscopy (TRES) measurement

In vitro time-gated emission fluorimetry were determined with a time-correlated single-photon counting (TCSPC) technique on a Horiba fluorescence spectrometer (FL3C–21). Under excitation at 340 nm (NanoLED), the emission signals over the indicated range were recorded with intervals of 10 nm. Cm460 and thioflavin S (THS) were used as fluorescent interferences. After they were added into the solution of
complex 2f (10 μM) in PBS buffer (pH 7.4), the time-resolved emission spectra of the mixture were acquired with the measurement range of 0–3.2 μs. For the in cellulo time-gated emission fluorimetry, the emission of 2f and a commercially available cellular dye (4’,6-diamidino-2-phenylindole (DAPI)) were compared in living A431 cells utilizing a multi-mode microplate reader (FlexStation 3-Molecular Devices). After incubating the cells with both complex 2f and DAPI for 2 h, the signal output from the cells was recorded with the time set to two different delay time. Measured intensity signals were converted into lifetime, \( \tau \), as follows:

\[
\tau = \frac{(t_2 - t_1)}{\ln(F_1/F_2)}
\]

where \( t_1 \) and \( t_2 \) refer to the two delay times, and \( F_1 \) and \( F_2 \) refer to corresponding intensity signals.

**Stability experiments**

For \(^1\)H NMR, complex 2f was dissolved in 90% \([d_6]\)DMSO/10% D\(_2\)O at 298 K over 7 days. \(^1\)H NMR measurements were carried out on a 400 MHz Bruker instrument. For UV–Visible spectrometry, complex 2f (2.5 μM) was dissolved in 90% acetonitrile/10% PBS buffer (pH = 7.4) at 298 K over 7 days. Absorption spectra were recorded on a Cary UV–100 Spectrophotometer at a range of 200 nm to 800 nm. The absorbance of complex 2f was corrected by subtraction of 90% acetonitrile/10% PBS buffer (pH = 7.4) as the background absorbance.

**Part D: Biological assays**

**Cell cultures**

The cells were cultivated in DMEM medium with 1% penicillin (100 units/mL)/streptomycin (100 μg/mL) and 10% fetal bovine serum (FBS). Cells were maintained at a density of 6 × 10\(^5\) cell/mL in 5% CO\(_2\) at 37 °C.

**MTT assay**

A431 and LO2 cells were seeded at 5000 cells per well in a 96–well plate and incubated overnight at 37 °C. The cells were treated with 2f (from a DMSO stock) at the indicated concentrations for 72 h. The final concentration of DMSO was less than 0.5%. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
reagent was added to each well at a final concentration of 0.5 mg/mL for a further 4 h. After that, the medium was replaced with 100 μL DMSO. The viability of the cells was measured by recording the absorbance of each well at 490 nm using a SpectraMax M5 microplate reader after shaking the plate for 10 min at room temperature in the dark.

**Confocal imaging**

Cells were seeded into a glass–bottomed dish (35 mm dish with 20 mm well). After 12 h, cells were incubated with complex 2f for the indicated periods and concentrations, followed by washing with PBS three times. The luminescence imaging of complexes in cells was carried out by a Leica TCS SP8 confocal laser scanning microscope system. The excitation wavelength was 405 nm.

**EGFR kinase assay**

The *in vitro* inhibition activity of complexes 2a–2h and reference EGFR inhibitor 1b against EGFR was measured by a fluorescence assay utilizing a commercially available EGFR kinase assay kit (BPS Bioscience, 40321). Briefly, after adding kinase buffer, PTK substrate, EGFR protein and complexes, the mixture was incubated for 30 min at 30 °C. Then, the reaction was initiated by adding ATP for 40 min incubation at 30 °C. Lastly, Kinase-Glo Max reagent was added to each well and the plate was incubated at room temperature for 15 minutes. The luminescence signal from the endogenous signaling luciferase (Ultra-Glo Luciferase) was measured using the microplate reader at 450 nm.

**Part E: Supporting information**

![Chemical structures of parent iridium(III) complex 1a and EGFR inhibitor 1b.](image)

*Figure S1* Chemical structures of parent iridium(III) complex 1a and EGFR inhibitor 1b.
**Figure S2** $^1$H NMR, $^{13}$C NMR, HRMS and HPLC spectra of complexes 2a–2h.

**Table S1** Lifetimes of complexes 2a–2h (5 μM) in PBS buffer containing 0.5% DMSO.

| Complex | $\tau_1$ (ns) | $\tau_2$ (ns) | $\tau_{ave}$ (ns) |
|---------|---------------|---------------|-------------------|
| 2a      | 143           | 35            | 79                |
| 2b      | 51            | 6             | 28                |
| 2c      | 376           | 16            | 102               |
| 2d      | 40            | 12            | 23                |
| 2e      | 575           | 33            | 525               |
| 2f      | 252           | 48            | 139               |
| 2g      | 123           | 23            | 66                |
| 2h      | 205           | 23            | 112               |

$^a$ $\tau_i$ and $\tau_{ave}$ refer to the lifetimes of the different decay components and average lifetime of the complex, respectively. The excitation wavelength was 340 nm. Decays were recorded at the peak emission wavelength.
**Figure S3** Emission spectra of complexes 2a–2h (5 μM) in PBS buffer containing 0.5% DMSO.

**Figure S4** Luminescence intensity of complex 2f (5 μM) in the absence and presence of EGFR (20 ng/μL) or various potentially interfering species including amino acids, anions or cations (20 μM).
Figure S5 (a) UV/Vis absorption of complex 2f (2.5 μM) in acetonitrile/H₂O (9:1) at t = 1 min and after incubation for 1, 2, 3, 4, 5, 6 and 7 days at 298 K. (b) ¹H NMR spectra of complex 2f in DMSO-d₆/D₂O (9:1) at t = 1 min and after incubation for 1, 2, 3, 4, 5, 6 and 7 days at 298 K.

Figure S6 Time-resolved spectra of complex 2f (10 μM) in PBS buffer containing 1% DMSO.

Figure S7 Time-resolved spectra of complex 2f (1 μM) and the nuclear dye DAPI (1 μM) in A431 cells with delay time set to (a) 50 μs and (b) 200 μs, respectively.
Figure S8 Cytotoxicity of complex 2f and the reference inhibitor 1b against normal LO2 cells and EGFR-overexpressing A431 cells. A431 and LO2 cells were treated with 2f and 1b for 72 h.

Figure S9 Effects of complex 2f on P-ERK, ERK, P-MEK, MEK and EGFR protein expression after EGFR knockdown by EGFR siRNA. After treatment by EGFR siRNA for 48 h, A431 cells were treated with 2f (5 μM) for 12 h. The protein levels were determined by Western blotting.

References
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