Electronic Supplementary Information

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

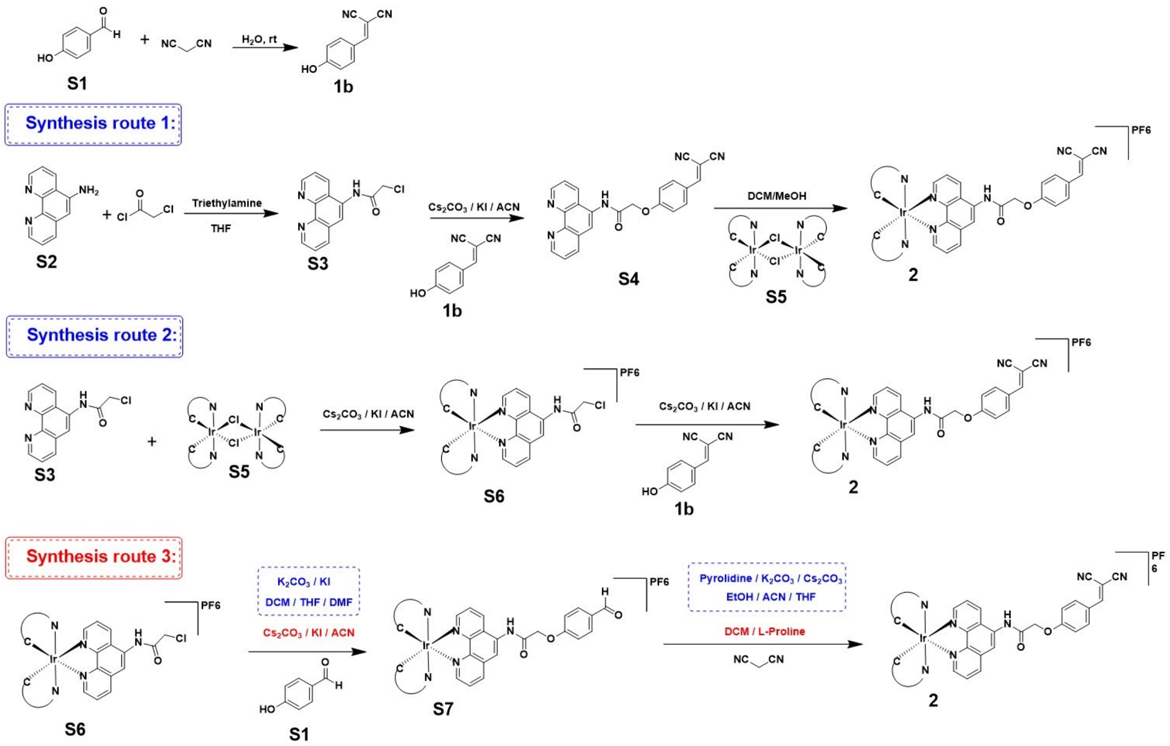
Iridium chloride hydrate ($IrCl_3 \cdot xH_2O$) was purchased from Precious Metals Online (Australia). Phosphate-buffered saline (10×) pH 7.4 (AM9625) was purchased from Life Technologies Ltd. (Hong Kong). Recombinant Human EGFR protein (10001–H08B) was purchased from Sino Biological Inc. (China). Unless 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 purcharsed from BPS Bioscience, Inc.

Part B. Synthesis experiment

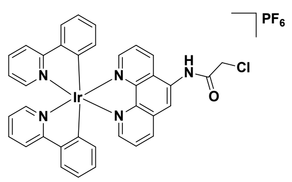
Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^1H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). ^1H and ^{13}C chemical shifts were referenced internally to solvent shift ($CDCl_3$–*d*: ^1H, 7.26, ^{13}C, 77.16; DMSO–d_6: ^1H, 2.50, ^{13}C, 39.52; acetone–d_6: ^1H, 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 ^1H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^1H–^1H and ±0.5 Hz for ^1H–^{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

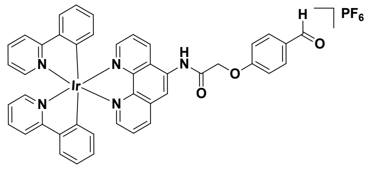
Complex **2a** was synthesized according to reported literatures.[1–3] Briefly, 2.1 equivalents of 2-phenylpyridine (ppy) and 1 equivalent of $IrCl_3 \cdot xH_2O$ were mixed together and further heated overnight at 120 °C in 12 mL of 2-methoxyethanol/H_2O (v/v, 3/1). Afterwards, the mixture was filtered and washed by excessive deionized water and then diethyl ether for three times, respectively to generate the dichloro-bridged dimer $[Ir(ppy)_2Cl]_2$. 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 (NH_4PF_6) 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 (Cs_2CO_3, 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 (CN**2**–CN**8**) of compound **S6**.



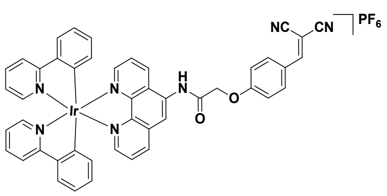
Scheme S1 Synthesis routes of iridium(III) complexes **2a**–**2h**. Conditions: (A) H_2O, rt; (B) triethylamine, THF; (C) Cs_2CO_3, KI, ACN; (D) DCM:MeOH (1:1, v/v); (E) Cs_2CO_3, KI, ACN; (F) Cs_2CO_3, KI, ACN; (G) Cs_2CO_3, KI, ACN; (H) DCM, L-Proline.



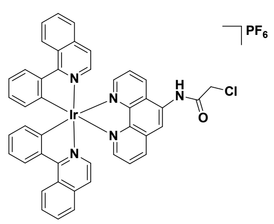
^{1}H NMR (400 MHz, Acetone) δ 9.96 (s, 1H), 8.91 (d, J = 8.4 Hz, 1H), 8.74 (d, J = 8.4 Hz, 1H), 8.58 (d, J = 5.2 Hz, 1H), 8.35 (d, J = 4.8 Hz, 1H), 8.25 (d, J = 4.8 Hz, 1H), 8.10 (d, J = 8.0 Hz, 2H), 7.97 (dd, J = 8.4, 5.2 Hz, 1H), 7.90 (dd, J = 8.6, 5.0 Hz, 1H), 7.81 – 7.75 (m, 4H), 7.57 (t, J = 6.0 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). ^{13}C 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, 131.20, 130.36, 127.61, 127.24, 126.63, 124.93, 123.49, 123.44, 122.60, 120.85, 119.83, 43.19. HRMS: Calcd. for $C_{36}H_{26}ClIrN_5OPF_6$ $[M–PF_6]^+$: 772.1443 Found: 772.1405.



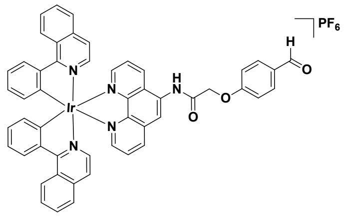
^{1}H NMR (400 MHz, Acetone) δ 10.26 (s, 1H), 9.80 (s, 1H), 8.96 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 7.6 Hz, 1H), 8.57 (s, 1H), 8.33 (d, J = 4.4 Hz, 1H), 8.23 (d, J = 4.0 Hz, 1H), 8.10 (d, J = 6.4 Hz, 2H), 7.95 – 7.86 (m, 2H), 7.84 – 7.74 (m, 6H), 7.56 (d, J = 5.2 Hz, 2H), 7.20 (d, J = 8.8 Hz, 2H), 6.94 (t, J = 7.2 Hz, 2H), 6.87 – 6.81 (m, 4H), 6.31 (d, J = 7.2 Hz, 2H), 5.02 (s, 2H). ^{13}C 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: Calcd. for $C_{43}H_{31}IrN_5O_3PF_6$ $[M–PF_6]^+$: 857.9549 Found: 857.8516.



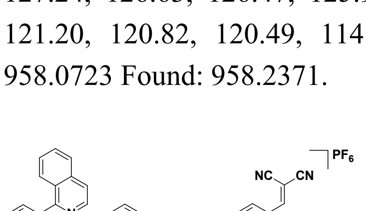
[1]H NMR (400 MHz, Acetone) δ 9.00 (dd, *J* = 8.8, 1.2 Hz, 1H), 8.72 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.67 (s, 1H), 8.46 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.35 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.21-8.18 (m, 3H), 8.08 (d, *J* = 8.8 Hz, 2H), 8.03 (dd, *J* = 8.8, 4.8 Hz, 1H), 7.94 – 7.83 (m, 5H), 7.68 (d, *J* = 5.6 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) δ 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, 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 $C_{46}H_{31}IrN_7O_2PF_6$ $[M–PF_6]^+$: 906.0010 Found: 906.0756.



[1]H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 126.92, 126.59, 126.28, 122.61, 122.19, 121.50, 43.31. HRMS: Calcd. for $C_{44}H_{30}ClIrN_5OPF_6$ $[M–PF_6]^+$: 872.4119 Found: 872.6614.

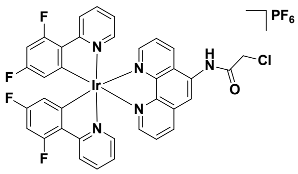


[1]H NMR (400 MHz, $CDCl_3$) δ 10.09 (s, 1H), 9.91 (s, 1H), 9.05 – 8.89 (m, 3H), 8.60 (s, 1H), 8.47 (d, *J* = 7.2 Hz, 1H), 8.31 (t, *J* = 7.2 Hz, 2H), 8.07 (dd, *J* = 5.0, 1.3 Hz, 1H), 8.03 (dd, *J* = 5.1, 1.4 Hz, 1H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.87 – 7.72 (m, 6H), 7.68 (dd, *J* = 8.4, 5.2 Hz, 4H), 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, CDCl3) δ 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, 131.07, 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 $C_{51}H_{35}IrN_5O_3PF_6$ $[M–PF_6]^+$: 958.0723 Found: 958.2371.

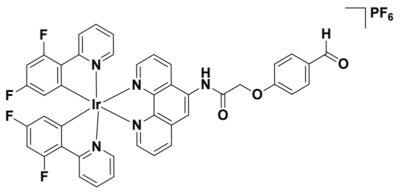


[1]H NMR (400 MHz, CDCl3) δ 9.90 (s, 1H), 9.05 – 9.01 (m, 3H), 8.70 (s, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.39 (t, *J* = 6.4 Hz, 2H), 8.16 (d, *J* = 4.4 Hz, 1H), 8.11 (d, *J* = 4.8 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.92 – 7.88 (m, 4H), 7.85 – 7.82 (m, 4H), 7.79 – 7.74 (m, 1H), 7.69 (d, *J* = 9.2 Hz, 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, CDCl3) δ 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,

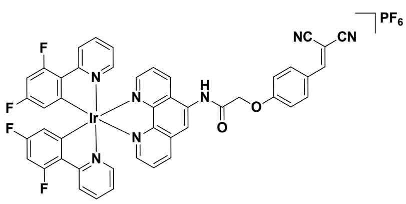
128.75, 128.19, 127.67, 127.52, 126.98, 126.83, 126.60, 126.43, 126.41, 126.29, 126.29, 125.00, 122.62, 122.53, 122.21, 121.86, 121.41, 121.41, 120.43, 116.13, 114.28, 113.18, 79.30, 67.25. HRMS: Calcd. for $C_{54}H_{35}IrN_7O_2PF_6$ $[M–PF_6]^+$: 1006.1184 Found: 1006.2544.



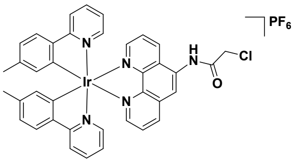
^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 9.01 (d, *J* = 8.8 Hz, 1H), 8.90 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.64 (s, 1H), 8.32 (dd, *J* = 5.2, 1.0 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 8.21 (dd, *J* = 5.2, 1.2 Hz, 1H), 8.08 (dd, *J* = 8.8, 5.2 Hz, 1H), 8.01 – 7.93 (m, 3H), 7.52 (d, *J* = 5.6 Hz, 2H), 7.06 (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). ^{13}C 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 $C_{36}H_{22}ClF_4IrN_5OPF_6$ $[M–PF_6]^+$: 844.1066 Found: 844.6542.



^{1}H 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 (d, *J* = 4.6 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). ^{13}C 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 $C_{43}H_{27}F_4IrN_5O_3PF_6$ $[M–PF_6]^+$: 930.1676 Found:930.1714.

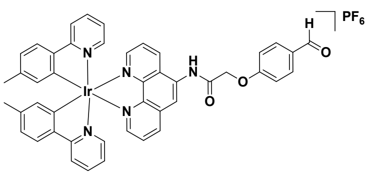


^{1}H NMR (400 MHz, CDCl3) δ 12.65 (s, 1H), 10.04 (d, *J* = 8.4 Hz, 1H), 8.82 (s, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 8.37 – 8.33 (m, 2H), 8.29 (d, *J* = 4.8 Hz, 1H), 8.19 (d, *J* = 4.8 Hz, 1H), 8.00 (dd, *J* = 8.4, 4.8 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.83 – 7.77 (m, 3H), 7.65 (s, 1H), 7.18 – 7.24 (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). ^{13}C 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 $C_{46}H_{27}F_4IrN_7O_2PF_6$ $[M–PF_6]^+$: 978.1792 Found: 978.1740.

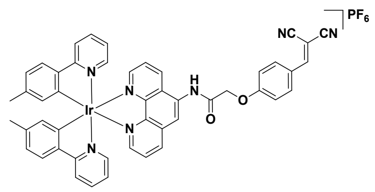


^{1}H NMR (400 MHz, Acetone) δ 10.08 (s, 1H), 9.04 (d, *J* = 8.4 Hz, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.71 (s, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.40 (d, *J* = 5.2 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 2H), 8.11 (dd, *J* = 8.4, 4.8 Hz, 1H), 8.07 – 8.00 (m, 1H), 7.89 – 7.83 (m, 4H), 7.66 (t, *J* = 5.9 Hz, 2H), 6.98 – 6.88 (m, 4H), 6.33 – 6.29 (m, 2H), 4.57

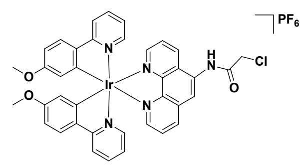
(s, 2H), 2.14 (s, 6H). [13]C NMR (101 MHz, Acetone) δ 168.73, 168.70, 167.11, 152.32, 151.45, 151.29, 150.97, 150.18, 150.16, 148.28, 145.99, 142.58, 142.54, 141.11, 141.09, 139.30, 139.20, 134.84, 134.49, 133.43, 133.39, 132.05, 128.50, 128.08, 127.50, 125.77, 125.75, 124.52, 124.51, 123.80, 123.77, 121.78, 120.33, 44.08, 21.88. HRMS: Calcd. for $C_{38}H_{30}ClIrN_5OPF_6$ $[M–PF_6]^+$: 800.3477 Found: 800.8413.



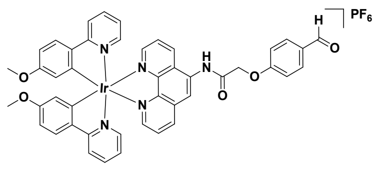
[1]H NMR (400 MHz, CDCl3) δ 12.57 (s, 1H), 9.88 (s, 1H), 8.74 (s, 1H), 8.41 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 4.8 Hz, 1H), 8.20 – 8.16 (m, 1H), 8.15 (d, *J* = 4.8 Hz, 1H), 7.91 – 7.79 (m, 6H), 7.71 – 7.66 (m, 3H), 7.64 – 7.60 (m, 2H), 7.31 – 7.28 (m, 2H), 7.21 (d, *J* =6.4 Hz, 1H), 6.93 – 6.91 (m, 2H), 6.85 – 6.77 (m, 2H), 6.19 (d, *J* = 8.8 Hz, 2H), 5.53 (s, 2H), 2.18 (s, 6H). [13]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 $C_{45}H_{35}IrN_5O_3PF_6$ $[M–PF_6]^+$: 886.2367 Found: 886.2370.



[1]H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.78 (d, *J* = 8.4 Hz, 1H), 8.54 (s, 1H), 8.42 (d, *J* = 8.0 Hz, 1H), 8.24 (dd, *J* = 18.3, 5.0 Hz, 2H), 7.89 – 7.82 (m, 5H), 7.71 – 7.64 (m, 3H), 7.60 (dd, *J* = 8.2, 5.6 Hz, 2H), 7.47 (s, 1H), 7.31 (d, *J* = 5.6 Hz, 1H), 7.27 – 7.24 (m, 1H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.87 – 6.79 (m, 4H), 6.18 (d, *J* = 7.2 Hz, 2H), 4.93 (s, 2H), 2.13 (s, 6H). [13]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 $C_{48}H_{35}IrN_7O_2PF_6$ $[M–PF_6]^+$: 934.0542 Found: 934.2518.

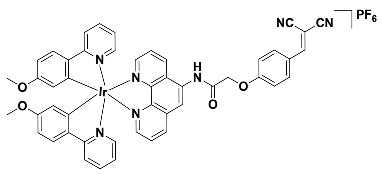


[1]H NMR (400 MHz, Acetone) δ 10.08 (s, 1H), 9.05 (d, *J* = 8.4 Hz, 1H), 8.87 (d, *J* = 8.0 Hz, 1H), 8.72 (s, 1H), 8.57 (d, *J* = 4.4 Hz, 1H), 8.46 (d, *J* = 4.4 Hz, 1H), 8.14 – 8.10 (m, 3H), 8.05 (dd, *J* = 8.1, 5.1 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.84 (t, *J* = 7.6 Hz, 2H), 7.62 (t, *J* = 5.3 Hz, 2H), 6.93 – 6.89 (m, 2H), 6.71 (dd, *J* = 8.7, 2.5 Hz, 2H), 5.96 (s, 2H), 4.57 (s, 2H), 3.63 (s, 6H). [13]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 $C_{38}H_{30}ClIrN_5O_3PF_6$ $[M–PF_6]^+$: 832.1656 Found: 832.5770.

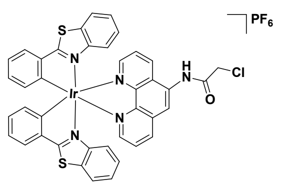


[1]H NMR (400 MHz, CDCl3) δ 12.56 (s, 1H), 9.96 (t, *J* = 8.8 Hz, 1H), 9.11 (d, *J* = 8.8 Hz, 1H), 8.75 (t, *J* = 8.8 Hz, 1H), 8.66 (d, *J* = 8.8 Hz, 2H), 8.41 (d, *J* = 3.6 Hz, 1H), 8.26 (t, *J* = 6.0 Hz, 1H), 8.23 – 8.16 (m, 2H), 8.07 (d, *J* = 7.6, Hz,

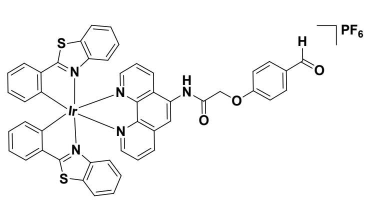
1H), 7.75 – 7.85 (m, 2H), 7.74 –7.58 (m, 5H), 7.23 –7.11 (m, 4H), 6.75 (t, J = 8.8 Hz, 2H), 6.67 (t, *J* = 7.2 Hz, 2H), 5.90 (d, *J* = 8.8 Hz, 1H), 5.65 (s, 2H), 3.63 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 191.06, 171.65, 169.01, 167.59, 161.32, 152.21, 151.88, 150.93, 150.36, 149.48, 149.08, 148.24, 138.39, 138.06, 137.94, 137.26, 136.77, 136.60, 134.69, 134.21, 132.06, 130.87, 126.53, 126.49, 126.17, 121.96, 120.02, 118.92, 117.39, 115.41, 107.78, 67.53, 54.87. HRMS: Calcd. for $C_{45}H_{35}IrN_5O_5PF_6$ [M–PF$_6$]$^+$: 918.0069 Found: 918.2212.



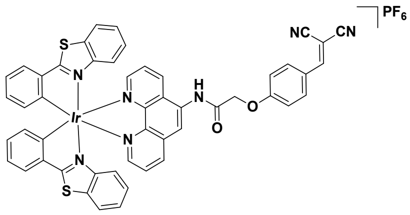
^{1}H NMR (400 MHz, CDCl3) δ 8.73 (d, *J* = 8.4 Hz, 1H), 8.52 – 8.50 (m, 2H), 8.32 (d, *J* = 5.2 Hz, 1H), 8.25 (d, *J* = 5.2 Hz, 1H), 7.82 (dd, *J* = 8.7, 5.0 Hz, 1H), 7.78 – 7.72 (m, 3H), 7.69 – 7.61 (m, 4H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 5.6 Hz, 1H), 7.22 (d, *J* = 6.0 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.83 (t, *J* = 5.6 Hz, 1H), 6.75 (t, *J* = 5.2 Hz, 1H), 6.68 – 6.64 (m, 2H), 5.92 – 5.90 (m, 2H), 5.65 (d, *J* = 8.8 Hz, 1H), 5.16 (d, *J* = 10.8 Hz, 1H), 4.90 (s, 2H), 3.64 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) 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, 137.96, 136.77, 136.38, 135.12, 133.53, 130.89, 129.93, 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, 113.21, 108.19, 107.75, 99.99, 79.35, 67.19, 54.85. HRMS: Calcd. for $C_{48}H_{35}IrN_7O_4PF_6$ [M–PF$_6$]$^+$: 966.0530 Found: 966.2416.



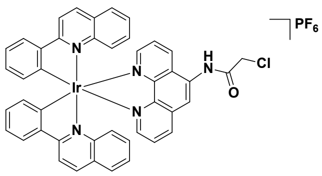
^{1}H NMR (400 MHz, Acetone) δ 9.89 (s, 1H), 8.93 (d, *J* = 8.8 Hz, 1H), 8.76 (d, *J* = 8.4 Hz, 1H), 8.56 – 8.54 (m, 2H), 8.44 (d, *J* = 5.2 Hz, 1H), 8.04 (dd, *J* = 8.4, 5.2 Hz, 1H), 7.99 – 7.87 (m, 3H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.19 (td, *J* = 7.8, 3.1 Hz, 2H), 7.03 (t, *J* = 7.5 Hz, 2H), 6.87 – 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). ^{13}C 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, 127.72, 126.93, 126.91, 124.97, 124.96, 124.28, 121.43, 118.19, 118.12, 44.04. . HRMS: Calcd. for $C_{40}H_{26}ClIrN_5OS_2PF_6$ [M–PF$_6$]$^+$: 884.4673 Found: 884.4271.



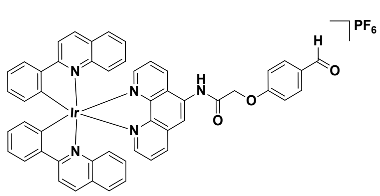
^{1}H NMR (400 MHz, Acetone) δ 12.50 (s, 1H), 9.89 (s, 1H), 8.84 (s, 1H), 8.81 (dd, *J* = 8.3, 1.3 Hz, 1H), 8.61 (d, *J* = 4.8 Hz, 1H), 8.48 (dd, *J* = 5.2, 1.2 Hz, 1H), 8.11 – 7.98 (m, 6H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.34 – 7.26 (m, 4H), 7.20 – 7.12 (m, 2H), 7.02 – 6.91 (m, 5H), 6.55 (d, *J* = 8.0 Hz, 2H), 6.02 (d, *J* = 8.0 Hz, 1H), 5.96 (d, *J* = 8.4 Hz, 1H), 5.54 (s, 2H). ^{13}C 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 $C_{47}H_{31}IrN_5O_3S_2PF_6$ [M–PF$_6$]$^+$: 970.1492 Found: 970.1486.



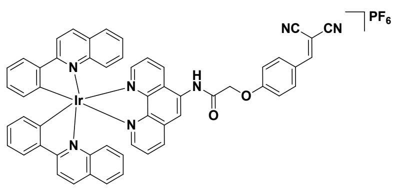
[1]H NMR (400 MHz, CDCl3) δ 9.38 (s, 1H), 8.96 (d, *J* = 8.4 Hz, 1H), 8.66 (s, 1H), 8.50 (d, *J* = 8.2 Hz, 1H), 8.43 (d, *J* = 5.2 Hz, 1H), 8.37 (d, *J* = 4.8 Hz, 1H), 7.98 (dd, *J* = 8.4, 5.2 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.88 – 7.73 (m, 6H), 7.61 (s, 1H), 7.23 (t, *J* = 3.6 Hz, 2H), 7.14 (dd, *J* = 12.8, 7.2 Hz, 2H), 7.05 (t, *J* = 8.0 Hz, 1H), 6.98 – 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 (s, 2H). [13]C NMR (101 MHz, CDCl3) δ 181.36, 180.97, 167.42, 162.44, 158.97, 151.23, 150.04, 149.53, 149.19, 149.15, 149.00, 147.92, 145.93, 140.31, 140.13, 138.08, 135.00, 133.66, 133.59, 133.51, 133.16, 132.45, 132.20, 131.25, 130.73, 129.91, 129.13, 128.03, 127.26, 126.76, 126.76, 126.63, 126.39, 126.33, 126.01, 124.97, 123.58, 123.53, 123.43, 123.17, 120.19, 117.44, 117.11, 116.08, 114.31, 113.19, 79.22, 67.03. HRMS: Calcd. for $C_{50}H_{31}IrN_7O_2S_2PF_6$ $[M–PF_6]^+$: 1018.1738 Found: 1018.1646.



[1]H NMR (400 MHz, $CDCl_3$) δ 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 Hz, 1H), 7.63 (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), 6.75 (t, *J* = 8.0 Hz, 1H), 6.65 (t, *J* = 6.4 Hz, 2H), 4.42 (s, 2H). [13]C NMR (101 MHz, CDCl3) δ 170.17, 167.38, 150.88, 150.48, 148.12, 147.85, 147.65, 147.01, 146.90, 145.75, 145.63, 144.90, 140.12, 139.80, 137.87, 135.08, 131.62, 131.14, 130.91, 130.86, 130.47, 129.25, 128.81, 127.58, 127.24, 127.11, 126.87, 126.20, 125.85, 124.00, 123.93, 123.35, 123.25, 119.52, 117.43, 117.25, 43.37. HRMS: Calcd. for $C_{45}H_{30}ClIrN_5OPF_6$ $[M–PF_6]^+$: 872.1756 Found: 872.5274.

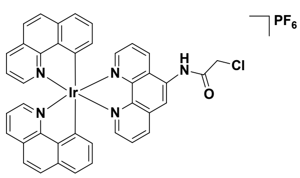


[1]H NMR (400 MHz, $CDCl_3$) δ 12.19 (s, 1H), 9.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 9.77 (d, *J* = 5.2 Hz, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.49 (dd, *J* = 4.0 Hz, 1H), 8.41 (d, *J* = 5.2 Hz, 1H), 8.30 (d, *J* = 5.2 Hz, 1H), 8.21 (d, *J* = 5.0 Hz, 1H), 8.15 – 8.03 (m, 5H), 8.02 – 7.95 (m, 2H), 7.80 (dd, *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* = 7.2 Hz, 2H), 6.75 – 6.60 (m, 2H), 6.58 (dd, *J* = 12.0, 8.4 Hz, 2H), 5.38 (s, 2H). [13]C NMR (101 MHz, CDCl3) δ 189.98, 189.98, 168.83, 168.09, 162.37, 149.80, 149.67, 146.66, 145.91, 145.18, 144.66, 144.60, 143.32, 139.06, 138.75, 136.55, 135.65, 134.06, 133.98, 133.53, 130.97, 130.63, 129.91, 129.77, 129.18, 128.29, 127.82, 126.47, 126.83, 126.32, 126.01, 125.85, 124.78, 123.03, 122.92, 122.21, 122.08, 118.94, 116.28, 114.31, 66.57. HRMS: Calcd. for $C_{51}H_{35}IrN_5O_3PF_6$ $[M–PF_6]^+$: 958.0723 Found: 958.2046.

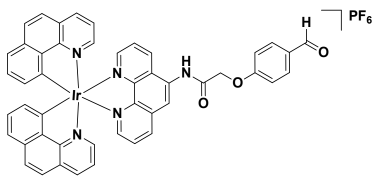


[1]H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.79 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.54 (dd, *J* = 5.2, 1.2 Hz, 1H), 8.44 (dd, *J* = 5.2, 1.6 Hz, 1H), 8.40 (s, 1H), 8.30 – 8.10 (m, 5H), 8.06 (dd, *J* = 7.6, 5.2 Hz, 2H), 7.89 (dd, *J* = 8.4, 5.2 Hz,

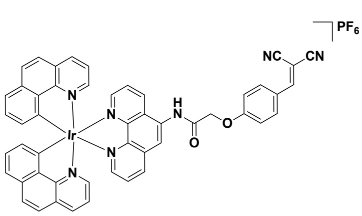
1H), 7.83 (d, *J* = 9.2 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.69 (dd, *J* = 8.2, 5.1 Hz, 1H), 7.63 – 7.59 (m, 1H), 7.54 (d, *J* = 5.6 Hz, 2H), 7.23 – 7.04 (m, 5H), 7.07 (d, *J* = 9.0 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 6.86 – 6.81 (m, 3H), 6.77 – 6.73 (m, 1H), 6.64 (d, *J* = 7.6 Hz, 2H), 4.92 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.93, 169.76, 167.33, 163.08, 162.45, 159.22, 158.99, 150.53, 150.26, 148.26, 147.69, 147.59, 147.15, 146.95, 145.70, 144.90, 140.14, 139.92, 137.93, 135.04, 134.16, 133.77, 133.50, 132.49, 131.53, 131.03, 130.91, 130.85, 130.28, 129.24, 128.88, 127.52, 127.30, 127.15, 127.01, 126.83, 126.65, 126.32, 126.01, 124.92, 124.00, 123.91, 123.37, 123.25, 119.81, 117.55, 117.38, 116.94, 116.02, 114.34, 113.23, 79.07, 66.99. HRMS: Calcd. for $C_{54}H_{35}IrN_7O_2PF_6$ $[M–PF_6]^+$: 1006.1184 Found: 1006.2522.



^{1}H NMR (400 MHz, Acetone) δ 9.11 (s, 1H), 8.83 (d, *J* = 8.8 Hz, 1H), 8.72 (d, *J* = 5.2 Hz, 1H), 8.50 (d, *J* = 8.0 Hz, 2H), 8.45 (d, *J* = 4.8 Hz, 1H), 8.35 (d, *J* = 4.9 Hz, 1H), 8.15 (t, *J* = 5.8 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 4H), 7.92 (dd, *J* = 8.4, 5.1 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.47 – 7.22 (m, 2H), 7.23 (t, *J* = 7.6 Hz, 2H), 6.47 (dd, *J* = 6.8, 4.4 Hz, 2H), 4.57 (s, 2H). ^{13}C NMR (101 MHz, Acetone) δ 166.36, 157.16, 151.85, 150.88, 148.95, 147.77, 146.80, 146.46, 145.31, 140.86, 138.23, 137.48, 134.57, 134.37, 133.86, 131.20, 129.76, 129.13, 129.09, 127.51, 127.26, 127.08, 126.46, 124.05, 122.39, 120.60, 120.20, 43.40. HRMS: Calcd. for $C_{40}H_{26}ClIrN_5OPF_6$ $[M–PF_6]^+$: 820.3373 Found: 820.1436.



^{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 $C_{47}H_{31}IrN_5O_3PF_6$ $[M–PF_6]^+$: 906.2053 Found: 906.2074.



^{1}H NMR (400 MHz, CDCl3) δ 8.61 (s, 1H), 8.45 (d, *J* = 7.6 Hz, 1H), 8.27 – 8.18 (m, 5H), 7.95 – 7.87 (m, 5H), 7.74 (d, *J* = 5.2 Hz, 1H), 7.76 – 7.65 (m, 6H), 7.61 – 7.47 (m, 3H), 7.32 – 7.27 (m, 3H), 7.23 (d, *J* = 7.6 Hz, 2H), 6.45 – 6.42 (m, 2H), 5.16 (s, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 167.96, 162.59, 158.93, 157.49, 157.18, 151.36, 150.12, 147.94, 147.46, 147.23, 146.02, 145.83, 145.53, 140.75, 140.56, 138.07, 137.20, 137.16, 134.42, 134.29, 133.91, 133.52, 130.97, 130.39, 130.24, 130.07, 129.95, 129.46, 129.16, 128.37, 127.29, 127.17, 126.45, 126.22, 124.91, 123.82, 123.69, 122.37, 121.80, 121.41, 120.94, 120.84, 116.14, 114.32, 113.21, 79.27, 67.26. HRMS: Calcd. for $C_{50}H_{31}IrN_7O_2PF_6$ $[M–PF_6]^+$: 954.0438 Found: 954.2146.

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):

$$F(t)=F_0e^{-t/\tau} \quad (1)$$

Where the lifetime τ is equal to the time after which the intensity F drops to 1/e of its initial value F_0. τ_{ave}, τ_1 and τ_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 (Φ_r = 0.062) and were calculated according to the following reported equation (2):

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r) \quad (2)$$

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 Φ 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 bu□er (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, τ, as follows:

$$\tau = (t_2 - t_1)/\ln(F_1/F_2)$$

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

Stability experiments

For ^{1}H NMR, complex **2f** was dissolved in 90% [d_6]DMSO/10% D_2O 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

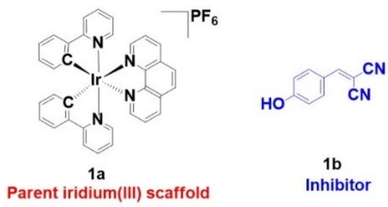
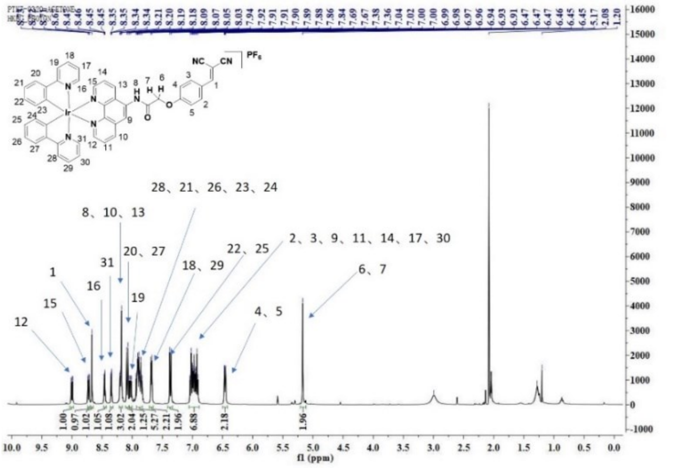


Figure S1 Chemical structures of parent iridium(III) complex **1a** and EGFR inhibitor **1b**.



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18、29
1
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19
15
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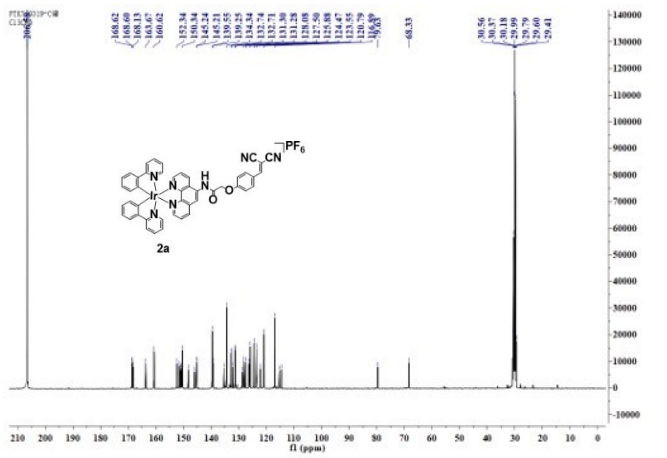
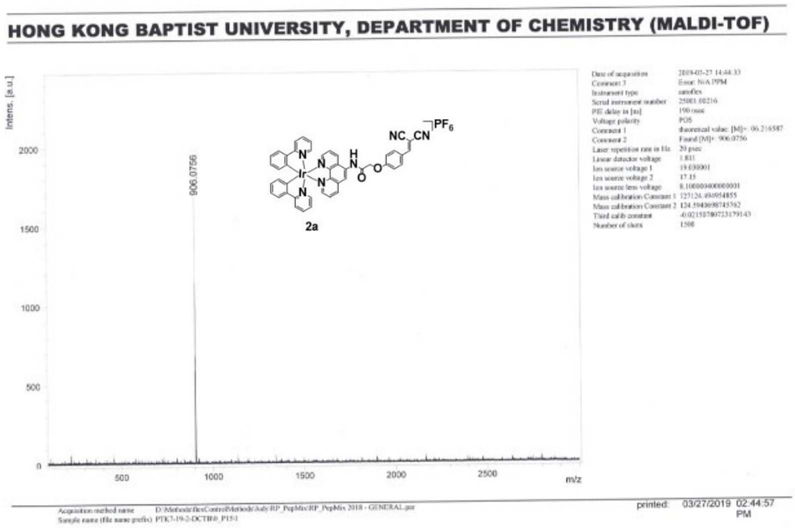
f1 (ppm)



2a
f1 (ppm)



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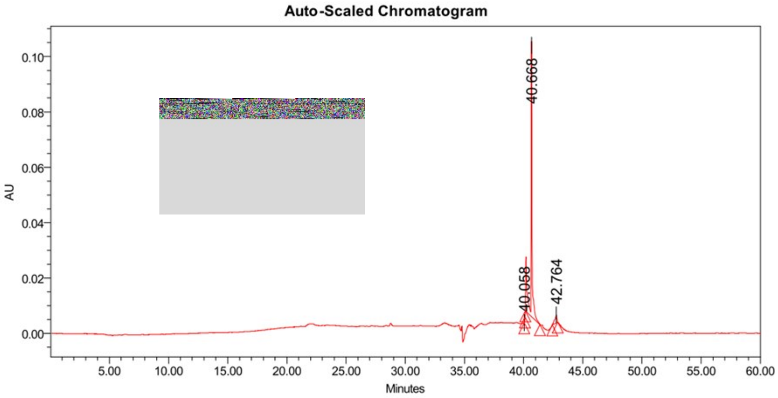
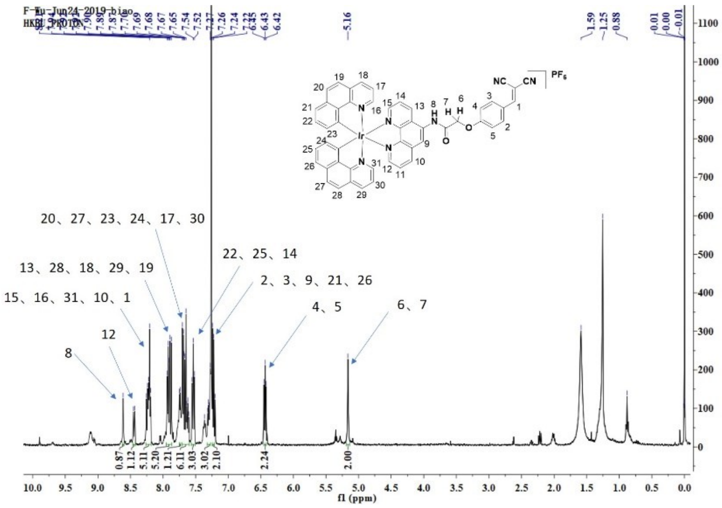
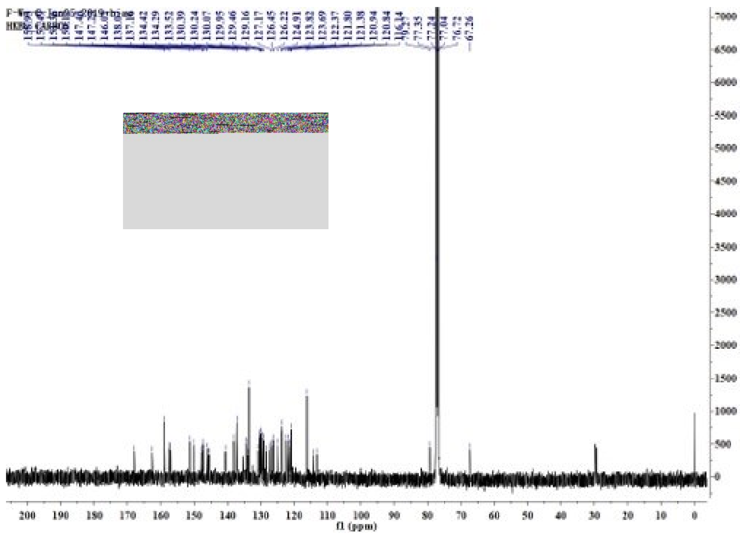
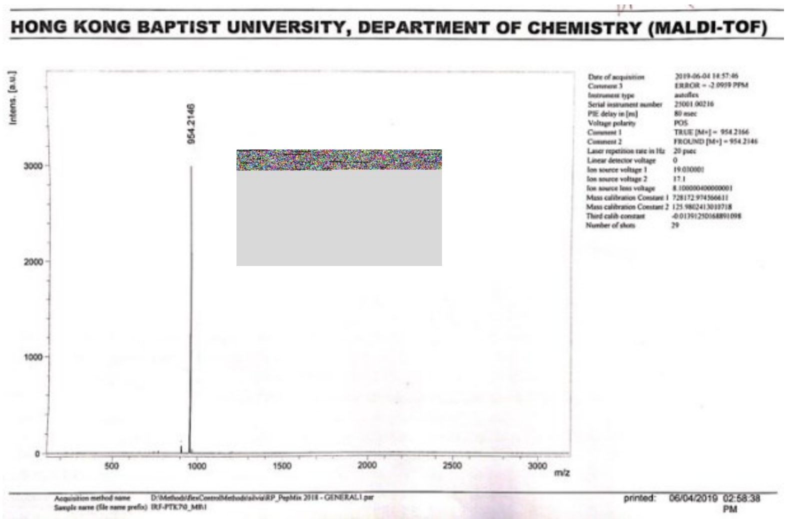


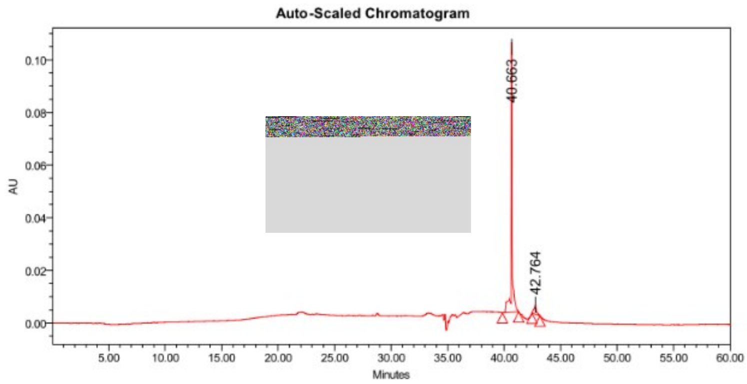
Peak Results

	Name	RT	Area	Height	Amount	Units	Total Area	% Area
1		40.058	1550	-130			674923	0.23
2		40.668	635960	96138			674923	94.23
3		42.764	37413	2940			674923	5.54









Peak Results

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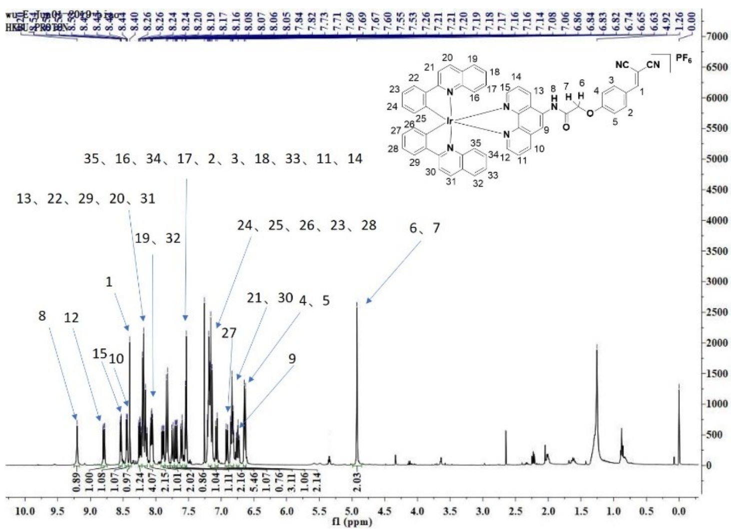
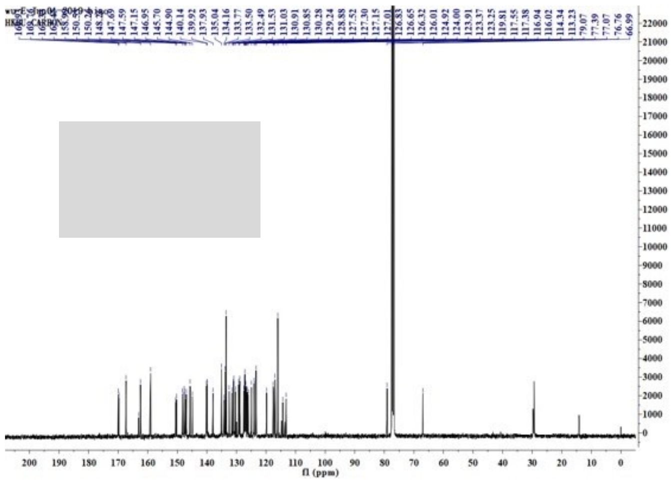
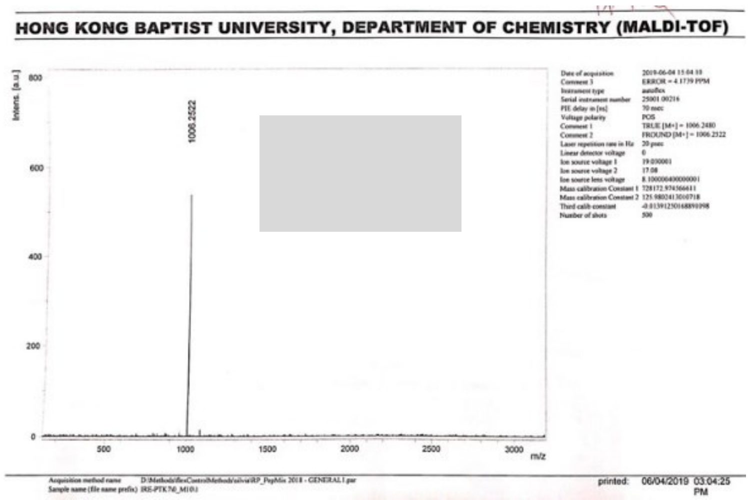
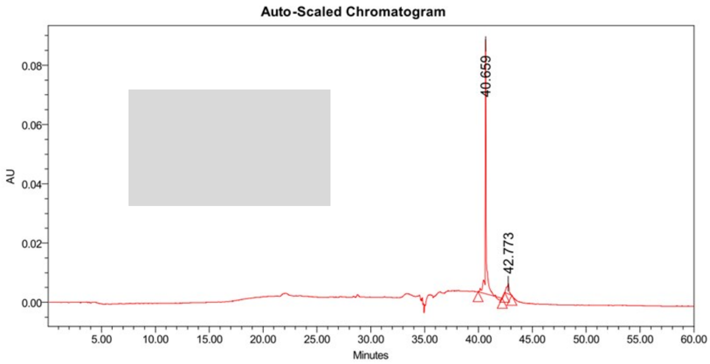
2		42.764	56554	3609			807568	7.00









Peak Results

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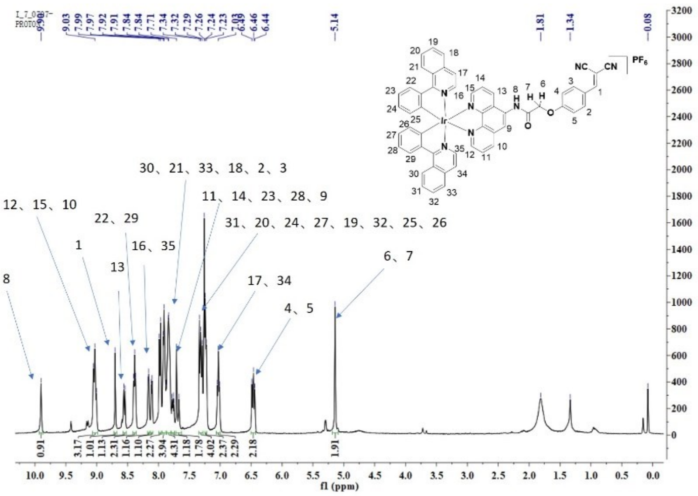
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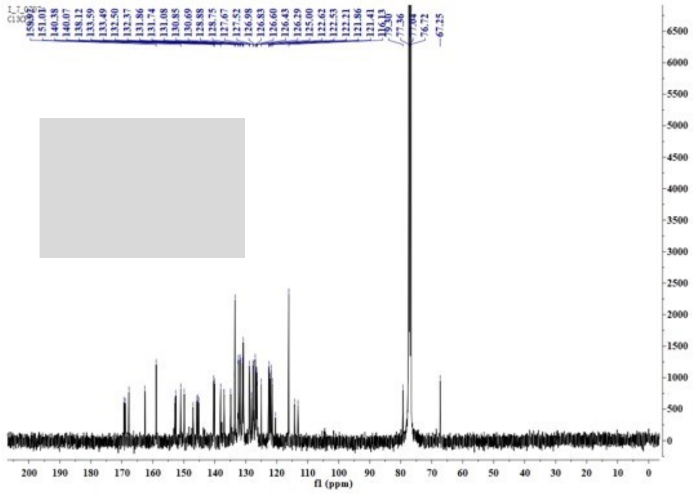
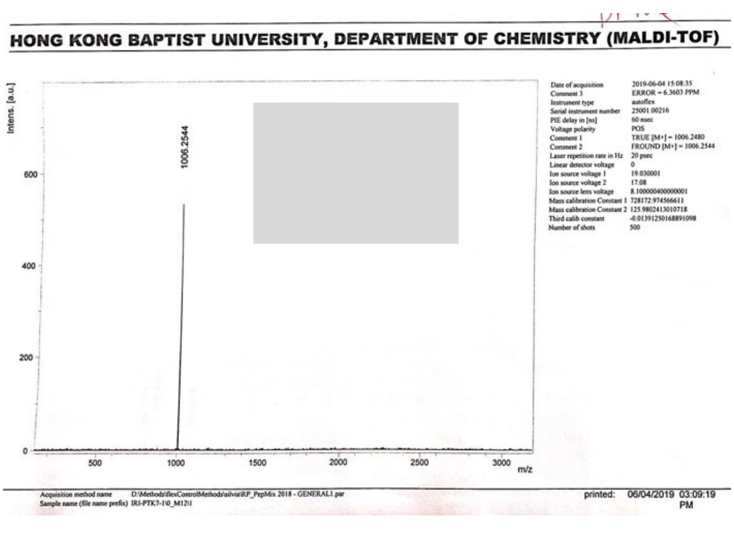
PF6
f1 (ppm)


f1 (ppm)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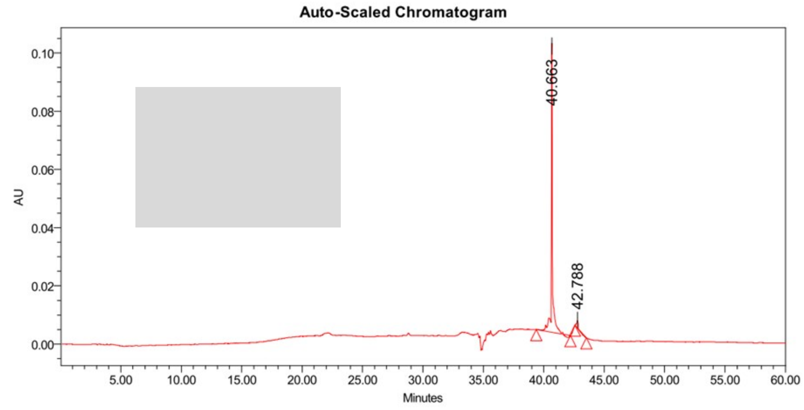

Peak Results

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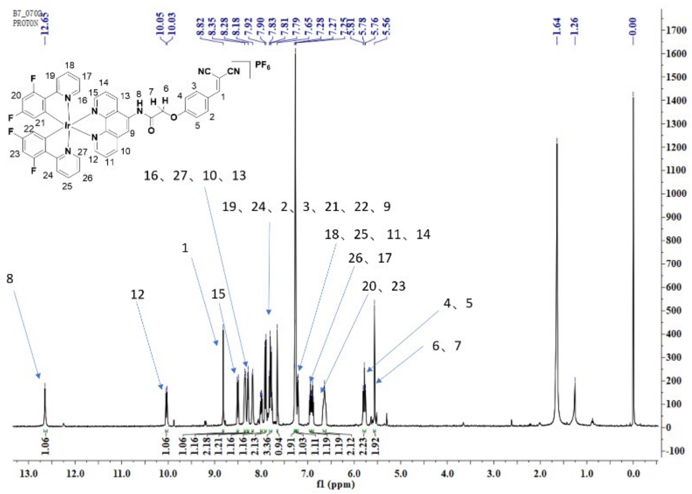
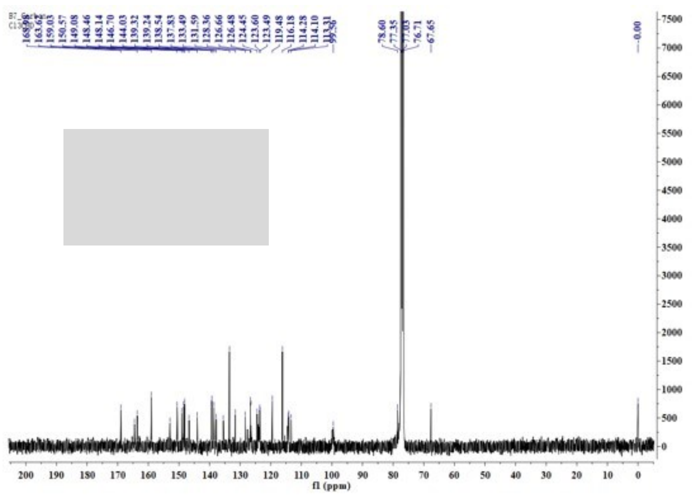
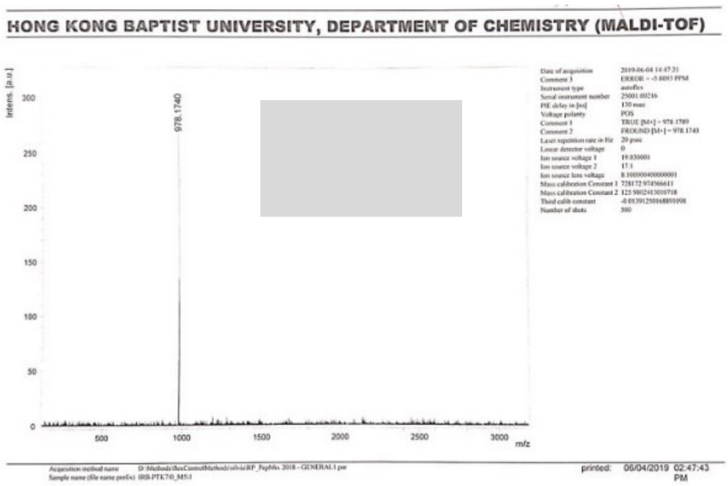
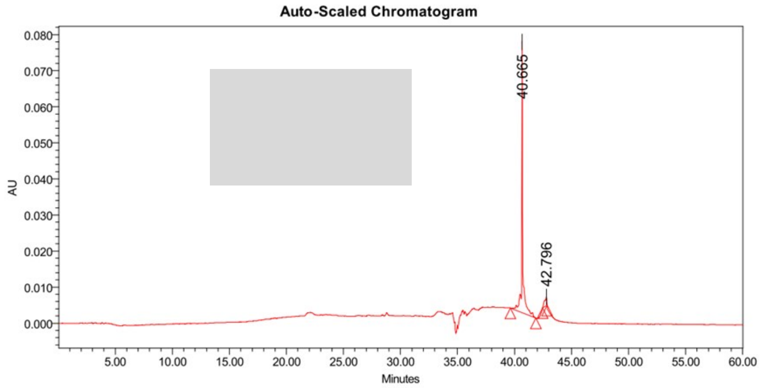
2		42.788	32159	2559			693558	4.64









Peak Results

	Name	RT	Area	Height	Amount	Units	Total Area	% Area
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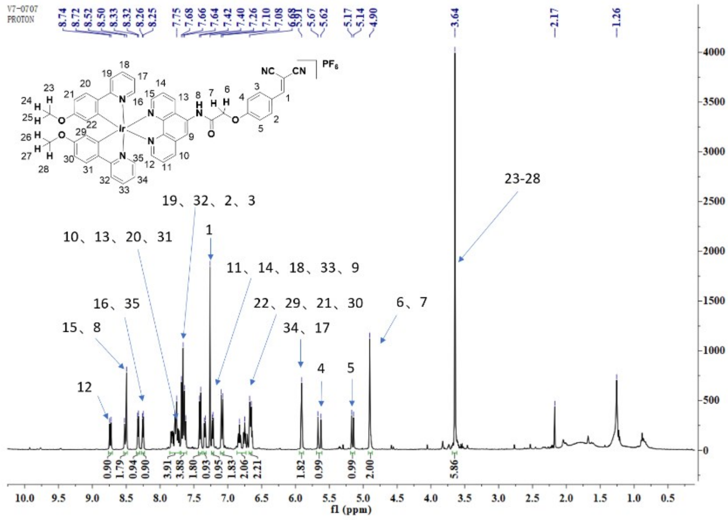
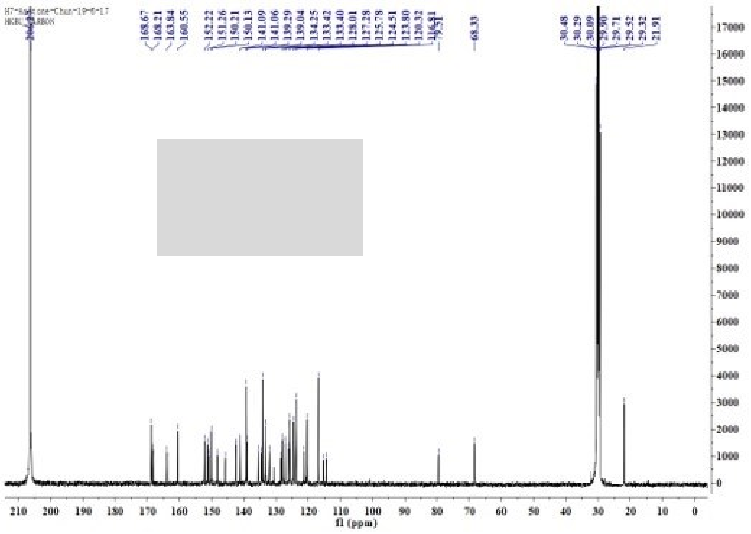
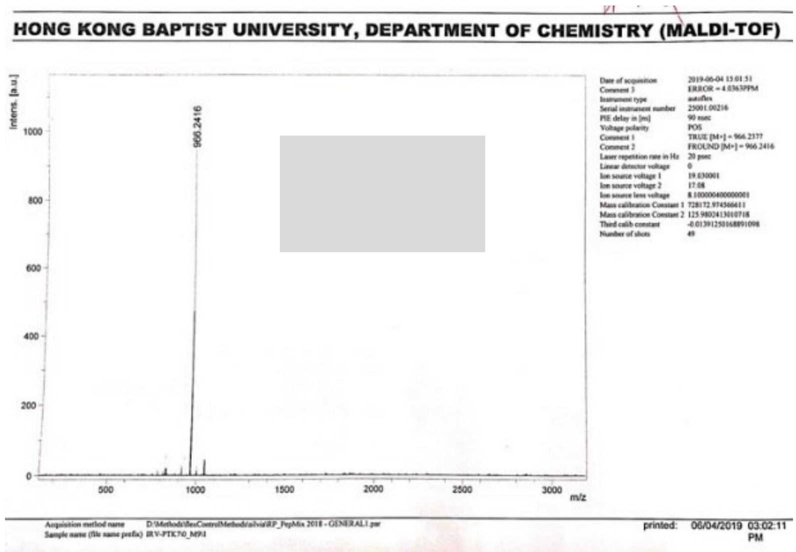
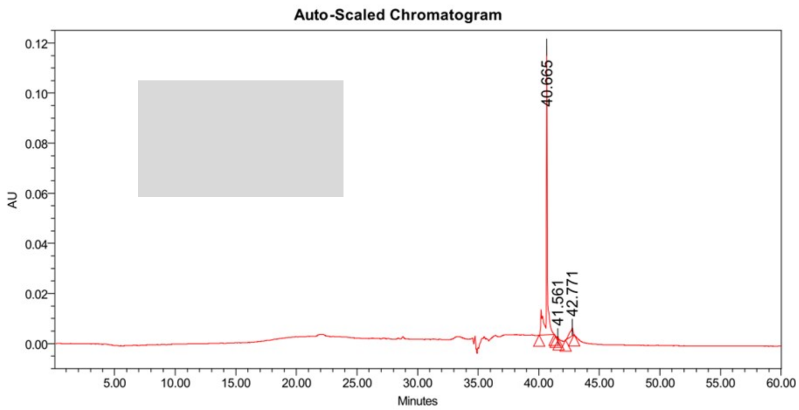
2		42.796	34453	2498			546927	6.30



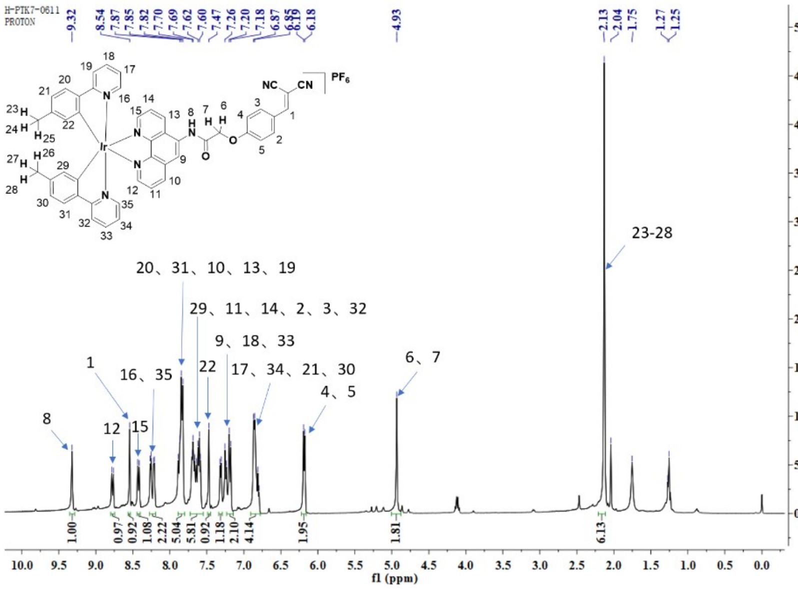






Peak Results

	Name	RT	Area	Height	Amount	Units	Total Area	% Area
1		40.665	712616	111296			779127	91.46
2		41.561	3746	540			779127	0.48
3		42.771	62765	3363			779127	8.06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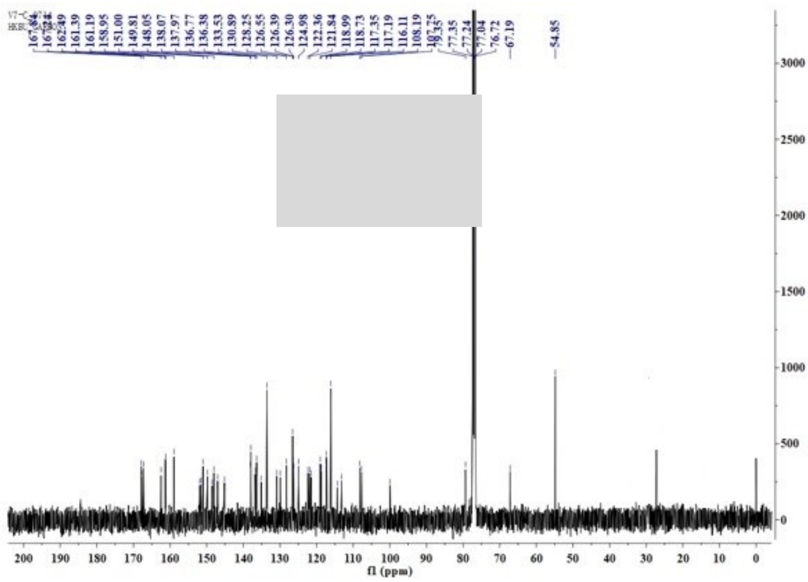
f1 (ppm)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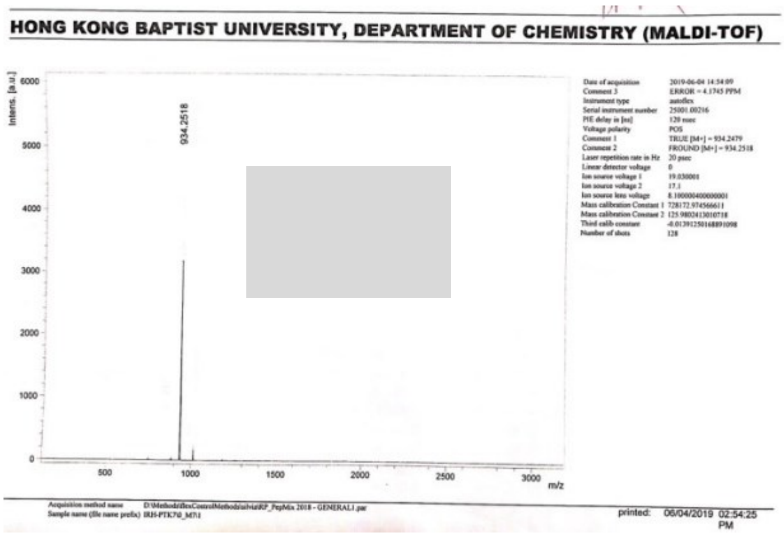
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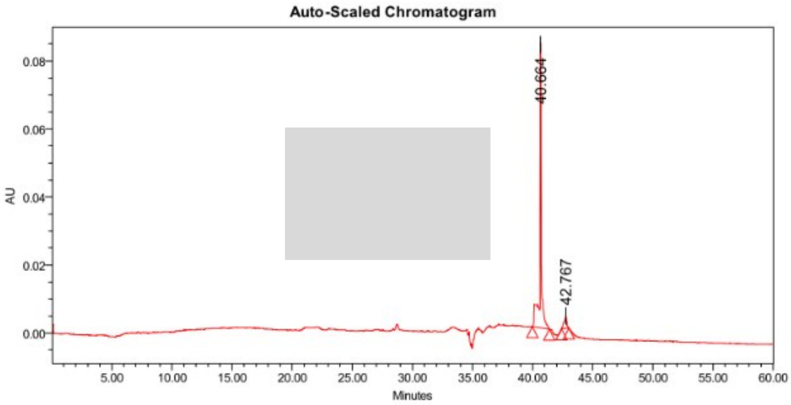

Peak Results

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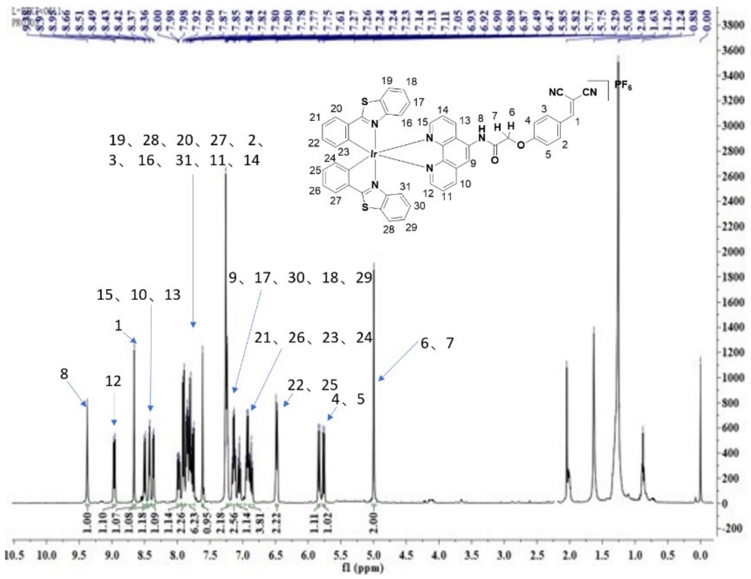
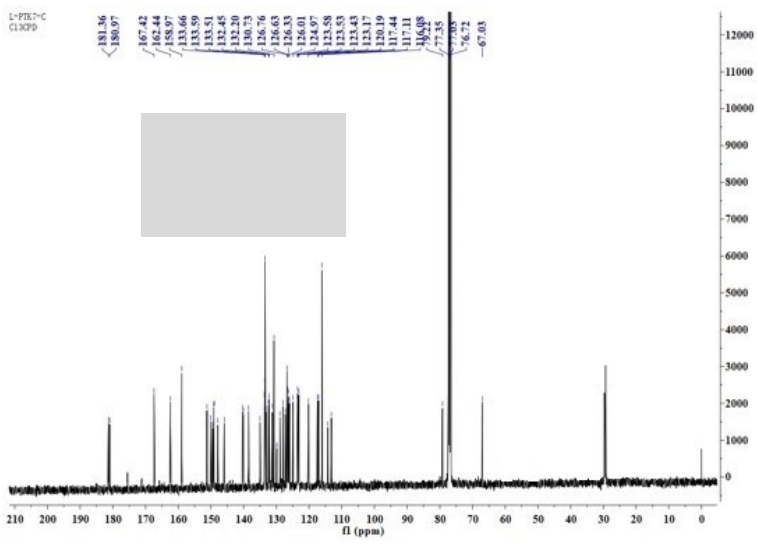
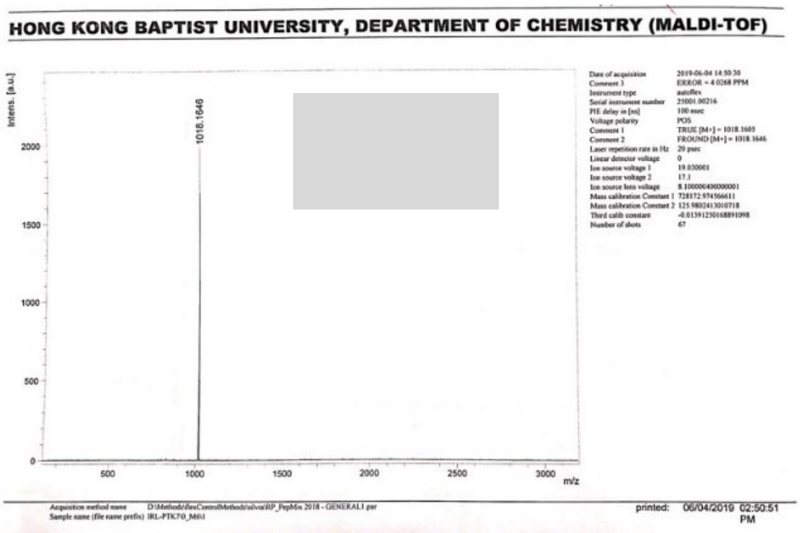
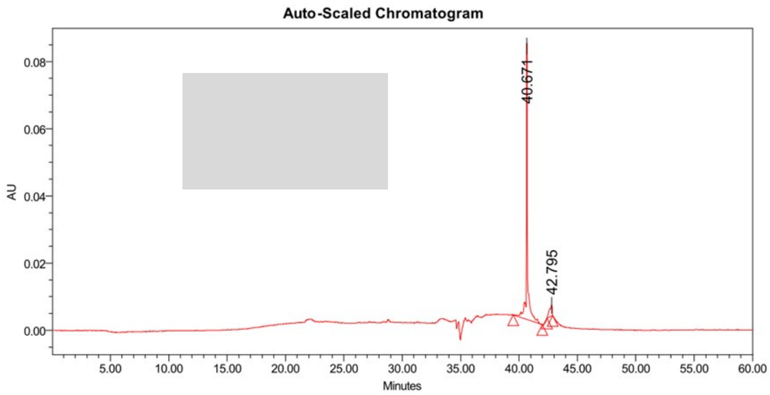
1		40.664	777481	80977			830994	93.56
2		42.767	53513	3601			830994	6.44









Peak Results

	Name	RT	Area	Height	Amount	Units	Total Area	% Area
1		40.671	554981	79415			608567	91.19
2		42.795	53585	3237			608567	8.81

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	τ_1 (ns)	τ_2 (ns)	τ_{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] τ_i and τ_{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.

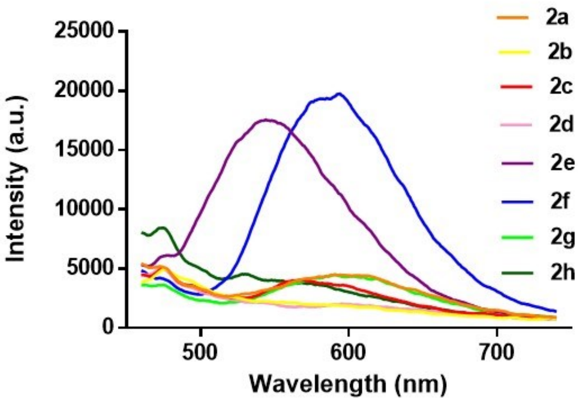


Figure S3 Emission spectra of complexes **2a**–**2h** (5 μM) in PBS buffer containing 0.5% DMSO.

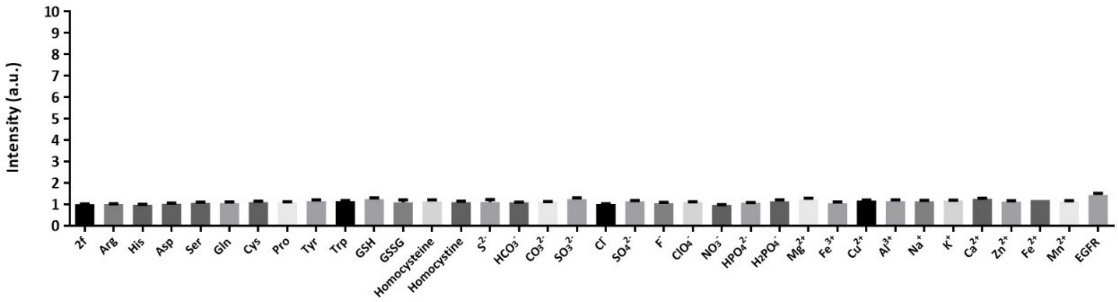
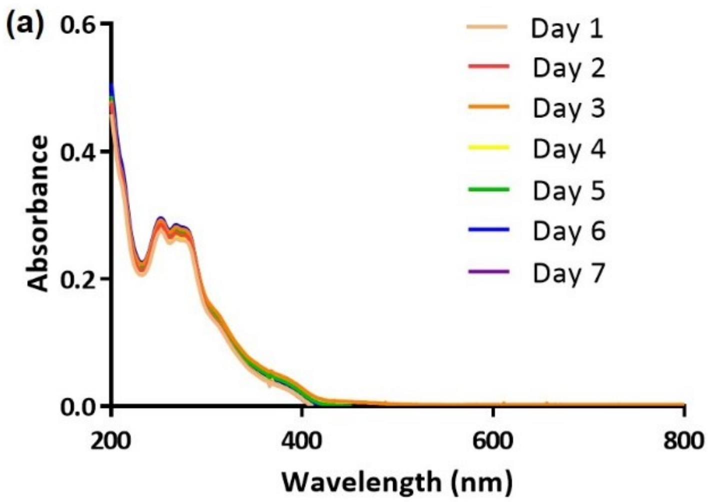


Figure S4 Luminescence intensity of complex **2f** (5 μM) in the absence and presence of EGFR (20 ng/μL) or various potentially interfering species including amino acids, anions or cations (20 μM).



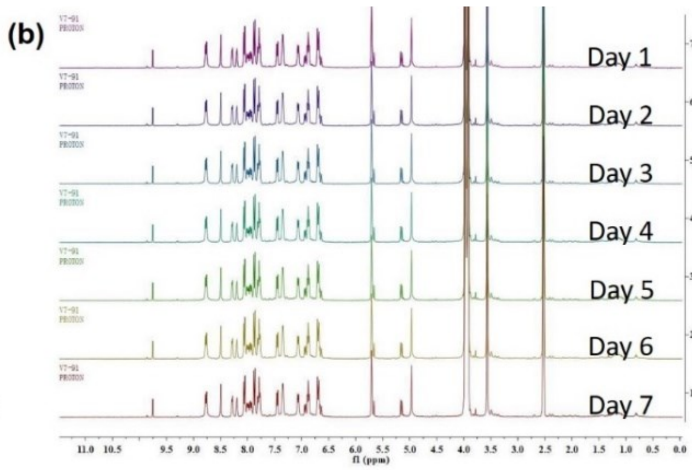


Figure S5 (a) UV/Vis absorption of complex **2f** (2.5 μM) in acetonitrile/H_2O (9:1) at t = 1 min and after incubation for 1, 2, 3, 4, 5, 6 and 7 days at 298 K. **(b)** ^1H NMR spectra of complex **2f** in DMSO–*d*6/D_2O (9:1) at t = 1 min and after incubation for 1, 2, 3, 4, 5, 6 and 7 days at 298 K.

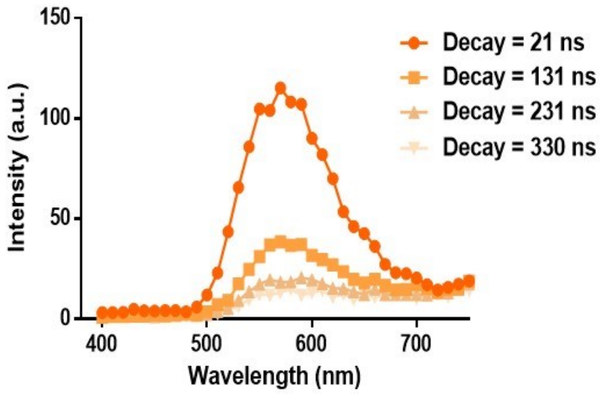


Figure S6 Time-resolved spectra of complex **2f** (10 μM) in PBS buffer containing 1% DMSO.

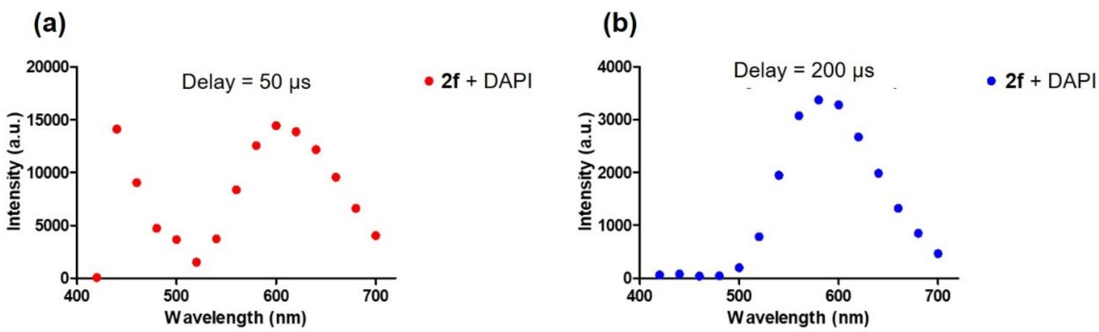


Figure S7 Time-resolved spectra of complex **2f** (1 μM) and the nuclear dye DAPI (1 μM) in A431 cells with delay time set to **(a)** 50 μs and **(b)** 200 μs, respectively.

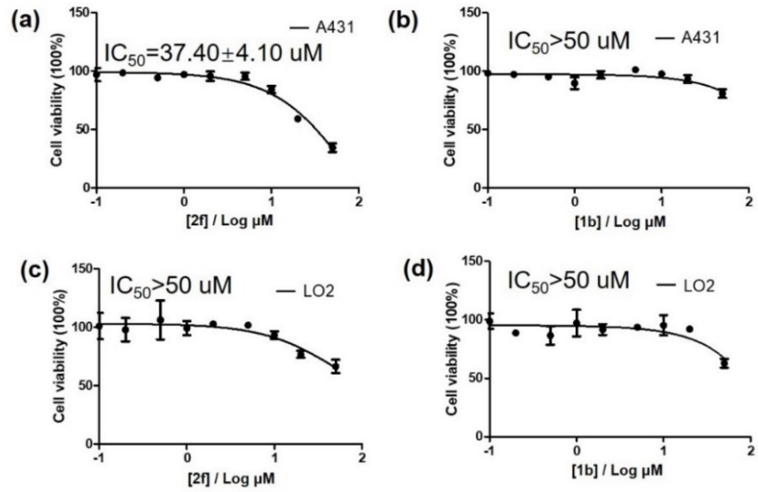


Figure S8 Cytotoxicity of complex **2f** and the reference inhibitor **1b** against normal LO2 cells and EGFR-overexpressing A431 cells. A431 and LO2 cells were treated with **2f** and **1b** for 72 h.

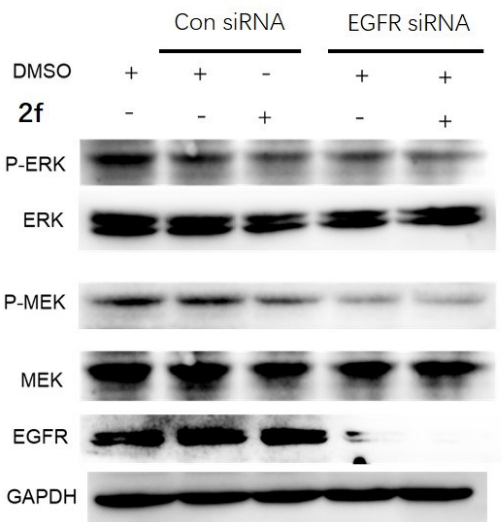


Figure S9 Effects of complex **2f** on P-ERK, ERK, P-MEK, MEK and EGFR protein expression after EGFR knockdown by EGFR siRNA. After treatment by EGFR siRNA for 48 h, A431 cells were treated with **2f** (5 μM) for 12 h. The protein levels were determined by Western blotting.

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