Electronic Supporting Information (ESI)

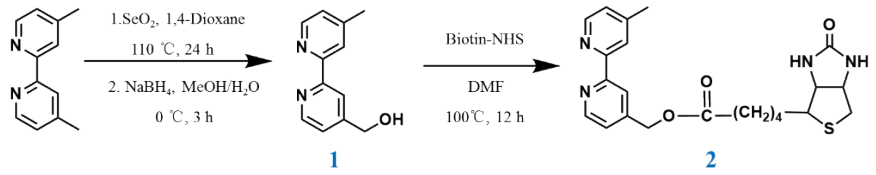
Highly X-ray sensitive iridium prodrug for visualized tumor radiochemotherapy

Zhennan Zhao, Pan Gao, Li Ma* and Tianfeng Chen*

Department of Chemistry, Jinan University, Guangzhou 510632, China.

Synthesis of compound 1:

The 4, 4'-dimethyl-2, 2'-dipyridyl (8g, 40 mmol) and SeO_2 (8g, 72.70 mmol) were suspended in the 350 mL 1, 4-dioxane and refluxed for 24 h under N_2 atmosphere. The solution was fileted immediately and the residue was washed by MeOH twice. The solvent was collected and evaporated to obtain the orange solid. The solid was suspended in 200 mL MeOH in a flask and maintained in ice bath. The fresh prepared $NaBH_4$ solution (2 mmol/mL, 20 mL) was added in the flask drop by drop to and then stirred for overnight. The resultant mixture was filtered and the filtrate was evaporated under reduced pressure to obtain the raw product. This raw product was suspended in the 50 mL saturated sodium carbonate solution and extracted by chloroform (50 mL x 3), and then the organic layer was desiccated by andydour $MgSO_4$ powder and concentrated to get the yellow solid. The product was further purified by silica gel chromatograph using DCM and MeOH (15:1, v/v) to give the white powder. Yield: 23.8%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.54 (d, J = 5.2 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.27 (s, 1H), 8.15 (s, 1H), 7.25 (d, J = 5.2 Hz, 1H), 7.13 (d, J = 5.2 Hz, 1H), 4.72 (s, 2H) 4.55 (s, 1H), 2.41 (s, 3H), ^{13}C NMR (101 MHz, DMSO-d_6, δ ppm) : 155.91. 155.74, 151.78, 149.09, 148.69, 148.56, 124.83, 122.36, 121.18, 118.74, 63.16, 21.20.



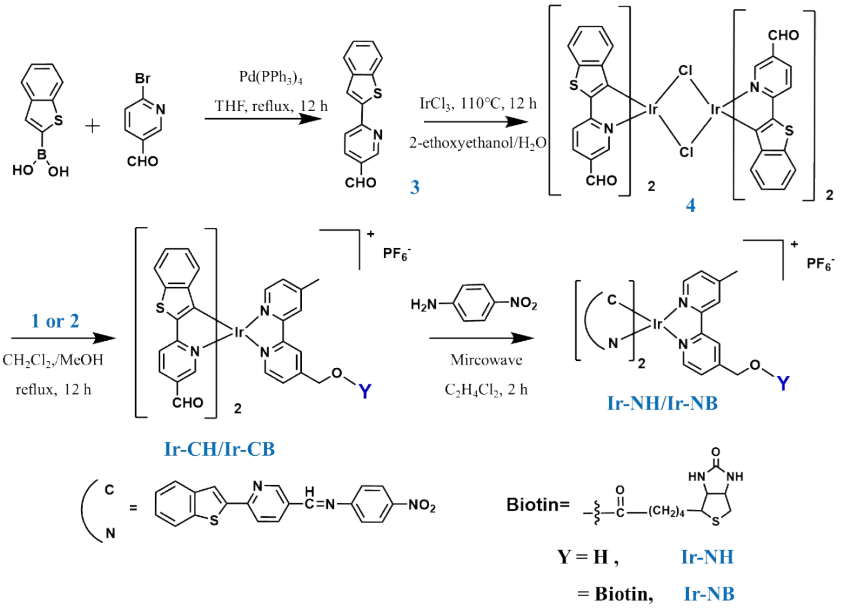
Scheme S1. Synthetic route of compound **1** and **2**.

Synthesis of compound 2:

Compound **1** (0.30 g, 1.5 mmol), EDCI (0.31 g, 1.6 mmol), NHS (0.18 g, 1.6 mmol), and biotin (0.36 g, 1.5 mmol) were dissolved in 10 mL dry DMF and stirred at 50 ℃for 12 h. The solution was poured into the ice water to allow the formation of white residue. The residue was filtered and purified by silica gel chromatograph using DCM and MeOH (20:1, v/v) to give the light yellow liquid. Yield: 50.1%. Anal. Calcd for $C_{22}H_{26}N_4O_3S$ (%): C, 61.95; H, 6.14; N, 13.14, Found (%): C, 61.88; H, 6.18; N, 13.07. ESI-TOFMS (CH_3OH): m/z 427.1336 $[M+H]^+$, 449.0345 $[M+Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.67 (d, J=5.2 Hz, 1H), 8.55 (d, J=5.2 Hz, 1H), 8.36 (s, 1H), 8.25 (s, 1H), 7.27 (d, J=5.2 Hz, 1H), 7.17 (d, J=5.2 Hz, 1H), 5.92 (s, 1H), 5.43 (s, 1H), 5.22 (s, 2H), 4.47 (t, J=7.6 Hz, 1H), 4.29 (t, J=7.6 Hz, 1H), 3.16 (m, 1H), 2.88 (m, 1H), 2.70 (d, J=12.8 Hz, 1H), 2.49-2.58 (m, 5H), 1.76-1.67 (m, 4H), 1.52-1.45 (m, 2H).

Synthesis of compound 3:

benzo[b]thiophen-2-ylboronic acid (2.67 g, 15 mmol), 6-bromo nicotinaldehyde (2.79 g, 15 mmol), tetrakis (triphenylphosphine) palladium (0.46 g, 0.4 mmol), sodium carbonate (3.18 g, 30 mmol), 50 mL THF and 15 mL water were added in a glass flask. The mixture was stirred and refluxed for 12 h under N_2 atmosphere. The resultant product was diluted in the 500 mL water to form the yellow residue, and then the mixture was extracted with 100 mL DCM for three times. The organic phase was washed with saturated salt water and desiccated by andydour Na_2SO_4 powder, filtered and concentrated. The raw product was further purified by silica gel column using DCM/MeOH as the eluent to obtain the yellow solid. Yield: 78.4%. ^{1}H NMR (300 MHz, DMSO) δ = 10.11 (s, 1H), 9.11 (s, 1H), 8.40 (s, 1H), 8.33 (s, 2H), 8.03 (t, J=6.0 Hz, 1H), 7.94 (t, J=6.0 Hz, 1H), 7.46- 7.43 (m, 2H). MALDI-TOF-MS (m/z): [M+H] 240.0301.



Scheme S2. Synthetic route of compounds and Ir(II) complexes

Synthesized of compound 4:

$IrCl_3$•$3H_2O$ (0.353 g, 1.0 mmol) and compound 3 were dissolved in a mixed solution of 2-ethoxyethanol (30 mL) and water (10 mL). The mixture was refluxed for 12 h under N_2 atmosphere to form the orange solid. The precipitate was filtered and washed with cold water and diethyl ether to give the compound **4**.

General route for synthesis of Ir(III) complex

Compound **4** (0.15 g, 0.20 mmol) and the ligand compound **1** (0.04 g, 0.20 mmol) or compound **2** (0.09 g, 0.20 mmol) were placed into the flask. A mixed solution of DCM and MeOH (1:1, v/v) was also added into the flask and refluxed for 12 h under N_2 atmosphere. After cooling to room temperature, NH_4PF_6 powder (0.16 g, 1 mmol) was added in the flask

and stirred for another 3 h. Next, the solution was filtered and evaporated under reduced pressure. The raw product was purified by silica gel chromatograph using DCM and MeOH (30:1, v/v) to give the orange-red powder. Yield: 76.4% for **Ir-CH** and 56.8% for **Ir-CB**.

Ir-CH: Anal. Calcd for $C_{40}H_{28}F_6IrN_4O_3PS_2$ (%): C, 47.38; H, 2.78; N, 11.24 Found (%): C, 50.71; H, 4.11; N, 16.13. ESI-TOFMS (CH_3OH): m/z calcd for: 869.1232 $[M\text{-}PF_6]^+$; found: 869.1225 $[M\text{-}PF_6]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9. ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9.66 (s, 2H), 9.25 (s, 2H), 8.70 (d, J=4.4 Hz, 1H), 8.62(d, J=4.4 Hz, 1H), 8.48 (s, 4H), 8.42 (s, 1H), 8.30 (s, 1H), 8.19 (t, J=6.0 Hz, 2H), 8.10 (t, J=6.0 Hz, 2H), 7.62-7.59 (m, 4H), 7.40 (d, J=4.8 Hz, 1H), 7.27 (d, J=4.8 Hz, 1H), 4.88 (s, 2H), 4.70 (s, 1H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6, δ ppm): 190.29, 190.10, 167.44, 167.35, 163.43, 163.34, 163.30, 156.88, 155.28, 154.71, 152.80, 152.72, 152.34, 151.49, 150.11, 149.85, 147.30, 147.11, 144.46, 141.03, 140.93, 139.84, 139.81, 136.73, 136.54, 133.50, 133.40, 131.45, 131.35, 130.82, 130.73, 130.12, 127.03, 126.61, 122.77, 121.51, 118.78, 118.74, 55.38, 21.35.

Ir-CB: Anal. Calcd for $C_{50}H_{42}F_6IrN_6O_5PS_3$ (%): C, 47.38; H, 2.78; N, 11.24 Found (%): C, 50.71; H, 4.11; N, 16.13. ESI-TOFMS (CH_3OH): m/z calcd for: 1095.2008 $[M\text{-}PF_6]^+$; found:1095.2014. ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9.67 (d, J=5.2 Hz, 2H), 9.31 (s, 1H), 9.16 (s, 1H), 8.86 (s, 2H), 8.69 (d, J=4.0 Hz, 1H), 8.54 (d, J=4.0 Hz, 1H), 8.26 (s, 1H), 8.01 (s, 2H), 7.94 (d, J=4.0 Hz, 1H), 7.81 (t, J=5.6 Hz, 2H), 7.75 (d, J=5.2 Hz, 2H), 7.43-7.39 (m, 3H), 7.26 (d, J=3.6 Hz, 1H), 6.97-6.93 (m, 2H), 6.41 (s, 2H), 4.93 (s, 2H), 4.30 (t, J=4.2 Hz 1H), 4.13 (t, J=4.2 Hz, 1H), 3.08 (m, 1H), 2.86 (t, J=4.4 Hz, 1H), 2.57(s, 4H), 2.06 (t, J=5.2 Hz, 2H), 1.57-1.33 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6, δ ppm): 193.36, 193.16, 172.33, 163.53, 163.43, 163.32, 163.21, 159.61, 159.15, 156.84, 155.23, 152.78, 152.71, 152.33, 151.50, 150.16, 150.10, 149.84, 147.24, 147.13, 144.67, 142.89, 142.82, 139.84, 139.81, 136.75, 136.55, 133.70, 133.24, 131.45, 131.34, 130.82, 130.74, 130.14, 126.91, 126.45, 122.75, 121.50, 118.79, 118.75, 61.53, 59.68, 57.27, 55.91, 55.37, 35.71, 29.32, 26.58, 26.55, 22.93.

Synthesis of imine compound

The synthetic route of imine compound was basing on the previous study. The 4-nitroaniline (20.6 mg, 0.1 mmol), magnesium sulfate (100 mg) and 0.1 mol Ir complex (101 mg **Ir-CH** or 124 mg **Ir-CB**) were dissolved in dry 50 mL1,2-dichloroethane and refluxed for 6 h. After the magnesium sulfate was removed, the raw product was concentrated under vacuum and recrystallized by diethyl ether to get the bright orange solid. The product was purified by silica gel chromatograph using DCM and MeOH (30:1, v/v) to give the powder Yield: 72.2% for **Ir-**

NH and 69.1% for **Ir-NB**.

Ir-NH: Anal. Calcd. for $C_{52}H_{36}F_6IrN_8O_5PS_2$ (%): C, 49.80; H, 2.89; N, 9.09 Found (%): C, 49.83; H, 2.94; N, 9.13. ESI-TOFMS (CH_3OH): ESI-TOFMS (CH_3OH): m/z calcd for: 1109.1879. $[M\text{-}PF_6]^+$; found: 1109.1873. ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9.26 (s, 2H), 8.72 (d, J=4.0 Hz,1H), 8.63 (d, J=4.0 Hz, 1H), 8.47 (s, 4H), 8.41 (s, 1H), 8.29 (s, 1H), 8.18 (t, J=6.0 Hz, 2H), 8.08 (t, J=6.0 Hz, 2H), 7.94 (s, 1H), 7.80 (d, J=12.4 Hz, 4H), 7.60 (m, 4H), 7.40 (t, J=4.0 Hz, 2H), 7.28 (t, J=4.0 Hz, 2H), 7.10 (d, J=12.4 Hz, 4H), 4.89 (s, 2H), 4.71 (s, 2H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6, δ ppm): 163.41, 163.29, 159.74, 159.54, 156.93, 155.22, 154.09, 152.73, 152.67, 152.34, 151.61, 150.15, 149.85, 147.90, 147.81, 146.69, 142.97, 141.09, 139.85, 139.82, 138.59, 138.50, 136.74, 136.54, 133.89, 133.20, 131.46, 131.36, 130.82, 130.72, 130.16, 126.94, 126.52, 122.77, 121.51, 118.79, 118.75, 118.16, 117.15, 116.04, 55.38, 21.38.

Ir-NB: Anal. Calcd for $C_{62}H_{50}F_6IrN_{10}O_7PS_3$ (%): C, 50.30; H, 3.40; N, 9.46. Found (%): C, 50.71; H, 4.11; N, 16.13. ESI-TOFMS (CH_3OH): m/z calcd for: 1335.2655 $[M\text{-}PF_6]^+$; found:1335.2650. ^1H NMR (DMSO-d_6, δ ppm): 9.13 (s, 1H), 9.03 (s, 1H), 8.86 (s, 1H), 8.46 (m, 2H), 8.32 (d, J=4.4 Hz, 1H), 8.25 (d, J=4.4 Hz, 1H), 8.15 (t, J=6.4 Hz, 2H), 8.08 (m, 3H), 7.82 (d, J=12.4 Hz, 4H), 7.69 (d, J=4.4 Hz, 1H), 7.65 (t, J=4.4 Hz, 2H), 7.61 (d, J=4.4 Hz, 2H), 7.53 (d, J=4.4 Hz, 1H), 7.31 (d, J=4.4 Hz, 1H), 7.28 (d, J=4.4 Hz, 1H), 7.14 (d, J=12.4 Hz, 4H), 6.98 (m, 2H), 6.40 (d, J=22.4 Hz, 2H),4.89 (s, 1H), 4.31 (t, 1H), 4.14 (t, 1H), 3.11 (q, J=4.4 Hz, 1H), 2.82 (dd, J=4.4, 10.0 Hz, 1H), 2.58 (s, 4H), 2.05 (s, 4H), 2.06 (t, J=5.2 Hz, 2H), 1.62-1.29 (m, 6H). ^{13}C NMR (DMSO-d_6, δ ppm): 172.69, 163.73, 163.41, 163.29, 163.18, 161.44, 161.02, 159.29, 159.09, 156.92, 155.20, 153.84, 152.72, 152.66, 152.34, 151.60, 150.17, 149.84, 147.77, 147.56, 145.94, 144.16, 141.69, 141.40, 140.97, 139.85, 139.82, 136.74, 136.54, 134.42, 133.99, 131.46, 131.35, 130.81, 130.72, 130.17, 126.90, 126.47, 122.77, 121.51, 118.79, 118.31, 117.03, 116.04, 61.49, 59.67, 55.87, 55.38, 54.07, 35.61, 28.48, 26.81, 25.69, 21.40.

Table S1. The radiotherapy sensitization efficacy of metal-based RSs in this work and previous reports.

RS	Metal ion	Cell line	Radiation dosage (Gy)	SER[a]	Reference
Ir-CH	Ir	A549 lung carcinoma cell line	4	1.52	this work
			8	6.65	
		WI38 normal lung cell line	4	1.14	
			8	3.65	
Ir-NH		A549 lung carcinoma cell line	4	1.96	
			8	6.81	
		WI38 normal lung cell line	4	1.26	
			8	3.72	
Ir-CB		A549 lung carcinoma cell line	4	4.29	
			8	10.50	
		WI38 normal lung cell line	4	2.75	
			8	3.32	
Ir-NB		A549 lung carcinoma cell line	4	4.19	
			8	10.35	
		WI38 normal lung cell line	4	2.99	
			8	3.69	
Cisplatin	Pt	A549 lung carcinoma cell line	4	1.34	
			8	4.36	
		WI38 normal lung cell line	4	1.17	
			8	4.08	
2c	Ru	A375 melanoma cell line	8	2.2	[1]
Cisplatin	Pt			1.5	
2a	Ru	A375 melanoma cell line	8	1.6	[2]
2b				2.4	
1a	Pt	A375 melanoma cell line	8	4.0	[3]
2a				7.0	
3a				3.5	
4a				4.0	
AH54	Ru	DLD1 colorectal cancer cell line		1.63 [b]	[4]
AH63				1.45 [b]	
AH108				1.52 [b]	
Ru-SR1#	Ru	PANC 1 pancreatic cancer cell line		2.01 [c]	[5]
Ru-SR3#				4.54 [c]	

a. Calculated by SER= (IC_{50} drug)/ IC_{50}(drug + IR)

b. The SER was calculated basing the provided IC_{50} value, SER= (IC_{50})/ (IC_{50} with IR)

c. The SER value was simulated using the multi-target single hit model.

Reference:

1. Z. Deng, L. Yu, W. Cao, W. Zheng and T. Chen, *Chem. Commun.*, 2015, **51**, 2637-2640.
2. Z. Q. Deng, L. L. Yu, W. Q. Cao, W. J. Zheng and T. F. Chen, *Chemmedchem*, 2015, **10**, 991-998.
3. Q. Xie, G. Lan, Y. Zhou, J. Huang, Y. Liang, W. Zheng, X. Fu, C. Fan and T. Chen, *Cancer Lett.*, 2014, **354**, 58-67.
4. R. Carter, A. Westhorpe, M. J. Romero, A. Habtemariam, C. R. Gallevo, Y. Bark, N. Menezes, P. J. Sadler and R. A. Sharma, *Sci. Rep.*, 2016, **6**, 20596.
5. Y. Zhou, Y. Xu, L. Lu, J. Ni, J. Nie, J. Cao, Y. Jiao and Q. Zhang, *Theranostics*, 2019, **9**, 6665-6675.

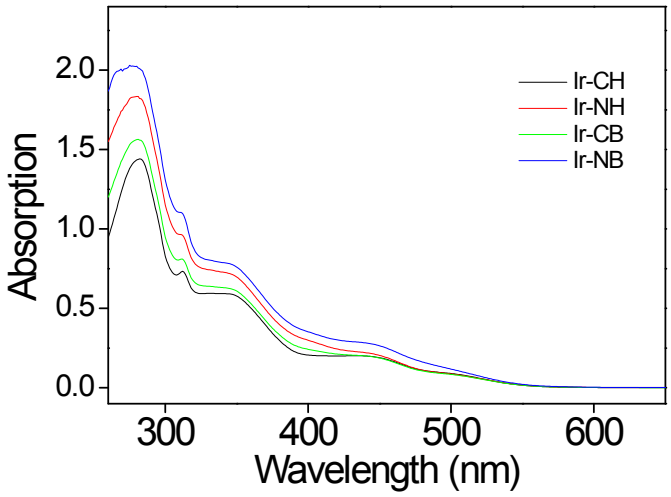


Figure S1. UV-Vis absorption spectrum of Ir(III) complexes.

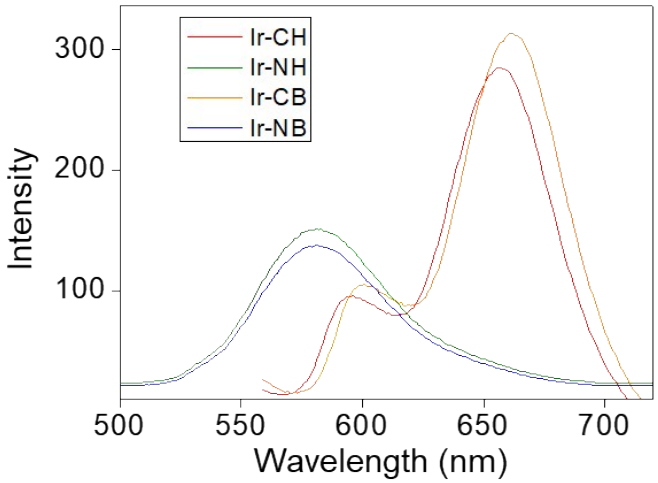


Figure S2. Emission spectrum of Ir(III) complexes in this work.

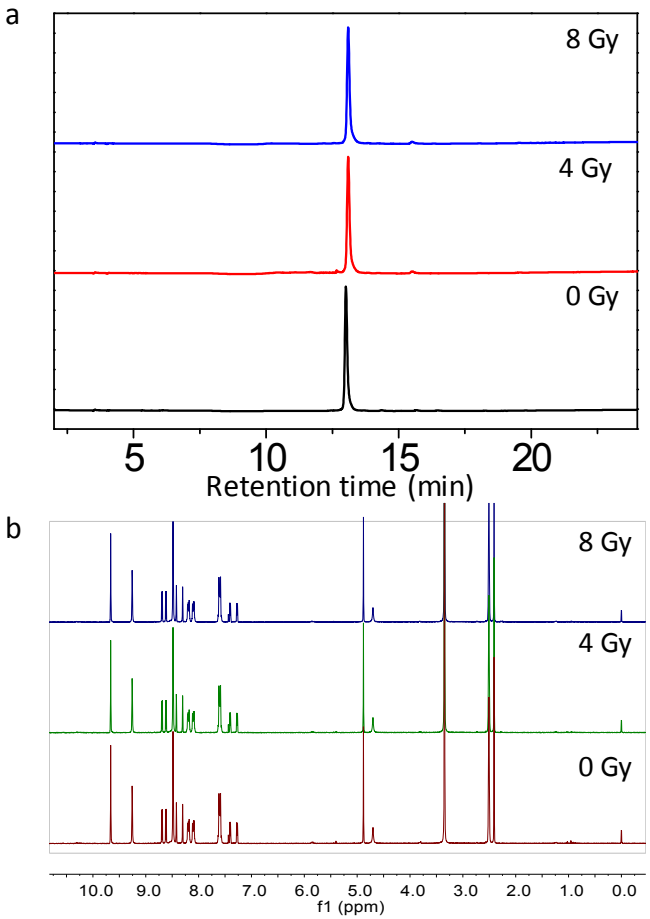


Figure S3. The stability of complex **Ir-CH** was examined upon X-ray radiation. (a) HPLC assay of **Ir-CH** after X-ray radiation. (b) ^{1}H NMR of **Ir-CH** before and after X-ray radiation.

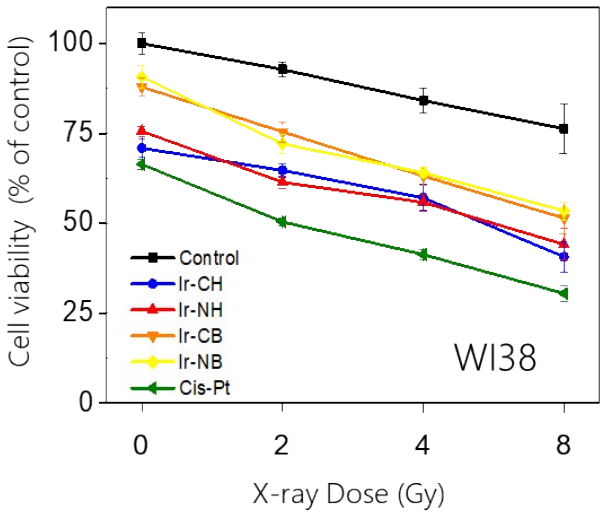


Figure S4. Cytotoxic effects of Ir complexes (10 μM) or cisplatin (10 μM) with or without the irradiation of X-ray at different dosage on WI38 cells.

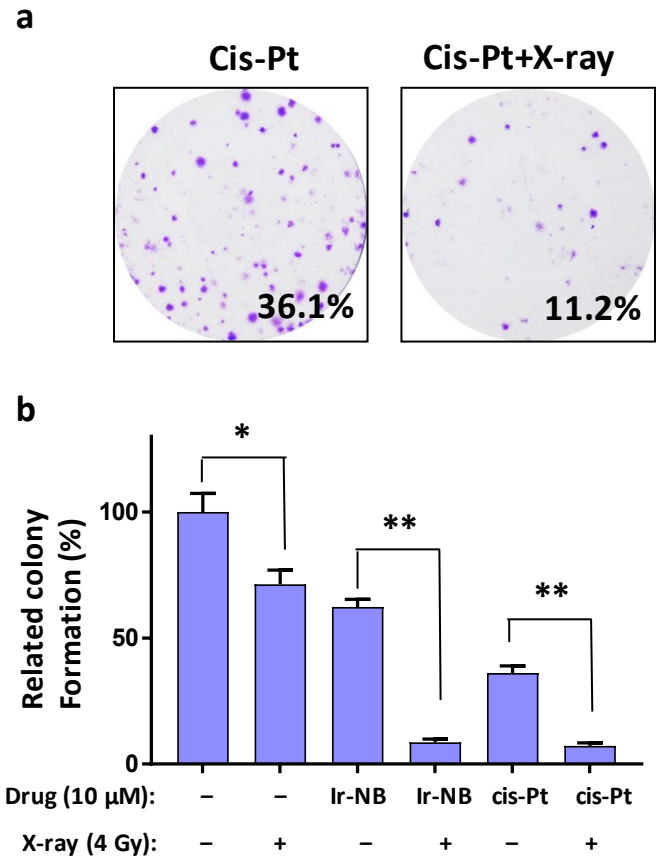


Figure S5. (a) Representative photographs of colony formation of A549 cells under the co-treatment of cisplatin and X-ray irradiation for 15 days. The numbers indicate the related colony formation in different groups. (b) Quantitative analysis of colony formation of A549 cells after various treatments for 15 days. Data were presented as mean ± sd. * means $p < 0.05$, ** means $p < 0.01$.

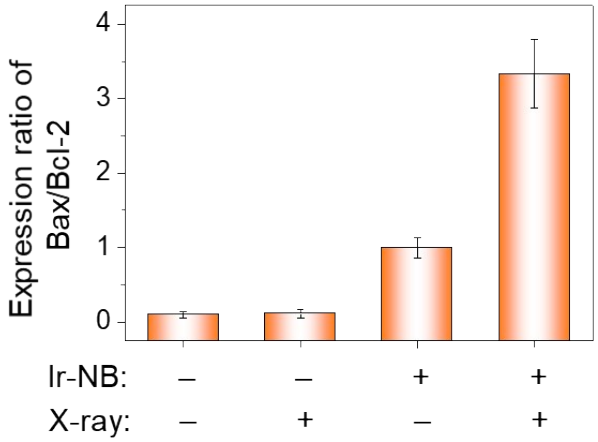


Figure S6. The expression ratio of Bax/Bcl-2 in A549 cells after the treatment of **Ir-NB** and X-ray (4 Gy). The quantitative analysis is corresponding to the representative bands in figure 3f.

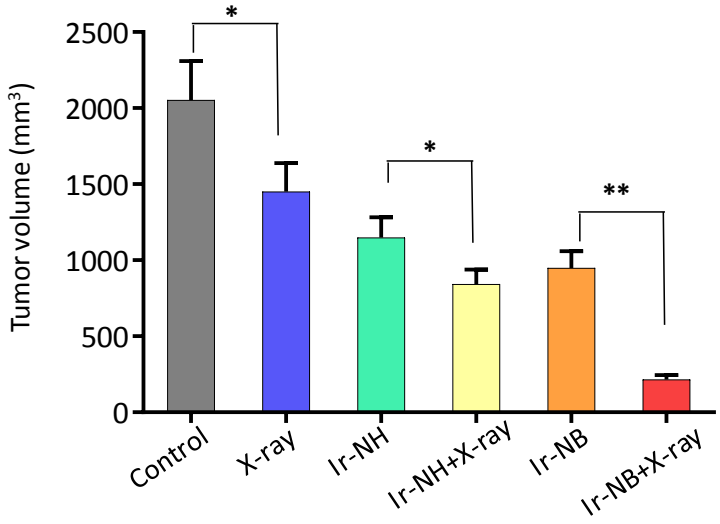


Figure S7. Quantitative analysis of the T2-weighted MR images of A549 tumor-bearing mice, corresponding to images in figure 5g. Data were presented as mean ± sd. * means $p < 0.05$, ** means $p < 0.01$.

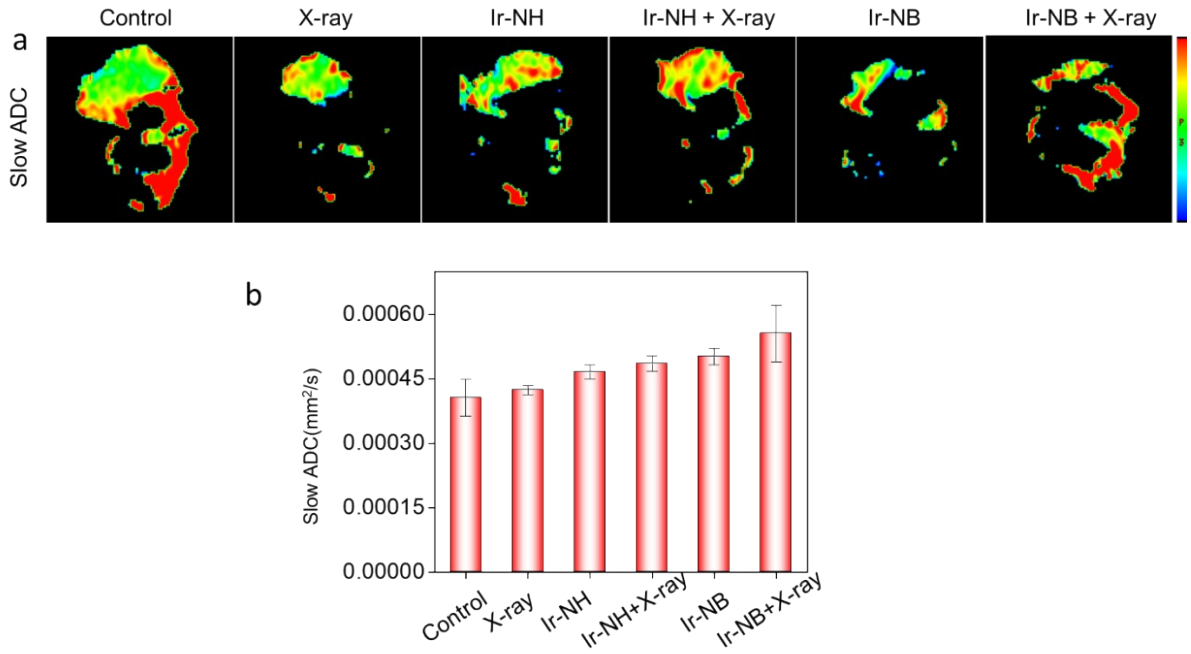


Figure S8. (a) Slow ADCs of A549 tumor-bearing mice after different treatments after 21 days. (b) Quantitative analysis of the pseudocolor signals of slow ADC. Each value represents means ± SD (n = 3).

Characterization of compounds

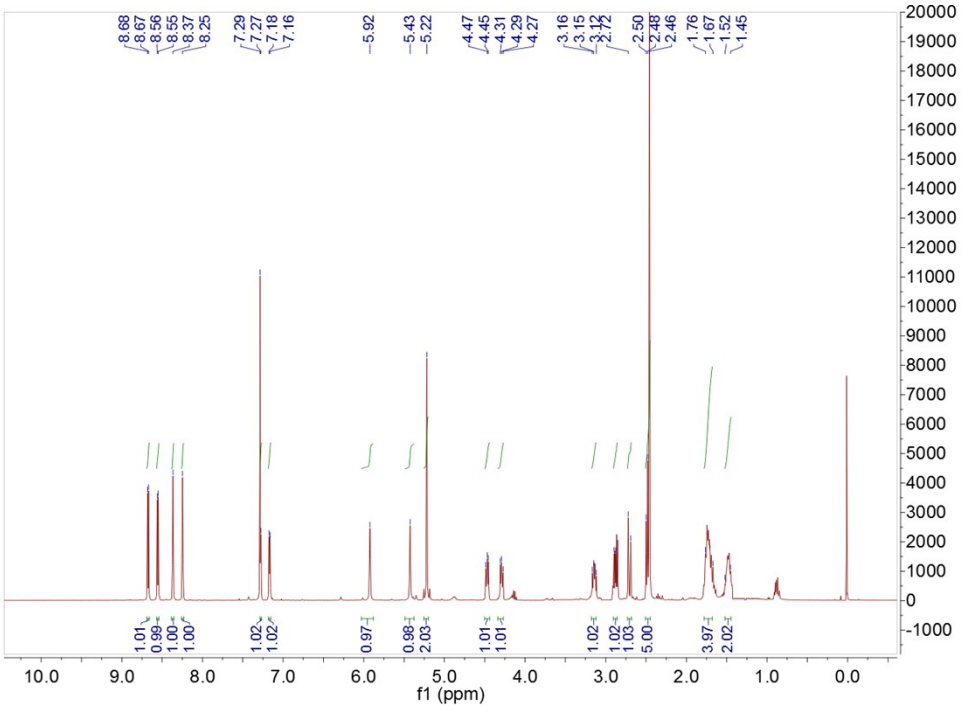


Figure S9. ^{1}H NMR of Compound 2

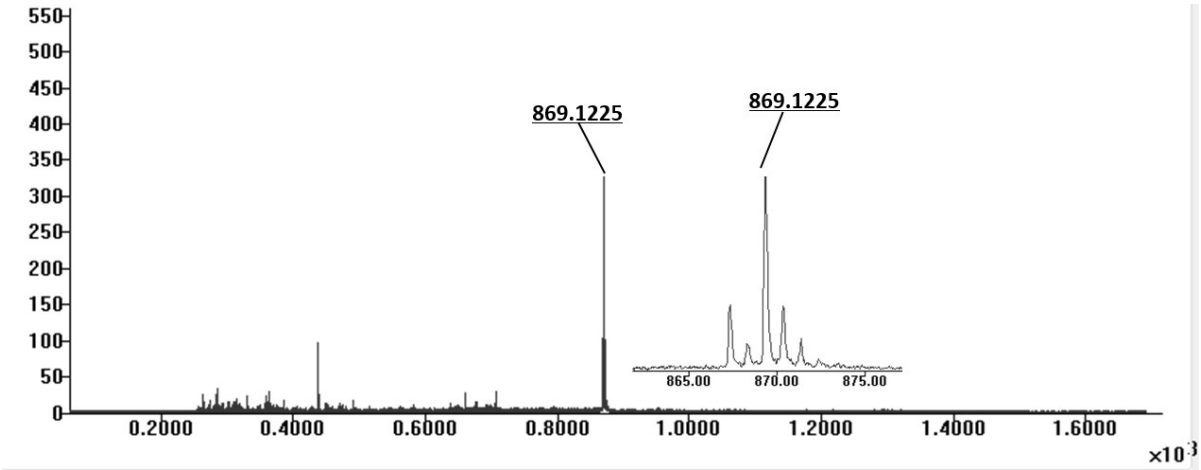


Figure S10. ESI-TOFMS of **Ir-CH**

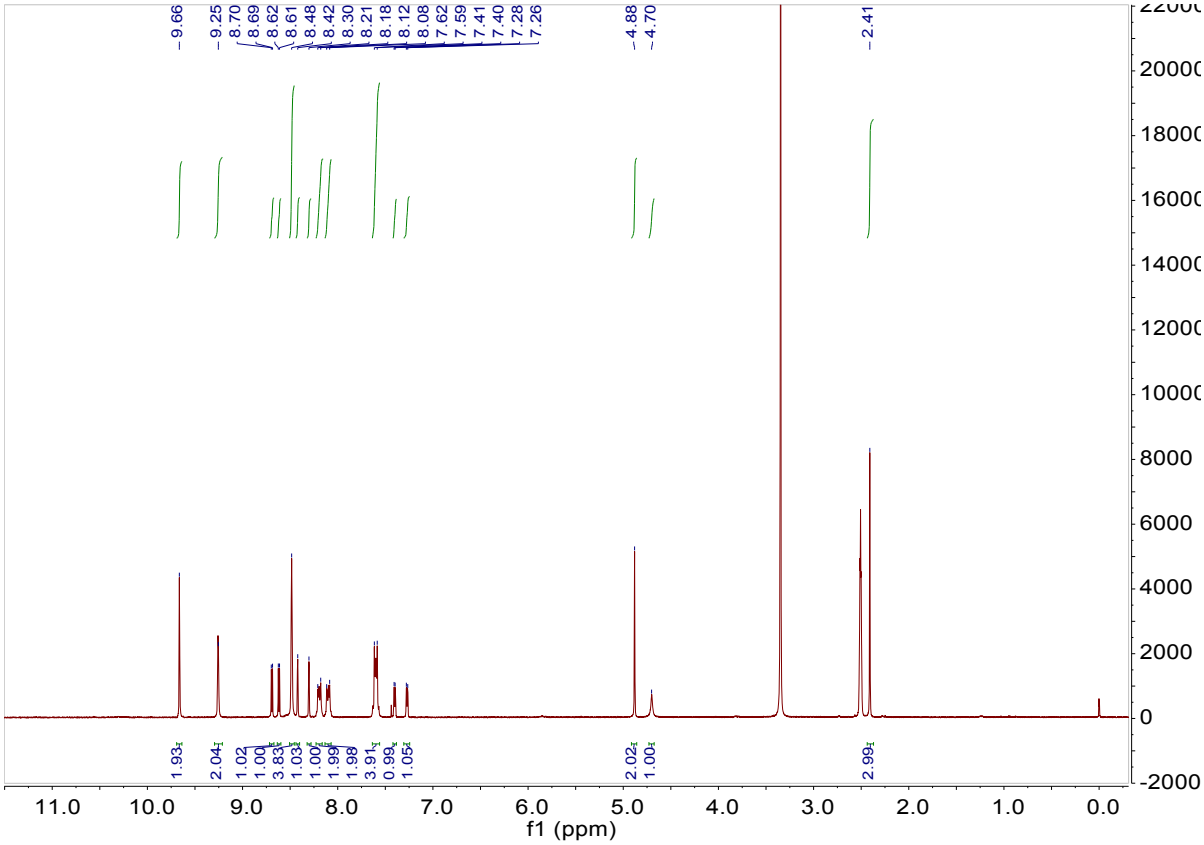


Figure S11. ^{1}H NMR of **Ir-CH**

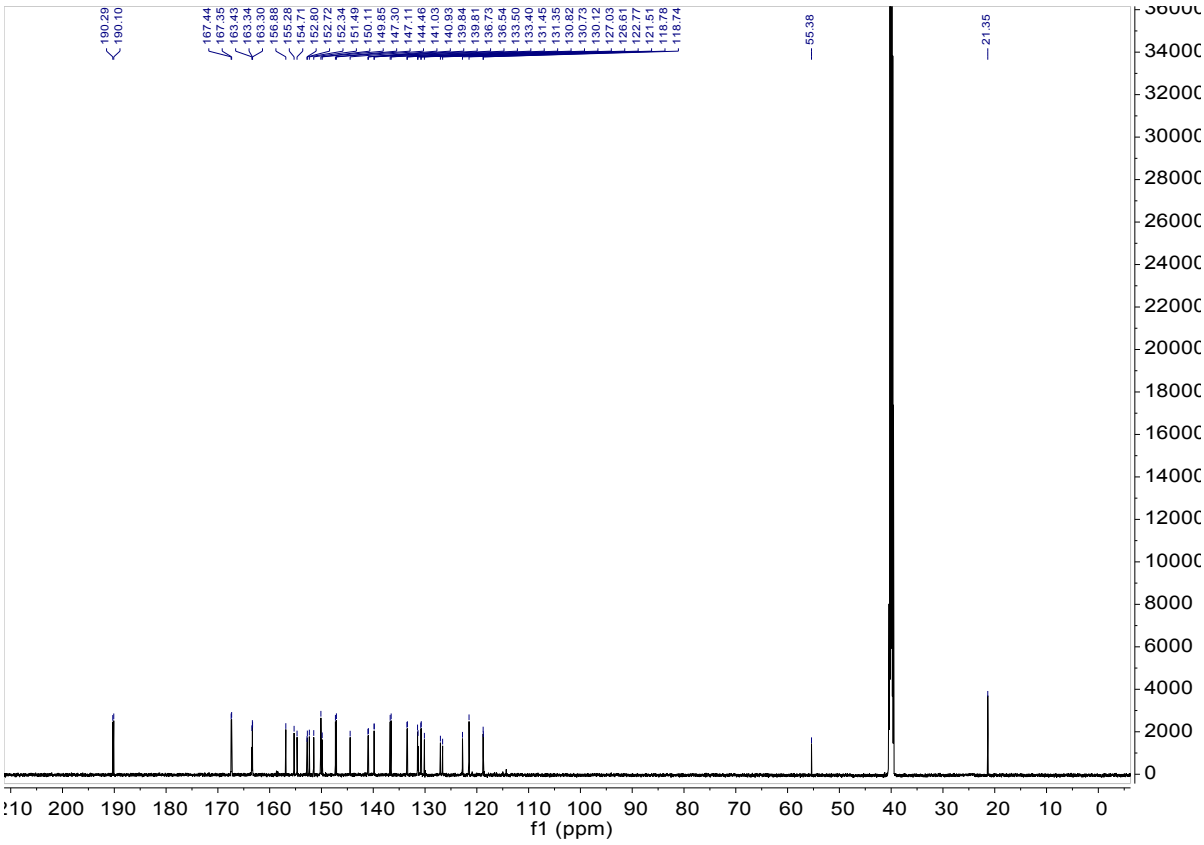


Figure S12. ^{13}C NMR of **Ir-CH**

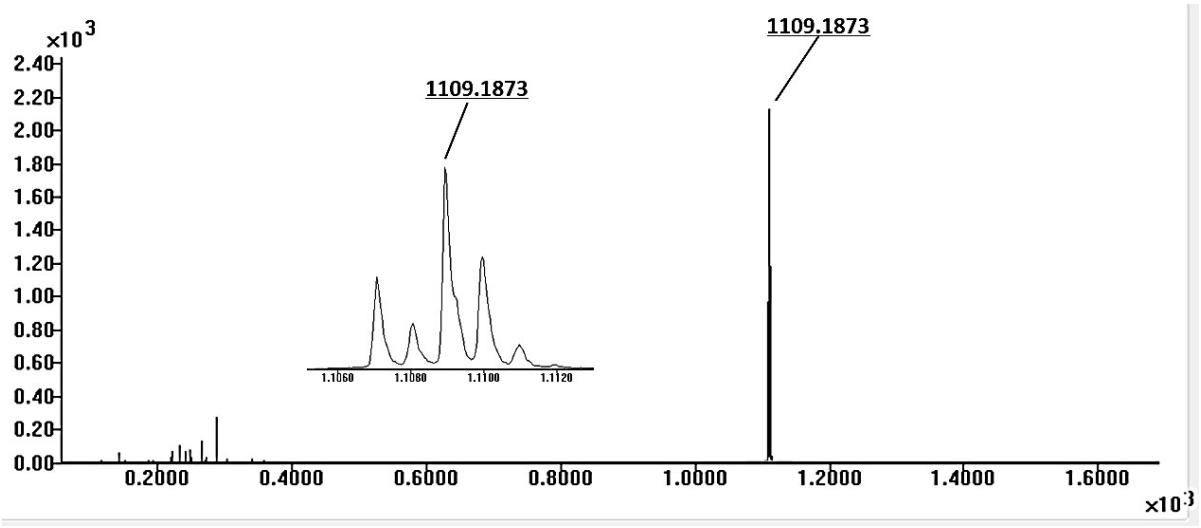


Figure S13. ESI-TOFMS of **Ir-NH**

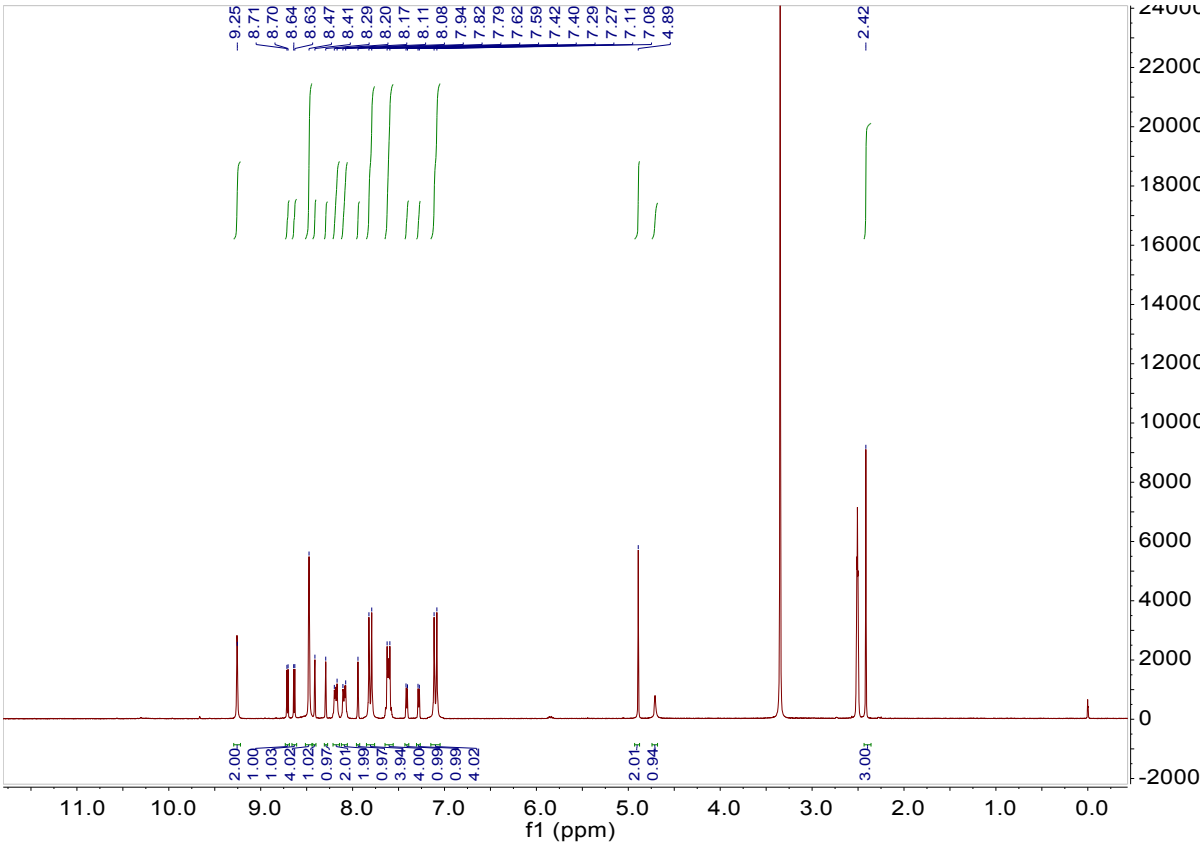


Figure S14. ^{1}H NMR of **Ir-NH**

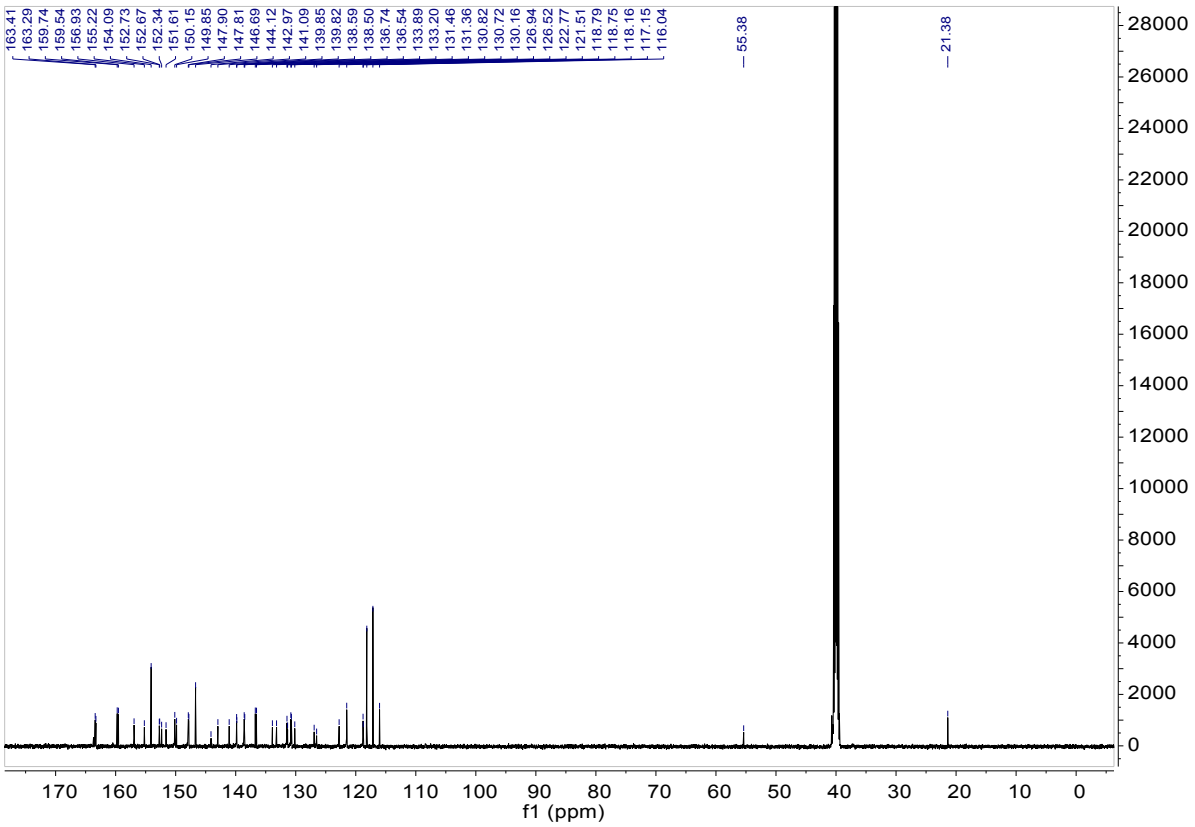


Figure S15. ^{13}C NMR of **Ir-NH**

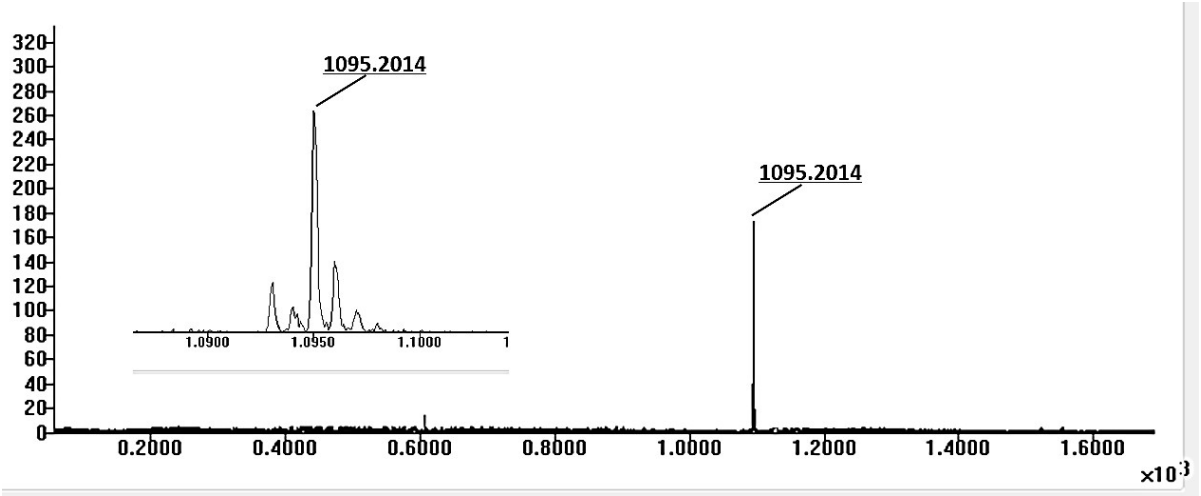


Figure S16. ESI-TOFMS of **Ir-CB**

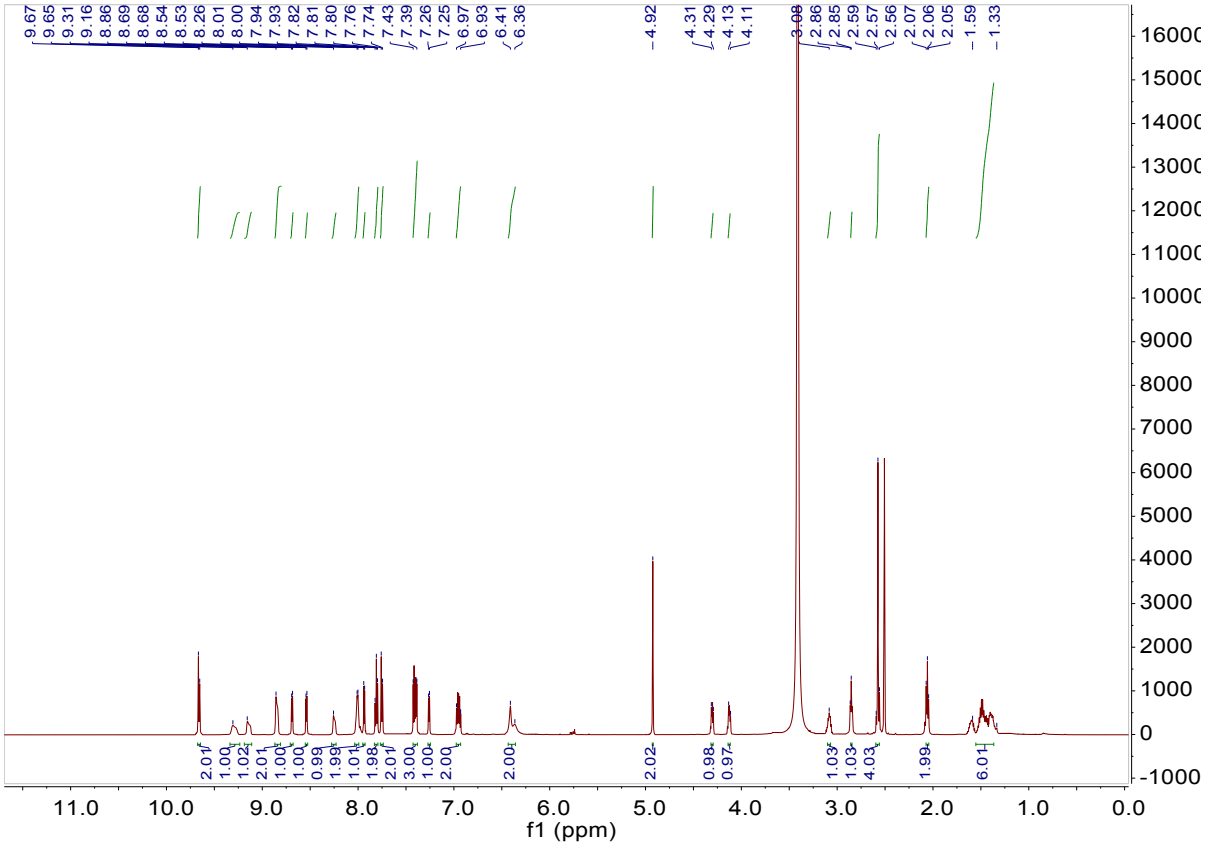


Figure S17. ^{1}H NMR of **Ir-CB**

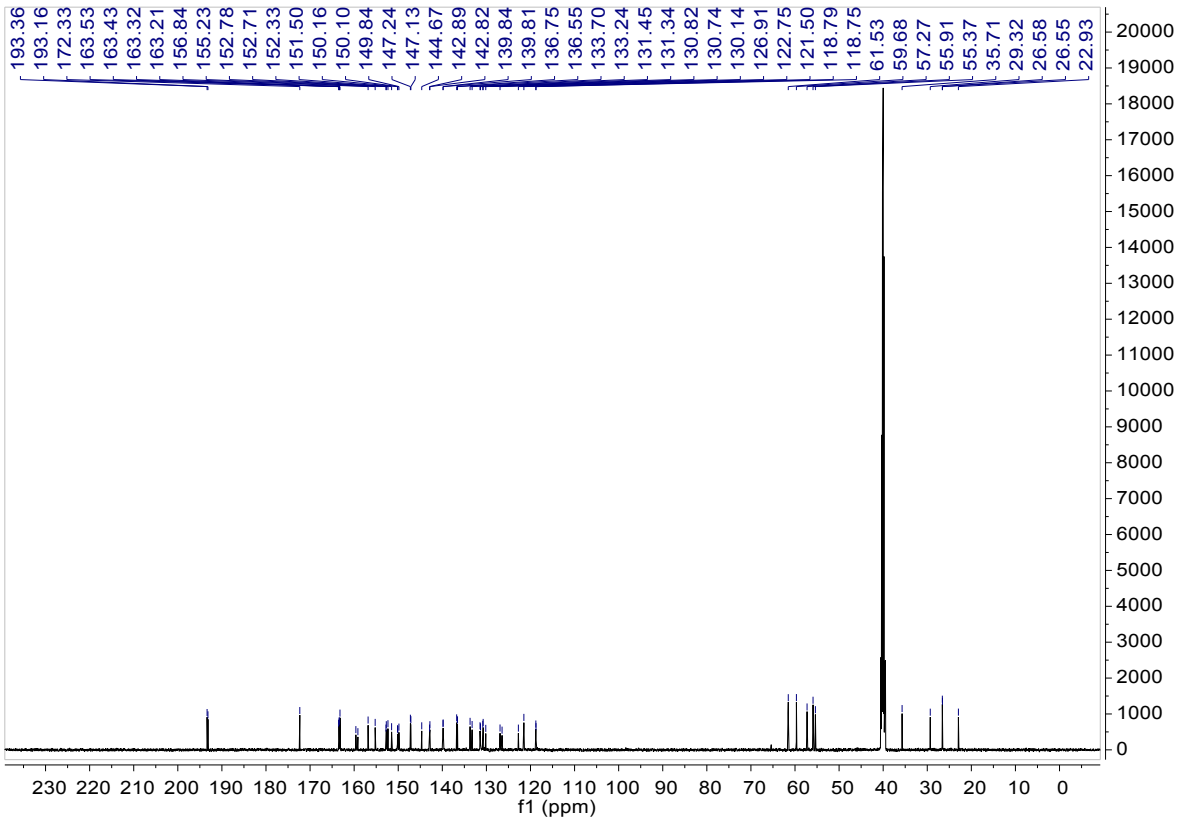


Figure S18.^{13}C NMR of **Ir-CB**

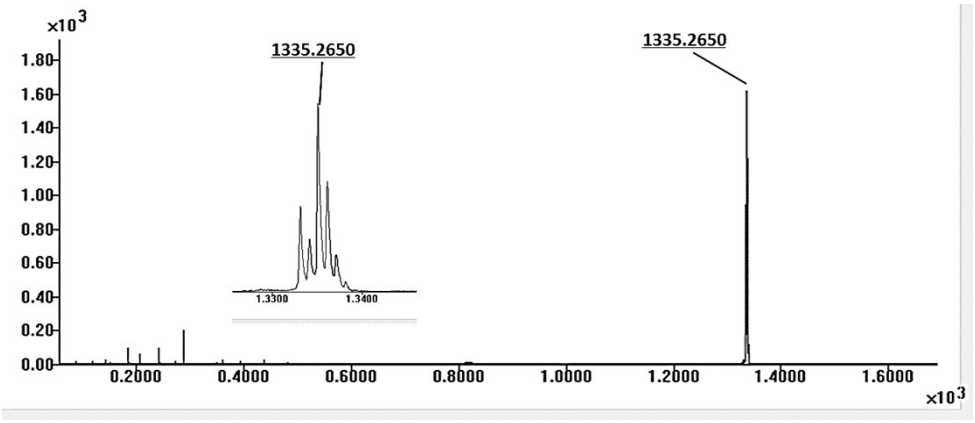


Figure S19.ESI-TOFMS of **Ir-NB**

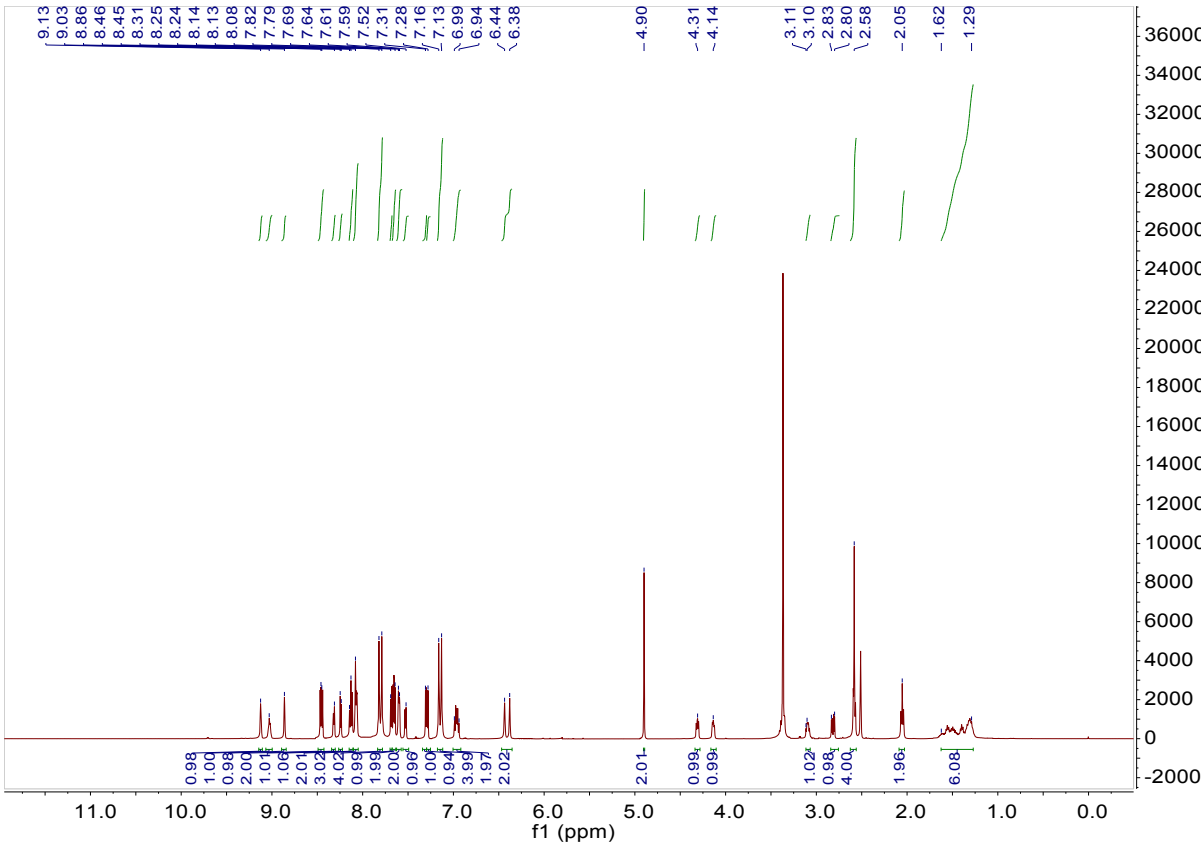


Figure S20.^{1}H NMR of **Ir-NB**

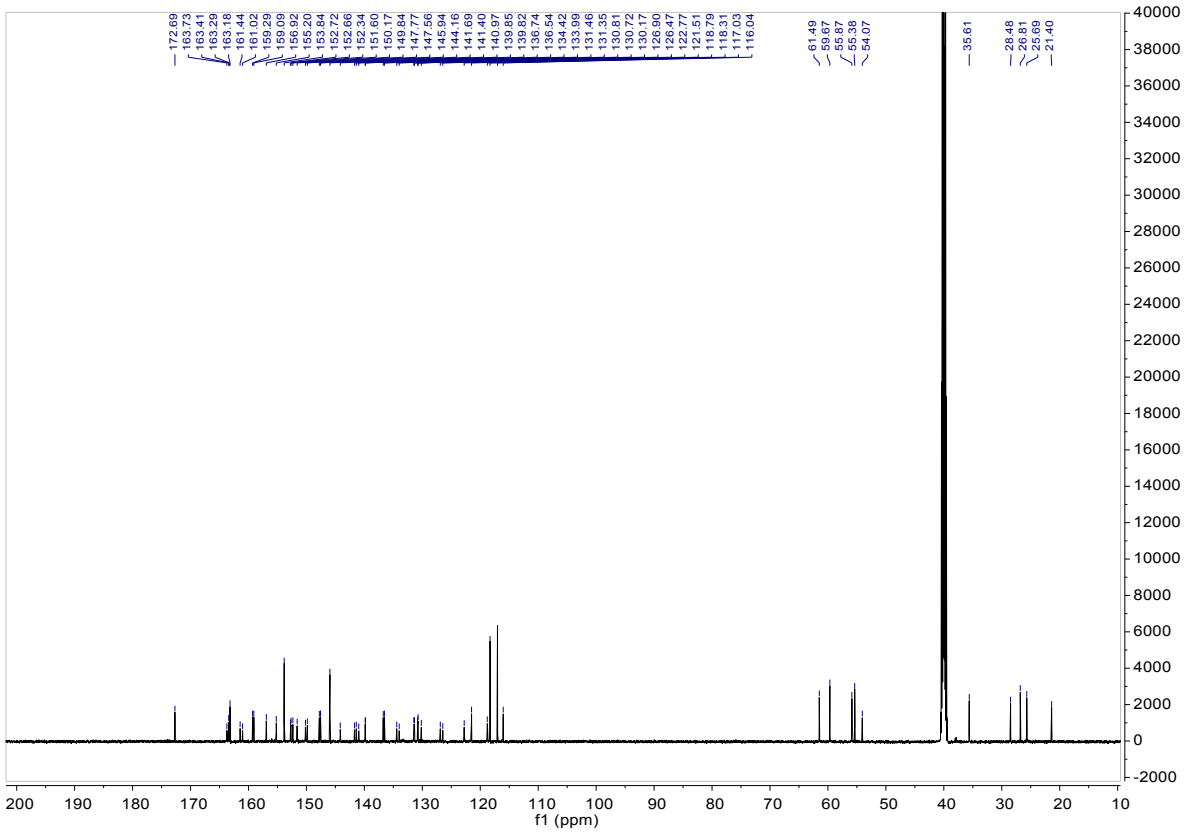


Figure S21. ^{13}C NMR of **Ir-NB**