Electronic Supplementary Information (ESI)

CX-5461-inspired monofunctional platinum RNA polymerase I selective inhibitors with selective lethality in BRCA1-deficient cancer cells

Zhen-Lei Zhang[a,1], Rui Rong[a,1], Xuan-Lin Ren[a], Ling-Wen Xu[a], Wen-Jing Lian[a], Xin Qiao[a,*], Jing-Yuan Xu[a,b,*]

[a] Department of Chemical Biology and Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University, Tianjin 300070, China.

[b] Key Laboratory of Immune Microenvironment and Disease of the Ministry of Education, Tianjin Medical University, Tianjin 300070, China.

[1] These authors contributed equally.

*Corresponding author:

E-mail: qiaoxin@tmu.edu.cn (X. Qiao), xujingyuan@tmu.edu.cn (J.-Y. Xu).

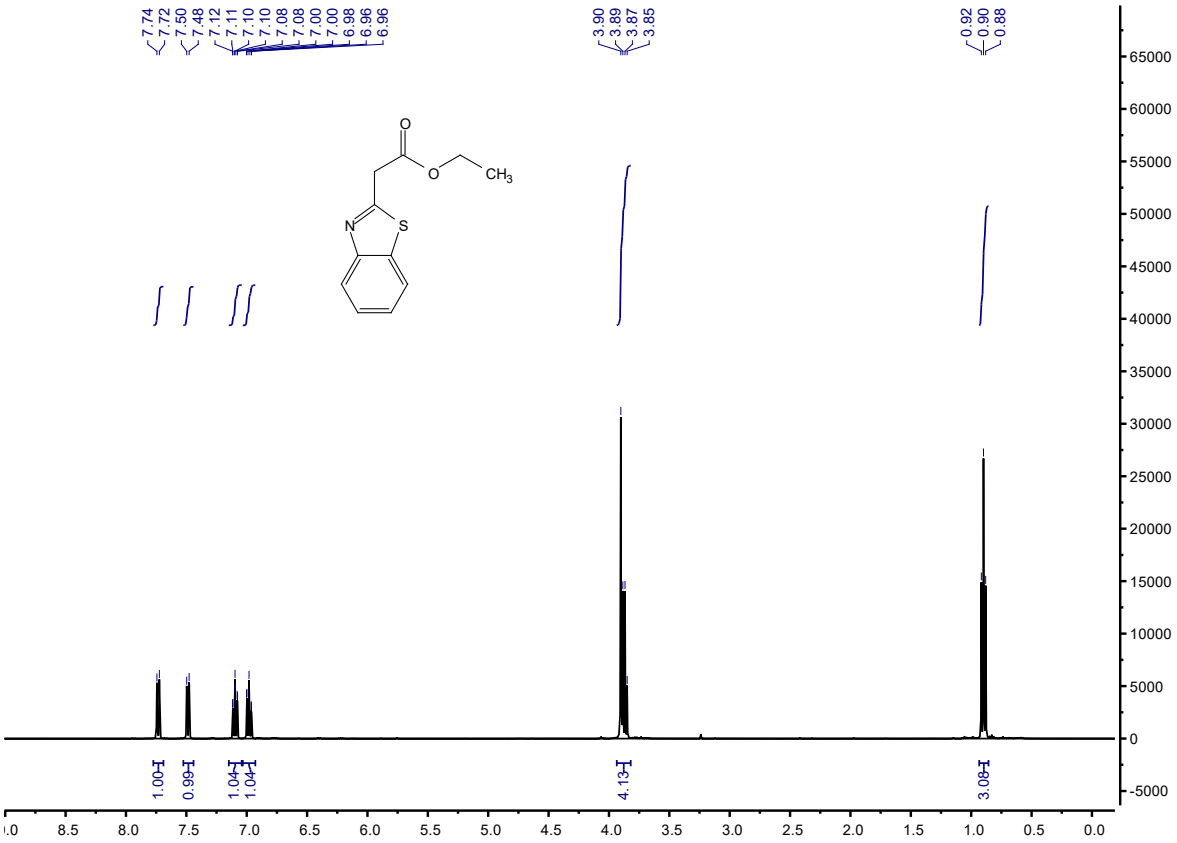


Figure S1. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

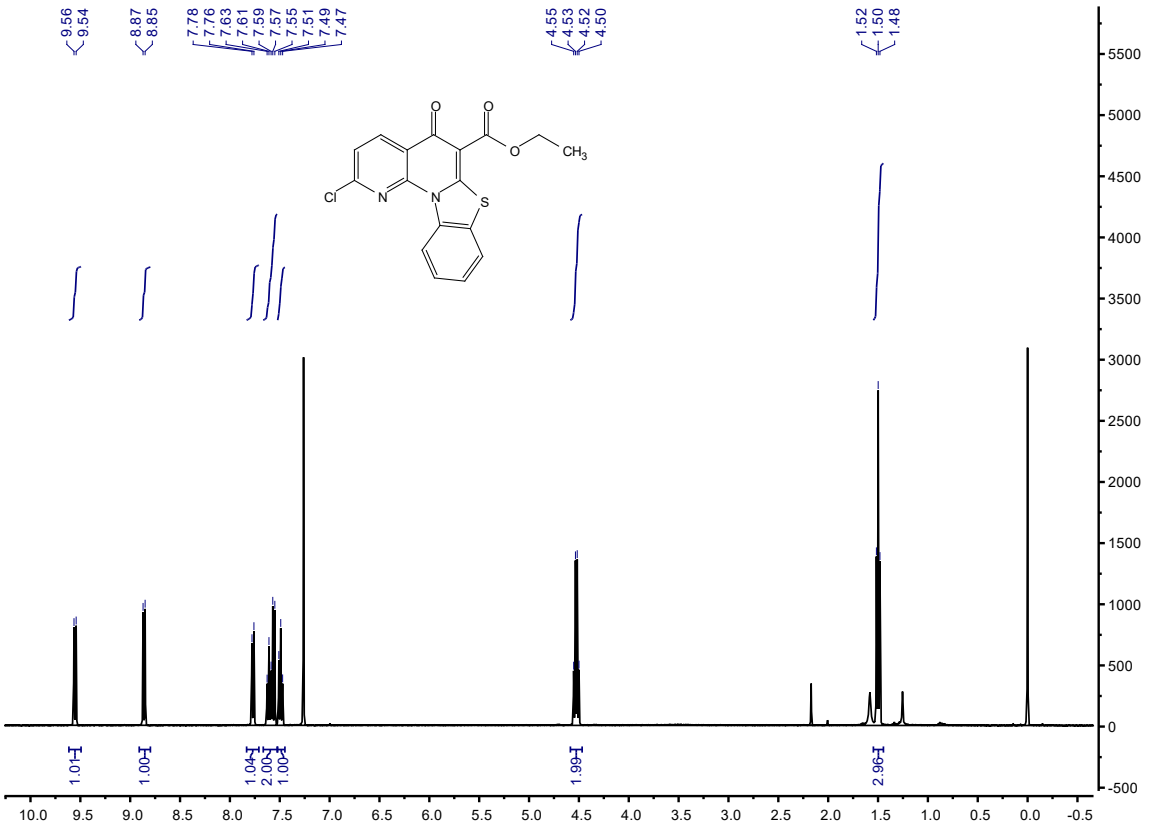


Figure S2. ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

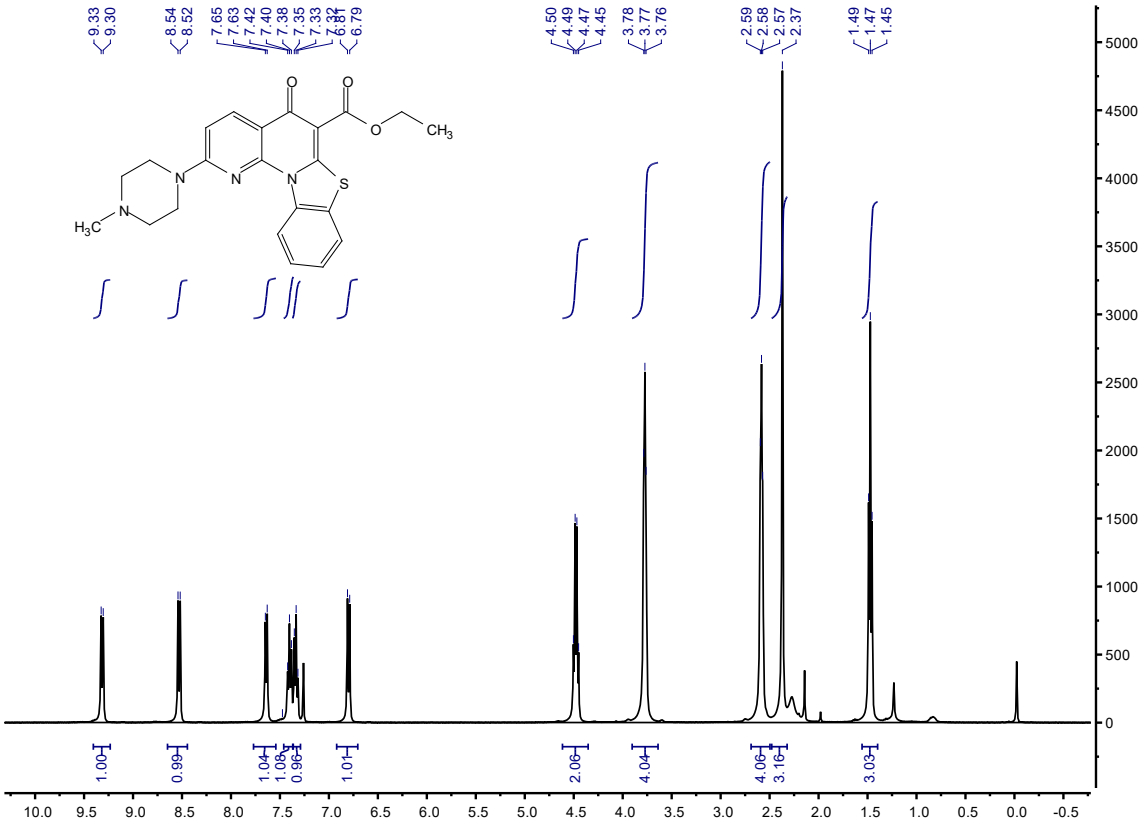


Figure S3. ^{1}H NMR spectrum of compound **4a** in $CDCl_3$.

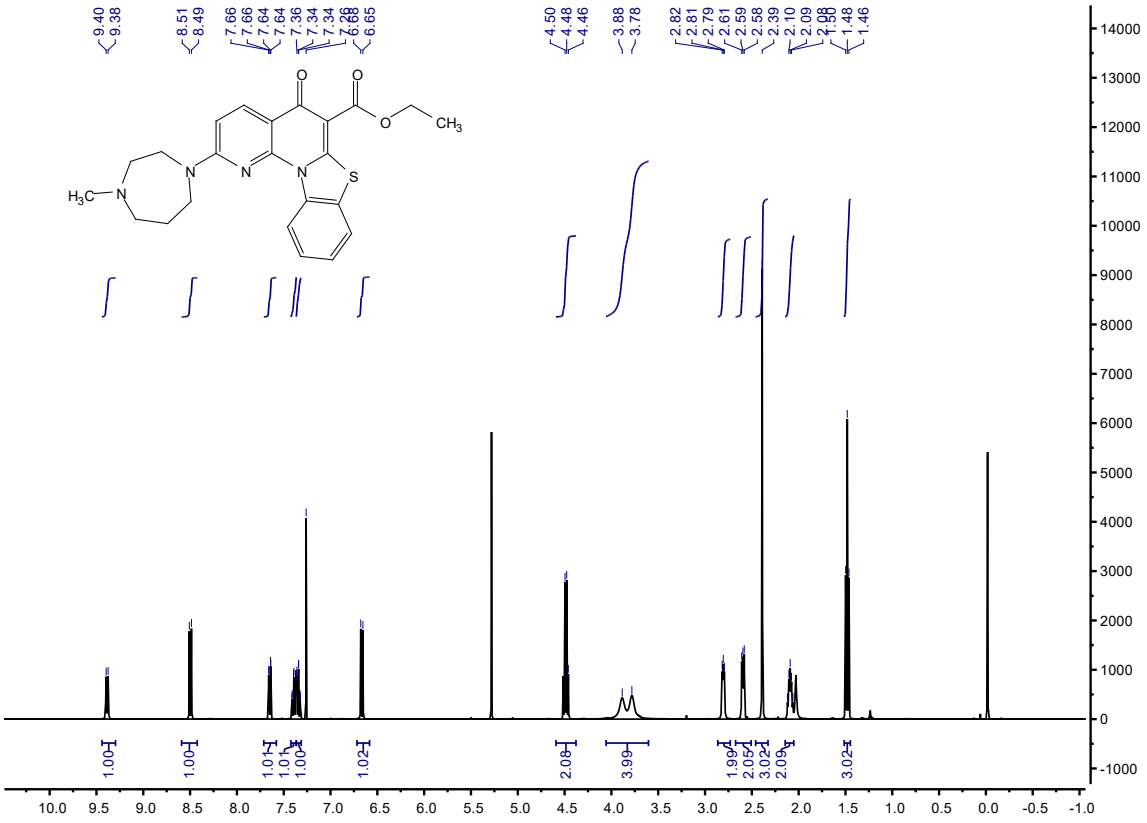


Figure S4. ^{1}H NMR spectrum of compound **4b** in $CDCl_3$.

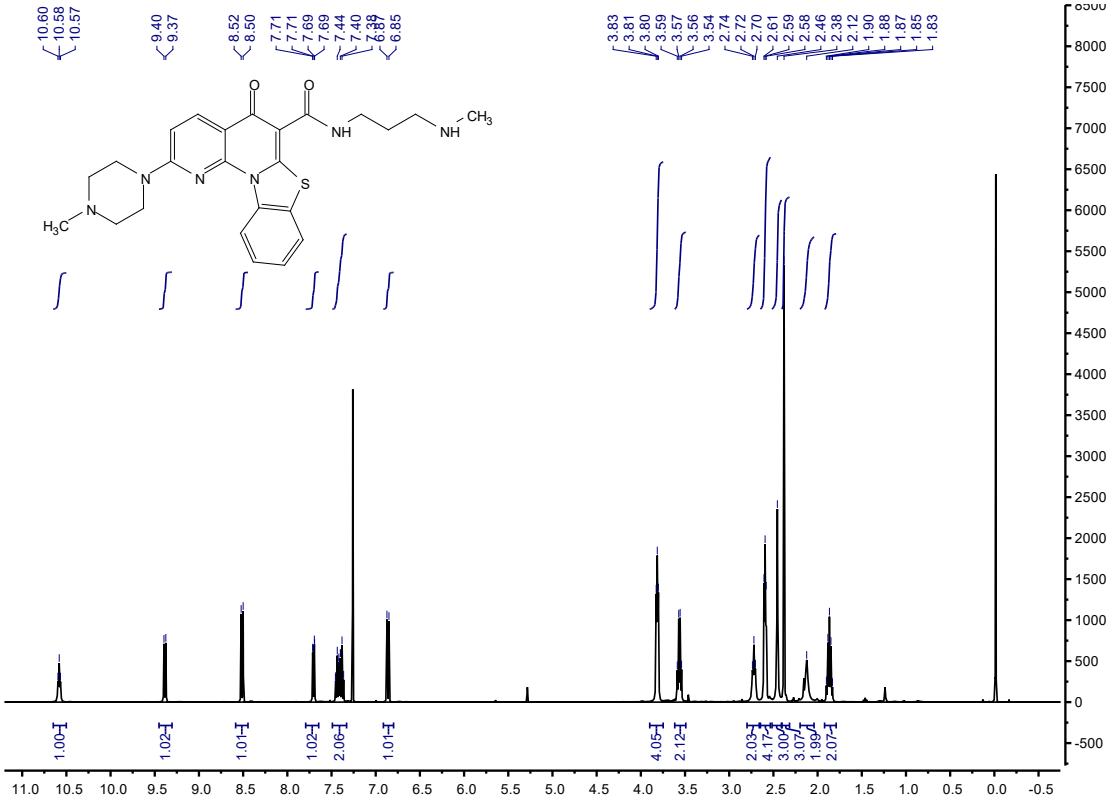


Figure S5. ^{1}H NMR spectrum of compound **Q1** in $CDCl_3$.

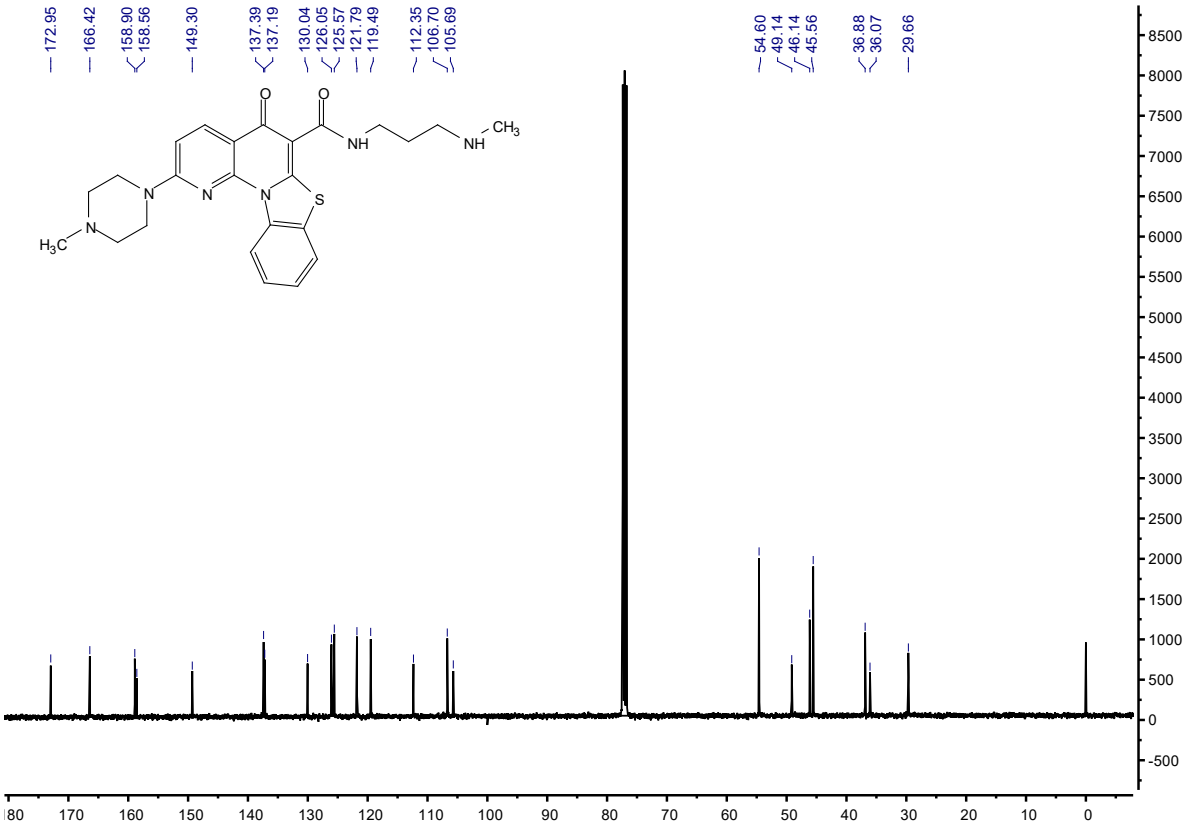
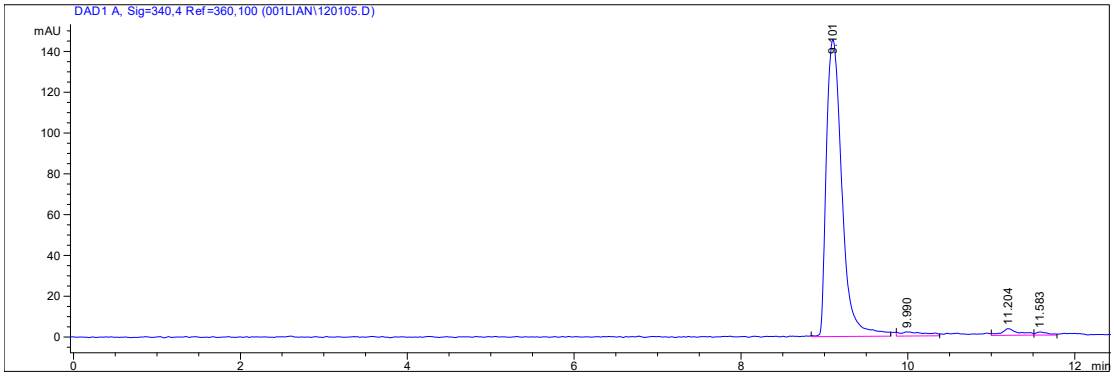


Figure S6. ^{13}C NMR spectrum of compound **Q1** in $CDCl_3$.



	Retention Time	Peak area	Concentration (%)
1	9.101	1887.3	95.504
2	9.99	46	1.304
3	11.204	48	2.405
4	11.583	15.7	0.787
Total		1997	100.000

Figure S7. Reverse-phase HPLC trace of compound **Q1**.

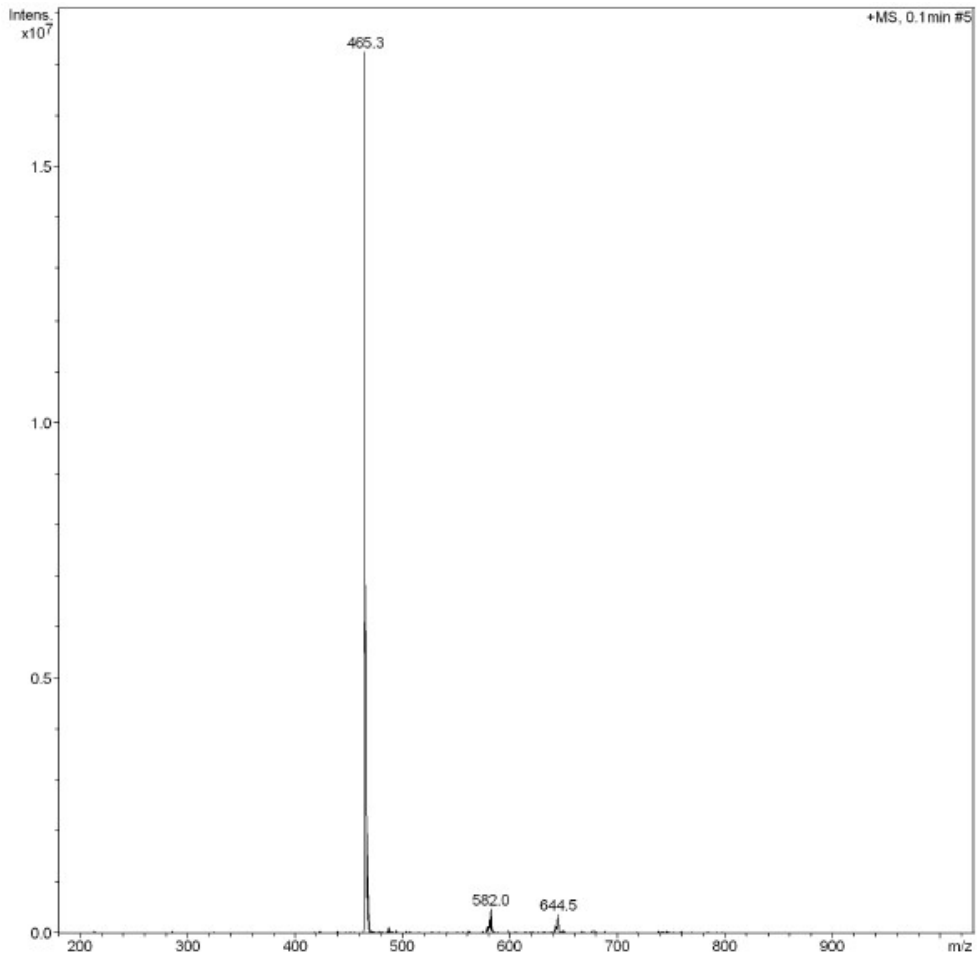


Figure S8. ESI-MS spectrum of **Q1** recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M+H]^+$: 465.2.

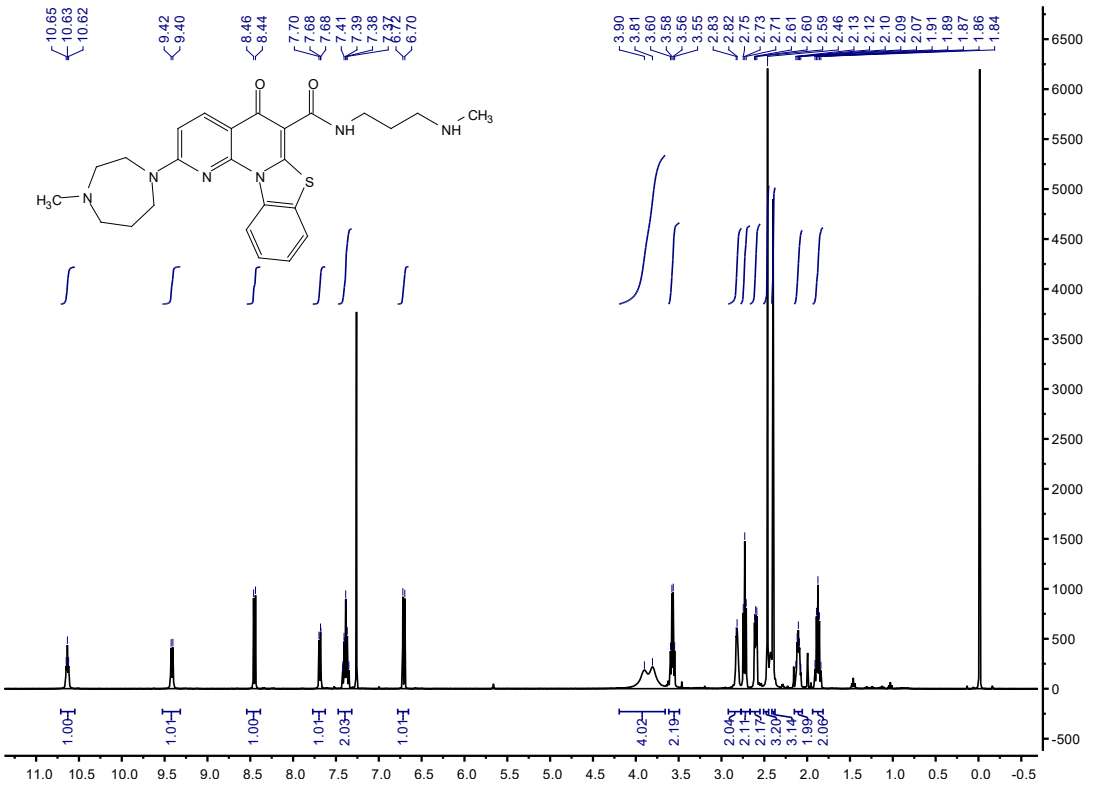


Figure S9. ^{1}H NMR spectrum of compound **Q2** in $CDCl_3$.

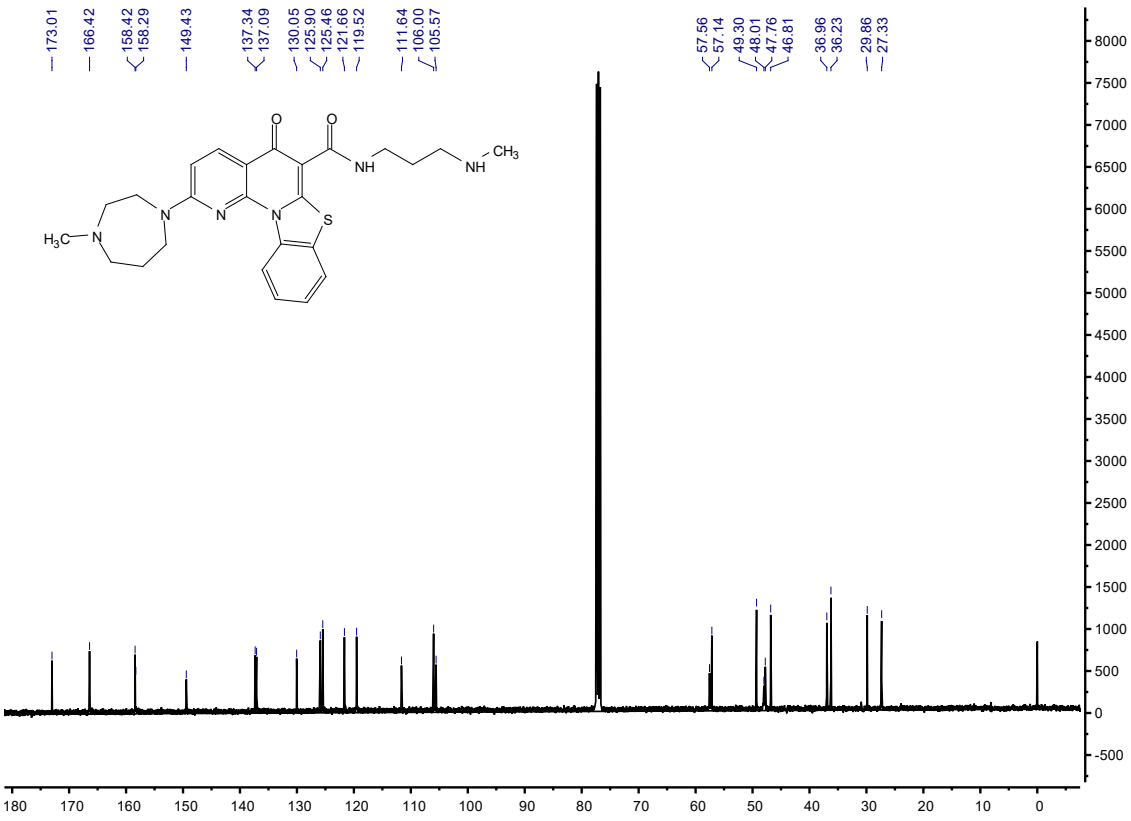
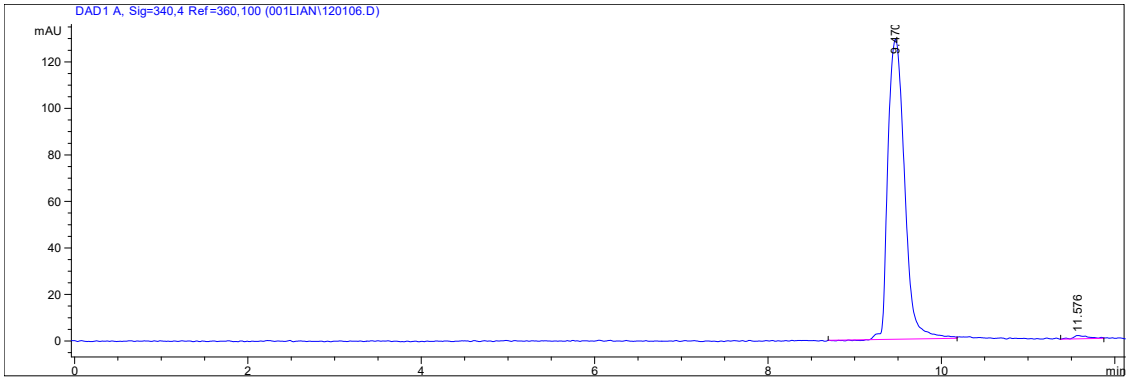


Figure S10. ^{13}C NMR spectrum of compound **Q2** in $CDCl_3$.



	Retention Time	Peak area	Concentration (%)
1	9.47	1716.5	98.999
2	11.576	17.4	1.001
Total		1733.9	100.000

Figure S11. Reverse-phase HPLC trace of compound **Q2**.

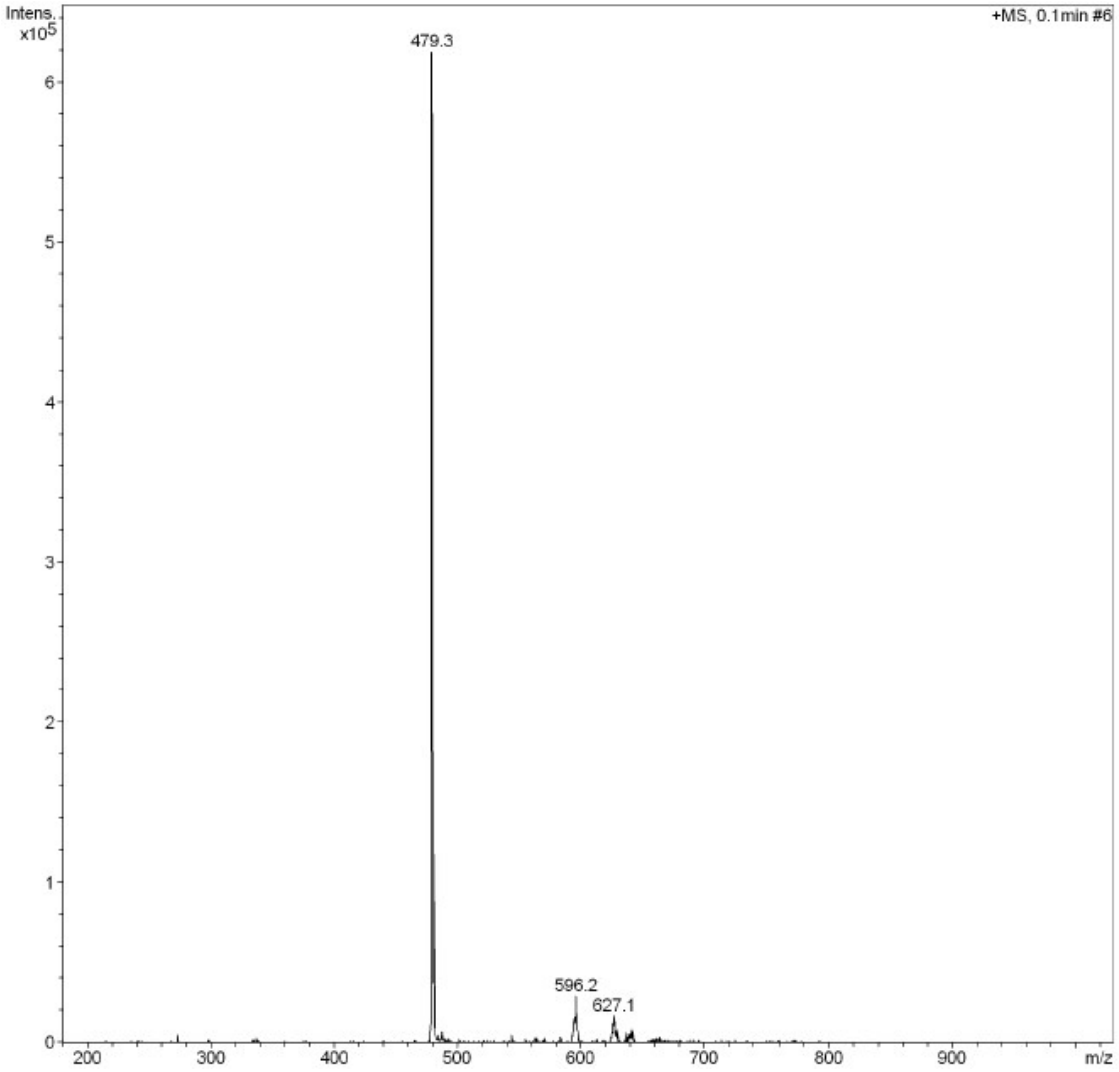


Figure S12. ESI-MS spectrum of **Q2** recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M+H]^+$: 479.2.

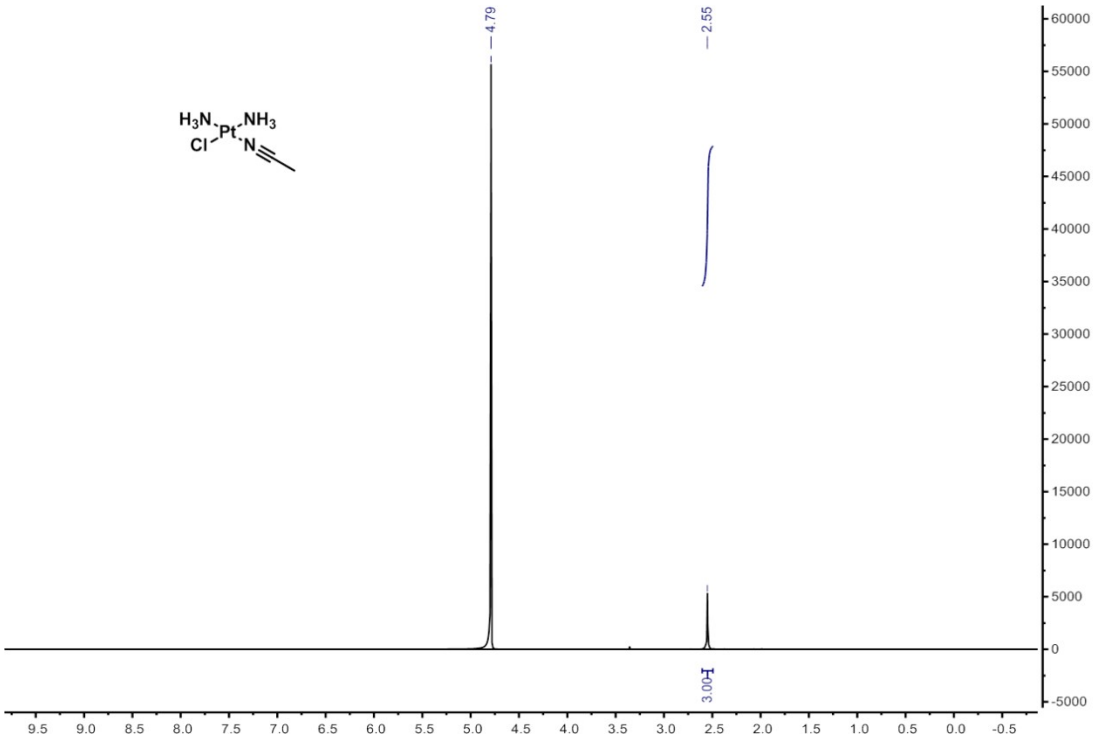


Figure S13. ^{1}H NMR spectrum of compound **P1** in D_2O.

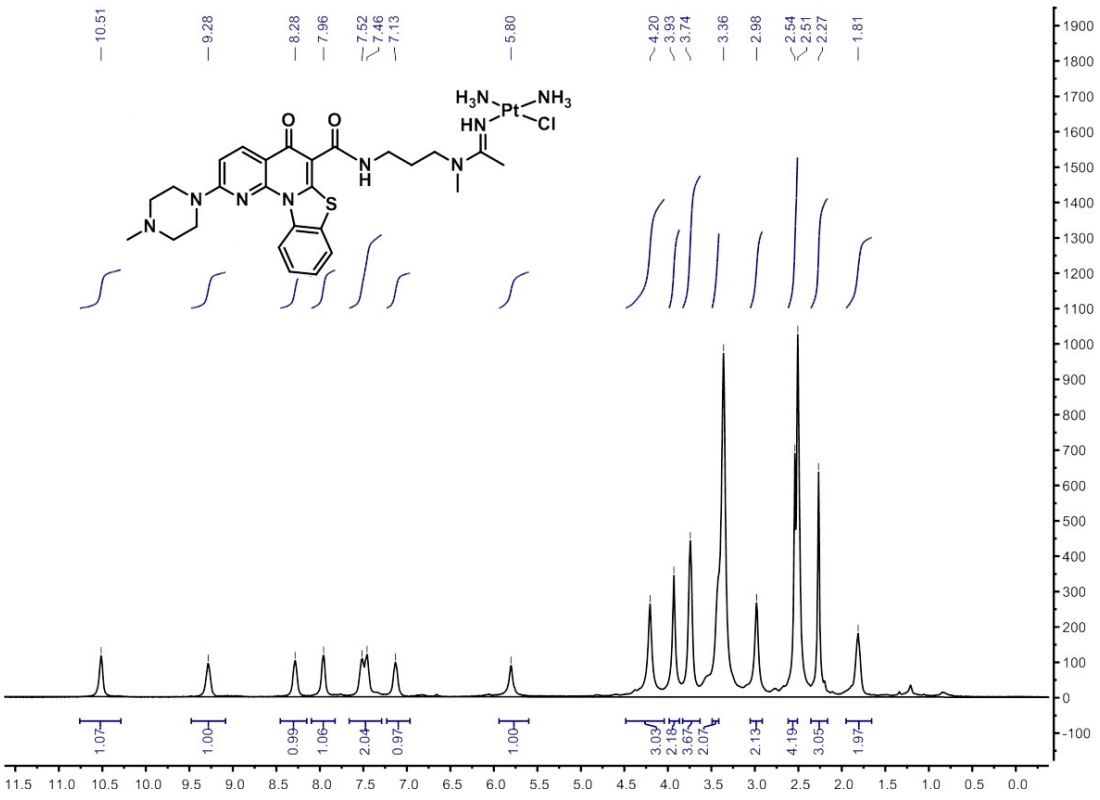


Figure S14. ^{1}H NMR spectrum of compound **P1-Q1** in DMSO-d_6.

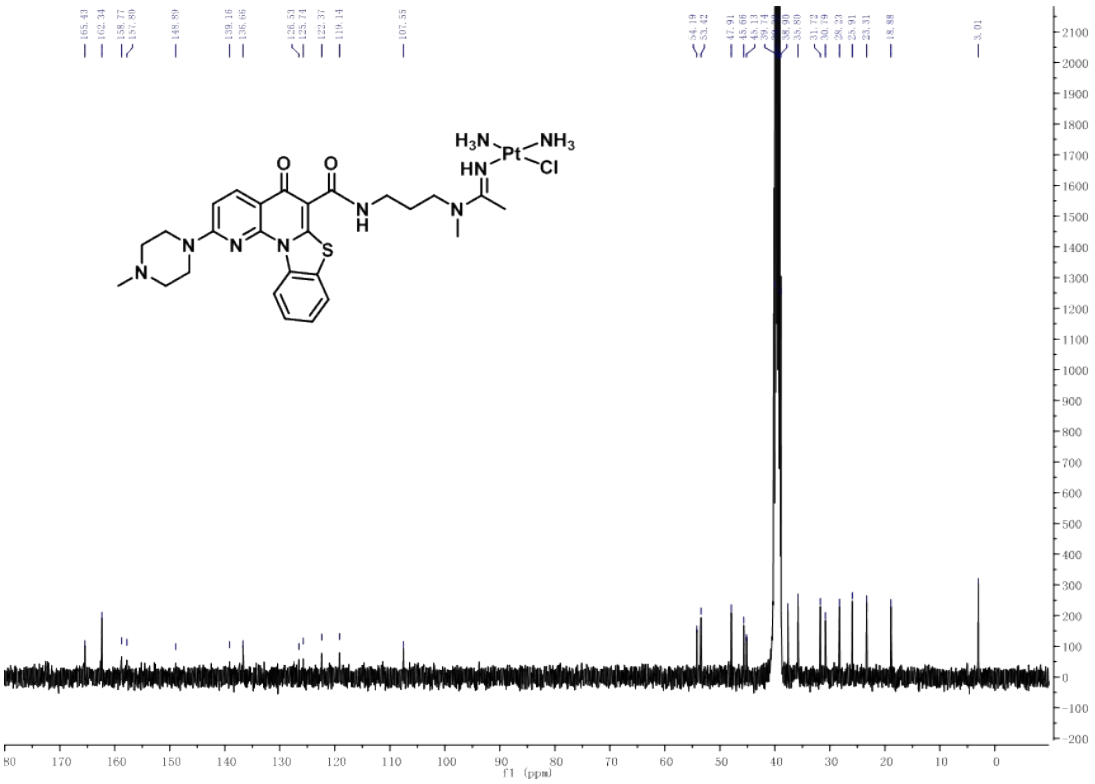
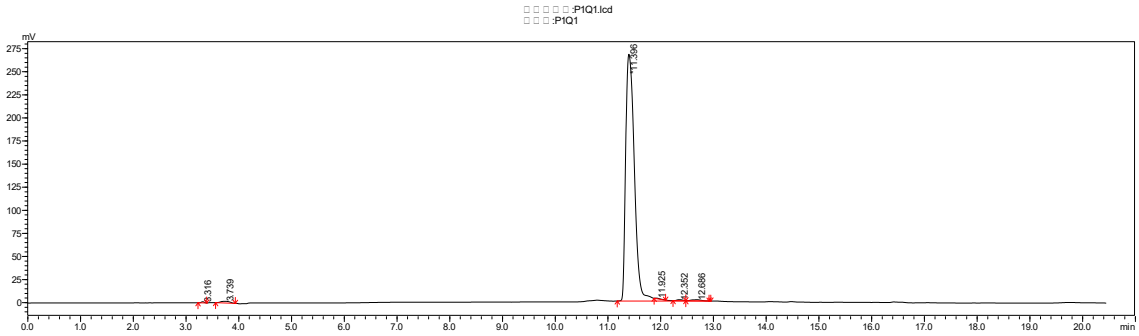
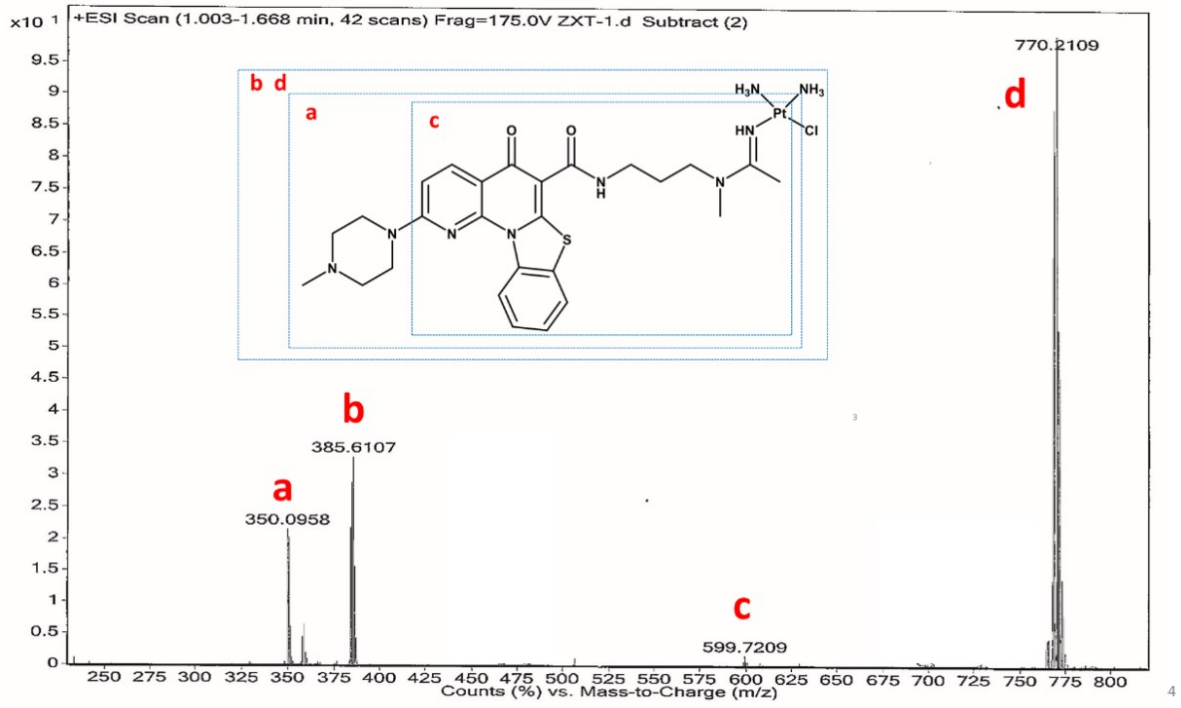


Figure S15. ^{13}C NMR spectrum of compound **P1-Q1** in DMSO-d_6.



	Retention Time	Peak area	Concentration (%)
1	3.316	4825	0.154
2	3.739	22550	0.718
3	11.396	3084313	98.237
4	11.925	3519	0.112
5	12.352	8709	0.277
6	12.686	15743	0.501
Total		3139659	100.000

Figure S16. Reverse-phase HPLC trace of compound **P1-Q1**.



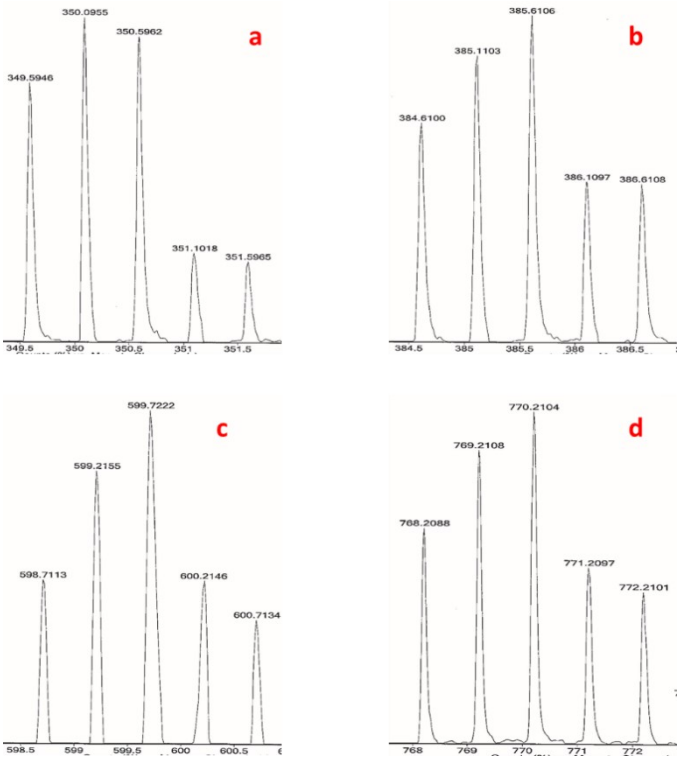


Figure S17. High-resolution ESI-MS spectrum of **P1-Q1** recorded in positive-ion mode. Characteristic molecular and fragment ions: $[M]^+$: 770.2109.

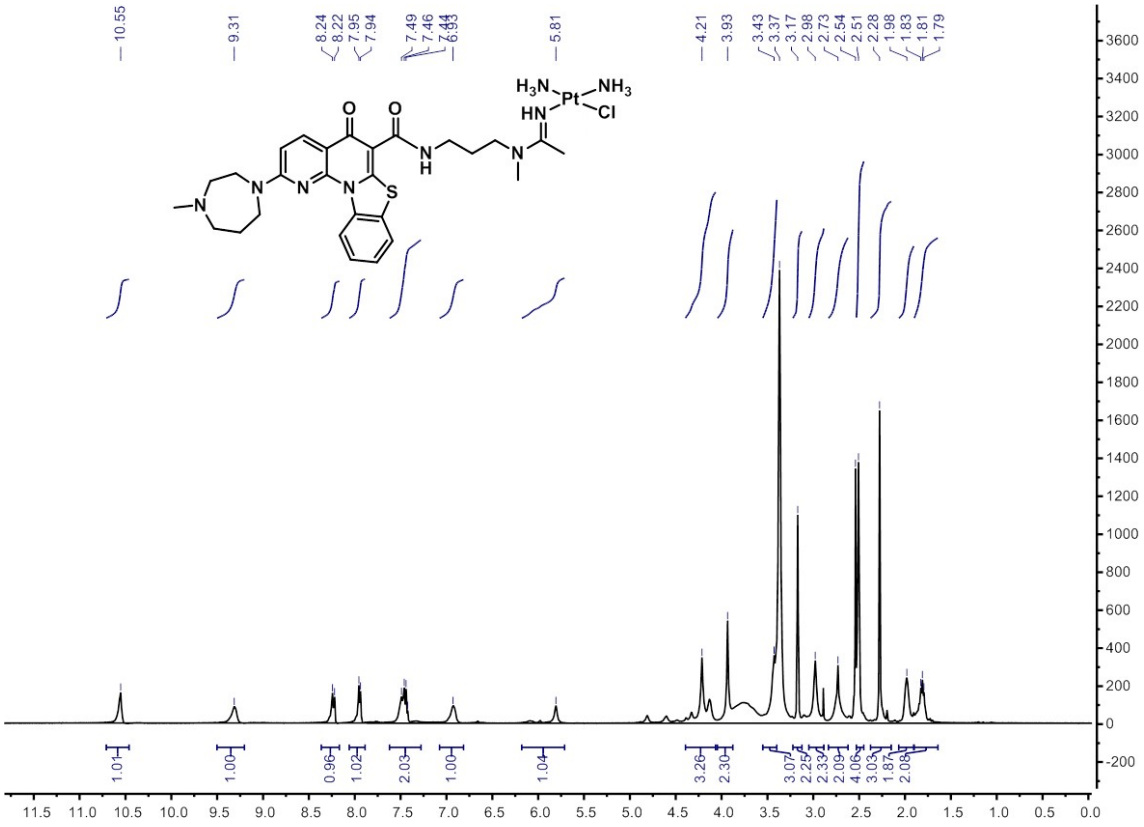


Figure S18. ^{1}H NMR spectrum of compound **P1-Q2** in DMSO-d_6.

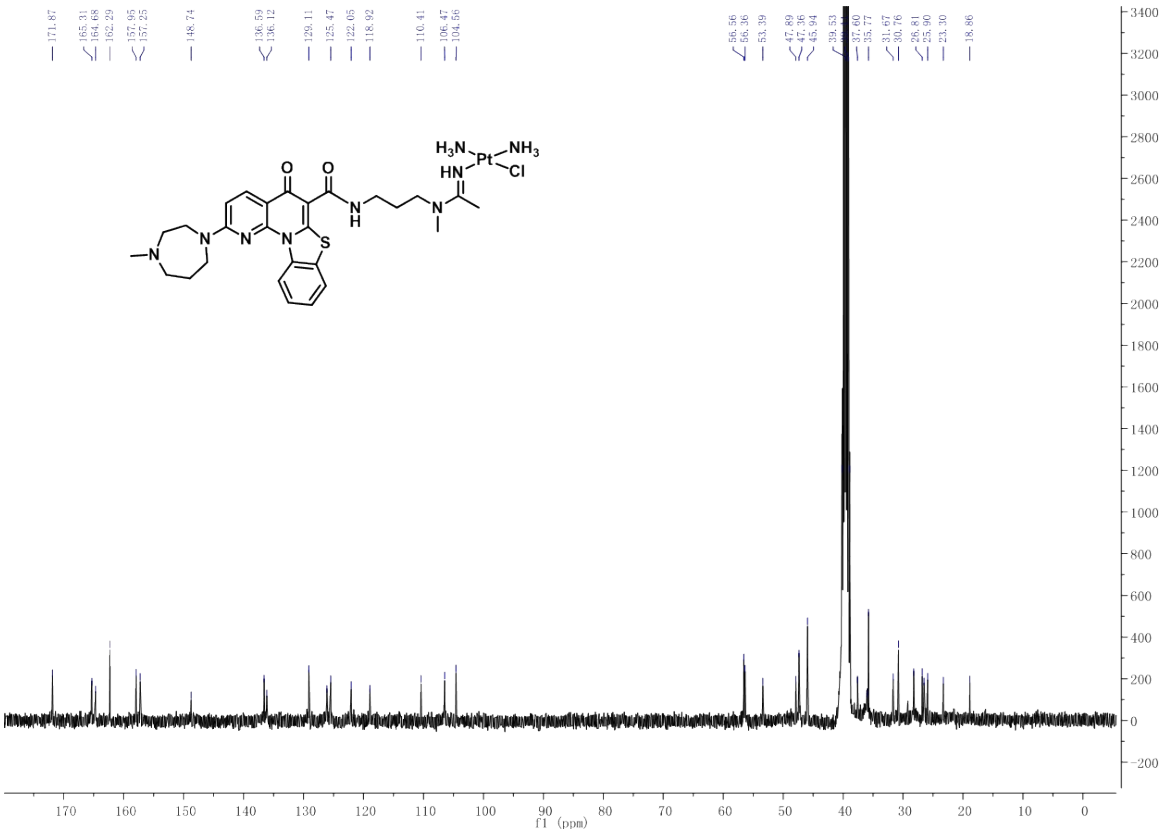
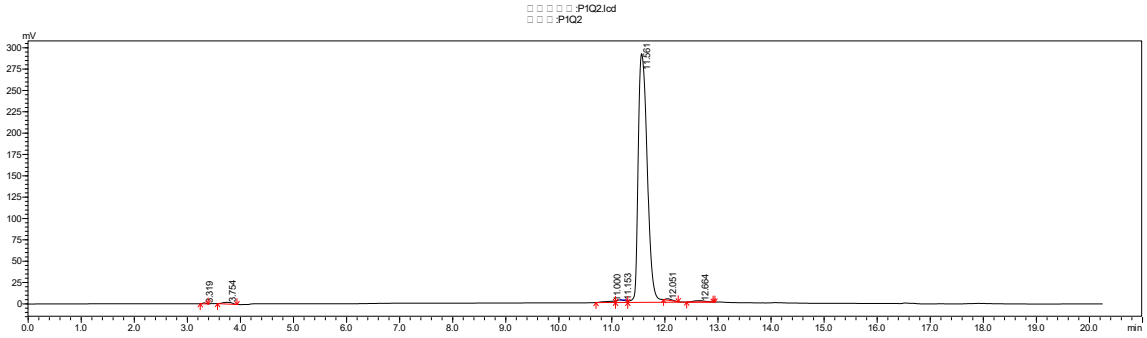


Figure S19. ^{13}C NMR spectrum of compound **P1-Q2** in DMSO-d_6.



	Retention Time	Peak area	Concentration (%)
1	3.319	4187	0.116
2	3.754	23872	0.662
3	11.000	21122	0.586
4	11.153	32261	0.895
5	11.561	3487863	96.737
6	12.051	12306	0.341
7	12.664	23916	0.663
Total		3605527	100.000

Figure S20. Reverse-phase HPLC trace of compound **P1-Q2**.

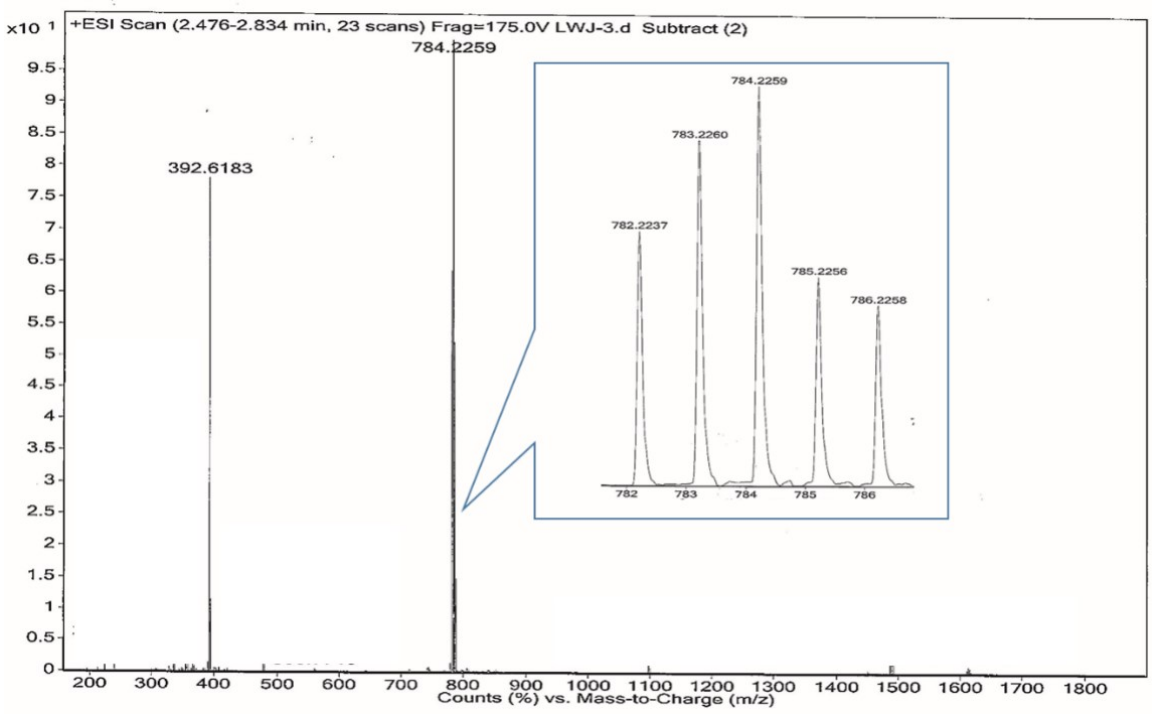


Figure S21. High-resolution ESI-MS spectrum of **P1-Q2** recorded in positive-ion mode. Characteristic molecular and fragment ions: ($[M + H]^{2+}$): 784.2259.

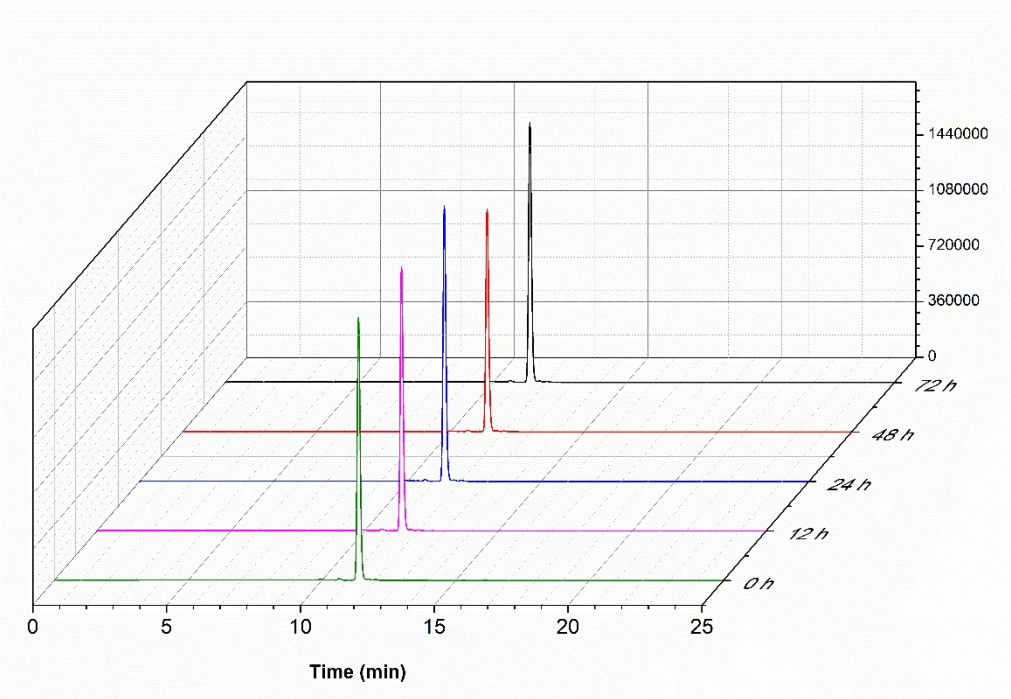


Figure S22. Stability of **P1-Q1** in PBS.

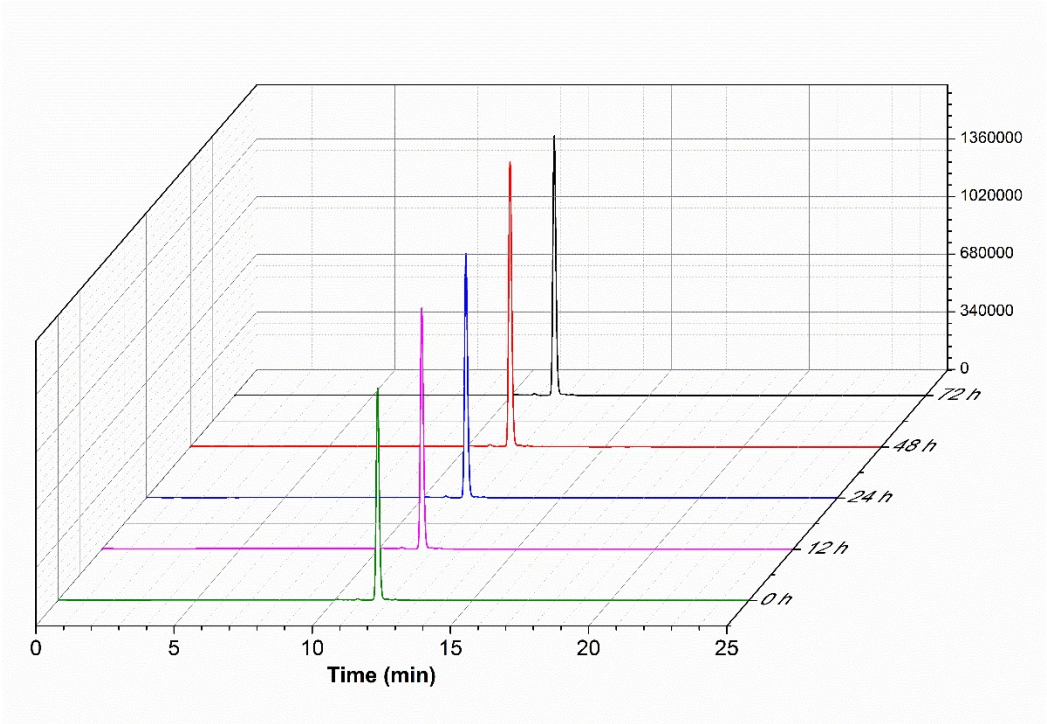


Figure S23. Stability of **P1-Q2** in PBS.

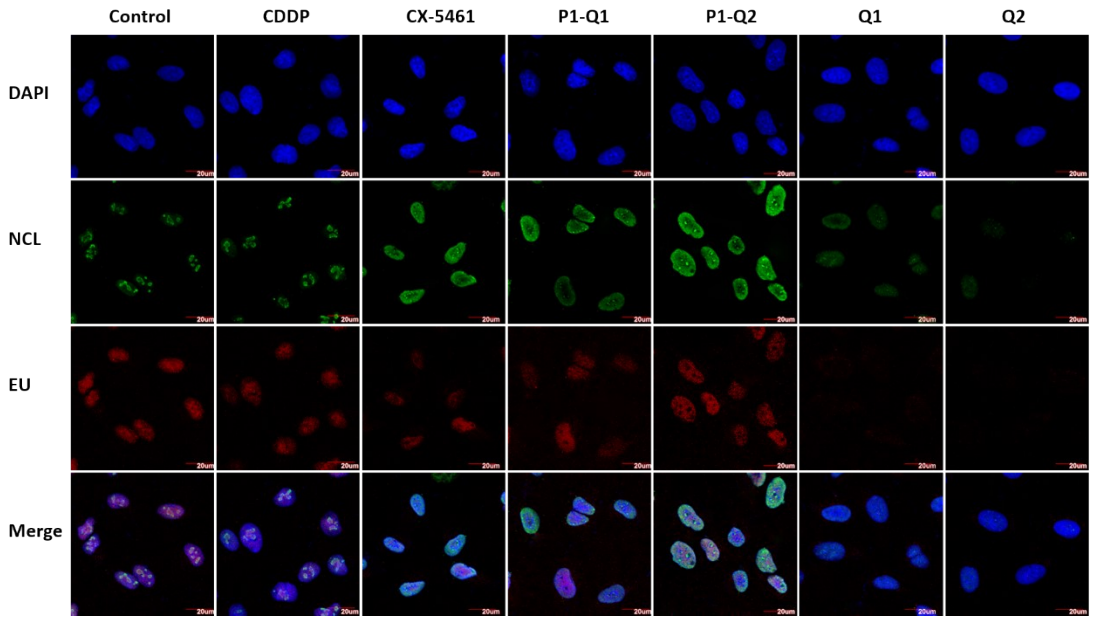


Figure S24. Representative cell images of global transcription in A549 cells. After 1 h EU incorporation, A549 cells were treated with CX-5461, cisplatin, **P1-Q1**, **P1-Q2** , **Q1**, or **Q2** at 10 μM for 4 h, respectively. Nucleolar protein NCL was stained by immunofluorescence detection. DAPI was used to label the nucleus.

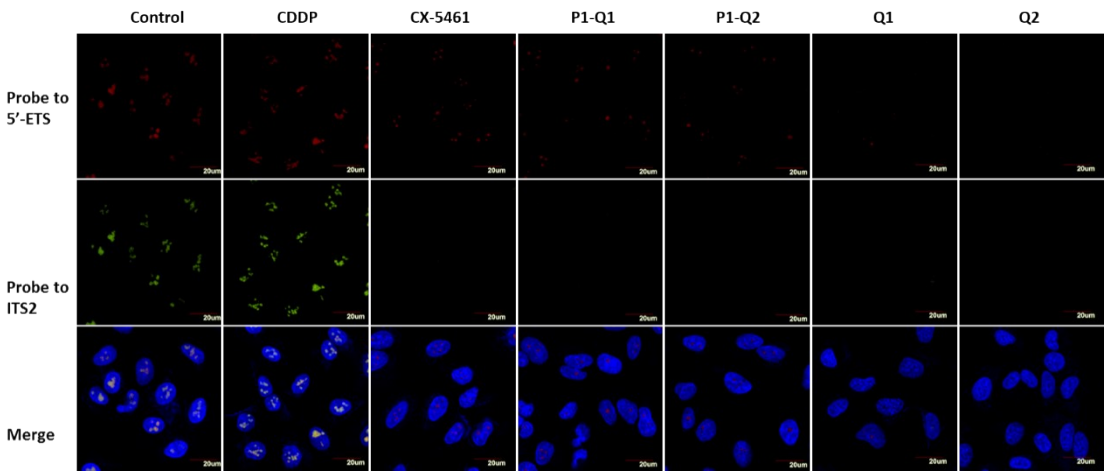


Figure S25. Disruption of 47S pre-rRNA expression in A549 cells detected by fluorescent *in situ* hybridization (FISH). Probes: 5'-ETS tagged by Cy5 (red), ITS2 tagged by Cy3 (green). Treatment conditions: cisplatin, CX-5461, **P1-Q1**, **P1-Q2** , **Q1**, or **Q2** at 10 μM for 3 h, respectively. DAPI was used to label the nucleus.

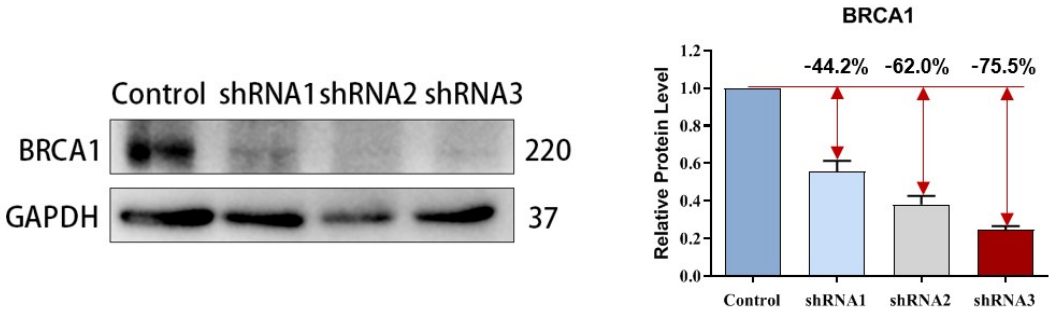


Figure S26. Western blot image shows knockdown of BRCA1 in A549 cells. Three independent lentiviral shRNAs against human BRCA1 were established and infected to A549 cells. The BRCA1 knockdown efficiency was verified by western blot. According to the western blot results, A549 cells that stably expressed lentiviral shRNA3 (against BRCA1) were selected as the model for mechanism studies.

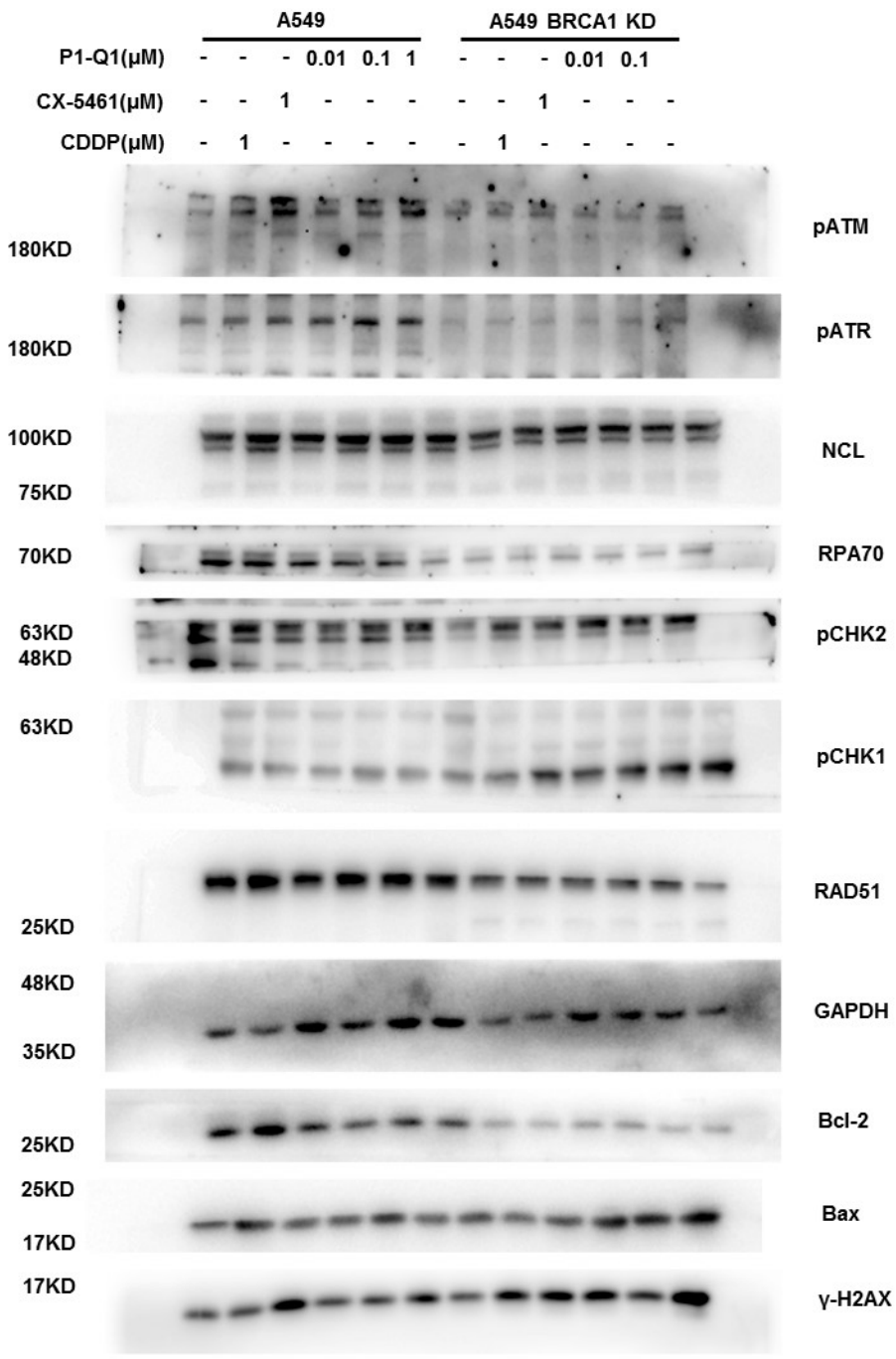


Figure S27. Un-cropped western blotting images of Figure 8.

Table S1. shRNA sequence information

	Forward (5'-3')	**Reverse (5'-3')**
shRNA1	CCGGCCCTTCTAAATGCCCATCA TTCTCGAGAATGATGGGCATTTA GAAGGGTTTTTG	AATTCAAAAACCCTTCTAAATGCCC ATCATTCTCGAGAATGATGGGCATT TAGAAGGG
shRNA2	CCGGGAGAATCCTAGAGATACT GAACTCGAGTTCAGTATCTCTAG GATTCTCTTTTTG	AATTCAAAAAGAGAATCCTAGAGA TACTGAACTCGAGTTCAGTATCTCT AGGATTCTC
shRNA3	CCGGGCCTACAAGAAAGTACGA GATCTCGAGATCTCGTACTTTCT TGTAGGCTTTTTG	AATTCAAAAAGCCTACAAGAAAGT ACGAGATCTCGAGATCTCGTACTTT CTTGTAGGC