Supplementary Information

Novel PROTAC Probes Targeting KDM3 Degradation to Eliminate Colorectal Cancer Stem Cells through Inhibition of Wnt/β-catenin Signaling

Shadid U. Zaman[1†], Piyusha P. Pagare[1†], Hongguang Ma[1], Rosalie G. Hoyle[1], Yan Zhang[1*], Jiong Li[1,2, 3*]

1. Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia 23298-0540, United States

2. Department of Oral and Craniofacial Molecular Biology, Virginia Commonwealth University, Richmond, Virginia 23298-0540, United States

3. Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia 23298-0540, United States

† These authors contributed equally

***Corresponding Authors:**

Jiong Li, Ph.D.

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia 23298-0540, United States

Email: jli29@vcu.edu

Yang Zhang, Ph.D.

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia 23298-0540, United States

Email: yzhang2@vcu.edu

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Table S1. The primers used for RT-qPCR.

Primer Name	Directions	Sequence
GAPDH	Forward	TCATTGACCTCAACTACATG
	Reversed	TCGCTCCTGGAAGATGGTGAT
AXIN2	Forward	CTGGCTTTGGTGAACTGTTG
	Reversed	AGTTGCTCACAGCCAAGACA
CCND1	Forward	CAATGACCCCGCACGATTTC
	Reversed	CATGGAGGGCGGATTGGAA
DKK1	Forward	TGCGTCACGCTATGTGCTG
	Reversed	CCATCCAAGGTGCTATGATC
ASCL2	Forward	GGCACCAACACTTGGAGATT
	Reversed	CCAGGTCAAGGGTTCTTTGT
RNF43	Forward	CATCAGCATCGTCAAGCTGGA
	Reversed	TTACCCCAGATCAACACCACT
ZNRF3	Forward	TCCGACTGTGCCATCTGTCTGGAGAA
	Reversed	CCCTTTTGTTCTATGATGTTGTGCCG
LGR5	Forward	CACCTCCTACCTAGACCTCAG
	Reversed	CGCAAGACGTAACTCCTCCAG
KDM3A	Forward	ACCTGCAGTTATTCTTCAGC
	Reversed	TAATGCCAGTCCTATGCCAT
KDM3B	Forward	TGTTCCCTGGGGACTCCTCT
	Reversed	GGGCACTACAGTACAGCTGG
KDM4A	Forward	CCTCACTGCGCTGTCTGTAT

	Reversed	CCAGTCGAAGTGAAGCACAT
KDM4B	Forward	ACTTCAACAAATACGTGGCCTAC
	Reversed	CGATGTCATCATACGTCTGCC
KDM4C	Forward	GATGAATGGAACATAGCTCGCC
	Reversed	GGTGTGCCATGCAAACGTG

Figure S1

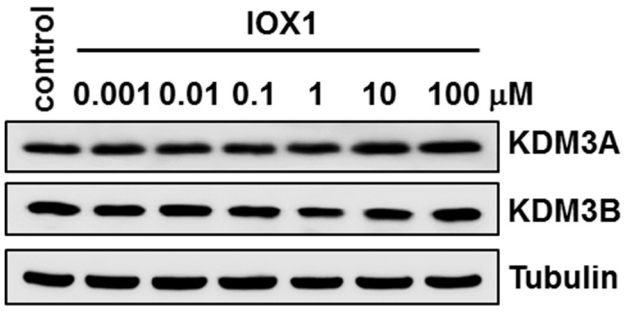


Figure S1. IOX1 could not induce KDM3A and KDM3B degradation in SW490 cells. SW480 cell were treated with IOX1 as indicated for 16 hours.

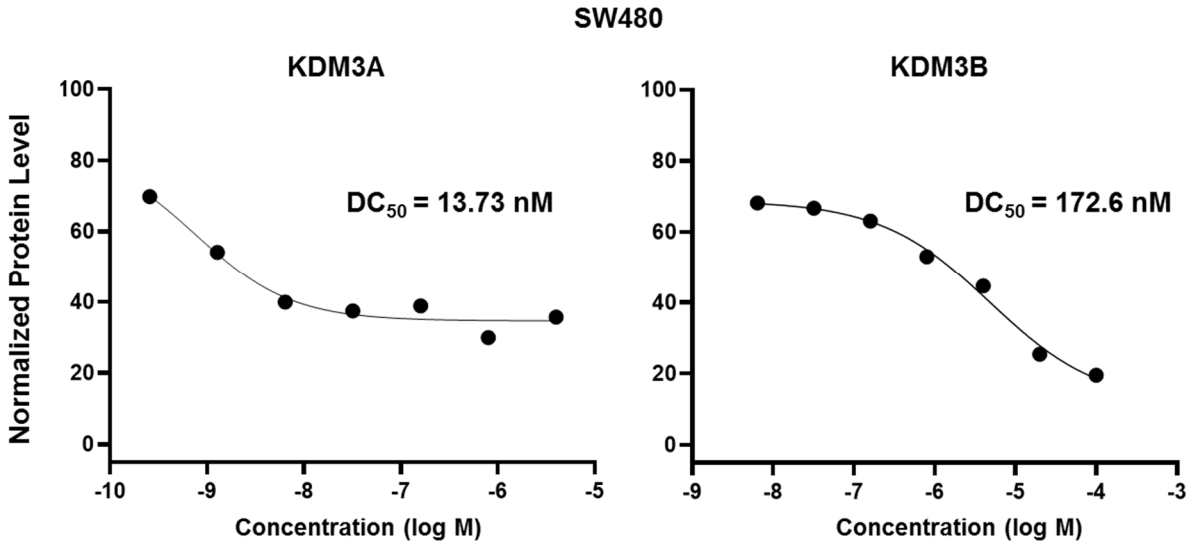


Figure S2. Examination of dose-dependent KDM3A or KDM3B degradation by compound 4 in SW480 cells. SW480 cells were treated with compounds **4** as indicated for 16 hours.

Figure S3

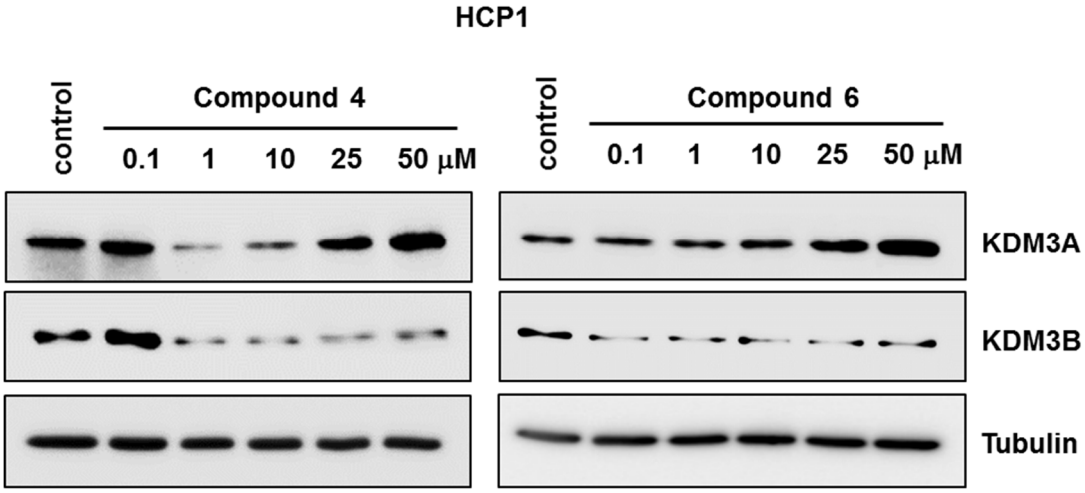


Figure S3. IOX1-PROTACs induced KDM3A and KDM3B degradation in HCP-1cells. HCP-1 cells were treated with compounds 4 or 6 as indicated for 16 hours.

Figure S4

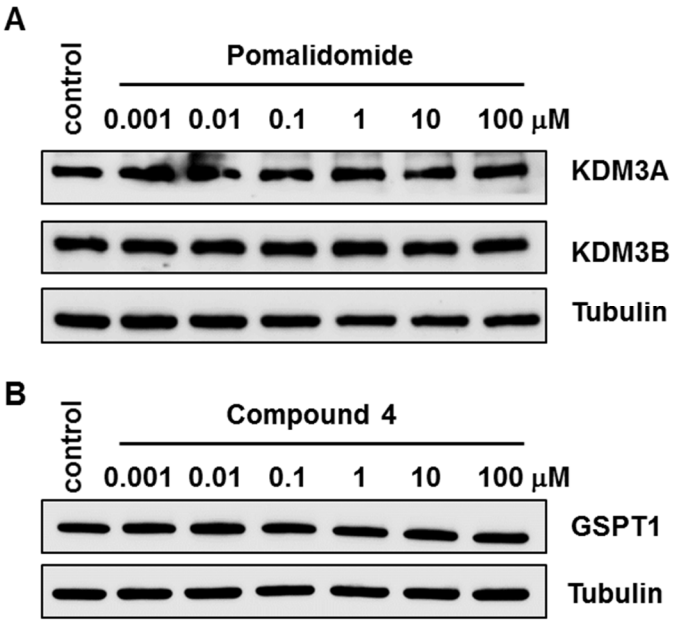


Figure S4. (A) Pomalidomide did not induce KDM3A and KDM3B degradation in SW480 cells. SW480 cells were treated with various concentration of pamalidomide as indicated for 16 hours. (B) Compound 4 did not induce GSPT1 degradation in SW480 cells. SW480 cells were treated with various concentration of compound 4 as indicated for 16 hours.

Figure S5

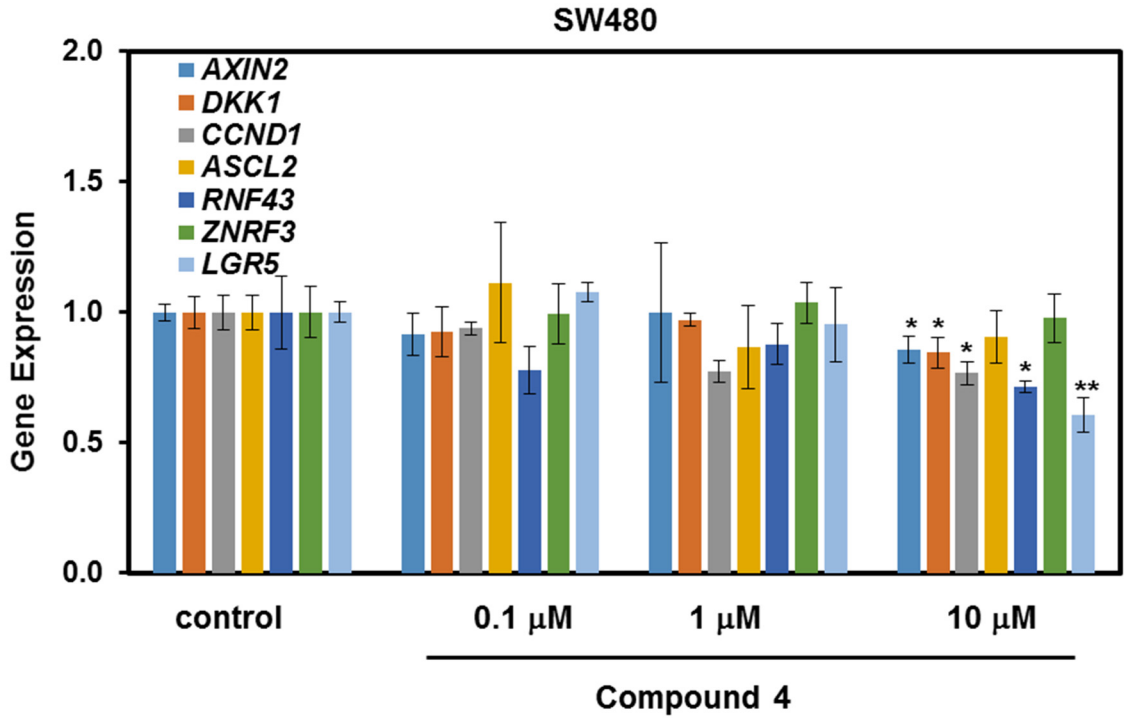
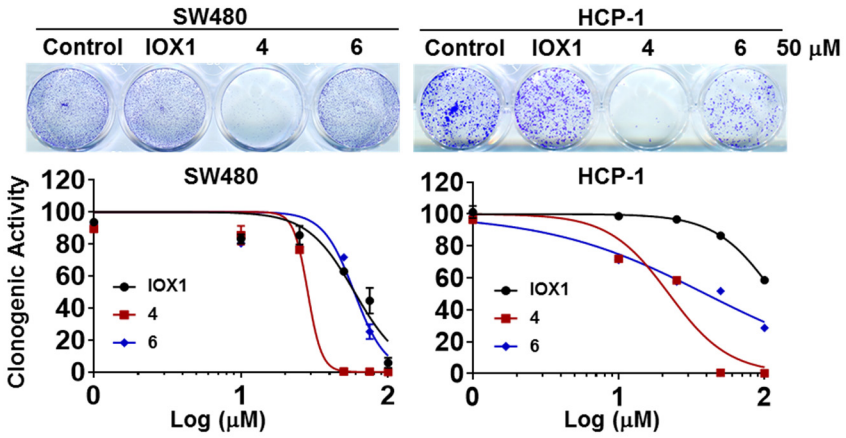


Figure S5. SW480 cells were treated with compounds **4** as indicated for 16 hours. Data represent mean±SD. *P<0.05; **P<0.01; unpaired two-tailed Student's t-Test.

Figure S6

A



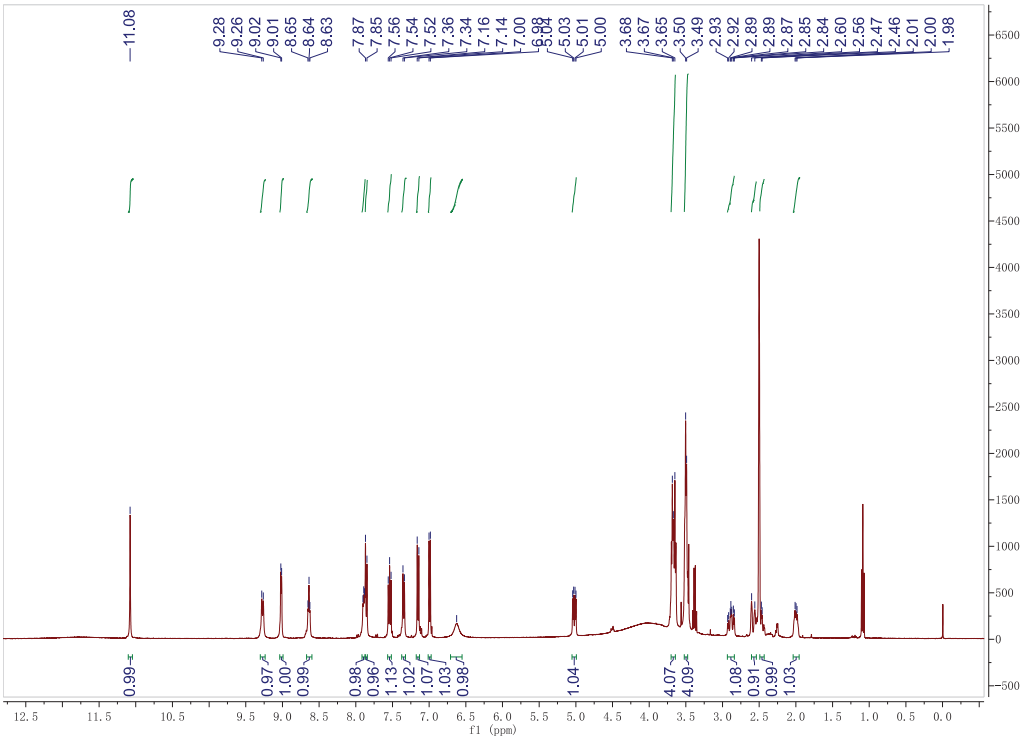
B

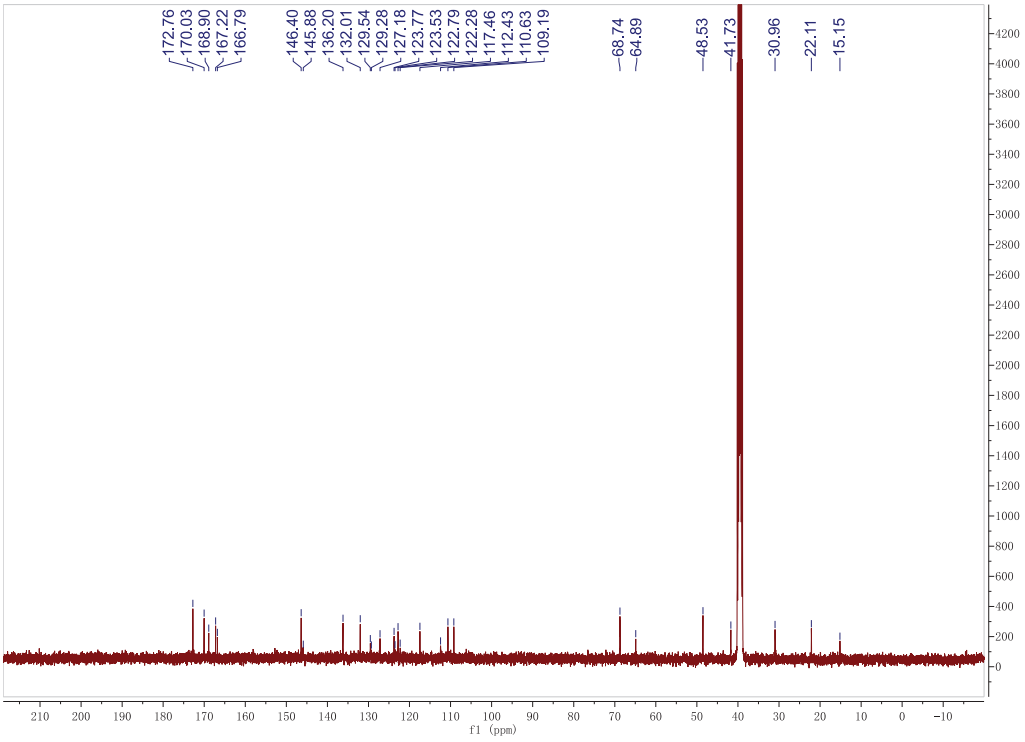
	ED_{50} (μM)	
	SW480	HCP-1
IOX1	59.5	> 100
4	28.4	22.1
6	58.8	39.4

Figure S6. IOX1-PROTACs suppresses cacogenic ability of CRC cells. (A). IOX1-PROTACs inhibited colony formation of SW480 and HCP-1 cells. (B) ED_{50} values of clonogenic assays.

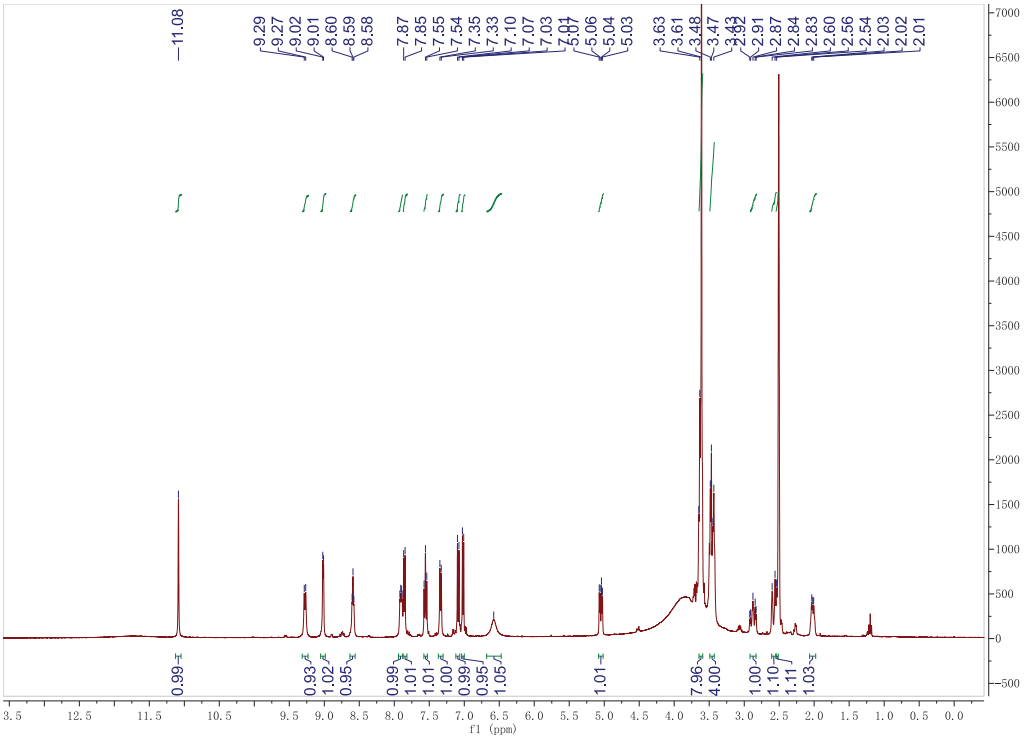
^{1}H and ^{13}C NMR spectra of final compounds

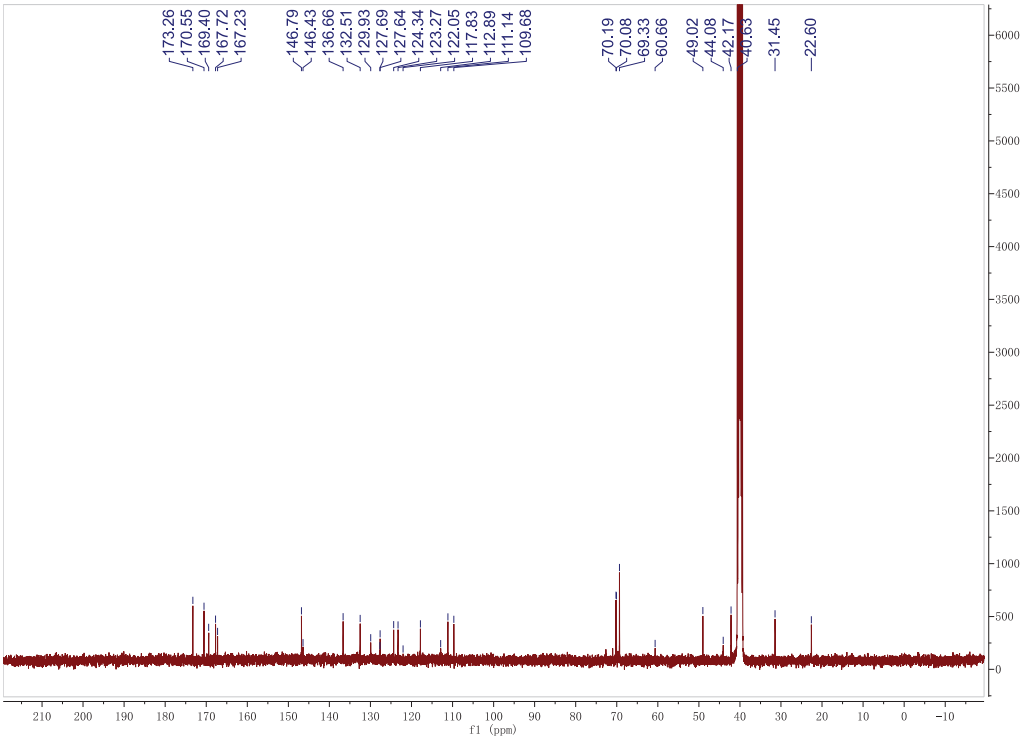
N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-8-hydroxyquinoline-5-carboxamide **(1)**



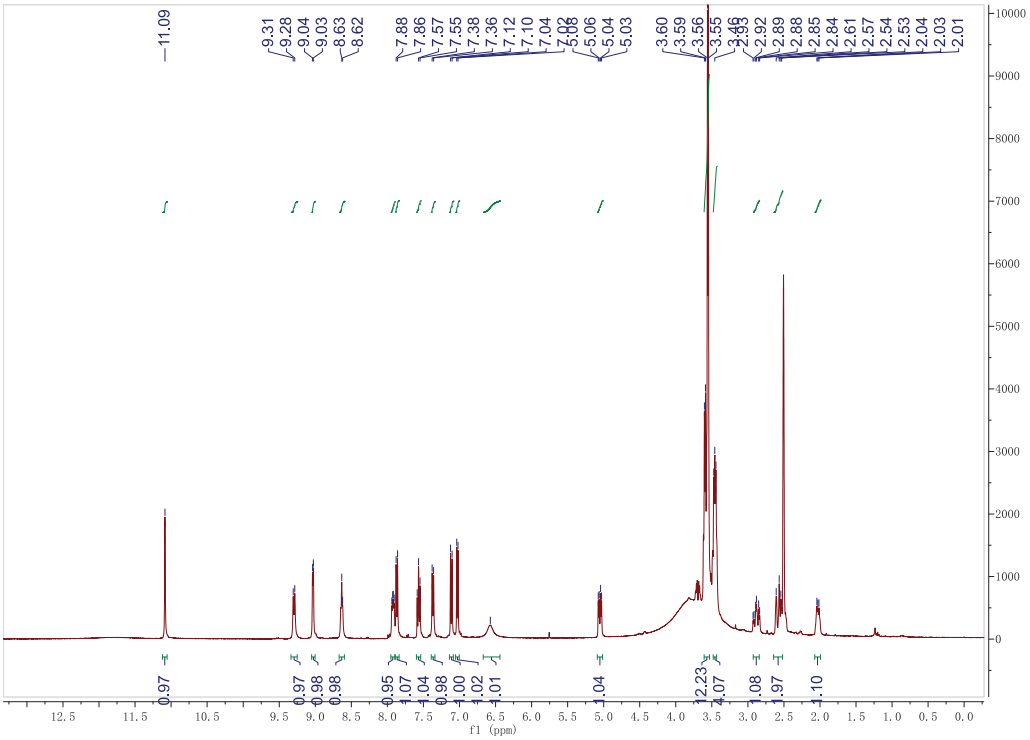


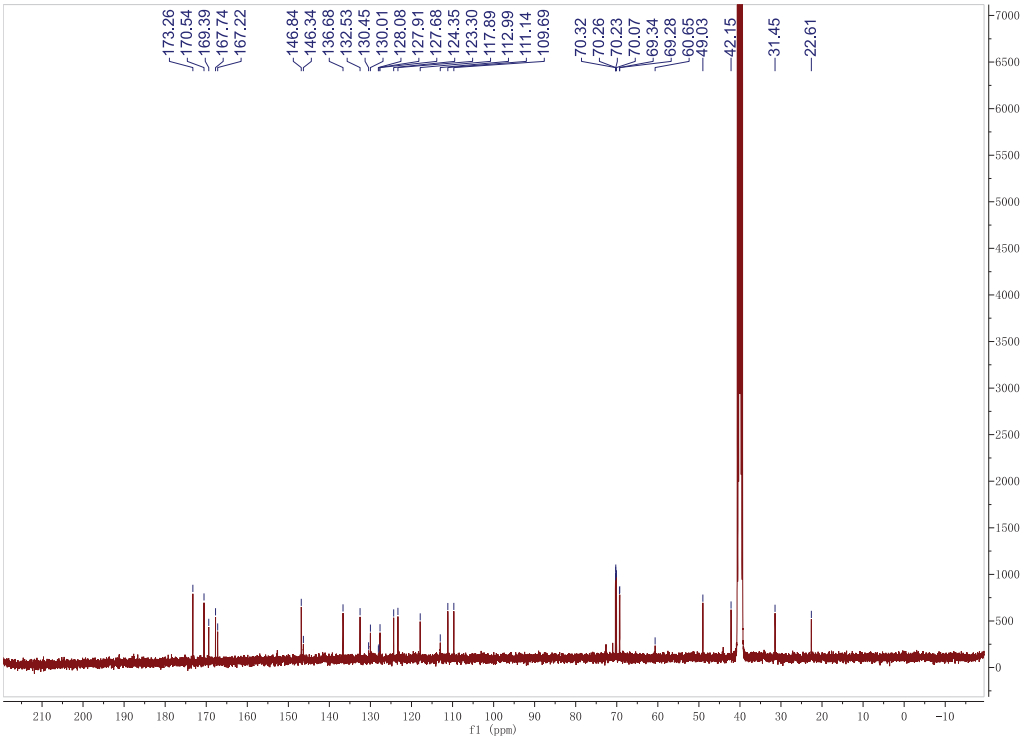
N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-8-hydroxyquinoline-5-carboxamide **(2)**



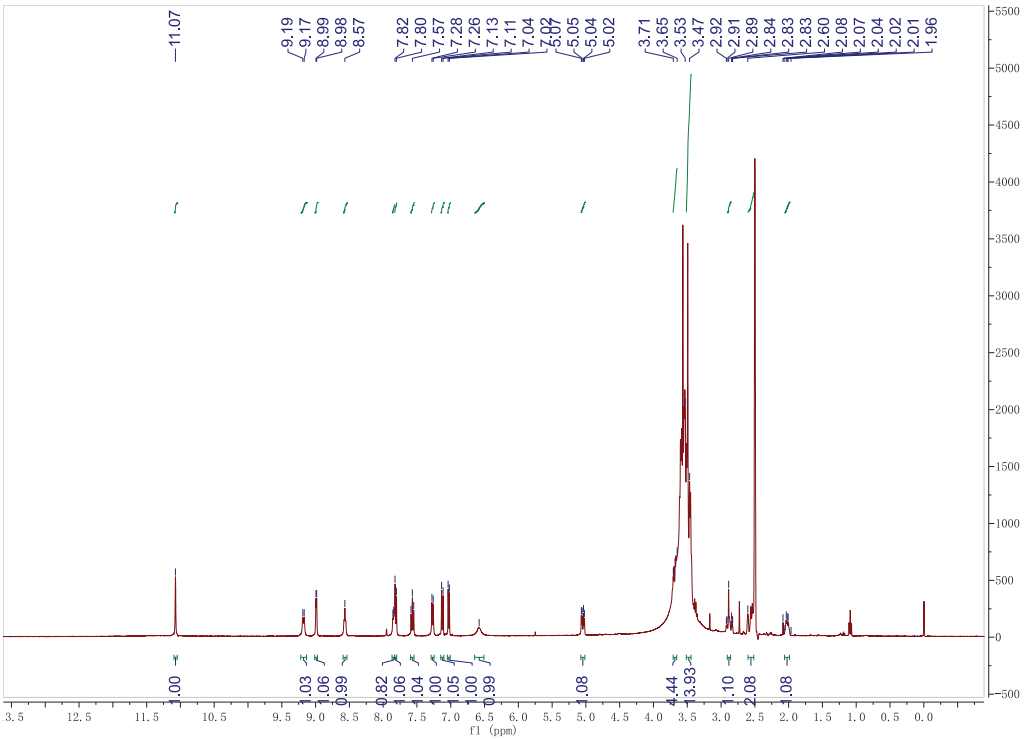


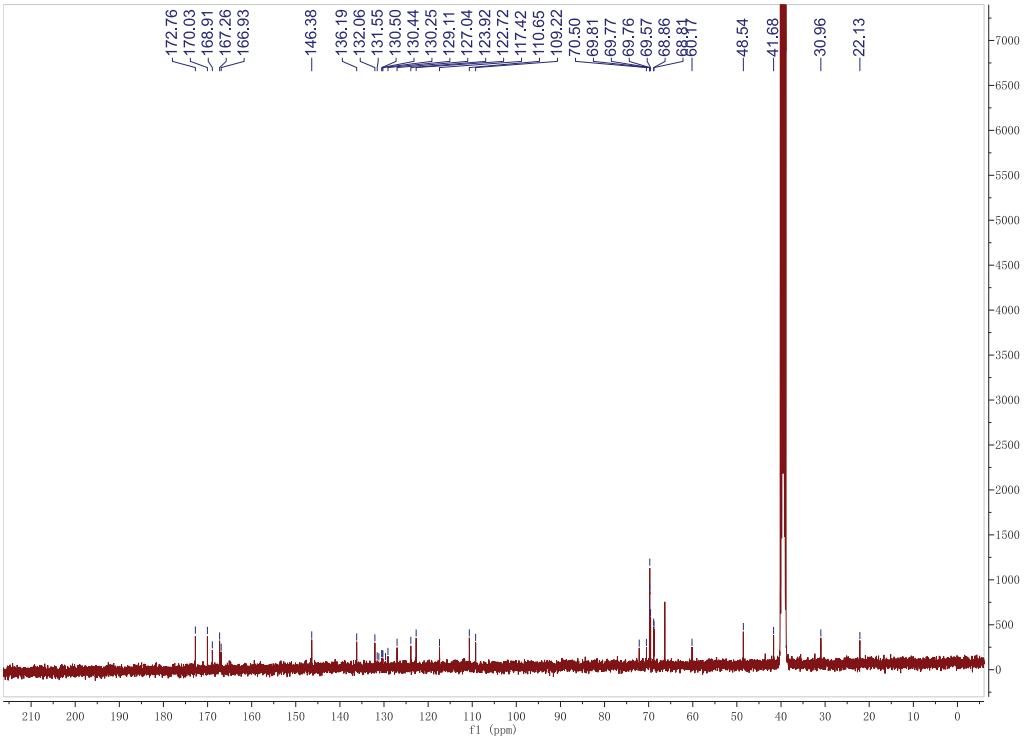
N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-8-hydroxyquinoline-5-carboxamide **(3):**



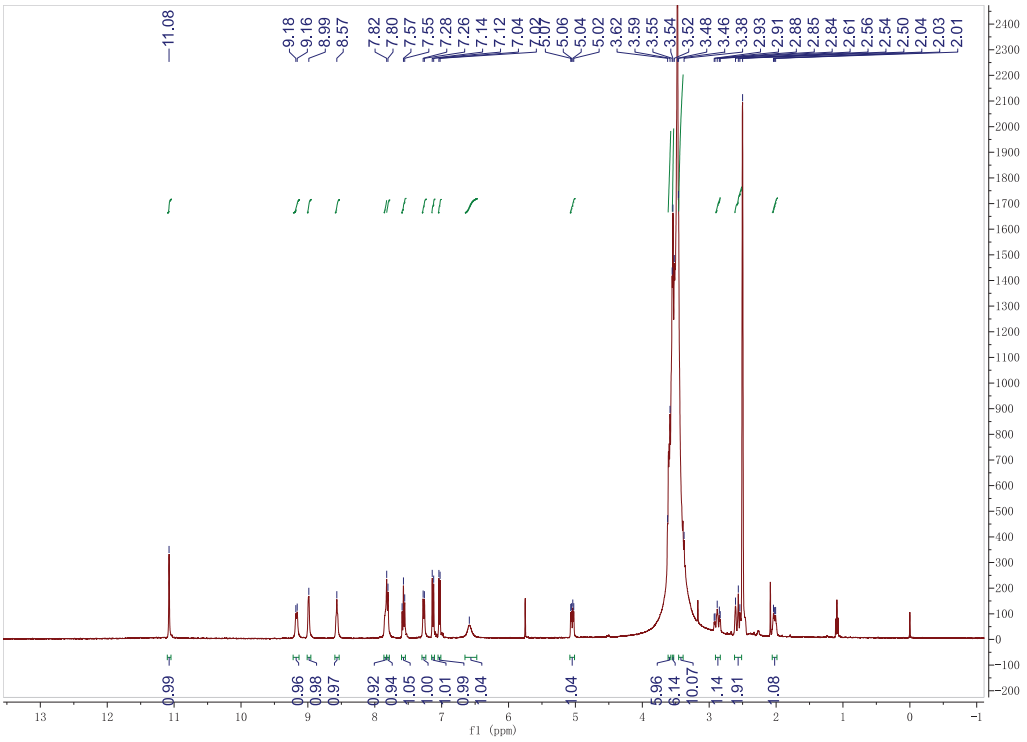


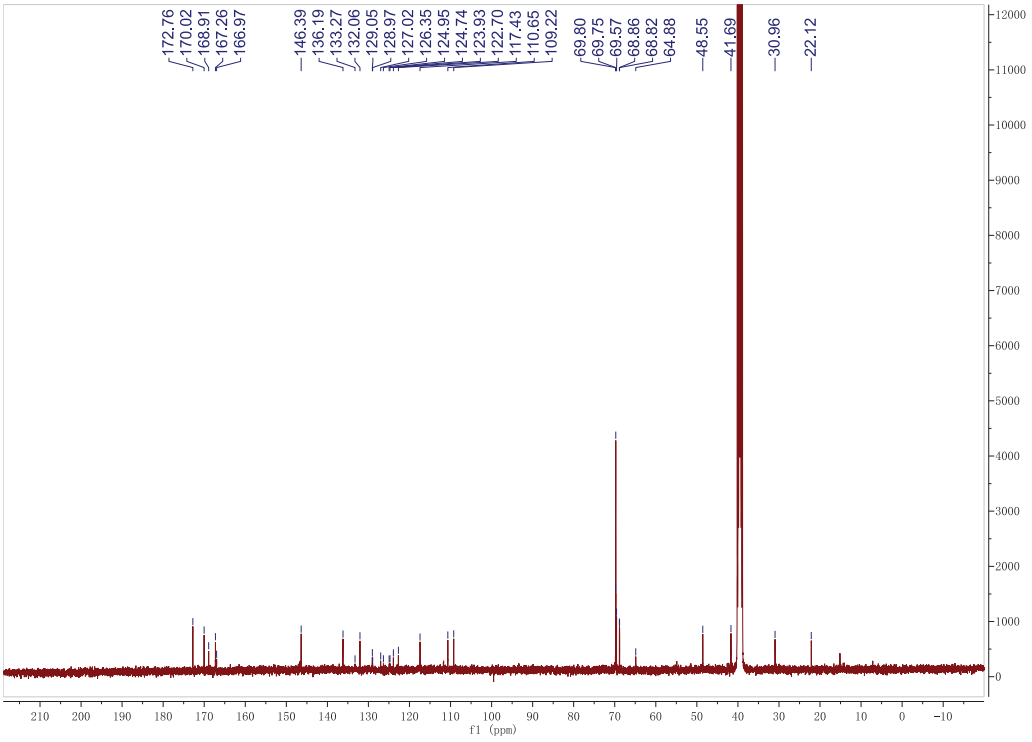
N-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-8-hydroxyquinoline-5-carboxamide **(4)**



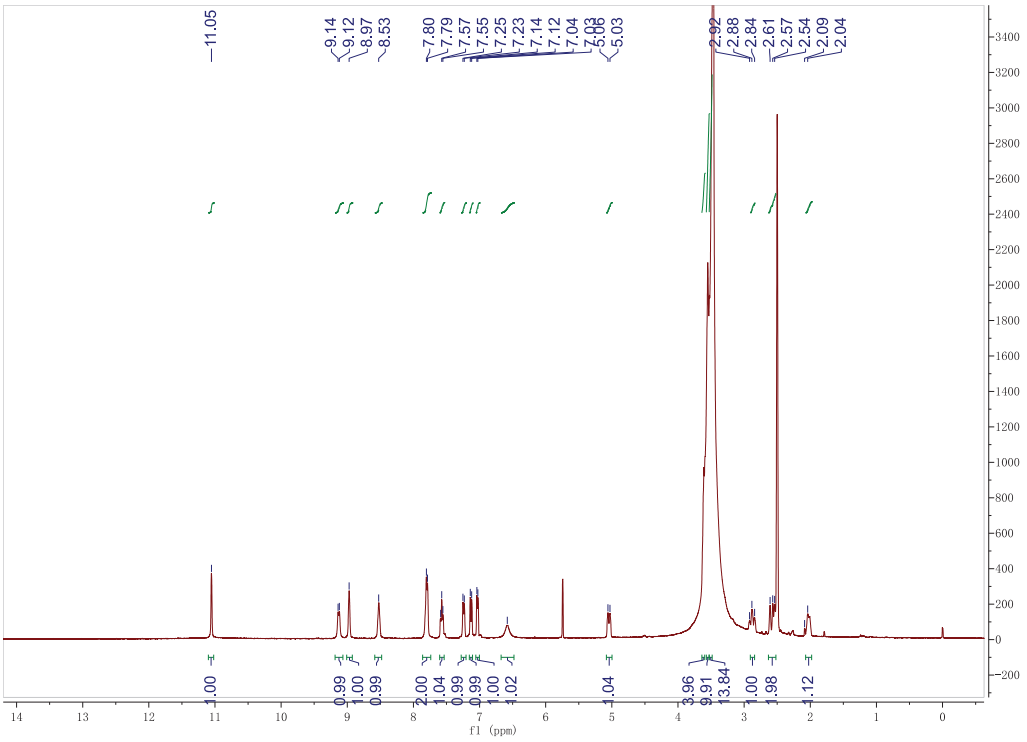


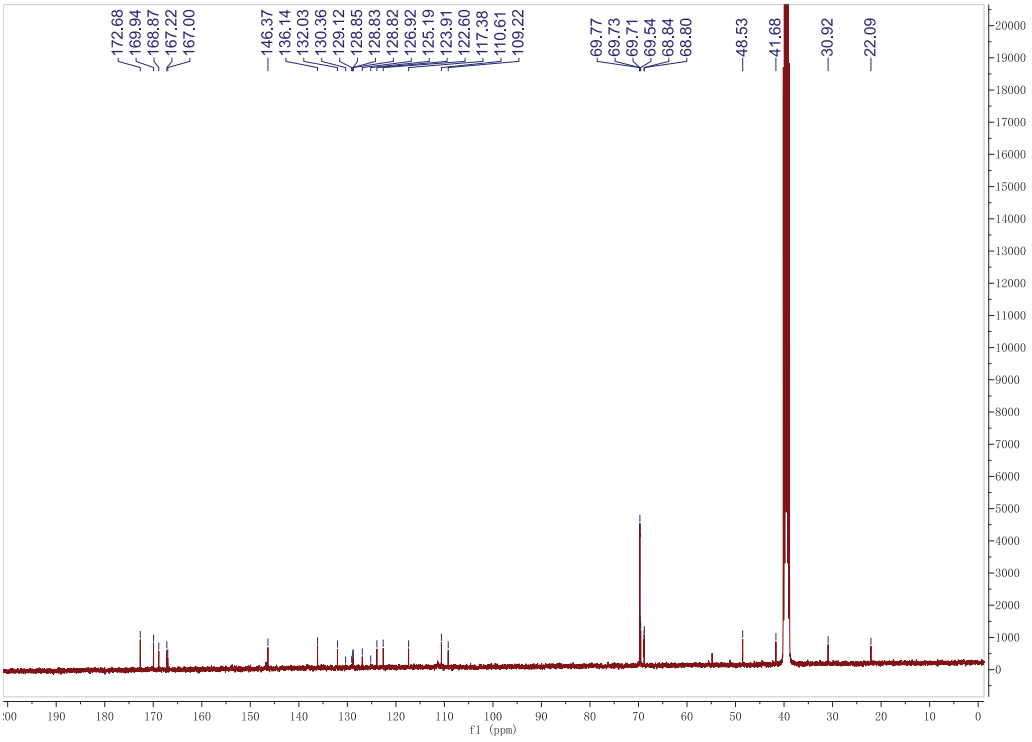
N-(17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaheptadecyl)-8-hydroxyquinoline-5-carboxamide **(5)**



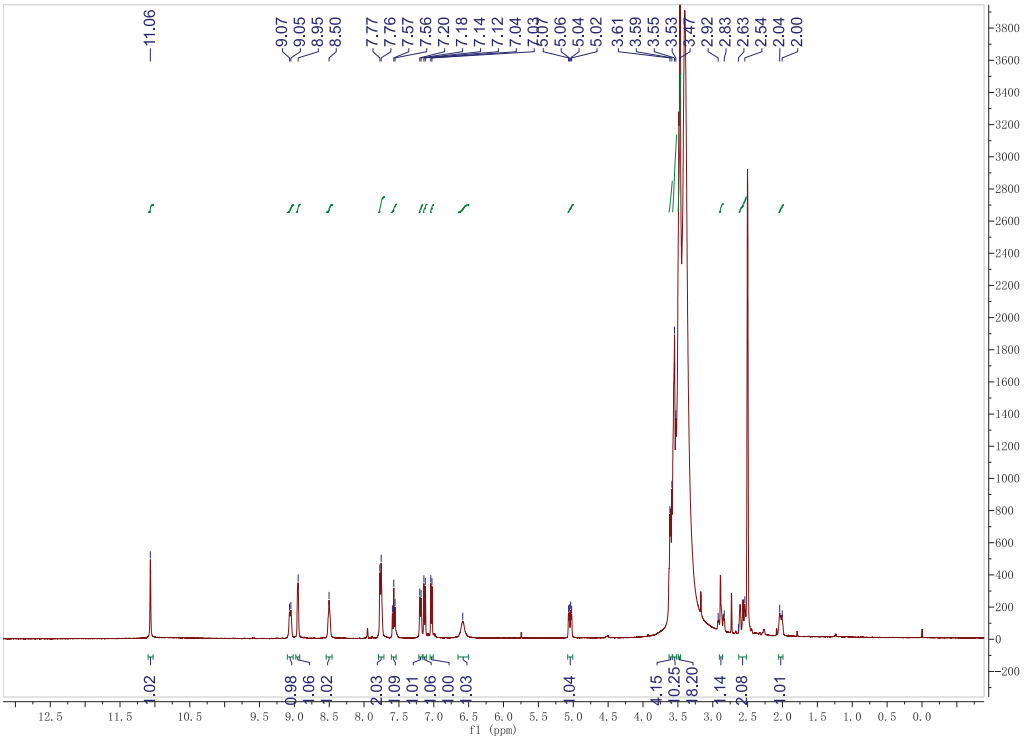


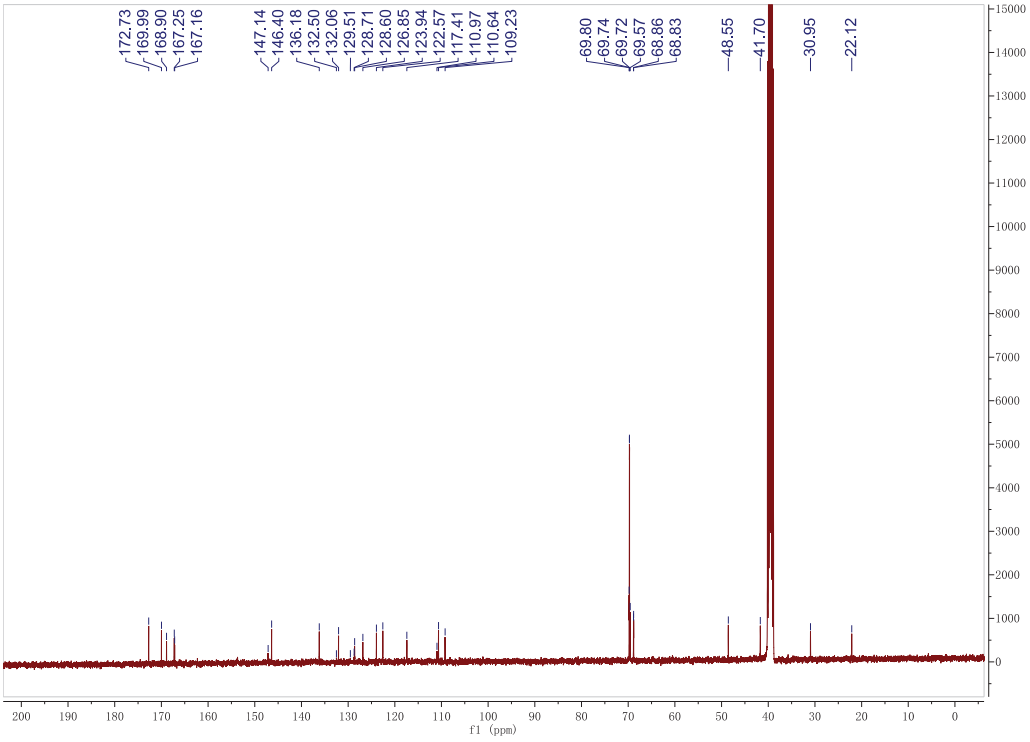
N-(20-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15,18-hexaoxaicosyl)-8-hydroxyquinoline-5-carboxamide **(6)**





N-(23-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15,18,21-heptaoxatricosyl)-8-hydroxyquinoline-5-carboxamide **(7)**





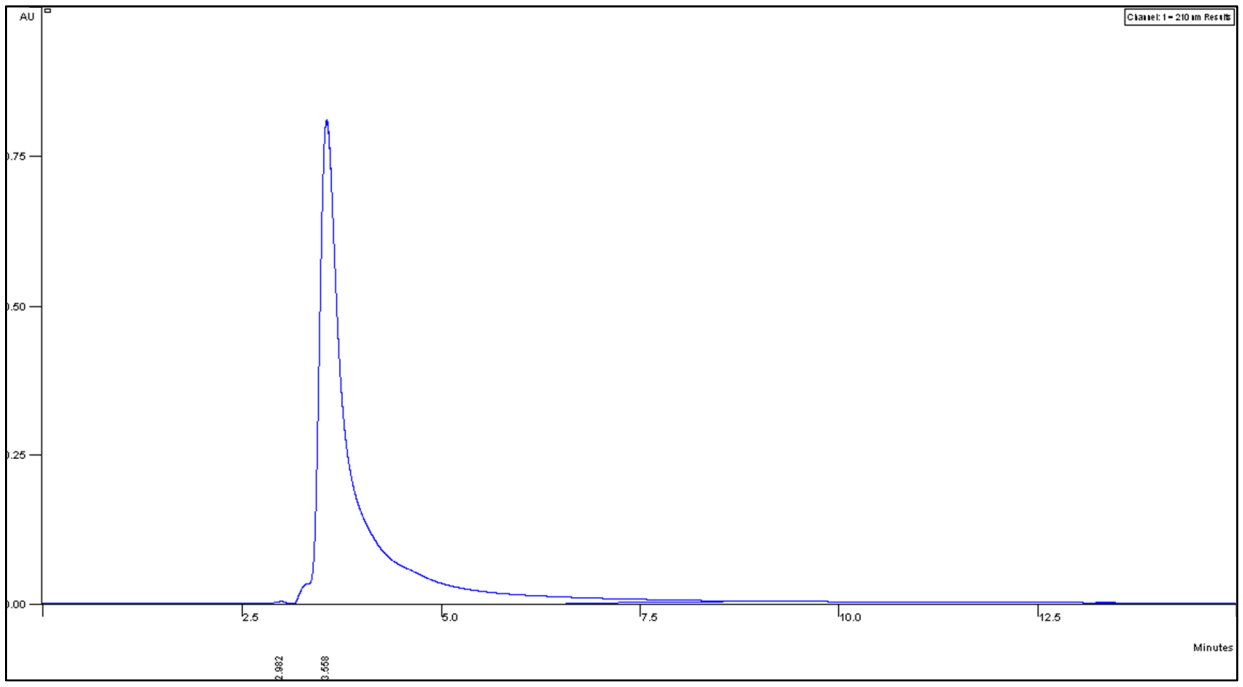
Purity data of final compounds.

HPLC System: Varian Prostar 210;
Column: Microsorb-MV100-5 C18 (250 * 4.6 mm);
Injection Volume: 5 μL;
Sample Concentration: 1mg/mL;
Single Wavelength: 210 nm

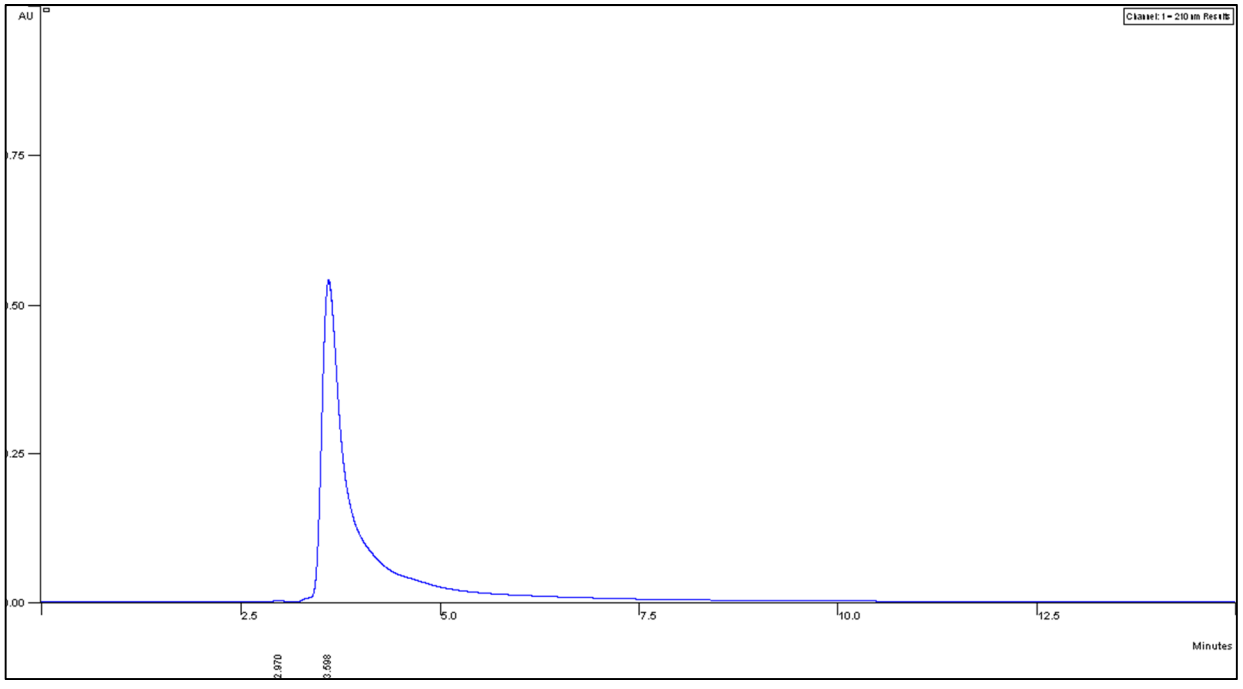
Table S2. HPLC Analysis of Target Compounds.

Compound	System	Retention Time (min)	Purity (%)
1	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.55	99.71
2	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.59	99.17
3	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.63	98.01
4	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.70	96.51
5	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.76	99.55
6	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.88	99.18
7	water/acetonitrile (30/70) with 0.1% trifluoroacetic acid at 1 mL/min for 15 minutes	3.92	99.85

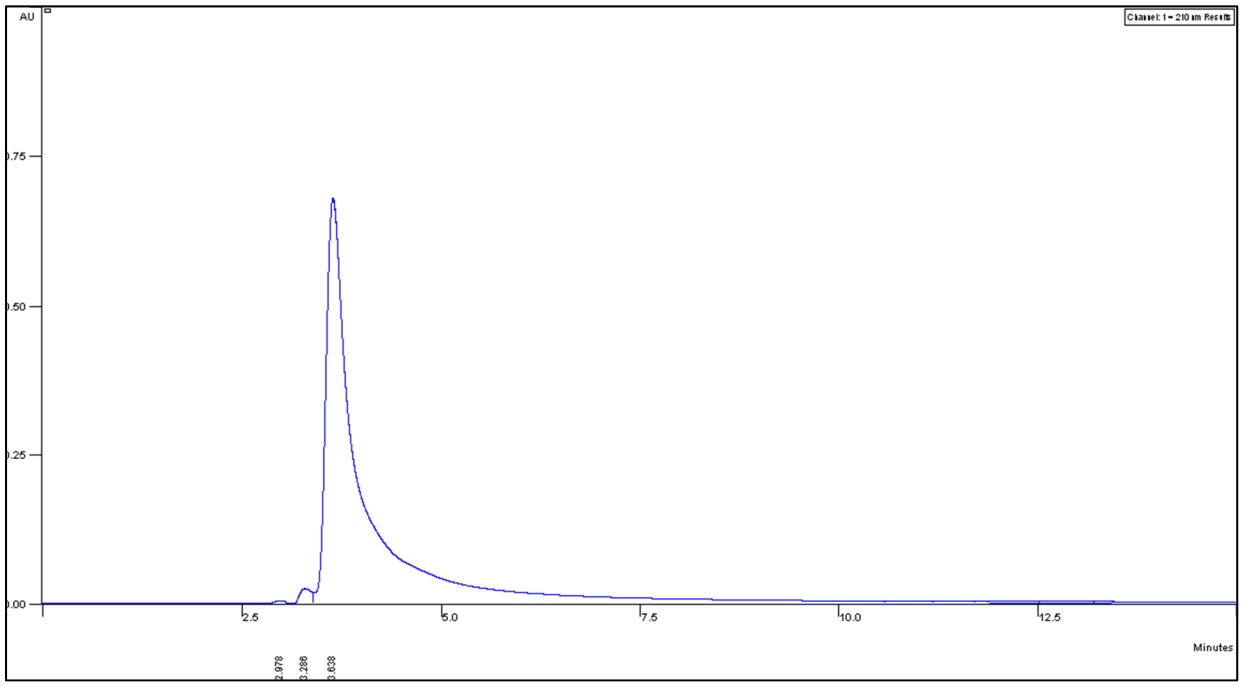
HPLC of Compound 1



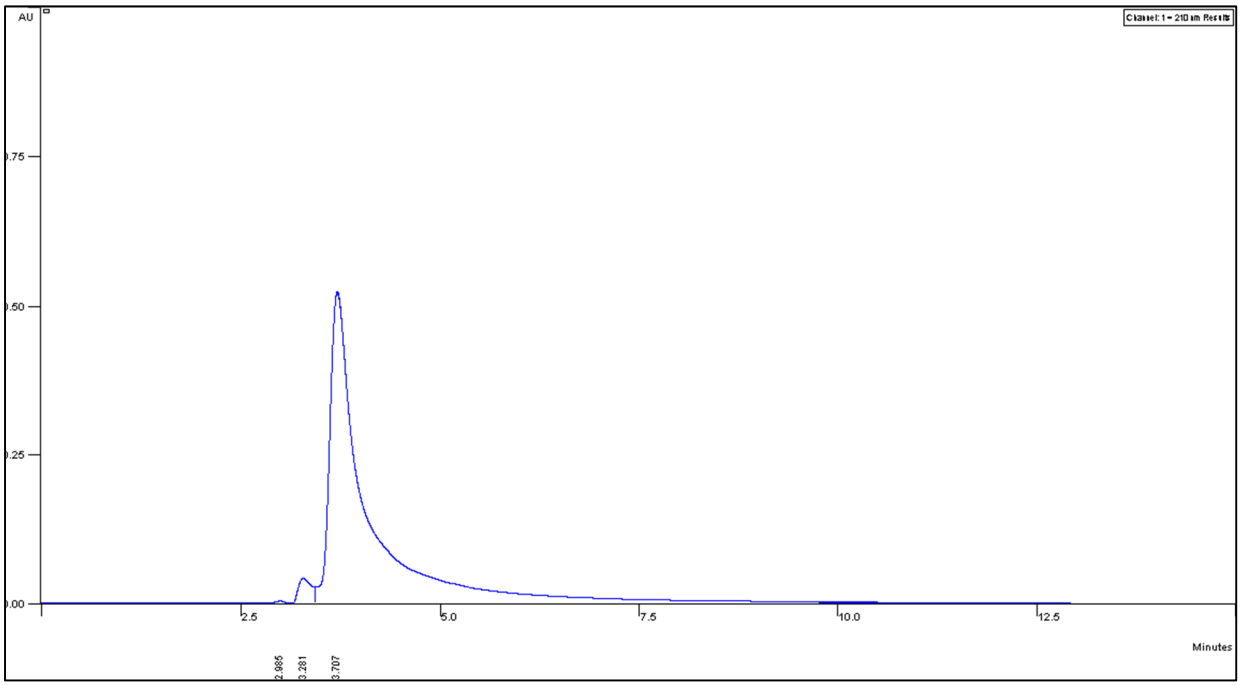
HPLC of Compound 2



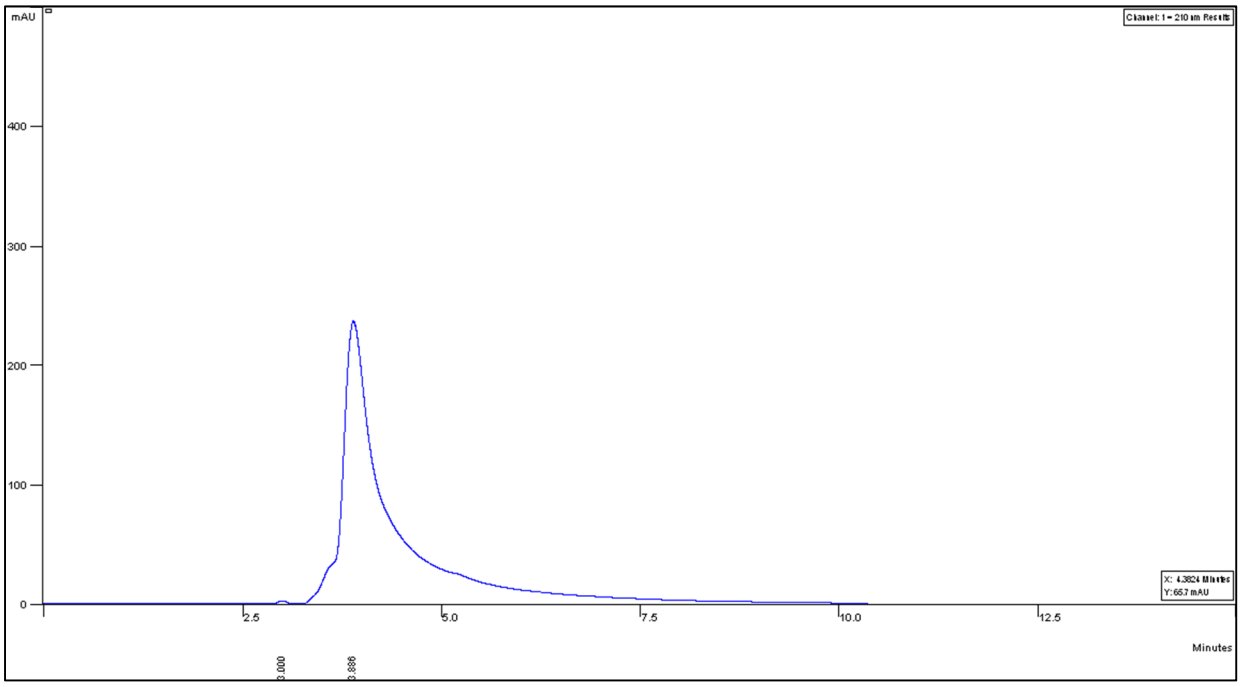
HPLC of Compound 3



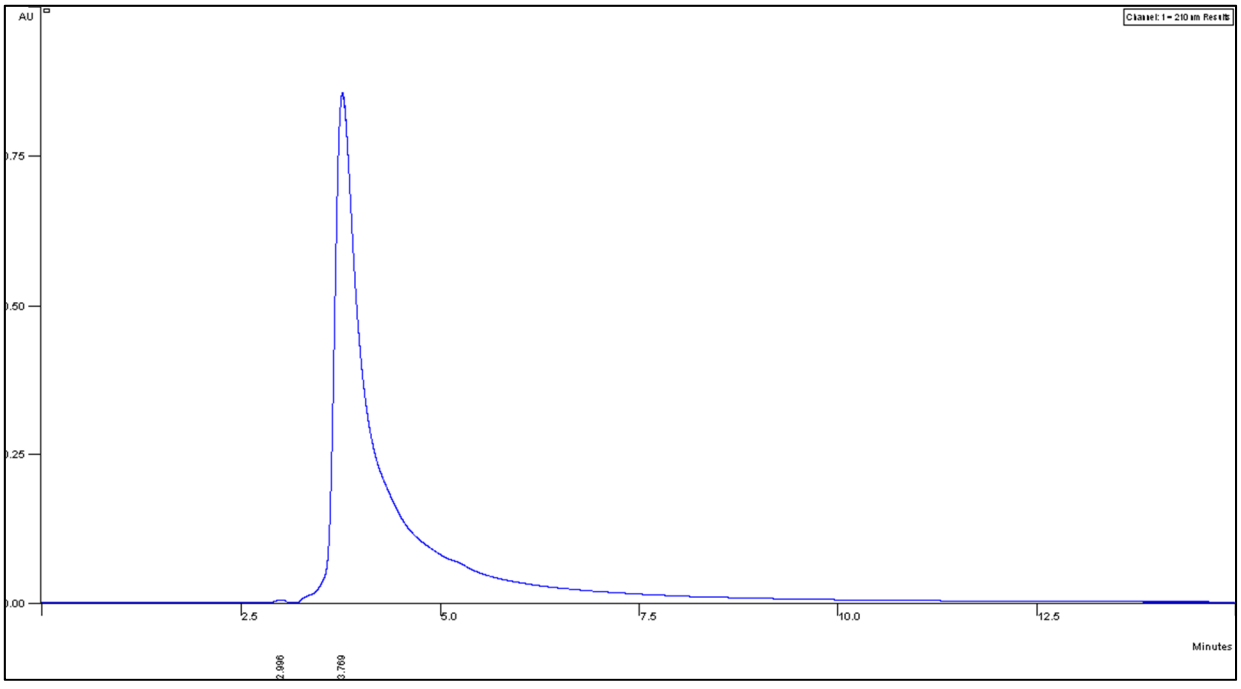
N HPLC of Compound 4



HPLC of Compound 5



HPLC of Compound 6



HPLC of Compound 7