Supporting Information

Discovery of Novel Schizocommunin Derivatives as Telomeric G-quadruplex Ligands that Trigger Telomere Dysfunction and the Deoxyribonucleic Acid (DNA) Damage Response

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Table S1. Oligomers used in this study.

Oligomer	Sequence
F21T	5′-FAM-d(GGGTTAGGGTTAGGGTTAGGG)-TAMRA-3′
F10T	5'-*FAM*-d(TATAGCTATA-HEG-TATAGCTATA)-*TAMRA*-3'
ds26	5'-GTTAGCCTAGCTTAAGCTAGGCTAAC-3'
biotin-HTG21	5'-biotin-d(GGGTTAGGGTTAGGGTTAGGG)-3'
biotin-pu22	5'-biotin-d(TGAGGGTGGGGAGGGTGGGGAA)-3'
biotin-c-kit1	5'-biotin-d(AGGGAGGGCGCTGGGAGGAGGG)-3'
biotin-bcl-2	5'-biotin-d(GGGCGCGGGAGGAAGGGGGCGGG)-3'
biotin-Hairpin	5'-biotin-d(TATAGCTATA-HEG-TATAGCTATA)-3'
HTG21	5'-GGGTTTGGGTTTGGGTTTGGG-3'
pu22	5'-TGAGGGTGGGGAGGGTGGGGAA-3'
c-kit1	5'-AGGGAGGGCGCTGGGAGGAGGG-3'
bcl-2	5'-GGGCGCGGGAGGAAGGGGGCGGG-3'
HRAS	5'-TCGGGTTGCGGGCGCAGGGCACGGGCG-3'
TBA	5'-GGTTGGTGTGGTTGG-3'

Table S2. The stabilization activity of compound**s** on telomeric G-quadruplex.

Compd.	ΔT_m (°C)	
	FRET melting [a]	CD melting [b]
7	8.4	3
8	11.8	11
9	10.0	8
16	23.5	20
22	9.0	7
27	23.1	20
30	24.6	21

[a] $\Delta T_m = T_m$ (DNA + ligand) - T_m (DNA). ΔT_m values for the incubation of 0.2 μM F21T with and without 1.0 μM of the compound in Tris-HCl buffer (10 mM, pH 7.4) containing 60 mM KCl. In the absence of a ligand, the T_m values of annealed F21T is 60 °C. [b] ΔT_m values for incubation of 2 μM HTG21 with and without 10 μM compound in Tris-HCl buffer (10 mM, pH 7.4) containing 60 mM KCl. In the absence of ligand, T_m values of annealed HTG21 is 67 °C.

Table S3. The stabilization activity of compound **16** on various G-quadruplexes.

DNA	T_m (°C)[a]		ΔT_m (°C)
	16 (0 μM)	**16** (10 μM)	
HTG21	67	87	20
pu22	82	95	13
c-kit1	64	78	14
bcl-2	87	95	8
HRAS	62	76	14
TBA	54	60	6

[a] Melting temperature (T_m) of DNA samples with and without compounds in 10 mM Tris-HCl (2.5 mM KCl for pu22, c-kit1 and bcl-2; 60 mM KCl for HTG21, HRAS and TBA) buffer detected by circular dichroism spectroscopy.

Table S4. Equilibrium binding constants (K_D) on various G-quadruplexes.

Compound	DNA	K_D (M)
16	HTG21	8.1×10^{-6}
	pu22	1.2×10^{-5}
	c-kit1	7.8×10^{-6}
	bcl-2	1.1×10^{-5}

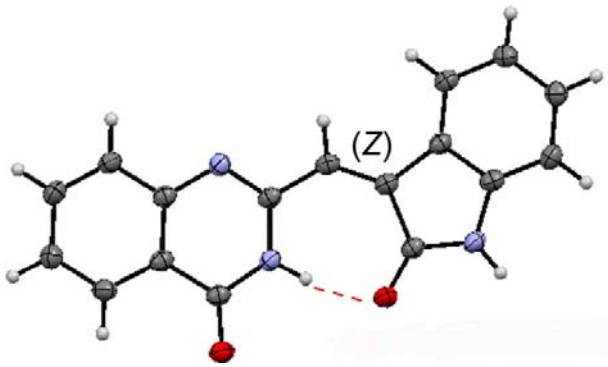


Figure S1. The X-ray crystallographic analysis of spectroscopy of compound **4**.
(reference: Uehata, K.; Kimura, N.; Hasegawa, K.; Arai, S.; Nishida, M.; Hosoe, T.; Kawai, K.; Nishida, A. Total synthesis of schizocommunin and revision of its structure. *J. Nat. Prod.* **2013**, *76*, 2034-2039.)

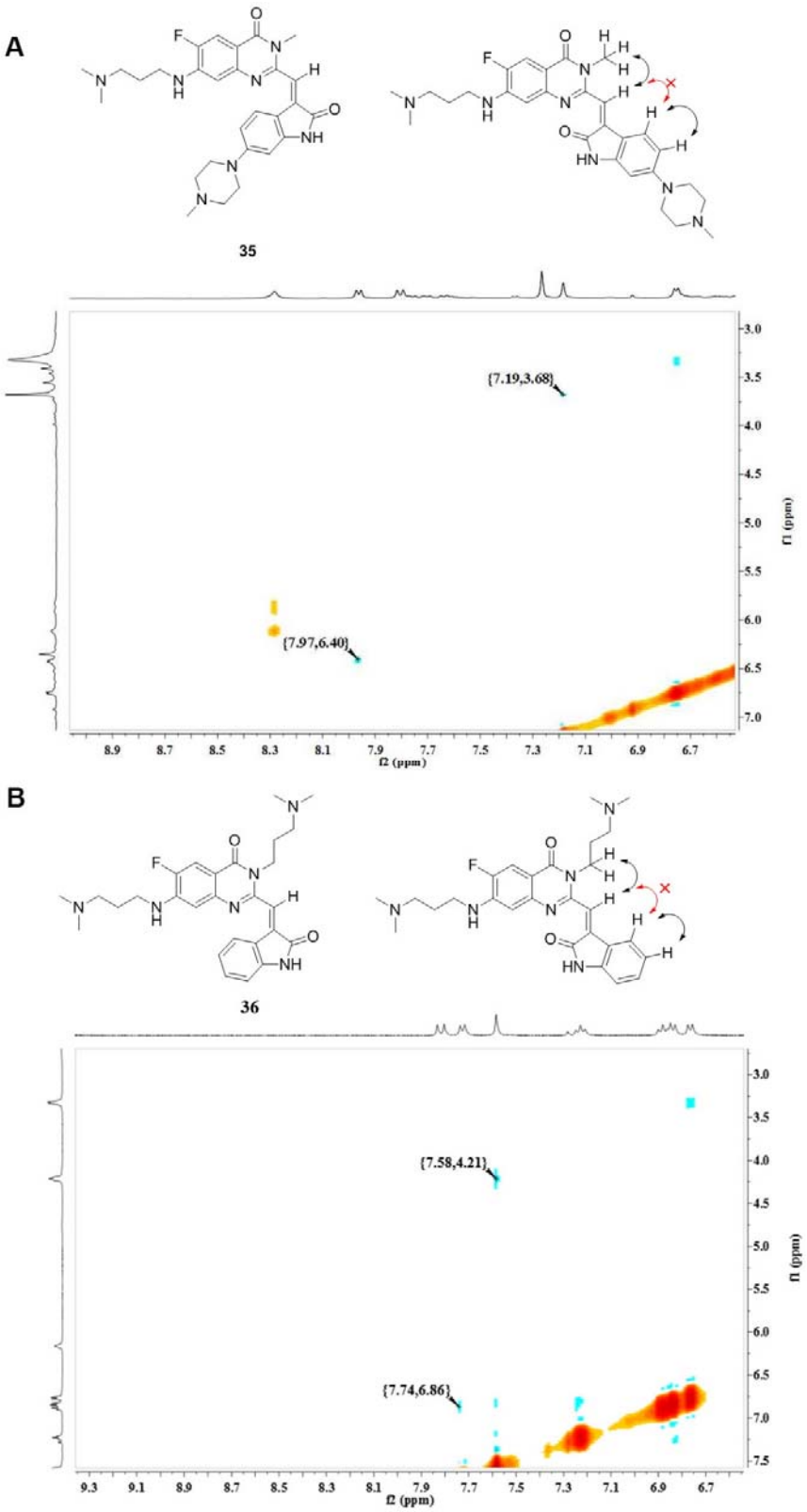


Figure S2. The NOE spectroscopy of compounds 35 (A) and 36 (B).

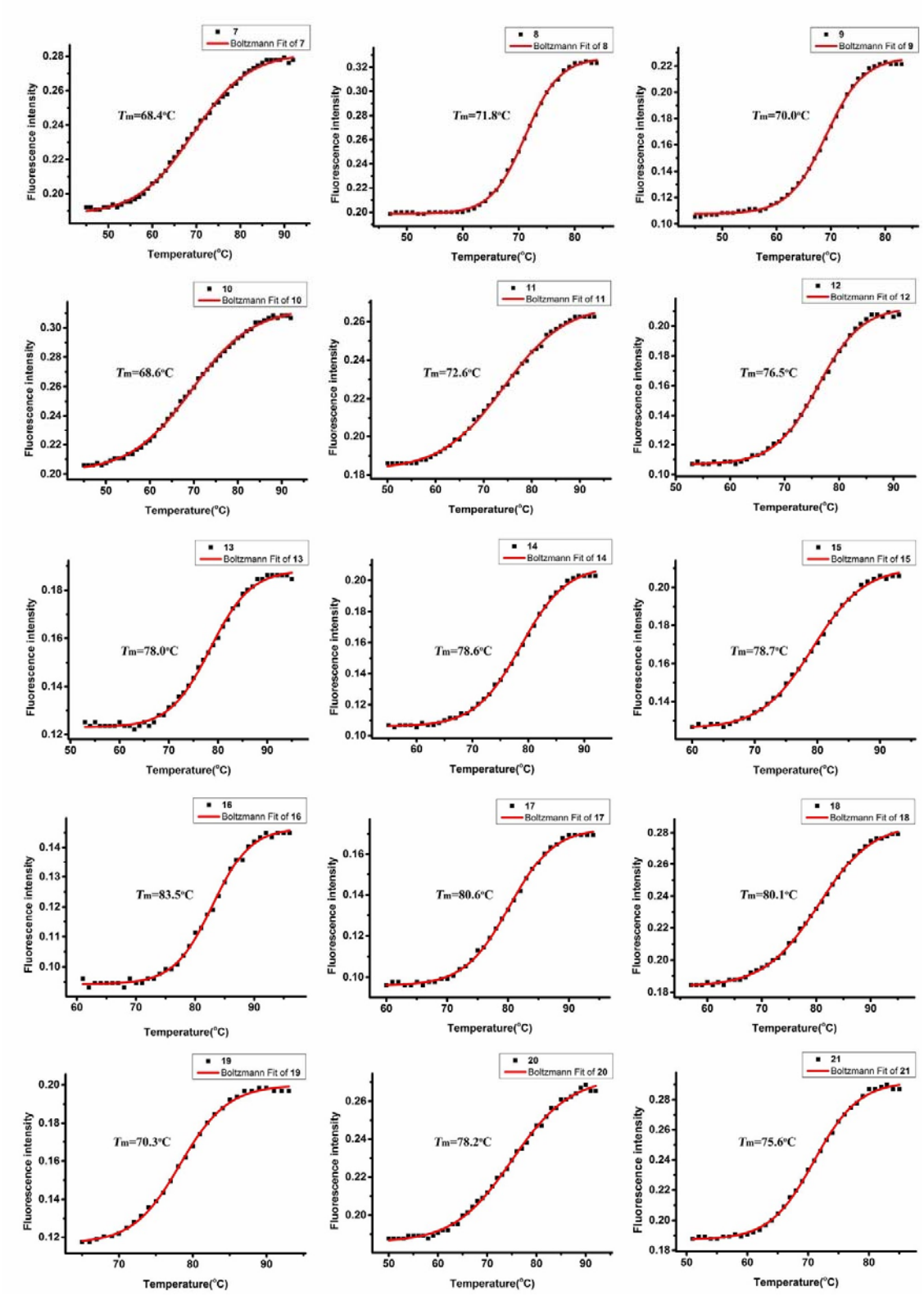


Figure S3. The melting curves of compounds **7** ~ **21** by using FRET melting assays.

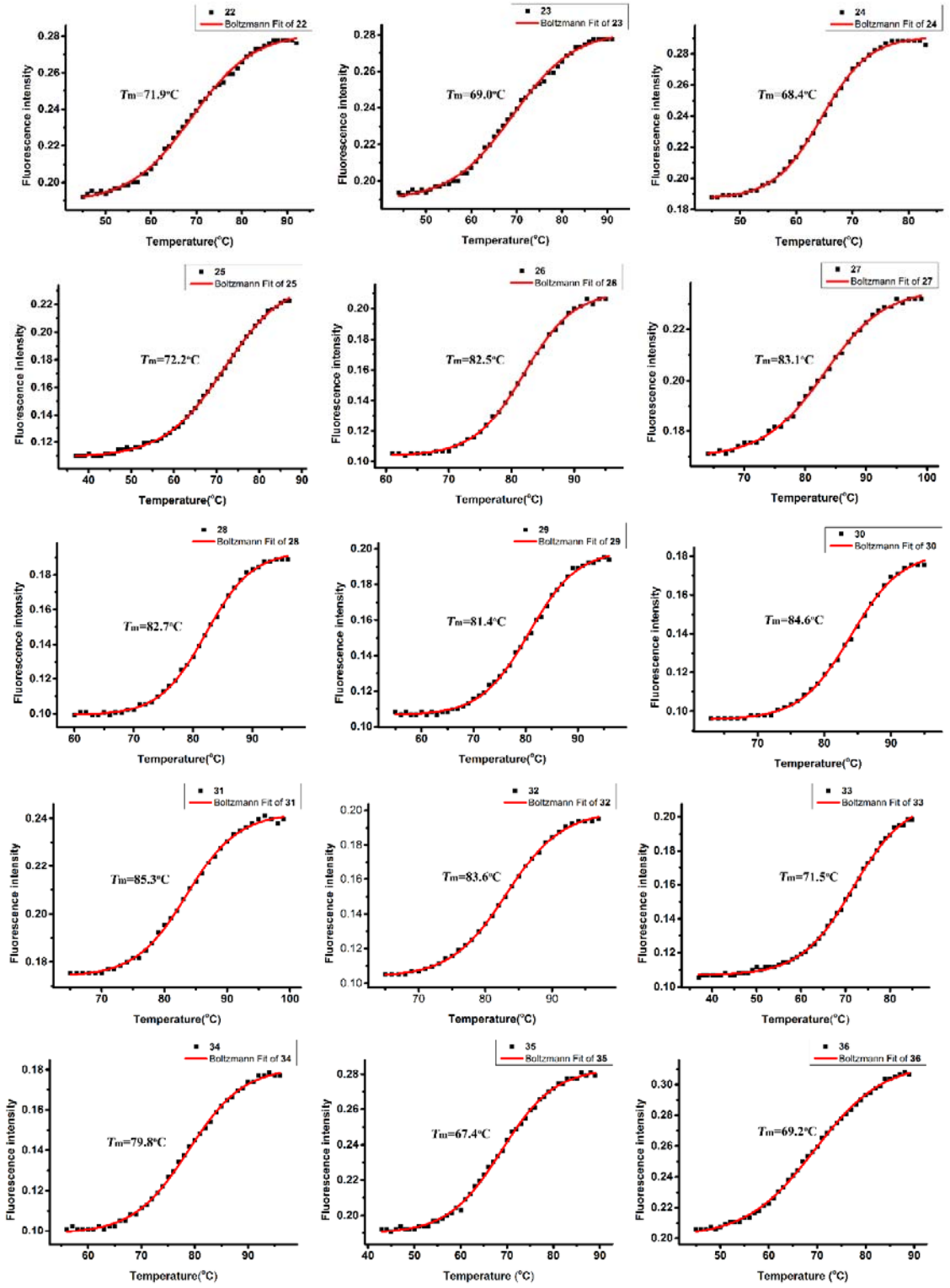


Figure S4. The melting curves of compounds **22** ~ **36** by using FRET melting assays.

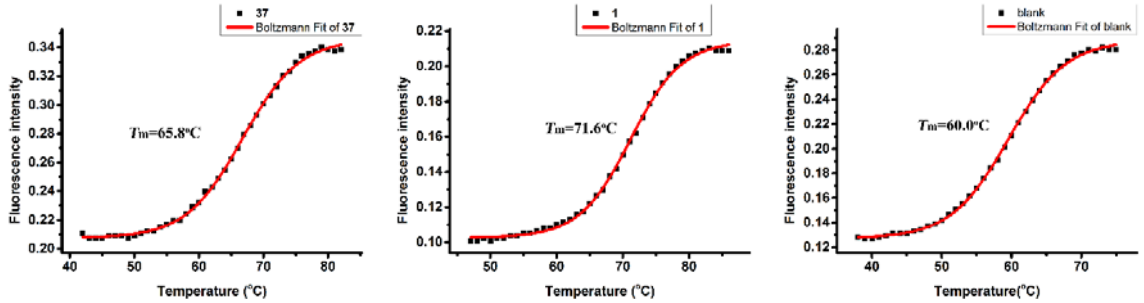


Figure S5. The melting curves of compounds **37** and the reference compound **1** by using FRET melting assays.

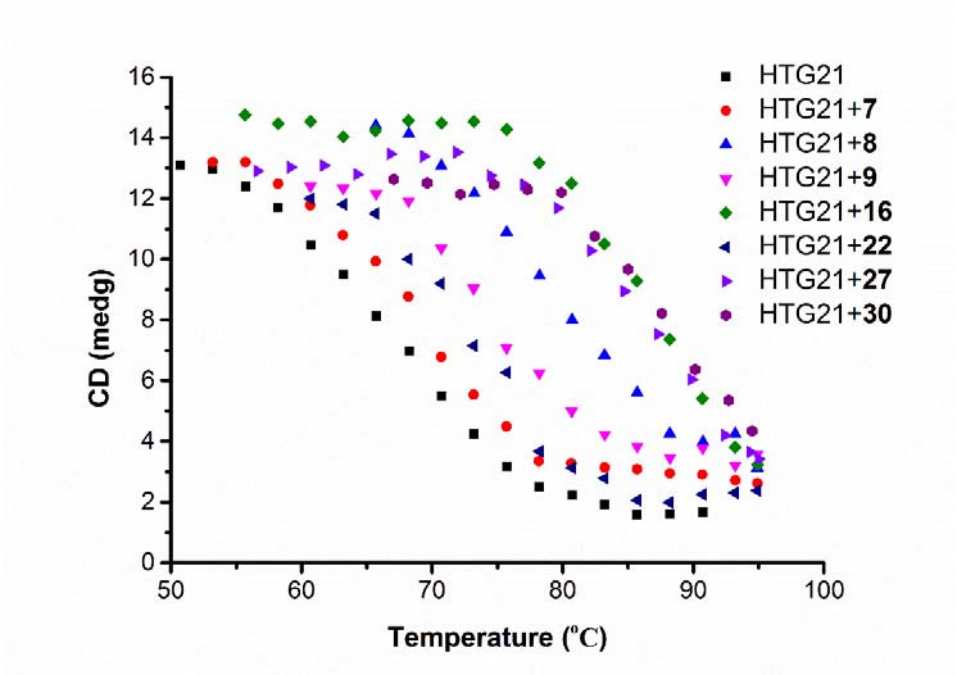


Figure S6. The melting curves of compounds **7**, **8**, **9**, **16**, **22**, **27**, **30** by using CD melting assays.

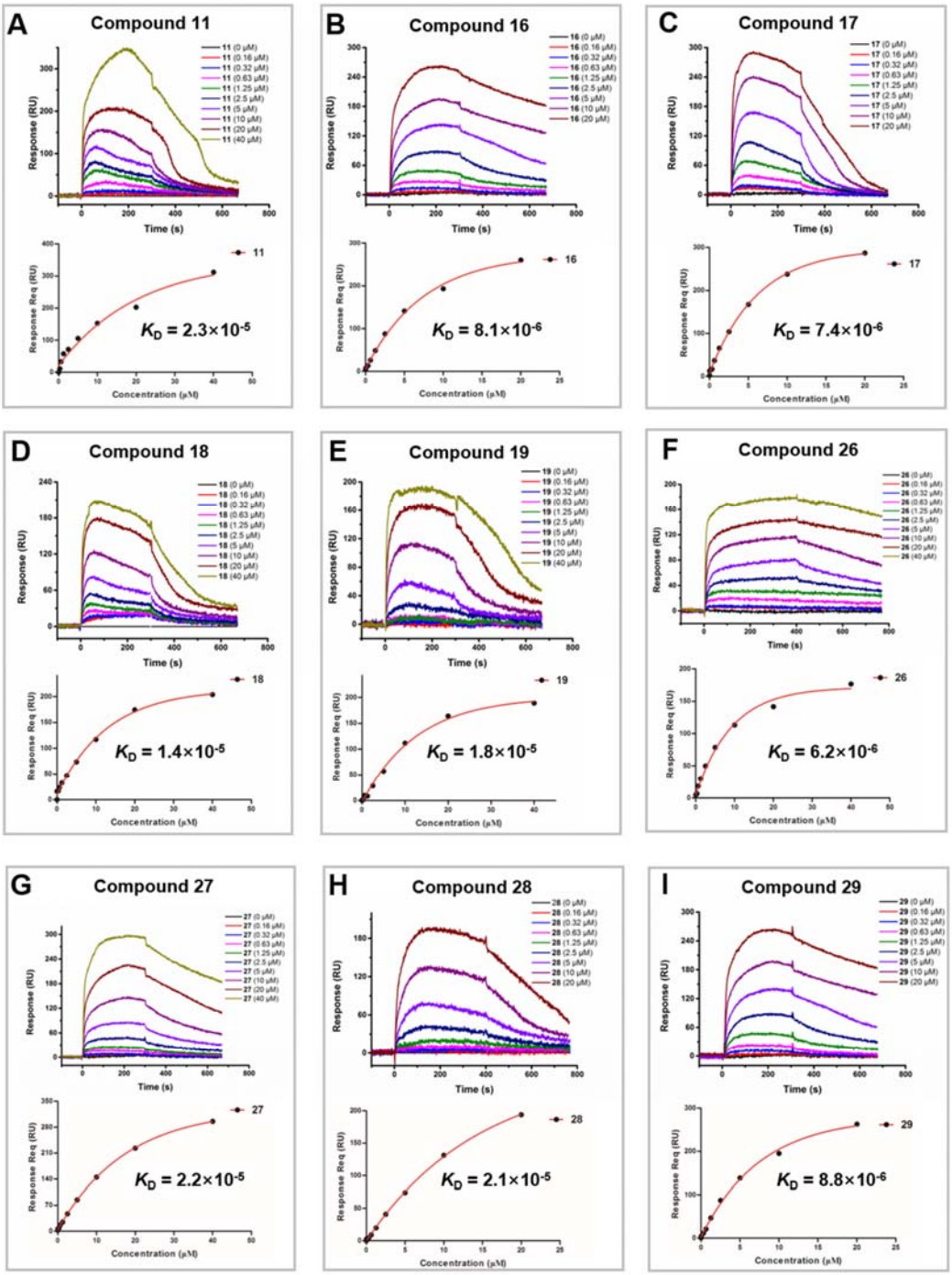


Figure S7. The SPR sensorgrams and fitting curves of compounds (A. compound **11**. B. compound **16**. C. compound **17**. D. compound **18**. E. compound **19**. F. compound **26**. G. compound **27**. H. compound **28**. I. compound **29**).

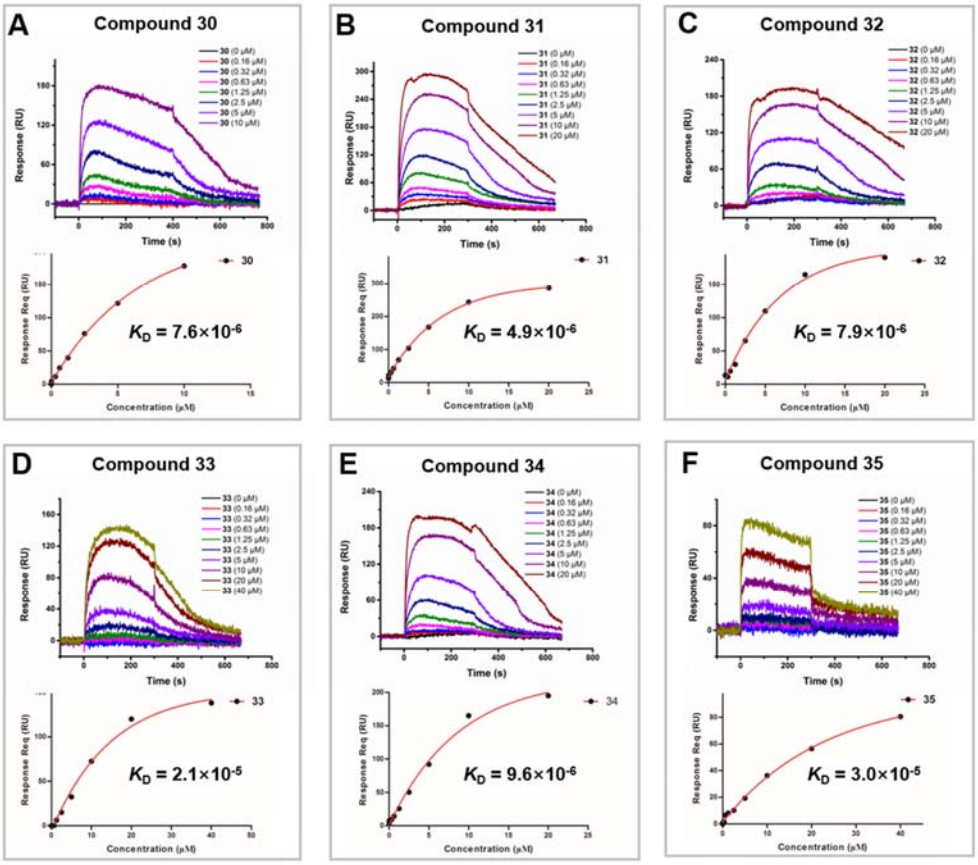


Figure S8. The SPR sensorgrams and fitting curves of compounds (A. compound **30**. B. compound **31**. C. compound **32**. D. compound **33**. E. compound **34**. F. compound **35**).

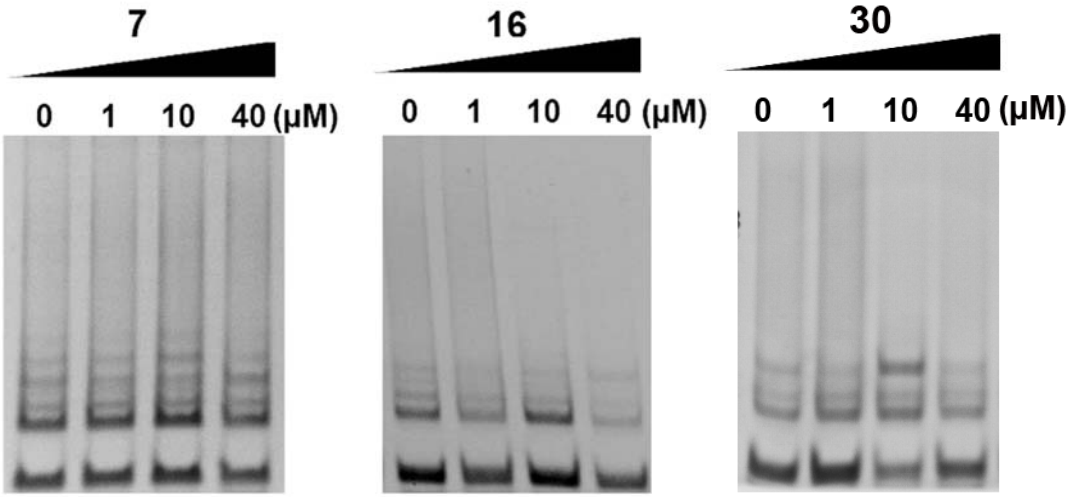


Figure S9. TRAP gel for compounds **7**, **16** and **30**.

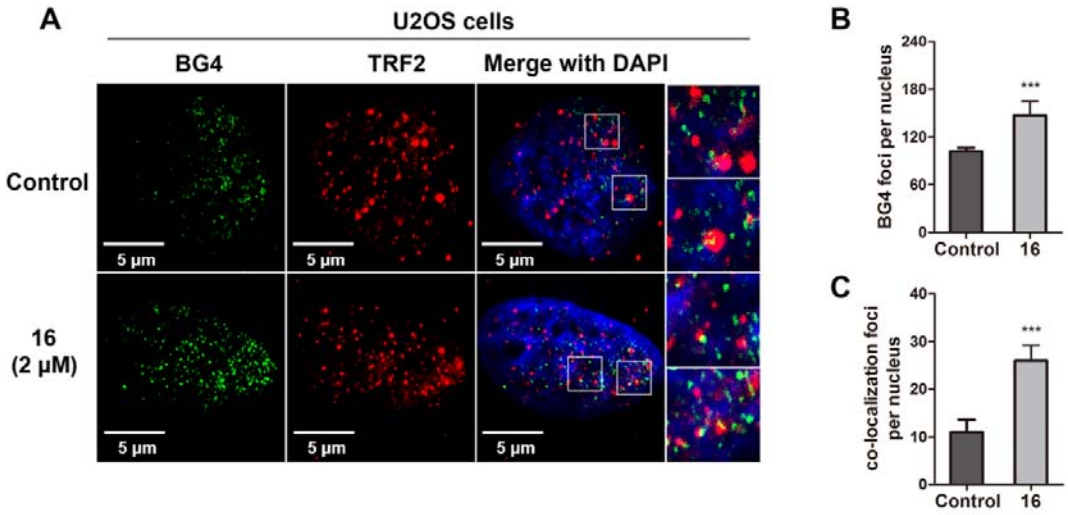


Figure S10. Compound **16** stabilized and induced the formation of telomeric G-quadruplexes in U2OS cells. (A) Representative immunofluorescence images of TRF2 (red) and BG4 (green) foci in U2OS cells (B) Quantification of the number of BG4 foci per nucleus in U2OS cells. (C) Quantification of the co-localization of BG4 and TRF2 per nucleus in U2OS cells.

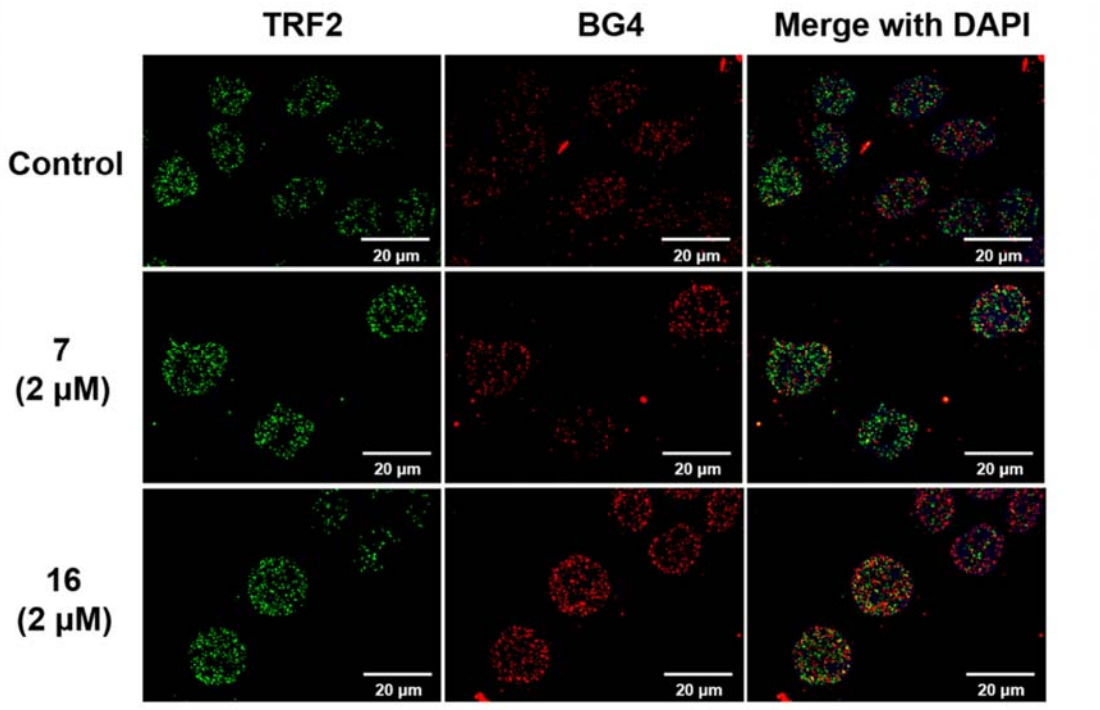


Figure S11. Multiple cells immunofluorescence images of TRF2 (green) and BG4 (red) foci in SiHa cells.

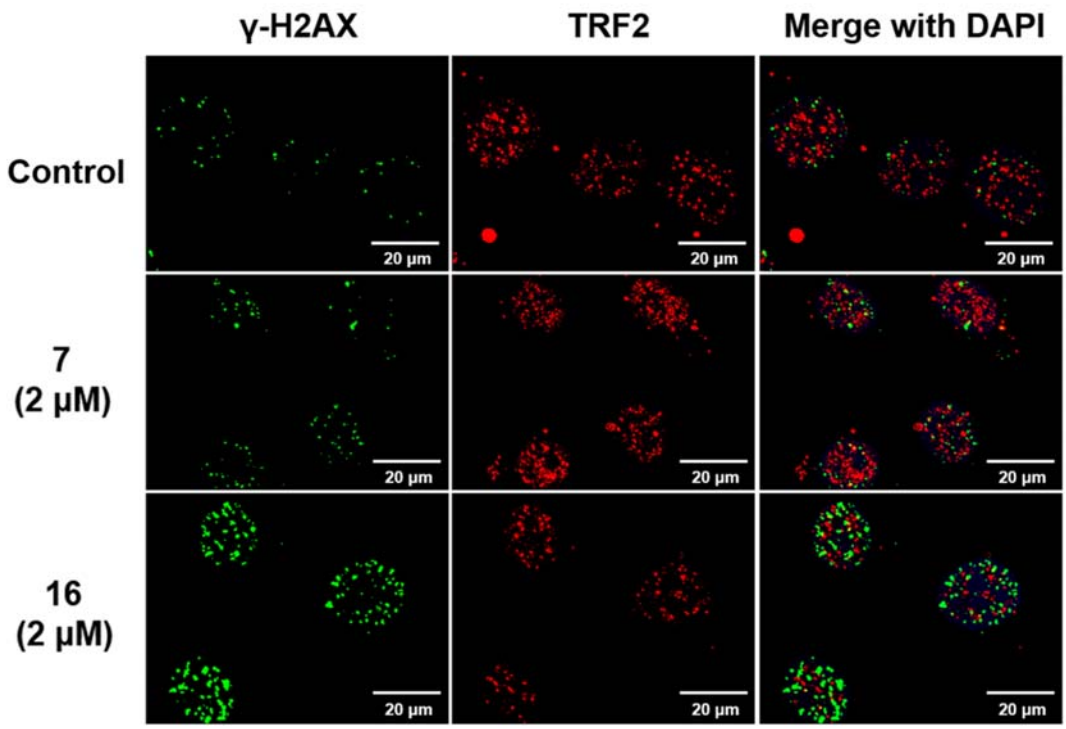


Figure S12. Multiple cells immunofluorescence images of γ-H2AX (green) and TRF2 (red) foci in SiHa cells.

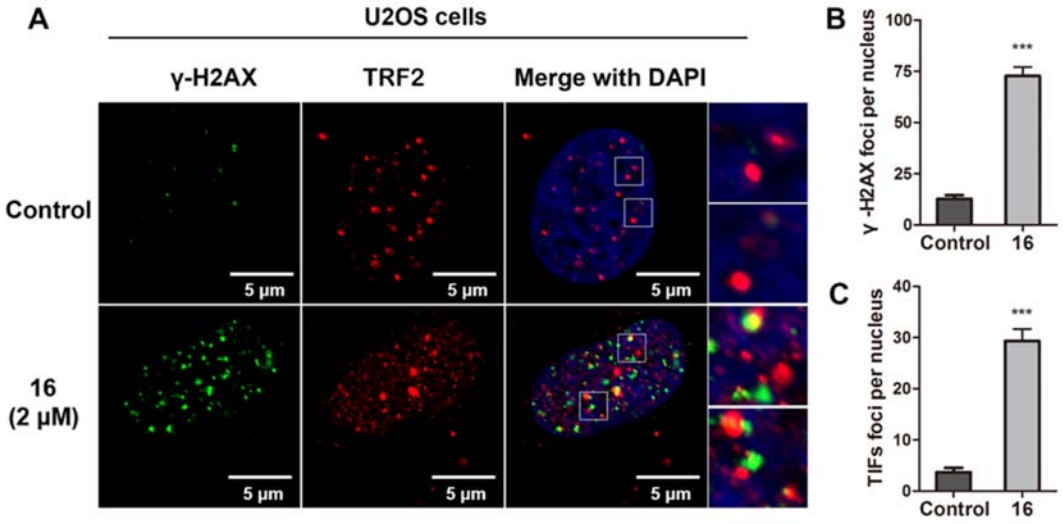


Figure S13. Compound **16** induced telomeric DNA damage in U2OS cells. (A) Representative immunofluorescence images of γ-H2AX (green) and TRF2 (red) foci in U2OS cells (B) Quantification of the number of γ-H2AX foci per nucleus in U2OS cells. (C) Quantification of the co-localization of γ-H2AX and TRF2 per nucleus in U2OS cells.

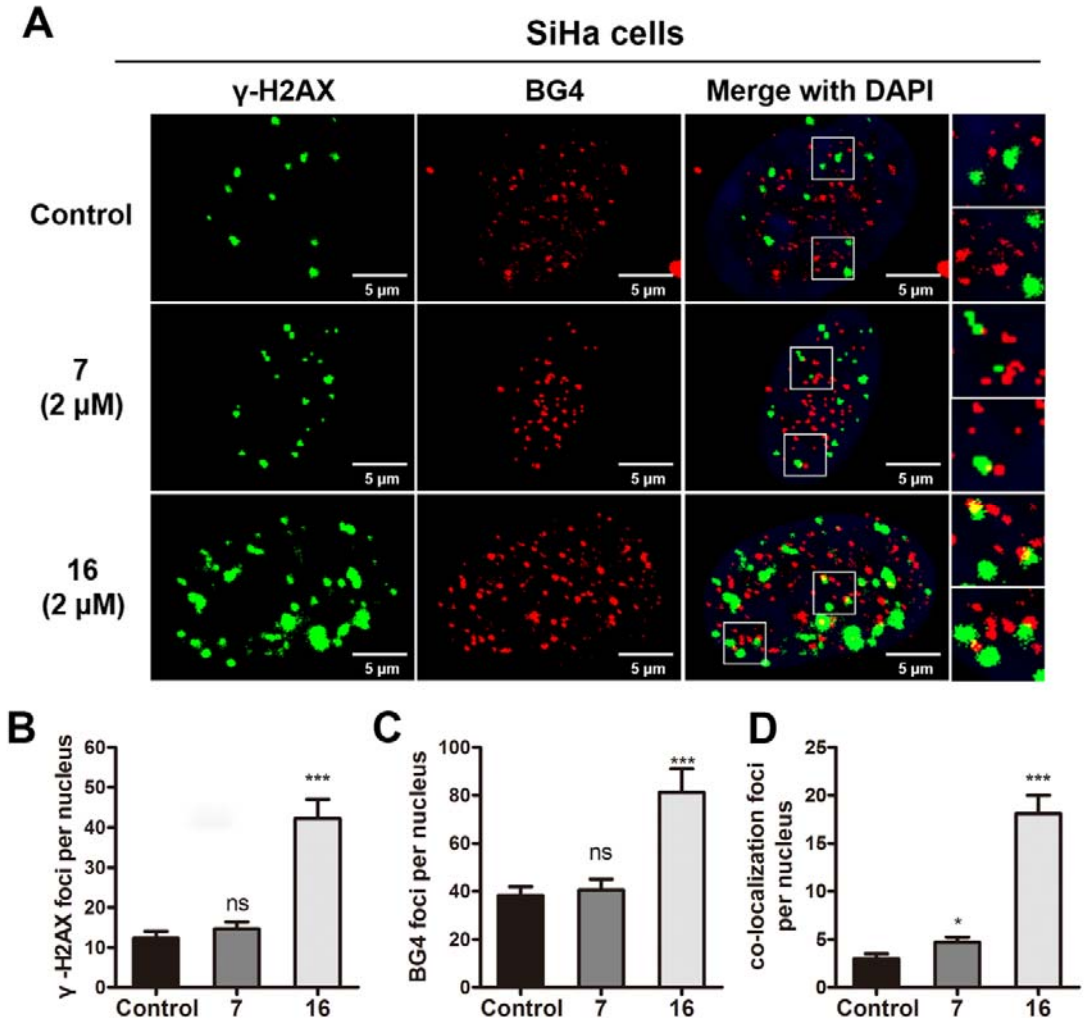


Figure S14. (A) Representative immunofluorescence images of γ-H2AX (green) and BG4 (red) foci in SiHa cells treated with 2 μM **7**, 2 μM **16** or 0.1% DMSO (control) for 24 h. The nuclei were stained with DAPI (blue). (B) The quantification of γH2AX foci number per nucleus. (C) The quantification of co-localization of BG4 and γH2AX per nucleus.

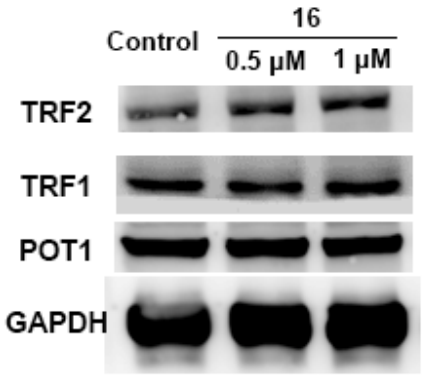


Figure S15. Expression of telomeric related proteins in SiHa cells. Cells were treated with 1 μM **16** or 0.1% DMSO for 48 h.

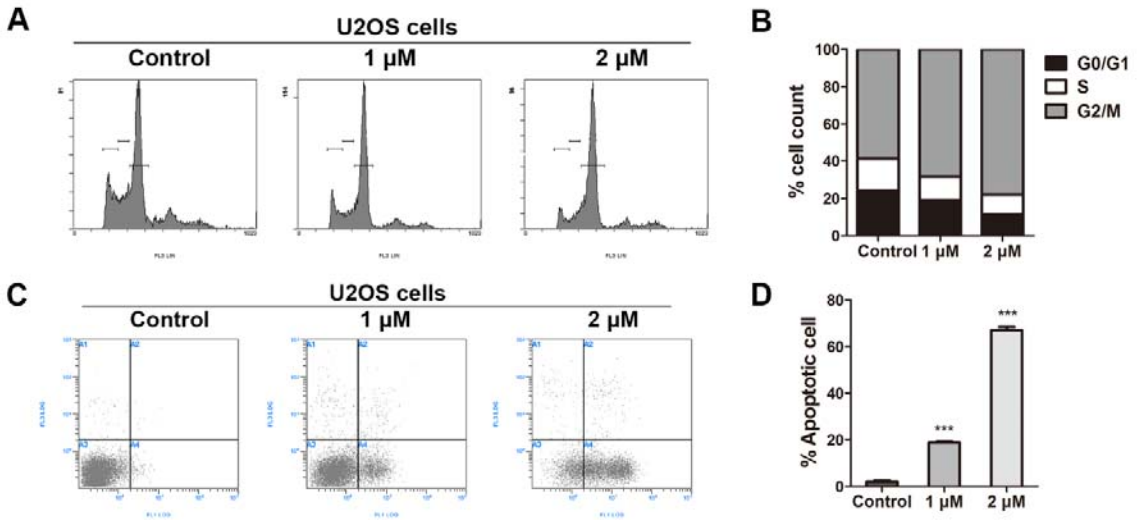


Figure S16. Compound **16** induced cell cycle arrest and apoptosis in U2OS cells. (A) Cell cycle analysis after propidium iodide (PI) staining after a 12-h treatment with **16** or 0.1% DMSO in U2OS cells. (B) The percentage of U2OS cells in different phases of the cell cycle, analyzed by EXPO32 ADC software. (C) Apoptosis evaluation of U2OS cells after a 96-h treatment with **16**. The cells were collected and stained with Annexin V-FITC and PI. (D) The percentage of apoptosis in U2OS cells, analyzed by EXPO32 ADC software.

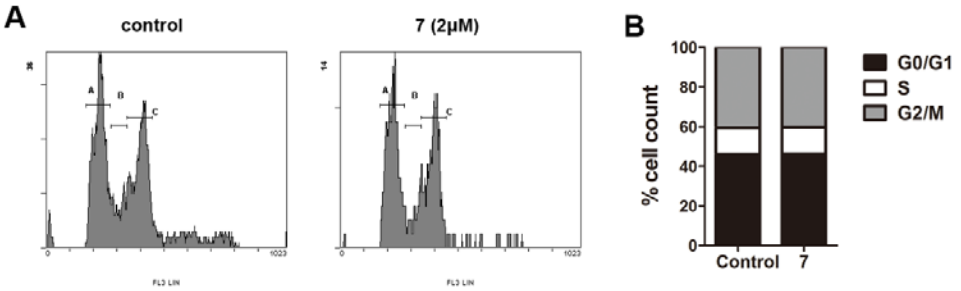


Figure S17. Cell cycle analysis of compound **7**. (A) Cell cycle analysis after PI staining after a 12-h treatment with **7** or 0.1% DMSO in SiHa cells. (B) The percentage of SiHa cells in different phases of the cell cycle, analyzed by EXPO32 ADC software.

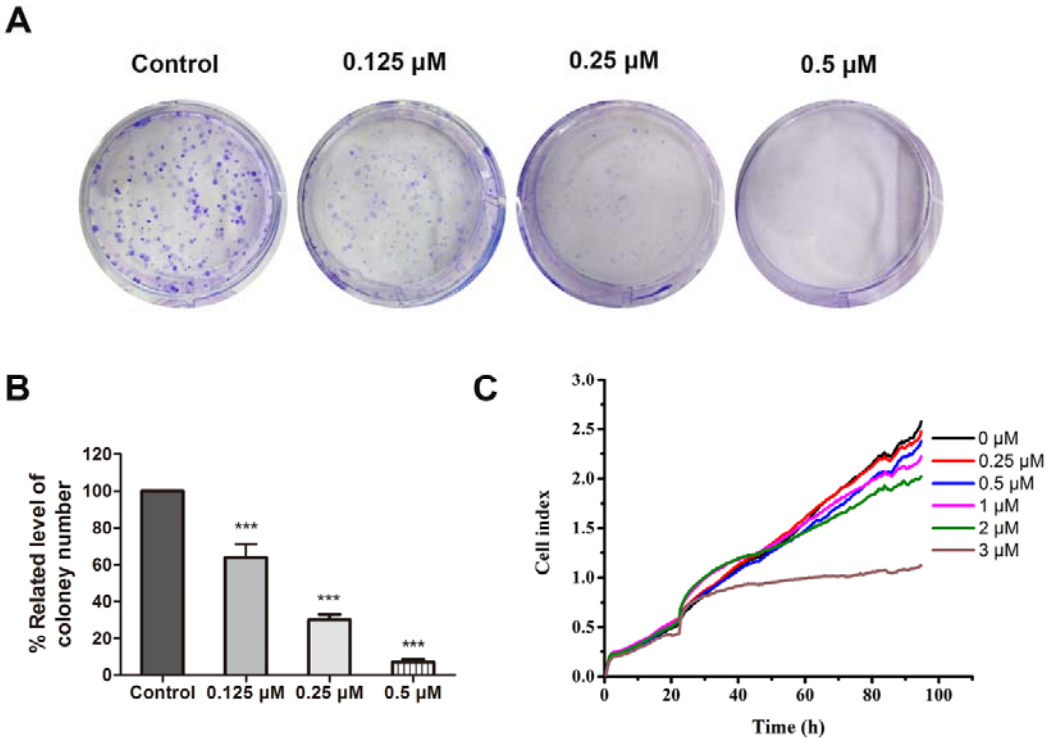


Figure S18. Antiproliferation activities of compound **16**. (A) Colony formation of SiHa cells in the presence of **16**. Cells were plated in a six wells dish with increasing concentrations of **16** for 12 days and stained with crystal violet before counting. (B) Quantification of colony numbers, the value of group without compound was set as 100%. (C) RTCA analysis of SiHa cells with **16** treatment. The growth curves of cells were obtained from the RTCA software 1.1.2.

Methods

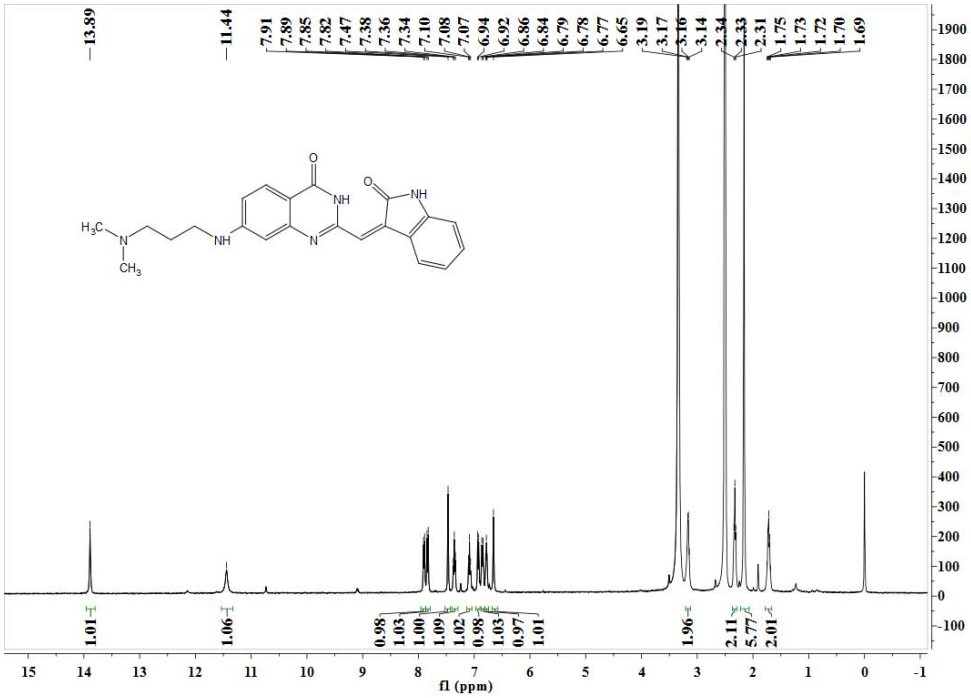
CD melting assay. CD studies were performed on a Chirascan circular dichroism spectrophotometer (Applied Photophysics, UK). A quartz cuvette with a 4 mm path length was used to record the spectra over a wavelength range of 230-330 nm with a 1nm bandwidth, 1 nm step size and a time of 0.5 s per point. A CD melting assay was performed at a fxed concentration of G-quadruplex (2 μM), either with or without a fixed concentration (10 μM) of compounds in Tris-HCl buffer (10 mM, pH 7.4) with 60 mM KCl. The data were recorded at intervals of 2.5 °C over the range 25-95 °C with a heating rate of 1°C/min. A buffer baseline was collected in the same cuvette and was subtracted from the sample spectra. The final analysis of the data was conducted using Origin 9.0 (OriginLab Corp.)

TRAP-LIG Assay. The ability of schizocommunin derivatives to inhibit telomerase in a cell-free system was assessed with the TRAP-LIG assay following previously published procedures.[47] Protein extracts from exponentially growing SiHa cells were used. Briefly, 0.1 μg of TS forward primer (5′-AATCCGTCGAGCAGAGTT-3′) was elongated by telomerase (500 ng protein extract) in TRAP buffer (20 mM Tris-HCl [pH 8.3], 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, and 0.05% Tween 20) containing 125 μM dNTPs and 0.05 μg BSA. The mix was added to tubes containing freshly prepared ligand at various concentrations and to a negative control containing no ligand. The initial elongation step was carried out for 30 min at 30 °C, followed by 94 °C for 5 min and a final maintenance of the mixture at 10 °C. Then the ligands were removed by phenol extraction and ethanol precipitation. The purified extended samples were then subject to PCR amplification. For this, a second PCR master mix was prepared consisting of 1 μM ACX reverse primer (5′-GCGCGG[CTTACC]$_3$CTAACC-3′), 0.1 μg TS forward primer (5′-AATCCGTCGAGCAGAGTT-3′), TRAP buffer, 5 μg BSA, 0.5 mM dNTPs, and 2 units of Taq polymerase. A 10 μL aliquot of the master mix was added to the purified telomerase extended samples and amplified for 35 cycles of 94 °C for 30 s, of 61 °C for 1 min, and of 72 °C for 1 min. Samples were separated on a 12% PAGE and visualized with EB. $^{Tel}IC_{50}$ values were then calculated from the optical density quantitated from the AlphaEaseFC software.

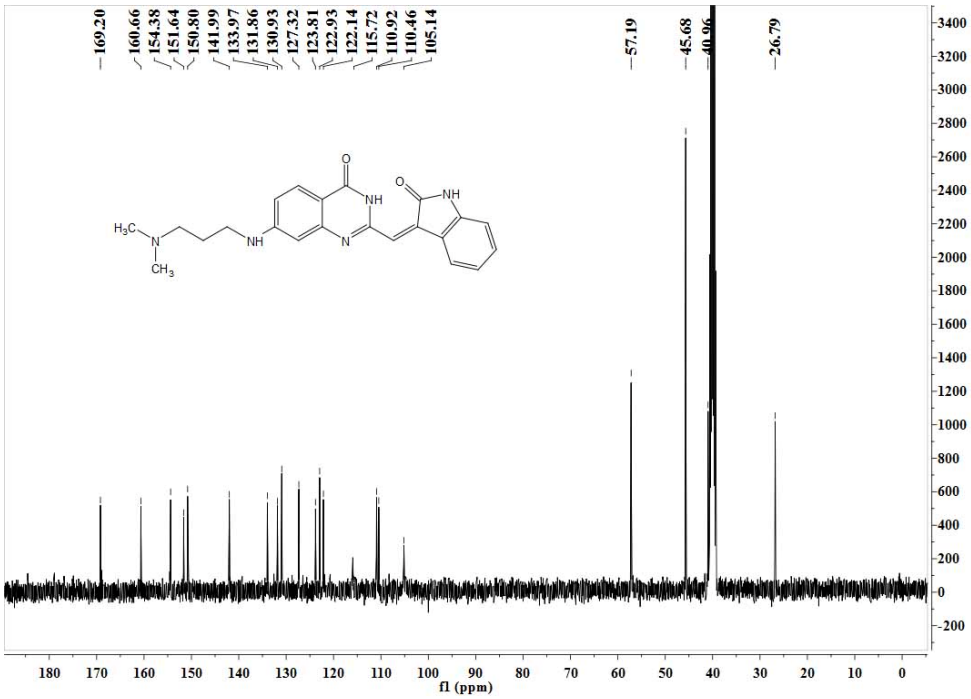
Colony formation assay. SiHa cells were seeded on 6-well plates (300/well) and treated with compound **16** or DMSO. DMEM was replaced and different concentrations of **16** were added every 2 days. Cells were fixed with methanol and dyed with crystal violet after cultured 12 days. The pictures were taken using a x40 magnification using a FL Auto Imaging System EVOS microscope (Life Technologies).

Real-Time Cellular Activity Assay. Compound-mediated cytotoxicity was monitored using a real-time cellular analysis (RTCA) multiplate instrument, xCELLigence RTCA DP instrument (Roche Applied Science, Indianapolis, IN), which was placed in a humidified incubator at 37 °C and 5% CO_2. This system monitors cellular events (cell viability, cell number, cell morphology, and degree of adhesion) in real time measuring electrical impedance across interdigitated microelectrodes integrated on the bottom of tissue culture E-Plates 16-well plates (Roche Applied Science, Indianapolis, IN). The evaluation of cellular events was performed on SiHa cells. For time-dependent cell response profiling, 50 µL of media was added to 16-well electric plates (E-Plates) to obtain background readings followed by the addition of 100 µL of cell suspension at the density of 20,000 cells/mL. After seeding, cells were allowed to settle for 20 min at room temperature before being inserted into the xCELLigence RTCA DP instrument. After 24 h, the cells were treated compounds or DMSO at various concentrations. The cells were monitored every 2 min for duration of 2 h after compounds addition to capture the short-term response and for every 15 min from 2 h after compound addition until the end of the treatment to capture the long-term response. Results were plotted using the RTCA software 1.1.2 (Roche Applied Science, Indianapolis, IN). The data expressed in cell index (CI) units was exported to Microsoft Excel software (Microsoft, Redmond, WA) for mathematical analysis and normalization.

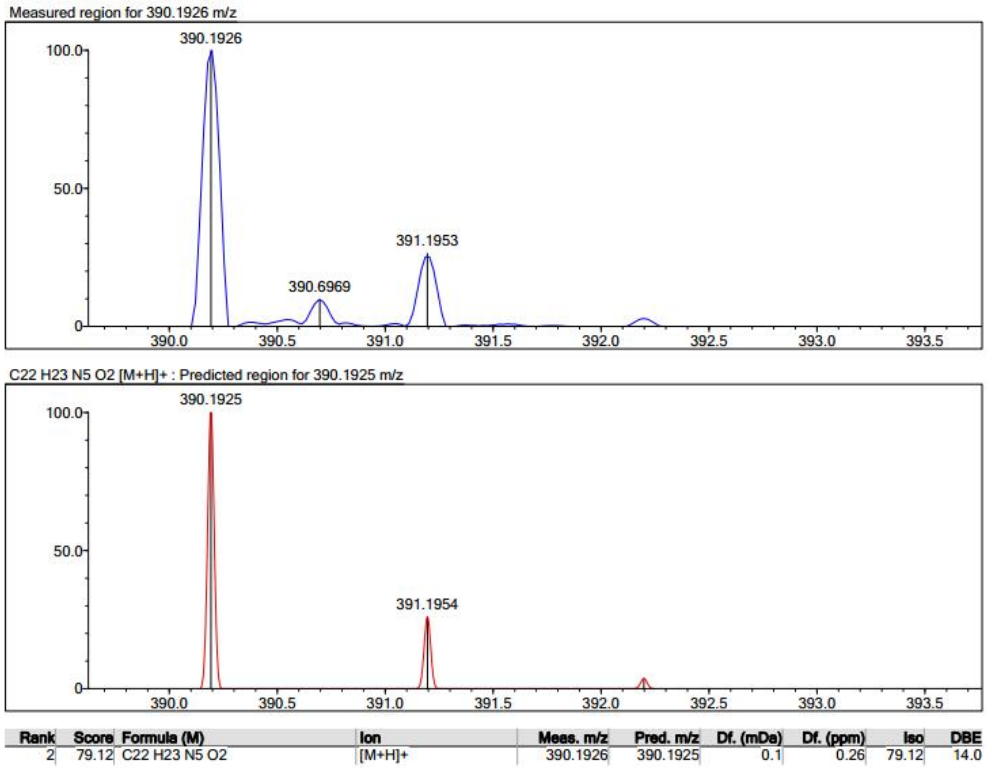
^{1}H NMR, ^{13}C NMR, HRMS and HPLC spectrum of final compounds



^{1}H NMR spectrum of **7**

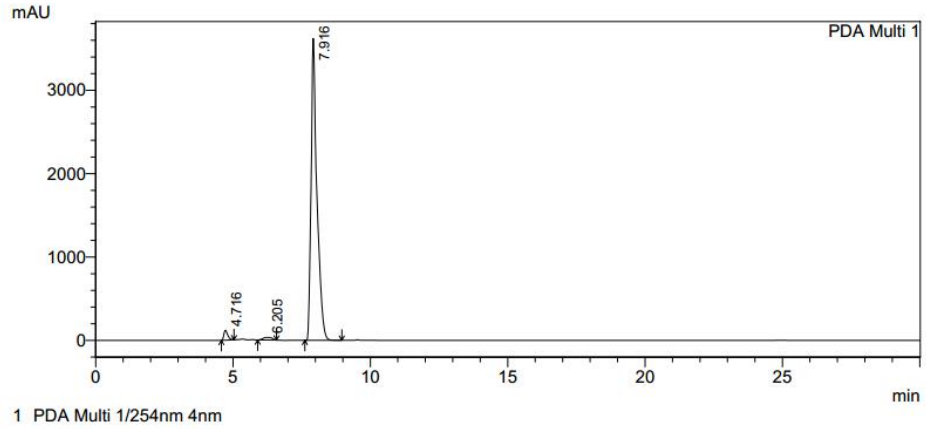


^{13}C NMR spectrum of **7**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	79.12	C22 H23 N5 O2	[M+H]+	390.1926	390.1925	0.1	0.26	79.12	14.0

HRMR spectrum of **7**



1 PDA Multi 1/254nm 4nm

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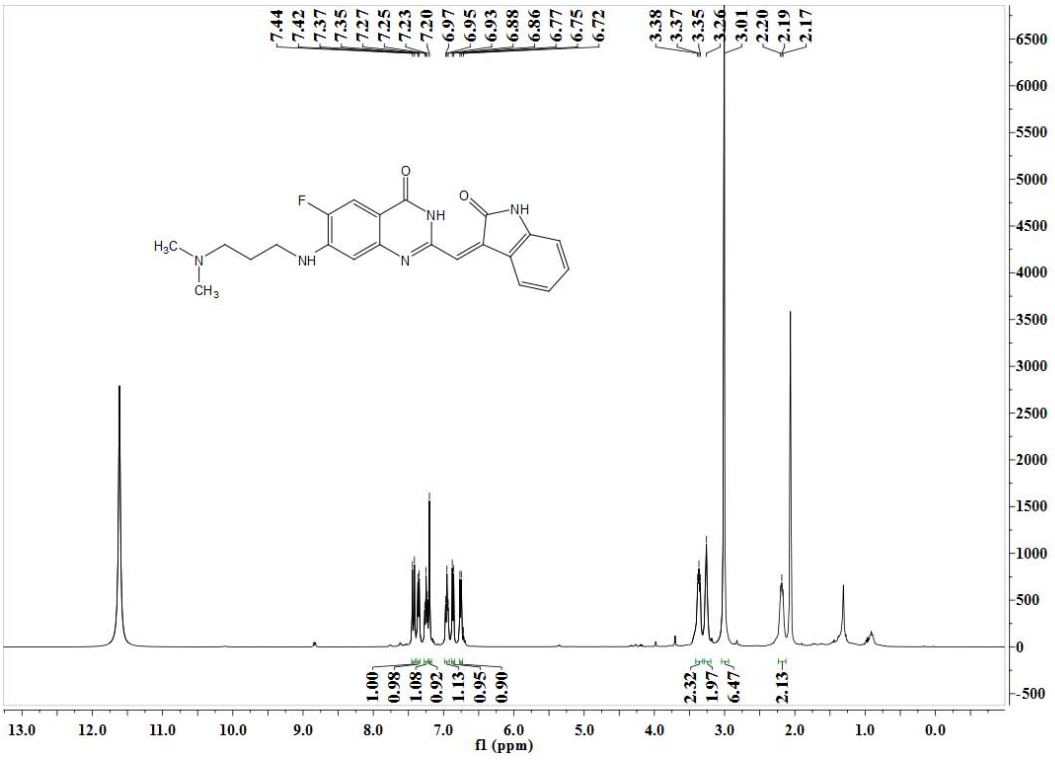
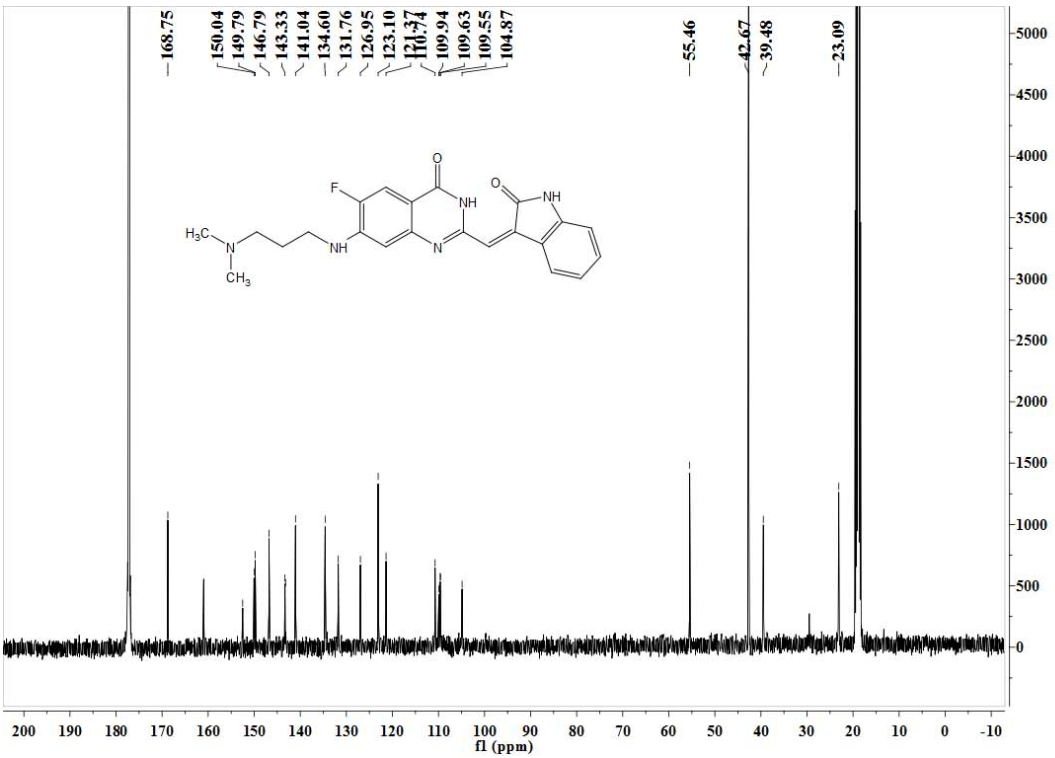
3	7.916	53789230	3621882	96.582	96.018
Total		55693055	3772099	100.000	100.000

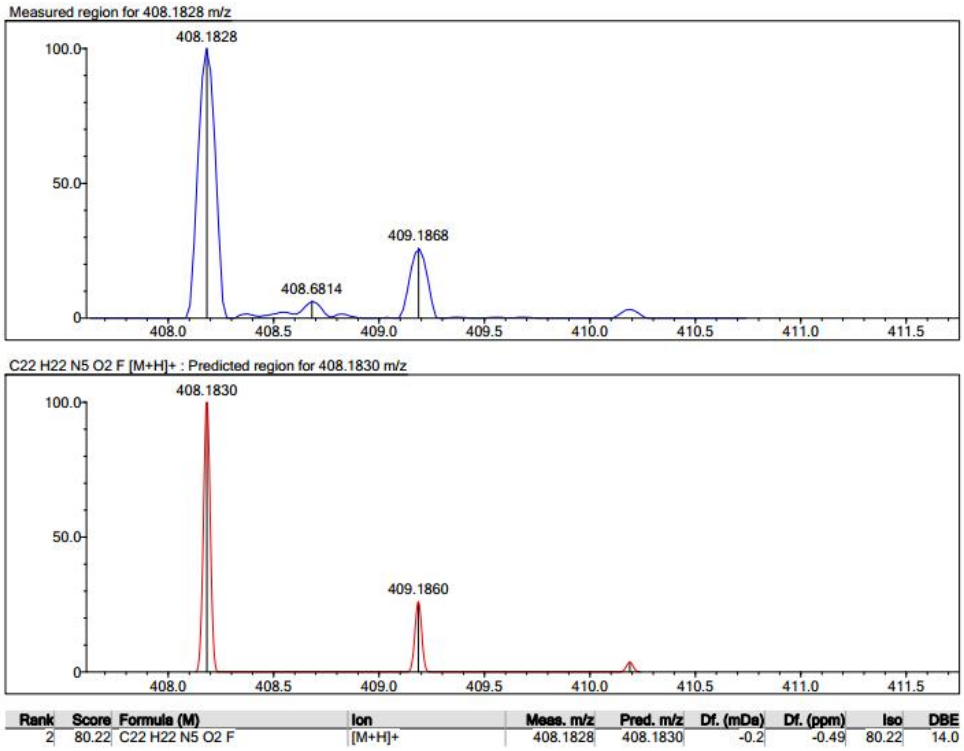
HPLC analysis of **7**



^{1}H NMR spectrum of **8**

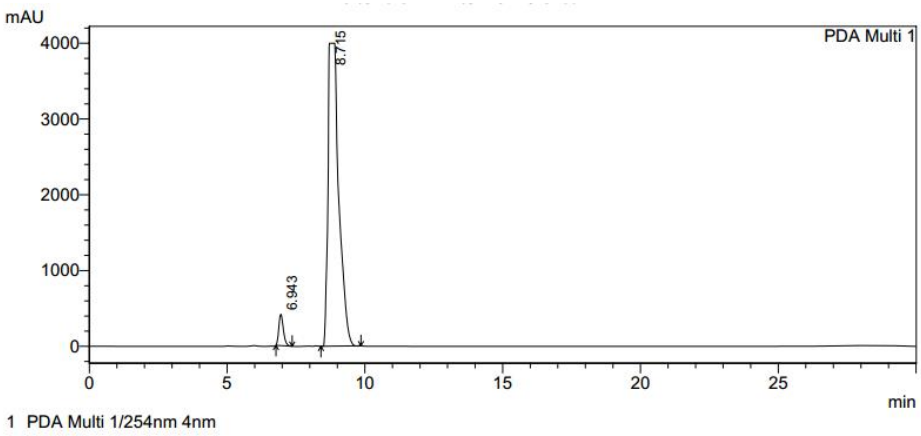


^{13}C NMR spectrum of **8**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	80.22	C22 H22 N5 O2 F	[M+H]+	408.1828	408.1830	-0.2	-0.49	80.22	14.0

HRMR spectrum of **8**



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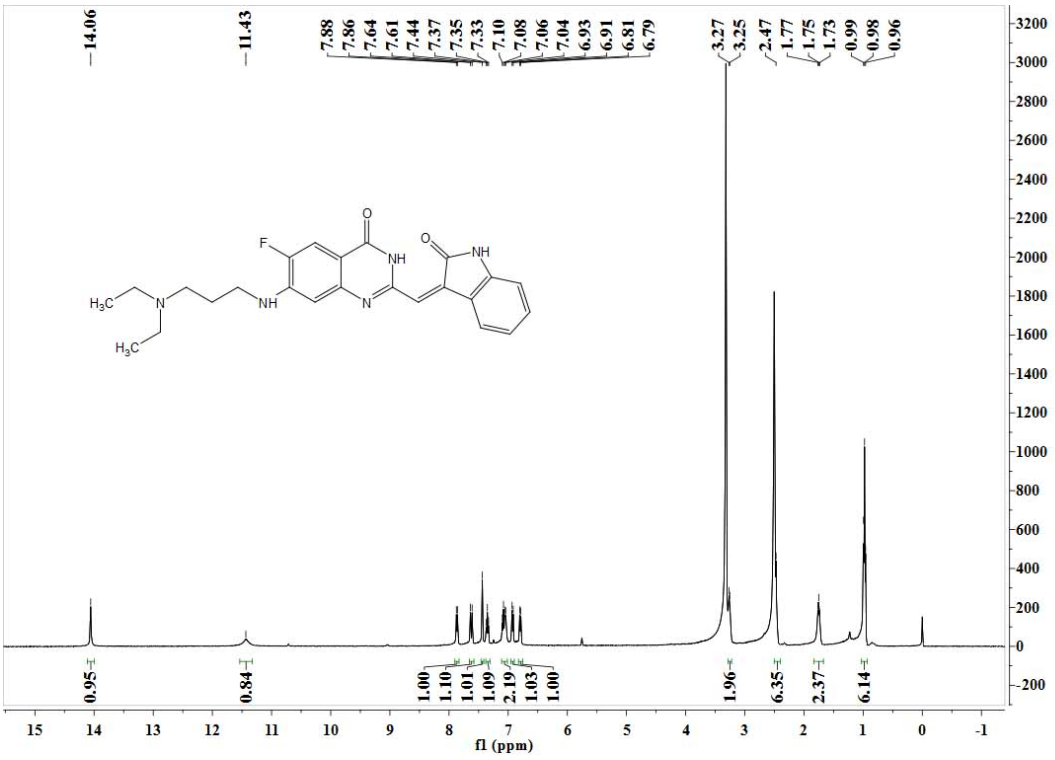
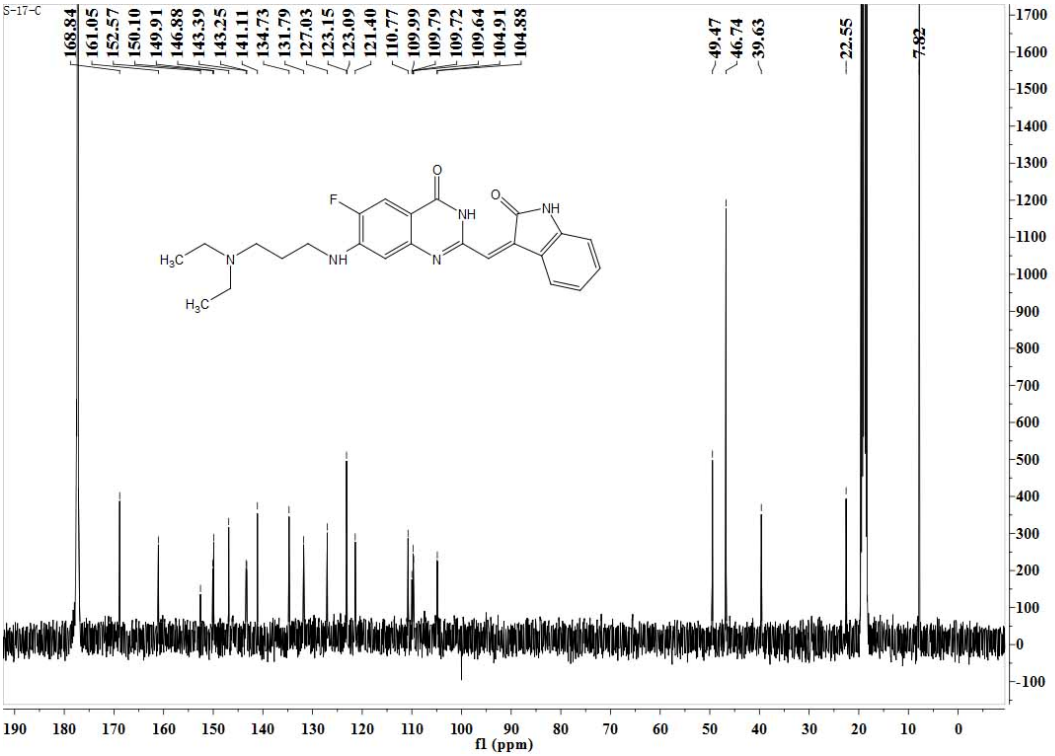
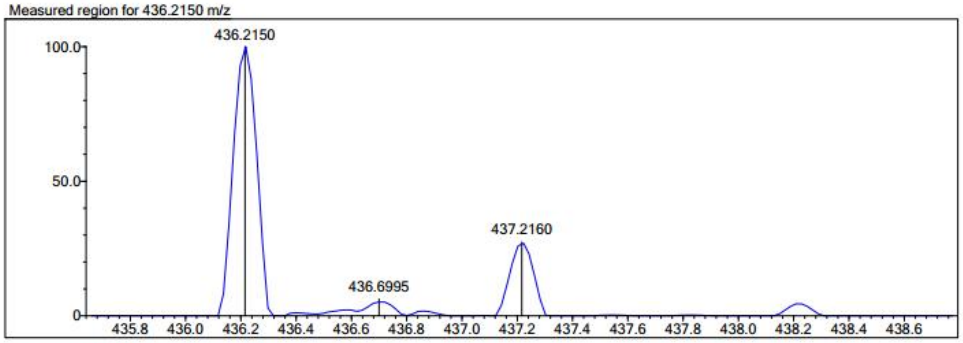
HPLC analysis of **8**

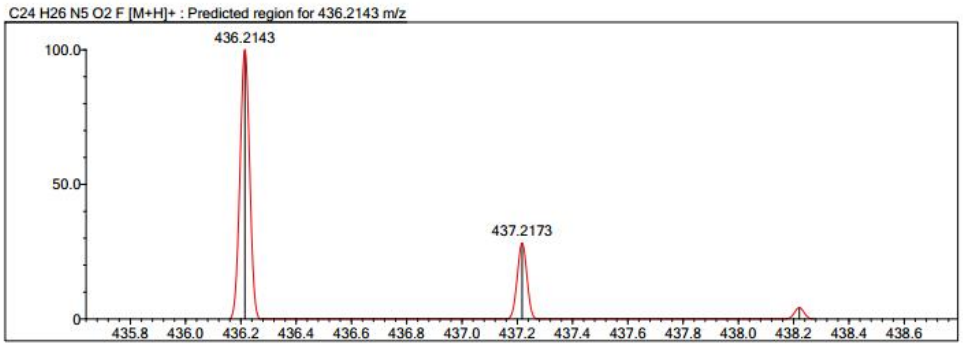


^{1}H NMR spectrum of **9**



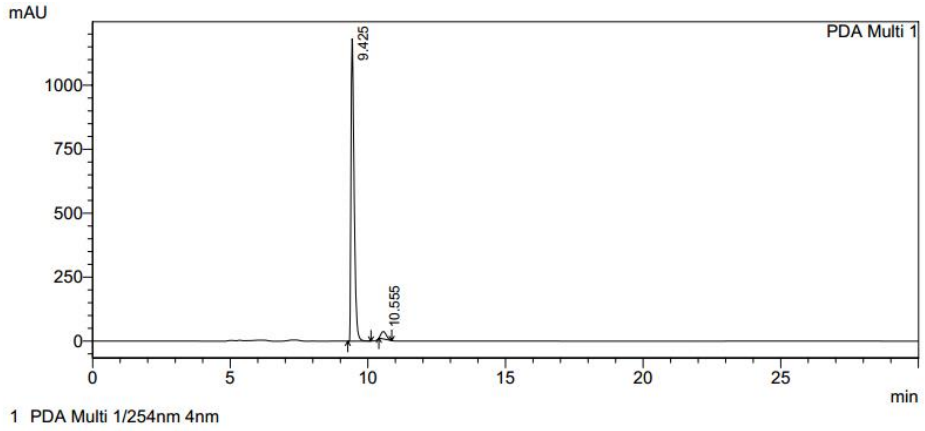
^{13}C NMR spectrum of **9**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	85.31	C24 H26 N5 O2 F	[M+H]+	436.2150	436.2143	0.7	1.60	86.61	14.0

HRMR spectrum of **9**



PeakTable

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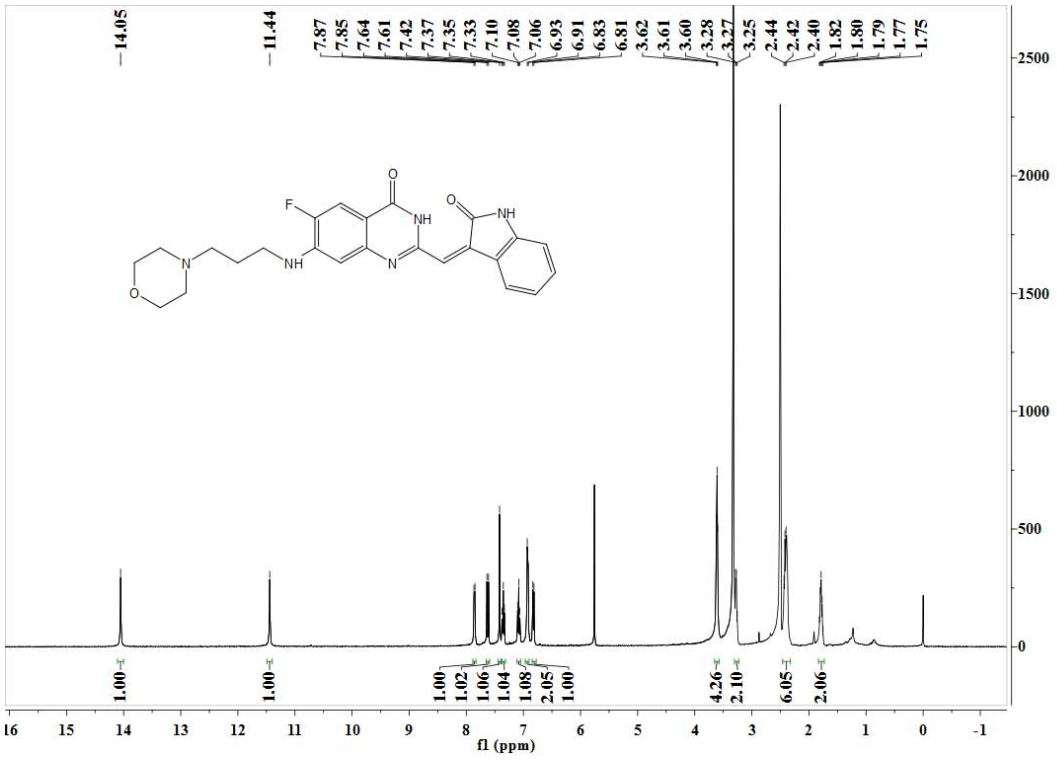
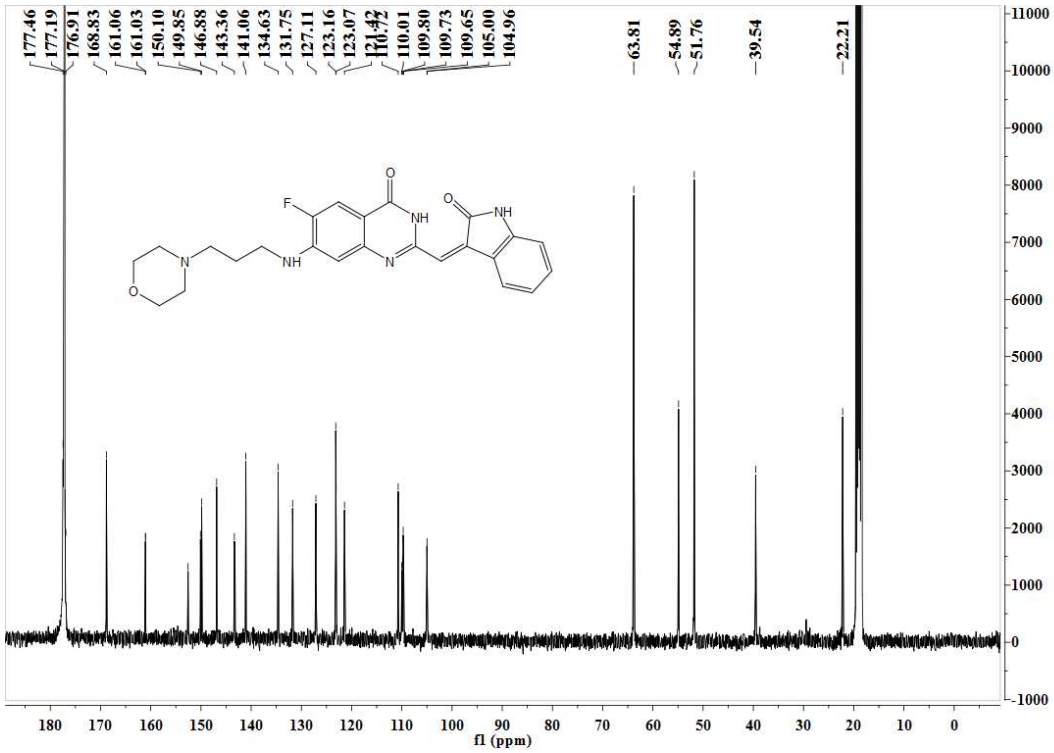
1	9.425	9313964	1182302	96.008	97.621
2	10.555	387291	28808	3.992	2.379
Total		9701255	1211110	100.000	100.000

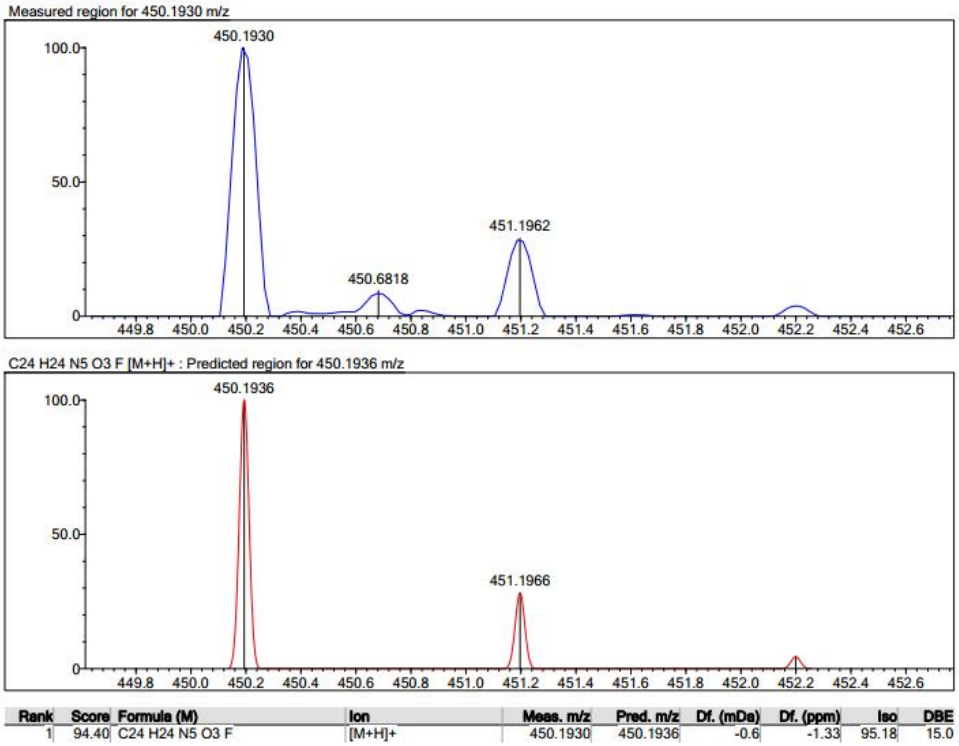
HPLC analysis of **9**



^{1}H NMR spectrum of **10**

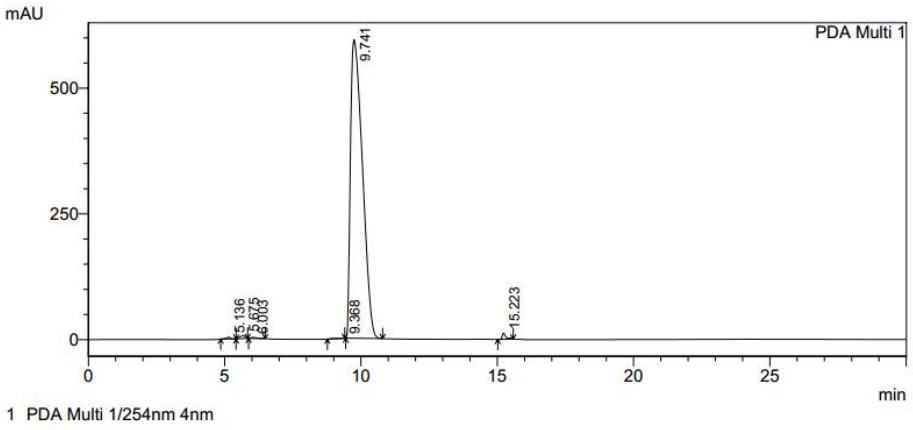


^{13}C NMR spectrum of **10**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	94.40	C24 H24 N5 O3 F	[M+H]+	450.1930	450.1936	-0.6	-1.33	95.18	15.0

HRMR spectrum of **10**

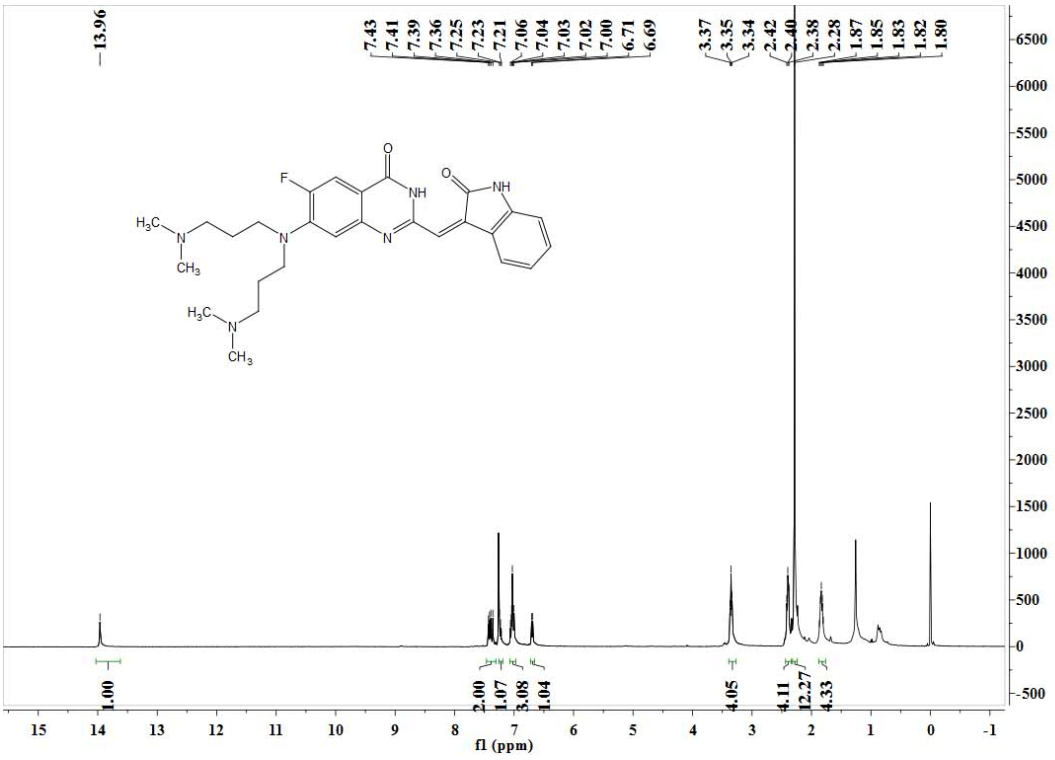


PeakTable

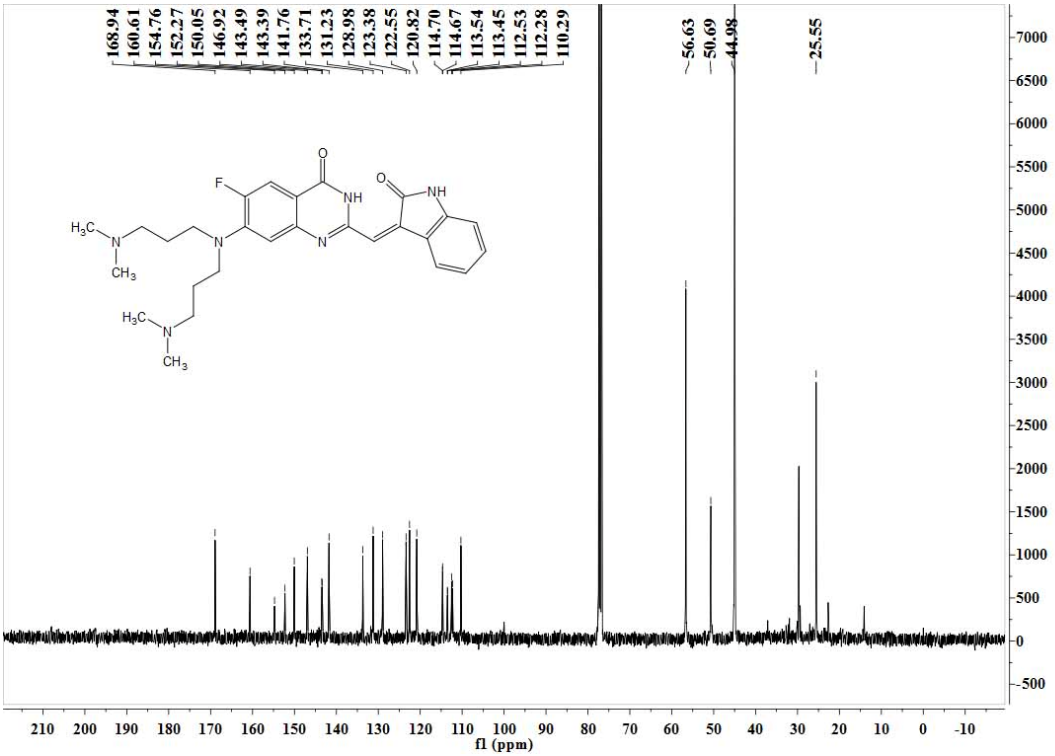
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.136	35991	4035	0.196	0.652
2	5.675	79626	6078	0.433	0.983
3	6.003	29897	2034	0.163	0.329
4	9.368	14481	102	0.079	0.016
5	9.741	18113646	594061	98.506	96.044
6	15.223	114810	12221	0.624	1.976
Total		18388450	618532	100.000	100.000

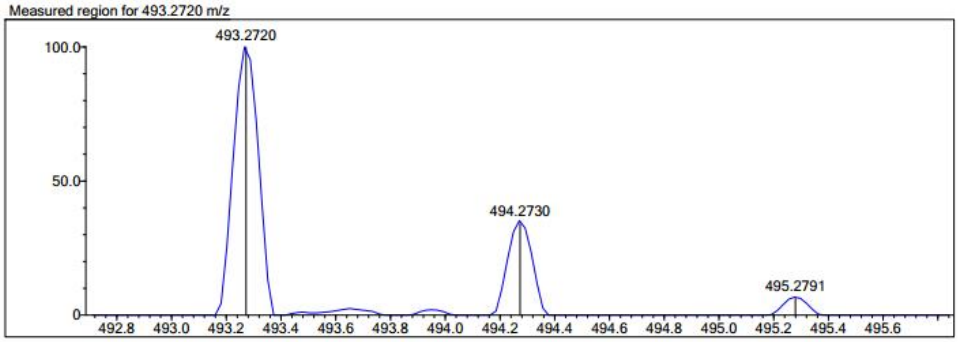
HPLC analysis of **10**



^{1}H NMR spectrum of **11**

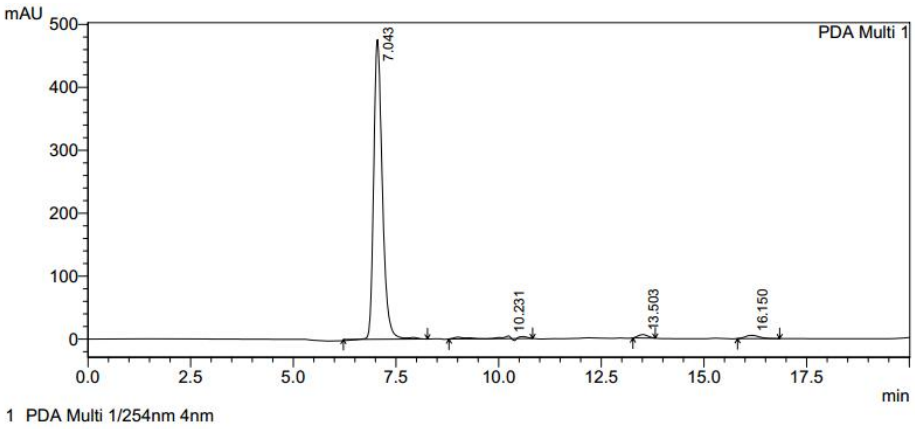


^{13}C NMR spectrum of **11**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	80.69	C27 H33 N6 O2 F	[M+H]+	493.2720	493.2722	-0.2	-0.41	80.69	14.0

HRMR spectrum of **11**

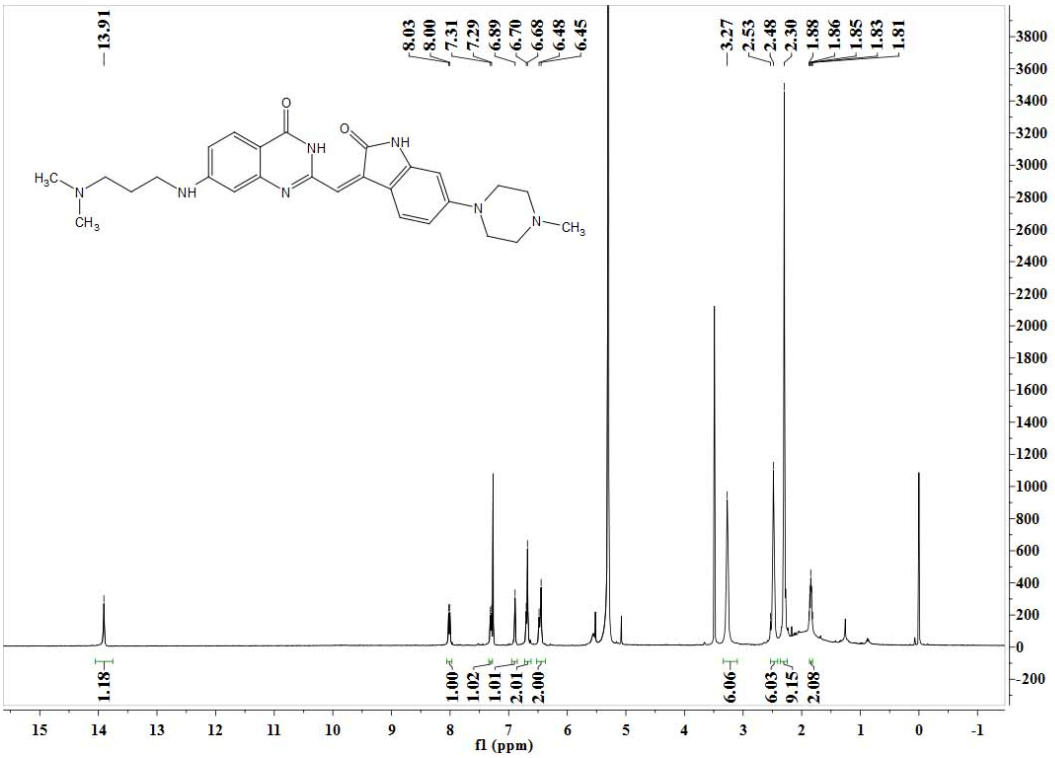


PeakTable

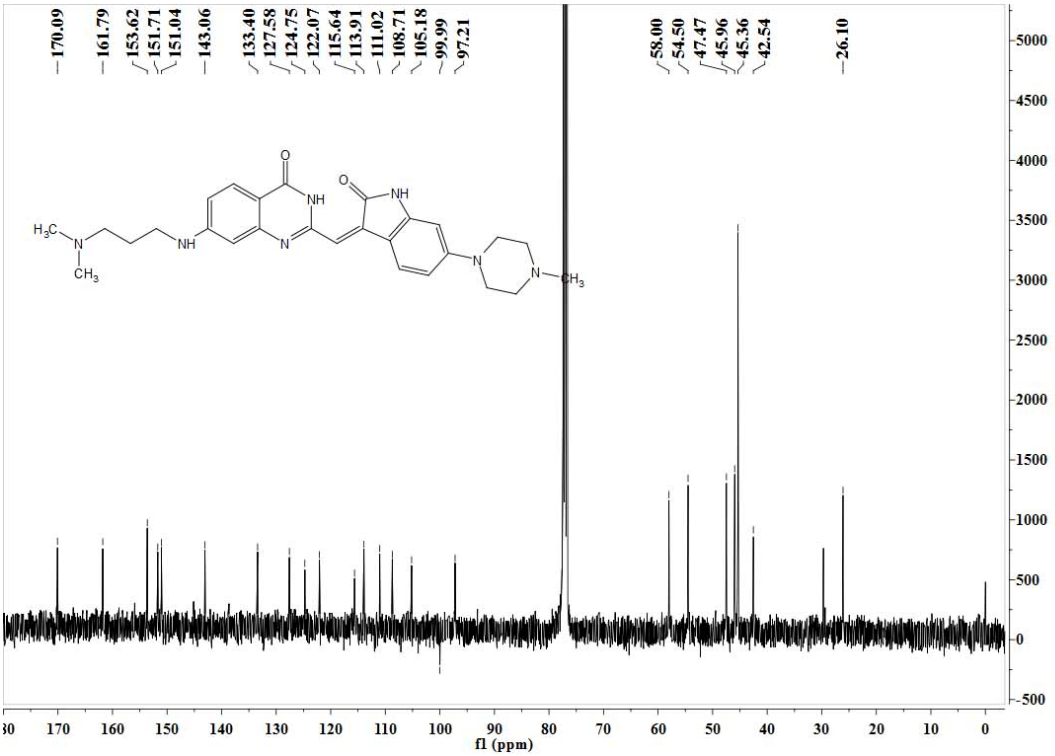
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.043	6992016	476396	95.065	96.952
2	10.231	146996	4287	1.999	0.872
3	13.503	91924	5473	1.250	1.114
4	16.150	124020	5217	1.686	1.062
Total		7354955	491372	100.000	100.000

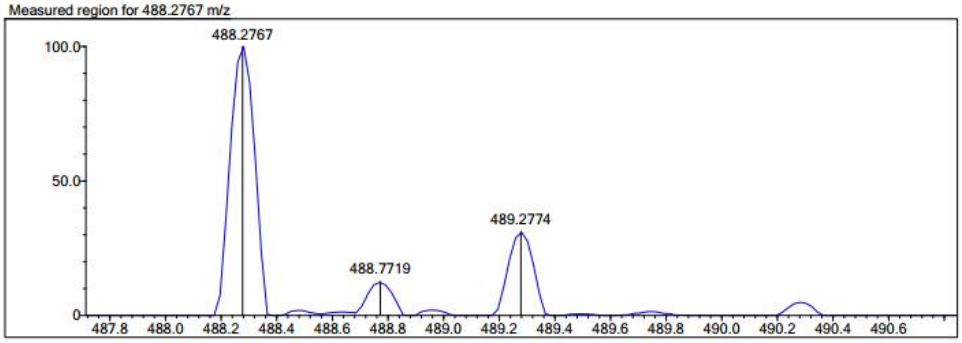
HPLC analysis of **11**



^{1}H NMR spectrum of **12**

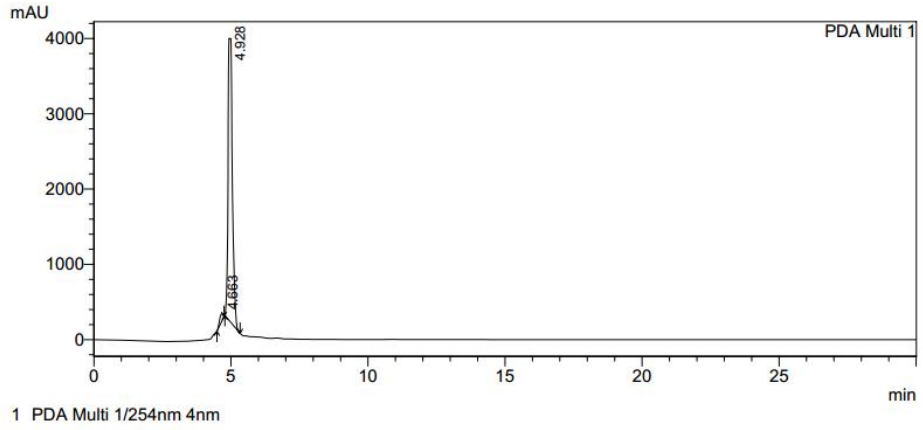


^{13}C NMR spectrum of **12**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	83.13	C27 H33 N7 O2	[M+H]+	488.2767	488.2768	-0.1	-0.20	83.13	15.0

HRMR spectrum of **12**

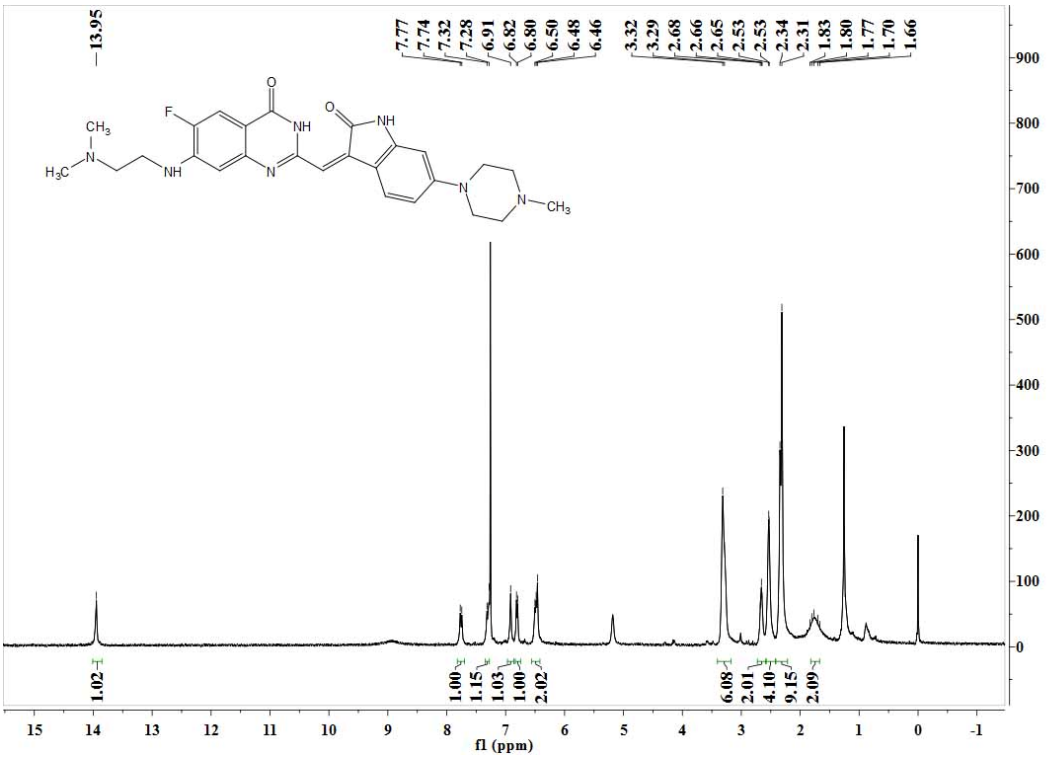


PeakTable

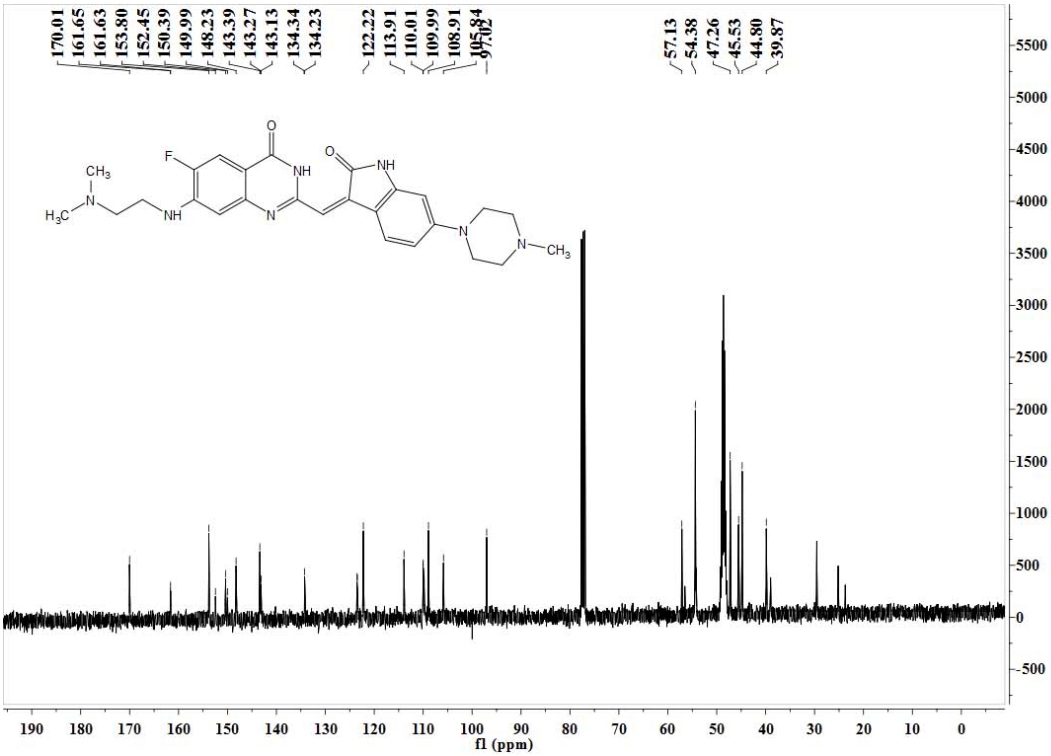
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.663	955157	101179	2.270	2.633
2	4.928	41119741	3741450	97.730	97.367
Total		42074898	3842629	100.000	100.000

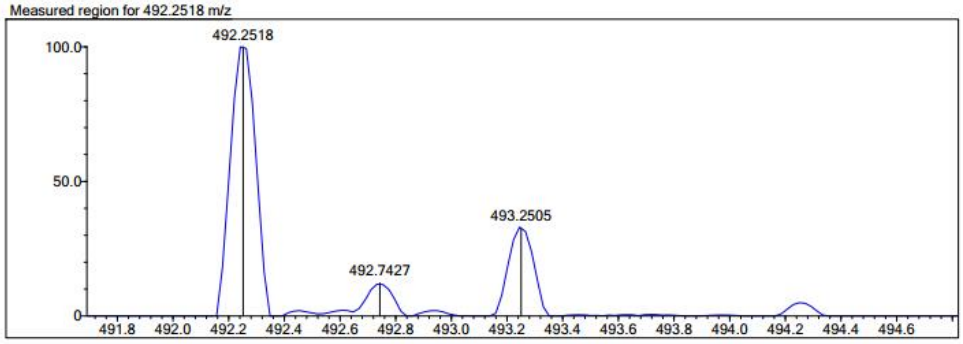
HPLC analysis of **12**



^{1}H NMR spectrum of **13**

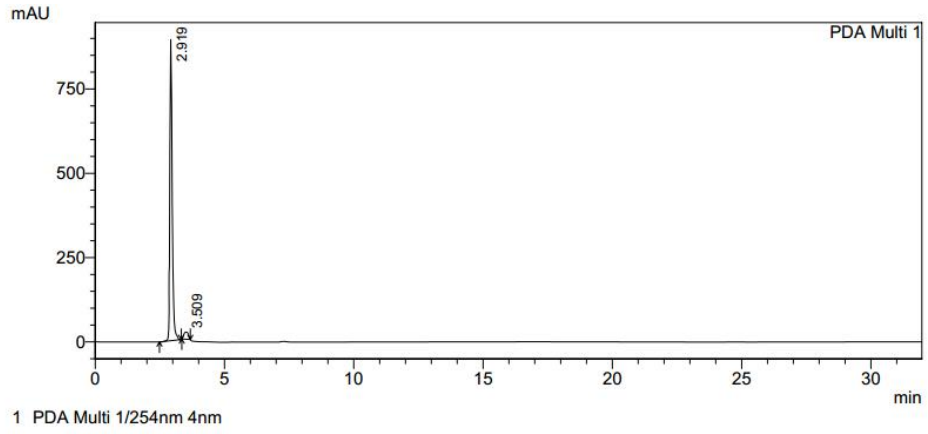


^{13}C NMR spectrum of **13**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.09	C26 H30 N7 O2 F	[M+H]+	492.2518	492.2518	0.0	0.00	90.09	15.0

HRMR spectrum of **13**

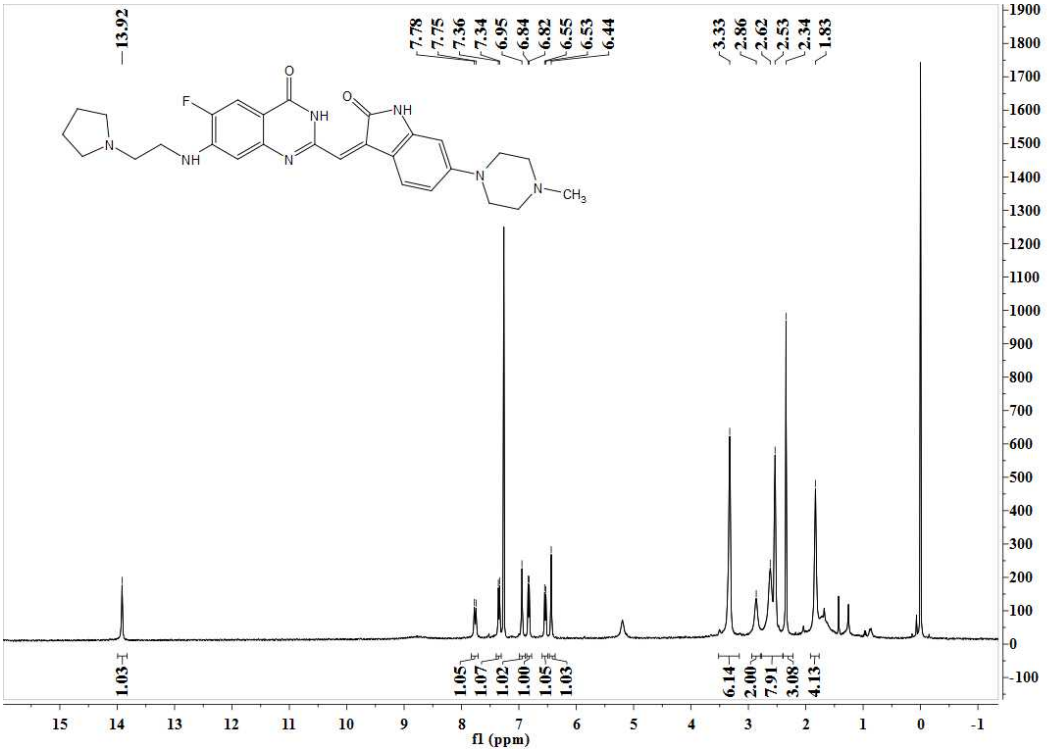


PeakTable

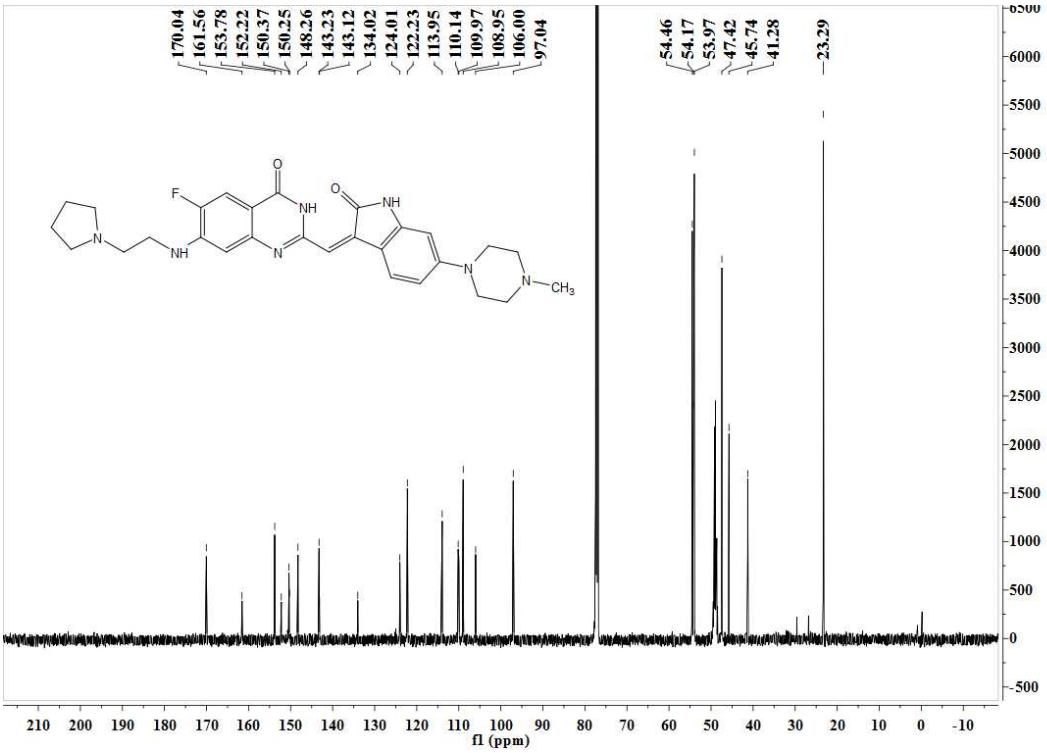
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.919	5662679	893424	95.541	97.701
2	3.509	264259	21022	4.459	2.299
Total		5926938	914445	100.000	100.000

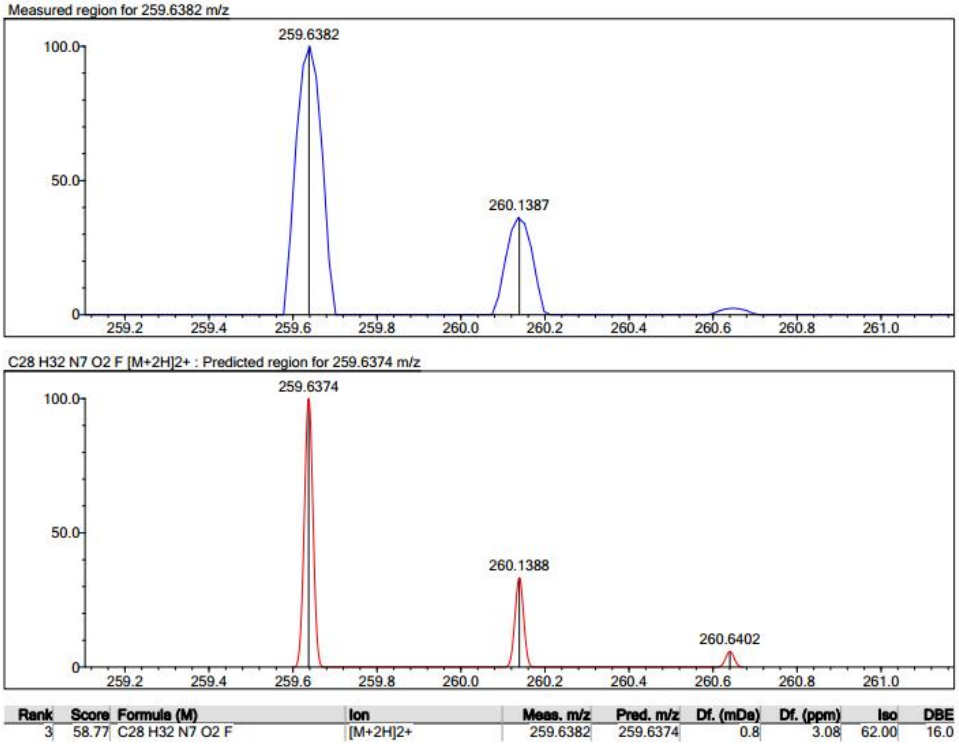
HPLC analysis of **13**



^{1}H NMR spectrum of **14**

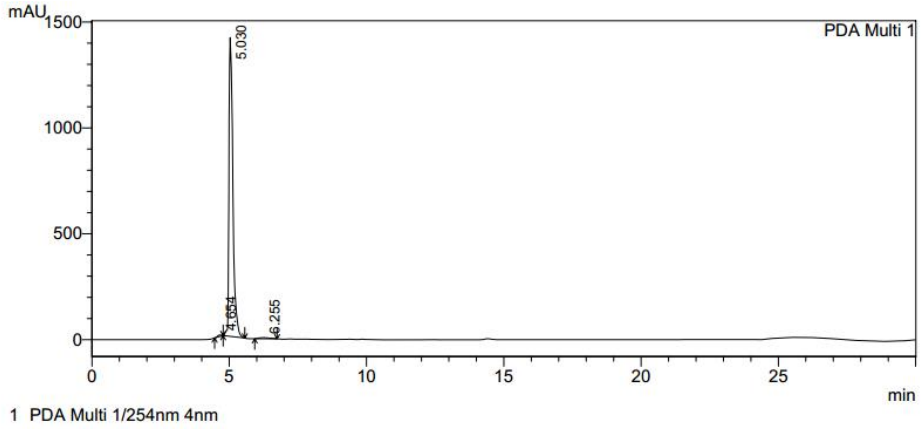


^{13}C NMR spectrum of **14**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	58.77	C28 H32 N7 O2 F	[M+2H]2+	259.6382	259.6374	0.8	3.08	62.00	16.0

HRMR spectrum of **14**

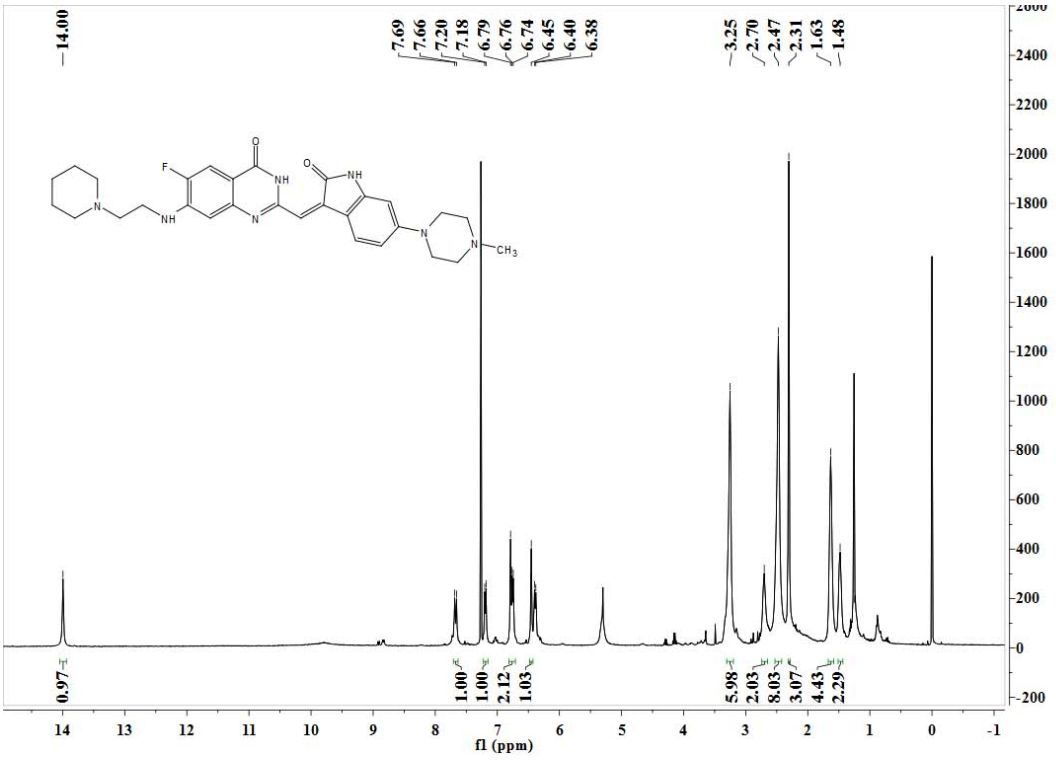


PeakTable

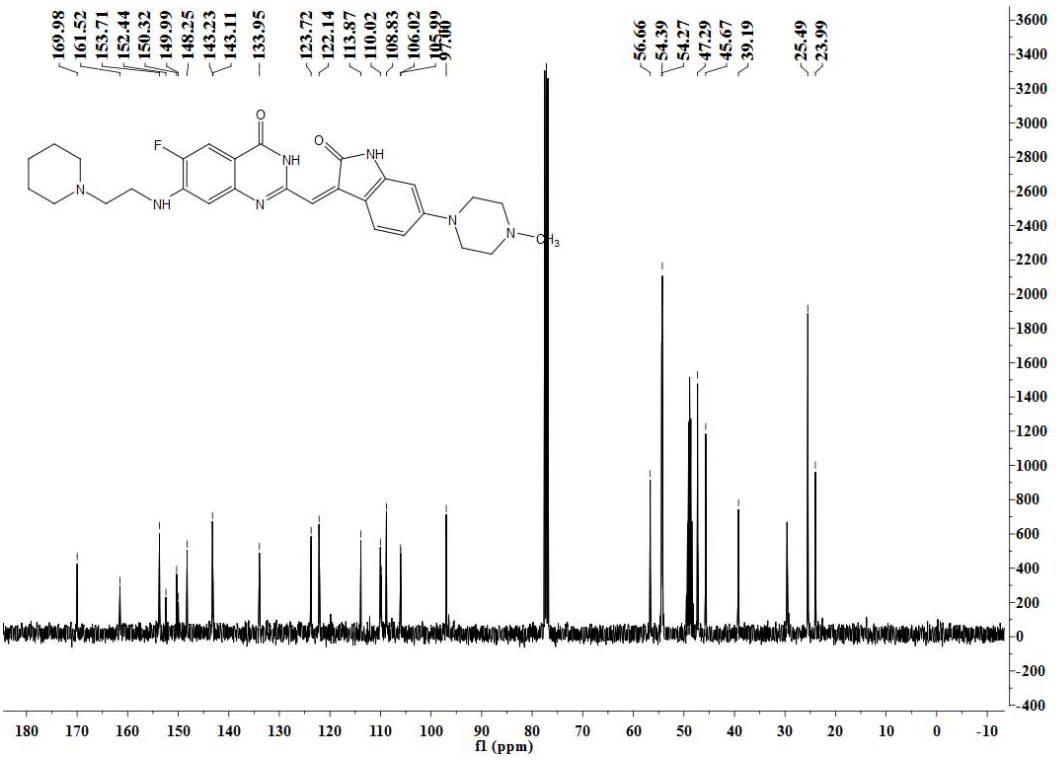
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.654	66759	7759	0.466	0.545
2	5.030	14102248	1410423	98.522	99.095
3	6.255	144761	5118	1.011	0.360
Total		14313769	1423299	100.000	100.000

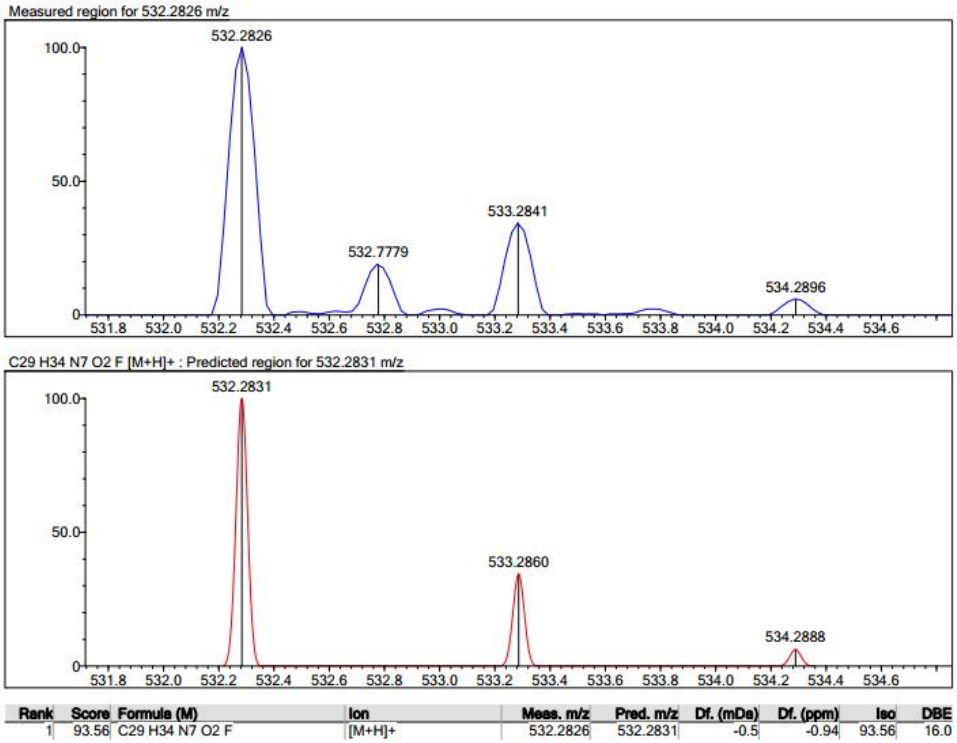
HPLC analysis of **14**



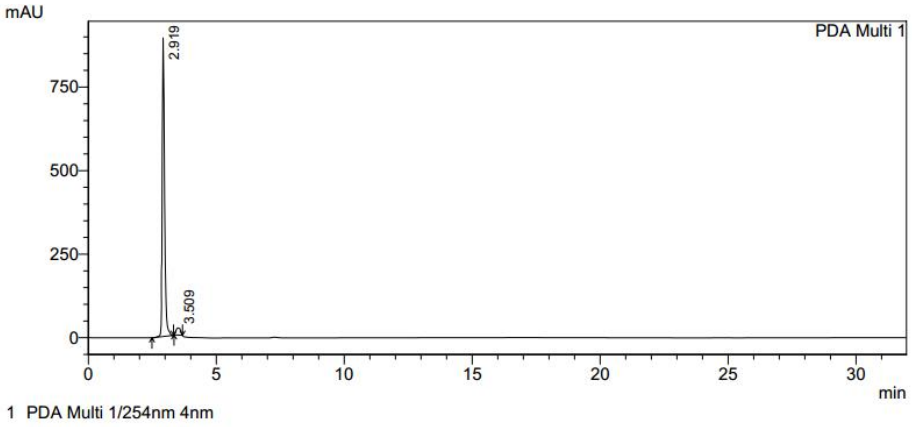
^{1}H NMR spectrum of **15**



^{13}C NMR spectrum of **15**



HRMR spectrum of **15**

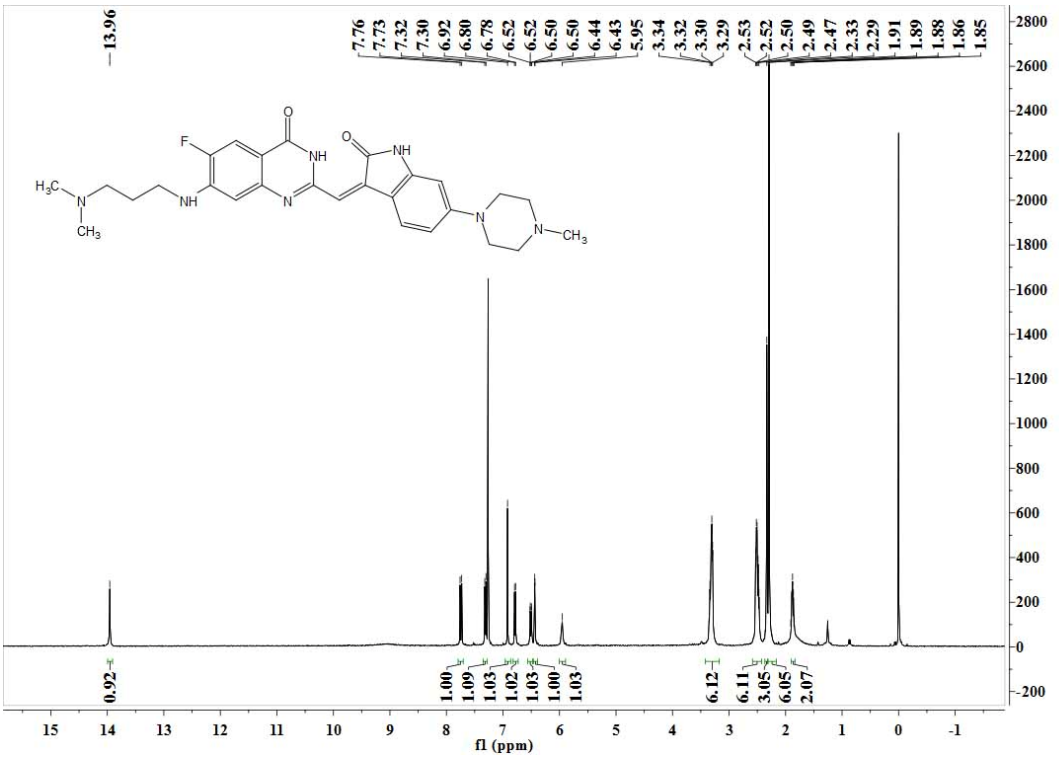


PeakTable

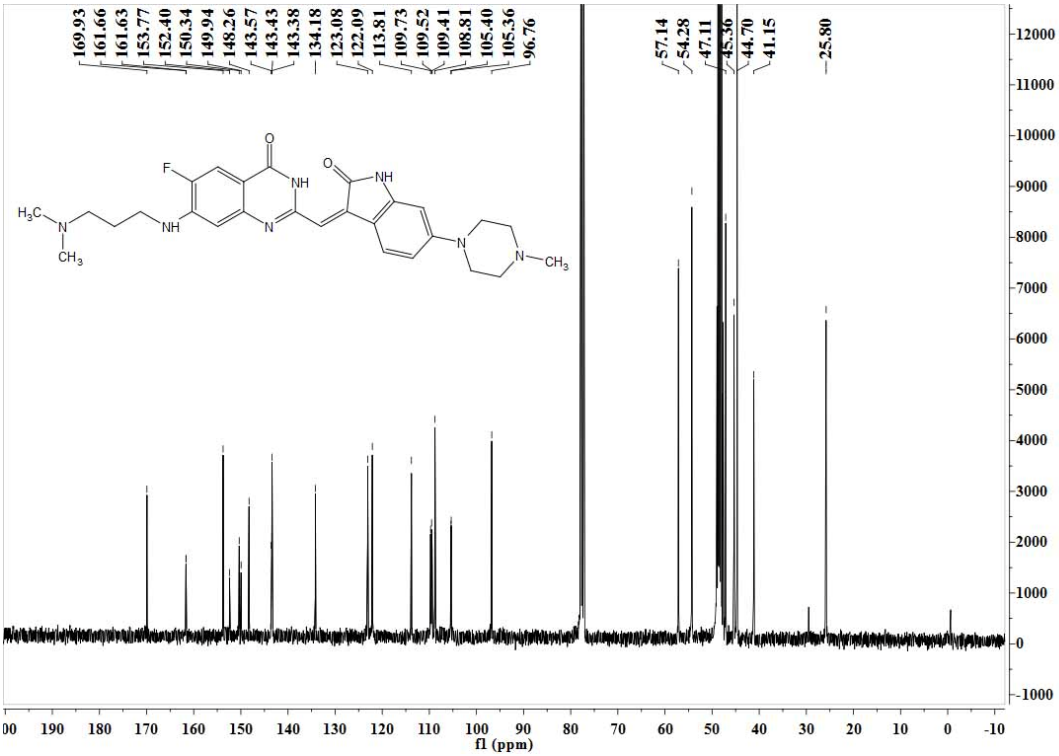
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.919	5662679	893424	95.541	97.701
2	3.509	264259	21022	4.459	2.299
Total		5926938	914445	100.000	100.000

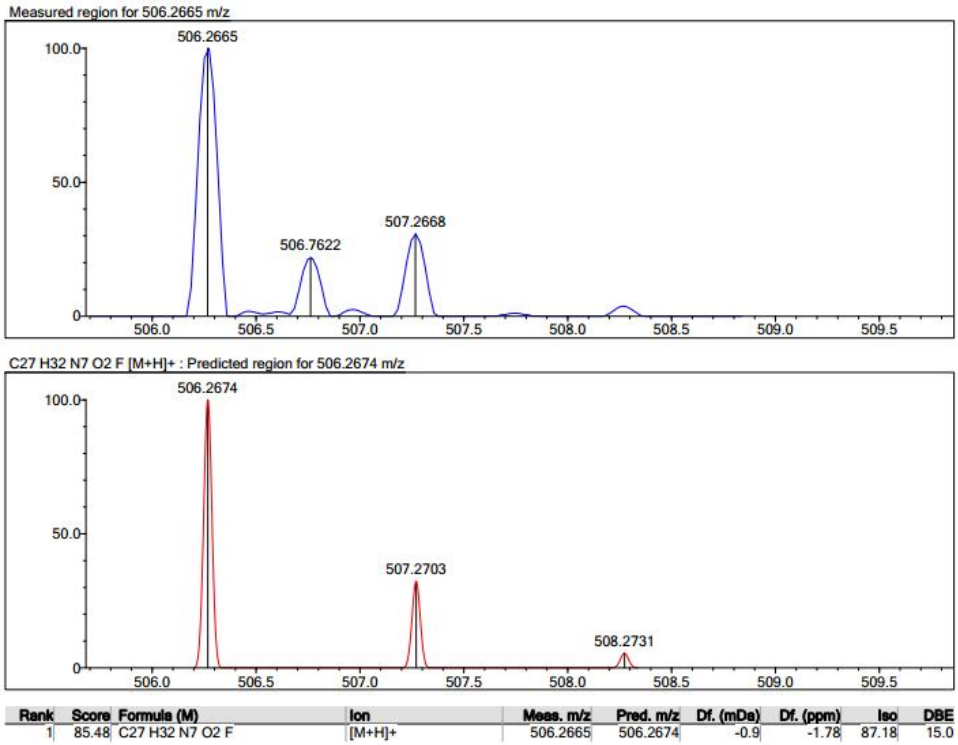
HPLC analysis of **15**



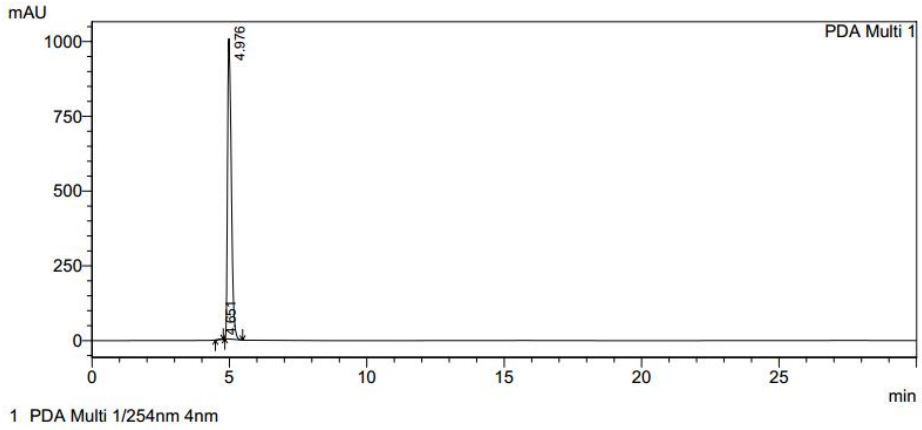
^{1}H NMR spectrum of **16**



^{13}C NMR spectrum of **16**



HRMR spectrum of **16**

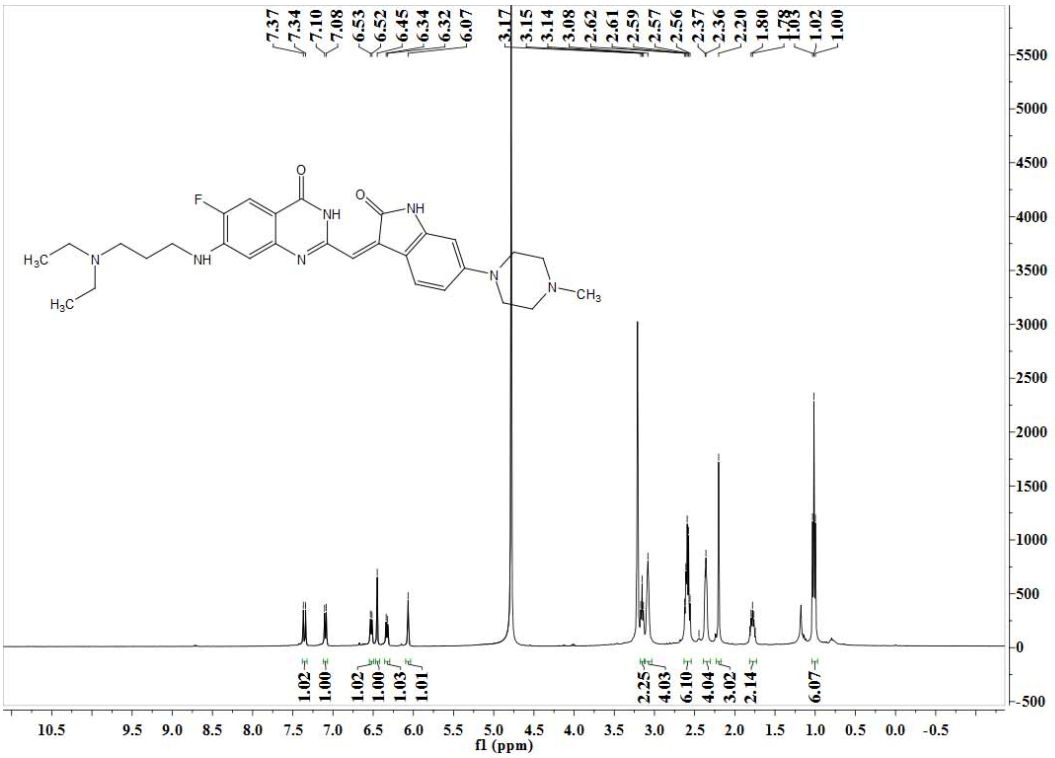


PeakTable

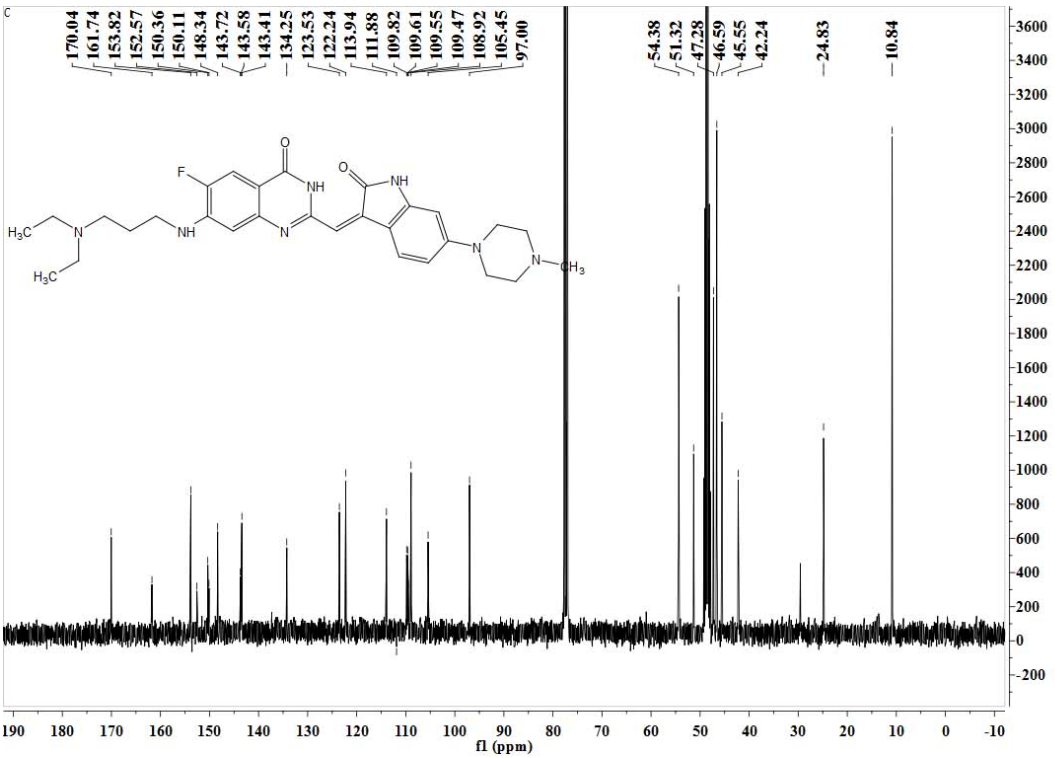
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.651	14071	2026	0.146	0.201
2	4.976	9641428	1004540	99.854	99.799
Total		9655499	1006566	100.000	100.000

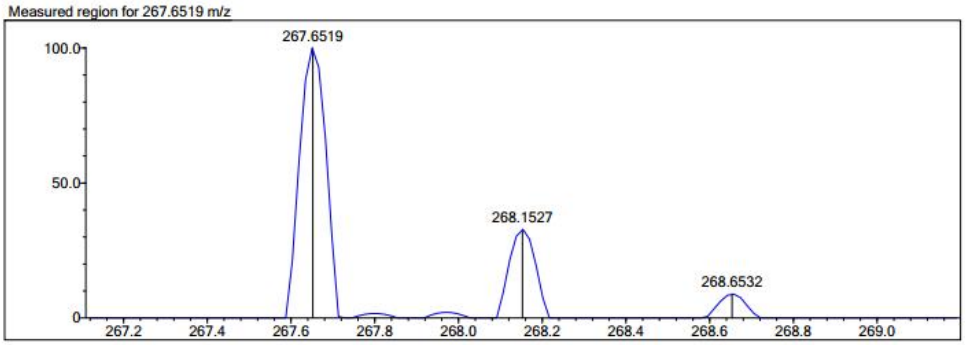
HPLC analysis of **16**

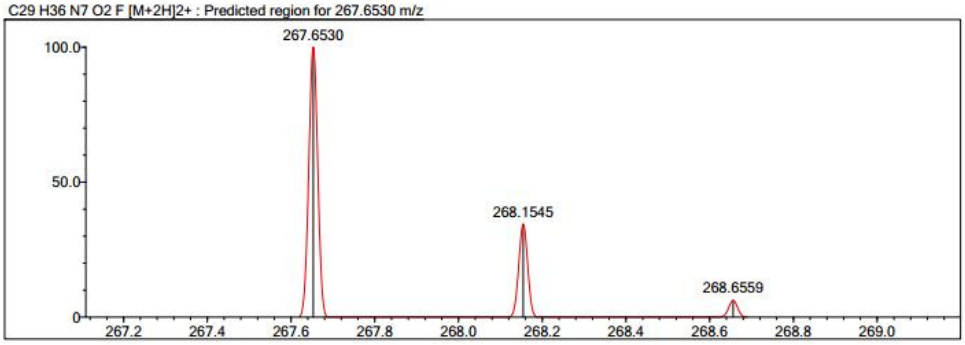


^{1}H NMR spectrum of **17**



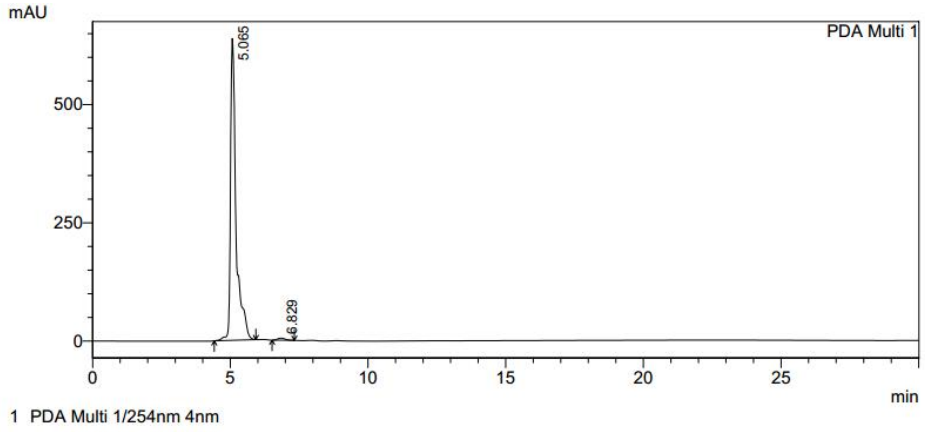
^{13}C NMR spectrum of **17**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	58.94	C29 H36 N7 O2 F	[M+2H]2+	267.6519	267.6530	-1.1	-4.11	63.90	15.0

HRMR spectrum of **17**

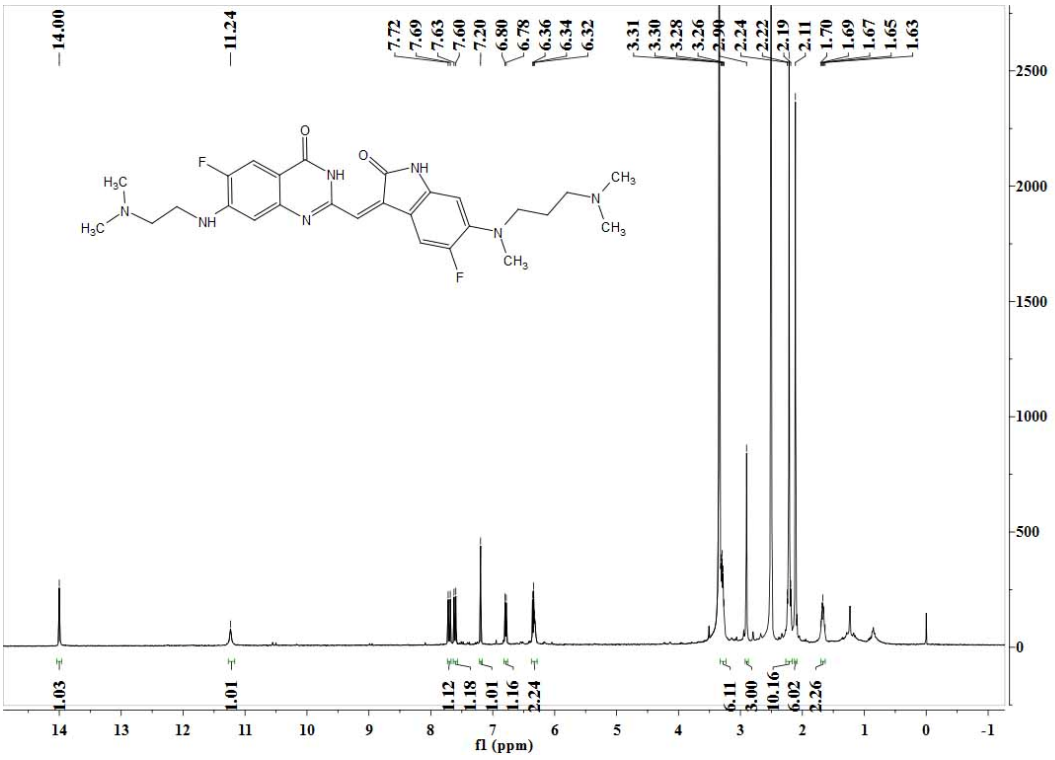


PeakTable

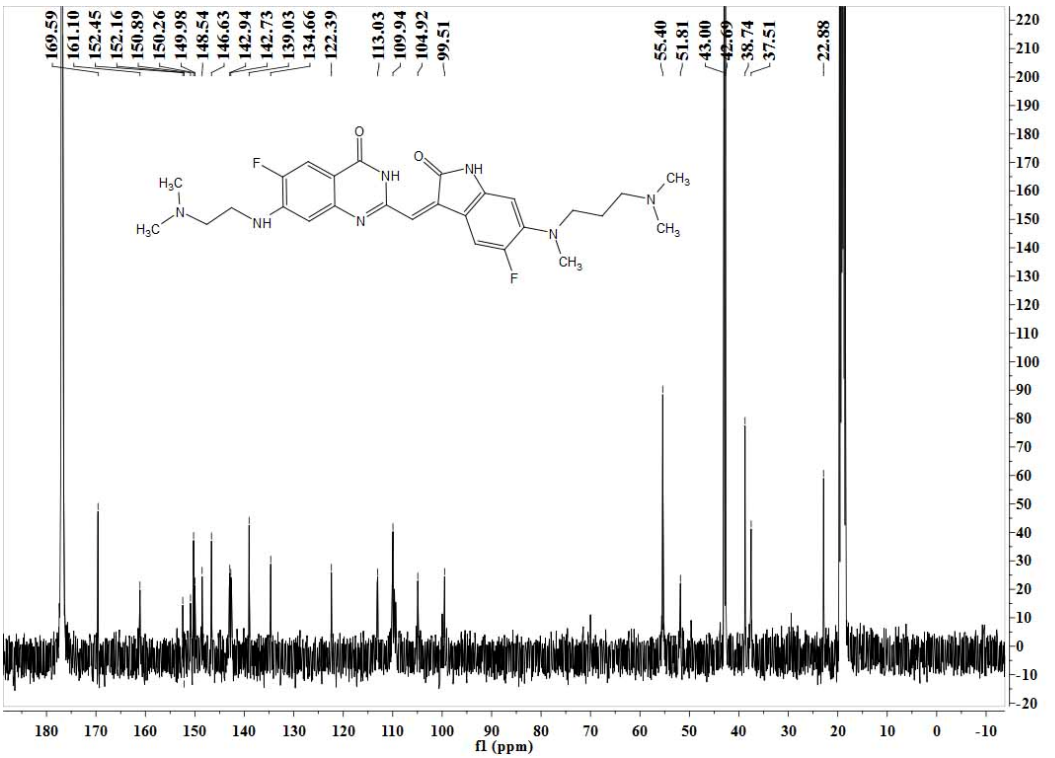
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.065	9031282	638328	99.046	99.279
2	6.829	86980	4634	0.954	0.721
Total		9118262	642961	100.000	100.000

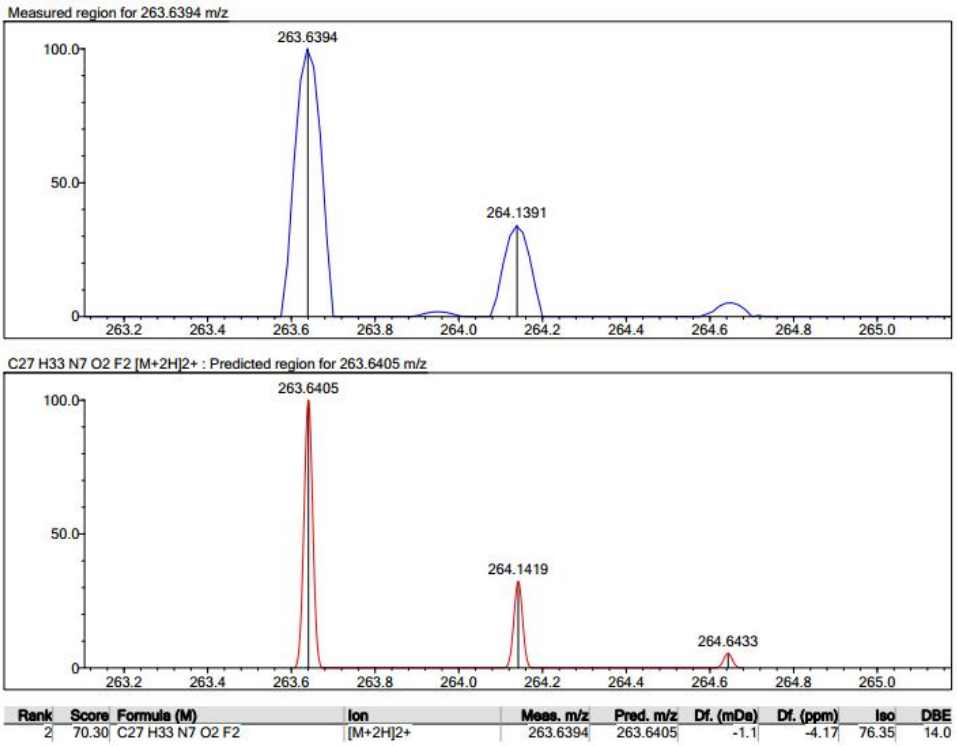
HPLC analysis of **17**



^{1}H NMR spectrum of **18**

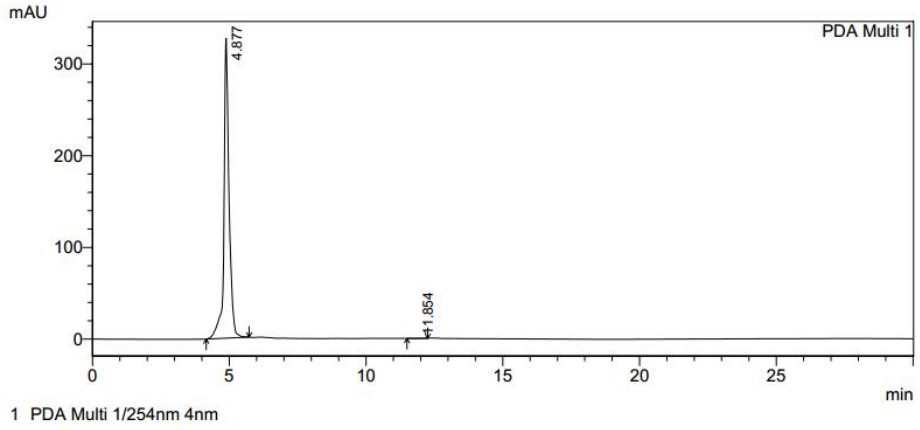


^{13}C NMR spectrum of **18**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	70.30	C27 H33 N7 O2 F2	[M+2H]2+	263.6394	263.6405	-1.1	-4.17	76.35	14.0

HRMR spectrum of **18**

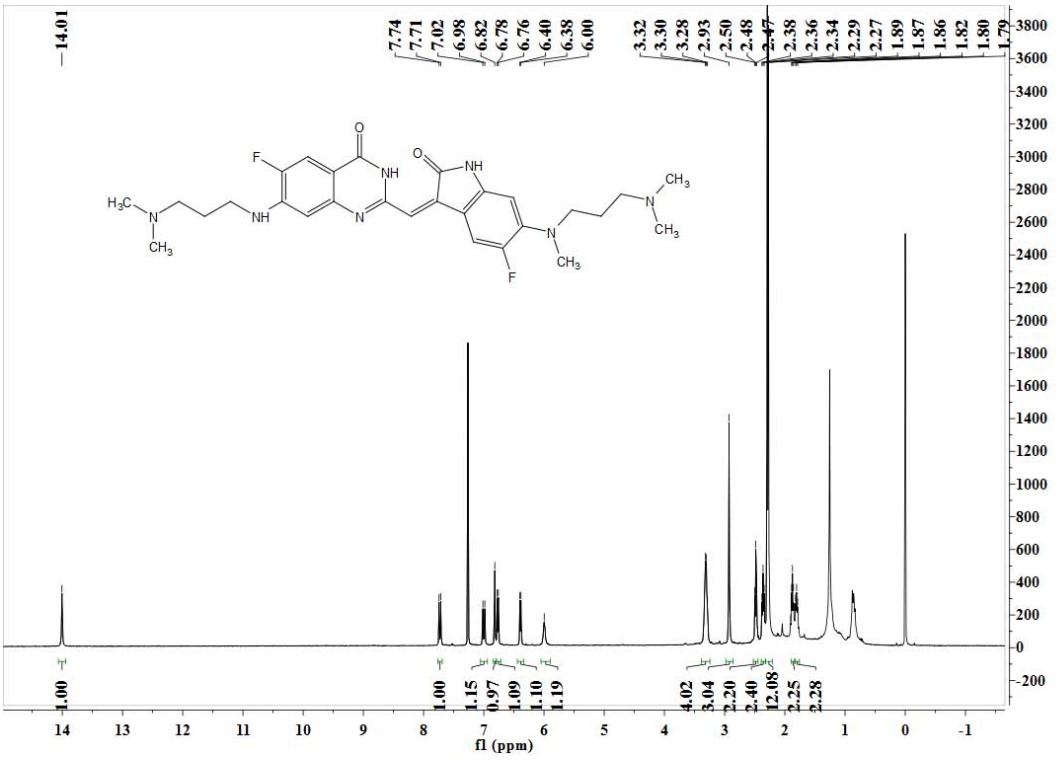


PeakTable

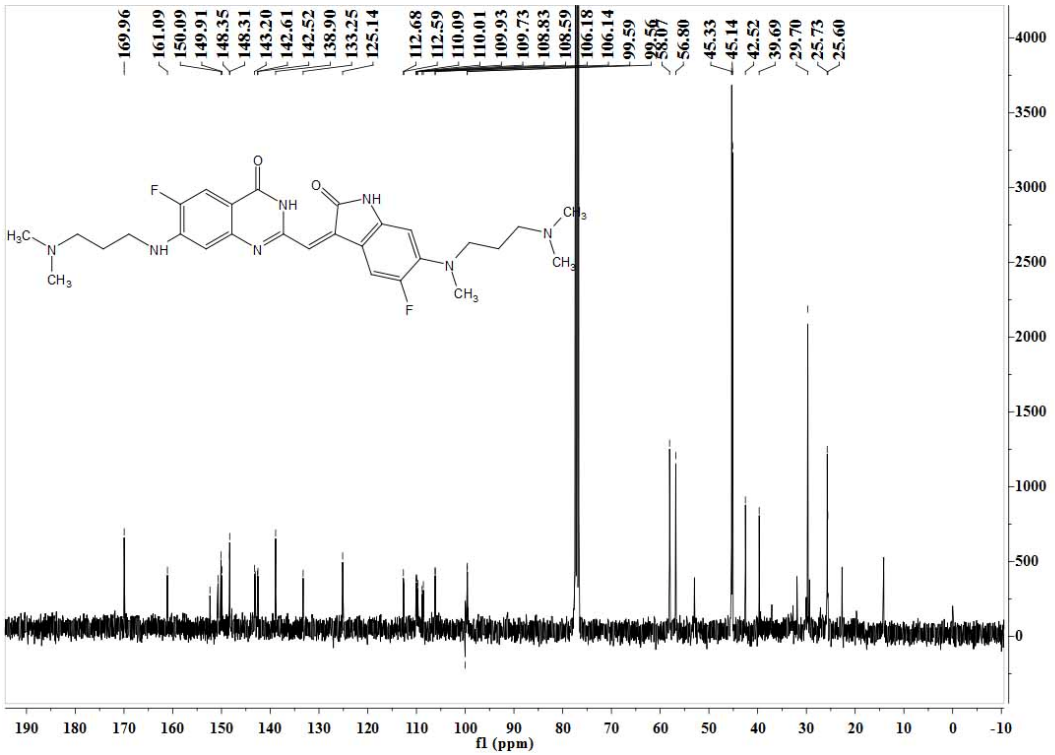
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.877	4247745	327085	99.946	99.971
2	11.854	2280	96	0.054	0.029
Total		4250025	327181	100.000	100.000

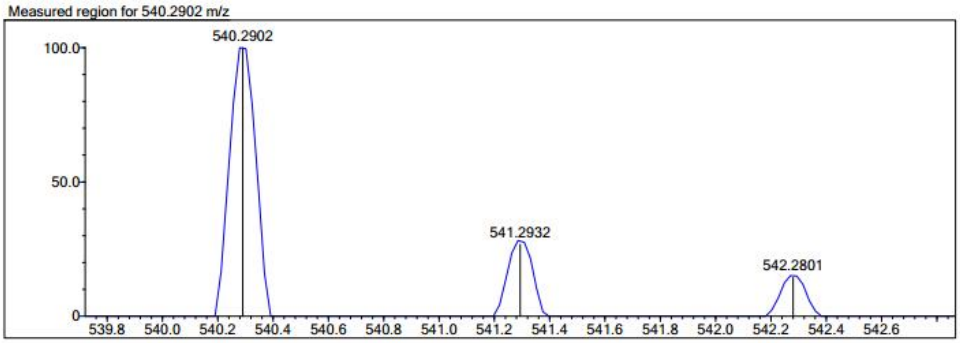
HPLC analysis of **18**



^{1}H NMR spectrum of **19**

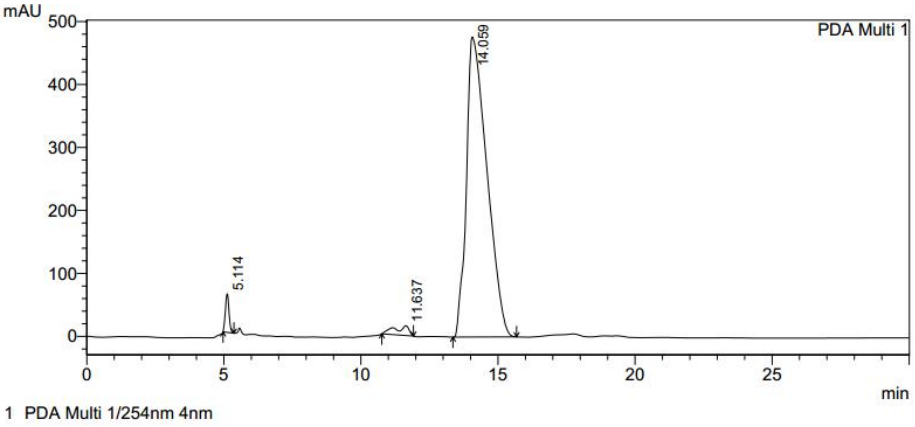


^{13}C NMR spectrum of **19**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	48.40	C28 H35 N7 O2 F2	[M+H]+	540.2902	540.2893	0.9	1.67	49.23	14.0

HRMR spectrum of **19**

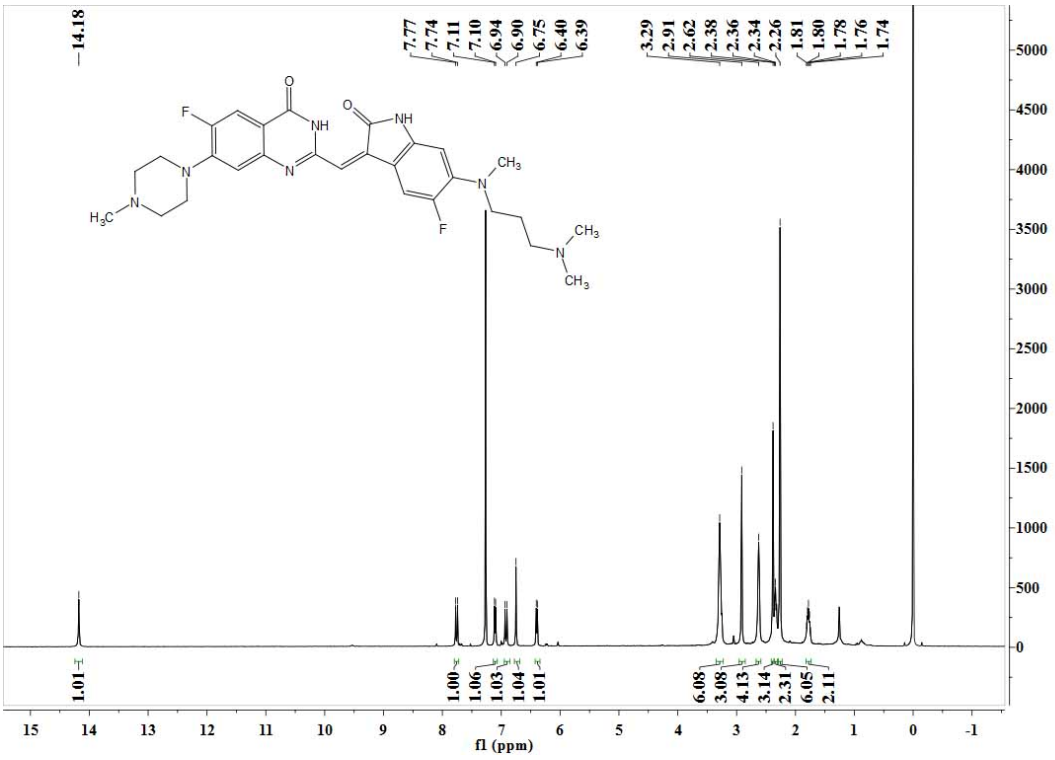


PeakTable

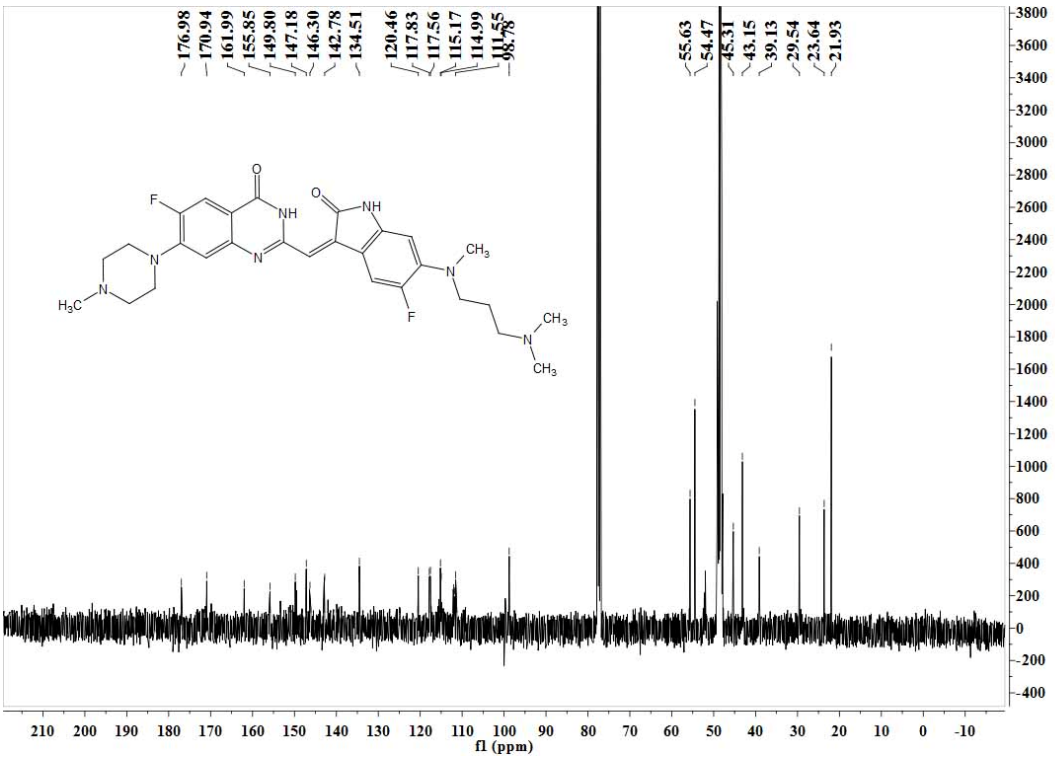
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.114	565835	61341	2.183	11.084
2	11.637	549367	15501	2.120	2.801
3	14.059	24799448	476566	95.697	86.115
Total		25914650	553409	100.000	100.000

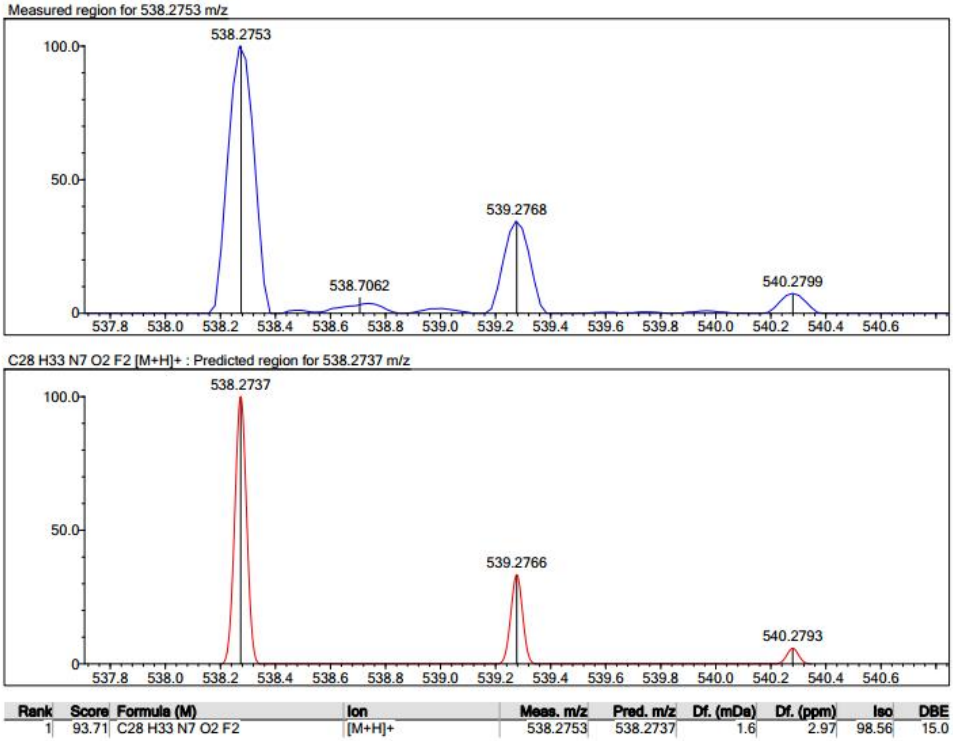
HPLC analysis of **19**



^{1}H NMR spectrum of **20**

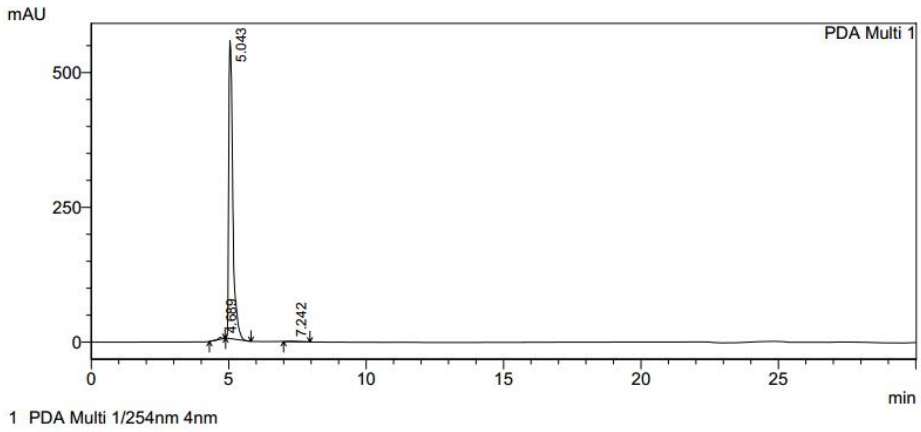


^{13}C NMR spectrum of **20**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	93.71	C28 H33 N7 O2 F2	[M+H]+	538.2753	538.2737	1.6	2.97	98.56	15.0

HRMR spectrum of **20**

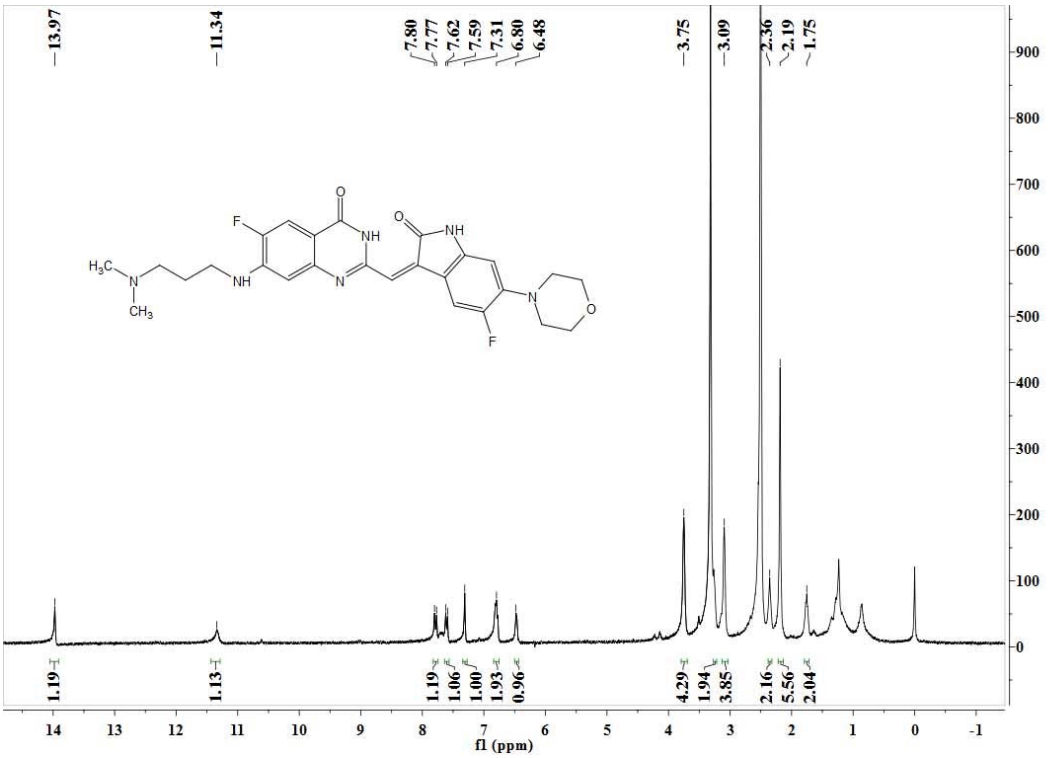


PeakTable

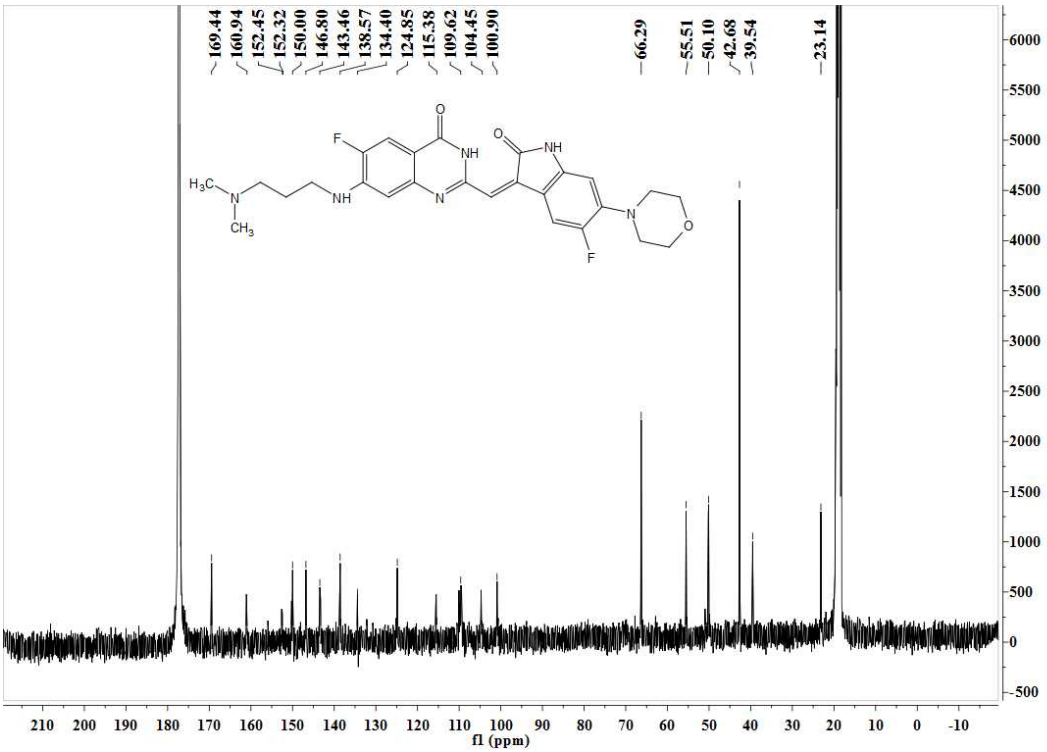
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.689	28606	3281	0.497	0.589
2	5.043	5708453	553530	99.166	99.283
3	7.242	19397	715	0.337	0.128
Total		5756456	557526	100.000	100.000

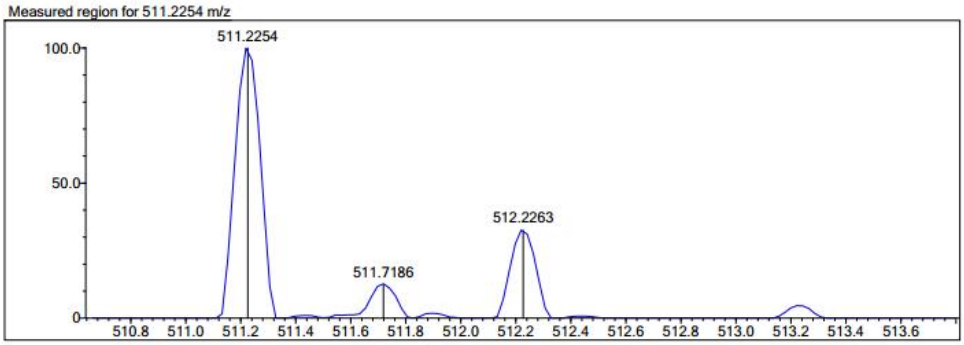
HPLC analysis of **20**



^{1}H NMR spectrum of **21**

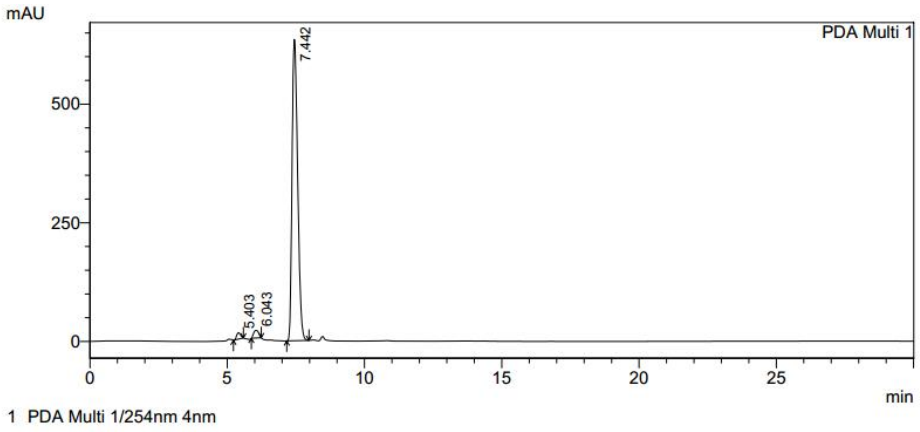


^{13}C NMR spectrum of **21**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	88.73	C26 H28 N6 O3 F2	[M+H]+	511.2254	511.2264	-1.0	-1.96	90.91	15.0

HRMR spectrum of **21**

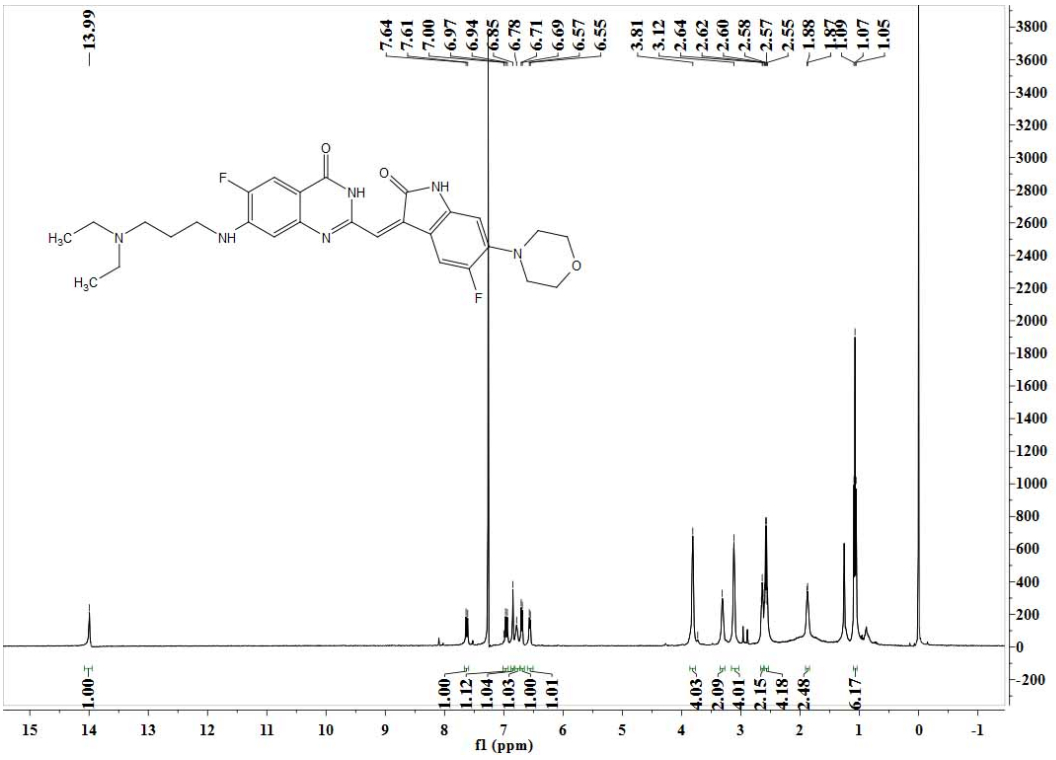


PeakTable

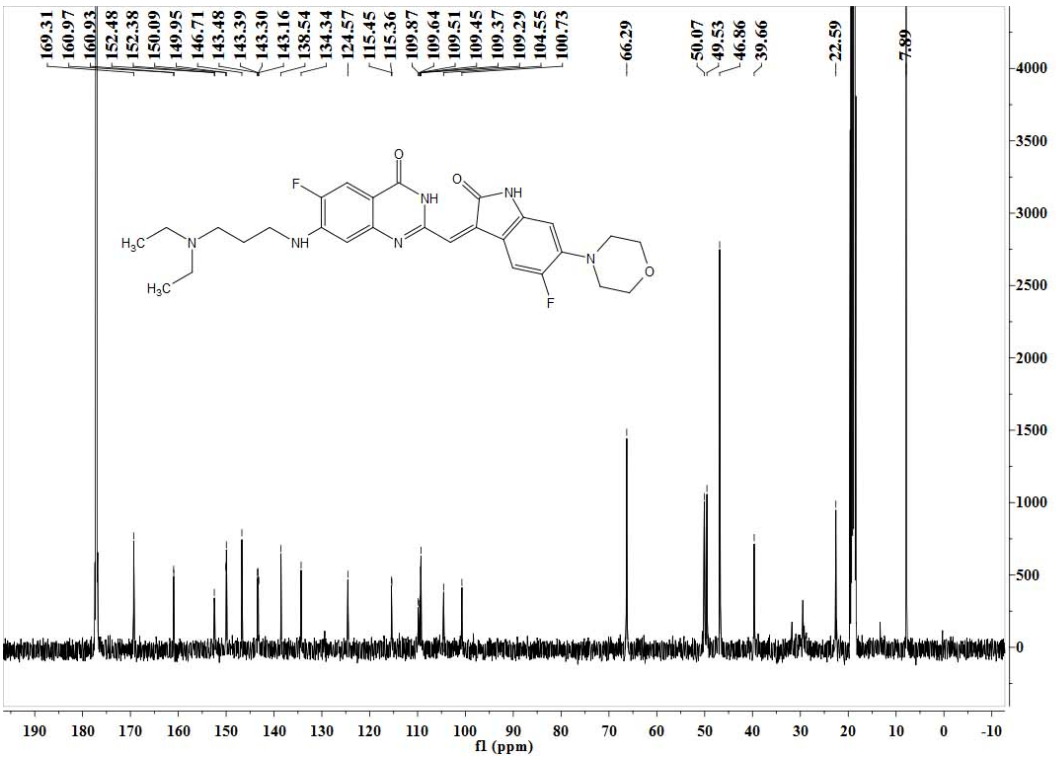
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.403	135067	13620	1.443	2.049
2	6.043	213676	16215	2.282	2.440
3	7.442	9014609	634837	96.275	95.511
Total		9363352	664672	100.000	100.000

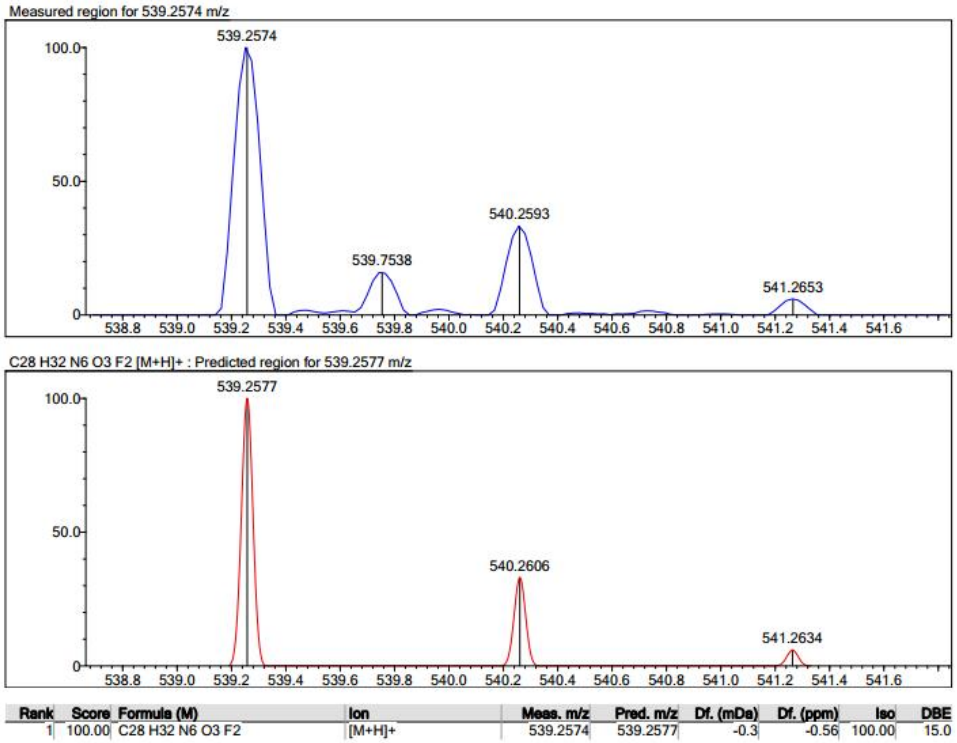
HPLC analysis of **21**



^{1}H NMR spectrum of **22**

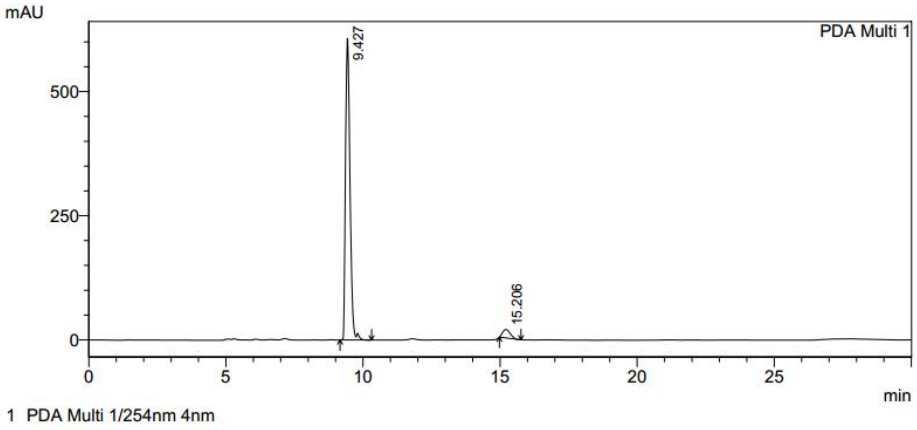


^{13}C NMR spectrum of **22**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	100.00	C28 H32 N6 O3 F2	[M+H]+	539.2574	539.2577	-0.3	-0.56	100.00	15.0

HRMR spectrum of **22**

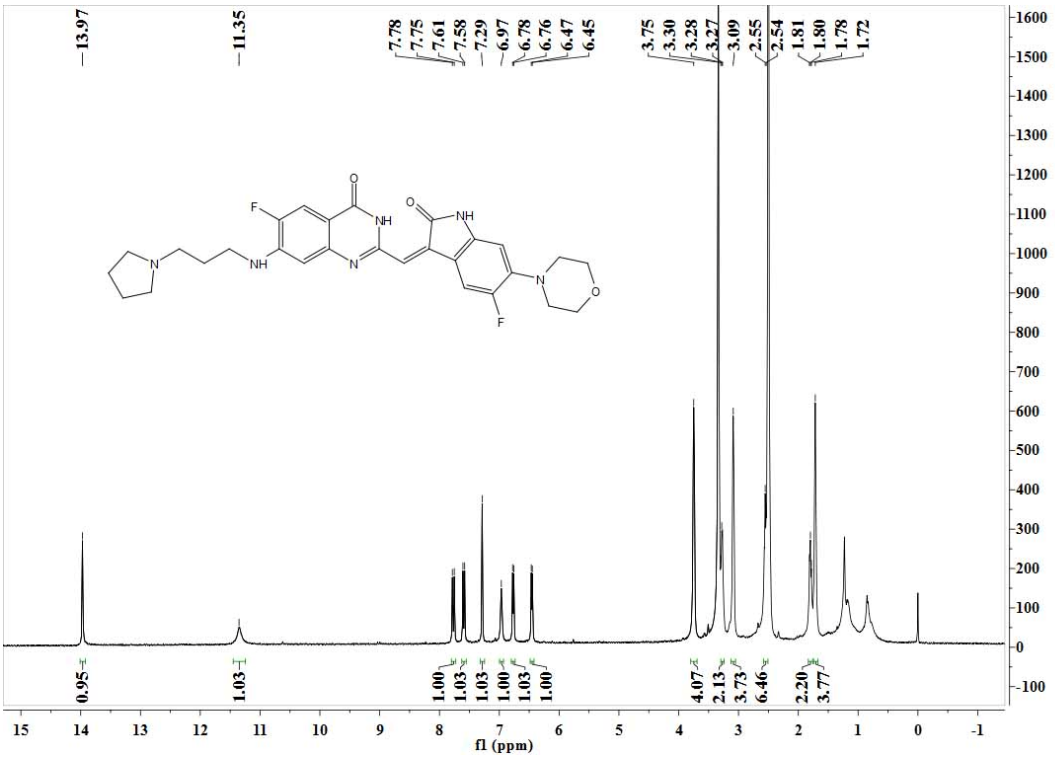


PeakTable

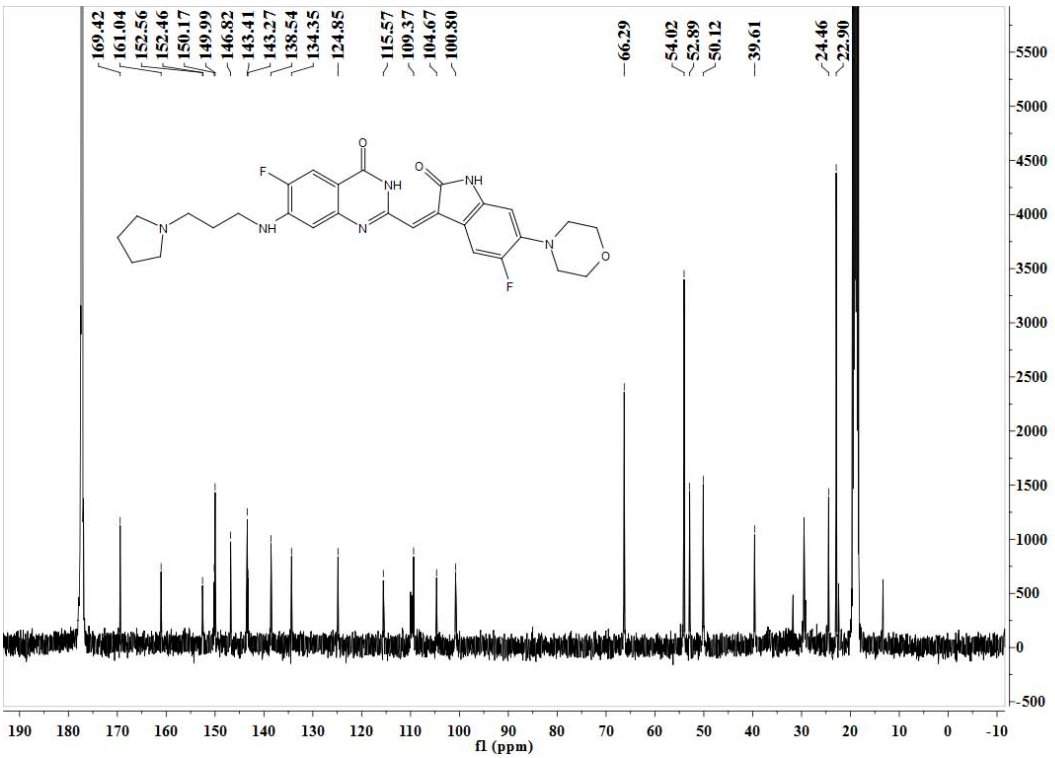
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.427	6889193	607130	95.474	97.275
2	15.206	326595	17007	4.526	2.725
Total		7215788	624137	100.000	100.000

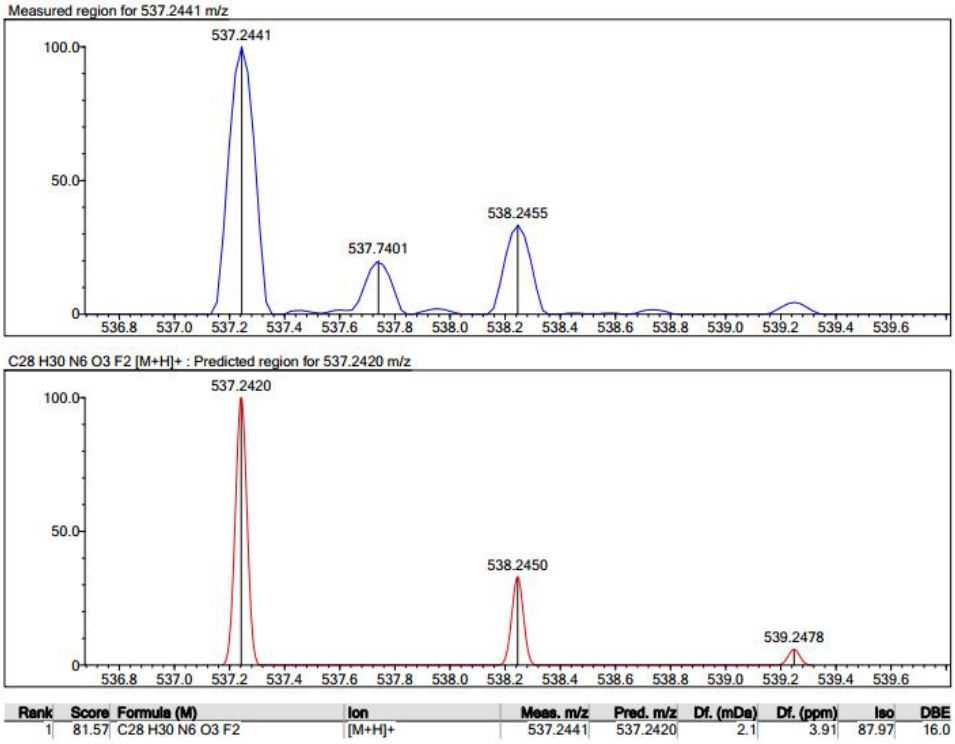
HPLC analysis of **22**



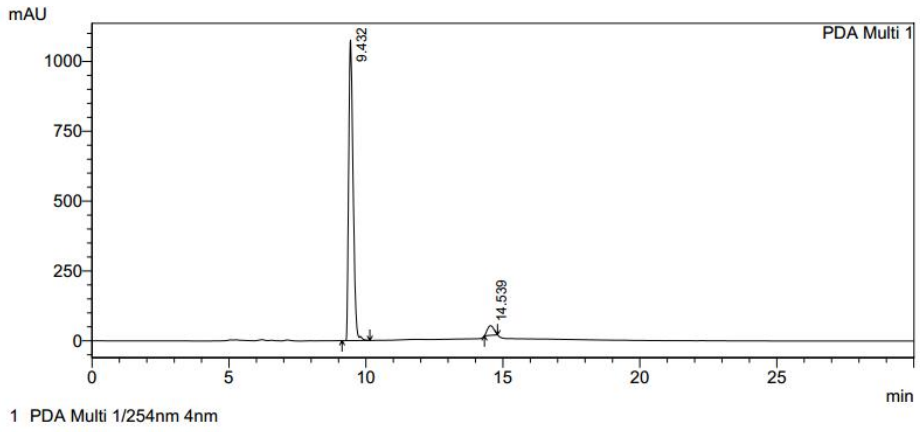
^{1}H NMR spectrum of **23**



^{13}C NMR spectrum of **23**



HRMR spectrum of **23**

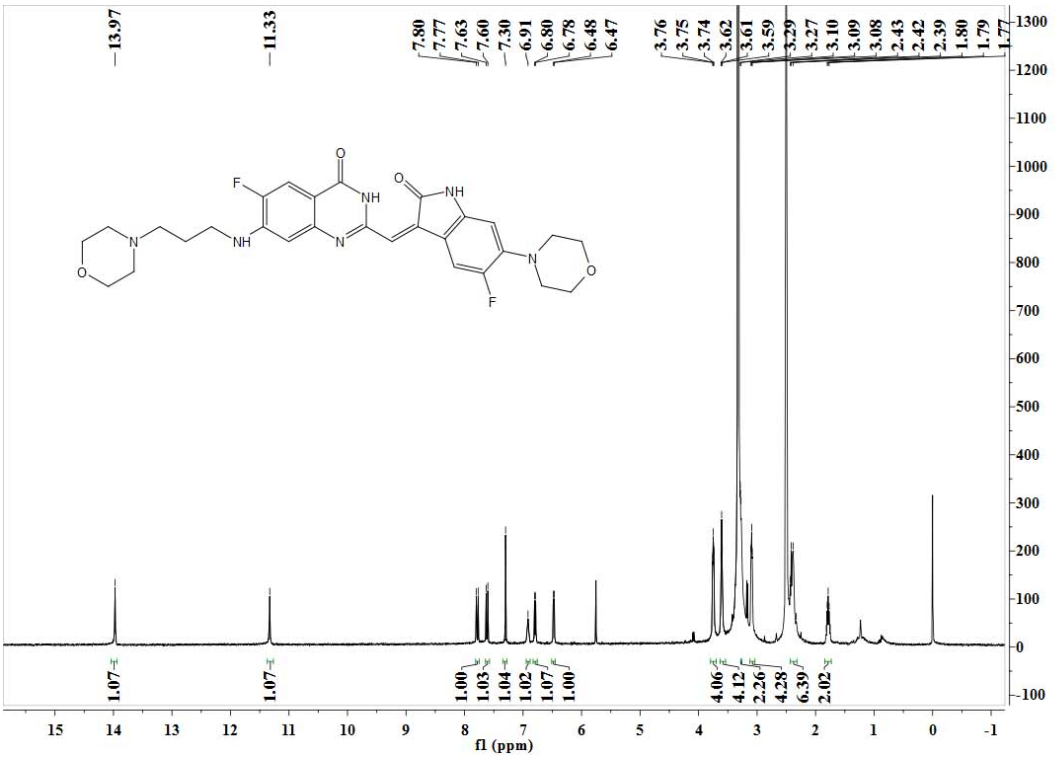


PeakTable

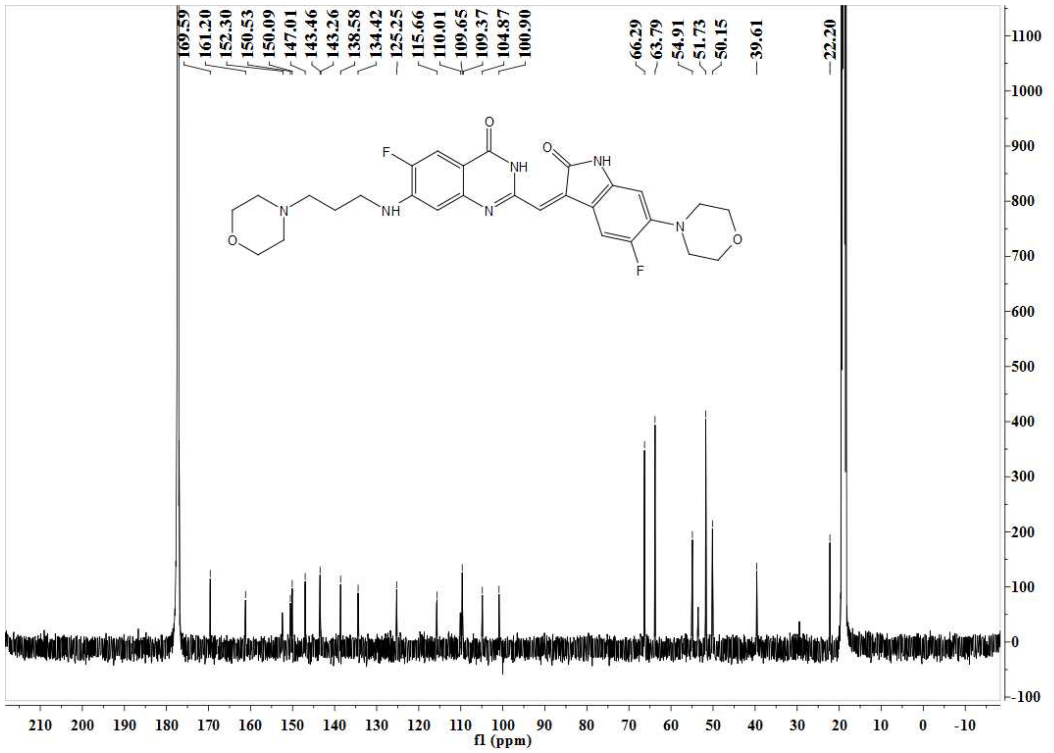
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.432	11993338	1075530	95.422	96.856
2	14.539	575425	34910	4.578	3.144
Total		12568764	1110440	100.000	100.000

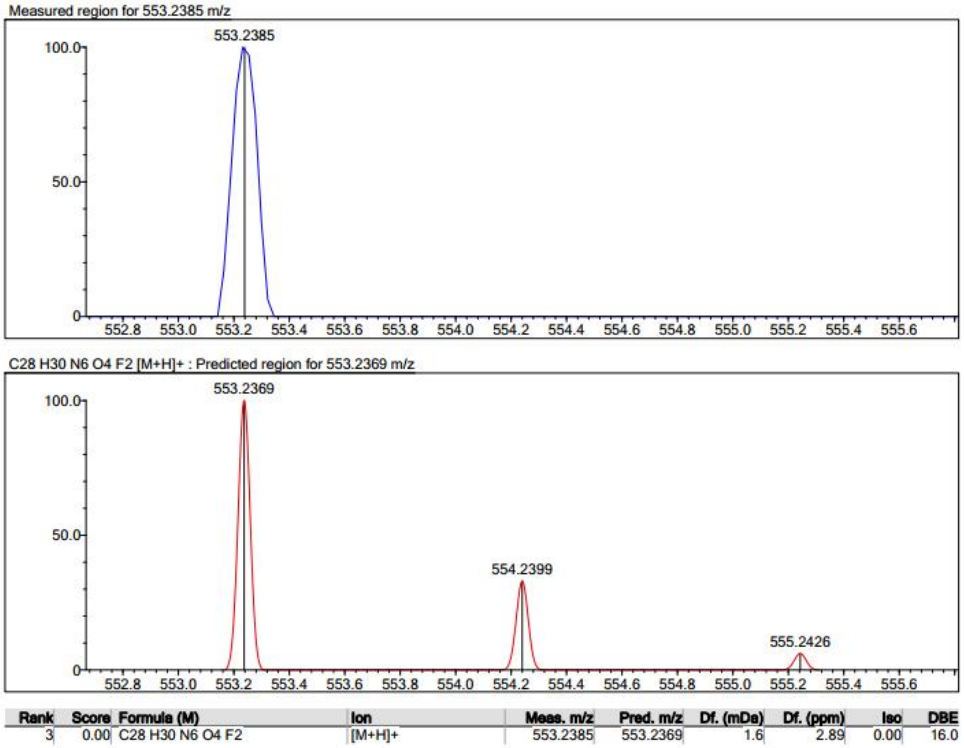
HPLC analysis of **23**



^{1}H NMR spectrum of **24**

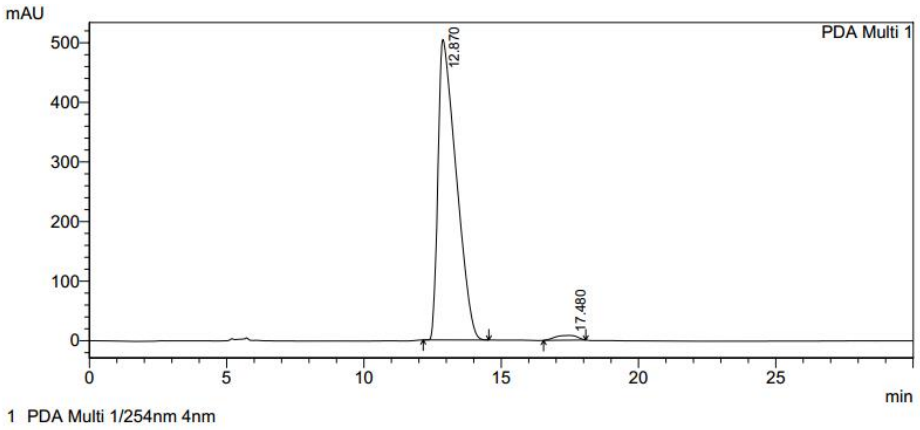


^{13}C NMR spectrum of **24**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	0.00	C28 H30 N6 O4 F2	[M+H]+	553.2385	553.2369	1.6	2.89	0.00	16.0

HRMR spectrum of **24**

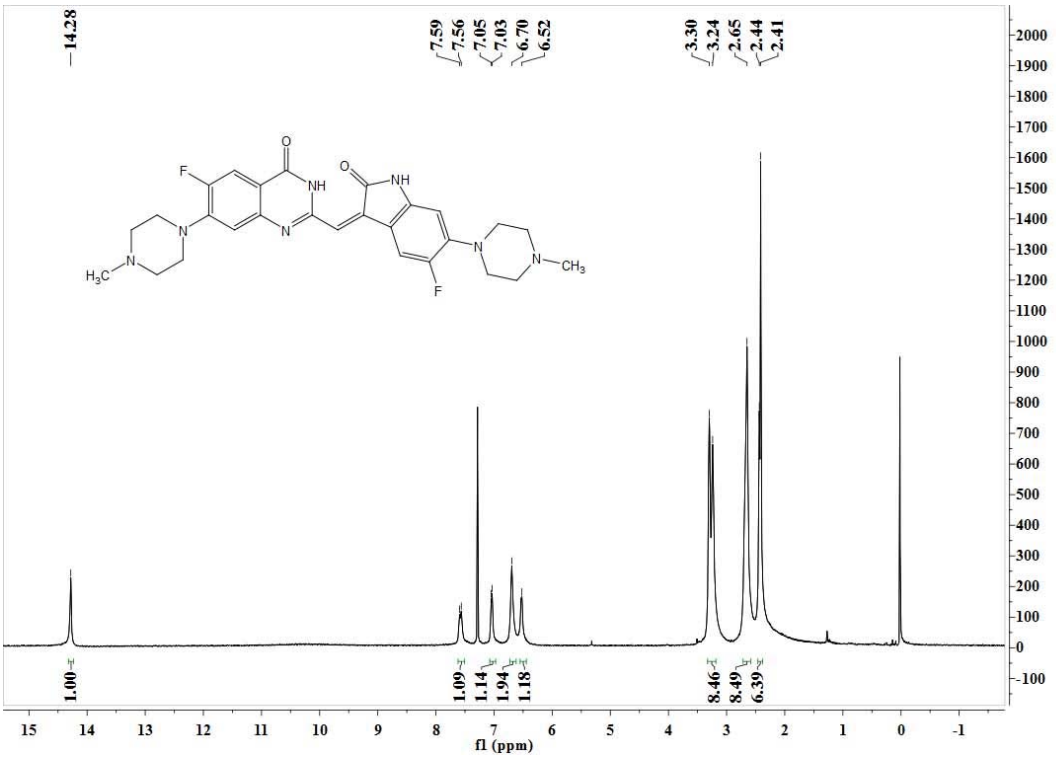


PeakTable

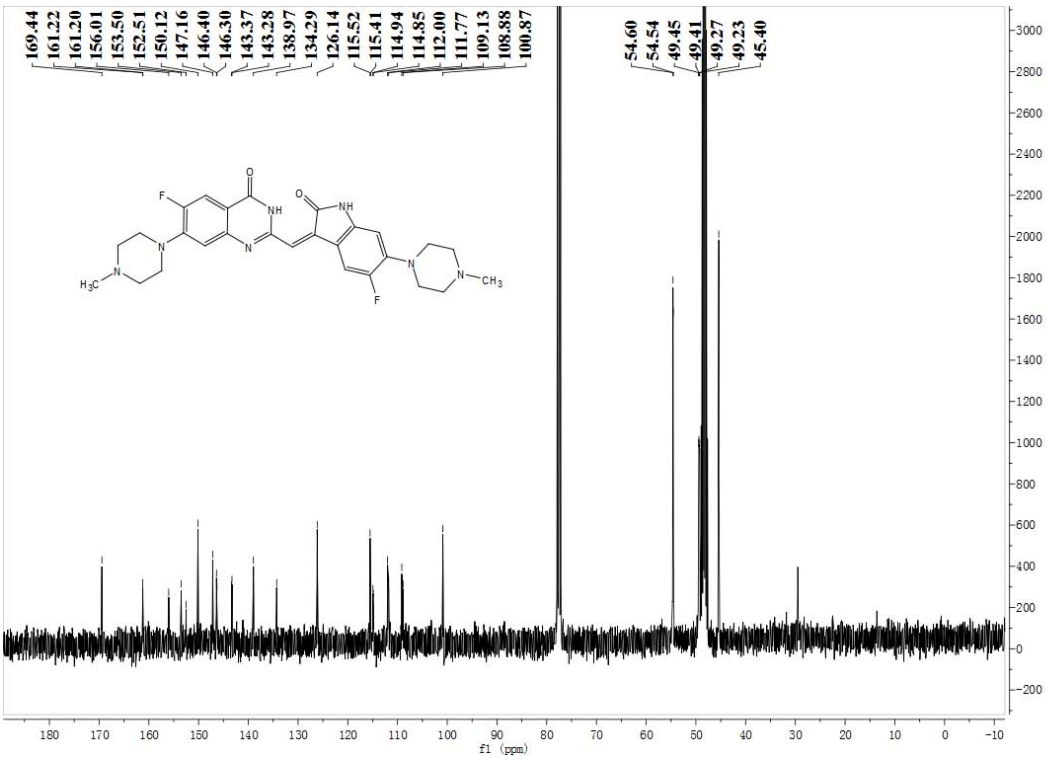
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	12.870	23185351	504358	98.124	98.464
2	17.480	443382	7869	1.876	1.536
Total		23628734	512227	100.000	100.000

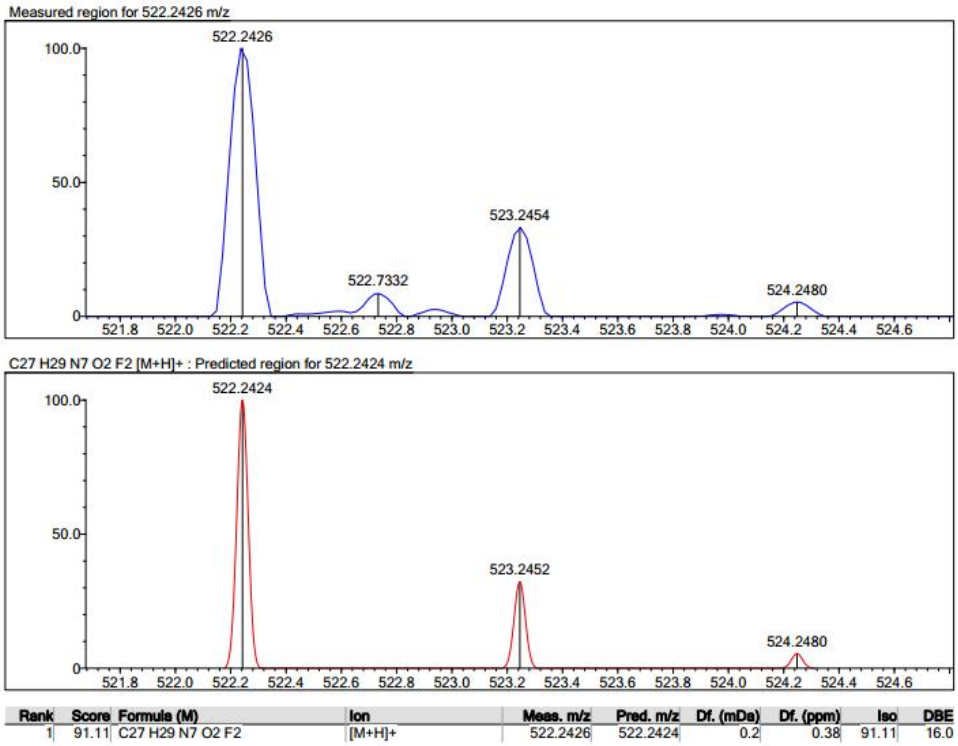
HPLC analysis of **24**



^{1}H NMR spectrum of **25**

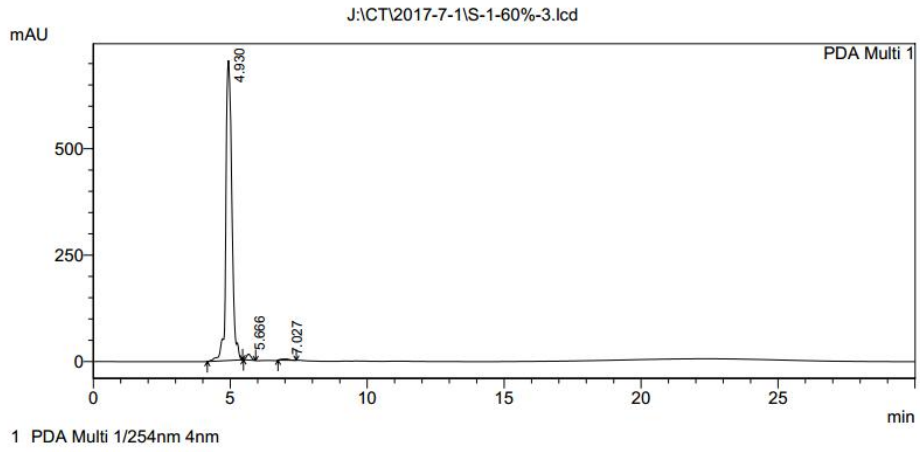


^{13}C NMR spectrum of **25**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.11	C27 H29 N7 O2 F2	[M+H]+	522.2426	522.2424	0.2	0.38	91.11	16.0

HRMR spectrum of **25**

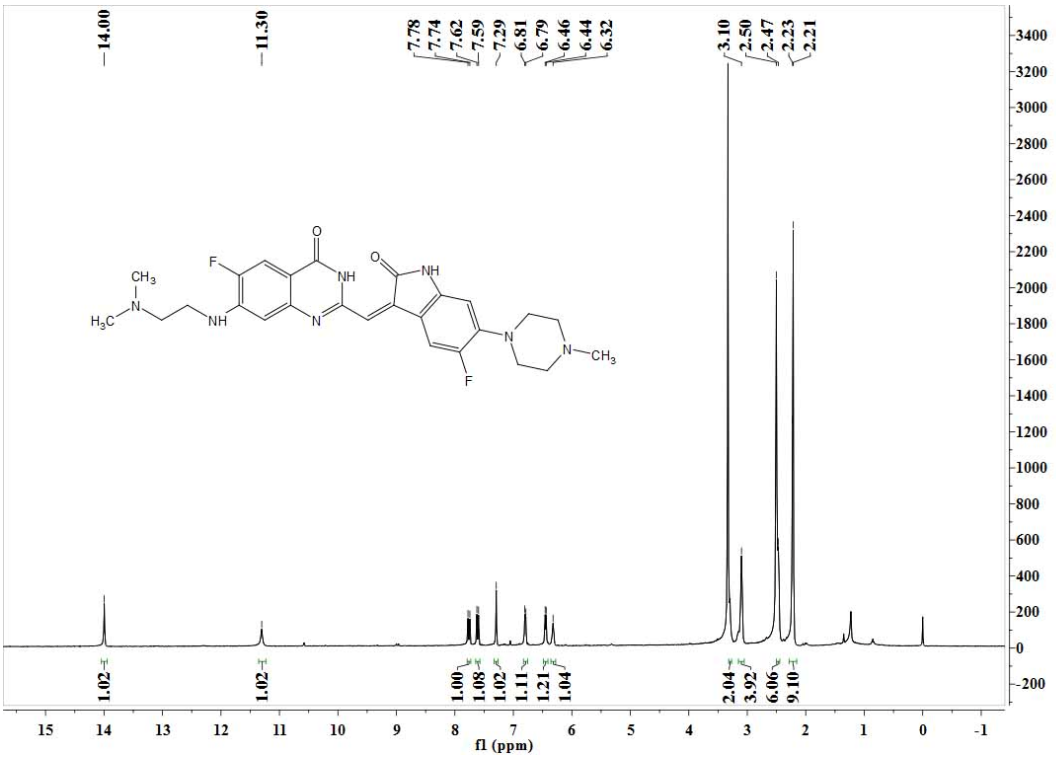


PeakTable

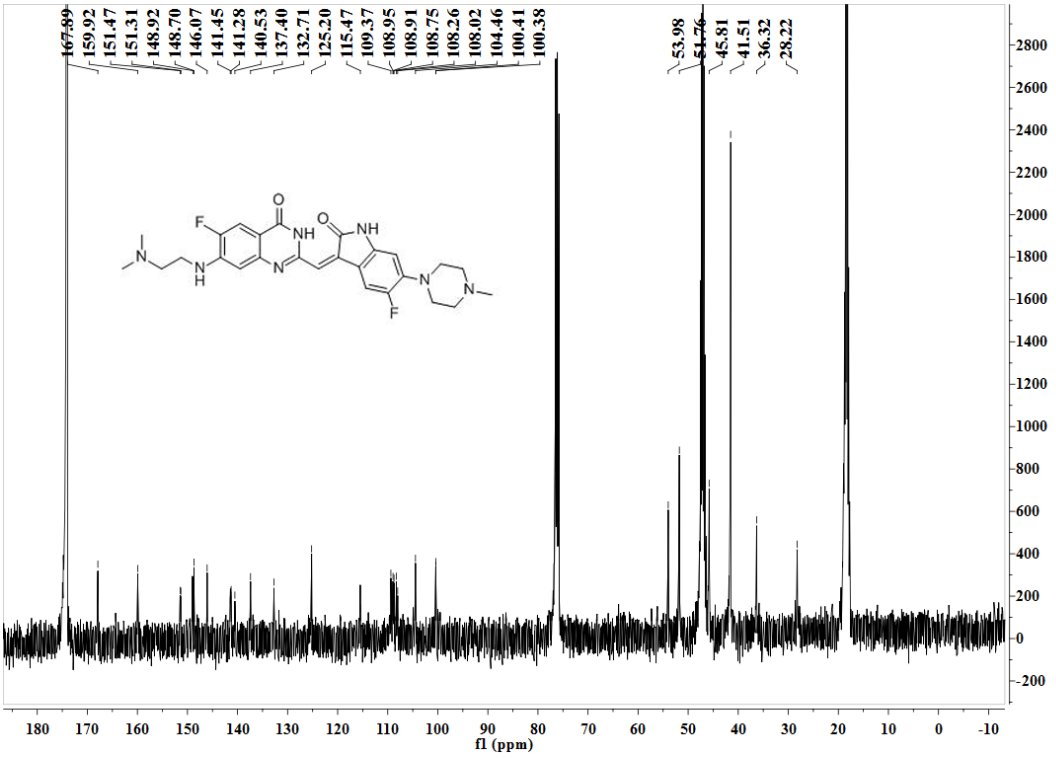
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.930	10698270	704776	98.000	97.678
2	5.666	160469	13893	1.470	1.925
3	7.027	57841	2863	0.530	0.397
Total		10916580	721532	100.000	100.000

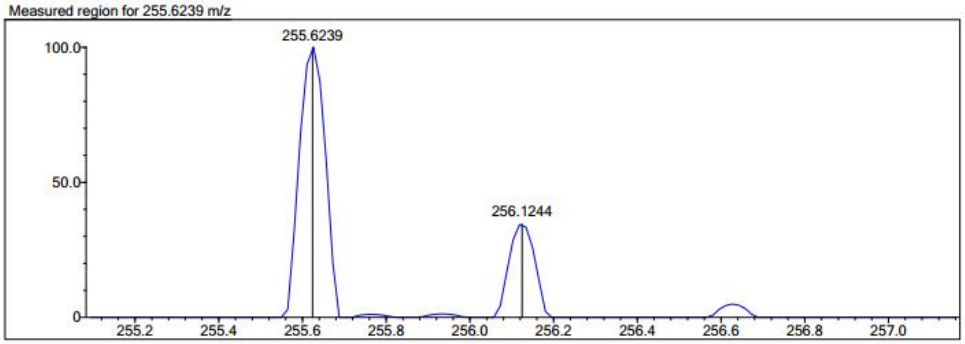
HPLC analysis of **25**

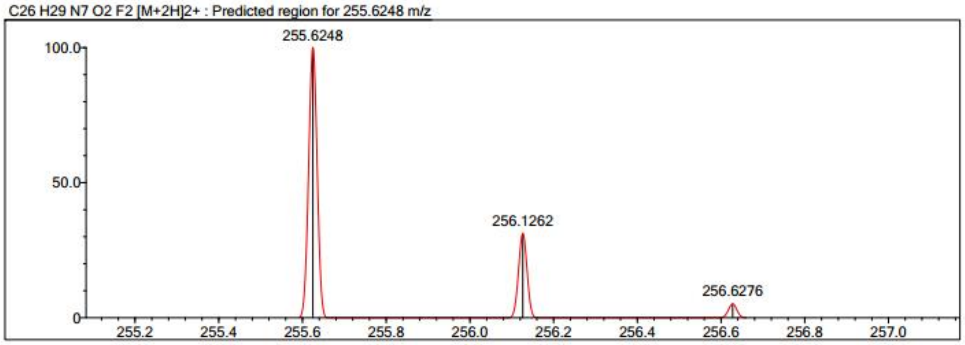


^{1}H NMR spectrum of **26**



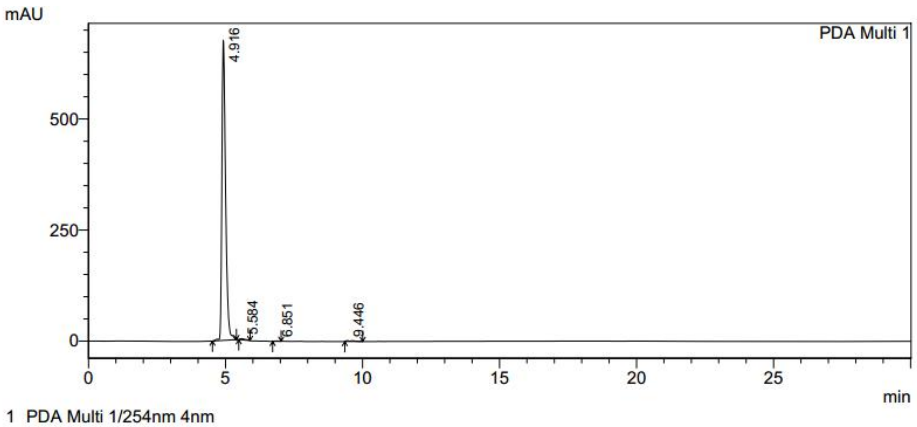
^{13}C NMR spectrum of **26**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	75.55	C26 H29 N7 O2 F2	[M+2H]2+	255.6239	255.6248	-0.9	-3.52	80.63	15.0

HRMR spectrum of **26**

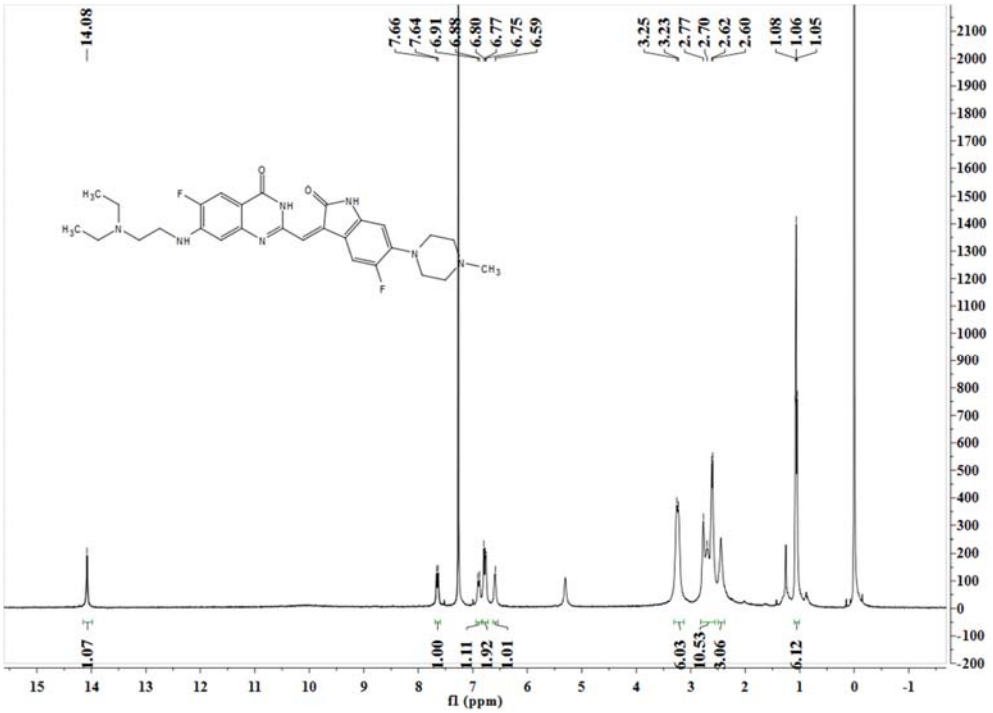


PeakTable

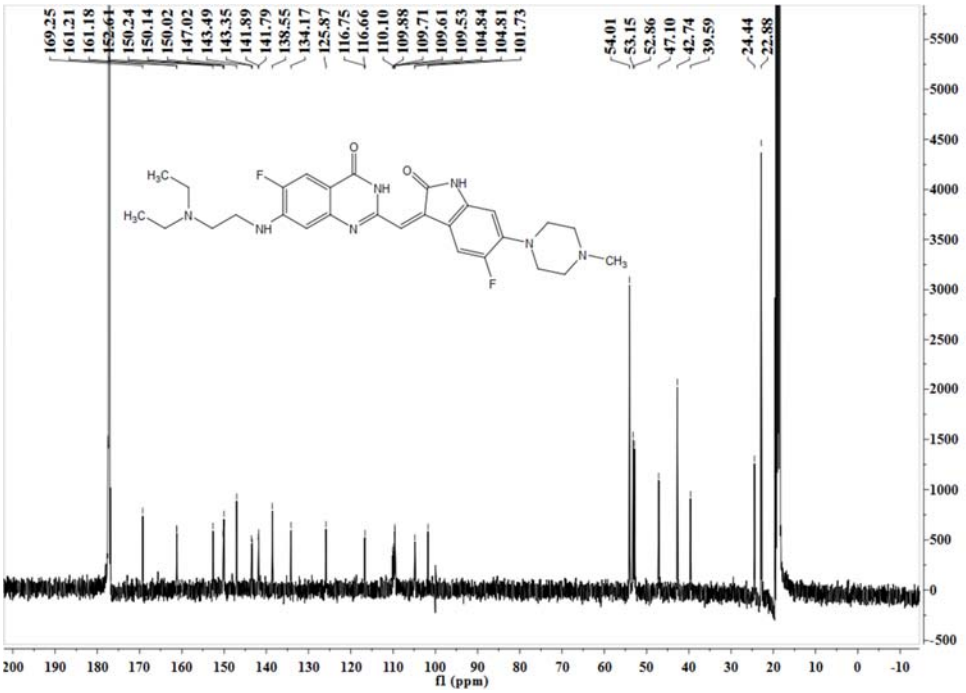
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.916	6353684	675894	98.852	99.239
2	5.584	33310	2501	0.518	0.367
3	6.851	7094	732	0.110	0.107
4	9.446	33356	1947	0.519	0.286
Total		6427445	681074	100.000	100.000

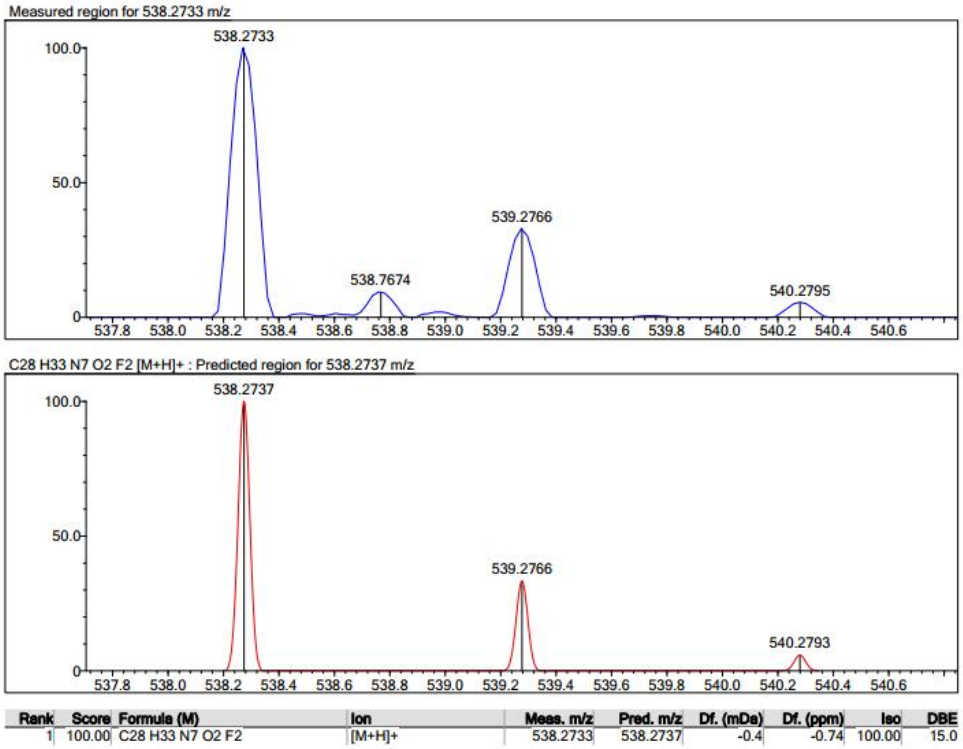
HPLC analysis of **26**



^{1}H NMR spectrum of **27**

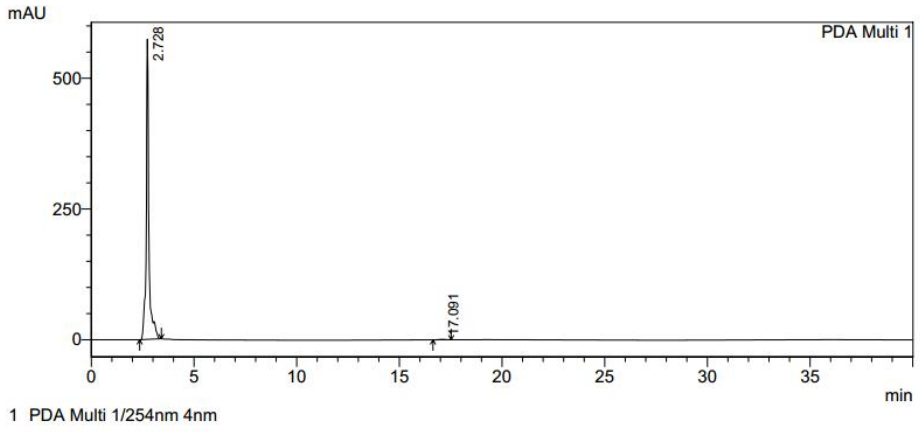


^{13}C NMR spectrum of **27**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	100.00	C28 H33 N7 O2 F2	[M+H]+	538.2733	538.2737	-0.4	-0.74	100.00	15.0

HRMR spectrum of **27**

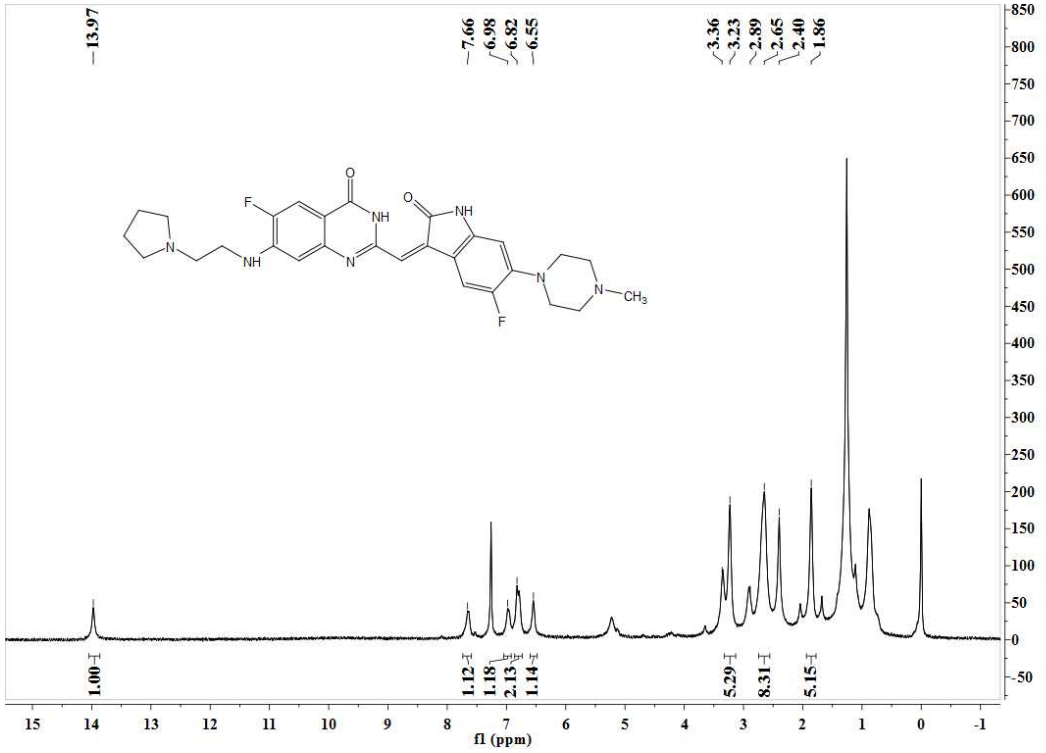


PeakTable

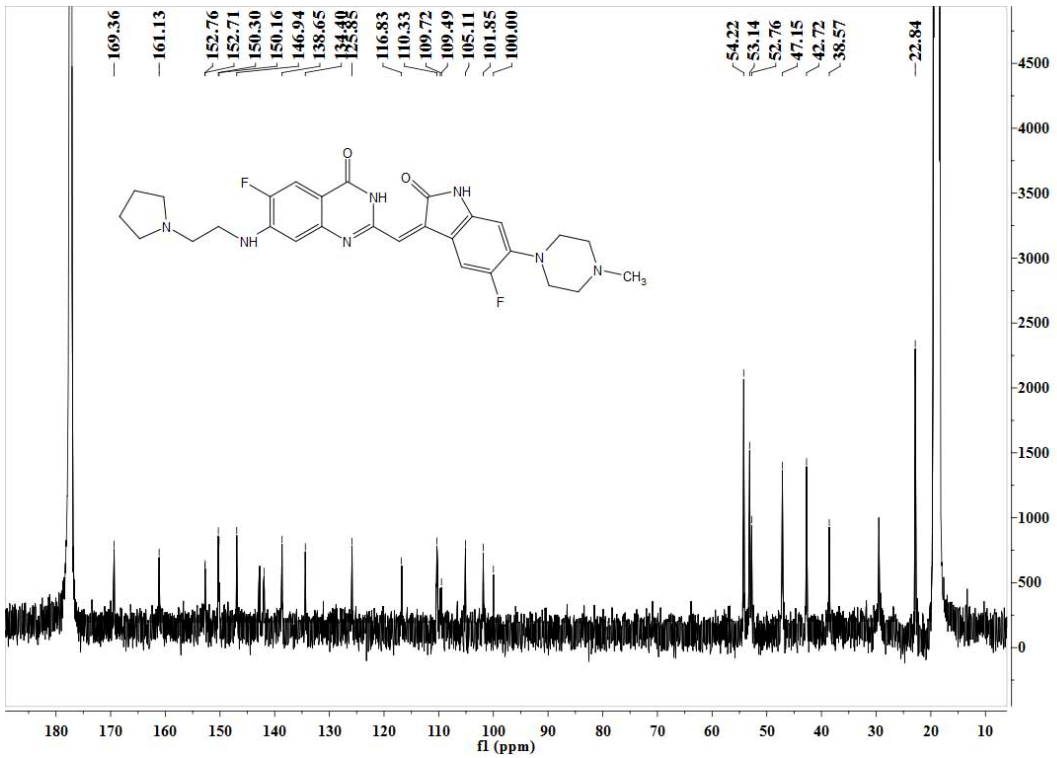
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.728	5145760	573991	99.628	99.853
2	17.091	19221	847	0.372	0.147
Total		5164981	574838	100.000	100.000

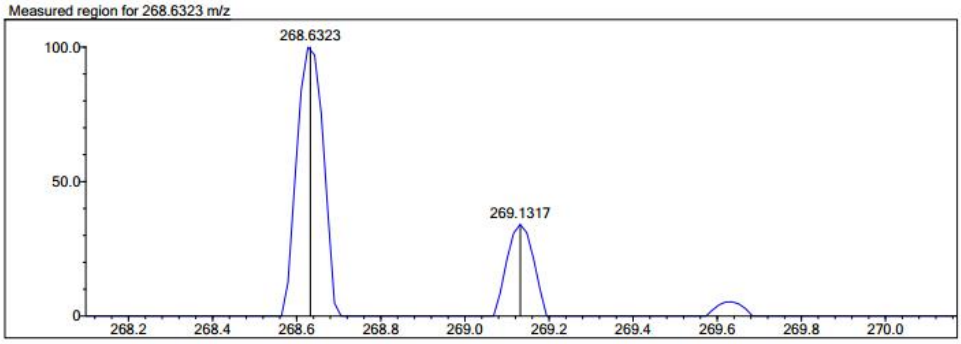
HPLC analysis of **27**

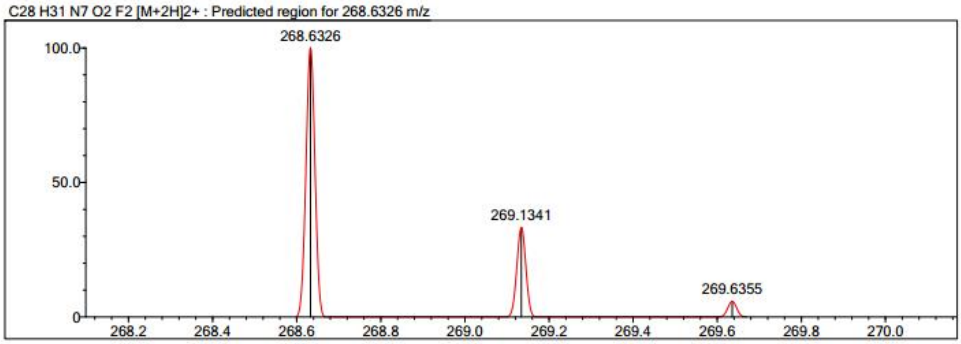


^{1}H NMR spectrum of **28**



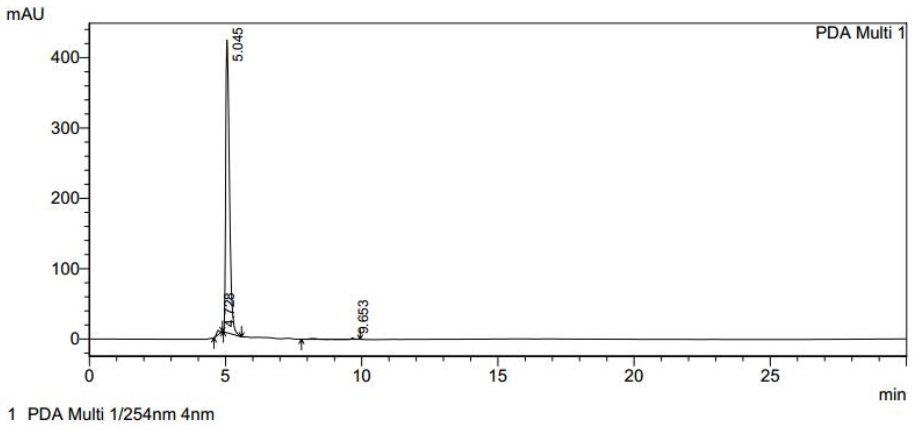
^{13}C NMR spectrum of **28**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	77.99	C28 H31 N7 O2 F2	[M+2H]2+	268.6323	268.6326	-0.3	-1.12	78.23	16.0

HRMR spectrum of **28**

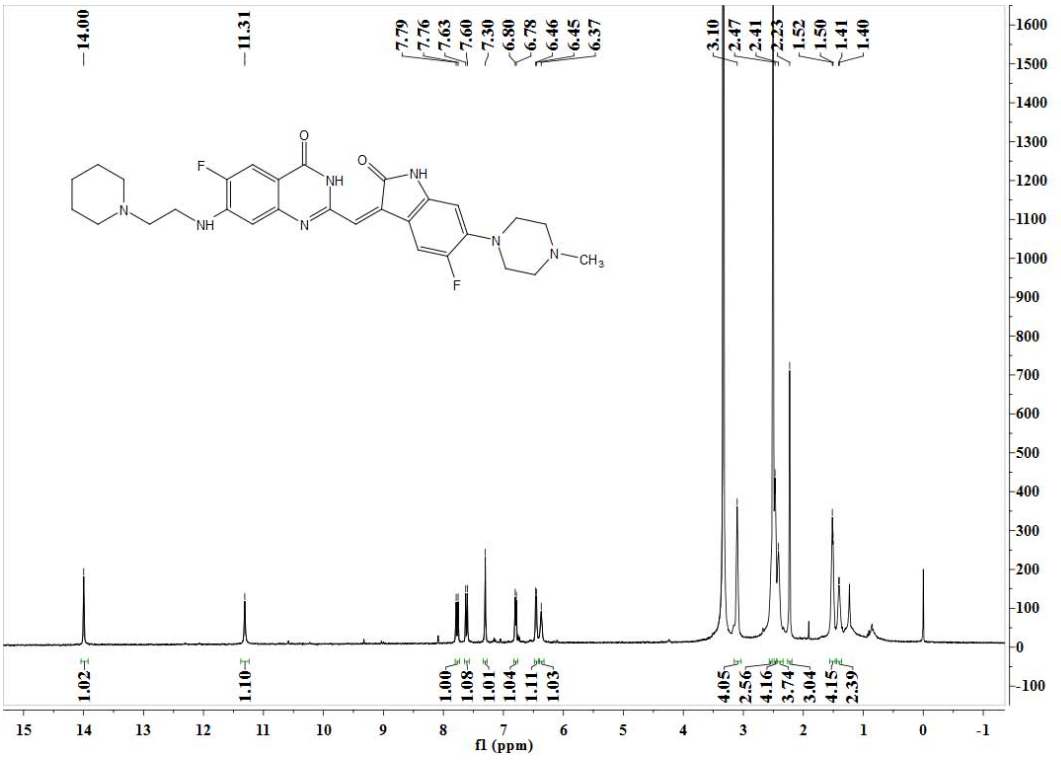


PeakTable

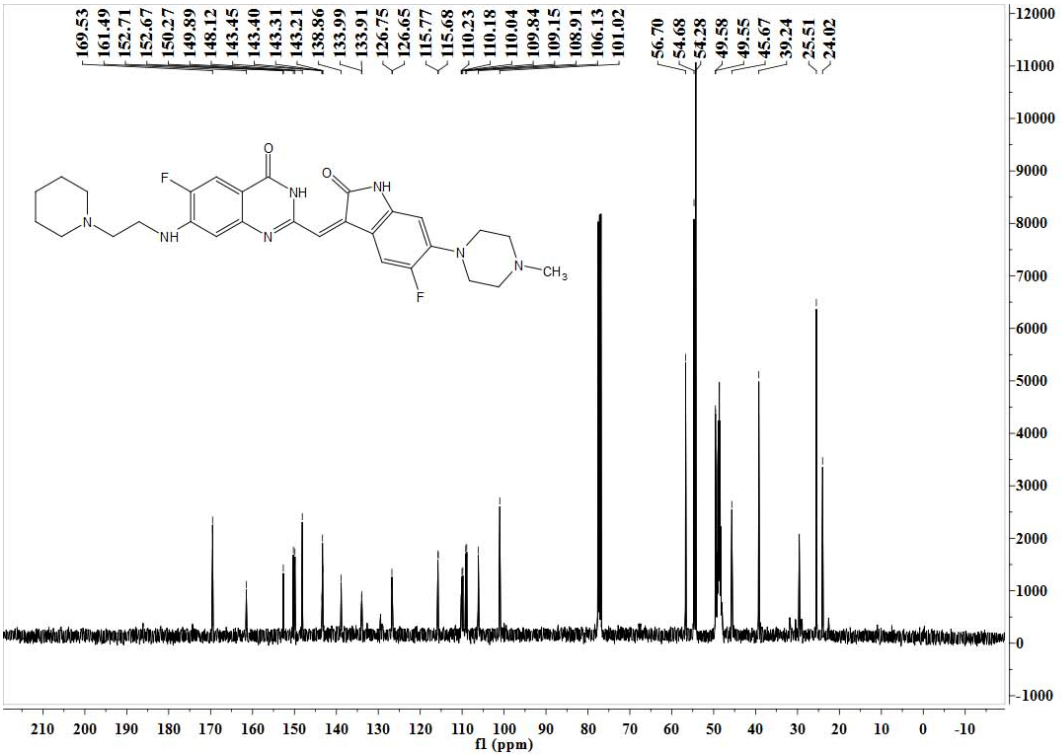
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.728	48308	6041	1.209	1.425
2	5.045	3939325	416190	98.574	98.156
3	9.653	8693	1777	0.218	0.419
Total		3996325	424008	100.000	100.000

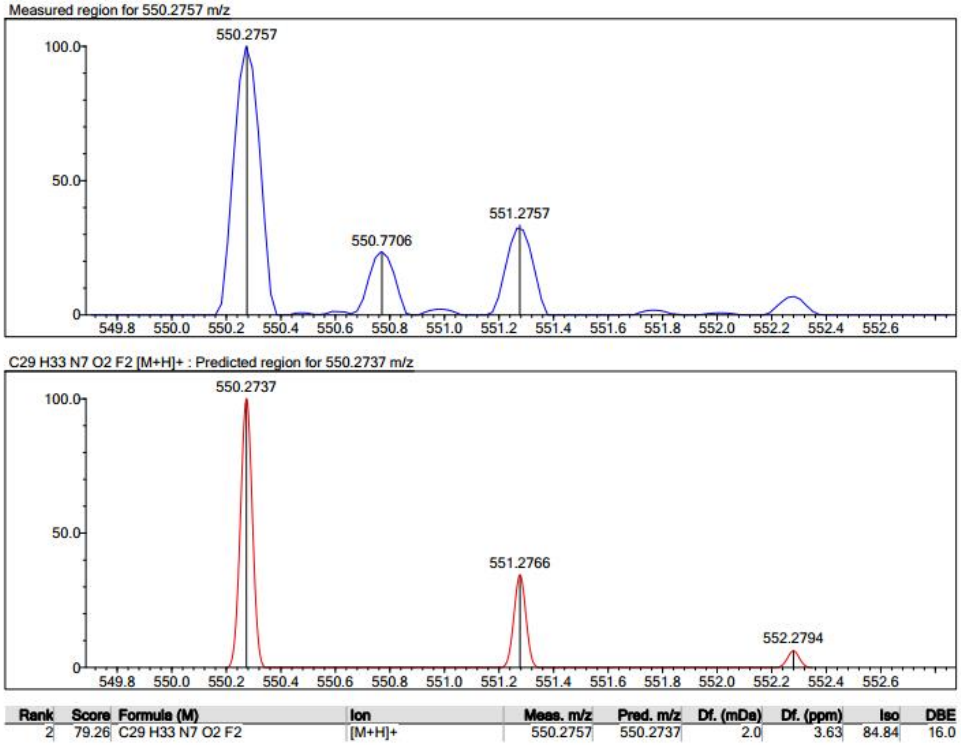
HPLC analysis of **28**



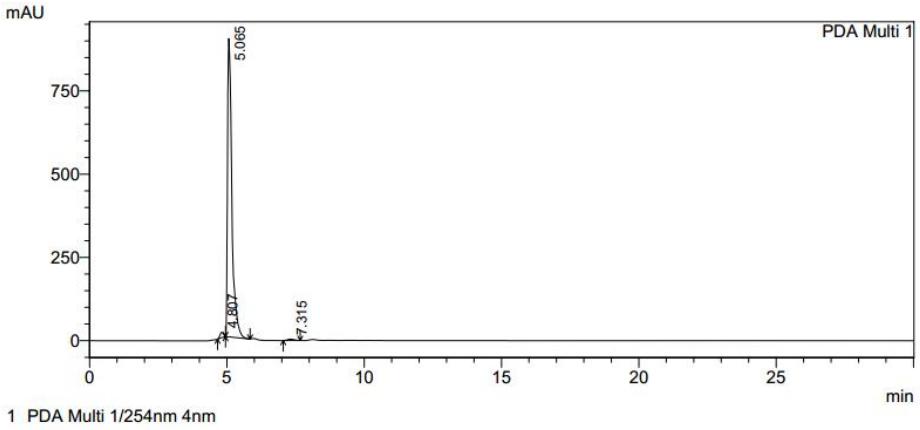
^{1}H NMR spectrum of **29**



^{13}C NMR spectrum of **29**



HRMR spectrum of **29**

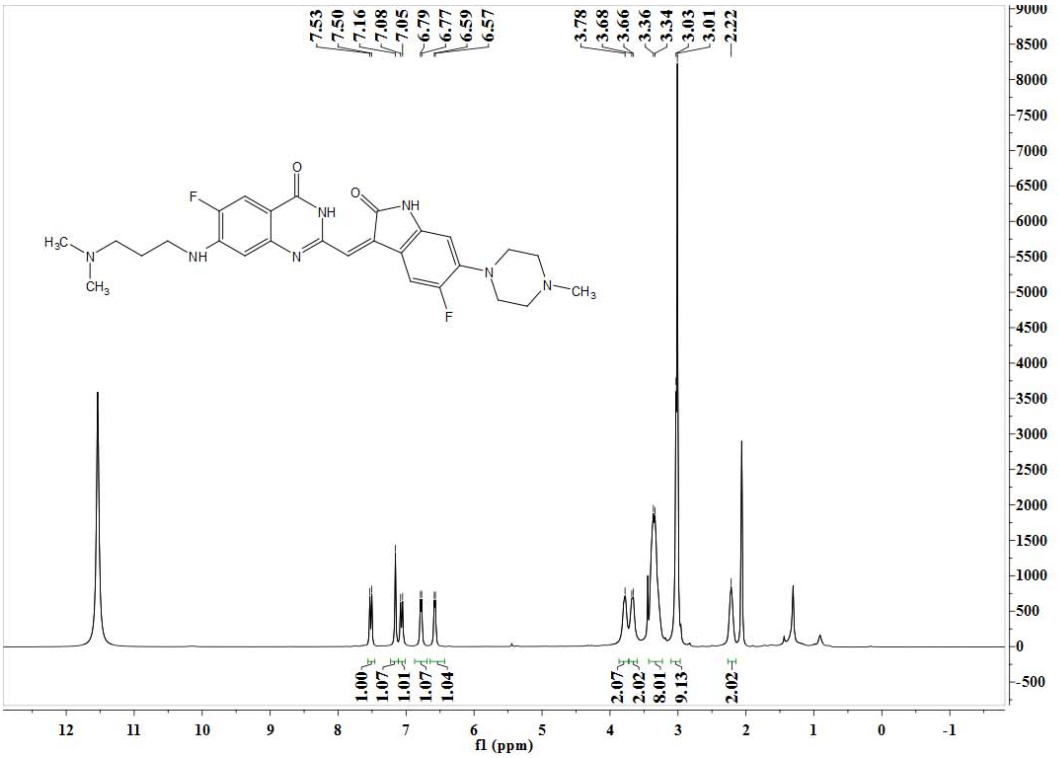


PeakTable

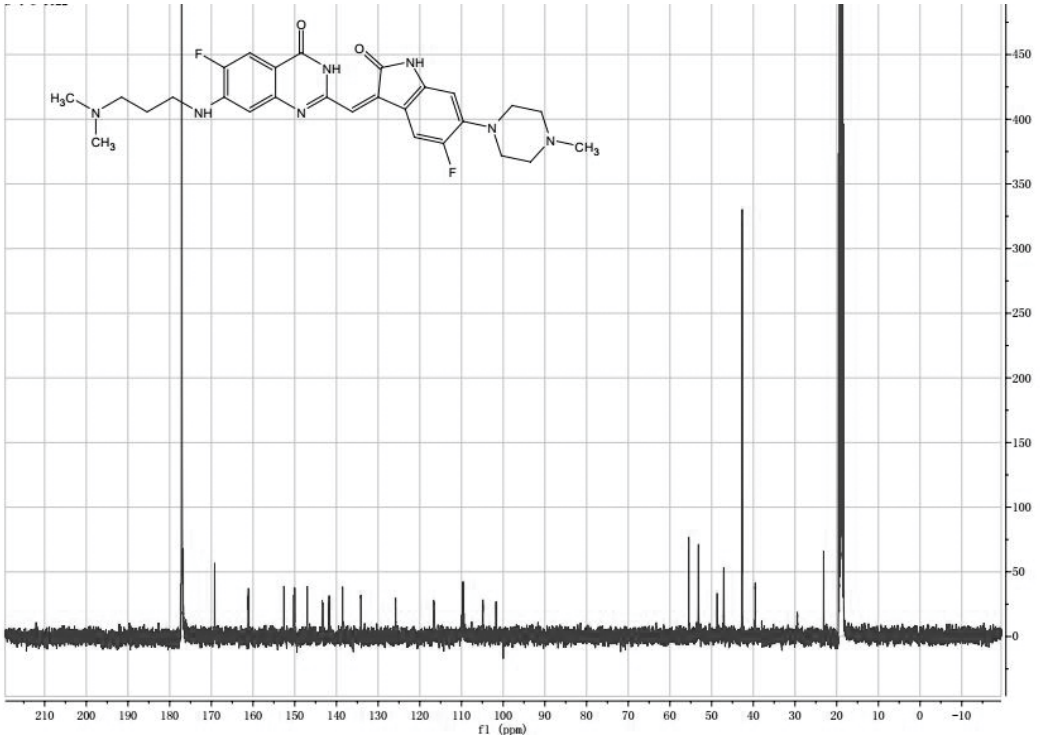
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.807	143057	16346	1.417	1.785
2	5.065	9885045	895604	97.910	97.782
3	7.315	67956	3968	0.673	0.433
Total		10096059	915918	100.000	100.000

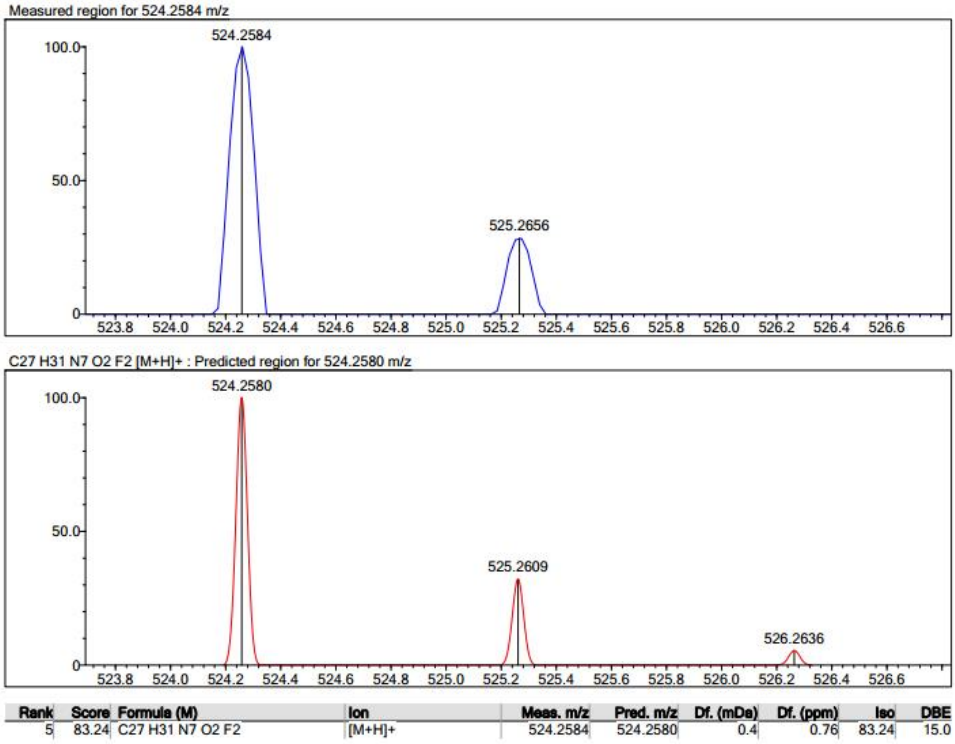
HPLC analysis of **29**



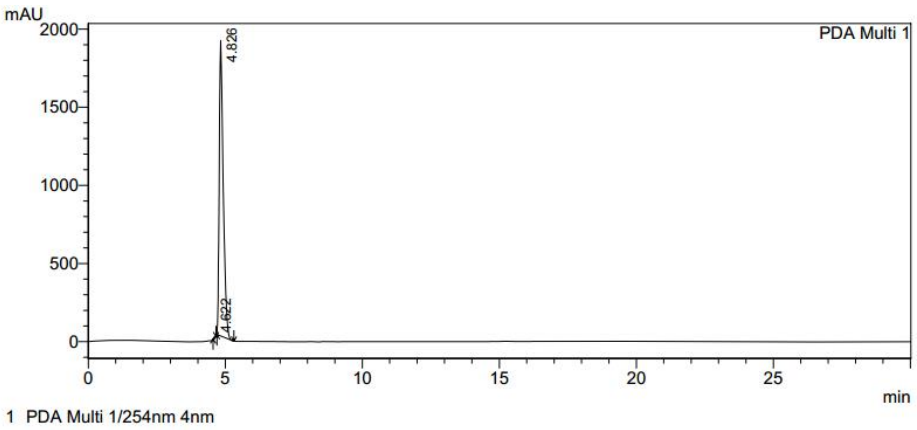
^{1}H NMR spectrum of **30**



^{13}C NMR spectrum of **30**



HRMR spectrum of **30**

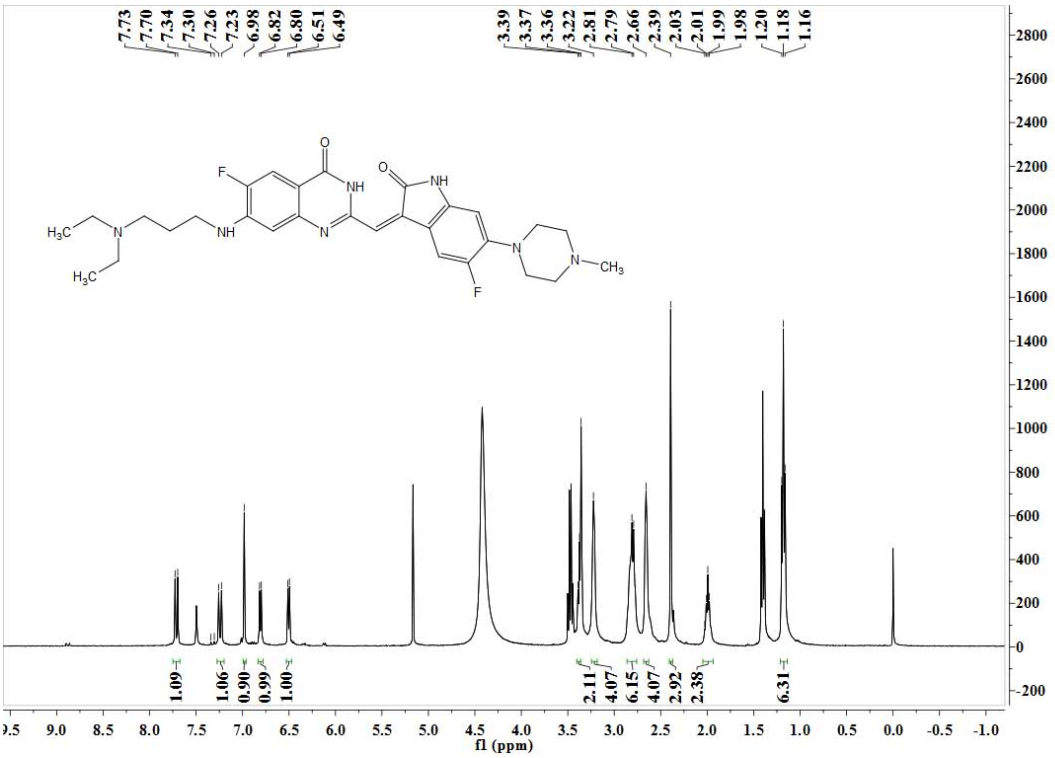


PeakTable

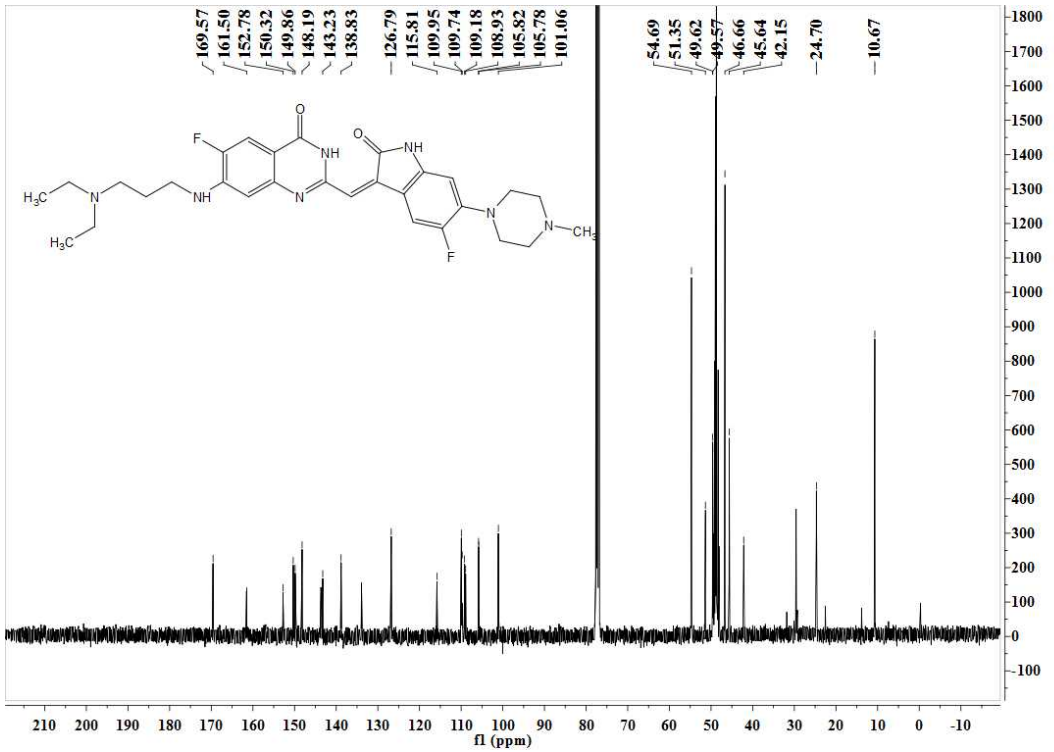
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.622	32466	6324	0.169	0.333
2	4.826	19181353	1891340	99.831	99.667
Total		19213819	1897663	100.000	100.000

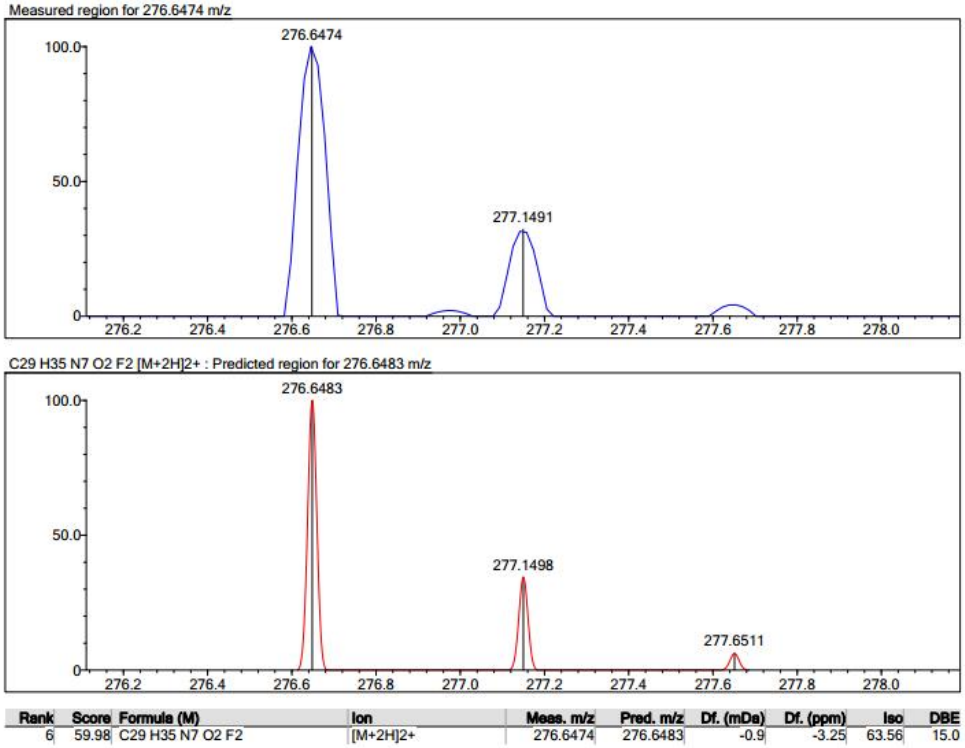
HPLC analysis of **30**



^{1}H NMR spectrum of **31**

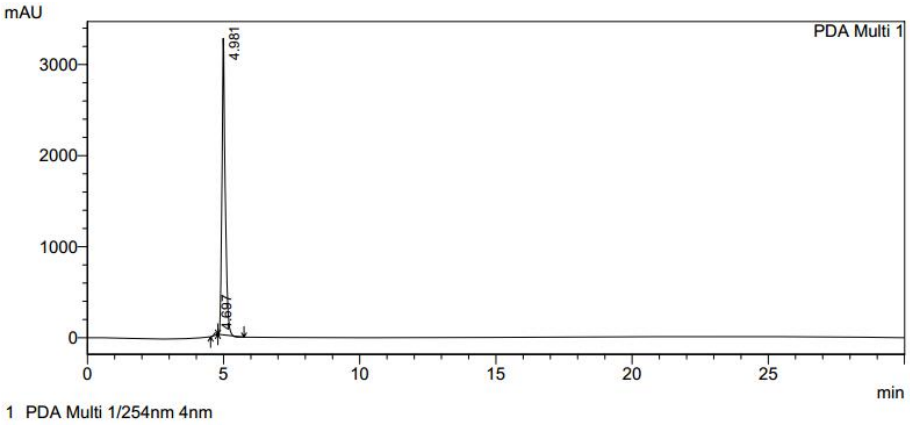


^{13}C NMR spectrum of **31**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
6	59.98	C29 H35 N7 O2 F2	[M+2H]2+	276.6474	276.6483	-0.9	-3.25	63.56	15.0

HRMR spectrum of **31**

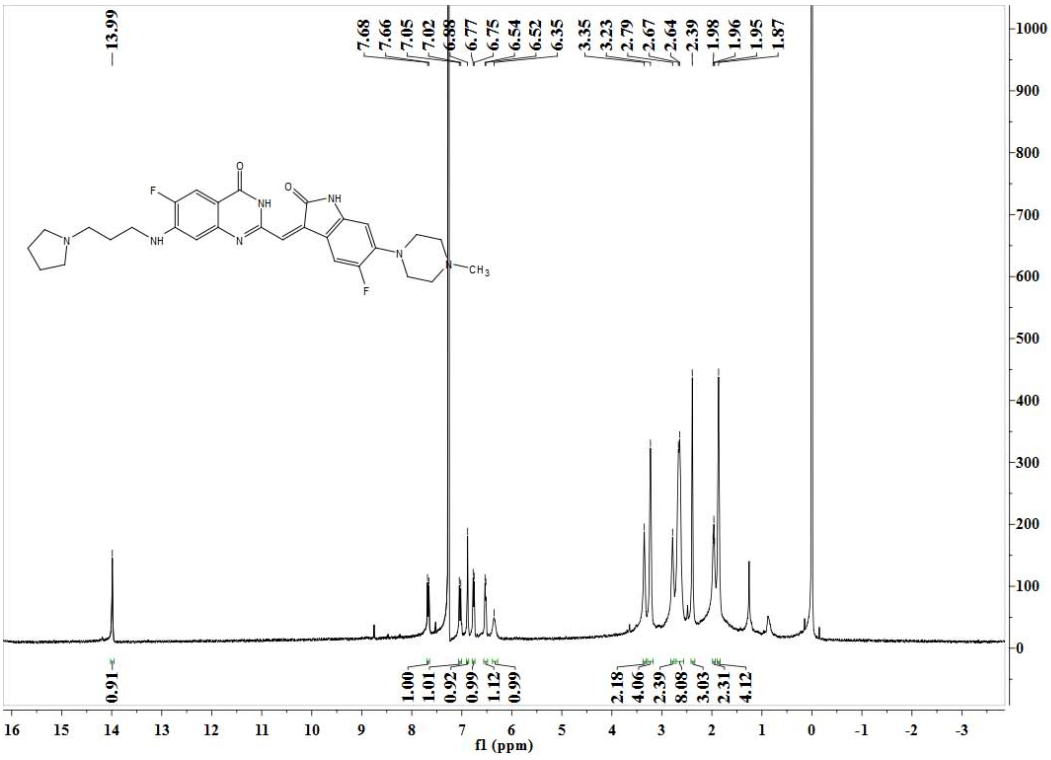


PeakTable

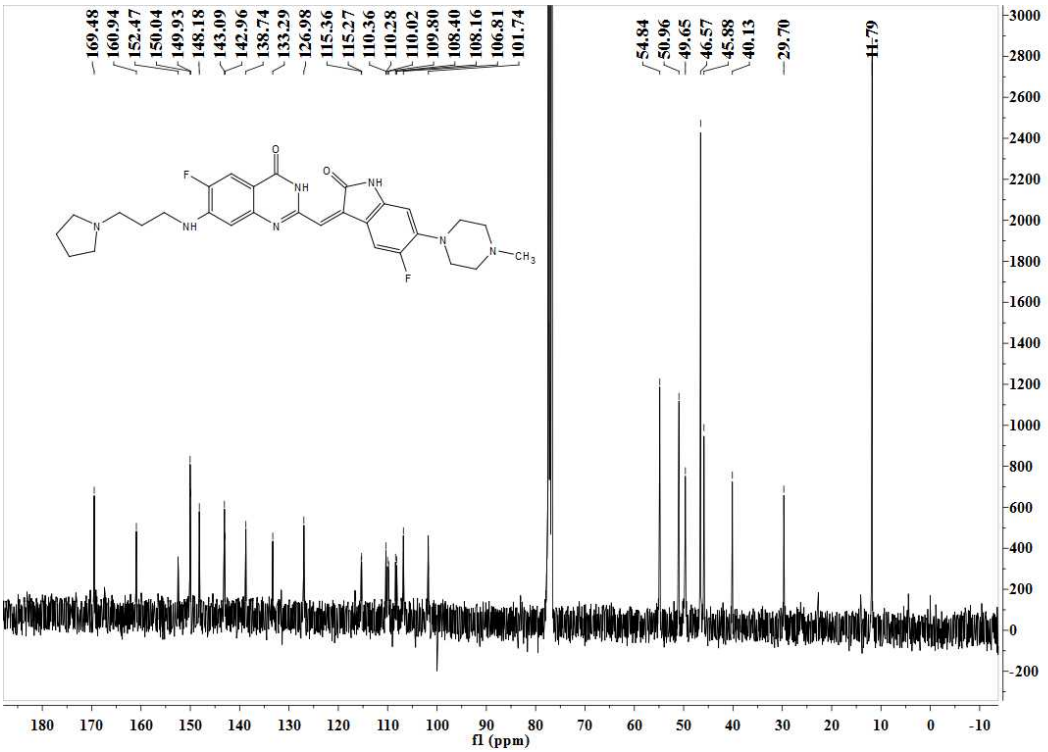
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.697	158382	22573	0.576	0.688
2	4.981	27341788	3257325	99.424	99.312
Total		27500169	3279898	100.000	100.000

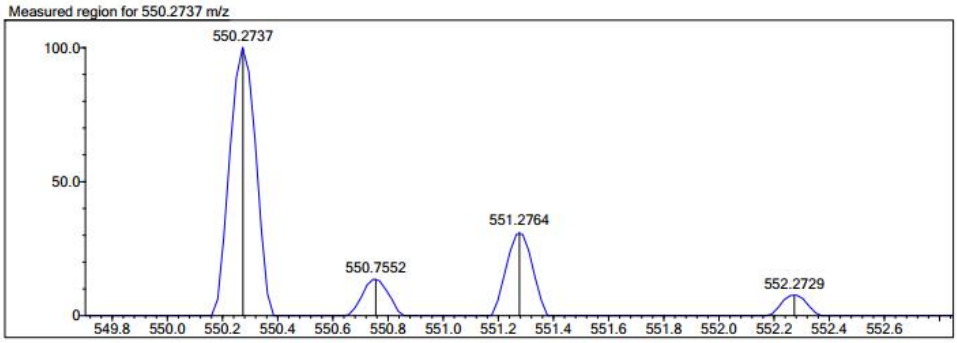
HPLC analysis of **31**

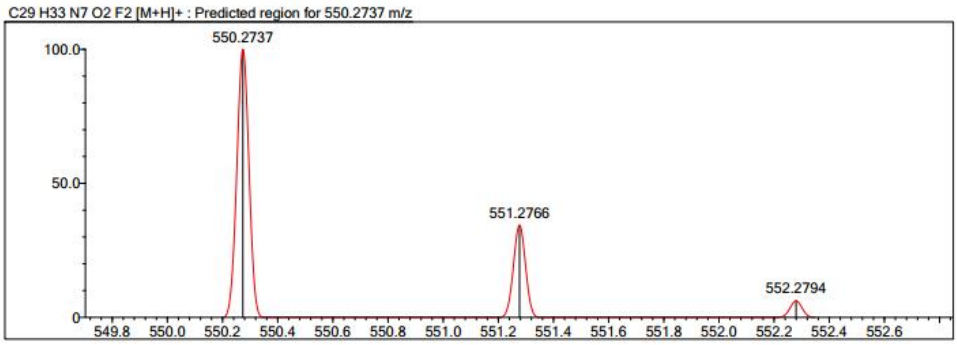


^{1}H NMR spectrum of **32**



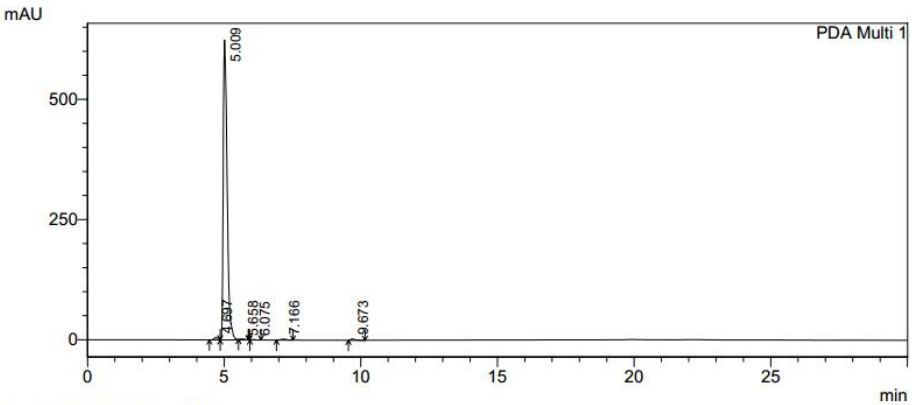
^{13}C NMR spectrum of **32**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	79.94	C29 H33 N7 O2 F2	[M+H]+	550.2737	550.2737	0.0	0.00	79.94	16.0

HRMR spectrum of **32**

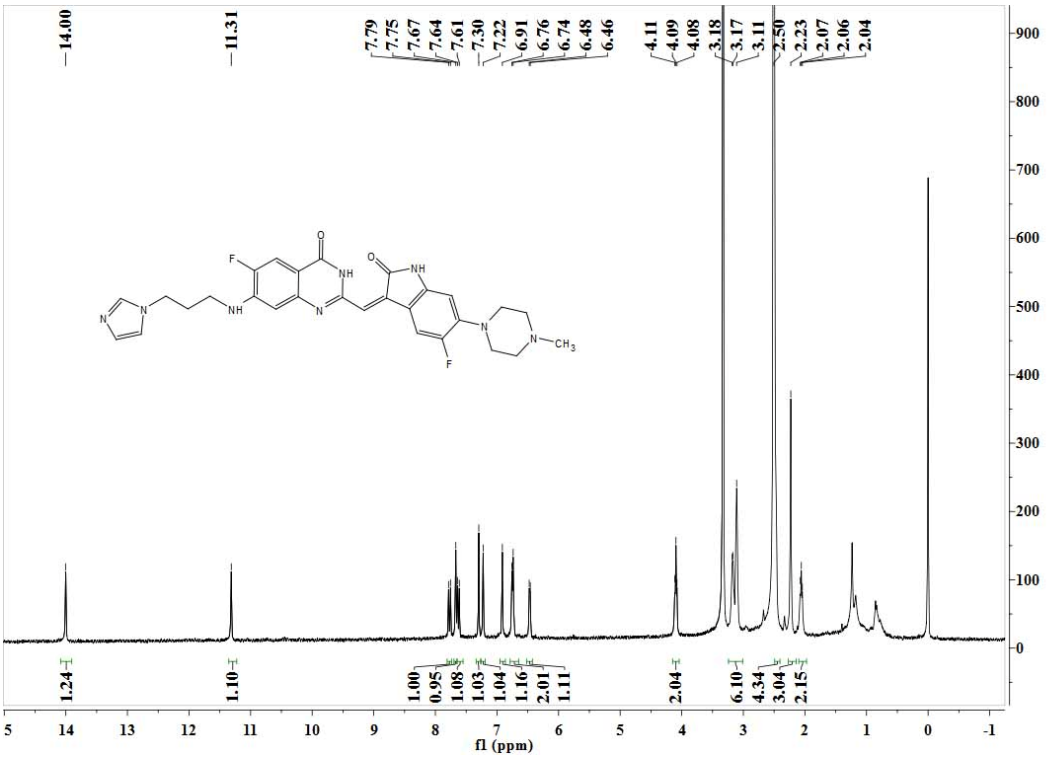


PeakTable

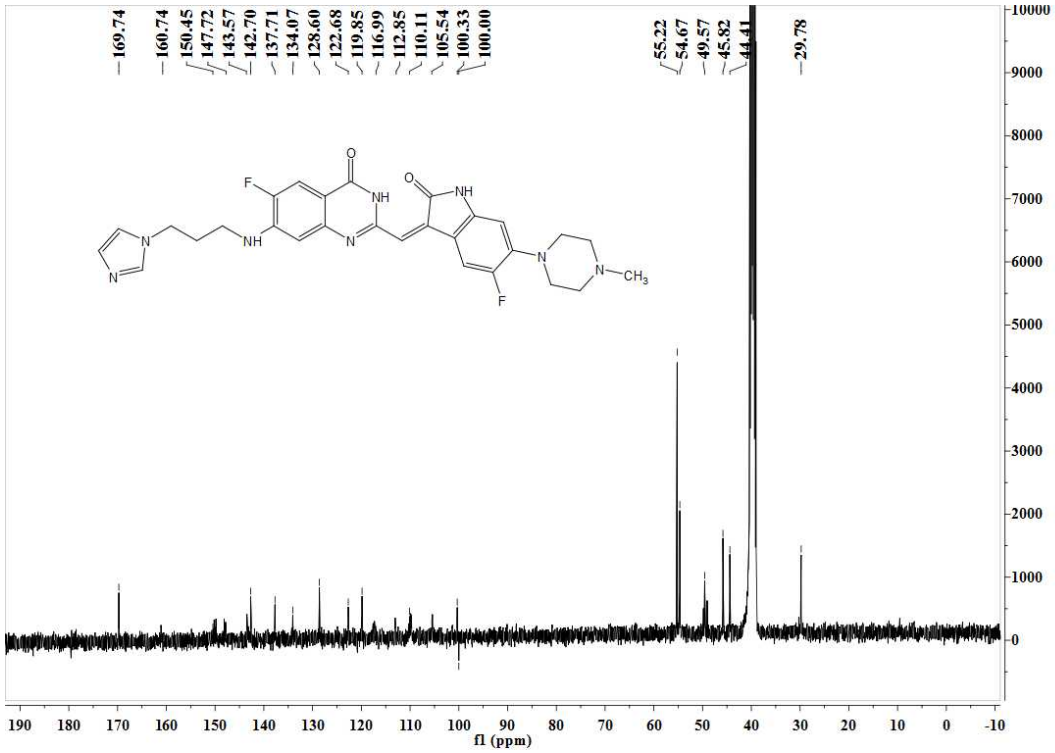
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.697	56410	5248	0.887	0.825
2	5.009	6223062	623804	97.868	98.076
3	5.658	9136	928	0.144	0.146
4	6.075	8020	864	0.126	0.136
5	7.166	28159	2068	0.443	0.325
6	9.673	33849	3132	0.532	0.492
Total		6358636	636044	100.000	100.000

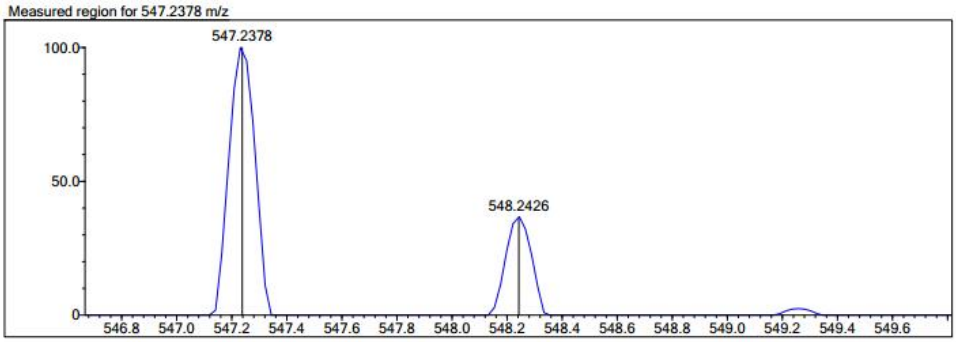
HPLC analysis of **32**

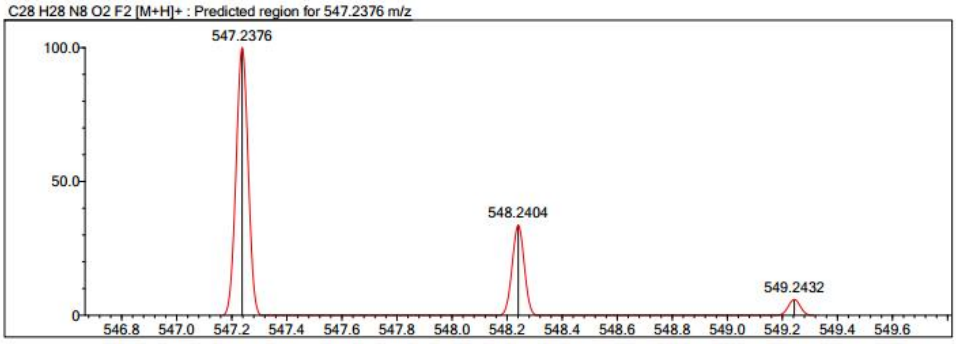


^{1}H NMR spectrum of **33**



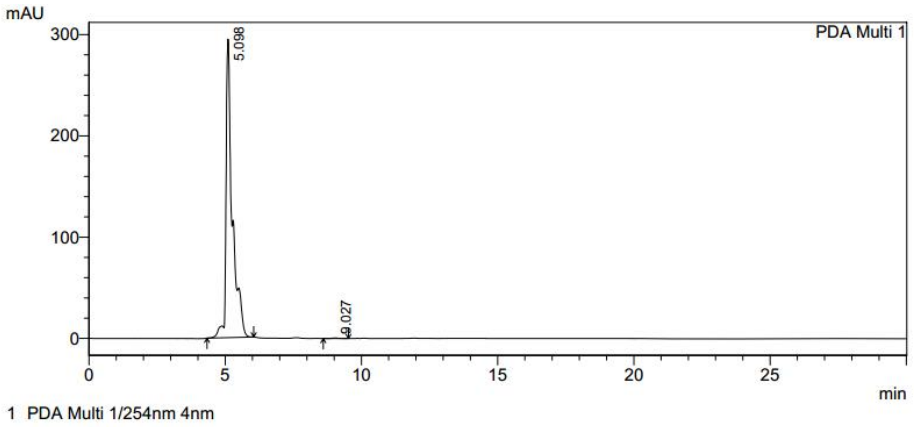
^{13}C NMR spectrum of **33**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	70.48	C28 H28 N8 O2 F2	[M+H]+	547.2378	547.2376	0.2	0.37	70.48	18.0

HRMR spectrum of **33**

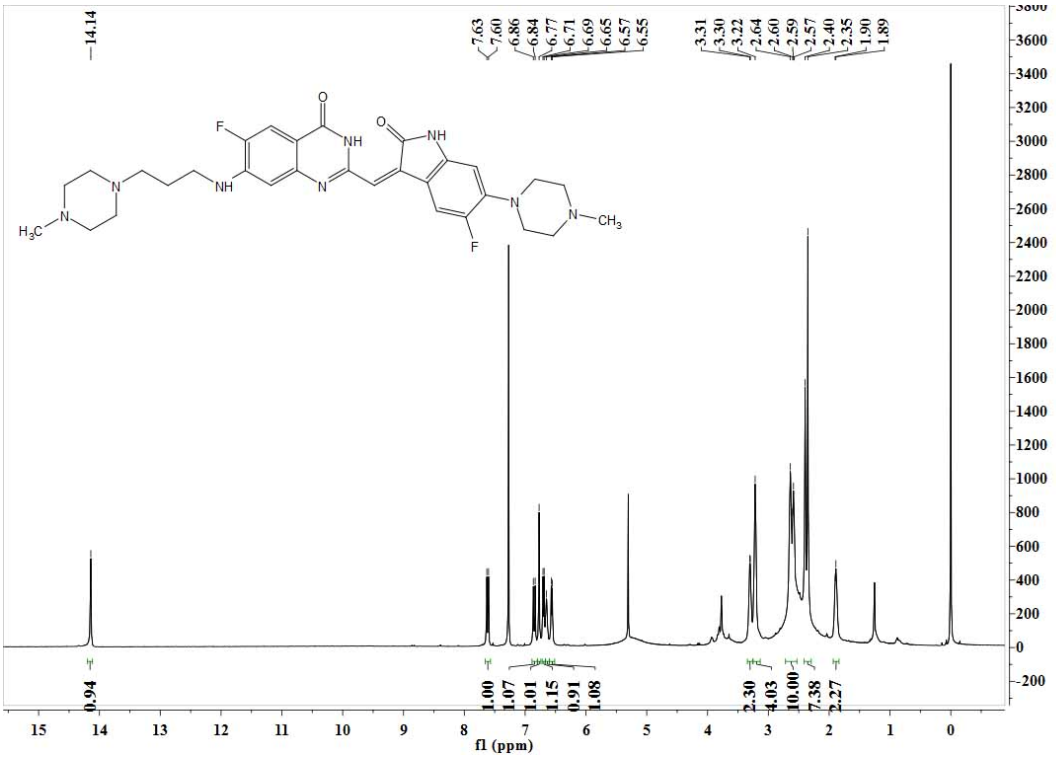


PeakTable

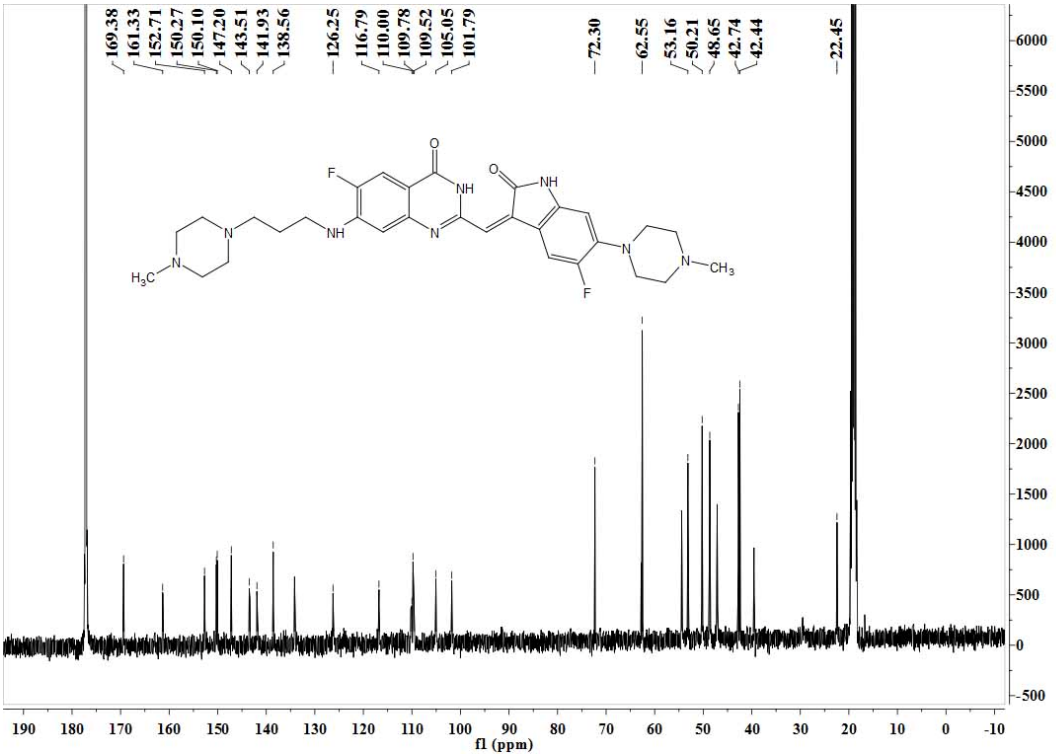
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.098	4738303	294765	99.848	99.823
2	9.027	7232	523	0.152	0.177
Total		4745535	295288	100.000	100.000

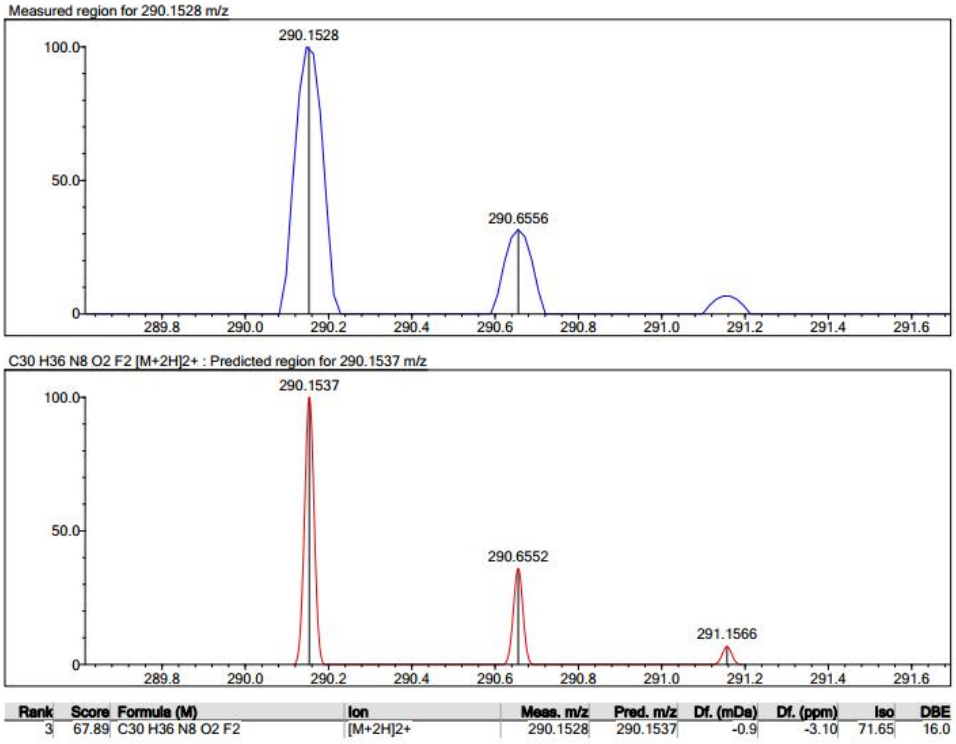
HPLC analysis of **33**



^{1}H NMR spectrum of **34**

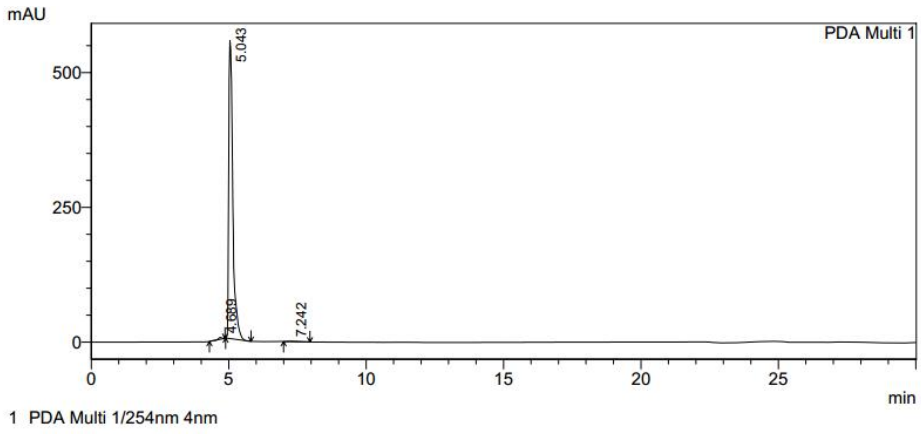


^{13}C NMR spectrum of **34**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	67.89	C30 H36 N8 O2 F2	[M+2H]2+	290.1528	290.1537	-0.9	-3.10	71.65	16.0

HRMR spectrum of **34**

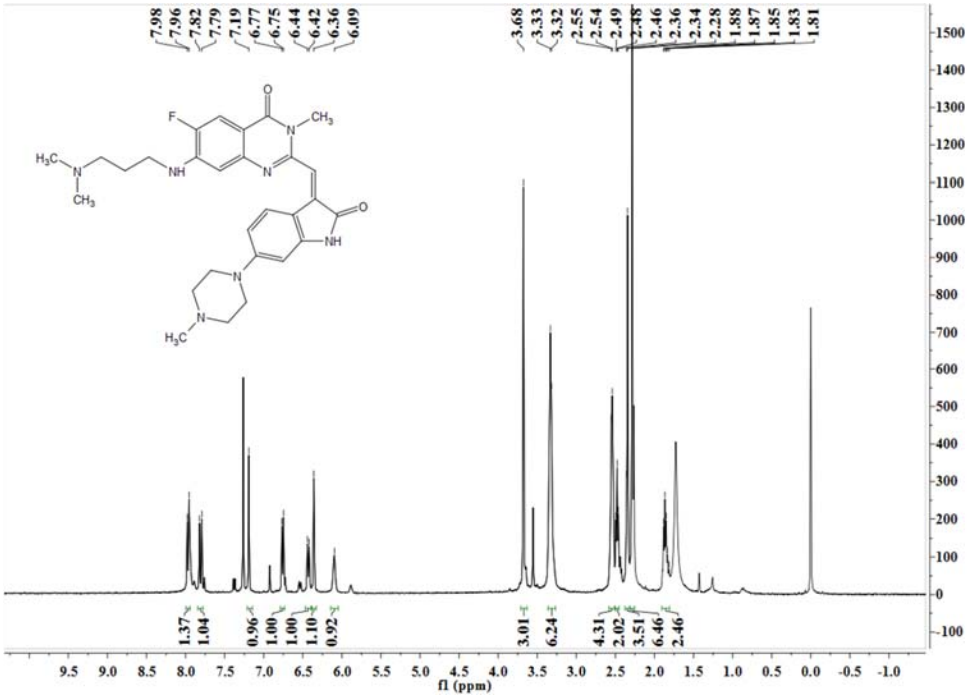


PeakTable

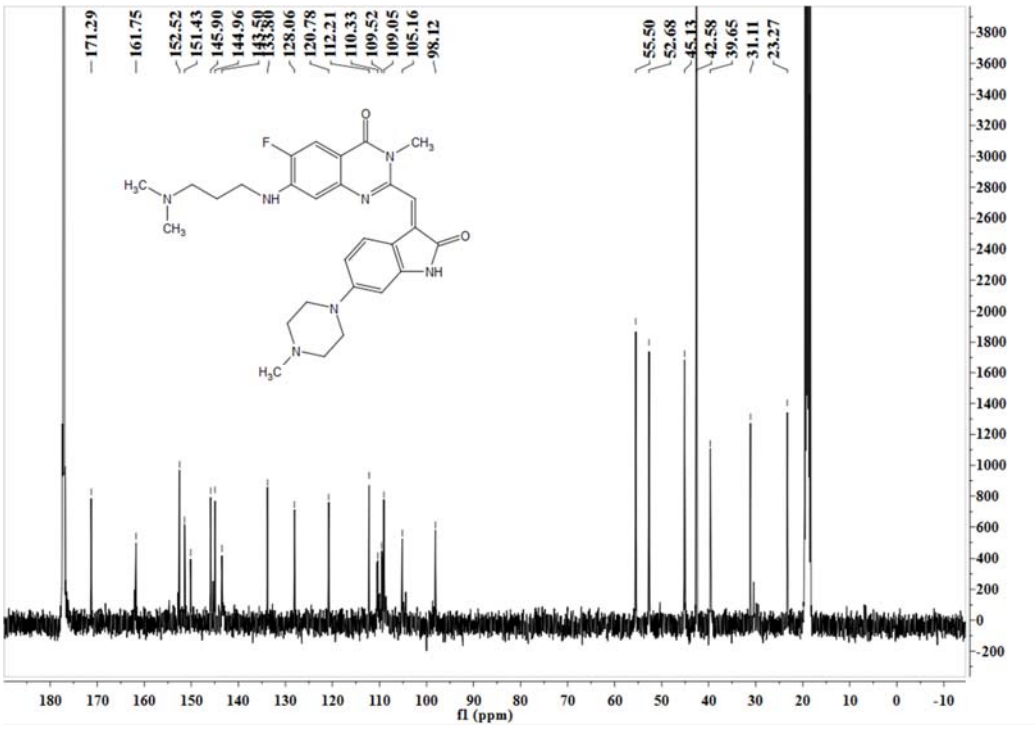
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.689	28606	3281	0.497	0.589
2	5.043	5708453	553530	99.166	99.283
3	7.242	19397	715	0.337	0.128
Total		5756456	557526	100.000	100.000

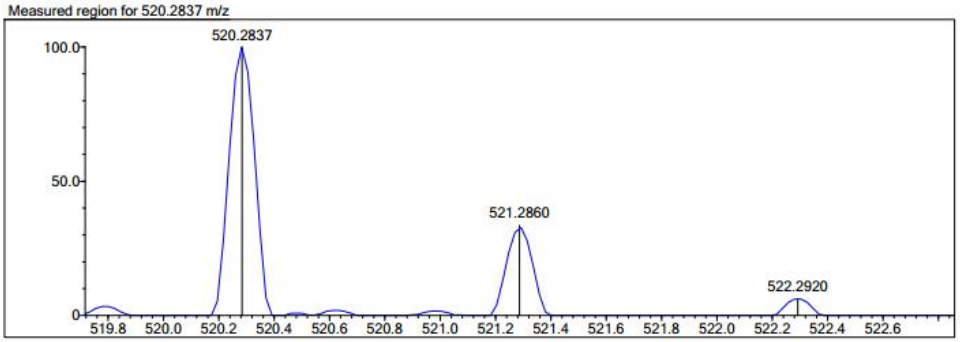
HPLC analysis of **34**

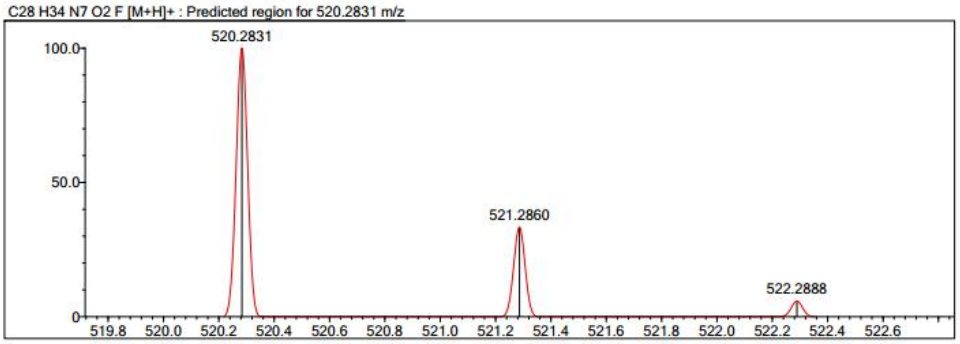


^{1}H NMR spectrum of **35**



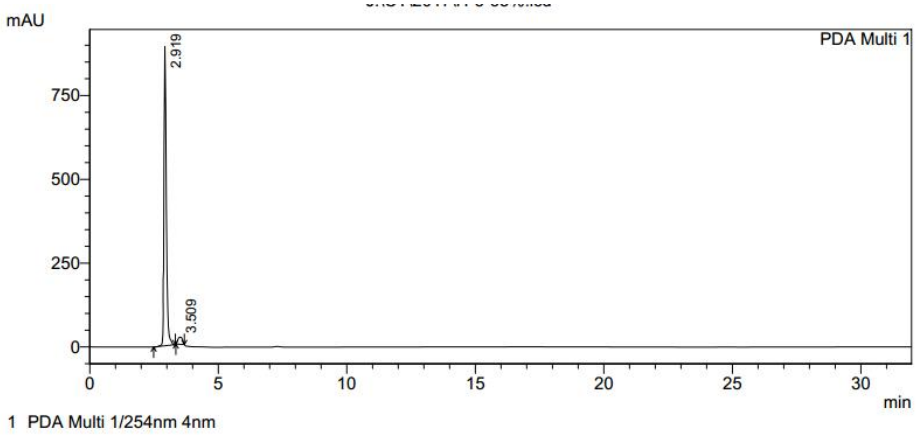
^{13}C NMR spectrum of **35**





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	98.89	C28 H34 N7 O2 F	[M+H]+	520.2837	520.2831	0.6	1.15	99.27	15.0

HRMR spectrum of **35**

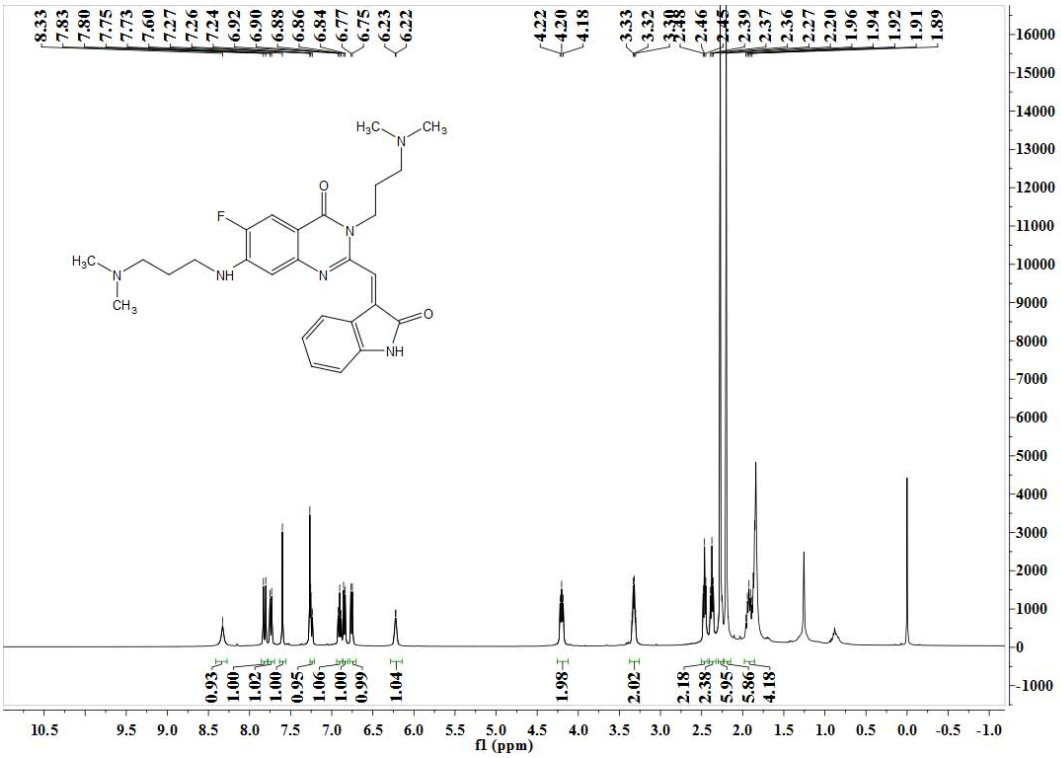


PeakTable

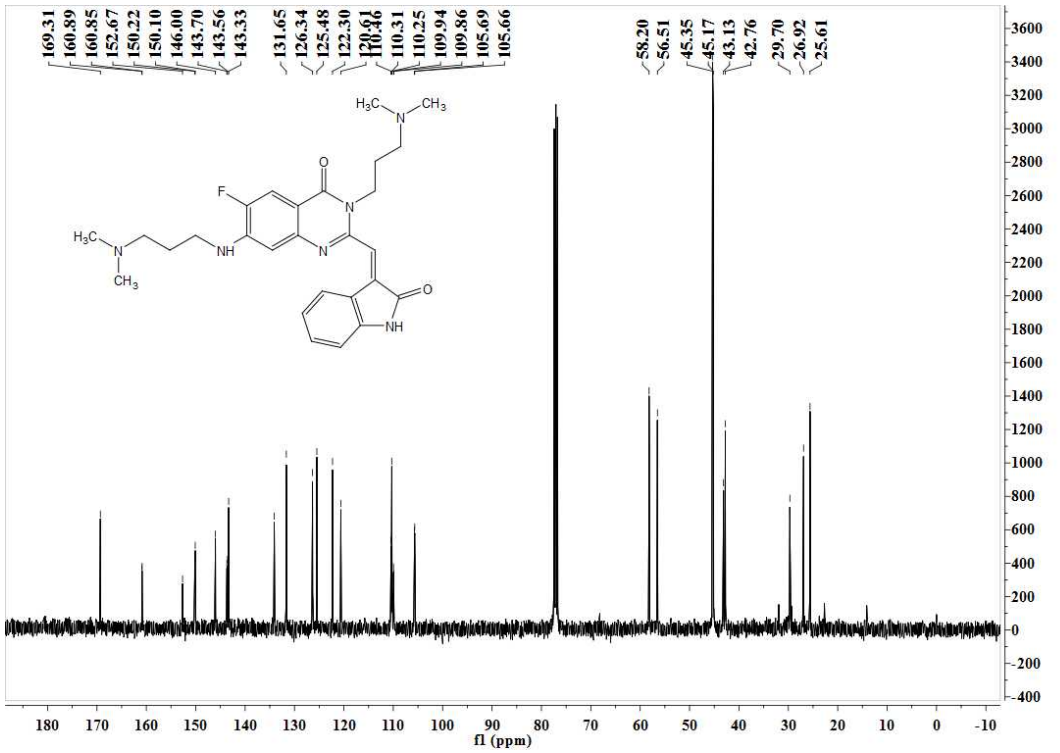
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.919	5662679	893424	95.541	97.701
2	3.509	264259	21022	4.459	2.299
Total		5926938	914445	100.000	100.000

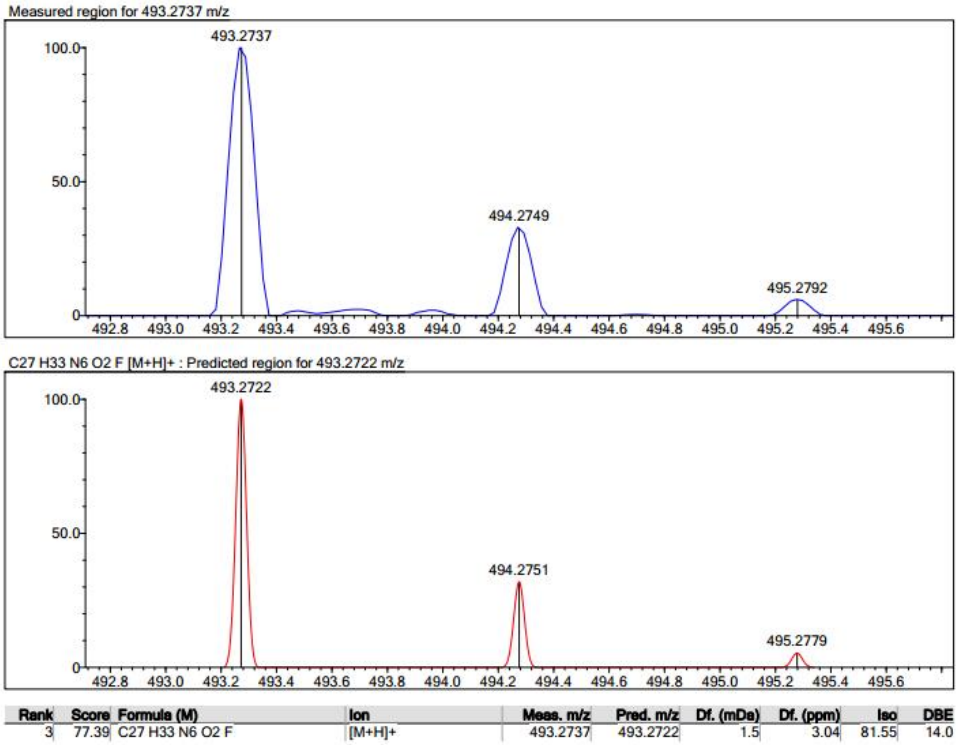
HPLC analysis of **35**



^{1}H NMR spectrum of **36**

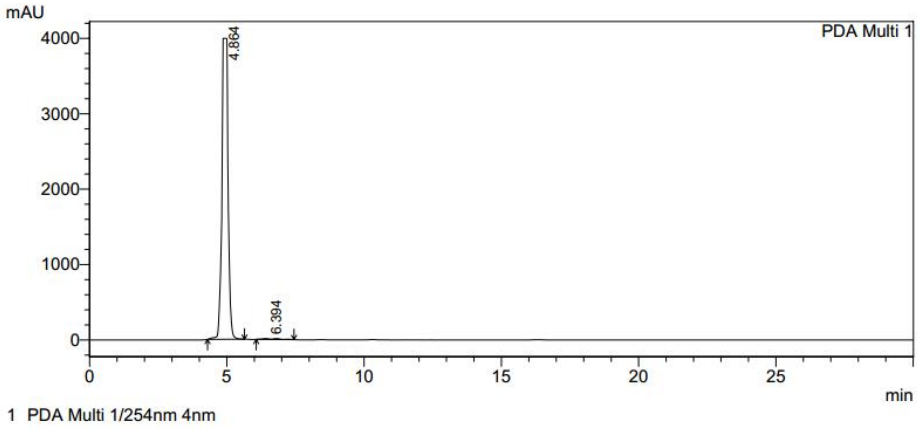


^{13}C NMR spectrum of **36**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	77.39	C27 H33 N6 O2 F	[M+H]+	493.2737	493.2722	1.5	3.04	81.55	14.0

HRMR spectrum of **36**

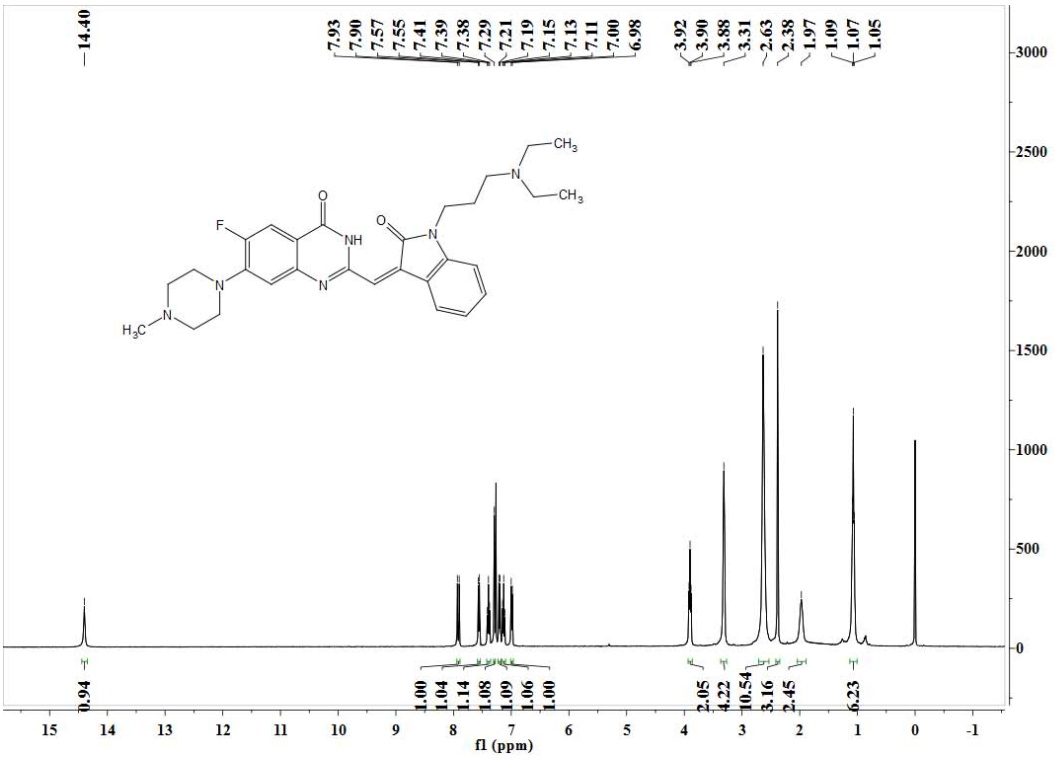


PeakTable

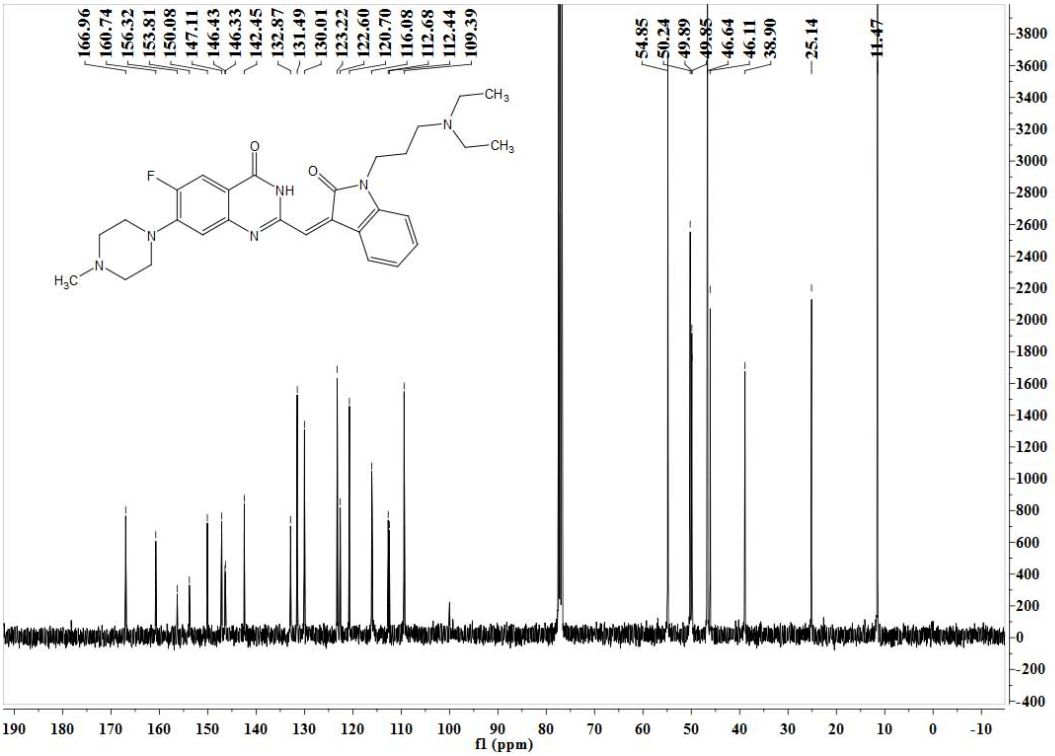
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.864	62872902	3991166	99.234	99.711
2	6.394	485643	11553	0.766	0.289
Total		63358545	4002719	100.000	100.000

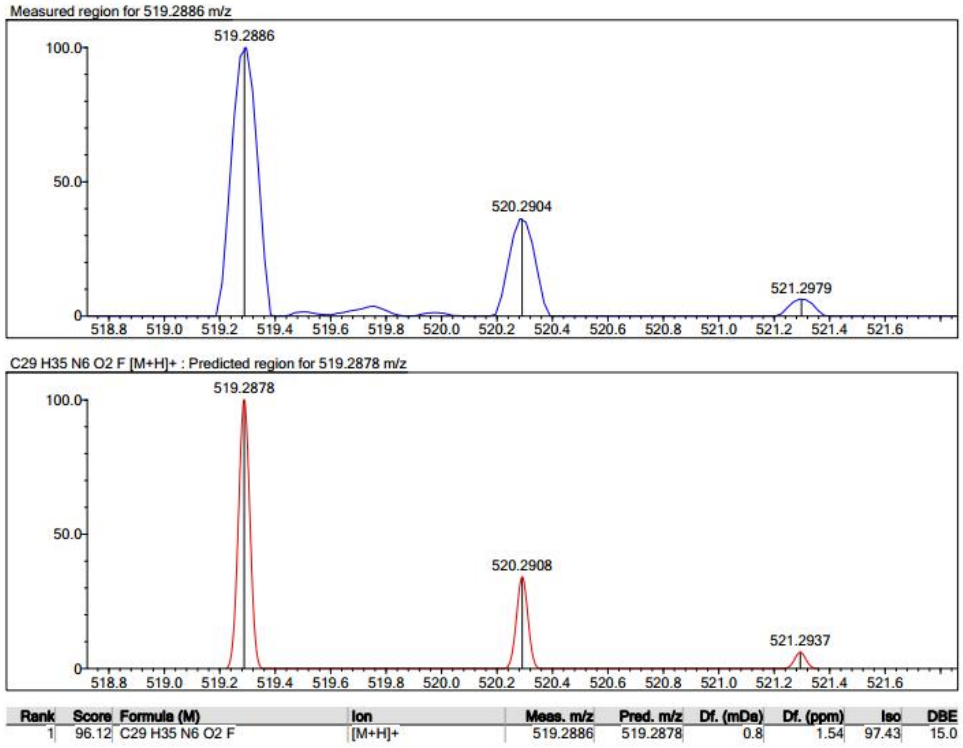
HPLC analysis of **36**



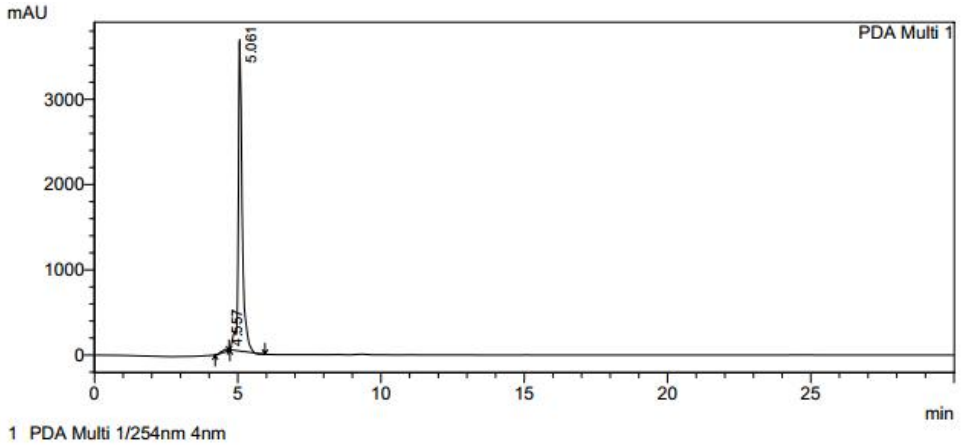
^{1}H NMR spectrum of **37**



^{13}C NMR spectrum of **37**



HRMR spectrum of **37**



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.557	373744	32690	1.052	0.888
2	5.061	35157843	3649530	98.948	99.112
Total		35531587	3682220	100.000	100.000

HPLC analysis of **37**