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Inhibition of O-GlcNAc transferase (OGT) by peptidic hybrids

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Molecular Modelling

Compound library. Fragment libraries from Asinex, ChemBridge, ChemDiv, Enamine, Life chemicals, Key Organics, Maybridge and Otava were downloaded in SDF format. These libraries were merged and duplicates removed, which resulted in a library containing 216,472 compounds. For these compounds a library of conformers was generated using OMEGA software (Release 2.5.1.4, OpenEye Scientific Software, Inc., Santa Fe, NM, USA; www.eyesopen.com)[1] using default settings, which resulted in a maximum of 200 conformers per ligand. The average number of conformers in the library was 51.

Structure-based virtual screening and docking. For docking with FRED software (Release 3.2.0.2, OpenEye Scientific Software, Inc., Santa Fe, NM, USA; www.eyesopen.com), OGT binding site (PDB entry: 4N39)[2] was prepared using MAKE RECEPTOR (Release 3.2.0.2, OpenEye Scientific Software, Inc., Santa Fe, NM, USA; www.eyesopen.com). The grid box around the UDP bound in the OGT crystal structure was generated automatically and was not adjusted. This resulted in a box with the following dimensions: 16.67 Å *15.00 Å * 20.33 Å and the volume of 5083 Å^3. For "Cavity detection" slow and effective "Molecular" method was used for detection of binding sites. Inner and outer contours of the grid box were also calculated automatically using "Balanced" settings for "Site Shape Potential" calculation. The inner contours were disabled. Ala896 was defined as hydrogen bond donor and acceptor constraint for the docking calculations. Fragment library, prepared by OMEGA, was then docked to the prepared UDP-binding site of OGT (PDB entry: 4N39)[2] using FRED (Release 3.2.0.2. OpenEye Scientific Software, Inc., Santa Fe, NM, USA; www.eyesopen.com)[3–5]. Docking resolution was set to high, other settings were set as default. A hit list of top 500 ranked molecules was retrieved and the best ranked FRED-calculated pose for each compound was inspected visually and used for analysis and representation.

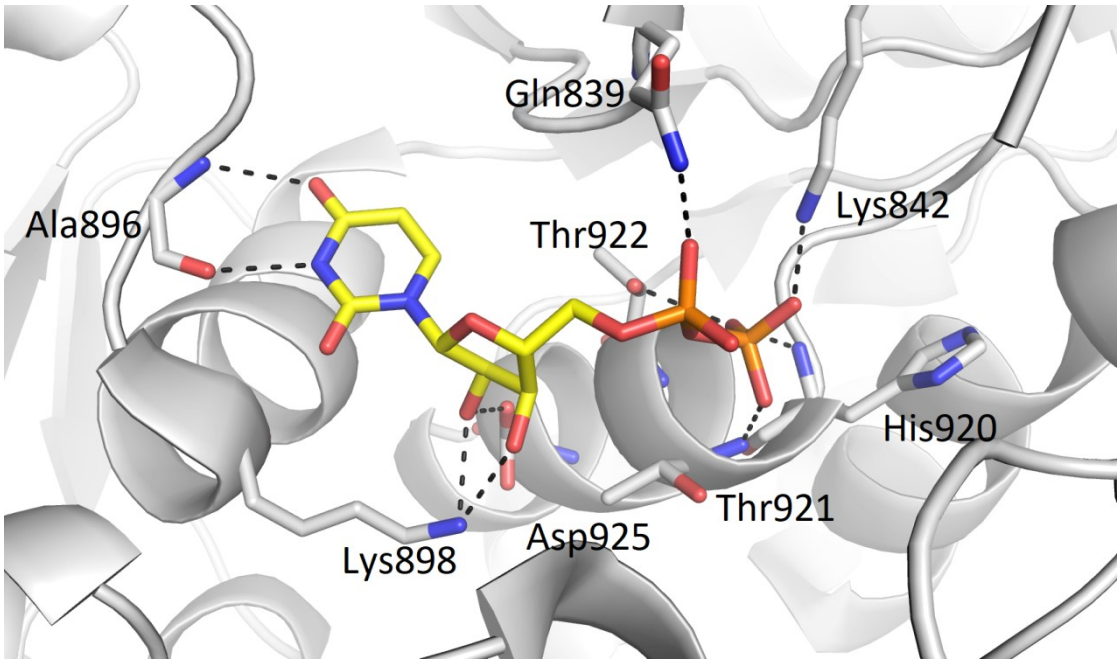


Figure S1. Binding mode of UDP (in yellow sticks) in OGT binding site (in grey cartoon, PDB entry: 4N39). The ligand and the neighbouring protein side-chains are shown as stick models, coloured according to the chemical atom type (blue, N; red, O; orange, S; green, Cl). Hydrogen bonds are indicated by black dotted lines.

General Procedures

Reagents, solvents and solutions. Unless stated otherwise, chemicals and solvents were obtained from commercial sources and used without further purification. Solvents for compounds synthesis were purchased from Biosolve (Valkenswaard, The Netherlands) and were stored on molecular sieves (4 Å). Fmoc-L-Glu-OtBu and Fmoc-L-Lys(Alloc)-OH were obtained from Iris Biotech GMBH (Marktredwitz, Germany) and other standard Fmoc amino acids were obtained from GL Biochem Ltd (Shanghai, China).

Purification Techniques. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F254). One or more of the following methods were used for visualization: 10% H_2SO_4 in MeOH, molybdenum blue, I_2 or ninhydrine followed by warming until spots could be visible. Flash chromatography was performed using Merck type 60, 230–400 mesh silica gel. Removal of solvent was performed under reduced pressure using a rotary evaporator.

Instrumentation for Compound Characterization. ^{1}H and ^{13}C NMR spectroscopy was carried out on an Agilent 400-MR spectrometer operating at 400 MHz for ^{1}H and 101 MHz for ^{13}C or on a Bruker AVANCE III spectrometer at 400 MHz and 101 MHz, respectively, in DMSO-d6 solution, with TMS as internal standard at 25°C. HSQC, TOCSY and NOESY (500 MHz) were performed on a VARIAN INOVA-500. Electrospray Mass experiments were performed on a Shimadzu LCMS QP-8000. High resolution mass spectrometry (HRMS) analysis was recorded using Bruker ESI-Q-TOF II or VGAnalytical AutoSpec Q Micromass mass spectrometer. Analytical LC-MS (electrospray ionization) was performed on Thermo-Finnigan LCQ Deca XP Max using the same buffers and protocol as described for analytical HPLC. Melting points were determined on a Reichert hot stage microscope and are uncorrected. IR spectra were recorded on a PerkineElmer Spectrum BX FT-IR spectrometer.

Analytical HPLC was performed on a Shimadzu-10AVP (Class VP) system using a Phenomenex Gemini C18 column (110 Å, 5 µm, 250×4.60 mm) at a flow rate of 1 mL min. The used buffers were 0.1 % trifluroacetic acid in CH_3CN: H_2O= 5: 95 (buffer A) and 0.1 % trifluroacetic acid in CH_3CN: H_2O= 95: 5 (buffer B). Runs were performed using standard protocol (a) or (b), (a): 100% buffer A for 2 min, then a linear gradient of buffer B (0-100% in 38 min) and UV-absorption was measured at 214 and 254 nm; (b) 100% buffer A for 2 min, then a linear gradient of buffer B (0-100% in 48 min) and UV-absorption was measured at 214 and 254 nm. Purification using preparative HPLC was performed on an Applied Biosystems workstation with a Phenomenex Gemini C18 column (10 µm, 110 Å, 250×21.2 mm) at a flow rate of 6.25 mL/min. Runs were performed by a standard protocol: buffer A for 5 min followed by a linear gradient of buffer B (0 – 100% in 70 min) with the same buffer as described for the analytical HPLC. HPLC analysis of fragments were performed on a Thermo Scientific Dionex UltiMate 3000 system (Thermo Fisher Scientific Inc., Waltham, MA, USA), using an Acquity UPLC® C8 column (1.7 µm, 2.1 × 50 mm), at a flow rate of 0.3 mL/min, temperature 25 °C and injection volume of 5 µL.

Method A: The eluent was a mixture of 0.1% TFA in water (A) and acetonitrile (B). The gradient was 10% B to 80% B in 12 min, then 80% B for 3 min.

Method B: The eluent was a mixture of 20 mM KH_2PO_4 (A) and acetonitrile (B). The gradient was 10% B to 40% B in 14 min, then 40% B for 2 min.

Method C: The eluent was a mixture of 20 mM KH_2PO_4 (A) and acetonitrile (B). The gradient was 5% B to 10% B in 12 min, then 10% B for 2 min.

The purity of the tested compounds was established to be ⩾95% for all fragments, except for F4, F12 and F13, where the purity was established to be ⩾90%. Some compounds have double peak in HPLC specter due to the amide-imidic acid tautomerism of quinolin-2-one fragment.
All literature compounds had ^{1}H NMR and mass spectra consistent with the assigned structures.

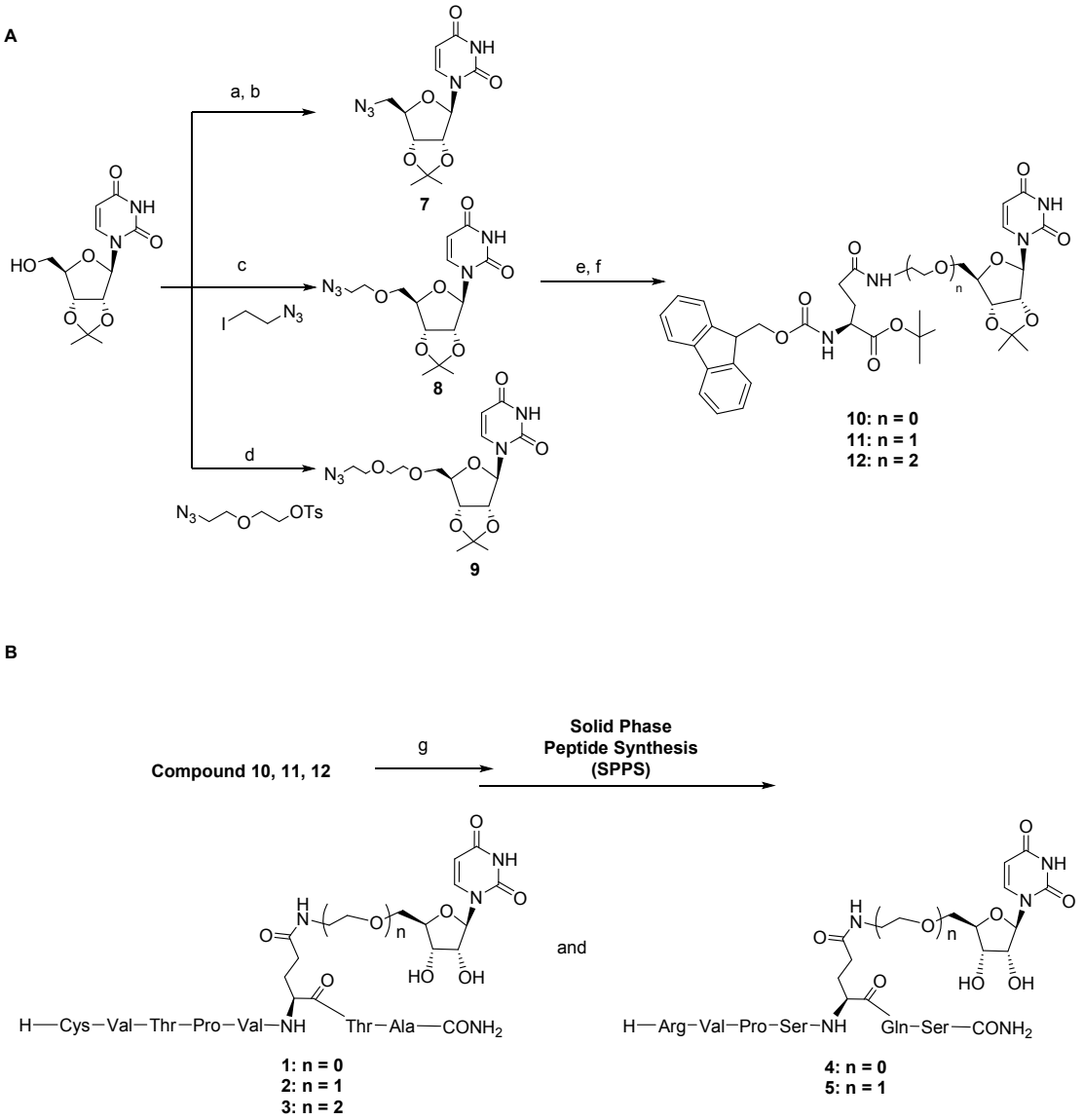
Experimental Details

Synthesis of all the peptides was achieved by following a standard Fmoc SPPS strategy on a Symphony Multiple Peptide Synthesizer and starting from Rink amide resin. The modified building blocks **7-9** were prepared and an on-resin approach for preparing compound **1-5** was established as described in Scheme 1S.
Peptides were assembled on Rink amide tentagel S resin (227 mg with a resin loading of 0.22 mmol/g). With the exception of the modified building block, peptide couplings were performed with protected Fmoc amino acid (4.0 eq), BOP reagent (4.0 eq), and DIPEA (8.0 eq) in DMF (total volume 5 mL) at ambient temperature for 1 hour.
Incorporation of the modified Glu were performed by using corresponding building block (2.0 eq), BOP reagent (4.0 equiv), and DiPEA (8 eq). Upon completion of SPPS, peptides were cleaved from the resin and deprotected by using a mixture of TFA/EDT/TIPS/H2O (9:0.25:0.25:0.5, V/V/V/V) followed by Et_2O precipitation to yield the crude peptides. **Pep1-15** were confirmed by LC/MS analysis, in each case providing the expected mass (table **S1**). Compounds **1-5** were purified by preparative hplc as described in the general method and their purities and identities were confirmed by analytical HPLC and high-resolution mass spectrometry.
For preparing **1-5**, modified "Glu" building blocks **7-9** were first synthesized following a method described in Scheme 1S (A). Briefly, a 2',3'-O-isopropylidene uridine precursor was converted into **7-9** which bear azido groups. For **7**, the 5'-hydroxy group was first converted into a p-toluenesulfonyl ester with p-toluenesulfonylchloride and pyridine as the catalyst/base. The following nucleophilic substitution with sodium azide gave **7** in high yield. The ensuing catalytic hydrogenation with Lindlar catalyst proceeded to completion within 3 h, and the desired amine derivative was found to be pure by LCMS and used without purification in the next reaction step due to its inherent instability. Treatment of with Fmoc-Glu-OtBu in dry dichloromethane, with DiPEA as the base, gave the **10** in relatively high yield. On the other hand, synthesis of the **8** and **9** began with the synthesis of azido linkers via a modified literature method[6,78]. Via the route, two linkers with hundred milligram quantities were readily prepared. These linkers were reacted with the 5'-hydroxy group of 2',3'-O-isopropylidene uridine precursor in CH_3CN with NaOMe under reflux, which produced the corresponding **8** and **9**. These intermediates subsequently transformed into the corresponding amine under reaction conditions similar to those used for **7**. The resulting compound confirmed by LCMS were reacted to Fmoc-L-Glu-OtBu to give **11** and **12** using the same condition as for **10**. Compounds **10**, **11** and **12** were deprotected by the treatment with TFA/DCM, and then were incorporated into **Pep6** and **Pep13** to replace the GlcNAc site residue Ala separately by standard automated SPPS strategy as described in Scheme 1S (B). After deprotection and cleavage from the resin, the crude peptides were precipitated in cold Et_2O, which was purified by preparative RP-HPLC. The purity and identity were confirmed via analytical HPLC and high-resolution mass spectrometry.

Table 1S. Analytical data of Pep1-Pep15

Compound	R_t (min)[a]	Molecular formula	Exact Mass	Measured Mass[b]
Pep1	8.94	$C_{55}H_{94}N_{16}O_{19}S$	1314.66	1313.95
Pep2	11.49	$C_{49}H_{82}N_{14}O_{18}S$	1186.57	1186.71
Pep3	9.20	$C_{44}H_{75}N_{13}O_{15}S$	1057.52	1057.29
Pep4	9.16	$C_{40}H_{69}N_{11}O_{13}S$	943.48	943.86
Pep5	9.58	$C_{37}H_{64}N_{10}O_{11}S$	856.45	856.80
Pep6	8.22	$C_{32}H_{57}N_9O_{10}S$	759.39	759.50
Pep7	7.64	$C_{29}H_{52}N_8O_9$	656.39	656.41
Pep8	6.05	$C_{24}H_{43}N_7O_8$	557.32	557.48
Pep9	11.00	$C_{80}H_{125}N_{25}O_{28}S$	1915.88	1915.80
Pep10	23.50	$C_{49}H_{79}N_{15}O_{16}$	1133.58	1133.65
Pep11	21.00	$C_{45}H_{74}N_{14}O_{13}$	1018.56	1018.74
Pep12	18.09	$C_{39}H_{63}N_{13}O_{12}$	905.47	905.55
Pep13	10.53	$C_{30}H_{54}N_{12}O_{10}$	742.41	742.51
Pep14	8.24	$C_{24}H_{42}N_8O_9$	586.31	586.94
Pep15	4.57	$C_{19}H_{33}N_7O_8$	487.24	487.73

[a]Retention time measured on a a Prosphere C18 column (250 × 4.6 mm, 300 Å, 10 µm) and an acetonitrile gradient (5-95%, 0.1% of TFA) in 40 minutes; flow rate of 1 ml/min.
[b] ESI-LRMS.



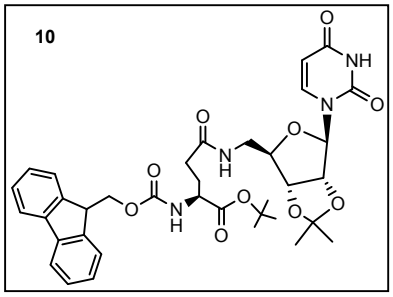
Scheme 1S. (**A**) Synthesis of **1-5**. Reagents and conditions: a) TsCl, pyridine, 0 ^{o}C→RT, 12h; b) NaN_3, DMF, RT, 12h; c) $ICH_2CH_2N_3$, NaOMe, CH_3CN, 85 ^{o}C, 12h; d) $TsOCH_2CH_2OCH_2CH_2N_3$, NaOMe, CH_3CN, 85 ^{o}C, 12h; e) Lindlar catalyst, H_2, RT, 3h; f) Fmoc-L-Glu-OtBu, Bop, DiPEA, CH_2Cl_2, RT, 20h; (**B**) Reagents and conditions: g) TFA/CH_2Cl_2, RT, 2h.

Preparative details and analytical data for compounds synthesized

The preparation of 2',3'-O-isopropylidene uridine and **7** were prepared according to established literature procedures and analytical data was in agreement with published values[9,10]. In addition, 1-azido-2-iodoethane was prepared based on the established procedure and the crude was used in next reaction without further purification because of its potential explosive character[6,11]. Similar method were used to produce 2-(2-azidoethoxy)ethyl 4-methylbenzenesulfonate and its analytical data were in agreement with published values[8].

Compound **7** (600 mg, 1.9 mmol) was dissolved in methanol (150 mL) and argon was bubbled through the solution for 10 min. Lindlar catalyst (200 mg) was added and the suspension was stirred under hydrogen. After 3h, TLC indicated the disappearance of the starting azide and the catalyst was filtered off. The solvent was evaporated under reduced pressure at no more than 40

°C and a colorless oil (550 mg) was obtained. LCMS indicated a high degree of purity so the product was used without purification for further synthesis.



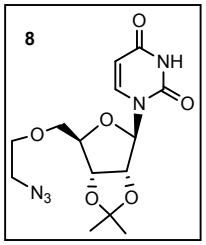
The crude (500 mg) was dissolved in dry DCM (50 mL), and N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-glutamic-acid alpha t-butyl ester (Fmoc-L-Glu-OtBu) (901 mg, 2.12 mmol), BOP (0.94 g, 2.12 mmol), and DiPEA (768 µl, 4.42 mmol) were added. The mixture was stirred for 20h. The solvent was evaporated and residue was dissolved in EtOAc (100 mL) and washed with 1 M $KHSO_4$ (3 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by evaporation. Silica gel chromatography (eluent: DCM/CH_3OH = 98.5/1.5 → 98/2) afforded **10** as a colorless oil (700 mg, 1.01 mmol, 57%). R_f = 0.6 (10% CH_3OH in DCM).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (t, *J* = 5.9 Hz, 1H), 7.86 (dt, *J* = 7.6, 0.9 Hz, 2H), 7.69 (d, *J* = 5.9 Hz, 1H), 7.67 (d, *J* = 6.4 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.38 (ddd, *J* = 8.3, 7.4, 1.0 Hz, 2H), 7.29 (tt, *J* = 7.4, 1.3 Hz, 2H), 5.72 (s, 1H), 5.71 (d, *J* = 2.1 Hz, 1H), 5.59 (d, *J* = 8.0 Hz, 1H), 4.99 (dd, *J* = 6.5, 2.3 Hz, 1H), 4.65 (dd, *J* = 6.6, 4.3 Hz, 1H), 4.26 (ddd, *J* = 16.4, 9.6, 6.6 Hz, 2H), 4.19 (d, *J* = 6.6 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.90 – 3.80 (m, 1H), 3.37 (dt, *J* = 13.7, 5.7 Hz, 1H), 3.22 (dt, *J* = 13.2, 6.2 Hz, 1H), 2.15 (t, *J* = 7.7 Hz, 2H), 1.98 – 1.80 (m, 1H), 1.81 – 1.68 (m, 1H), 1.43 (s, 3H), 1.35 (s, 9H), 1.24 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.99, 171.80, 170.75, 163.65, 156.49, 150.66, 144.21, 143.54, 141.14, 128.06, 127.49 , 125.70 , 125.67 , 120.54 , 113.73 , 102.21 , 92.53 , 85.31 , 83.86 , 81.87 , 80.97 , 66.05 , 60.18 , 55.33 , 54.56 , 47.07 , 41.10 , 31.94 , 28.07 , 27.41 , 27.09 , 25.61.

HRMS (EI) m/z, calculated for $C_{36}H_{42}N_4O_{10}H^+$ [M + H^+]: 691.2974, found 691.2983.

The deprotection of **10** was conducted using 10 mL of TFA/DCM (1:1). After evaporation, the residue was used for SPPS directly without additional purification.

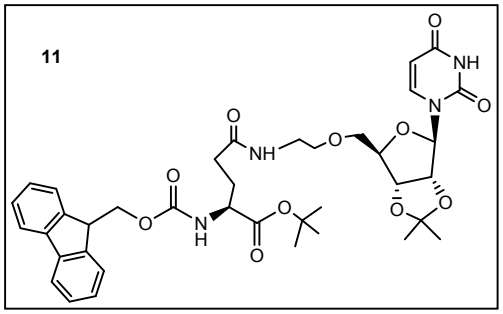


2',3'-O-isopropylidene uridine (0.50 g, 1.8 mmol) was dissolved in CH_3CN (50 mL). After addition of 1-azido-2-iodoethane (0.35 g, 1.8 mmol) and NaOMe (0.28 g, 5.2 mmol) at room temperature, the solution was refluxed and stirred overnight. After judging by TLC (CH_2Cl_2: MeOH, 10:1), the solution was concentrated in vacuo, then the crude mixture was dissolved in EtOAc (200 mL) and washed with brine (1 × 200 mL) and water (2 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting yellow oil was purified by silica gel chromatography eluting with a gradient of CH_2Cl_2: MeOH (100:1 → 50:1) to yield **8** (340 mg, 0.96 mmol, 53%). In the meantime, 90 mg of starting material was collected.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 5.75 (d, *J* = 8.1 Hz, 1H), 5.63 (d, *J* = 2.7 Hz, 1H), 4.95 (dd, *J* = 6.4, 2.7 Hz, 1H), 4.92 (dd, *J* = 6.4, 3.0 Hz, 1H), 4.34 – 4.27 (m, 1H), 4.14 (t, *J* = 6.2 Hz, 2H), 3.90 (dd, *J* = 12.0, 2.6 Hz, 1H), 3.78 (dd, *J* = 12.0, 3.4 Hz, 1H), 3.51 (t, *J* = 6.2 Hz, 2H), 2.97 (d, *J* = 24.5 Hz, 1H), 1.56 (s, 3H), 1.34 (s, 3H), .

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.58 , 151.00 , 140.81 , 114.39 , 101.87 , 96.26 , 87.07 , 84.28 , 80.50 , 62.71 , 48.30 , 39.74 , 27.33 , 25.34 .

HRMS (EI, m/z): calculated for $C_{14}H_{19}N_5O_6Na^+$ ([M+Na]$^+$): 376.1228, found: 376.1254.



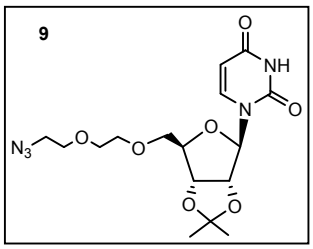
8 (340 mg, 0.96 mmol) was dissolved in methanol (150 mL) and argon was bubbled through the solution for 10 min. Lindlar catalyst (300 mg) was added and the suspension stirred under hydrogen. After 3 h, TLC indicated the disappearance of the starting azide and the catalyst was filtered off. The solvent was evaporated under reduced pressure at no more than 40 °C and a colorless oil (315 mg) dried is obtained. LCMS indicated a high degree of purity so the product was used without purification for further synthesis. The residue was dissolved in dry DCM (50 mL), and Fmoc-L-Glu-OtBu (491 mg, 1.15 mmol), BOP (512 mg, 1.15 mmol), and DiPEA (419 µL, 2.41 mmol) were added. The mixture was stirred for 20 h. The solvent was evaporated and residue was dissolved in EtOAc (100 mL) and washed with 1 M $KHSO_4$ (3 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL). The organic layer was dried over Na_2SO_4 and filtered and the solvent was removed by evaporation. Silica gel chromatography (eluent: DCM/CH_3OH = 98 /2 → 30/1) afforded product **11** as a colorless oil (420 mg, 0.57 mmol, 59% yield in two steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (dt, *J* = 7.6, 0.9 Hz, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.69 (d, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.38 (td, *J* = 7.5, 1.1 Hz, 2H), 7.29 (tt, *J* = 7.5, 1.2 Hz, 2H), 5.83 (d, *J* = 2.5 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 5.10 – 5.02 (m, 1H), 4.86 (dd, *J* = 6.3, 2.6 Hz, 1H), 4.72 (dd, *J* = 6.3, 3.4 Hz, 1H), 4.33 – 4.14 (m, 3H), 4.08 (q, *J* = 4.2 Hz, 1H), 3.93 (t, *J* = 6.3 Hz, 2H), 3.90 – 3.81 (m, 2H), 3.56 (tt, *J* = 9.8, 4.7 Hz, 2H), 3.49 (t, *J* = 6.3 Hz, 2H), 3.38 (t, *J* = 5.8 Hz, 2H), 3.13 (q, *J* = 5.8 Hz, 2H), 2.14 (t, *J* = 7.7 Hz, 2H), 1.97 – 1.83 (m, 1H), 1.73 (m, 1H), 1.45 (s, 3H), 1.35 (s, 9H), 1.25 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.86, 171.77, 162.53, 156.48, 150.96, 144.22 , 141.13 , 140.74 , 129.34 , 128.07 , 127.71 , 127.49 , 125.70 , 121.81 , 120.54 , 120.45 , 113.31 , 101.29 , 92.64 , 87.24 , 84.35 , 80.95 , 80.87 , 66.07 , 61.64 , 54.63 , 47.07 , 36.50 , 32.09 , 28.14 , 28.08 , 27.45 , 27.02 , 25.58.

HRMS (EI, m/z): calculated for $C_{38}H_{46}N_4O_{11}Na^+$ ([M+Na]$^+$): 757.3055, found: 757.4896.

The deprotection of **11** was conducted using 10 of TFA/DCM (1:1, 10 mL). After evaporation, the residue was used for SPPS directly without additional purification.

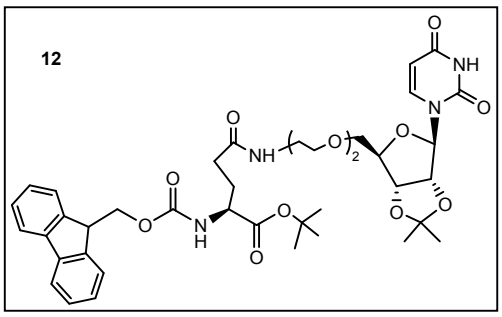


2',3'-O-isopropylidene uridine (0.60 g, 2.1 mmol) was dissolved in CH_3CN (50 mL). After addition of 2-(2-azidoethoxy)ethyl 4-methylbenzenesulfonate (0.72 g, 2.5 mmol) and NaOMe (0.34 g, 6.3 mmol) at room temperature, the solution was refluxed and stirred for 2 days. After judging by TLC (CH_2Cl_2: MeOH = 5:1), the solution was concentrated in vacuo, then the crude mixture was dissolved in EtOAc (200 mL) and washed with brine (1 × 200 mL) and water (2 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting yellow oil was purified by silica gel chromatography eluting with a gradient of CH_2Cl_2: MeOH (50:1) to yield compound **9** (620 mg, 1.56 mmol, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.1 Hz, 1H), 5.75 (d, *J* = 8.0 Hz, 1H), 5.54 (d, *J* = 2.8 Hz, 1H), 5.04 (dd, *J* = 6.5, 2.8 Hz, 1H), 4.96 (dd, *J* = 6.5, 3.5 Hz, 1H), 4.29 (td, *J* = 3.5, 2.5 Hz, 1H), 4.23 – 4.11 (m, 2H), 3.91 (dd, *J* = 12.1, 2.5 Hz, 1H), 3.80 (d, *J* = 11.9 Hz, 1H), 3.75 – 3.71 (m, 2H), 3.66 (t, *J* = 5.0 Hz, 2H), 3.33 (td, *J* = 4.7, 1.2 Hz, 2H), 2.83 (s, 1H), 1.57 (s, 3H), 1.36 (d, *J* = 0.9 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.93 , 114.43 , 102.20 , 97.14 , 87.25 , 84.03 , 80.41 , 69.64 , 67.58 , 62.86 , 50.86 , 39.98 , 27.40 , 25.40 .

HRMS (EI, m/z): calculated for $C_{16}H_{23}N_5O_7Na^+$ ([M+Na]$^+$): 420.1490, found: 420.1493.



9 (360 mg, 0.91 mmol) was dissolved in methanol (150 mL) and argon was bubbled through the solution for 10 min. Lindlar catalyst (200 mg) was added and the suspension stirred under hydrogen. After 3 h, TLC indicated the disappearance of the starting azide and the catalyst was filtered off. The solvent was evaporated under reduced pressure at no more than 40 °C and a colorless oil (350 mg) was obtained. LCMS indicated a high degree of purity so the product was used without purification for further synthesis. The residue was dissolved in dry DCM (50 mL), and Fmoc-L-Glu-OtBu (441 mg, 1.04 mmol), BOP (502 mg, 1.13 mmol), and DiPEA (410 µL, 2.35 mmol) were added. The mixture was stirred for 20 h. The solvent was evaporated and theresidue was dissolved in EtOAc (100 ml) and washed with 1 M KHSO4 (3 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL). The organic layer was dried over Na_2SO_4 and filtered and the solvent was removed by evaporation. Silica gel chromatography (eluent: DCM/CH3OH = 98.5 /1.5 → 95/5) afforded product **12** as a colorless oil (510 mg, 0.65 mmol, 70 % yield in two steps).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (dt, *J* = 7.6, 0.9 Hz, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.69 (d, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.38 (td, *J* = 7.5, 1.1 Hz, 2H), 7.29 (tt, *J* = 7.5, 1.2 Hz, 2H), 5.83 (d, *J* = 2.5 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 5.10 – 5.02 (m, 1H), 4.86 (dd, *J* = 6.3, 2.6 Hz, 1H), 4.72 (dd, *J* = 6.3, 3.4 Hz, 1H), 4.33 – 4.14 (m, 3H), 4.08 (q, *J* = 4.2 Hz, 1H), 3.93 (t, *J* = 6.3 Hz, 2H), 3.90 – 3.81 (m, 2H), 3.56 (tt, *J* = 9.8, 4.7 Hz, 2H), 3.49 (t, *J* = 6.3 Hz, 2H), 3.38 (t, *J* = 5.8 Hz, 2H), 3.13 (q, *J* = 5.8 Hz, 2H), 2.14 (t, *J* = 7.7 Hz, 2H), 1.97 – 1.83 (m, 1H), 1.73 (ddd, *J* = 16.2, 11.6, 7.7 Hz, 1H), 1.45 (s, 3H), 1.35 (s, 9H), 1.25 (s, 3H).

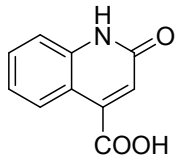
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.81, 171.71, 156.48, 150.85, 144.22, 141.14 , 140.85 , 128.05 , 127.48 , 125.69 , 125.67 , 120.52 , 113.31 , 101.24 , 92.66 , 87.26 , 84.34 , 80.95 , 80.90 , 69.13 , 66.78 , 66.06 , 61.66 , 54.60 , 47.08 , 45.50, 38.99 , 31.96 , 28.07 , 27.45 , 27.13 , 25.59 .

HRMS (EI, m/z): calculated for $C_{40}H_{50}N_4O_{12}Na^+$ ([M+Na]$^+$): 801.3317, found: 801.3325.

The deprotection of **12** was conducted using TFA/DCM (1:1, 10 mL). After evaporation, the residue was used for SPPS directly without additional purification.

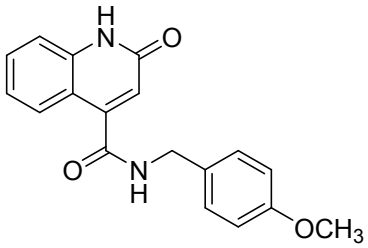
Preparative details and analytical data for fragments synthesized

F01 was synthesized according to established literature procedure[12]. Malonic acid (25.44 g, 244.48 mmol) with isatin (11.99 g, 81.49 mmol) were suspended in acetic acid (250 mL). The reaction mixture was stirred overnight under reflux. Acetic acid was partly evaporated and precipitated solid was filtered. Residue solid was suspended in water (300 mL) and filtered. The brown solid was suspended in saturated sodium hydrogen carbonate (400 mL) and the violet insoluble residues were filtered out. The liquid fraction was acidified with concentrated HCl to pH 1-2. The resulting grey solid was filtered and recrystallized from ethanol to give yellow solid **F01** (15.42 g, 78%). mp 248.2-250.2 °C;



^{1}H NMR (400 MHz, DMSO-d_6) δ 14.10 (bs, 1H), 12.09 (s, 1H), 8.13 (dd, J = 8.3, 1.4 Hz, 1H), 7.54 (ddd, J = 8.3, 7.1, 1.4 Hz, 1H), 7.41 (dd, J = 8.3, 1.4 Hz, 1H), 7.22 (ddd, J = 8.3, 7.1, 1.4 Hz, 1H), 6.80 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.78, 160.99, 141.15, 139.39, 130.85, 126.09, 123.38, 122.22, 115.74, 115.72; HRMS (EI, m/z): calculated for $C_{10}H_6NO_3$ ([M-H]$^-$): 188.0348, found: 188.0350; IR cm^{-1} 2961, 2861, 2582, 1710, 1649, 1615, 1544, 1509, 1477, 1435, 1354, 1323, 1280, 1258, 1229, 1183, 1164, 1156, 1142, 1042, 1007, 938, 872, 852, 793, 773, 759, 740, 711, 656, 635, 623, 547, 527, 506.

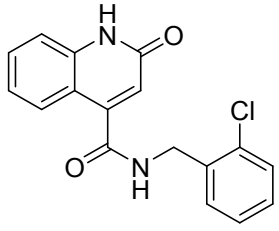
General procedure for the synthesis of F02-F06, F08, F10, F12, F14-F19 and F21 through a coupling reaction as described in the Scheme 2S (A).



Starting material **F01** (200 mg, 1.06 mmol) and the appropriate amine (1.06 mmol, 1 equiv) were dissolved in anhydrous DMF (20 mL) and stirred for 10 minutes in an ice bath. To the solution HOBt (1.2 equiv), triethylamine (4 equiv) and EDC (1.3 equiv) were added. The reaction mixture was stirred at room temperature overnight. After evaporation, the residue was dissolved in EtOAc (40 mL) and washed with a 20% solution of citric acid (20 mL) and saturated solution of $NaHCO_3$ (20 mL). The organic phase was dried over Na_2SO_4, the drying agent filtered, and the solvent evaporated to obtain carboxamides **F02-F06, F08, F10, F12, F14-F19** and **F21.**

N-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F02**). White solid. (193 mg, 59%); mp 264.7-265.3°C;

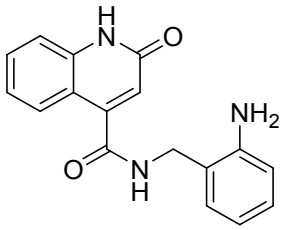
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 9.24 (t, *J* = 6.0 Hz, 1H) 7.70 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.3, 7. 1, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.33–7.25 (m, 2H), 7.19 (ddd, *J* = 8.3, 7.1, 1.4 Hz, 1H), 6.98-6.89 (m, 2H), 6.52 (s, 1H), 4.43 (d, *J* = 5.9 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.61, 161.21, 158.29, 146.10, 139.18, 130.82, 128.62, 125.80, 122.02, 119.71, 116.13, 115.63, 113.78, 55.03, 41.80; HRMS (EI, m/z): calculated for $C_{18}H_{15}N_2O_3$ ([M-H]$^-$): 307.1083, found: 307.1080; HPLC purity, 99.7% at 254 nm (method A, t_R = 4.577 min); IR cm^{-1} 3263, 2997, 2835, 1661, 1631, 1602, 1528, 1512, 1475, 1437, 1396, 1356, 1303, 1246, 1225, 1175, 1165, 1143, 1110, 1071, 1038, 983, 952, 913, 878, 825, 797, 777, 754, 724, 682, 655, 638, 620, 556, 511.



N-(2-chlorobenzyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F03**). Pale yellow solid. (188 mg, 57%); mp 286.6-288.8°C;

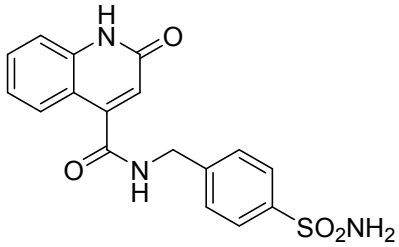
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.32 (t, *J* = 5.8 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.53-7.43 (m, 2H), 7.43-7.29 (m, 3H), 7.21 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 6.62 (d, *J* = 1.4

Hz, 1H), 4.58 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.87, 161.19, 145.81, 139.21, 135.62, 132.17, 130.85, 129.24, 129.06, 128.89, 127.34, 125.83, 122.06, 119.98, 116.09, 115.66, 40.42; HRMS (EI, m/z): calculated for $C_{17}H_{12}N_2O_2Cl$ ([M-H]$^-$): 311.0587, found: 311.0585; HPLC purity, 98.7% at 254 nm (method A, t_R = 5.350 min); IR cm^{-1} 3267, 2962, 2851, 1665, 1634, 1604, 1572, 1551, 1525, 1506, 1477, 1460, 1442, 1397, 1359, 1326, 1298, 1262, 1193, 1168, 1165, 1143, 1131, 1054, 1036, 982, 949, 918, 878, 796, 778, 753, 680, 656, 626, 593, 555, 530, 522, 512.



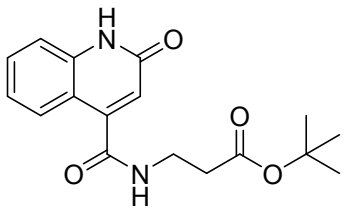
N-(2-aminobenzyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F04**). Yellow solid. (176 mg, 57%); mp 268.3-271.1°C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.32 (t, *J* = 5.8 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.19 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.06 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.00 (td, *J* = 7.5, 1.6 Hz, 1H), 6.67 (d, *J* = 1.4 Hz, 1H), 6.63-6.50 (m, 2H), 5.14 (s, 2H), 4.35 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.96, 161.17, 146.14, 146.00, 139.20, 130.84, 128.93, 128.00, 125.85, 122.00, 121.27, 119.81, 116.08, 115.87, 115.64, 114.69. – peak for CH_2 is covered by DMSO; HRMS (EI, m/z): calculated for $C_{17}H_{14}N_3O_2$ ([M-H]$^-$): 292.1086, found: 292.1080; HPLC purity, 90.8% at 254 nm (method A, t_R = 2.227 min); IR cm^{-1} 3429, 3356, 3251, 3006, 2844, 1667, 1634, 1603, 1541, 1496, 1477, 1463, 1435, 1396, 1357, 1312, 1288, 1265, 1227, 1190, 1154, 1061, 1037, 979, 948, 912, 876, 800, 776, 751, 735, 689, 654, 631, 607, 552, 526, 509.



2-oxo-N-(4-sulfamoylbenzyl)-1,2-dihydroquinoline-4-carboxamide (**F05**). Yellow solid. (211 mg, 56%); mp 281.3-283.6°C;

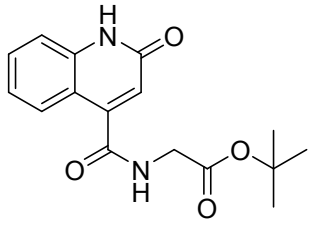
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.40 (t, *J* = 6.0 Hz, 1H), 7.87-7.78 (m, 2H), 7.71 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.60–7.51 (m, 3H), 7.40–7.33 (m, 1H), 7.36 (s, *2*H), 7.21 (ddd, *J* = 8.2, 7.1, 1.4 Hz, 1H), 6.63 (d, *J* = 1.3 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.91, 161.20, 145.81, 142.94, 142.75, 139.21, 130.87, 127.55, 125.82, 122.07, 119.94, 116.05, 115.66, 42.04; HRMS (EI, m/z): calculated for $C_{17}H_{14}N_3O_4S$ ([M-H]$^-$): 356.0705, found: 356.0698**;** HPLC purity, 95.1% at 254 nm (method A, t_R = 2.693 min); IR cm^{-1} 3274, 2995, 2854, 1665, 1634, 1604, 1572, 1551, 1525, 1506, 1477, 1460, 1442, 1397, 1359, 1326, 1298, 1262, 1193, 1168, 1165, 1143, 1131, 1054, 1036, 982, 949, 918, 878, 796, 778, 753, 680, 656, 626, 593, 555, 530, 522, 512.



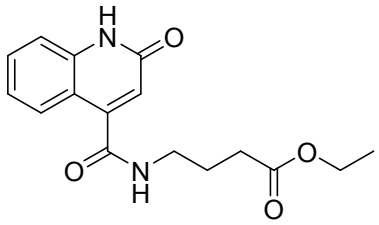
tert-butyl 3-(2-oxo-1,2-dihydroquinoline-4-carboxamido)propanoate (**F06**). Pale yellow solid. (228 mg, 68%); mp 208.8-210.8°C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s, 1H), 8.83 (t, *J* = 5.5 Hz, 1H), 7.71 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.18 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.46 (s, 1H), 3.47 (td, *J* = 6.8, 5.5 Hz, 2H), 2.58-2.48 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.47, 165.67, 161.22, 146.22, 139.17, 130.82, 125.92, 121.93, 119.66, 116.06, 115.58, 79.97, 35.26, 34.64, 27.72; HRMS (EI, m/z): calculated for $C_{17}H_{19}N_2O_4$ ([M-H]$^-$): 315.1345, found: 315.1343; HPLC

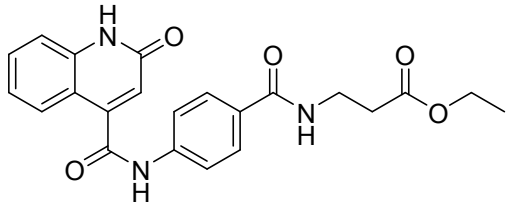
purity, 95.7% at 254 nm (method A, t_R = 4.867 min); IR cm^{-1} 3301, 2979, 2852, 1725, 1671, 1640, 1605, 1541, 1508, 1474, 1435, 1392, 1366, 1328, 1299, 1266, 1219, 1155, 1138, 1072, 1036, 1005, 985, 949, 892, 845, 796, 775, 751, 724, 682, 654, 629, 585, 576, 557, 527, 509.



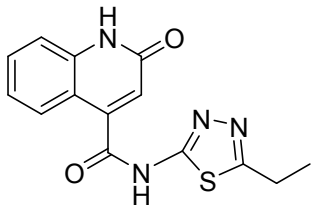
tert-butyl (2-oxo-1,2-dihydroquinoline-4-carbonyl)glycinate (**F08**). Pale yellow solid. (155 mg, 49%); mp 269.6-272.4°C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.14 (t, *J* = 6.0 Hz, 1H), 7.81 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.36 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.22 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.50 (s, 1H), 3.93 (d, *J* = 6.0 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.52, 166.25, 161.14, 145.96, 139.18, 130.93, 125.97, 121.98, 119.73, 116.04, 115.60, 80.99, 41.65, 27.71; HRMS (EI, m/z): calculated for $C_{16}H_{17}N_2O_4$ ($[M-H]^-$): 301.1188, found: 301.1181; HPLC purity, 95.0% at 254 nm (method B, t_R = 8.147 min); IR cm^{-1} 3301, 2974, 2853, 1740, 1728, 1668, 1645, 1605, 1546, 1508, 1473, 1434, 1396, 1367, 1303, 1276, 1263, 1226, 1158, 1099, 1044, 984, 949, 890, 869, 850, 798, 776, 751, 730, 710, 682, 653, 632, 574, 555, 525, 508.



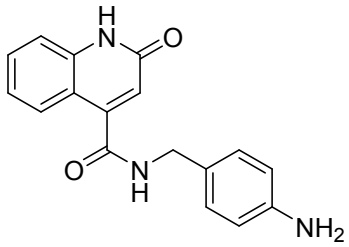
ethyl 4-(2-oxo-1,2-dihydroquinoline-4-carboxamido)butanoate (**F10**). Pale beige solid. (198 mg, 62%); mp 214.6-216.6°C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H), 8.77 (t, *J* = 5.7 Hz, 1H), 7.71 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.51 (s, 1H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.30 (td, *J* = 6.9, 5.6 Hz, 2H), 2.39 (t, *J* = 7.4 Hz, 2H), 1.80 (p, *J* = 7.1 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.56, 165.72, 161.29, 146.22, 139.22, 130.77, 125.86, 121.96, 119.65, 116.13, 115.63, 59.79, 38.13, 30.85, 24.18, 14.09; HRMS (EI, m/z): calculated for $C_{16}H_{17}N_2O_4$ ($[M-H]^-$): 301.1188, found: 301.1183; HPLC purity, 97.2% at 254 nm (method B, t_R = 6.253 min); IR cm^{-1} 3278, 2962, 2857, 1730, 1661, 1639, 1603, 1541, 1507, 1475, 1442, 1397, 1374, 1353, 1326, 1290, 1263, 1235, 1177, 1165, 1098, 1065, 1034, 979, 913, 876, 795, 777, 754, 733, 683, 653, 624, 581, 560, 526, 513.



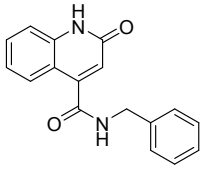
(ethyl 3-(4-(2-oxo-1,2-dihydroquinoline-4-carboxamido)benzamido)propanoate) (**F12**). Pale yellow solid (99 mg, 23%); mp 275.11-278.0°C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 2H), 8.51 (t, *J* = 5.5Hz, 1H), 7.89-7.78 (m, 4H), 7.74 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.38 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.19 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.71 (s, 1H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.49 (td, *J* = 7.0, 5.5 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.32, 165.63, 164.54, 161.25, 145.60, 141.12, 139.35, 130.98, 129.70, 128.02, 125.66, 122.21, 120.17, 119.17, 115.87, 115.81, 59.91, 35.49, 33.80, 14.06; HRMS (EI, m/z): calculated for $C_{22}H_{20}N_3O_5$ ($[M-H]^-$): 406.1403, found: 406.1409; HPLC purity, 90.3% at 254 nm (method B, t_R = 8.293 min); IR cm^{-1} 3289, 2992, 2845, 1722, 1654, 1632, 1609, 1593, 1548, 1520, 1473, 1435, 1410, 1393, 1365, 1321, 1261, 1182, 1153, 1117, 1077, 1040, 1019, 981, 949, 916, 900, 856, 841, 791, 775, 753, 715, 681, 652, 630, 584, 557, 541, 521, 510.



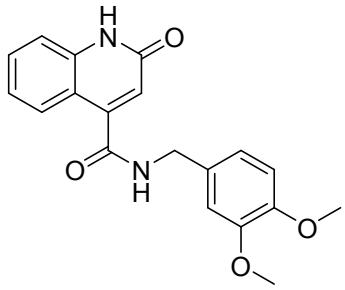
N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F14**). Beige solid (223 mg, 70%); mp > 300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 12.09 (s, 1H), 7.67 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.58 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.39 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.22 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.85 (s, 1H), 3.06 (q, *J* = 7.5 Hz, 2H), 1.34 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 167.49, 166.67, 164.94, 161.31, 143.75, 139.67, 131.59, 125.97, 122.81, 122.22, 116.27, 116.07, 23.22, 14.40; HRMS (EI, m/z): calculated for $C_{14}H_{11}N_4O_2S$ ([M-H]$^-$): 299.0603, found: 299.0606; HPLC purity, 98.9% at 254 nm (method A, t_R = 4.403 min);IR cm^{-1} 3085, 2964, 2844, 1667, 1553, 1525, 1485, 1429, 1389, 1380, 1350, 1307, 1267, 1248, 1231, 1184, 1153, 1134, 1104, 1040, 1007, 981, 942, 897, 835, 782, 772, 763, 746, 705, 651, 620, 603, 555, 539, 517, 507.



N-(4-aminobenzyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F15**). Pale beige solid (178 mg, 57%); mp 288.3-289.0°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H), 9.10 (t, *J* = 5.9 Hz, 1H), 7.69 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.53 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.19 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.06-6.98 (m, 2H), 6.58–6.51 (m, 2H), 6.48 (s, 1H), 5.01 (s, 2H), 4.31 (d, *J* = 5.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.44, 161.24, 147.67, 146.28, 139.17, 130.76, 128.32, 125.83, 125.68, 121.97, 119.59, 116.19, 115.60, 113.73, 42.13; HRMS (EI, m/z): calculated for $C_{17}H_{14}N_3O_2$ ([M-H]$^-$): 292.1086, found: 292.1090; HPLC purity, 98.2% at 254 nm (method C, t_R = 3.883 min); IR cm^{-1} 3430, 3341, 3267, 3003, 2851, 1666, 1633, 1600, 1541, 1519, 1475, 1440, 1395, 1361, 1311, 1280, 1267, 1230, 1190, 1184, 1163, 1131, 1067, 1039, 982, 952, 912, 878, 844, 825, 804, 775, 754, 733, 686, 655, 627, 564, 526, 509.

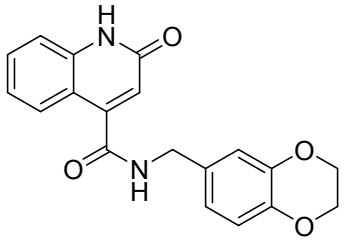


N-benzyl-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F16**). Pale yellow solid (214 mg, 73%); mp 297.1-298.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 9.32 (t, *J* = 6.0 Hz, 1H), 7.71 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.46–7.33 (m, 5H), 7.33–7.24 (m, 1H), 7.20 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 6.56 (d, *J* = 1.4 Hz, 1H), 4.51 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.76, 161.21, 146.03, 139.20, 138.89, 130.83, 128.41, 127.22, 126.95, 125.80, 122.03, 119.77, 116.11, 115.65, 42.33; HRMS (EI, m/z): calculated for $C_{17}H_{13}N_2O_2$ ([M-H]$^-$): 277.0977, found: 277.0978; HPLC purity, 97.7% at 254 nm (method A, t_R = 4.583 min); IR cm^{-1} 3257, 2961, 2854, 1662, 1633, 1603, 1534, 1508, 1476, 1454, 1440, 1397, 1361, 1328, 1277, 1263, 1190, 1165, 1144, 1072, 1041, 1030, 981, 951, 912, 876, 833, 795, 778, 752, 693, 683, 655, 623, 584, 555, 526, 512.



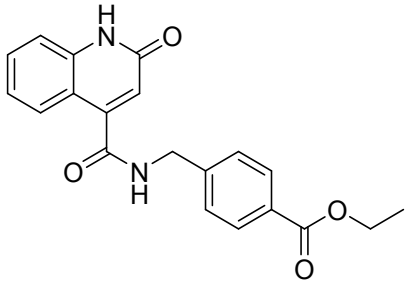
N-(3,4-dimethoxybenzyl)-2-oxo-1,2-dihydroquinoline-4-carboxamide (**F17**). Beige solid (182 mg, 51%); mp 238.1245.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 9.23 (t, *J* = 6.0 Hz, 1H), 7.70 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.19 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.00–6.91 (m, 2H), 6.88 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.52 (d, *J* = 1.9 Hz, 1H) , 4.43 (d, *J* = 6.0 Hz, 2H), 3.75 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (101 MHz,

DMSO-d_6) δ 165.64, 161.23, 148.65, 147.82, 146.16, 139.21, 131.26, 130.83, 125.78, 121.99, 119.69, 119.34, 116.15, 115.66, 111.70, 111.16, 55.50, 55.35, 42.12; HRMS (EI, m/z): calculated for $C_{19}H_{17}N_2O_4$ ([M-H]$^-$): 337.1188, found: 337.1182; HPLC purity, 97.7% at 254 nm (method A, t_R = 4.033 min); IR cm^{-1} 3301, 2956, 2846, 1670, 1636, 1604, 1542, 1516, 1467, 1452, 1434, 1396, 1371, 1296, 1282, 1262, 1240, 1199, 1154, 1143, 1026, 982, 949, 942, 891, 865, 810, 795, 772, 752, 735, 721, 687, 661, 650, 616, 558, 525, 508.



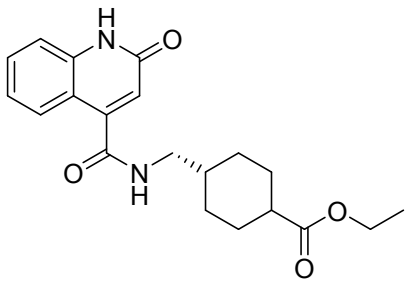
N-((2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methyl)--2-oxo-1,2-dihydro-quinoline-4-carboxamide (**F18**). Pale yellow solid (211 mg, 59%); mp 287.3-290.6 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 9.23 (t, *J* = 6.0 Hz, 1H), 7.70 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.36 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.00–6.91 (m, 3H), 6.51 (s, 1H) , 4.37 (d, *J* = 6.0 Hz, 2H), 4.26-4.20 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.62, 161.21, 146.02, 143.15, 142.36, 139.21, 131.90, 130.81, 125.80, 122.01, 120.16, 119.75, 116.93, 116.12, 116.04, 115.66, 64.05, 63.98, 41.79; HRMS (EI, m/z): calculated for $C_{19}H_{15}N_2O_4$ ([M-H]$^-$): 335.1032, found: 335.1031; HPLC purity, 99.0% at 254 nm (method A, t_R = 4.437 min); IR cm^{-1} 3254, 2947, 2856, 1661, 1635, 1604, 1592, 1532, 1507, 1477, 1457, 1442, 1432, 1397, 1358, 1311, 1279, 1260, 1244, 1205, 1191, 1167, 1155, 1144, 1125, 1104, 1067, 1040, 984, 952, 918, 883, 875, 862, 821, 806, 777, 755, 715, 683, 659, 646, 629, 556, 526, 512.



Ethyl 4-((2-oxo-1,2-dihydroquinoline-4-carboxamido)methyl) benzoate (**F19**). Pale beige solid (235 mg, 65%); mp 269.4-271.4 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 10.01 (s, 1H), 9.42 (t, *J* = 6.0 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.59–7.49 (m, 3H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 6.61 (s, 1H) , 4.58 (d, *J* = 6.0 Hz, 2H), 4.32 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.91, 165.56, 161.19, 145.85, 144.46, 139.21, 130.86, 129.33, 128.55, 127.38, 125.80, 122.09, 119.90, 116.06, 115.66, 60.65, 42.14, 14.16; HRMS (EI, m/z): calculated for $C_{20}H_{17}N_2O_4$ ([M-H]$^-$): 349.1188, found: 349.1183; HPLC purity, 97.6% at 254 nm (method B, t_R = 11.930 min); IR cm^{-1} 3283, 2987, 2828, 1701, 1664, 1639, 1611, 1578, 1542, 1508, 1479, 1439, 1423, 1415, 1397, 1363, 1276, 1263, 1191, 1177, 1160, 1127, 1109, 1078, 1036, 1021, 988, 951, 912, 881, 860, 843, 797, 781, 772, 764, 752, 746, 714, 690, 654, 642, 625, 561, 522, 510.

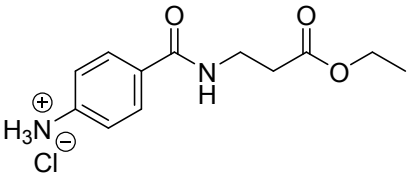


Ethyl 4-((2-oxo-1,2-dihydroquinoline-4-carboxamido)methyl)cyclohexane-1-carboxylic acid (**F21**). Beige solid (158 mg, 42%); mp 239.1-244.0 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H), 8.75 (t, *J* = 5.8 Hz, 1H), 7.69 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 6.49 (d, *J* = 1.4 Hz, 1H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.14 (t, *J* = 6.3 Hz, 2H), 2.25 (tt, *J* = 12.1, 3.5 Hz, 1H), 1.92 (dt, *J*

= 12.1, 3.5 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.56 – 1.48 (m, 1H), 1.39 – 1.25 (m, 2H), 1.18 (t, *J* = 7.1 Hz, 3H), 1.09 – 0.94 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.49, 166.25, 161.77, 146.91, 139.67, 131.26, 126.35, 122.51, 120.06, 116.68, 116.11, 60.11, 45.30, 42.84, 37.28, 29.77, 28.69, 14.16; HRMS (EI, m/z): calculated for $C_{20}H_{23}N_2O_4$ ([M-H]$^-$): 355.1658, found: 355.1657; HPLC purity, 98.9% at 254 nm (method A, t_R = 5.457 min); IR cm^{-1} 3296, 2930, 2855, 1728, 1662, 1633, 1603, 1533, 1506, 1469, 1458, 1440, 1397, 1378, 1319, 1270, 1253, 1229, 1216, 1186, 1165, 1147, 1133, 1072, 1045, 1026, 979, 914, 900, 887, 875, 800, 777, 765, 750, 702, 676, 655, 629, 555, 527, 512.

Synthesis of starting amine of F12

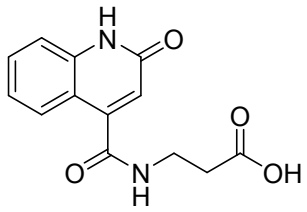


N-(4-Aminobenzoyl)-beta-alanine (500 mg, 2.40 mmol) was dissolved in anhydrous ethanol (40 mL) and cooled to 0 °C. A mixture of thionyl chloride (0.52 mL, 3 equiv.) in anhydrous ethanol (1 mL) was added dropwise to the solution of the starting compound. The reaction mixture was refluxed for 5 h under argon atmosphere. Ethanol was evaporated and remaining crystals were washed twice with pure ethanol (yield 93%). The resulting compound was used in the coupling reaction to form fragment **F12**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 3H), 8.57 (t, *J* = 5.6 Hz, 1H), 7.88-7.80 (m, 2H), 7.27-7.19 (m, 2H), 4.07 (q, *J* = 7.2 Hz, 2H), 3.48 (td, *J* = 7.0, 5.4 Hz, 2H), 2.57 (t, J = 7.0 Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H).

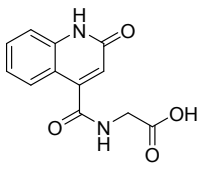
Synthesis of F07 and F09 as described in the Scheme 2S (B).

F06 (200 mg, 0.63 mmol) was dissolved in 1 mL of trifluoroacetic acid and stirred for 10 min at room temperature. Trifluoroacetic acid was evaporated and the crude **F07** was crystalized from ether. The same procedure was conducted on **F08** (130 mg, 0.43 mmol) to produce **F09**.



3-(2-oxo-1,2-dihydroquinoline-4-carboxamido)propanoic acid (**F07**). Pale yellow solid (164 mg, 99.7%); mp 256.5-257.8°C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s, 1H), 8.82 (t, *J* = 5.5 Hz, 1H), 7.72 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.19 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.47 (s, 1H), 3.48 (td, *J* = 6.8, 5.5 Hz, 2H), 2.55 (t, *J* = 6.8 Hz, 2H) – COOH peak is missing; ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.77, 165.71, 161.25, 146.22, 139.16, 130.79, 125.96, 121.98, 119.69, 116.11, 115.57, 35.21, 33.50; HRMS (EI, m/z): calculated for $C_{13}H_{11}N_2O_4$ ([M-H]$^-$): 259.0719, found: 259.0717; HPLC purity, 95.0% at 254 nm (method C, t_R = 4.140 min); IR cm^{-1} 3270, 3088, 2955, 2863, 1673, 1634, 1545, 1504, 1446, 1421, 1376, 1358, 1335, 1296, 1264, 1193, 1136, 1074, 1036, 988, 920, 890, 866, 849, 800, 776, 755, 719, 688, 650, 632, 605, 557, 524, 508.



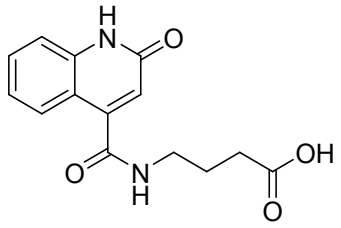
(2-oxo-1,2-dihydroquinoline-4-carbonyl)glycine (**F09**). Pale yellow solid (100 mg, 94.5%); mp 279.8-282.8°C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 11.97 (s, 1H), 9.12 (t, *J* = 6.0 Hz, 1H), 7.83 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.55 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.36 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.51 (s, 1H), 3.96 (d, *J* = 6.1 Hz, 2H) - COOH peak is missing; ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.82, 166.30, 161.16, 146.01, 139.18, 130.89, 126.05, 121.97, 119.70, 116.10, 115.56, 40.68; HRMS (EI, m/z): calculated for $C_{12}H_9N_2O_4$ ([M-H]$^-$

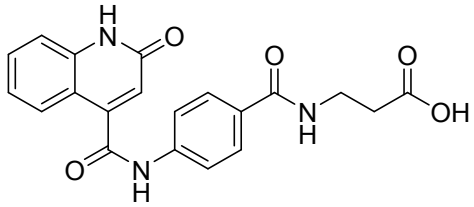
): 245.0562, found: 245.0564; HPLC purity, 96.7% at 254 nm (method C, t_R = 3.407 min); IR cm^{-1} 3527, 3338, 2985, 2862, 2524, 1732, 1637, 1527, 1505, 1470, 1446, 1417, 1352, 1328, 1266, 1220, 1160, 1110, 1042, 1021, 996, 962, 933, 892, 863, 848, 797, 768, 756, 636, 616, 544, 529, 507.

Synthesis of F11, F13, F20 and F22 as described in the Scheme 2S (C).

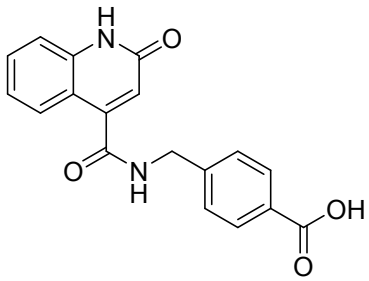
F10 (170 mg, 0.56 mmol) was dissolved in ethanol (40 mL), 1 M NaOH (6 eq.) was added and the reaction mixture was stirred for 4-5 h at room temperature. Ethanol was evaporated. The residue was dissolved in water (20 mL) and the pH was adjusted to 1~2. After filtration and drying, **F11** was obtained. The same procedure was conducted on F12 (60 mg, 0.15 mmol), **F19** (100 mg, 0.29 mmol) and **F21** (100 mg, 0.28 mmol) separately and the corresponding products **F13**, **F20** and **F22** were obtained.



4-(2-oxo-1,2-dihydroquinoline-4-carboxamido)butanoic acid (**F11**). White solid (156 mg, 89%); mp 283.5-285.7°C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 11.93 (s, 1H), 8.76 (t, *J* = 5.6 Hz, 1H), 7.71 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.20 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.51 (s, 1H), 3.30 (q, *J* = 6.6 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.77 (p, *J* = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.14, 165.70, 161.27, 146.31, 139.17, 130.77, 125.88, 122.00, 119.62, 116.14, 115.59, 38.28, 31.00, 24.24; HRMS (EI, m/z): calculated for $C_{14}H_{13}N_2O_4$ ($[M-H]^-$): 273.0875, found: 273.0879; HPLC purity, 99.1% at 254 nm (method B, t_R = 2.473 min); IR cm^{-1} 3356, 2953, 2875, 2591, 1720, 1670, 1642, 1537, 1508, 1484, 1471, 1422, 1361, 1339, 1285 1270, 1192, 1161, 1093, 1051, 1036, 997, 902, 855, 797, 779, 757, 654, 548, 531, 510.

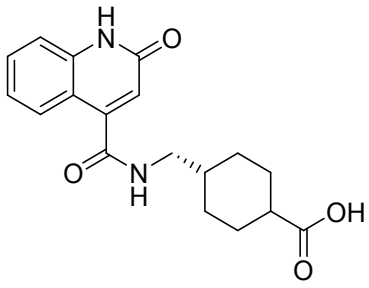


3-(4-(2-oxo-1,2-dihydroquinoline-4-carboxamido)benzamido)propanoic acid (**F13**). Pale beige solid (40 mg, 77%); mp 269.8-272.3°C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 12.05 (s, 1H), 10.96 (s, 1H), 8.51 (t, *J* = 5.5 Hz, 1H), 7.92-7.76 (m, 4H), 7.73 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.58 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.23 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 6.75 (s, 1H), 3.49 (q, *J* = 7.0 Hz, 2H), 2.54 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.92, 165.58, 164.50, 161.19, 145.61, 141.04, 139.26, 131.00, 129.77, 128.02, 125.67, 122.25, 120.18, 119.14, 115.86, 115.75, 35.54, 33.79; HRMS (EI, m/z): calculated for $C_{20}H_{16}N_3O_5$ ($[M-H]^-$): 378.1090, found: 378.1097; HPLC purity, 91.2% at 254 nm (method B, t_R = 4.997 min); IR cm^{-1} 3286, 2989, 2886, 1723, 1645, 1593, 1541, 1518, 1431, 1394, 1317, 1257, 1233, 1180, 1154, 1115, 1071, 1040, 1019, 975, 953, 915, 872, 853, 840, 812, 793, 776, 755, 722, 683, 652, 630, 595, 584, 559, 538, 522, 511.



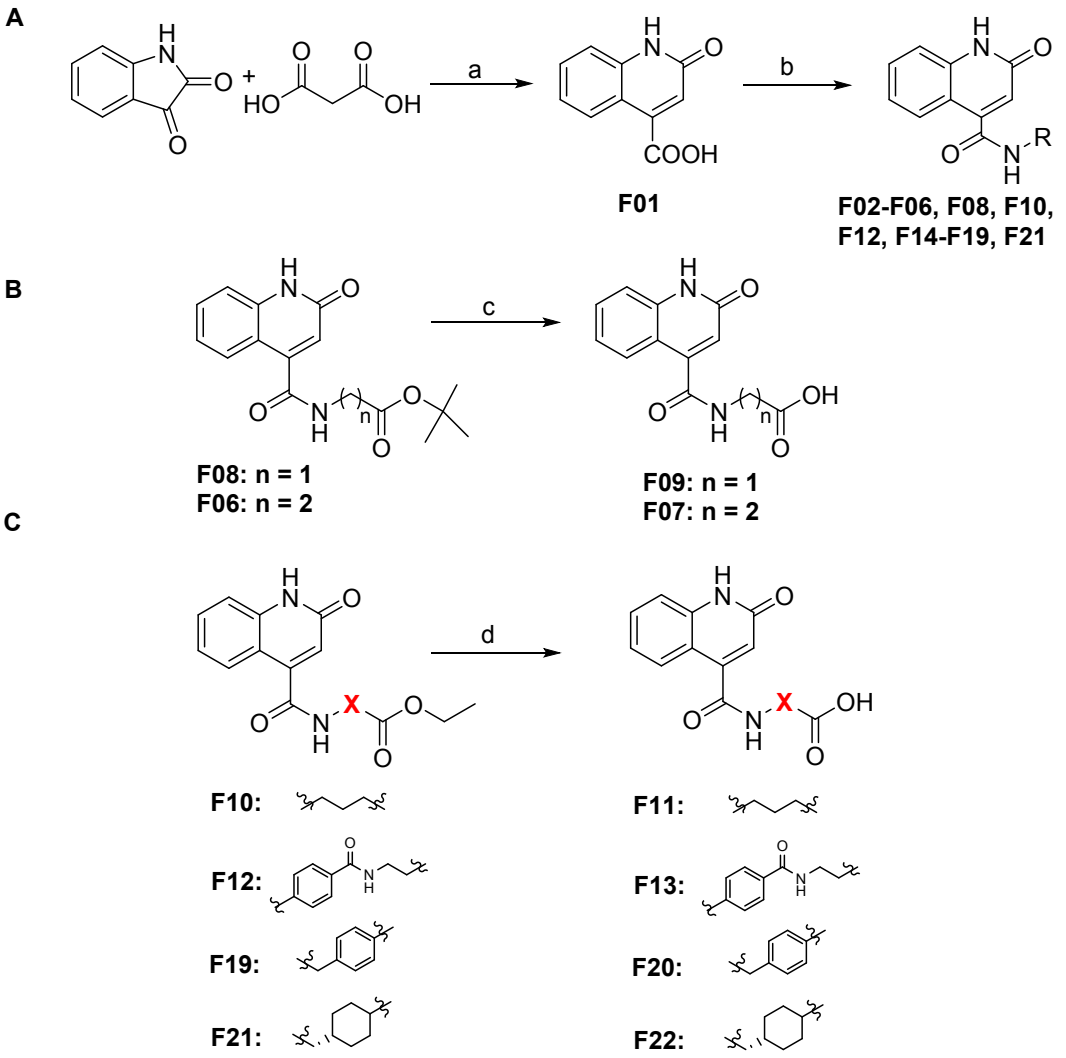
4-((2-oxo-1,2-dihydroquinoline-4-carboxamido)methyl)benzoic acid (**F20**). Beige solid (30 mg, 33%); mp >300 °C;
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 9.43 (t, *J* = 6.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.71 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.55 (td, *J* = 7.1, 1.4 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.21 (td, *J* = 7.1, 1.4 Hz, 1H), 6.61 (s, 1H), 4.57 (d, *J* = 6.0 Hz, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 170.06, 165.76, 161.24, 146.05, 139.72, 139.24, 138.20, 130.78, 129.20, 126.14, 125.77, 122.01, 119.76, 116.14, 115.72, 42.24; HRMS (EI, m/z): calculated for $C_{18}H_{13}N_2O_4$ ([M-H]$^-$): 321.0875, found: 321.0875; HPLC purity, 99.7% at 254 nm (method A, t_R = 3.360 min); IR cm^{-1} 3421, 3263, 2925, 2888, 1638, 1594, 1541, 1506, 1472, 1429, 1389, 1302, 1282, 1266, 1256, 1228, 1194, 1176, 1158, 1140, 1102, 1077, 1040, 1033, 1016, 982, 949, 884, 869, 851, 800, 774, 760, 754, 730, 708, 689, 655, 622 592, 582, 556, 544, 536, 518, 505.



4-((2-oxo-1,2-dihydroquinoline-4-carboxamido)methyl)cyclohexane-1-carboxylic acid (**F22**), Beige solid (47 mg, 51%); mp >300 °C;

^{1}H NMR (400 MHz, DMSO-d_6 + 1 % CD_3COOD) δ 7.68 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.48 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.17 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 6.53 (d, *J* = 1.4 Hz, 1H), 3.12 (d, *J* = 6.8 Hz, 2H), 2.20-2.06 (m, 1H), 1.94–1.87 (m, 2H), 1.81–1.73 (m, 2H), 1.55–1.42 (m, 1H), 1.34–1.20 (m, 2H), 1.05–0.90 (m, 2H) – 3 peaks are missing (COOH, 2 x NH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.68, 165.70, 161.55, 146.62, 138.74, 130.65, 125.74, 122.10, 119.14, 116.23, 115.61, 44.69, 42.27, 36.72, 29.28, 28.08; HRMS (EI, m/z): calculated for $C_{18}H_{19}N_2O_4$ ([M-H]$^-$): 327.1345, found: 327.1342; HPLC purity, 96.7% at 254 nm (method A, t_R = 3.347 min); IR cm^{-1} 3441, 3283, 2922, 2854, 1664, 1641, 1551, 1545, 1508, 1476, 1418, 1329, 1291, 1263, 1135, 926, 878, 847, 778, 753, 730, 684, 670, 653, 632, 585, 569, 544, 527, 512.



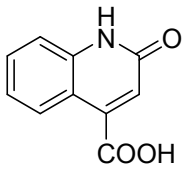
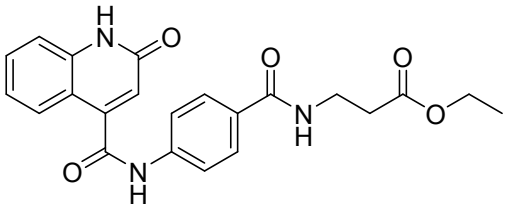
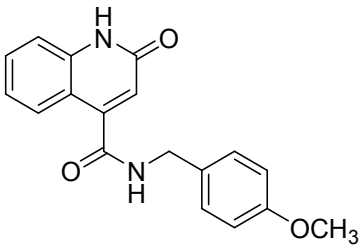
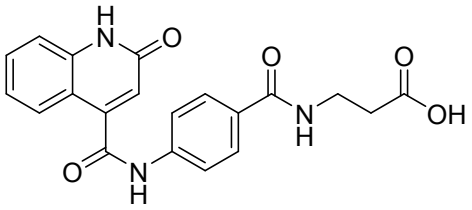
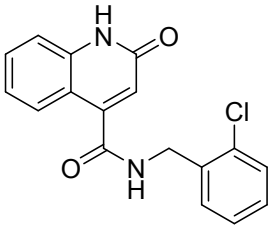
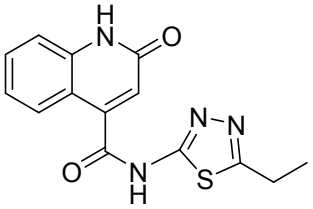
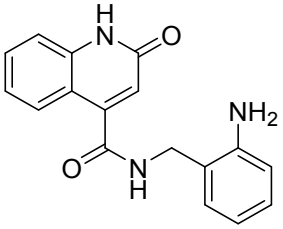
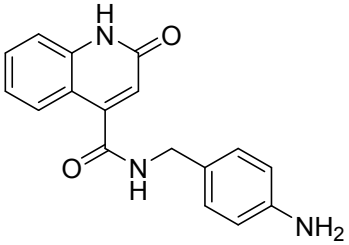
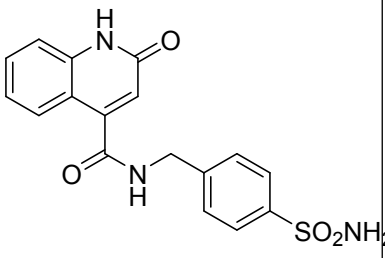
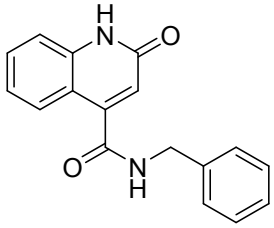
Scheme 2S. (**A**) Synthesis of **F01**-**F06**, **F08**, **F10**, **F12**, **F14**-**F19** and **F21**. (**B**) Synthesis of **F09** and **F07** through deprotection of tert-butyl esters **F08** and **F06**. (**C**) Synthesis of **F11**, **F13**, **F20** and **F22** through hydrolysis of ethyl esters **F10** and **F12**, **F19** and **F21**. Reagents and conditions: a) acetic acid, reflux, overnight, 72%; b) corresponding amine, HOBt, EDC, TEA, DMF, RT, overnight; c) TFA, RT, 10 min; d) 1 M NaOH, EtOH, RT, 4-5 h.

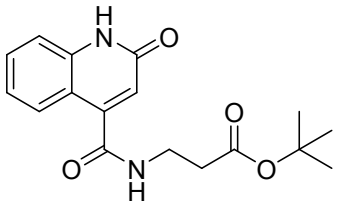
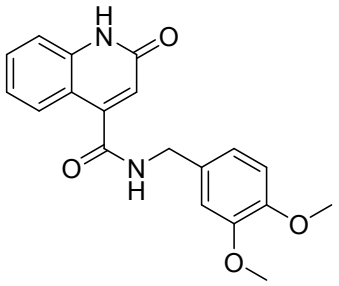
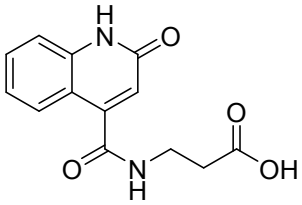
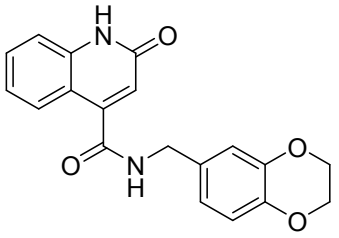
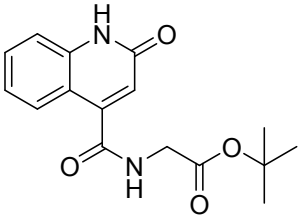
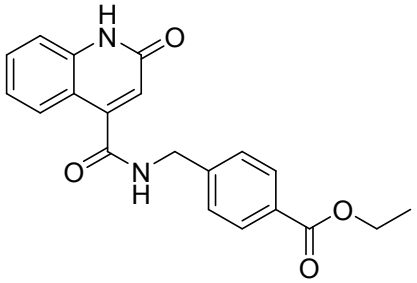
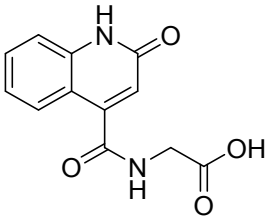
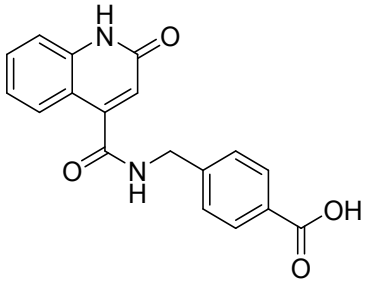
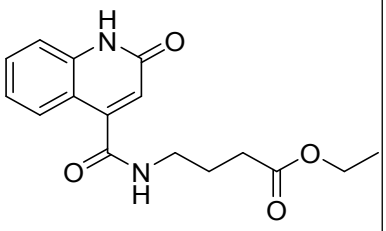
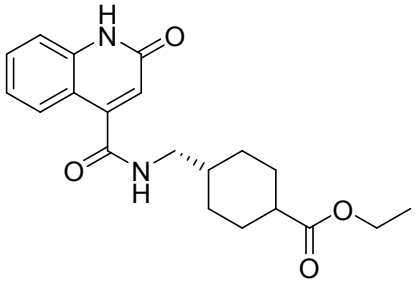
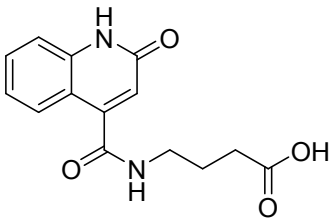
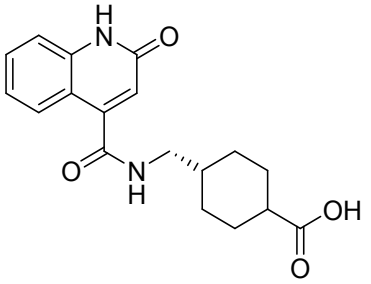
Calculation of LogP and Polar Surface Area (PSA) of ionized compounds at neutral pH

	LogP[a]	***PSA***[a]
Goblin-1 (ref. 13):	-6.05	500.2 Å^2
1	-5.82	439.7 Å^2
6	-4.77	397.2 Å^2

[a]logP & tPSA– calculated using Molinspiration property engine v2016.10 (©2017 Molinspiration Cheminformatics)

Table 2S: Structures of prepared fragments F01-F22.

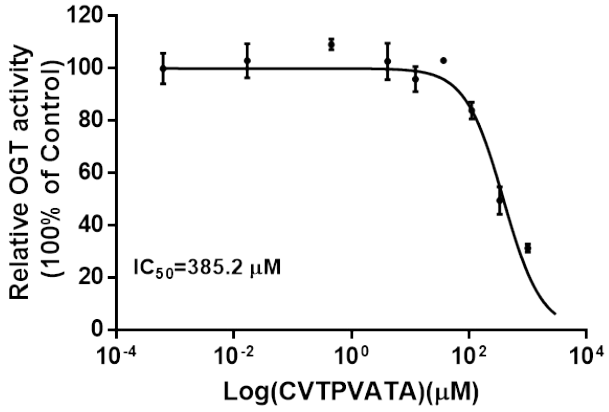
Cpd.		Cpd.	
F01		F12	
F02		F13	
F03		F14	
F04		F15	
F05		F16	

F06		F17	
F07		F18	
F08		F19	
F09		F20	
F10		F21	
F11		F22	

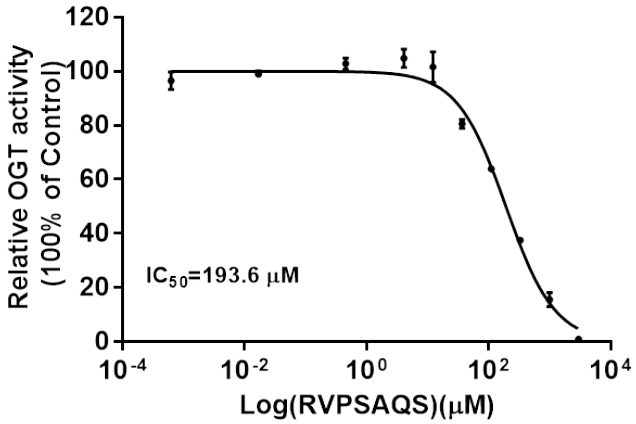
Biochemical Assays: *In vitro* UDP-Glo assay

This assay evaluates O-GlcNAcylation through monitoring UDP formation in glycosyltransferase reactions by luminescence. Briefly, OGT reactions were carried out in a 100 µL final volume containing 0.5 mM UDP-GlcNAc, 6 µg purified or 60 µg lysate enzyme, 100 µM peptide in OGT reaction buffer (50 mM Tris-HCl, pH 7.5;1 mM DTT;12.5 mM $MgCl_2$). Reactions were incubated at room temperature for 2 h. At the end of that period, each reaction was transferred in triplicate into a 96-well white microplate and was mixed with a 1:1 ratio of the UDP-Glo Detection Reagent. After incubation at room temperature for 1h, the luminescence was recorded with a Mithras LB940 Multimode microplate reader using Mikro Win 2000 software (Berthold Technology, Germany).

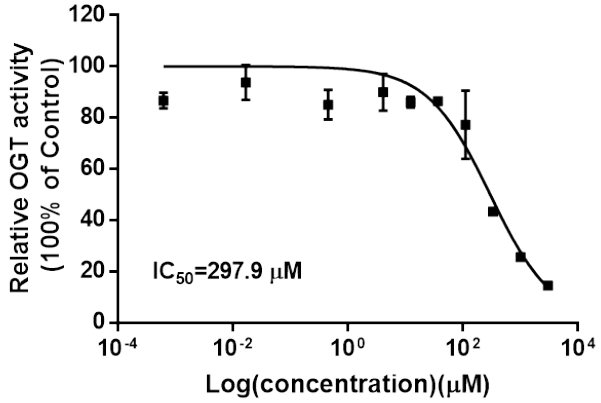
Inhibition of OGT for Pep6



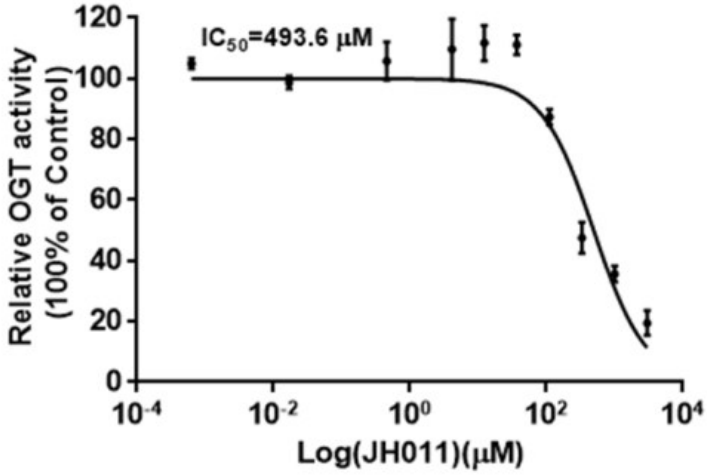
Inhibition of OGT for Pep13



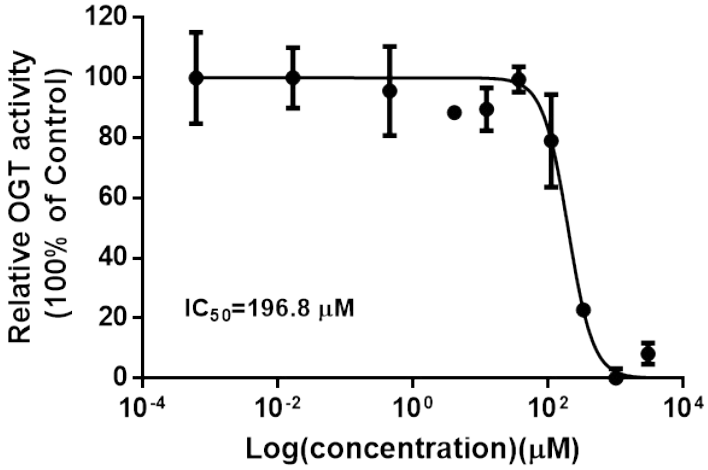
Inhibition of OGT for Compound 1



Inhibition of OGT for Compound 3

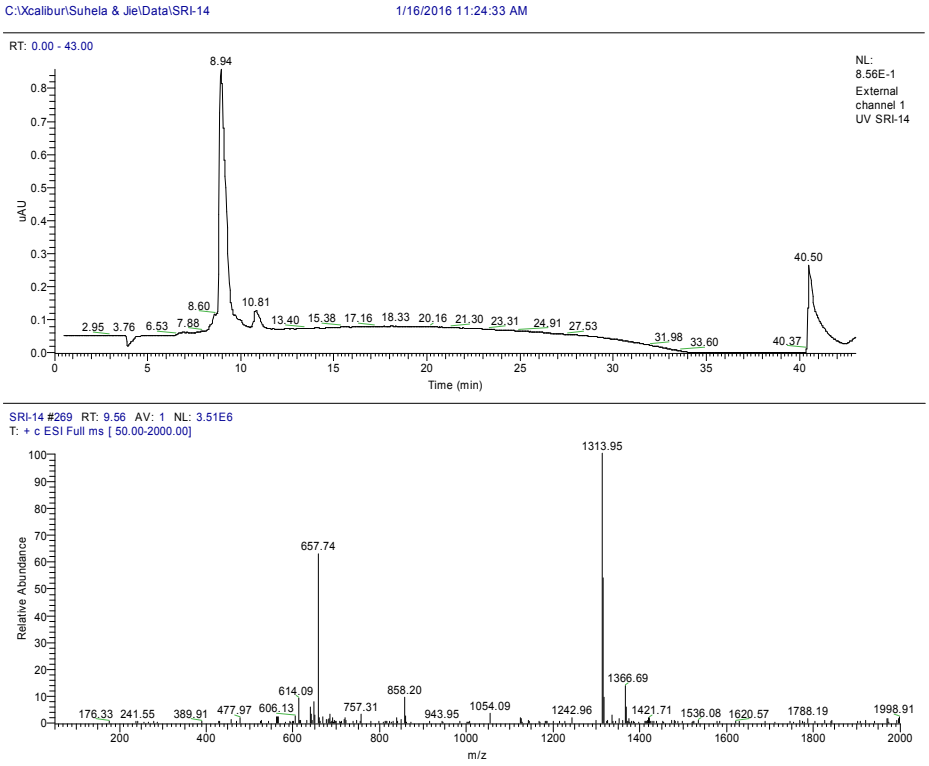


Inhibition of OGT for Compound 6

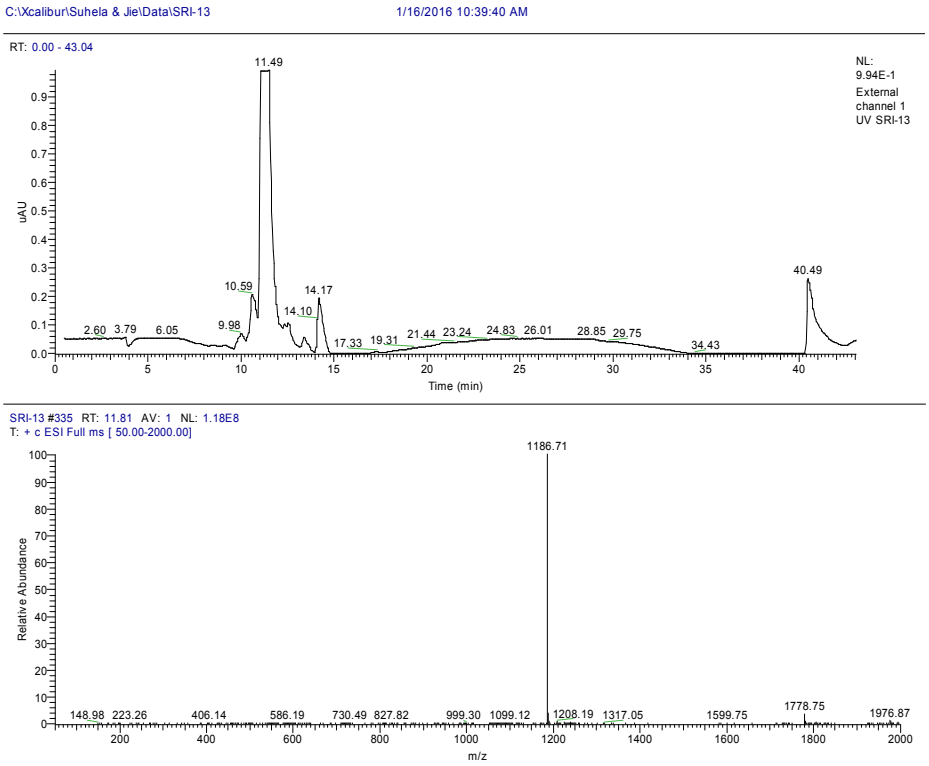


Analytical LCMS results for Pep1-15

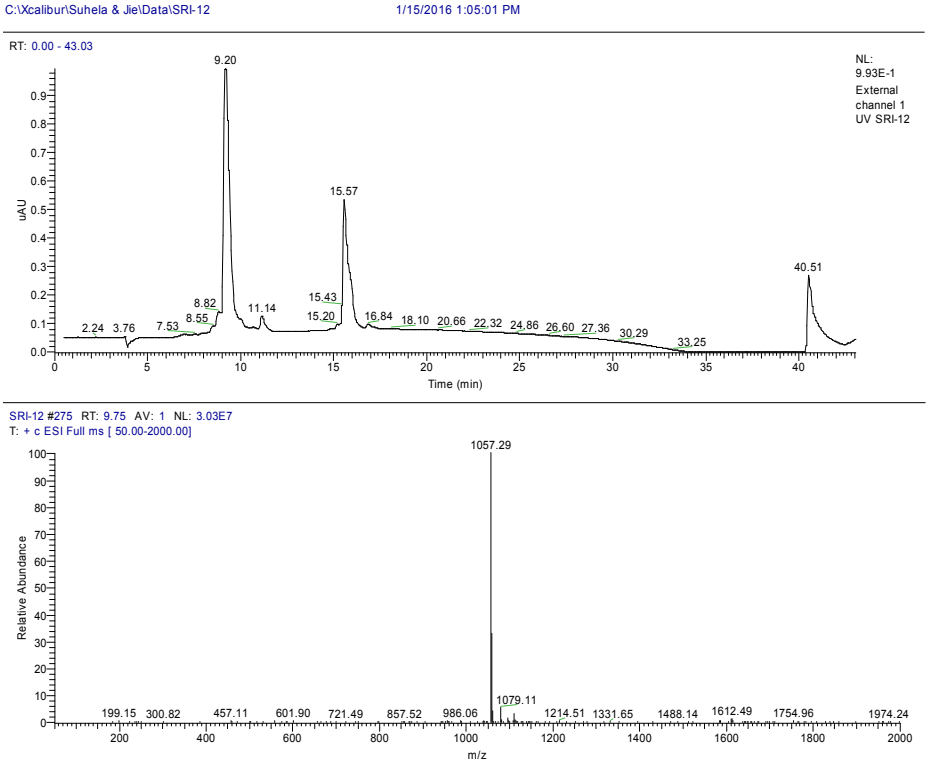
Pep1



Pep2



Pep3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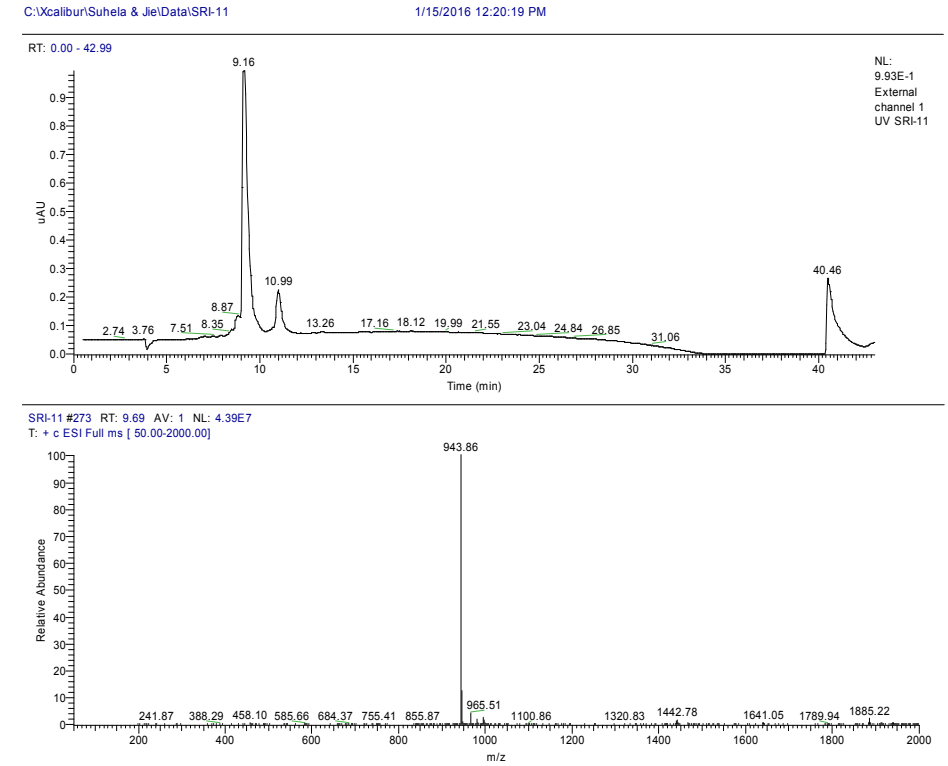
NL:
9.93E-1
External
channel 1
UV SRI-12
uAU
Time (min)
SRI-12 #275 RT: 9.75 AV: 1 NL: 3.03E7
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z

Pep4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Time (min)
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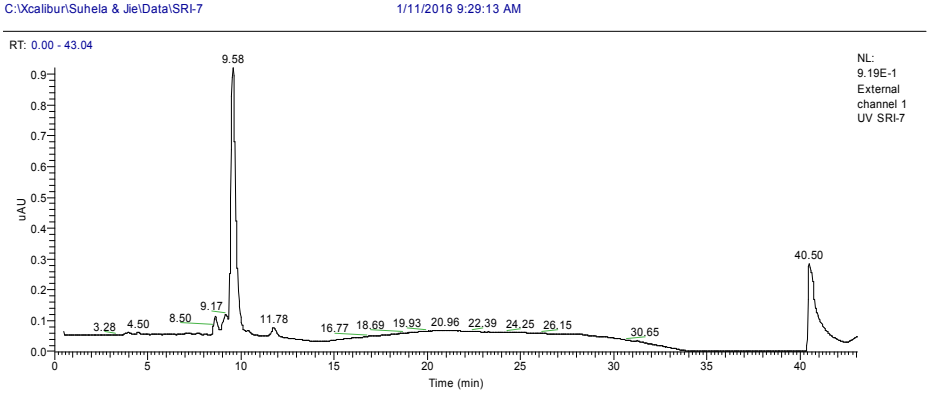
Relative Abundance
m/z

Pep5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NL:
9.19E-1
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channel 1
UV SRI-7
uAU
Time (min)
9.58
40.50
3.28
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9.17
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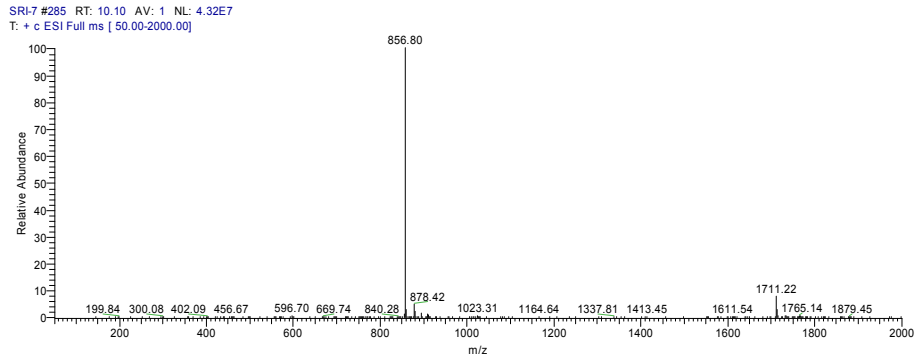
16.77
18.69
19.93
20.96
22.39
24.25
26.15
30.65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T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
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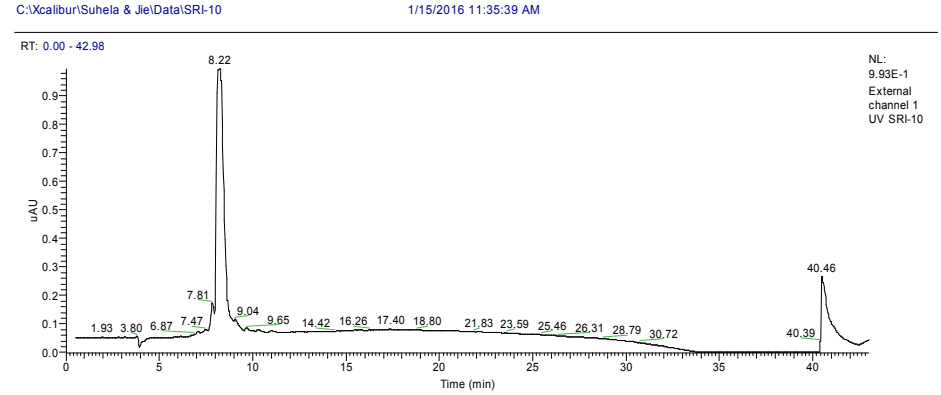
878.42
1711.22
199.84
300.08
402.09
456.67
596.70
669.74
840.28
1023.31
1164.64
1337.81
1413.45
1611.54
1765.14
1879.45

Pep6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channel 1
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Time (min)
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40.46
7.81
9.04
1.93
3.80
6.87
7.47
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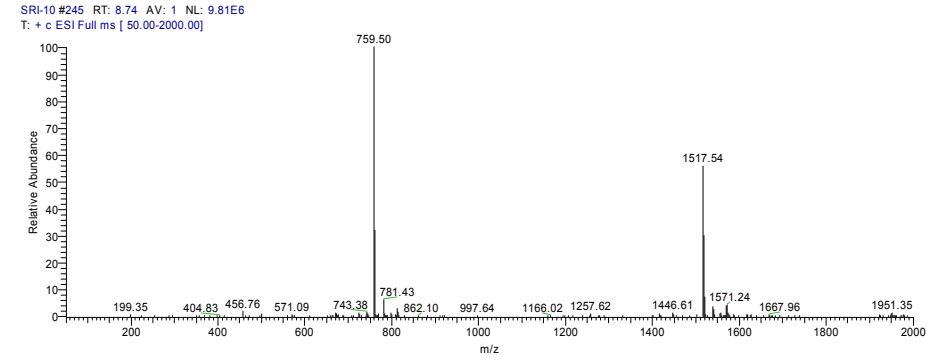
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m/z
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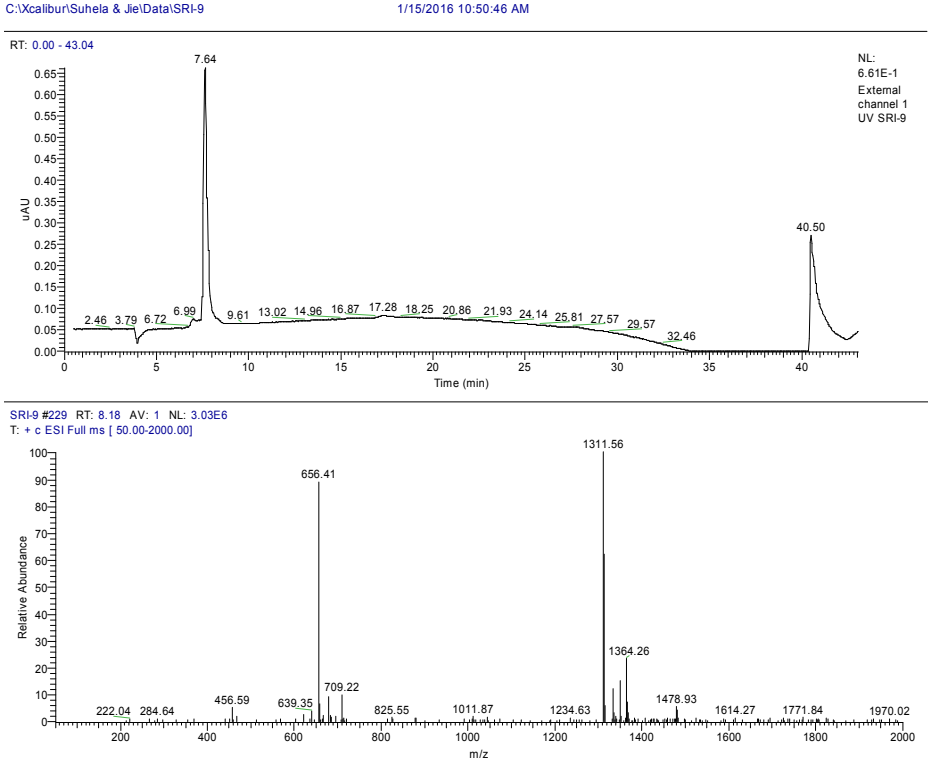
1517.54
781.43
1571.24
199.35
404.83
456.76
571.09
743.38
862.10
997.64
1166.02
1257.62
1446.61
1667.96
1951.35

Pep7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uAU
Time (min)
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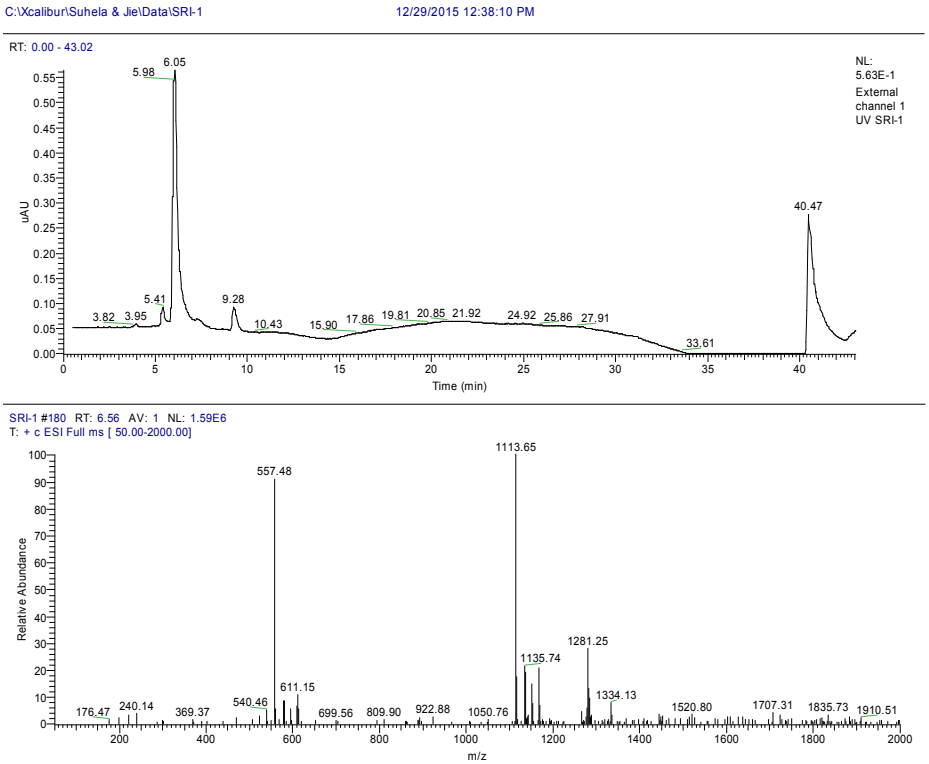
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z

Pep8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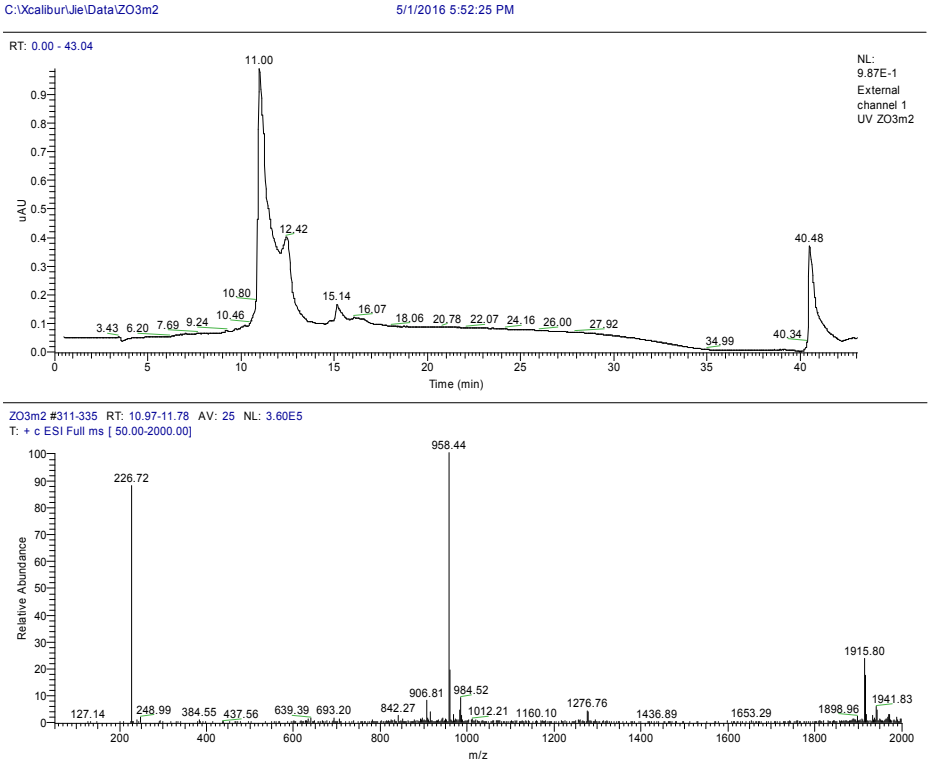
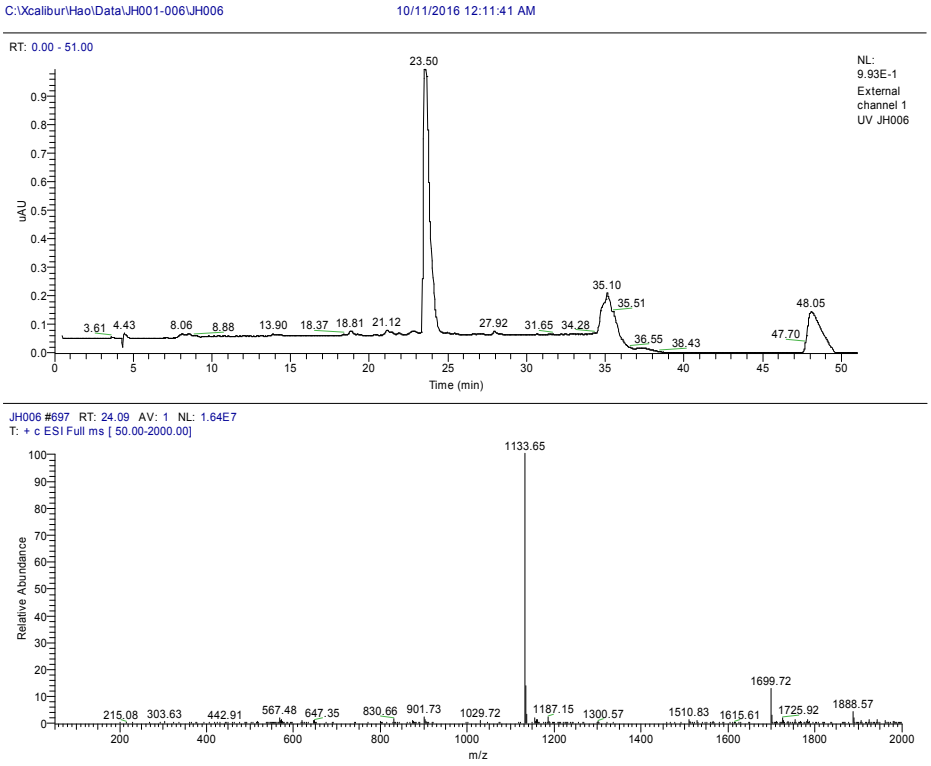
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z

Pep9


C:\Xcalibur\Jie\Data\ZO3m2 5/1/2016 5:52:25 PM
RT: 0.00 - 43.04
NL: 9.87E-1 External channel 1 UV ZO3m2
uAU
Time (min)
ZO3m2 #311-335 RT: 10.97-11.78 AV: 25 NL: 3.60E5
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z

Pep10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uAU
Time (min)
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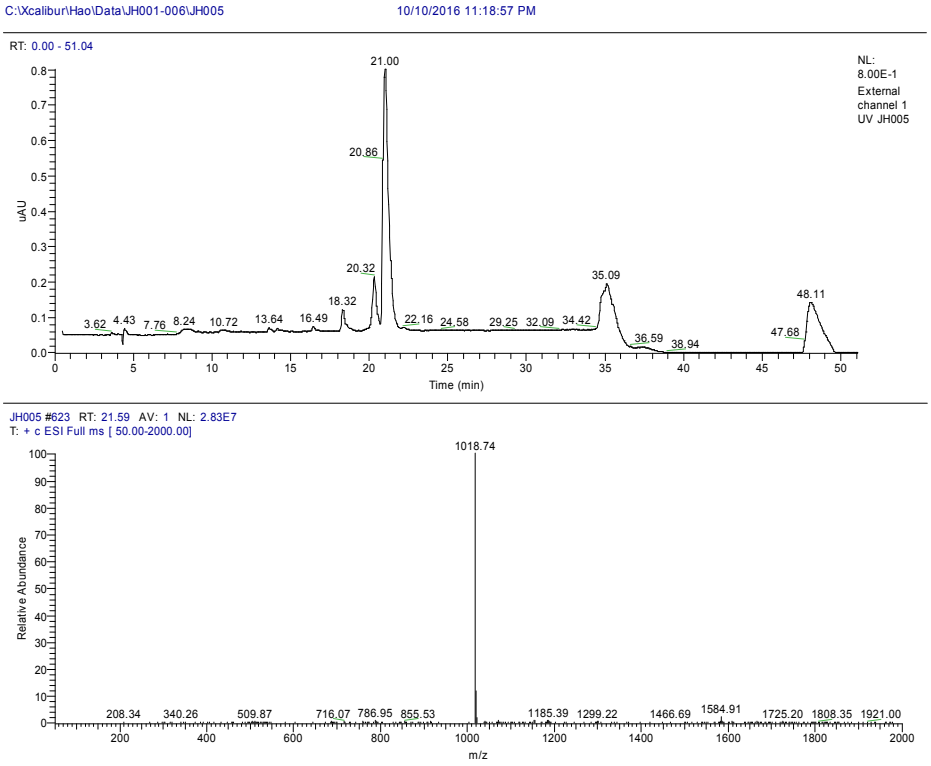
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z

Pep11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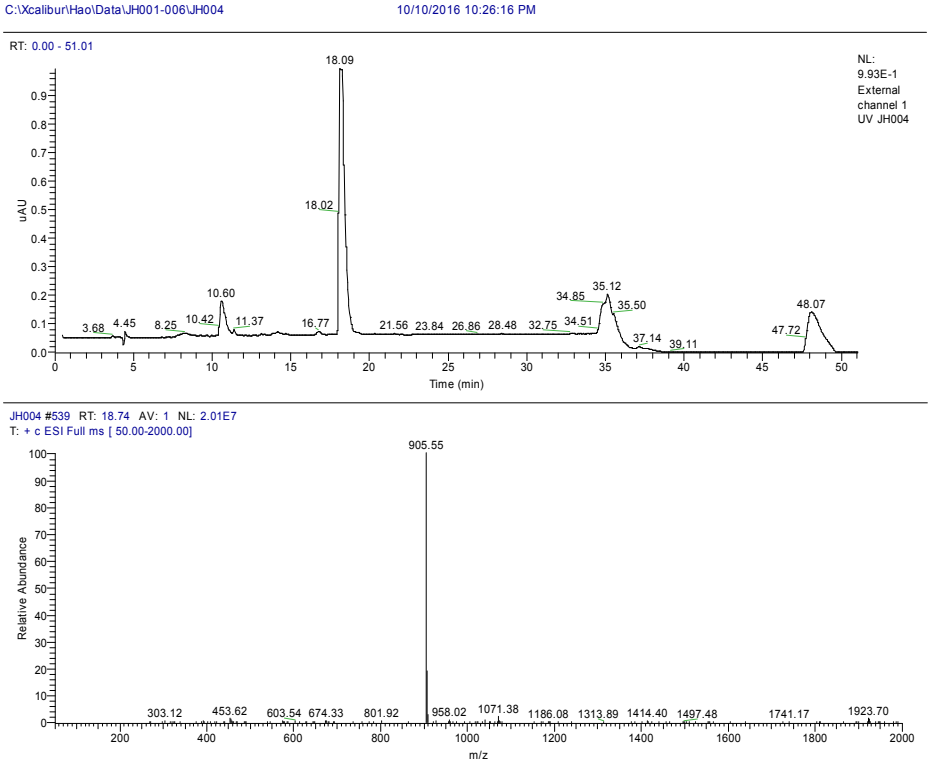
NL:
8.00E-1
External
channel 1
UV JH005
uAU
Time (min)
21.00
20.86
20.32
18.32
35.09
48.11
47.68
JH005 #623 RT: 21.59 AV: 1 NL: 2.83E7
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
1018.74

Pep12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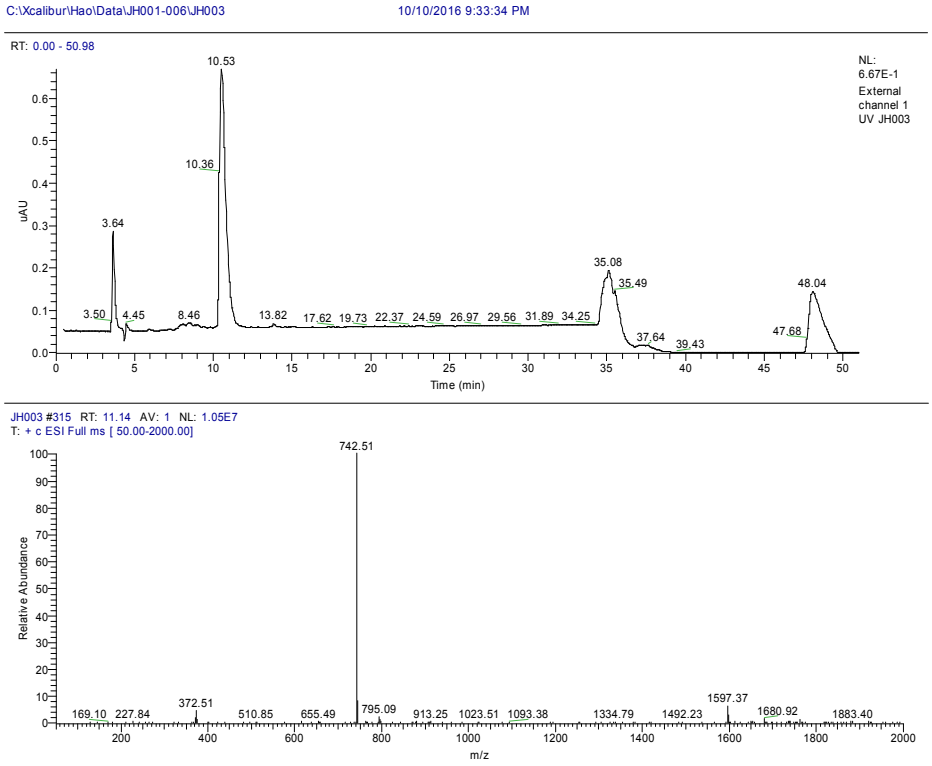
9.93E-1
External
channel 1
UV JH004
uAU
Time (min)
18.09
18.02
10.60
35.12
48.07
JH004 #539 RT: 18.74 AV: 1 NL: 2.01E7
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
905.55

Pep13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RT: 0.00 - 50.98
NL:
6.67E-1
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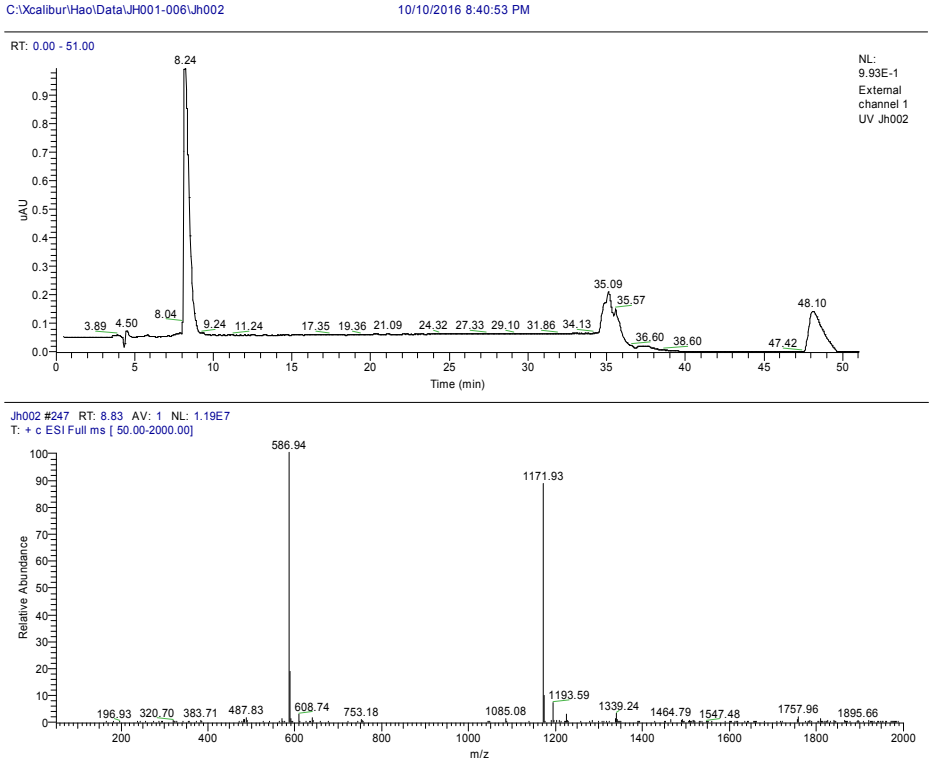
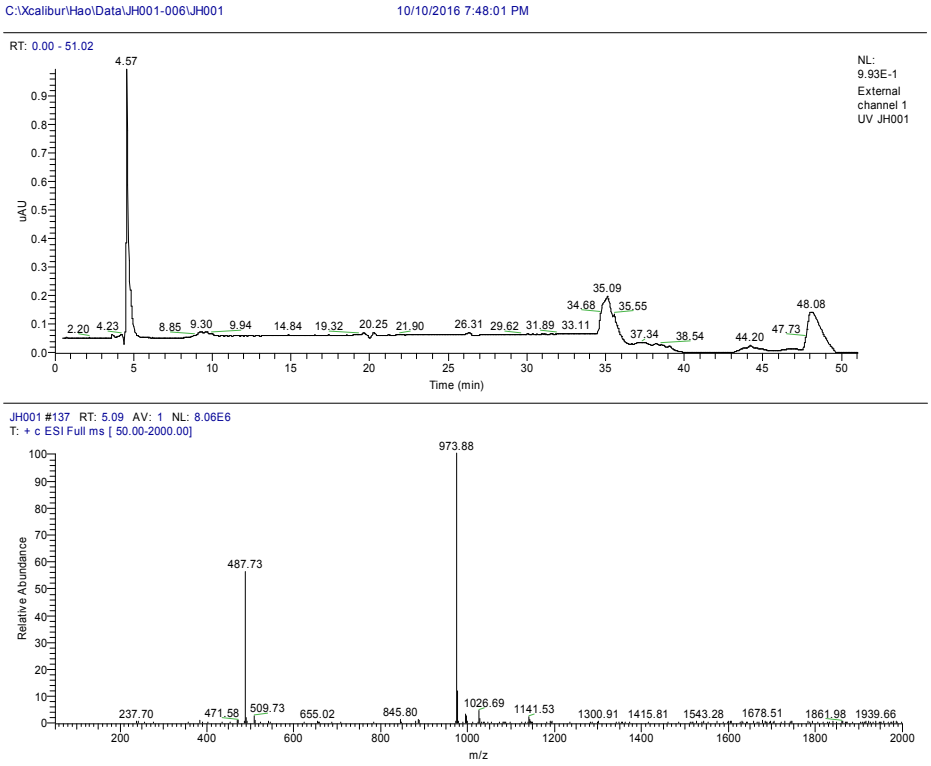
channel 1
UV JH003
uAU
Time (min)
10.53
10.36
3.64
35.08
35.49
48.04
47.68
37.64
39.43
3.50
4.45
8.46
13.82
17.62
19.73
22.37
24.59
26.97
29.56
31.89
34.25
JH003 #315 RT: 11.14 AV: 1 NL: 1.05E7
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
742.51
1597.37
372.51
795.09
169.10
227.84
510.85
655.49
913.25
1023.51
1093.38
1334.79
1492.23
1680.92
1883.40

Pep14


C:\Xcalibur\Hao\Data\JH001-006\Jh002
10/10/2016 8:40:53 PM
RT: 0.00 - 51.00
NL:
9.93E-1
External
channel 1
UV Jh002
uAU
Time (min)
8.24
35.09
35.57
48.10
8.04
3.89
4.50
9.24
11.24
17.35
19.36
21.09
24.32
27.33
29.10
31.86
34.13
36.60
38.60
47.42
Jh002 #247 RT: 8.83 AV: 1 NL: 1.19E7
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
586.94
1171.93
1193.59
196.93
320.70
383.71
487.83
608.74
753.18
1085.08
1339.24
1464.79
1547.48
1757.96
1895.66

Pep15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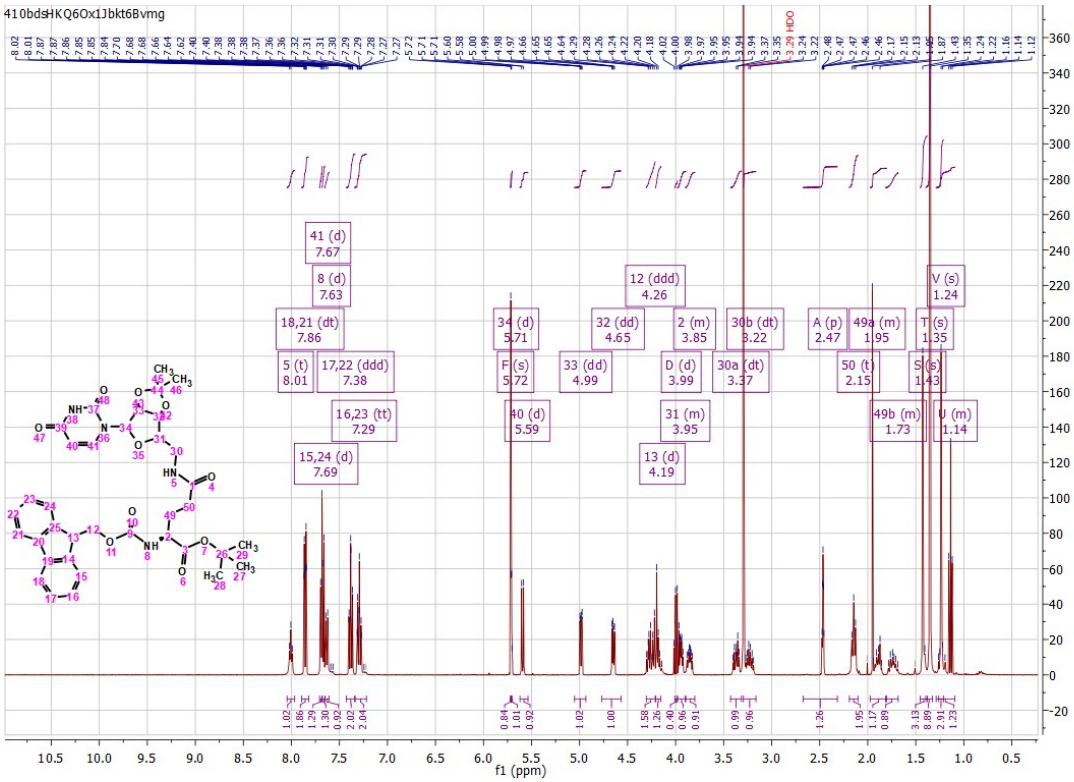
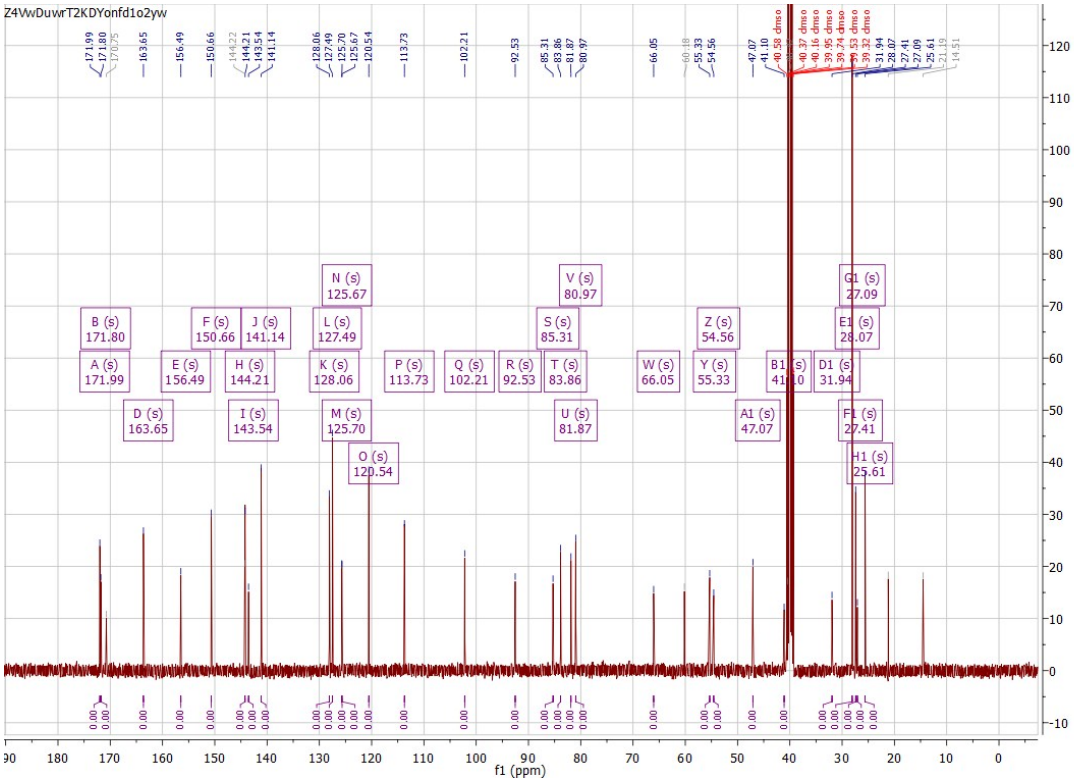
RT: 0.00 - 51.02
NL: 9.93E-1
External channel 1 UV JH001
uAU
Time (min)
4.57
2.20 4.23 8.85 9.30 9.94 14.84 19.32 20.25 21.90 26.31 29.62 31.89 33.11 34.68 35.09 35.55 37.34 38.54 44.20 47.73 48.08
JH001 #137 RT: 5.09 AV: 1 NL: 8.06E6
T: + c ESI Full ms [50.00-2000.00]
Relative Abundance
m/z
973.88
487.73
237.70 471.58 509.73 655.02 845.80 1026.69 1141.53 1300.91 1415.81 1543.28 1678.51 1861.98 1939.66

^{1}H and ^{13}C NMR spectra for new compounds

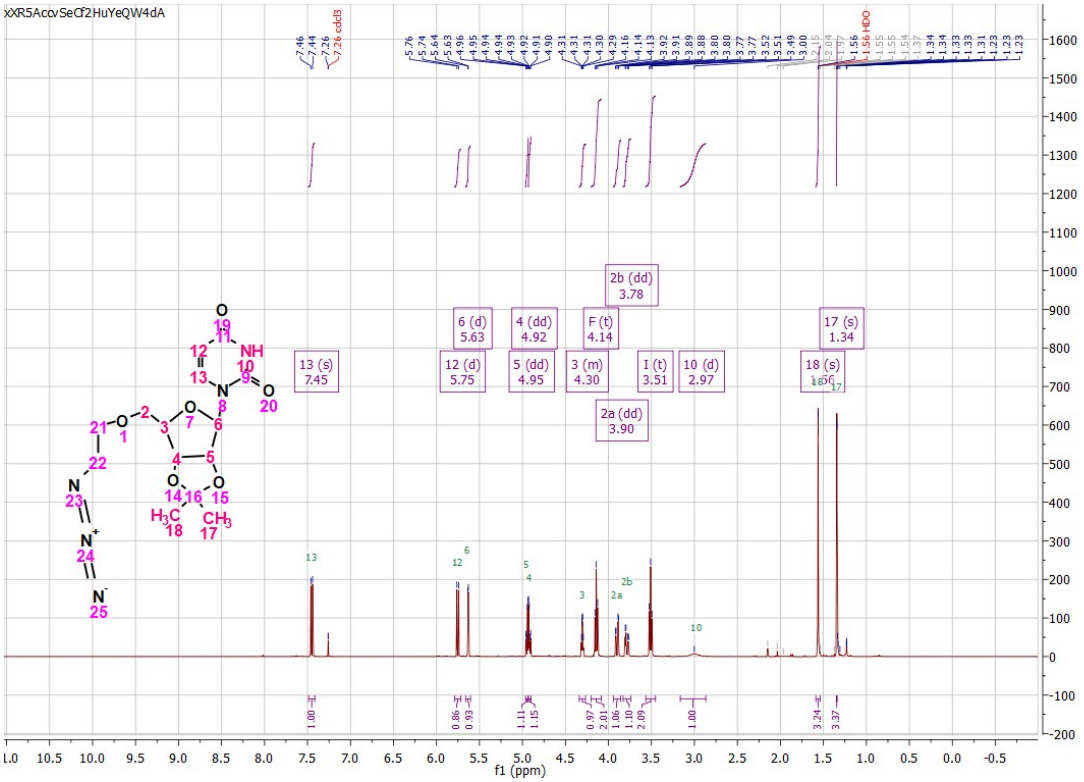
Compound **10**: ^{1}H NMR (400 MHz, DMSO-d_6)



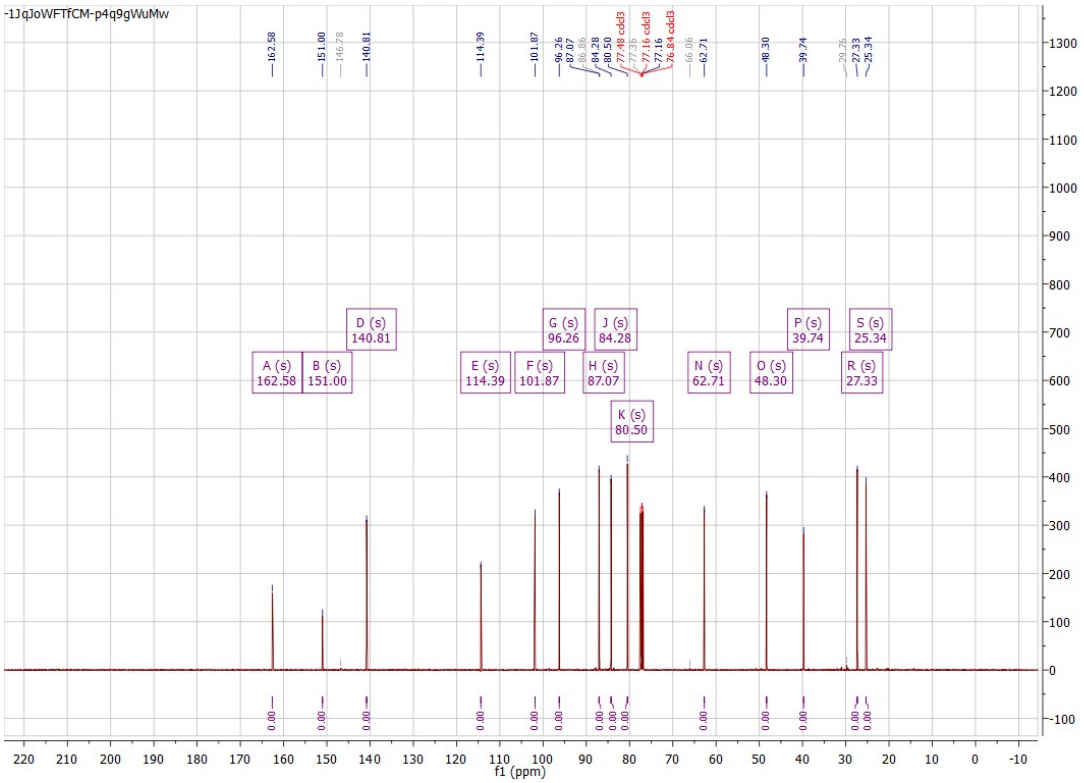
Compound **10**: ^{13}C NMR (101 MHz, DMSO-d_6)



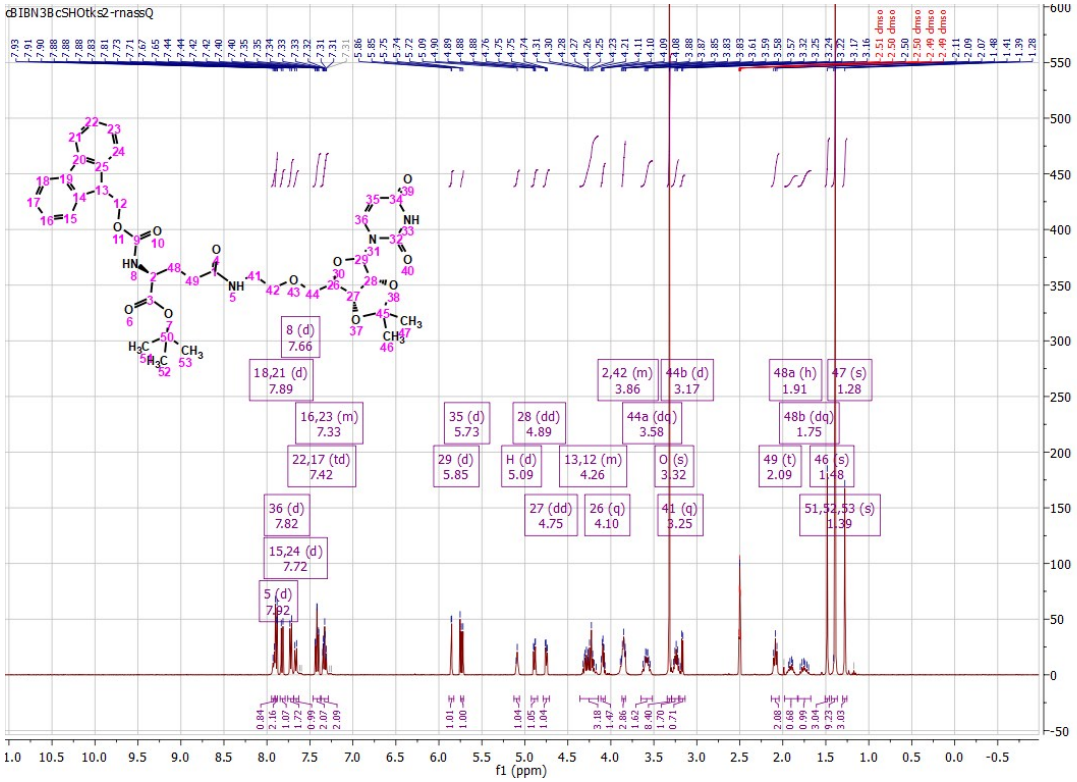
Compound **8**: ^{1}H NMR (400 MHz, Chloroform-*d*)



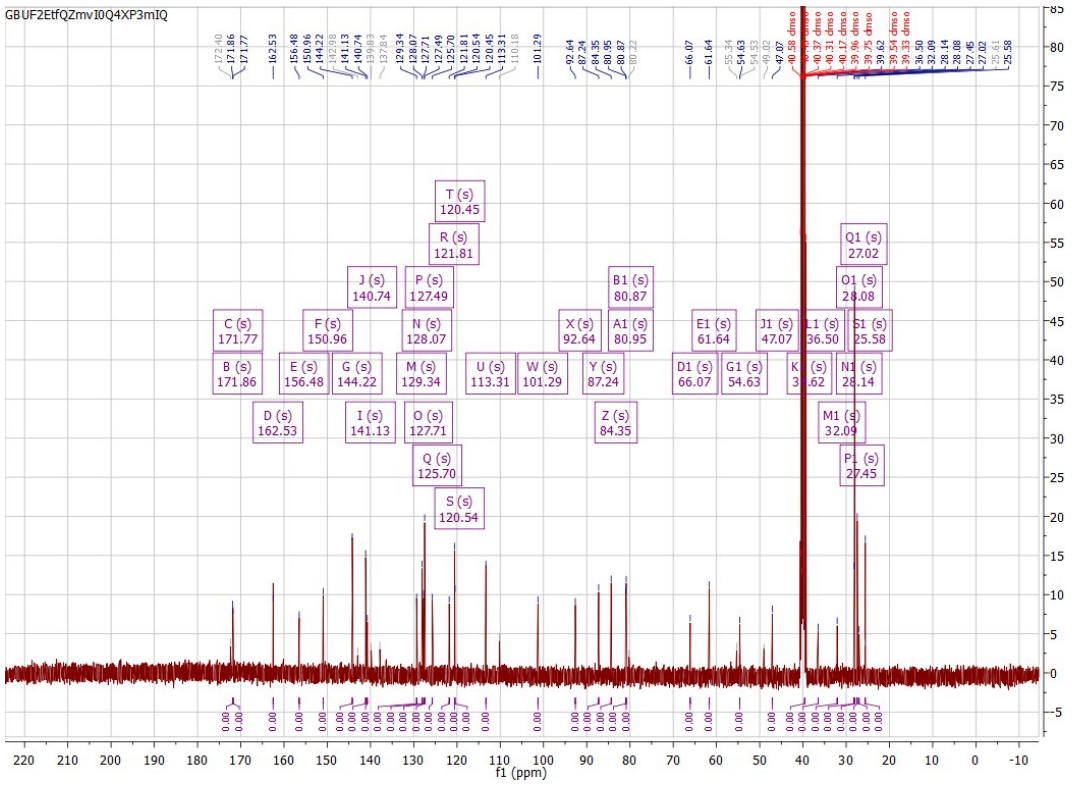
Compound **8**: ^{13}C NMR (101 MHz, Chloroform-*d*)



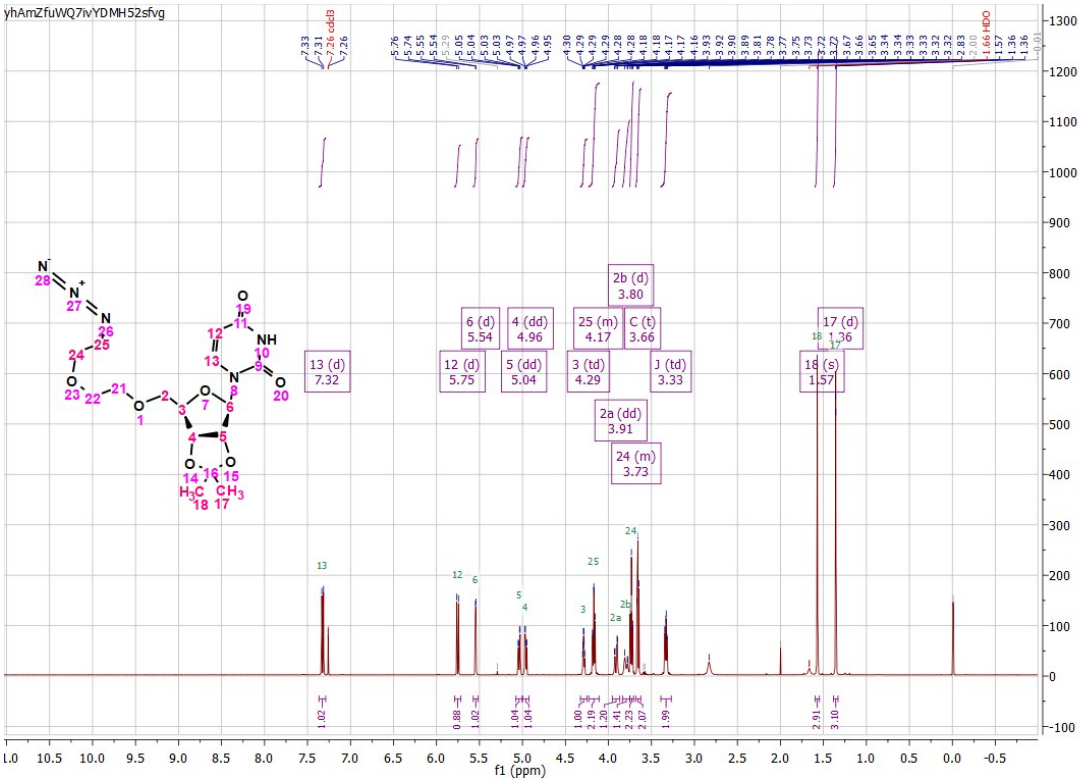
Compound **11**: ^{1}H NMR (400 MHz, DMSO-d_6)



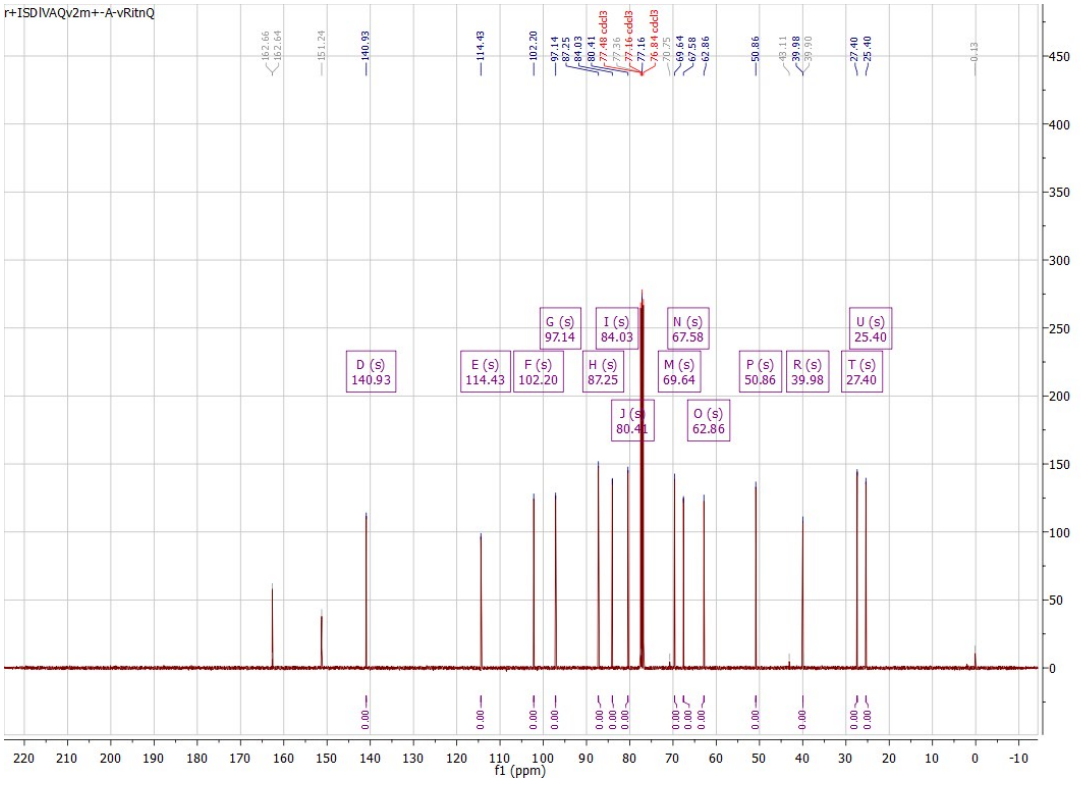
Compound **11**: ^{13}C NMR (101 MHz, DMSO-d_6)



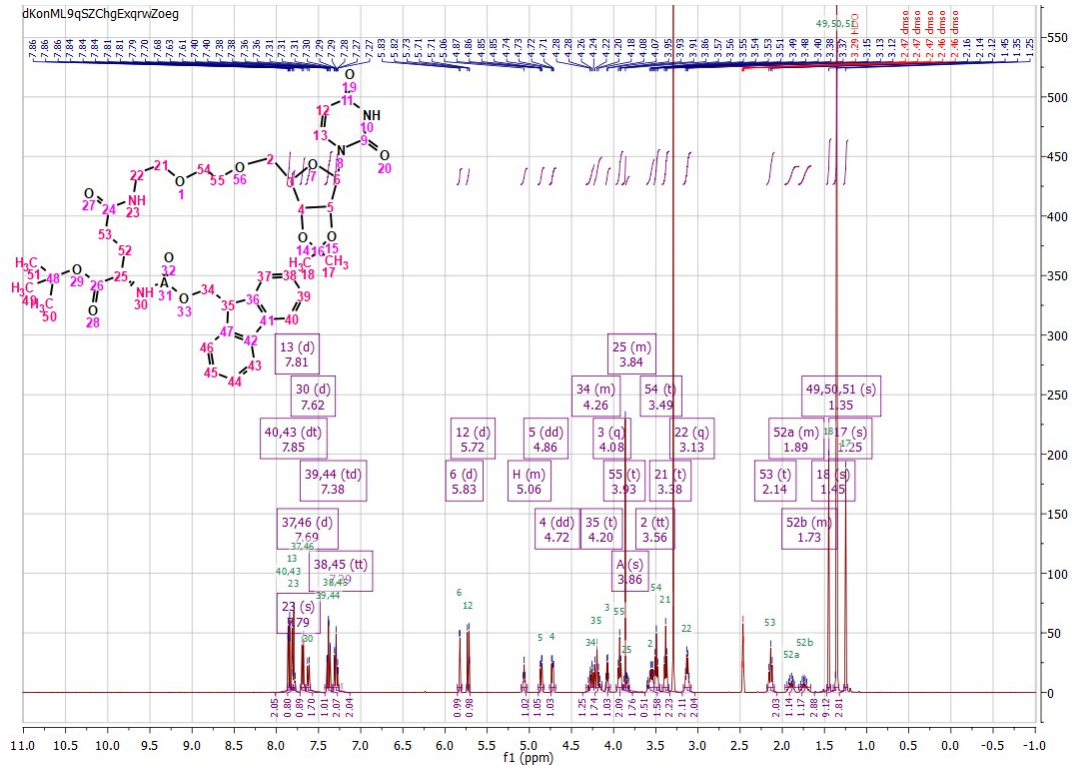
Compound **9**: ^{1}H NMR (400 MHz, Chloroform-*d*)



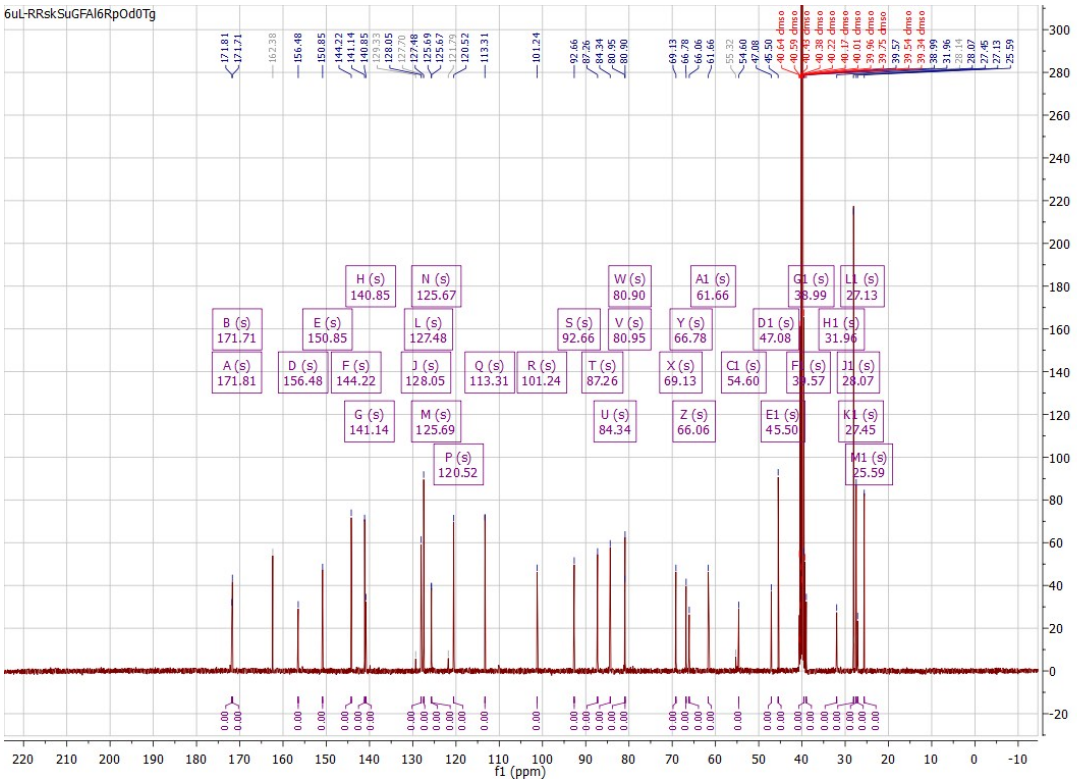
Compound **9**: ^{13}C NMR (101 MHz, Chloroform-*d*)



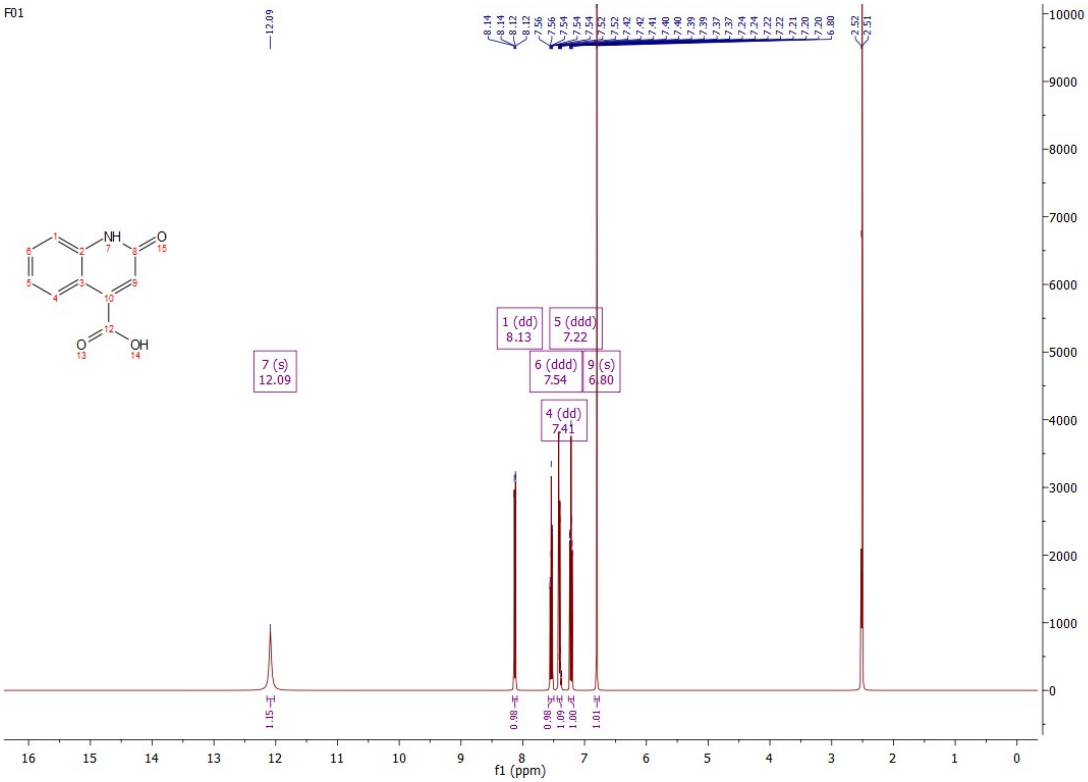
Compound **12**: ^{1}H NMR (400 MHz, DMSO-d_6)



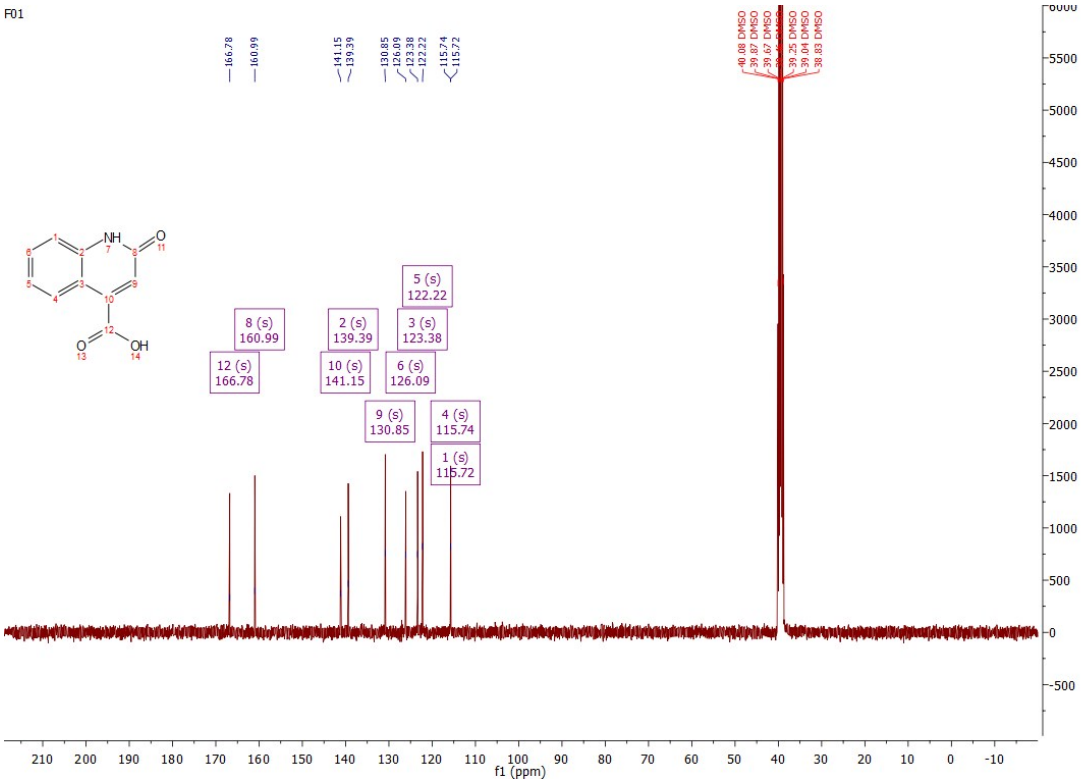
Compound **12**: ^{13}C NMR (101 MHz, DMSO-d_6)



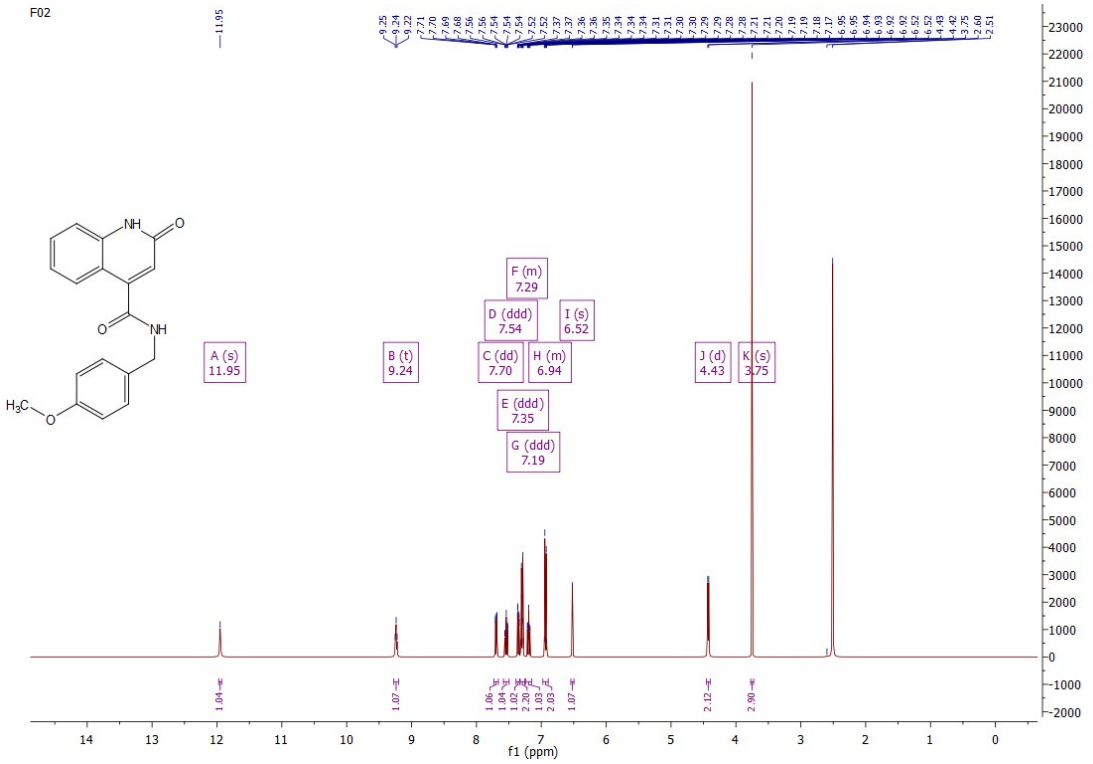
Fragment **F01**: ^{1}H NMR (400 MHz, DMSO-d_6)



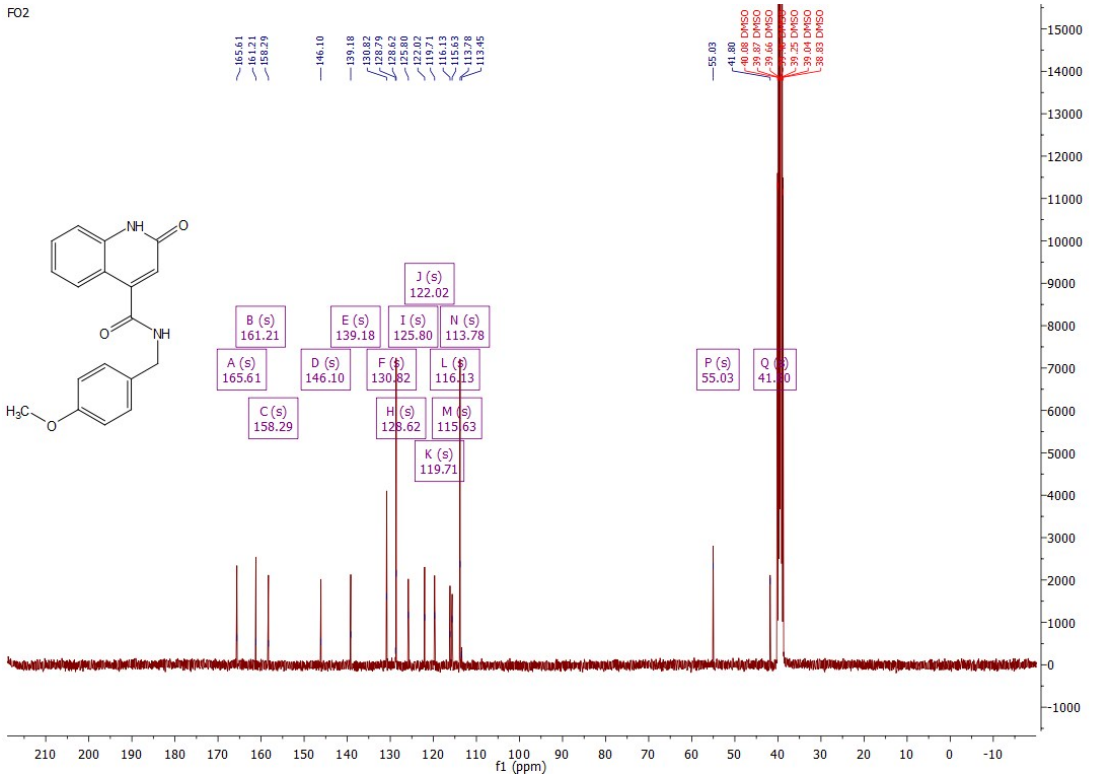
Fragment **F01**: ^{13}C NMR (101 MHz, DMSO-d_6)



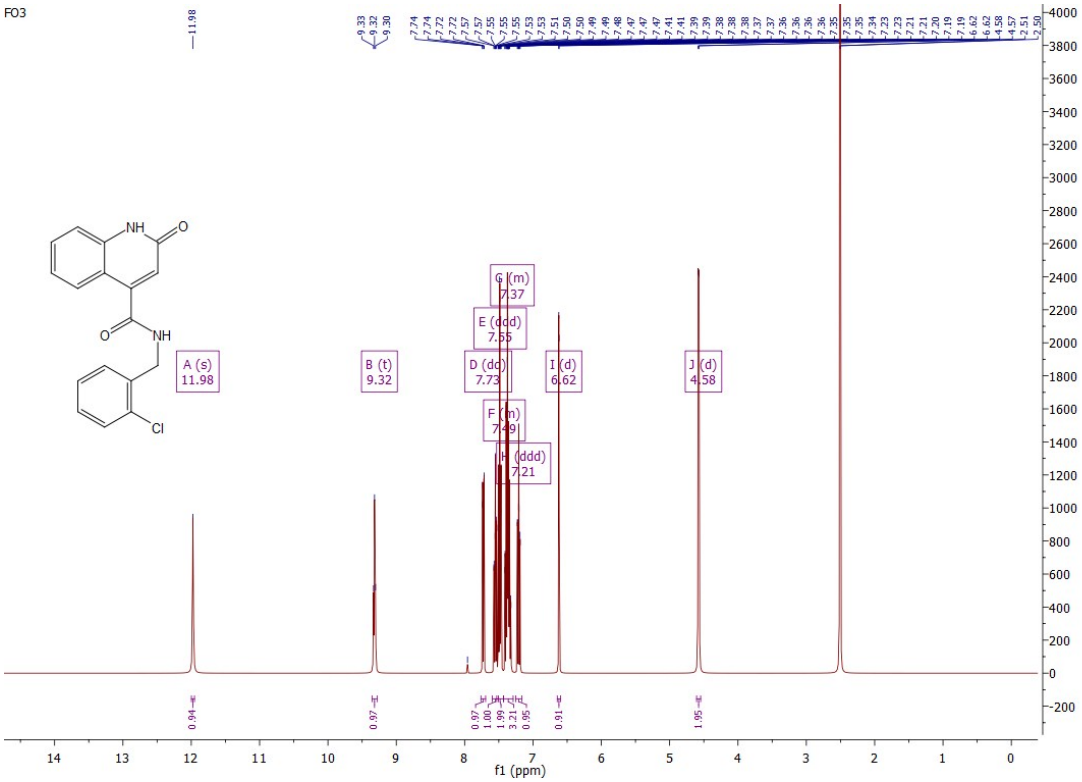
Fragment **F02**: ^{1}H NMR (400 MHz, DMSO-d_6)



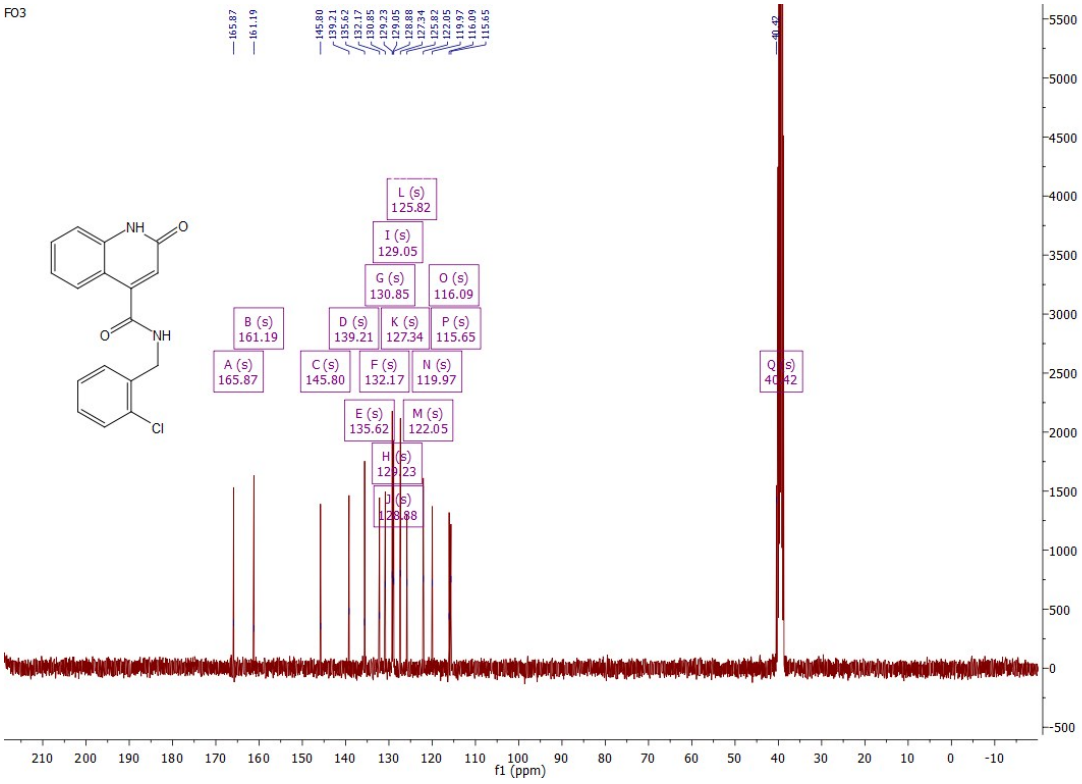
Fragment **F02**: ^{13}C NMR (101 MHz, DMSO-d_6)



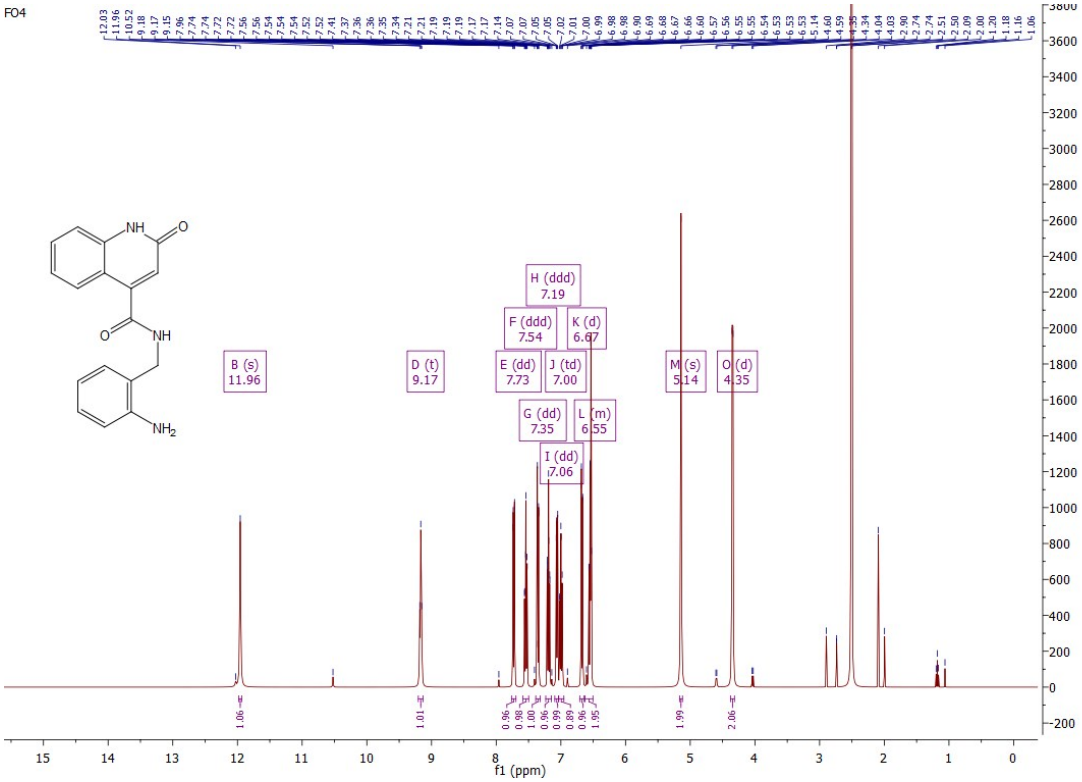
Fragment **F03**: ^{1}H NMR (400 MHz, DMSO-d_6)



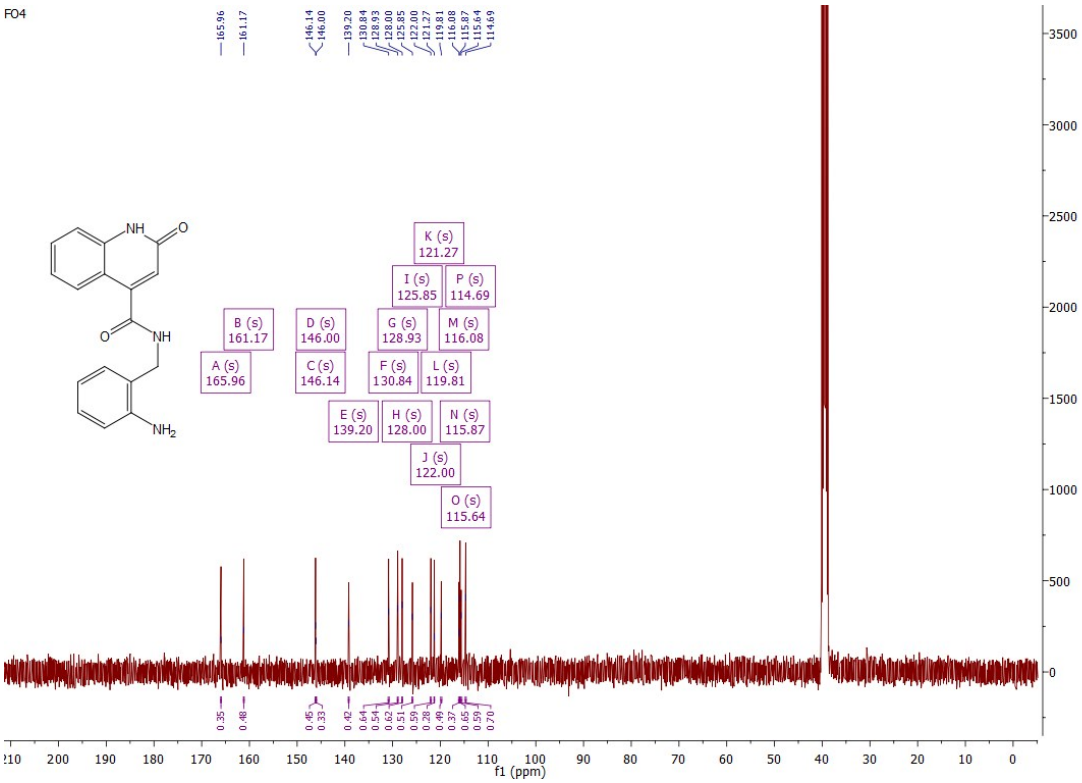
Fragment **F03**: ^{13}C NMR (101 MHz, DMSO-d_6)



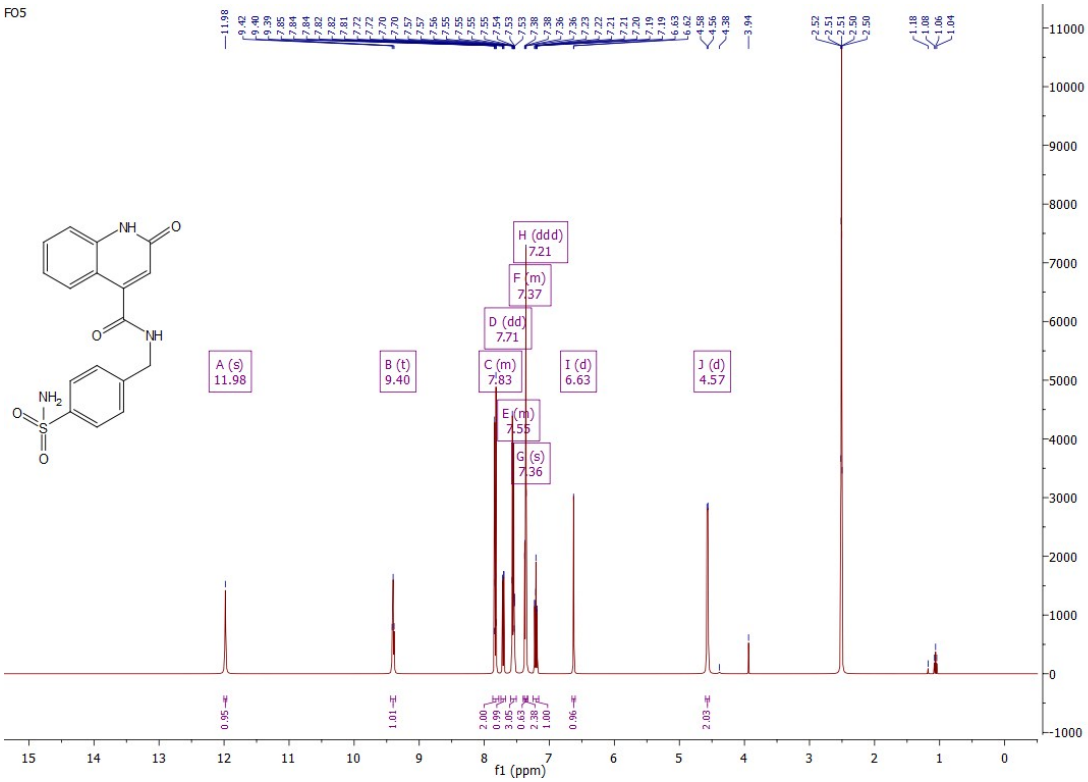
Fragment **F04**: ^{1}H NMR (400 MHz, DMSO-d_6)



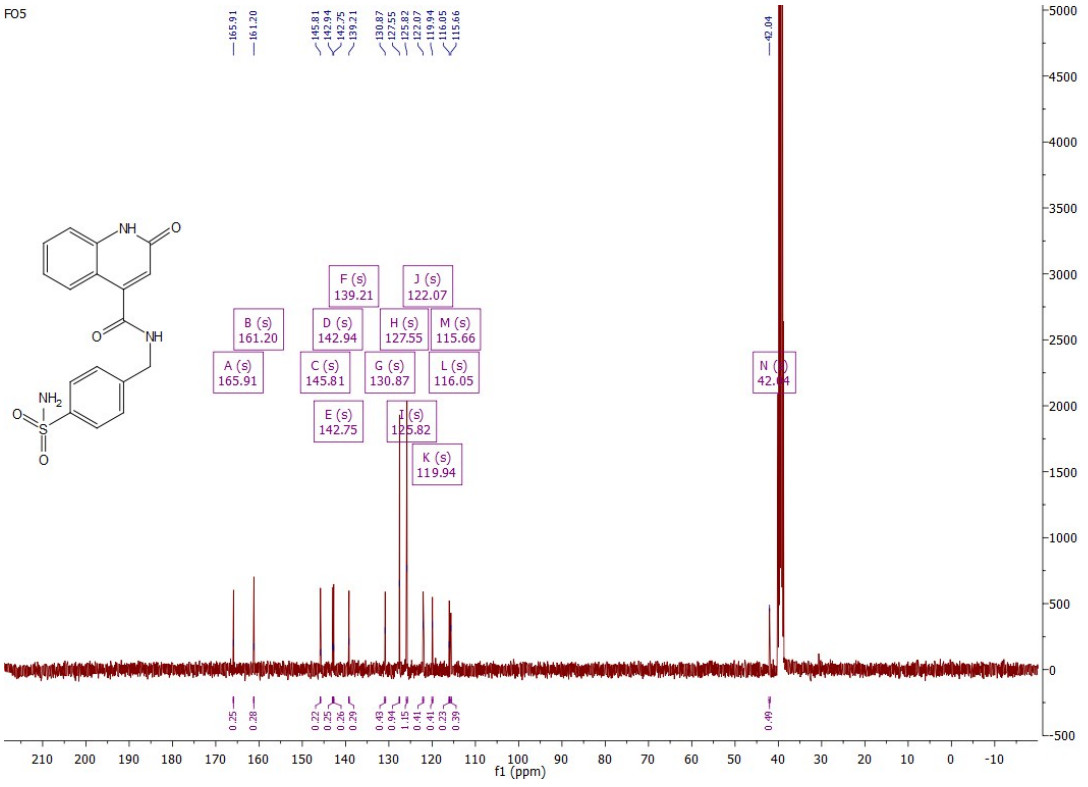
Fragment **F04**: ^{13}C NMR (101 MHz, DMSO-d_6)



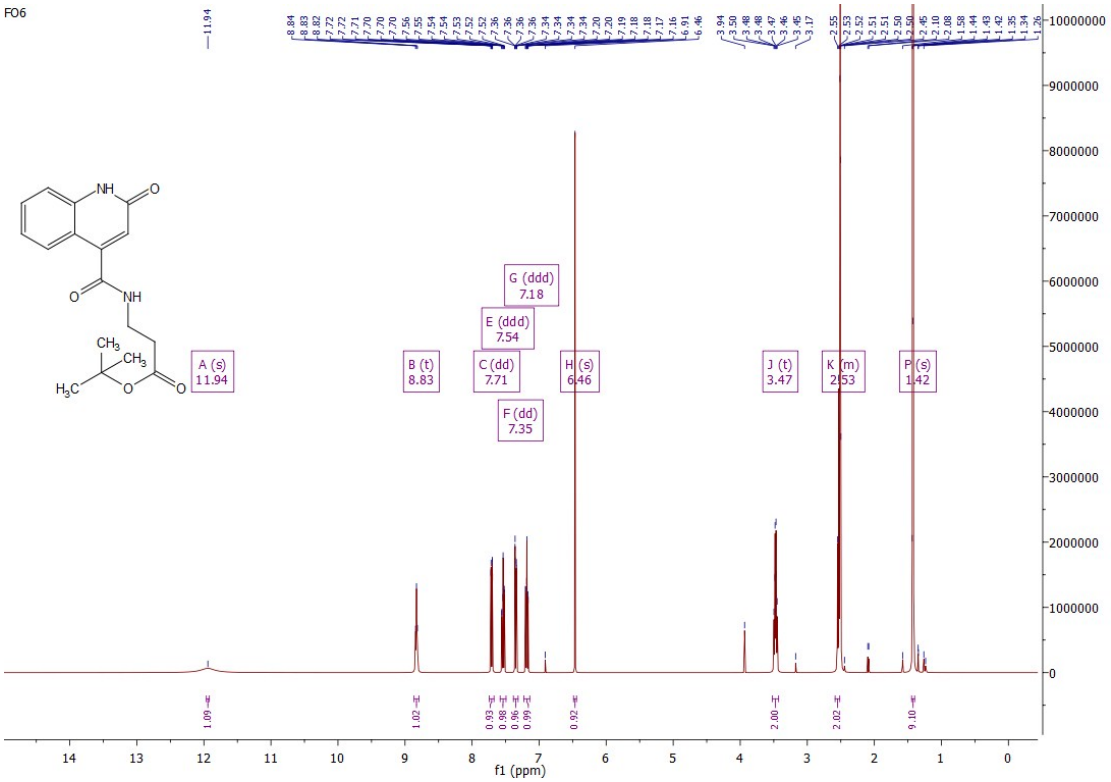
Fragment **F05**: ^{1}H NMR (400 MHz, DMSO-d_6)



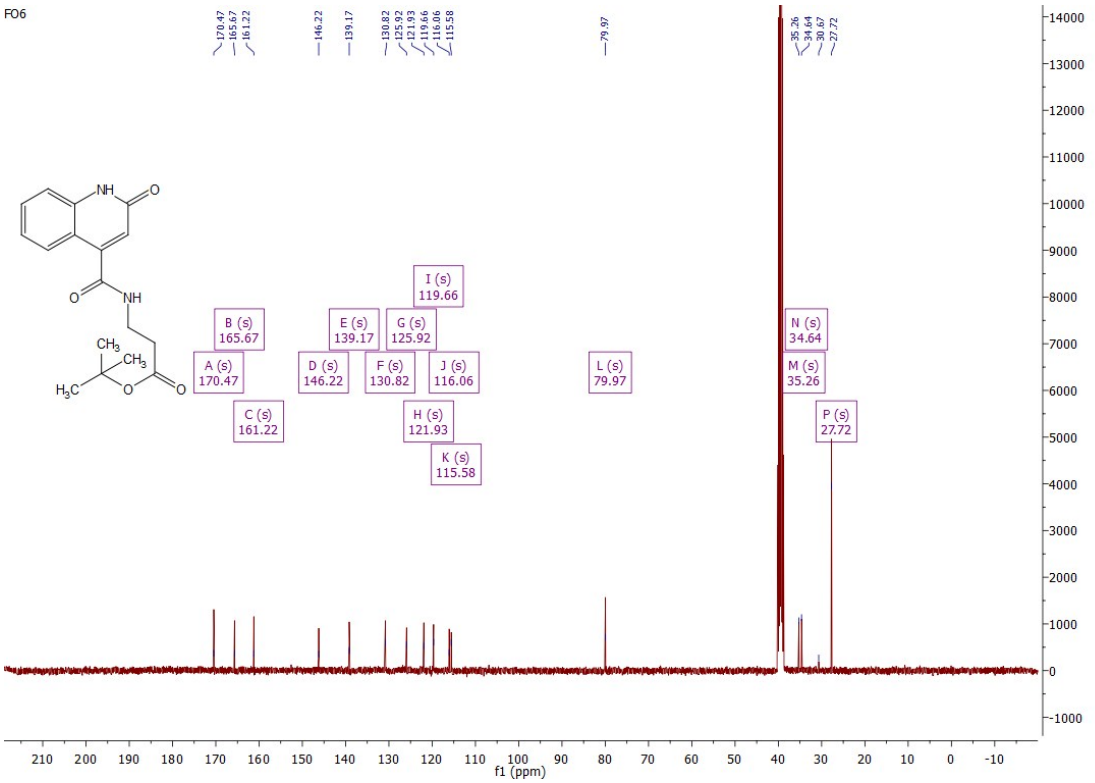
Fragment **F05**: ^{13}C NMR (101 MHz, DMSO-d_6)



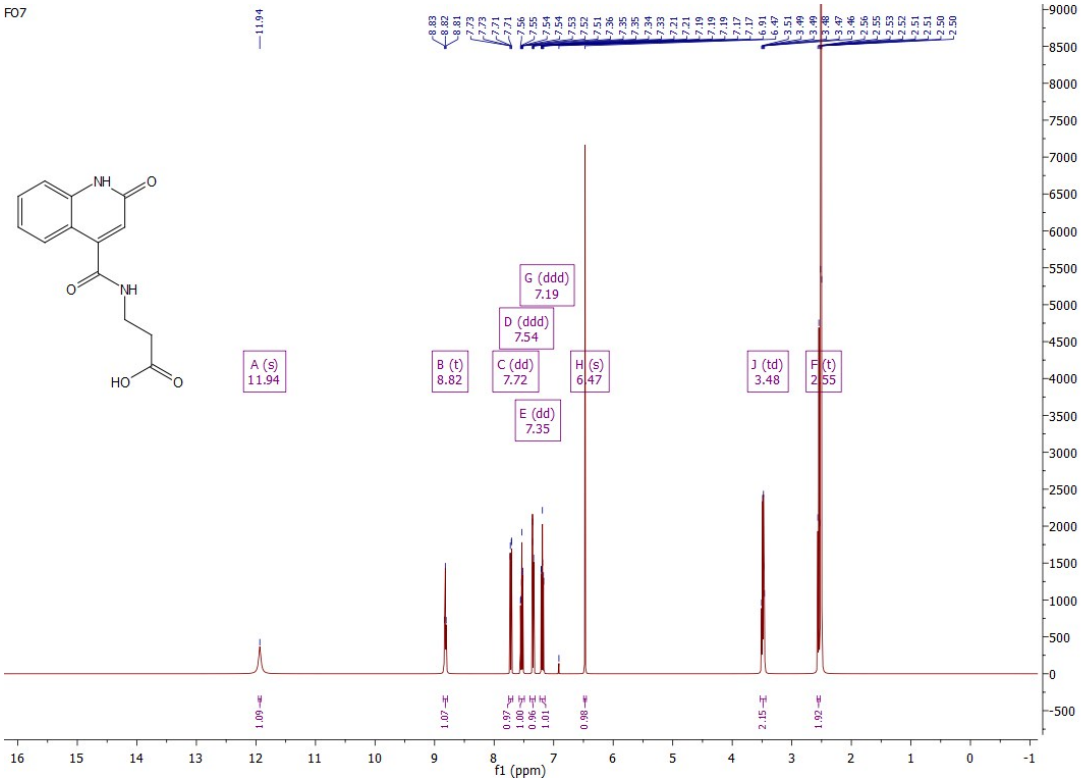
Fragment **F06**: ^{1}H NMR (400 MHz, DMSO-d_6)



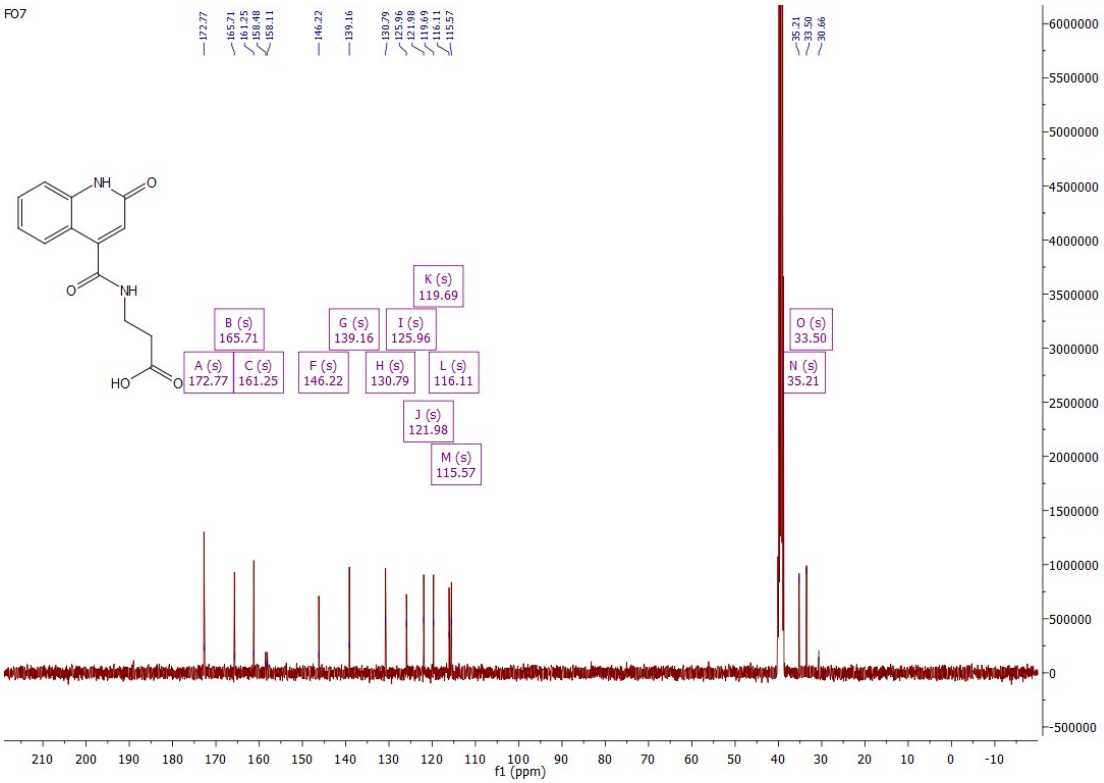
Fragment **F06**: ^{13}C NMR (101 MHz, DMSO-d_6)



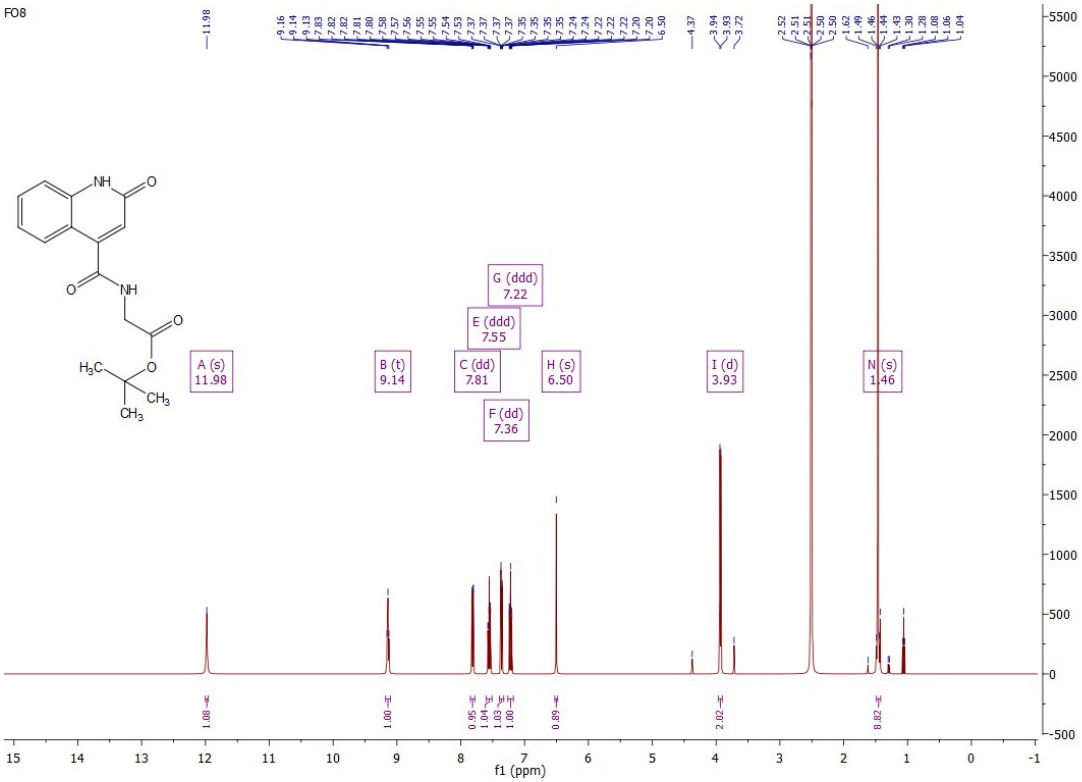
Fragment **F07**: ^{1}H NMR (400 MHz, DMSO-d_6)



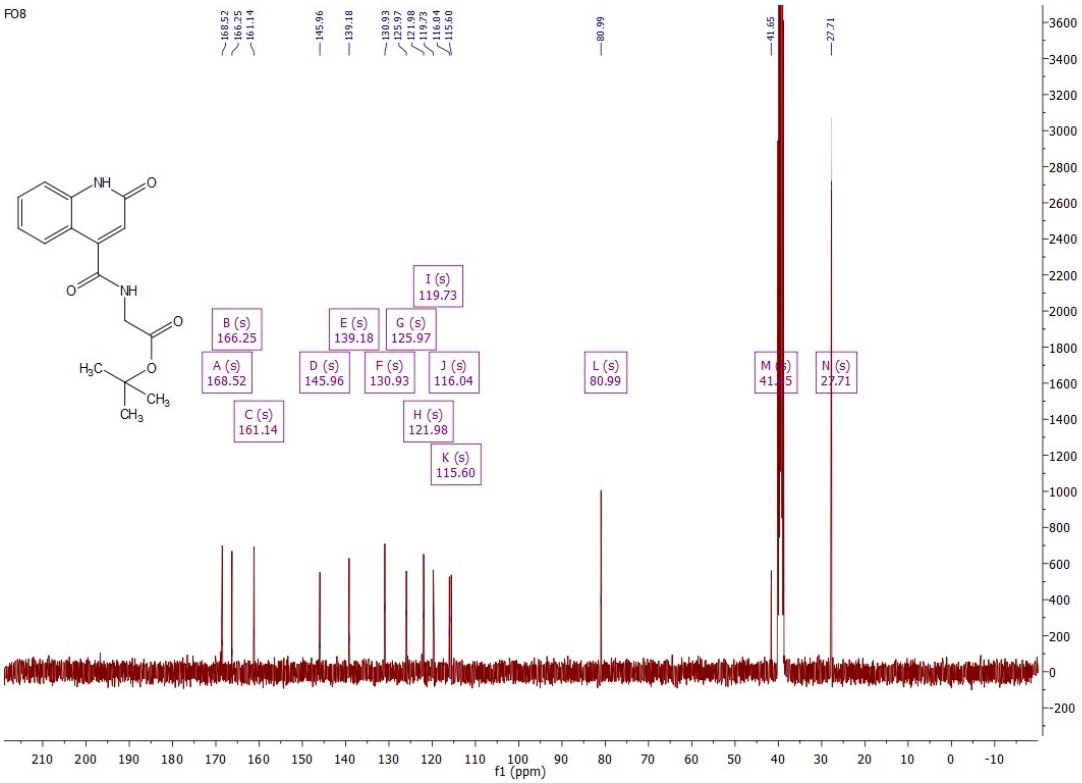
Fragment **F07**: ^{13}C NMR (101 MHz, DMSO-d_6)



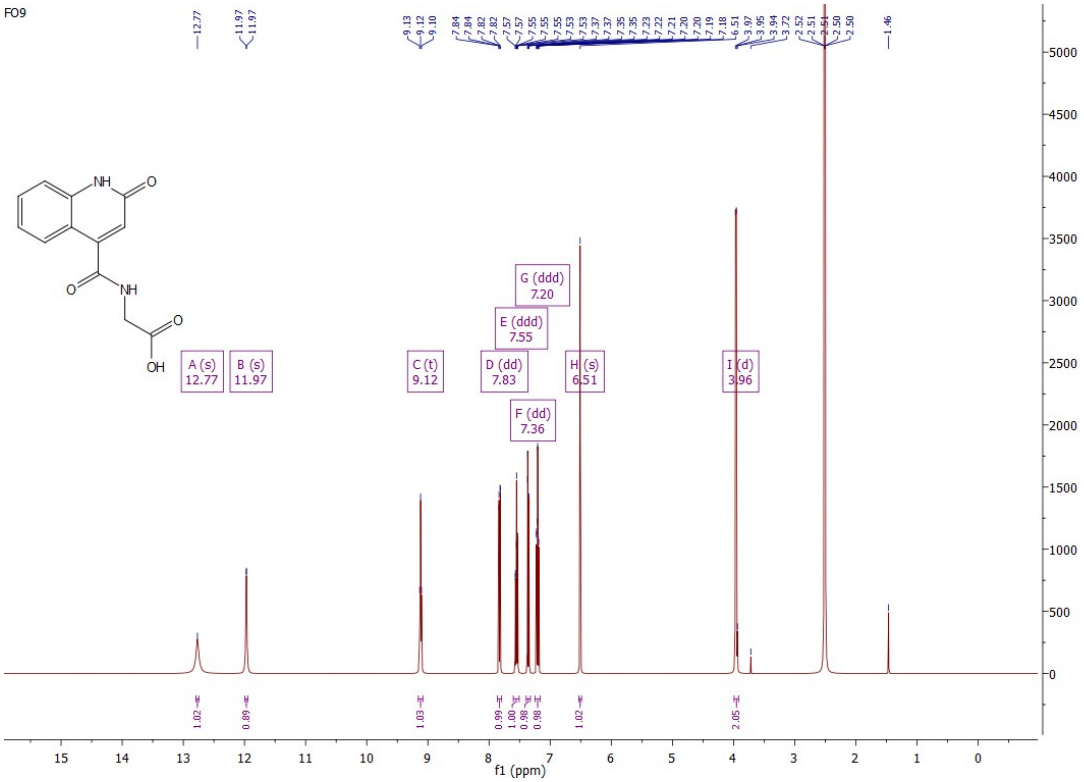
Fragment **F08**: ^{1}H NMR (400 MHz, DMSO-d_6)



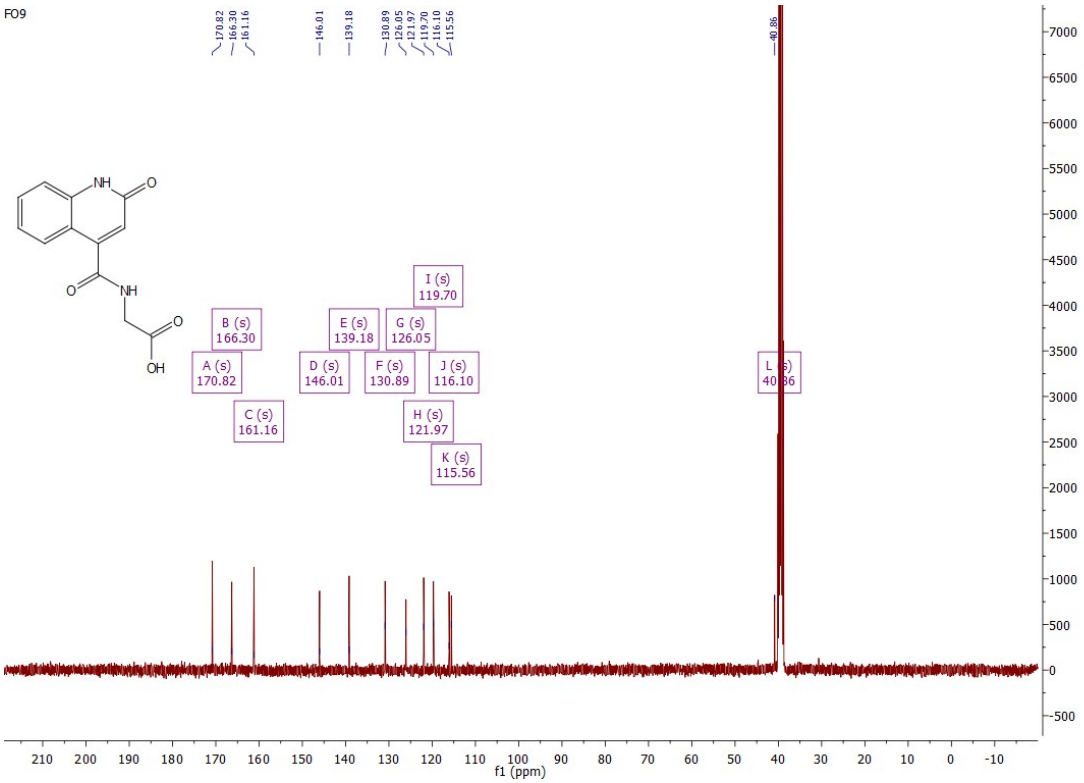
Fragment **F08**: ^{13}C NMR (101 MHz, DMSO-d_6)



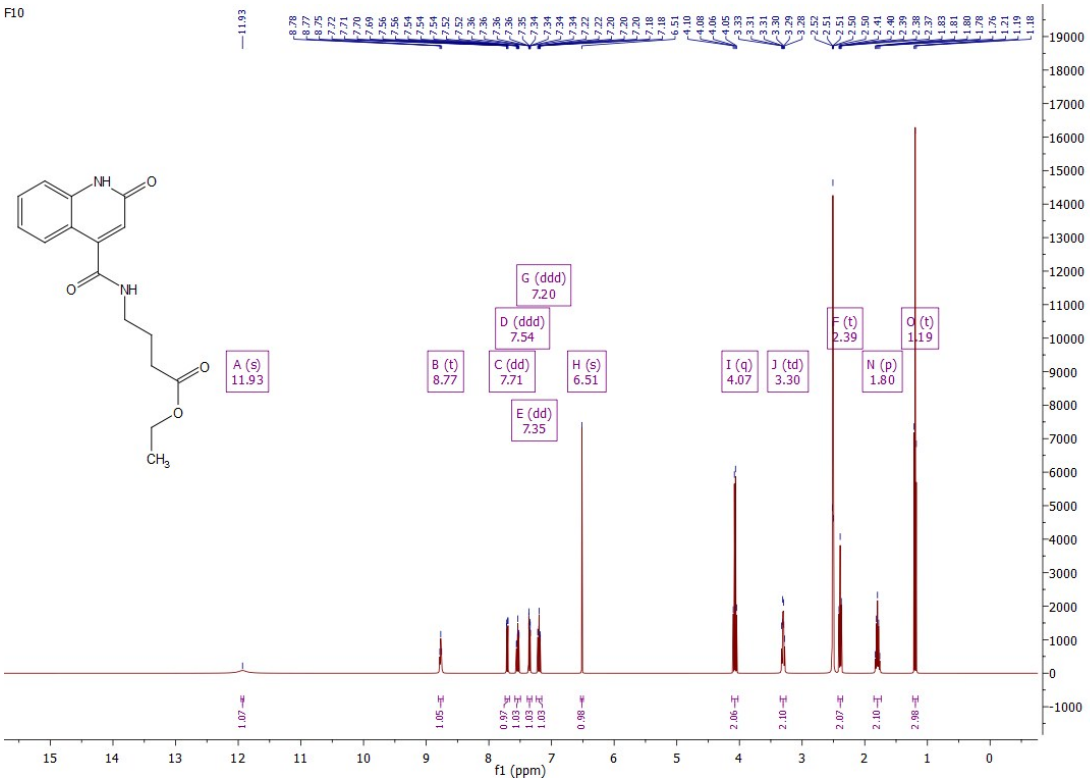
Fragment **F09**: ^{1}H NMR (400 MHz, DMSO-d_6)



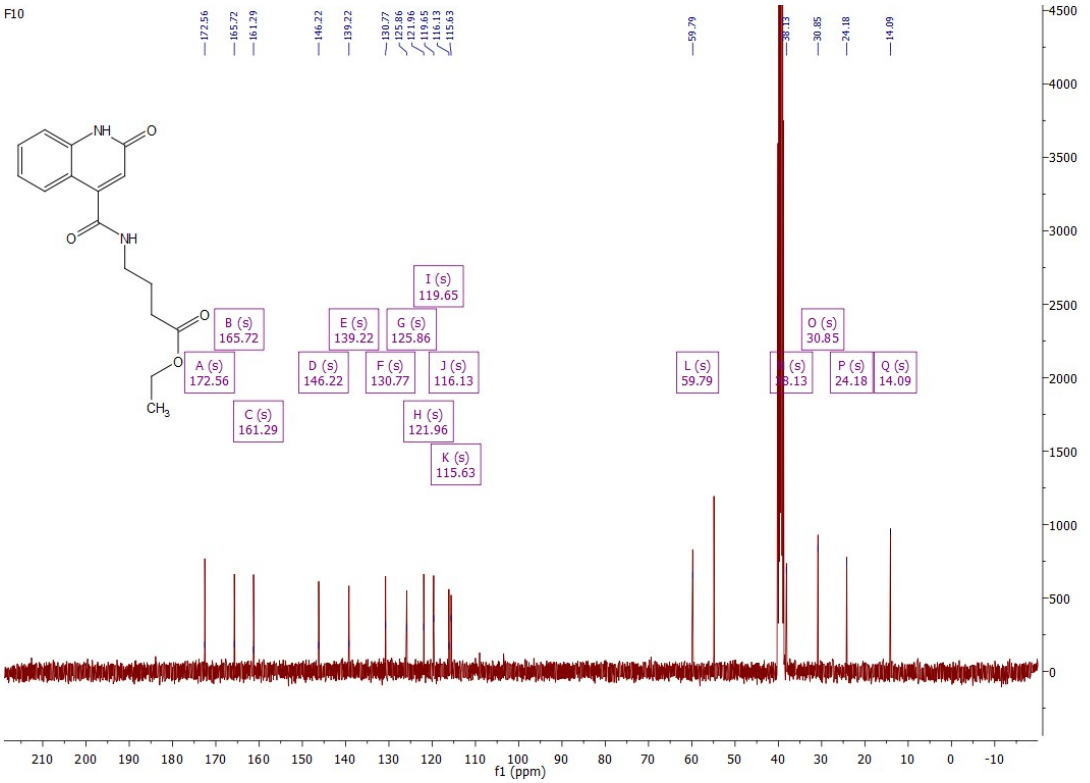
Fragment **F09**: ^{13}C NMR (101 MHz, DMSO-d_6)



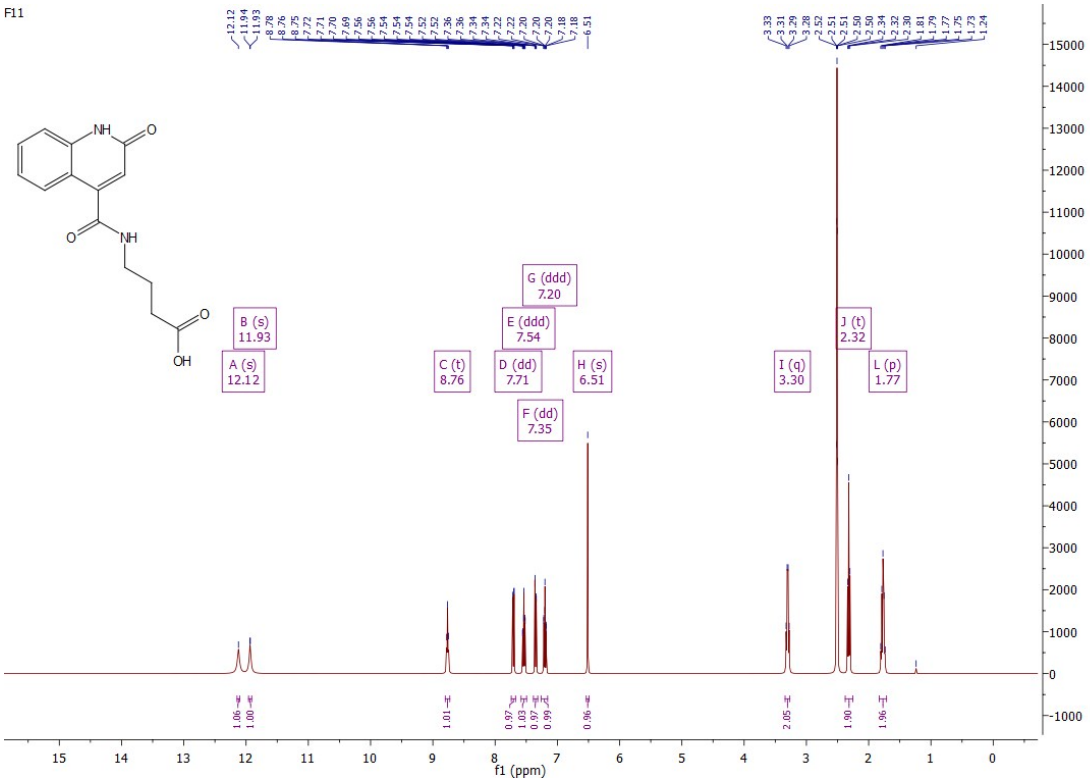
Fragment **F10**: ^{1}H NMR (400 MHz, DMSO-d_6)



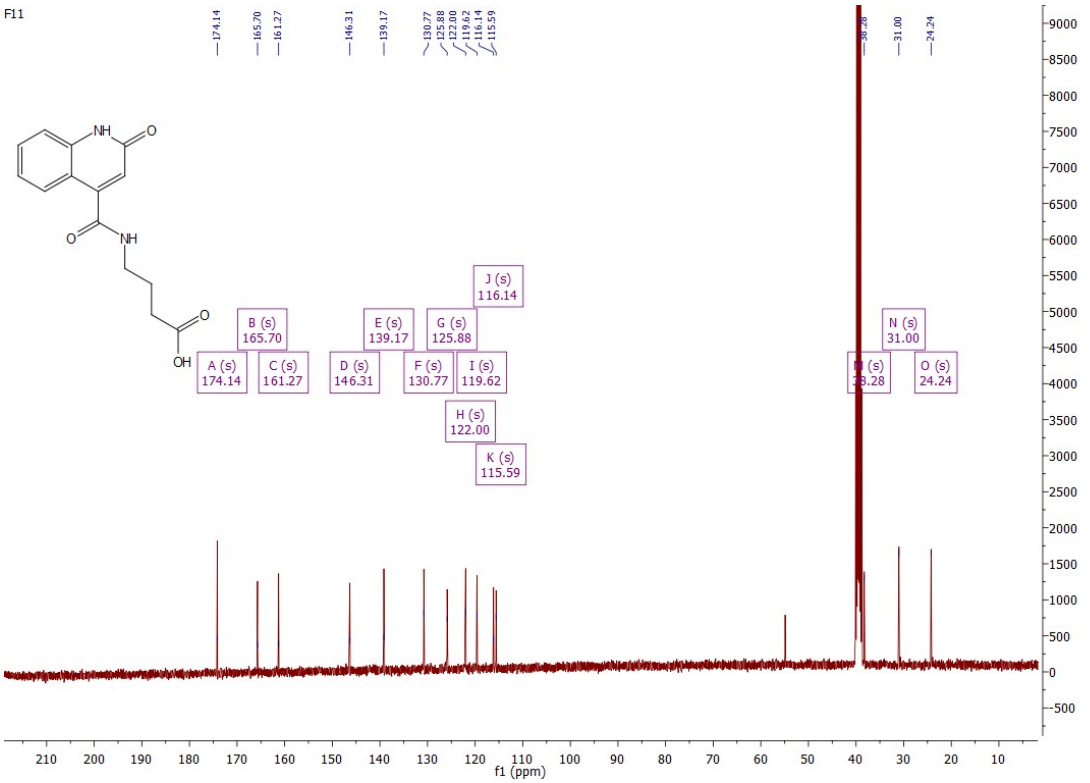
Fragment **F10**: ^{13}C NMR (101 MHz, DMSO-d_6)



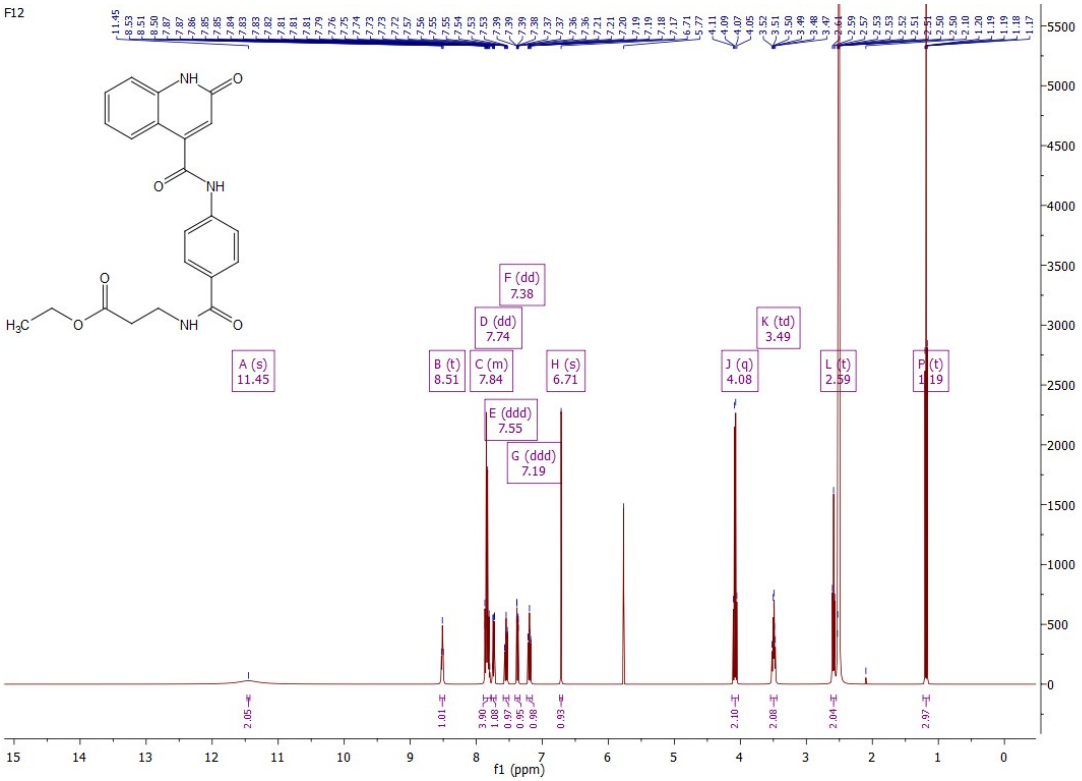
Fragment **F11**: ^{1}H NMR (400 MHz, DMSO-d_6)



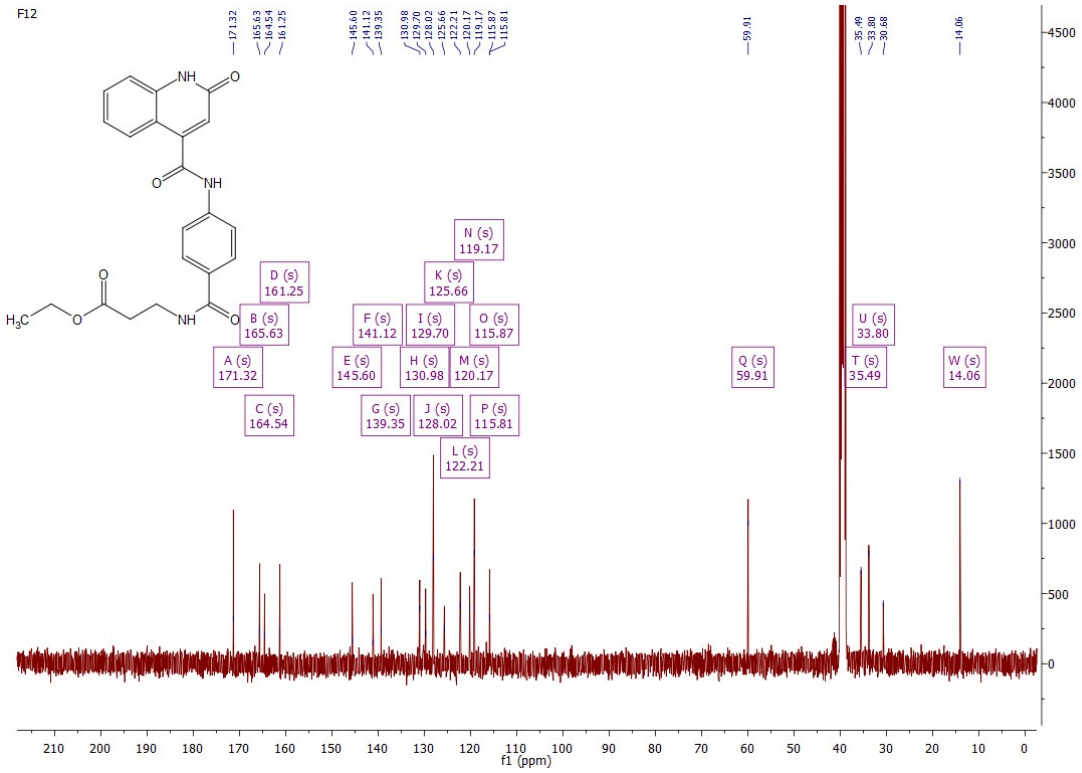
Fragment **F11**: ^{13}C NMR (101 MHz, DMSO-d_6)



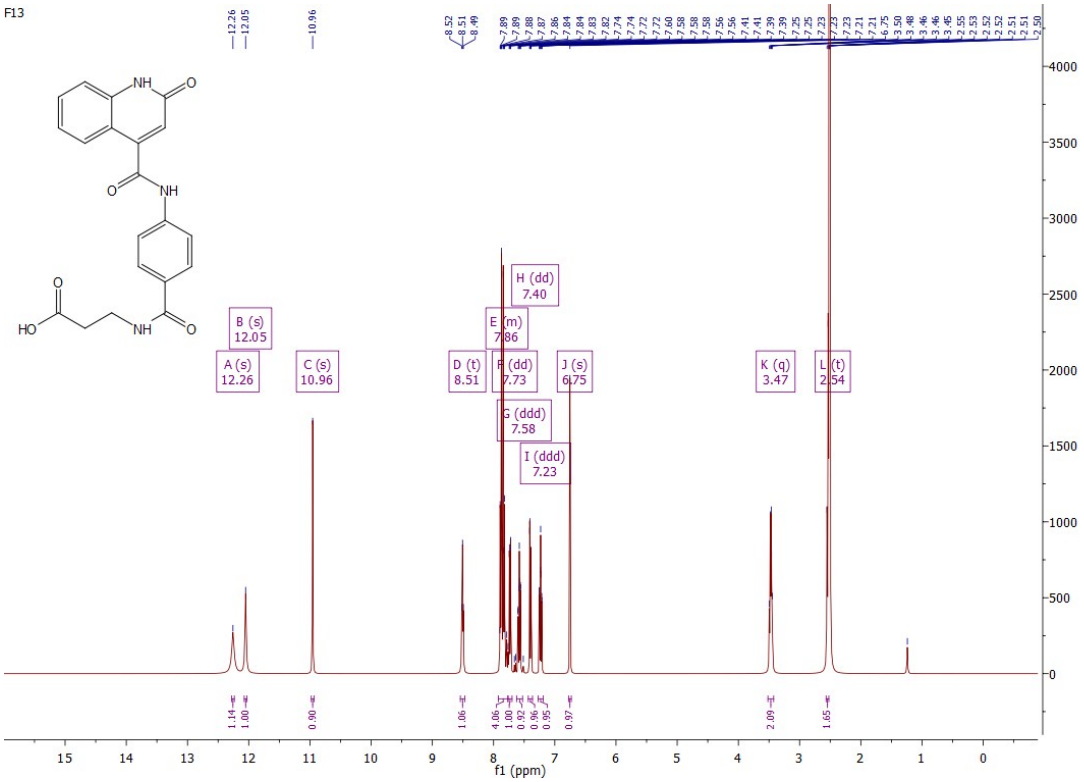
Fragment **F12**: ^{1}H NMR (400 MHz, DMSO-d_6)



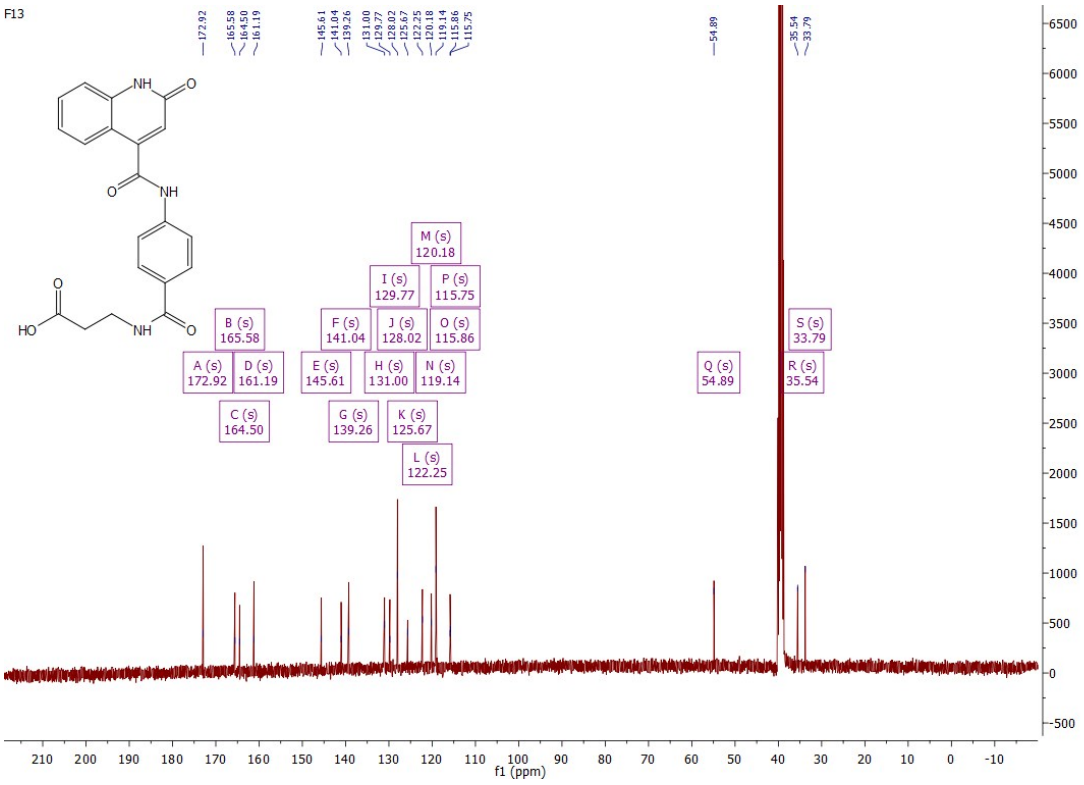
Fragment **F12**: ^{13}C NMR (101 MHz, DMSO-d_6)



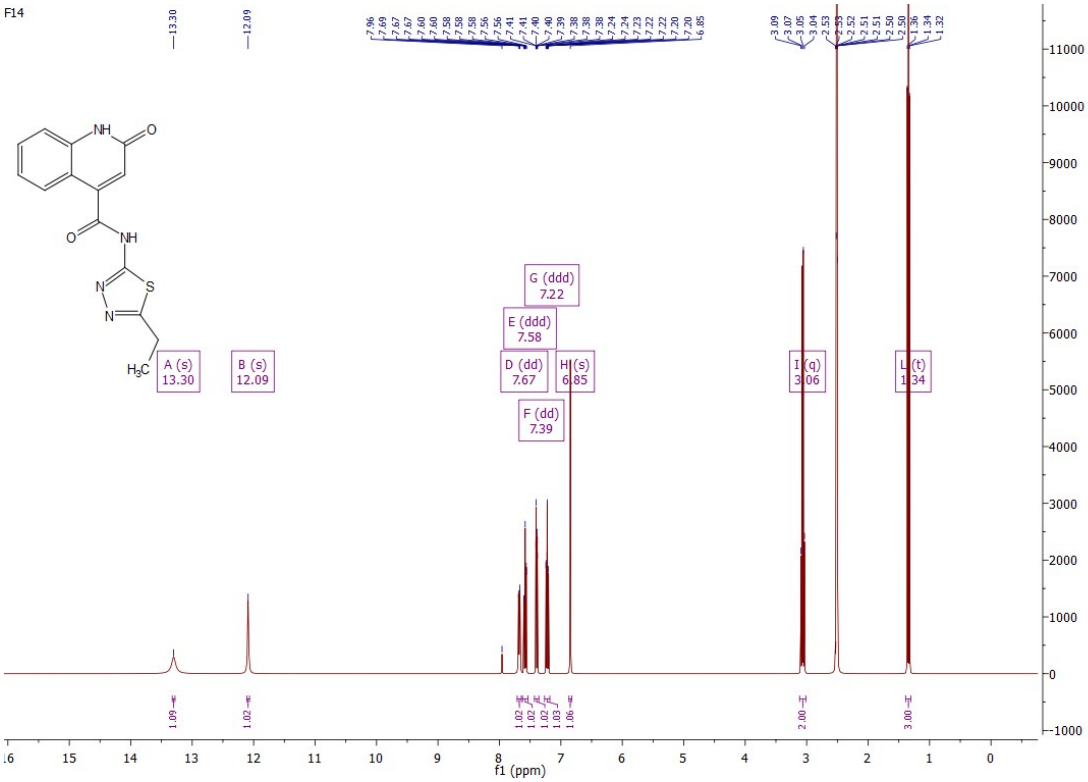
Fragment **F13**: ^{1}H NMR (400 MHz, DMSO-d_6)



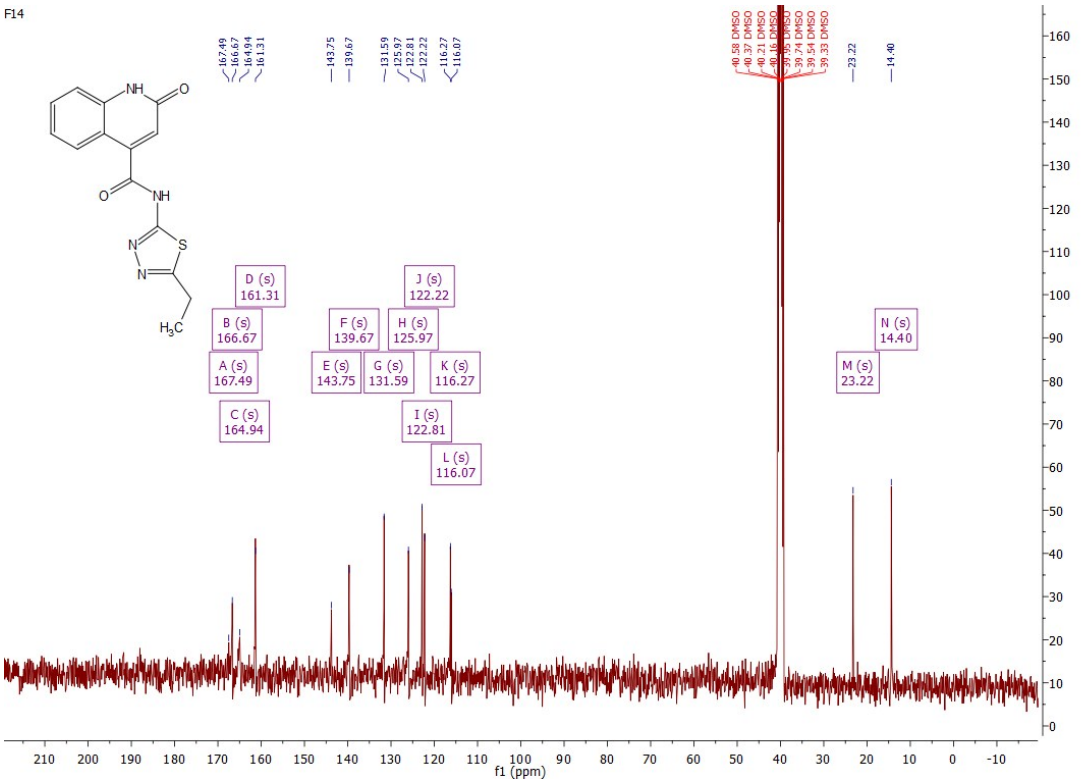
Fragment **F13**: ^{13}C NMR (101 MHz, DMSO-d_6)



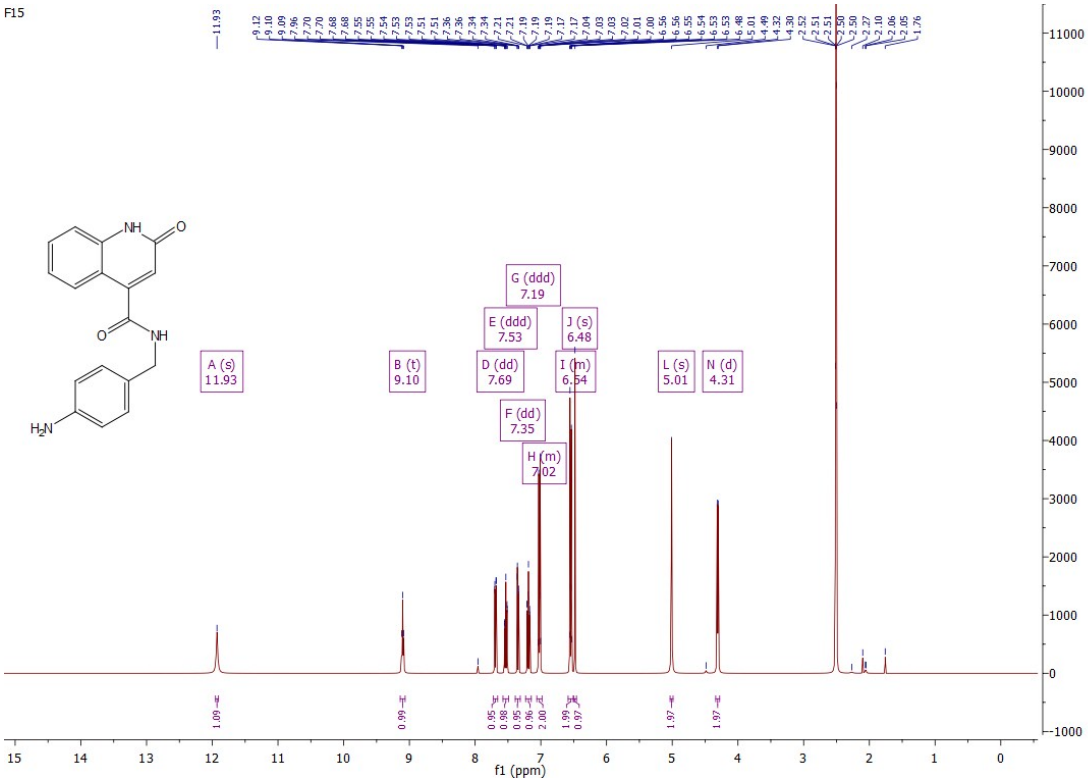
Fragment **F14**: ^{1}H NMR (400 MHz, DMSO-d_6)



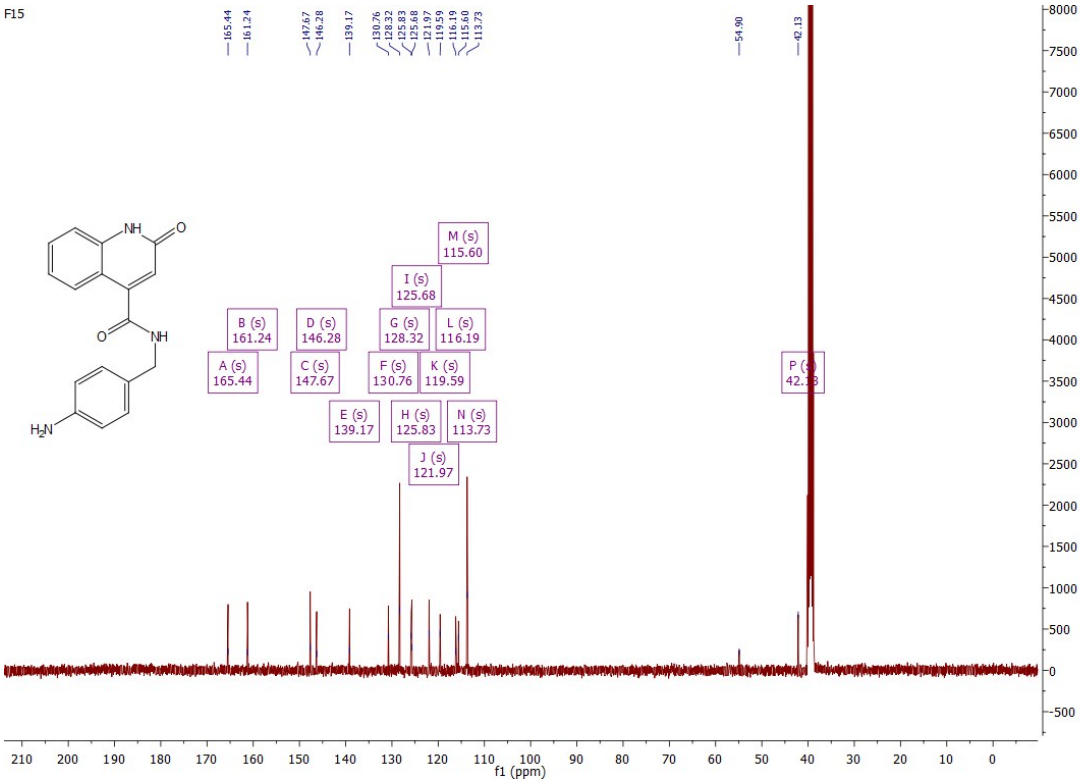
Fragment **F14**: ^{13}C NMR (101 MHz, DMSO-d_6)



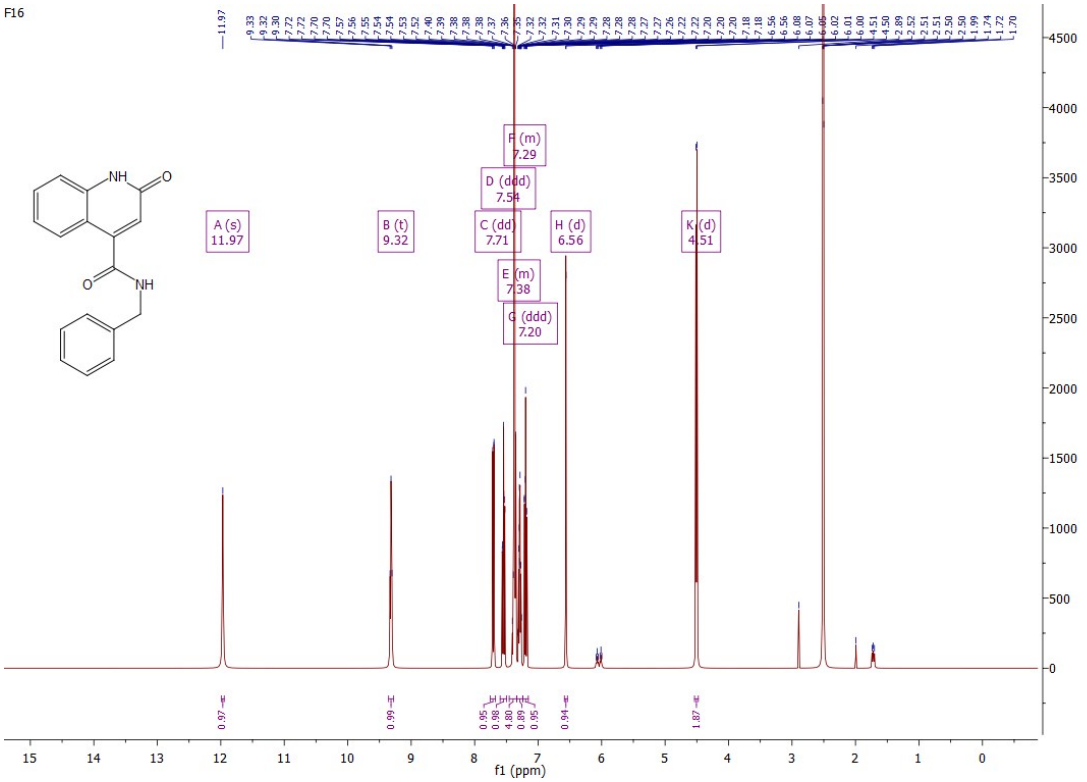
Fragment **F15**: ^{1}H NMR (400 MHz, DMSO-d_6)



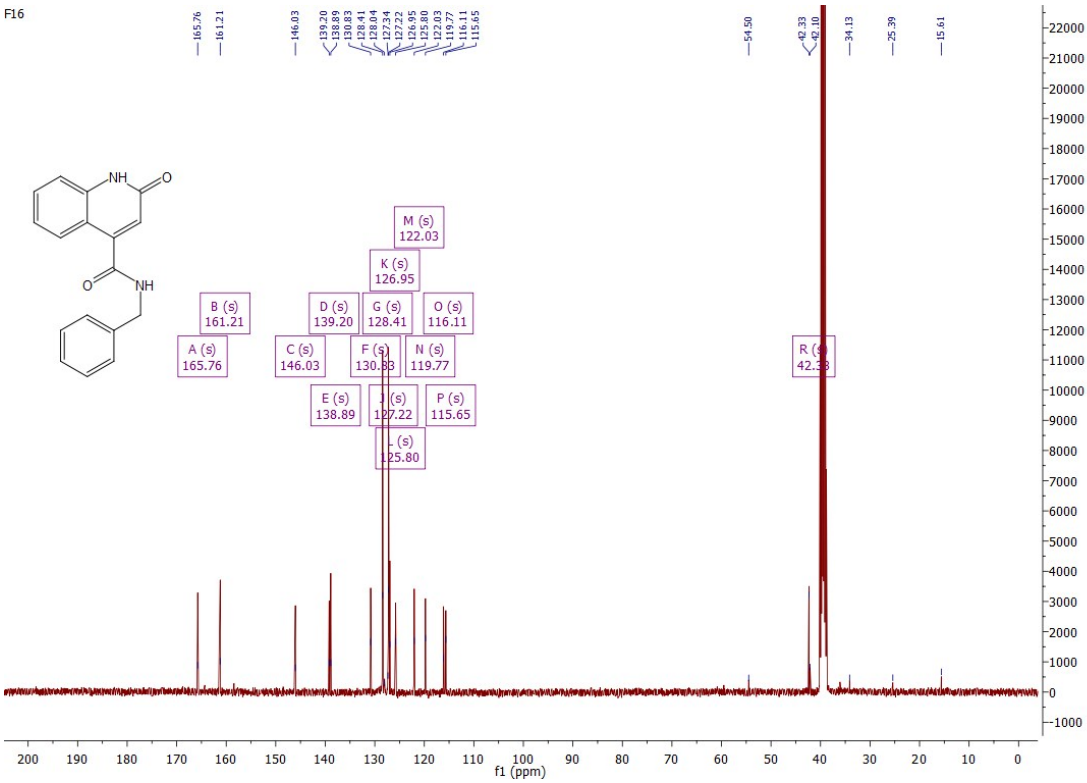
Fragment **F15**: ^{13}C NMR (101 MHz, DMSO-d_6)



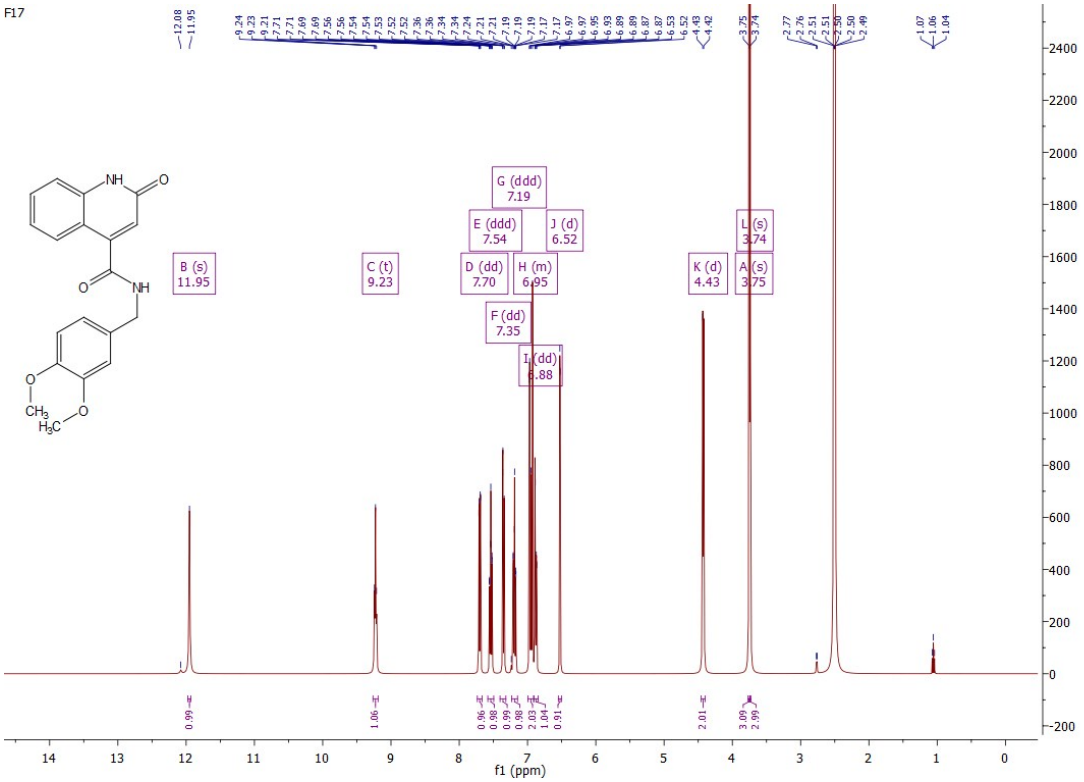
Fragment **F16**: ^{1}H NMR (400 MHz, DMSO-d_6)



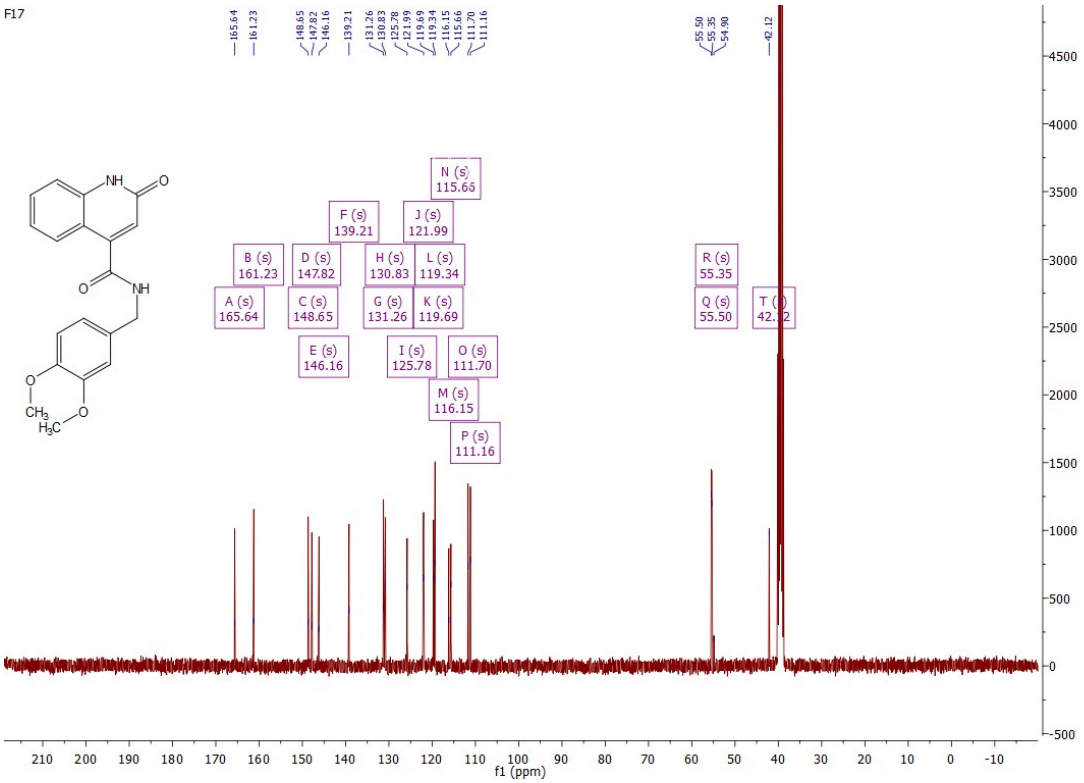
Fragment **F16**: ^{13}C NMR (101 MHz, DMSO-d_6)



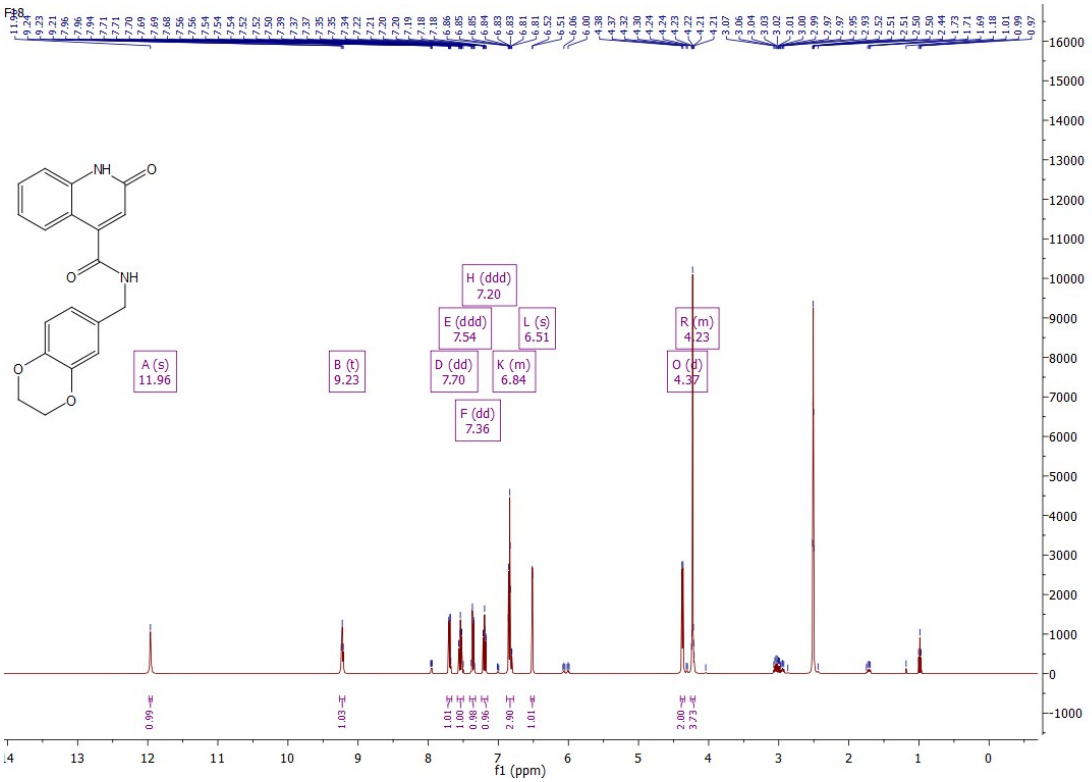
Fragment **F17**: ^{1}H NMR (400 MHz, DMSO-d_6)



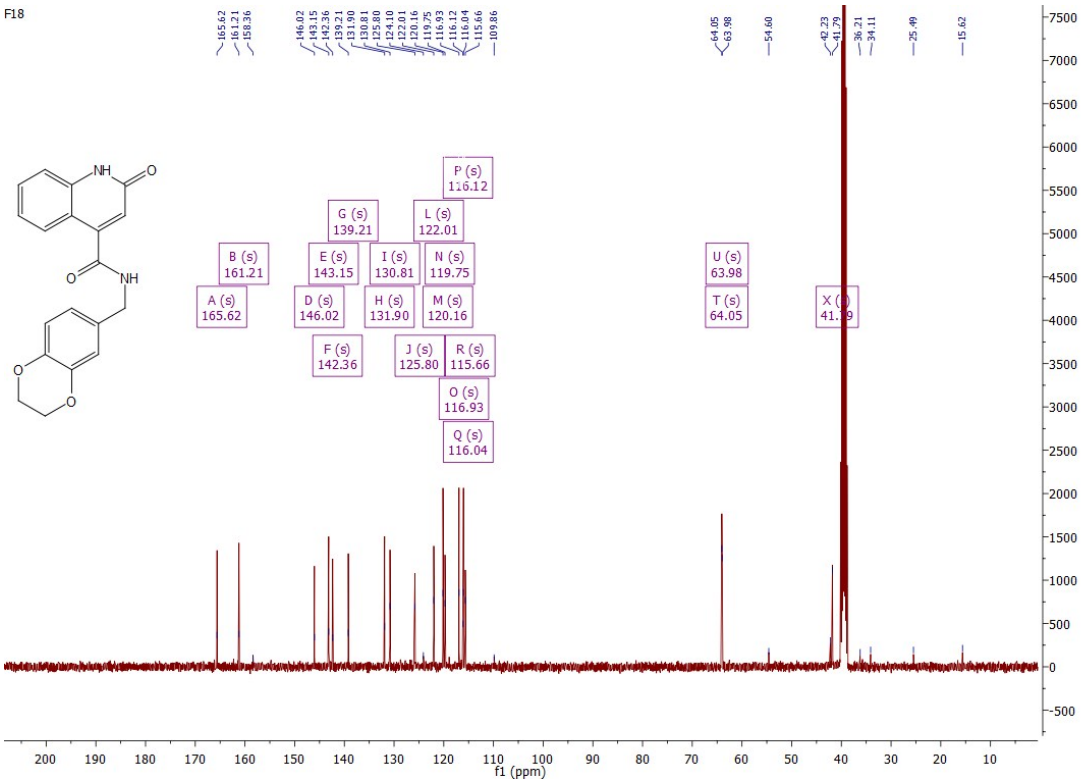
Fragment **F17**: ^{13}C NMR (101 MHz, DMSO-d_6)



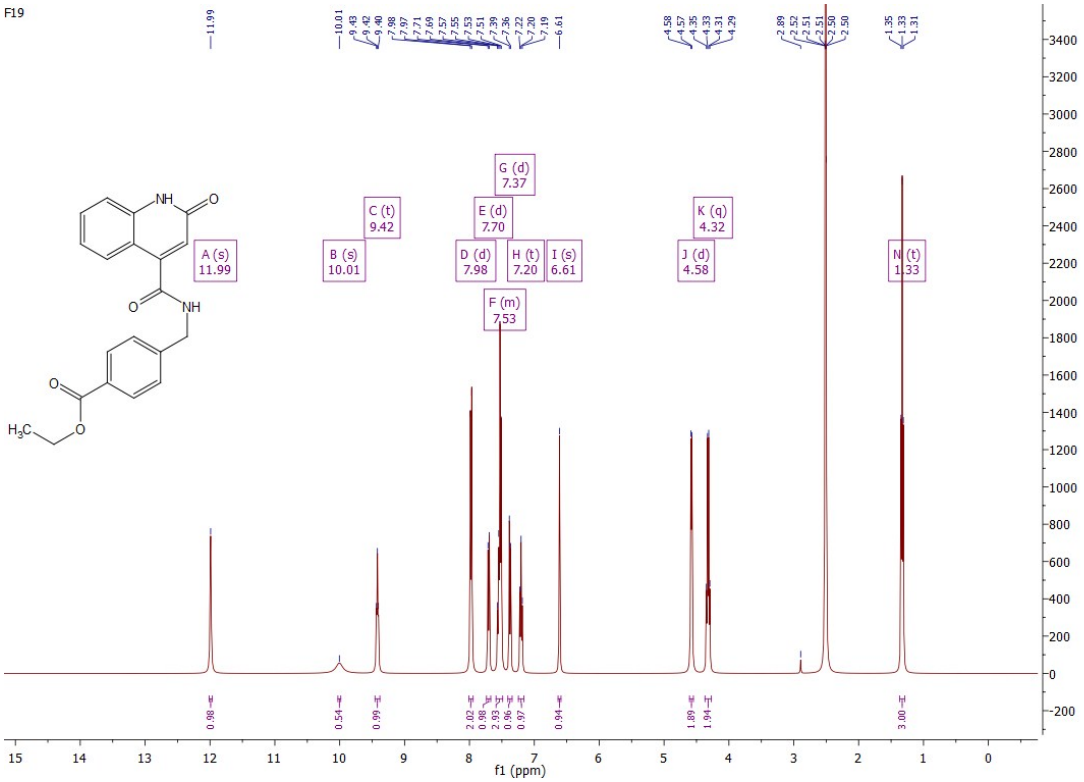
Fragment **F18**: ^{1}H NMR (400 MHz, DMSO-d_6)



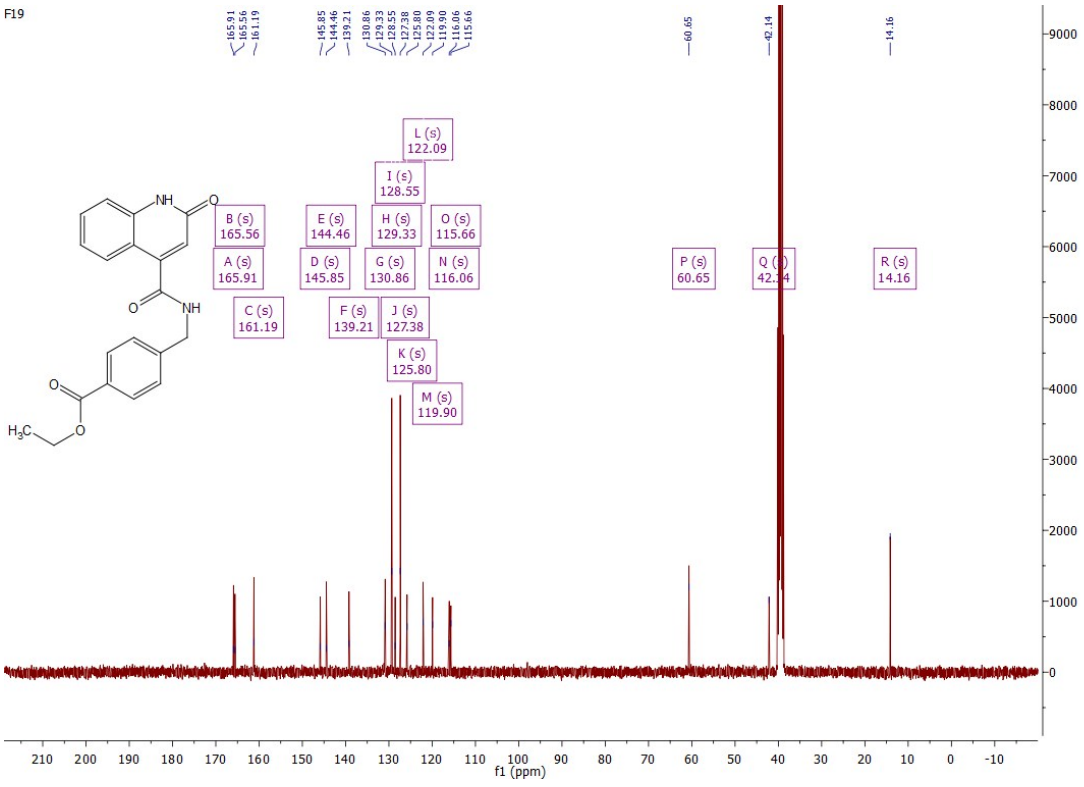
Fragment **F18**: ^{13}C NMR (101 MHz, DMSO-d_6)



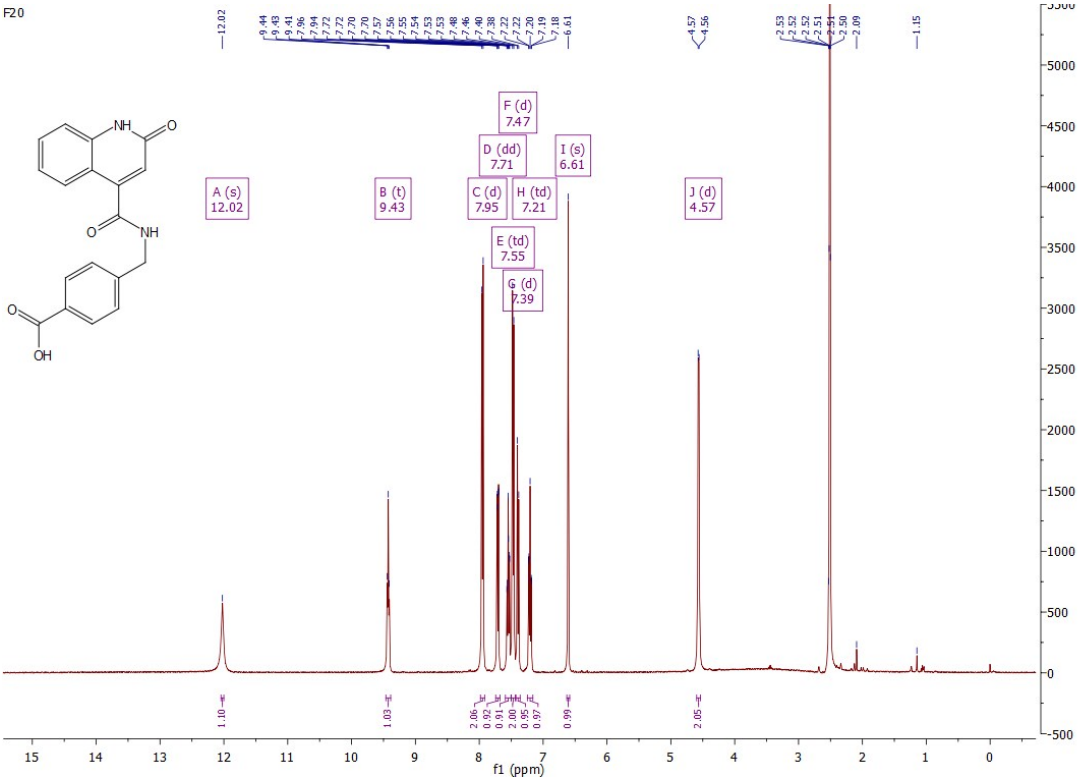
Fragment **F19**: ^{1}H NMR (400 MHz, DMSO-d_6)



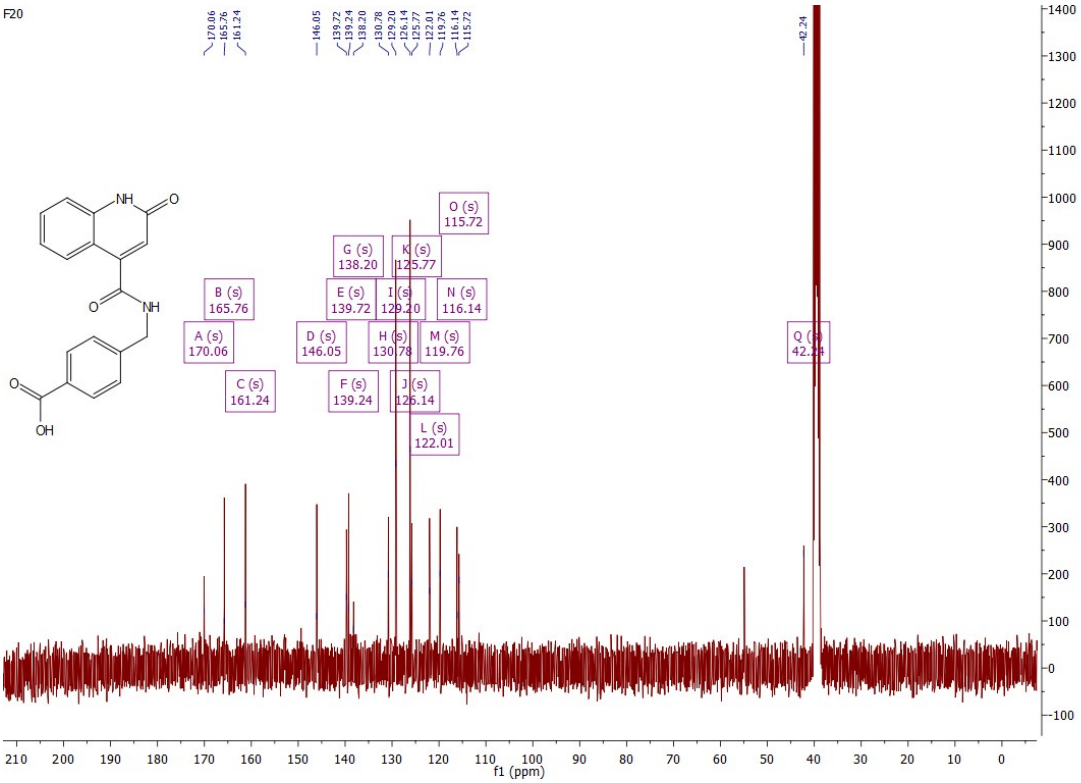
Fragment **F19**: ^{13}C NMR (101 MHz, DMSO-d_6)



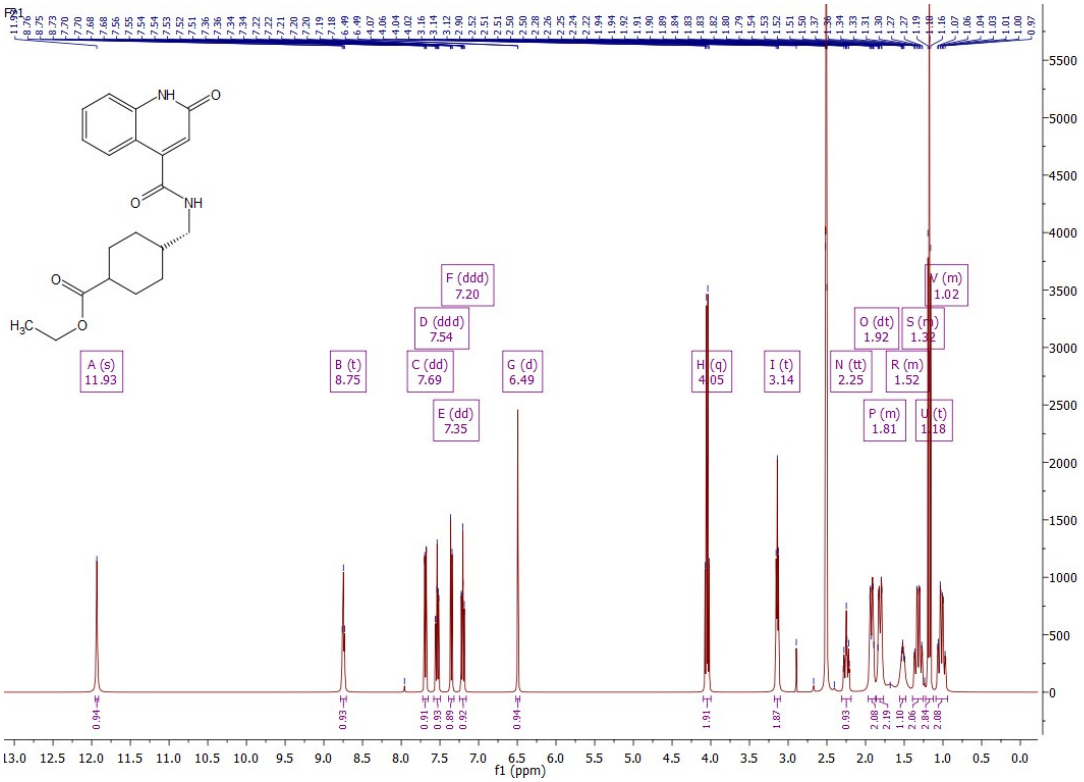
Fragment **F20**: ^{1}H NMR (400 MHz, DMSO-d_6)



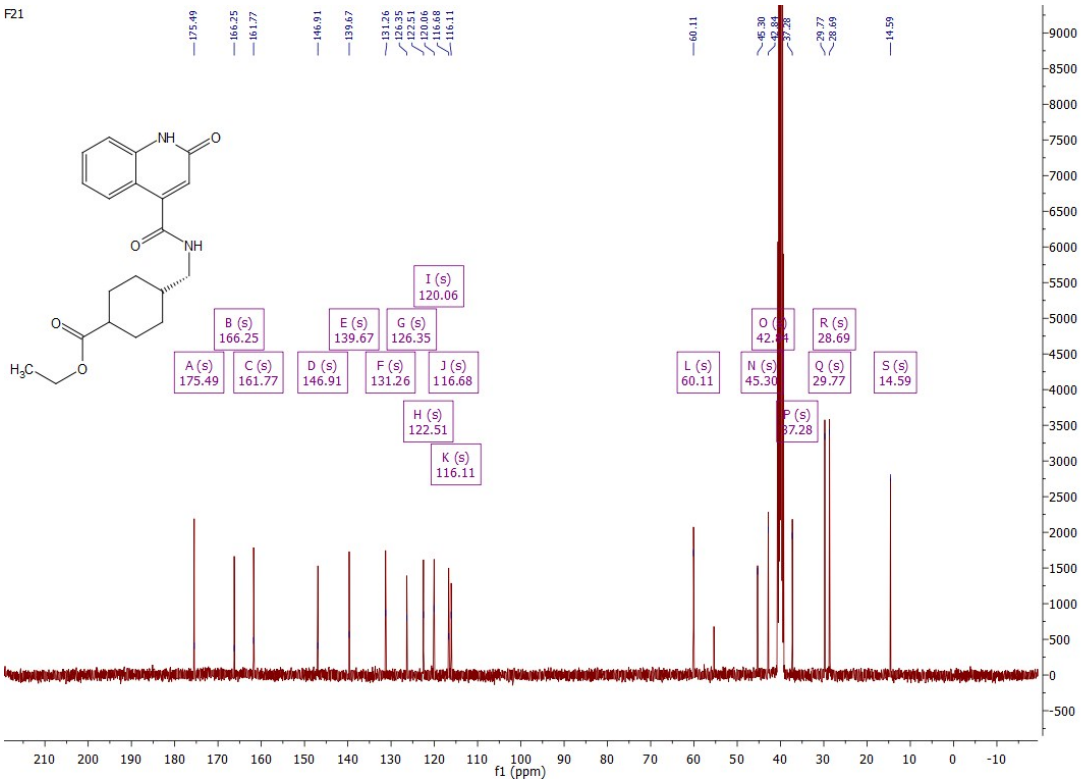
Fragment **F20**: ^{13}C NMR (101 MHz, DMSO-d_6)



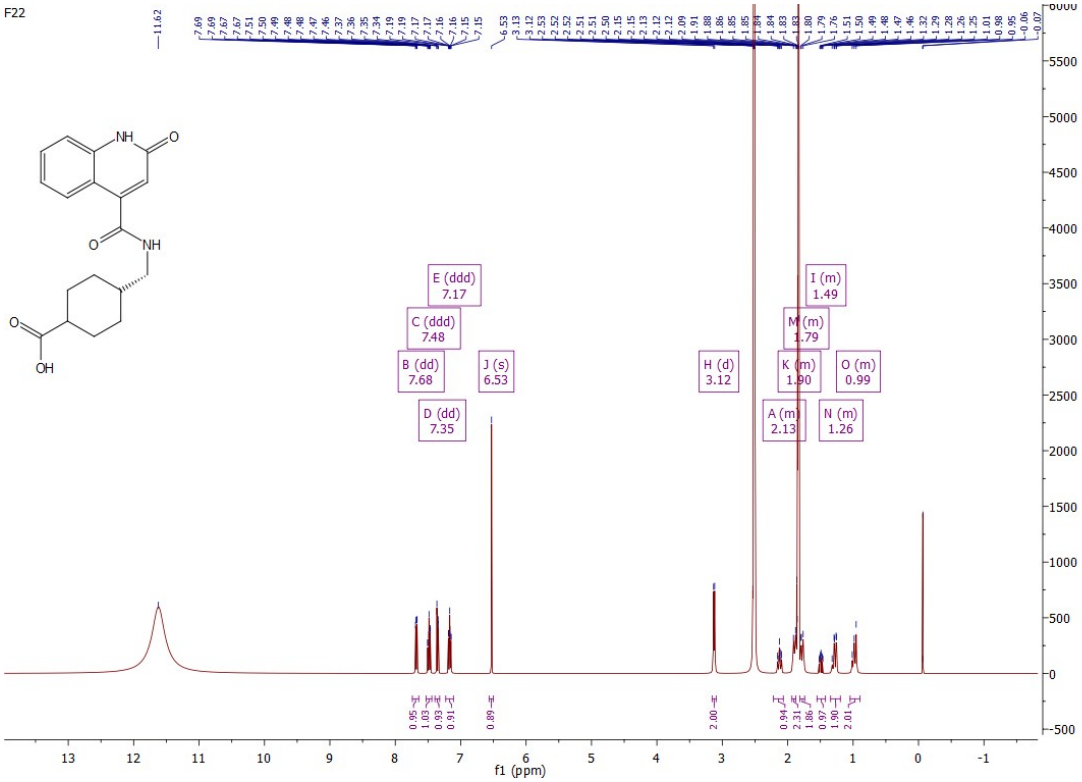
Fragment **F21**: ^{1}H NMR (400 MHz, DMSO-d_6)



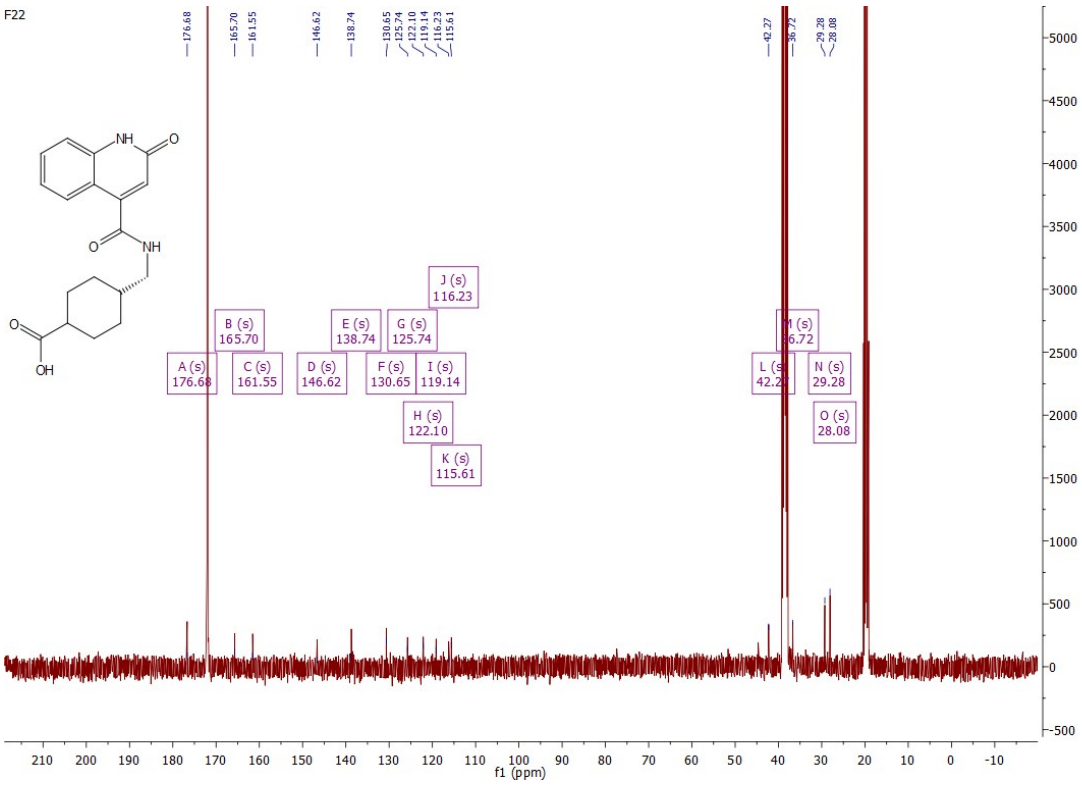
Fragment **F21**: ^{13}C NMR (101 MHz, DMSO-d_6)



Fragment **F22**: ^{1}H NMR (400 MHz, DMSO-d_6 + 1 % CD_3COOD)



Fragment **F22**: ^{13}C NMR (101 MHz, DMSO-d_6 + 1 % CD_3COOD)



Analytical HRMS and HPLC traces for Pep6, Pep13 and compounds 1-6.

Pep6: HRMS (EI, m/z): calculated for $C_{32}H_{57}N_9O_{10}SH^+$ ([M+H]$^+$): 760.4022, found: 760.4026.

Pe13: HRMS (EI, m/z): calculated for $C_{30}H_{54}N_{12}O_{10}H^+$ ([M+H]$^+$): 743.4164, found: 743.4144.

1: HRMS (EI, m/z): calculated for $C_{43}H_{70}N_{12}O_{16}SH^+$ ([M+H]$^+$): 1043.4826, found: 1043.4816.

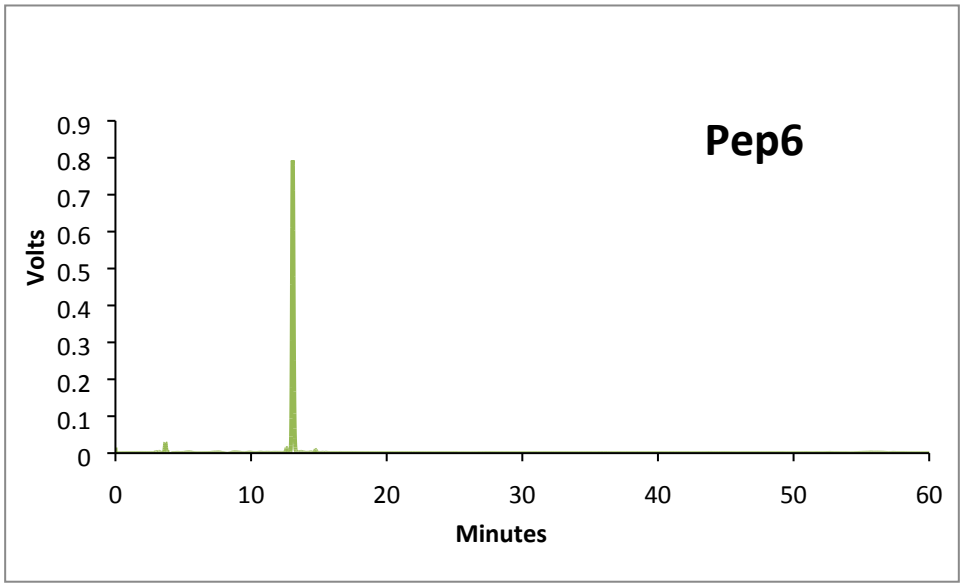
2: HRMS (EI, m/z): calculated for $C_{45}H_{74}N_{12}O_{17}SH^+$ ([M+H]$^+$): 1087.5088, found: 1087.5096.

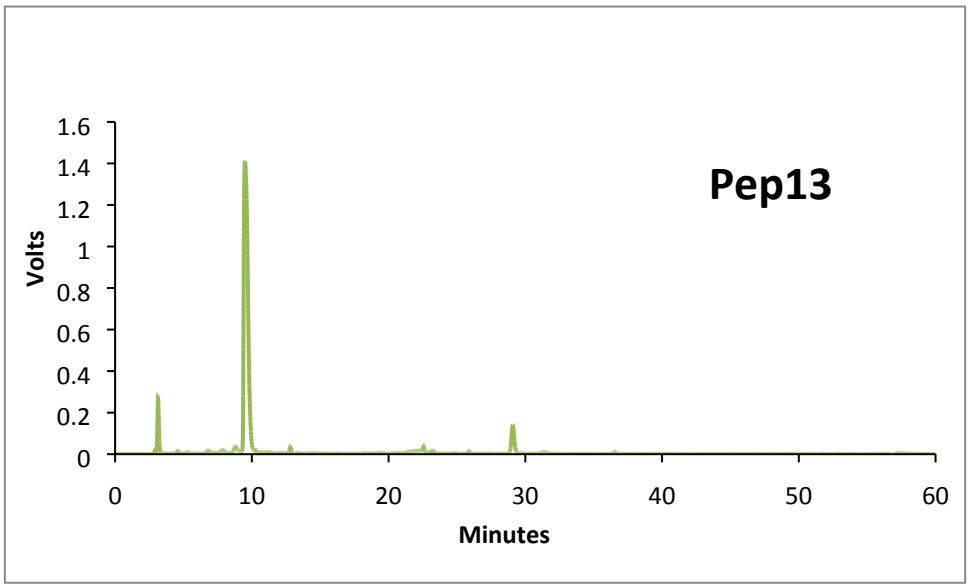
3: HRMS (EI, m/z): calculated for $C_{47}H_{78}N_{12}O_{18}SH^+$ ([M+H]$^+$): 1131.5351, found: 1131.5306.

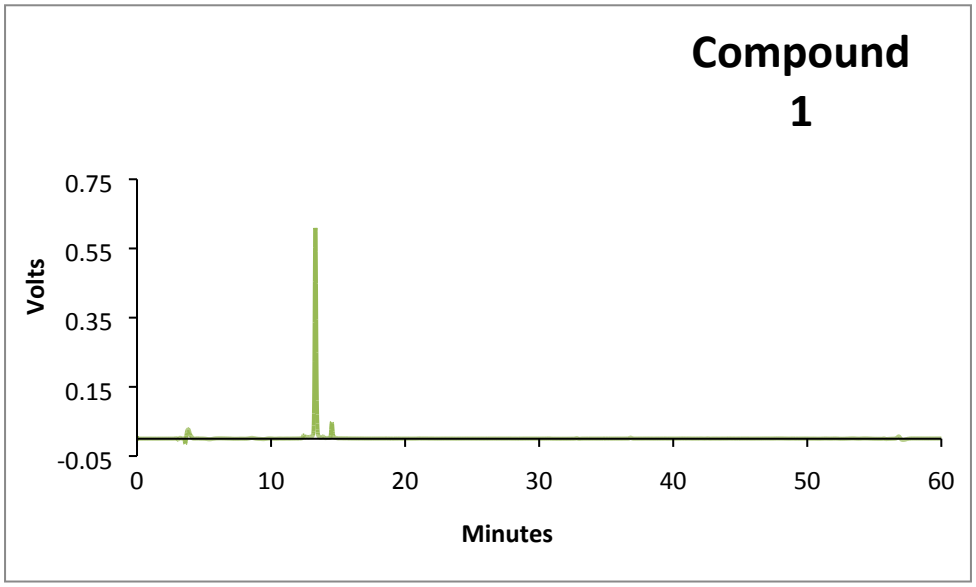
4: HRMS (EI, m/z): calculated for $C_{41}H_{67}N_{15}O_{16}H^+$ ([M+H]$^+$): 1026.4963, found: 1026.4960.

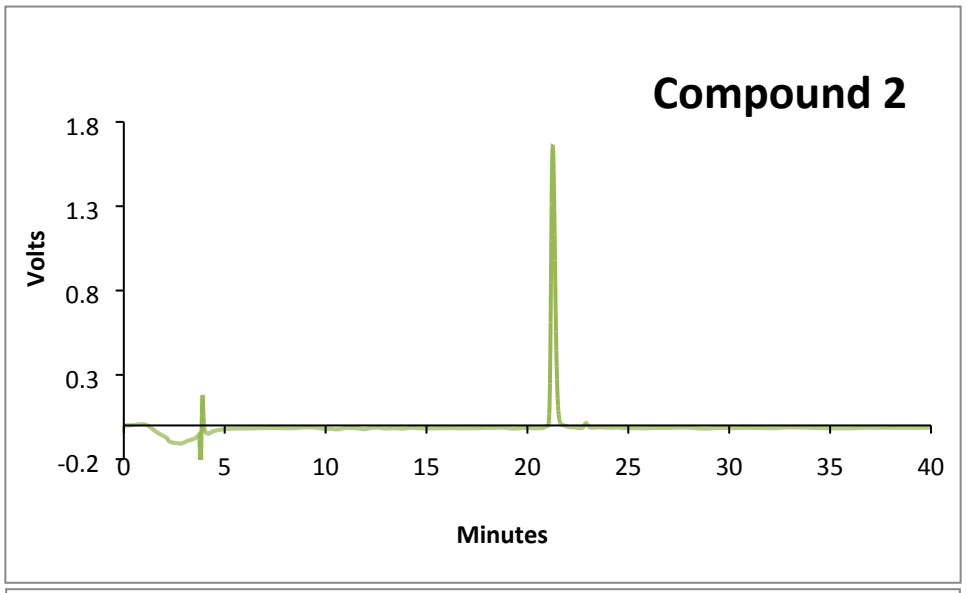
5: HRMS (EI, m/z): calculated for $C_{43}H_{71}N_{15}O_{17}H^+$ ([M+2H]$^{2+}$/2): 535.7649, found: 535.7656.

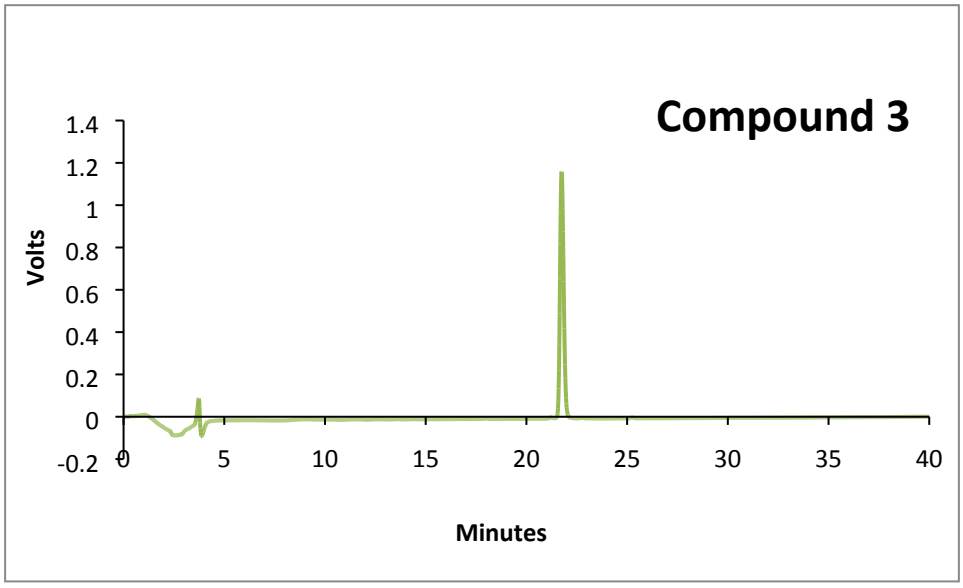
6: HRMS (EI, m/z): calculated for $C_{53}H_{76}N_{12}O_{13}SH^+$ ([M+H]$^+$): 1121.5454, found: 1121.5455.

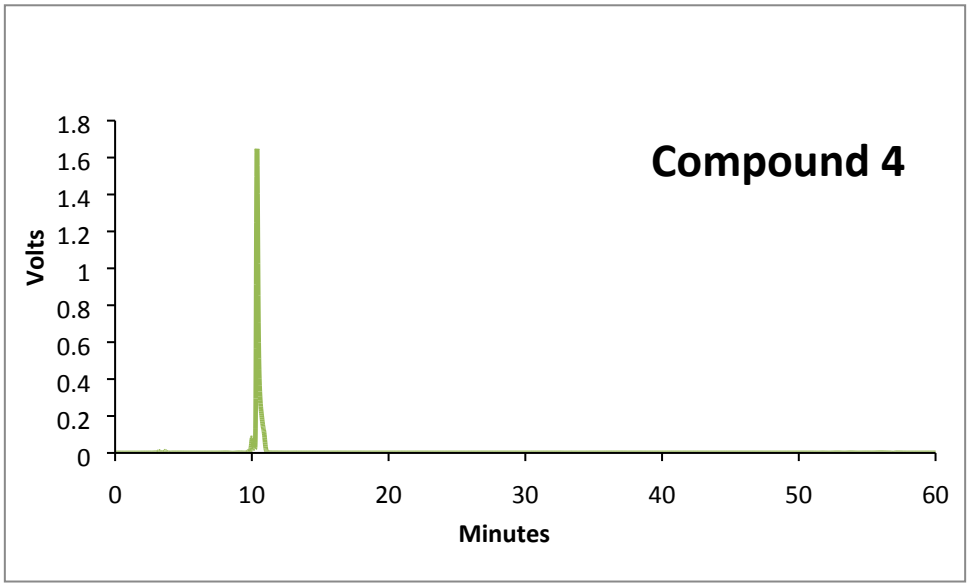


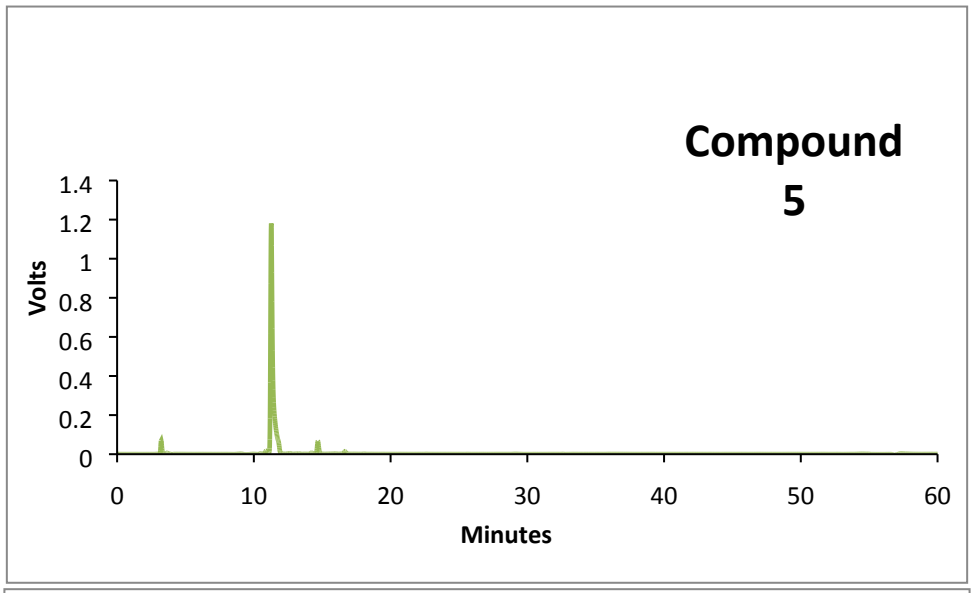


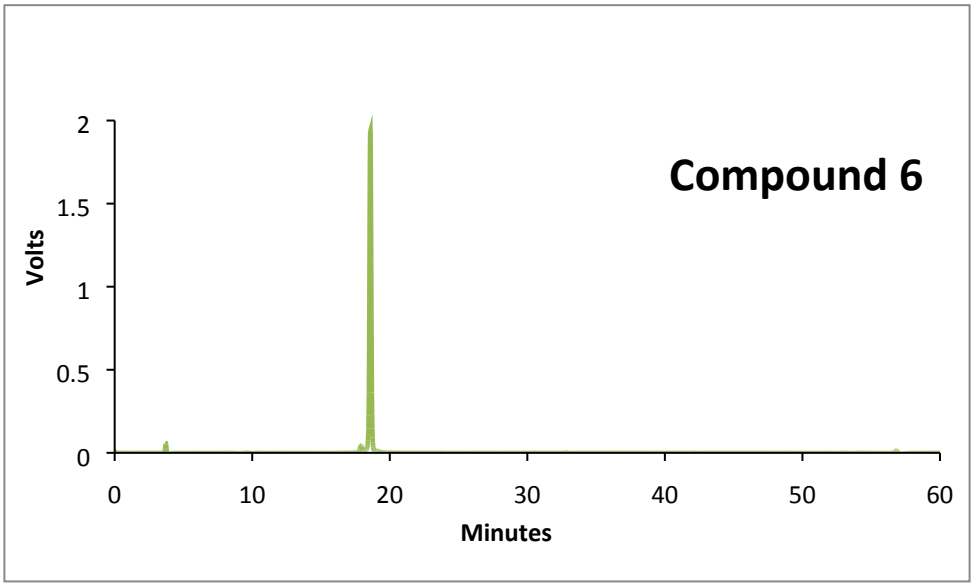

Compound 1
Volts
0.75
0.55
0.35
0.15
-0.05
0
10
20
30
40
50
60
Minutes


Compound 2
Volts
1.8
1.3
0.8
0.3
-0.2
0
5
10
15
20
25
30
35
40
Minutes

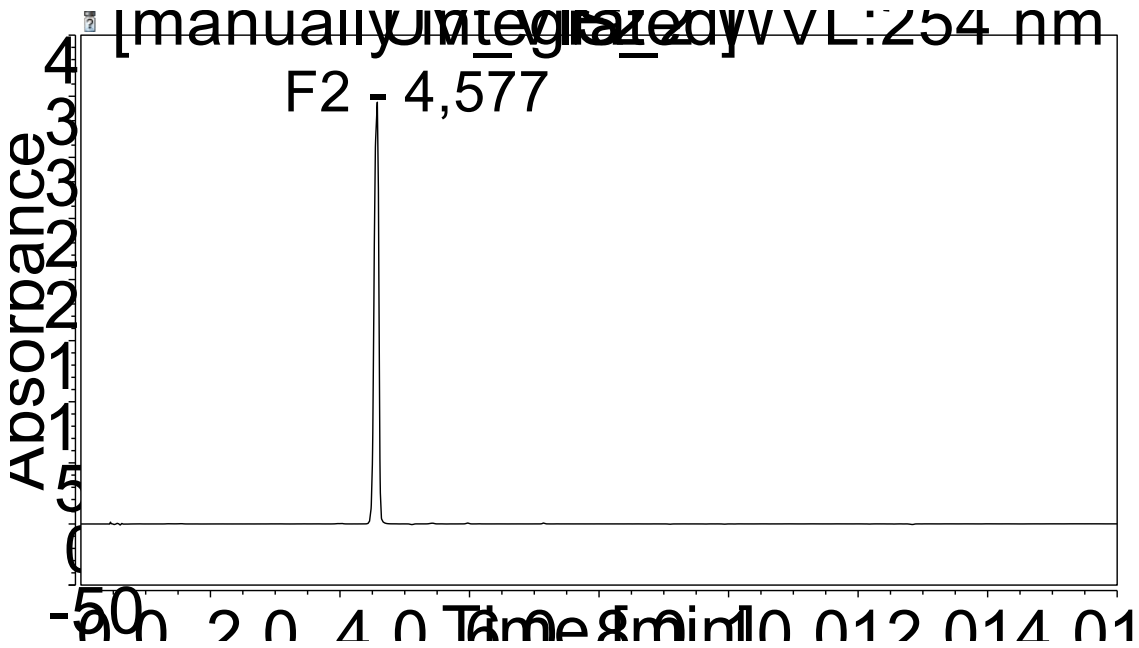

Compound 3
Volts
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
5
10
15
20
25
30
35
40
Minutes

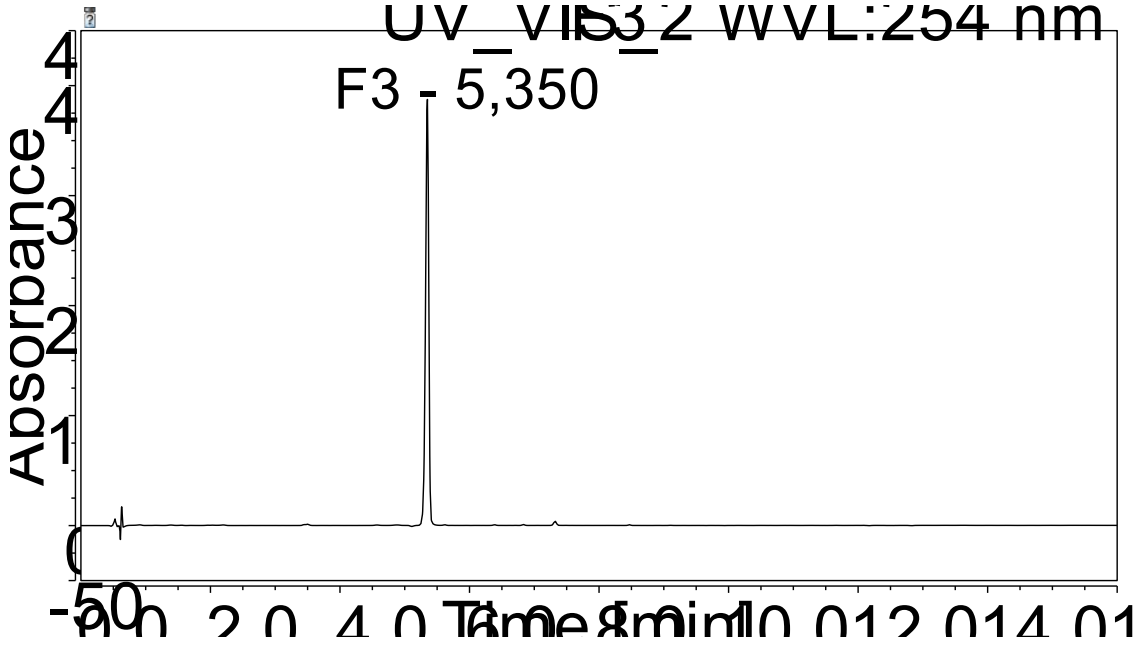

Compound 4
Volts
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Minutes

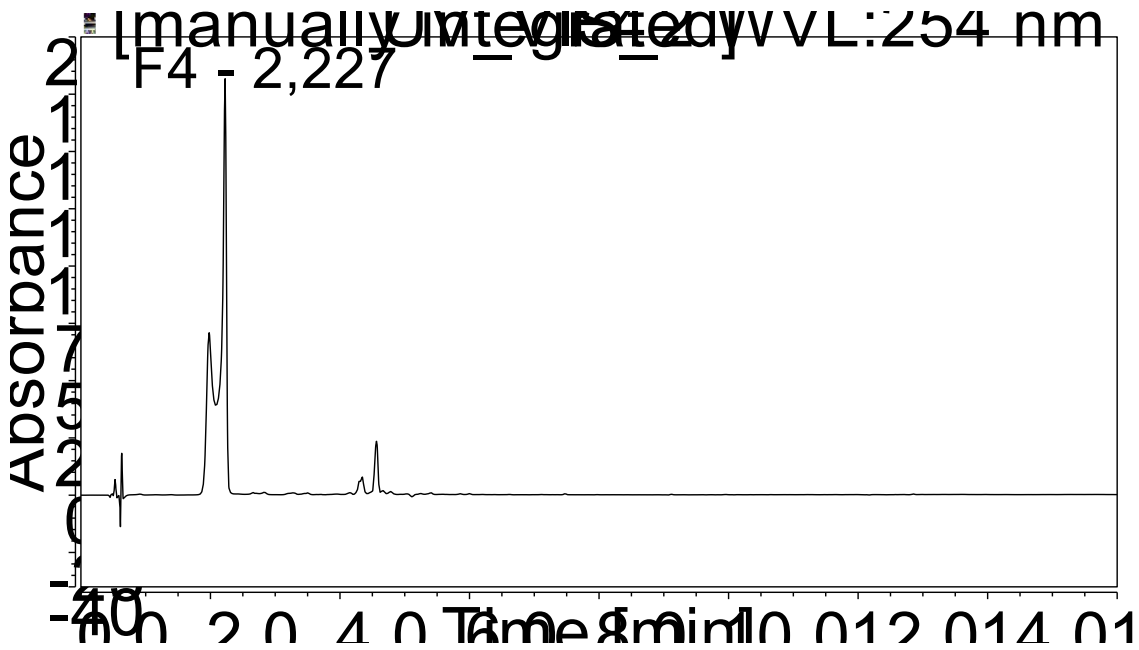

Compound 5
Volts
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Minutes

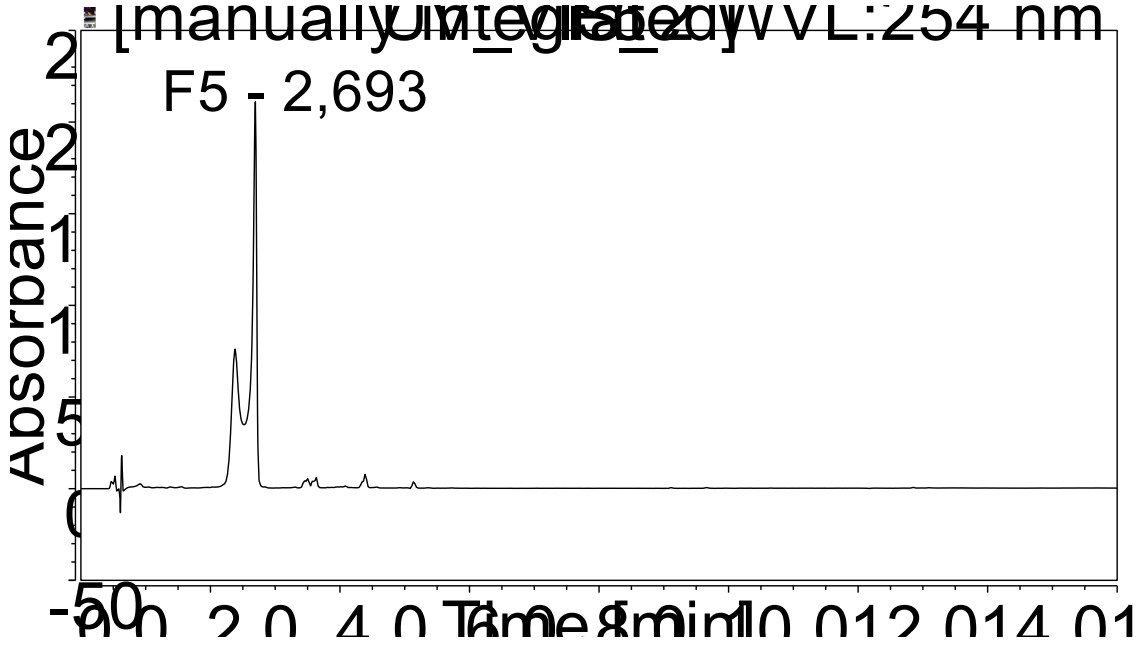

Compound 6
Volts
2
1.5
1
0.5
0
0
10
20
30
40
50
60
Minutes

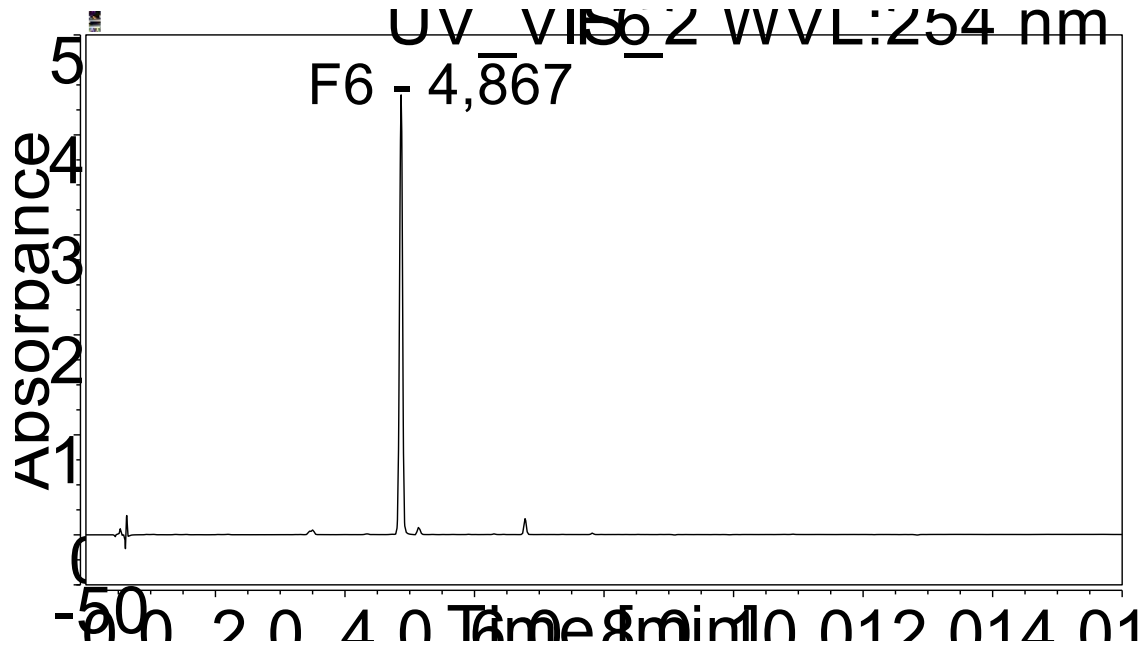
HPLC analysis of Fragments 2-22

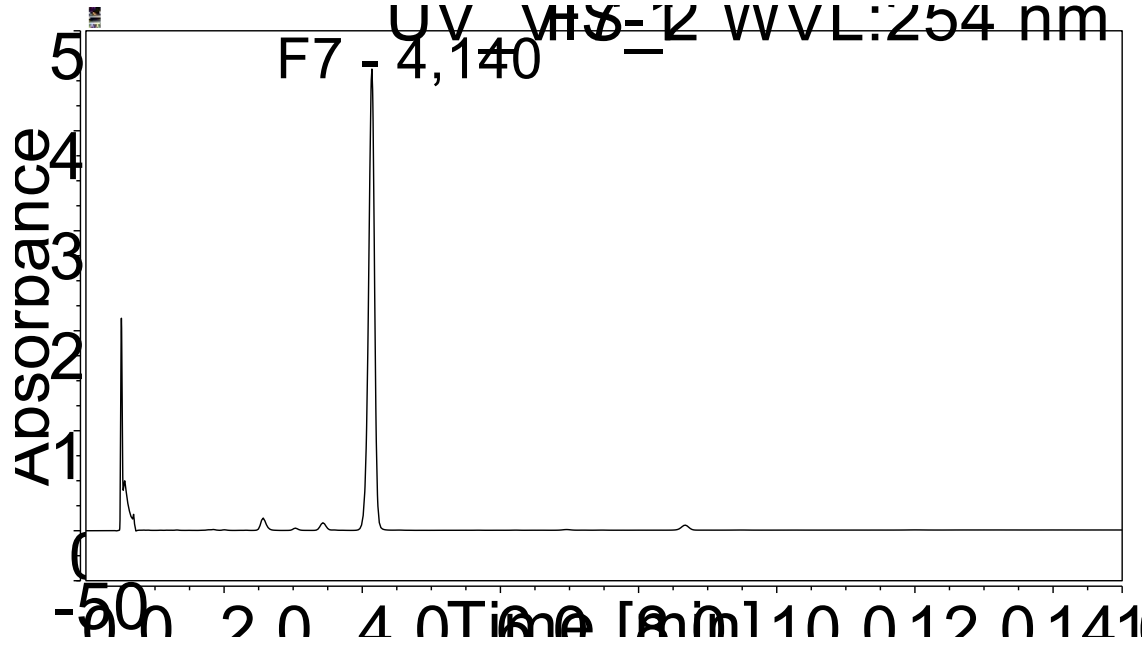


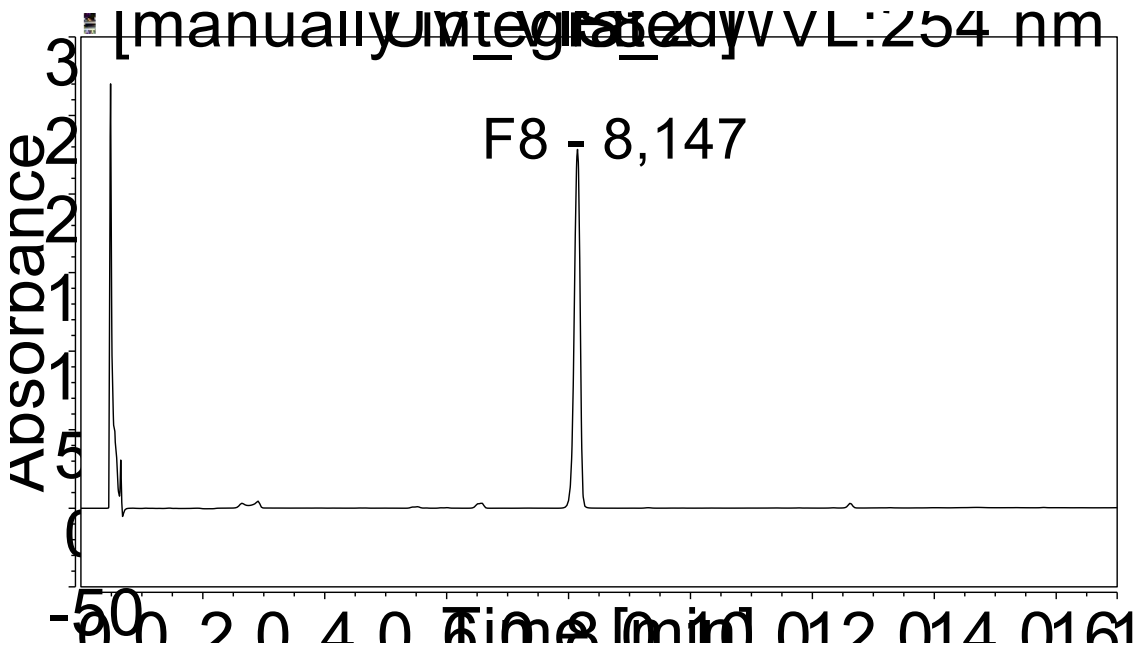


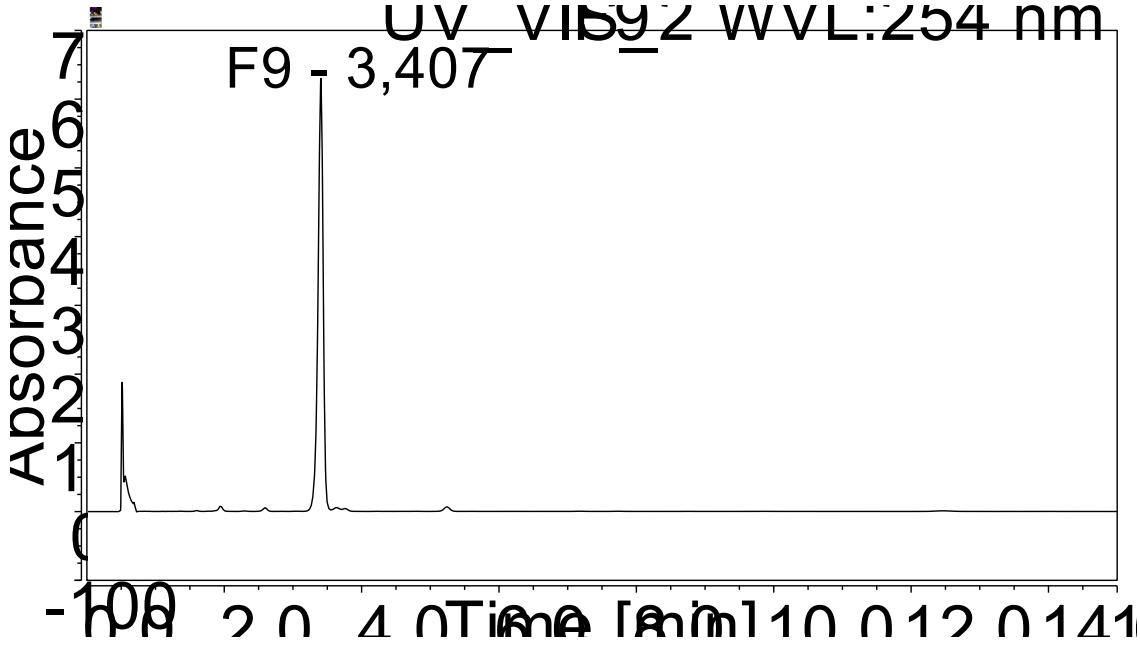

F4 - 2,227
Absorbance
Time [min]

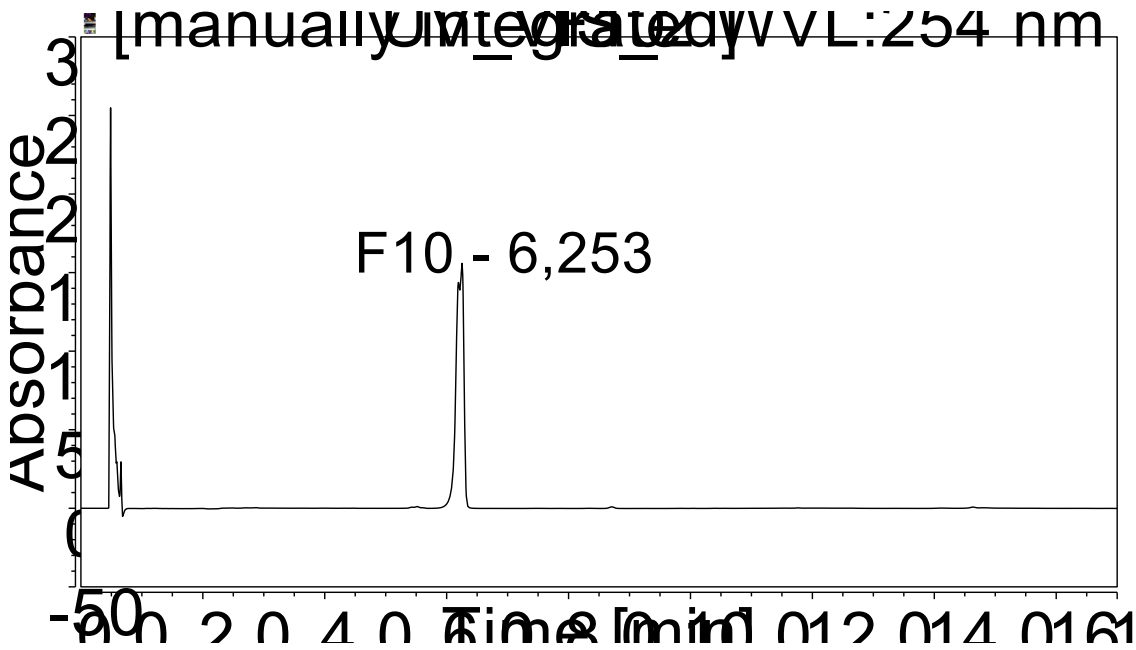

F5 - 2,693
Absorbance
Time [min]

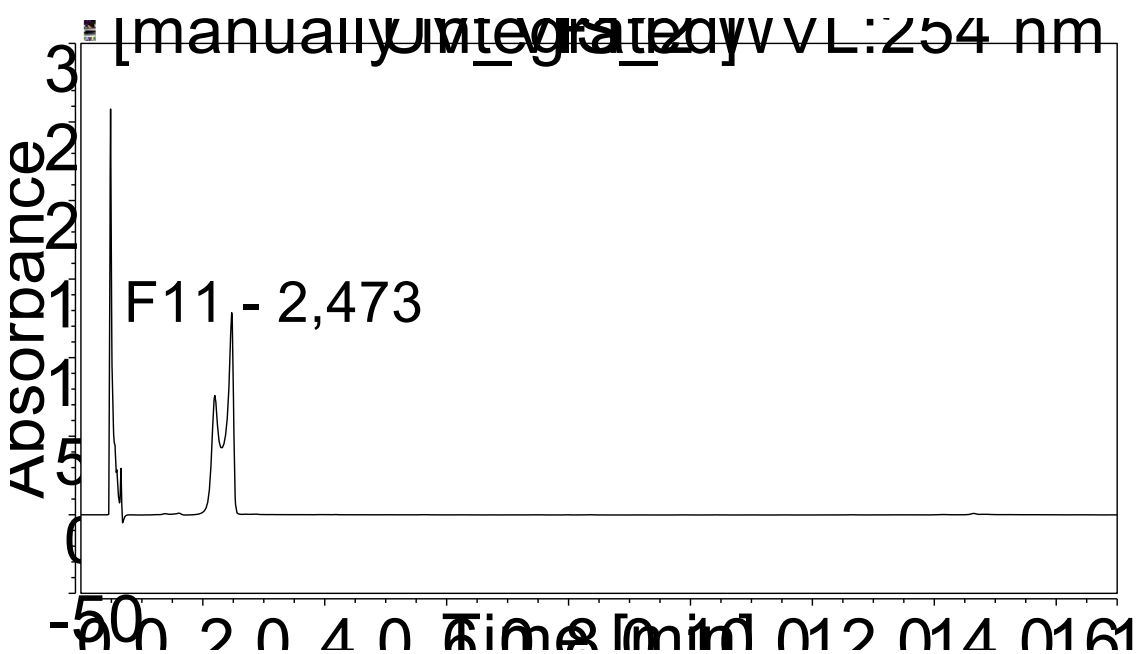

F6 - 4,867
Absorbance
Time [min]

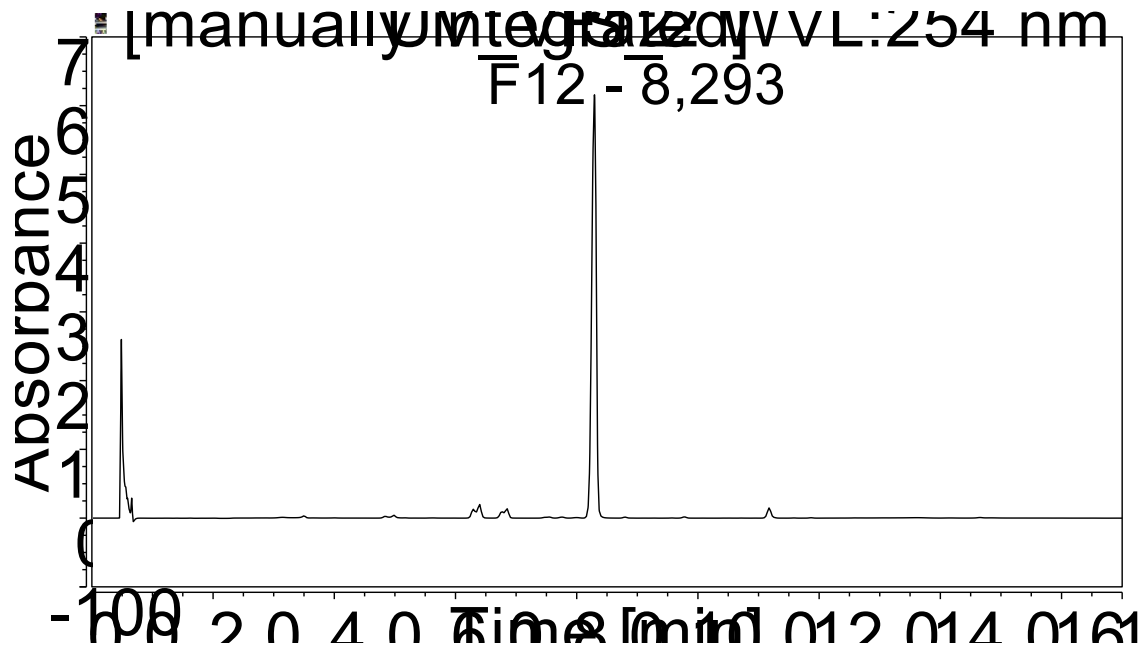

F7 - 4,140
Absorbance
Time [min]

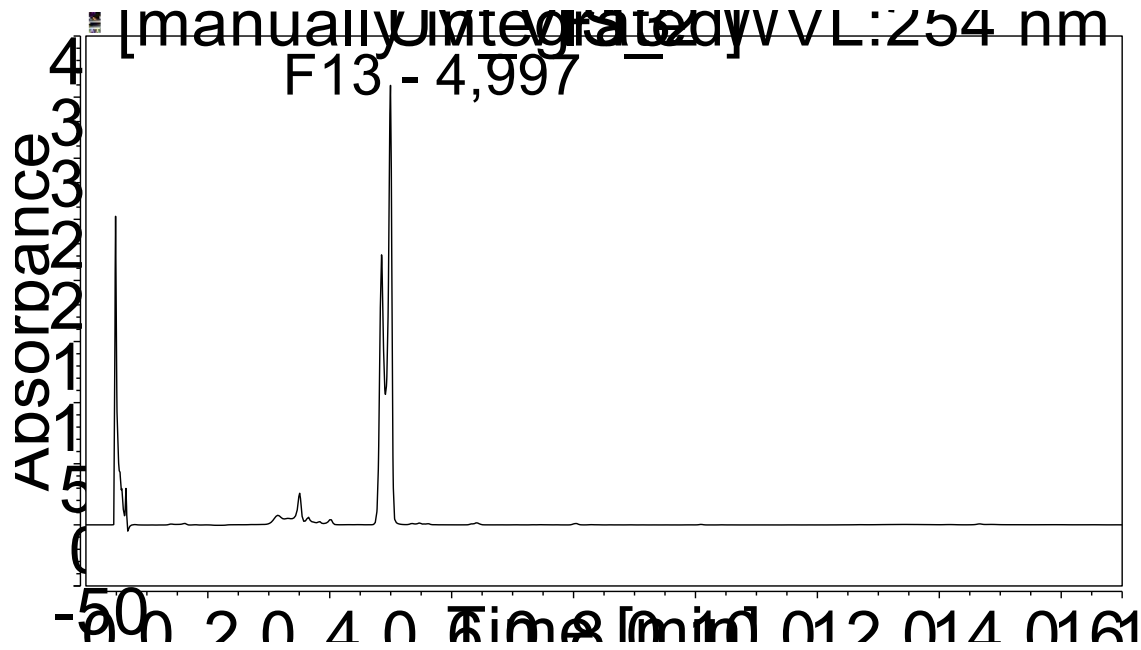

[manually integrated]
F8 - 8,147
Absorbance
Time [min]

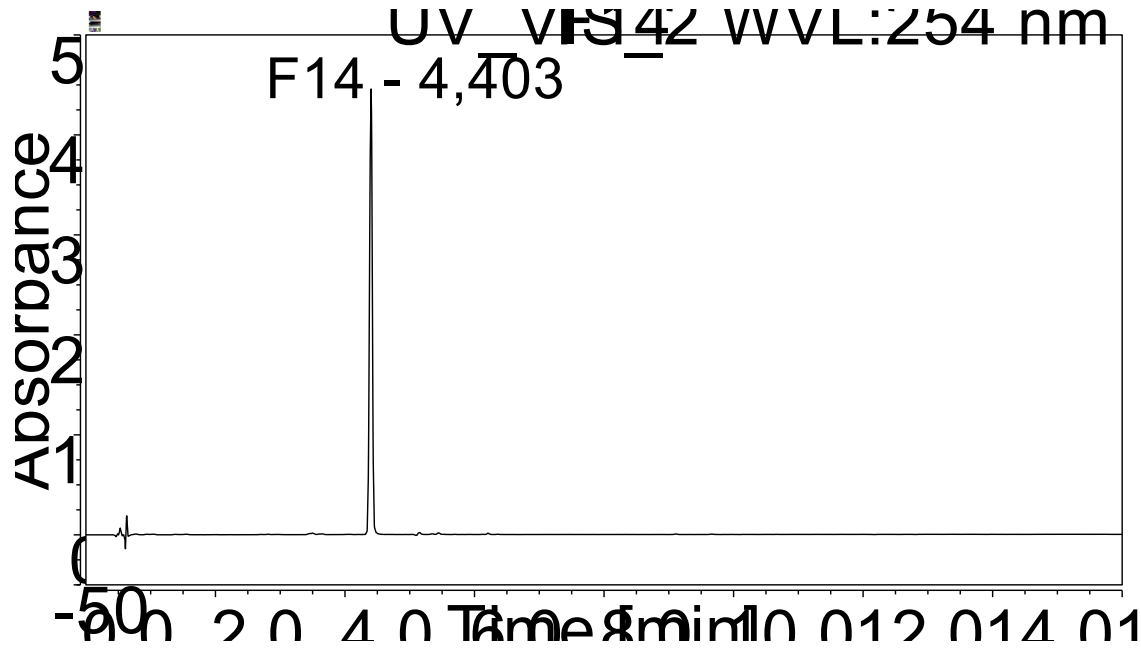

F9 - 3,407
Absorbance
Time [min]

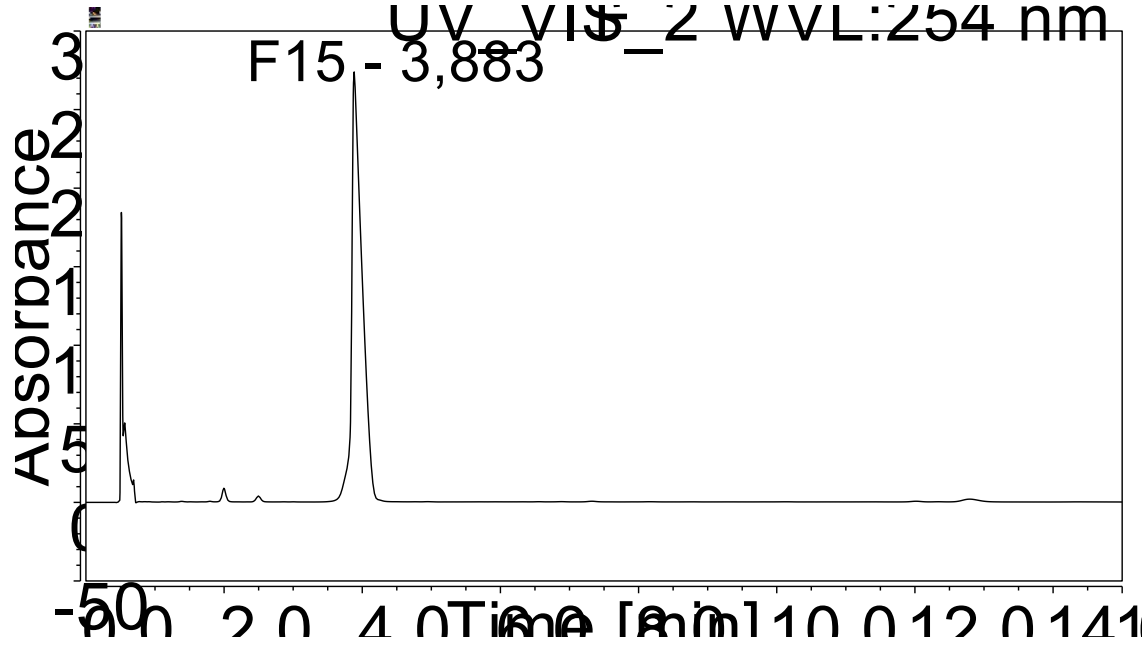

F10 - 6,253

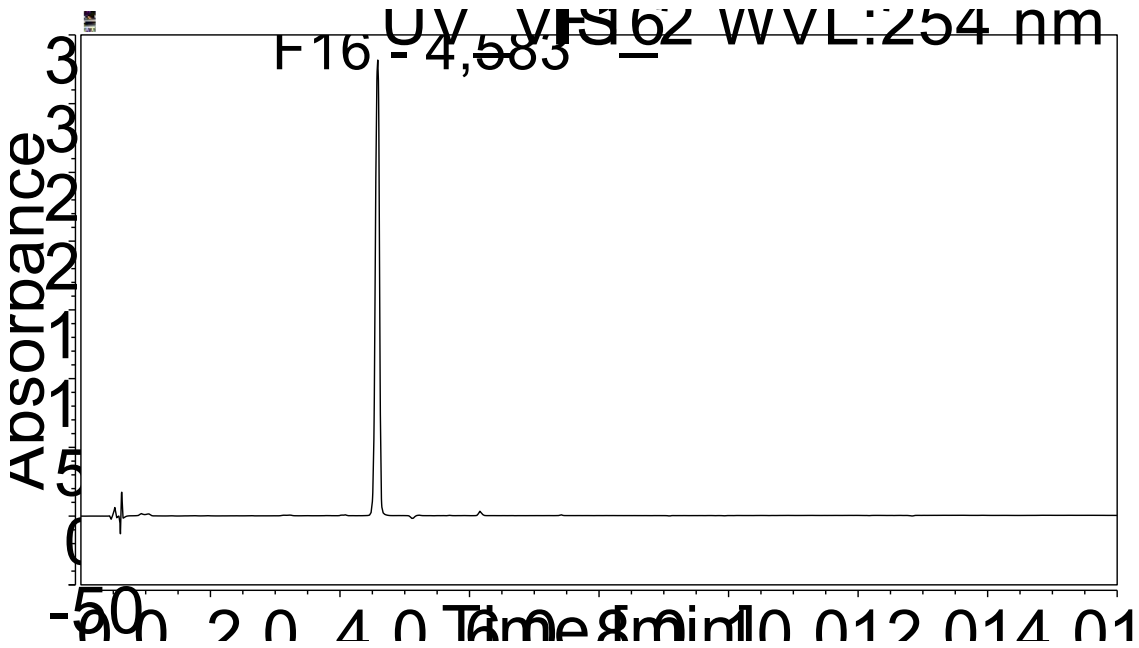

F11 - 2,473

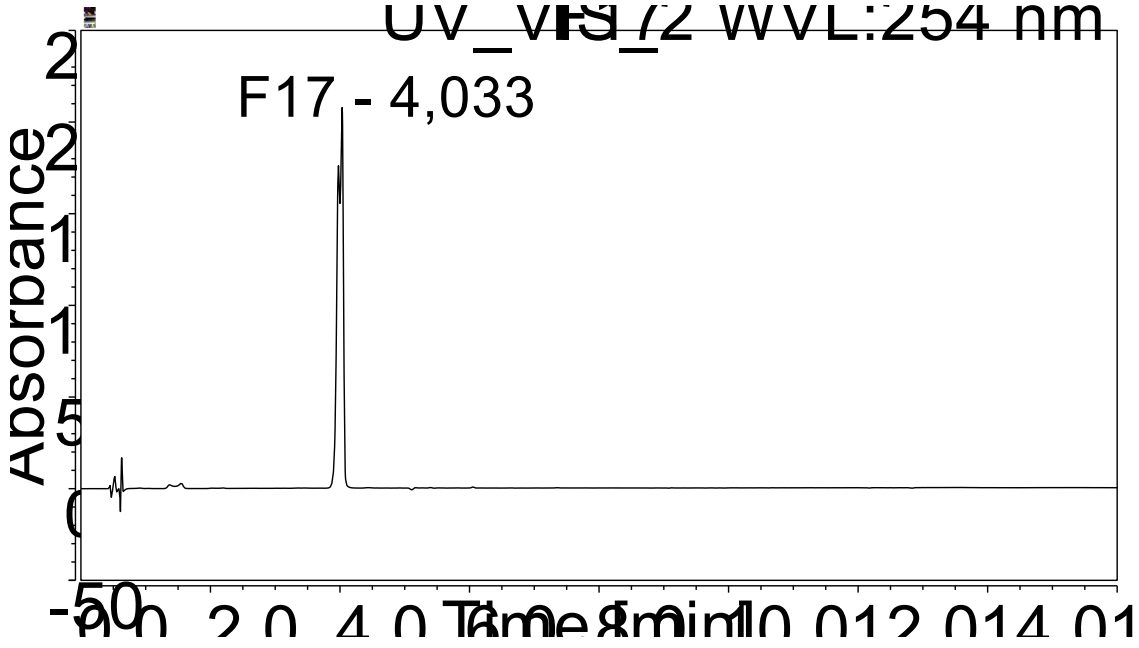

[manually integrated]
F12 - 8,293
Absorbance
Time [min]

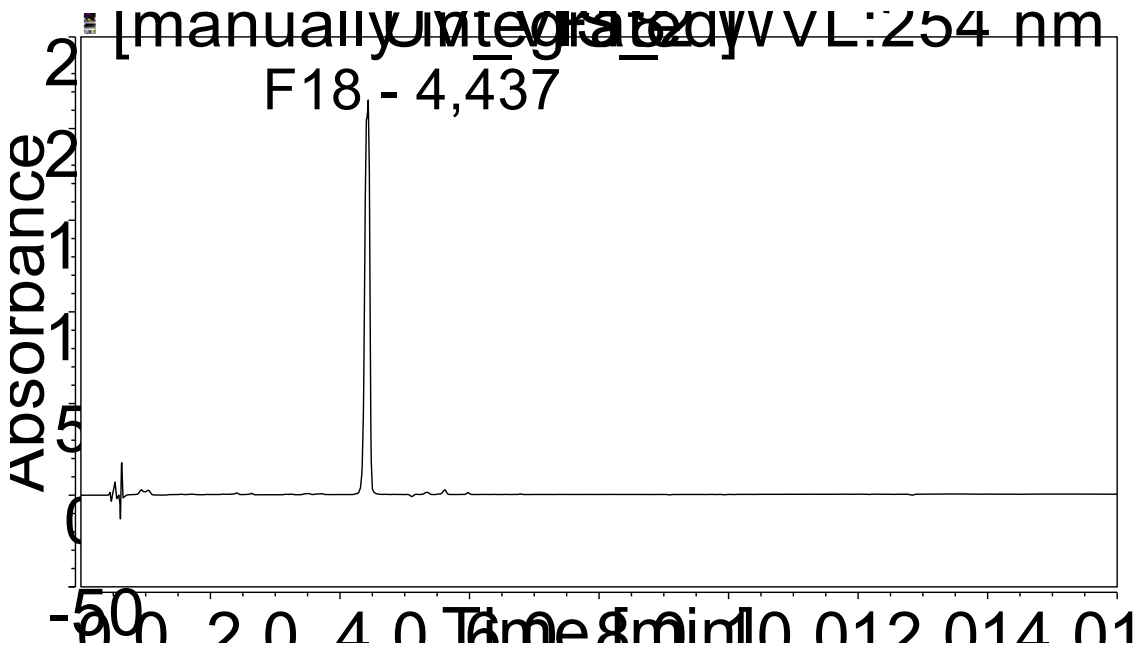

[manually integrated]
F13 - 4,997
Absorbance
Time [min]

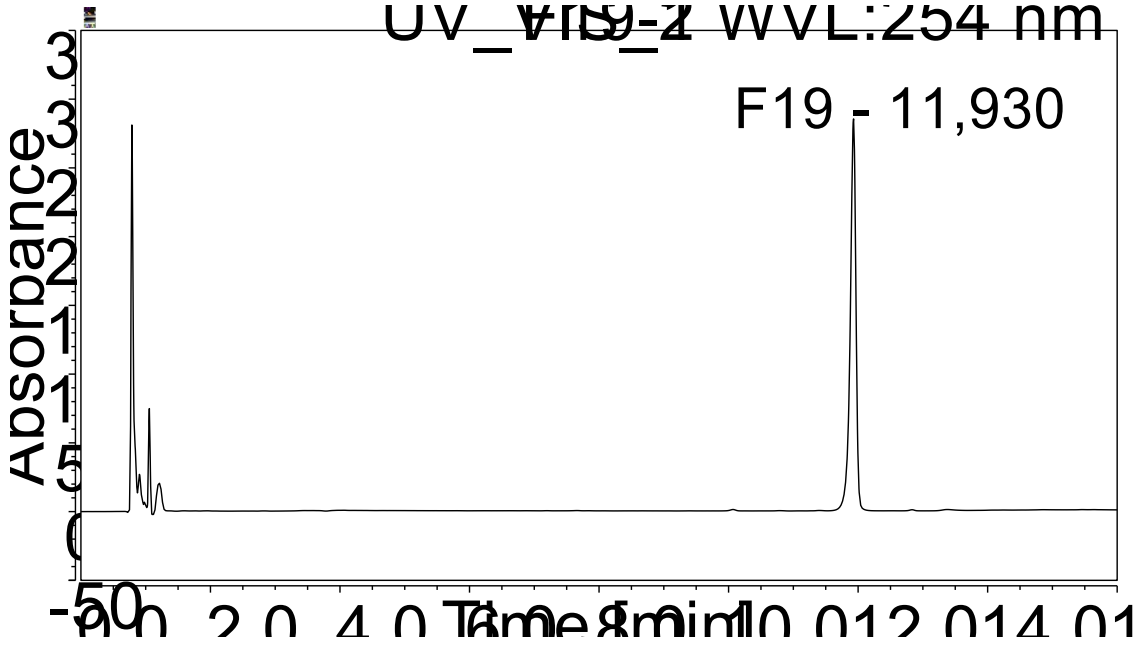

F14 - 4,403
Absorbance
Time [min]

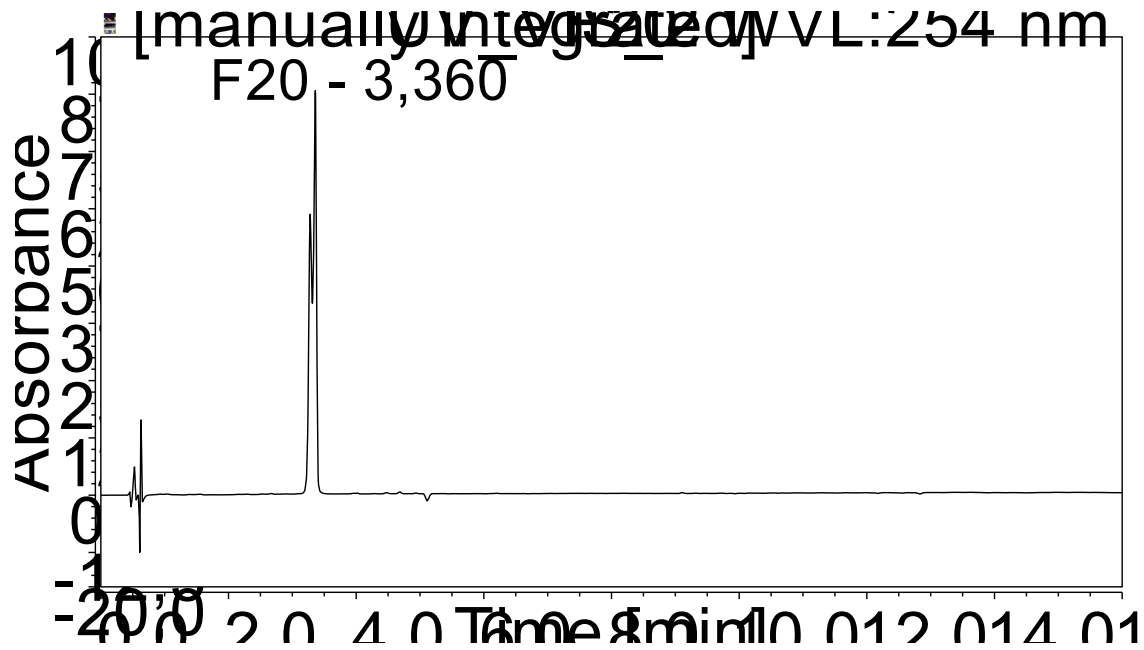

F15 - 3,883
Absorbance
Time [min]

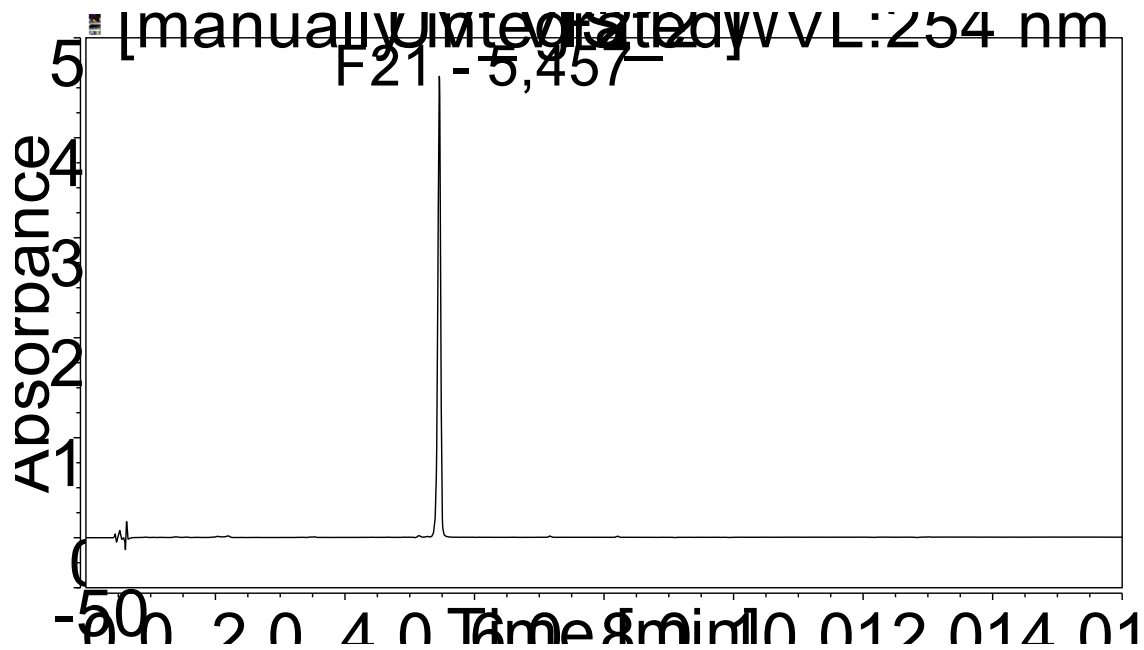

F16 - 4,583
Absorbance
Time [min]

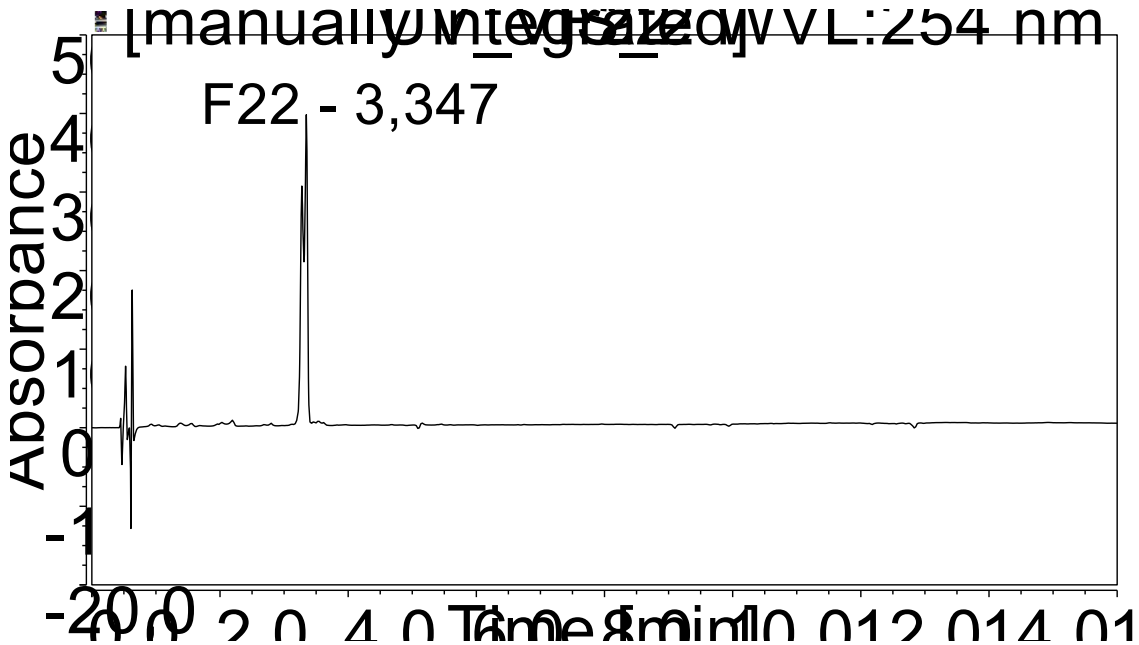

F17 - 4,033
Absorbance
Time [min]


[manually integrated]
F18 - 4,437
Absorbance
Time [min]


F19 - 11,930
Absorbance
Time [min]


[manually integrated] VVL:254 nm
F20 - 3,360
Absorbance
Time [min]


[manually integrated] VVL:254 nm
F21 - 5,457
Absorbance
Time [min]



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