Supplementary Information (SI) for RSC Advances.
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Rational design and synthesis of non-competitive transcription inhibitors targeting a conserved RNA polymerase-σ^A interface

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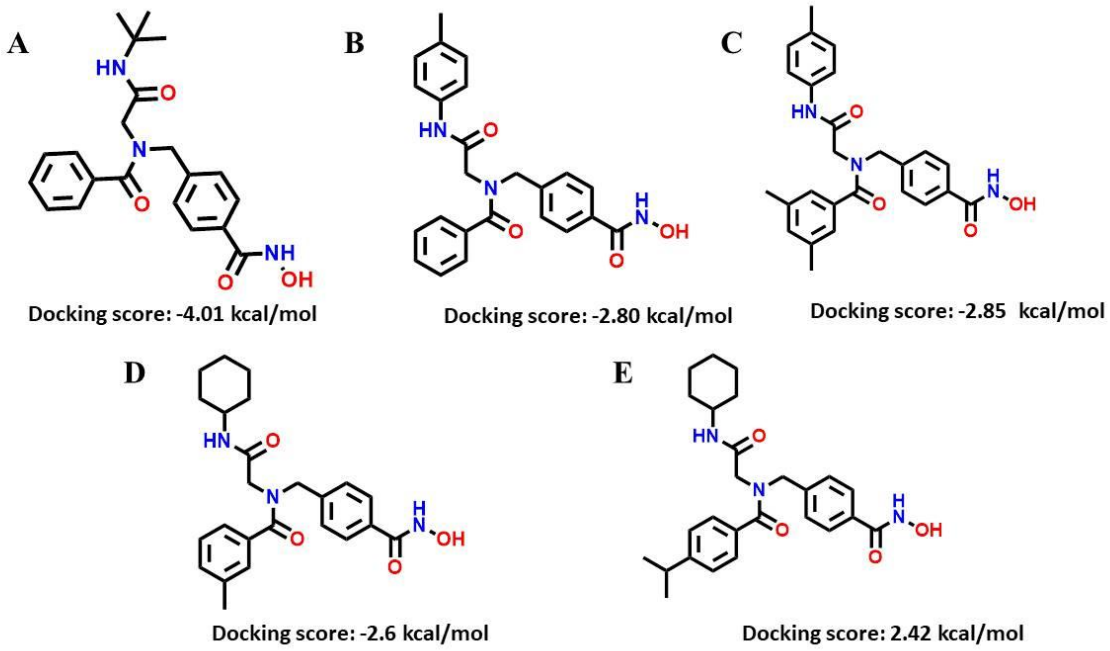
(a)



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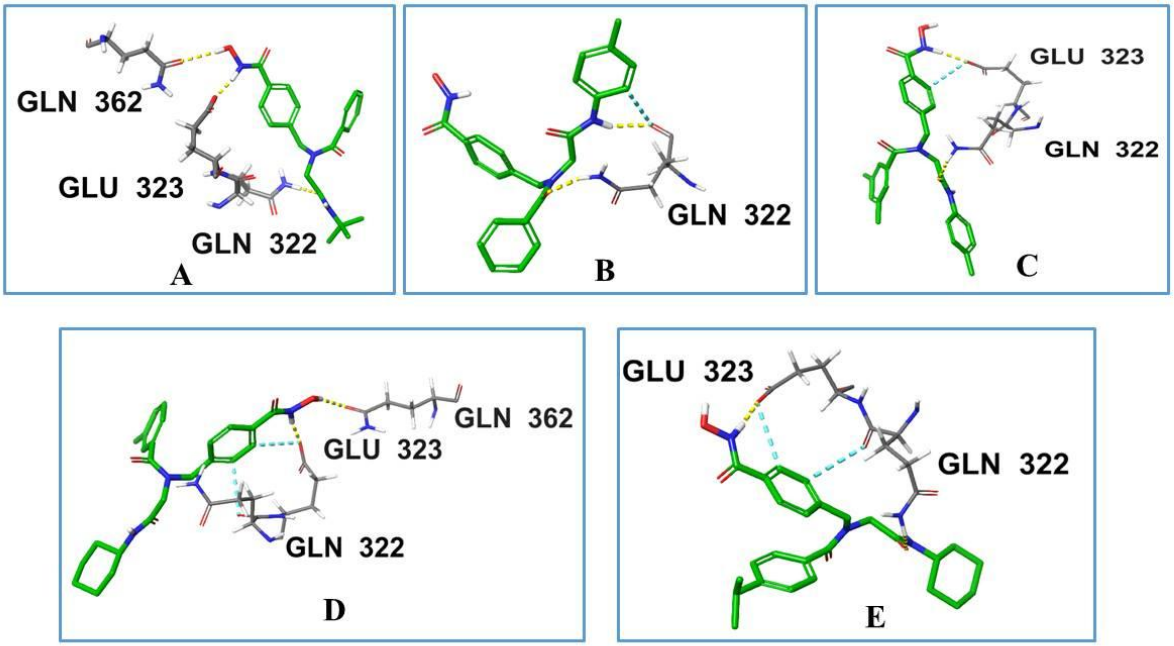


Figures S1: (a) Top five molecules from HTVS (b) 3D Interactions between ligands and $\sigma^{A}{}_{2}$.

Set of NMR signals due to the cis-trans amide bond rotation

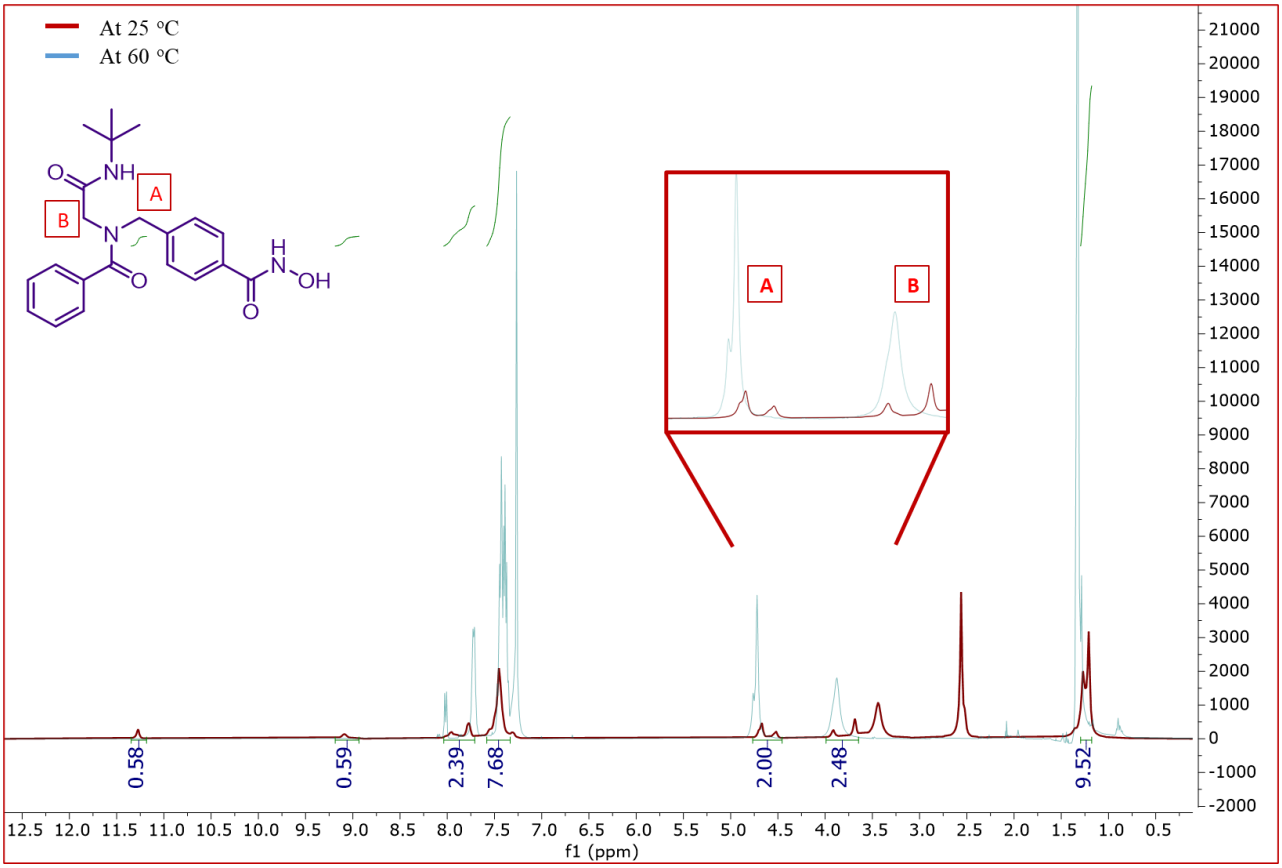


Figure S2. 1D NMR spectra of **2a** (a) room temperature spectra (red color) shows two peaks for A and B protons. (b) spectra acquired at 60 °C (blue color) shows merging of peaks due to rapid equilibration of isomers at higher temperature.

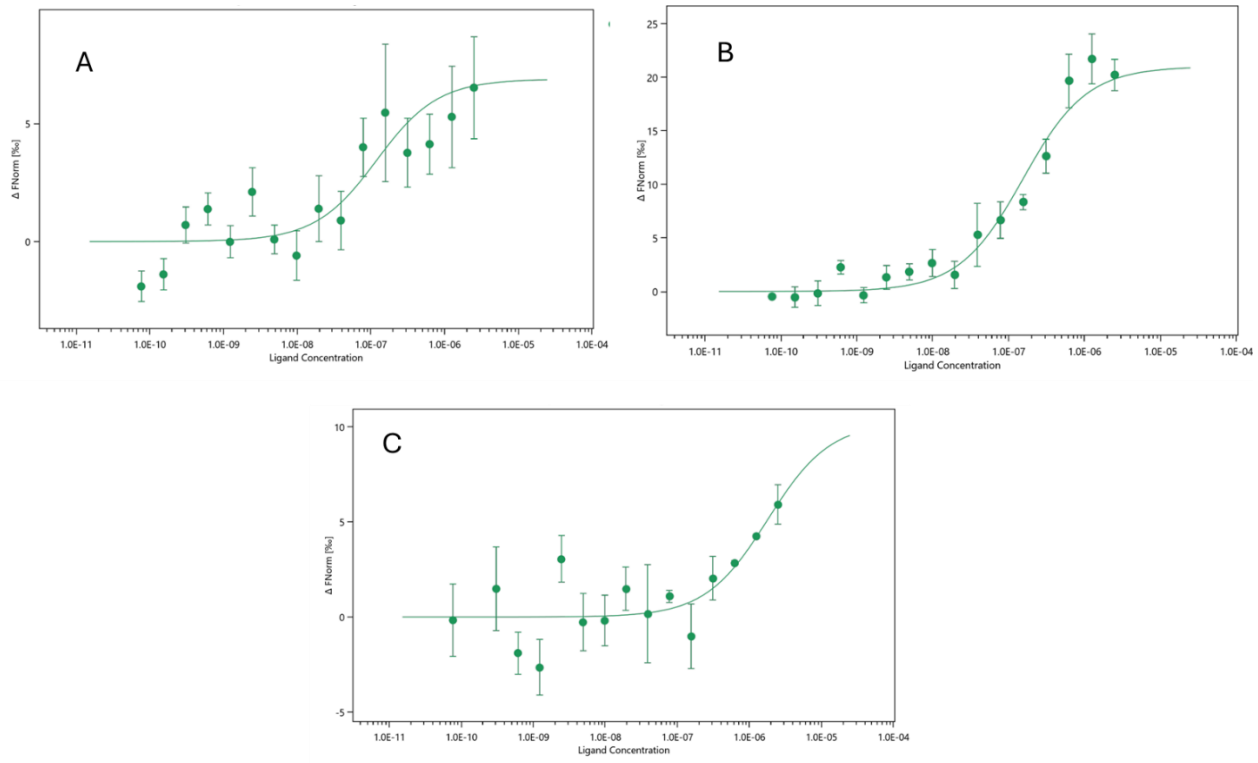


Figure S3. Binding affinity between RNAP and σ^A under different conditions **(A))** MST plot showing ΔF_{norm} values for RNAP-σ^A binding in the presence of DMSO (vehicle control) **(B)** MST plot showing ΔF_{norm} values for RNAP-σ^A binding in the presence of **2p** negative control **(C)** MST plot showing ΔF_{norm} values for RNAP-σ^A binding in the presence of **2u** ligand. See Table S1 and main manuscript for further details.

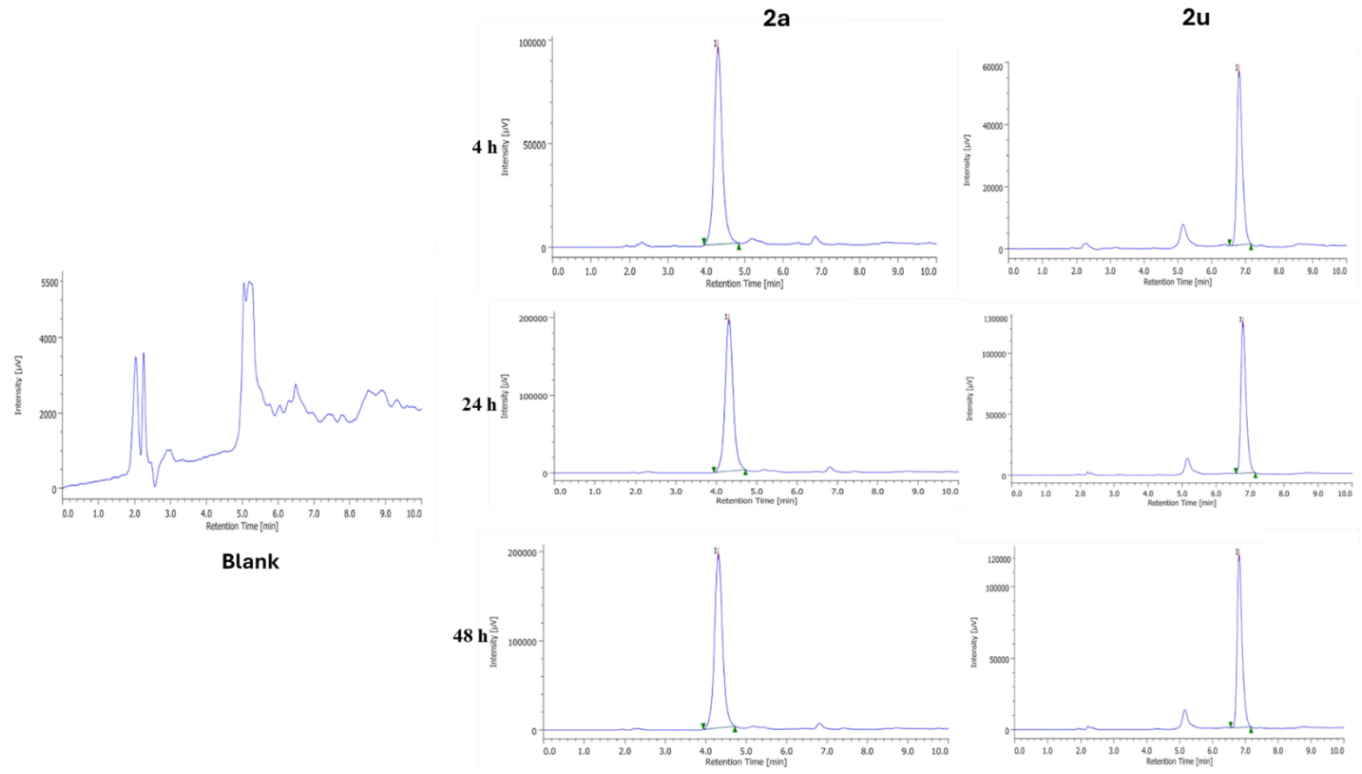


Figure S4. HPLC chromatograms of blank, compounds **2a,** and **2u** to elucidate the stability in phosphate buffer saline (PBS) from 4 h to 48 h. Blank was prepared using 10 µL DMSO into 990 µL PBS, from which 50 µL was taken and diluted up to 1 mL using ACN. Sample **2a** and **2u** were prepared by dissolving 2 mg compound in 10 µL DMSO and diluted up to 1 mL using PBS, from which 50 µL was taken and diluted up to 1 mL using ACN. **Method 1** was used for HPLC analysis

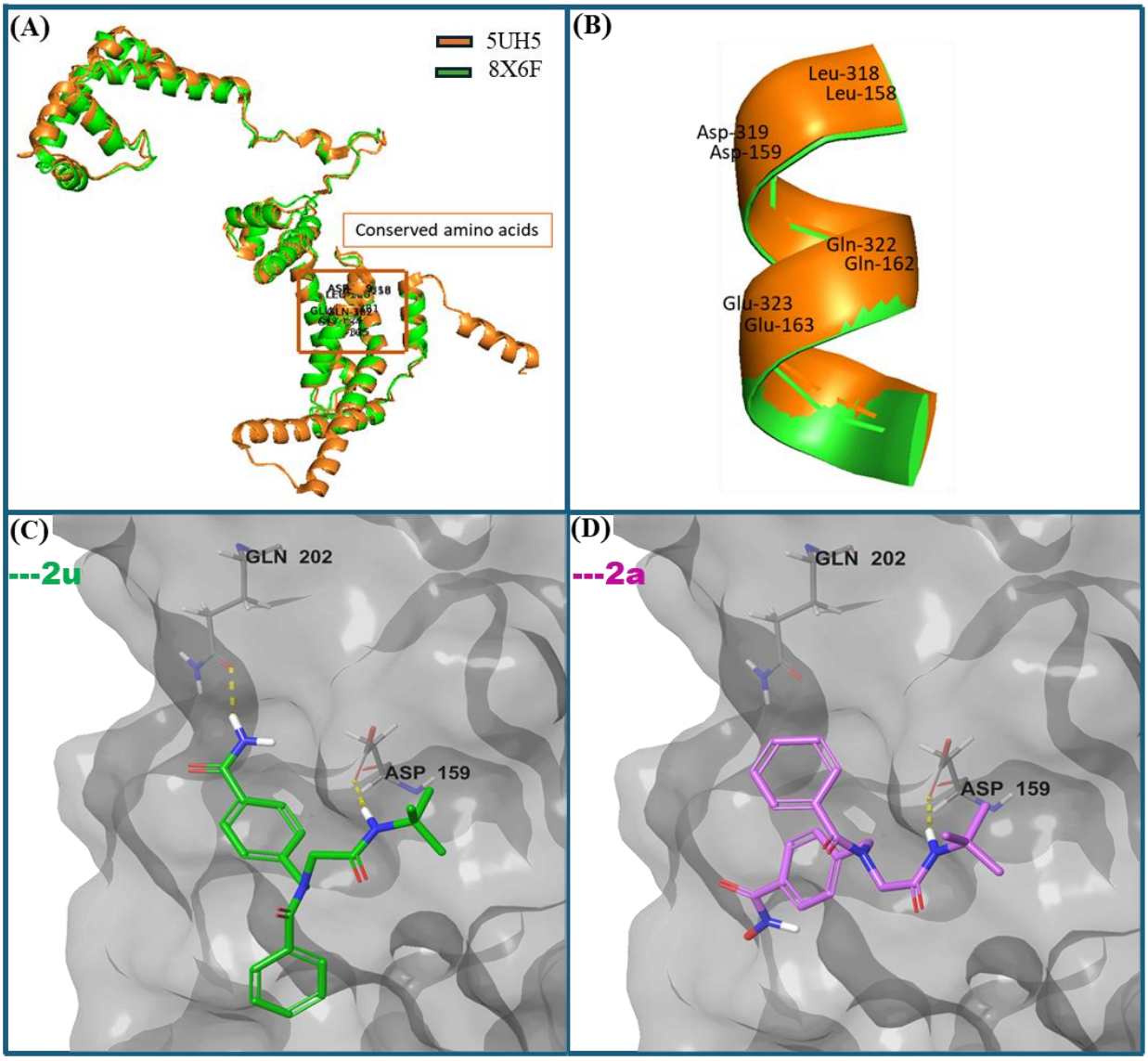


Figure S5. (A) Superimposed structures of chain F of 5UH5 (*M. tuberculosis*) onto the chain E of 8X6F (*S. aureus*) (B) Sequence of conserved interacting amino acids of *M. tuberculosis* and *S. aureus* (C) Binding pose of **2u** and (D) Binding pose of **2a**.

Table S1. *In vitro* binding affinity of RNAP-σ^A in various conditions

Condition	K_d	Response Amplitude	Standard Error	Reduced Chi-Square	Signal-to-Noise Ratio

σ^A–RNAP (+ DMSO)	109 ± 50 nM	6.8	1.2	1.9	5.5
σ^A–2p–RNAP	143 ± 22 nM	21	1.5	2.4	14.2
σ^A–2u–RNAP	1.78 ± 2.7 µM	10.23	1.5	1.8	7.2

Table S2. Components of *in-vitro* transcription assay and their concentration

Component	Stock	Working
DNA (rrnAP3 Promoter)	413.9 nM	20 nM
RNAP	411 nM	100 nM
σ^A	11.8 $\boldsymbol{\mu}$M	100 nM
CarD	203.8 µM	4 µM
RbpA	80 µM	2 µM
Tris-HCl (pH 7.9)	1 M	10 mM
KCl	1 M	70 mM
DTT(Dithiothreitol)	100 mM	1 mM
$MnCl_2$	100 mM	2 mM
$MgCl_2$	100 mM	5 mM
Glycerol	100%	5%
NTPs	100 mM	2 mM
DMSO(Dimethylsulfoxide)	100%	5%
DEPC(Diethylpyrocarbonate) treated water	-	-

Computational details

Protein preparation

The X-ray crystal structure of *M. tuberculosis* transcription initiation complex of RNA polymerase-sigma factor (σ) was retrieved from the protein data bank (PDB ID: 5UH5). The protein 3D structure was modified using the protein preparation wizard to make up for any missing information, such as bond orders, charges, and connectivity, as incorporated in Maestro (Schrodinger suite, Version 22-2). The protein preparation further included the assignment of polar hydrogen bonds and the removal of water molecules and metal ligands. The protein structure has different chains (A, B, C, D, E, F) among which only the Chain F (σ^A) was retained (Figure 2A).

Ligand preparation

The chemical structures of all the ligands acquired from URDL were subjected to energy minimization by the LigPrep module (which uses Epik) of Maestro in the Schrodinger suite using the OPLS2005 force field. All possible states at pH 7.0 ± 2.0 were generated using an ionizer module. Both the rotamers (cis and trans) was generated during ligand preparation and the Glide program samples both rotamer during docking.

High-throughput virtual screening (HTVS)

The grid box was generated by defining the centroid of the conserved amino acids (Asp319, Gln322, and Glu323) (Figure 1A). The final dimensions of the grid box were center_x = 138.73, center_y = 3.97, center_z = 57.64 and the box dimensions along x, y, and z co-ordinate = 10 x 10 x 10 Å. HTVS wizard of Glide program (Schrodinger suite, Version 22-2) was used for carrying out virtual screening using the grid file. HTVS is designed for speed, using a simplified scoring function to rapidly eliminate molecules that cannot fit the protein binding site. The hits from the HTVS stage (top 10%) were then re-docked using the Standard Precision (SP) mode. SP docking performs a more exhaustive sampling of ligand poses and uses a more sophisticated scoring function (GlideScore) than HTVS to better estimate binding affinity. The top 10% hits from SP docking were again subjected to Extra Precision (XP) docking mode. XP mode applies extensive sampling and severe scoring penalties for violations (such as desolvation penalties) to eliminate false positives. The top scoring poses from XP mode were manually visualized for interactions with the conserved residues.

Molecular docking in *S. aureus*

Protein preparation

The crystal structure of *S. aureus* RNAP-σ^A was retrieved from the protein data bank (PDB 8X6F), the structure alignment with crystal structure of *M. tuberculosis* (PDB 5UH5) (Figure S3A) was performed using PyMol (open-source system) and conserved amino acids were identified (Figure S3B). Chain-E from PDB 8X6F were taken and utilized for protein preparation using similar methodology mentioned above.

Ligand Preparation and Molecular Docking

Previously prepared ligands for docking against *M. tuberculosis* were used. The grid box was generated defining the centroid of conserved amino acids (Asp159, Gln162, and Glu163). The final dimensions of the grid box were center_x = 172.32, center_y = 114.36, center_z = 118.78 and the box dimensions along x, y, and z co-ordinate = 10 x 10 x 10 Å. The prepared ligands were subject to extra precision (XP) docking and the top scoring poses were visualized for interaction with conserved amino acids.

Experimental methodologies

General Chemistry information

All chemicals and solvents were obtained from commercial suppliers (Sigma-Aldrich, TCI, Spectrochem, and CDH) and used as purchased without further purification. The progress of all reactions was monitored by thin layer chromatography (TLC) using Merck precoated silica gel plates (with fluorescence indicator UV254). Components were visualized by irradiation with ultraviolet light (254 nm) or staining in potassium permanganate solution following heating. Compounds were purified over silica gel (230-400 mesh) using a solvent mixture specified in individual experiments. All solvents used for chromatographic purification were distilled before use. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance 400 using deuterated solvents, 1H NMR. Signals marked with an asterisk (*) correspond to peaks assigned to the minor rotamer conformation. Chemical shifts are given in parts per million (ppm), relative to the residual solvent peak for ^{1}H and ^{13}C. Analytical HPLC analysis was carried out by: **Method 1**- JASCO HPLC system equipped with an AS-4550 Compact Autosampler, LC-4000 pumps, and a 190-800 nm (PDA-detector). Using a Zodiac C18, 5 µm particle (250 mm × 4.6 mm) column, UV absorption was detected at 254 nm with an gradient flow of 40-70% HPLC-grade acetonitrile to HPLC-grade water + 0.1% formic acid + 0.2M SLS in 10 min for elution at a flow rate of 1 mL/min. **Method 2**- Shimadzu system with SIL-40 (autosampler), LC-40D (pumps), and 190-800 nm (PDA-detector). A SunFireTM C18, 5 µm particle (250 mm × 4.6 mm) column, UV absorption was detected at 254 nm with isocratic flow of 60% acetonitrile and 40% HPLC-grade water + 0.1% formic acid + 0.2M SLS in 10 min for elution at a flow rate of 1 mL/min. The purity of final compounds was more than 95%. High-resolution mass spectrometry (HRMS) analysis was performed using Q-TOF Agilent system in ESI positive or negative modes.

*4.1 General synthesis procedure of compound **1a-1r** (Ugi reaction)*

A mixture of 4-aminomethyl benzoic acid methyl ester hydrochloride (100 mg, 0.5 mmol, 1 eq.), different aldehydes (0.5 mmol, 1 eq.), triethylamine (0.5 mmol, 1 eq.), and 100 mg of crushed molecular sieves (MS) 4 Å was stirred in dry methanol (4 mL) at 15℃ temperature for 30 min. Next, the appropriate carboxylic acid (0.5 mmol, 1.0 eq.) and isocyanide (0.5 mmol, 1.0 eq.) were added sequentially. The reaction mixture was stirred at 15-25℃ temperature for 48 h. After completion of the reaction (monitored using TLC), the reaction mixture was filtered, and the solvent was removed under reduced pressure. Water (10 mL) was added, and the pH was adjusted to a pH 4 using 1M HCl. The mixture was extracted with dichloromethane (3 x 20 mL), and the combined organic layers were dried over anhydrous sodium sulphate, filtered, and concentrated in a vacuum. The crude products were purified by column chromatography (n-Hexane/ Ethyl acetate: 60:40) with 50-80% overall yield (Scheme 1).

*Methyl 4-((N-(2-(tert-butylamino)-2-oxoethyl)benzamido)methyl)benzoate **(1a)***: Synthesized from 4-aminomethyl benzoic acid methyl ester hydrochloride (200 mg, 0.99 mmol, 1 eq.), triethylamine (137 µL, 0.99 mmol, 1eq.), paraformaldehyde (29 mg, 0.99 mmol, 1 eq.), benzoic acid (120 mg, 0.99 mmol, 1 eq.) and *tert*-butyl isocyanide (111 µL,0.99 mmol, 1 eq.). White solid, yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16-7.94 (m, 3H), 7.52-7.37 (m, 6H), 6.24 (s, 1H), 4.86*/4.70 (2 x s, 2H), 3.96/3.69* (2 x s, 2H), 3.91 (s, 3H), 1.34/1.29* (2 x s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.48, 130.19, 130.12, 128.68, 128.43, 126.77, 52.22, 28.71. HRMS (+ESI) *m/z* calculated for $C_{22}H_{26}N_2O_4$: 382.188, found 383.1963 $[M+H]^+$. Purity 100% [Rt 9.1 min (Method 1)].

*4.2 General synthesis procedure of compound **2a-2r** (Hydroxyaminolysis)*

4.2.1Preparation of fresh hydroxylamine solution

To a solution of potassium hydroxide (5.6 g, 49.9 mmol) in methanol (7 mL), the methanolic solution of hydroxylamine hydrochloride (2.33 g, 33.6 mmol) was added at 0 °C and stirred for 30 min. The resultant precipitate was removed by filtration. The filtrate was collected to provide free hydroxylamine solution which was stored in a refrigerator before use. The preformed intermediates (**1a**-**1r**) were further used to react with freshly prepared methanolic hydroxylamine solution for 4 h at 0 ˚C. After the completion of the reaction (monitored using TLC), the reaction mixture was neutralized with 1M HCl. The precipitates so formed were filtered dried and purified using column chromatography (Methanol/ Dichloromethane: 12:88) with 30-60% overall yield (Scheme 1).

N-2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2a)*****:** Yellow solid, yield 56%. ^{1}H NMR (400 MHz, DMSO) δ 11.16 (s, 1H), 9.06 (s, 1H), 7.96-7.69 (m, 2H), 7.58-7.19 (m, 7H), 4.67/4.52* (2 x s, 2H), 3.91*/3.68 (2 x s, 2H), 1.27*/1.21 (2 x s, 9H).). ^{13}C NMR (101 MHz, DMSO) δ 171.94, 167.42, 164.54, 141.04, 136.66, 130.02, 128.76, 128.10, 127.57, 127.07, 51.73, 50.70, 49.23, 28.92. HRMS (+ESI) *m/z* calculated for $C_{21}H_{25}N_3O_4$: 383.1845, found: 384.1900 $[M+H]^+$. Purity 95% [Rt 4.42 min (Method 1)].

N-(2-(tert-butylamino)-1-(4-hydroxyphenyl)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2b)*****:** White solid, yield 40%. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 9.47 (s, 1H), 8.96 (s, 1H), 7.46 (d, *J* = 34.2 Hz, 7H), 7.12-6.85 (m, 4H), 6.61 (d, *J* = 8.3 Hz, 2H), 5.33/4.88* (2 x s, 2H), 4.26 (s, 1H), 1.23 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 169.59, 157.50, 137.27, 130.90, 130.70, 129.89, 129.06, 126.96, 126.67, 126.58, 115.50, 50.78, 28.72. HRMS (+ESI) *m/z* calculated for $C_{27}H_{29}N_3O_5$: 475.2107, found: 476.218 $[M+H]^+$. Purity 94.7% [Rt 2.98 min (Method 1)].

N-(2-(tert-butylamino)-2-oxo-1-(pyridin-2-yl)ethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2c)*****:** Yellow solid, yield 57%. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 8.95 (s, 1H), 8.43 (s, 1H), 7.66 (s, 3H), 7.55-7.42 (m, 6H), 7.30 (s, 2H), 7.18 (d, *J* = 7.1 Hz, 2H), 7.07 (s, 2H), 5.54 (s, 1H), 4.92/4.48 (2 x s, 2H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 172.62, 172.49, 168.68, 136.91, 133.06, 131.45, 131.39, 130.01, 129.10, 128.67, 126.68, 126.65, 50.91, 28.61. HRMS (-+ESI) *m/z* calculated for $C_{26}H_{28}N_4O_4$: 460.2134, found: 461.2209 $[M+H]^+$. Purity 96.80% [Rt 3.7 min (Method 1)].

N-(2-(tert-butylamino)-1-(4-chlorophenyl)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2d)*****:** White solid, yield 44%. ^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.98 (s, 1H), 7.96-7.67 (m, 1H), 7.52-6.93 (m, 12H), 6.08*/5.41 (2 x s, 1H), 4.92/4.28 (2 x s, 2H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 168.71, 136.92, 133.07, 131.42, 128.71, 126.70, 50.92, 28.63. HRMS (+ESI) *m/z* calculated for $C_{27}H_{28}ClN_3O_4$: 493.1771, found: 494.1846 $[M+H]^+$. Purity 95.0% [Rt 4.7 min (Method 1)].

4-((N-(2-(tert-butylamino)-2-oxoethyl)acetamido)methyl)-N-hydroxybenzamide ***(2e)*****:** Brown semi-solid**,** yield 54%. ^{1}H NMR (400 MHz, DMSO) δ 11.21 (s, 1H), 9.04 (s, 1H), 7.79-7.55 (m, 2H), 7.41-7.26 (m, 2H), 4.61*/4.46 (2 x s, 2H), 3.82/3.78* (2 x s, 2H), 2.04/2.02/1.91* (3 x s, 3H),

1.31/1.26/1.22 (3 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 171.70, 171.48, 170.87, 169.22, 167.66, 167.64, 166.59, 141.66, 141.23, 132.19, 131.86, 127.95, 127.70, 127.35, 127.22, 52.62, 51.43, 50.78, 50.58, 49.46, 48.74, 28.99, 28.85, 21.82, 21.75 HRMS (+ESI) *m/z* calculated for $C_{16}H_{23}N_3O_4$**:** 321.1696, found: 322.1769 $[M+H]^+$. Purity 96.0% [Rt 5.8 min (Method 2)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)nicotinamide ***(2f)*****:** Yellow-solid, yield 46%. ^{1}H NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 10.35 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.58 (s, 1H), 7.41 (dd, *J* = 22.6, 8.0 Hz, 2H), 7.21-7.07 (m, 2H), 4.74 (s, 2H), 3.97 (s, 2H), 1.25/1.24* (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 168.16, 167.52, 166.57, 141.67, 136.08, 131.98, 127.53, 127.15, 122.50, 121.07, 120.67, 112.28, 109.65, 50.74, 29.49, 28.91, 19.93. HRMS (+ESI) *m/z* calculated for $C_{20}H_{24}N_4O_4$: 384.1798, found 385.1861 $[M+H]^+$. Purity 99.50% [Rt 4.3 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)-4-nitrobenzamide ***(2g)*****:** Yellow-solid, yield 60%. ^{1}H NMR (400 MHz, DMSO) δ 11.24 (s, 1H), 9.07 (s, 1H), 8.29 (d, *J* = 8.7 Hz, 2H), 7.80-7.65 (m, 3H), 7.61-7.28 (m, 3H), 4.69/4.45* (2 x s, 2H), 3.94*/3.66 (2 x s, 2H), 1.26/1.24*/1.17(3 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 170.43, 167.01, 148.22, 142.97, 140.61, 128.63, 128.28, 128.20, 127.56, 124.43, 124.10, 65.40, 51.79, 50.83, 50.77, 49.43, 29.02, 28.76, 15.64. HRMS (+ESI) *m/z* calculated for $C_{21}H_{24}N_4O_6$: 428.1696, found: 429.1789 $[M+H]^+$. Purity 99% [Rt 3.48 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-2-chloro-N-(4-(hydroxycarbamoyl)benzyl)nicotinamide ***(2h)*****:** White solid**,** yield 37%. ^{1}H NMR (400 MHz, DMSO) 8.55-8.42 (m, 1H), 7.96-7.79 (m, 4H), 7.52-7.48 (ddd, *J* = 60.8, 26.7, 7.7, 4H), 7.39 (s, 1H), 4.19*/3.97 (2 x s, 2H), 3.62 (s, 2H), 1.14 (s, 9H)**.** ^{13}C NMR (101 MHz, DMSO) δ 167.43, 166.81, 164.43, 150.81, 150.76, 145.98, 140.51, 138.34, 132.31, 128.19, 127.55, 123.66, 55.36, 50.75, 49.37, 28.75. HRMS (+ESI) *m/z* calculated for $C_{20}H_{23}ClN_4O_4$**:** 418.1408, found: 419.1473 $[M+H]^+$. Purity 96.6% [Rt 3.7 min (Method 2)].

N-(2-(tert-butylamino)-2-oxoethyl)-2-chloro-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2i):*** Yellow solid*,* yield 54%. ^{1}H NMR (400 MHz, DMSO) 7.93 (d, *J* = 8.3, 1H), 7.54-7.44 (m, 3H), 7.42-7.27 (m, 4H), 5.32 (d, *J* = 15.2, 1H), 4.24/3.88 (2 x s, 2H), 3.54*/3.52 (2 x s, 2H), 1.23*/1.13 (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 172.50, 168.82, 166.79, 142.56, 141.53, 135.85, 131.15, 131.04, 130.29, 130.00, 129.96, 129.81, 129.37, 128.73, 128.32, 127.96, 127.72, 50.84,

50.70, 49.12, 28.74. HRMS (-ESI) *m/z* calculated for $C_{21}H_{24}ClN_3O_4$**:** 417.1455, found: 416.1357 $[M-H]^-$. Purity 98.9% [Rt 4.3 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)-1H-indole-3-carboxamide ***(2j)*****:** Yellow solid**,** yield 36%. ^{1}H NMR (400 MHz, DMSO) δ 11.56 (s, 1H), 11.21 (s, 1H), 9.04 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.57 (s, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 7.9 Hz, 2H), 7.20-7.08 (m, 2H), 4.74 (s, 2H), 3.97 (s, 2H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 168.17, 167.51, 164.43, 141.68, 136.07, 132.00, 127.56, 127.44, 127.15, 122.48, 121.06, 120.65, 112.27, 109.65, 65.39, 55.38, 50.74, 40.59, 40.38, 40.17, 39.96, 39.75, 39.54, 39.34, 28.91, 19.94, 15.64. HRMS (+ESI) *m/z* calculated for $C_{23}H_{26}N_4O_4$: 422.1966, found: 423.2039 $[M+H]^+$. Purity 95% [Rt 4.1 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)-1H-pyrrole-3-carboxamide ***(2k)*****:** White solid, yield 40%. ^{1}H NMR (400 MHz, DMSO) δ 11.21 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.52 (s, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.07 (s, 1H), 6.76 (s, 1H), 6.24 (s, 1H), 4.65 (s, 2H), 3.93 (s, 2H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 167.93, 167.54, 143.41, 129.98, 128.00, 121.41, 118.57, 117.76, 109.17, 70.23, 50.66, 28.92. HRMS (+ESI) *m/z* calculated for $C_{19}H_{24}N_4O_4$: 372.1798, found: 373.1877 $[M+H]^+$. Purity 95% [Rt 3.7 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)thiazolidine-4-carboxamide ***(2l)*****:** White solid**,** yield 27%. ^{1}H NMR (400 MHz, DMSO) δ 11.24 (d, *J* = 24.4 Hz, 1H), 9.05 (d, *J* = 9.9 Hz, 1H), 7.78-7.66 (m, 1H), 7.46-7.20 (m, 1H), 4.90-3.60 (m, 6H), 3.13-3.00 (m, 1H), 2.72 (dd, *J* = 9.8, 7.8 Hz, 1H), 1.24*/1.23 (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 198.12, 168.07, 167.26, 141.93, 130.14, 129.93, 128.28, 70.23, 58.71, 56.13, 50.90, 50.80, 50.58, 50.01, 28.67. HRMS (+ESI) *m/z* calculated for $C_{18}H_{26}N_4O_4S$: 394.1680, found: 395.1739 $[M+H]^+$. Purity 95.8% [Rt 3.6 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)-1H-pyrazole-3-carboxamide ***(2m)*****:** White solid, yield 54%. ^{1}H NMR (400 MHz, DMSO) δ 13.16 (s, 1H), 7.92 (d, *J* = 8.2 Hz, 2H), 7.82-7.70 (m, 2H), 7.50 (d, *J* = 15.9 Hz, 1H), 7.37 (dd, *J* = 18.6, 8.0 Hz, 2H), 6.65-6.59 (m, 1H), 5.16*/4.67 (2 x s, 2H), 4.22*/3.84 (2 x s, 2H), 1.24/1.21 (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 167.78, 167.66, 167.26, 143.18, 129.96, 128.22, 127.77, 127.49, 107.91,

65.39, 52.93, 51.77, 50.64, 50.23, 29.02, 28.91, 15.64. HRMS (-ESI) *m/z* calculated for $C_{18}H_{23}N_5O_4$ 373.1731, found: 372.1669 $[M-H]^-$. Purity 100.0% [Rt 5.9 min (Method 1)].

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)furan-3-carboxamide ***(2n)*****:** White solid**,** yield 46%. ^{1}H NMR (400 MHz, DMSO) δ 13.04 (s, 1H), 7.83-7.70 (m, 2H), 7.55-7.47 (m 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 6.62 (s, 1H), 5.14*/4.65 (2 x s, 2H), 4.33*/3.84 (2 x s, 2H), 1.25*/1.21 (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 167.61, 167.18, 165.38, 162.44, 156.71, 154.66, 150.45, 145.09, 144.51, 144.04, 143.88, 129.91, 128.22, 127.46, 110.95, 51.83, 50.82, 49.06, 28.79. HRMS (+ESI) *m/z* calculated for $C_{19}H_{23}N_3O_5$: 373.1638, found: 374.1726 $(M+H)^+$. Purity 96.4% [Rt 5.3 min (Method 2)].

N-(2-(tert-butylamino)-2-oxoethyl)-3-hydroxy-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2o)*****:** White solid, yield 60%. ^{1}H NMR (400 MHz, DMSO) δ 9.72 (s, 1H), 7.95-7.12 (m, 4H), 6.89-6.78 (m, 3H), 4.65/4.52* (2 x s, 2H), 3.87*/3.67 (2 x s, 2H), 1.27*/1.25 (2 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 182.77, 179.02, 171.89, 167.67, 167.37, 157.68, 142.93, 142.44, 137.78, 130.16, 130.01, 129.89, 128.17, 127.45, 117.37, 116.87, 114.01, 51.80, 50.74, 49.20, 28.82. HRMS (+ESI) *m/z* calculated for $C_{21}H_{25}N_3O_5$: 399.1794, found: 400.1892 $(M+H)^+$. Purity 95.0% [Rt 3.1 min (Method 1)].

N-(2-(benzylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2p)*****:** Yellow semi solid**,** yield 42%. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (s, 1H), 9.06 (s, 1H), 8.43 (s, 1H), 7.75 (dd, *J* = 8.1, 2H), 7.43 (qd, *J* = 3.5 Hz, 6H), 7.33 (m, 4H), 7.17 (d, *J* = 7.5 Hz, 1H), 4.70/4.58* (2 x s, 2H), 4.30 (dd, *J* = 26.1, 5.6, 2H), 4.02*/3.81 (2 x s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 171.91, 171.86, 168.20, 140.90, 139.74, 139.46, 136.42, 132.18, 130.03, 129.70, 128.92, 128.85, 128.77, 128.12, 127.70, 127.61, 127.37, 127.26, 127.06, 51.55, 49.26, 42.64. HRMS (+ESI) *m/z* calculated for $C_{24}H_{23}N_3O_4$**:** 417.1689, found: 418.1784 $(M+H)^+$. Purity 98.6% [Rt 2.9 min (Method 2)].

N-(2-(cyclohexylamino)-2-oxoethyl)-N-(4-(hydroxycarbamoyl)benzyl)benzamide ***(2q)*****:** White solid**,** yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 26.1, 7.9 Hz, 1H), 7.70 (s, 1H), 7.54-7.31 (m, 5H), 7.17 (d, *J* = 27.5 Hz, 1H), 4.75*/4.72//4.61 (3 x s, 2H), 4.18-3.94 (m, 1H), 3.78*/3.69 (2 x s, 2H), 1.95-1.49 (m, 6H), 1.44-0.91 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 172.60, 171.89, 166.99, 152.47, 140.93, 136.60, 132.09, 130.06, 128.80, 128.14, 127.55, 127.08, 51.52, 49.19,

48.09, 32.69, 24.89, 21.85. HRMS (+ESI) *m/z* calculated for $C_{23}H_{27}N_3O_4$: 409.2002, found 410.2074 $(M+H)^+$. Purity 95.9.0% [Rt 5.3 min (Method 1)].

N-(4-(hydroxycarbamoyl) benzyl)-N-(2-(naphthalen-2-ylamino)-2-oxoethyl) benzamide ***(2r)*****:** Yellow solid**,** yield 30%. ^{1}H NMR (400 MHz, DMSO) δ 11.24 (s, 1H), 10.21 (d, *J* = 61.6 Hz, 1H), 9.06 (s, 1H), 8.31 (d, *J* = 35.9 Hz, 1H), 7.89-7.77 (m, 5H), 7.58-7.38 (m, 9H), 5.76 (s, 1H), 4.78/4.65 (2 x s, 2H), 4.23/4.06 (2 x s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 172.68, 152.49, 129.01, 128.98, 128.93, 128.36, 128.14, 127.92, 127.76, 127.60, 127.29, 127.04, 126.94, 120.23, 115.92, 21.85, 21.64, 15.01. HRMS (+ESI) *m/z* calculated for $C_{27}H_{23}N_3O_4$**:** 453.1689 found: 454.1761 $(M+H)^+$. Purity 96.0% [Rt 3.78 min (Method 1)].

4.3 General synthetic procedure for compound ***2s***

Mixture of substituted methyl benzoate (150 mg, 0.39 mmol) and hydrazine hydrate (294 mg, 5.889 mmol, 80%) in 3 mL of ethanol was refluxed for 6 h. After completion of the reaction, excess ethanol was distilled off, and the mixture was allowed to cool. The resulting crude product was filtered to obtain a solid, which was then washed with water. The obtained precipitate after washing was filtered and dried and purified using column chromatography.

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(hydrazinecarbonyl)benzyl)benzamide ***(2s)*****:** White semi-solid**,** yield 43%. ^{1}H NMR (400 MHz, CDCl3) δ 7.76 (d, *J* = 7.8 Hz, 2H), 7.70 (s, 1H), 7.47 – 7.36 (m, 6H), 7.26 (d, *J* = 7.7 Hz, 1H), 6.26/5.35* (2 x s, 1H), 4.86*/4.71 (2 x s, 2H), 3.89 (s, 2H), 3.97/3.70* (2 x s, 2H), 1.36/1.27* (2 x s, 9H).^{13}C NMR (101 MHz, DMSO) δ 172.88, 172.71, 167.64, 140.20, 134.94, 132.27, 130.29, 128.34, 128.69, 128.57, 127.53, 127.09, 126.77, 53.81, 51.44, 50.24, 28.72. HRMS (+ESI) *m/z* calculated for $C_{21}H_{26}N_4O_3$: 382.2005, found: 383.2078 $(M+H)^+$. Purity 99.0% [Rt 3.0 min (Method 1)].

4.4 General synthetic procedure for compound ***2t***

The solution of potassium hydroxide (5.6 g, 49.9 mmol) in methanol (7 mL) was added to the methanolic solution of *O*-methylhydroxylamine hydrochloride (2.33 g, 33.6 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The resultant precipitate was removed by filtration. The filtrate was collected to provide free hydroxylamine solution which was stored in a refrigerator before use. The preformed intermediate was further used to react with freshly prepared methanolic

hydroxylamine solution for 4 h at 0 ˚C. After the completion of the reaction, the reaction mixture was neutralized with acetic acid. The precipitate so formed was filtered dried and purified using column chromatography.

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-(methoxycarbamoyl)benzyl)benzamide ***(2t):*** White solid**,** yield 53%. ^{1}H NMR (400 MHz, DMSO) δ 11.97 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 2H), 7.44 (q, *J* = 4.3 Hz, 7H), 4.69/4.53* (2 x s, 2H), 3.70/3.57* (2 x s, 2H), 3.38 (s, 3H), 1.26*/1.20 (2 x s, 9H). ^{13}C (101 MHz, DMSO) δ 171.97, 171.74, 167.76, 167.39, 166.95, 142.78, 142.28, 136.62,136.39, 130.17, 130.02, 129.97, 128.97, 128.78, 128.20, 127.42, 127.08, 126.86, 51.87, 50.75, 49.35, 47.77, 29.02, 28.80. HRMS (+ESI) *m/z* calculated for $C_{22}H_{27}N_3O_4$: 397.20, found: 398.2076 $(M+H)^+$. Purity 96.24% [Rt 5.5 min (Method 1)].

4.5 General synthetic procedure for compound ***2u***

The mixture of substituted methyl benzoate (150 mg, 0.3926 mmol) and ammonium methanol (1-3 ml), NaOMe (70 mg, 2.944 mmol) in 3 mL of methanol was stirred at 25-30 ºC for 24 h. After the completion of the reaction, the reaction mixture was neutralized with acetic acid. The precipitate so formed was filtered dried and purified using column chromatography.

N-(2-(tert-butylamino)-2-oxoethyl)-N-(4-carbamoylbenzyl)benzamide ***(2u):*** White solid, yield 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00-7.84 (m, 2H), 7.53-7.29 (m, 7H), 4.68/4.52* (2 x s, 2H), 3.90*/3.66 (2 x s, 2H), 1.25/1.23*/1.19 (3 x s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 185.15, 171.94, 167.82, 167.37, 142.50, 136.61, 130.12, 129.97, 128.94, 128.75, 128.13, 127.32, 127.05, 126.85, 51.83, 50.73, 49.31, 28.78. HRMS (+ESI) *m/z* calculated for $C_{21}H_{25}N_3O_3$: 367.1896, found: 368.1969 $(M+H)^+$. Purity 95.0% [Rt 6.8 min (Method 1)].

4.6 General synthetic procedure for compound ***2v***

The mixture of substituted methyl benzoate (100 mg, 0.2617 mmol) and methanolic solution of NaOH (31 mg, 0.785 mmol) was stirred for 4 h at 25-30 ºC. After the completion of the reaction, the reaction mixture was neutralized with acetic acid. The precipitate formed was filtered and dried. Further purification was performed using silica gel column chromatography.

4-((N-(2-(tert-butylamino)-2-oxoethyl)benzamido)methyl)benzoic acid ***(2v)*****:** White solid**,** yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 2H), 7.53-7.37 (m, 5H), 7.28 (s, 1H), 6.45/5.29* (2 x s, 1H), 4.92*/4.76 (2 x s, 2H), 4.08/3.76* (2 x s, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 171.97, 167.36, 142.74, 136.58, 130.14, 130.00, 129.70, 128.96, 128.76, 128.18, 127.40, 127.05, 126.84, 51.86, 50.74, 49.34, 28.77. HRMS (+ESI) *m/z* calculated for $C_{21}H_{24}N_2O_4$: 368.1736, found: 369.1809 $(M+H)^+$. Purity 99.0% [Rt 6.5 min (Method 1)].

Microscale thermophoresis (MST)

Freshly purified σ^A was buffer exchanged in 25 mM HEPES (pH-8.0), 200 mM NaCl and 5% glycerol, and labelled using the Monolith protein labelling kit RED-NHS (NanoTemper Technologies) according to the manufacturer's instructions. For protein labelling, 10 µM of protein mixed with 30 µM dye (diluted from 600 µM dye stock provided by the manufacturer) was incubated for 30 min at 25-30 °C. The dilution of the protein was performed by the addition of buffer with no glycerol (25 mM HEPES (pH 8.0), 200 mM NaCl). For the purification of the labelled σ^A, pre-packed columns (Sephadex G50) were used as provided by the manufacturer. The labelled σ^A was used at 5 nM (diluted accordingly with 25 mM HEPES (pH 8.0)) and was titrated with different concentrations of small molecules. 0.05% Tween 20 and 10 µg/ml of Bovine serum albumin (BSA) were added to avoid non-specific interactions. The measurement was obtained at an LED/excitation power setting of 100%. Data were analyzed using the MO Affinity Analysis software (Version 2.2.5; NanoTemper Technologies) at different standard MST-off times (Summarized in Tables 1, 2, 3, and 4).

In vitro **transcription assay**

The inhibition of *M. tuberculosis* RNAP by the designed compounds was experimentally validated using an *in vitro* transcription assay. The components and their respective concentrations used in the assay are mentioned in Table S2. The reaction mixture was prepared by combining the *in vitro* transcription buffer (1X PBS, pH 7.5, 70 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 5 mM $MgCl_2$, 5% glycerol) and σ^A (100 nM) in a microcentrifuge tube. The synthetic ligand (for example **2a** at 1 mM) was added to the mixture and incubated at 37 °C for 15 min. Subsequently, 100 nM RNAP was added and incubated at 37 °C for 15 min. Further, the DNA template (the rRNA *AP3* promoter)

was introduced at a final concentration of 20 nM and was incubated at 37 °C for 15 min. Next, RbpA (2 μM) and CarD (4 μM) was added to the reaction mixture and incubated at 37 °C for 15 min. Transcription was initiated by adding rNTPs to a final concentration of 2 mM and incubating for 1 h at 37 °C. Post-transcription treatment to degrade the DNA template was performed using DNase I (1 μL) in 10 X DNase1 buffer (1 μL) with 90 min incubation at 37 °C. To detect the amount of mRNA formed after transcription, 80 μL of diluted (1:200) RiboGreen (Quant-iT™ RiboGreen® RNA Reagent) was added to 20 μL of the reaction mixture and incubated for 20 min at room temperature. The excitation wavelength was 480 nm, while the emission wavelength was 535 nm. Fluorescence was measured using a 96-well plate reader (Tecan Infinite 200 PRO) and the transcriptional activity of the mixture was subsequently analyzed.

To investigate whether the strong transcriptional inhibition observed with compounds **2a** and **2u** resulted from direct disruption of the RNAP–σ^A interaction as opposed to indirect effects, we used a microscale thermophoresis (MST) assay to quantitatively assess formation of the RNAP holo-enzyme complex under controlled conditions. Freshly purified σ^A and RNAP were prepared as described above and these proteins were subsequently buffer-exchanged into an MST-compatible buffer consisting of 25 mM HEPES (pH 7.5) and 250 mM NaCl to ensure identical ionic and buffer conditions. For fluorescent detection, σ^A was labeled using the RED-NHS Protein Labeling Kit (NanoTemper Technologies), in which the NHS-ester reactive dye covalently couples to primary amines of lysine residues, forming stable amide bonds without disrupting protein integrity. In this assay, σ^A was first exchanged into labeling buffer (25 mM HEPES, 250 mM NaCl) using the A-column provided in the kit to remove interfering components. Subsequently, 90 μL of 10 μM σ^A was incubated with 10 μL of 300 μM RED-NHS dye for 30 min at room temperature in the dark with gentle mixing. Excess unreacted dye was removed using the B-column supplied with the kit, and the labeling efficiency was verified by fluorescence measurement on the NanoTemper instrument. For MST measurements, labeled σ^A was used at a final concentration of 20 nM, while RNAP was serially diluted in a 16-point 1:1 dilution series with a maximum concentration of 2.5 μM. Given that RNAP is a multi-subunit complex prone to heat-induced adsorption, protein concentrations and buffer conditions were extensively optimized to achieve binding saturation while minimizing capillary wall interactions. The final MST buffer additionally contained 0.01 mg/mL BSA and 0.05% Tween-20 to reduce surface adsorption artifacts. All samples were

equilibrated prior to measurement to ensure that the apo RNAP complex could be stably assembled prior to the assay. Thermophoresis was performed using a Monolith NT.115 instrument (NanoTemper Technologies) with standard capillaries (Cat# MO-K022-1000), employing low MST power and 40% LED power to balance signal intensity and thermal stability. Normalized fluorescence (ΔFnorm) was plotted against RNAP concentration and fitted using a 1:1 binding model to determine the apparent dissociation constant (Kd). For competition experiments, σ^A was pre-incubated with compound **2u** or **2p** prior to RNAP titration, allowing assessment of compound-mediated disruption of the RNAP–σA interaction under equilibrium conditions.

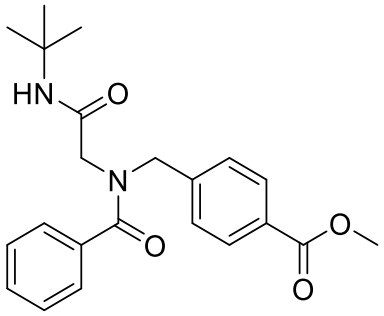
Antibacterial assay

Virulent *M. tuberculosis* H37Rv was inoculated from the frozen stock in 7H9-OADS medium and cultured at 37 ºC with shaking at 150 rpm until a turbid growth is obtained. Subsequently, cultures were diluted in 7H9-OADS medium to an optical density at 600 nm (OD_{600}) of 0.01 and dispensed as 100 µl/well in different wells of a U-bottom 96-well plate (Costar) containing 2.5 µl of respective drugs prepared as 10 mM stock solution in dimethyl sulfoxide (DMSO) to achieve the final concentration of 250 µM in each well. Pathogenic *S. aureus* was cultured from the frozen stock in the LB medium and incubated at 37 ºC with shaking at 200 rpm for overnight to achieve turbid growth. Cultures were diluted in LB medium to OD_{600} of 0.01 that were subsequently dispensed as 100 µl/well in different wells of a U-bottom 96-well plate (Costar). One microliter of the respective drugs was added in each well from the 10 µM stock solution so that the final concentration of 100 µM is achieved in each well. Simultaneously, wells without drug (UT) or with equivalent volume of DMSO were used as controls. Growth was determined by monitoring pellet formation at the bottom of the well by visually inspecting plate after 10 days (*M. tuberculosis*) or 48 h (*S. aureus*) of static incubation at 37 ºC.

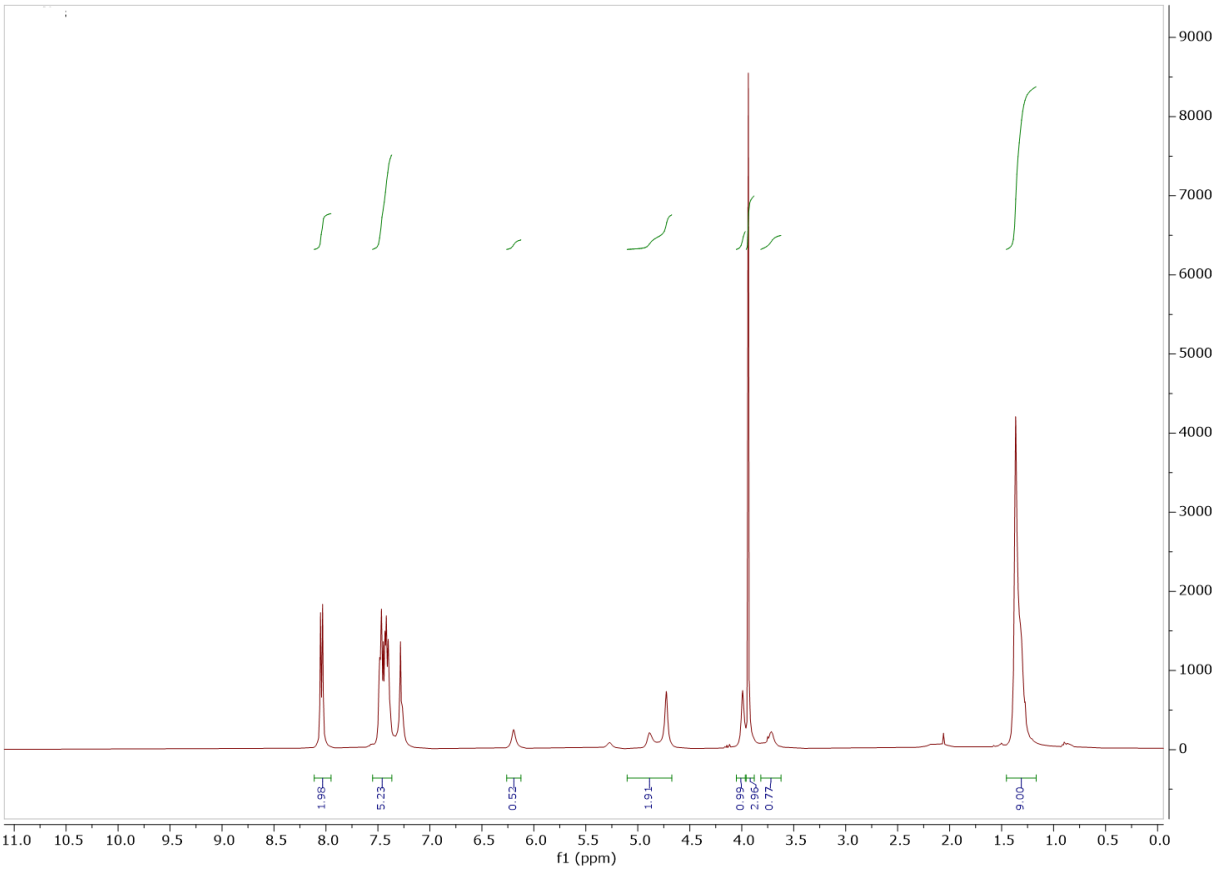
For MIC determination against *S. aureus*, serial dilutions of the inhibitor were prepared in LB medium and 50 µl was dispensed in each well of a U-bottom 96-well plate (Costar) containing equal volume of bacterial culture at OD_{600} of 0.02. As mentioned above, wells without drug (UT) or with 1 µl DMSO were simultaneously used as controls. Growth was determined by monitoring pellet formation at the bottom of the well by visually inspecting plate after 48 h of static incubation at 37 ºC. At the end of incubation, 10 µl cultures from the drug-treated or untreated wells were spread on LB agar plate to determine killing.

Spectral data

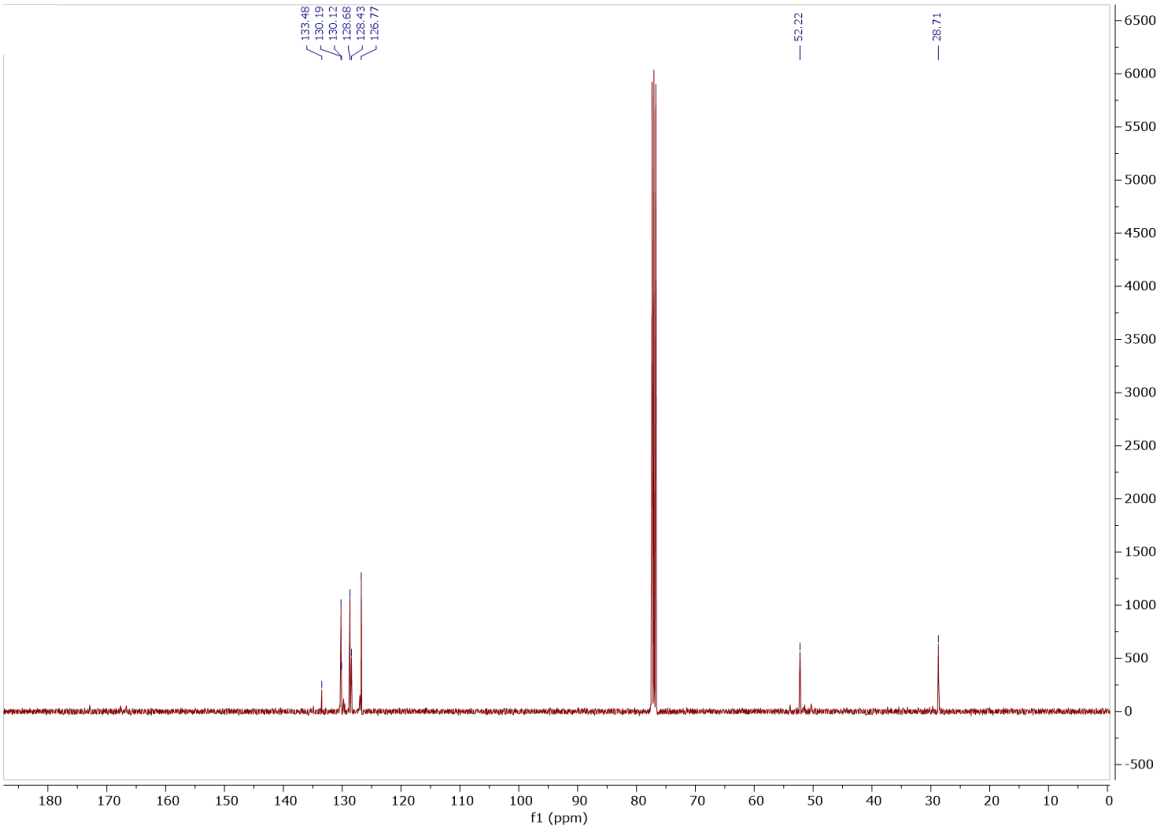
Spectral data of compound **1a**



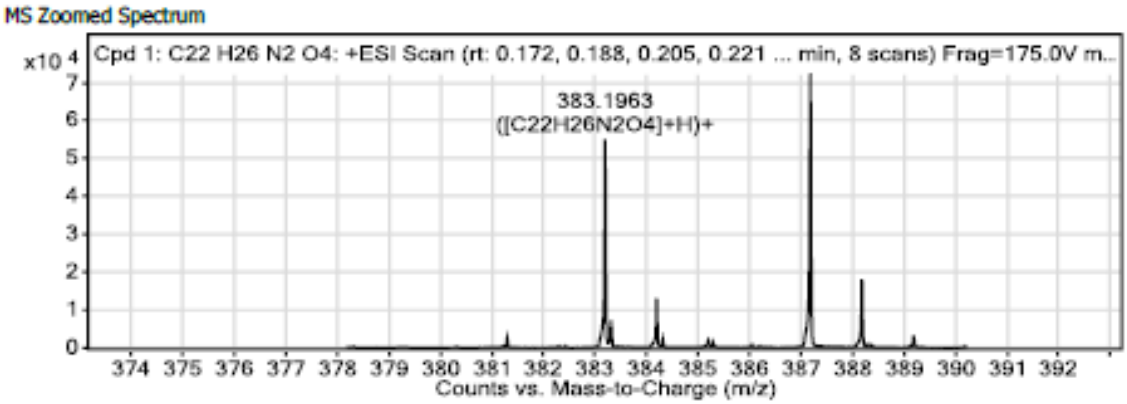
^{1}H NMR



^{13}C NMR



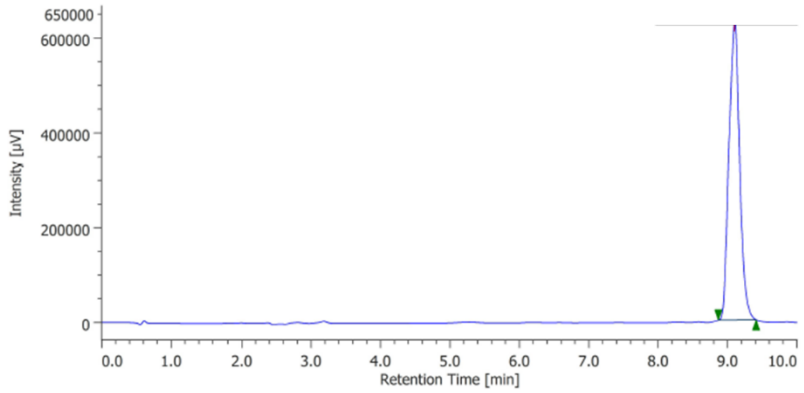
HRMS



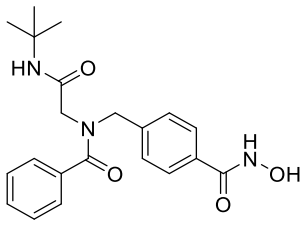
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
383.1963	383.1965	0.53	1	55307.7	C22H26N2O4	(M+H)+
384.1998	384.1997	-0.02	1	13518.85	C22H26N2O4	(M+H)+
385.2002	385.2025	6.01	1	2291.1	C22H26N2O4	(M+H)+

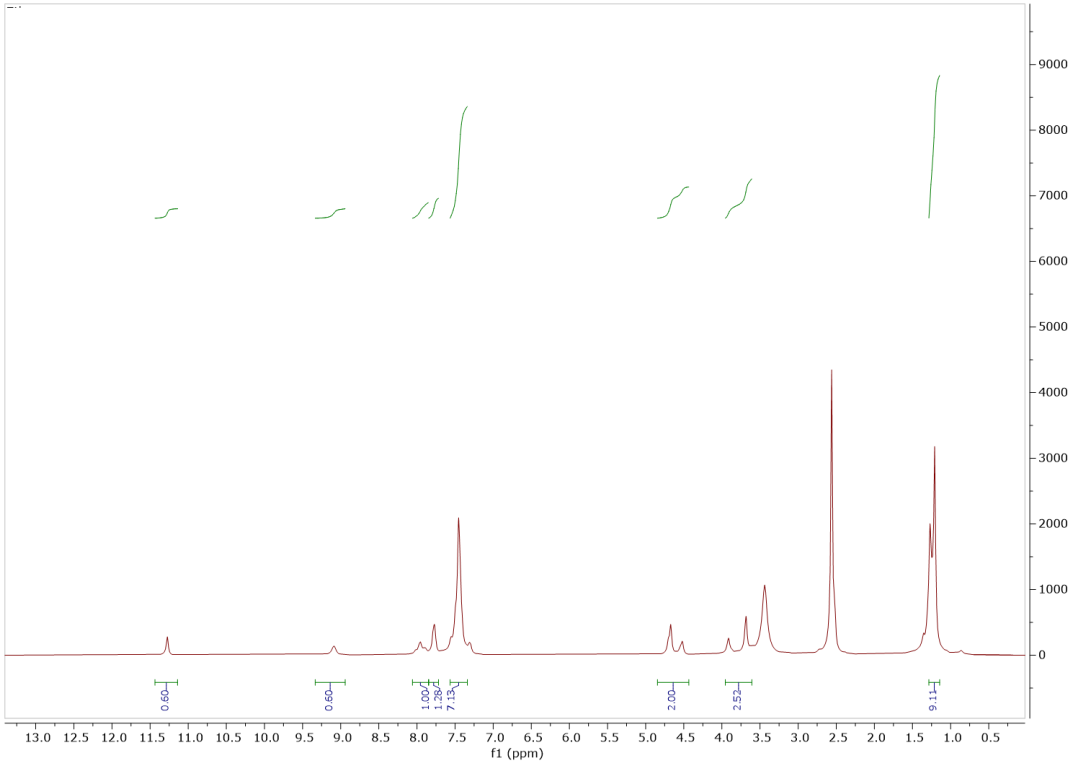
HPLC



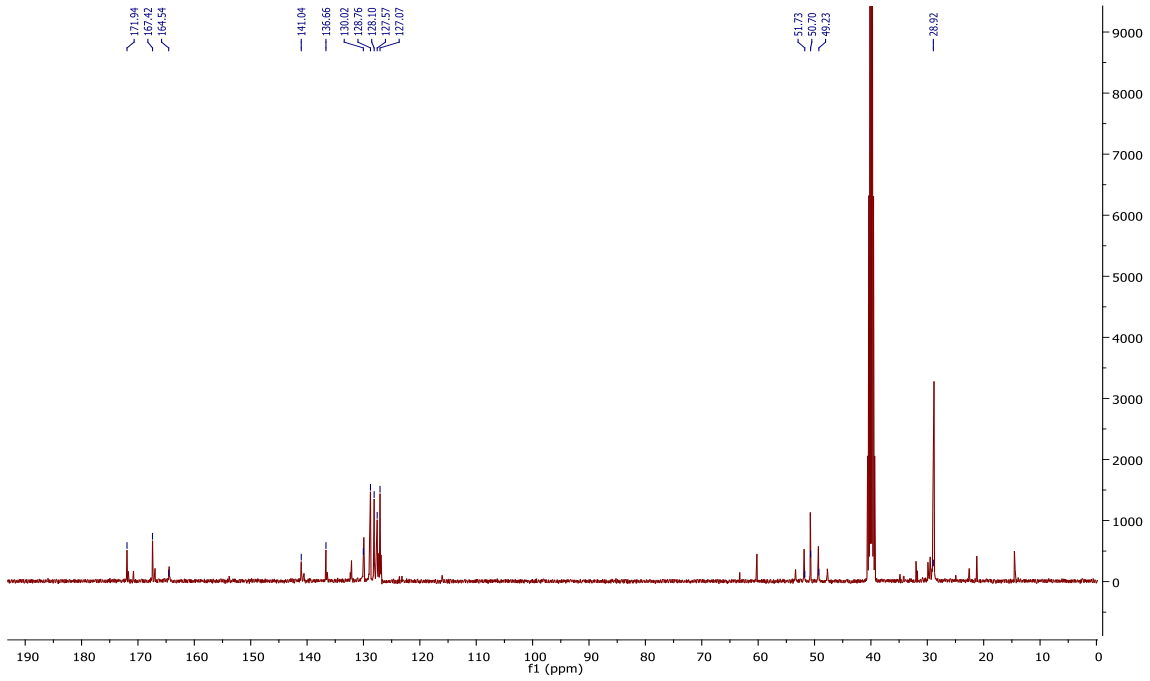
Spectral data of compound **2a**



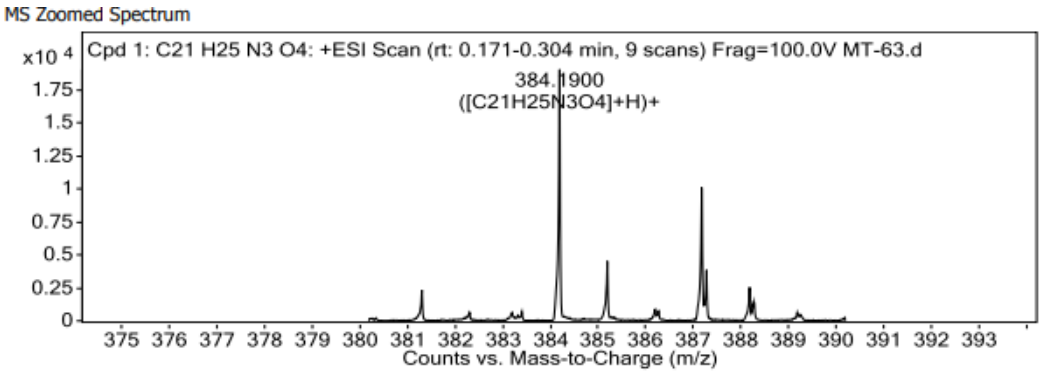
^{1}H NMR



^{13}C NMR



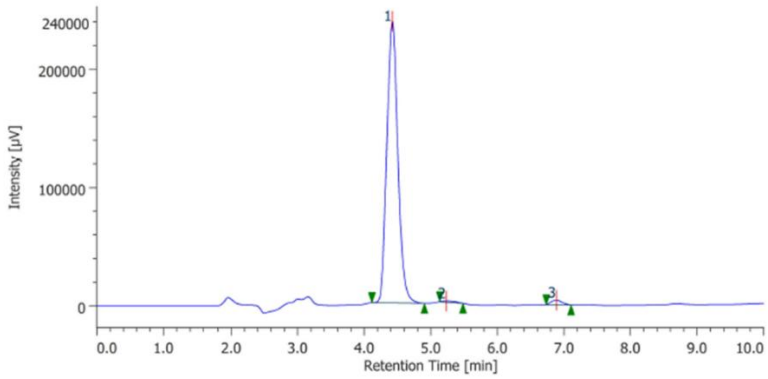
HRMS



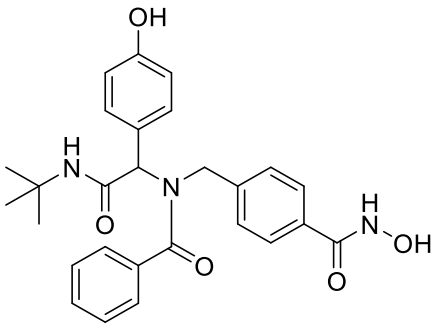
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
384.19	384.1918	4.6	1	19078.53	C21H25N3O4	(M+H)+
385.1921	385.1949	7.24	1	4536.5	C21H25N3O4	(M+H)+
386.1955	386.1975	5.2	1	884.18	C21H25N3O4	(M+H)+

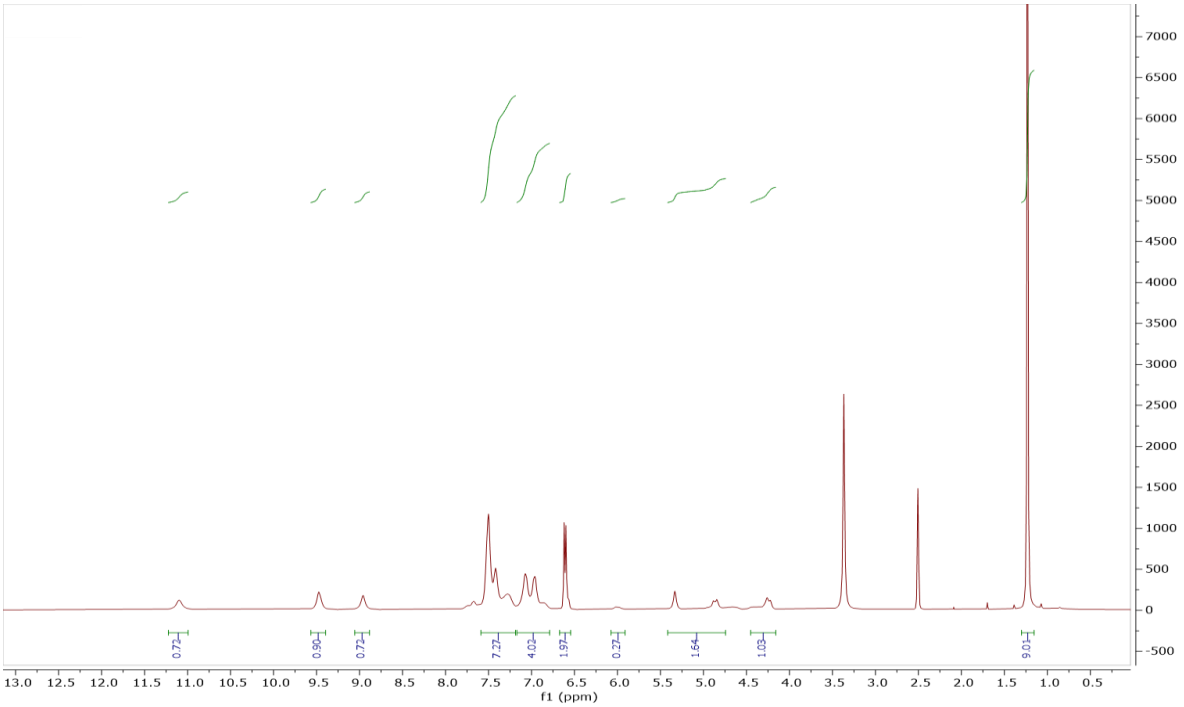
HPLC



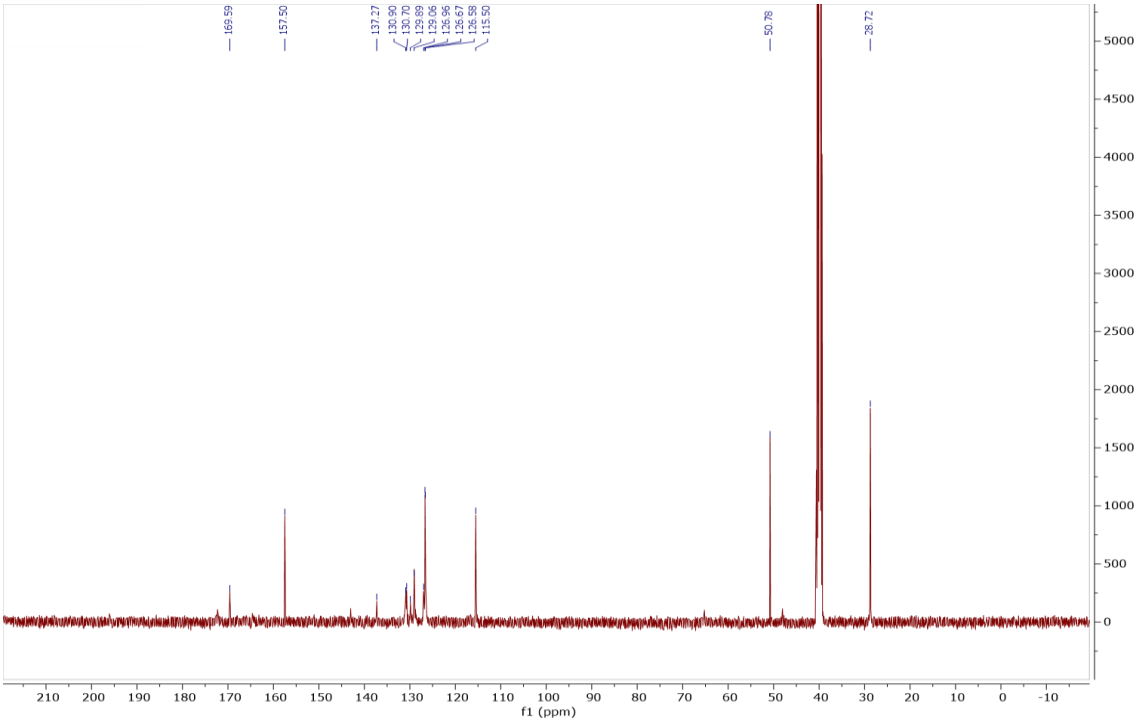
Spectral data of compound **2b**



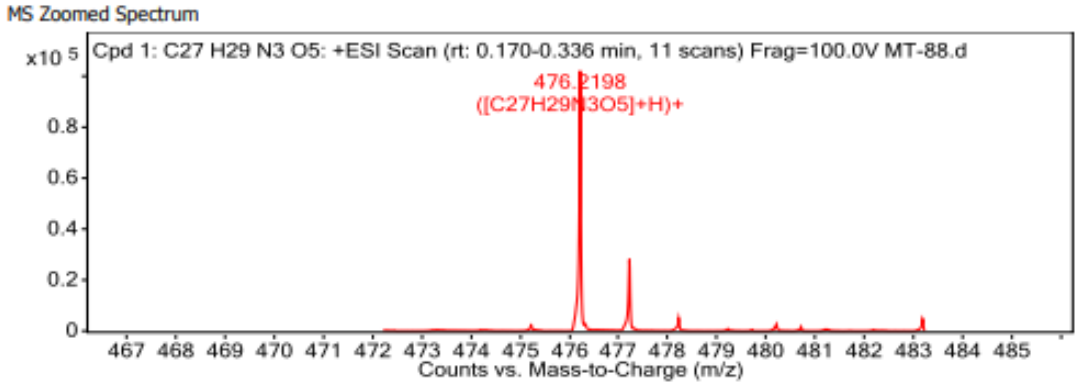
^{1}H NMR



^{13}C NMR



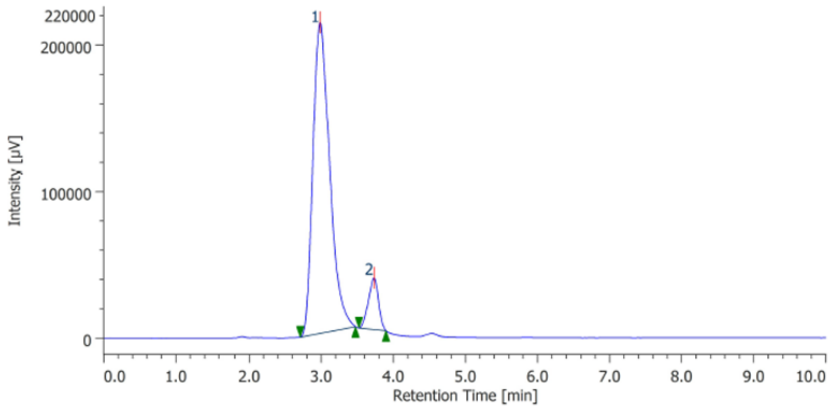
HRMS



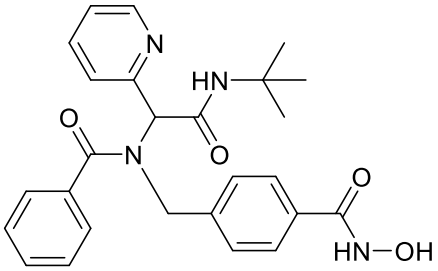
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
476.2198	476.218	-3.74	1	102842.55	C27H29N3O5	(M+H)+
477.2223	477.2212	-2.42	1	28258.16	C27H29N3O5	(M+H)+
478.2249	478.2239	-2.07	1	5009.19	C27H29N3O5	(M+H)+
479.2265	479.2266	0.2	1	897.2	C27H29N3O5	(M+H)+

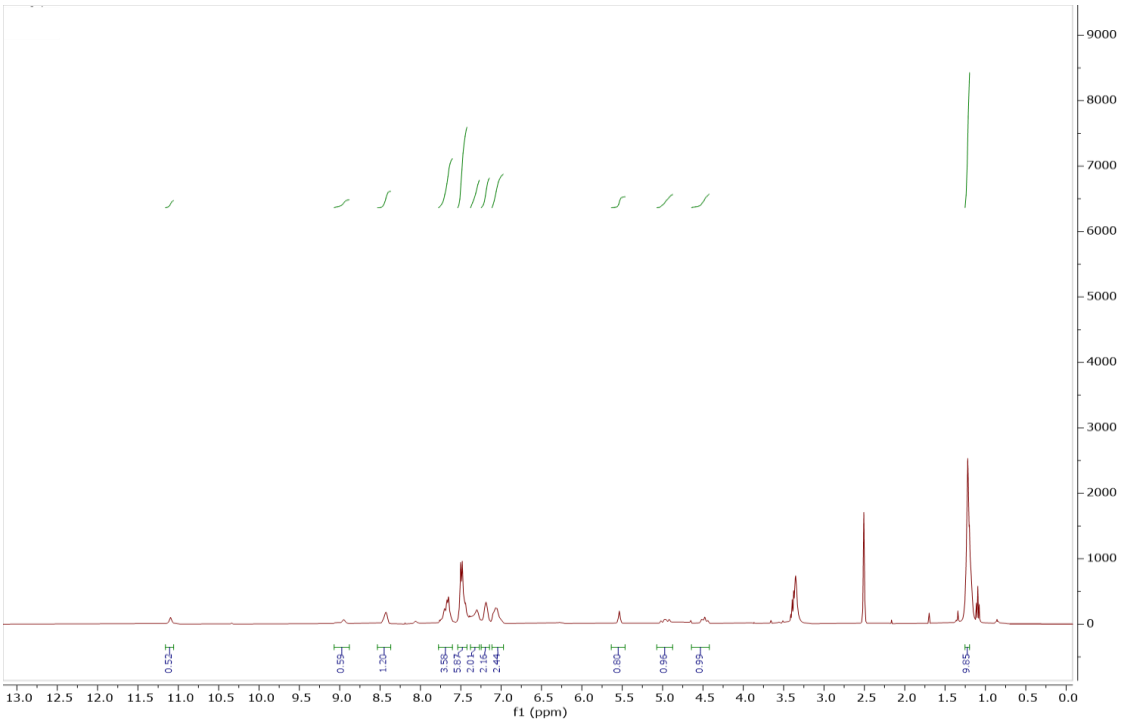
HPLC



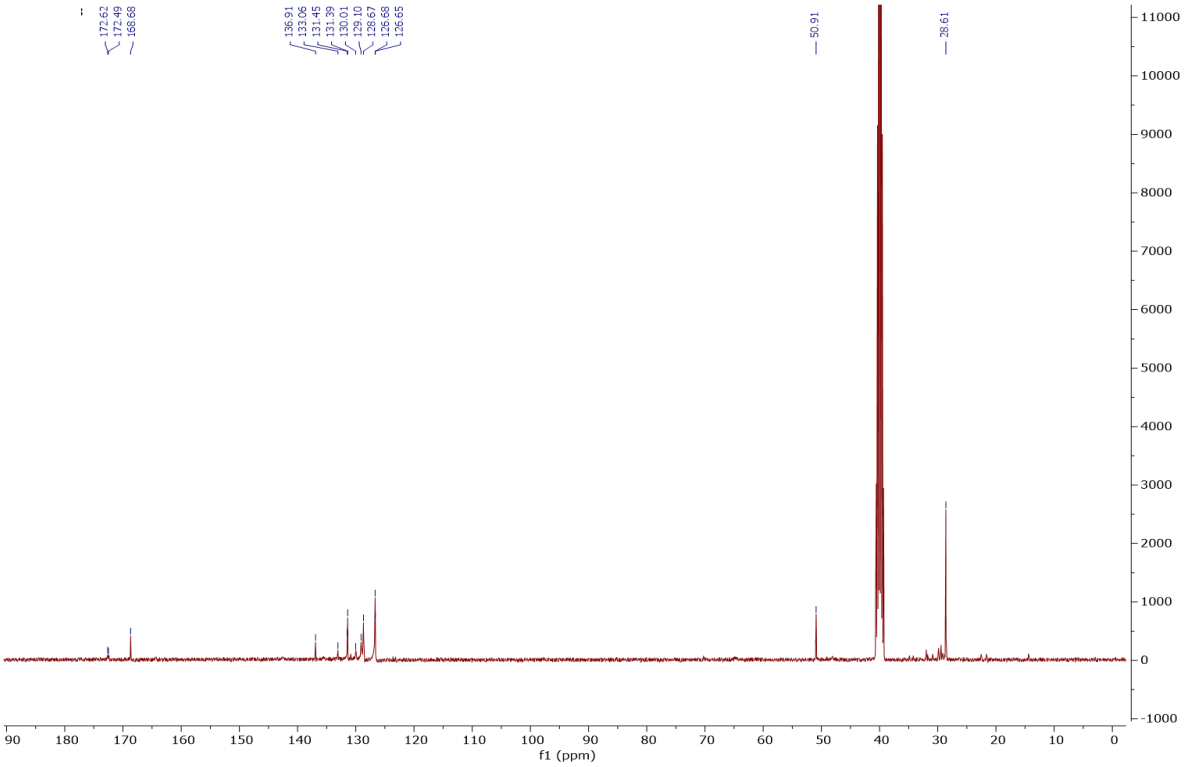
Spectral data of compound **2c**



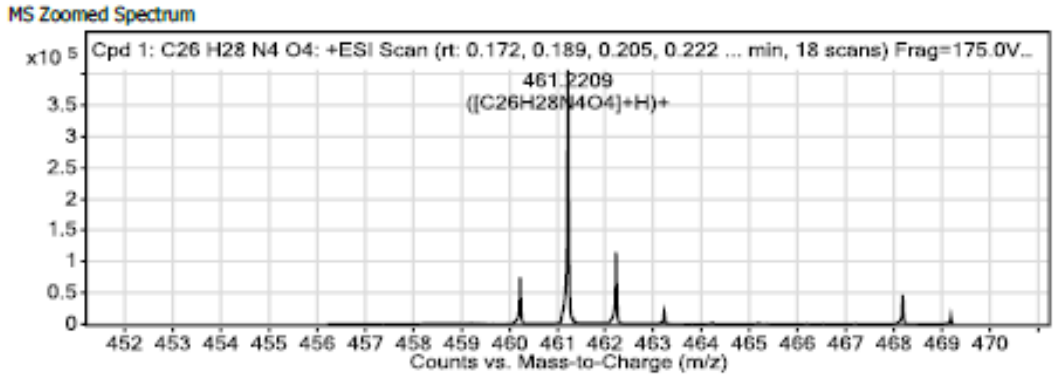
^{1}H NMR



^{13}C NMR



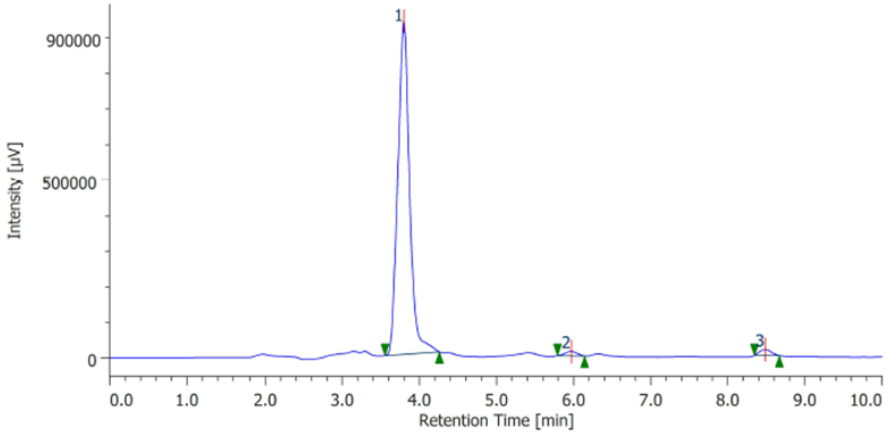
HRMS



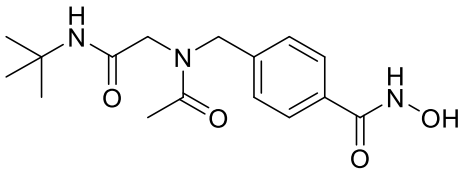
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
461.2209	461.2183	-5.5	1	411996.28	C26H28N4O4	(M+H)+
462.2235	462.2214	-4.47	1	117809.13	C26H28N4O4	(M+H)+
463.2257	463.2242	-3.17	1	18671.12	C26H28N4O4	(M+H)+
464.2278	464.2268	-2.1	1	2353.69	C26H28N4O4	(M+H)+

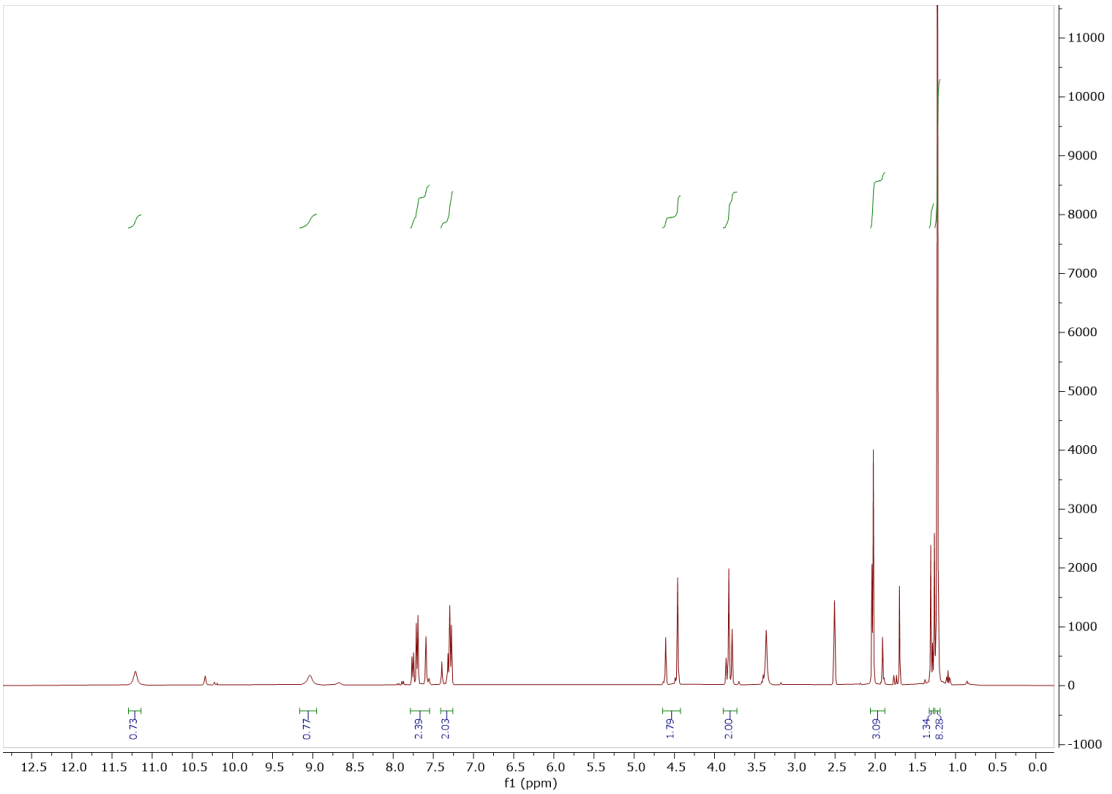
HPLC



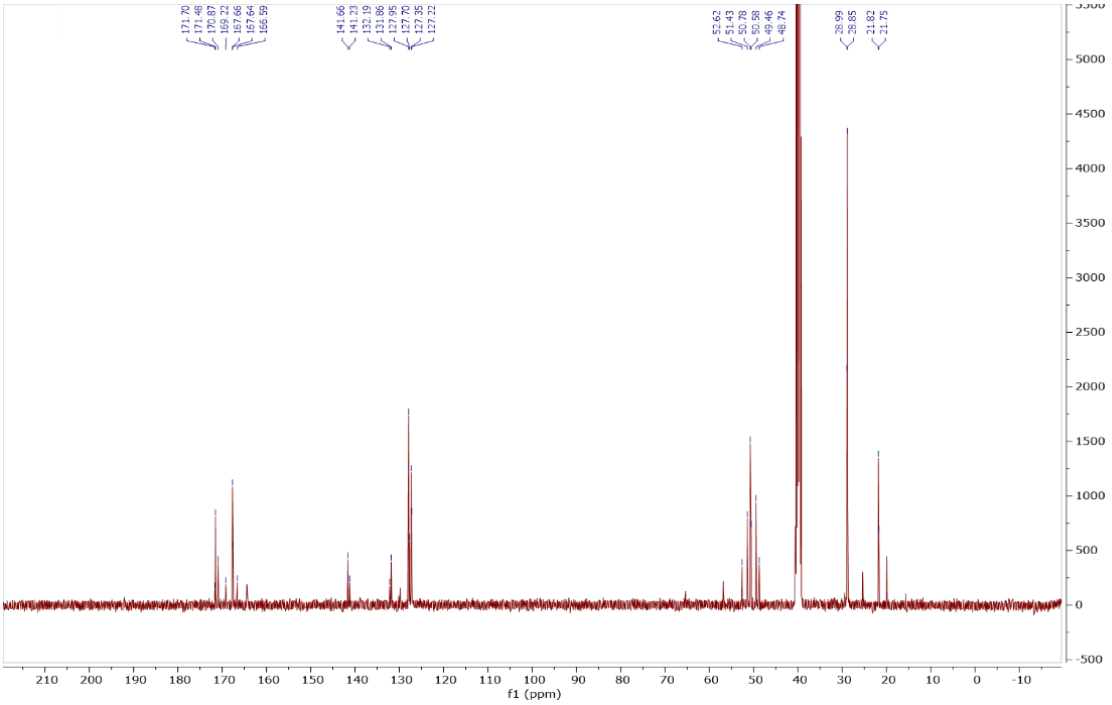
Spectral data of compound **2e**



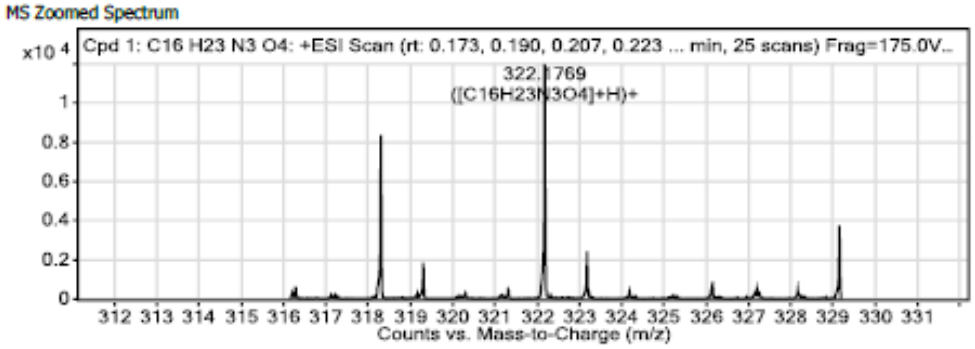
^{1}H NMR



^{13}C NMR



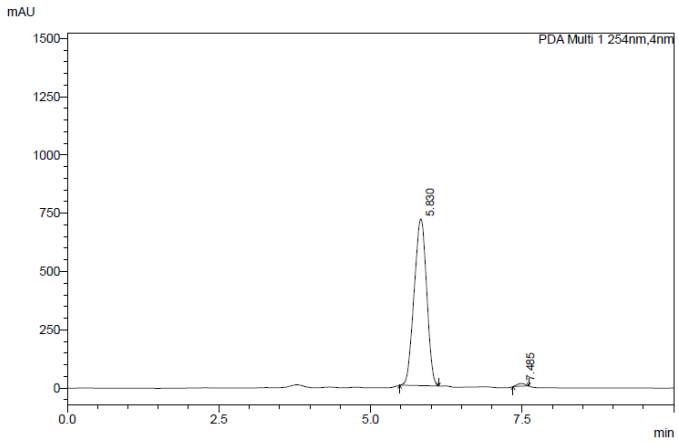
HRMS



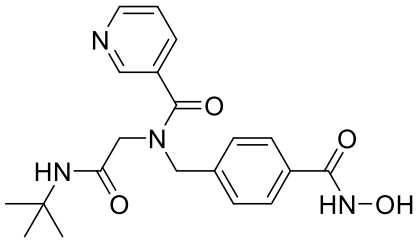
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
321.1684	321.1683	-0.23	1	207.42	C16H23N3O4	M+
322.1769	322.1761	-2.49	1	12352.89	C16H23N3O4	(M+H)+
323.1797	323.1792	-1.71	1	2437.83	C16H23N3O4	(M+H)+
324.1814	324.1816	0.52	1	386.82	C16H23N3O4	(M+H)+

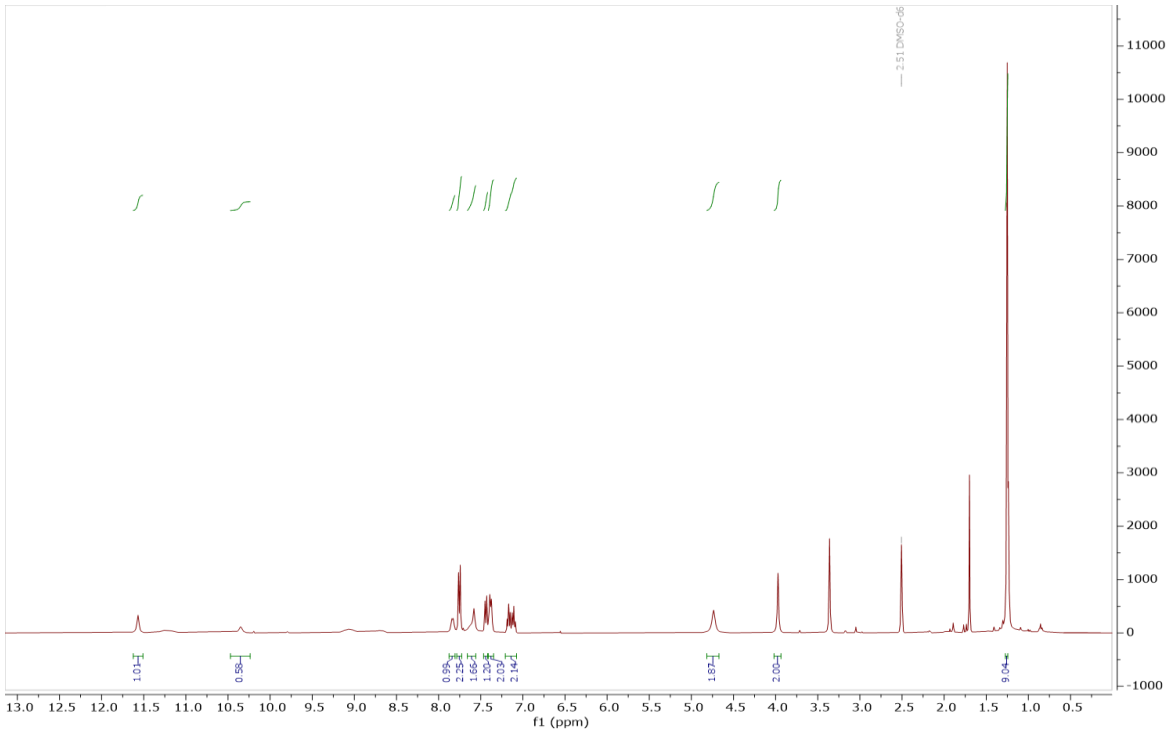
HPLC



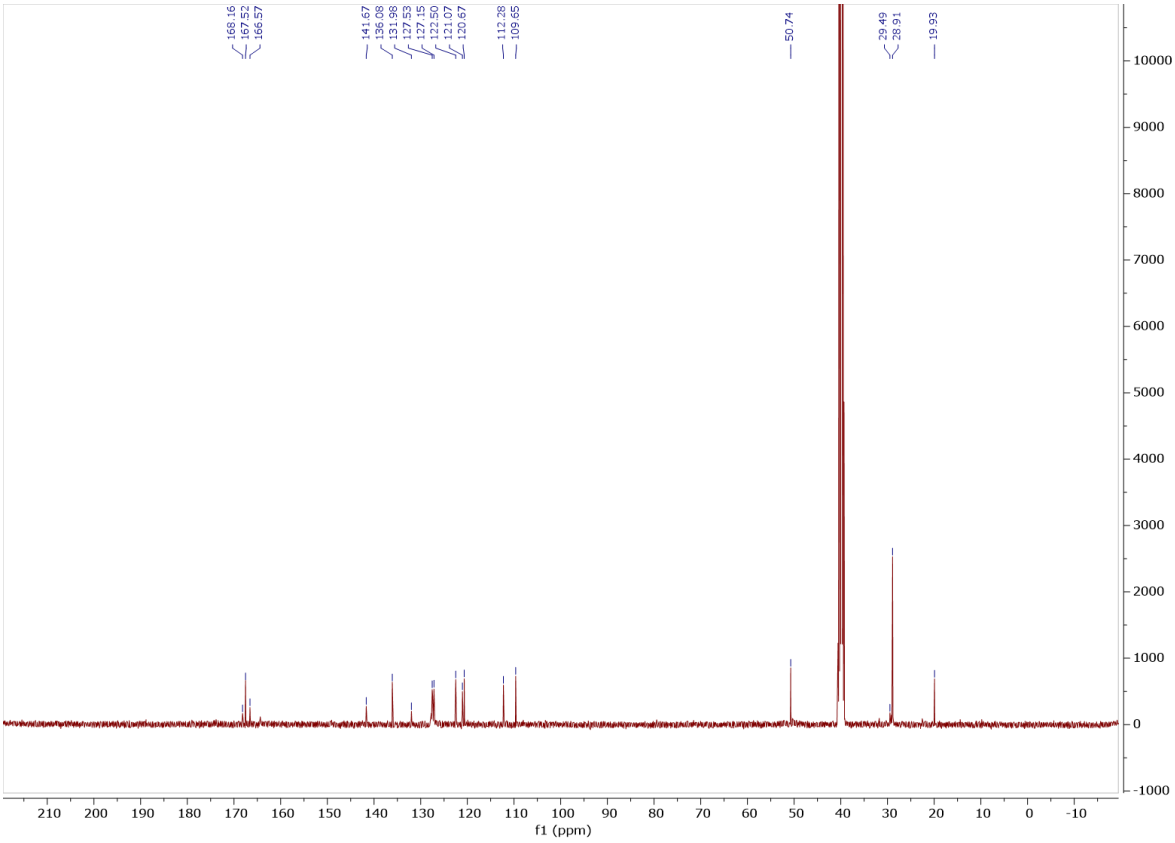
Spectral data of compound **2f**



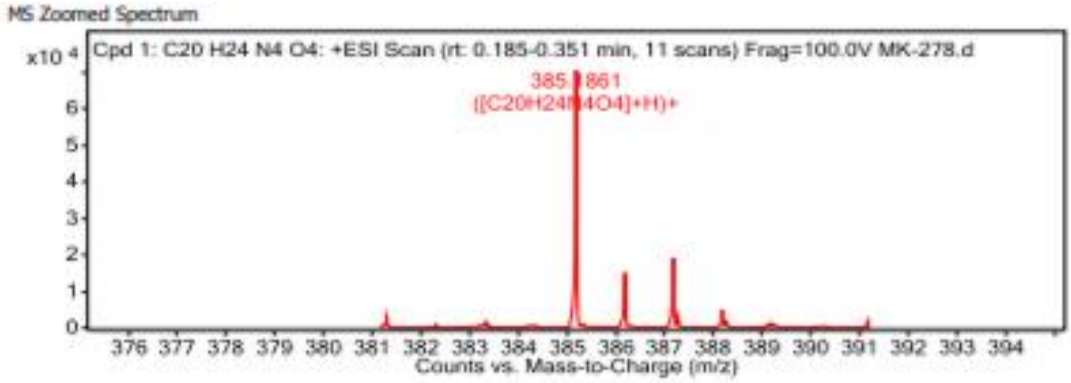
^{1}H NMR



^{13}C NMR



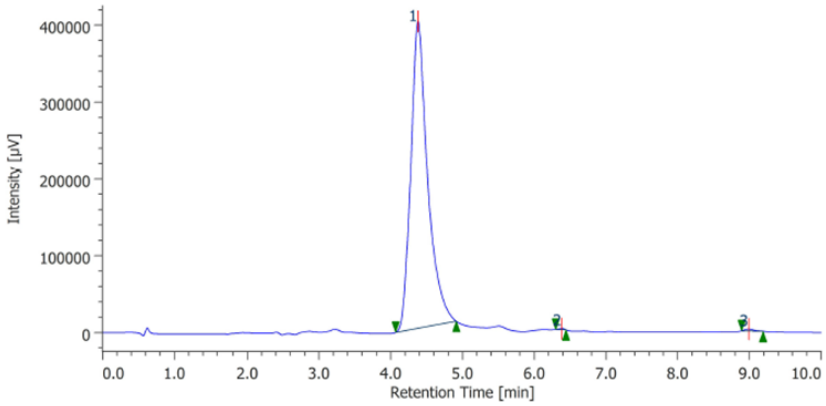
HRMS



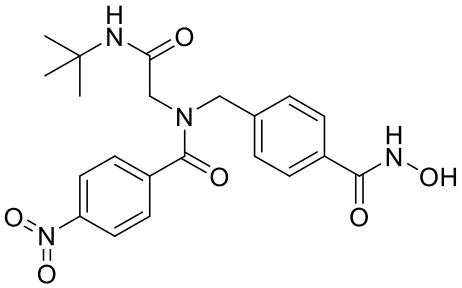
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
385.1861	385.187	2.4	1	70782.25	C20H24N4O4	(M+H)+
386.1885	386.19	3.98	1	15210.81	C20H24N4O4	(M+H)+
387.1799	387.1926	32.92	1	19584.57	C20H24N4O4	(M+H)+

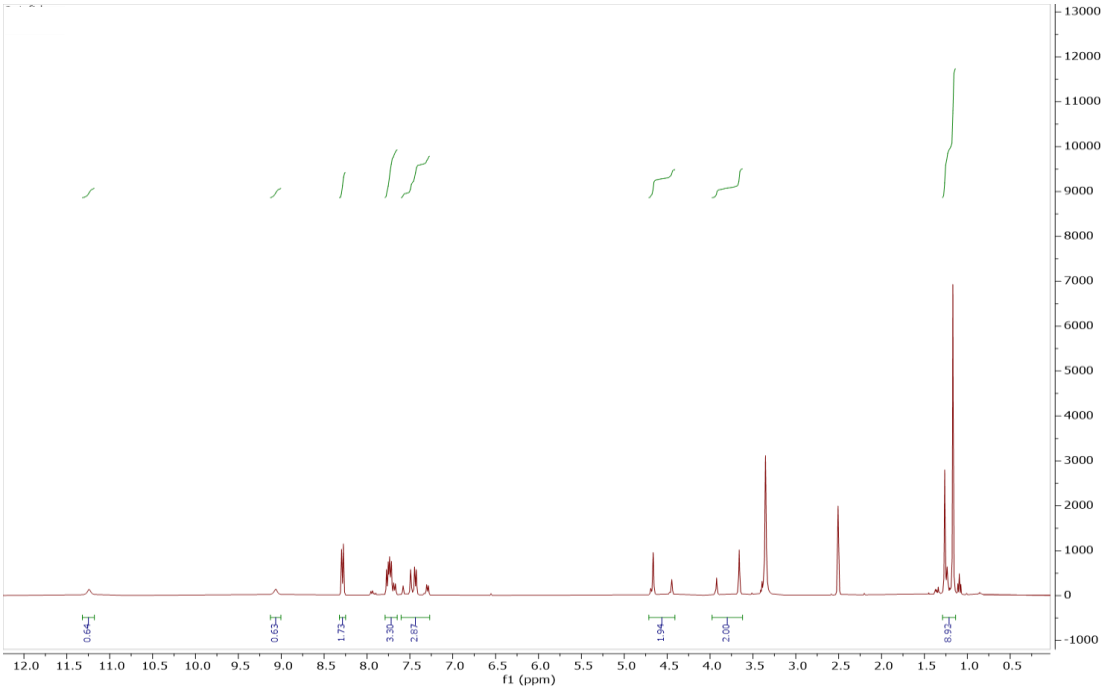
HPLC



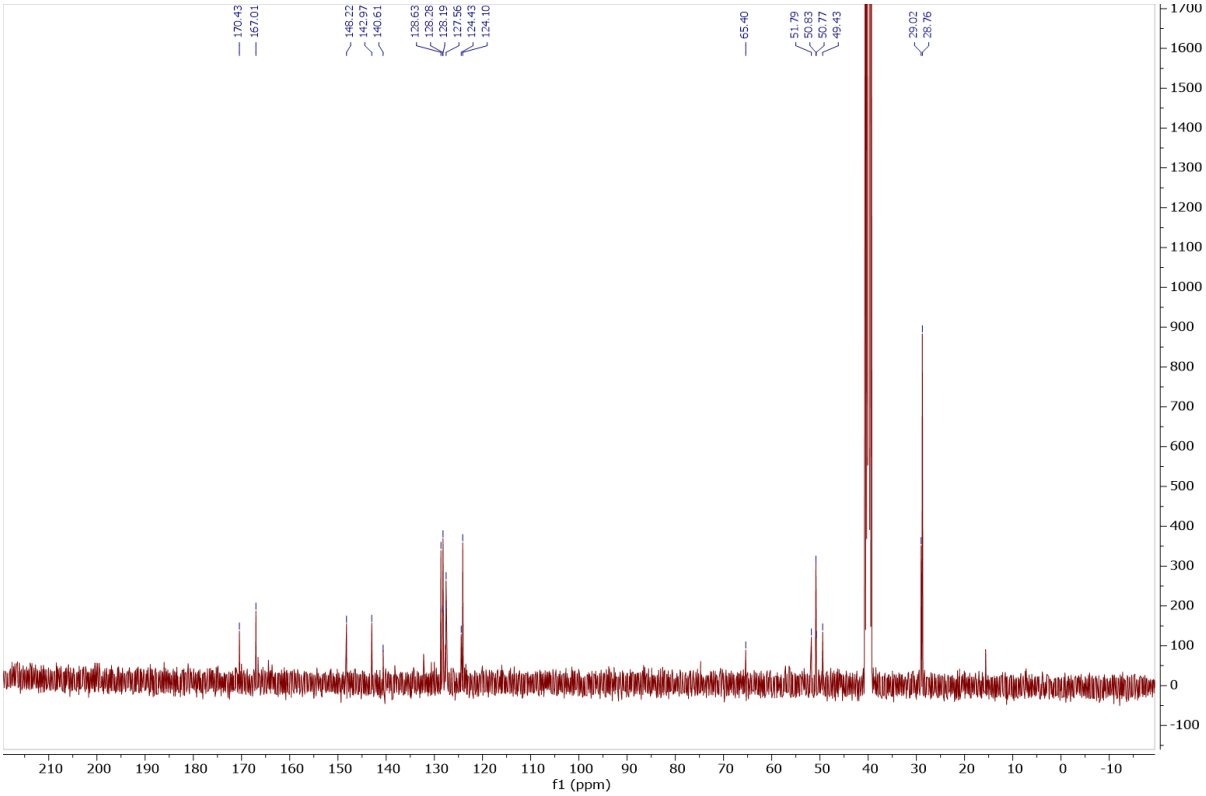
Spectral data of compound **2g**



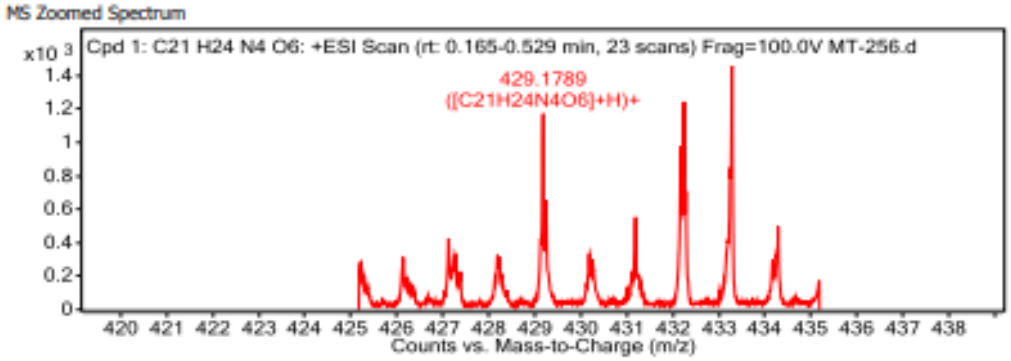
^{1}H NMR



^{13}C NMR



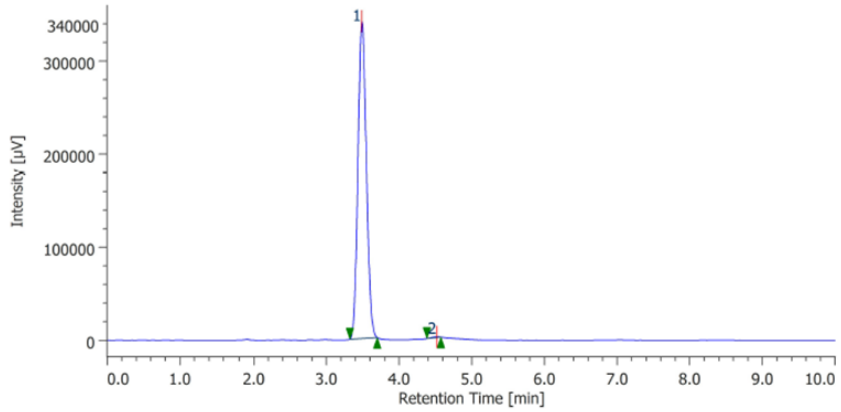
HRMS



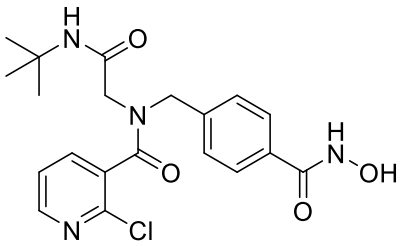
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
429.1789	429.1769	-4.69	1	1178.61	C21H24N4O6	(M+H)+
430.1856	430.1799	-13.3	1	336.28	C21H24N4O6	(M+H)+
431.1839	431.1824	-3.65	1	558.9	C21H24N4O6	(M+H)+

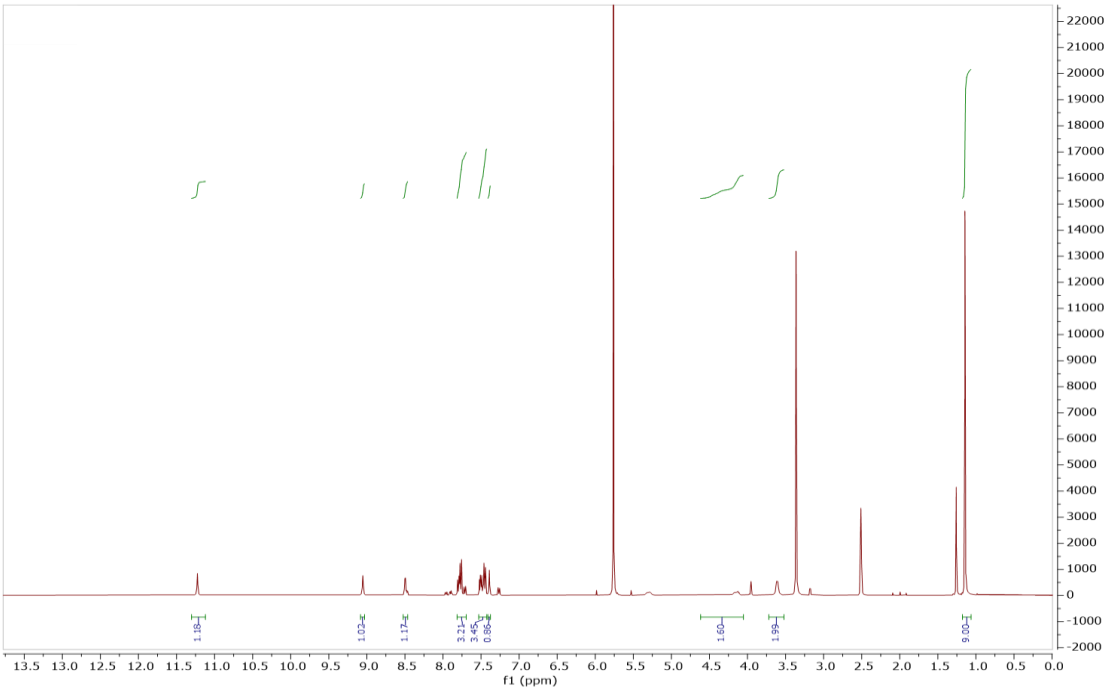
HPLC



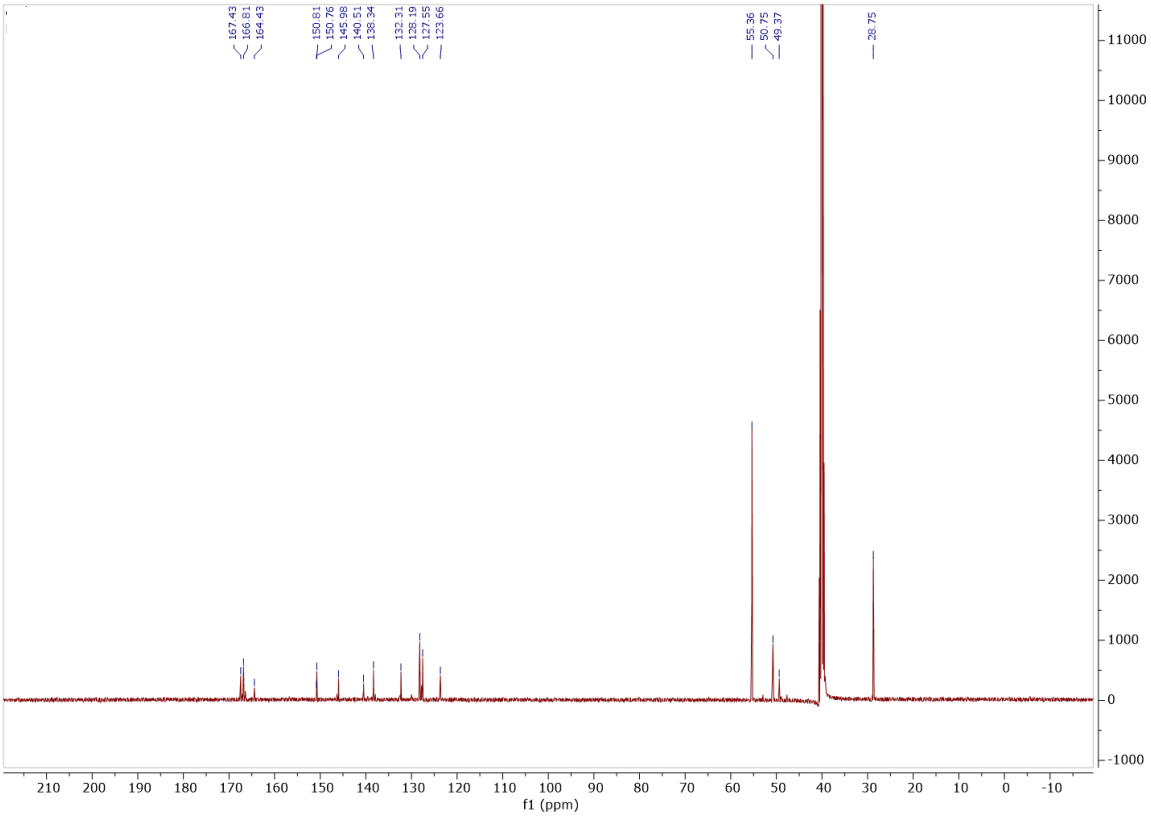
Spectral data of compound **2h**



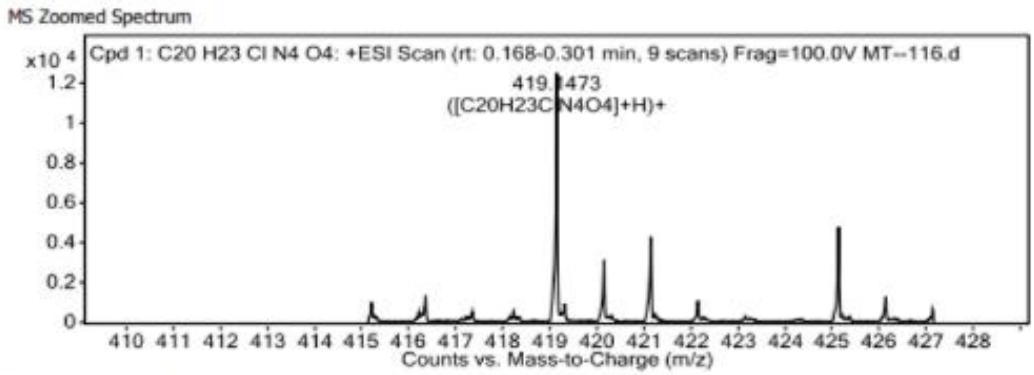
^{1}H NMR



^{13}C NMR



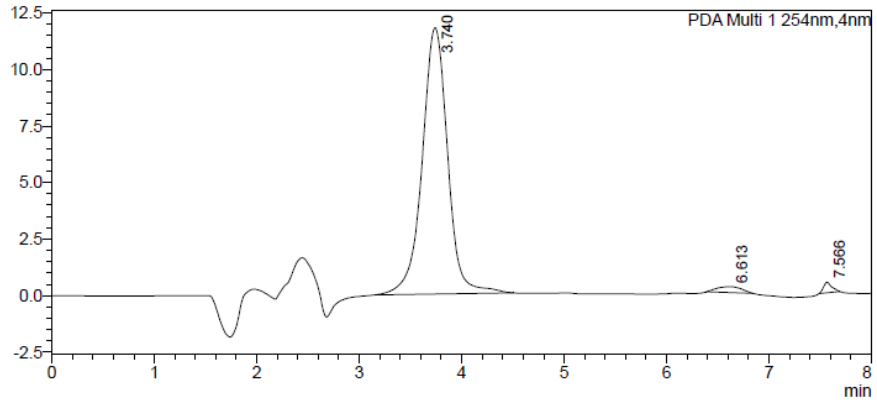
HRMS



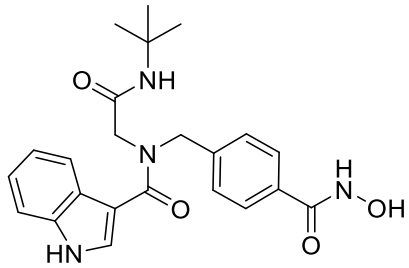
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
419.1473	419.1481	1.84	1	12851.17	C20H23ClN4O4	(M+H)+
420.1492	420.1511	4.36	1	3114.94	C20H23ClN4O4	(M+H)+
421.1442	421.1459	4.2	1	4377.02	C20H23ClN4O4	(M+H)+
422.1487	422.1485	-0.41	1	1100.85	C20H23ClN4O4	(M+H)+
423.1535	423.1509	-6.1	1	291.34	C20H23ClN4O4	(M+H)+

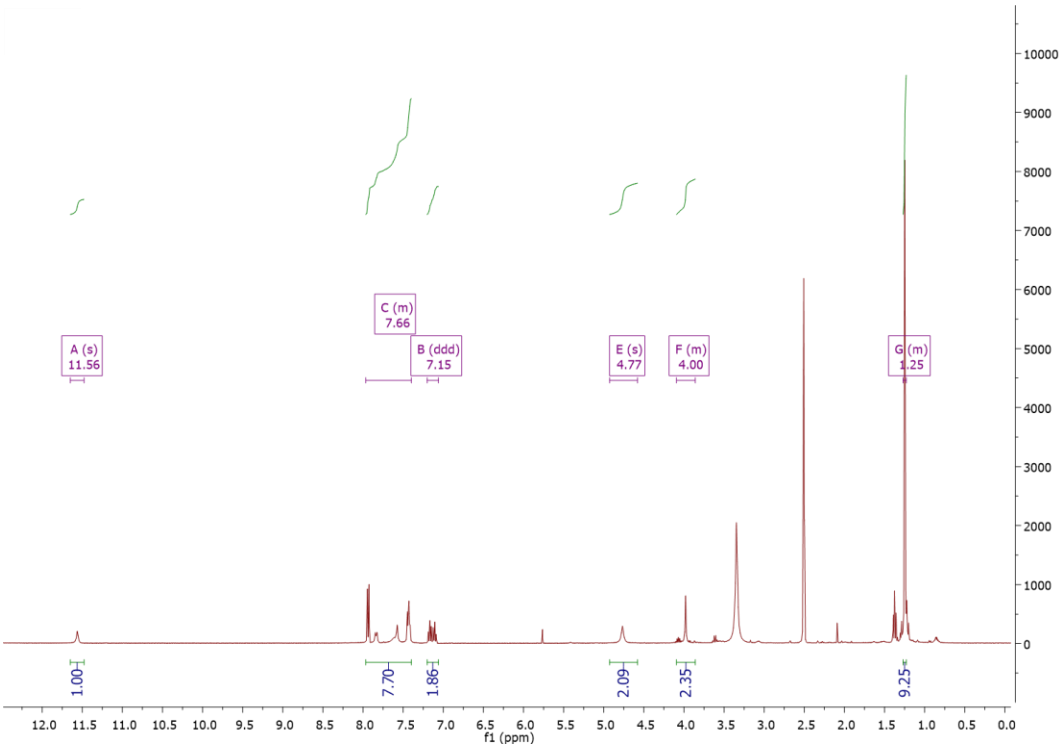
HPLC



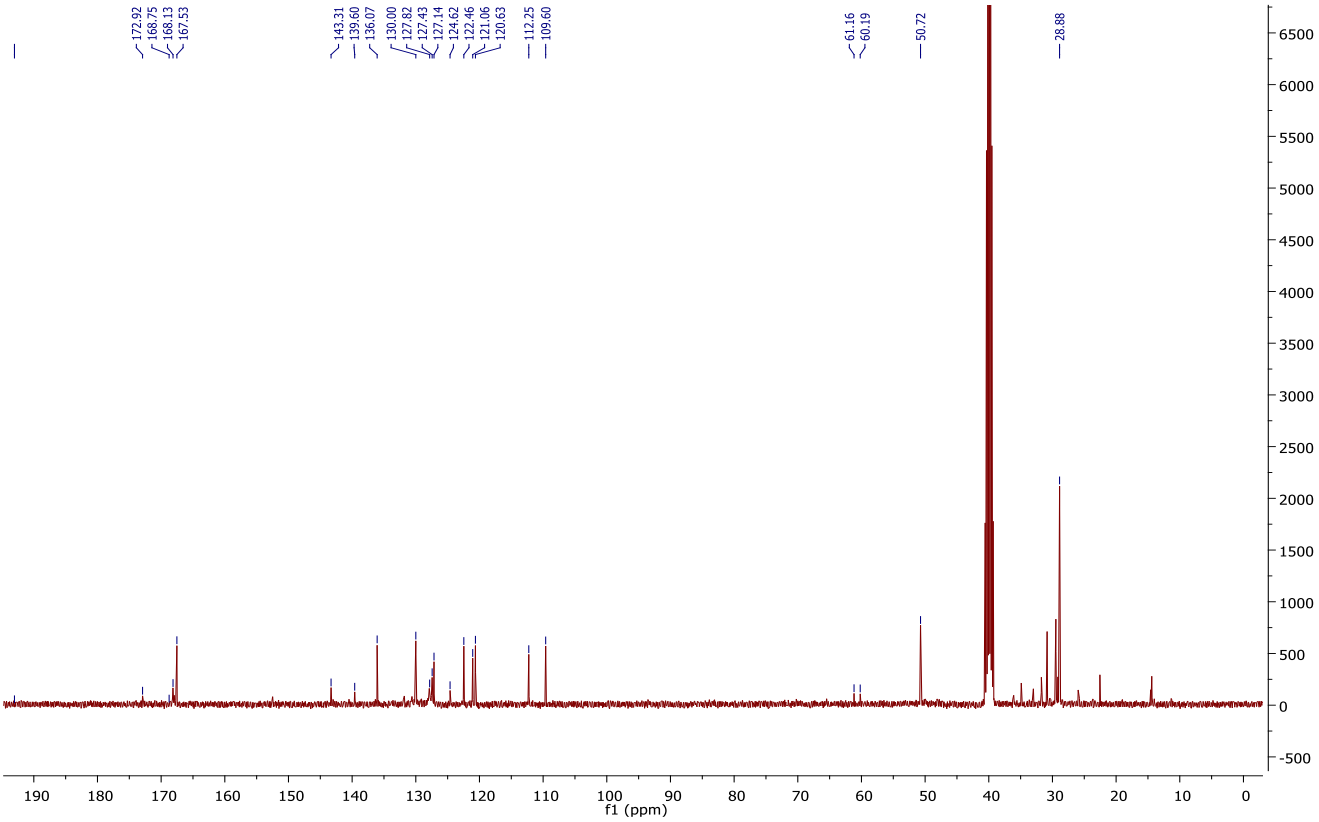
Spectral data of compound **2j**



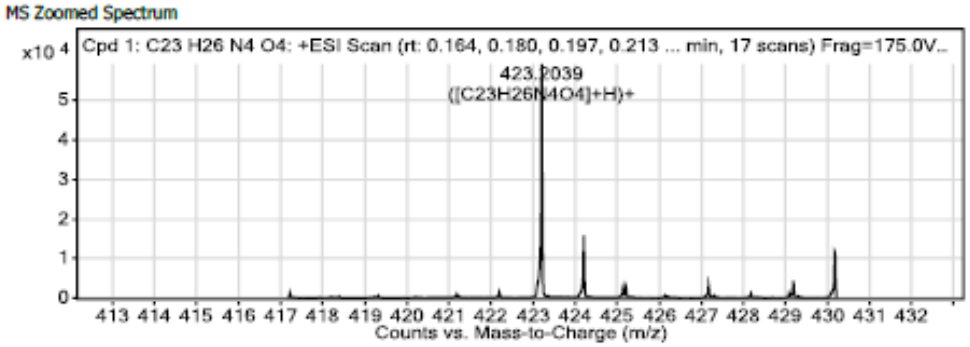
^{1}H NMR



^{13}C NMR



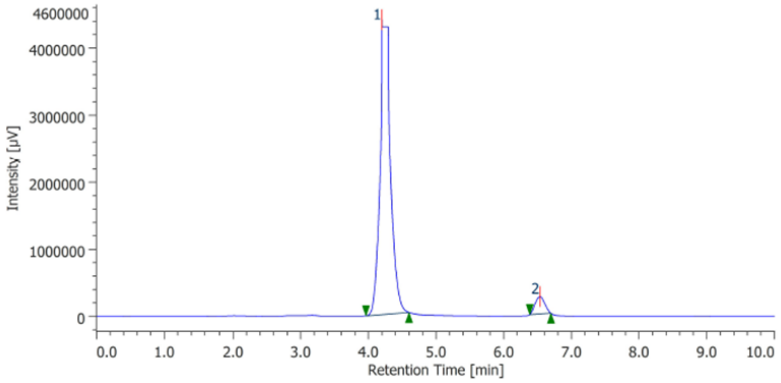
HRMS



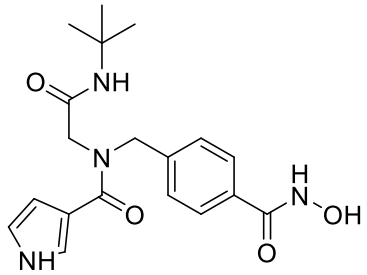
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
422.1963	422.1949	-3.5	1	1469.76	C23H26N4O4	M+
423.2039	423.2027	-2.94	1	59295.91	C23H26N4O4	(M+H)+
424.2068	424.2057	-2.63	1	15834.59	C23H26N4O4	(M+H)+
425.2093	425.2084	-2.06	1	2549.44	C23H26N4O4	(M+H)+

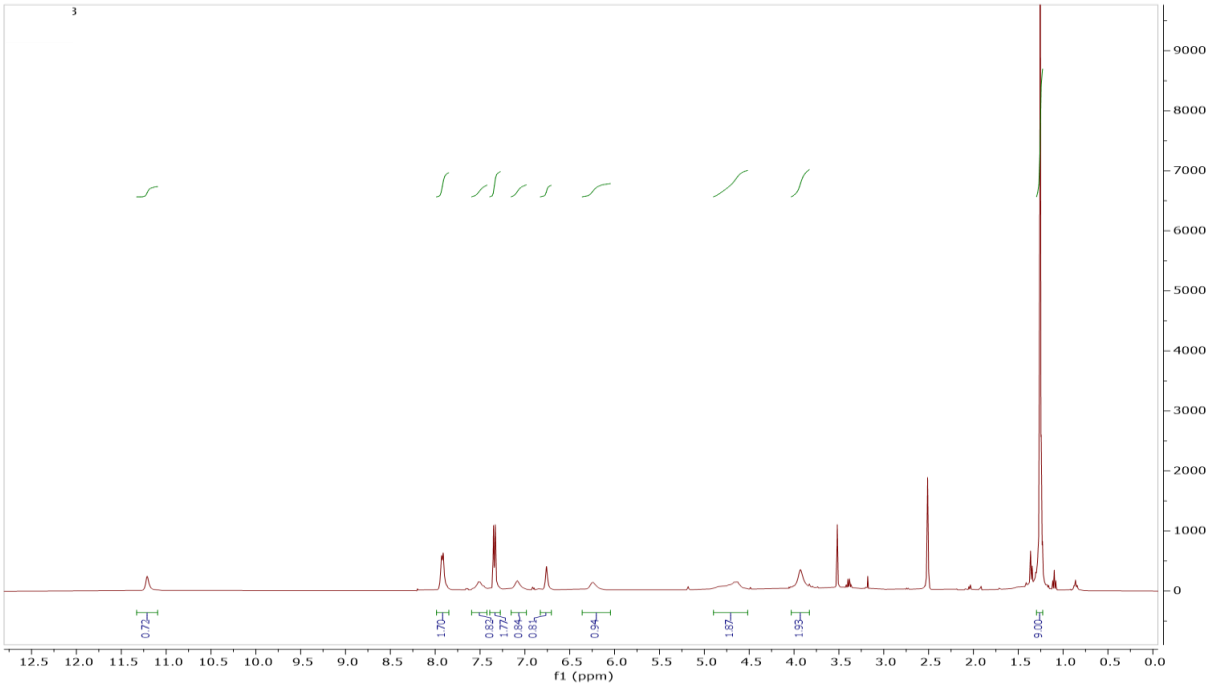
HPLC



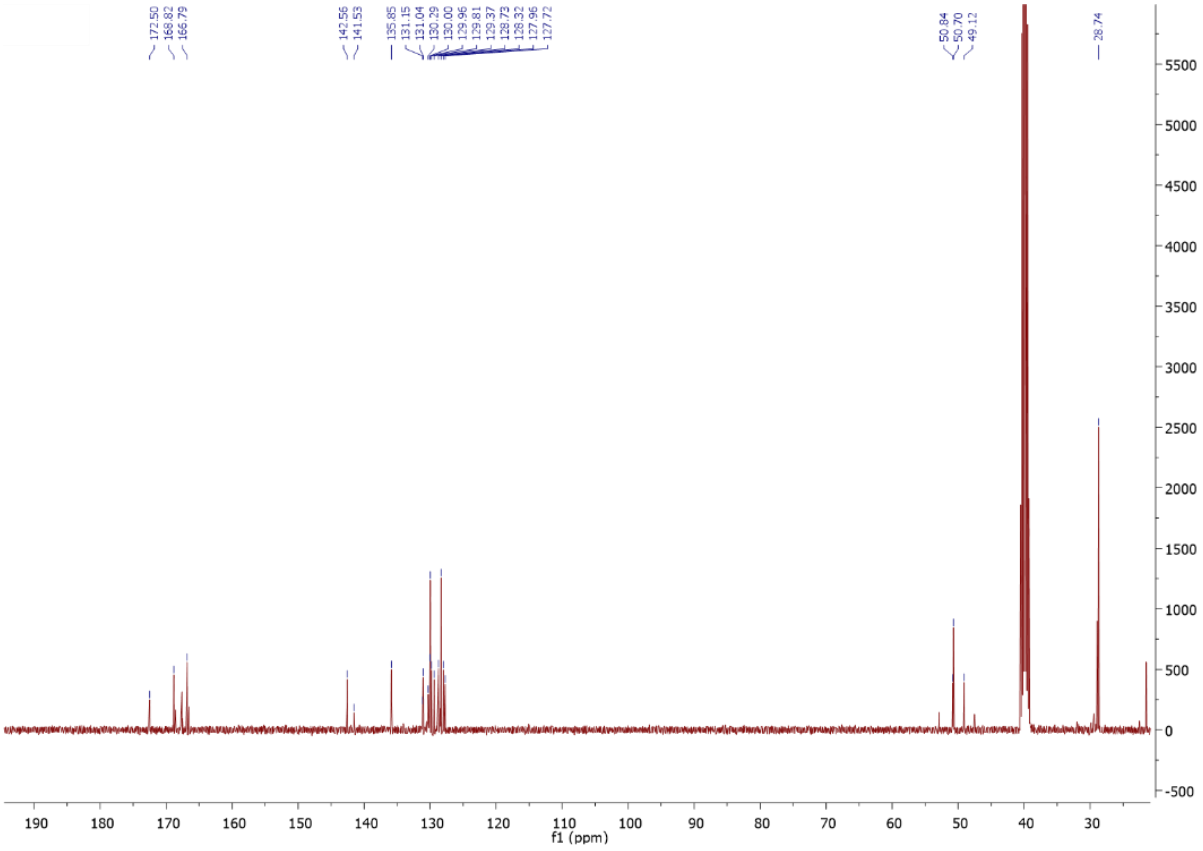
Spectral data of compound **2k**



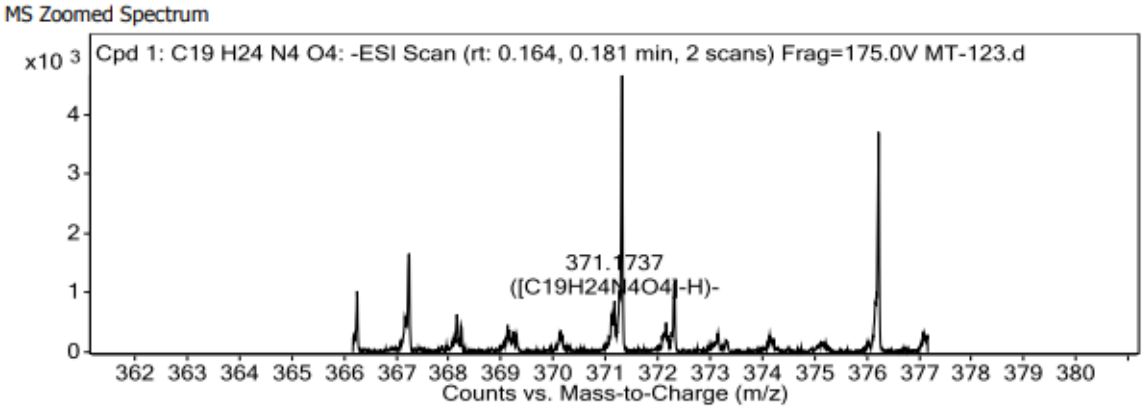
^{1}H NMR



^{13}C NMR



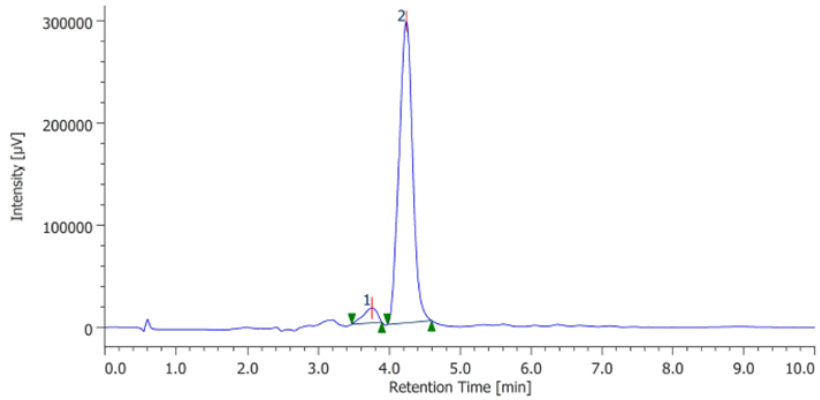
HRMS



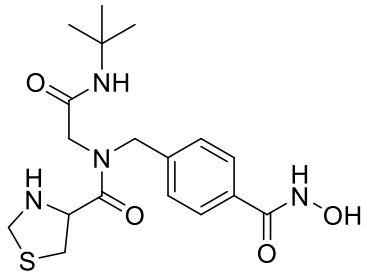
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
371.1737	371.1725	-3.36	1	858.55	C19H24N4O4	(M-H)-
372.164	372.1755	30.92	1	247.52	C19H24N4O4	(M-H)-

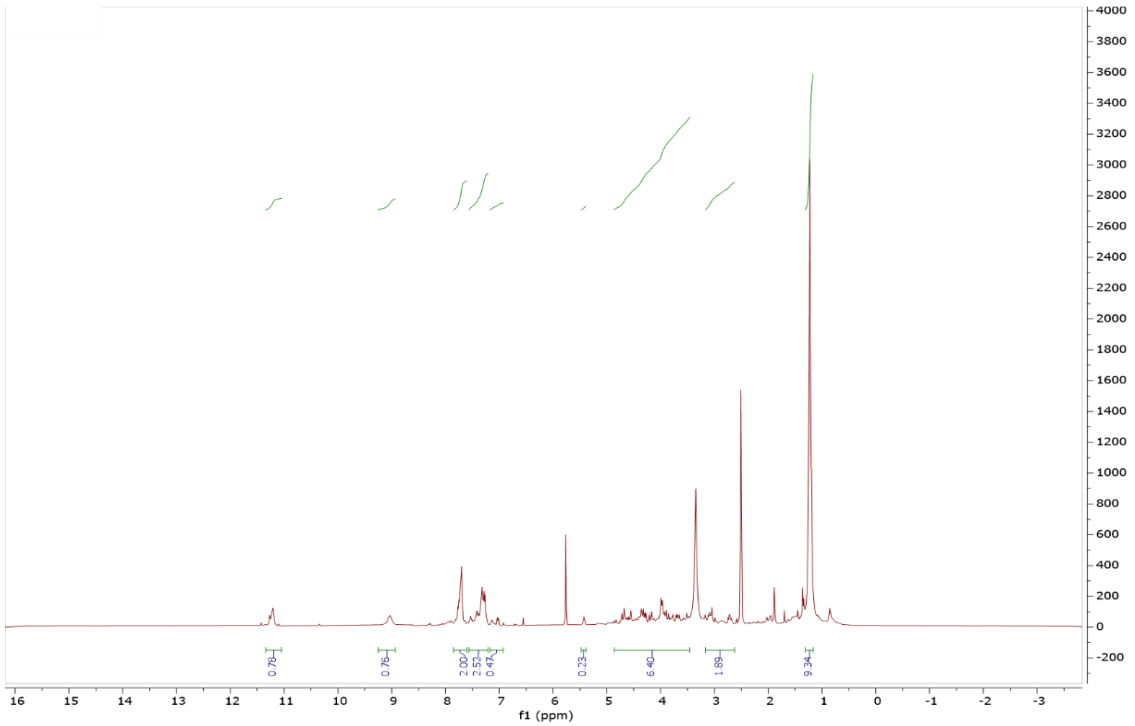
HPLC



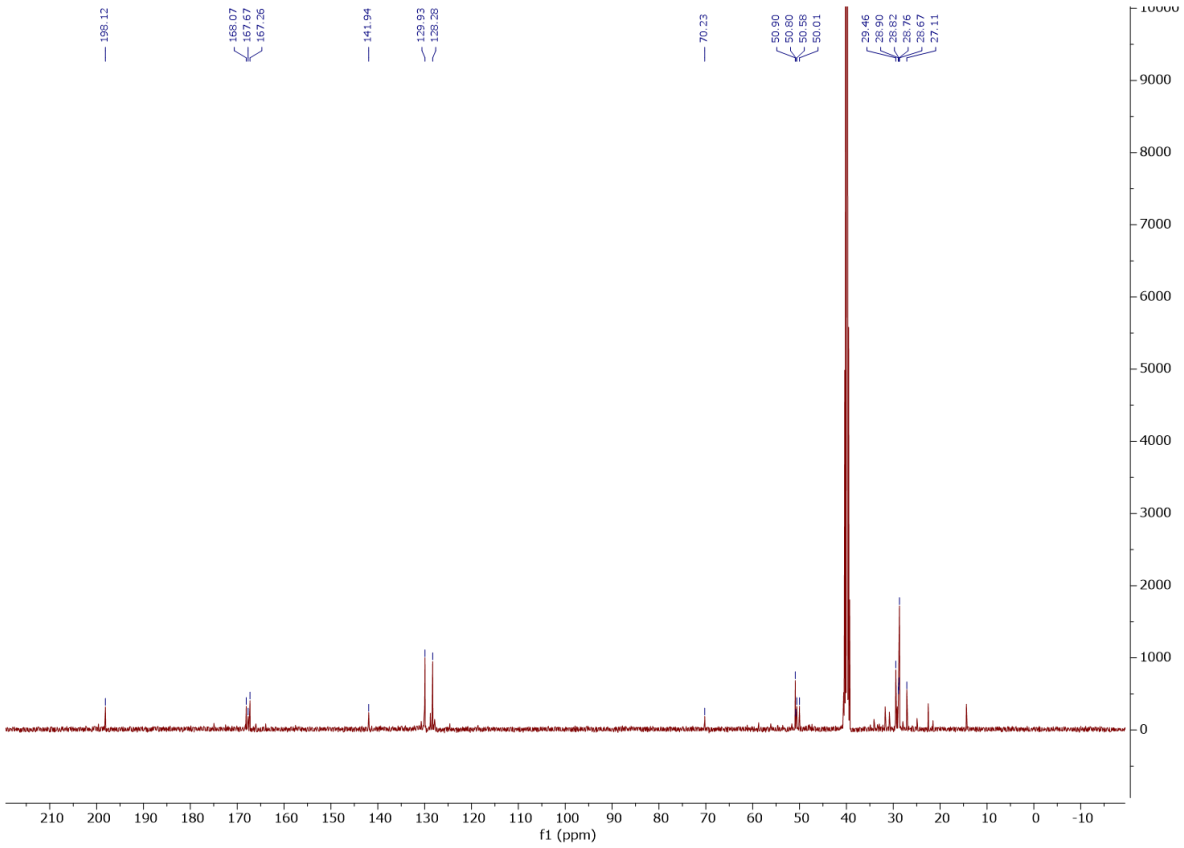
Spectral data of compound **2l**



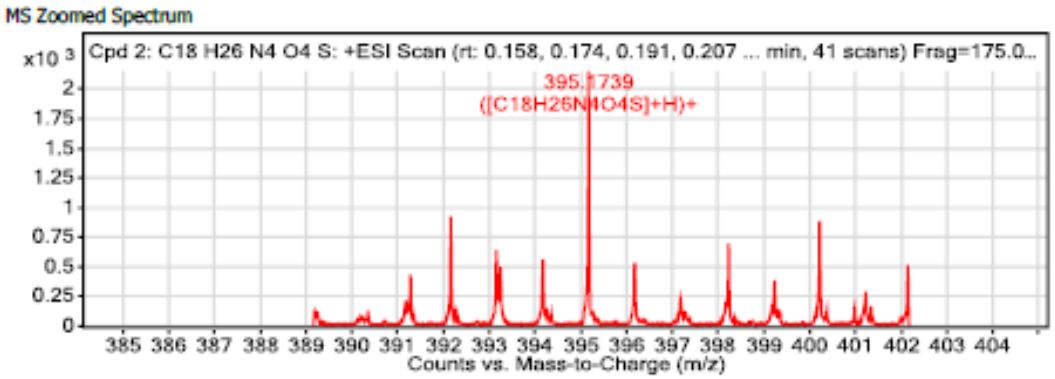
^{1}H NMR



^{13}C NMR



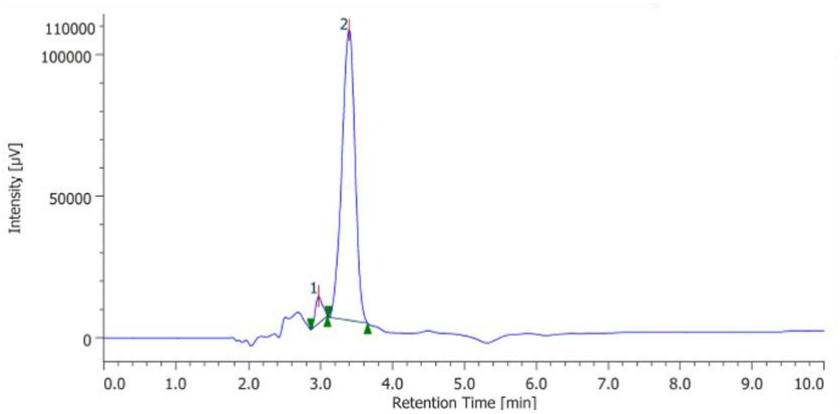
HRMS



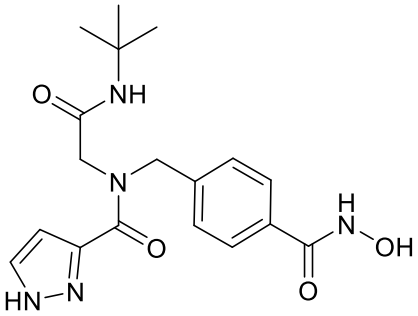
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
394.1745	394.1669	-19.21	1	575.6	C18H26N4O4S	M+
395.1739	395.1748	2.28	1	2173.8	C18H26N4O4S	(M+H)+
396.1778	396.1776	-0.57	1	553.91	C18H26N4O4S	(M+H)+
397.1716	397.1745	7.25	1	189.4	C18H26N4O4S	(M+H)+

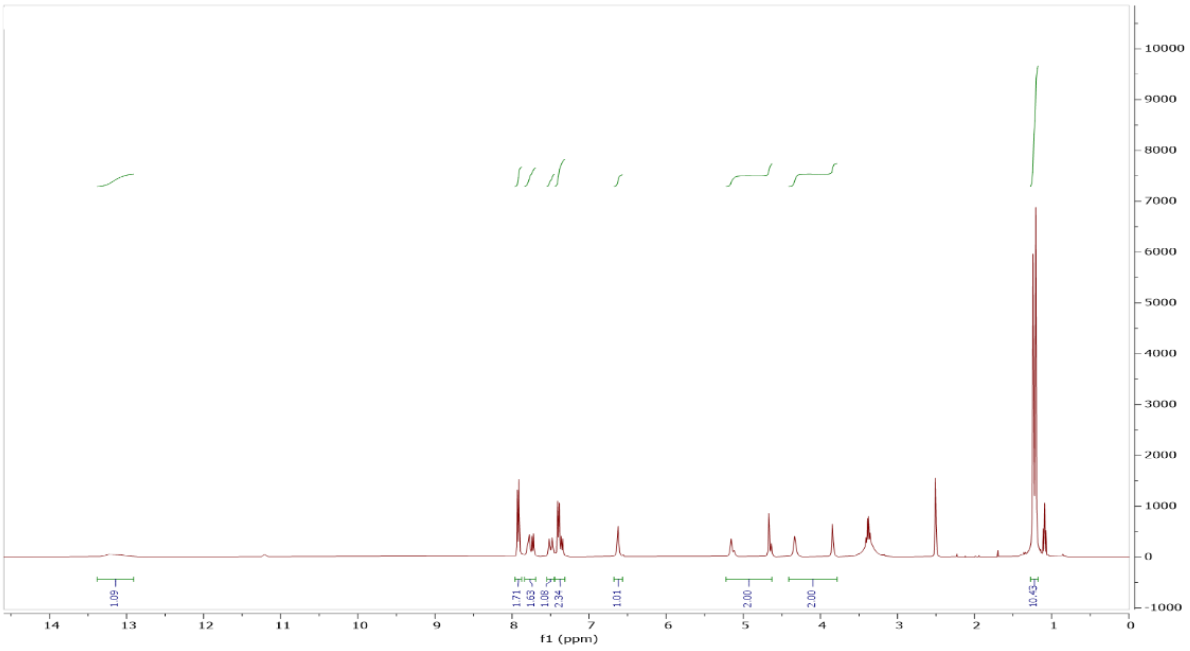
HPLC



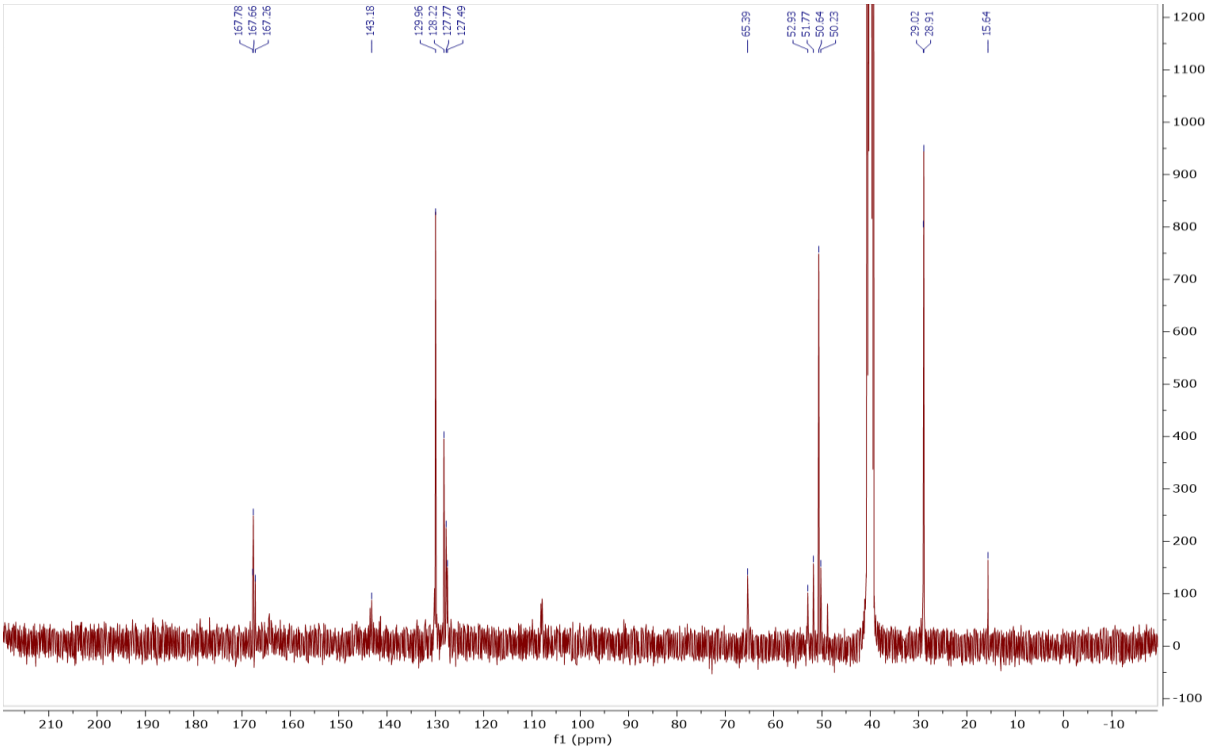
Spectral data of compound **2m**



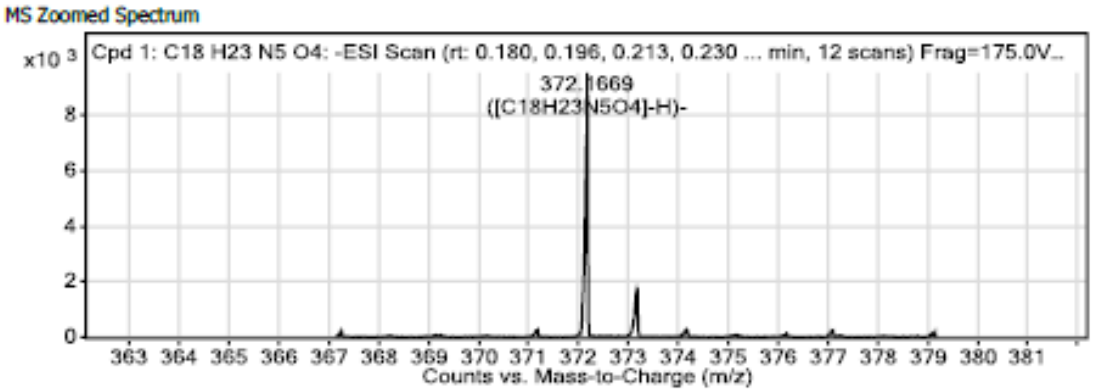
^{1}H NMR



^{13}C NMR



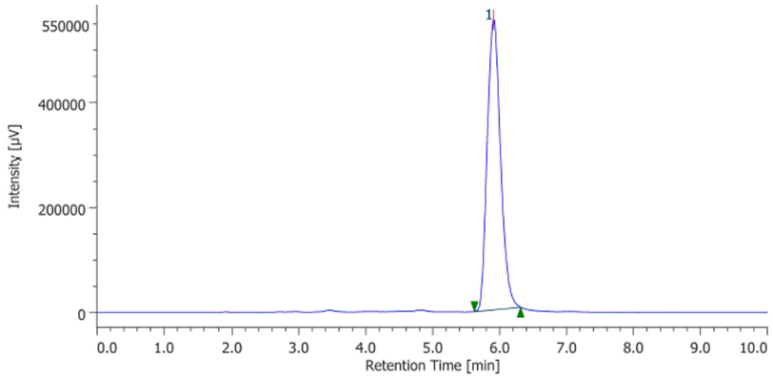
HRMS



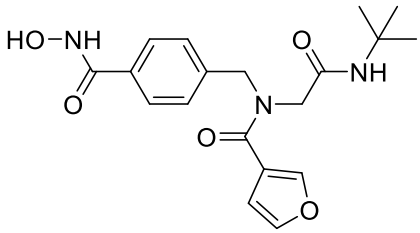
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
372.1669	372.1677	2.33	1	9569.51	C18H23N5O4	(M-H)-
373.1657	373.1706	13.19	1	1940.57	C18H23N5O4	(M-H)-
374.1534	374.173	52.39	1	215.38	C18H23N5O4	(M-H)-

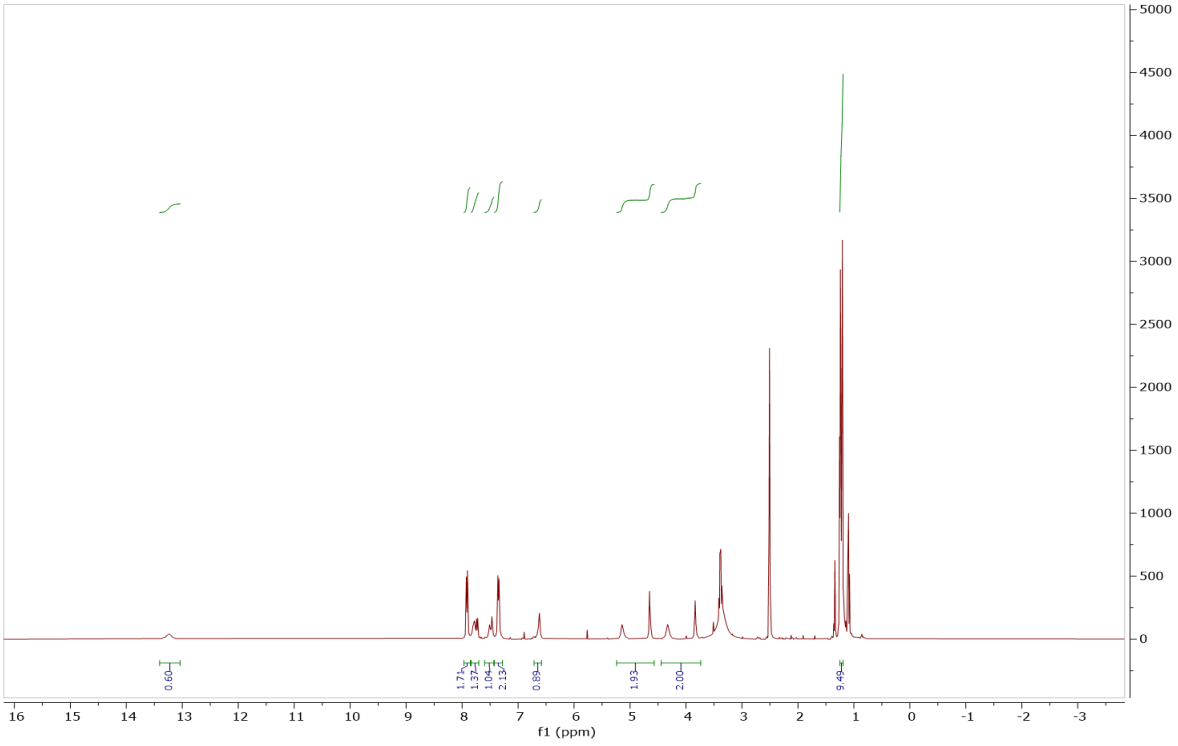
HPLC



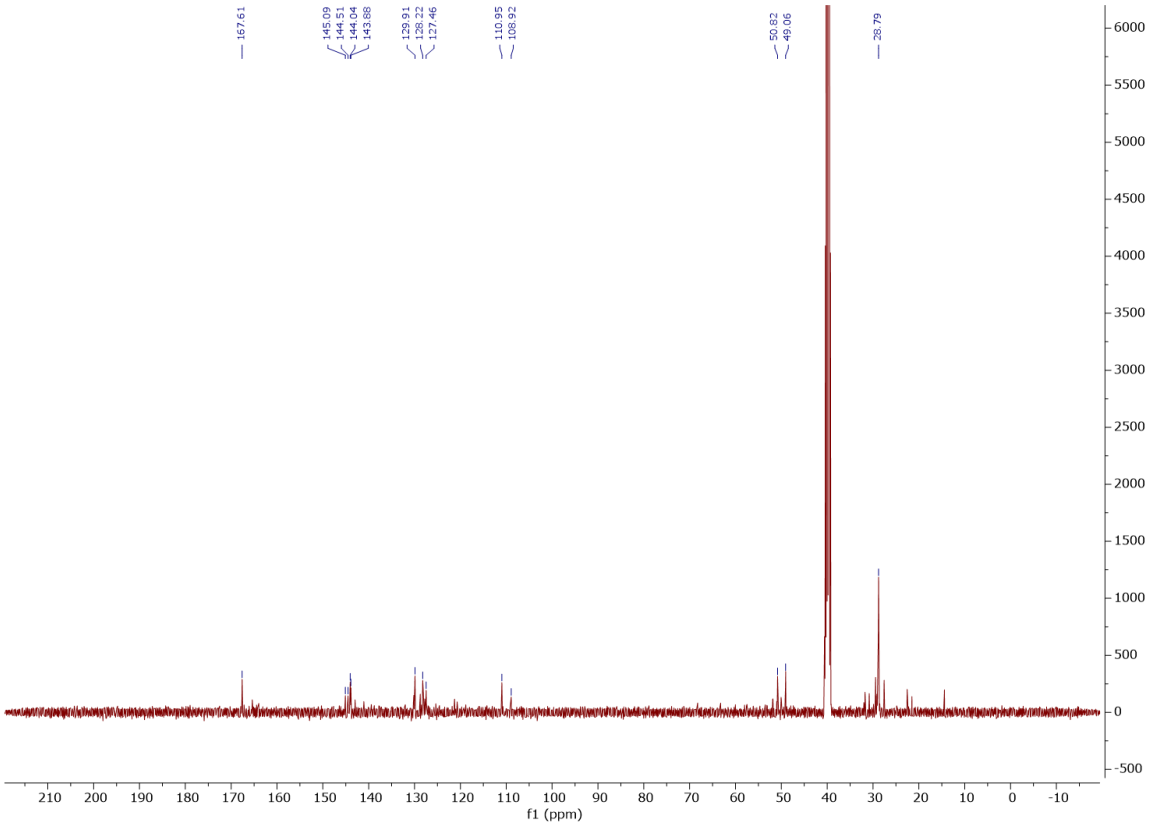
Spectral data of compound **2n**



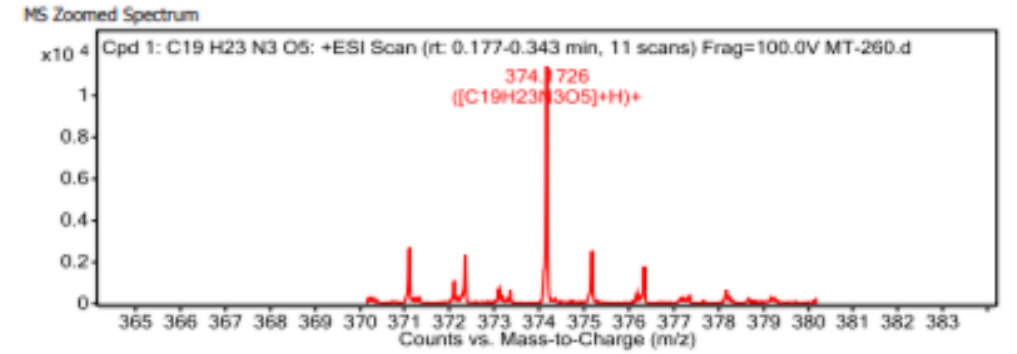
^{1}H NMR



^{13}C NMR



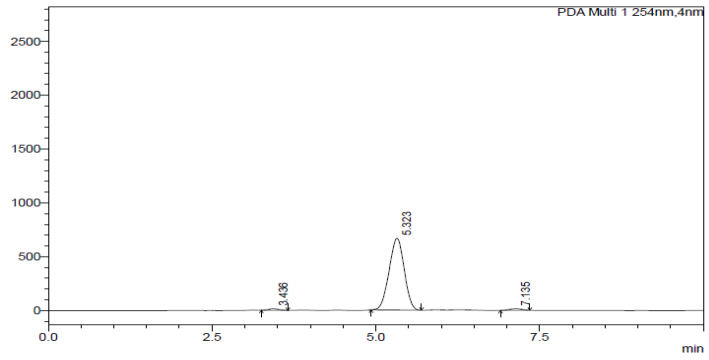
HRMS



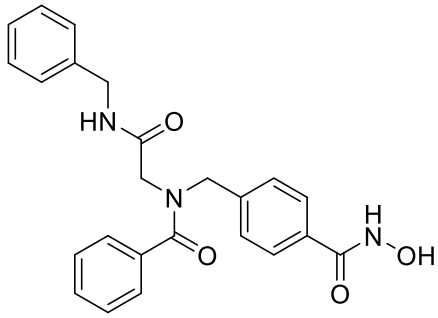
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
374.1726	374.171	-4.19	1	11522.73	C19H23N3O5	(M+H)+
375.176	375.1741	-4.88	1	2557.7	C19H23N3O5	(M+H)+
376.1803	376.1766	-9.77	1	497.95	C19H23N3O5	(M+H)+

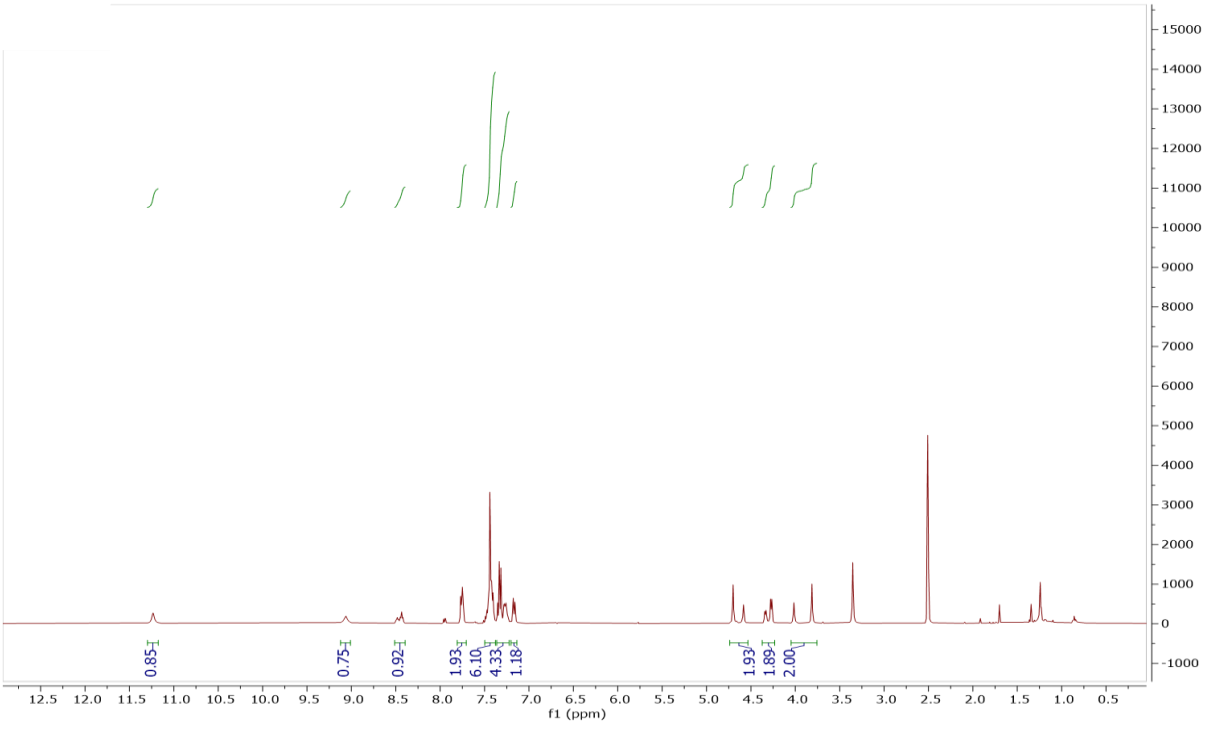
HPLC



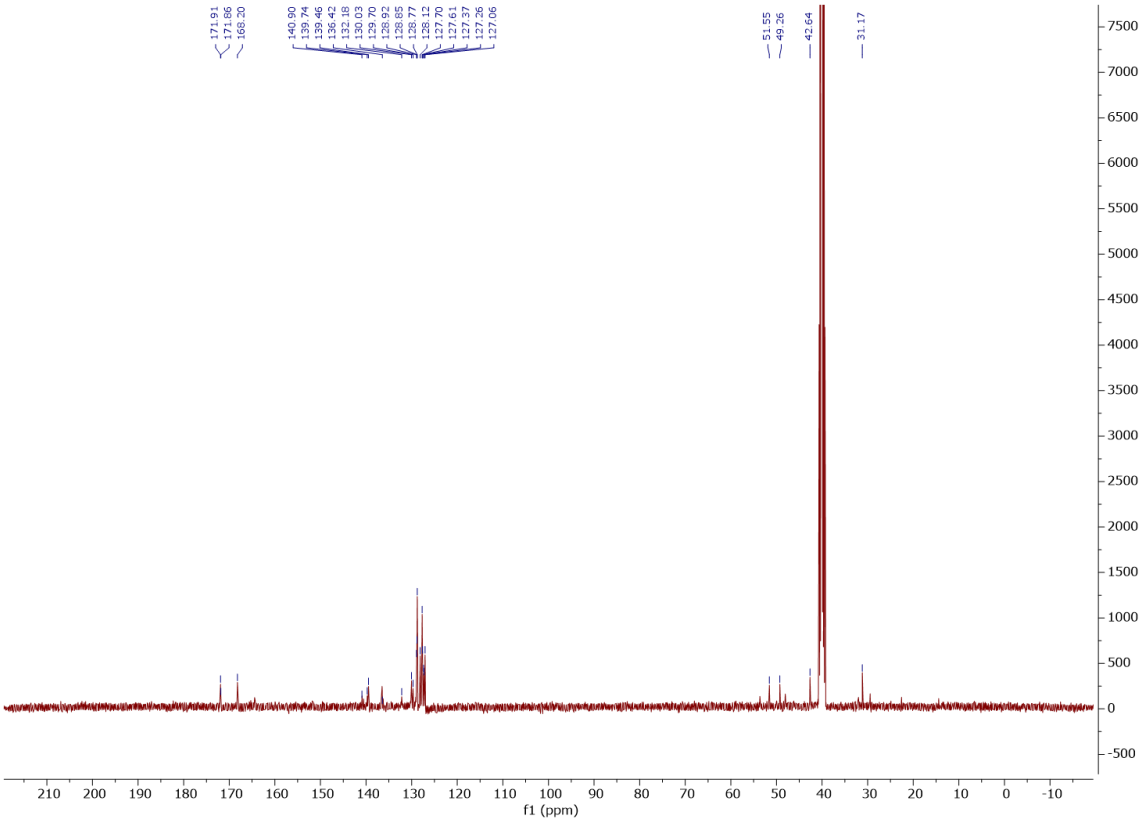
Spectral data of compound **2p**



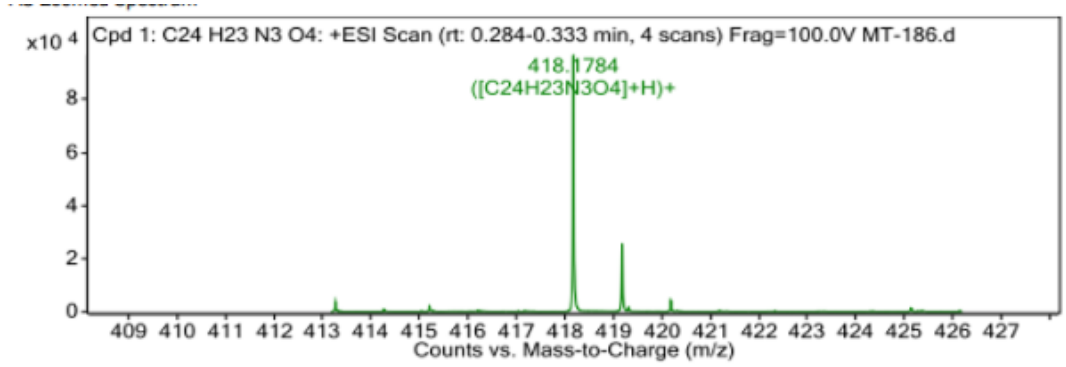
^{1}H NMR



^{13}C NMR



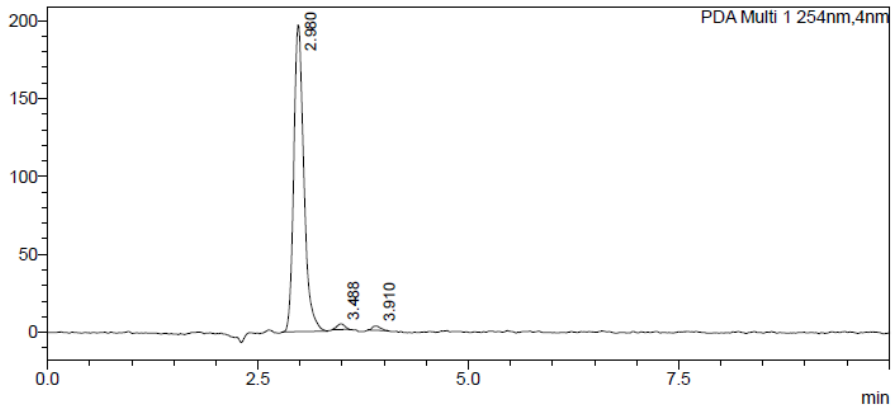
HRMS



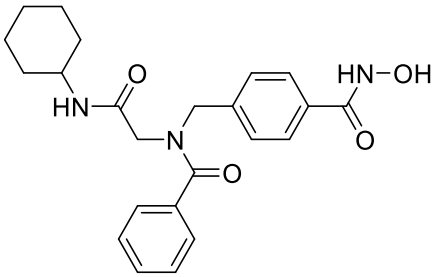
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
418.1784	418.1761	-5.39	1	99072.7	C24H23N3O4	(M+H)+
419.1813	419.1793	-4.77	1	25573.32	C24H23N3O4	(M+H)+
420.1841	420.182	-4.9	1	4318.35	C24H23N3O4	(M+H)+
421.1873	421.1847	-6.28	1	562.83	C24H23N3O4	(M+H)+

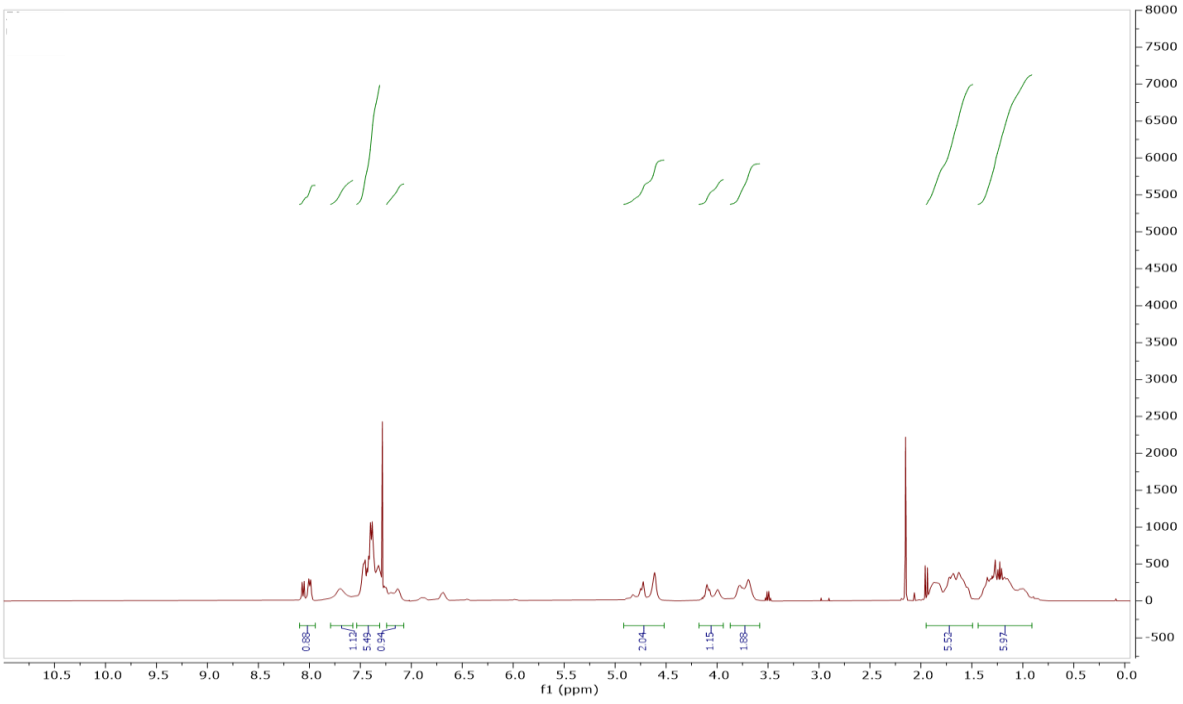
HPLC



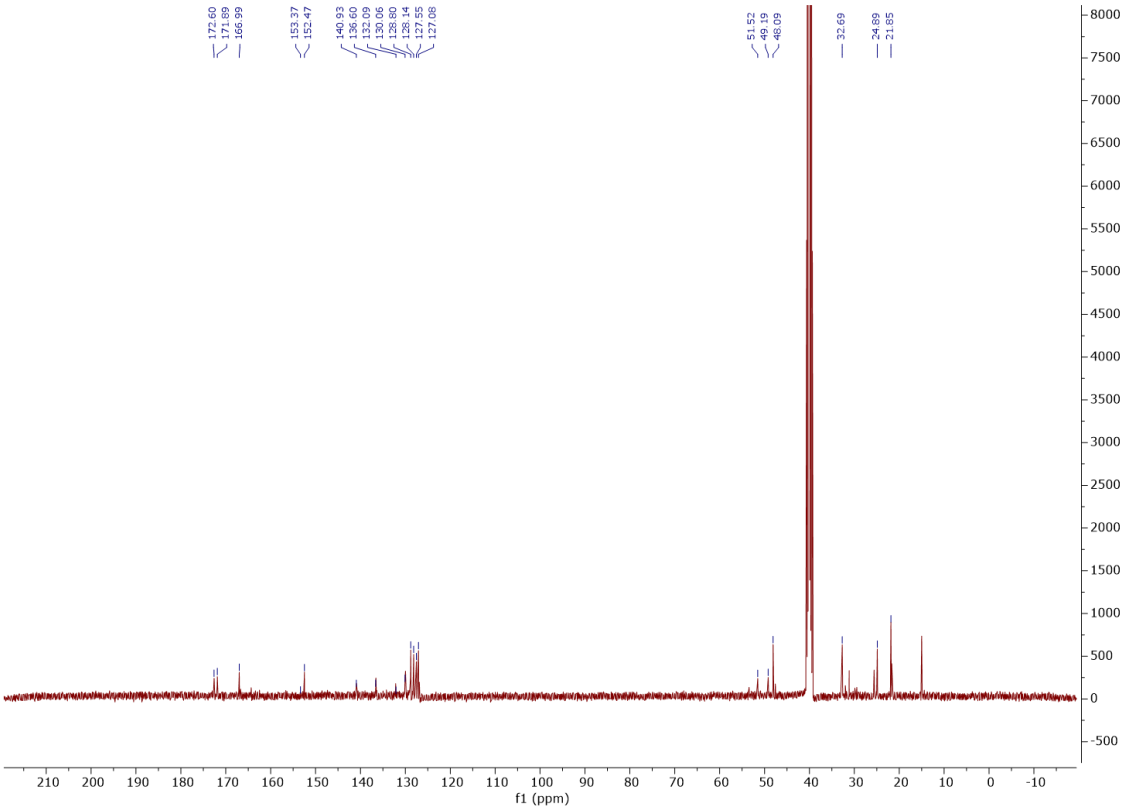
Spectral data of compound **2q**



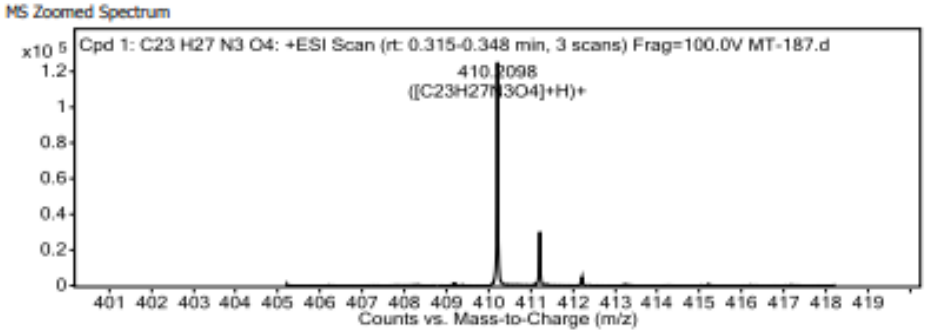
^{1}H NMR



^{13}C NMR



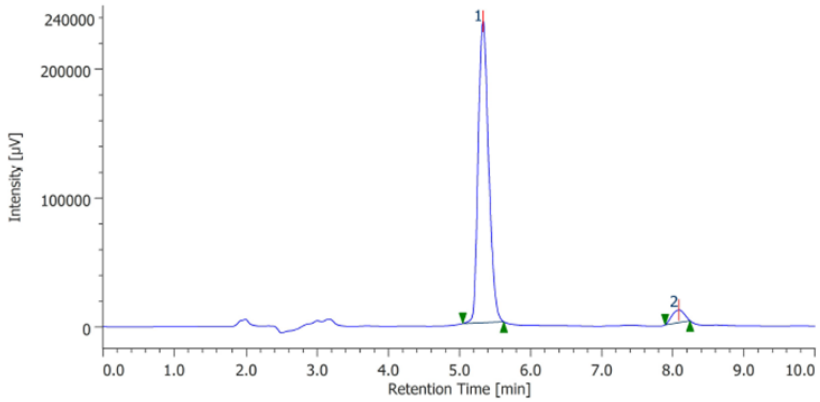
HRMS



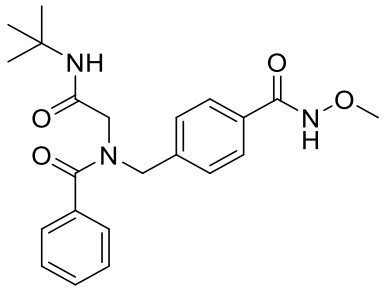
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
410.2098	410.2074	-5.7	1	126872.13	C23H27N3O4	(M+H)+
411.2126	411.2106	-4.99	1	30330.87	C23H27N3O4	(M+H)+
412.2155	412.2133	-5.36	1	4957.22	C23H27N3O4	(M+H)+
413.218	413.2159	-4.93	1	650.25	C23H27N3O4	(M+H)+

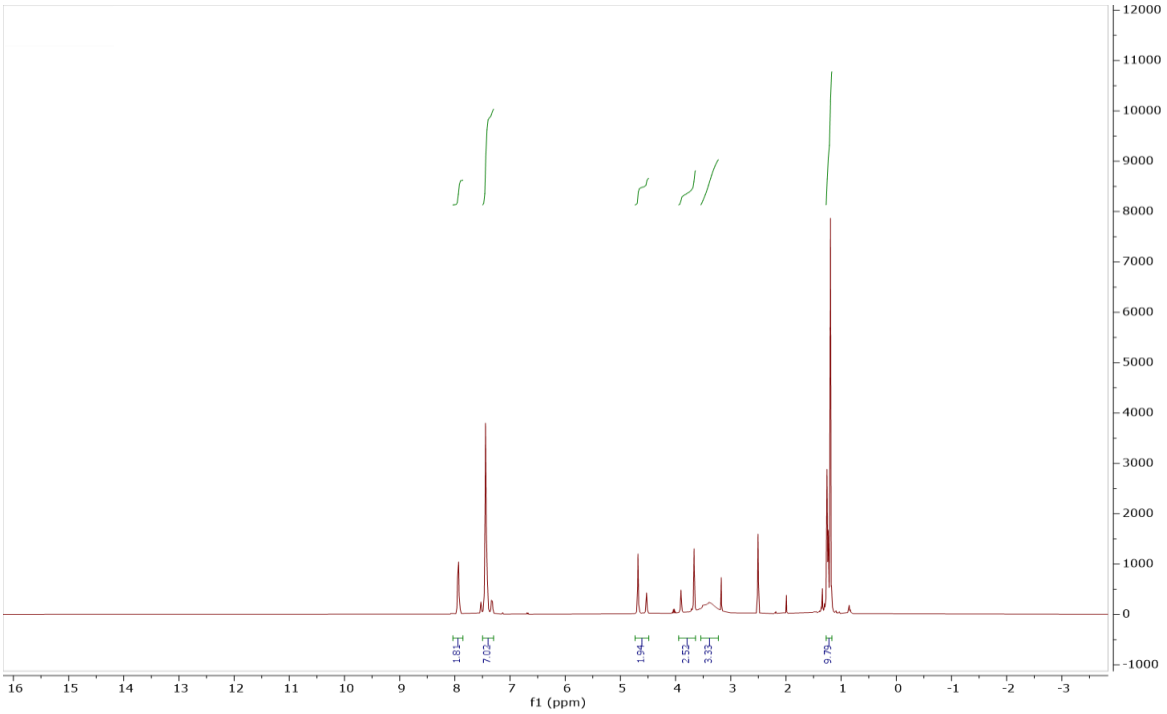
HPLC



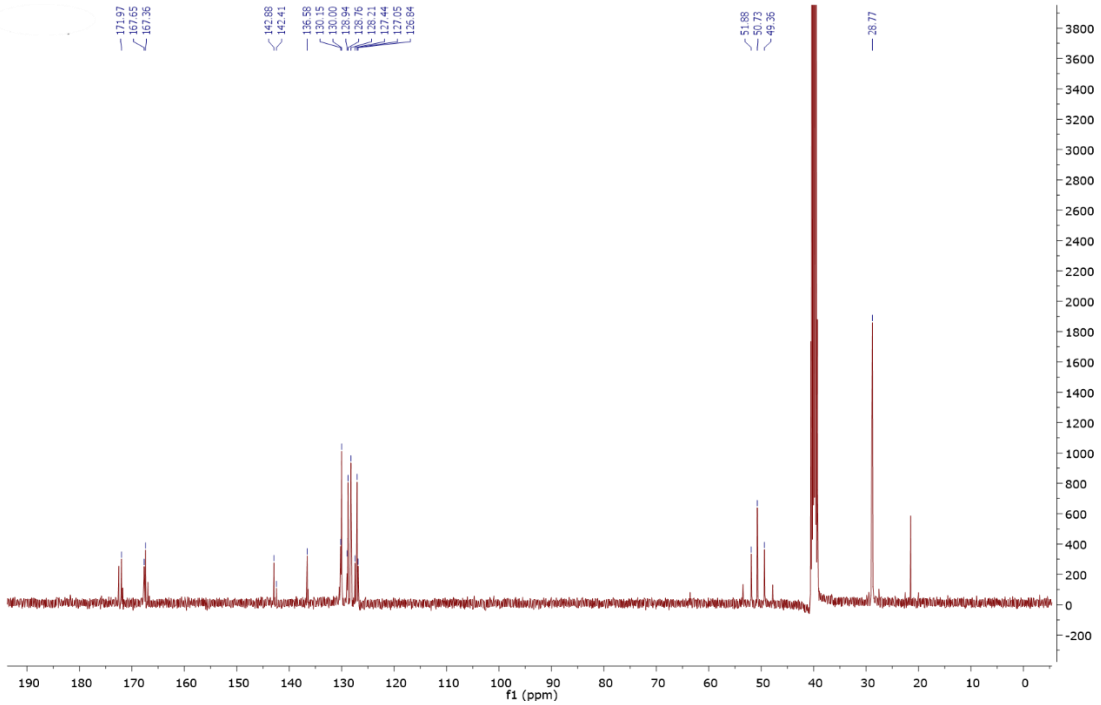
Spectral data of compound **2t**



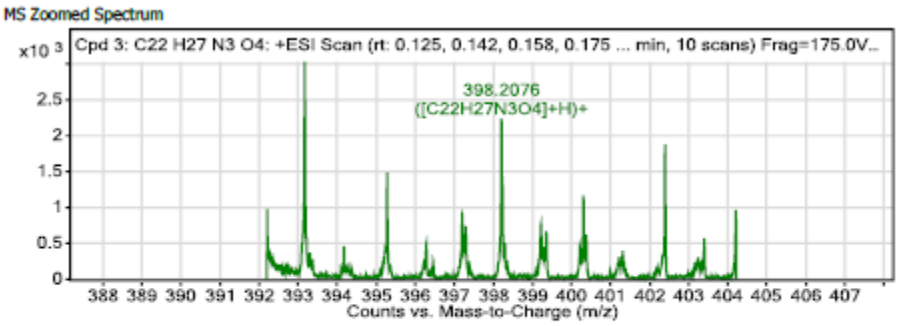
^{1}H NMR



^{13}C NMR



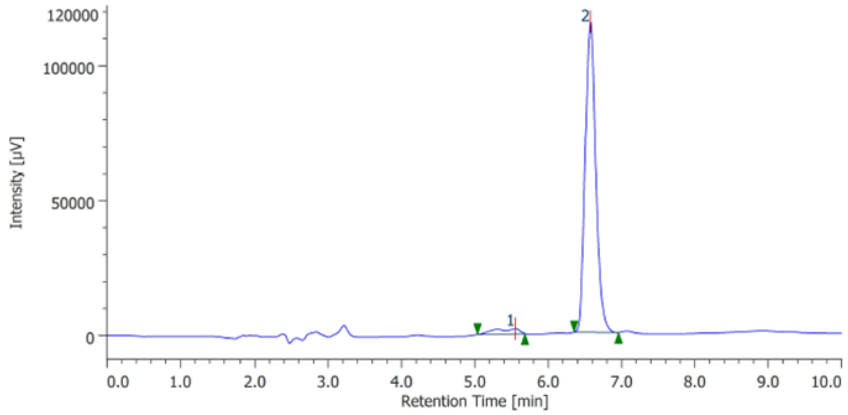
HRMS



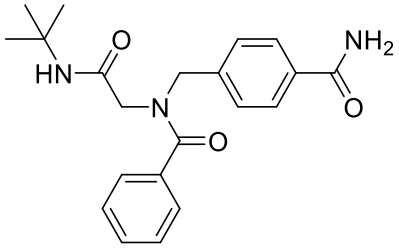
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
397.2	397.1996	-0.87	1	954.25	C22H27N3O4	M+
398.2076	398.2074	-0.52	1	2219.99	C22H27N3O4	(M+H)+
399.213	399.2106	-6.16	1	752.18	C22H27N3O4	(M+H)+

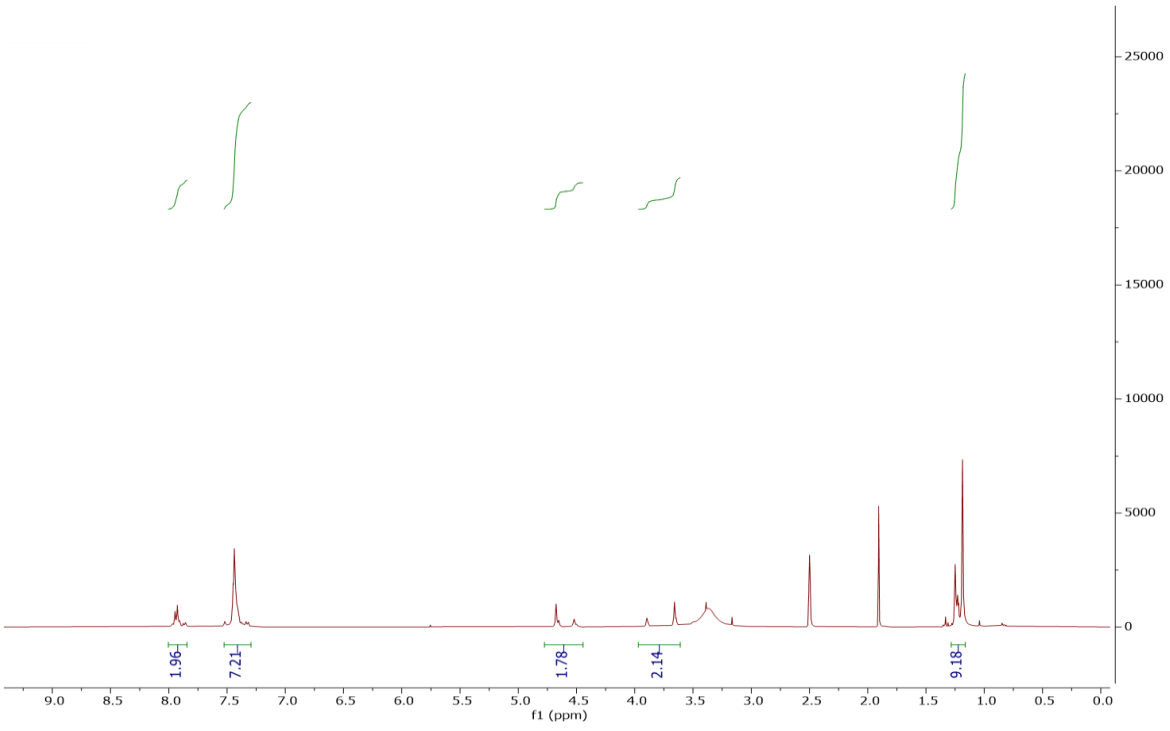
HPLC



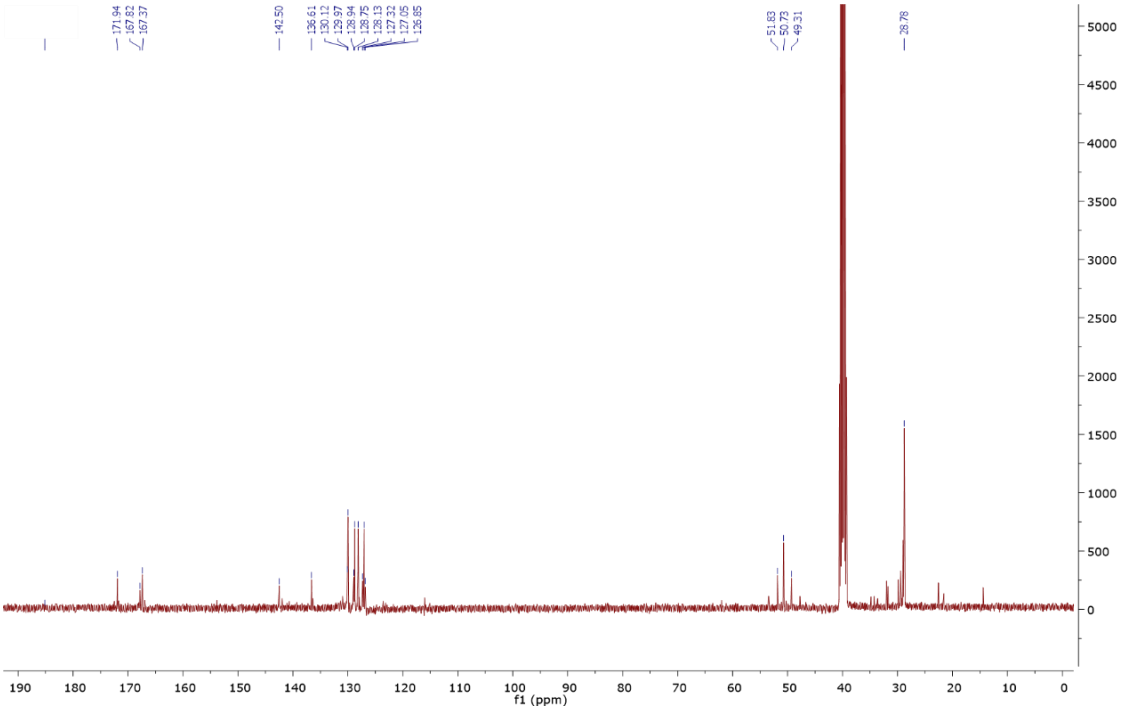
Spectral data of compound **2u**



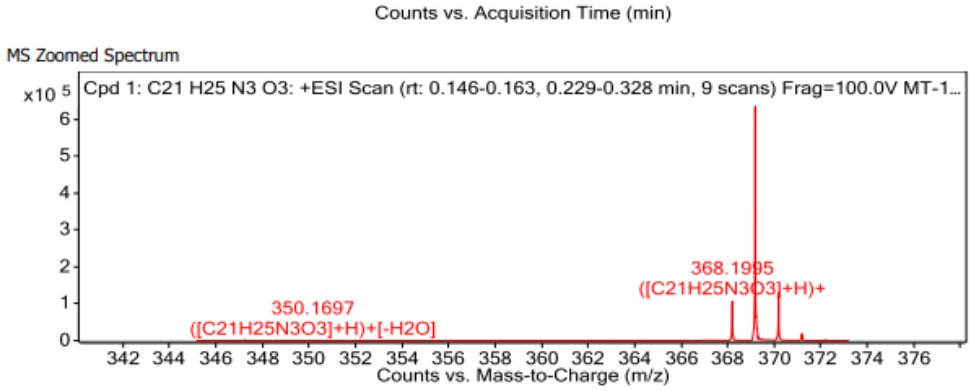
^{1}H NMR



^{13}C NMR



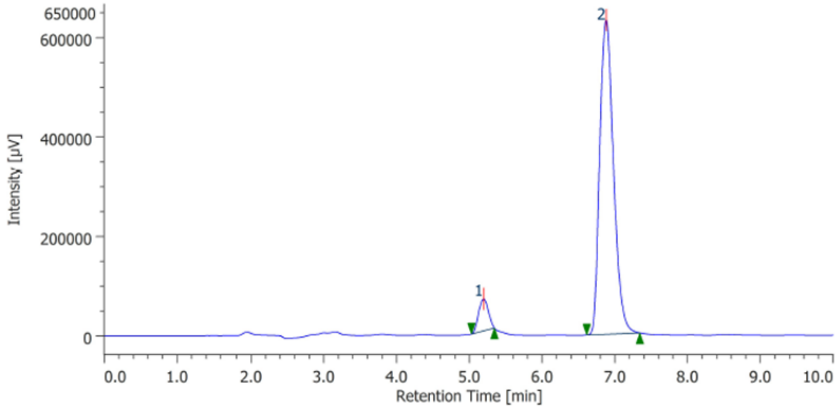
HRMS



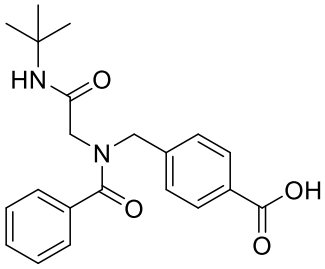
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
350.1697	350.1863	47.32	1	111.75	C21H25N3O3	(M+H)+[-H2O]
368.1995	368.1969	-7.07	1	108624.77	C21H25N3O3	(M+H)+

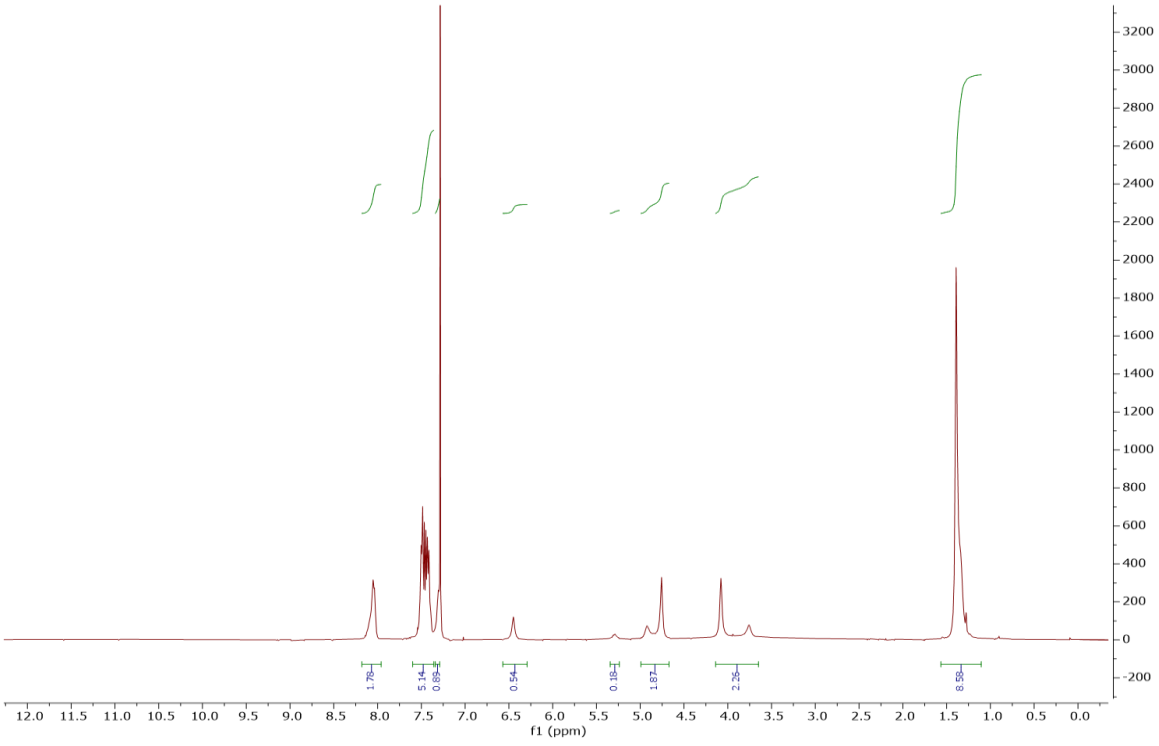
HPLC



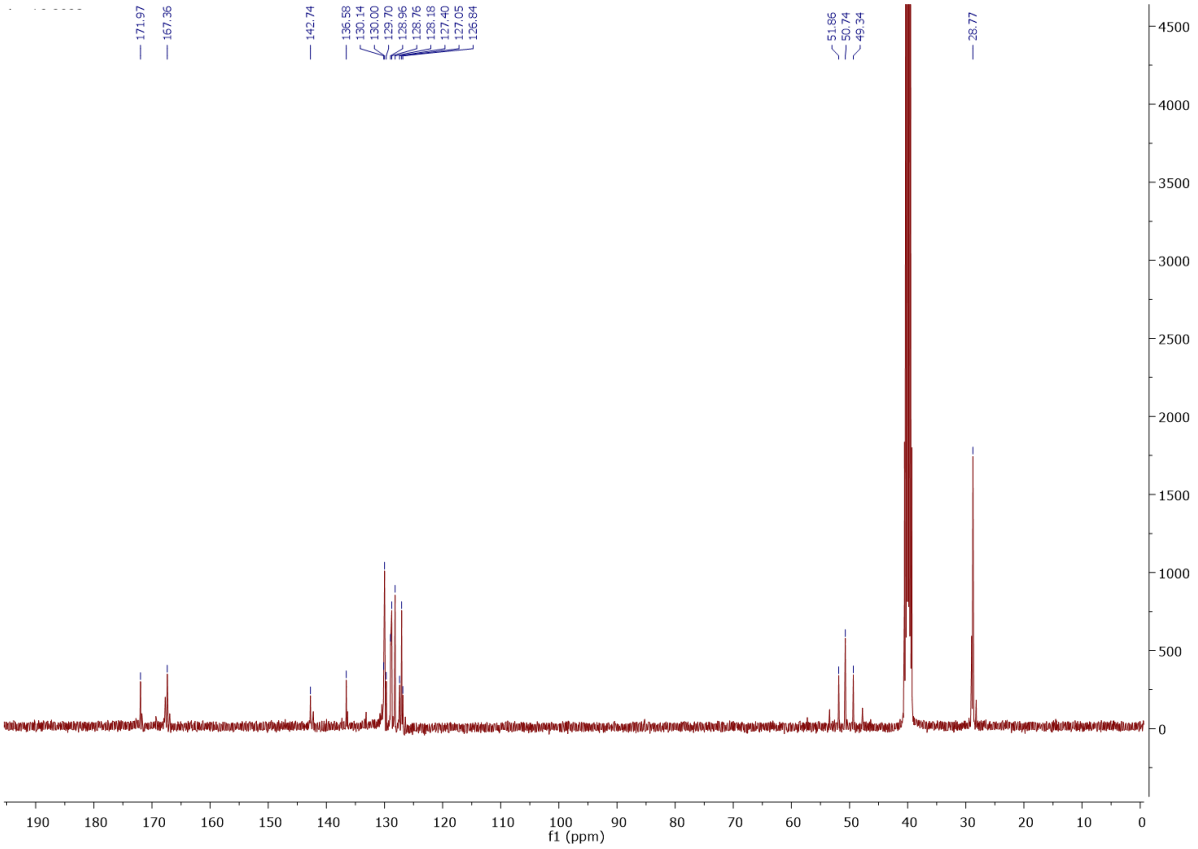
Spectral data of compound **2v**



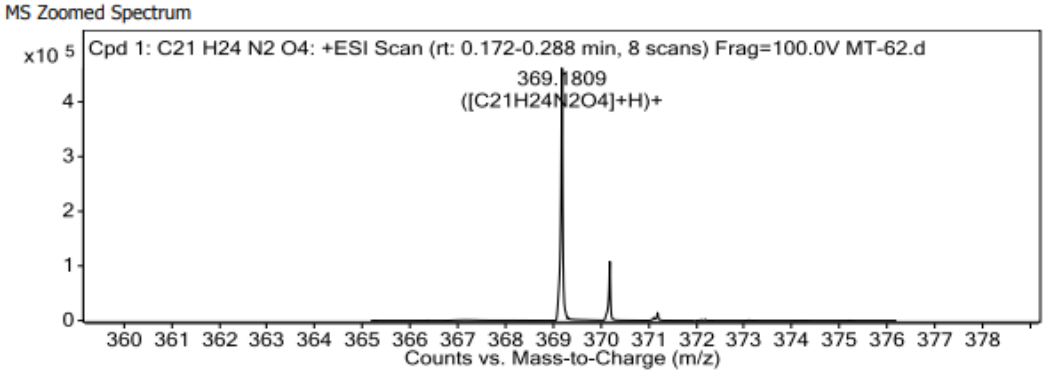
^{1}H NMR



^{13}C NMR



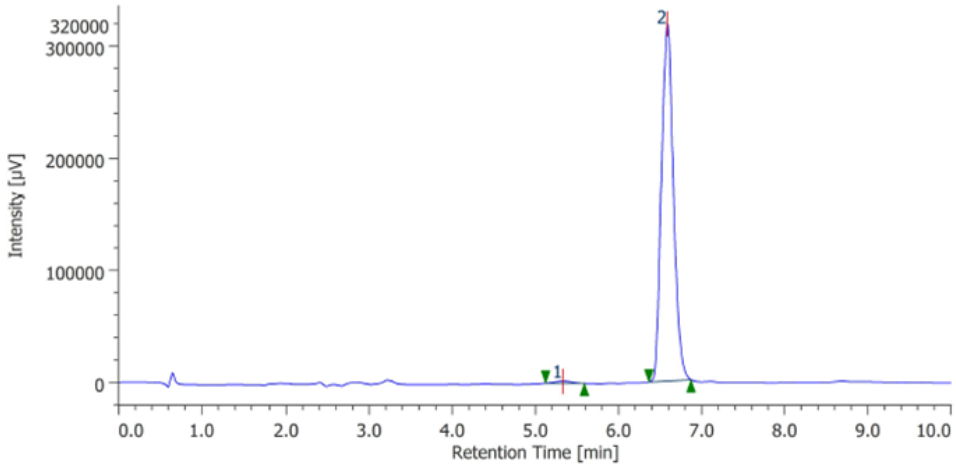
HRMS



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
369.1809	369.1809	0.08	1	464883.38	C21H24N2O4	(M+H)+
370.1837	370.1841	0.97	1	109572.41	C21H24N2O4	(M+H)+
371.1863	371.1868	1.37	1	14192.03	C21H24N2O4	(M+H)+
372.1887	372.1894	1.93	1	1934.29	C21H24N2O4	(M+H)+

HPLC


320000
300000
200000
100000
0
Intensity [µV]
1
2
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Retention Time [min]