

Supplementary data

Development of Combretastatin A-4 Analogues as Potential Anticancer Agents with Improved Aqueous Solubility

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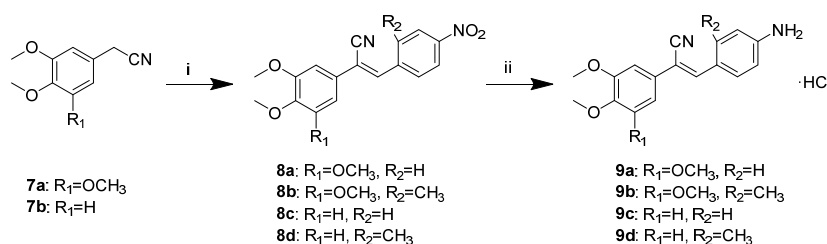
1. Experimental section

1.1. Reagents and instruments

All the chemicals used in this study were purchased from Aladdin, all reagents were obtained commercially and used were of analytical grade and THF were dehydrated by 4Å molecular sieve. Melting points were determined with the SGWX-4 melting-point measurement apparatus and were uncorrected. The NMR spectra were recorded on a Bruker AV-300 spectrometer in DMSO-d₆ using tetramethylsilane as the internal standard, chemical shifts were recorded as δ (ppm). High resolution mass spectra were obtained using MALDI-TOF/TOF mass spectrometer (Bruker DARTONIK, Germany). Flash chromatography was carried out on silica gel (200–300 mesh), and chromatographic solvent proportions are expressed on a volume: volume basis.

1.2. General procedure for the synthesis of compounds 9a-d

Scheme 1. Synthesis of compounds 9a-d [1-5]

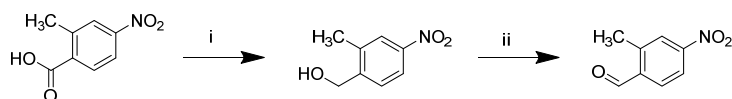


Reagents and conditions: (i) 4-Nitrobenzaldehyde or 2-methyl-4-nitrobenzaldehyde, EtOH, 5% NaOH, r.t, 0.5 h. (ii) SnCl₂, EtOH, 80°C, 0.5 h.

The specific synthesis steps are described in "Experimental section" in Chapter 4 of the text .

1.3. Procedure for the synthesis of 2-methyl-4-nitrobenzaldehyde

Scheme 2. Synthesis of the intermediate 2-methyl-4-nitrobenzaldehyde

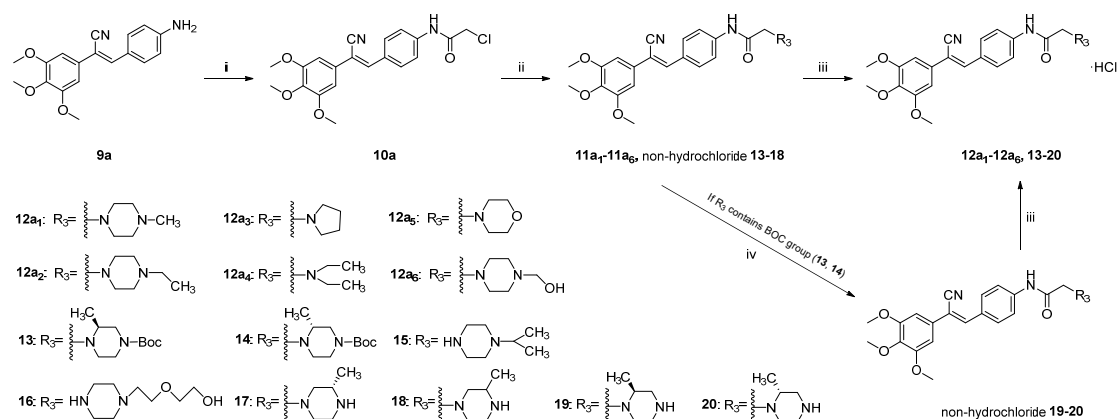


Reagents and conditions: (i) Borane dimethyl sulfide complex, THF, 70°C , 3 h. (ii) PCC, DCM, r.t., 2 h.

$\text{BH}_3 \cdot \text{DMS}$ (10 mmol) was added dropwise to a stirred solution of nitrobenzoic acid (5 mmol) in THF (25 mL) at r.t. The solution was heated at reflux temperature for 3 h and cooled to 20°C . The reaction was quenched carefully with $\text{MeOH}:\text{H}_2\text{O}$ (1:1, 10 mL), the mixture stirred for 10 min. HCl (5 mol/L) 5 mL was added and the mixture heated at 50°C for 30 min. The solvent was removed under reduced pressure, the residue partitioned between EtOAc and water. The organic fraction was dried, the solvent removed under reduced pressure to give (2-methyl-4-nitrophenyl)methanol [6]. Then, to a stirred CH_2Cl_2 solution (200 mL) containing pyridinium chlorochromate 2.06 g (9.56 mmol) and anhydrous magnesium sulfate 3.50 g, 1.41 g of (2-methyl-4-nitrophenyl)methanol (4.79 mmol) was added. The mixture was stirred for 2 h at ambient temperature. The organic solution was washed with water ($100\text{ mL} \times 3$) and brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated to dryness. The residue was purified on a silica gel column (PE:EA = 9:1) to afford the compound 2-methyl-4-nitrobenzaldehyde (0.73 g, 92% yield).

1.4. General procedure for the synthesis of compounds 12a-d, 13-20.

Scheme 3. Synthesis of compounds **12a-d**, **13-20** [7, 8].



Reagents and conditions: (i) Chloroacetyl chloride, THF, tetraethylammonium hydroxide (TEA), 0°C, 2-3 h. (ii) CH₃CN, R₃, KI, K₂CO₃, 80 °C, 3 h. (iii) Hydrochloric acid, THF, rt, 8 h. (iv) 20% TFA, DCM, rt, 3 h and then hydrochloric acid, THF, rt, 8 h.

The specific synthesis steps are described in "Experimental section" in Chapter 4 of the text.

1.5. Solubility Assessment

The aqueous solubility was determined as previously reported using the high throughput solubility screens procedure published by Bruno Bard et al [9, 10]. Put simply, a certain amount of powdered compound was placed in a 2 ml centrifuge tube and mixed with an appropriate volume of aqueous phosphate buffer (0.01 M, pH 7.4) to make the whole system supersaturated. The mixtures were sonicated (KQ5200DE numerical control ultrasonic cleaner, Kunshan Ultrasonic Instrument Co., Ltd., China) for 20 min. After sonication, the mixture was allowed to equilibrate at room temperature (22 ± 2°C) for 1.5 h with vortex shaking (1200 rpm). Then the centrifuge tube was put into the centrifuge (1-14k, sigma, Germany), centrifuged with 3000 rpm for 15 minutes, 0.22 μM filter membrane filtration supernatant, then take 150 μl filtrate and add it to 96-well UV plate, with three well for each compound. Draw 150 μL blank solution were added into 96 well-plate as blank control. The 96-well UV

plate prepared above was read on a SynergyHTX Multifunctional microplate reader (BioTek, USA) at 338 nm (compounds **12a-12d** and **15-20**) or 370 nm (compounds **9a-d**). The absorbance of the sample was corrected according to the UV response of the sample and blank solution, and the corrected UV responses value were used for solubility calculations by bring into the standard curve of the compound.

1.6. Plasma stability assessment

Prepare plasma: blood was collected from SD rats (200-250g, male and female, Animal Experimental Center of Yanbian University) into heparin tubes, and supernatant plasma was collected after centrifugation.

A certain amount of **9a** and **12a₁** were accurately weighed and mixed with rat plasma. After incubation at 37°C for 0 h, 6 h, 12 h, 24 h and 36 h, the plasma samples were treated with methanol and analyzed by HPLC to determine their stability in plasma.

Analytical instrument and conditions:

A HITACHI Primaide HPLC liquid chromatography system equipped was used for all analyses. Chromatographic separation was performed on a Welch Ultimate XB-C18 column (3.9 mm × 150 mm, 5 μm; Welch, China). The analytes were eluted using a gradient method with a flow rate of 1 mL/min. Mobile phase A was Ultra pure water, and mobile phase B was methanol according to the following gradient: (1). The gradient elution conditions of **9a**: 0 to 8 minutes, 55% to 67% B; 8.1 to 15 minutes, 67% to 90% B; 15.1 to 25 minutes, 55% B. (2). The gradient elution conditions of **12a₁**: 0 to 9 minutes, 55% to 65% B; 9.1 to 12 minutes, 65% to 75% B; 12.1 to 15

minutes, 75% to 85% B. 15.1 to 20 minutes, 90% B; 20.1 to 30 minutes, 55% B. The injection volume was 3 μ L. The column temperature was set at 40°C.

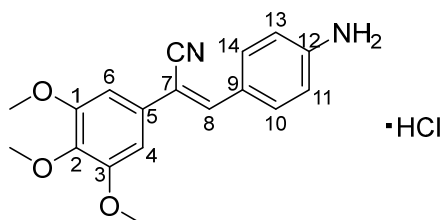
1.7. The drug-plasma protein binding rate assessment

Compound **12a₁** at 90 μ M concentration were mixed with rats plasma. The mixtures were subjected to equilibrium dialysis versus phosphate buffer pH 7.4 buffer at 4°C for 48 hour using equilibrium dialysis. The dialysis membrane of molecular weight cutoff 1 kDa was used. Dialysis experiments were done in triplicate. On completion of the dialysis period, absorb a certain amount of buffer and plasma respectively, and add methanol to them to treat the sample. Dialysis samples were analyzed by HPLC, The plasma protein binding rate of the compound was calculated as follows: protein binding rate / % = (plasma drug concentration inside dialysis bag – dialysate drug concentration outside dialysis bag) / plasma drug concentration inside dialysis bag \times 100%.

2. Identification data of compounds

(Z)-3-(4-Aminophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile monohydrochloride

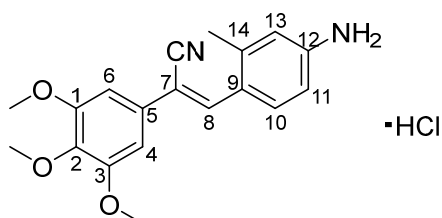
(9a)



Yellow powder, mp. 203-207°C, yield 48%. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.86 (d, *J* = 6.0 Hz, 2H, C11, C13-H), 7.82 (s, 1H, C8-H), 7.37 (s, 3H, C12-NH₃⁺), 7.00 (d,

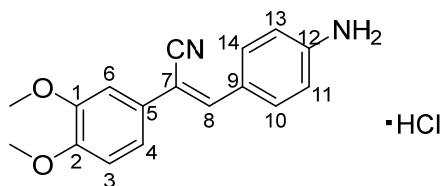
$J = 6.0$ Hz, 2H, C10, C14-H), 6.96 (s, 2H, C4, C6-H), 3.86 (s, 6H, C1, C3-OCH₃), 3.70 (s, 3H, C2-OCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 153.68 (s, C1, C3), 142.50 (s, C12), 138.60 (s, C2), 131.21 (s, C10, C14), 130.22 (s, C8), 128.60 (s, C5), 119.90 (s, C9, C11, C13), 118.89 (s, C7-CN), 107.72 (s, C7), 103.59 (s, C4, C6), 60.62 (s, C2-OCH₃), 56.57 (s, C1-OCH₃, C3-OCH₃). HRMS calcd. for C₁₈H₁₉N₂O₃⁺ ([M+H]⁺): 311.13902, found: 311.13889.

***(Z)*-3-(4-Amino-2-methylphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile monohydrochloride(9b)**



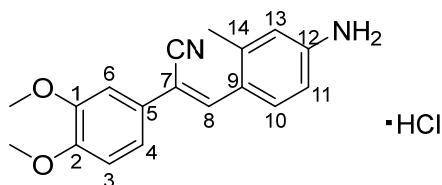
Yellow powder, mp: 224-230°C, yield 48%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.02 (s, 1H, C8-H), 7.81 (d, $J = 8.0$ Hz, 1H, C13-H), 7.06 (s, 2H, C10, C11-H), 7.00 (s, 2H, C4, C6-H), 3.86 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 2.38 (s, 3H, C14-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 153.71 (s, C1, C3), 141.69 (s, C12), 140.11 (s, C2), 138.96 (s, C14), 130.60 (s, C8), 129.76 (s, C10), 129.71 (s, C9), 122.84 (s, C5), 119.03 (s, C7-CN), 118.29 (s, C13), 112.34 (s, C7, C11), 104.00 (s, C4, C6), 60.64 (s, C2-OCH₃), 56.60 (s, C1, C3-OCH₃), 20.05 (s, C14-CH₃). HRMS calcd. for C₁₉H₂₁N₂O₃⁺ ([M+H]⁺): 325.15467, found: 325.15460.

***(Z)*-3-(4-Aminophenyl)-2-(3,4-dimethoxyphenyl)acrylonitrile monohydrochloride (9c)**



Yellow powder, mp: 214-220°C, yield 50%. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.85 (s, 1H, C8-H), 7.83 (s, 2H, C10, C14-H), 7.29 (d, $J = 2.2$ Hz, 1H, C6-H), 7.22 (dd, $J = 8.4, 2.3$ Hz, 1H, C4-H), 7.07 (s, 2H, C11, C13-H), 7.05 (s, 1H, C3-H), 3.85 (s, 3H, C2-OCH₃), 3.80 (s, 3H, C1-OCH₃). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ 150.15 (s, C1), 149.61 (s, C2), 140.58 (s, C12), 130.93 (s, C9, C10, C14), 127.03 (s, C5), 120.73 (s, C4, C7-CN), 119.26 (s, C11, C13), 118.82 (s, C8), 112.30 (s, C6), 108.96 (s, C3), 108.58 (s, C7), 56.20 (s, C2-OCH₃), 56.10 (s, C1-OCH₃). HRMS calcd. for $\text{C}_{17}\text{H}_{17}\text{N}_2\text{O}_2^+$ ($[\text{M}+\text{H}]^+$): 281.12845, found: 281.12851.

(Z)-3-(4-Amino-2-methylphenyl)-2-(3,4-dimethoxyphenyl)acrylonitrile monohydrochloride (9d)

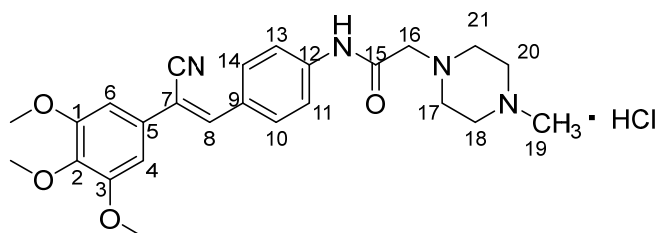


Yellow powder, mp: 236-239°C, yield 50%. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.96 (s, 1H, C8-H), 7.79 (d, $J = 8.1$ Hz, 1H, C10-H), 7.30 (s, 1H, C4-H), 7.26 (d, $J = 8.5$ Hz, 1H, C13-H), 7.06 (m, 3H, C3, C6, C11-H), 3.83 (d, $J = 12.1$ Hz, 6H, C1, C2-OCH₃), 2.37 (s, 3H, C14-CH₃). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ 150.43 (s, C1, C12), 149.61 (s, C2), 139.91 (s, C8), 139.74 (s, C14), 129.69 (s, C10), 126.64 (s, C7), 123.30 (s, C4), 119.58 (s, C5, C9, C13), 118.29 (s, C7-CN), 112.72 (s, C6), 112.32 (s, C3),

109.36 (s, C11), 56.23 (s, C2-OCH₃), 56.13 (s, C1-OCH₃), 20.05 (s, C14-CH₃).

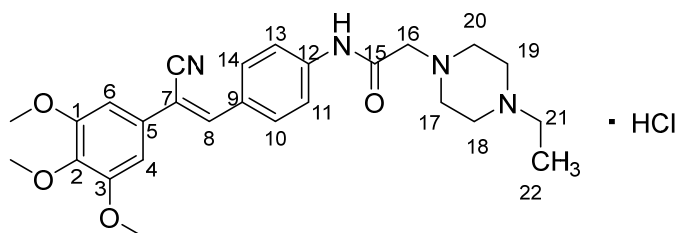
HRMS calcd. for C₁₈H₁₉N₂O₂⁺ ([M+H]⁺): 295.14410, found: 295.14389.

***(Z)*-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(4-methylpiperazin-1-yl)acetamide monohydrochloride (12a₁)**



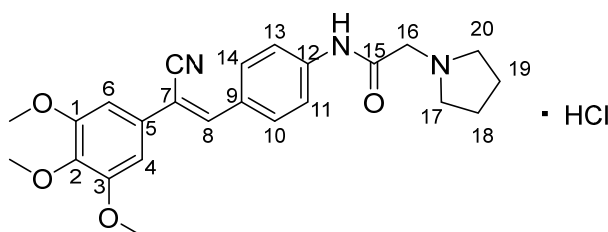
Yellow powder, mp: 230-232°C, yield 30%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.72 (s, 1H), 11.09 (s, 1H), 8.00 (s, 1H, C8-H), 7.95 (d, 2H, C11, C13-H), 7.83 (d, 2H, C10, C14-H) 7.01 (s, 2H, C4, C6-H), 4.10 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.61 (s, 4H, C17, C18, C20, C21-H), 3.40 (s, 4H, C17, C18, C20, C21-H), 2.82 (s, 3H, C19-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 165.07 (s, C15), 153.70 (s, C1, C3), 142.37 (s, C12), 140.59 (s, C2), 138.81 (s, C8), 130.56 (s, C10, C14), 130.04 (s, C9), 129.68 (s, C5), 119.81 (s, C11, C13), 118.68 (s, C7-CN), 109.07 (s, C7), 103.77 (s, C4, C6), 60.64 (s, C2-OCH₃), 57.76 (s, C16), 56.60 (s, C1, C3-OCH₃), 49.96 (d, C17, C18, C20, C21), 42.15 (s, C19). HRMS calcd. for C₂₅H₃₁N₄O₄⁺ ([M+H]⁺): 451.23398, found: 451.23396.

***(Z)*-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(4-ethylpiperazin-1-yl)acetamide monohydrochloride (12a₂)**



Yellow powder, mp: 232-234°C, yield 30%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.23 (s, 1H), 10.78 (s, 1H), 7.99 (s, 1H, C8-H), 7.94 (d, $J = 8.9$ Hz, 2H, C11, C13-H), 7.83 (d, $J = 8.8$ Hz, 2H, C10, C14-H), 7.01 (s, 2H, C4, C6-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.57 (m, 2H, C16-H), 3.49 – 2.87 (m, 10H, C17, C18, C19, C20, C21-H), 1.27 (t, $J = 7.3$ Hz, 3H, C22-H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 153.70 (s, C1, C3), 142.43 (s, C12), 140.87 (s, C2), 138.77 (s, C8), 130.49 (s, C10, C14), 130.07 (s, C5), 129.40 (s, C9), 119.80 (s, C11, C13), 118.71 (s, C7-CN), 108.87 (s, C7), 103.74 (s, C4, C6), 67.48 (s, C16), 60.64 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 50.94 (s, C21), 49.36 (s, C17, C18, C19, C20), 9.24 (s, C22). HRMS calcd. for C₂₆H₃₃N₄O₄⁺ ([M+H]⁺) 465.24963, found: 465.24979.

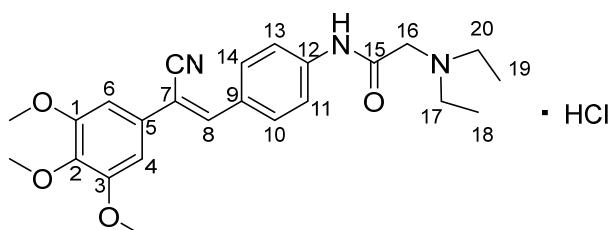
(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(pyrrolidin-1-yl)acetamide monohydrochloride (12a₃)



Yellow powder, mp: 238-240°C, yield 30%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.21 (s, 1H), 10.38 (s, 1H), 7.99 (s, 1H, C8-H), 7.95 (d, 2H, C11, C13-H), 7.80 (d, 2H, C10, C14-H), 7.01 (s, 2H, C4, C6-H), 4.32 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.27 (d, 4H, C17, C20-H), 1.98 (s, 4H, C18, C19-H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 164.46 (s, C15), 153.69 (s, C1, C3), 142.35 (s, C12), 140.60 (s, C2), 138.78 (s, C8), 130.58 (s, C10, C14), 130.03 (s, C5), 129.68 (s, C9), 119.70 (s, C11, C13), 118.68 (s, C7-CN), 109.05 (s, C7), 103.75 (s, C4, C6), 60.62 (s,

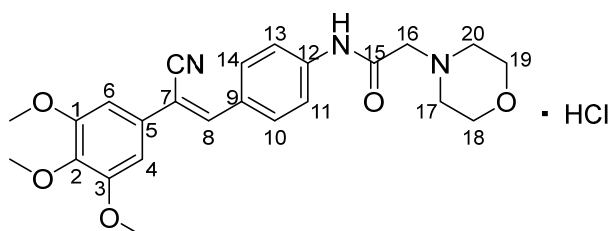
C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 56.16 (s, C16), 54.45 (s, C17, C20), 23.31 (s, C18, C19). HRMS calcd. for C₂₄H₂₈N₃O₄⁺ ([M+H]⁺): 422.20743, found: 422.20746.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(diethylamino)acetamide monohydrochloride (12a₄)



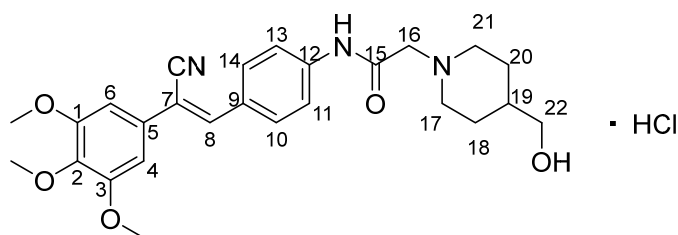
Yellow powder, mp: 230-232°C, yield 30%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.51 (s, 1H), 9.95 (s, 1H), 8.00 (s, 1H, C8-H), 7.96 (d, 2H, C11, C13-H), 7.83 (d, 2H, C10, C14-H), 7.02 (s, 2H, C4, C6-H), 4.25 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.27 (s, 4H, C17, C20-H), 1.27 (t, *J* = 7.2 Hz, 6H, C18, C19-H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.27 (s, C15), 153.69 (s, C1, C3), 142.33 (s, C12), 140.52 (s, C2), 138.79 (s, C8), 130.55 (s, C10, C14), 130.01 (s, C5), 129.85 (s, C9), 119.80 (s, C11, C13), 118.66 (s, C7-CN), 109.17 (s, C7), 103.76 (s, C4, C6), 60.62 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 53.20 (s, C16), 48.89 (s, C17, C20), 9.41 (s, C18, C19). HRMS calcd. for C₂₄H₃₀N₃O₄⁺ ([M+H]⁺): 424.22308, found: 424.22314.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-morpholinoacetamide monohydrochloride (12a₅)



Yellow powder, mp: 140-142°C, yield 30%. ^1H NMR (300 MHz, DMSO- d_6): δ 11.34 (s, 1H), 10.81 (s, 1H), 8.00 (s, 1H, C8-H), 7.96 (d, 2H, C11, C13-H), 7.82 (d, 2H, C10, C14-H), 7.01 (s, 2H, C4, C6-H), 4.25 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.38 (s, 8H, C17, C18, C19, C20-H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 163.72 (s, C15), 153.71 (s, C1, C3), 142.34 (s, C12), 140.45 (s, C8), 138.82 (s, C2), 130.59 (s, C10, C14), 130.03 (s, C5), 129.85 (s, C9), 119.84 (s, C7-CN, C11, C13), 109.19 (s, C7), 103.78 (s, C4, C6), 63.59 (s, C18, C19), 60.64 (s, C16), 57.50 (s, C2-OCH₃), 56.60 (s, C1, C3-OCH₃), 52.29 (s, C17, C20). HRMS calcd. for C₂₄H₂₈N₃O₅⁺ ([M+H]⁺): 438.20235, found: 438.20209.

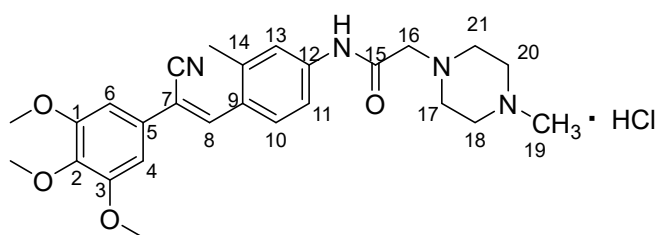
(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(4-(hydroxymethyl)piperizin-1-yl)acetamide monohydrochloride (12a₆)



Yellow powder, mp: 94-96°C, yield 28%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.27 (s, 1H), 9.93 (s, 1H), 7.99 (s, 1H, C8-H), 7.96 (d, 2H, C10, C14-H), 7.82 (d, 2H, C11, C13-H), 7.02 (s, 2H, C4, C6-H), 4.22 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.57 (d, J = 10.9 Hz, 2H, C22-H), 3.28 (d, J = 5.6 Hz, 2H, C17, C21-H), 3.12 (d, J = 11.5 Hz, 2H, C17, C21-H), 1.85 (d, J = 12.5 Hz, 2H, C18, C20-H), 1.58 (m, 3H, C18, C19, C20-H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 163.87 (s, C15), 153.69 (s, C1, C3), 142.34 (s, C12), 140.49 (s, C2), 138.80 (s, C8), 130.57 (s, C10, C14), 130.02 (s, C5), 129.82 (s, C9), 119.81 (s, C11, C13), 118.67 (s, C7-CN),

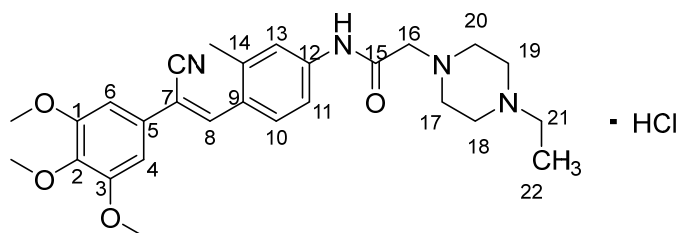
109.16 (s, C7), 103.76 (s, C4, C6), 65.34 (s, C22), 60.63 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 53.03 (s, C17, C21), 36.16 (s, C19), 26.19 (s, C18, C20). HRMS calcd. for C₂₆H₃₂N₃O₅⁺ ([M+H]⁺): 466.23365, found: 466.23389.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)-3-methylphenyl)-2-(4-methylpiperazin-1-yl)acetamide monohydrochloride (12b₁)



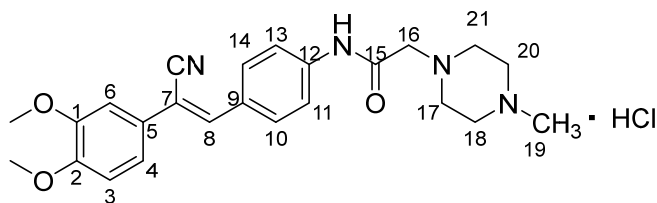
Yellow powder, mp: 232-237°C, yield 35%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.63 (s, 1H), 10.89 (s, 1H), 8.06 (s, 1H, C8-H), 7.80 (d, *J* = 8.2 Hz, 1H, C10-H), 7.63 (d, *J* = 8.4 Hz, 2H, C11, C13-H), 7.01 (s, 2H, C4, C6-H), 4.08 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.59 (s, 4H, C17, C18, C20, C21-H), 3.38 (s, 4H, C17, C18, C20, C21-H), 2.82 (s, 3H, C19-H), 2.39 (s, 3H, C14-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 153.71 (s, C1, C3), 141.93 (s, C9), 140.24 (s, C12), 139.29 (s, C14), 138.96 (s, C2), 129.86 (s, C5), 129.33 (s, C8), 129.09 (s, C10), 121.17 (s, C13), 118.37 (s, C7-CN), 117.28 (s, C11), 112.30 (s, C7), 104.02 (s, C4, C6), 60.65 (s, C2-OCH₃), 56.60 (s, C1, C3-OCH₃), 49.09 (s, C19), 20.29 (s, C14-CH₃). HRMS calcd. for C₂₆H₃₃N₄O₄⁺ ([M+H]⁺): 465.24963, found: 465.24985.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)-3-methylphenyl)-2-(4-ethylpiperazin-1-yl)acetamide monohydrochloride (12b₂)



Yellow powder, mp: 226-230°C, yield 35%. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 11.68 (s, 1H), 10.95 (s, 1H), 8.06 (s, 1H, C8-H), 7.80 (d, $J = 8.3$ Hz, 1H, C10-H), 7.63 (d, $J = 8.1$ Hz, 2H, C11, C13-H), 7.01 (s, 2H, C4, C6-H), 4.11 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.67 (d, $J = 11.5$ Hz, 4H, C17, C18, C19, C20-H), 3.39 (d, 4H, C17, C18, C19, C20-H), 3.19 (d, $J = 7.3$ Hz, 2H, C21-H), 2.39 (s, 3H, C14-CH₃), 1.28 (t, $J = 7.2$ Hz, 3H, C22-CH₃). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ 164.13 (s, C15), 153.73 (s, C1, C3), 141.93 (s, C14), 139.33 (s, C2, C12), 129.50 (s, C5, C10), 129.12 (s, C8, C9), 117.29 (s, C7-CN, C11), 112.38 (s, C7, C13), 104.03 (s, C4, C6), 60.62 (s, C2-OCH₃), 56.63 (s, C1, C3-OCH₃), 50.80 (s, C17, C20), 48.95 (s, C21), 47.70 (d, C18, C19), 20.30 (s, C14-CH₃), 9.18 (s, C22). HRMS calcd. for $\text{C}_{27}\text{H}_{35}\text{N}_4\text{O}_4^+$ ($[\text{M}+\text{H}]^+$): 479.26528, found: 479.26563.

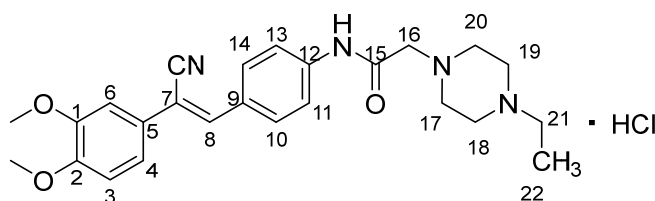
(Z)-N-(4-(2-cyano-2-(3,4-dimethoxyphenyl)vinyl)phenyl)-2-(4-methylpiperazin-1-yl)acetamide monohydrochloride (12c1)



Yellow powder, mp: 243-245°C, yield 35%. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 11.48 (s, 1H), 10.89 (s, 1H), 7.94 (s, 1H, C8-H), 7.91 (s, 2H, C11, C13-H), 7.80 (d, $J = 8.8$ Hz, 2H, C10, C14-H), 7.33 (d, $J = 2.3$ Hz, 1H, C6-H), 7.25 (dd, $J = 8.4, 2.2$ Hz, 1H, C4-H), 7.08 (d, $J = 8.5$ Hz, 1H, C3-H), 3.97 (s, 2H, C16-H), 3.86 (s, 3H, C1-OCH₃),

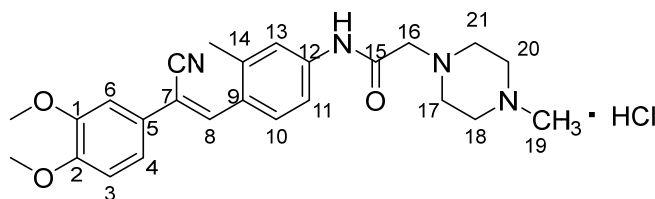
3.81 (s, 3H, C2-OCH₃), 3.54 (s, 4H, C17, C18, C20, C21-H), 3.33 (s, 4H, C17, C18, C20, C21-H), 2.82 (s, 3H, C19-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 150.24 (s, C1), 149.61 (s, C2), 140.56 (s, C12), 140.30 (s, C8), 130.34 (s, C10, C14), 129.93 (s, C5), 126.97 (s, C9), 119.80 (s, C11, C13), 119.34 (s, C4), 118.76 (s, C7-CN), 112.30 (s, C7), 109.09 (s, C3), 109.00 (s, C6), 56.22 (s, C2-OCH₃), 56.11 (s, C1-OCH₃), 48.90 (s, C17, C18, C20, C21), 42.22 (s, C19). HRMS calcd. for C₂₄H₂₉N₄O₃⁺ ([M+H]⁺): 421.22342, found: 421.22333.

(Z)-N-(4-(2-cyano-2-(3,4-dimethoxyphenyl)vinyl)phenyl)-2-(4-ethylpiperazin-1-yl)acetamide monohydrochloride (12c₂)



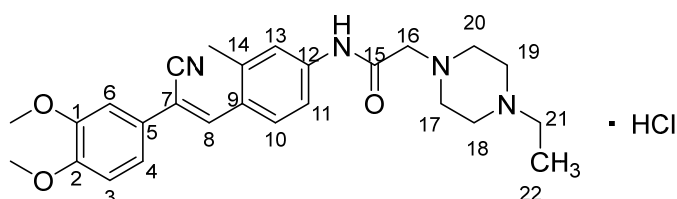
Yellow powder, mp: >250°C, yield 35%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.39 (s, 1H), 10.90 (s, 1H), 7.94 (s, 1H, C8-H), 7.92 (s, 2H, C11, C13-H), 7.80 (d, *J* = 8.9 Hz, 2H, C10, C14-H), 7.33 (d, *J* = 2.3 Hz, 1H, C6-H), 7.25 (dd, *J* = 8.4, 2.2 Hz, 1H, C4-H), 7.08 (d, *J* = 8.6 Hz, 1H, C3-H), 4.01 (s, 2H, C16-H), 3.86 (s, 3H, C1-OCH₃), 3.81 (s, 3H, C2-OCH₃), 3.68 – 3.57 (m, 6H, C17, C20, C21-H), 3.17 (s, 4H, C18, C19-H), 1.28 (t, *J* = 7.2 Hz, 3H, C22-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 150.25 (s, C1), 149.62 (s, C2), 140.57 (s, C12), 140.32 (s, C8), 130.35 (s, C10, C14), 129.90 (s, C5), 126.97 (s, C9), 119.81 (s, C11, C13), 119.35 (s, C4), 118.76 (s, C7-CN), 112.32 (s, C7), 109.10 (s, C3), 109.01 (s, C6), 56.21 (s, C2-OCH₃), 56.11 (s, C1-OCH₃), 49.05 (s, C21), 9.23 (s, C22). HRMS calcd. for C₂₅H₃₁N₄O₃⁺ ([M+H]⁺): 435.23907, found: 435.23883.

***(Z)*-N-(4-(2-cyano-2-(3,4-dimethoxyphenyl)vinyl)-3-methylphenyl)-2-(4-methylpiperazin-1-yl)acetamide monohydrochloride (12d₁)**



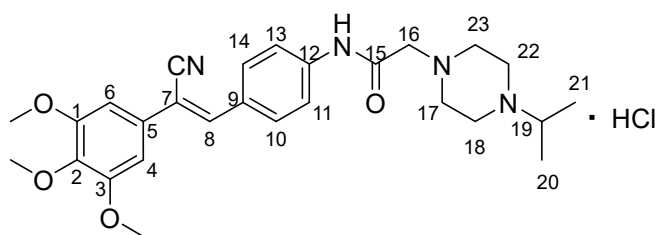
Yellow powder, mp: 234-240°C, yield 35%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.10 (s, 1H), 10.52 (s, 1H), 7.98 (s, 1H, C8-H), 7.78 (d, *J* = 8.4 Hz, 1H, C10-H), 7.61 (d, *J* = 8.3 Hz, 2H, C11, C13-H), 7.32 (d, *J* = 2.2 Hz, 1H, C6-H), 7.27 (dd, *J* = 8.3, 2.3 Hz, 1H, C4-H), 7.08 (d, *J* = 8.3 Hz, 1H, C3-H), 3.86 (s, 3H, C1-OCH₃), 3.82 (s, 3H, C2-OCH₃), 3.76 (s, 2H, C16-H), 3.43 (d, *J* = 42.3 Hz, 4H, C17, C18, C19, C20-H), 3.20 (d, *J* = 39.1 Hz, 4H, C17, C18, C19, C20-H), 2.80 (s, 3H, C19-H), 2.39 (s, 3H, C14-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 150.33 (s, C1), 149.60 (s, C2), 140.18 (s, C12), 140.05 (s, C14), 139.04 (s, C8), 129.36 (s, C5), 128.94 (s, C9), 126.84 (s, C10), 121.17 (s, C11), 119.48 (s, C4), 118.49 (s, C13), 117.27 (s, C7-CN), 112.32 (s, C7), 112.11 (s, C3), 109.36 (s, C6), 56.21 (s, C2-OCH₃), 56.13 (s, C1-OCH₃), 51.60 (s, C16), 49.28 (s, C18, C20), 49.04 (s, C17, C21), 42.35 (s, C19), 20.29 (s, C14-CH₃). HRMS calcd. for C₂₅H₃₁N₄O₃⁺ ([M+H]⁺): 435.23907, found: 435.23877.

***(Z)*-N-(4-(2-cyano-2-(3,4-dimethoxyphenyl)vinyl)-3-methylphenyl)-2-(4-ethylpiperazin-1-yl)acetamide monohydrochloride (12d₂)**



White powder, mp: 248-250°C, yield 40%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.51 (s, 1H), 10.84 (s, 1H), 7.99 (s, 1H, C8-H), 7.79 (d, J = 8.8 Hz, 1H, C10-H), 7.61 (d, J = 7.7 Hz, 2H, C11, C13-H), 7.32 (d, J = 2.2 Hz, 1H, C6-H), 7.27 (dd, J = 8.3, 2.2 Hz, 1H, C4-H), 7.08 (d, J = 8.5 Hz, 1H, C3-H), 4.06 (s, 2H, C16-H), 3.86 (s, 3H, C1-OCH₃), 3.82 (s, 3H, C2-OCH₃), 3.63 (s, 6H, C17, C18, C19, C20, C21-H), 3.37 (s, 4H, C17, C18, C19, C20-H), 2.39 (s, 3H, C14-CH₃), 1.28 (t, J = 7.2 Hz, 3H, C22-H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 150.38 (s, C1), 149.61 (s, C2), 139.99 (s, C12), 139.88 (s, C14), 139.14 (s, C8), 129.68 (s, C5), 129.07 (s, C9), 126.79 (s, C10), 121.16 (s, C4), 119.52 (s, C13), 118.45 (s, C7-CN), 117.28 (s, C11), 112.33 (s, C7), 109.38 (s, C3, C6), 56.23 (s, C2-OCH₃), 56.14 (s, C1-OCH₃), 49.05 (s, C21), 20.31 (s, C14-CH₃), 9.20 (s, C22). HRMS calcd for C₂₆H₃₃N₄O₃⁺ ([M+H]⁺): 449.25472, found: 449.25452.

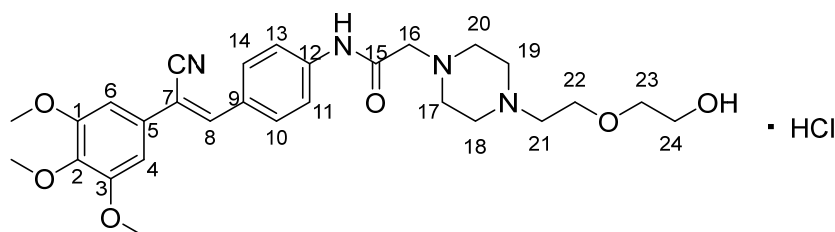
(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(4-isopropylpiperazin-1-yl)acetamide monohydrochloride (15)



Yellow powder, m.p.: 234-236°C, yield 40%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.48 (s, 1H), 10.99 (s, 1H), 7.99 (s, 1H, C8-H), 7.95 (d, J = 8.5 Hz, 2H, C11, C13-H), 7.82 (d, J = 8.6 Hz, 2H, C10, C14-H), 7.01 (s, 2H, C4, C6-H), 4.08 (s, 2H, C16-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.53 (q, J = 32.4, 17.4 Hz, 9H, C17, C18, C19, C22, C23-H), 1.32 (d, J = 6.5 Hz, 6H, C20, C21-CH₃). ^{13}C NMR (75 MHz,

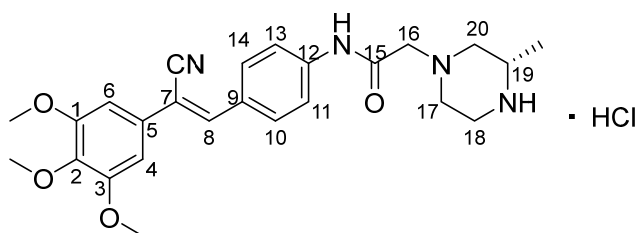
DMSO-*d*₆) δ 164.49 (s, C15), 153.19 (s, C1, C3), 142.02 (s, C12), 139.35 (s, C2), 138.16 (s, C8), 130.38 (s, C10, C14), 129.86 (s, C5), 129.75 (s, C9), 120.09 (s, C11, C13), 118.62 (s, C7-CN), 109.09 (s, C7), 103.19 (s, C4, C6), 60.77 (s, C2-OCH₃), 58.68 (s, C16), 57.90 (s, C19), 56.24 (s, C1-OCH₃, C3-OCH₃), 49.61 (s, C17, C23), 45.55 (s, C18, C22), 16.45 (s, C20, C21). HRMS calcd. for C₂₇H₃₅N₄O₄⁺ ([M+H]⁺): 479.26528, found: 479.26569.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(4-(2-(2-hydroxyethoxy)ethyl)piperazin-1-yl)acetamide monohydrochloride (16)



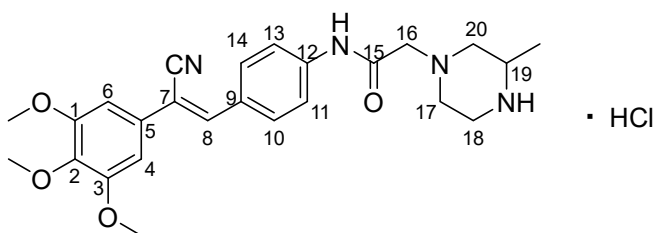
Yellow powder, mp: 240-243°C, yield 40%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.30 (s, 1H), 10.98 (s, 1H), 7.99 (s, 1H, C8-H), 7.95 (d, *J* = 8.3 Hz, 2H, C11, C13-H), 7.82 (d, *J* = 8.4 Hz, 2H, C10, C14-H), 7.01 (d, *J* = 1.2 Hz, 2H, C4, C6-H), 4.11 (s, 1H, C24-OH), 4.03 (s, 2H, C16-H), 3.88 – 3.86 (m, 6H, C1, C3-OCH₃), 3.83 (s, 2H, C22), 3.71 (d, *J* = 1.0 Hz, 3H, C2-OCH₃), 3.57 – 3.47 (m, 8H, C17, C18, C19, C20-H), 3.39 (s, 6H, C21, C23, C24-H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.68 (s, C15), 153.24 (s, C1, C3), 142.10 (s, C2, C12), 139.41 (s, C8), 138.22 (s, C5), 130.40 (s, C10, C14), 129.87 (s, C9), 120.09 (s, C11, C13), 118.64 (s, C7-CN), 109.11 (s, C7), 103.24 (s, C4, C6), 72.05 (s, C24), 63.67 (s, C22), 60.77 (s, C2-OCH₃), 60.29 (s, C23), 57.95 (s, C16), 56.27 (s, C1, C3-OCH₃), 55.84 (s, C21), 49.41 (s, C17, C20), 49.18 (s, C18, C19). HRMS calcd. for C₂₈H₃₇N₄O₆⁺ ([M+H]⁺): 525.27076, found: 525.27106.

(S,Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(3-methylpiperazin-1-yl)acetamide monohydrochloride (17)



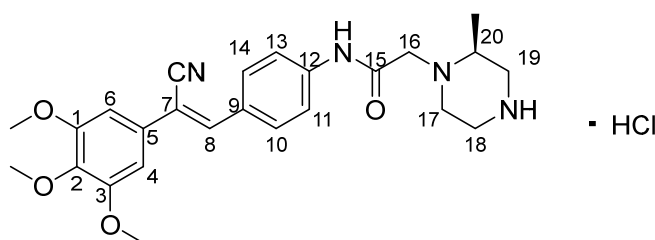
Yellow powder, mp: 196-200°C, yield 40%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.92 (s, 1H), 9.87 (s, 1H), 9.60 (s, 1H), 7.99 (s, 1H, C8-H), 7.95 (d, *J* = 8.3 Hz, 2H, C10, C14-H), 7.82 (d, *J* = 8.4 Hz, 2H, C11, C13-H), 7.01 (d, *J* = 1.3 Hz, 2H, C4, C6-H), 4.01 (s, 2H, C16-H), 3.87 (d, *J* = 1.3 Hz, 6H, C1, C3-OCH₃), 3.71 (d, *J* = 1.4 Hz, 3H, C2-OCH₃), 3.61 (d, *J* = 6.6 Hz, 2H, C18-H), 3.48 (s, 2H, C17-H), 3.28 (s, 2H, C20-H), 3.07 (s, 1H, C19-H), 1.30 (d, *J* = 6.4 Hz, 3H, C19-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 164.08 (s, C15), 153.69 (s, C1, C3), 142.32 (s, C12), 140.45 (s, C2), 138.79 (s, C8), 130.56 (s, C10, C14), 130.02 (s, C9), 129.90 (d, *J* = 16.8 Hz, C5), 119.83 (s, C11, C13), 118.66 (s, C7-CN), 109.13 (s, C7), 103.75 (s, C4, C6), 67.48 (s, C16), 60.63 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 54.03 (s, C17, C18), 48.53 (s, C19, C20), 15.74 (s, C19-CH₃). HRMS calcd. for C₂₅H₃₁N₄O₄⁺ ([M+H]⁺): 451.23398, found: 451.23395.

(Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(3-methylpiperazin-1-yl)acetamide monohydrochloride (18)



Yellow powder, mp: 195-200°C, yield 40%. ^1H NMR (300 MHz, DMSO- d_6) δ 10.92 (s, 1H), 9.89 (s, 1H), 9.62 (s, 1H), 7.99 (s, 1H, C8-H), 7.95 (d, J = 8.4 Hz, 2H, C10, C14-H), 7.82 (d, J = 8.3 Hz, 2H, C11, C13-H), 7.01 (d, J = 1.4 Hz, 2H, C4, C6-H), 4.01 (s, 2H, C16-H), 3.87 (d, J = 1.3 Hz, 6H, C1, C3-OCH₃), 3.71 (d, J = 1.4 Hz, 3H, C2-OCH₃), 3.62 (s, 2H, C18-H), 3.49 (s, 2H, C17-H), 3.29 (s, 2H, C20-H), 3.07 (s, 1H, C19-H), 1.30 (d, J = 6.3 Hz, 3H, C19-CH₃). ^{13}C NMR (75 MHz, DMSO- d_6) δ 164.17 (s, C15), 153.68 (s, C1, C3), 142.32 (s, C2), 140.46 (s, C8), 138.78 (s, C5), 130.56 (s, C10, C14), 130.01 (s, C9), 129.78 (s, C12), 119.82 (s, C11, C13), 118.66 (s, C7-CN), 109.12 (s, C7), 103.75 (s, C4, C6), 60.63 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 54.07 (s, C19), 48.53 (s, C17, C18, C20), 15.75 (s, C19-CH₃). HRMS calcd. for C₂₅H₃₁N₄O₄⁺ ([M+H]⁺): 451.23398, found: 451.23407.

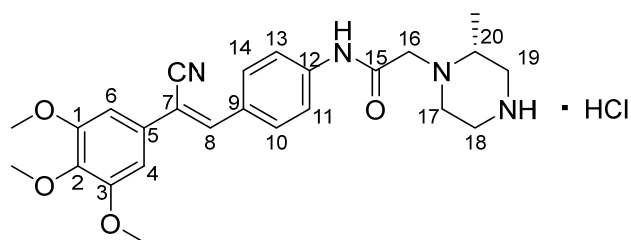
(S,Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(2-methylpiperazin-1-yl)acetamide monohydrochloride (19)



Yellow powder, mp: 200-202°C, yield 40%. ^1H NMR (300 MHz, DMSO- d_6) δ 11.07 (s, 1H), 9.74 (s, 3H), 7.99 (s, 1H, C8-H), 7.95 (d, J = 8.6 Hz, 2H, C11, C13-H), 7.82 (d, J = 8.6 Hz, 2H, C10, C14-H), 7.01 (s, 2H, C4, C6-H), 3.87 (s, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.54 (s, 7H, C17, C18, C19, C20-H), 1.30 (d, J = 7.1 Hz, 3H, C20-CH₃). ^{13}C NMR (75 MHz, DMSO- d_6) δ 153.68 (s, C1, C3), 142.31 (s, C8), 140.42 (s, C2), 138.78 (s, C12), 130.55 (s, C10, C14), 130.00 (s, C9), 129.86 (s, C5),

119.86 (s, C11, C13), 118.65 (s, C7-CN), 109.17 (s, C7), 103.75 (s, C4, C6), 60.63 (s, C2-OCH₃), 56.59 (s, C1, C3-OCH₃), 55.11 (s, C16), 53.78 (s, C20), 49.04 (s, C17, C19), 45.59 (s, C18), 14.40 (s, C20-CH₃). HRMS calcd. for C₂₅H₃₁N₄O₄⁺ ([M+H]⁺): 451.23398, found: 451.23419.

(R,Z)-N-(4-(2-cyano-2-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-2-(2-methylpiperazin-1-yl)acetamide monohydrochloride (20)



Yellow powder, mp: 200-202°C, yield 40%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.04 (s, 1H), 9.73 (s, 2H), 7.99 (s, 1H, C8-H), 7.98 – 7.92 (m, 2H, C11, C13-H), 7.82 (dd, *J* = 8.8, 1.8 Hz, 2H, C10, C14-H), 7.01 (d, *J* = 1.8 Hz, 2H, C4, C6-H), 4.12 (d, *J* = 43.9 Hz, 2H, C16-H), 3.87 (d, *J* = 1.8 Hz, 6H, C1, C3-OCH₃), 3.71 (s, 3H, C2-OCH₃), 3.46 (d, *J* = 13.6 Hz, 7H, C17, C18, C19, C20-H), 1.32 (d, *J* = 6.4 Hz, 3H, C20-CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 165.13 (s, C15), 154.49 (s, C1, C3), 143.11 (s, C2, C12), 141.20 (s, C8), 139.60 (s, C5), 131.37 (s, C10, C14), 130.75 (d, *J* = 8.7 Hz, C9), 120.67 (s, C11, C13), 119.46 (s, C7-CN), 110.00 (s, C7), 104.56 (s, C4, C6), 61.44 (s, C2-OCH₃), 57.40 (s, C1, C3-OCH₃), 55.98 (s, C16), 54.64 (s, C20), 49.82 (s, C17, C19), 46.42 (s, C18), 15.19 (s, C20-CH₃). HRMS calcd. for C₂₅H₃₁N₄O₄⁺ ([M+H]⁺): 451.23398, found: 451.23404.

3. References

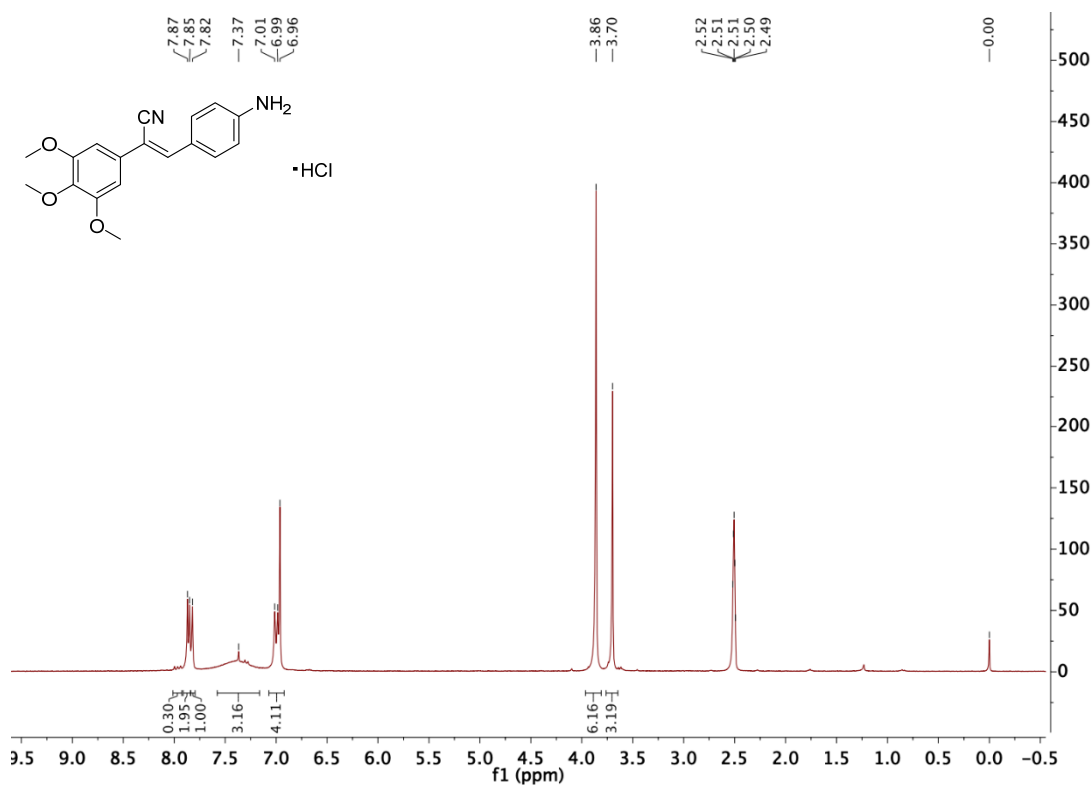
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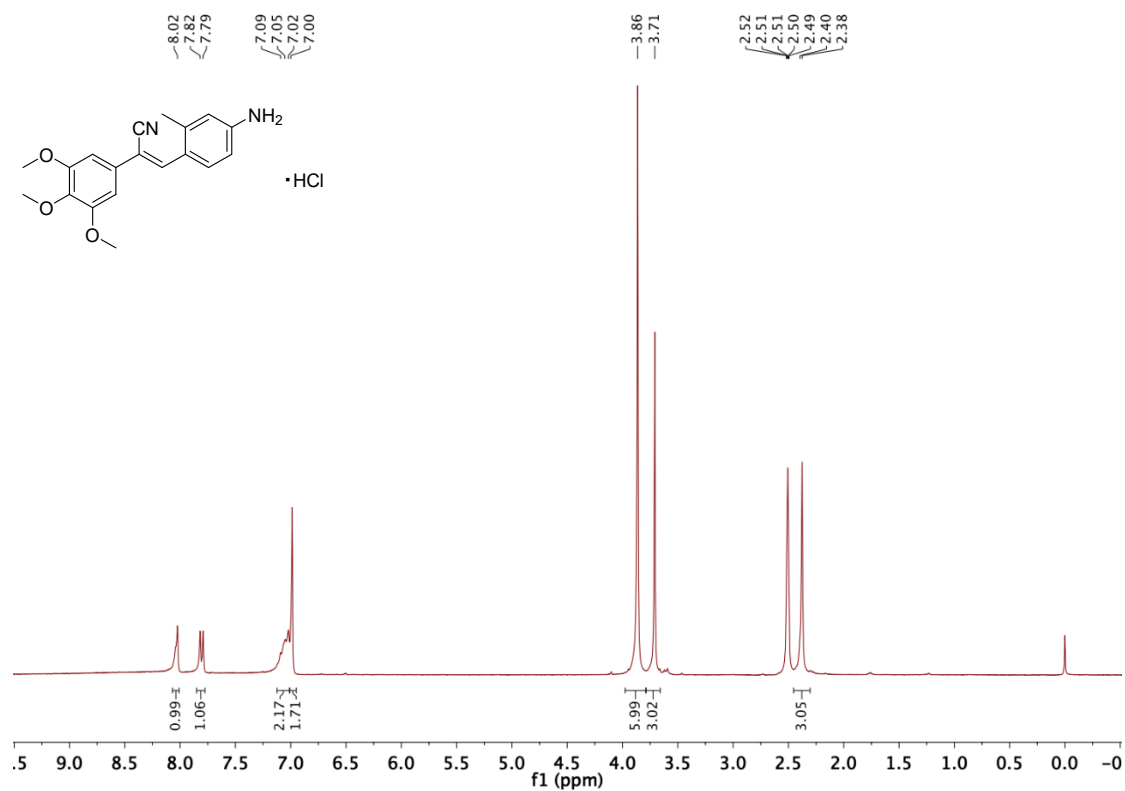
4. The spectra for the new compounds synthetized and the mass spectra

4.1. ^1H NMR

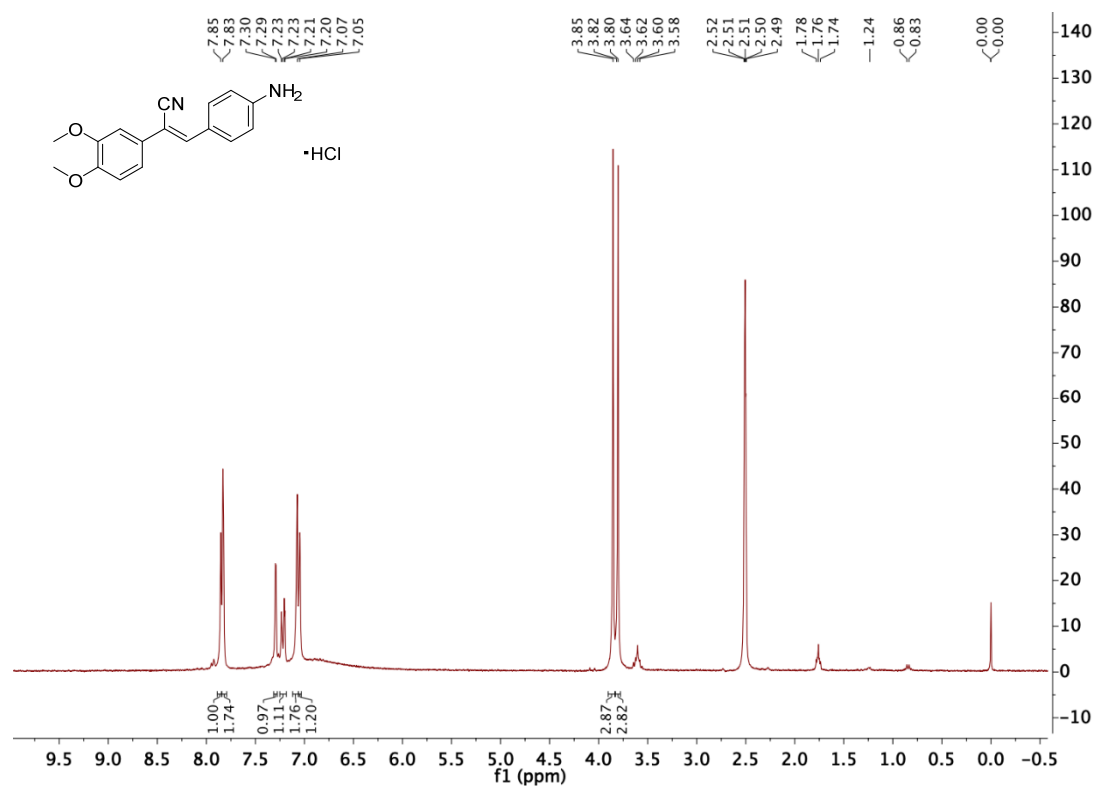
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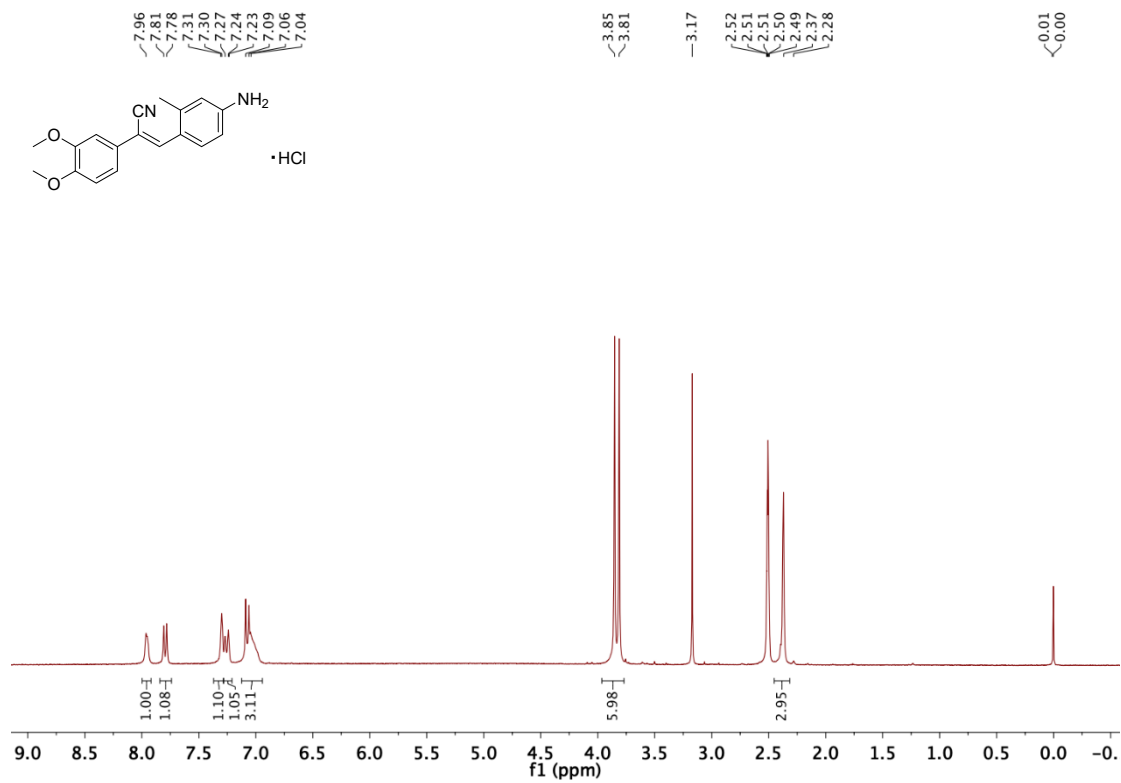
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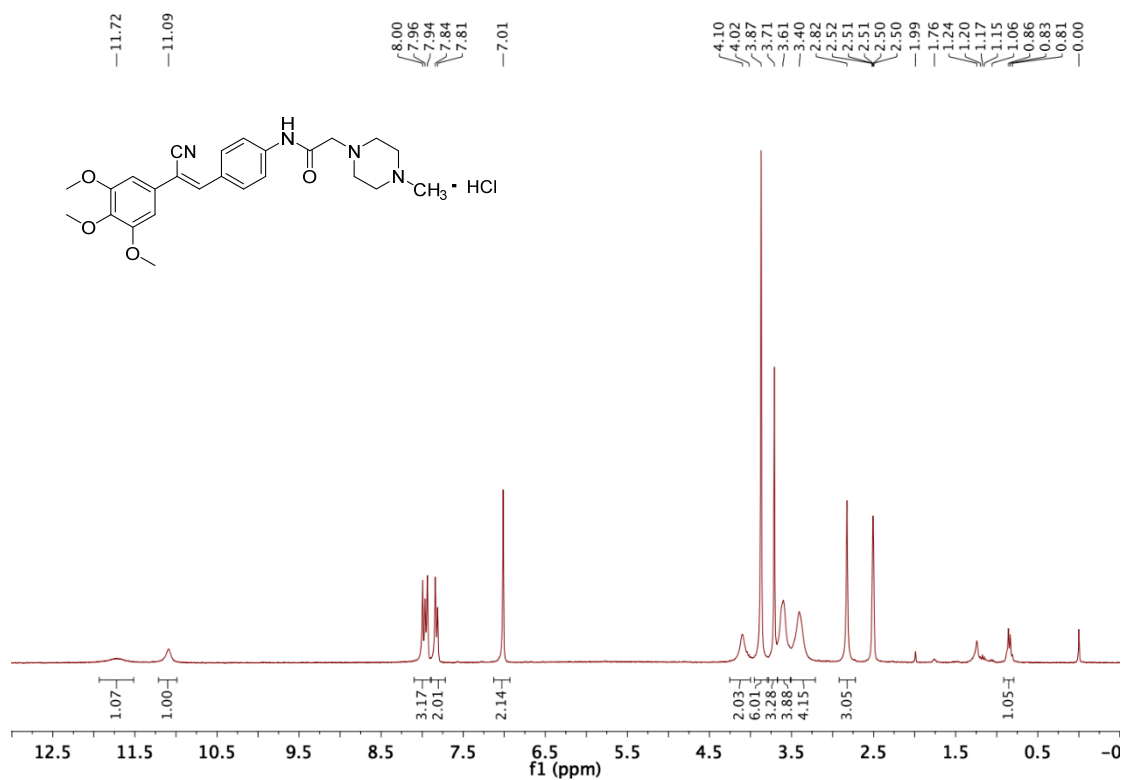
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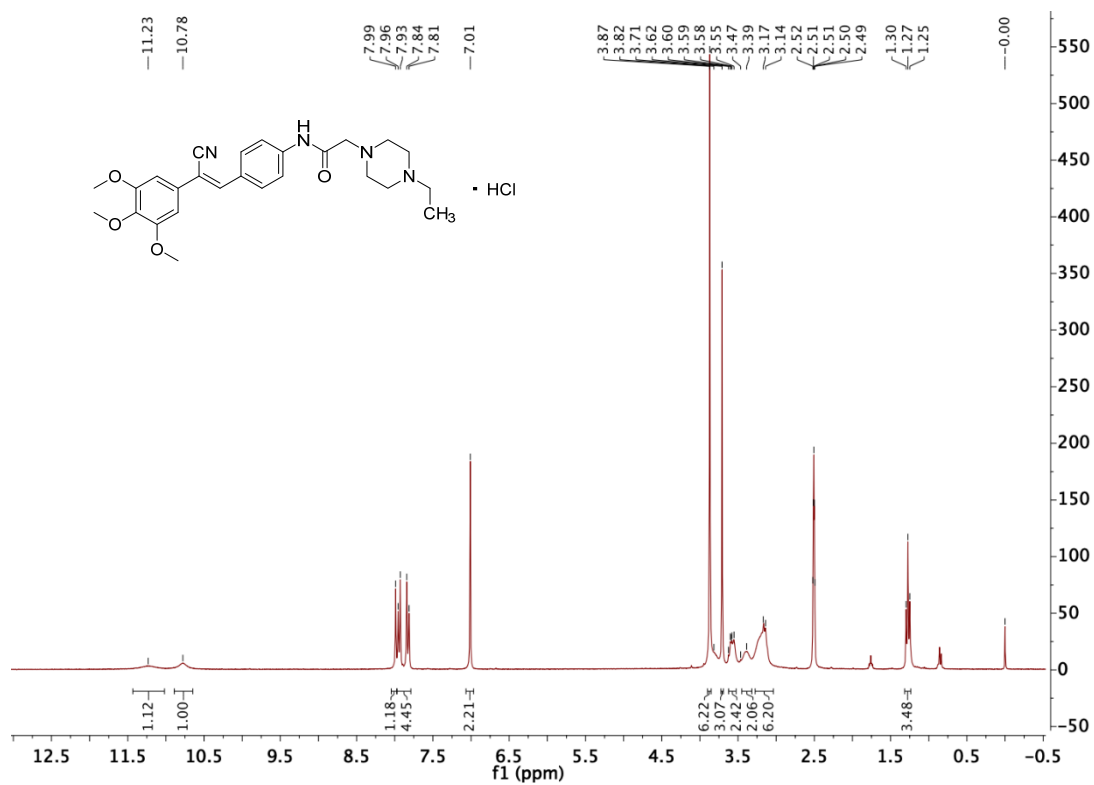
9d.



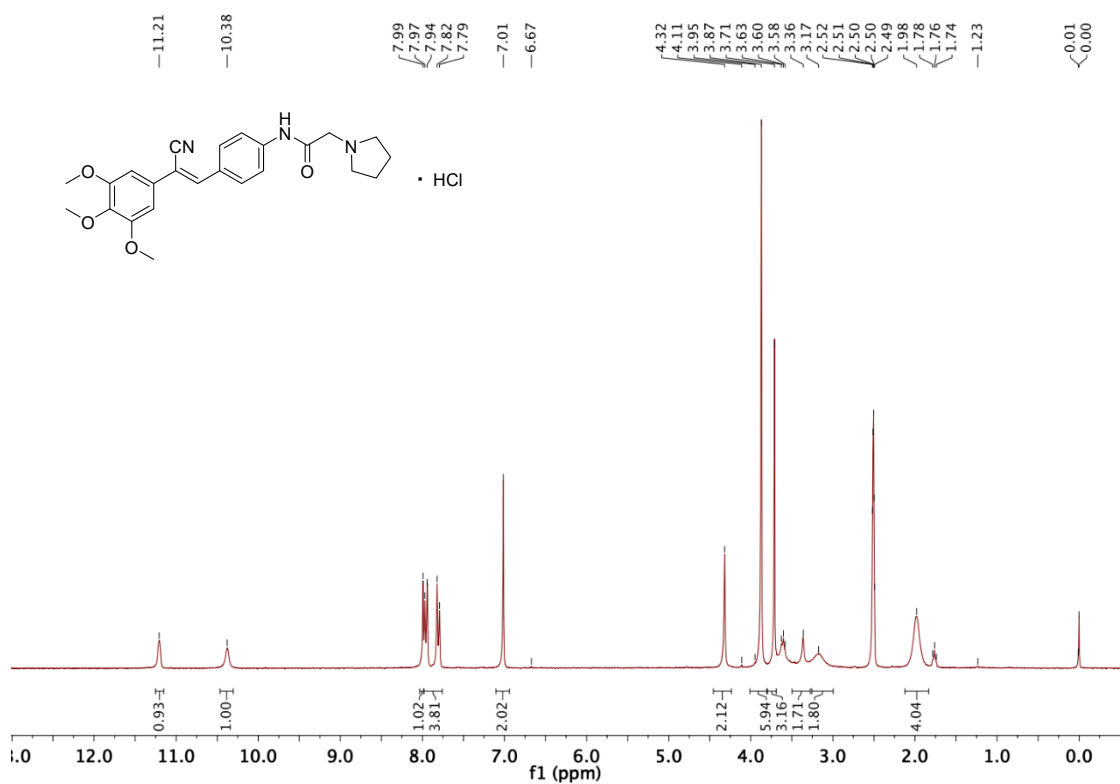
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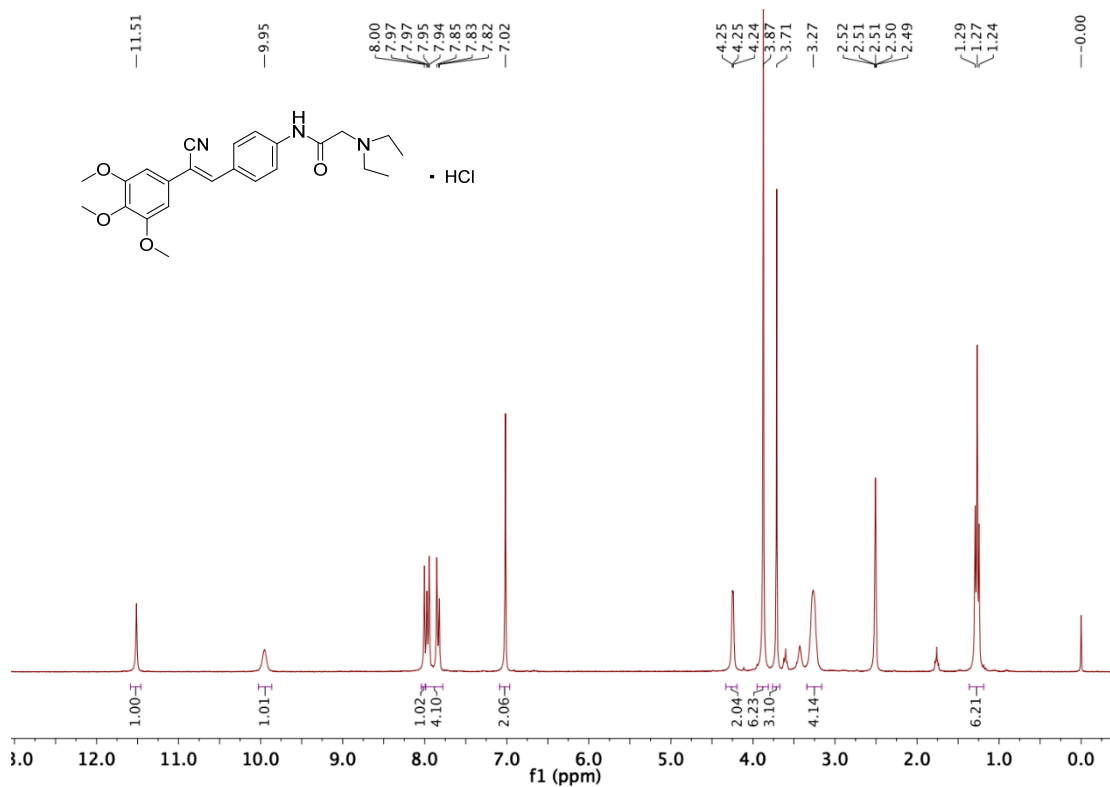
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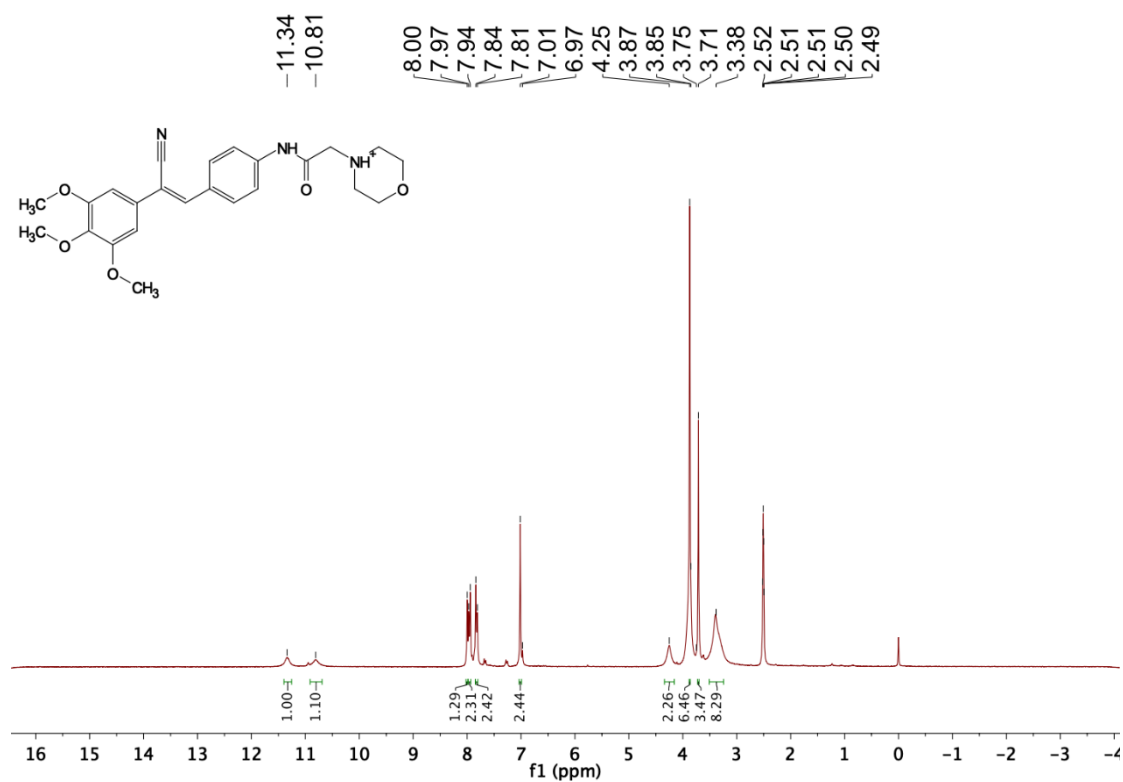
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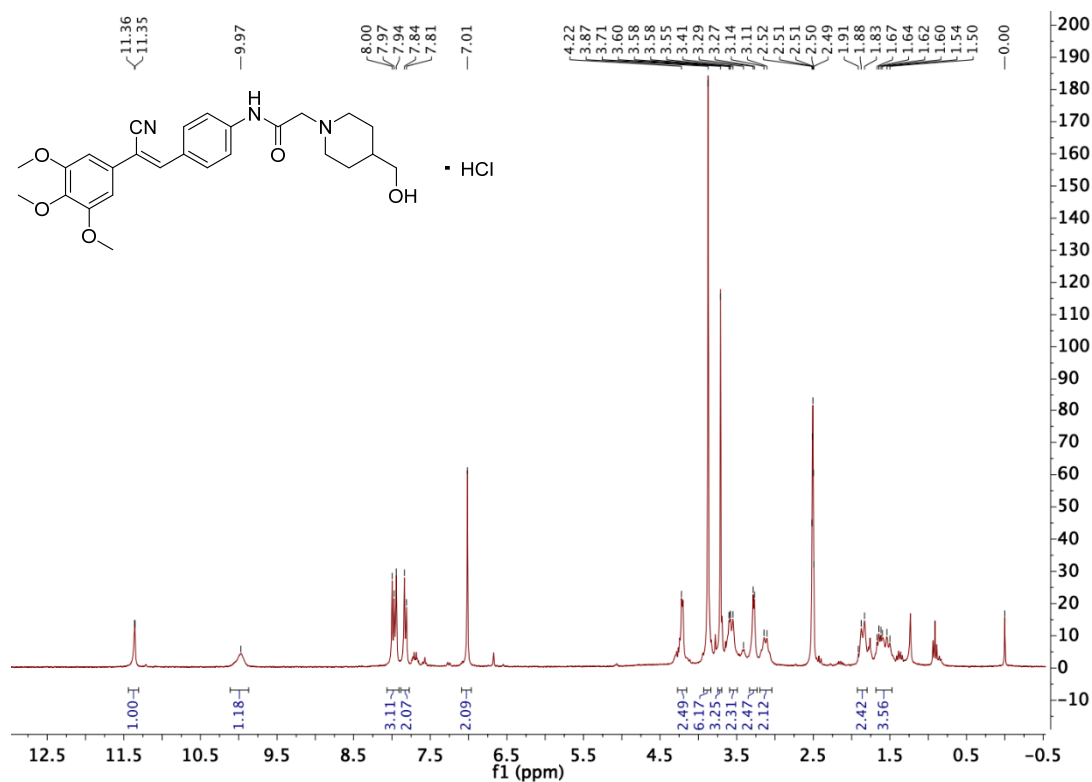
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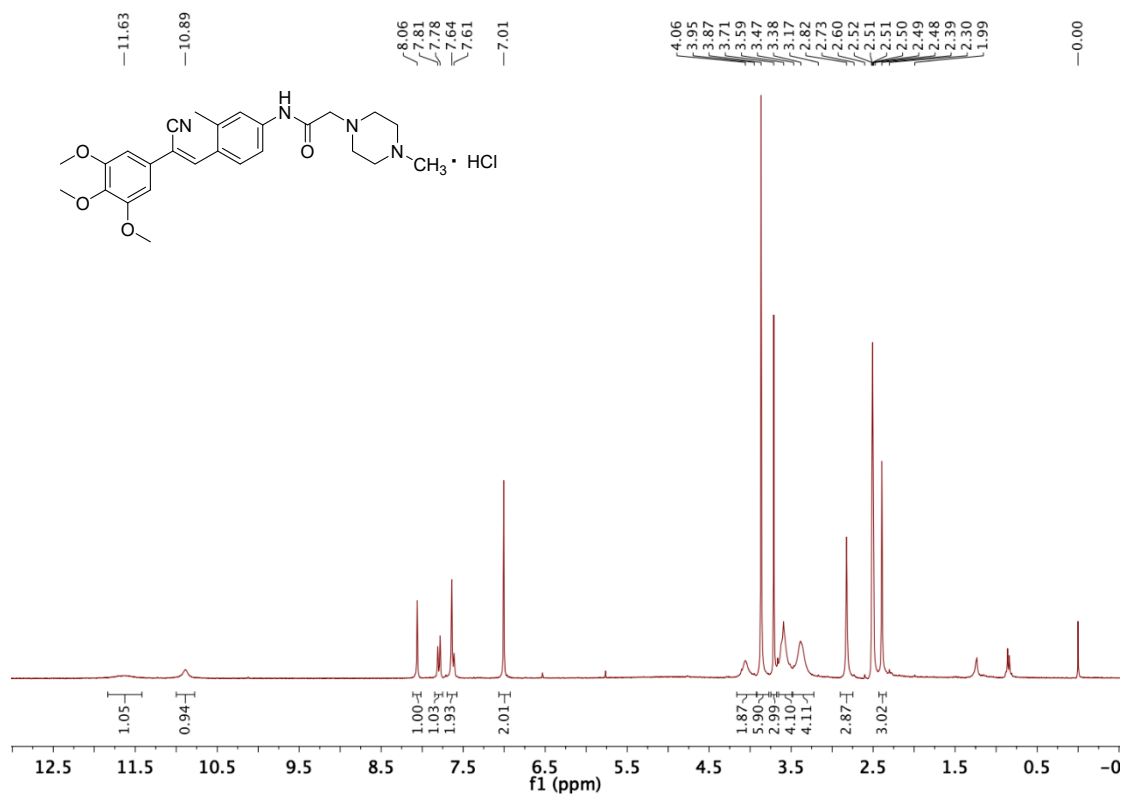
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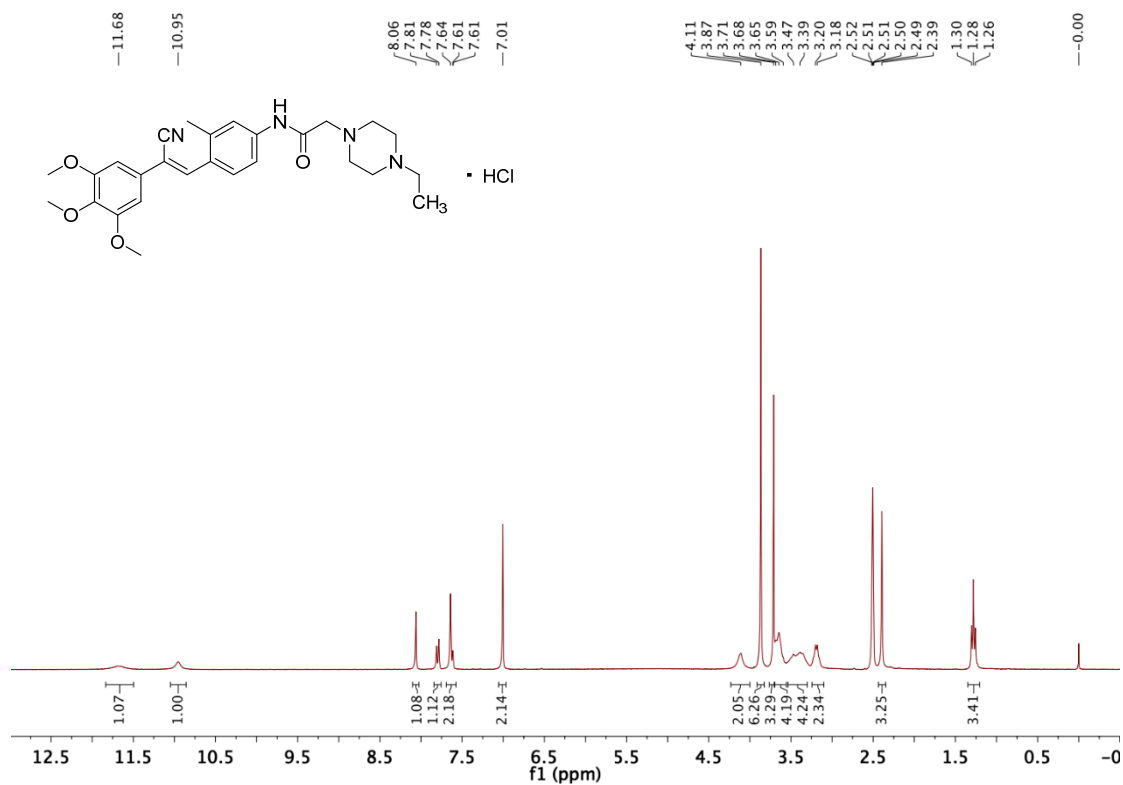
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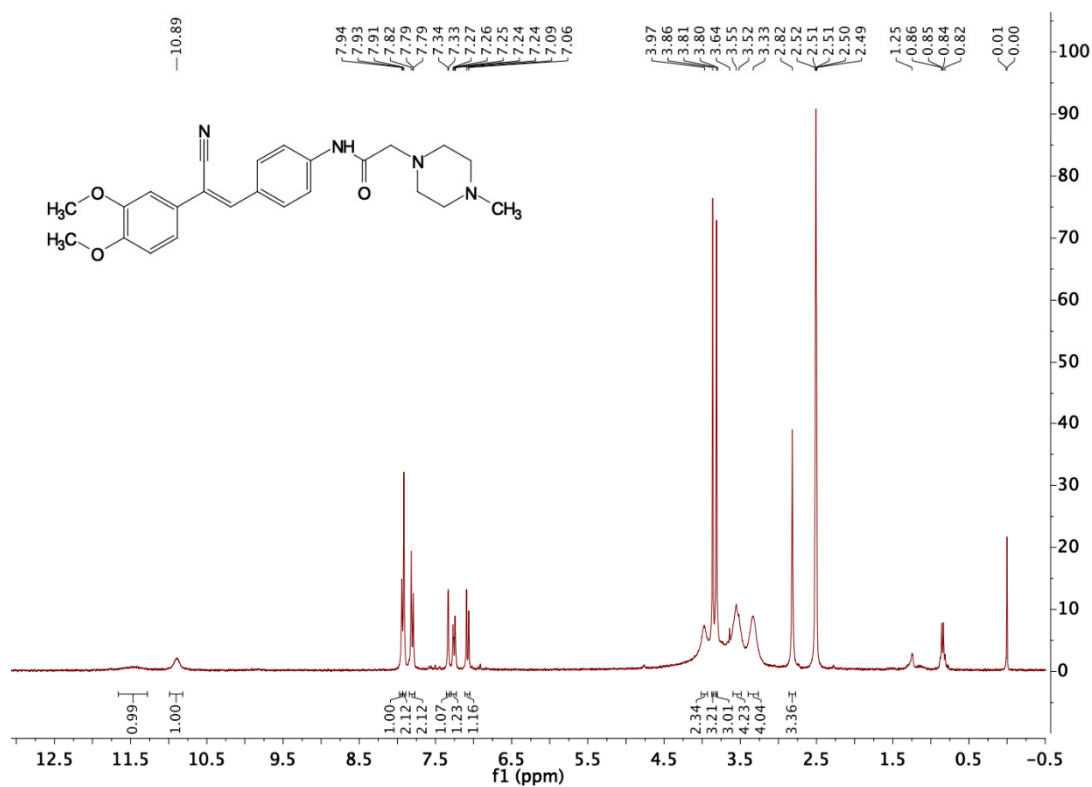
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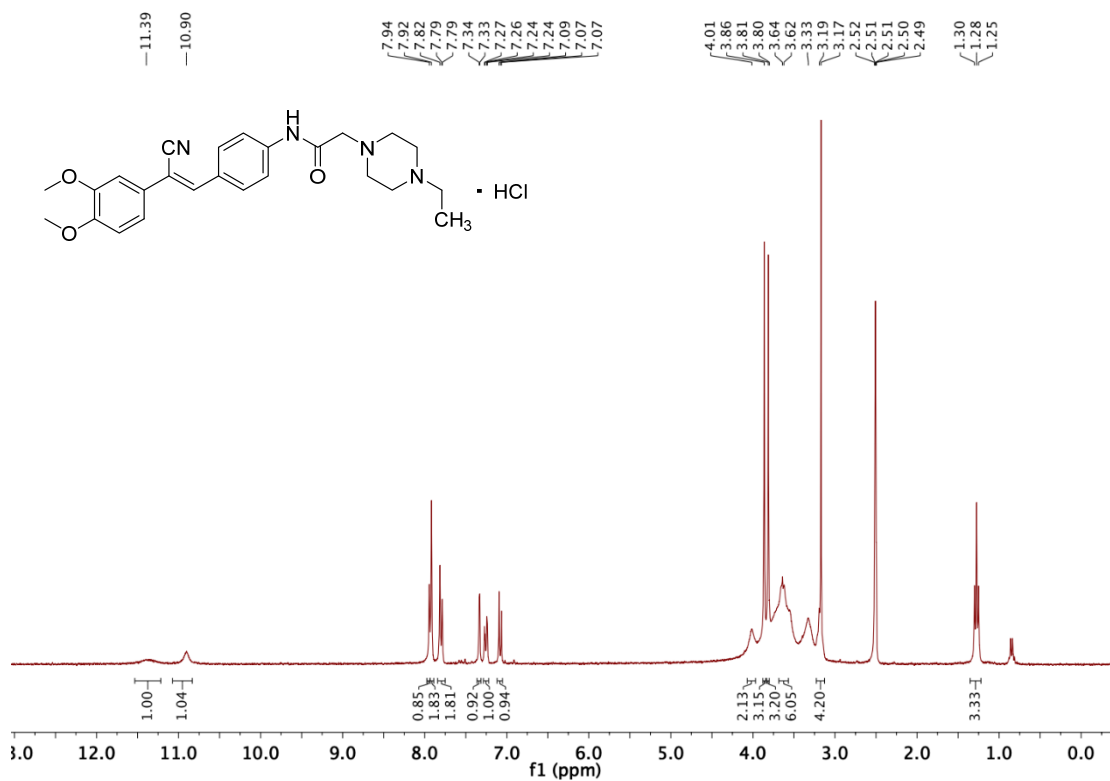
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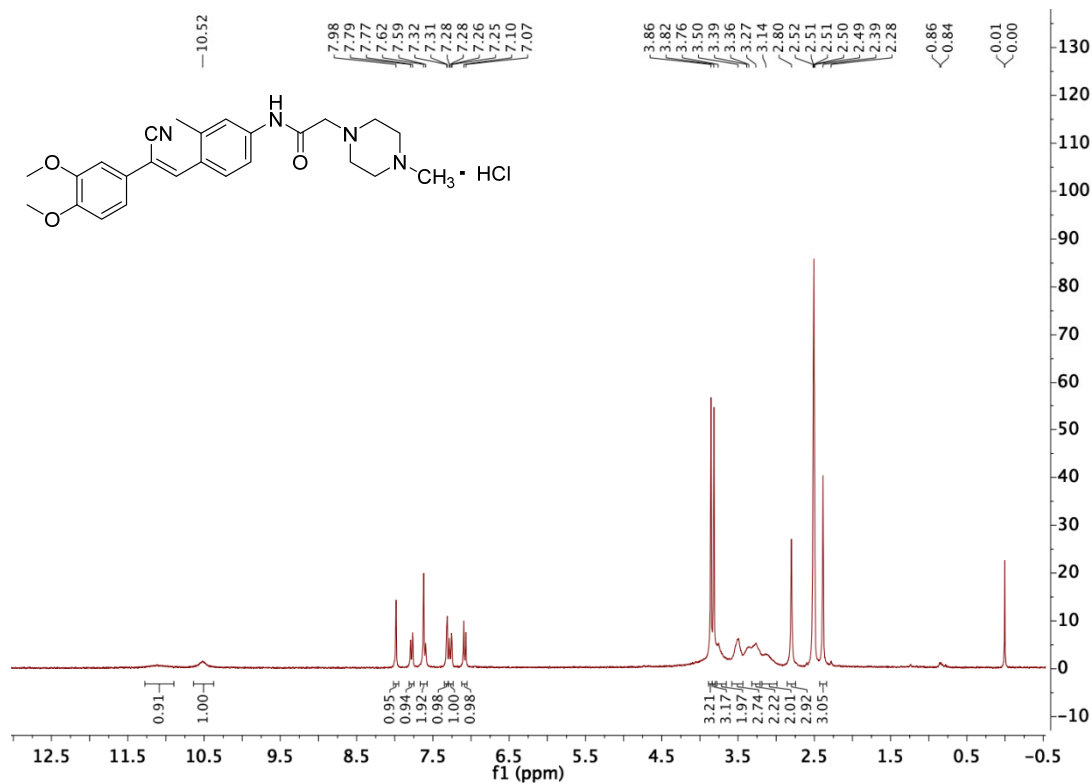
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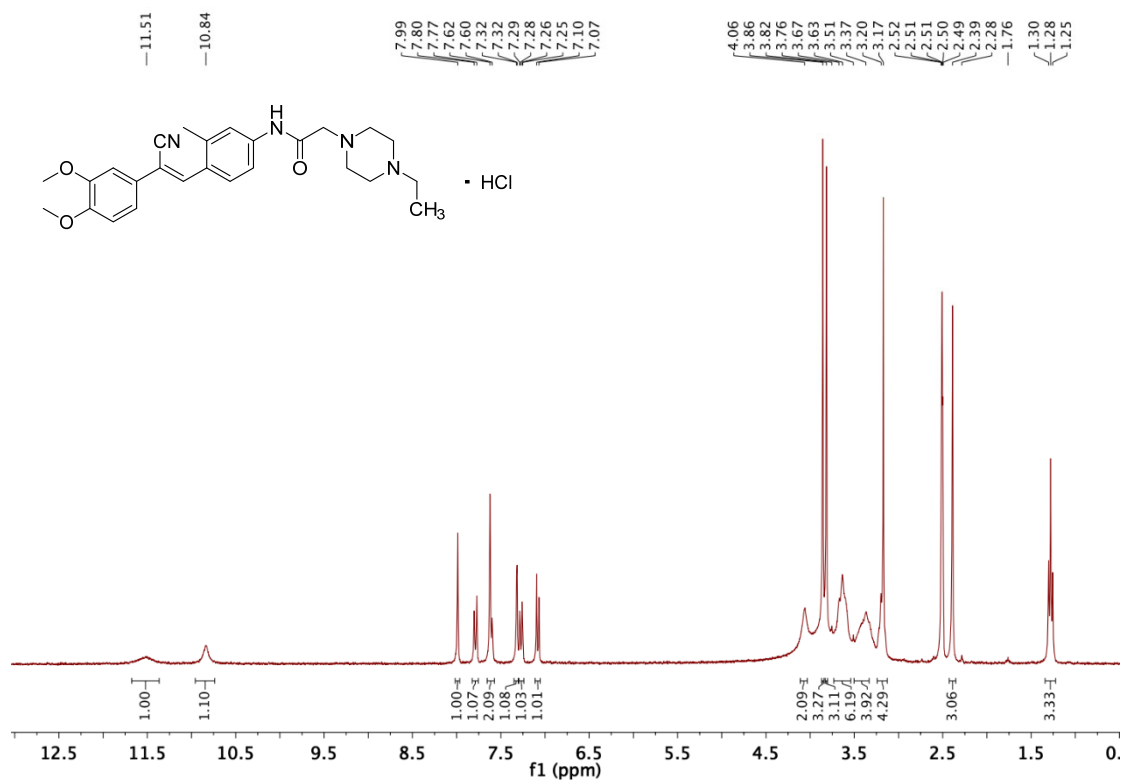
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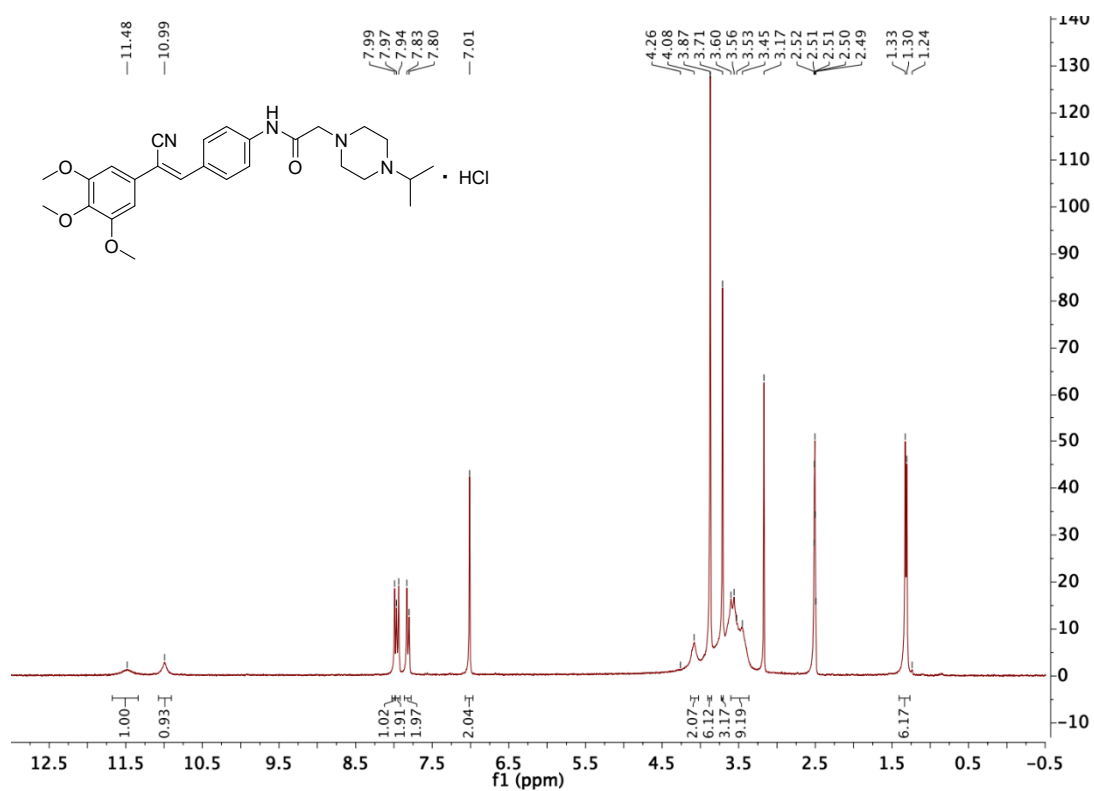
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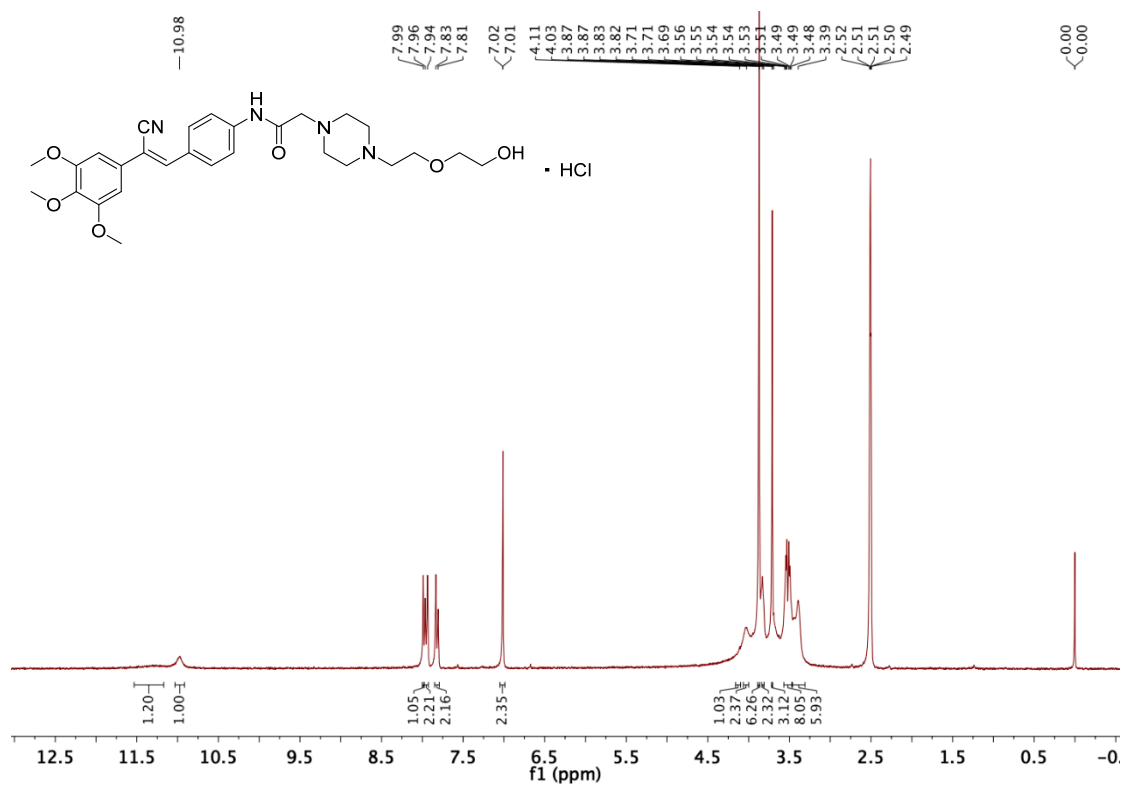
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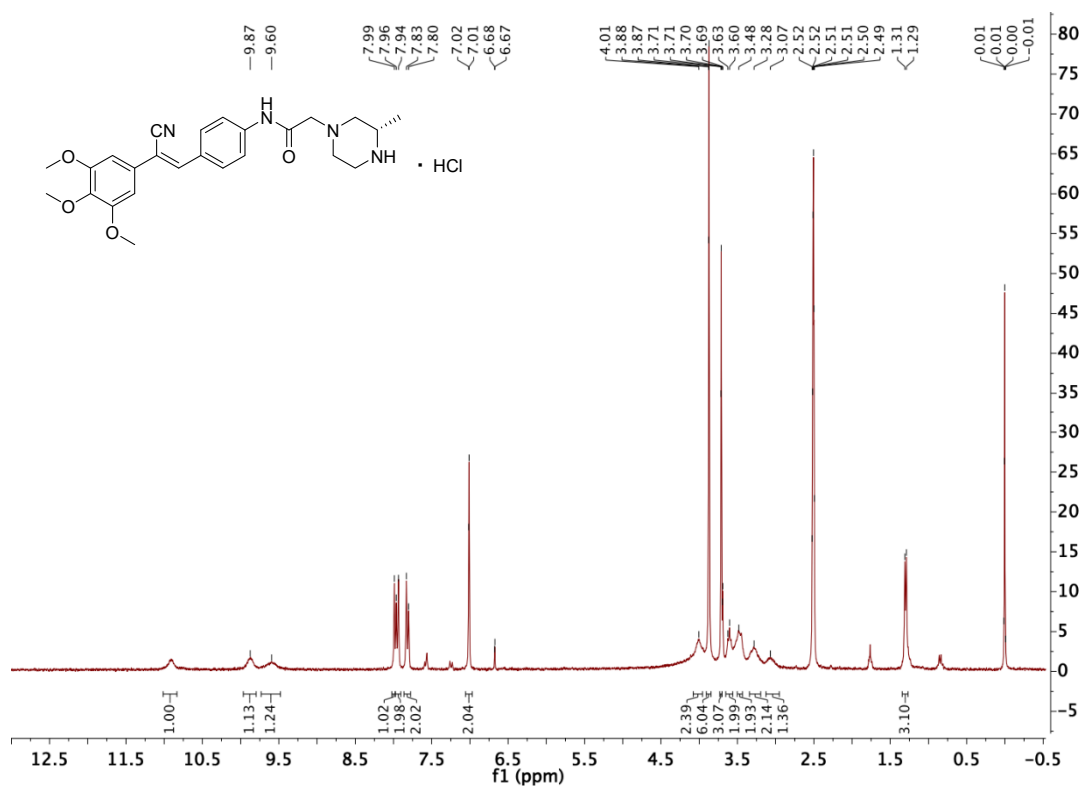
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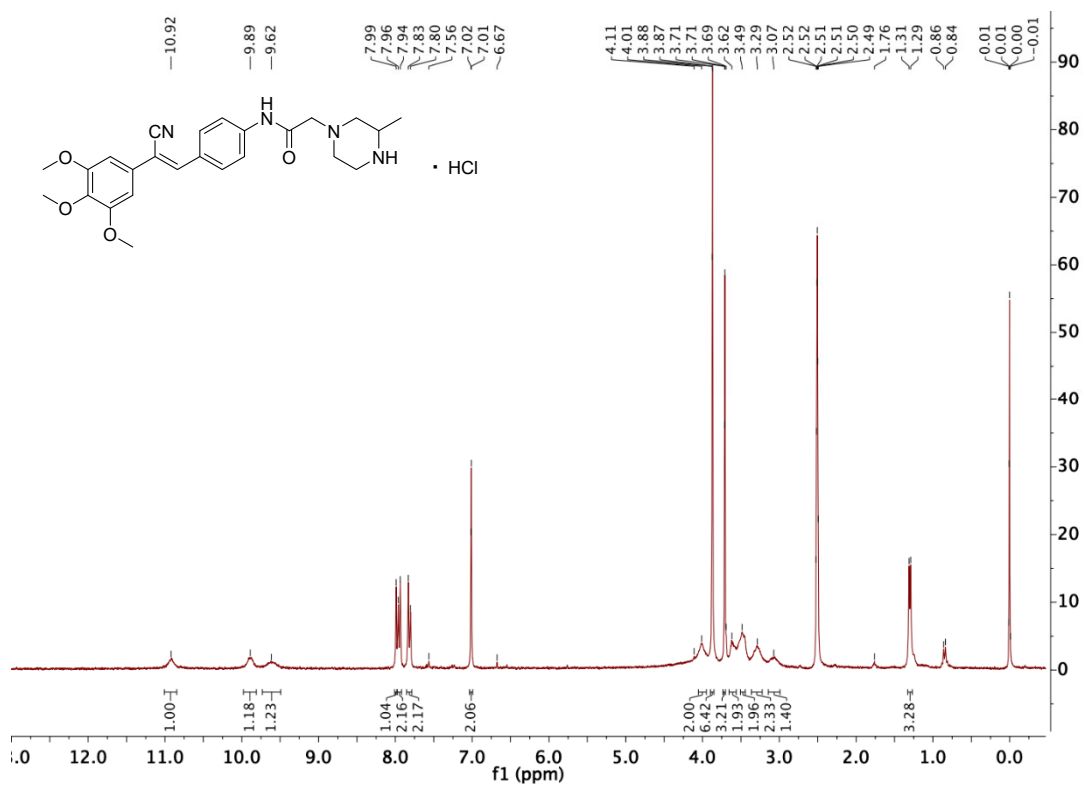
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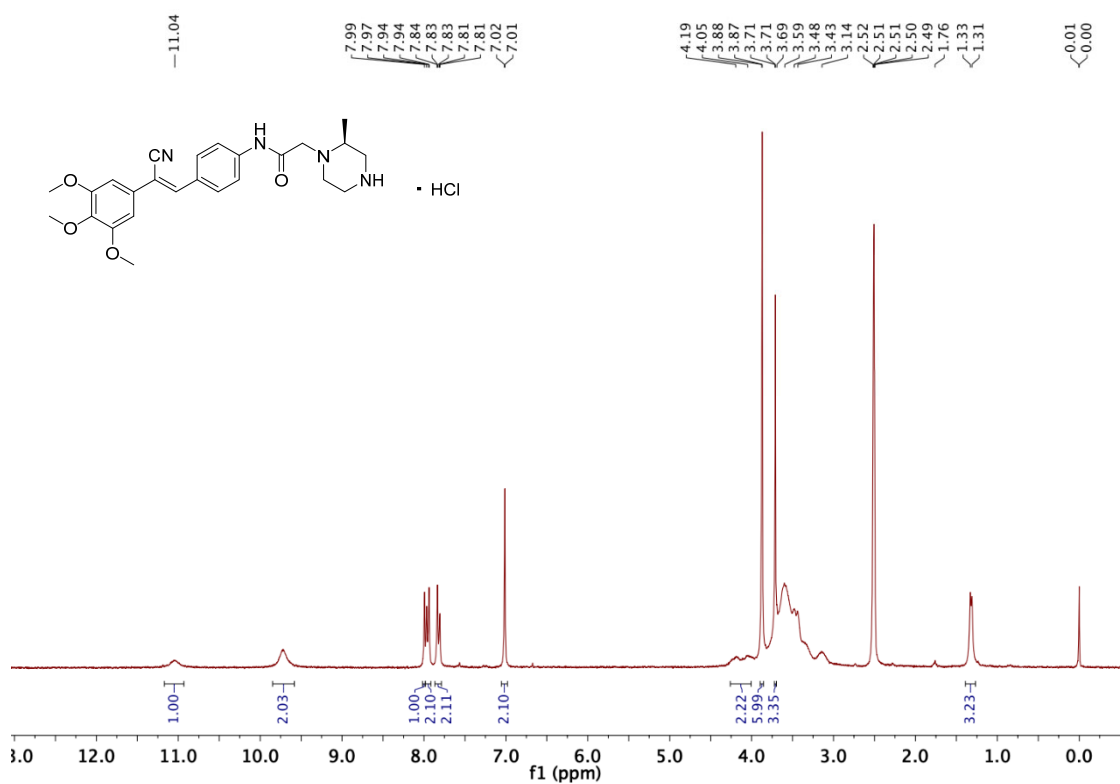
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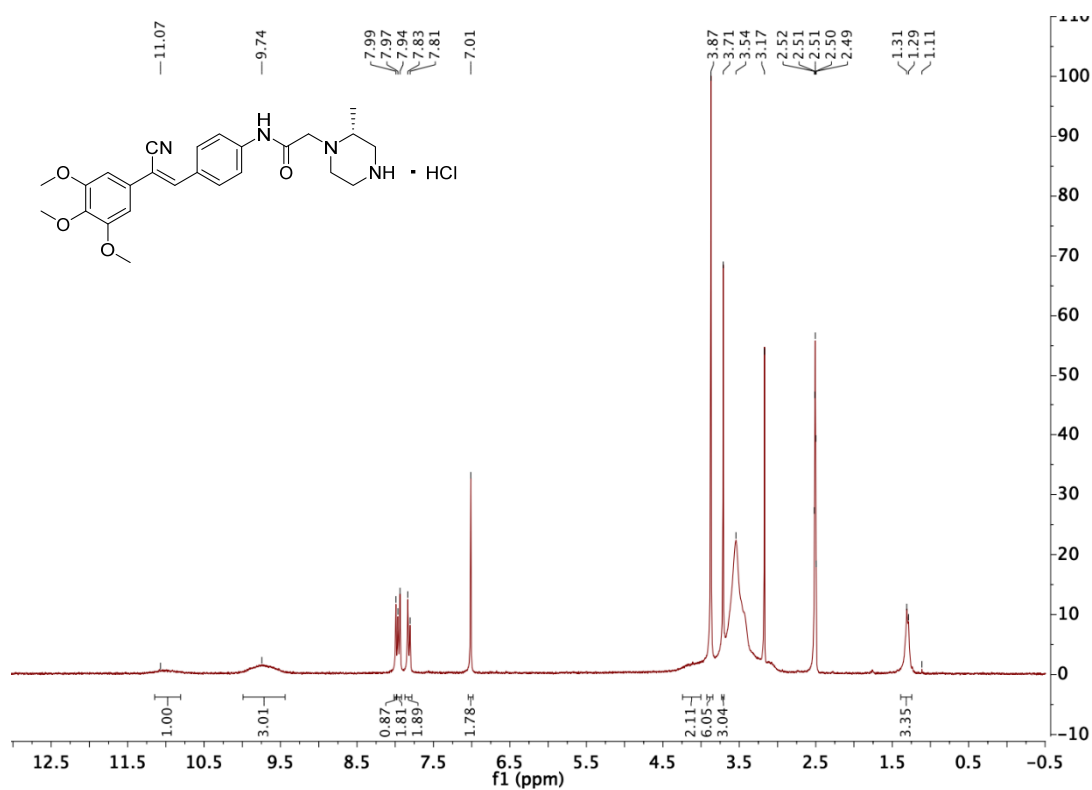
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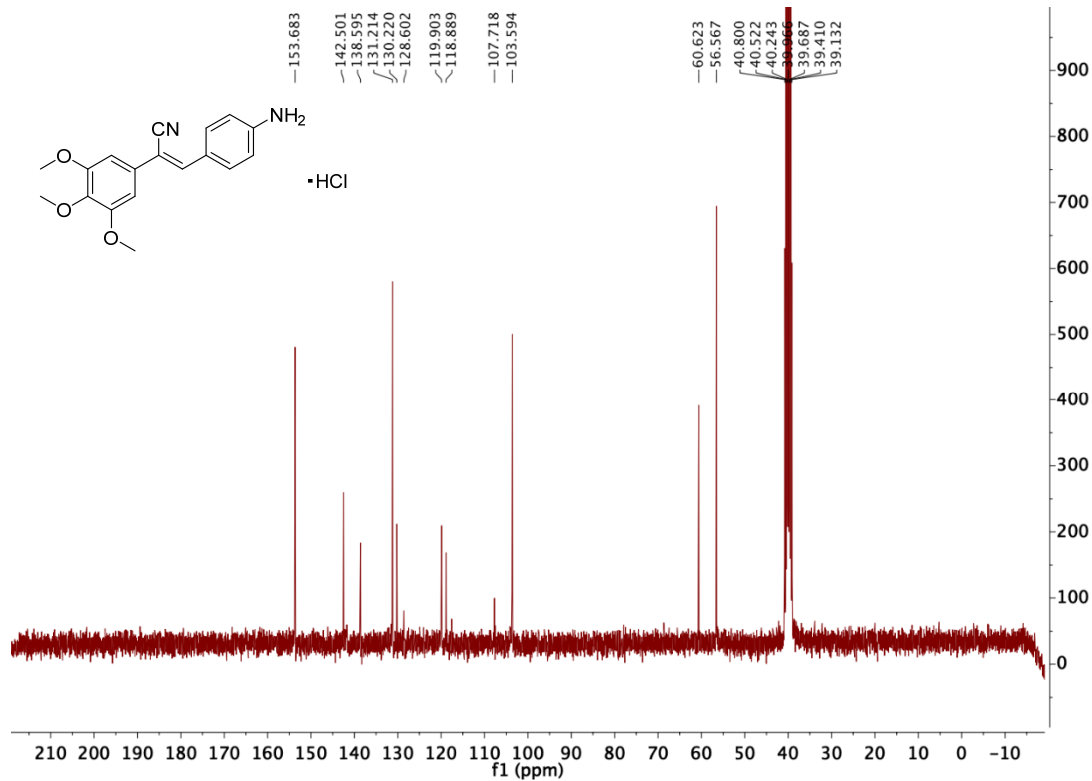


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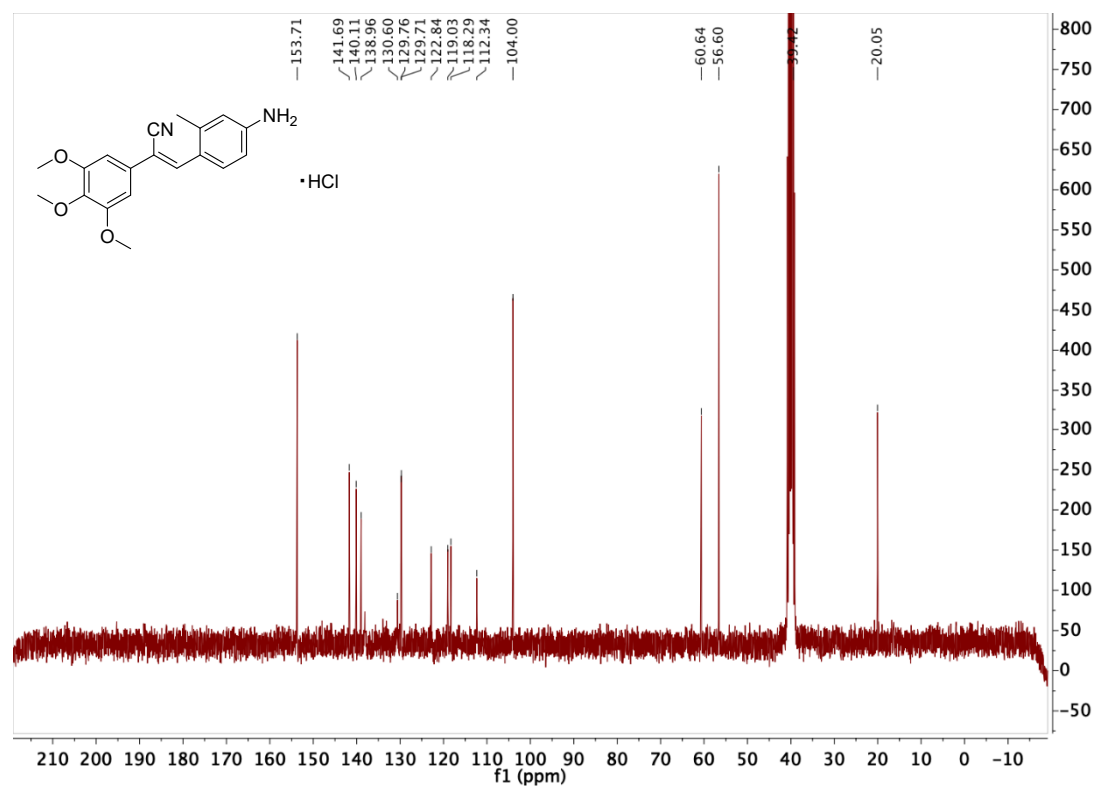


4.2. ¹³C NMR

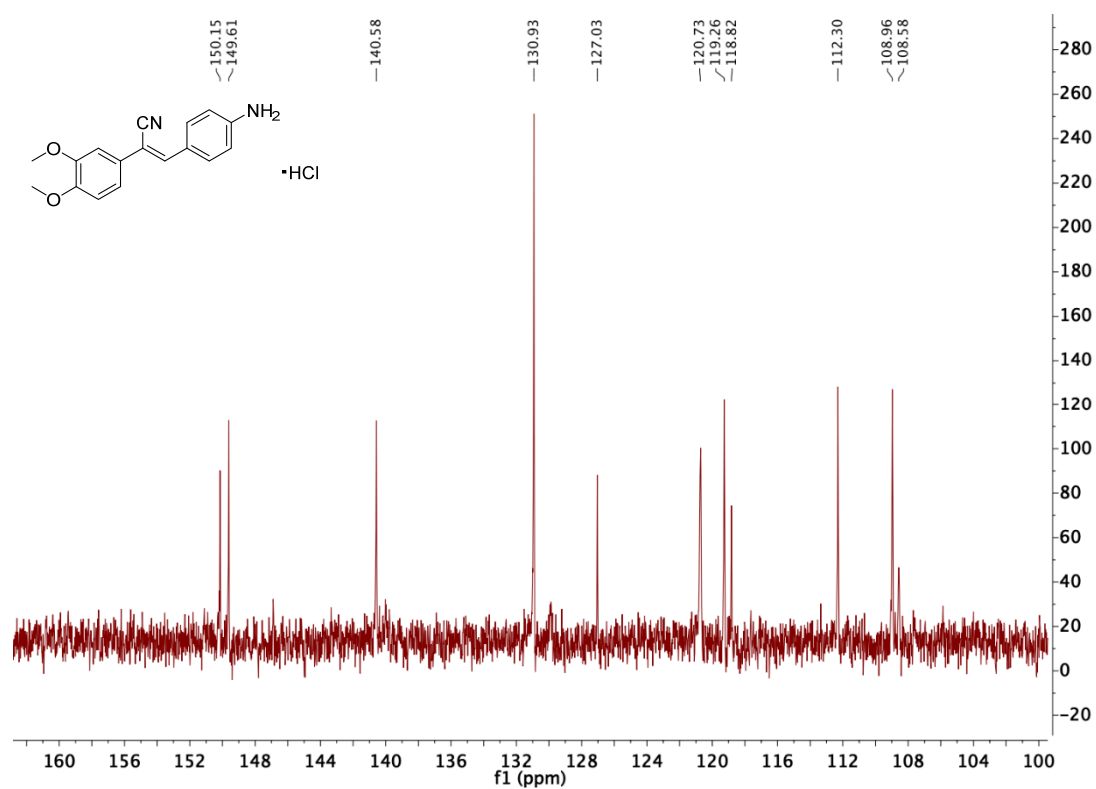
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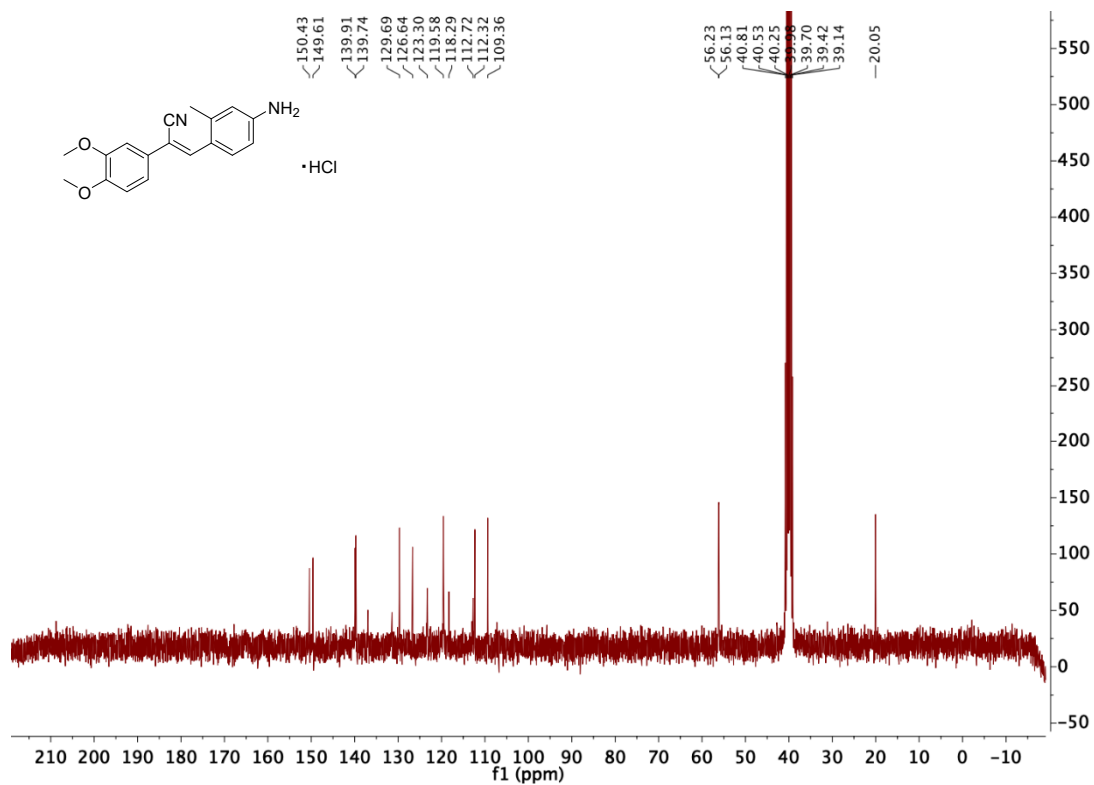
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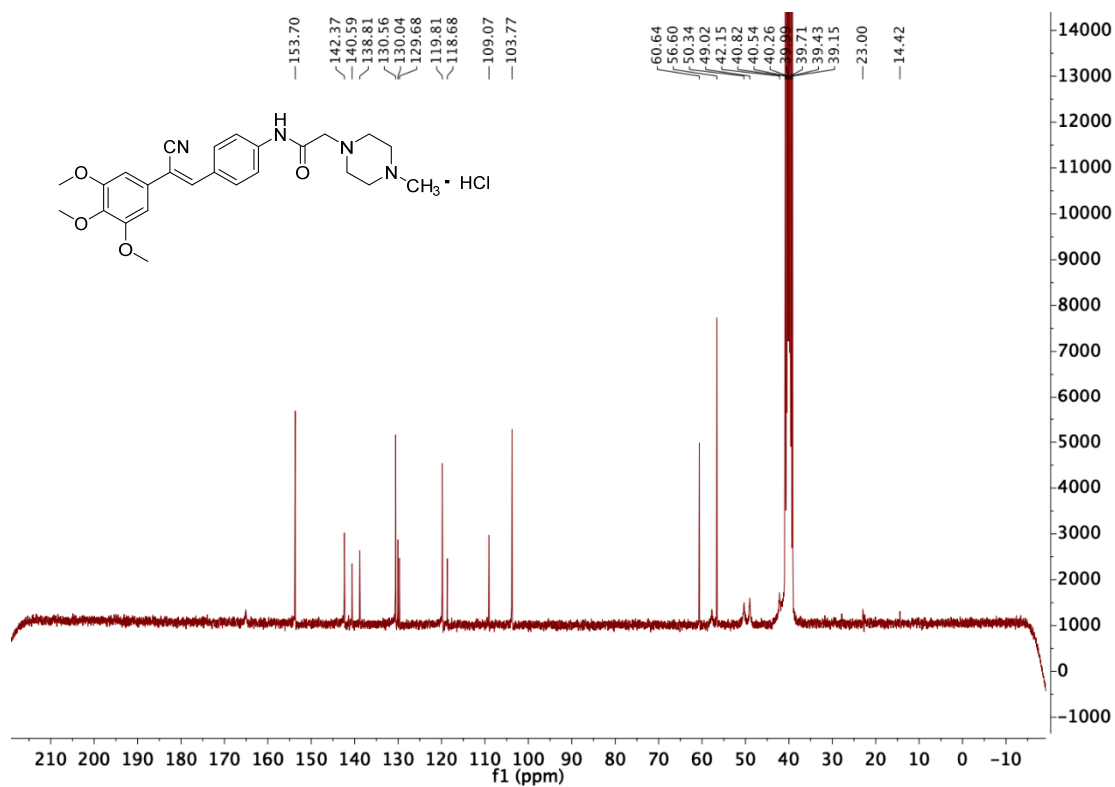
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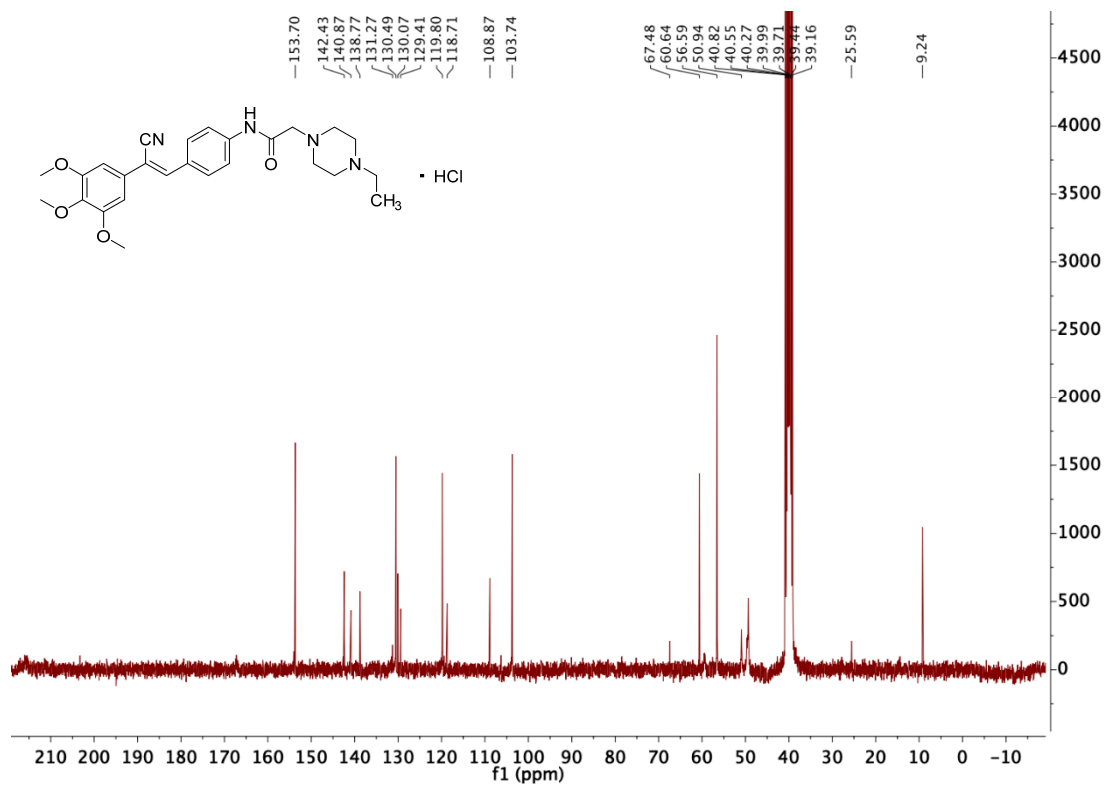
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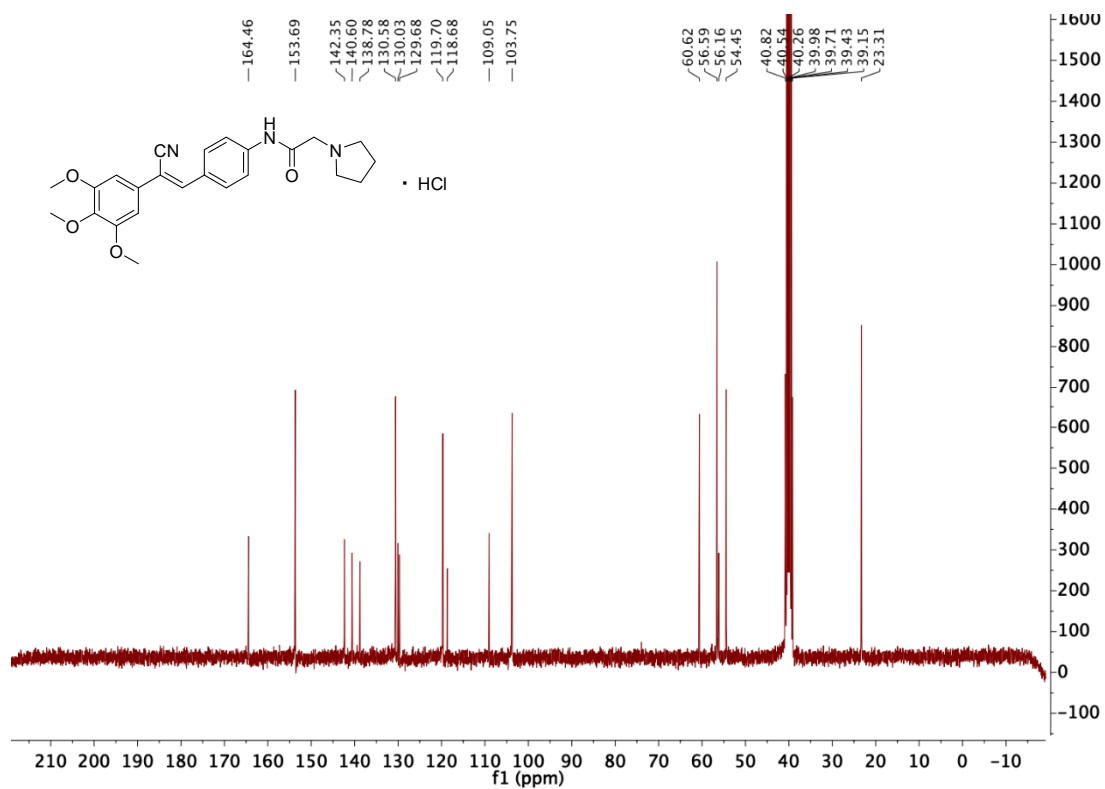
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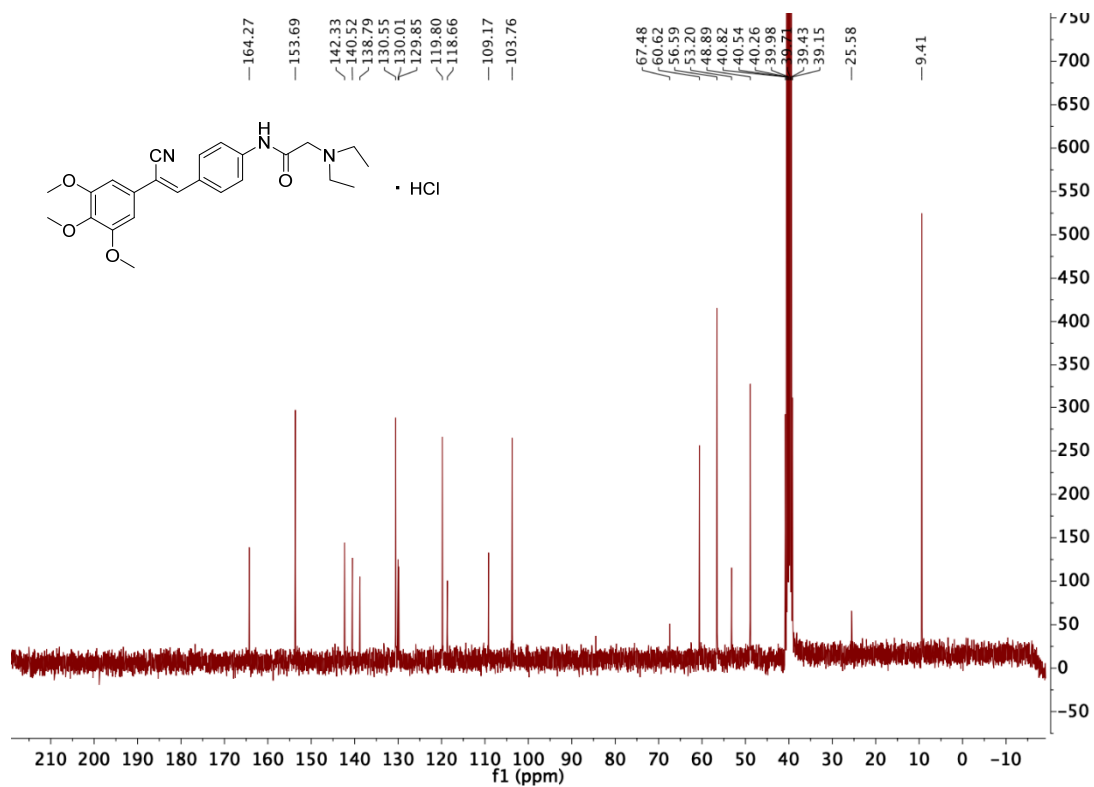
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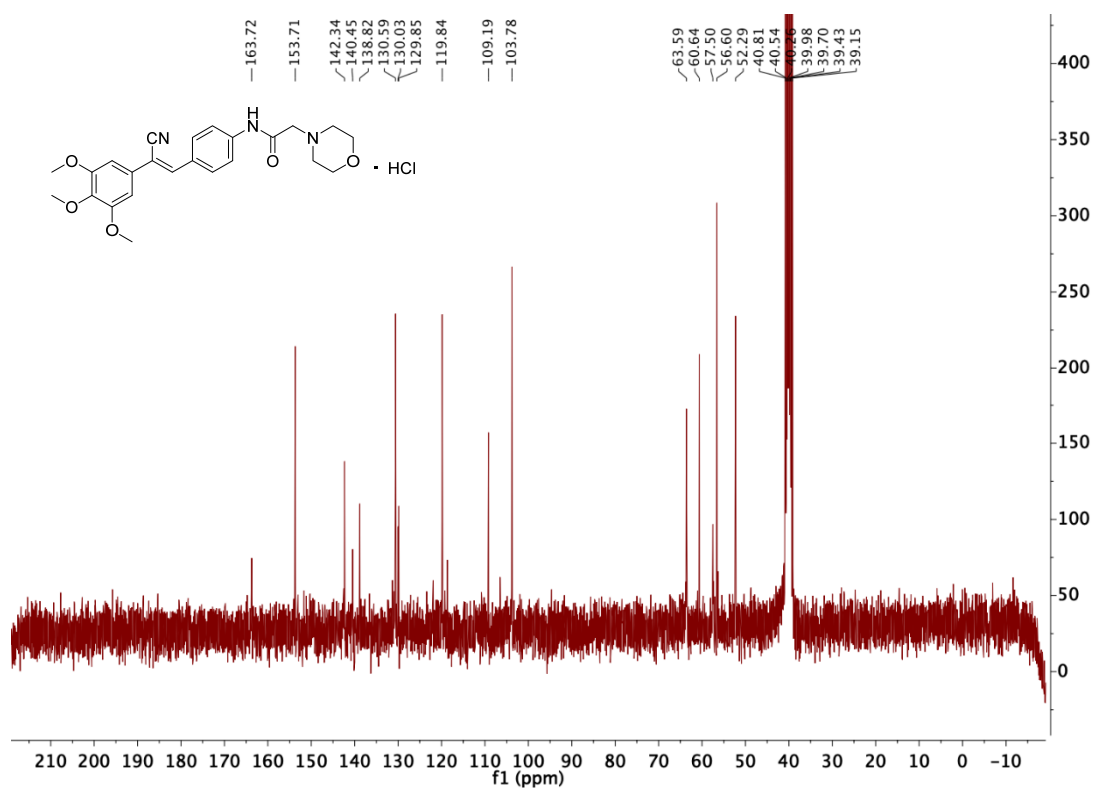
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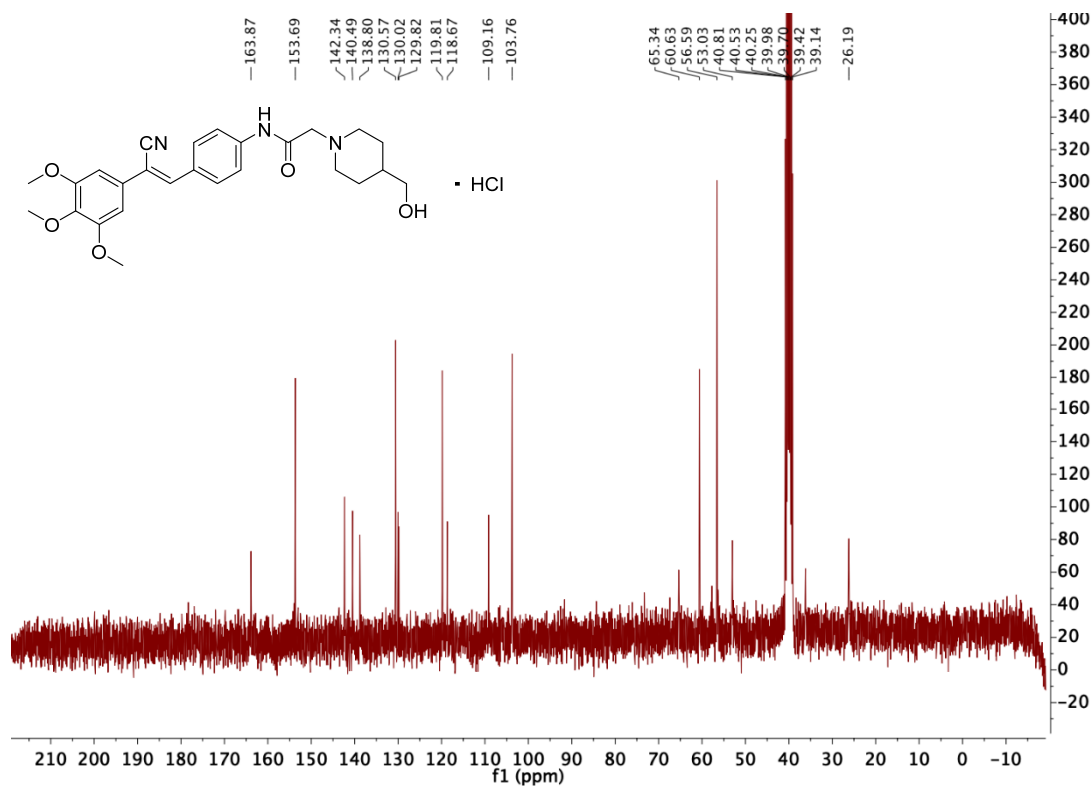
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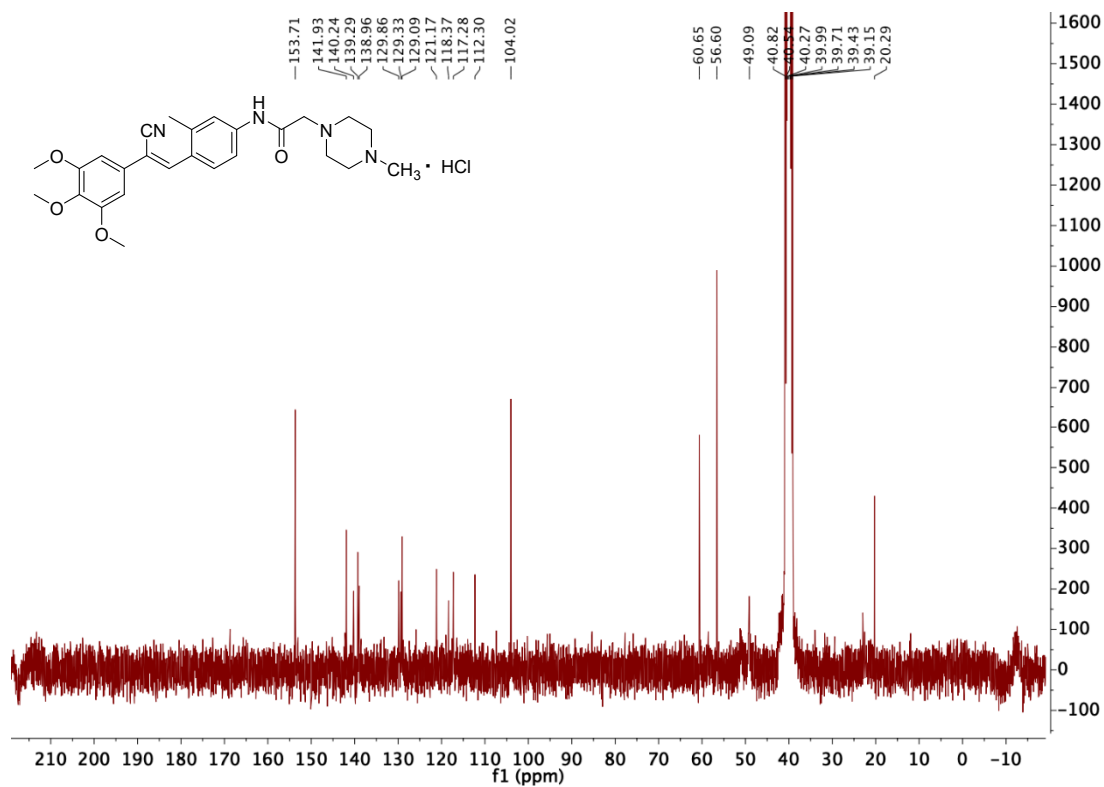
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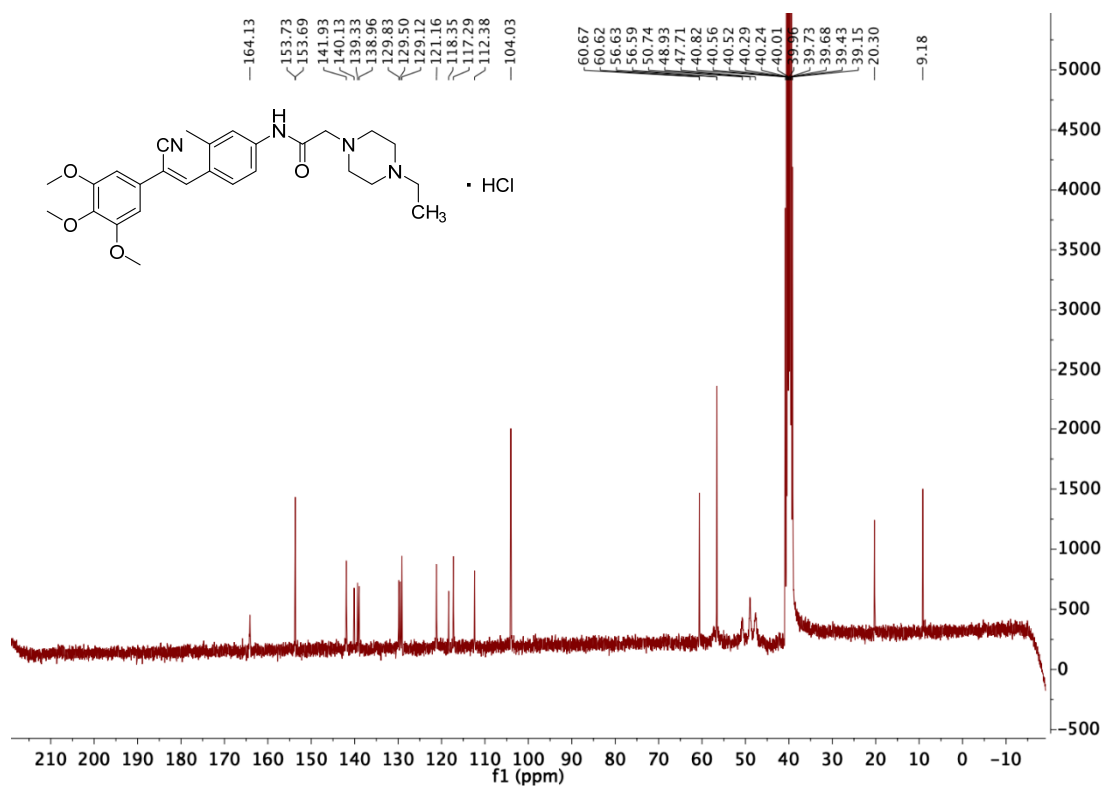
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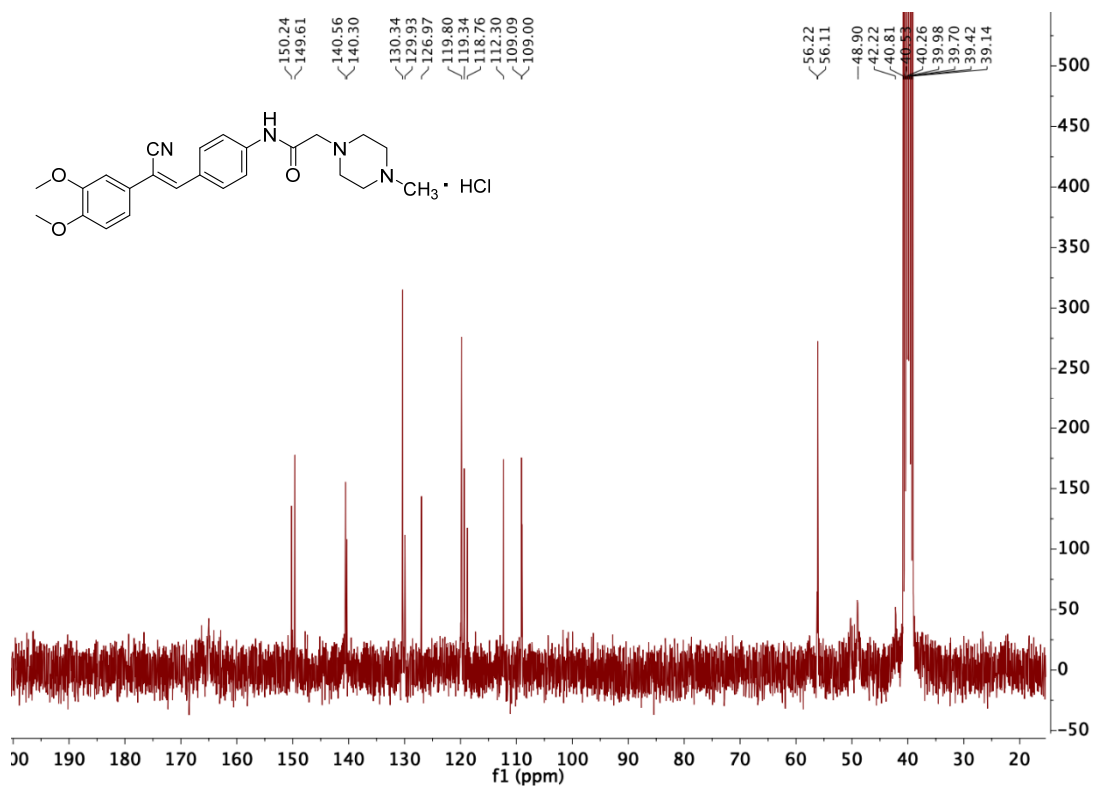
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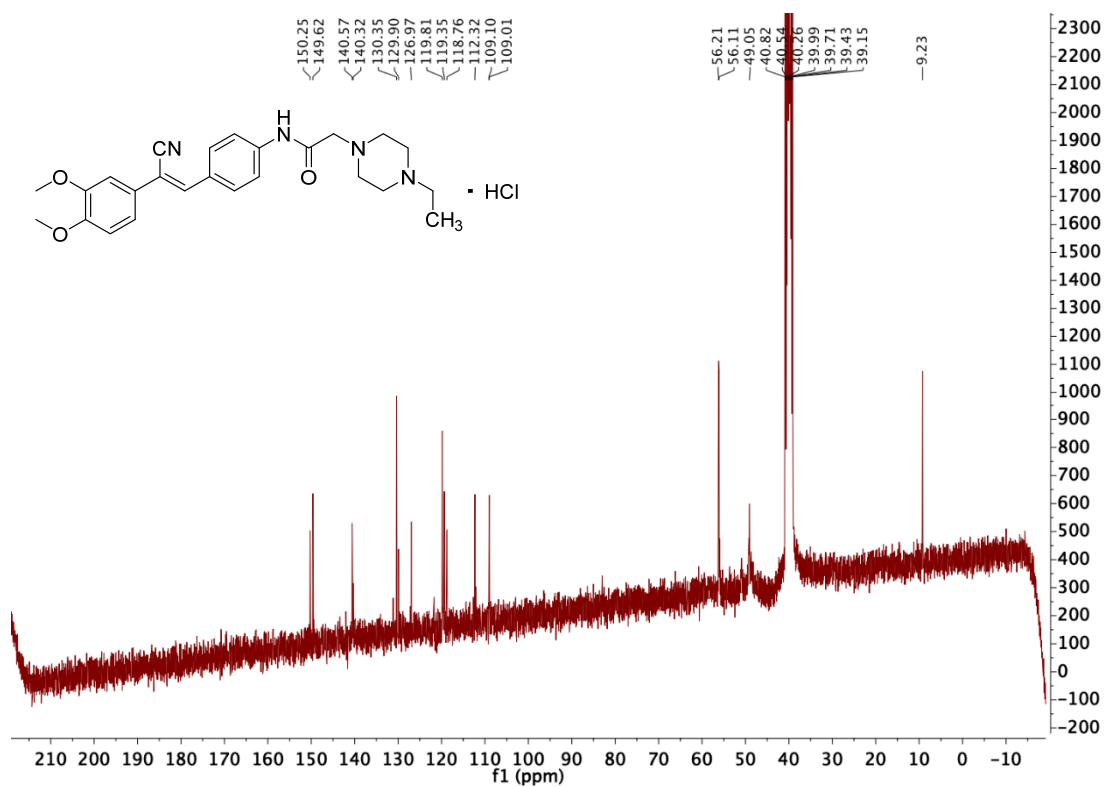
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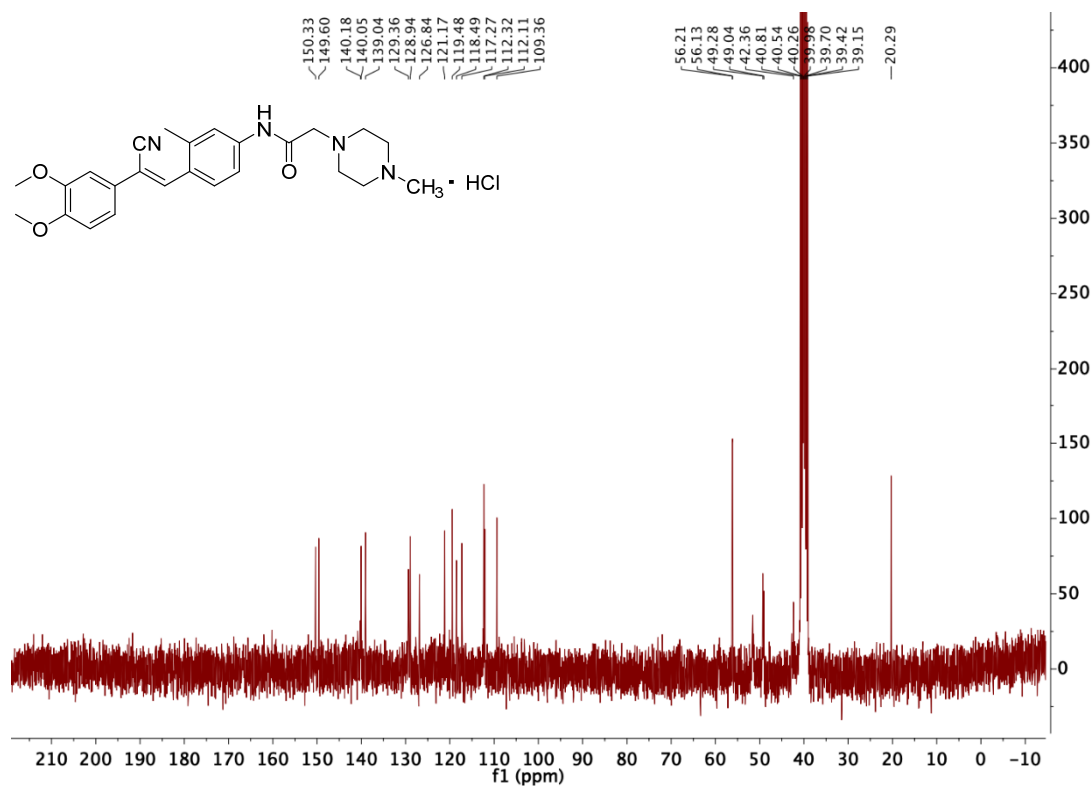
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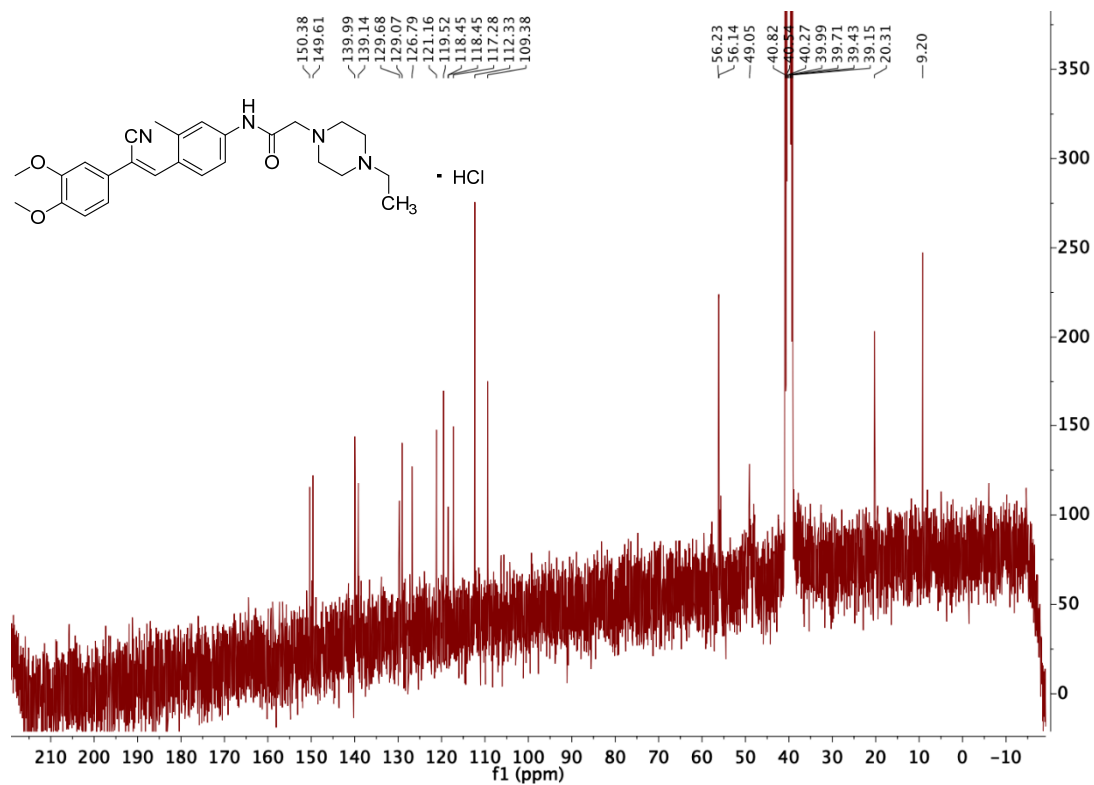
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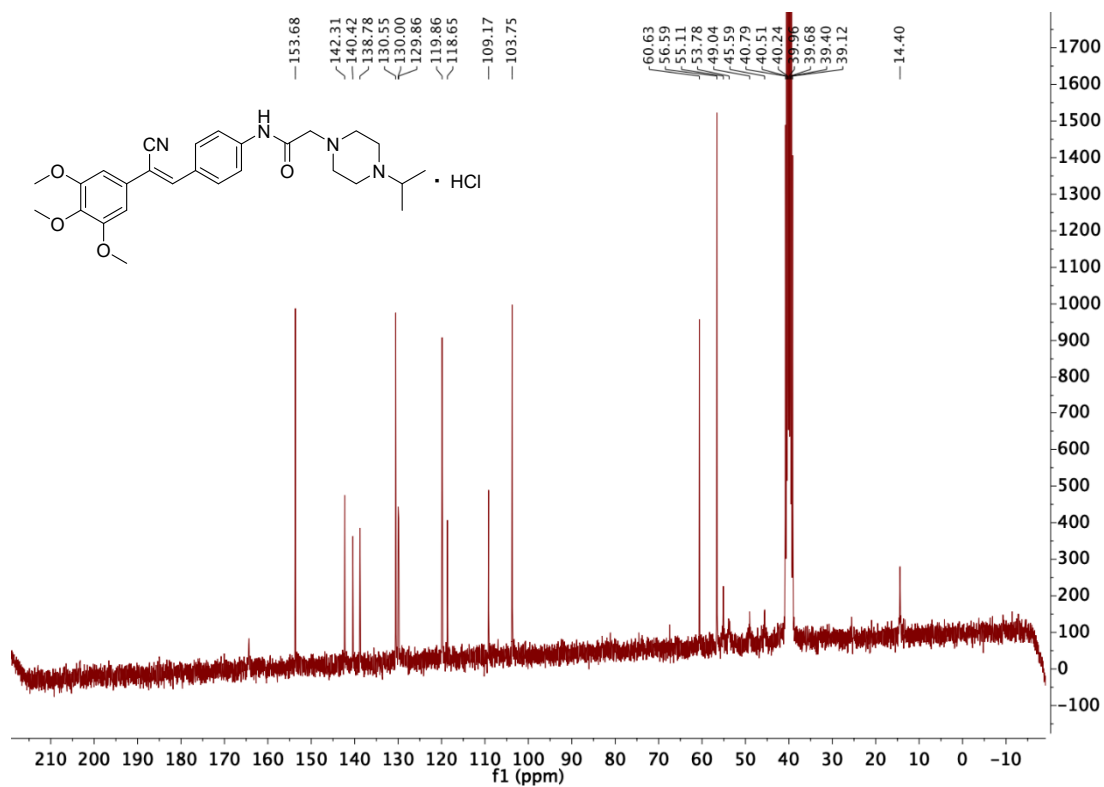
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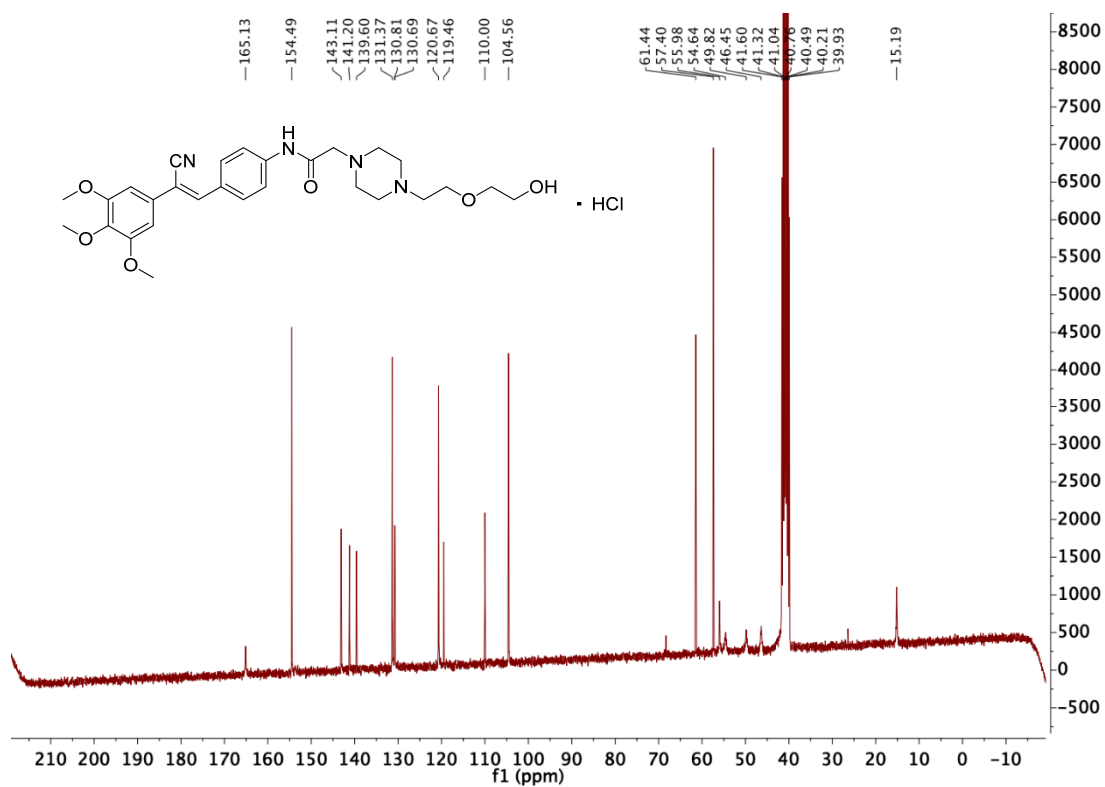
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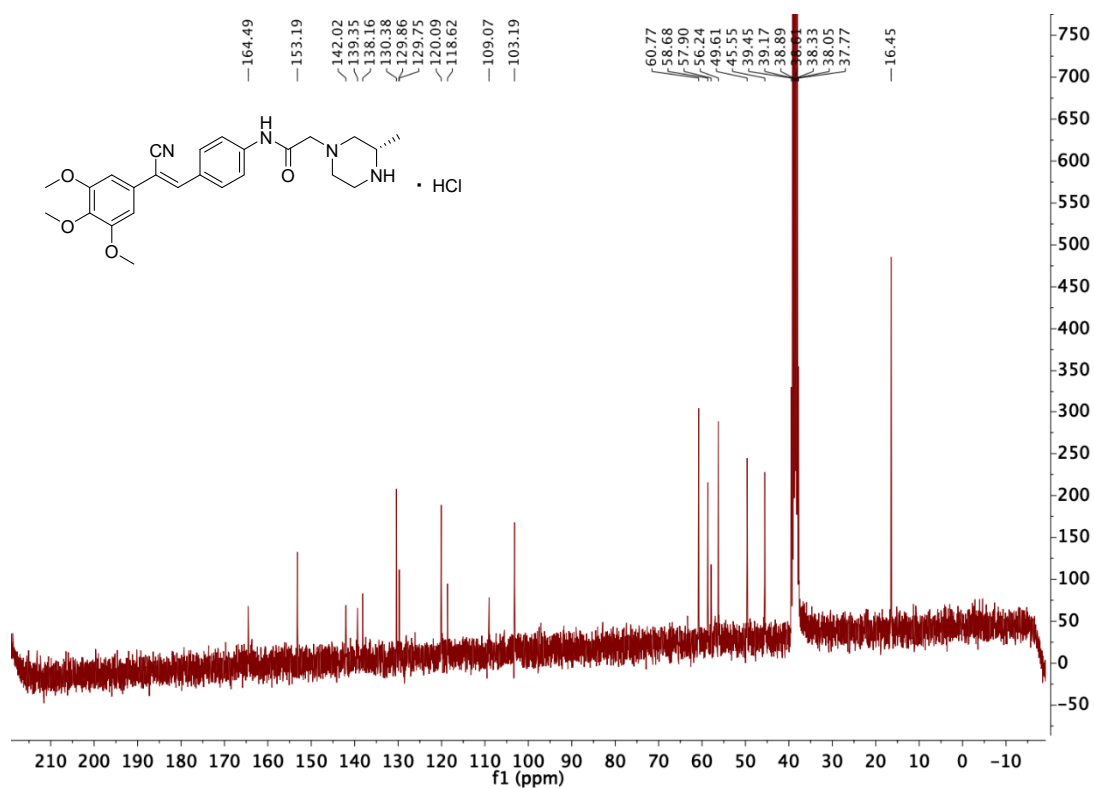
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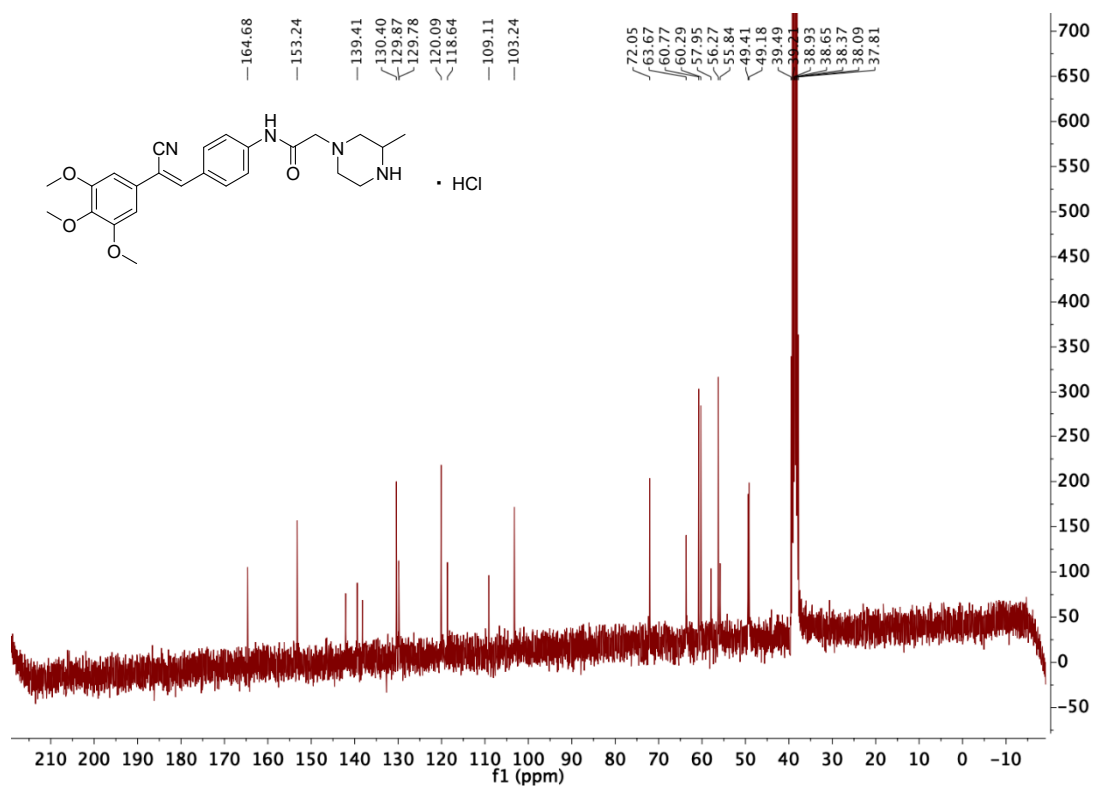
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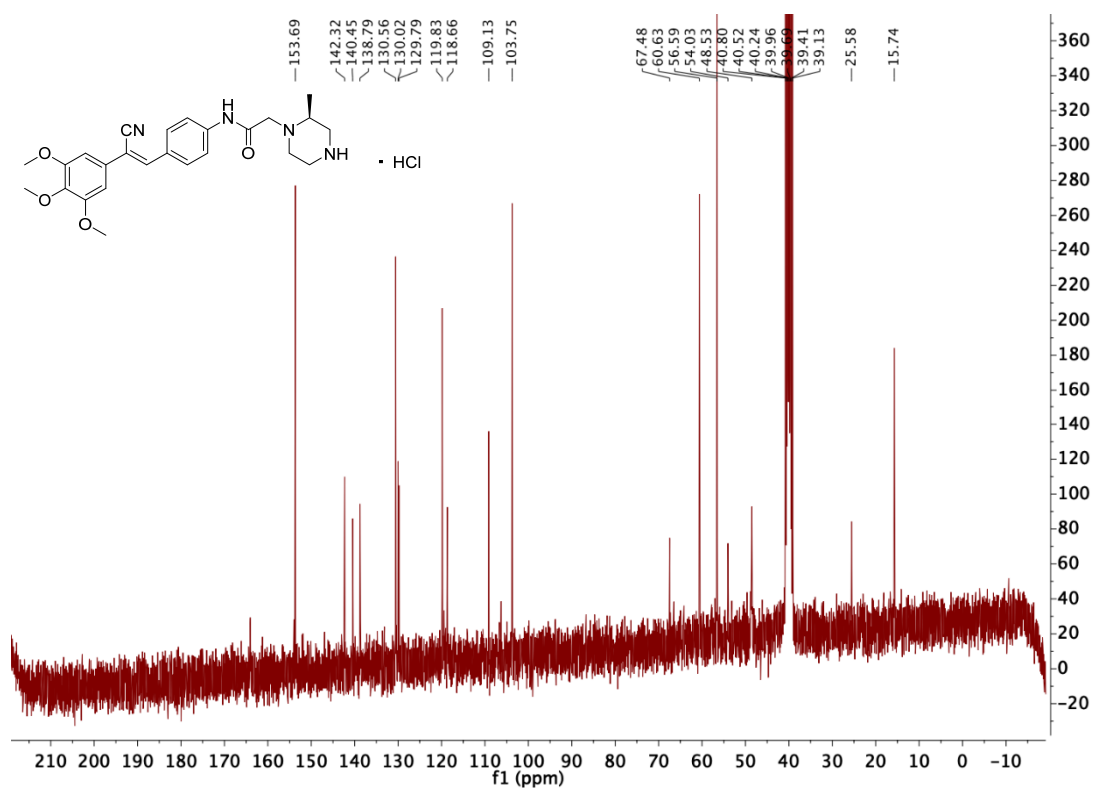
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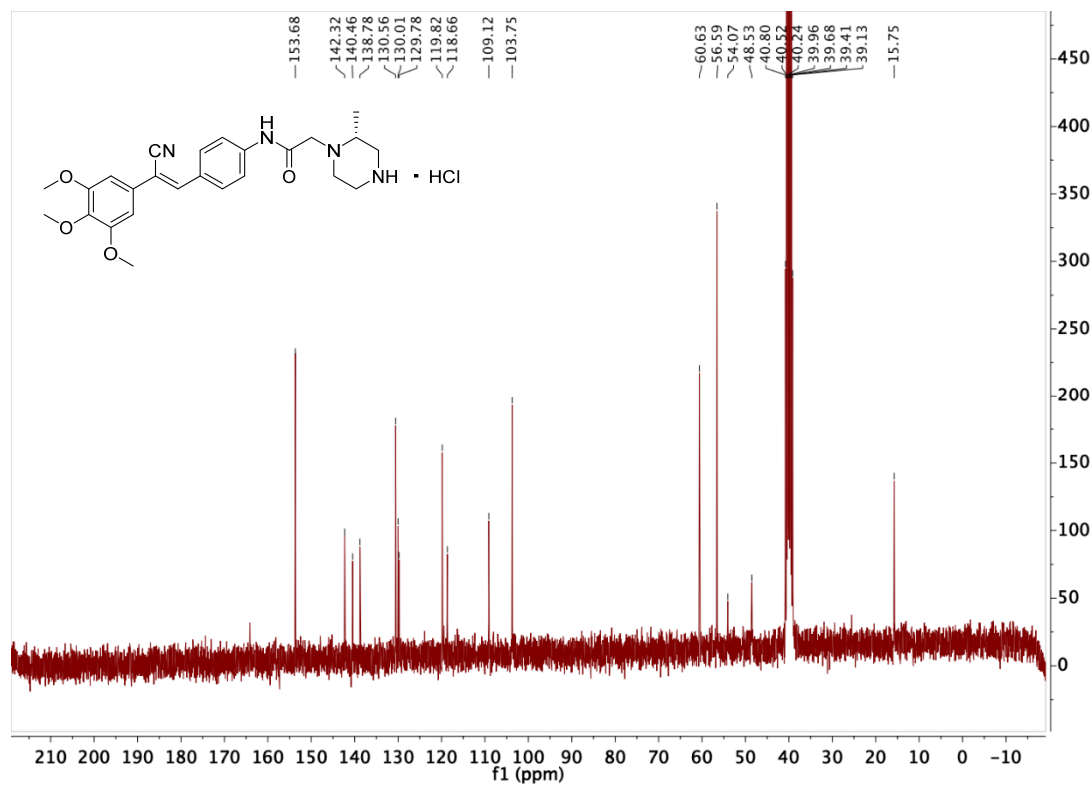
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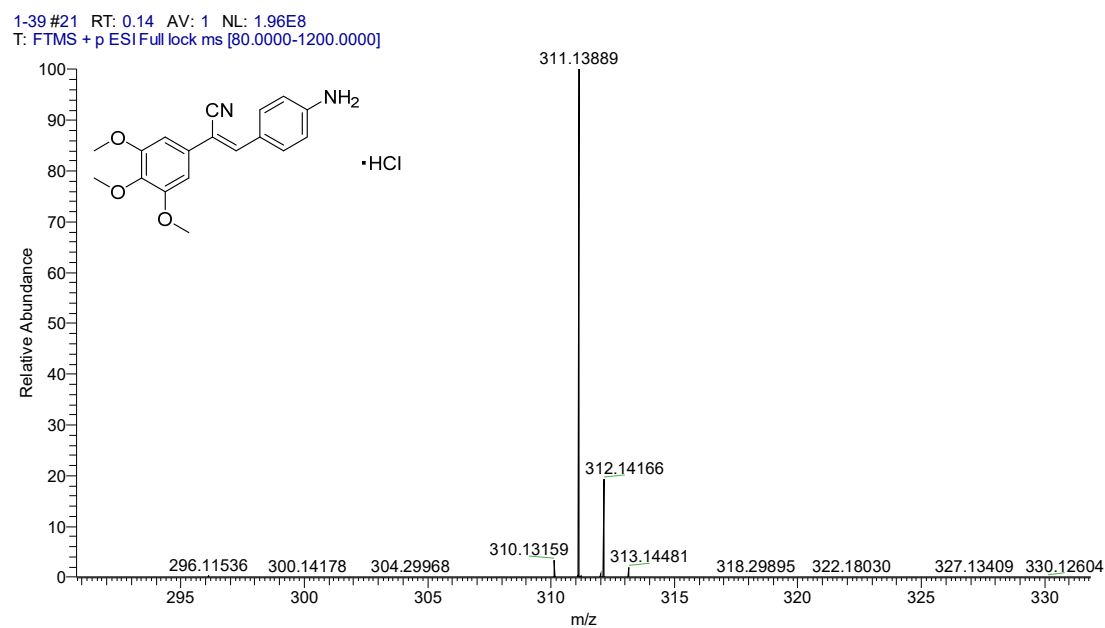


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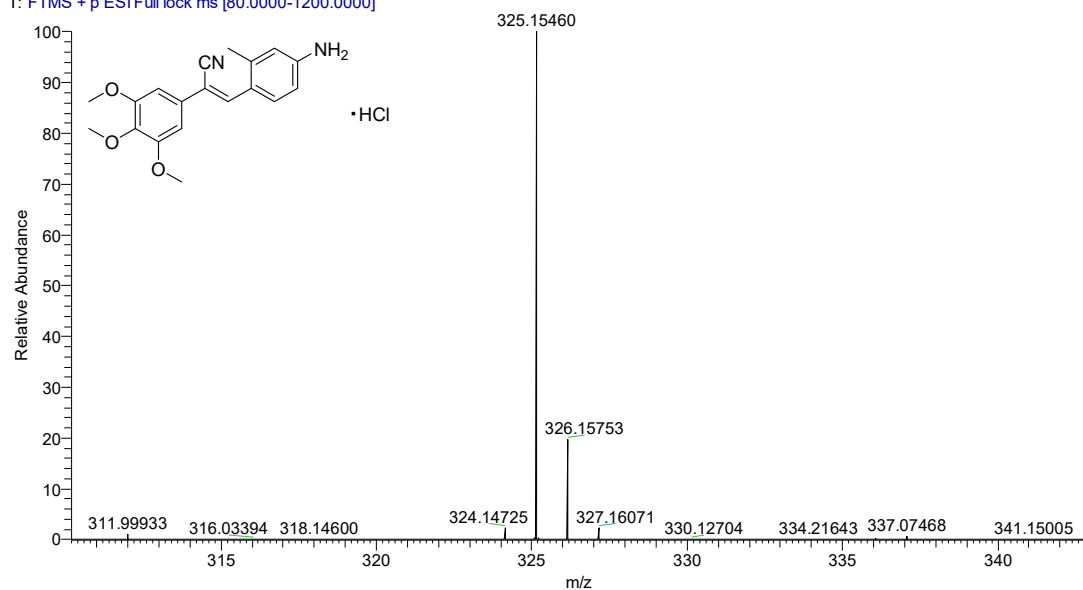
4.3. HRMS

9a. HRMS (ESI) m/z calcd for $C_{18}H_{19}N_2O_3^+$ ($M+H$) $^+$ 311.13902, found 311.13889.



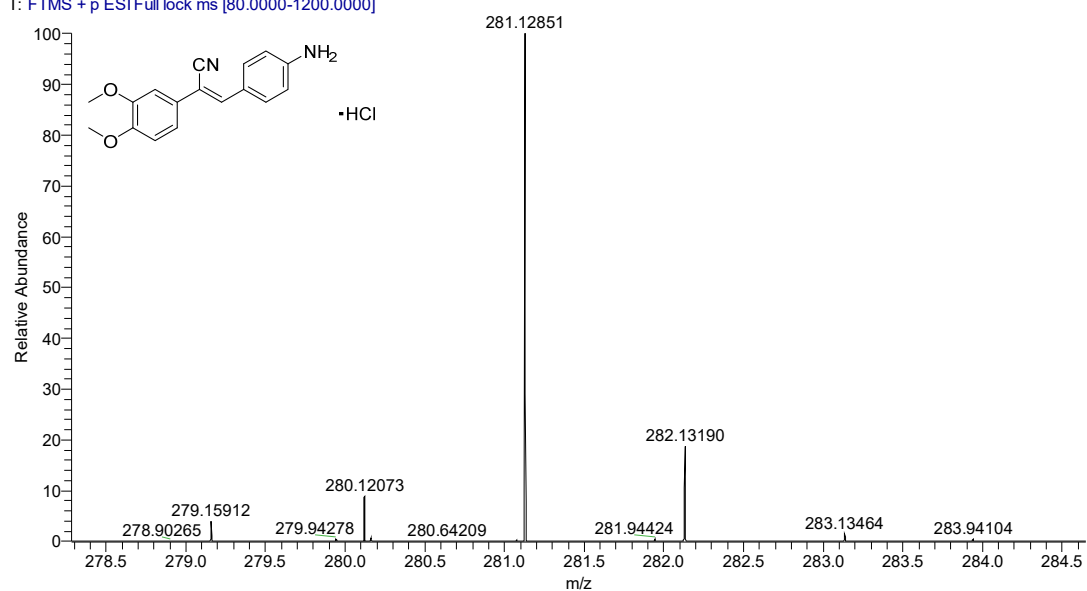
9b. HRMS (ESI) m/z calcd for $C_{19}H_{21}N_2O_3^+$ ($M+H$) $^+$ 325.15467, found 325.15460.

1-40 #17 RT: 0.11 AV: 1 NL: 1.66E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



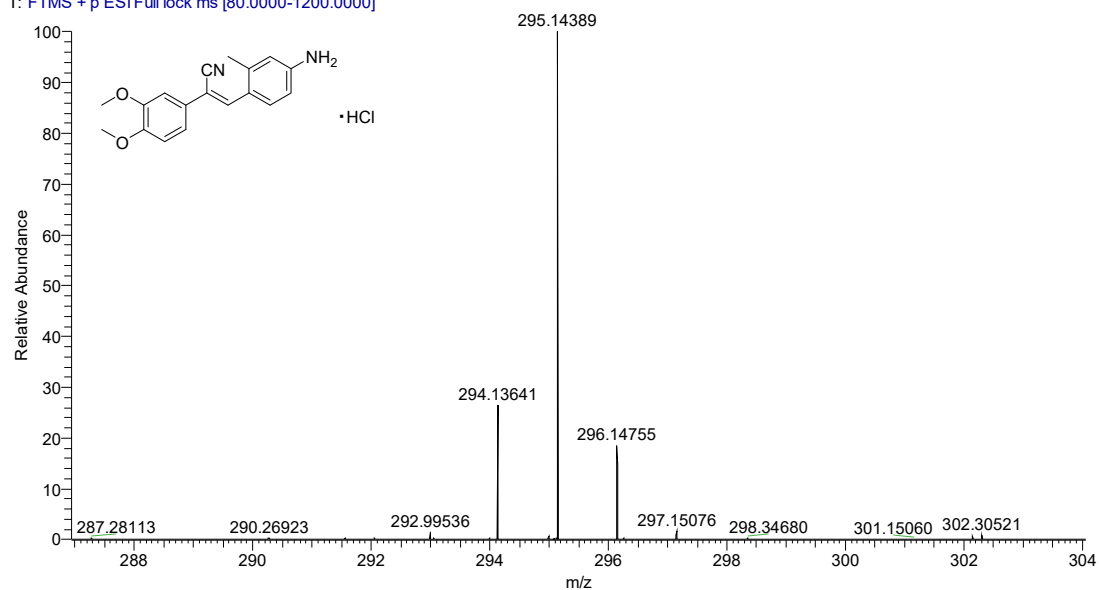
9c. HRMS (ESI) m/z calcd for $C_{17}H_{17}N_2O_2^+$ ($M+H$) $^+$ 281.12845, found 281.12851.

1-41 #36 RT: 0.22 AV: 1 NL: 7.60E6
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



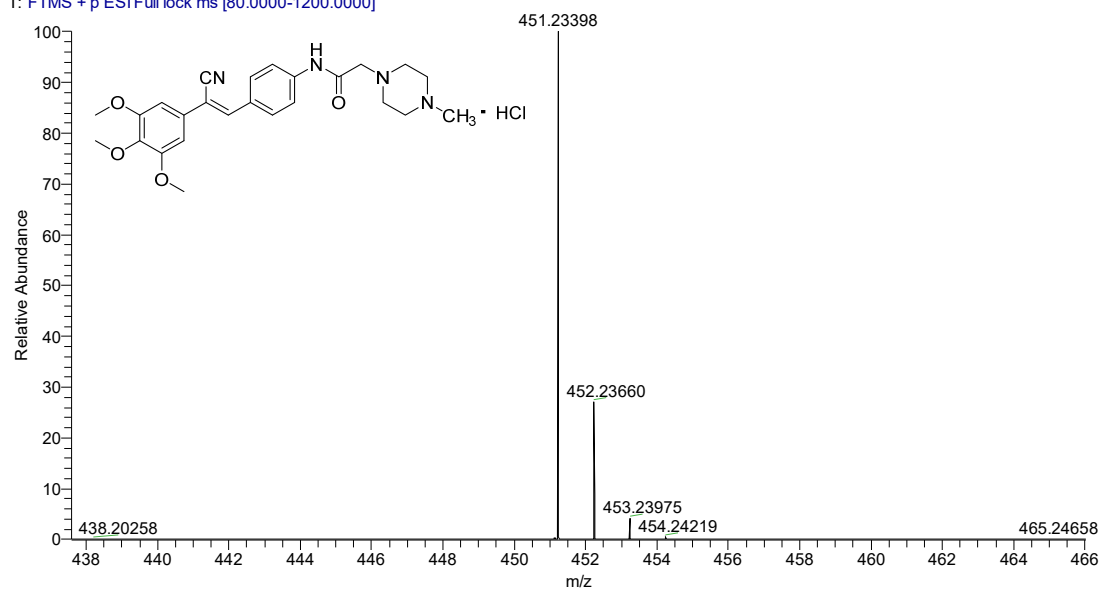
9d. HRMS (ESI) m/z calcd for $C_{18}H_{19}N_2O_2^+$ (M+H) $^+$ 295.14410, found 295.14389.

1-42 #16 RT: 0.11 AV: 1 NL: 2.80E7
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



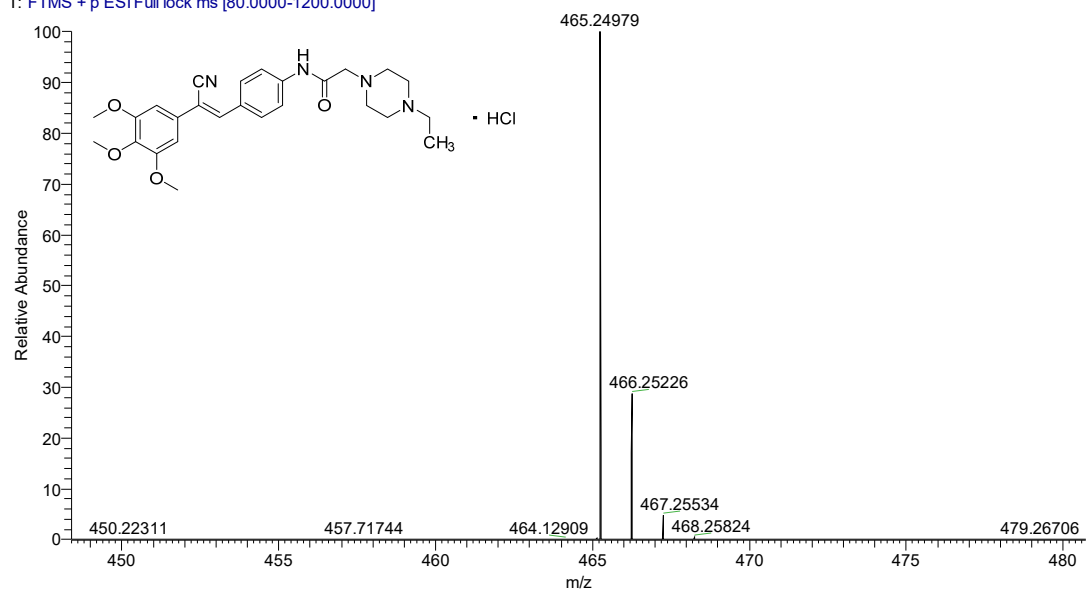
12a₁. HRMS (ESI) m/z calcd for $C_{25}H_{31}N_4O_4^+$ (M+H) $^+$ 451.23398, found 451.23396.

1-44 #19 RT: 0.13 AV: 1 NL: 2.51E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



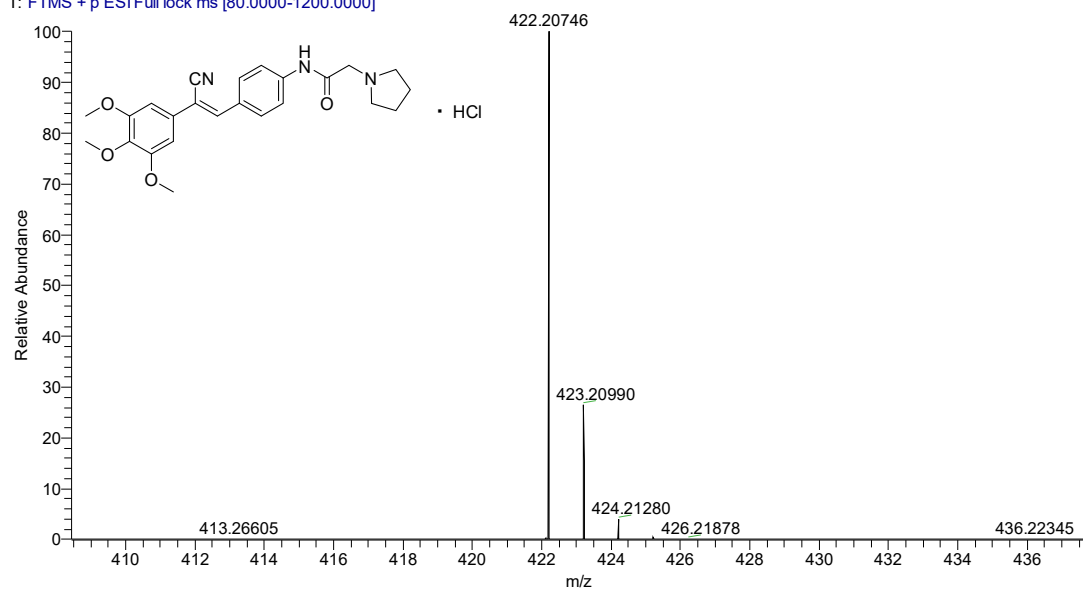
12a₂. HRMS (ESI) m/z calcd for C₂₆H₃₃N₄O₄⁺ (M+H)⁺ 465.24963, found 465.24979.

1-48 #13 RT: 0.09 AV: 1 NL: 4.12E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



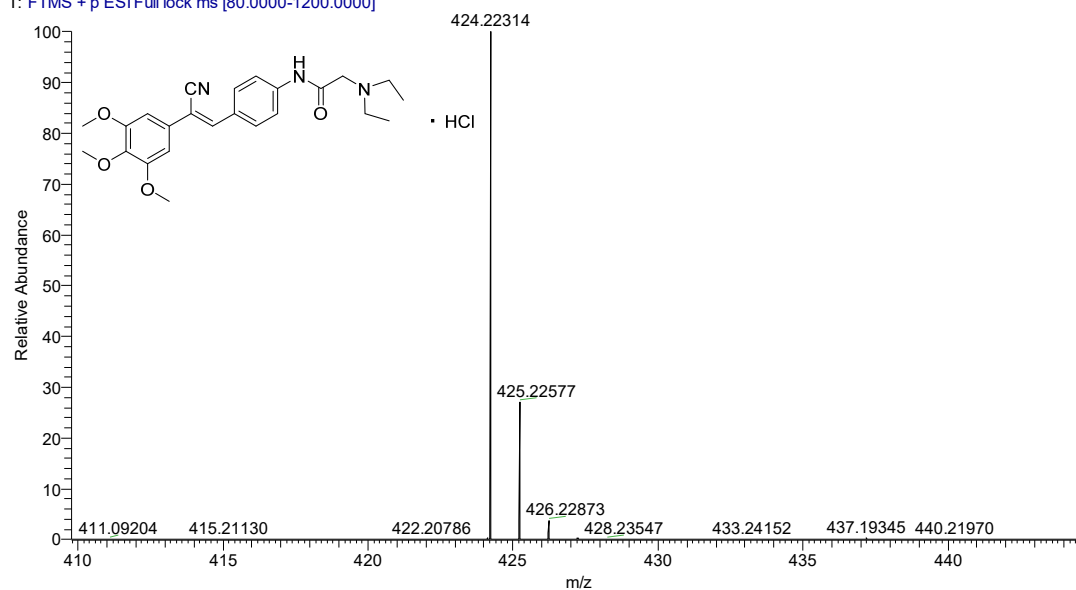
12a₃. HRMS (ESI) m/z calcd for C₂₄H₂₈N₃O₄⁺ (M+H)⁺ 422.20743, found 422.20746.

1-45 #12 RT: 0.09 AV: 1 NL: 6.04E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



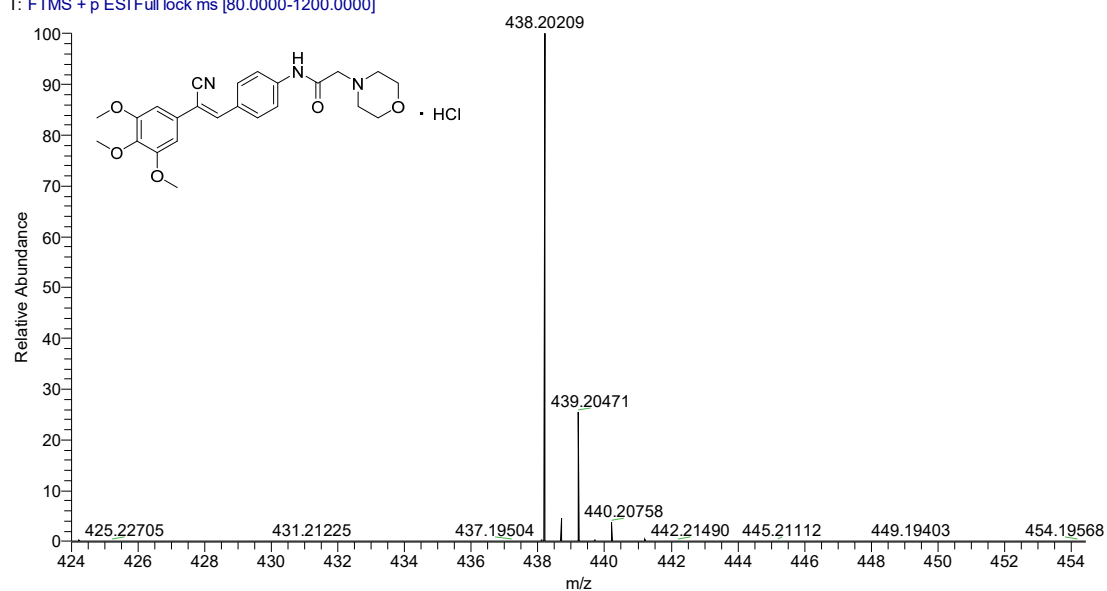
12a4. HRMS (ESI) m/z calcd for $C_{24}H_{30}N_3O_4^+$ (M+H) $^+$ 424.22308, found 424.22314.

1-47 #16 RT: 0.11 AV: 1 NL: 2.69E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



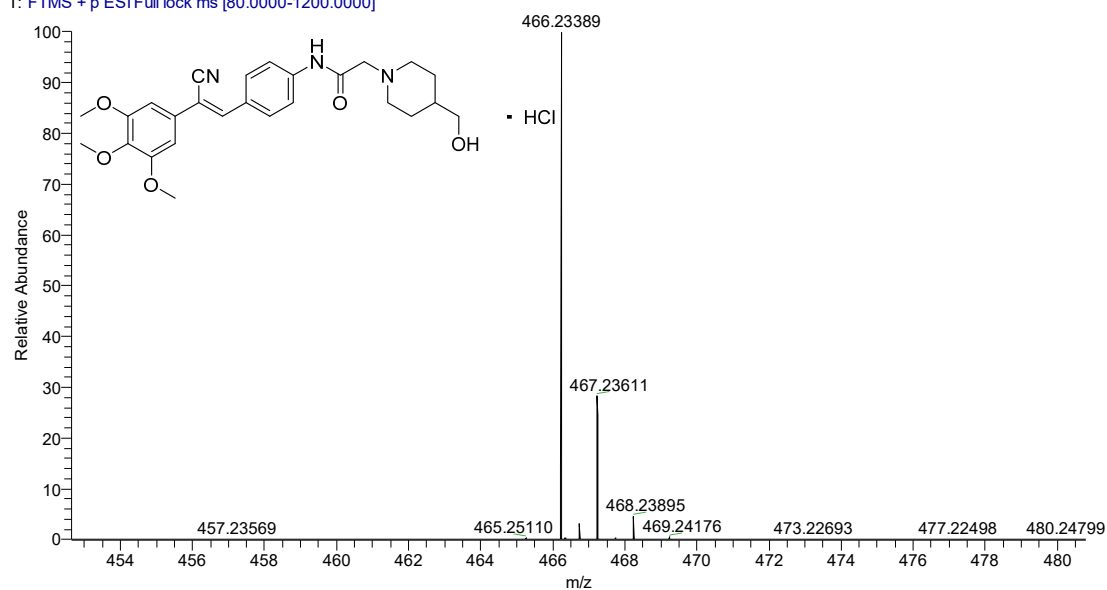
12a5. HRMS (ESI) m/z calcd for $C_{24}H_{28}N_3O_5^+$ (M+H) $^+$ 438.20235, found 438.20209.

1-43 #18 RT: 0.12 AV: 1 NL: 3.54E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



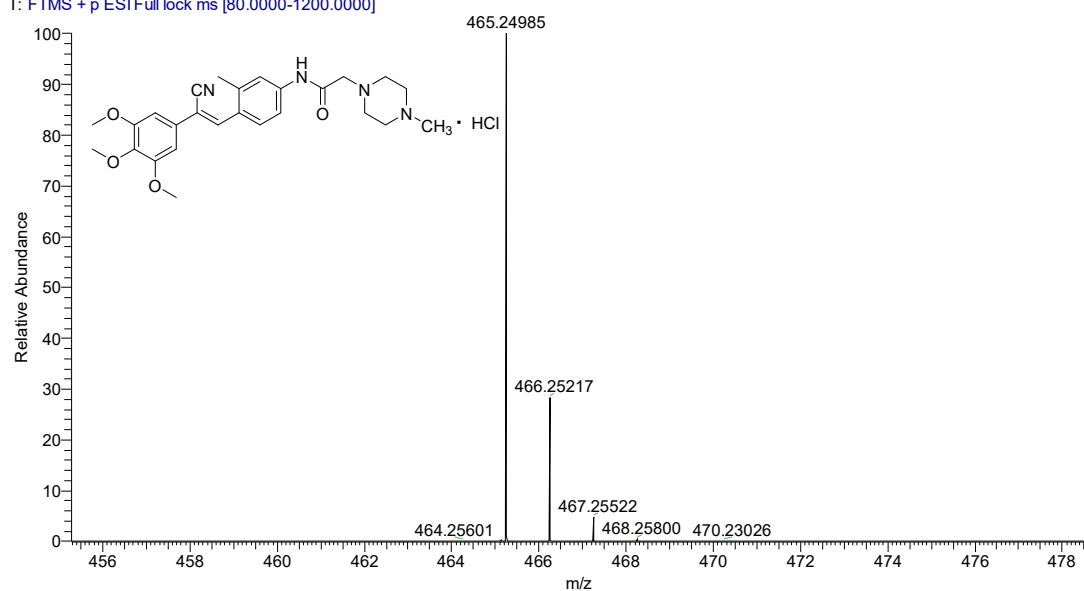
12a₆. HRMS (ESI) m/z calcd for C₂₆H₃₂N₃O₅⁺ (M+H)⁺ 466.23365, found 466.23389.

1-49 #17 RT: 0.11 AV: 1 NL: 1.76E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



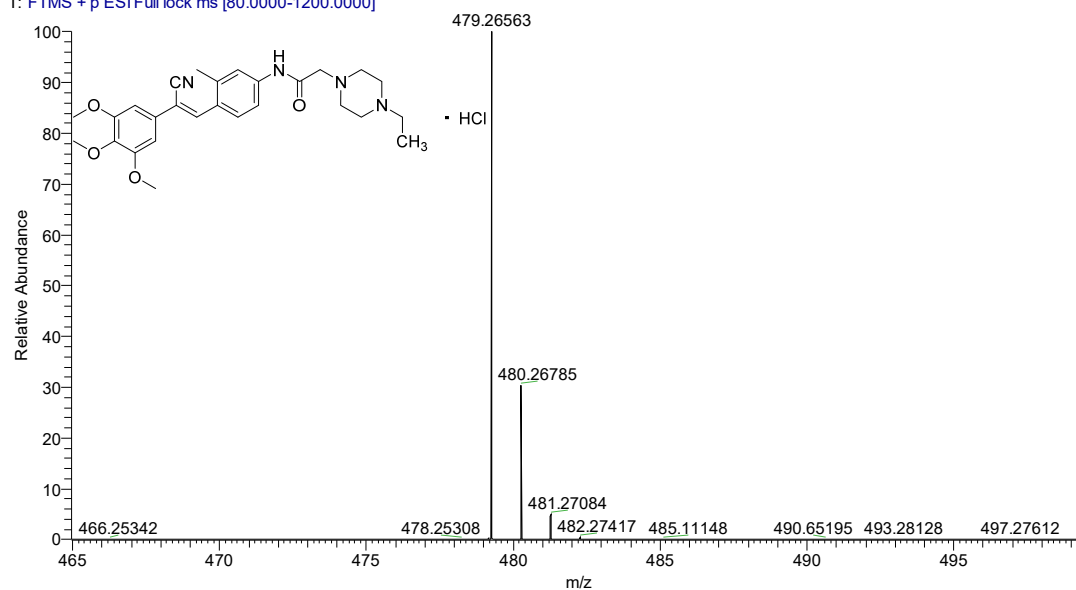
12b₁. HRMS (ESI) m/z calcd for C₂₆H₃₃N₄O₄⁺ (M+H)⁺ 465.24963, found 465.24985.

1-50 #18 RT: 0.12 AV: 1 NL: 1.75E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



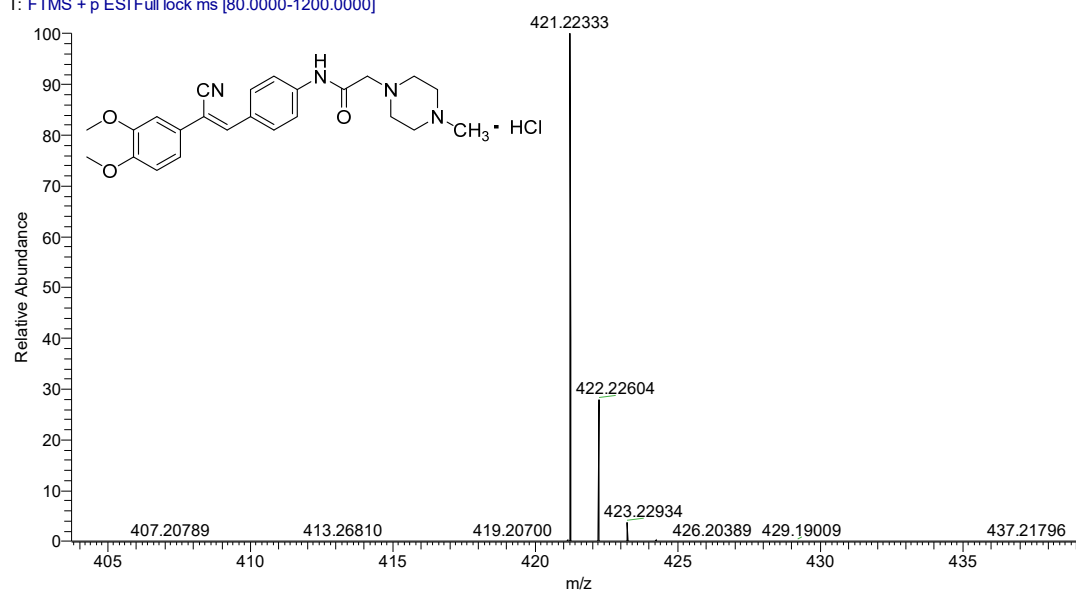
12b₂. HRMS (ESI) m/z calcd for C₂₇H₃₅N₄O₄⁺ (M+H)⁺ 479.26528, found 479.26563.

1-51 #17 RT: 0.11 AV: 1 NL: 3.81E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



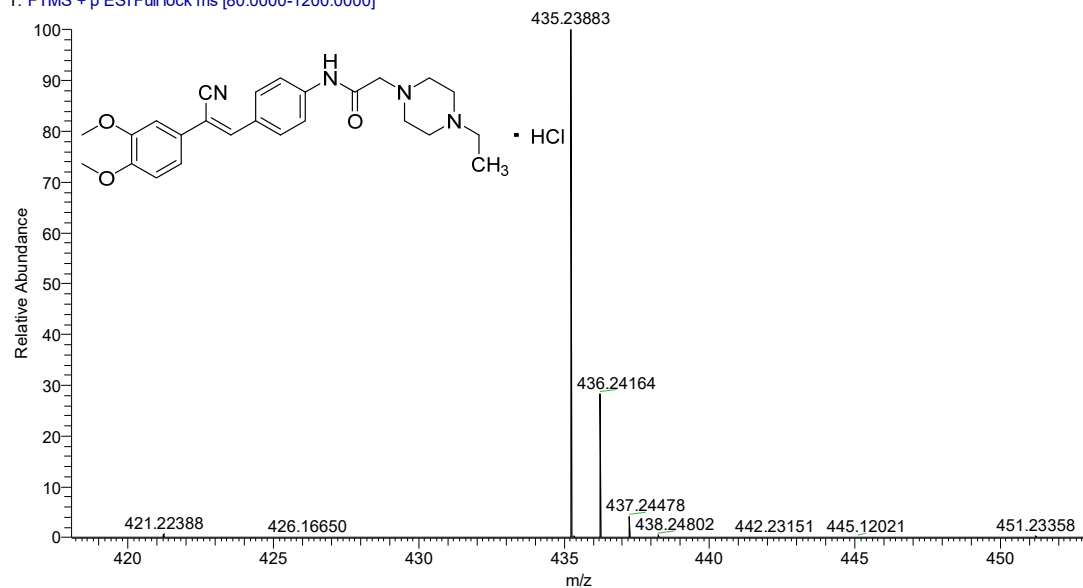
12c₁. HRMS (ESI) m/z calcd for C₂₄H₂₉N₄O₃⁺ (M+H)⁺ 421.22342, found 421.22333.

1-52 #17 RT: 0.11 AV: 1 NL: 1.95E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



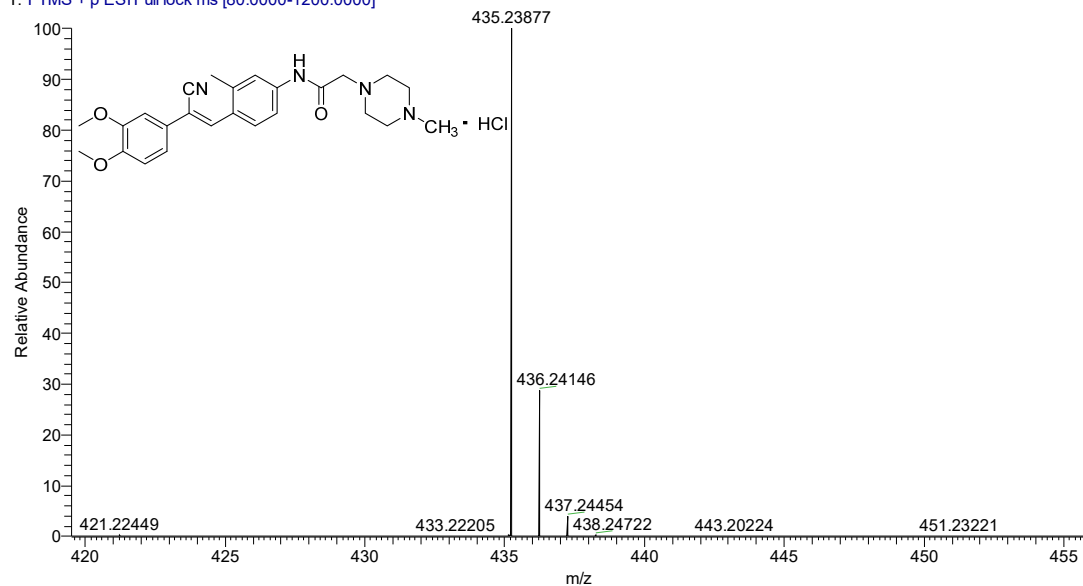
12c₂. HRMS (ESI) m/z calcd for C₂₅H₃₁N₄O₃⁺ (M+H)⁺ 435.23907, found 435.23883.

1-53 #16 RT: 0.11 AV: 1 NL: 1.45E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



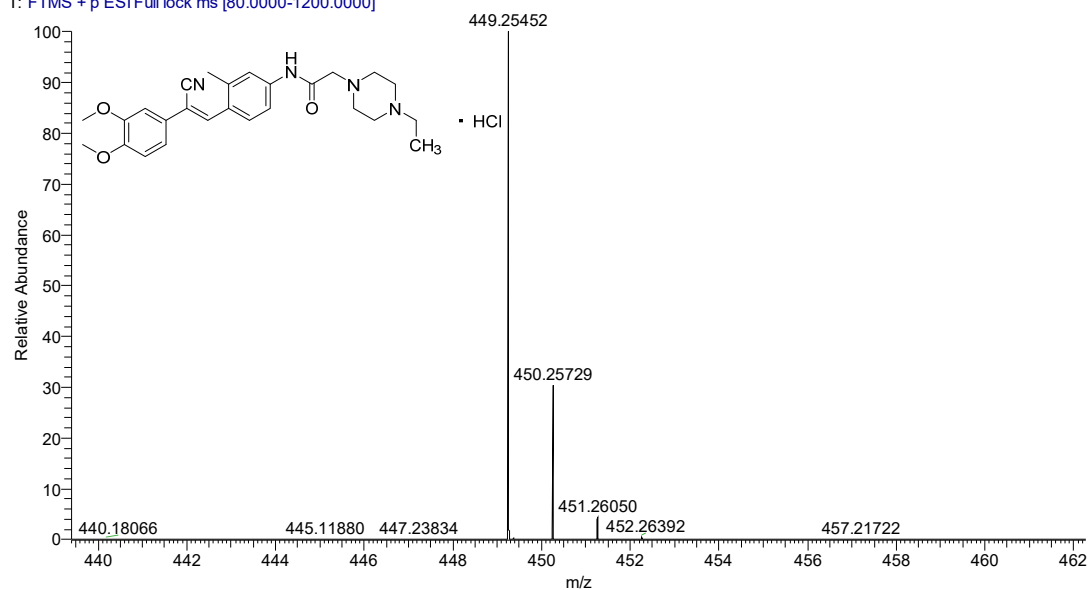
12d₁. HRMS (ESI) m/z calcd for C₂₅H₃₁N₄O₃⁺ (M+H)⁺ 435.23907, found 435.23877.

1-54 #24 RT: 0.15 AV: 1 NL: 2.70E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



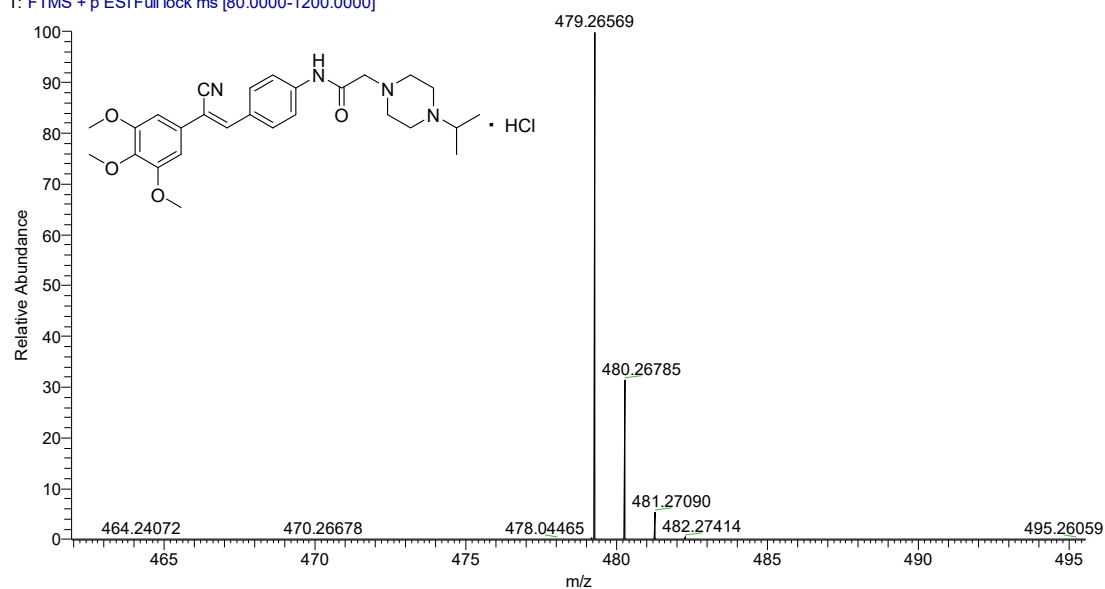
12d₂. HRMS (ESI) m/z calcd for C₂₆H₃₃N₄O₃⁺ (M+H)⁺ 449.25472, found 449.25452.

1-55 #16 RT: 0.11 AV: 1 NL: 3.11E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



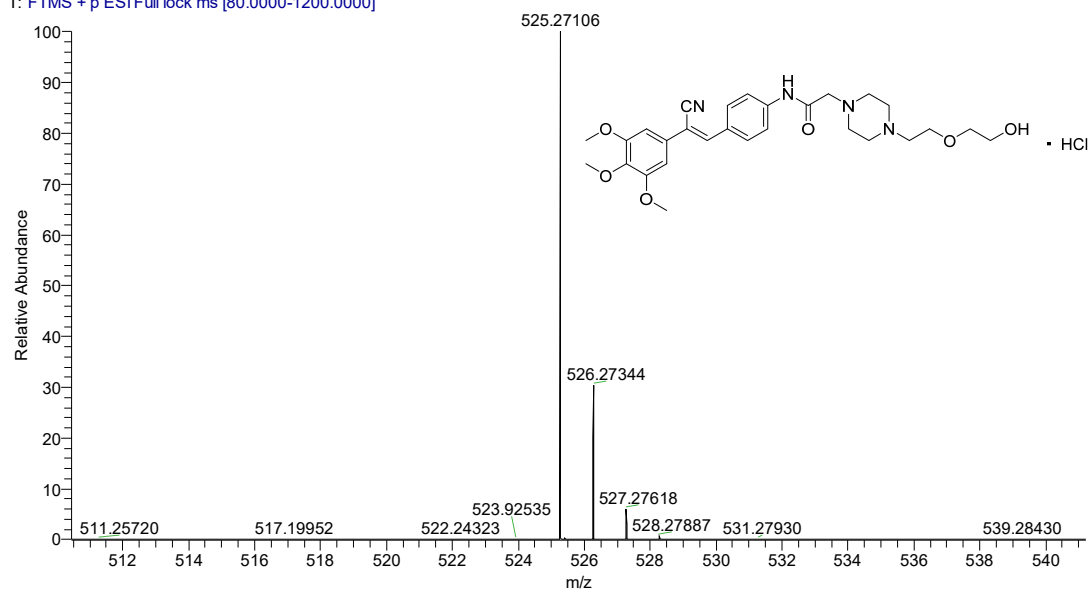
15. HRMS (ESI) m/z calcd for C₂₇H₃₅N₄O₄⁺ (M+H)⁺ 479.26528, found 479.26569.

1-60 #17 RT: 0.11 AV: 1 NL: 4.31E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



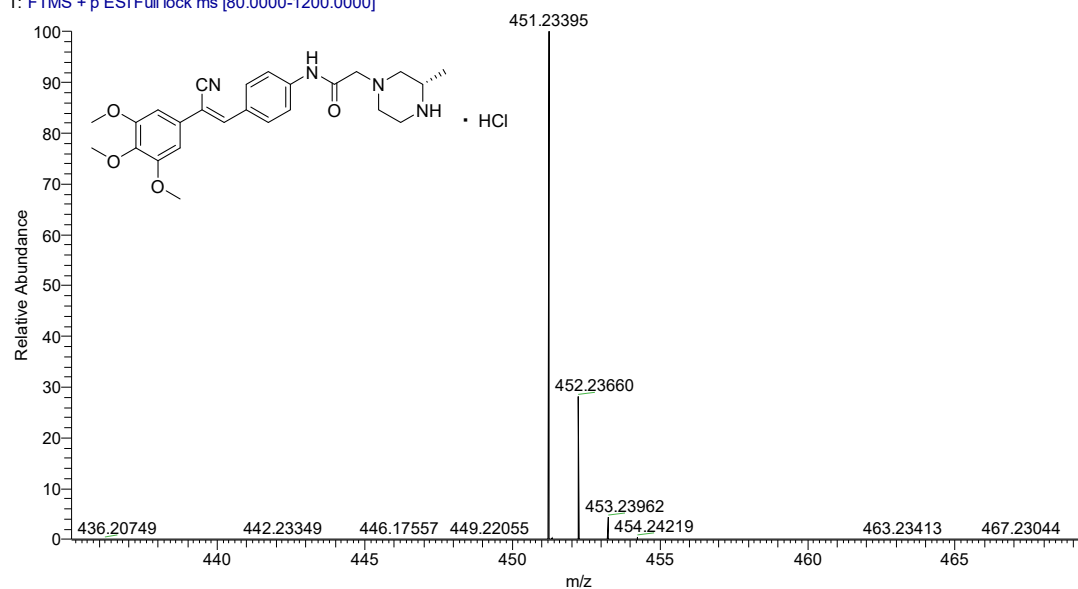
16. HRMS (ESI) m/z calcd for $C_{28}H_{37}N_4O_6^+$ (M+H) $^+$ 525.27076, found 525.27106.

1-61 #19 RT: 0.12 AV: 1 NL: 3.46E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



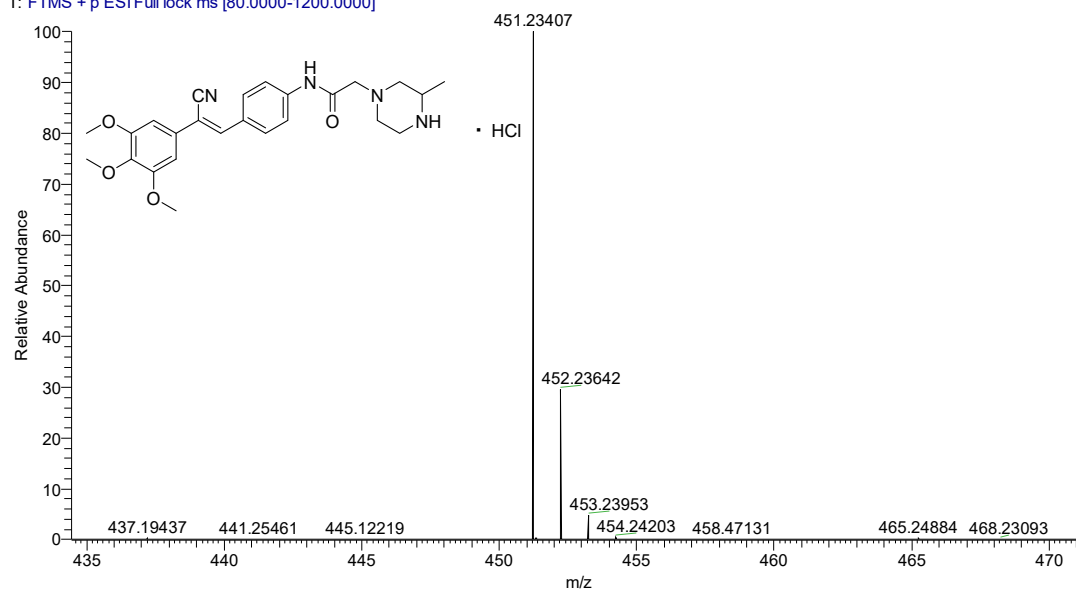
17. HRMS (ESI) m/z calcd for $C_{25}H_{31}N_4O_4^+$ (M+H) $^+$ 451.23398, found 451.23395.

1-58 #17 RT: 0.11 AV: 1 NL: 3.47E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



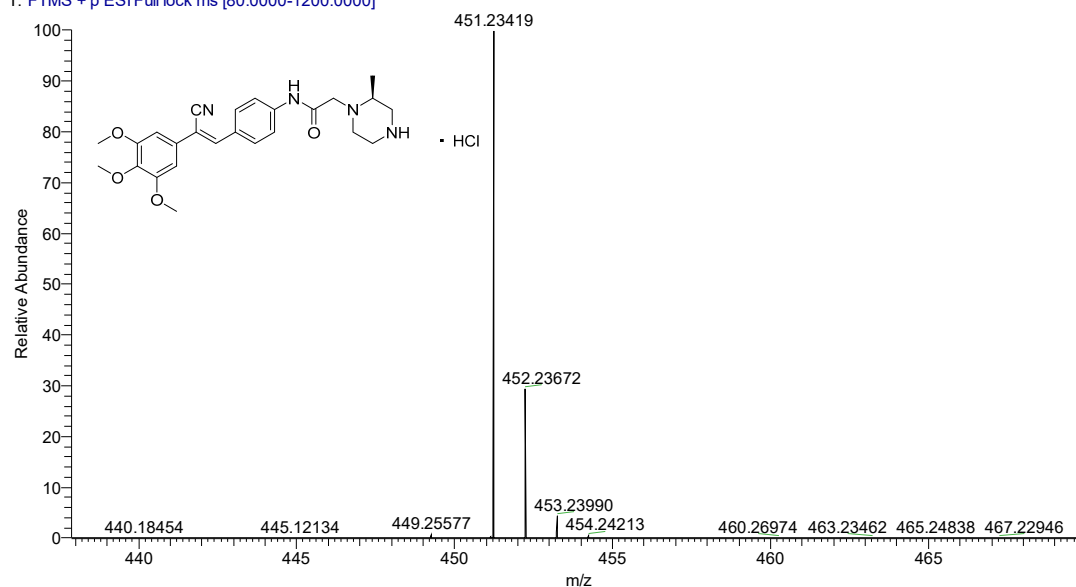
18. HRMS (ESI) m/z calcd for $C_{25}H_{31}N_4O_4^+$ ($M+H$) $^+$ 451.23398, found 451.23407.

1-59 #17 RT: 0.11 AV: 1 NL: 1.48E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



19. HRMS (ESI) m/z calcd for $C_{25}H_{31}N_4O_4^+$ ($M+H$) $^+$ 451.23398, found 451.23419.

1-56 #13 RT: 0.09 AV: 1 NL: 1.16E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]



20. HRMS (ESI) m/z calcd for C₂₅H₃₁N₄O₄⁺ (M+H)⁺ 451.23398, found 451.23404.

1-57 #14 RT: 0.10 AV: 1 NL: 1.30E8
T: FTMS + p ESI Full lock ms [80.0000-1200.0000]

