

Supplementary Materials

p97 Inhibitors Possessing Antiviral Activity Against SARS-CoV-2 and Low Cytotoxicity

Rui Ding¹, Tiffany C. Edwards¹, Prithwish Goswami¹, Daniel J. Wilson¹, Christine D. Dreis¹, Yihong Ye², Robert J. Geraghty^{1,*} and Liqiang Chen^{1,*}

¹ Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA

² Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Supplementary Materials and Methods

3.1.20. tert-Butyl benzyl(6-chloro-2-(methylsulfonyl)pyrimidin-4-yl)carbamate (27). A solution of **25** [1] (4.46 g, 16.8 mmol), Boc₂O (4.53 g, 20.8 mmol), and DMAP (12 mg, 0.10 mmol) in anhydrous THF (50 mL) was allowed to stir at rt for 16 h. After the organic solvent was removed in vacuo, the crude **26** was used for next reaction without purification. To the residue in CH₂Cl₂ (100 mL) at 0 °C was added mCPBA (55% purity, 11.7 g, 37.3 mmol) and the mixture was allowed to warm to rt and stir at rt for 3 h. The reaction was quenched with saturated NaHCO₃ and the mixture was extracted with CH₂Cl₂. The organic layer was concentrated and the residue was purified by flash column chromatography (30% EtOAc/hexanes) to give compound **27** as a white solid (6.39 g, 96% for 2 steps). ¹H NMR (400 MHz, CDCl₃) δ 8.37 (s, 1H), 7.30 (d, *J* = 4.4 Hz, 4H), 7.26–7.23 (m, 1H), 5.30 (s, 2H), 3.16 (s, 3H), 1.51 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 164.5, 162.6, 162.0, 152.7, 137.1, 128.7, 127.7, 127.5, 114.3, 85.0, 49.5, 39.1, 28.1. HRMS (ESI⁺) calcd for C₁₇H₂₁ClN₃O₄S (M+H)⁺ 398.0936, found 398.0940.

3.1.21. 2-Methyl-1H-indole-4-carboxamide (29). A solution of 2-methyl-1H-indole-4-carboxylic acid (**28** [2], 1.16 g, 6.63 mmol), Boc₂O (5.71 g, 26.2 mmol), Et₃N (2.0 mL, 14.3 mmol), and DMAP (7.1 mg, 0.058 mmol) in anhydrous THF (30 mL) was allowed to stir at rt for 45 min and 7N NH₃ in MeOH (10 mL) was added. The resulting white suspension was allowed to stir for additional 25 min and concentrated. The residue was purified by flash column chromatography (MeOH/CH₂Cl₂ = 10:1) to give compound **29** as a yellow solid (632 mg, 54%). ¹H NMR (400 MHz, CD₃OD) δ 7.42 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.06 (td, *J* = 7.8, 1.1 Hz, 1H), 6.57 (s, 1H), 2.45 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 174.6, 138.9, 138.8, 128.7, 125.4, 120.5, 120.4, 114.9, 100.8, 13.5. HRMS (ESI⁻) calcd for C₁₀H₉N₂O (M-H)⁻ 173.0720, found 173.0721.

3.1.22. tert-Butyl benzyl(2-(4-carbamoyl-2-methyl-1H-indol-1-yl)-6-chloropyrimidin-4-yl)carbamate (30). A solution of 2-methyl-1H-indole-4-carboxamide (**29**, 210 mg, 1.21 mmol) and LDA (1 N in THF, 3.0 mL) in anhydrous THF (20 mL) was allowed to stir at rt for 15 min and **27** (820 mg, 2.06 mmol) was added. The resulting red solution was allowed to stir at rt for 40 min and additional LDA (1 N in THF, 1.0 mL) was added. The dark red solution was stirred for 30 min and quenched with saturated NH₄Cl. After the mixture was extracted with CH₂Cl₂, the organic layer was concentrated and the residue was purified by flash column chromatography (50% EtOAc/hexanes) to give compound **30** as a light yellow solid (280 mg, 47%). ¹H NMR (400 MHz, CDCl₃) δ 8.56 (brs, 1H), 8.20 (brs, 1H), 7.89 (s, 1H), 7.56 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.45 (dt, *J* = 8.0, 0.9 Hz, 1H), 7.35–7.32 (m, 2H), 7.29–7.26 (m, 1H), 7.25–7.24 (m, 1H), 7.23–7.21 (m, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.70–6.69 (m, 1H), 5.33 (s, 2H), 2.47 (s, 3H), 1.46 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 165.7, 162.1, 161.8, 156.4, 153.3, 138.6, 138.1, 137.0, 128.4, 127.8, 127.4, 127.2, 125.0, 120.6, 120.4, 114.5, 107.3, 100.7, 83.5, 48.8, 28.1, 14.0. HRMS (APCI⁺) calcd for C₂₆H₂₇ClN₅O₃ (M+H)⁺ 492.1797, found 492.1796.

3.1.23. tert-Butyl benzyl(2-(4-carbamoyl-2-methyl-1H-indol-1-yl)-6-(piperidin-1-yl)pyrimidin-4-yl)carbamate (11). A solution of **30** (29 mg, 0.059 mmol) and piperidine (30 μL, 0.30 mmol) in dioxane (1 mL) was heated at 100 °C for 1.5 h and the organic solvent was removed in vacuo. The resulting crude **31** was used directly for next reaction. The residue was dissolved in anhydrous CH₂Cl₂ (3 mL) and TFA (1 mL) was added. The mixture was allowed to stir at rt for 2 h, quenched

with saturated NaHCO₃, and concentrated. The residue was purified by flash column chromatography (EtOAc) to give compound **11** as a white solid (12 mg, 45% for 2 steps). ¹H NMR (400 MHz, CDCl₃) δ 8.28 (s, 2H), 7.56 (dd, *J* = 7.5, 0.8 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.33 (d, *J* = 4.6 Hz, 3H), 7.31–7.26 (m, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.75 (s, 1H), 5.25 (brs, 1H), 5.21 (s, 1H), 4.42 (d, *J* = 5.8 Hz, 2H), 3.49 (t, *J* = 5.4 Hz, 4H), 2.46 (s, 3H), 1.64–1.59 (m, 2H), 1.57–1.52 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 166.5, 164.5, 163.8, 157.0, 138.6, 137.5, 137.0, 128.8 (2C), 127.54, 127.47, 127.3, 126.4, 120.5, 120.1, 113.8, 100.8, 46.3, 45.4, 25.6, 24.9, 14.0. HRMS (ESI⁻) calcd for C₂₆H₂₇N₆O (M-H)⁻ 439.2252, found 439.2255.

3.1.24. *tert*-Butyl benzyl(2-(4-carbamoyl-2-methyl-1*H*-indol-1-yl)-6-(cyclohex-1-en-1-yl)pyrimidin-4-yl)carbamate (**32**). A mixture of **30** (70 mg, 0.14 mmol), cyclohex-1-en-1-ylboronic acid (23 mg, 0.18 mmol), Pd(PPh₃)₂Cl₂ (6 mg, 0.008 mmol), Na₂CO₃ (39 mg, 0.37 mmol) in dioxane (2 mL) and H₂O (1 mL) was heated at 100 °C for 1 h. After concentration, the residue was purified by flash column chromatography (30% EtOAc/hexanes) to give compound **32** as a white solid (65 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 8.47 (s, 1H), 8.13 (brs, 1H), 7.74 (s, 1H), 7.58 (d, *J* = 7.4 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.33–7.30 (m, 2H), 7.27–7.26 (m, 1H), 7.25–7.23 (m, 1H), 7.21–7.19 (m, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 7.10–7.08 (m, 1H), 6.70–6.69 (m, 1H), 5.29 (s, 2H), 2.48–2.45 (m, 5H), 2.30–2.25 (m, 2H), 1.80–1.74 (m, 2H), 1.69–1.63 (m, 2H), 1.45 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 167.4, 166.3, 162.0, 156.4, 153.7, 139.3, 137.7, 137.0, 135.1, 133.0, 128.3, 127.7, 127.4, 126.9, 126.0, 120.6, 120.3, 114.0, 103.3, 100.7, 82.6, 48.8, 28.3, 26.2, 25.5, 22.7, 22.1, 14.0. HRMS (ESI⁺) calcd for C₃₂H₃₆N₅O₃ (M+H)⁺ 538.2813, found 538.2817.

3.1.25. 1-(4-(Benzylamino)-6-cyclohexylpyrimidin-2-yl)-2-methyl-1*H*-indole-4-carboxamide (**12**). A mixture of **32** (38 mg, 0.071 mmol), 10 % Pd/C (4 mg) in MeOH (4 mL) was hydrogenated (balloon) at rt for 5 h. After filtration, the filtrate was concentrated and the obtained **33** was treated with 10% TFA/CH₂Cl₂ for 4 h. After the reaction was quenched with sat NaHCO₃, the mixture was extracted with CH₂Cl₂. The organic layer was concentrated and the residue was purified by flash column chromatography (10% MeOH/CH₂Cl₂) to give compound **12** as a white solid (19 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 8.64 (s, 1H), 8.48 (brs, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.35–7.33 (m, 1H), 7.31–7.30 (m, 3H), 7.29–7.27 (m, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.73 (s, 1H), 5.92 (s, 1H), 5.45 (brs, 1H), 4.48 (d, *J* = 5.7 Hz, 2H), 2.44 (s, 3H), 2.42–2.38 (m, 1H), 1.91–1.88 (m, 2H), 1.82–1.78 (m, 2H), 1.73–1.68 (m, 1H), 1.44–1.40 (m, 1H), 1.37–1.34 (m, 2H), 1.32–1.28 (m, 1H), 1.26–1.19 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 166.5, 164.1, 157.5, 138.1, 137.8, 137.0, 128.8, 127.7, 127.6, 127.4, 125.8, 120.3, 120.1, 114.2, 100.8, 95.3, 45.9, 32.1, 26.4 (2C), 26.1, 14.0. HRMS (ESI⁺) calcd for C₂₇H₃₀N₅O (M+H)⁺ 440.2445, found 440.2434.

References

1. Cervi, G.; Magnaghi, P.; Asa, D.; Avanzi, N.; Badari, A.; Borghi, D.; Caruso, M.; Cirila, A.; Cozzi, L.; Felder, E.; et al. Discovery of 2-(cyclohexylmethylamino)pyrimidines as a new class of reversible valosine containing protein inhibitors. *J. Med. Chem.* **2014**, *57*, 10443-10454, doi:10.1021/jm501313x.
2. Torisu, K.; Kobayashi, K.; Iwahashi, M.; Nakai, Y.; Onoda, T.; Nagase, T.; Sugimoto, I.; Okada, Y.; Matsumoto, R.; Nanbu, F.; et al. Development of prostaglandin D2 receptor

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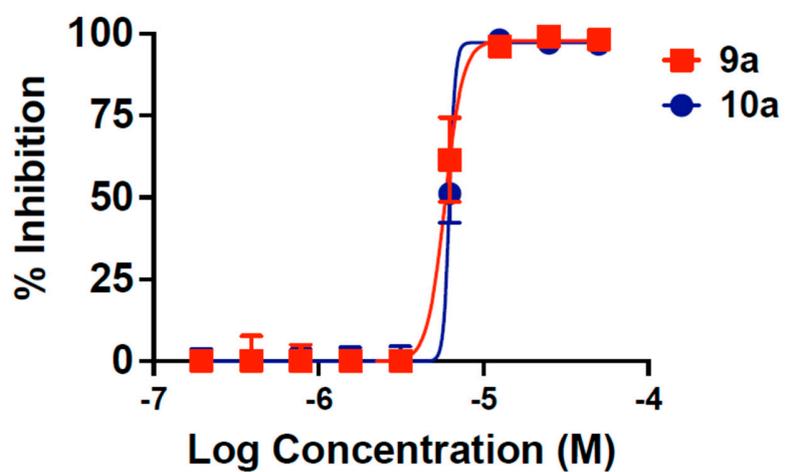
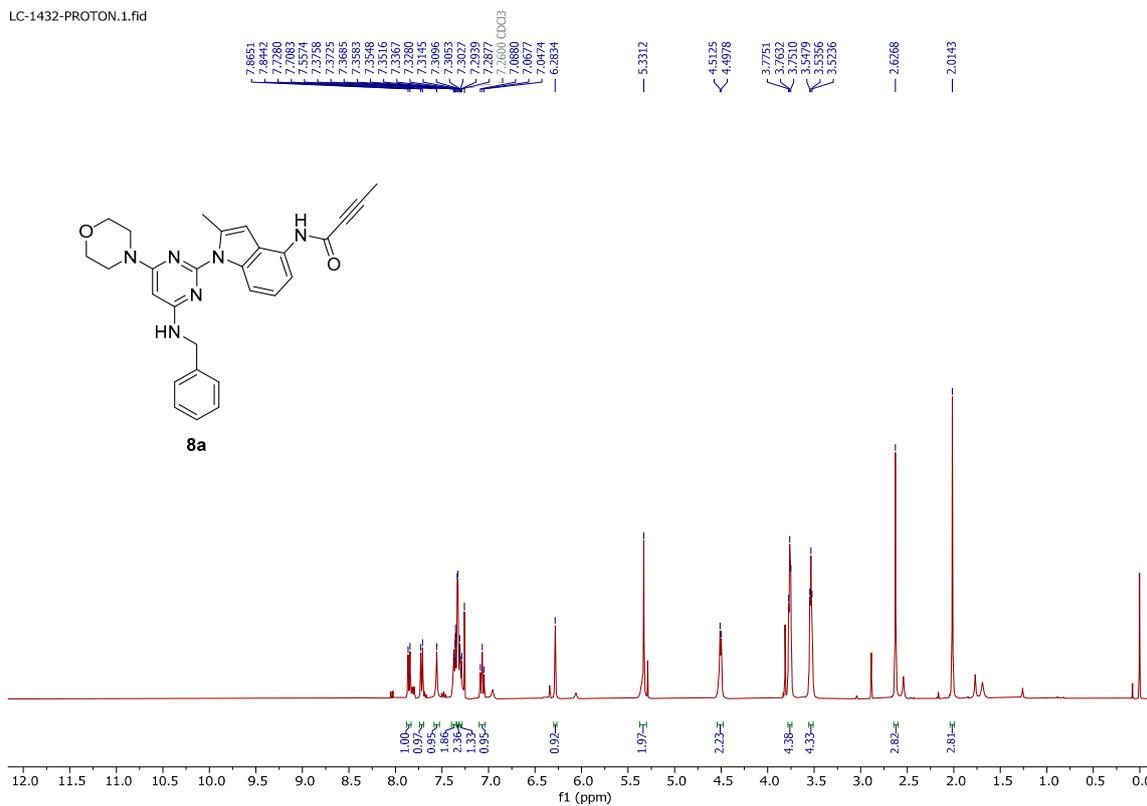
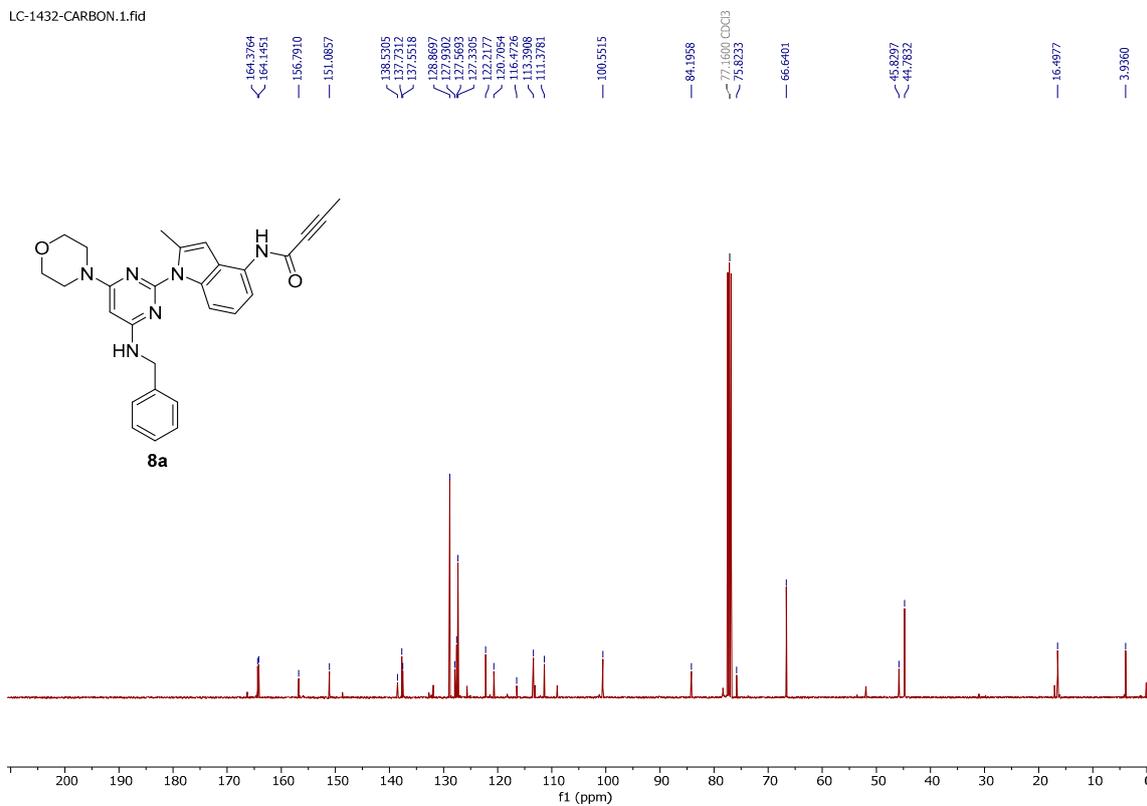


Figure S1. Dose-response curves of compounds **9a** and **10a** against SARS-CoV-2.

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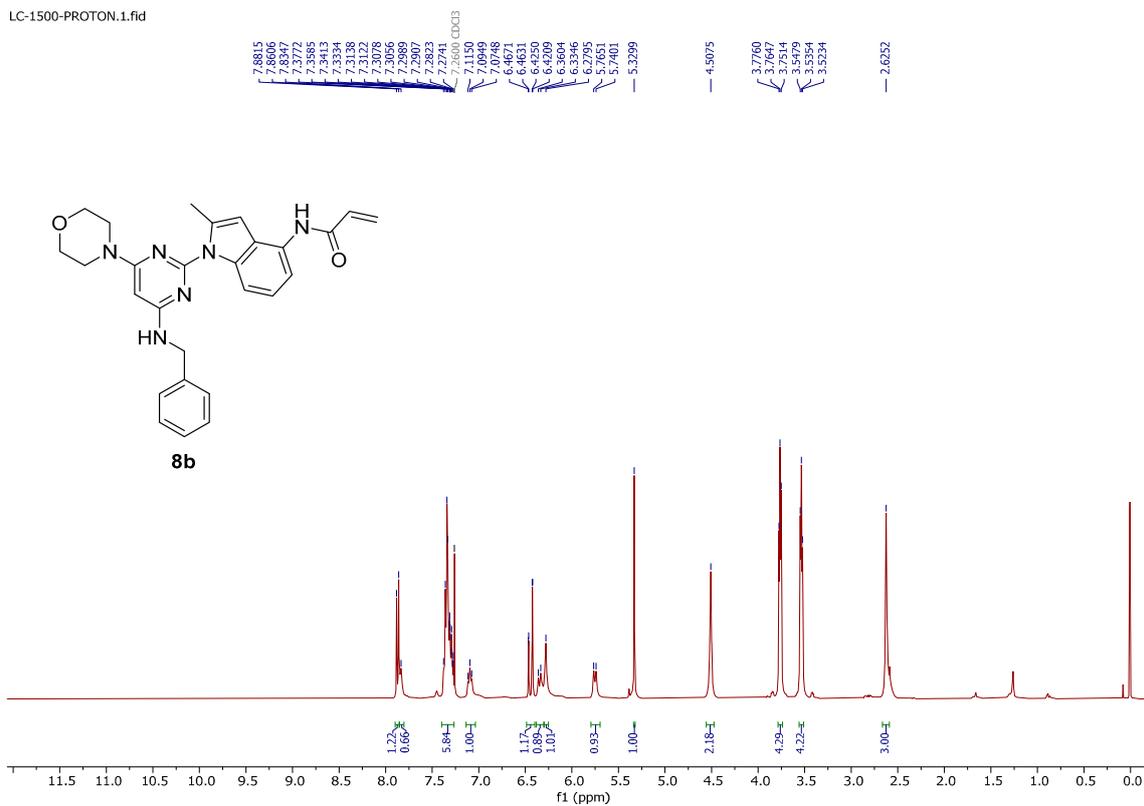


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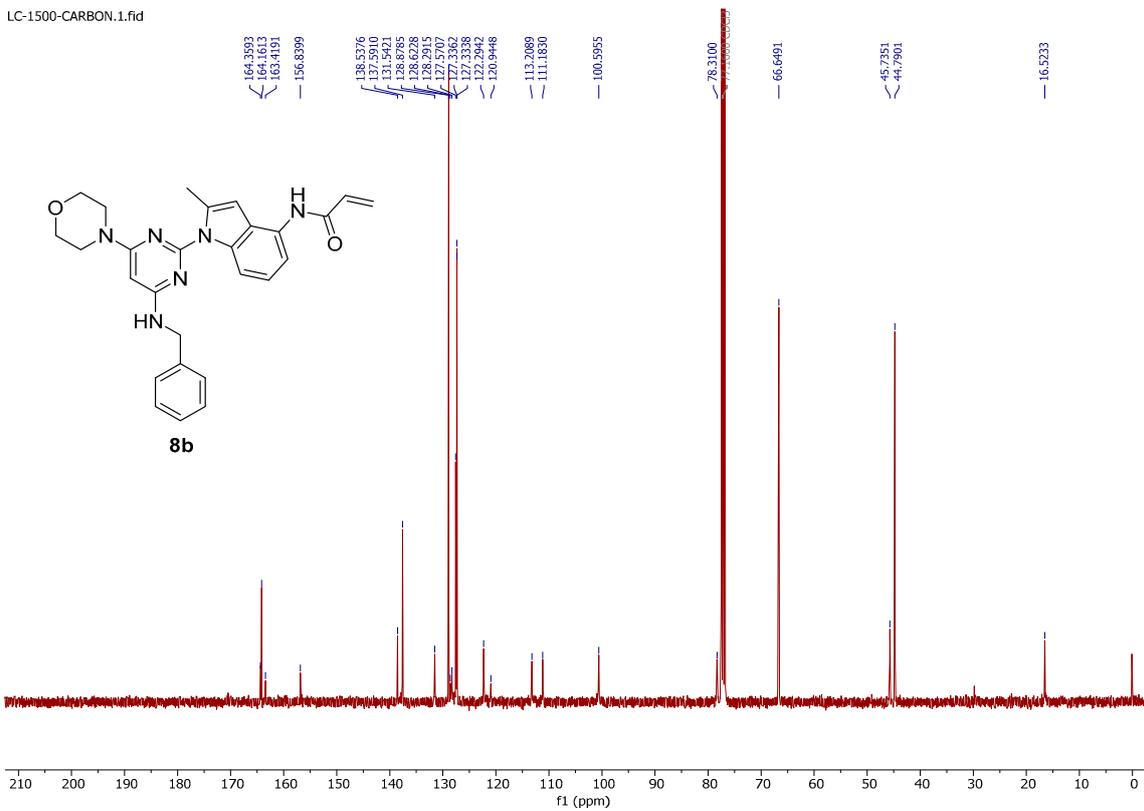


^1H and ^{13}C NMR spectra of compound **8a**

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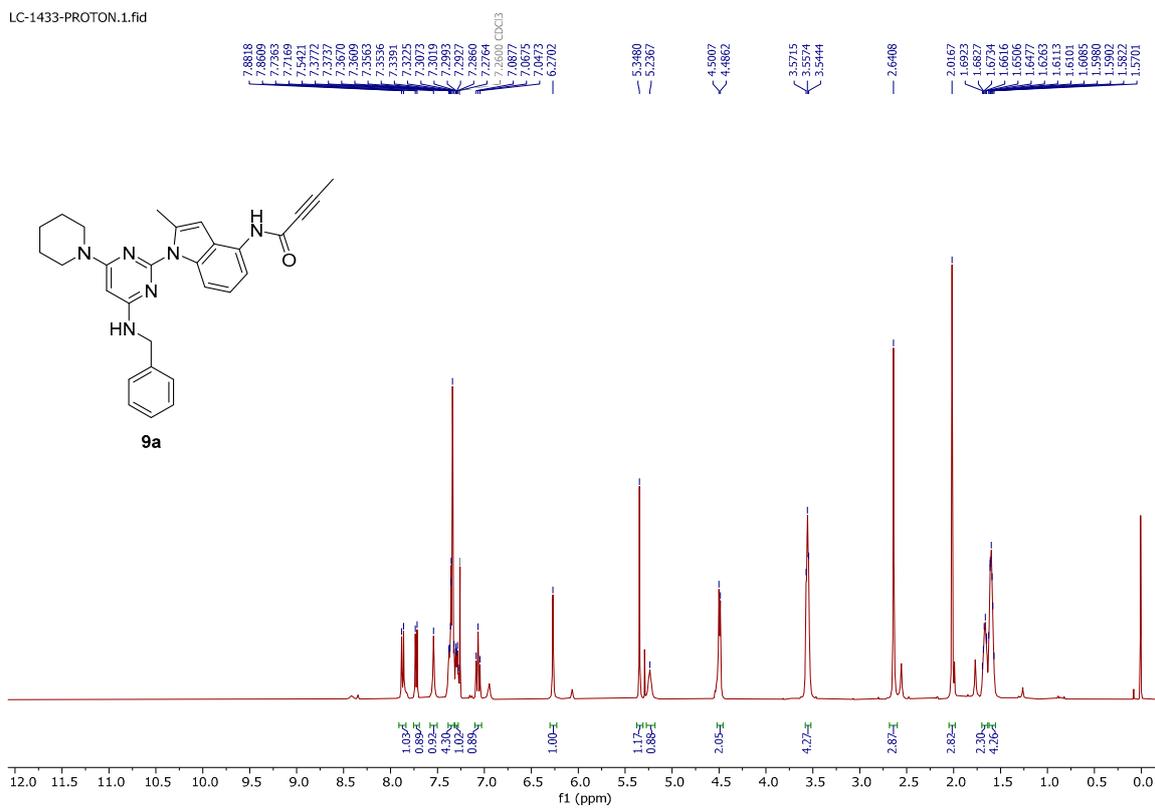


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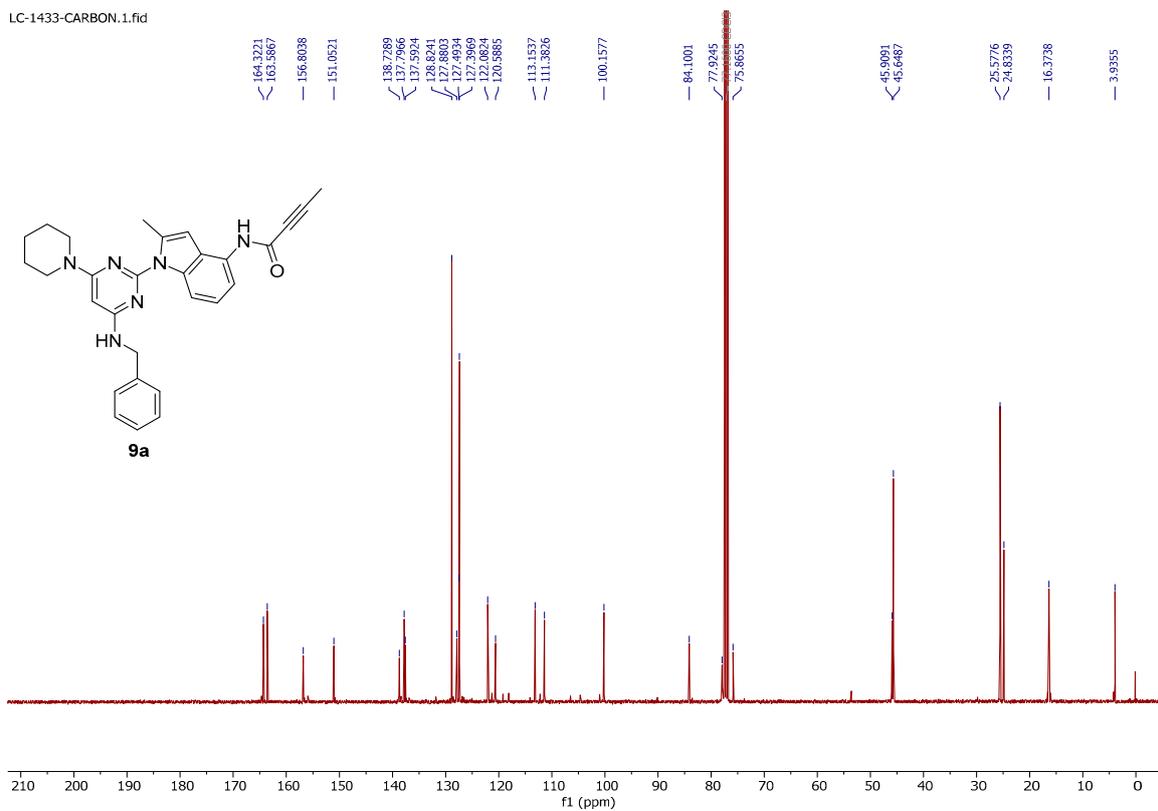


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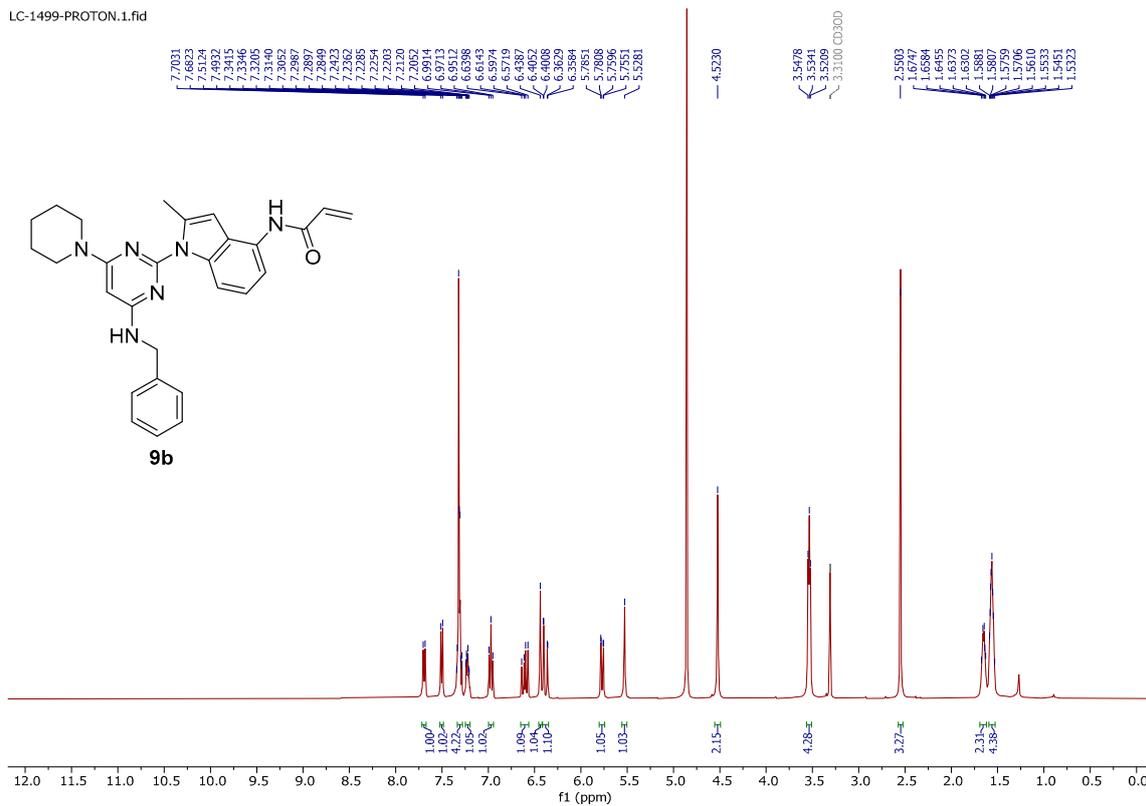


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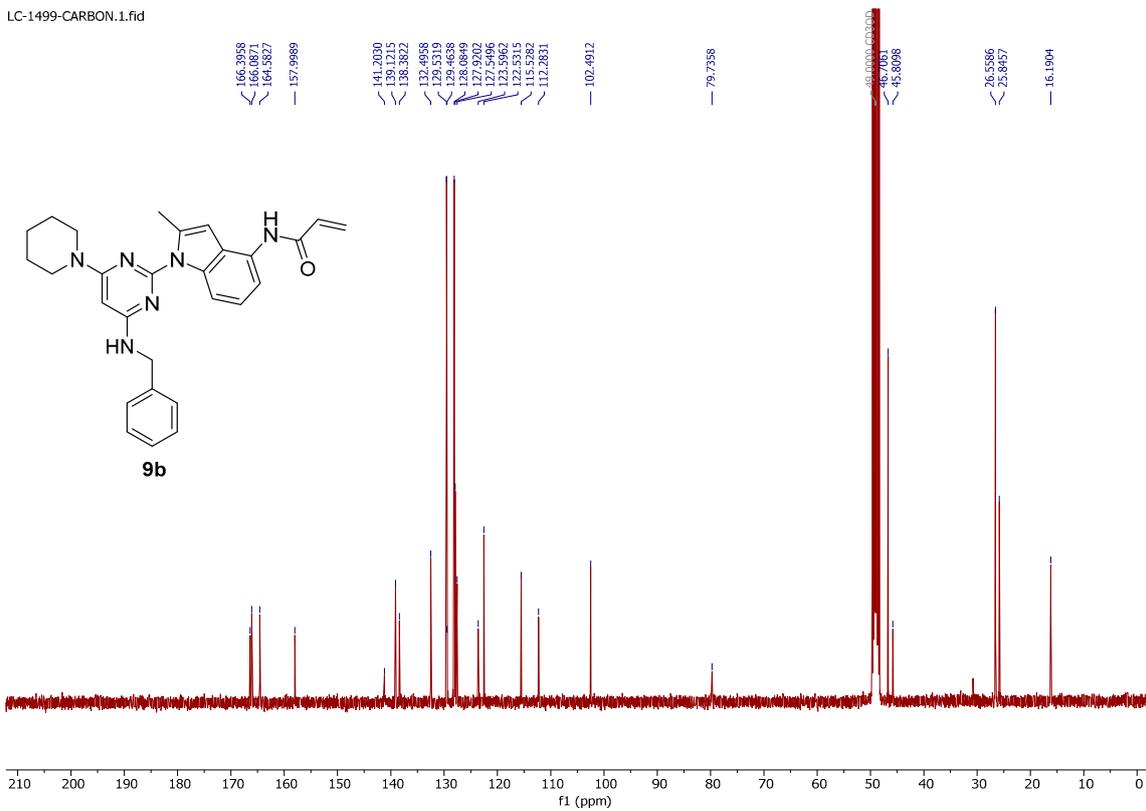


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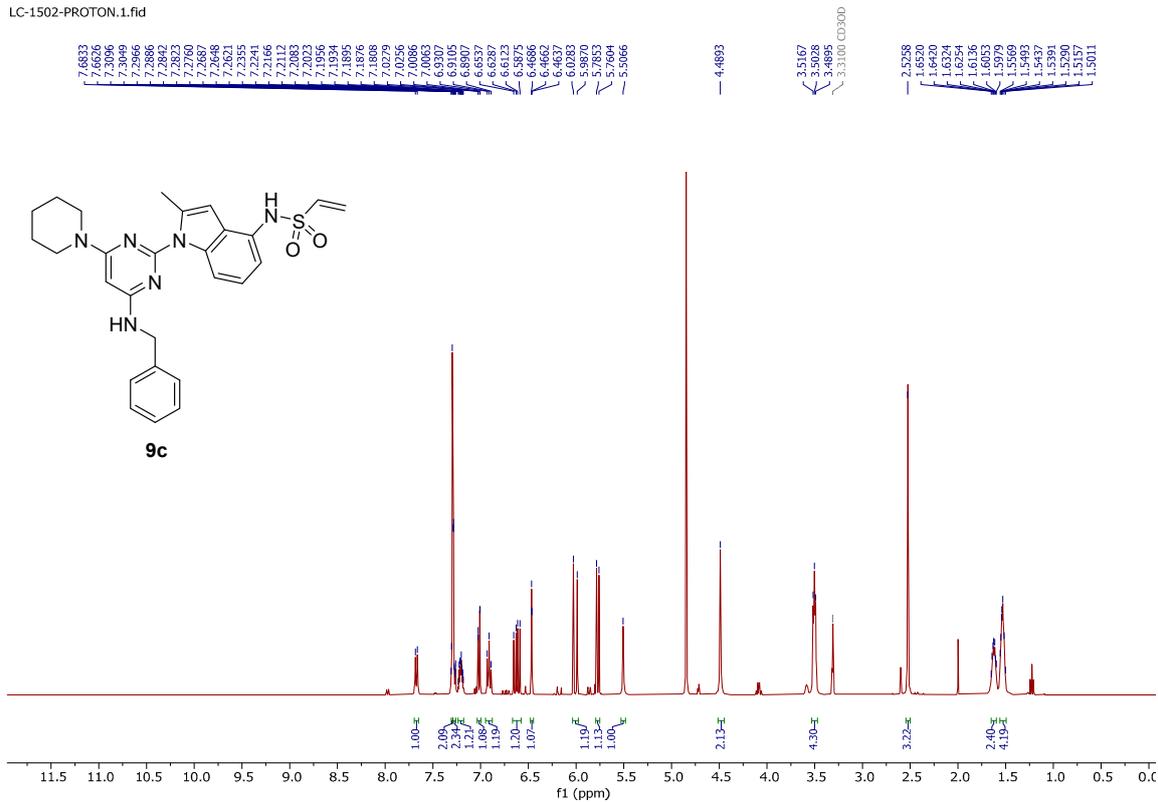


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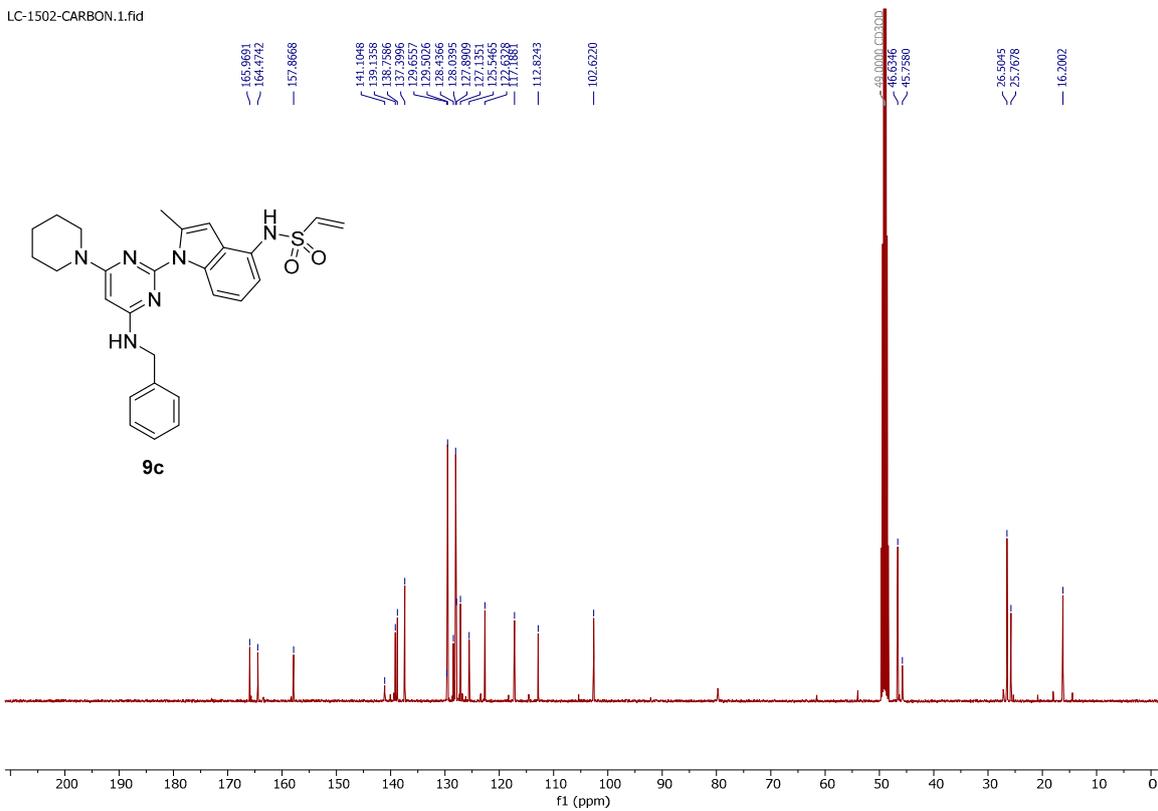


¹H and ¹³C NMR spectra of compound **9b**

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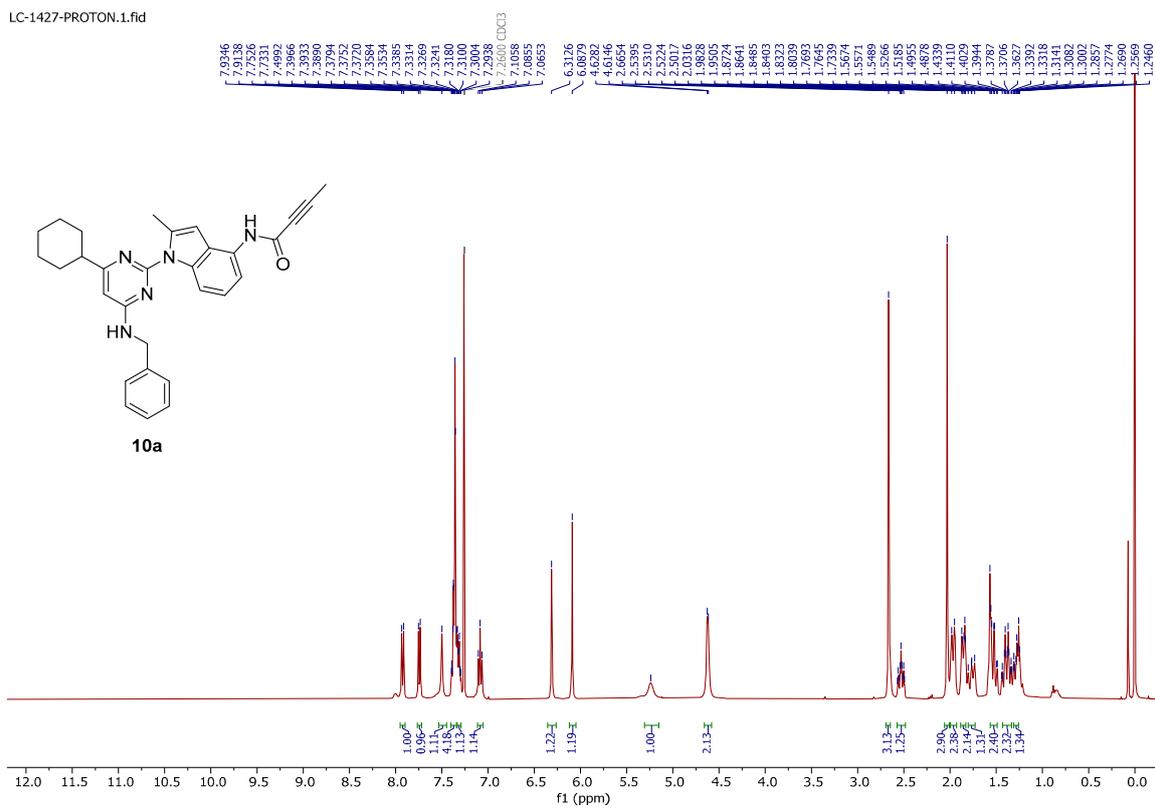


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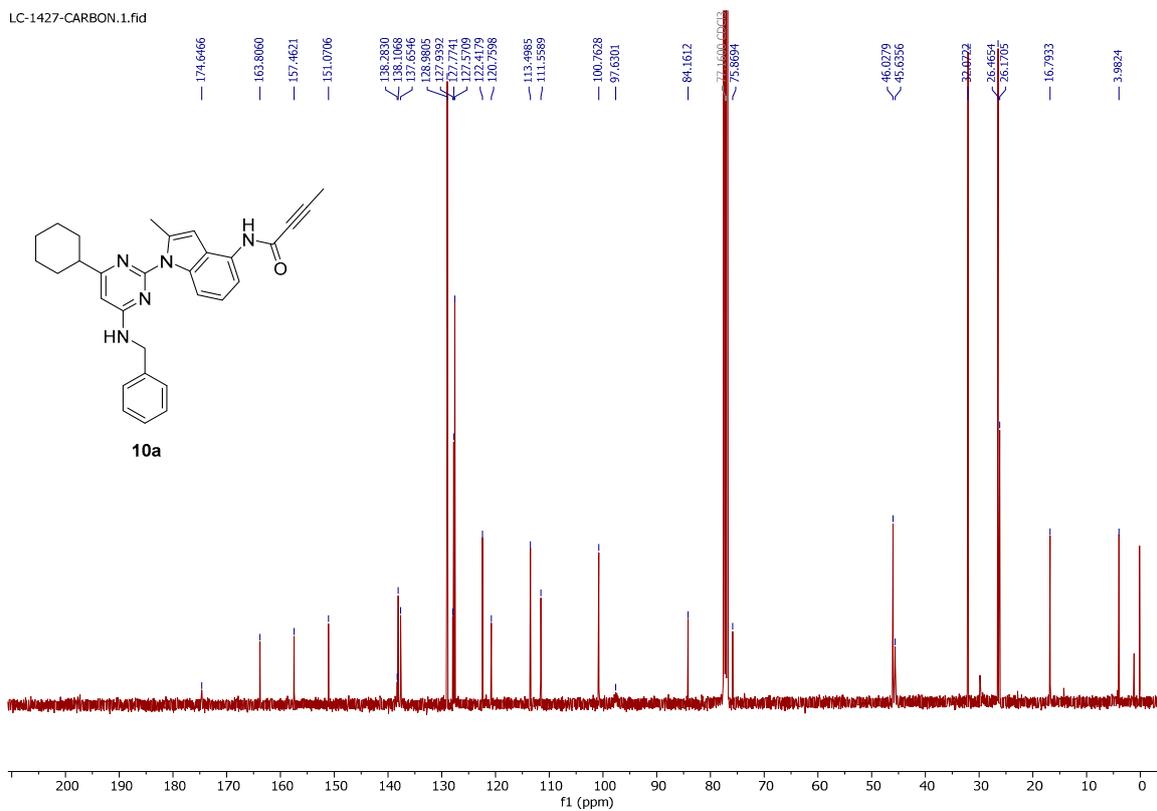


¹H and ¹³C NMR spectra of compound **9c**

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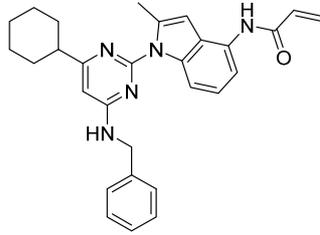


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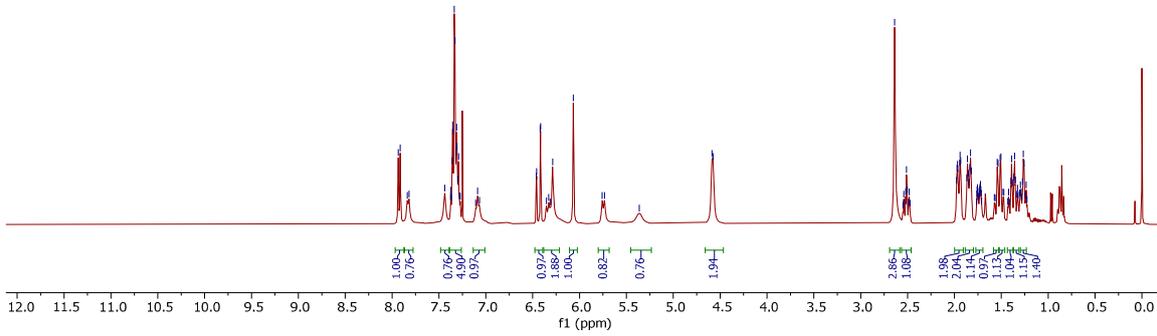


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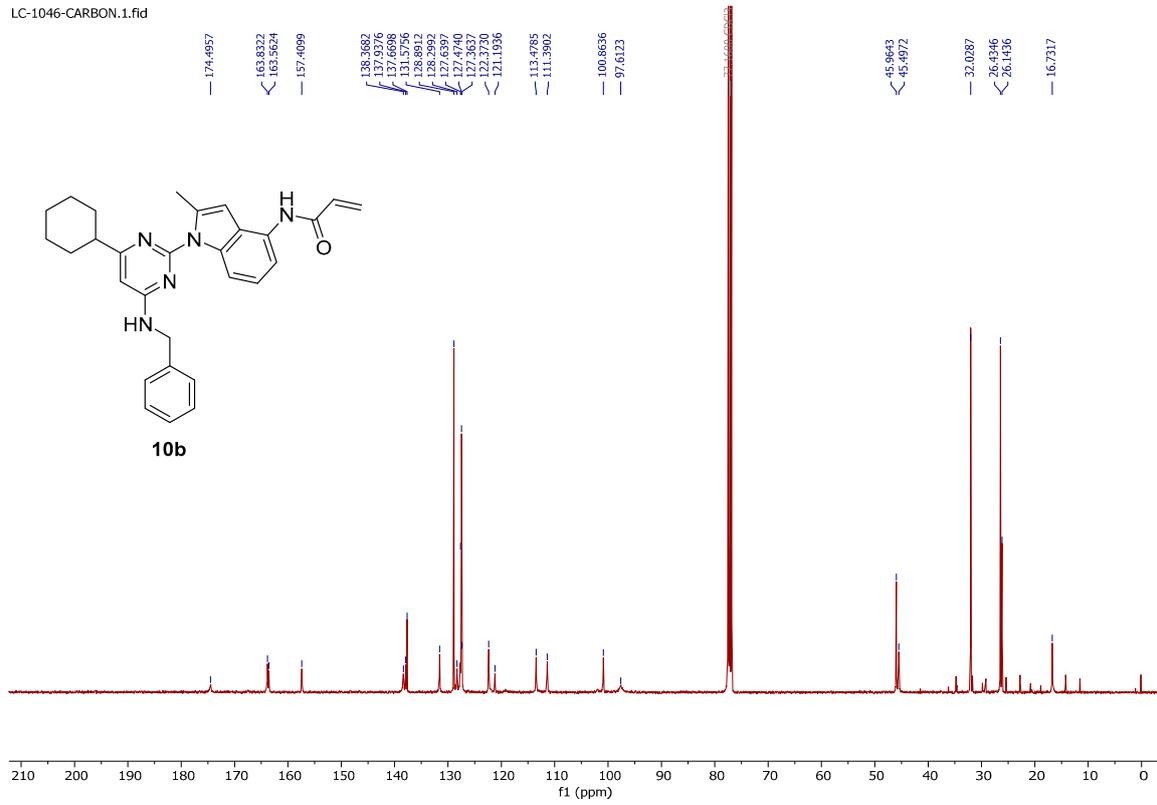
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10b

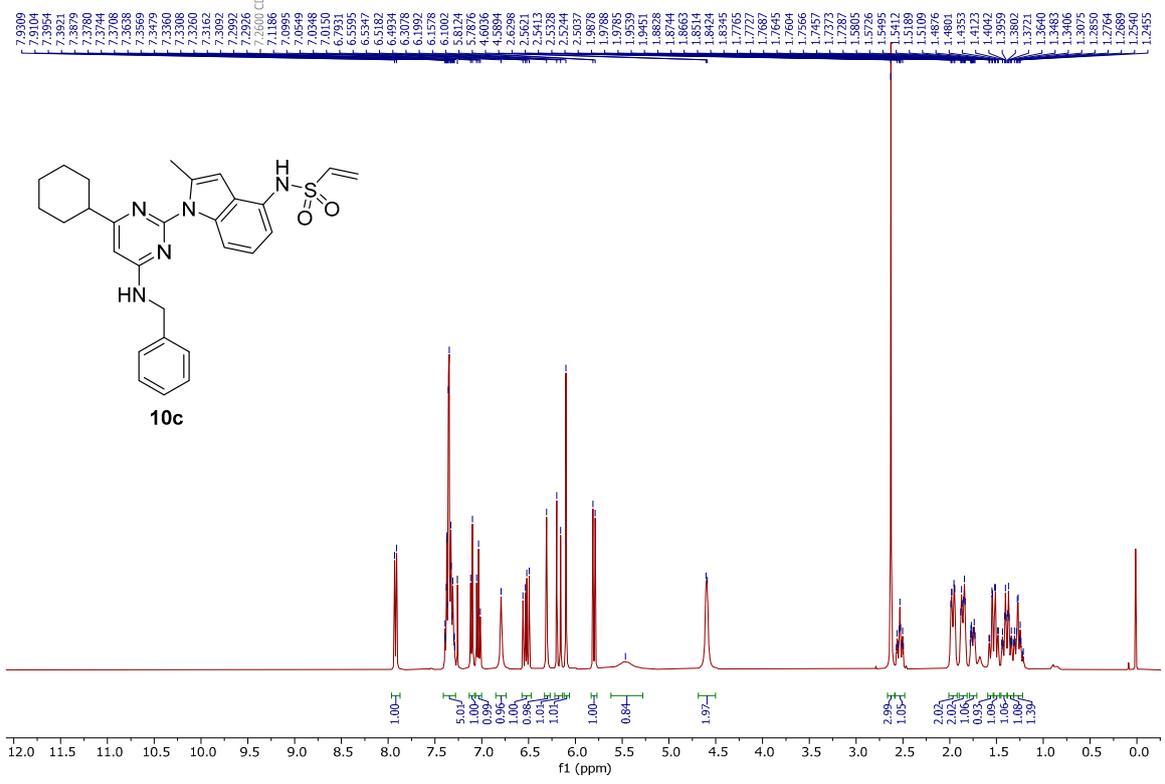


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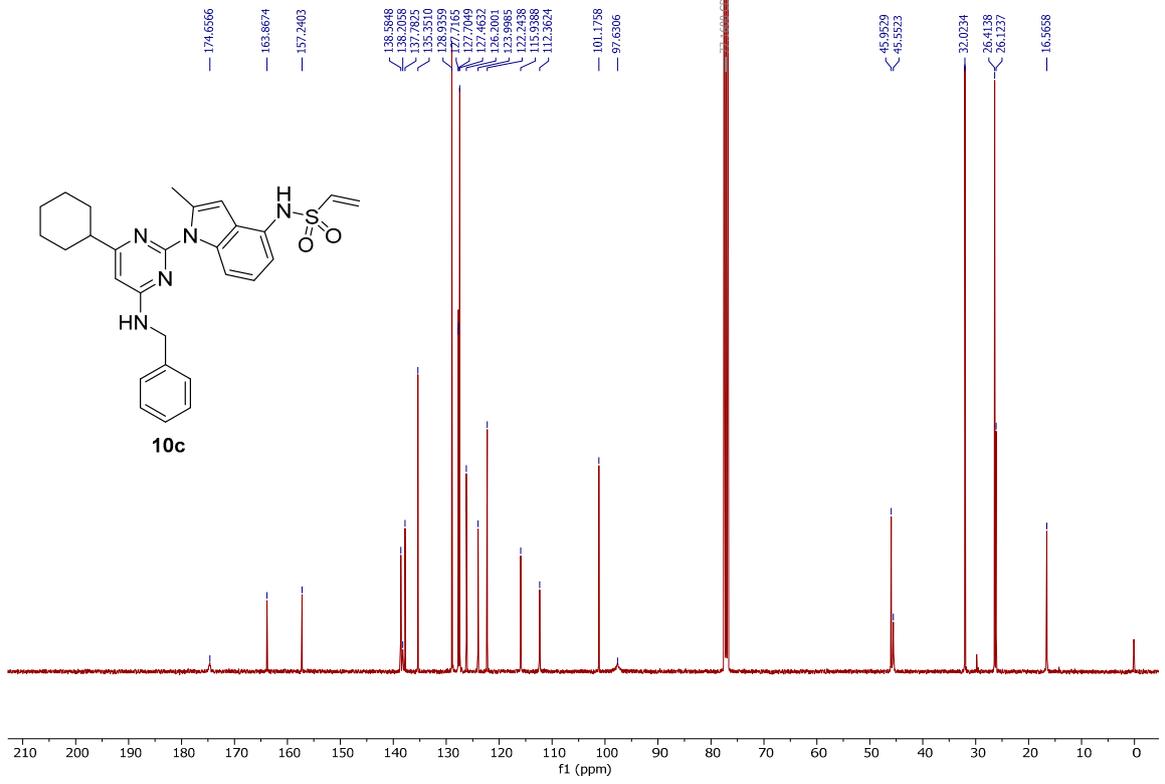


¹H and ¹³C NMR spectra of compound 10b

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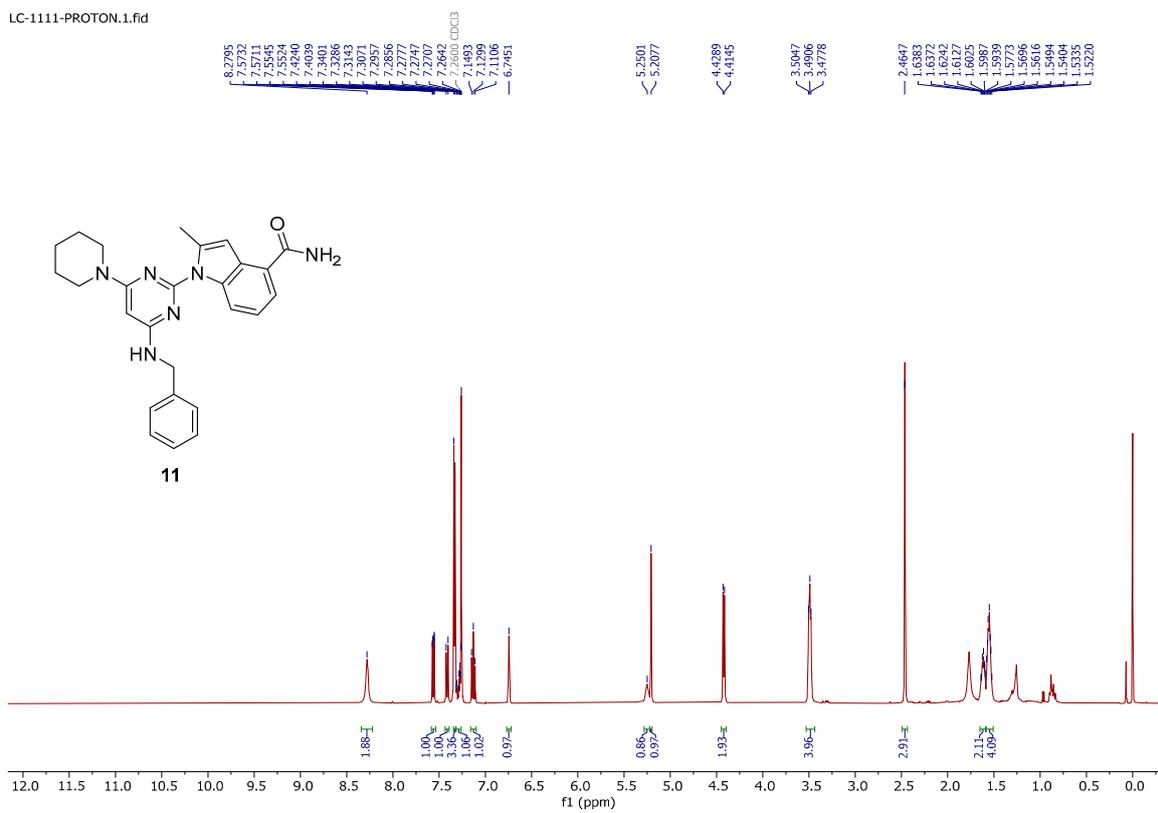


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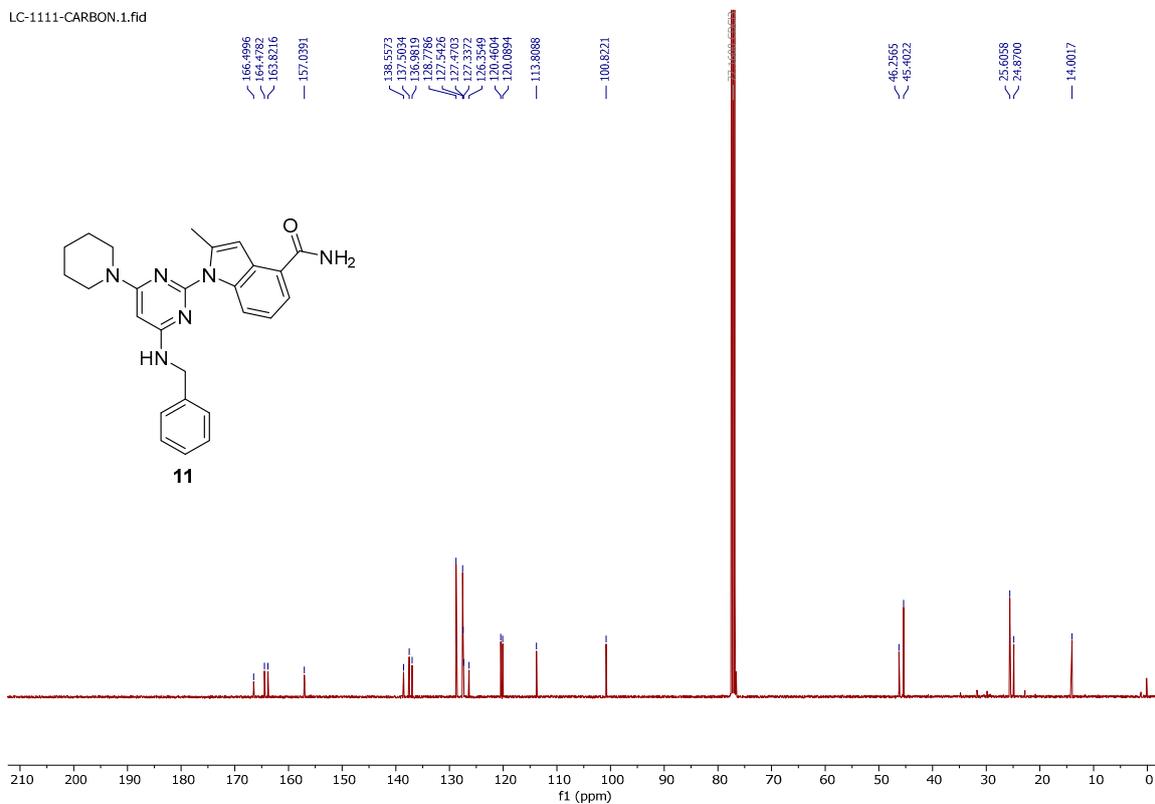


¹H and ¹³C NMR spectra of compound **10c**

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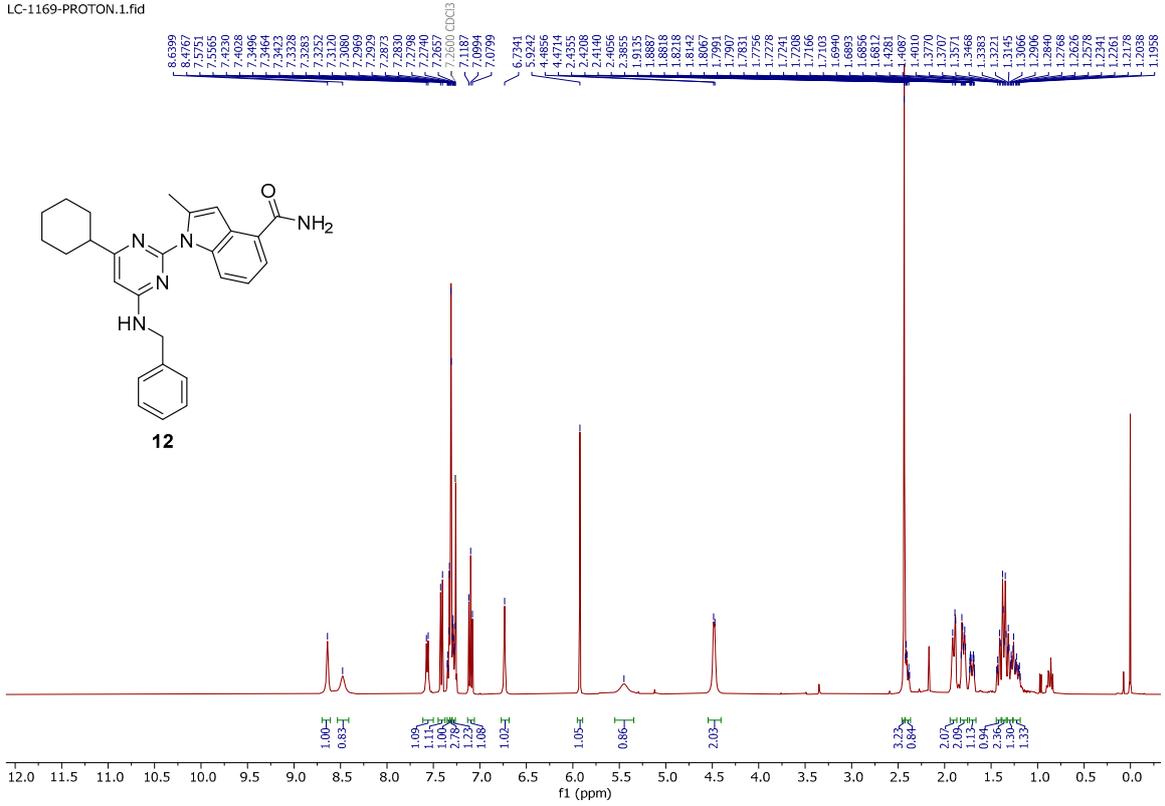


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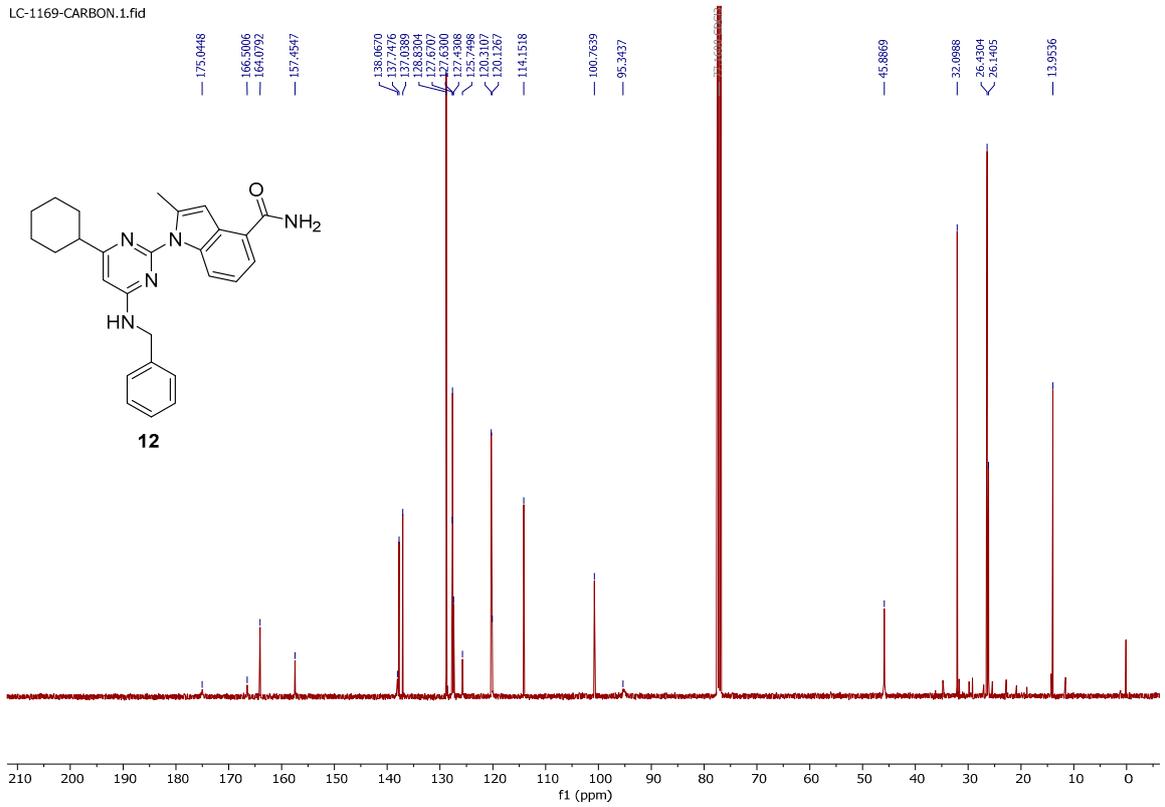


^1H and ^{13}C NMR spectra of compound **11**

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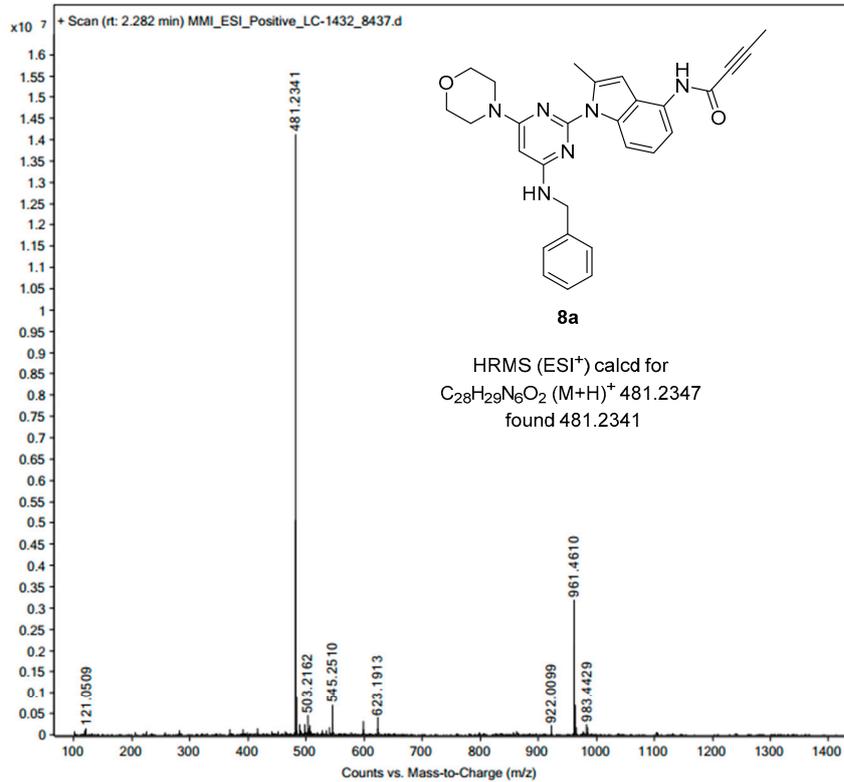


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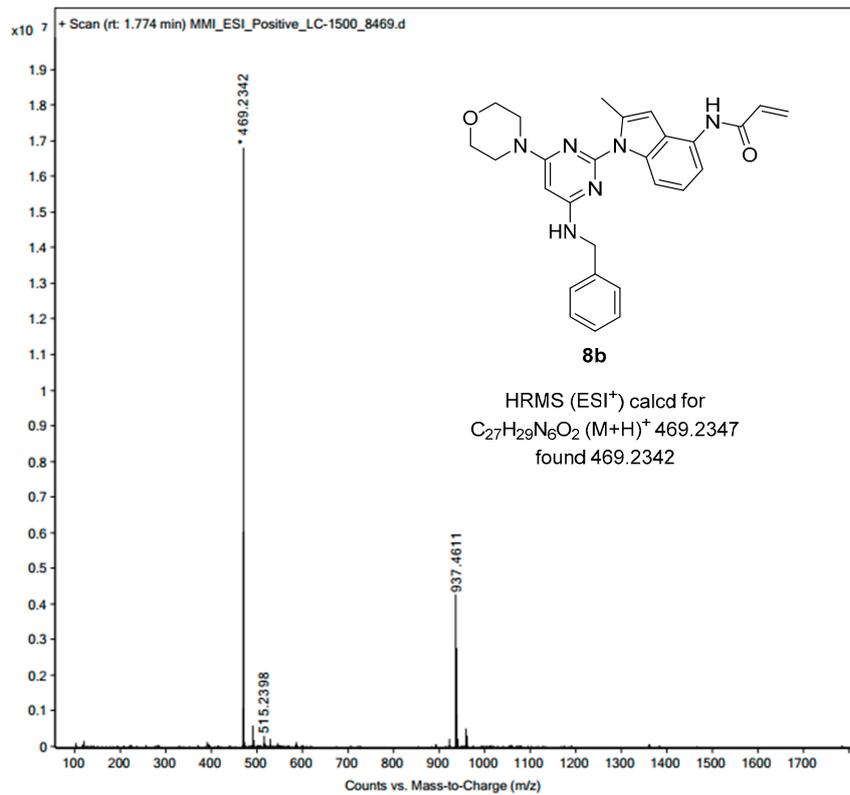
¹H and ¹³C NMR spectra of compound 12

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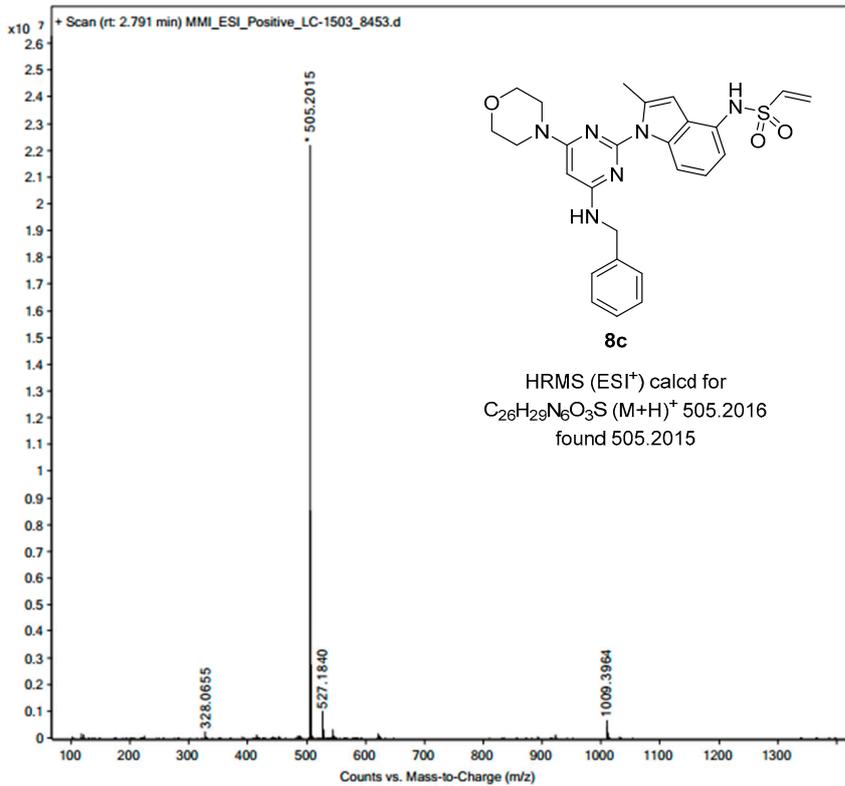
HRMS spectrum of compound 8a

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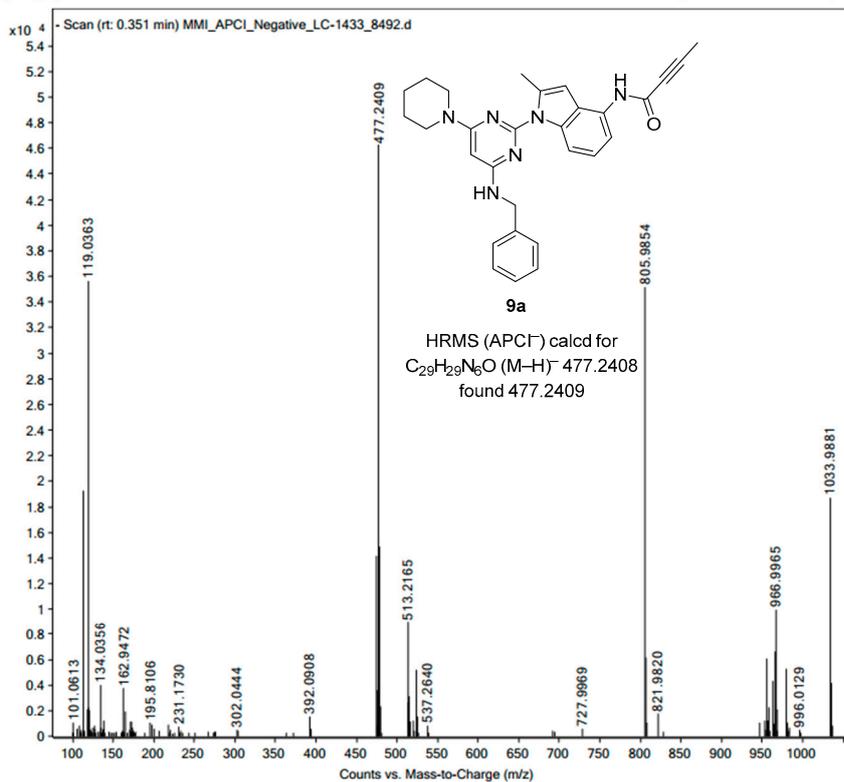
HRMS spectrum of compound 8b

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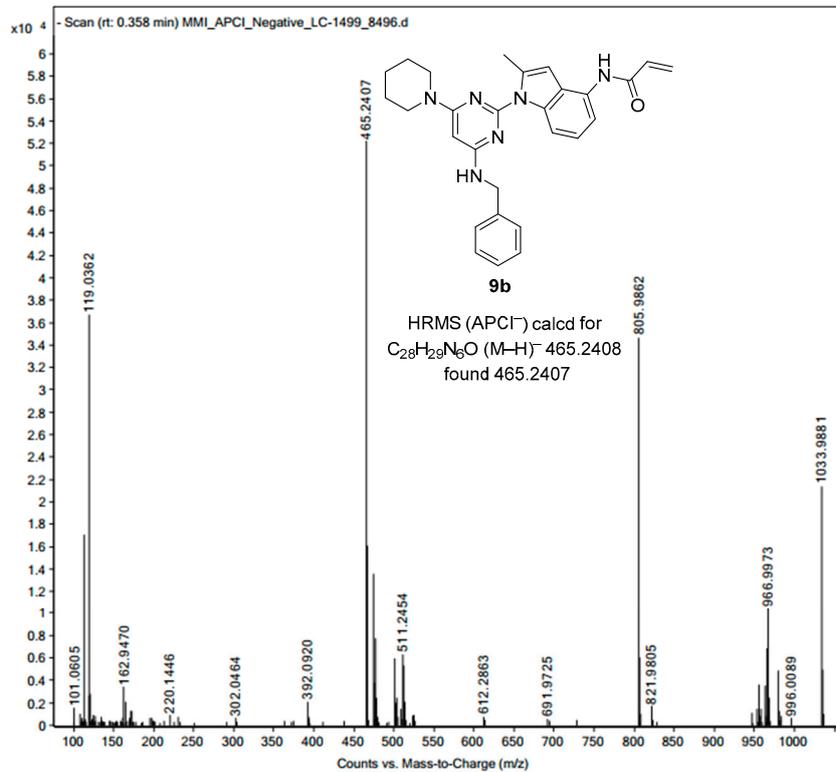
HRMS spectrum of compound 8c

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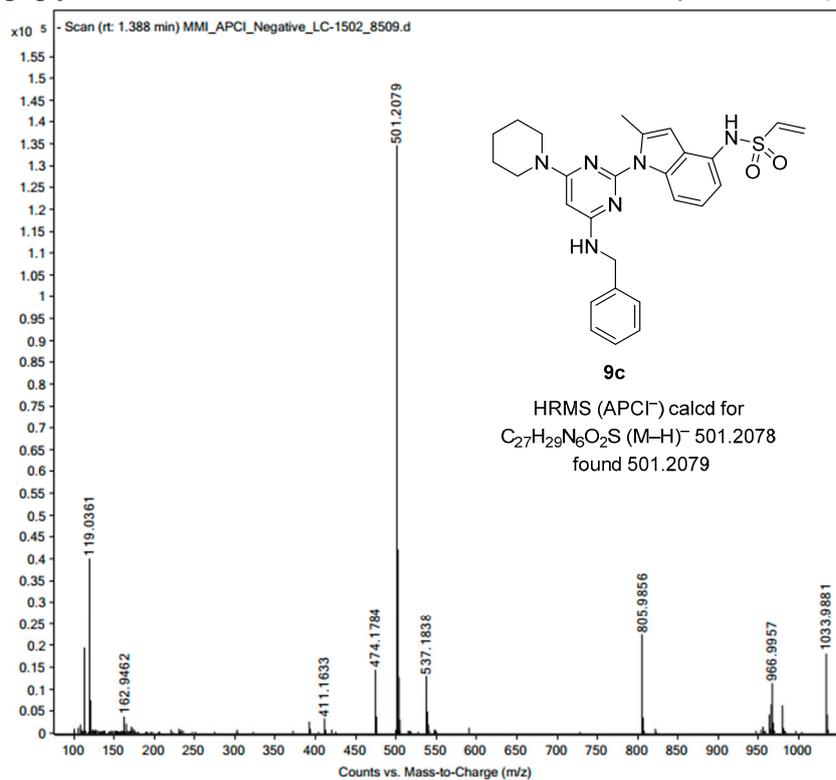
HRMS spectrum of compound 9a

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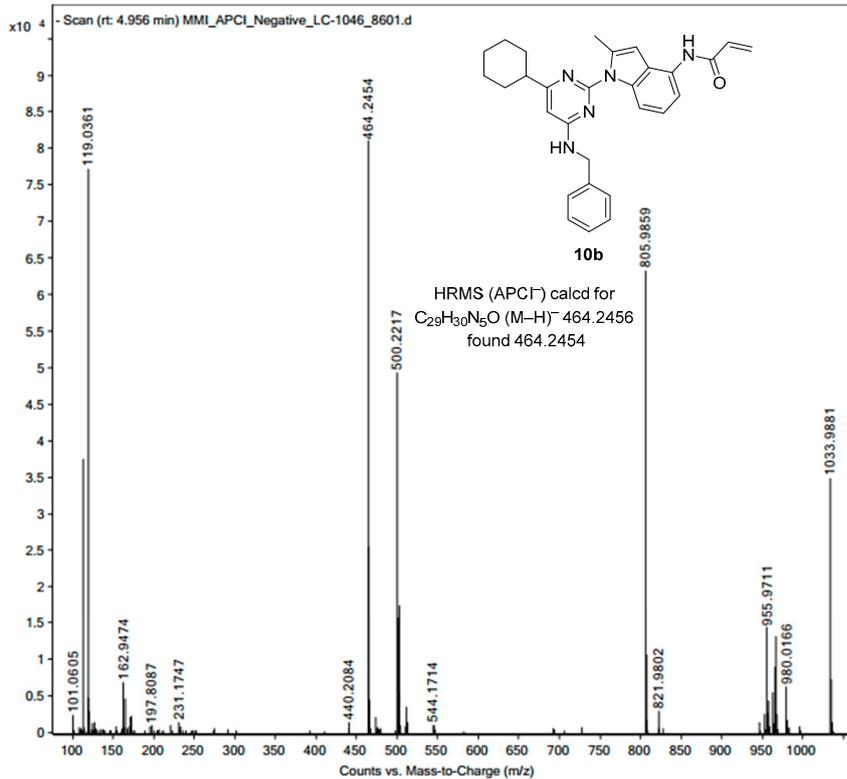
HRMS spectrum of compound 9b

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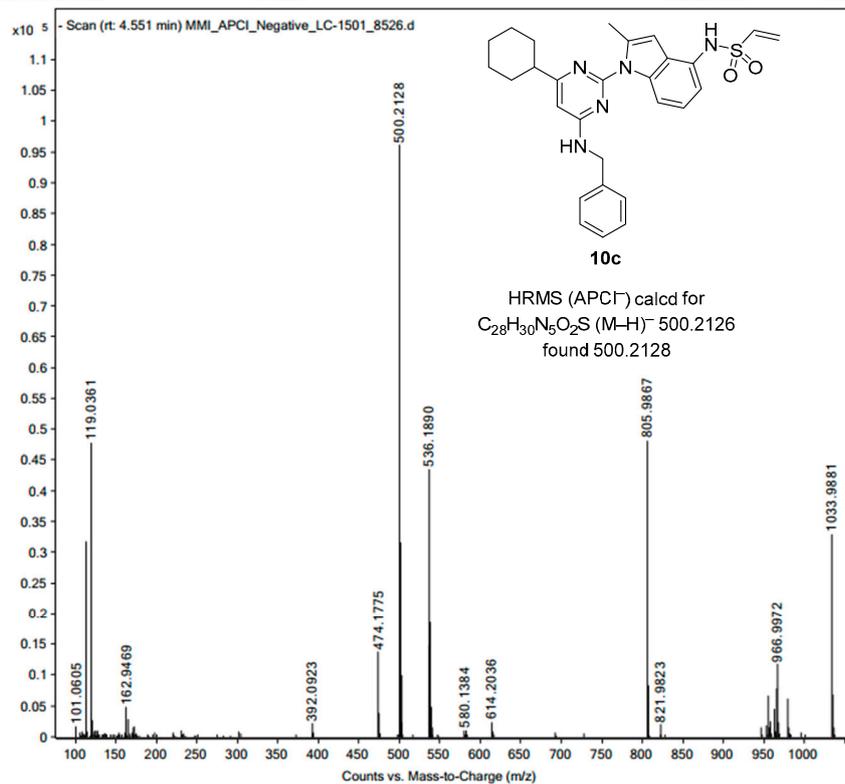
HRMS spectrum of compound 9c

Sample Name	LC-1046	Position	P2-E4	Instrument Name	TOF Walk Up
User Name	PRITHWISH GOSWAMI	Inj Vol	1	InjPosition	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	MMI_APCI_Negative_LC-1046_8601.d
ACQ Method	MMI_APCI_Negative.m	Comment		Acquired Time	11/1/2024 3:36:38 PM (UTC-05:00)



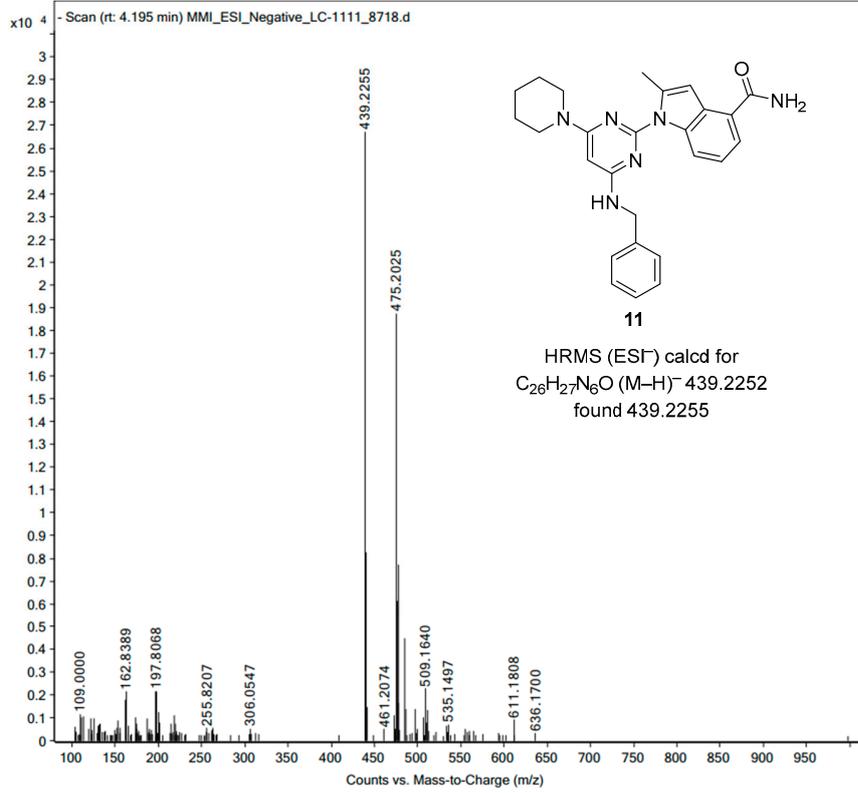
HRMS spectrum of compound 10b

Sample Name	LC-1501	Position	P2-C4	Instrument Name	TOF Walk Up
User Name	PRITHWISH GOSWAMI	Inj Vol	1	InjPosition	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	MMI_APCI_Negative_LC-1501_8526.d
ACQ Method	MMI_APCI_Negative.m	Comment		Acquired Time	10/31/2024 5:24:50 PM (UTC-05:00)



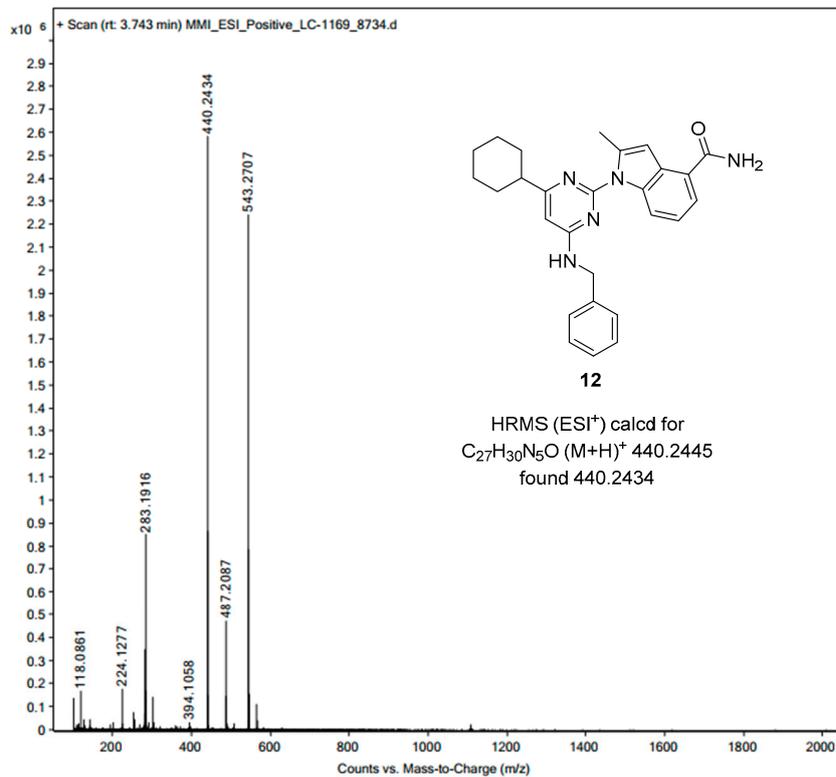
HRMS spectrum of compound 10c

Sample Name	LC-1111	Position	P1-B1	Instrument Name	TOF Walk Up
User Name	PRITHWISH GOSWAMI	Inj Vol	1	InjPosition	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	MML_ESI_Negative_LC-1111_8718.d
ACQ Method	MML_ESI_Negative.m	Comment		Acquired Time	11/5/2024 12:56:13 PM (UTC-06:00)



HRMS spectrum of compound 11

Sample Name	LC-1169	Position	P1-B7	Instrument Name	TOF Walk Up
User Name	PRITHWISH GOSWAMI	Inj Vol	1	Inj Position	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	MMI_ESI_Positive_LC-1169_8734.d
ACQ Method	MMI_ESI_Positive.m	Comment		Acquired Time	11/5/2024 2:30:39 PM (UTC-06:00)



HRMS spectrum of compound 12