Supplementary Information

Antituberculosis activity of α-aminoacyl amide derivatives

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1. Antituberculosis activity of compounds

Mycobacterium tuberculosis H37Rv was grown in MB7H9 medium supplemented with 0.2% tween and 1XADC for media enrichment till logarithmic phase. The *M. tb* H37Rv cells with A_{600nm} of 0.02 (~2 x 10^6 cfu/ml) were incubated with varied concentration (25 – 0.1 µM) of each compound for 7 days at 37^{o}C in 96 well U-bottom microtitre plate. After that, 30 µl of 0.01% resazurin was added to each well and incubated at 37 °C for 24 hours. Following that, plates were observed for any change in the color of dye and the fluorescent intensity of the wells was measured using spectrofluorimeter at an excitation/emission wavelength of 530nm/590nm. As a control, only *M. tb* H37Rv cells, only media and rifampicin were also be employed. For calculating the MIC of compound, following formula was used: (fluorescence intensity in the presence of compound - florescence intensity in the absence of compound)/ fluorescence intensity in the absence of compound *100. By using this formula, percentage inhibition at each concentration of compound was determined. For determination of MIC_{99} value (It is defined as the concentration of compound that will result in 99% inhibition of growth of the bacteria), drop plating was performed. Briefly, 5 µl of each sample from 96 well plate was spotted on MB7H11 agar plates containing oleic acid-albumin-dextrose-catalase (OADC) and incubated at 37°C for 2-3 weeks till the colonies of *M. tb* appears.

2. Screening of compounds against a panel of ESKAP pathogens

2.1.1 Chemicals and reagents

All bacterial culture media and supplements, including Middlebrook 7H9 broth, Middlebrook 7H11 Agar, albumin, dextrose and catalase supplement (ADC), oleic acid, albumin, dextrose, and catalase (OADC) supplements, were purchased from BD (Franklin Lakes, NJ, USA). All other chemicals and antibiotics were procured from Sigma–Aldrich (St Louis, MO, USA). RPMI and FBS were purchased from Lonza (USA) from Becton-Dickinson (NJ, USA).

2.1.2 Bacterial strains

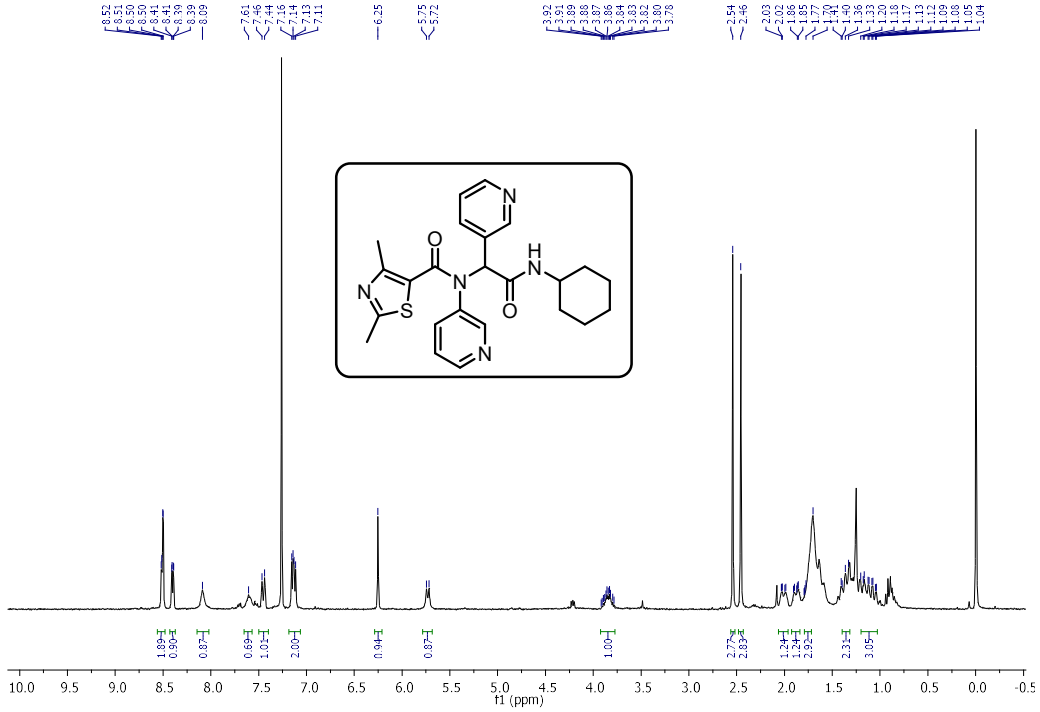
Compounds were tested against a bacterial panel consisting of ESKAPE pathogens, including *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA-1605), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213). Further, *M. abscessus* (ATCC49093)*, M. abscessus* (ATCC44266)*, M. abscessus* (ATCC 19977)*, M. fortuitum* (ATCC 6841)*, M. chelonae* (ATCC 35752) were used for the studies. The bacteria were typically cultured on Mueller-Hinton Agar (MHA). The mycobacterial strains were cultured in Middlebrook 7H9broth supplemented with glycerol, ADC and 0.1% tyloxapol in a 37°C roller bottle incubator (ThermoFisher Scientific Inc., USA) at 20 rpm.

2.1.3 Antibacterial susceptibility testing

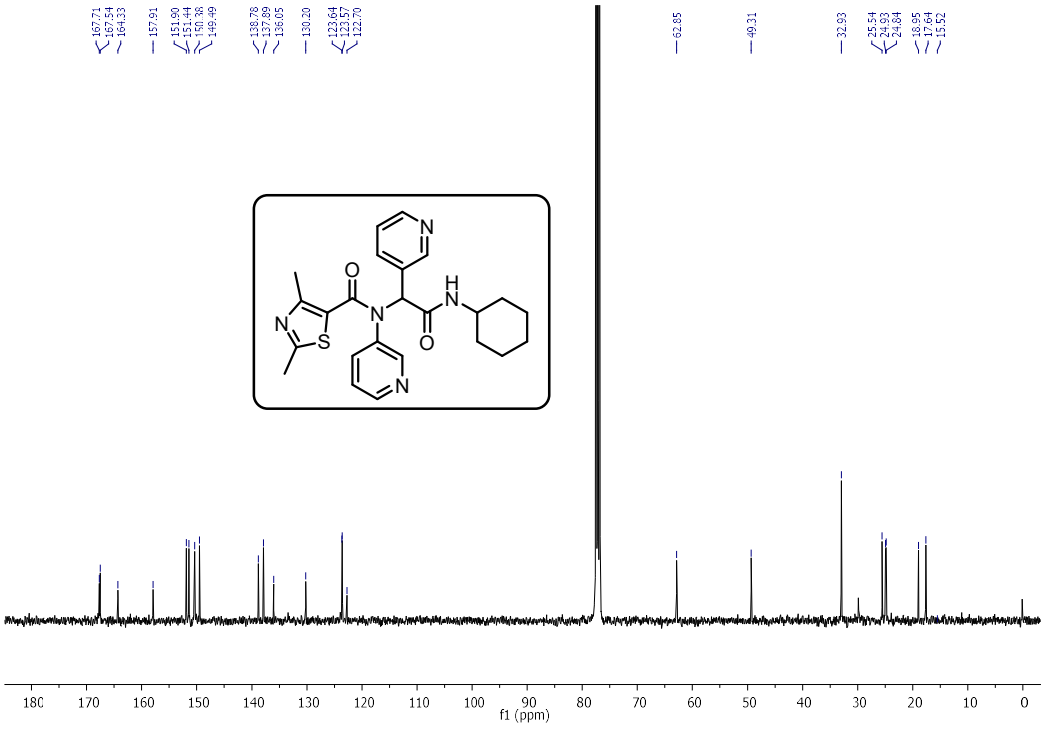
Microplate Alamar Blue Assay (MABA)

Antibacterial susceptibility testing was performed according to CLSI guidelines utilizing broth microdilution assay by using 96 well round bottom plate [1]. Stock solutions of the test compounds were prepared at a concentration of 10 mg/mL in dimethyl sulfoxide (DMSO) and stored at 0℃. Bacterial cultures were grown in 7H9OADC broth, and their optical density (OD) at 600 nm was measured, then diluted to achieve approximately 10^5 CFU/ml. The compounds were evaluated in concentrations ranging from 64 to 0.5 mg/L in a two-fold serial dilution, with 2.5 µL of each concentration added to the wells of a 96-well round-bottom microtiter plate. Subsequently, 97.5 µL of the bacterial suspension was added to each well containing the test compound, along with appropriate controls. The plates were incubated at 37°C for 48 hours, following which bacterial growth was measured and the minimum inhibitory concentration (MIC) was determined. The MIC is defined as the lowest concentration of the compound where there is no visible bacterial growth. For each compound, MIC determinations were carried out independently three times using duplicate samples.

3. ^{1}H, ^{13}C NMR, mass spectra of the compounds



^{1}H NMR of compound 16a



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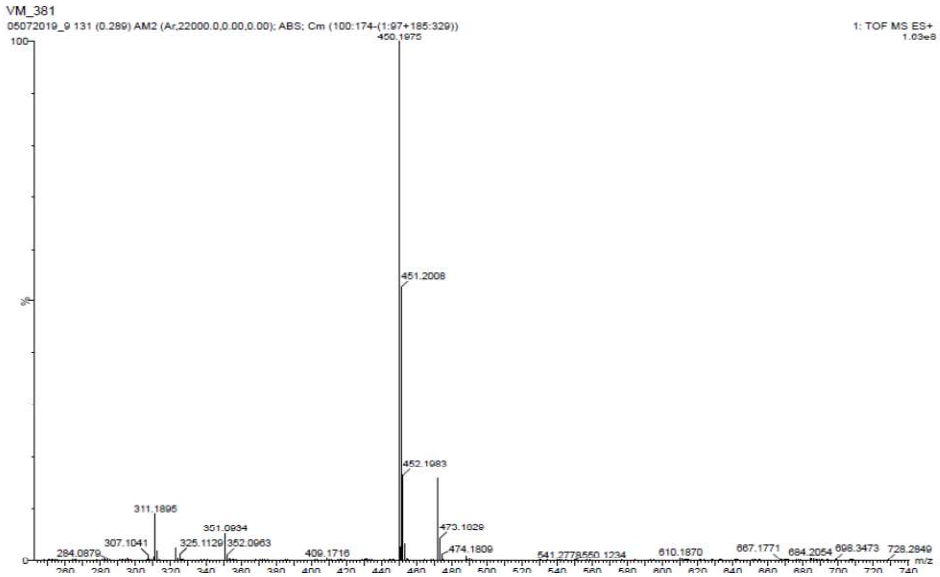
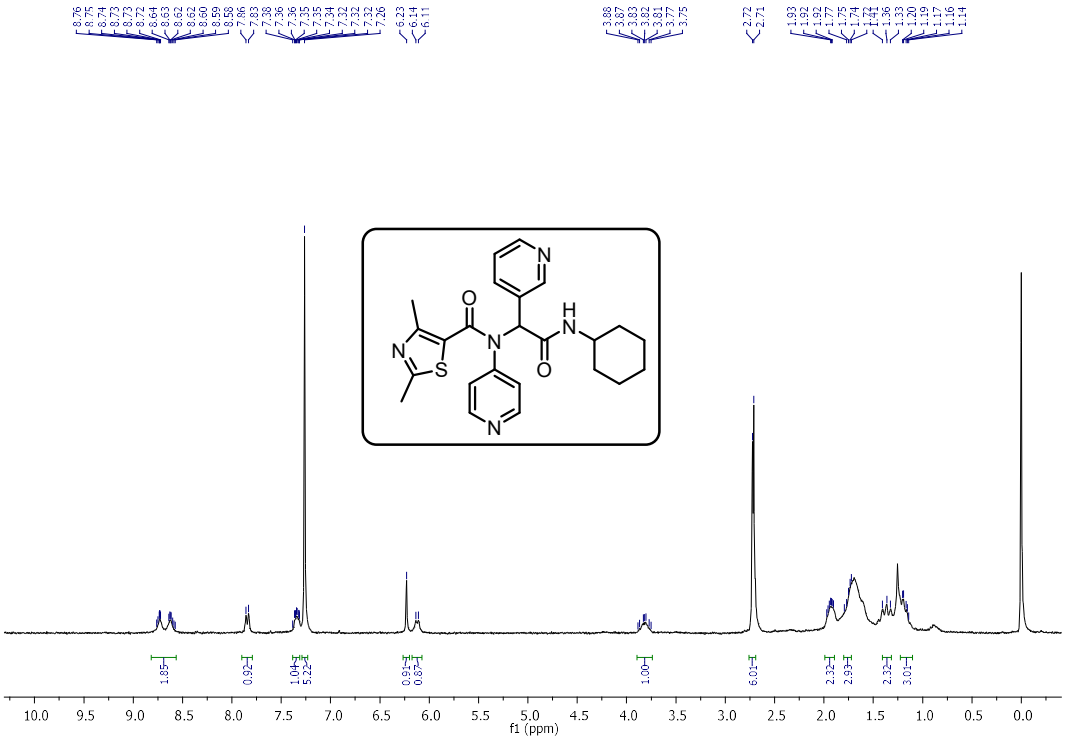


High Resolution Mass Spectra of compound 16a



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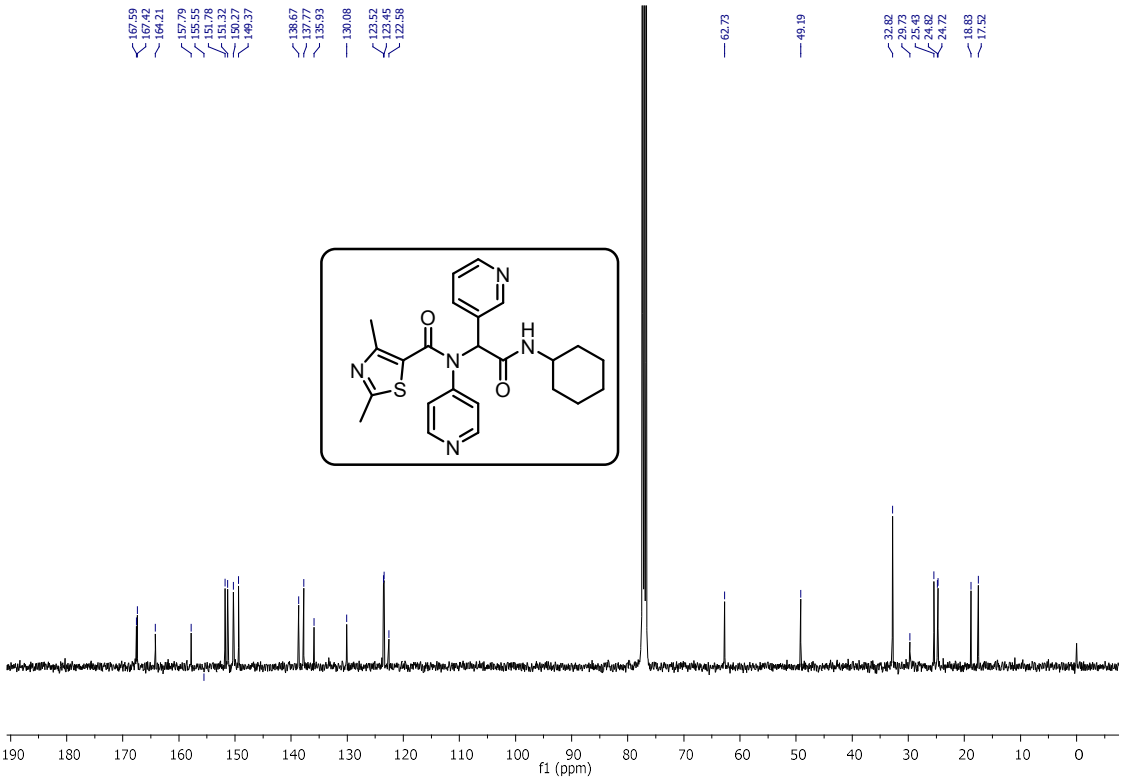

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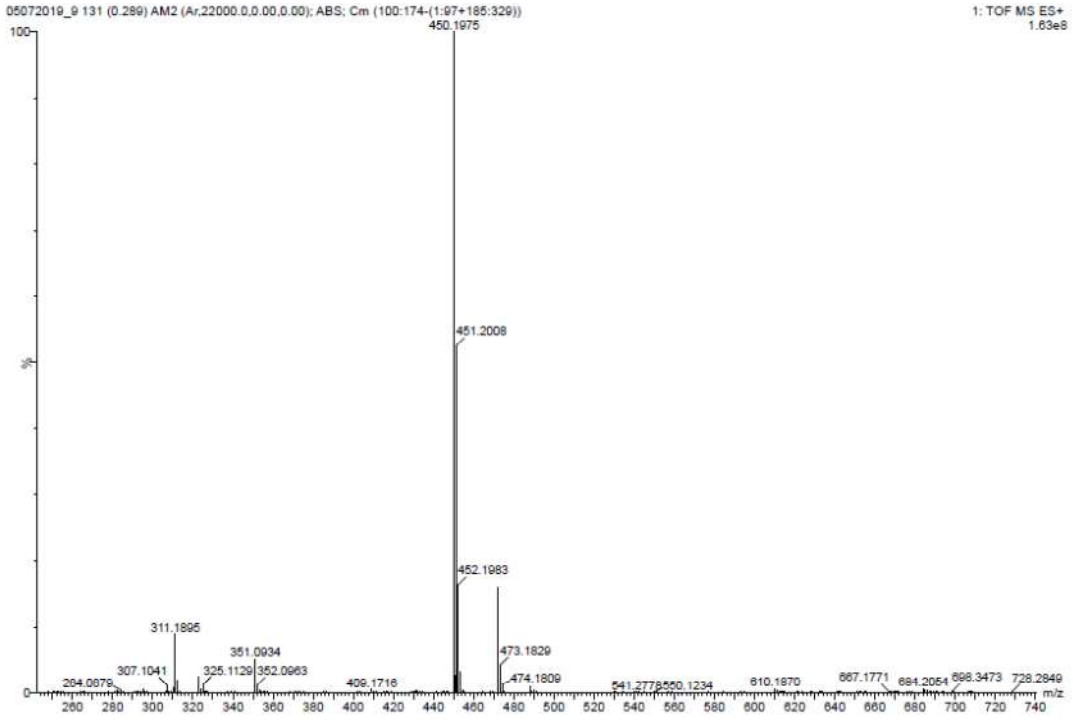

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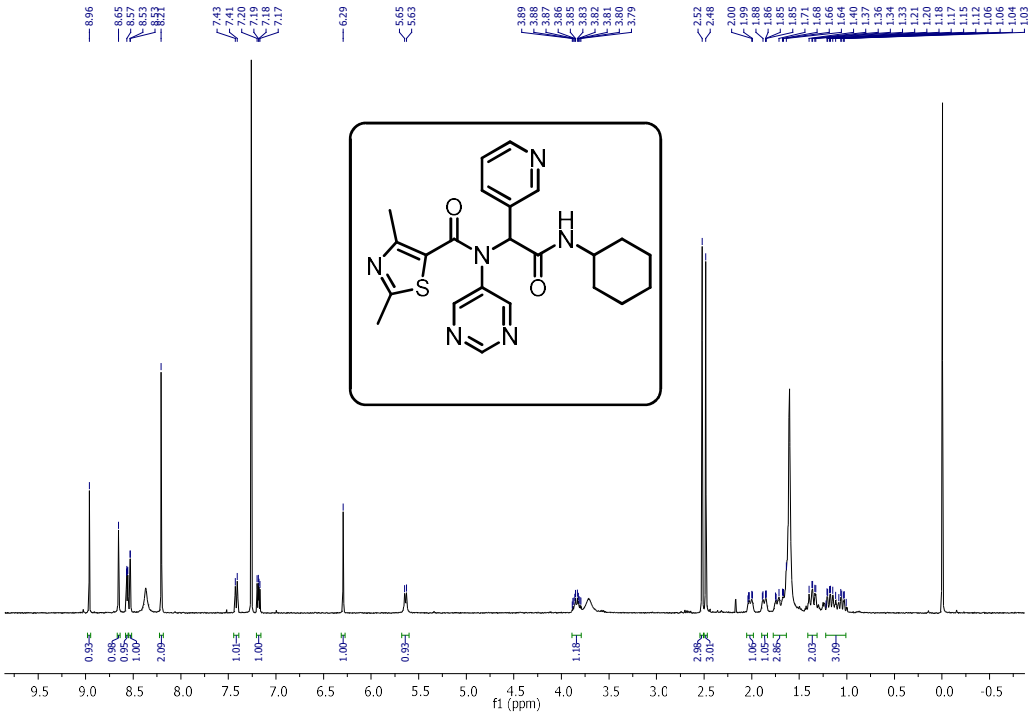
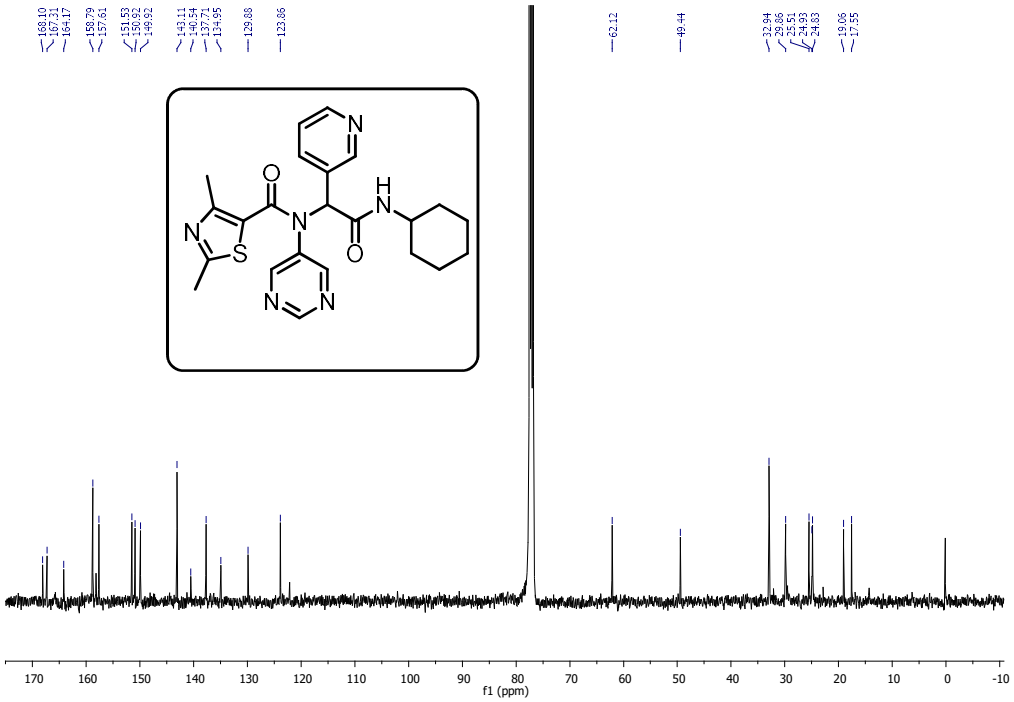


^{1}H NMR of compound 16c



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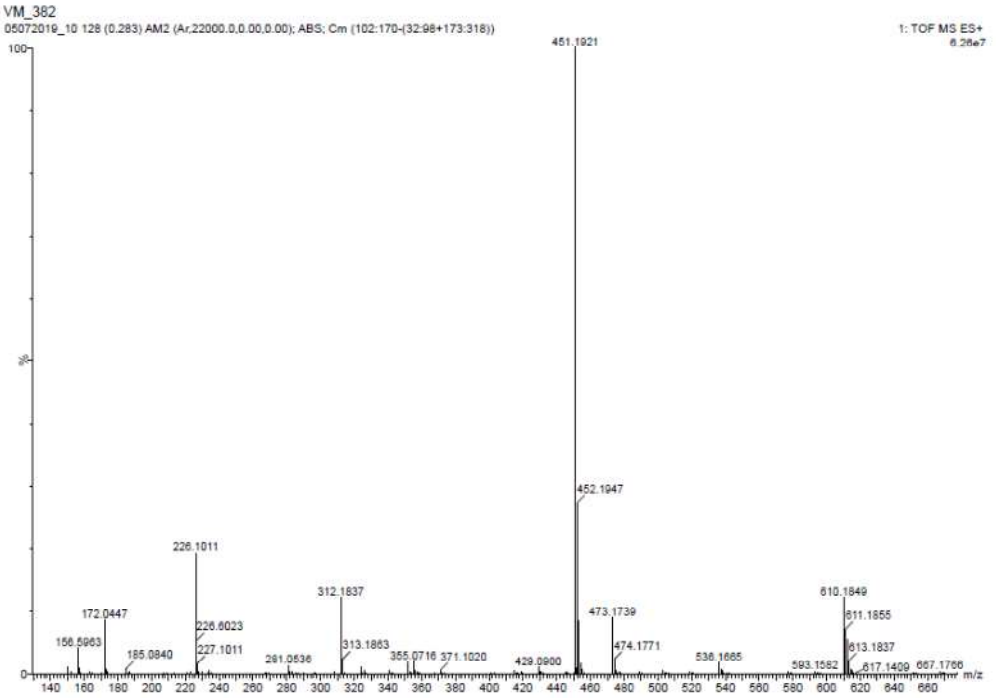

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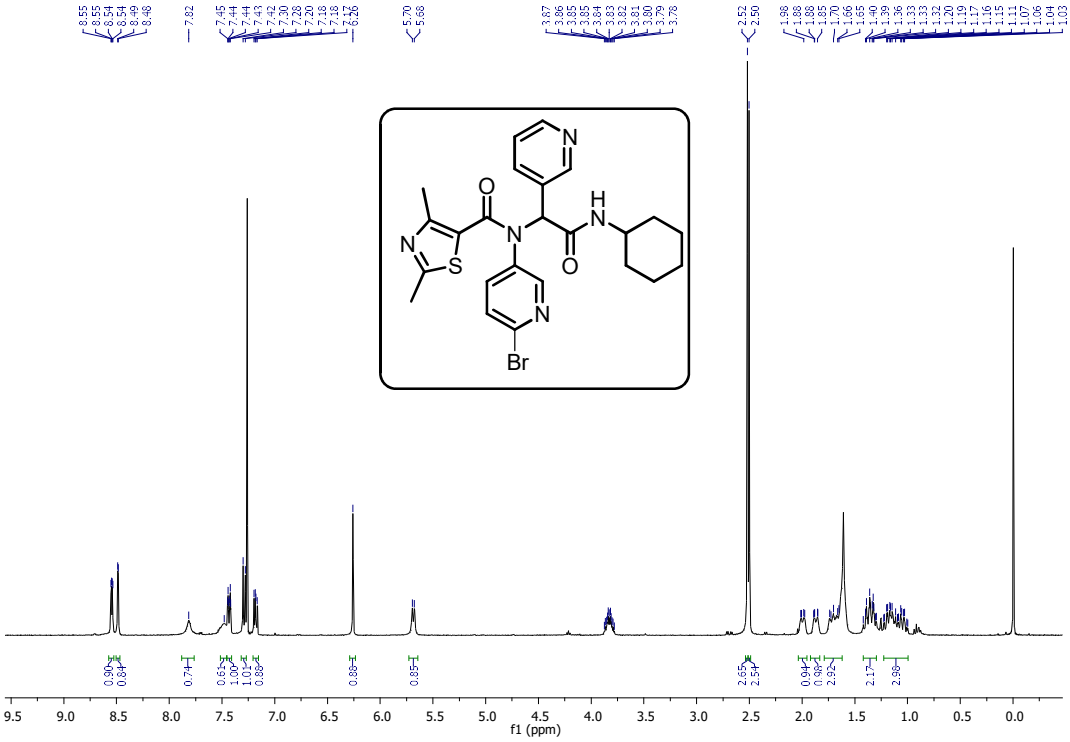

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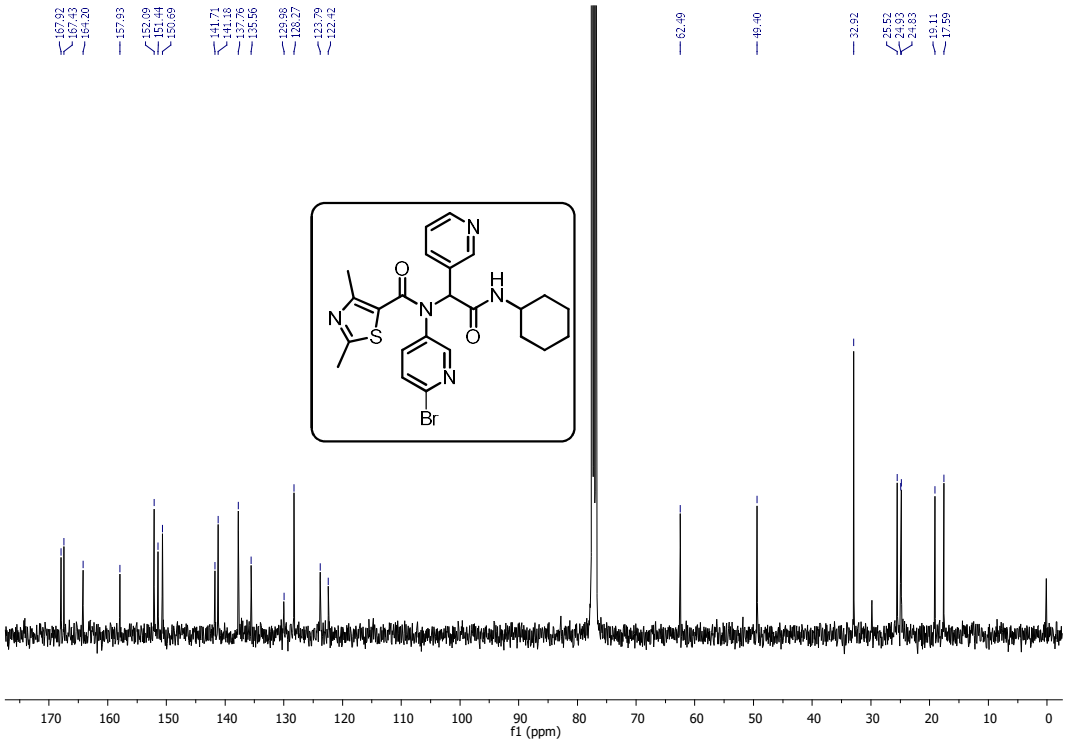
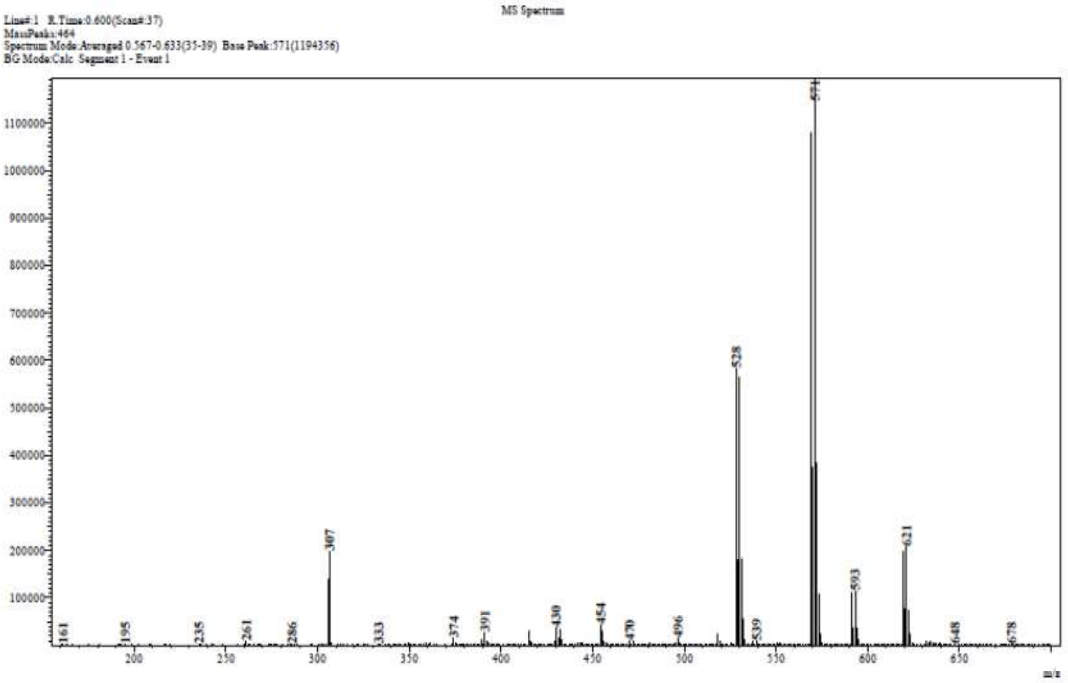
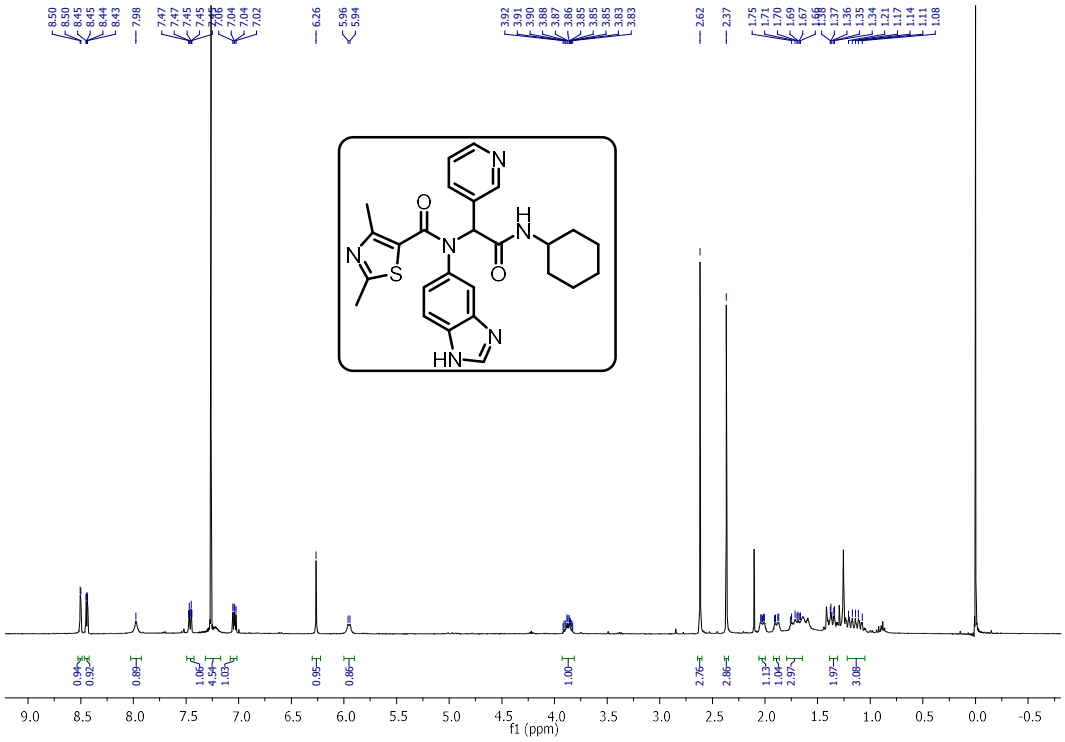


^{13}C NMR of compounds 16d



Electron Ionization Mass Spectra of compound 16d



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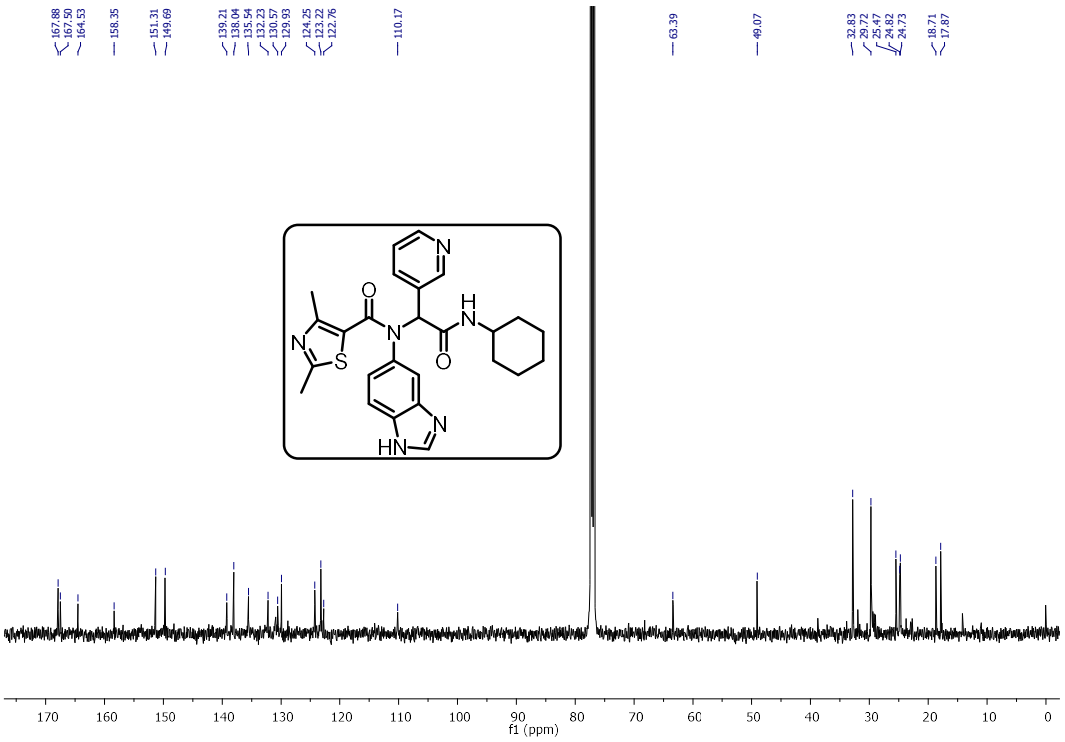

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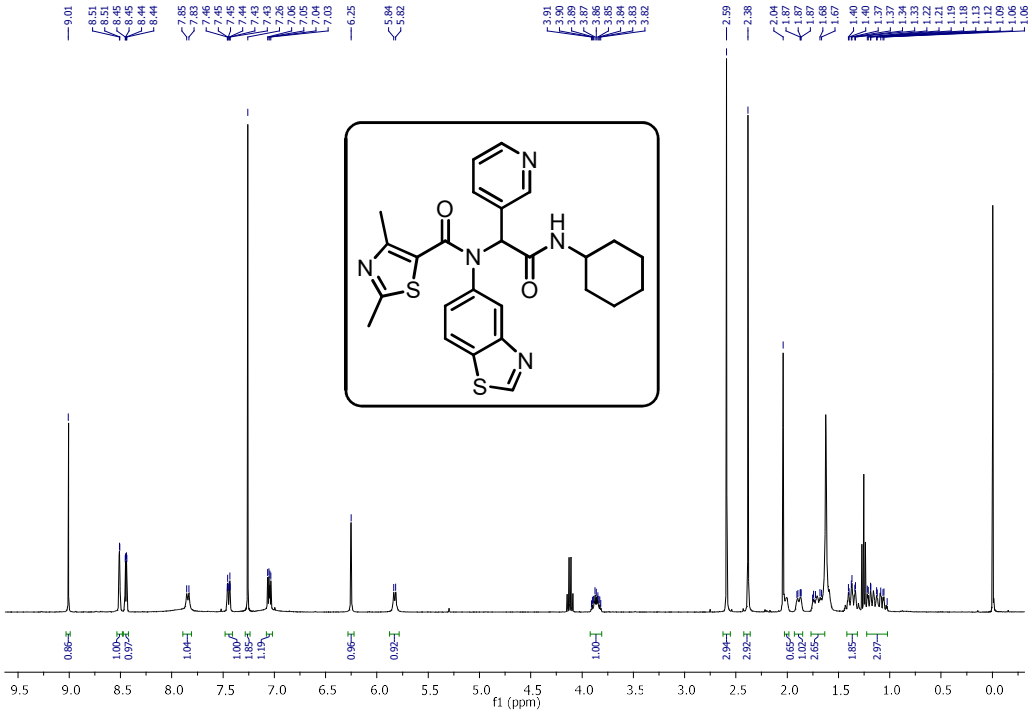
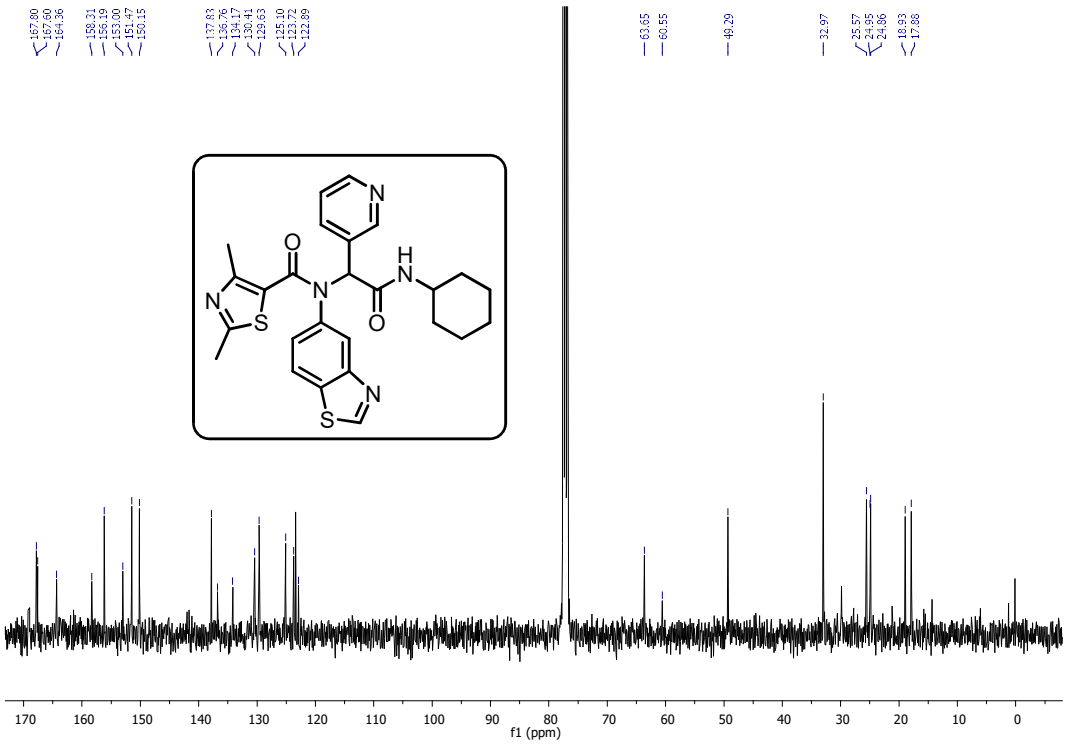


^{1}H NMR of compound 16f



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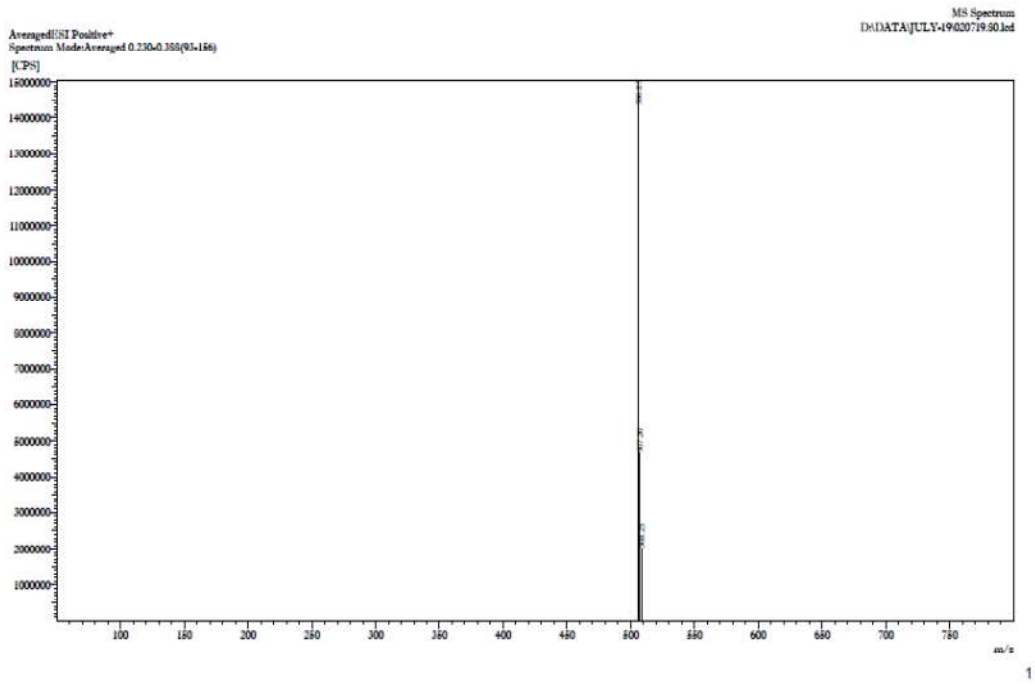
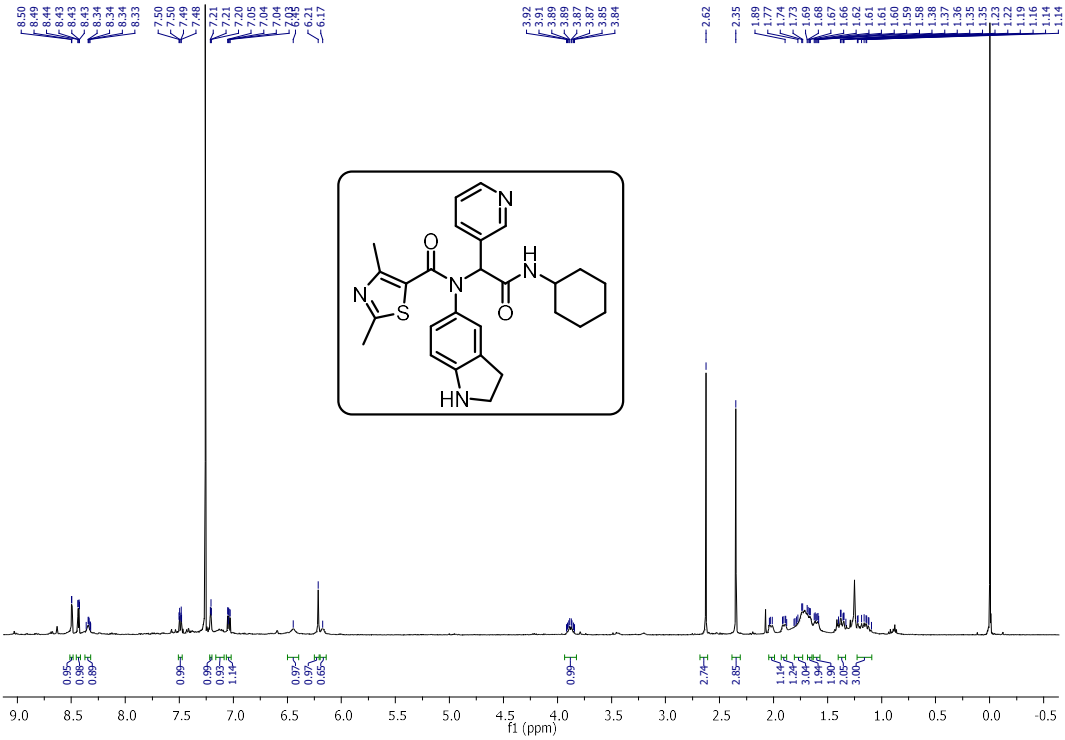


Electron Ionization Mass Spectra of compound 16f



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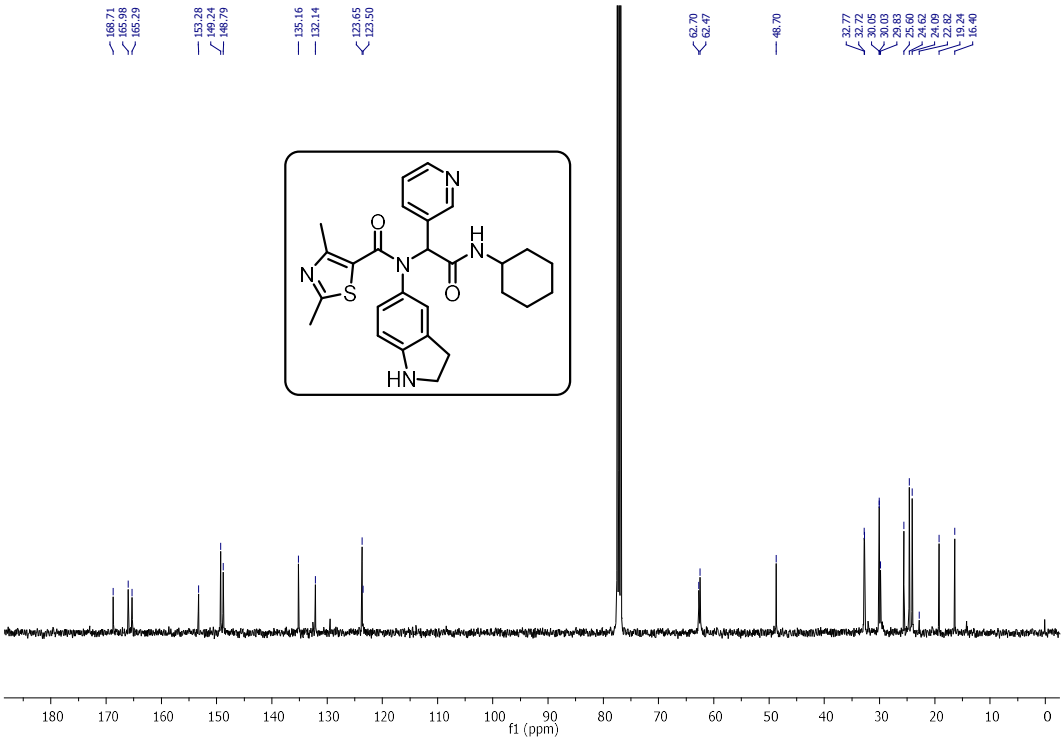

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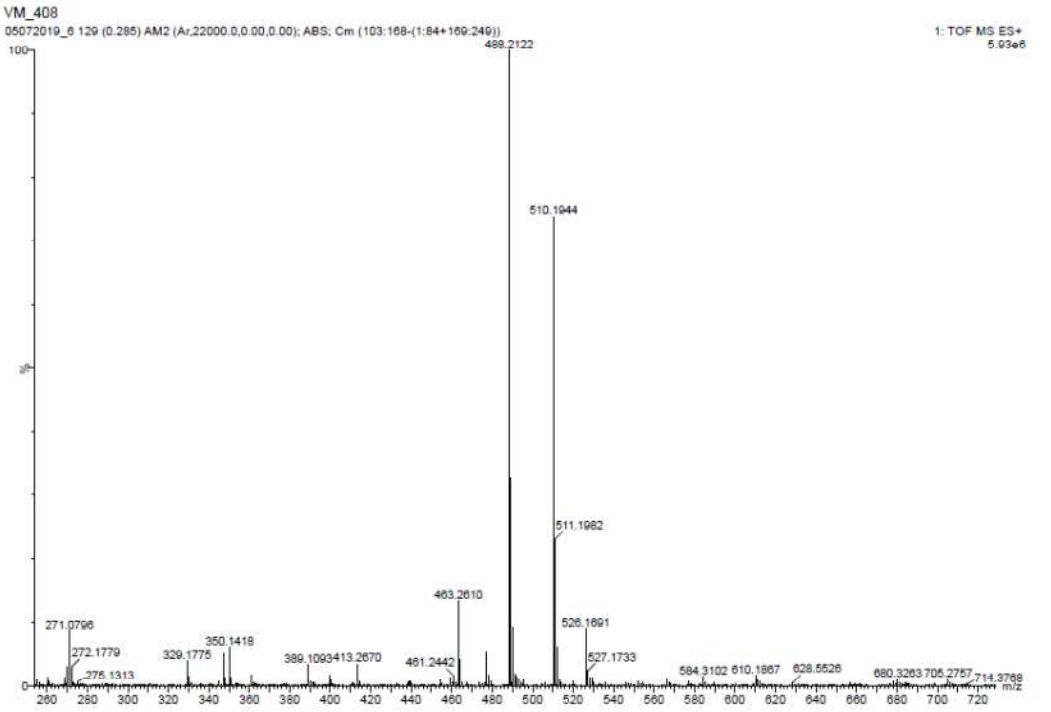
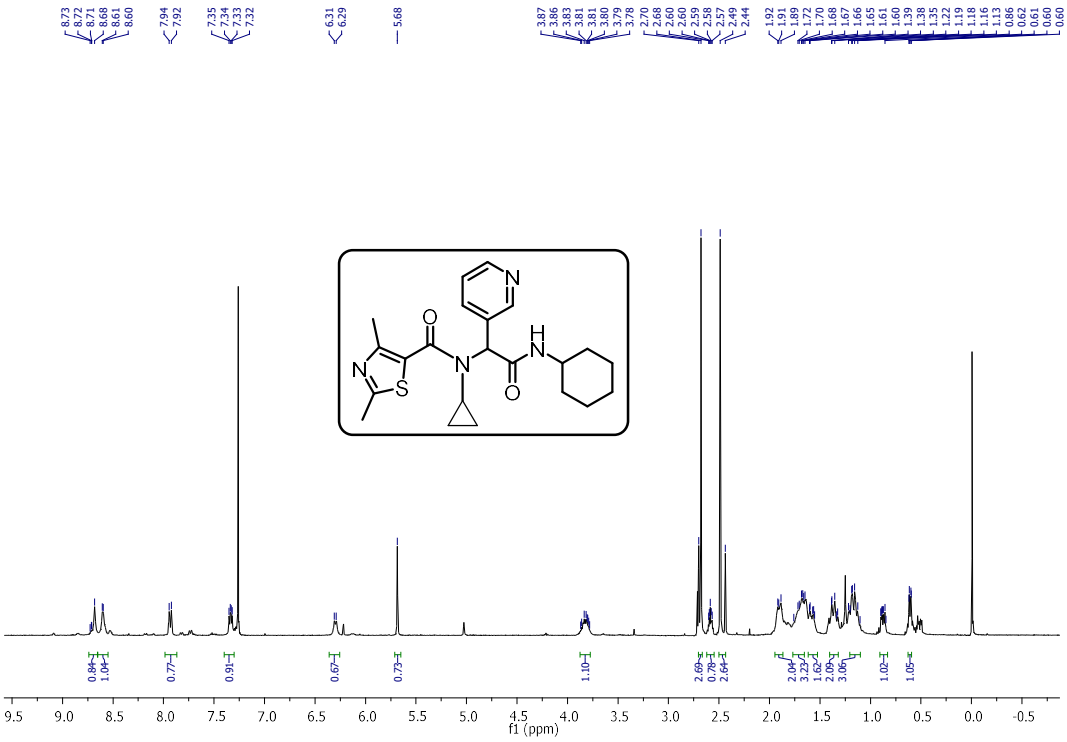


High Resolution Mass Spectra of compound 16g



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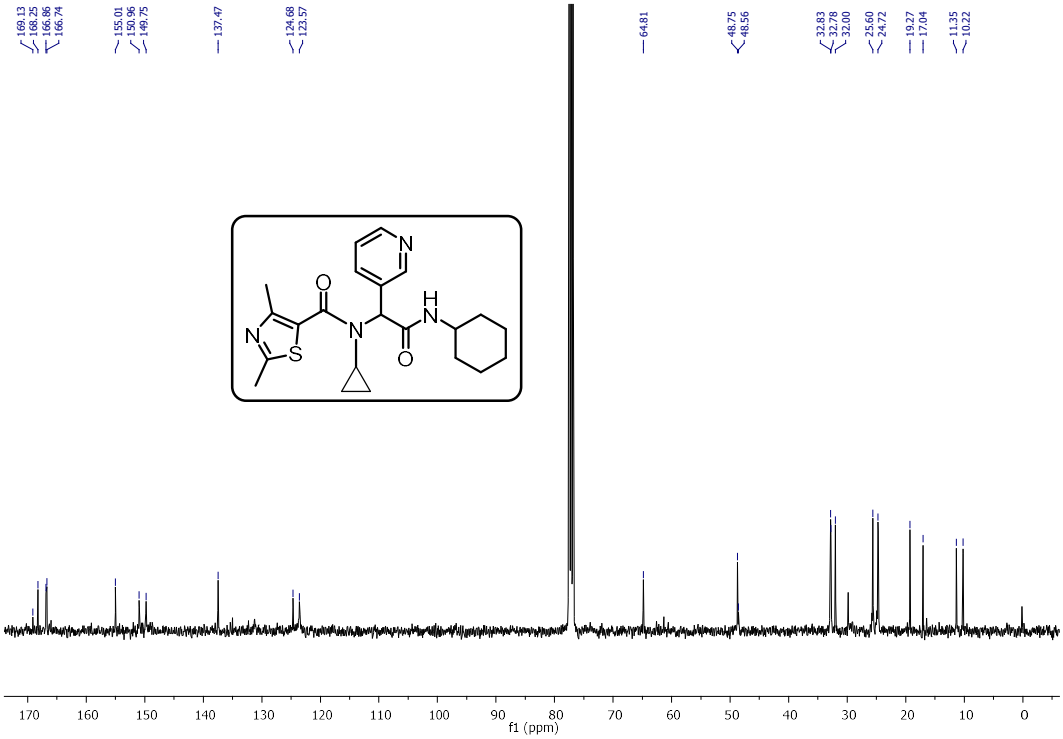

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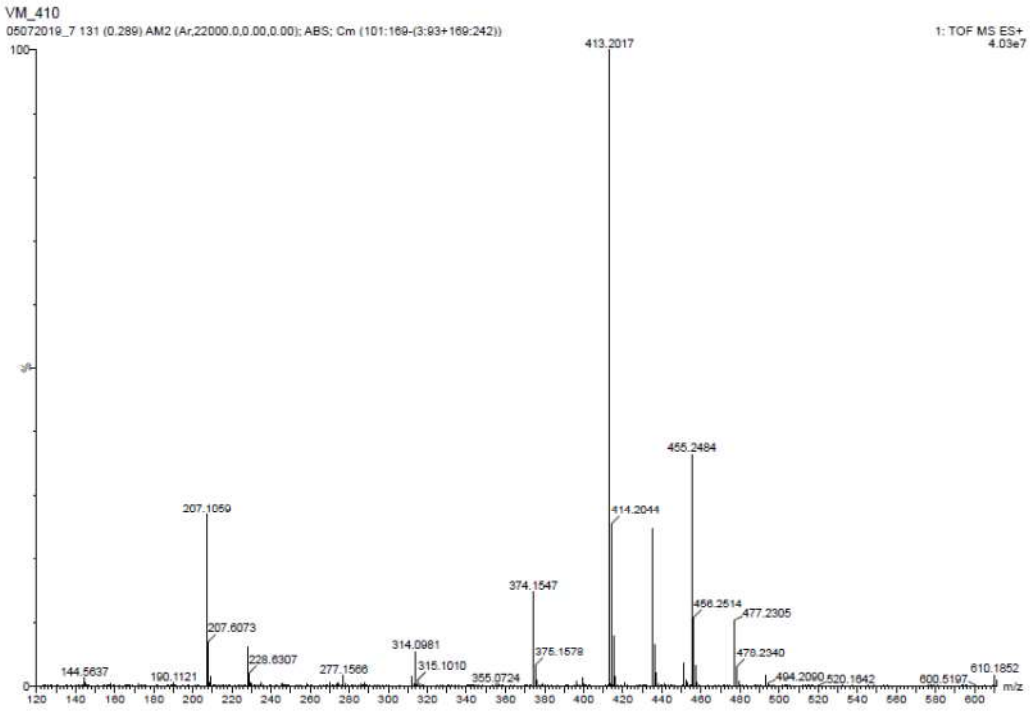

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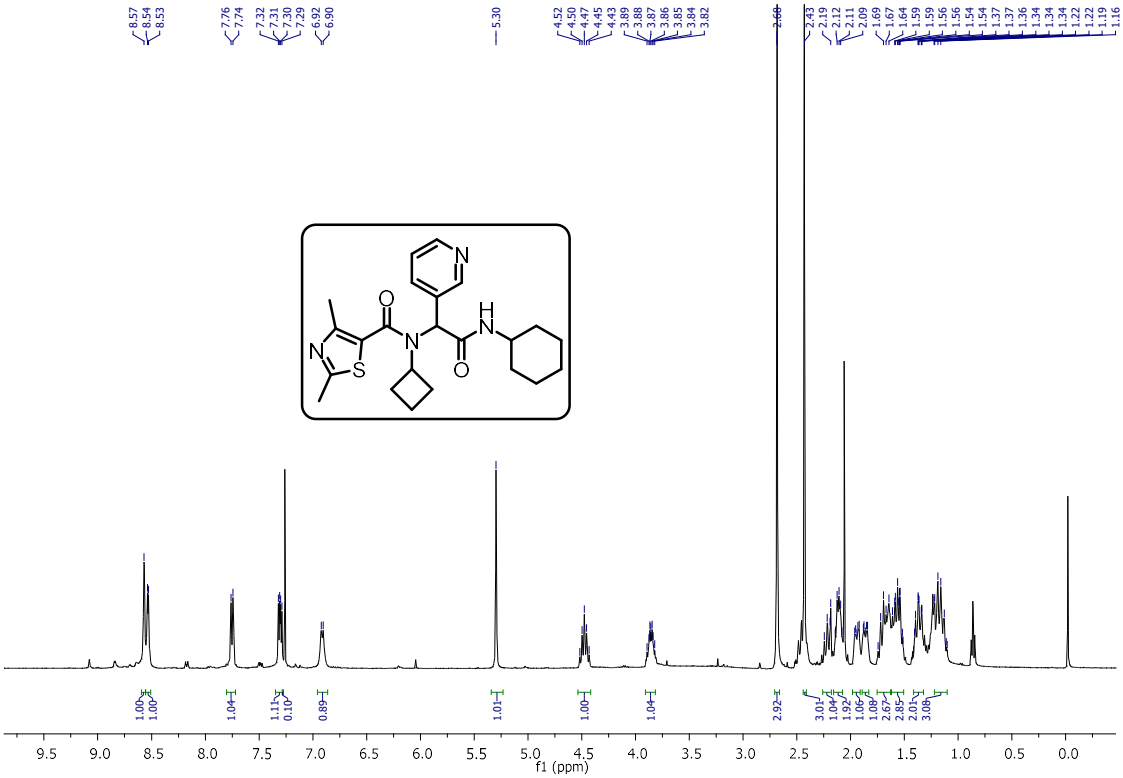
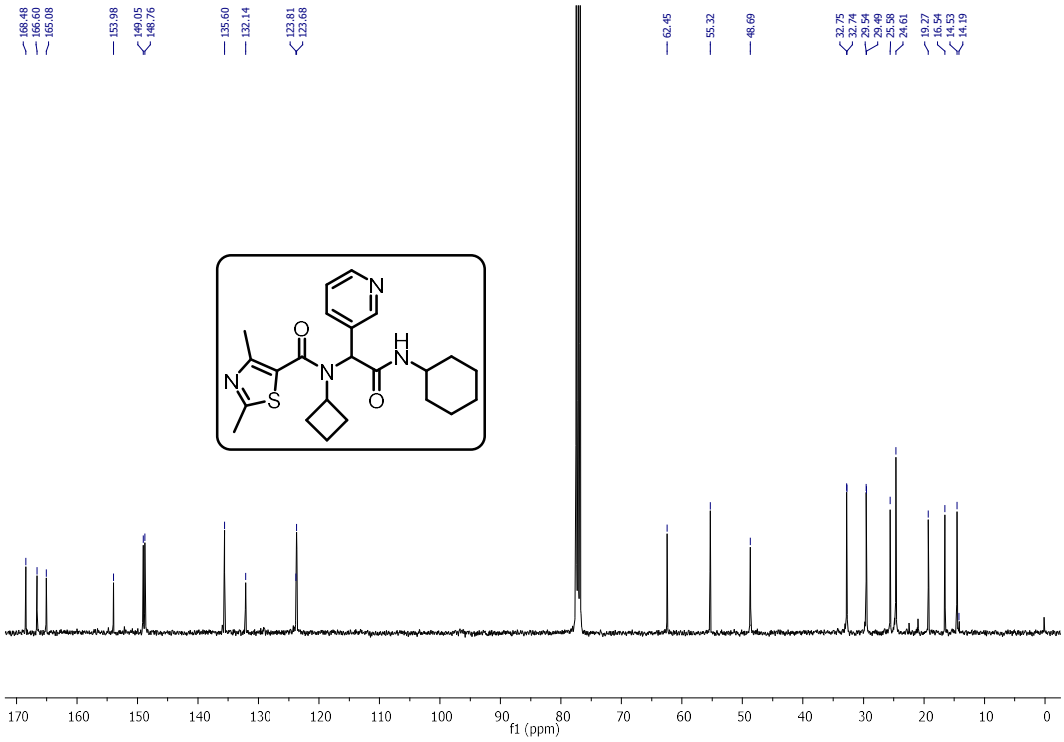


^{1}H NMR of compound 16i



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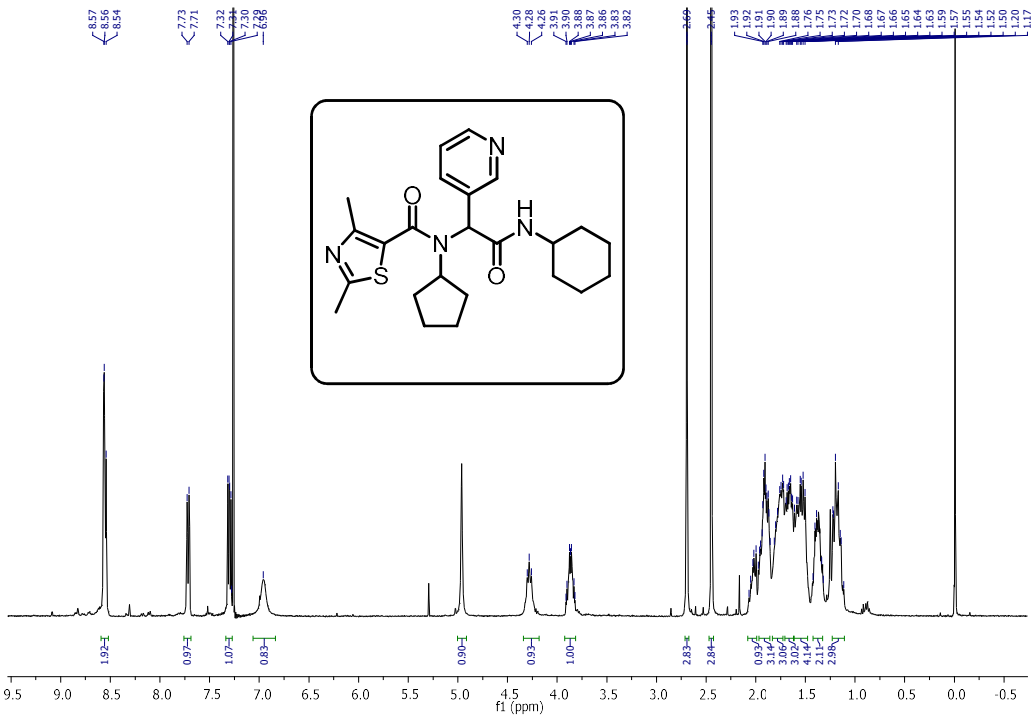

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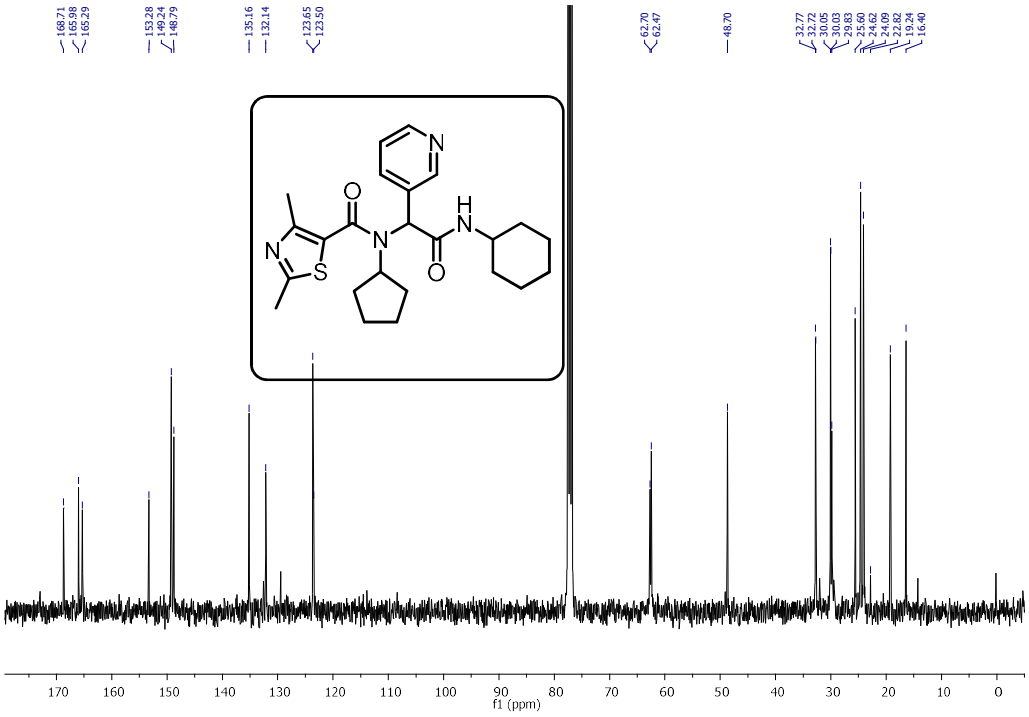
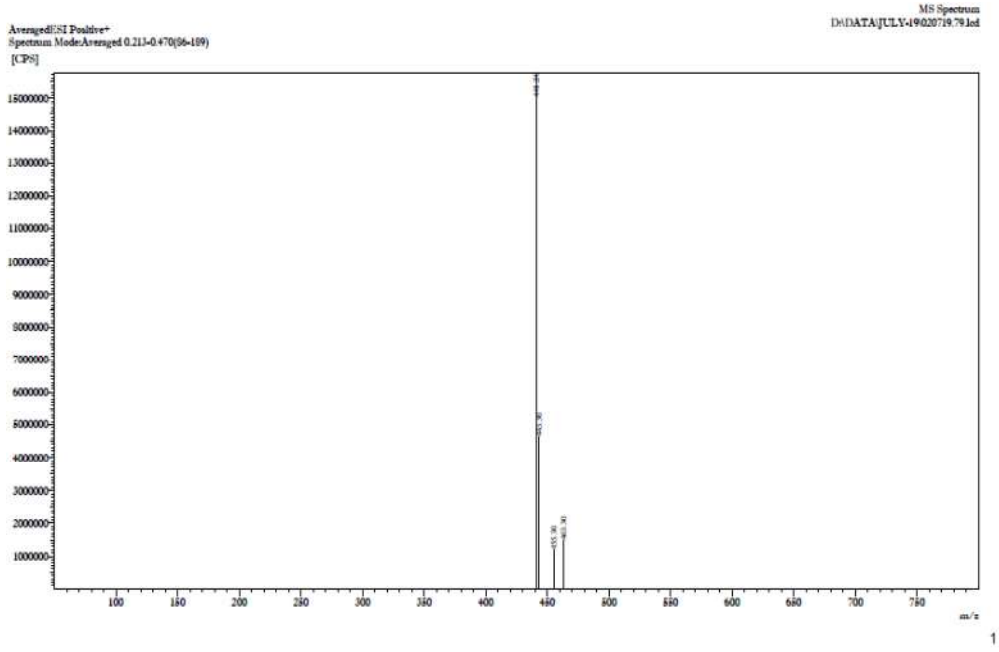
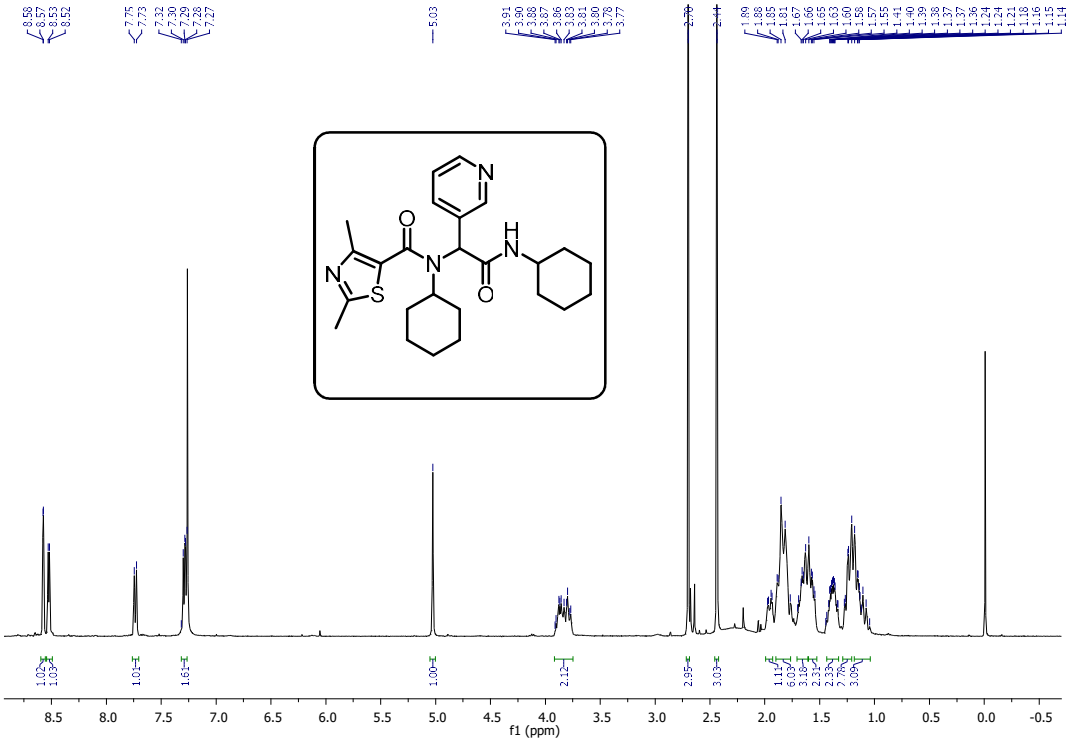


^{13}C NMR of compounds 16j



Electron Ionization Mass Spectra of compound 16j



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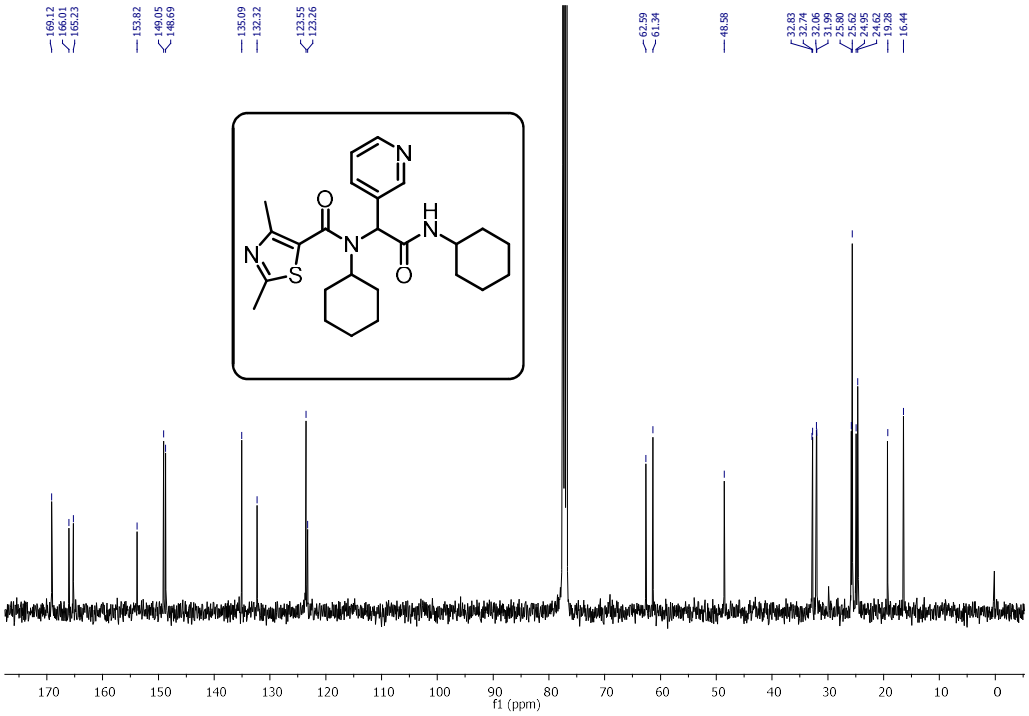

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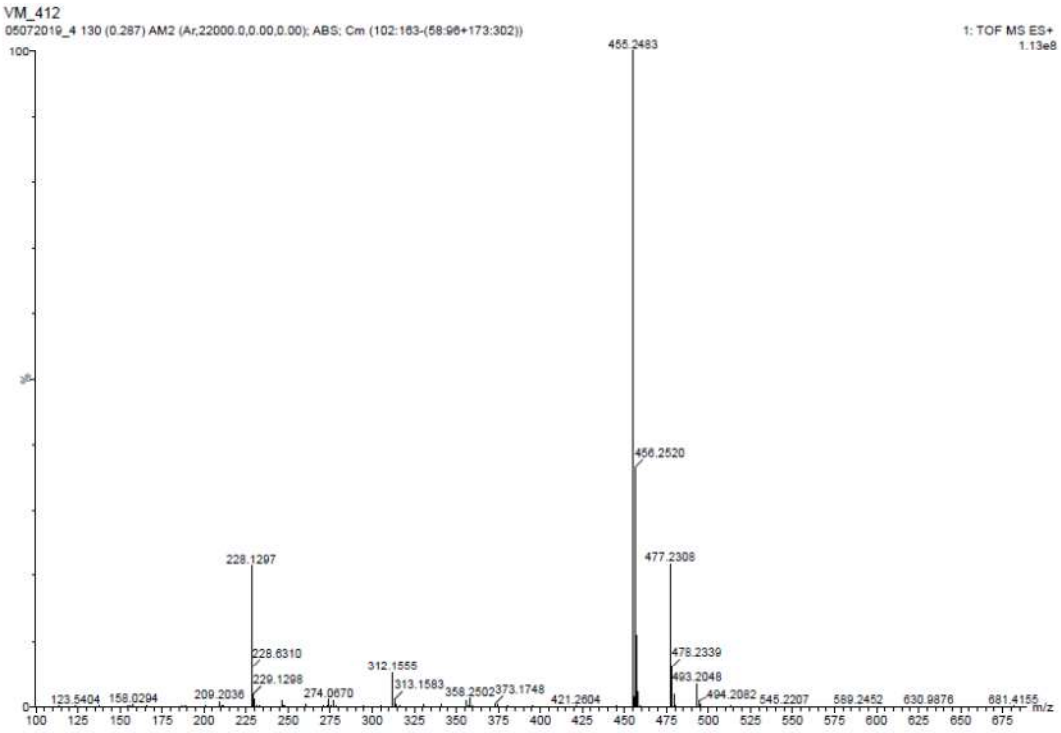

High Resolution Mass Spectra of compound 16k

4. References

1. G.L. Woods, B.A. Brown-Elliott, P.S. Conville, E.P. Desmond, G.S. Hall, G. Lin, G. E. Pfyffer, J.C. Ridderhof, S.H. Siddiqi, R.J. Wallace, N.G. Warren, F.G. Witebsky, Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes, second ed., Clinical and Laboratory Standards Institute, Wayne (PA), 2011. http://www.ncbi.nlm.nih.gov/books/NBK544374/.