**Supporting Information**

# Structural modification of zolpidem resulted potent antimicrobial activity in imidazo[1,2-*a*]pyridine/pyrimidine-1,2,3-triazoles

Rajkumar Reddyrajula [a], Udayakumar Dalimba [a*]

[a]Organic Chemistry Laboratory, Department of Chemistry, National Institute of Technology Karnataka, Surathkal, Srinivasanagar, Mangalore-575025, India.

[*]Corresponding author email: udayaravi80@gmail.com, udayakumar@nitk.ac.in

**Table of contents**



## 1. Materials and instruments

All the required reagents and solvents were purchased from Sigma Aldrich, Alfa-aesar, Merk and Spectrochem chemical companies, and were used as such without any purification. Silica gel (merck, 60-120 mesh) was used in column chromatography for purification of reaction products. Reaction was monitored with TLC (Merck KGaA) coated with 60 F254 silica gel and accomplished under UV light. Melting point was recorded on a Stuart SMP3 melting point instrument and is uncorrected. $^{1}$H-NMR and $^{13}$C-NMR spectra of synthesized compounds were recorded on Bruker (400 MHz: $^{1}$H-NMR, 100 MHz: $^{13}$C-NMR) spectrometer with TMS as an internal reference. The ESI mass spectra were recorded on waters micro mass Q-Tofmicrospectrometer with an ESI source. Elemental analysis was done using a Thermo electron corporation EA-112 series C, H, N, S analyzer.

**2. Antitubercular activity**

All the title compounds were screened for their antitubercular activity against *M. tuberculosis* using microplate alamar blue assay (MABA) method, which is non-toxic and uses a thermally stable cell permeable reagent (resarzurin). Briefly, to reduce the medium evaporation in the test wells while incubation, 200 µL of sterile deionized water was added to all test wells of sterile 96-well plates. The target compounds and standard drugs were prepared in two-fold dilutions (50.0, 25.0, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.4 µg/mL) by dissolving in DMSO. 100 µL of the Middlebrook 7H9 broth (MB) with OADC(oleic acid, albumin, dextrose and catalase; Difco) and 100 µL of *M. tuberculosis* H37Rv (ATCC27294) inoculum was supplemented into 7H9 broth wells containing 10-fold serial dilutions of drugs per mL. The tubes were covered with para film and incubated at 37 °C for five days. To this, 10% tween 80 (1:1 mixture) and 25µL of a freshly prepared alamar blue reagent was added. Then incubated for further 24 hours. The minimal inhibition concentration (MIC) was defined as the lowest concentration of a compound, which prevented a visible growth of bacteria by colour change from blue to pink. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

**3. Antibacterial activity**

The final compounds were evaluated for antibacterial activity against several bacterial strains using the disc diffusion method by measuring the zone of inhibition. Three bacterial strains namely *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were cultivated in brain heart infusion agar medium for 24 h. The culture suspensions were prepared and adjusted by comparing against 0.5 McFarland turbidity standard tubes. The hollow tube of 5 mm diameter was taken and heated. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate. All the compounds were dissolved in DMSO and appropriate dilutions (75, 50, 25 and 10 µL) were made with the help of micropipette and added to respective wells. DMSO was used as a solvent and as control. After the inoculation of organism and compound, the petri plates were incubated for 18-24 h at 37 ˚C. Inhibition zones formed on the medium were evaluated in millimeter (mm). The negative solvent control (DMSO) did not show any antimicrobial activity. Studies were performed in triplicate and the average reading was taken. Inhibition zones were compared with those of the reference discs.

**4. Antifungal activity.**

The final compounds were screened for antifungal activity against *c. albicans*, *a. niger* and *a. flavus* fungal strains using the disc diffusion method by measuring the zone of inhibition. All these fungal strains were cultivated in Sabouraud agar medium for 24 h. Similar protocol was followed as antibacterial study. For Facultative anaerobes, incubate plates were taken in the Co2 Jar and kept the jar in the incubator at 37 ºC whereas for Anaerobic organisms, incubate plates were kept in the Anaerobic jar and kept the jar in the incubator at 37 ºC. As like bacterial study, the plates were then examined for the presence of zones of inhibition and the results were recorded.

**5. I*n vitro* cytotoxicity**

The VERO cell lines (African green monkey kidney: Cat. no. 11965-092) were purchased from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines were seeded in 96 well flat-bottom microtiter plates accommodating DMEM media which was supplemented with 10% heat inactivated fetal calf serum (FBS) and also added 1% Antibiotic-Antimycotic 100X solution. The cells were incubated at 37 °C (95% humidity and 5% $CO_2$) for 24 hours. The test compounds were prepared in different concentrations (500, 250, 125, 62.5, 31.25 µg/mL) by dissolving in distilled DMSO. The cells were then treated to different concentrations of drug and were incubated for another 72 hours. The cells in well were washed twice with phosphate buffer solution, stock solution of MTT (20 µL, 5mg/mL in sterile PBS) was added to each well and cells were incubated for additional 4h at 5% $CO_2$ atmosphere. After the supernatant was flicked off from the incubator, 100 µL of dimethyl sulfoxide was added to dissolve the formazan crystals. Absorbance of wells containing cells and blanks was recorded with a 570 nm using micro plate reader. Percentage of growth inhibition was calculated from below equation. IC50 value of the compounds was calculated by graph Pad Prism Version5.1.

$$\% \text{ Growth Inhibition} = \frac{\text{Mean OD of test compounds}}{\text{Mean OD of Negative control}} \times 100$$

## 6. Molecular docking

The 3D crystal structure of InhA (PDB Id: 4TZK) of M. tuberculosis were obtained from Protein Data Bank for docking explorations. The proteins for docking were prepared by removing the co-crystallized ligand, heteroatoms and water molecules etc. The molecules were docked within the active site of InhA using Auto-dock Vina 1.1.2 software.

**Table S1. Docking score and interacting amino acid residues of the active compounds.**

| Comp. | 4TZK | |
|---|---|---|
| | **Docking score Kcal/mol** | **Interactions** |
| **4a** | -9.6 | Lys 165, Tyr 158, Phe 149 |
| **4b** | -9.8 | Lys 165, Tyr 158, Phe 149, Ser 94 |
| **4c** | -9.2 | Tyr 158, Lys 165, Gly 96, Leu 197, Ala 198 |
| **4e** | -9.8 | Lys 165, Tyr 158, Ser 94, Ser 20 |
| **4f** | -9.9 | Lys 165, Phe 149, Gly 104 |
| **4h** | -9.5 | Tyr 158, Phe 149, Ser 194 |
| **4j** | -9.6 | Tyr 158, Phe 149, Gly 104, Ser 94 |
| **4l** | -9.0 | Lys 165, Tyr 158, Phe 149, Gly 104, Ser 94 |
| **8a** | -9.4 | Tyr 158, Gly 96, Val 65 |
| **8b** | -9.4 | Tyr 158, Phe 149, Val 65 |
| **8c** | -9.4 | Ala 198, Tyr 158, Gly 96, Thr 17 |
| **8d** | -9.2 | Tyr 158, Gly 96 |
| **8e** | -10.1 | Thr 196, Ser 20 |
| **8h** | -9.9 | Phe 149, Lys 165 |
| **8j** | -9.3 | Lys 165, Tyr 158, Phe 149, Ser 94 |
| **8k** | -9.4 | Thr 196, Tyr 158, Phe 149, Ser 20 |
| **13a** | -10.2 | Tyr 158, Phe 149, Thr 17 |
| **13b** | -10.4 | Ile 194, Tyr 158 |
| **13f** | -10.0 | Tyr 158, Thr 17 |
| **13h** | -10.1 | Ile 194, Tyr 158 |

## 7. Representative NMR and ESI-Mass spectra of compounds.

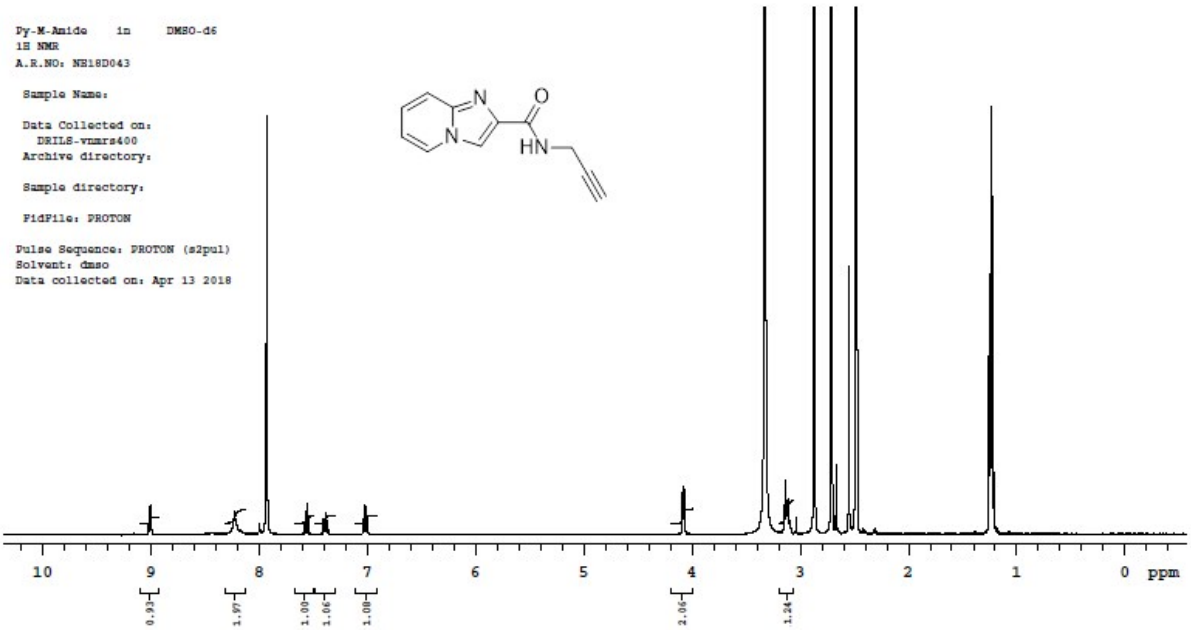


**Figure S1**. [1]H-NMR Spectrum of Compound **3.**

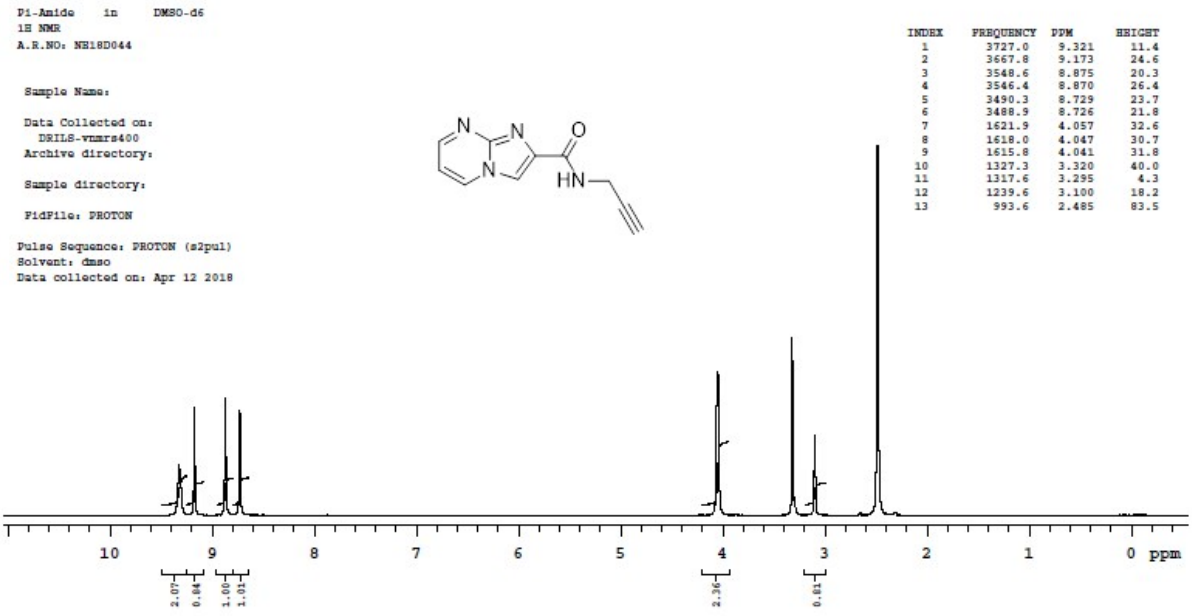


**Figure S2**. [1]H-NMR Spectrum of Compound **3a.**

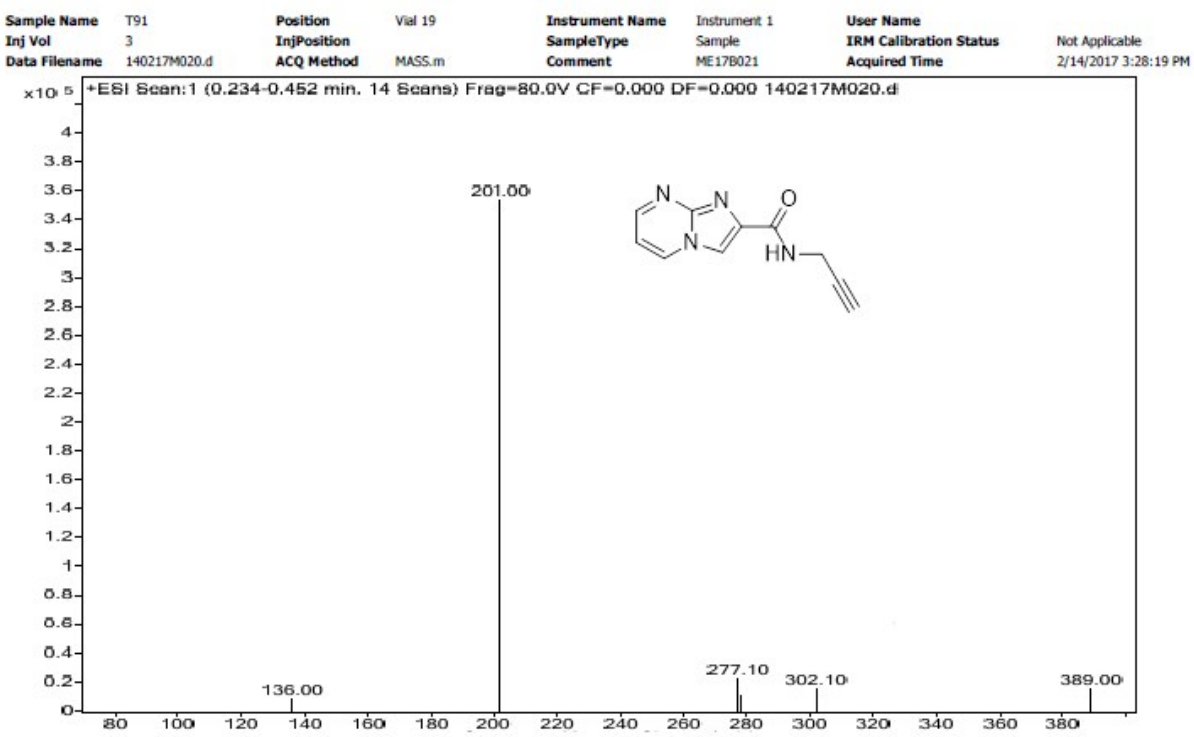


**Figure S3**. ESI-MS Spectrum of Compound **3a.**

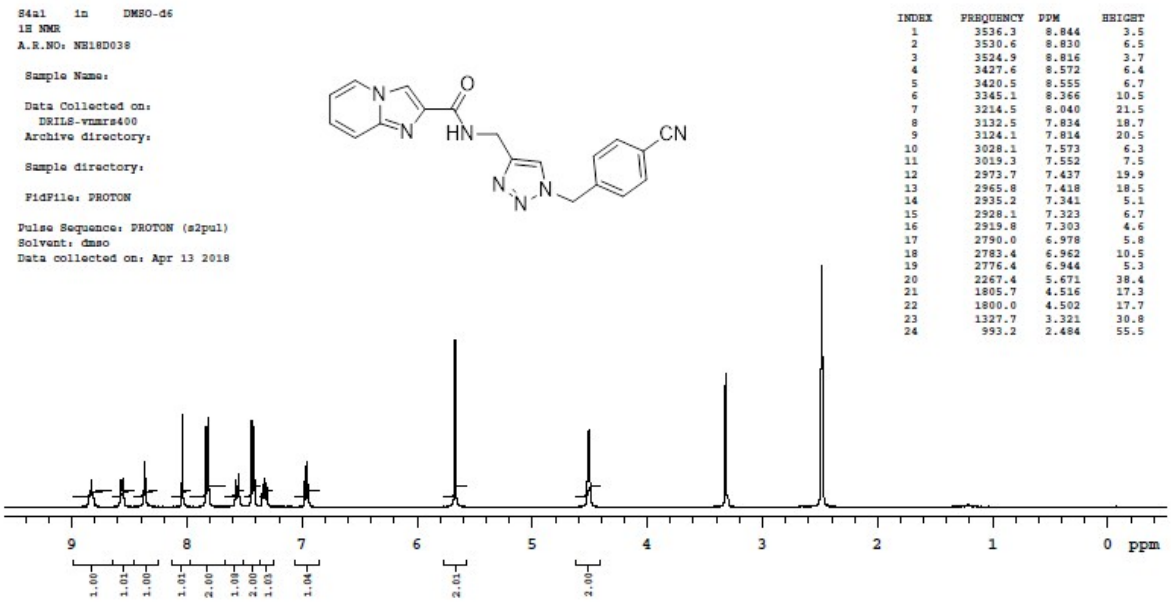


**Figure S4**. $^{1}$H-NMR Spectrum of Compound **4a.**

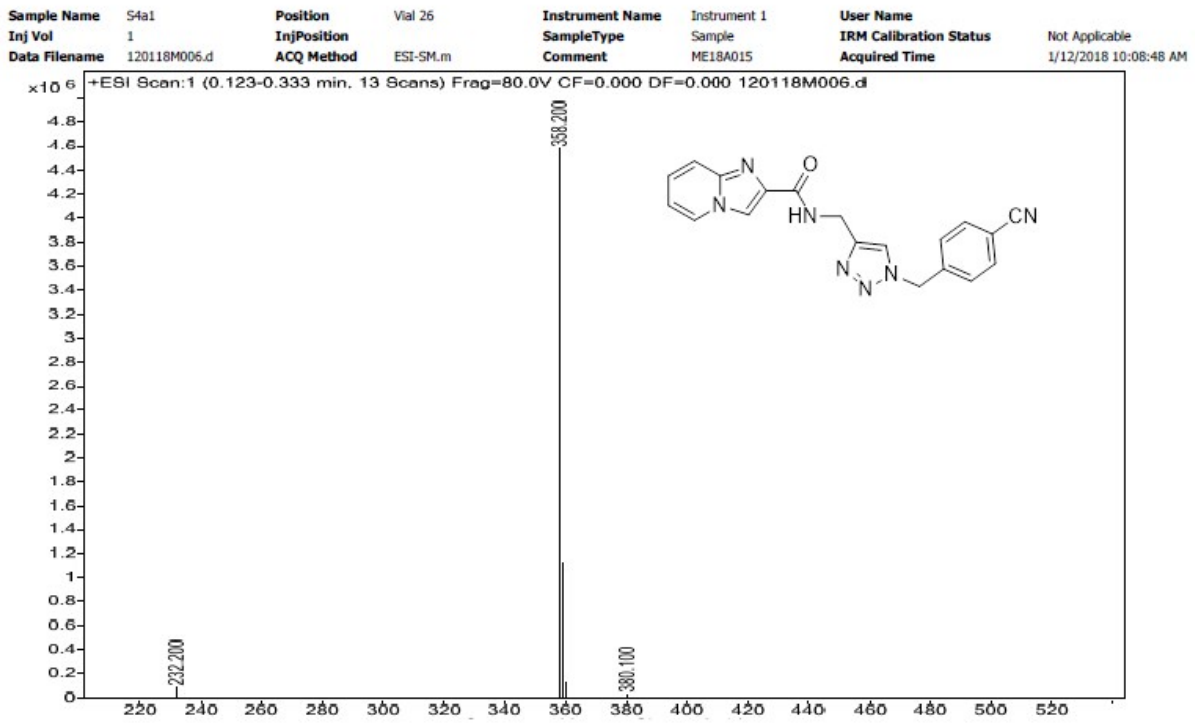


**Figure S5**. ESI-MS Spectrum of Compound **4a.**

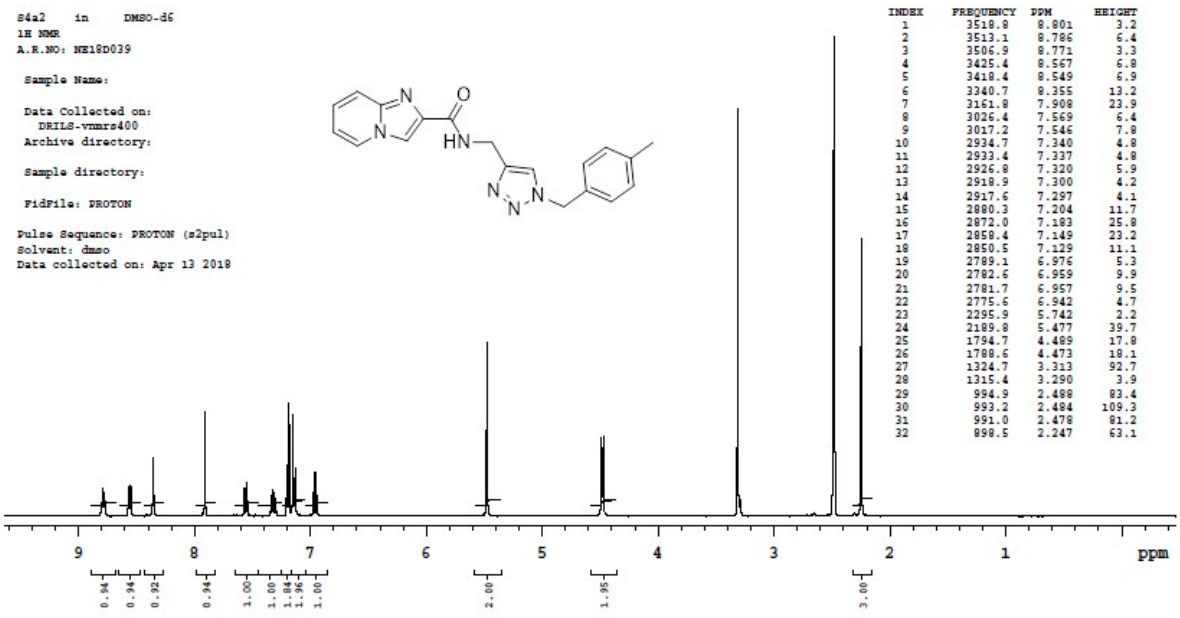


**Figure S6**. [1]H-NMR Spectrum of Compound **4b.**

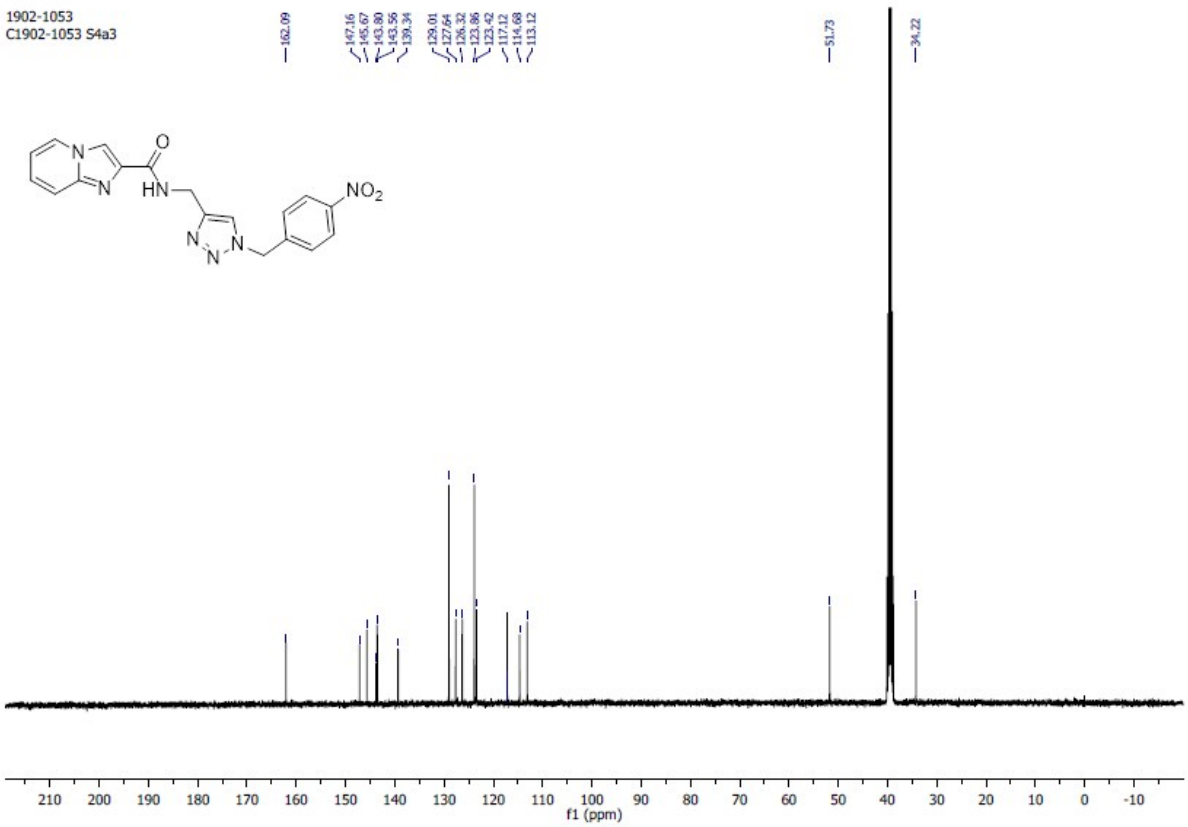


**Figure S7**. $^{13}$C-NMR Spectrum of Compound **4c.**

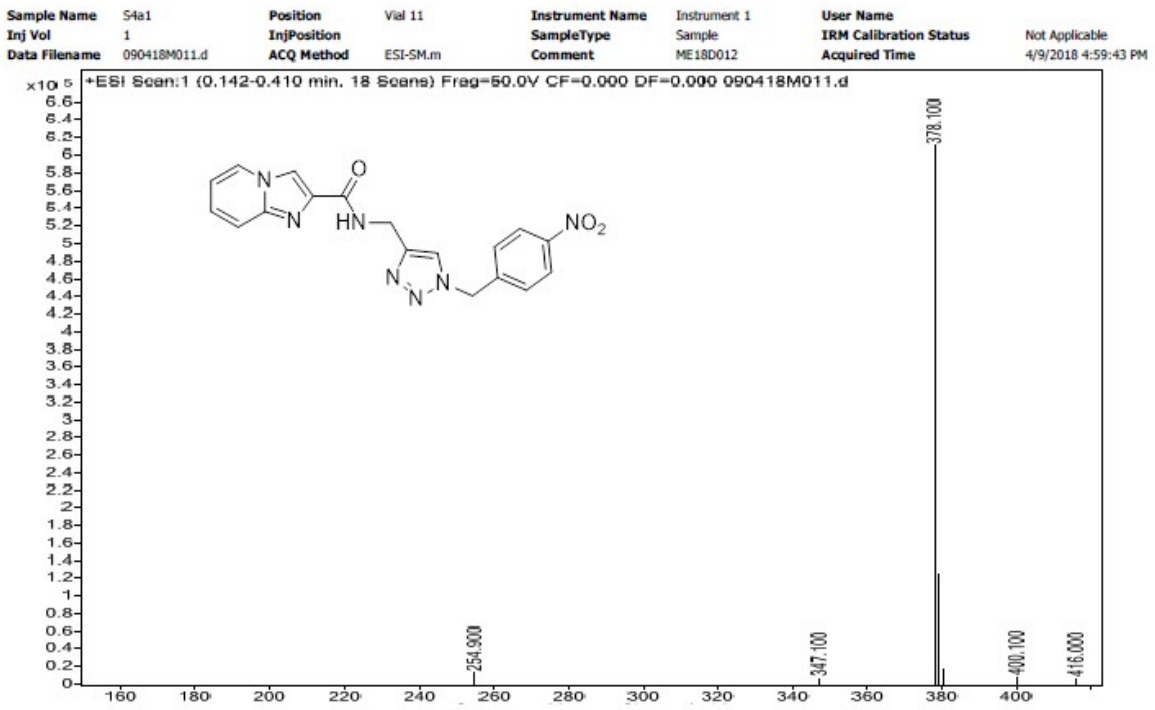


**Figure S8**. ESI-MS Spectrum of Compound **4c.**

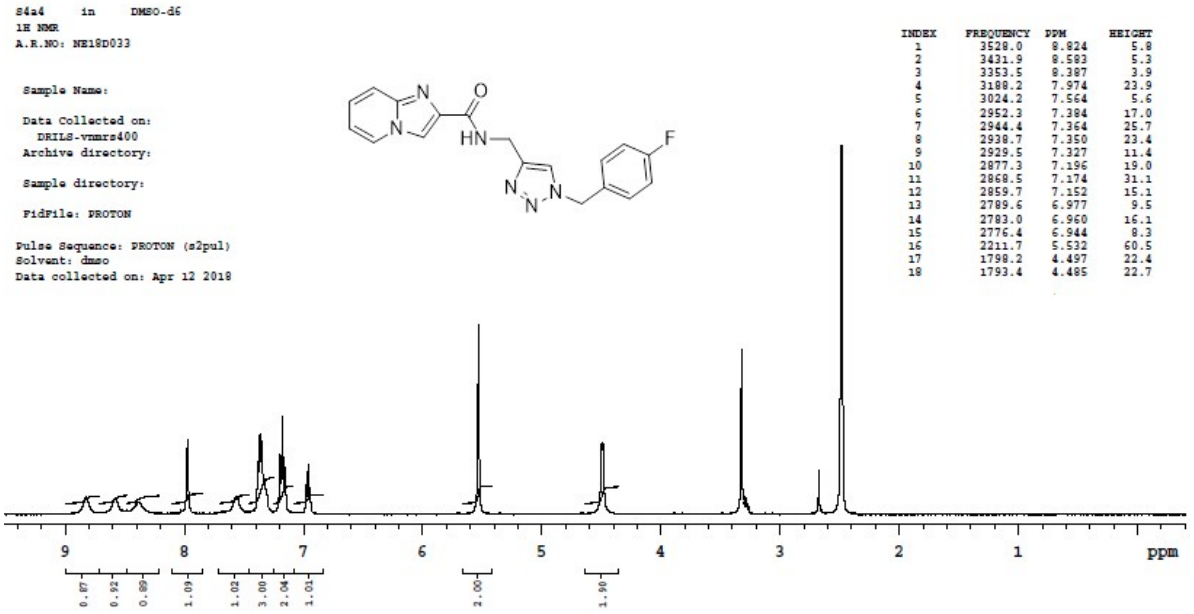


**Figure S9**. [1]H-NMR Spectrum of Compound **4d.**

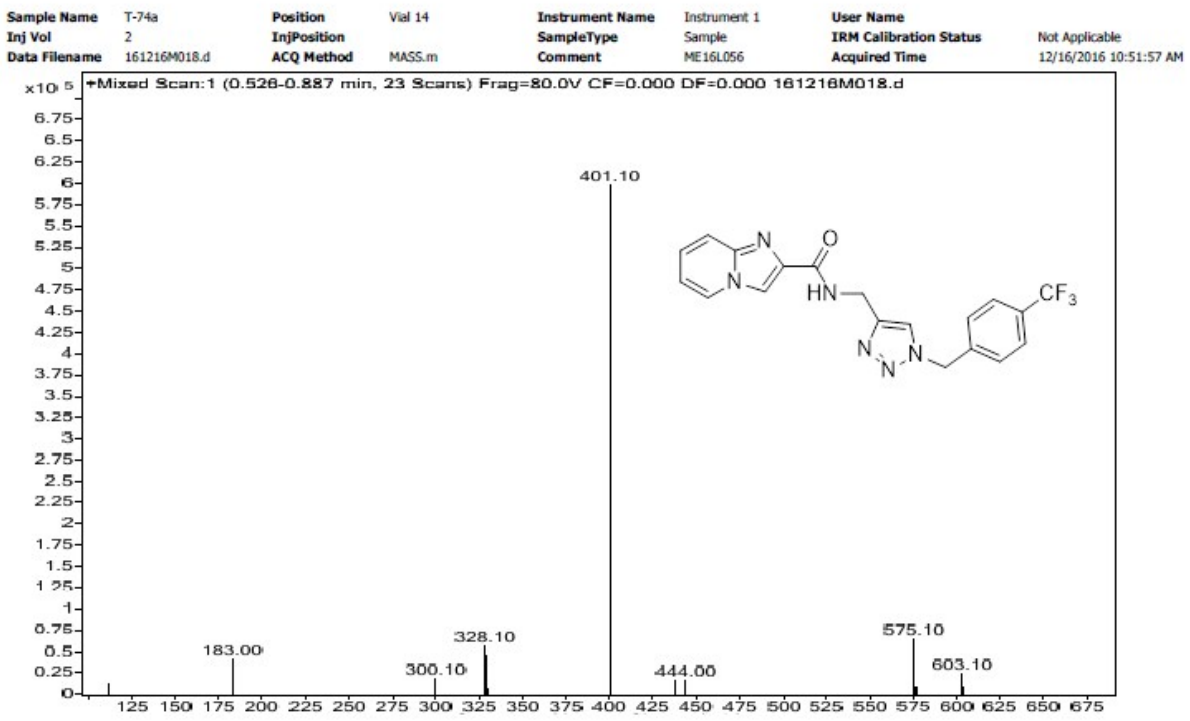


**Figure S10**. ESI-MS Spectrum of Compound **4e.**

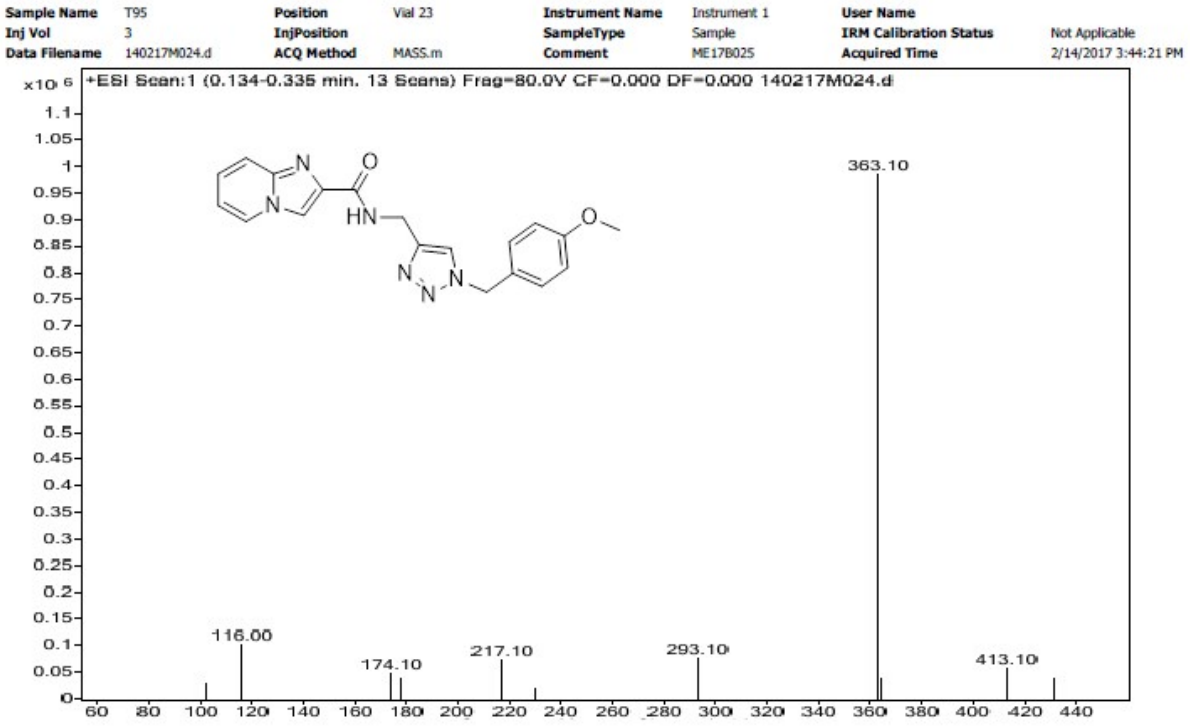


**Figure S11**. ESI-MS Spectrum of Compound **4f.**

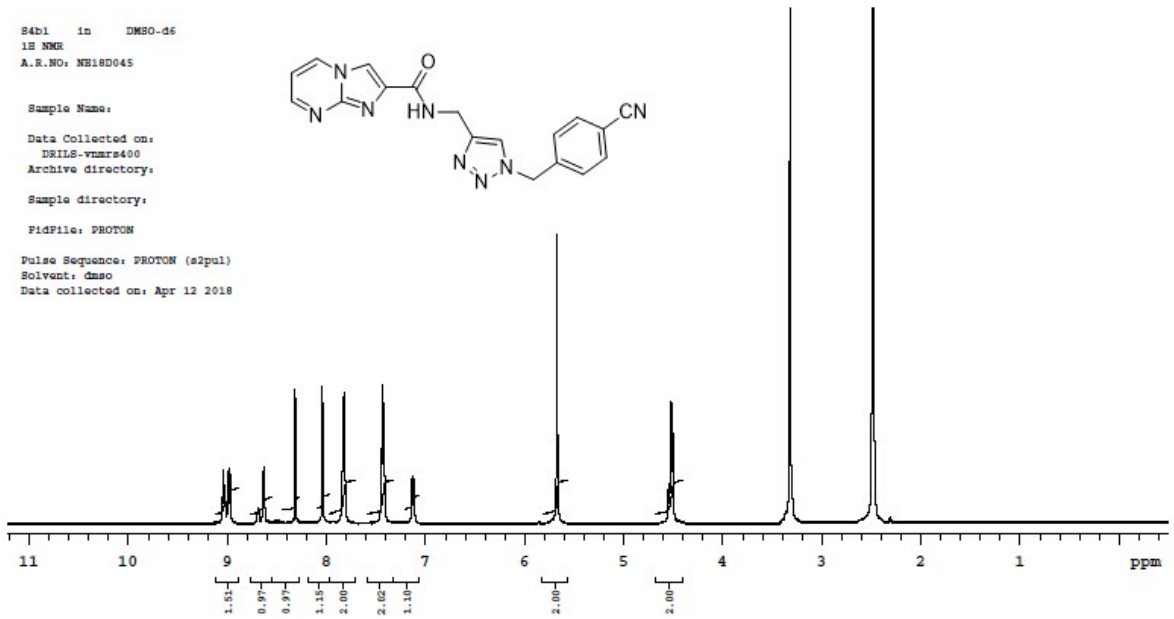


**Figure S12**. [1]H-NMR Spectrum of Compound **4j.**

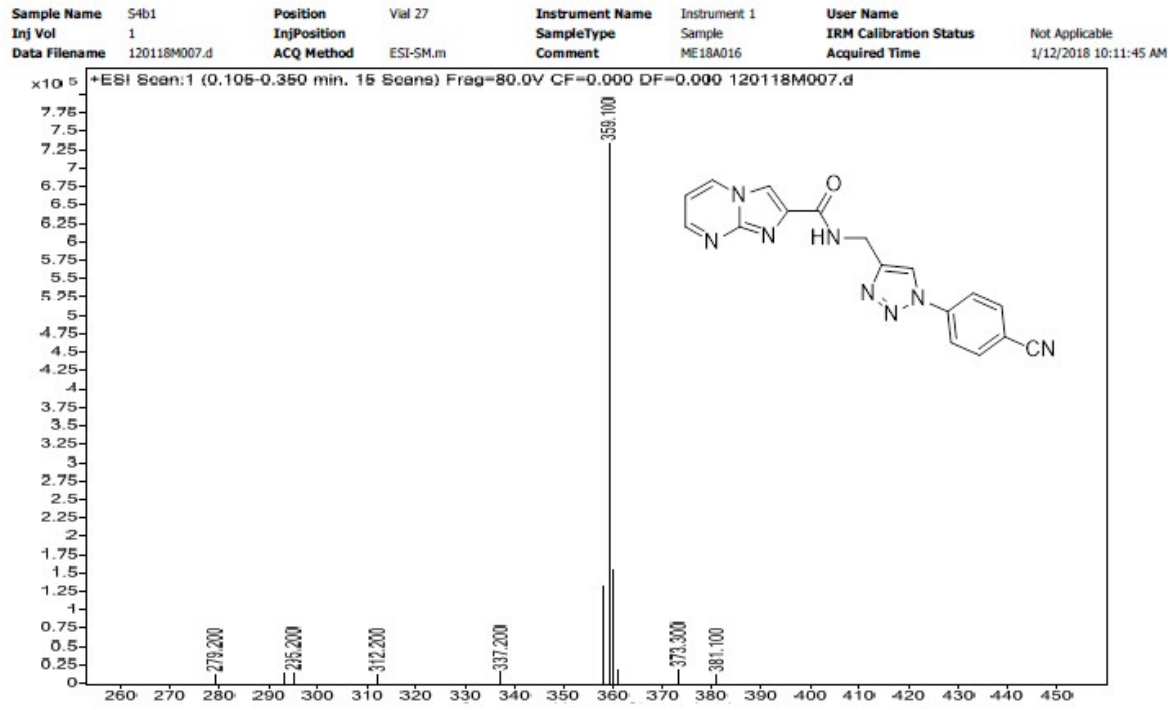


**Figure S13**. ESI-MS Spectrum of Compound **4j.**

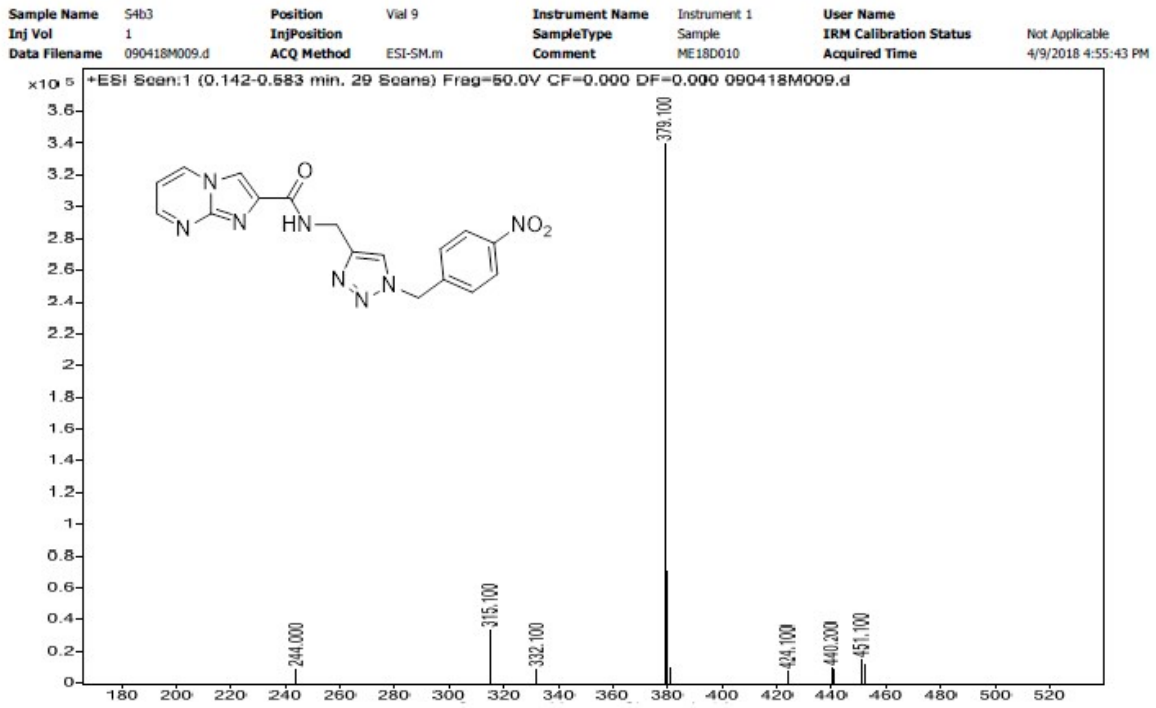


**Figure S14**. ESI-MS Spectrum of Compound **4l.**

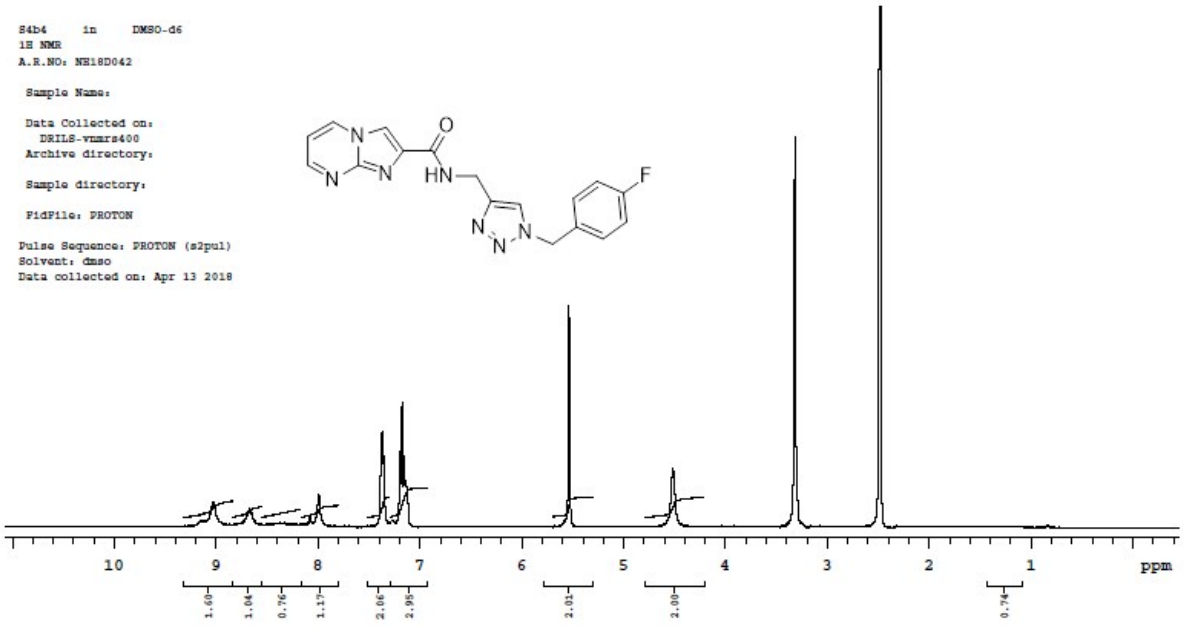


**Figure S15**. [1]H-NMR Spectrum of Compound **4m.**

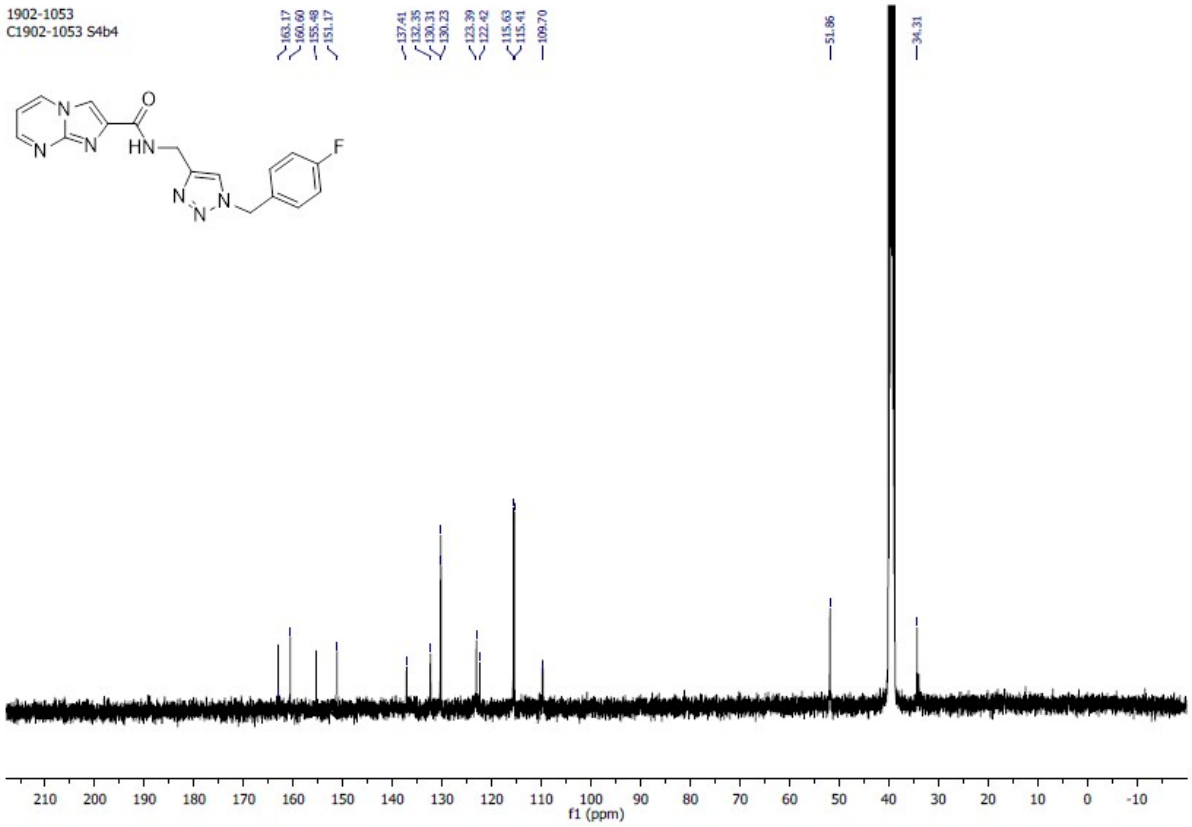


**Figure S16**. [13]C-NMR Spectrum of Compound **4m.**

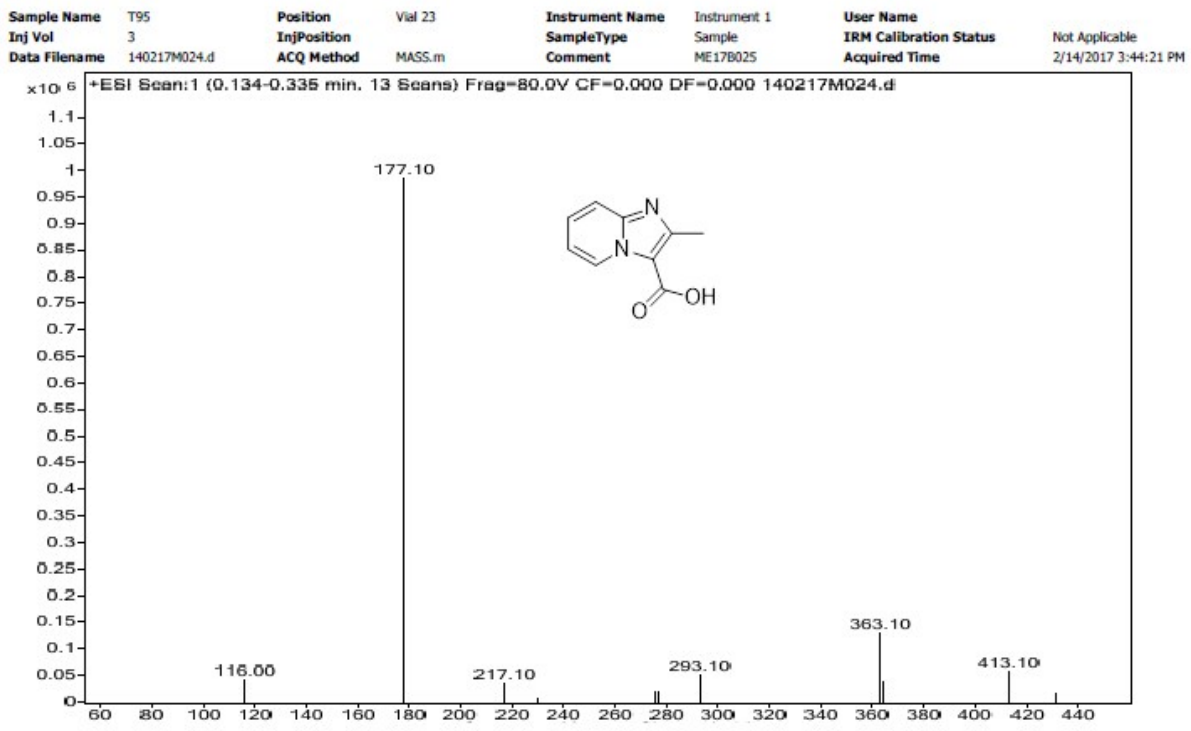


**Figure S17**. ESI-MS Spectrum of Compound **6.**

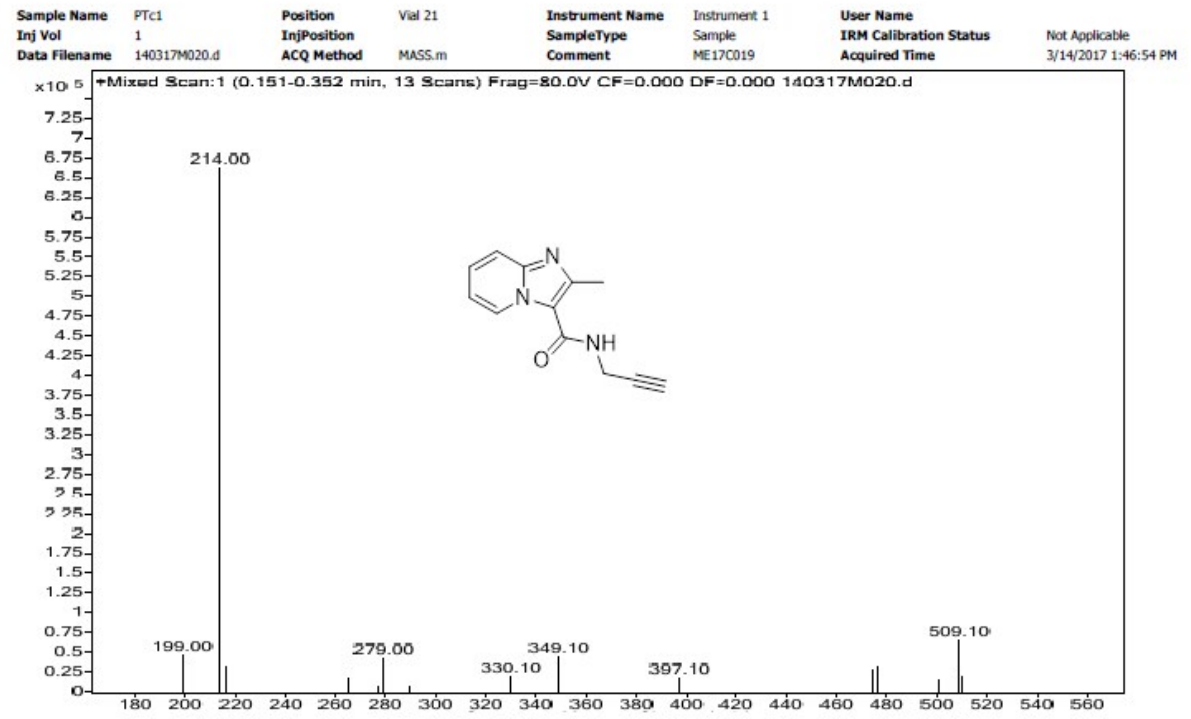


**Figure S18**. ESI-MS Spectrum of Compound **7.**

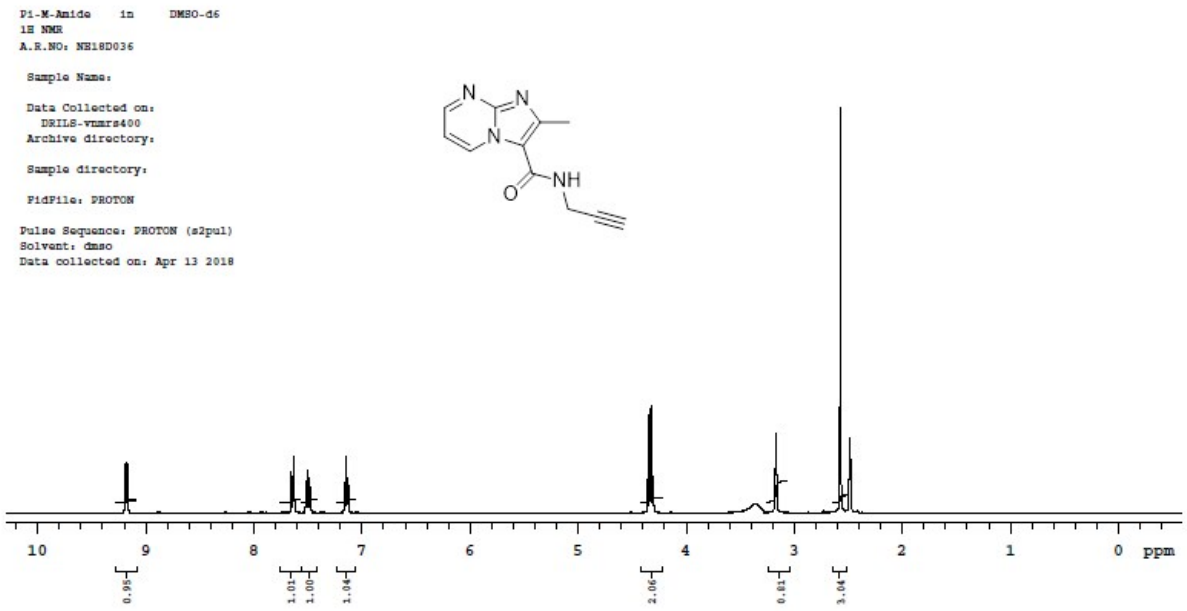


**Figure S19**. [1]H-NMR Spectrum of Compound **7a.**

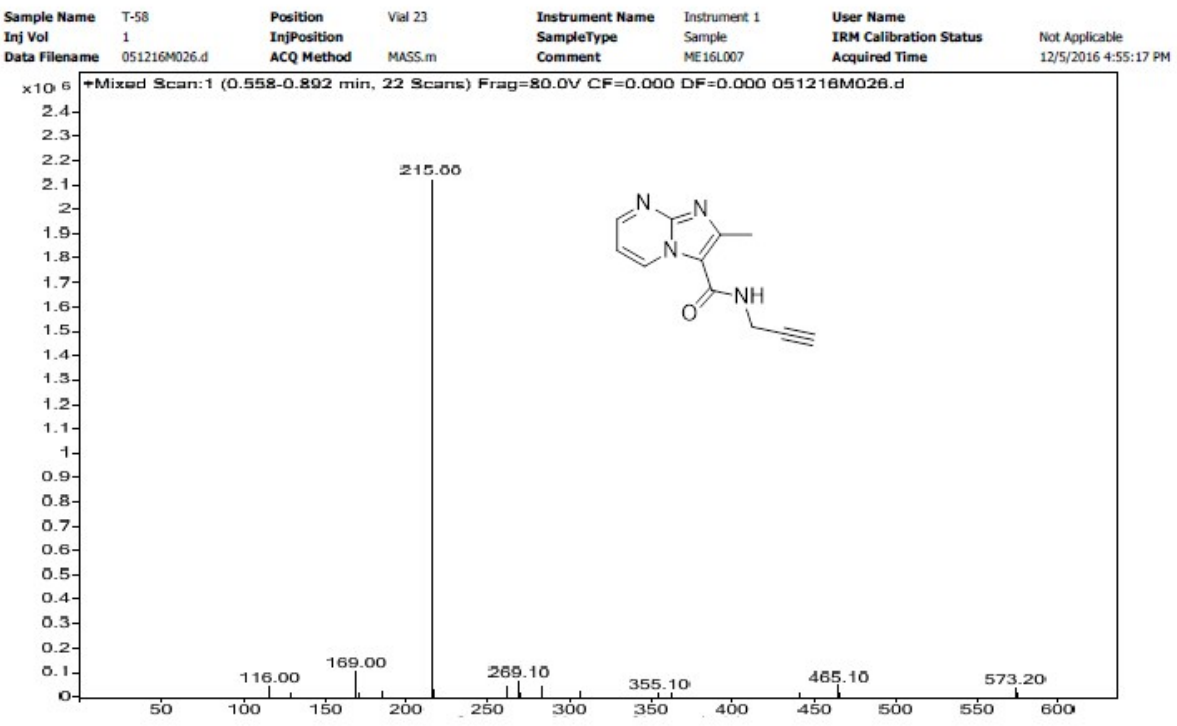


**Figure S20**. ESI-MS Spectrum of Compound **7a.**

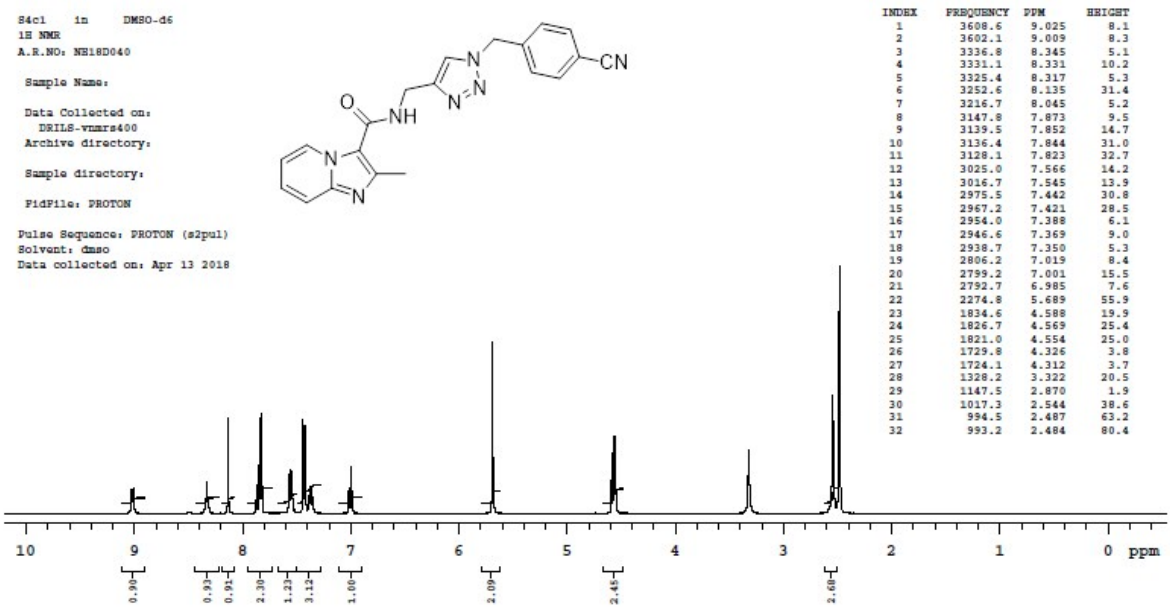


**Figure S21**. [1]H-NMR Spectrum of Compound **8a.**

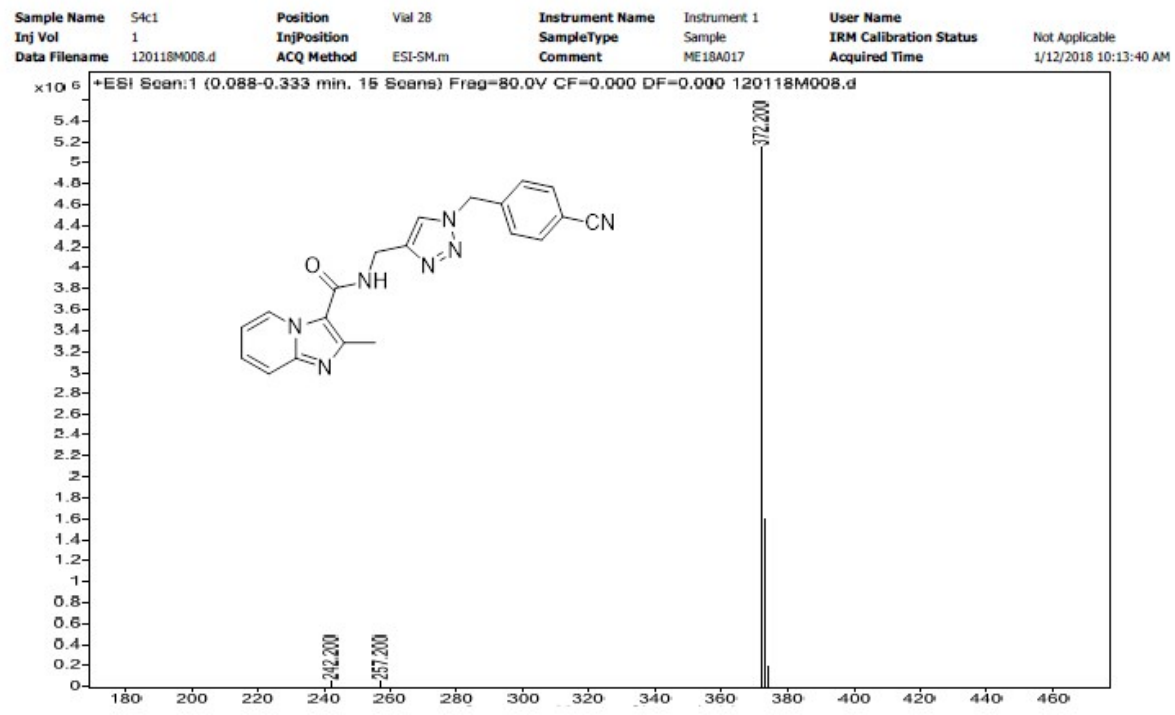


**Figure S222**. ESI-MS Spectrum of Compound **8a.**

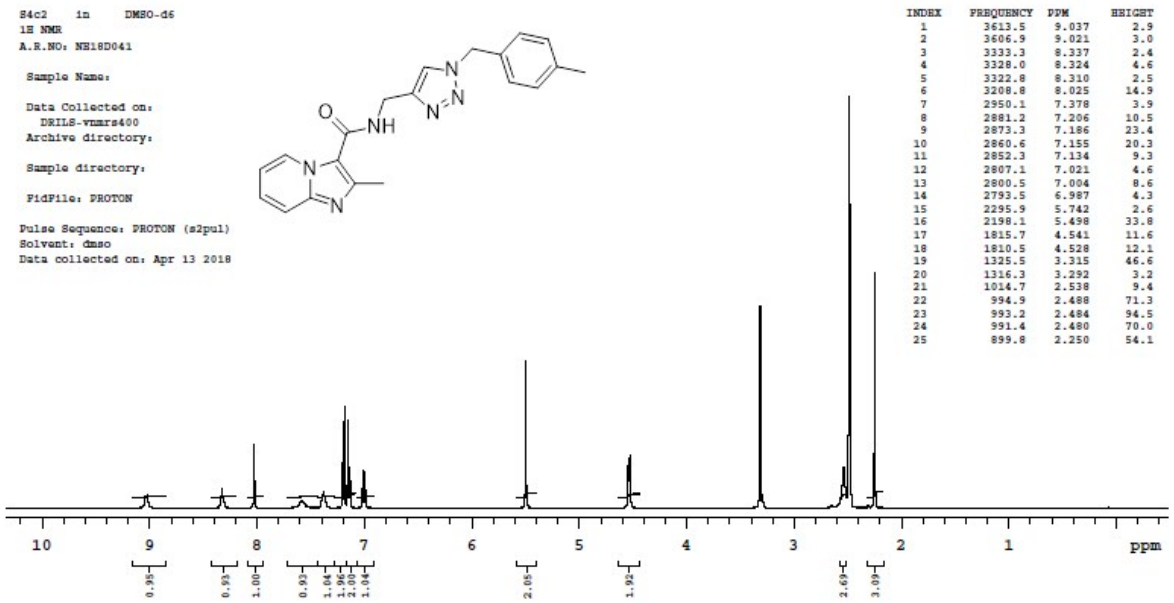


**Figure S23**. [1]H-NMR Spectrum of Compound **8b.**

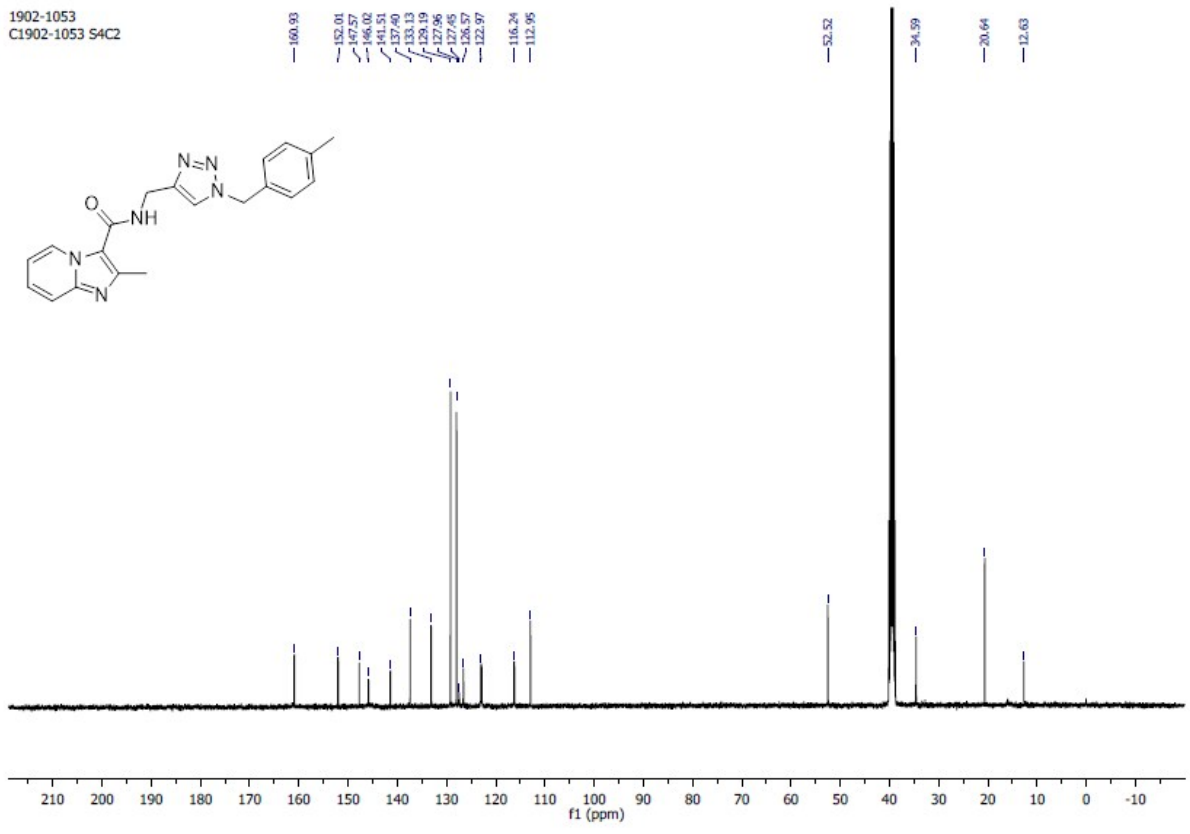


**Figure S24**. [13]C-NMR Spectrum of Compound **8b.**

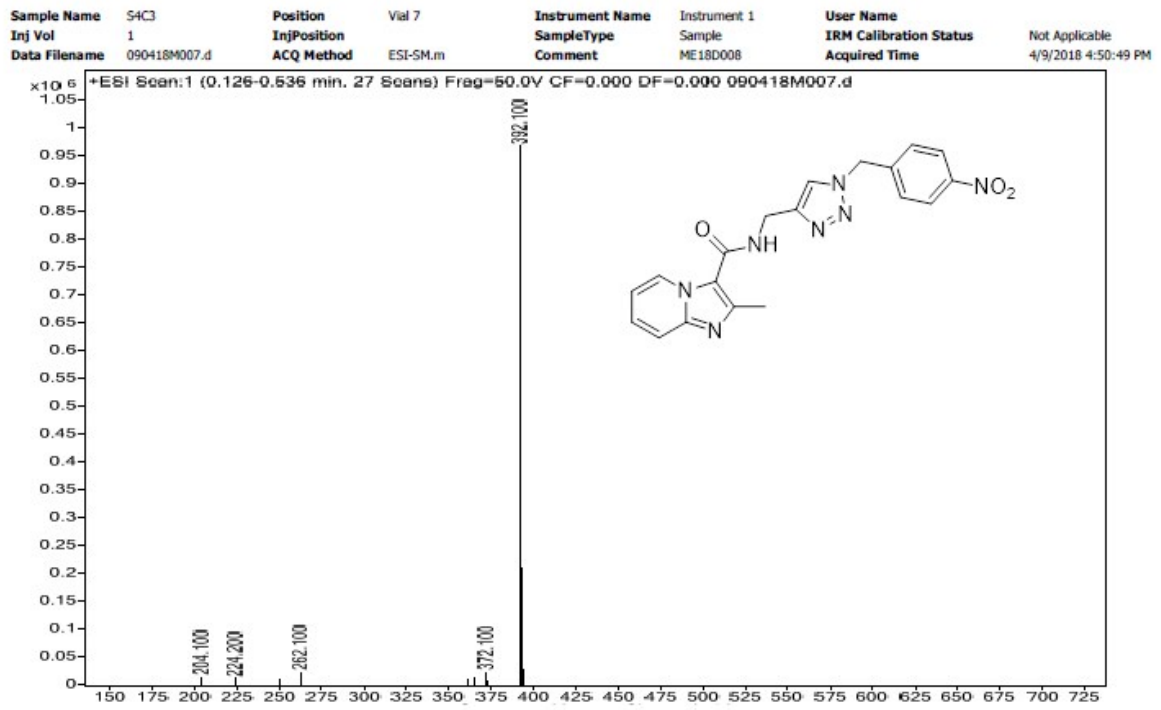


**Figure S25**. ESI-MS Spectrum of Compound **8c.**

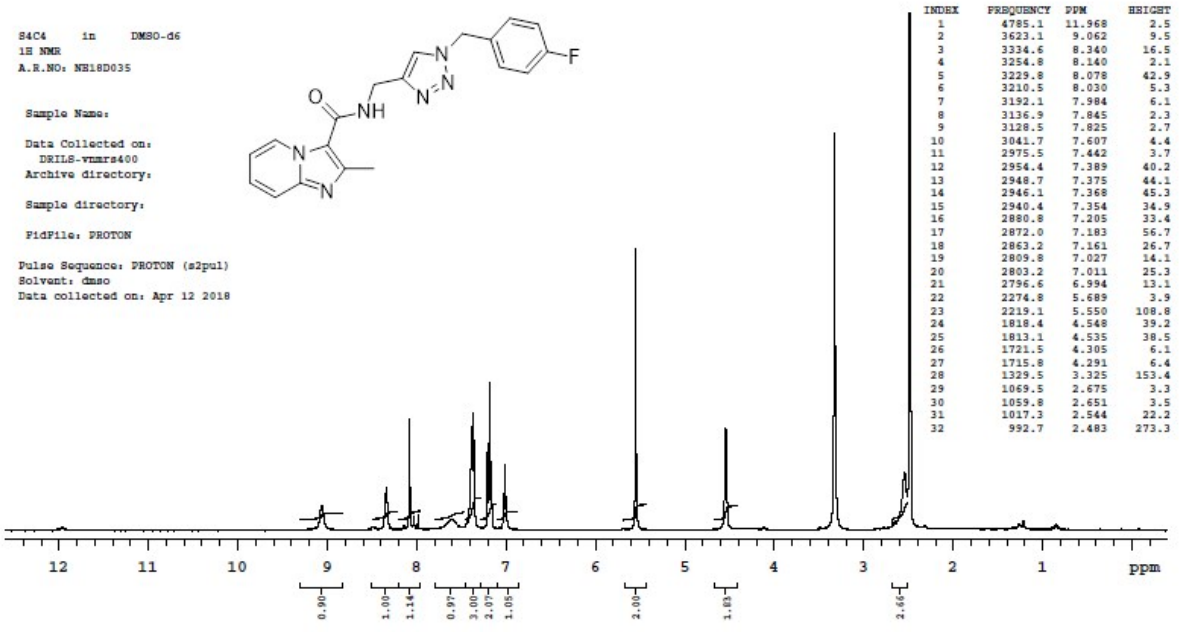


**Figure S26**. $^{1}$H-NMR Spectrum of Compound **8d.**

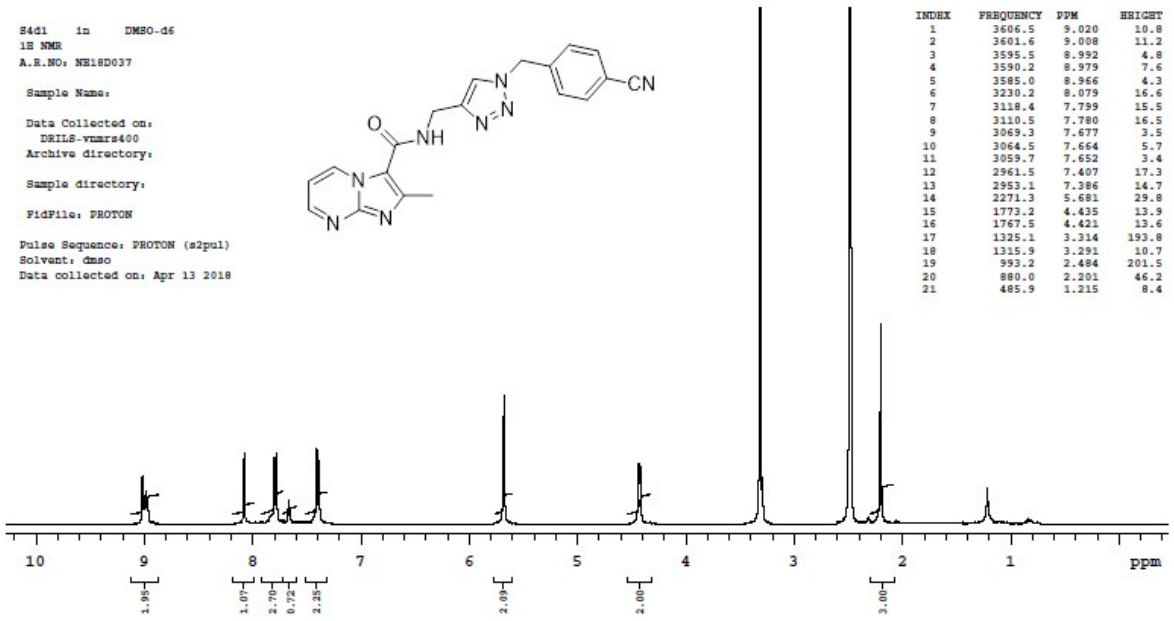


**Figure S27**. $^{1}$H-NMR Spectrum of Compound **8j.**

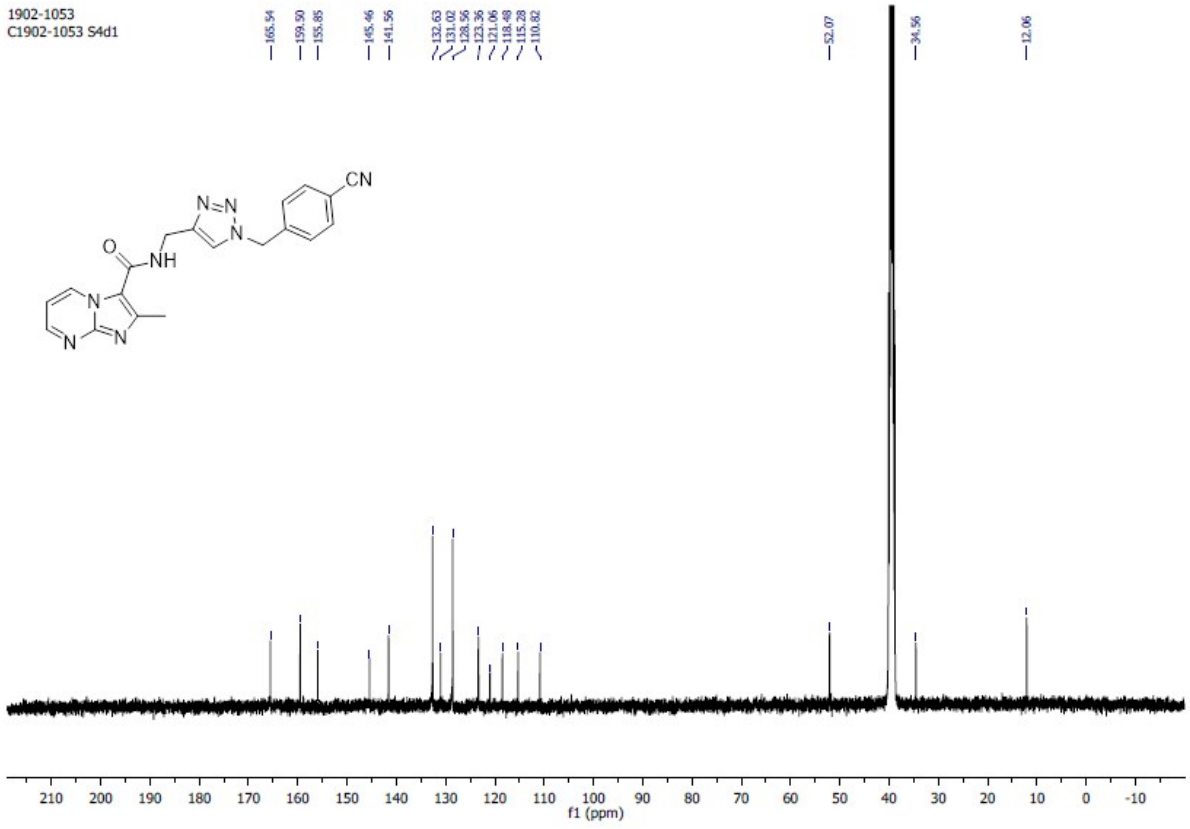


**Figure S28**. $^{13}$C-NMR Spectrum of Compound **8j.**

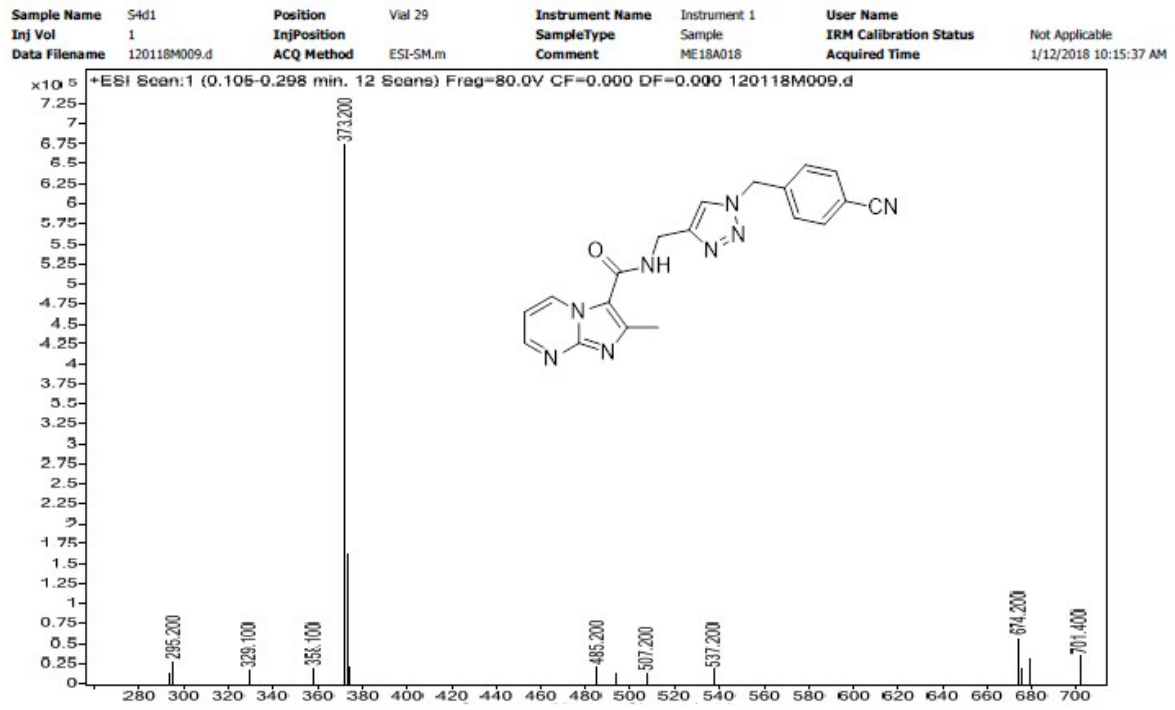


**Figure S29**. ESI-MS Spectrum of Compound **8j.**

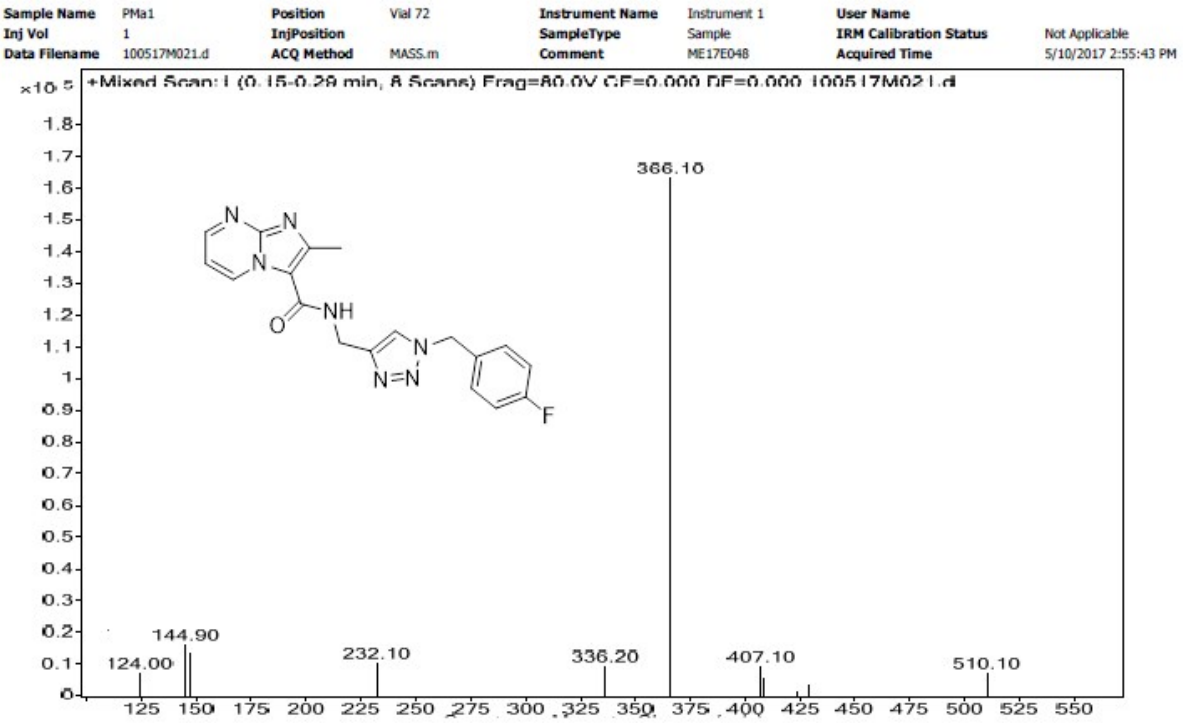


**Figure S30**. ESI-MS Spectrum of Compound **8m.**

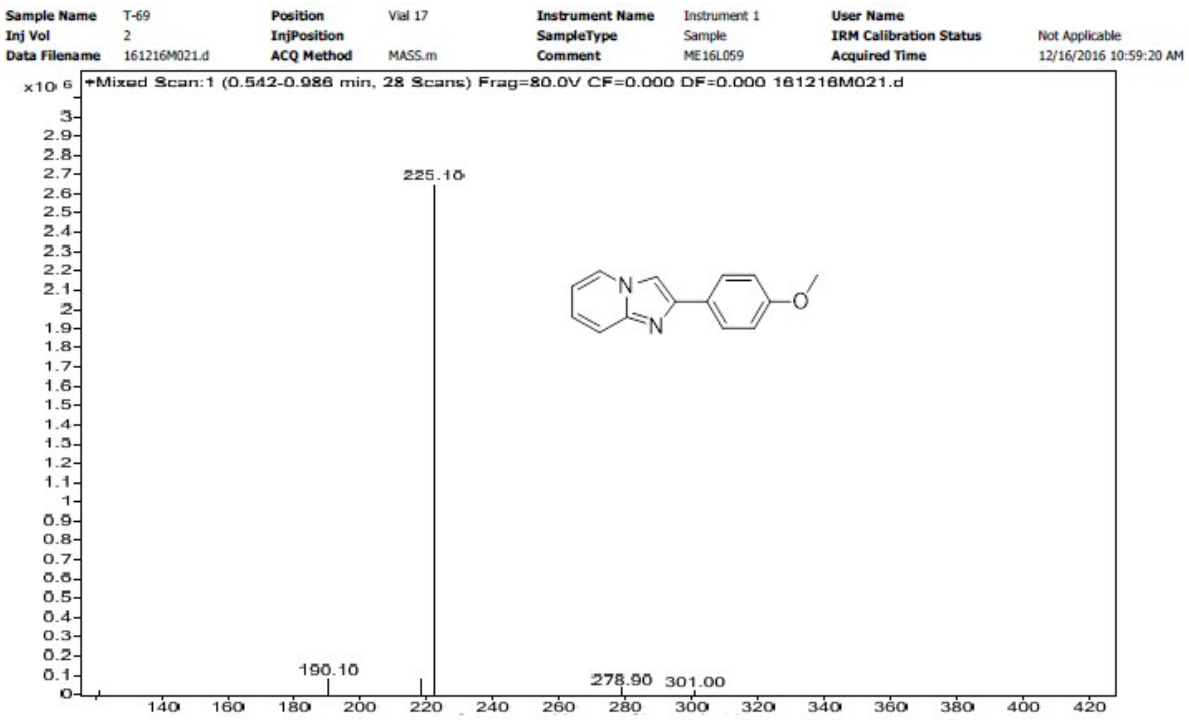


**Figure S31**. ESI-MS Spectrum of Compound **9.**

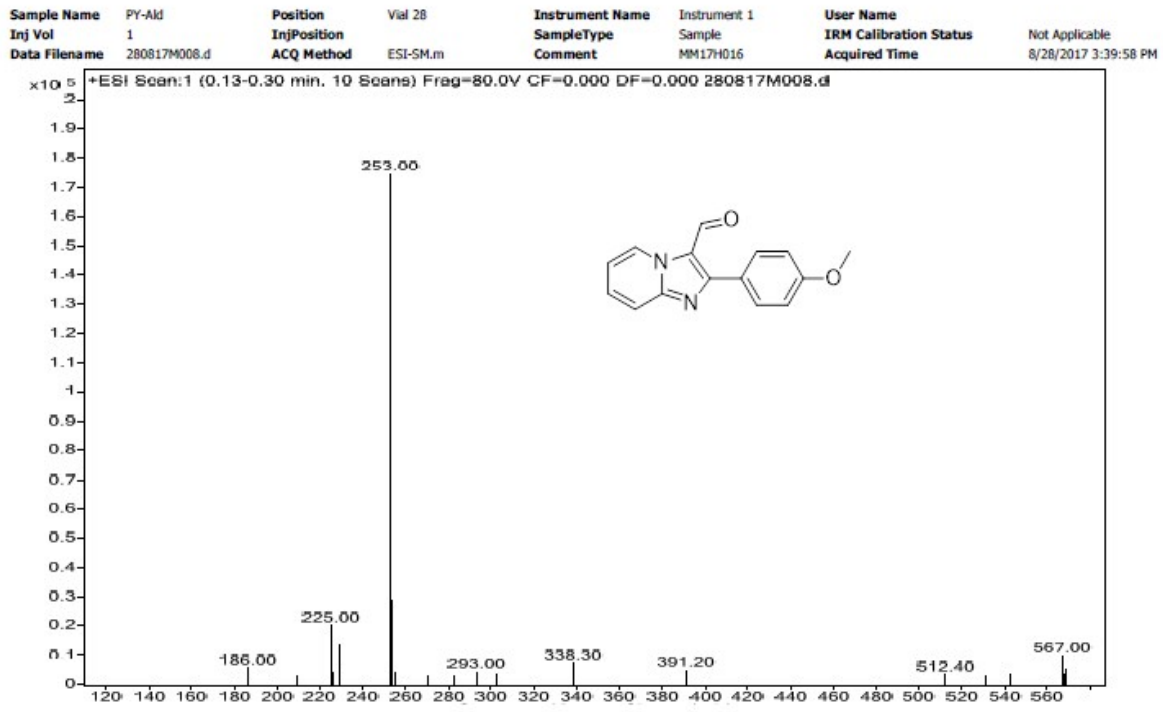


**Figure S32**. ESI-MS Spectrum of Compound **10.**

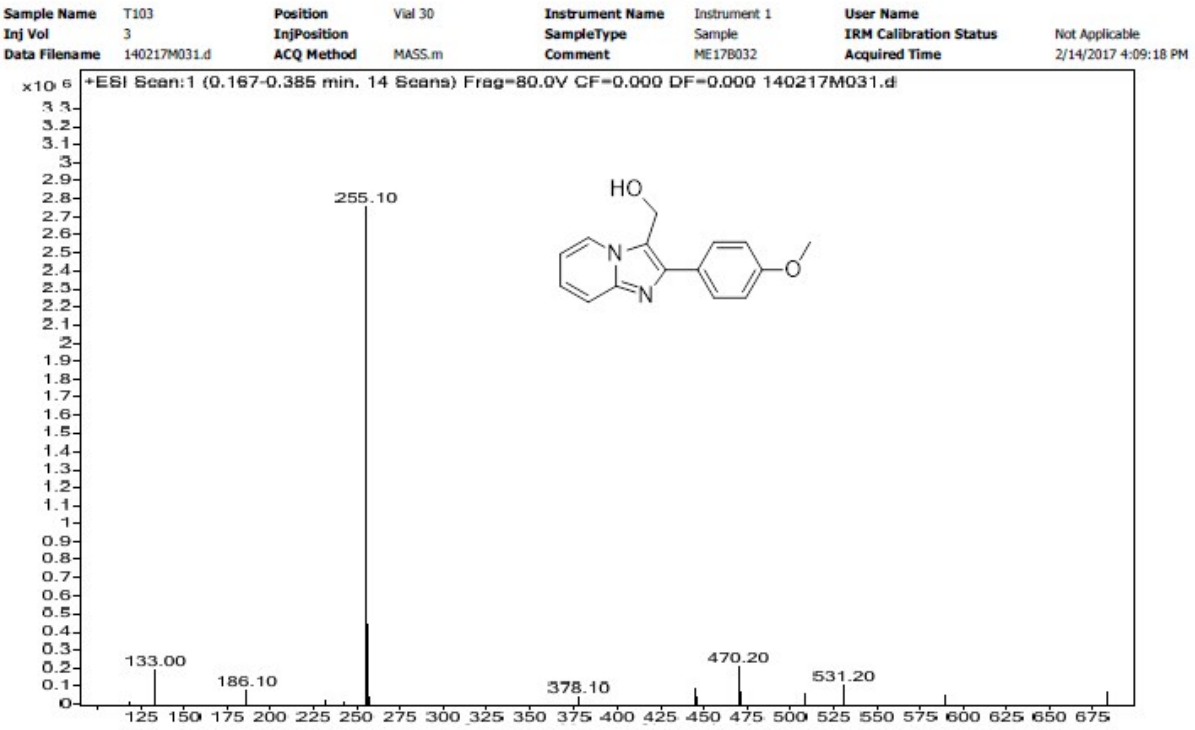


**Figure S33**. ESI-MS Spectrum of Compound **11.**

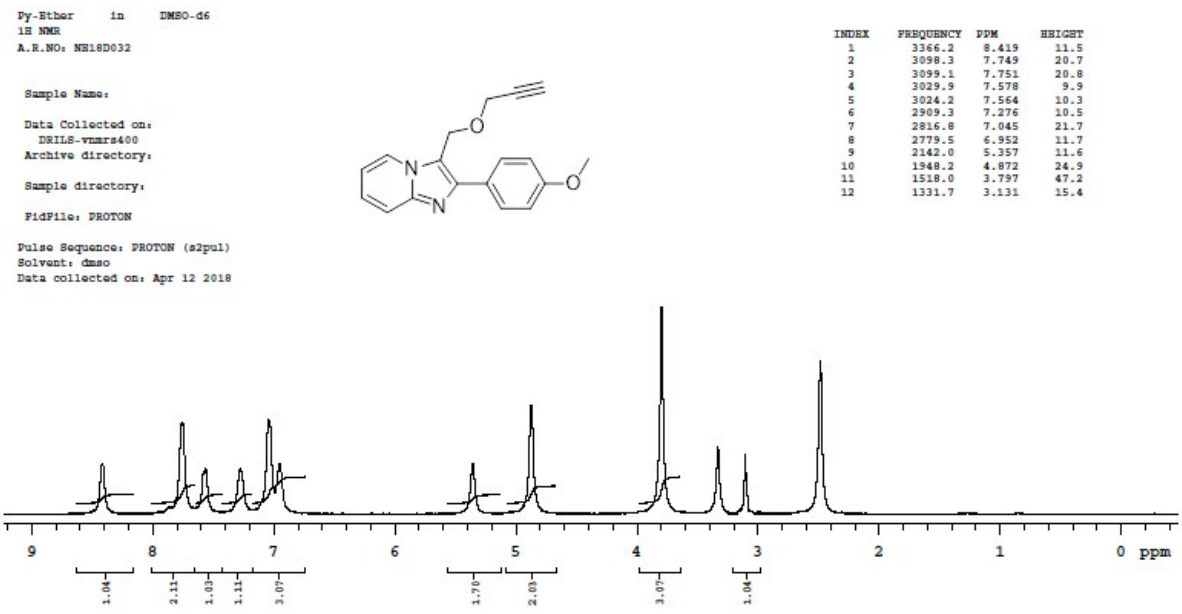


**Figure S34**. [1]H-NMR Spectrum of Compound **12.**

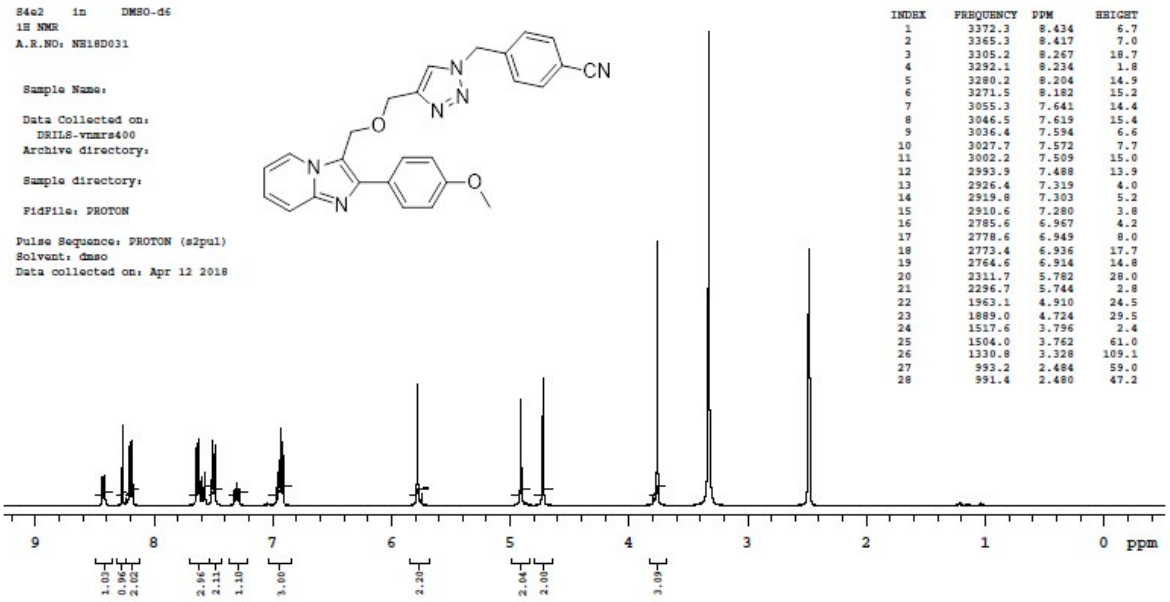


**Figure S35**. [1]H-NMR Spectrum of Compound **13a.**

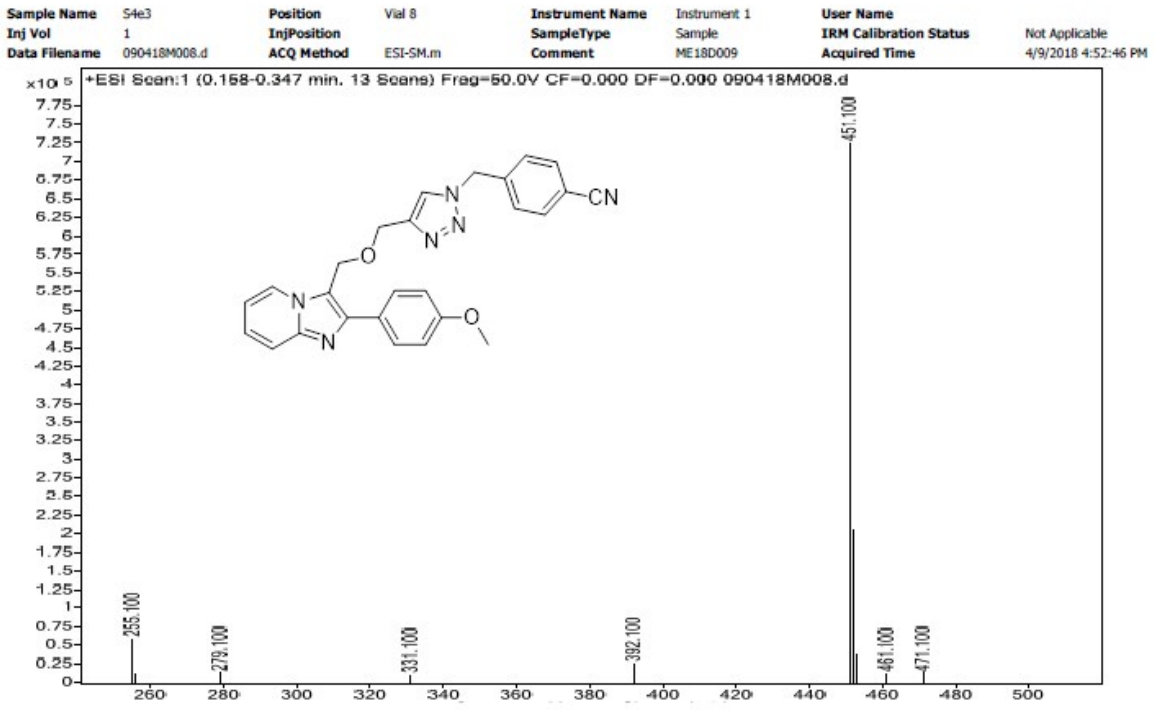


**Figure S36**. ESI-MS Spectrum of Compound **13a.**

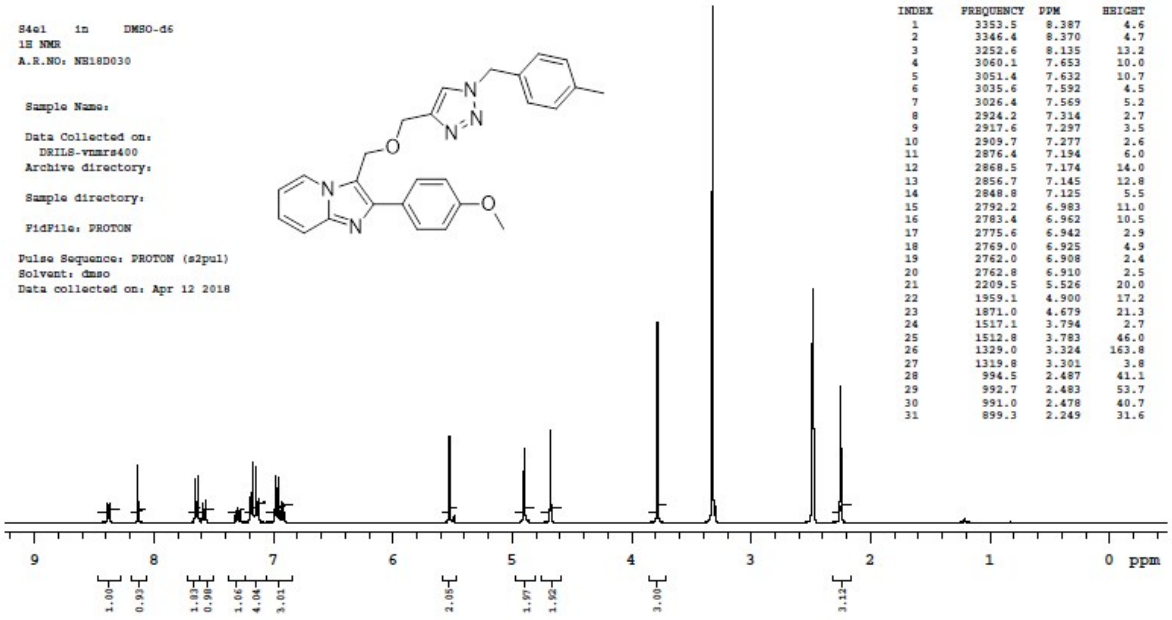


**Figure S37**. $^{1}$H-NMR Spectrum of Compound **13b.**

.

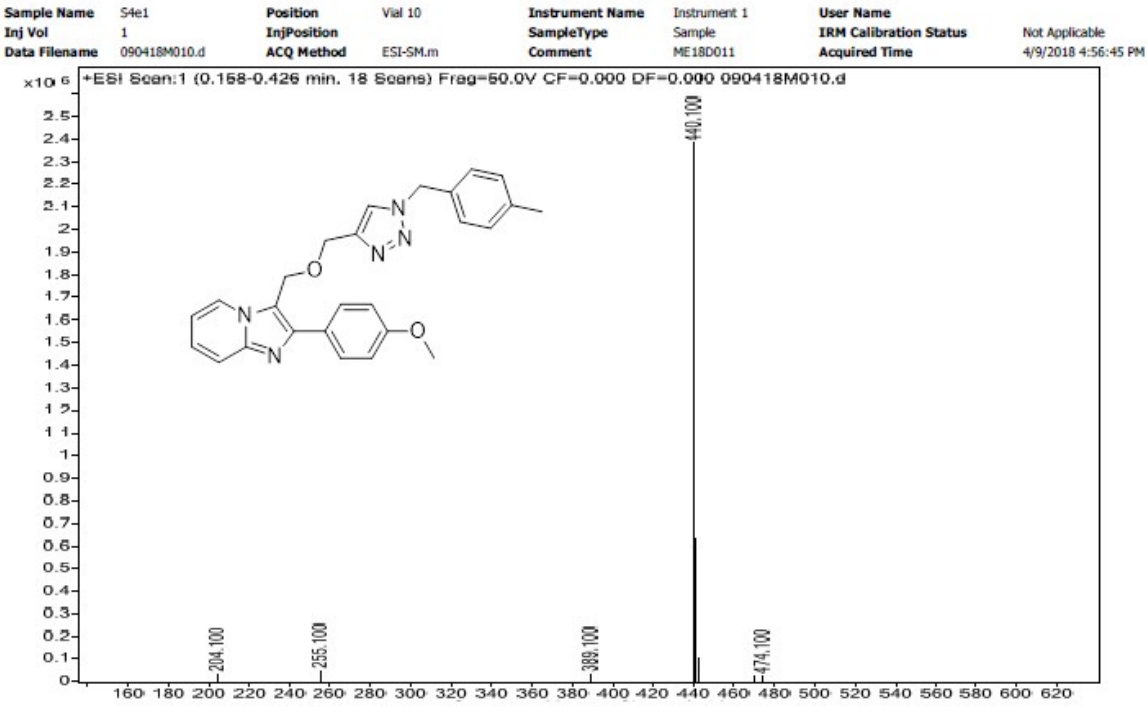


**Figure S38**. ESI-MS Spectrum of Compound **13b.**