

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*}

^aOrganic 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

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1. Materials and instruments

All the required reagents and solvents were purchased from Sigma Aldrich, Alfa-aesar, Merck 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. ¹H-NMR and ¹³C-NMR spectra of synthesized compounds were recorded on Bruker (400 MHz: ¹H-NMR, 100 MHz: ¹³C-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 (resazurin). 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 $\mu\text{g}/\text{mL}$) 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 25 μL 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 °C whereas for Anaerobic organisms, incubate plates were kept in the Anaerobic jar and kept the jar in the incubator at 37 °C. As like bacterial study, the plates were then examined for the presence of zones of inhibition and the results were recorded.

5. In 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% CO₂) 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% CO₂ 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.

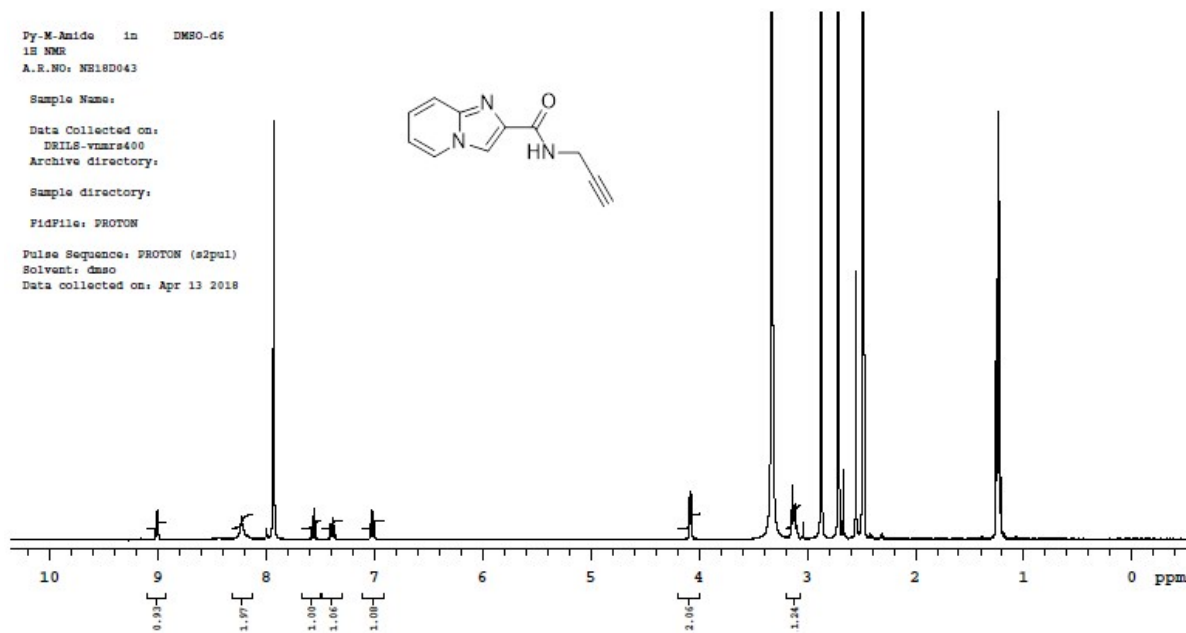


Figure S1. ¹H-NMR Spectrum of Compound 3.

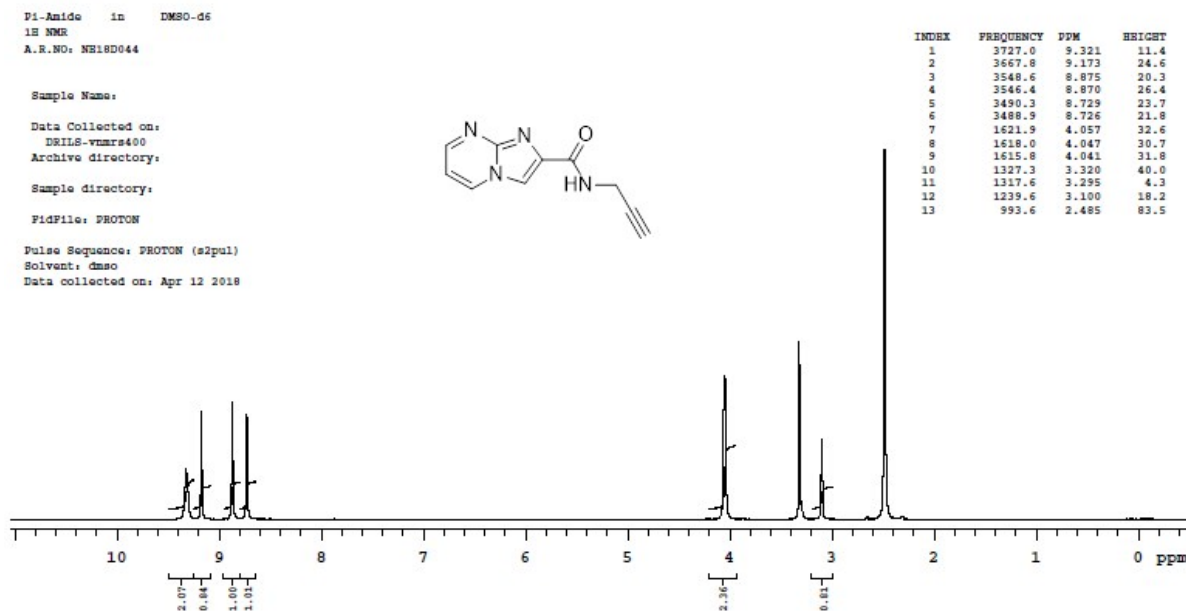


Figure S2. ¹H-NMR Spectrum of Compound 3a.

Sample Name T91 Position Vial 19 Instrument Name Instrument 1 User Name
 Inj Vol 3 InjPosition SampleType Sample IRM Calibration Status Not Applicable
 Data Filename 140217M020.d ACQ Method MASS.m Comment ME17B021 Acquired Time 2/14/2017 3:28:19 PM

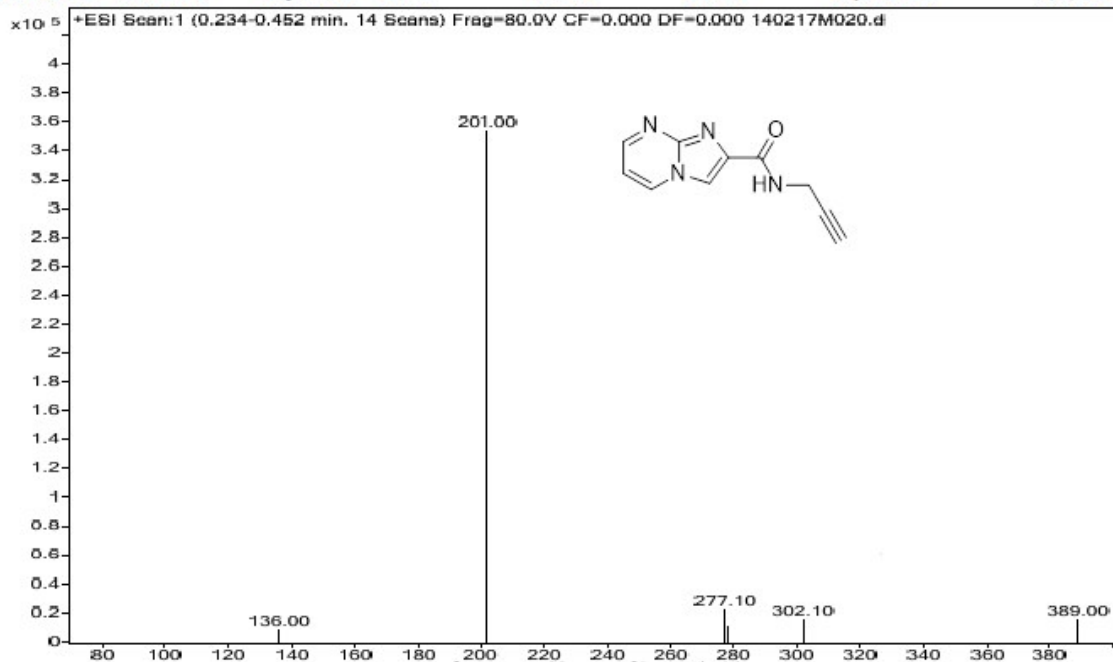


Figure S3. ESI-MS Spectrum of Compound 3a.

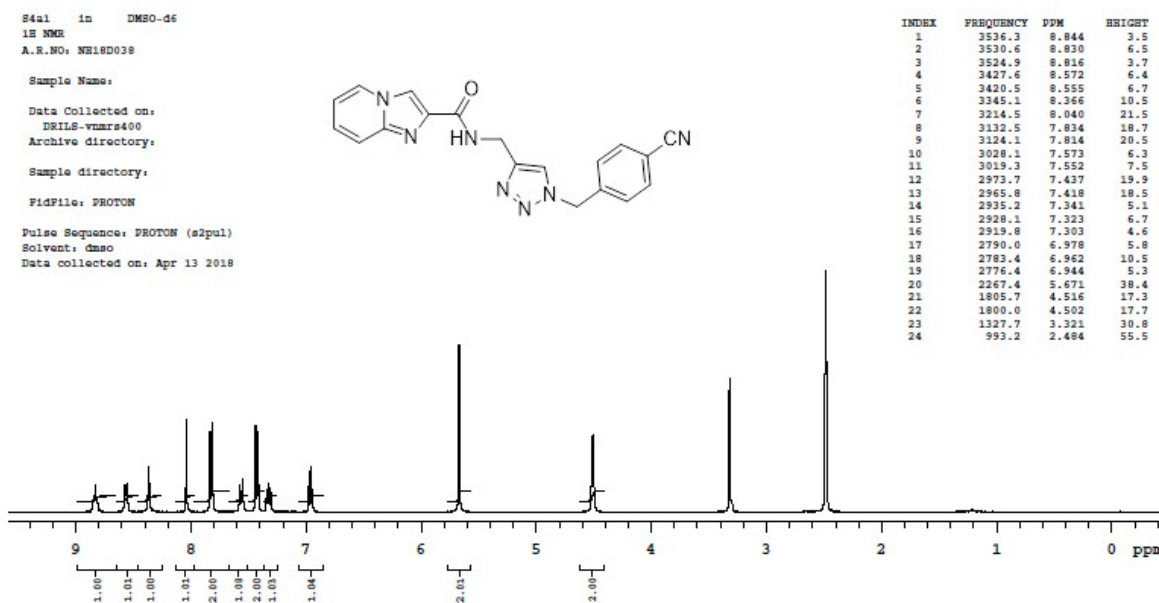


Figure S4. ¹H-NMR Spectrum of Compound 4a.

Sample Name S4a1 **Position** Vial 26 **Instrument Name** Instrument 1 **User Name**
Inj Vol 1 **InjPosition** **SampleType** Sample **IRM Calibration Status** Not Applicable
Data Filename 120118M006.d **ACQ Method** ESI-SM.m **Comment** ME18A015 **Acquired Time** 1/12/2018 10:08:48 AM

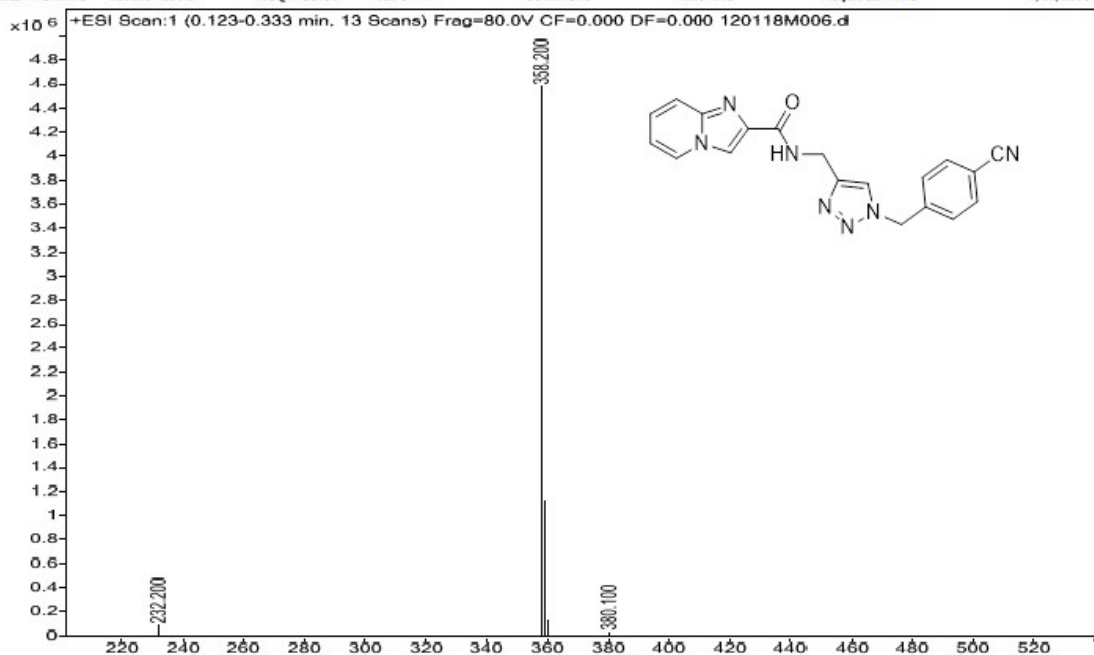


Figure S5. ESI-MS Spectrum of Compound 4a.

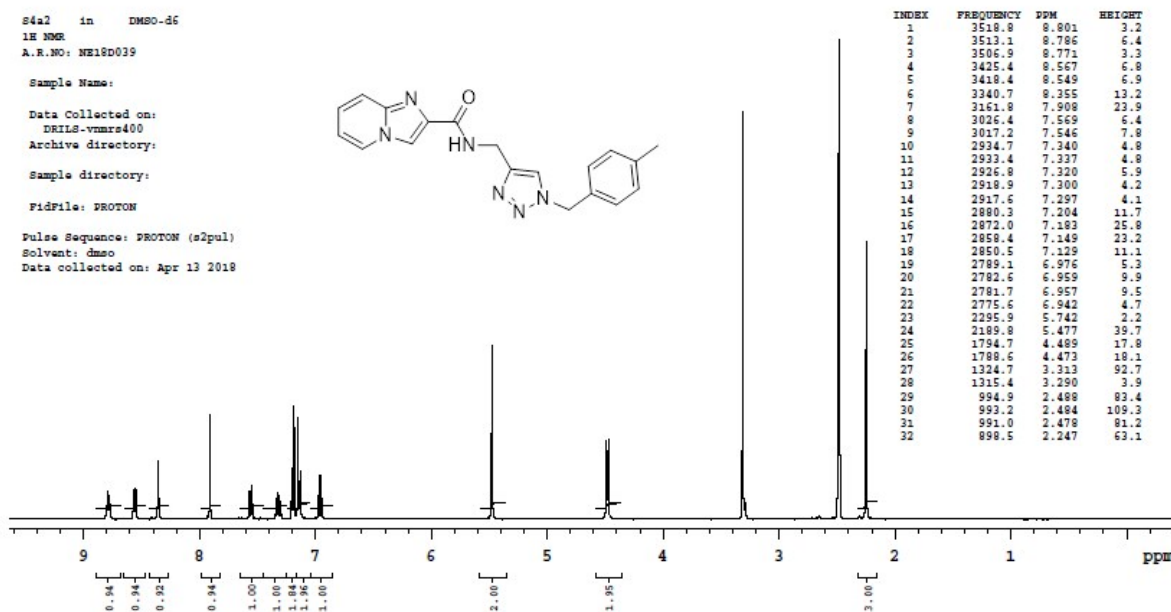


Figure S6. ¹H-NMR Spectrum of Compound 4b.

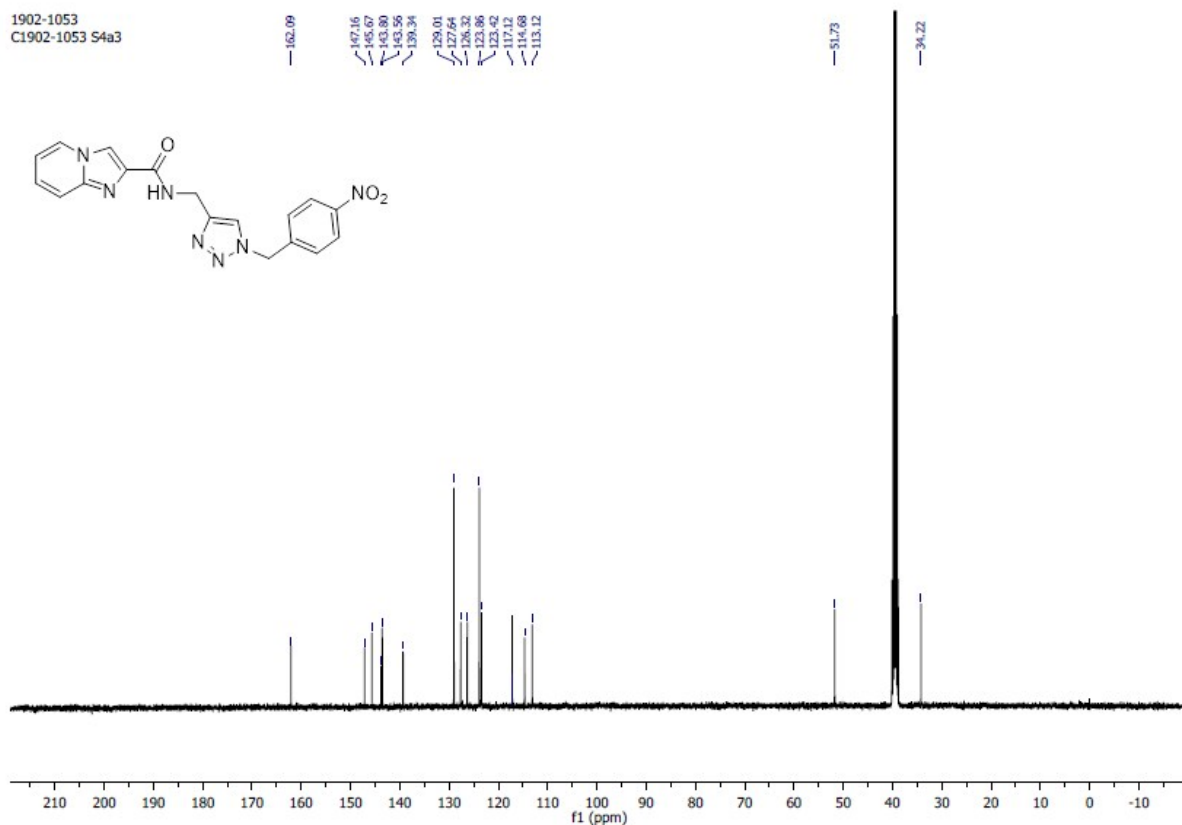


Figure S7. ^{13}C -NMR Spectrum of Compound 4c.

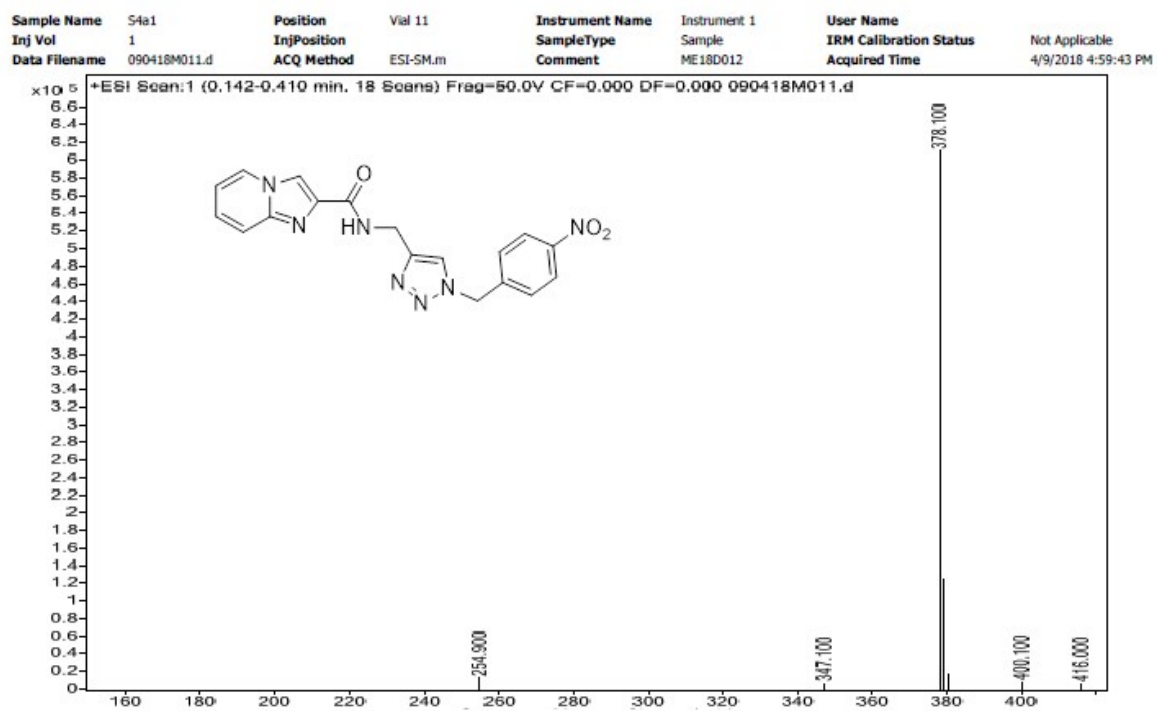


Figure S8. ESI-MS Spectrum of Compound 4c.

S4a4 in DMSO-d6
 1H NMR
 A.R.NO: NE18D033

Sample Name:

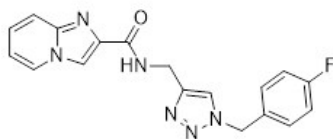
Data Collected on:
 DRILS-vmr400

Archive directory:

Sample directory:

Fidfile: PROTON

Pulse Sequence: PROTON (s2pul)
 Solvent: DMSO
 Data collected on: Apr 12 2018



INDEX	FREQUENCY	PPM	HEIGHT
1	3528.0	8.824	5.8
2	3431.9	8.583	5.3
3	3353.5	8.387	3.9
4	3188.2	7.974	23.9
5	3024.2	7.564	5.6
6	2952.3	7.384	17.0
7	2944.4	7.364	25.7
8	2938.7	7.350	23.4
9	2929.5	7.327	11.4
10	2877.3	7.196	19.0
11	2868.5	7.174	31.1
12	2859.7	7.152	15.1
13	2789.6	6.977	9.5
14	2783.0	6.960	16.1
15	2776.4	6.944	8.3
16	2211.7	5.532	60.5
17	1798.2	4.497	22.4
18	1793.4	4.485	22.7

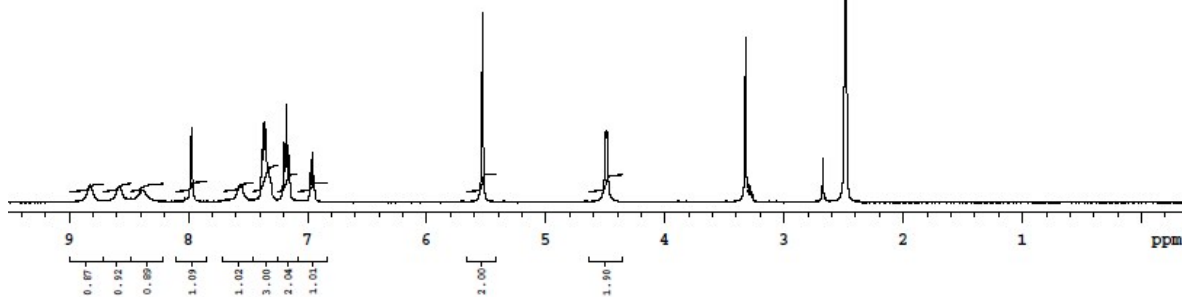


Figure S9. ¹H-NMR Spectrum of Compound 4d.

Sample Name	T-74a	Position	Vial 14	Instrument Name	Instrument 1	User Name	
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	161216M018.d	ACQ Method	MASS.m	Comment	ME16L056	Acquired Time	12/16/2016 10:51:57 AM

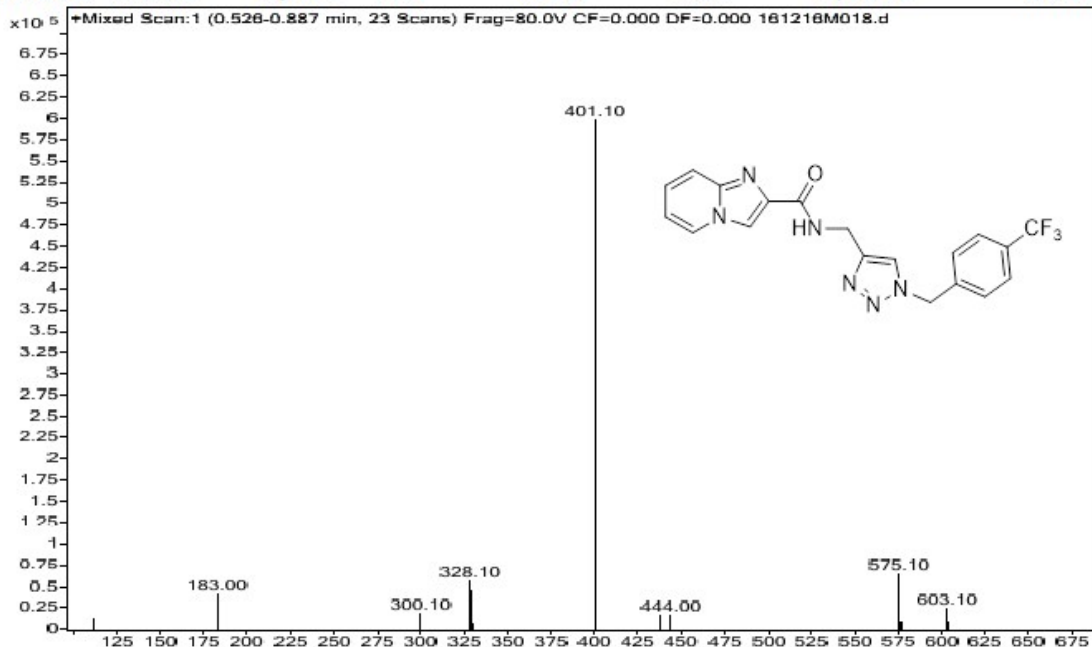


Figure S10. ESI-MS Spectrum of Compound 4e.

Sample Name	T95	Position	Vial 23	Instrument Name	Instrument 1	User Name	
Inj Vol	3	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	140217M024.d	ACQ Method	MASS.m	Comment	ME17B025	Acquired Time	2/14/2017 3:44:21 PM

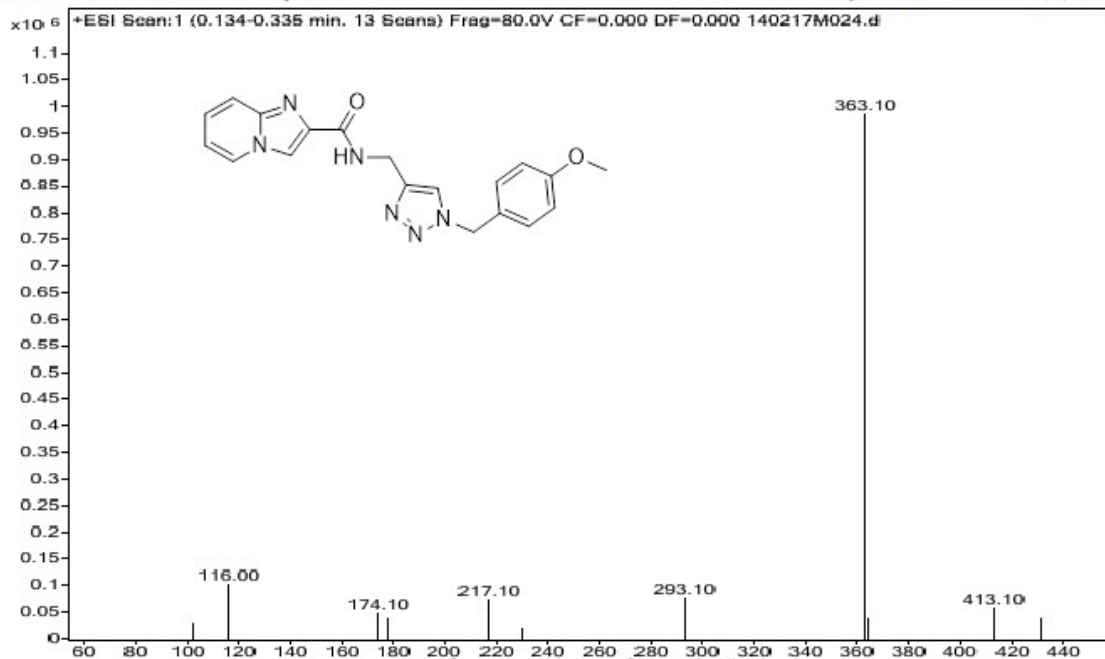


Figure S11. ESI-MS Spectrum of Compound 4f.

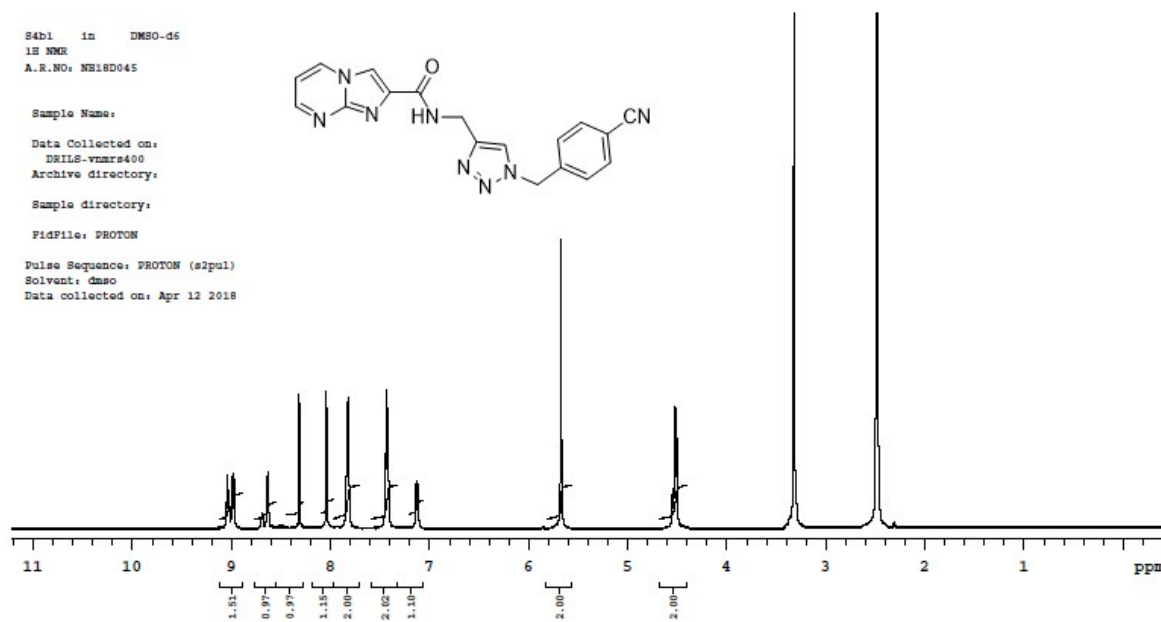


Figure S12. ¹H-NMR Spectrum of Compound 4j.

Sample Name	S4b1	Position	Vial 27	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	120118M007.d	ACQ Method	ESI-SM.m	Comment	ME18A016	Acquired Time	1/12/2018 10:11:45 AM

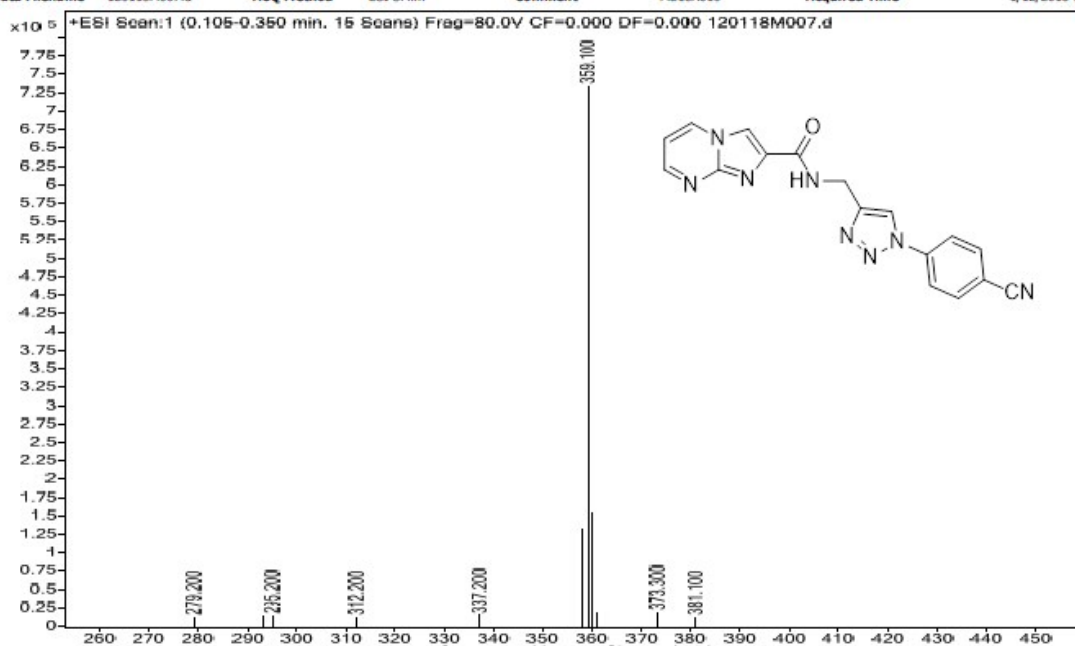


Figure S13. ESI-MS Spectrum of Compound 4j.

Sample Name	S4b3	Position	Vial 9	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	090418M009.d	ACQ Method	ESI-SM.m	Comment	ME18D010	Acquired Time	4/9/2018 4:55:43 PM

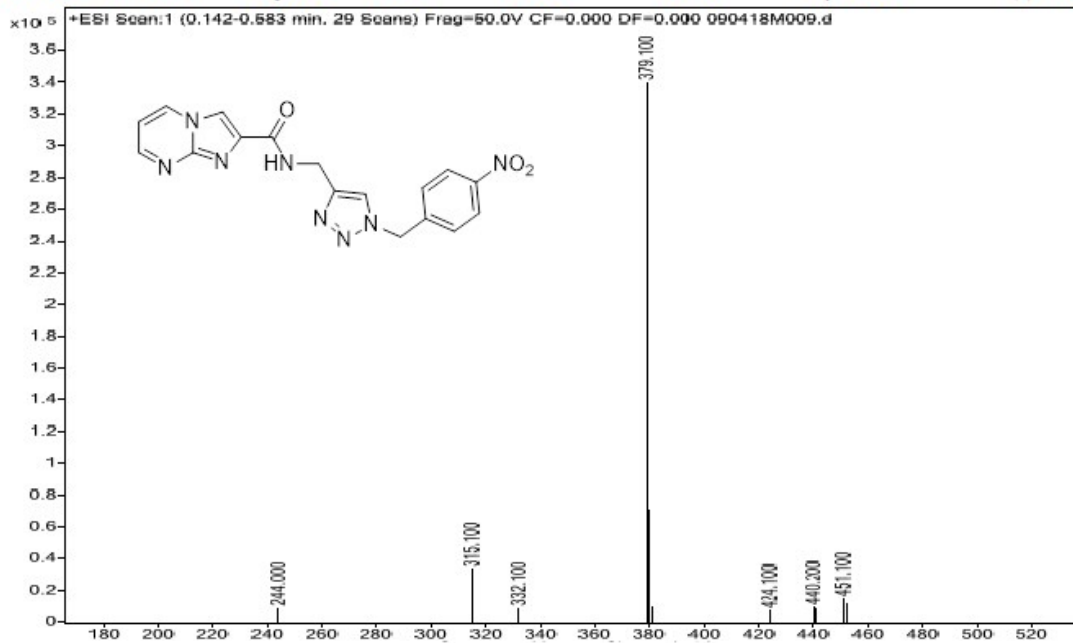


Figure S14. ESI-MS Spectrum of Compound 4l.

S4b4 in DMSO-d6
1H NMR
A.R.NO: NH18D042
Sample Name:
Data Collected on:
DRILS-vnmrs400
Archive directory:
Sample directory:
FidFile: PROTON
Pulse Sequence: PROTON (s2pul)
Solvent: dmsd
Data collected on: Apr 13 2018

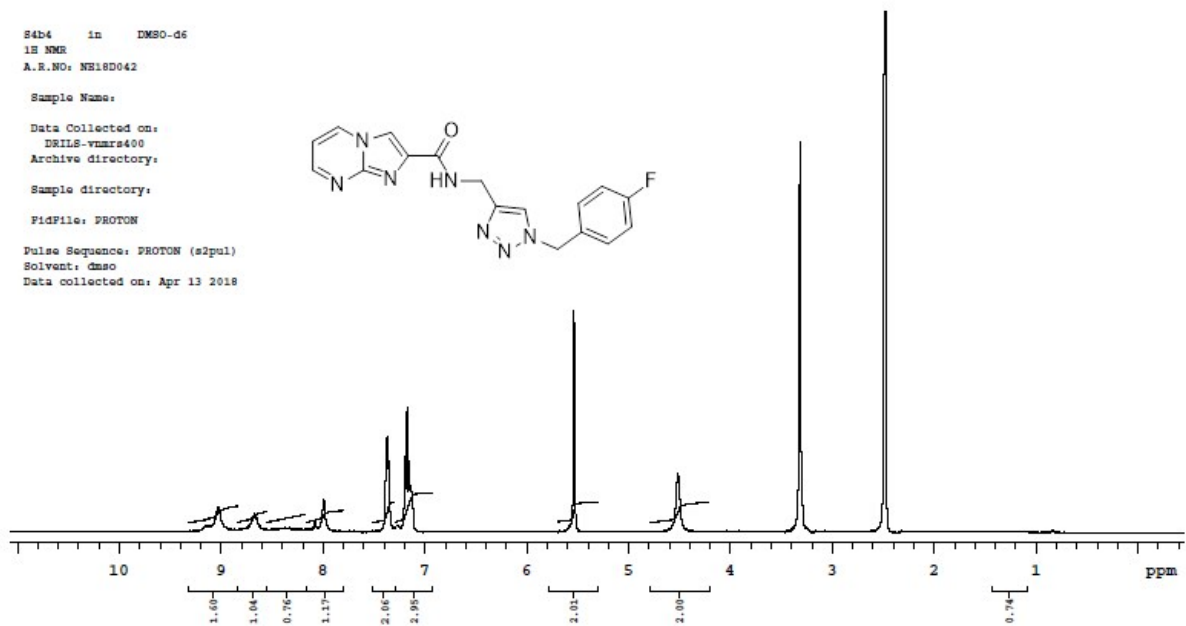


Figure S15. ¹H-NMR Spectrum of Compound 4m.

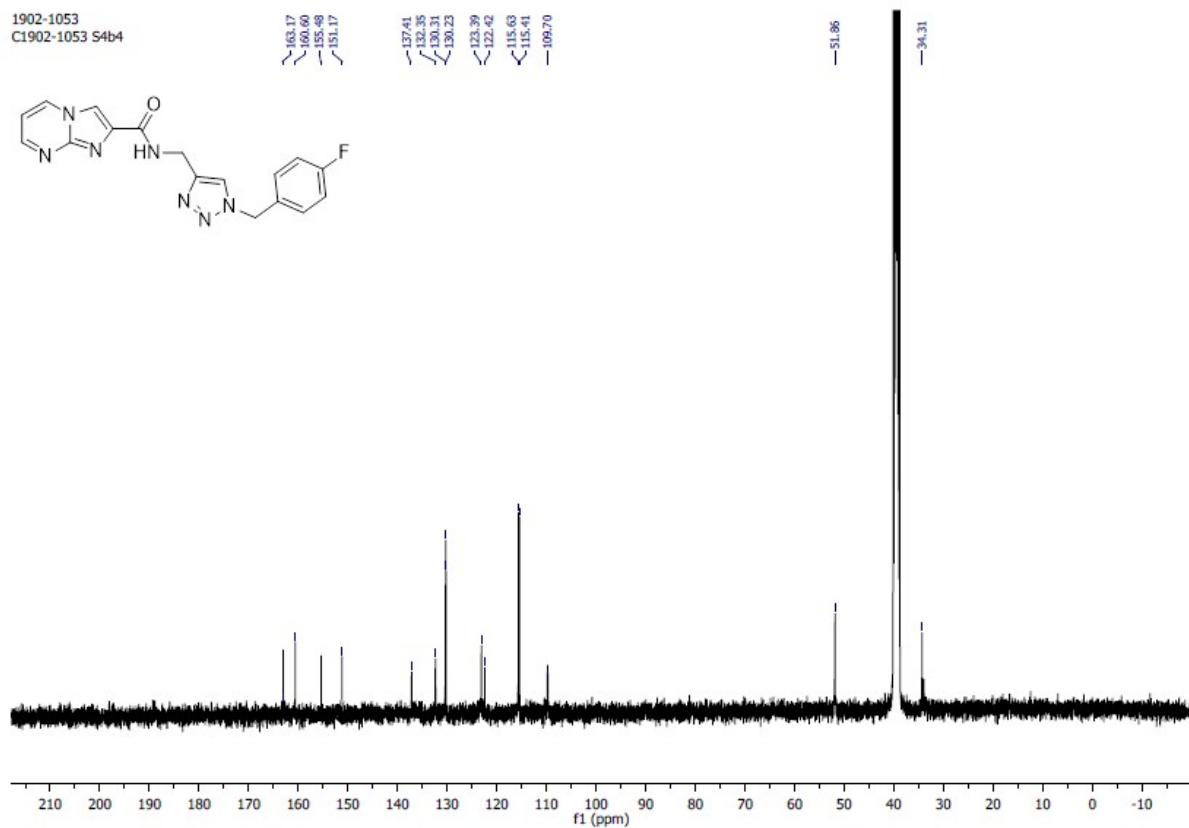


Figure S16. ¹³C-NMR Spectrum of Compound 4m.

Sample Name	T95	Position	Vial 23	Instrument Name	Instrument 1	User Name	
Inj Vol	3	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	140217M024.d	ACQ Method	MASS.m	Comment	ME17B025	Acquired Time	2/14/2017 3:44:21 PM

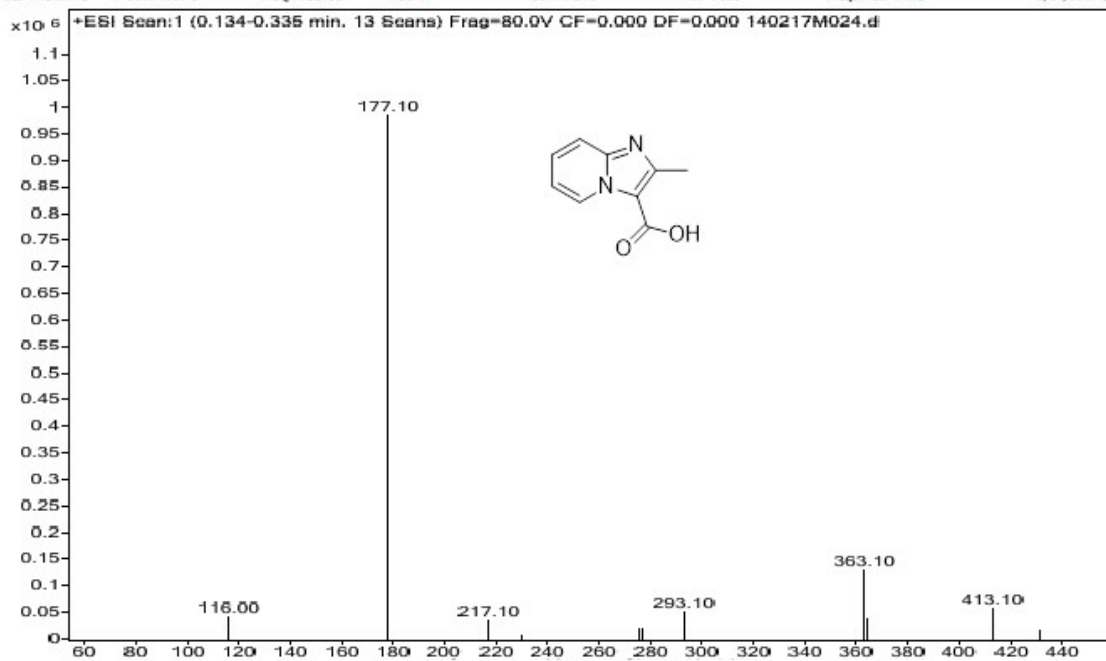


Figure S17. ESI-MS Spectrum of Compound 6.

Sample Name	PTC1	Position	Vial 21	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	140317M020.d	ACQ Method	MASS.m	Comment	ME17C019	Acquired Time	3/14/2017 1:46:54 PM

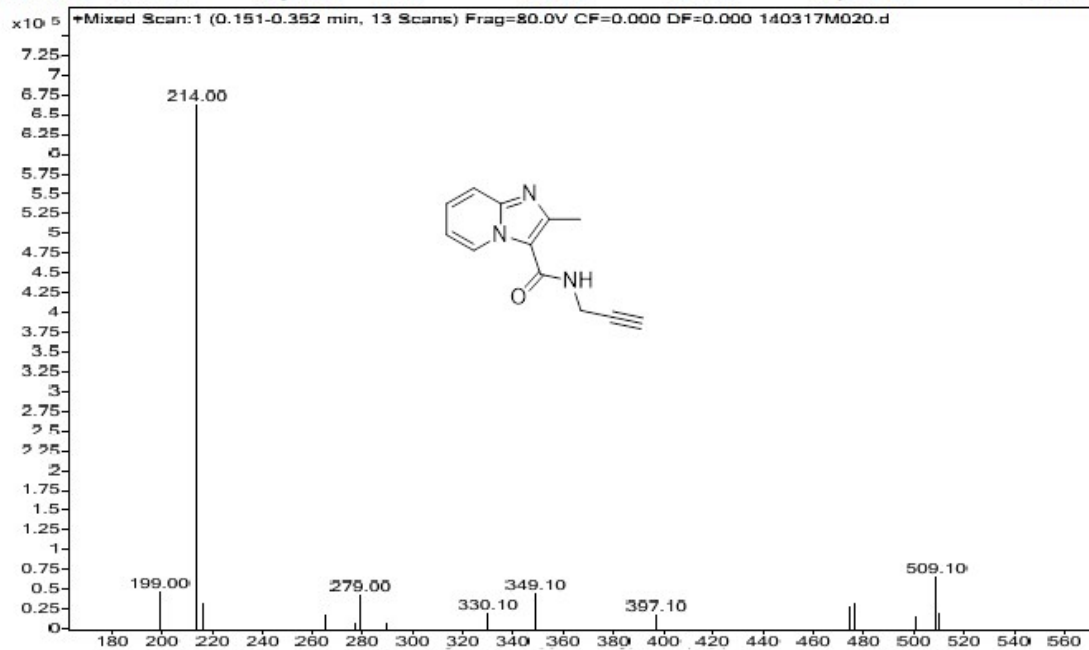


Figure S18. ESI-MS Spectrum of Compound 7.

Pi-M-Amide in DMSO-d6
 1H NMR
 A.R.NO: NH18D036
 Sample Name:
 Data Collected on:
 BRILS-vnmrs400
 Archive directory:
 Sample directory:
 FidFile: PROTON
 Pulse Sequence: PROTON (s2pul)
 Solvent: dmsc
 Data collected on: Apr 13 2018

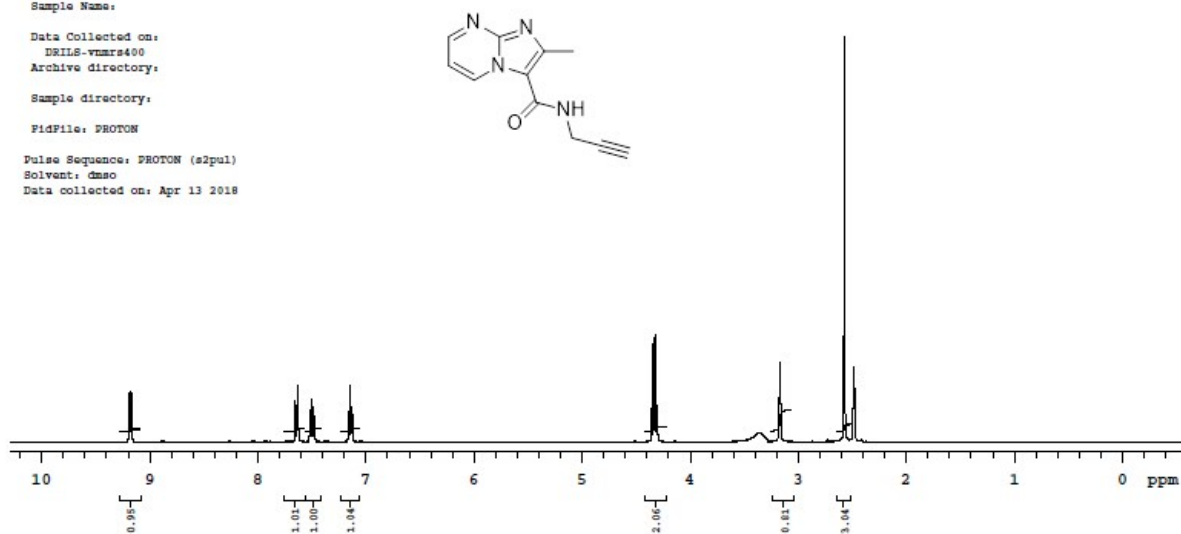


Figure S19. ¹H-NMR Spectrum of Compound 7a.

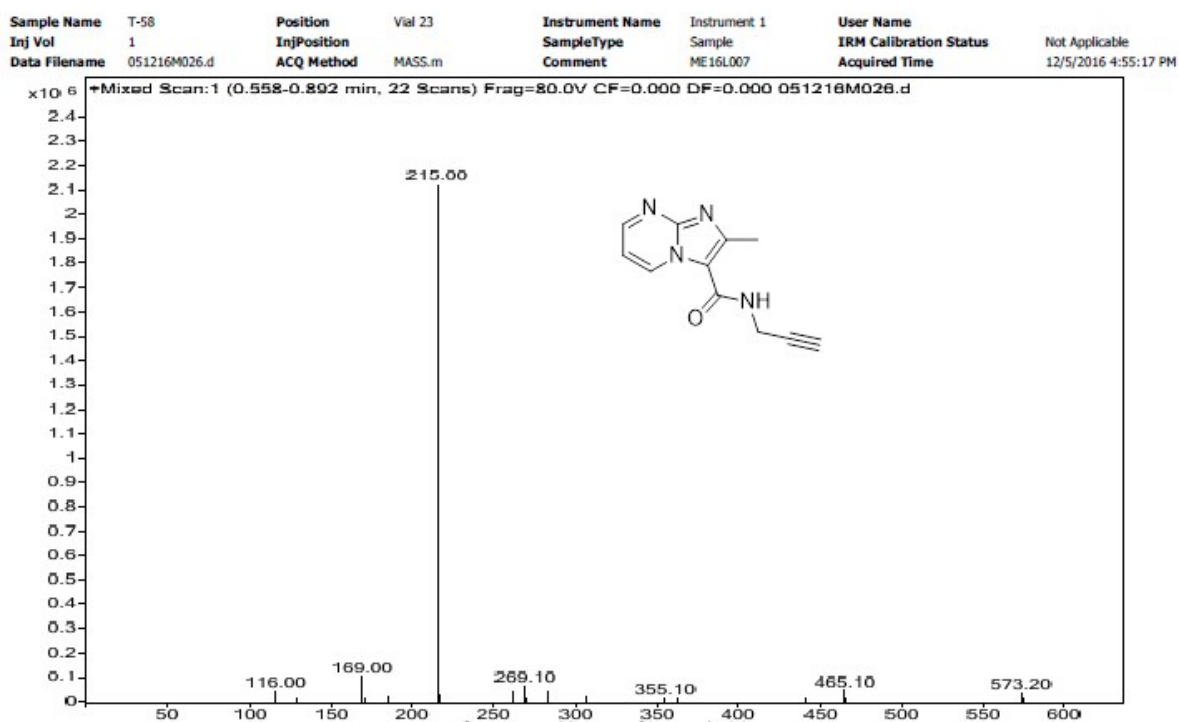


Figure S20. ESI-MS Spectrum of Compound 7a.

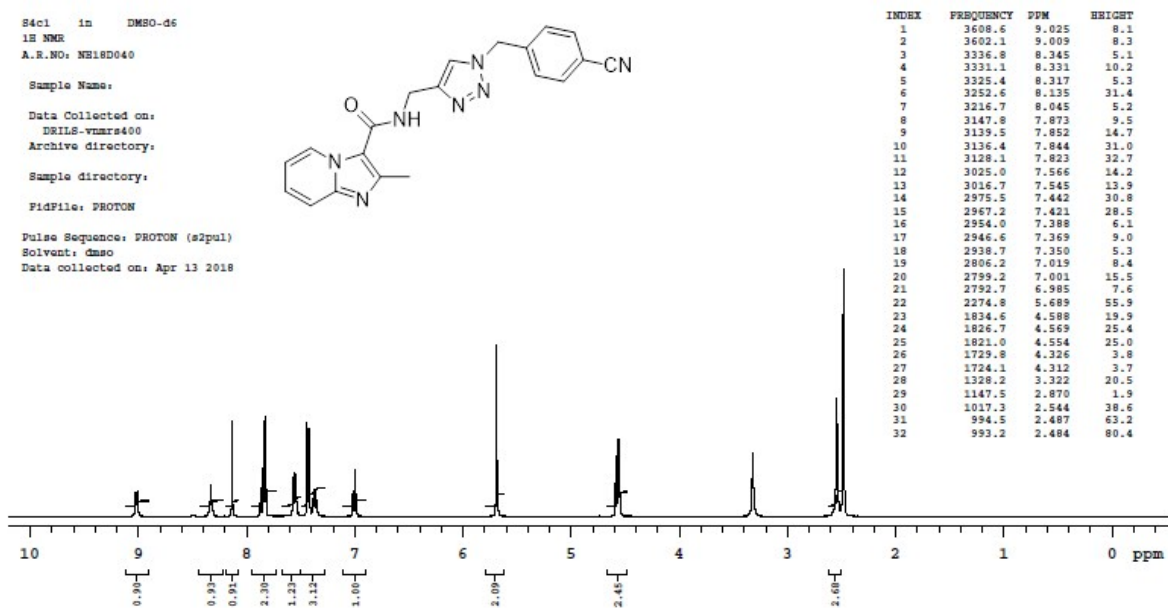


Figure S21. ¹H-NMR Spectrum of Compound 8a.

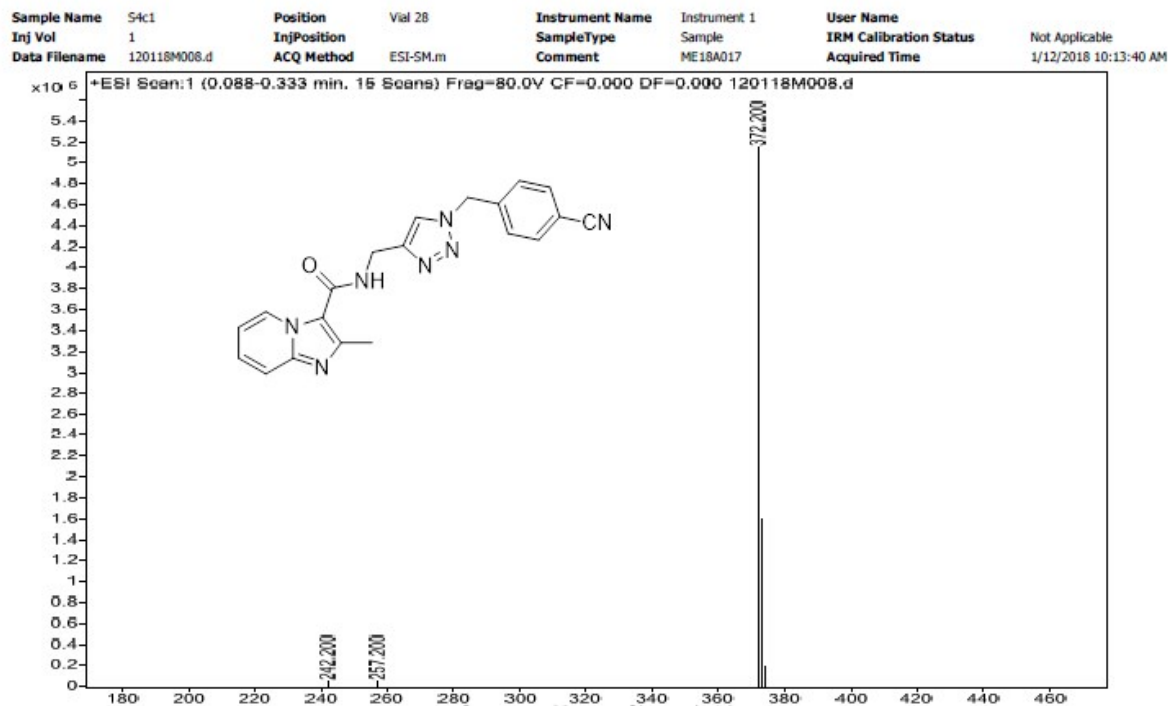


Figure S22. ESI-MS Spectrum of Compound 8a.

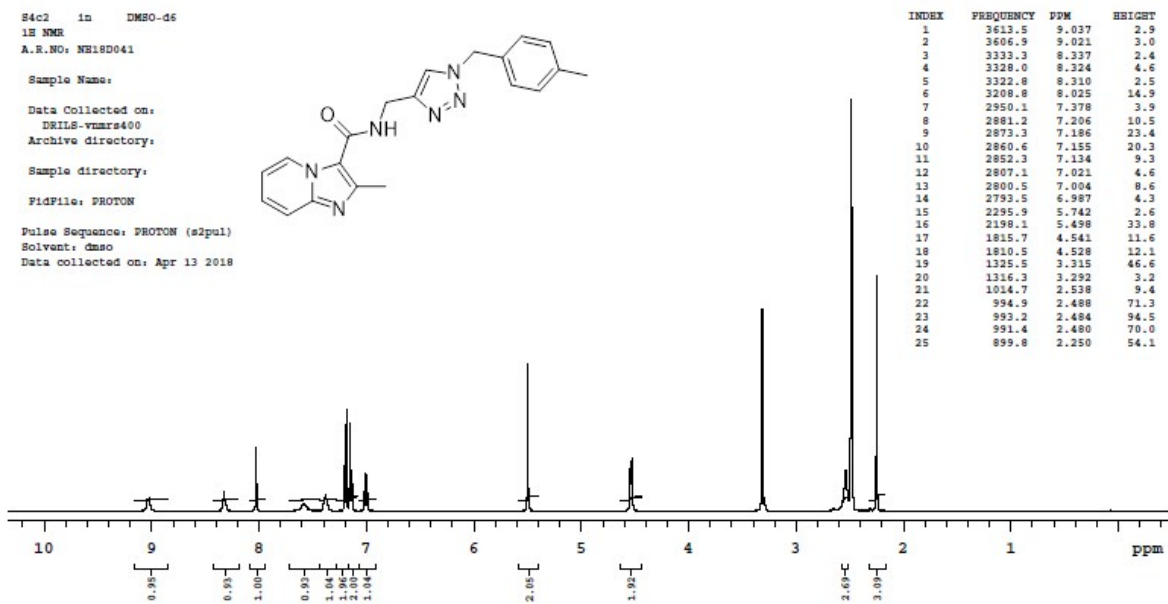


Figure S23. ¹H-NMR Spectrum of Compound **8b**.

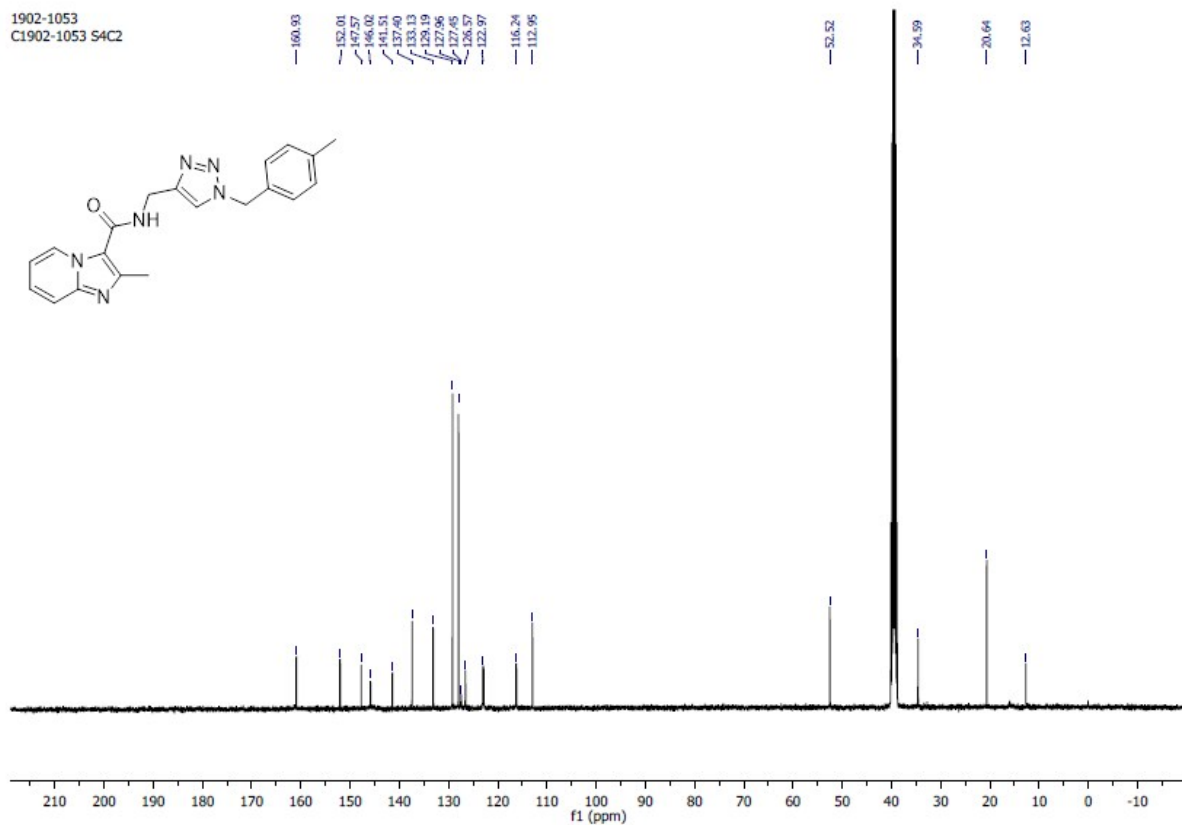


Figure S24. ¹³C-NMR Spectrum of Compound **8b**.

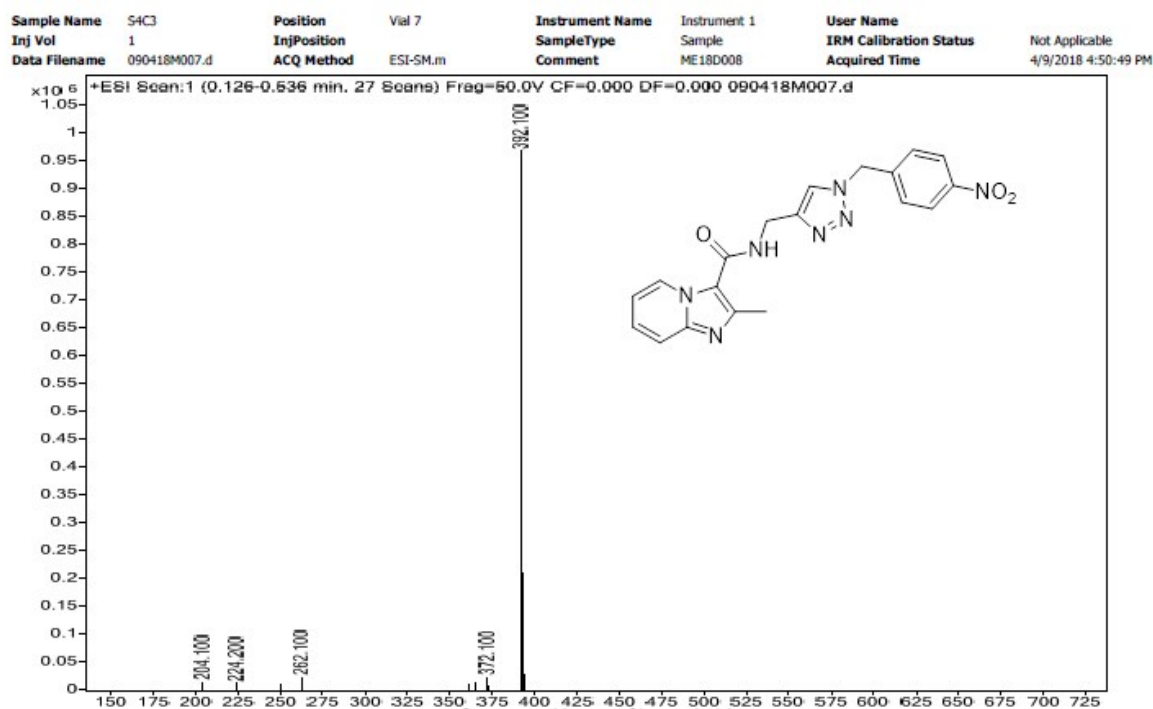


Figure S25. ESI-MS Spectrum of Compound 8c.

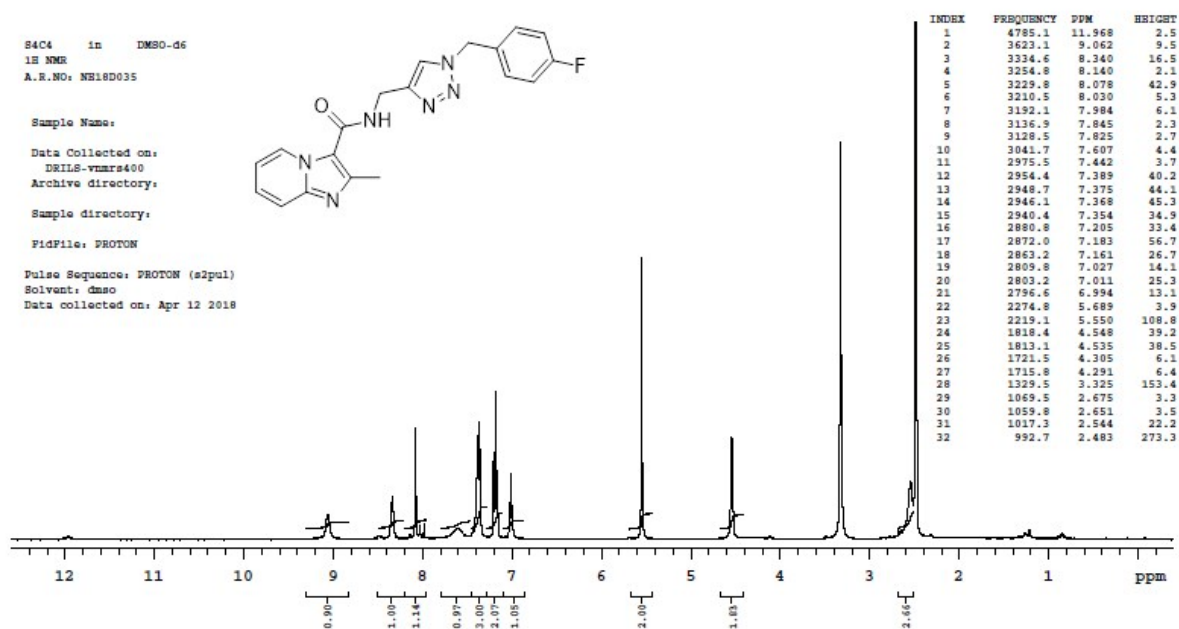


Figure S26. ¹H-NMR Spectrum of Compound 8d.

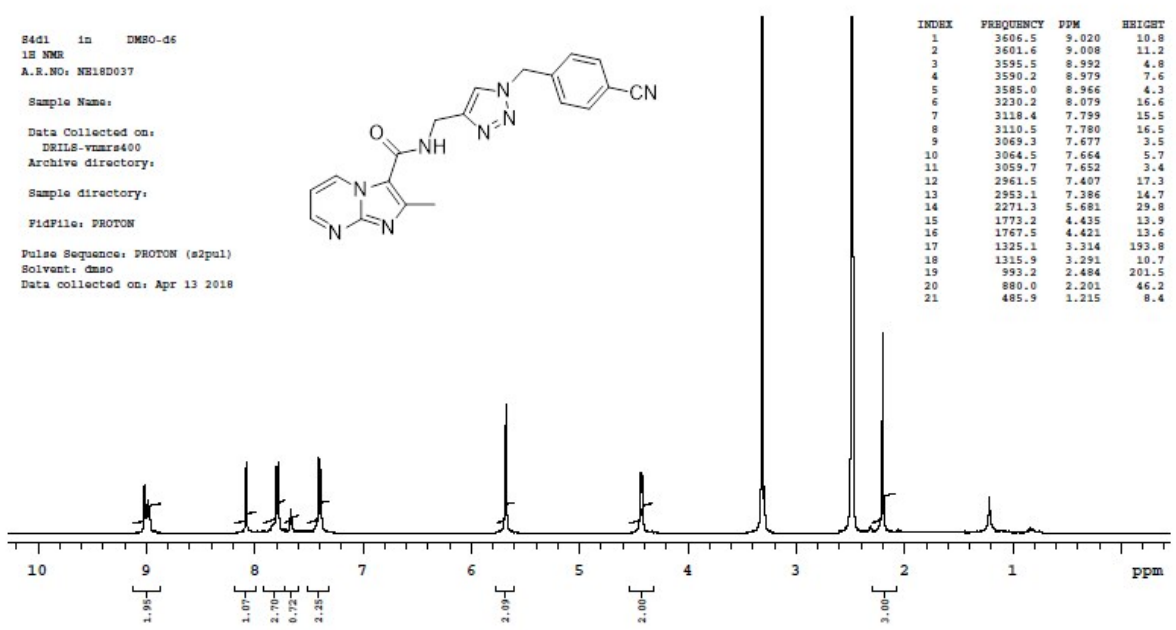


Figure S27. ¹H-NMR Spectrum of Compound 8j.

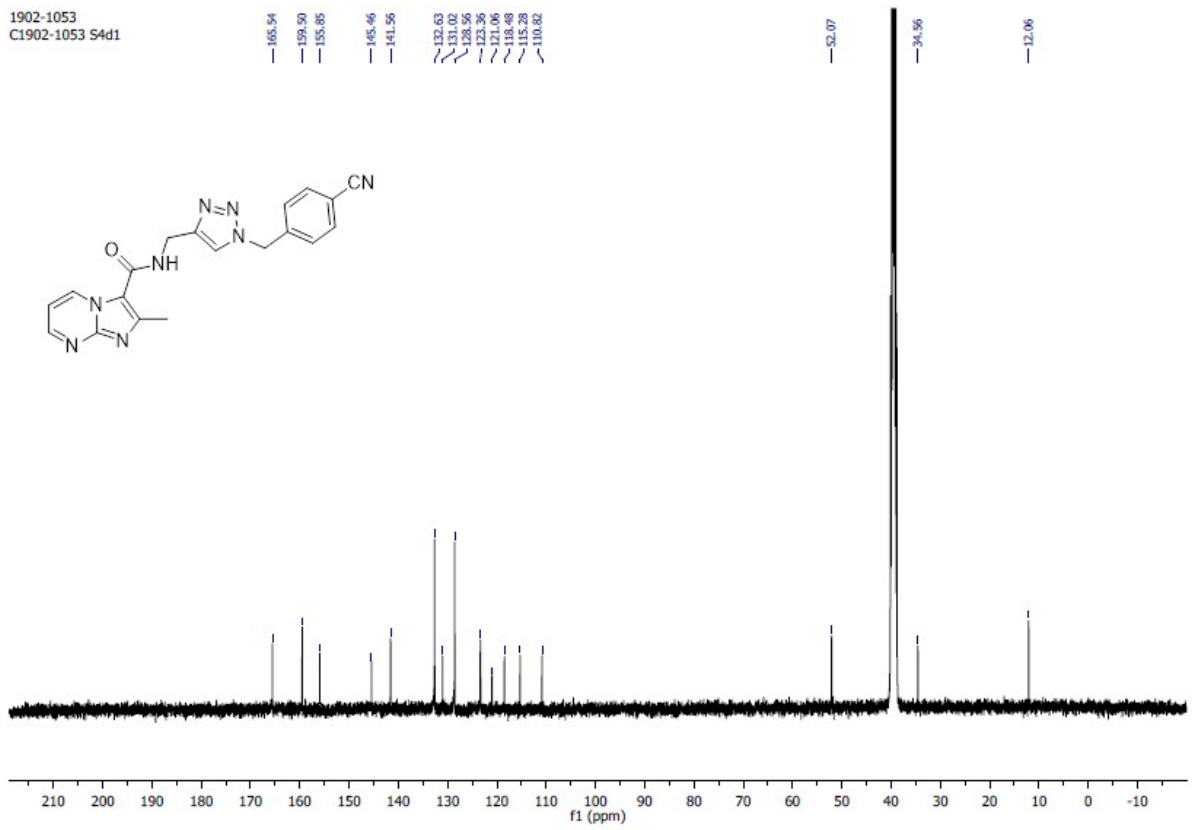


Figure S28. ¹³C-NMR Spectrum of Compound 8j.

Sample Name	S4d1	Position	Vial 29	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	120118M009.d	ACQ Method	ESI-SM.m	Comment	ME18A018	Acquired Time	1/12/2018 10:15:37 AM

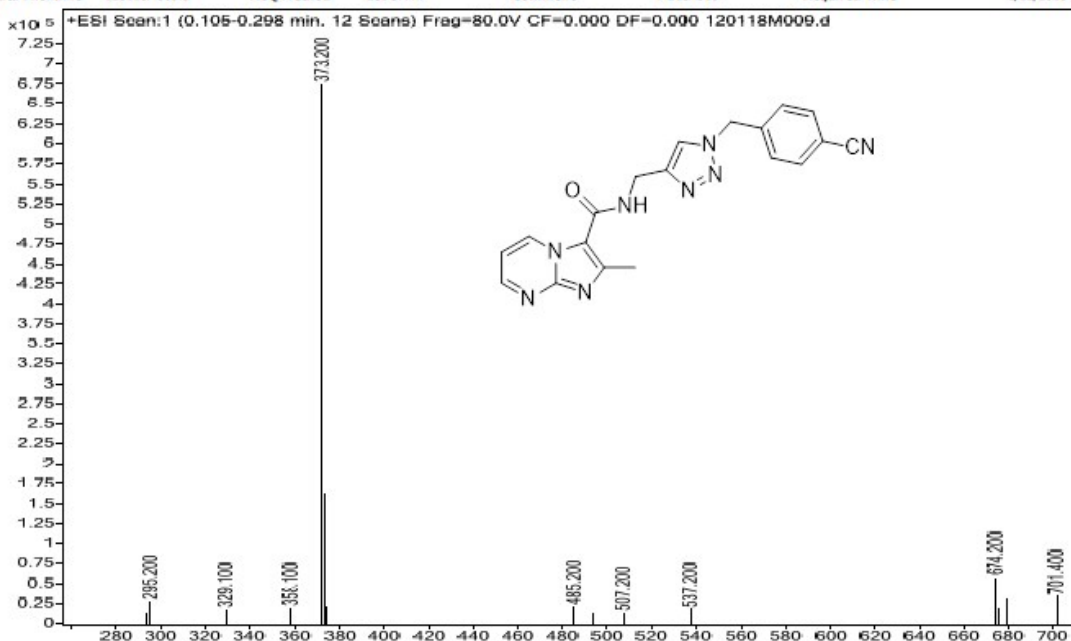


Figure S29. ESI-MS Spectrum of Compound 8j.

Sample Name	PMA1	Position	Vial 72	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	100517M021.d	ACQ Method	MASS.m	Comment	ME17E048	Acquired Time	5/10/2017 2:55:43 PM

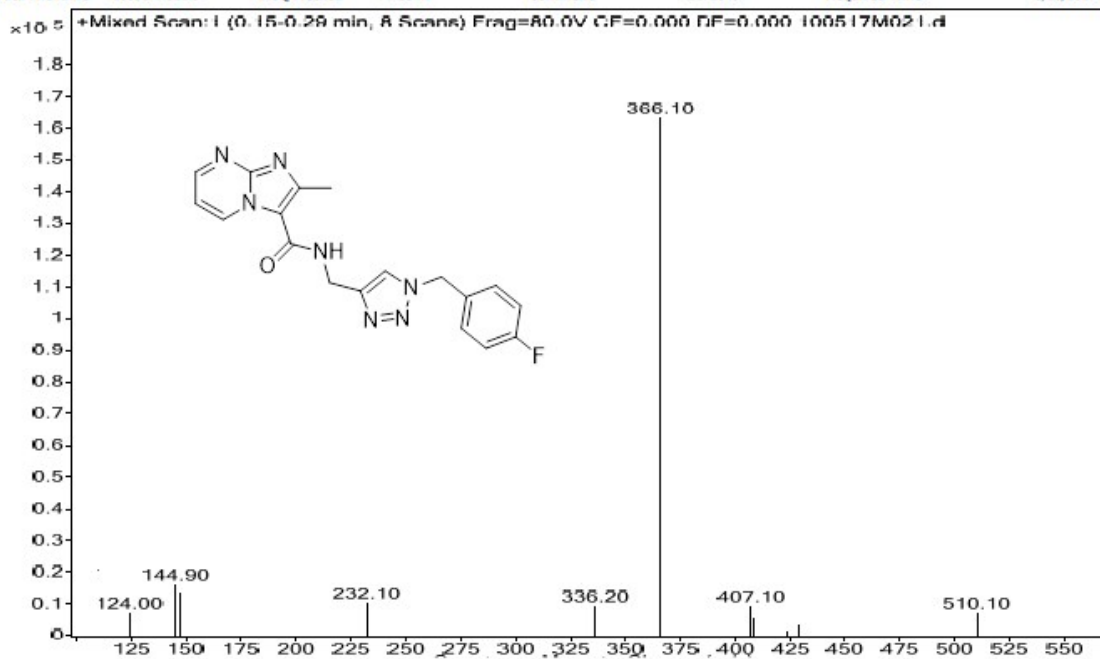


Figure S30. ESI-MS Spectrum of Compound 8m.

Sample Name	T-69	Position	Vial 17	Instrument Name	Instrument 1	User Name	
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	161216M021.d	ACQ Method	MASS.m	Comment	ME16L059	Acquired Time	12/16/2016 10:59:20 AM

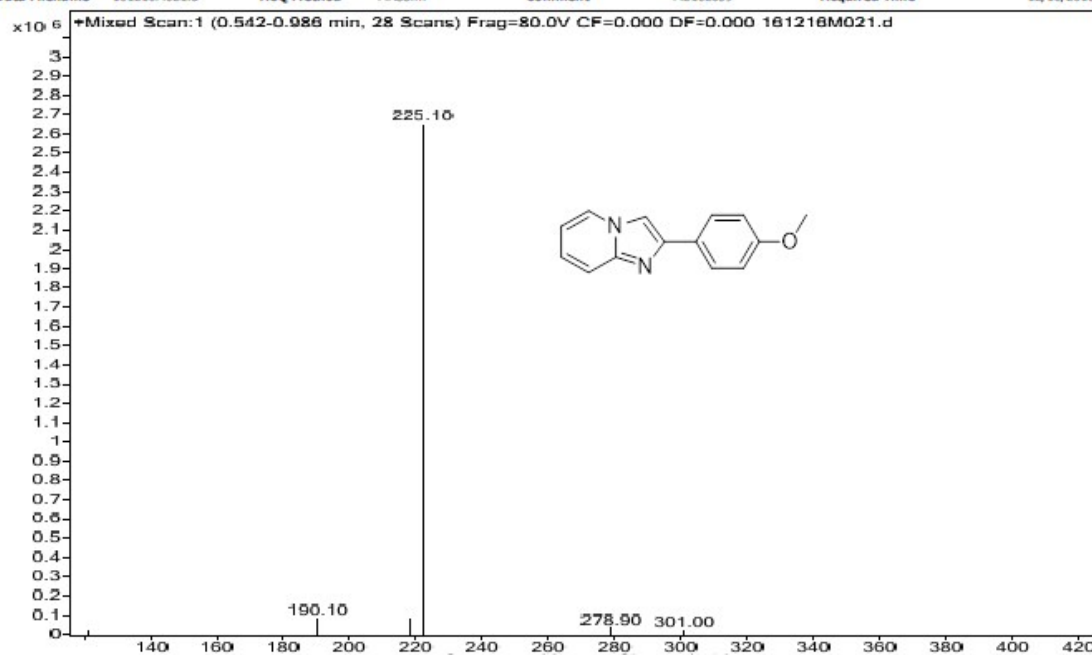


Figure S31. ESI-MS Spectrum of Compound 9.

Sample Name	PY-Ald	Position	Vial 28	Instrument Name	Instrument 1	User Name	
Inj Vol	1	InjPosition		SampleType	Sample	IRM Calibration Status	Not Applicable
Data Filename	280817M008.d	ACQ Method	ESI-SM.m	Comment	MM17H016	Acquired Time	8/28/2017 3:39:58 PM

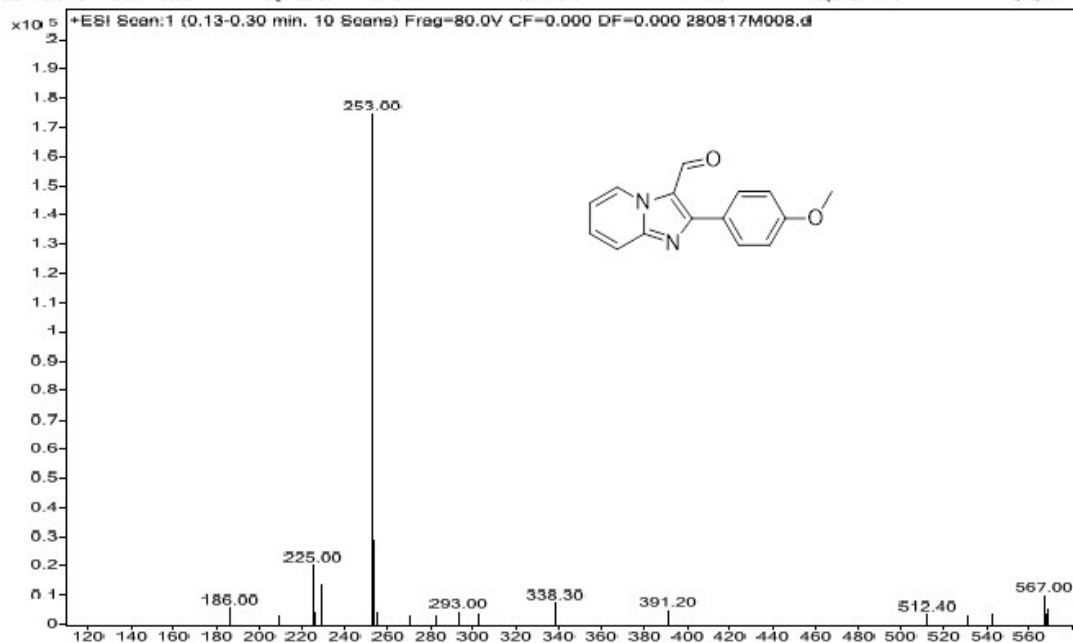


Figure S32. ESI-MS Spectrum of Compound 10.

Sample Name T103 Position Vial 30 Instrument Name Instrument 1 User Name
 Inj Vol 3 InjPosition SampleType Sample IRM Calibration Status Not Applicable
 Data Filename 140217M031.d ACQ Method MASS.m Comment ME17B032 Acquired Time 2/14/2017 4:09:18 PM

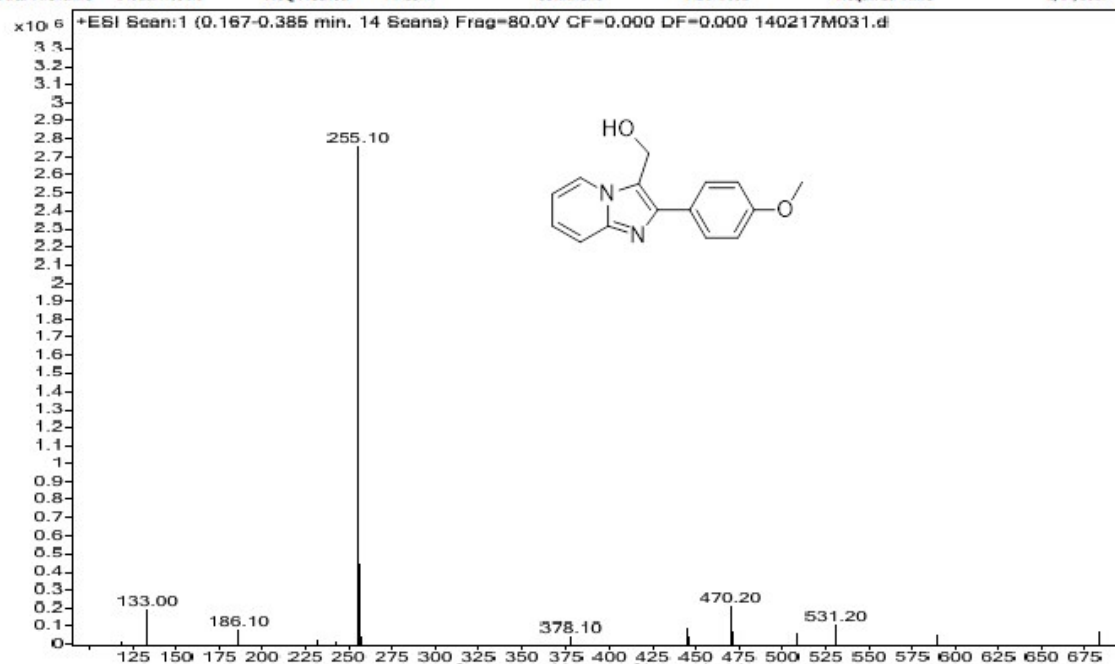


Figure S33. ESI-MS Spectrum of Compound 11.

Py-Ether in DMSO-d6
 1H NMR
 A.R.NO: NE18D032

Sample Name:

Data Collected on:
 DRILLS-vnmrs400
 Archive directory:

Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
 Solvent: dmsc
 Data collected on: Apr 12 2018

INDEX	FREQUENCY	DDM	BRIGHT
1	3366.2	8.419	11.5
2	3098.3	7.749	20.7
3	3099.1	7.751	20.8
4	3029.9	7.578	9.9
5	3024.2	7.564	10.3
6	2909.3	7.276	10.5
7	2816.8	7.045	21.7
8	2779.5	6.952	11.7
9	2142.0	5.357	11.6
10	1948.2	4.872	24.9
11	1518.0	3.797	47.2
12	1331.7	3.131	15.4

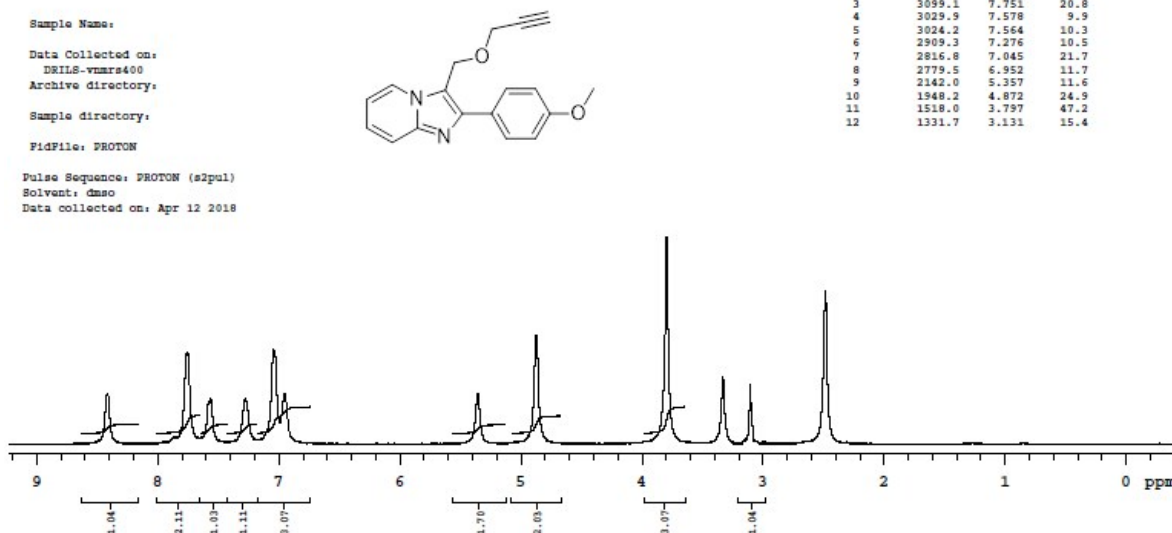


Figure S34. ¹H-NMR Spectrum of Compound 12.

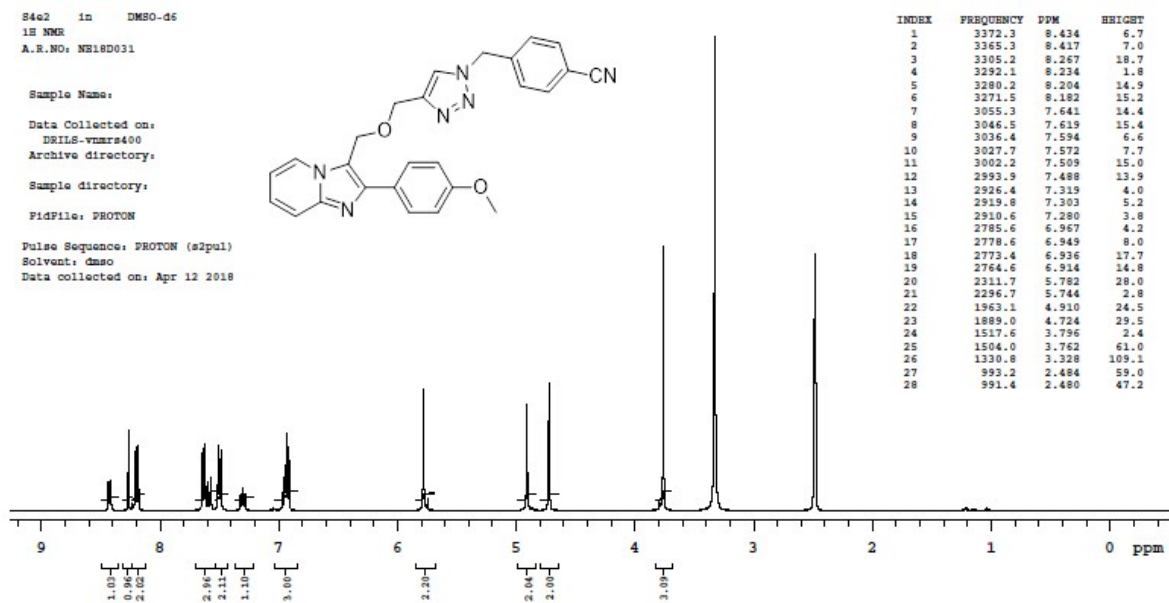


Figure S35. ¹H-NMR Spectrum of Compound 13a.

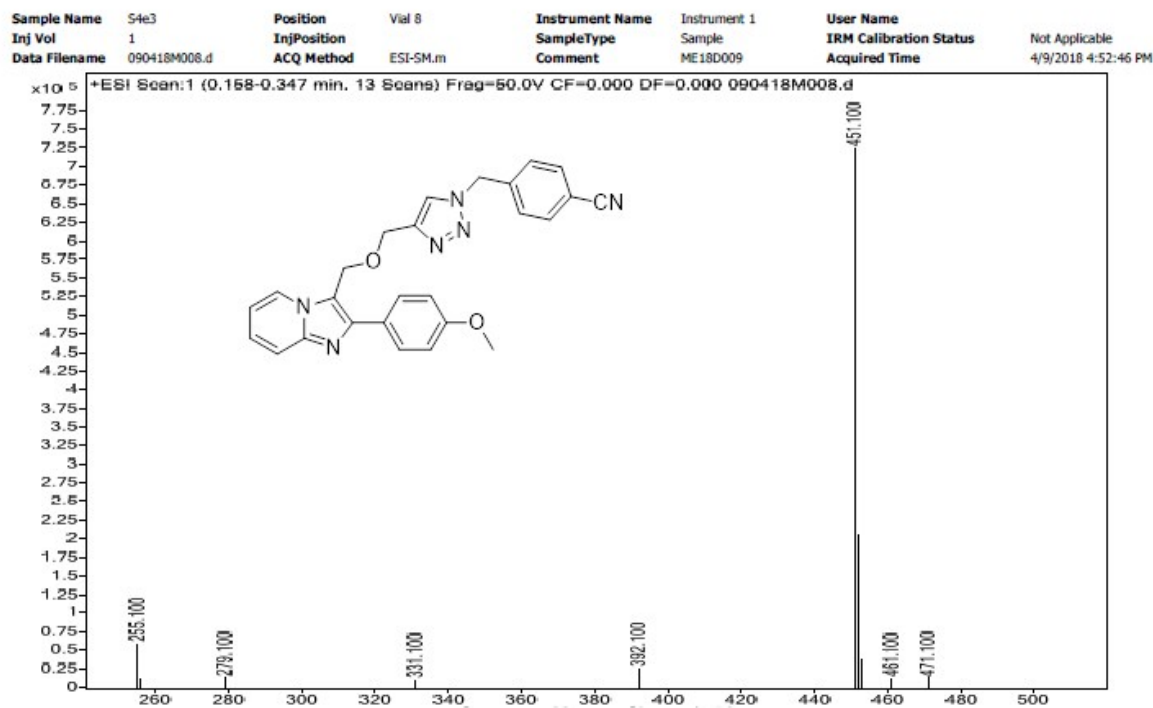


Figure S36. ESI-MS Spectrum of Compound 13a.

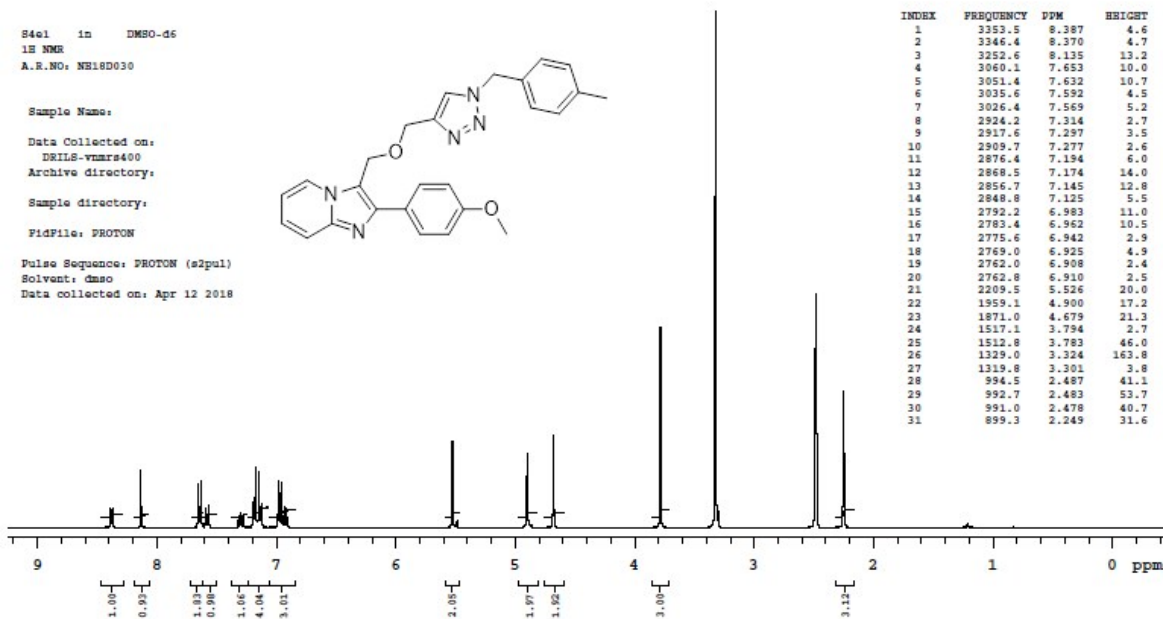


Figure S37. ¹H-NMR Spectrum of Compound 13b.

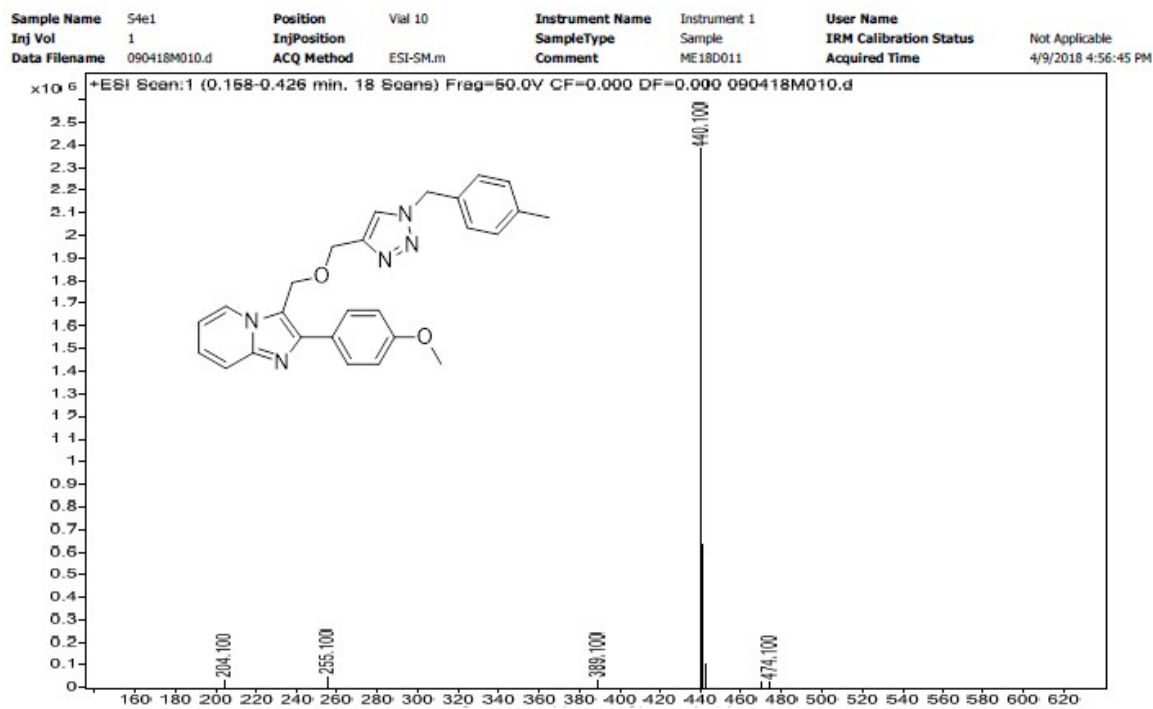


Figure S38. ESI-MS Spectrum of Compound 13b.