Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Microwave assisted copper (*I*) catalyzed A^3 cascade coupling of imidazo[1,2-*a*]pyridines *via* C-H bond functionalization as selective COX-2 inhibitors, antioxidants and *in silico* studies

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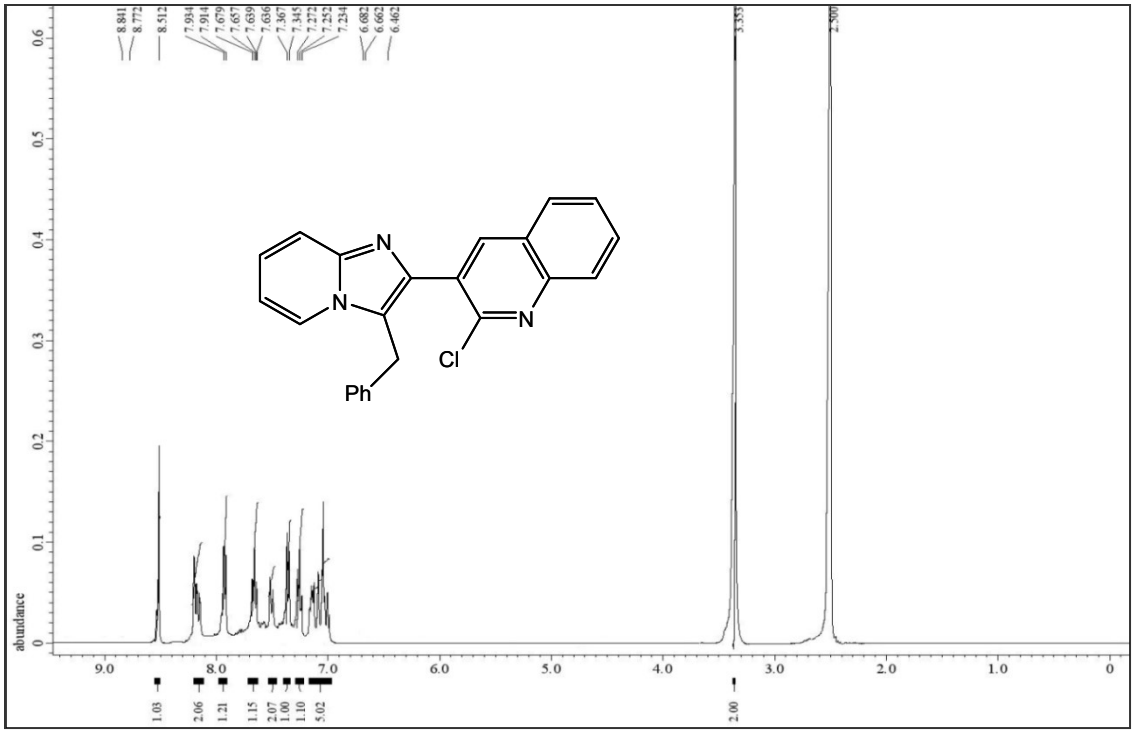
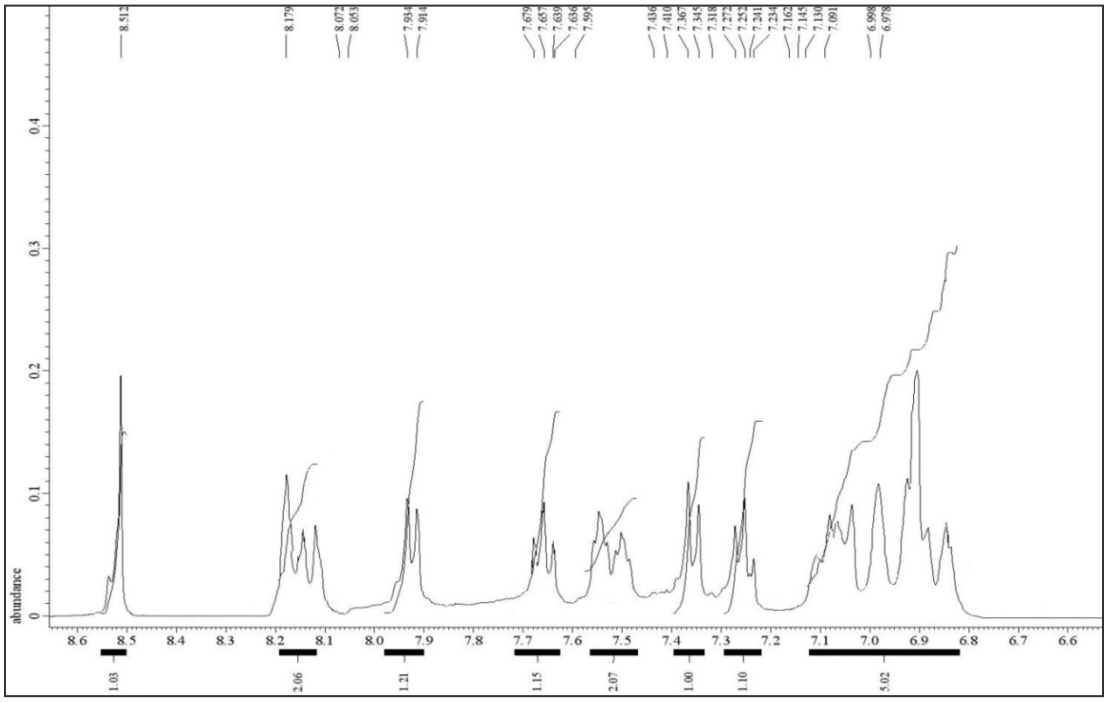


Spectrum No. 1 ^{1}H NMR spectrum of compound 8e (DMSO- $d_{6,}$ 400 MHz)



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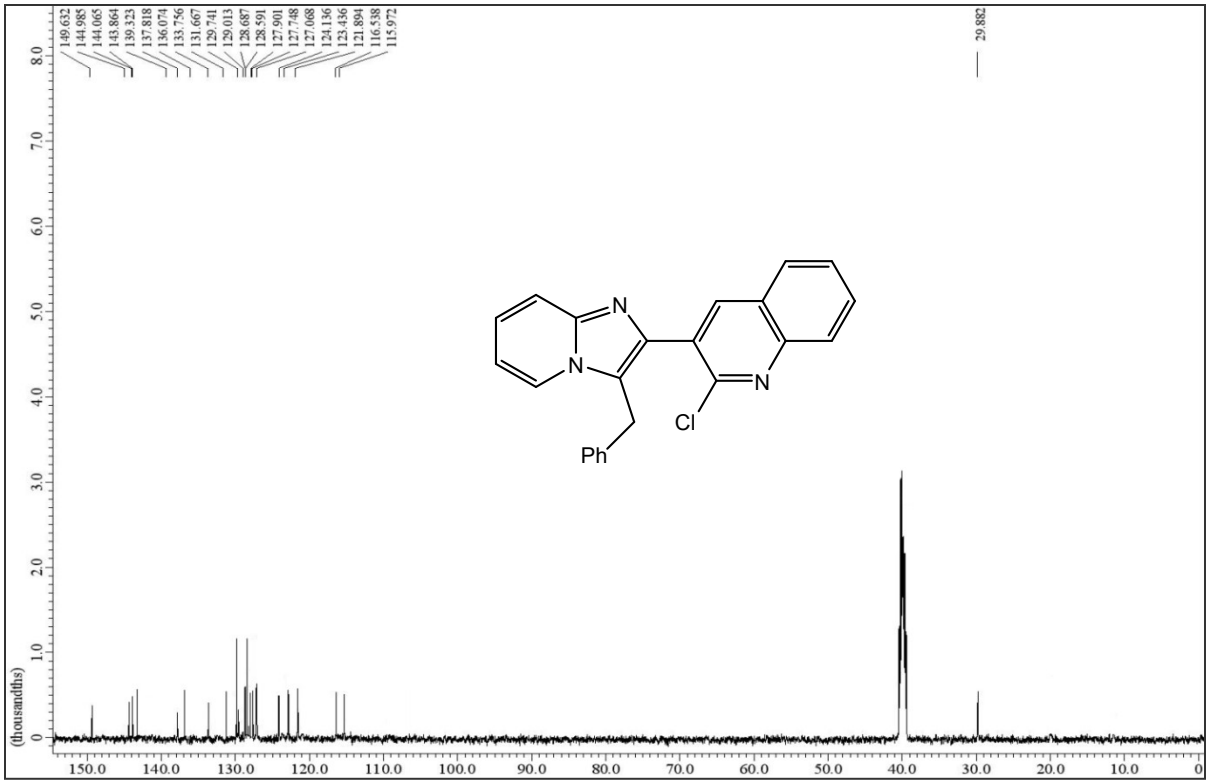
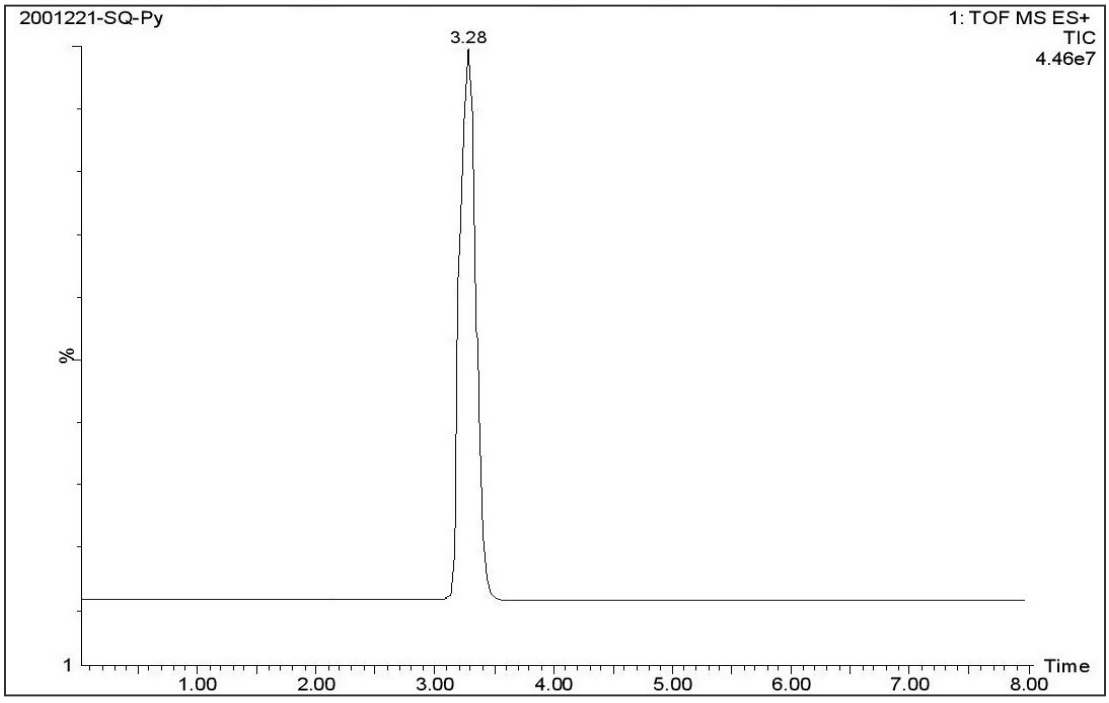


Spectrum No. 3 ^{13}C NMR spectrum of compound 8e (DMSO- d_6, 100 MHz)



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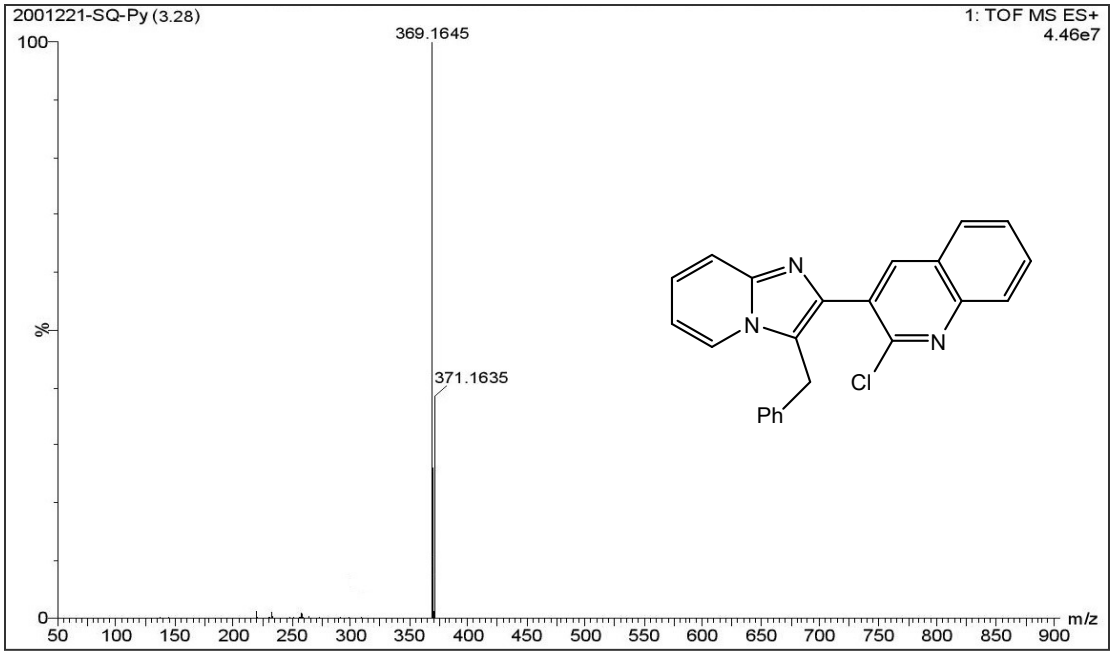

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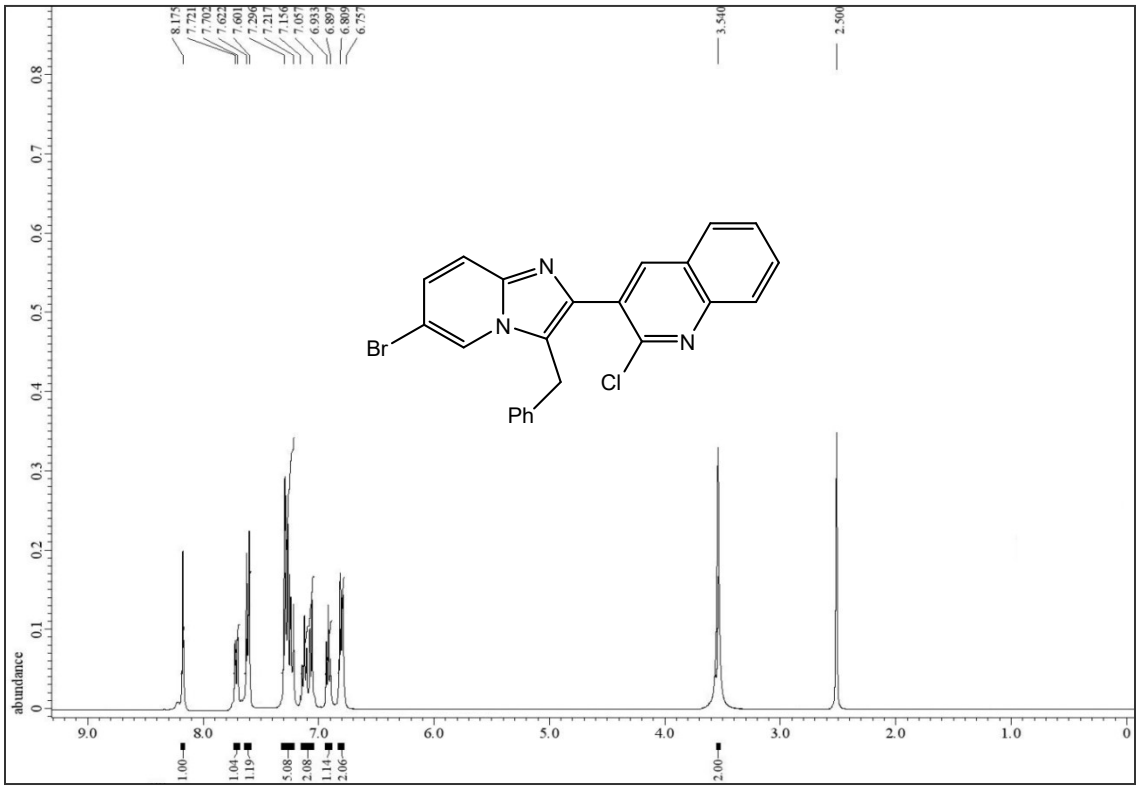

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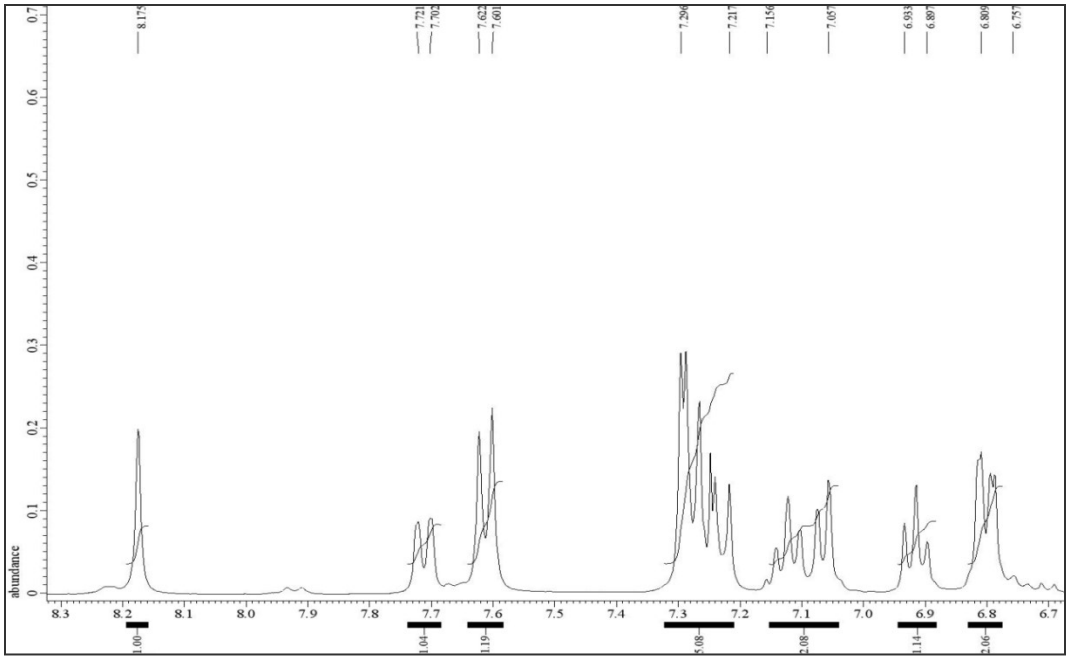

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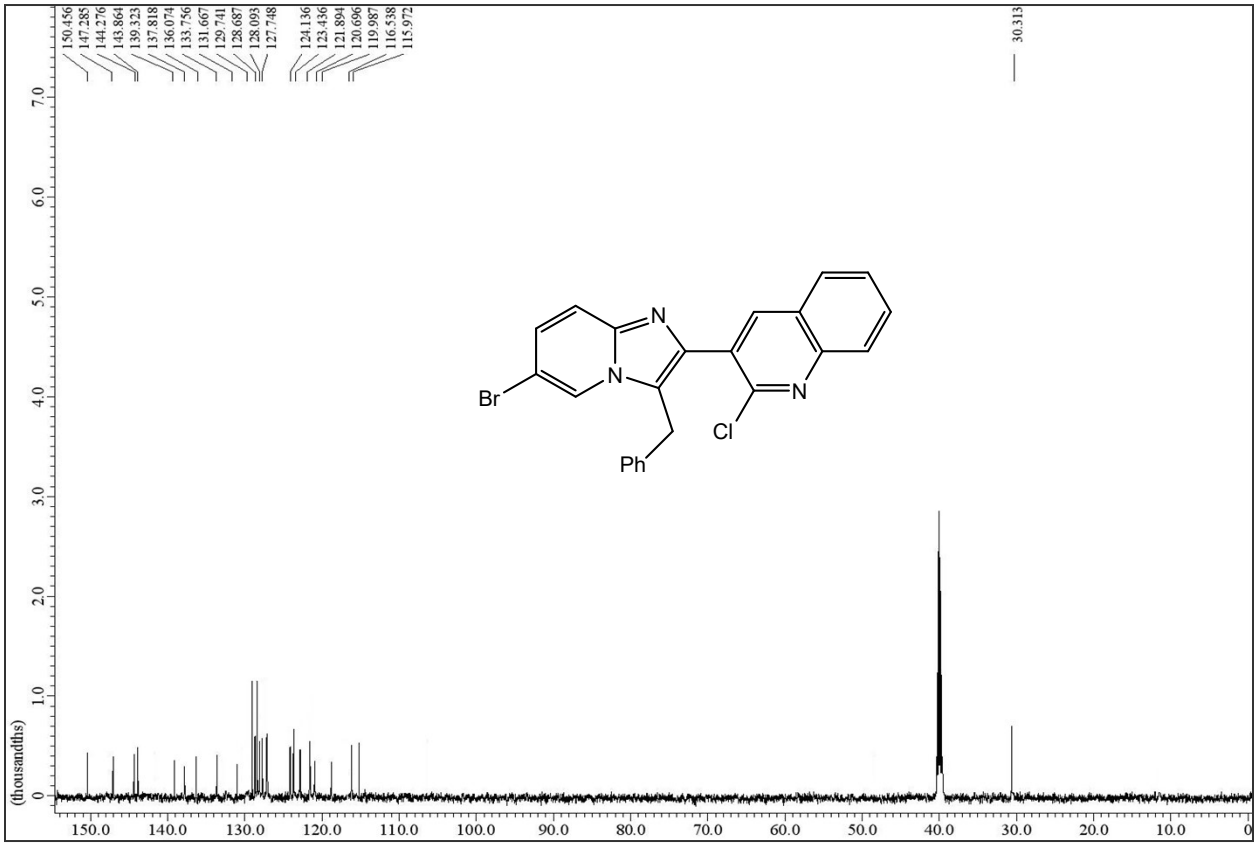

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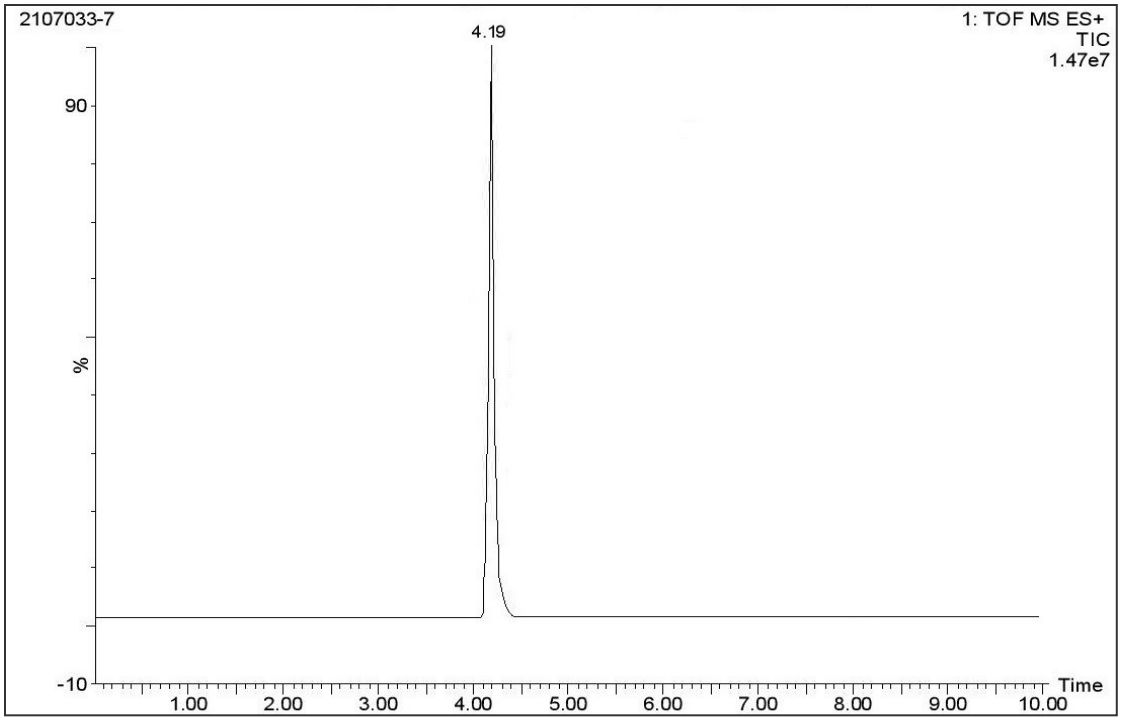

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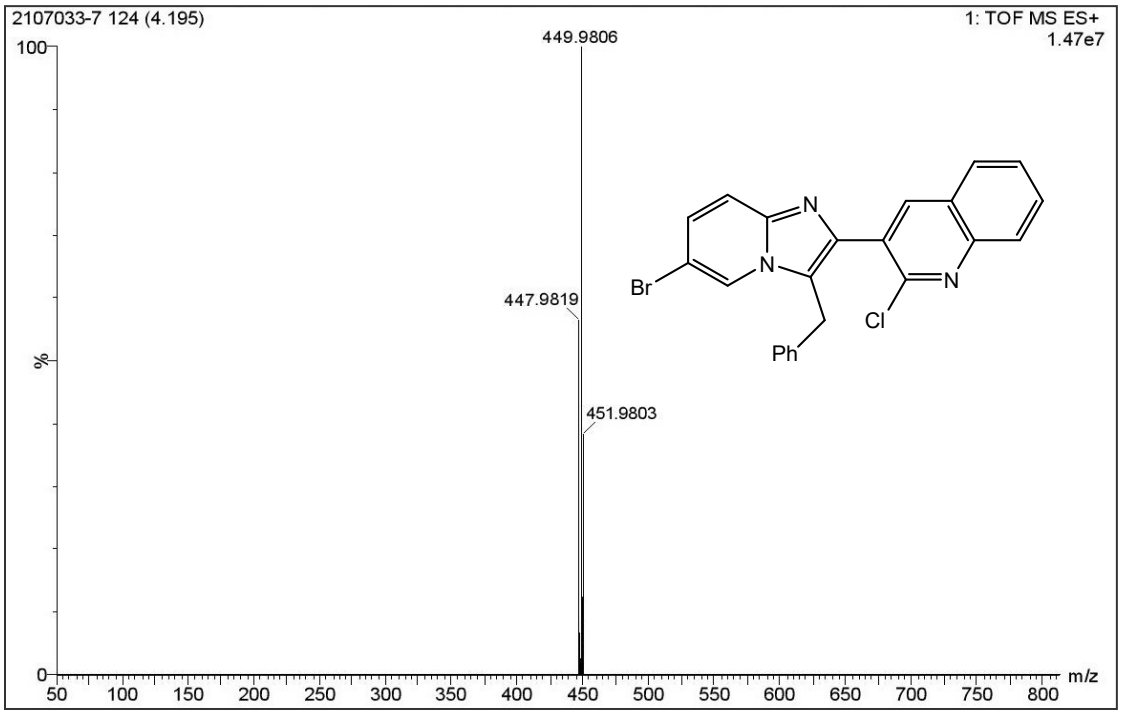

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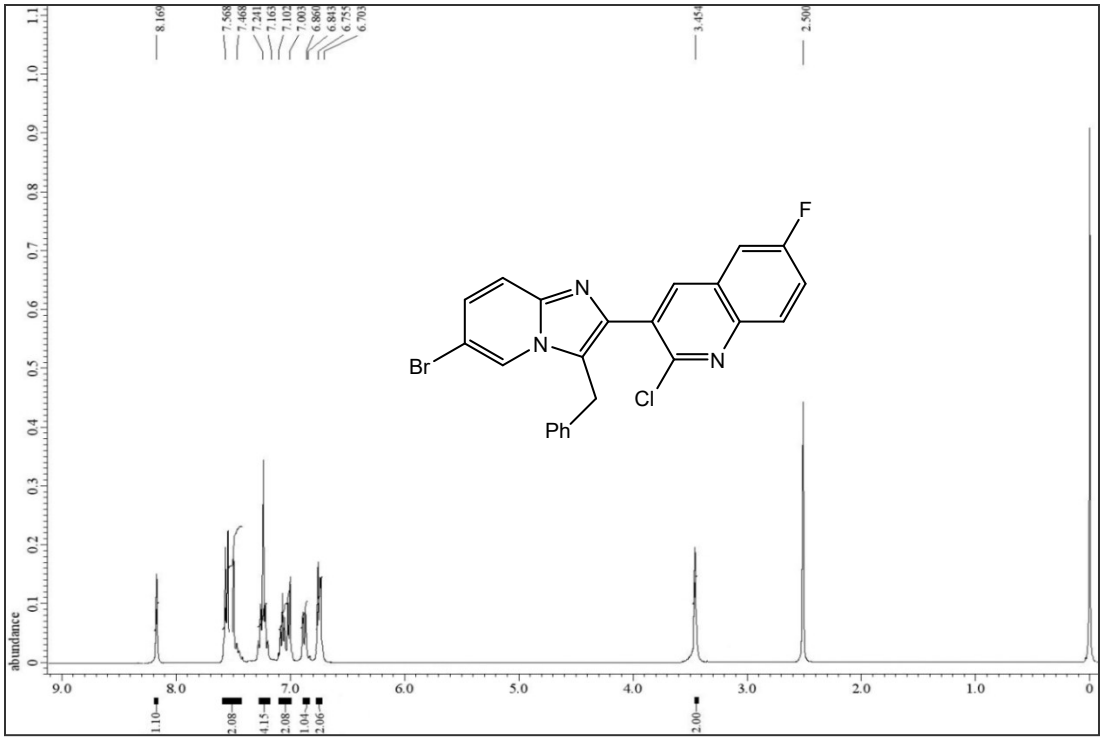

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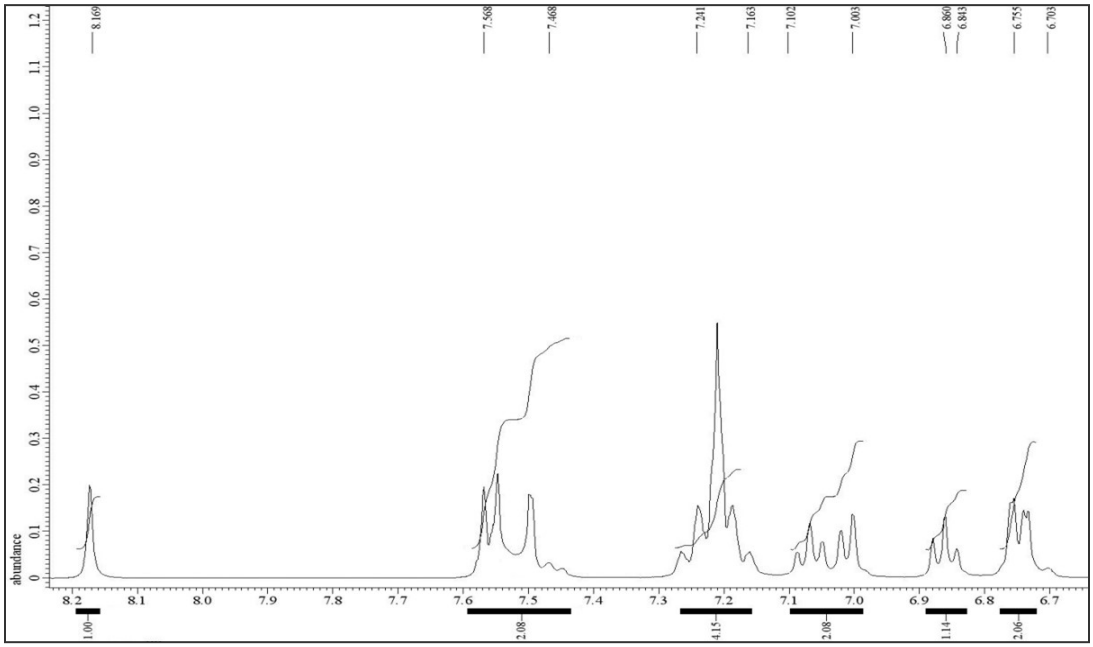

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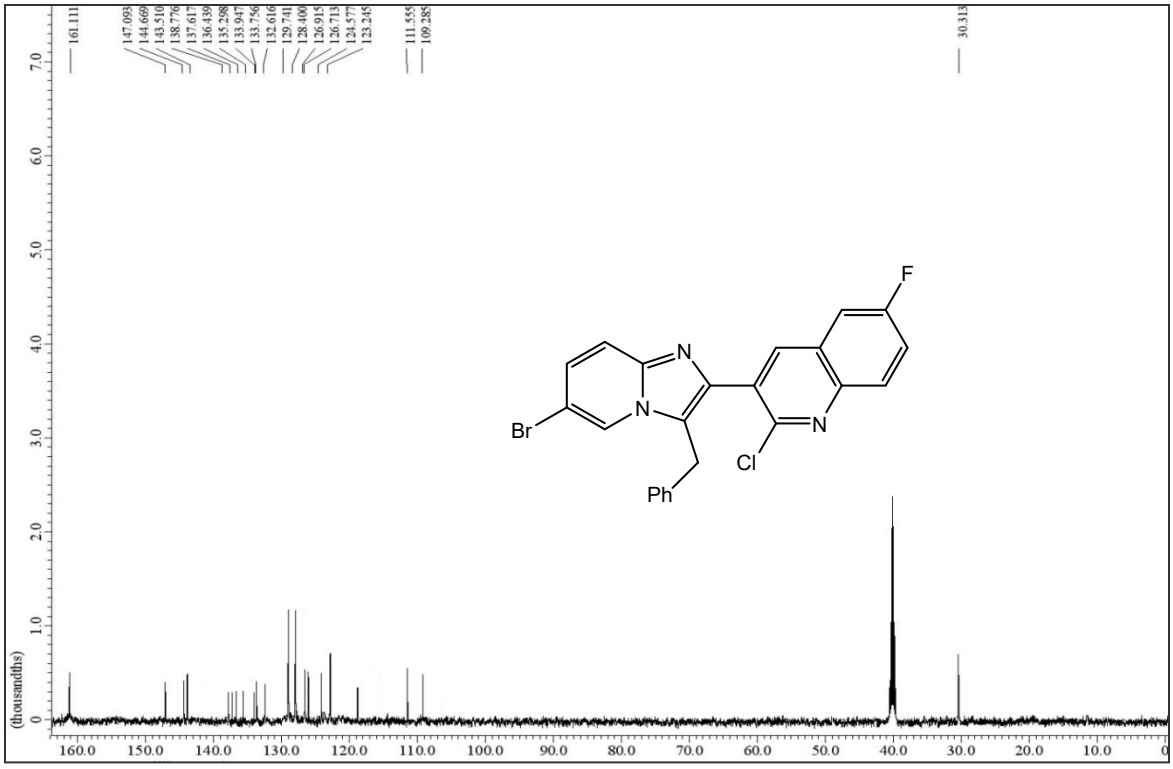

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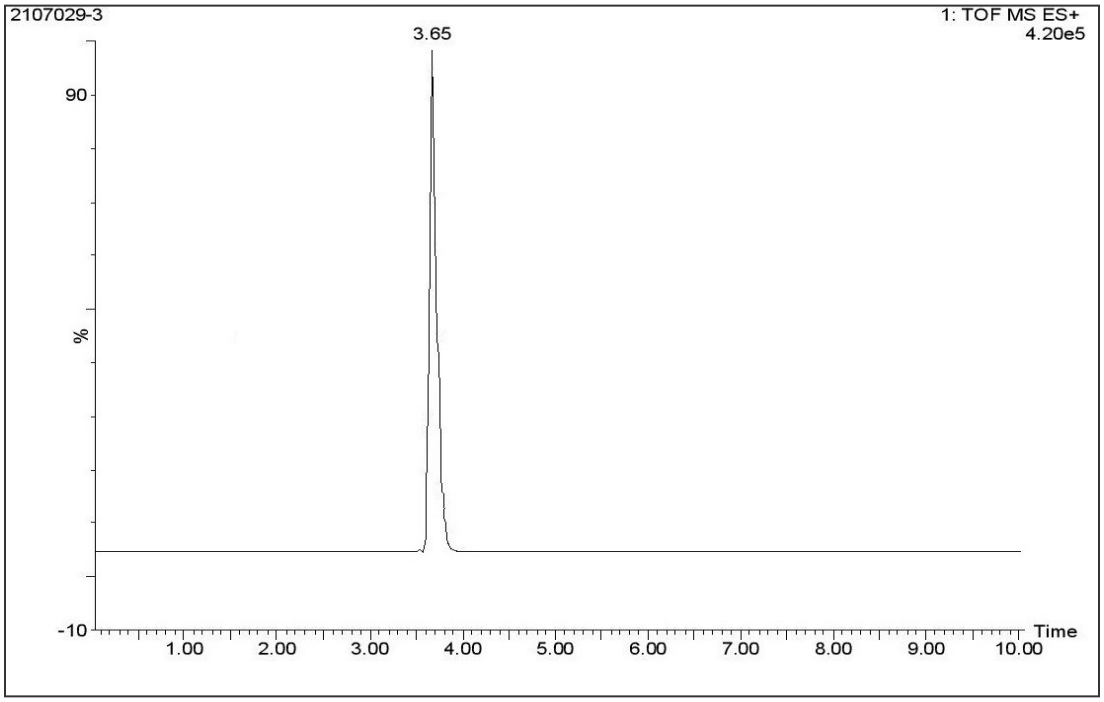

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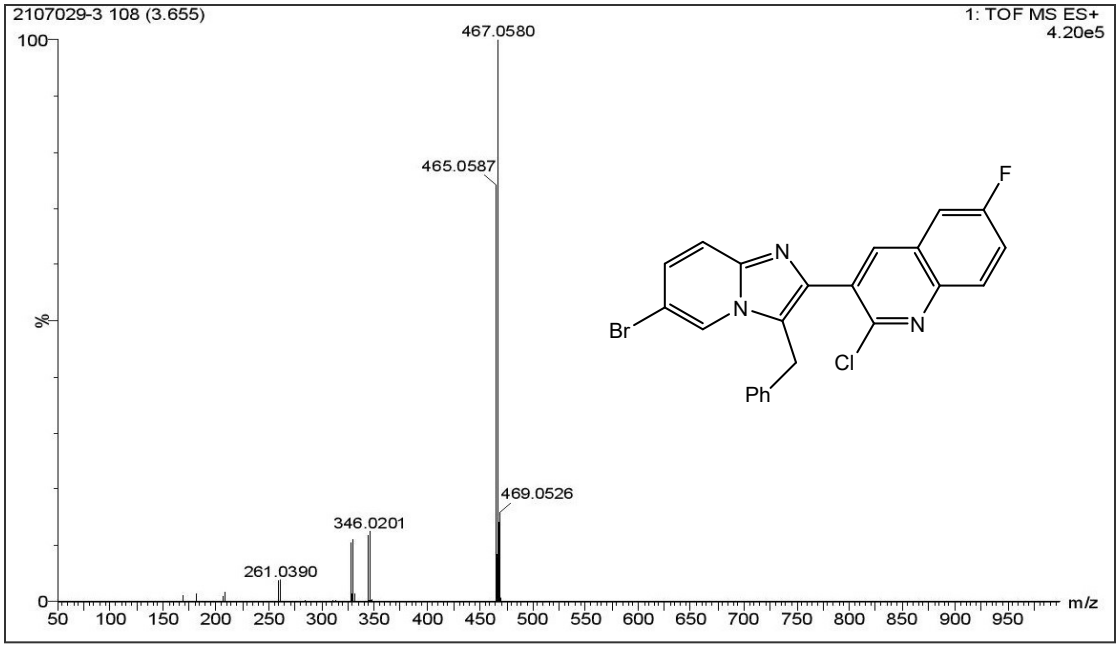
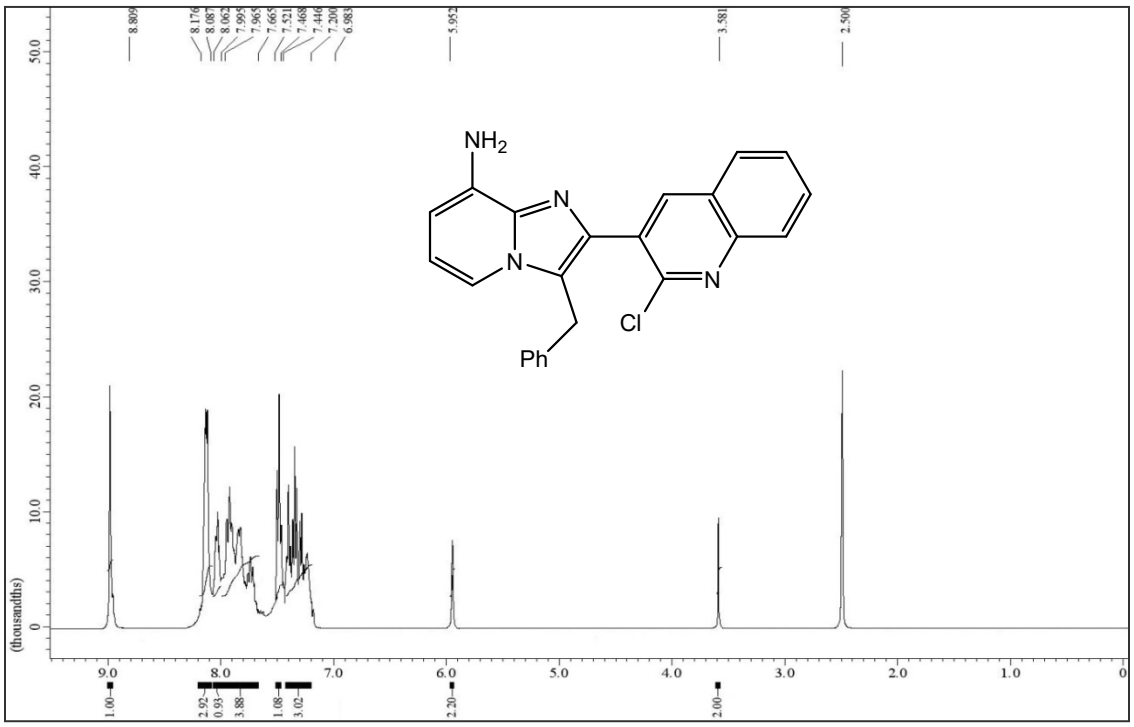
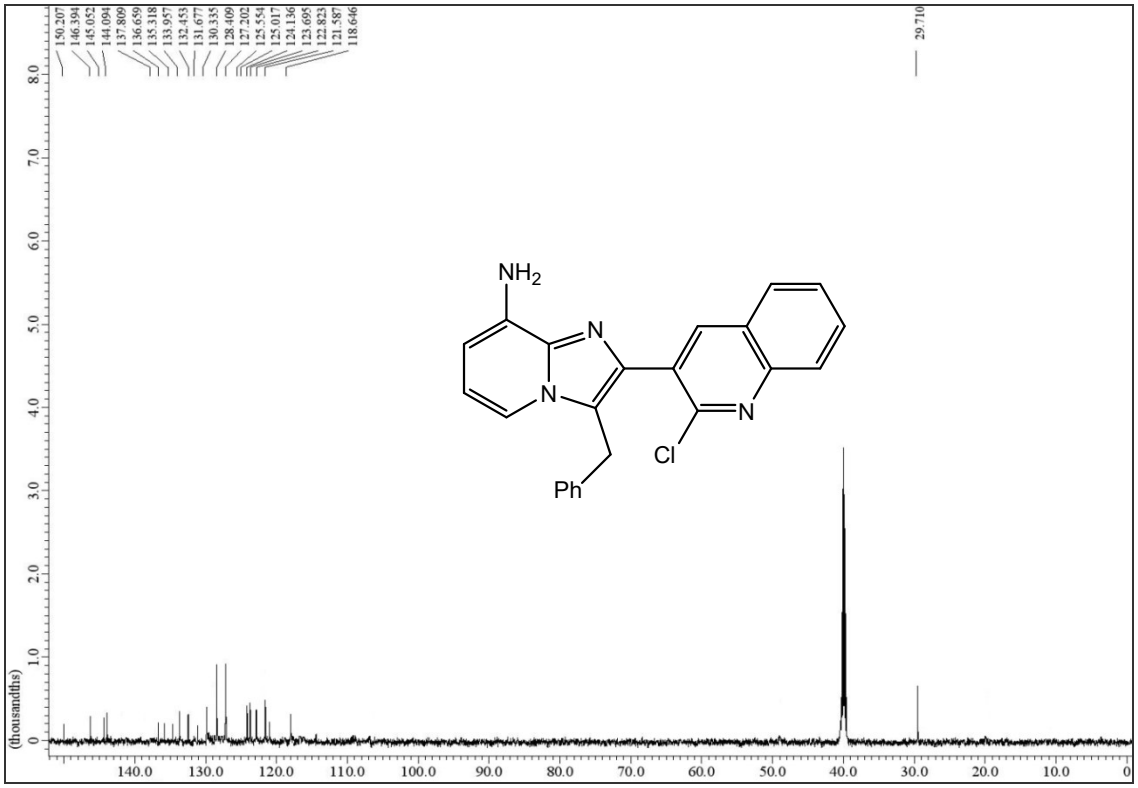


Spectrum No. 15 Mass spectrum of compound 8k



Spectrum No. 16 ^{1}H NMR spectrum of compound 8m (DMSO- $d_{6,}$ 400 MHz)



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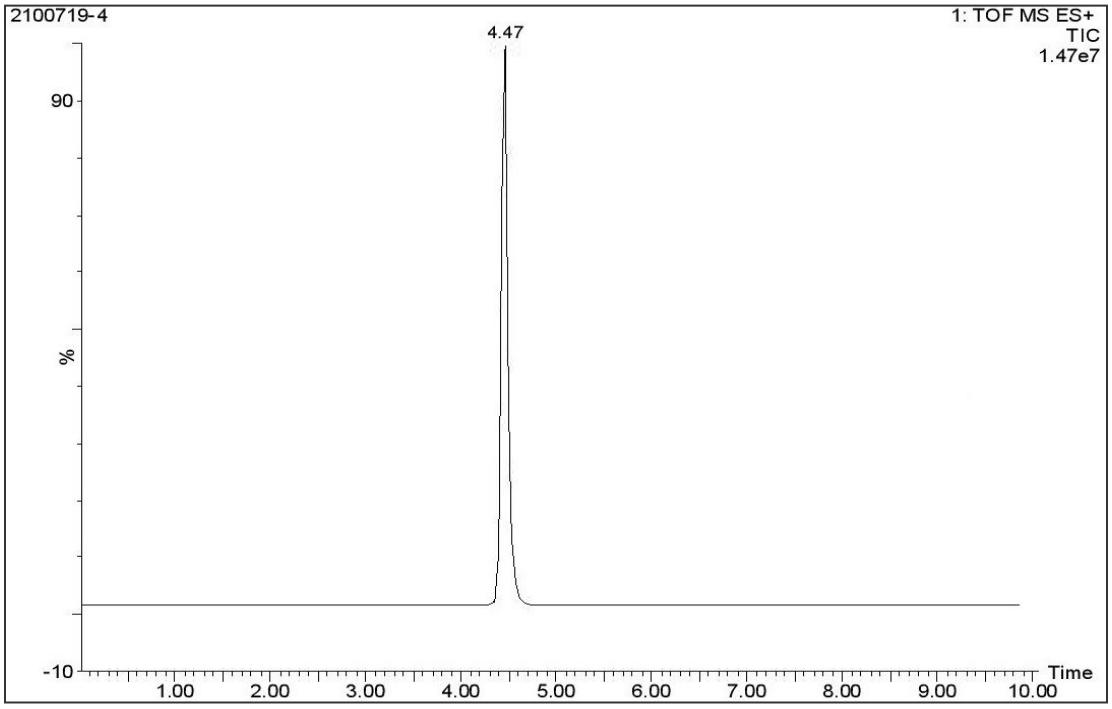

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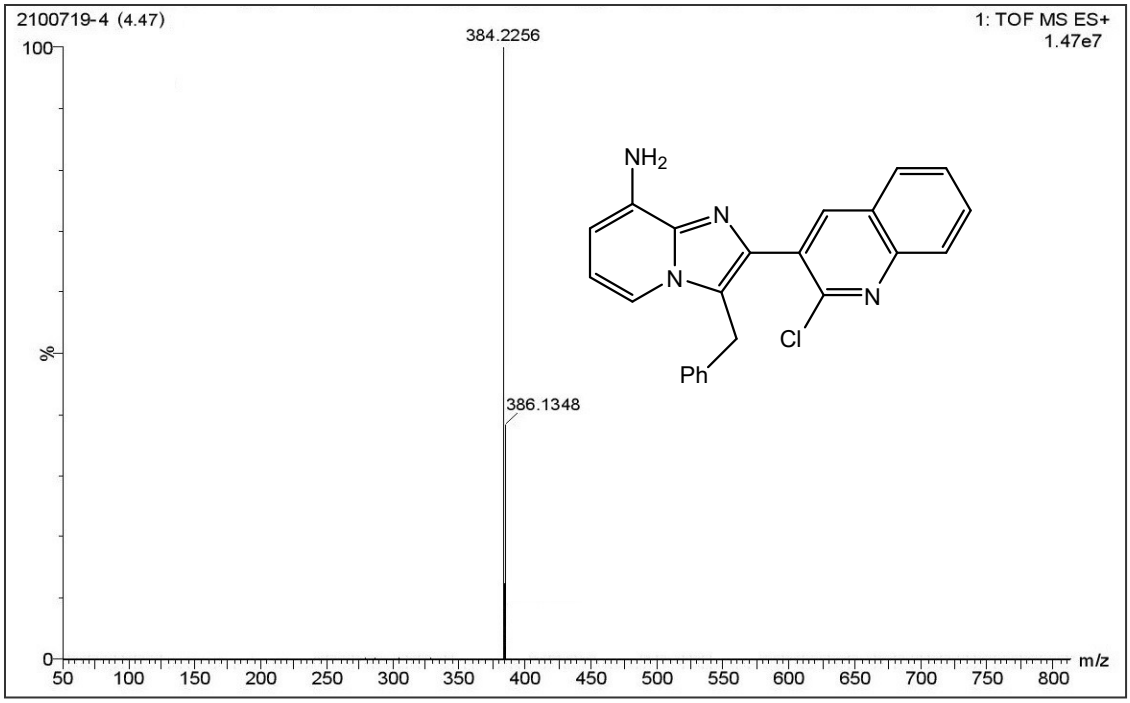

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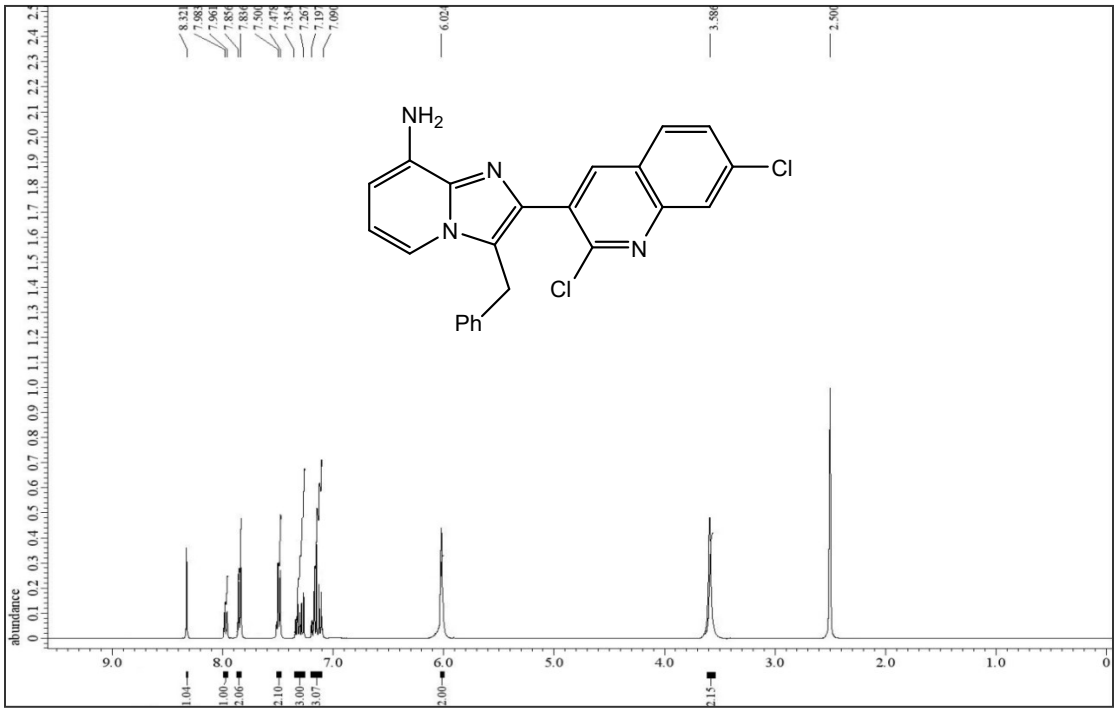

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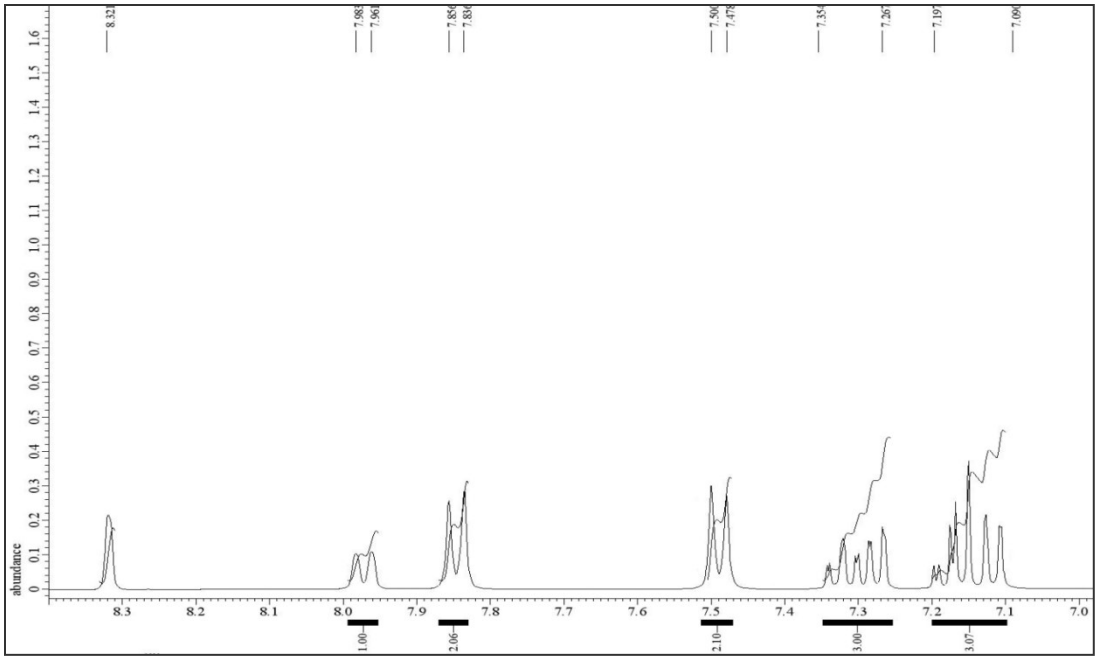

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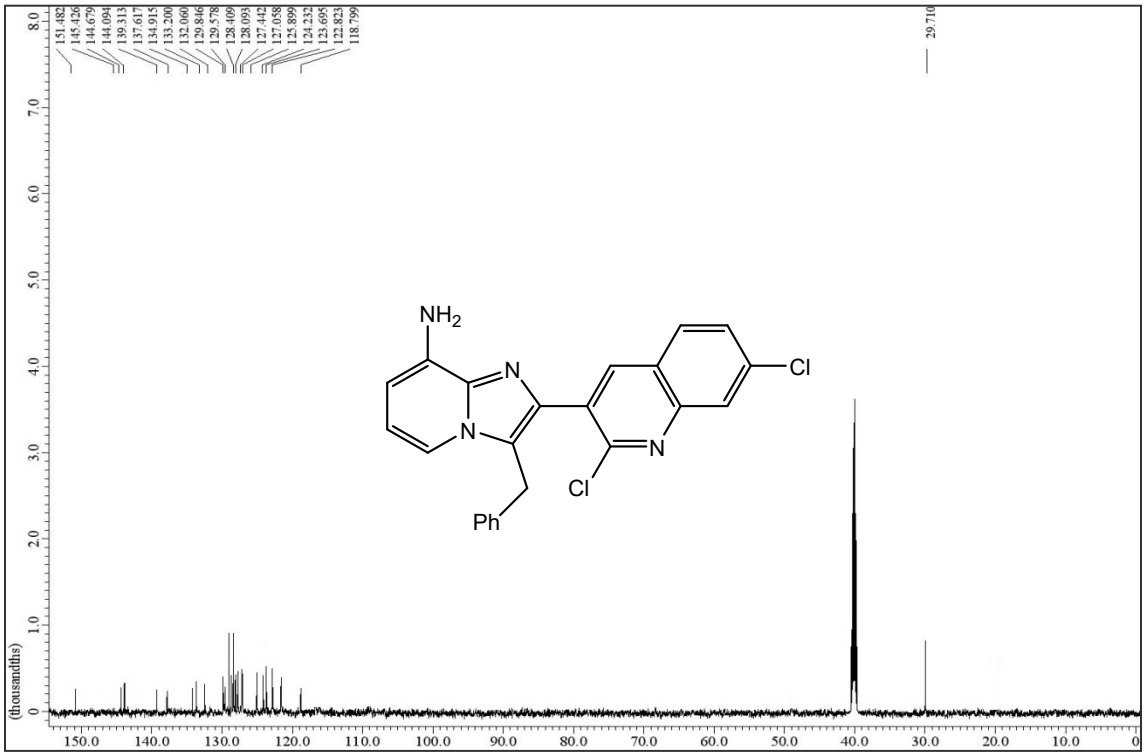

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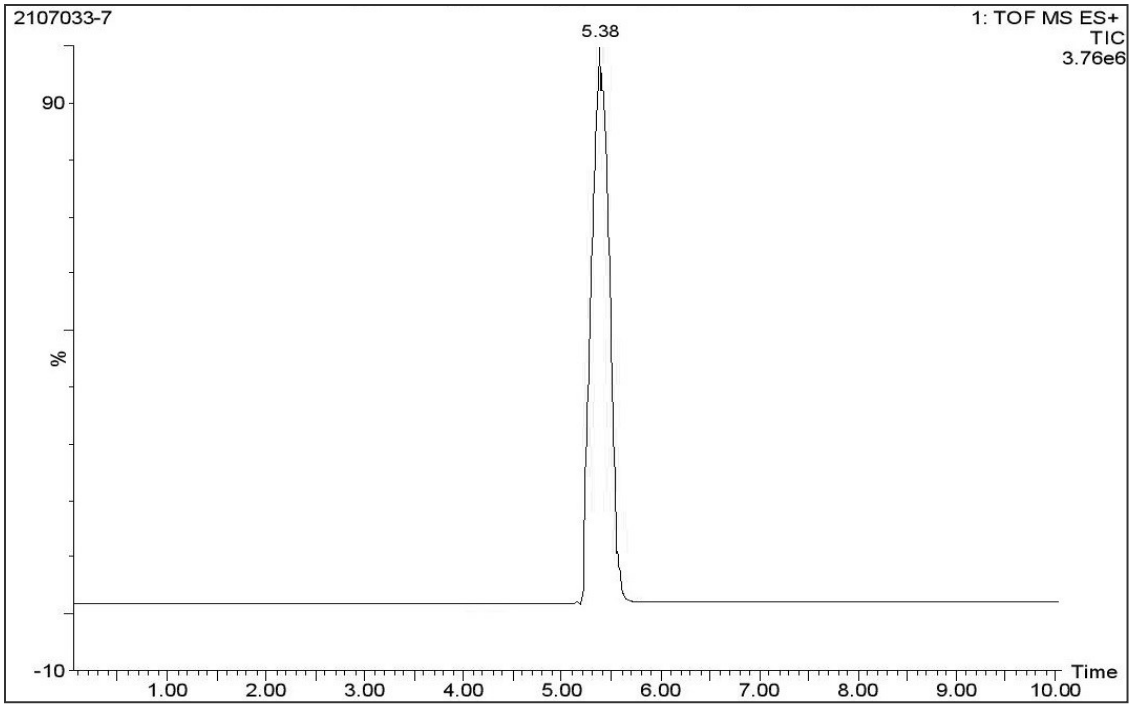

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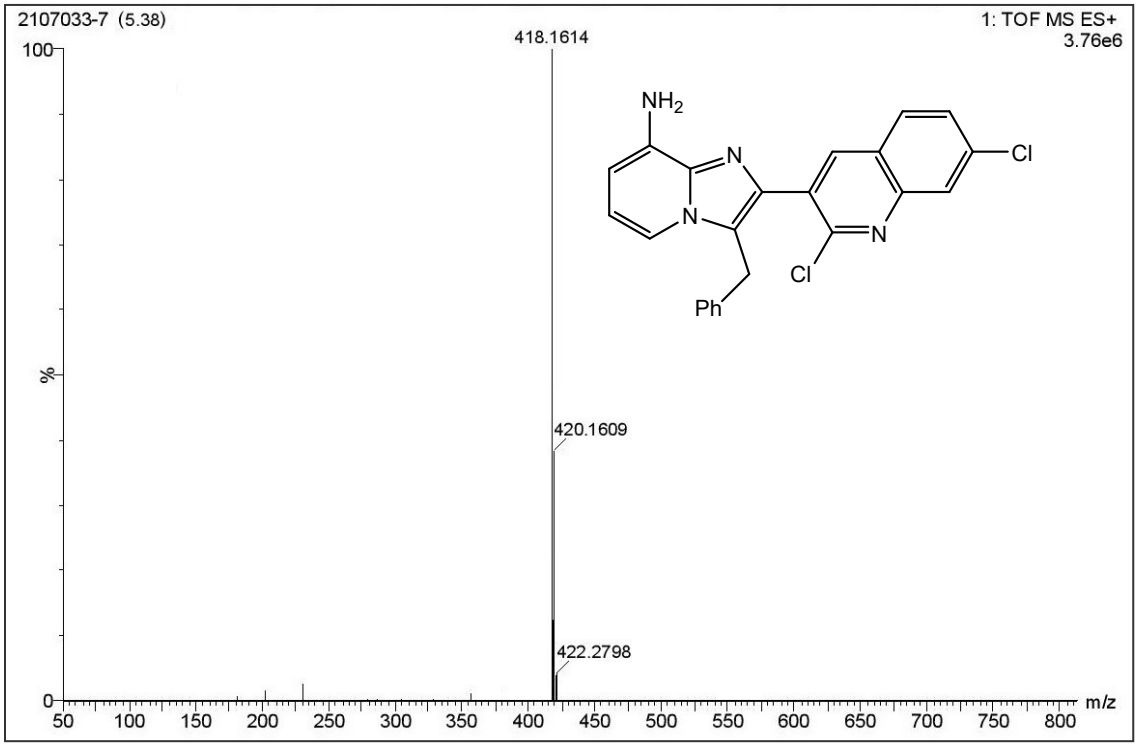

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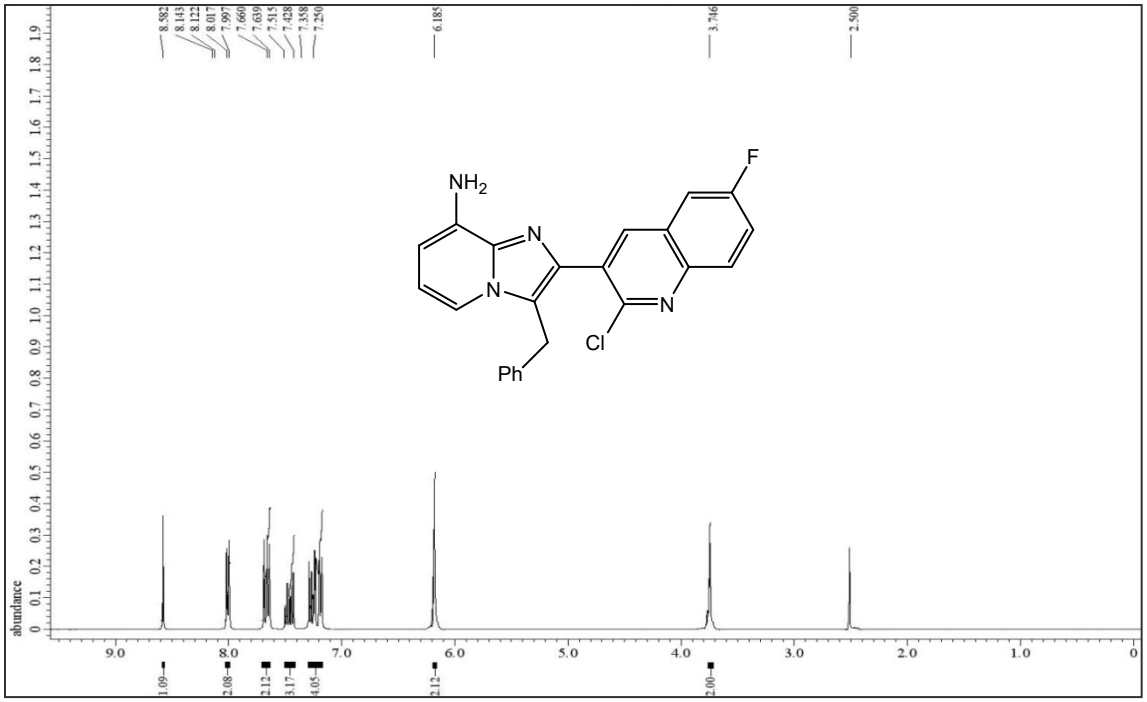

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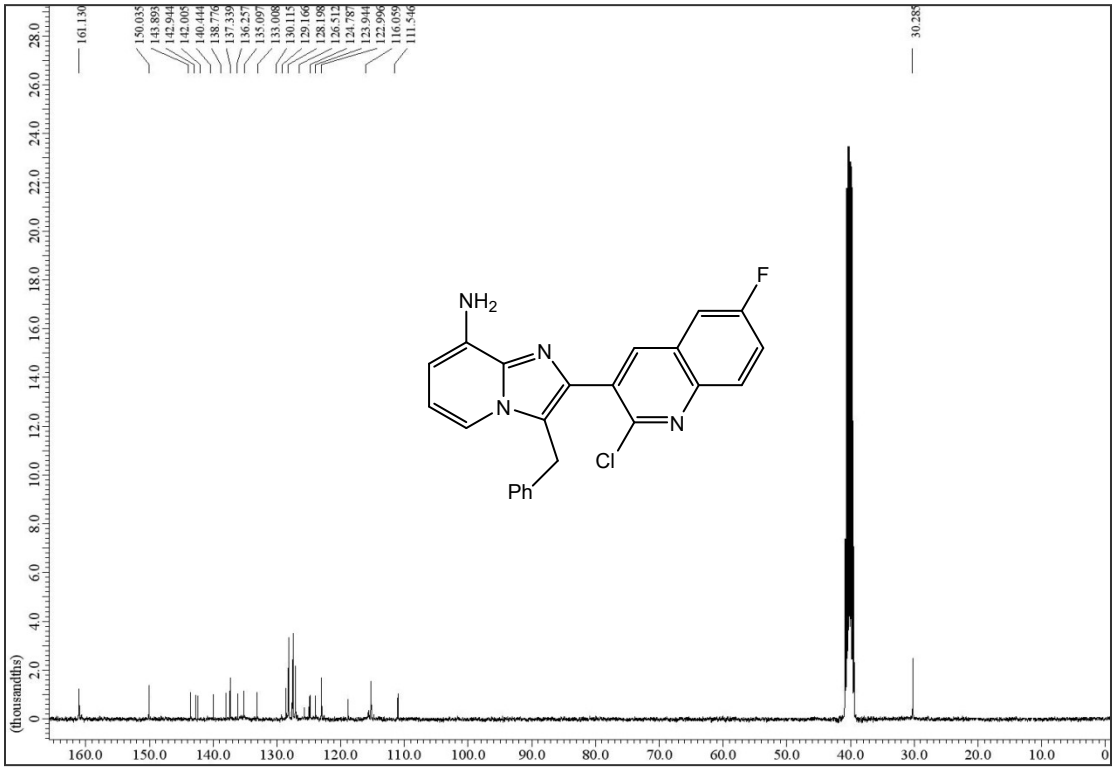

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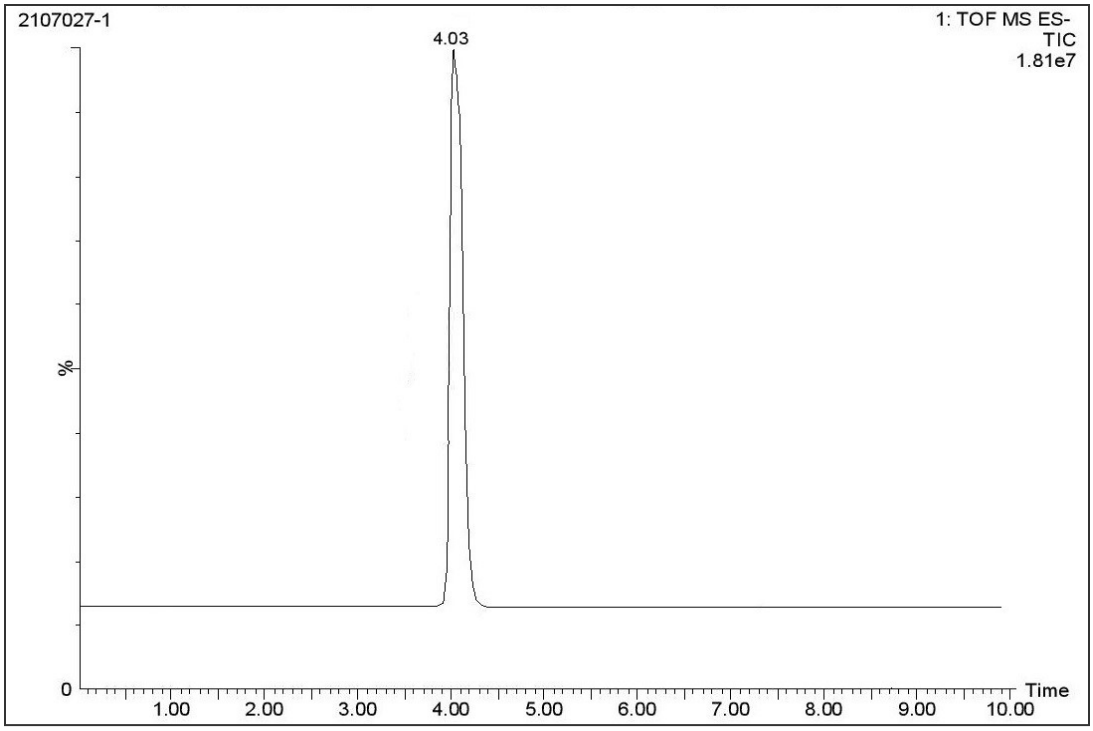

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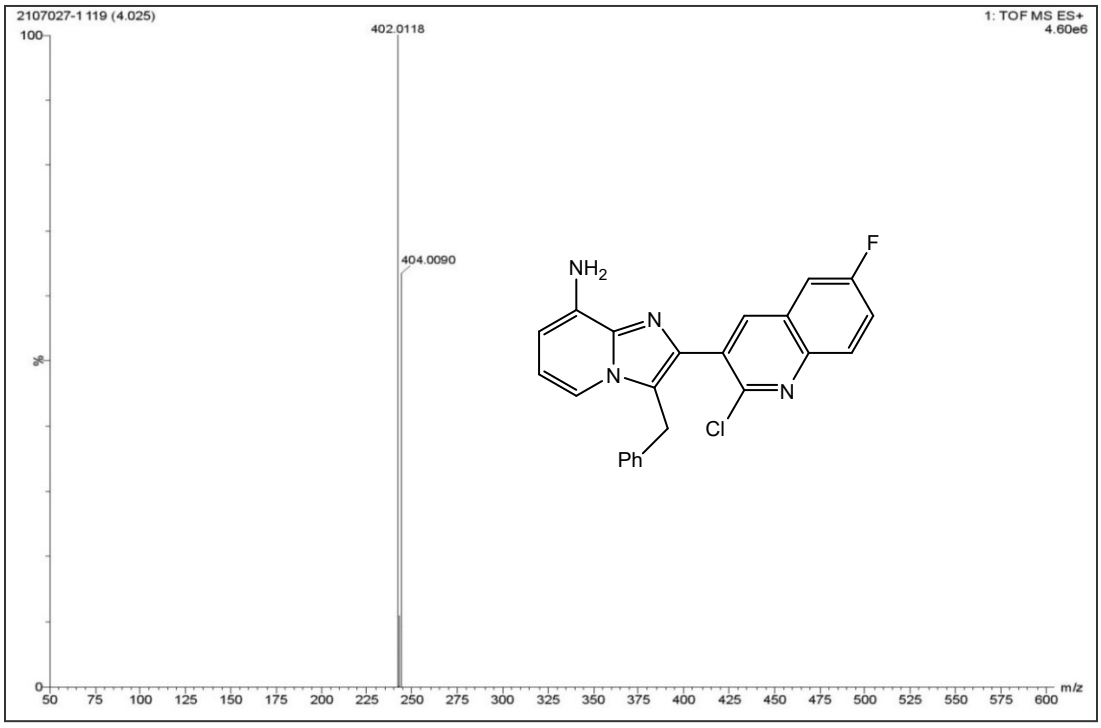

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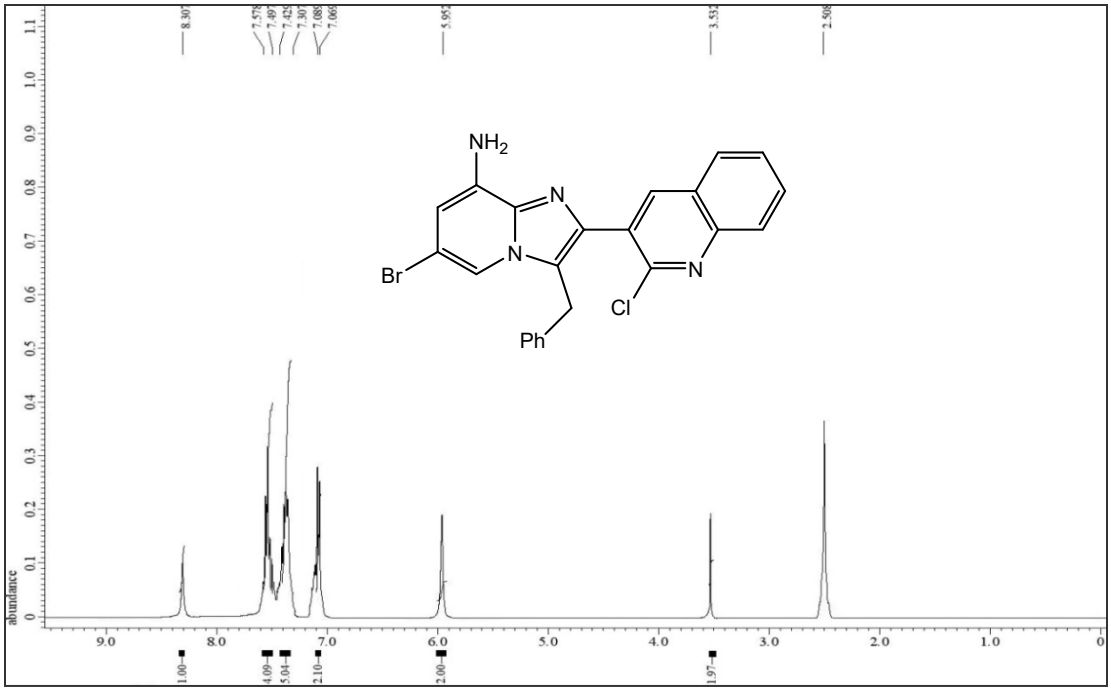

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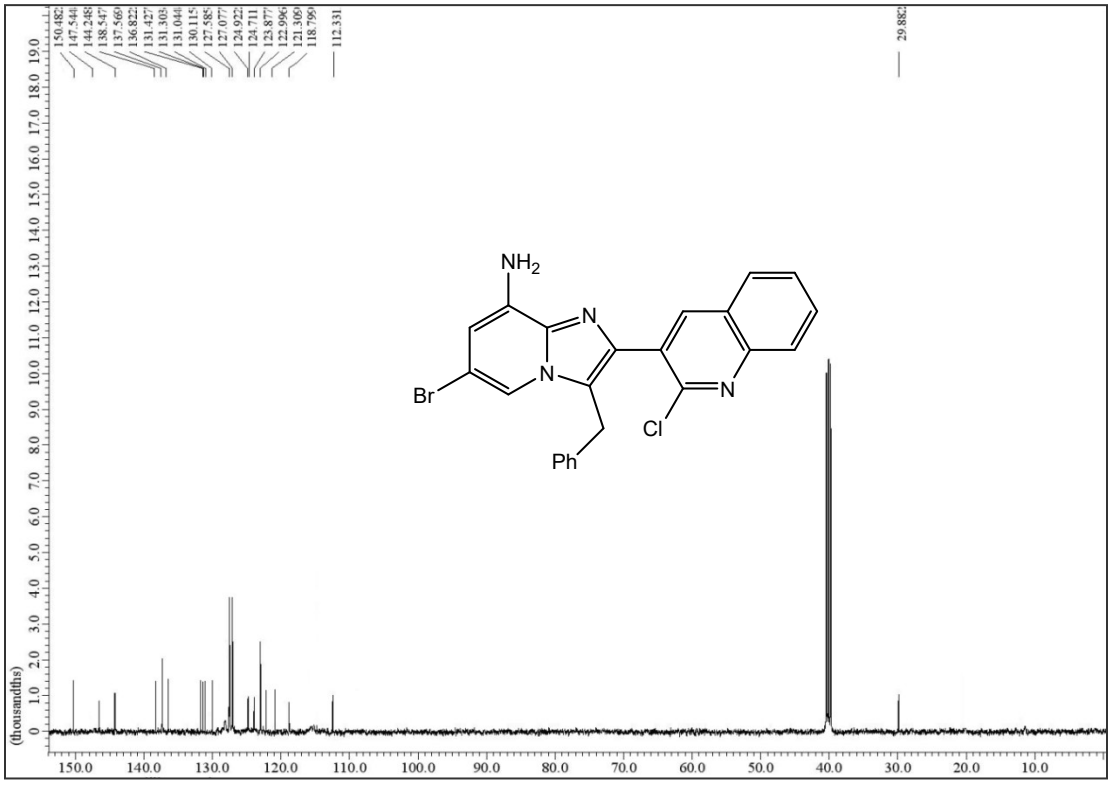

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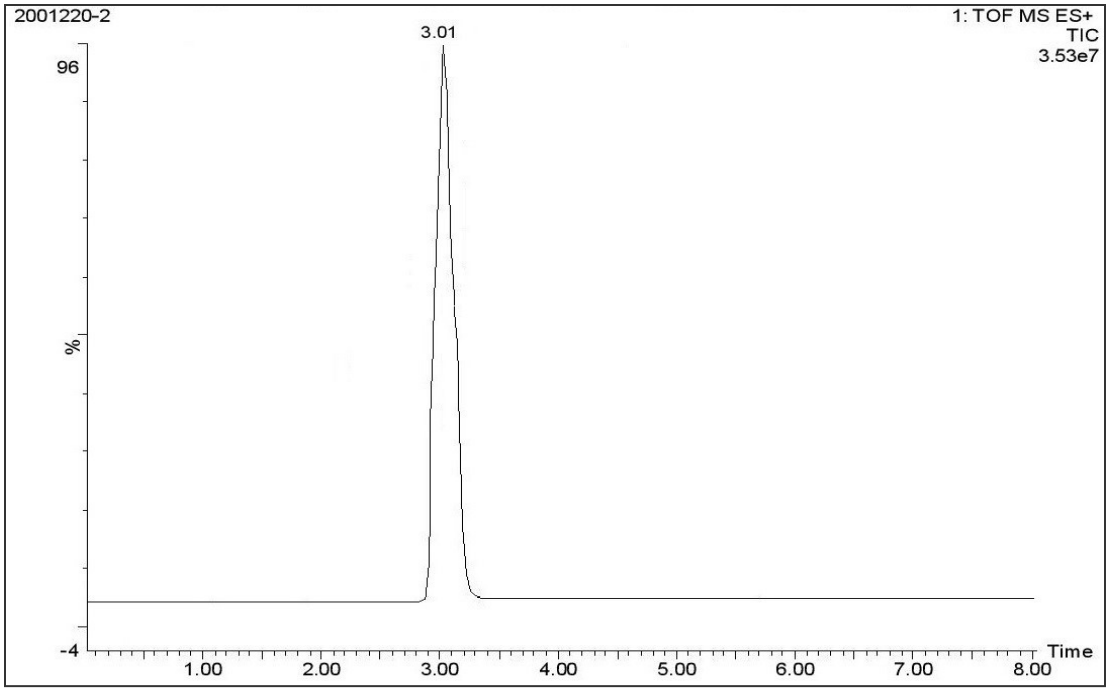

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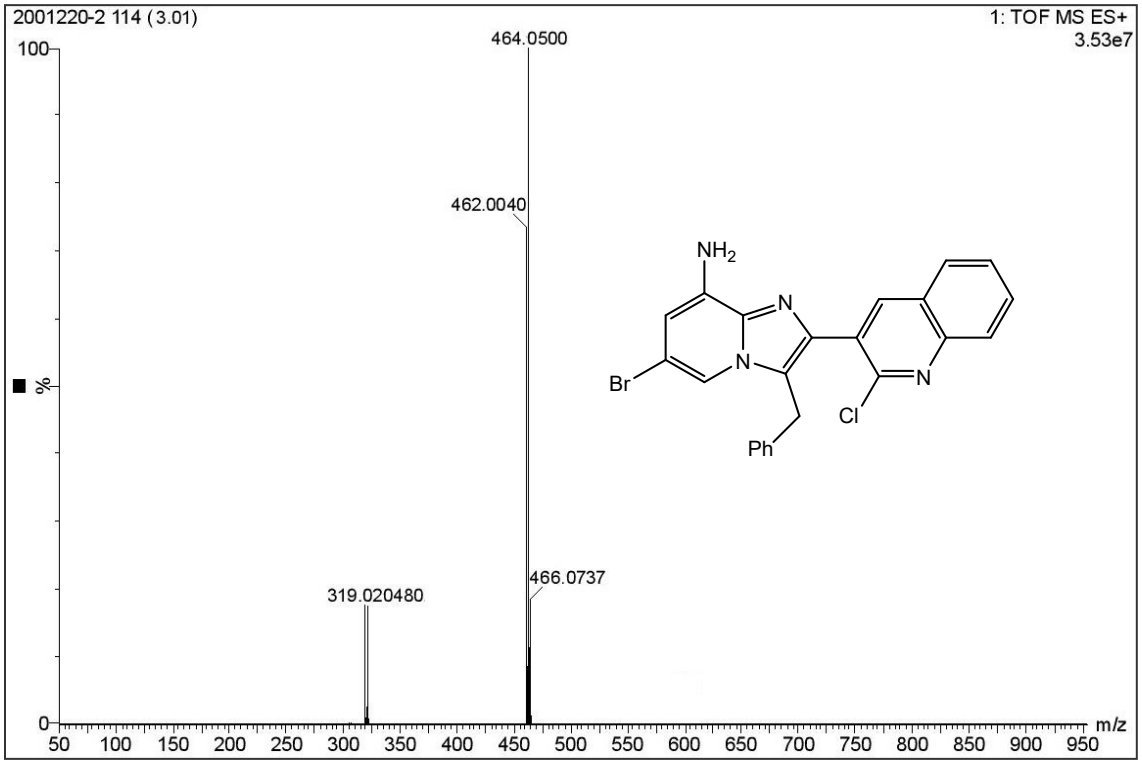
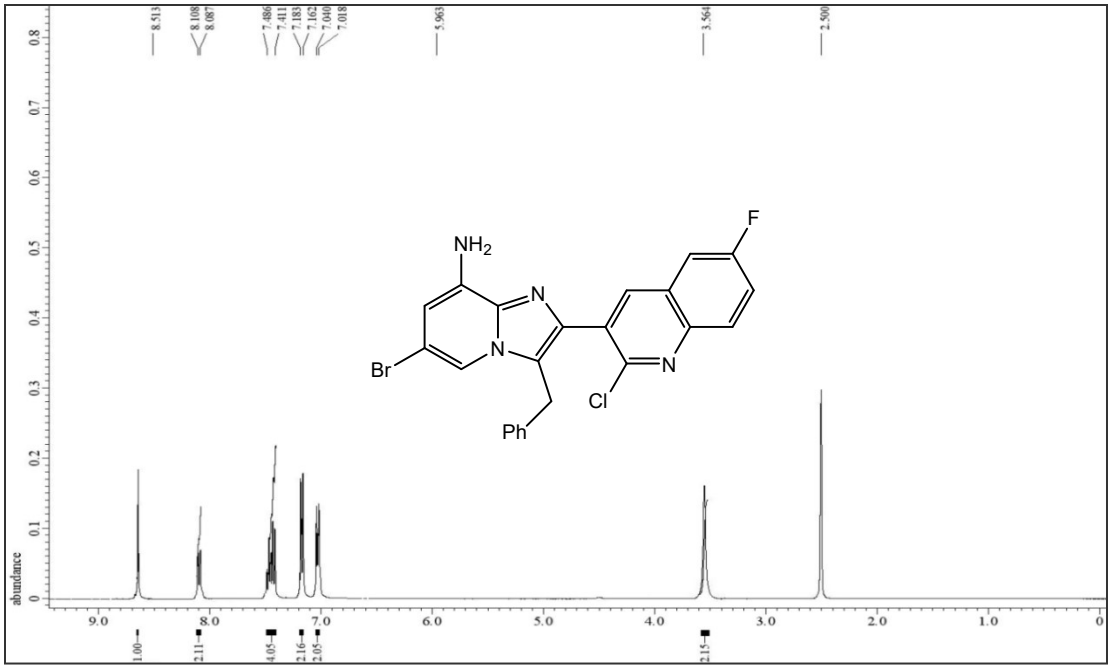
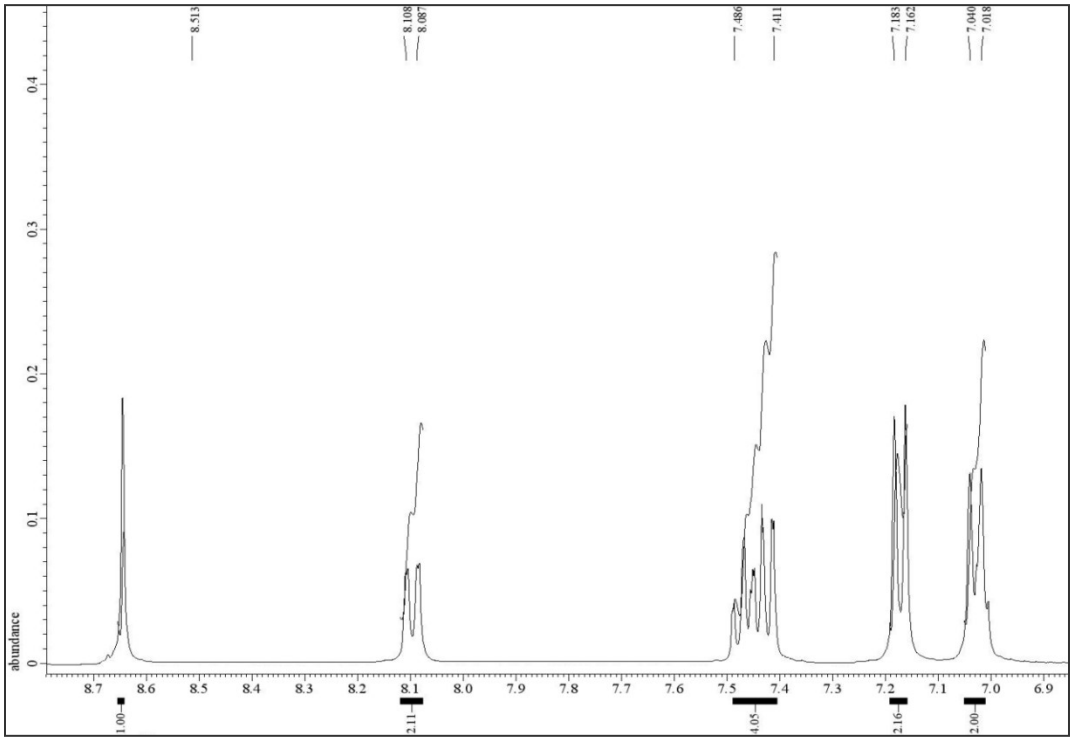


Spectrum No. 32 Mass spectrum of compound 8q



Spectrum No. 33 ^{1}H NMR spectrum of compound 8s (DMSO- $d_{6,}$ 400 MHz)



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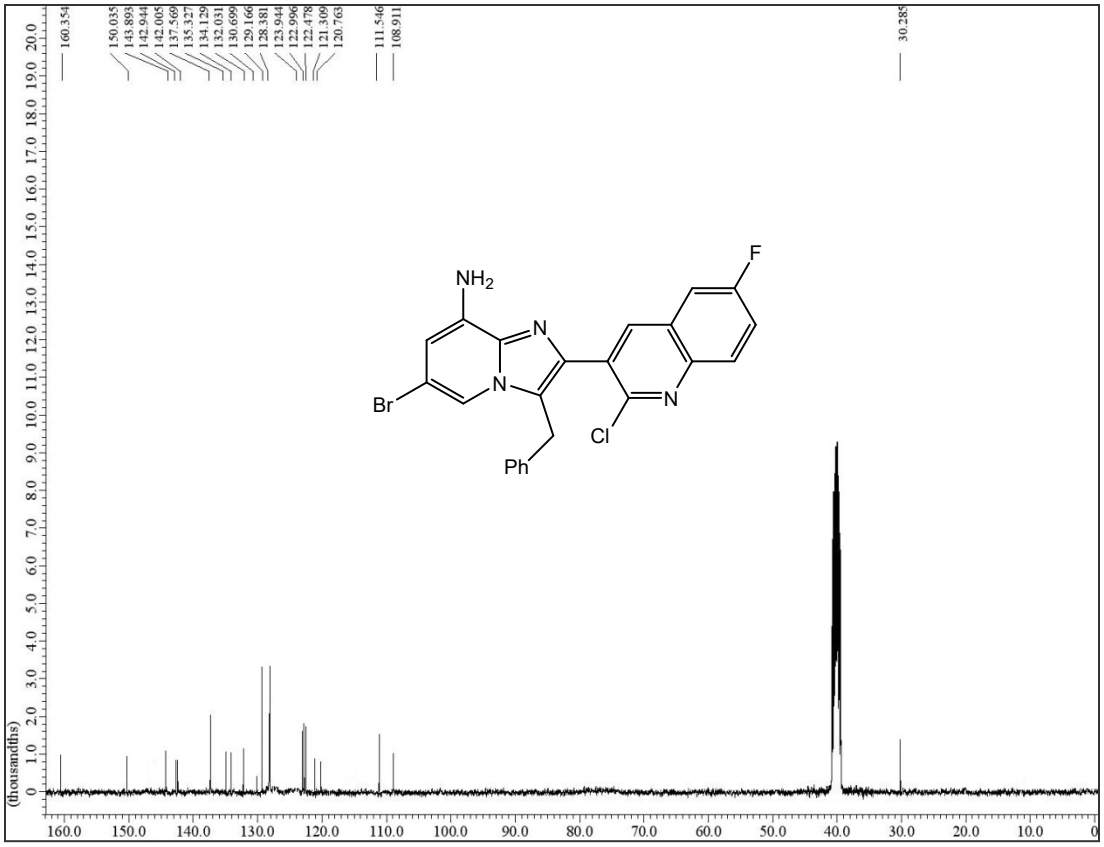

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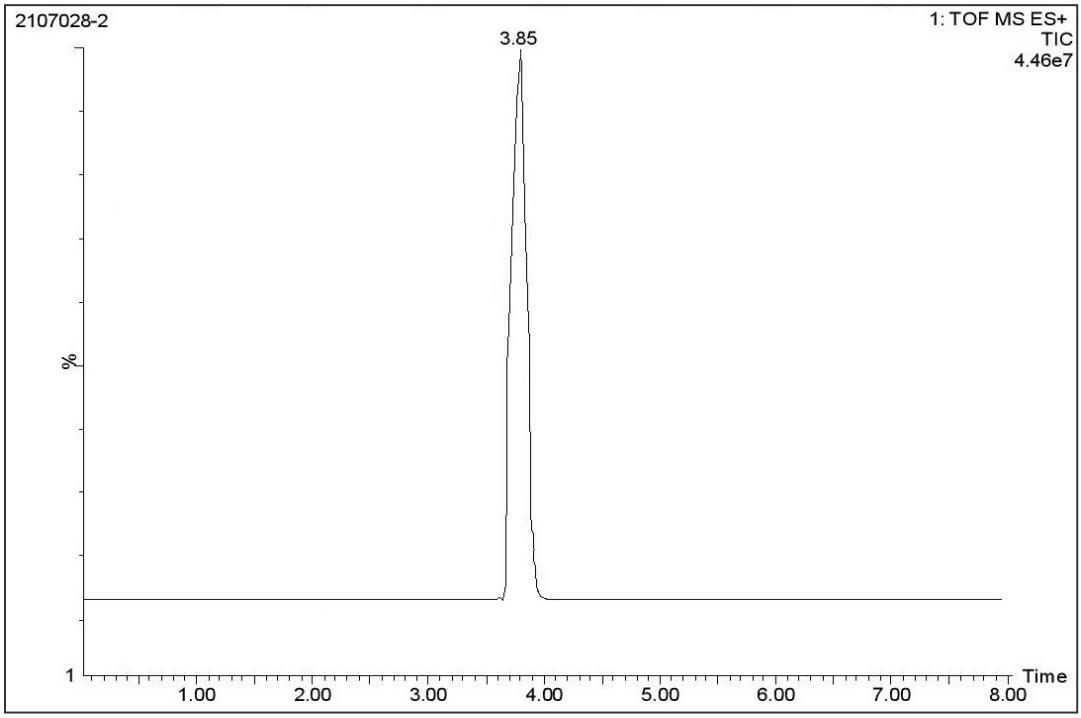
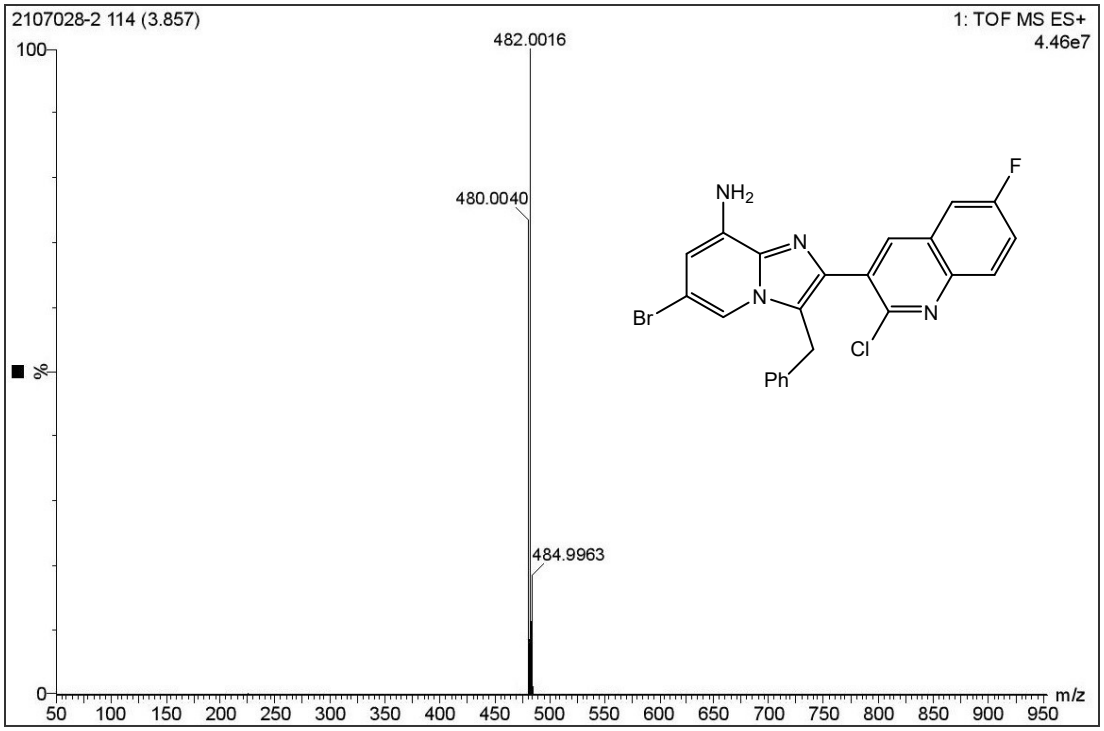
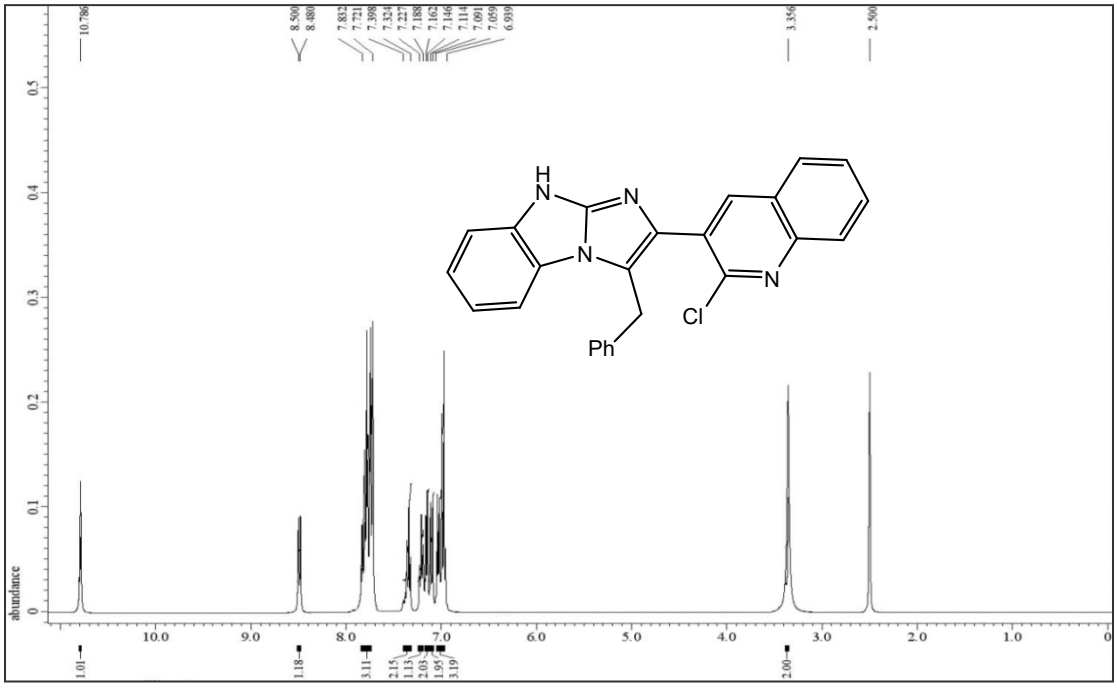


Spectrum No. 36 LC of compound 8s



Spectrum No. 37 Mass spectrum of compound 8s



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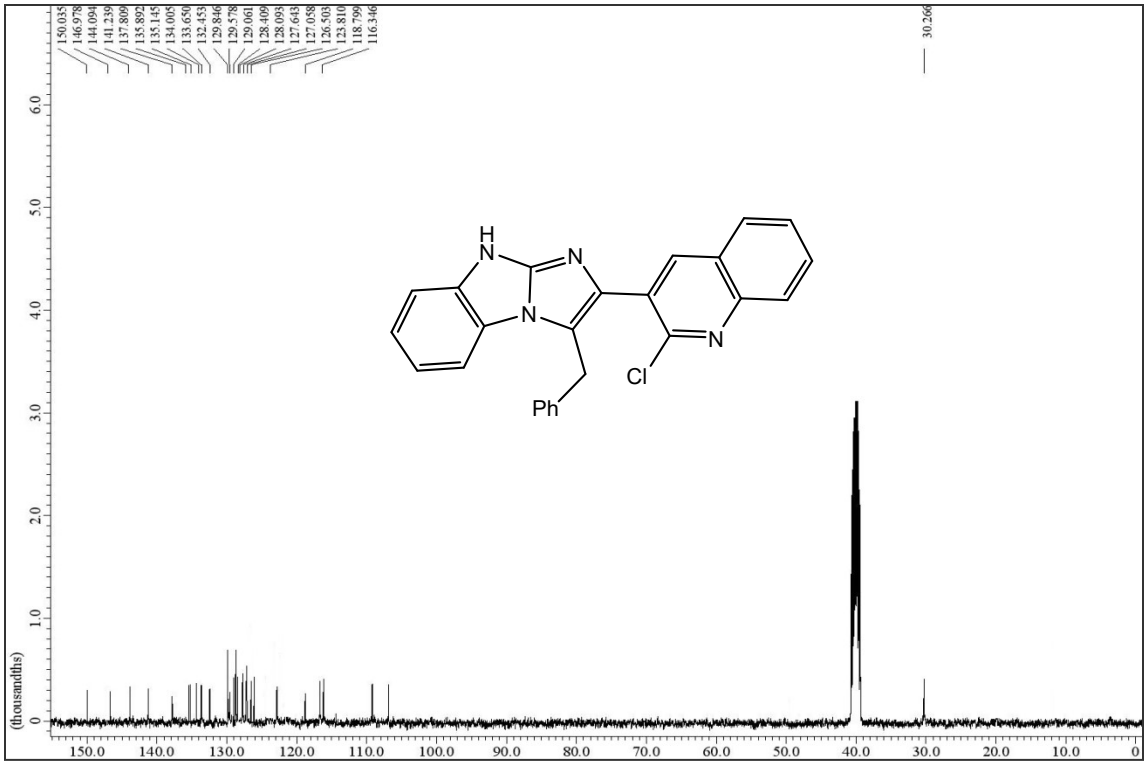

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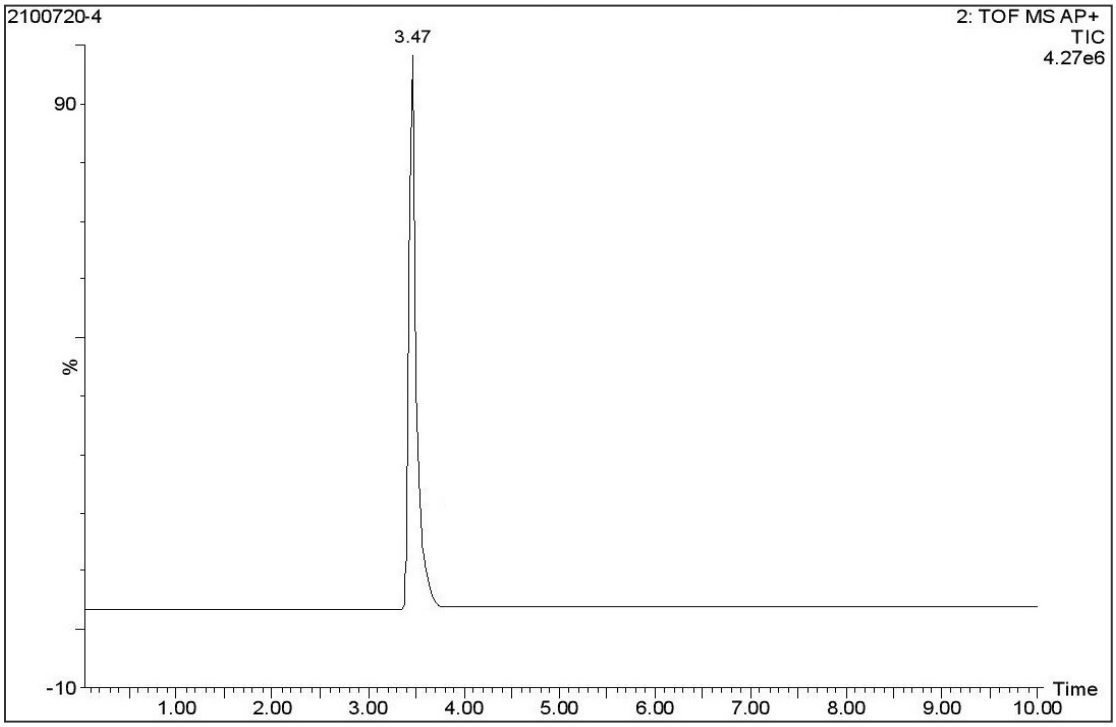
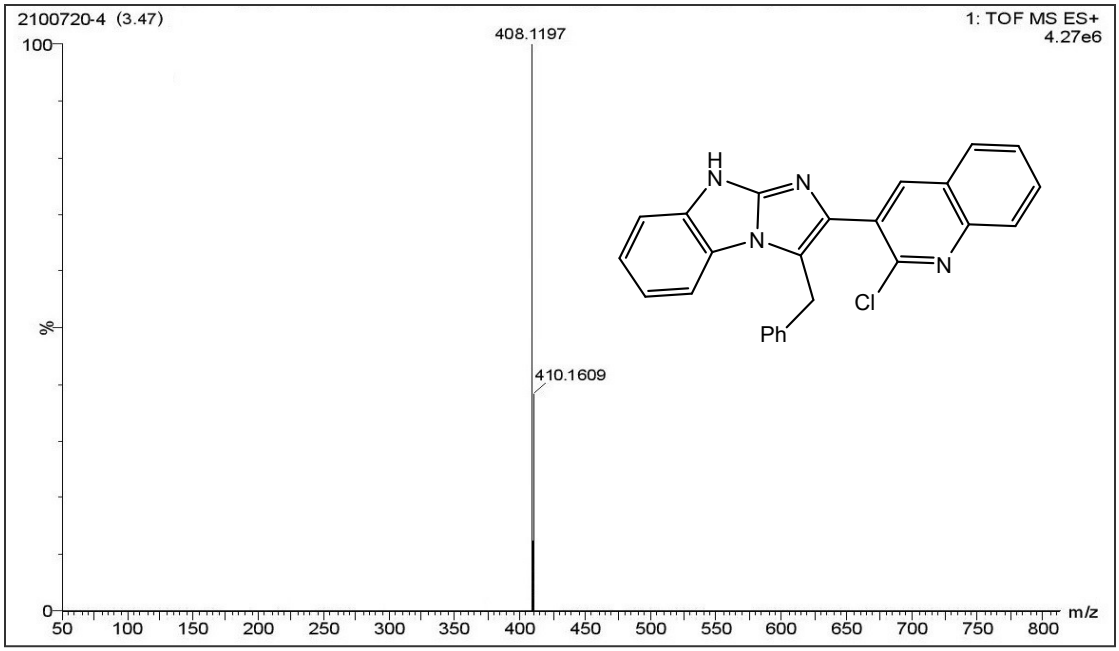
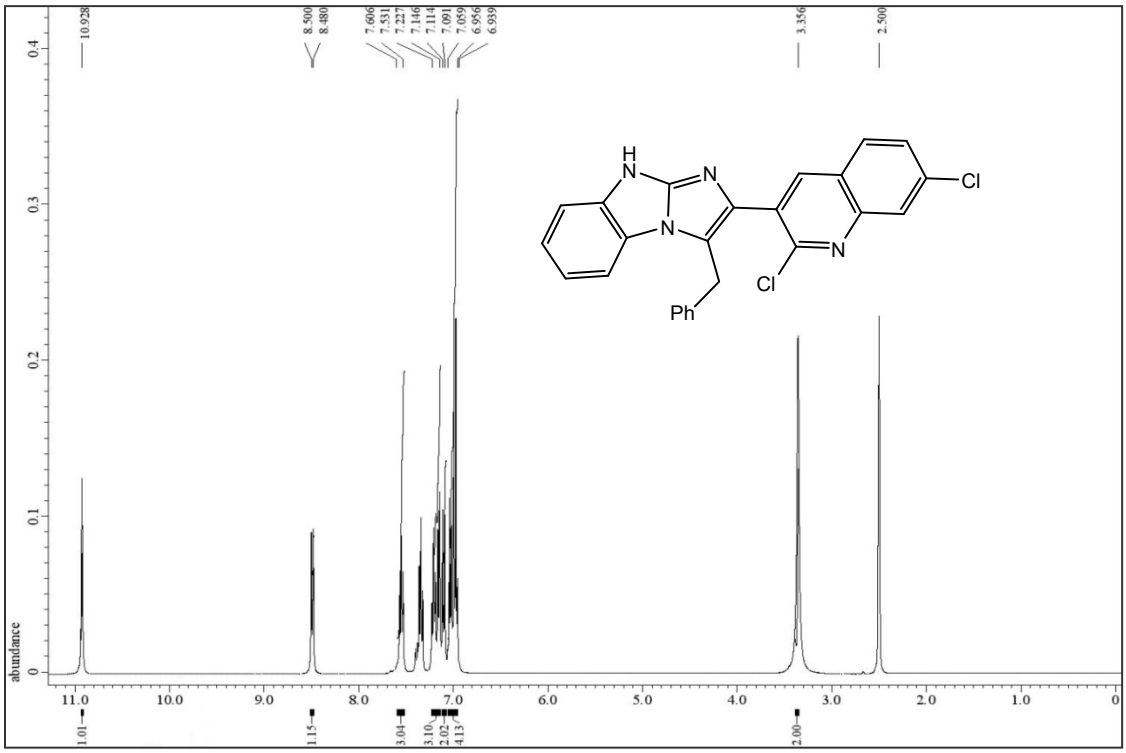


Spectrum No. 40 LC of compound 8u



Spectrum No. 41 Mass spectrum of compound 8u



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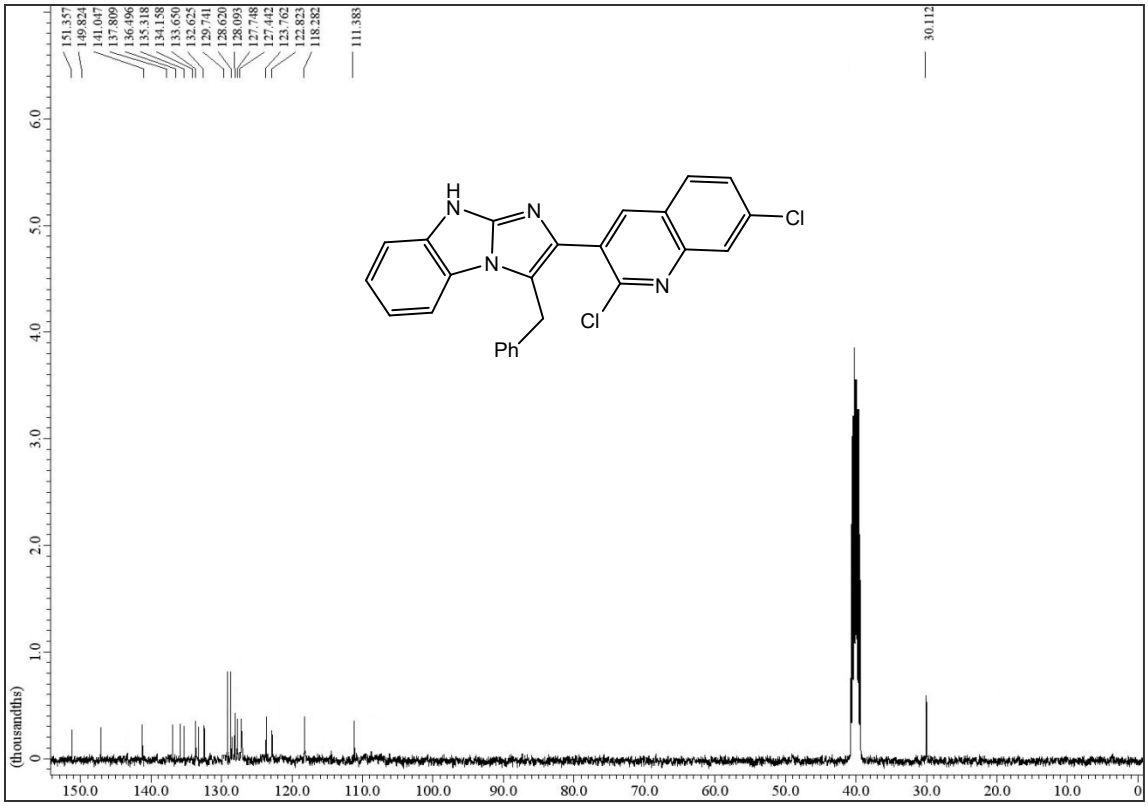

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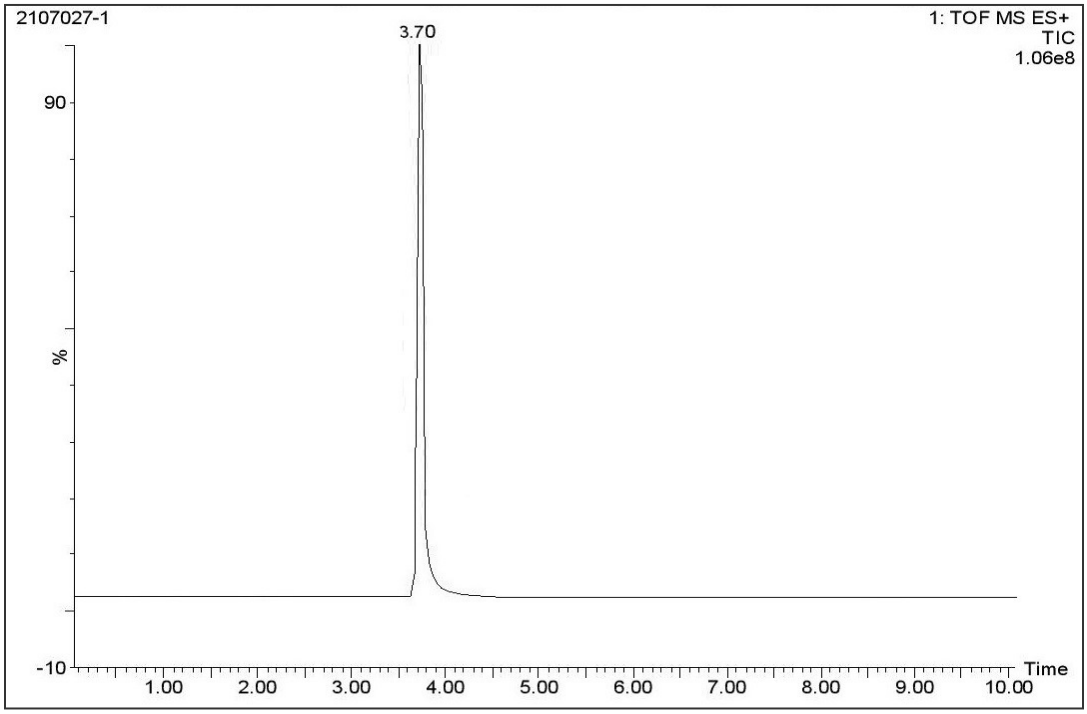
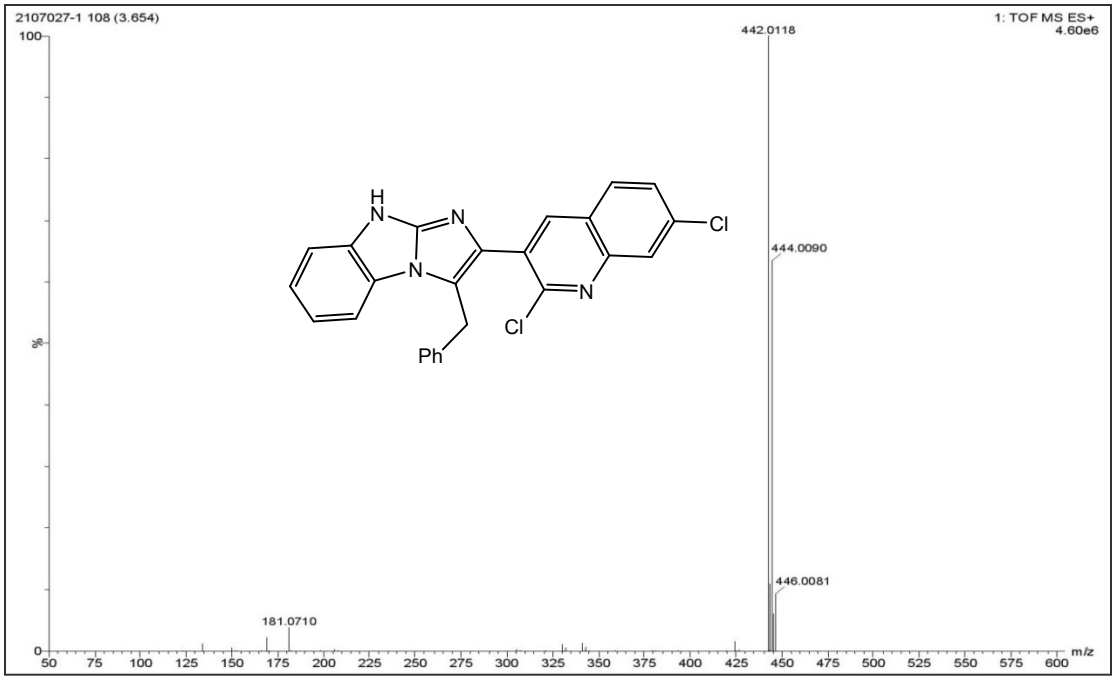


Spectrum No. 44 LC of compound 8v



Spectrum No. 45 Mass spectrum of compound 8v

Biological Evaluation

In vitro COX-2 assay

In vitro COX-2 inhibition was carried out by using human COX-2 Elisa kit and following the manufacture's instructions. Supernatant liquid (100 µL) collected was added into each well in duplicate (control and treated sample) and incubated for 2.5 hours at room temperature. The solution was discarded and washed 4 times with 1x wash solution. After the last wash, the remaining wash buffer was removed by pirating or decanting. The plate was inverted and blotted against clean paper towels. 1x prepared biotinylated antibody (100 µL) was added to each well and incubated for 1 hour at room temperature with gentle shaking and the solution was discarded. Washing step was repeated as described above. Streptavidin solution (100 µL) was added to each well and incubated for 45 minutes at room temperature with gentle shaking and the solution was discarded. The washing step was repeated and TMB One-Step Substrate Reagent (100 µL) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. Stop Solution (50 µL) was added to each well and read at 450 nm immediately.

ATP-2Na solution (40 mM, 10 µL) was added into each well and mixed thoroughly followed by incubation for 30 min at 37ºC. The samples were removed and wells were washed 3

times with wash buffer. Blocking solution (100 µL) was added into each well and incubated for 30 min at 37ºC. Blocking solution was discarded and then 50 µl of Anti-phosphotyrosine - HRP solution was added into each well and incubated for 30 min at 37ºC. The antibody solution was discarded and each well was washed 4 times using washing buffer. Then HRP substrate solution (100 µl) (TMBZ) was added into each well and incubated it for 30 min at 37ºC and stop solution (100 µl) was added into each well in the same order as HRP substrate solution. The absorbance was measured at 450 nm with a plate reader. % Inhibition was calculated as follows:

% Inhibition = (Abs of sample /Abs of control) × 100

Using graph Pad Prism Version 5.1 the IC_{50} of compounds was calculated by taking percentage of inhibition v/s COX-2 at five different concentrations of treatment.

Scavenging activity using the DPPH method

The stock solution of DPPH (3.94 mg in 100ml methanol) stored at 4° C until use. Then, DPPH (2 ml) solution was mixed with 1 ml of five different concentrations (20, 40, 60, 80 and 100 µg mL^{-1}) of the samples and standard respectively. A mixture of distilled water (1 ml) and DPPH (2 ml) solution was used as the control. The reaction mixture was mixed and kept in the dark for 30 min and incubated at room temperature. The absorbance was recorded spectrophotometrically at 517 nm. An antioxidant activity was calculated based on the percentage of DPPH radical scavenged using the following equation.

$$\text{Scavenging effect \%} = \frac{[\text{Control absorbance - Sample absorbance}]}{[\text{Control absorbance}]} \times 100$$

Molecular docking assay

The crystal structures of Mefenamic Acid Bound to Human Cyclooxygenase-2 (PDB ID: 5IKR; A chain) for the docking studies, obtained from the Protein Data Bank (X-Ray Diffraction, 2.34 Å). The protein was prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel [29] charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was acquired with the help of Tripos force field using Gasteiger-Huckel charges and molecular docking was performed with Surflex-Dock program which is interfaced with Sybyl-X 2.0 [30] and other miscellaneous parameters were assigned with the default values provided by the software.