Supplementary Material

Pyrazole-tethered isoxazoles: hypervalent iodine-mediated, metal-free synthesis and biological evaluation

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S1. Single crystal structure of compound **2a**

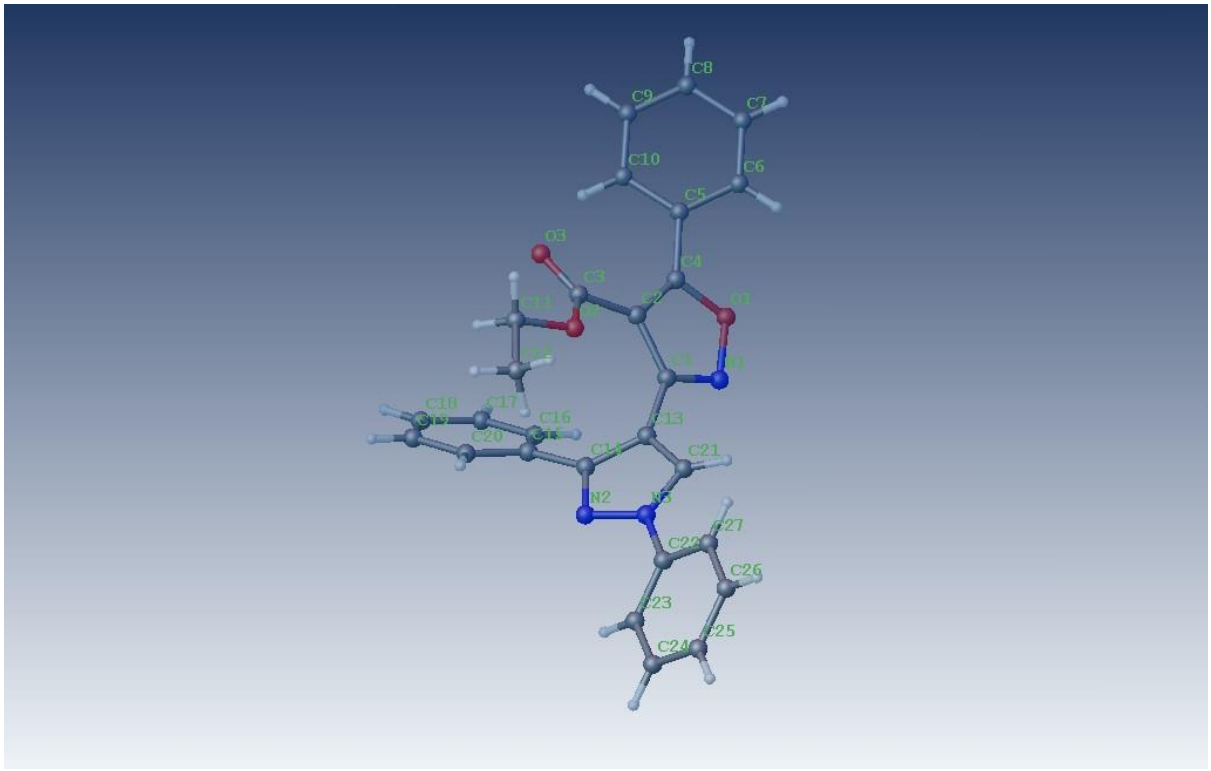

Figure S1. Single crystal structure of compound **2a** (CCDC no. 2343634)

S2. Biological Assay

Antimicrobial assay

The antimicrobial activity of synthesized compounds was screened against a range of gram-positive and gram-negative bacterial strains as well against one fungal strain under in-vitro conditions using double-dilution method. From glycerol stock solution (20% v/v), all the bacterial and fungal strains were pipetted out and cultured in nutrient agar and potato dextrose agar at -20°C. The potential of all synthesized compounds against tested microbial strains was determined by using Mueller Hinton broth. The microbes were incubated for 24 h at 37°C and 30°C for bacterial and fungal strain respectively. The estimation of growth was observed at wavelength (λ_{max} 690 nm) in ELISA plate reader. The results of all the tested compounds as well as control drug were taken in minimum inhibitory concentration (MIC, µM) against tested microbial strains. Amoxicillin and Fluconazole were used as standard drugs against antibacterial and antifungal pathogens respectively.[1]

Antioxidant assay

Antioxidant properties of the synthesized molecules were investigated *via* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Ascorbic acid was taken as a reference compound. The stock solutions of DPPH and synthesized compounds were prepared in methanol having concentrations of 0.1 mM and 0.5 mg/ml respectively. 1000 ml of the DPPH solution and 2500mL of synthesized compounds were mixed and placed in an incubator for 30 min at 37°C and λ_{max} was measured at 517 nm. All the experiments were repeated three times and % Radical Scavenging Activity (%RSA values) was calculated using the equation: % RSA = $(A_0-A_t)/A_0$ x 100, where A_0 and A_t corresponds to the absorbance of the blank and tested compound at λ_{517}.[2]

Cytotoxicity assay

The cytotoxicity assay was performed on mouse-fibroblast and Vigna radiata seed cells using 1mg/mL (DMSO) solutions of synthesized compounds. Both the cells were incubated for 24 h separately in 96 well microplate. After 24 h, each target compound was treated with both cell lines and incubated for 48 h and control represents the untreated cells. Further, an MTT solution of a concentration of 5 mg/ mL was prepared in PBS (phosphate-butter saline). MTT solution (20 µL) was added to each well keeping total volume 100 µL. The resulting wells were incubated for 4 h at 37°C to get formazan crystals. Further, DMSO was added to each well to solubilize formazan crystals and the absorbance was measured at wavelength λ=570 nm on a microplate reader. The percentage cell viability was calculated by comparing the absorbance of the cells treated with the synthesized one and that of untreated one.[3]

References:

1. Humphries, R. M.; Ambler, J.; Mitchell, S. L.; Castanheira, M.; Dingle, T.; Hindler, J. A.; Koeth, L.; Sei, K. *J. Clin. Microbiol.* **2018**, *56*, e01934. https://doi.org/10.1128/JCM.01934-17.
2. Sharma, O. P.; Bhat, T. K. *Food Chem.* **2009**, *113*, 1202. https://doi.org/10.1016/j.foodchem.2008.08.008.
3. Ghasemi, M.; Turnbull, T.; Sebastian, S.; Kempson, I. *Int. J. Mol. Sci.* **2021**, *22*, 12827. https://doi.org/10.3390/ijms222312827.

S2. ^{1}H, ^{13}C and HRMS data of isoxazoles derivatives 1a-g and 2a-f

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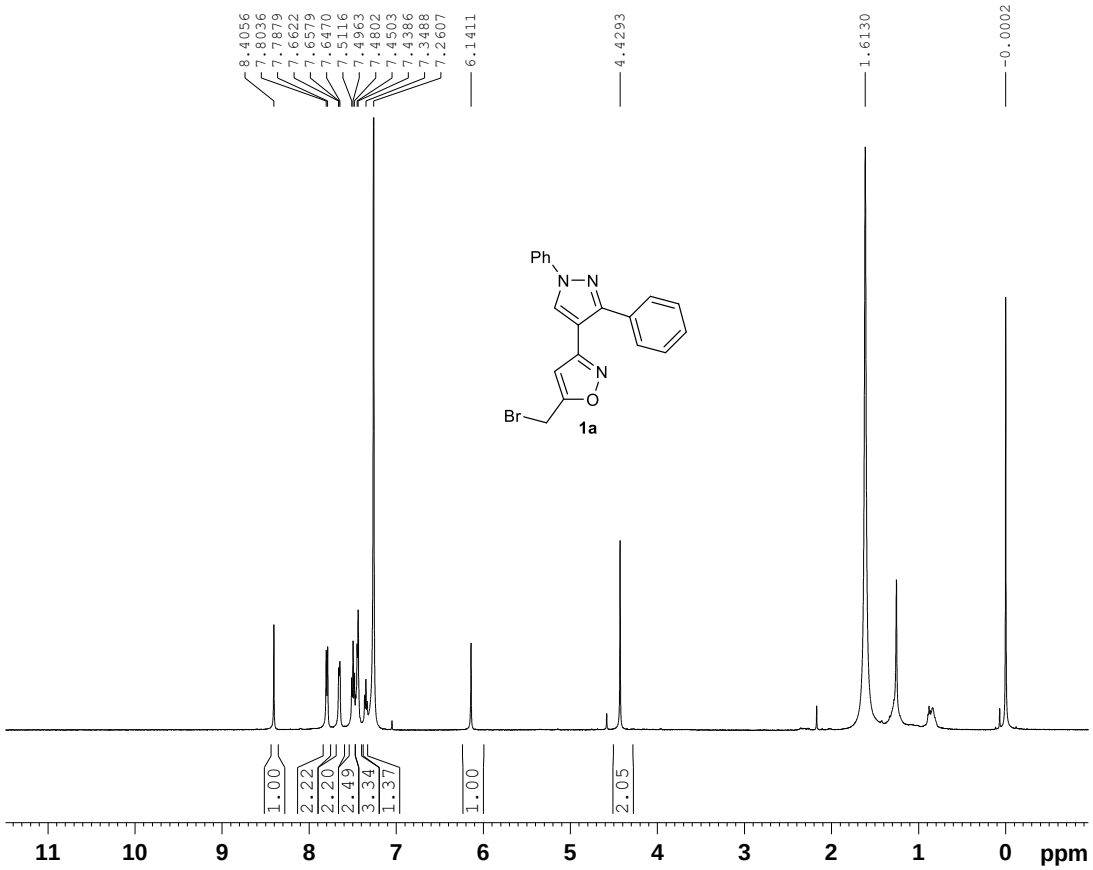

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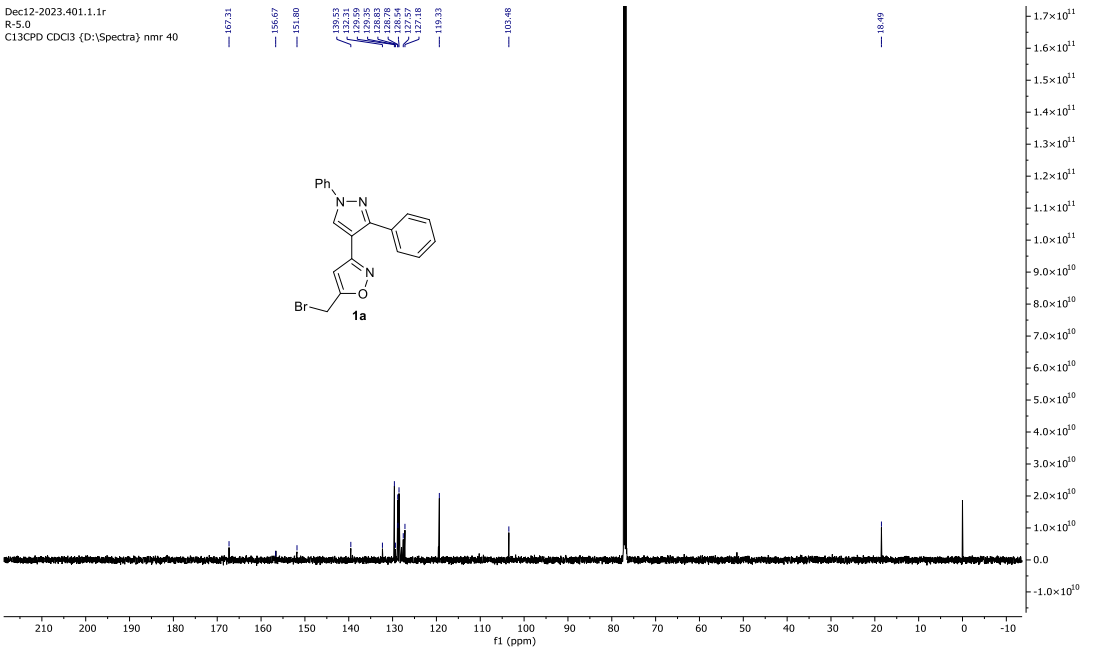

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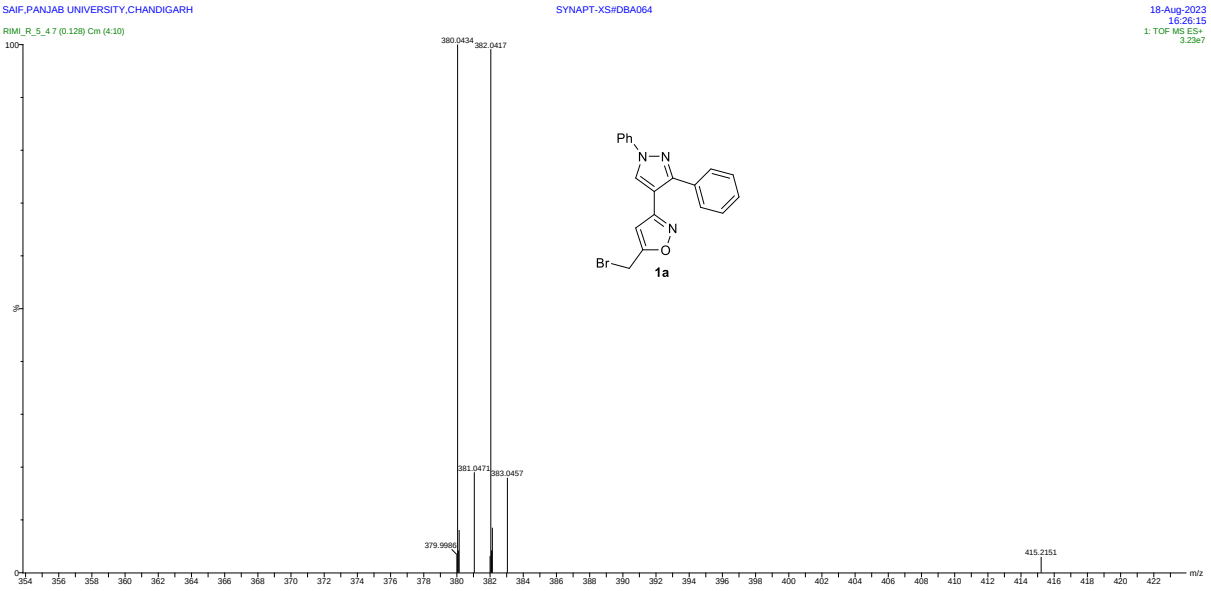
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m/z

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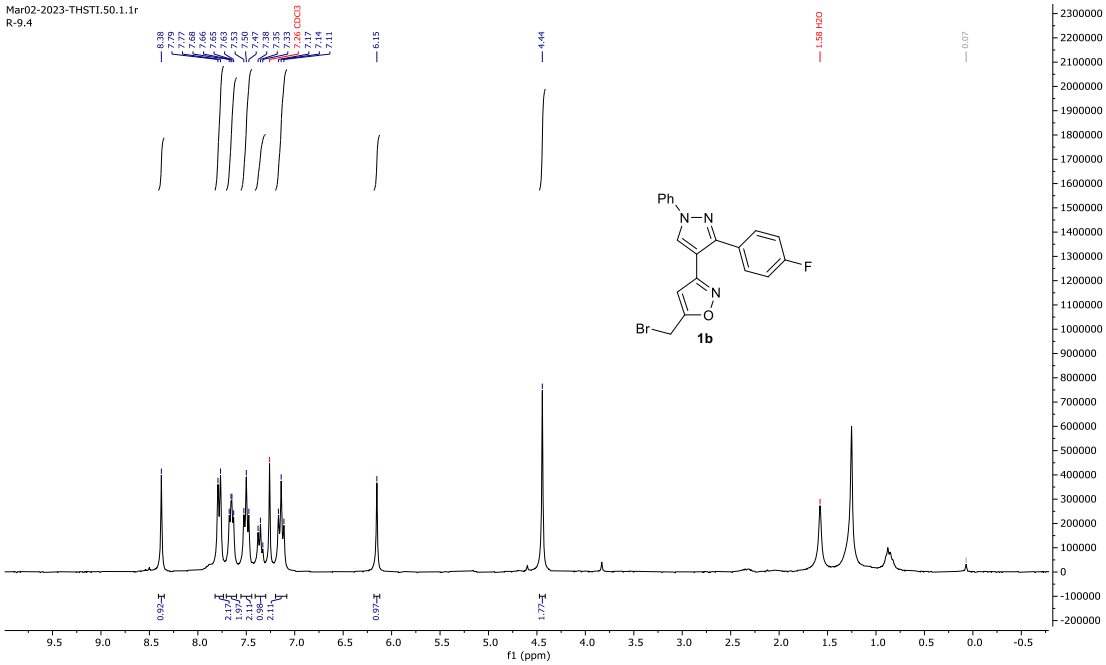

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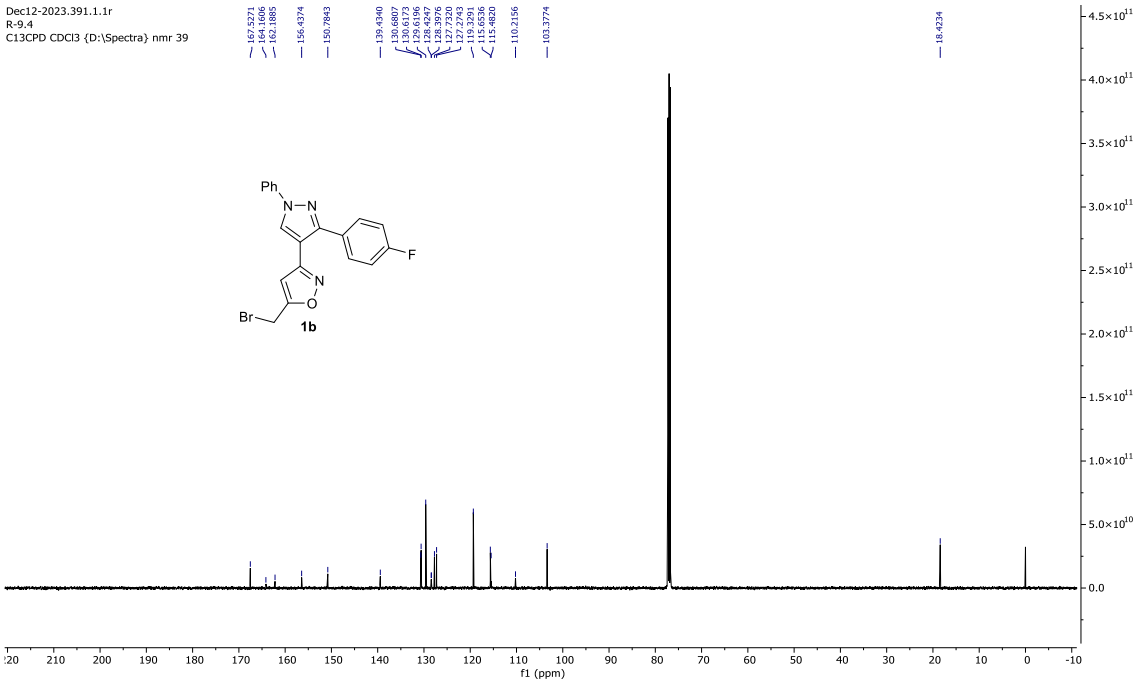

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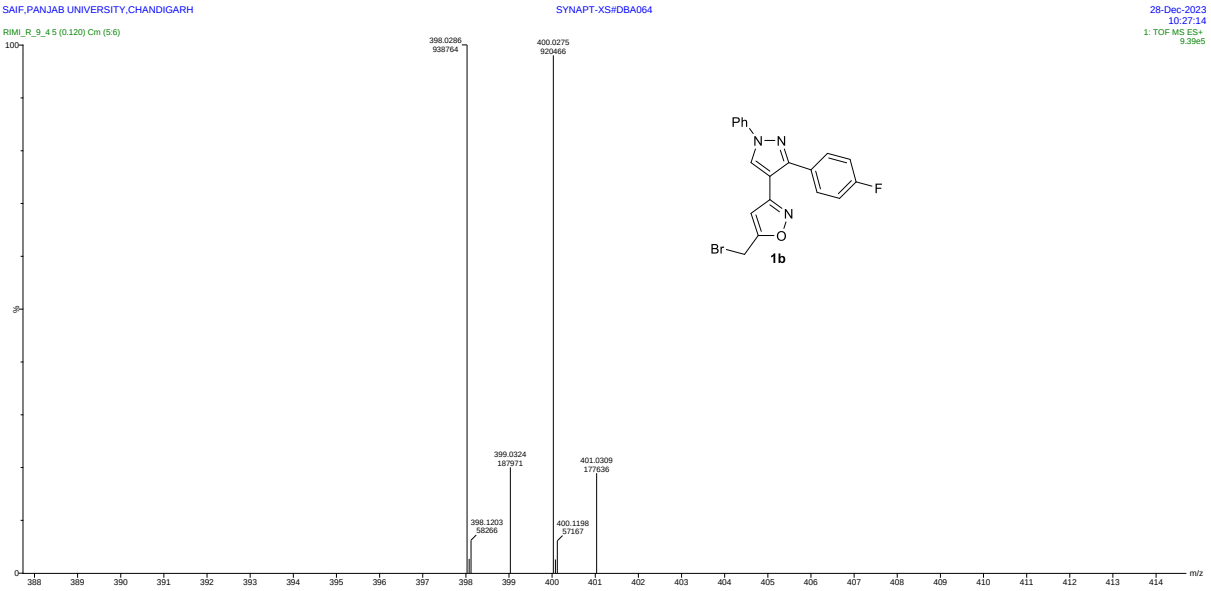
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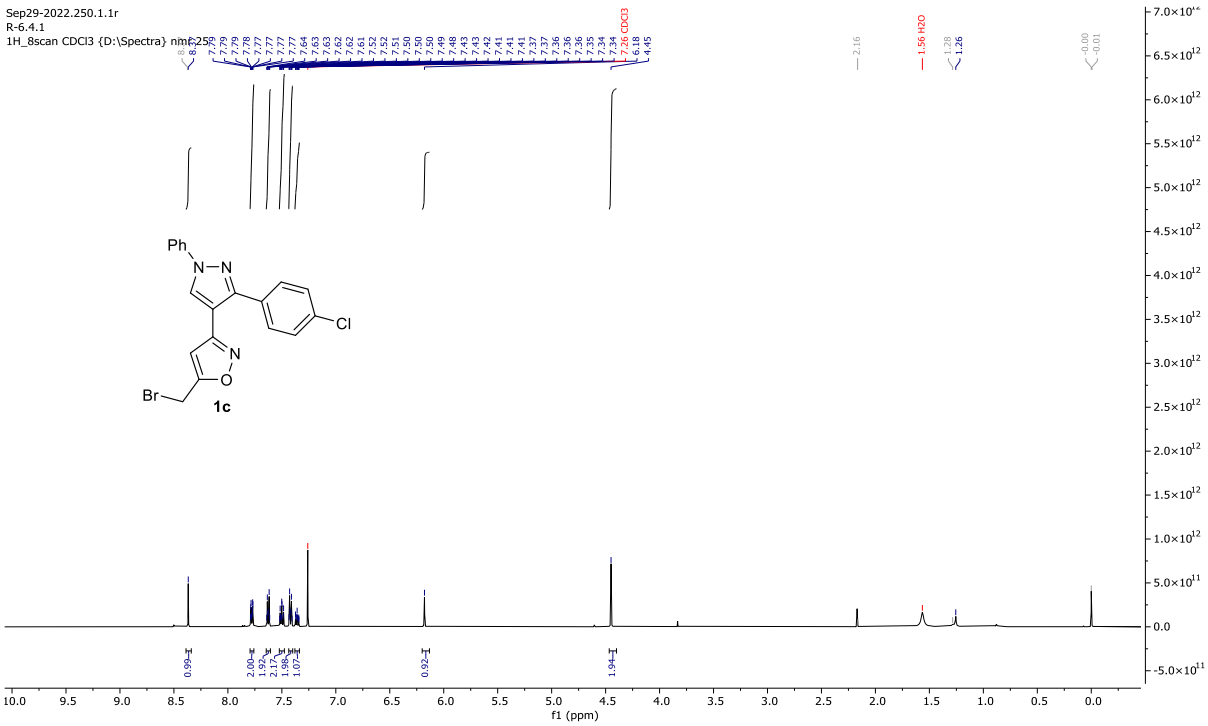

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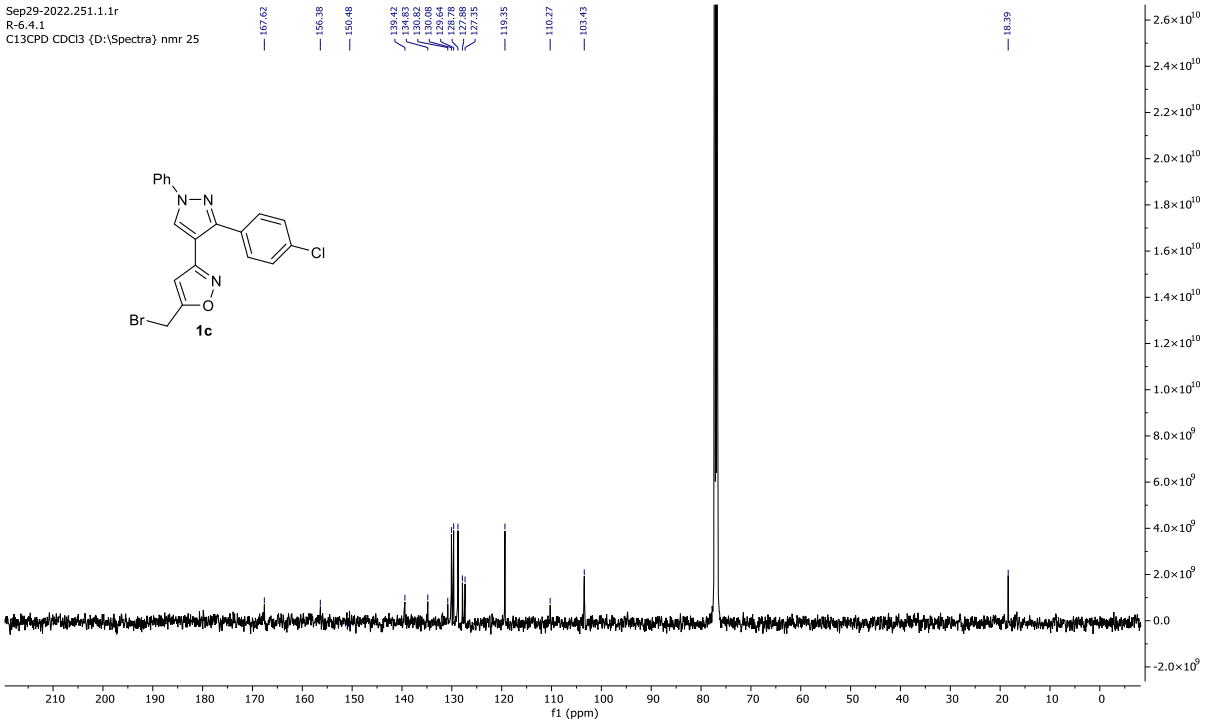

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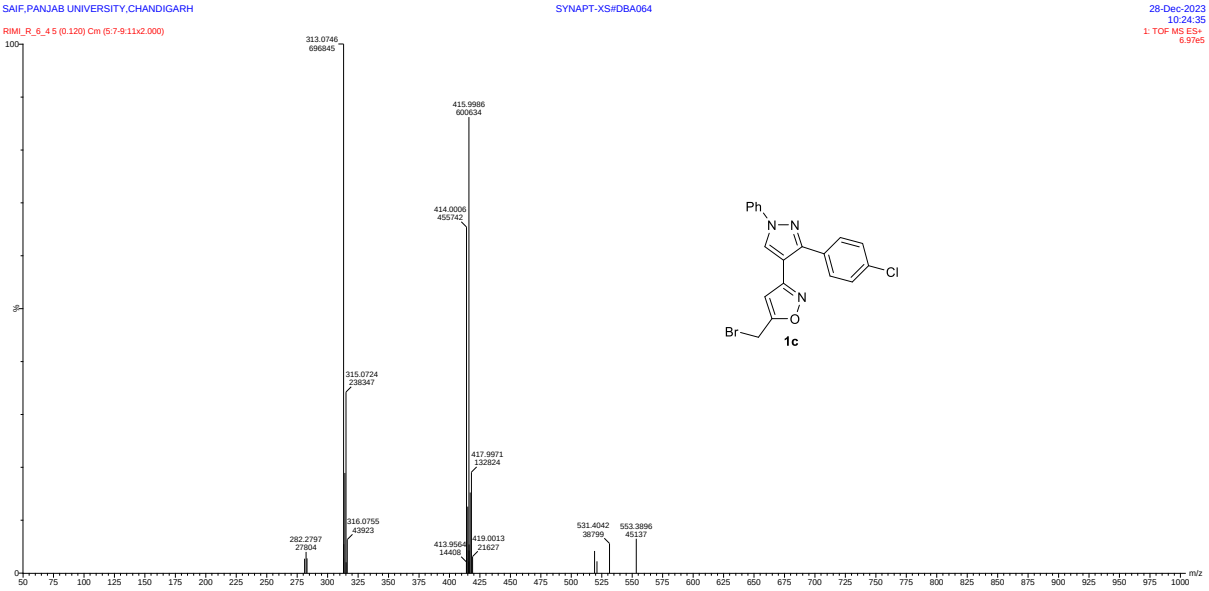
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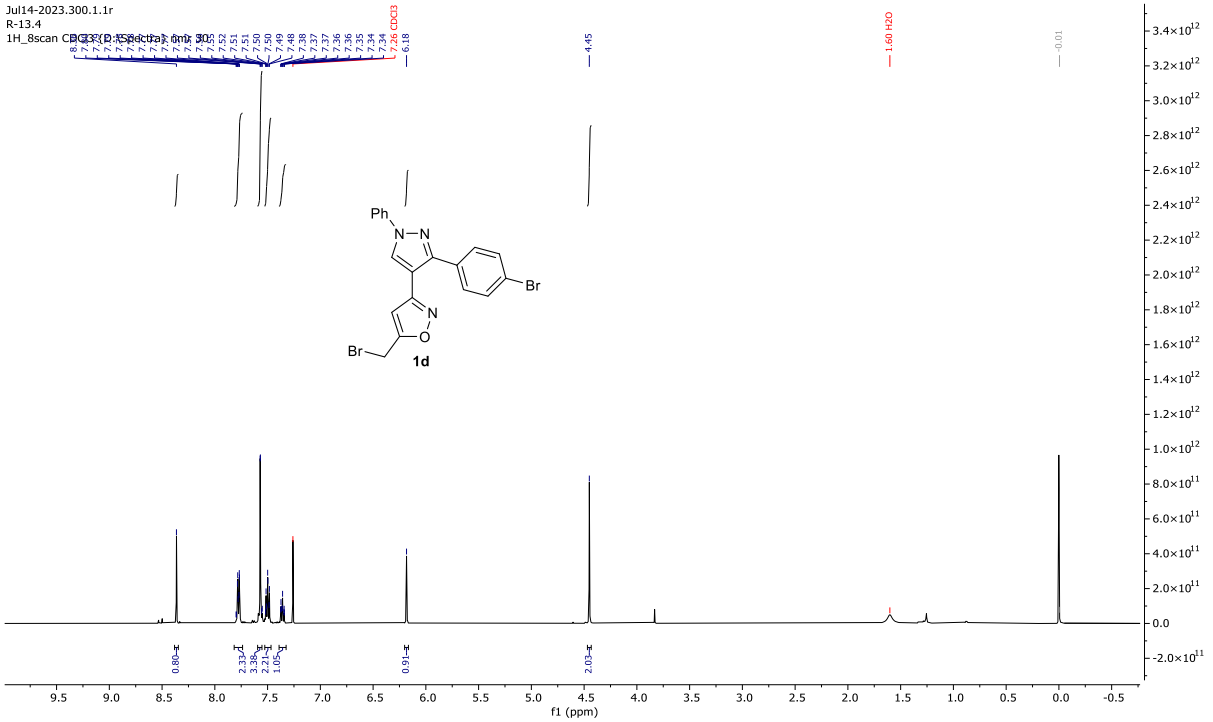

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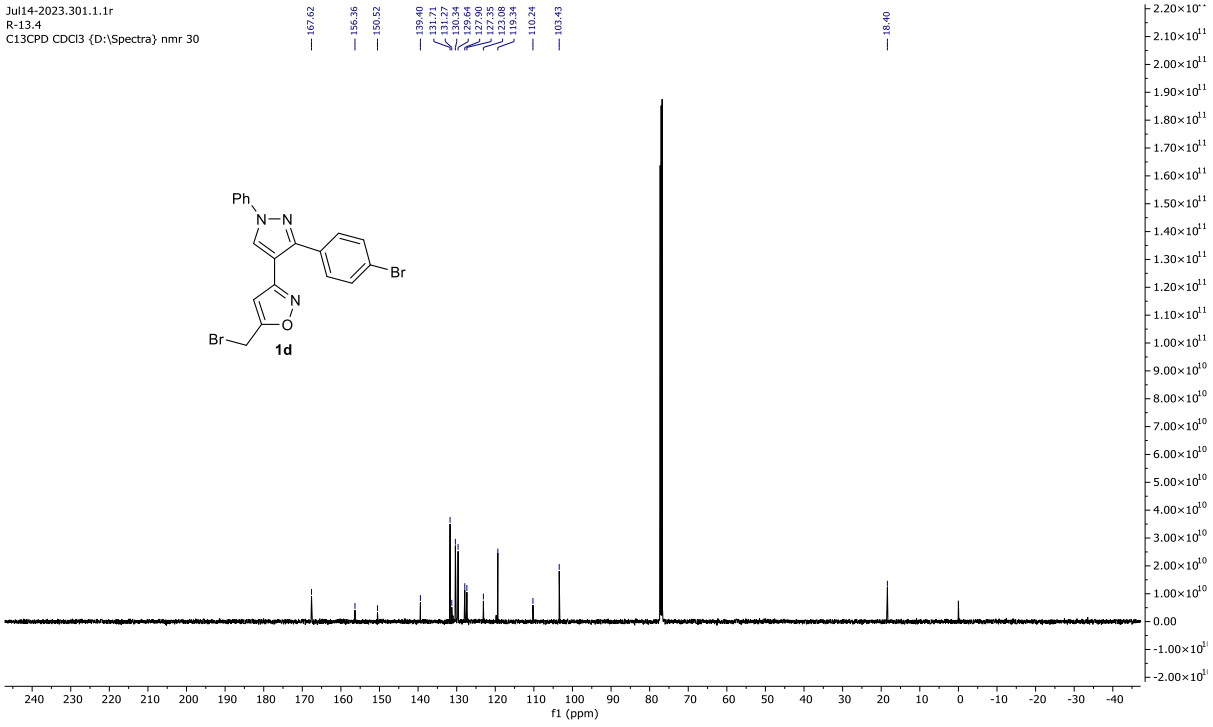

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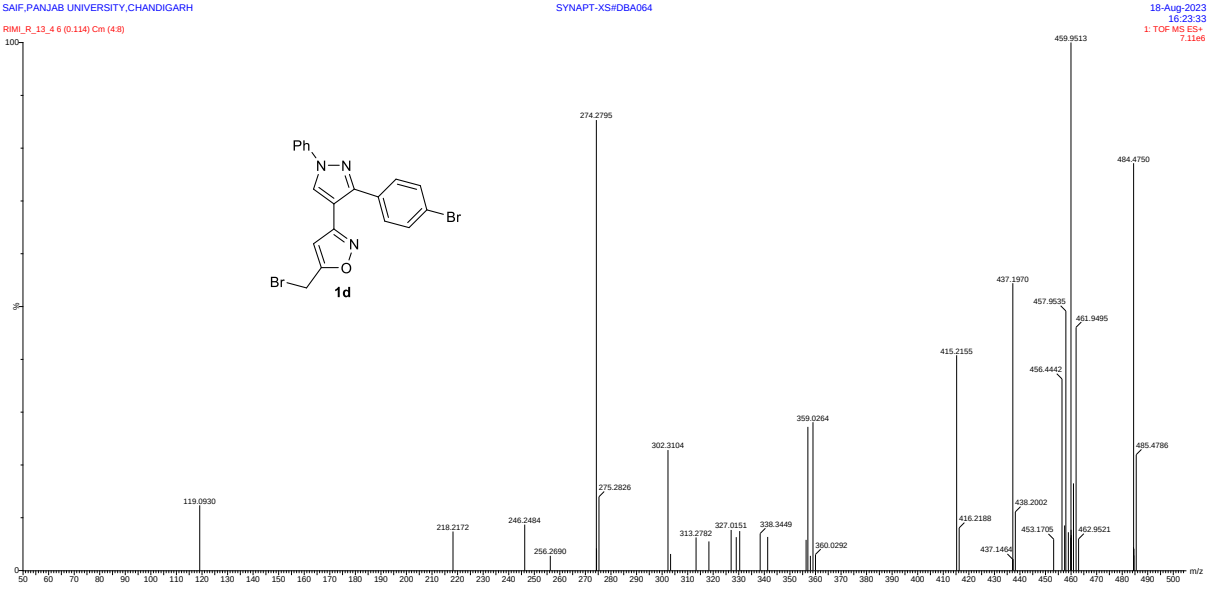
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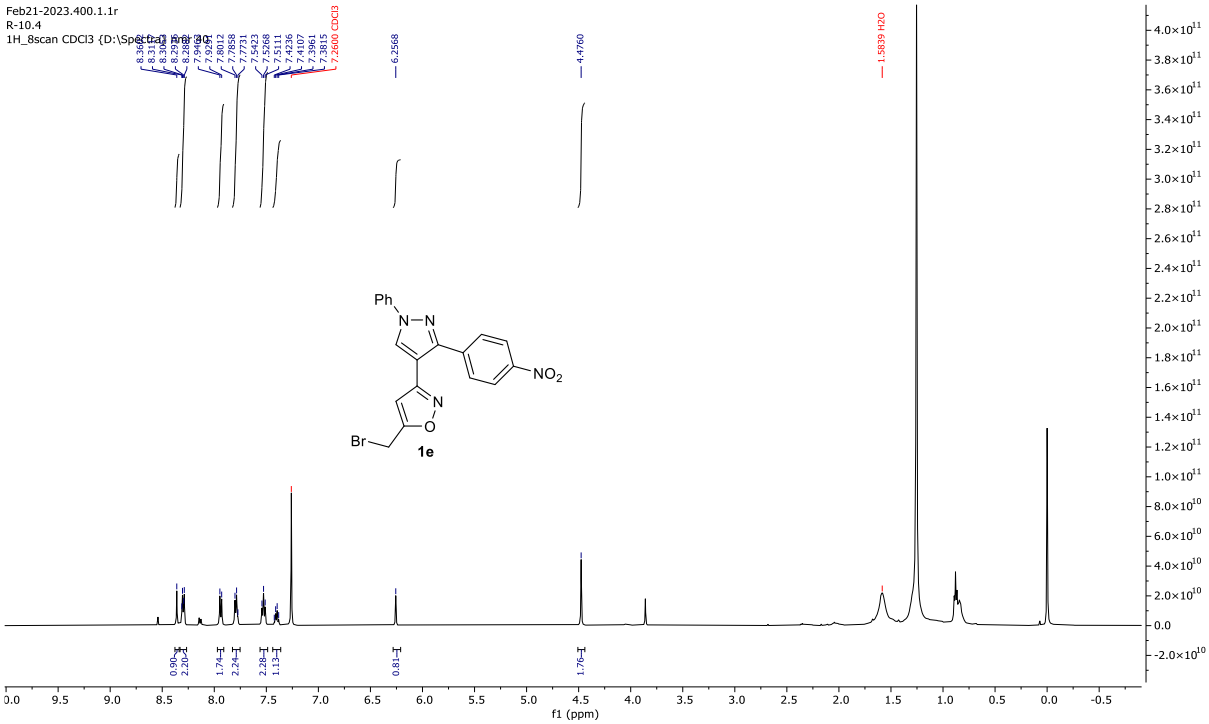

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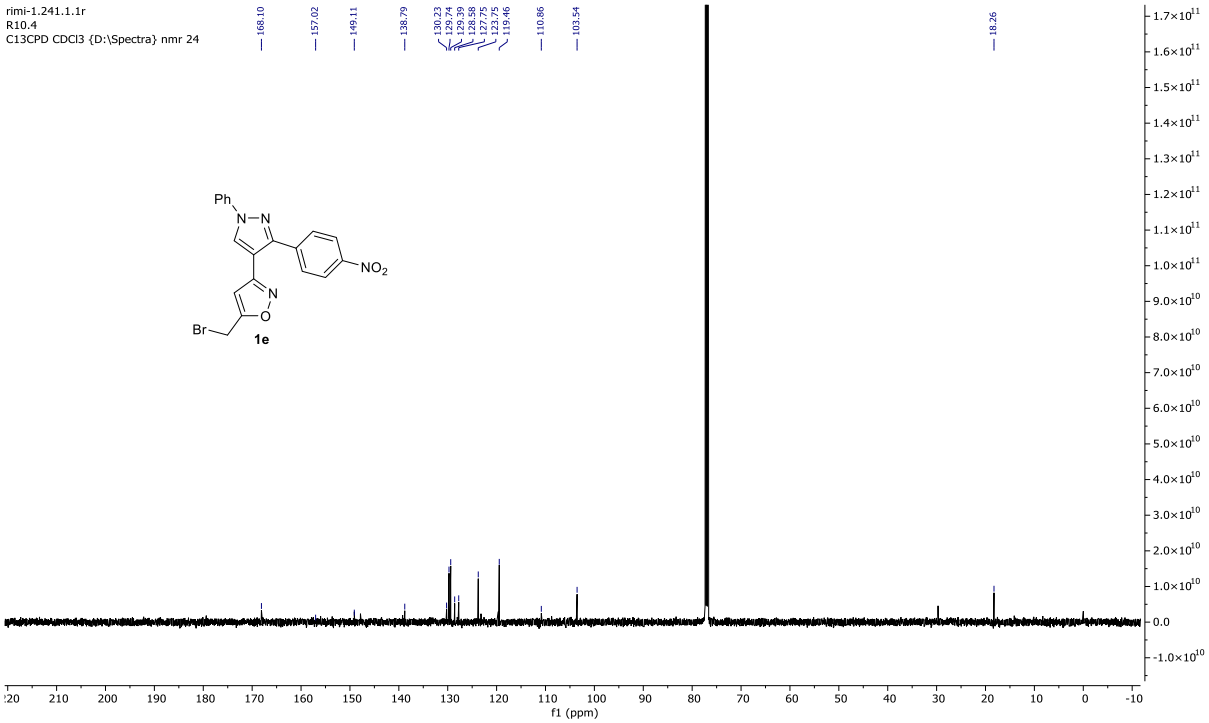

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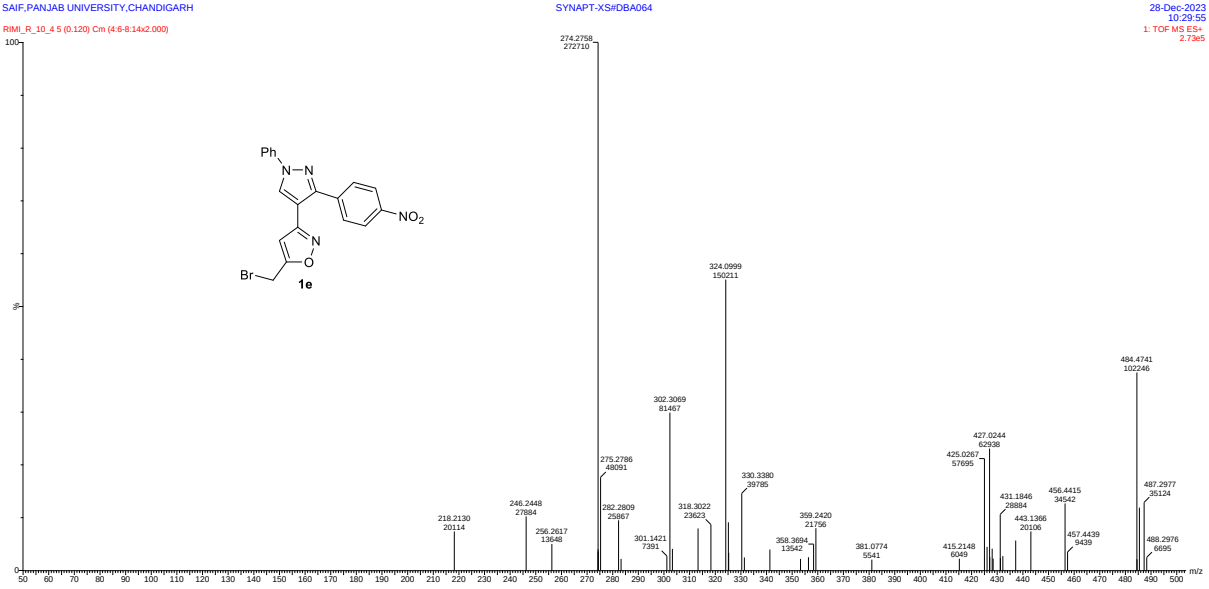

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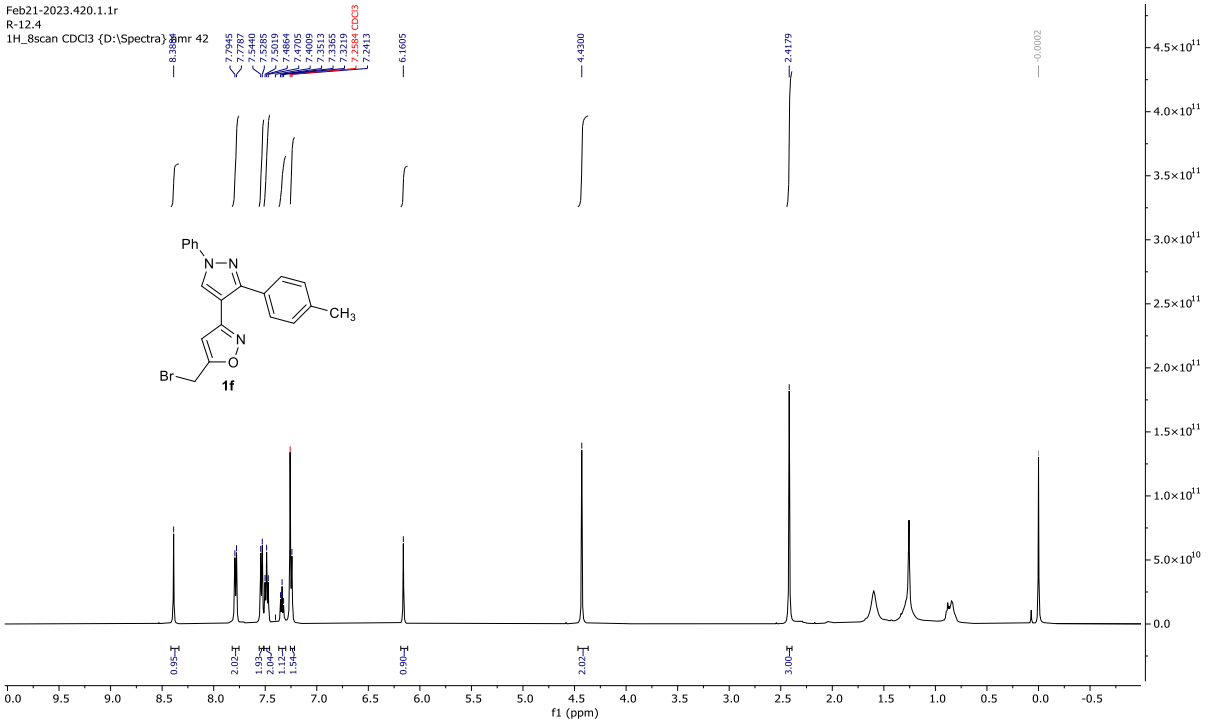

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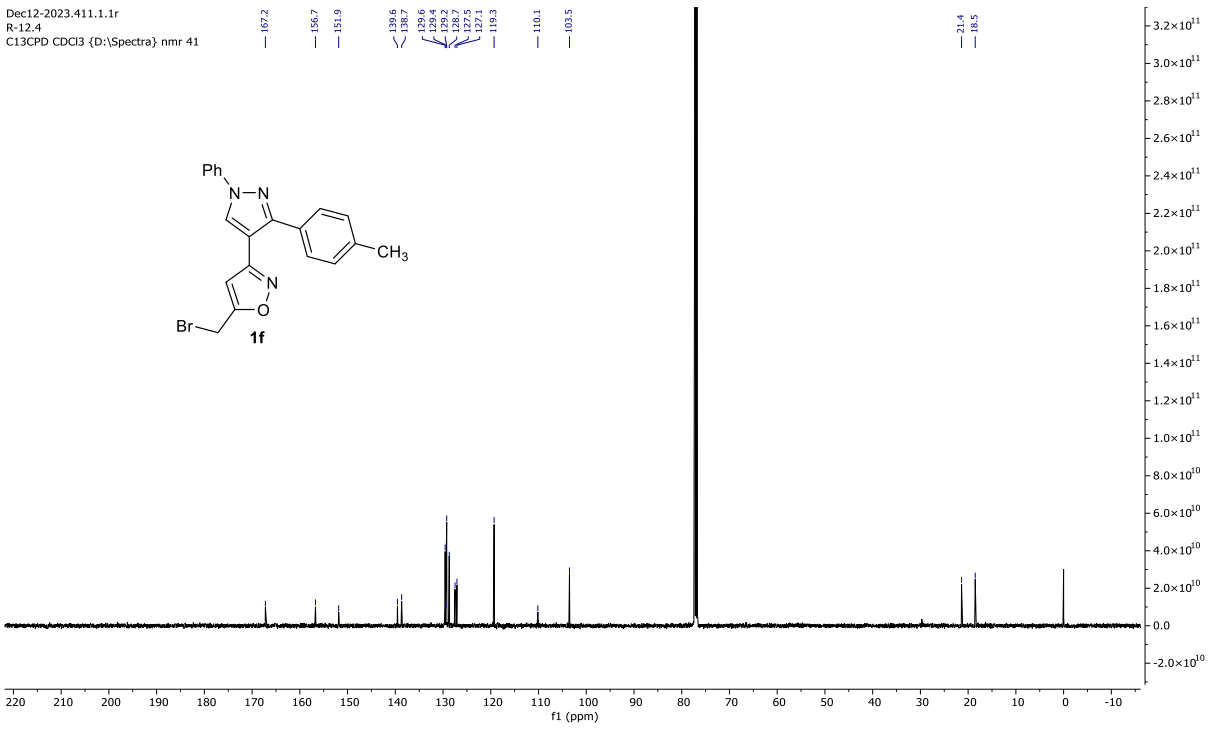

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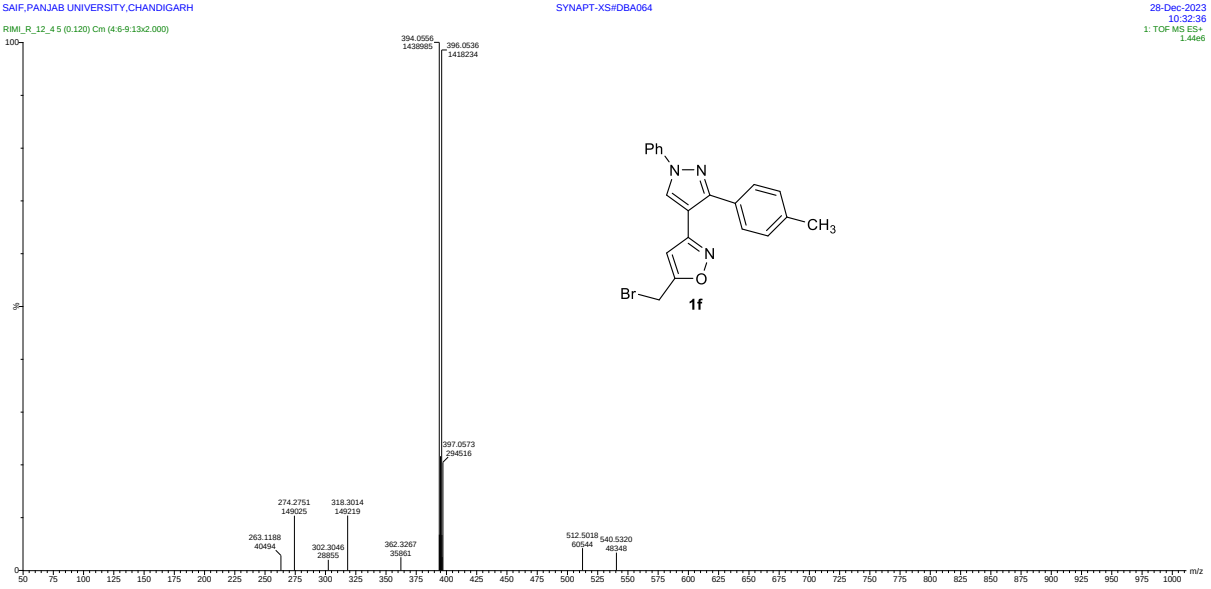
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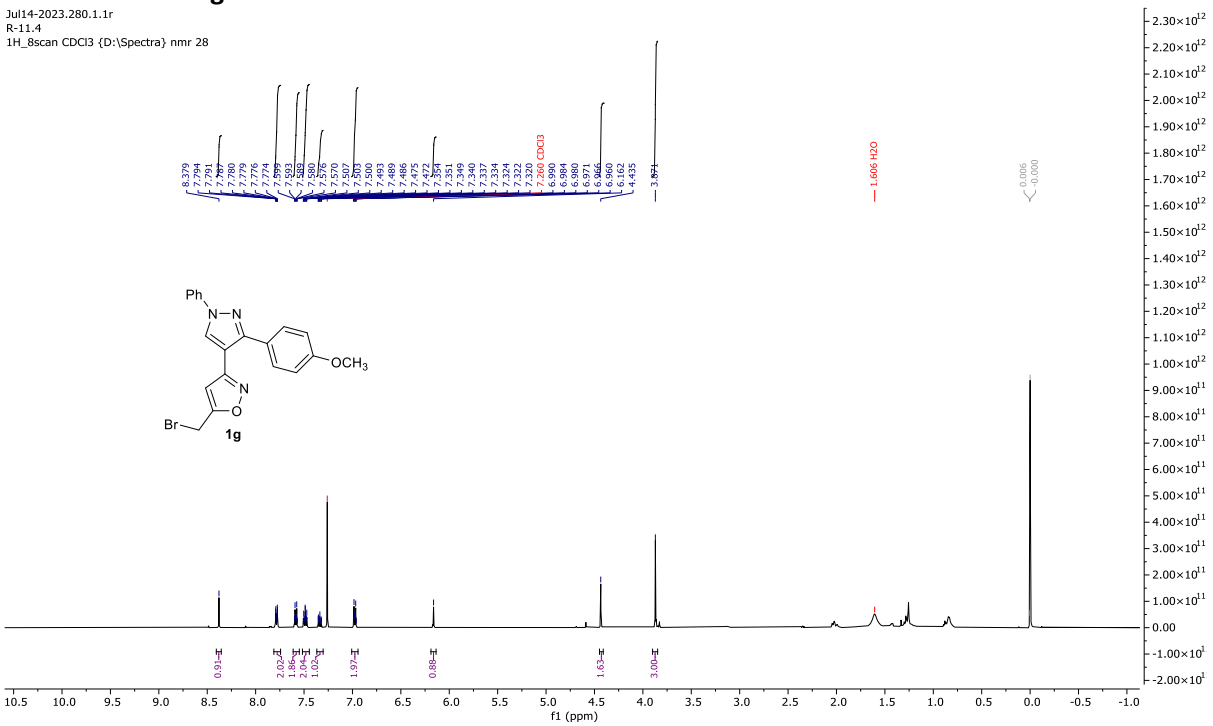

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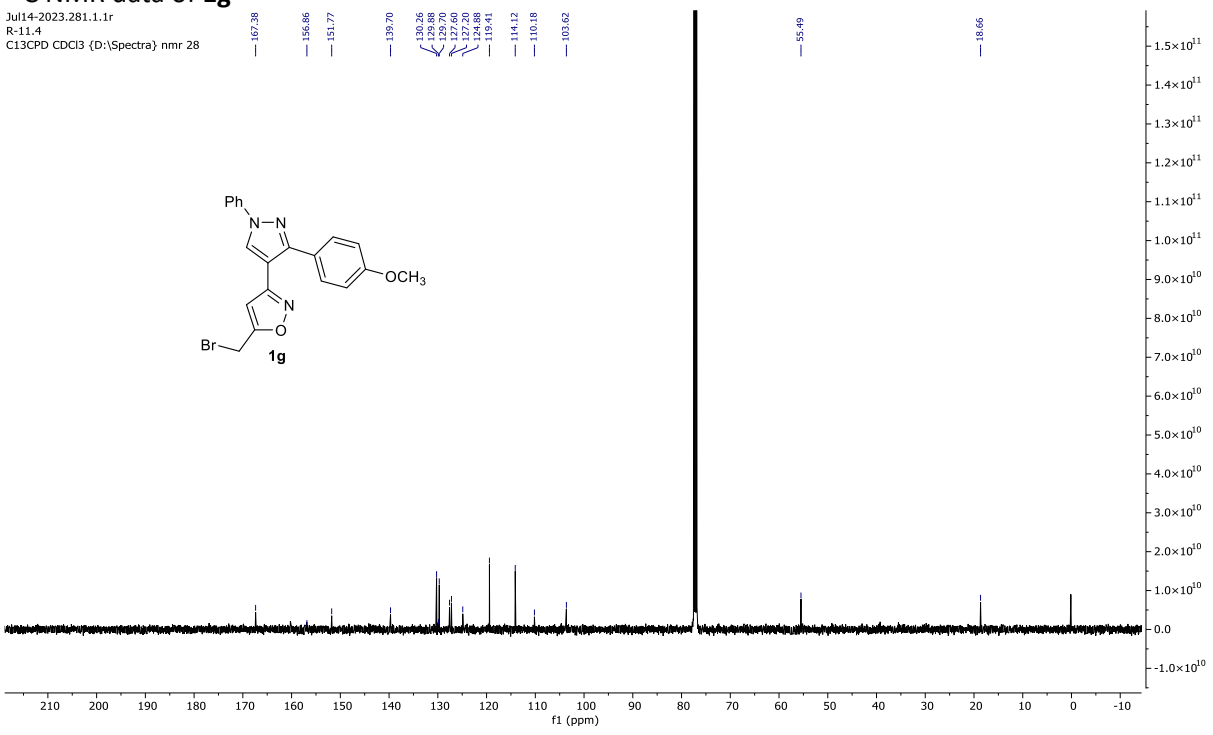

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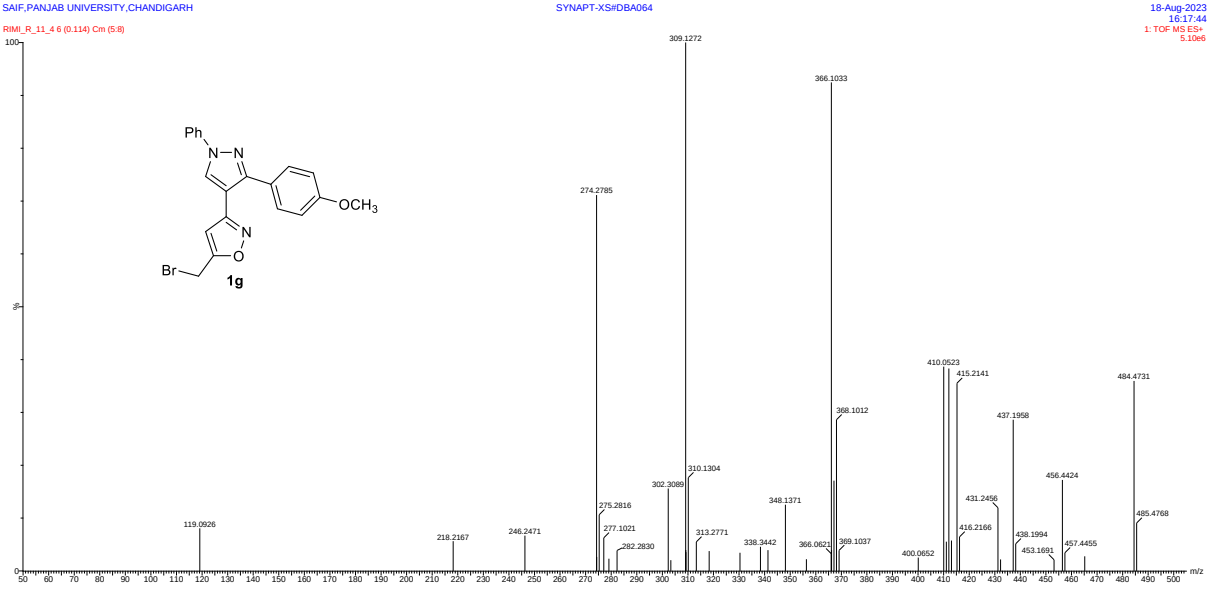
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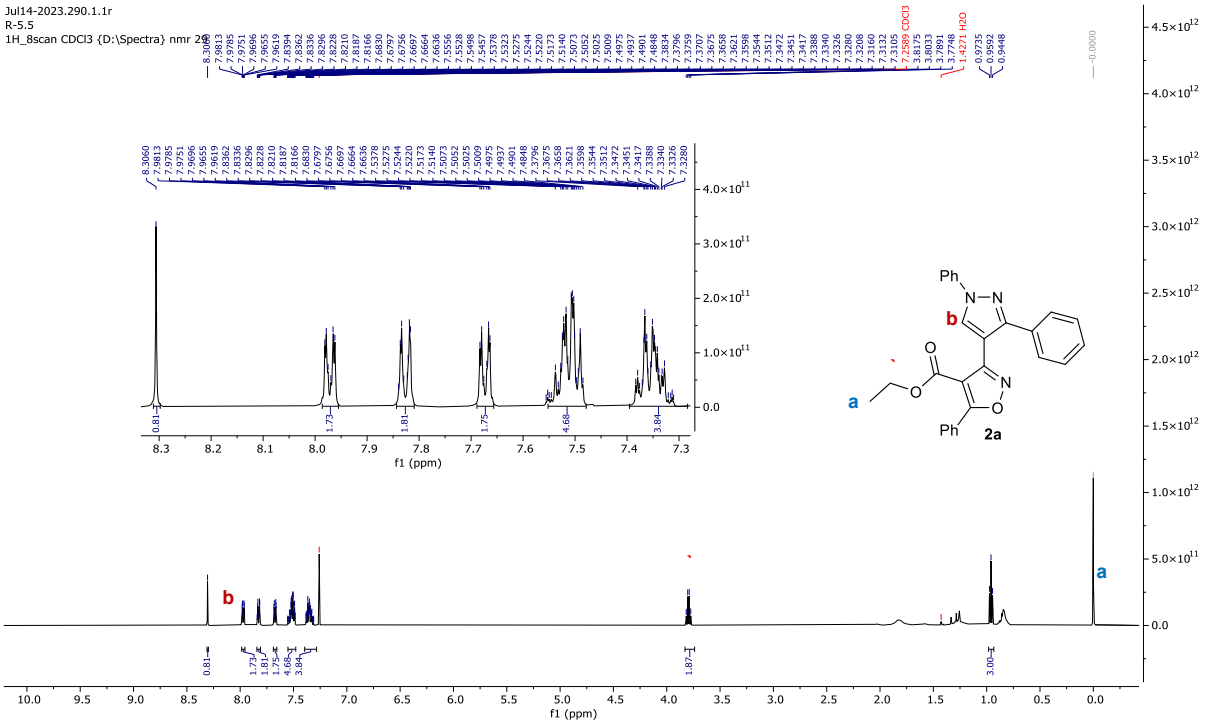

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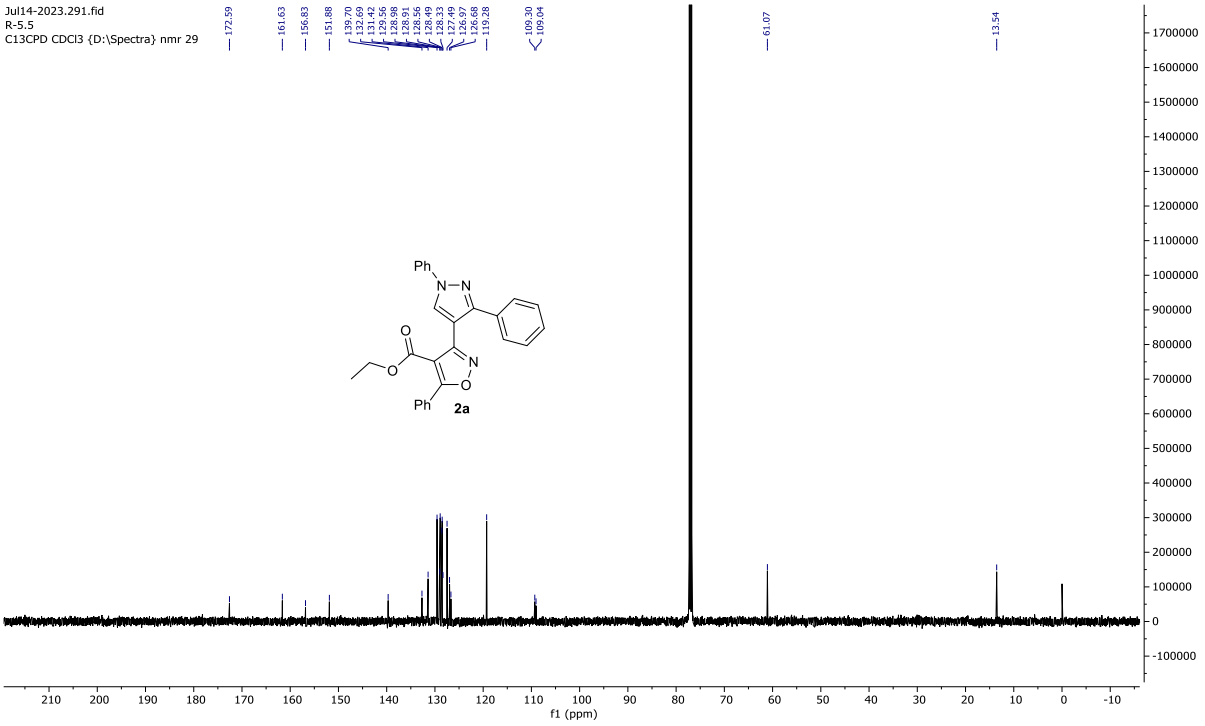

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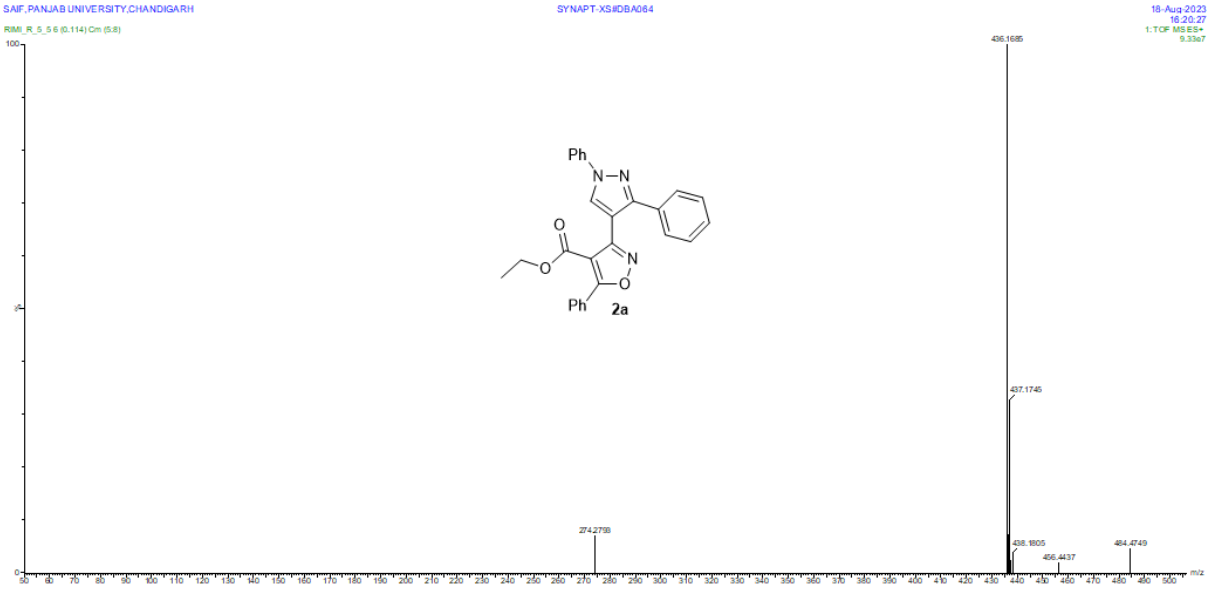
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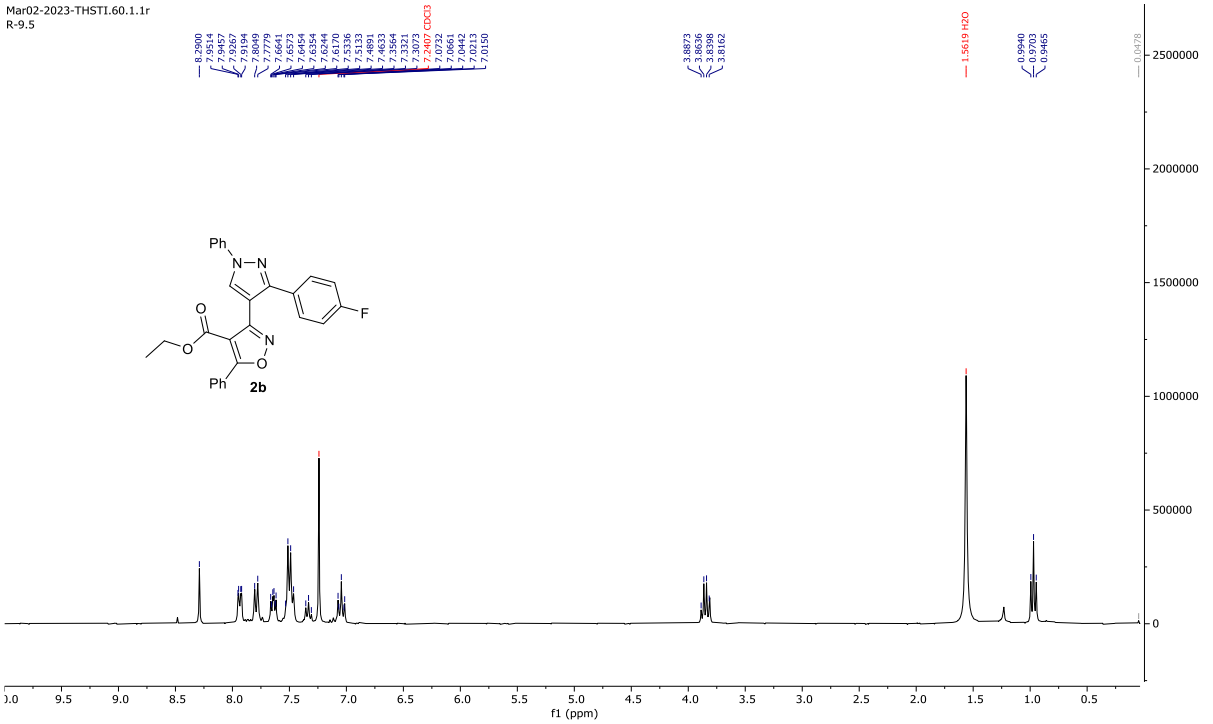

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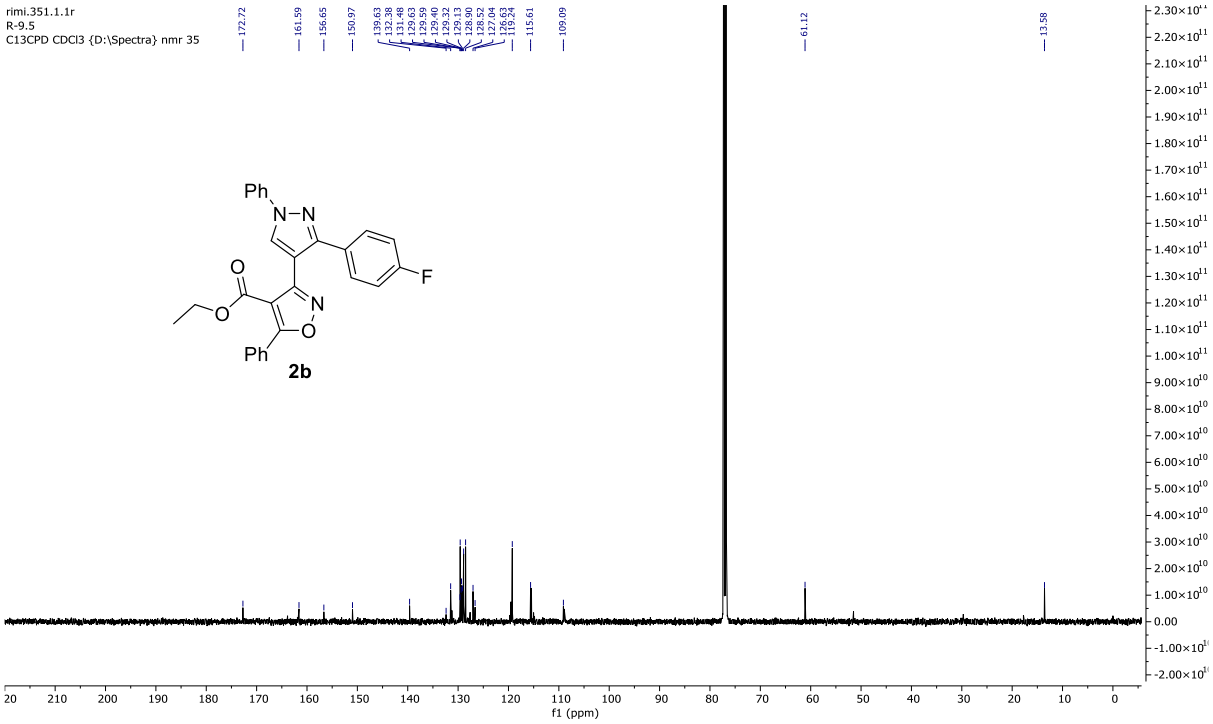

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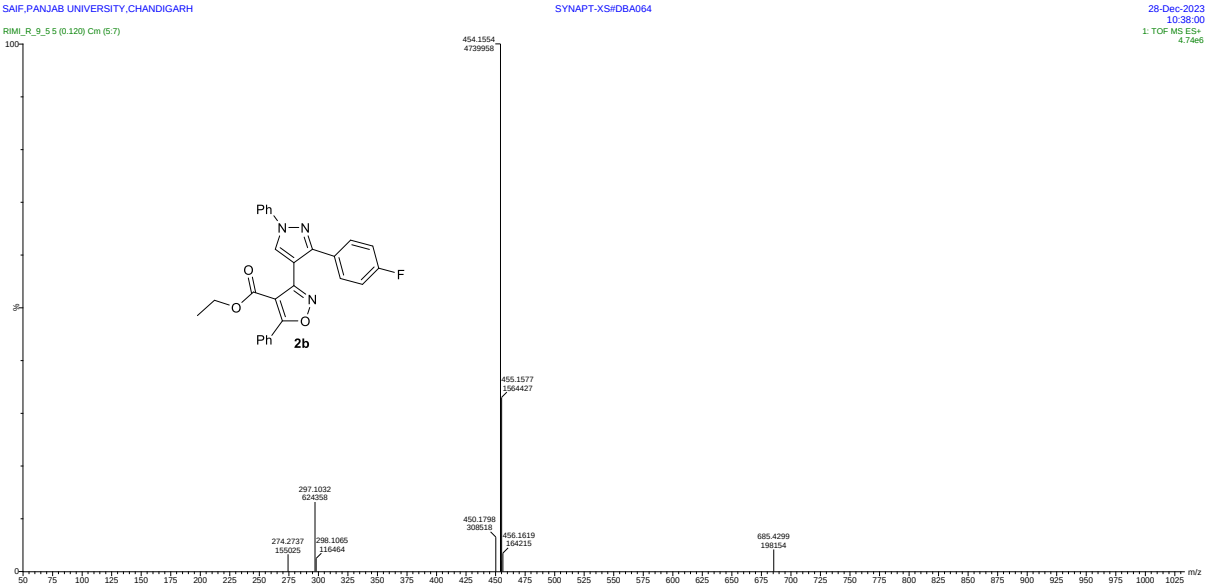
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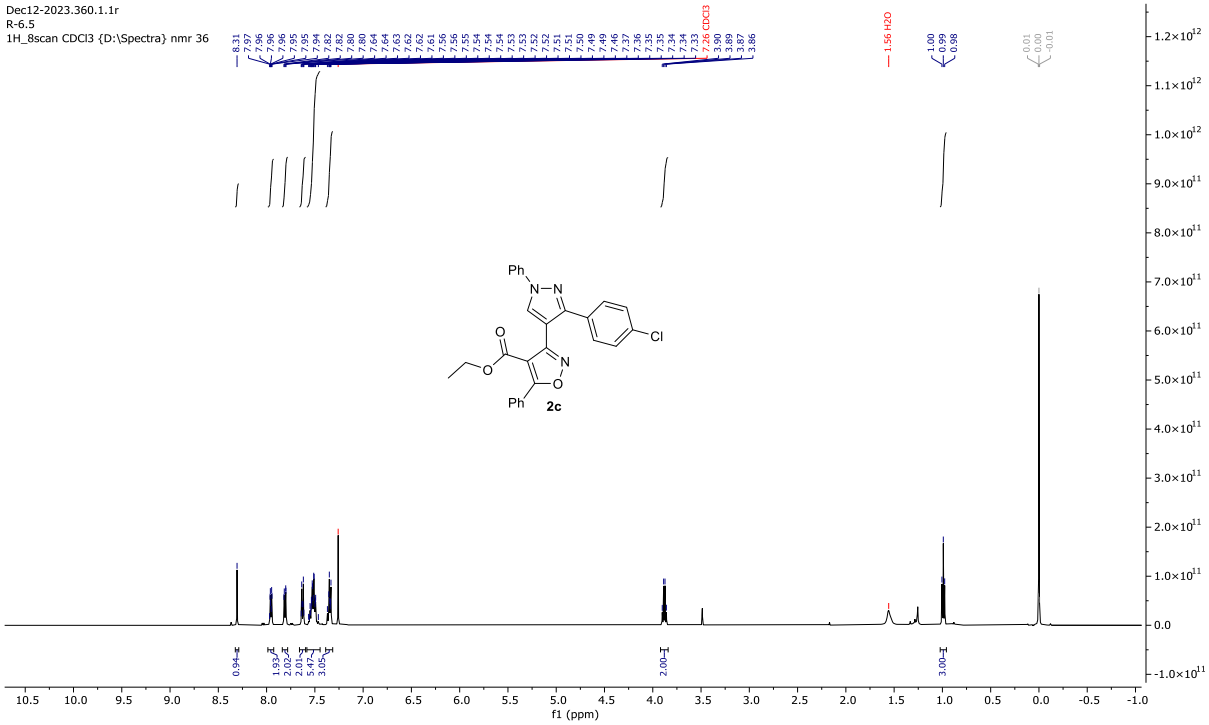

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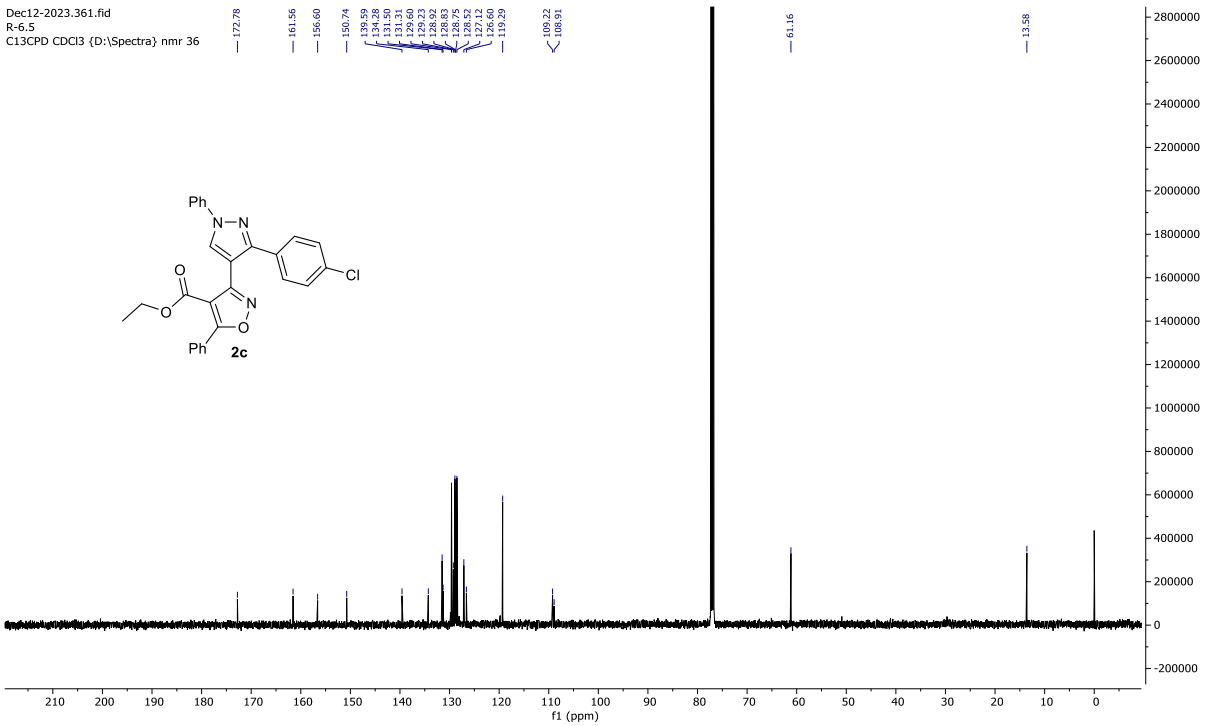

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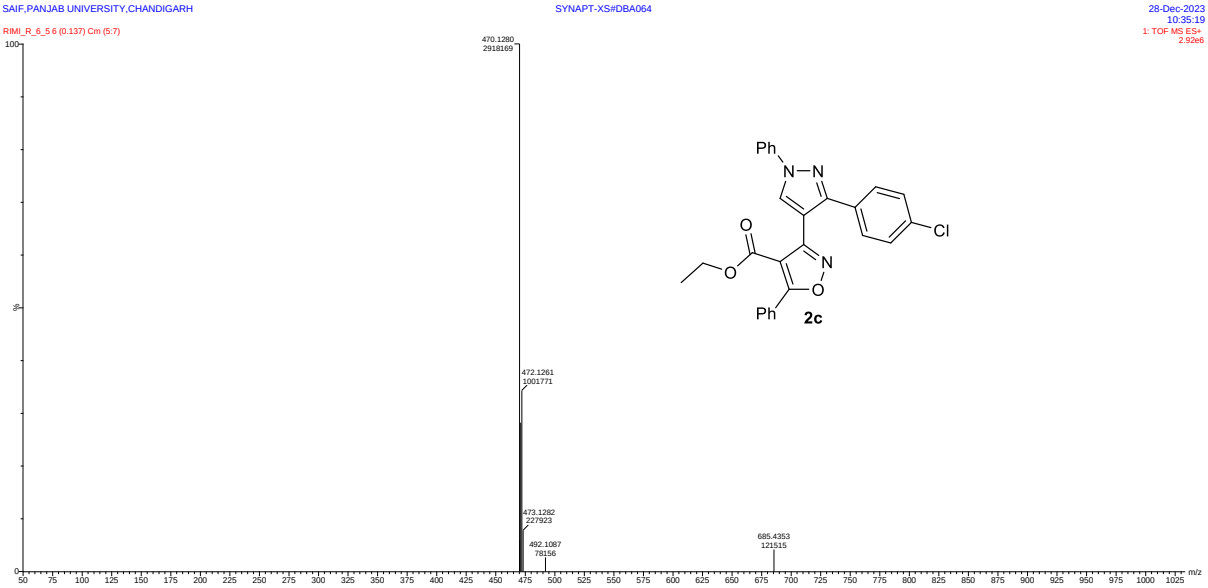
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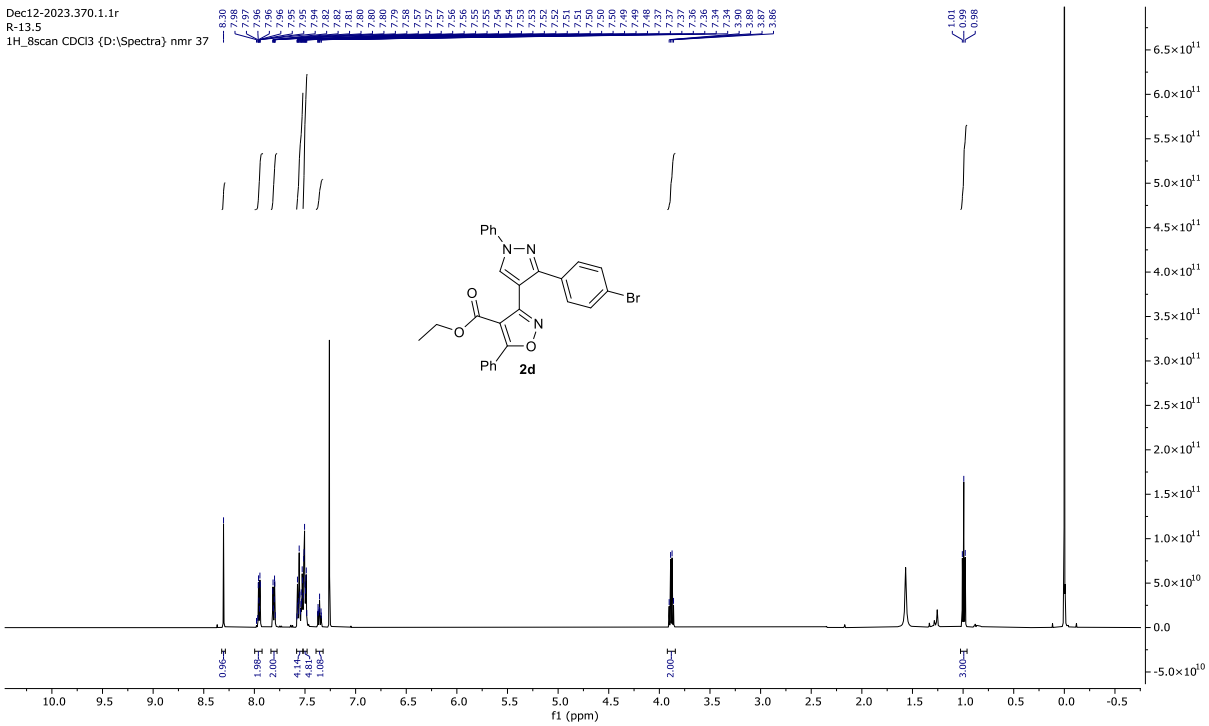

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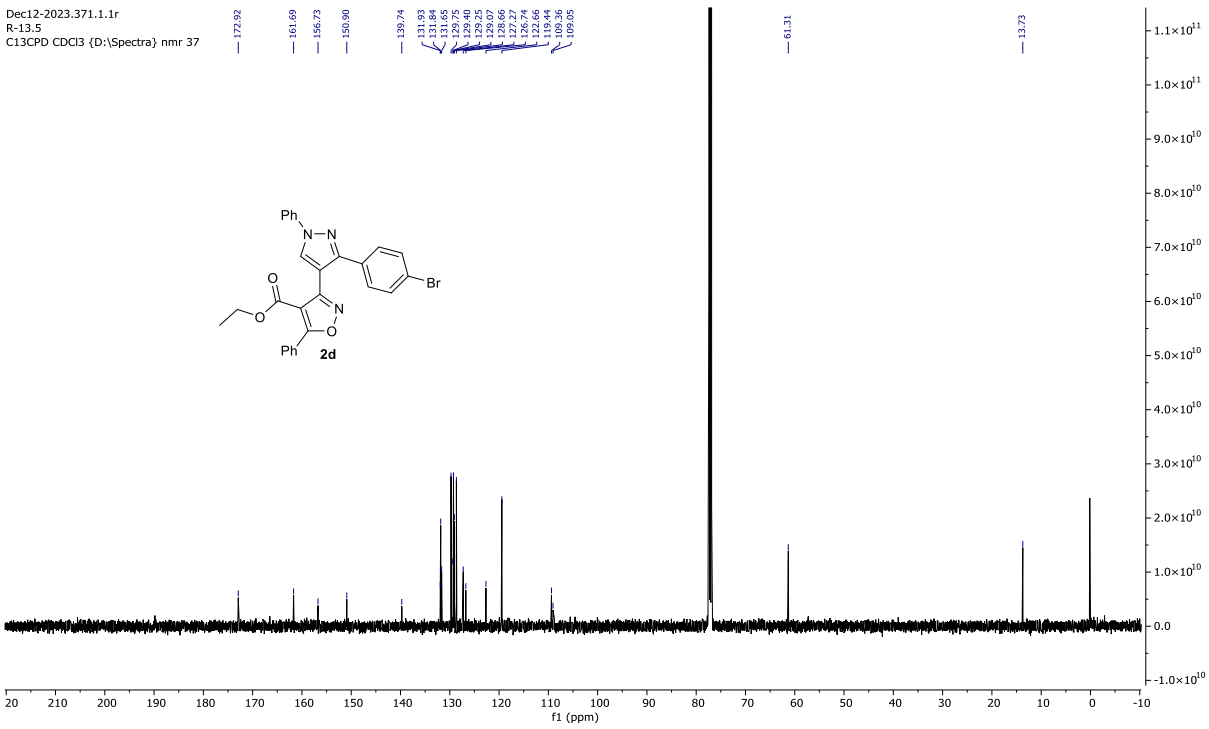

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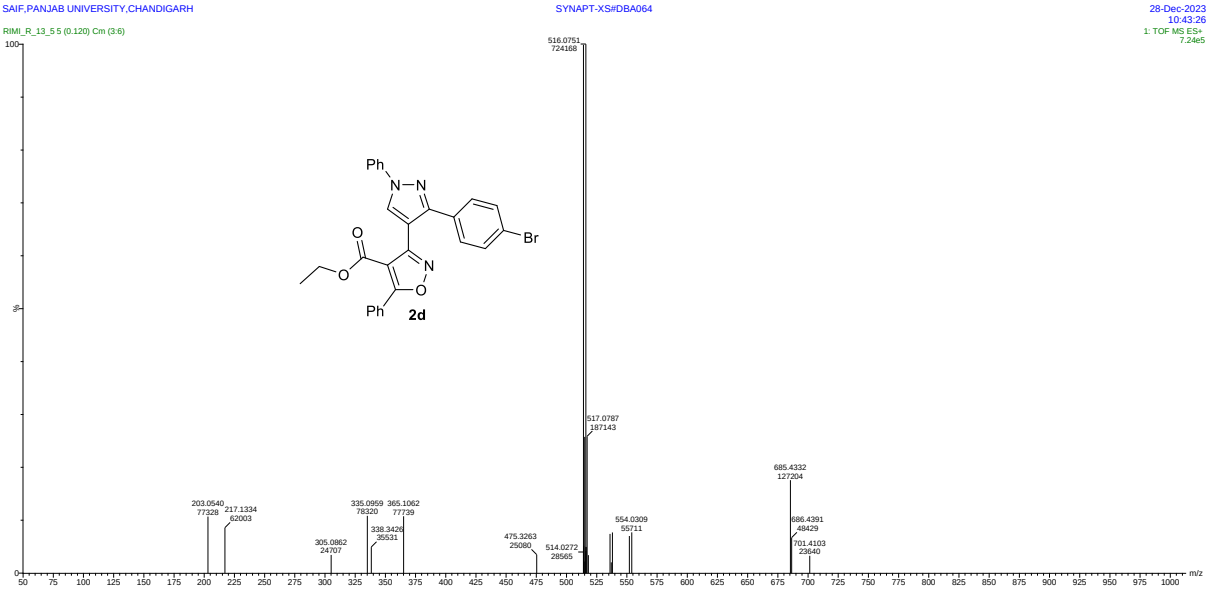
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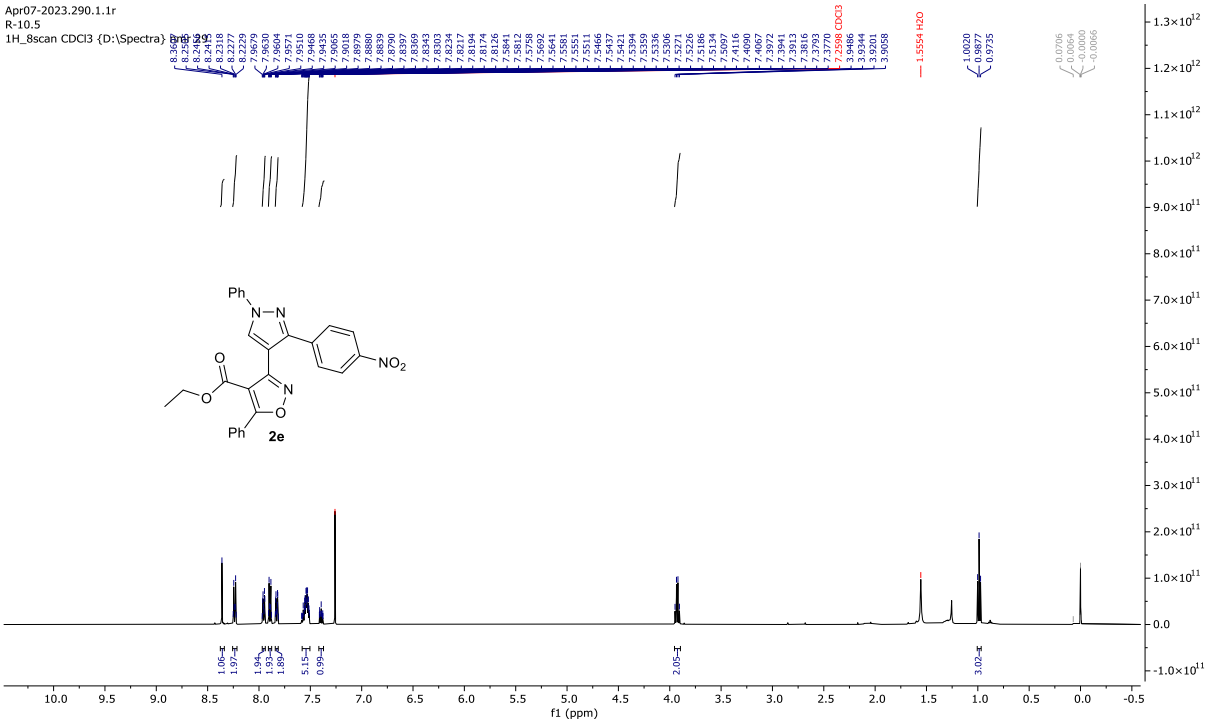
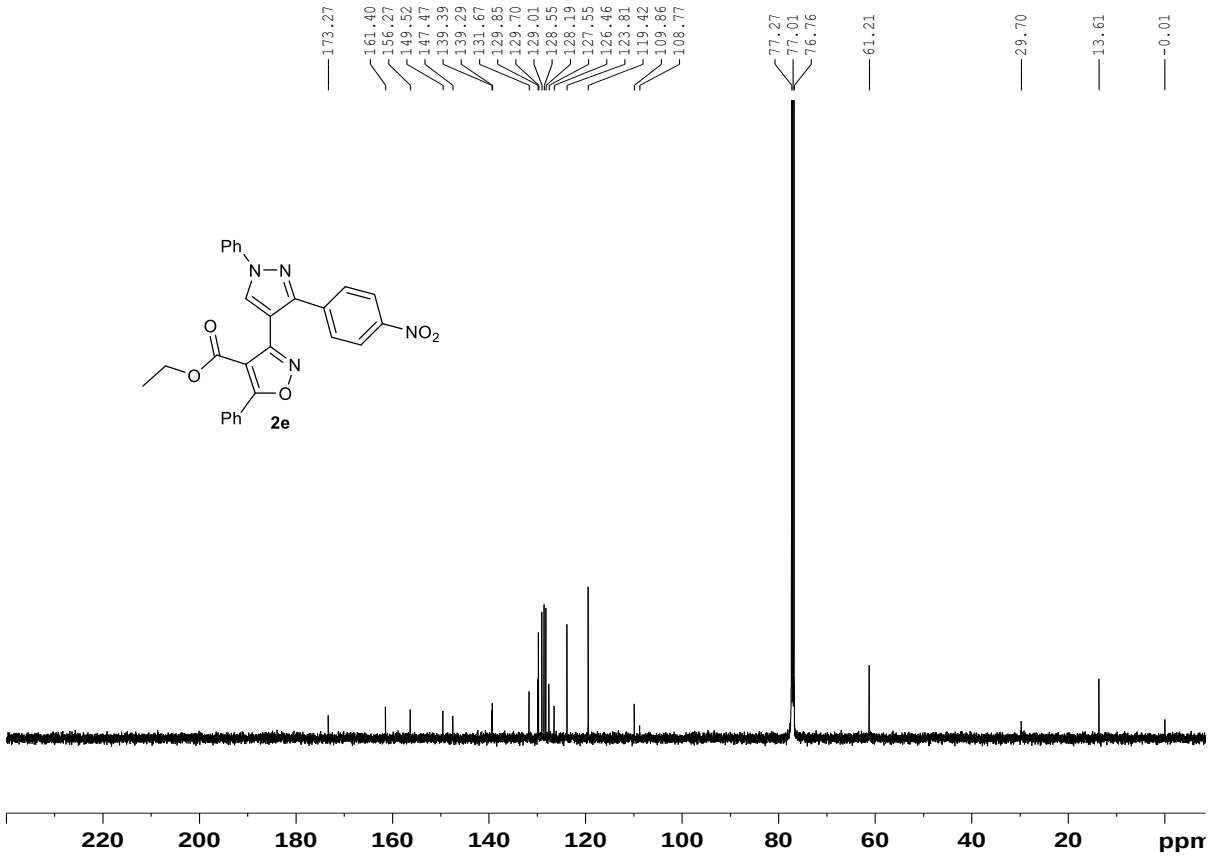
%
m/z

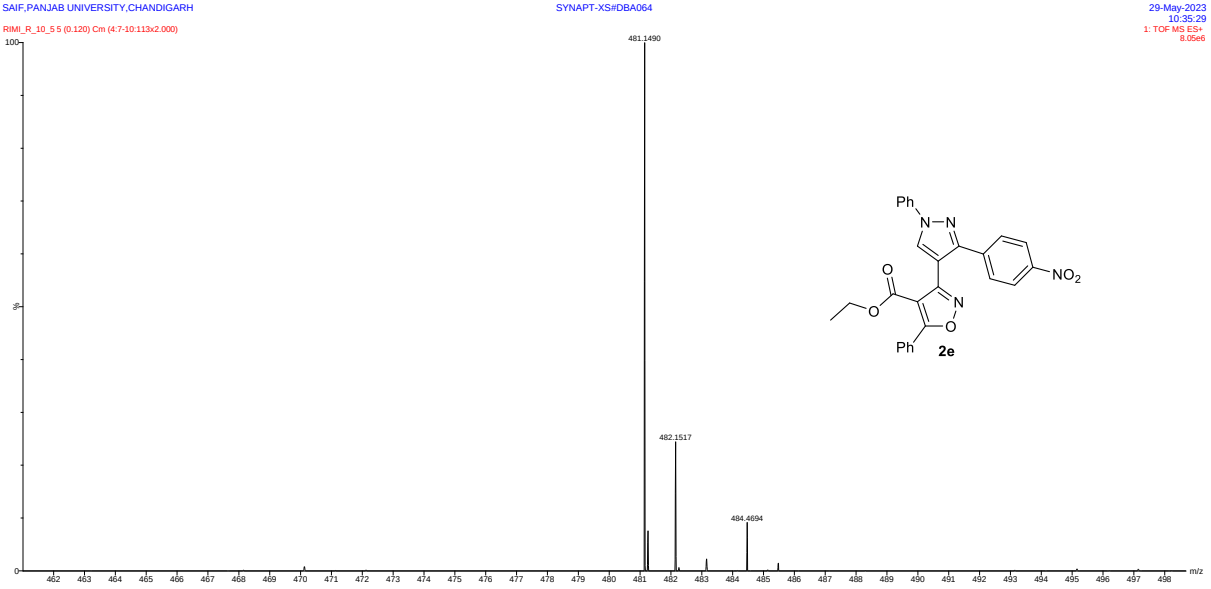
^{1}H NMR data of **2e**



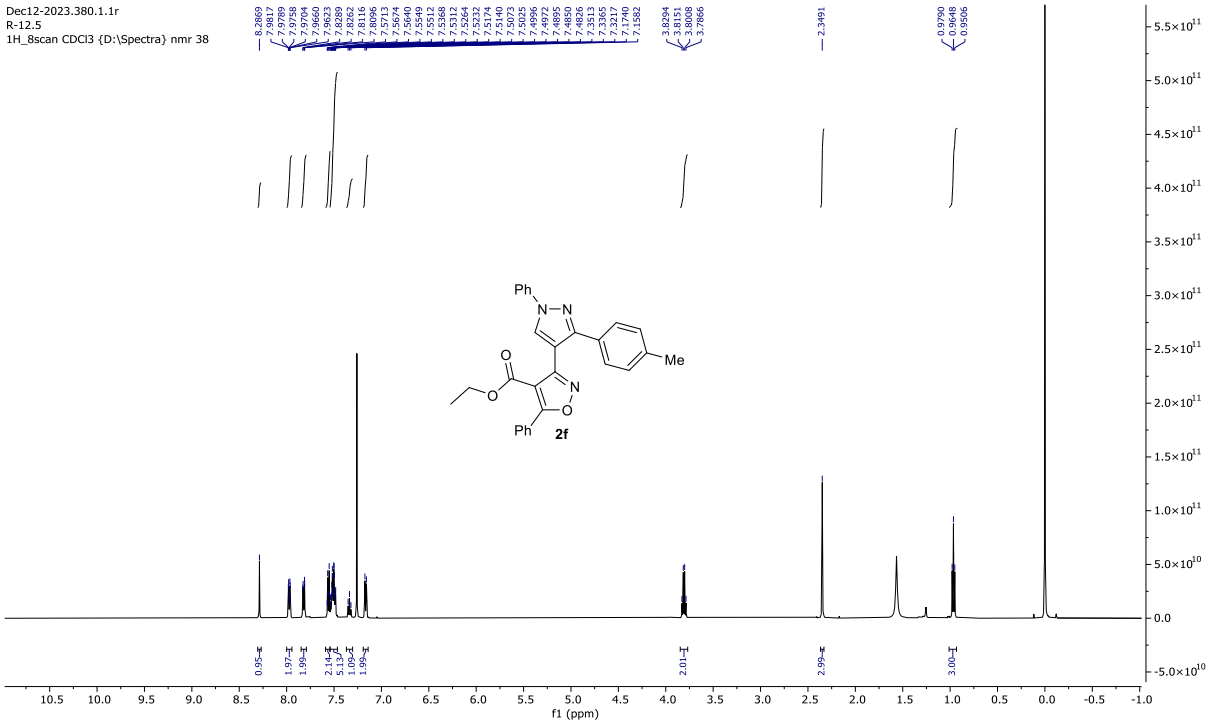
^{13}C NMR data of **2e**



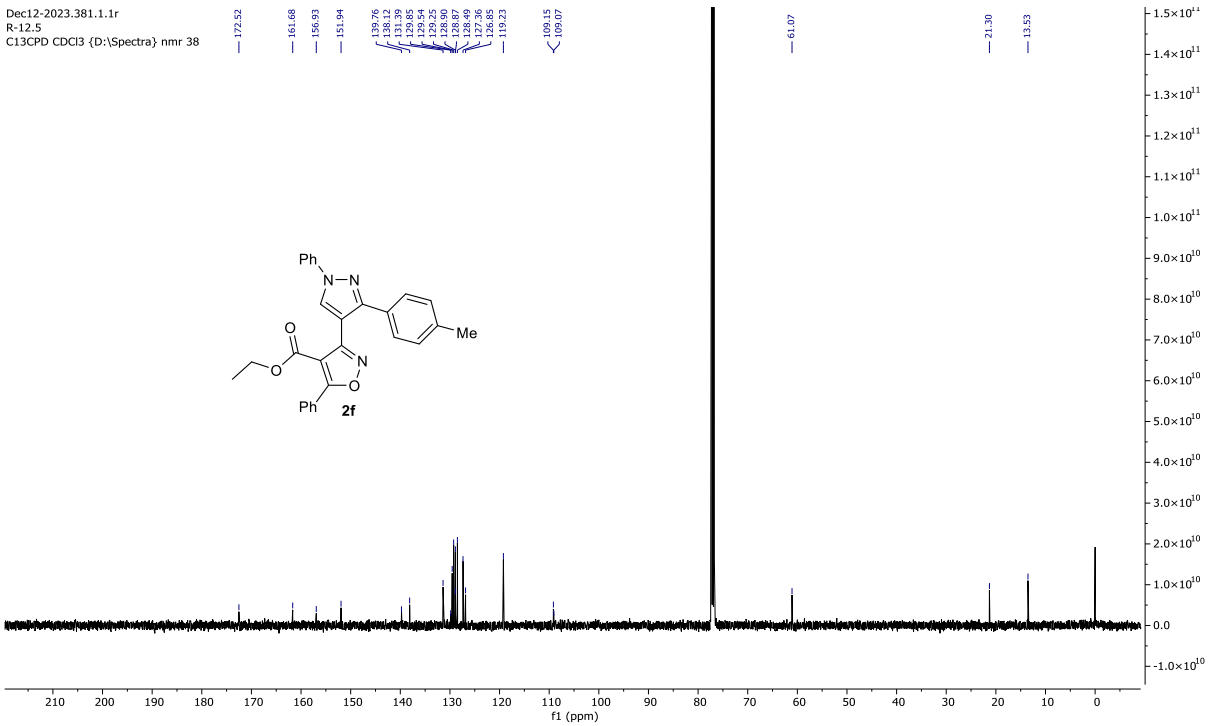
HRMS of **2e**


SAIF,PANJAB UNIVERSITY,CHANDIGARH
SYNAPT-XS#DBA064
29-May-2023
10:35:29
RIMI_R_10_5 5 (0.120) Cm (4:7-10:113x2.000)
1: TOF MS ES+
8.05e6
481.1490
482.1517
484.4694
Ph
N
N
O
O
N
O
Ph
NO2
2e
%
m/z

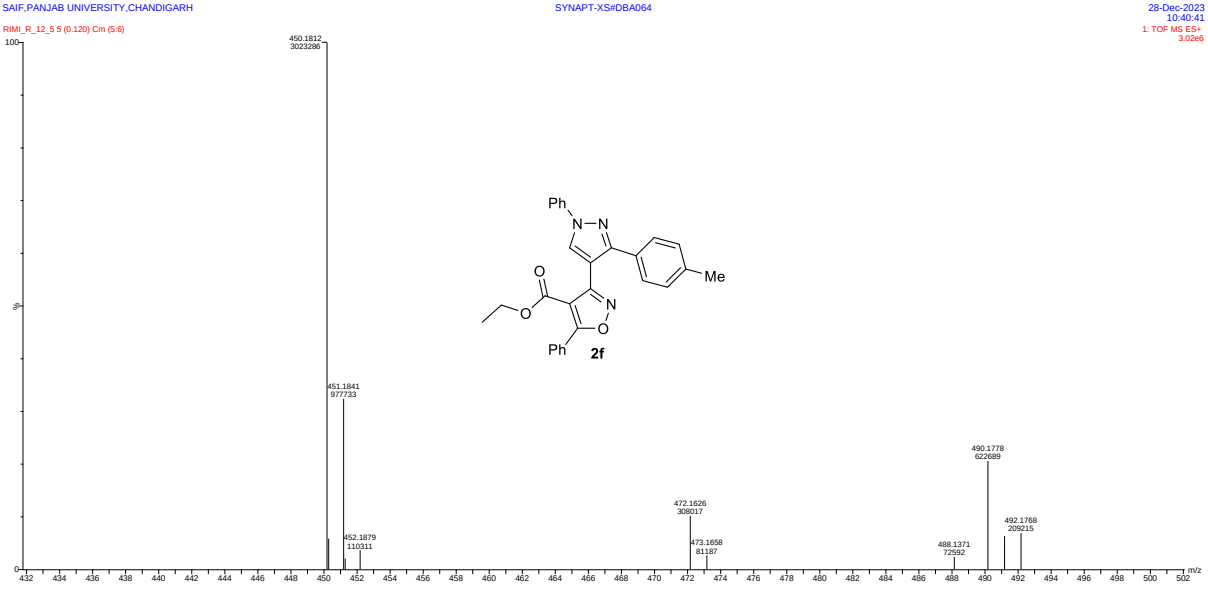
^{1}H NMR data of **2f**



^{13}C NMR data of **2f**



HRMS of **2f**


SAIF,PANJAB UNIVERSITY,CHANDIGARH
SYNAPT-XS#DBA064
28-Dec-2023
10:40:41
RIMI_R_12_5 5 (0.120) Cm (5:6)
1: TOF MS ES+
3.02e6
450.1812
3023286
451.1841
977733
452.1879
110311
472.1626
308017
473.1658
81187
488.1371
72592
490.1778
622689
492.1768
209215
Ph
N—N
Me
O
O
N
O
Ph
2f
%
m/z