Supplementary Material

Metal-free hypervalent iodine-mediated synthesis and biological evaluation of 2-[(3-aryl-isoxazol-5-yl)methoxy)]benzaldehydes

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S1. Biological Assay

Antimicrobial assay[1]

The samples were examined in vitro to determine potential against one fungal pathogen strain and seven bacterial strains by using double dilution method. The strains were cultured in potato dextrose agar and in nutrient agar made from a 20% volume by volume glycerol stock, prior to use in the study. The temperature was kept at -20 °C when these strains were cultured. MIC values were determined by using the Mueller Hinton broth protocol utilizing 96-well ELISA plates. Microbial growth in all samples and in control sample were incubated for 24 hours at 37°C for bacterial strains and, for fungal strain the temperature was kept 30°C. The estimation of growth was recorded at 690 nm in ELISA plate reader. Amoxycillian, a broad-spectrum antibacterial agent, and flucanazole, a broad spectrum anti-fungal agent was taken as control samples.

Antioxidant assay[2]

Antioxidant behaviour of the synthesized compounds was checked by using the DPPH method taking ascorbic acid as reference compound. Both DPPH and ascorbic acid were procured from Merck. Methanol was used as solvent to prepare the samples of both DPPH solution and the target compounds 1a-m. The reaction mixture was prepared by combining 2.5 mL of the target compound solution with per ml of DPPH solution. The absorbance was calculated at 517nm in triplicates considering the mean values by keeping reaction mixture at 37°C in an incubator for half an hour.

Cytotoxicity assay[3]

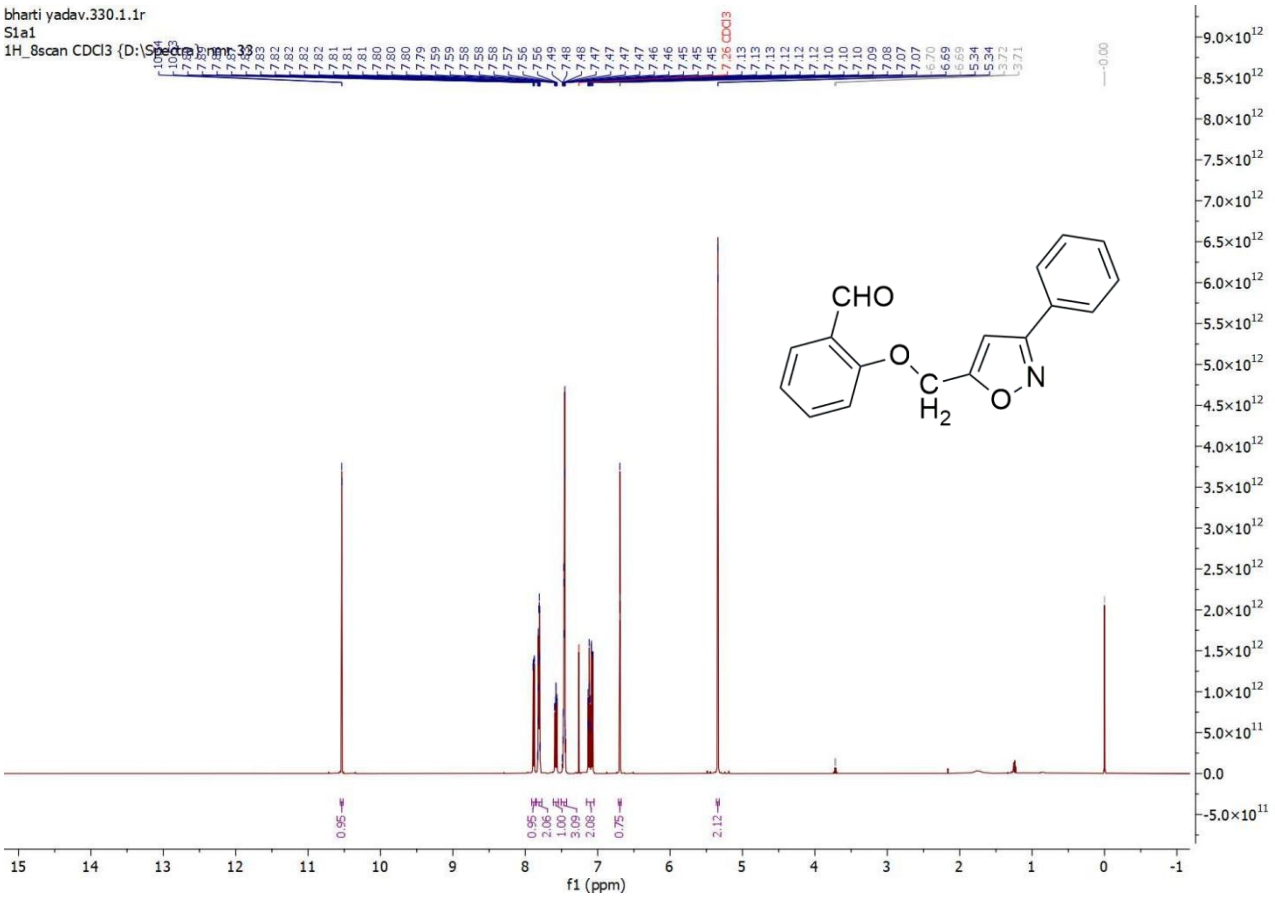
The synthesized compounds were screened using cytotoxicity assay performed on mouse-fibroblast and Vigna radiata seed cells. Both these cells were incubated in 96-well microplate for 24 h. The solution of synthesized compounds (1 mg/ML in DMSO) was treated with these cell lines and incubated for 48 h. MTT solution of conc. 5 mg/mL, prepared in PBS (phosphate-butter saline) was added to each cell maintain 100 µL total volume. After incubation for 4h at 37°C, formazan crystals were obtained which get solubilized after further addition of DMSO. Thereafter, absorbance was recorded at 570nm using a microplate reader. From the comparison of the absorbance of the treated cells with that of the untreated cells, the percentage cell viability of the synthesized compounds was calculated.

References

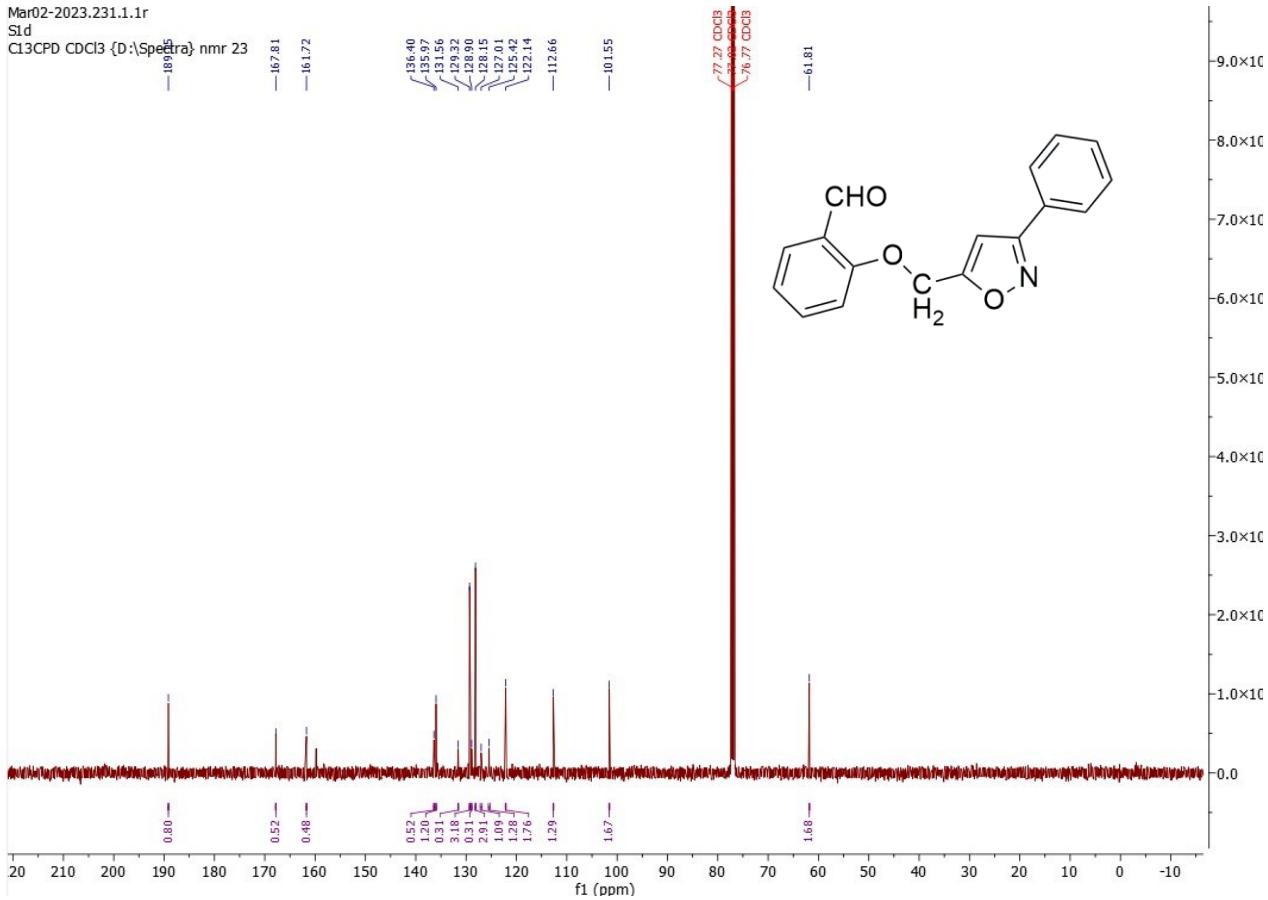
1. Humphries, R. M.; Ambler, J.; Mitchell, S. L.; Castanheira, M.; Dingle, T.; Hindler, J. A.; Koeth, L.; Sei, K. *J. Clin. Microbiol*. **2018**, *56*, e01934
2. Sharma, O. P.; Bhat, T. K. *Food Chem*. **2009**, *113*, 1202
3. Ghasemi, M.; Turnbull, T.; Sebastian, S.; Kempson, I. *Int. J. Mol. Sci*. **2021**, *22*, 12827

S2. ^{1}H NMR and ^{13}C NMR and HRMS spectra of 1a-m

^{1}HNMR spectrum of **1a**



^{13}CNMR of compound **1a**



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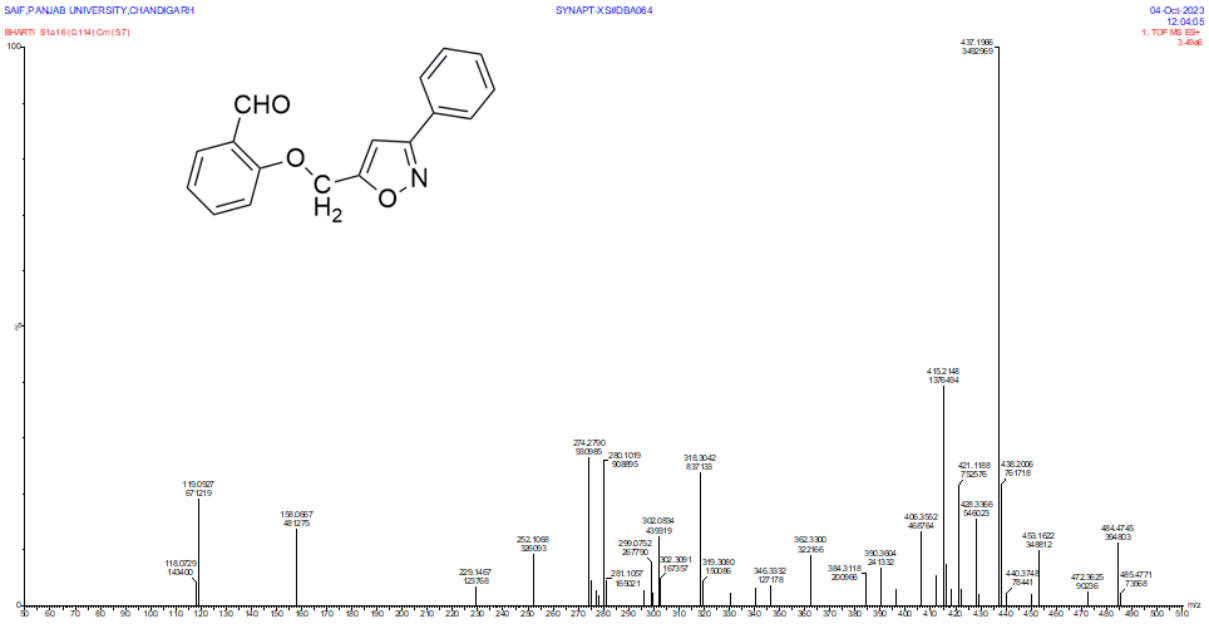

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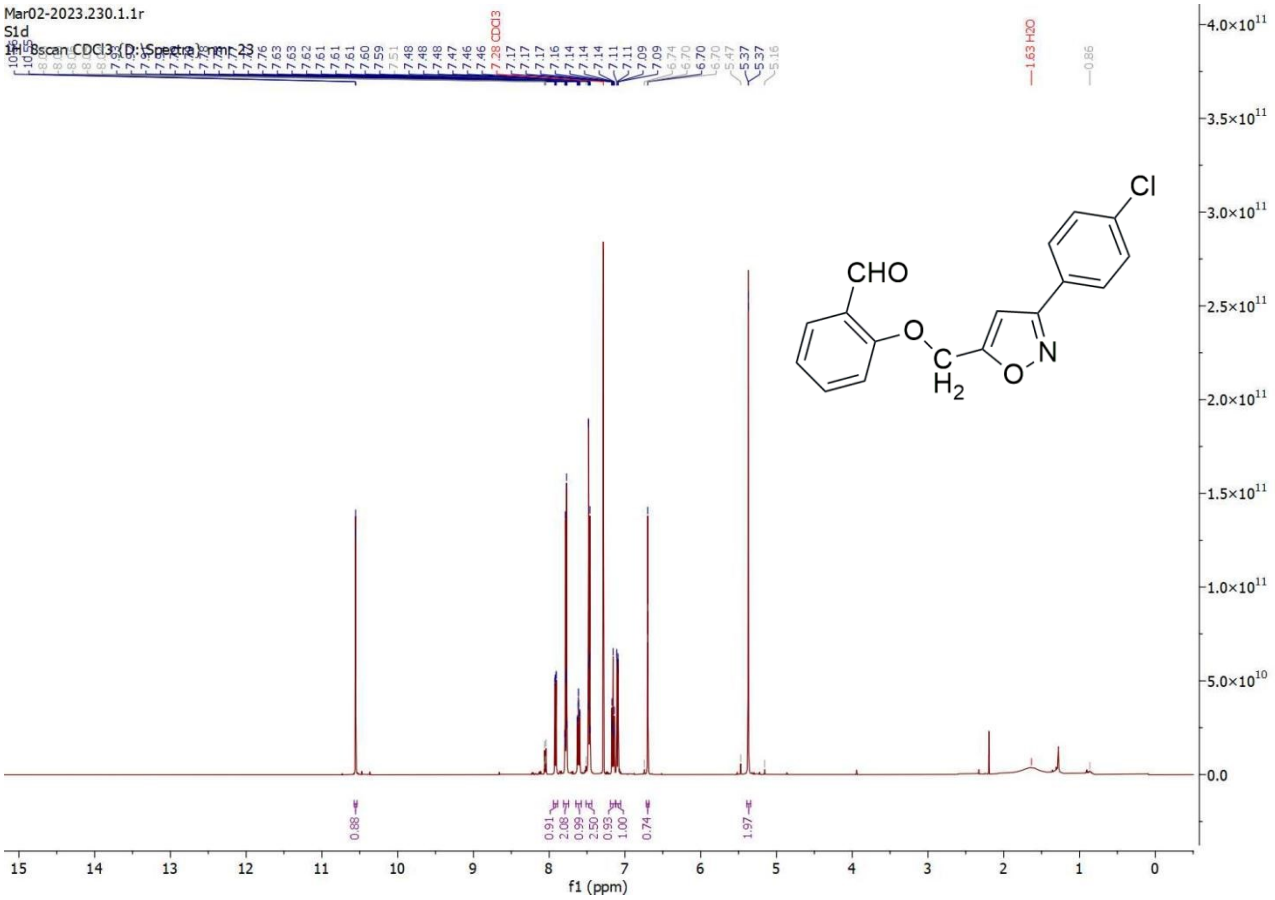

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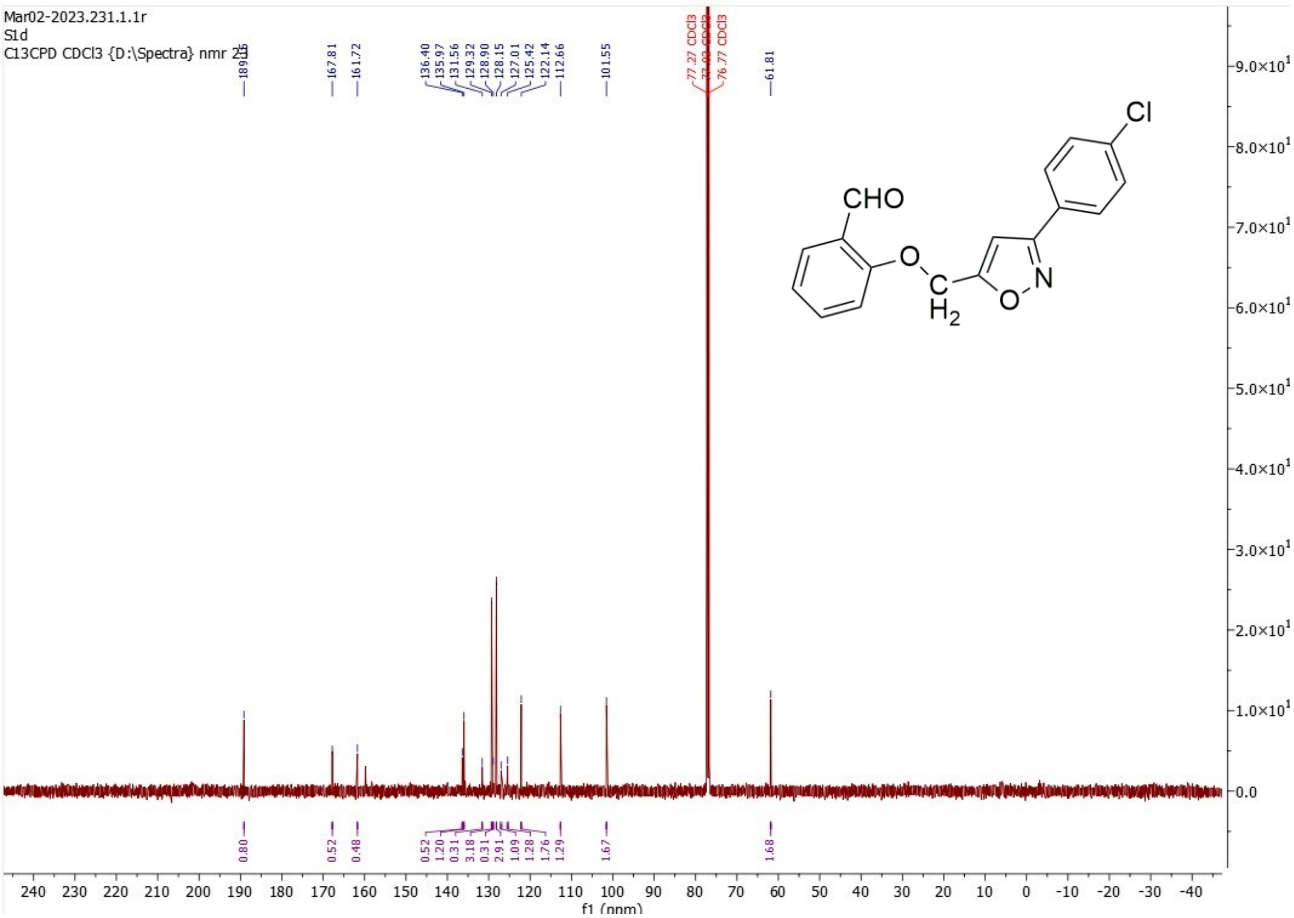

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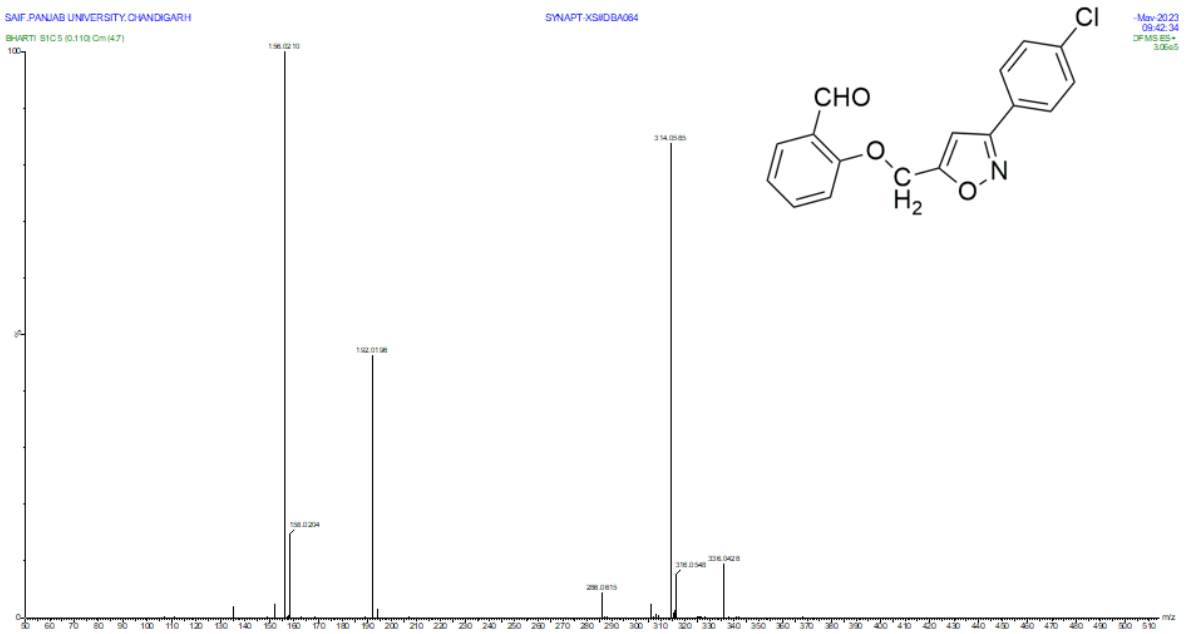

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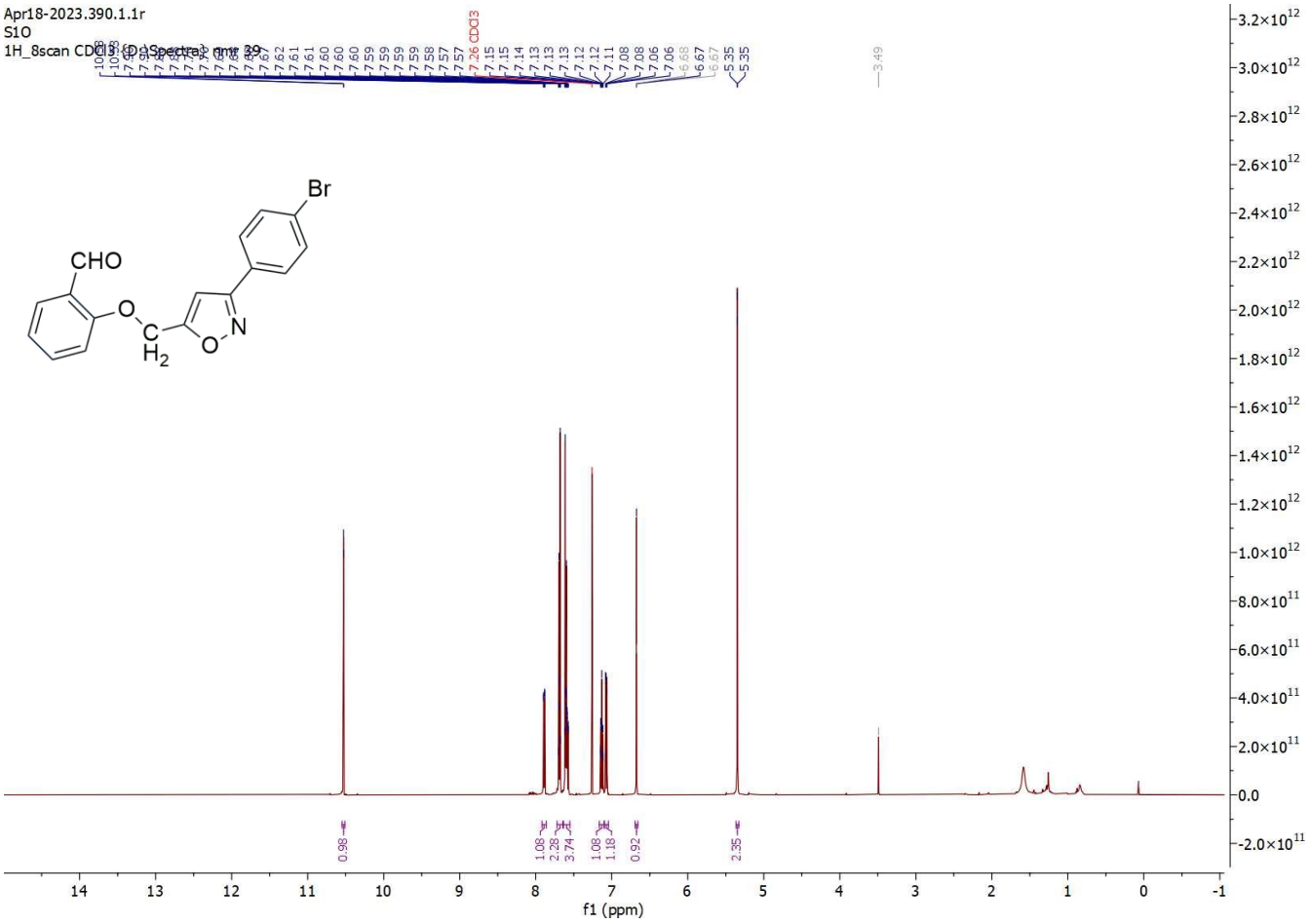

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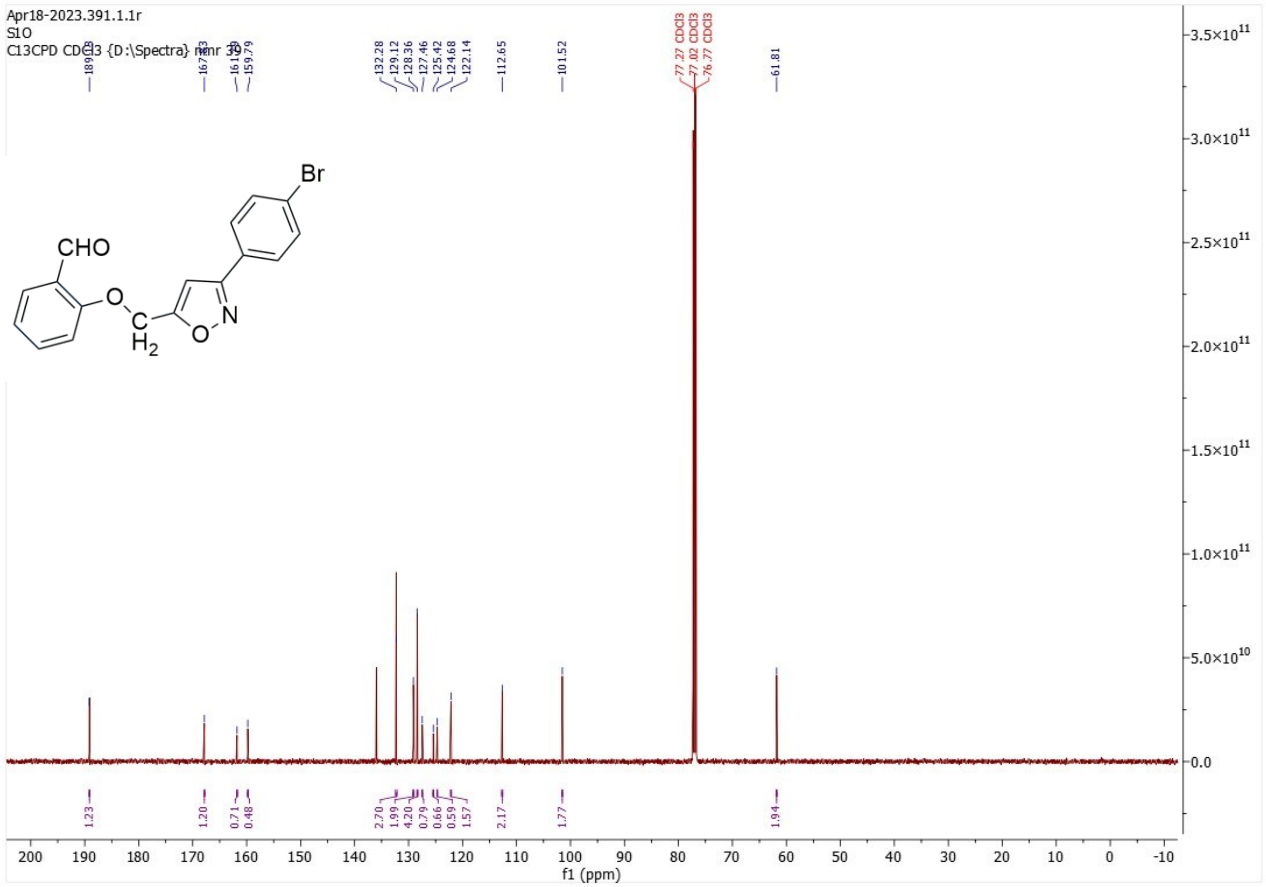

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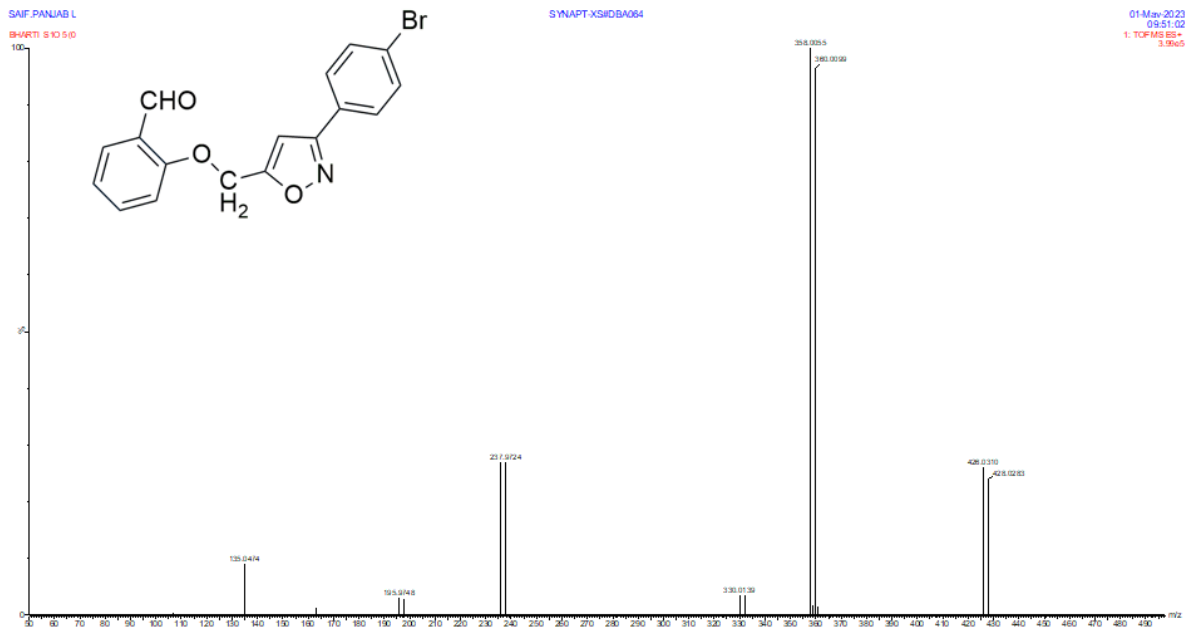

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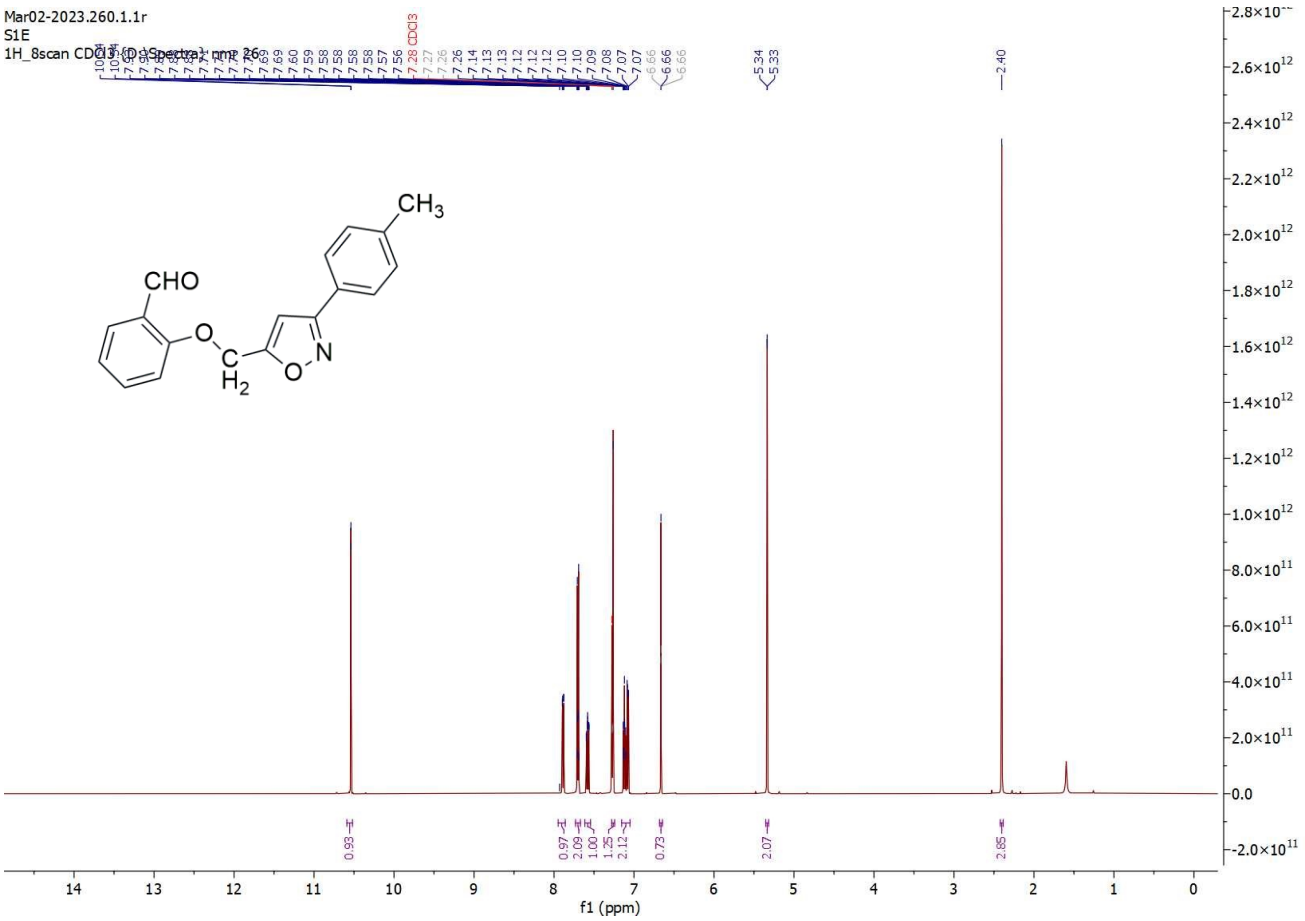

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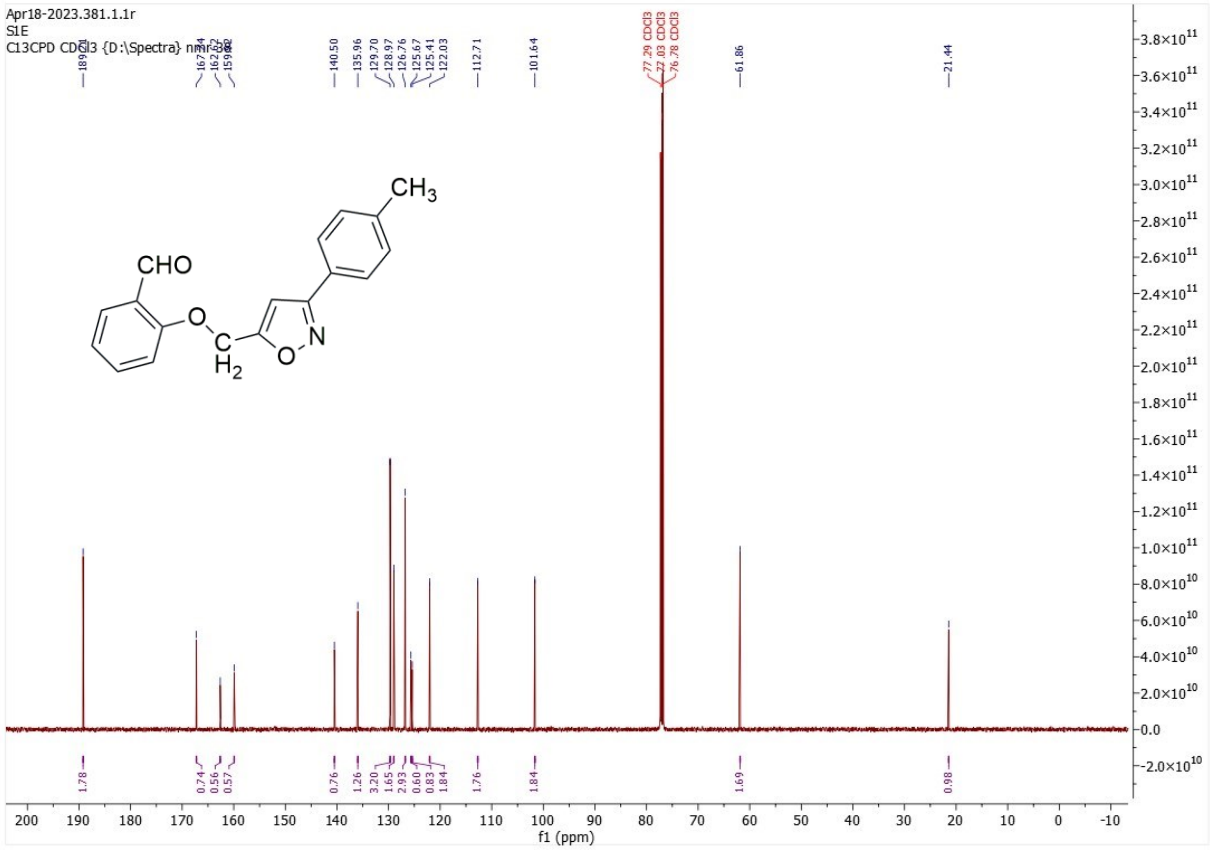

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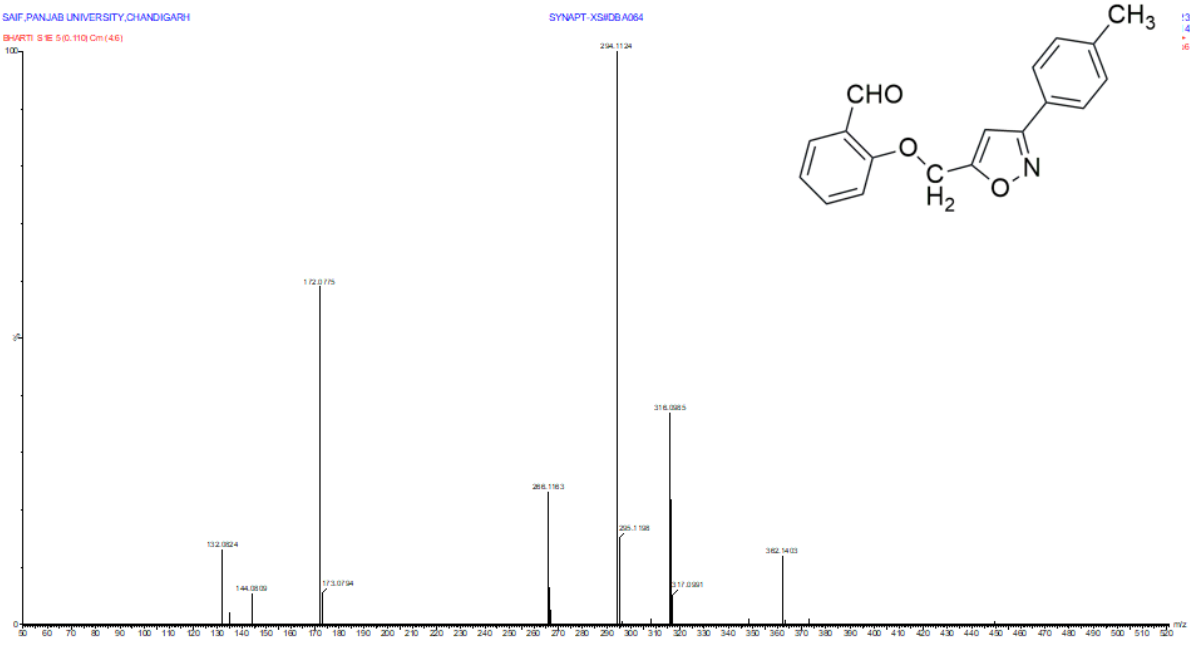

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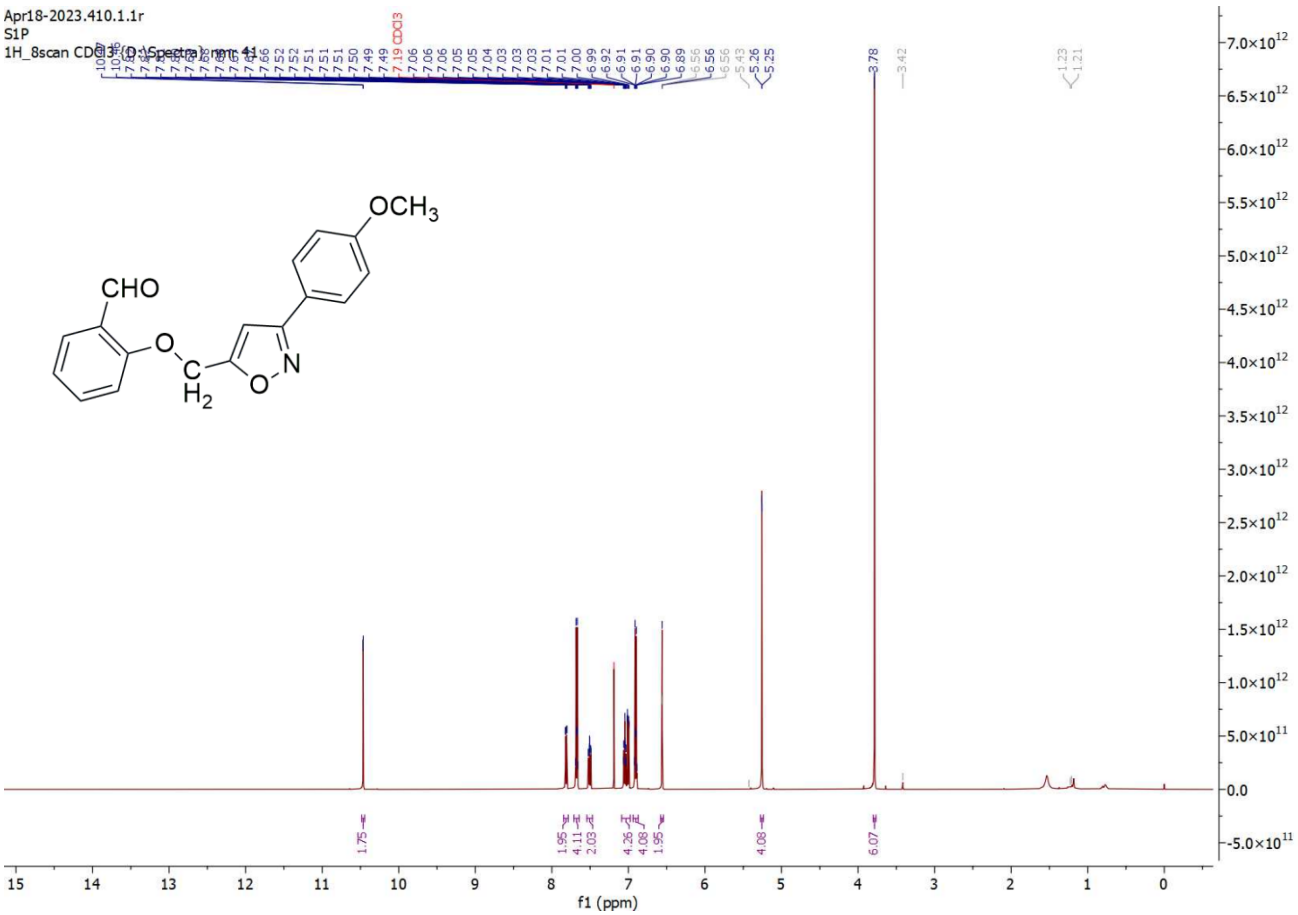

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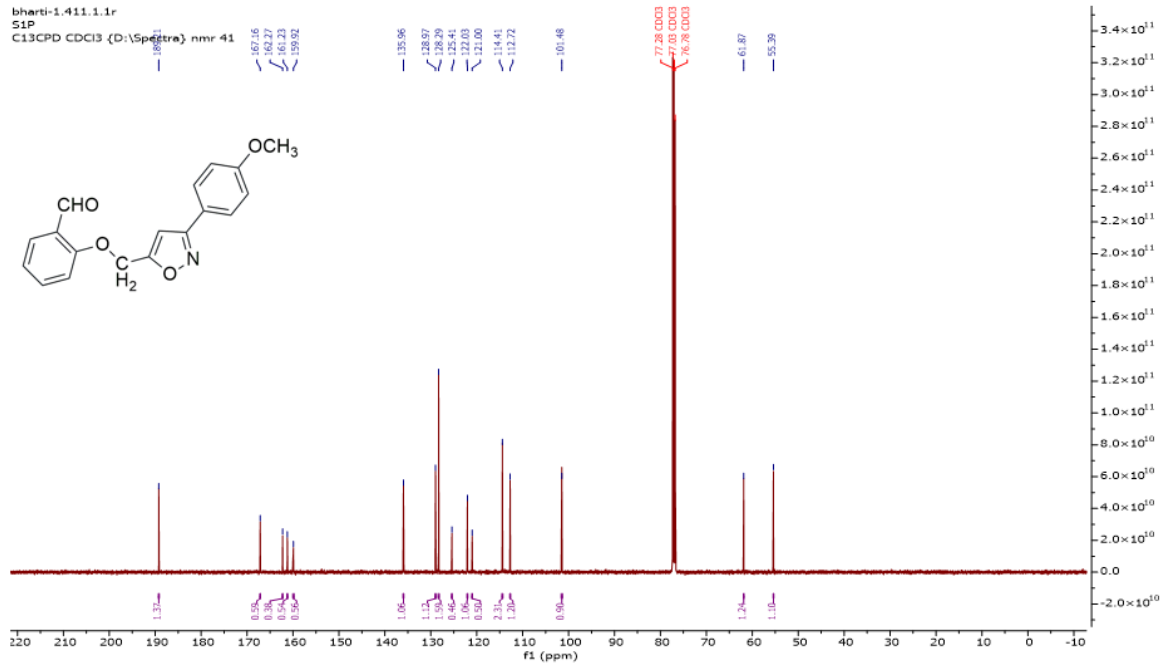

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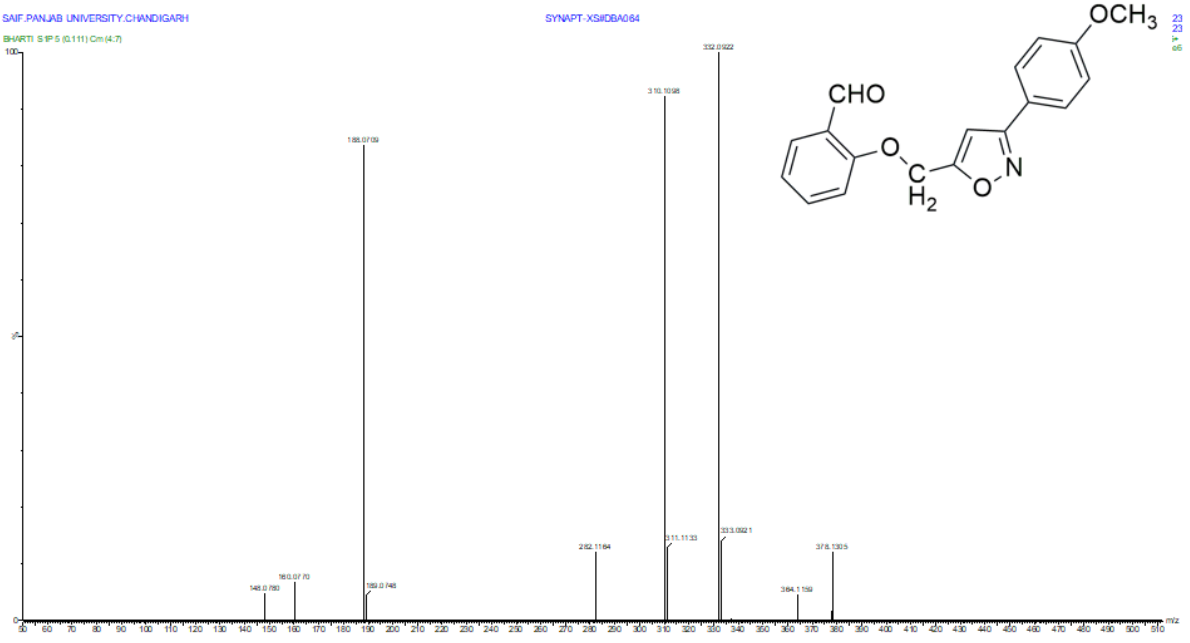

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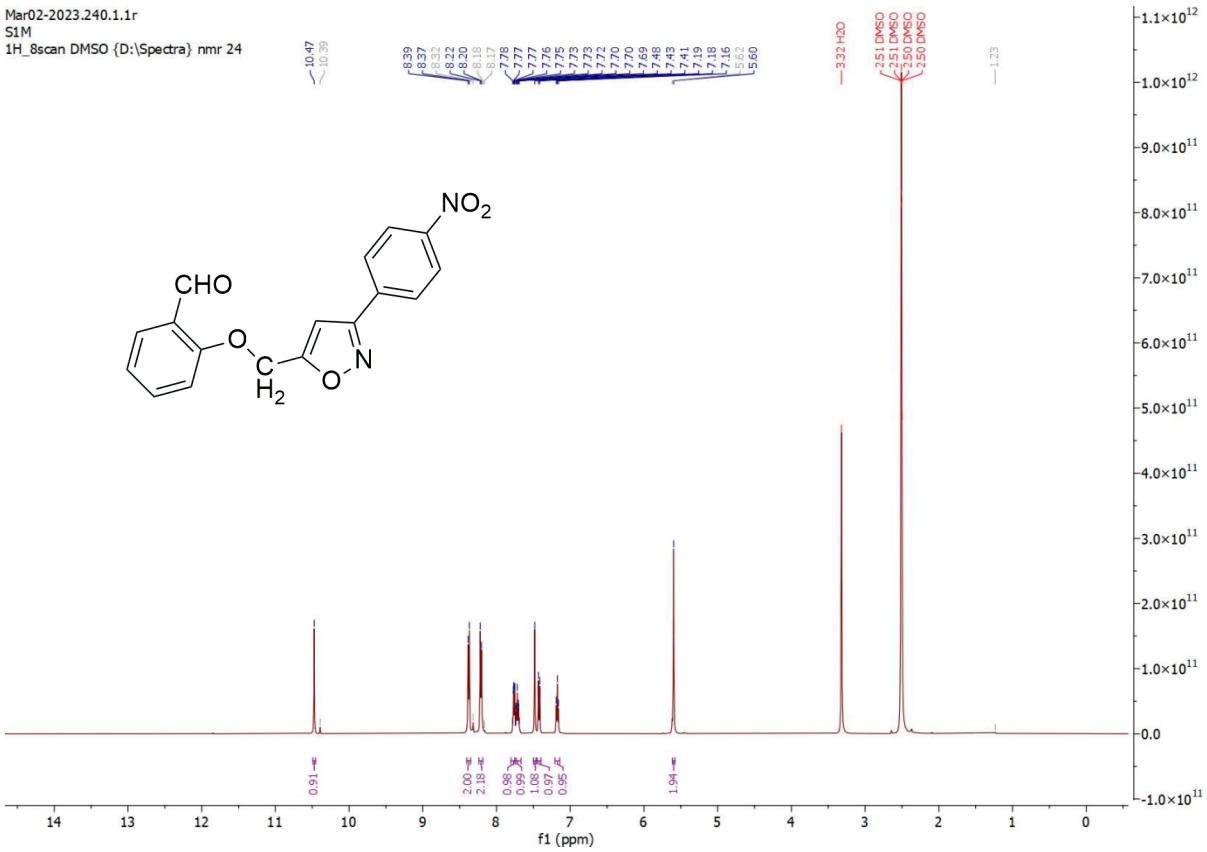

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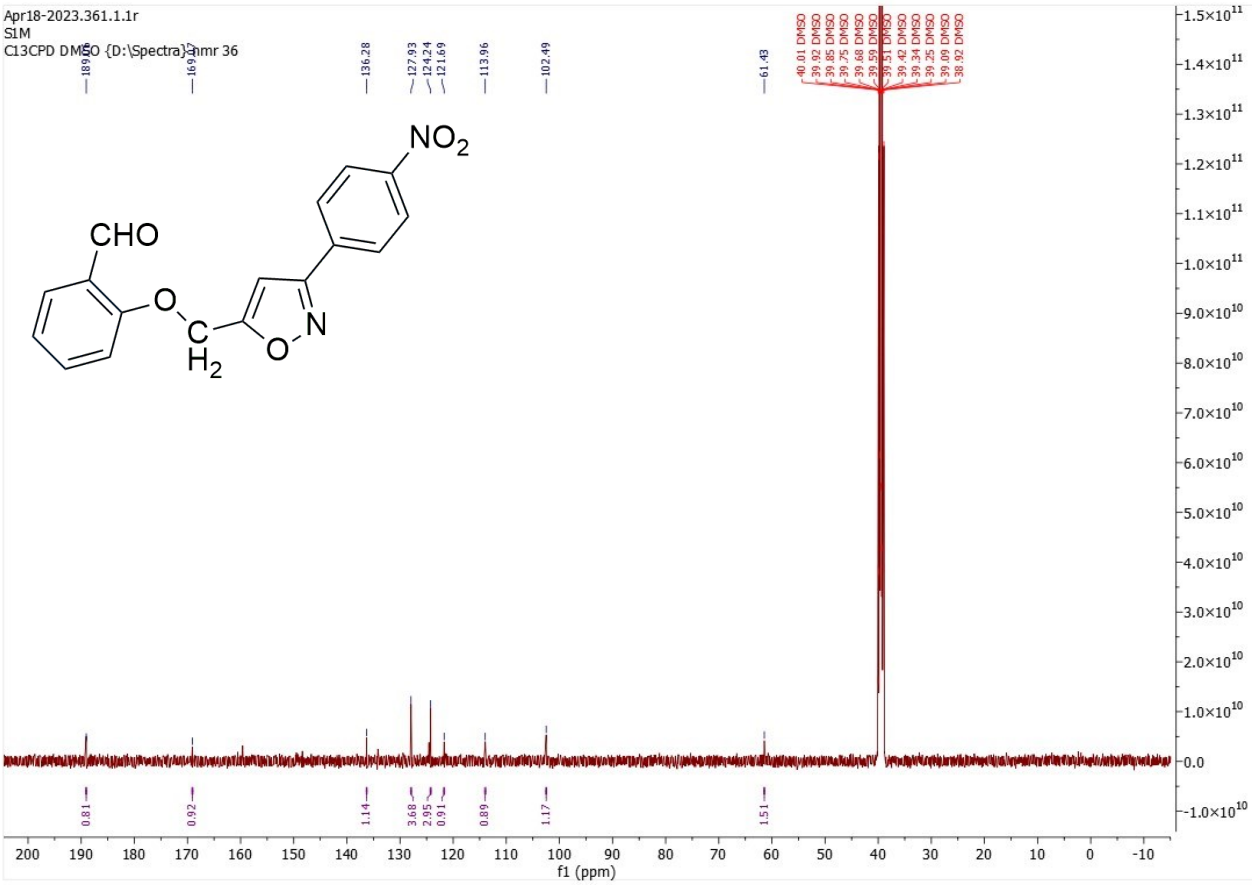

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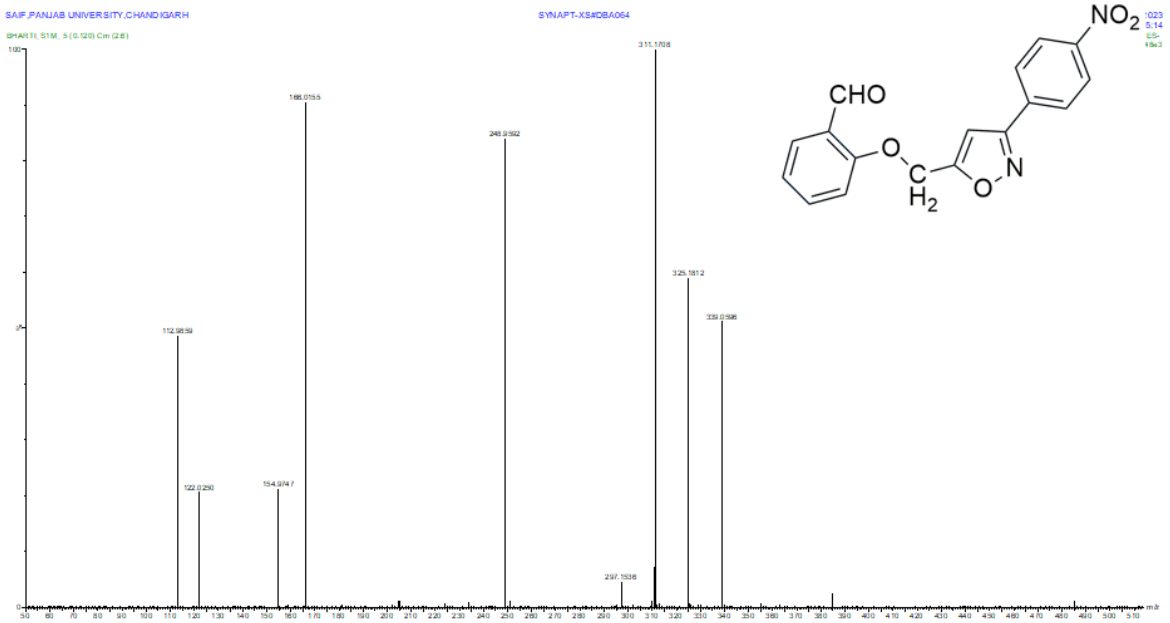
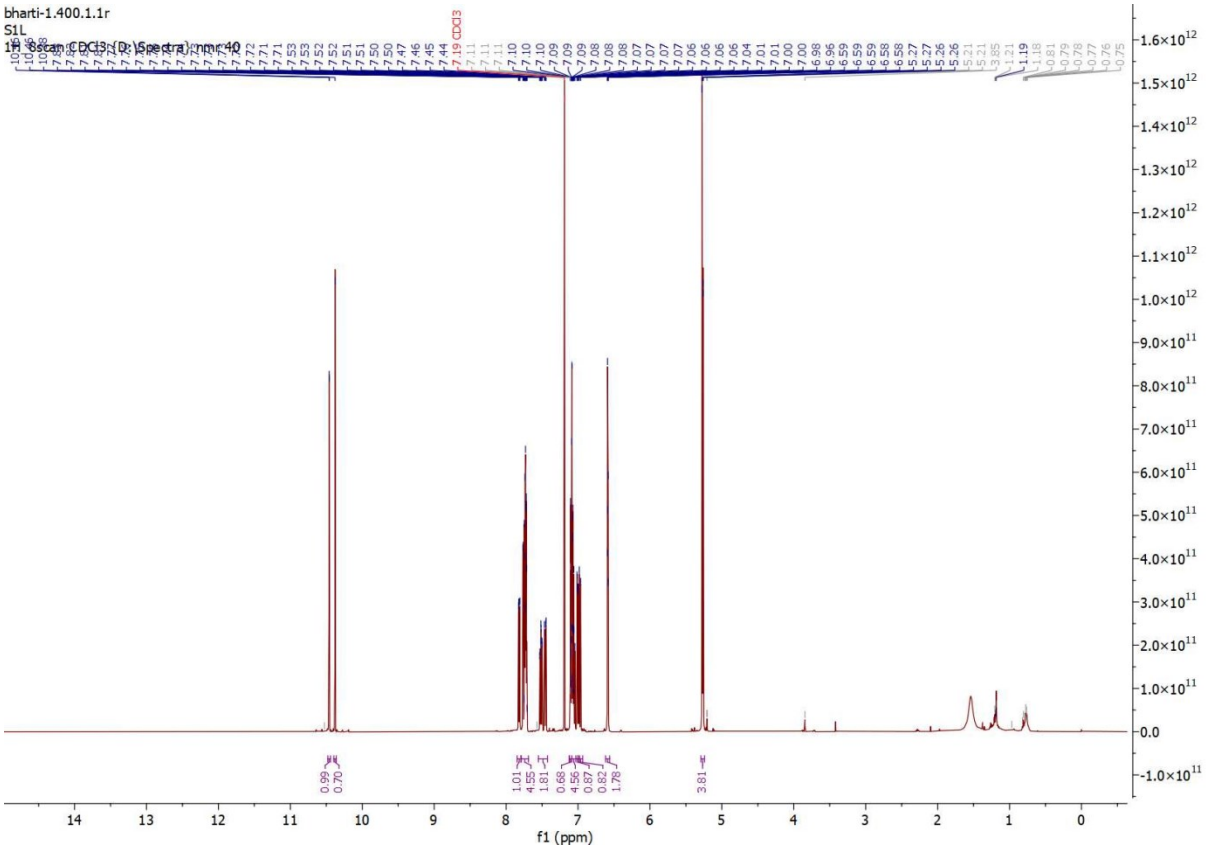


^{1}HNMR spectrum of **1g+1g'**



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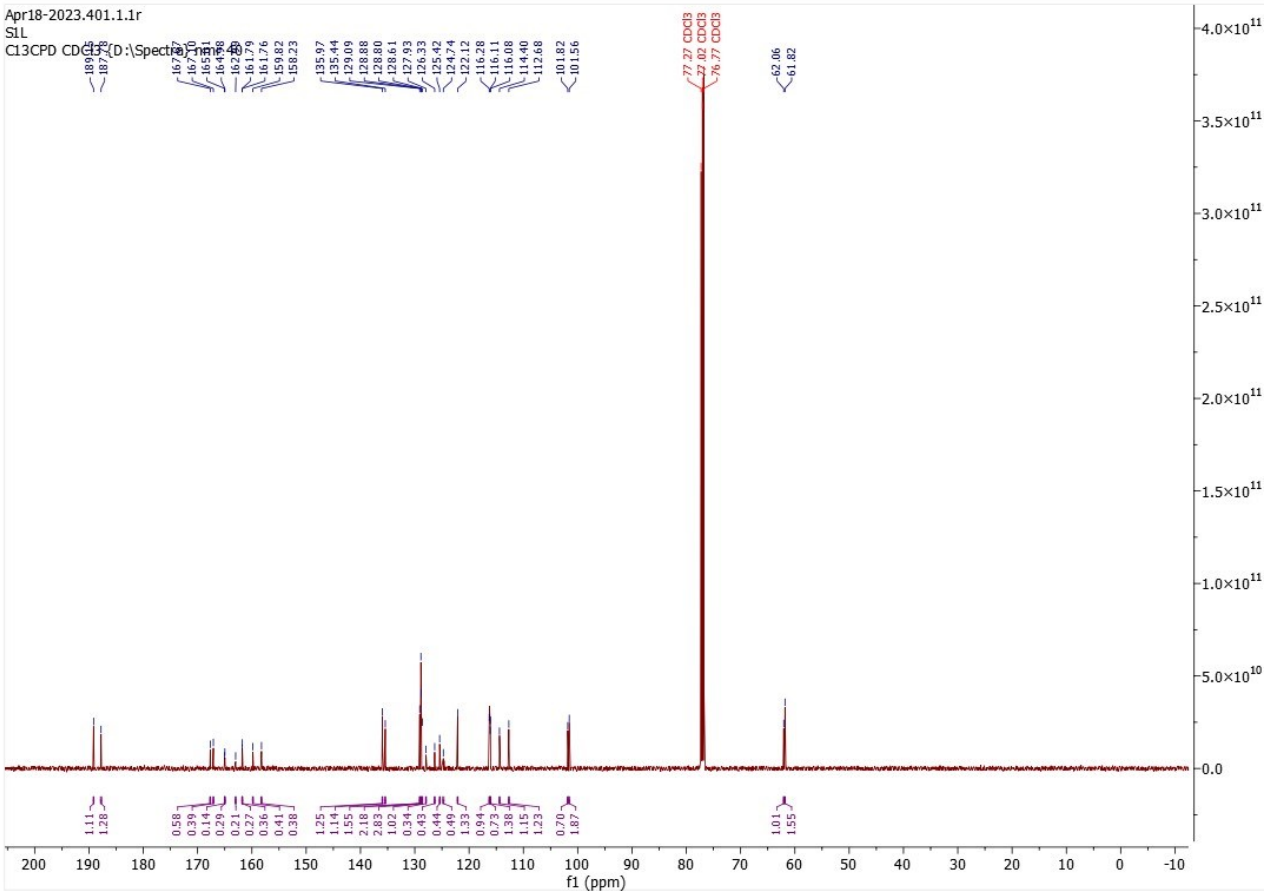

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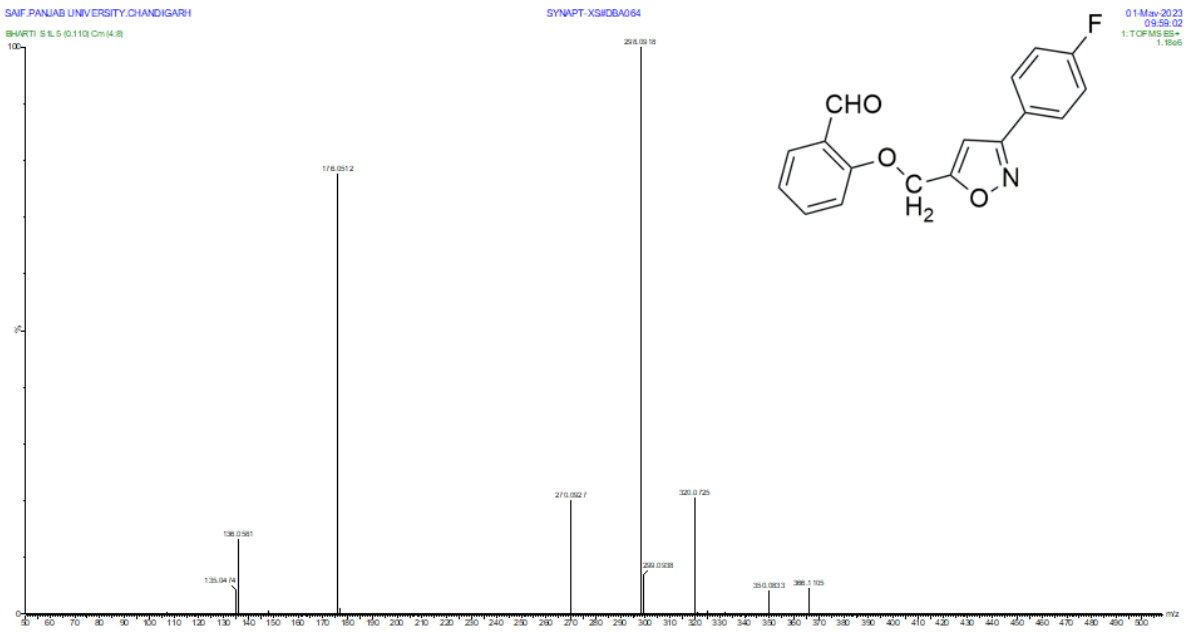

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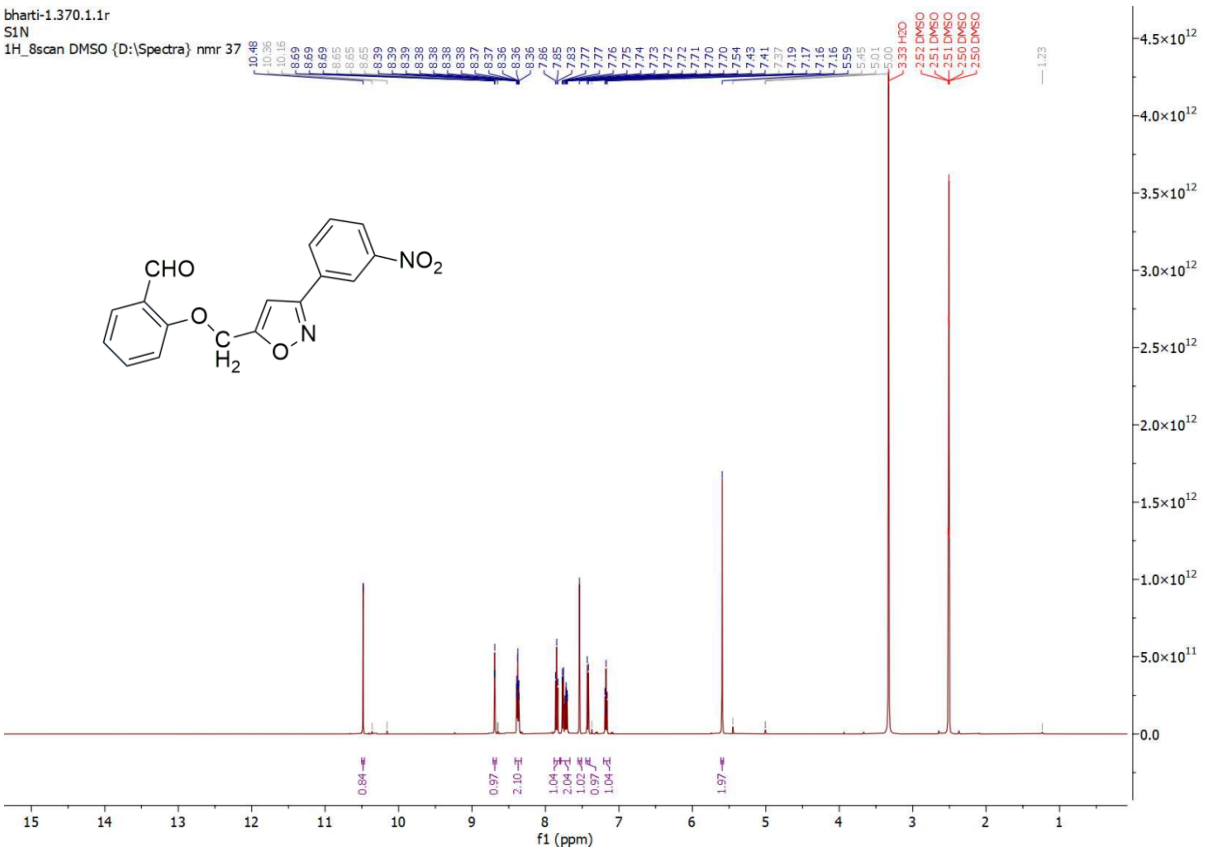

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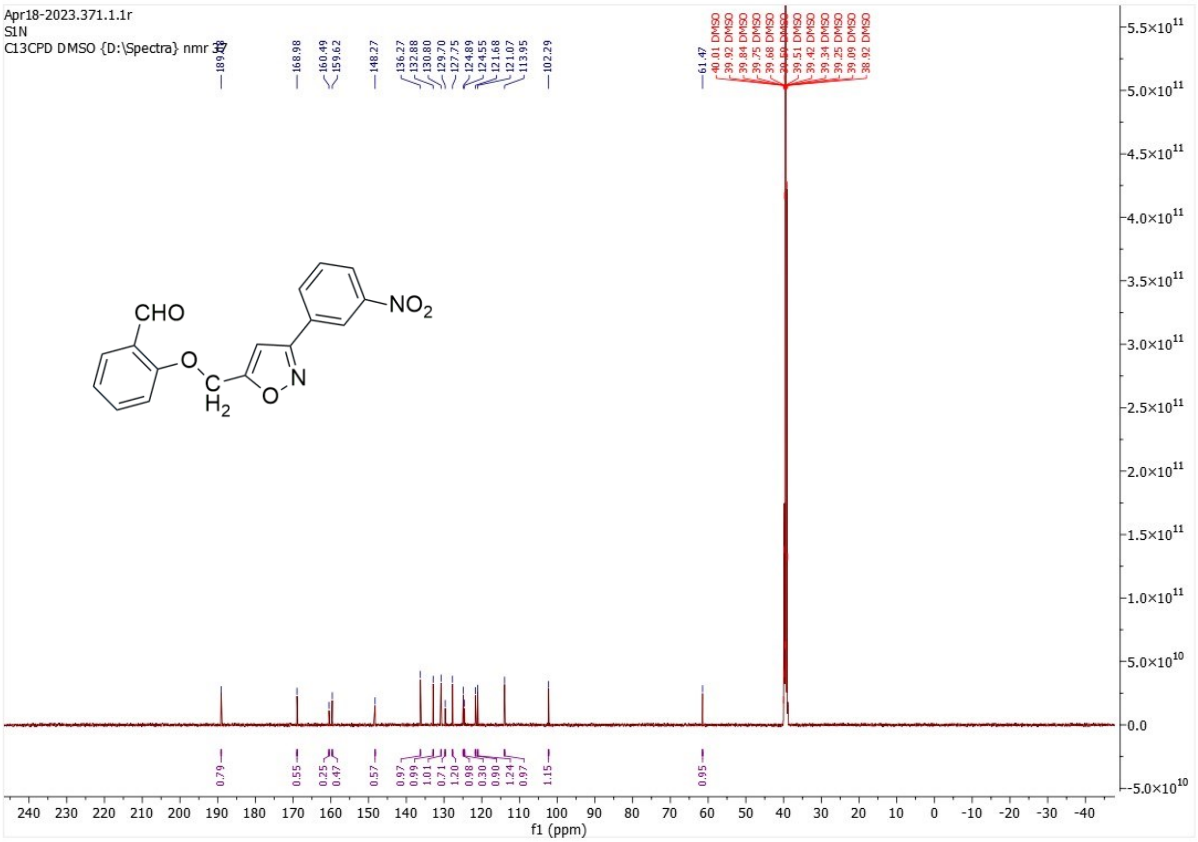

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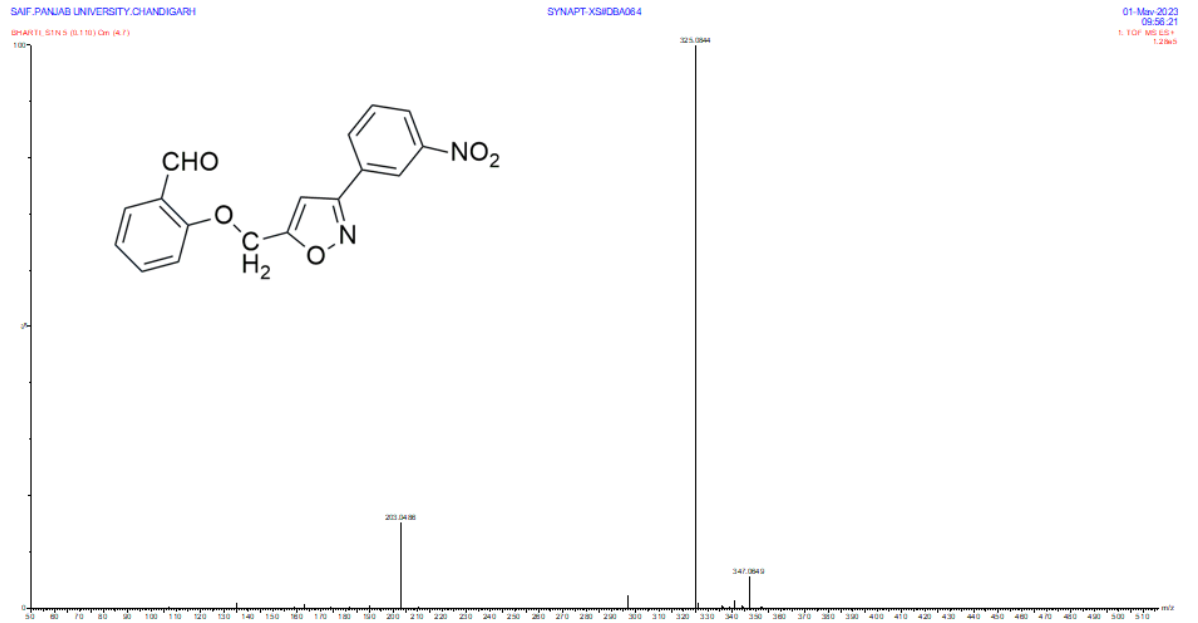

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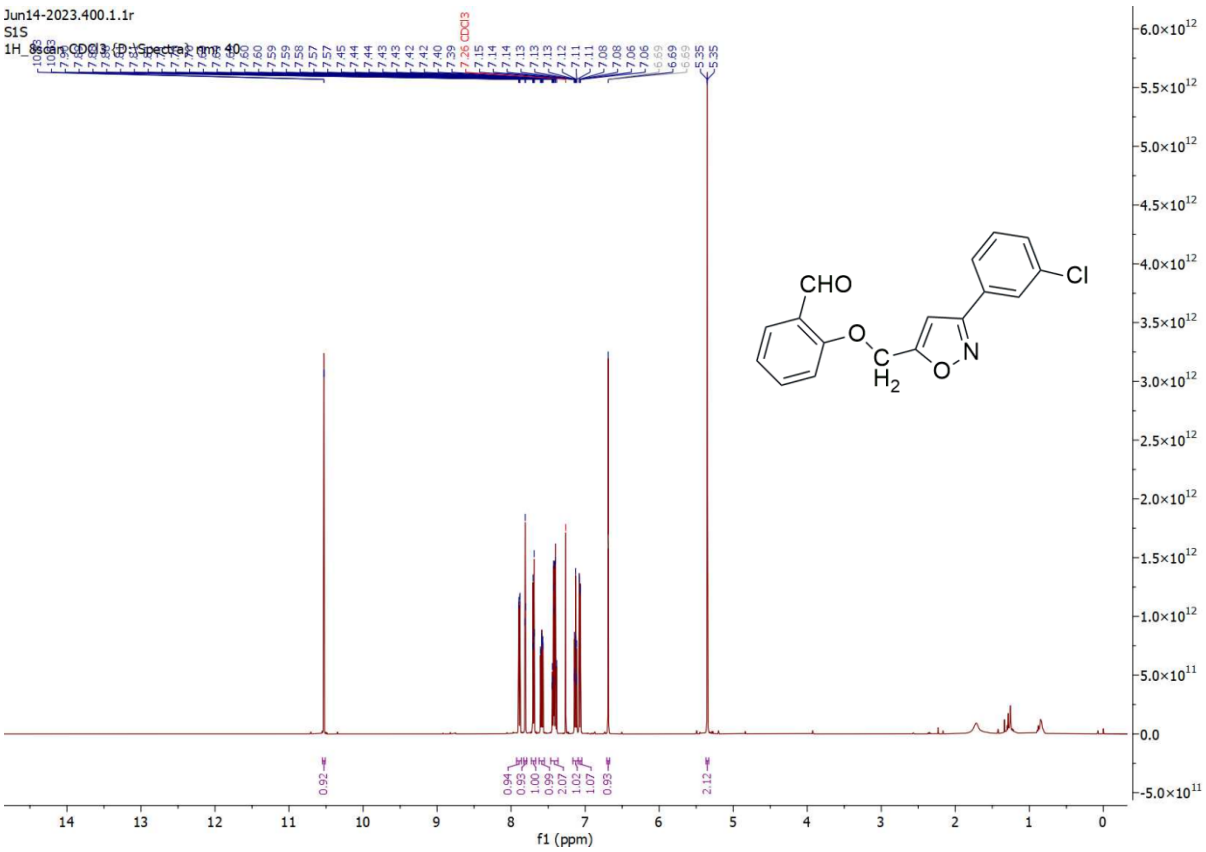

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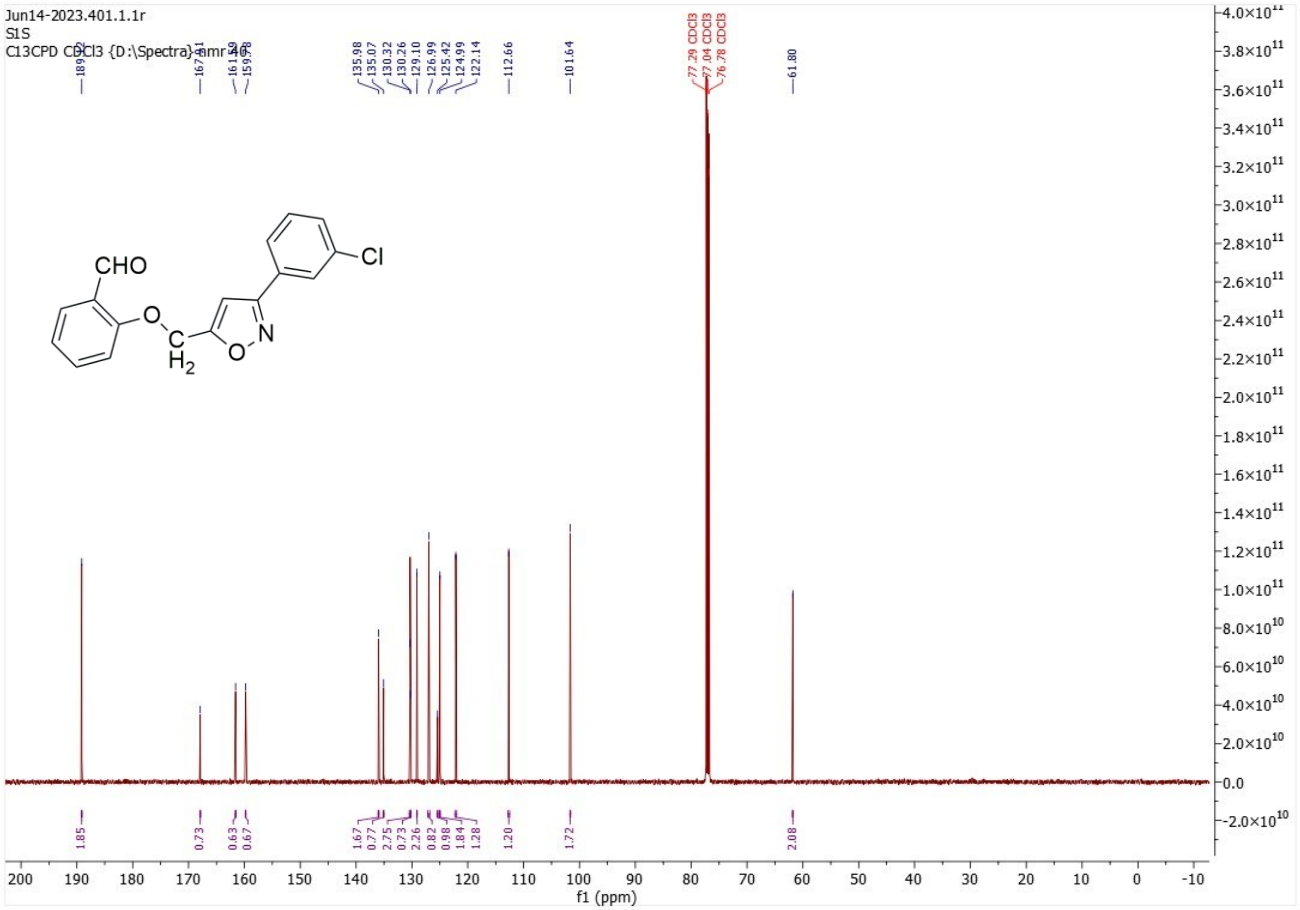

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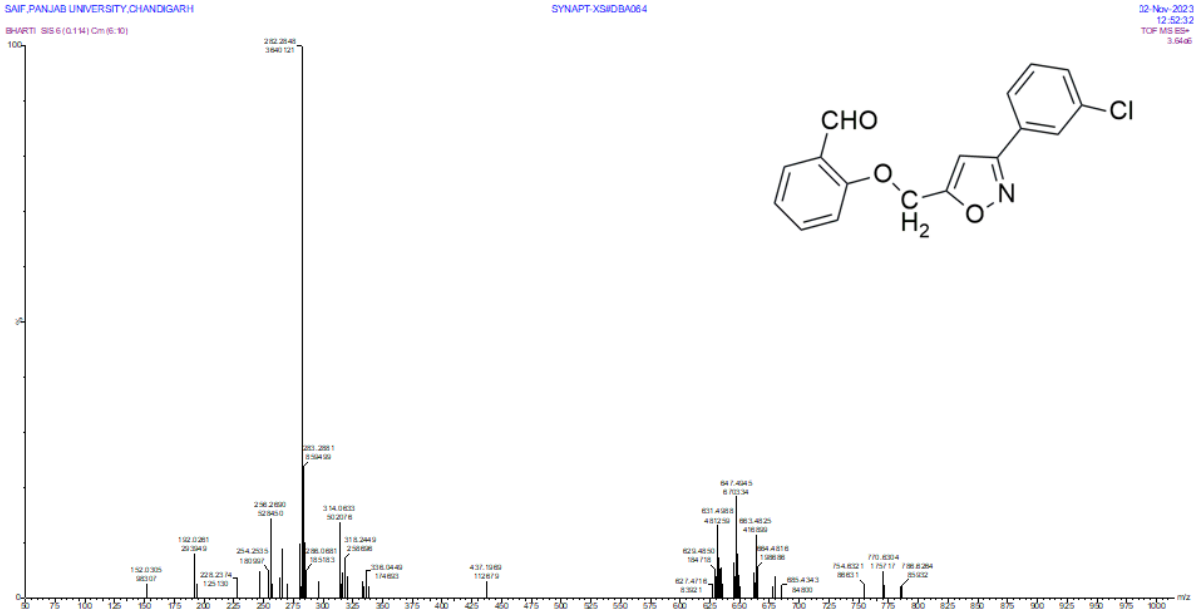

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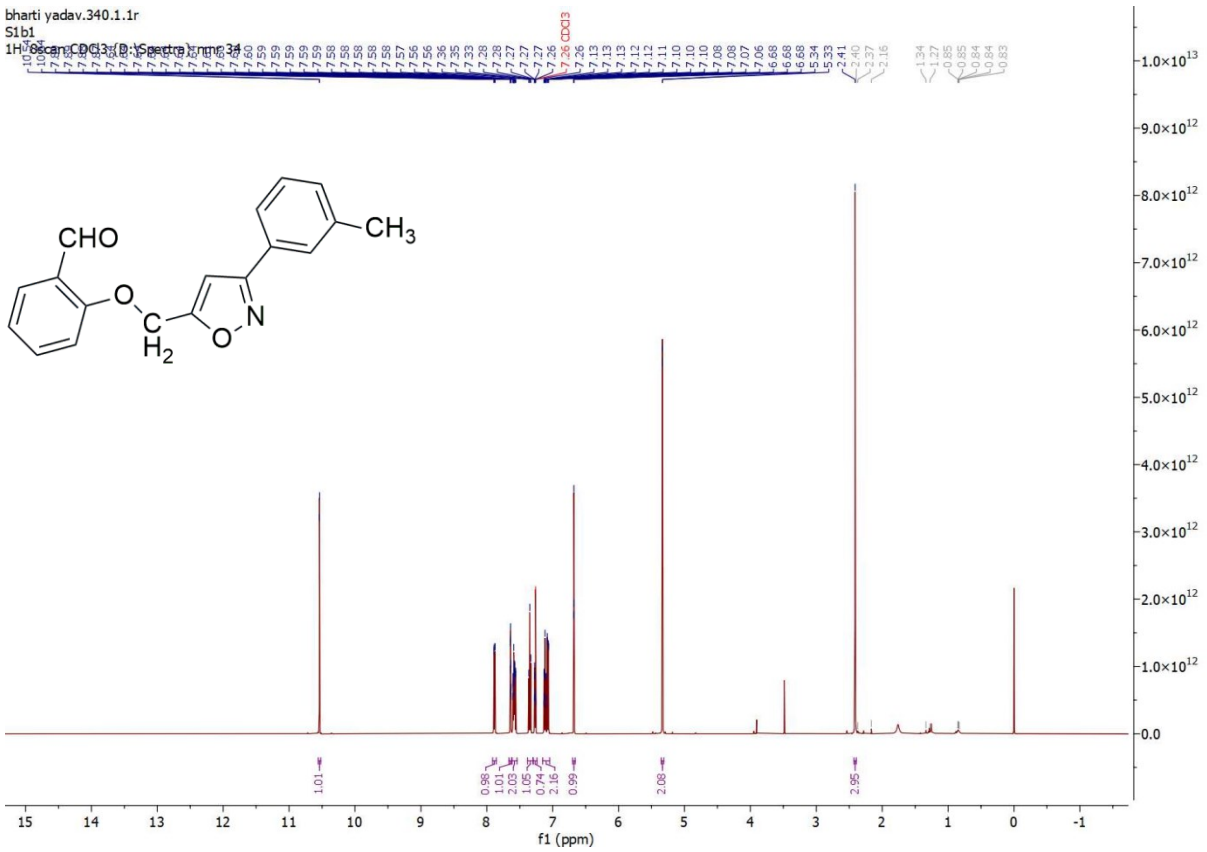

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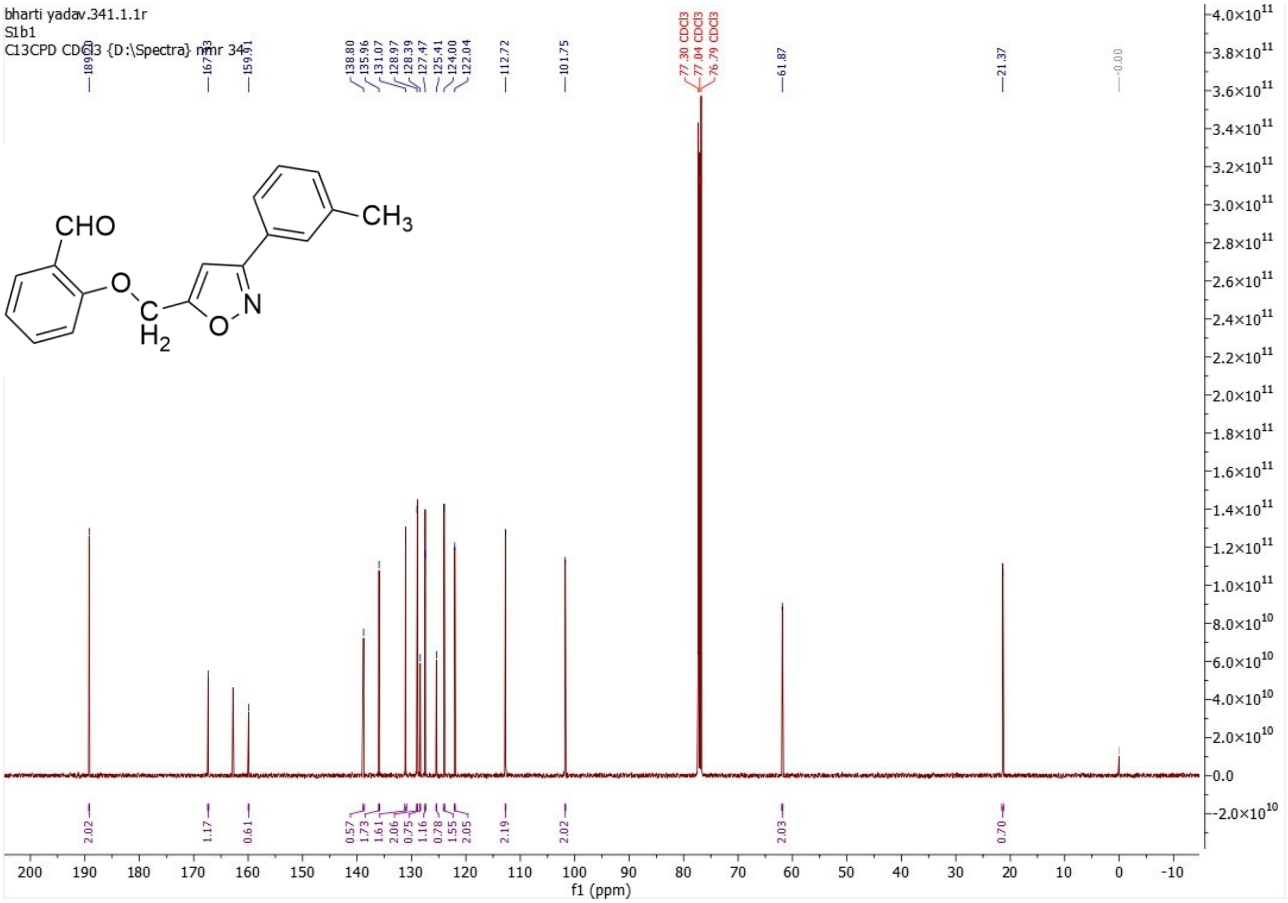

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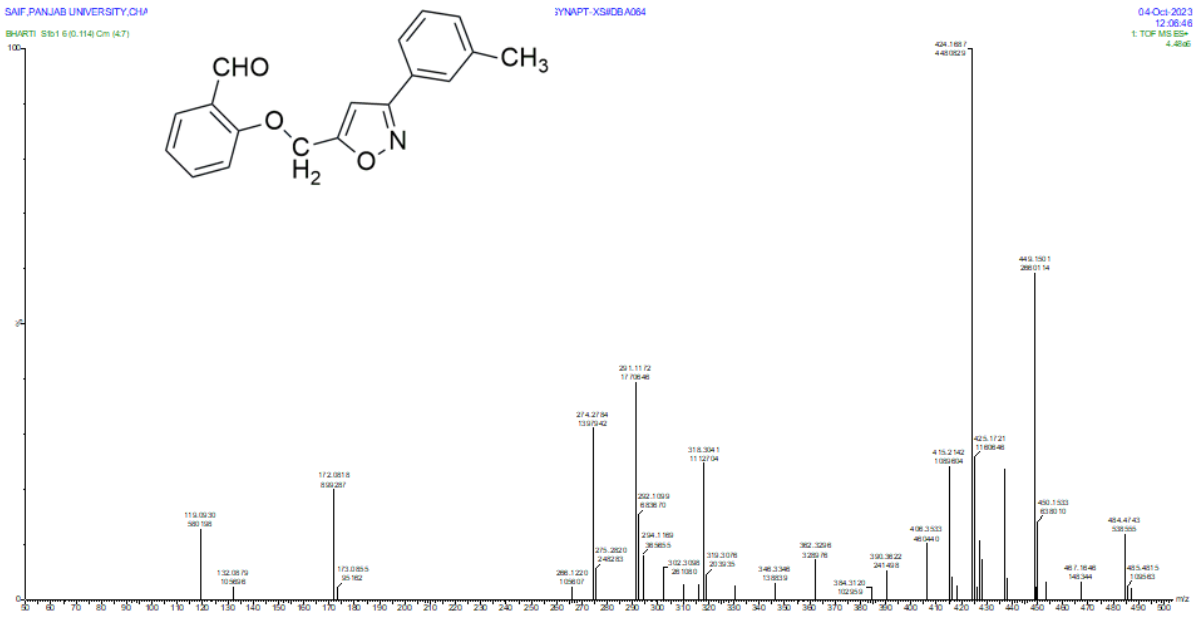

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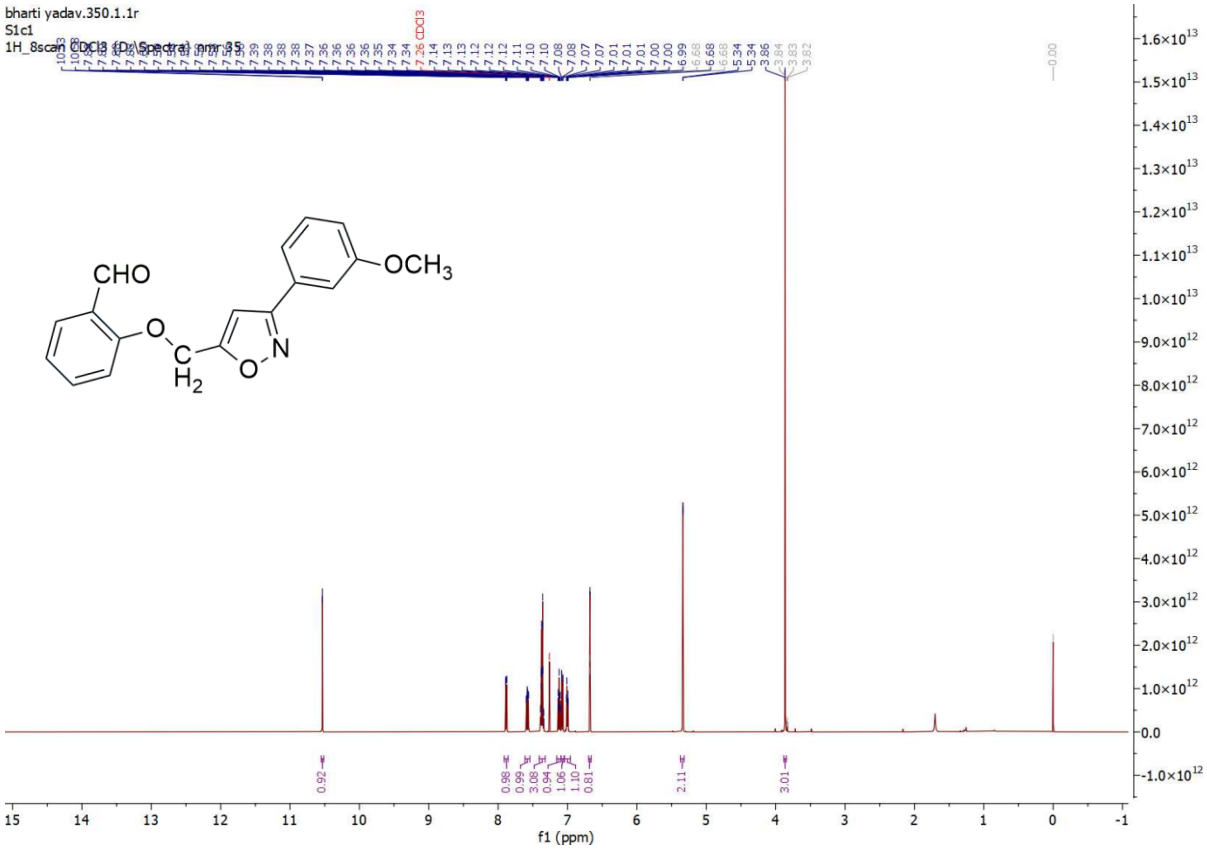

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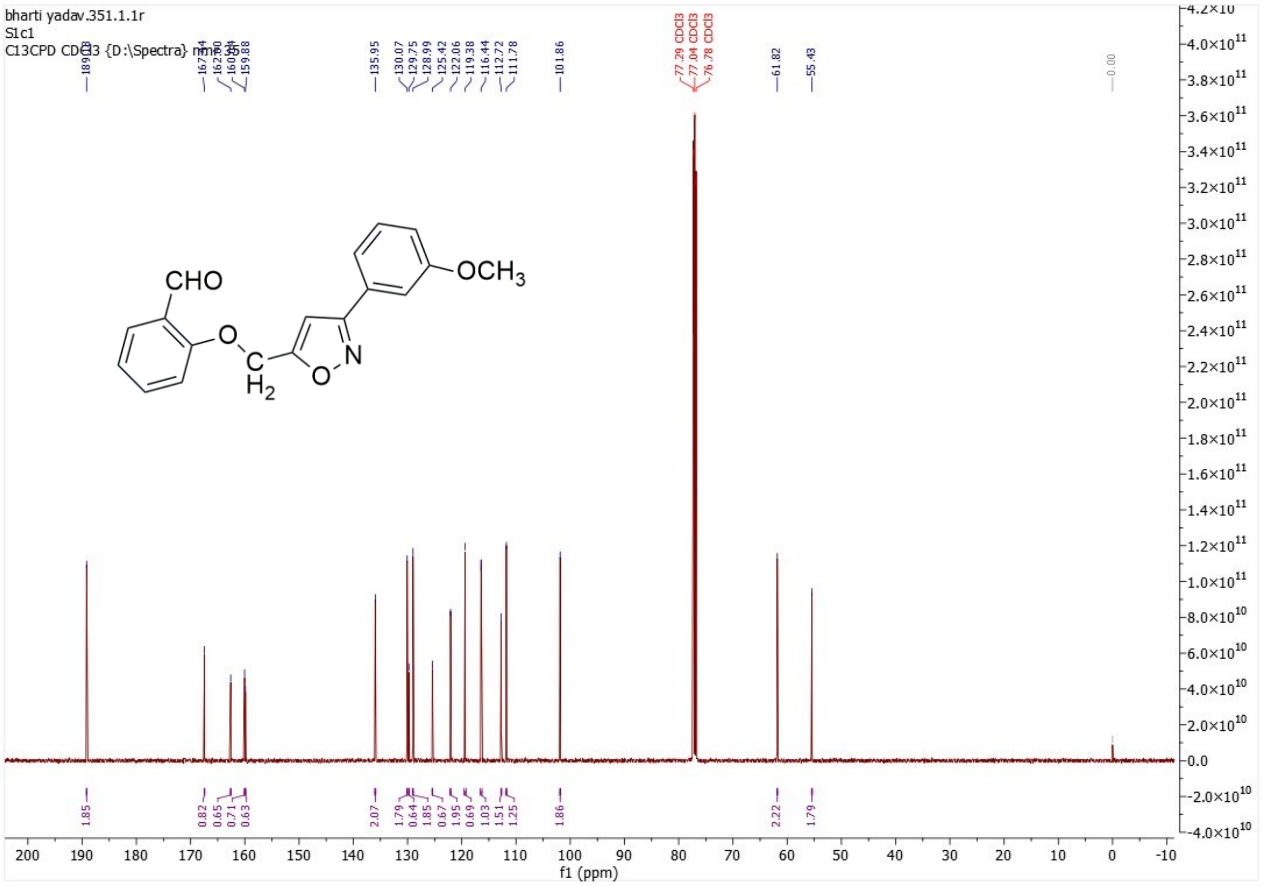

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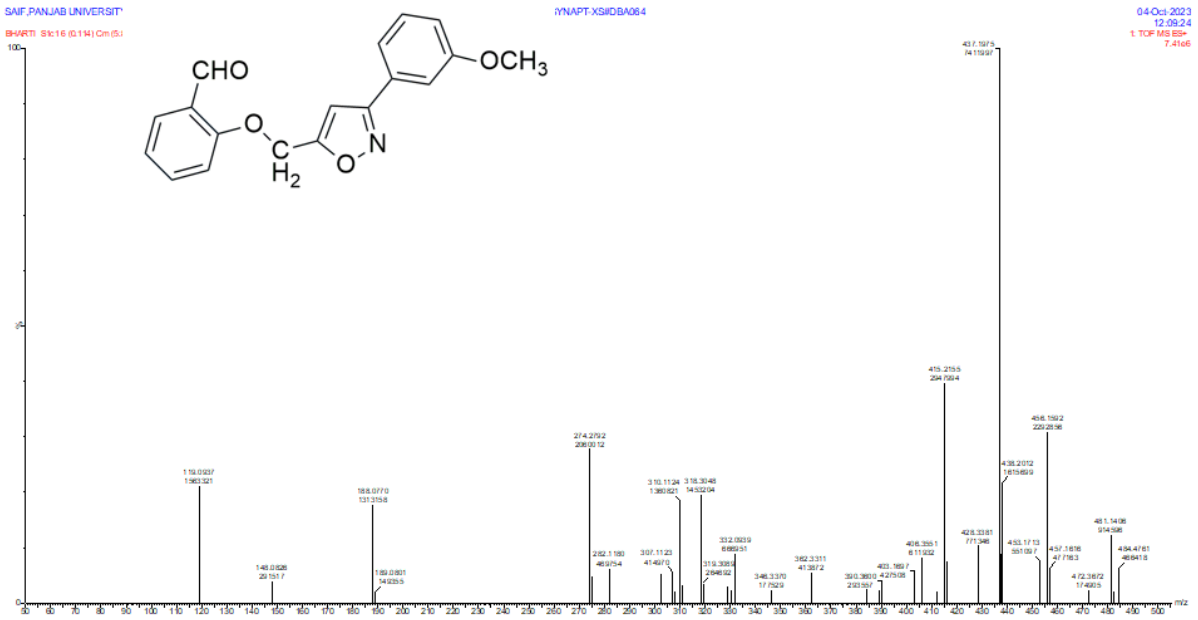

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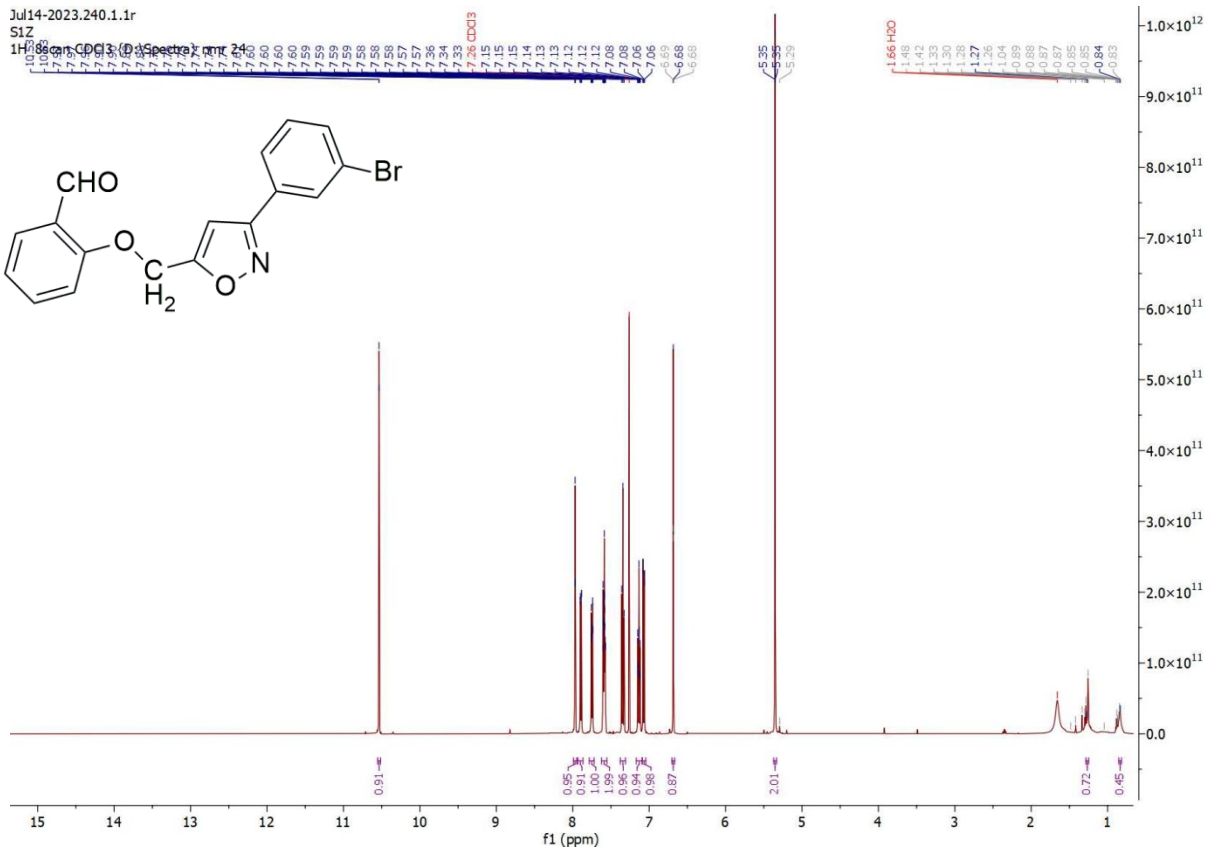

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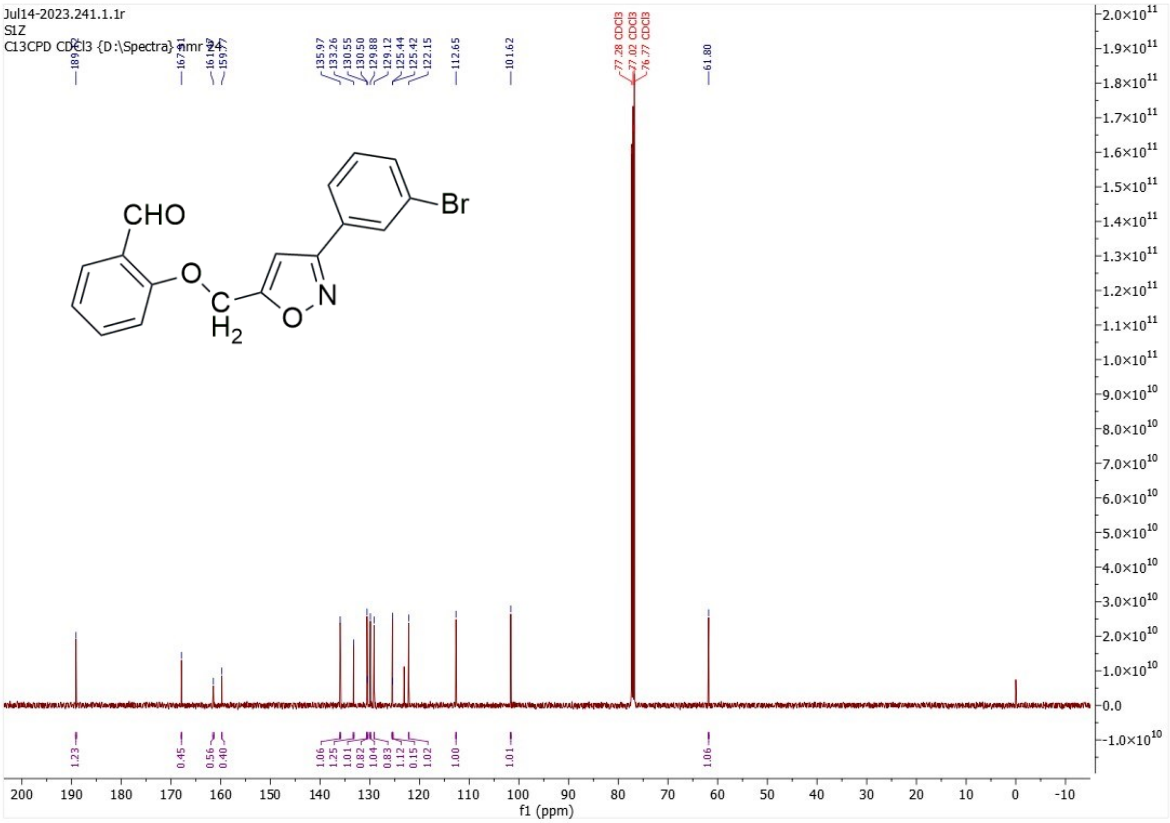

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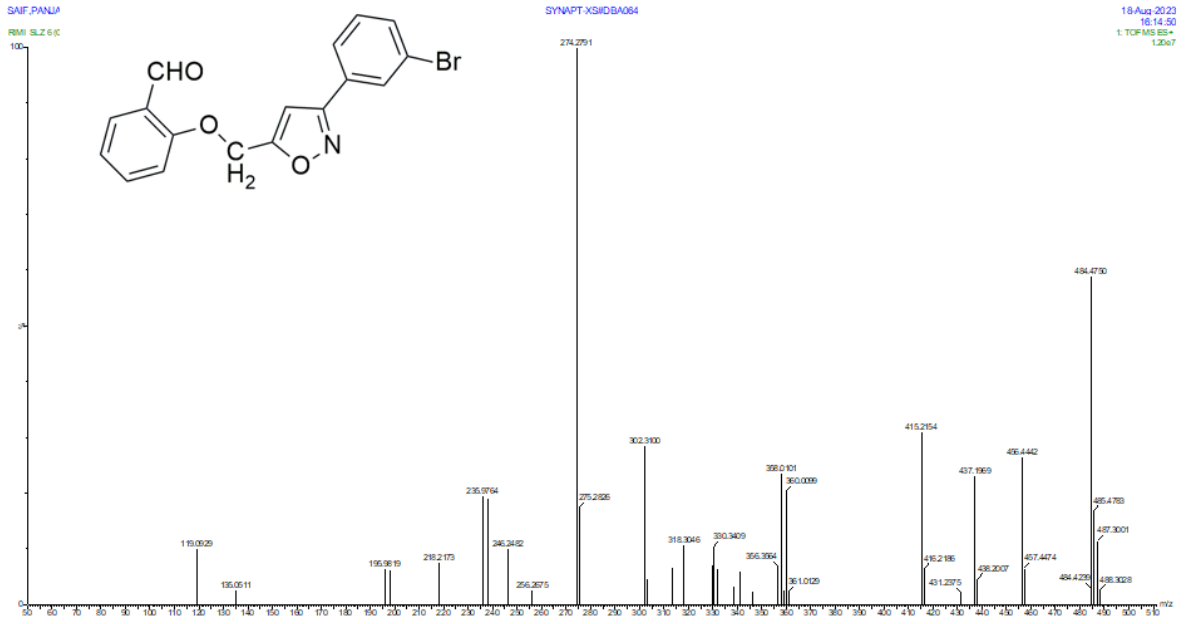

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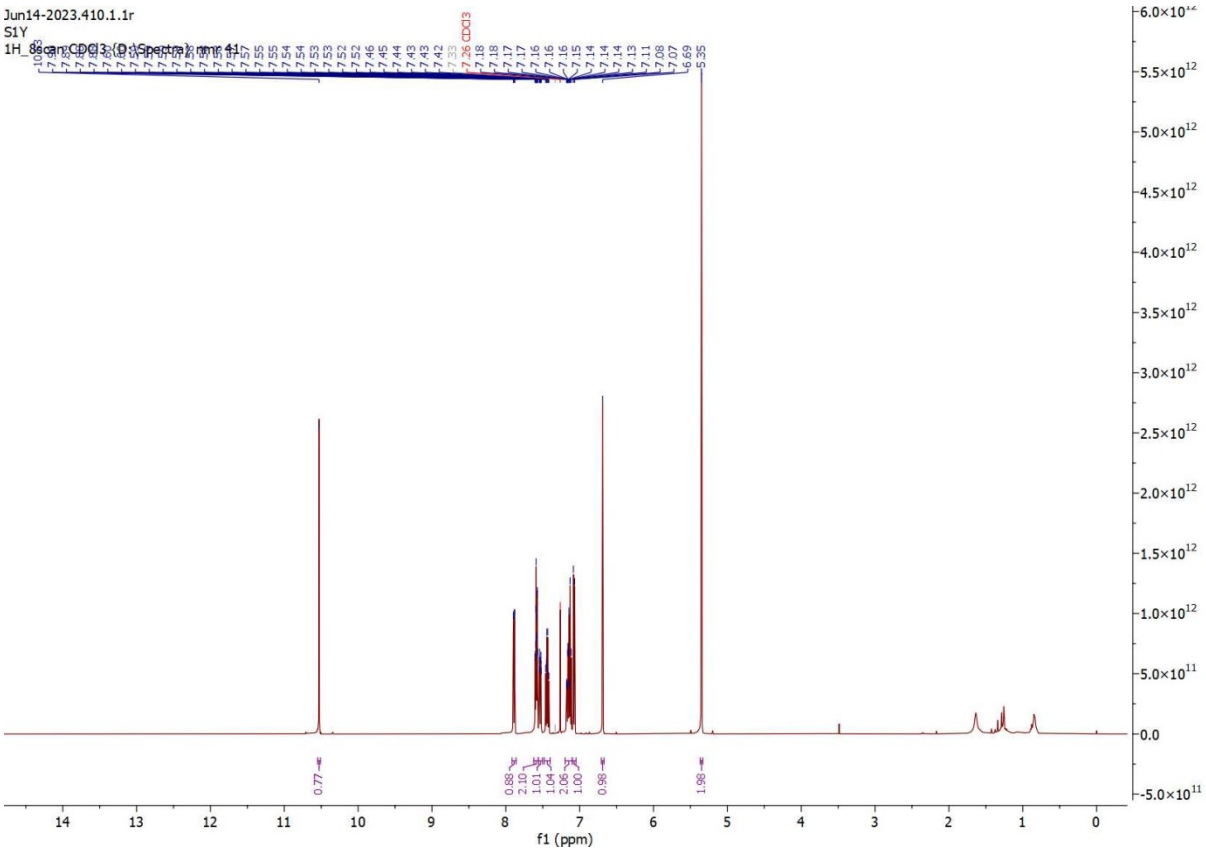

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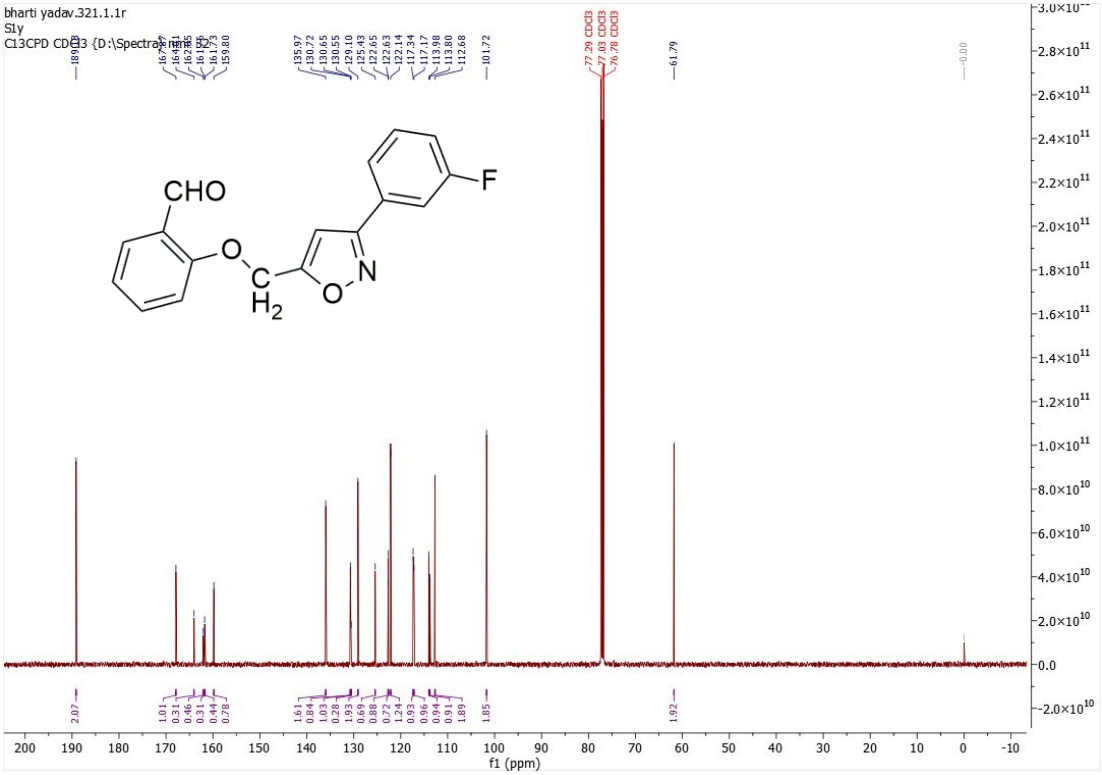

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