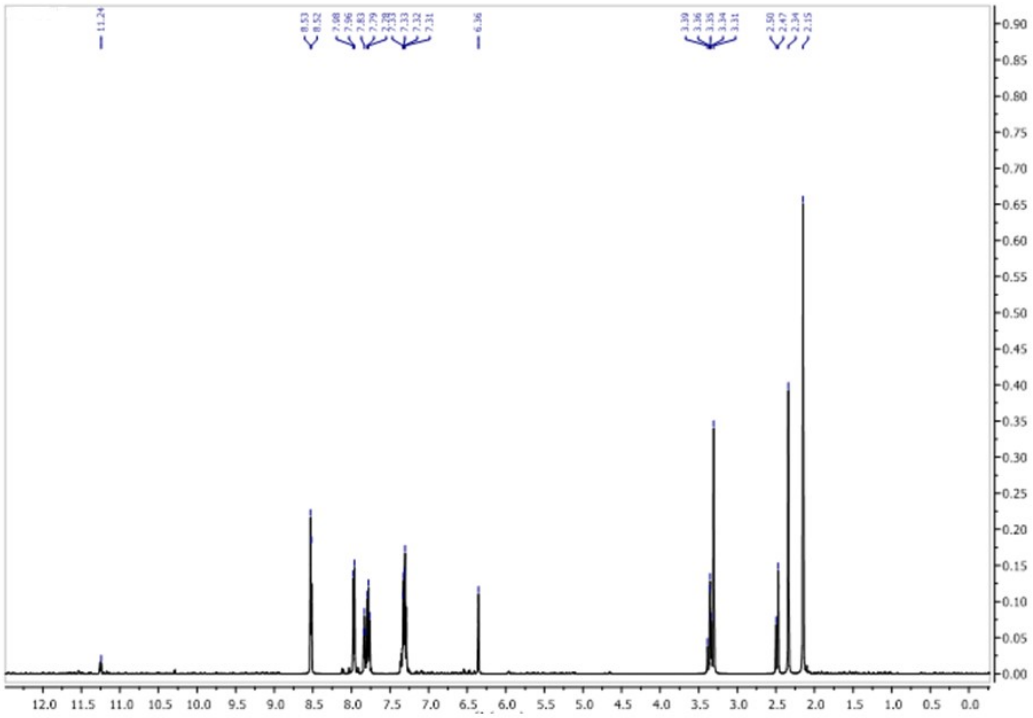
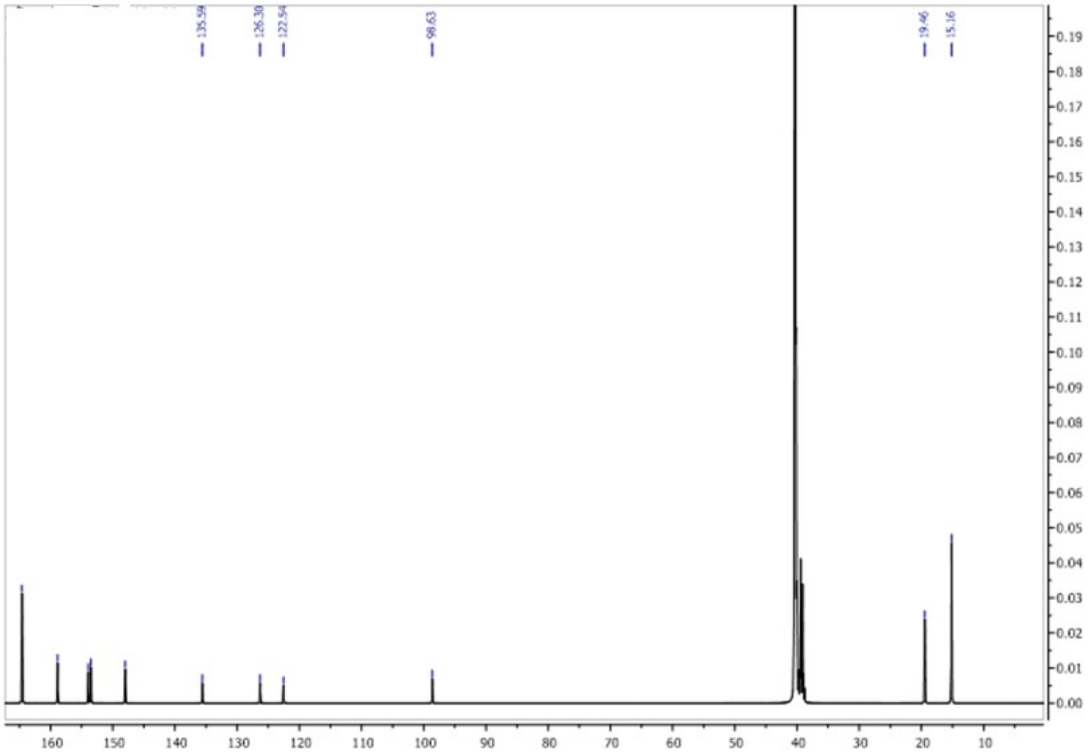
S1. Experimental

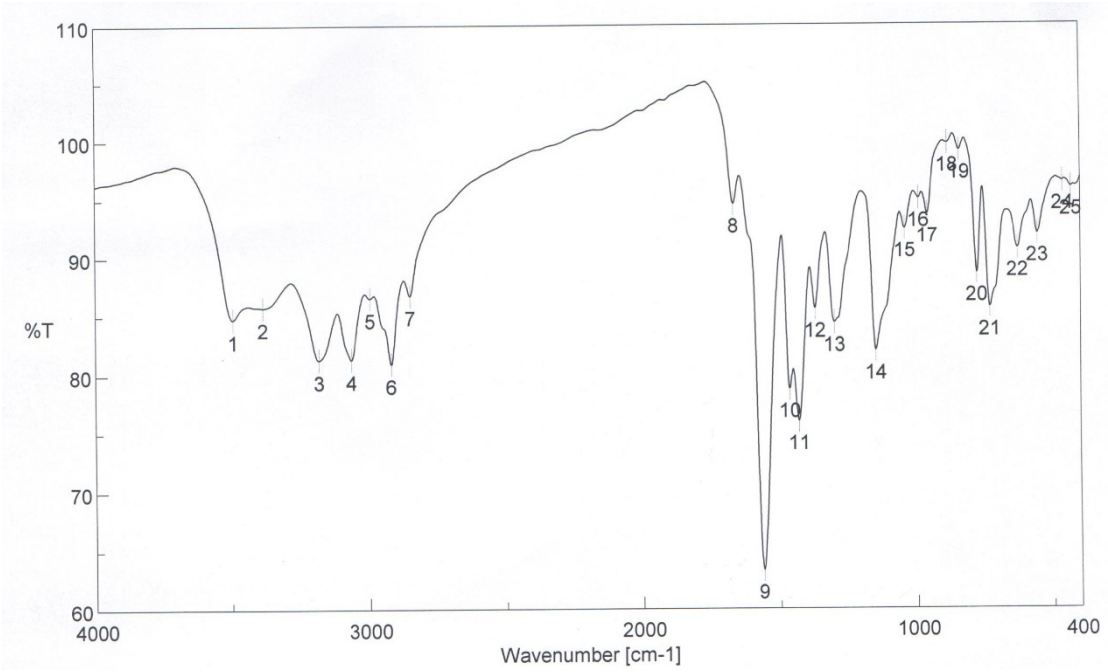
S.2. Copies of IR, ^{1}H NMR and ^{13}C NMR spectra of final compounds



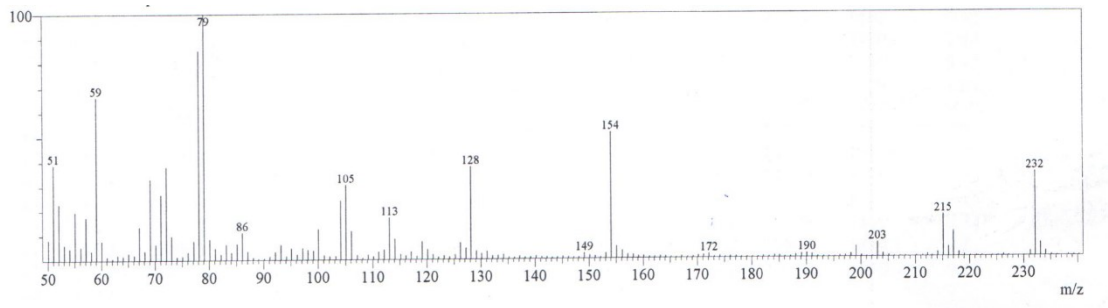
^{1}H NMR Spectrum of Compound 2a



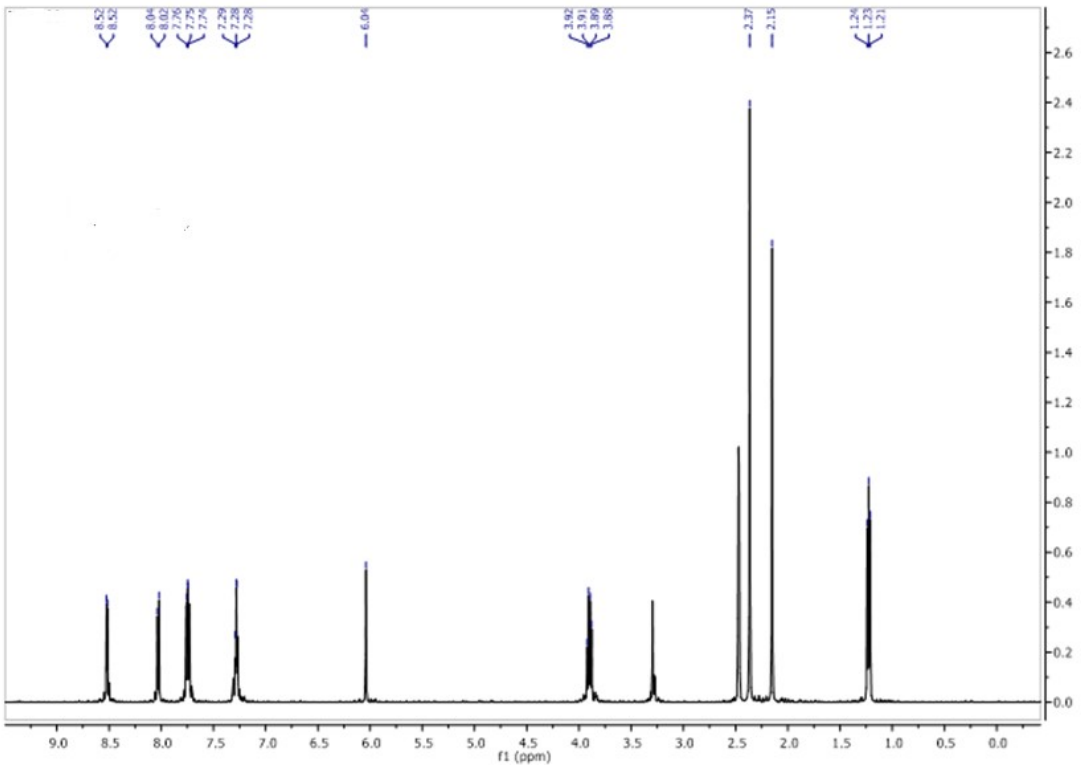
^{13}C NMR Spectrum of Compound 2a



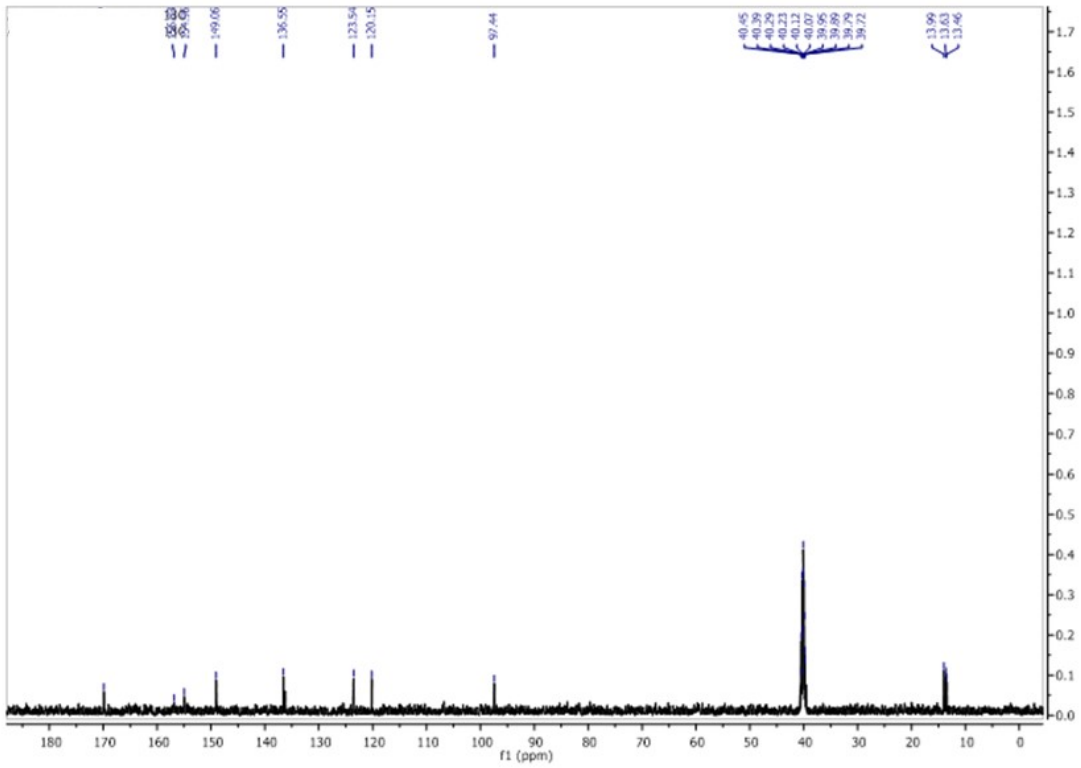
IR Spectrum of Compound 2a



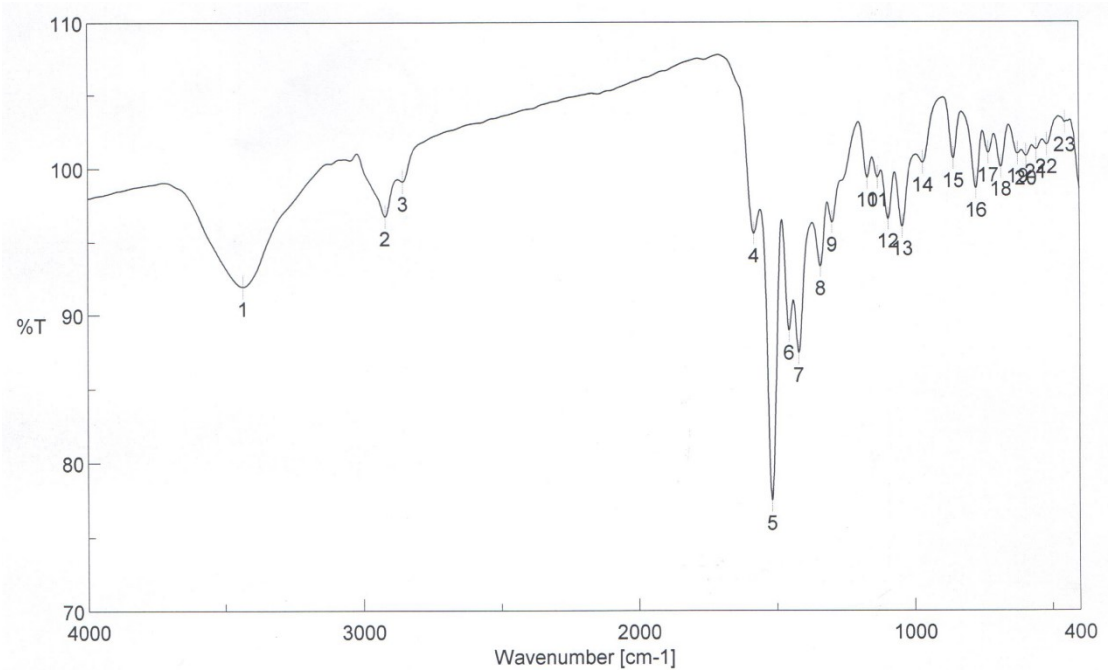
Mass Spectrum of Compound 2a



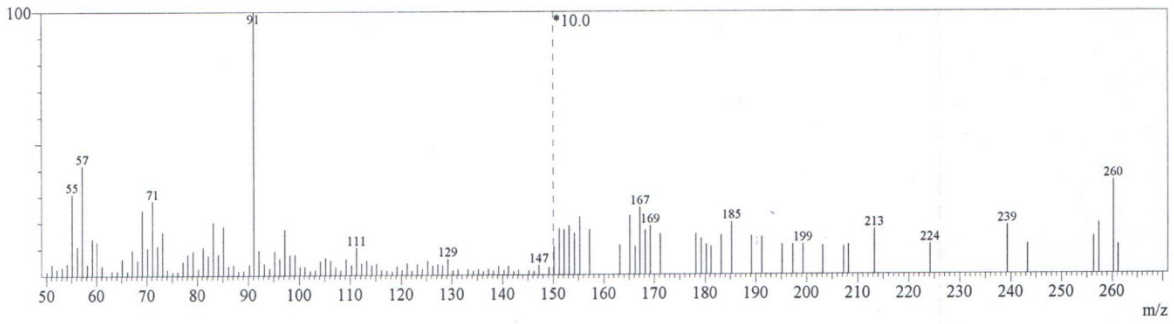
^{1}H NMR Spectrum of Compound 2b



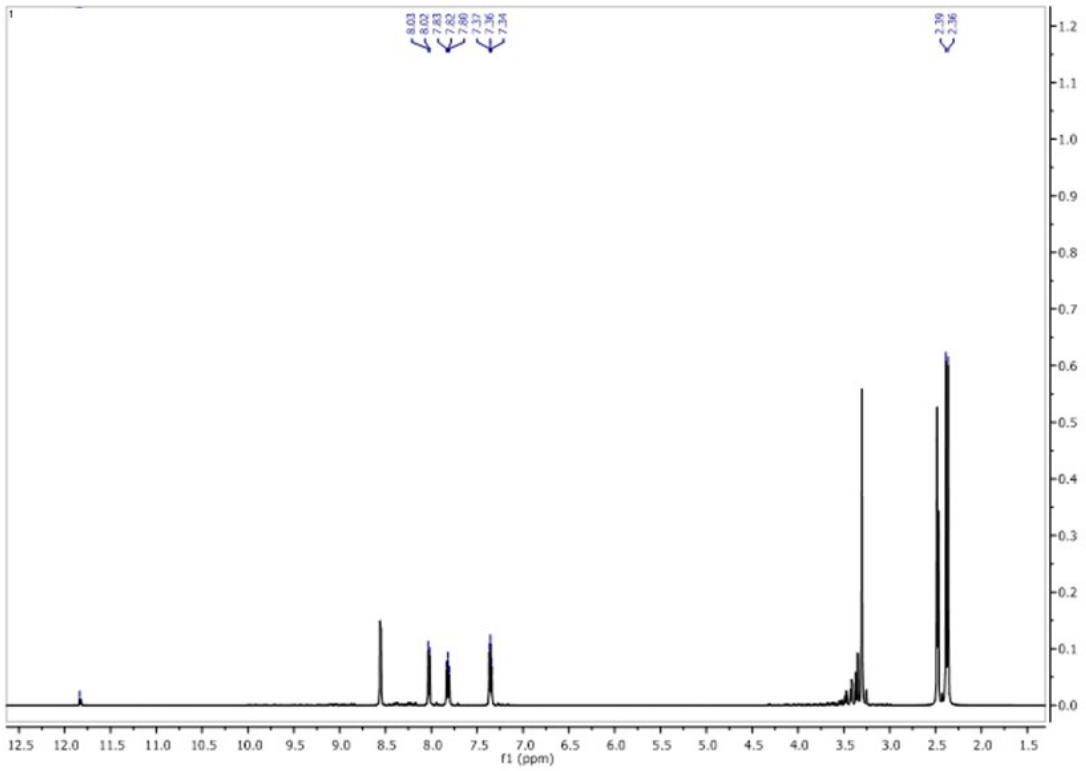
^{13}C NMR Spectrum of Compound 2b



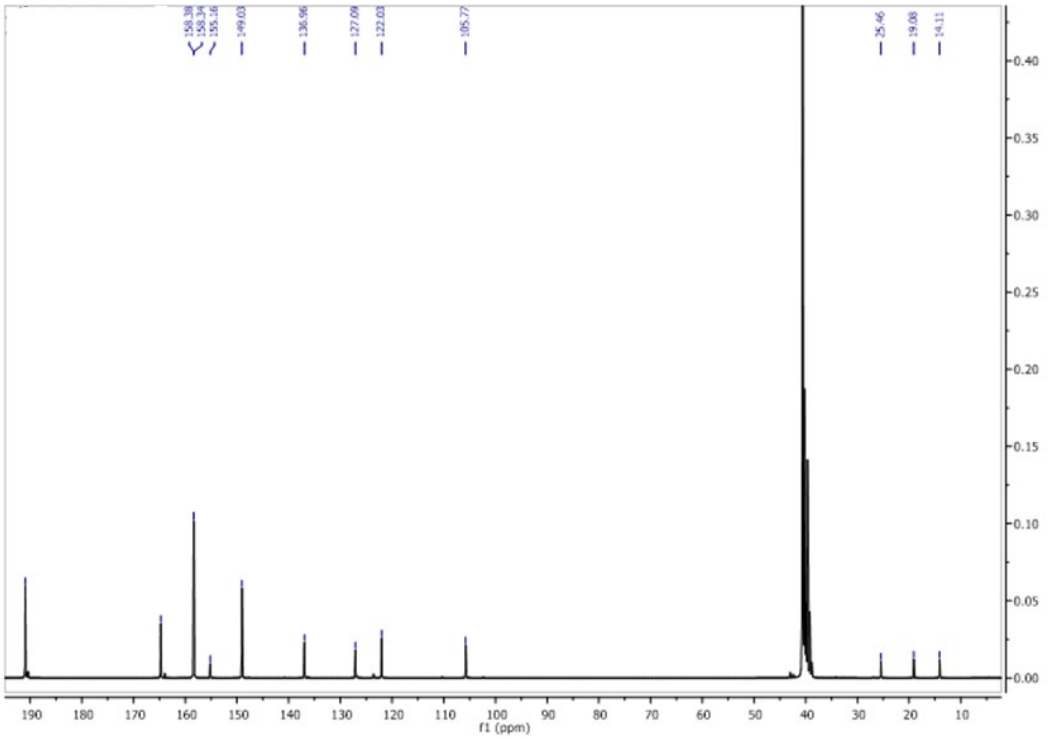
IR Spectrum of Compound 2b



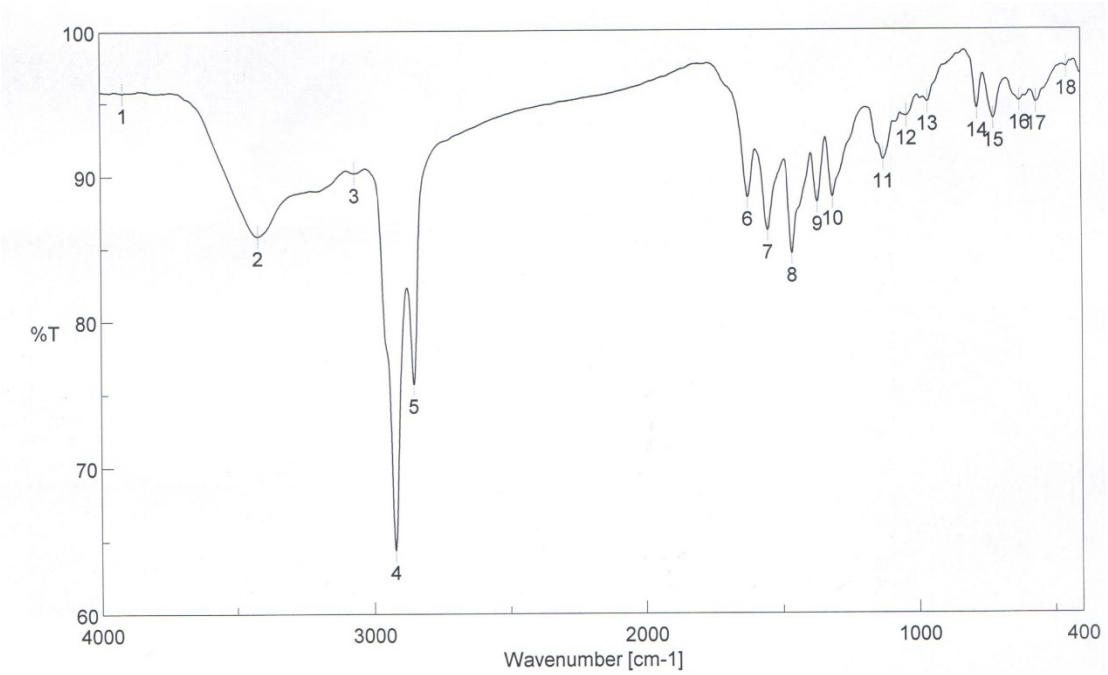
Mass Spectrum of Compound 2b



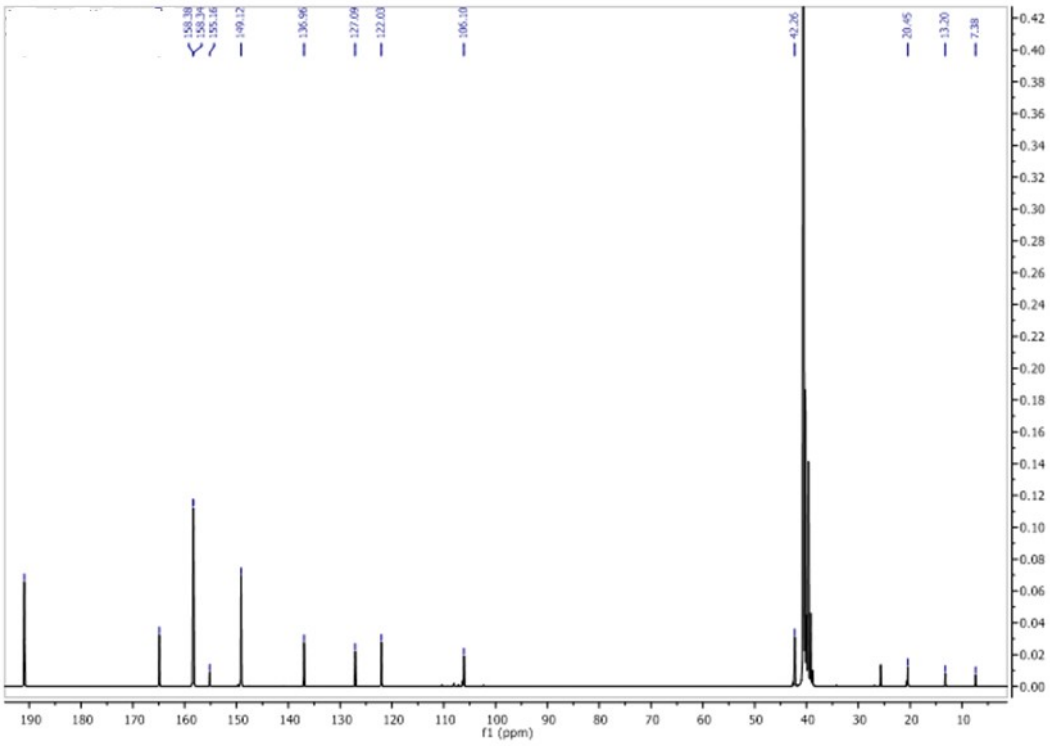
^{1}H NMR Spectrum of Compound 3a



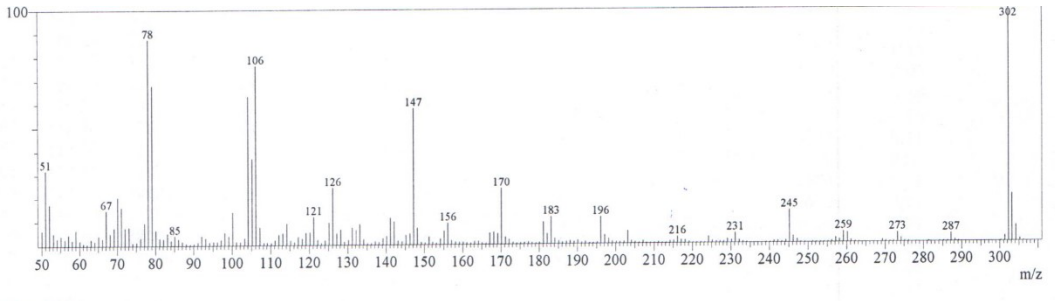
^{13}C NMR Spectrum of Compound 3a



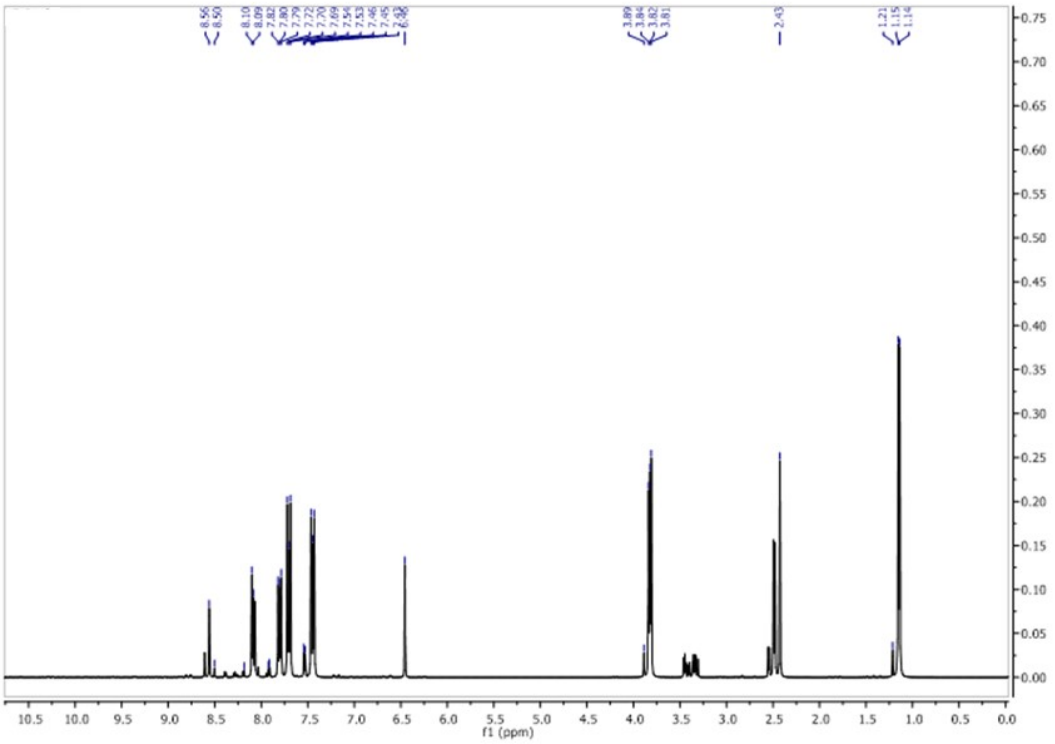
IR Spectrum of Compound 3a



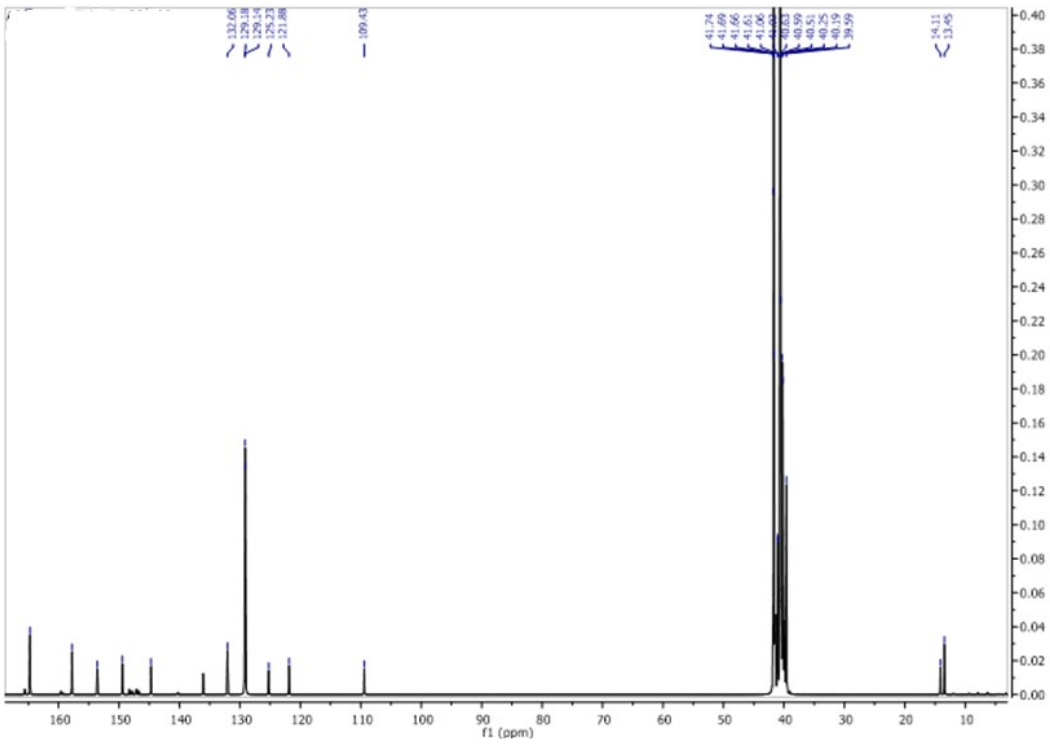
^{13}C NMR Spectrum of Compound 3b



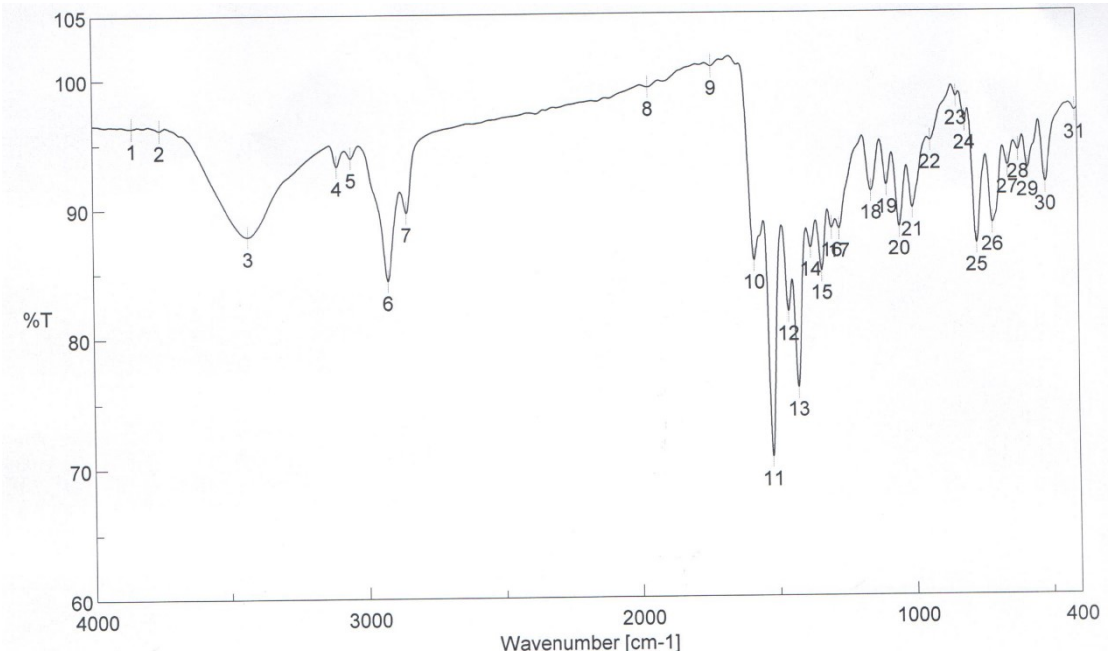
Mass Spectrum of Compound 3b



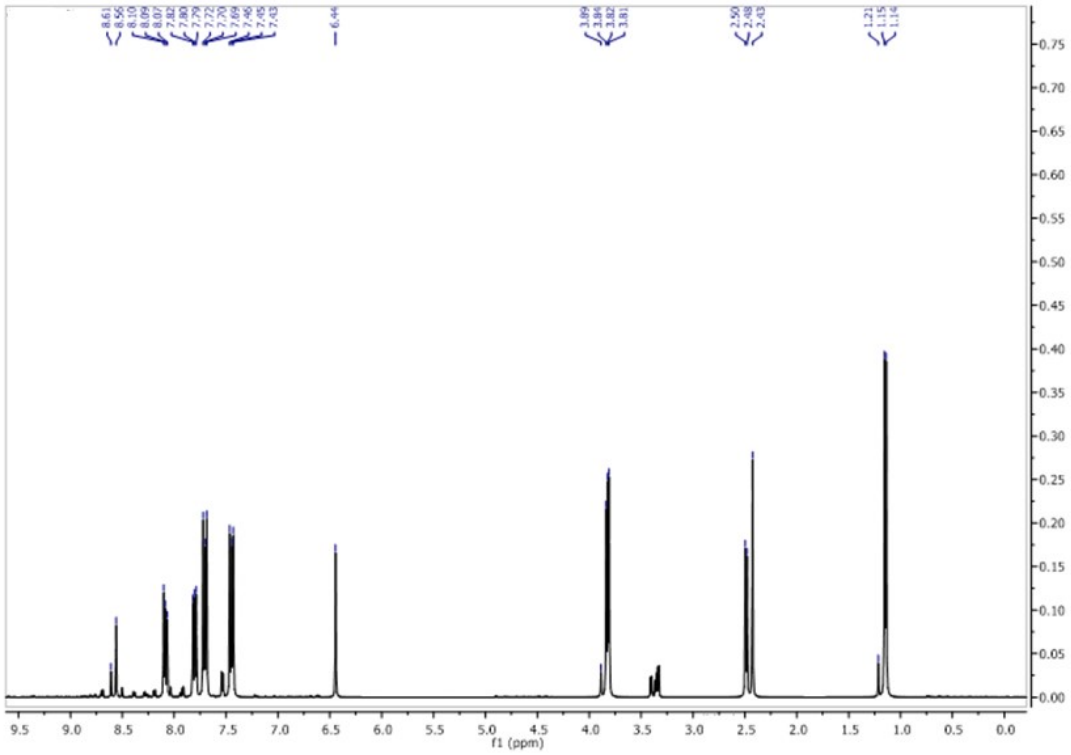
^{1}H NMR Spectrum of Compound 4b



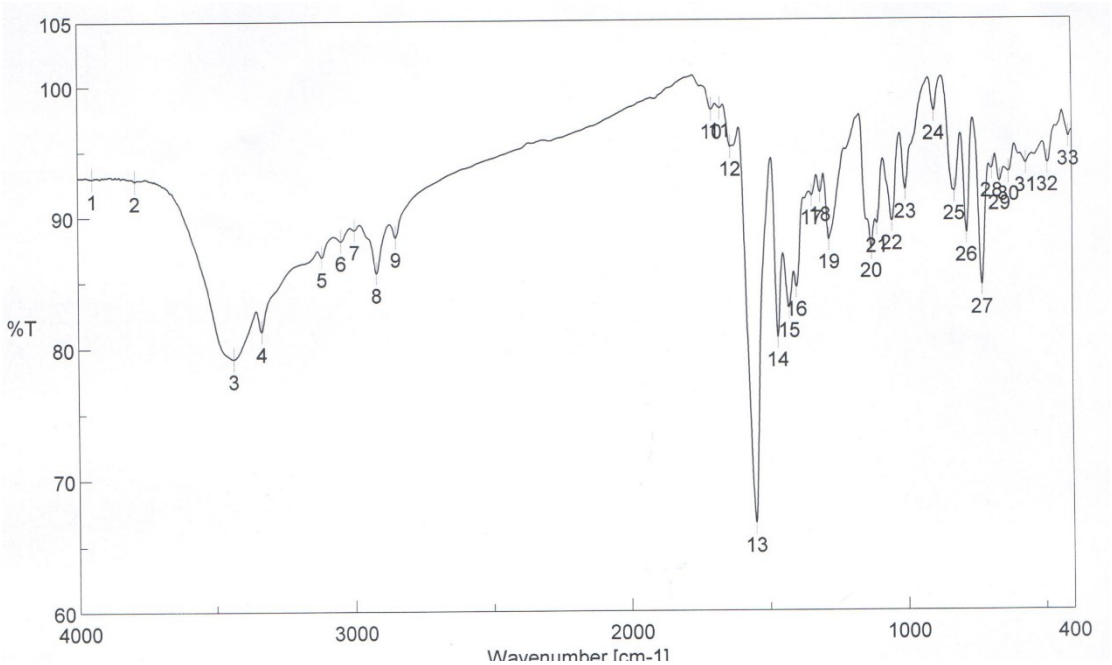
^{13}C NMR Spectrum of Compound 4b



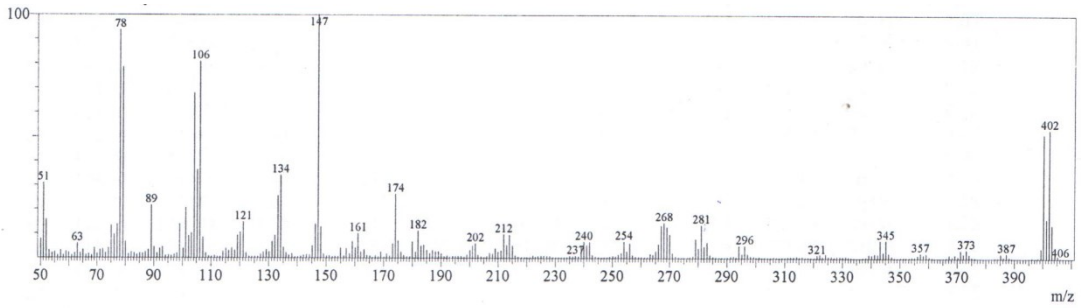
IR Spectrum of Compound 4b



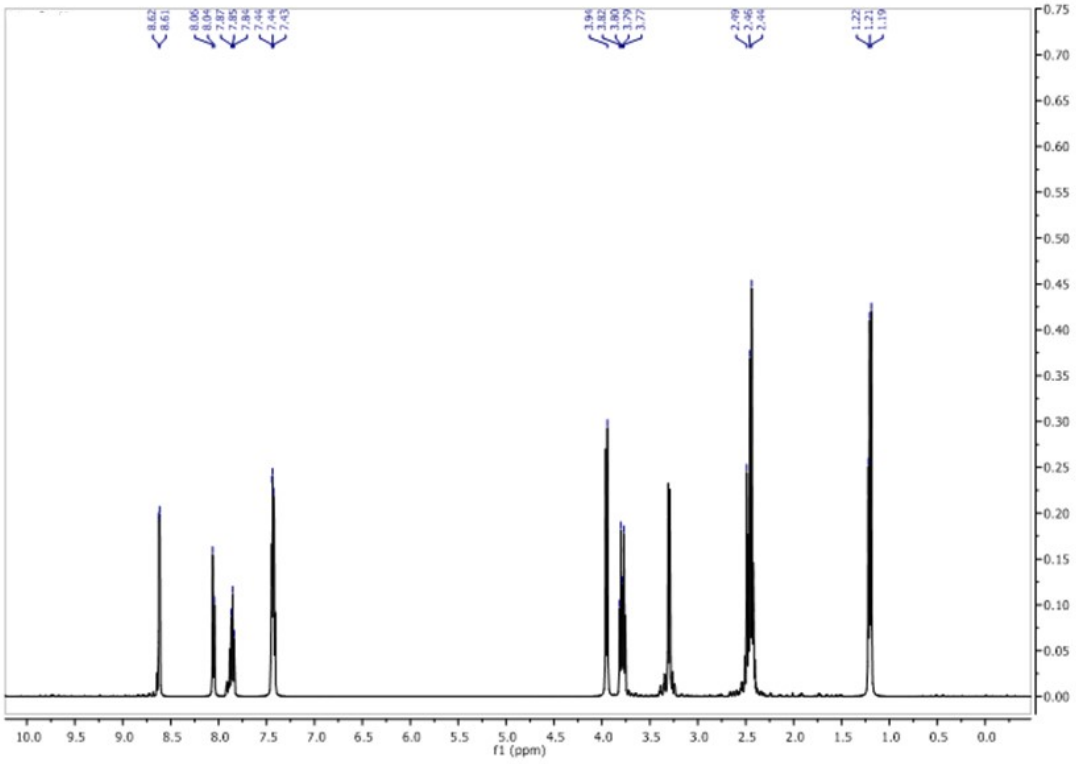
^{1}H NMR Spectrum of Compound 5b



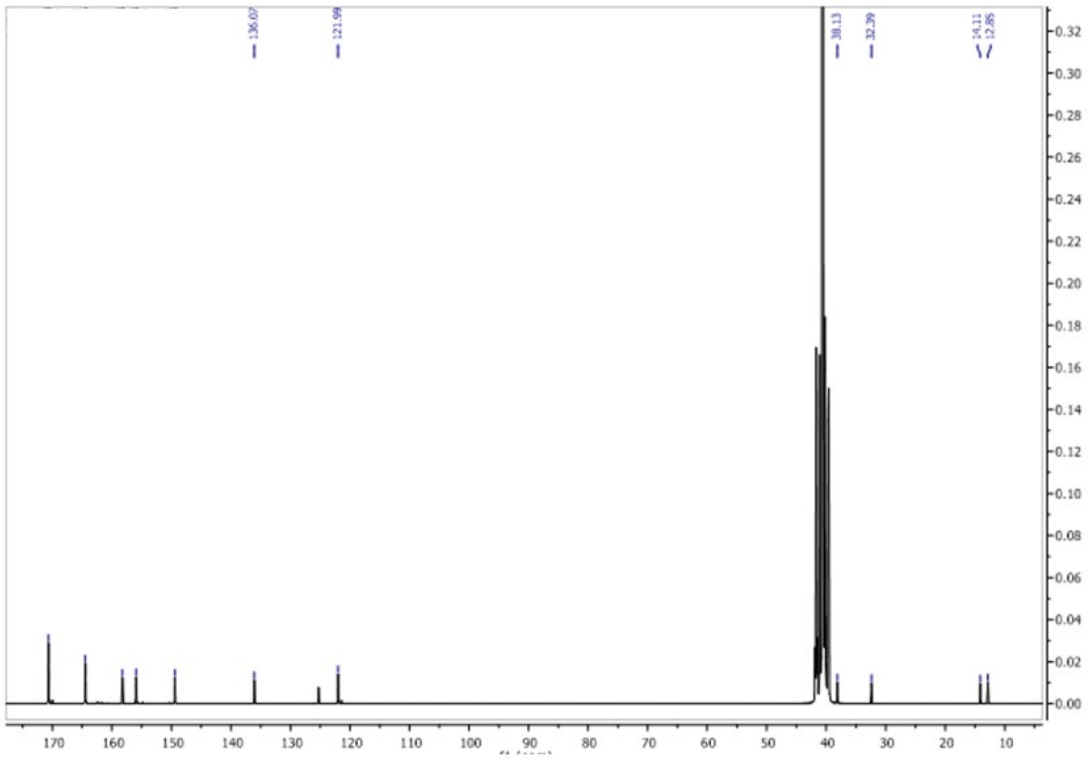
IR Spectrum of Compound 5b



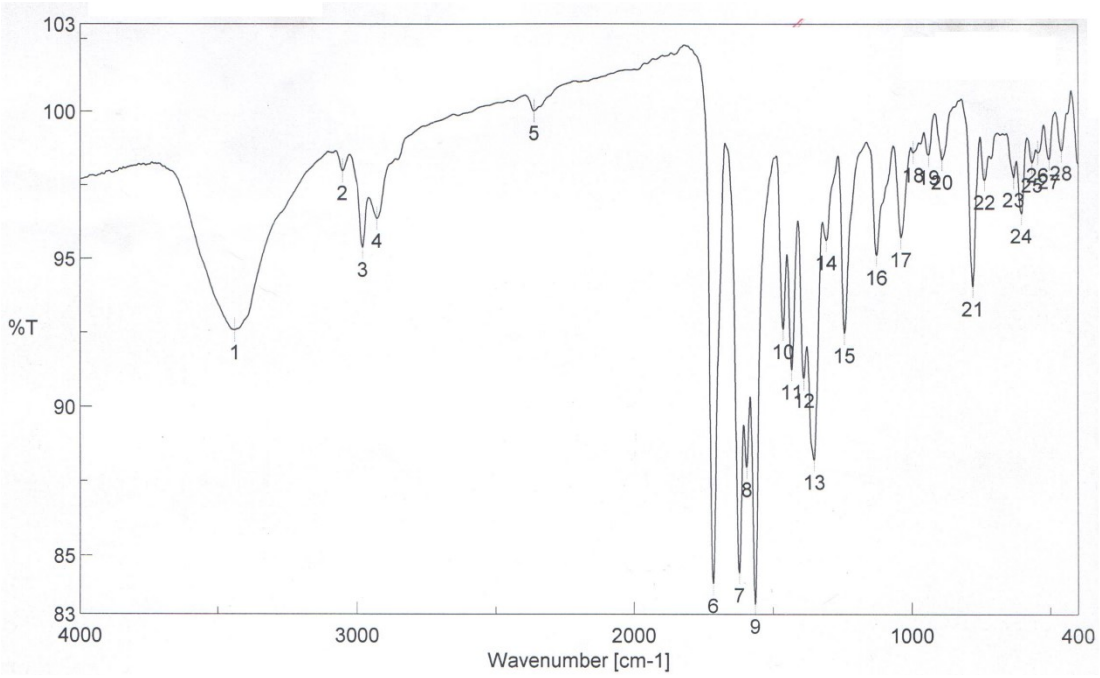
Mass Spectrum of Compound 5b



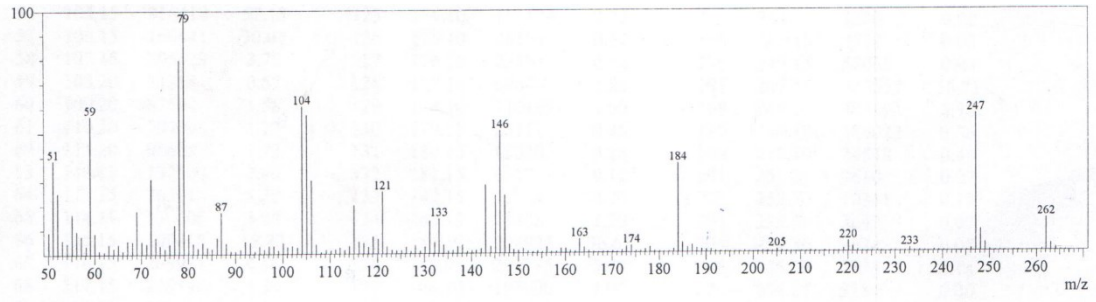
^{1}H NMR Spectrum of Compound 6b



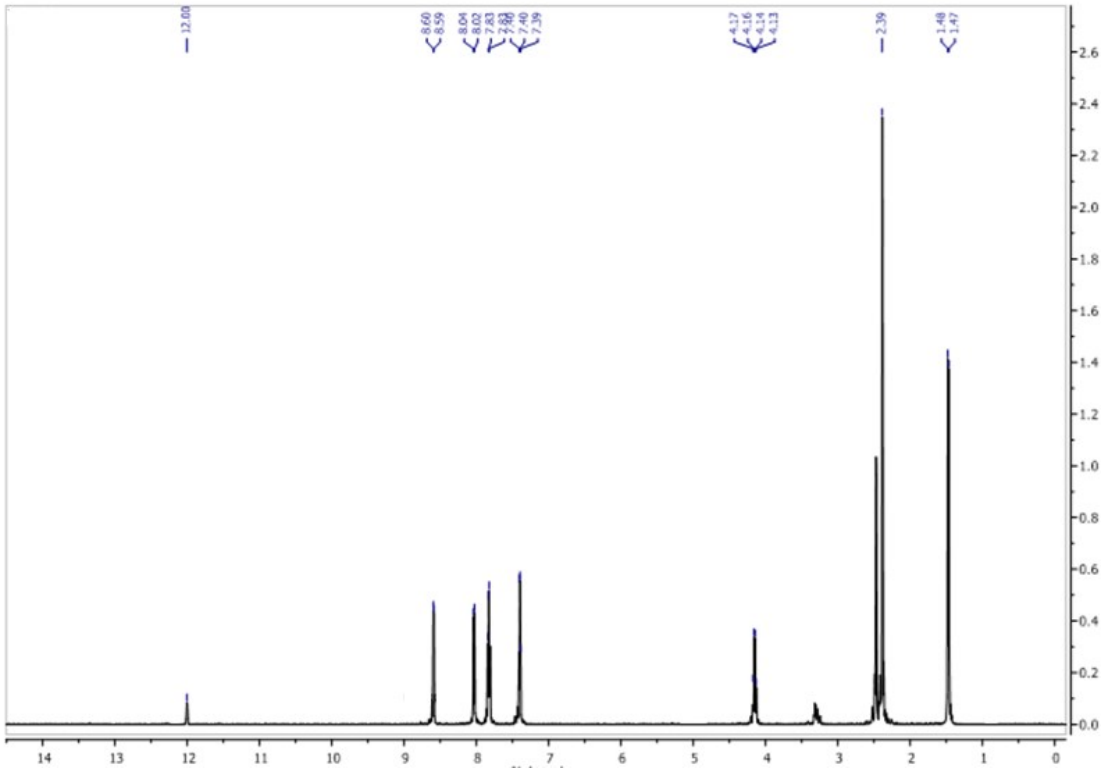
^{13}C NMR Spectrum of Compound 6b



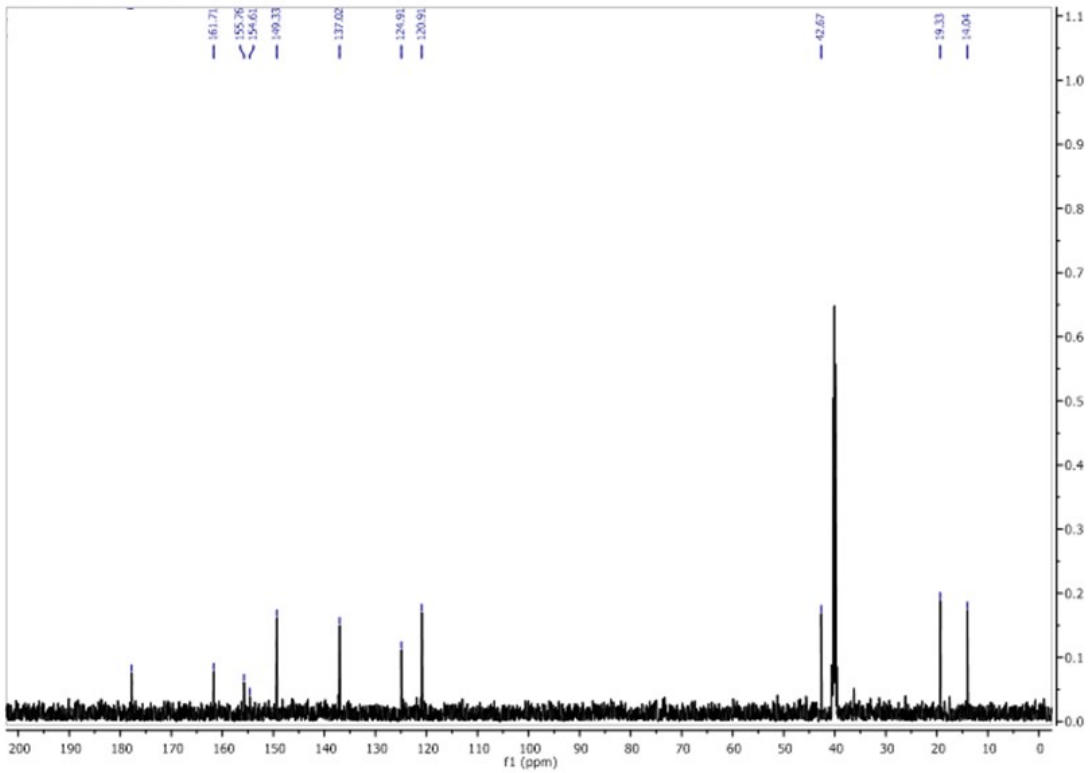
IR Spectrum of Compound 6b



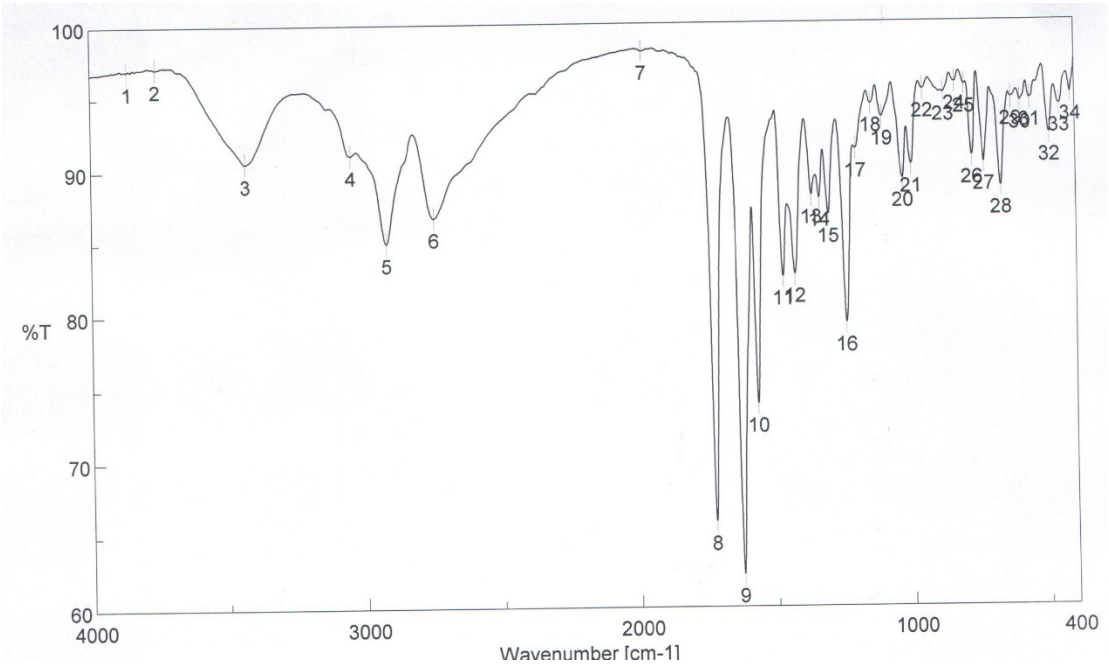
Mass Spectrum of Compound 6b



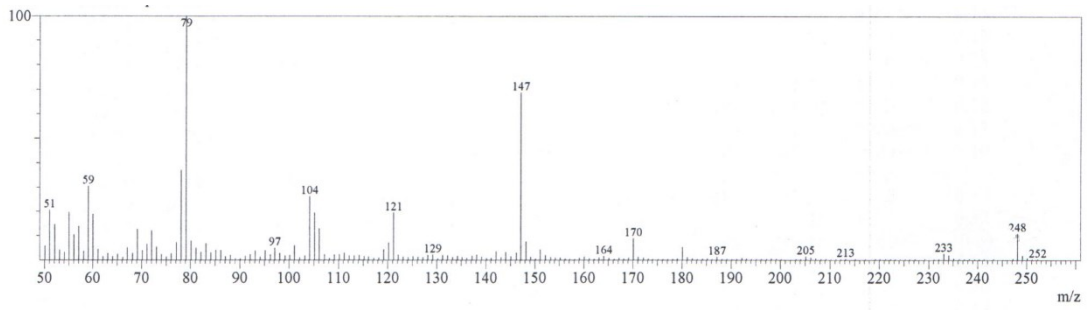
^{1}H NMR Spectrum of Compound 7a



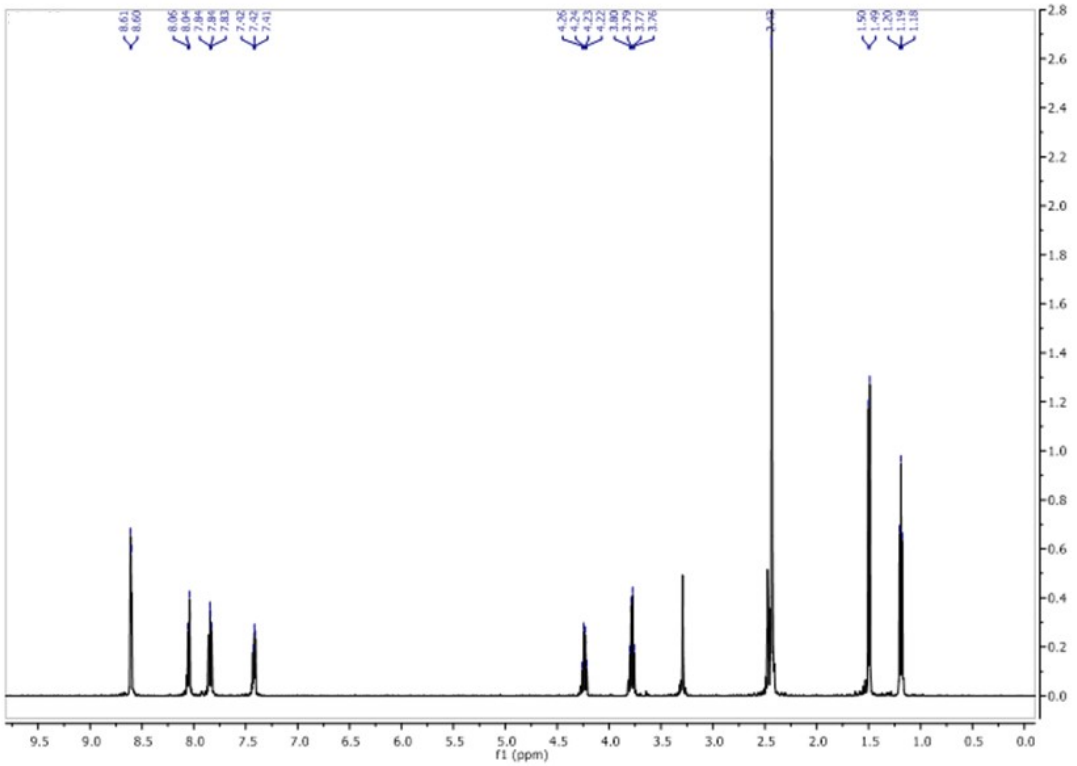
^{13}C NMR Spectrum of Compound 7a



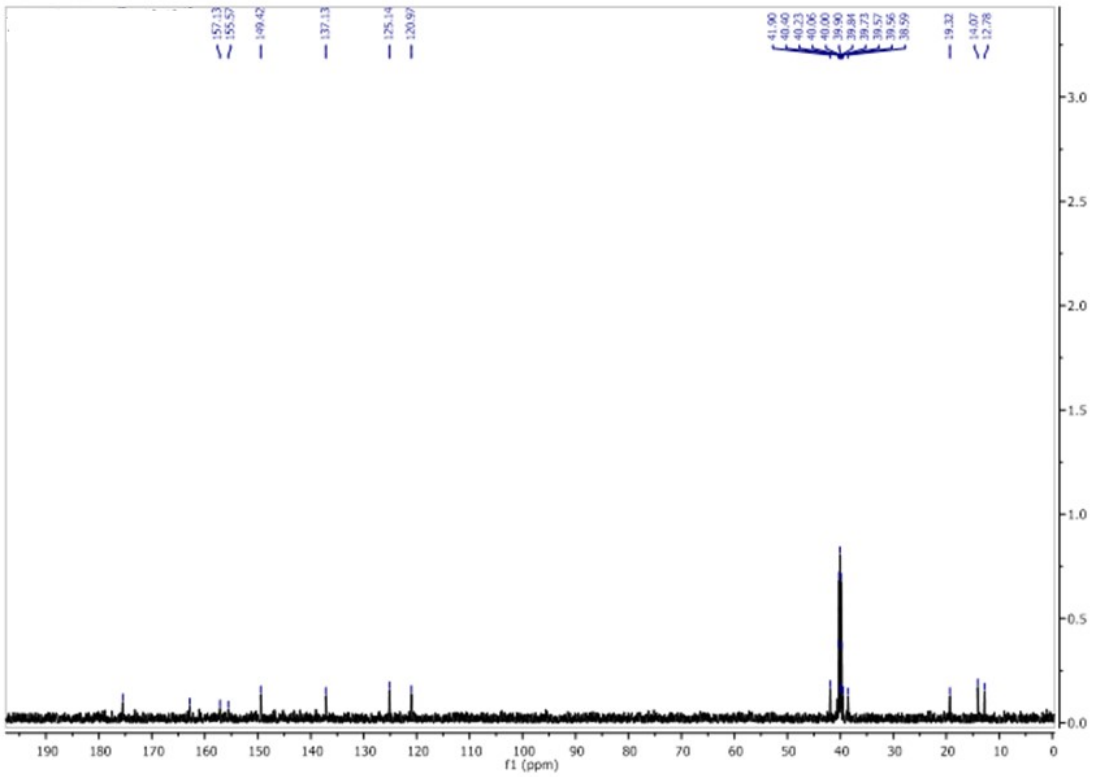
IR Spectrum of Compound 7a



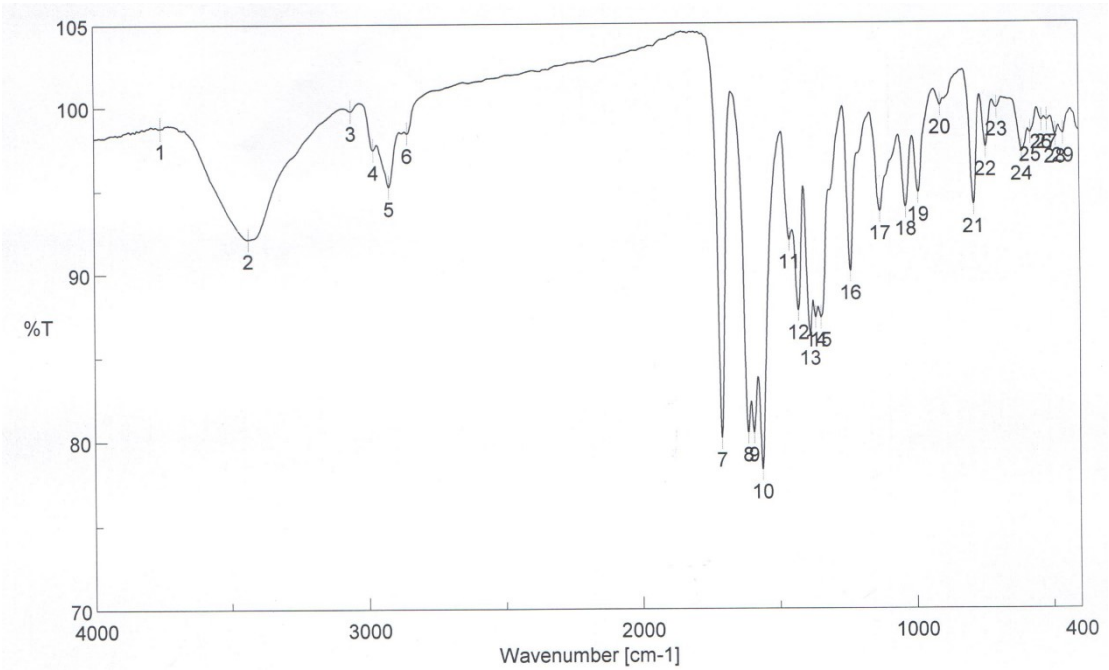
Mass Spectrum of Compound 7a



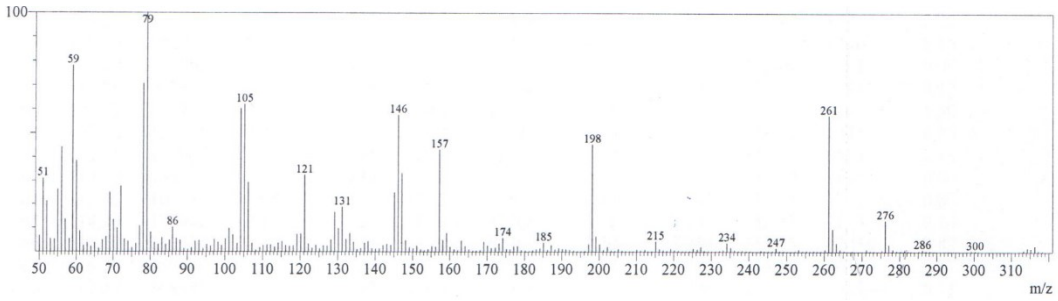
^{1}H NMR Spectrum of Compound 7b



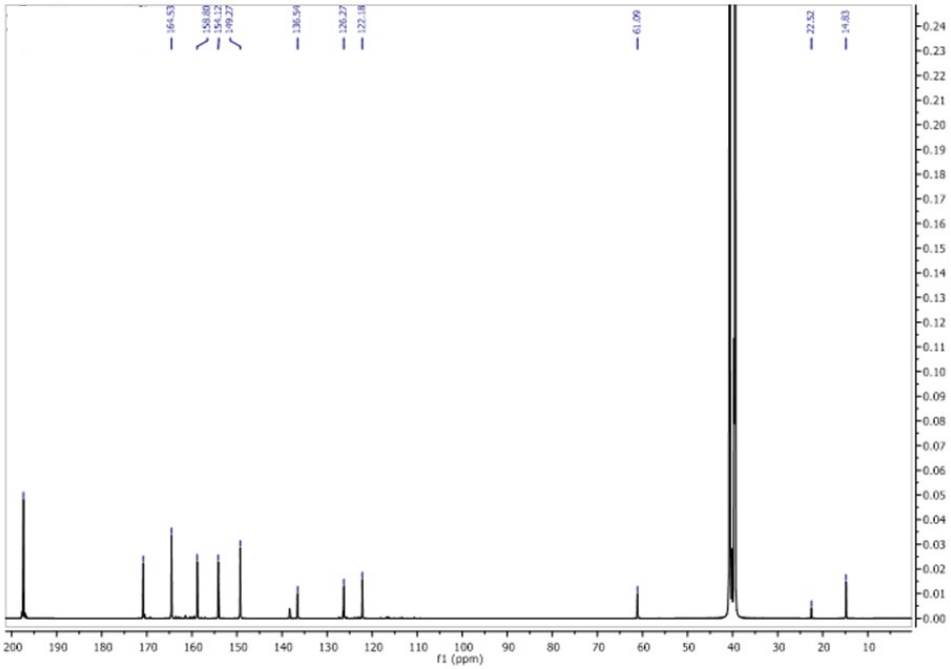
^{13}C NMR Spectrum of Compound 7b



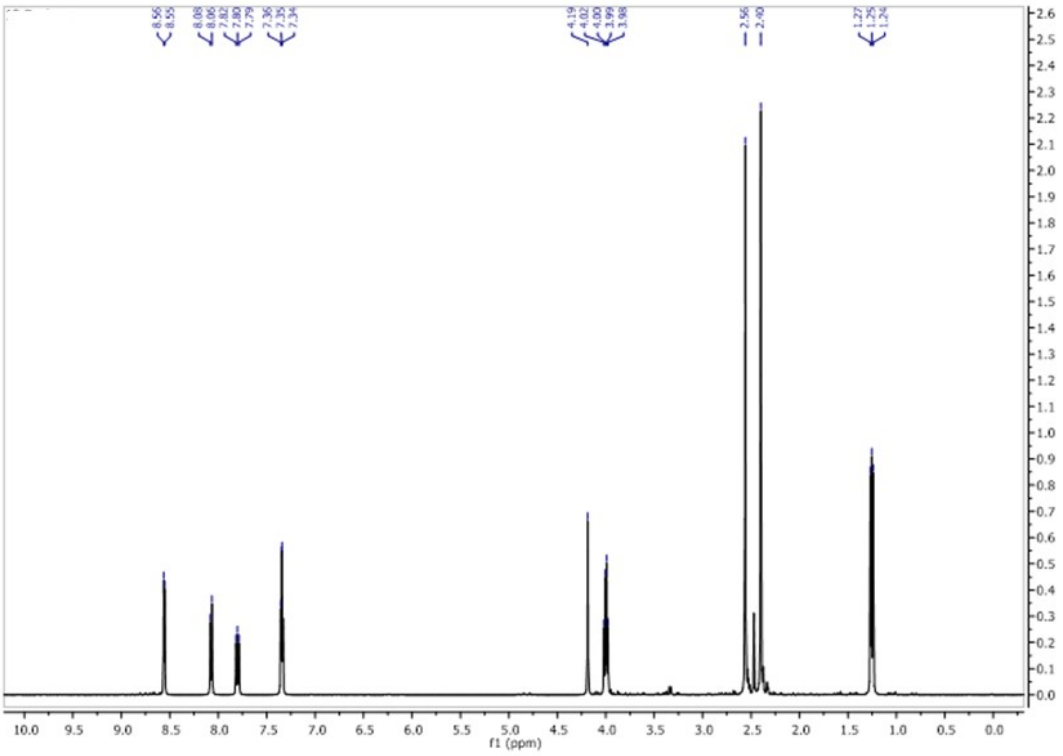
IR Spectrum of Compound 7b



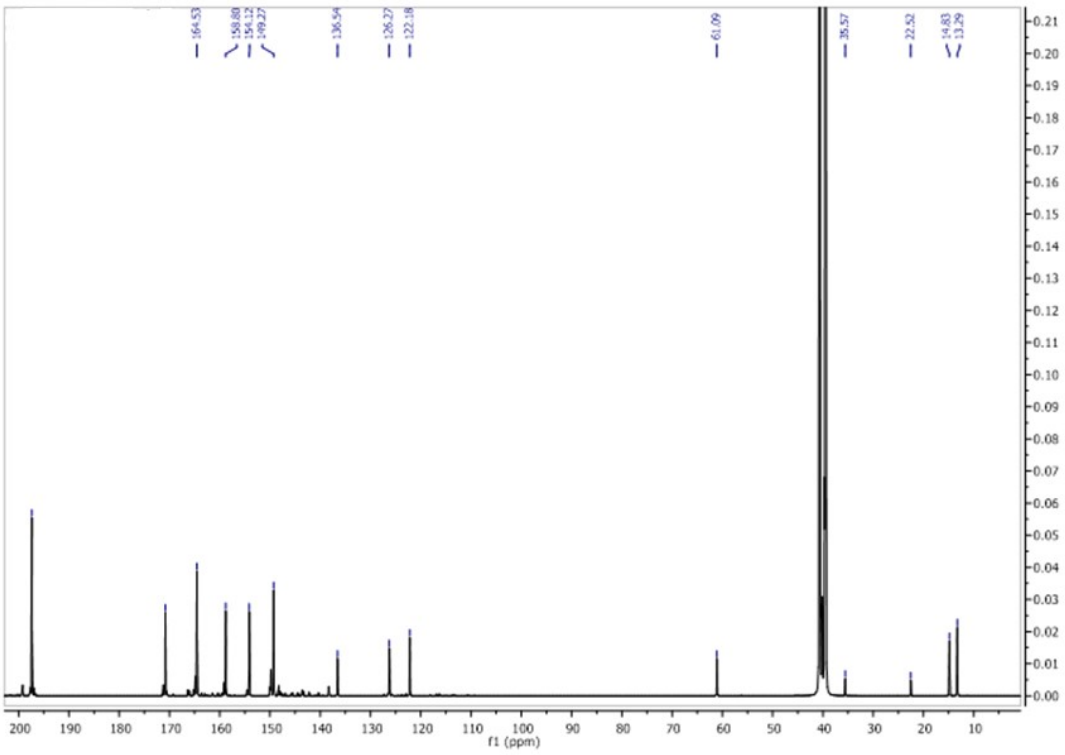
Mass Spectrum of Compound 7b



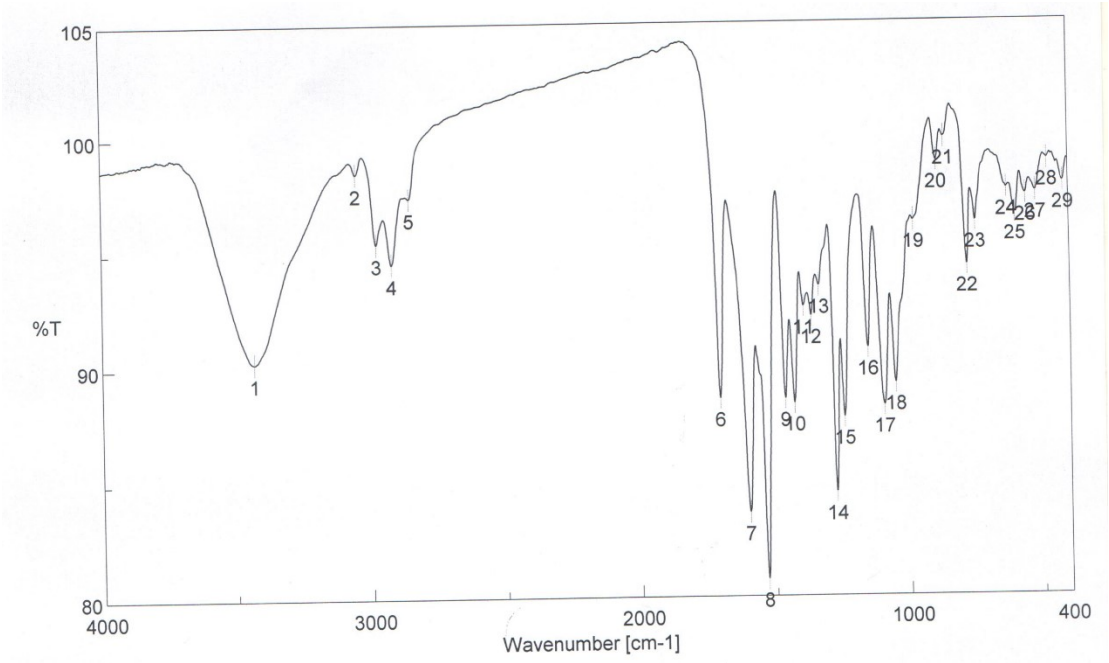
^{13}C NMR Spectrum of Compound 8a



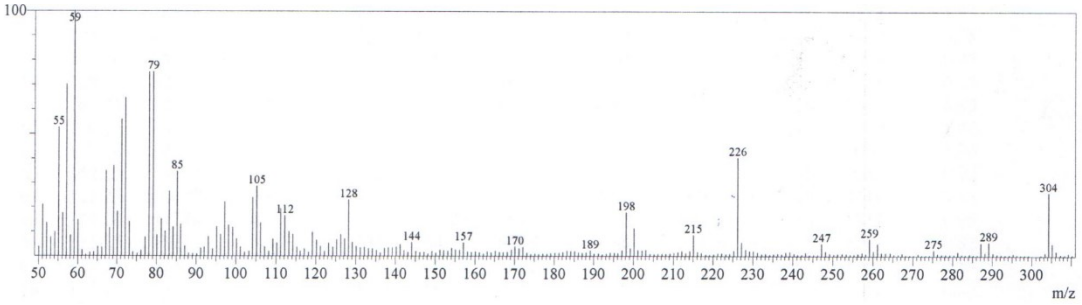
^{1}H NMR Spectrum of Compound 8b



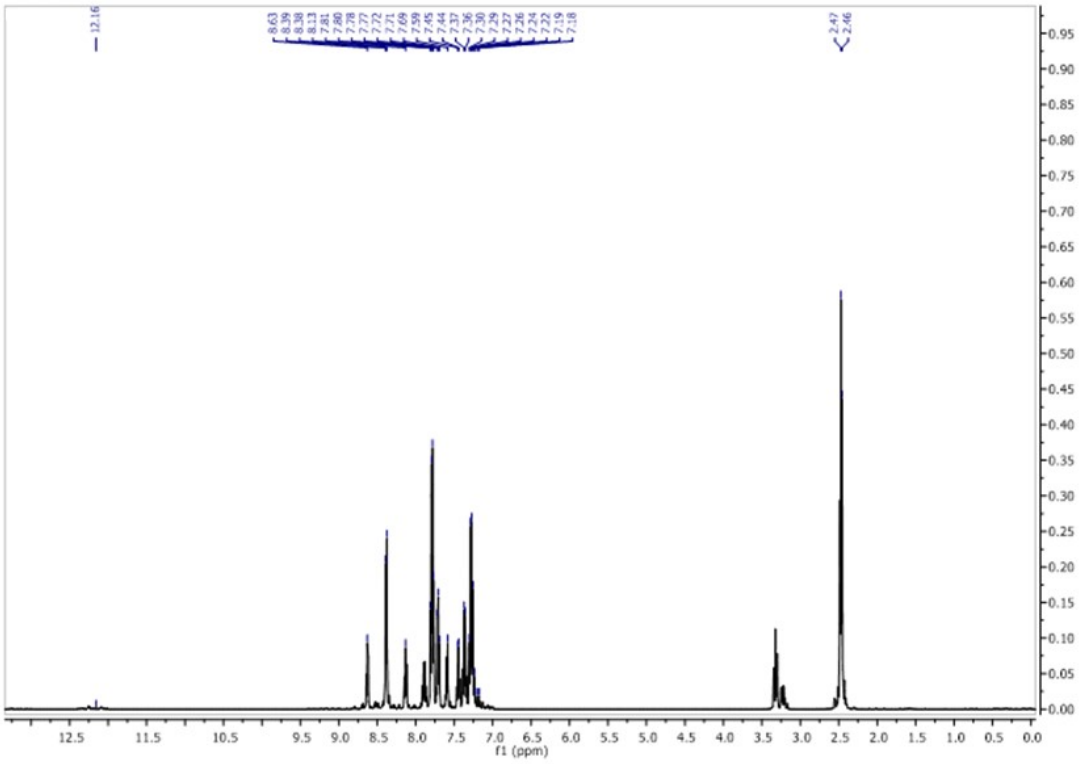
^{13}C NMR Spectrum of Compound 8b



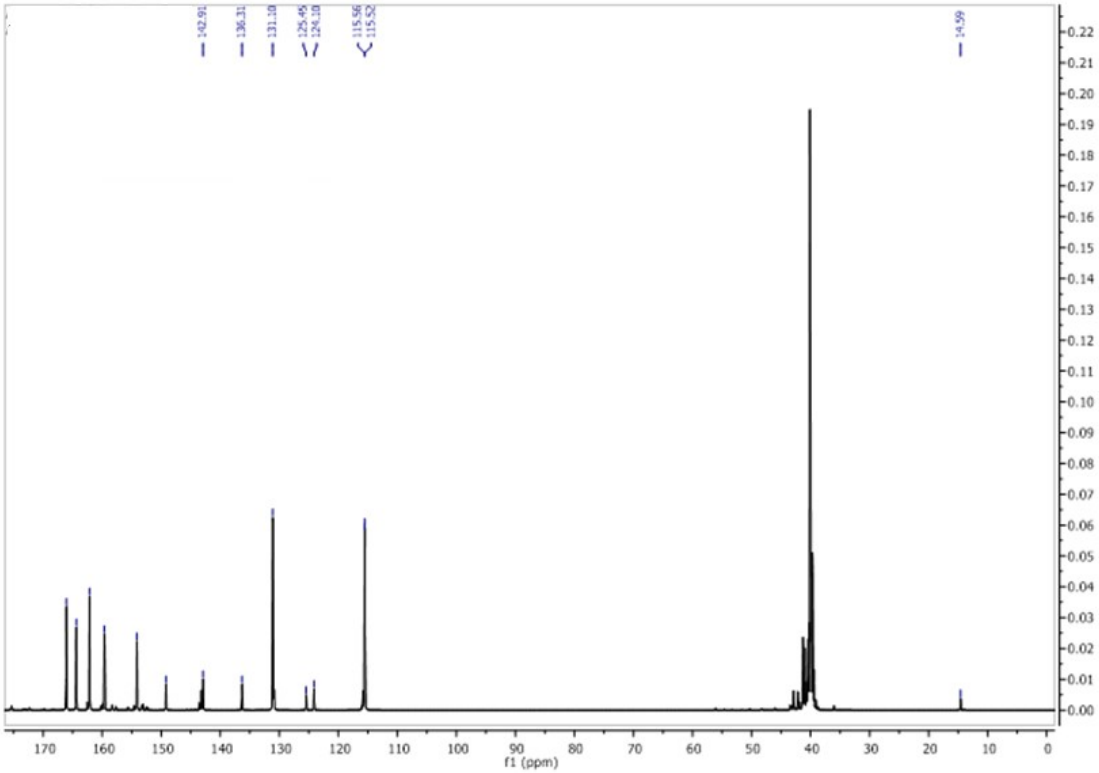
IR Spectrum of Compound 8b



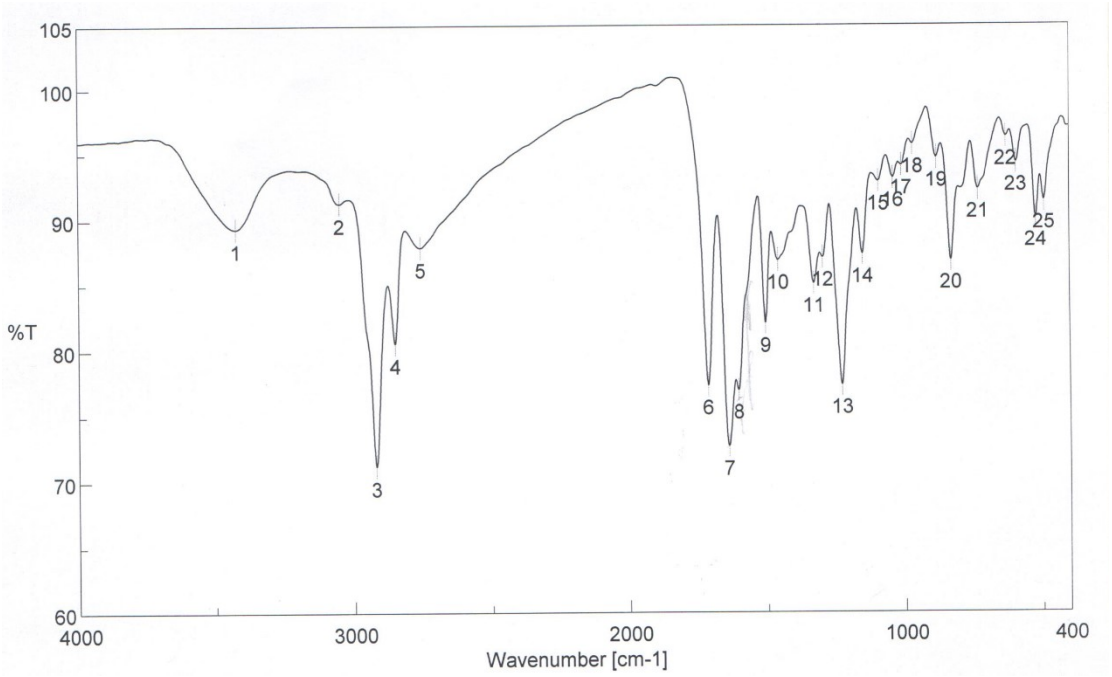
Mass Spectrum of Compound 8b



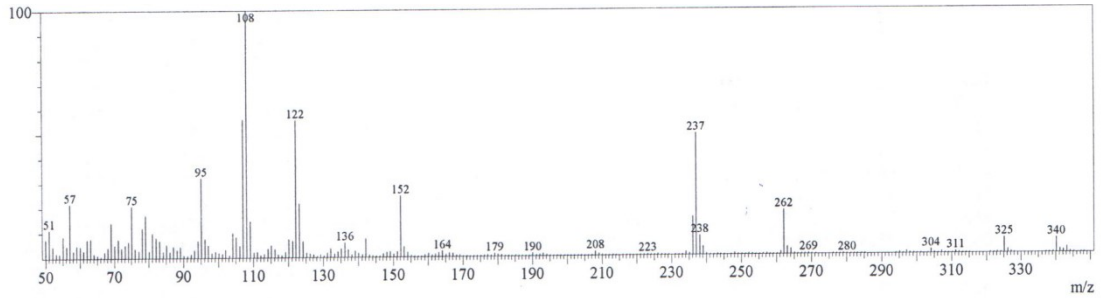
^{1}H NMR Spectrum of Compound 9a



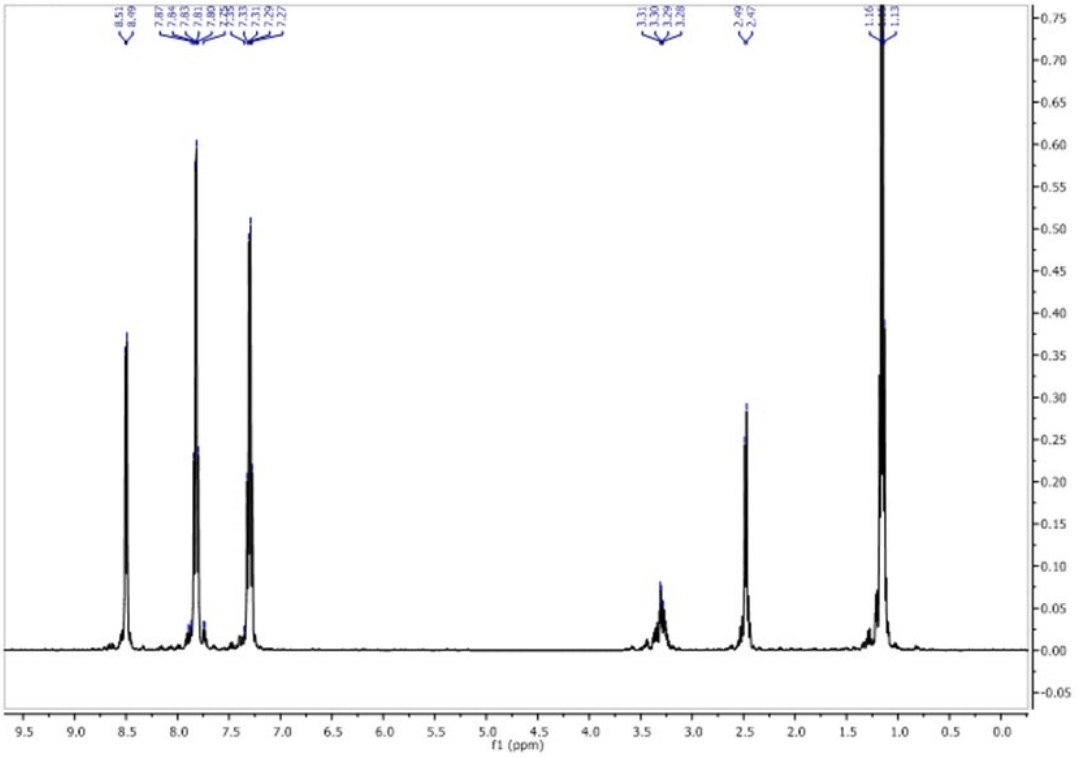
^{13}C NMR Spectrum of Compound 9a



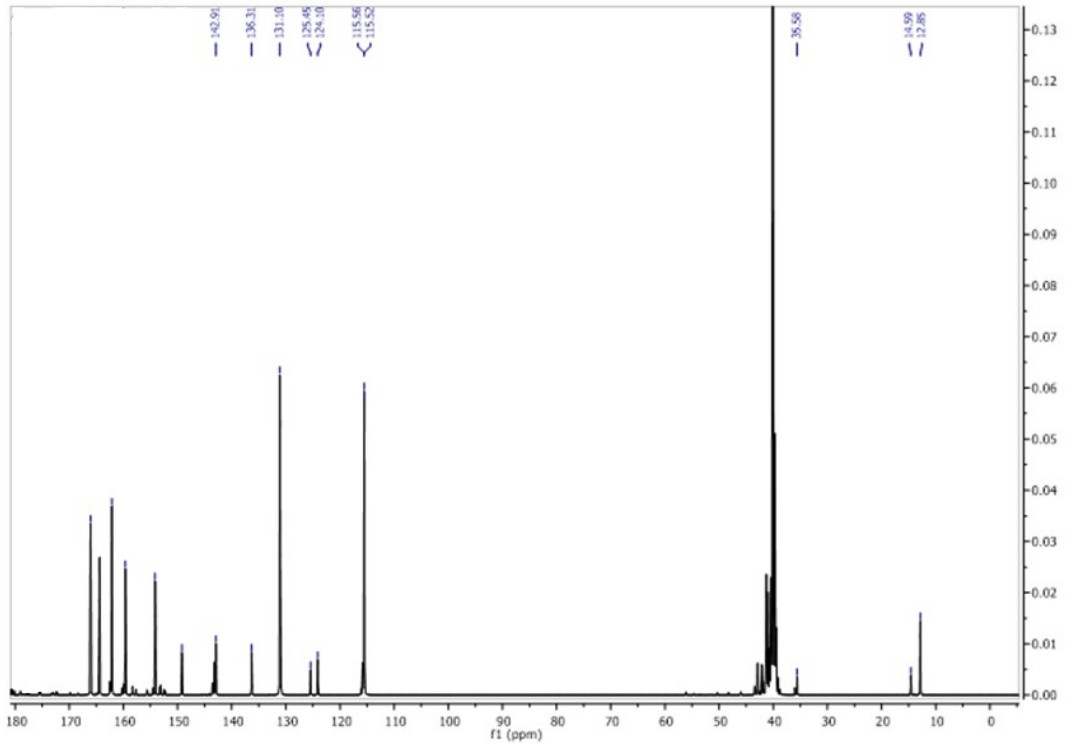
IR Spectrum of Compound 9a



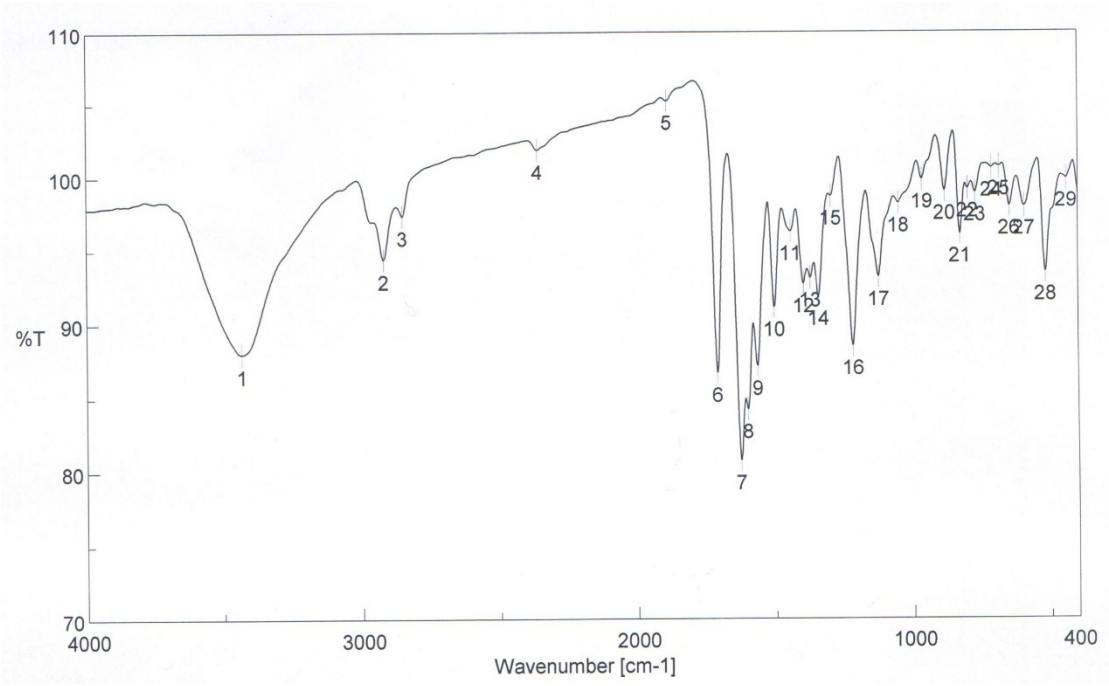
Mass Spectrum of Compound 9a



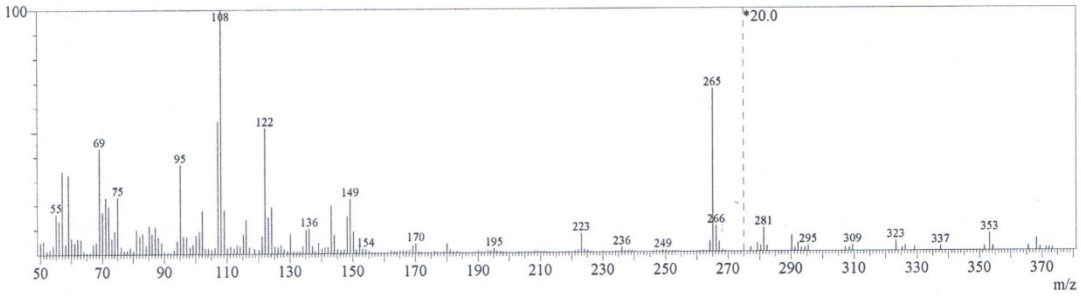
^{1}H NMR Spectrum of Compound 9b



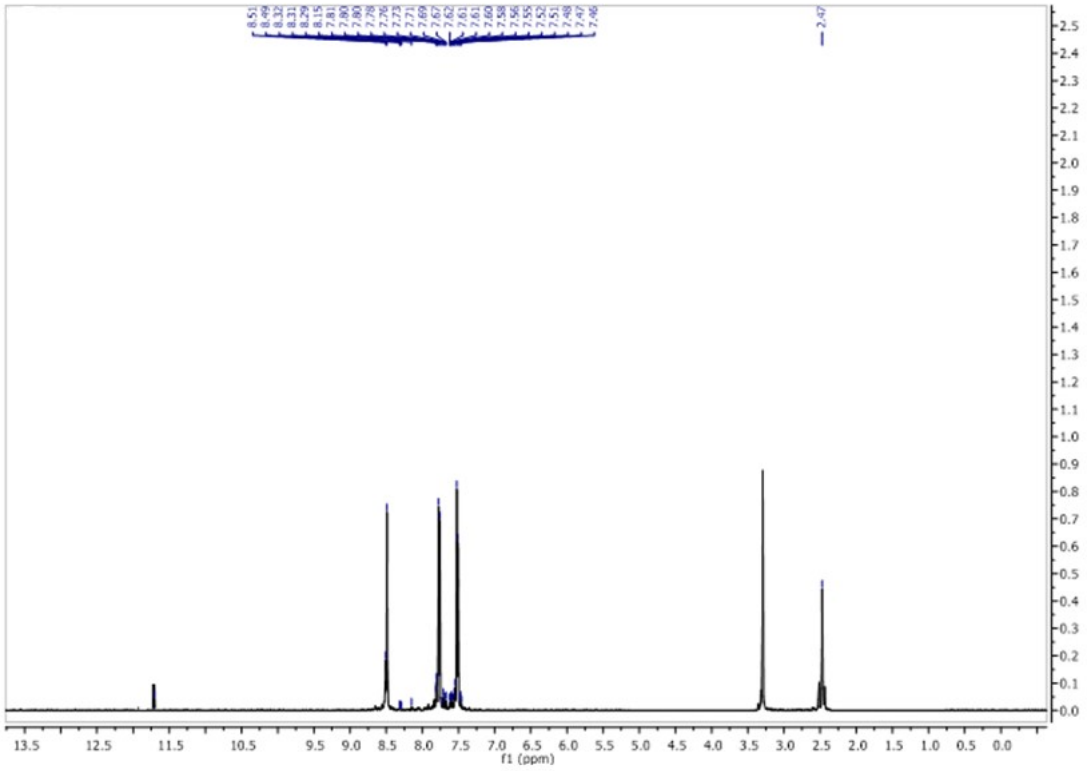
^{13}C NMR Spectrum of Compound 9b



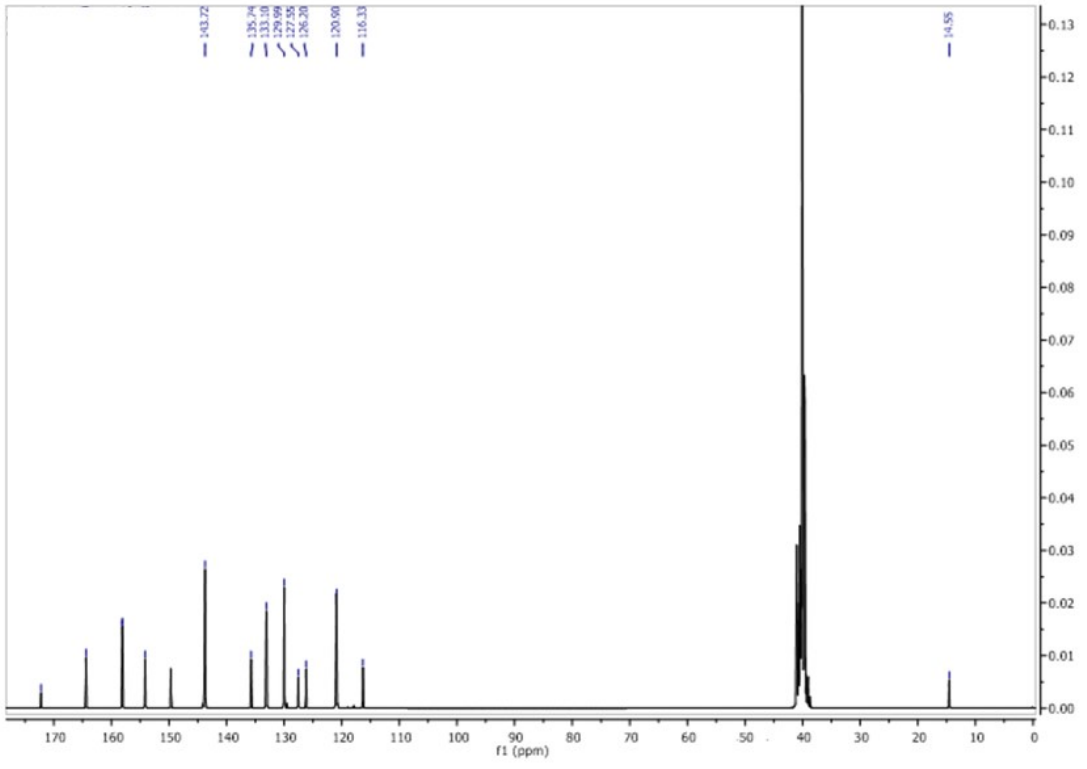
IR Spectrum of Compound 9b



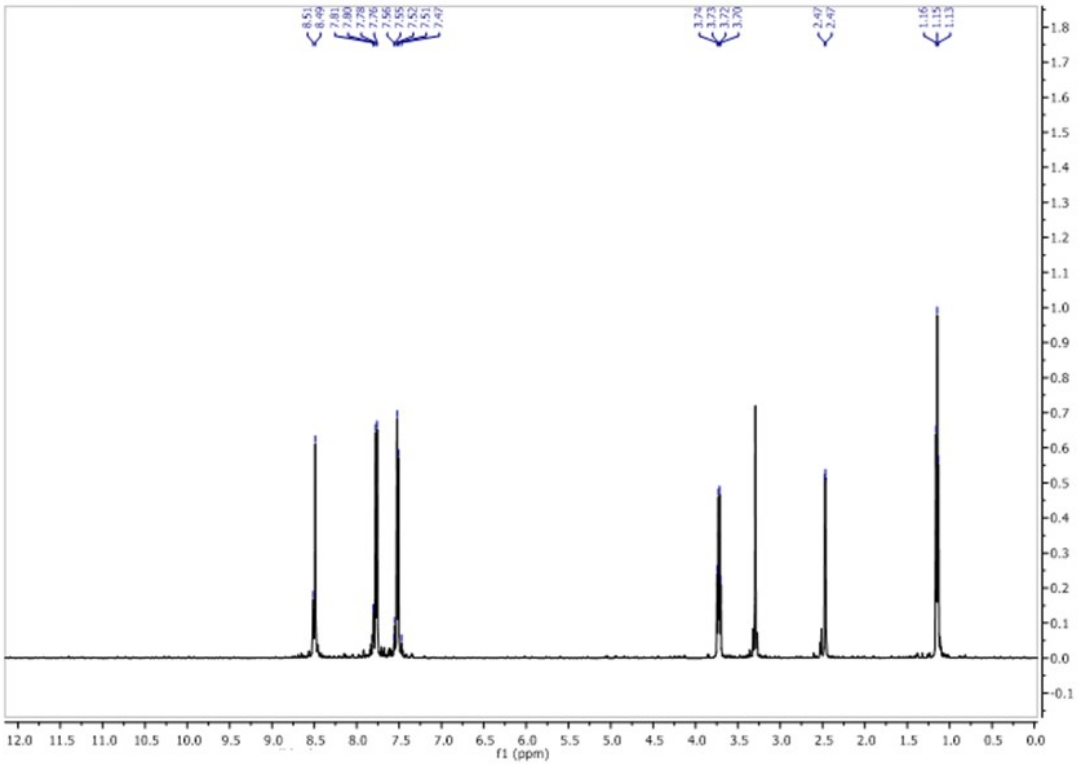
Mass Spectrum of Compound 9b



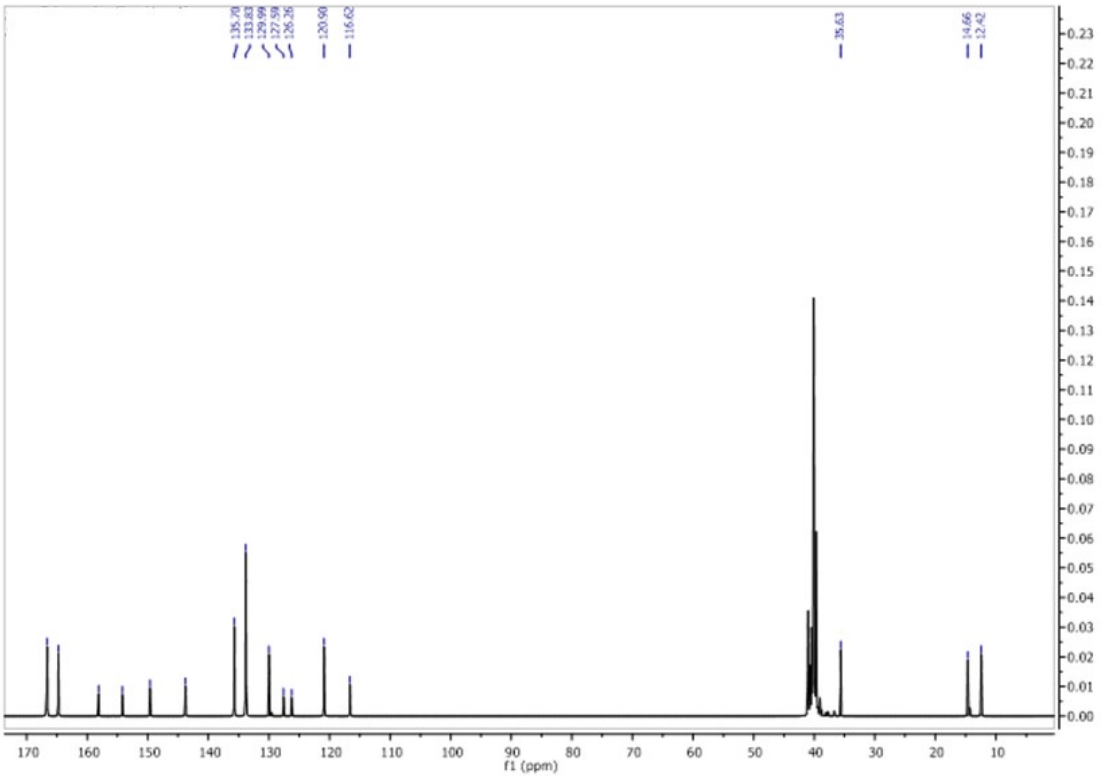
^{1}H NMR Spectrum of Compound 10a



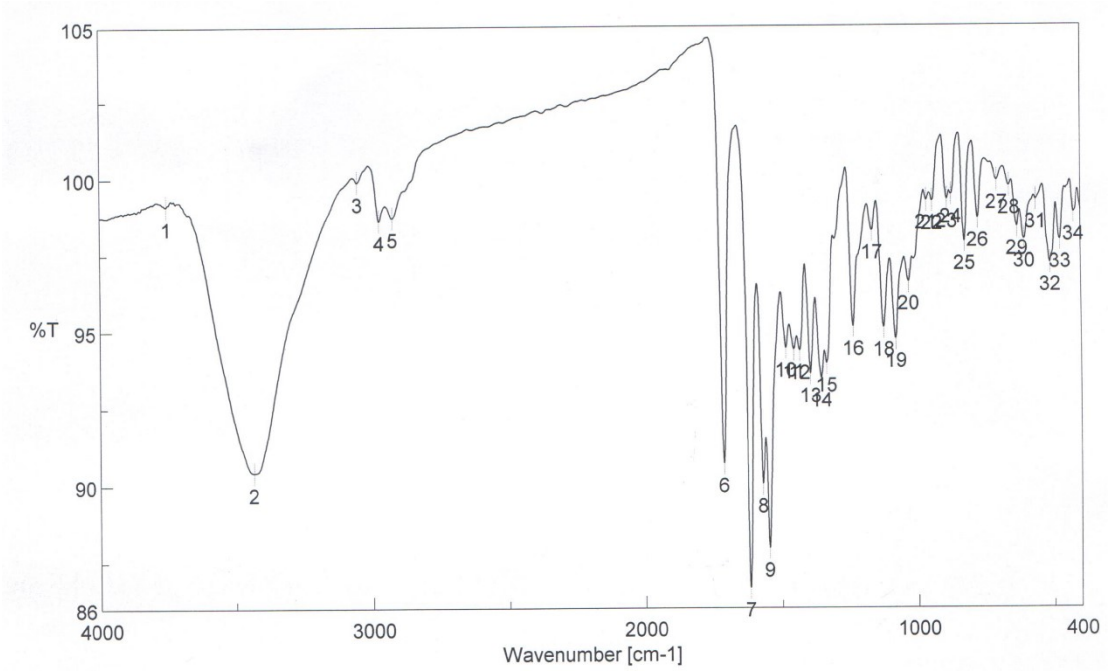
^{13}C NMR Spectrum of Compound 10a



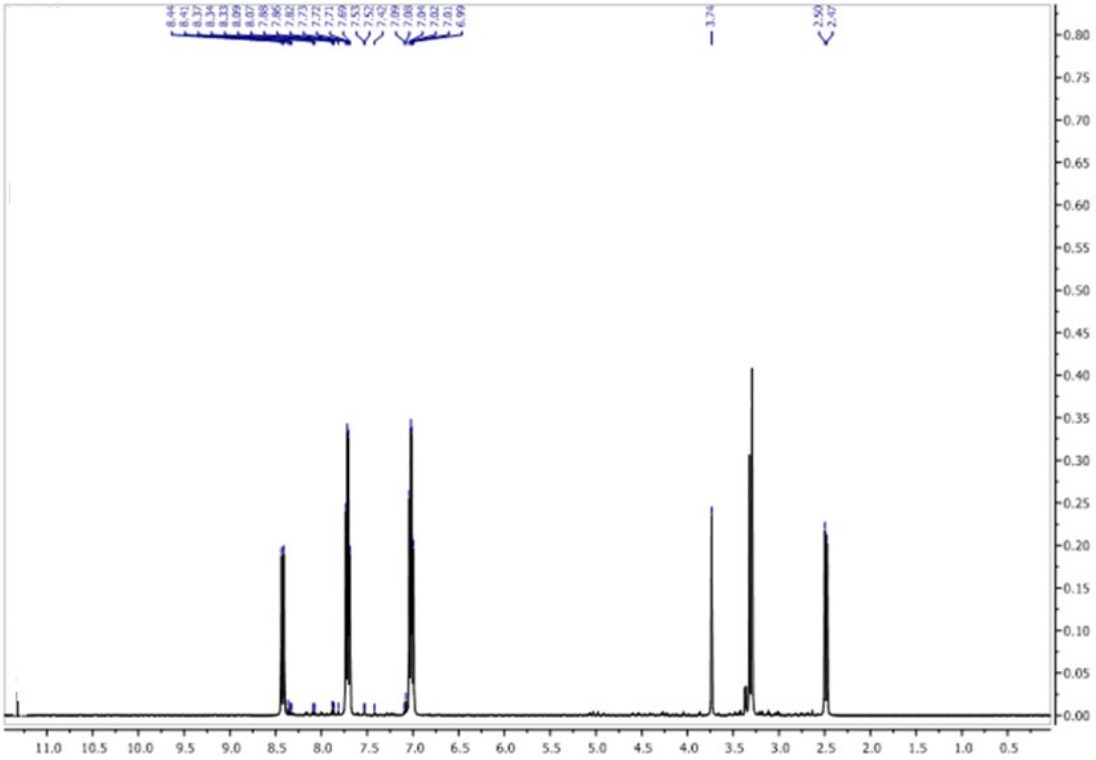
^{1}H NMR Spectrum of Compound 10b



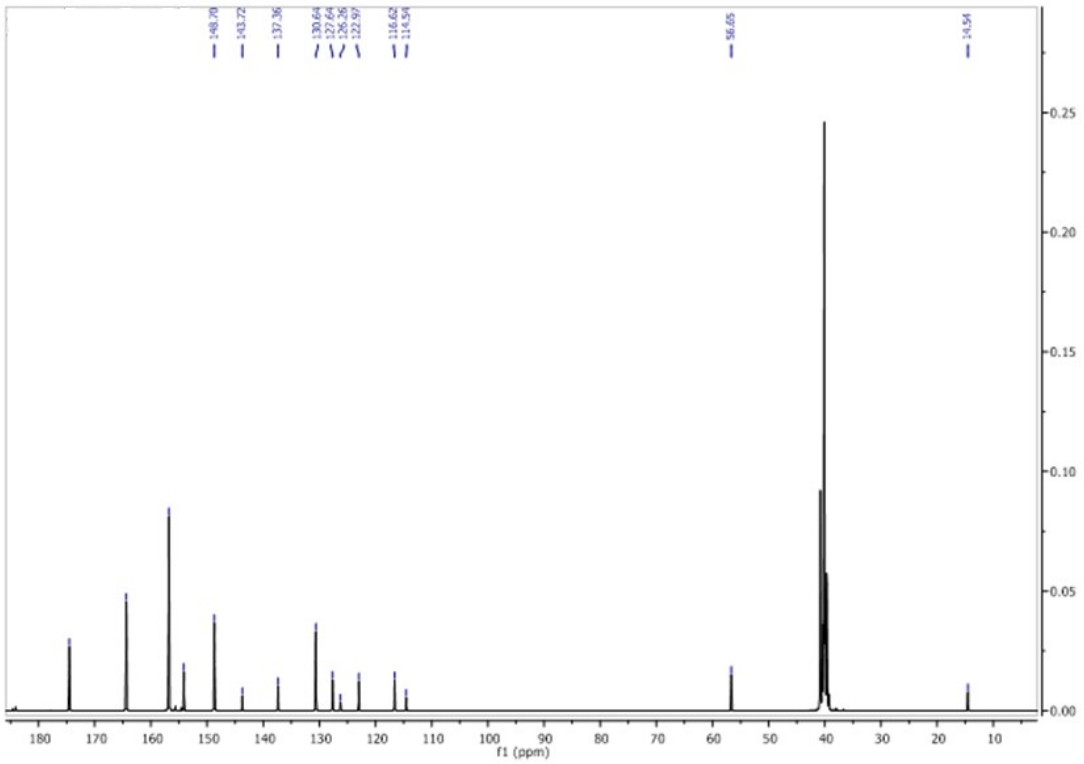
^{13}C NMR Spectrum of Compound 10b



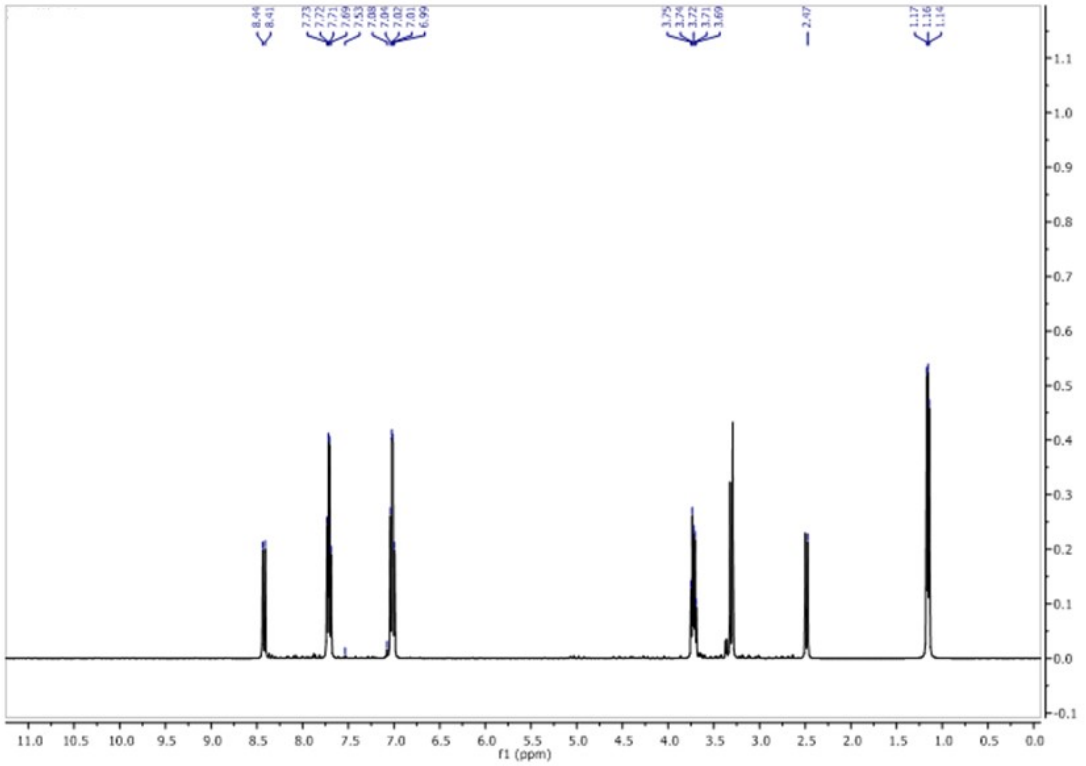
IR Spectrum of Compound 10b



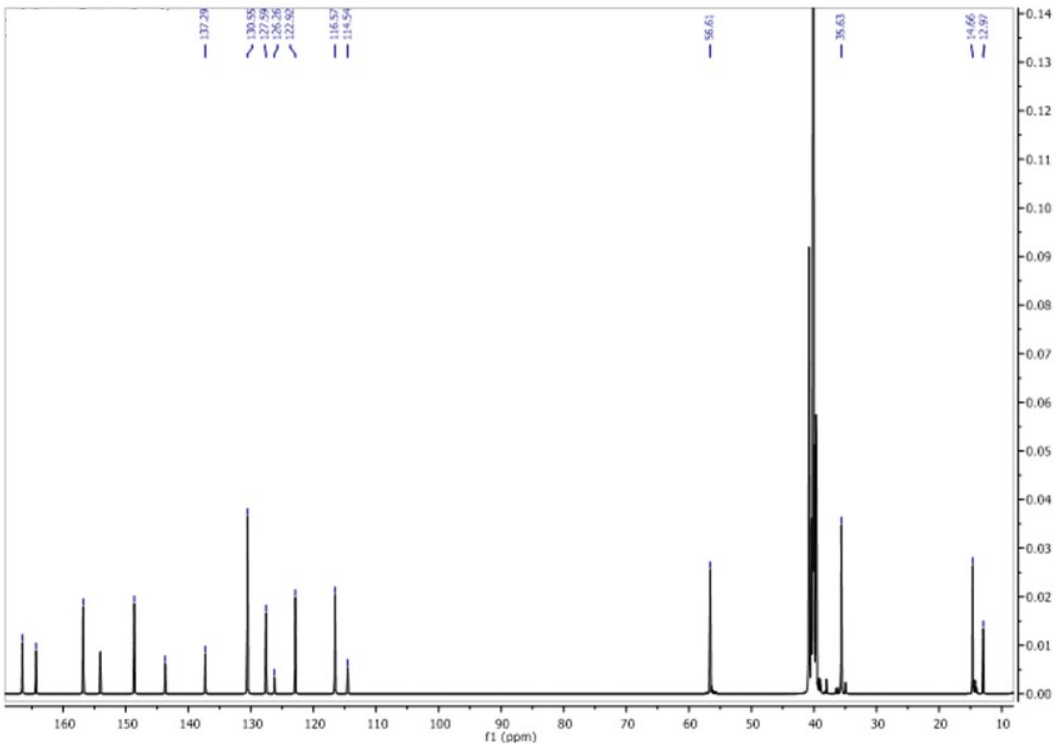
^{1}H NMR Spectrum of Compound 11a



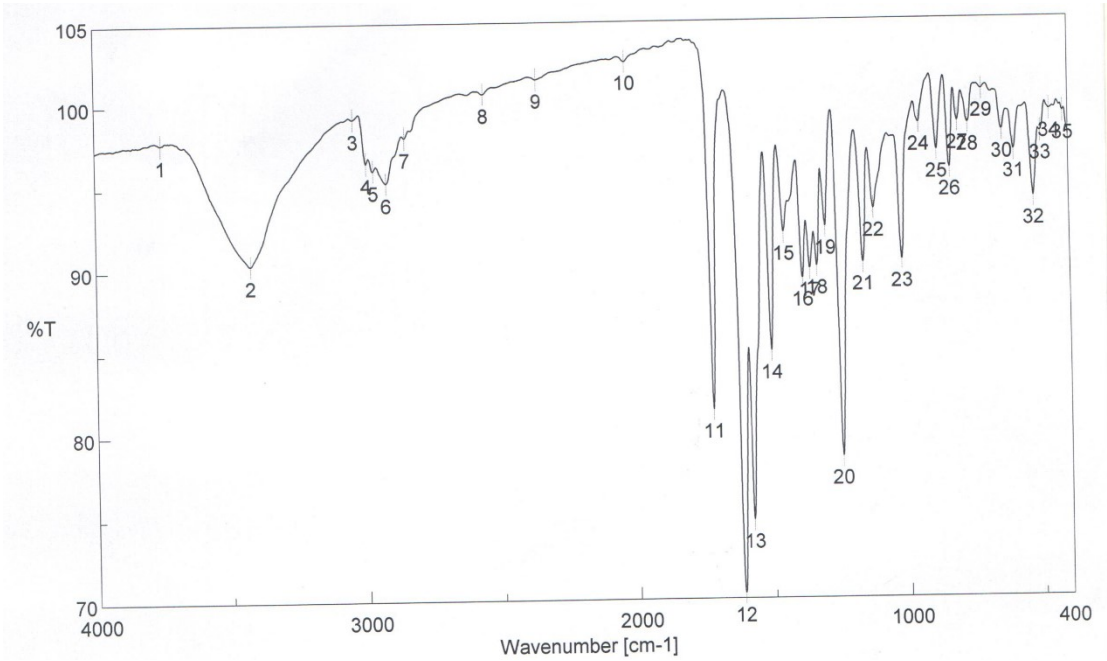
^{13}C NMR Spectrum of Compound 11a



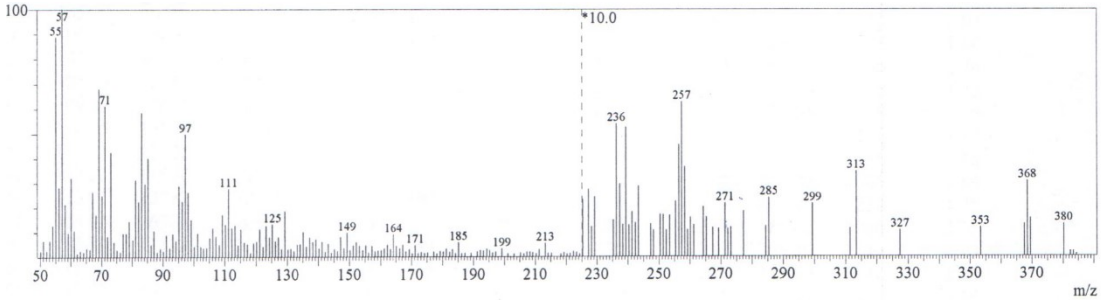
^{1}H NMR Spectrum of Compound 11b



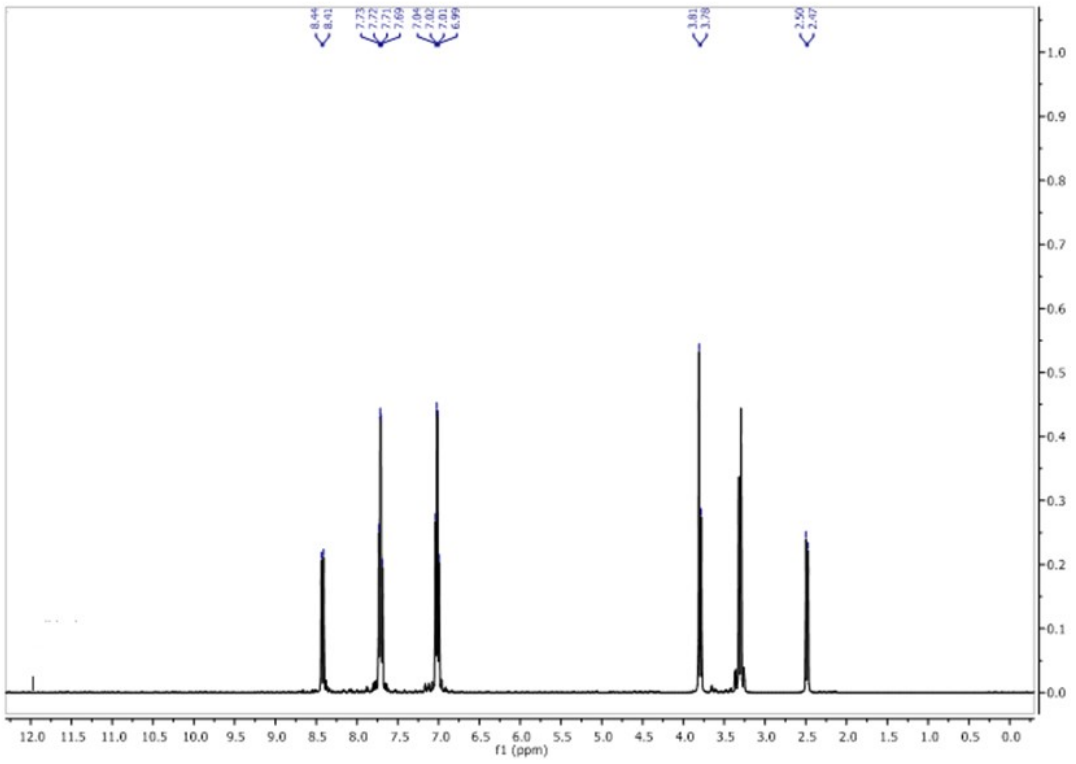
^{13}C NMR Spectrum of Compound 11b



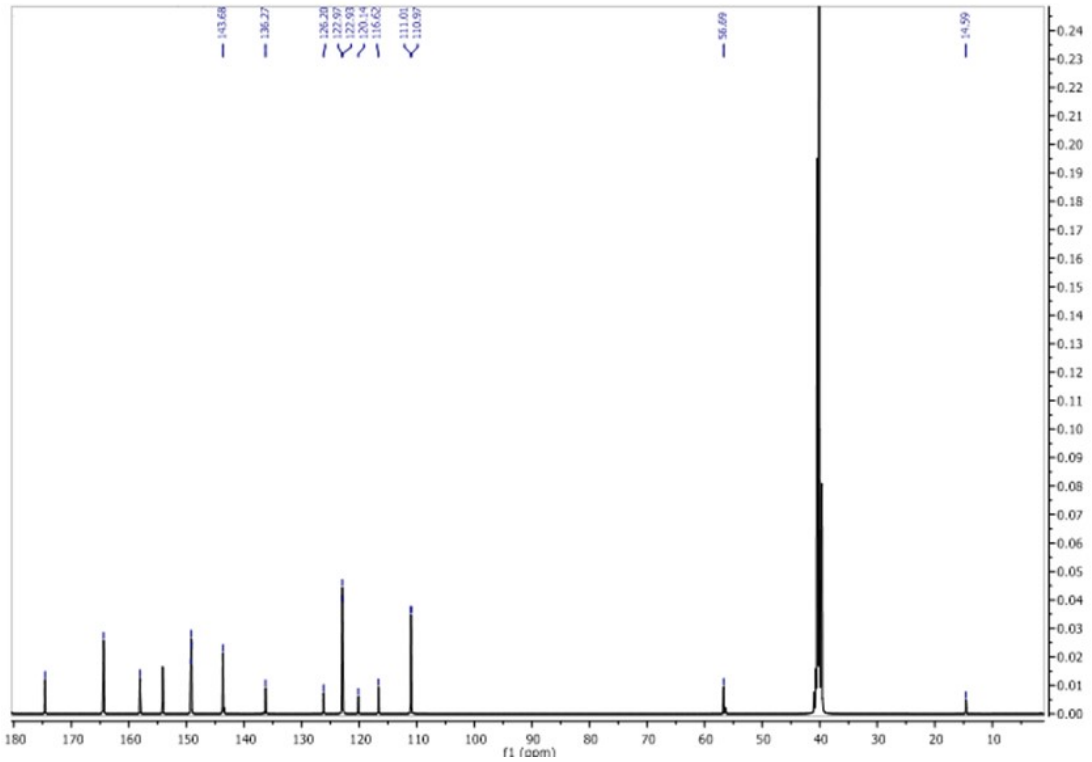
IR Spectrum of Compound 11b



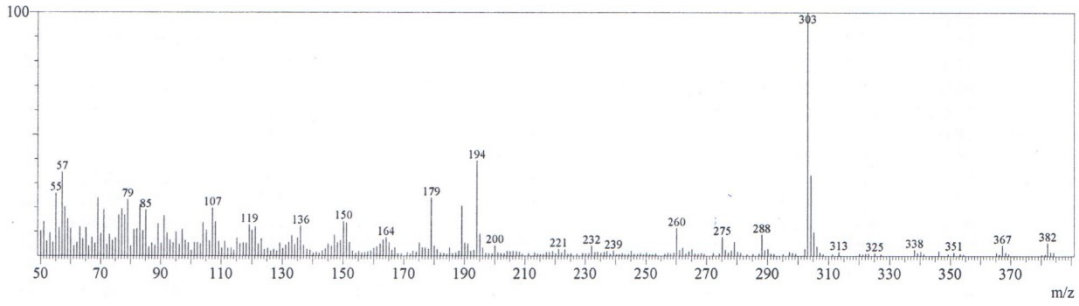
Mass Spectrum of Compound 11b



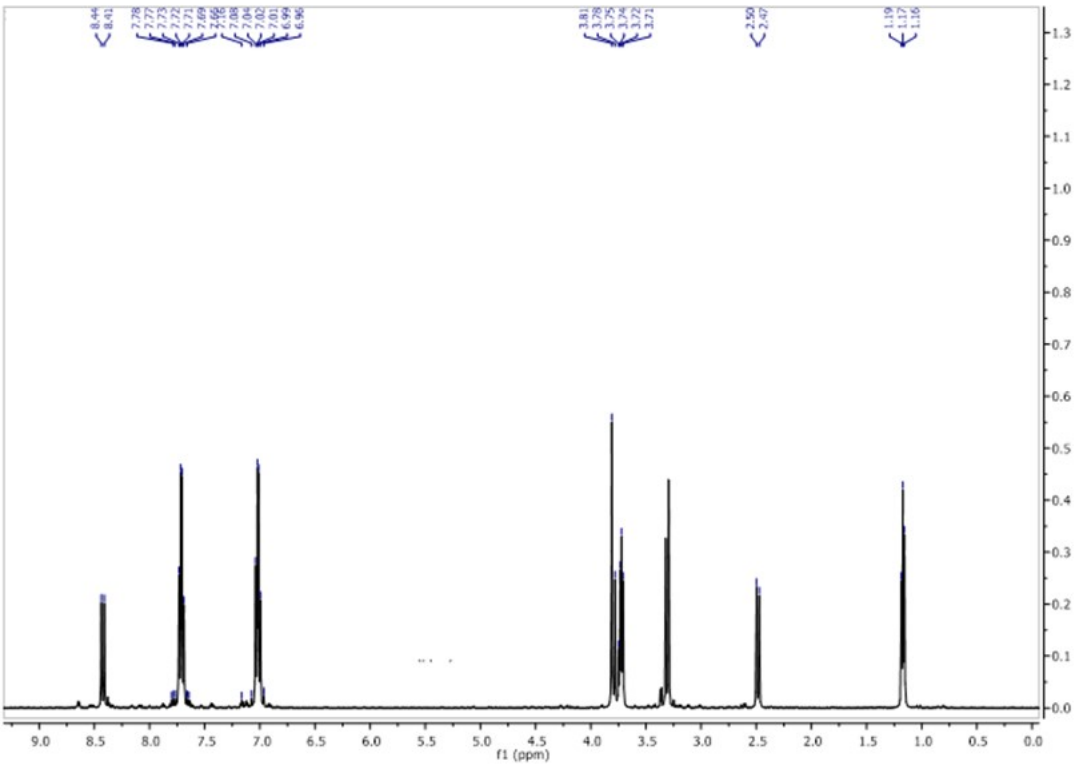
^{1}H NMR Spectrum of Compound 12a



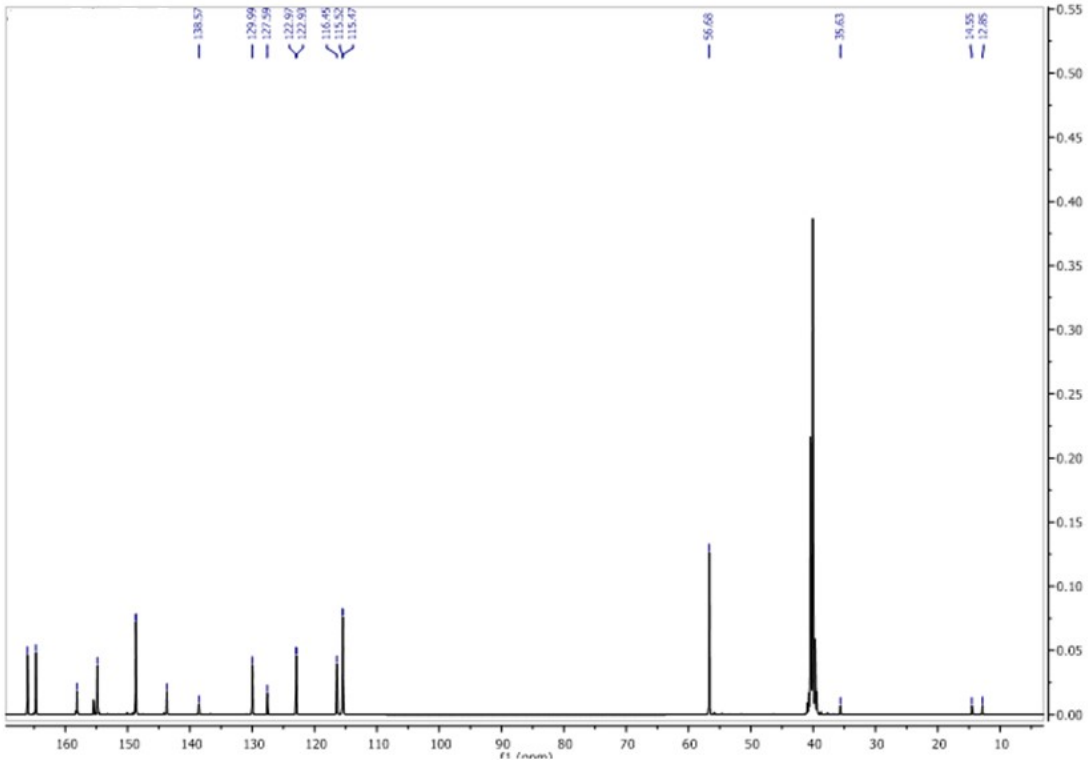
^{13}C NMR Spectrum of Compound 12a



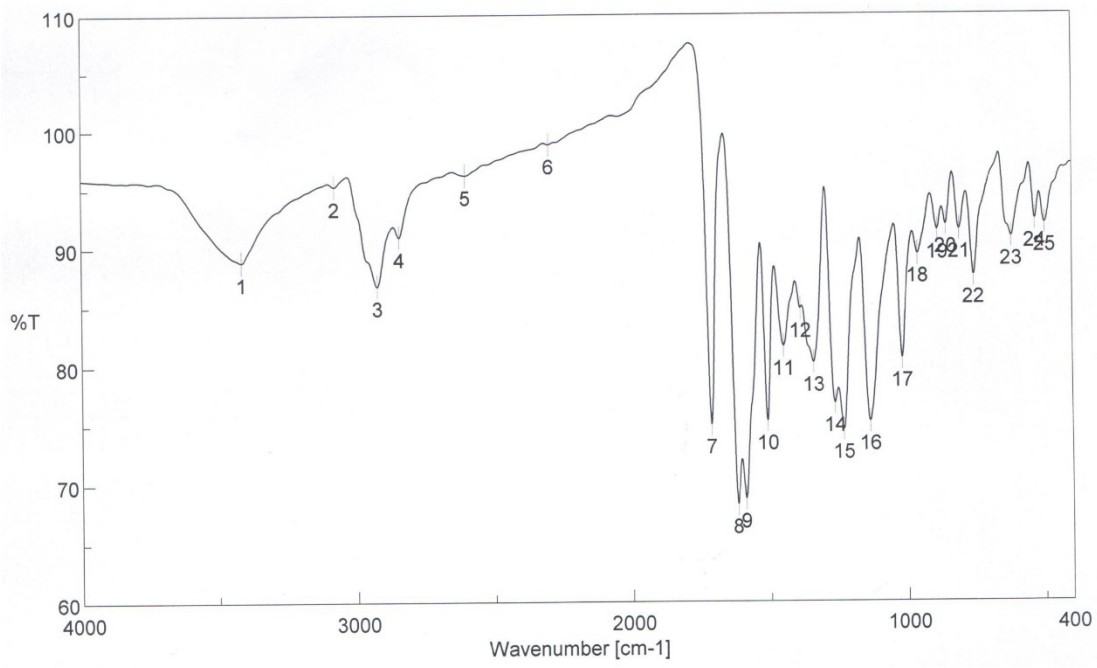
Mass Spectrum of Compound 12a



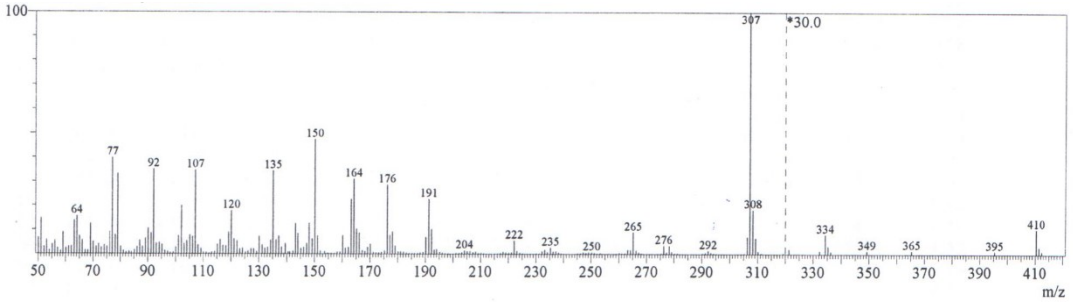
^{1}H NMR Spectrum of Compound 12b



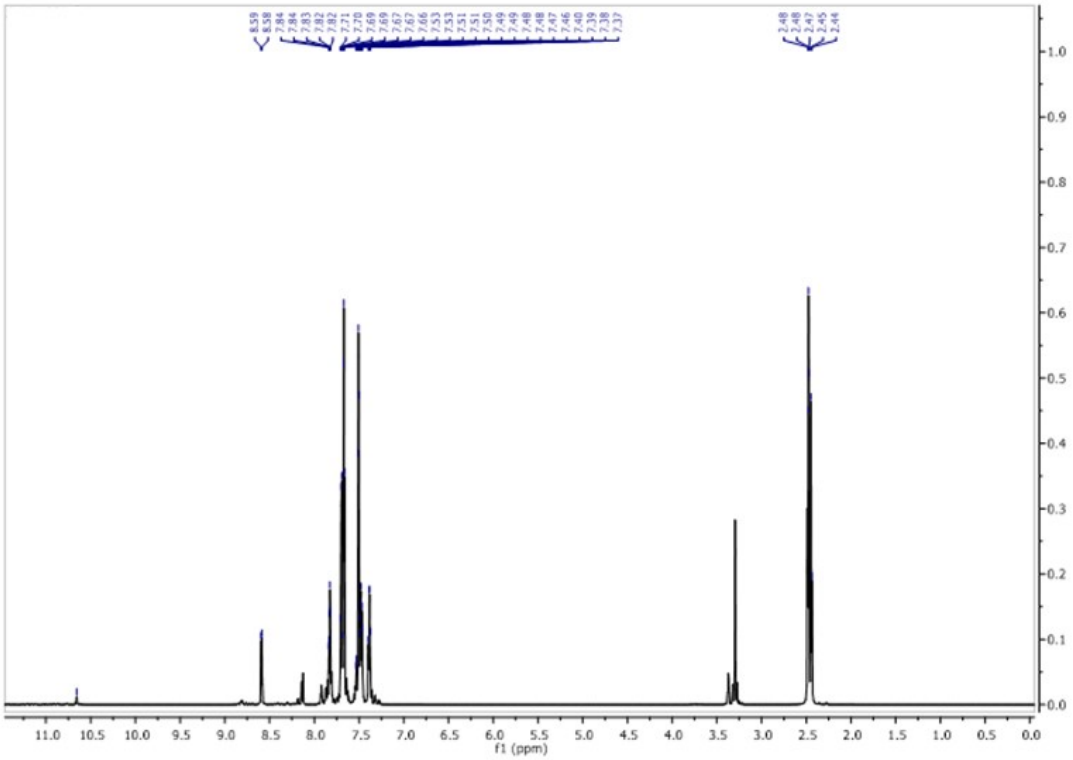
^{13}C NMR Spectrum of Compound 12b



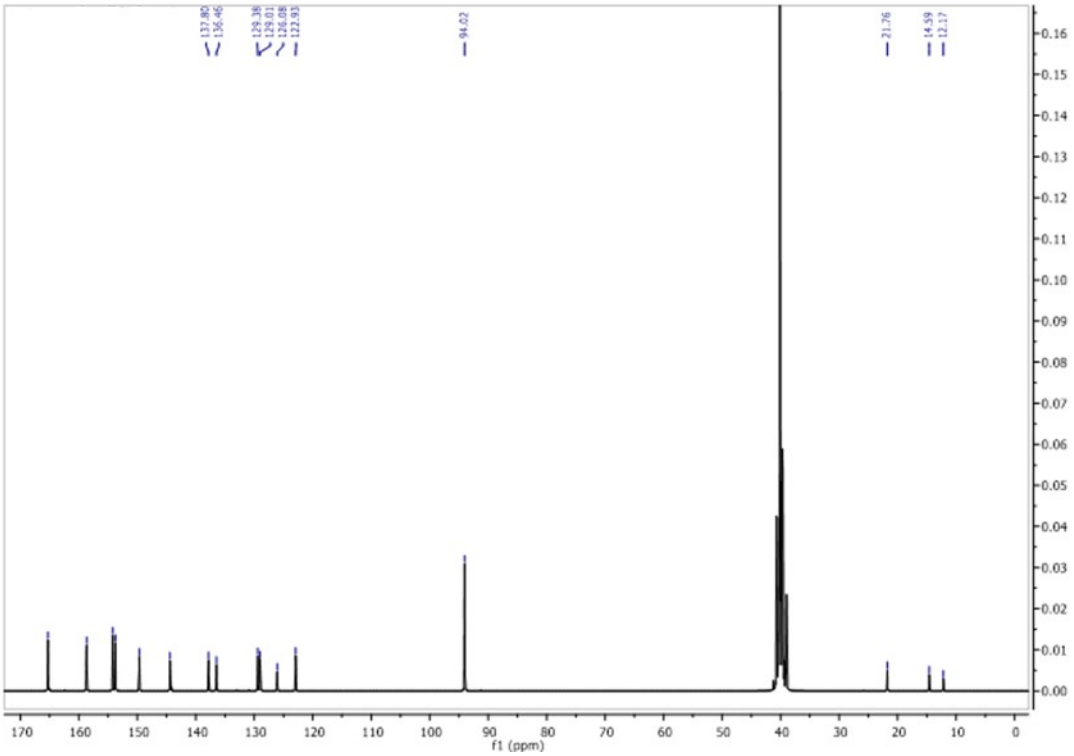
IR Spectrum of Compound 12b



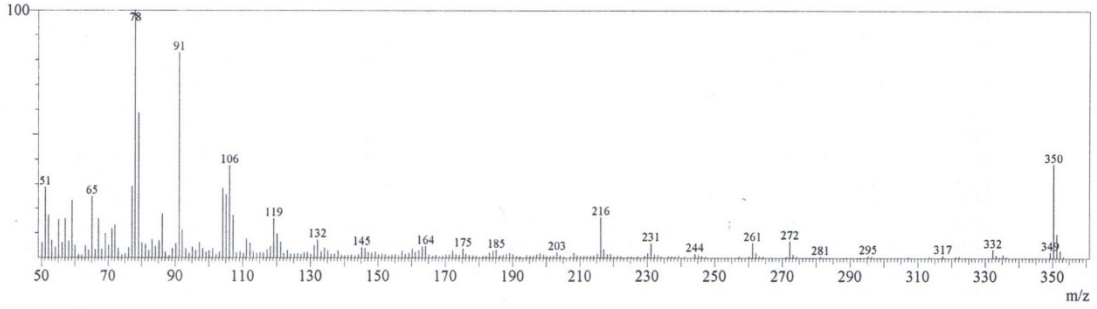
Mass Spectrum of Compound 12b



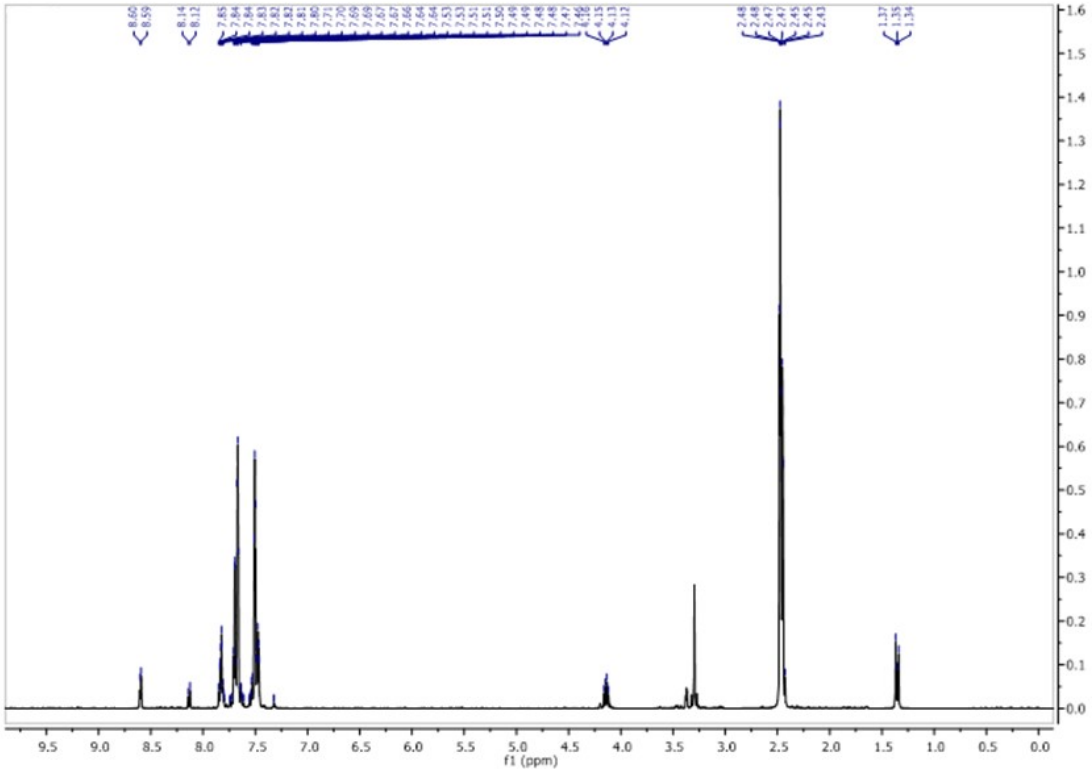
^{1}H NMR Spectrum of Compound 13a



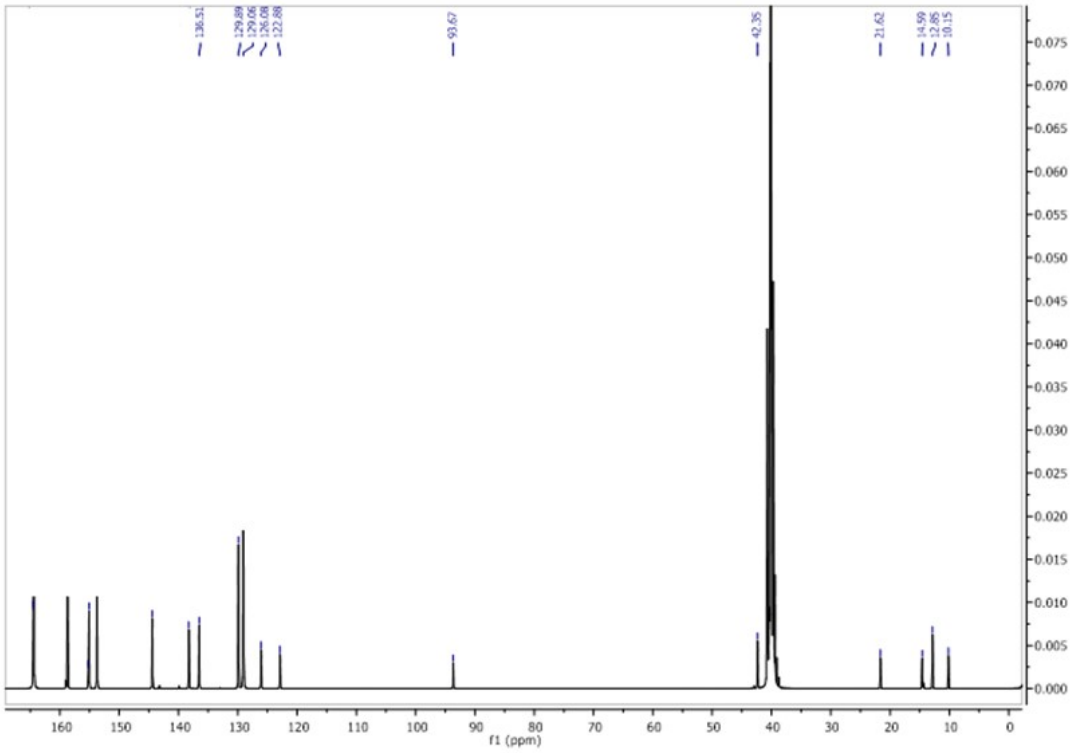
^{13}C NMR Spectrum of Compound 13a



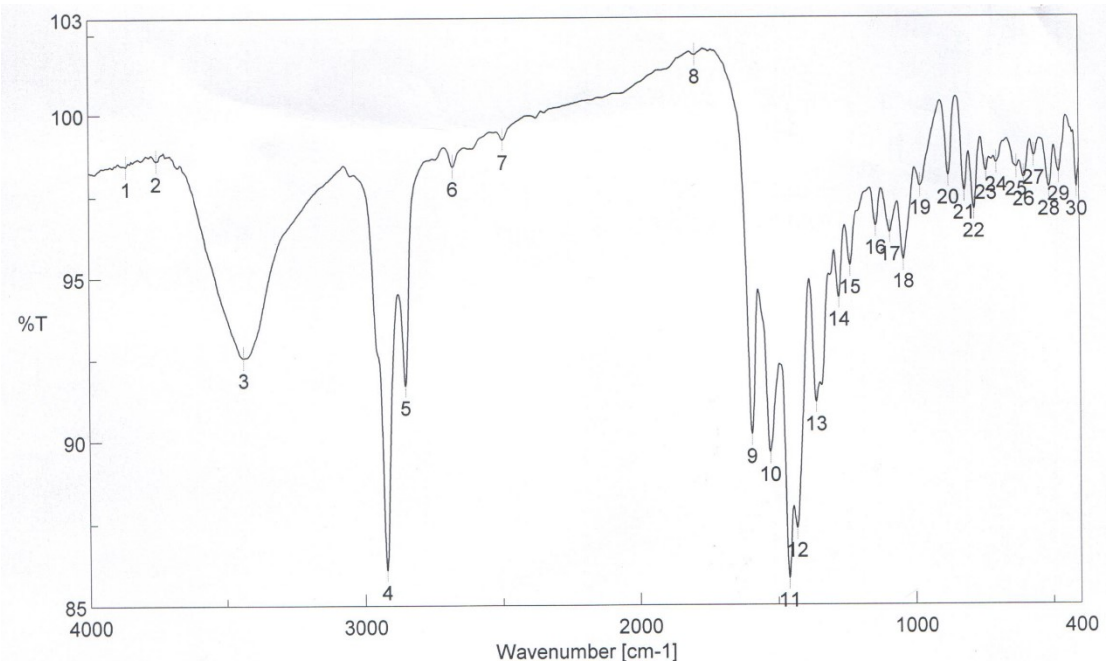
Mass Spectrum of Compound 13a



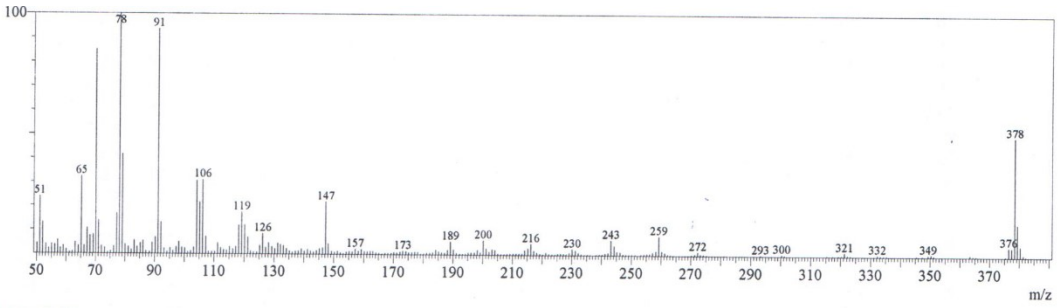
^{1}H NMR Spectrum of Compound 13b



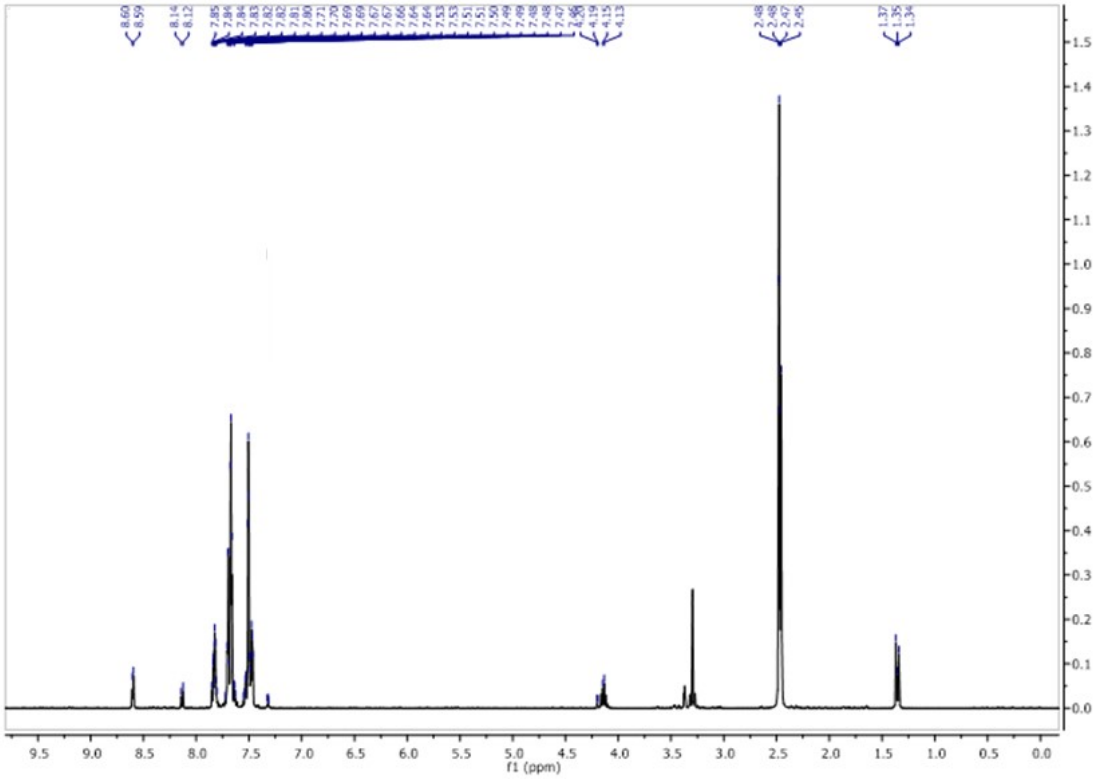
^{13}C NMR Spectrum of Compound 13b



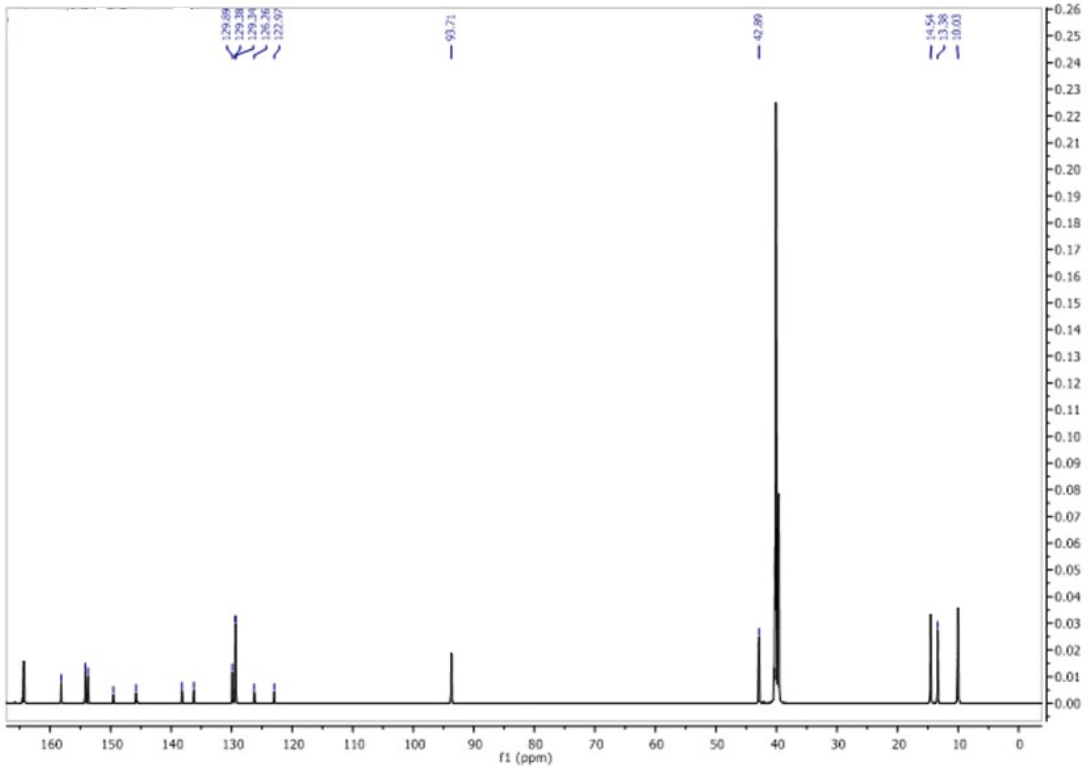
IR Spectrum of Compound 13b



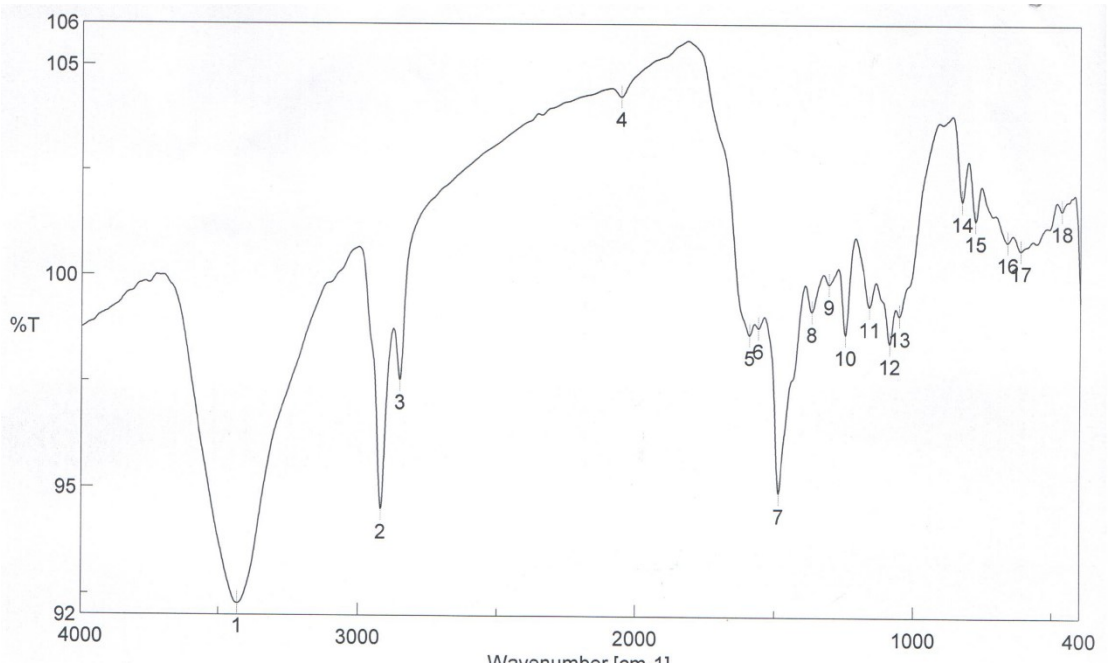
Mass Spectrum of Compound 13b



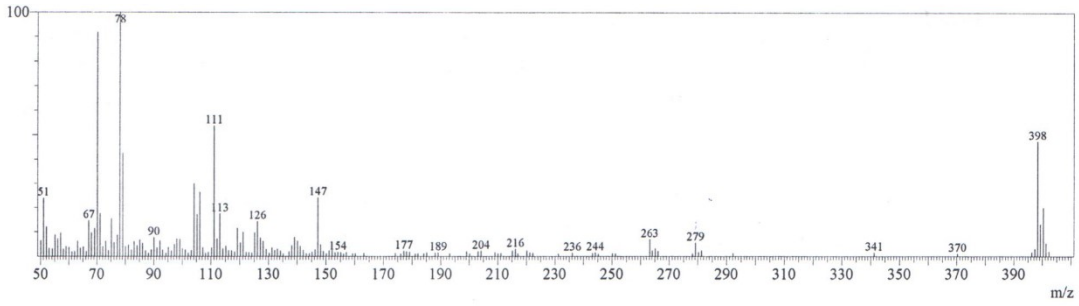
^{1}H NMR Spectrum of Compound 14b



^{13}C NMR Spectrum of Compound 14b



IR Spectrum of Compound 14b



Mass Spectrum of Compound 14b

S.2.Biological evaluation:

***S.2.1. In vitro* anti-proliferative assay:**

A panel of human cancer cell lines was tested for their chemosensitivity to 26 newly synthesized derivatives: normal African Green monkey kidney cell line (VERO), human Hepatocellular carcinoma cell line (HEPG-2), human breast carcinoma cell line (MCF-7), human laryngeal carcinoma cell line (HEP-2), were obtained frozen in liquid nitrogen (-180^{o}C) from American Type Culture Collection (ATCC; Washington, DC, USA) and were maintained at National Cancer Institute as monolayer cultures in RPMI-1640 supplemented with 10% FBS and 1% penicillin- streptomycin.

The anti-proliferative activity was determined using sulforhodamine-B (SRB) method.[1] Cells were seeded in 96-well microtiter plates at a concentration of 3×10^3 cells/well. They were left to attach for 24 h before incubation with drugs. The cells were treated for 48 h with single dose (100 ug/ml) of all the compounds and for IC_{50} the cells were treated by different concentrations (0, 5, 12.5, 25 and 50 ug/ml) of compounds 1a,8a on HEP-2 cell line and 13a on HEPG-2 cell line. The optical density (O.D) of each well was measured spectrophotometrically at 570 nm using ELISA microplate reader (TECAN Sunrise TM, Germany).The mean values were estimated as percentage of cell viability as follows: O.D (treated cells) / O.D (control cells) × 100. The IC50 value (the concentration that produces 50% inhibition of cell growth) of each drug was calculated using dose response curve-fitting models (Graph-Pad Prism software, version 5).

S.2.2. The determination of the oxidative stress of the promising compounds 8a and 13a

S.2.2.1 For the preparation of cell-free media and cell lysate

Cells of HEPG-2 and HEP-2 cell lines were cultured in T75 flasks, left for 24 h, and then treated with IC50 concentration of the compounds 13a and 8a, respectively for 48 h. The medium was collected and used for the determination of NOx level. Cell

pellets were prepared by removing the cells from the flasks by trypsi- nization and used for the determination of glutathione content. The treated and control cell pellet were collected, washed, and suspended in cold lysis buffer, then sonicated and centrifuged, and the clear supernatant was taken into another Eppendorf.

S.2.2.2Determination of protein concentration

Protein concentration was assessed in the medium and cell lysate by using the Bradford method.[2] The method based on the binding of Coomassie brilliant blue G-250 dye with protein and forming a complex which can be detected spectrophotometrically at 595nm then the concentration was determined using a standard calibration curve.

S.2.2.2. Determination of non-protein reduced thiols content (glutathione content)

Reduced glutathione (GSH) in cell lysate was determined according to the method of Ellman, [3]it is based on the reduction of Ellman's reagent [5,5′-dithio-bis- (2-nitrobenzoic acid)] by SH groups to form 1 mol of 2-nitro-5- mercaptobenzoic acid per mole of SH. The optical density was measured at 412 nm against a reagent blank and the results were expressed as μmol/mg protein.

S.2.2.3. Determination of total nitrate/nitrite (NOx)

Total nitrate/nitrite (NOx) was measured in cell culture media as a stable end product, nitrite, according to the method of Miranda. [4]The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction. The diazotiza- tion of sulfanilic acid with nitrite at acidic pH is subsequent coupling with N-(10-naphthyl) ethylenediamine to an intensely colored product that is determined spectrophotometrically at 540 nm and expressed as nmol/mg protein.

S.2.3. *In vitro* enzymatic inhibitory evaluation against CDK-2 and GSK3β

The CDK2 assay kit purchased from Bioscience company was designed to measure CDK2/CyclinA2 activity using Kinase-Glo MAX as a detection reagent. The CDK2 assay Kit was performed by using white 96-well plate, with enough purified recombinant CDK2/CyclinA2 enzyme, CDK substrate peptide, ATP and kinase assay buffer. The Kinase-Glo MAX assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light that was detected spectrophotometrically.[5]

The GSK3β Assay Kit obtained from Bioscience company was performed in white 96-well plate, with enough purified recombinant GSK3β enzyme, GSK3β substrate (GSK substrate peptide), ATP, and kinase assay buffer. Kinase-Glo reagent (Promega) was used as a detection reagent for the enzyme activity.The inhibition or the remaining activity of the enzyme was detected by measuring luminescence using the micro-plate reader representing the ATP amount liberated during the reaction.[6]

S.2.4.Cell cycle analysis and apoptosis of compound 13a

Cell cycle phases is determined by using a fluorescent dye to stain the DNA followed by measuring its intensity. Staining of DNA displays a clear differentiation of the cells in different stages as G0/G1 phase, S, G2 and M phases in addition to the evaluation of aneuploid sets of cells.

Cell cycle analysis was performed by (Beckman Coulter, Brea, CA, USA). [7]Apoptosis detection was carried out using a FITC Annexin-V/PI commercial kit (Becton Dickenson, Franklin Lakes, NJ, USA) according to the manufacturer's kit.

S.2.5.The effect of compounds 13a on the levels of Bax, Bcl-2 and Caspase-3 in HepG-2 cells

Apoptotic markers were estimated by real time polymerase chain reaction assay (RT-PCR) according to the method reported for Bax[8], Bcl-2[9] and Caspase-3.[10]

Principle:

cDNA of each gene separately was mixed with fixed amount of SYBER green master mix in addition to a known volume of the primer of the selected gene (as shown in table 1). The mixture was subjected to several thermal conditions in a constant time.

The change determined in each gene was expressed as fold change compared to glyceraldhyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene.

Table (1): The sequences of the primers of the selected genes used as determined in the gene bank.

No.	Primer name	Primer sequence
1	Bax	F 5'- TCAGGATGCGTCCACCAAGAAG 3', R 5'- TGTGTCCACGGCGGCAATCATC -3'.
2	Bcl2	F 5'- ATCGCCCTGTGGATGACTGAGT -3' R 5'- GCCAGGAGAAATCAAACAGAGGC -3'.
3	Caspase-3	F 5'- GGAAGCGAATCAATGGACTCTGG-3', R 5'- GCATCGACATCTGTACCAGACC-3'.
4	GAPDH	F 5'- GTCTCCTCTGACTTCAACAGCG-3' R 5'- ACCACCCTGTTGCTGTAGCCAA-3'

References:

1 V. Vichai and K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112–1116.

2 M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248–254.

3 G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70–77.

4 D. Baskić, I. Jovanović, P. Ristić, V. Jakovljević, D. Delibašić and N. Arsenijević, in *Nitric Oxide*, 2005, vol. 6, pp. 49–52.

5 U. Asghar, A. K. Witkiewicz, N. C. Turner and E. S. Knudsen, *Nat. Rev. Drug Discov.*, 2015, 14, 130–146.

6 J. A. Mccubrey, L. S. Steelman, F. E. Bertrand, N. M. Davis, M. Sokolosky, S. L. Abrams, G. Montalto, A. B. D'Assoro, M. Libra, F. Nicoletti, R. Maestro, J. Basecke, D. Rakus, A. Gizak, Z. Demidenko, L. Cocco, A. M. Martelli and M. Cervello, *Oncotarget*, 2014, **5**, 2881–2911.

7 M. B. Labib, J. N. Philoppes, P. F. Lamie and E. R. Ahmed, *Bioorg. Chem.*, 2018, **76**, 67–80.

8 H. Kalkavan and D. R. Green, *Cell Death Differ.*, 2018, **25**, 46–55.

9 S. Qian, Z. Wei, W. Yang, J. Huang, Y. Yang and J. Wang, *Front. Oncol.*, 2022, 12.

10 E. Eskandari and C. J. Eaves, *J. Cell Biol.*, 2022, 221.