Supplementary Material

Synthesis and biological screening of diethyl [*N*-(thiazol-2-yl)carbamoyl]methylphosphonates

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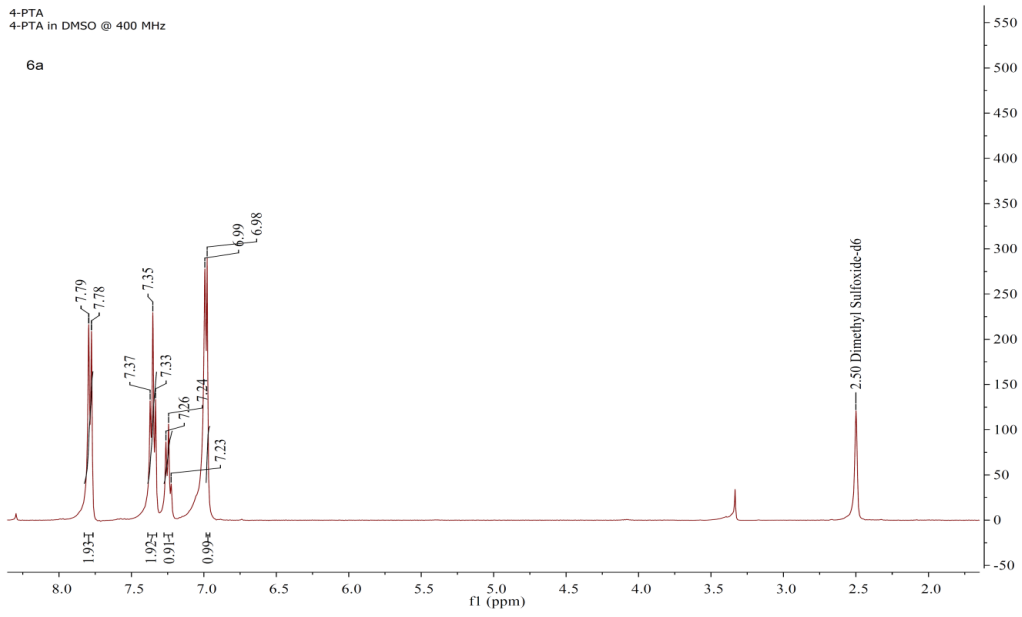

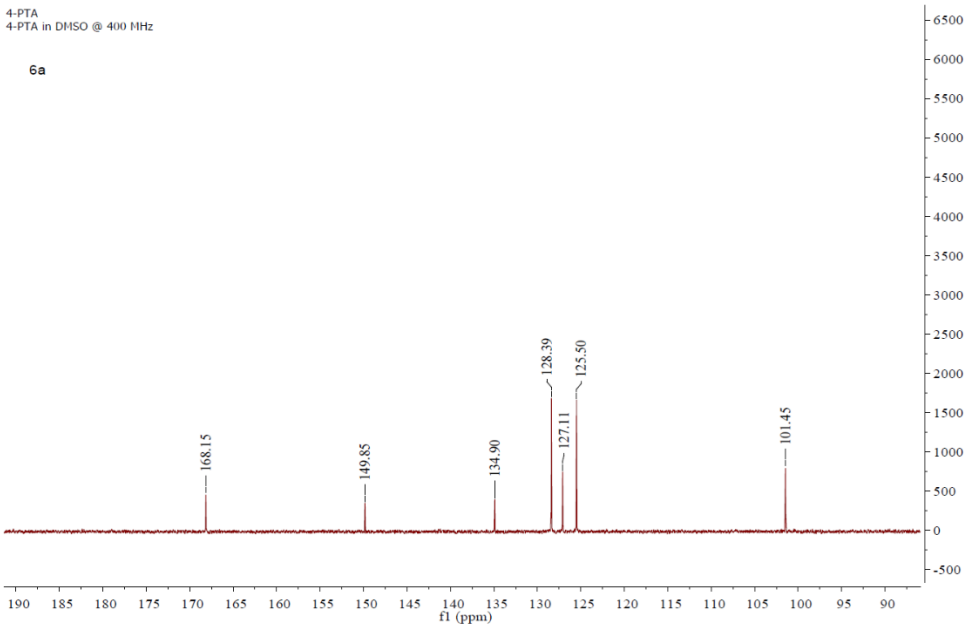

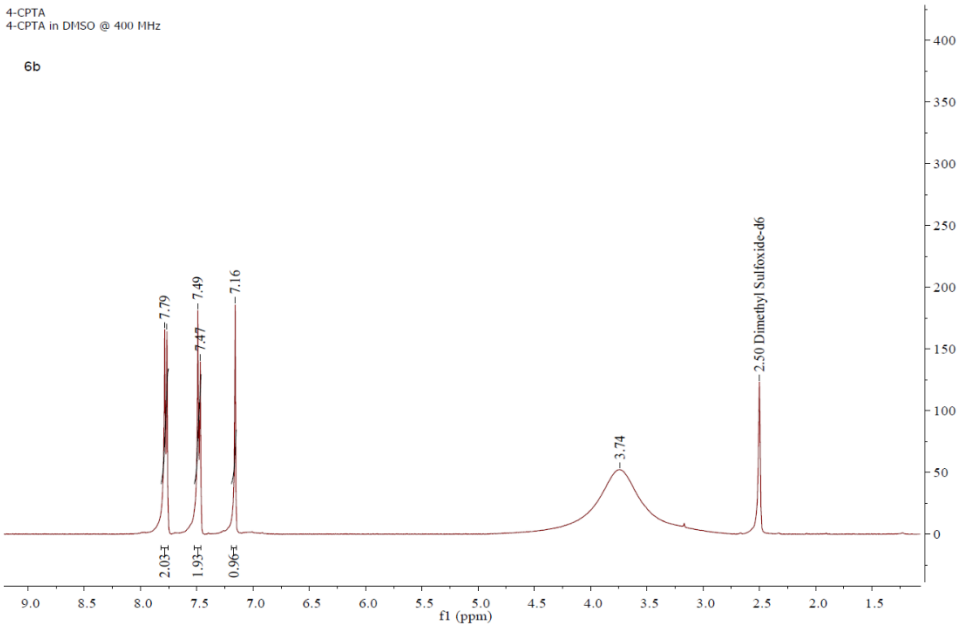

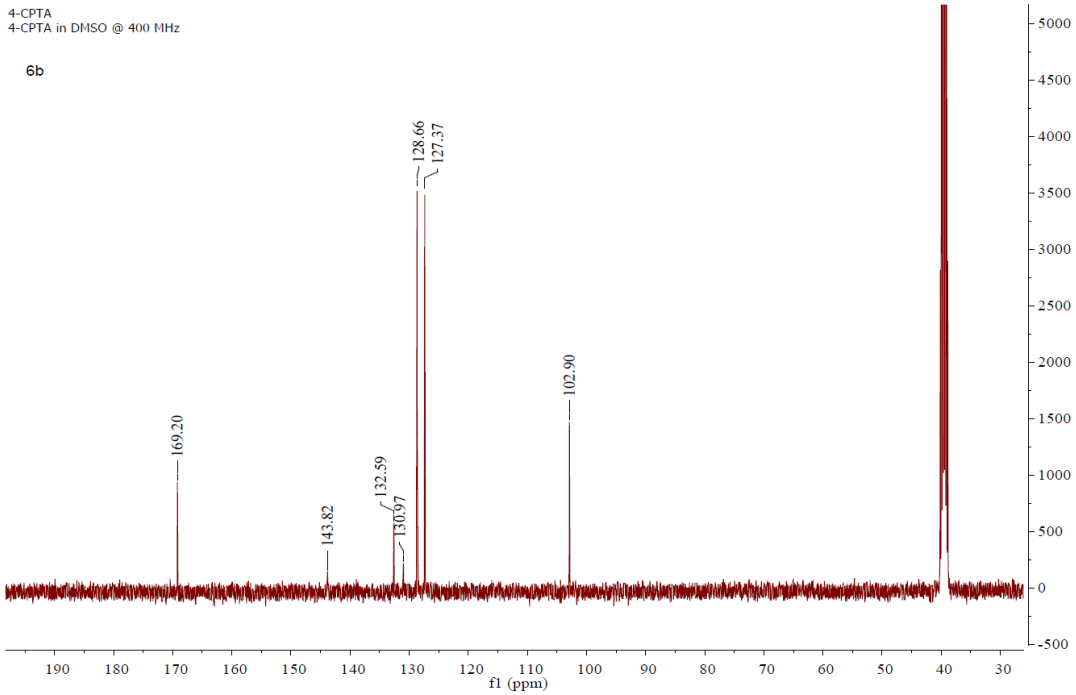

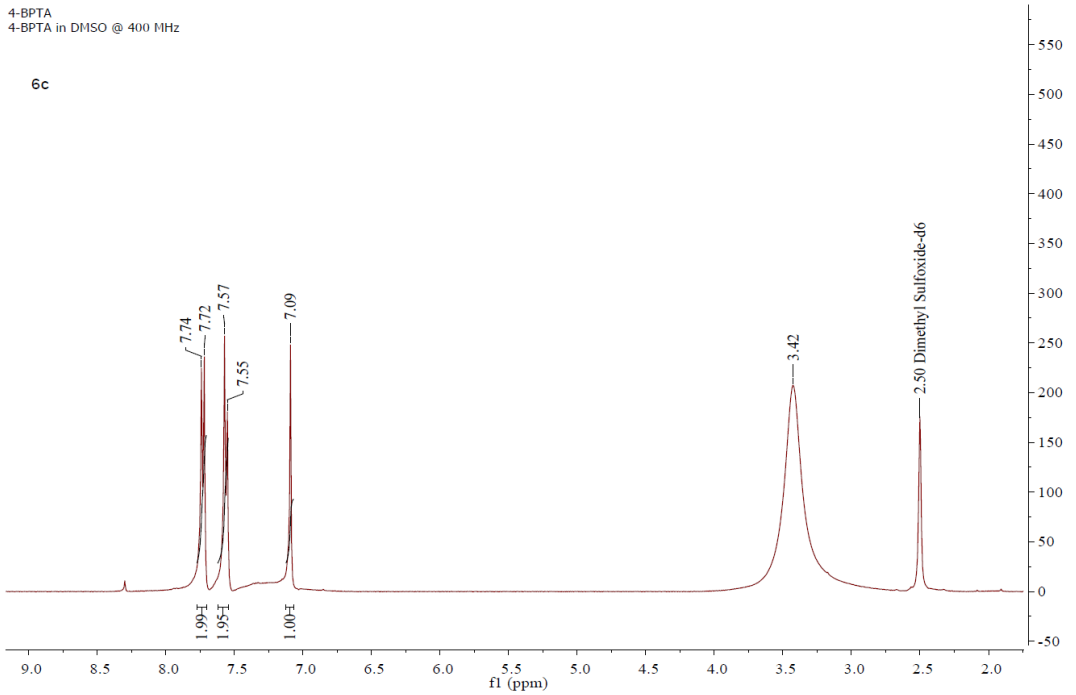

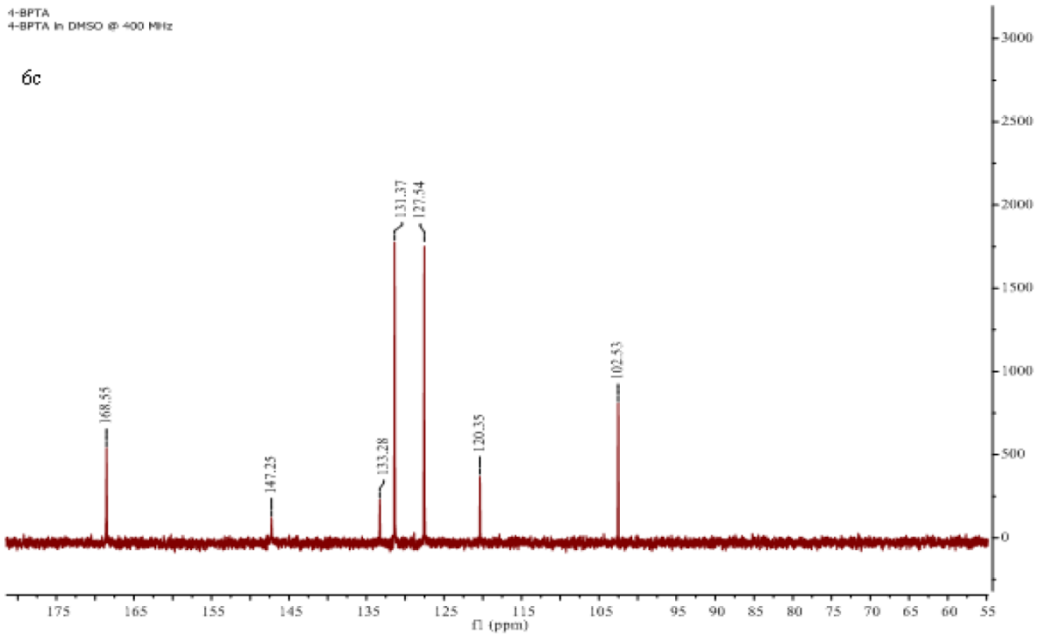

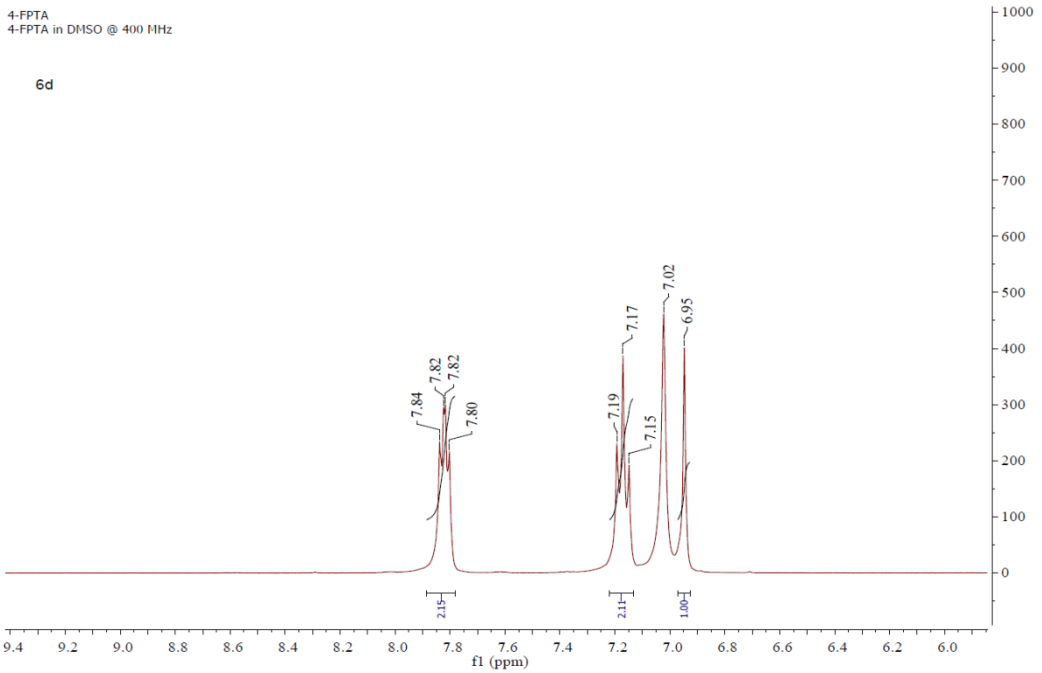

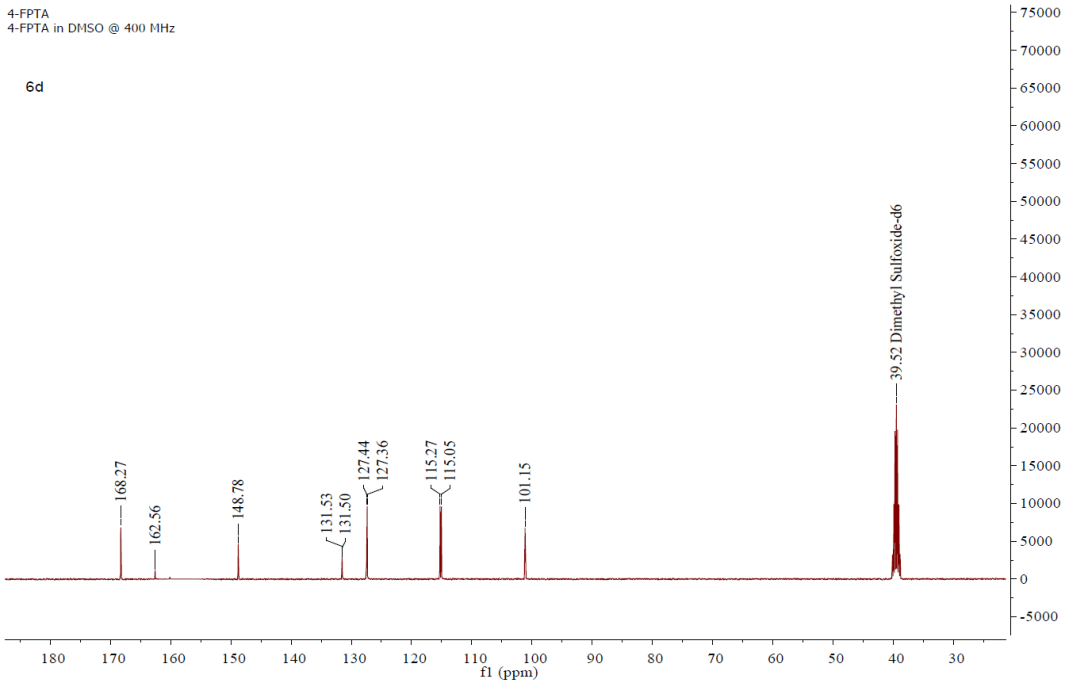

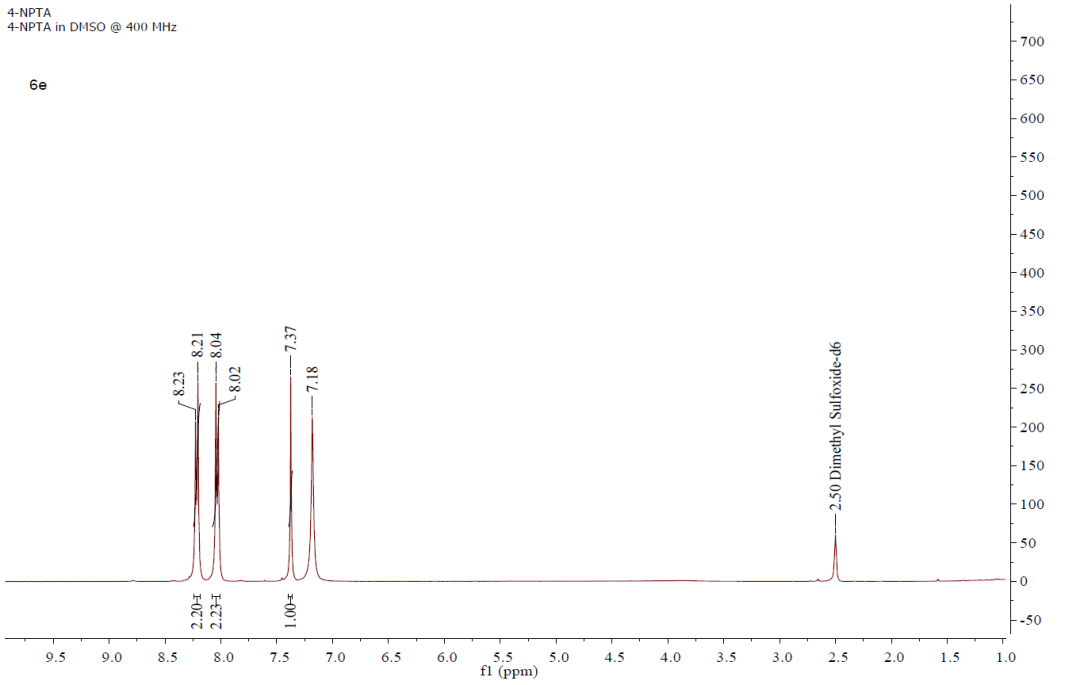

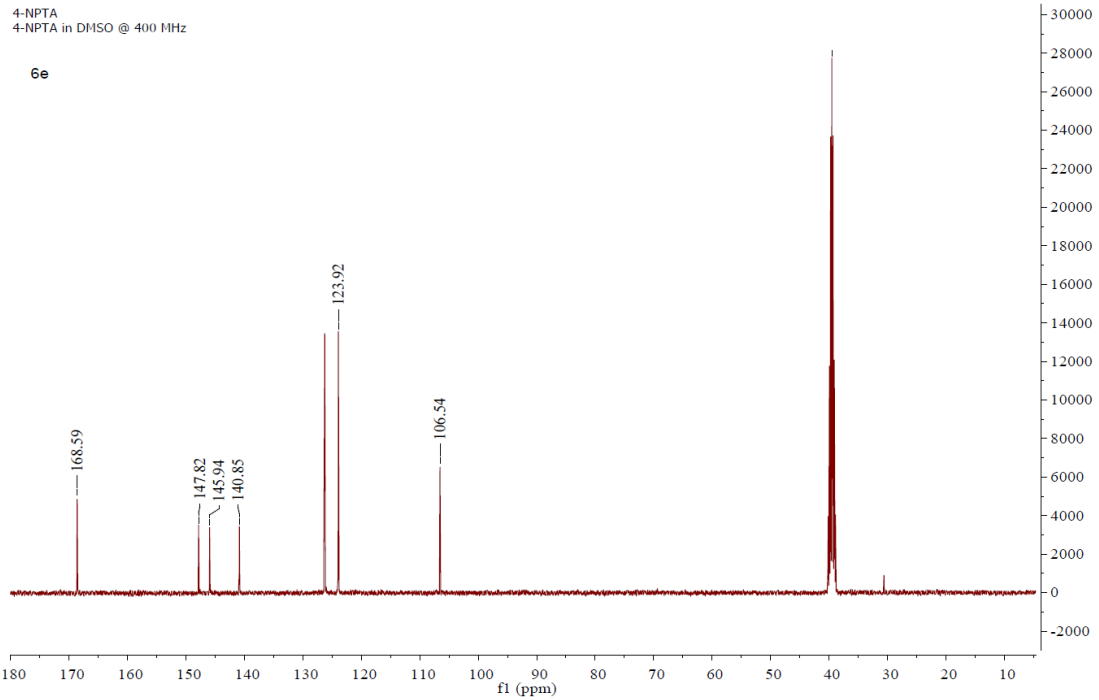

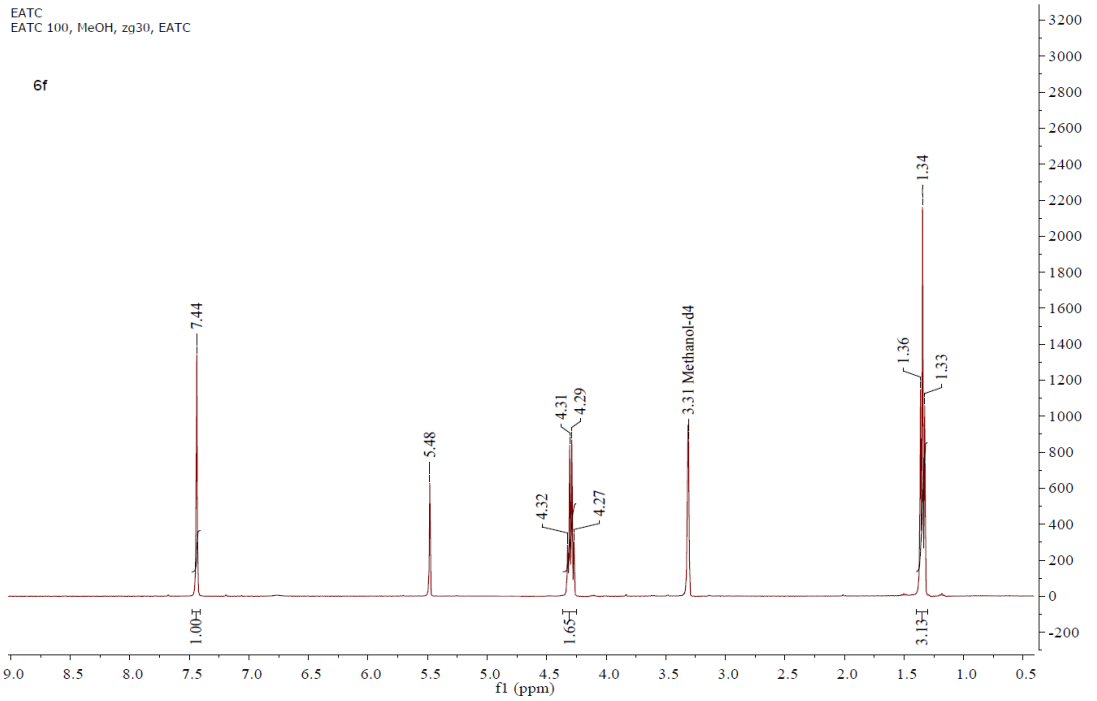

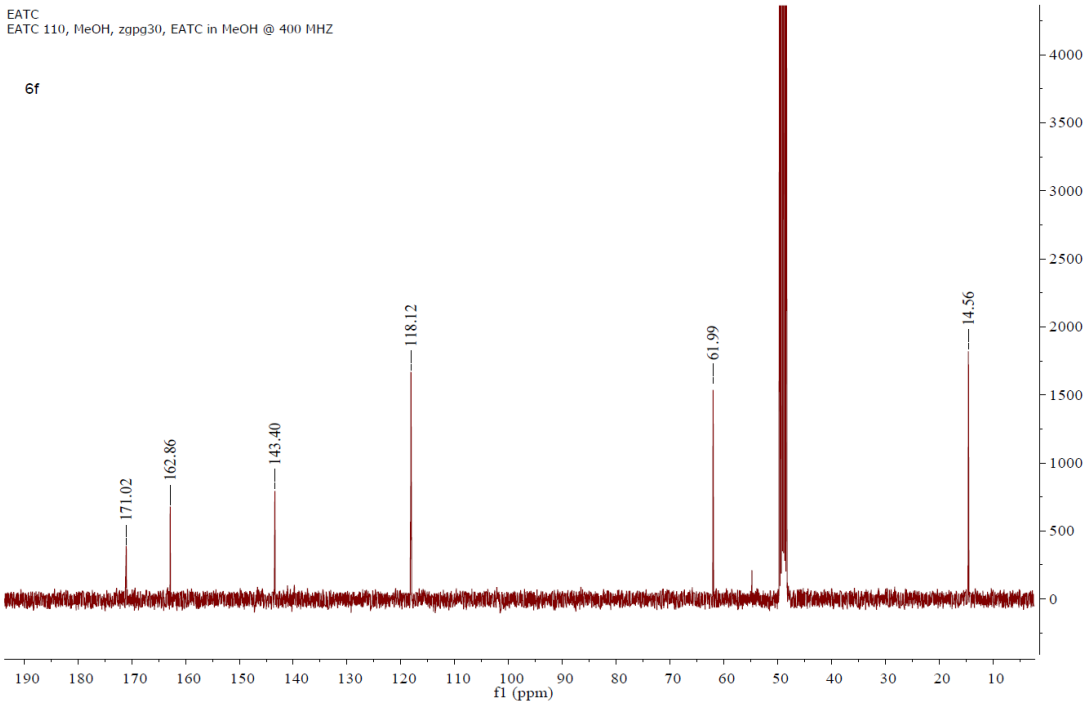

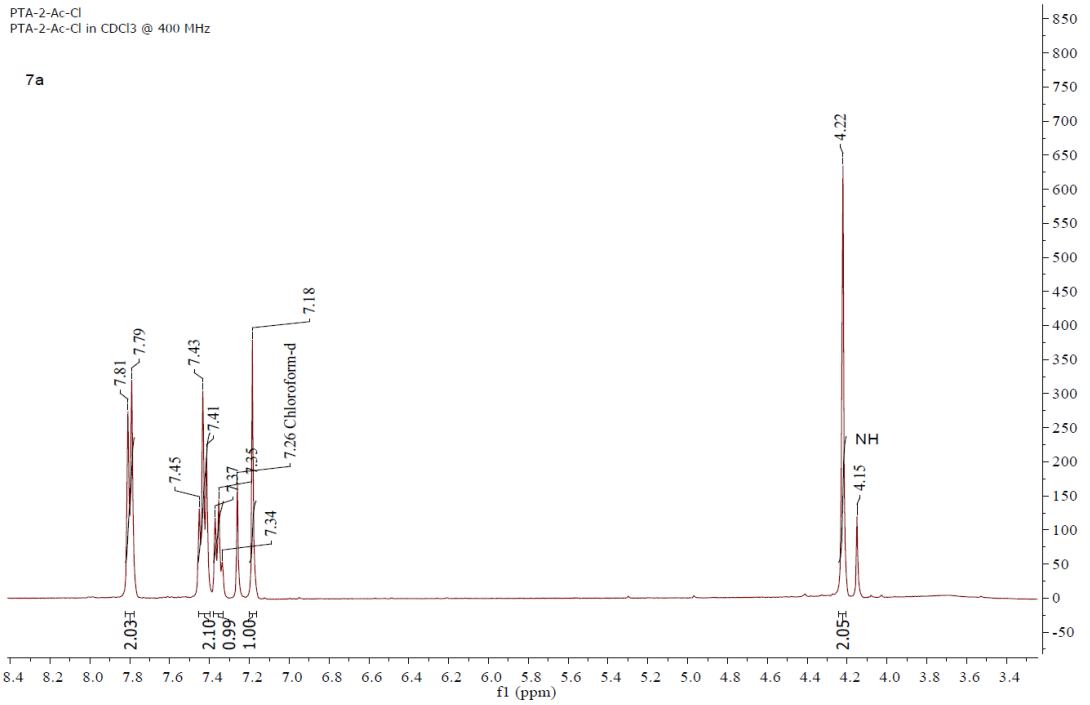

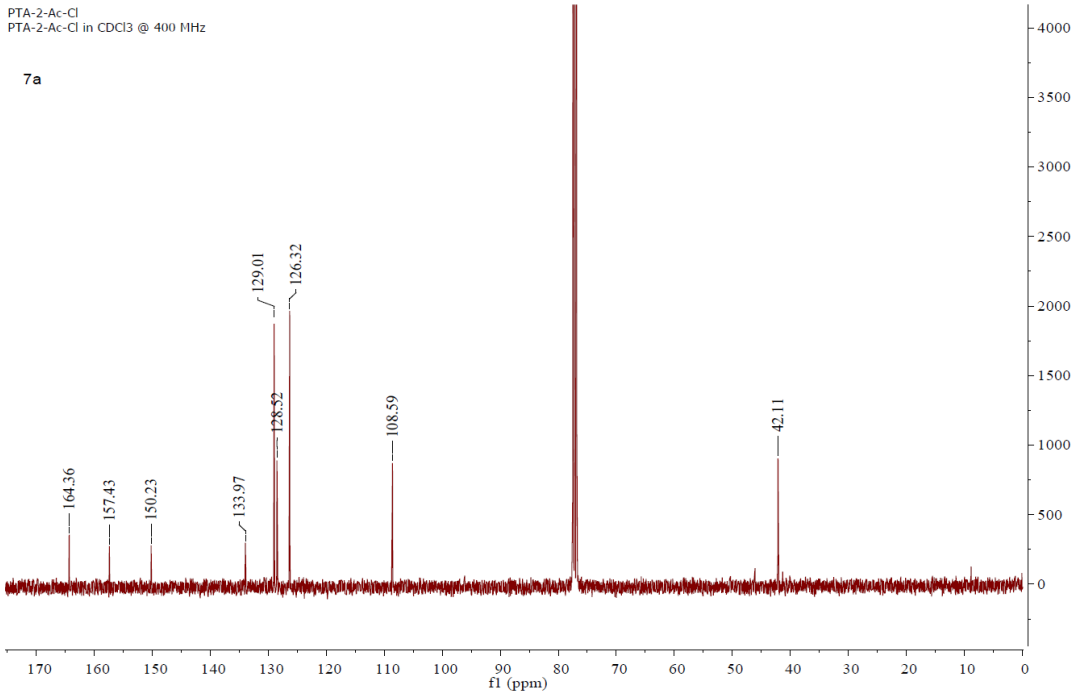

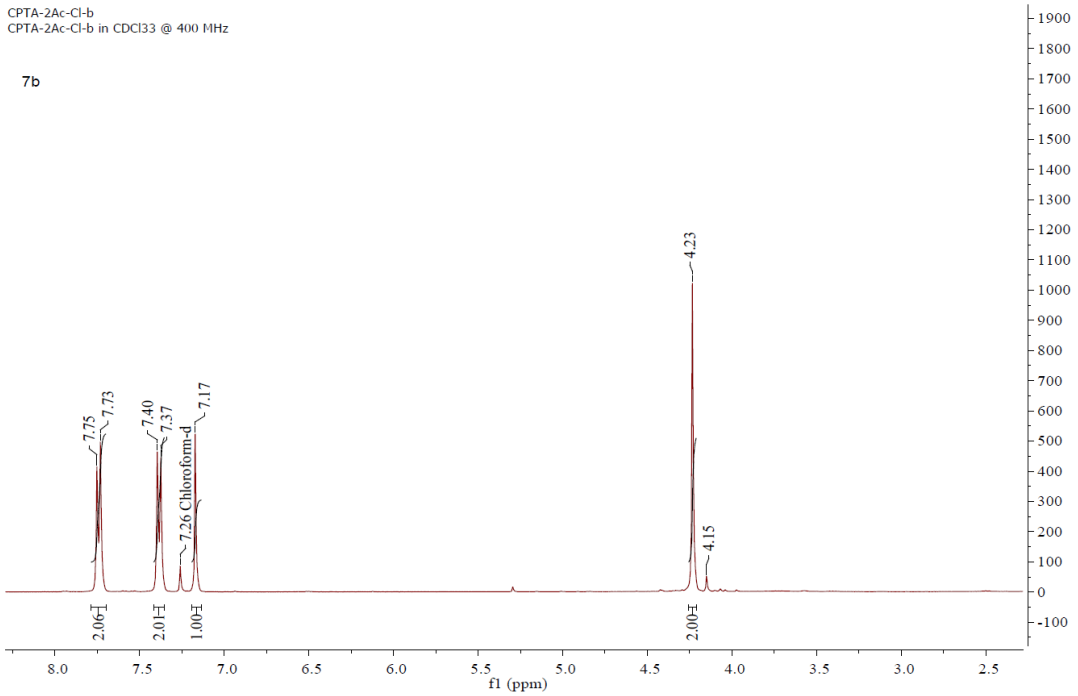

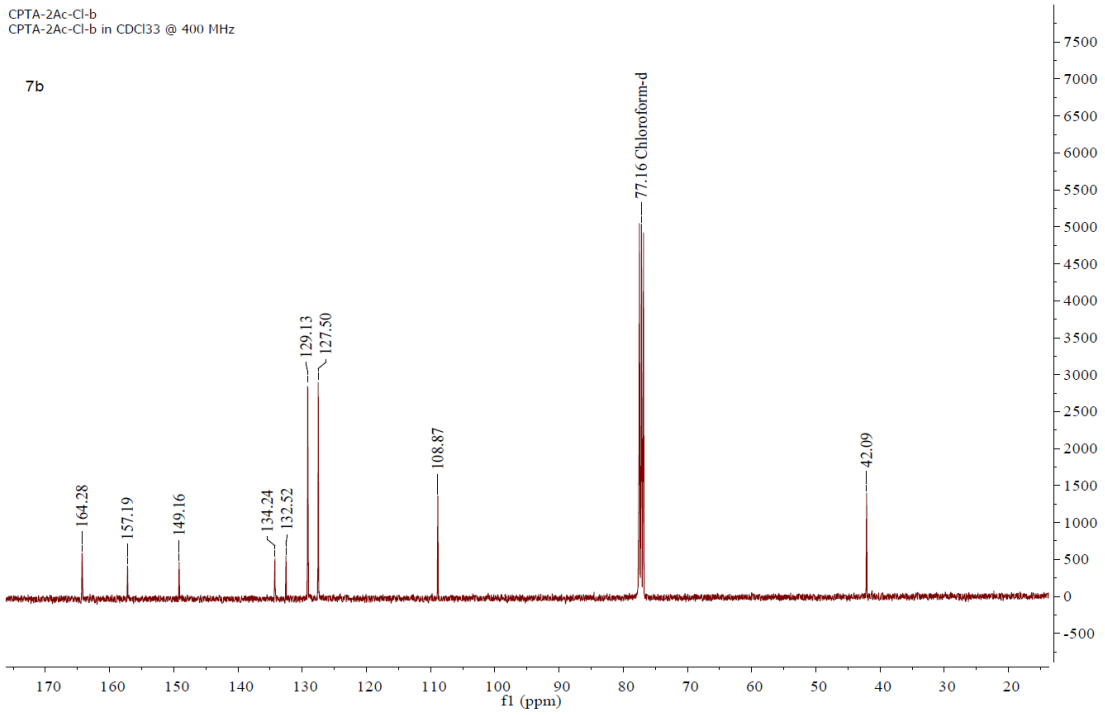

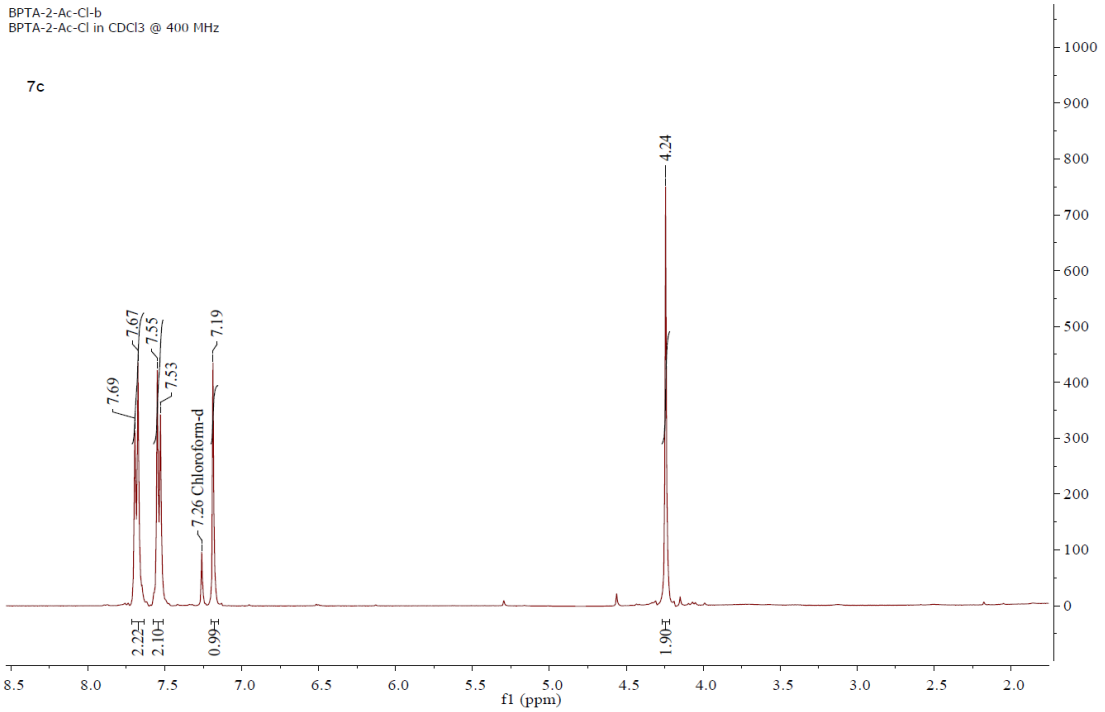

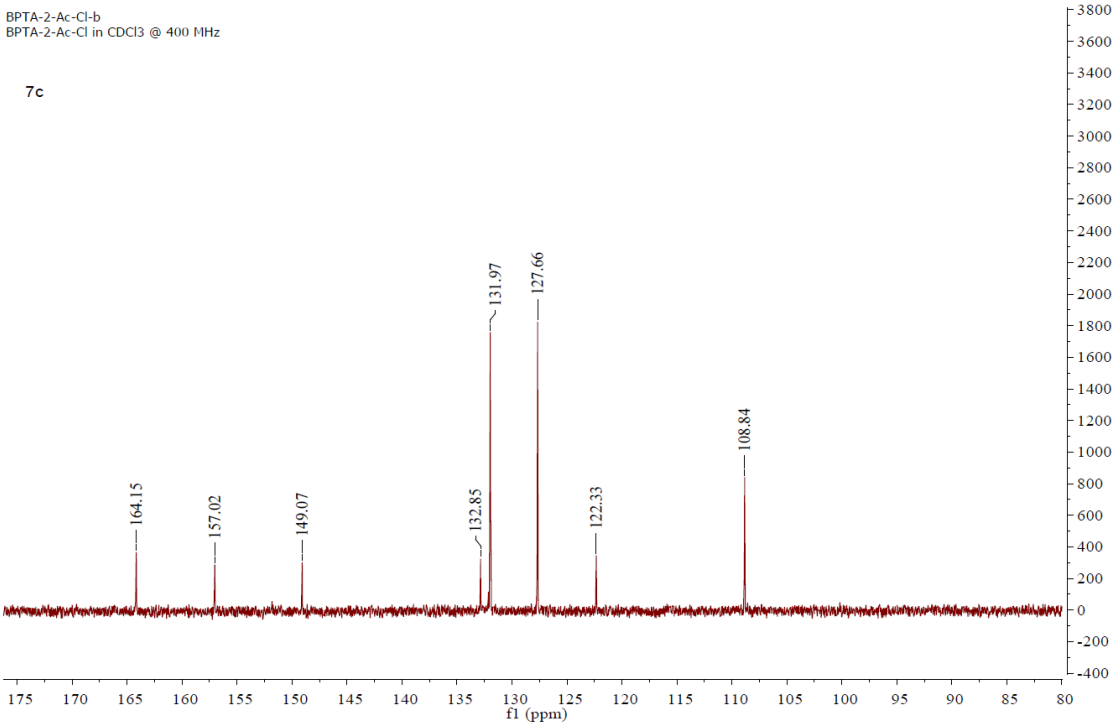

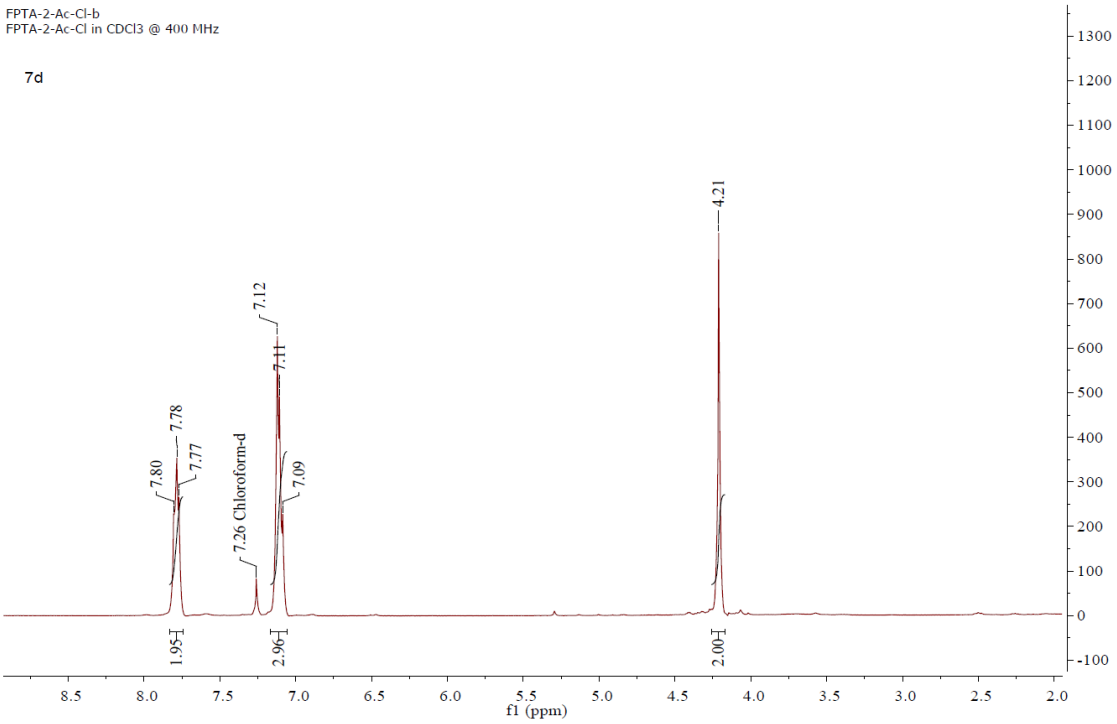

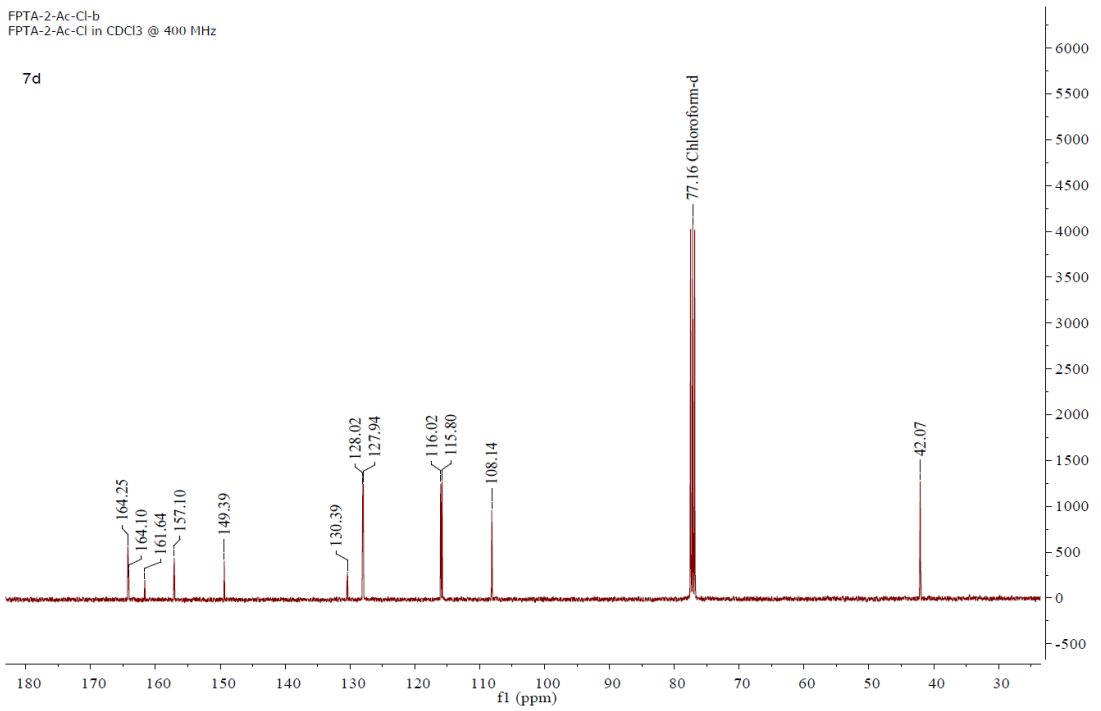

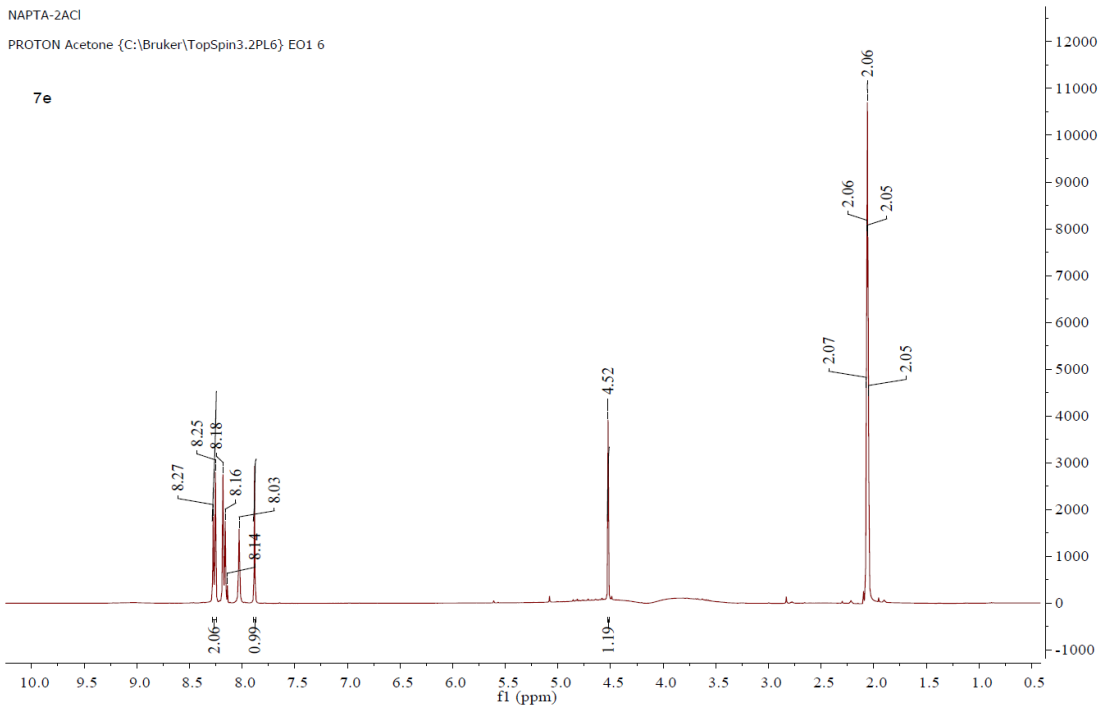

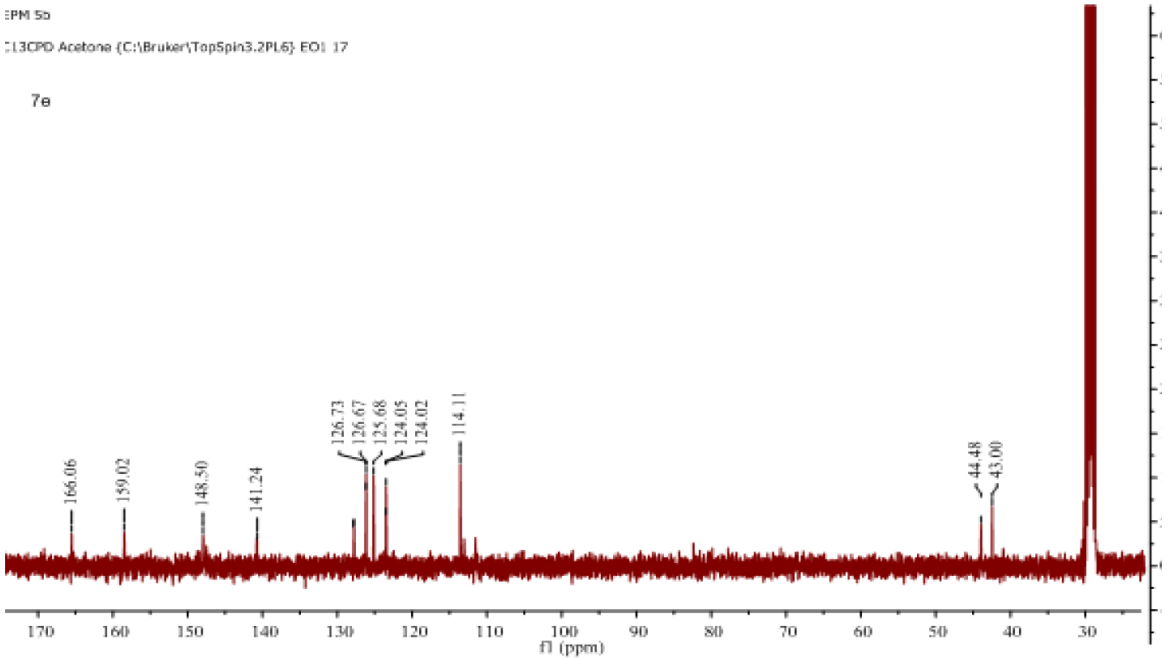

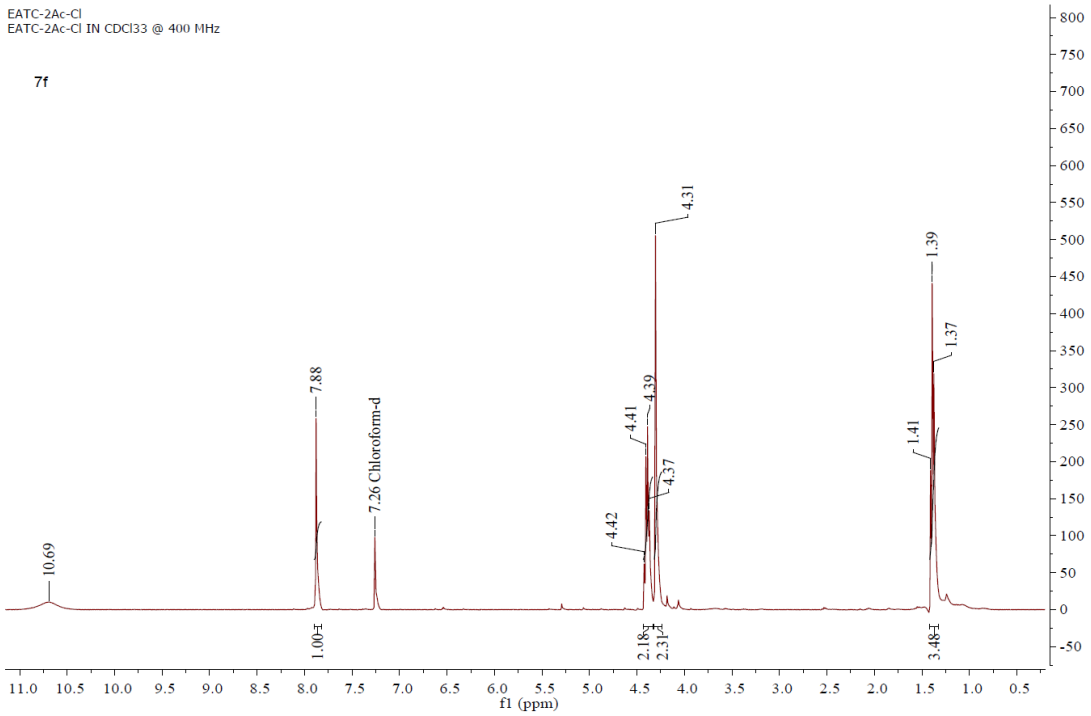

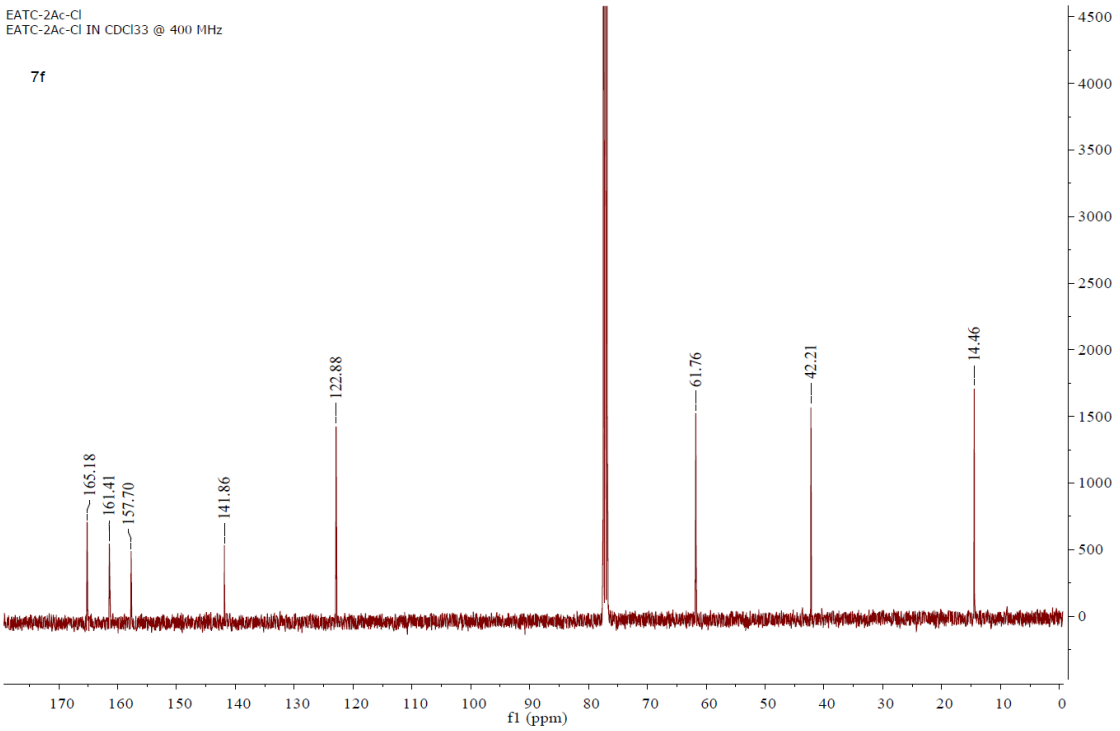

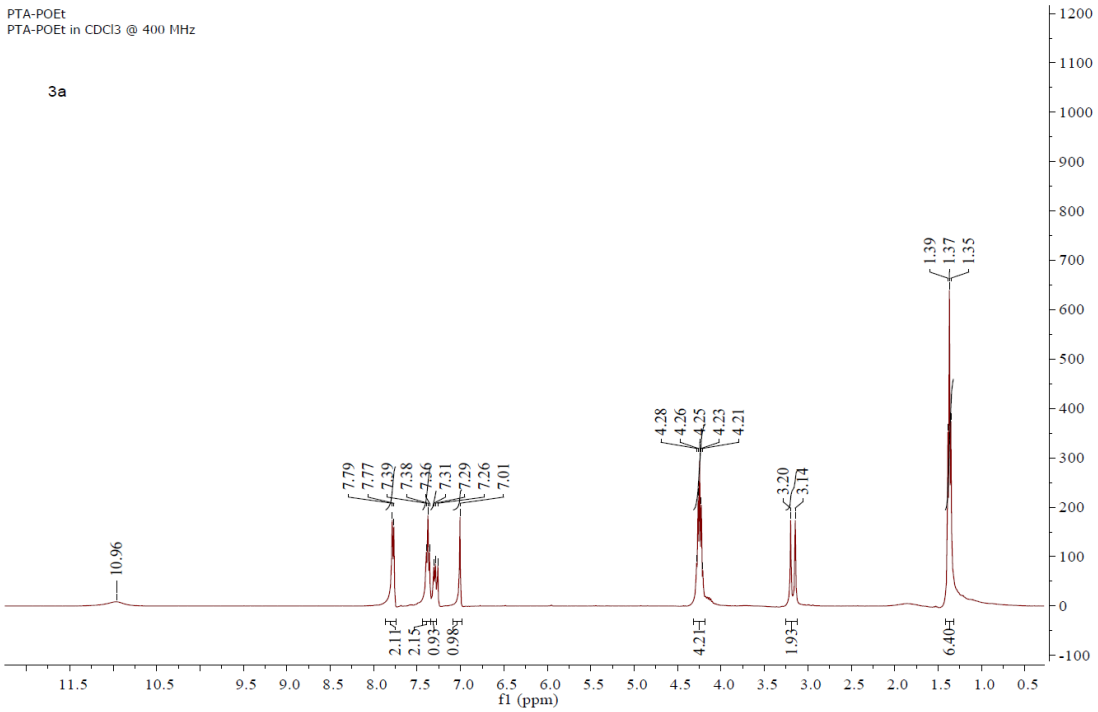

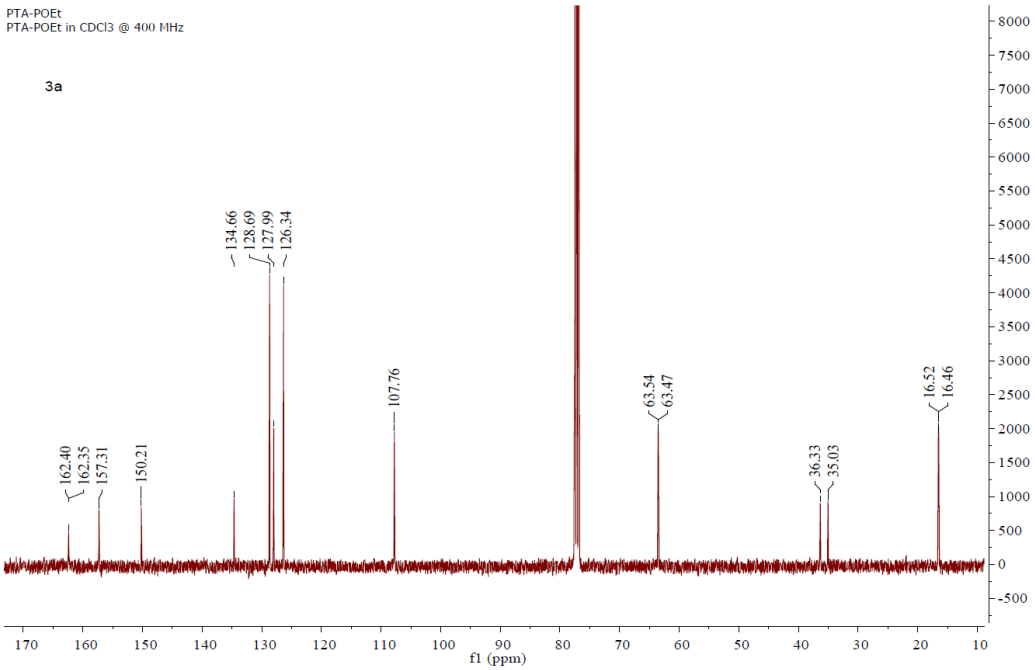

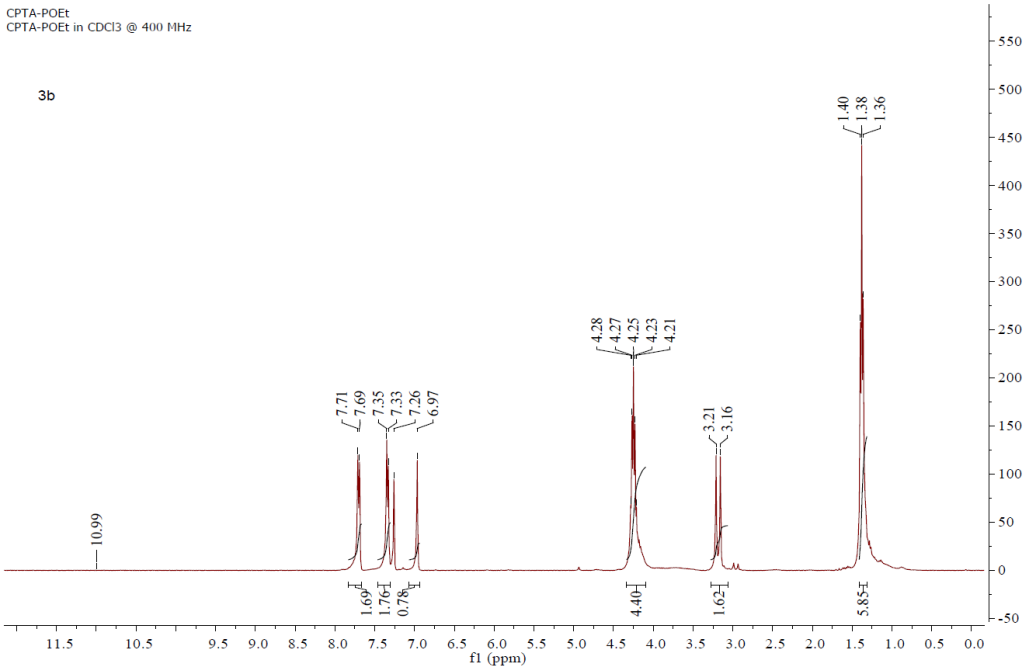

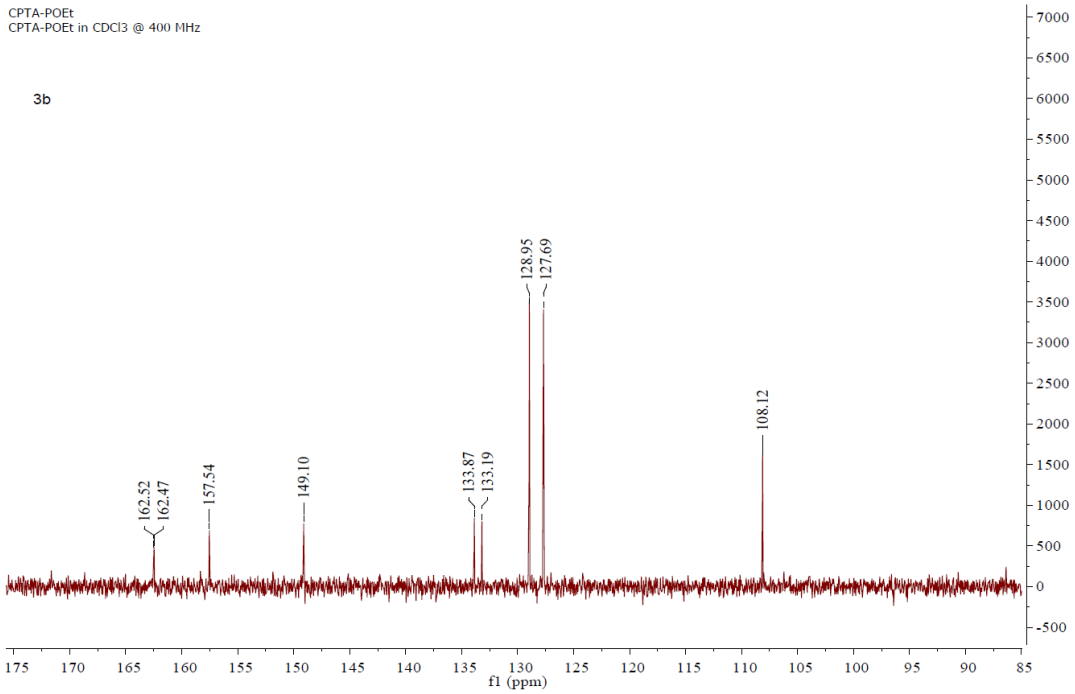

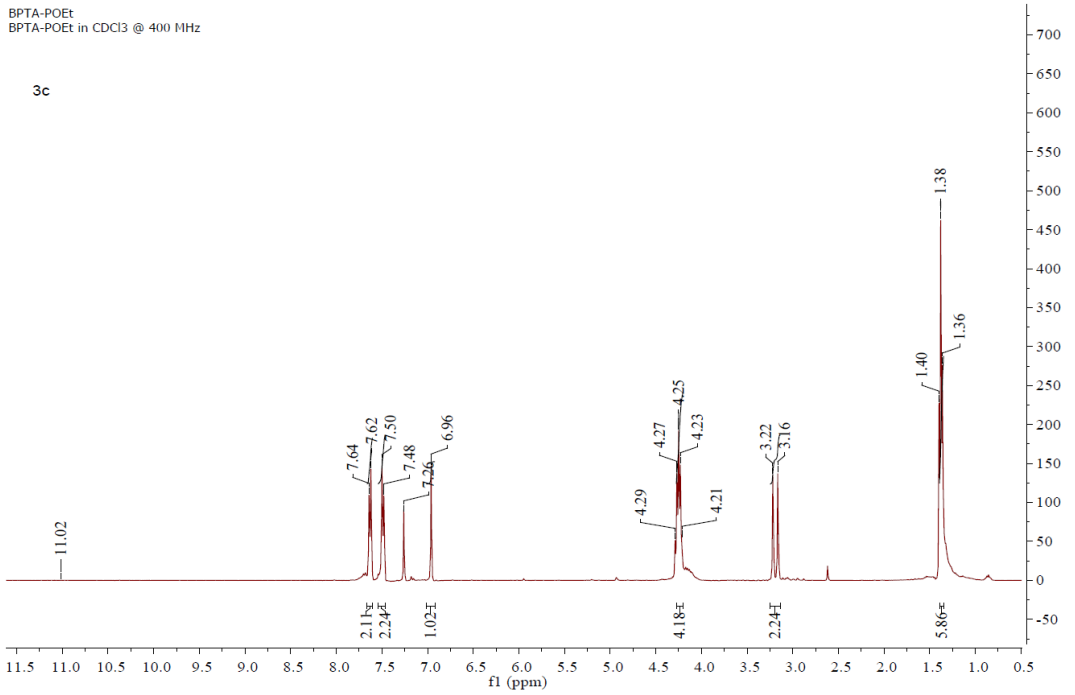

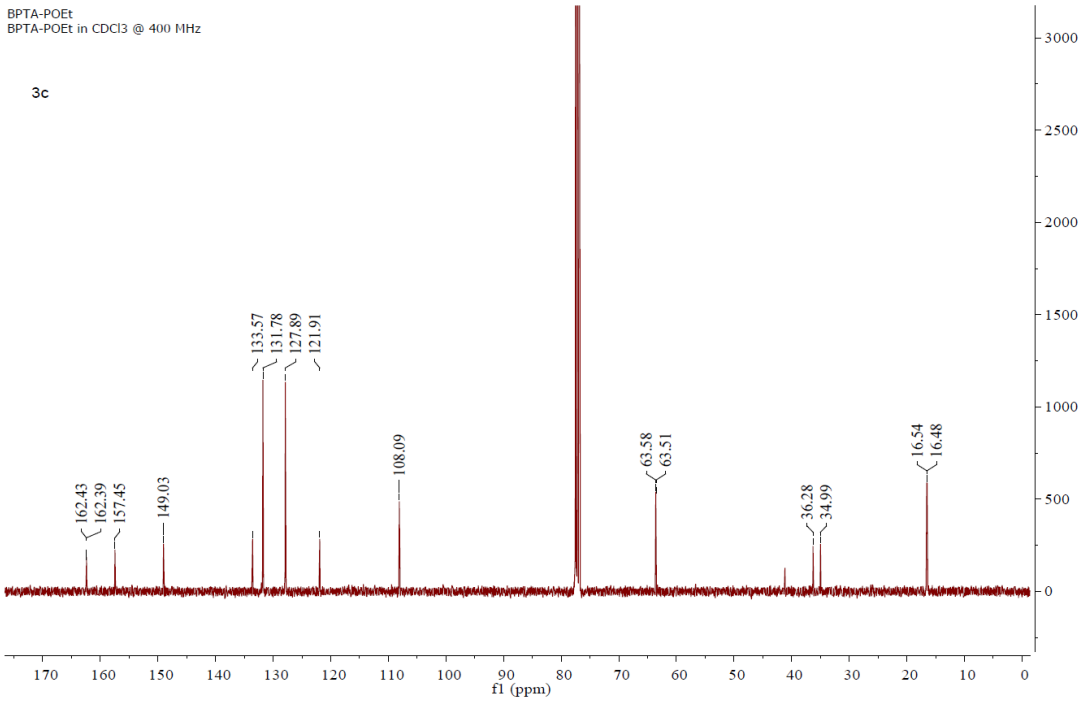

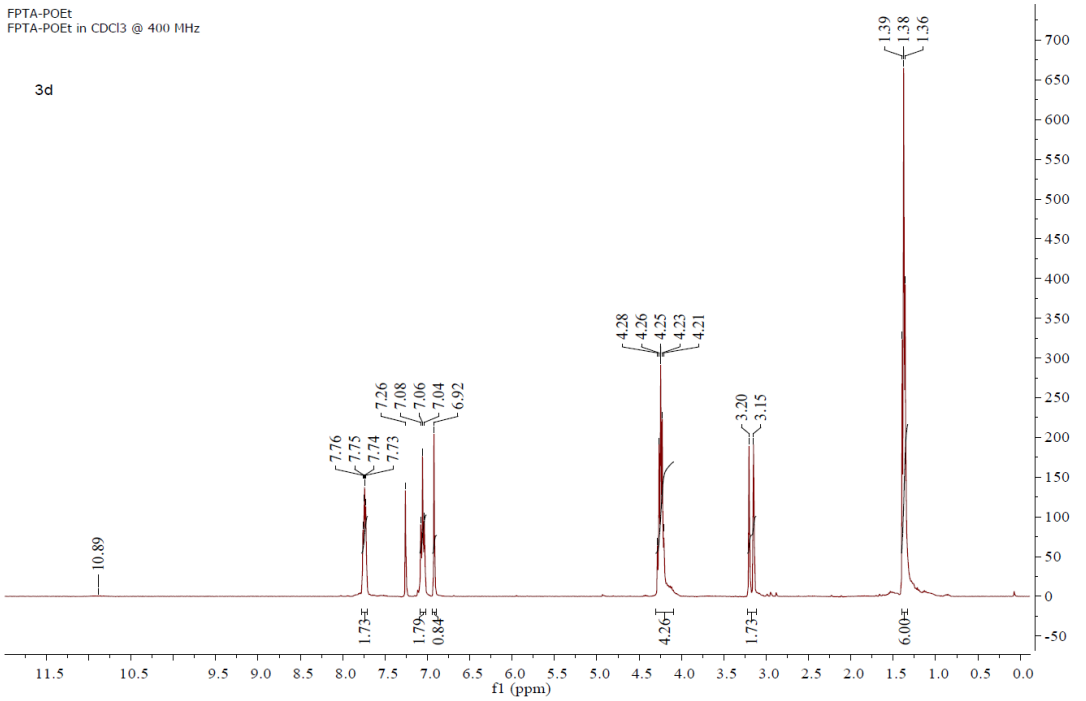

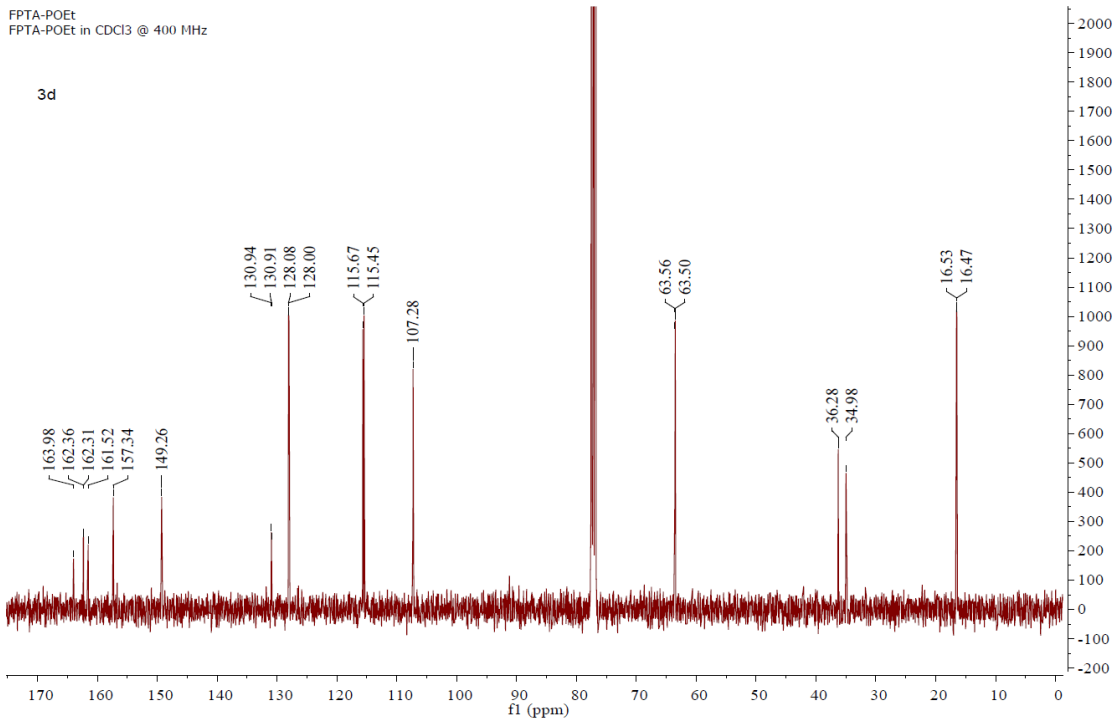

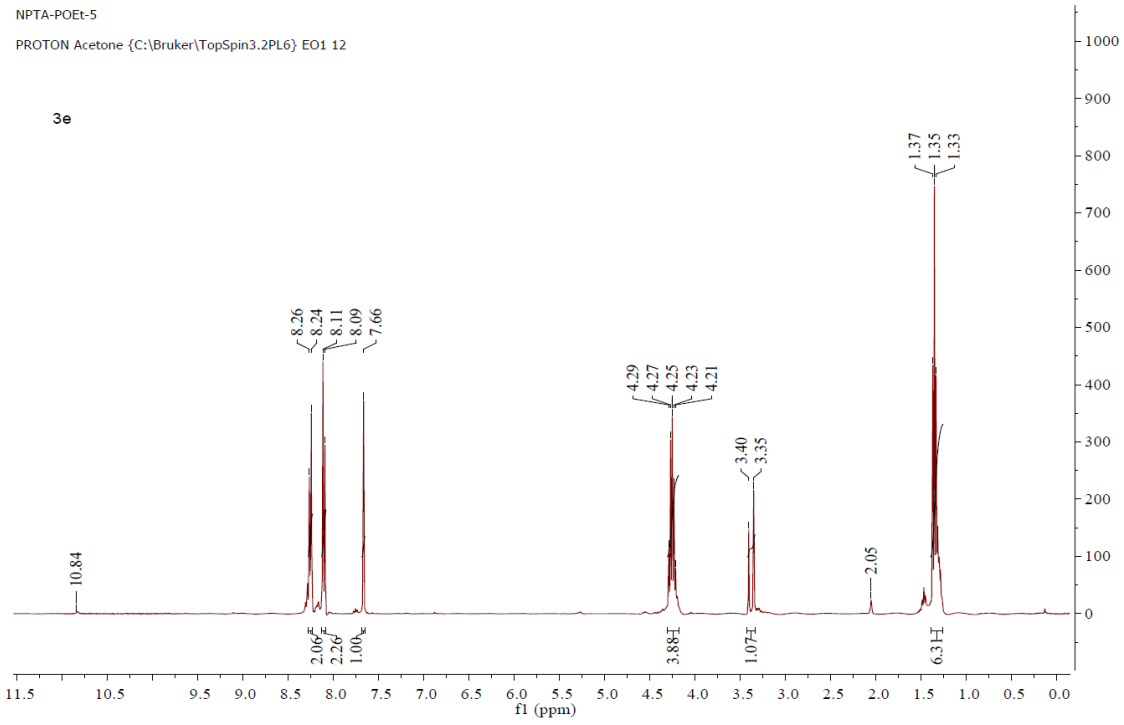

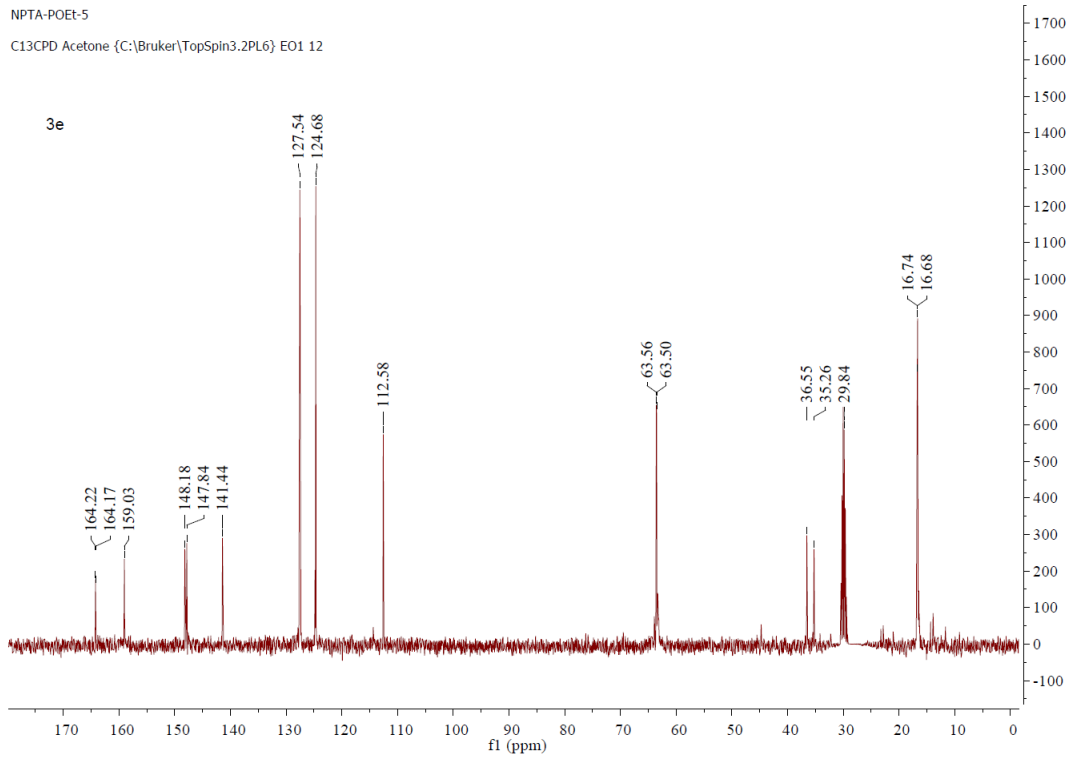

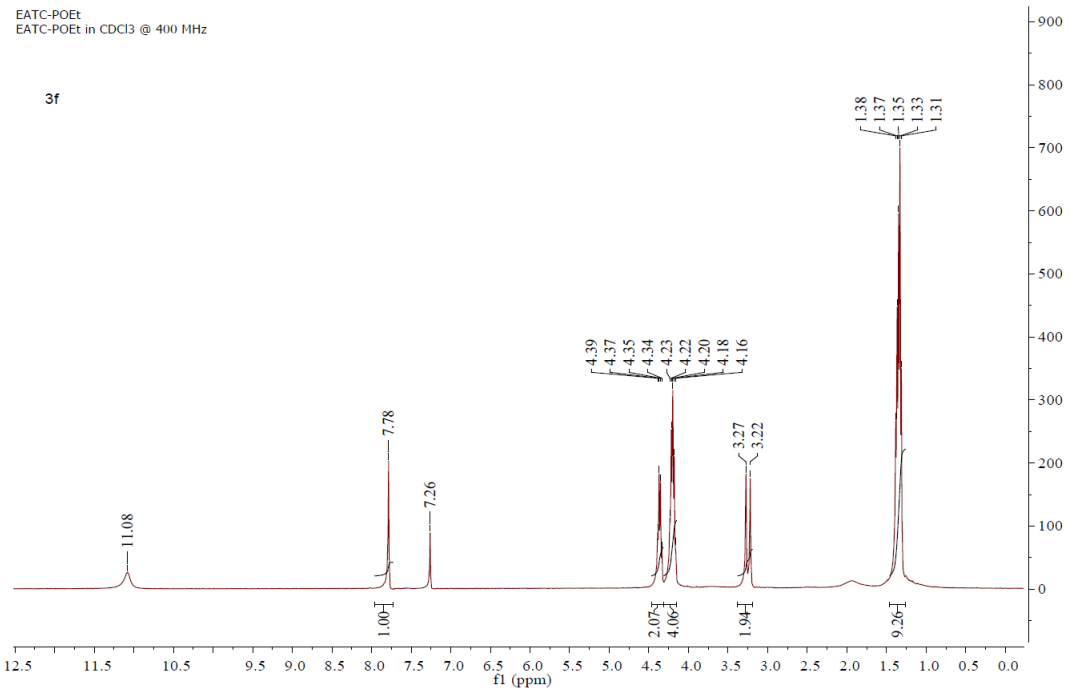

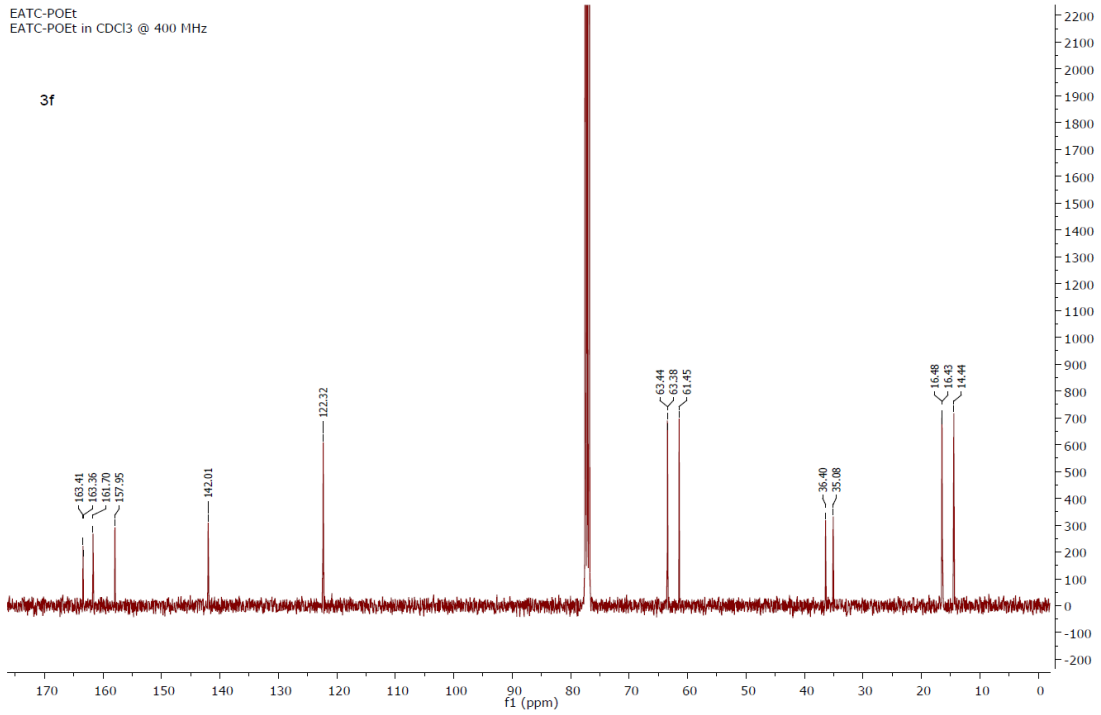

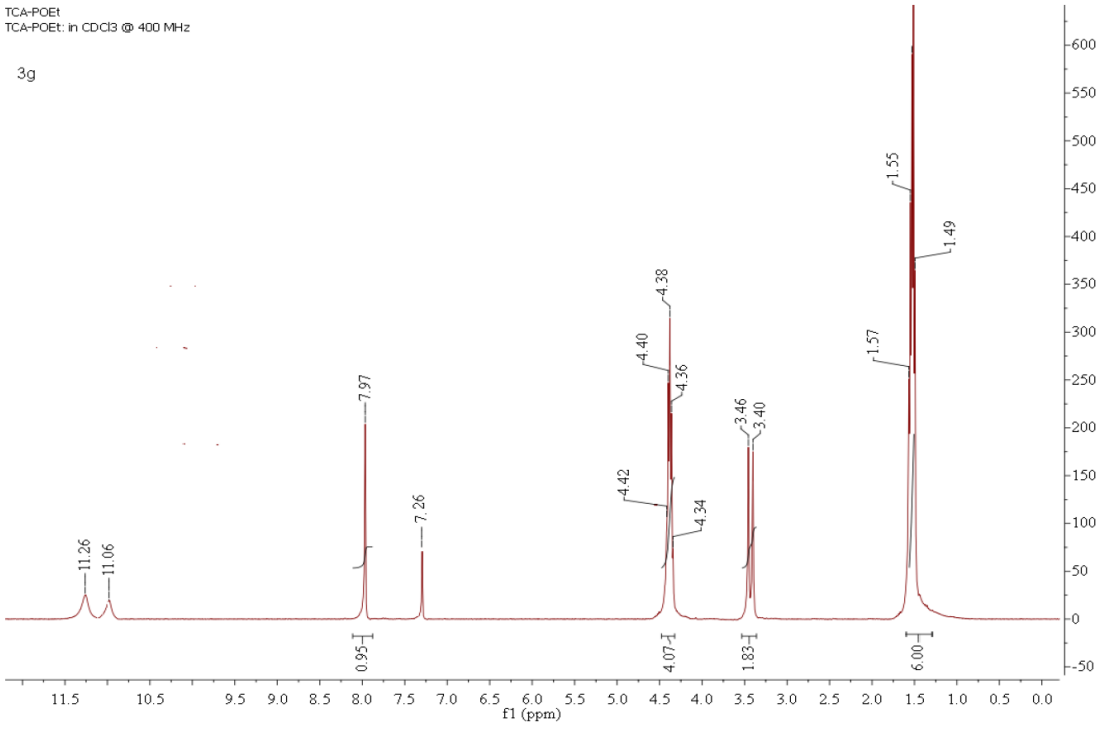

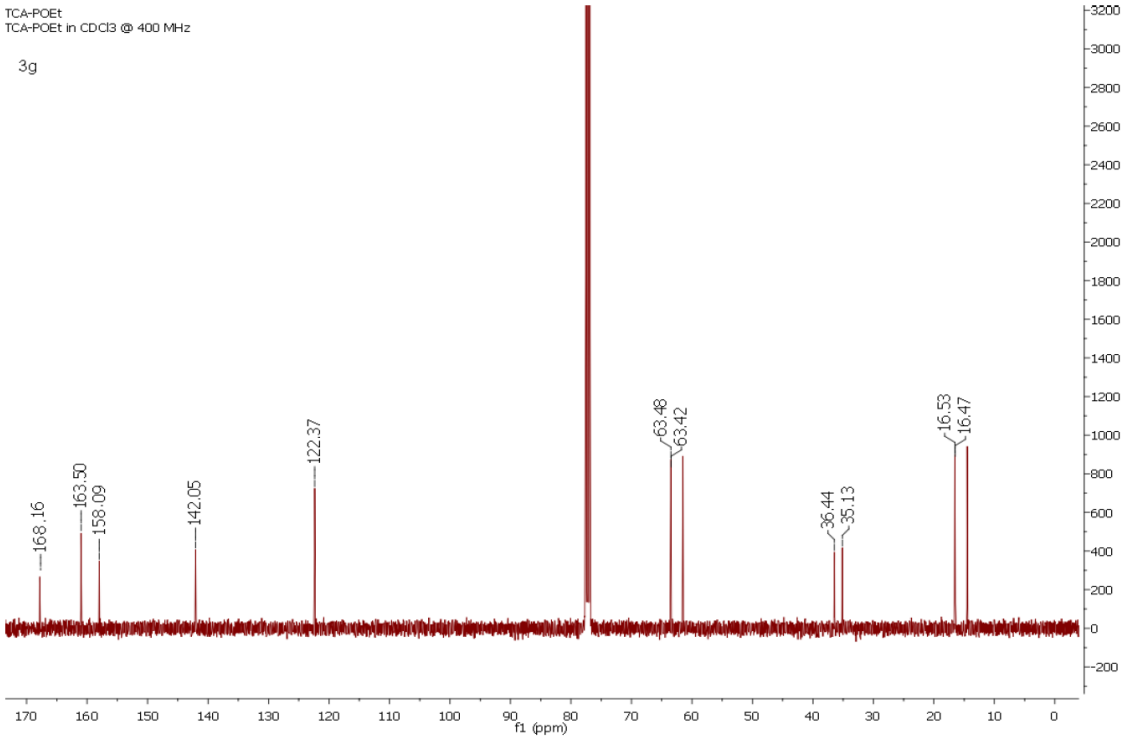

SUPPORTING INFORMATION II – *Bioassay Methods*

1. Anti-cancer (cytotoxicity) bioassays

1.1. *Resazurin-based in vitro cytotoxicity test using HeLa cells*

The screening was conducted using multi-well plates which are suited for HeLa cells in the log phase of growth with final cell density > 10 cells/cm. Each experiment normally includesa blank control, containing medium without the cells.[1-3] Non-contaminated HeLa cells (6.57x 10^5 cells per well) in media were allowed to grow in the incubator under an atmosphere of5% CO_2 at 37 ^oC for 24 h. To each well was dispensed 200 µL of HeLa culture, containing6.57 x 10^5 cells under LabE Air laminar flow hood (Vivid Air, South Africa); 20 µL of resazurin dye (Sigma TOX-8) and test compound (50 µL) were added, which were then incubated in the presence of 5% CO2 at 37 ^oC for 24 hours in a shaker, to enhance the distribution of the dye. The absorbance of each well was measured with Bio-tek Power Wave X fluorometer (Beijing, China), and increases in fluorescence were monitored at a wave length of 590 nm, using an excitation wavelength of 560 nm.[4]

2. Anti-cancer (cytotoxicity) xCELLigence RTCA assays using SH-SY5Y cells

2.1. SH-SY5Y cell culturing (thawing) and sub-culturing

Dulbecco's modified eagle medium (DMEM) supplemented with 1% ($^{v}/_{v}$) L-glutamine (2mM), 10% ($^{v}/_{v}$) Fetal Calf Serum (FCS) and 1% ($^{v}/_{v}$) of PBS (penicillin-streptomycin-amphotericin) was prepared and warmed at 37 $^{\circ}$C in a humidified atmosphere under 5%CO_2. [5] The SH-SY5Y culture vessel, containing complete growth medium, was equally warmed to 37 °C in the incubator for 2 minutes to thaw the cells. The surfaces of all the vials were decontaminated by spraying with 70% ($^{v}/_{v}$) ethanol, and subsequent steps were carried out under a strict aseptic condition in a laminar flow cabinet. The cells were aseptically transferred to a 15 mL centrifuge tube, containing 9 mL of DMEM, centrifuged at 1200 rpm at 4 $^{\circ}$C for 2 minutes. The cell pellets were re-suspended in the DMEM in a T25 flask and incubated at 37 $^{\circ}$C under 5% CO_2 and the cells were fed every 4 days by replacing the medium with a fresh DMEM.[5] The SH-SY5Ycells were sub-cultured from a T25 flask at a passage of 75% confluence into a T75 flask, in a ratio of 1:2-1:5. The floating cells were discarded with the spent medium, adherent cells rinsed with 3 mL of PBS, followed by the addition of 1 mL of trypsin solution, and were then placed in the incubator under 5% CO_2 at37 $^{\circ}$C for 2 minutes until the cells were detached. Fresh medium (1 mL) was then added, aspirated and centrifuged at 2000 rpm at 4 $^{\circ}$C for 2 minutes. The cell pellets were re-suspended in the medium, transferred into a new T75 flask, incubated under 5% CO_2 at 37 $^{\circ}$C and new medium was added after 4 days.[5]

2.2. Cytotoxicity assay using xCELLigence RTCA SP instrument

The optimal seeding concentration for the proliferation experiments of the SH-SY5Y was first determined.[5] Prior to seeding of the SH-SY5Y cells to each well, 100 µL of the medium(DMEM) was added to each well, and scanned by the xCelligence RTCA system to determine the baseline values.[5] Ten thousand (1 x 10^4) SH-SY5Y cells in 50 µl culture medium (DMEM Ham supplemented with 1% L-glutamine (2 mM), 10% Fetal Calf Serum(FCS) and 1% PBS were seeded into each well of the microelectronic censored E-Plate 96,and incubated at 37°C under 5% CO_2 in a humidified atmosphere.[5,6] The culture proliferation, attachment, and spreading of the cells were monitored every 30 minutes for 24hours by the xCELLigence, after seeding. The cells at the log growth phase were exposed induplicate to 50 µL of different concentrations of the test compounds in the medium, incubated at 37 ^{o}C under 5% CO_2 at relative humidity of up to 98%, and scanned every 15minutes for 24 hours.[5] The controls wells received either SH-SY5Y cells (normal cell growth), medium only, medium-DMSO at a concentration of 0.20% ($^{v}/_{v}$) or blank / PBS solvent.[7-9] All experiments were run for a total of 48 h.[5,6]

2.3. Statistical and data analysis of xCelligence RTCA data

The calculations were automatically computed by the RTCA-integrated software of the xCELLigence RTCA system.[10] The RTCA software also performs a non-linear regression analysis of selected sigmoidal dose-response

for each test compound in comparison to the experimental data points, and calculates the linear or logarithmic half maximum inhibitory concentrations (IC_{50} or log IC_{50}) at a given time point that produce 50% reduction of cell index (CI), relative to the SH-SY5Y control CI (100%).49 All data have been generated by the RTCA software, and are presented as mean (mmol/L) ± SEM (standard error of mean).The cytotoxic effects of the test compounds were evaluated by plotting a dose response curve (DRC) of the Cell index (CI) at a time point against the linear- or Log-concentration.[5]

3. Antimalarial bioassay

3.1. Procedure for antimalarial resasurin-based PfLDH bioassay

3.1.1. *Preparation of solutions*

i) **Malstat solution**: Triton X-100 (400 µL), L-lactic acid (4g), Trizma base (1.32 g) and acetylpyridine adenine dinucleotide; APAD (22 mg) were dissolved in H_2O, and the pH was adjusted to 9 using a basic Tris Buffer (Tris–C).

ii**) NBT/PES solution**: Nitro blue tetrazolium salt (160 mg) and phenazineethosulphate (8 mg)were dissolved in H_2O (100 mL). The bottle was covered with aluminium foil, and thesolution was stored at 4 ^oC. The solution was used within a month of storage.

3.1.2. *Antimalarial whole cell PfLDH-based inhibition assay*

Giemsa stain is a mixture of malstat and NBT/PES solutions. Malstat solution was prepared by mixing triton X-100 (400 µL), L-lactic acid (4 g), Trizma base (1.32 g) and acetylpyridineadenine dinucleotide; APAD (22 mg) were dissolved in H_2O, and the pH was adjusted to 9 using a basic Tris Buffer (Tris-C). Also, NBT/PES solution was obtained from Nitro bluetetrazolium salt (160 mg) and phenazineethosulphate (8 mg) were dissolved in H_2O (100mL), the bottle was covered with aluminium foil, and the solution was stored at 4 ^oC. The two solutions were used within a month of storage.

3.1.3. *Antimalarial PfLDH-based single concentration screening*

The solutions of all of the test compounds (20 mM in DMSO) were stored in a deep freezer at
-40 ^oC prior to the screening. Stock solutions were diluted with the media (blood plasma /haemolysed red blood cells) to obtain a concentration of 20 µM that was used for the test. Chloroquine (CQ) was used as a positive control (reference or standard antimalarial drug). A 100 µL of each compound in triplicate was placed in a 96 well plate, and control wells contained only the media (blank). The parasite culture was removed from the T75 flask, and transferred into an Eppendorff tube, then centrifuged at 2000 rpm for 5 minutes (Eppendorff AG centrifuge 5810R, New Jersey, U.S.A). The supernatant was discarded, and the culture pellets were retained at the bottom of the EPPENDORFF tube. Blood smear viable cell count assays were performed using Giemsa stain

to determine the percentage parasitemia and 2%haematocrit, 2% parasitemia suspension was made in a 10 µL culture media.[3] The parasite viability of a test sample = Abs test compound x 100 Abs positive control 4. In vitro antimycobacterial green fluorescent protein microplate assay (GFPMA).

4. Anti-tuberculosis green fluorescent protein microplate assay (GFPMA)

MIC90 and MIC99, which are the concentration of the thiazole-based compounds, inhibit 90% and 99% of the growth of the virulent M. tuberculosis H_{37}Rv (Mtb H_{37}Rv), were determined using the GFPMA in a microplate-based fluorometric assay. A stock culture of MtbH37Rv was grown to observe the density at 600 nm (i.e., OD600) of 0.6-0.7 in the Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween-80, 0.2% glycerol, and albumin-NaCl-glucose (ADC) complex. Culture dilutions were made in the medium (1:500) and 50 µL was dispensed into each microwell of a 96-well plate (row 2-12). In order to determine the MIC90 and MIC99, the test compounds were dissolved in DMSO to make stock solutions of 12.8 mM, and serial dilutions was done up to a final concentration of 640µM. Each compound (100 µL) was added to the first row of the wells of the 96-well plate, and two fold serial dilutions were made, to provide ten dilutions of each compound (160-0.078 µM). Rifampicin was used for positive controls, while 5% DMSO and the Middlebrook 7H9-based media were employed as negative controls. The plates were incubated for 14 days at 37 ˚C, and the MIC90 and MIC99 values were read using an inverted fluorometric plate reader on Day 7 and Day 14, post-inoculation. The lowest test and reference drug concentrations that inhibit the growth of more than 90% and 99% of the MtbH_{37}Rv at day 7, scored on a scale of 10, were considered as the MIC90 and MIC99 values respectively (Figure 1).[11]

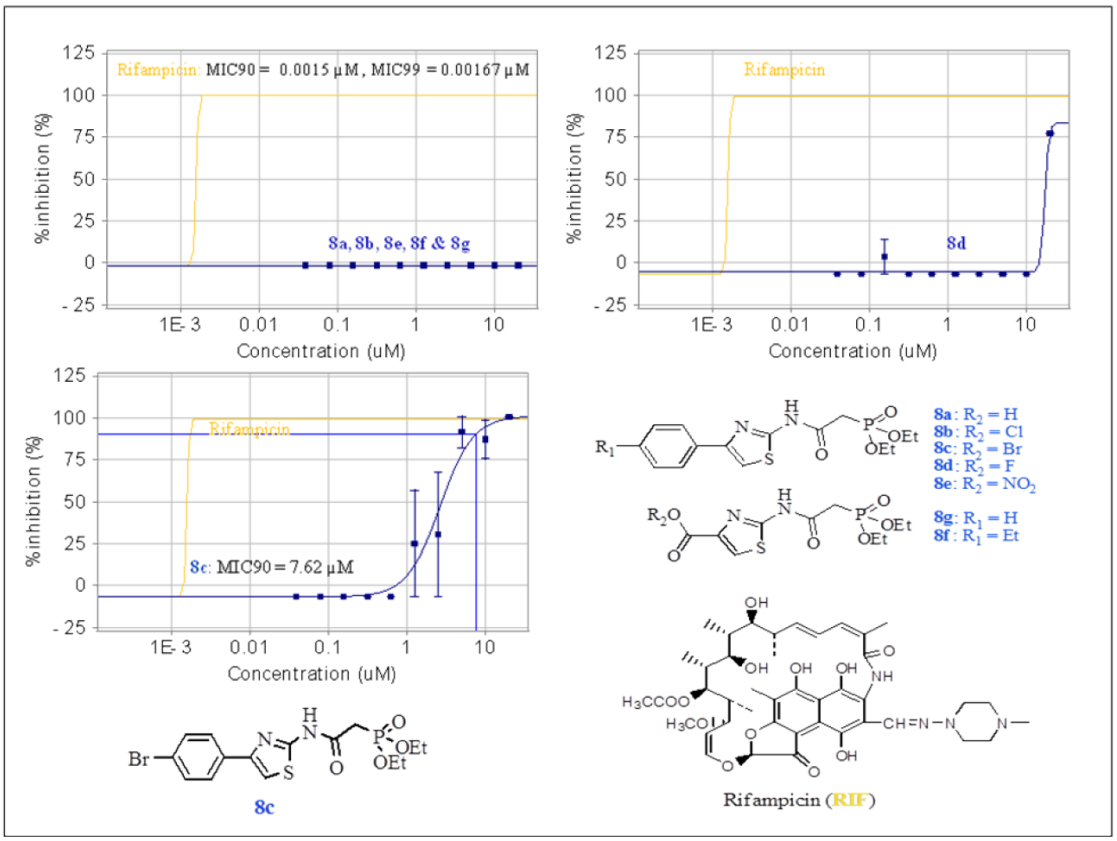

Figure 1: Dose-response plot of GFP-based anti-TB activities of **8a-g** and rifampicin.

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