Supporting Information

Stereoselective Synthesis of the Di-Spirooxindole Analogs Based Oxindole and Cyclohexanone Moieties as Potential Anticancer Agents

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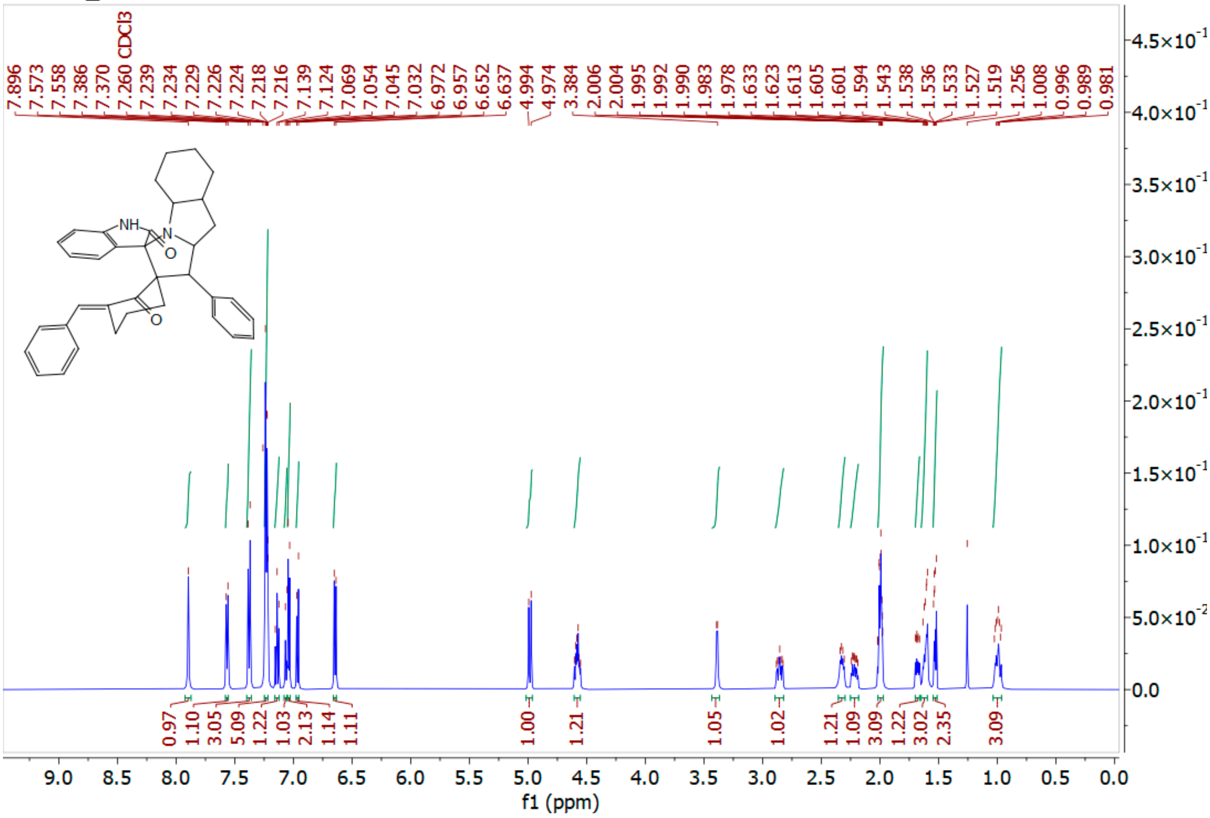
NMR SPECTRA

Figure S1: ^{1}H-NMR and ^{13}C-NMR for compound-**4a**

AB1171_1H



AB1171_13C

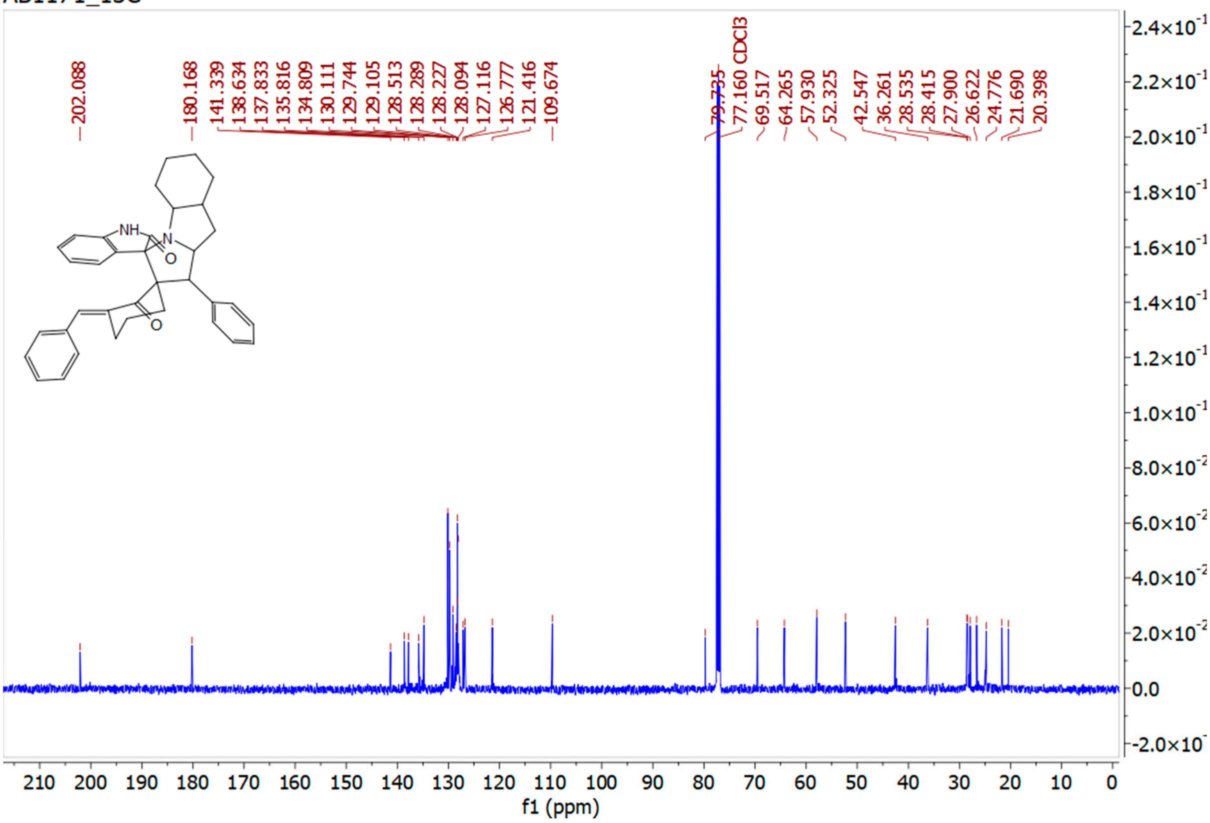
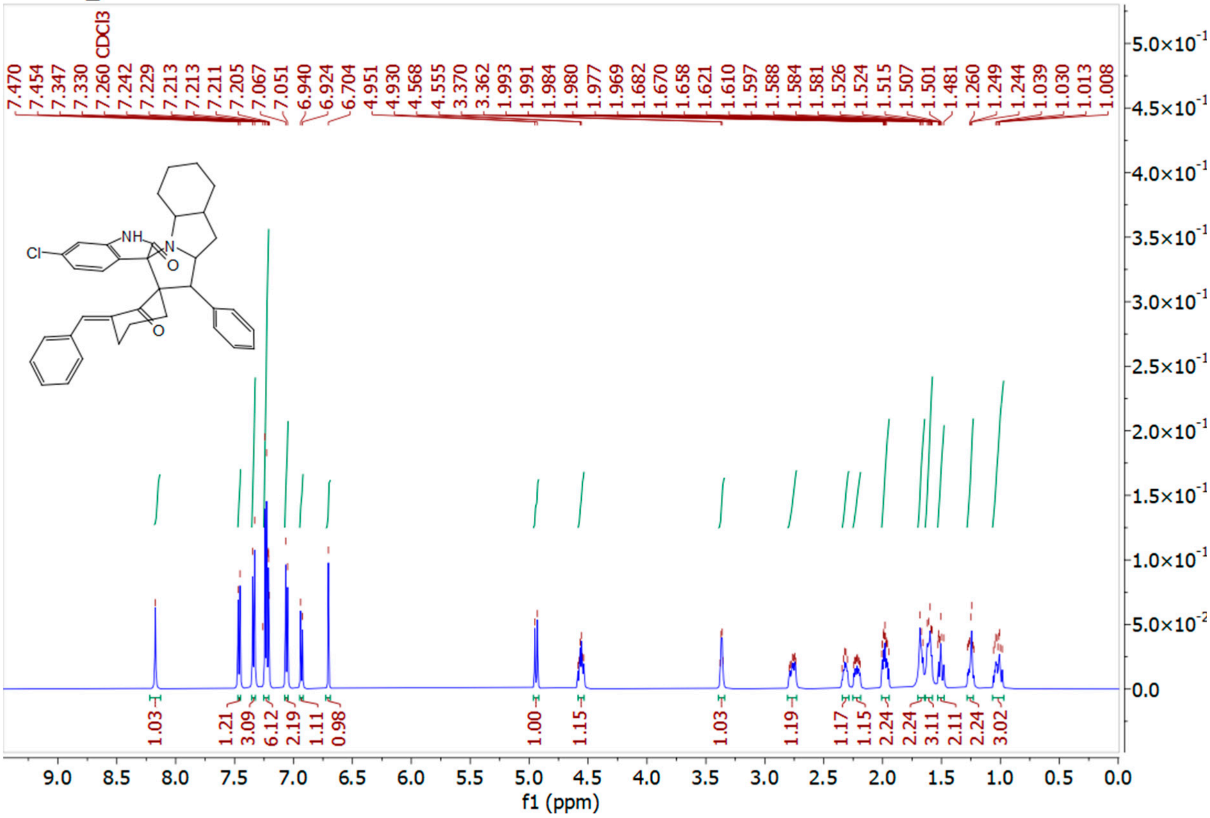


Figure S2: ^{1}H-NMR and ^{13}C-NMR for compound-**4b**

AB1172_1H



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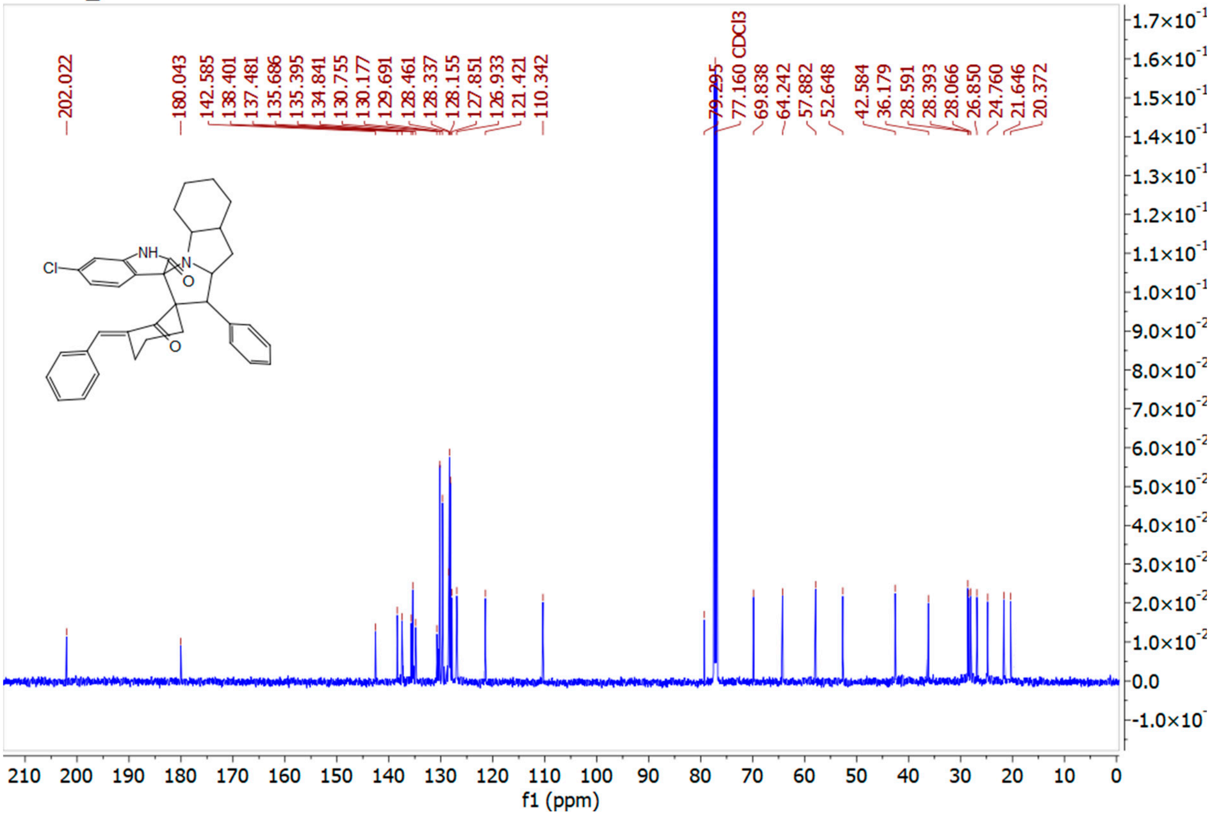
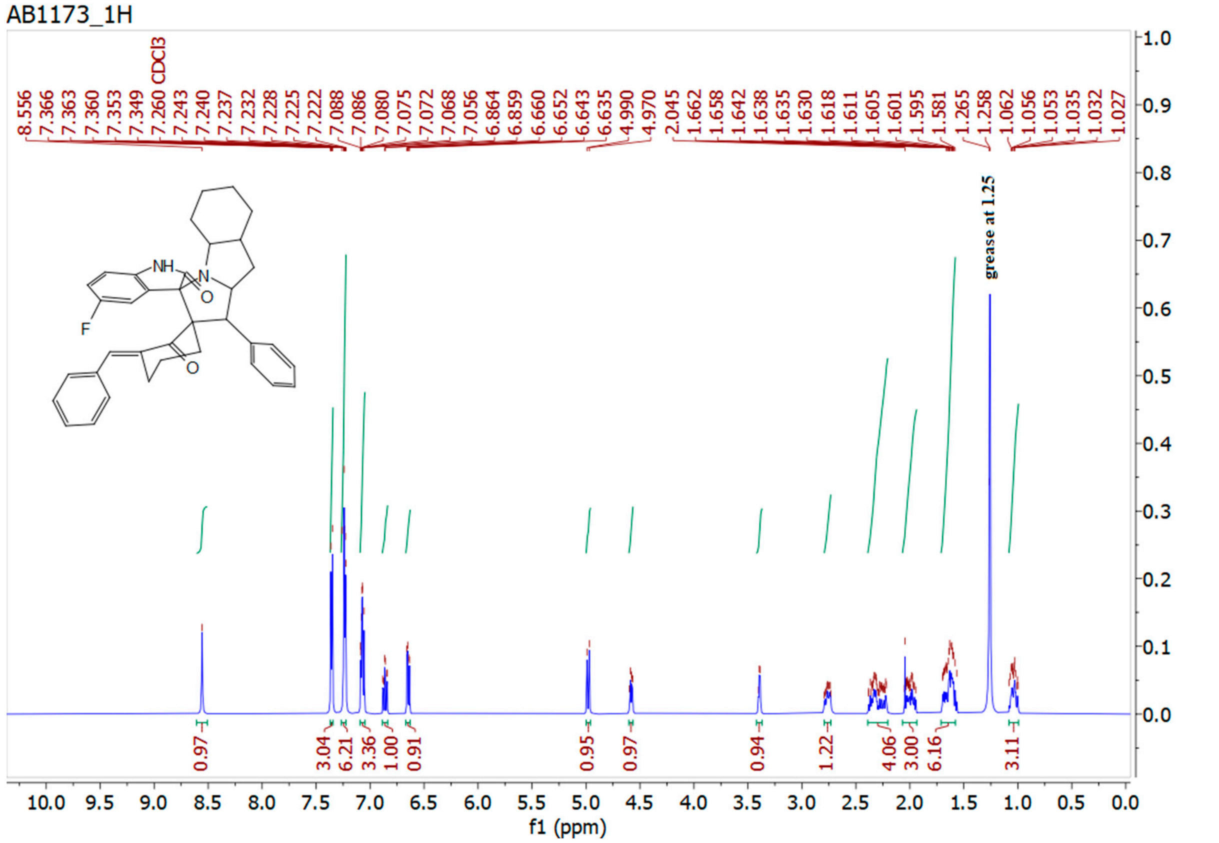


Figure S3: ^{1}H-NMR and ^{13}C-NMR for compound-**4c**



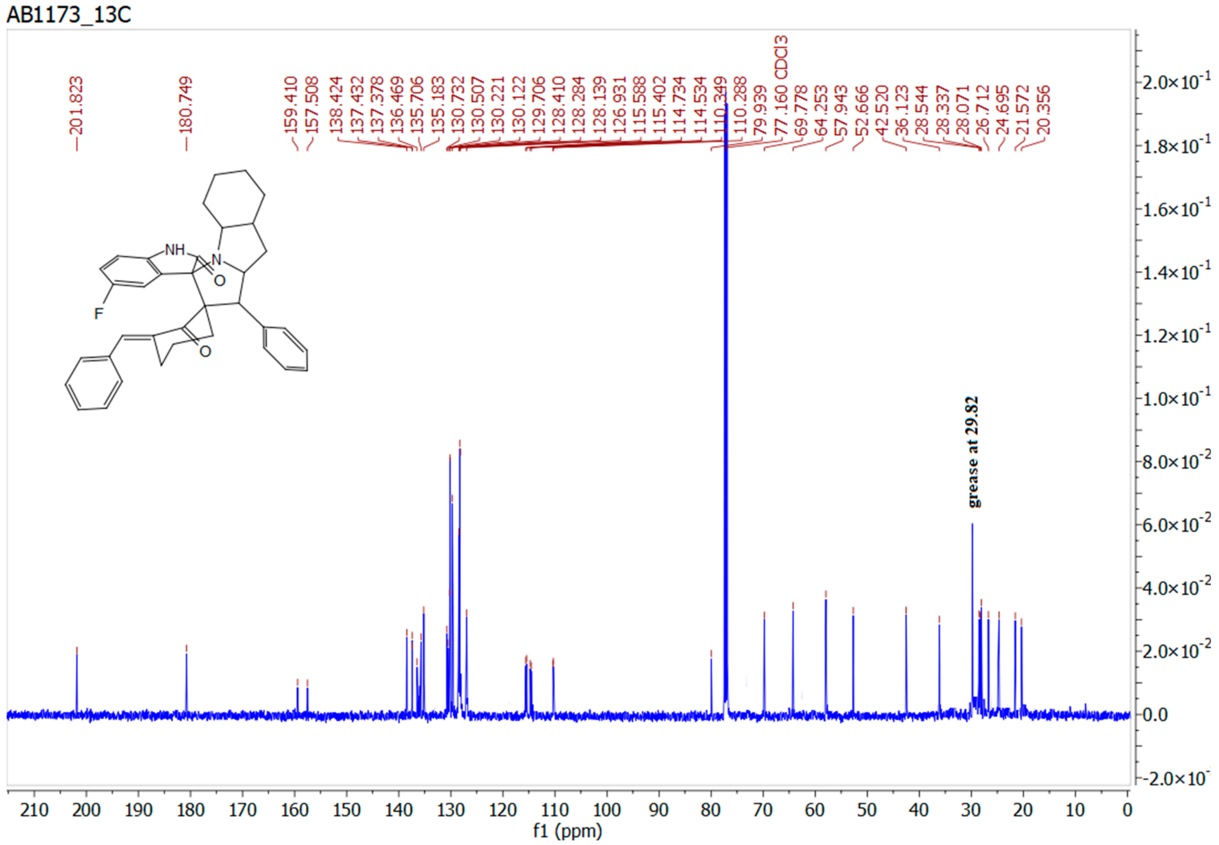
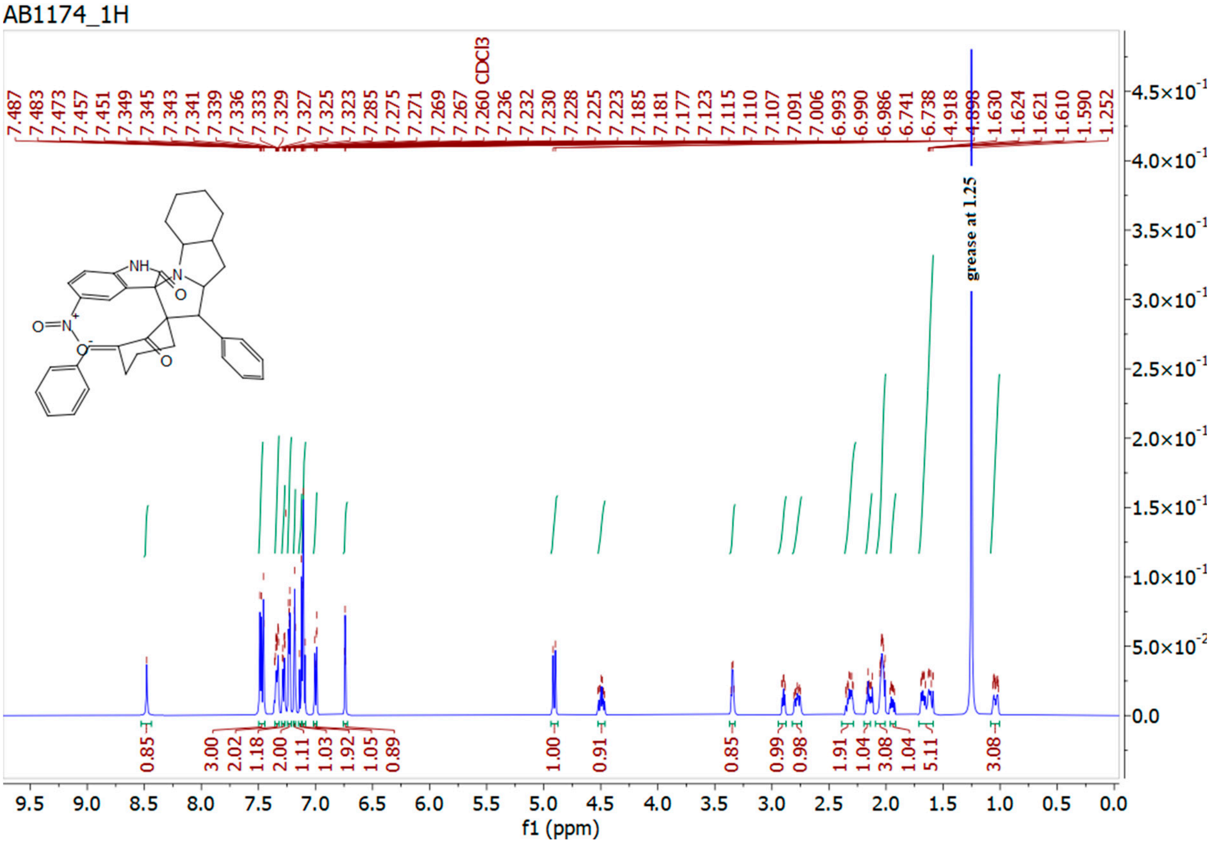


Figure S4: ^{1}H-NMR and ^{13}C-NMR for compound-**4d**



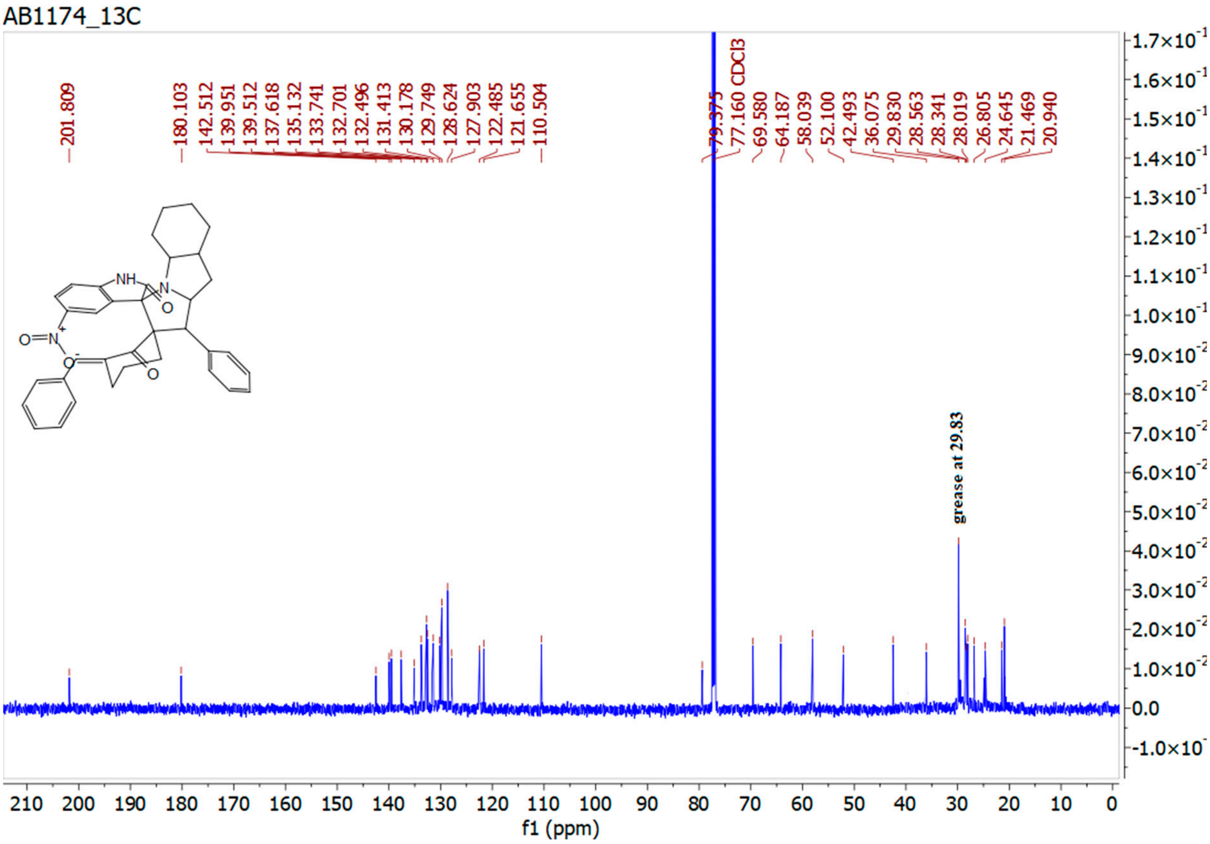
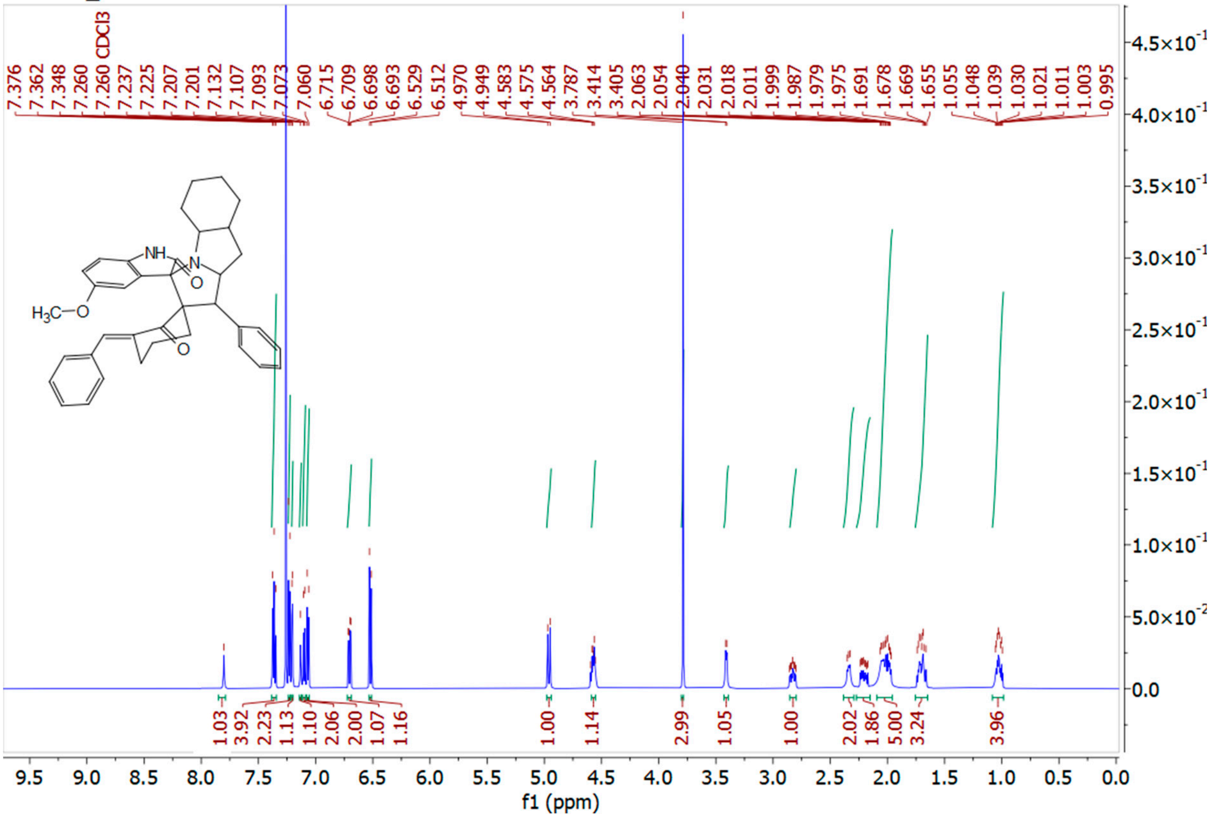


Figure S5: ^{1}H-NMR and ^{13}C-NMR for Compound-**4e**

AB1175_1H



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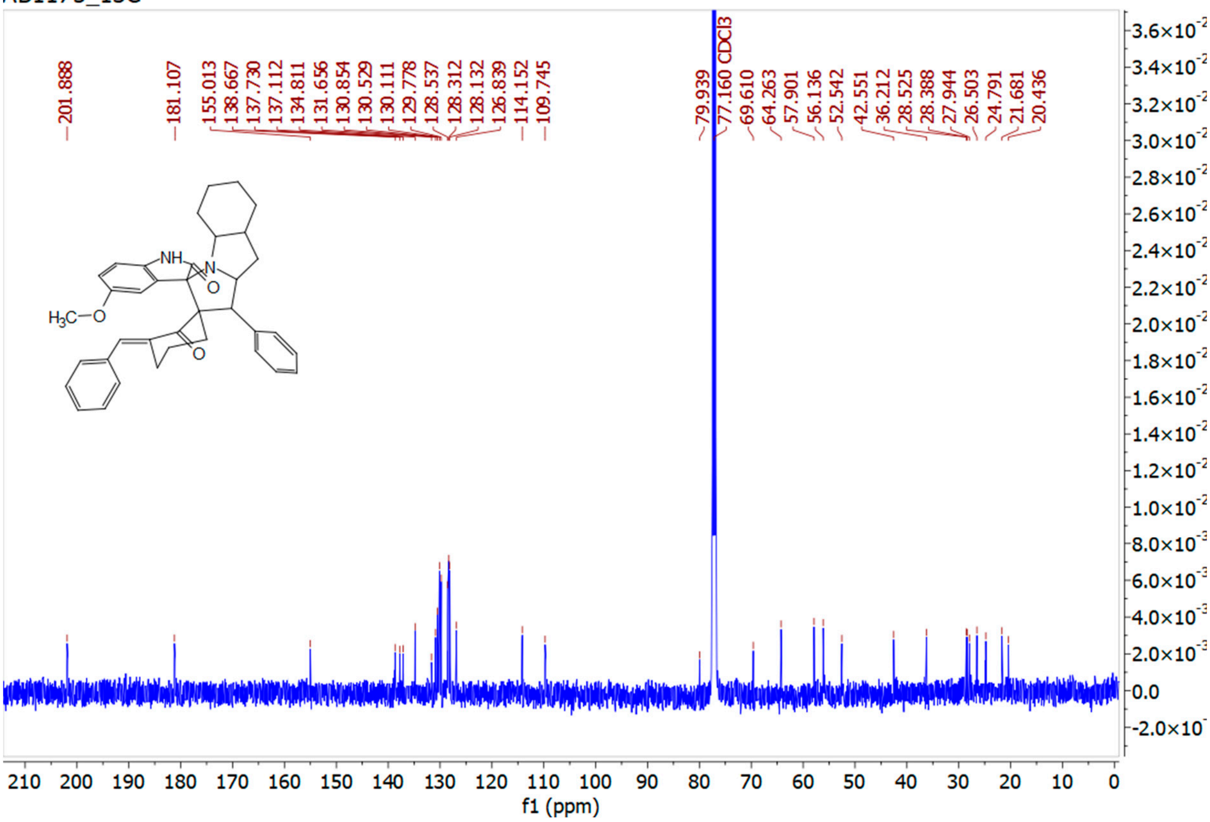
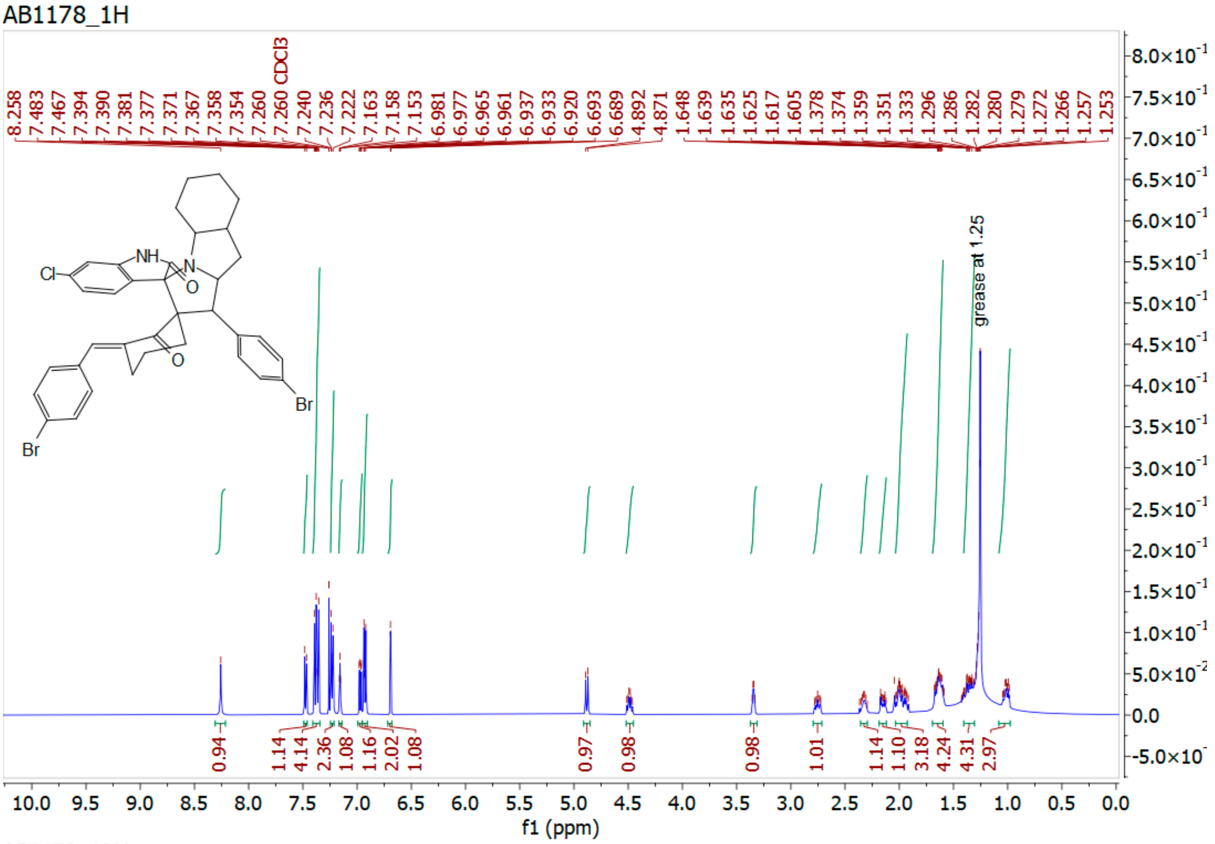


Figure S6: ^{1}H-NMR and ^{13}C-NMR for compound-**4f**



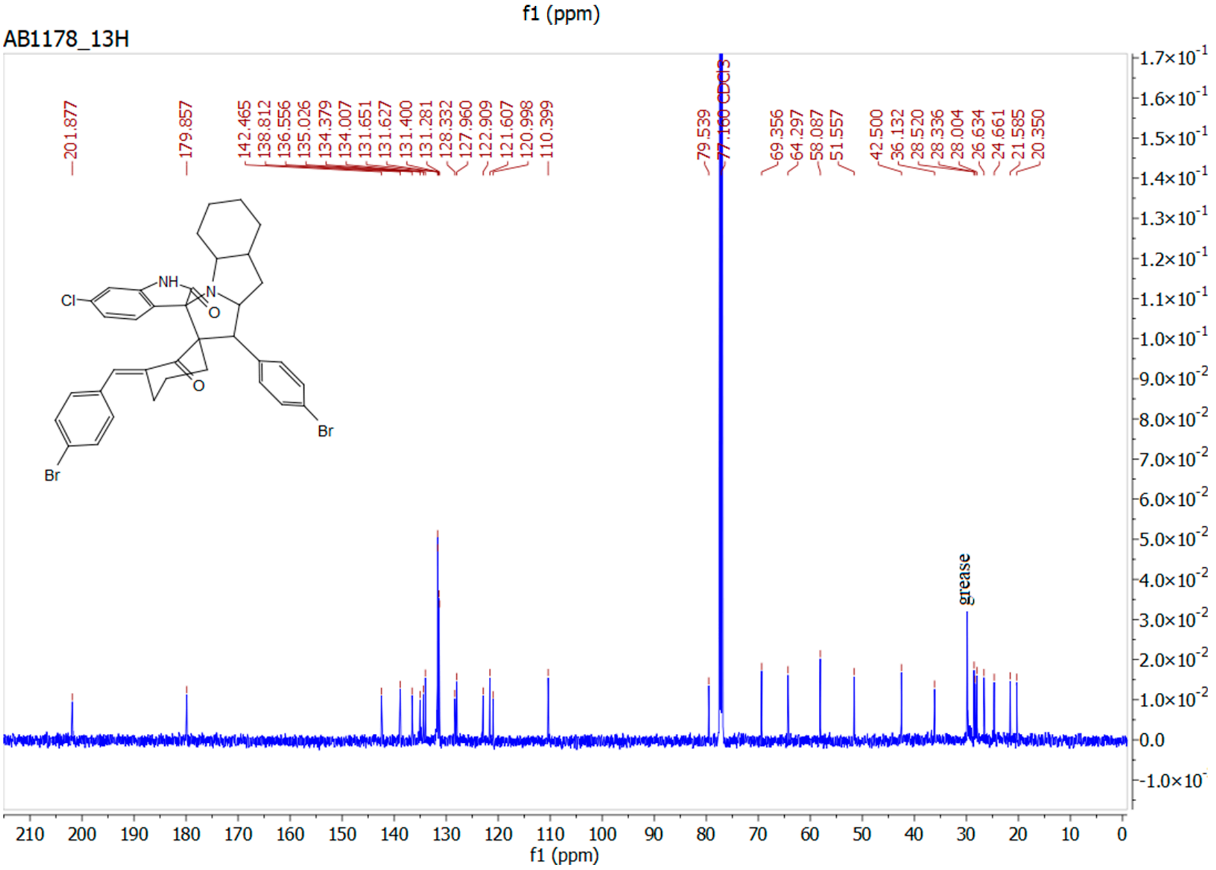
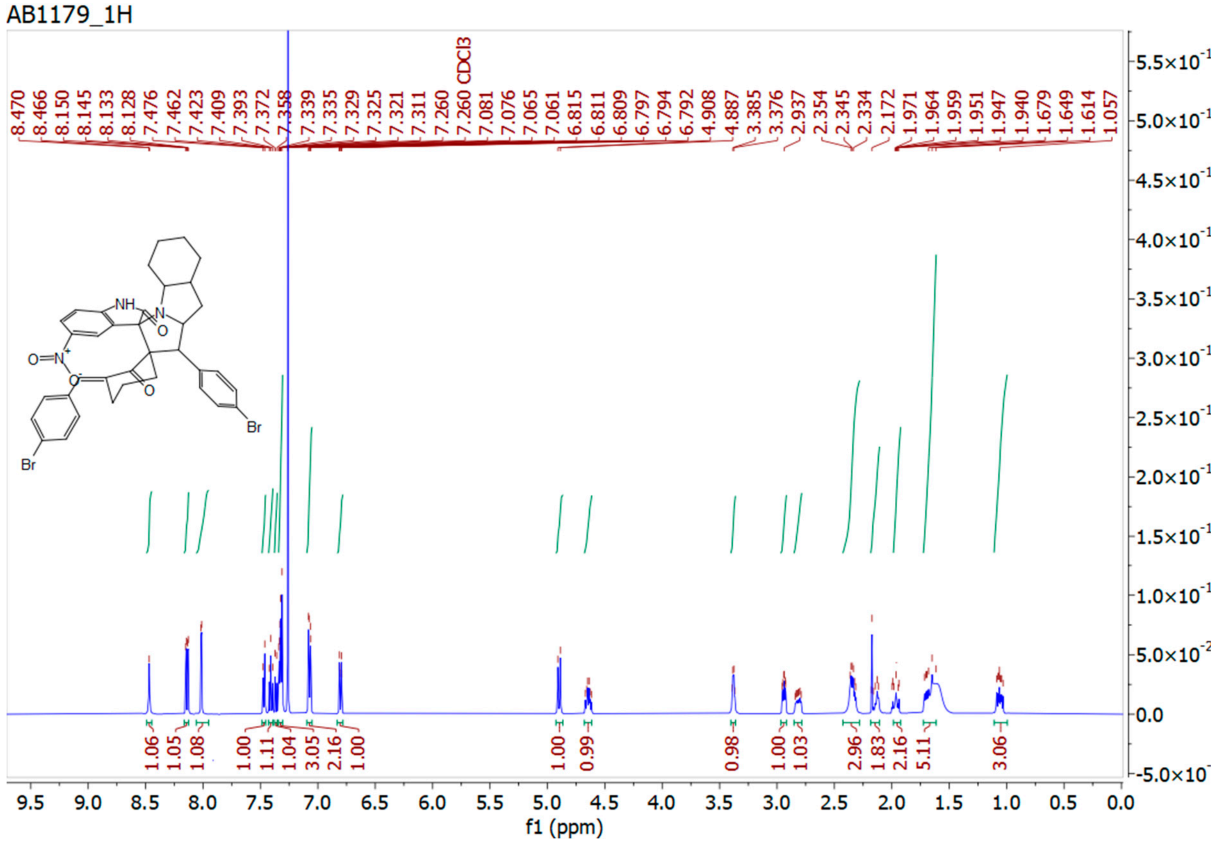


Figure S7: ^{1}H-NMR and ^{13}C-NMR for compound-**4g**



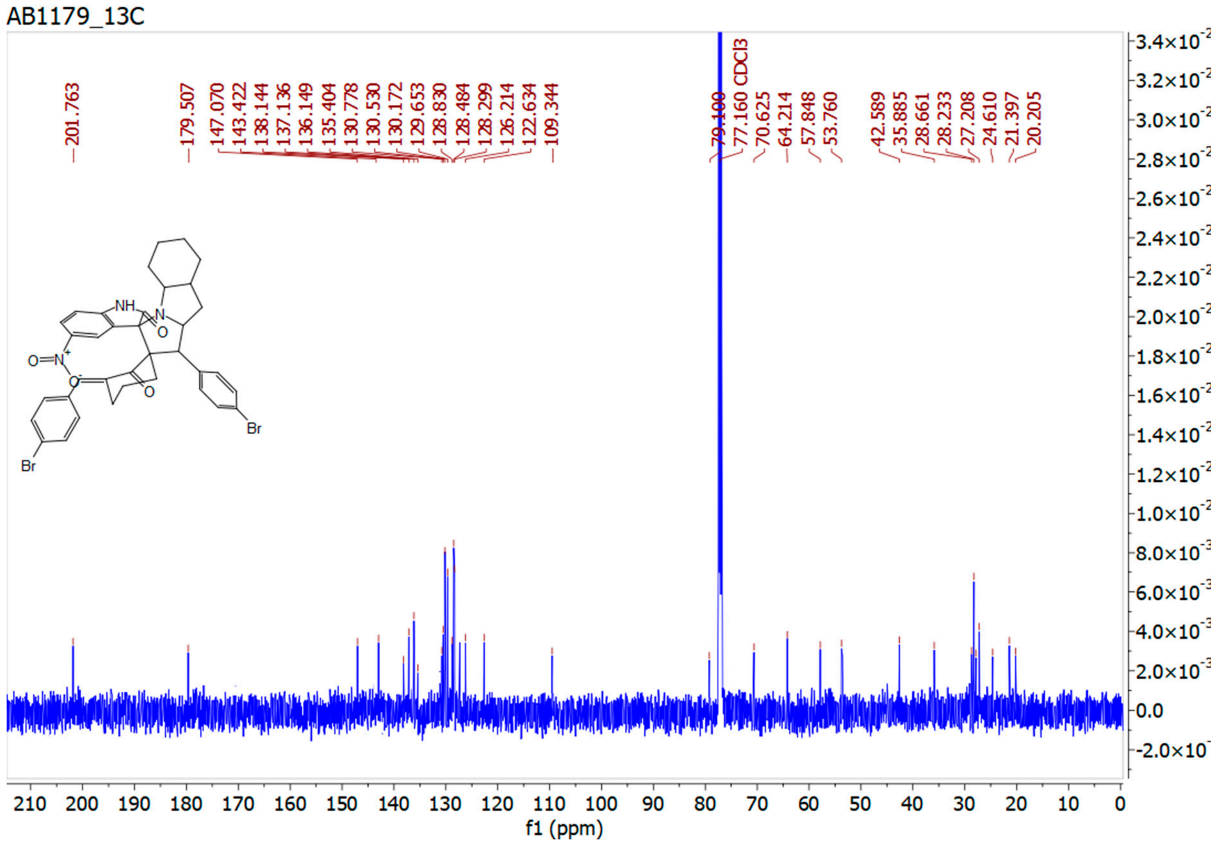
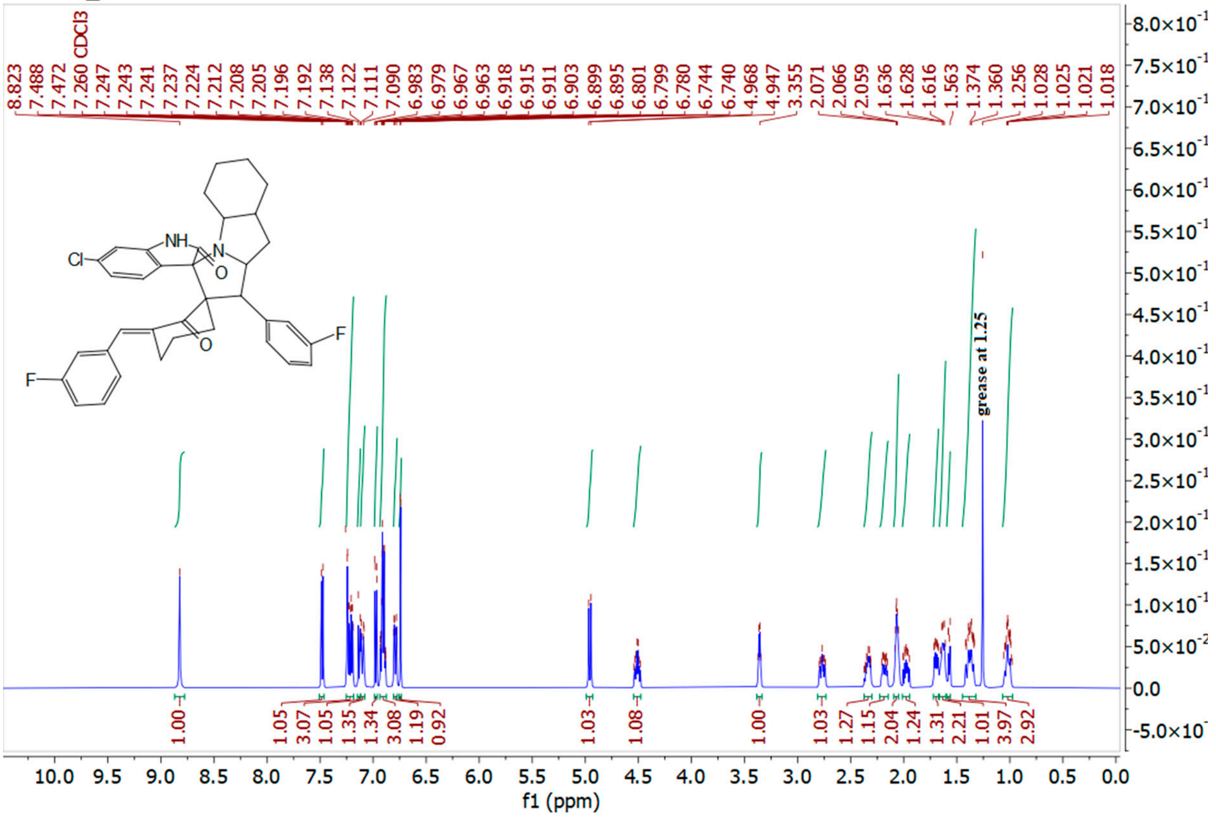


Figure S8: ^{1}H-NMR and ^{13}C-NMR for compound-**4h**

AB1181_1H



AB1181_13C

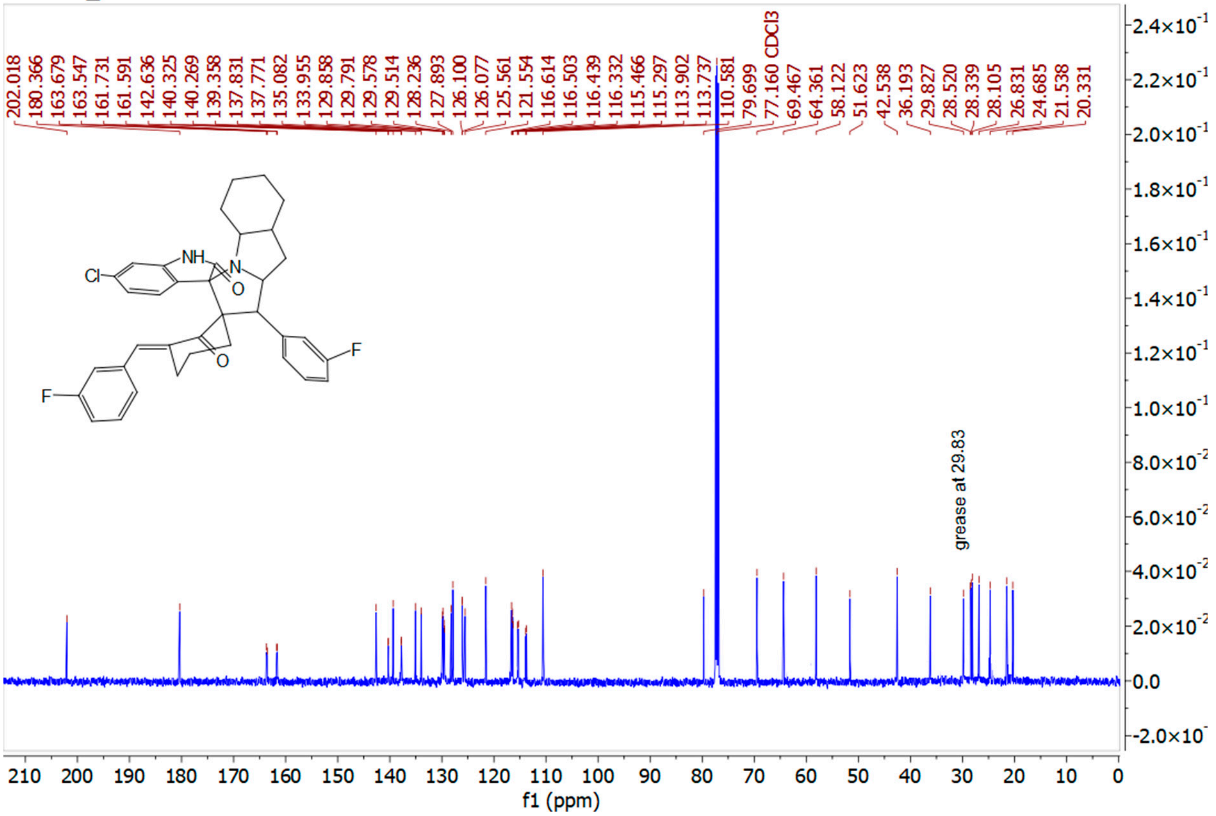
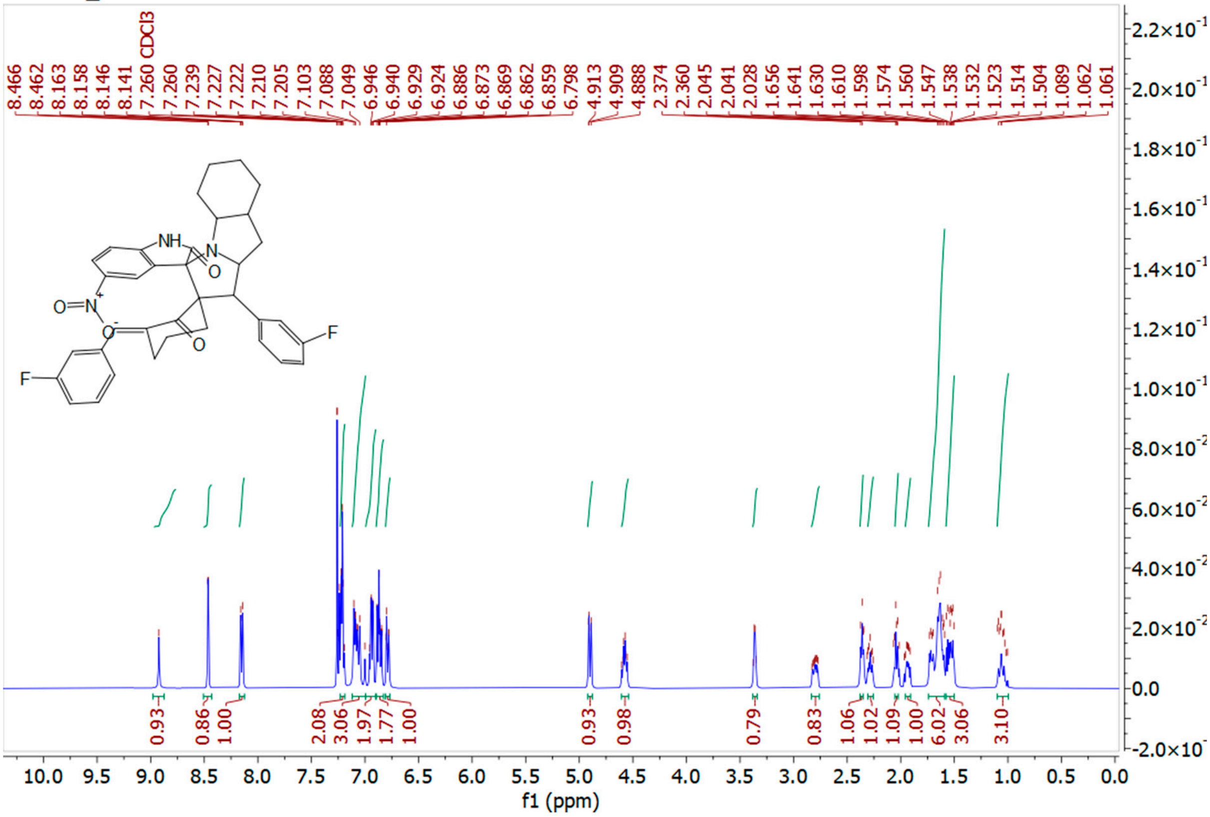


Figure S9: ^{1}H-NMR and ^{13}C-NMR for compound-**4i**

AB1182_1H



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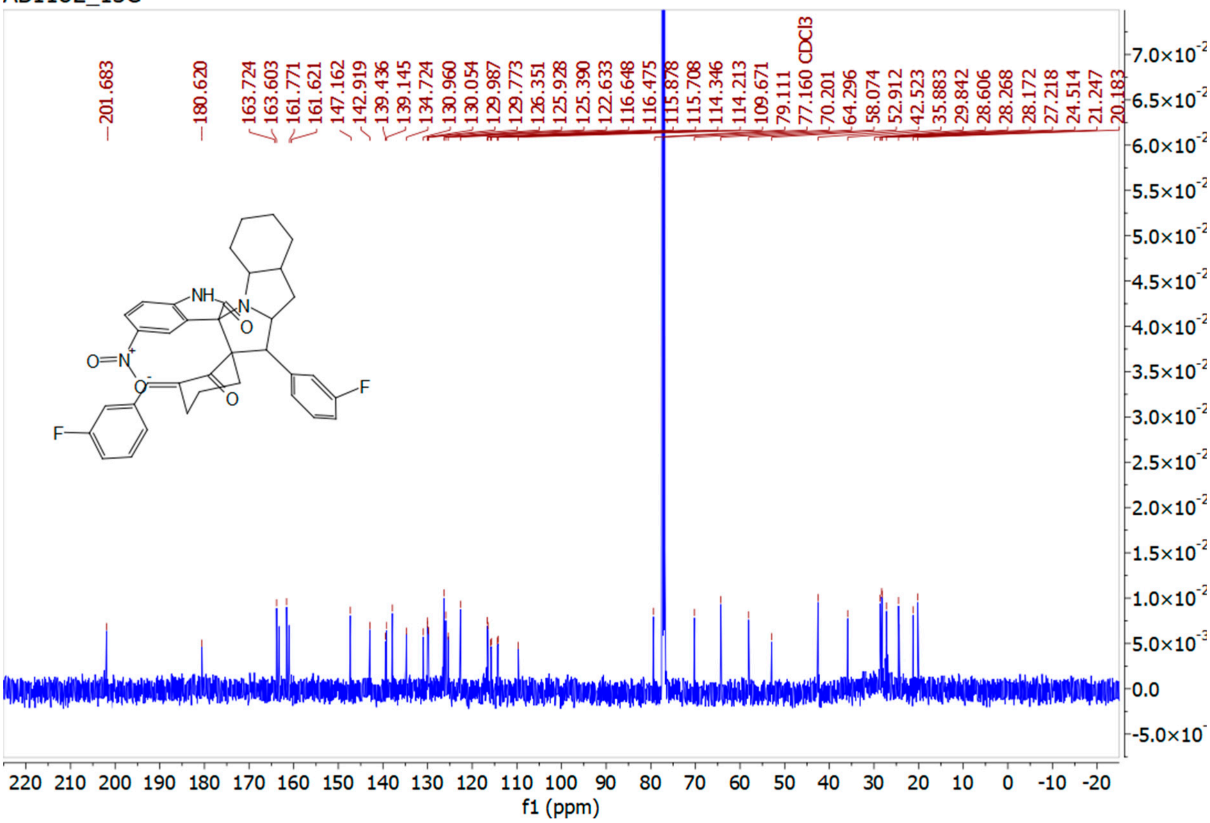
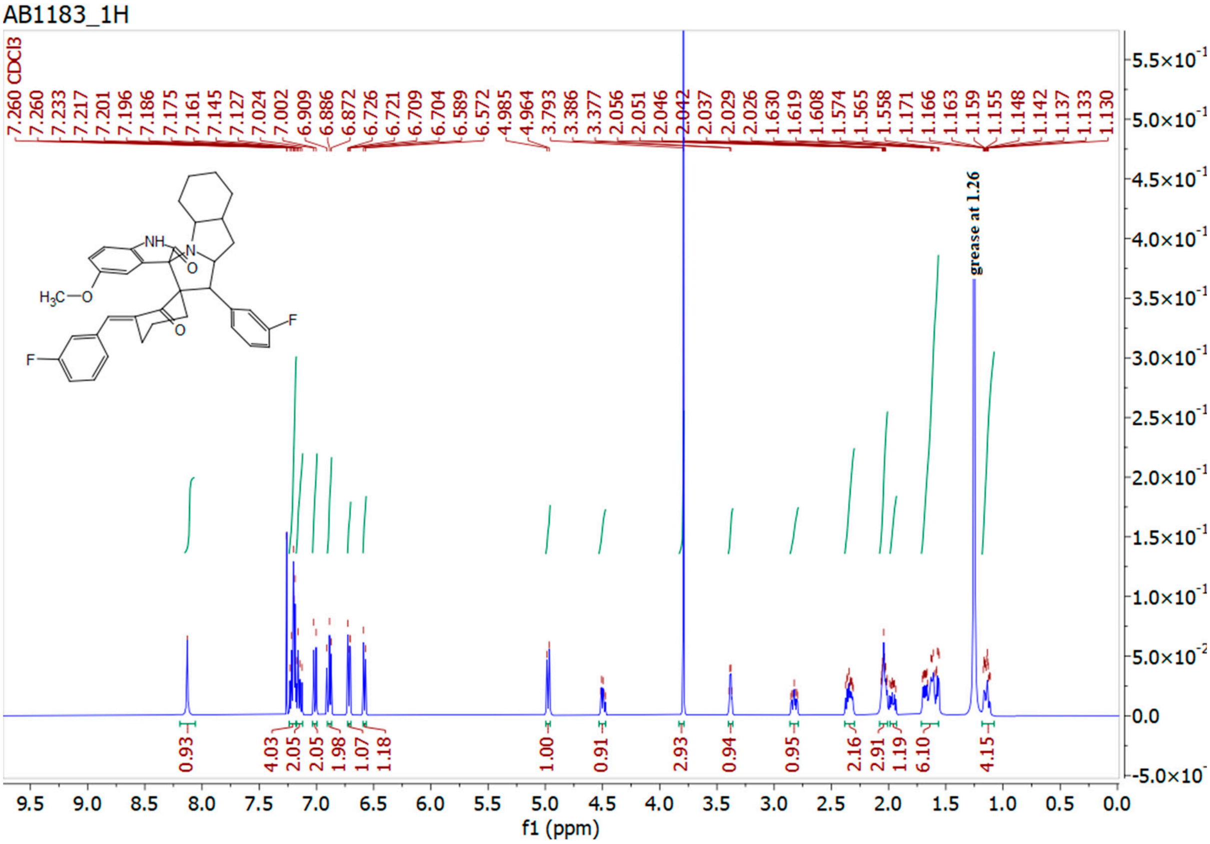


Figure S10: ^{1}H-NMR and ^{13}C-NMR for Compound-**4j**



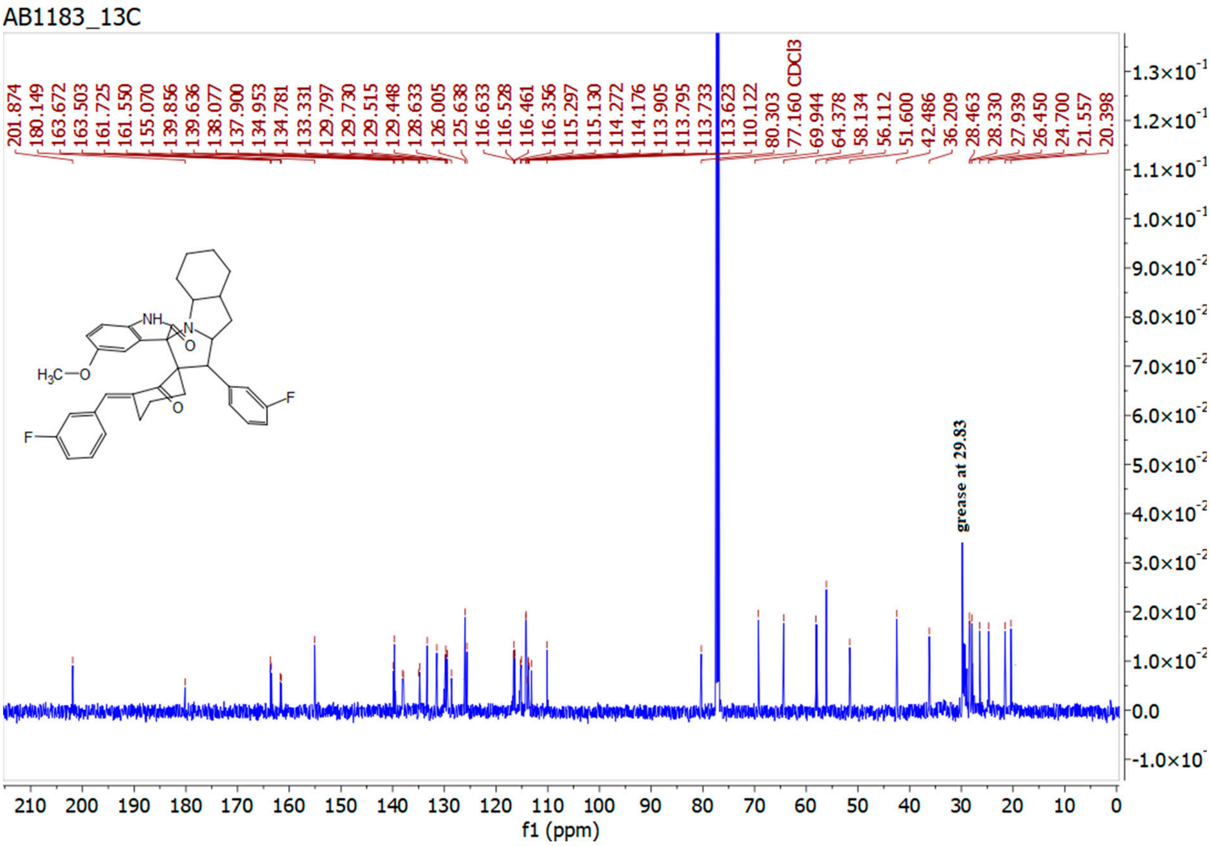
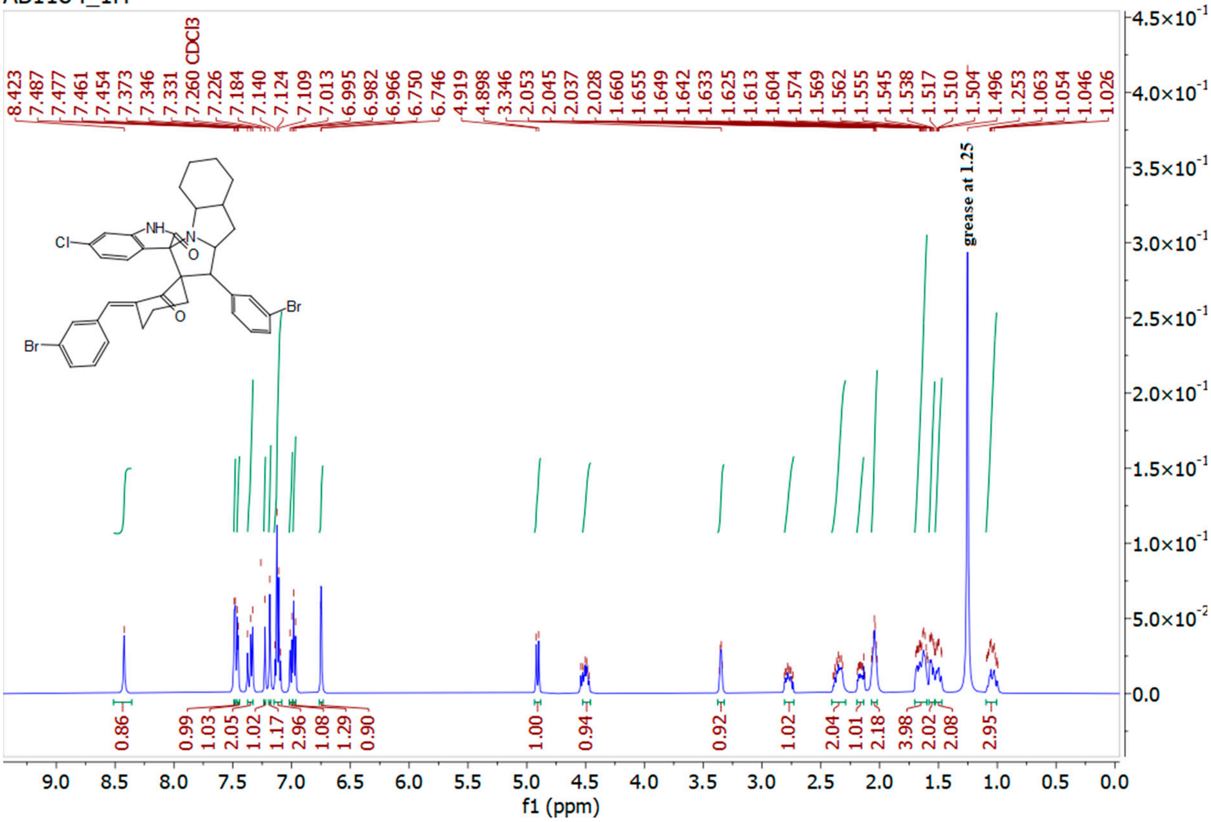


Figure S11: ^{1}H-NMR and ^{13}C-NMR for compound-**4k**

AB1184_1H



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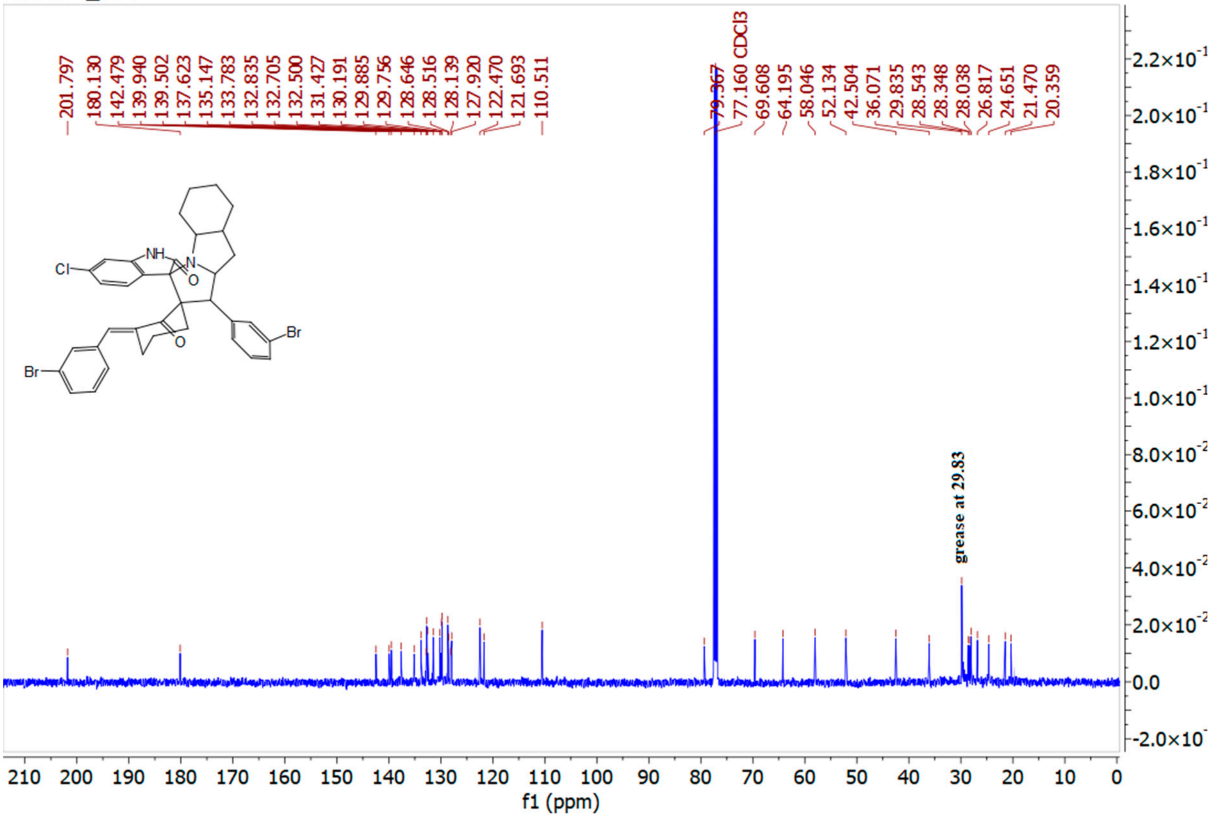
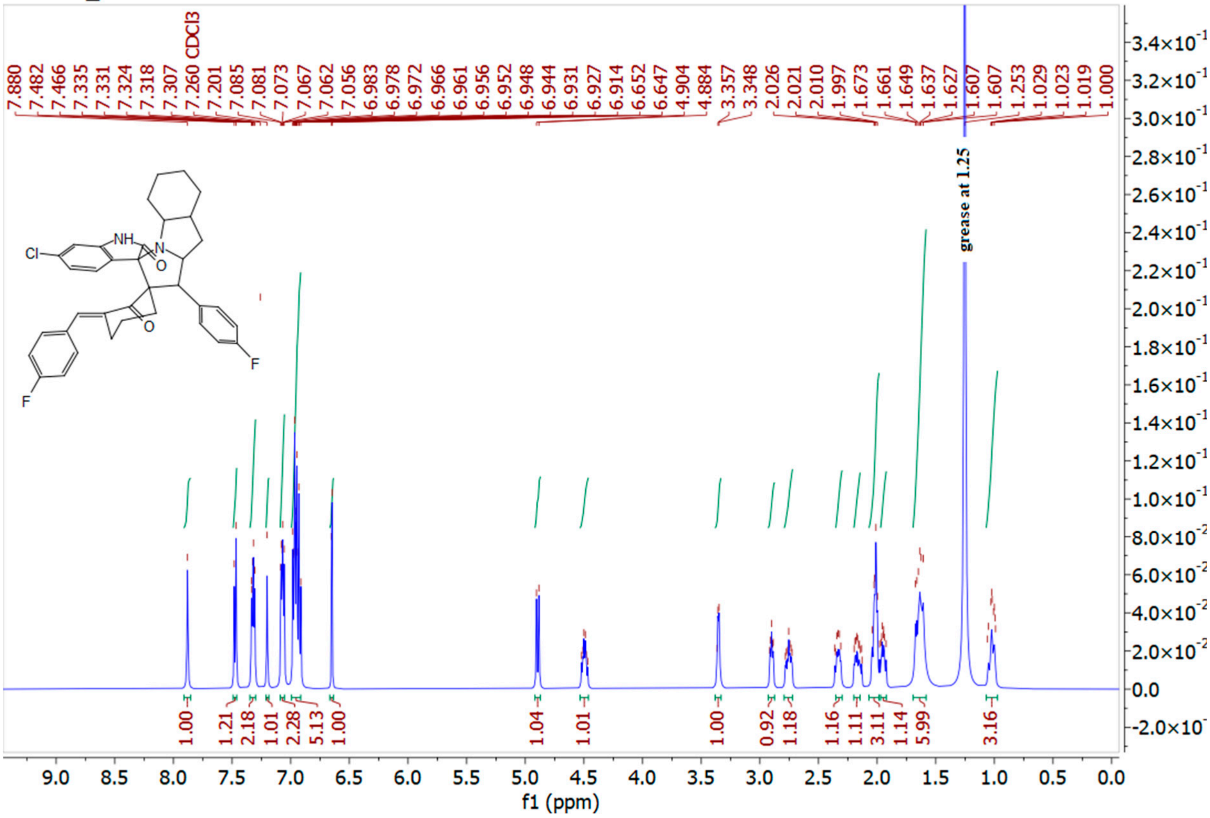


Figure S12: ^{1}H-NMR and ^{13}C-NMR for compound-**4l**

AB1187_1H



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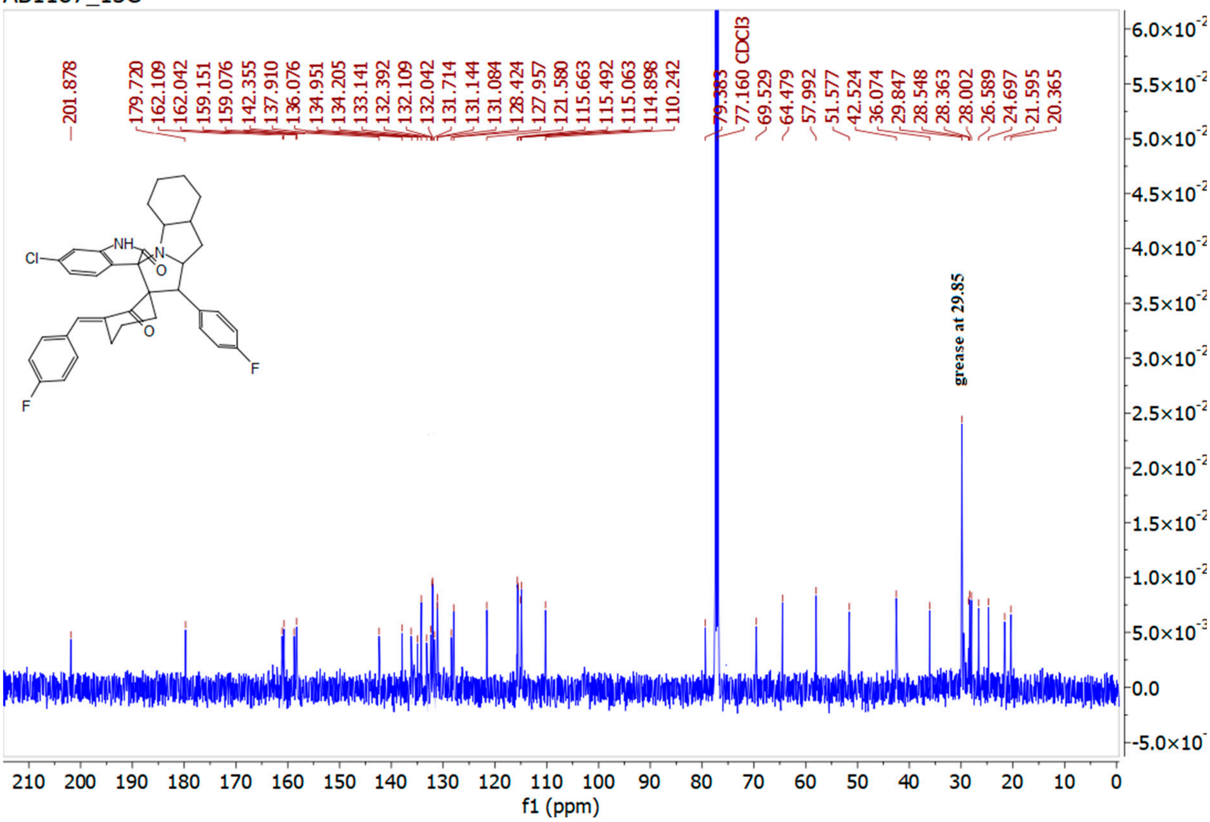
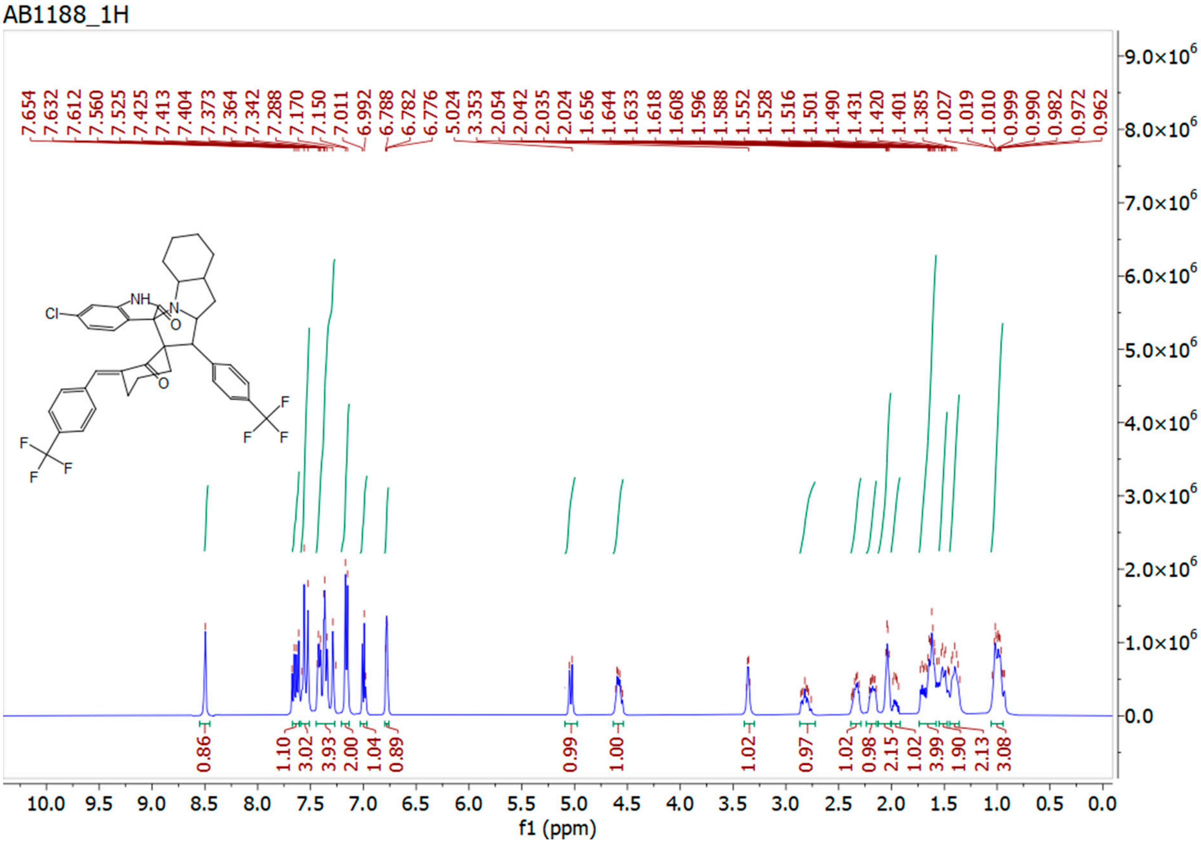


Figure S13: ^{1}H-NMR and ^{13}C-NMR for compound-**4m**



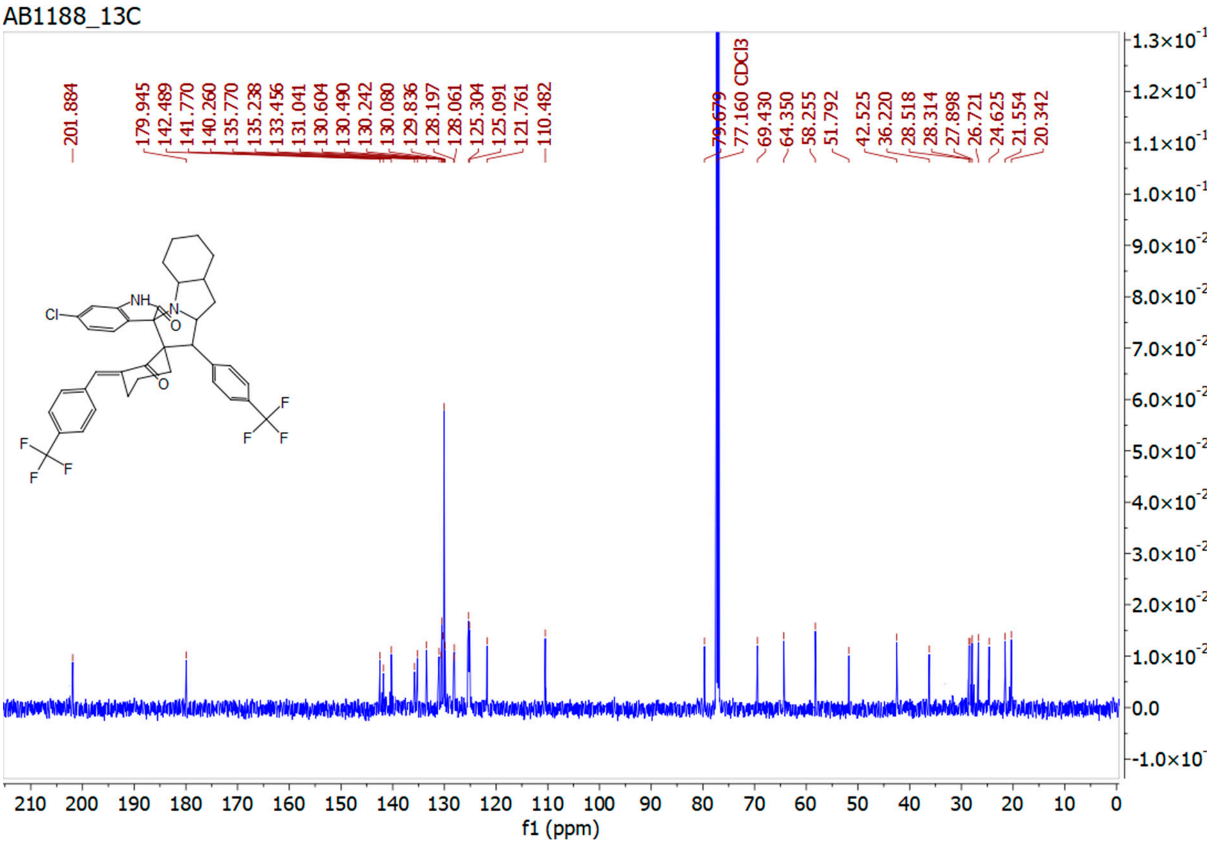
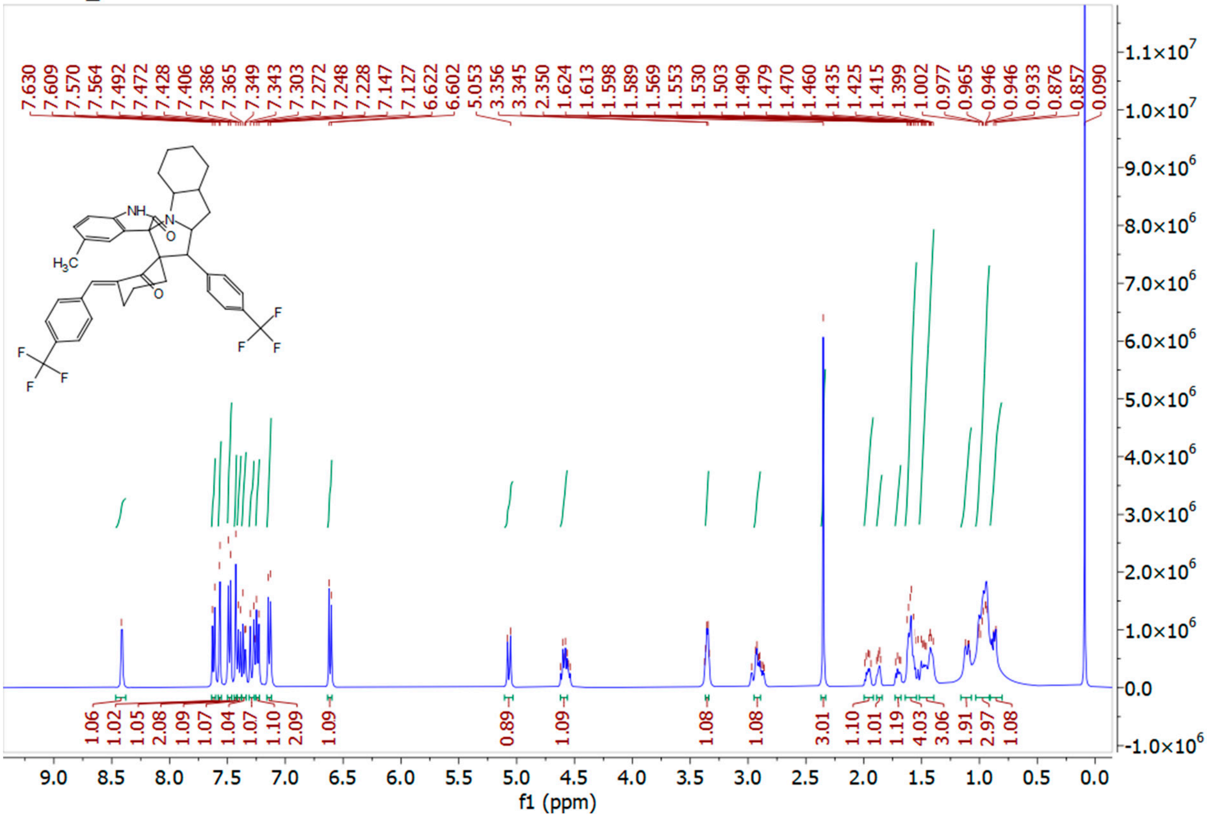


Figure S14: ^{1}H-NMR and ^{13}C-NMR for compound-**4n**

AB1189_1H



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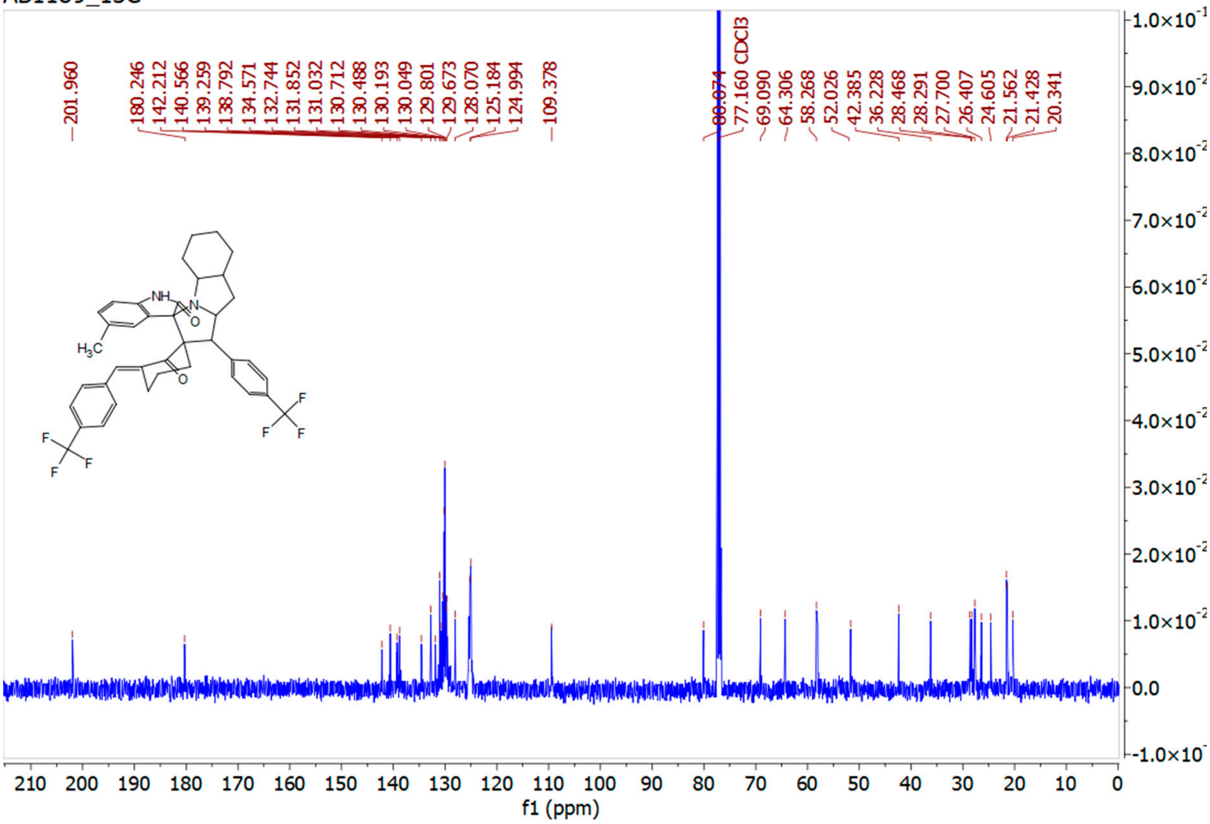
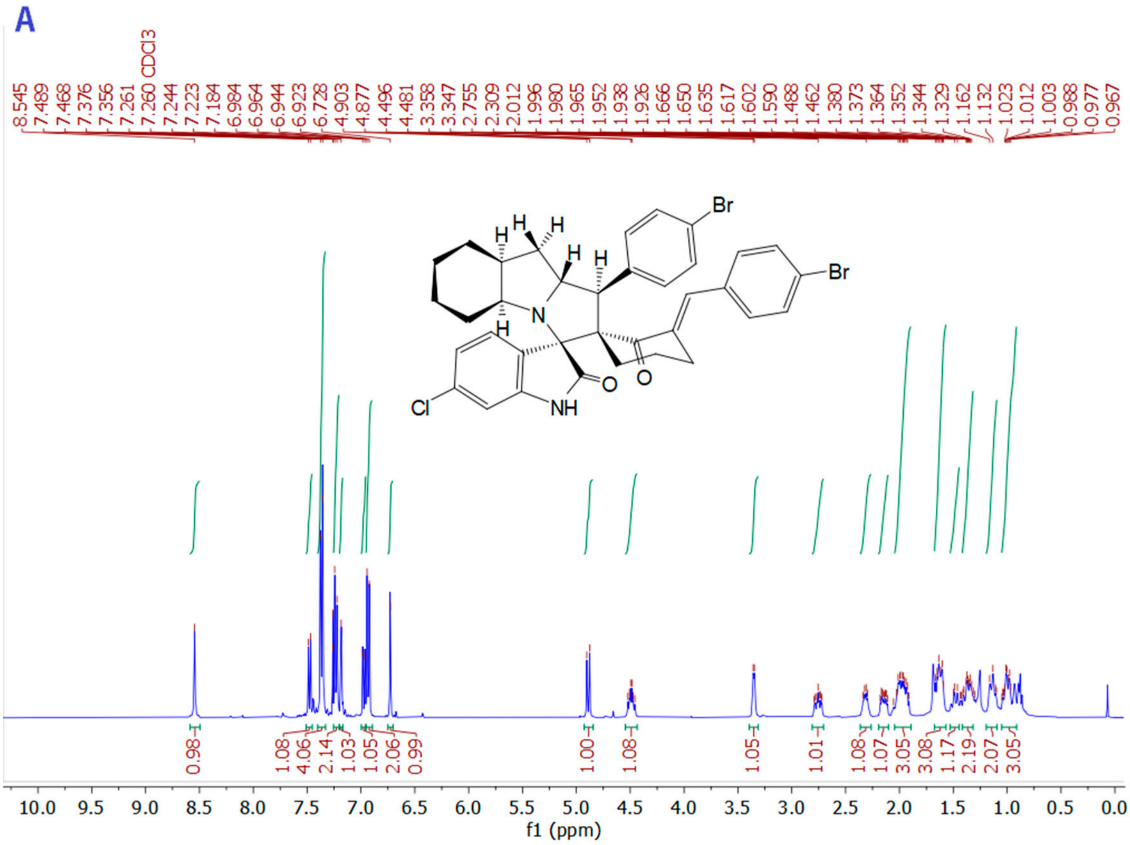


1D- &2D-NMR deep investigation for compound **4f**

Figure S15: ^{1}H-NMR and ^{13}C-NMR for compound-**4f**



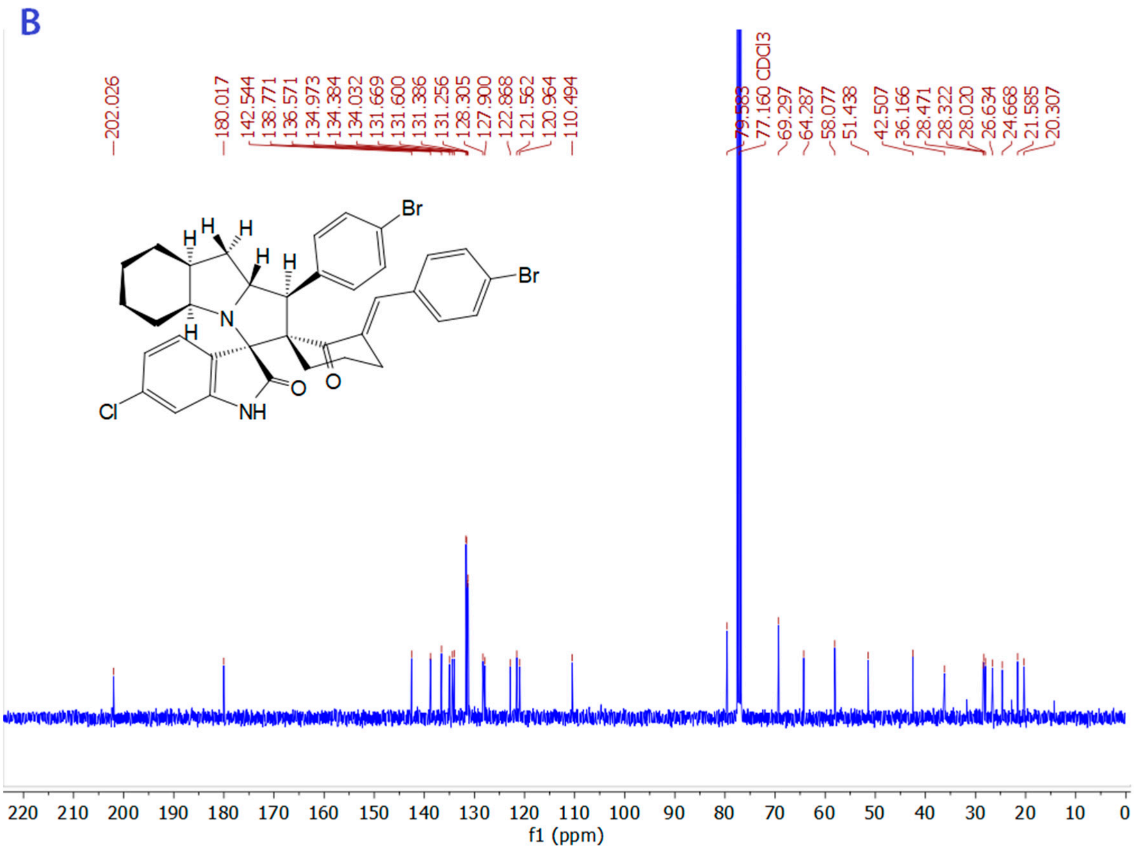


Figure S16: DEPT-135- NMR for compound **4f** expanded aliphatic region.

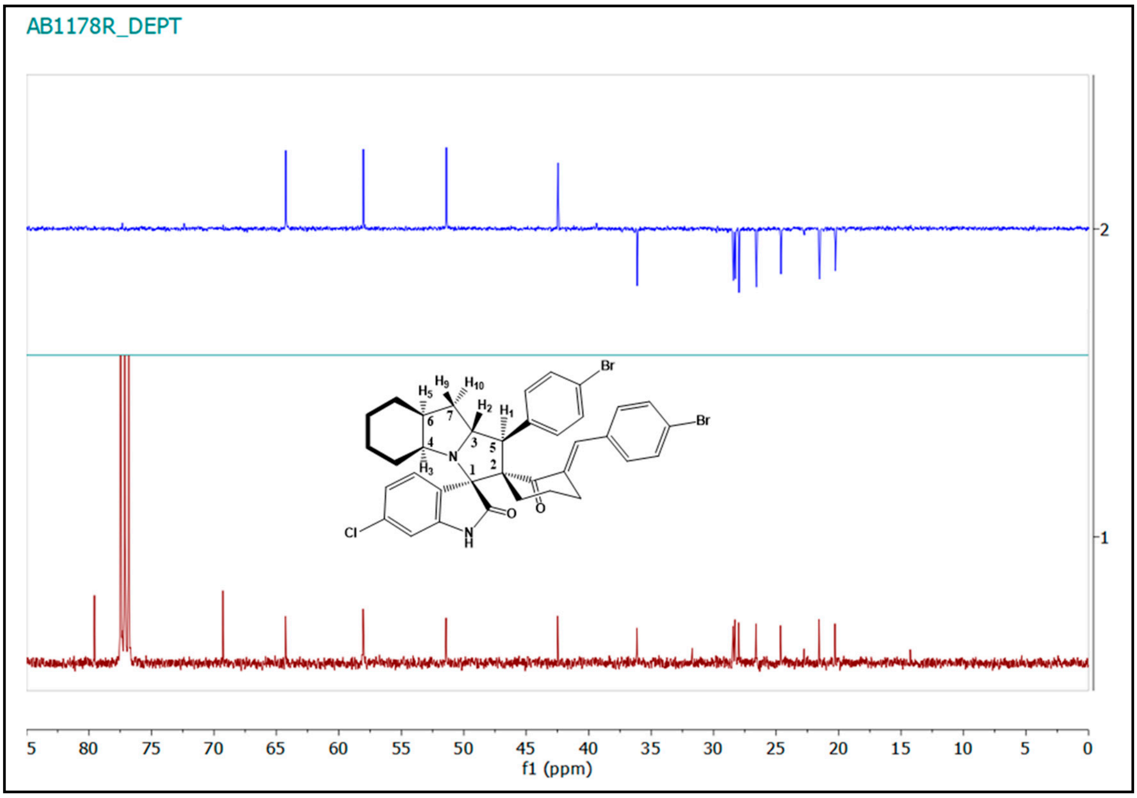
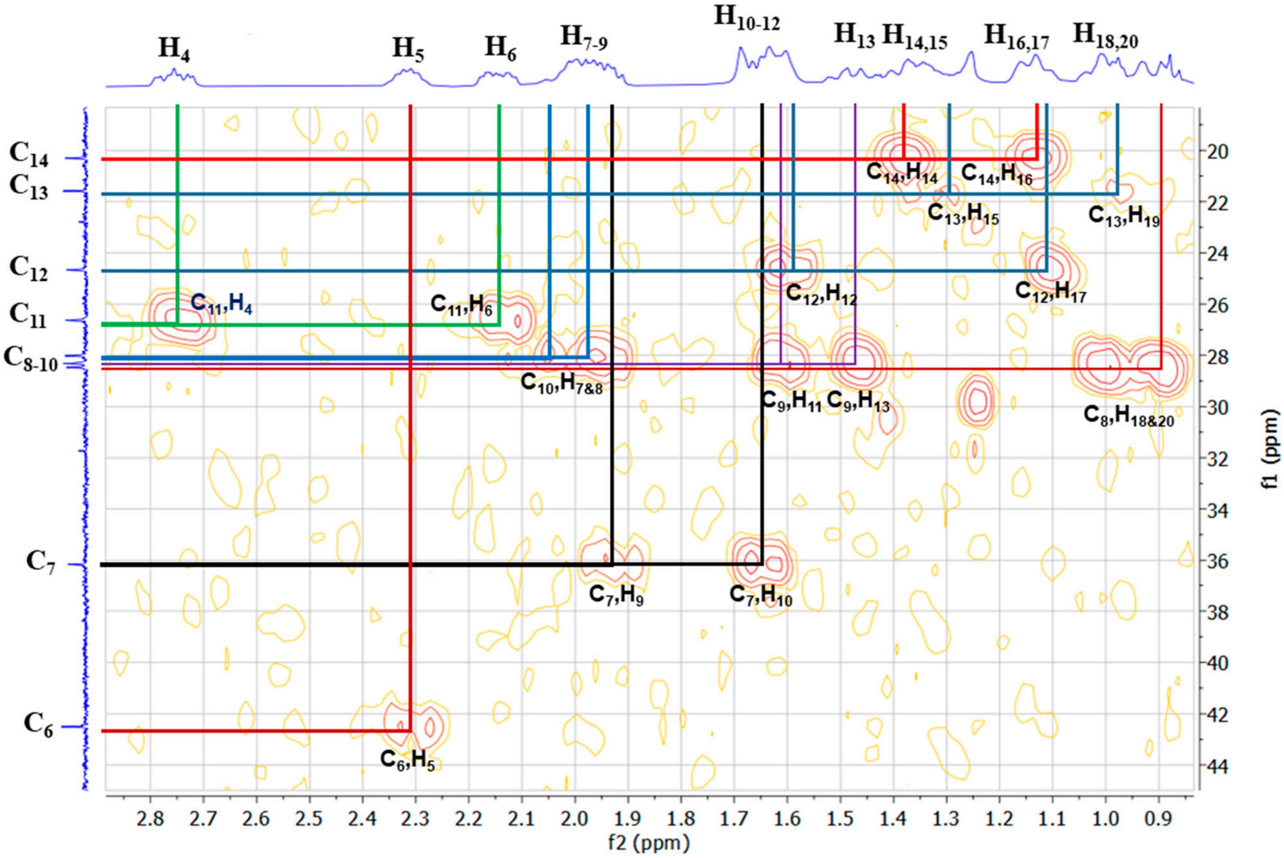


Figure S17: HMQC- NMR for compound **4f** more expanded aliphatic region.



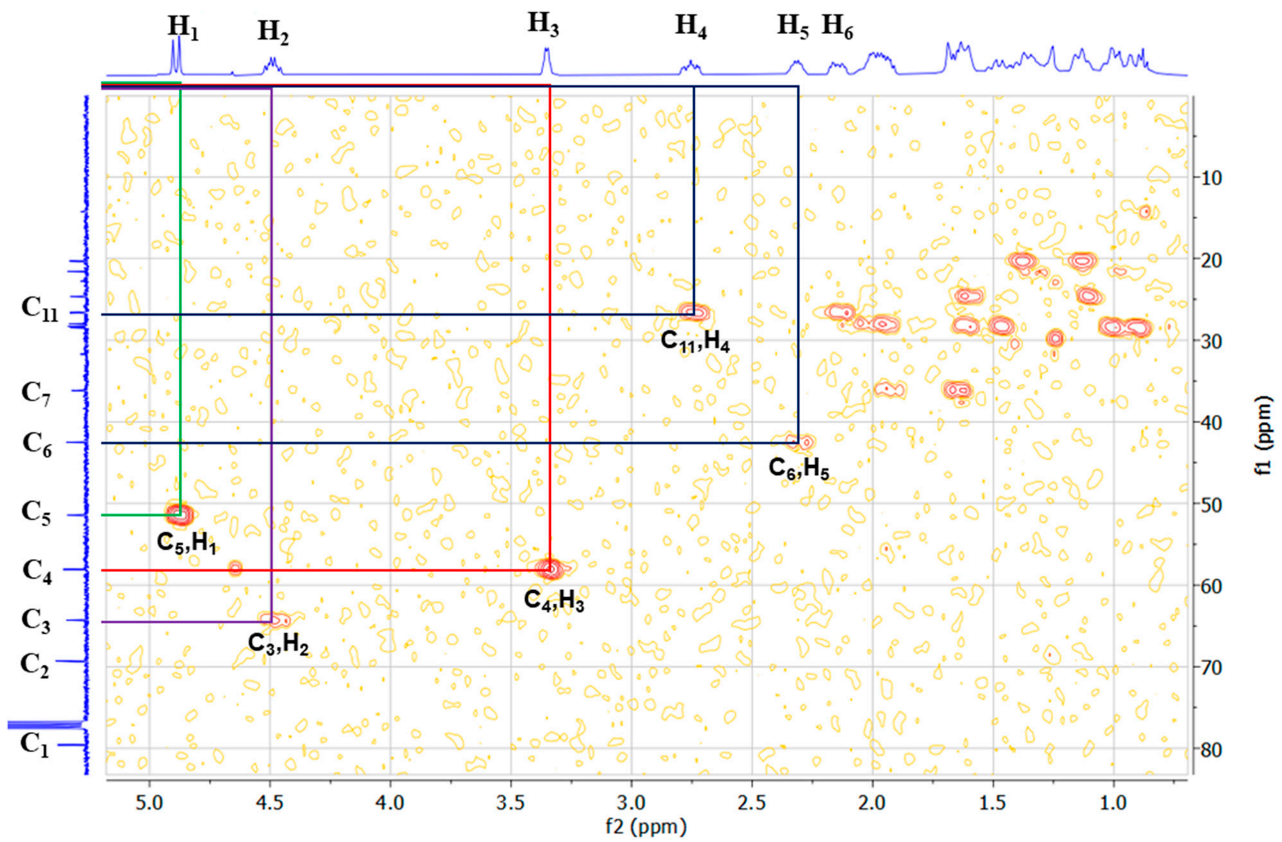
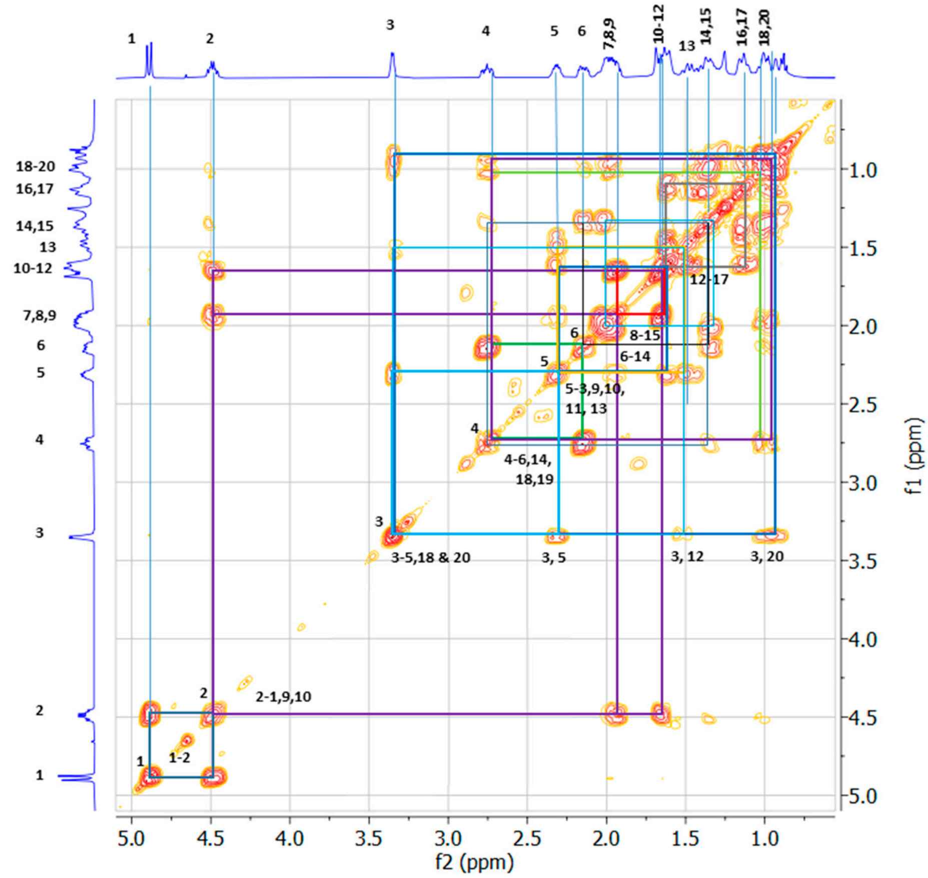


Figure S18: COSY- NMR for compound **4f** more expanded aliphatic region.



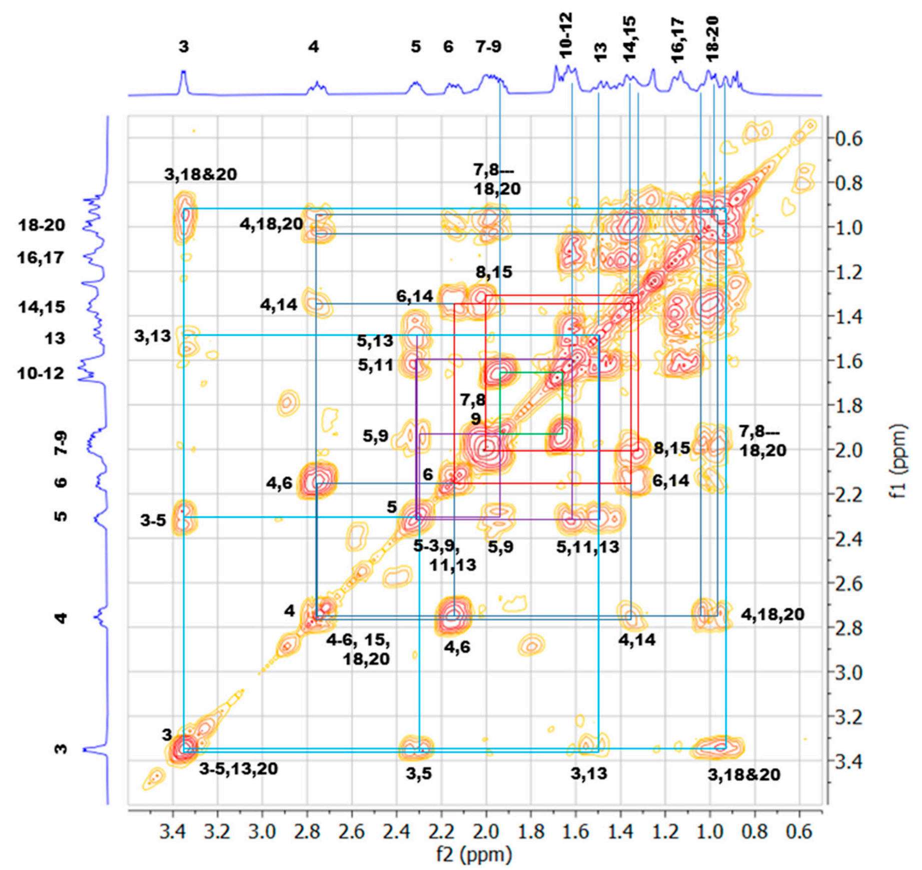
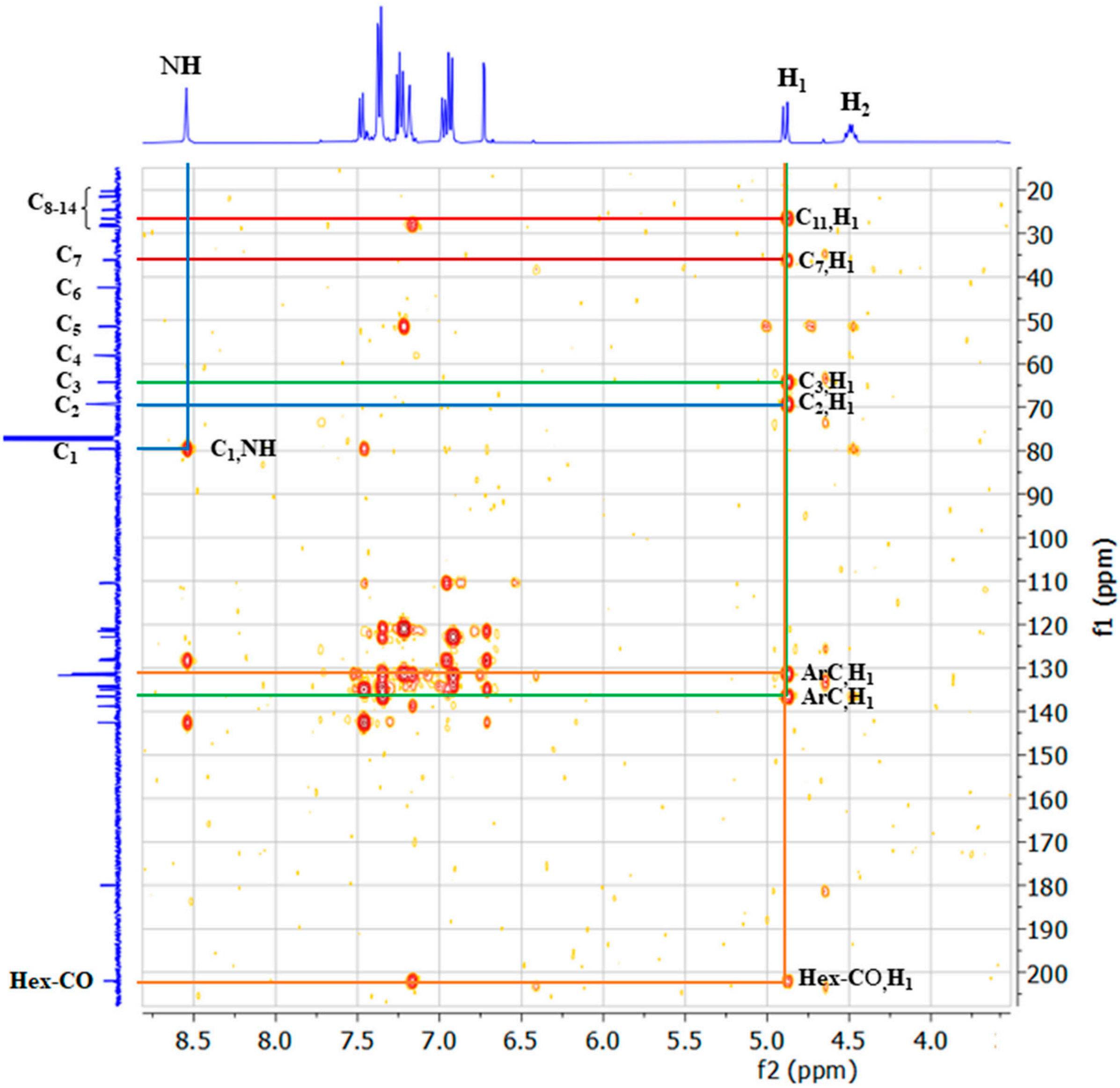


Figure S19: HMBC- NMR for compound **4f**.



The Biological Activity Assays Protocols

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, PC3 cells (Prostate Cancer) / BJ *Human fibroblast* cells / HeLa cells (Cervical Cancer) / MCF-7 and MDA-MB-231 breast cancer cell line were cultured in Dulbecco's Modified Eagle Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 μg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $1x10^5$ cells/ml was prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1-30μM). After 48 hrs, 200 μL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for PC3 cells. The percent inhibition was calculated by using the following formula:

% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

The results (% inhibition) were processed by using Soft- Max Pro software (Molecular Device, USA).

STANDARD DRUG:

Standard drug used in the MTT assay was doxorubicin.

References

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3. Price, P.; McMillan1, T.J. Use of the tetrazolium assay in measuring the response of human tumor cells to ionizing radiation. *Cancer Research* **1990**, *50*, 1392–1396. Retrieved from https://cancerres.aacrjournals.org/content/canres/50/5/1392.full.pdf. (access date: 1 June 2020).
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5. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63, doi:10.1016/0022-1759(83)90303-4.