SUPPORTING INFORMATION

Targeting Triple-negative Breast Cancer by a Novel Proteolysis Targeting Chimera (PROTAC) Degrader of Enhancer of Zeste Homolog 2 (EZH2)

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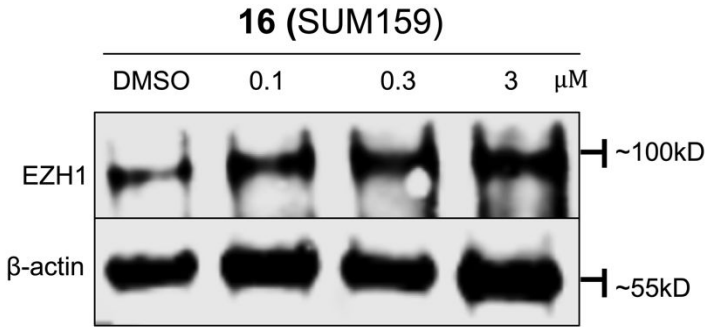
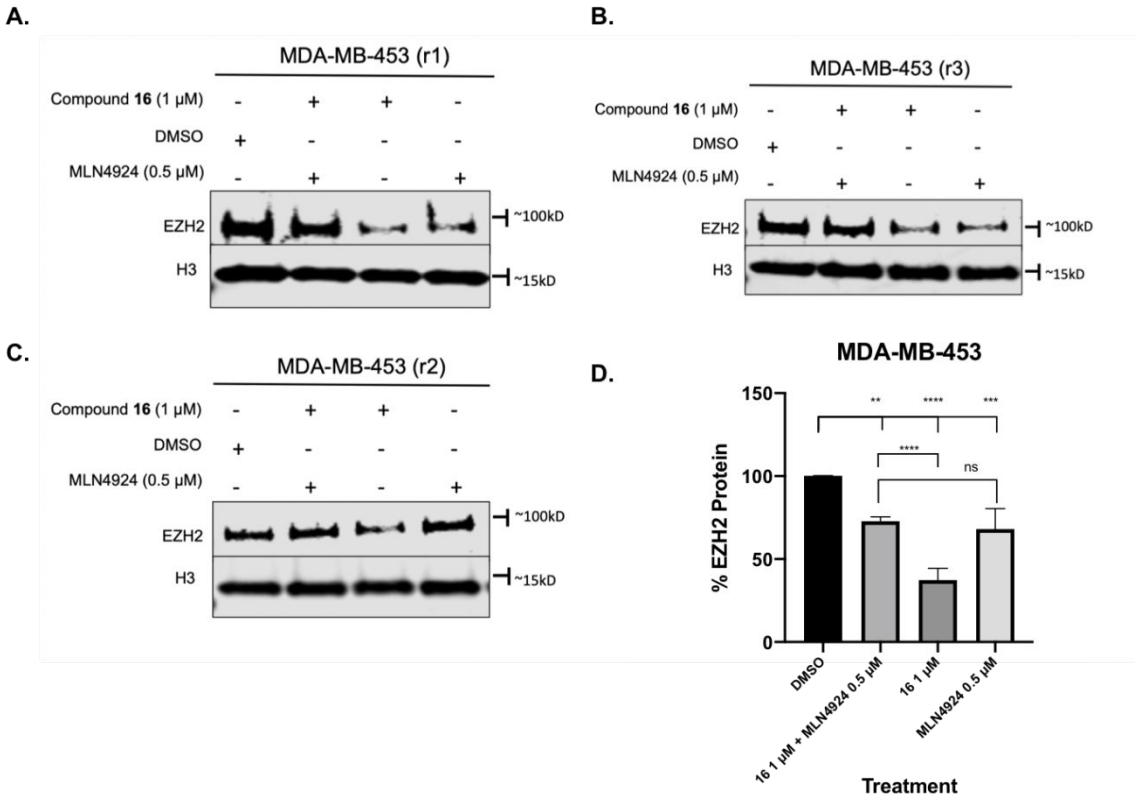
1. **Figure S1**. The effect of **16** on reducing the EZH1 protein level in SUM159 cells. SUM159 cells were treated with DMSO or **16** at the indicated concentration for 48 h. The cell lysates were analyzed by western blotting to examine the EZH1 protein level. β-Actin was used as the loading control. Results shown are representative of at least two independent experiments.

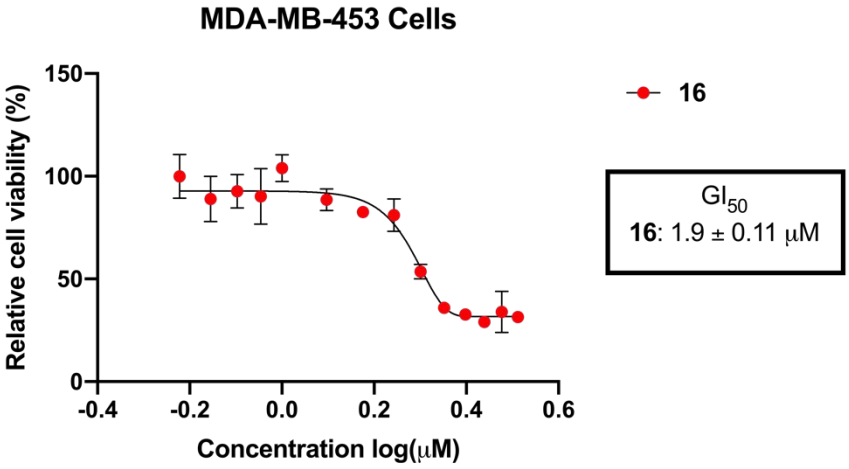


2. **Figure S2.** EPZ-6438 effectively reduces the H3K27me3 in MDA-MB-453 cells. MDA-MB-453 cells were treated with DMSO or EPZ-6438 at indicated concentrations for 48 h. Cells were harvested and the H3K27me3 protein level was assessed by western blotting. β-Actin was utilized as the loading control. Results are representative of at least two independent experiments.

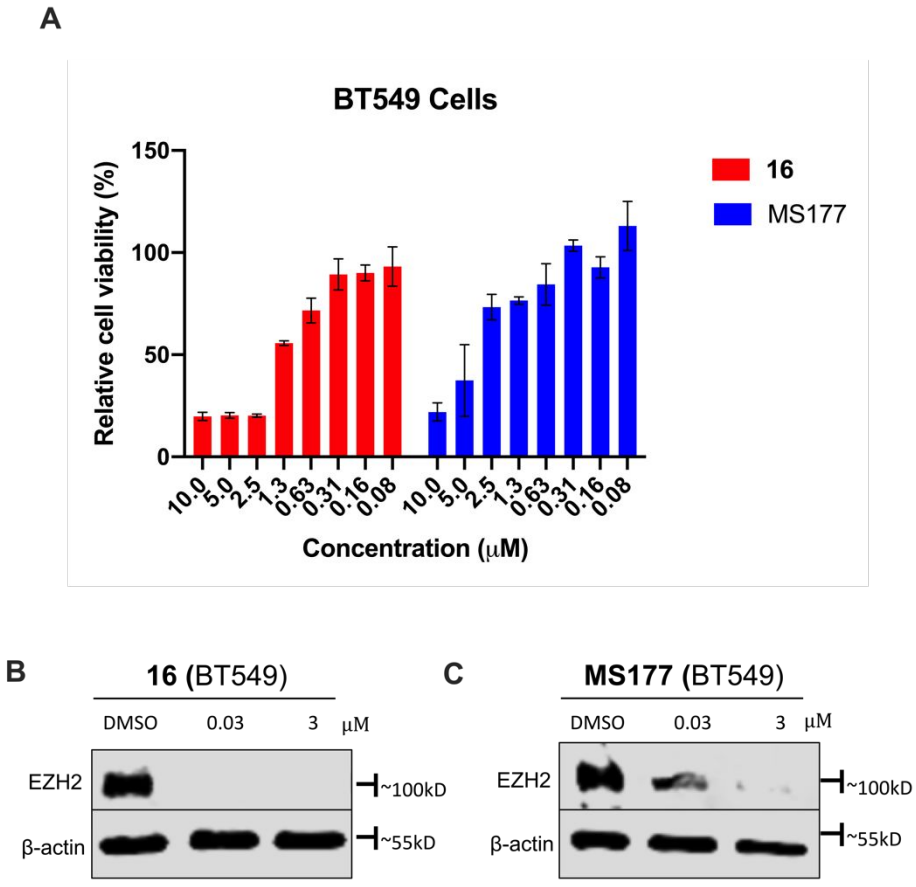
3. **Figure S3.** The EZH2 degradation induced by compound **16** is rescued by NEDD8 activating E1 enzyme (NAE) inhibitor MLN4924. (A-C) MDA-MB-453 cells were pretreated with MLN4924 (0.5 μM) for 30 min and then co-incubated with compound **16** (1 μM) for 24 h. The EZH2 protein level was determined by western blot analysis. H3 was used as the loading control. (D) Quantification of western blot replicates A-C and Figure 7C.



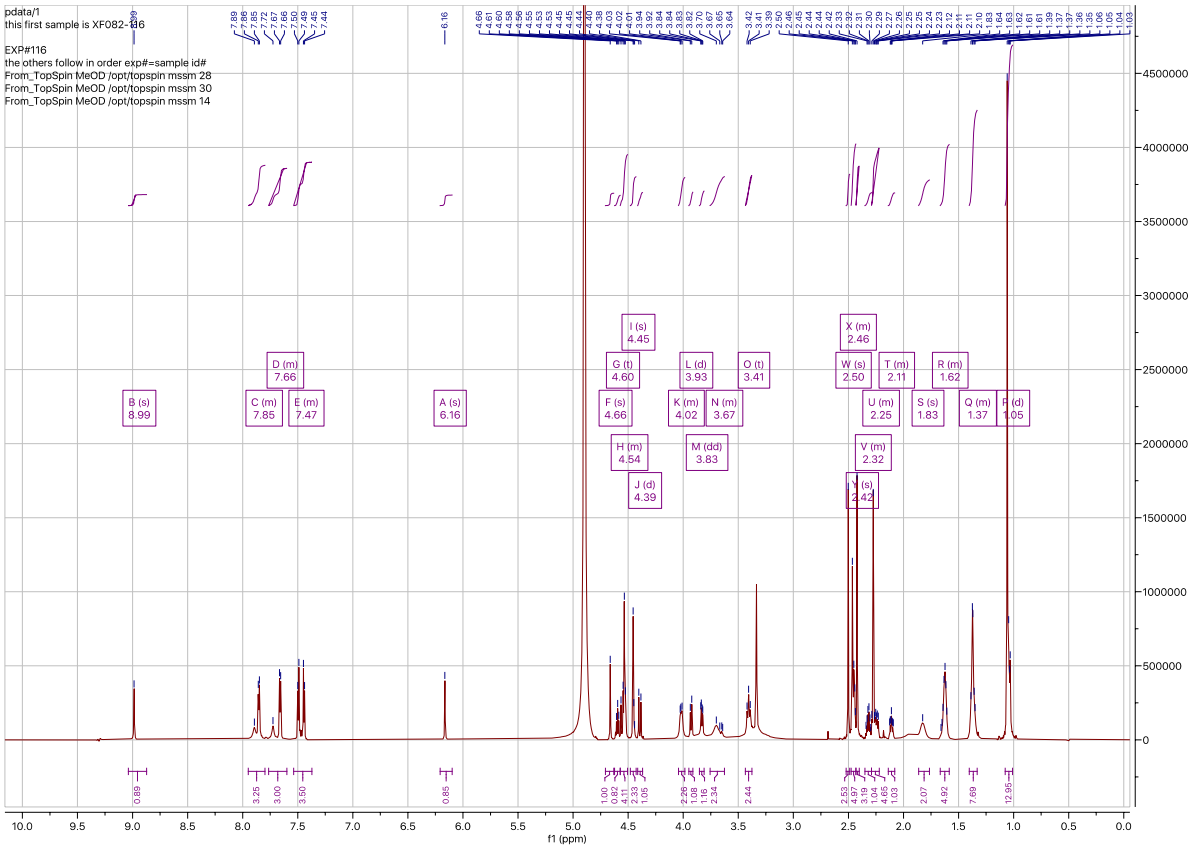
4. **Figure S4**. Compound **16** inhibits the growth of MDA-MB-453 cells. MDA-MB-453 cells were treated with serial dilution (from 3.2 to 0.6 μM, with 1.1-fold dilution, 14 points) of **16** for 5 days. Cell viability was determined using a WST-8 assay. GI_{50} result shown is the mean values ± SD from three independent experiments.



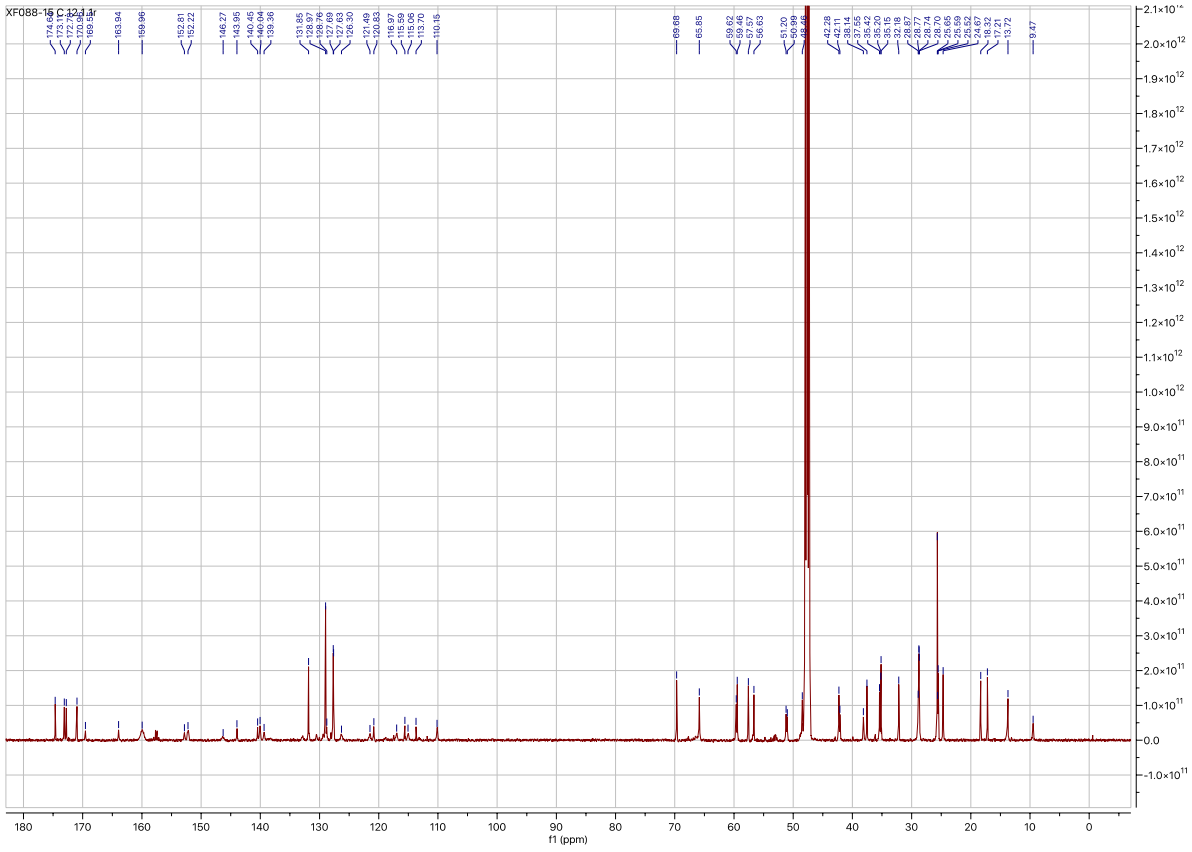
5. **Figure S5.** Compound **16** displays superior growth inhibition and EZH2 degradation to MS177 in BT549 cells. (A) BT549 cells were treated with MS177 or **16** for 5 d and then evaluated for cellular growth inhibition utilizing the WST-8 assay. Results shown are the mean values ± SD from three independent experiments. BT549 cells were treated with **16** (B) or MS177 (C) for 48 h at indicated concentrations and then analyzed by western blotting to assess for the EZH2 protein level. β-Actin was used as the loading control. Results shown are representative of two independent experiments.



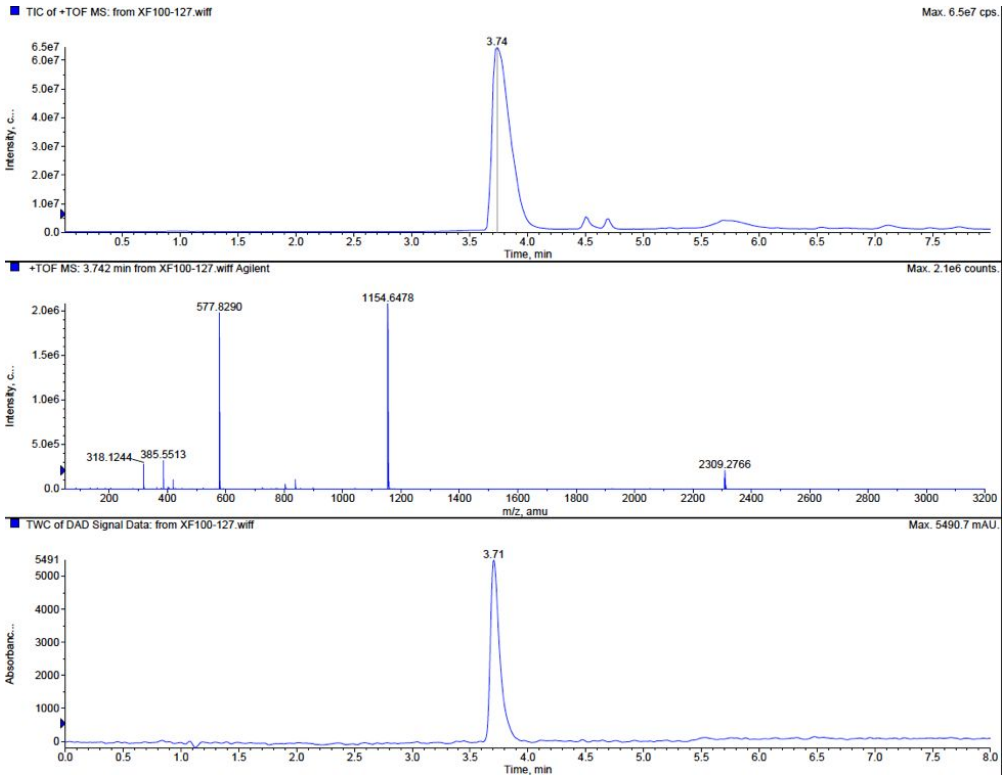
6. ^{1}H NMR spectrum of compound **16**.



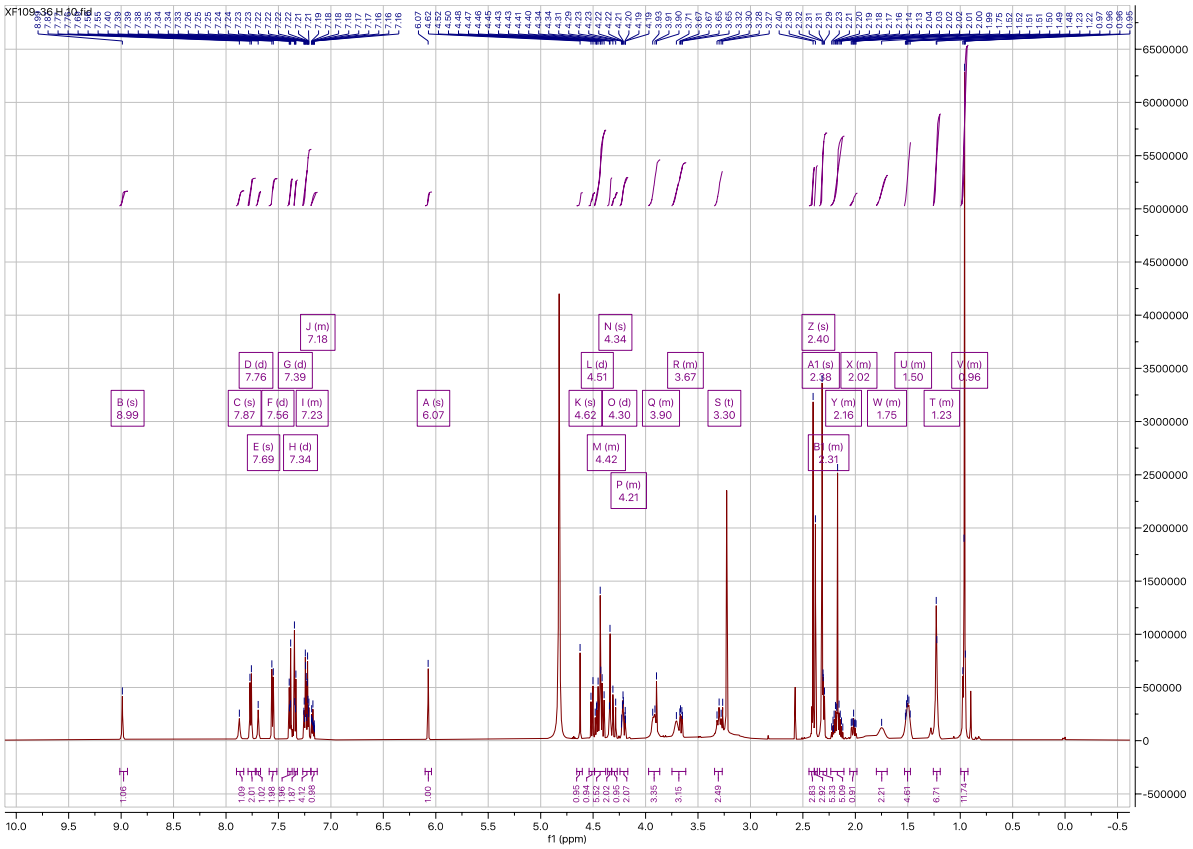
7. ^{13}C NMR spectrum of compound **16**.



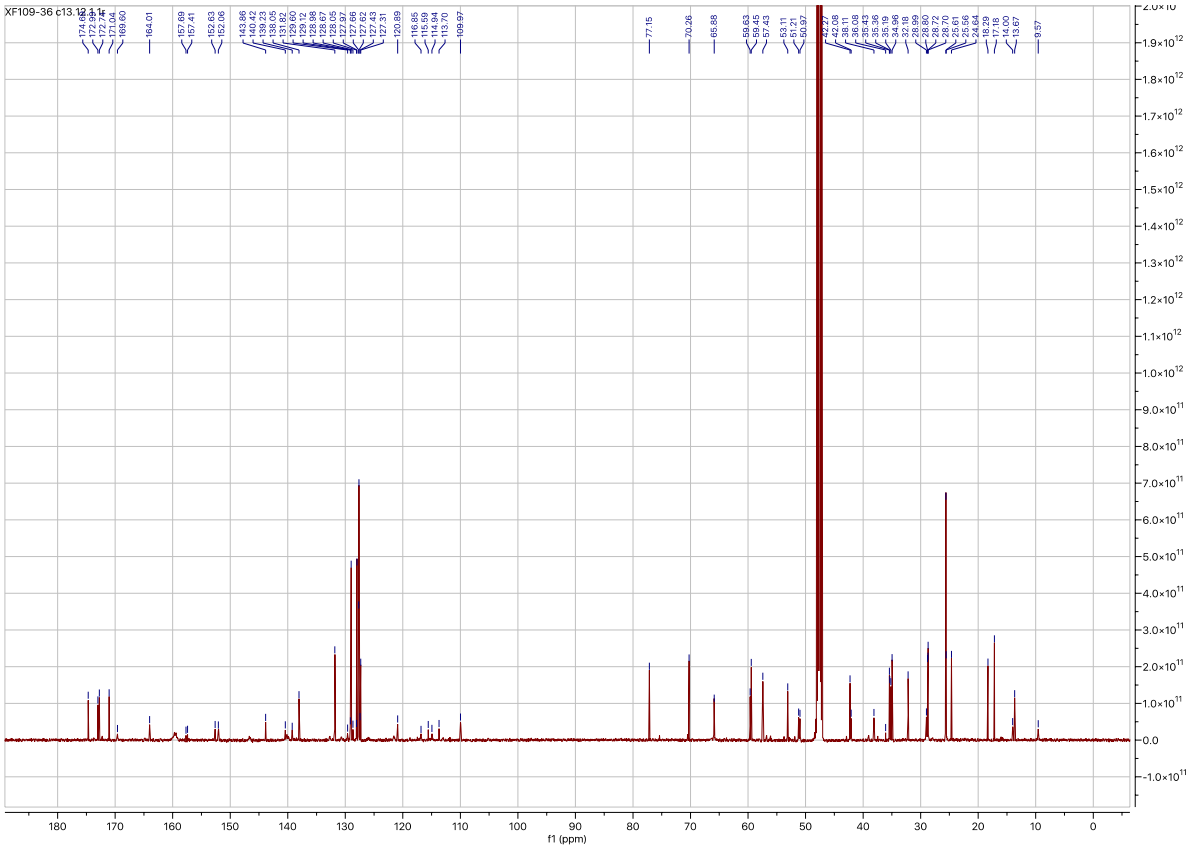
8. HPLC-HRMS spectrum of compound **16**.



9. ^{1}H NMR spectrum of compound **17**.



10. ^{13}C NMR spectrum of compound **17**.



11. HPLC-HRMS spectrum of compound **17**.