

# In Silico Discovery and Optimisation of a Novel Structural Class of Hsp90 C-Terminal Domain Inhibitors

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Representative  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR spectra and HPLC chromatograms

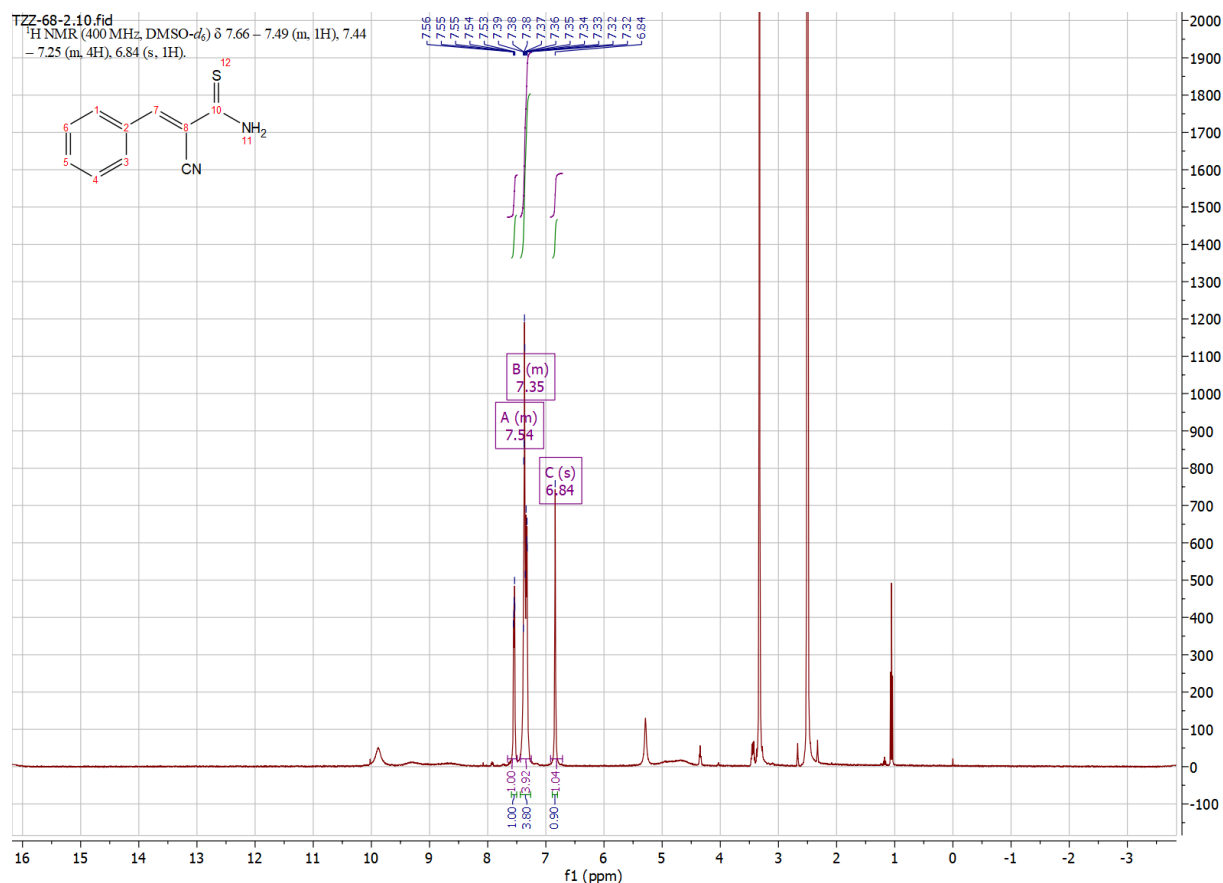


Figure S1:  $^1\text{H}$  NMR spectrum of compound 3a

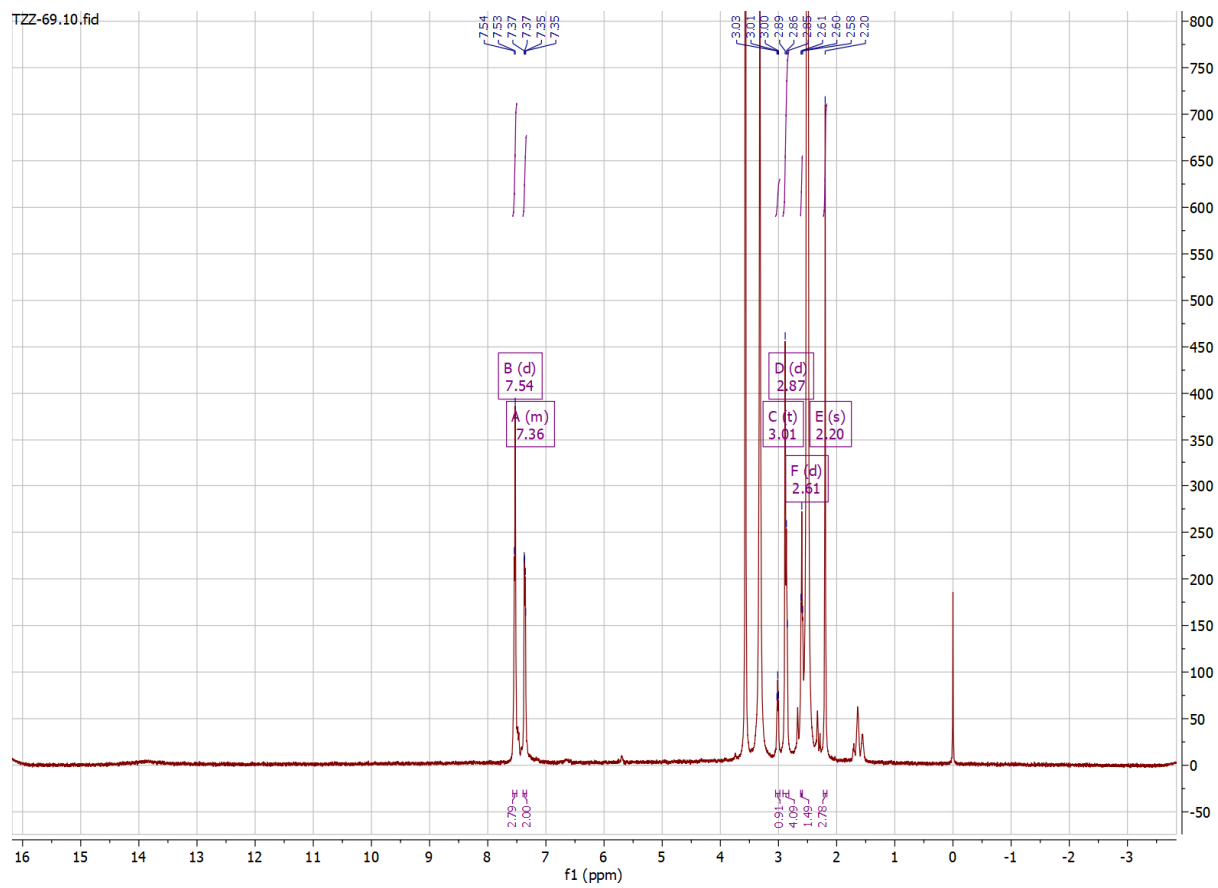
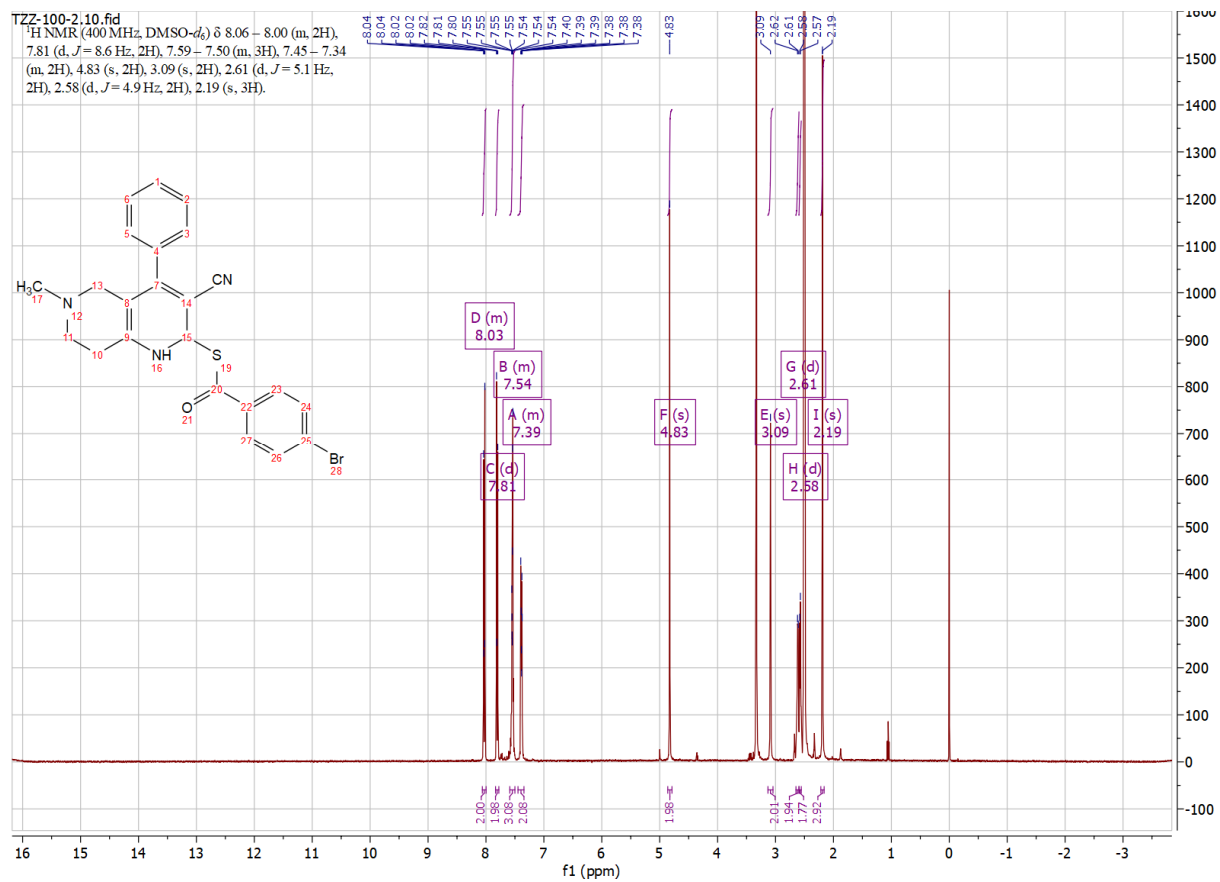
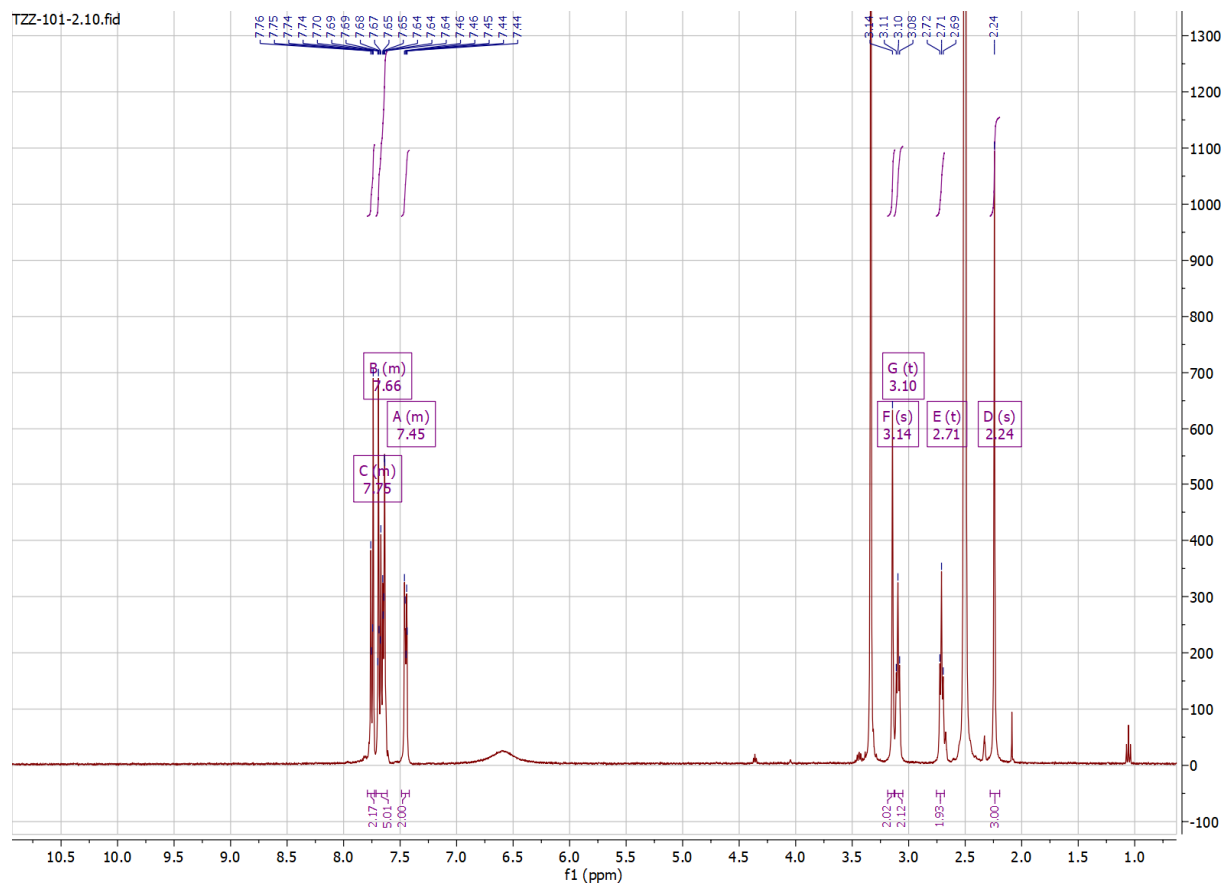


Figure S2:  $^1\text{H}$  NMR spectrum of compound **4a**



**Figure S3:** <sup>1</sup>H NMR spectrum of compound 5a



**Figure S4:**  $^1\text{H}$  NMR spectrum of compound **6a**

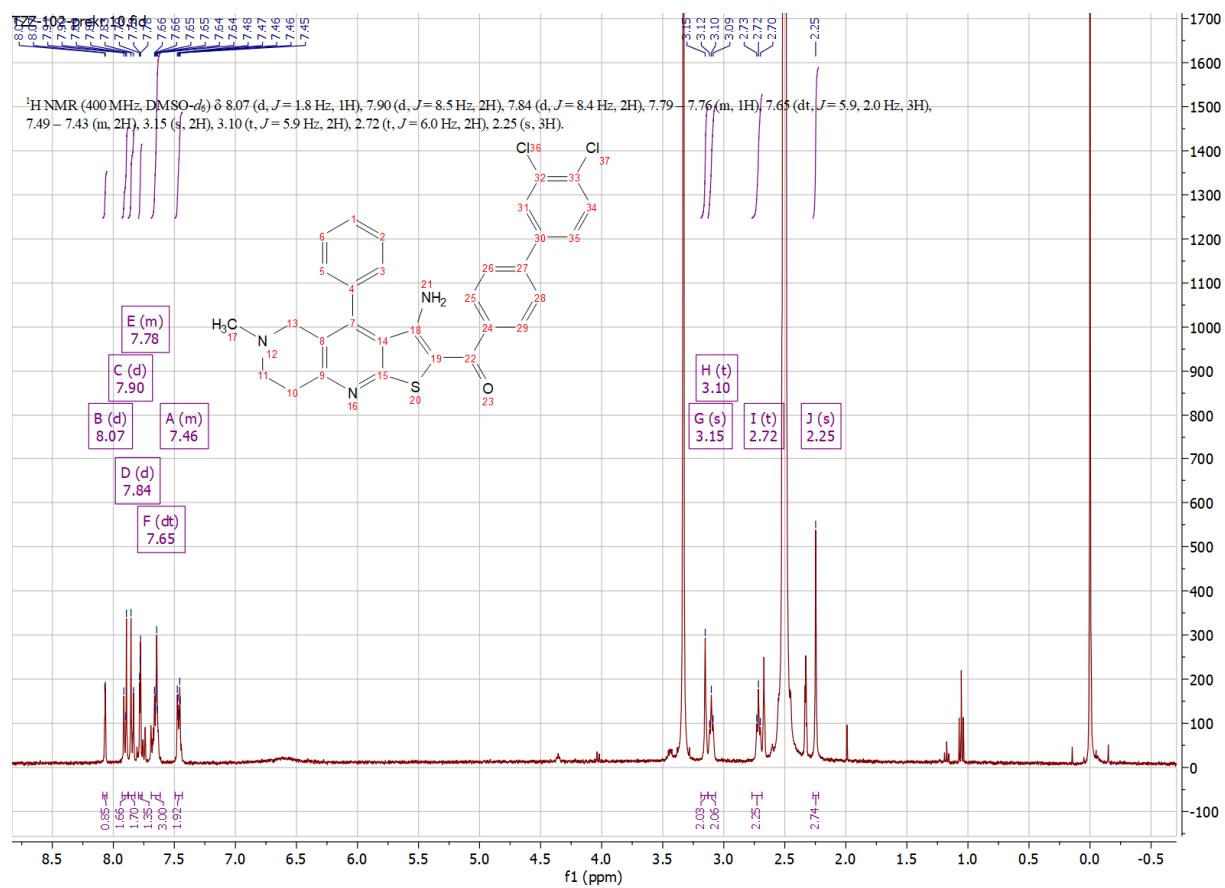
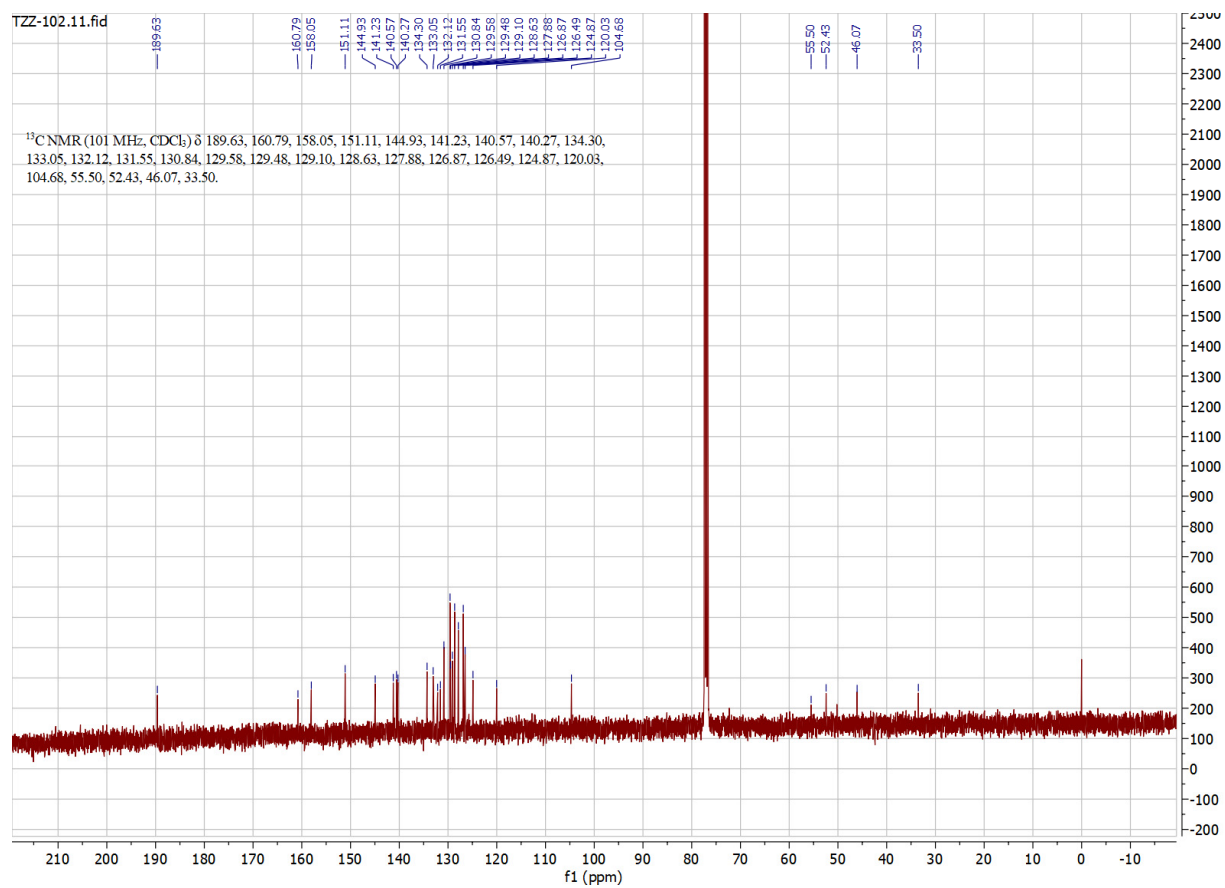
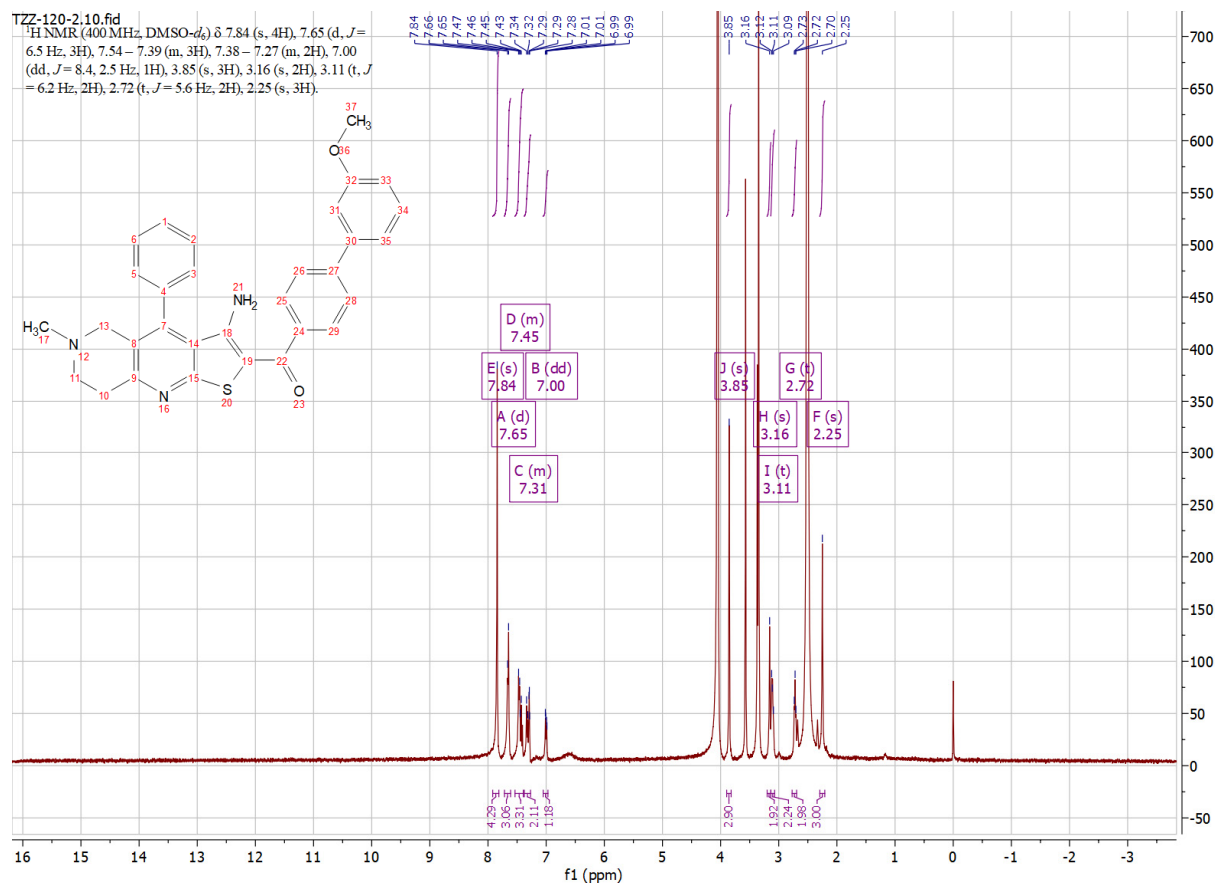


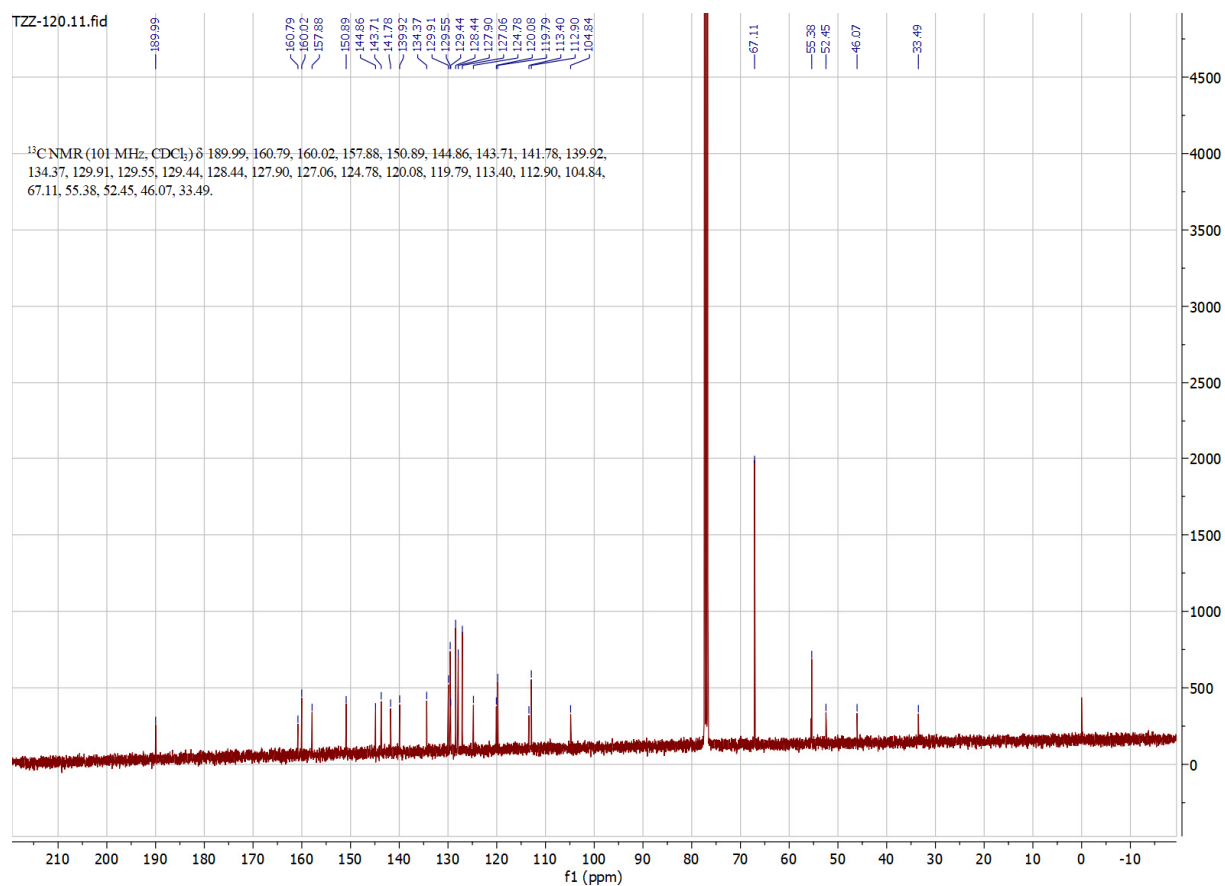
Figure S5: <sup>1</sup>H NMR spectrum of compound 7a



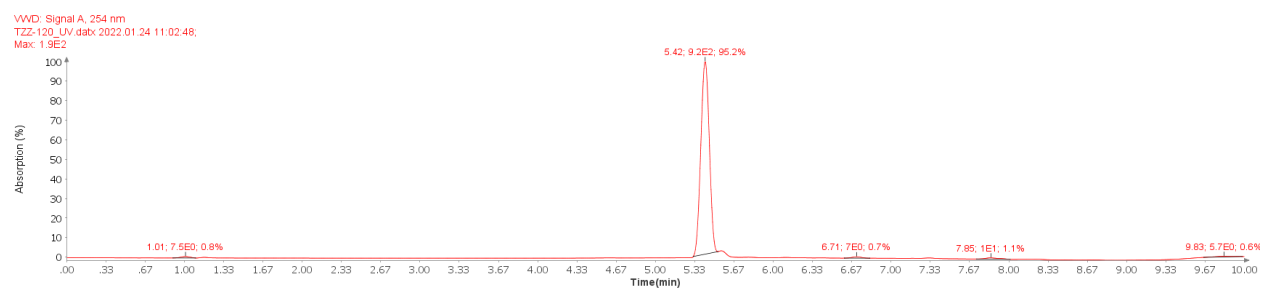
**Figure S6:**  $^{13}\text{C}$  NMR spectrum of compound **7a**



**Figure S7:** <sup>1</sup>H NMR spectrum of compound **7b**

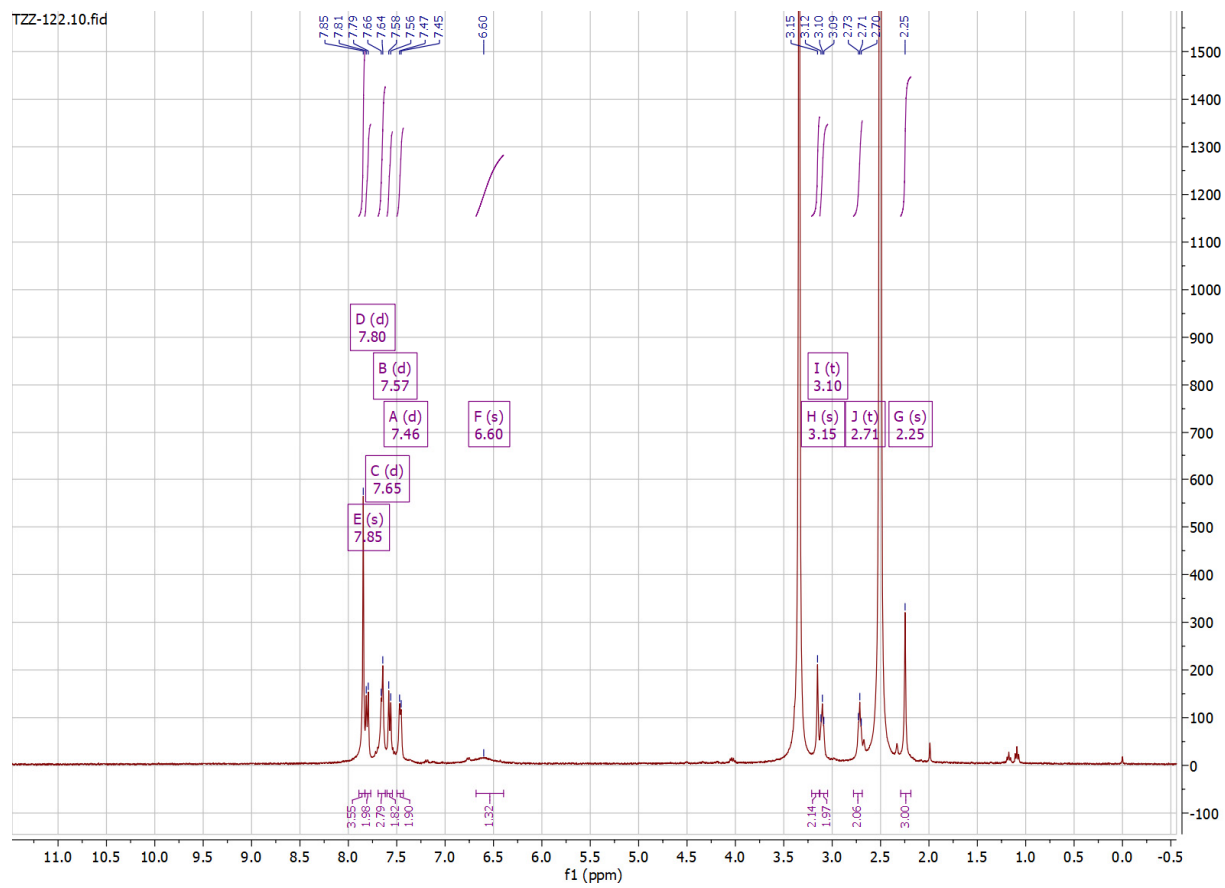


**Figure S8:**  $^{13}\text{C}$  NMR spectrum of compound **7b**

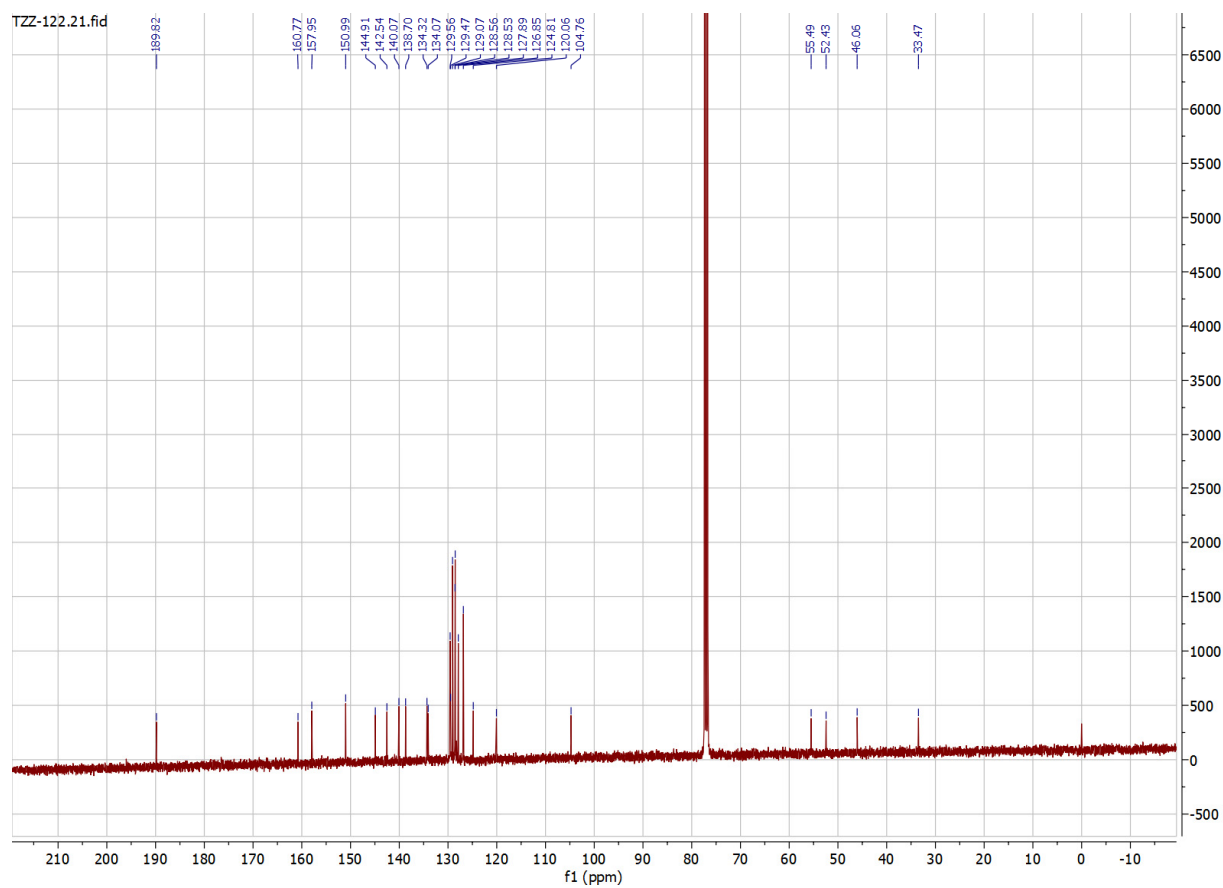


**Figure S9:** HPLC spectrum of **7b**

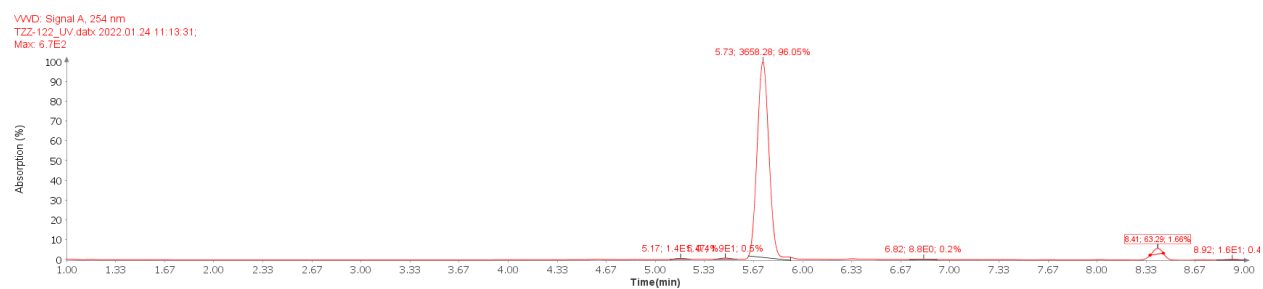




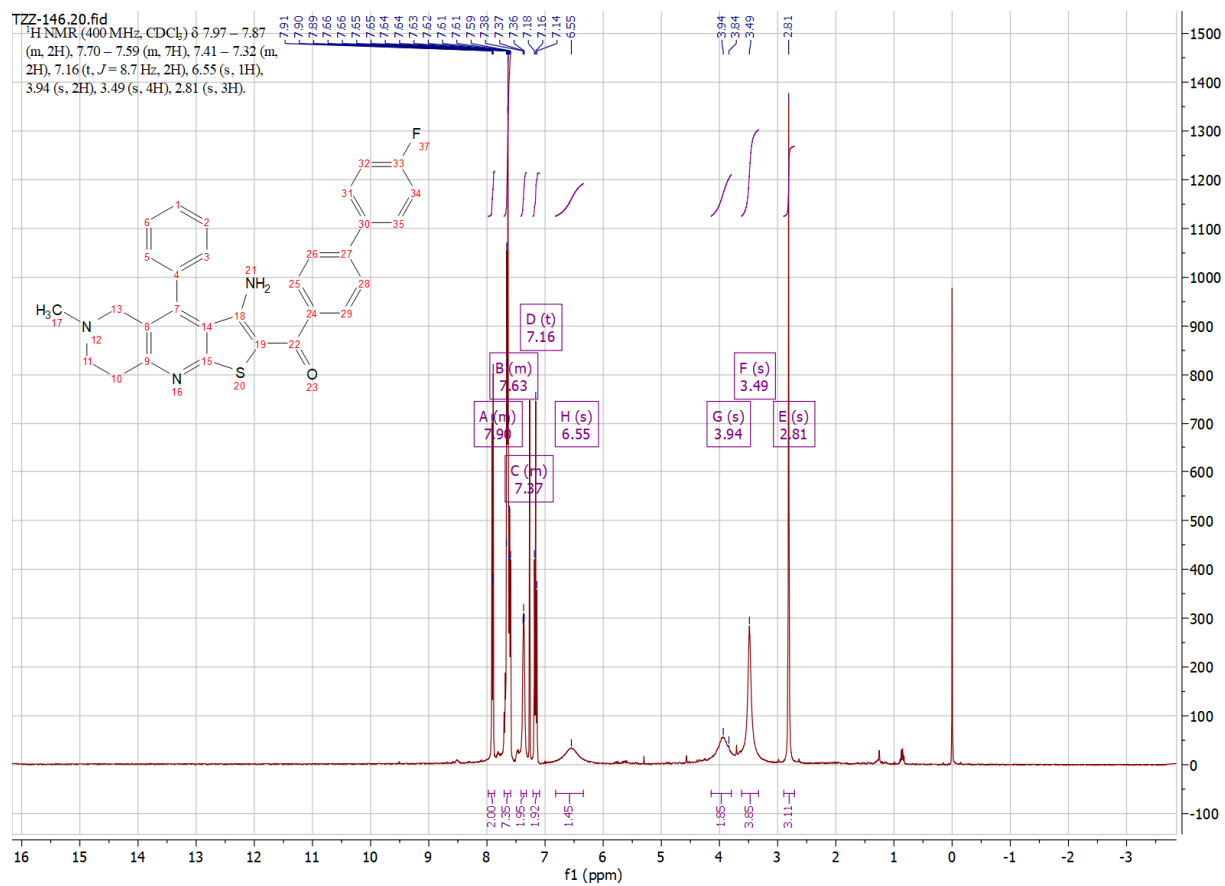
**Figure S10:**  $^1\text{H}$  NMR spectrum of compound **7c**



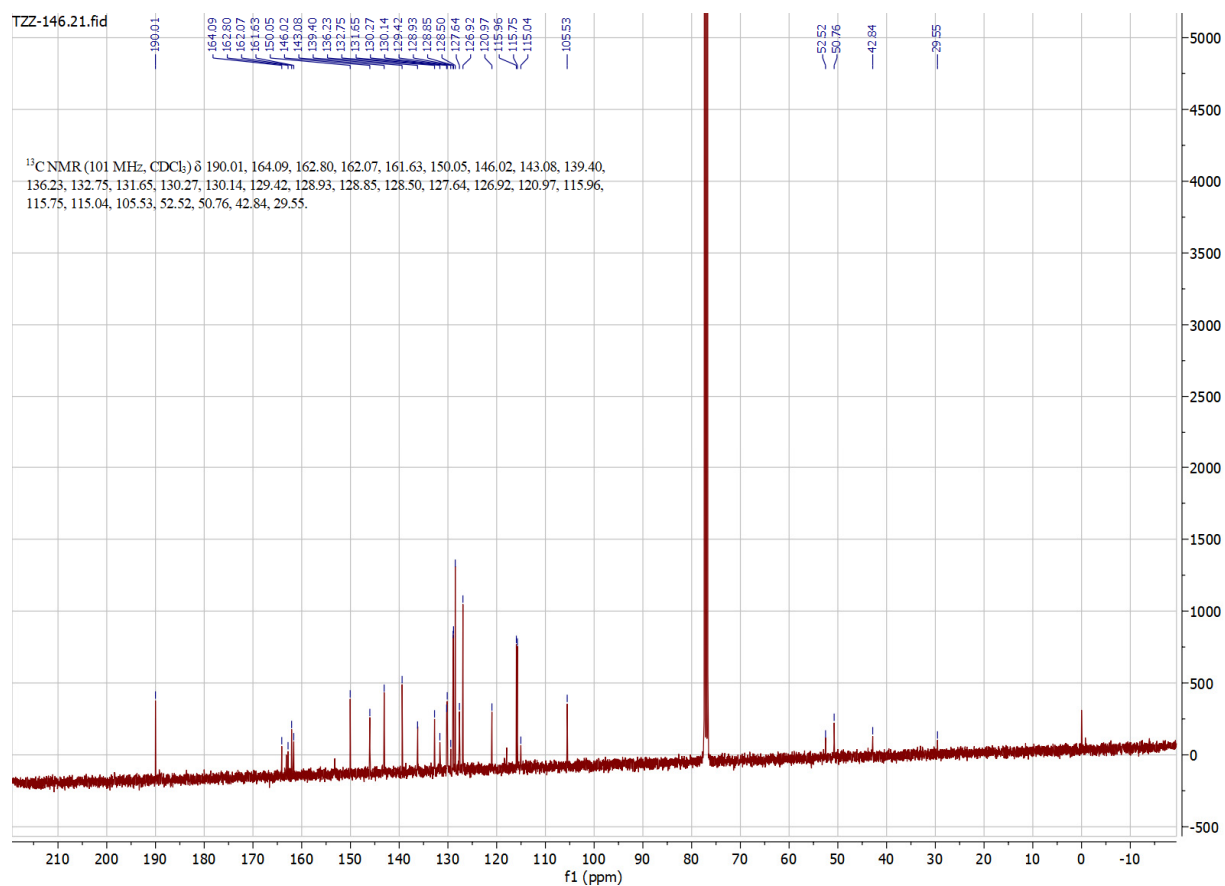
**Figure S11:**  $^{13}\text{C}$  NMR spectrum of compound **7c**



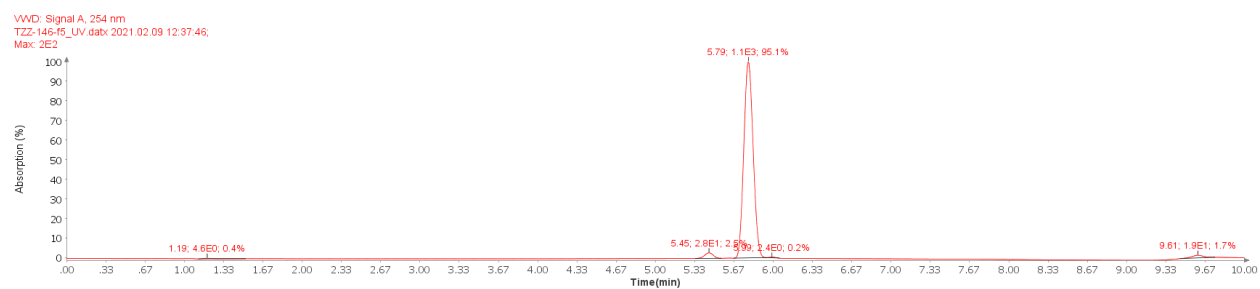
**Figure S12:** HPLC spectrum of compound **7c**



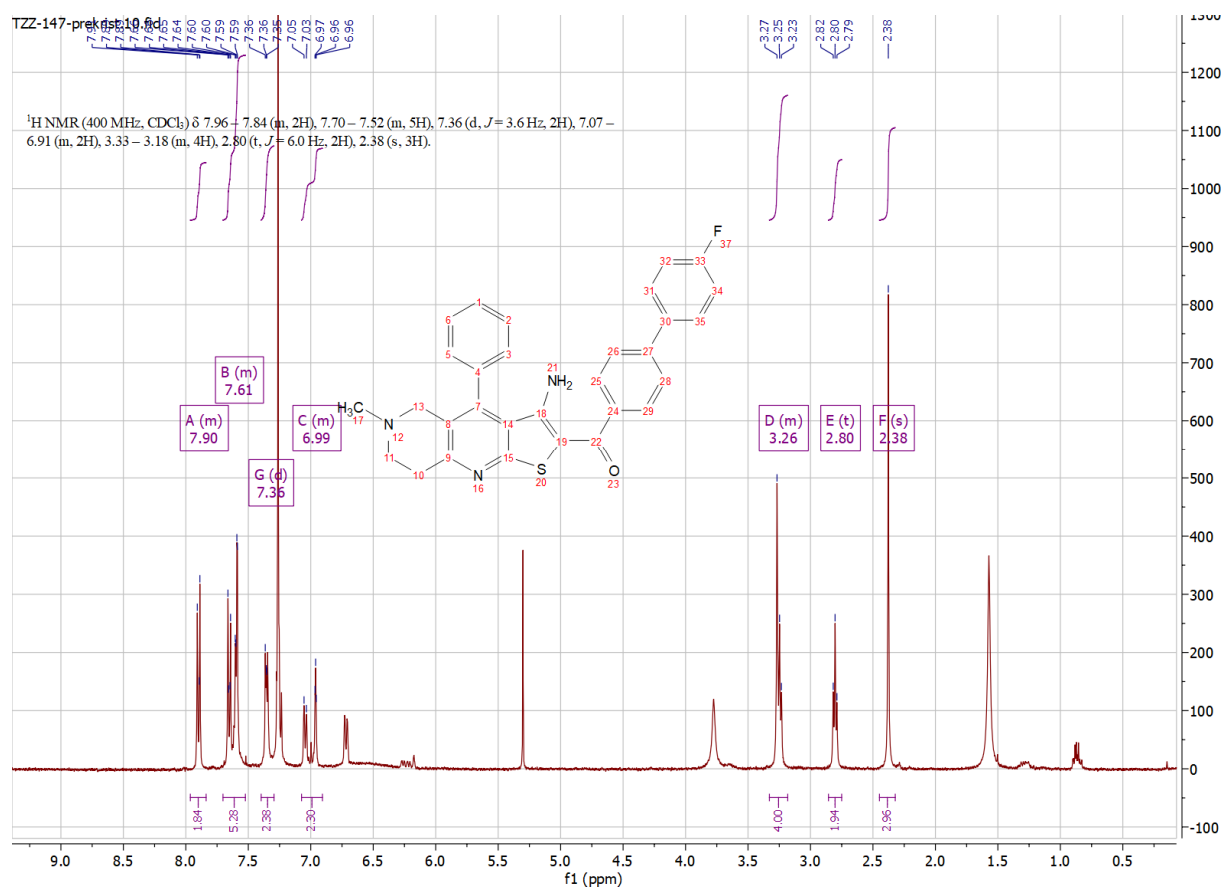
**Figure S13:** <sup>1</sup>H NMR spectrum of compound 7d



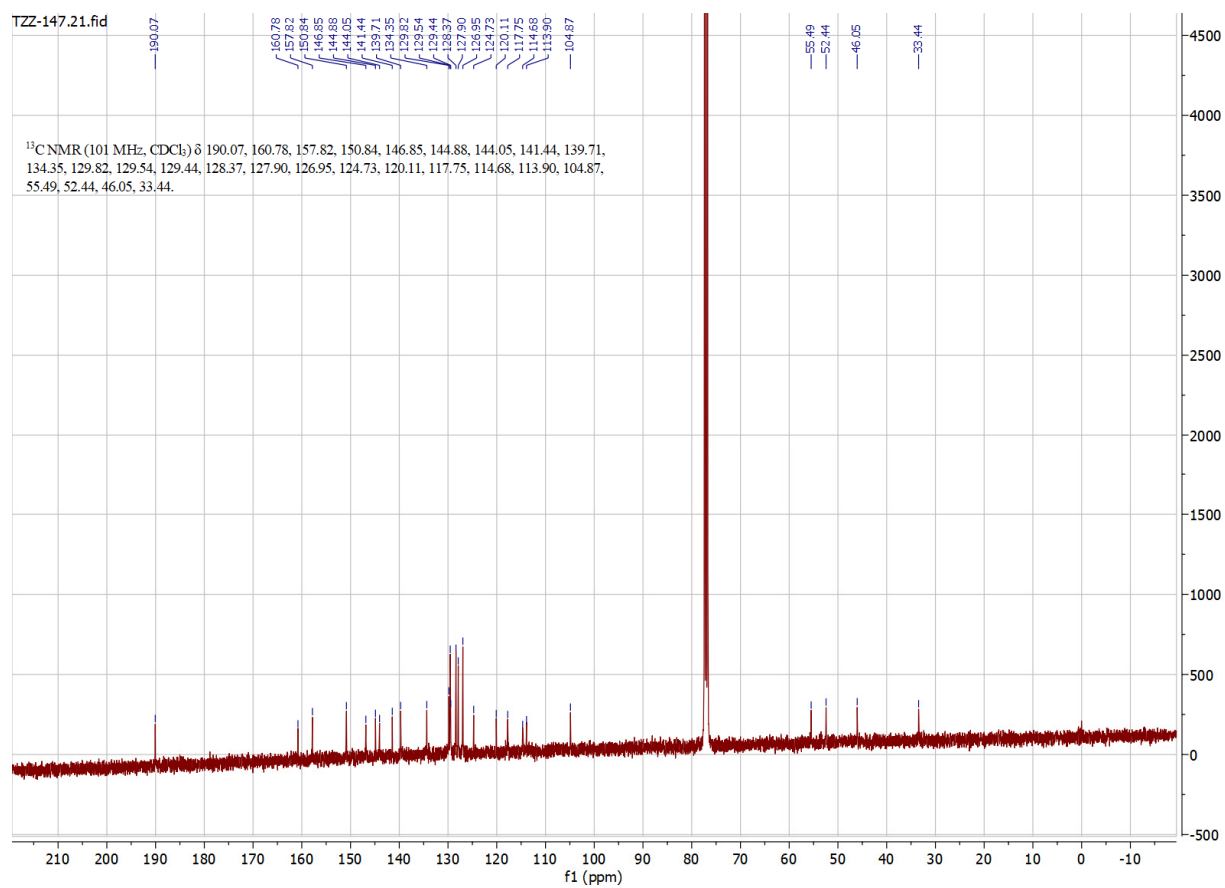
**Figure S14:**  $^{13}\text{C}$  NMR spectrum of compound **7d**



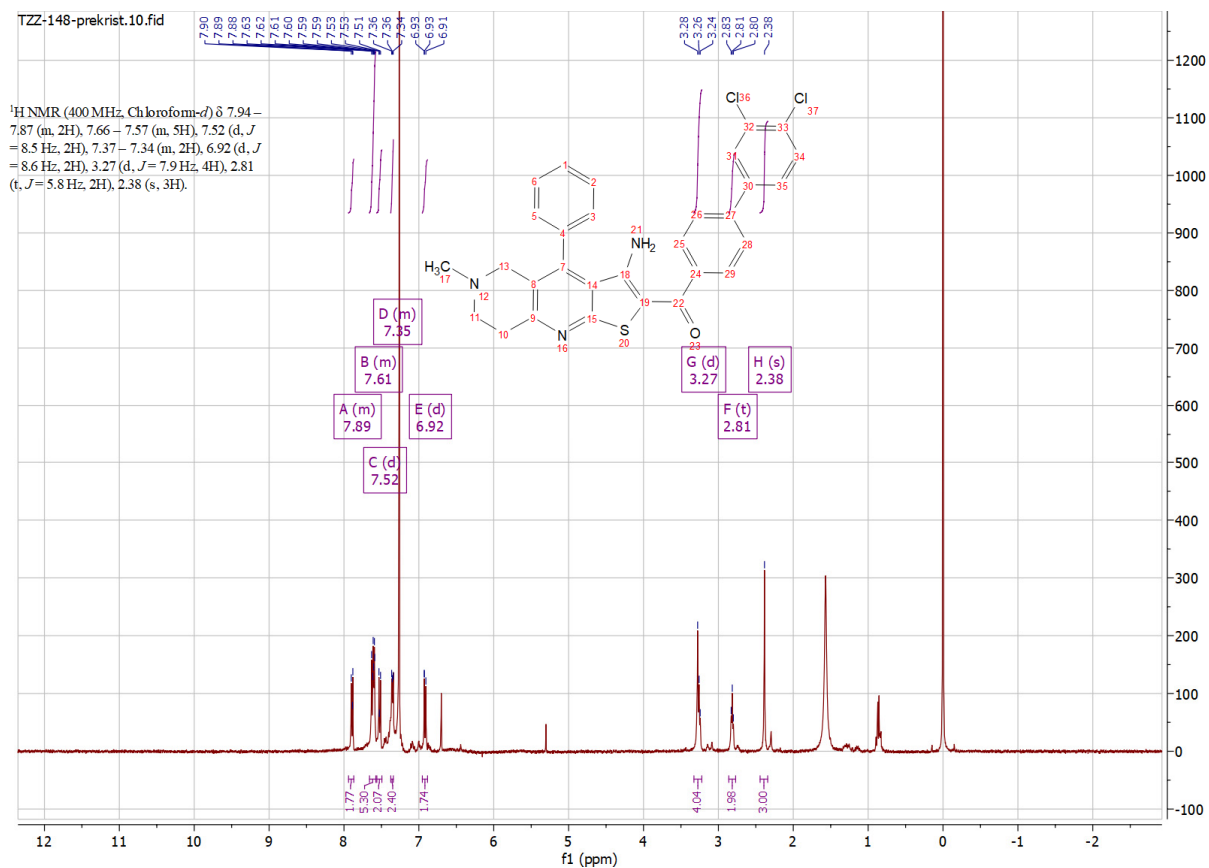
**Figure S15:** HPLC spectrum of compound **7d**



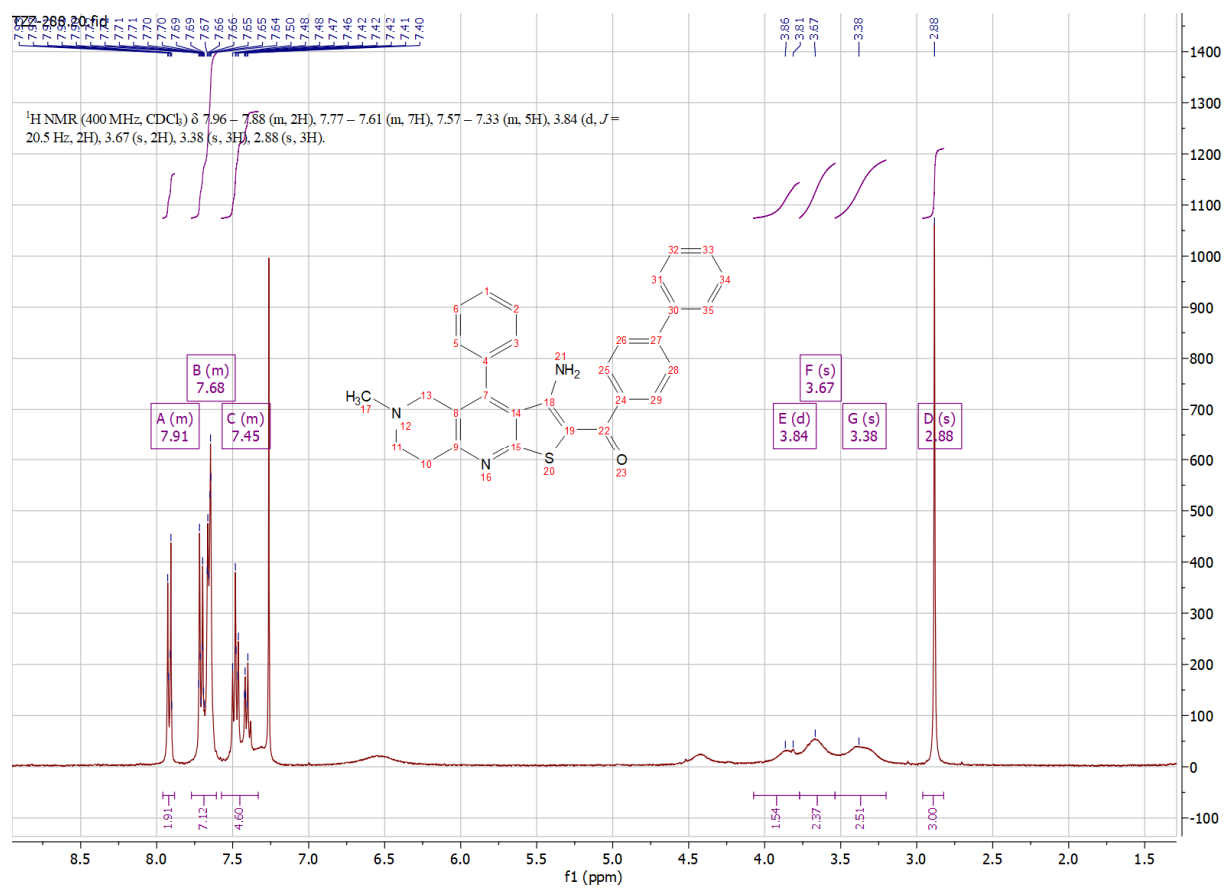
**Figure S16:** <sup>1</sup>H NMR spectrum of compound 7f



**Figure S17:**  $^{13}\text{C}$  NMR spectrum of compound **7f**

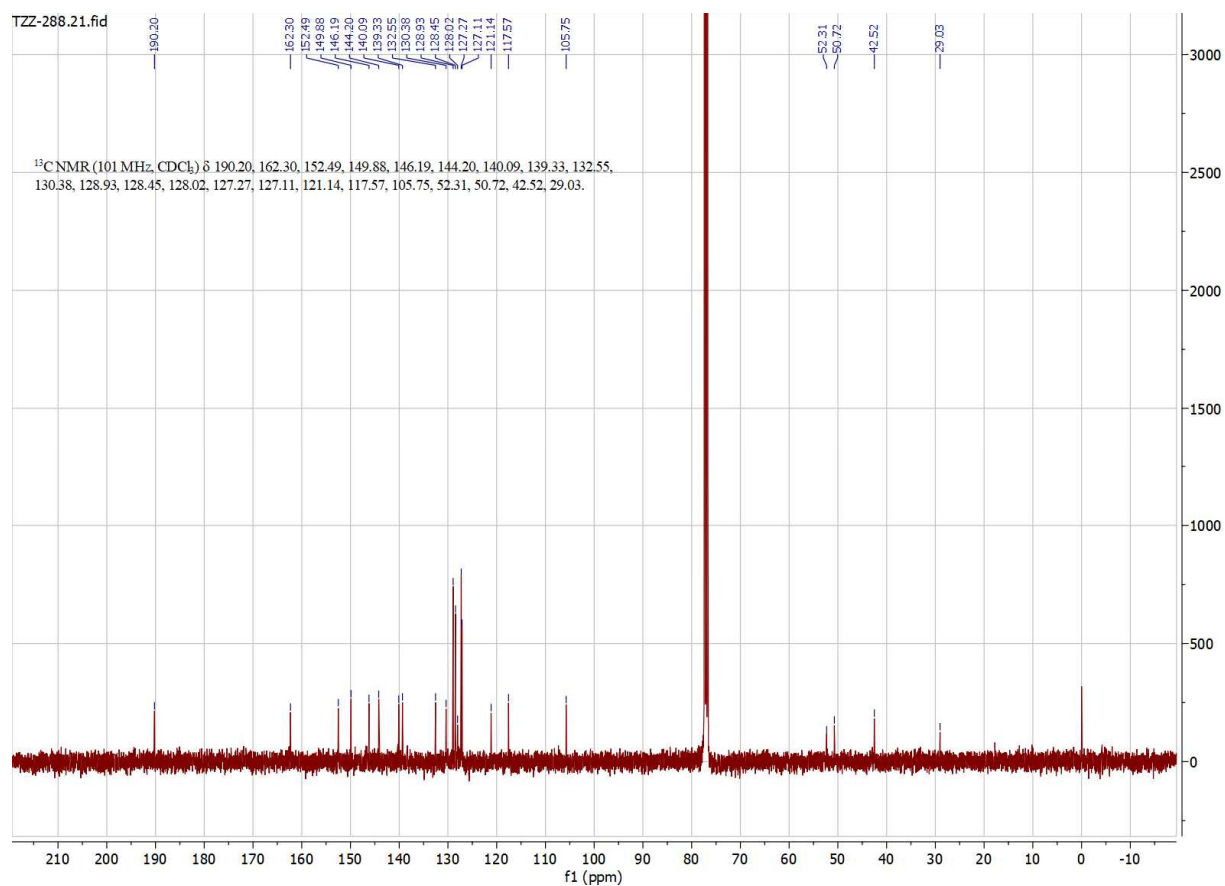


**Figure S18:** <sup>1</sup>H NMR spectrum of compound 7g

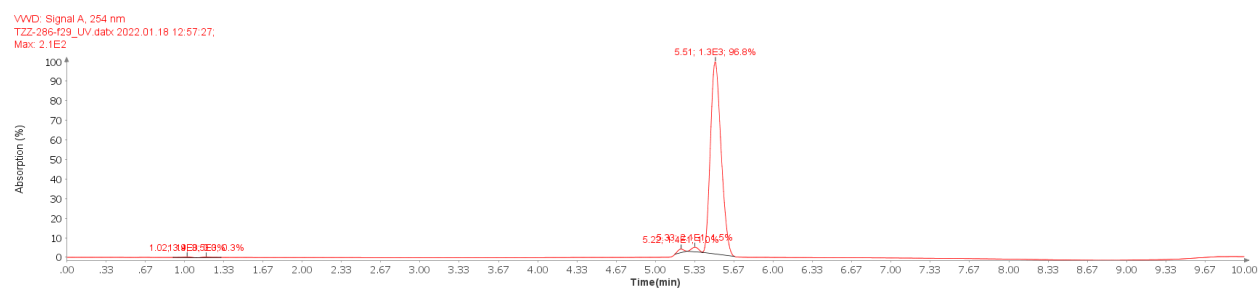


**Figure S19:** <sup>1</sup>H NMR spectrum of compound 7h

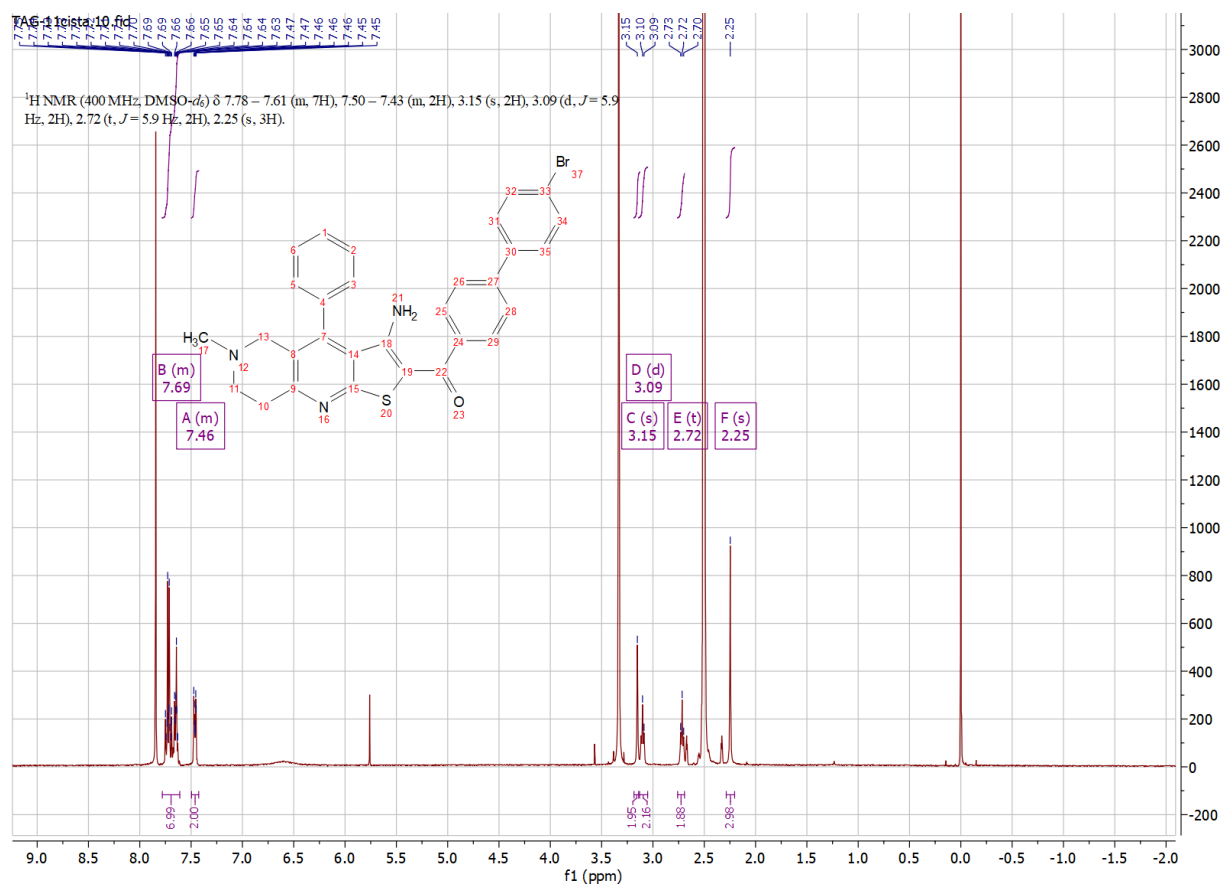




**Figure S20:**  $^{13}\text{C}$  NMR spectrum of compound **7h**



**Figure S21:** HPLC spectrum of compound **7h**



**Figure S22:** <sup>1</sup>H NMR spectrum of compound 7e

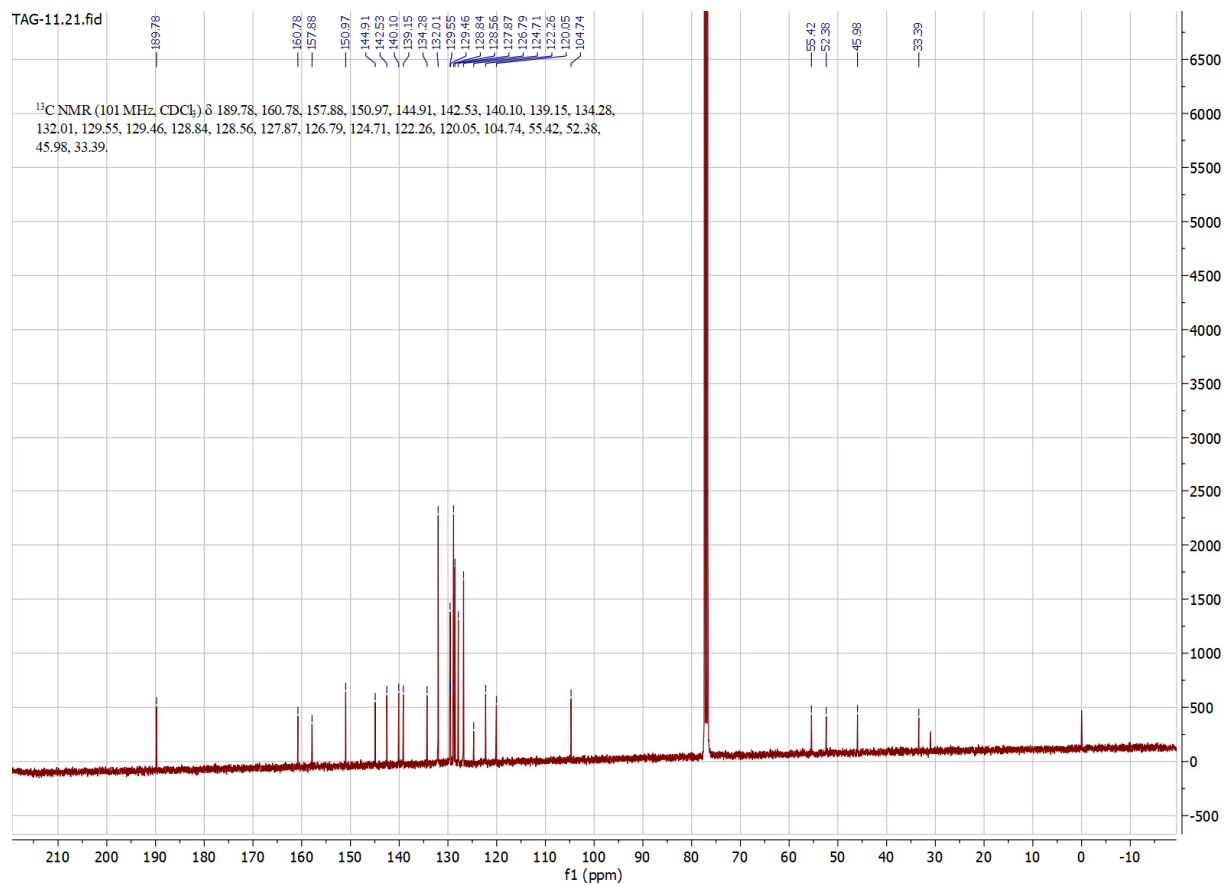


Figure S23:  $^{13}\text{C}$  NMR spectrum of compound 7e

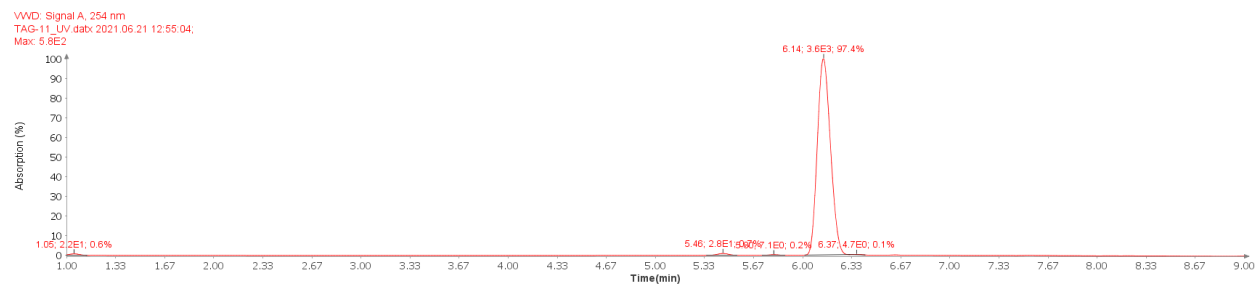
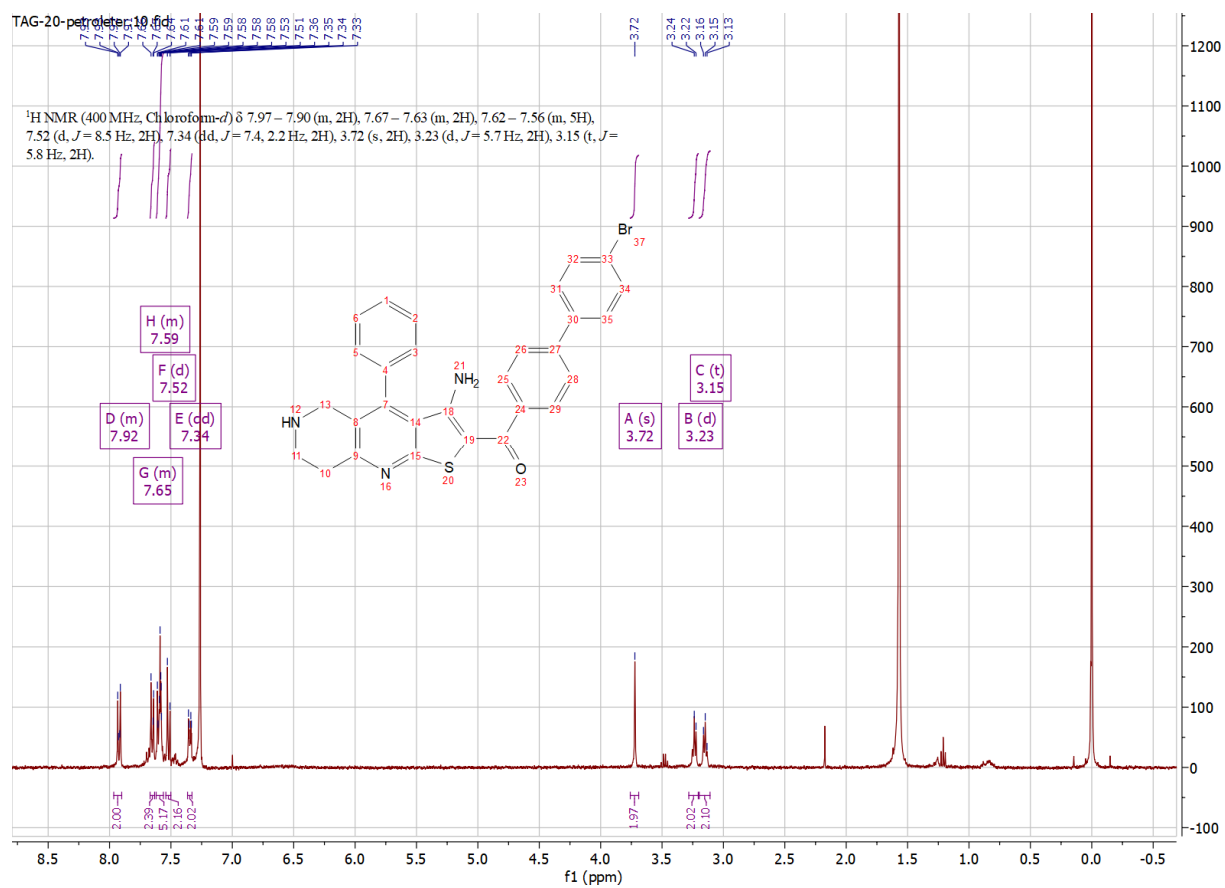
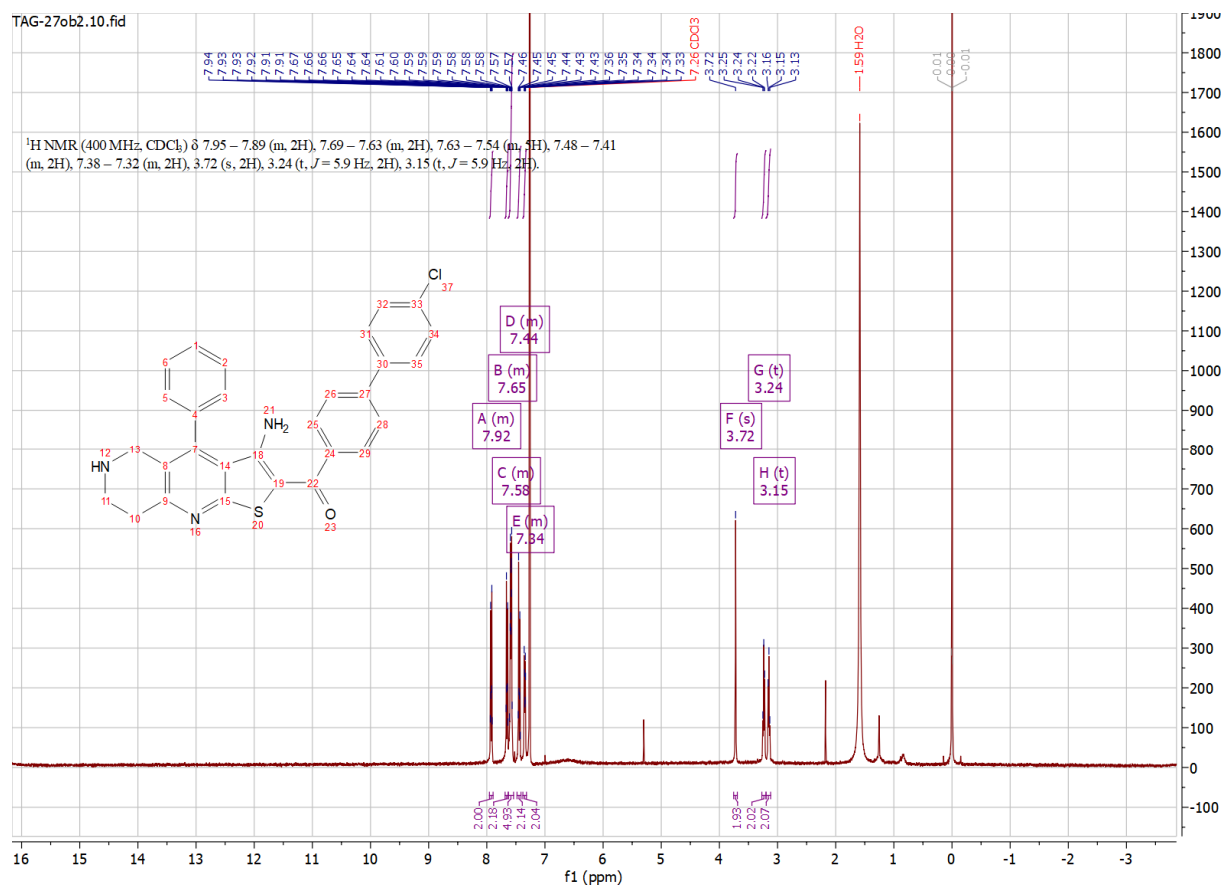
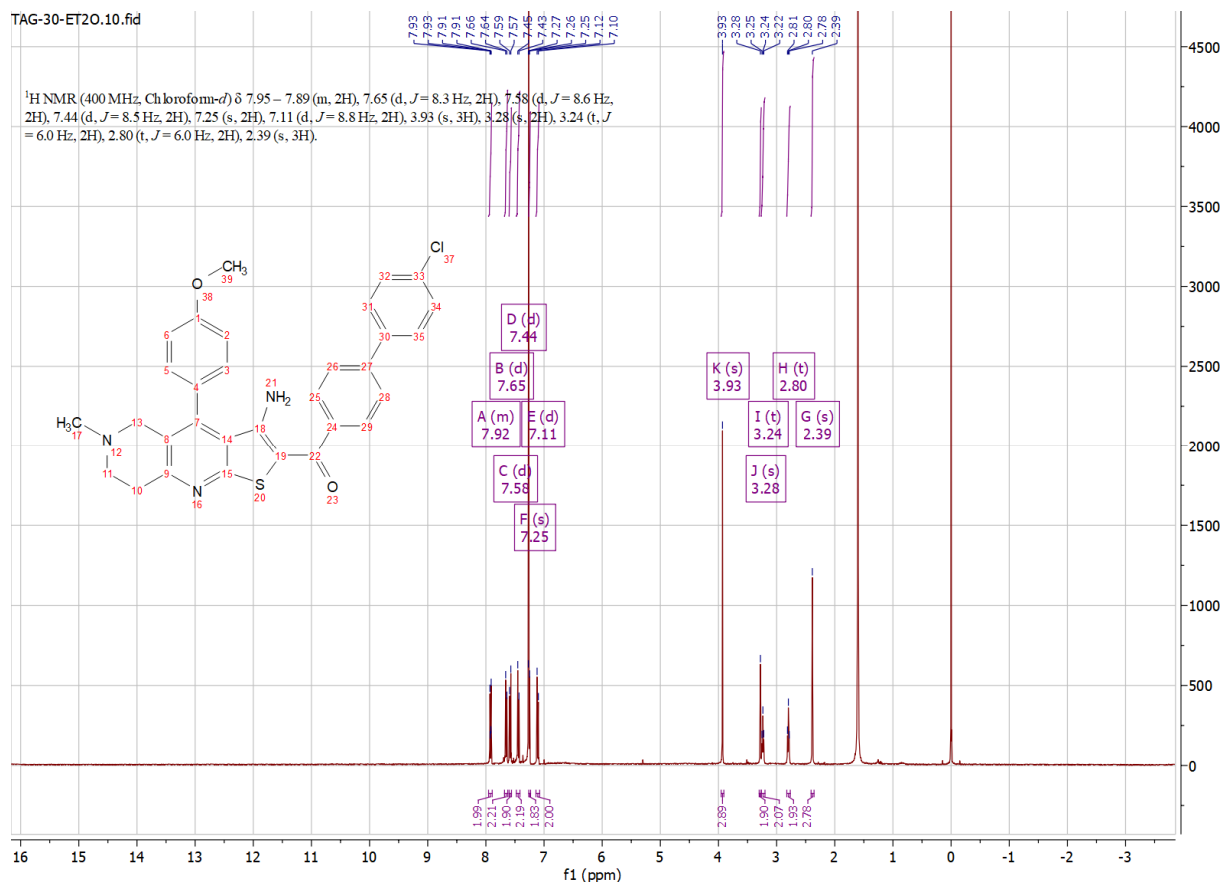


Figure S24: HPLC spectrum of compound 7e



**Figure S25:** <sup>1</sup>H NMR spectrum of compound 71





**Figure S27:** <sup>1</sup>H NMR spectrum of compound 7k

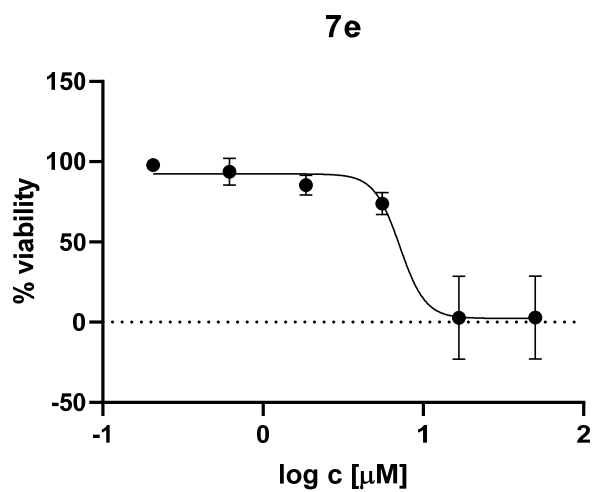
## Biological evaluation

### Cell culture

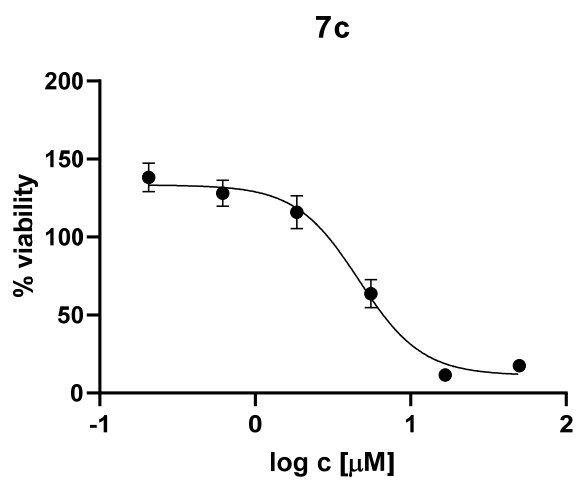
The MCF-7 cells were cultured in Dubecco's modified Eagle's MEM medium (Sigma – Aldrich, St. Louis, MO, USA), which was supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin (Sigma-Aldrich, St. Louis, MO, USA), 100 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA). Cells were plated on 96-well plates at density 4000 cells/well and were allowed to attach. Cells were treated with final compounds (6 concentrations, 3-fold dilution), positive control 17-DMAG or vehicle control (0.5% DMSO) and incubated for 72 hours.

SK-NM-C cells were cultured in RPMI 1640, HEPES modification medium (Sigma – Aldrich, St. Louis, MO, USA), supplemented with which was supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin (Sigma-Aldrich, St. Louis, MO, USA), 100 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA). Cells were plated on 96-well plates at density 2000 cells/well and were allowed to attach. Cells were treated with final compounds (6 concentrations, 3-fold dilution), positive control 17-DMAG or vehicle control (0.5% DMSO) and incubated for 72 hours.

Representative  $IC_{50}$  curves

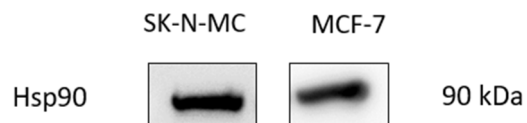


**Figure S28:**  $IC_{50}$  curve of compound **7e** in MCF-7 cell line



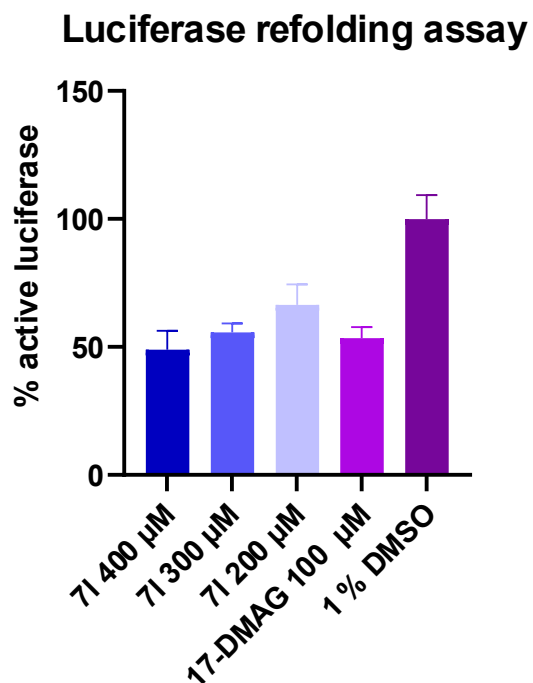
**Figure S29:**  $IC_{50}$  curve of compound **7c** in SK-NM-C cell line

Validation of chosen cell lines for expression of Hsp90



**Figure S30:** Western blot of validation of overexpression of Hsp90 in MCF-7 and SK-N-MC cell lines

Luciferase refolding assay, screening of compound 7l



**Figure S31:** Percentage of active luciferase in luciferase refolding assay after treatment with compound 7l, 17-DMAG (positive control) or 1 % DMSO (vehicle control)