Electronic Supplementary Material (ESI) for RSC Advances.

Design, Synthesis and Cytotoxic Activity of Molecular Hybrids Based on Quinolin-8-yloxy and Cinnamide Hybrids and Their Apoptosis Inducing Property

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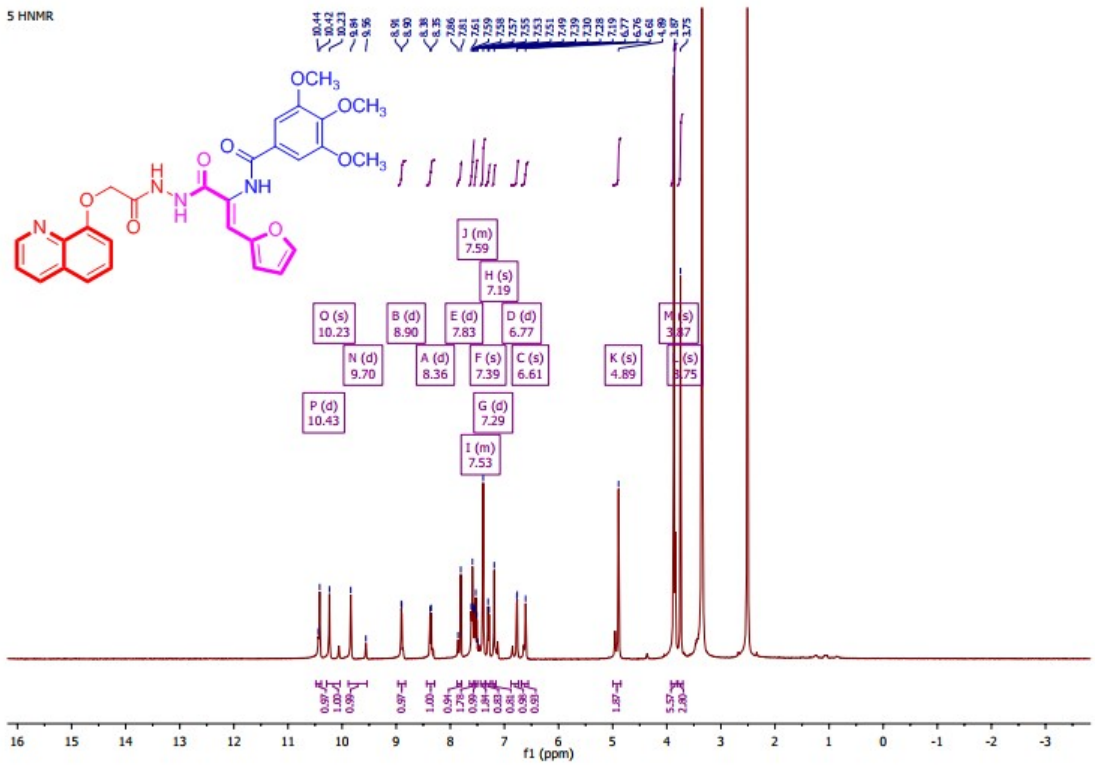

Figure S1: ^{1}H-NMR spectrum of compound **5**

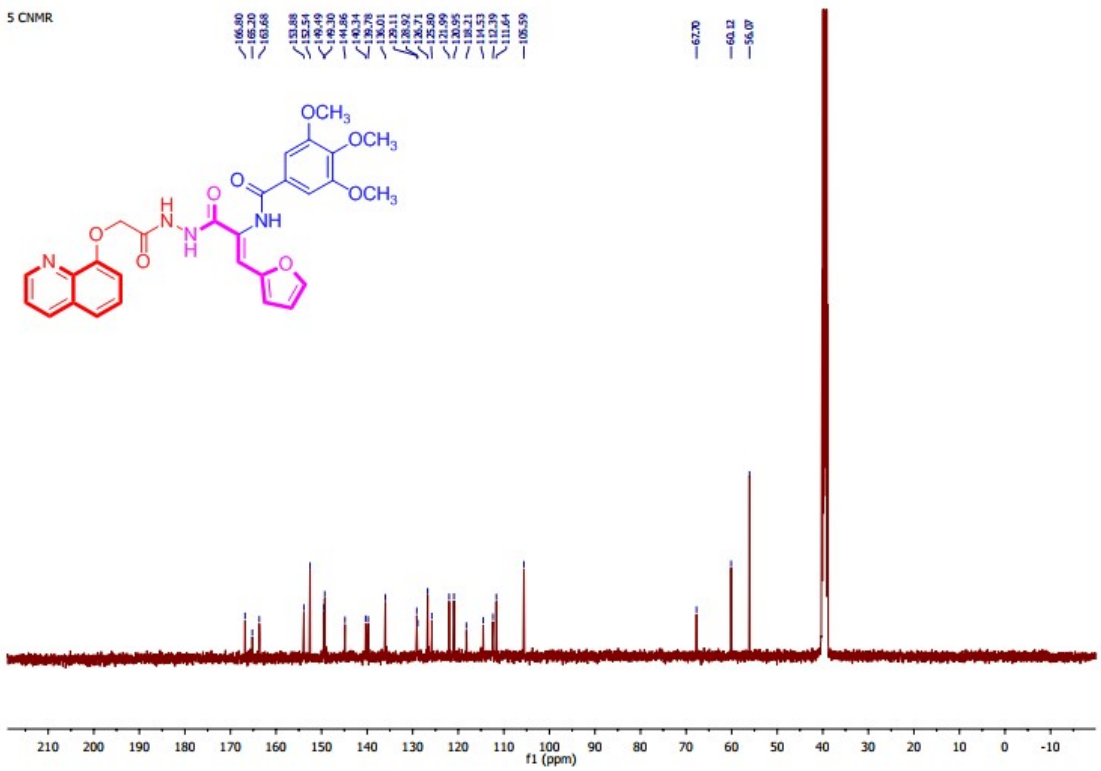

Figure S2: ^{13}C-NMR spectrum of compound **5**

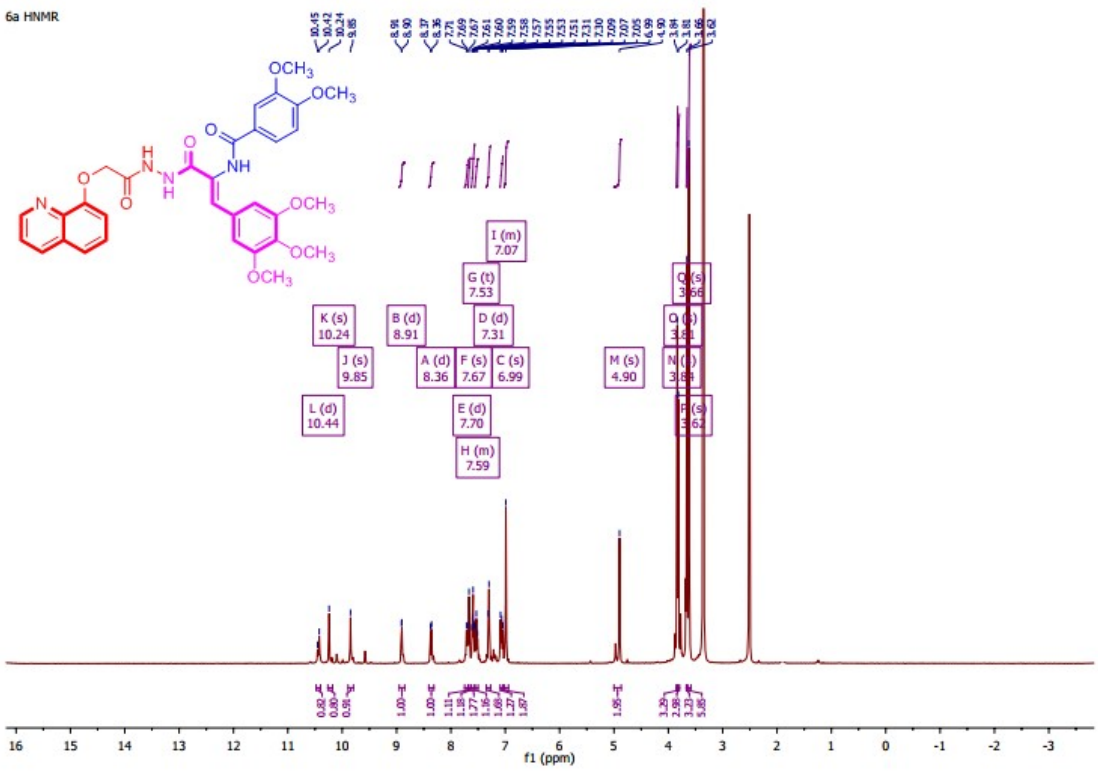

Figure S3: ^{1}H-NMR spectrum of compound **6a**

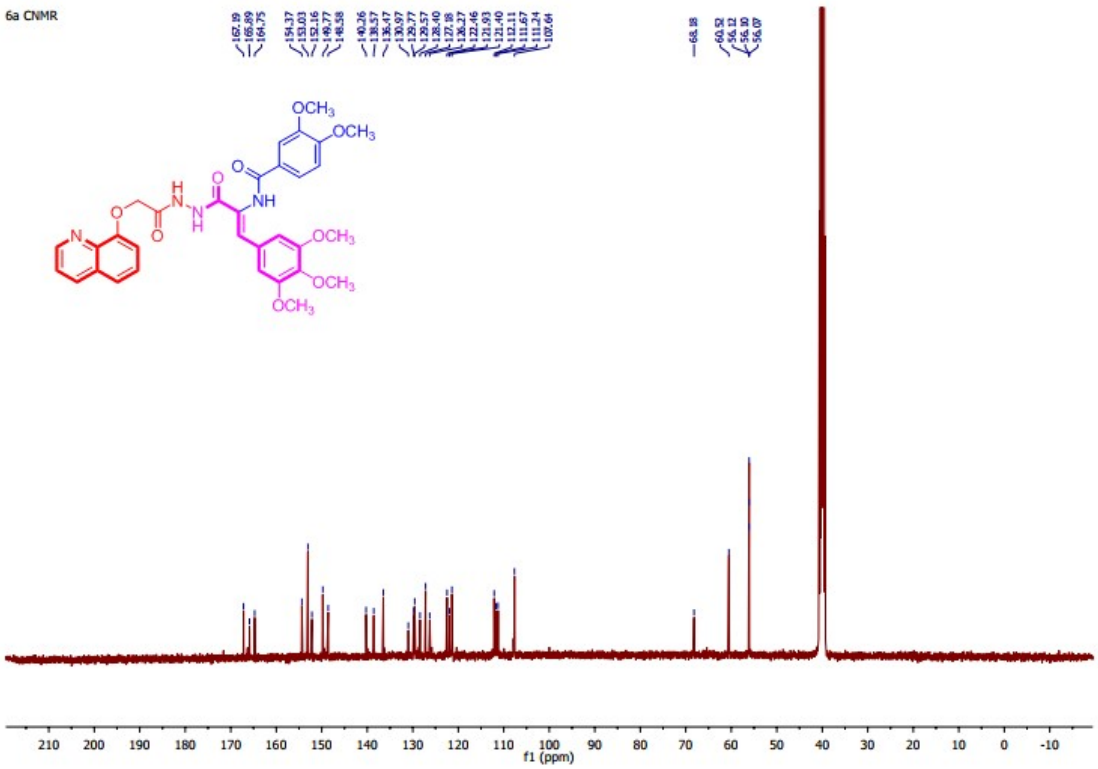

Figure S4: ^{13}C-NMR spectrum of compound **6a**

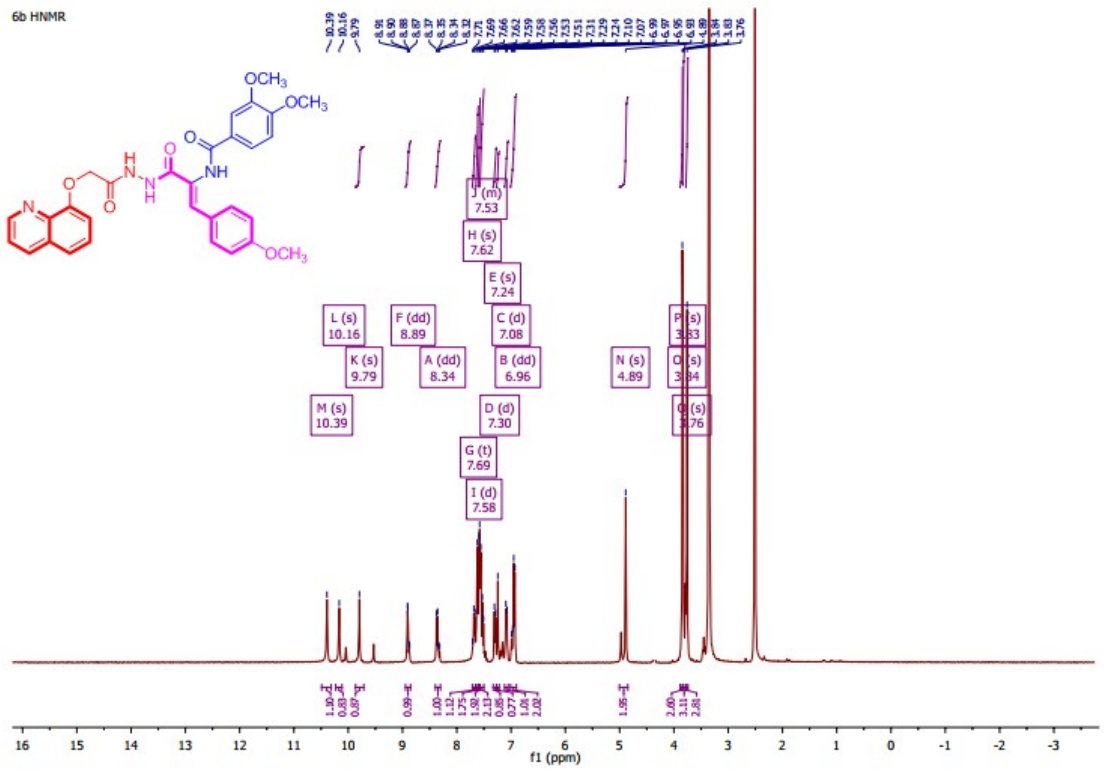

Figure S5: ^{1}H-NMR spectrum of compound **6b**

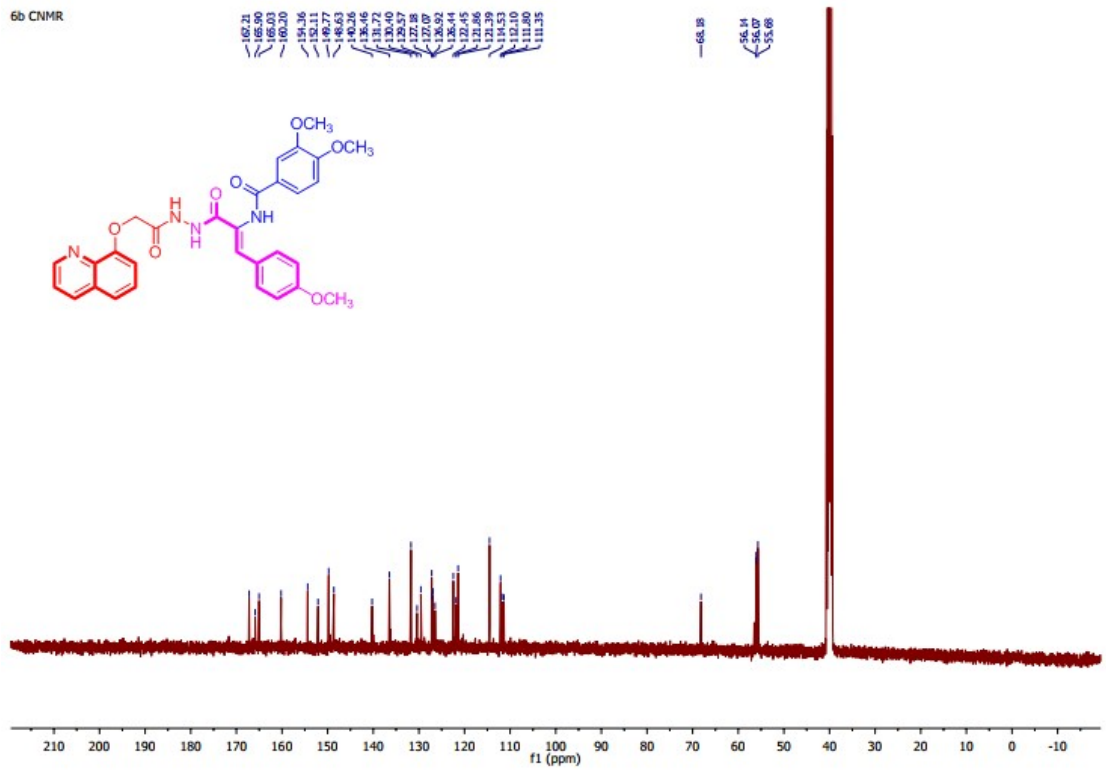

Figure S6: ^{13}C-NMR spectrum of compound **6b**

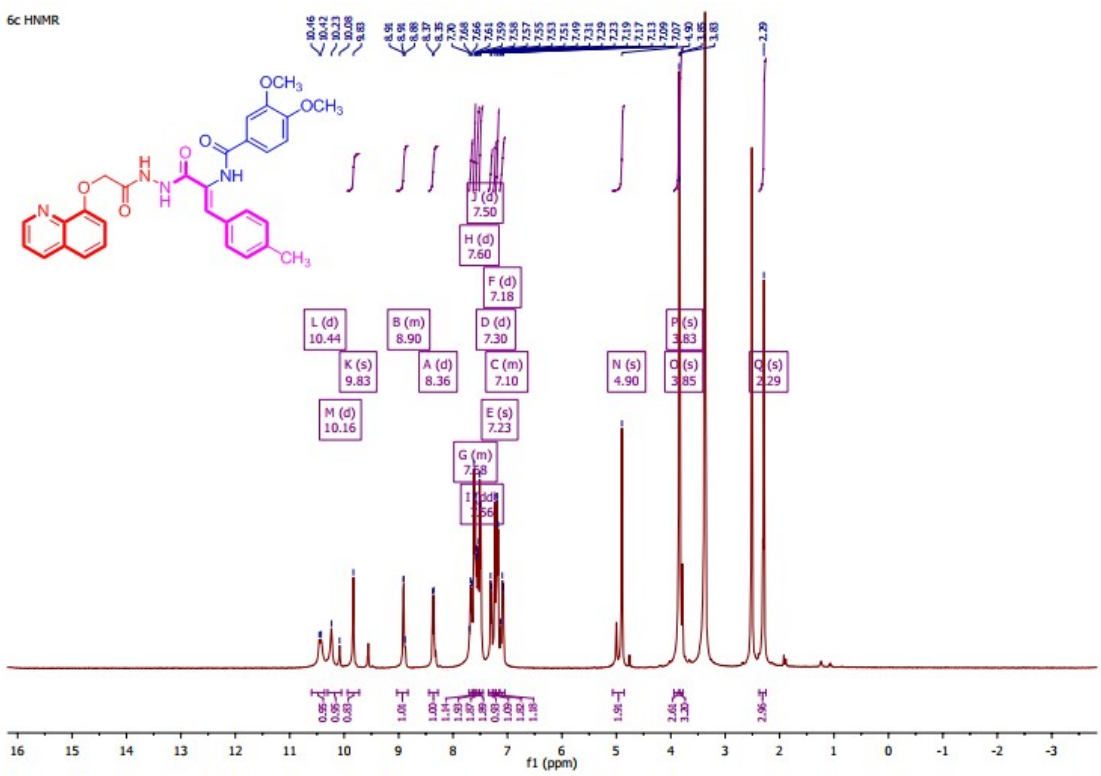

Figure S7: ^{1}H-NMR spectrum of compound **6c**

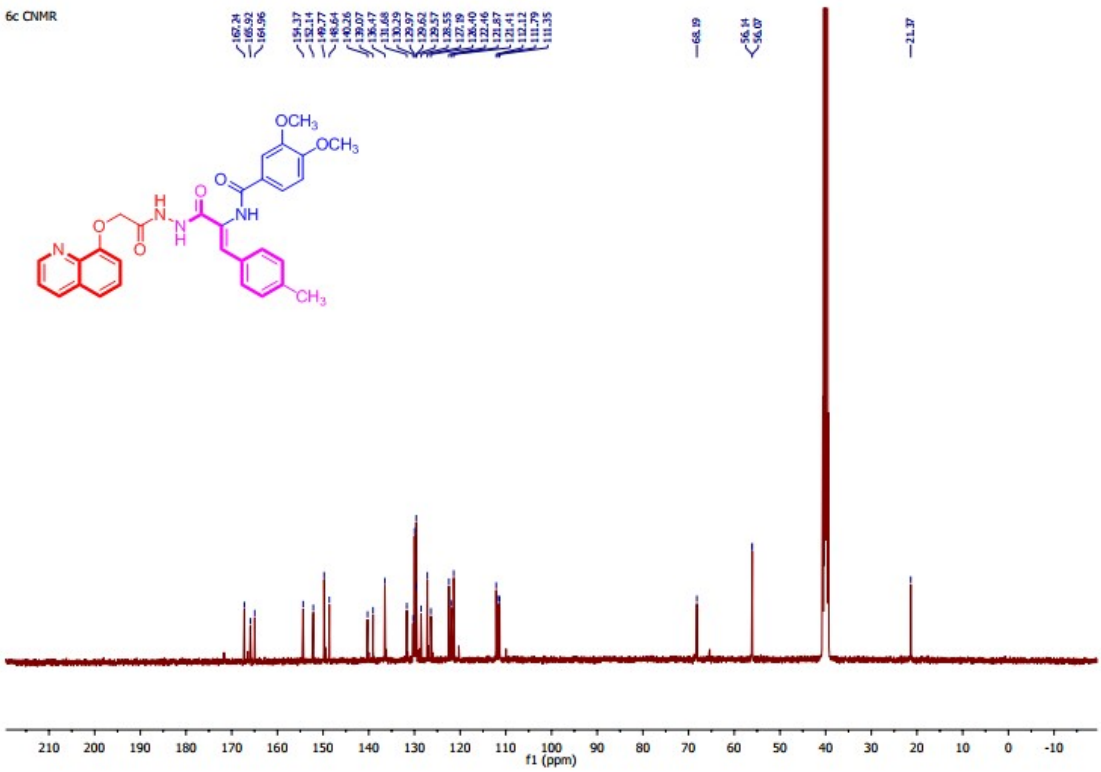

Figure S8: ^{13}C-NMR spectrum of compound **6c**

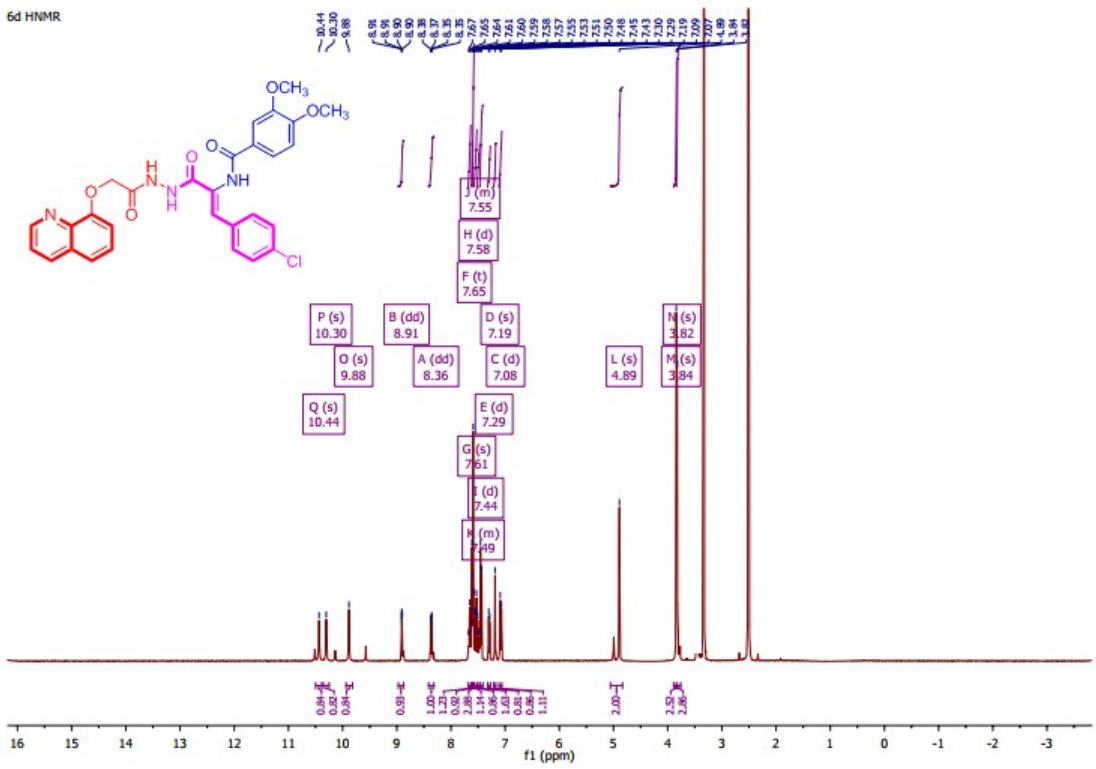

Figure S9: ^{1}H-NMR spectrum of compound **6d**

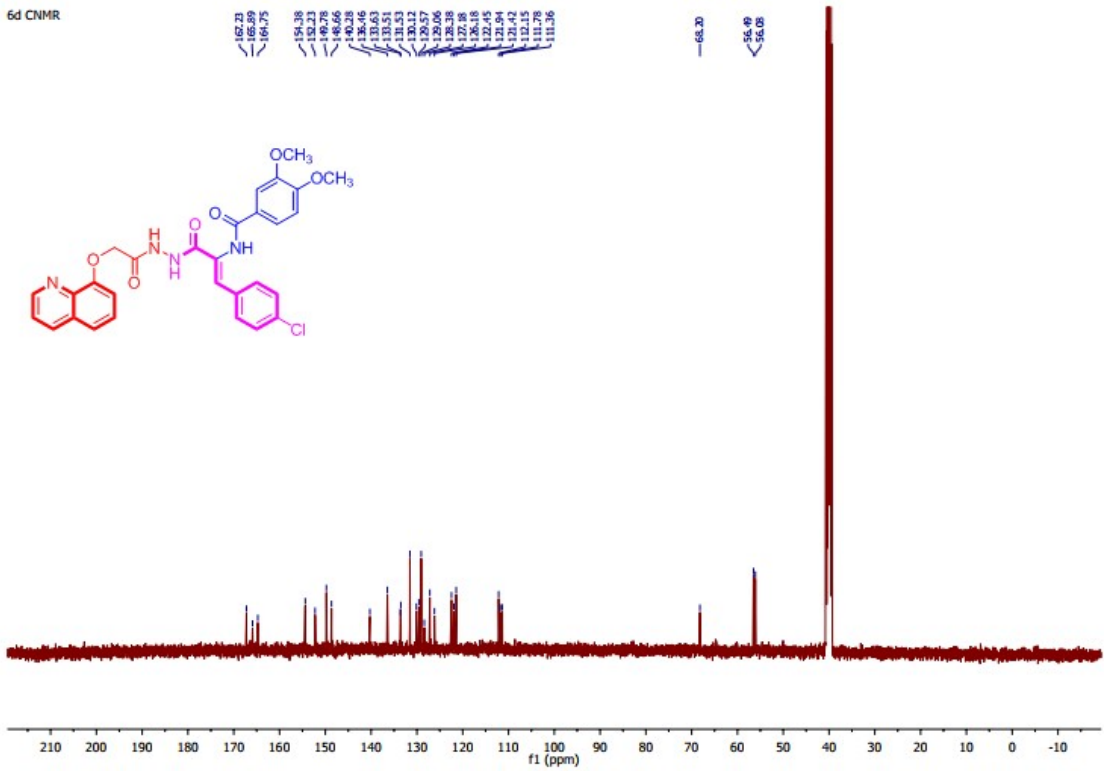

Figure S10: ^{13}C-NMR spectrum of compound **6d**

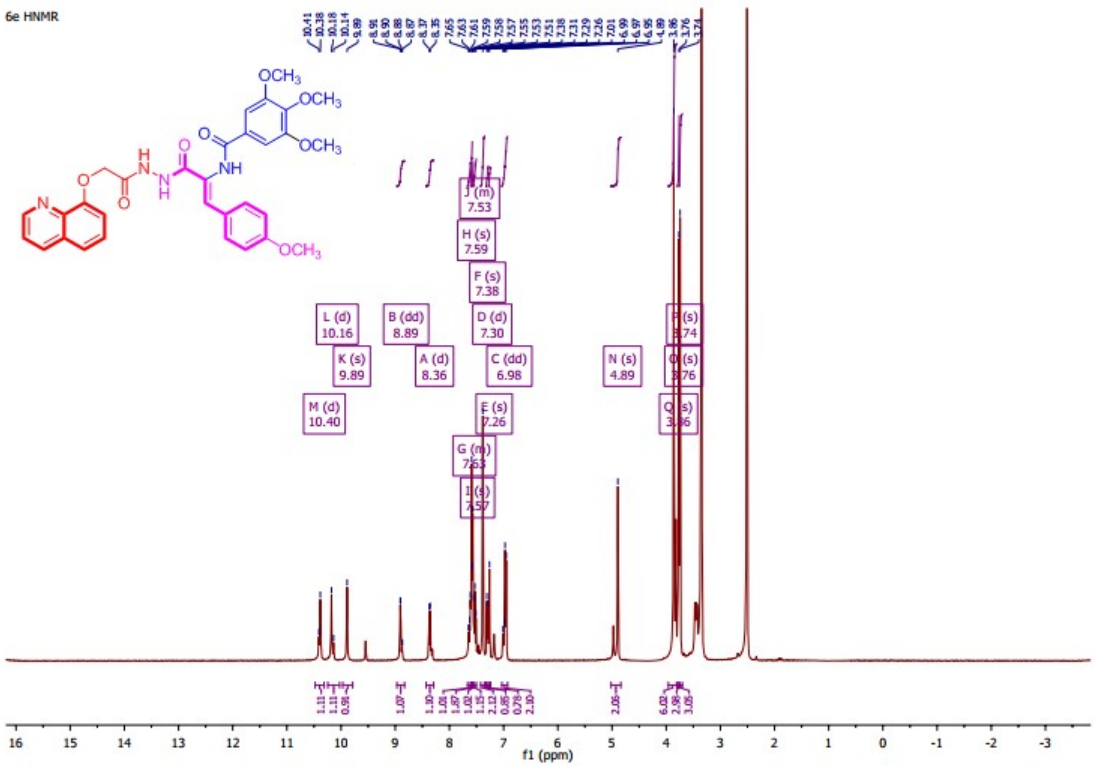

Figure S11: ^{1}H-NMR spectrum of compound **6e**

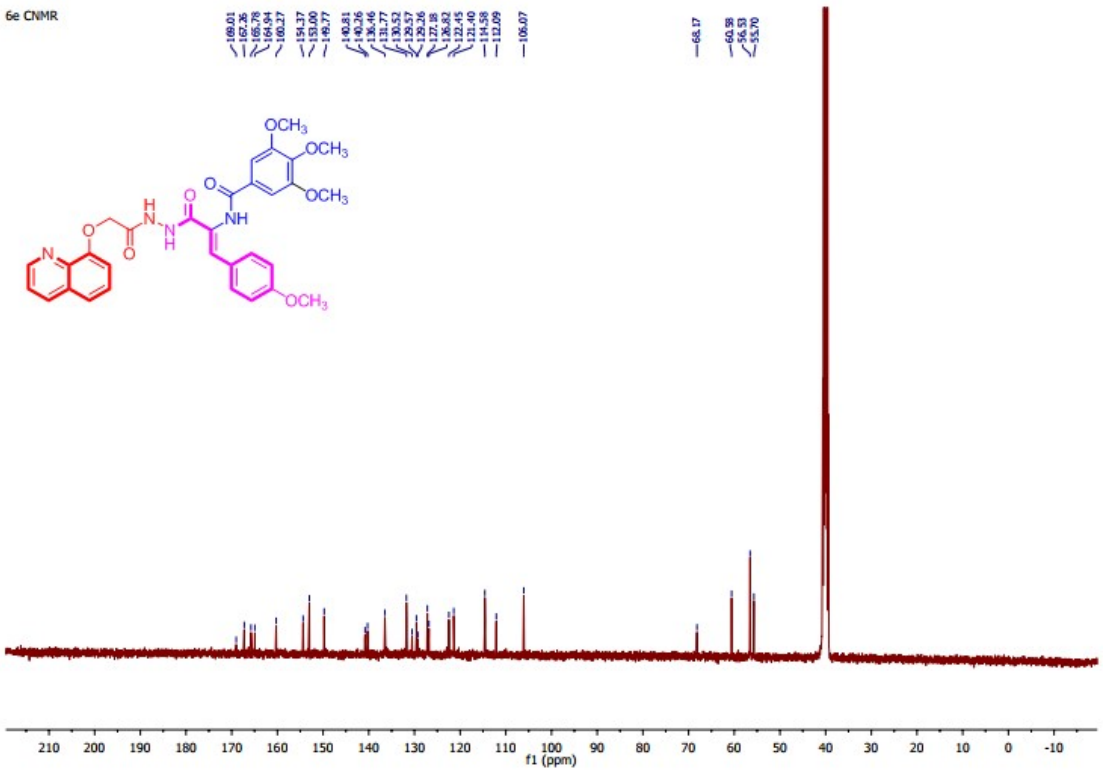

Figure S12: ^{13}C-NMR spectrum of compound **6e**

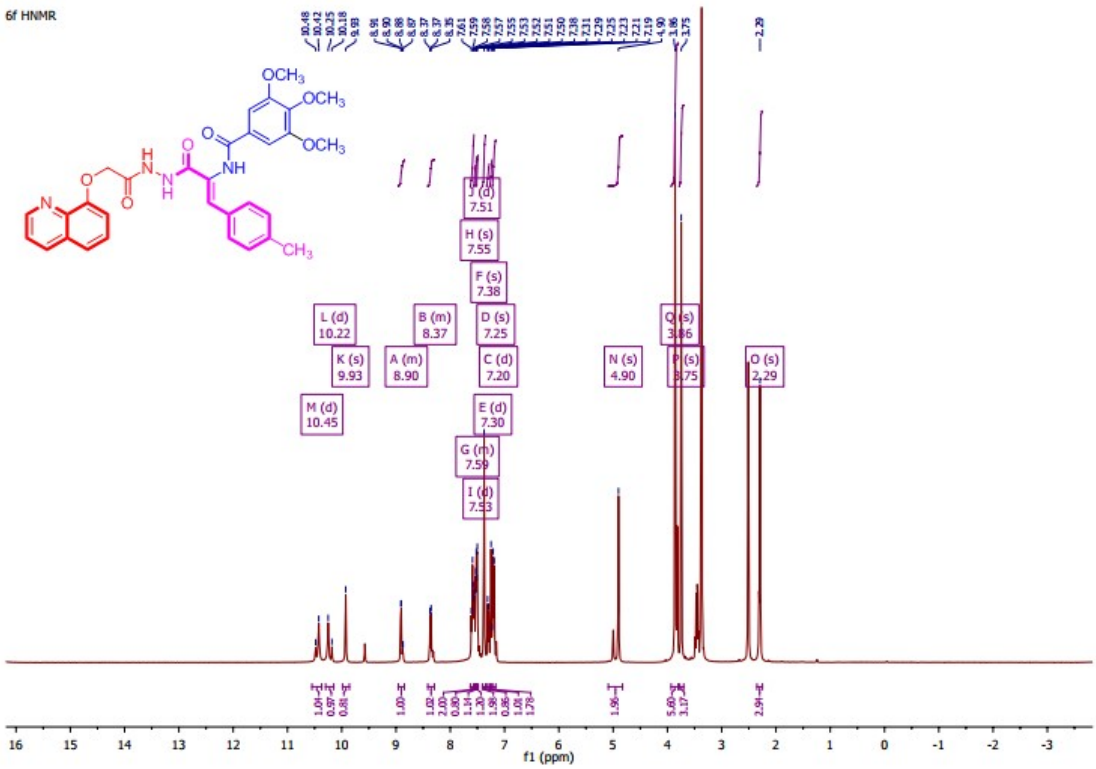

Figure S13: ^{1}H-NMR spectrum of compound **6f**

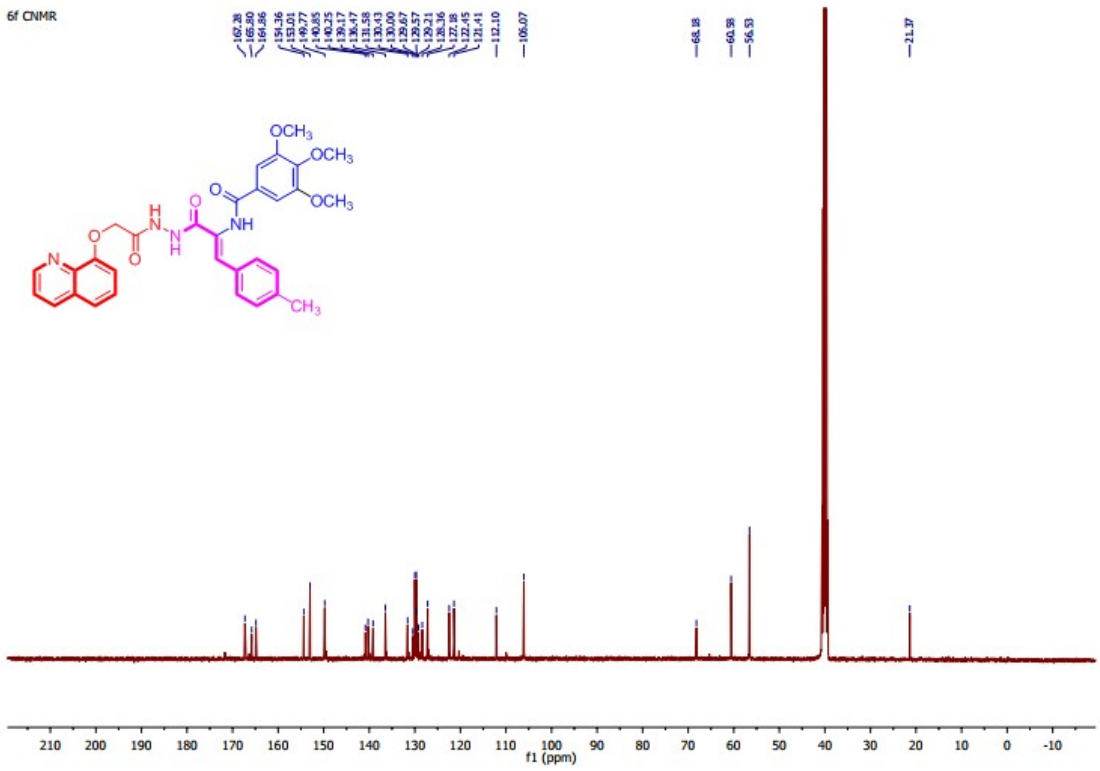

Figure S14: ^{13}C-NMR spectrum of compound **6f**

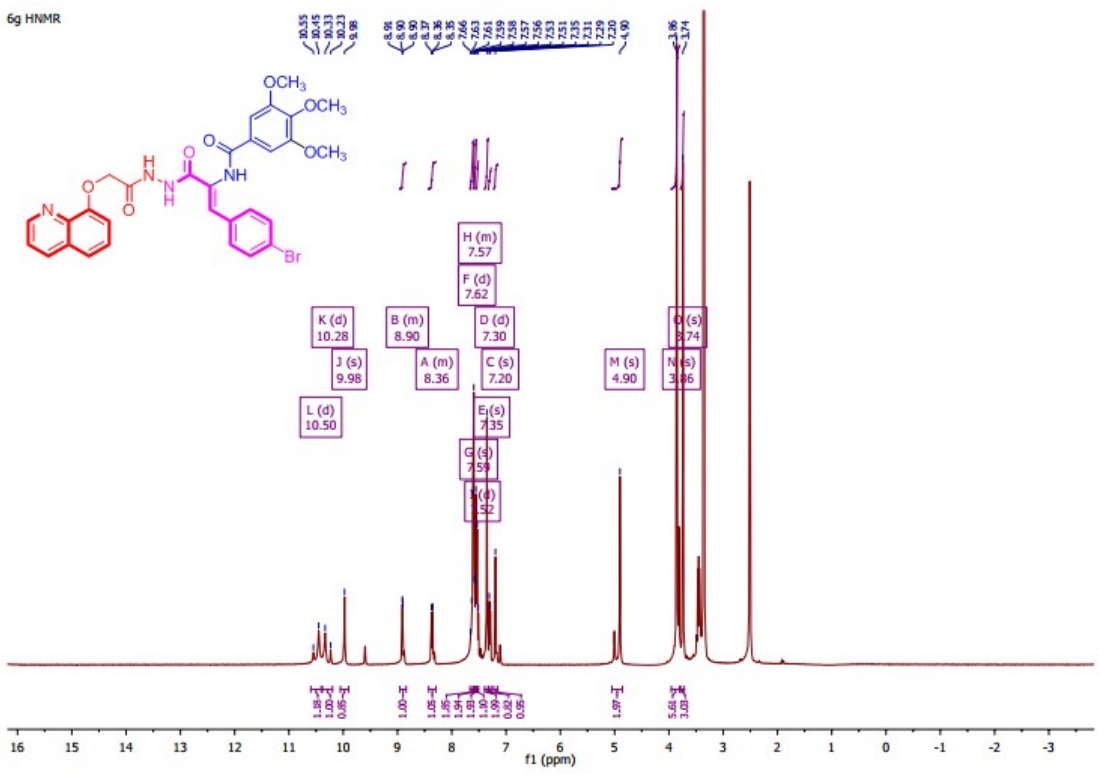

Figure S15: ^{1}H-NMR spectrum of compound **6g**

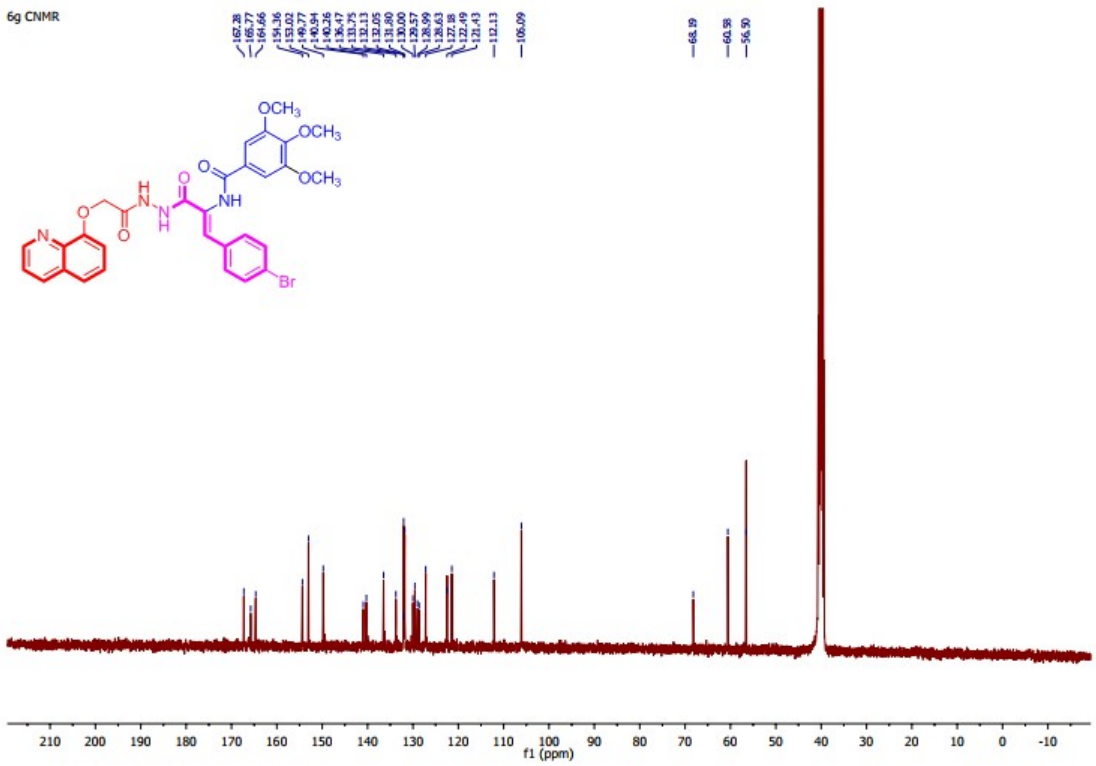

Figure S16: ^{13}C-NMR spectrum of compound **6g**

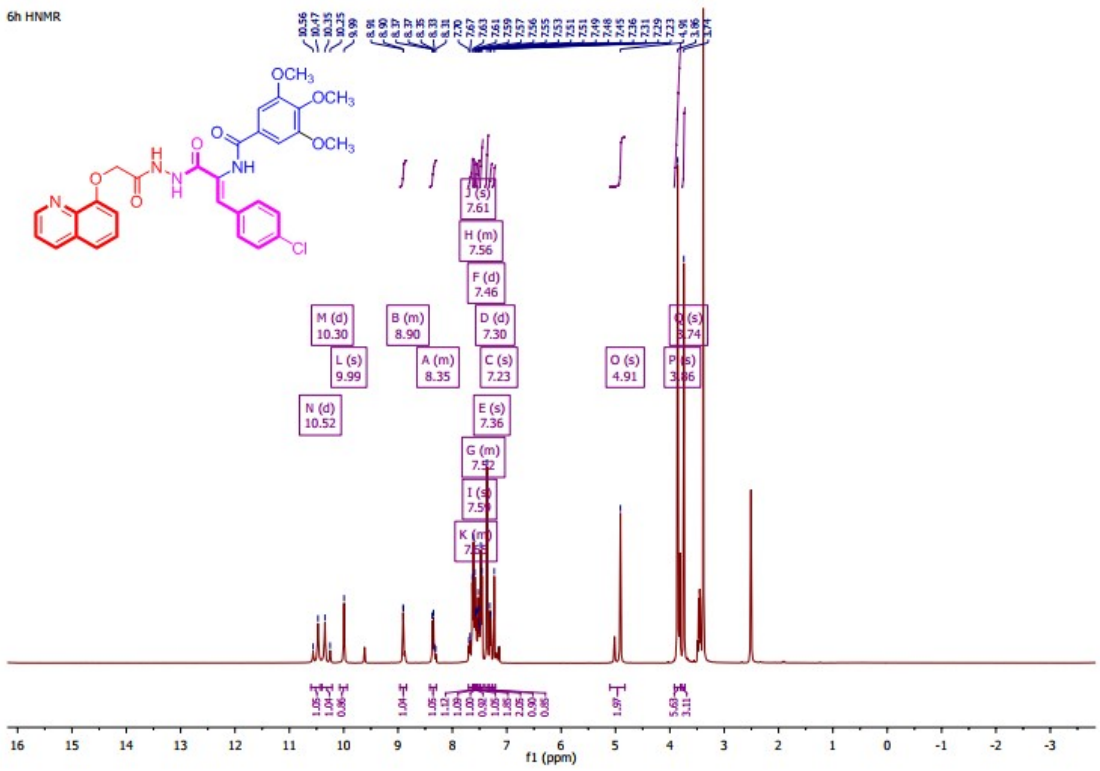

Figure S17: ^{1}H-NMR spectrum of compound **6h**

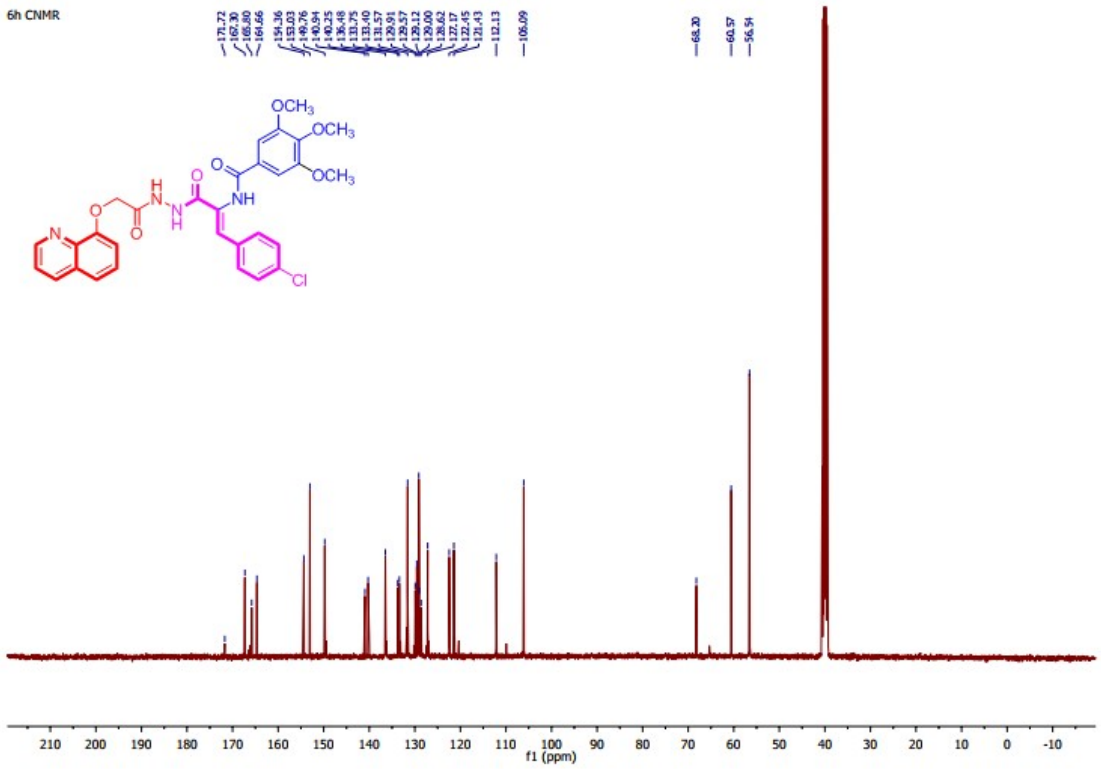

Figure S18: ^{13}C-NMR spectrum of compound **6h**

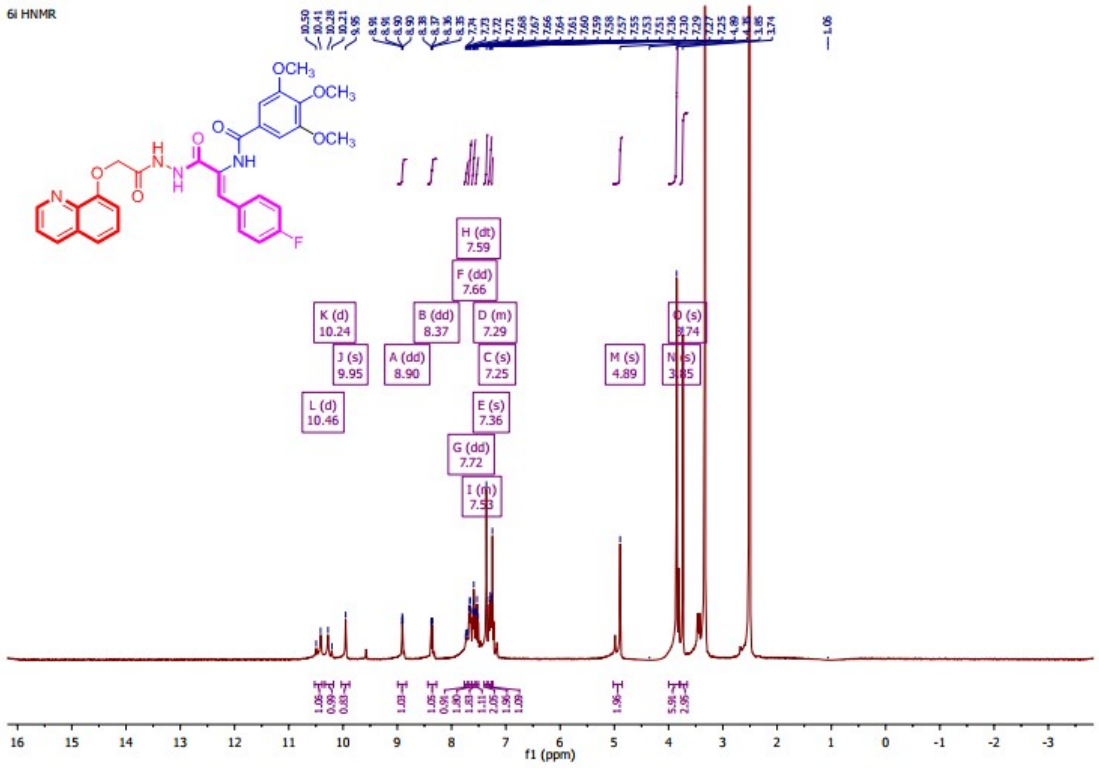

Figure S19: ^{1}H-NMR spectrum of compound **6i**

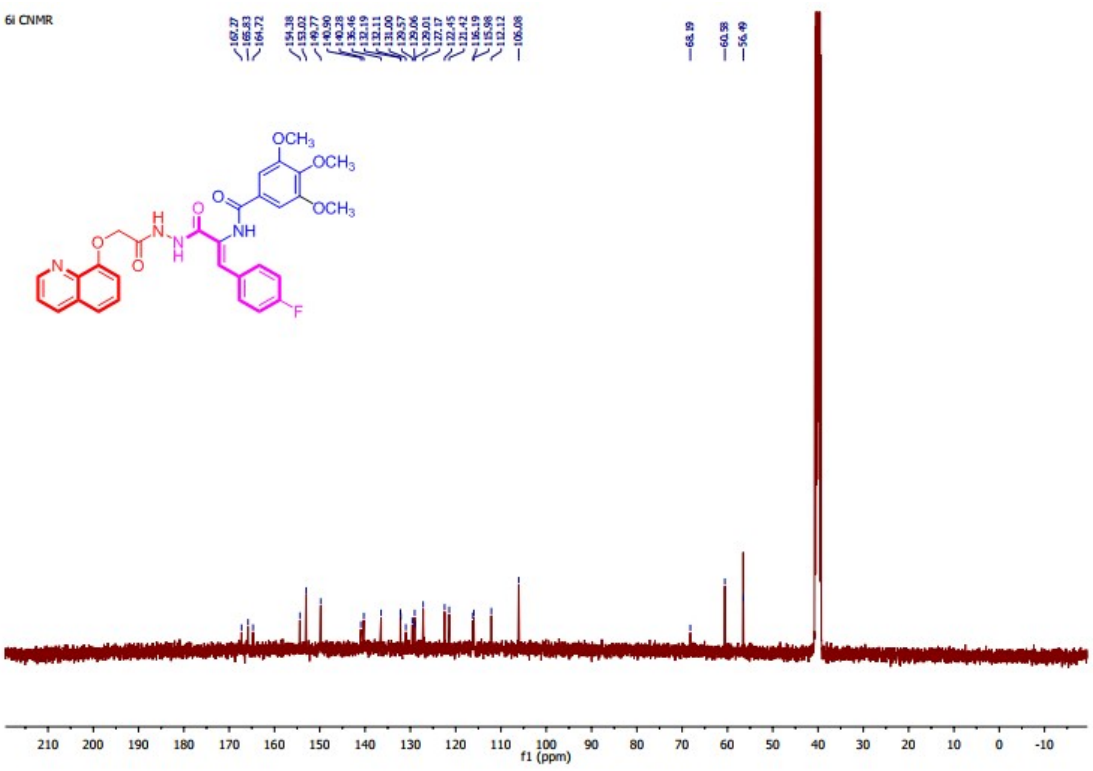

Figure S20: ^{13}C-NMR spectrum of compound **6i**

Appendix A

S4.2. Biological Studies

S4.2.1. Cytotoxic activity evaluation

To measure the cytotoxic actvity of the synthesized quinolin-8-yloxy-cinnamide Hybrids **5** and **6a-i** in liver (HepG2) cell line. Cell viability assay was assessed using MTT assay method. Cells at density of 1 x 10^4 were seeded in a 96-well plate at 37 ^oC for 24 h under 5% CO_2. After incubation, the cells were treated with different concentrations of the test quinoline cinnamide derivatives **5** and **6a-i** and incubated for 24 h, then 20 µl of MTT solution at 5 mg/mL was applied and incubated for 4 h at 37 °C. Dimethyl sulphoxide (DMSO) in volume of 100 µl was added to each well to dissolve the purple formazan that had formed. The color intensity of the formazan product, which represents the growth condition of the cells, is quantified by using an ELISA plate reader (EXL 800, USA) at 570 nm absorbance. The experimental conditions were carried out with at least three replicates, and the experiments were repeated at least three times.

S4.2.2. Tubulin polymerization Assay

Compound **6e** and colchicine (Col) were evaluated for their tubulin inhibitory activity according to manufacturer's instructions using # abcam Human Beta-tubulin simplestep ELISA Kit ab245722.

Version 1 Last updated 7 April 2020

ab245722
Human Beta-Tubulin SimpleStep ELISA® Kit

For the quantitative measurement of Beta-Tubulin in human cell and tissue homogenate extract samples.

This product is for research use only and is not intended for diagnostic use.

S4.2.3. Cell cycle analysis of compound 6e

Cell cycle analysis in HepG2 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* EZCell™ Cell Cycle Analysis Kit Catalog #K920) by flow cytometry assay. HepG2 cells at a density of 2×10^5 per well were harvested and washed twice in PBS. After that, the cells were incubated at 37 °C and 5% CO_2. The medium was incubated with the tested compound **6e** at the IC_{50} (μM) for 48 h, washed twice in PBS, fixed with 70% ethanol, rinsed again with PBS. Afterward, medium was stained with DNA fluorochrome PI for 15 min at 37 °C. The samples were immediately analyzed using Facs Calibur flow cytometer (Becton and Dickinson, Heidelberg, Germany).

S4.2.4. Apoptosis assay for compound 6e

Apoptosis in HepG2 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* Annexin V-FITC Apoptosis Detection Kit, Catalog #: K101) by flow cytometry assay. HepG2 cells at a density of 2×10^5 per well were treated

with compound **6e** at the IC_{50} (μM) for 48 h, then the cells were harvested and stained with Annexin V-FITC/ PI dye for 15 min in the dark at 37 °C. The samples were immediately analyzed using *FACS Calibur* flow cytometer (Becton and Dickinson, Heidelberg, Germany).