Supporting Information

Design, Synthesis and Investigation of Cytotoxic Activity of *cis*-Vinylamide Linked Combretastatin Analogues as Potential Anticancer Agents

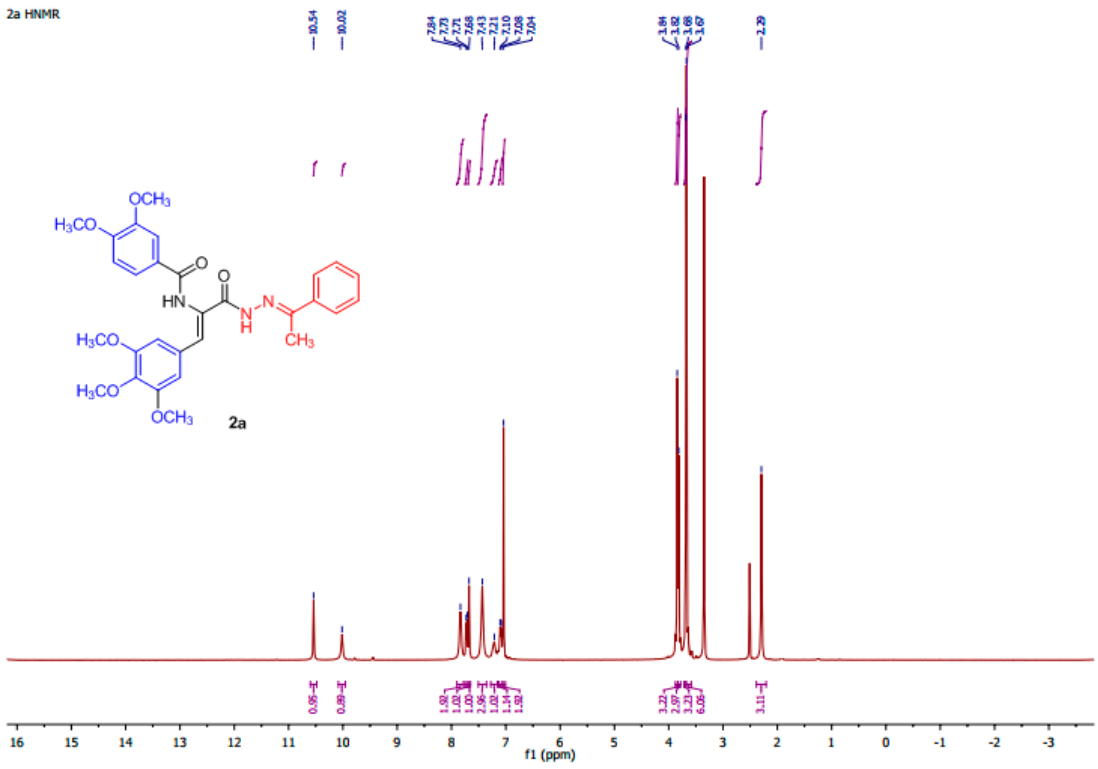


Figure S1: ^{1}H-NMR spectrum of compound **2a**

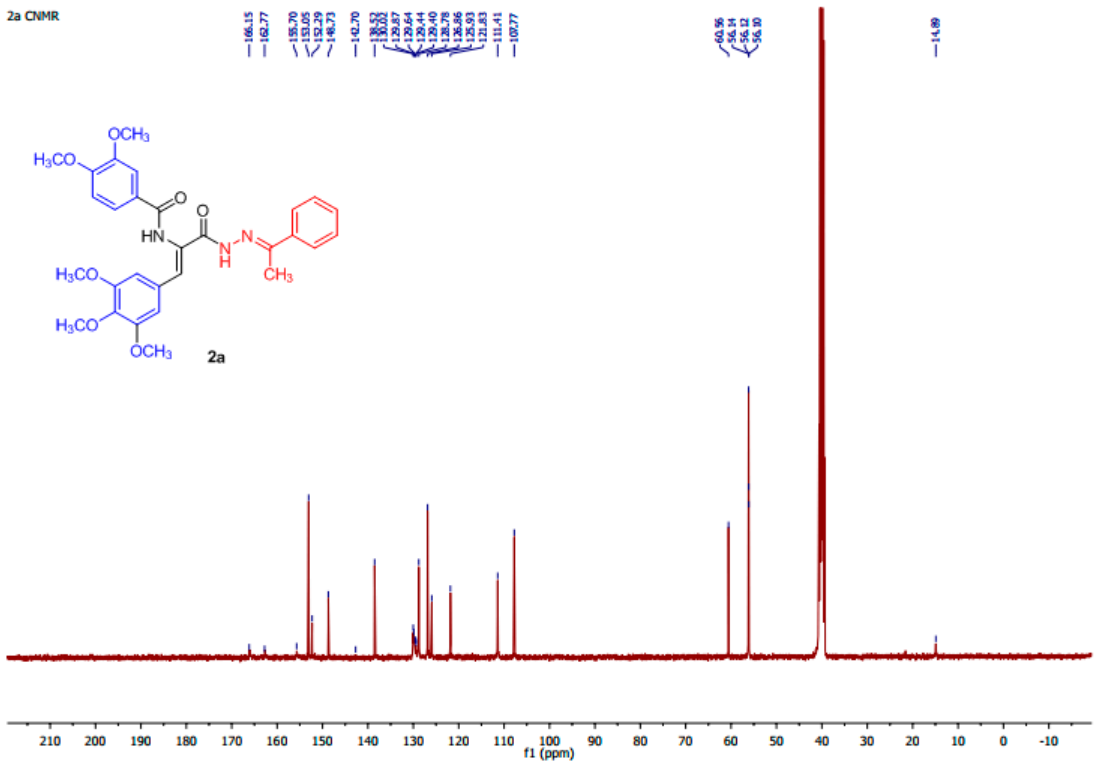


Figure S2: ^{13}C-NMR spectrum of compound **2a**

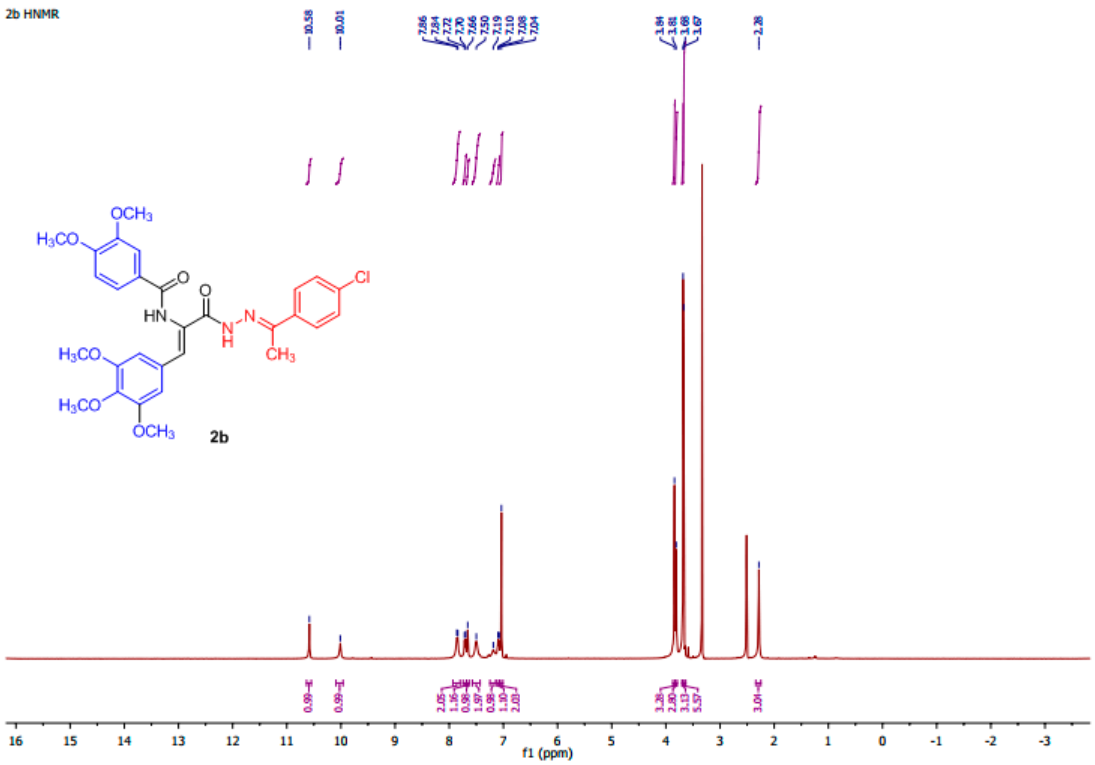


Figure S3: ^{1}H-NMR spectrum of compound **2b**

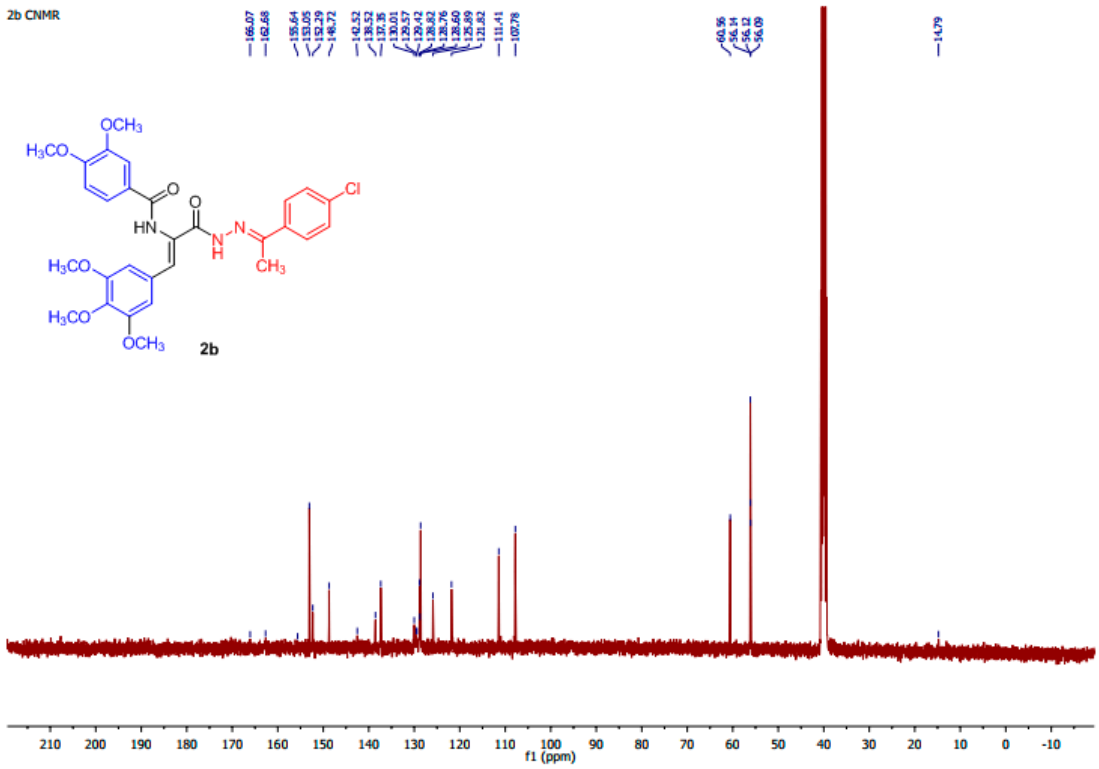


Figure S4: ^{13}C-NMR spectrum of compound **2b**

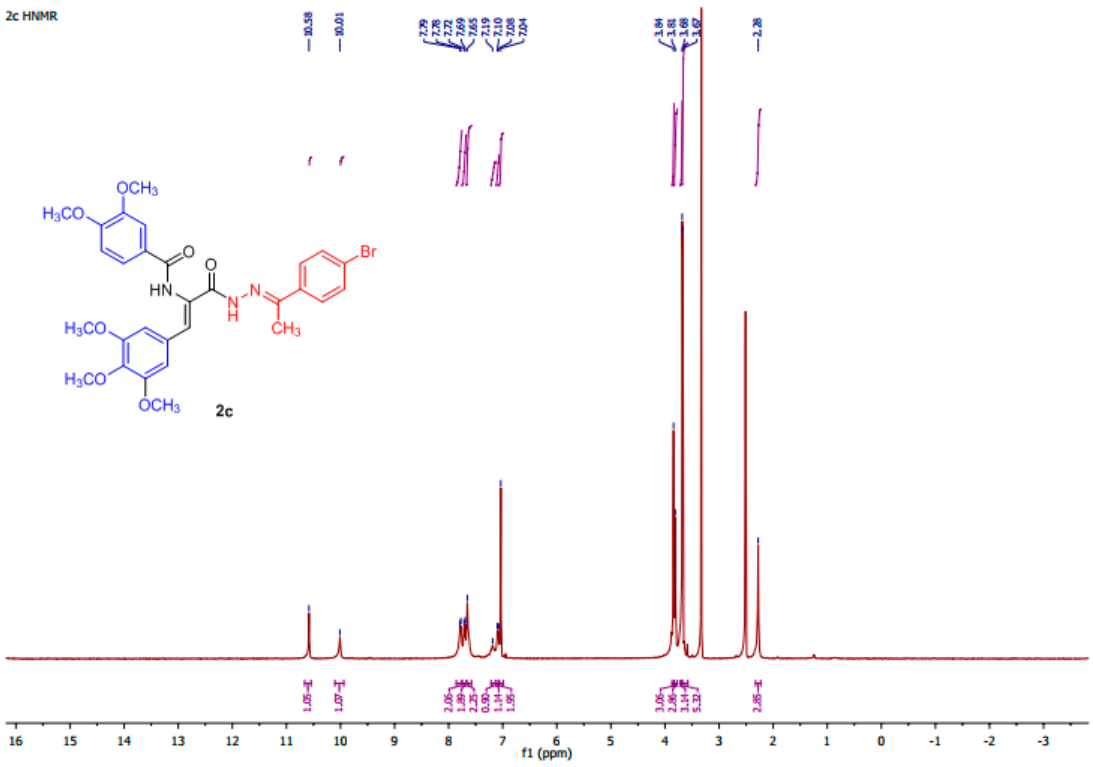


Figure S5: ^{1}H-NMR spectrum of compound **2c**

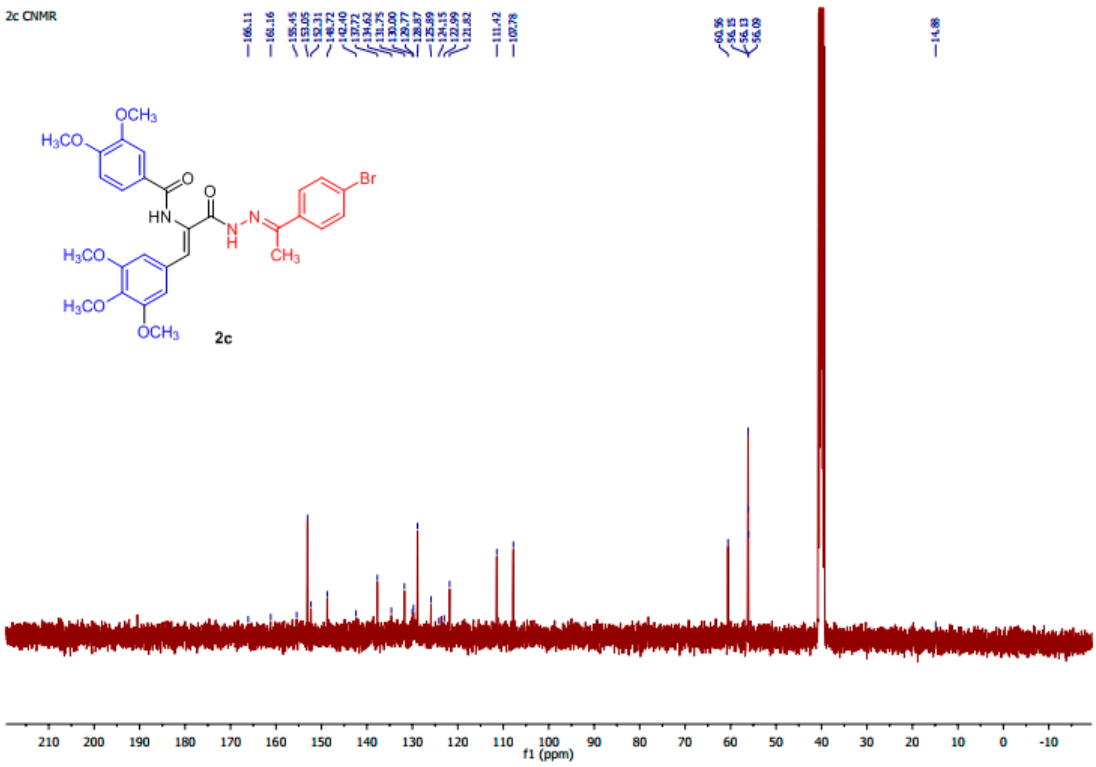


Figure S6: ^{13}C-NMR spectrum of compound **2c**

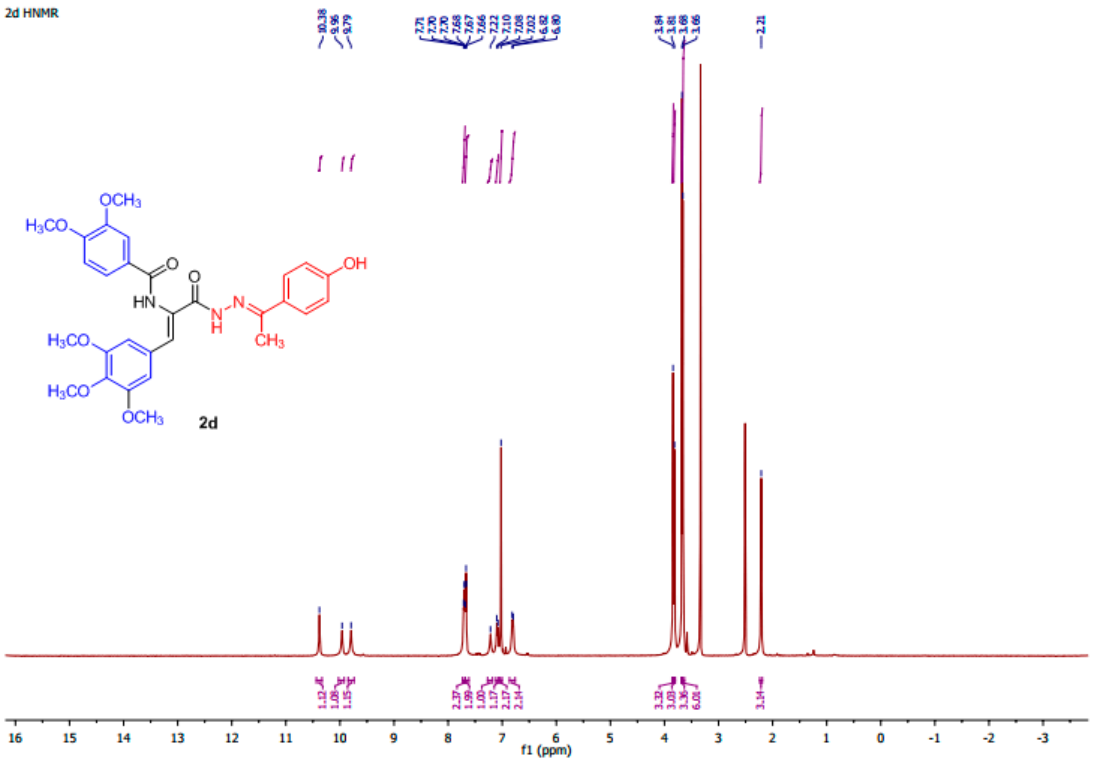


Figure S7: ^{1}H-NMR spectrum of compound **2d**

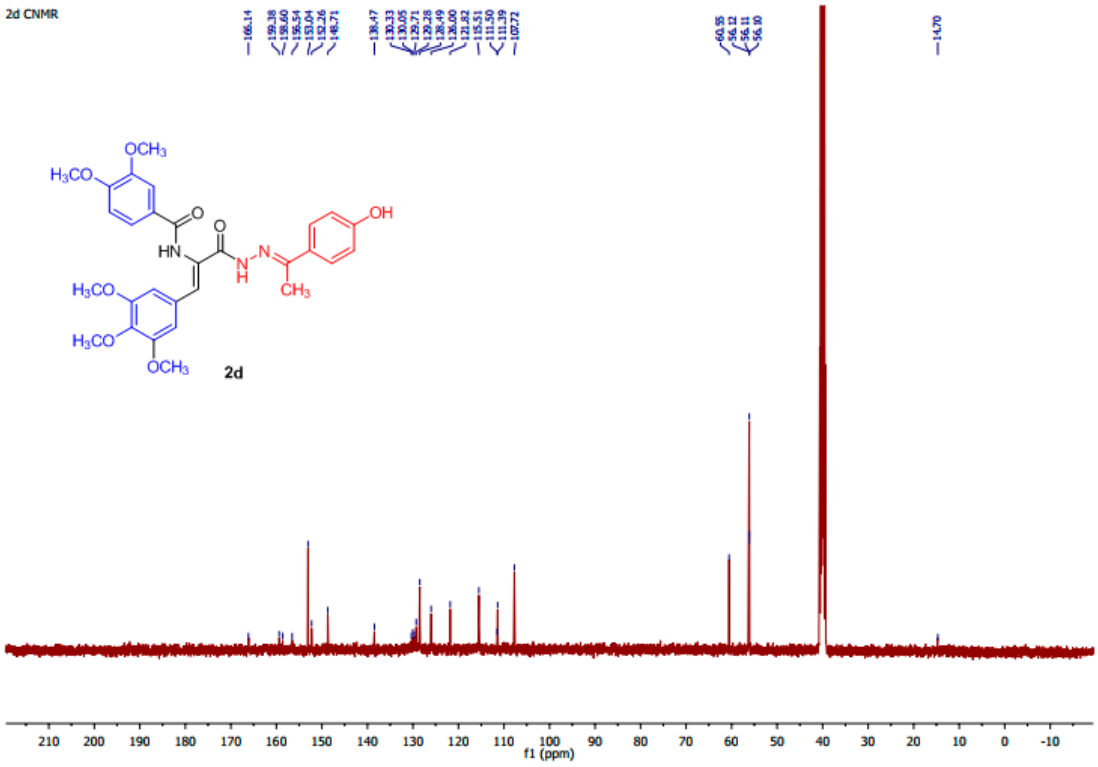


Figure S8: ^{13}C-NMR spectrum of compound **2d**

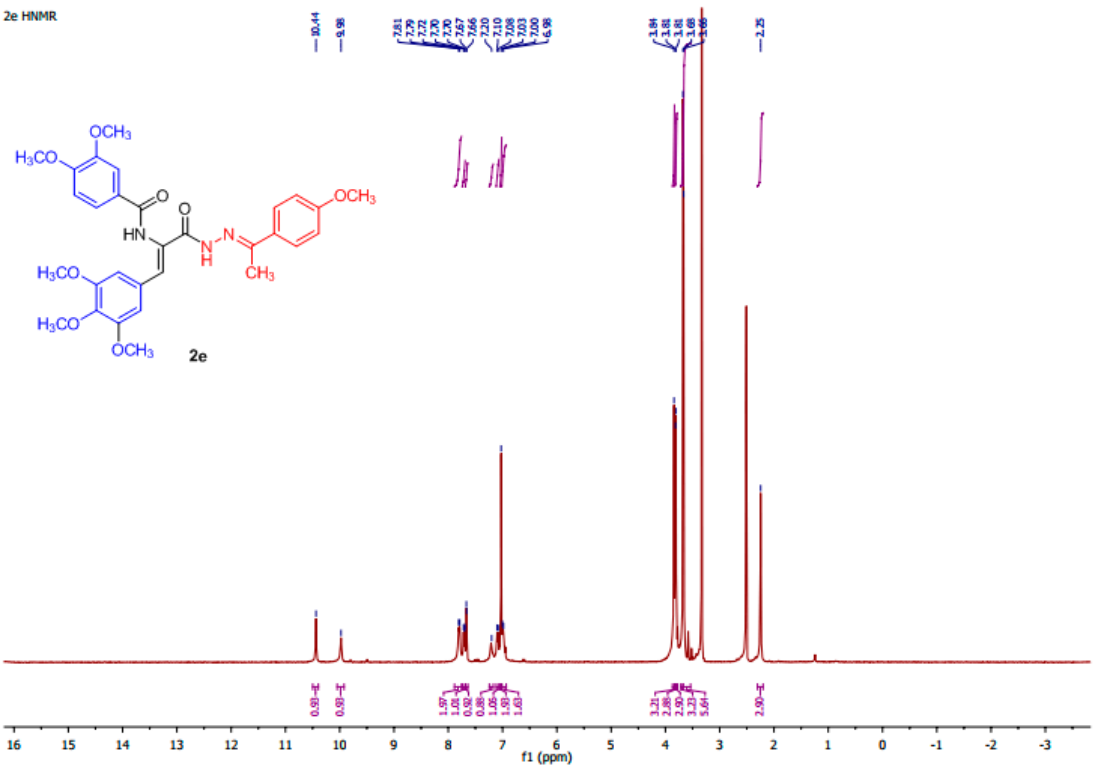


Figure S9: ^{1}H-NMR spectrum of compound **2e**

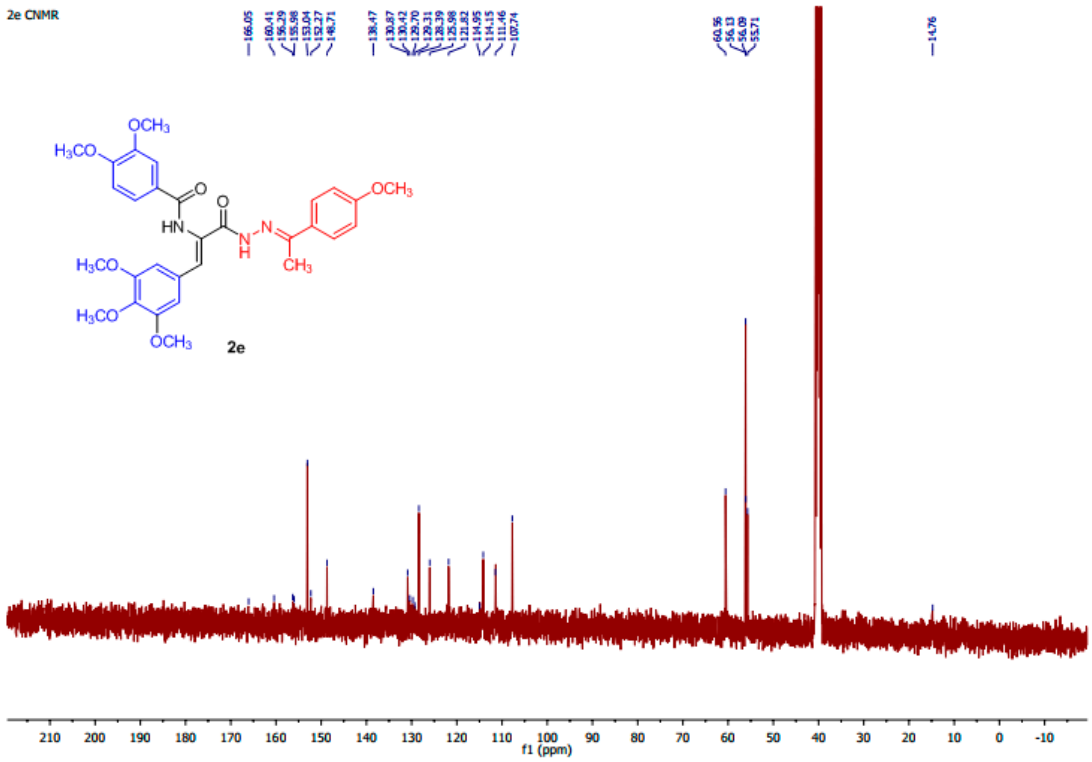


Figure S10: ^{13}C-NMR spectrum of compound **2e**

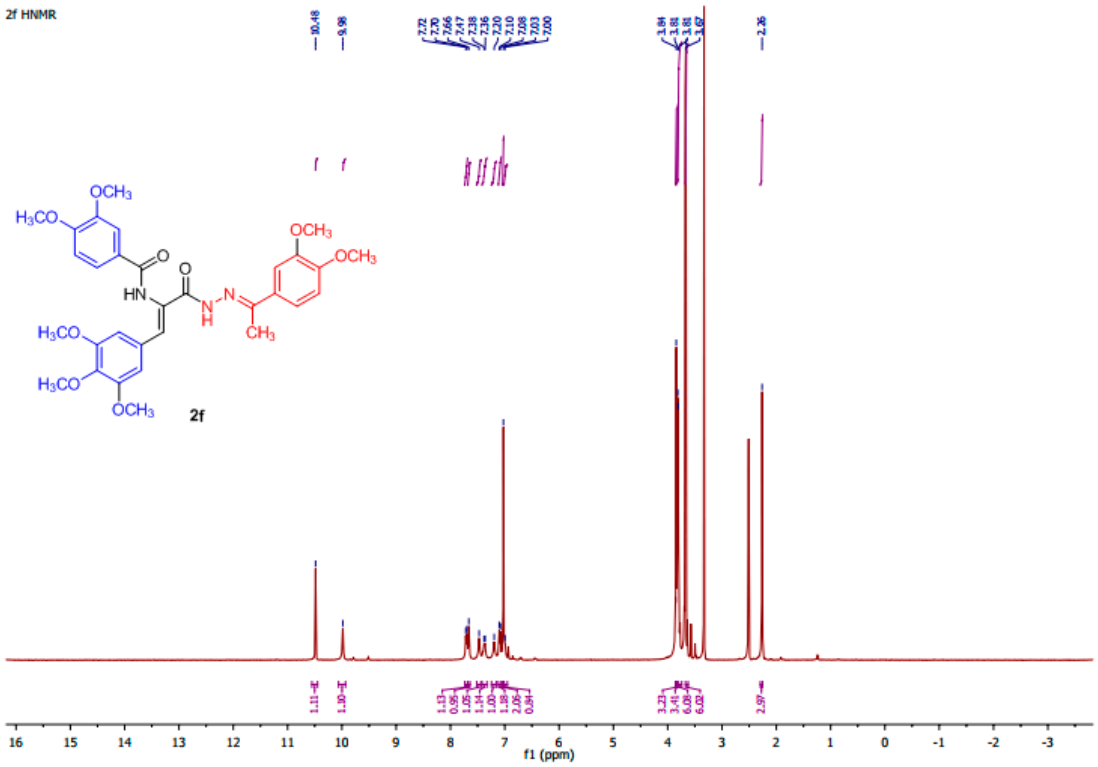


Figure S11: ^{1}H-NMR spectrum of compound **2f**

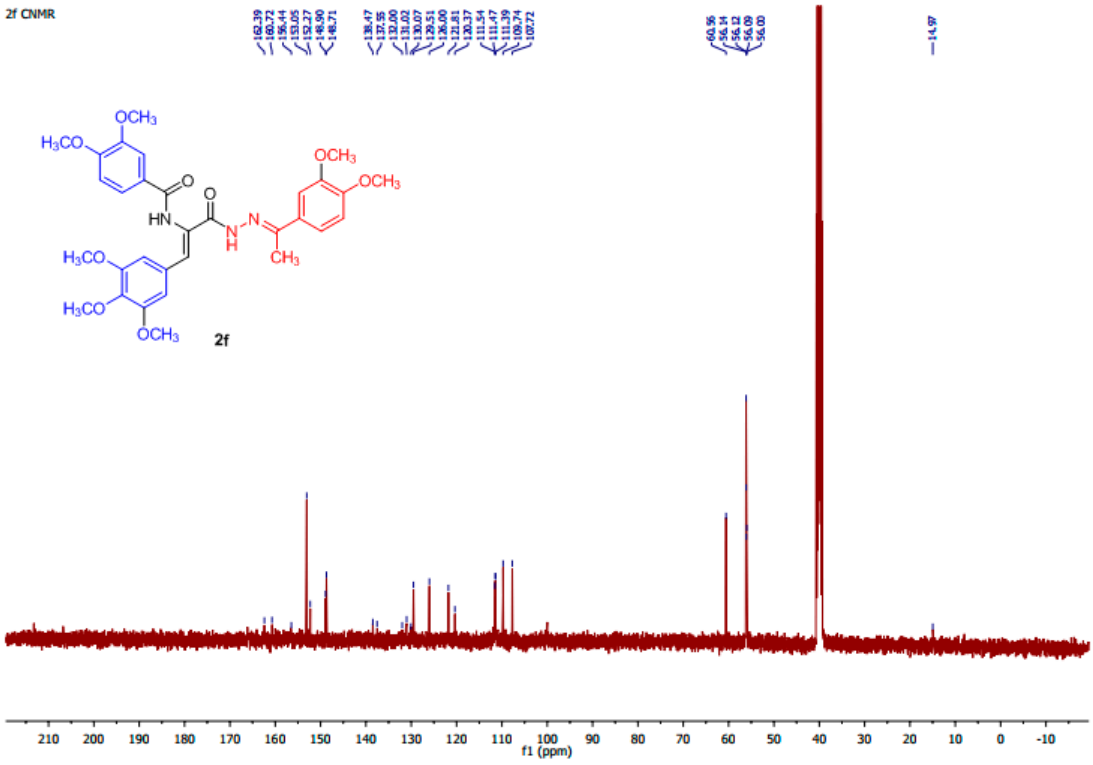


Figure S12: ^{13}C-NMR spectrum of compound **2f**

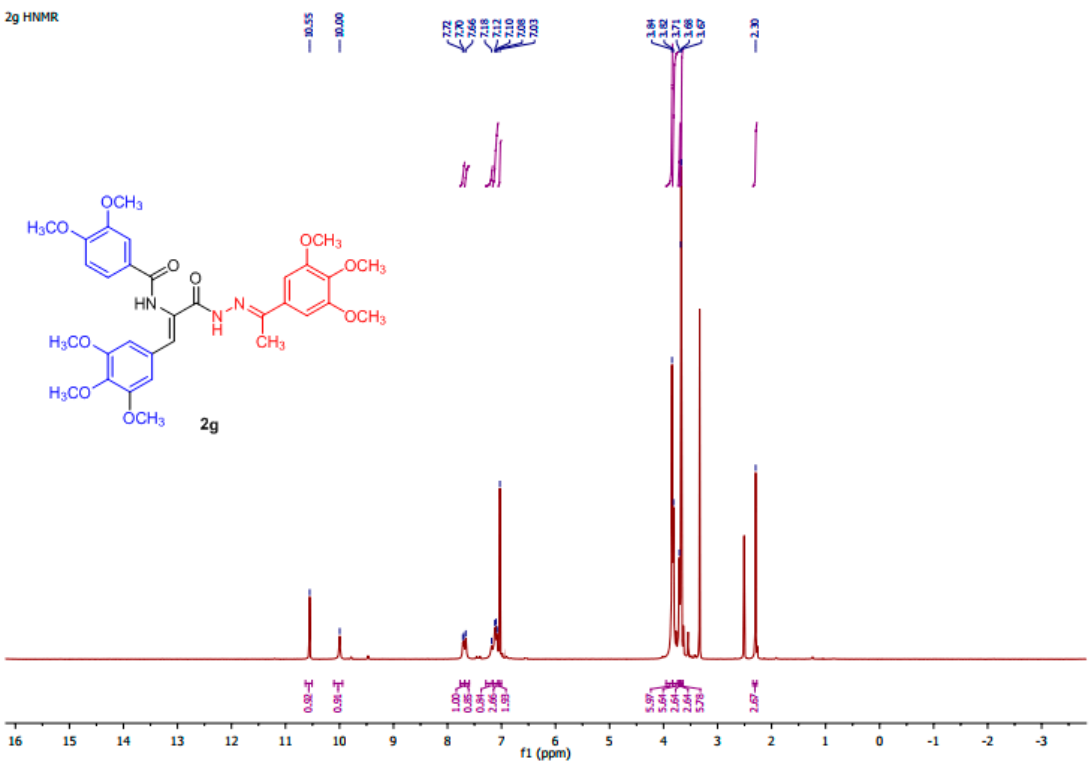


Figure S13: ^{1}H-NMR spectrum of compound **2g**

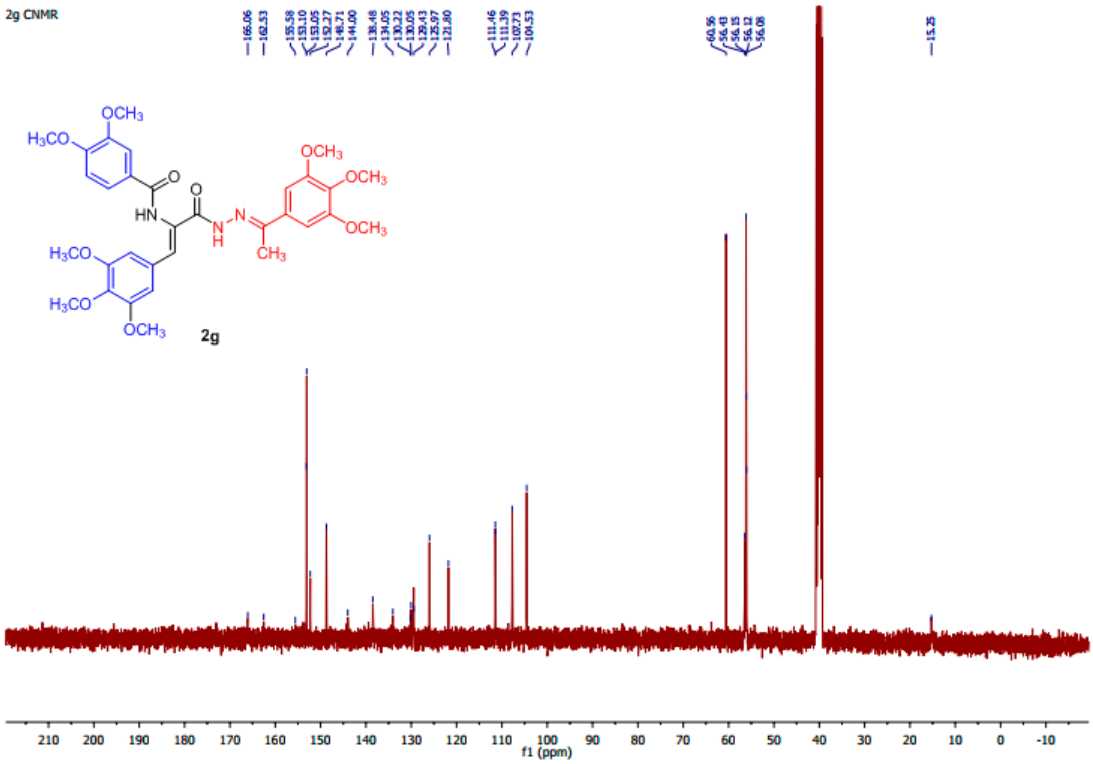


Figure S14: ^{13}C-NMR spectrum of compound **2g**

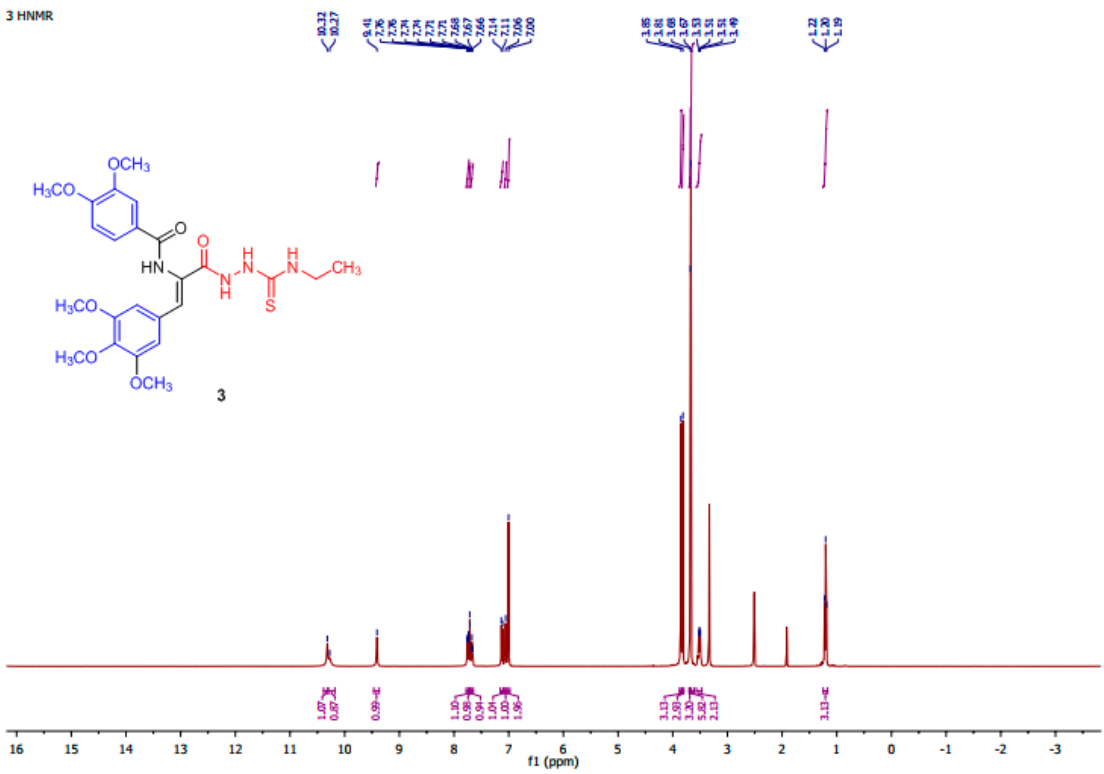


Figure S15: ^{1}H-NMR spectrum of compound **3**

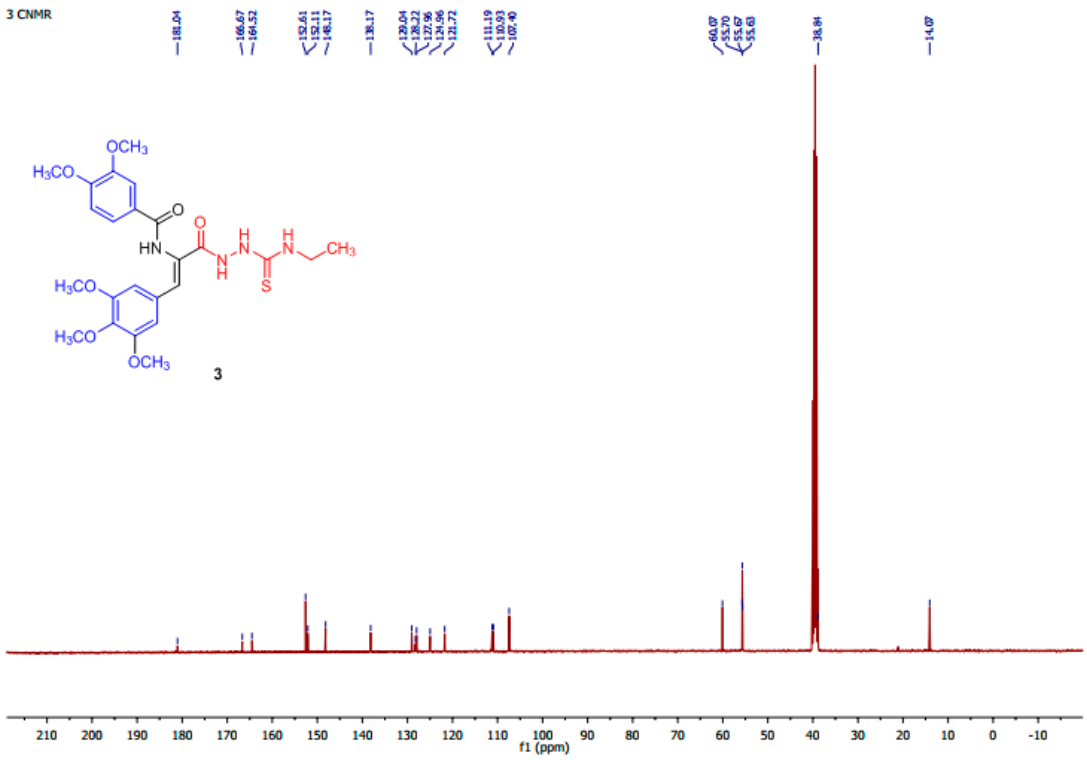


Figure S16: ^{13}C-NMR spectrum of compound **3**

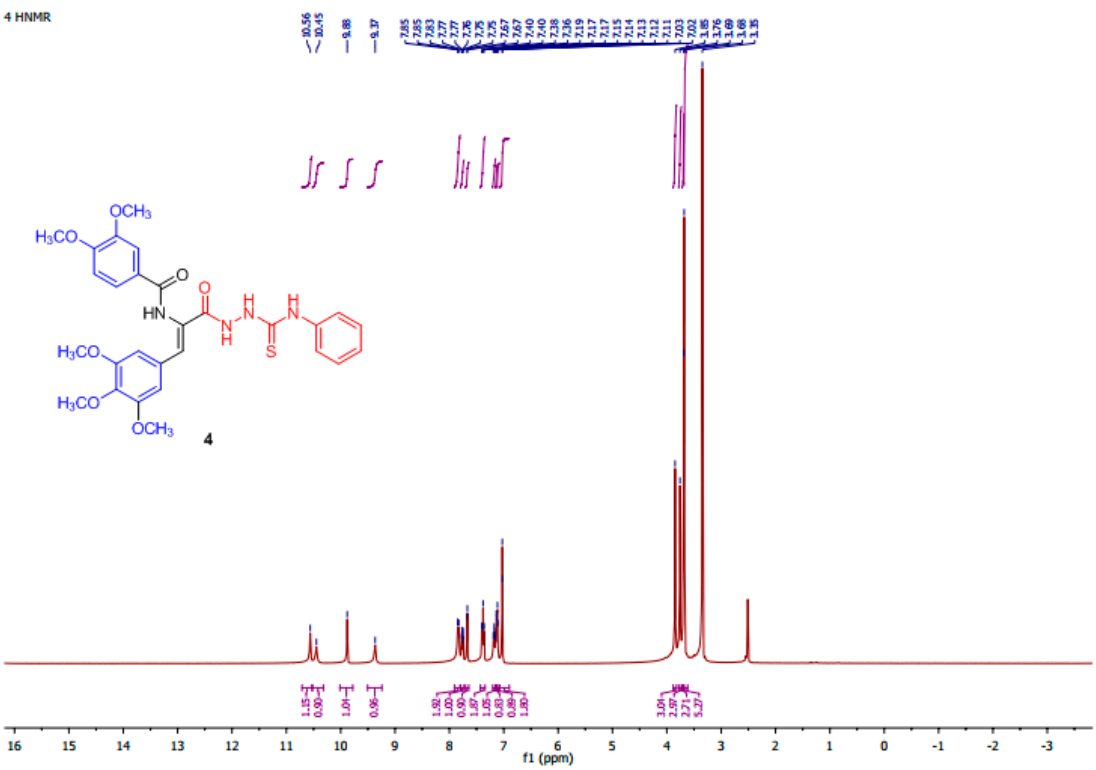


Figure S17: ^{1}H-NMR spectrum of compound **4**

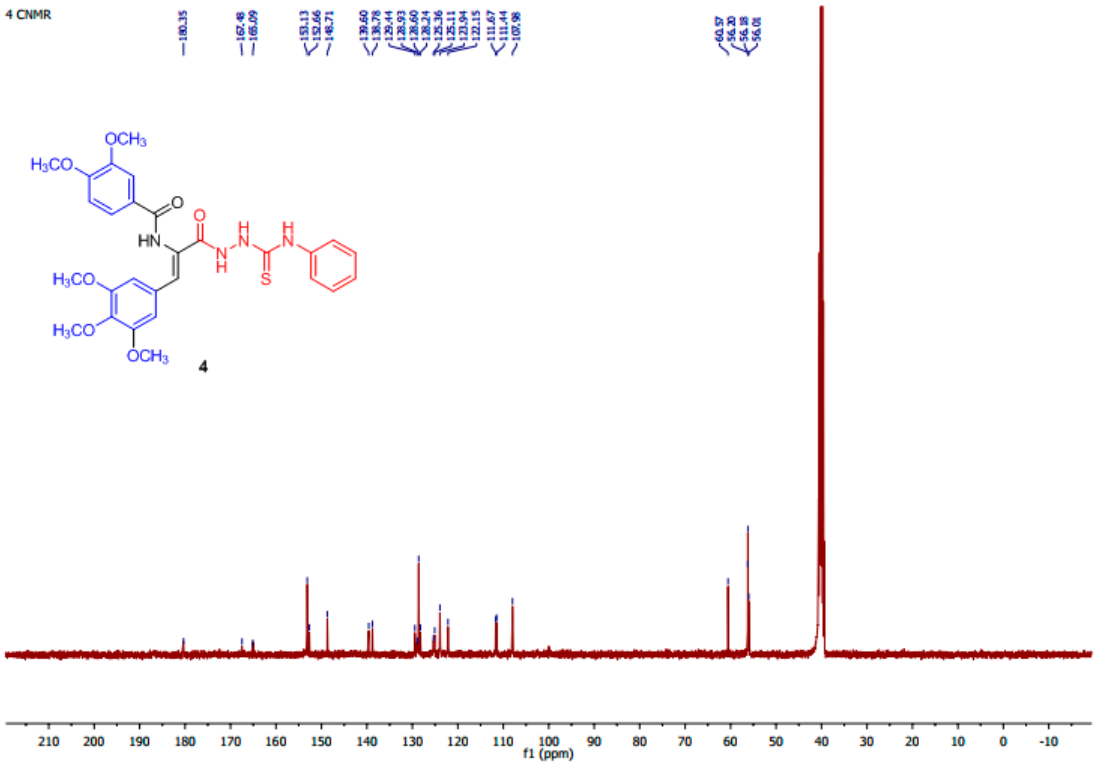


Figure S18: ^{13}C-NMR spectrum of compound **4**

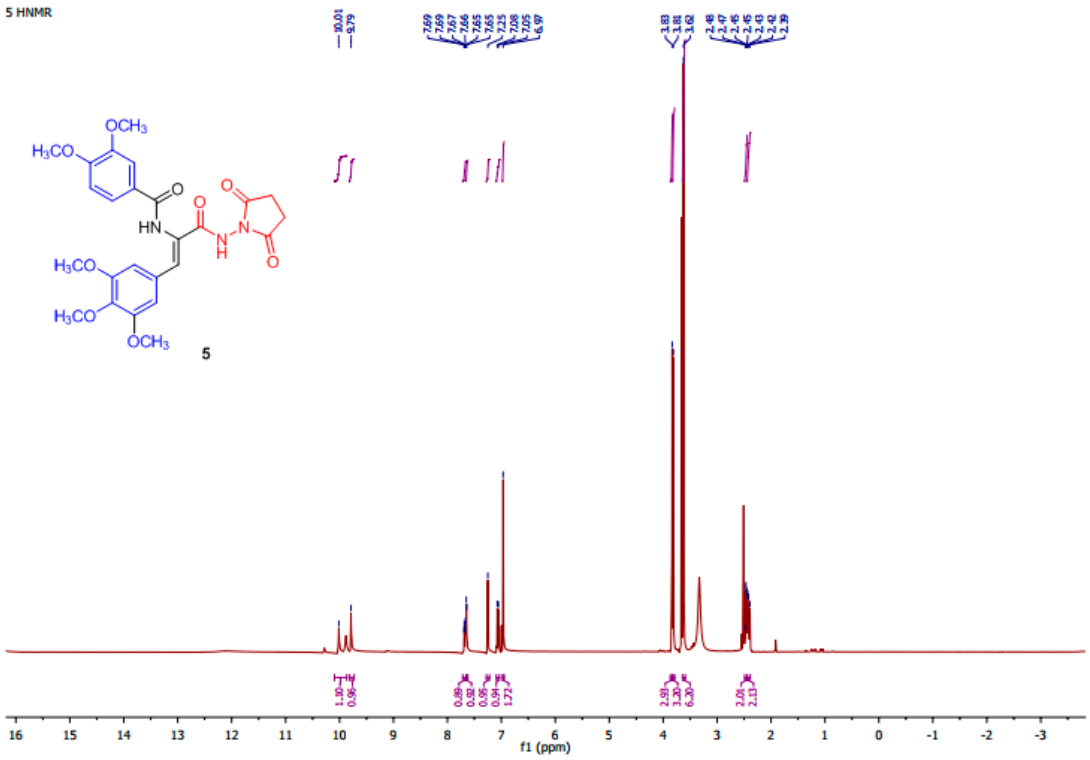


Figure S19: ^{1}H-NMR spectrum of compound **5**

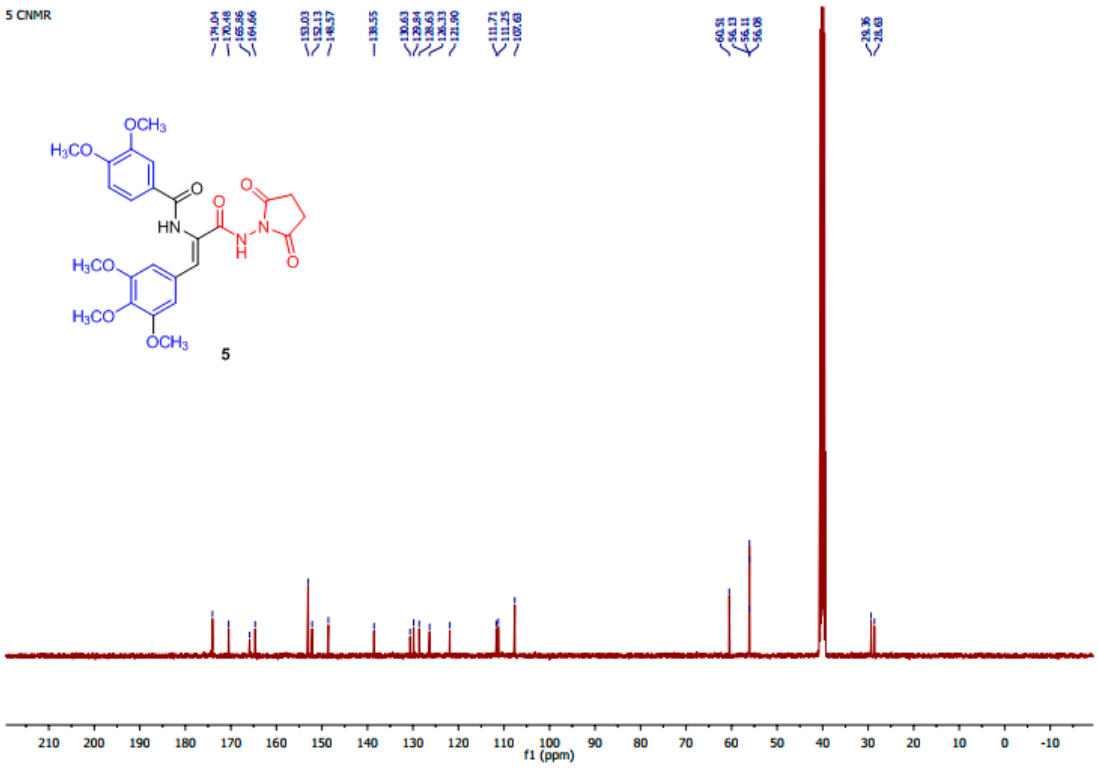


Figure S20: ^{13}C-NMR spectrum of compound **5**

4.1.1. Chemistry: General

Melting points were determined in open capillaries tube using Electrothermal Digital melting point apparatus and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a Bruker 400 MHz DRX-Avance NMR spectrometer, peaks positions are given in ppm downfield from tetramethylsilane (TMS) as the internal standard. Elemental analyses were performed on Elementar, Vario El, Microanalytical unit, Cairo, Egypt and were found within ±0.4% of the theoretical values.

4.2. Biological studies

4.2.1. Cytotoxic activity evaluation

To measure the cytotoxic actvity of the newly synthesized *cis*-vinylamide derivatives **2a-5** in breast carcinoma (MCF-7) 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 compounds **2a-5** 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.

4.2.2. Tubulin inhibitions Assay

Compounds **2f, 3, 5** and CA-4 were evaluated for its tubulin inhibitory activity according to manufacturer's instructions using # abcam Human Beta-tubulin simplestep ELISA Kit ab245722.

4.2.3. Cell cycle analysis of compound 3

Cell cycle analysis in MCF-7 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* EZCell^{TM} Cell Cycle Analysis Kit Catalog #K920) by flow cytometry assay. MCF-7 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 **3** at its 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).

4.2.4. Apoptosis assay for compound 3

Apoptosis in MCF-7 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* Annexin V-FITC Apoptosis Detection Kit, Catalog #: K101) by flow cytometry assay. MDA-MB-231 cells at a density of 2 × 10^5 per well were treated with compound **3** at its 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).

4.2.5. *C*aspase 3 assay for compound 3

To determine the effect of the synthesized *cis*-vinylamide molecule **3** on apoptosis, the active caspase 3 level was measured using ELISA analysis according to manufacturer's instructions. Briefly, MCF-7 cells at a density of 2×10^5 per well were treated with compound **3** at its IC_{50} (μM) for 48 h, then the cells were lysed with cell extraction buffer. This lysate was diluted in standard diluent buffer over the range of the assay. The optical density of each well was determined within 30 min using a microplate plate reader set at 450 nm to determine the human active caspase 3 level.