

New Schiff Base–TMB Hybrids: Design, Synthesis and Antiproliferative Investigation as Potential Anticancer Agents

Najiah M. Alyamani

Department of Biology, College of Science, University of Jeddah, Jeddah 21493, Saudi Arabia; nmalyamani@uj.edu.sa

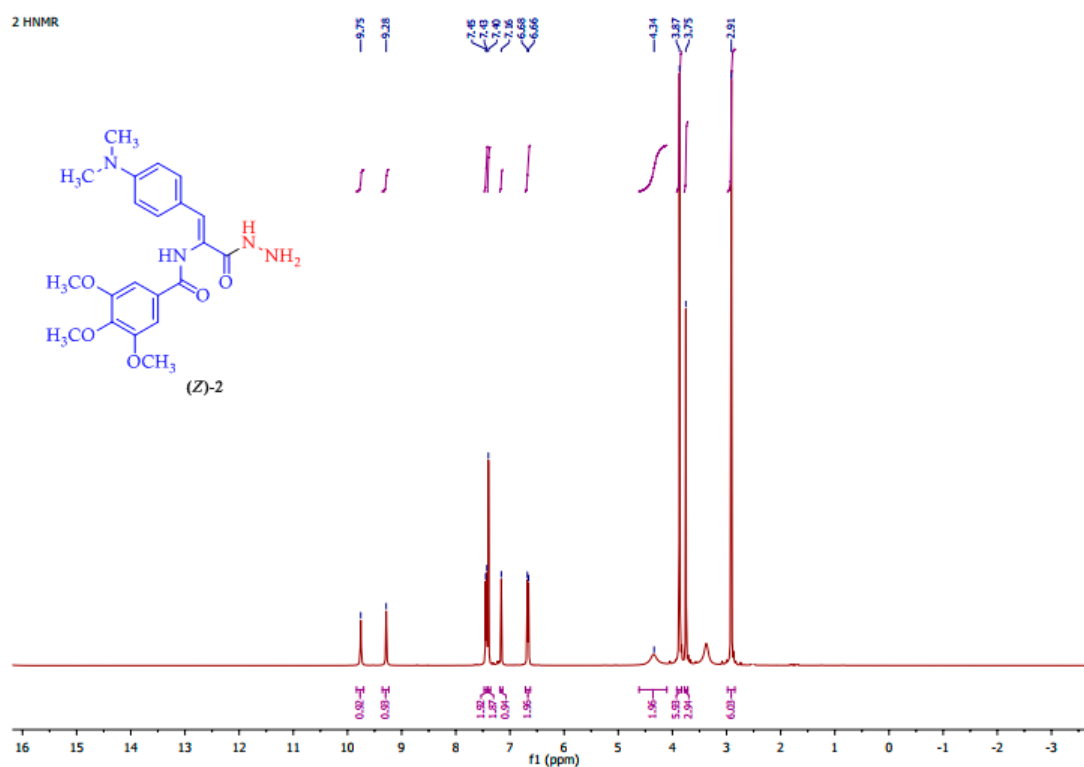


Figure S1: ¹H-NMR spectrum of compound 2

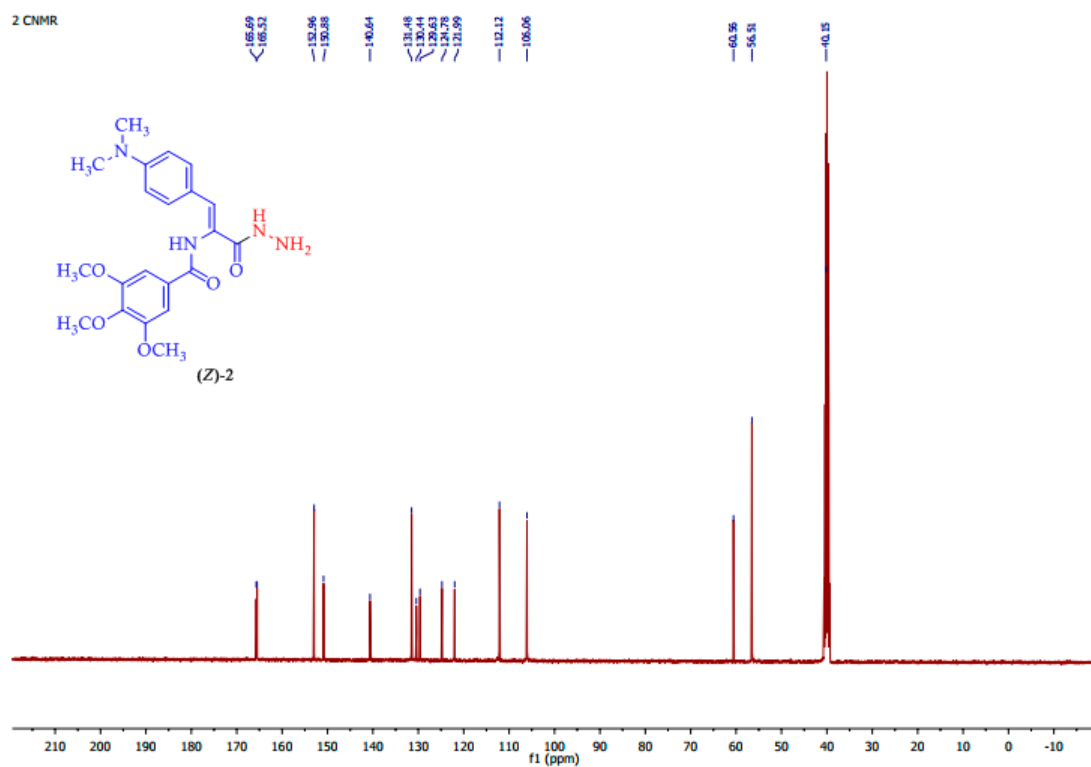


Figure S2: ¹³C-NMR spectrum of compound 2

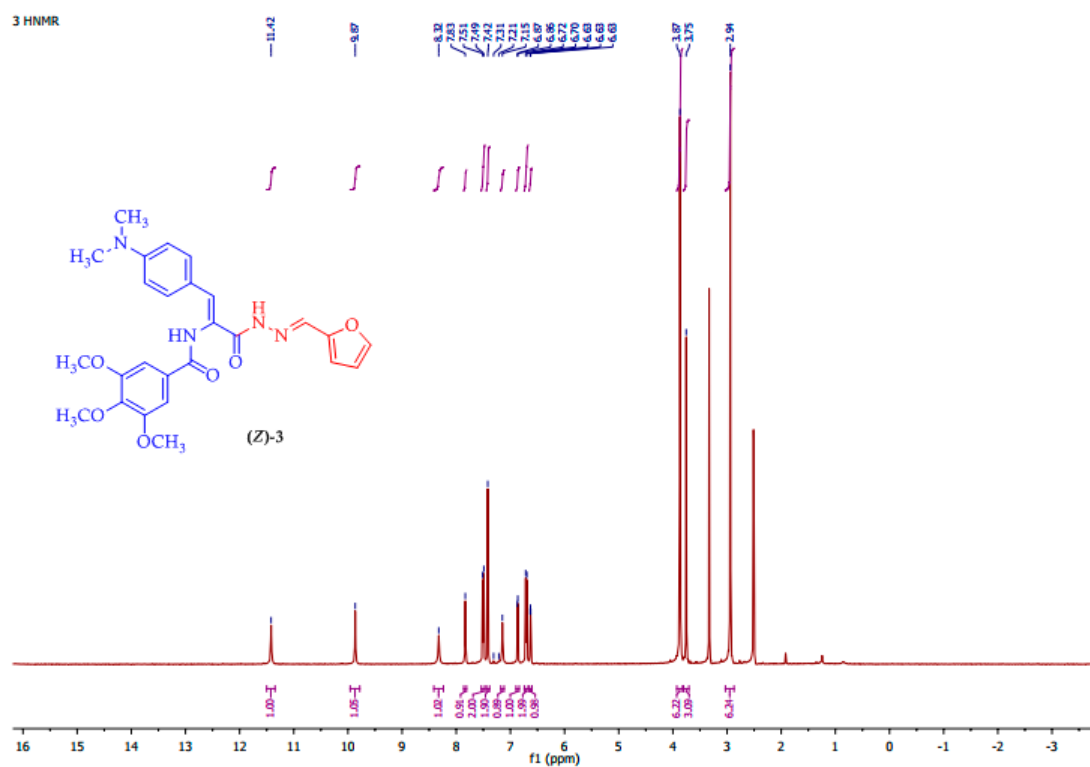


Figure S3: ^1H -NMR spectrum of compound 3

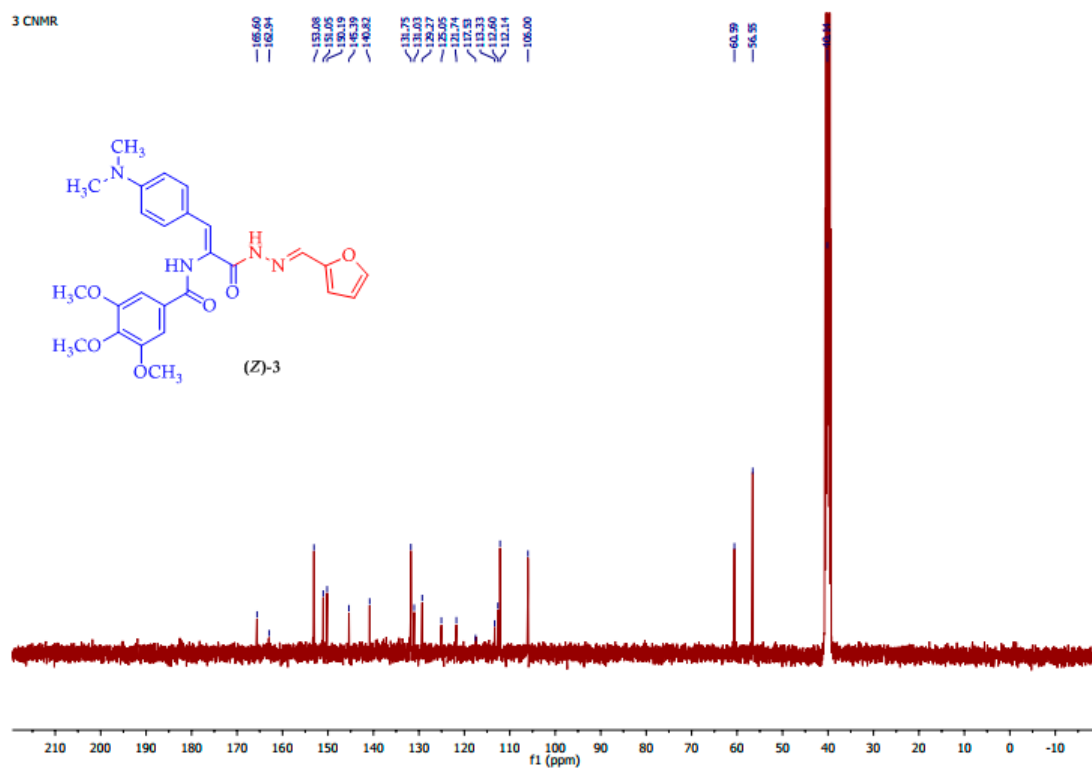


Figure S4: ¹³C-NMR spectrum of compound 3

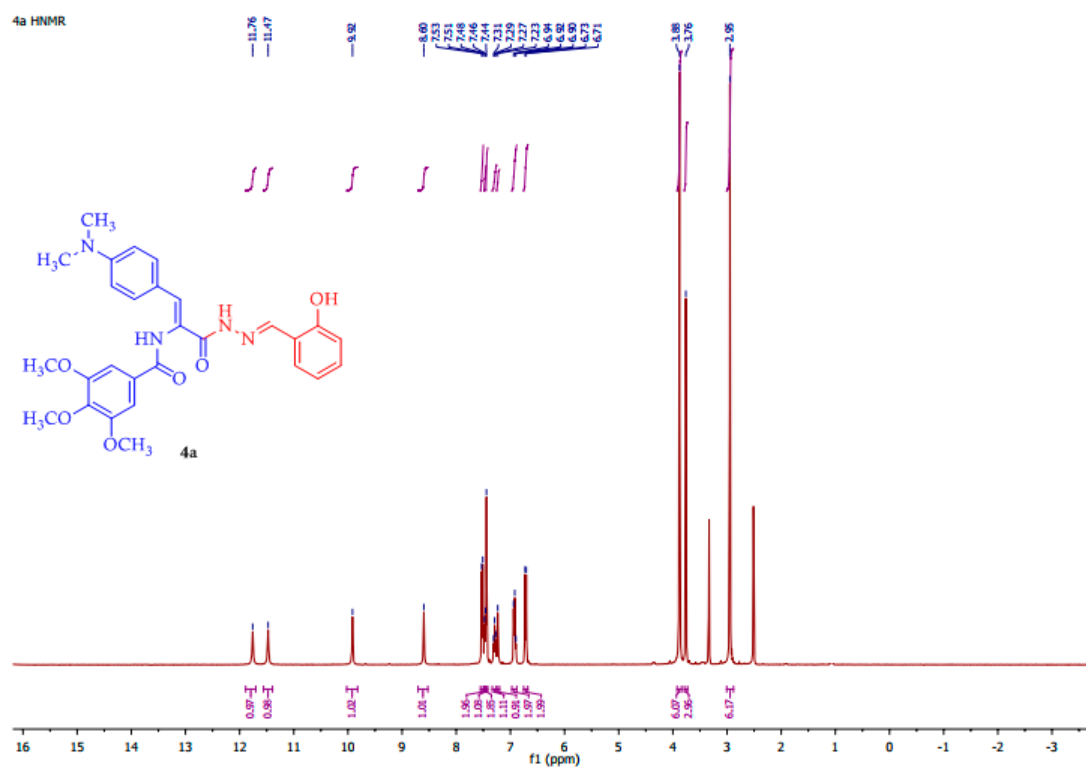


Figure S5: ¹H-NMR spectrum of compound 4a

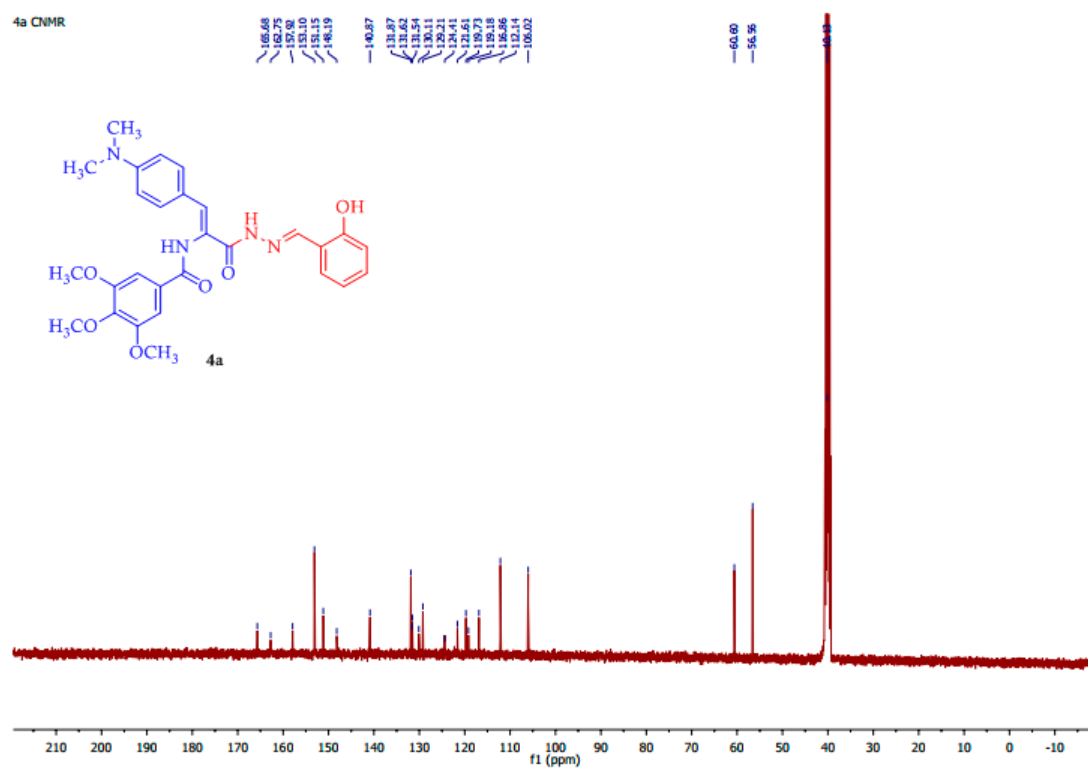


Figure S6: ¹³C-NMR spectrum of compound 4a



Figure S7: ^1H -NMR spectrum of compound **4b**

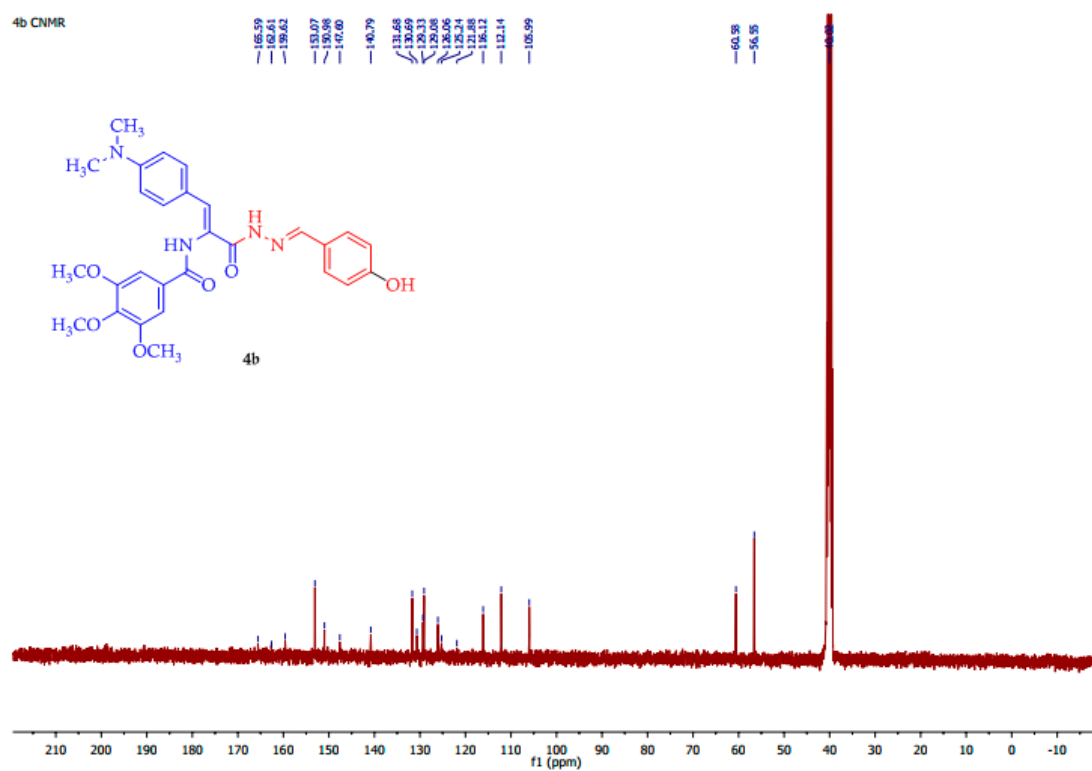


Figure S8: ¹³C-NMR spectrum of compound **4b**

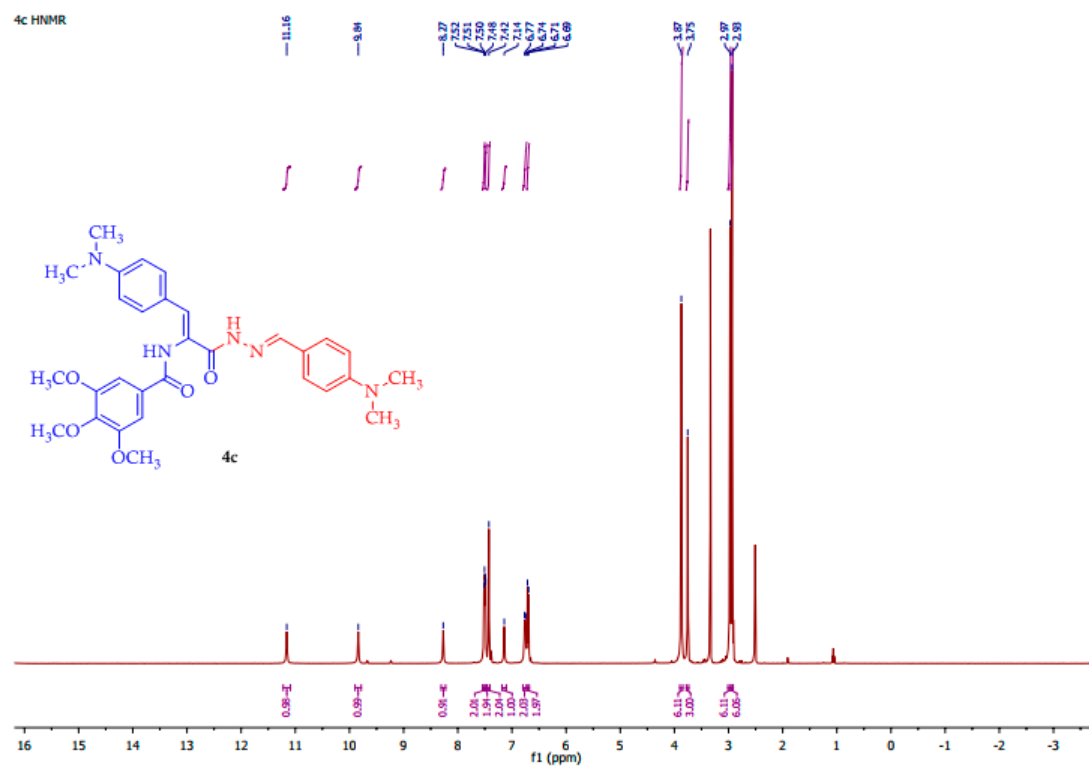


Figure S9: ^1H -NMR spectrum of compound 4c

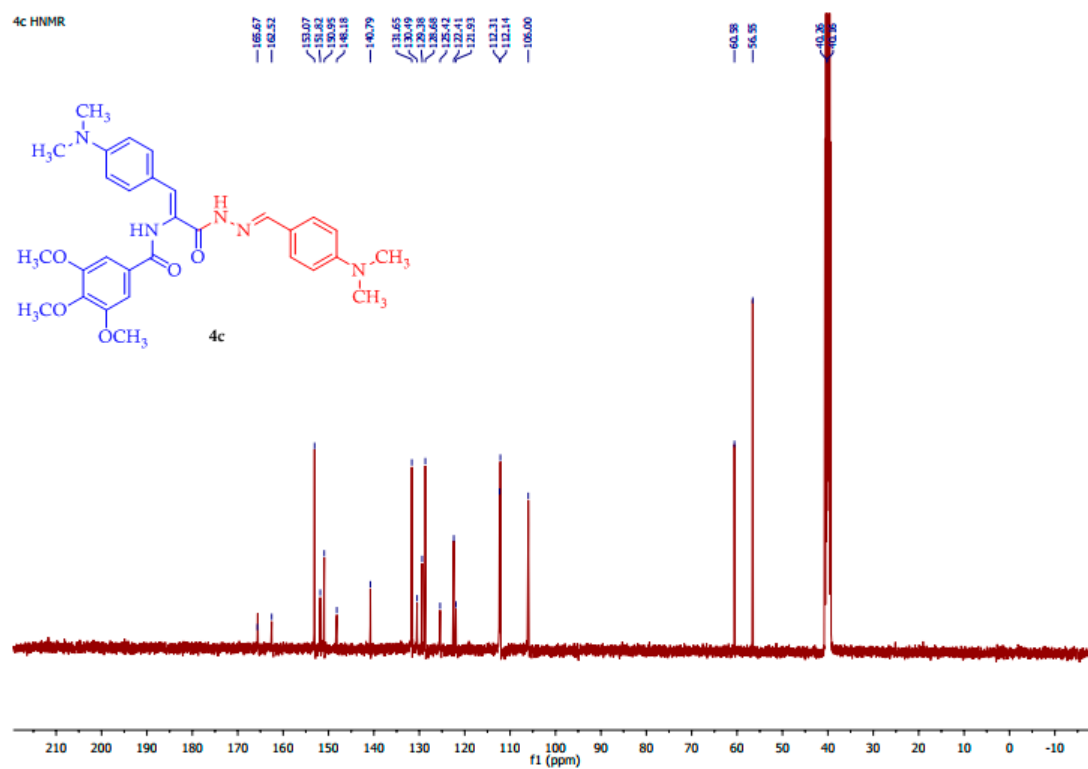


Figure S10: ¹³C-NMR spectrum of compound 4c

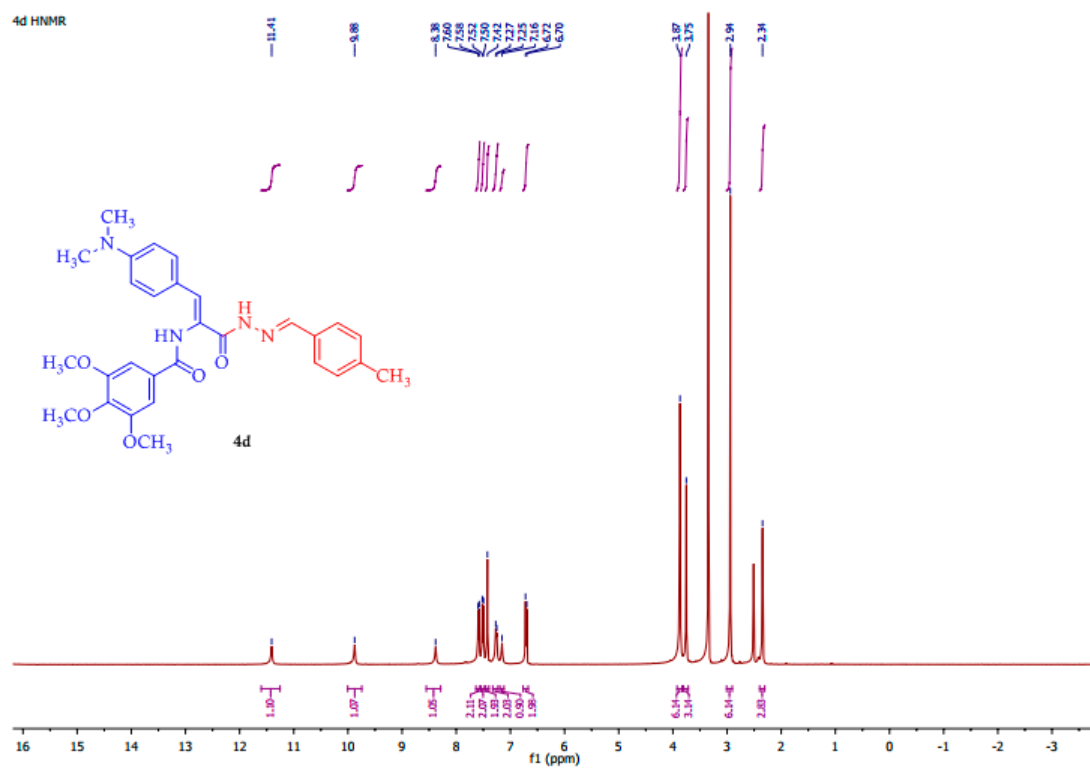


Figure S11: ^1H -NMR spectrum of compound 4d

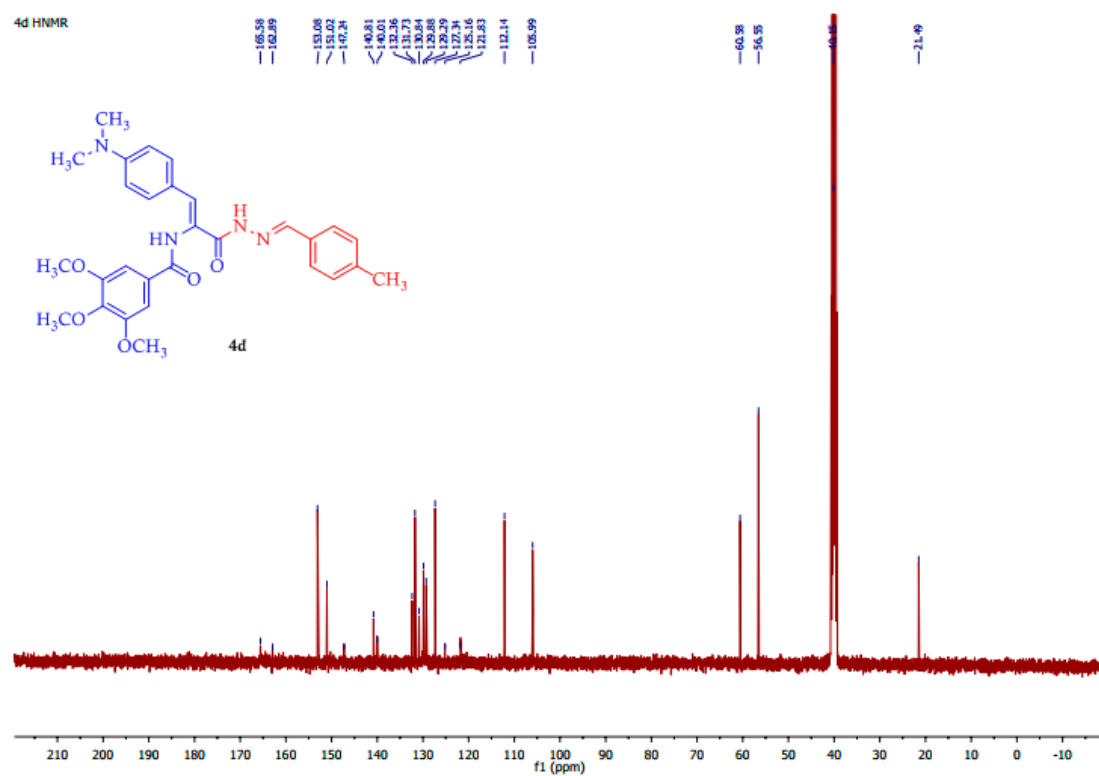


Figure S12: ¹³C-NMR spectrum of compound **4d**

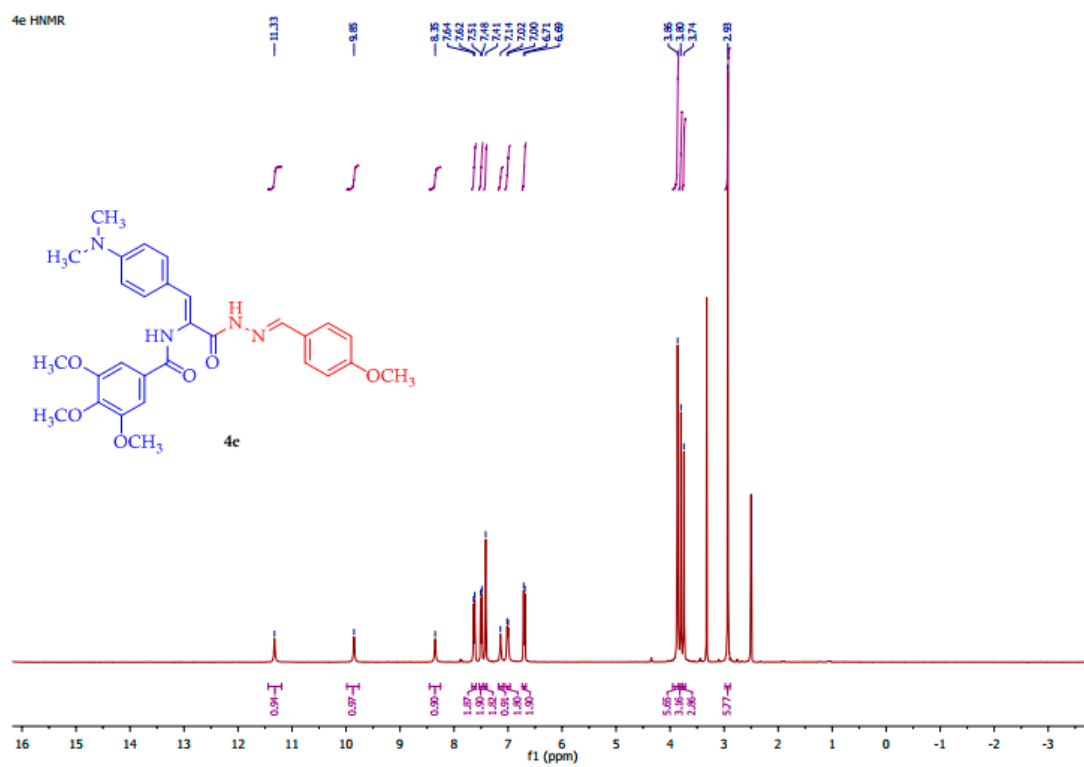


Figure S13: ¹H-NMR spectrum of compound **4e**

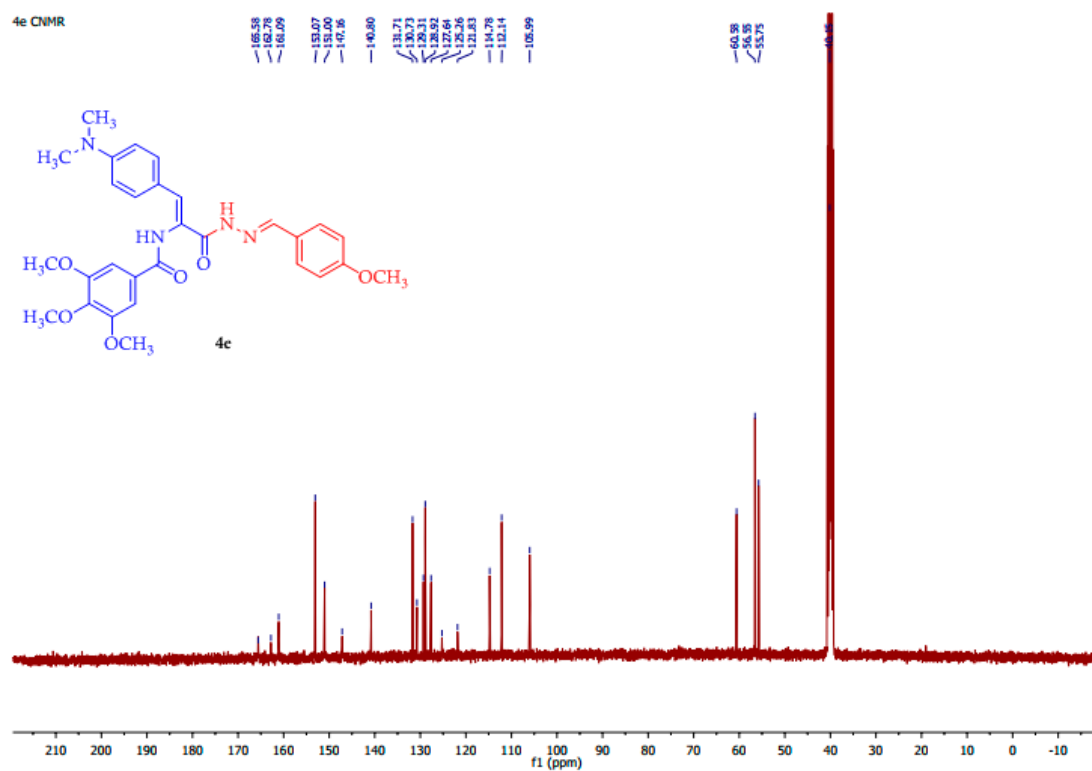


Figure S14: ¹³C-NMR spectrum of compound **4e**

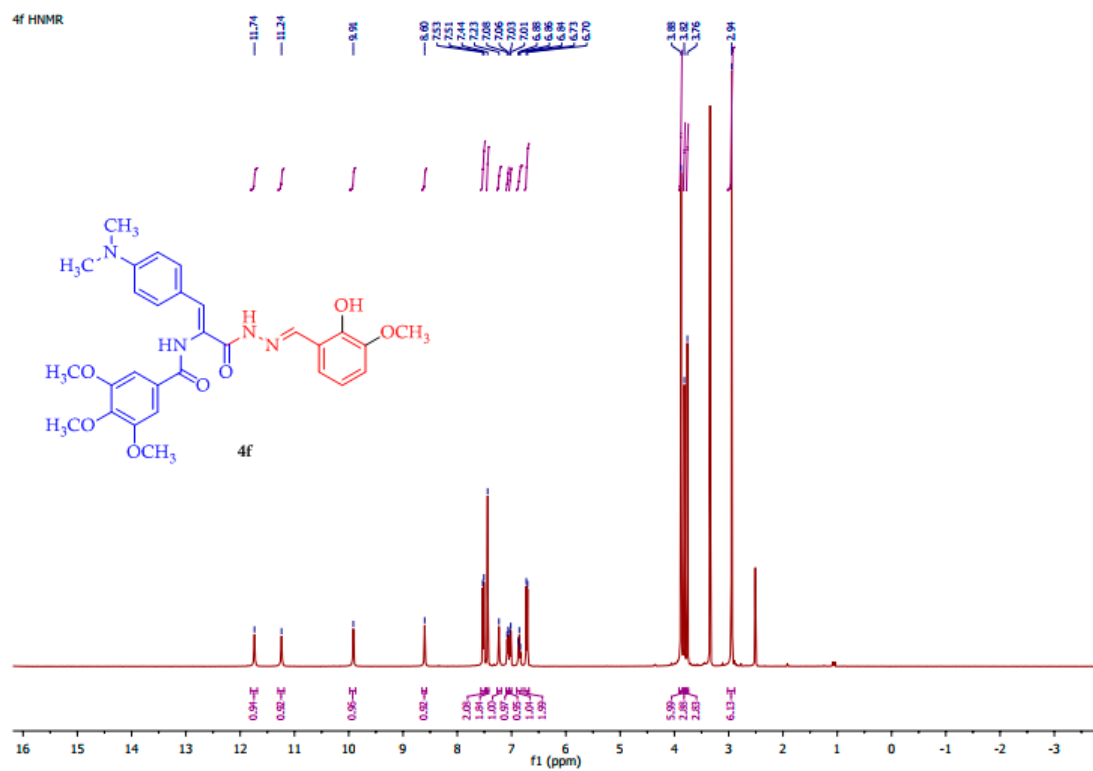


Figure S15: ^1H -NMR spectrum of compound 4f

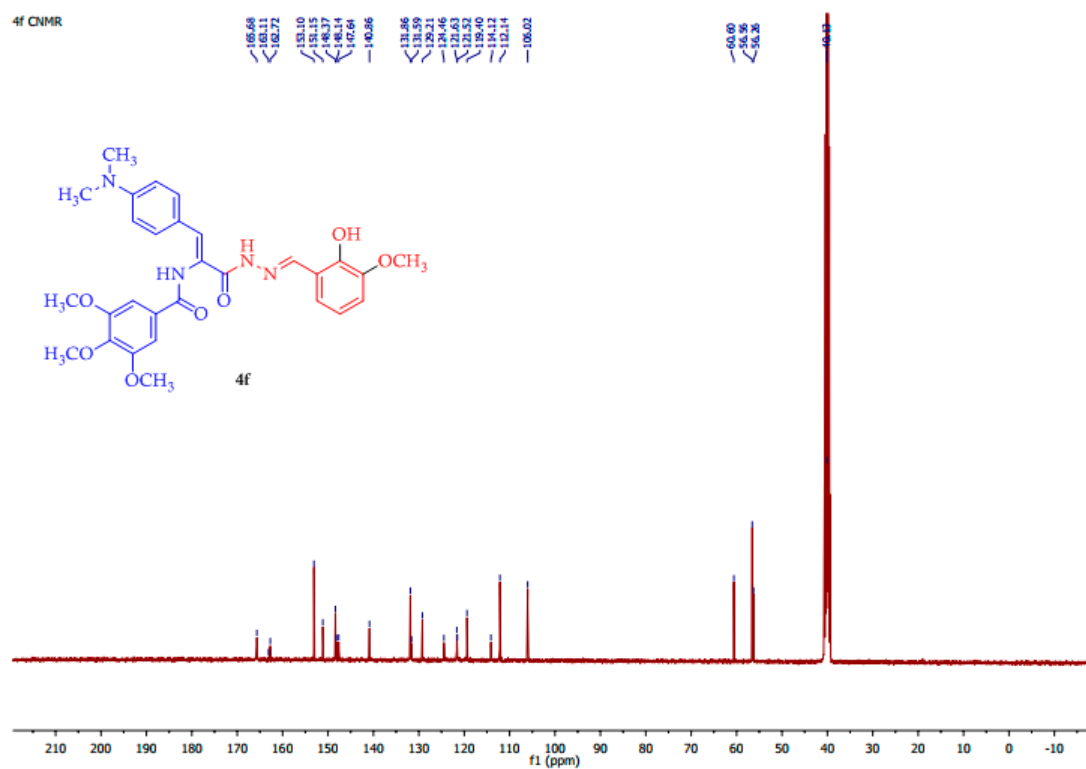


Figure S16: ^{13}C -NMR spectrum of compound **4f**

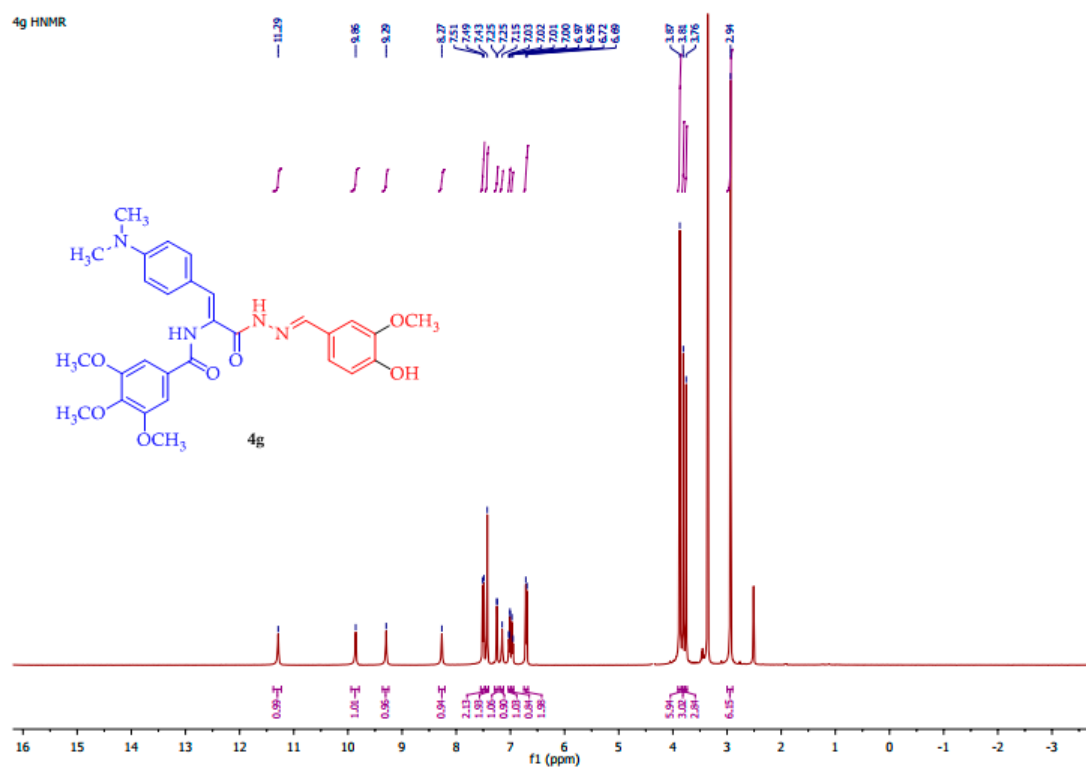


Figure S17: ^1H -NMR spectrum of compound **4g**

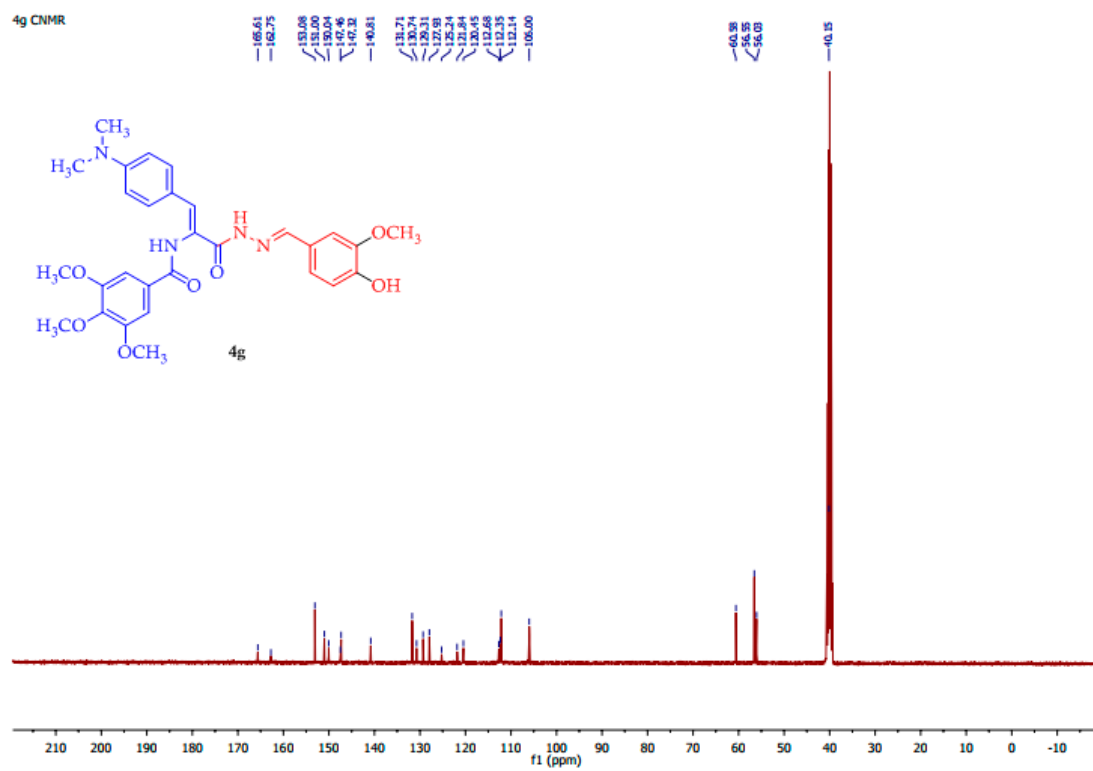


Figure S18: ^{13}C -NMR spectrum of compound **4g**

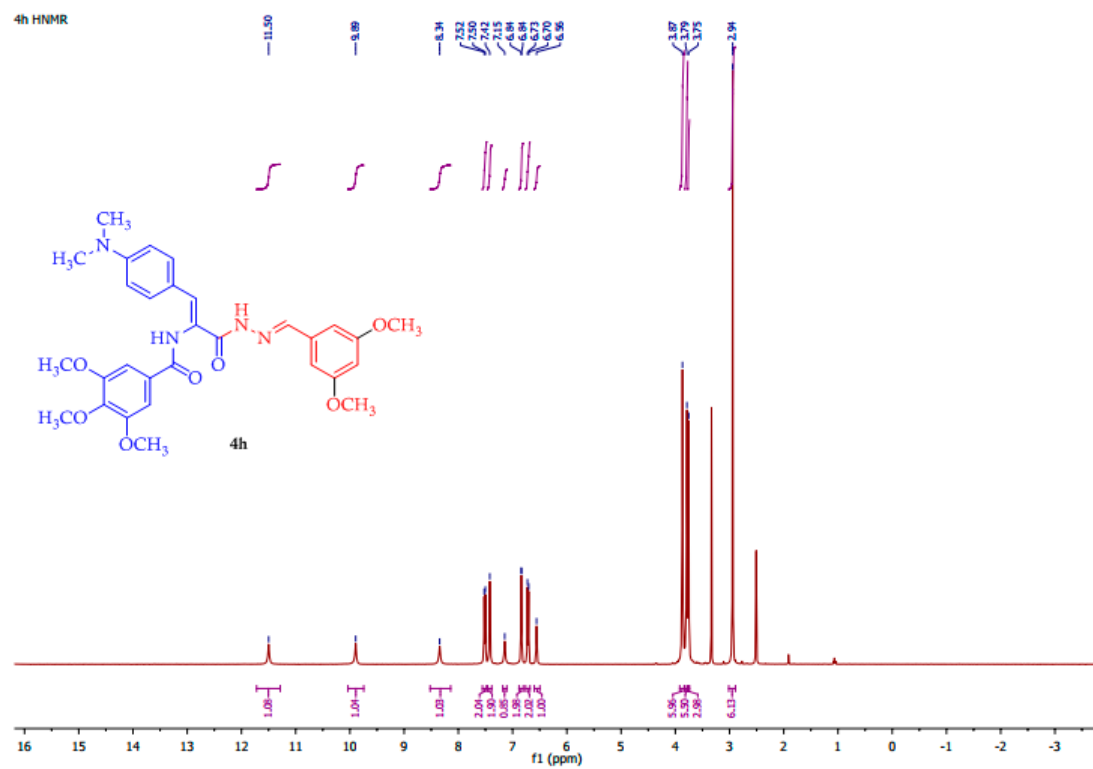


Figure S19: ¹H-NMR spectrum of compound **4h**

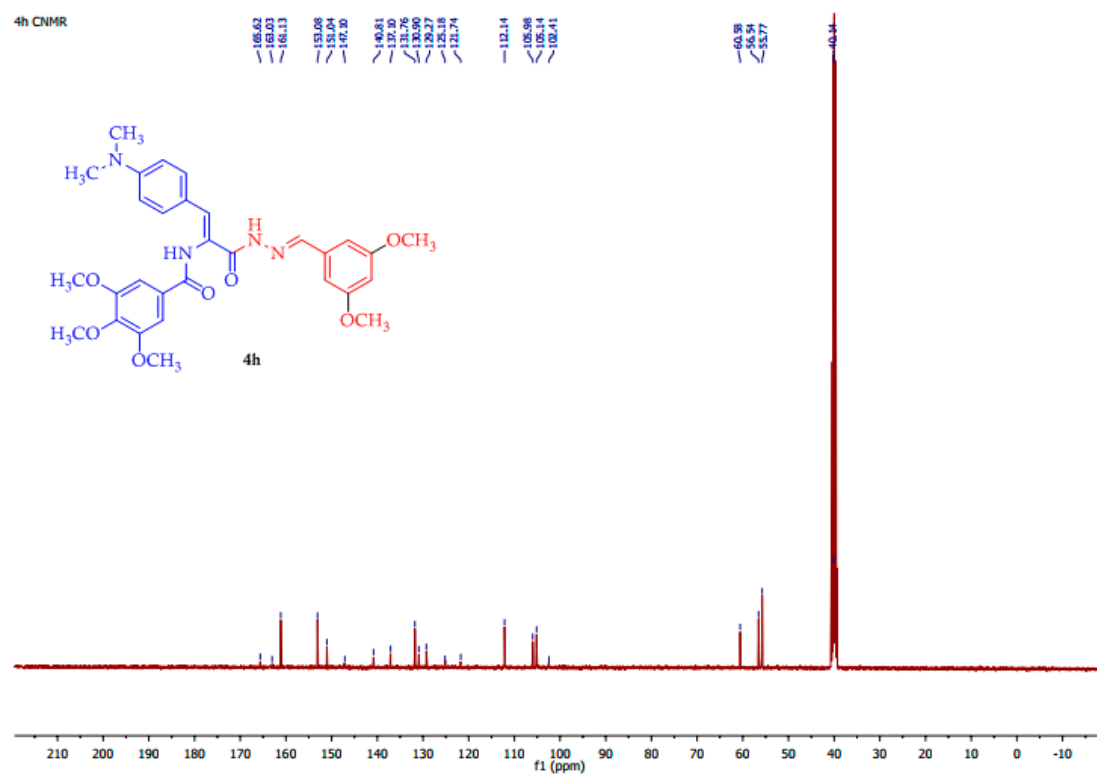


Figure S20: ¹³C-NMR spectrum of compound **4h**

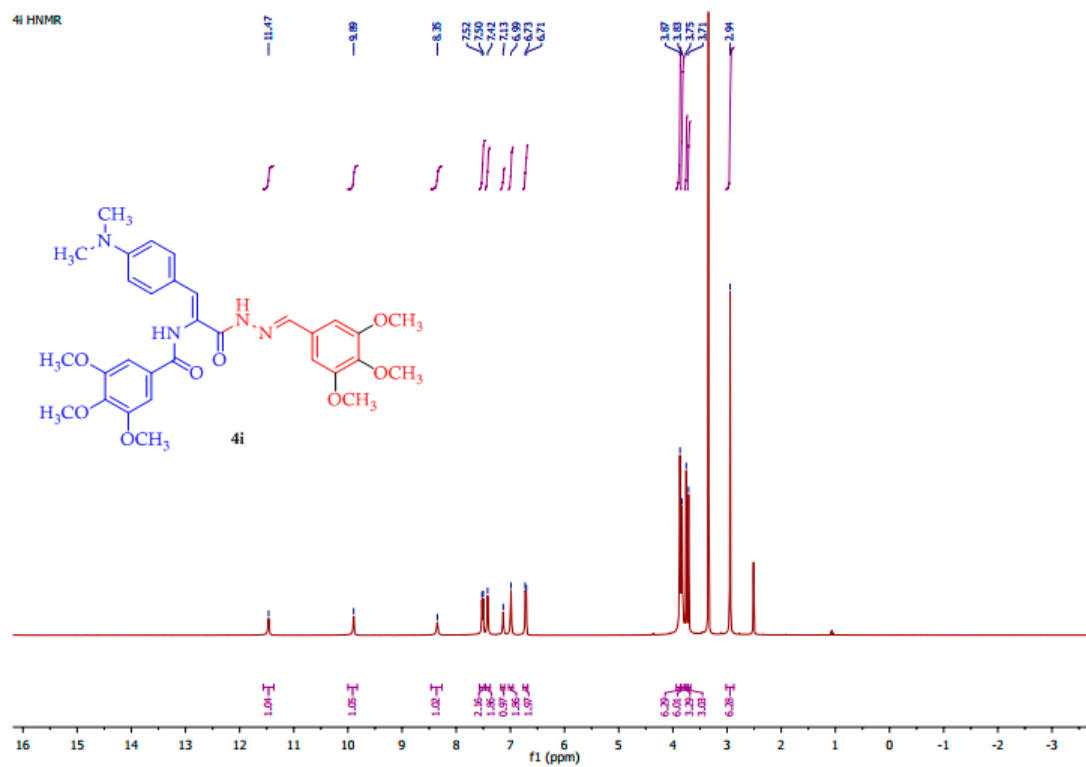


Figure S21: ^1H -NMR spectrum of compound **4i**

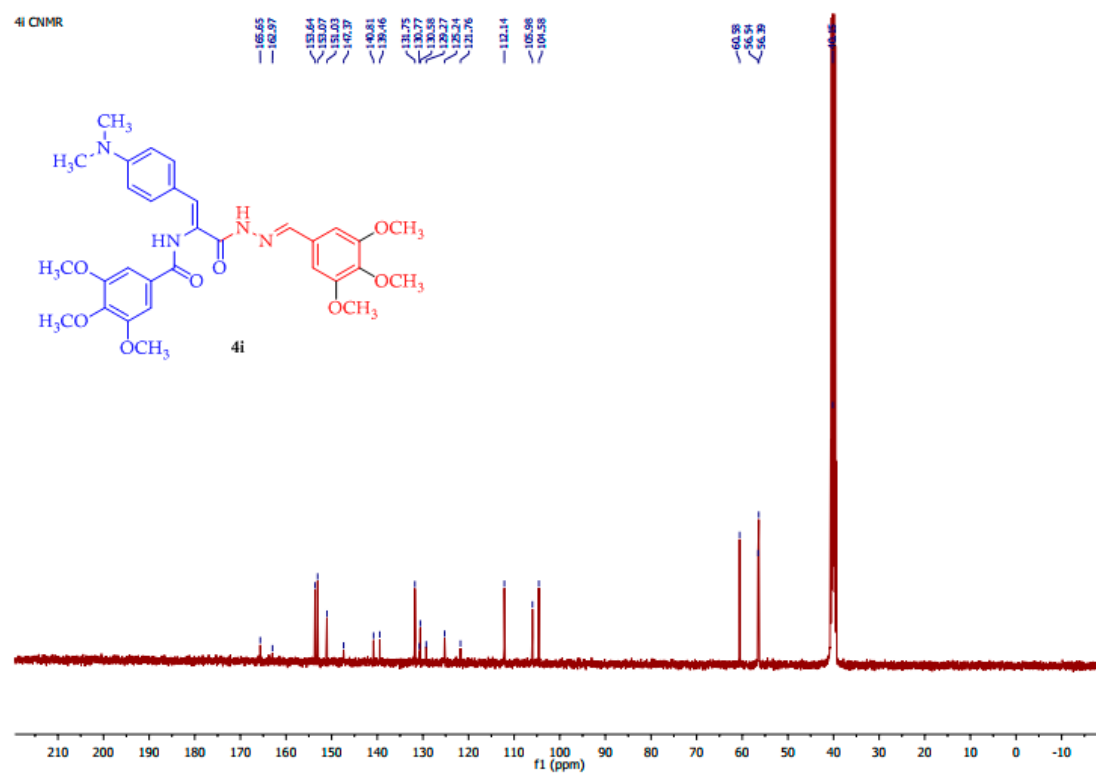


Figure S22: ^{13}C -NMR spectrum of compound **4i**

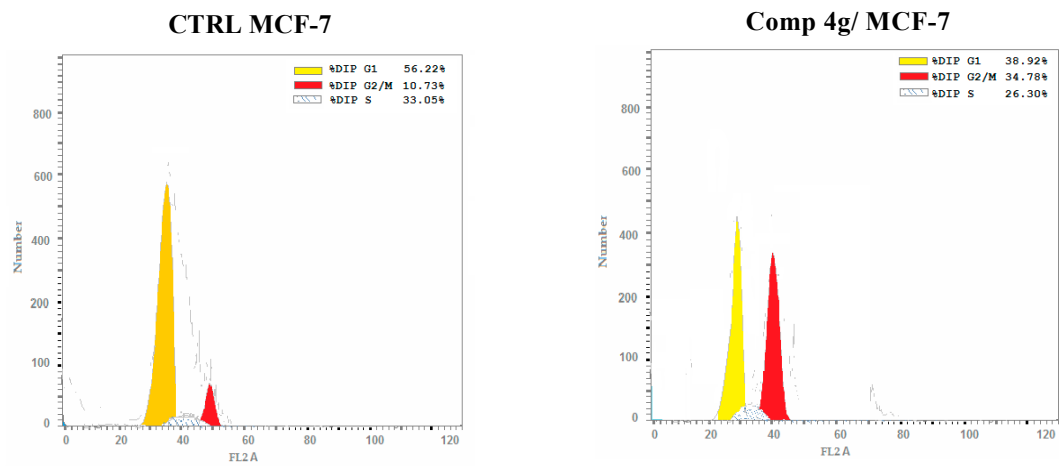


Figure S23: Compound 4g induce G2/M phase disturbance in MCF-7 cells

S4.1.1. Chemistry: General

Melting points were determined in open capillaries tube using Electrothermal Digital melting point apparatus and were uncorrected. The ^1H -NMR and ^{13}C -NMR were performed on a Bruker 400 MHz (Bruker comp., MA, USA) at Applied Research Center, College of Science, University of Jeddah, Saudi Arabia. Elemental analyses were determined using model 2400 CHNS/O elemental analyzer (PerkinElmer) at College of Science, University of Jeddah, Saudi Arabia. All the values were within $\pm 0.4\%$ of the theoretical values. All chemical, reagents and solvents were purchased from Sigma-Aldrich and Across organics chemical companies and used without further purification.

S4.2. Biological Studies

S4.2.1. Cytotoxic Activity against breast carcinoma MCF-7 Cell Line

To measure the cytotoxic activity of schiff base-TMB hybrids **3** and **4a-i** in MCF-7 cancer cell line, cell viability assay was assessed using MTT assay method. *P*-vanillin Schiff bas **4g** and Col were screened for their effects on normal breast cell line MCF-10A (ATCC Cat. No. CRL-10317). Cells at density of 1×10^4 were seeded in a 96-well plate at 37 °C for 24 h under 5% CO_2 . After incubation, the cells were treated with different concentrations (0.40, 1.56, 6.25, 25 and 100 μM) of the test schiff base-TMB hybrids **3** and **4a-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. β -Tubulin inhibition Assay

Compound **4g** and colchicine were evaluated for their tubulin inhibitory activity in MCF-7 breast cancer cells according to manufacturer's instructions using # abcam Human Beta-tubulin simplestep ELISA Kit ab245722.

S4.2.3. Cell Cycle Analysis of Compound 4g

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% CO₂. The medium was incubated with the tested compound **4g** at its IC₅₀ (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. Annexin V FITC/PI Staining Assay for Compound 4g

MCF-7 cells at a density of 2×10^5 per well were treated with compound **4g** at its IC₅₀ (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).

S4.2.5. qRT-PCR measurements of p53, Bax and Bcl2 for compound 4g

Real-time PCR for p53, BAX and Bcl-2 genes expression was done using commercial Qiagen RNA extraction/ BioRad SYBER green PCR master mix according manufacturer's instructions. Briefly, 2 μl of cDNA template, 10 pMol of each forward and reverse primer, 10 μl of 2X Master Mix and to 20 μl total reaction mixture volume by nuclease free water and then was introduced to thermal cycler instrument (Thermo Scientific, USA). The cycling parameters for the PCR amplification were achieved by initial denaturation at 95 °C for 3 minutes followed by 40 cycles of 94 °C for 15 seconds and annealing/extension step at 60 °C for 1 min. Relative quantification of target genes was run on Rotor-Gene 6000 Series Software 1.7 (Build 87).

Table S1: The primer sequences for Real Time PCR assay

Gene	Sequences (5' -3')
P53	F: AGAGTCTATAGGCCACCCC R: GCTCGACGCTAGGATCTGAC
Bax	F: GAGGAAGTGGACAGTAACATGGAGCT R: CGGCCCCAGTTGAAGTTGC
Bcl2	F: GCCGGTTCAGGTACTCAGTCATC R: GTCACCTTCACCGTTCCA
GAPDH	F: GCACCGTCAAGGCTGAGAAC R: ATGGTGGTGAAGACGCCAGT