Supplementary Information

Design, synthesis and antimicrobial evaluation of benzimidazole containing 4-thiazolidinone based 5-arylidene derivatives

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EXPERIMENTAL SECTION

Materials and methods

Melting point was determined using open capillary methods.TLC is used to examine the reaction.Infrared spectra were recorded in KBr using a Shimadzu IR Prestige-21 (CE) Fourier transform infrared spectrophotometer; spectral data were given in cm^{-1}. The percentage of C, H, and N had been analyzed by using a Perkin-Elmer 2400 CHN analyzer. NMR spectra were recorded on a Bruker instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in DMSO-d_6 solution using TMS as an internal standard. Chemical shifts were given in ppm, and coupling constants (*J*) were given in Hz.Mass spectra were obtained using the electron impact (EI) ionization method.

Preparation of *N*-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-1-phenylmethanimine (3)

Benzaldehyde (0.01 mol) and compound (1) (0.01 mol) were dissolved in dry methanol (30 ml). A catalytic amount of glacial CH_3COOH was added to the mixture after 10 to 15 minutes of warming the mixture. Reflux of the reaction mixture at 60 ºC for 5-6 hours was conducted. After cooling at room temperature, the reaction mixture was filtered to remove the brown crystals that formed. Cold methanol was used to wash the product, and it was dried and recrystallized from ethanol to produce a compound (3). Yield 74%; solid; M.P. 210–211 °C; IR(KBr, cm^{-1}): 3321 (N–H str., benzimidazole), 3055 (C–H str., aromatic), 3030(C–H str., aromatic), 2997 (C–H str., aliphatic), 2875(C–H str., aliphatic), 1610 (C=N str.), 1531–1448 (C=C str., aromatic); ^{1}H NMR (500 MHz, DMSO–d_6):δ=12.10 (s, 1H of >NH), 8.11 (s, 1H of –N=C<u>H</u>–), 7.67–7.75 (m, 2H, Ar–H), 7.58 (t, *J* = 5.6 Hz, 1H of Benz–H), 7.34–7.47 (m, 4H of Ar–H and Benz–H), 7.15 (d, *J* = 5.6 Hz, 2H of Benz–H), 4.77 (s, 2H of –C<u>H</u>$_2$–);^{13}C NMR (100 MHz, DMSO–d_6):δ= 161.49 (–N=<u>C</u><), 153.28 (Benz–C), 141.07 (Benz–C), 136.88 (Benz–C), 135.37 (Ar–C), 129.79 (Ar–C),

128.53 (2) (Ar–C), 127.88 (2) (Ar–C), 126.07 (Benz–C), 122.99 (Benz–C), 117.83 (Benz–C), 114.26 (Benz–C), 54.38 (Benz–C). LCMS (*m/z*): 236.21 $[M+H]^+$; Anal. calcd. For $C_{15}H_{13}N_3$: C, 76.57; H, 5.57, N, 17.86%; Found: C, 76.51; H, 5.53, N, 17.80%.

Preparation of 3-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-2-phenylthiazolidin-4-one (4)

A solution of compound (3) (0.01 mole) and thioglycolic acid (0.03 mole) was prepared in 1,4-dioxane (25 ml). After the addition of the catalytic amount of $ZnCl_2$, the reaction mixture was stirred at 110-120°C for 10-12 hours. The reaction mixture was then allowed to cool at room temperature. In a cooled dil. $NaHCO_3$ solution, the reaction mixture was poured. As a result of the extraction, the light grey product was filtered, washed with dil $NaHCO_3$ solution and then with cold methanol. It was dried and recrystallized from methanol to obtain a compound (4).Yield 61 %; M.P. 227–228°C; IR (KBr, cm^{-1}): 3327 (N–H str.), 3070 (C–H str. Aromatic), 2947 (C–H str. $–CH_2–$), 1686 (N=C str.), 1655 (C=O str.), 1598 (C=C str.), 1320 (N–C str. 2º amine), 1190 (N–C str. 3º amine), 735 (C–S str.); ^{1}H NMR (500 MHz, DMSO–d_6):δ= 12.10 (s, 1H of >NH), 7.55–7.62 (m, 1H of Benz–H), 7.44–7.50 (m, 1H of Benz–H), 7.39 (d, J = 6.8 Hz, 2H of Ar–H), 7.26–7.36 (m, 3H of Ar–H), 7.08–7.17 (m, 2H of Benz–H), 5.86 (d, J = 0.8 Hz, 1H of C_2–H thiazolidinone), 4.65 (s, 2H of $–CH_2–$),3.55 (s, 2H of C_5–H thiazolidinone); ^{13}C NMR (100 MHz, DMSO–d_6):δ= 172.51 (>C=O), 150.97 (Benz–C), 140.87 (Benz–C), 140.56 (Ar–C), 136.64 (Benz–C), 128.84 (2) (Ar–C), 127.41 (Ar–C), 127.04 (2) (Ar–C), 125.08 (Benz–C), 122.92 (Benz–C), 118.60 (Benz–C), 114.33 (Benz–C), 62.81 ($C_2$of thiazolidinone), 44.00 ($–CH_2–$), 34.88 (C_5 of thiazolidinone); LCMS (*m/z*): 310.13$[M+H]^+$; Anal. calcd. For $C_{17}H_{15}N_3OS$: C, 66.00; H, 4.89, N, 13.58%; Found: C, 65.95; H, 4.82, N, 13.50%

BIOLOGICAL ASSAY:

Antibacterial assay

The activity of compounds was determined as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller Hinton Broth (Becton Dickinson, USA). Primary screening was done first for antibacterial activity in six sets against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes* at different concentrations of 1000, 500, 250 μg/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, 12.5 and 6.25 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to 10^6 CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as a nutrient medium to grow and dilute the compound suspension for test organisms. 2 % DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Synthesized compounds were diluted to 1000 μg/mL concentration, as stock solution. The control tube containing no antibiotic was immediately subcultured [before inoculation] by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. 10 μg/mL suspensions were further inoculated on an appropriate media and growth was noted after 24 h and 48 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Solvent had no influence on strain growth. The result of this was greatly affected by the size of inoculum. The test mixture contained 10^6

CFU/mL organisms. DMSO and sterilized distilled water were used as negative control while ciprofloxacin (1 U strength) was used as positive control.

Antifungal assay

The newly prepared compounds **5a-o** were screened for their antifungal activity as primary screening in six sets against *C. albicans*, *A. niger* and *A. clavatus* at various concentrations of 1000, 500, 250 μg/mL. The primary active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilution against all fungi. For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water were used as negative control while griseofulvin (1 U strength) was used as positive control.

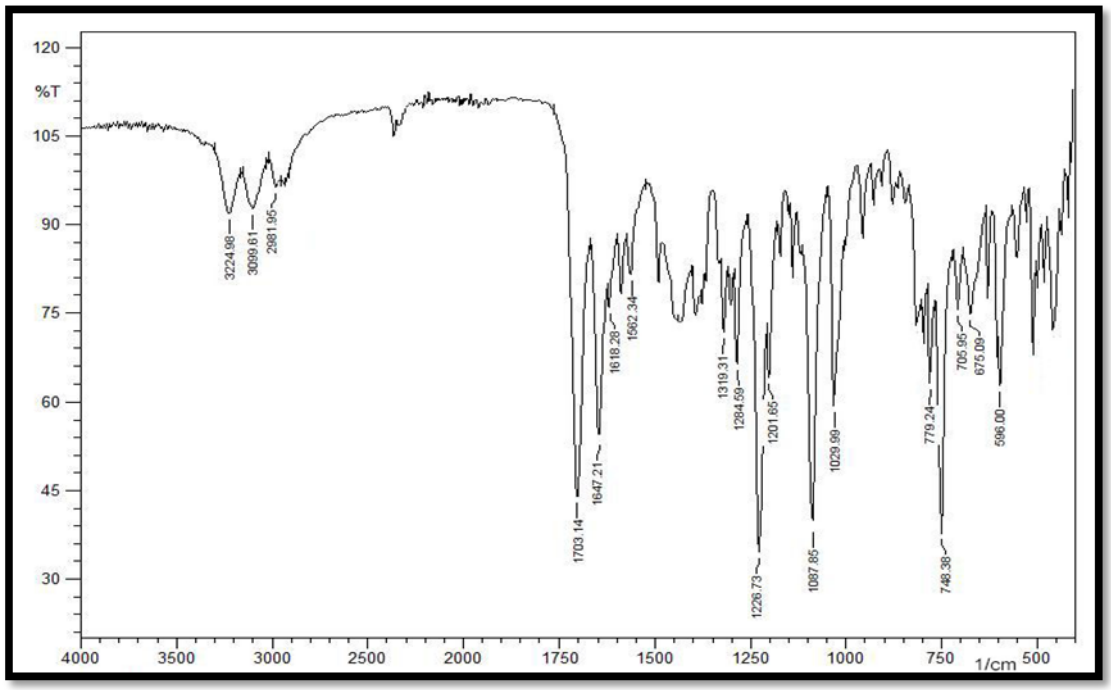

Fig. S1 — IR spectrum of compound 5a

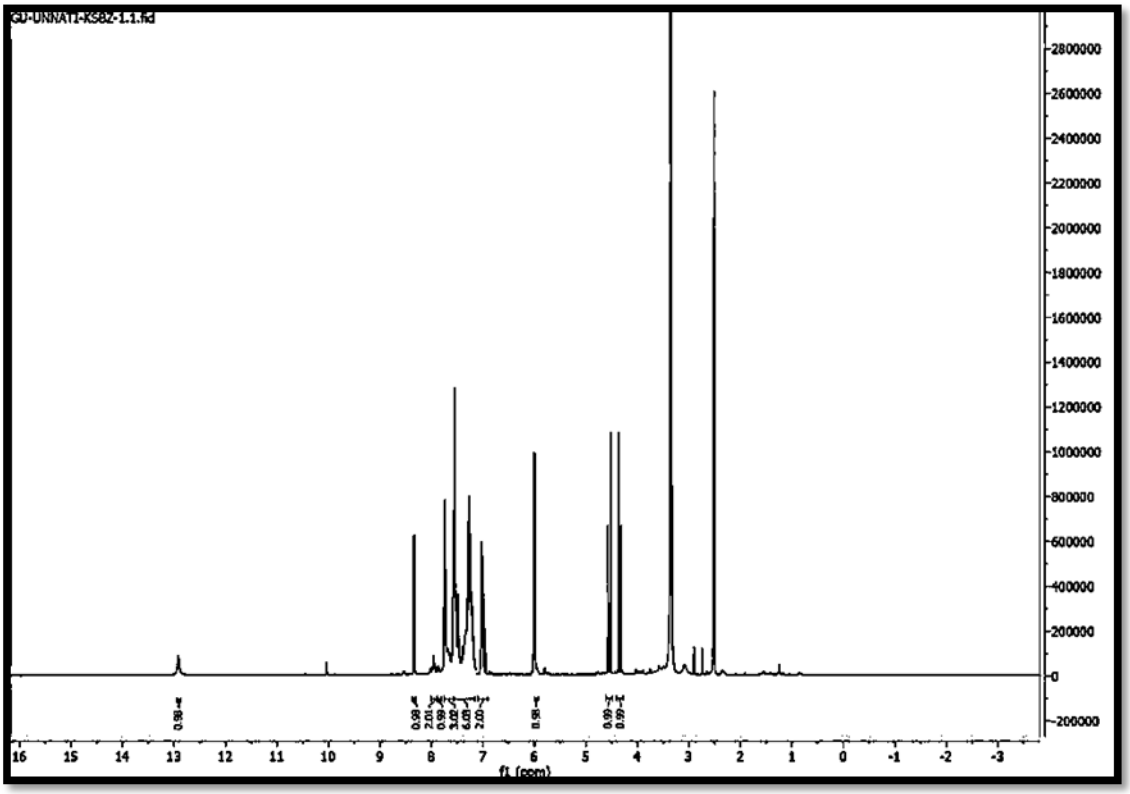

Fig. S2 — ^{1}H NMR spectrum of compound 5a

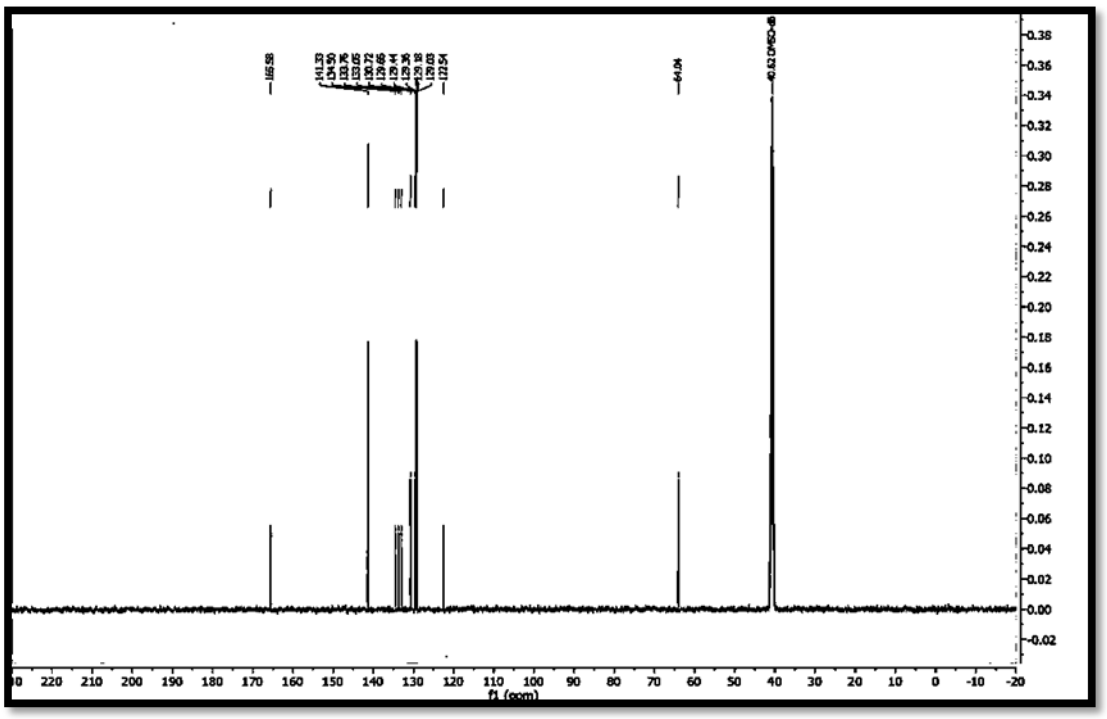

Fig. S3 — ^{13}C NMR spectrum of compound 5a

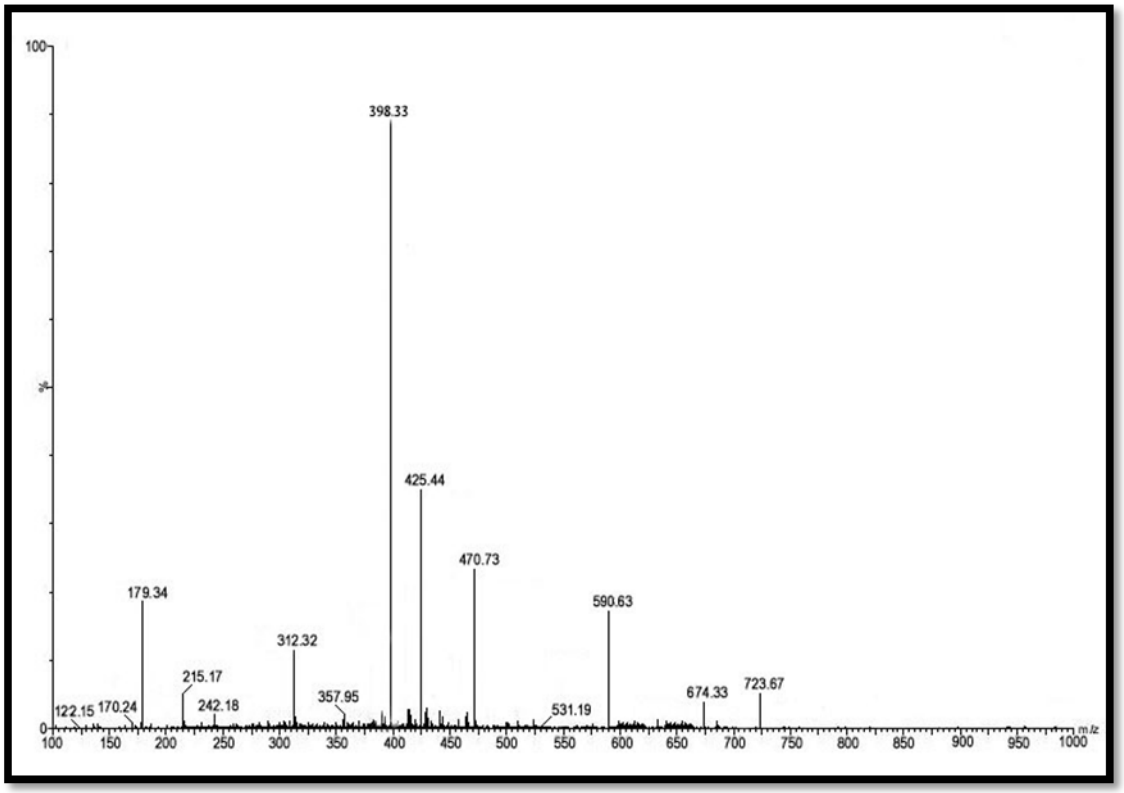

Fig. S4 — Mass spectrum of compound 5a

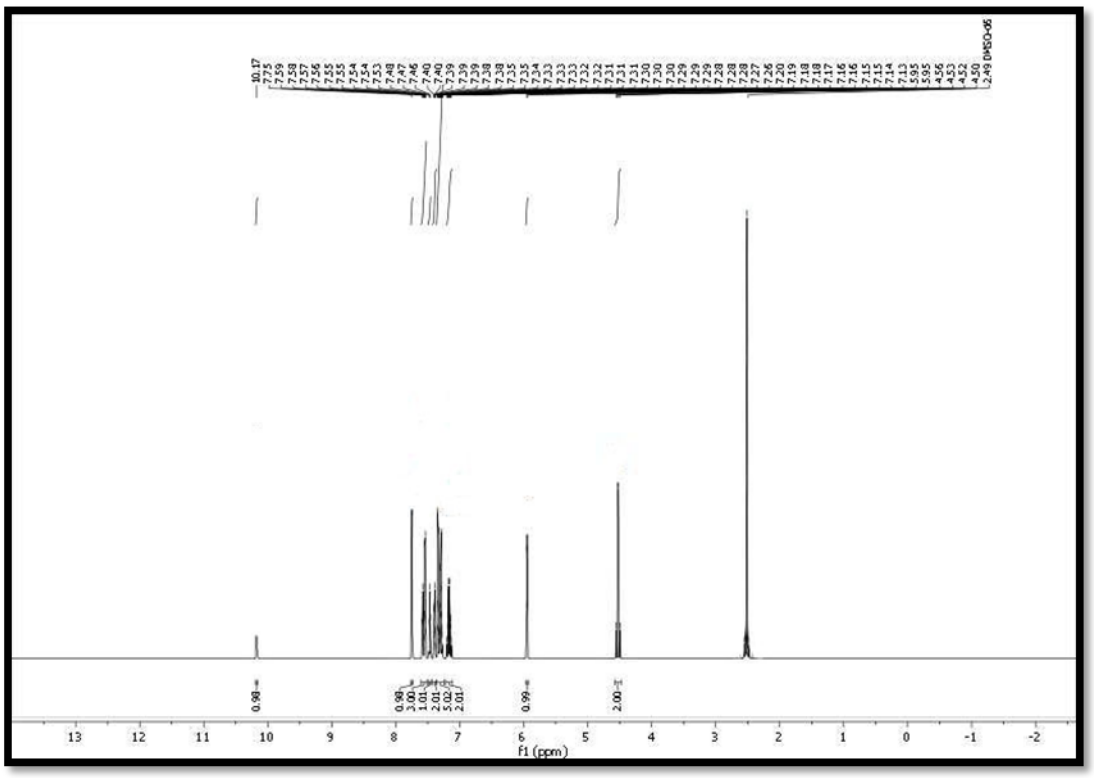

Fig. S5 — ^{1}H NMR spectrum of compound 5d

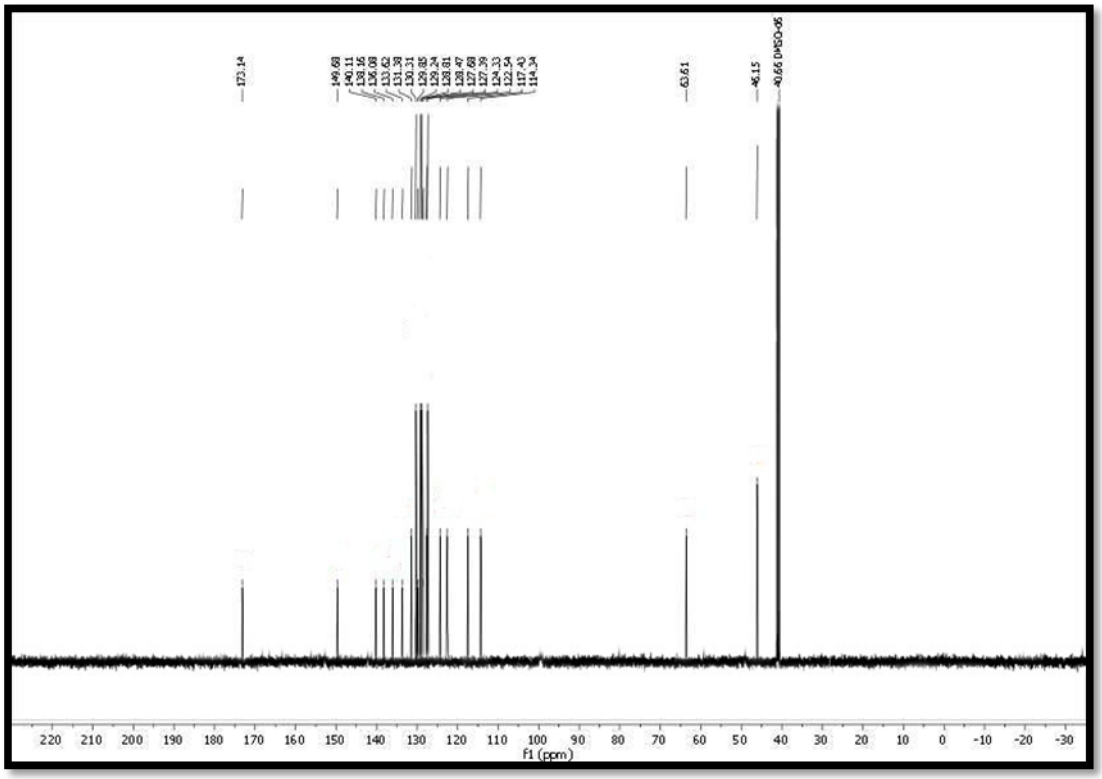

Fig. S6 — ^{13}C NMR spectrum of compound 5d

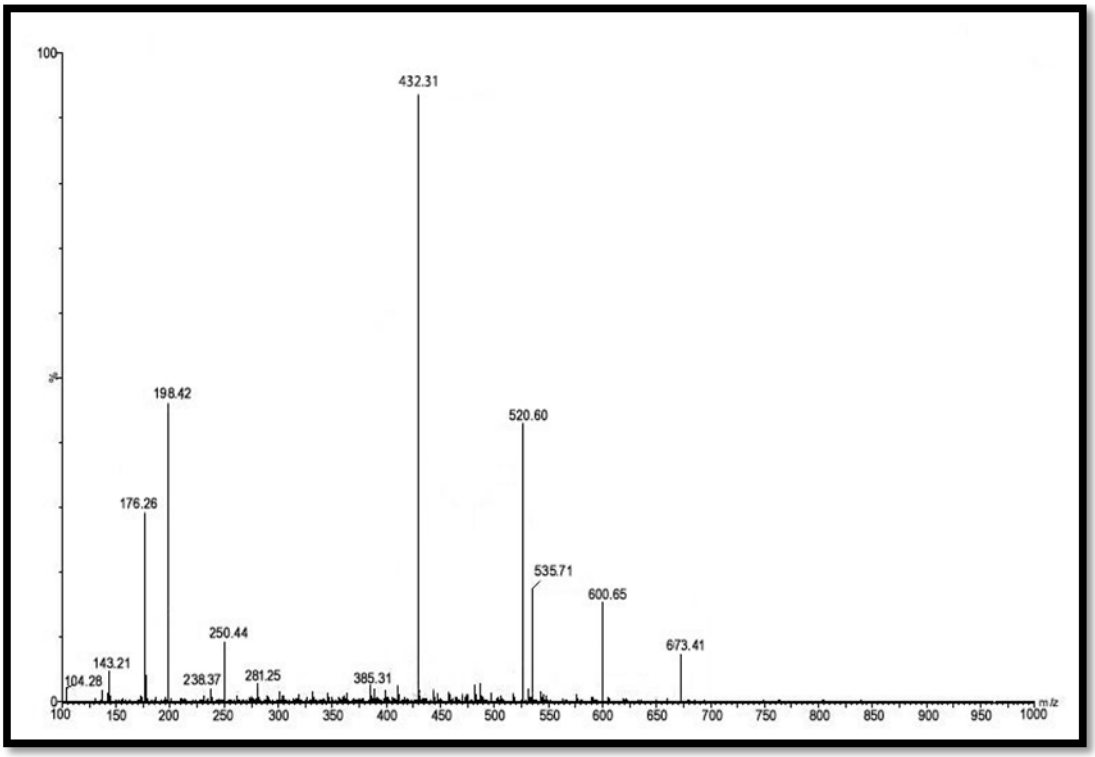

Fig. S7 — Mass spectrum of compound 5d

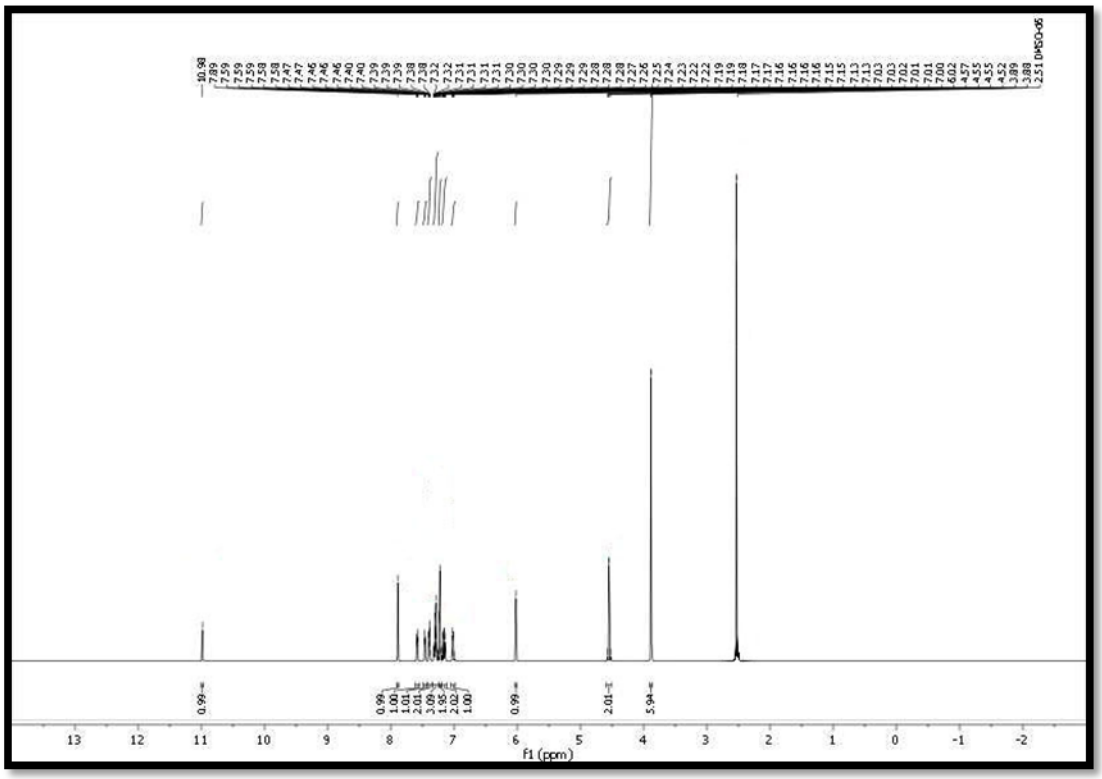

Fig. S8 — ^{1}H NMR spectrum of compound 5j

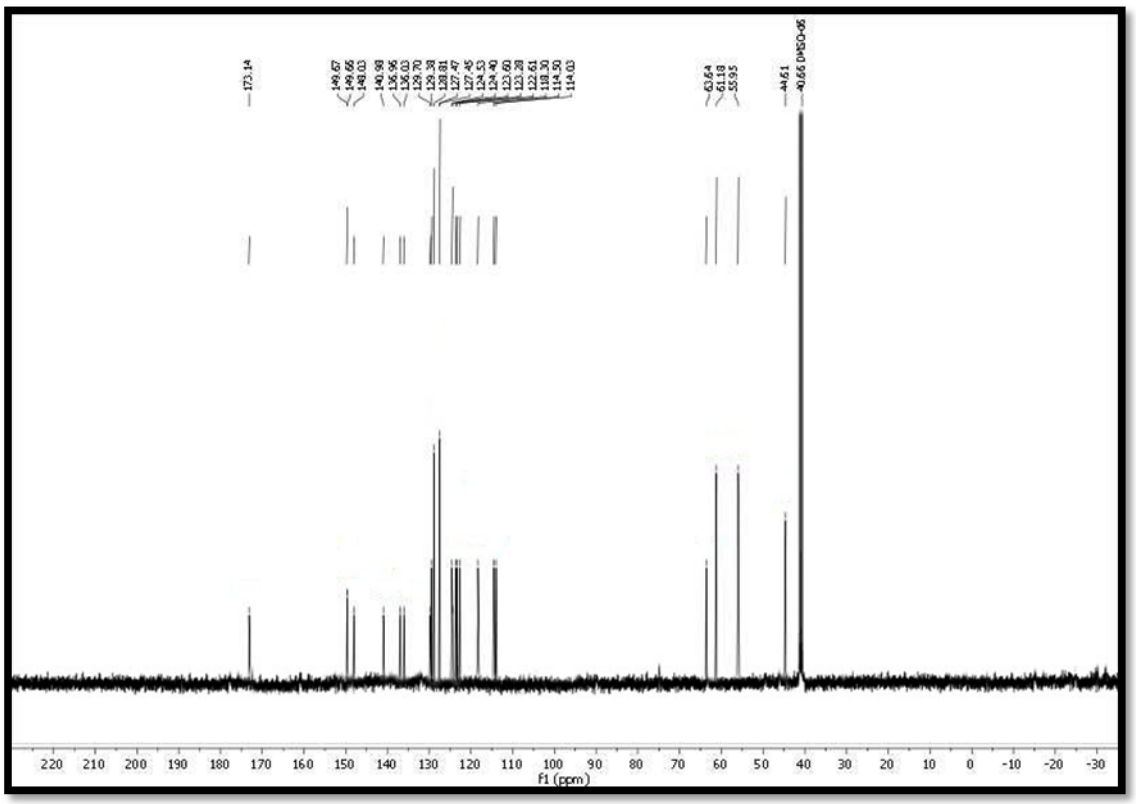

Fig. S9 — ^{13}C NMR spectrum of compound 5j

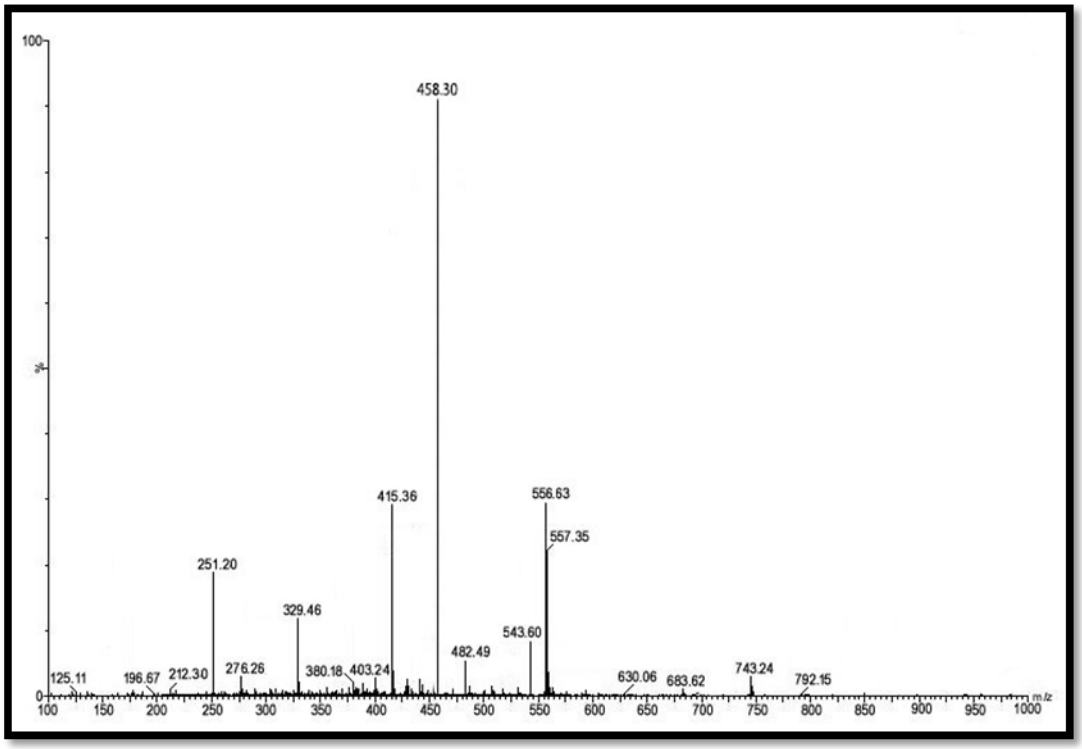

Fig. S10 — Mass spectrum of compound 5j

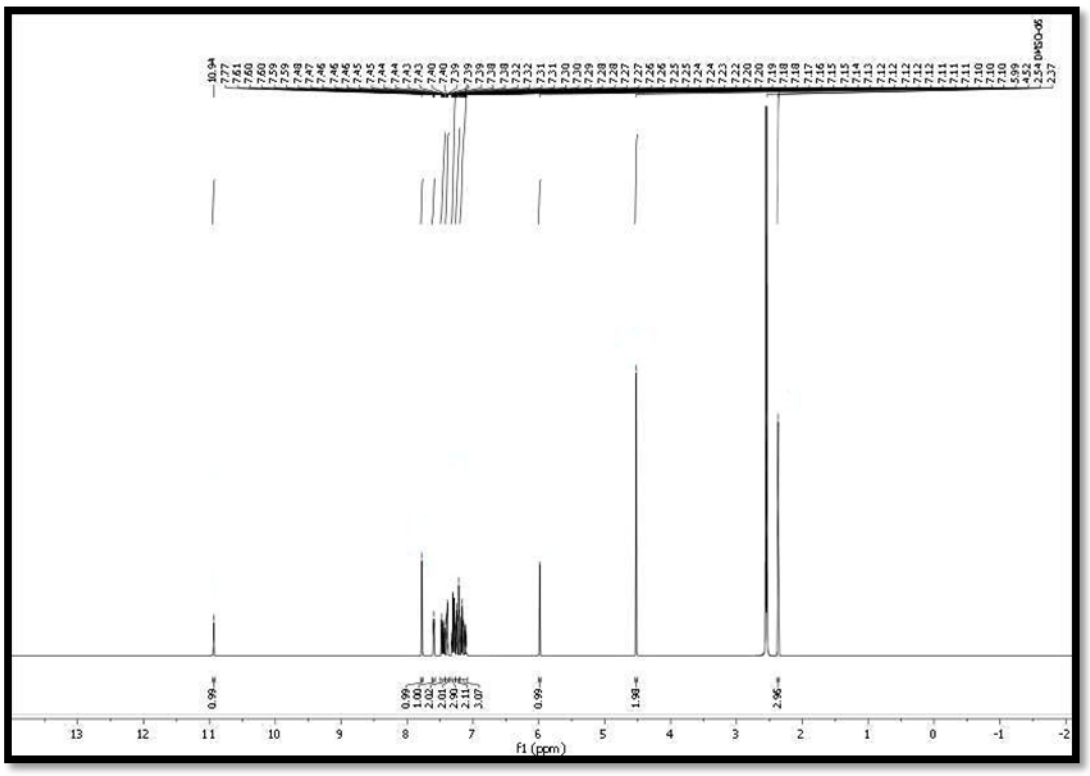

Fig. S11 — ^{1}H NMR spectrum of compound 5k

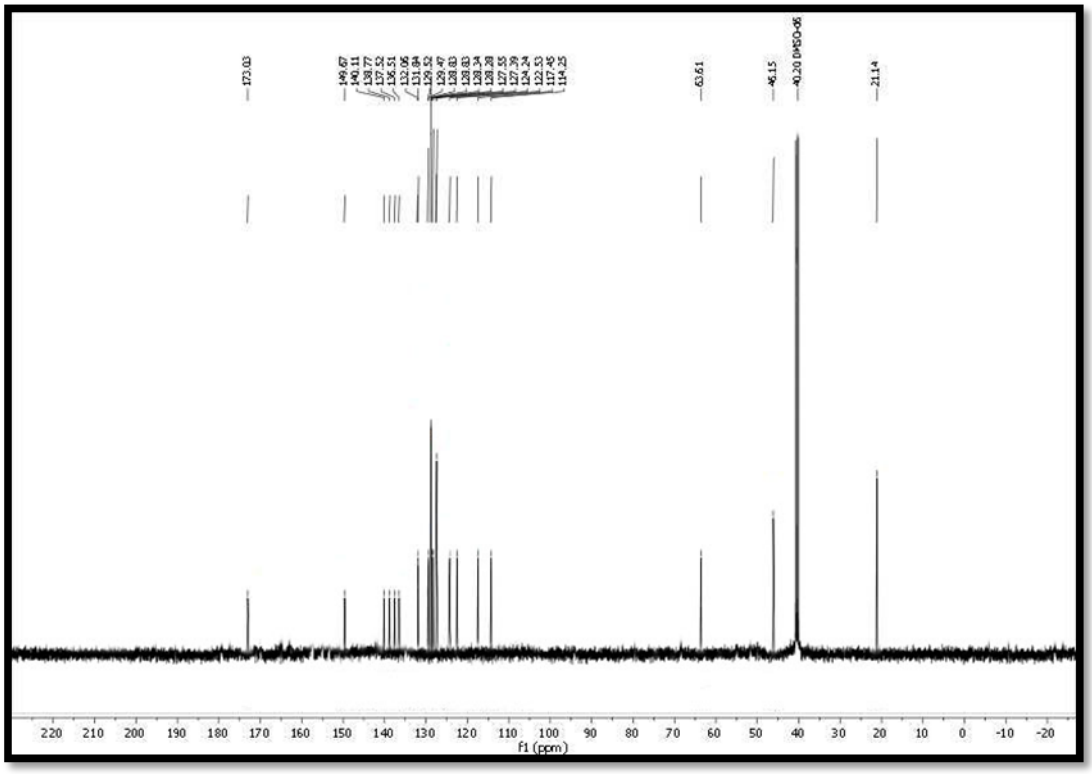

Fig. S12 — ^{13}C NMR spectrum of compound 5k

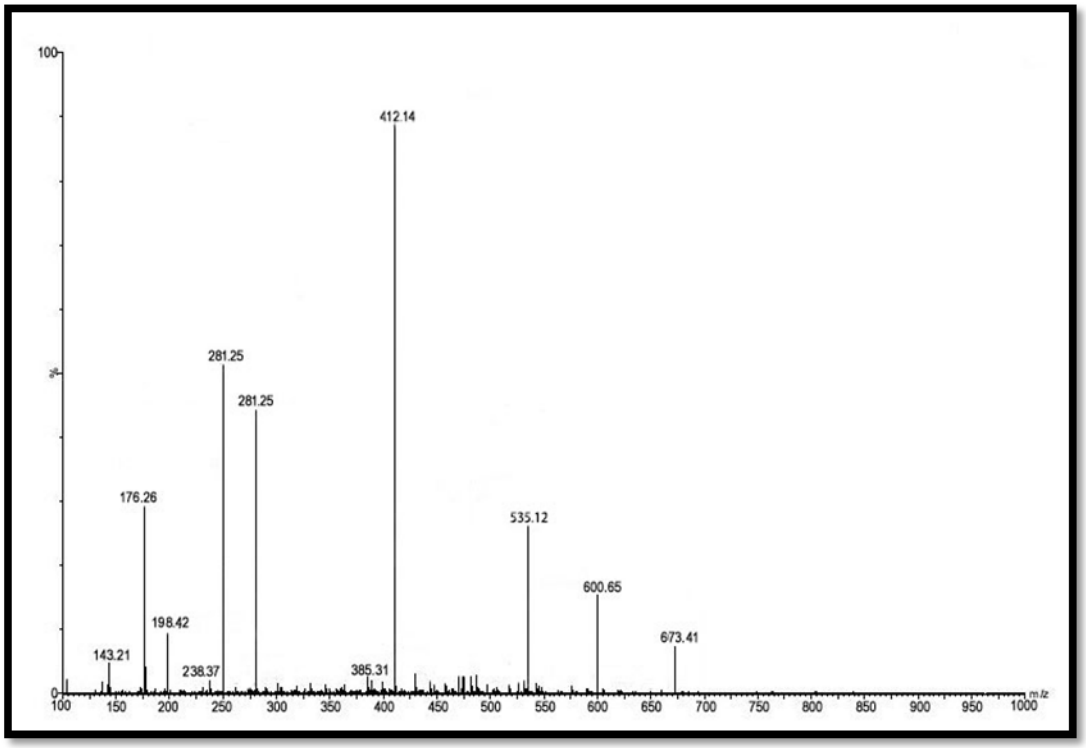

Fig. S13 — Mass spectrum of compound 5k

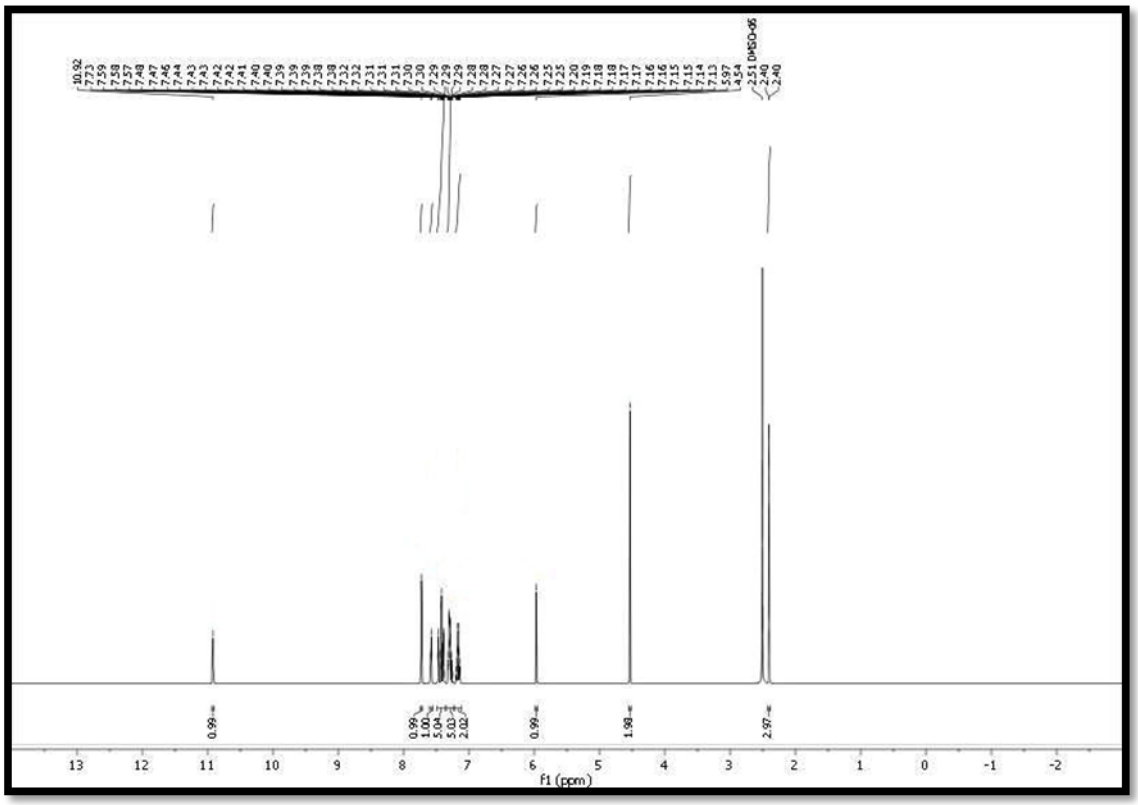

Fig. S14 — ^{1}H NMR spectrum of compound 5l

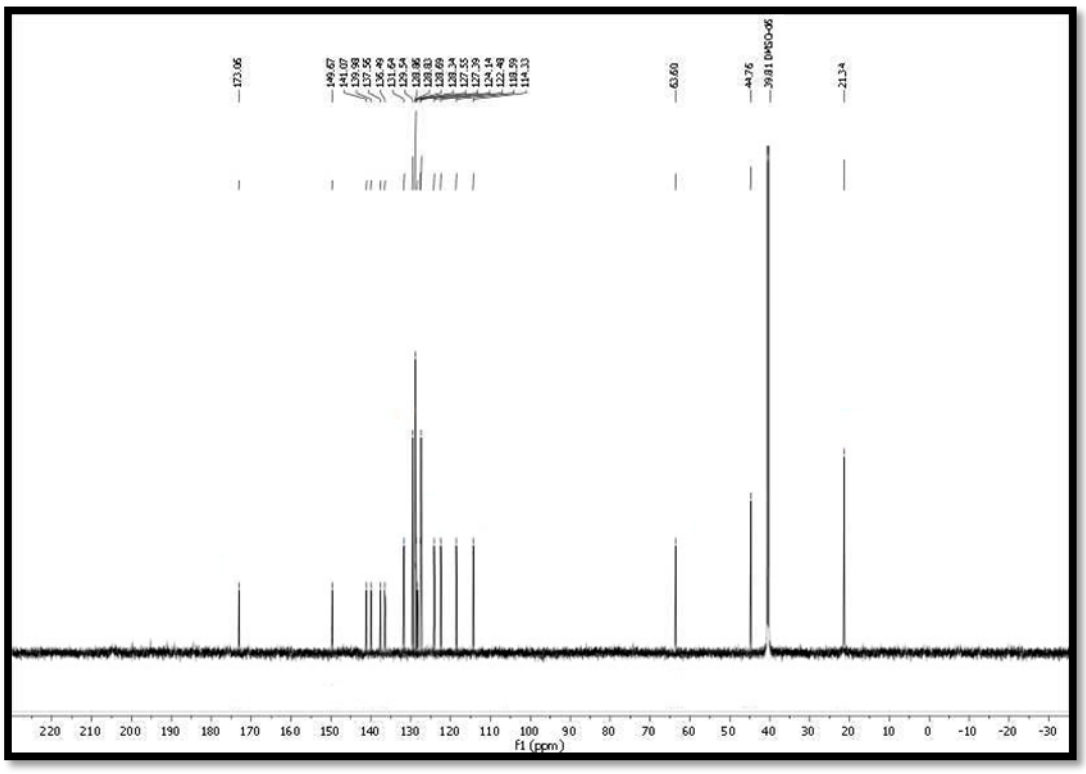

Fig. S15 — ^{13}C NMR spectrum of compound 5l

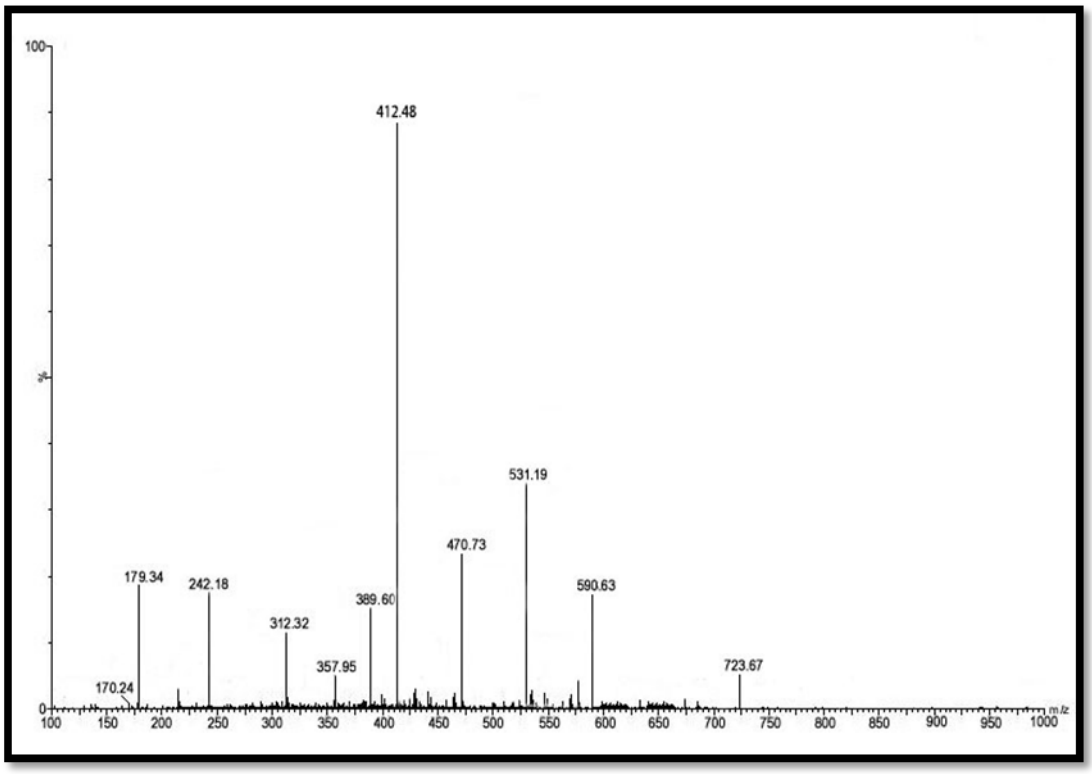

Fig. S16 — Mass spectrum of compound 5l

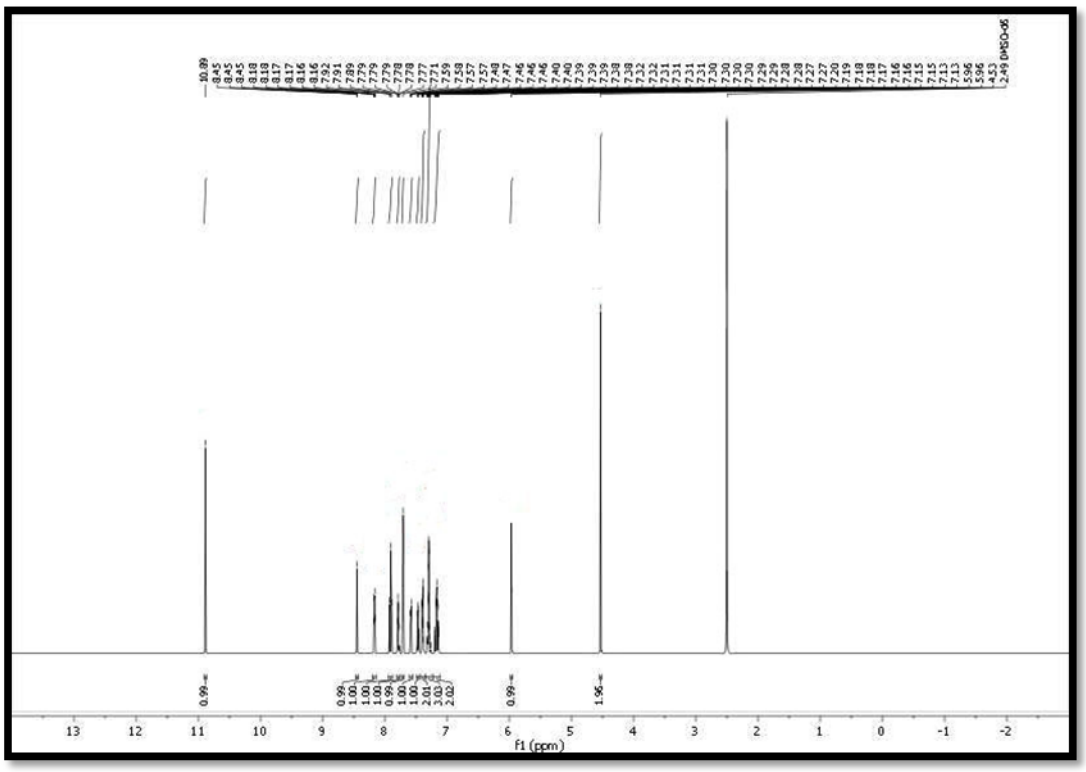

Fig. S17 — ^{1}H NMR spectrum of compound 5n

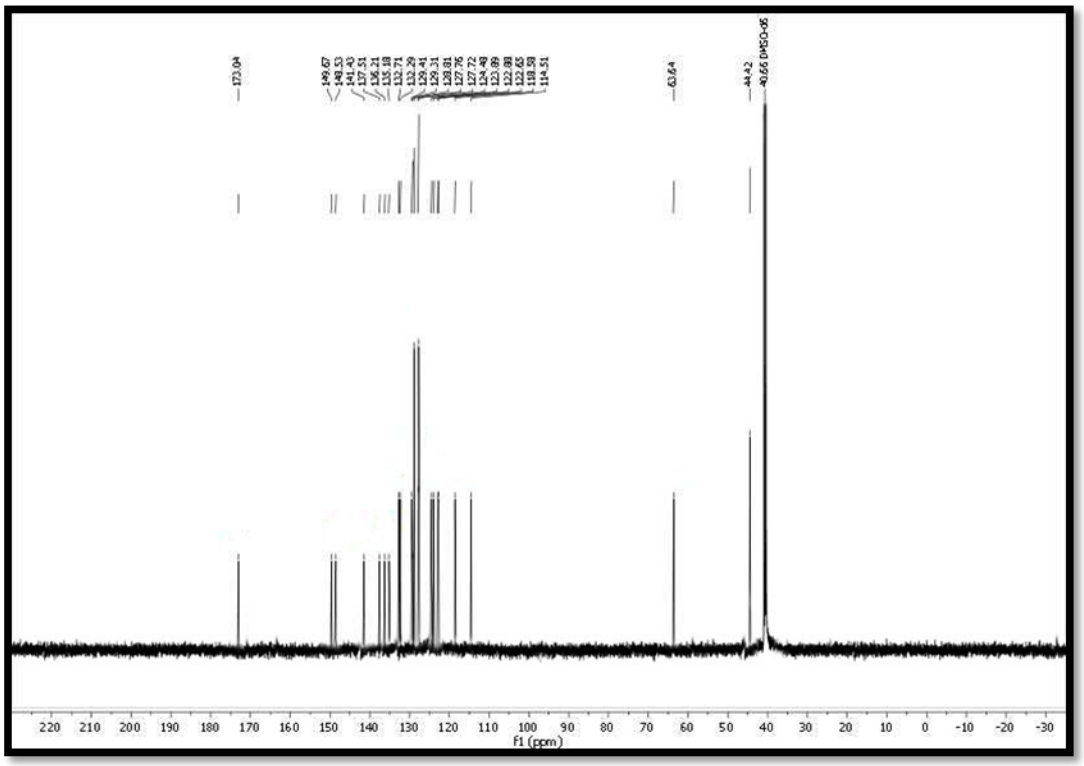

Fig. S18 — ^{13}C NMR spectrum of compound 5n

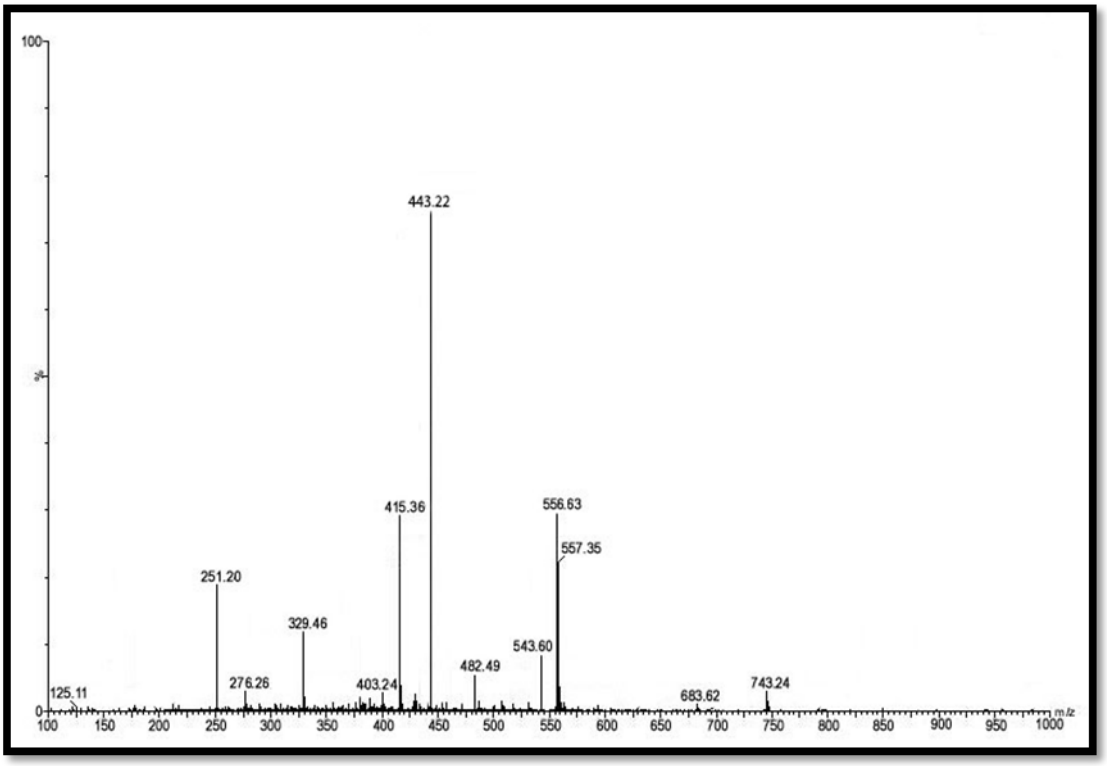

Fig. S19 — Mass spectrum of compound 5n