Supplementary Information

Synthesis and screening for antioxidant and cytotoxic activities of novel 2-thioxo-benzo[f]chromeno[2,3-*d*]pyrimidin-4-ones derived by cetylpyridinium chloride catalyzed multicomponent reactions in aqueous micellar media

Dini Ahanthem[a], Medhabati Thiyam[a], Reena Haobam[b] & Warjeet S Laitonjam*[a]
[a] Department of Chemistry, Manipur University, Canchipur, Imphal 795 003, India
[b] Department of Biotechnology, Manipur University, Canchipur, Imphal 795 003, India
E-mail: warjeet@yahoo.com; warjeet@manipuruniv.ac.in

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Experimental Section

General experimental procedures

The melting points were determined on a Buchi M-560 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Shimadzu FT-IR spectrophotometer in the range of 200 cm^{-1} to 4000 cm^{-1}. All the samples were run on a sodium chloride plate as a liquid film. Absorption maxima were recorded in wave numbers (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on BRUCKER–ACF-300 (300 MHz). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on BRUCKER-ACF-300 (75 MHz). All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS), reference to the chemical shifts of residual solvent resonances (^1H and ^{13}C NMR). Coupling constants are given in Hz. All samples are run in deutero-chloroform ($CDCl_3$) and DMSO. The FAB mass spectra were recorded at 6000 Mass Spectrometed data systems using Argon/Xenon (6KV, 10mA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature.

*General procedure for the synthesis of 2-thioxo-bezochromenopyrimidin-4-ones (**4a-4o**)*

In a typical experiment, *p*-nitrobenzaldehyde **3** (1.0 mmol), β-napthol **2** (0.144g, 1.0 mmol), thiobarbituric acid **1** (1.0 mmol) and CPC (0.015 mmol %) were taken in a round bottom flask using water (2.5 mL) and acetonitrile (2.5 mL) as solvent in 1:1 ratio. The reaction mixture was refluxed and the progress of the reaction was monitored by TLC. After completion of reaction (8 h), the solid obtained was collected by filtration and washed successively with water (3 x 10 mL) and with acetonitrile (3 x 5 mL). The crude product was recrystallized from ethanol to afford the pure compound **4** which required no further purification.

*2-Thioxo-5-(4-nitrophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one (**4a**)*. Yellow solid; m.p. 207-210^{0}C; IR (KBr): ν_{max} 3541, 1651, 1537, 1445, 1350, 1198, 1132, 1015, 849 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 5.70 (s, 1H), 6.72 (m, 1H), 6.85 (m, 1H), 7.01 (d, 2H, *J*=7.6 Hz), 7.24 (m, 4H), 7.67 (d, 2H, *J*=7.6 Hz), 10.90 (br s, 1H), 11.20 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 173.6, 152.9, 145.7, 128.3, 123.5, 95.6, 31.6; Anal. Calcd. for $C_{21}H_{13}N_3O_4S$: C 62.52, H 3.25, N 10.42; Found: C 62.83, H 3.64, N 10.54.

*2-Thioxo-5-(4-chlorophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one (**4b**)*. White solid; m.p: 198-203^{0}C; IR (KBr): ν_{max} 3524, 1660, 1537, 1443, 1359, 1201, 1134, 1013, 866 cm^{-1}; ^1H NMR

(DMSO-d_6, 300 MHz): ∂ 5.80 (s, 1H), 6.97-7.22 (m, 10H), 11.32 (br s, 1H), 11.69 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂173.4, 164.0, 163.0, 142.7, 129.8, 128.9, 128.0, 96.0, 30.6; HRMS (EI) cald. 393.0464; found 393.0478. Anal. Calcd. for $C_{21}H_{13}ClN_2O_2S$: C 64.20, H 3.34, N 7.13; Found: C 64.13, H 3.75, N 7.37.

2-Thioxo-5-(1H-indol-3-yl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4c)***. White solid; m.p: 188-192^{0}C; IR (KBr): ν_{max} 3538, 1655, 1528, 1377, 1277, 1213, 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 5.80 (s, 1H), 6.97-7.22 (m, 10H), 12.28 (br s, 1H), 12.32 (br s, 1H), 13.01 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.2, 163.3, 161.5, 145.1, 141.5, 137.1,129.5, 124.5, 123.6, 118.4, 113.9, 112.9, 109.2, 29.5; Anal. Calcd. for $C_{23}H_{15}N_3O_2S$: C 69.50, H 3.80, N 10.57; Found: C 69.65, H 3.98, N 10.86.

2-Thioxo-5-(3-hydroxynaphthalen-1-yl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4d).*** White solid; m.p: 195-198^{0}C; IR (KBr): ν_{max} 3452, 3032, 1678, 1564, 1458, 1211, 1138, 814 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 5.56 (s, 1H), 7.15 (m, 2H), 7.22 (m, 2H), 7.55 (m, 2H), 7.60 (m, 2H), 7.88 (m, 2H), 8.22 (m, 2H), 11.55 (br s, 1H), 12.20 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 174.9, 173.5, 160.3, 156. 8, 148.3, 131.4, 129.8, 128.0, 126.6, 126.5, 123.1, 119.1, 109.1, 43.2, 29.6; Anal. Calcd. for $C_{25}H_{16}N_2O_3S$: C 70.74, H 3.80, N 6.60; Found: C 70.46, H 3.62, N 6.33.

2-Thioxo-5-(4-N,N-dimethylanilino)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4e).*** White solid; m.p: 205-208^{0}C; IR (KBr): ν_{max} 3457, 3119, 1634, 1537, 1495, 1373, 1194, 1142, 1011 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 3.00 (s, 3H), 3.02 (s, 3H), 5.90 (s, 1H), 6.75 (m, 2H), 7.22 (m, 2H), 7.40 (m, 2H), 8.22 (m, 2H), 8.48 (m, 2H), 11.98 (br s, 1H), 12.05 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.0, 173.5, 163.5, 161.0, 156.7, 155.3, 140.3, 128.6, 120.9, 112.0, 109.8, 95.9, 46.1, 30.9; Anal. Calcd. for $C_{23}H_{19}N_3O_2S$: C 68.81, H 4.77, N 10.47; Found: C 68.69, H 4.54, N 10.23.

2-Thioxo-5-(4-carbaldehydophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4f)***. White solid; m.p: 201-203^{0}C; IR (KBr): ν_{max} 3522, 3148, 1670, 1574, 1518, 1433, 1298, 1207, 1148 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 4.50 (s, 1H), 5.90 (s, 1H), 7.12-8.25 (m, 10H), 11.62 (br s, 1H), 12.25 (br s, 1H), 12.42 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.4, 173.5, 162.5, 145.2, 132.7, 128.6, 61.3, 29.6; Anal. Calcd. for $C_{22}H_{14}N_2O_3S$: C 68.38, H 3.65, N 7.25; Found: C 68.61, H 3.48, N 7.46.

2-Thioxo-5-propyl-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4g).*** White solid; m.p: 188-192^{0}C; IR (KBr): ν_{max} 3178, 1686, 1582, 1522, 1316, 1165 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 0.82 (m, 3H), 1.18 (m, 2H), 2.28 (m, 2H), 5.78 (m, 1H), 7.12 (m, 2H), 7.32-7.45 (m, 2H), 7.65-7.72 (m, 2H), 11.98 (br s, 1H), 12.05 (br s, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 178.2, 157.3, 145.5, 135.3, 129.8, 128.6, 128.0, 126.6, 126.5, 123.1, 119.1, 109.1, 31.8, 29.6, 22.4, 14.5; Anal. Calcd. for $C_{24}H_{20}N_2O_2S$: C 71.98, H 5.03, N 6.99; Found: C 71.87, H 4.92, N 6.55.

2-Thioxo-5-(2,4-dimethoxyphenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4h).*** White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.73 (s, 3H), 3.85 (s, 3H), 4.56 (s, 1H), 7.08-7.23 (m, 5H), 7.40-8.13 (m, 4H), 12.34 (br s, 1H), 12.48 (br s, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 178.5, 161.7, 159.4, 155.2, 154.1, 150.1, 145.4, 129.2, 128.0, 126.1, 125.9, 122.6, 121.6, 121.2, 118.4, 117.0, 56.3, 55.5, 28.8; Anal. Calcd. for $C_{23}H_{18}N_2O_4S$: C 66.01, H 4.34, N 6.69; Found: C 66.23, H 4.62, N 6.83.

2-Thioxo-5-(3-nitrophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4i).*** White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 5.75 (s, 1H), 7.38-7.43 (m, 4H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.88-8.07 (m, 4H), 8.17 (d, *J* = 9.2 Hz, 1H), 12.14 (br s, 1H), 12.38 (br s, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 173.1, 163.8, 153.6, 148.7, 138.4, 133.8, 131.3, 125.6, 123.1, 116.9, 89.4, 33.8; Anal. Calcd. for $C_{21}H_{13}N_3O_4S$: C 62.52, H 3.25, N 10.42; Found: C 62.74, H 3.42, N 10.61.

2-Thioxo-5-(4-methoxyphenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4j).*** White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.63 (s, 3H), 5.56 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 2H), 7.11-7.26 (m, 4H), 7.32-7.88 (m, 4H), 11.34 (br s, 1H), 12.18 (br s, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 177.8, 164.3, 158.2, 153.8, 150.4, 146.7, 132.2, 131.0, 129.8, 129.2, 127.6, 125.8, 124.2, 118.7, 114.5, 89.6, 56.3, 55.6, 33.8; Anal. Calcd. for $C_{22}H_{16}N_2O_3S$: C 68.02, H 4.15, N 7.21; Found: C 68.17, H 4.27, N 7.45.

1,3-Dimethyl-2-thioxo-5-phenyl-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4k).*** White solid; m.p: 215-217^{0}C; IR (KBr): ν_{max} 2978, 1688, 1584, 1526, 1322, 1095 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.32 (s, 3H), 3.48 (s, 3H), 5.72 (m, 1H), 7.12-7.28 (m, 4H), 7.36-7.44 (m, 5H), 7.72 (m, 2H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 175.1, 162.2, 152.3, 147.5, 135.8, 131.8, 130.6,

128.4, 126.9, 126.3, 124.7, 123.1, 119.3, 116.2, 91.8, 36.6, 28.4, 27.5; Anal. Calcd. for $C_{23}H_{18}N_2O_2S$: C 71.48, H 4.69, N 7.25; Found: C 71.62, H 4.96, N 7.48.

1,3-Dimethyl-2-thioxo-5-(4-nitrophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4l).*** White solid; m.p: 272-275^{0}C; IR (KBr): ν_{max} 2924, 1678, 1588, 1516, 1338, 1227, 1118 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.28 (s, 3H), 3.53 (s, 3H), 5.84 (m, 1H), 7.38 (d, *J* = 9.1 Hz, 1H), 7.42-7.48 (m, 2H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.78-8.02 (m, 4H), 8.14 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 175.4, 161.8, 152.6, 150.3, 147.2, 139.7, 131.2, 130.4, 128.7, 127.3, 125.8, 124.1, 123.5, 118.1, 116.6, 90.4, 36.2, 29.3, 28.4; Anal. Calcd. for $C_{23}H_{17}N_3O_4S$: C 64.03, H 3.97, N 9.74; Found: C 64.21, H 4.02, N 9.88.

1,3-Dimethyl-2-thioxo-5-(4-methoxyphenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4m).*** White solid; m.p: 257-259^{0}C; IR (KBr): ν_{max} 3032, 2962, 1686, 1574, 1528, 1432, 1328, 1205 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.30 (s, 3H), 3.58 (s, 3H), 3.88 (s, 3H), 5.77 (m, 1H), 6.78 (d, *J* = 8.9 Hz, 2H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.34-7.48 (m, 3H), 7.68-7.82 (m, 2H), 8.10 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 174.8, 161.4, 159.4, 158.1, 153.2, 135.6, 133.4, 129.8, 128.3, 127.8, 125.3, 123.1, 122.5, 118.2, 116.3, 82.1, 56.4, 29.2, 28.7, 28.1;
Anal. Calcd. for $C_{24}H_{20}N_2O_3S$: C 69.21, H 4.84, N 6.73; Found: C 69.04, H 4.92, N 6.84.

1,3-Dimethyl-2-thioxo-5-(4-methylphenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4n).*** White solid; m.p: 212-215^{0}C; IR (KBr): ν_{max} 3011, 2954, 1692, 1635, 1483, 1344, 1216 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 2.19 (s, 3H), 3.31 (s, 3H), 3.64 (s, 3H), 5.69 (m, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.31-7.51 (m, 3H), 7.70-7.84 (m, 2H), 7.96 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 175.1, 161.6, 152.8, 147.3, 140.2, 135.9, 132.1, 130.3, 129.3, 128.6, 127.5, 125.3, 124.4, 122.5, 117.8, 116.5, 91.3, 36.2, 29.4, 28.2, 20.7; Anal. Calcd. for $C_{24}H_{20}N_2O_2S$: C 71.98, H 5.03, N 6.99; Found: C 71.83, H 5.31, N 7.07.

1,3-Dimethyl-2-thioxo-5-(4-chlorophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one ***(4o).*** White solid; m.p: 250-252^{0}C; IR (KBr): ν_{max} 3054, 2958, 1690, 1650, 1583, 1474, 1211 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, 300 MHz): ∂ 3.33 (s, 3H), 3.56 (s, 3H), 5.71 (m, 1H), 7,12 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.38-7.48 (m, 3H), 7.75-7.80 (m, 2H), 8.06 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (DMSO-d$_6$, 75 MHz): ∂ 174.8, 161.9, 152.2, 150.3, 147.3, 142.4, 133.2, 131.7, 130.8, 129.4, 128.5, 127.8, 125.6, 123.8, 116.8, 116.1, 90.6, 35.7, 29.0, 28.4; Anal. Calcd. for $C_{23}H_{17}ClN_2O_2S$: C 65.63, H 4.07, N 6.66; Found: C 65.46, H 4.16, N 6.96.

Antioxidant assay

Determination of DPPH free radical scavenging activity is based on the reduction of methanolic DPPH in the presence of a hydrogen donating antioxidant. Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. The adsorption and intense violet DPPH solution was reduced by an antioxidant compound. Lowering absorbance of the reaction mixture inferred higher free-radical scavenging effect. Each compound (3ml. at 0.025g/ml) was mixed with DPPH (Sigma) solution (45µg/ml) in HPLC grade methanol (Merck), vortexed well at room temperature and was left standing for 10 mins exactly and the absorbance was measured at 517 nm spectrophotometrically. A solution of BHT (Butylated hydroxy toluene, Sigma) of 125 µg/ml in MeOH was used as reference and the methanol without DPPH as a blank solution. Radical scavenging activity was calculated on percentage basis using the following equation,

$$\% \text{ Scavenging activity} = (A_0 - A_{10})/ (R_0 - R_{10}) \times 100,$$

where, A_0 and A_{10} are the absorbance values of (DPPH + Sample) solution at 0 min. and 10 min. respectively. R_0 and R_{10} are the absorbance values of (DPPH + BHT) solution at 0 min. and 10 min. respectively.

The results indicated that the tested compounds contained antioxidants which reduced the stable DPPH free radical to the corresponding yellow coloured diphenyl-picrylhydrazine. The antioxidant activity of the compound was expressed as IC_{50}. The IC_{50} value was defined as the which was graphically determined from the trend line of the curve of percent inhibition (Y-axis) against concentration (µg/mL) (X-axis), where the straight line form the 50% inhibition on the Y-axis intersected the trend line gave the IC (µg/mL) on the X-axis.

Cytotoxicity assay

Synthesized compounds (**4a**-**4o**) were tested for cytotoxic activity against HEPG2 cells. Each compound was dissolved with DMSO and diluted with water to obtain a concentration of 20 mM. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.005% in the highest concentration). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)- 2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product after 48 h. In brief, the cells were seeded in a 96-well plate at a cell concentration of 1 x 10^4 cell per well in 100 µL of growth medium and fresh medium

containing different concentrations of the test sample was added after 24 h of seeding. Serial twofold dilution of the tested chemical compound was added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates using a multichannel pipette. The microtiter plates were incubated at 37^{o}C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The low percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37^{o}C, various concentrations of each sample (50, 25, 12.5, 6.25, 3.125 & 1.56 μg) were added, and the incubation was continued for 48 h and viable cell yield was determined by a colorimetric method. After the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbances of the plates were measured after gently shaking on microplate reader, using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate and the cell cytotoxic effect of each tested compound was calculated.

Figure S1. IR spectrum of **4a**

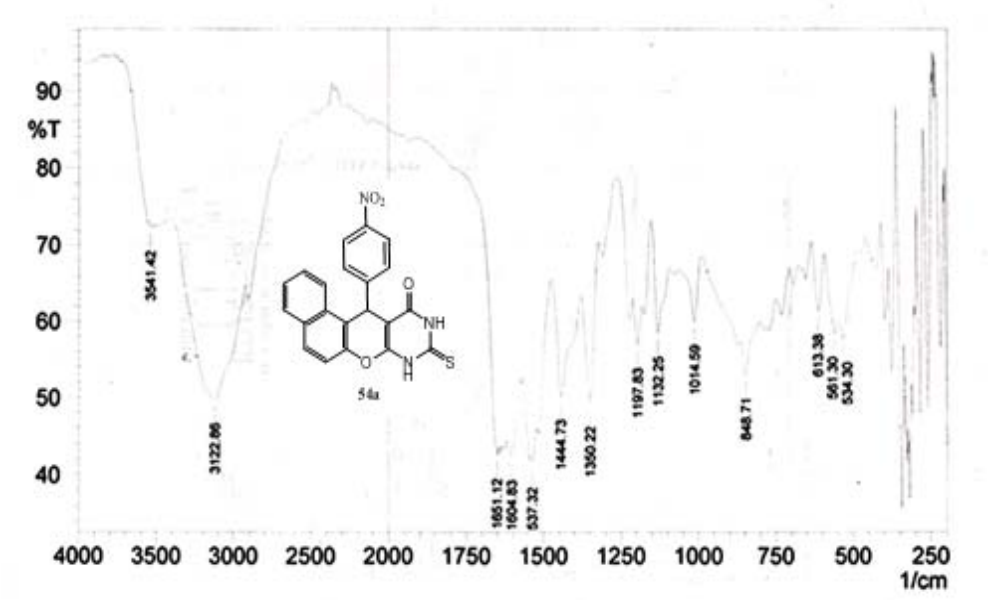

Figure S2. ^{1}H NMR spectrum of **4a**

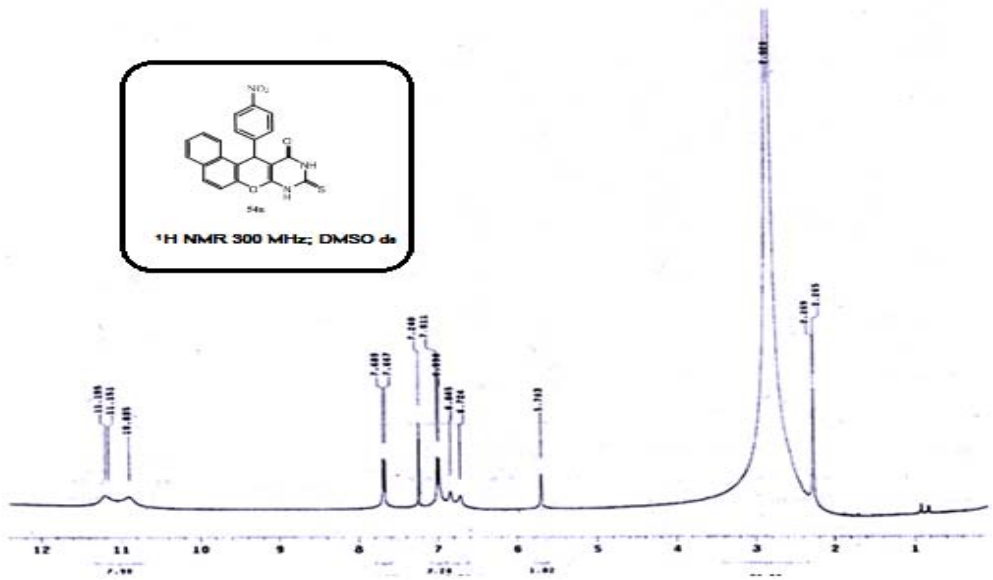

Figure S3. ^{13}C NMR spectrum of **4a**

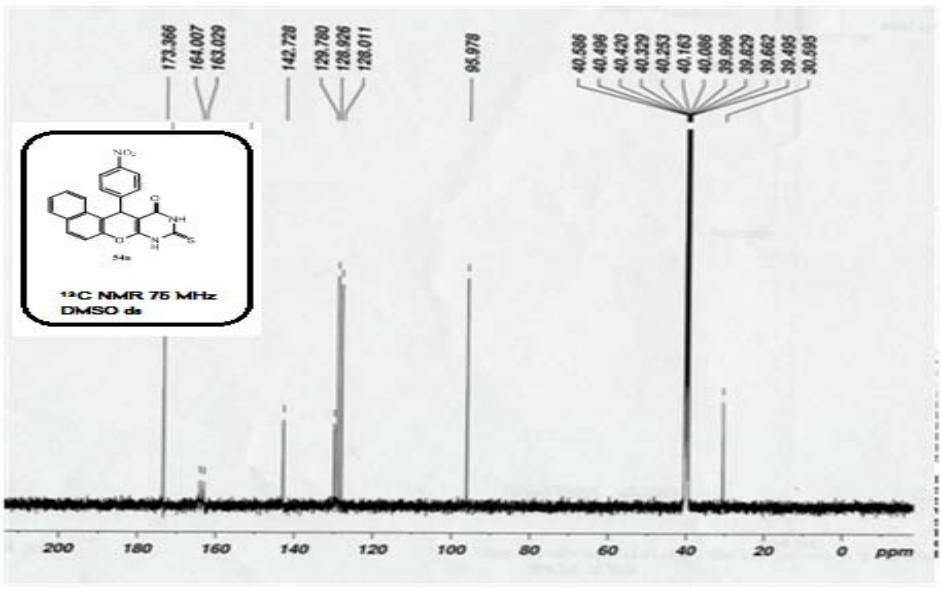

Figure S4. IR spectrum of **4b**

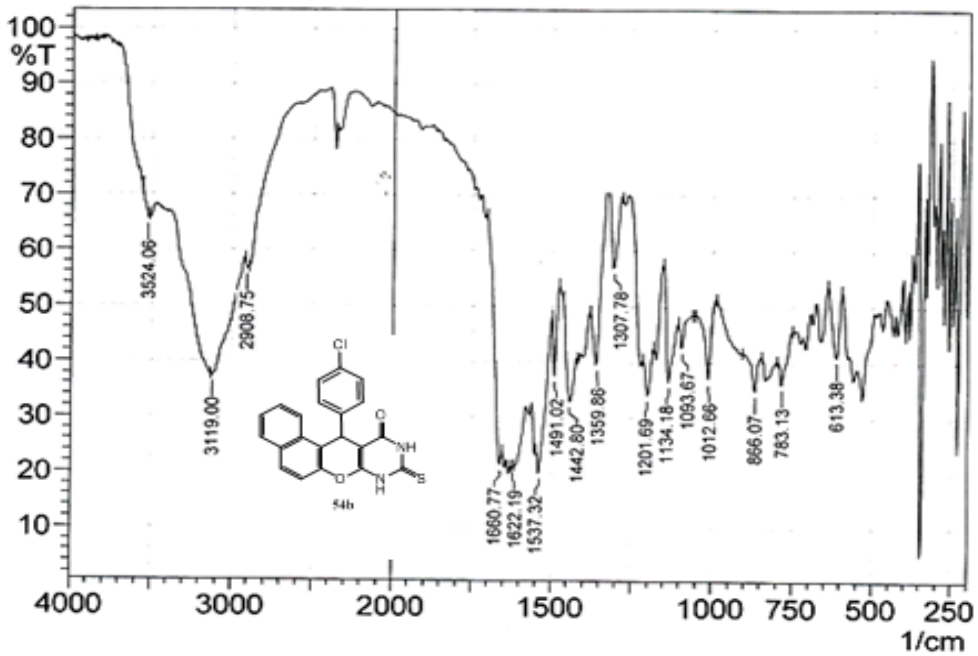

Figure S5. ^{1}H NMR spectrum of **4b**

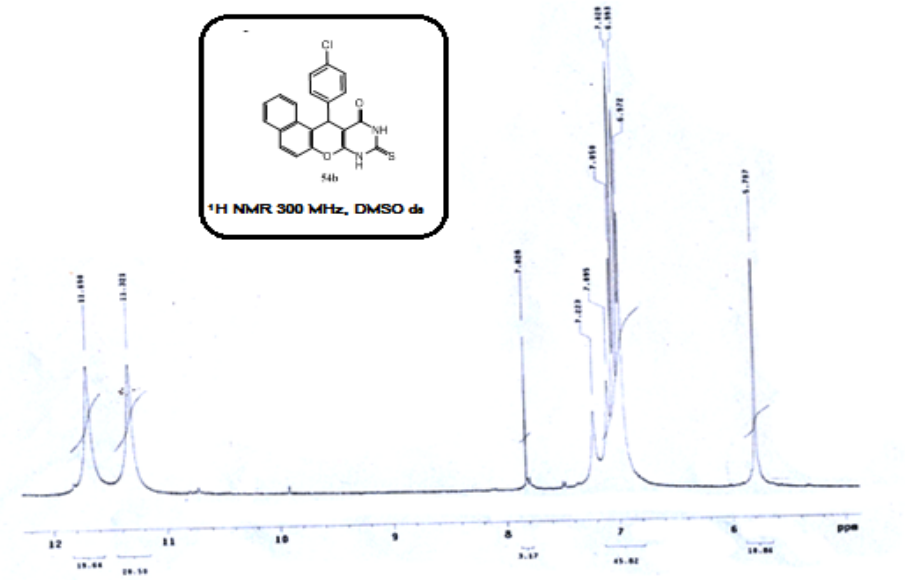

Figure S6. ^{13}C NMR spectrum of **4b**

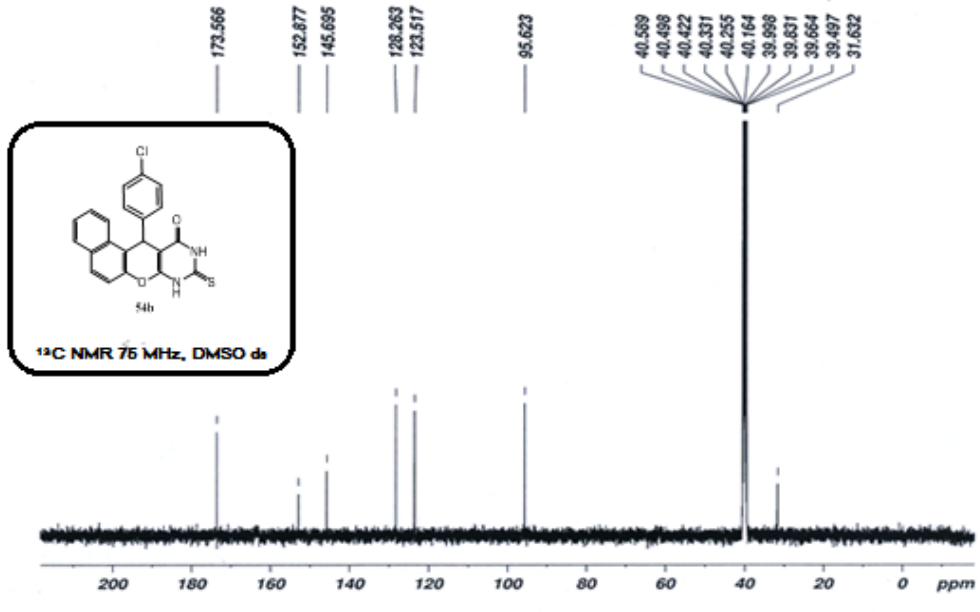

Figure S7. Mass spectrum of **4b**

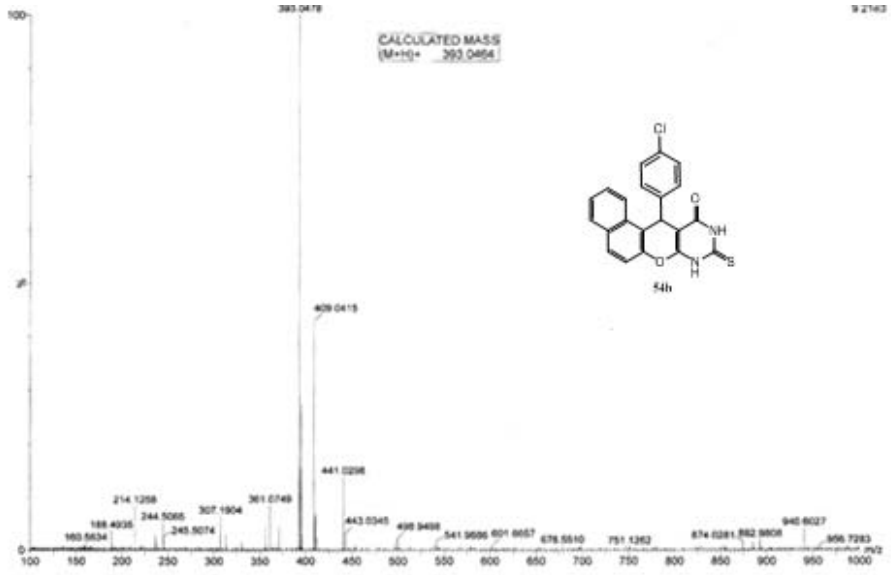

Figure S8. IR spectrum of **4c**

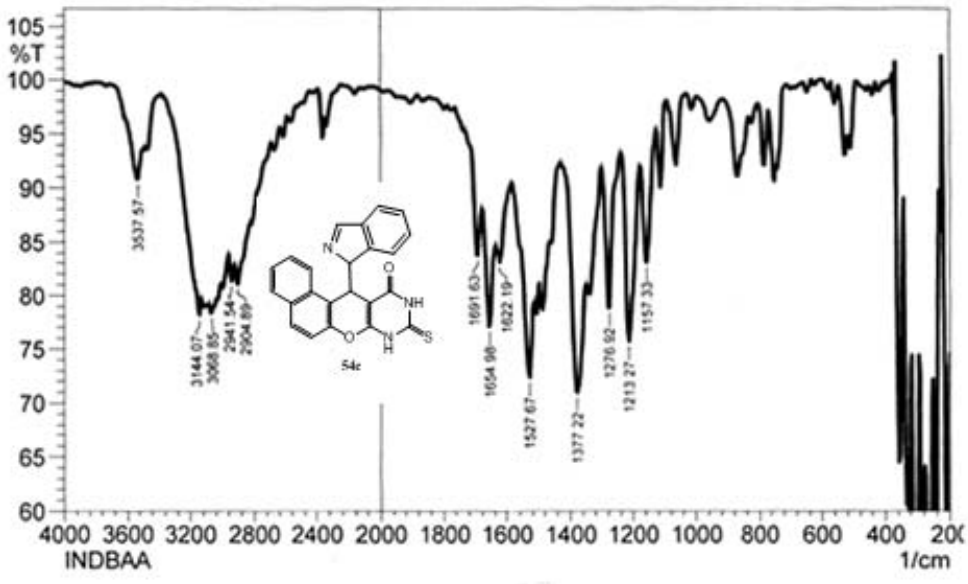

Figure S9. [1]H NMR spectrum of **4c**

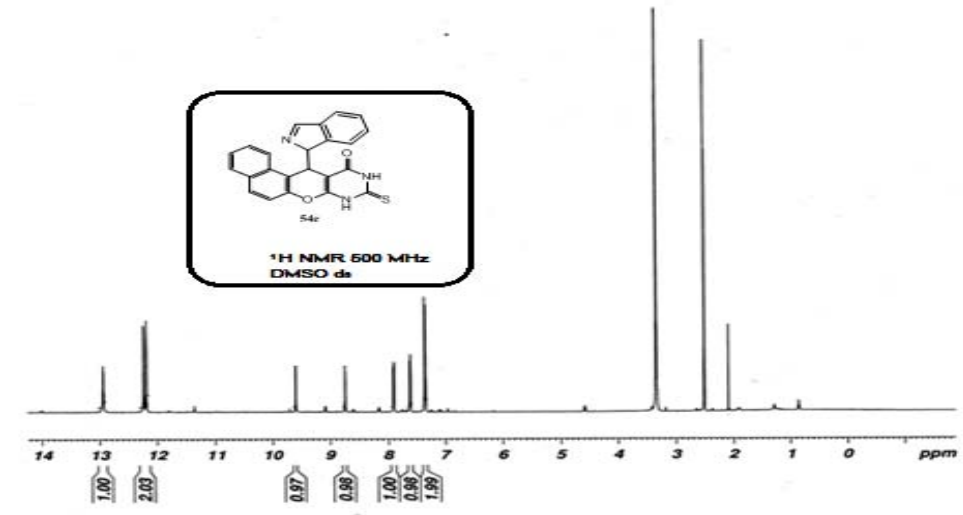

Figure S10. [13]C NMR spectrum of **4c**

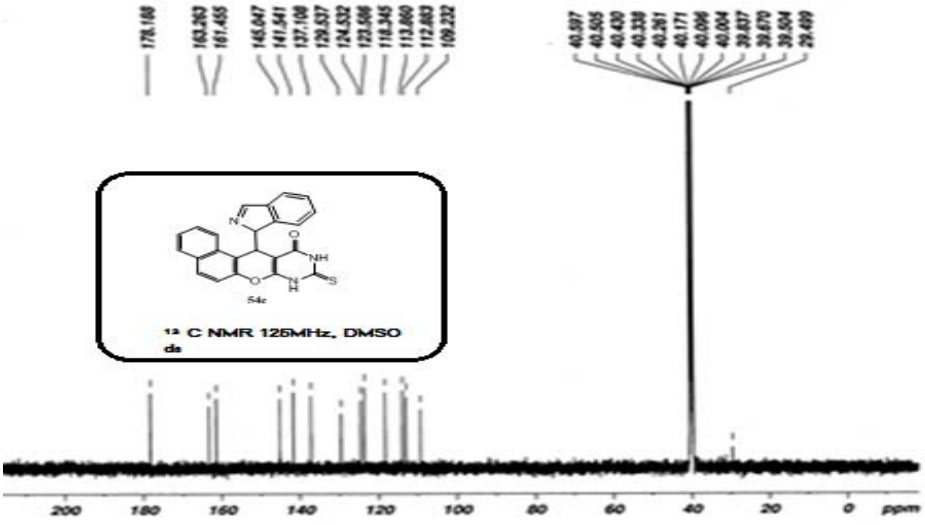

Figure S11. IR spectrum of **4e**

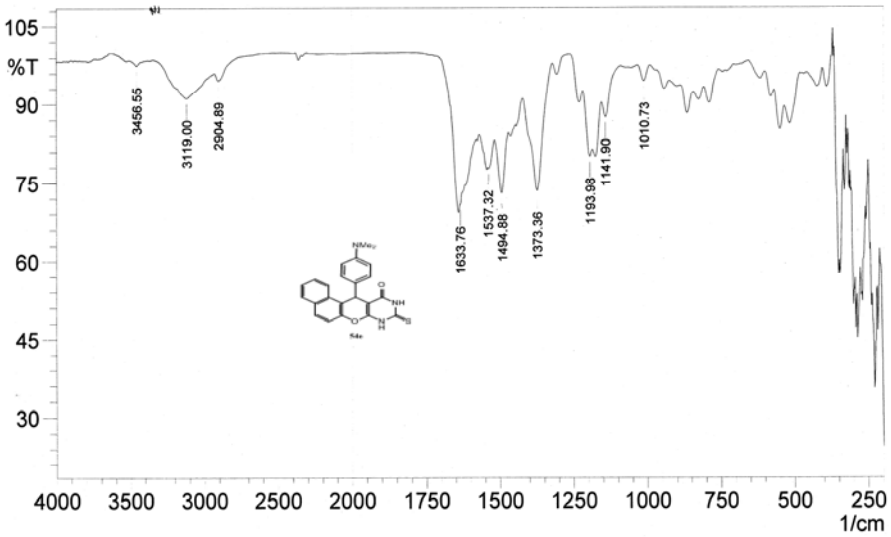

Figure S12. ^{1}H NMR spectrum of **4e**

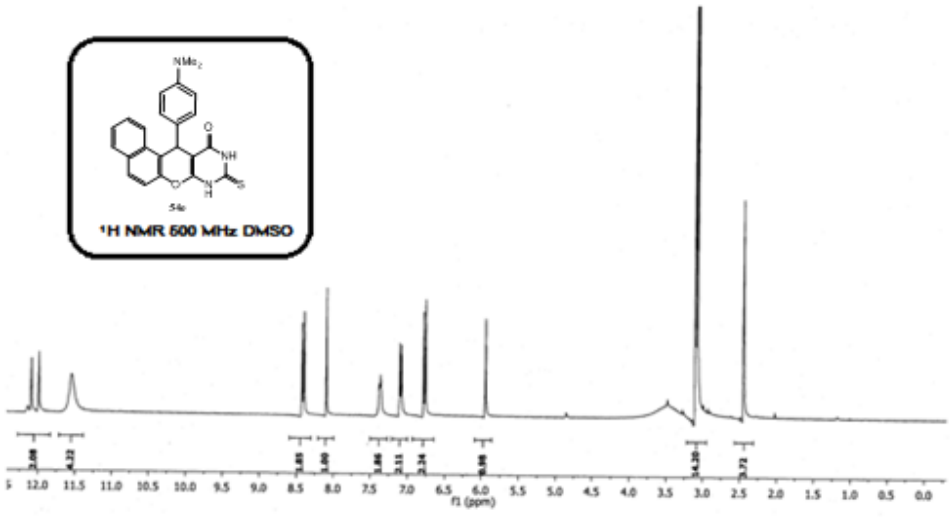

Figure S13. ^{13}C NMR spectrum of **4e**