Electronic Supporting Information

Biphenyl urea derivatives as selective CYP1B1 inhibitors

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S1. Experimental procedures

All reactions were carried out under dry conditions under nitrogen atmosphere. Acetone was distilled prior to use. All the chemicals were purchased from Sigma Aldrich or SpectroChem and solvents from Rankem and used as it is or otherwise it is specified accordingly.

General procedure for the synthesis of compounds 5a-p: To the solution of substituted aniline (1 equivalent) in distilled acetone was added corresponding phenyl isocyanate (1 equivalent). After 1 h, a precipitate began to settle down. After stirring for 12 -16 hrs, the precipitate was filtered and washed with dichloromethane (10 ml) and dried under vacuum to obtain desired product.

1,3-Diphenyl urea (5a):[1] White solid; 0.453 g (90% yield); m.p. 230-232 °C (lit. 231-235 °C). ESIMS: *m/z* 213.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.96 (2H, m, Ar-H), 7.28 (4H, m, Ar-H), 7.44 (4H, d, *J* = 7.6 Hz, Ar-H), 8.62 (2H, s, 2 x CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 118.71 (Ar-C-), 122.49 (Ar-C-), 129.32 (Ar-C-), 140.24 (-C-N), 153.08 (-C=O).

1-Phenyl-3-o-tolylurea (5b):[1] White solid; 0.831 g (78% yield); mp. 208-209 °C (Lit. 212 °C);[1] ESIMS: *m/z* 227.01 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (3H, s, -CH_3), 6.97 (2H, m, Ar-H), 7.15 (2H, m, Ar-H), 7.29 (2H, t, *J* = 7.7 Hz, Ar-H), 7.48 (2H, d, *J* = 6.8 Hz, Ar-H), 7.85 (1H, d, *J* = 8.1 Hz, Ar-H), 7.93 (1H, s, CO-NH), 9.03 (1H, s, CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 18.45, -(-CH_3), 118.52 (Ar-C-), 121.52 (Ar-C-), 123.16 (Ar-C-), 126.69 (Ar-C-), 127.78(Ar-C-), 129.36 (Ar-C-), 130.71 (Ar-C-CH_3), 137.96 (-C-N), 140.44 (-C-N), 153.20 (-C=O):

1-Phenyl 3-m-tolylurea (5c):[2] White solid; 0.741 g (70% yield); m.p. 168-170 °C (Lit. 173-174 °C);[2] ESIMS: *m/z* 227.11 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (3H, s, -CH_3), 6.79 (1H, d, *J* = 7 Hz, Ar-H), 6.96 (1H, t, *J* = 7.7 Hz, Ar-H), 7.15 (1H, m, Ar-H), 7.27 (4H, m, Ar-H), 7.46 (2H d, *J* = 7.7 Hz, Ar-H), 8.59 (1H, s, CO-NH), 8.65 (1H, s, CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 21.57 (-CH_3), 115.89 (Ar-C-) , 118.49 (Ar-C-), 119.21 (Ar-C-), 122.29 (Ar-C-), 123.08 (Ar-C-), 129.15 (Ar-C-CH_3), 138.47 (-C-N), 140.39 (-C-N), 153.28 (-C=O).

1-(2-Methoxyphenyl)-3-phenylurea (5d):[3] White solid; 0.740 g (75% yield); m.p. 140-143 °C (Lit. 146.2-146.8 °C);[3] ESIMS: *m/z* 243.1 $[M+H]^+$; ^{1}H NMR (400 MHz,

DMSO-d_6): δ 3.86 (3H, s, -OC$\underline{H}_3$), 6.91 (4H, m, Ar-$\underline{H}$), 7.26 (2H, t, *J* = 7.2 Hz, Ar-$\underline{H}$), 7.44 (2H, m, Ar-$\underline{H}$), 8.11 (1H, m, Ar-$\underline{H}$), 8.20 (1H, s, CO-N$\underline{H}$), 9.28 (1H, s, CO-N$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6): δ 56.17 (-O$\underline{C}H_3$), 111.19 (Ar-$\underline{C}$-), 118.42 (Ar-$\underline{C}$-), 121.07 (Ar-$\underline{C}$-) (Ar-$\underline{C}$-), 122.29 (Ar-$\underline{C}$-), 129.32 (-$\underline{C}$-N), 140.38 (-$\underline{C}$-N), 148.12 (-$\underline{C}$=O), 152.93 (Ar-$\underline{C}$-OCH_3).

1-(3-Methoxyphenyl)-3-phenylurea (5e):[4] White solid; 0.637 g (64% yield); mp. 148-152 °C (lit 155°C);[4] ESIMS: *m/z* 243.10 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (3H, s, -OC$\underline{H}_3$), 6.57 (1H, m, Ar-$\underline{H}$), 6.96 (2H, m, 6H, Ar-$\underline{H}$), 7.18 (2H, m, Ar-$\underline{H}$), 7.28 (2H, m, Ar-$\underline{H}$), 7.45 (2H, d, *J* = 7.7 Hz, Ar-$\underline{H}$), 8.67 (2H, d, *J* = 8.4 Hz, 2-CO-N$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6): δ 55.42 (-O$\underline{C}H_3$), 104.48 (Ar-$\underline{C}$-), 107.70 (Ar-$\underline{C}$-), 111.02 (Ar-$\underline{C}$-), 118.74 (Ar-$\underline{C}$-), 122.39 (Ar-$\underline{C}$-), 129.31 (Ar-$\underline{C}$-), 140.17 (-$\underline{C}$-N), 141.47 (-$\underline{C}$-N), 153.00 (-$\underline{C}$=O), 160.45 (Ar-$\underline{C}$-OCH_3).

1-(4-Methoxyphenyl)-3-phenylurea (5f):[1] White solid; 0.741 g (72%); mp. 180-182 °C (lit 186-190°C);[1] ESIMS: *m/z* 243.10 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.69 (3H, s, -OC$\underline{H}_3$), 6.84 (2H, d, *J* = 2.4 Hz, Ar-$\underline{H}$), 6.86 (1H, t, *J* = 3.6 Hz, Ar-$\underline{H}$), 7.24 (2H, t, *J* = 7.6 Hz, Ar-$\underline{H}$), 7.33 (2H, m, Ar-$\underline{H}$), 7.42 (2H, d, *J* = 7.2 Hz, Ar-$\underline{H}$), 8.42 (1H, s, CO-N$\underline{H}$), 8.53 (1H, s, CO-N$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6): δ 55.68 (-O$\underline{C}H_3$), 114.51 (Ar-$\underline{C}$-), 118.43 (Ar-$\underline{C}$-), 120.54 (Ar-$\underline{C}$-), 122.13 (Ar-$\underline{C}$-), 129.04 (Ar-$\underline{C}$-), 133.33 (-$\underline{C}$-N), 140.43 (-$\underline{C}$-N), 153.36 (-$\underline{C}$=O), 154.98 (Ar-$\underline{C}$-OCH_3).

1-(2-Chloroyphenyl)-3-phenylurea (5g):[1] White solid; 0.610 g (63%); mp. 182-184 °C (lit 180-182 °C);[1] ESIMS: *m/z* 247.10 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.98 (2H, m, Ar-$\underline{H}$), 7.27 (4H, m, Ar-$\underline{H}$), 7.44 (3H, m, Ar-$\underline{H}$), 8.62 (2H, s, CO-N$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6): δ 118.71 (Ar-$\underline{C}$-), 121.79 (Ar-$\underline{C}$-), 153.07 (Ar-$\underline{C}$-), 122.33 (Ar-$\underline{C}$-), 122.65 (Ar-$\underline{C}$-), 123.77 (Ar-$\underline{C}$-), 128.10 (Ar-$\underline{C}$-Cl), 129.31 (-$\underline{C}$-N) , 129.73 (-$\underline{C}$-N), 140.25 (-$\underline{C}$=O).

1-(3-Chloroyphenyl)-3-phenylurea (5h):[1] White solid; 0.636g (65%), mp. 182-185 °C (lit 188-189 °C); ESIMS: *m/z* 247.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.96 (2H, m, Ar-$\underline{H}$), 7.26 (4H, m, Ar-$\underline{H}$), 7.31 (2H, d, *J* = 8 Hz, Ar-$\underline{H}$), 7.69 (1H, s, Ar-$\underline{H}$), 8.71 (1H, s, CO-N$\underline{H}$), 8.84 (1H, s, CO-N$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6): δ 117.13 (Ar-$\underline{C}$), 118.07 (Ar-$\underline{C}$-), 118.92 (Ar-$\underline{C}$-), 121.93 (Ar-$\underline{C}$-), 122.32 (Ar-$\underline{C}$-), 122.62 (Ar-$\underline{C}$-), 129.32 (Ar-$\underline{C}$-), 130.89 (Ar-$\underline{C}$-), 133.76 (Ar-$\underline{C}$-Cl), 139.94(-$\underline{C}$-N), 141.83 (-$\underline{C}$-N), 152.92 (-$\underline{C}$=O).

1-(4-Chloroyphenyl)-3-phenylurea (5i):[5] White solid; 0.718 g (74%); mp. 230-234 °C (lit 237-238 °C);[5] ESIMS: *m/z* 247.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (1H, m, Ar-H), 7.30 (4H, m, Ar-H), 7.45 (4H, m, Ar-H), 8.661 (1H, s, CO-NH), 8.77 (1H, s, CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 118.82 (Ar-C-), 120.21 (Ar-C-), 122.49 (Ar-C-), 125.84 (Ar-C-), 129.15 (Ar-C-), 129.32 (Ar-C-Cl), 139.26 (-C-N), 140.14 (-C-N), 152.95 (-C=O).

1-(2-Nitroyphenyl)-3-phenylurea (5j):[5] Reddish yellow powder; 0.670 g (72%); mp. 166-168 °C (lit 170 °C);[5] ESIMS: *m/z* 258.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (1H, m, Ar-H), 6.96 (2H, m, Ar-H), 7.26 (2H, m, Ar-H), 7.37 (3H, m, Ar-H), 7.43 (2H, d, *J* = 7.6 Hz, Ar-H), 7.94 (1H, d, *J* = 1.2 Hz, CO-NH), 8.62 (1H, s, CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 115.96 (Ar-C-), 118.70 (Ar-C-), 119.70 (Ar-C-), 122.32 (Ar-C-), 125.90 (Ar-C-), 129.30 (Ar-C-), 130.79 (Ar-C-), 136.21 (-C-N), 140.24 (-C-N), 146.74 (Ar-C-NO_2), 153.15 (-C=O).

1-(3-Nitroyphenyl)-3-phenylurea (5k):[4] Reddish yellow powder; 0.724 g (77%); mp. 193-194 °C (lit 195 °C);[4] ESIMS: *m/z* 258.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.78 (2H, s, Ar-H), 6.87 (2H, m, Ar-H), 7.28 (4H, m, Ar-H), 7.63 (1H, s, Ar-H), 7.44 (2H, m, 2 x CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 107.54 (Ar-C-), 110.06 (Ar-C-), 112.84 (Ar-C-), 116.64 (Ar-C-), 118.70 (Ar-C-), 120.47 (Ar-C-), 122.32 (Ar-C-), 124.79 (Ar-C-), 129.33 (Ar-C-), 130.63 (Ar-C-), 140.24 (Ar-C-), 141.76 (-C-N), 149.15 (-C-N), 150.64 (Ar-C-NO_2), 152.95 (-C=O).

1-(4-Nitroyphenyl)-3-phenylurea (5l):[6] Yellow powder; (0.713 g, 76%); mp. 253-256 °C (lit 255-257 °C);[6] ESIMS: *m/z* 258.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.57 (3H, m, Ar-H), 6.69 (1H, s, Ar-H), 6.94 (1H, m, Ar-H), 7.26 (2H, t, *J* = 7.2 Hz, Ar-H), 7.43 (2H, d, *J* = 7.6 Hz, Ar-H), 7.92 (2H, d, *J* = 9.2 Hz, 2 x CO-NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 112.90 (Ar-C-), 118.71 (Ar-C-), 122.33 (Ar-C-), 126.92 (Ar-C-), 129.30 (Ar-C-), 135.97 (-C-N), 140.32 (-C-N), 153.07 (Ar-C-NO_2), 156.22 (-C=O).

1-(4-Methoxyphenyl)-3-phenylthiourea (5m):[6] White powder; 1.213 g (79%); mp. 151-153°C (lit 159-161°C);[6] ESIMS: *m/z* 259.08 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (3H, s, OCH_3), 6.88 (2H, d, *J* = 2.4 Hz, Ar-H), 7.90 (1H, m, Ar-H), 7.29 (4H, m, Ar-H), 7.45 (2H, d, *J* = 7.2 Hz, Ar-H), 9.59 (2H, d, *J* = 9.2 Hz, CS-NH); ^{13}C NMR (101

MHz, DMSO-d_6): δ 55.76 (-O<u>C</u>H_3), 114.20 (Ar-<u>C</u>-), 124.20 (Ar-<u>C</u>-), 126.79 (Ar-<u>C</u>-), 128.93 (Ar-<u>C</u>-), 132.89 (-<u>C</u>-N), 140.08 (-<u>C</u>-N), 157.08 (Ar-<u>C</u>-OCH_3), 180.13 (-<u>C</u>=S).

1-(3-Chlorophenyl)-3-(4-methoxyphenyl)thiourea (5n):[6] White powder; 0.861 g (74%); mp. 122-123 °C (lit 120 °C);[6] ESIMS: *m/z* 293.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO- d_6): δ 3.73 (3H, s, OC<u>H</u>$_3$), 6.89 (2H, d, *J* = 9.2 Hz, Ar-<u>H</u>), 7.12 (1H, m, Ar-<u>H</u>), 7.28 (4H, m, Ar-<u>H</u>), 7.68 (1H, s, Ar-<u>H</u>), 9.76 (2H, d, *J* = 13.1 Hz, CS-N<u>H</u>); ^{13}C NMR (101 MHz, DMSO-d_6): δ 55.77 (-O<u>C</u>H_3), 114.30 (Ar-<u>C</u>-), 122.39 (Ar-<u>C</u>-), 123.45 (Ar-<u>C</u>-), 124.58 (Ar-<u>C</u>-), 126.44 (Ar-<u>C</u>-), 130.45 (Ar-<u>C</u>-), 132.38 (-<u>C</u>-N), 132.98 (Ar-<u>C</u>-Cl), 141.85 (-<u>C</u>-N), 157.25 (Ar-<u>C</u>-OCH_3), 180.35 (-<u>C</u>=S).

1-(4-Chlorophenyl)-3-(4-methoxyphenyl)thiourea (5o): White powder; 0.956 g (83.2%); mp. 151-153 °C (lit 150-152 °C);[6] ESIMS: *m/z* 293.04 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (3H, s, OC<u>H</u>$_3$), 6.89 (2H, m, Ar-<u>H</u>), 7.29 (2H, d, *J* = 8.8 Hz, Ar-<u>H</u>), 7.34 (2H, m, Ar-<u>H</u>), 7.48 (2H, d, *J* = 8.8 Hz, Ar-<u>H</u>), 9.67 (2H, s, 2 x CS-N<u>H</u>); ^{13}C NMR (101 MHz, DMSO-d_6): δ 55.25 (-O<u>C</u>H_3), 113.75 (Ar-<u>C</u>-), 125.31 (Ar-<u>C</u>-), 126.07 (Ar-<u>C</u>-), 128.12 (Ar-<u>C</u>-), 128.23 (-<u>C</u>-N), 131.95 (Ar-<u>C</u>-Cl), 138.62 (-<u>C</u>-N), 156.67 (Ar-<u>C</u>-OCH_3), 179.93 (-<u>C</u>=S).

1-(4-Methoxyphenyl)-3-o-tolylthiourea (5p): White powder; 1.01 g (79%); mp 190-192 °C (lit 195 °C);[6] ESIMS: *m/z* 273.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.22 (3H, s, C<u>H</u>$_3$), 3.72 (3H, s, OC<u>H</u>$_3$), 6.88 (2H, d, *J* = 9.2 Hz, Ar-<u>H</u>), 7.15 (2H, m, Ar-<u>H</u>), 7.22 (2H, m, Ar-<u>H</u>), 7.30 (2H, d, *J* = 8.8 Hz, Ar-<u>H</u>), 9.15 (1H, s, CS-N<u>H</u>), 9.45 (1H, s, CS-N<u>H</u>); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.90 (-<u>C</u>H_3), 55.24 (-O<u>C</u>H_3), 113.70 (Ar-<u>C</u>-), 126.08, 126.25 (Ar-<u>C</u>-), 126.41 (Ar-<u>C</u>-), 128.05 (Ar-<u>C</u>-), 130.32 (Ar-<u>C</u>-), 132.19 (-<u>C</u>-N), 134.82 (-<u>C</u>-N), 137.91 (Ar-<u>C</u>-CH_3), 156.59 (Ar-<u>C</u>-OCH_3), 180.63 (-<u>C</u>=S).

In-vitro CYP450 enzyme inhibition

All CYP enzymes (Sacchrosomes^{TM}; human CYP enzymes bound to yeast microsomal membranes) used in this study were manufactured by CYP Design Ltd (Leicester, UK). This method was used to measure the percentage inhibition of a CYP450 by a compound or to determine the IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited) of a compound. Both percentage inhibition and IC_{50} values effectively reflect the inhibitory potential of a compound and hint at the possible effectiveness of a compound in a biological process. Percentage inhibition is determined at a particular

concentration of the compound which is usually 10 µM. An assay which determines IC_{50} values includes the yeast microsomes that bear the cytochrome P450 enzymes (i.e. Sacchrosomes™), a chosen chemical compound in six serial dilutions in DMSO (with DMSO concentration never exceeding 0.5%), 96-well flat-bottomed microtitre plate, substrates such as ER (7-ethoxyresorufin) or CEC (3-cyano-7-ethoxycoumarin) or EOMCC (7-ethoxy-methyloxy-3-cyanocoumarin) or DBF (dibenzylfluorescein), depending on the CYP450 used in the assay. The substrates form fluorescent compounds upon CYP metabolism. A fluorescent plate reader is used to monitor fluorescence emitted which ultimately determines IC_{50} values via measurement of fluorescence units at each endpoint (i.e. at each concentration of compound used).

A typical CYP450 end point assay, for inhibition of CYP1B1

Regenerating system consists of: 5 µl Solution A (183 mg of $NADP^+$ + 183 mg of glucose-6-phosphate + 654 µl of 1.0 M magnesium chloride solution + 9.15 ml of sterile ultra-pure water) + 1 µl Solution B (250 Units of glucose-6-phosphate dehydrogenase + 6.25 ml of 5 mM sodium citrate; mixed in a tube and made up to 10 ml with sterile ultra-pure water) + 39 µl 0.2 M phosphate buffer (KPi; 0.6 ml of 1.0M K2HPO4 + 9.4 ml of 1.0 M KH_2PO_4 mixed and made up to 50 ml with sterile ultra-pure water) + 5 µl potential inhibitory compound. Enzyme system consists of: 0.5 µl CYP1B1 (0.5 pmoles; CYP Design Ltd) + 1.7 µl control protein (denatured proteins from yeast cells that do not contain recombinant CYP450 proteins) + 5 µl 0.1 mM 7-ER (7-ethoxyresorufin substrate) + 42.8 µl 0.1M Kpi (0.3 ml of 1.0 M K_2HPO_4 + 4.7 ml of 1.0 M KH_2PO_4 were mixed and made up to 50 ml with sterile ultra-pure water. The assay is performed using (a) sensitivity (Gain): 65/70/75 of the Biotek Synergy plate reader (this would differ from one instrument to the other) and (b) Filter: 530/590 nm that monitors fluorescence excitation/ emission of resorufin, the metabolite of 7-ethoxyresorufin substrate (ER); the excitation/ emission differs with the substrate that is used. Similar assays were performed with Sacchrosomes™ bearing the other human CYPs using appropriate fluorescent substrates, as detailed above.

Procedure for IC_{50} determination using Sacchrosomes™

The plate reader (BioTek) was warmed at 37°C. Compounds were serially diluted to six different concentrations with 10% DMSO in a Sero-Wel white microplate. Serial dilutions were made with a dilution factor of 1:20. 45 µl of regenerating system was prepared and pre-warmed at 37°C, as detailed in Table S1.

Table S1. The constitution of the regenerating system used per reaction in each single well for different CYPs was as follows.

Enzyme	Solution A	Solution B	Inhibitor	KPi buffers	water
CYP1A1	5 µl	1 µl	5 µl	39 µl 0.2 M	-
CYP1B1	5 µl	1 µl	5 µl	39 µl 0.2 M	-
CYP1A2	5 µl	1 µl	5 µl	20 µl 0.5 M	19 µl
CYP2D6	5 µl	1 µl	5 µl	25 µl 0.2 M	14 µl
CYP3A4	5 µl	1 µl	5 µl	25 µl 0.2 M	14 µl

Meanwhile, 50 µl of enzyme substrate mix reaction was prepared and incubated at 37°C for 10 min (Table S2).

Table S2. The constitution of enzyme-substrate mixtures was as follows.

Enzyme	P450 conc. in Sacchrosomes™	Control Microsome	Substrate	KPi buffers	water
CYP1A1	0.5 µl (0.5 pmole)	2 µl	5 µl 0.1 mM E.R.	42.5 µl 0.1 M	-
CYP1B1	0.5 µl (0.5 pmole)	1.7 µl	5 µl 0.1 mM E.R.	42.8 µl 0.1 M	-
CYP1A2	1 µl (1 pmole)	1.6 µl	5 µl 320 µM CEC	42.4 µl 0.1 M	-
CYP2D6	2.5 µl (2.5 pmole)	0.4 µl	0.5 µl 2 mM EOMCC	25 µl 0.2 M	21.6 µl
CYP3A4	1.1 µl (1 pmole)	10.102 µl	0.1 µl 2 mM	25 µl 0.2 M	23.96 µl

In wells of a black 96-well flat-bottomed microplate, 45 µl of regenerating system, 5 µl serial dilutions of inhibitor were pipetted out from the dilution plate and then 50 µl of enzyme/substrate was added except in control well (positive control); for this well, instead of inhibitor 5 µl of 10% DMSO was added. In the background well (negative control), only 45 µl regenerating system and 5 µl 10% DMSO were added with no enzyme; the microplate was then vortexed for a few seconds. The microplate was incubated for 10 min.

which was followed by addition of 75 µl of Tris-acetonitrile to all wells, using an eight-channel multi-pipette, to stop the reaction; after that 50 µl of enzyme/substrate reaction was added into the ‘ negative control’ well. The plate was left to shake for 10 sec and the fluorescence units for each endpoint were monitored at appropriate settings (for assay parameters and plate layout) selected on the KC4 software of the BioTek plate reader.

Calculation of IC_{50} values

To calculate IC_{50} values, a series of dose-response data, for example, drug concentrations (x_1, x_2, ...,x_n) at which specific growth inhibition occurs (y_1, y_2, ...,y_n) were generated. The values of y were in the range of 0-1. The simplest estimate of IC_{50} is to plot x-y and fit the data with a straight line (via linear regression). IC_{50} values are then estimated using the fitted line, i.e.

$Y = a * X + b,$

$IC_{50} = (0.5 - b)/a.$

Raw data was imported and computed in Microsoft Excel. The maximum change in relative fluorescence units (RFU) relative to positive control with 0.5% DMSO was calculated. The enzyme inhibition was plotted using sigmoidal curve (4 parameter variable slope equation) and half inhibitory concentration (IC_{50}) values were analysed statistically using Graph-Pad Prism Software (Version 6.0).

Transfection of mammalian expression plasmids that encode human *CYP1A1* & *CYP1B1* genes isolated from a human liver cDNA library in HEK293 cells grown in suspension cells

HEK293 ‘suspension’ cells (1 x 10^6 per mL), obtained from CYP Design Ltd, were counted using improved Neubauer counting chamber and the cell viability (≥ 90% viability) was determined using trypan blue dye exclusion. Actively dividing suspension cells in log phase were seeded in appropriate volumes in Erlenmeyer flask (Corning #431143) and incubated at 37°C, 8% CO_2 and shaken at 130 rpm on an orbital shaker (Panasonic). Before transfection, the mammalian expression plasmids (pcDNA3.1/CYP1B1 and pcDNA3.1/CYP1A1) containing human *CYP1A1* and *CYP1B1* genes (isolated from a human liver cDNA library) were propagated in *E. coli* DH5α, grown in LB medium in presence of ampicillin (50 µg/mL). The endotoxin-free plasmids were prepared using Zymo PURE™ Plasmid Maxiprep Kit as per manufacturer’s

instructions (#D4202, Zymo Pure). The quantity and purity of plasmid DNA ($A_{260/280} \geq$ 1.9) was determined by Bio Spectrophotometer (Eppendorf). The quality of plasmids DNA was determined using 1% agarose gel electrophoresis.

To initiate transfection, the respective plasmid DNA – cationic lipid complexes were prepared as per manufacturer's instructions (Invitrogen #16447-100) in OptiPRO SFM reduced serum medium (Invitrogen #12309-09). Further, the aseptic preparation of DNA-lipid complexes was added slowly to the respective flasks containing HEK293 suspension cells. The negative control was prepared by adding OptiPRO SFM reduced serum media without plasmid DNA. The suspension cells were incubated at 37°C and checked for optimal expression of CYP enzymes at regular intervals. 24 to 48 h post transfection, the cells were counted and the cell viability was determined. The transfected cells in sufficient volumes were spun at 200 x g for 5 minutes. The supernatant was discarded and the cells were washed once with pre-warmed phosphate buffered saline. The cells were once again spun at 200 x g for 5 min at room temperature and the supernatant was discarded. The cells were gently re-suspended in pre-warmed growth media to obtain cell density 4 x 10^6 cells per mL for CYP1B1 and 2 x 10^6 cells per mL for CYP1A1 transfected HEK293 cells, respectively.

Screening of potential CYP inhibitors using recombinant human live cells

For screening of potential compounds, recombinant HEK293 cells (100 - 200 x 10^3 cells per well) expressing CYP enzymes were seeded in 50 µL volume in triplicates in black 96-well plates with transparent bottom (Corning #3904). The test compounds either at single point concentrations (10 µM) or at various concentrations (ranging from 1 nM to 30 µM) for determination of IC_{50} values were added in 25 µL volume to the wells followed by incubation at 37°C, 8% CO_2 for 30 min. After incubation, the fluorogenic substrate 7-ethoxyresorufin was added at 5 µM in 25 µL to the wells and contents were mixed homogenously by shaking to perform the 7-ethoxyresorufin-O-deethylase (EROD) assay. The plate was read on a 96 well plate-reader (Biotek, Synergy HT) for 60 min using suitable wavelengths for emission (530/30) and excitation (590/40) of fluorescence. IC50 values were calculated as described above using Graph-Pad Prism Software (Version 6.0).

Growth of yeast strains for expression of CYP1B1 enzyme

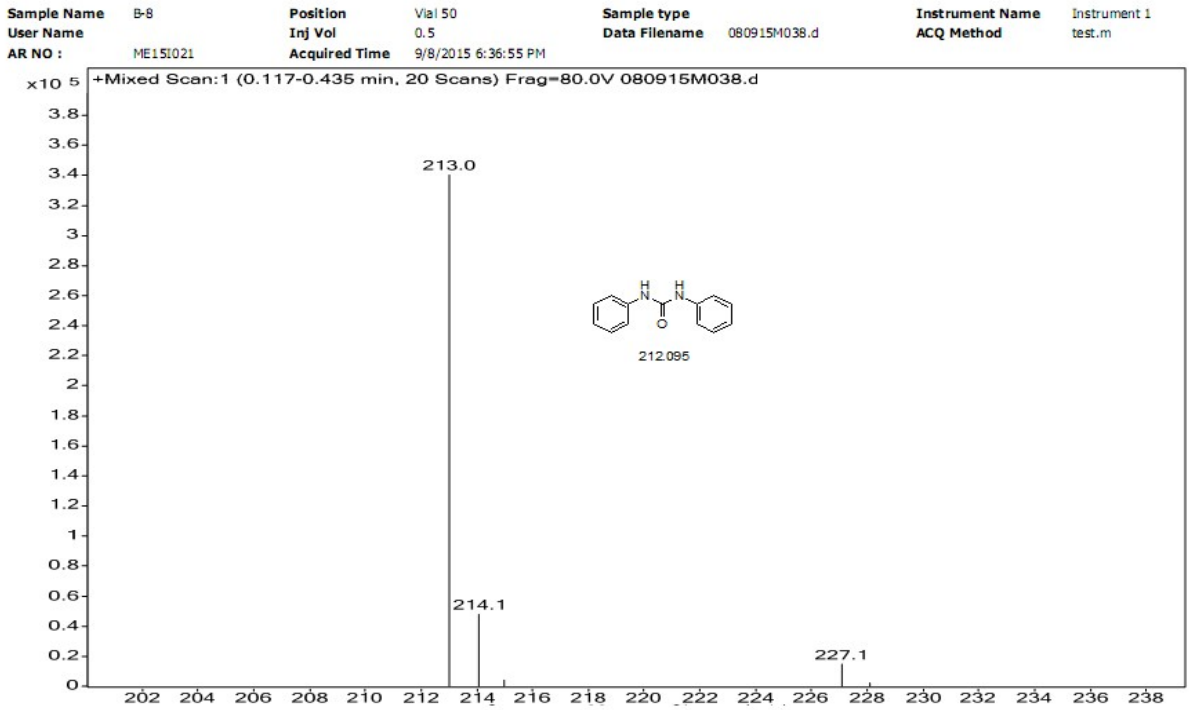
A yeast strain (CYP Design Ltd), harbouring a human *CYP1B1* gene expression cassette, was streaked out from a glycerol stock and grown on SD-minimal medium agar plates with the required supplements, at 30ºC for 3 days. Single colonies were then picked and grown, at first, as pre-cultures in minimal medium and then grown in full YPD medium for expression of the CYP1B1 enzyme.

Determination of IC_{50} values using recombinant yeast live cells

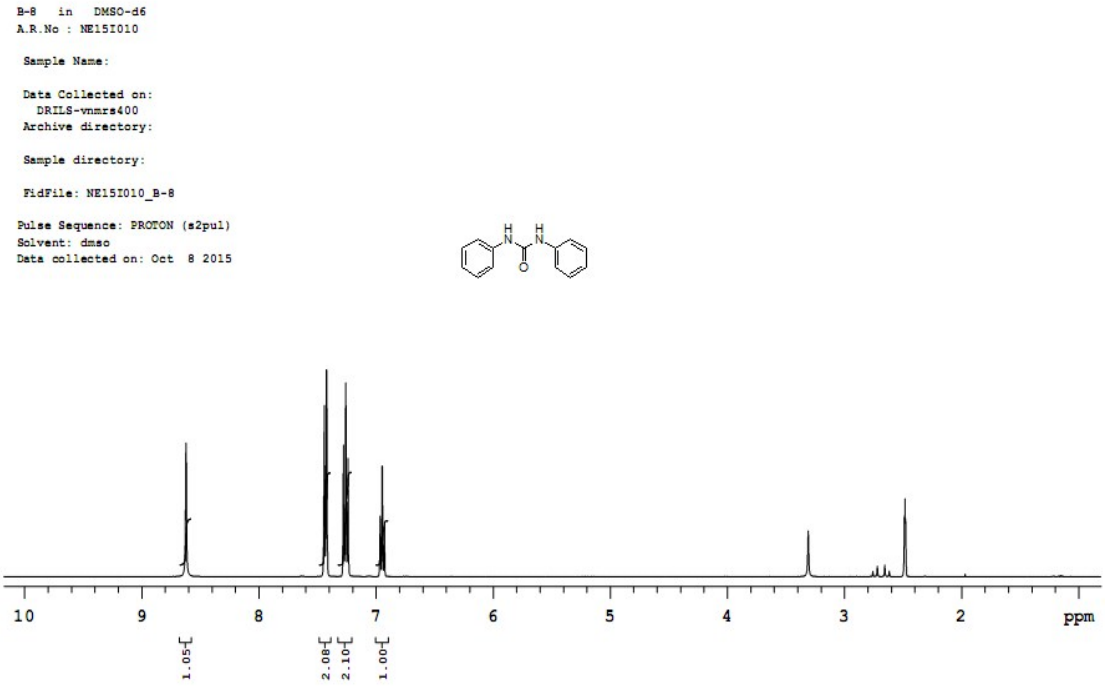
For IC_{50} determinations, 4×10^8 cells (equivalent to approximately 25 OD_{600}) were taken from the exponential growth phase, approximately 16-20 h after induction of CYP1B1 protein. These cells, enough for 100 assays, were aliquoted appropriately into eppendorf tubes. Cells were centrifuged on a bench top microfuge for 30 sec at 13,000 rpm (15.7 g). The supernatants were removed carefully so as to not dislodge the pellet. The cell pellets were then re-suspended in 650 µl of TE buffer (50 mM Tris-HCl pH. 7.4, 1 mM EDTA). The cells were diluted 1:10 before carrying out an IC_{50} determination using a protocol similar to the determination of IC_{50}s in Sacchrosomes™. IC50 values were calculated as described above using Graph-Pad Prism Software (Version 6.0).

Molecular modelling: The human CYP family of enzymes are oxidoreductases involved in the metabolism of xenobiotics, mainly hydroxylation of unreactive carbon atoms in aromatic and aliphatic rings or aliphatic chains. The crystal structures of CYP enzymes were retrieved from the protein data bank: CYP1A1 (PDB ID: 4I8V),[7] CYP1B1 (PDB ID:3PMO)[8], CYP1A2 (PDB ID:2HI4)[9], CYP3A4 (PDB ID: 4NY4)[10] and CYP2D6 (PDB ID: 4WNT)[11]. The structures were subjected to protein preparation wizard facility under default conditions implemented in Maestro v9.0 and Impact program v5.5 (Schrodinger, Inc., New York, NY, 2009). The prepared protein was further utilized to construct grid file by selecting co-crystallized ligand as centroid of grid box. For standardization of molecular docking procedure co-crystallized ligands such as ANF (CYP1A1, CYP1B1 and CYP1A2), ajmalicine (CYP2D6) and bromocriptine (CYP3A4) were extracted from prepared enzyme-ligand complexes and re-docked The rest of the chemical structures were sketched, minimized and docked using GLIDE XP. The ligand-protein complexes were minimized using macromodel. In order to determine selectivities, the corresponding binding sites of CYP enzymes 1A1, 1A2, 2D6 and 3A4 were aligned and analysed with respect to CYP1B1.

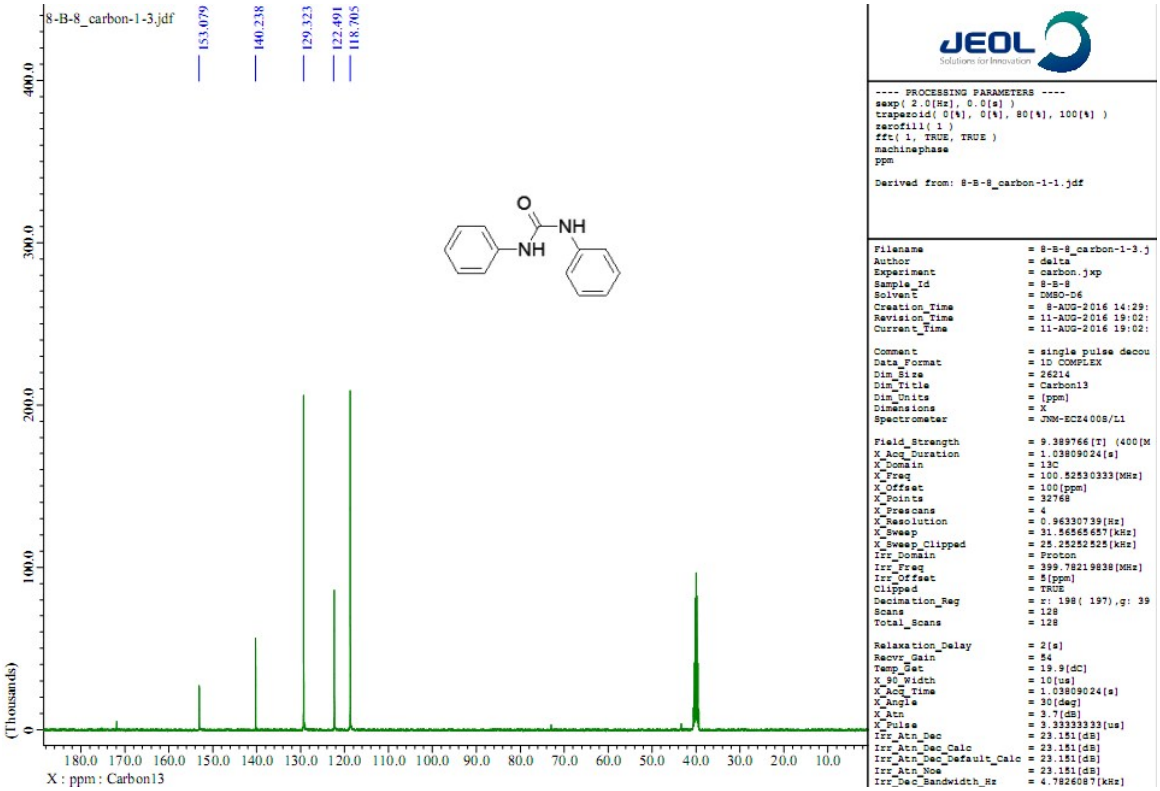
S2. Scanned Spectra:



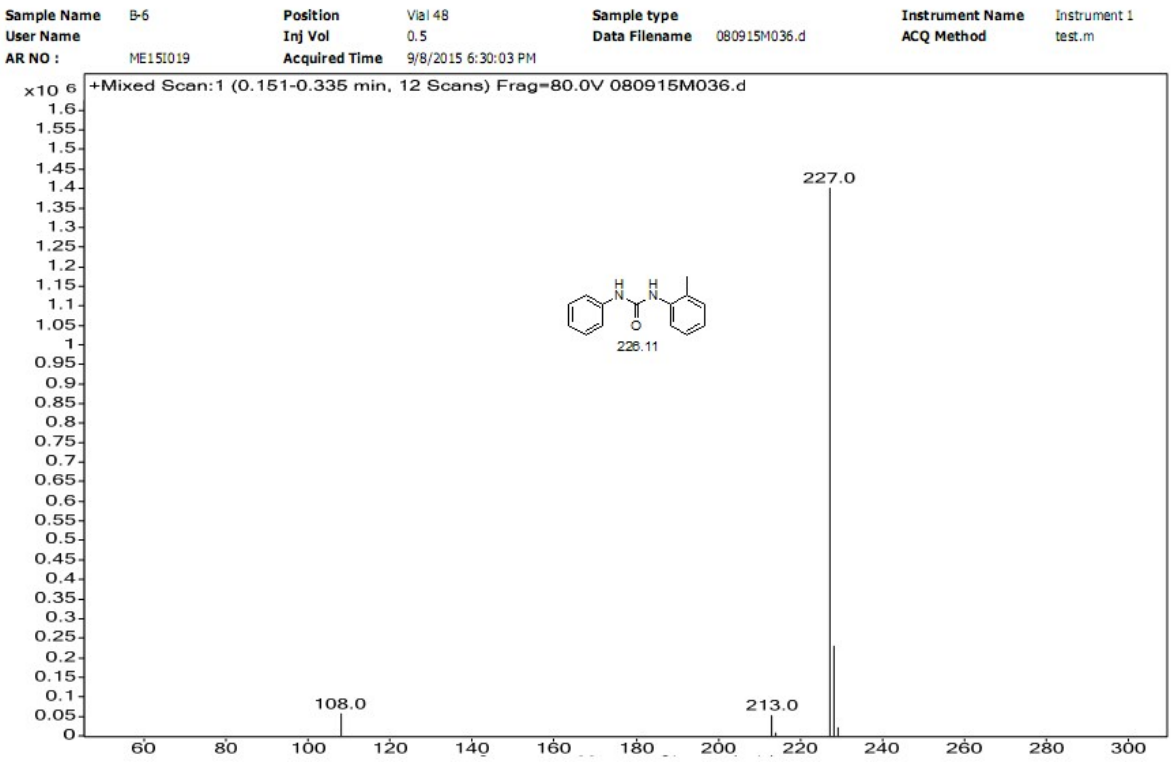
S2.1: ESI+ Mass spectrum of compound 5a



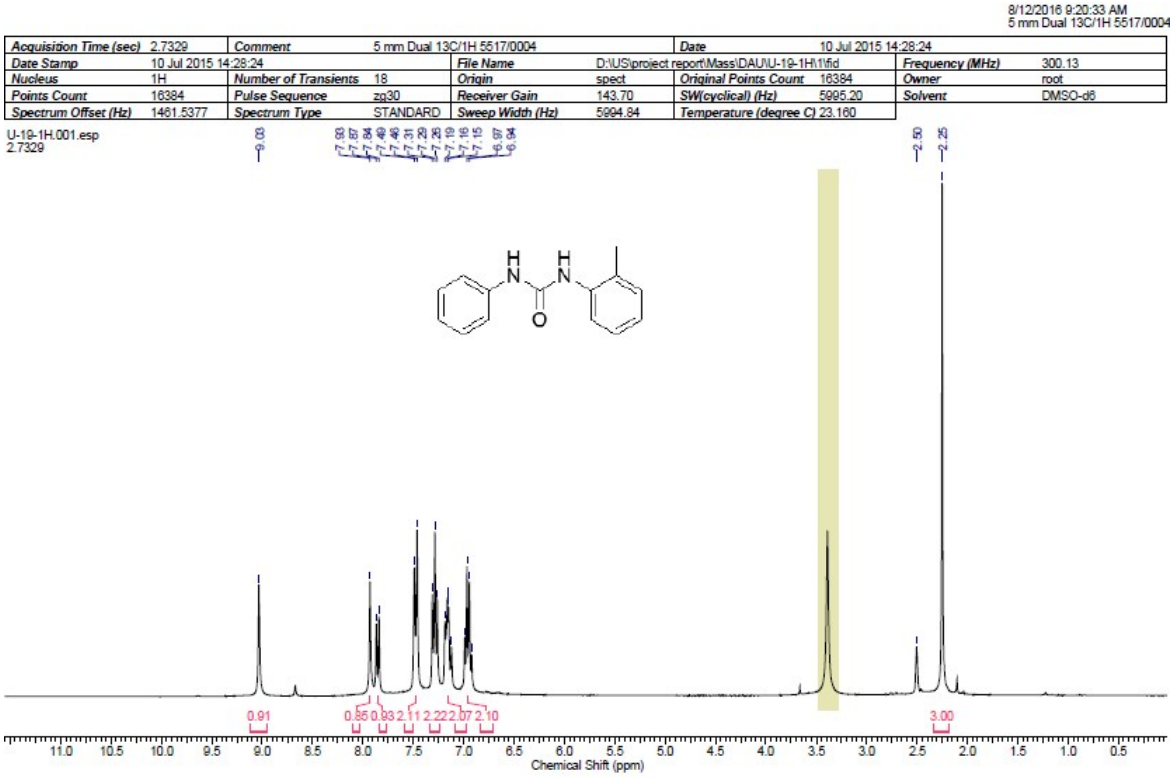
S2.2: 400 MHz 1H-NMR spectrum of compound 5a



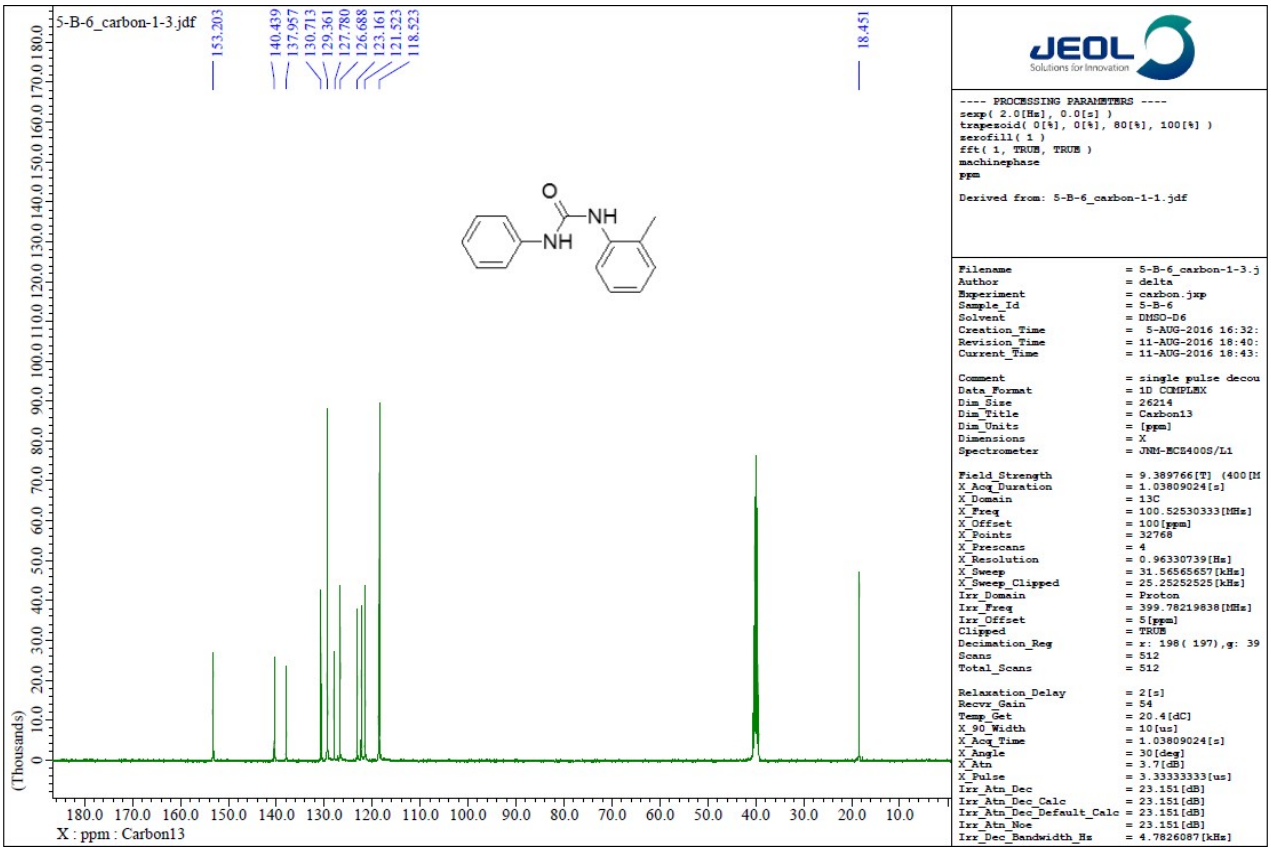
S2.3: 13C NMR Spectrum of compound 5a



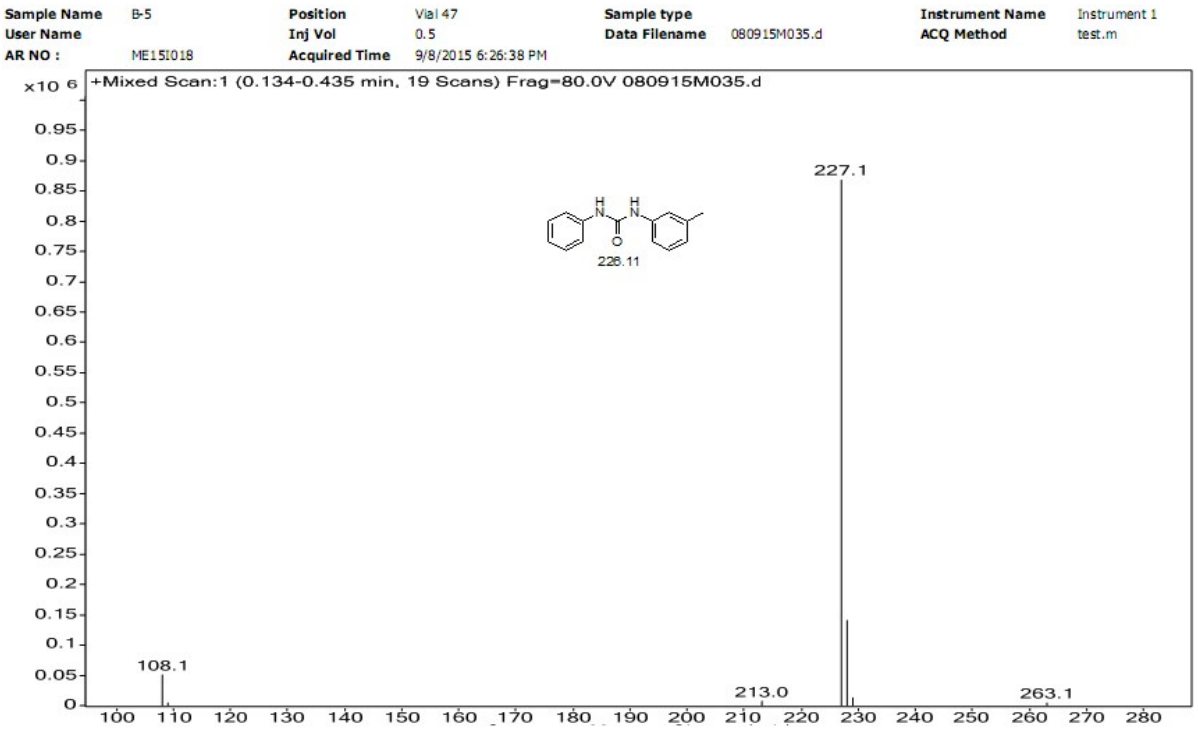
S2.4: ESI⁺ Mass spectrum of compound 5b



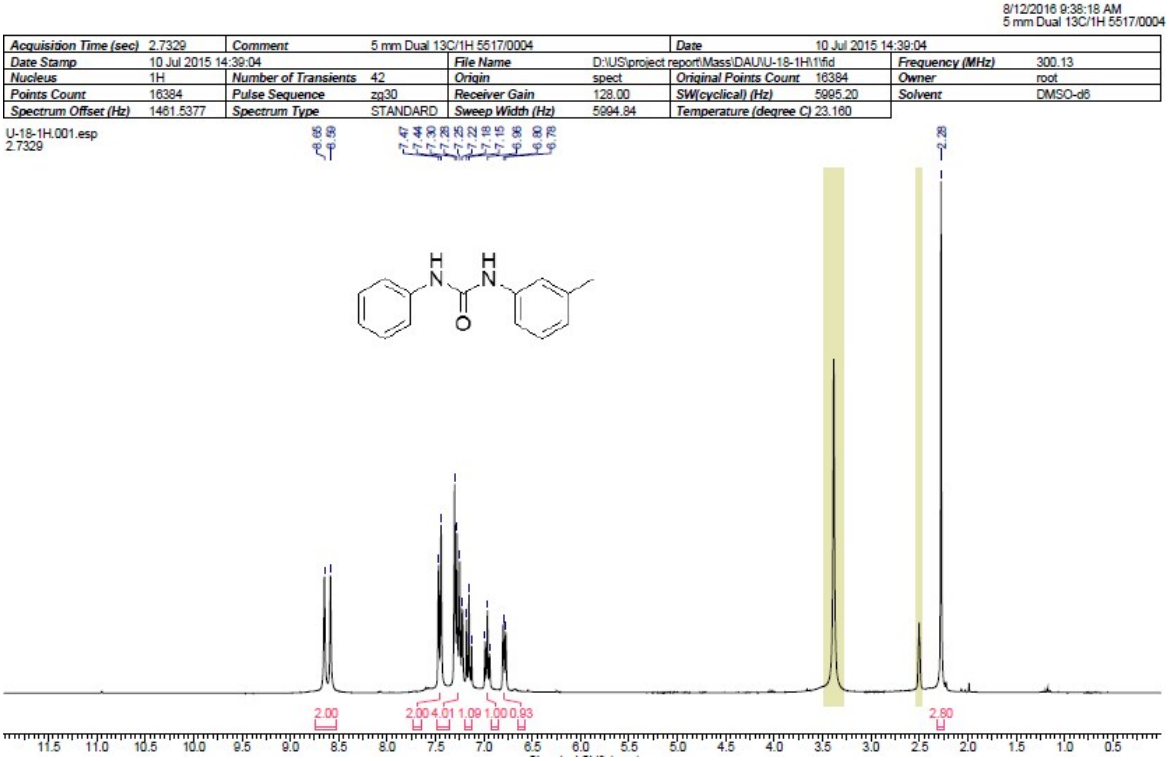
S2.5: 300 MHz 1H-NMR spectrum of compound 5b



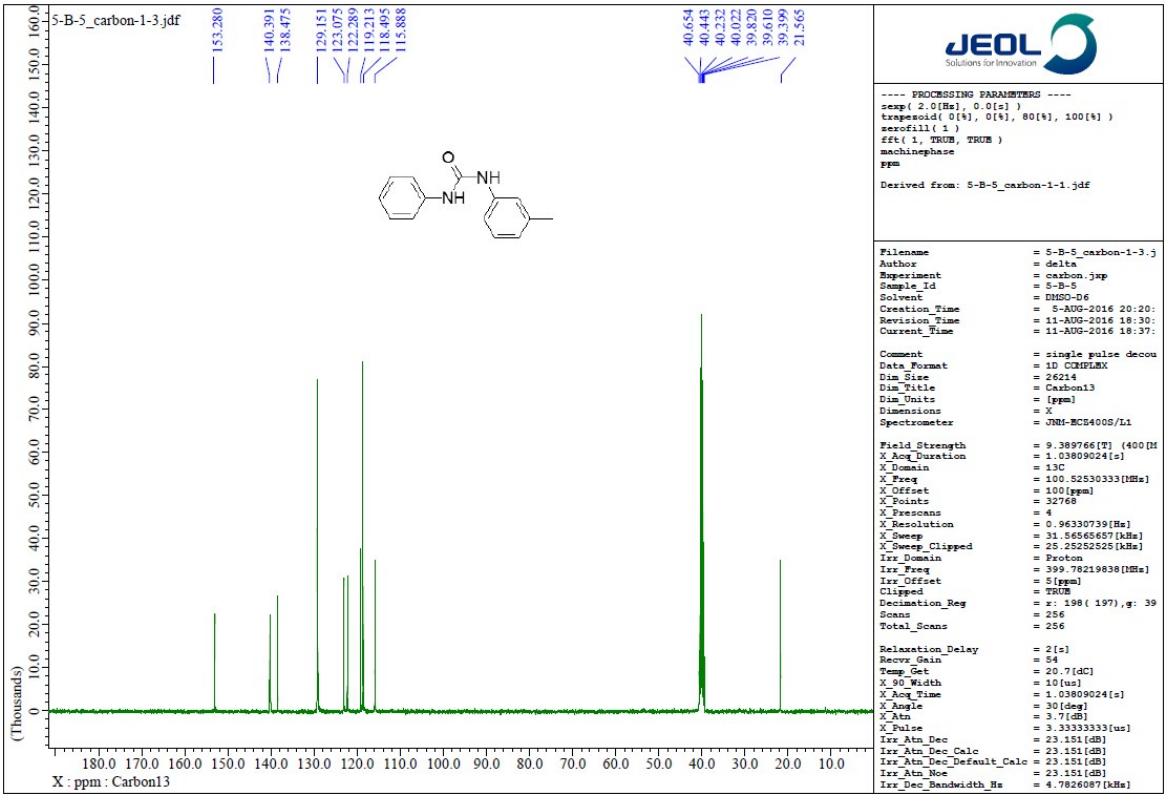
S2.6: 13C NMR Spectrum of compound 5b



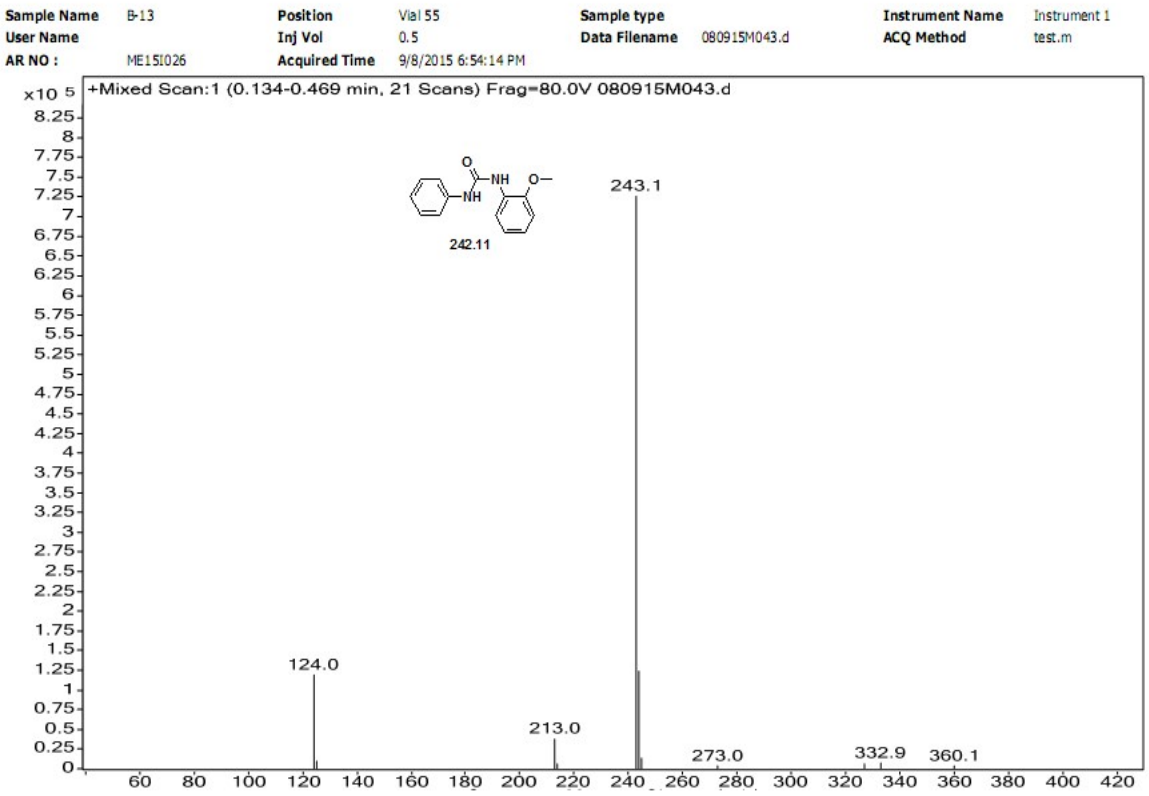
S2.7: ESI+ Mass spectrum of compound 5c



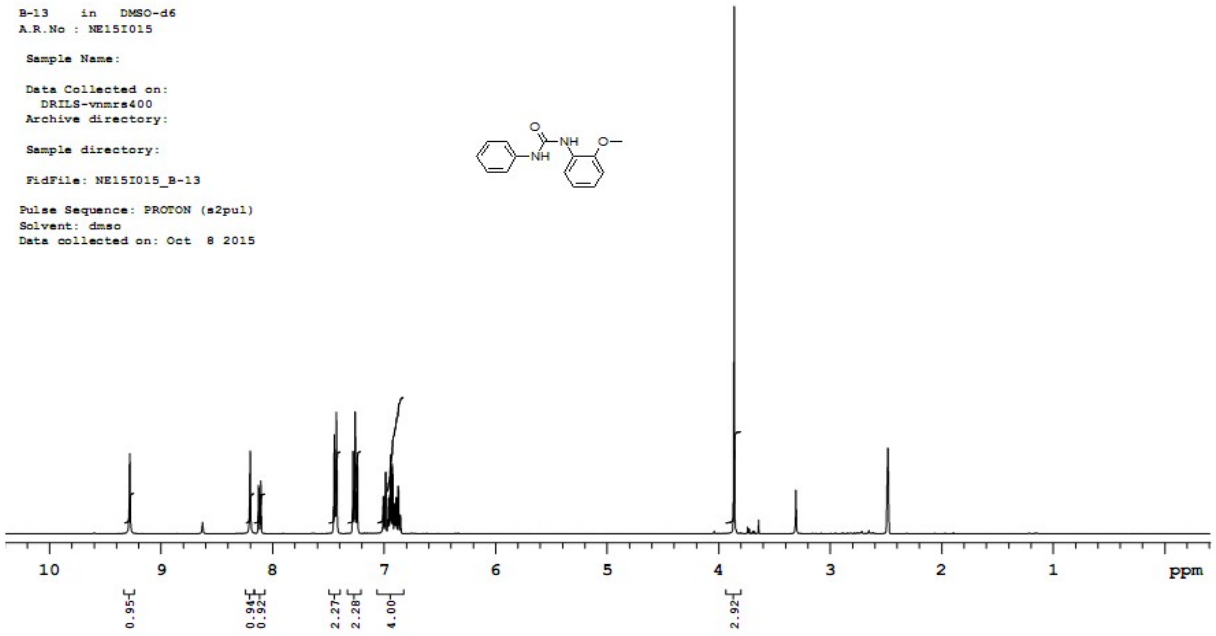
S2.8: 300 MHz 1H-NMR spectrum of compound 5c



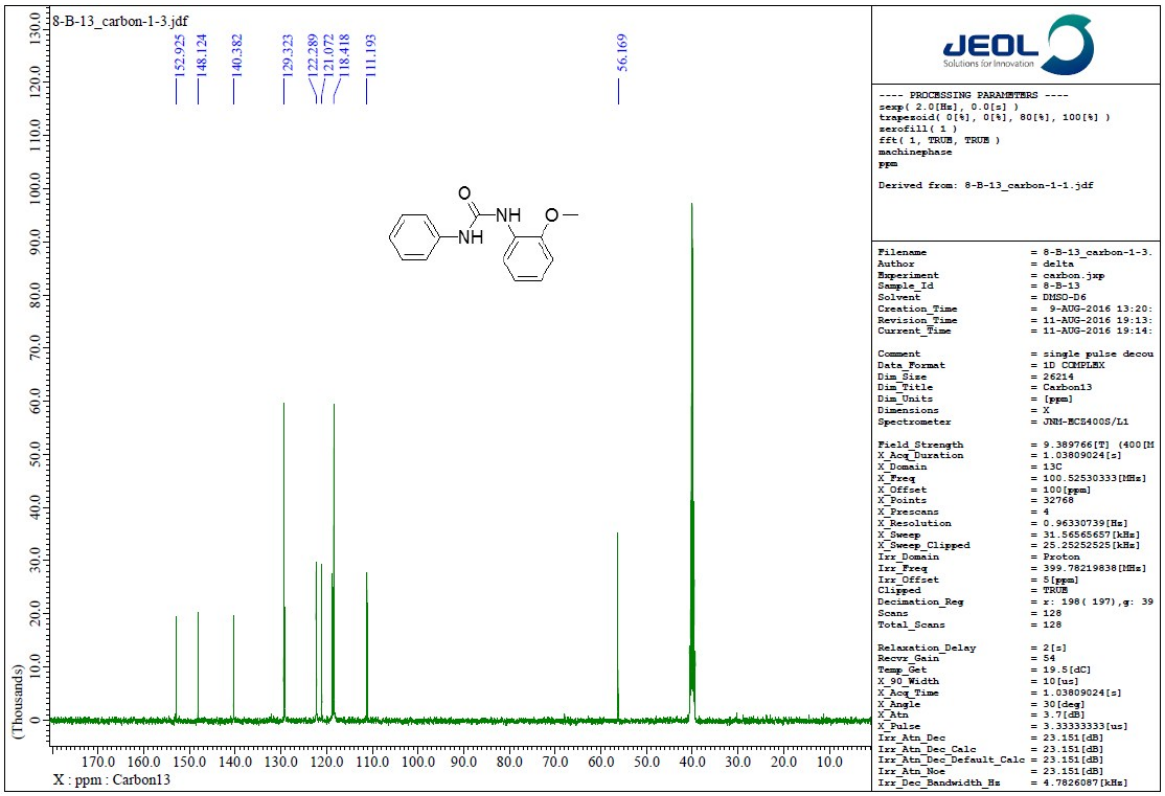
S2.9: 13C NMR Spectrum of compound 5c



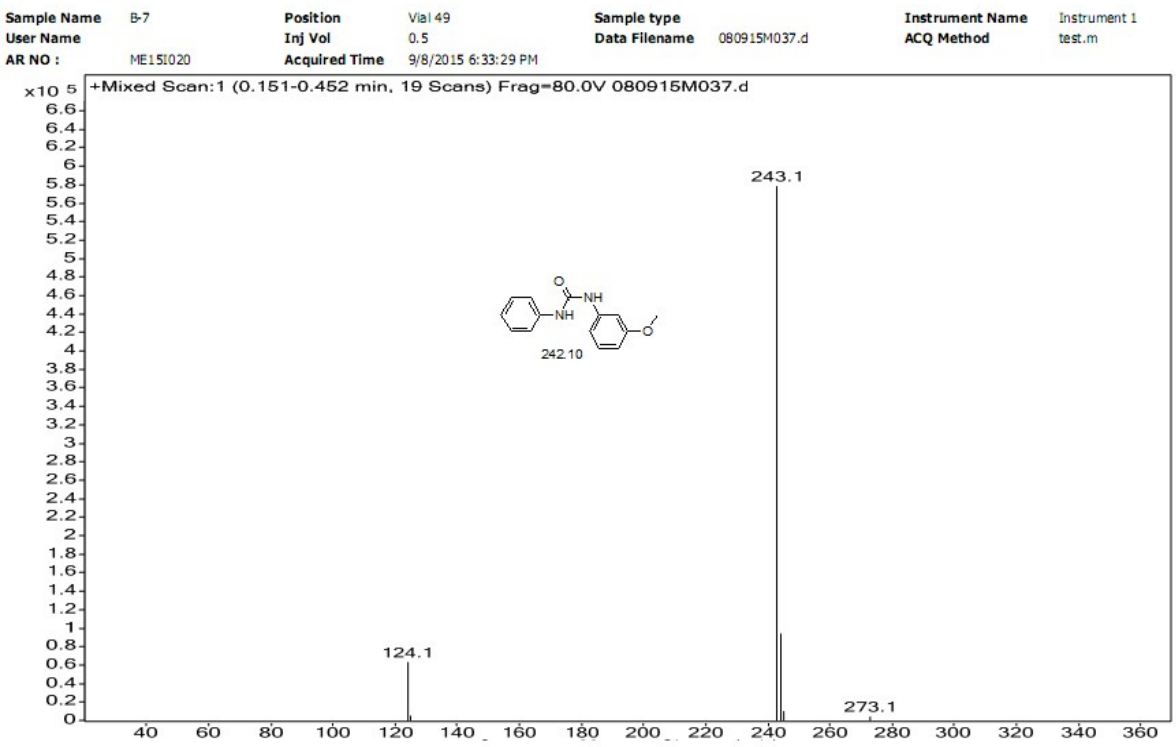
S2.10: ESI+ Mass spectrum of compound 5d



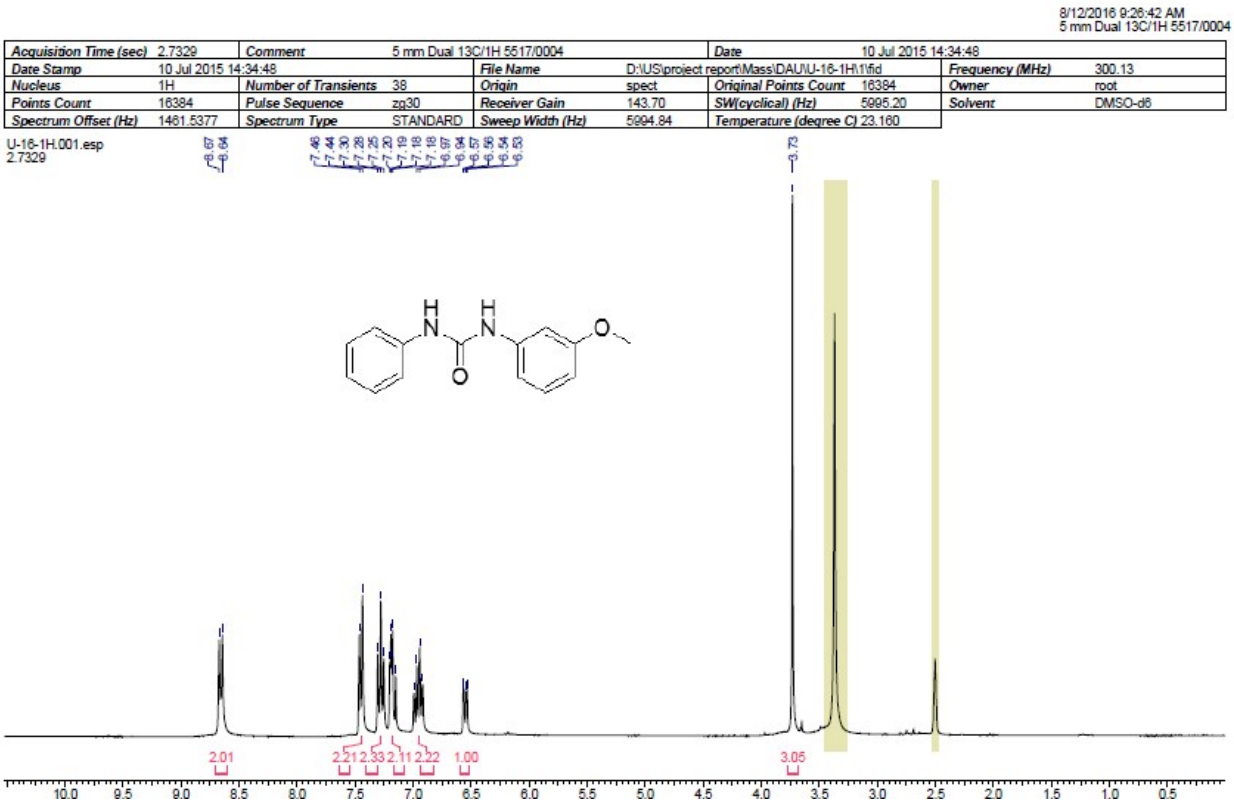
S2.11: 400 MHz 1H-NMR spectrum of compound 5d



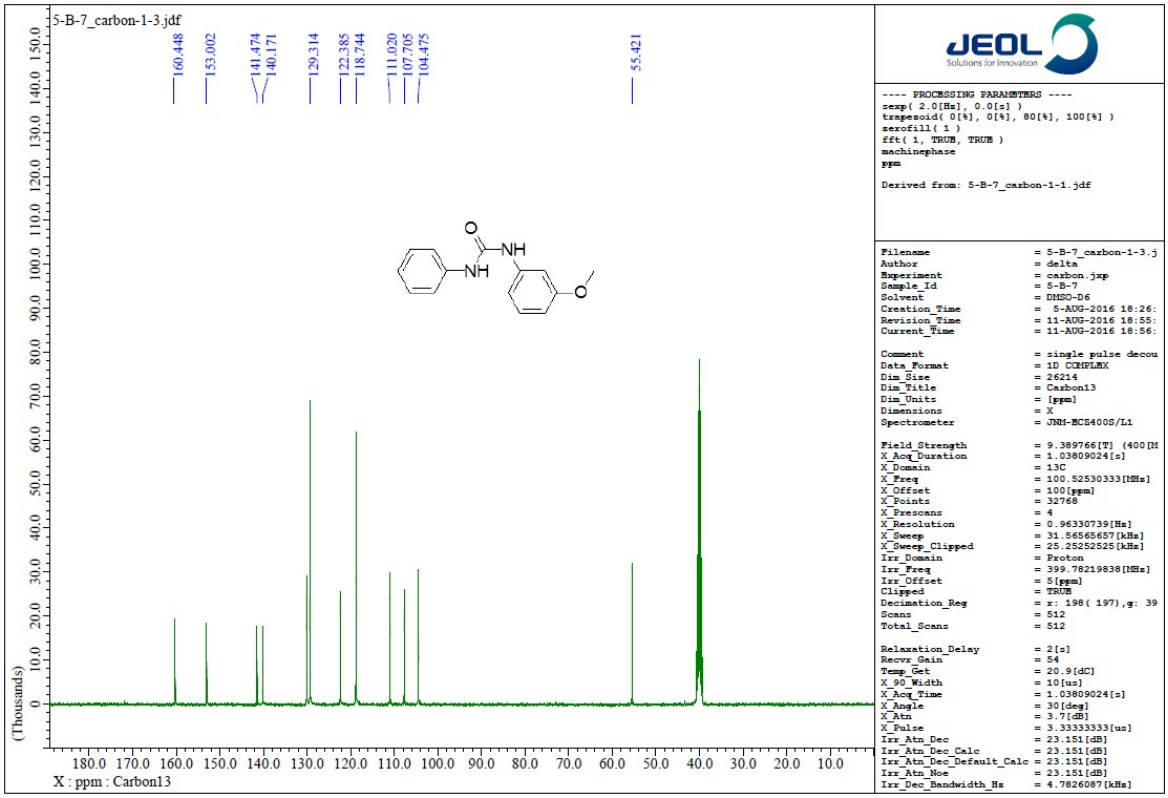
S2.12: ^{13}C NMR Spectrum of compound 5d



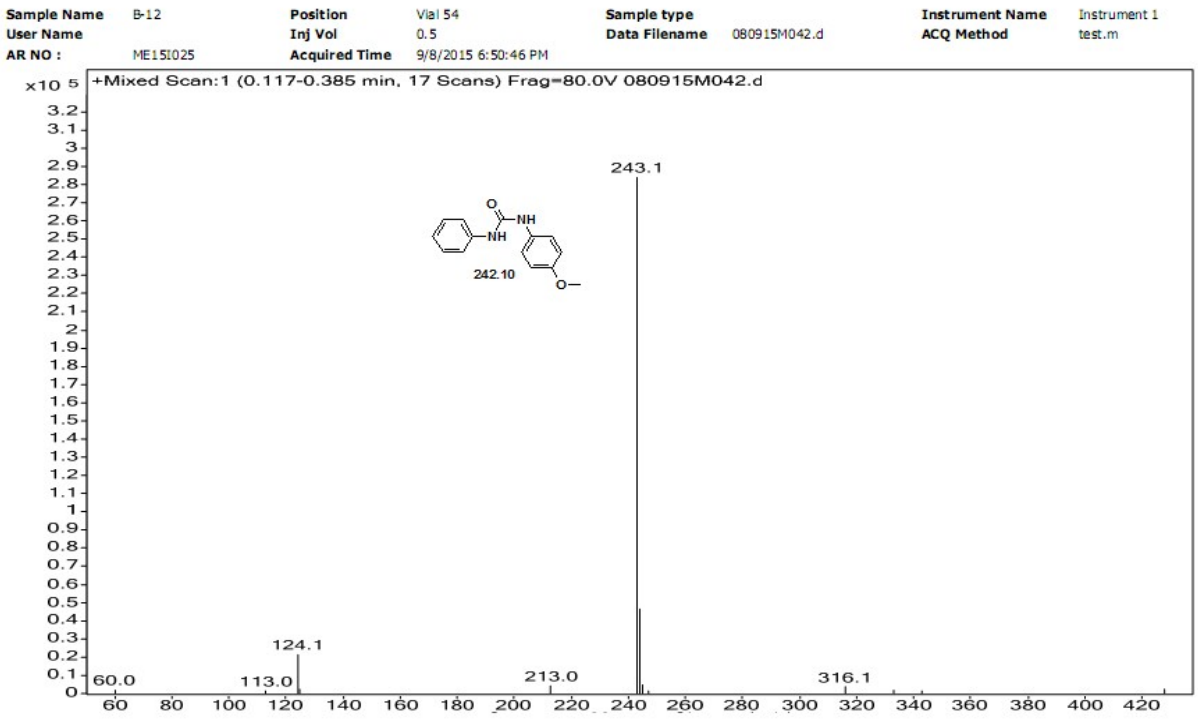
S2.13: ESI+ Mass spectrum of compound 5e



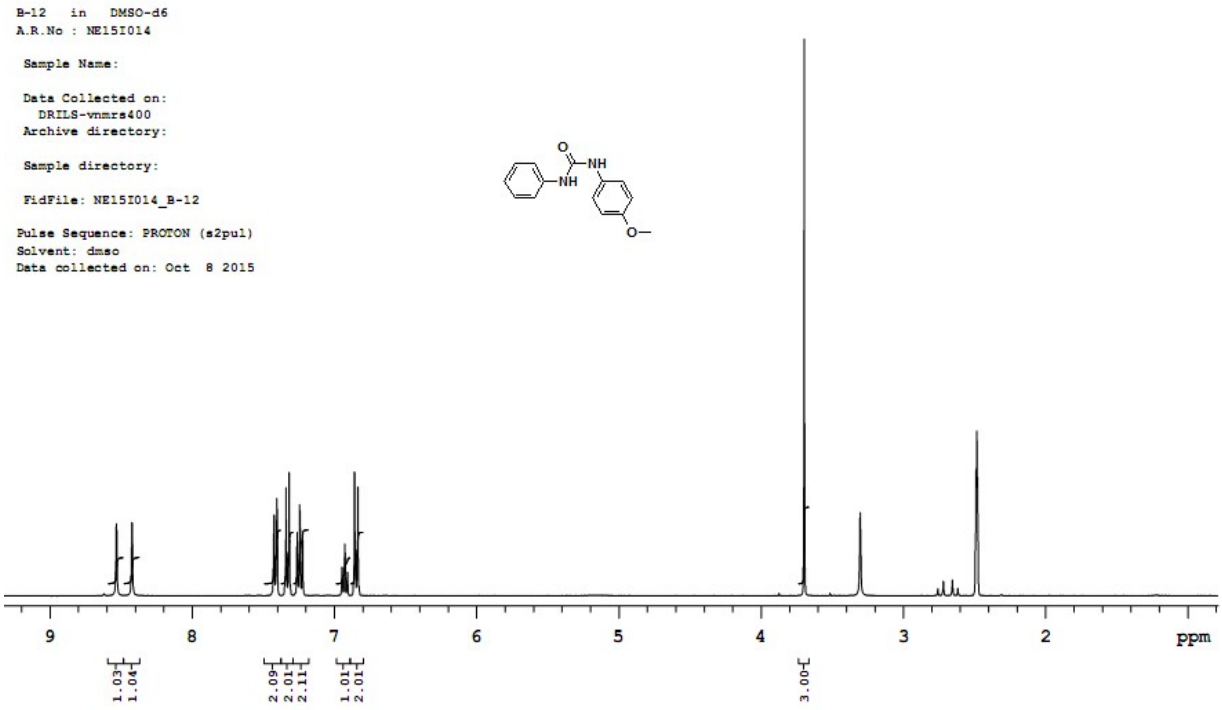
S2.14: 300 MHz 1H-NMR spectrum of compound 5e



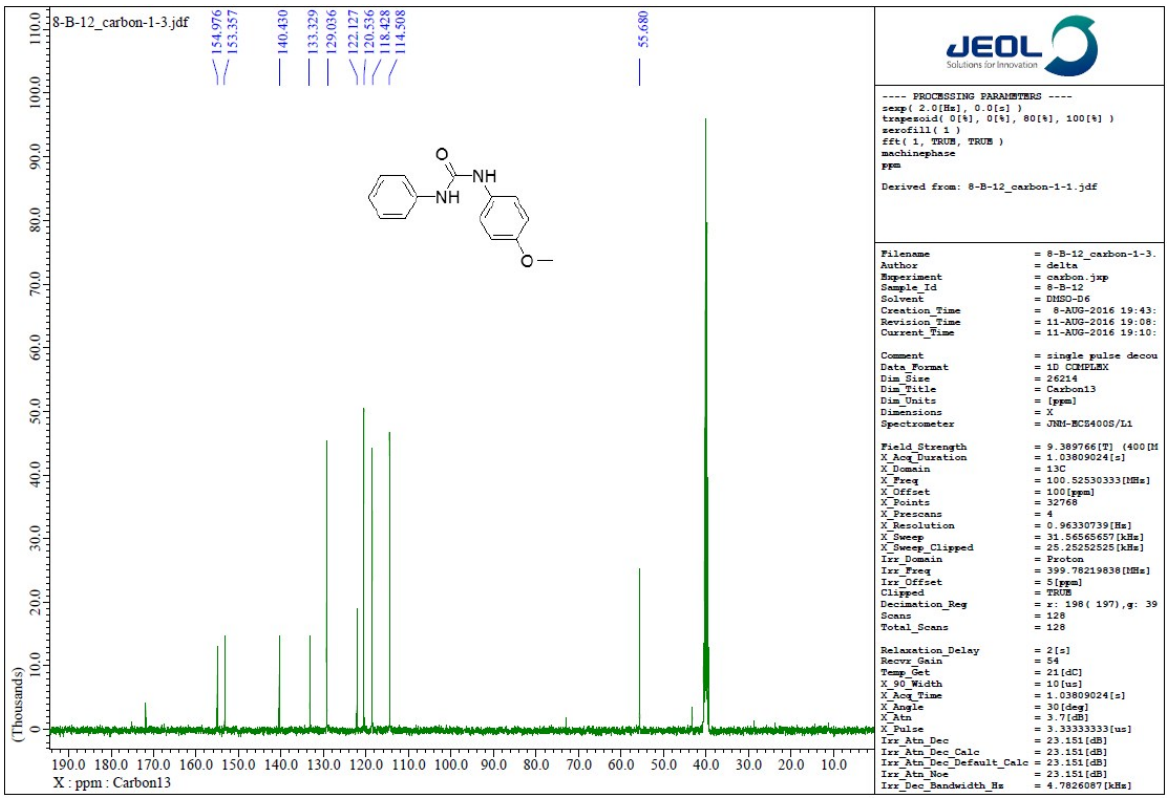
S2.15: 13C NMR Spectrum of compound 5e



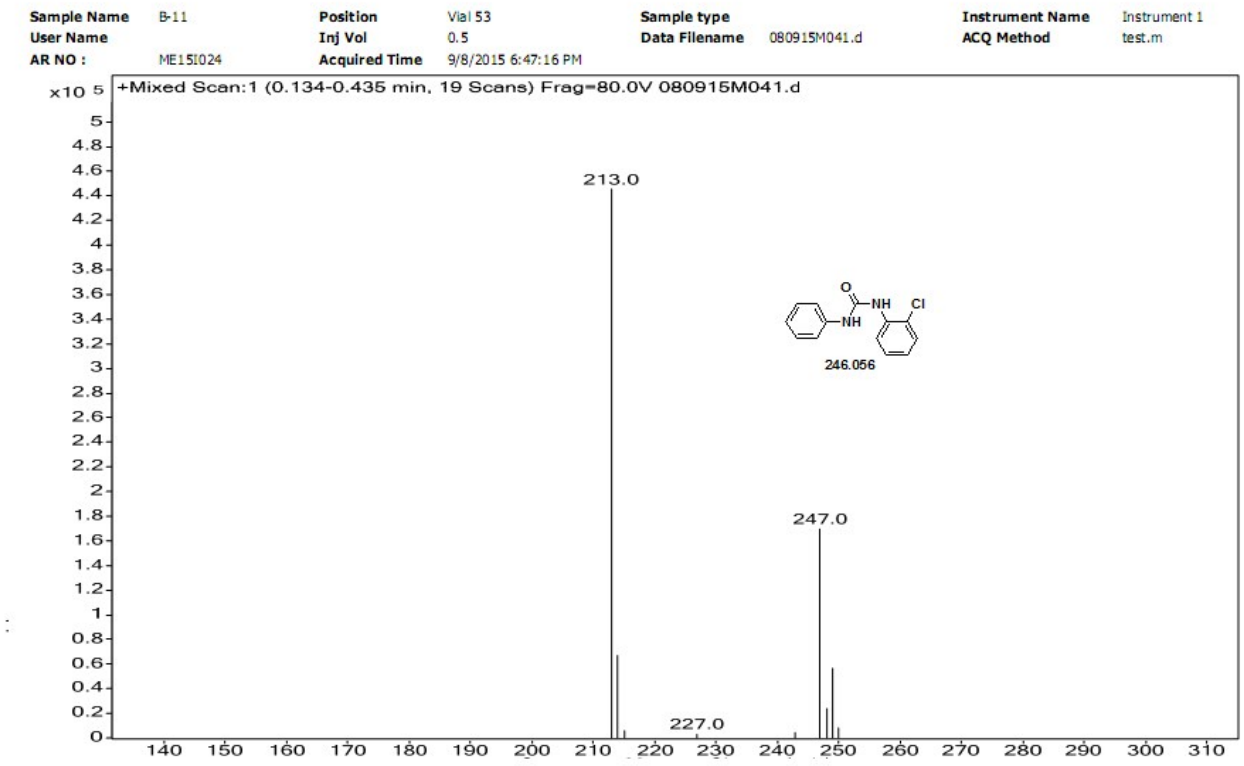
S2.16: ESI+ Mass spectrum of compound 5f



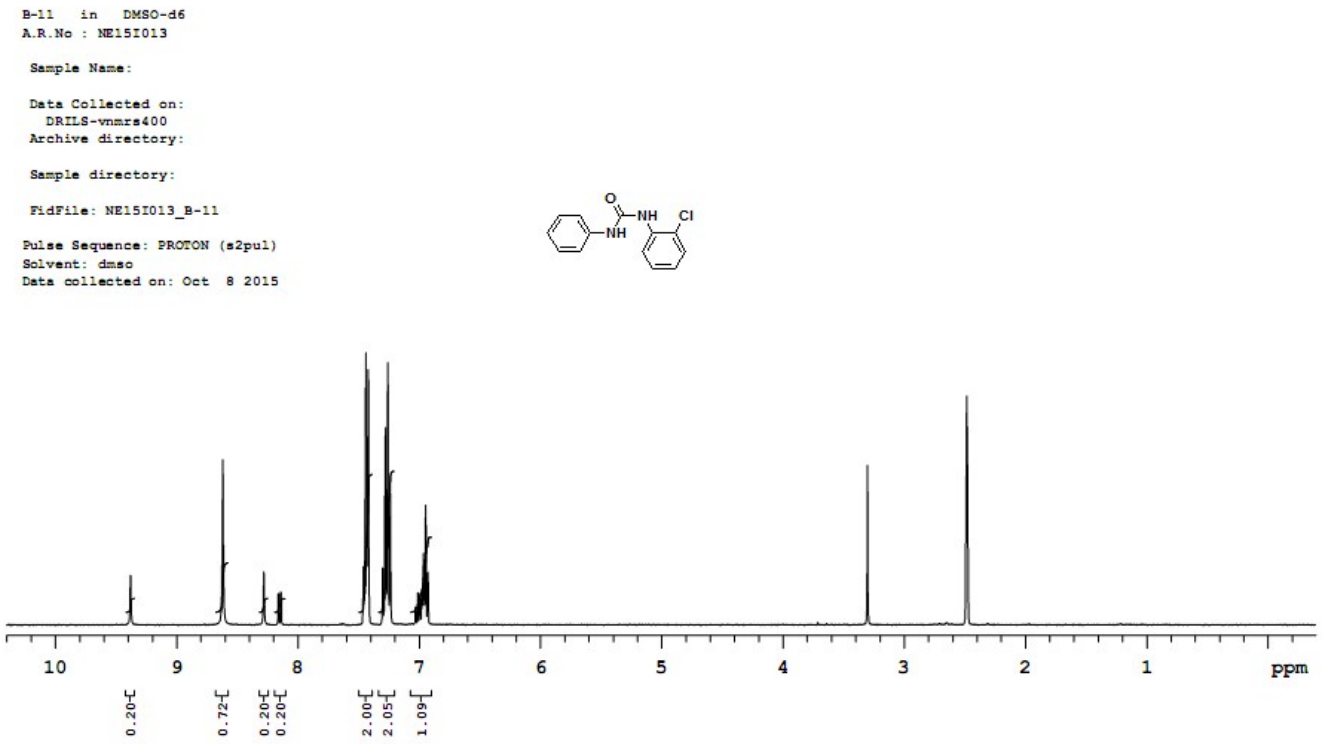
S2.17: 400 MHz ^{1}H-NMR spectrum of compound 5f



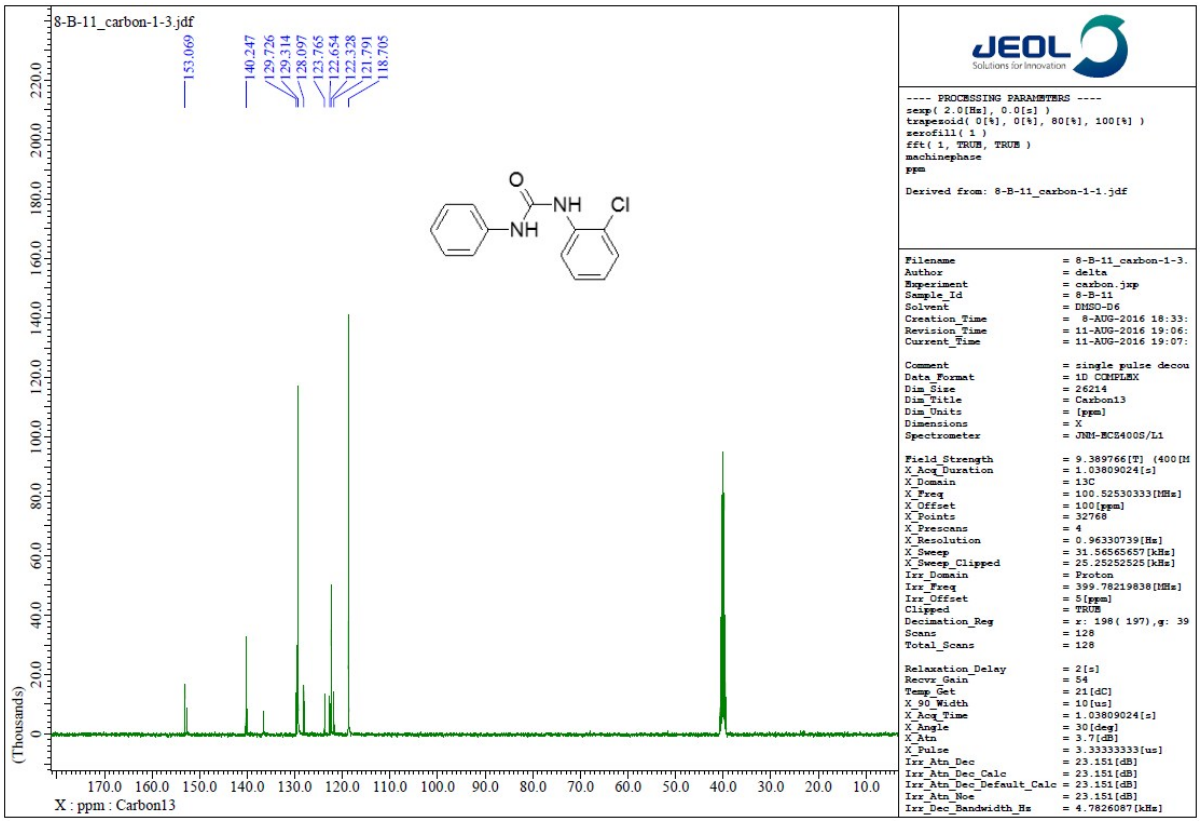
S2.18: 13C NMR Spectrum of compound 5f



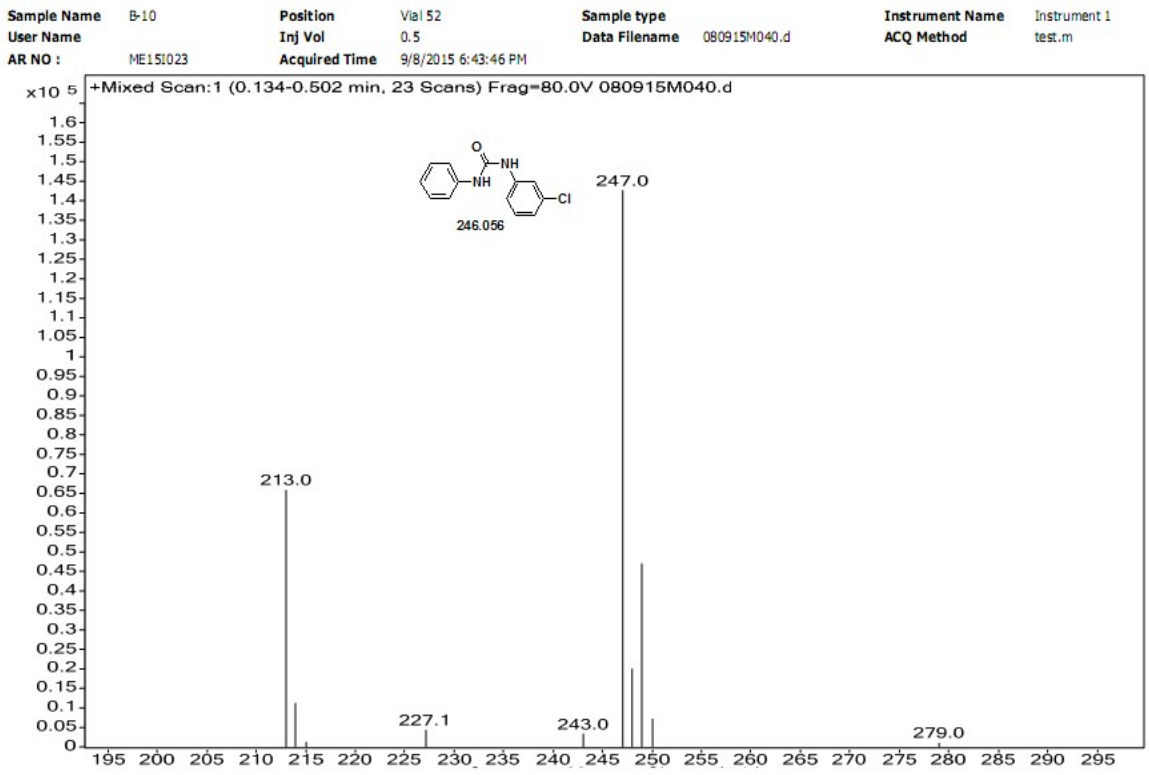
S2.19: ESI^{+} Mass spectrum of compound 5g



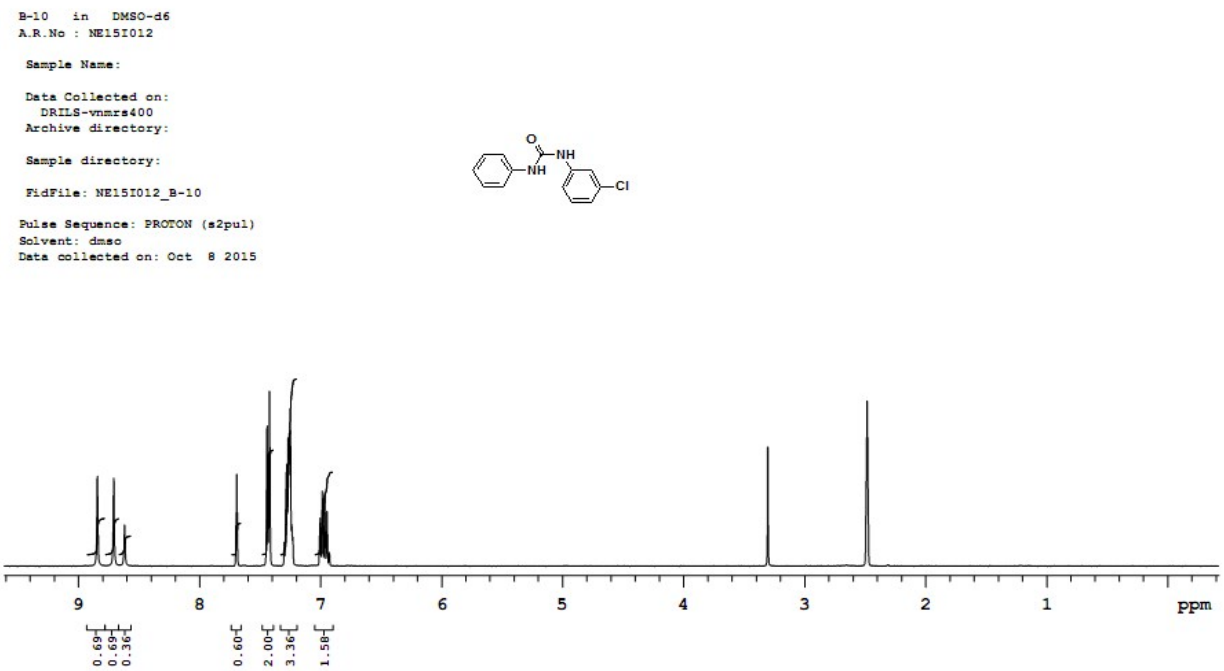
S2.20: 400 MHz 1H-NMR spectrum of compound 5g



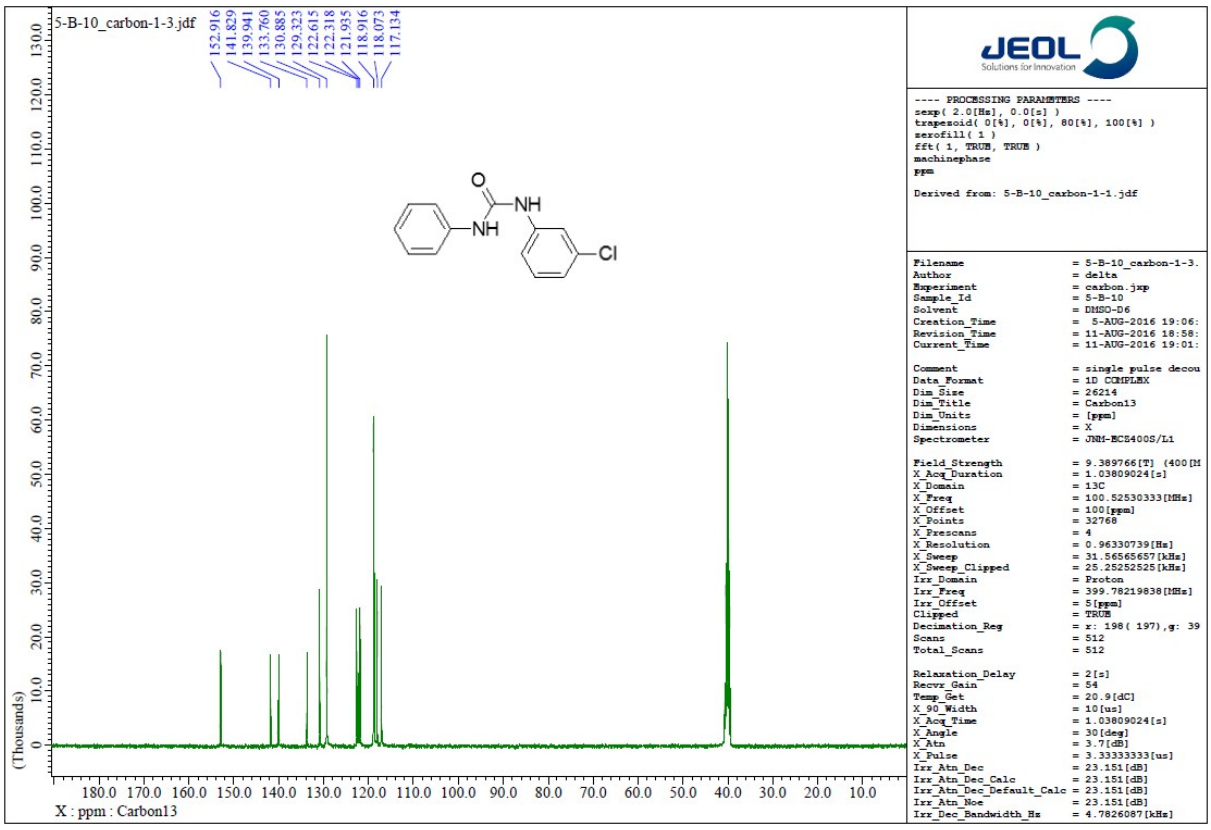
S2.21: 13C NMR Spectrum of compound 5g



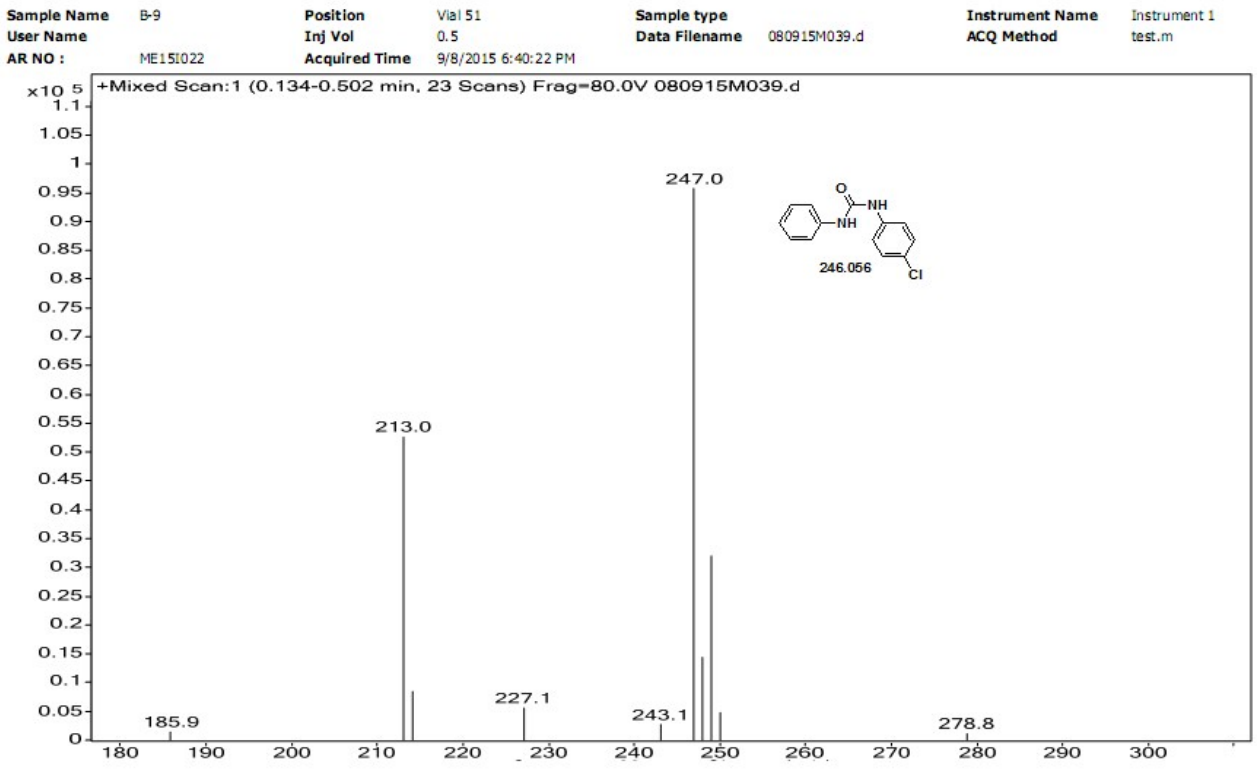
S2.22: ESI$^+$ Mass spectrum of compound 5h



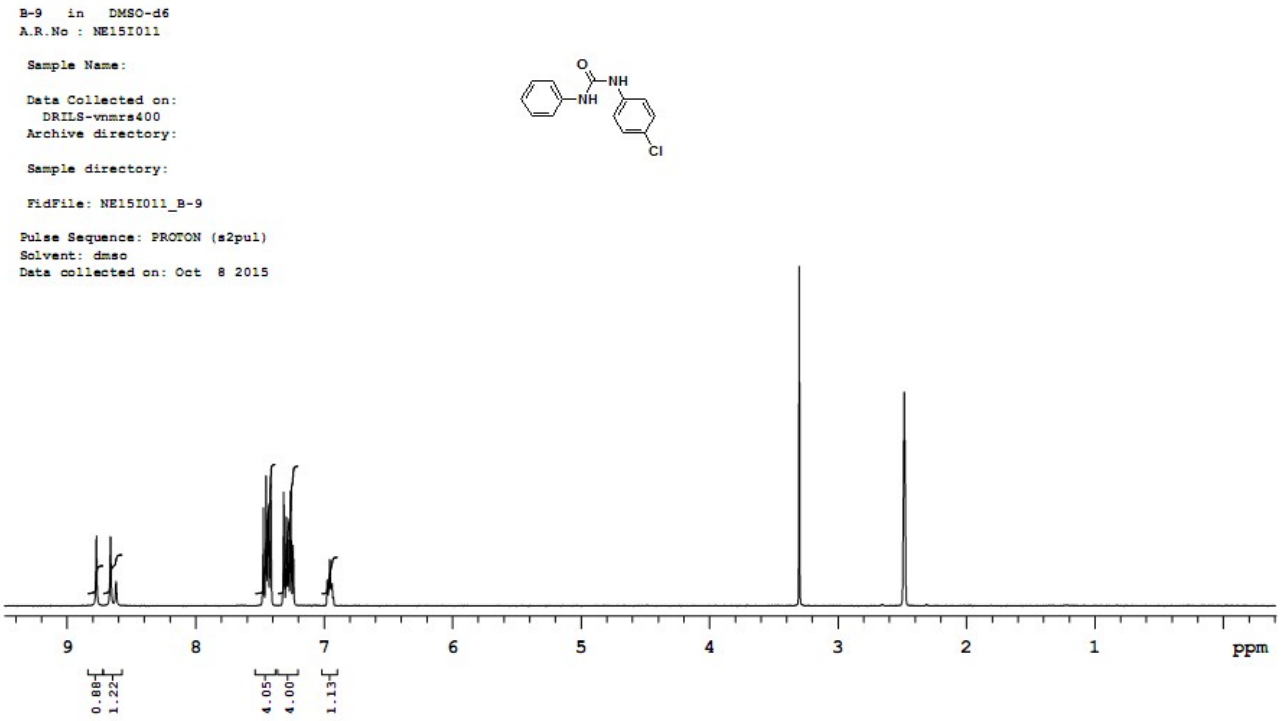
S2.23: 400 MHz ^{1}H-NMR spectrum of compound 5h



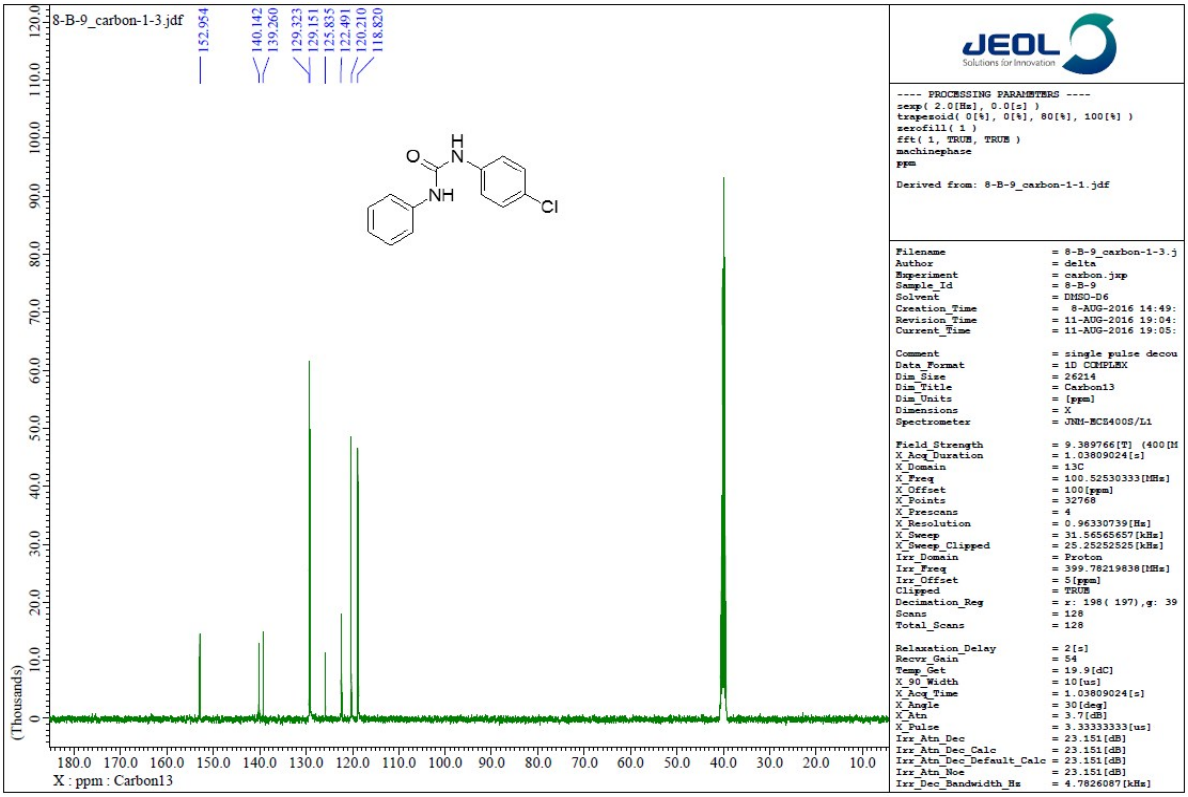
S2.24: 13C NMR Spectrum of compound 5h



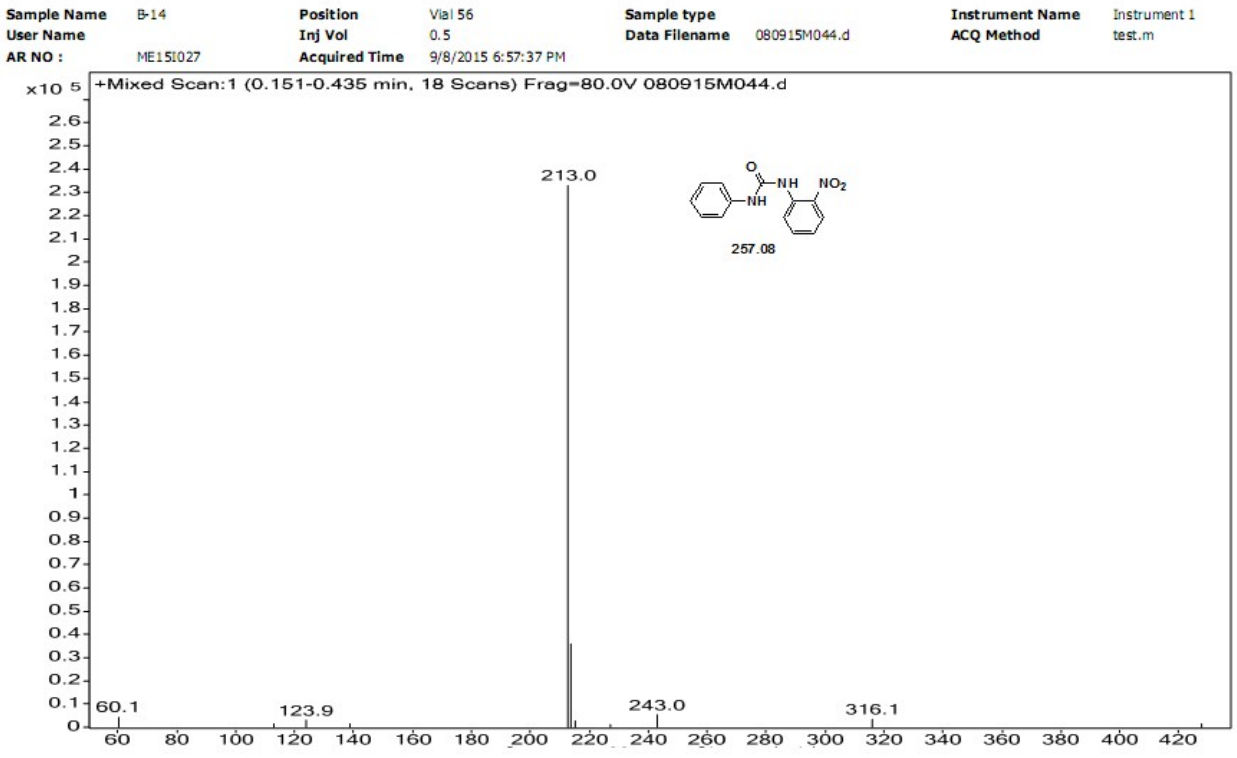
S2.25: ESI$^+$ Mass spectrum of compound 5i



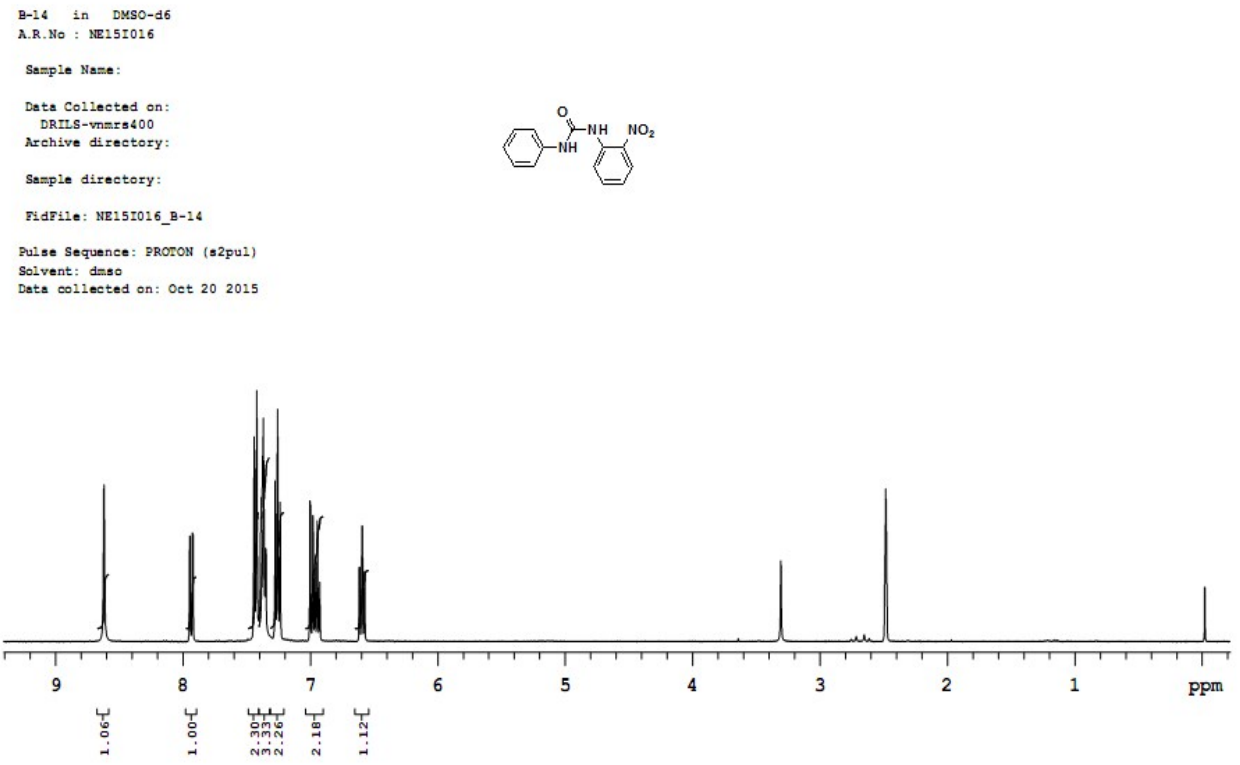
S2.26: 400 MHz ^{1}H-NMR spectrum of compound 5i



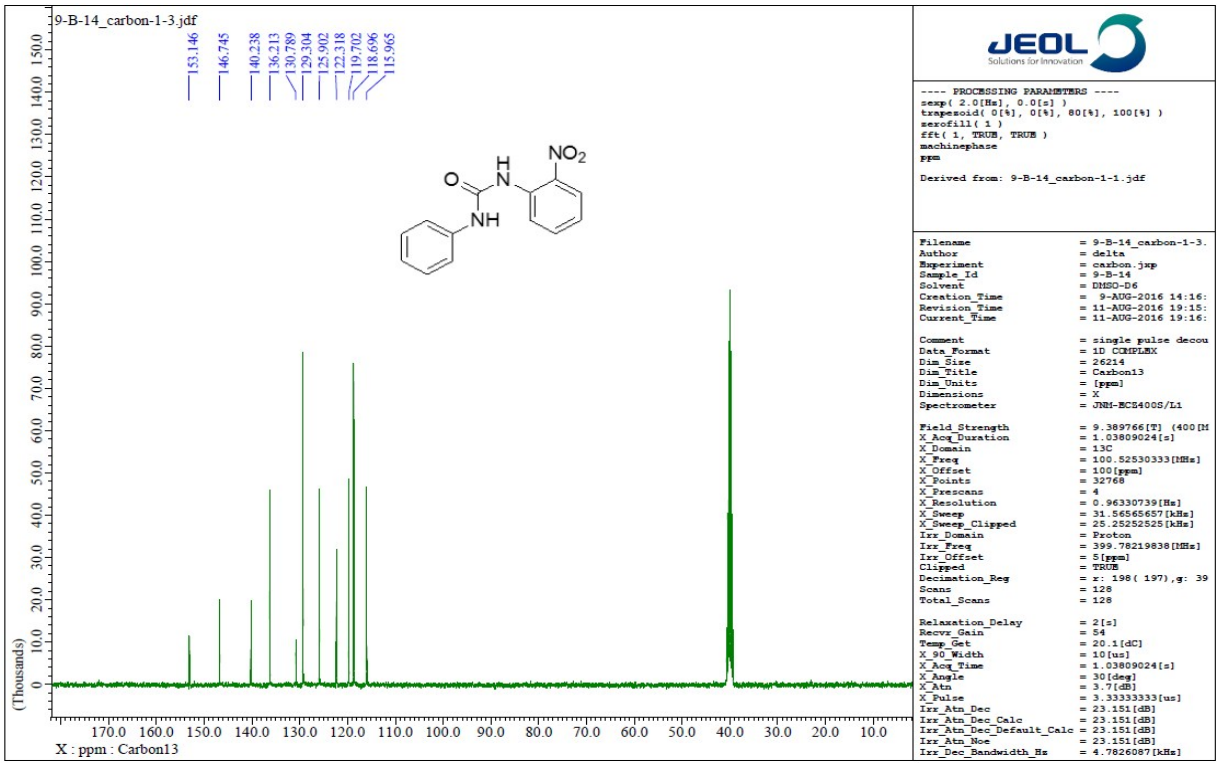
S2.27: 13C NMR Spectrum of compound 5i



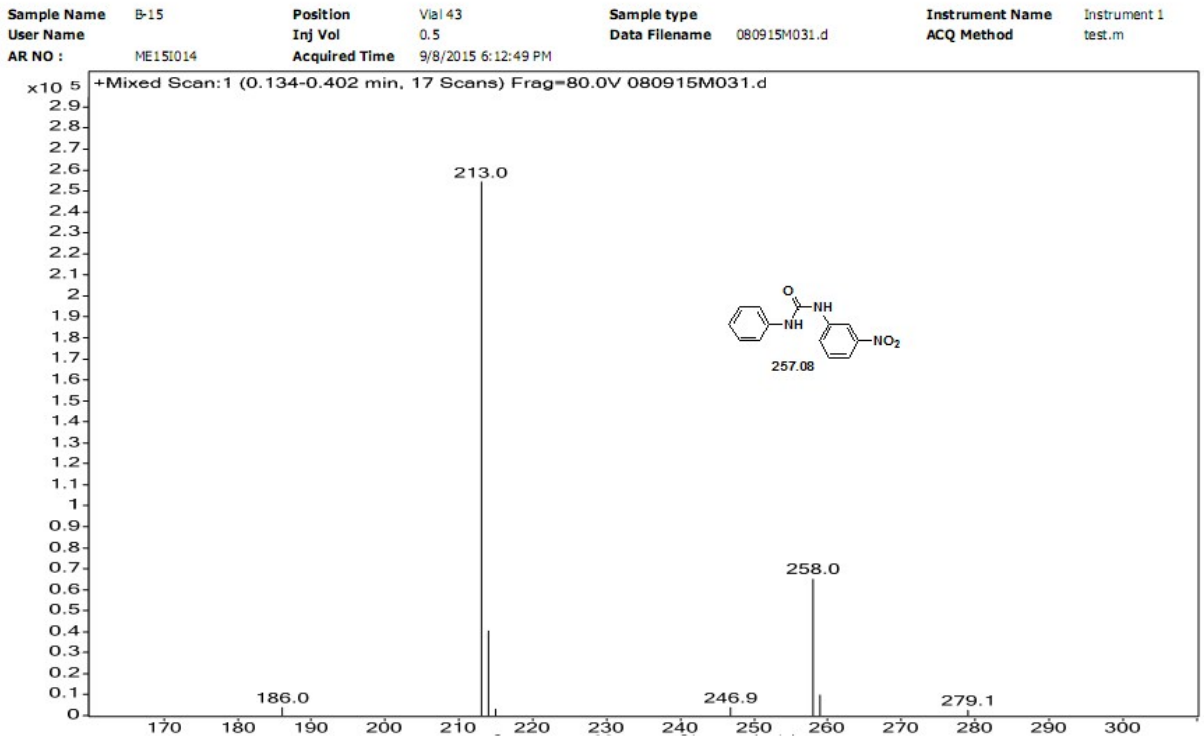
S2.28: ESI$^+$ Mass spectrum of compound 5j



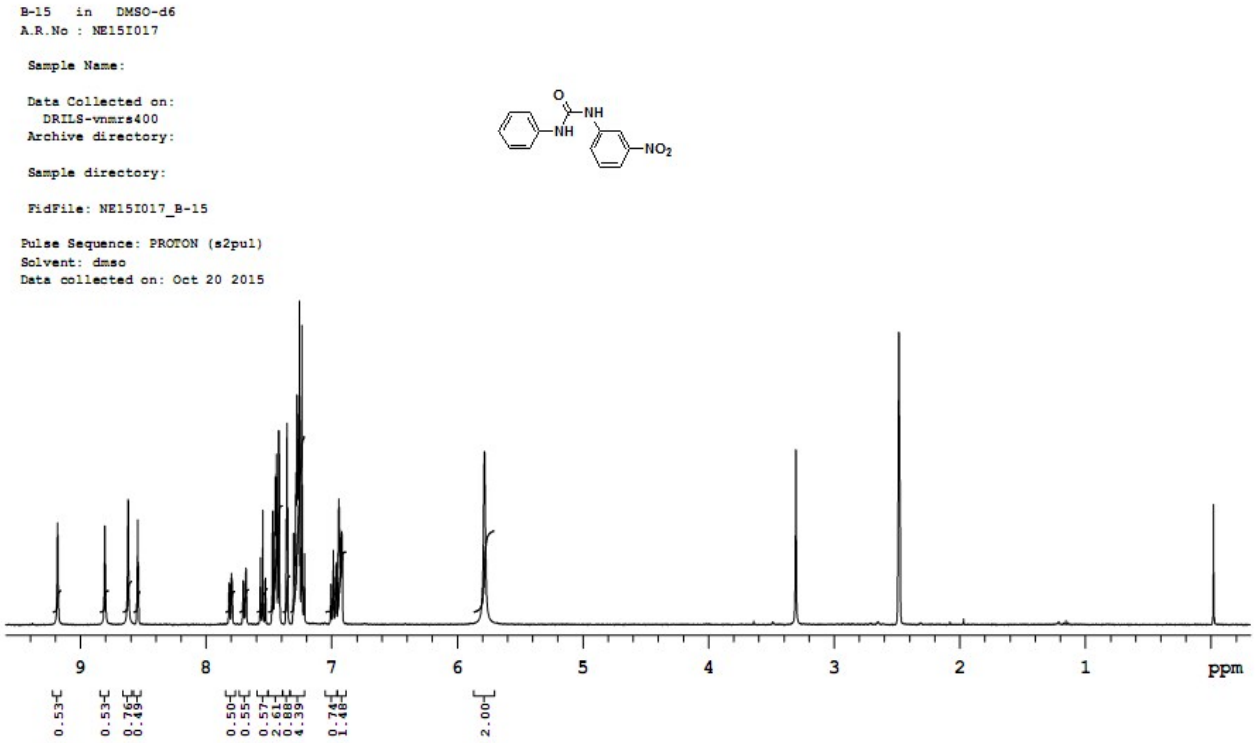
S2.29: 400 MHz ^{1}H-NMR spectrum of compound 5j



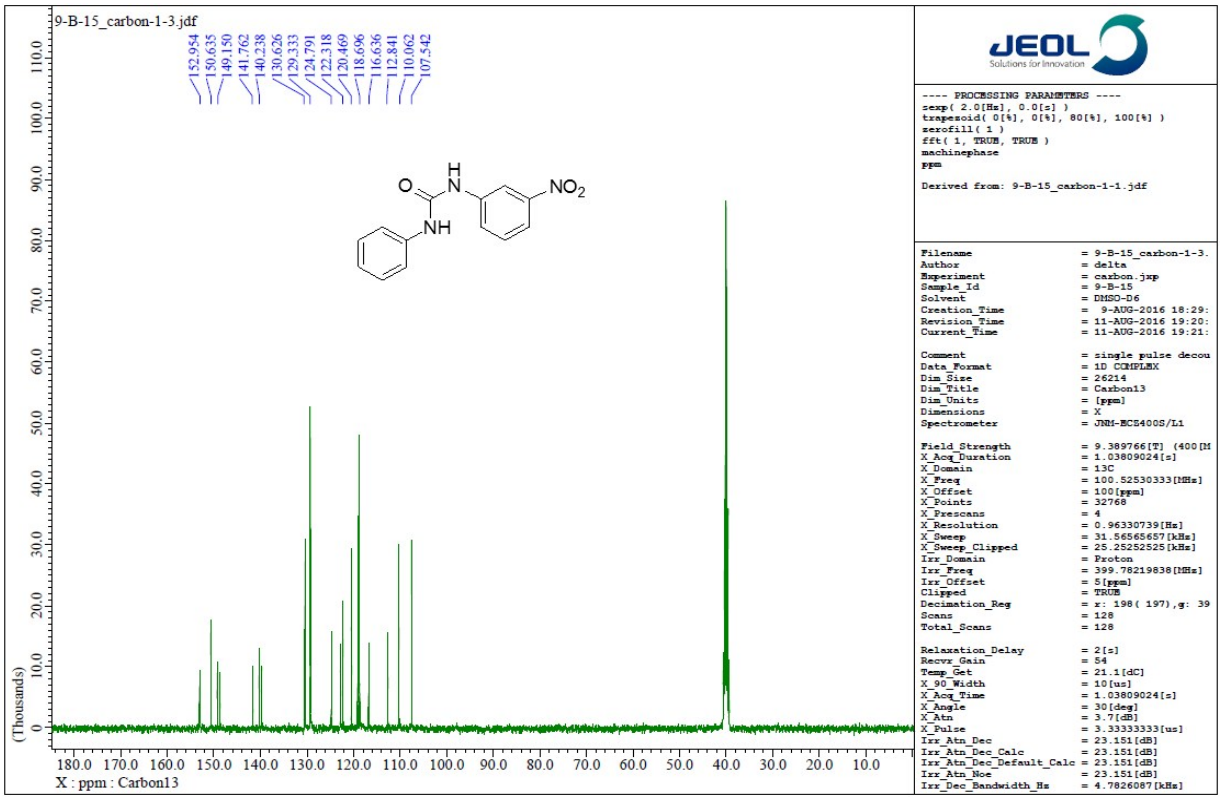
S2.30: 13C NMR Spectrum of compound 5j



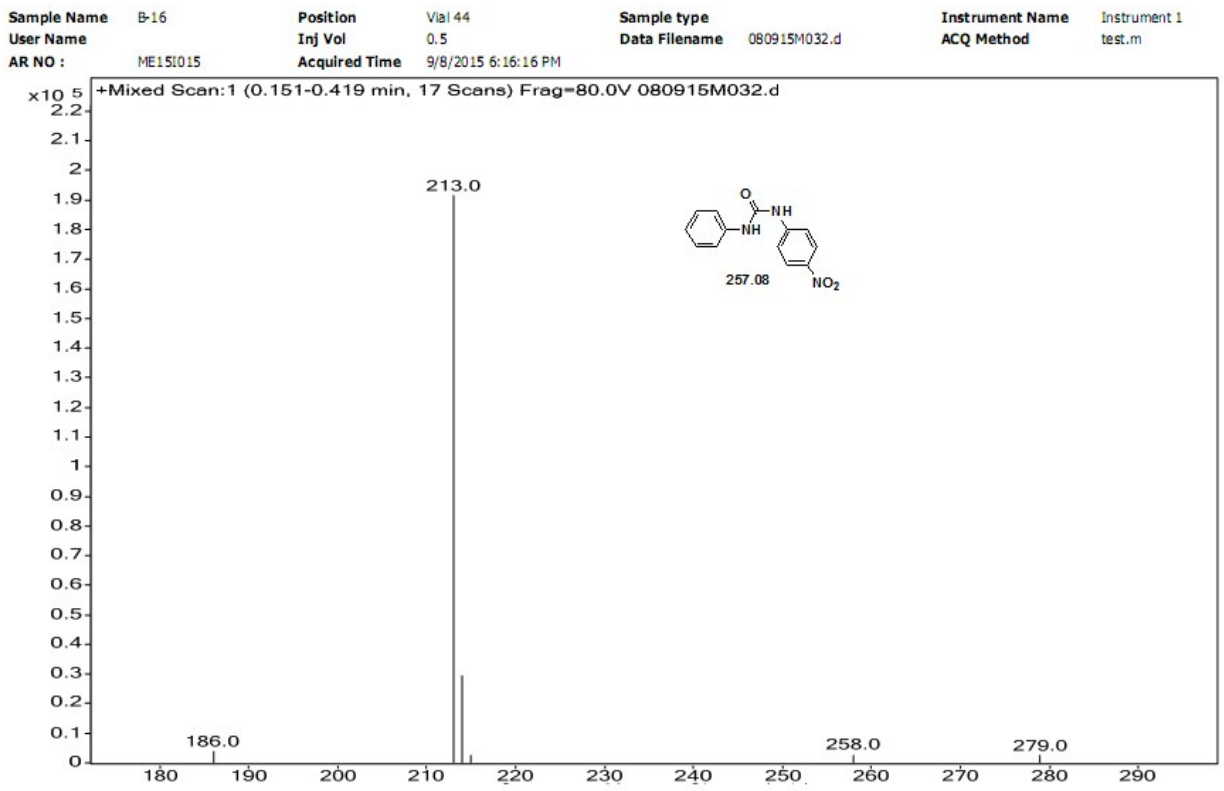
S2.31: ESI^{+} Mass spectrum of compound 5k



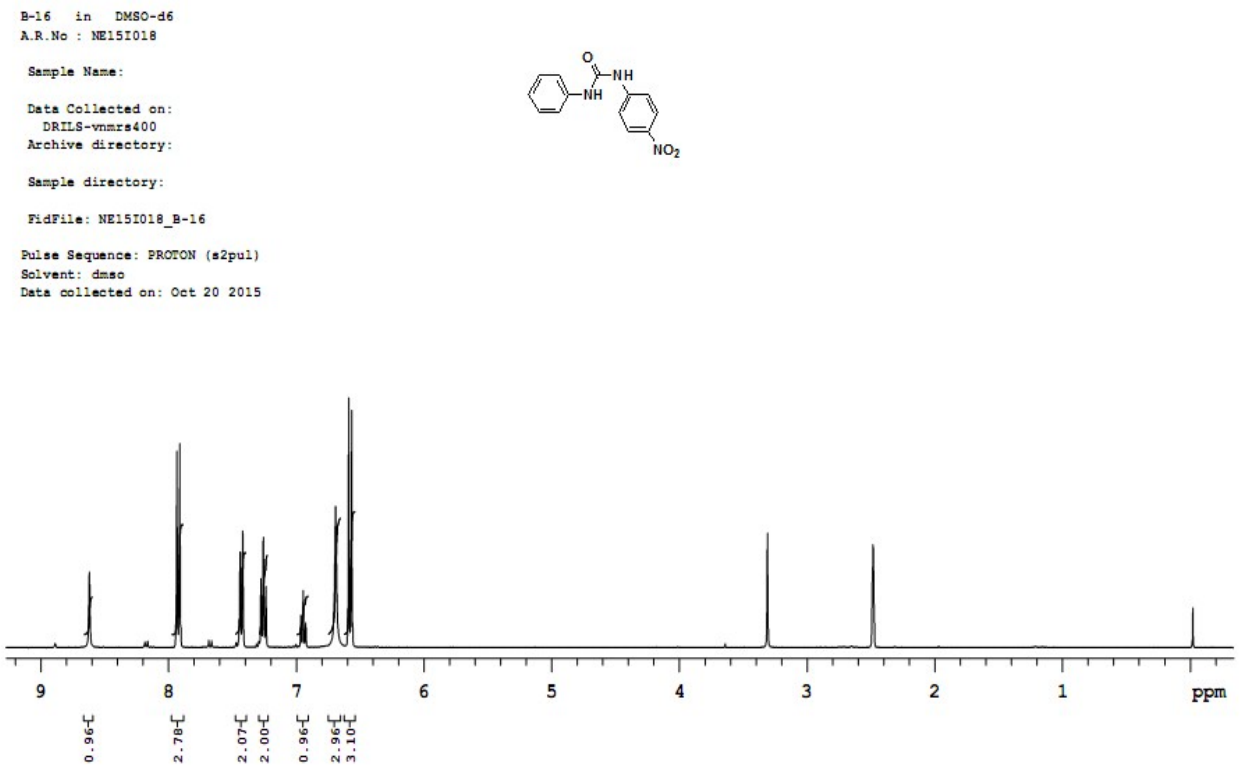
S2.32: 400 MHz ^{1}H-NMR spectrum of compound 5k



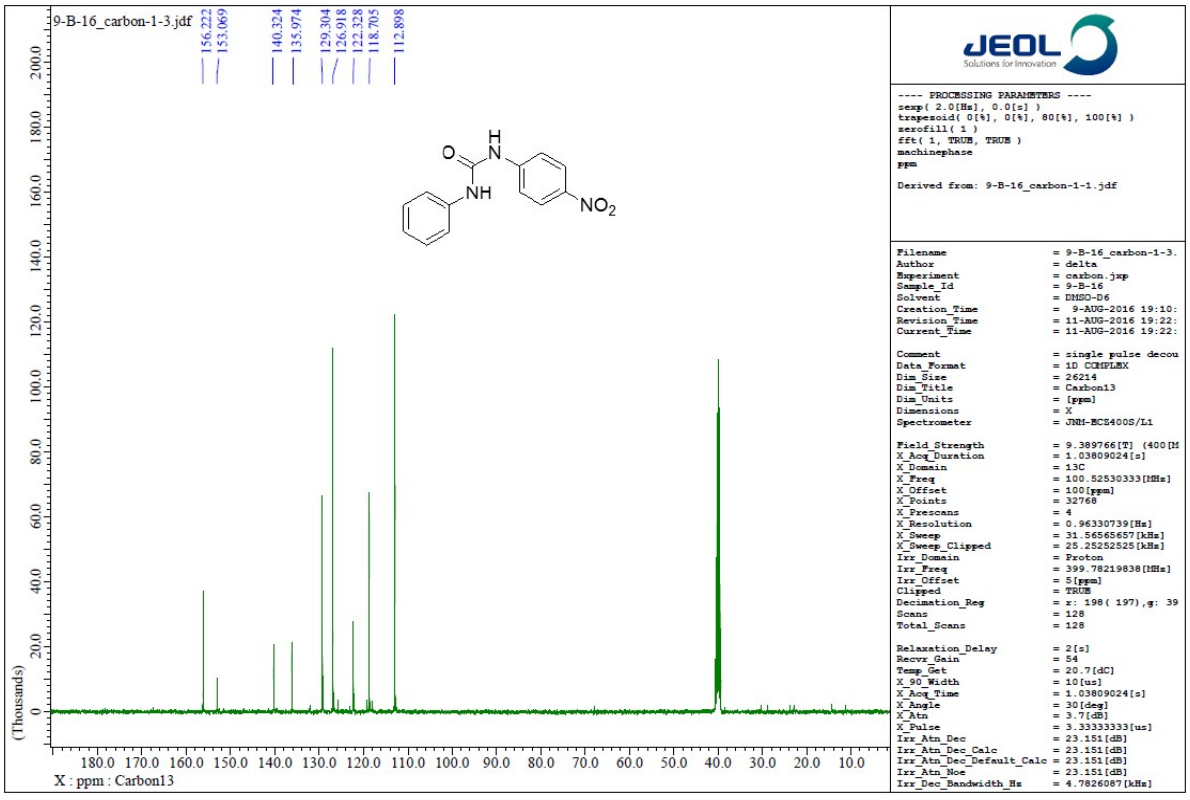
S2.33: 13C NMR Spectrum of compound 5k



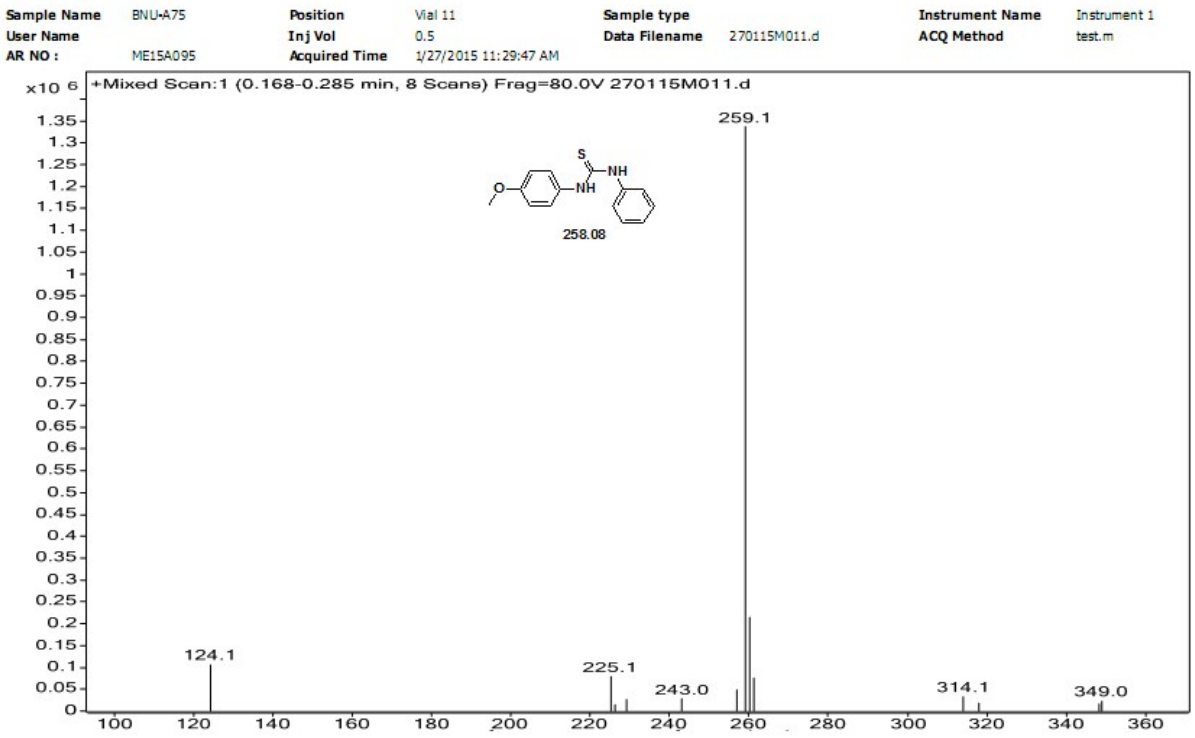
S2.34: ESI$^+$ Mass spectrum of compound 5l



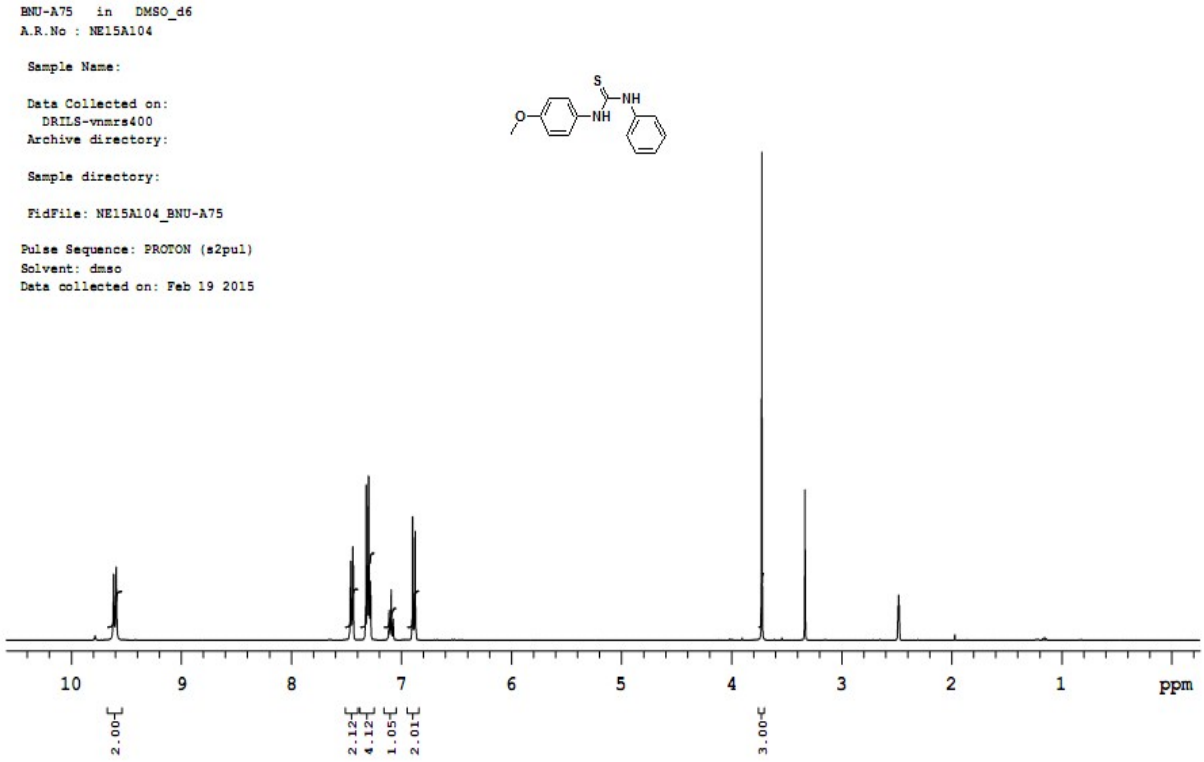
S2.35: 400 MHz ^{1}H-NMR spectrum of compound 5l



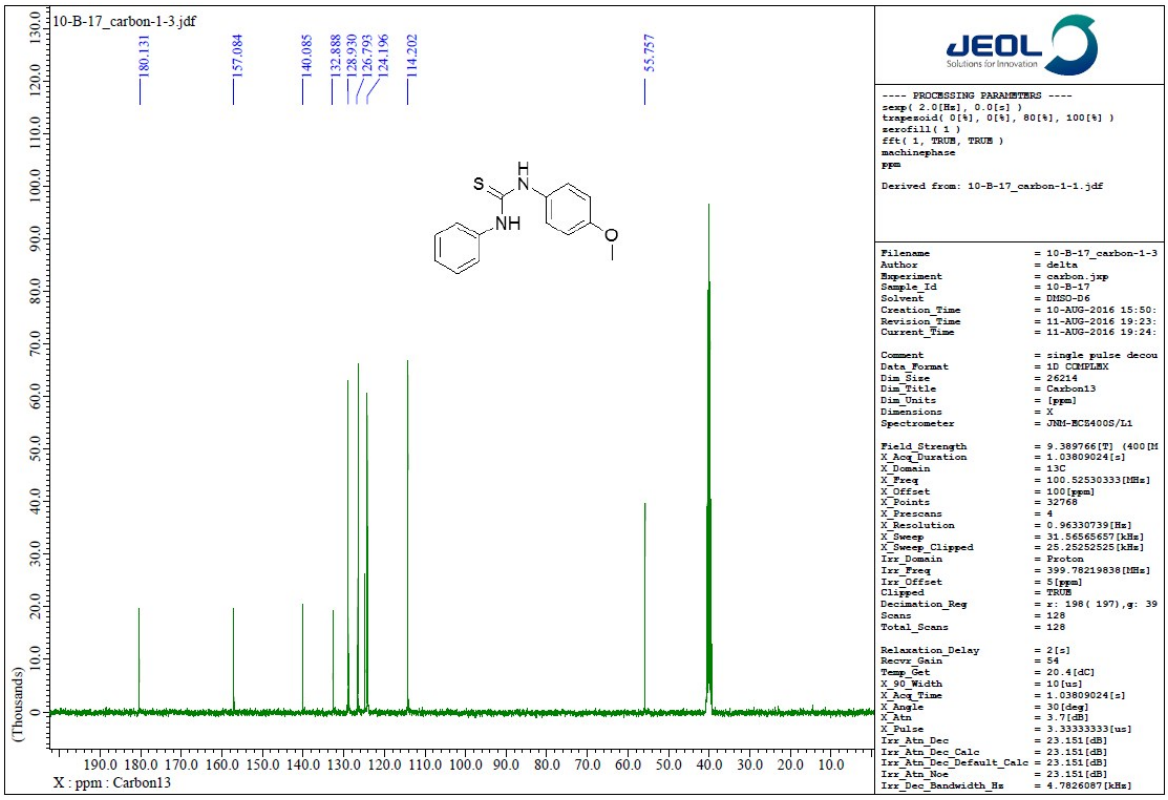
S2.36: 13C NMR Spectrum of compound 5l



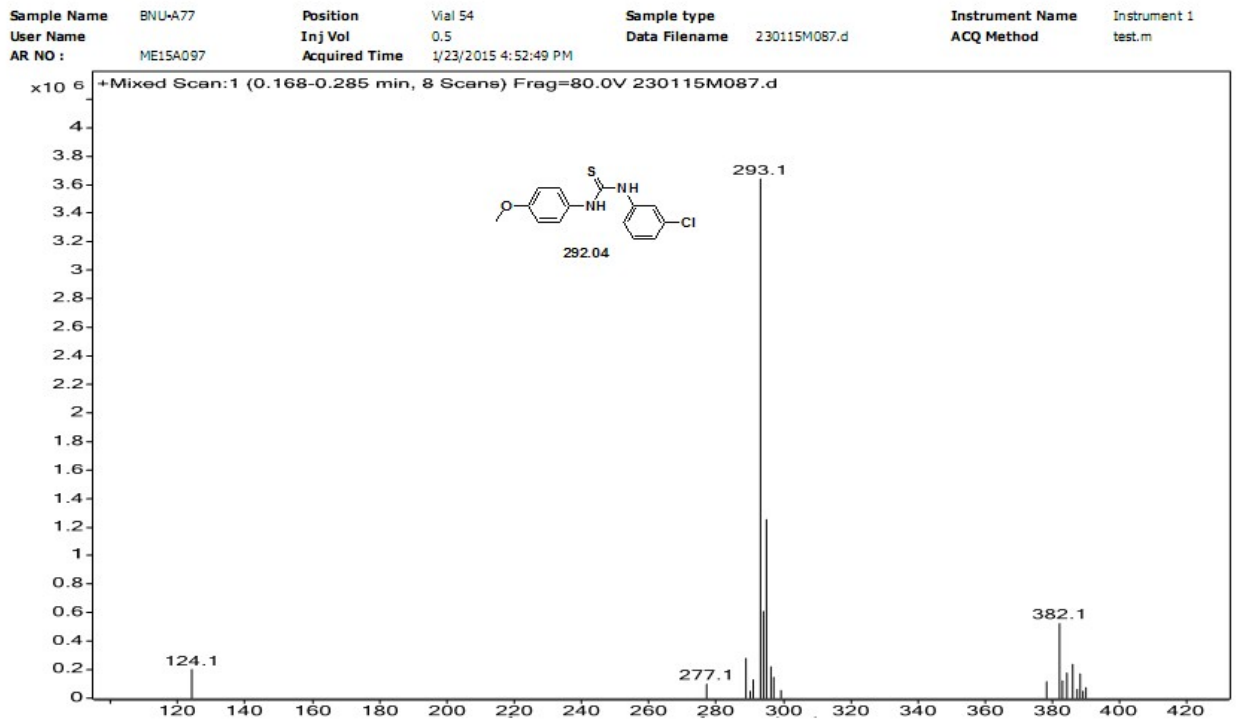
S2.37: ESI$^+$ Mass spectrum of compound 5m



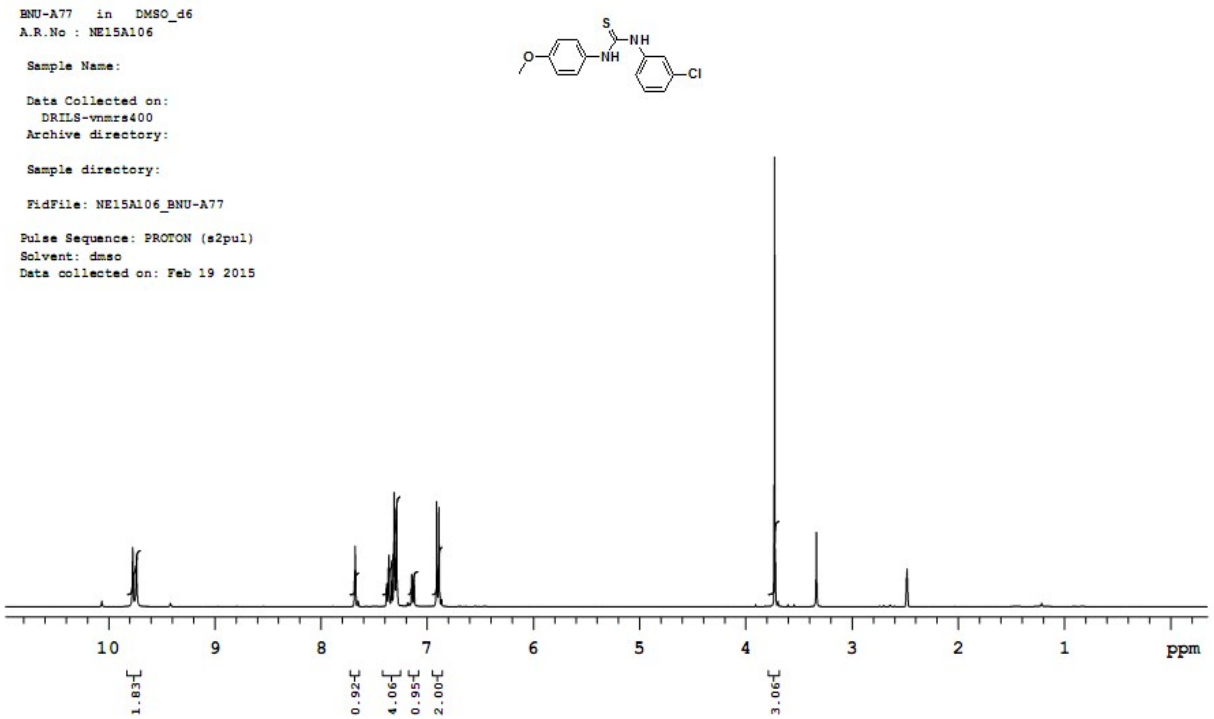
S2.38: 400 MHz 1H-NMR spectrum of compound 5m



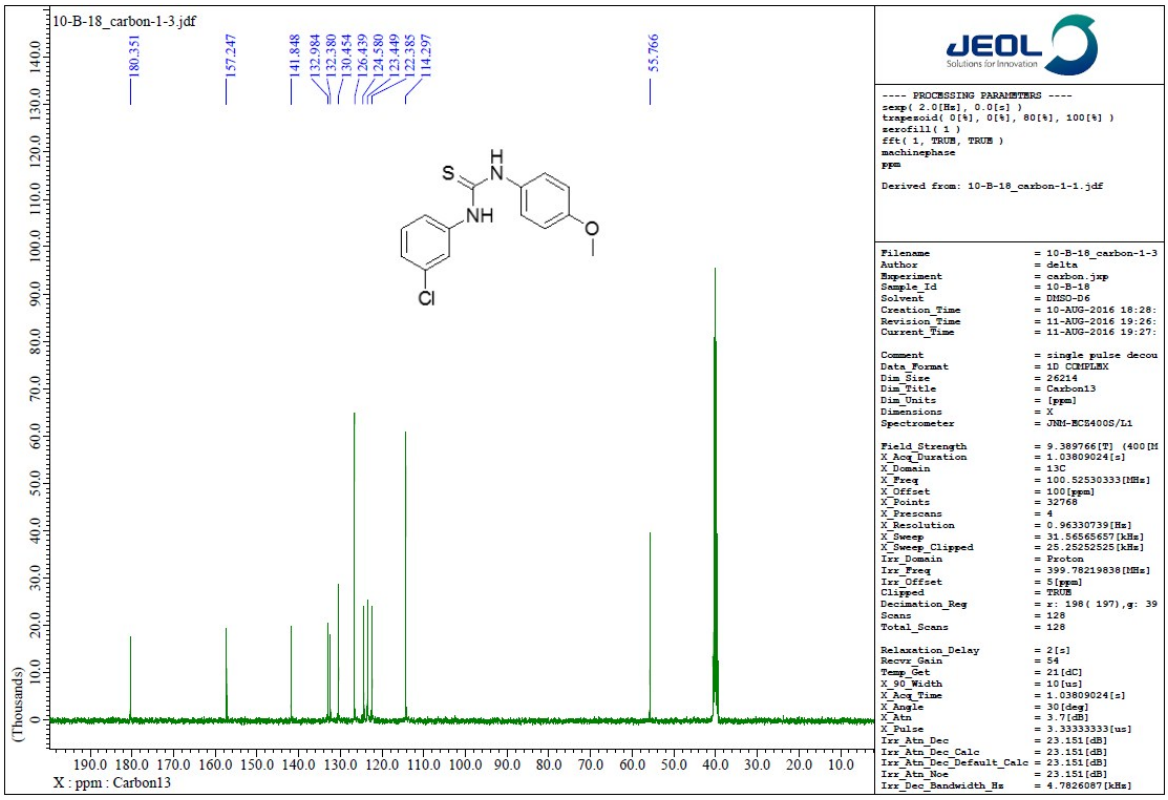
S2.39: 13C NMR Spectrum of compound 5m



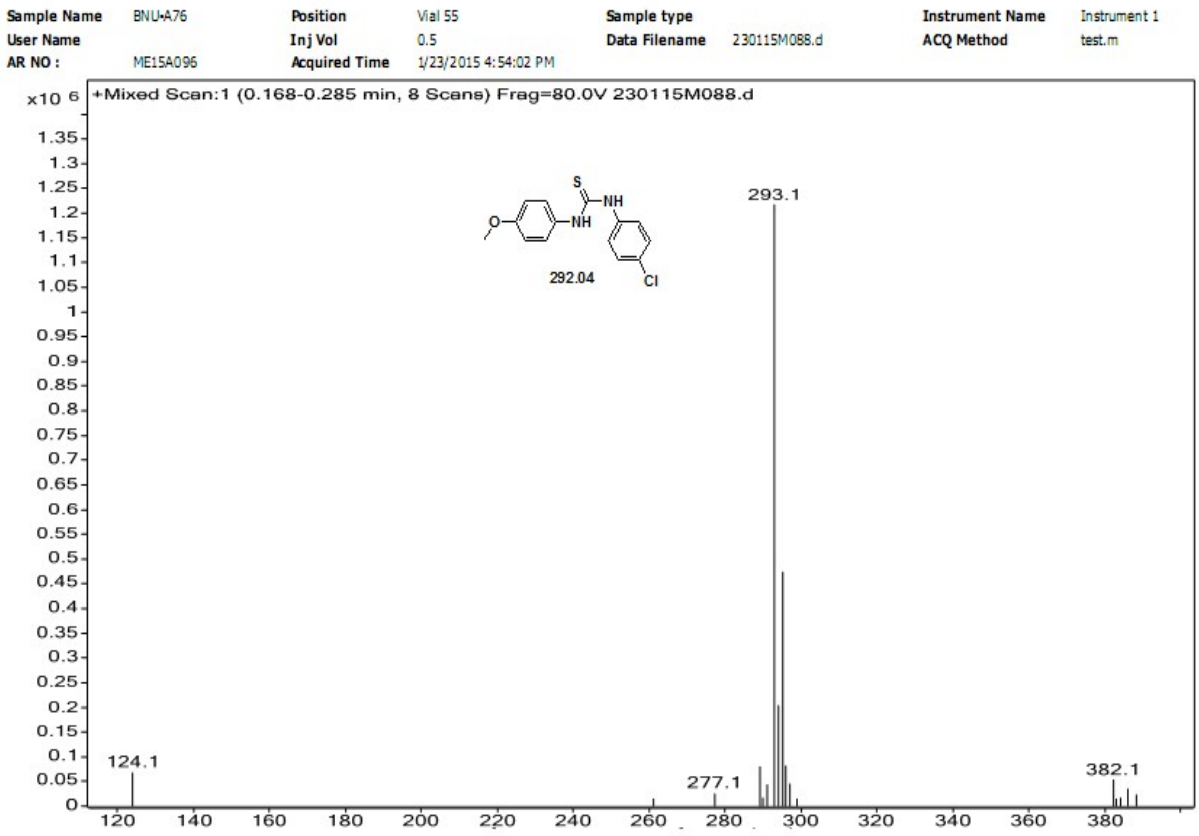
S2.40: ESI^{+} Mass spectrum of compound 5n



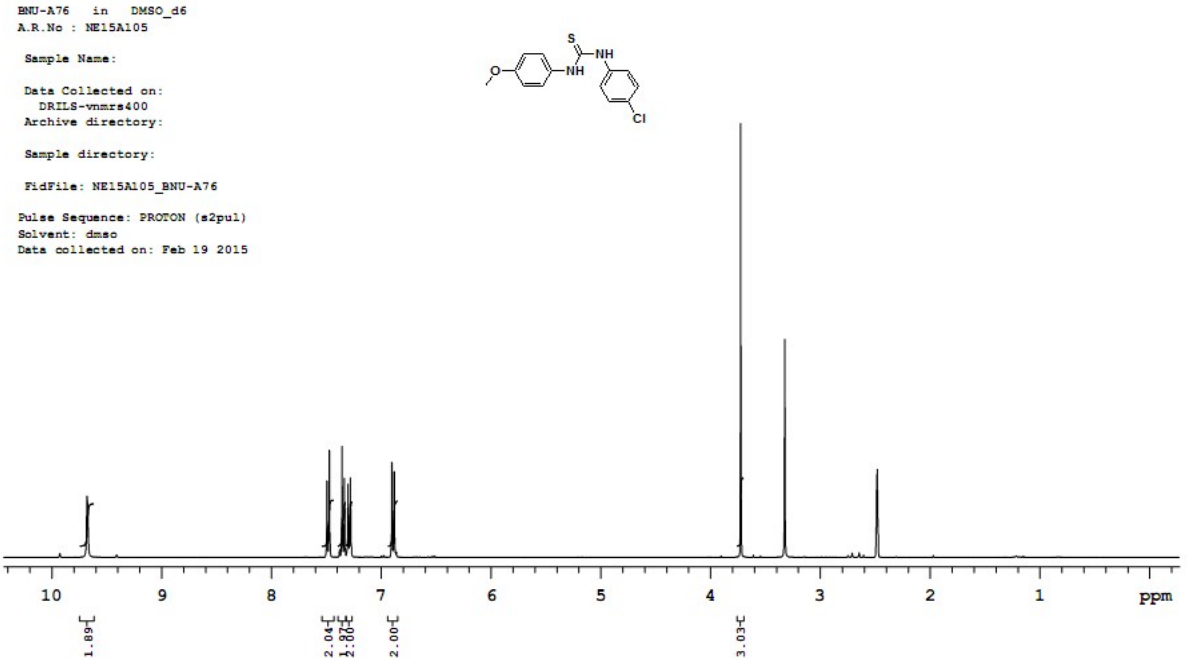
S2.41: 400 MHz ^{1}H-NMR spectrum of compound 5n



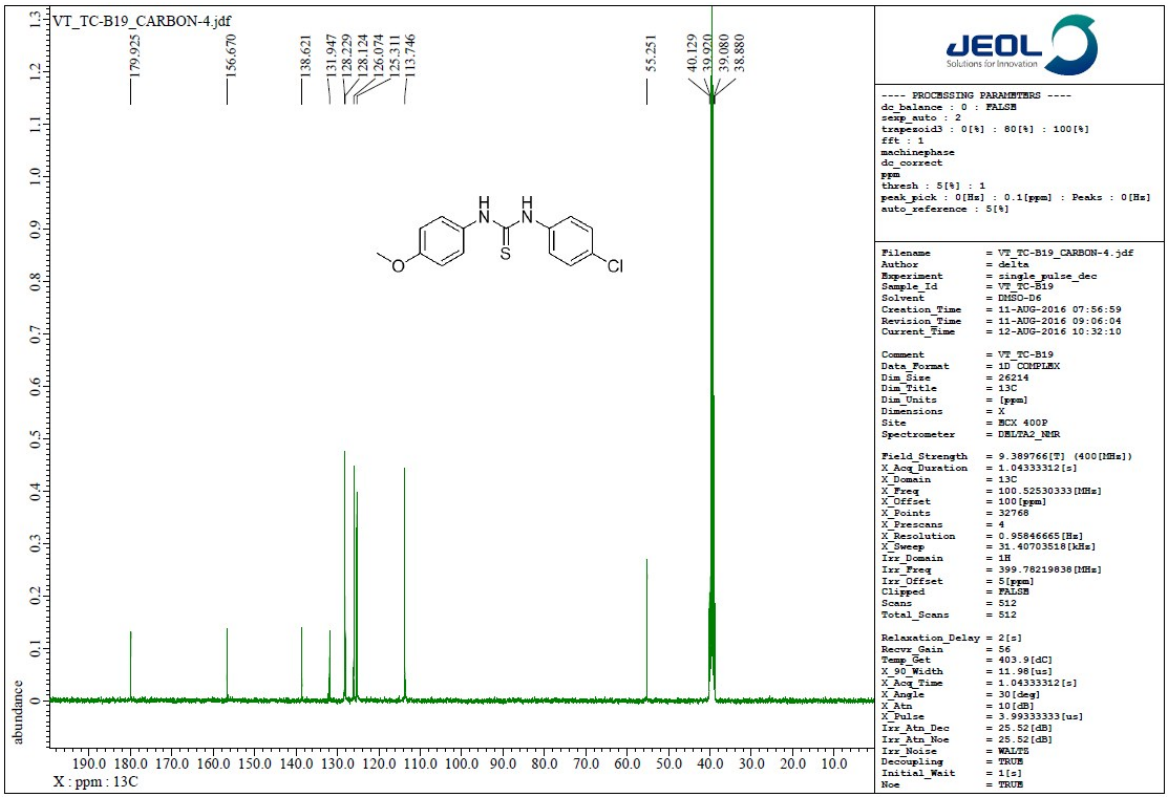
S2.42: 13C NMR Spectrum of compound 5n



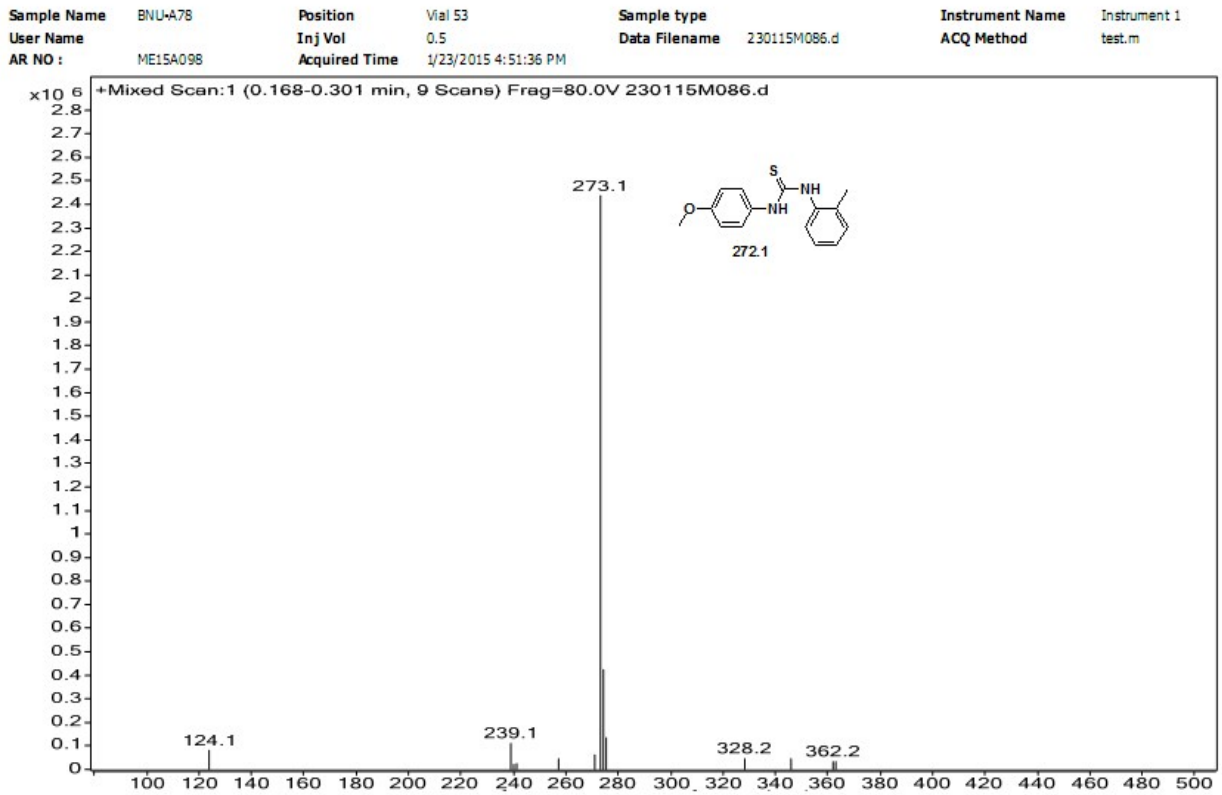
S2.43: ESI+ Mass spectrum of compound 5o



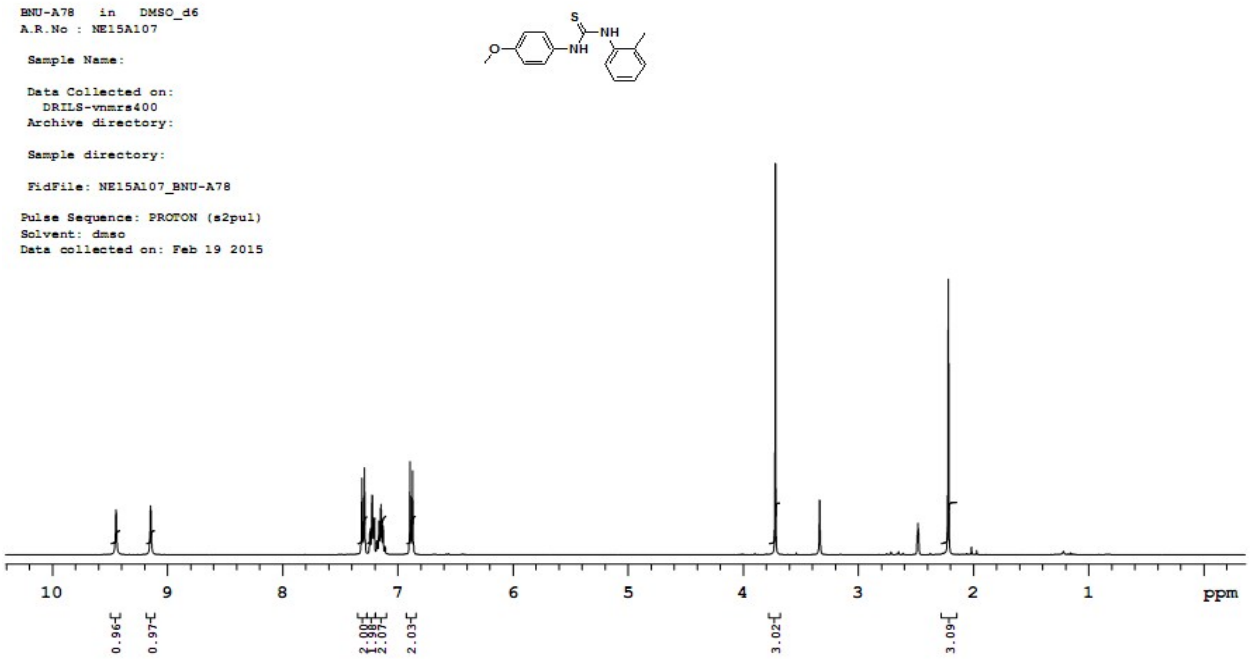
S2.44: 400 MHz ^{1}H-NMR spectrum of compound 5o



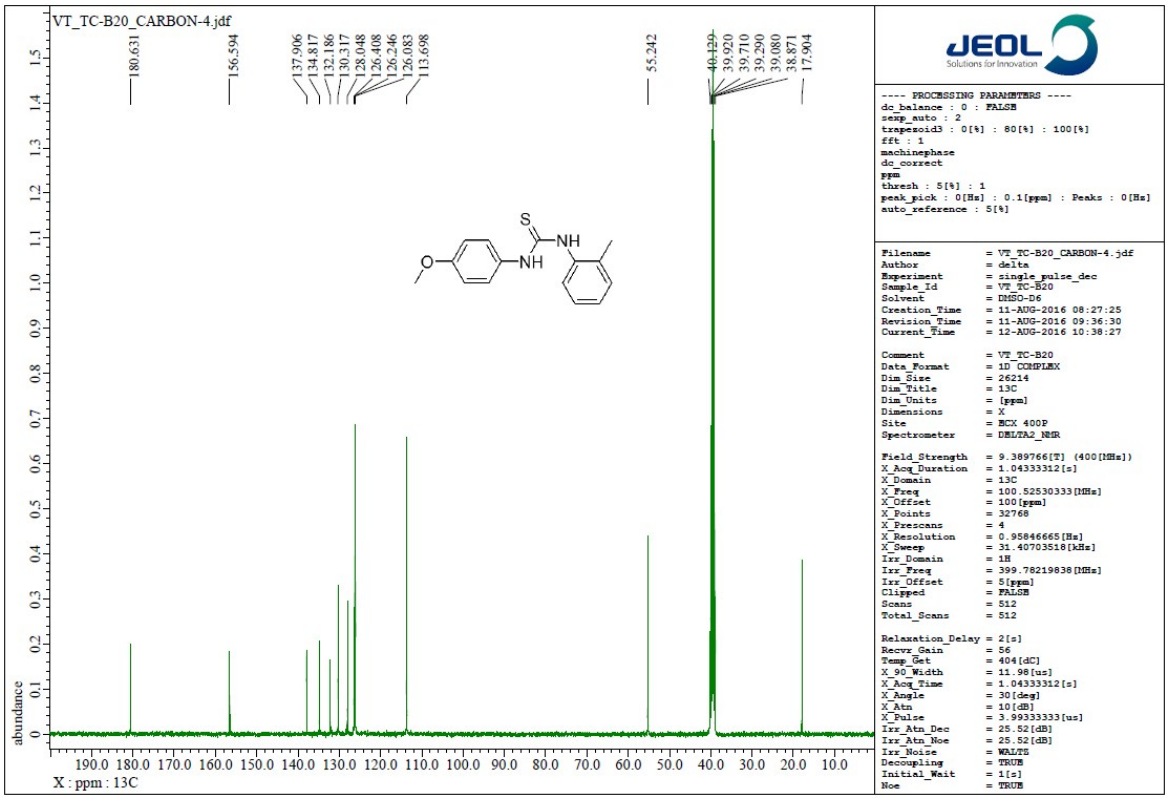
S2.45: 13C NMR Spectrum of compound 5o



S2.46: ESI^{+} Mass spectrum of compound 5p

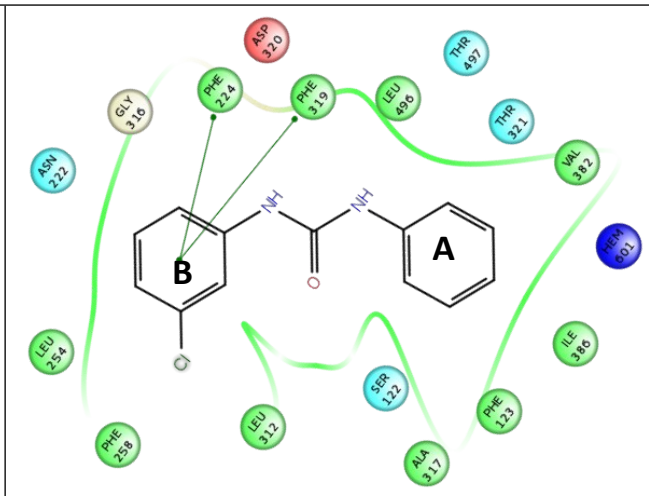
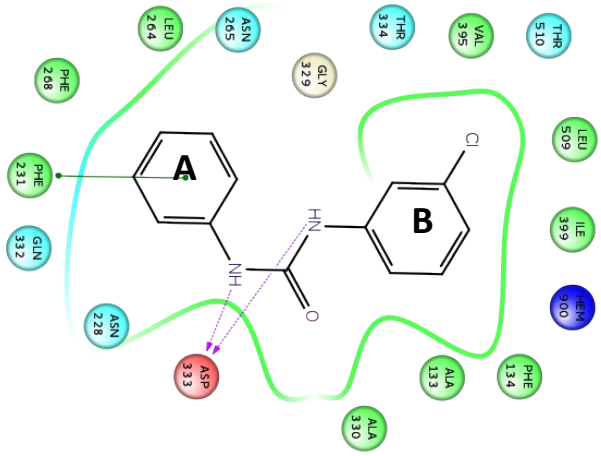
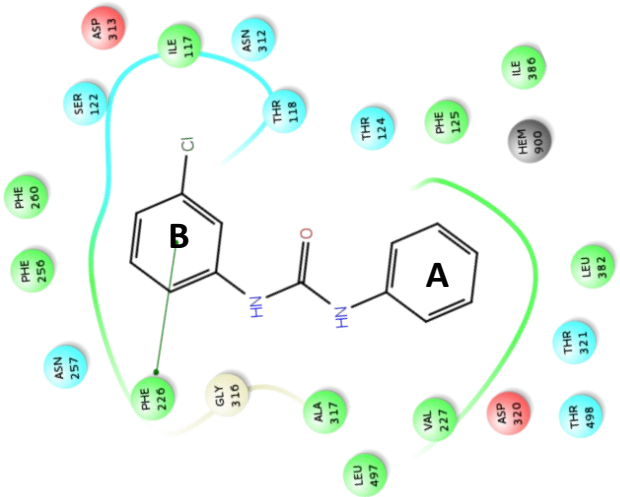


S2.47: 400 MHz ^{1}H-NMR spectrum of compound 5p



S2.48: 13C NMR Spectrum of compound 5p

S3. Molecular modeling images (2D) of compound 5h with CYP1A1, CYP1B1 and CYP1A2

5h with CYP1A1	
5h with CYP1B1	
5h with CYP1A2	

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