SUPPORTING INFORMATION

Design and Discovery of 2-Arylquinazolinones as Potent and Selective Inhibitors of the Tankyrases

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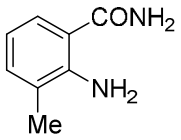
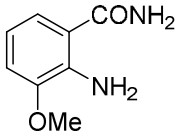
Section A: General synthetic methods

Chemical reagents were purchased from Sigma, Aldrich, Fluka, Acros, Lancaster and Novabiochem. Anhydrous CH_2Cl_2 was obtained by distillation over calcium hydride, anhydrous THF was obtained by distillation over sodium / benzophenone. All other solvents were purchased from Fisher Scientific. Analytical TLC was performed using silica gel 60 F_{254} pre-coated on aluminium sheets (0.25 mm thickness). Column chromatography was performed on silica gel 60 (35-70 micron) from Fisher Scientific. Melting points were recorded on a Reichert-Jung Kofler block apparatus and are uncorrected. ^{1}H and ^{13}C NMR were recorded using a Bruker Advance DPX 500 MHz and 400 MHz (^{1}H) instruments. High resolution mass spectra were determined using the electrospray ionization or electron impact techniques and were calibrated with sodium formate using a Bruker Daltonics MicroTOF instrument. The brine was saturated. Experiments were conducted at ambient temperature, unless otherwise stated. Solutions in organic solvents were dried with anhydrous $MgSO_4$. Solvents were evaporated under reduced pressure.

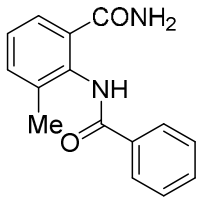
Section B: Chemistry experimental



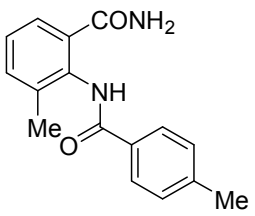
2-Amino-3-methylbenzamide (5a). 2-Amino-3-methylbenzoic acid **4a** (2.93 g, 19.8 mmol) in dry DMF (78 mL) was treated with 1,1-carbonyl-diimidazole (3.14 g, 19.4 mmol) at 70°C under Ar for 1 h, after which aq. NH_3 (35%, 49 mL) was added dropwise and the mixture was stirred for 16 h. The mixture was allowed to cool to 20°C and was diluted with EtOAc (100 mL). The mixture was washed with water (2 × 40 mL) and brine (2 × 40 mL). The organic solution was dried and the solvent was evaporated to give **5a** (2.14 g, 98%) as a white solid: mp 150-152°C (lit.[1] mp 150-152°C); ^{1}H NMR ($(CD_3)_2SO$) 2.05 (3 H, s, Me), 6.35 (2 H, br, $ArNH_2$), 6.41 (1 H, brt, J = 7.6 Hz, 5-H), 7.00 (1 H, br, CONH), 7.04 (1 H, d, J = 6.8 Hz, 6-H), 7.34 (1 H, dd, J = 8.0, 0.8 Hz, 4-H), 7.67 (1 H, br, CONH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) 17.56 (Me), 113.59 (1-C), 114.17 (5-C), 122.99 (3-C), 126.61 (6-C), 132.67 (4-C), 148.21 (2-C), 171.73 (C=O).



2-Amino-3-methoxybenzamide (5b). 2-Amino-3-methoxybenzoic acid **4b** (3.00 g, 17.9 mmol) in dry DMF (80 mL) was treated with 1,1-carbonyl-diimidazole (3.19 g, 19.7 mmol) at 70°C under Ar for 1 h, after which aq. NH_3 (35%, 50 mL) was added dropwise and the mixture was stirred for 16 h. The mixture was allowed to cool to 20°C and was diluted with EtOAc (100 mL). The mixture was washed with water (2 × 40 mL) and brine (2 × 40 mL). The organic solution was dried and the solvent was evaporated to give **5b** (2.38 g, 80%) as a white solid: mp 139-141°C (lit.[1] mp 139-141°C); ^{1}H NMR ($(CD_3)_2SO$) 3.77 (3 H, s, Me), 6.23 (2 H, br s, Ar-NH_2), 6.44 (1 H, t, J = 8.0, 5-H), 7.04 (1 H, dd, J = 7.6, 0.8, 6-H), 7.03 (1 H, br s, CONH), 7.16 (1 H, dd, J = 8.0, 1.2, 4-H), 7.67 (1 H, br s, CONH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) 55.53 (Me), 111.98 (6-C), 113.38 (3-C), 113.64 (5-C), 120.42 (4-C), 140.19 (1-C), 146.88 (2-C), 171.19 (C=O).

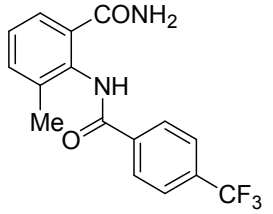


2-Benzamido-3-methylbenzamide (6a). Dry pyridine (134 mg, 1.7 mmol) was added to **5b** (200 mg, 1.3 mmol) in dry THF (5.0 mL), followed by benzoyl chloride (210 mg, 1.5 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 4:1) gave **6a** (280 mg, 83%) as a white solid: mp 193-197°C (lit.[2] 190-193°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.22 (3 H, s, Me), 7.26 (1 H, t, J = 7.5 Hz, 5-H), 7.39-7.44 (3 H, m, 4,6-H_2 +N*H*H), 7.53 (2 H, m, Ph 3,5-H_2), 7.58-7.60 (1 H, m, Ph 4-H), 7.71 (1 H, s, NH*H*), 7.96 (2 H, m, Ph 2,6-H_2), 10.20 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.34 (Me), 125.80 (6-C), 125.99 (5-C), 127.48 (Ph 2,6-C_2), 128.49 (Ph 3,5-C_2), 131.63 (Ph 4-CH), 132.14 (4-C), 132.88 (1-C), 134.26 (2-C), 134.49 (Ph 1-C), 135.98 (3-C), 165.02 (NHCO), 169.70 ($CONH_2$).

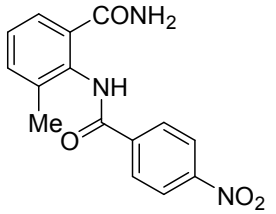


3-Methyl-2-(4-methylbenzamido)benzamide (6b). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-methylbenzoyl chloride (340 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 1:1 → 4:1) gave **6b** (380 mg, 71%) as a white solid: mp 237-239°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.27 (3 H, s, 3-Me), 2.44 (3 H, s, Ph 4-Me), 7.31 (1 H, t, J = 7.6 Hz, 5-H), 7.39 (2 H, d, J = 7.9 Hz, Ph 3,5-H_2), 7.44-7.50 (3 H, m, 4,6-H_2 + N*H*H), 7.75 (1 H, s, NH*H*), 7.91 (2 H, d, J = 7.9 Hz, Ph 2,6-H_2), 10.22 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$)

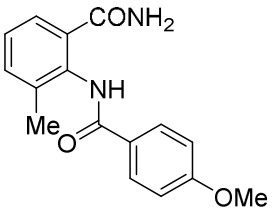
(HSQC / HMBC) δ 18.38 (3-Me), 20.98 (Ph 4-Me), 125.80 (5-C), 125.87 (6-C), 127.51 (Ph 2,6-C_2), 129.01 (Ph 3,5-C_2), 131.48 (Ph 1-C), 132.17 (4-C), 132.68 (1-C), 134.65 (2-C), 135.94 (3-C), 141.63 (Ph 4-C), 164.92 (NHCO), 169.77 ($CONH_2$); MS (EI) *m/z* 269.1261 $(M)^+$ ($C_{16}H_{17}N_2O_2$ requires 269.1290).



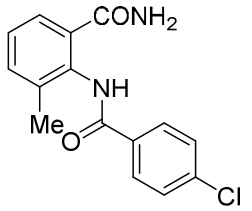
c**3-Methyl-2-(4-trifluoromethylbenzamido)benzamide (6c).** Dry pyridine (205 mg, 2.6 mmol) was added to (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-trifluoromethylbenzoyl chloride (460 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 7:3) gave **6c** (460 mg, 71%) as a white solid: mp 259-261°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.23 (3 H, s, Me), 7.28 (1 H, t, *J* = 7.5 Hz, 5-H), 7.38-7.44 (3 H, m, 4,6-H_2 + N*H*H), 7.71 (1 H, s, NH*H*), 7.92 (2 H, d, *J* = 8.0 Hz, Ph 3,5-H_2), 8.15 (2 H, d, *J* = 8.0 Hz, Ph 2,6-H_2), 10.33 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.17 (Me), 123.92 (q, *J* = 270.8 Hz, CF_3), 125.50 (q, *J* = 3.6 Hz, Ph 3,5-H_2), 125.86 (6-C), 126.33 (5-C), 128.45 (Ph 2,6-H_2), 131.41 (q, *J* = 27.7 Hz, Ph 4-C), 132.08 (4-C), 133.38 (1-C), 134.01 (2-C), 136.07 (3-C), 138.20 (Ph 1-C), 164.01 (NHCO), 169.50 ($CONH_2$); MS (ES) *m/z* 345.0828 $(M + Na)^+$ ($C_{16}H_{13}F_3N_2NaO_2$ requires 345.0827).



3-Methyl-2-(4-nitrobenzamido)benzamide (6d). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-nitrobenzoyl chloride (390 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 1:1 → 4:1) gave **6d** (380 mg, 83%) as a pale yellow solid: mp 191-193°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.23 (3 H, s, Me), 7.29 (1 H, t, *J* = 7.5 Hz, 5-H), 7.37 (1 H, s, N*H*H), 7.42-7.44 (2 H, m, 4,6-H_2), 7.73 (1 H, s, NH*H*), 8.18 (2 H, m, Ph 2,6-H_2), 8.38 (2 H, d, *J* = 7.5 Hz, Ph 3,5-H_2), 10.39 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.13 (Me), 123.67 (Ph 3,5-H_2), 125.87 (6-C), 126.43 (5-C), 129.04 (Ph 2,6-H_2), 132.07 (4-C), 133.48 (1-C), 133.86 (2-C), 136.08 (3-C), 140.12 (Ph 1-C), 149.19 (Ph 4-C), 163.61 (NHCO), 169.45 ($CONH_2$); MS (ES) *m/z* 322.0796 $(M + Na)^+$ ($C_{15}H_{13}N_3NaO_4$ requires 322.0803).

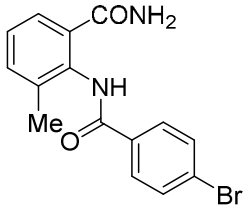


2-(4-Methoxybenzamido)-3-methylbenzamide (6e). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-methoxybenzoyl chloride (380 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 7:3) gave **6e** (160 mg, 28%) as a white solid: mp 182-185°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.26 (3 H, s, 3-Me), 3.89 (3 H, s, OMe), 7.11 (2 H, m, Ph 3,5-H_2), 7.30 (1 H, t, *J* = 7.6 Hz, 5-H), 7.43-7.45 (2 H, m, 4-H + N*H*H), 7.48 (1 H, m, 6-H), 7.74 (1 H, s, NH*H*), 7.99 (2 H, m, Ph 2,6-H_2) 10.18 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.41 (3-Me), 55.43 (OMe), 113.74 (Ph 3,5-C_2), 125.78 (6-C), 126.42 (5-C), 129.39 (Ph 2,6-C_2), 132.17 (4-C), 132.62 (1-C), 134.79 (2-C), 135.93 (3-C), 140.90 (Ph 1-C), 161.96 (Ph 4-C), 164.54 (NHCO), 169.81 ($CONH_2$); MS *m/z* 285.1235 (EI) $(M)^+$ $C_{16}H_{17}N_2O_3$ requires 285.1239).

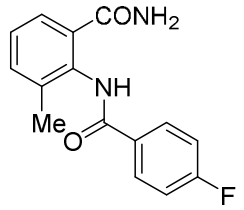


2-(4-Chlorobenzamido)-3-methylbenzamide (6f). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-chlorobenzoyl chloride (380 mg, 2.2 mmol)

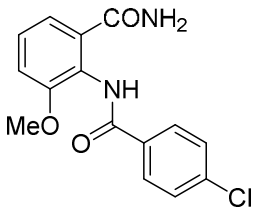
in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 7:3) gave **6f** (400 mg, 69%) as a white solid: mp 220-223°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.27 (3 H, s, Me), 7.32 (1 H, t, *J* = 7.6 Hz, 5-H), 7.42-7.50 (3 H, m, 4,6-H_2 + N*H*H), 7.66 (2 H, d, *J* = 8.6 Hz, Ph 3,5-H_2), 7.74 (1 H, s, NH*H*), 8.03 (2 H, d, *J* = 8.6 Hz, Ph 2,6-H_2), 10.27 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.23 (Me), 125.81 (6-C), 126.15 (5-C), 128.56 (Ph 3,5-H_2), 129.45 (Ph 2,6-H_2), 132.09 (4-C), 133.11 (1-C), 133.14 (Ph 1-C), 134.25 (2-C), 136.04 (3-C), 136.43 (Ph 4-C), 164.09 (NHCO), 169.59 ($CONH_2$); MS (ES) *m/z* 313.0505 $(M + Na)^+$ ($C_{15}H_{13}{}^{37}ClN_2NaO_2$ requires 313.0527), 311.0533 $(M + Na)^+$ ($C_{15}H_{13}{}^{35}ClN_2NaO_2$ requires 311.0563).



2-(4-Bromobenzamido)-3-methylbenzamide (6g). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-bromobenzoyl chloride (483 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 7:3) gave **6g** (410 mg, 62%) as a white solid: mp 221-224°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.27 (3 H, s, Me), 7.32 (1 H, t, *J* = 7.6 Hz, 5-H), 7.42-7.49 (3 H, m, 4,6-H_2 + N*H*H), 7.73 (1 H, s, NH*H*), 7.80 (2 H, d, *J* = 8.6 Hz, Ph 3,5-H_2), 7.95 (2 H, d, *J* = 8.6 Hz, Ph 2,6-H_2), 10.27 (1 H, s NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.23 (Me), 125.34 (Ph 4-C), 125.82 (6-C), 126.15 (5-C), 129.63 (Ph 2,6-C_2), 131.50 (Ph 3,5-C_2), 132.09 (4-C), 133.13 (1-C), 133.49 (Ph 1-C), 134.24 (2-C), 136.03 (3-C), 164.22 (NHCO), 169.58 ($CONH_2$); MS (ES) *m/z* 357.0033 $(M + Na)^+$ ($C_{15}H_{13}{}^{81}BrN_2NaO_2$ requires 359.0078), 355.0052 $(M + Na)^+$ ($C_{15}H_{13}{}^{79}BrN_2NaO_2$ requires 355.0058).

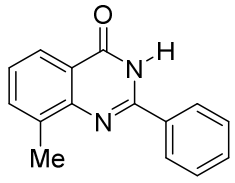


2-(4-Fluorobenzamido)-3-methylbenzamide (6h). Dry pyridine (205 mg, 2.6 mmol) was added to **5a** (300 mg, 2.0 mmol) in dry THF (5.0 mL), followed by 4-fluorobenzoyl chloride (349 mg, 2.2 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc / petroleum ether 7:3) gave **6h** (520 mg, 95%) as a white solid: mp 286-288°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.21 (3 H, s, Me), 7.26 (1 H, t, *J* = 8.0 Hz, 5-H), 7.34-7.43 (5 H, m, 4,6-H_2 + N*H*H + Ph 3,5-H_2), 7.70 (1 H, s, NH*H*), 8.03-8.04 (2 H, m, Ar 2,6-H_2), 10.19 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 18.27 (Me), 115.43 (d, *J* = 21.6 Hz, Ph 3,5-H_2), 125.81 (6-C), 125.10 (5-C), 130.23 (d, *J* = 9.1 Hz, Ph 2,6-H_2), 130.80 (d, *J* = 2.6 Hz, Ph 1-C), 132.09 (4-C), 133.13 (2-C), 134.35 (1-C), 136.04 (3-C), 164.04 (NHCO), 164.10 (d, *J* = 247.5 Hz, Ph 4-C), 169.65 ($CONH_2$); MS (ES) *m/z* 295.8043 $(M + Na)^+$ ($C_{15}H_{13}FN_2NaO_2$ requires 295.0859).

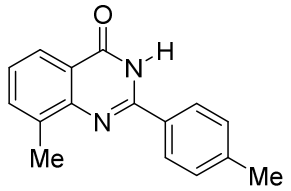


2-(4-Chlorobenzamido)-3-methoxybenzamide (6i). Dry pyridine (213 mg, 2.7 mmol) was added to **5b** (350 mg, 2.1 mmol) in dry THF (5.0 mL), followed by 4-chlorobenzoyl chloride (410 mg, 2.3 mmol) in dry THF (5.0 mL). The mixture was stirred for 16 h. Evaporation and chromatography (EtOAc) gave **6i** (536 mg, 83%) as a white solid: mp 220-223°C; ^{1}H NMR ($(CD_3)_2SO$) δ 3.78 (3 H, s, Me), 7.15 (1 H, dd, *J* = 8.0, 1.5 Hz, 6-H), 7.20 (1 H, dd, *J* = 8.0, 1.5 Hz, 4-H), 7.31-7.34 (2 H, m, N*H*H + 5-H), 7.52 (1 H, s, NH*H*), 7.59 (2 H, d, *J* = 8.5 Hz, Ph 3,5-H_2), 7.96 (2 H, d, *J* = 8.5 Hz, Ph 2,6-H_2), 9.79 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 55.95 (Me), 113.48 (4-C), 119.84 (6-C), 124.03 (2-C), 127.29 (5-C), 128.48 (Ph 3,5-C_2), 129.57 (Ph 3,5-C_2), 133.08 (Ph 1-C), , 135.08 (1-C), 136.35 (Ph 4-C), 154.96 (3-C),

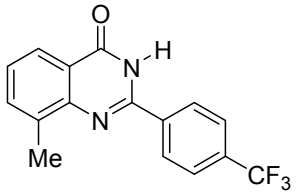
164.39 (NHCO), 168.83 ($CONH_2$); MS (EI) *m/z* 305.0677 $(M)^+$ ($C_{15}H_{14}{}^{35}ClN_2O_3$ requires 305.0692).



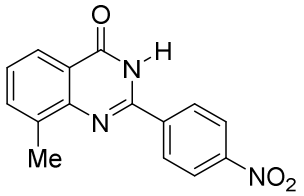
8-Methyl-2-phenylquinazolin-4-one (7a). Compound **6a** (93 mg, 0.37 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7a** (39 mg, 43%) as a white solid: mp 215-217°C (lit.[2] 206-209°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.62 (3 H, s, Me), 7.39 (1 H, t, *J* = 7.6 Hz, 6-H), 7.54-7.60 (3 H, m, Ph 3,4,5-H_3), 7.69 (1 H, d, *J* = 7.1 Hz, 7-H), 8.00 (1 H, dd, *J* = 7.8, 1.2 Hz, 5-H), 8.23 (2 H, dd, *J* = 7.8, 1.2 Hz, Ph 2,6-H_2), 12.51 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.14 (Me), 120.87 (4a-C), 123.47 (5-C), 126.02 (6-C), 127.71 (Ph 2,6-C_2), 128.59 (Ph 3,5-C_2), 131.29 (Ph 4-C), 132.96 (Ph 1-C), 134.88 (7-C), 135.58 (8-C), 147.10 (8a-C), 151.03 (2-C), 162.54 (4-C).



8-Methyl-2-(4-methylphenyl)quinazolin-4-one (7b). Compound **6b** (100 mg, 0.37 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7b** (75 mg, 81%) as a white solid: mp 269-271°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.39 (3 H, s, PhMe), 2.61 (3 H, s, 8-Me), 7.35-7.39 (3 H, m, Ph 3,5-H_2 + 6-H), 7.69 (1 H, dt, *J* =7 .0, 1.0 Hz, 7-H), 7.97 (1 H, dd, *J* = 8.0, 1.0 Hz, 5-H), 8.14 (2 H, d, *J* = 8.0 Hz, Ph 2,6-H_2), 12.44 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.16 (8-Me), 20.99 (PhMe), 120.78 (4a-C), 123.47 (5-C), 125.85 (6-C), 127.65 (Ph 2,6-C_2), 129.19 (Ph 3,5-C_2), 130.14 (8-C), 134.86 (7-C), 135.48 (Ph 1-C), 141.37 (Ph 4-C), 147.18 (8a-C), 150.97 (2-C), 162.56 (4-C); MS (EI) *m/z* 251.1115 $(M)^+$ ($C_{16}H_{15}N_2O$ requires 251.1106).



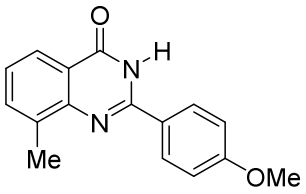
8-Methyl-2-(4-trifluoromethylphenyl)quinazolin-4-one (7c). Compound **6c** (100 mg, 0.30 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7c** (71 mg, 78%) as a white solid: mp 258-259°C (lit.[2] 255-257°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.69 (3 H, s, Me), 7.44 (1 H, t, *J* = 7.5 Hz, 6-H), 7.72 (1 H, d, *J* = 7.5 Hz, 7-H), 7.93 (2 H, d, *J* = 8.0 Hz, Ph 3,5-H_2), 8.00 (1 H, d, *J* = 8.0 Hz, 5-H), 8.41 (2 H, d, *J* = 8.5 Hz, Ph 2,6-H_2), 12.74 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.13 (Me), 123.97 (d, *J* = 271.3 Hz, CF_3), 123.54 (5-C), 125.53 (q, *J* = 3.6 Hz, Ph 3,5-C_2), 126.67 (6-C), 128.67 (Ph 2,6-C_2), 131.05 (q, *J* = 31.8 (Ph 4-C), 135.06 (7-C), 135.85 (8-C), 136.85 (Ph 1-C), 146.82 (8a-C), 149.90 (2-C), 162.41 (4-C).



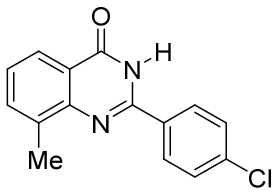
8-Methyl-2-(4-nitrophenyl)quinazolin-4-one (7d). Compound **6d** (100 mg, 0.33 mmol) was heated with aq. NaOH (2.5 M, 15 mL) at 100°C for 16 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7d** (90 mg, 97%) as a yellow solid: mp >300°C (lit.[2] 317-319°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.59 (3 H, s, Me), 7.18 (1 H, t, *J* = 7.5 Hz, 6-H), 7.47 (1 H, dd, *J* = 6.5, 0.5 Hz, 7-H), 7.90 (1 H, dd, *J* = 6.5, 0.5 Hz, 5-H), 8.30 (2 H, d, *J* = 9.0 Hz, Ph 3,5-H_2), 8.63 (2 H, d, *J* = 9.0 Hz, Ph 2,6-H_2); 12.80 (1H, br s, 12.80. ^{13}C NMR ($(CD_3)_2SO$) (HSQC /

HMBC) δ 17.34 (Me), 121.56 (4a-C), 123.11 (Ph 3,5-C_2), 123.54 (5-C), 123.59 (6-C), 128.83 (Ph 2,6-C_2), 132.02 (7-C), 134.31 (8-C), 145.22 (Ph 1-C), 149.35 (8a-C), 152.03 (Ph 4-C), 156.05 (2-C), 169.33 (4-C).

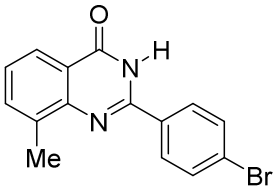
2-(4-Methoxyphenyl)-8-methylquinazolin-4-one (7e). Compound **6e** (100 mg, 0.35 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7e** (68 mg, 73%) as a white solid: mp 225-228°C (lit.[2] 227-229°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.60 (3 H, s, 8-Me), 3.48 (3 H, s, OMe), 7.09 (2 H, d, *J* = 8.5 Hz, Ph 3,5-H_2), 7.35 (1 H, t, *J* = 7.5 Hz, 6-H), 7.66 (1 H, d, *J* = 7.0 Hz, 7-H), 7.97 (1 H, dd, *J* = 8.0, 1.0 Hz, 5-H), 8.23 (2 H, d, *J* = 8.5 Hz, Ph 2,6-H_2), 12.39 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.16 (8-Me), 55.45 (OMe) , 114.00 (Ph 3,5-C_2), 120.55 (4a-C), 123.45 (5-C), 125.07 (Ph 1-C), 125.56 (6-C), 129.39 (Ph 2,6-C_2), 134.81 (7-C), 135.30 (8-C), 147.30 (8a-C), 150.61 (2-C), 161.82 (Ph 4-C), 162.60 (4-C).



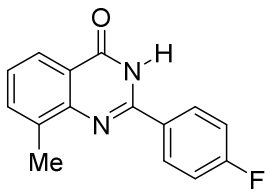
2-(4-Chlorophenyl)-8-methylquinazolin-4-one (7f). Compound **6f** (100 mg, 0.35 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7f** (80 mg, 85%) as a white solid: mp >300°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.61 (3 H, s, Me), 7.41 (1 H, t, *J* = 7.5 Hz, 6-H), 7.64 (2 H, d, *J* = 9.0 Hz, Ph 3,5-H_2), 7.70 (1 H, d, *J* = 7.0 Hz, 7-H), 7.98 (1 H, d, *J* = 8.0 Hz, 5-H), 8.25 (2 H, d, *J* = 9.0 Hz, Ph 2,6-H_2), 12.59 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.14 (Me), 120.91 (4a-C), 123.51 (7-C), 126.25 (6-C), 128.70 (Ph 3,5-C_2), 129.56 (Ph 2,6-H_2), 131.78 (Ph 1-C), 134.98 (5-C), 135.64 (8-C), 136.24 (Ph 4-C), 146.94 (8a-C), 150.06 (2-C), 162.46 (4-C); MS (ES) *m/z* 295.0418 $(M + Na)^+$ ($C_{15}H_{12}{}^{37}ClN_2NaO$ requires 295.0426), 293.0438 $(M + Na)^+$ ($C_{15}H_{12}{}^{35}ClN_2NaO$ requires 293.0458).



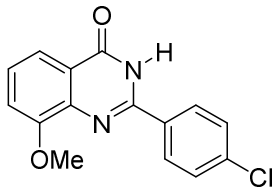
2-(4-Bromophenyl)-8-methylquinazolin-4-one (7g). Compound **6g** (100 mg, 0.30 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7g** (77 mg, 81%) as a white solid: mp >300°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.61 (3 H, s, Me), 7.41 (1 H, t, *J* = 7.5 Hz, 6-H), 7.70 (1 H, d, *J* = 7.0 Hz, 7-H), 7.77 (2 H, d , *J* = 8.5 Hz, Ph 3,5-H_2), 7.99 (1 H, dd, *J* = 8.5, 1.5 Hz, 5-H), 8.17 (2 H, d, *J* = 8.5 Hz, Ph 2,6-H_2), 12.58 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.16 (Me), 120.91 (4a-C), 123.50 (5-C), 125.16 (Ph 1-C), 126.25 (6-C), 129.74 (Ph 3,5-C_2), 131.62 (Ph 2,6-C_2), 132.13 (Ph 4-C), 134.97 (7-C), 135.63 (8-C), 146.92 (8a-C), 150.17 (2-C), 162.44 (4-C); MS (ES) *m/z* 338.9934 $(M + Na)^+$ ($C_{15}H_{11}{}^{81}BrN_2NaO$ requires 338.9967), 336.9953 $(M + Na)^+$ ($C_{15}H_{11}{}^{79}BrN_2NaO$ requires 336.9952).



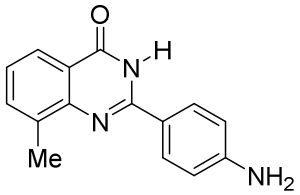
2-(4-Fluorophenyl)-8-methylquinazolin-4-one (7h). Compound **6h** (100 mg, 0.37 mmol) was heated with aq. NaOH (0.5 M, 15 mL) at 60°C for 3.5 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7h** (80 mg, 86%) as a white solid: mp



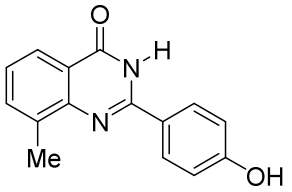
>300°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.61 (3 H, s, Me), 7.38-7.42 (3 H, m, Ph 3,5-H_2 + 6-H), 7.69 (1 H, d, J = 7.0 Hz, 7-H), 7.99 (1 H, m, 5-H), 8.30 (2 H, m, Ph 2,6-H_2), 12.55 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.14 (Me), 115.64 (d, J = 21.8 Hz, Ph 3,5-C_2), 120.77 (4a-C), 123.48 (5-C), 126.07 (6-C), 129.44 (Ph 1-C), 130.30 (d, J = 9.0 Hz, Ph 2,6-C_2), 134.94 (7-C), 135.55 (8-C), 147.01 (8a-C), 150.09 (2-C), 162.77 (d, J = 248.0 Hz, Ph 4-C), 165.01 (4-C); MS (EI) *m/z* 255.0919 $(M)^+$ ($C_{15}H_{12}FN_2O$ requires 255.0933).



2-(4-Chlorophenyl)-8-methoxyquinazolin-4-one (7i). Compound **6i** (409 mg, 1.3 mmol) was heated with aq. NaOH (0.5 M, 80 mL) at 60°C for 6 h. The mixture was acidified by addition of aq, HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7i** (330 mg, 87%) as a white solid: mp 297-299°C; ^{1}H NMR ($(CD_3)_2SO$) δ 3.94 (3H, s, Me), 7.39 (1 H, dd, J = 8.0, 1.0 Hz, 7-H), 7.45 (1 H, t, J = 8.0 Hz, 6-H), 7.63 (2 H, d, J = 8.5 Hz, Ph 3,5-H_2), 7.70 (1 H, dd, J = 7.5, 1.0 Hz, 5-H), 8.20 (2 H, d, J = 8.5 Hz, Ph 2,6-H_2), 12.59 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 56.03 (Me), 115.21 (7-C), 116.86 (5-C), 122.04 (4a-C), 127.06 (6-C), 128.64 (Ph 3,5-C_2), 129.55 (Ph 2,6-C_2), 131.77 (Ph 1-C), 136.08 (Ph 4-C), 149.90 (2-C), 154.70 (8-C), 162.11 (4-C); MS (ES) *m/z* 311.0371 $(M + Na)^+$ ($C_{15}H_{11}{}^{37}ClN_2NaO_2$ requires 311.0407), 309.0391 $(M + Na)^+$ ($C_{15}H_{11}{}^{35}ClN_2NaO_2$ requires 309.0407], 287.0569 $(M)^+$ ($C_{15}H_{12}{}^{35}ClN_2O_2$ requires 287.0587).

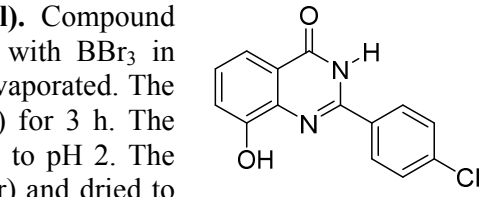


2-(4-Aminophenyl)-8-methylquinazolin-4-one (7j). Compound **7d (**74 mg, 0.26 mmol) was stirred with Pd/C (10%, 10 mg) and ammonium formate (170 mg, 2.6 mmol) in MeOH (6 mL) and DMF (6 mL) under Ar for 3 h. The mixture was filtered through Celite. Evaporation and chromatography (EtOAc) gave **7j** (41 mg, 62%) as a white solid: mp 256-258°C (lit.[2] 254-256°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.57 (3 H, s, Me), 5.82 (2 H, s, NH_2), 6.64 (2 H, d, J = 8.5 Hz, Ph 3,5-H_2), 7.28 (1 H, t, J = 7.5 Hz, 6-H), 7.62 (1 H, d, J = 7.0 Hz, 7-H), 7.92 (1 H, dd, J = 7.0, 0.5 Hz, 5-H), 8.00 (2 H, d, J = 8.5 Hz, Ph 2,6-H_2), 12.05 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.20 (Me), 113.03 (Ph 3,5-C_2), 119.09 (Ph 1-C), 120.13 (4a-C), 123.41 (5-C), 124.69 (6-C), 129.08 (Ph 2,6-C), 134.61 (7-C), 134.86 (8-C), 147.74 (8a-C), 151.22 (2-C), 152.03 (Ph 4-C), 162.68 (4-C).

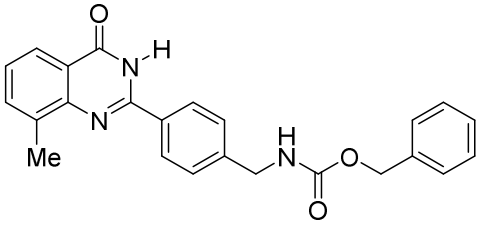


2-(4-Hydroxyphenyl)-8-methylquinazolin-4-one (7k). Compound **7e** (25 mg, 0.094 mmol) was boiled under reflux with BBr_3 in CH_2Cl_2 (1.0 M, 0.56 mL) for 3 h. The solvent was evaporated. The residue was stirred with aq. NaOH (2.5 M, 10 mL) for 3 h. The mixture was acidified by addition of aq. HCl (9 M) to pH 2. The mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried and the solvent was evaporated to give **7k** (20 g, 83%) as a white solid: mp 262-265°C (lit.[2] 258-261°C); ^{1}H NMR ($(CD_3)_2SO$) δ 2.60 (3 H, s, Me), 6.90 (2 H, d, J = 9.0 Hz, Ph 3,5-H_2), 7.34 (1 H, t, J = 7.5 Hz, 6-H), 7.66 (1 H, d, J = 7.0 Hz, 7-H), 7.95 (1 H, d, J = 7.5 Hz, 5-H), 8.13 (2 H, d, J = 9.0 Hz, Ph 2,6-H_2), 12.28 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.89 (Me), 116.06 (Ph 3,5-C_2), 121.16 (4a-C), 124.16 (5-C), 124.20 (Ph 1-C), 126.07 (6-C), 130.24 (Ph 2,6-C_2), 135.48 (7-C), 135.92 (8-C), 148.13 (8a-C), 151.59 (2-C), 161.21 (Ph 4-C), 163.34 (4-C).

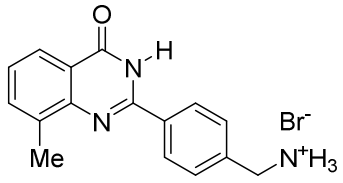
2-(4-Chlorophenyl)-8-hydroxyquinazolin-4-one (7l). Compound **7i** (100 mg, 0.35 mmol) was boiled under reflux with BBr_3 in CH_2Cl_2 (1.0 M, 1.4 mL) for 16 h. The solvent was evaporated. The residue was stirred with aq. NaOH (2.5 M, 15 mL) for 3 h. The mixture was acidified by addition of aq, HCl (9 M) to pH 2. The precipitate was collected by filtration, washed (water) and dried to give **7l** (85 mg, 89%) as a white solid: mp 260-263°C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.23 (1 H, dd, *J* = 7.5, 1.0 Hz, 7-H), 7.34 (1 H, t, *J* = 8.0 Hz, 6-H), 7.57 (1 H, dd, *J* = 7.5, 1.0 Hz, 5-H), 7.62 (2 H, d, *J* = 7.0 Hz, Ph 3,5-H_2), 8.45 (2 H, d, *J*= 7.0 Hz, Ph 2,6-H_2), 9.66 (1 H, s, OH), 12.52 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 115.67 (5-C), 118.47 (7-C), 121.74 (4a-C), 127.33 (6-C), 128.51 (Ph 3,5-C_2), 129.84 (Ph 2,6-C), 131.36 (Ph 1-C), 136.24 (Ph 4-C), 137.45 (8a-C), 149.31 (2-C), 153.05 (8-C), 162.20 (4-C); MS (ES) *m/z* 297.0227 $(M + Na)^+$ ($C_{14}H_9{}^{37}ClN_2NaO_2$ requires 297.0250), 295.0218 $(M + Na)^+$ ($C_{14}H_9{}^{35}ClN_2NaO_2$ requires 295.0250).



8-Methyl-2-(4-(phenylmethoxycarbonylamino-methyl)phenyl)quinazolin-4-one (10). Compound **8** (4.00 g, 26.5 mmol) was stirred vigorously with benzyl chloroformate (4.52 g, 26.5 mmol) and aq. K_2CO_3 (1.0 M, 144 mL) for 16 h. The precipitate was collected by filtration and dried. This material was stirred with thionyl chloride (10 mL) under Ar for 16 h. The thionyl chloride was evaporated. The residue was suspended in CH_2Cl_2 and filtered. The solvent was evaporated to give the acyl chloride (6.52 g, 81%) as a colorless gum, which was used immediately without purification. This compound (700 mg, 2.3 mmol) was stirred with **5a** (290 g, 1.9 mmol) and dry pyridine (316 mg, 4.0 mmol) in dry THF (20 mL) under Ar for 16 h. The solvent was evaporated. The residue, in EtOAc (30 mL) was washed twice with water and twice with brine. Drying and evaporation gave crude **9** (480 mg) as a white solid. This material (100 mg, 0.24 mmol) was suspended in aq. K_2CO_3 (1.0 M, 58 mL) and the mixture was stirred vigorously for 16 h at 100°C. The mixture was cooled to 20°C and acidified to pH~1 by addition of aq. HCl (9 M). The precipitate was collected by filtration and dried. Chromatography (EtOAc / petroleum ether 3:2) gave **10** (86 mg, 89%) as a white solid: mp 280-283°C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.60 (3 H, s, Me), 4.29 (2 H, d, *J* = 6.5 Hz, Ar 4-CCH_2), 5.07 (2 H, s, Cbz-CH_2), 7.23-7.39 (8 H, m, 6-H + Ar 3,5-H_2 + Ph-H_5), 7.59 (1 H, d, *J* = 7.0 Hz, 7-H), 7.93 (1 H, br, Ar 4-CH_2NH), 8.22 (2 H, d, *J* = 8.0 Hz, Ar 2,6-H_2), 12.50 (1 H, s, NH); ^{13}C NMR ($(CD_3)_2SO$) (HSQC / HMBC) δ 17.96 (Me), 44.36 (Ar 4-CCH_2), 66.17 (Cbz-CH_2), 121.78 (4a-C), 124.22 (5-C), 125.43 (6-C), 127.58 (Ar 3,5-C_2), 128.48 (Ar 2,6-C_2 + Ph 2,6-C_2), 128.53 (Cbz 4-C), 129.09 (Ph 3,5-C_2), 134.43 (7-C), 135.64 (8-C), 137.87 (Ar 1-C + Ph 1-C), 143.10 (Ar 4-C), 148.79 (8a-C), 157.15 (Cbz-CO), 165.60 (4-C); MS *m/z* (ES) 422.1497 $(M + Na)^+$ ($C_{24}H_{21}N_3NaO_3$ requires 422.1481).

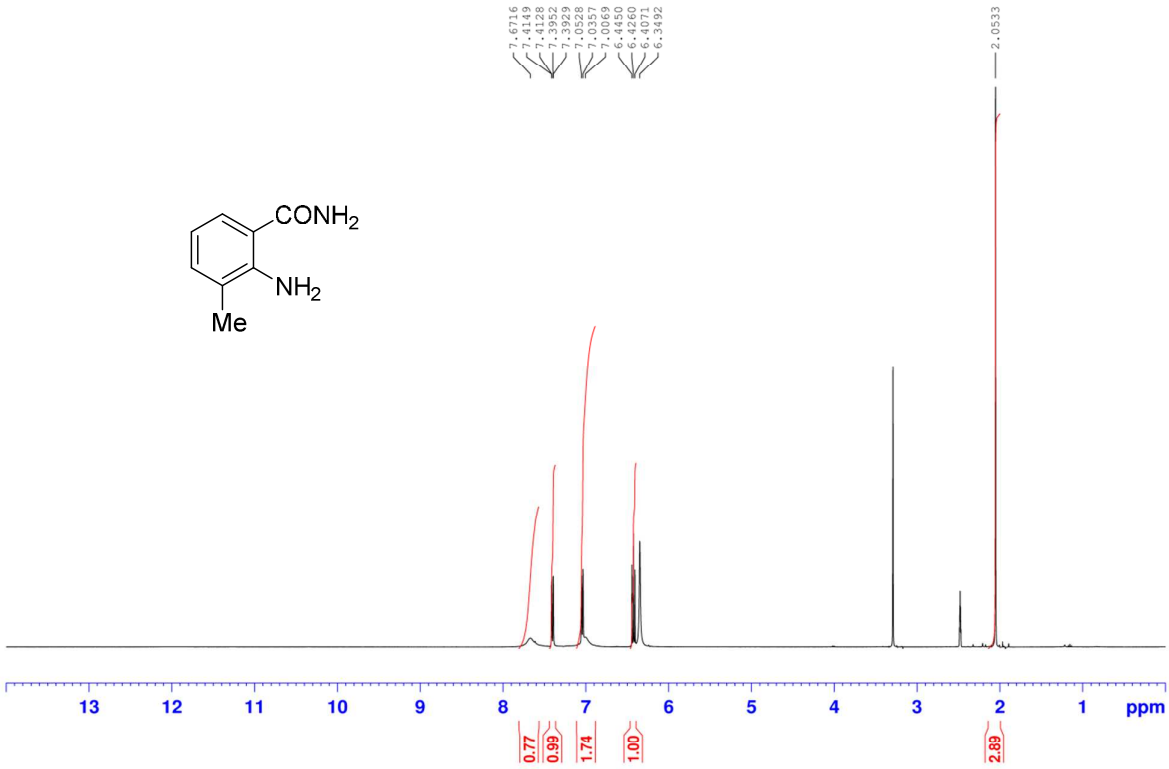


2-(4-Aminomethylphenyl)-8-methylquinazolin-4-one hydrobromide (11). Compound **10** (200 mg, 0.62 mmol) was treated with HBr in AcOH (33%, 1.5 mL) for 16 h. Evaporation gave **11** (190 mg, 93%) as a white solid: mp >300°C; ^{1}H NMR ($(CD_3)_2SO$ / CD_3OD 1:1) δ 2.69 (3 H, s, Me), 4.14 (2 H, m, CH_2), 7.41 (1 H, t, *J* = 7.5 Hz, 6-H), 7.64 (2 H, d, *J* = 8.0 Hz, Ph 3,5-H_2), 7.71 (1 H, d, *J* = 7.5 Hz, 7-H), 8.06 (1 H, d, *J* = 7.5 Hz, 5-H), 8.26 (2 H, d, *J* = 8.0 Hz, Ar 2.6-H_2), 8.28 (3 H, br, $^+NH_3$), 12.59 (1 H, s, NH); ^{13}C NMR

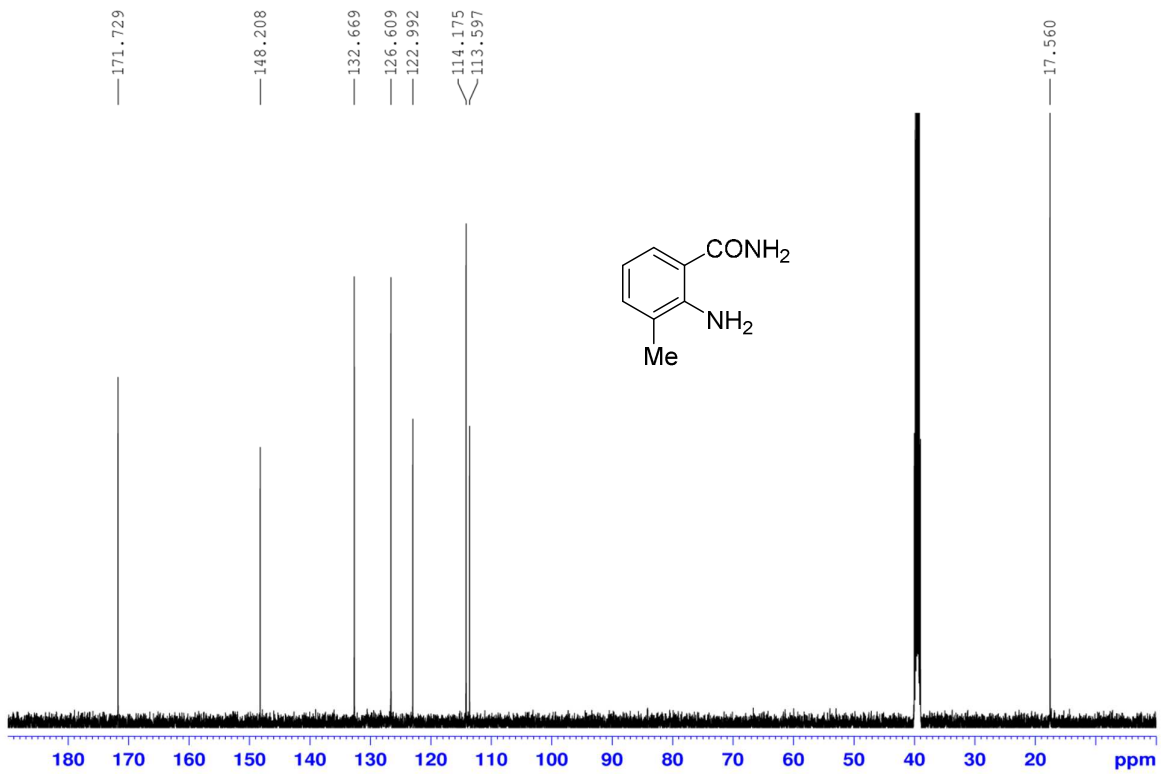


($(CD_3)_2SO$) (HSQC / HMBC) δ 17.16 (Me), 41.89 (CH_2), 120.92 (4a-C), 123.52 (5-C), 126.25 (6-C), 127.93 (Ph 2,6-C_2), 129.00 (Ph 3,5-C_2), 130.35 (Ph 1-C), 135.01 (7-C), 135.64 (8-C), 137.00 (Ph 4-C), 147.00 (8a-C), 150.08 (2-C), 162.04 (4-C); MS (ES) *m/z* 288.1101 $(M + Na)^+$ ($C_{16}H_{15}N_3NaO$ requires 288.1113).

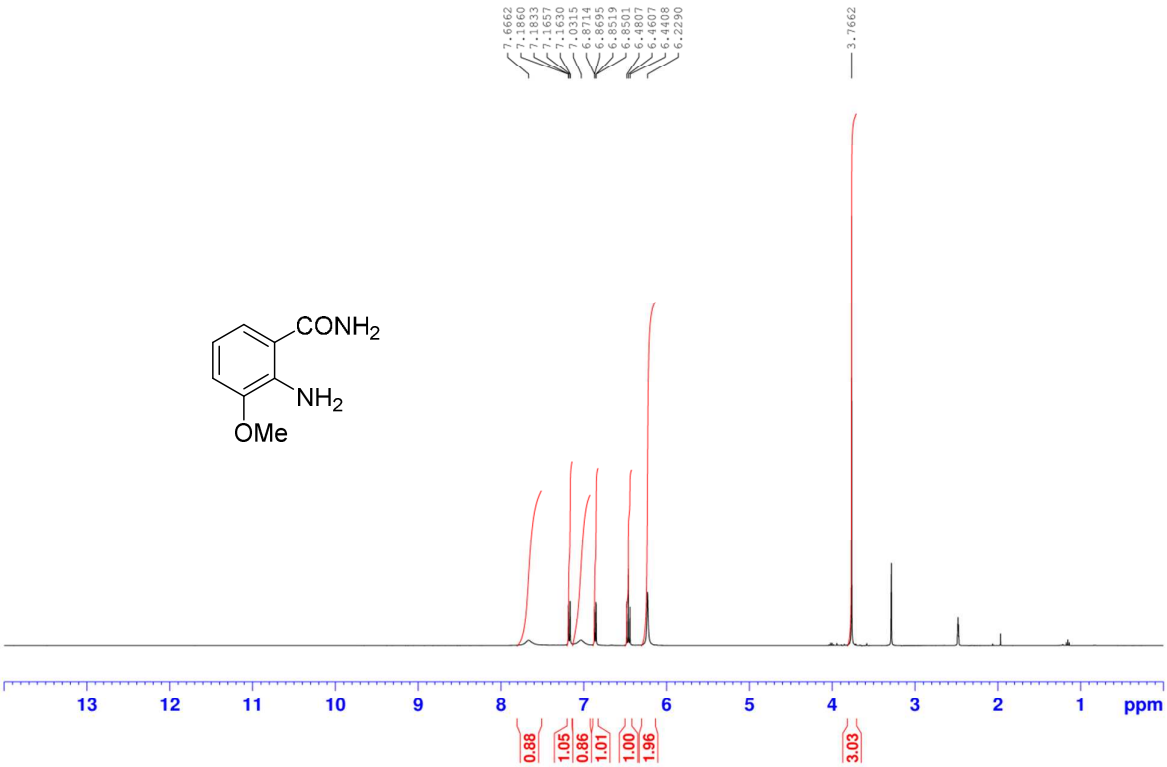
Section C: NMR spectra for compounds synthesized



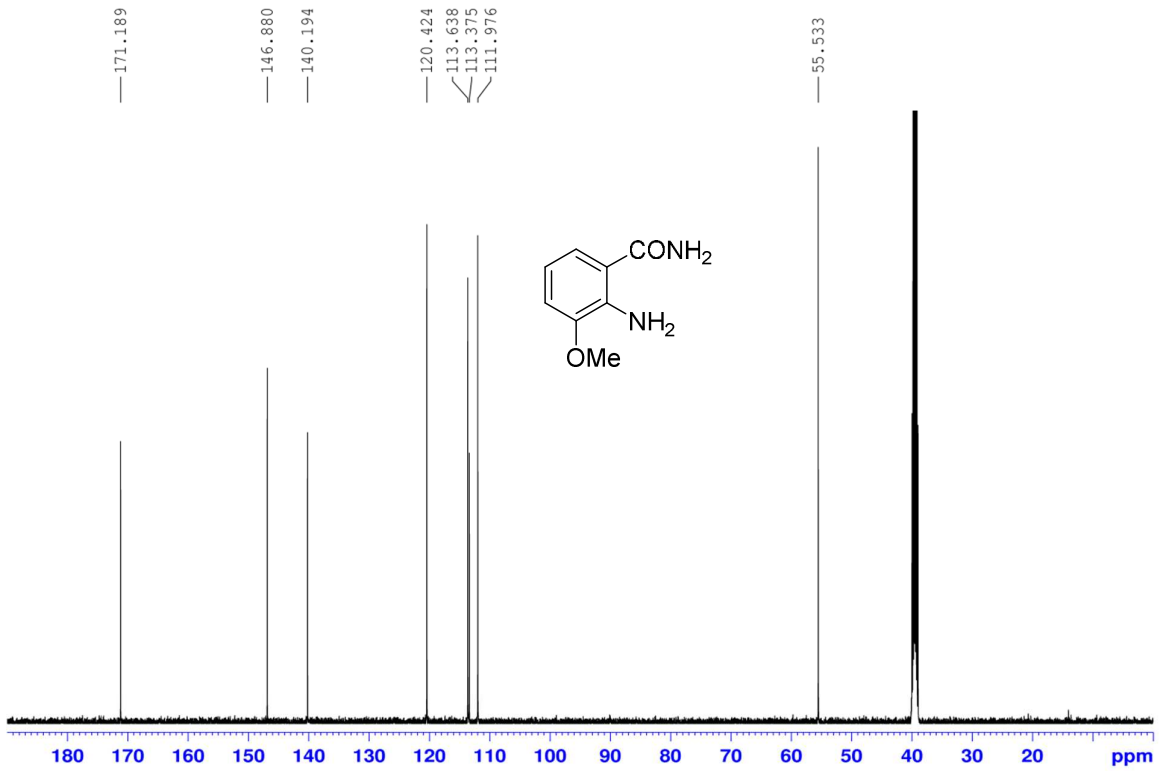
^{1}H NMR spectrum of 5a



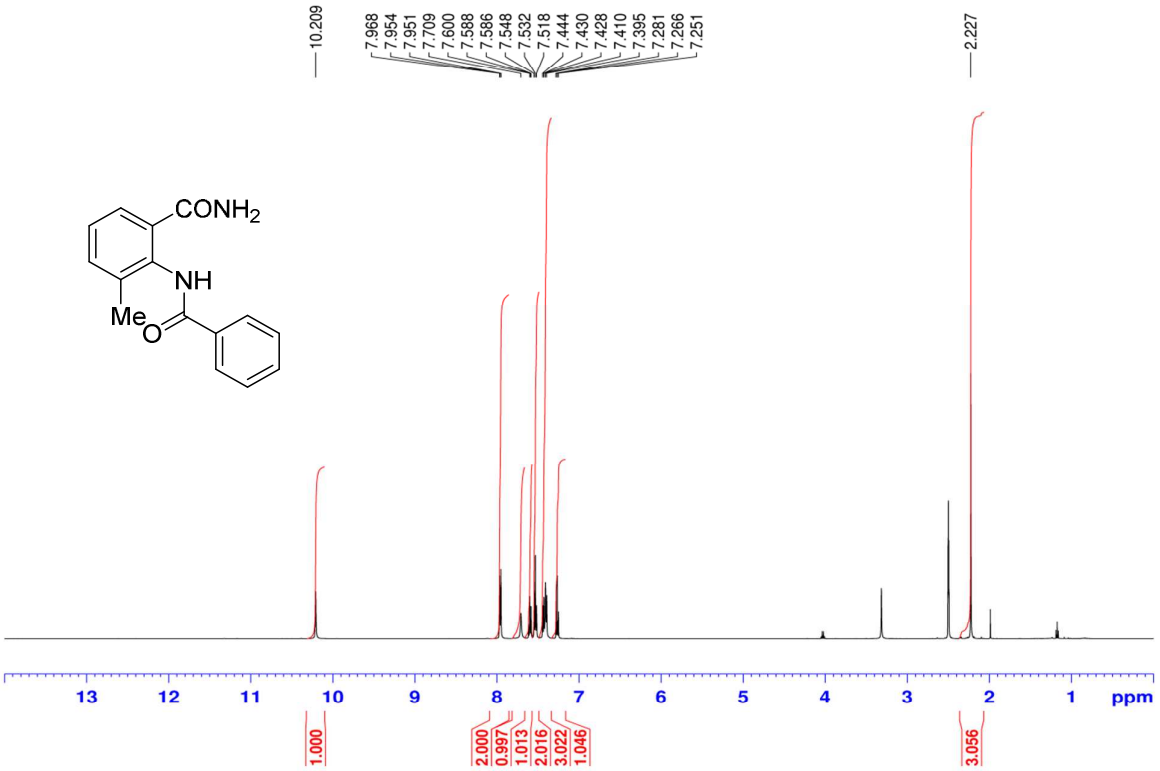
^{13}C NMR spectrum of 5a



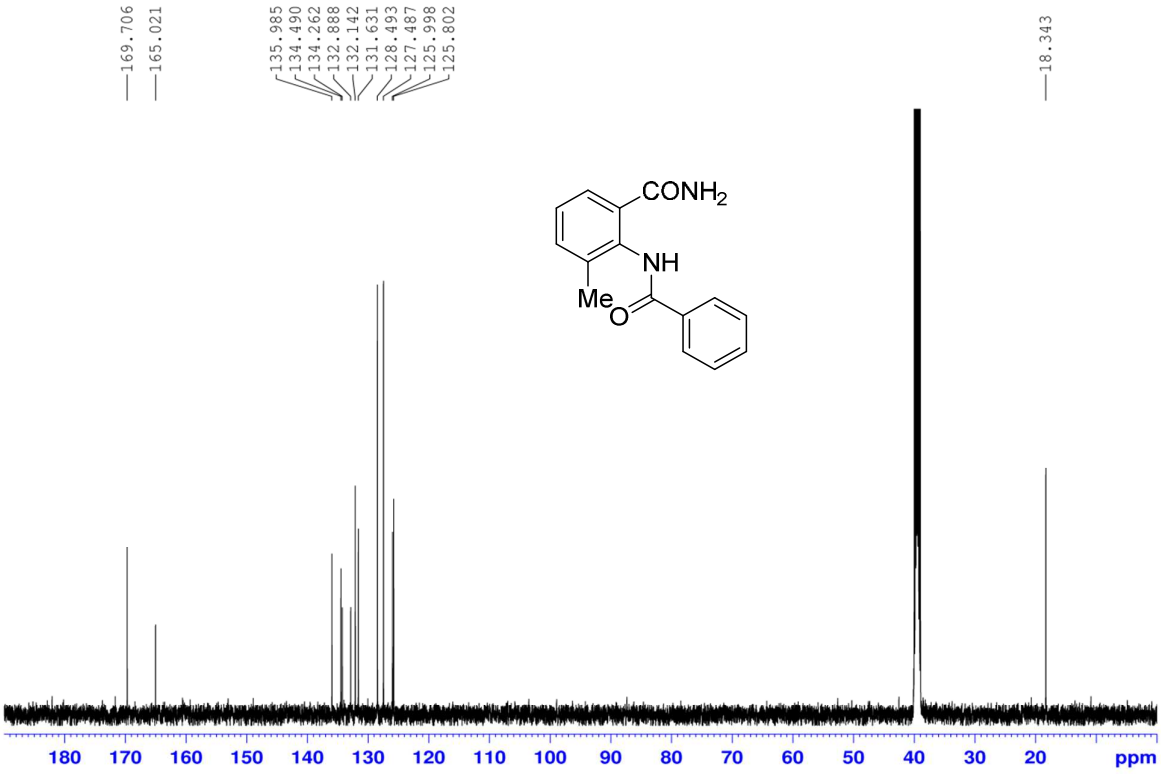
^{1}H NMR spectrum of 5b



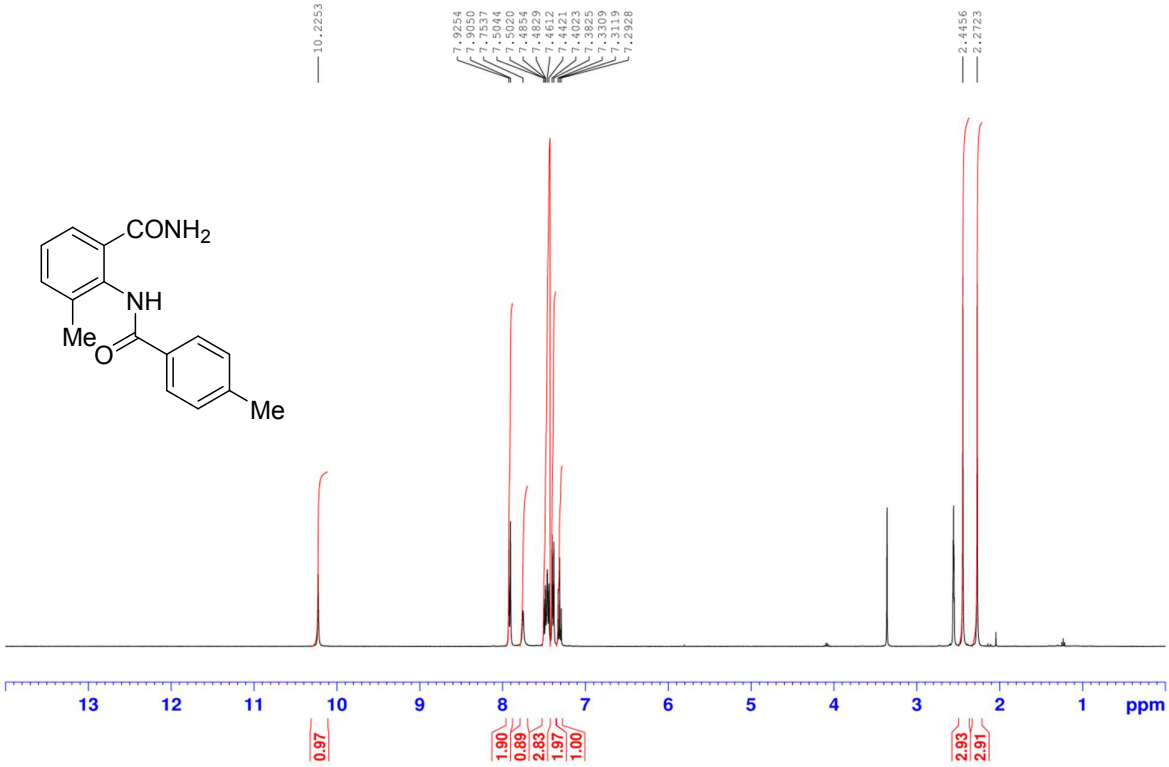
^{13}C NMR spectrum of 5b



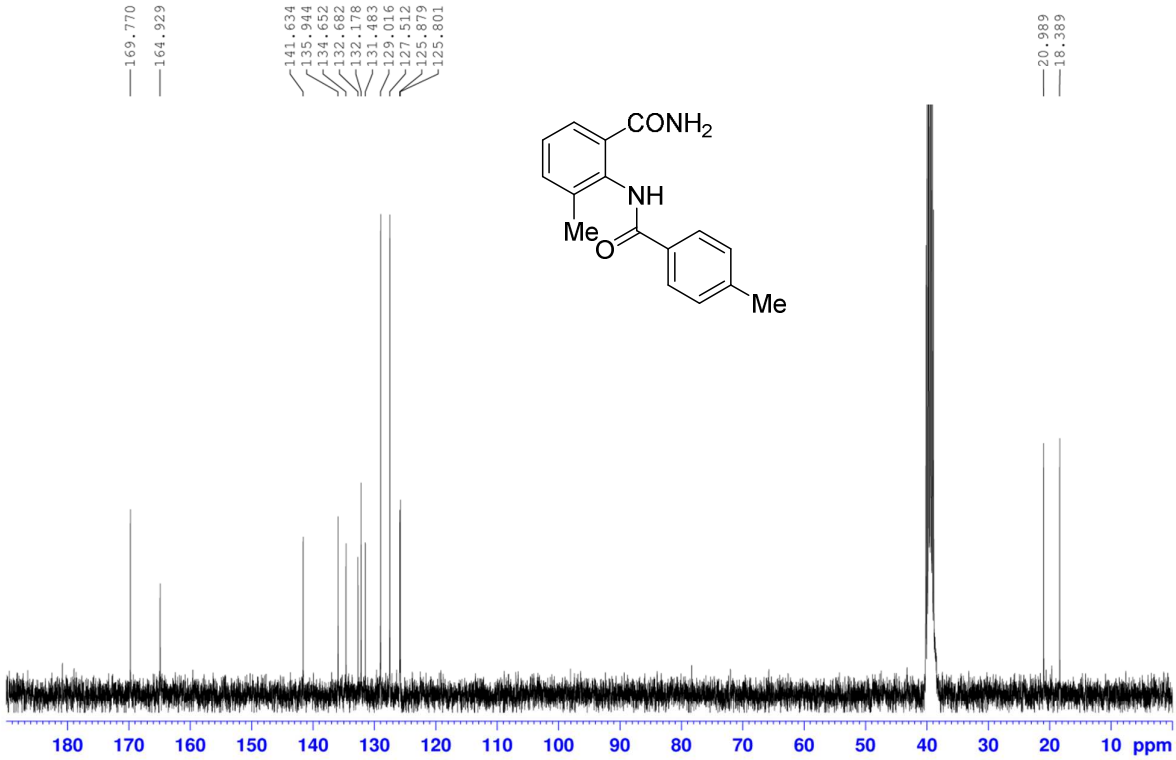
^{1}H NMR spectrum of 6a



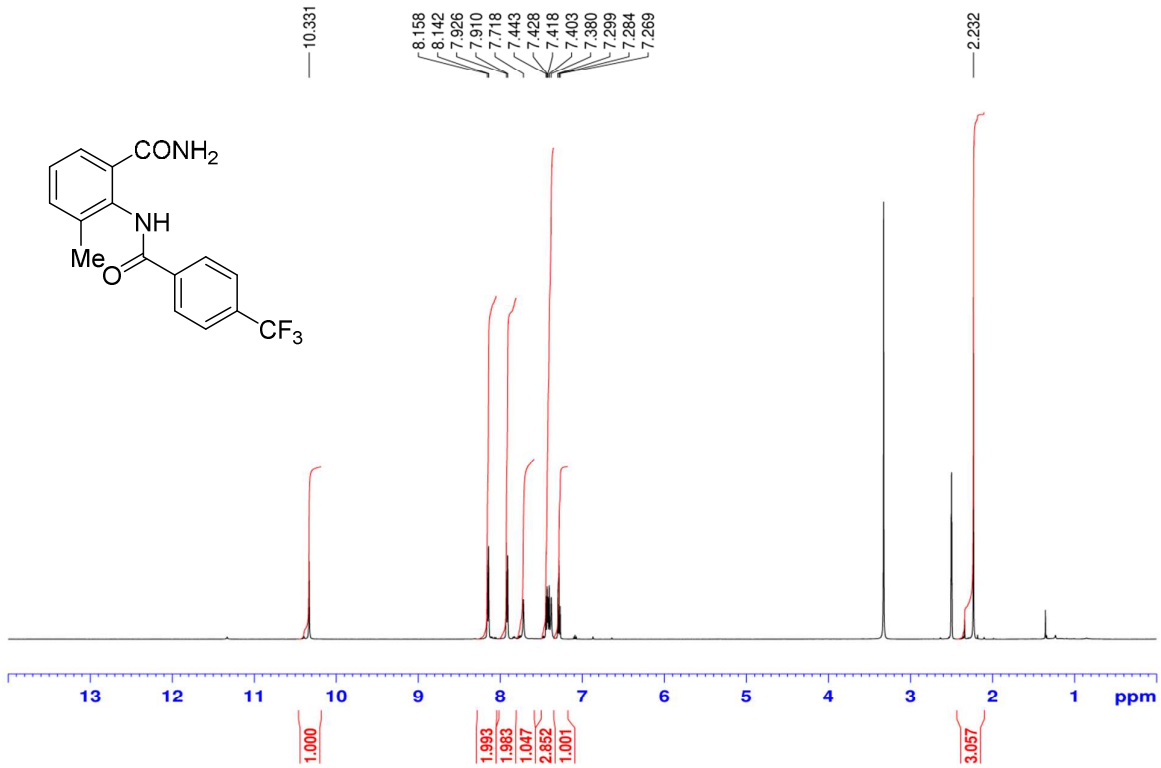
^{13}C NMR spectrum of 6a



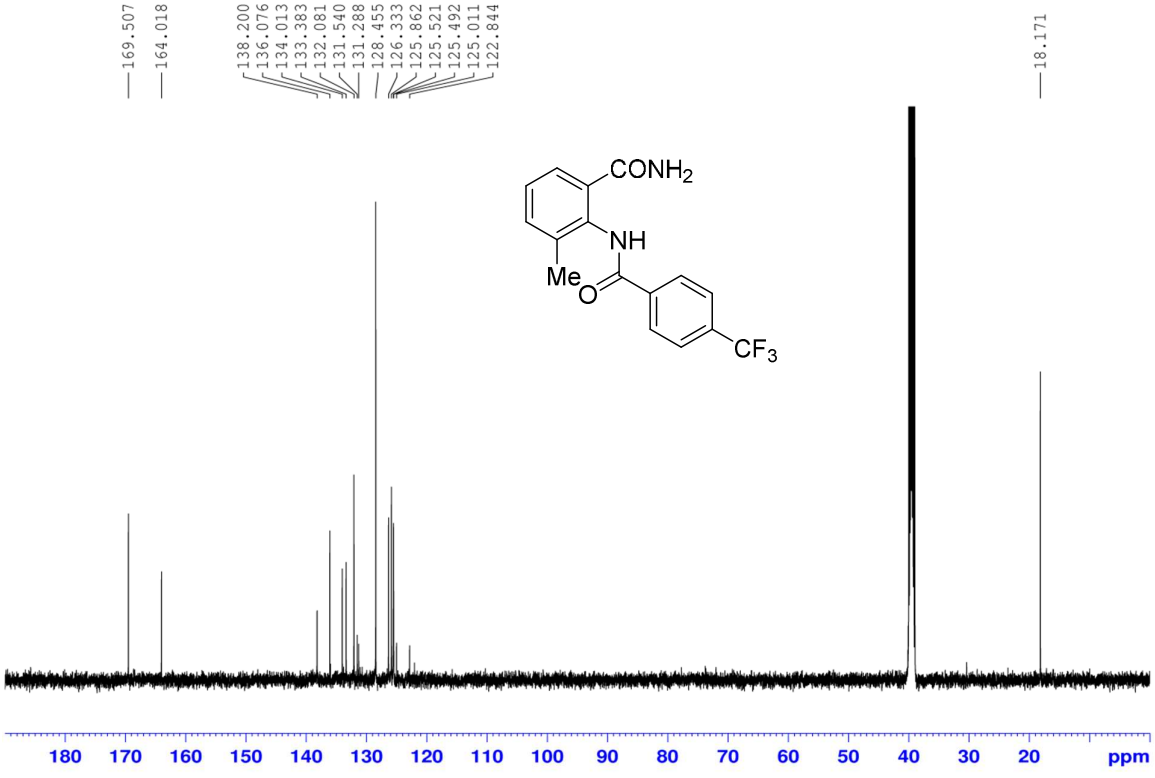
[1]H NMR spectrum of 6b



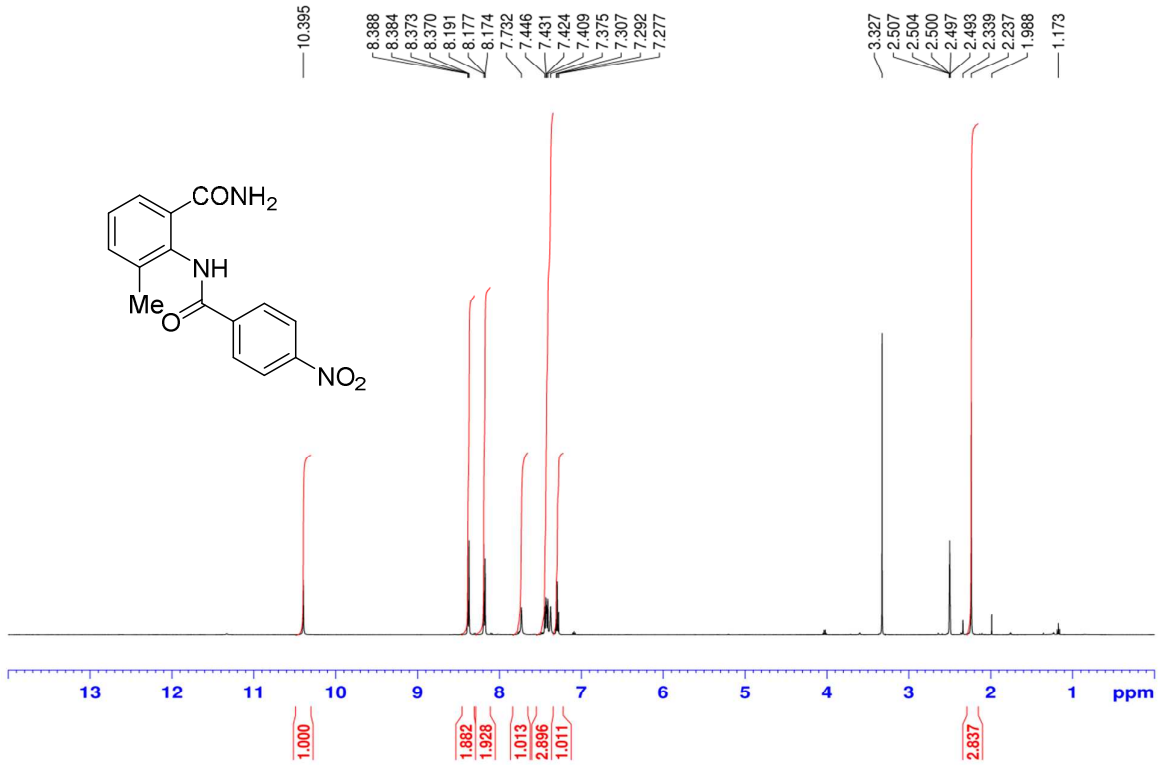
[13]C NMR spectrum of 6b



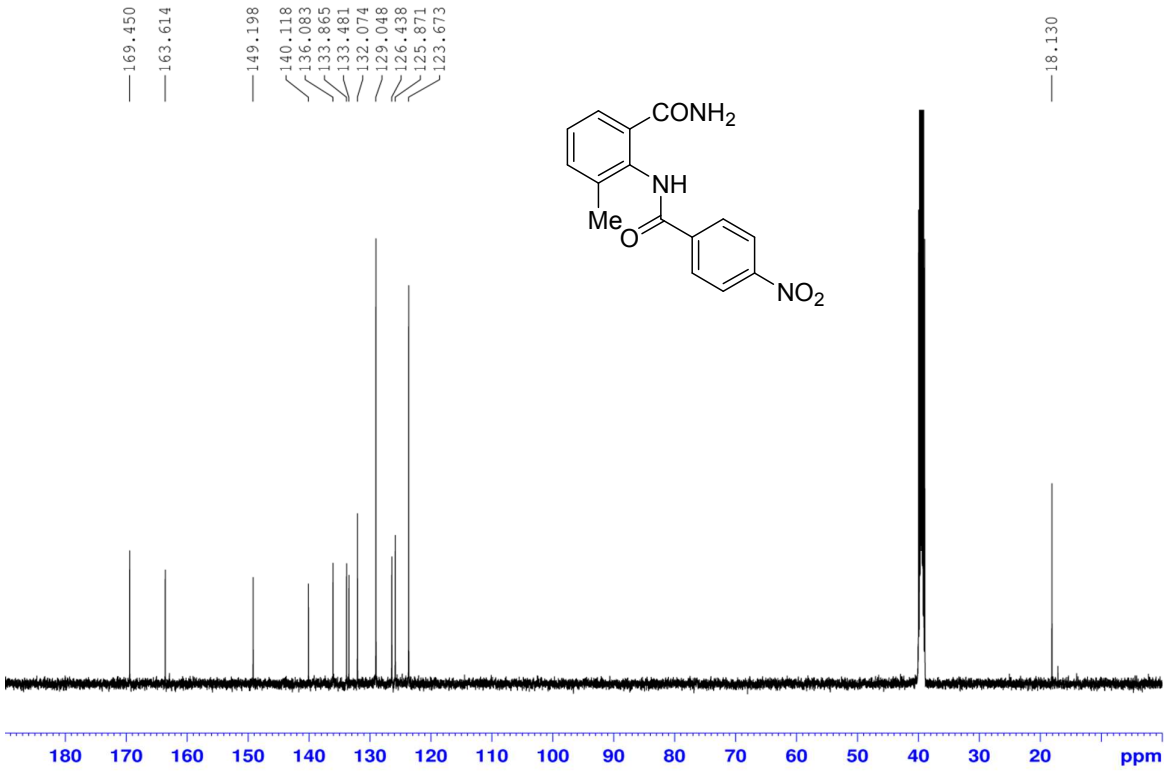
^{1}H NMR spectrum of 6c



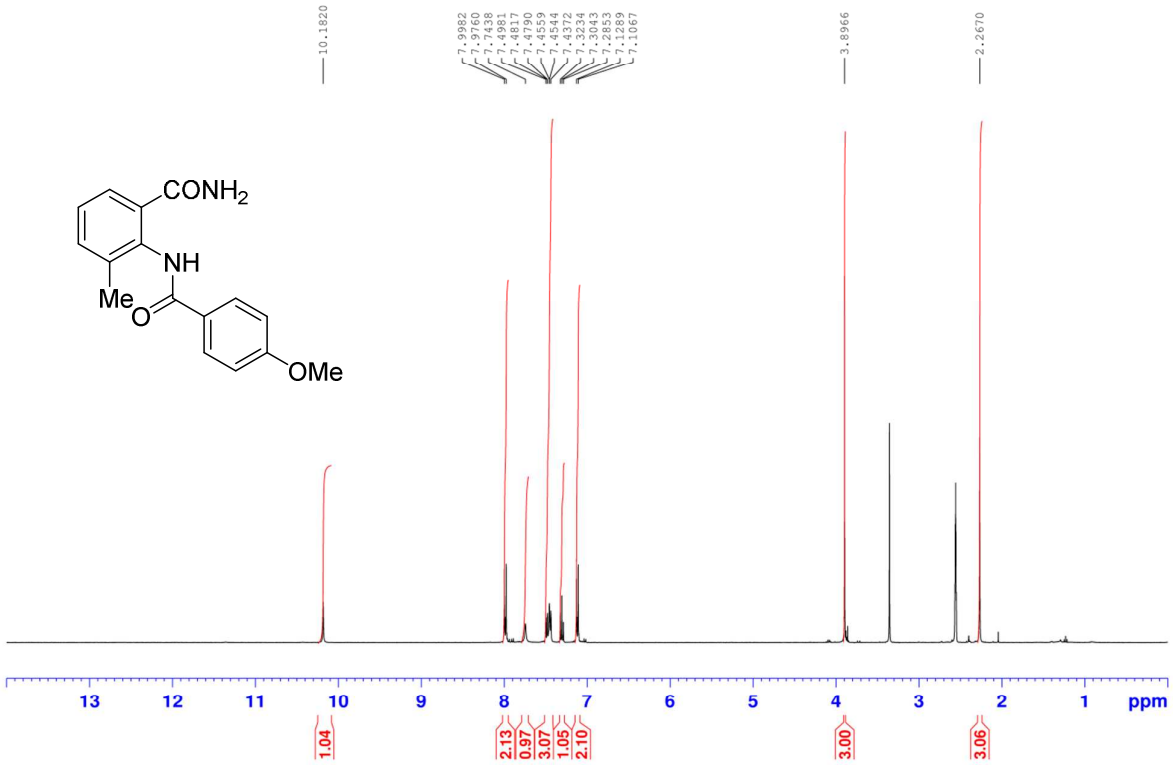
^{13}C NMR spectrum of 6c



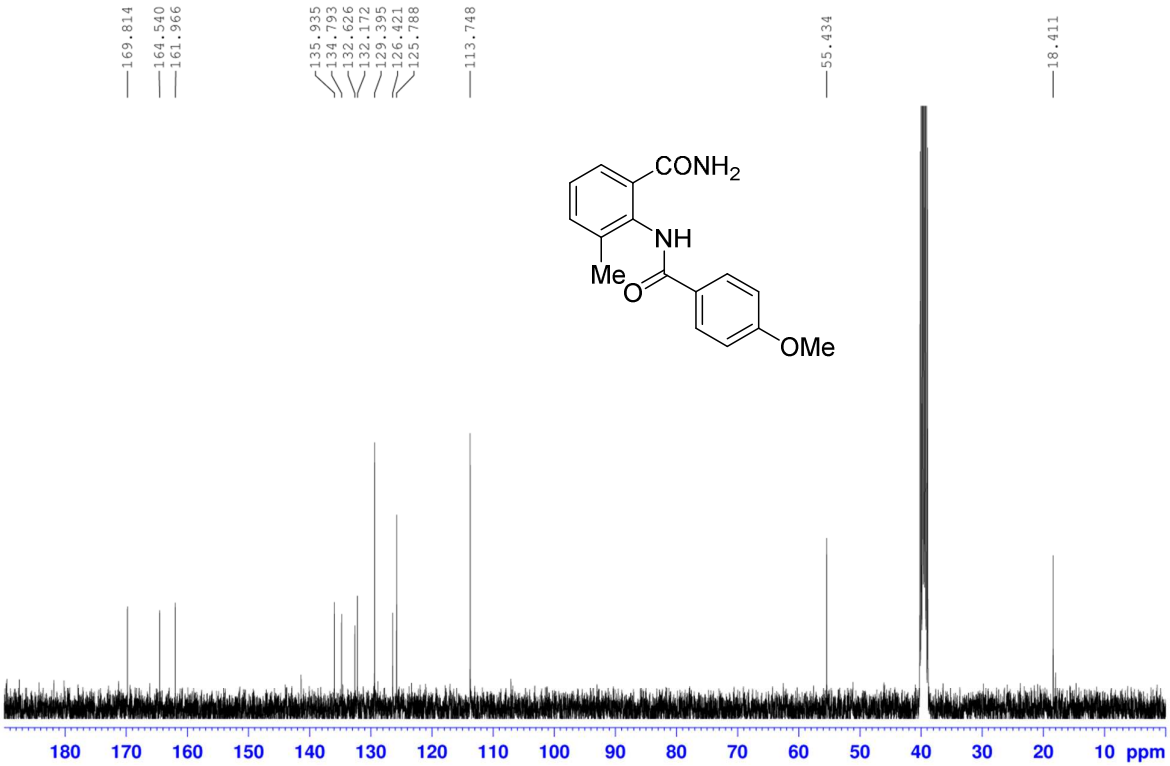
^{1}H NMR spectrum of 6d



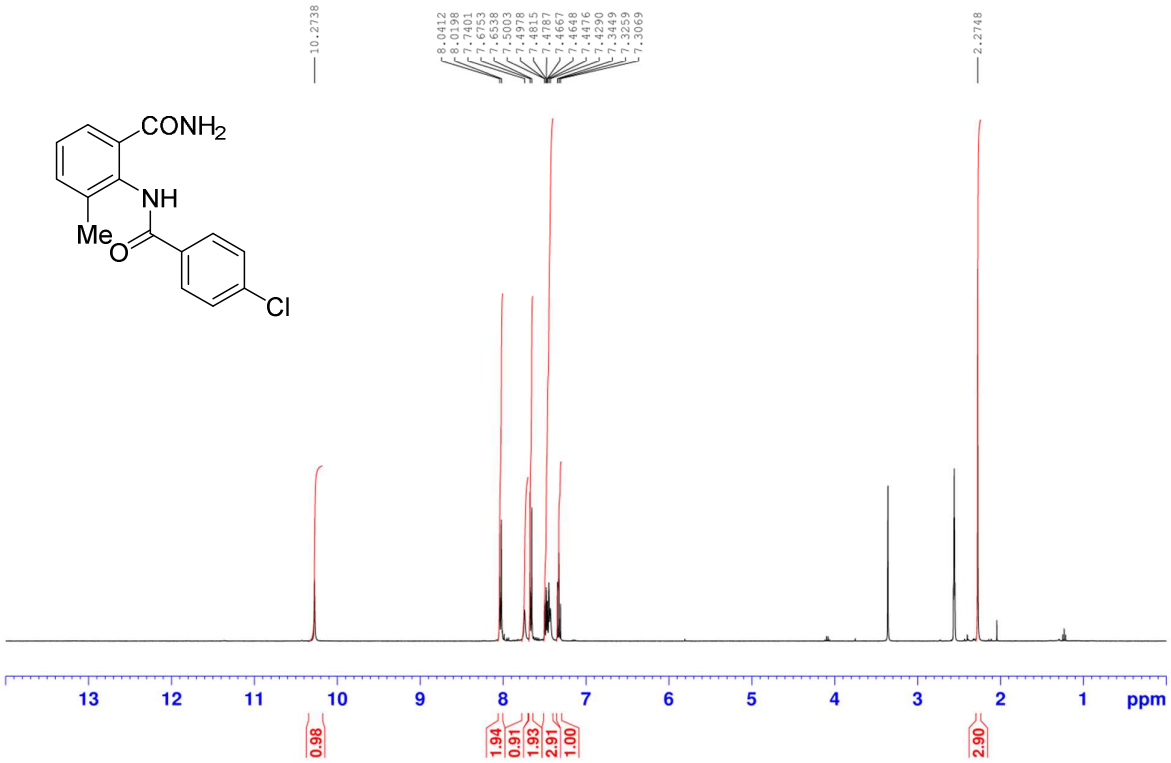
^{13}C NMR spectrum of 6d



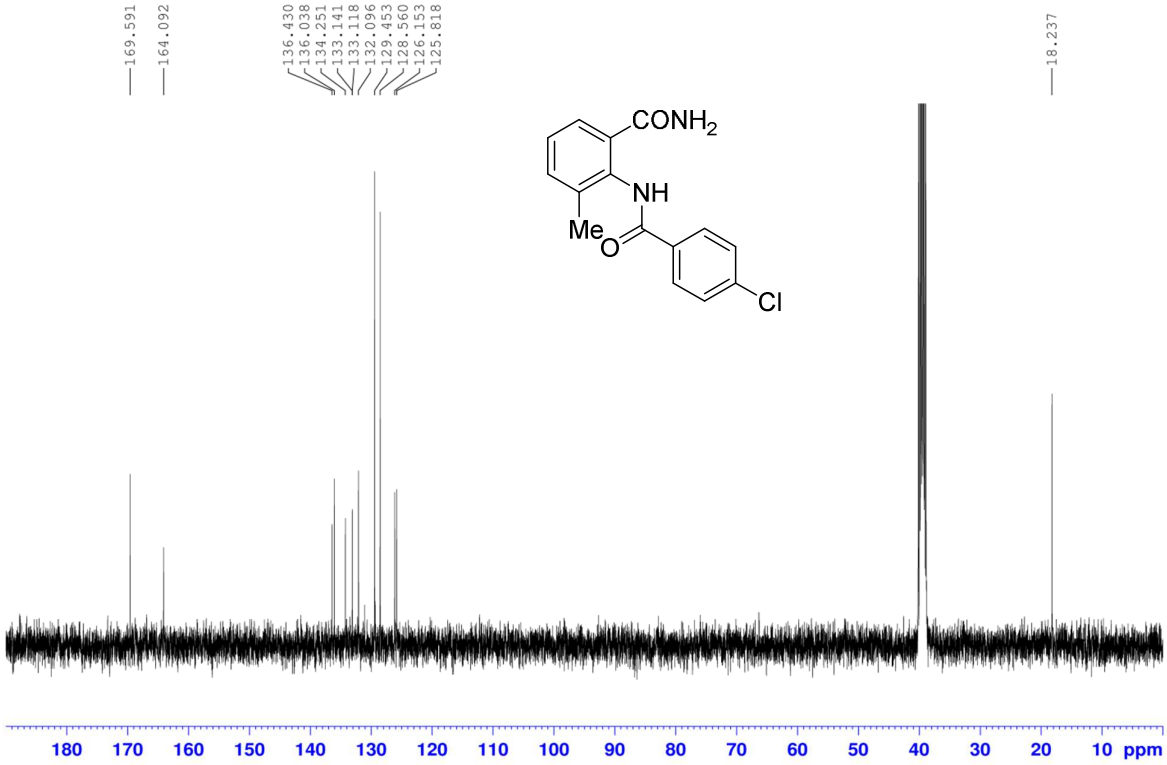
^{1}H NMR spectrum of 6e



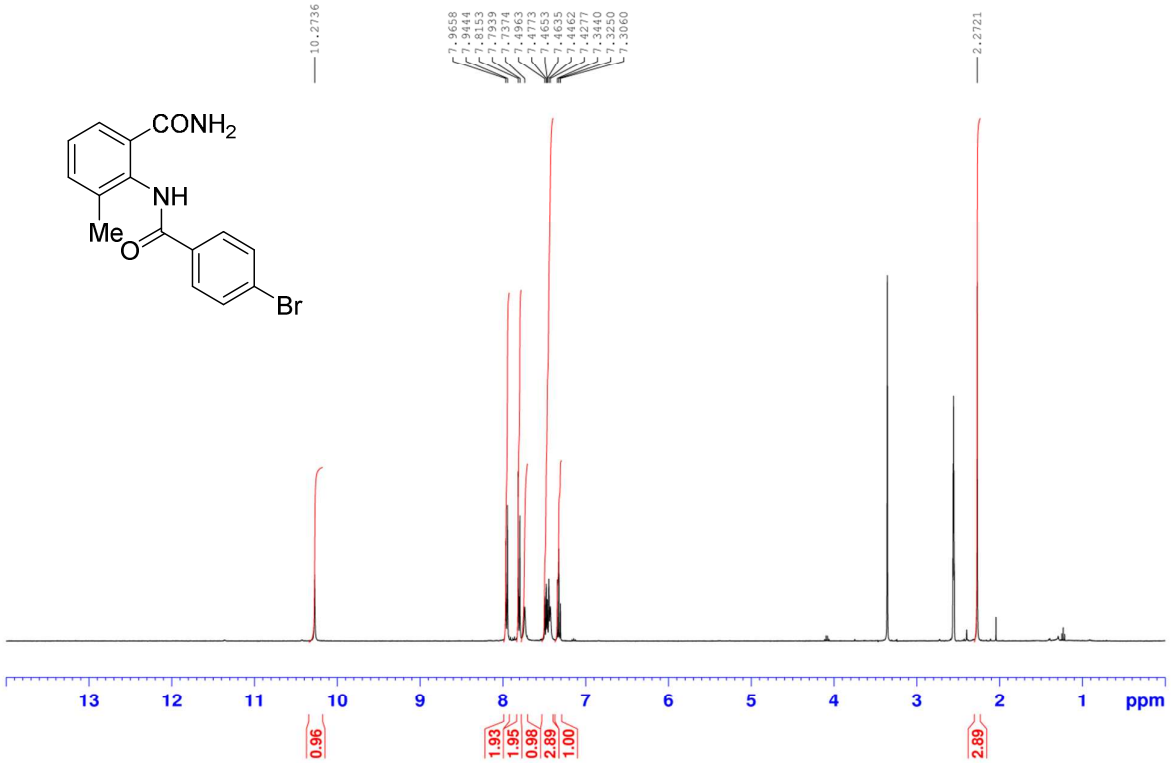
^{13}C NMR spectrum of 6e



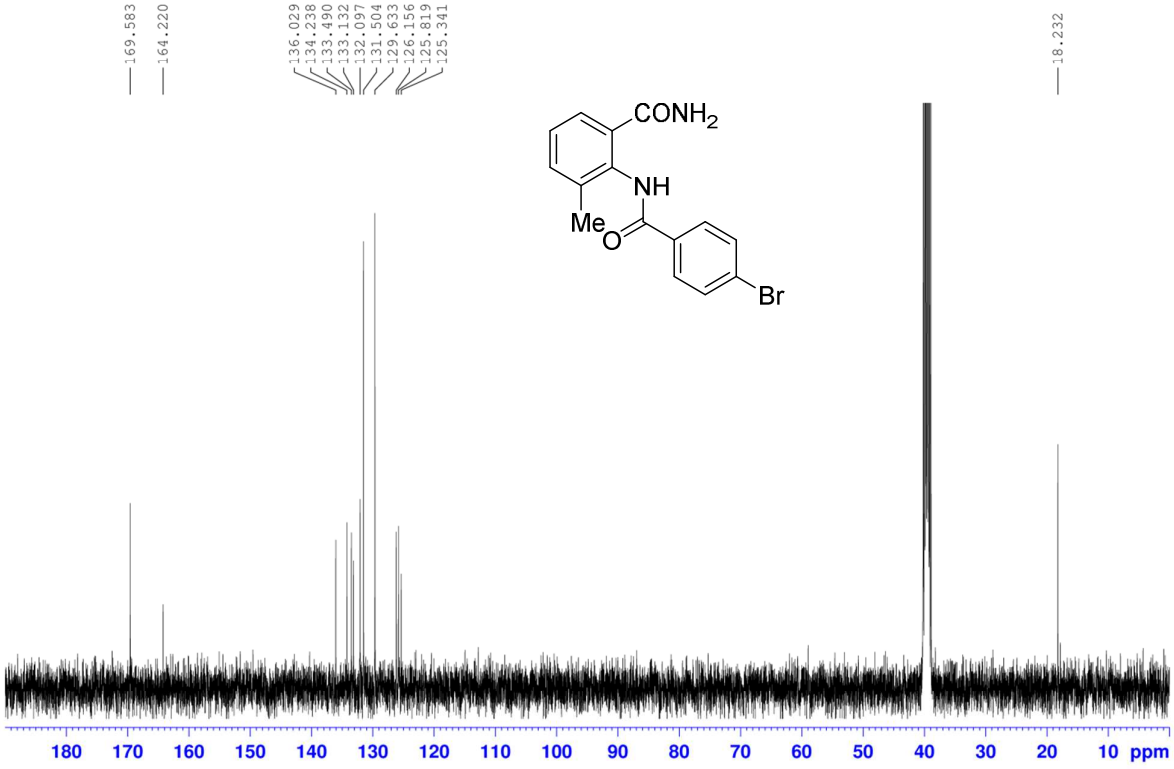
^{1}H NMR spectrum of 6f



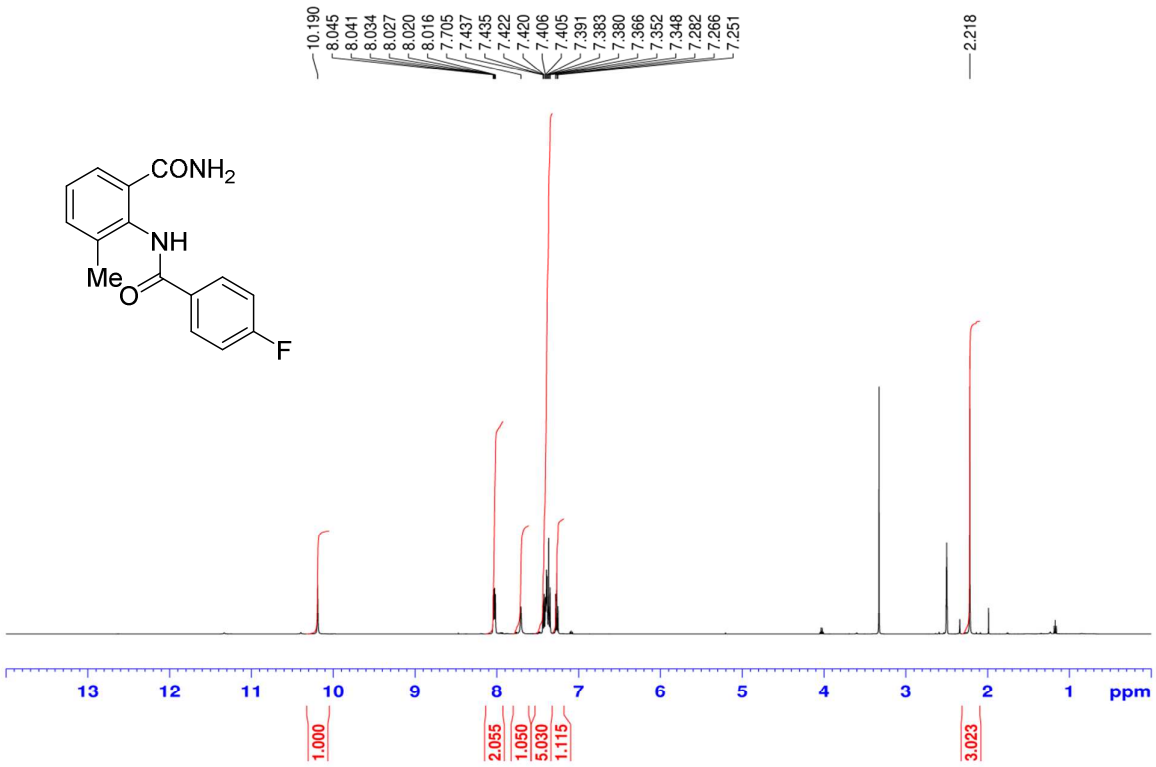
^{13}C NMR spectrum of 6f



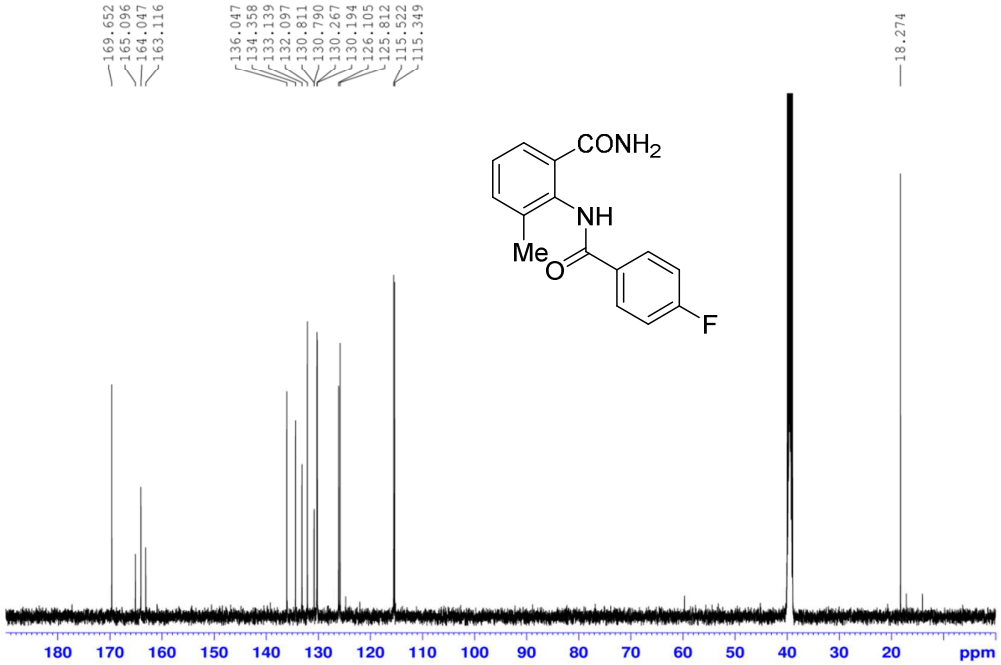
^{1}H NMR spectrum of 6g



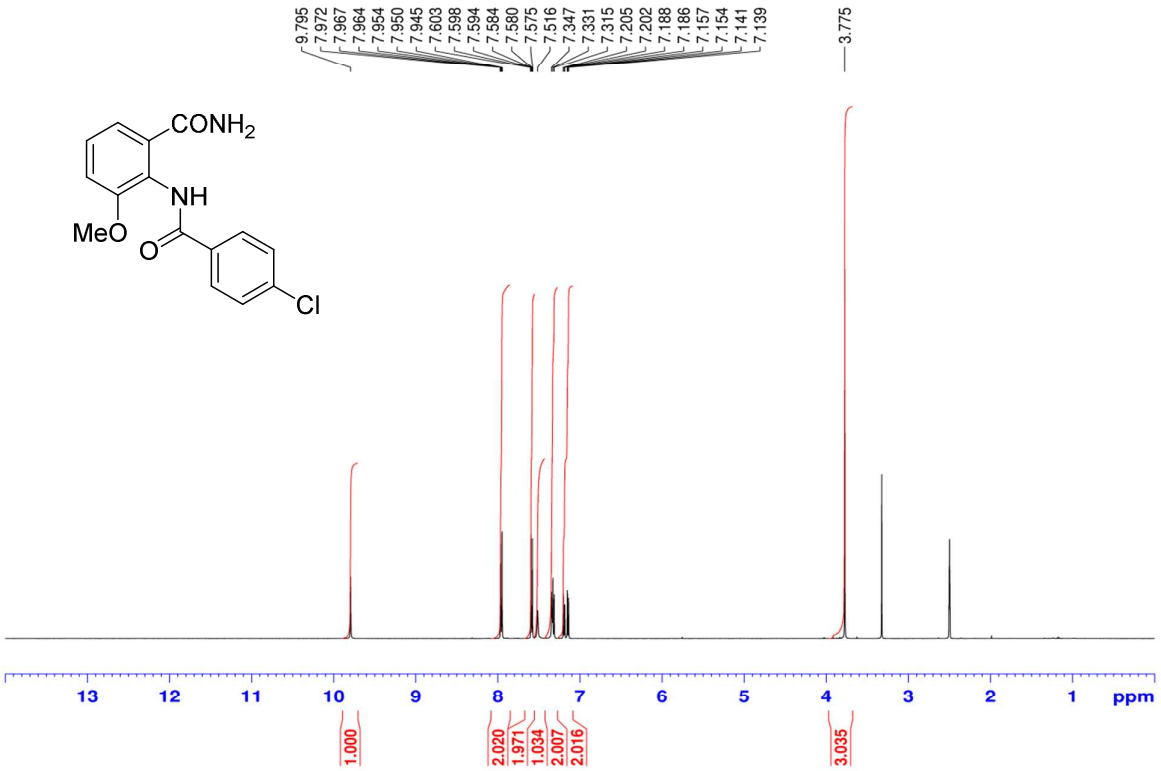
^{13}C NMR spectrum of 6g



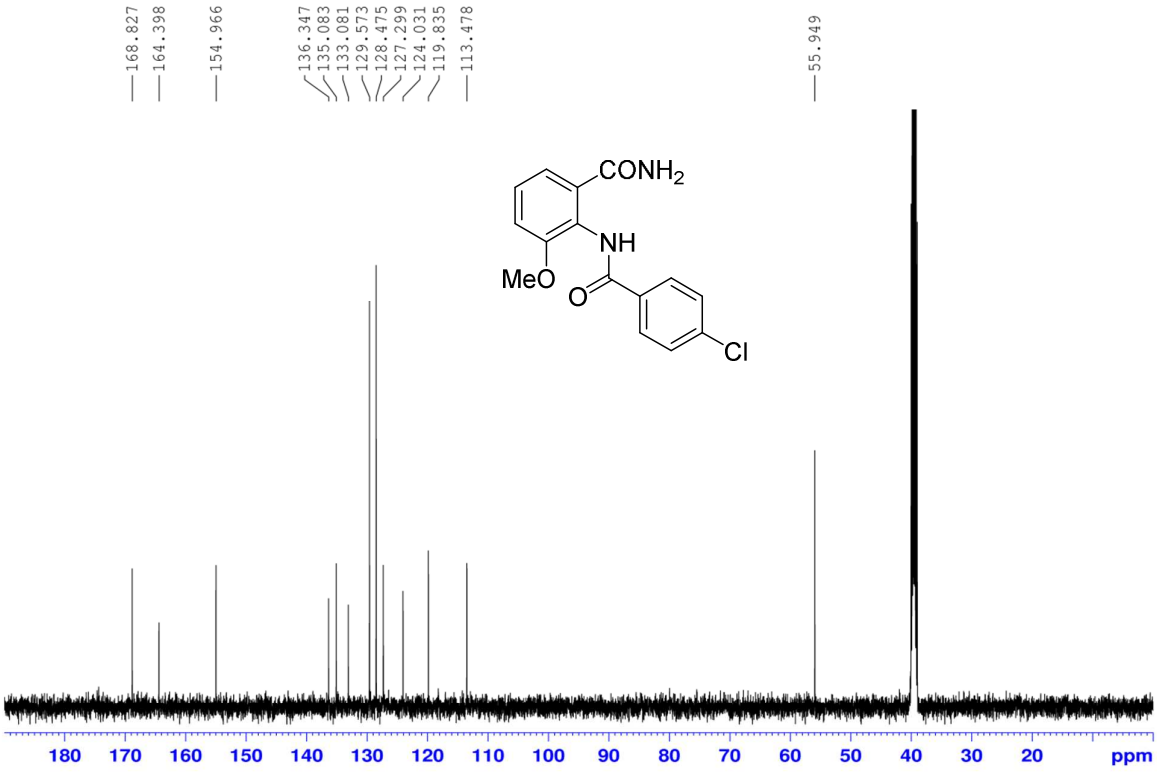
^{1}H NMR spectrum of 6h



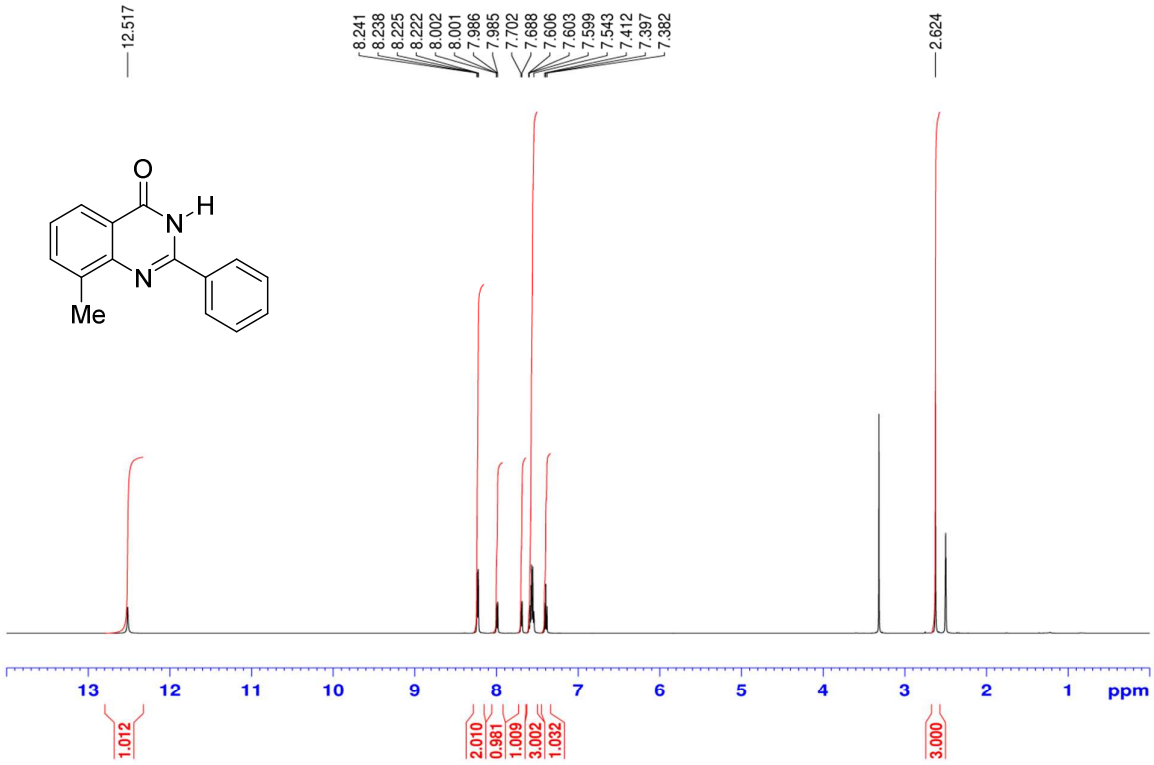
^{13}C NMR spectrum of 6h



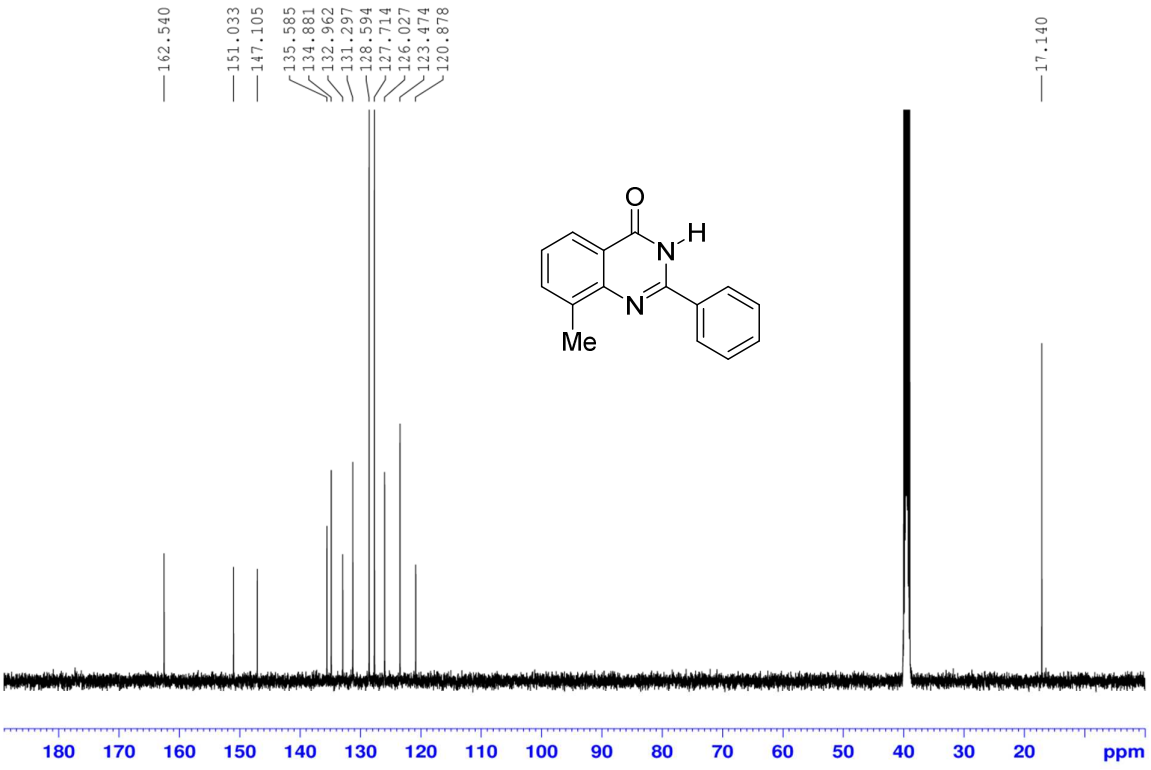
^{1}H NMR spectrum of 6i



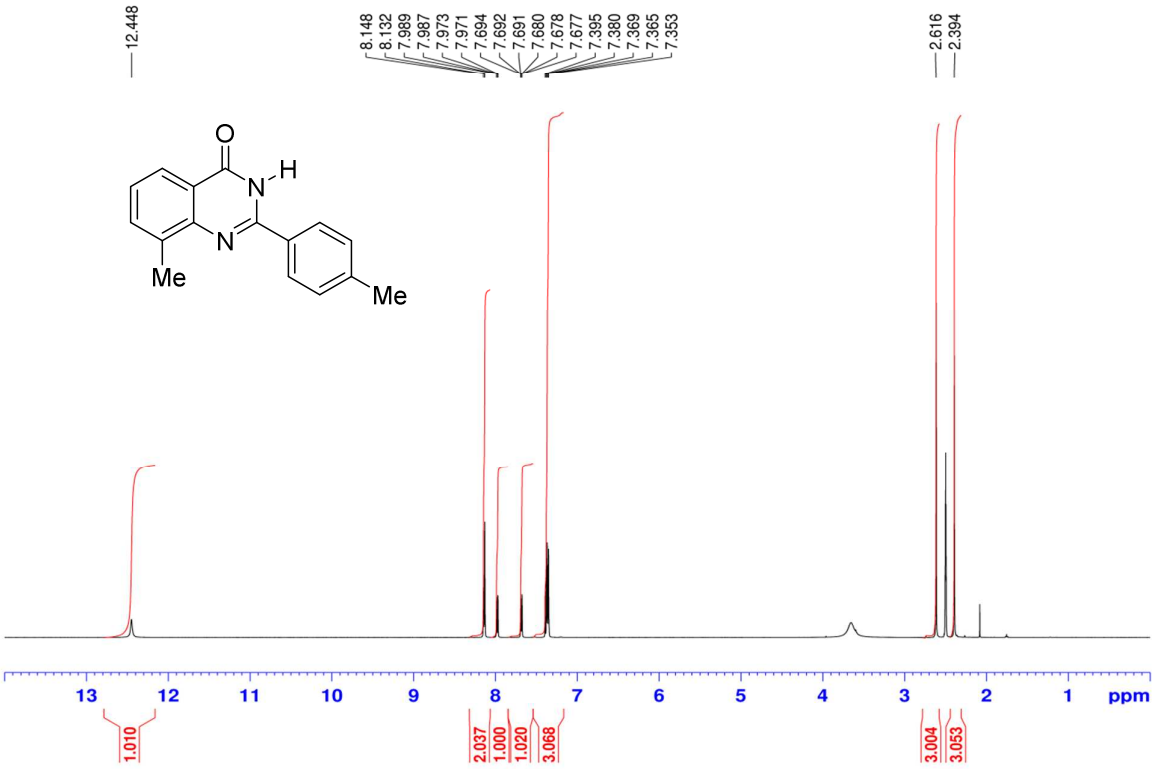
^{13}C NMR spectrum of 6i



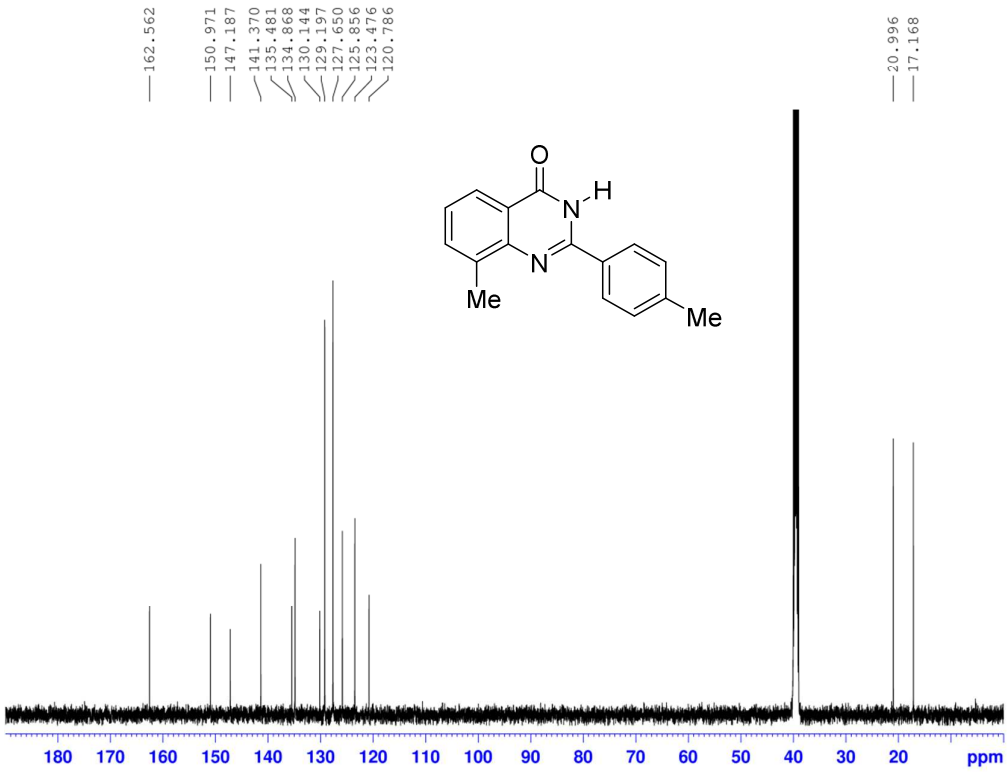
^{1}H NMR spectrum of 7a



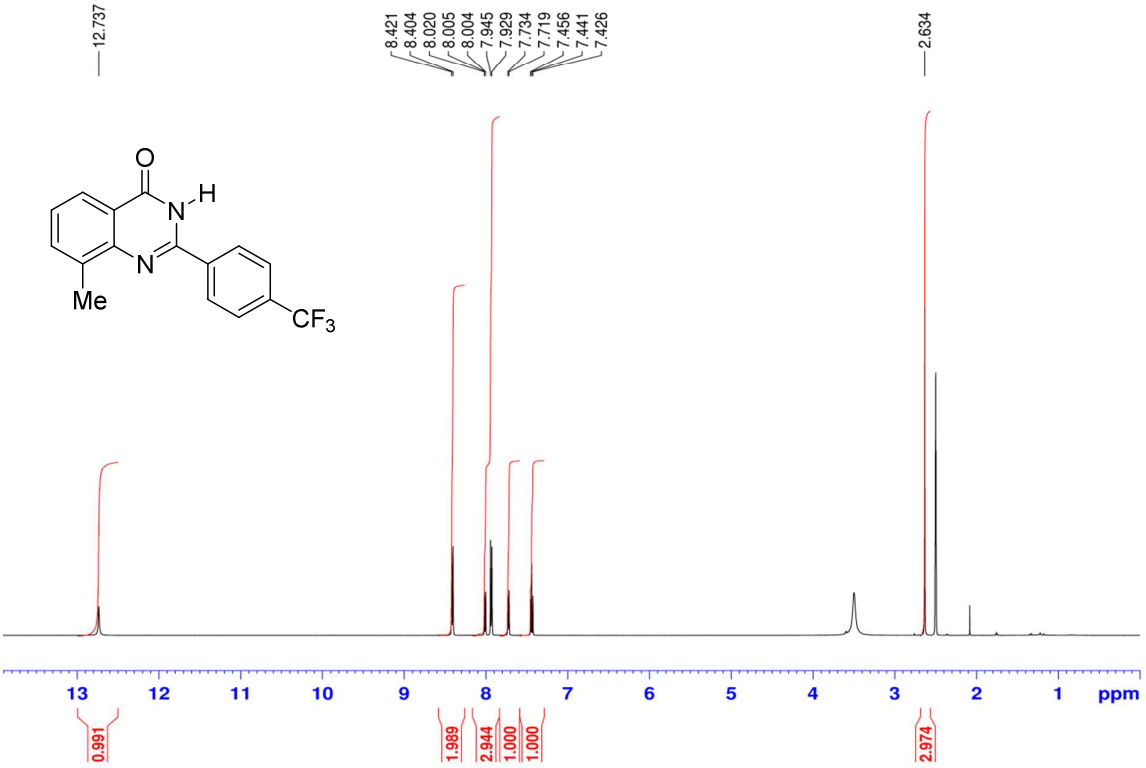
^{13}C NMR spectrum of 7a



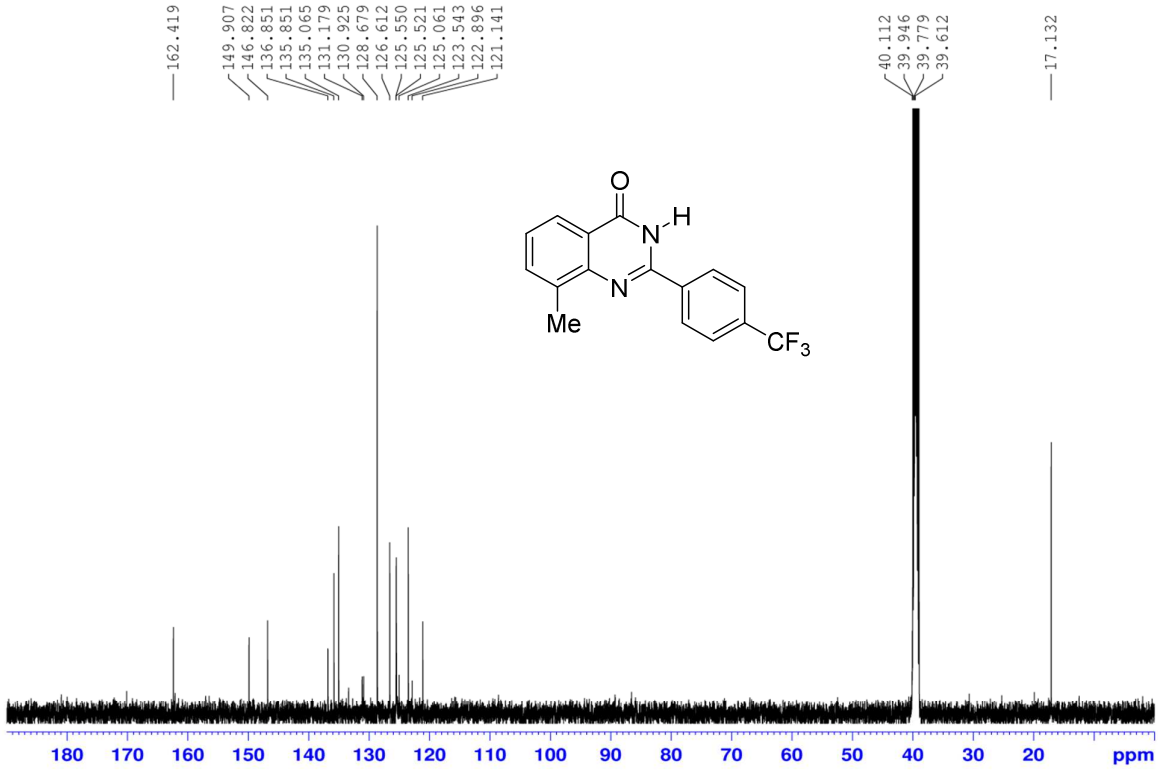
F ^{1}H NMR spectrum of 7b



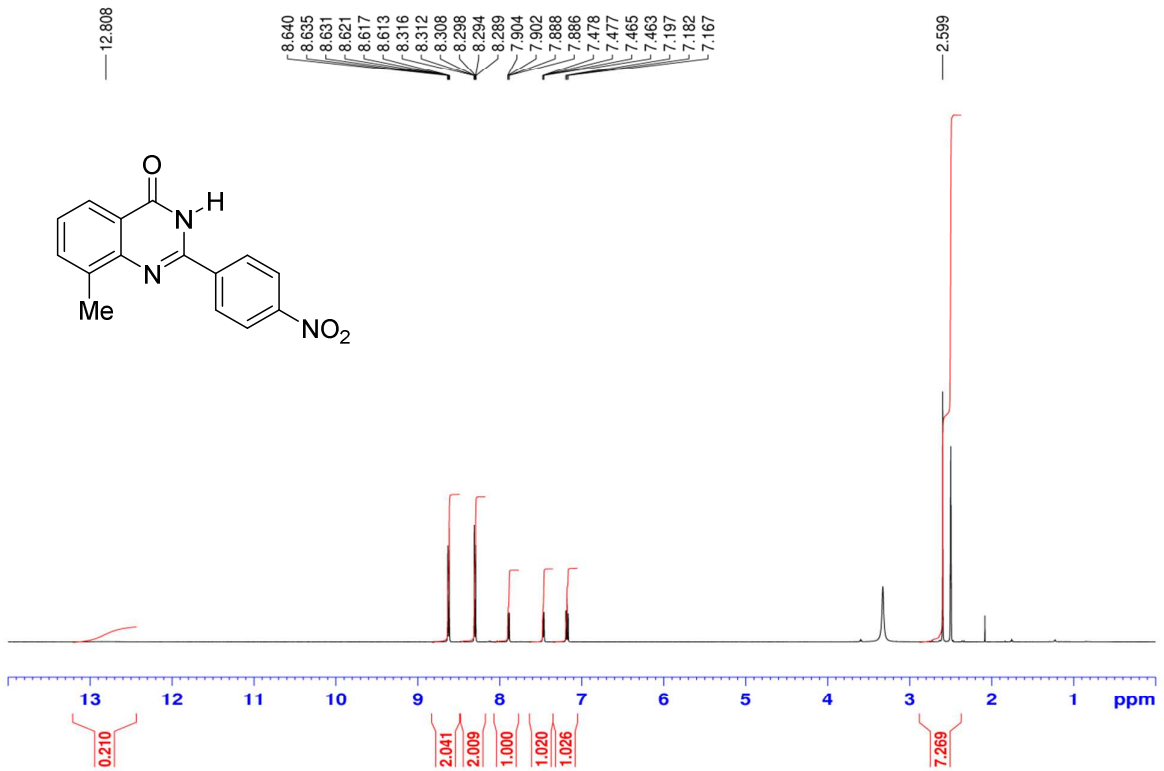
^{13}C NMR spectrum of 7b



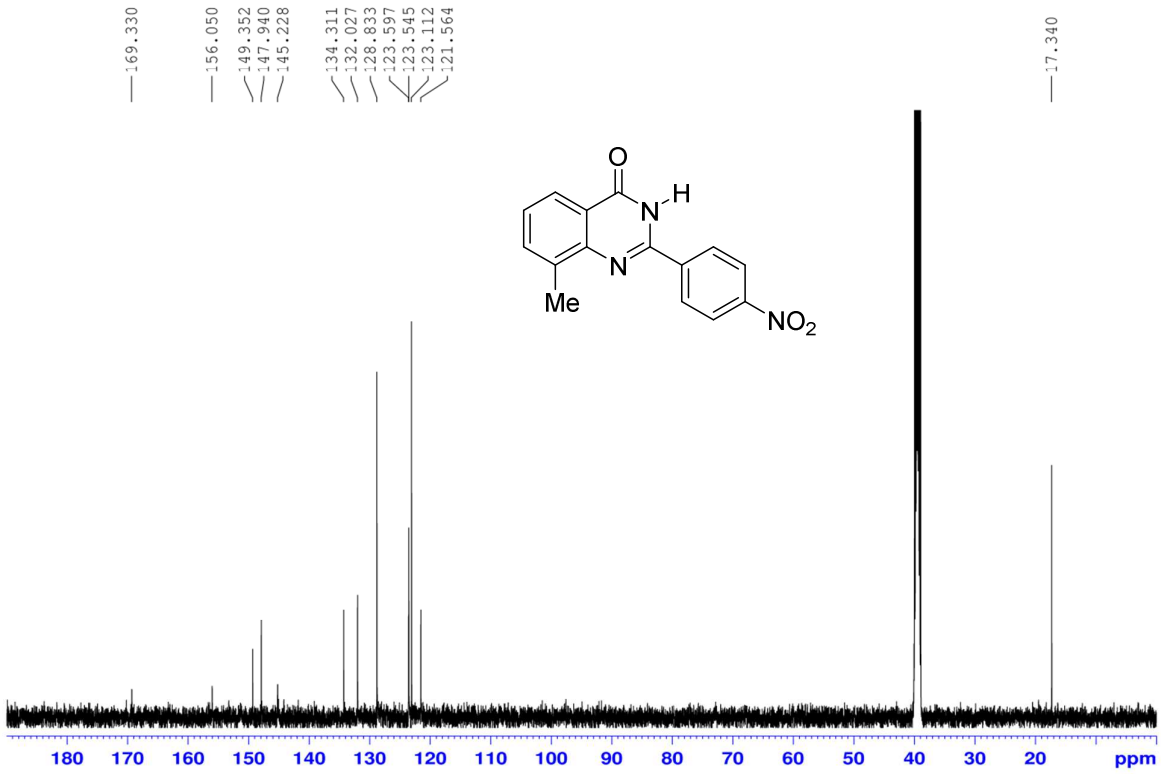
^{1}H NMR spectrum of 7c



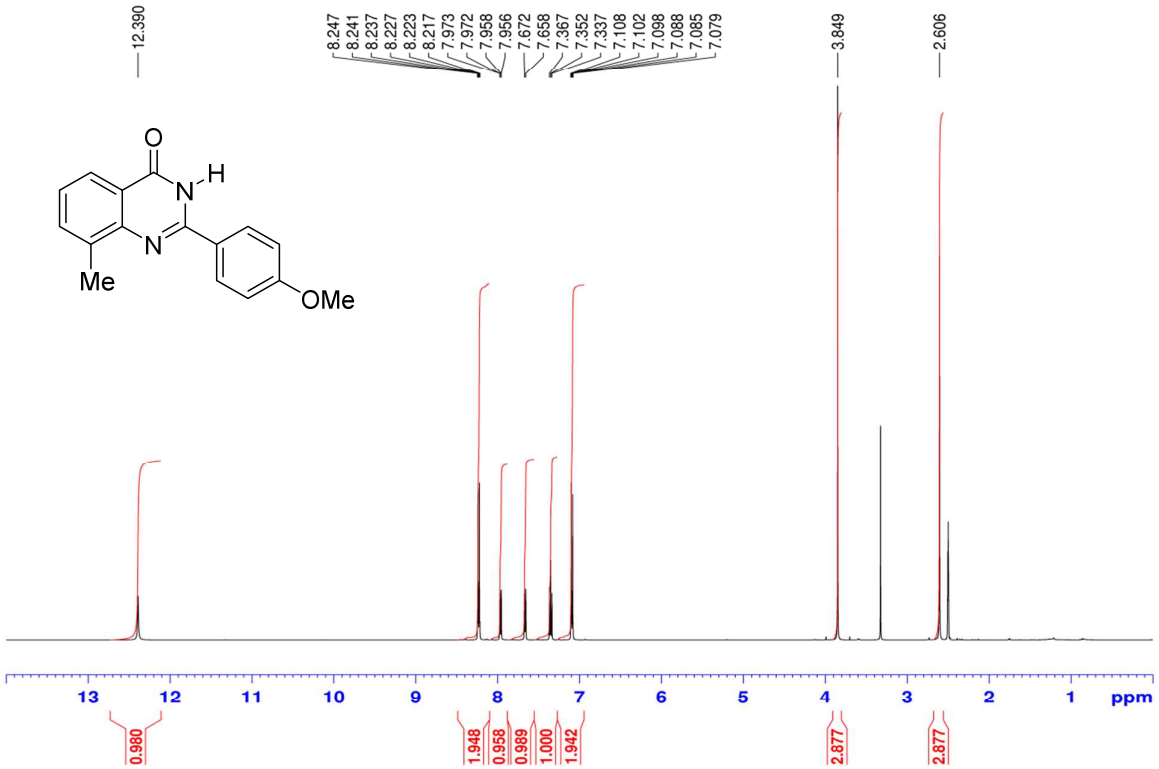
^{13}C NMR spectrum of 7c



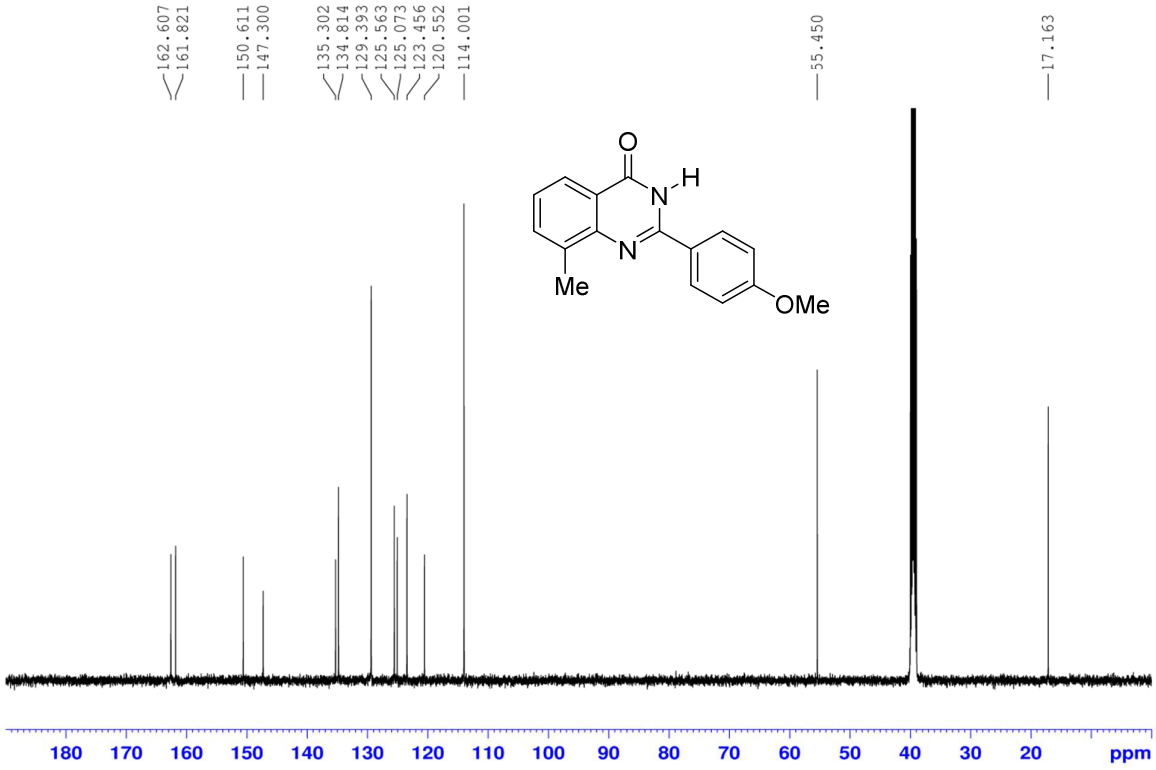
^{1}H NMR spectrum of 7d



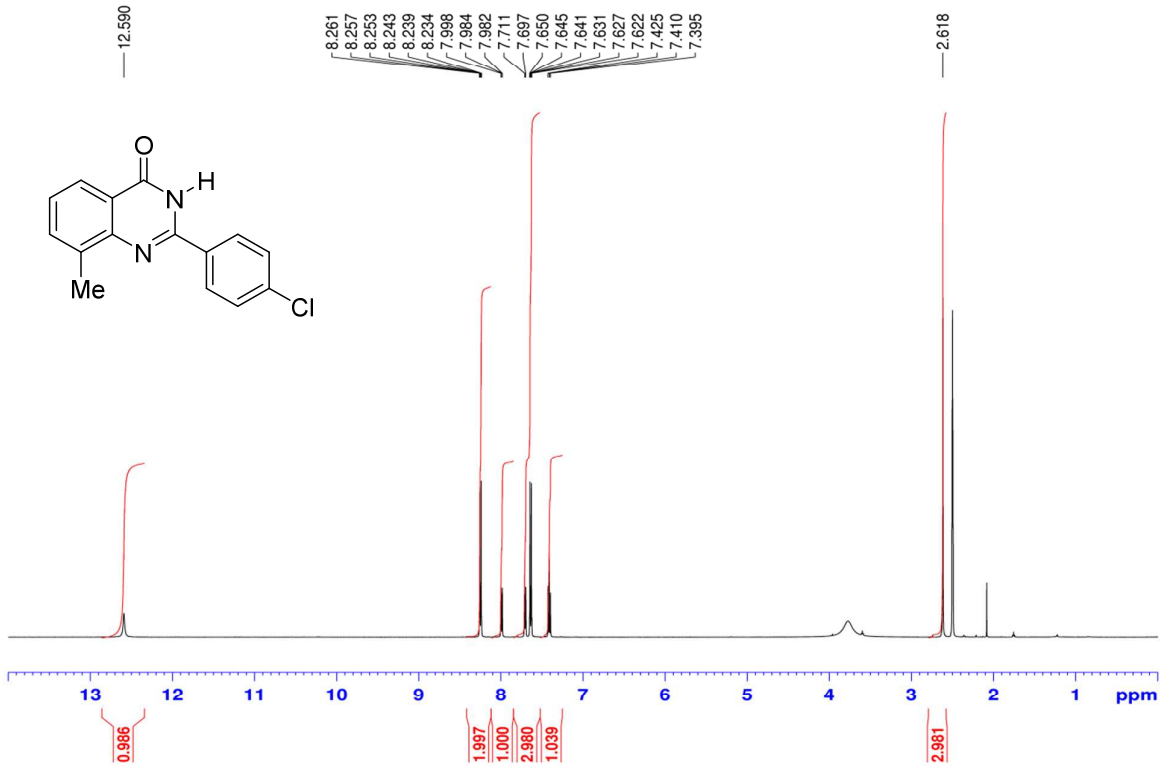
^{13}C NMR spectrum of 7d



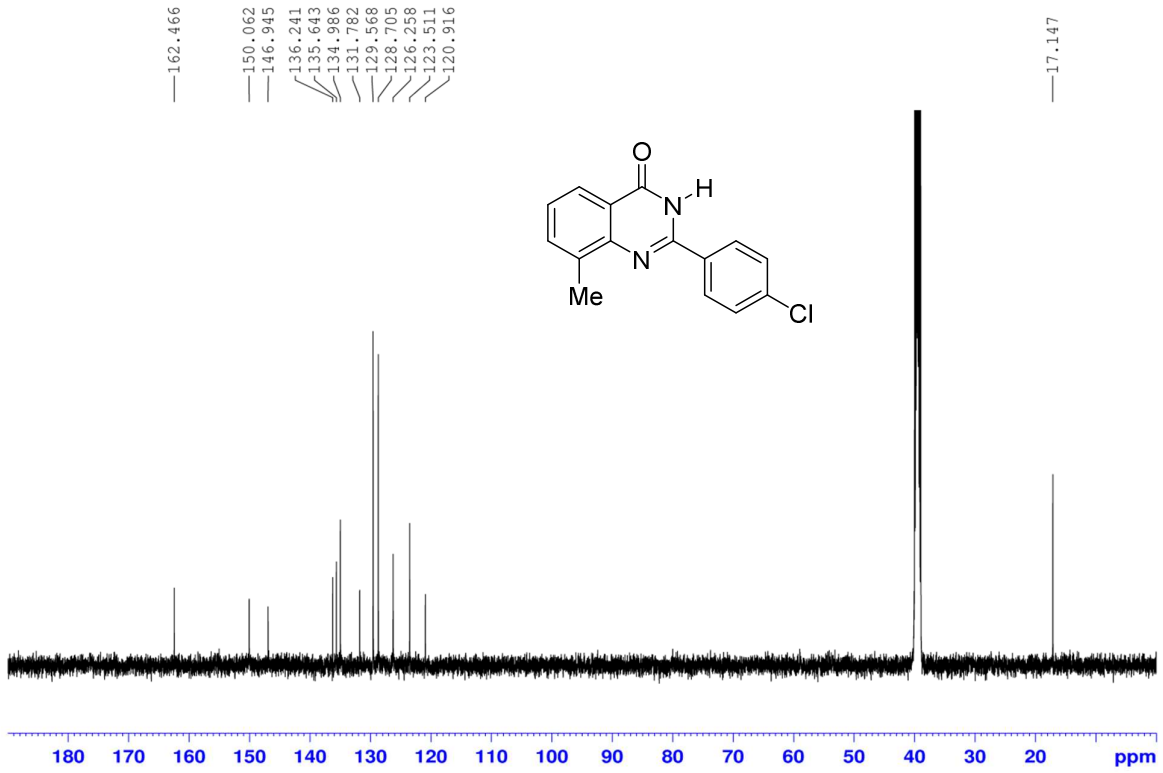
^{1}H NMR spectrum of 7e



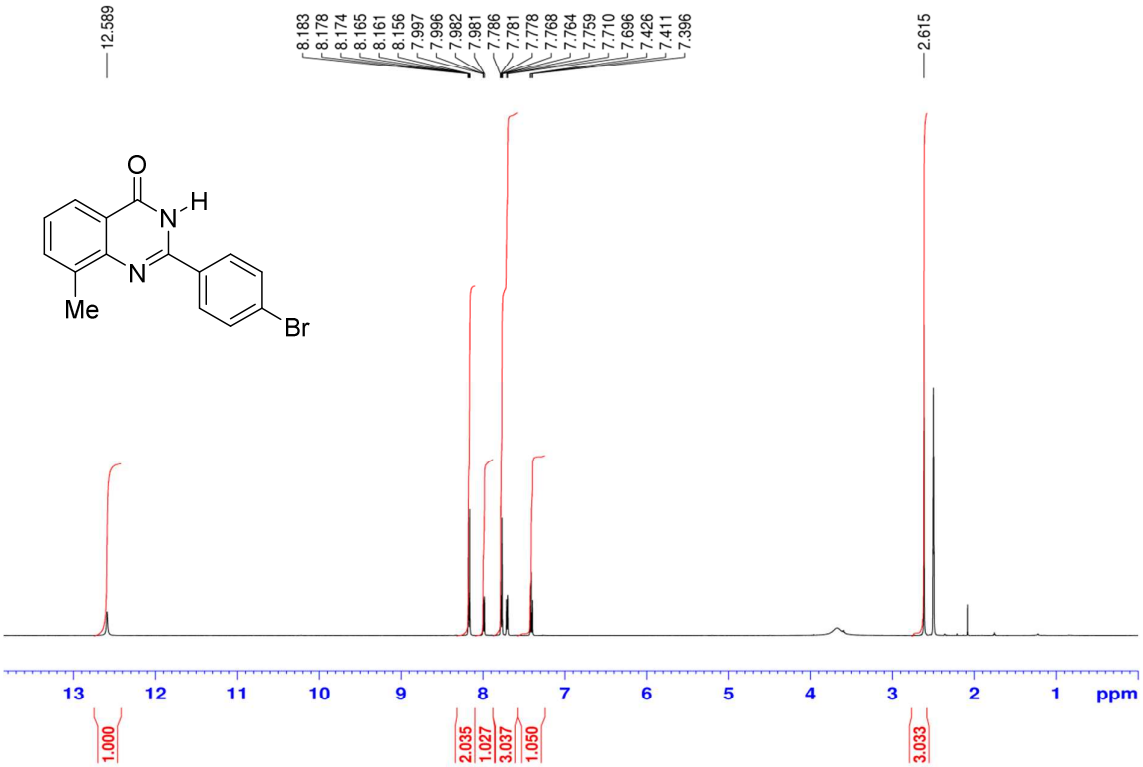
^{13}C NMR spectrum of 7e



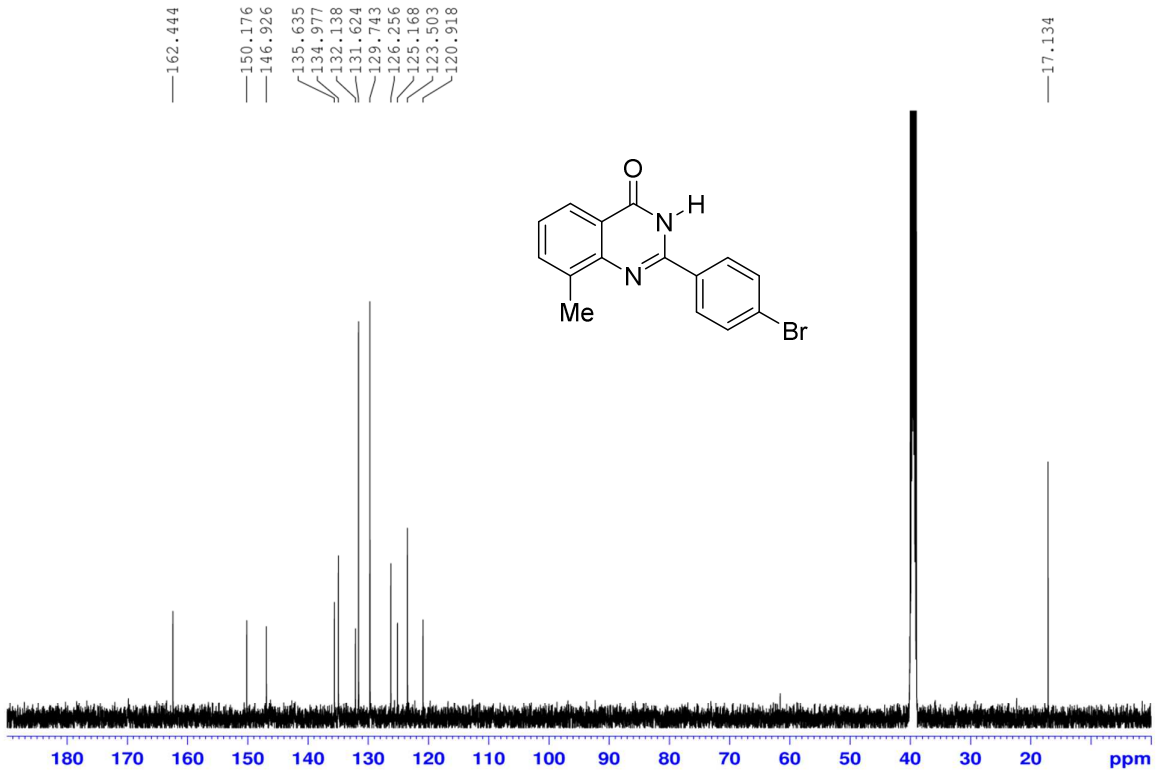
^{1}H NMR spectrum of 7f



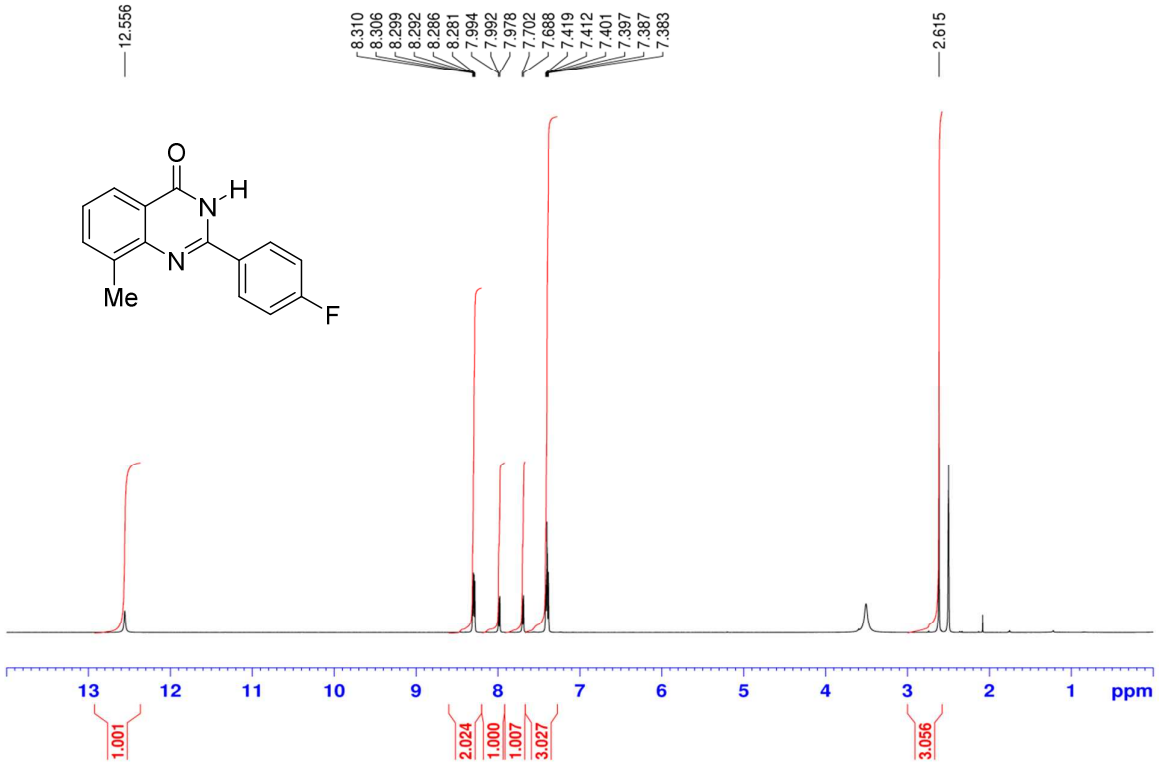
^{13}C NMR spectrum of 7f



^{1}H NMR spectrum of 7g



^{13}C NMR spectrum of 7g



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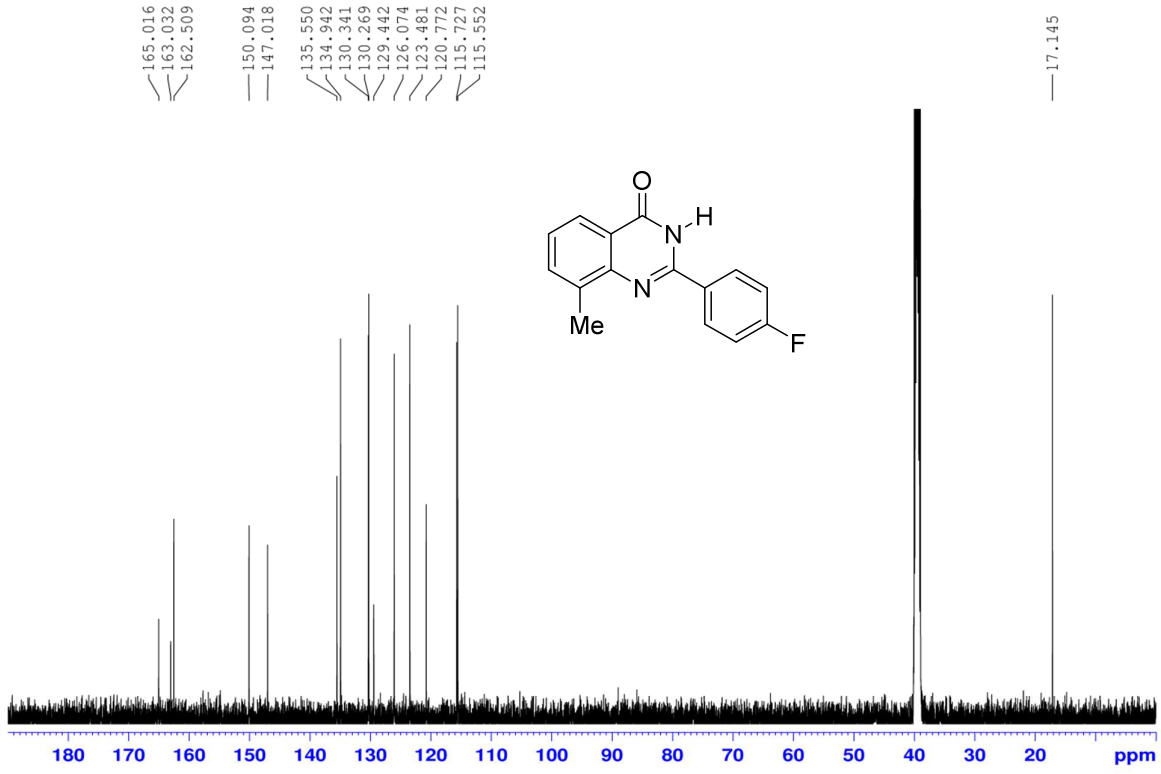

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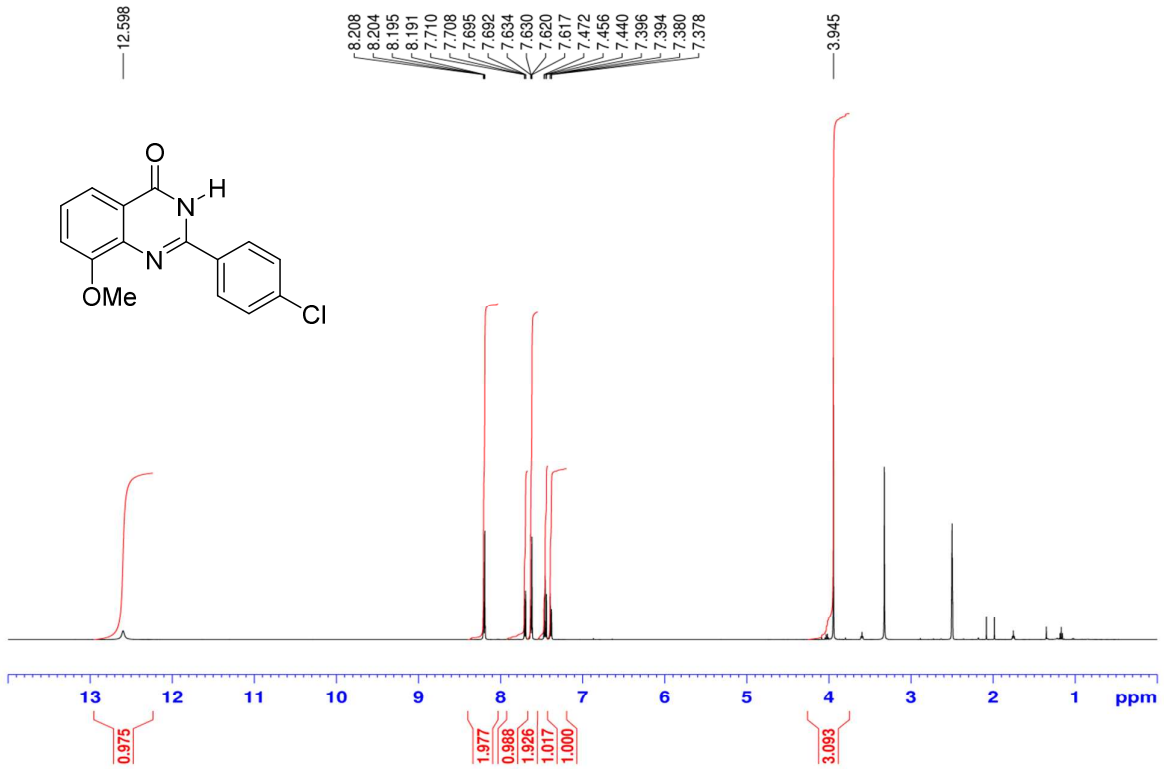
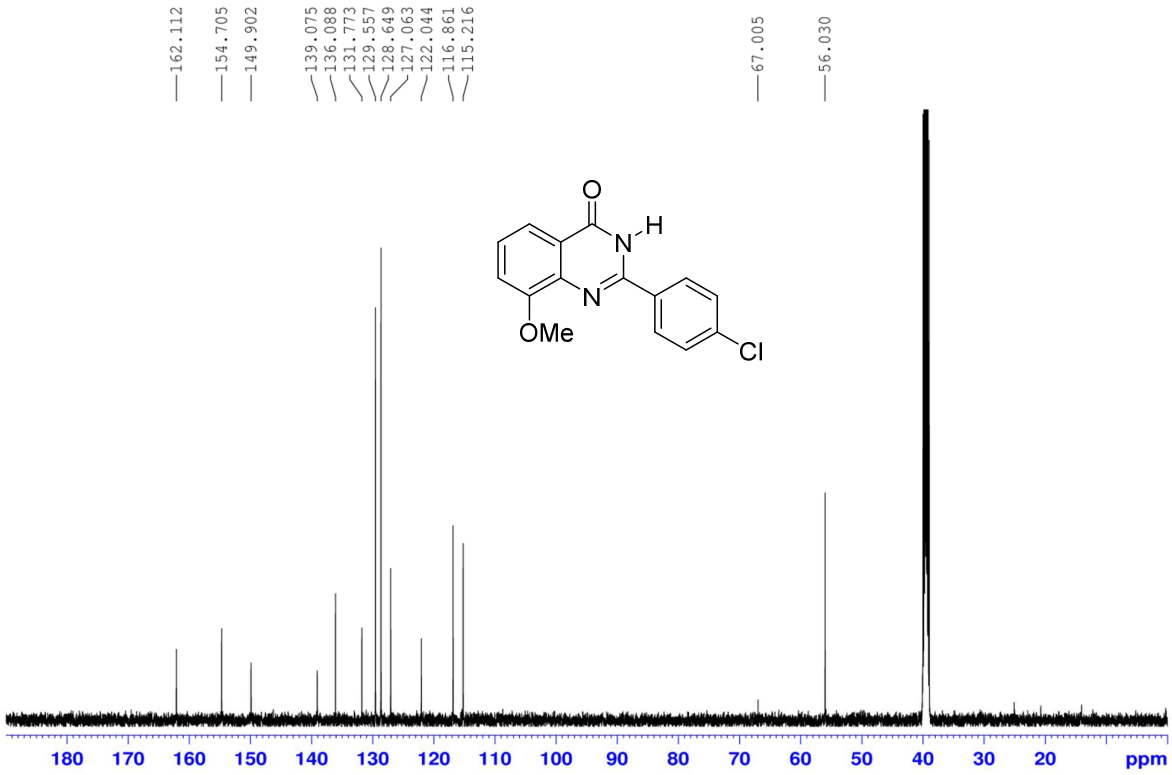
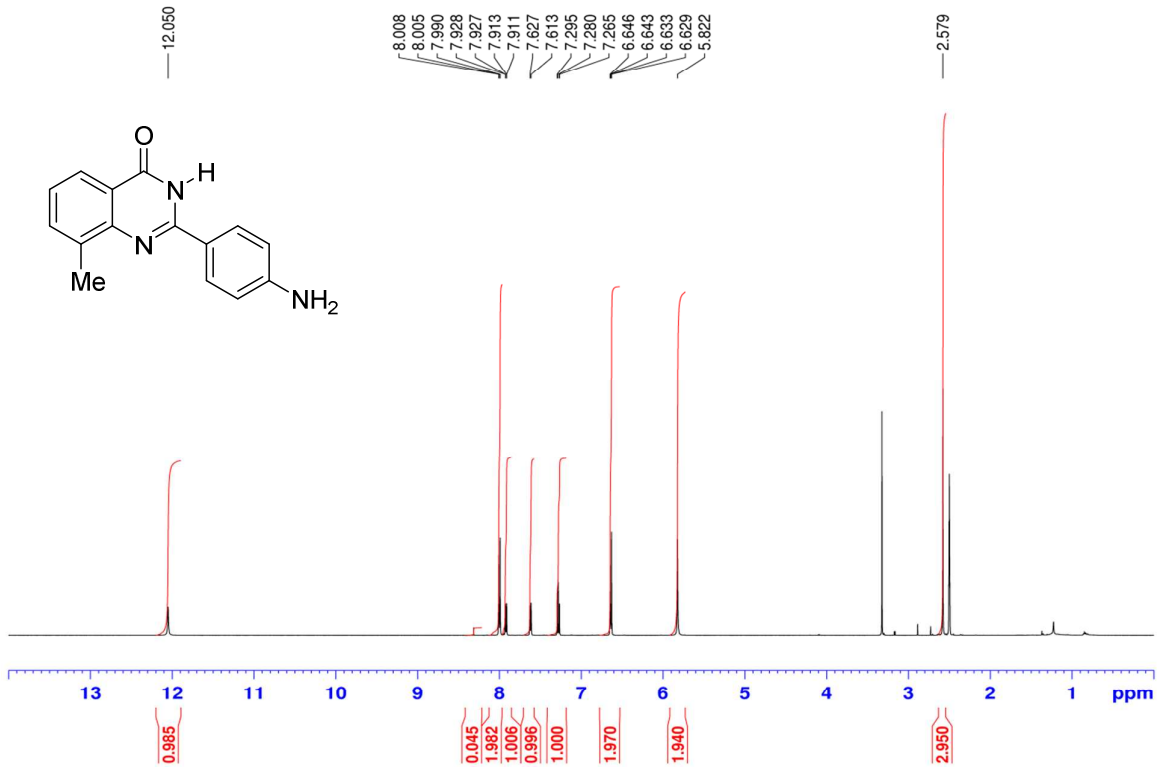


^{1}H NMR spectrum of compound 7i



^{13}C NMR spectrum of compound 7i



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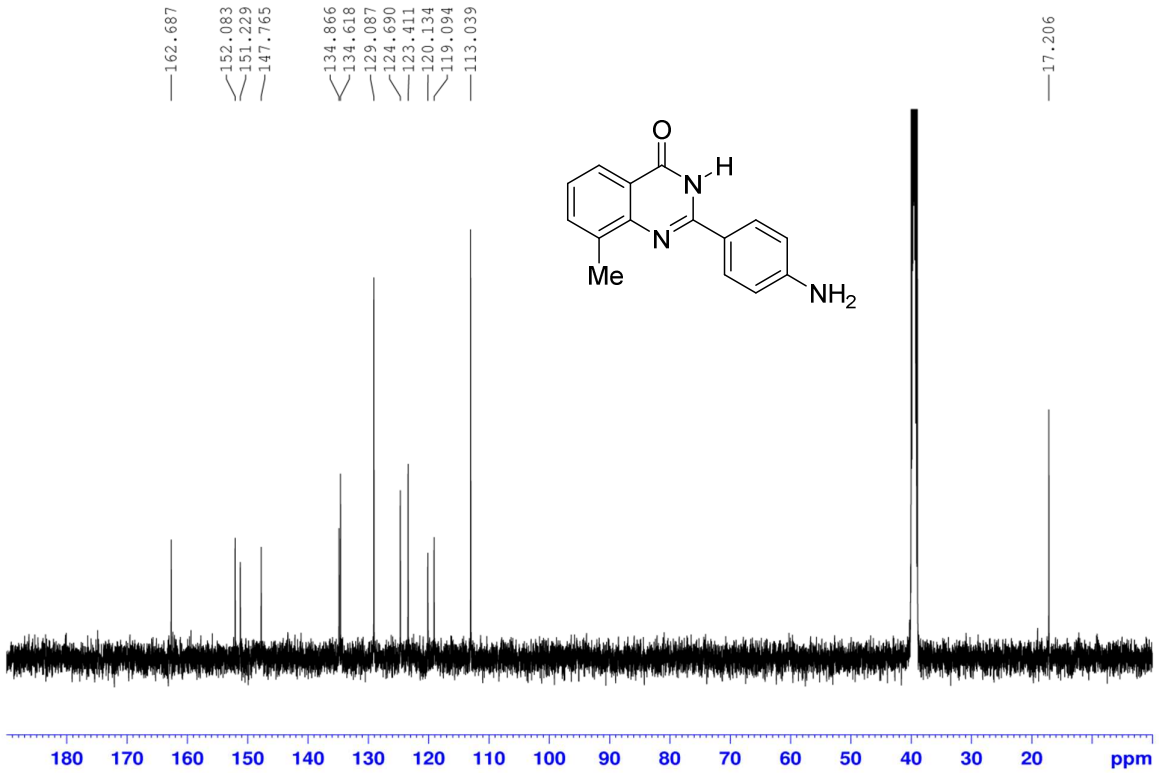

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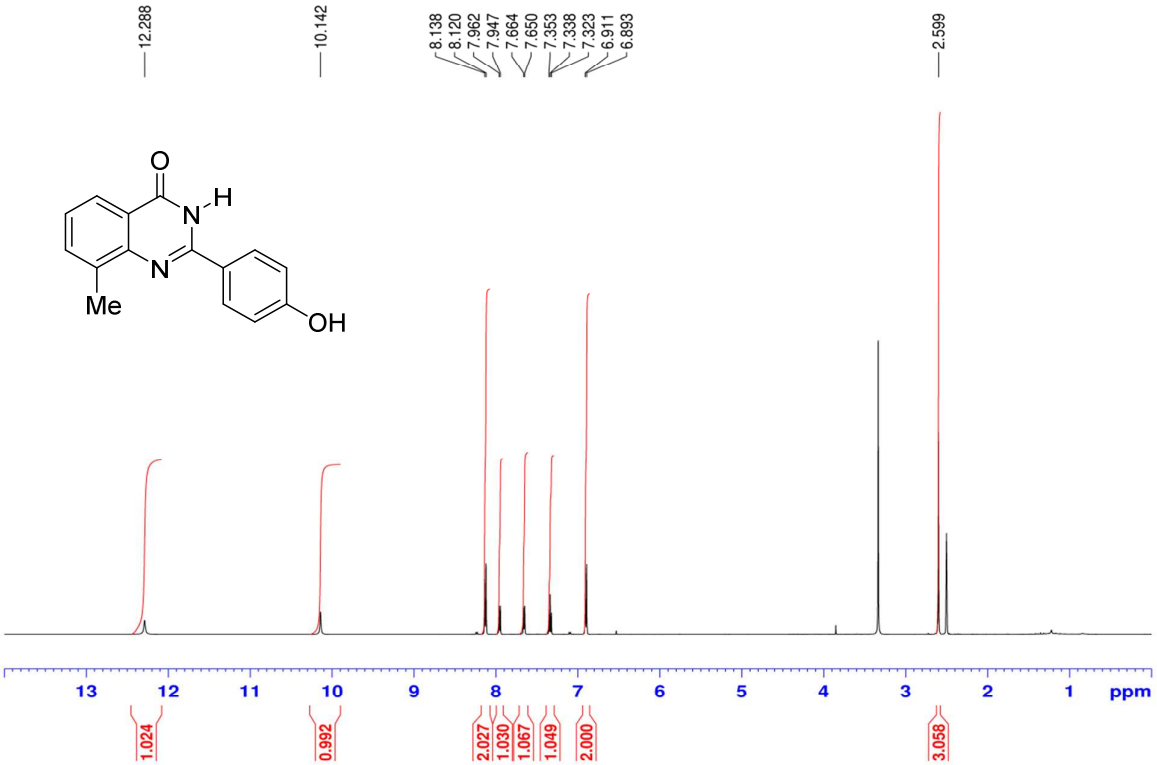

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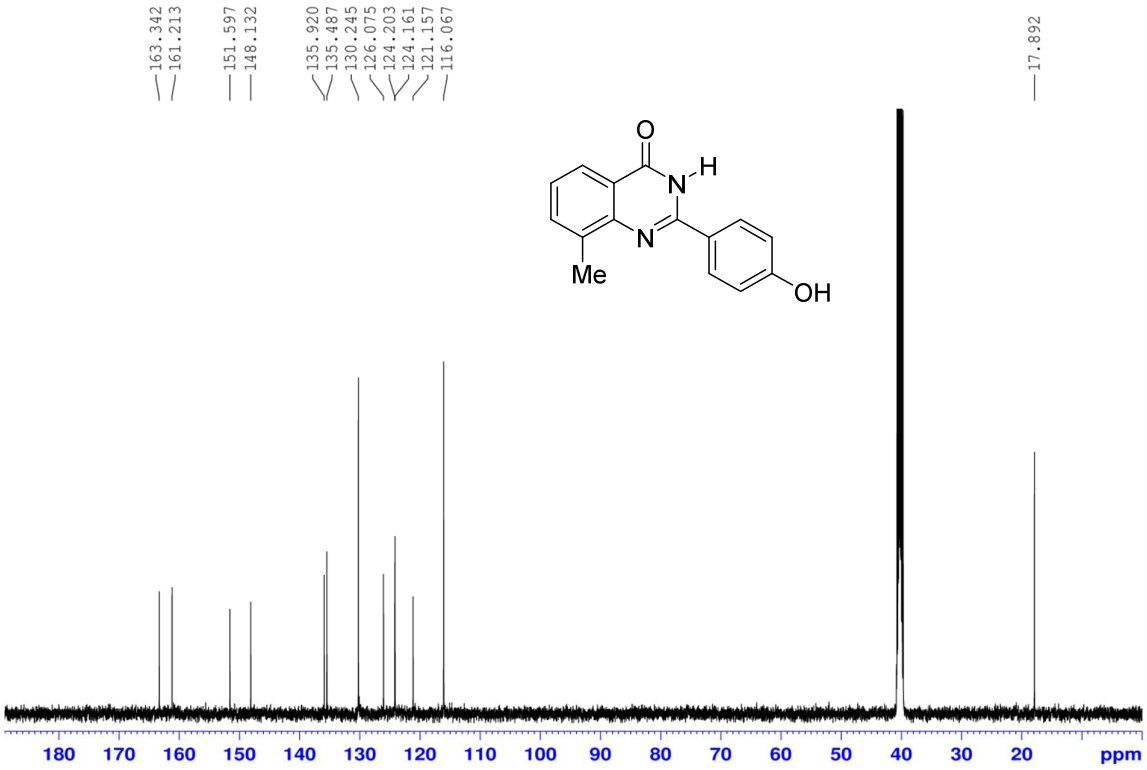
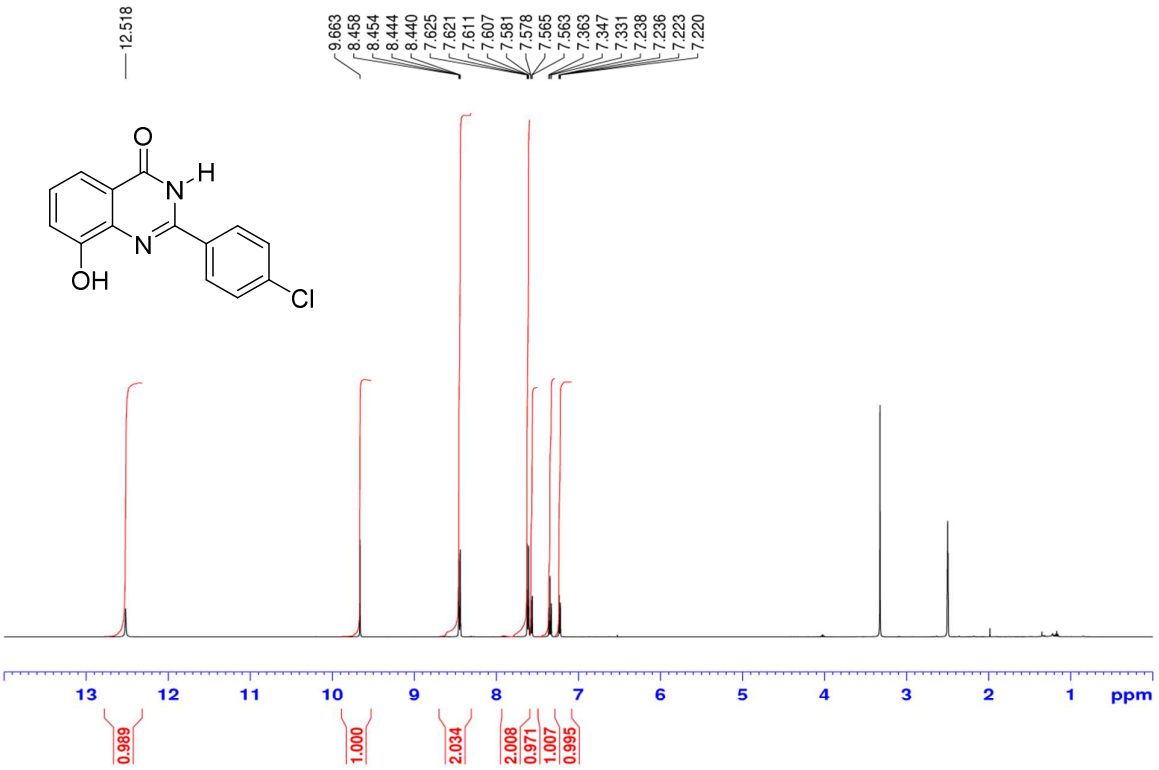
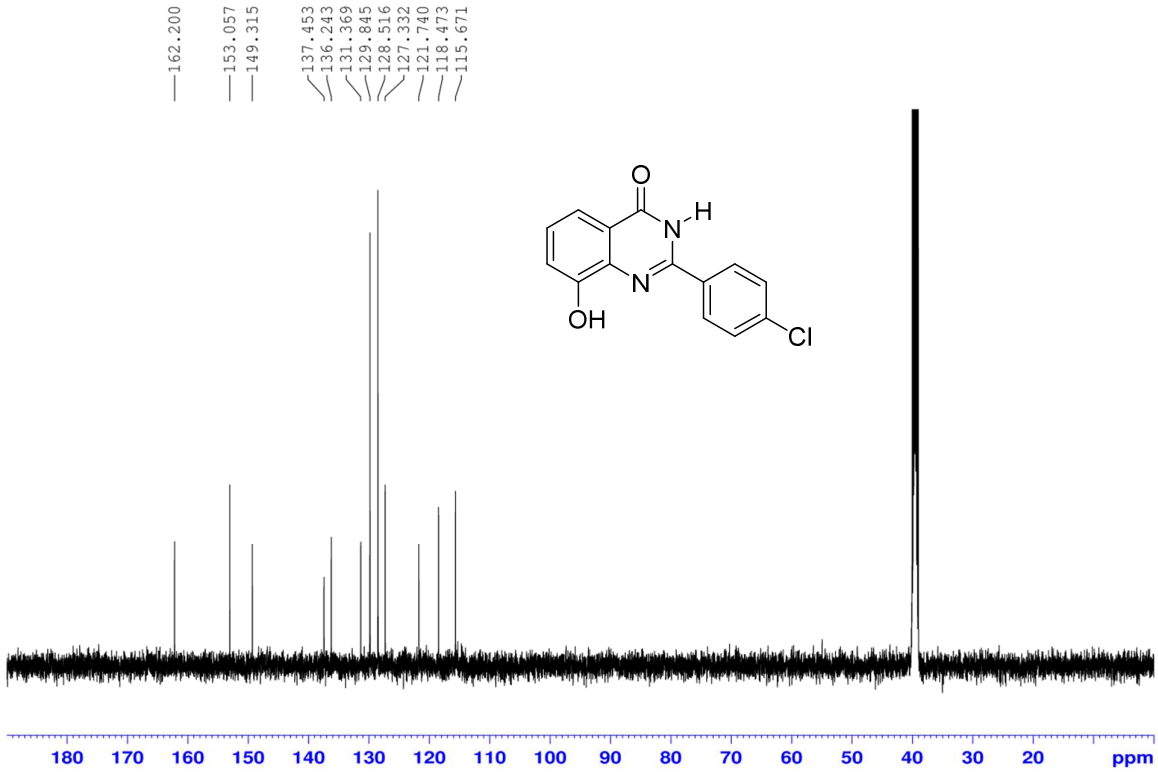


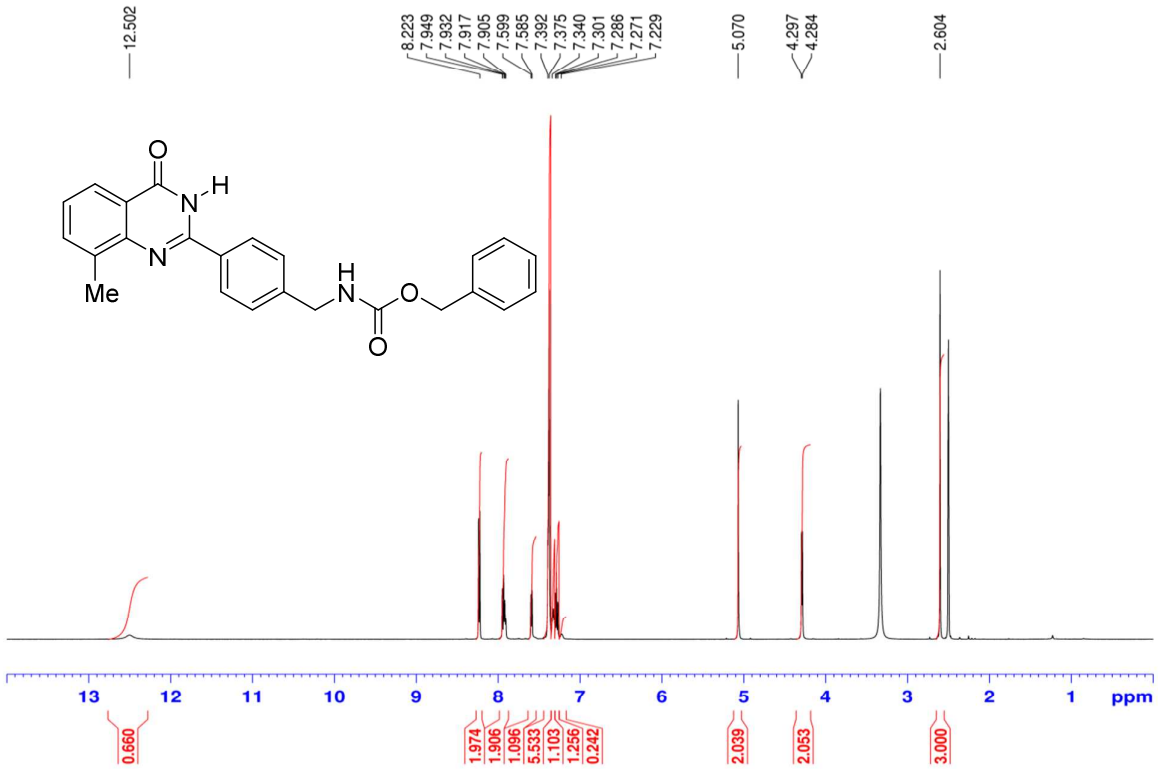
[13]C NMR spectrum of 7k



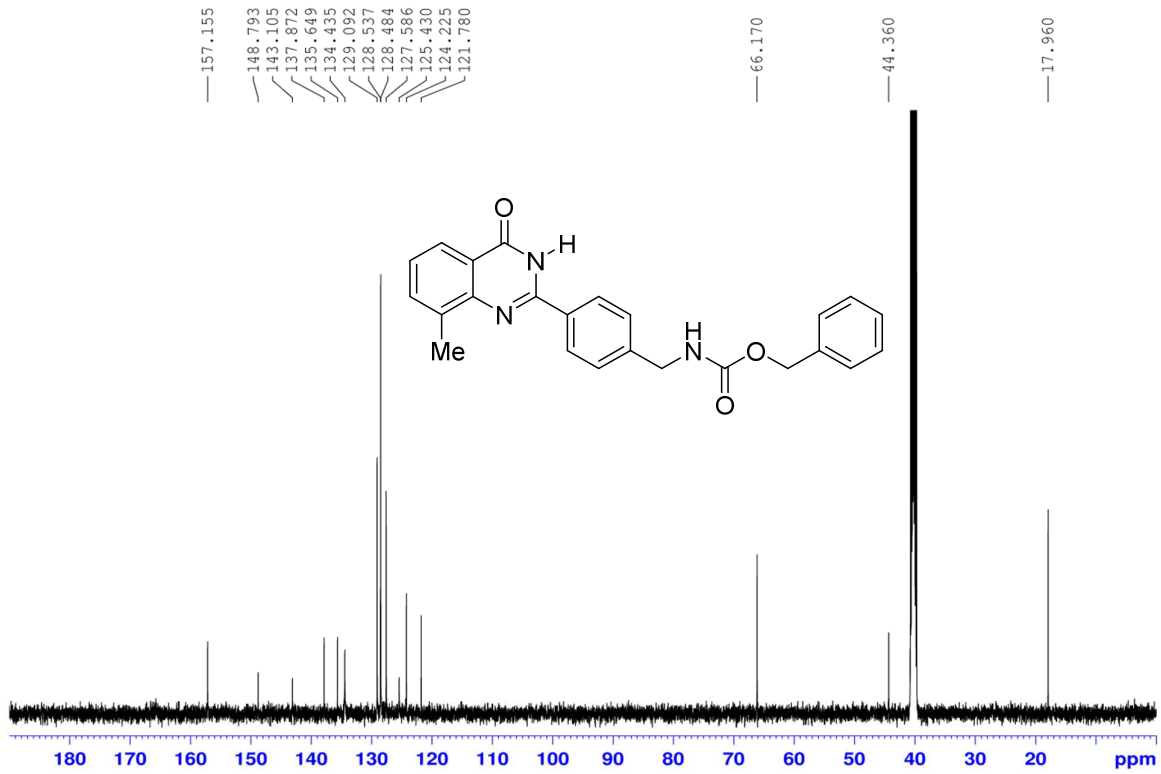
^{1}H NMR spectrum of 7l



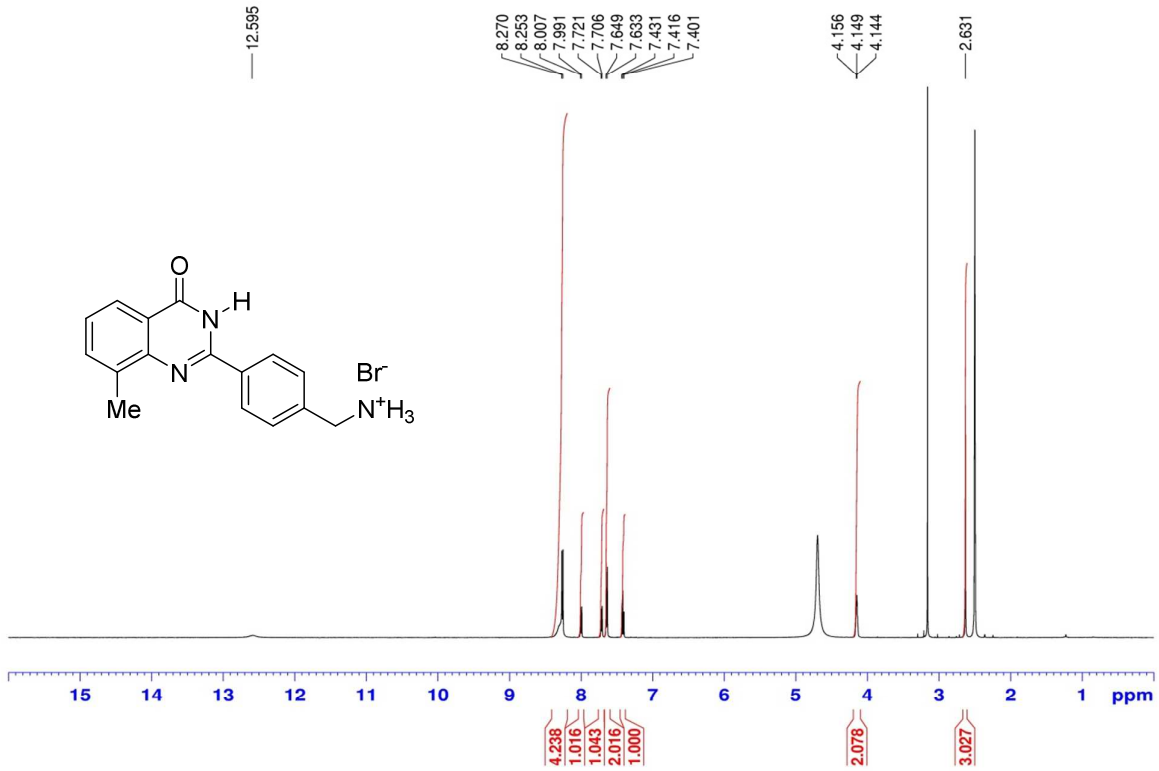
^{13}C NMR spectrum of 7l



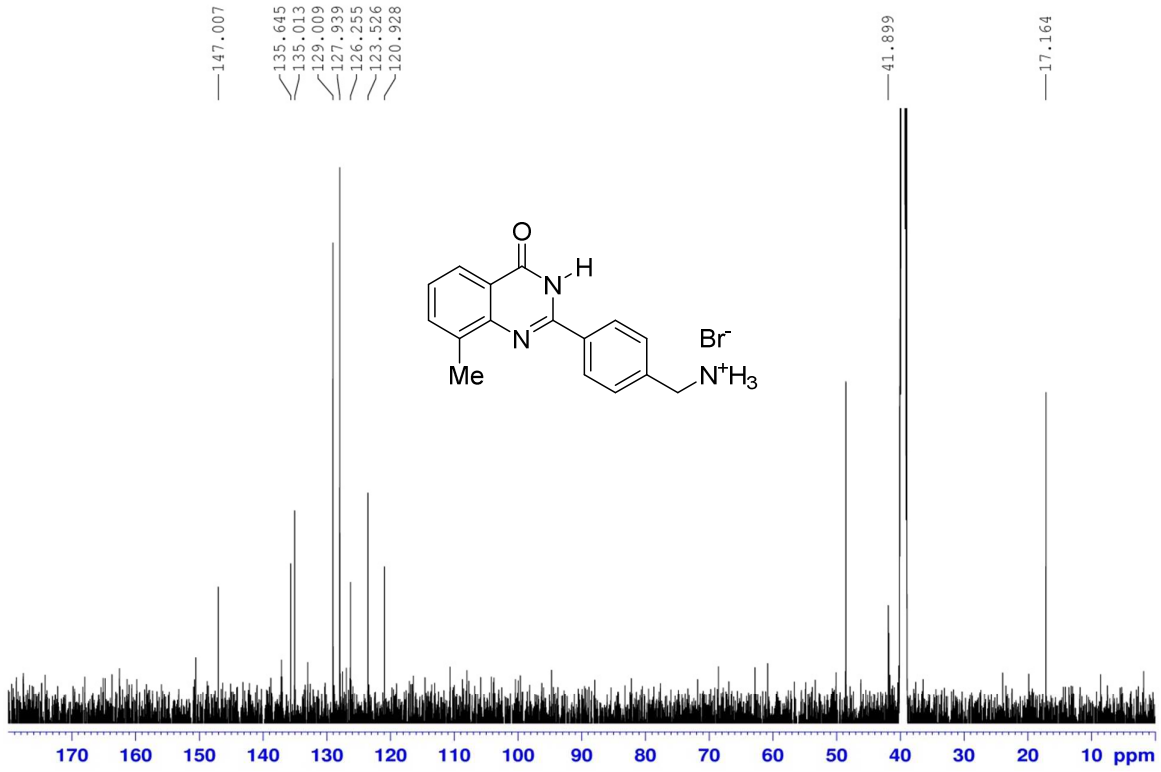
^{1}H NMR spectrum of 10



^{13}C NMR spectrum of 10



^{1}H NMR spectrum of compound 11



^{13}C NMR spectrum of compound 11

Section D: Enzyme assay methods

Tankyrase-1 assay

Tankyrase-1 assays were performed using a commercial kit (Amsbio Europe Ltd. Catalogue # 4700-096-K), using pre-coated histone well plates. The 20× I-PAR assay buffer (catalogue # 4684-096-07) was diluted 1 in 20 with distilled H_2O. This buffer (50 μL) was added to rehydrate the histone-coated wells (30 min at room temperature), then removed by aspiration. The reaction volume (50 μL) consisted of I-PAR assay buffer with tankyrase-1 protein (5 mU in 25 μL I-PAR assay buffer), solutions of test inhibitors (5 μL) in I-PAR buffer prepared from stock solutions in DMSO to give a final concentration of 1% DMSO, assay substrate (15 μL) (catalogue # 4700-096-02). Background wells were treated with I-PAR assay buffer alone. Maximum enzyme activity was established using wells containing enzyme only + 1% DMSO. The plates were held for 30 min at room temperature. The wells were washed with 2× PBS-T (as described above) and 2× PBS. The antibody diluent was prepared from 5× stock solution (catalogue # 4684-096-03) using distilled H_2O. The anti-PAR monoclonal antibody (catalogue # 4684-096-04) was diluted 1000-fold with 1× antibody diluent and 50 μL were added per well. The reaction was held for 30 min at room temperature. The wells were washed with 2 × PBS-T and 2 × PBS. Goat anti-mouse IgG-HRP conjugate (catalogue # 4684-096-05) was diluted 1000-fold with 1× antibody diluent and 50 μL was added per well. The reaction was held for 30 min at room temperature. The wells were washed with 2 × PBS-T and 2 × PBS. Pre-warmed TACS-Sapphire™ (50 μL) was added per well and held at room temperature in the dark for 30 min. The reaction was stopped by the addition of HCl (0.20 M, 50 μL) and the absorbance at 450nm was read within 20 min. The IC_{50} values were calculated using a four-parameter logistic curve and SigmaPlot 12.0 software.

Tankyrase-2 assay

A suspension of tankyrase-2 protein (catalytic + SAM domains) (7.5 ng, BPS Bioscience and AMS Bio Europe Ltd. Catalogue # 80515) in reaction buffer (25 μL, 50 mM TRIS-HCl pH 8.0, 5.0 mM $MgCl_2$, 20 μM $ZnCl_2$) was loaded into ELISA-quality, half-volume, high binding 96-well plates (Greiner bio-one) and these were held at 4°C for 16 h. The wells were then washed four times with phosphate-buffered saline solution pH 7.4 (+ 0.05% v/v tween 20 (PBS-T) (250 μL)). Skimmed milk (Marvel) in reaction buffer (5%, 100 μL) was added per well and the mixture was allowed to stand for 1 h. The wells were then washed with PBS-T (4 × 250 μL). A reaction volume of 25 μL was used and consisted of reaction buffer with 5 μL of varying concentrations of inhibitor from stock solutions in DMSO, to give a final DMSO concentration of 1%, and 5 μL of a solution of biotinylated NAD^+ (12.5 μM, BioLog Life Science Institute) and NAD^+ (12.5 μM, Enzo Life Sciences) to give a final reaction concentration (total NAD^+ and derivatives) of 5 μM. The plates were incubated at 30°C for 2 h. The wells were then washed with PBS-T (4 × 250 μL), then streptavidin / HRP solution (100 μL, R & D systems) was added per well and the plates were held at room temperature for 2 h. The wells were then washed with PBS-T (4 × 250 μL), a 1:1 mixture of substrate solutions A and B (100 μL, R & D systems) was added per well and the plates were held for 30 min. The reaction was stopped by the addition of aq. H_2SO_4 (1.0 M, 25 μL) and the absorbance at 450 nm was read within 20 min. The IC_{50} values for inhibitors were determined using a four-parameter logistic curve and SigmaPlot 12.0 software.

PARP-1 assay

PARP-1 assays were performed using a commercial kit (Amsbio Europe Ltd. Catalogue # 4676-096-K) using pre-coated histone well plates. A solution of 20× PARP assay buffer (catalogue # 4671-096-02) was diluted to 1× with distilled water. PARP assay buffer was used to rehydrate the histone-coated wells (50 μL per well) for 30 min, then this solution was removed by aspiration. A reaction volume of 50 μL was used and consisted of PARP assay buffer with PARP-1 protein (25 μL, 0.5 mU in 1× PARP assay buffer), 5 μL of inhibitor solutions in PARP assay buffer prepared from stock solutions in DMSO to give a final concentration of 1% DMSO and 20 μL of substrate (2.5 μL 10× PARP cocktail (catalogue # 4671-096-03), 2.5 μL 10× activated DNA (catalogue # 4671-096-06), 15 μL PARP assay buffer). The plates were held for 1 h at room temperature. The wells were washed twice with PBS-T and twice with PBS (250 μL each). Streptavidin-HRP solution (catalogue # 4800-30-06) was diluted 500-fold with 1× streptavidin-HRP diluent (catalogue # 4671-096-04), 50 μL was added to each well and the plates were held for 1 h at room temperature. The wells were washed with PBS-T (2 ×) and PBS (2 ×). Pre-warmed TACS-Sapphire™ (50 μL) was added per well and the mixtures were left at room temperature in the dark for 30 min. The reaction was quenched by the addition of HCl (0.2 M, 50 μL) and the absorbance at 450 nm was measured within 20 min. The IC_{50} values were calculated using a four-parameter logistic curve and SigmaPlot 12.0 software.

IMPDH2 assay

Lyophilized human IMPDH-2 recombinant protein (Novocib SAS, catalogue # E-Nov1) was suspended in storage buffer (40 mM Tris-HCl, pH 8.0, 110 mM NaCl, 2.2 mM KCl, 3.0 mM dithiothreitol, 4.0 mM glutathione and 20% glycerol) to make a 100 μM stock solution. Kinetic assays were performed at 37°C in assay buffer (100 mM Tris-HCl, pH 9.0, 100 mM KCl and 5.0 mM dithiothreitol) using final concentrations of 1 μM IMPDH-2, 0.5 mM NAD^+ (Enzo Life Sciences, catalogue # BML-KI282-0500) 1.0 mM inosine monophosphate (Sigma Aldrich, catalogue # I4625) and varying concentrations of inhibitor (prepared from stock solutions in DMSO, to give a final DMSO concentration of 1%) in a total reaction volume of 100 μL. A known inhibitor, 6-thioinosine monophosphate (Carbosynth, catalogue # NT10843) was used at varying concentrations as a positive control. Reactions were monitored at 340 nM using a BMG LABTECH FLUOstar Omega™ plate reader. Linear regression fit (r^2) and rates were calculated using Omega MARS™ LABTECH software.

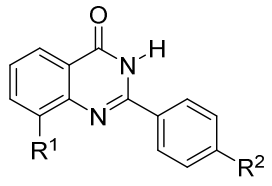
Section E: Modeling method

Modeling was performed with the SYBYL software suite with UCSF Chimera for visualisation. Ligands were constructed and charged within SYBYL, using the conformation of XAV939 as a starting orientation for the rings.

Ligands were manually docked to the TNKS-2 enzyme structure generated by removing the XAV939 ligand from the published 3KR7 structure[3]. Once docked, the smaller ligands (of similar size and shape to XAV939) were minimized while restraining the hydrogen bonds onto the amide-binding motif (C=O to Ser^{1068} and Gly^{1032}; NH to Gly^{1032}) and the conformation of the receptor pocket. Once a favourable binding conformation had been established for the ligand, the complete receptor / ligand complex was then minimized (without restraints) to give the final structures. When modelling **10**, the receptor pocket was manipulated to open the channel from the XAV939-binding site to the surface of the TNKS-2

enzyme (in which the extended ligands could bind). These ligands were again minimized whilst in the binding pocket and the amide-receptor interactions were fixed. The generated structures were then subjected to further refinement by molecular dynamics and mechanics (unrestrained) to give the final structures and allow us to investigate additional interactions between the side chains and receptor.

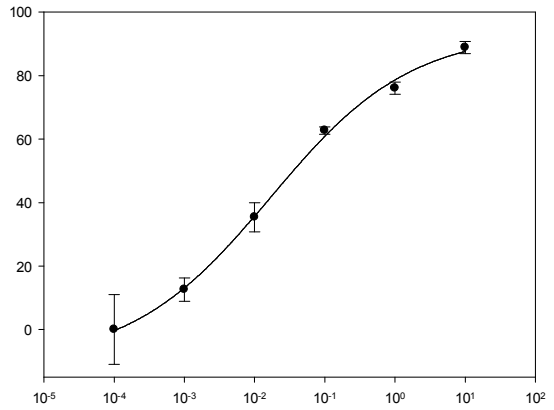
Section F. Preliminary screen for inhibitory activity against tankyrase-2 at 10 nM



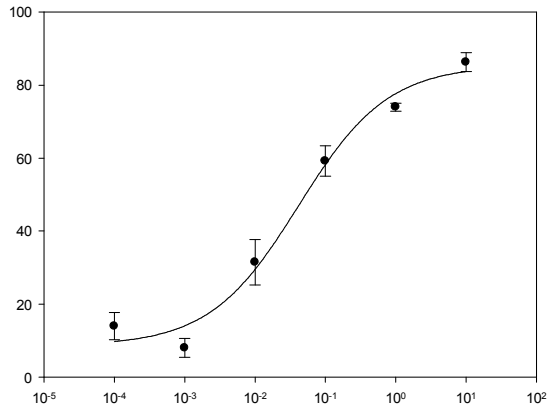
Compound	R^1	R^2	% Inhibition ± standard error
1	-	-	59 ± 9
3	H	Cl	2 ± 5
7a	Me	H	41 ± 6
7b	Me	CH_3	74 ± 1
7c	Me	CF_3	62 ± 8
7d	Me	NO_2	63 ± 9
7e	Me	OCH_3	77 ± 2
7f	Me	Cl	49 ± 14
7l	OH	Cl	32 ± 2
7i	OMe	Cl	25 ± 5
7g	Me	Br	77 ± 7
7h	Me	F	49 ± 8
7j	Me	NH_2	68 ±3
7k	Me	OH	68 ± 3
10	Me	Cbz	70 ± 9
11	Me	$N^+H_3\ Br^-$	5 ± 2

Section G: %Inhibition *vs.* concentration graphs for tankyrase-1 assay

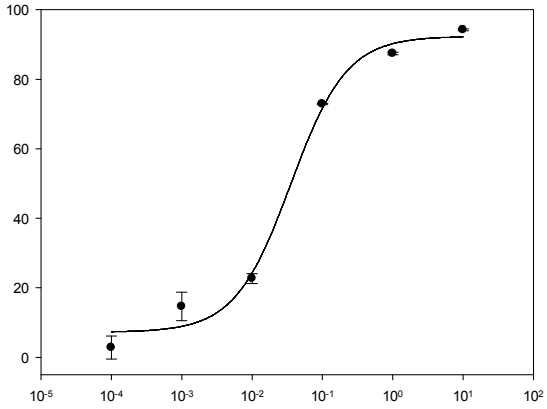
X-axes: Concentration of compound (μM); Y-axes: % Inhibition of enzyme activity



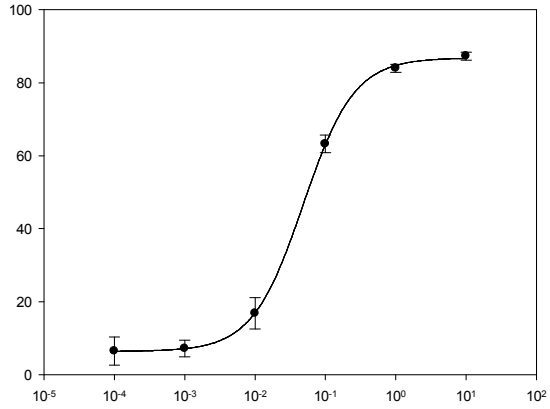
Inhibition of tankyrase-1 activity by 1



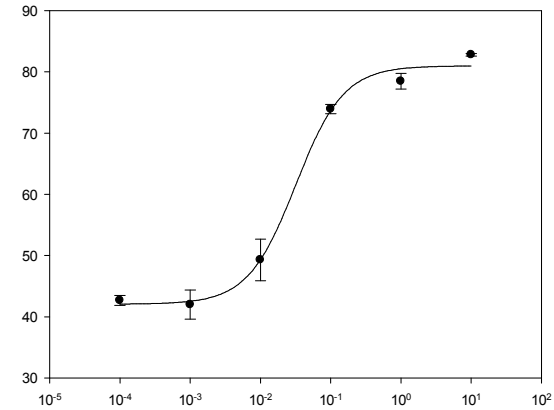
Inhibition of tankyrase-1 activity by 7a



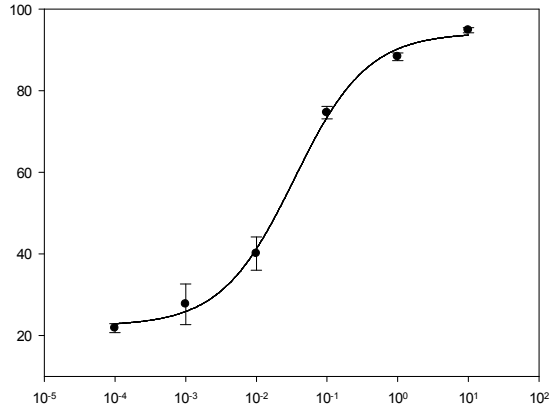
Inhibition of tankyrase-1 activity by 7b



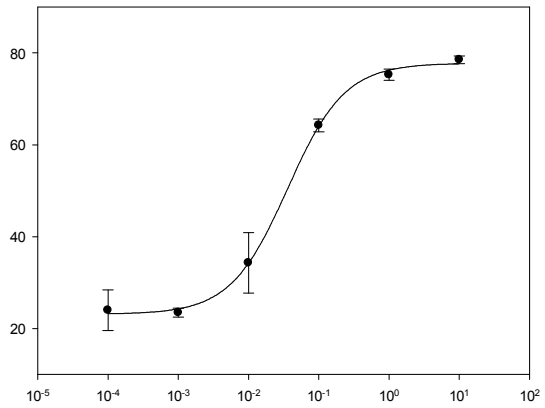
Inhibition of tankyrase-1 activity by 7c



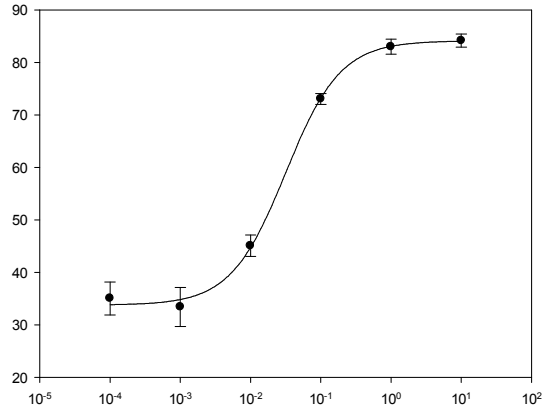
Inhibition of tankyrase-1 activity by 7d



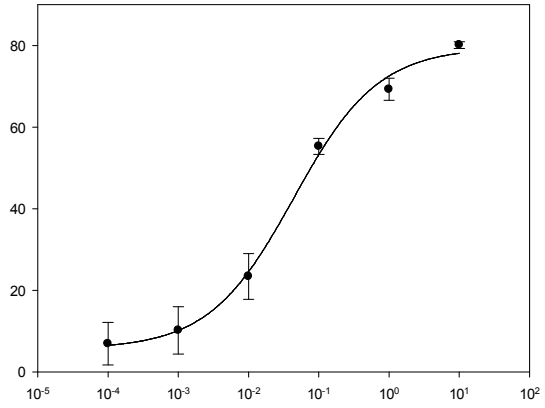
Inhibition of tankyrase-1 activity by 7e



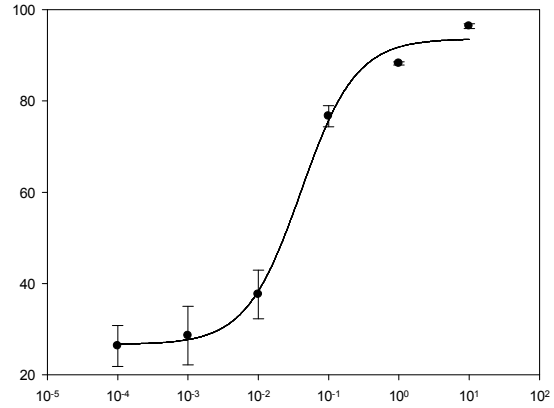
Inhibition of tankyrase-1 activity by 7f



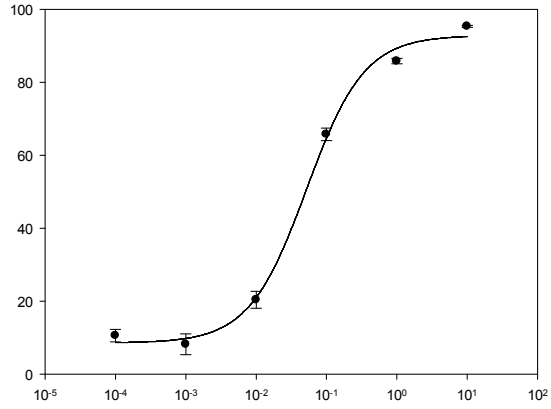
Inhibition of tankyrase-1 activity by 7g



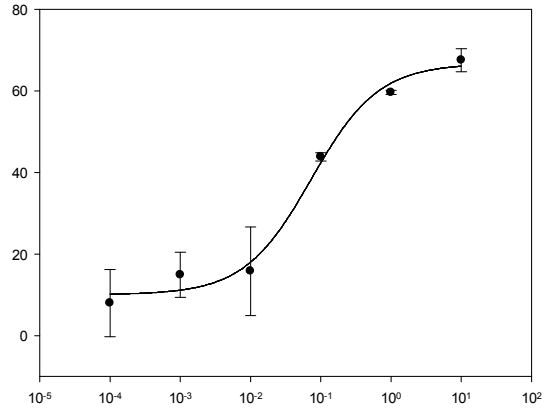
Inhibition of tankyrase-1 activity by 7h



Inhibition of tankyrase-1 activity by 7j



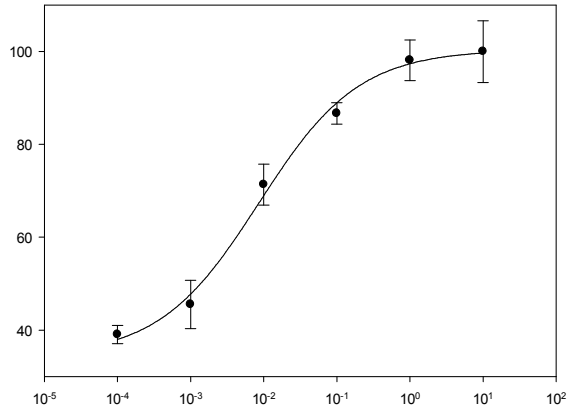
Inhibition of tankyrase-1 activity by 7k



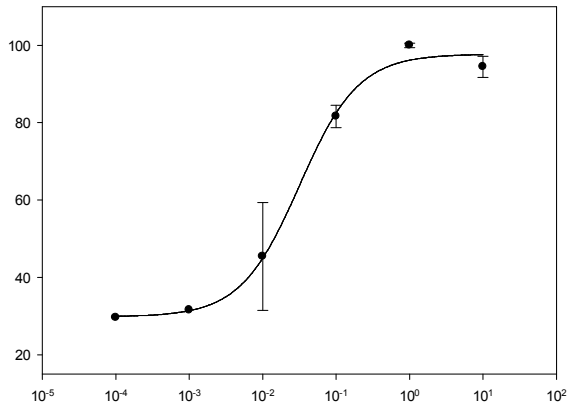
Inhibition of tankyrase-1 activity by 10

Section H: %Inhibition *vs.* concentration graphs for tankyrase-2 assay

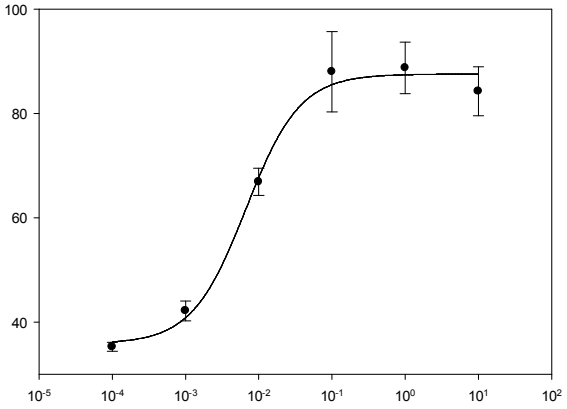
X-axes: Concentration of compound (μM); Y-axes: % Inhibition of enzyme activity



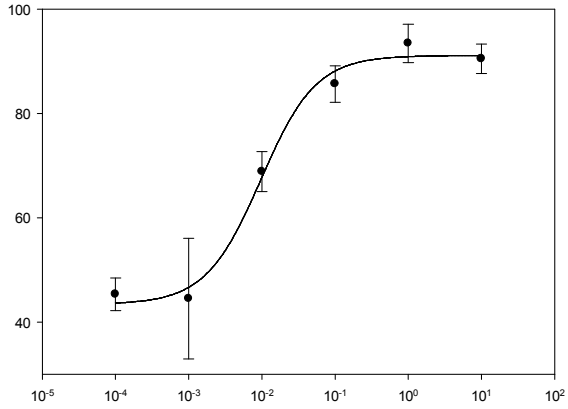
Inhibition of tankyrase-2 activity by 1



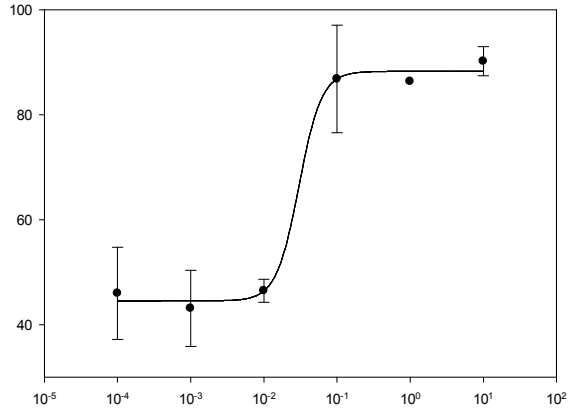
Inhibition of tankyrase-2 activity by 7a



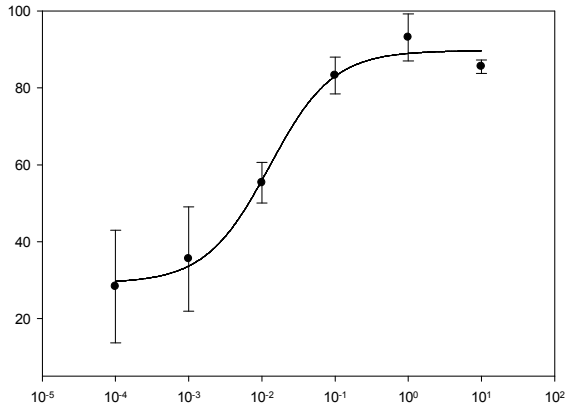
Inhibition of tankyrase-2 activity by 7b



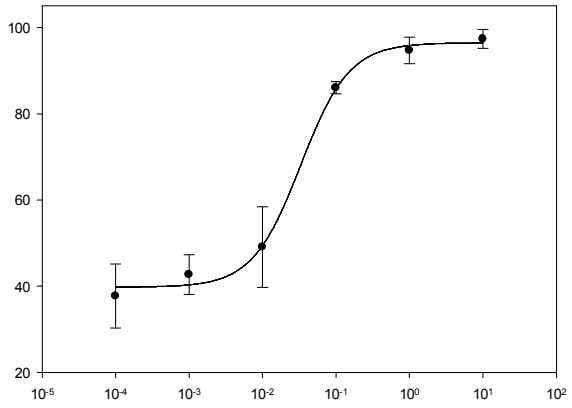
Inhibition of tankyrase-2 activity by 7c



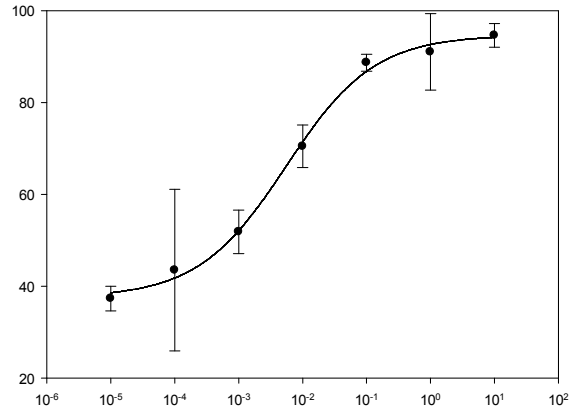
Inhibition of tankyrase-2 activity by 7d



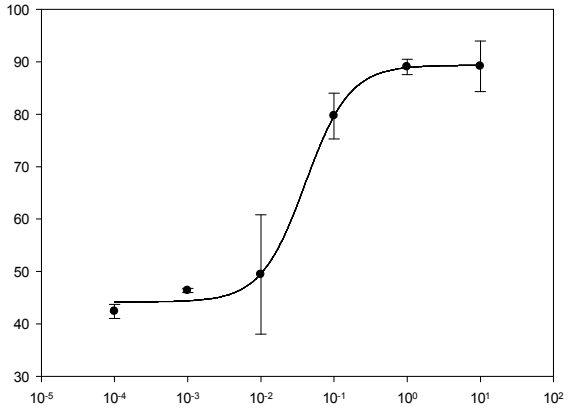
Inhibition of tankyrase-2 activity by 7e



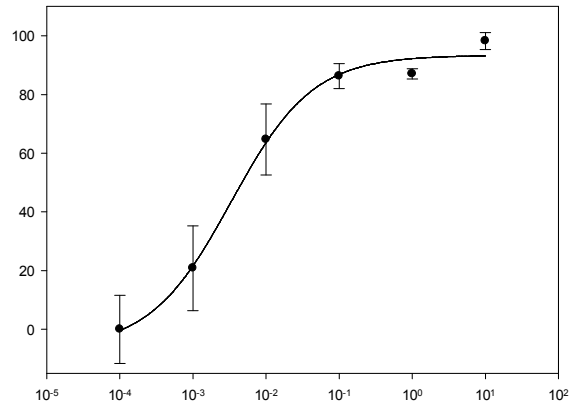
Inhibition of tankyrase-2 activity by 7f



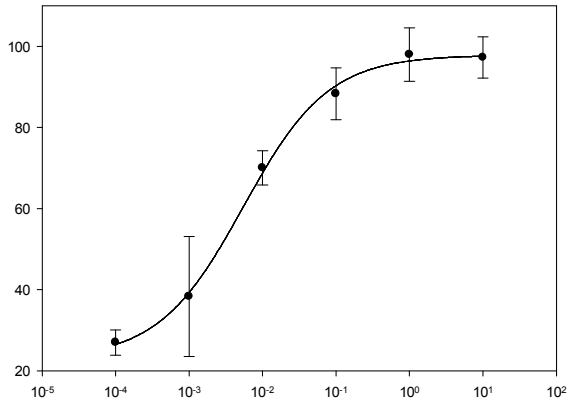
Inhibition of tankyrase-2 activity by 7g



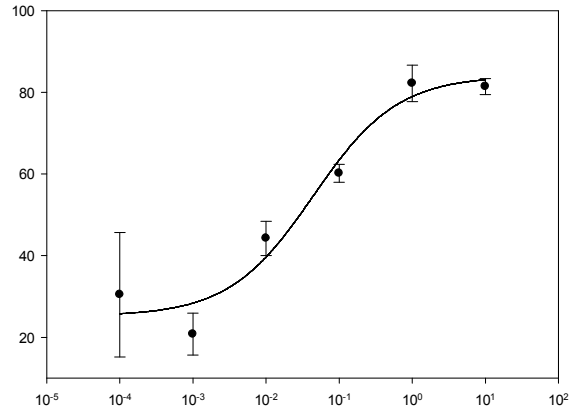
Inhibition of tankyrase-2 activity by 7h



Inhibition of tankyrase-2 activity by 7j



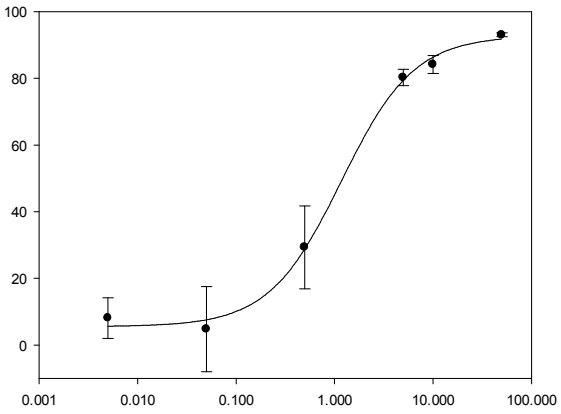
Inhibition of tankyrase-2 activity by 7k



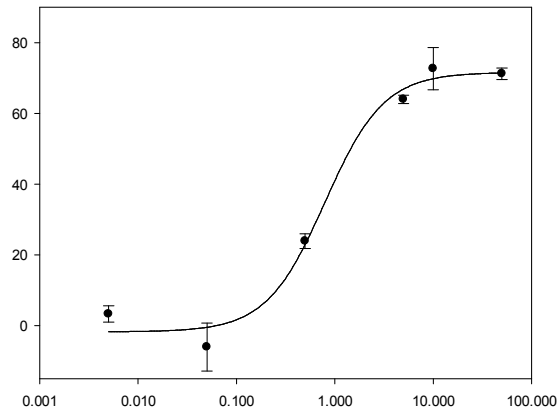
Inhibition of tankyrase-2 activity by 71

Section I: %Inhibition *vs.* concentration graphs for PARP-1 assay

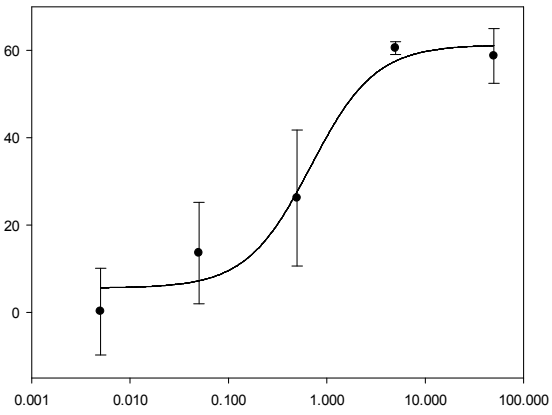
X-axes: Concentration of compound (μM); Y-axes: % Inhibition of enz yme activity



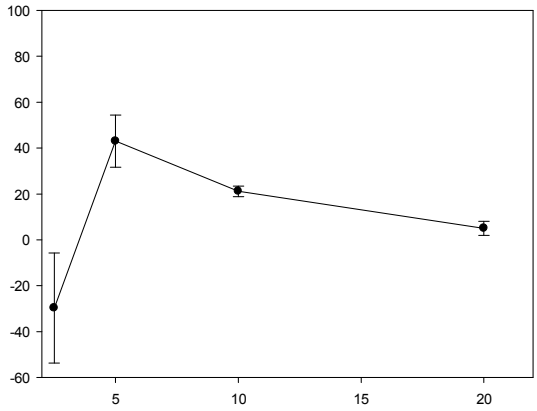
Inhibition of PARP-1 activity by 1



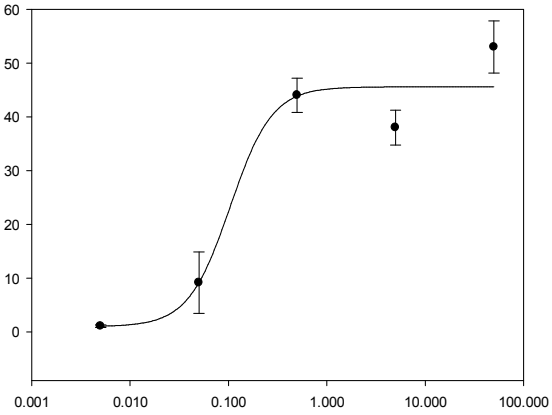
Inhibition of PARP-1 activity by 7a



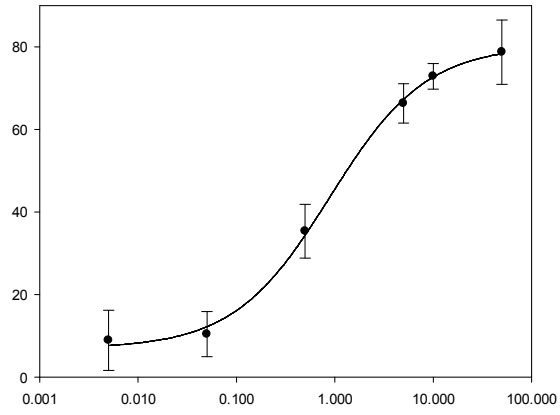
Inhibition of PARP-1 activity by 7b



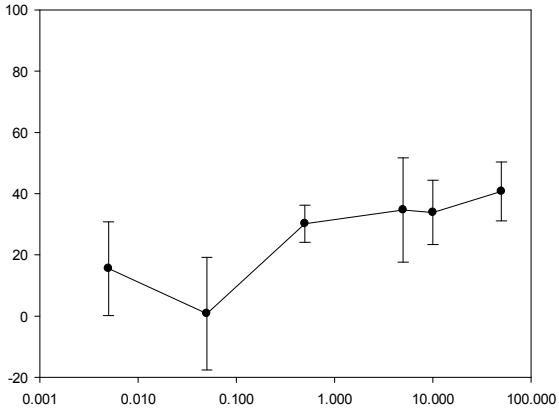
Inhibition of PARP-1 activity by 7c



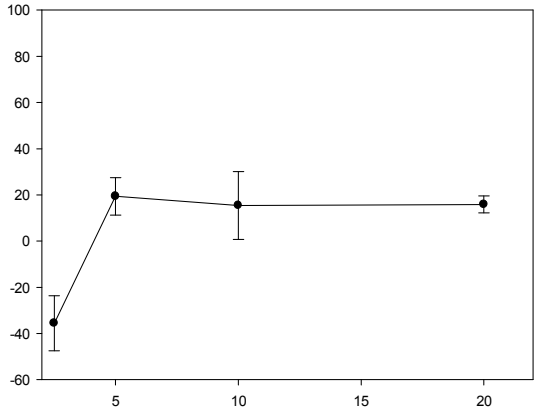
Inhibition of PARP-1 activity by 7d



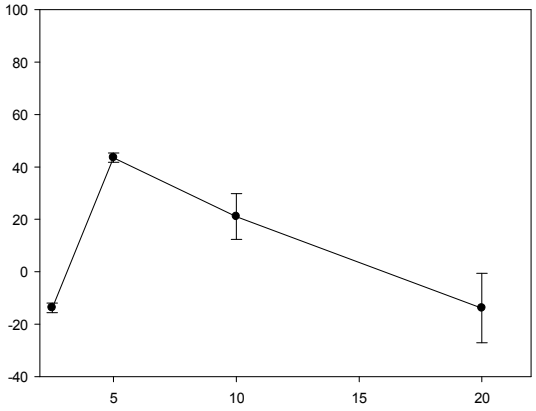
Inhibition of PARP-1 activity by 7e



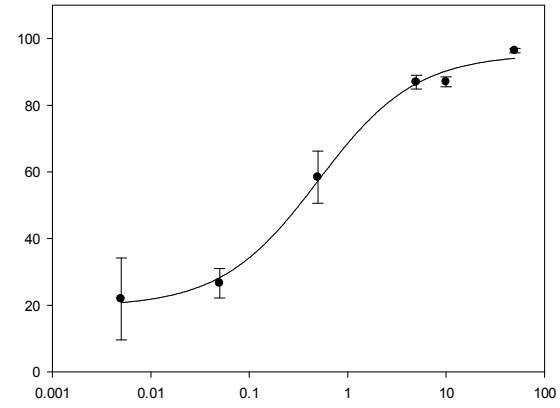
Inhibition of PARP-1 activity by 7f



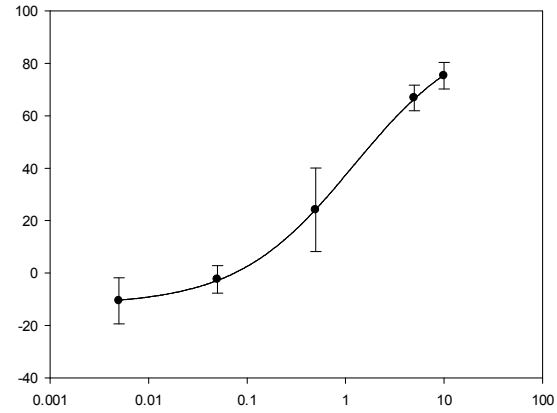
Inhibition of PARP-1 activity by 7g



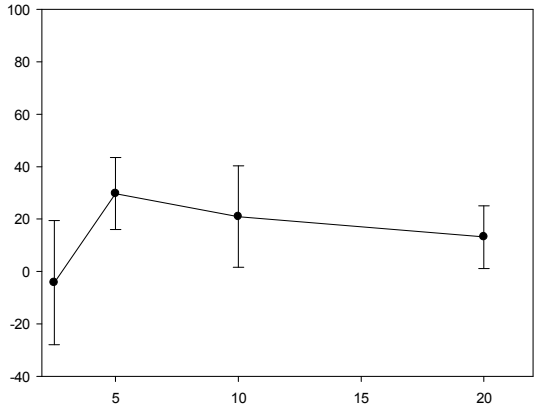
Inhibition of PARP-1 activity by 7h



Inhibition of PARP-1 activity by 7j



Inhibition of PARP-1 activity by 7k

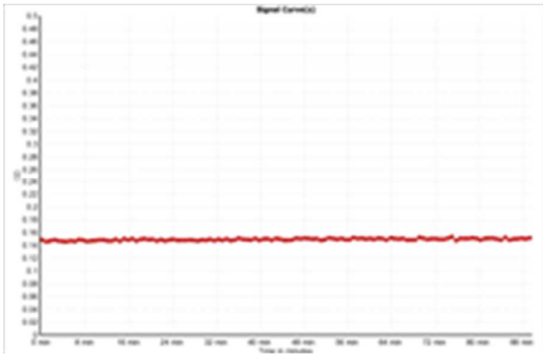


Inhibition of PARP-1 activity by 10

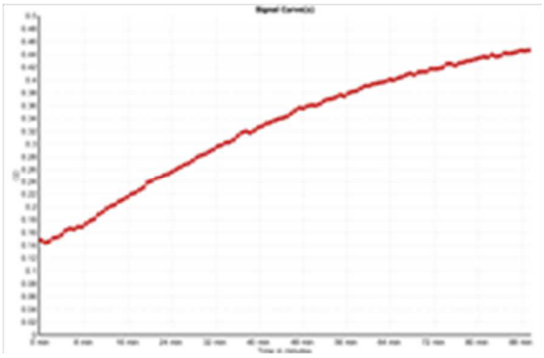
Section J: Absorbance *vs.* time curves for IMPHD2 assay

X-axes: Time (min); Y-axes: Absorbance (340 nM)

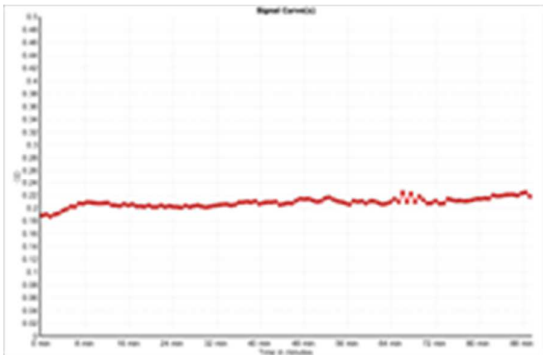
Experiment 1. 6-ThioIMP.



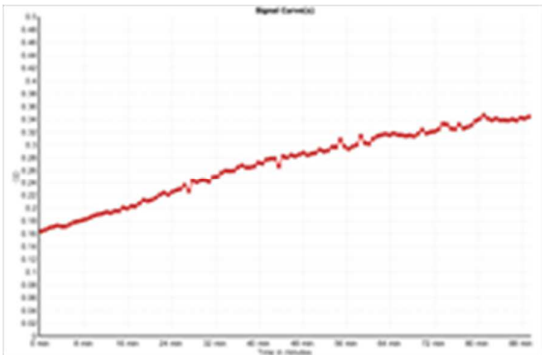
No enzyme



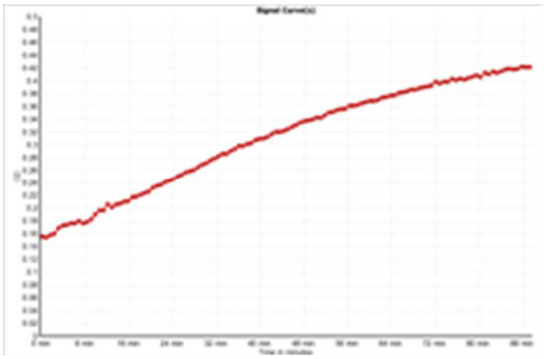
IMPDH2 + 1% DMSO



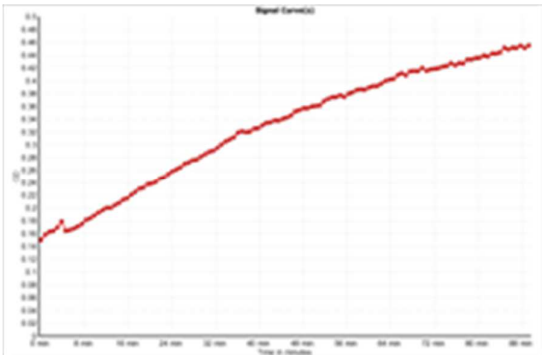
IMPDH2 + 1% DMSO + 6-thioIMP (100 μM)



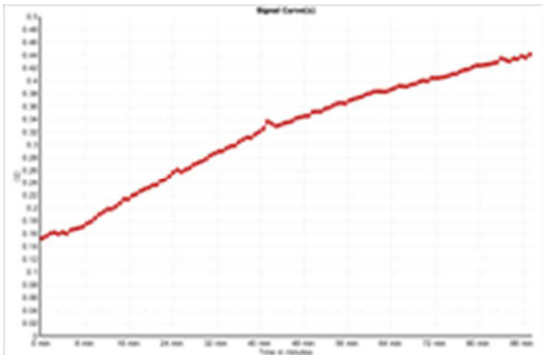
IMPDH2 + 1% DMSO + 6-thioIMP (10 μM)



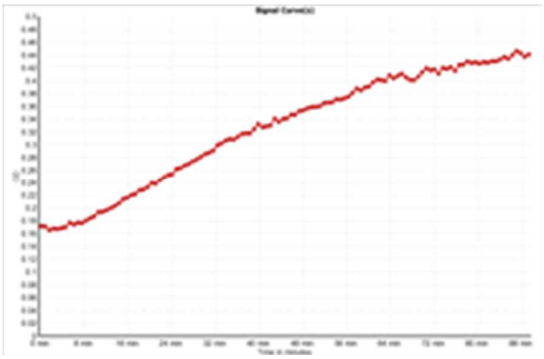
IMPDH2 + 1% DMSO + 6-thioIMP (1.0 μM)



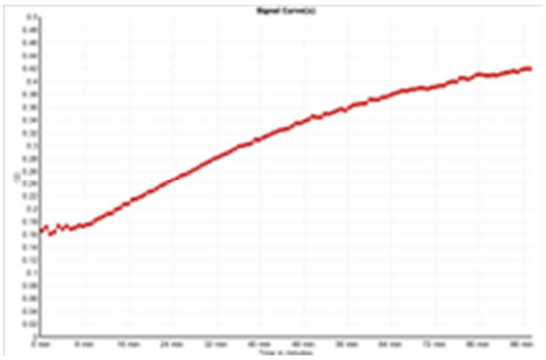
IMPDH2 + 1% DMSO + 6-thioIMP (100 nM)



IMPDH2 + 1% DMSO + 6-thioIMP (10 nM)

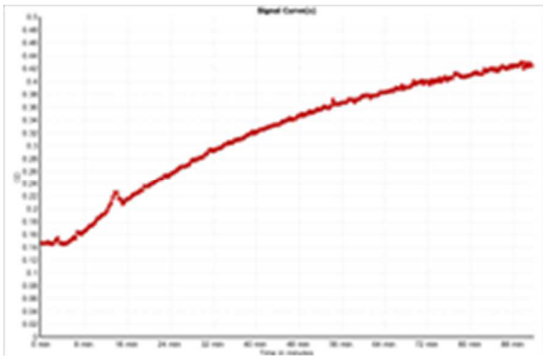


IMPDH2 + 1% DMSO + 6-thioIMP (1.0 nM)

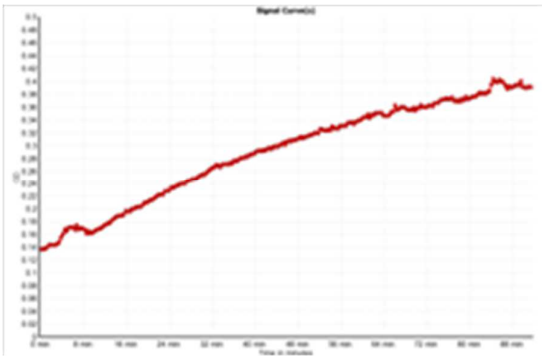


IMPDH2 + 1% DMSO + 6-thioIMP (100 pM)

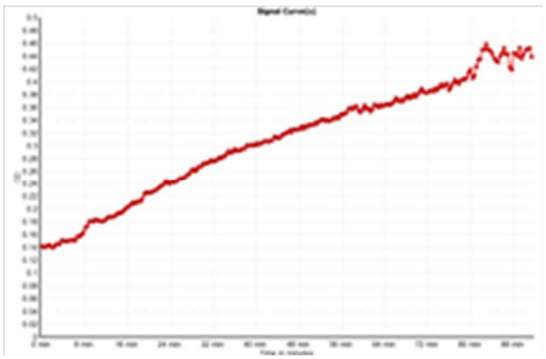
Experiment 2. Compounds 1 and 7a.



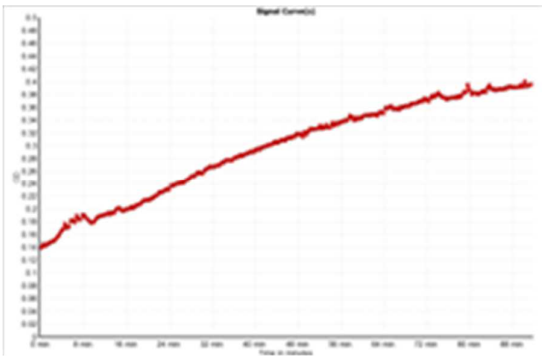
IMPDH2 + 1% DMSO



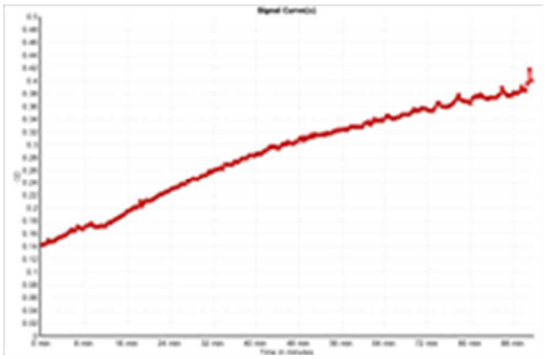
IMPDH2 + 1% DMSO + 1 (10 μM)



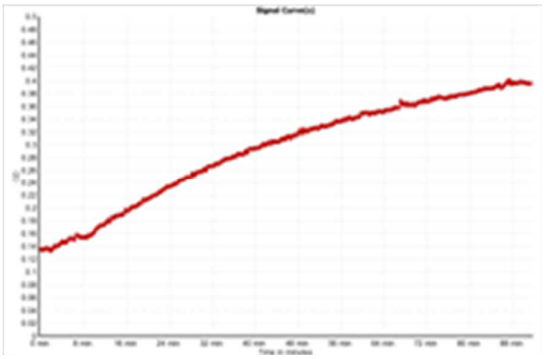
IMPDH2 + 1% DMSO + 1 (1.0 μM)



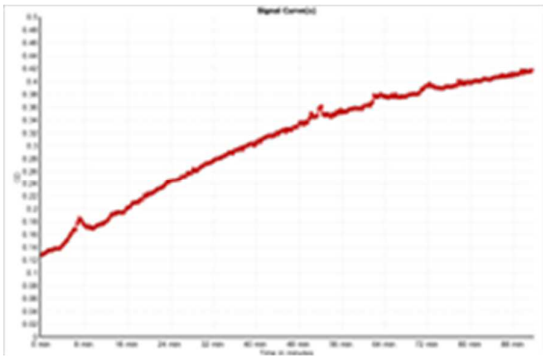
IMPDH2 + 1% DMSO + 1 (100 nM)



IMPDH2 + 1% DMSO + 7a (10 μM)

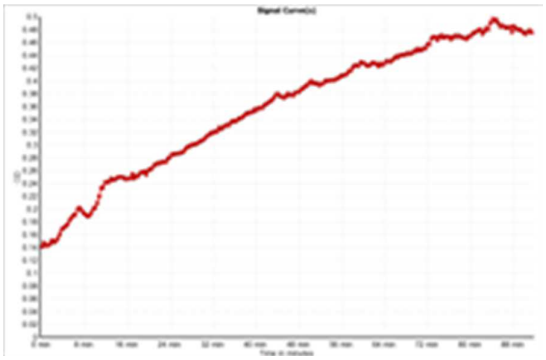


IMPDH2 + 1% DMSO + 7a (1.0 μM)

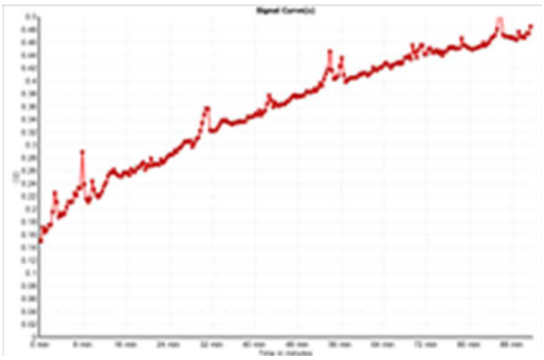


IMPDH2 + 1% DMSO + 7a (100 nM)

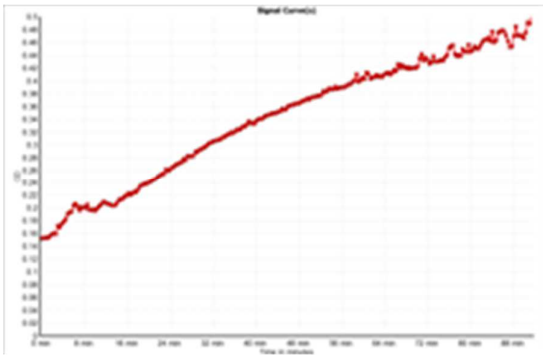
Experiment 3. Compounds 7g,j.



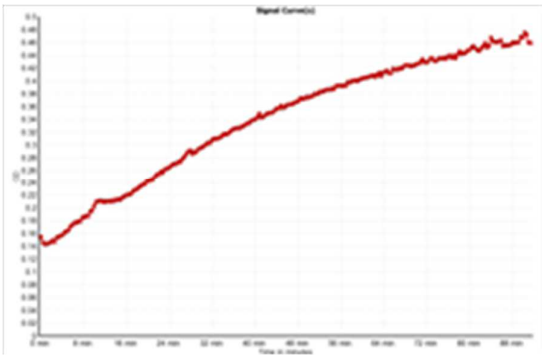
IMPDH2 + 1% DMSO



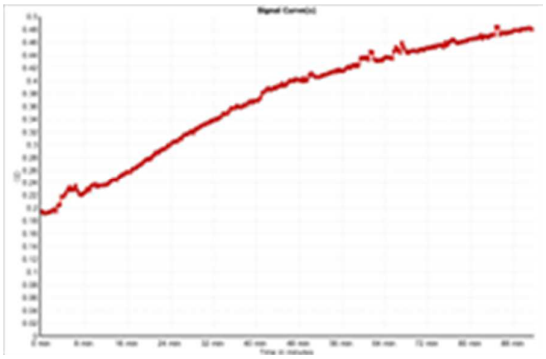
IMPDH2 + 1% DMSO + 7g (10 μM)



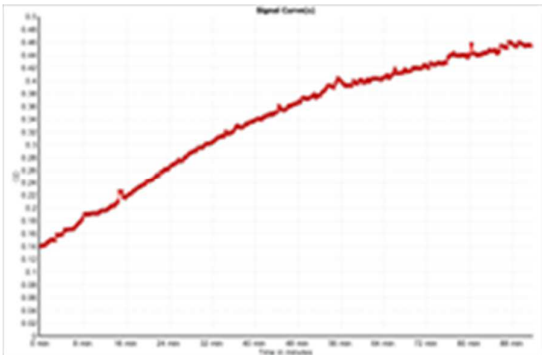
IMPDH2 + 1% DMSO + 7g (1.0 μM)



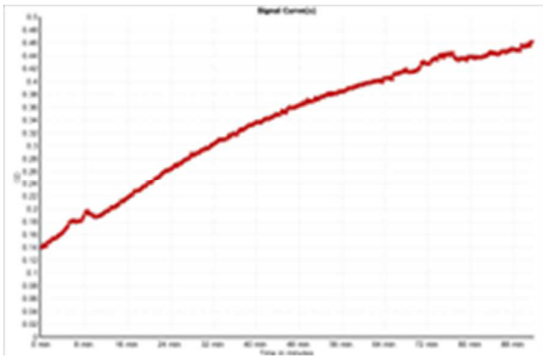
IMPDH2 + 1% DMSO + 7g (100 nM)



IMPDH2 + 1% DMSO + 7j (10 μM)

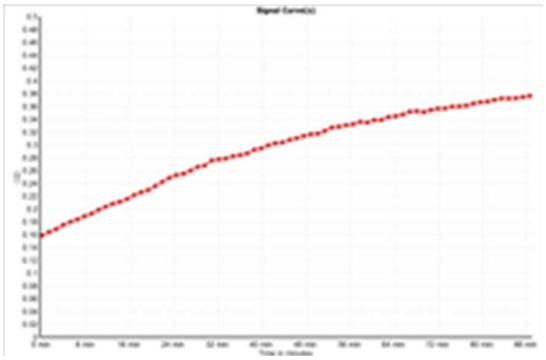


IMPDH2 + 1% DMSO + 7j (1.0 μM)

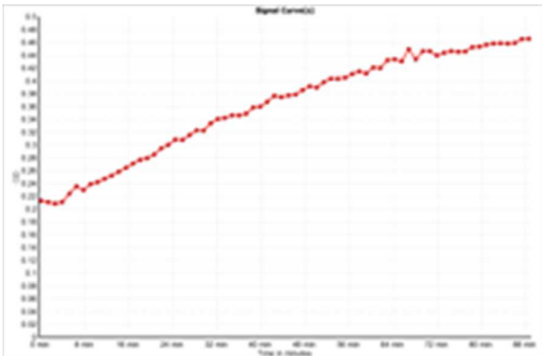


IMPDH2 + 1% DMSO + 7j (100 nM)

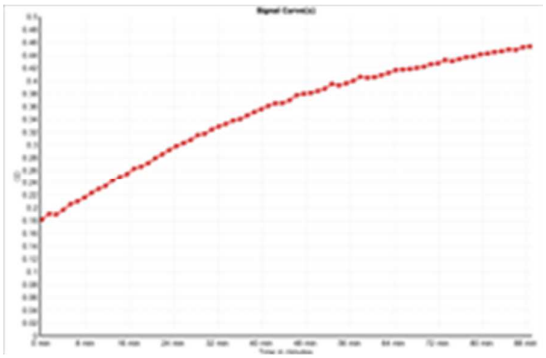
Experiment 4. Compounds 7b-f,h,k and 10.



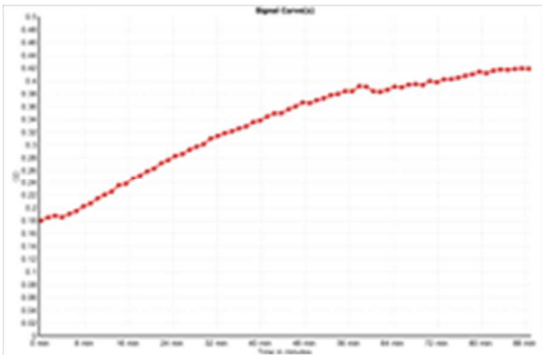
IMPDH2 + 1% DMSO



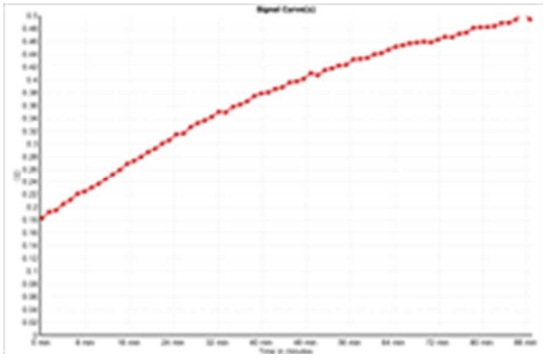
IMPDH2 + 1% DMSO + 7b (10 μM)



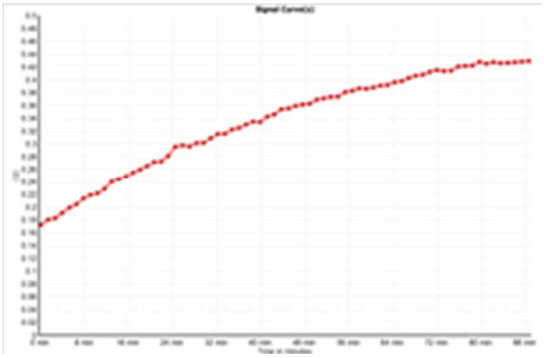
IMPDH2 + 1% DMSO + 7b (1.0 μM)



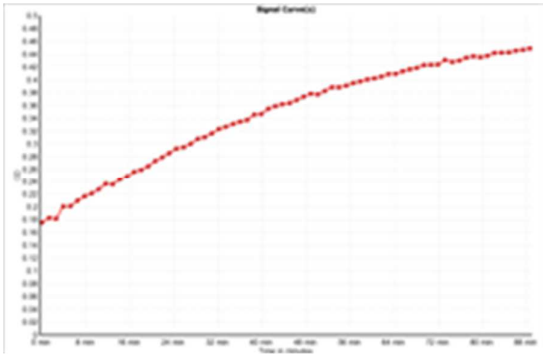
IMPDH2 + 1% DMSO + 7b (100 nM)



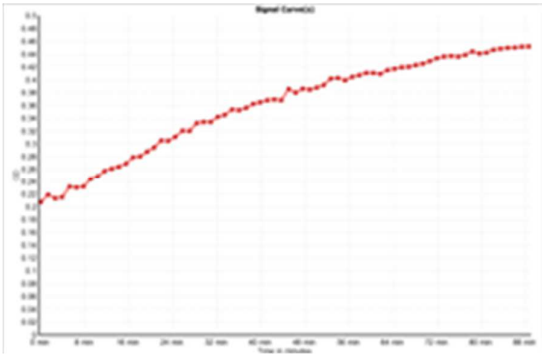
IMPDH2 + 1% DMSO + 7c (10 μM)



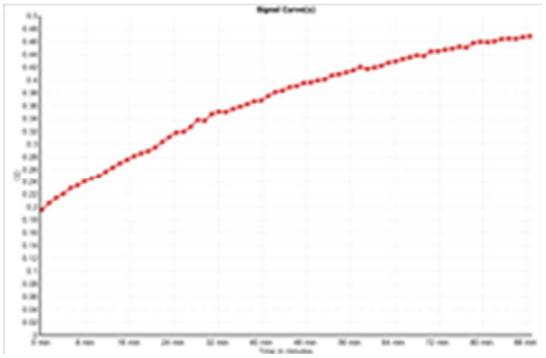
IMPDH2 + 1% DMSO + 7c (1.0 μM)



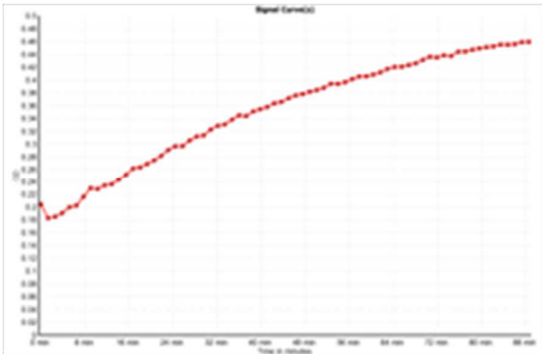
IMPDH2 + 1% DMSO + 7c (100 nM)



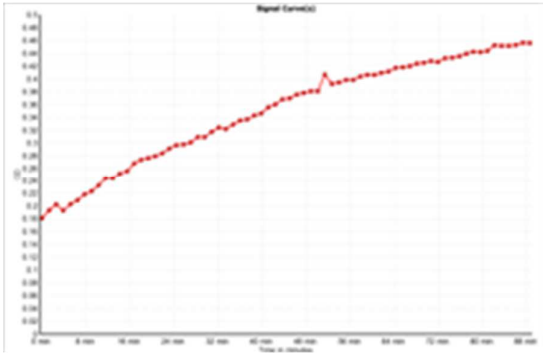
IMPDH2 + 1% DMSO + 7d (10 μM)



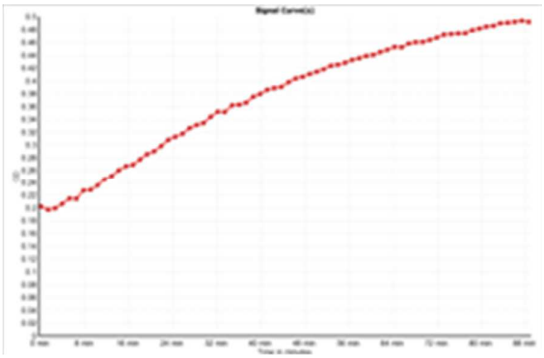
IMPDH2 + 1% DMSO + 7d (1.0 μM)



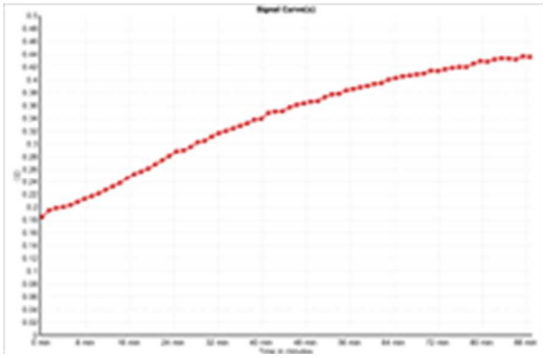
IMPDH2 + 1% DMSO + 7d (100 nM)



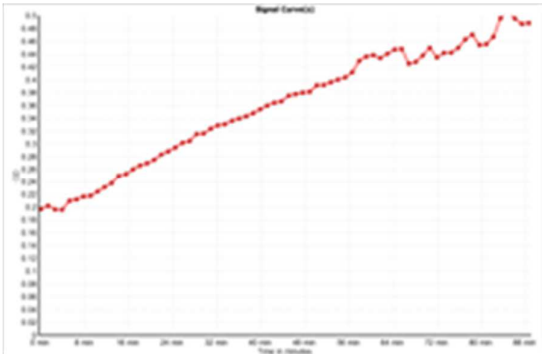
IMPDH2 + 1% DMSO + 7e (10 μM)



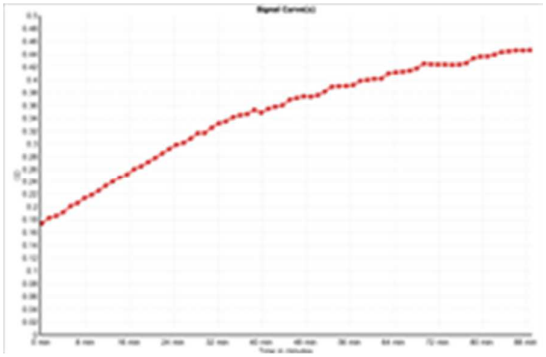
IMPDH2 + 1% DMSO + 7e (1.0 μM)



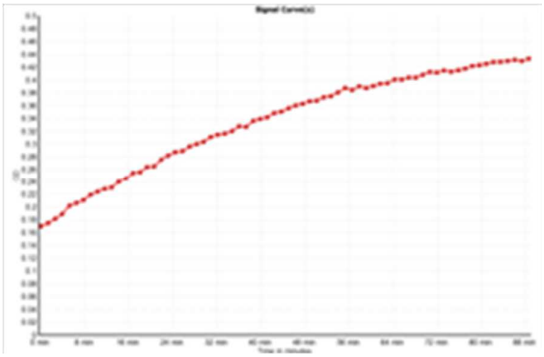
IMPDH2 + 1% DMSO + 7e (100 nM)



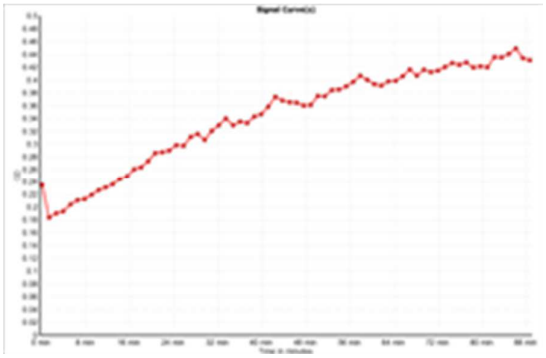
IMPDH2 + 1% DMSO + 7f (10 μM)



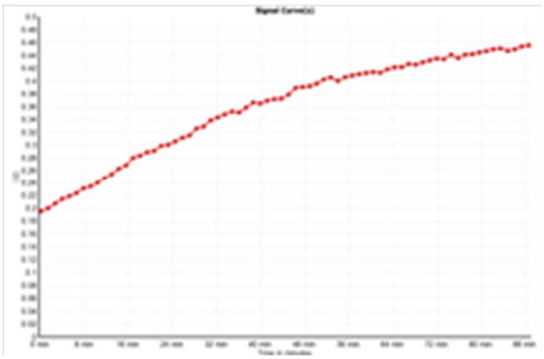
IMPDH2 + 1% DMSO + 7f (1.0 μM)



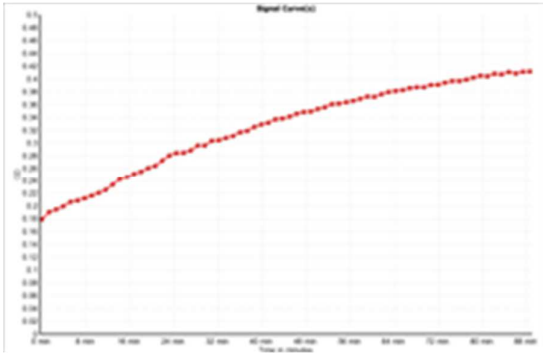
IMPDH2 + 1% DMSO + 7f (100 nM)



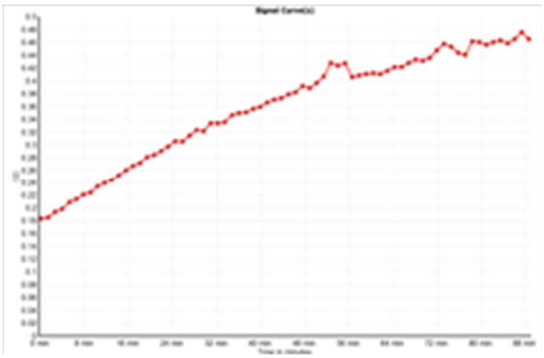
IMPDH2 + 1% DMSO + 7h (10 μM)



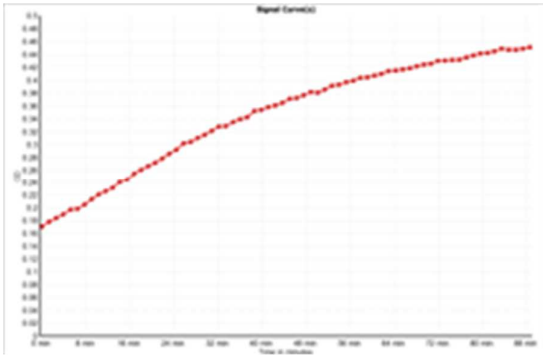
IMPDH2 + 1% DMSO + 7h (1.0 μM)



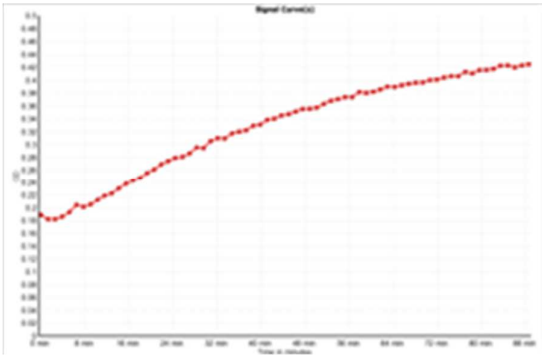
IMPDH2 + 1% DMSO + 7h (100 nM)



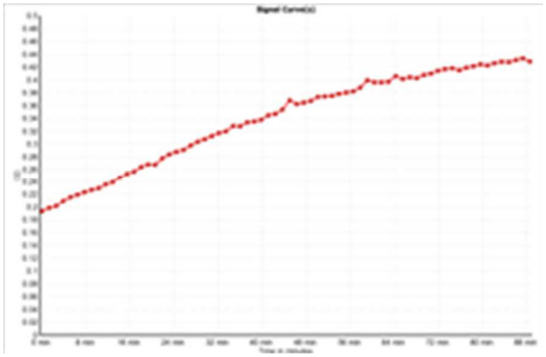
IMPDH2 + 1% DMSO + 7k (10 μM)



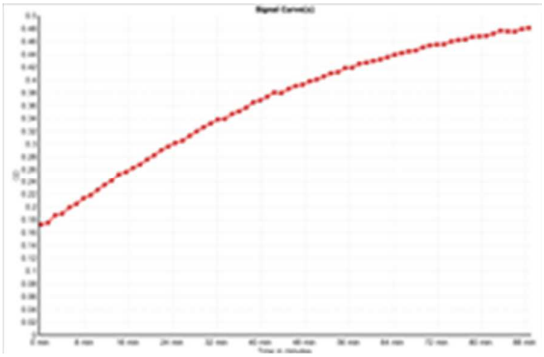
IMPDH2 + 1% DMSO + 7k (1.0 μM)



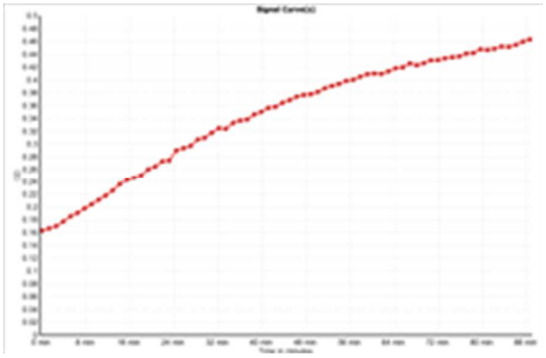
IMPDH2 + 1% DMSO + 7k (100 nM)



IMPDH2 + 1% DMSO + 10 (10 μM)



IMPDH2 + 1% DMSO + 10 (1.0 μM)



IMPDH2 + 1% DMSO + 10 (100 nM)

Section K. Initial rates of IMPDH2-catalysed formation of NADH in the presence of compounds 1,7a-h,j,k and 10.

Experiment	Rate (Absorbance min^{-1})	r^2
IMPDH2 + 1% DMSO	4.90×10^{-3}	0.975
IMPDH2 + 1% DMSO + 6-thioIMP (1.0 mM)	3.12×10^{-4}	N/A
IMPDH2 + 1% DMSO + 6-thioIMP (100 μM)	3.84×10^{-4}	N/A
IMPDH2 + 1% DMSO + 6-thioIMP (10 μM)	2.67×10^{-3}	0.957
IMPDH2 + 1% DMSO + 6-thioIMP (1.0 μM)	4.24×10^{-3}	0.996
IMPDH2 + 1% DMSO + 6-thioIMP (100 nM)	4.68×10^{-3}	0.995
IMPDH2 + 1% DMSO + 6-thioIMP (10 nM)	4.74×10^{-3}	0.995
IMPDH2 + 1% DMSO + 6-thioIMP (1.0 nM)	4.47×10^{-3}	0.977
IMPDH2 + 1% DMSO + 6-thioIMP (100 pM)	4.29×10^{-3}	0.997
IMPDH2 + 1% DMSO + **1** (10 μM)	4.44×10^{-3}	0.987
IMPDH2 + 1% DMSO + **1** (1.0 μM)	4.44×10^{-3}	0.983
IMPDH2 + 1% DMSO + **1** (100 nM)	4.04×10^{-3}	0.989
IMPDH2 + 1% DMSO + **7a** (10 μM)	4.73×10^{-3}	0.988
IMPDH2 + 1% DMSO + **7a** (1.0 μM)	4.58×10^{-3}	0.993
IMPDH2 + 1% DMSO + **7a** (100 nM)	4.76×10^{-3}	0.964
IMPDH2 + 1% DMSO + **7b** (10 μM)	4.45×10^{-3}	0.994
IMPDH2 + 1% DMSO + **7b** (1.0 μM)	4.45×10^{-3}	0.997
IMPDH2 + 1% DMSO + **7b** (100 nM)	4.41×10^{-3}	0.994
IMPDH2 + 1% DMSO + **7c** (10 μM)	4.59×10^{-3}	0.987
IMPDH2 + 1% DMSO + **7c** (1.0 μM)	4.51×10^{-3}	0.976
IMPDH2 + 1% DMSO + **7c** (100 nM)	4.21×10^{-3}	0.983
IMPDH2 + 1% DMSO + **7d** (10 μM)	4.51×10^{-3}	0.988

IMPDH2 + 1% DMSO + **7d** (1.0 μM)	4.46×10^{-3}	0.966
IMPDH2 + 1% DMSO + **7d** (100 nM)	4.47×10^{-3}	0.993
IMPDH2 + 1% DMSO + **7e** (10 μM)	4.14×10^{-3}	0.954
IMPDH2 + 1% DMSO + **7e** (1.0 μM)	4.97×10^{-3}	0.997
IMPDH2 + 1% DMSO + **7e** (100 nM)	4.24×10^{-3}	0.994
IMPDH2 + 1% DMSO + **7f** (10 μM)	4.65×10^{-3}	0.994
IMPDH2 + 1% DMSO + **7f** (1.0 μM)	5.15×10^{-3}	0.996
IMPDH2 + 1% DMSO + **7f** (100 nM)	5.75×10^{-3}	0.997
IMPDH2 + 1% DMSO + **7g** (10 μM)	4.69×10^{-3}	0.986
IMPDH2 + 1% DMSO + **7g** (1.0 μM)	4.72×10^{-3}	0.973
IMPDH2 + 1% DMSO + **7g** (100 nM)	4.70×10^{-3}	0.989
IMPDH2 + 1% DMSO + **7h** (10 μM)	4.63×10^{-3}	0.984
IMPDH2 + 1% DMSO + **7h** (1.0 μM)	4.53×10^{-3}	0.988
IMPDH2 + 1% DMSO + **7h** (100 nM)	4.10×10^{-3}	0.993
IMPDH2 + 1% DMSO + **7j** (10 μM)	4.53×10^{-3}	0.993
IMPDH2 + 1% DMSO + **7j** (1.0 μM)	4.75×10^{-3}	0.978
IMPDH2 + 1% DMSO + **7j** (100 nM)	4.66×10^{-3}	0.992
IMPDH2 + 1% DMSO + **7k** (10 μM)	4.81×10^{-3}	0.995
IMPDH2 + 1% DMSO + **7k** (1.0 μM)	4.62×10^{-3}	0.968
IMPDH2 + 1% DMSO + **7k** (100 nM)	4.87×10^{-3}	0.946
IMPDH2 + 1% DMSO + **10** (10 μM)	4.11×10^{-3}	0.961
IMPDH2 + 1% DMSO + **10** (1.0 μM)	4.92×10^{-3}	0.997
IMPDH2 + 1% DMSO + **10** (100 nM)	4.27×10^{-3}	0.995

Section L: References for Supplementary Information

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