Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Operative conversions of 3-carboxy-4-quinolones into 3-nitro-4-quinolones via ipso-nitration: Potential antifilarial agents as inhibitor of *Brugia malayi* thymidylate kinase.

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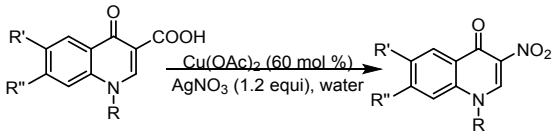
General Information

Reagent grade solvents were used for the extraction and flash chromatography. All the reagents and chemicals were purchased from Sigma Aldrich Chemical Co., Lancaster and were used directly without further purification. The progress of reactions was checked by analytical thin-layer chromatography (TLC, Merck silica gel 60F-254 plates). The plates were visualized by UV illumination. Flash column chromatography was performed using silica gel (230-400 mesh). The solvent compositions reported for all chromatographic separations are on a volume/volume (v/v) basis. All glassware's were dried in an open flame before use in connection with an inert atmosphere. Solvents were evaporated under reduced pressure. Tetramethylsilane (0.0 ppm) was used as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) was used in ^{13}C NMR. The abbreviations used to indicate the peak multiplicity were; s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; Hz, Hertz. FAB MS was recorded on Jeol (Japan)/SX-102. Infrared spectrum was taken with KBr on Perkin-Elmer RX-1. Melting points were determined on a Buchi 535 digital melting point apparatus and were uncorrected. Elemental analysis was performed on Perkin-Elmer 2400 C, H, N analyzer and values were within ±0.4% of the calculated values.

Experimental procedure:



Procedure 1 : In a typical experiment, the quinolone acid (**12**) (1 mmol)[1], copper acetate (60 mol %) and Silver nitrate (1.2 mmol) were taken in 50 ml round-bottom flask containing deionised water (10 mL) and the reaction mixture was refluxed, till the completion of the reaction (monitored by TLC). After completion the reaction mixture was poured in 100gm cursed ice and the solid compound separated and filtered, in some case solid is not separated in such case the compound was extracted with ethyl acetate, dried over sodium sulphate and evaporated under vacuum to give crude product (**13**). Crude was purified by silica gel (60-120 mesh) column chromatography to afford the corresponding product.

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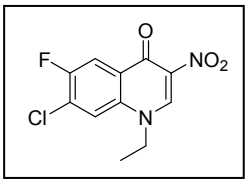
1. Note: All the precursors were synthesized according to the published literature. (Koga, H.; Itoh A.; Murayama, S.; Suzue S. and Irikura T., *J. Med. Chem.*; 1980, **23**, 1358)
2. Raghavan, K.; Lang, S. A. and Marshall M. S., *J. Heterocycl. Chem*, 1986, **23**, 1801.

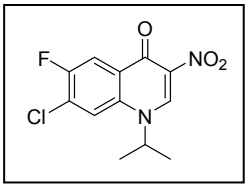
Procedure 2: A mixture of The ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 15 (269.66mg, 1mmol) and LiCl 1.5 equivalent (1.5mmol) in DMSO (15ml) was refluxed for 12 h, and then DMSO is removed in vacuum. The residue was repeatedly washed with water (20ml x 3) and was dissolved in 10 ml of deionised water and copper acetate (60 mol %) and silver nitrate (1.3 mmol) were added. The reaction mixture was than refluxed for about 12hrs. The reaction mixture was poured in water (50 ml), and the separated compound 15b was filtered, crystalized in DMSO to give white needle crystal 145.59mg (The spectral data is accord to the published literature). The product was arylated with 4-fluoronitrobenze (2 equivalent) in K_2CO_3 (4 equivalent)/DMF (8ml) by heating at 100-100^oC for 10 hrs. The crude product thus obtained was purify using column chromatography (25% EtOAc/hexane) to give the title compound 169.81mg (70 %) as yellow solid.

7-chloro-1-ethyl-6-fluoro-3-nitroquinolin-4(1H)-one (13a)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (269.66 mg, 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 235.45 mg (87 %) as pale yellow solid; mp 270-272^oC (uncorrected). IR (ν_{max}, Neat, cm-1): 3444, 3278, 1660, 1434, 1309, 1249, 839, 456, 180. ^1H NMR (300 MHz,) δ 9.71 (s, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 5.9 Hz, 1H), 4.50 (q, *J* = 6.9 Hz, 2H), 1.56 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.94 (d, *J* = 6.5 Hz), 157.95 (s), 152.75 (s), 146.18 (s), 135.61 (d, *J* = 8.5 Hz), 131.89 (s), 126.12 (*m*), 117.85 (d, *J* = 11 Hz), 113.22, 112.82, 48.66 (s), 13.64 (s). Elemental Analysis calculated for $C_{11}H_8ClFN_2O_3$: C, 48.82; H, 2.98; N, 10.35; Found C, 48.62; H, 3.01; N, 10.11. ES-MS (M+H): 271.0 m/z.

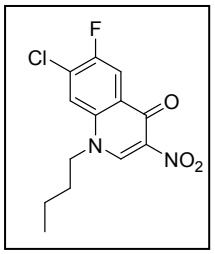
7-chloro-6-fluoro-1-isopropyl-3-nitroquinolin-4(1H)-one (13b)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (284.67mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 273.28 mg (96 %) as pale yellow solid; mp 263-256^oC (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3432, 3258, 1655, 1480, 1320, 1232, 842, 460; ^1H NMR (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.11 (m, 1H), 7.70 (dd, J = 3.4, 2.5 Hz, 1H), 4.74 (m, 1H), 1.56 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.88 (d, *J* = 8.7 Hz),

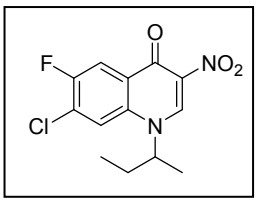
157.98 (s), 152.78 (s), 147.24 (s), 137.47 (d, J = 8.5 Hz), 132.12(s), 127.80 (d, J = 10 Hz), 126.19, 125.82, 118.89 (d, J = 13 Hz), 113.08, 112.69, 54.41 (s), 19.83 (s). Anal. calcd for $C_{12}H_{10}ClFN_2O_3$: C, 50.63; H, 3.54; N, 9.84; Found C, 50.42; H, 3.52; N, 9.74; ES-MS (M+H): 285.0 m/z.

1-butyl-7-chloro-6-fluoro-3-nitroquinolin-4(1H)-one (13c)



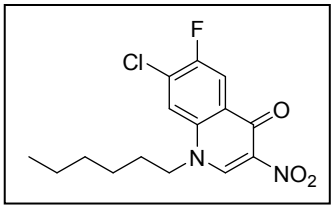
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (298.70mg , 1 mmol), following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 259.86 mg (87 %) as pale yellow solid; mp 275-278^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3461, 3260, 1649, 1474, 1322, 1232, 1211, 851. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.13 (m, 1H), 7.79 (d, J = 5.7 Hz, 1H), 4.10 (t, J = 7.6 Hz, 2H), 1.81 (m, 2H), 1.59 (m, 2H), 1.10 (dd, J = 8.8, 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.83 (d, J = 6.5 Hz), 157.90 (s), 152.70 (s), 145.21 (s), 135.4 (d, J = 6.5 Hz), 131.70 (s), 126.37, 125.9943, 124.60 (d, J = 11 Hz), 116.31 (d, J = 11 Hz), 113.26, 112.88, 48.21 (s), 29.21 (s), 18.99 (s), 13.28 (s). Anal. calcd for $C_{13}H_{12}ClFN_2O_3$: C, 52.27; H, 4.05; N, 9.38; Found C, 52.21; H, 4.12; N, 9.21. ES-MS (M+H): 299.1 m/z.

1-(sec-butyl)-7-chloro-6-fluoro-3-nitroquinolin-4(1H)-one (13d)



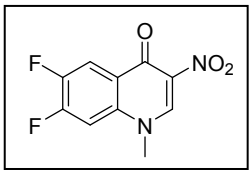
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (298.70mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 250.9 mg (84 %) as pale yellow solid; mp 265-270^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3421, 3269, 1644, 1452, 1325, 1238, 867, 380, 190. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.11 (m, 1H), 7.75 (d, J = 5.6 Hz, 1H), 4.78 (m, 1H), 1.48 (m, 2H), 1.17 (d, J = 6.1 Hz, 3H), 0.92 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.88 (d, J = 9 Hz), 157.63 (s), 152.43 (s), 145.59 (s), 137.97 (d, J = 9 Hz), 132.26 (s), 127.44 (d, J = 12 Hz), 126.25 (s), 125.88 (s), 118.92 (d, J = 11 Hz), 113.14 (s), 112.75 (s), 50.46 (s), 26.77 (s), 19.91 (s), 8.00 (s). Elemental Analysis calculated for $C_{13}H_{12}ClFN_2O_3$: C, 52.27; H, 4.05; N, 9.38; Found C, 52.11; H, 4.10; N, 9.29. ES-MS (M+H): 299.1 m/z.

7-chloro-6-fluoro-1-hexyl-3-nitroquinolin-4(1H)-one (13e)



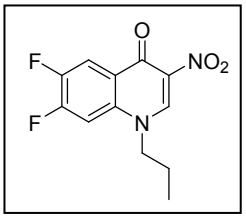
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (325.75 mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 261.4 mg (80 %) as yellow solid; mp 278-280^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3410, 3259, 1633, 1412, 1319, 1241, 854. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.62 (s, 1H), 8.12 (m, 1H), 7.78 (d, *J* = 5.6 Hz, 1H), 4.07 (td, *J* = 7.5, 0.6 Hz, 2H), 1.45 (m, 2H), 1.19 (m, 6H), 0.85 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.81 (d, *J* = 9Hz), 157.91 (s), 152.71 (s), 145.31 (s), 135.1 (d, *J* = 9Hz), 131.68 (s), 126.37 (s), 125.99 (s), 124.6 (d, *J* = 13Hz), 116.30 (d, *J* = 11Hz), 113.25 (s), 112.87 (s), 48.68 (s), 30.89 (s), 27.76 (s), 26.30 (s), 21.49 (s), 13.21 (s). Anal. calcd for $C_{15}H_{16}ClFN_2O_3$: C, 55.14; H, 4.94; N, 8.57; Found C, 55.11; H, 4.32; N, 8.39. ES-MS (M+H): 327.9 m/z.

6,7-difluoro-1-methyl-3-nitroquinolin-4(1H)-one (13f)



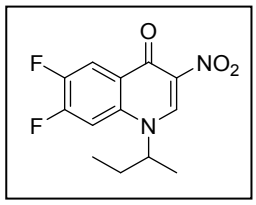
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (240.17mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 196.93 mg (82 %) as canary yellow solid; mp 256-259^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 33265, 3061, 1653, 1514, 1463, 1321, 1205, 843. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.36 (t, *J* = 9.4 Hz, 1H), 7.51 (dd, *J* = 11.2, 6.5 Hz, 1H), 3.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 161.43 (d, *J* = 8 Hz), 155.19 (s), 154.82 (s), 150.81 (s), 150.42 (s), 150.0 (s), 149.62 (s), 145.60 (s), 145.23 (s), 144.99 (s), 138.11 (m), 122.81 (m), 112.97 (d, *J* = 12.5 Hz), 112.60 (d, *J* = 11 Hz), 103.25 (d, *J* = 13 Hz), 102.87 (d, *J* = 11.5 Hz) 44.04 (s). Elemental Analysis calculated for $C_{10}H_6F_2N_2O_3$: C, 50.01; H, 2.52; N, 11.66; Found C, 49.98; H, 2.32; N, 11.69. ES-MS (M+H): 241.0 m/z.

6,7-difluoro-3-nitro-1-propylquinolin-4(1H)-one (13g)



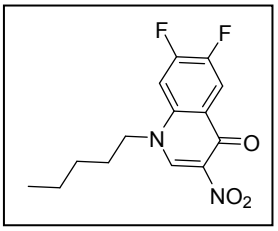
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (268.22mg, 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 217.25 mg (81 %) as pale yellow solid; mp 262-263^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3286, 3050, 1644, 1534, 1454, 1311, 1211, 831. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (m, 1H), 8.30 (t, *J* = 9.5 Hz, 1H), 7.42 (dd, *J* = 11.0, 6.5 Hz, 1H), 3.59 (t, *J* = 7.4 Hz, 2H), 1.55 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 161.32 (d, *J* = 9 Hz), 155.71 (s), 154.93(s), 150.49 (d, *J* = 1.2 Hz), 150.11 (d, *J* = 2 Hz), 149.73 (s), 145.28 (s), 144.90 (m), 136.04 (m), 132.00 (s), 123.84 (m), 112.90 (d, *J* = 11 Hz), 112.52 (d, *J* = 11.5 Hz) 114.20 (d, *J* = 13 Hz) 104.01 (d, *J* = 13 Hz), 50.83 (s), 21.73 (s), 9.96 (s). Anal. calcd for $C_{12}H_{10}F_2N_2O_3$: C, 53.74; H, 3.76; N, 10.44; Found C, 53.14; H, 3.62; N, 10.16. ES-MS (M+H): 269.8 m/z.

1-(sec-butyl)-6,7-difluoro-3-nitroquinolin-4(1H)-one (13h)



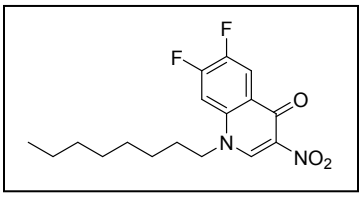
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (282.25mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 231.43 mg (82 %) as yellow solid; mp 265-266^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3276, 3105, 1631, 1510, 1432, 1331, 1232, 852. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.31 (t, J = 9.4 Hz, 1H), 7.43 (m, 1H), 4.79 (m, 1H), 1.49 (m, 2H), 1.17 (d, J = 6.0 Hz, 3H), 0.94 (dd, J = 9.4, 5.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 161.23 (d, *J* = 6.5 Hz), 154.96 (s), 154.57 (s), 150.48 (s), 150.11 (s), 149.74 (s), 147.40 (d, *J* = 0.6 Hz), 145.43 (s), 145.1 (d, *J* = 0.5 Hz), 144.90 (d, *J* = 1 Hz), 136.68 (m), 132.20 (s), 124.36 (m), 122.75 (d, *J* = 11 Hz), 12.37 (d, *J* = 13 Hz), 105.05 (d, *J* = 14 Hz) 104.67 (d, *J* = 11.5 Hz), 50.29 (s), 27.38 (s), 19.73 (s), 8.00 (s). Anal. calcd for $C_{13}H_{12}F_2N_2O_3$: C, 55.32; H, 4.29; N, 9.93; Found C, 55.26; H, 4.09; N, 10.01. ES-MS (M+H): 283.0 m/z.

6,7-difluoro-3-nitro-1-pentylquinolin-4(1H)-one (13i) This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (296.27mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 263.68 mg (89 %) as pale yellow solid; mp 269-271^{o}C



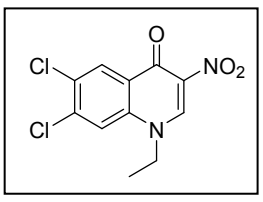
(uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3406, 3209, 3109, 1648, 1551, 1469, 1325, 1212, 867. ^1H NMR (300 MHz, $CDCl_3$) δ 9.67 (s, 1H), 8.31 (t, J = 9.5 Hz, 1H), 7.58 (dd, J = 11.0, 6.4 Hz, 1H), 4.03 (t, J = 7.5 Hz, 2H), 1.30 (m, 6H), 0.87 (dd, J = 8.5, 4.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 161.21 (d, *J* = 9 Hz), 155.29 (s), 154.91 (s), 150.77 (s), 150.40 (s), 150.08 (s), 149.71 (d, *J* = 0.75 Hz), 145.58 (s), 145.33 (s), 145.19 (s), 135.35 (m), 131.63 (s), 122.01 (m), 122.85 (d, *J* = 11 Hz), 122.49 (d, *J* = 11 Hz), 102.55 (d, *J* = 11 Hz), 102.16 (d, *J* = 13 Hz), 48.55 (s), 30.54 (s), 27.94 (s), 21.15 (s), 12.30 (s). Elemental Analysis calculated for $C_{14}H_{14}F_2N_2O_3$: C, 56.76; H, 4.76; N, 9.46; Found: C, 56.34; H, 4.52; N, 9.49. ES-MS (M+H): 297.1 m/z.

6,7-difluoro-3-nitro-1-octylquinolin-4(1H)-one (13j)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (338.35mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 294.36 mg (87 %) as pale yellow solid; mp 276-279°C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3434, 3221, 3089, 1659, 1532, 1473, 1362, 1239, 856. ^1H NMR (300 MHz, $CDCl_3$) δ 9.67 (s, 1H), 8.31 (t, *J* = 9.5 Hz, 1H), 7.59 (dd, *J* = 11.0, 6.4 Hz, 1H), 4.04 (dd, *J* = 11.5, 4.1 Hz, 2H), 1.42 (m, 2H), 1.22 (m, 10H), 0.84 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 161.19 (d, *J* = 9 Hz), 155.26 (s), 154.88 (s), 150.76 (s), 150.39 (s), 150.07 (s), 149.68 (s), 145.95 (s), 145.56 (s), 145.18 (s), 135.36 (m), 131.62 (s), 122.02 (m), 112.85 (d, *J* = 13 Hz), 112.48 (d, *J* = 13 Hz), 102.52 (d, *J* = 11 Hz), 102.14 (d, *J* = 11 Hz), 49.77 (s), 30.40 (s), 28.68 (s), 28.59 (s), 27.71 (s), 26.67 (s), 21.91 (s), 13.37 (s); Anal. calcd for $C_{17}H_{20}F_2N_2O_3$: C, 60.35; H, 5.96; N, 8.28; Found C, 59.98; H, 6.01.; N, 8.32. ES-MS (M+H): 339.1 m/z.

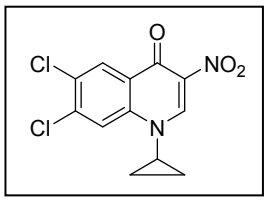
6,7-dichloro-1-ethyl-3-nitroquinolin-4(1H)-one (13k)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (287.1mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 241.16 mg (84 %) as pale yellow solid; mp 266-267°C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3421, 3211, 3090, 1651, 1512, 1485,

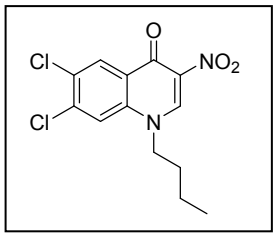
1352, 1231, 856, 660; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, 1H), 8.49 (s, 1H), 7.97 (s, 1H), 4.53 (q, *J* = 6.7 Hz, 2H), 1.56 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 163.41, 146.26, 136.80, 136.45, 132.07, 129.76, 128.04, 126.82, 117.46, 48.05, 13. 65; Anal. calcd for $C_{11}H_8Cl_2N_2O_3$: C, 46.02; H, 2.81; N, 9.76; Found C, 46.22; H, 2.74; N, 9.68. ES-MS (M+H): 286.9 m/z.

6,7-dichloro-1-cyclopropyl-3-nitroquinolin-4(1H)-one (13l)



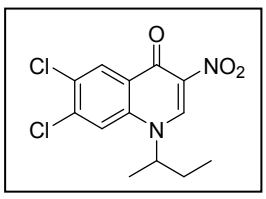
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (299.11mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 248.26 mg (83 %) as pale yellow solid; mp 271-271^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3440, 3321, 3101, 1644, 1521, 1467, 1331, 1242, 866, 649. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.47 (s, 1H), 7.89 (s, 1H), 3.67 (dd, *J* = 2.9, 1.9 Hz, 1H), 1.02 (m, 2H), 0.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 163.01, 147.23, 136.17, 135.26, 132.60, 129.79, 127.79, 127.75, 118.75, 33.42, 10.32. Anal. calcd for $C_{12}H_8Cl_2N_2O_3$: C, 48.19; H, 2.70; N, 9.37; Found C, 48.12; H, 2.72; N, 9.52. ES-MS (M+H): 298.9 m/z.

1-butyl-6,7-dichloro-3-nitroquinolin-4(1H)-one (13m)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (315.15mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (20%EtOAc/hexane) to give the title compound 286.78 mg (91 %) as yellow solid; 268-270^{o}C (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3412, 3287, 3089, 1639, 1532, 1471, 1328, 1220, 869, 598. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (s, 1H), 8.49 (s, 1H), 7.95 (s, 1H), 4.13 (t, *J* = 7.6 Hz, 2H), 1.81 (m, 2H), 1.60 (m, 2H), 1.09 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 163.27, 145.26, 137.15, 136.48, 131.86, 129.70, 128.05, 125.14, 115.71, 47.60, 29.21, 18.96, 13.27. Anal. calcd for $C_{13}H_{12}Cl_2N_2O_3$: C, 49.55; H, 3.84; N, 8.89; Found C, 49.43; H, 3.54; N, 8.73. ES-MS (M+H): 315.02 m/z.

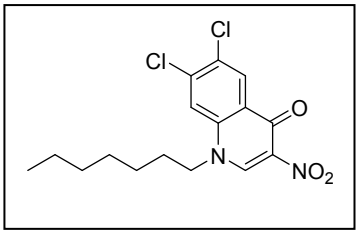
1-(sec-butyl)-6,7-dichloro-3-nitroquinolin-4(1H)-one (13n)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (315.15mg , 1 mmol) with 10ml of conc.HNO_3 and

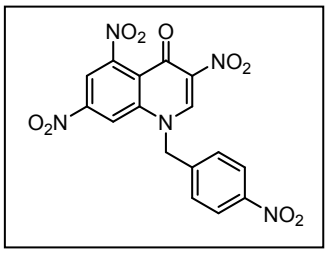
conc. H_2SO_4 (3:1), following the procedure 1. The crude product thus obtained was purify using column chromatography (20% EtOAc/hexane) to give the title compound 258.42 mg (82%) as pale yellow solid; mp 265-266^oC (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3367, 3298, 3083, 1661, 1541, 1469, 1373, 1249, 841, 582, 421. ^1H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.45 (s, 1H), 7.97 (s, 1H), 4.79 (m, 1H), 1.49 (m, 2H), 1.16 (d, *J* = 6.1 Hz, 3H), 0.91 (dd, *J* = 9.6, 5.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 163.30, 145.65, 137.82, 136.37, 132.45, 129.43, 128.00, 127.96, 118.37, 49.83, 26.76, 19.90, 8.01 . Anal. calcd for $C_{13}H_{12}Cl_2N_2O_3$: C, 49.55; H, 3.84; N, 8.89; Found C, 49.51; H, 3.91; N, 8.73. ES-MS (M+H): 315.02 m/z.

6,7-dichloro-1-heptyl-3-nitroquinolin-4(1*H*)-one (13o)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative (356.07mg , 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography(20% EtOAc/hexane) to give the title compound 307.21 mg (86 %) as pale yellow solid; mp 280-282^oC (uncorrected). IR (ν_{max}, Neat, cm^{-1}): 3421, 3321, 3211, 3108, 1659, 1488, 1392, 1241, 860, 637, 487. ^1H NMR (300 MHz, $CDCl_3$) δ 9.75 (m, 1H), 8.48 (s, 1H), 7.95 (s, 1H), 4.10 (td, *J* = 7.5, 0.6 Hz, 2H), 1.45 (m, 4H), 1.21 (m, 6H), 0.85 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 163.25, 146.18, 137.11, 136.47, 131.86, 129.69, 128.05, 125.13, 115.70, 48.26, 30.99, 27.78, 27.32, 26.65, 21.98, 13.37. Anal. calcd. for $C_{16}H_{18}Cl_2N_2O_3$: C, 53.80; H, 5.08; N, 7.84; Found C, 53.72; H, 5.11; N, 7.99. ES-MS (M+H): 357.07 m/z.

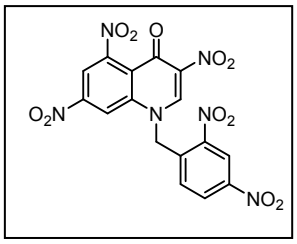
3,5,7-trinitro-1-(4-nitrobenzyl)quinolin-4(1*H*)-one (14a)



This compound was prepared *via* interaction of corresponding 3-carboxylic derivative **14** (324.29mg , 1 mmol), following the procedure **1**. The crude product thus obtained was purify using column chromatography (40% EtOAc/hexane) to give the firstly title compound **14a** 145.27mg (35 %) as canary yellow solid; mp >300^oC. IR (ν_{max}, Neat, cm^{-1}): 3132, 1660, 1532, 1329, 1261, 869. ^1H NMR (300 MHz, $CDCl_3$) δ 10.05 (m, 1H), 8.99 (d, *J* = 0.9 Hz, 1H), 8.22 (d, *J* = 8.5 Hz, 2H), 8.00 (d, *J* = 1.0 Hz, 1H), 7.69 (s, 2H), 5.88

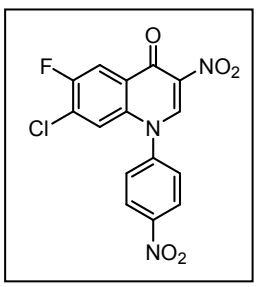
(s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.69, 150.40, 146.38, 146.30, 144.65, 143.84, 141.63, 130.14, 128.17, 123.83, 122.93, 122.89, 117.61, 113.49, 54.97. Elemental Analysis calculated for $C_{16}H_9N_5O_9$: C, 46.28; H, 2.18; N, 16.86; Found C, 46.16; H, 2.15; N, 16.91. ES-MS (M+H): 416.04 m/z.

1-(2,4-dinitrobenzyl)-3,5,7-trinitroquinolin-4(1*H*)-one (14b)



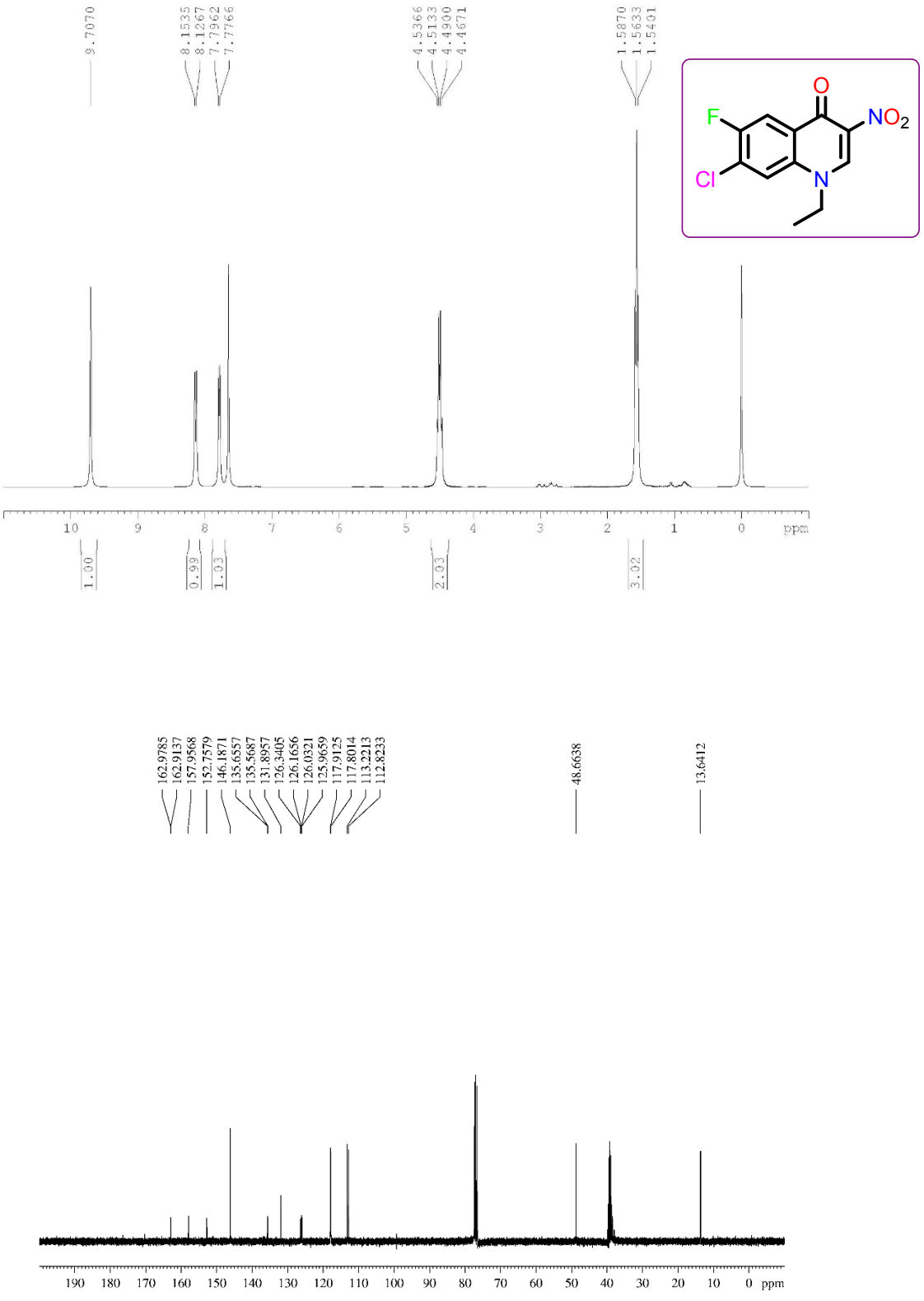
This compound was prepared *via* interaction of corresponding 3-carboxylic derivative **14** (324.29mg, 1 mmol) following the procedure **1**. The crude product thus obtained was purify using column chromatography (40% EtOAc/hexane) to give the compound **14a** and then title compound **14b** 138.08mg (30 %) as dark yellow solid; mp >300^{o}C. IR (ν_{max}, Neat, cm^{-1}): 3214, 1656, 1521, 1341, 1269, 860. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.96 (d, *J* = 0.9 Hz, 1H), 8.70 (m, 1H), 8.42 (d, *J* = 7.7 Hz, 1H), 8.03 (s, 1H), 7.77 (m, 1H), 6.05 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.69, 150.40, 147.80, 147.78, 146.37, 145.69, 141.81, 141.65, 140.27, 131.17, 129.34, 127.80, 123.57, 119.20, 117.60, 113.23, 52.61. Anal. calcd for $C_{16}H_8N_6O_{11}$: C, 41.75; H, 1.75; N, 18.26; Found C, 41.56; H, 1.65; N, 18.12. ES-MS (M+H): 461.03 m/z.

7-chloro-6-fluoro-3-nitro-1-(4-nitrophenyl)quinolin-4(1*H*)-one (16)



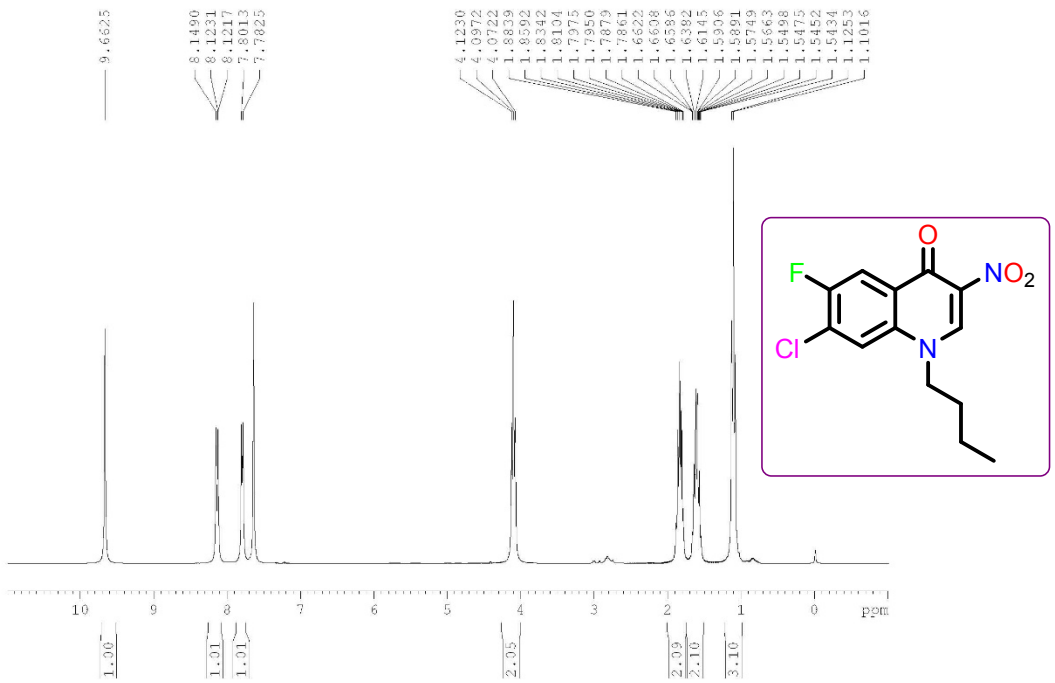
This compound was prepared *via* interaction of corresponding 3-deacrboxylative (Krapcho decarboxylation, Procedure **2**) derivative **15a** (197.59mg, 1 mmol) following the procedure 1, and then product so got after purification arylated with 4-fluoronitrobenze (2 equivalent) in K_2CO_3 (4equivalnet)/DMF (8ml). The crude product thus obtained was purify using column chromatography (25% EtOAc/hexane) to give the title compound

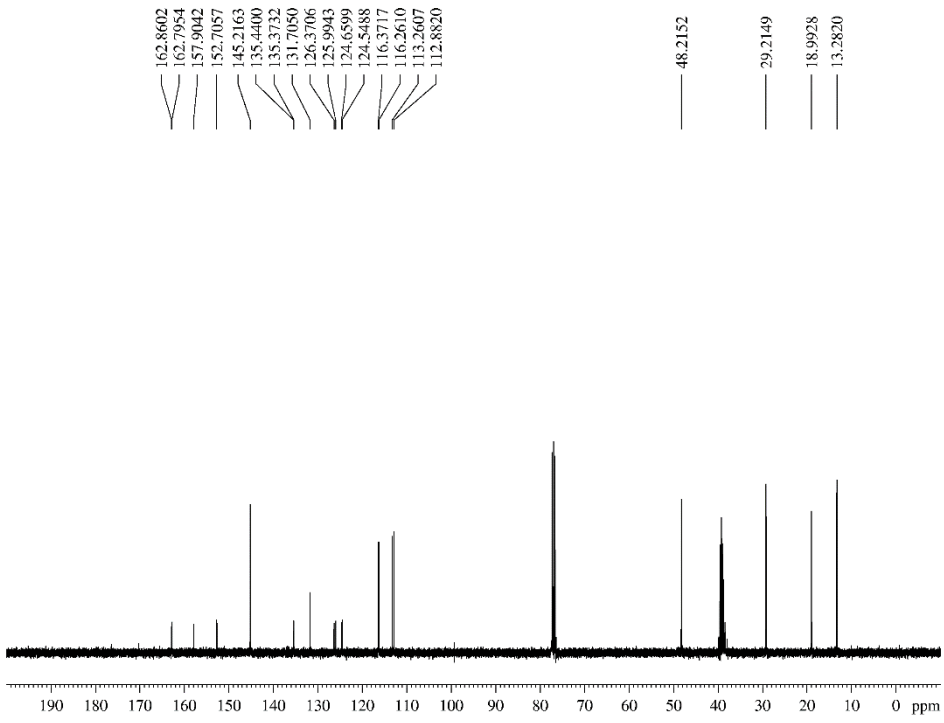
16 169.81mg (70 %) as yellow solid; mp 298-300^{o}C. IR (ν_{max}, Neat, cm^{-1}): 3312, 3114, 1653, 1511, 1340, 1222, 869, 740. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.51 (s, 1H), 8.36 (d, *J* =8.6 Hz, 2H), 8.18 (m, 1H), 7.74 (m, 2H), 7.63 (m, 1H): ^{13}C NMR (100 MHz, $CDCl_3$ + DMSOd_6) δ 162.53 (d, *J* = 7 Hz), 157.18 (s), 151.99 (s), 143.09 (s), 142.55 (s), 141.09 (s), 137.77 (s), 136.74 (d, *J* = 7.5 Hz), 126.91 (s), 126.52 (s), 123.70 (s), 120.71(d, *J* = 11 Hz), 119.55 (s), 115.28 (s), 114.91 (s. Elemental Analysis calculated for $C_{16}H_8N_6O_{11}$: C, 41.75; H, 1.75; N, 18.26; Found C, 41.32; H, 1.83; N, 18.25. ES-MS (M+H): 461.03 m/z.



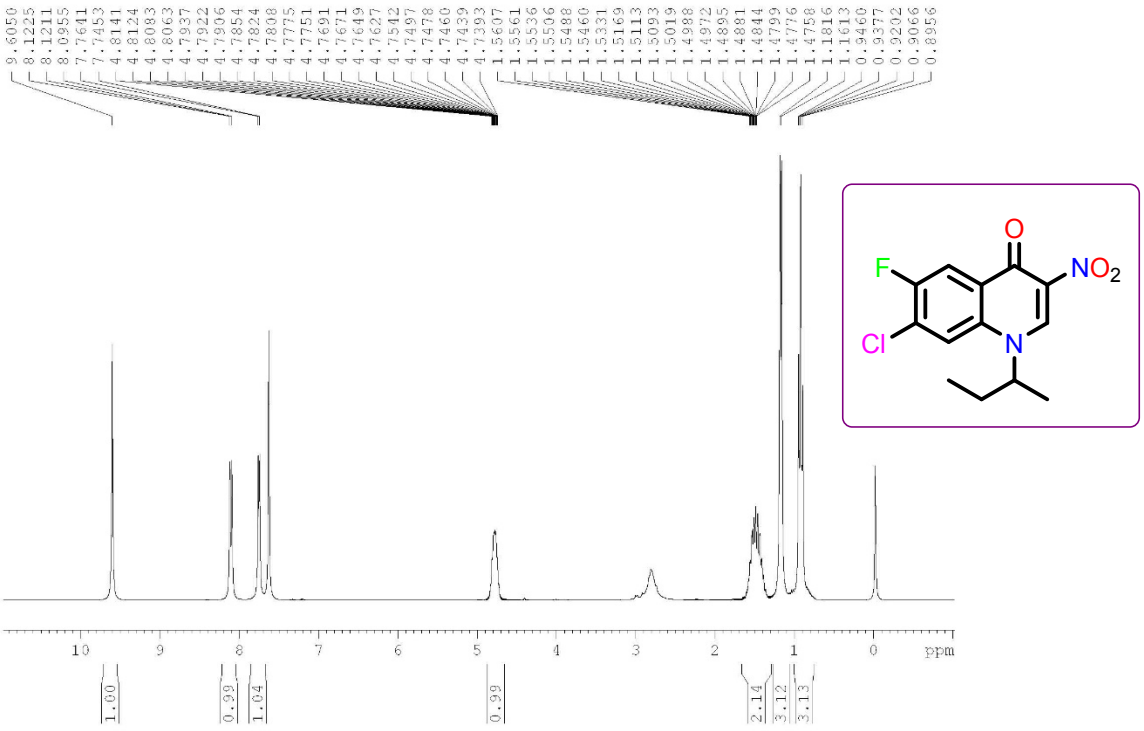
^{1}H and **^{13}C** NMR Spectra of compound **13a**

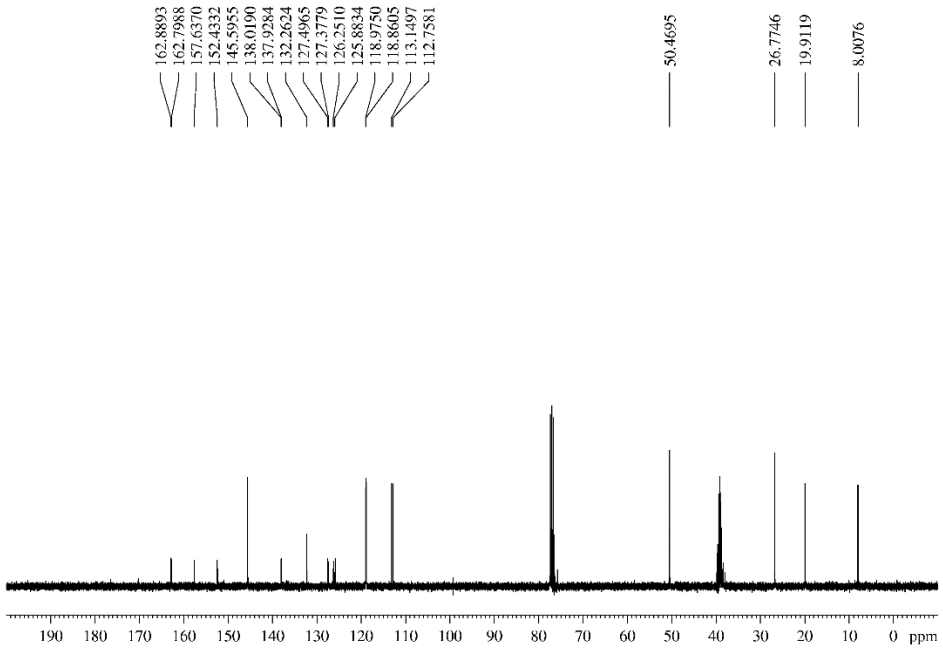
^{1}H and **^{13}C** NMR Spectra of compound **13b**



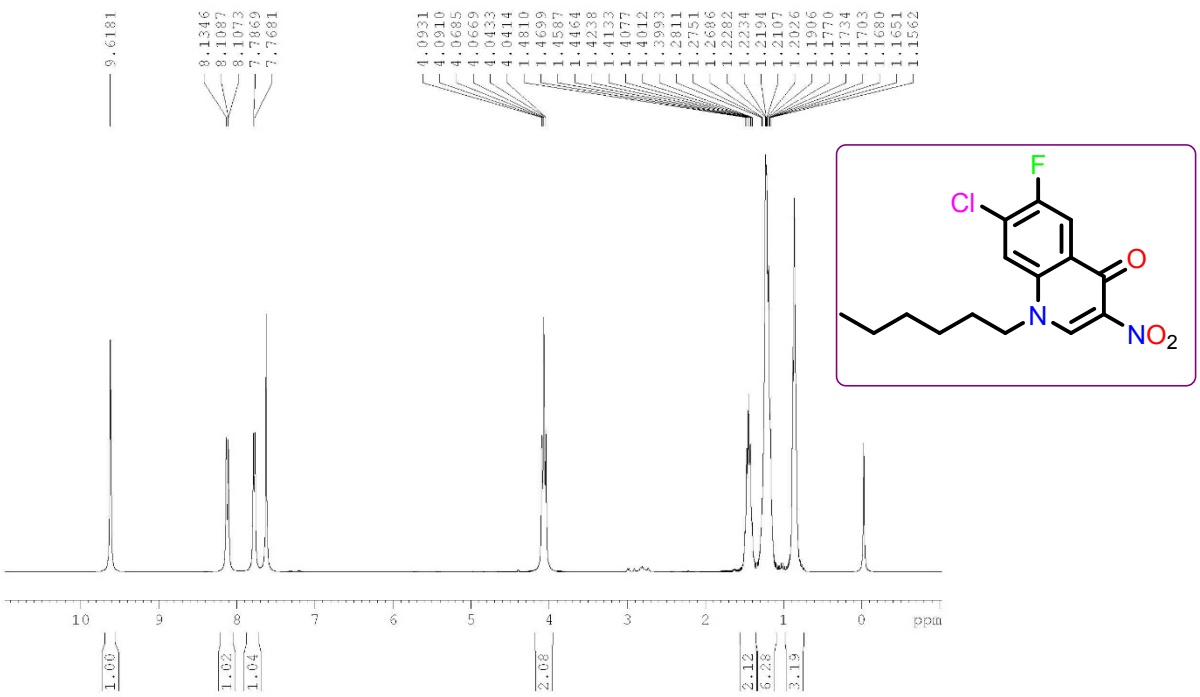


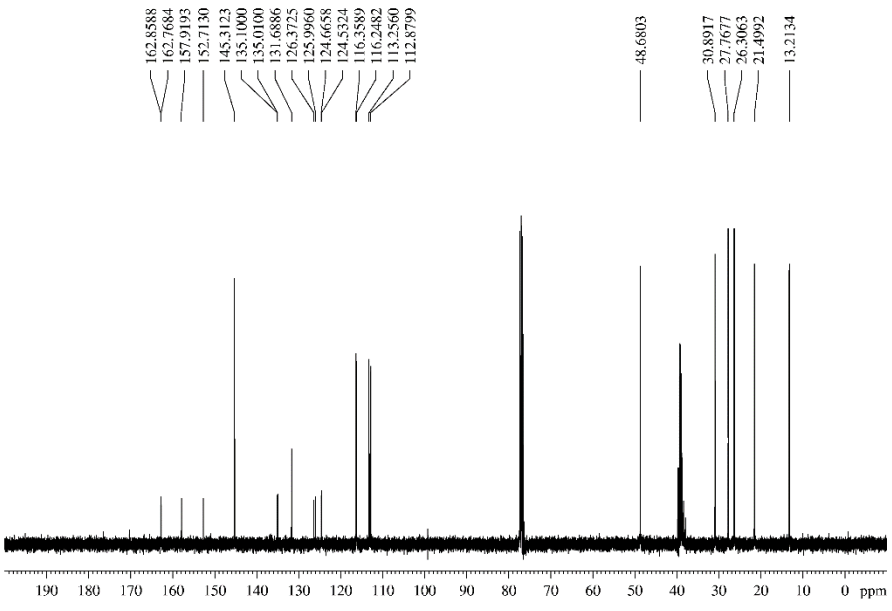
^{1}H and **^{13}C** NMR Spectra of compound **13c**



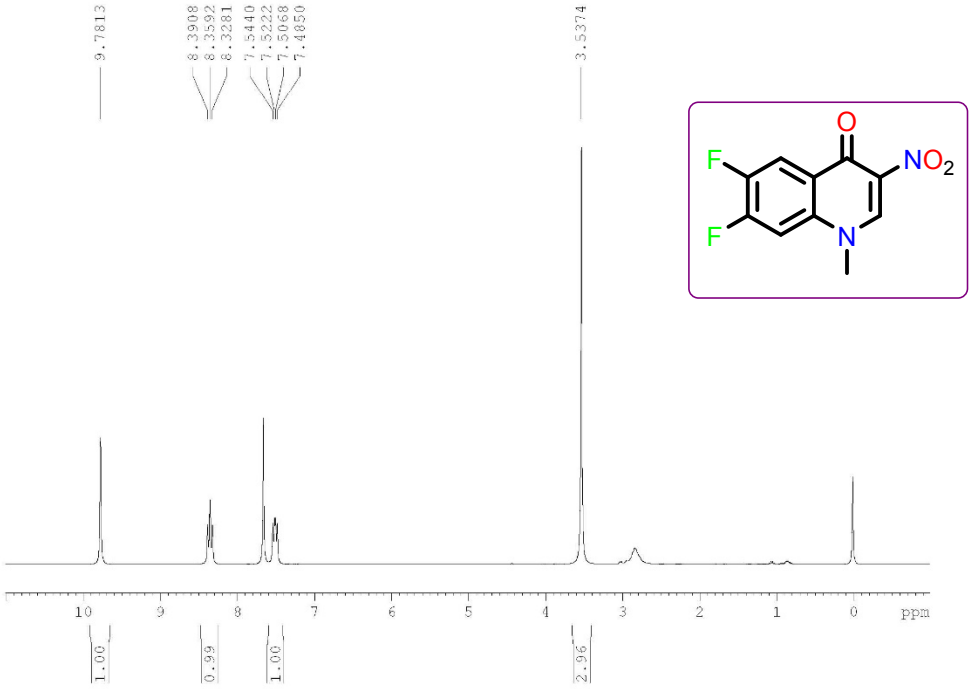


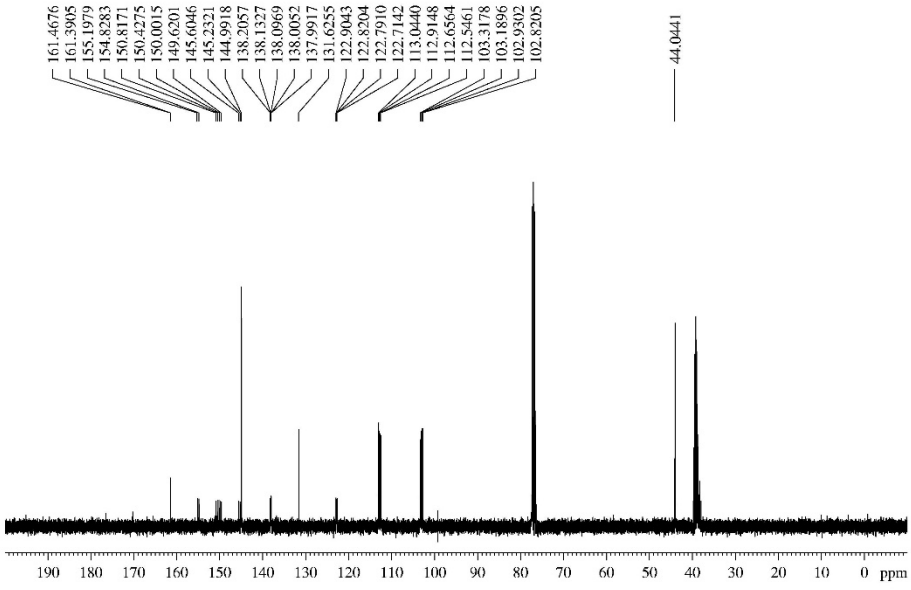
^{1}H and **^{13}C** NMR Spectra of compound **13d**



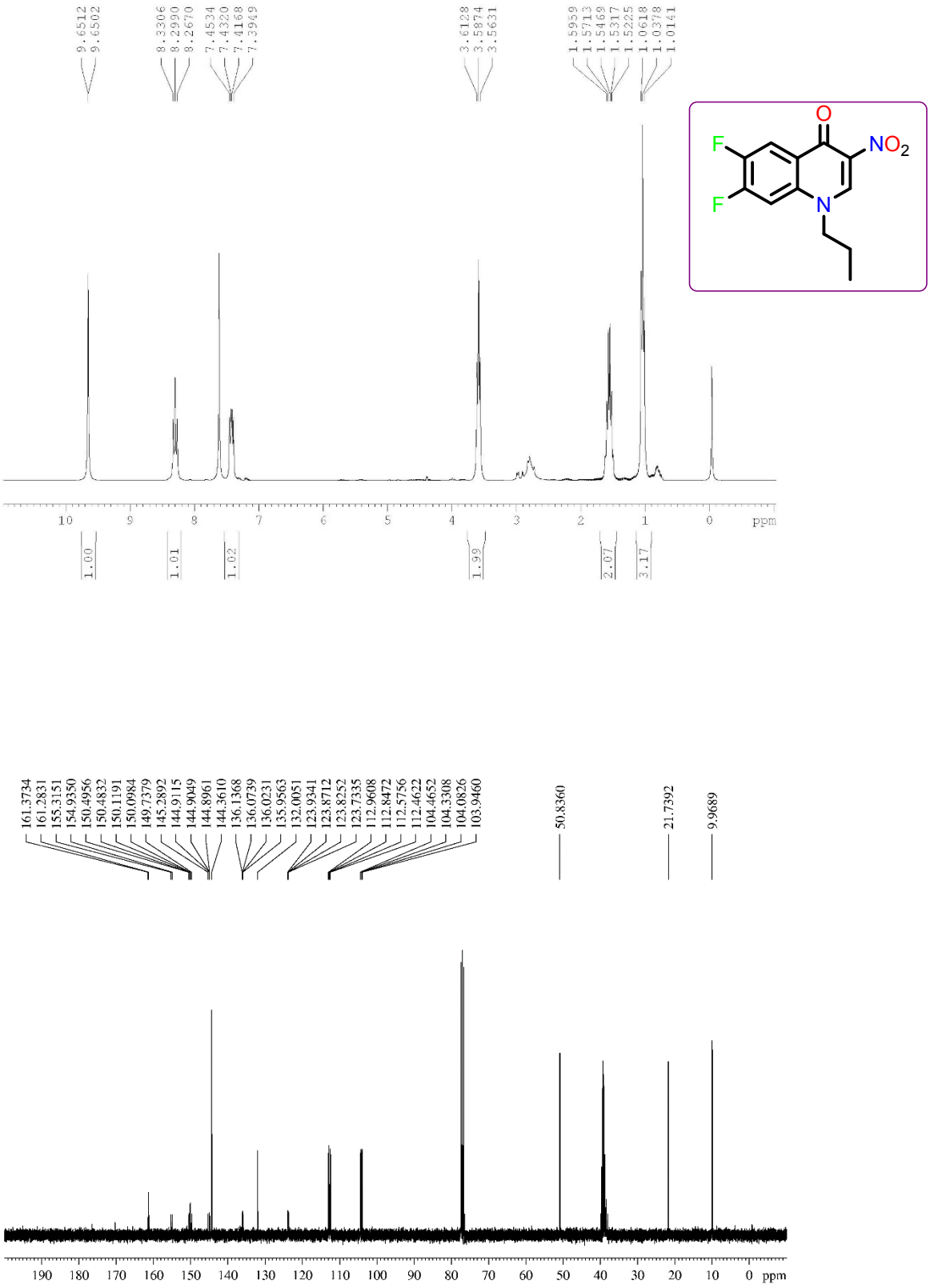


^{1}H and **^{13}C** NMR Spectra of compound **13e**

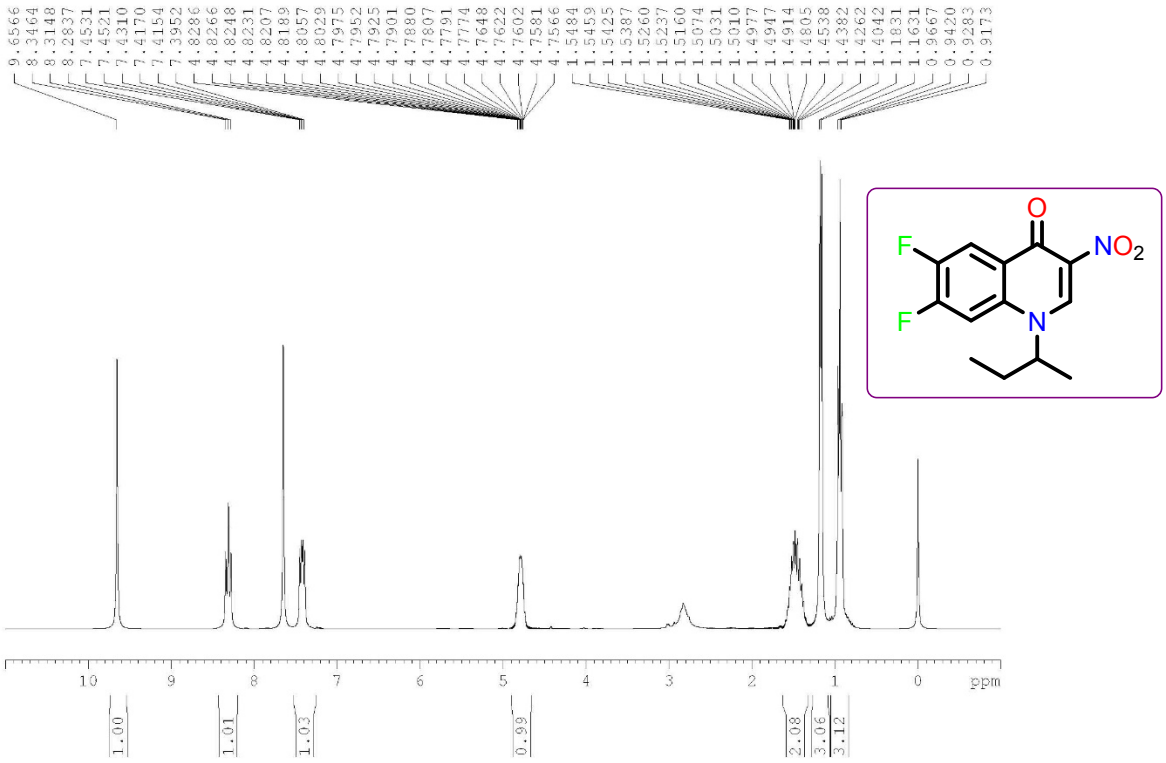


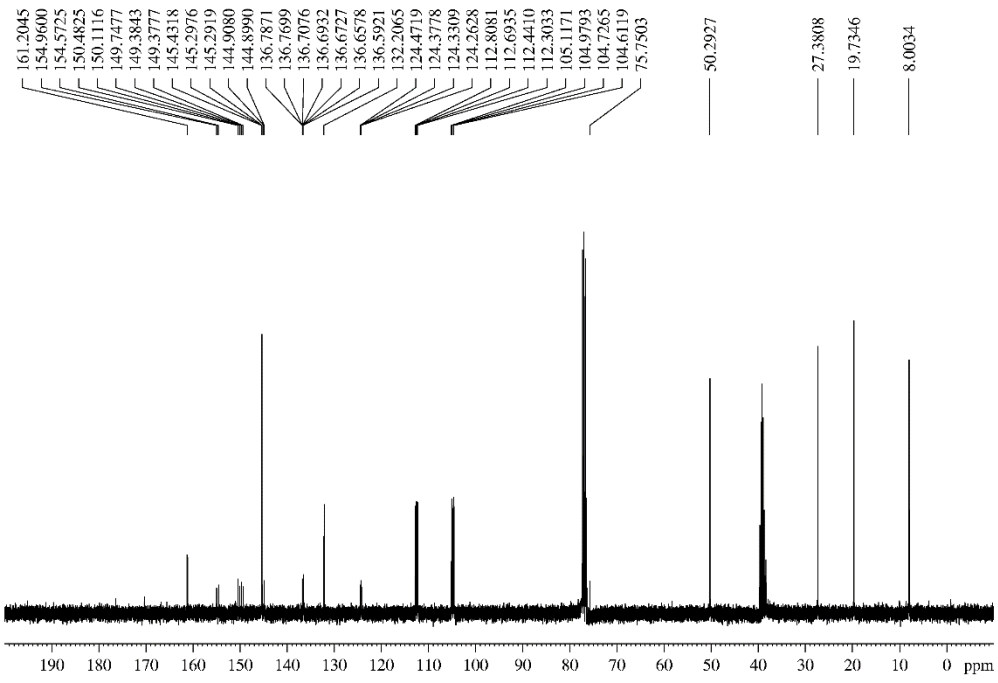


^{1}H and **^{13}C** NMR Spectra of compound **13f**

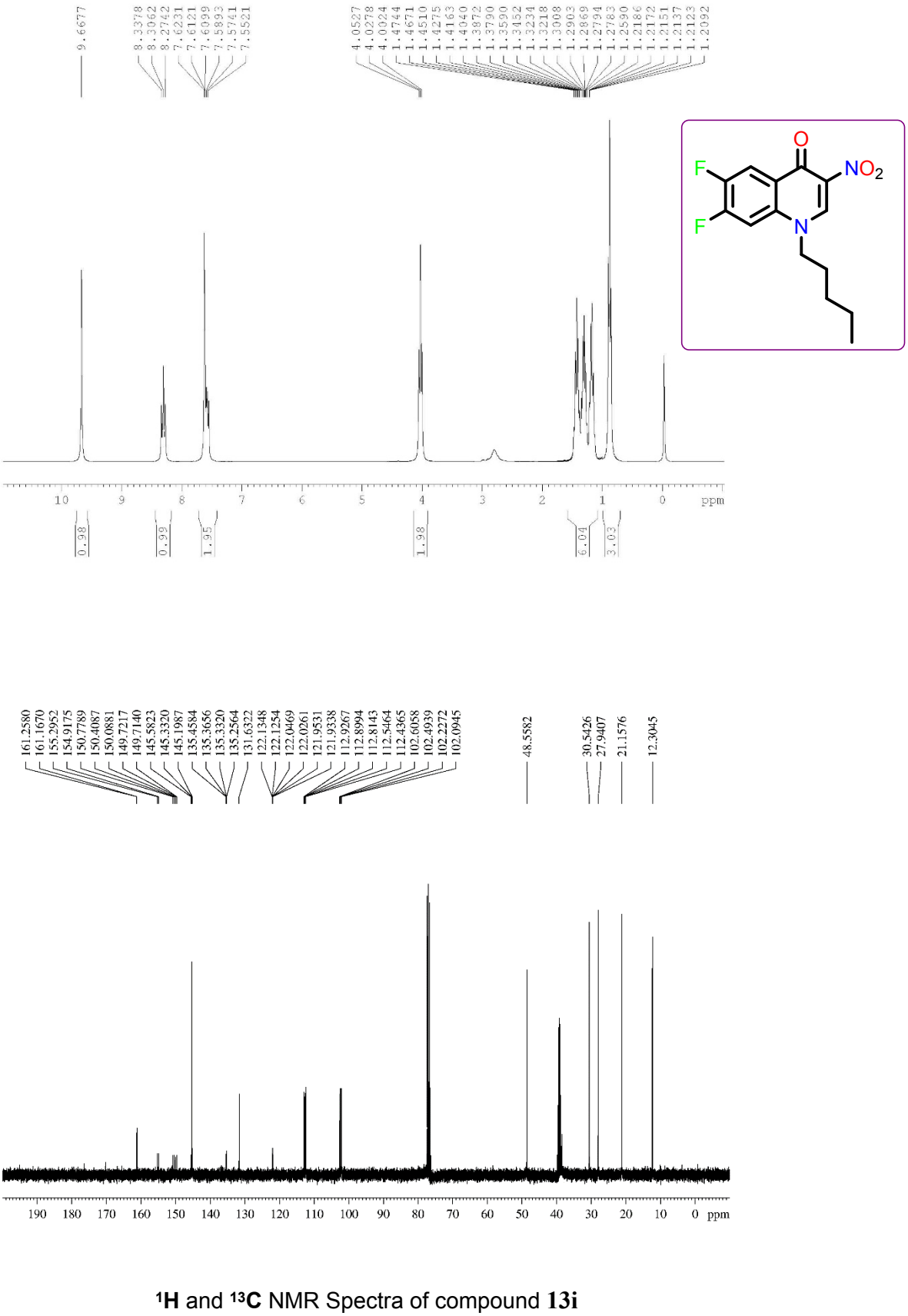


^{1}H and **^{13}C** NMR Spectra of compound **13g**

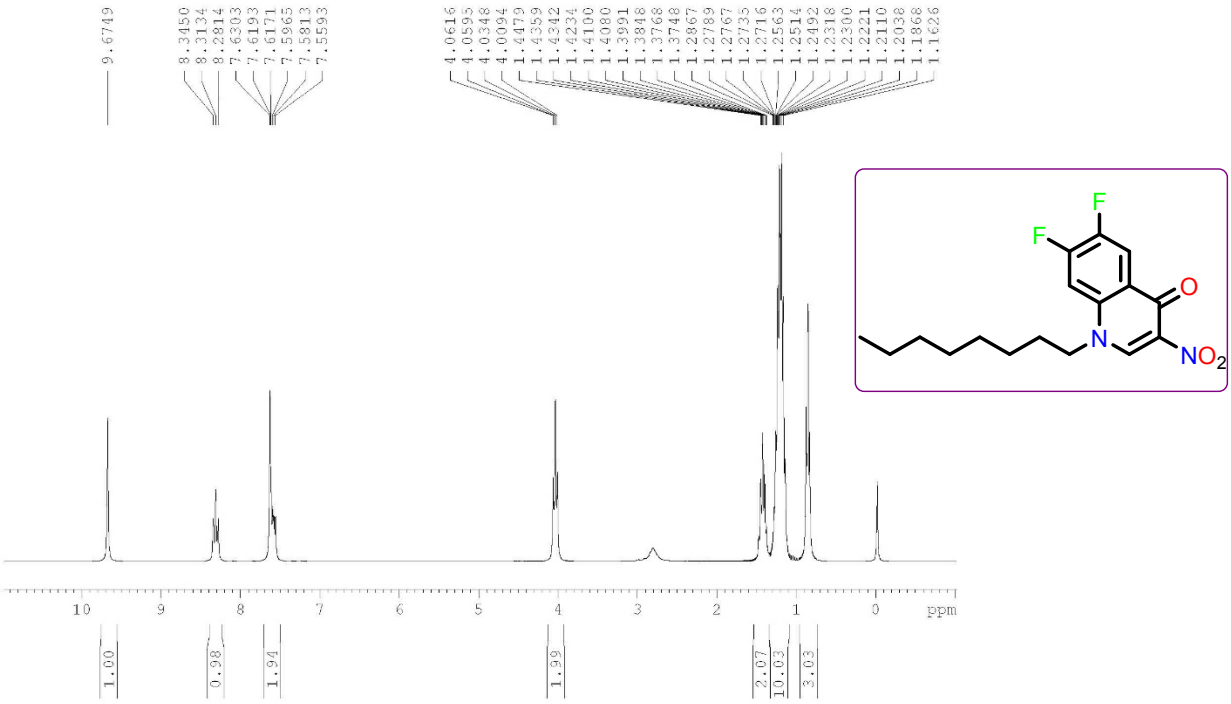


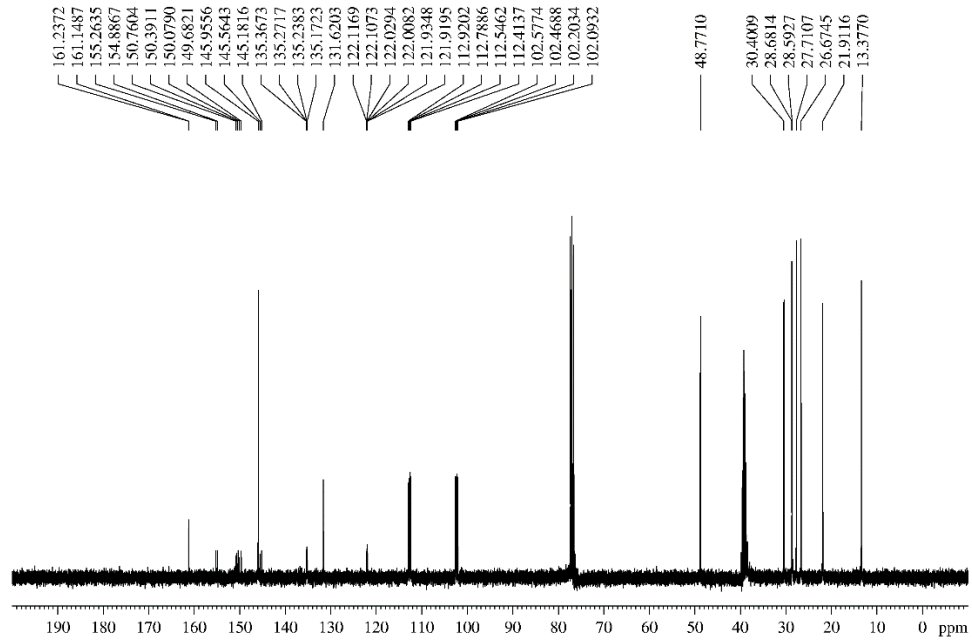


^{1}H and **^{13}C** NMR Spectra of compound **13h**

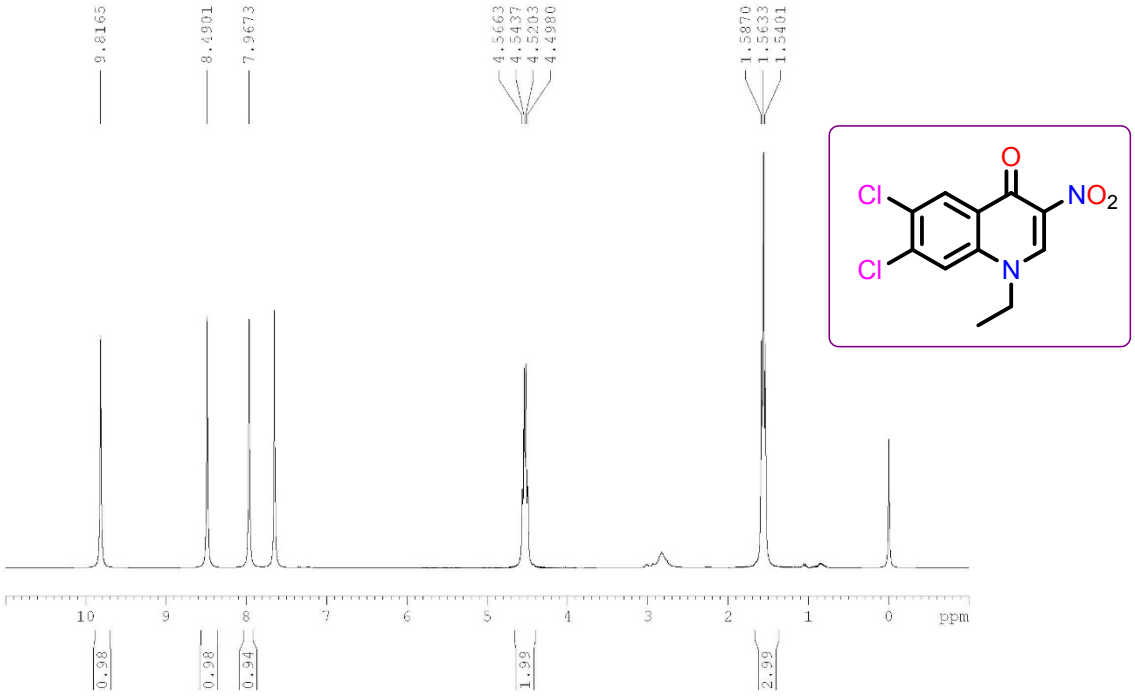


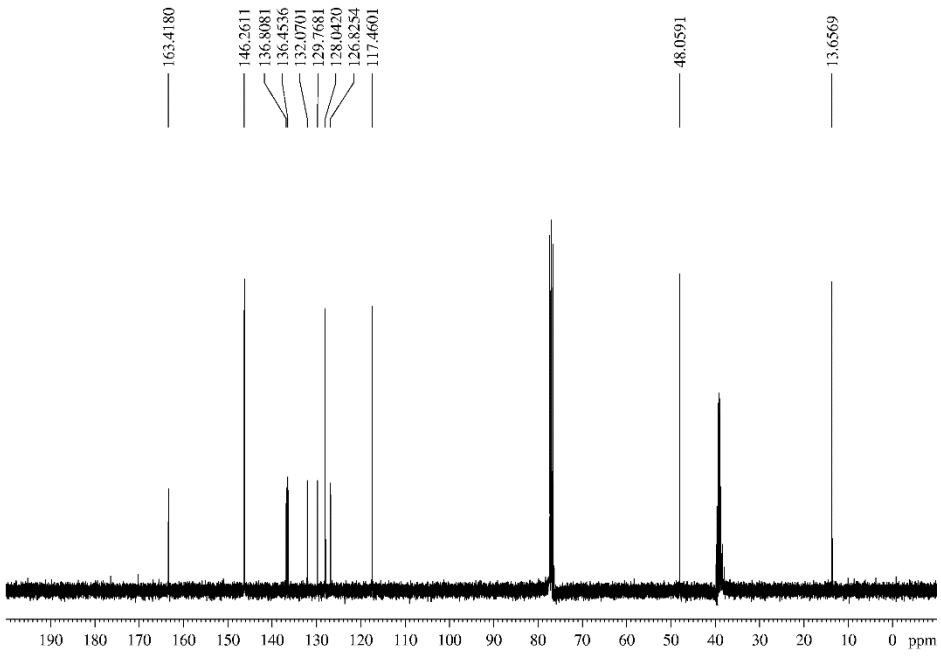
^{1}H and **^{13}C** NMR Spectra of compound **13i**



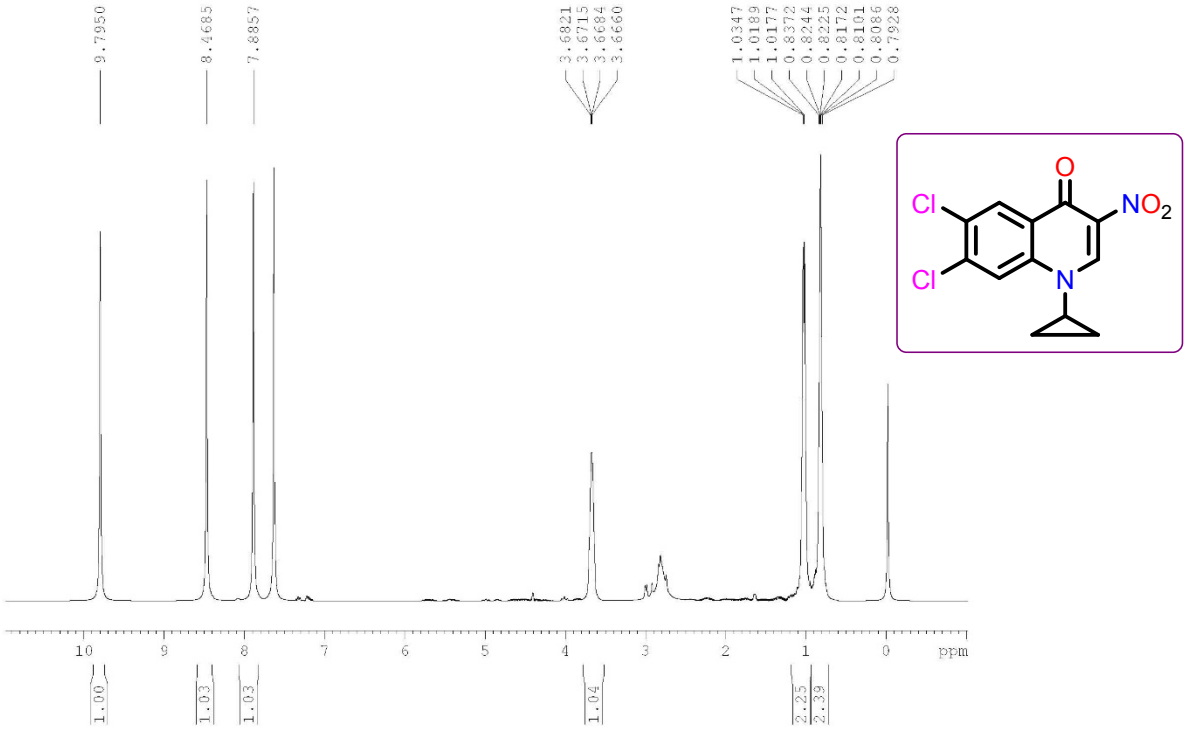


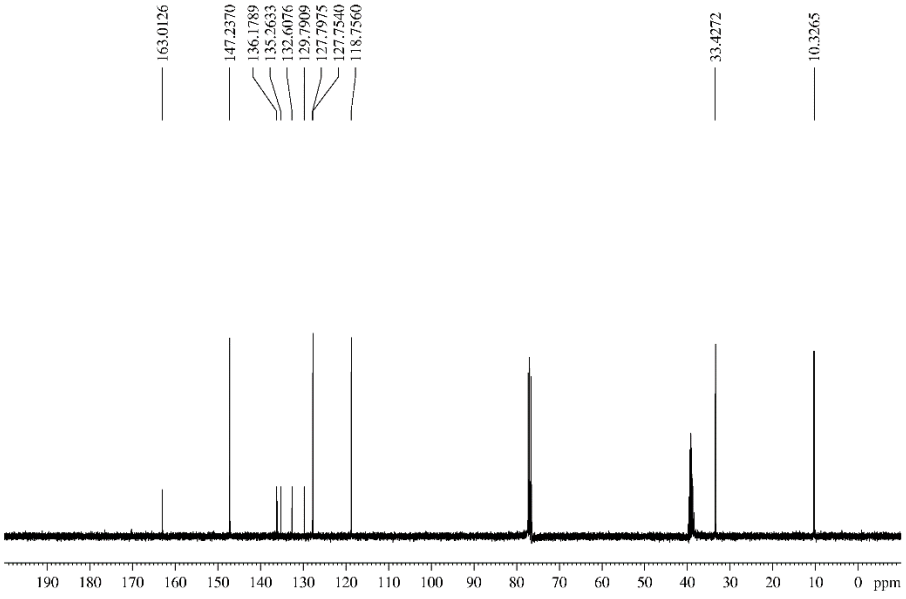
^{1}H and **^{13}C** NMR Spectra of compound **13j**



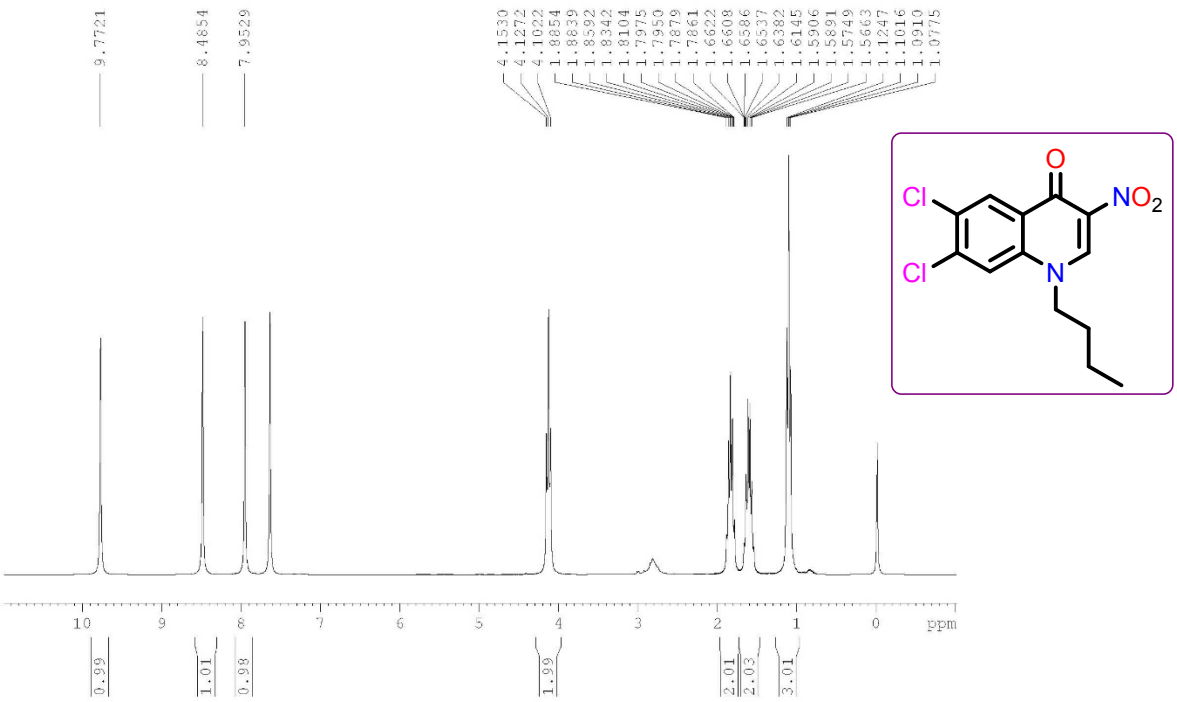


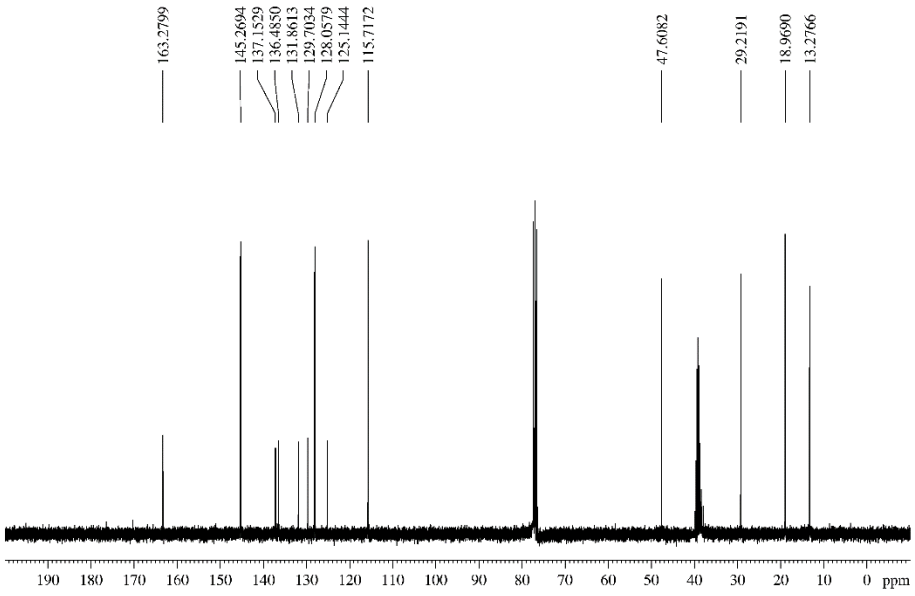
^{1}H and **^{13}C** NMR Spectra of compound **13k**



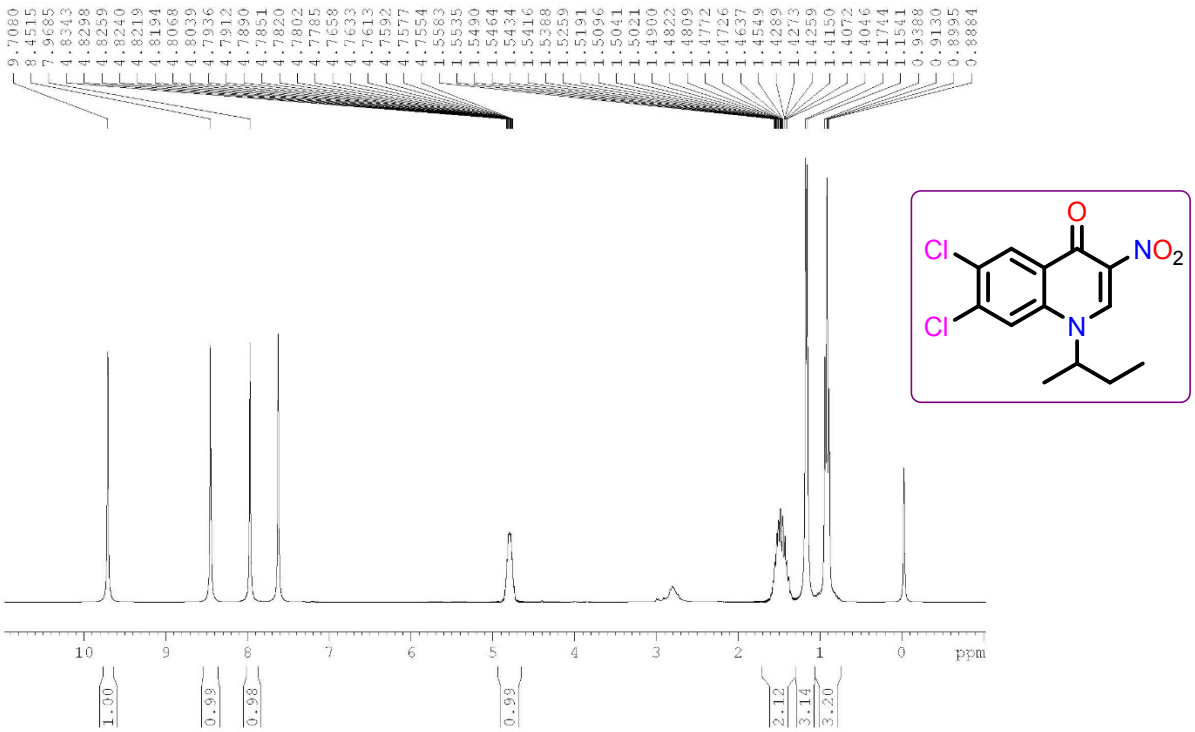


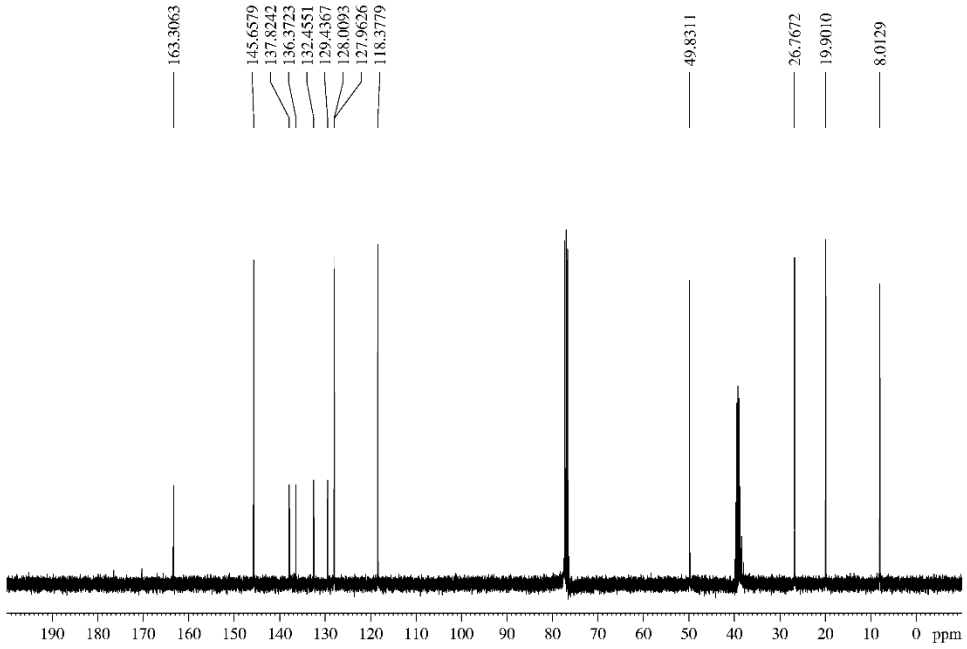
^{1}H and **^{13}C** NMR Spectra of compound **13l**



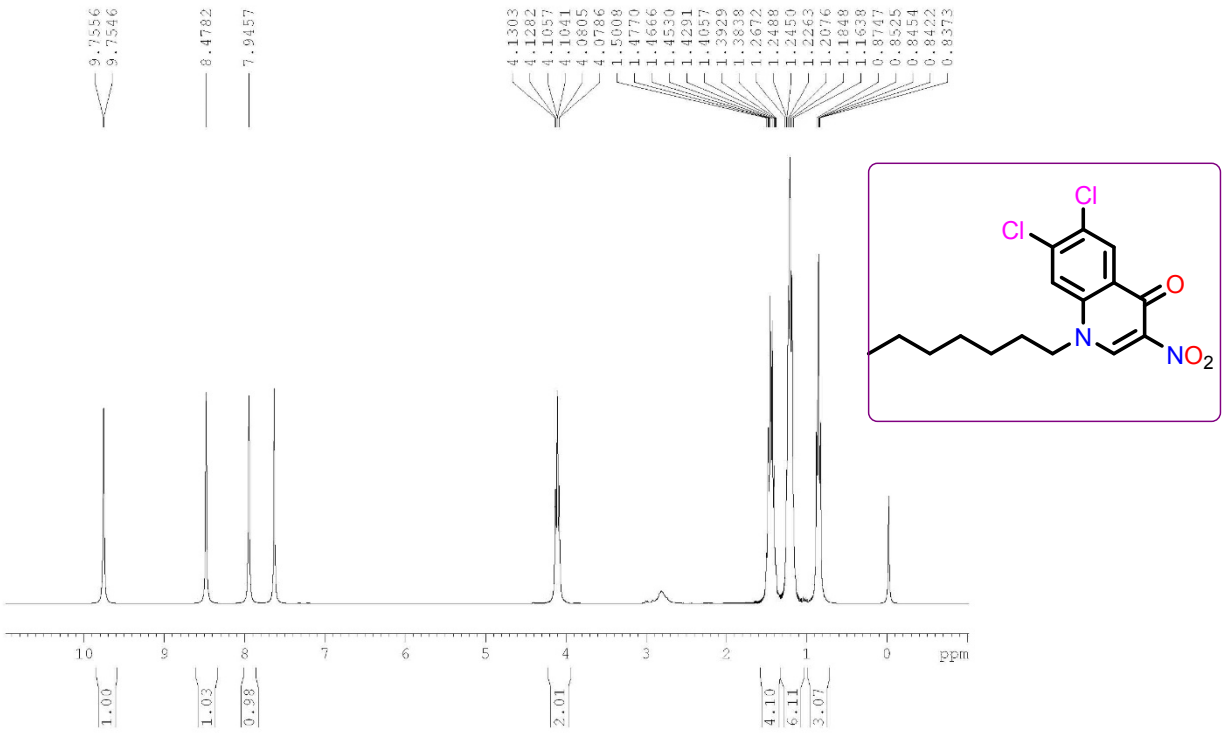


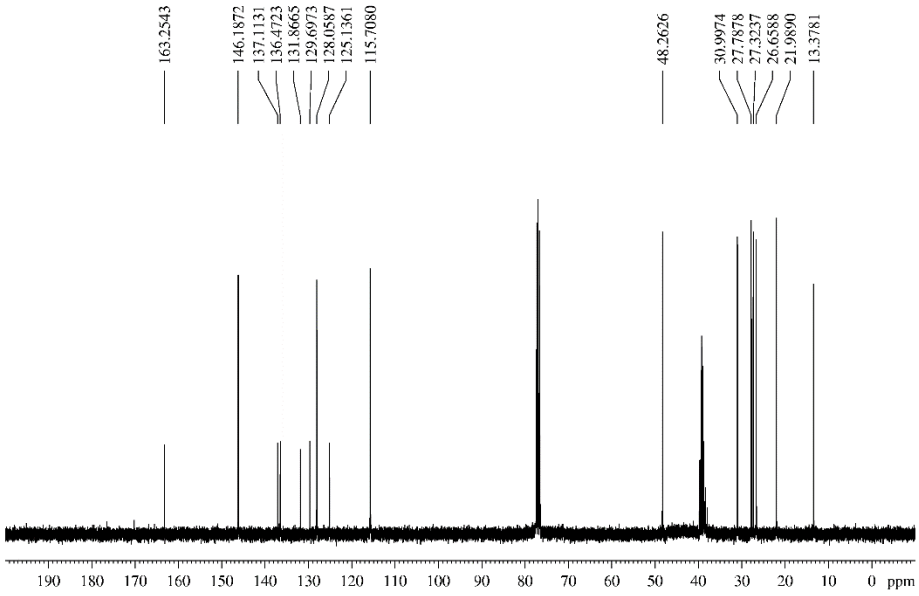
^{1}H and **^{13}C** NMR Spectra of compound **13m**



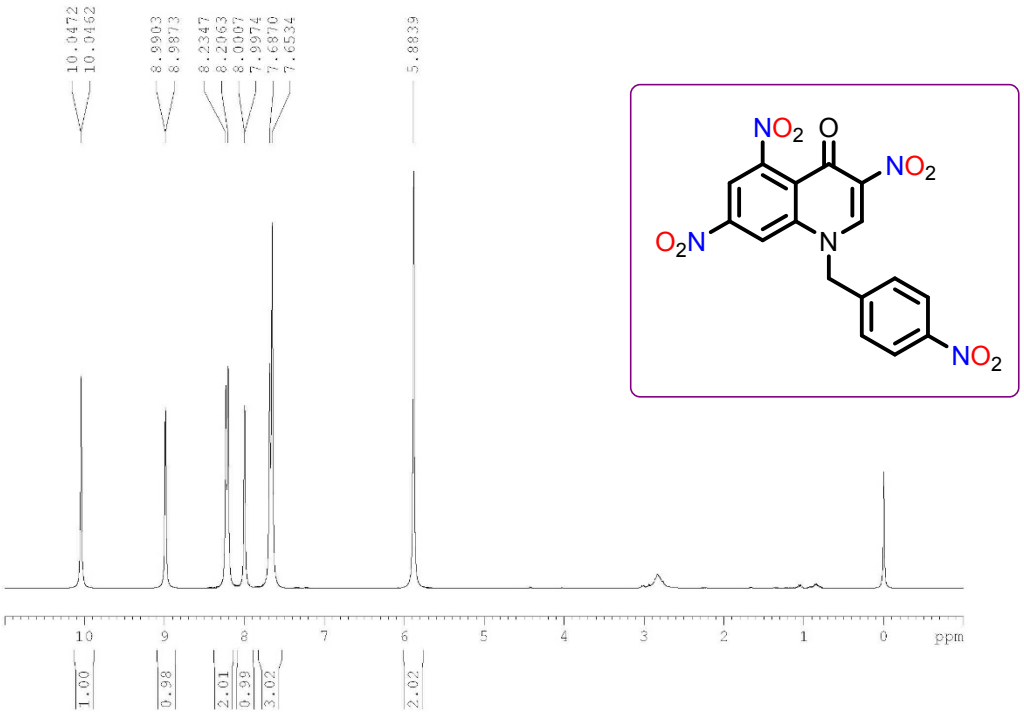


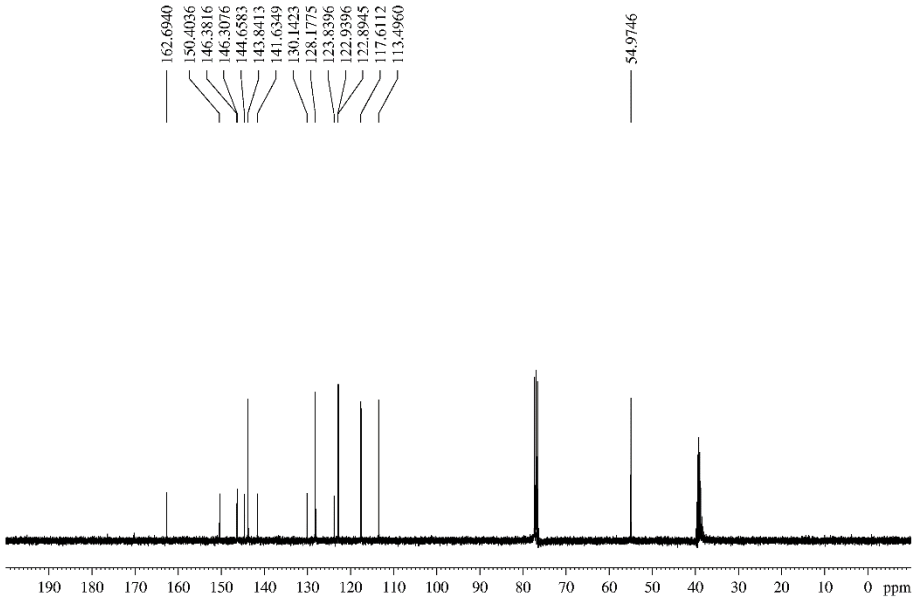
^{1}H and **^{13}C** NMR Spectra of compound **13n**



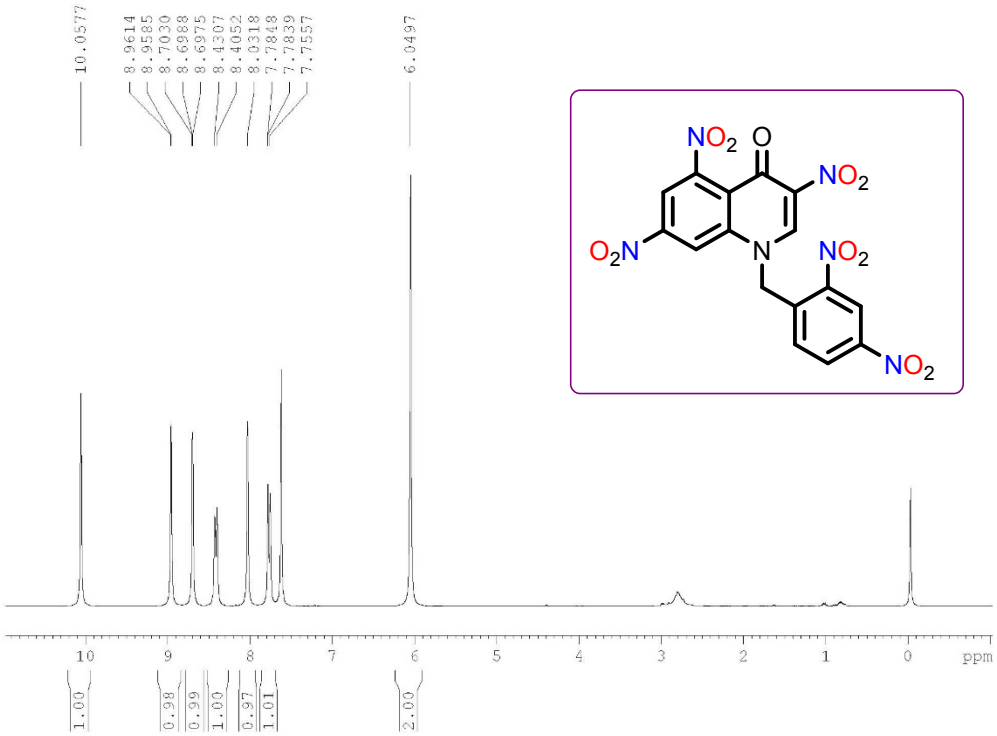


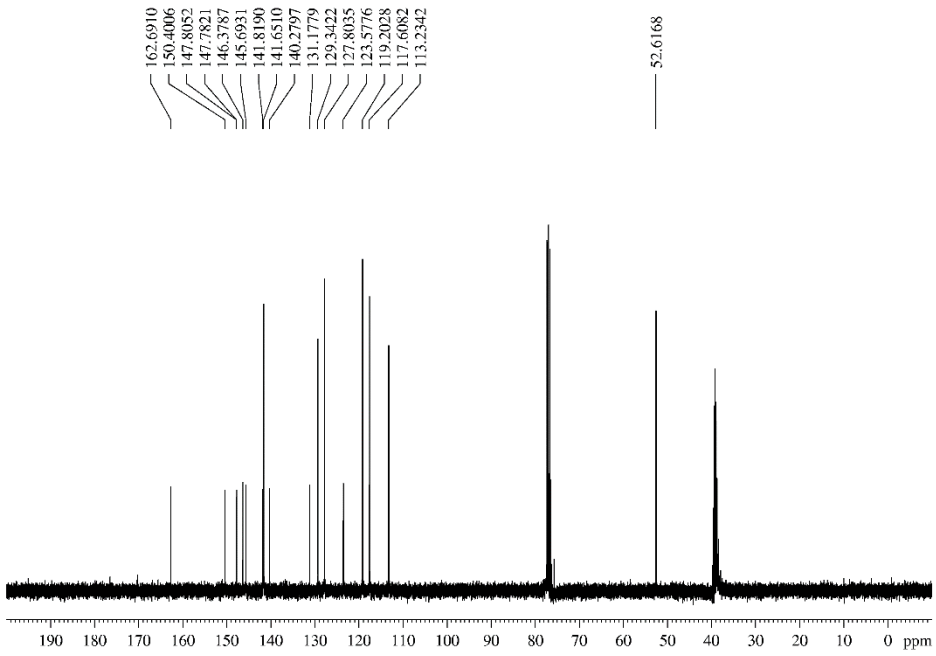
^{1}H and **^{13}C** NMR Spectra of compound **13o**



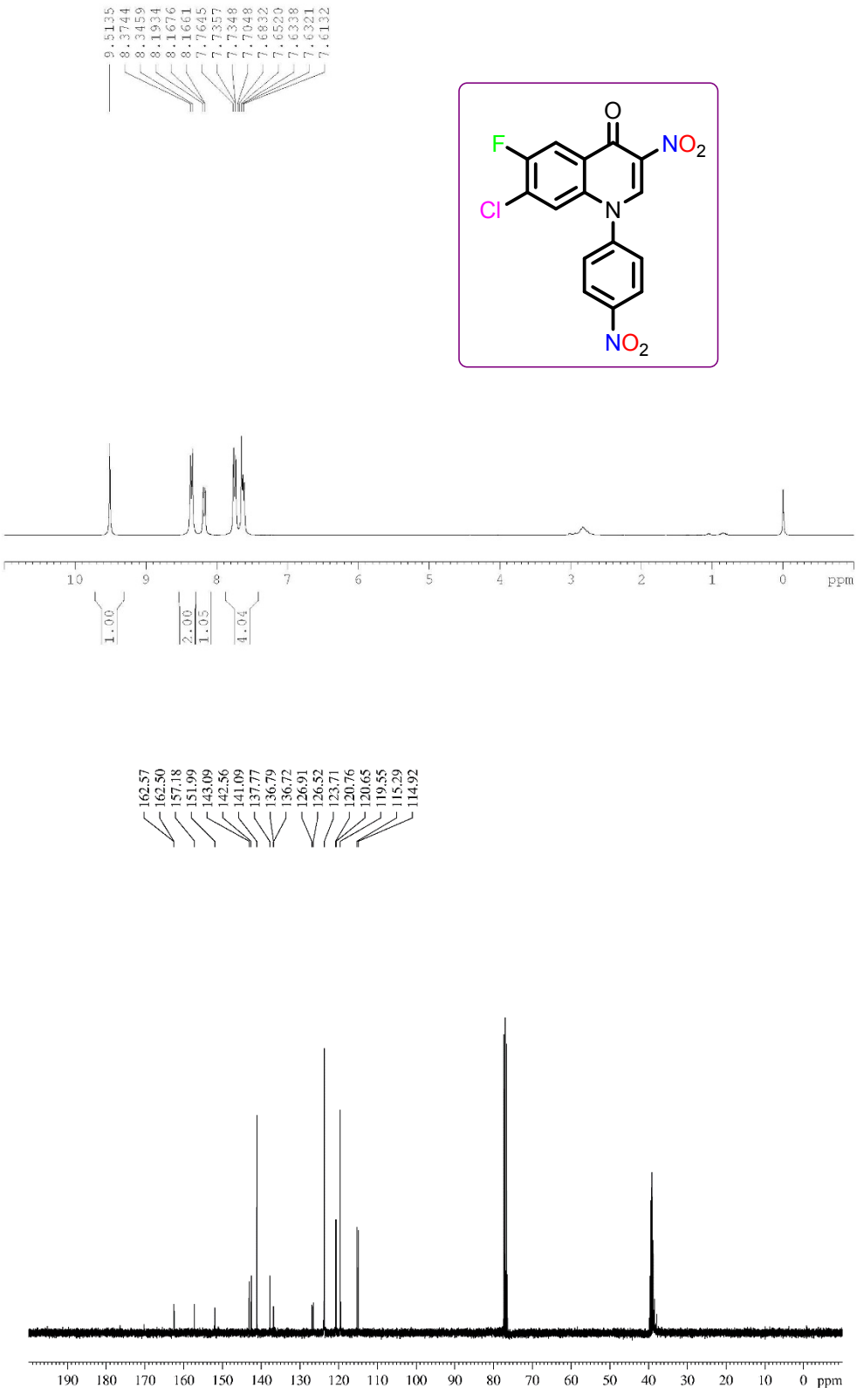


^{1}H and **^{13}C** NMR Spectra of compound **14a**





^{1}H and **^{13}C** NMR Spectra of compound **14b**



^{1}H and **^{13}C** NMR Spectra of compound **16**

Biological Evaluation

All the synthesized quinolone based molecules were evaluated for their BmTMK inhibitory activity using a spectrophotometric assay. Some of these hybrid compounds showed significant inhibition of BmTMK. The results have been summarized in Table 1. Among the all tested hybrids, compound 14a and 14b were found to be the most potent compound of the series with IC_{50} value of 3.11 and 2.9 μM . Three compounds of the series (14a, 14b and 16) showed IC_{50} values in the range of 2.9-3.4 μM.

Docking Studies

In order to validate the selectivity of these compounds for filarial BmTMK as compared to human thymidylate kinase (hTK) the comparative docking was carried out. In this experiment the all designed compounds showed good binding affinity or selectivity towards BmTMK as compared to hTK enzyme. These compounds were also compared with the standard substrate TMP for validating the docking scores using template docking protocol from Molegro Virtual Docker 4.0. The docking study clearly supports the observations that these compounds selectively inhibit the filarial protein. The comparative docking scores and in vitro IC50 results were represented in the table 1. The compound 14b also showed one hydrogen bond interaction with the Arg-98. The compound also showed hydrogen bond interactions with Gly-103, His-70 and Thr-107 which may contribute its higher binding scores than the remaining molecules. It is also showing “pi-pi” stacking interactions with the Tyr-106, Phe-73, Phe-43 along with the hydrophobic interactions with these amino acids. In case of the 14a it showed important interactions with amino acids viz. Arg-98, Lys-20 and Ser-21 along with the additional hydrophobic interactions with Ser-21, Tyr-106, Phe-73. The phenyl ring of the pheny ring on the quinolone part of the molecule also forms “pi-pi” stacking interactions with the amino acids Phe-73 and Tyr-107 these interactions may contribute for the higher binding scores and affinities for filarial enzyme.

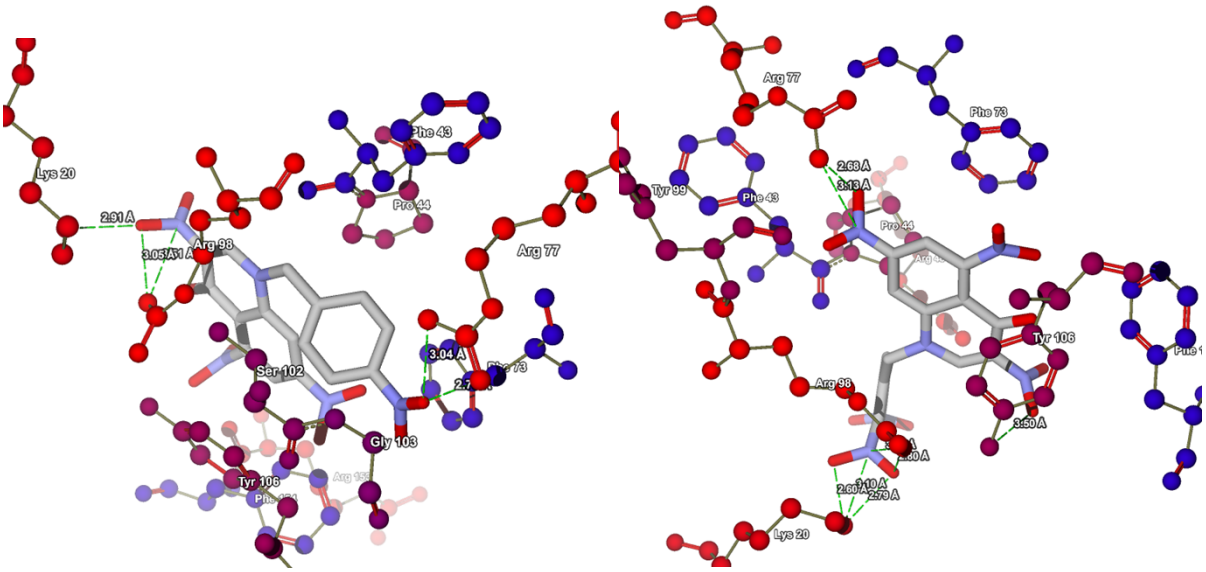


Figure 1. The docking of compound **14a** and **14b** in the active site of BmTK.

Table 1. The comparative docking analysis of scores obtained during docking of BmTMK and hTK enzyme.

Ligand	MolDock Score	Rerank Score	Docking Score	Similarity Score	MolDock Score	Rerank Score	Docking Score	Similarity Score	IC_{50}
		hTK					**BmTK**		
13a	-79.5965	-68.852	-328.308	-247.343	-100.796	-83.8764	-430.527	-329.753	24.22
13b	-85.2614	-70.873	-336.683	-250.241	-99.0792	-85.9896	-431.742	-333.967	15.32
13c	-68.0744	-36.0696	-346.07	-279.184	-113.09	-94.3778	-460.435	-342.805	19.24
13d	-89.1156	-64.172	-344.36	-256.293	-108.633	-86.2709	-442.552	-335.148	20.54
13e	-89.3988	-68.9648	-376.423	-288.243	-139.087	-102.635	-448.893	-311.807	15.3
13f	-57.5574	-40.8753	-331.346	-272.241	-94.1705	-80.7184	-426.444	-332.535	21.23
13g	-80.2563	-67.2831	-329.625	-248.522	-105.036	-76.9323	-440.751	-336.38	23.21
13h	-88.0055	-70.1535	-344.857	-258.116	-116.368	-94.1081	-436.464	-317.872	19.21
13i	-81.7409	-64.9249	-359.099	-278.868	-120.394	-95.2358	-465.952	-342.523	28.11
13j	-109.655	-94.2736	-362.447	-248.796	-125.381	-69.3578	-445.277	-321.491	29.11
13k	-80.7085	-69.2156	-328.066	-246.039	-99.1663	-82.5334	-428.199	-329.081	18.01
13l	-72.0231	-58.011	-342.572	-265.101	-104.166	-77.4672	-449.312	-346.608	25.28
13m	-76.8326	-60.6093	-345.951	-270.038	-113.9	-97.0834	-460.398	-342.249	31.21
13n	-88.6888	-65.8386	-339.951	-250.383	-114.454	-62.5448	-429.623	-316.428	22.81
13o	-97.5435	-77.8008	-377.247	-280.961	-130.126	-79.6063	-452.032	-323.499	21.2
14a	**-116.417**	**-94.1023**	**-440.055**	**-309.038**	**-137.051**	**-111.361**	**-551.506**	**-407.706**	**3.11**
14b	**-138.402**	**-101.46**	**-480.995**	**-329.518**	**-146.743**	**-106.238**	**-584.918**	**-435.866**	**2.9**
16	-91.4008	-67.6985	-406.978	-311.933	-125.723	-103.016	-531.386	-399.827	3.4
TMP[5]	**-126.393**	**-102.275**	**-622.611**	**-492.54**	**-122.112**	**-101.210**	**-620.603**	**-482.34**	**17**[5]

Materials and Methods

Biological assays

Thymidylate kinase assay:

The TMP kinase activity was measured spectrophotometrically using an enzyme coupling assay [1]. The reaction mixture of 1.0 ml contained the following: 50 mM Tris-HCl pH 7.4, 50 mM KCl, 1.0 mM phosphoenolpyruvate, 1.0 mM $MgCl_2$, 0.05 mM NADH, 2U pyruvate kinase, 2U lactate dehydrogenase 0.05 mM TMP and 0.5 mM ATP. The molar ratio of $MgCl_2$: ATP was kept constant at 2. The reaction was initiated by the addition of the recombinant enzyme and the decrease in absorbance at 340 nm was monitored at 37°C using a thermostated UV-2450 PC spectrophotometer (Shimadzu, Japan). Activities were calculated using a molar extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as the amount of enzyme catalyzing the production of 1.0 µmol nucleoside diphosphate min^{-1} under the above conditions. All kinetic parameters were obtained from at least three measurements.

BmTMK inhibition assay:

Compounds were screened against recombinant BmTMK using a coupled assay with pyruvate kinase and lactate dehydrogenase.[1] Activity was measured spectrophotometrically by following the change in absorption at 340 nm due to the oxidation of NADH. The assay was carried out using TMP as substrate (Km = 17 µM). Control spectrophotometric assays were performed to verify that the compounds were inhibitors of PfTMPK and not of the coupling enzymes, by using ADP as substrate and the two coupling enzymes pyruvate kinase and lactate dehydrogenase, but no BmTMK or TMP.[2] Percentage inhibitions were determined at different concentrations of inhibitors against BmTMK and percentage inhibition data were fit to the standard IC_{50} equation to calculate IC_{50}.

Molecular Docking

The molecular docking studies were carried out using the Molegro Virtual Docker 4.0 [3] which is based on the hybrid guided differential evolution (DE) algorithm developed by Storn and Price in 1995 [4]. It is based on the use of the predictive cavties during the search process which permits its fast and accurate prediction of the binding modes as docking output which may be represented by scoring function of MolDock which is based on a piecewise linear potential (PLP) introduced by Gehlhaar et al. [5]. The docking algorithm in MolDock was guided with new term considering the hydrogen bond interactions in account. The re-ranking procedure was also applied to increase the precision in different runs. Before docking runs the protein preparation wizard from DS 2.0

was sued to minimize the protein structure using the default parameters using the earlier reported protocol [**6-9**]. The pharmacophore based protocol from Molegro Virtual Docker 4.0 was used to dock these ligands in the binding site. This information was used for docking of these ligands using the homology modeled protein reported previously by our group. All other settings were kept default as reported earlier [**7-11**].

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