Supporting information

Design, synthesis and *in-vitro* evaluation of coumarin-imidazo[1,2-*a*]pyridine derivatives against cancer induced osteoporosis

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Analytical Data of Compounds 3a-d

4-hydroxy-5-methylisophthalaldehyde (3a): White solid; Yield: 60%; mp.: 125 - 127 ºC; IR (neat): 3262, 2865, 1703, 1626, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 11.82 (s, 1H), 9.97 (s, 1H), 9.90 (s, 1H), 7.97 (d, J = 1.8 Hz, 1H), 7.93 (brs, 1H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 196.4, 189.9, 165.0, 137.2, 134.8, 128.7, 125.2, 119.7, 15.1; ESI-MS: (m/z): 164 $[M+H]^+$.

4-hydroxy-5-isopropylisophthalaldehyde (3b): White solid; yield; 89%; mp.: 164-163 ºC; IR (KBr): 3028, 1677, 1665, 1615, 1229, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 11.94 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 7.99 (brs, 1H), 7.96 (d, J =1.47 Hz,1H), 3.43-3.33 (m, 1H), 1.27 (d, J =5.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 196.6, 189.9, 164.3, 138.9, 134.8, 133.2, 129.0, 119.9, 26.5, 22.1 ; ESI-MS: (*m/z*) 193 $[M+H]^+$.

5-sec-butyl-4-hydroxy-benzene-1,3-dicarbaldehyde (3c): Oily; Yield: 64%; IR (neat): 3267, 2862, 1709, 1622, 1018 cm^{-1}; 1H NMR ($CDCl_3$, 200 MHz): δ 12.00 (s, 1H), 10.51 (s, 1H), 9.96 (s, 1H), 8.08 (brs, 1H), 8.00 (s, 1H), 3.27-3.10 (m, 1H), 1.74-1.58 (m, 2H), 1.25 (d, J = 7.0 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H); 13C NMR (CDCl3, 75 MHz): δ 196.5, 189.4, 164.0, 137.2, 134.5, 133.5, 128.7, 119.6, 32.8, 28.9, 19.4, 11.6; ESI-MS (m/z): 207 $[M+H]^+$.

5-tert-butyl-4-hydroxyisophthalaldehyde (3d): Oily; Yield: 65%; IR (neat): 3252, 2865, 1703, 1626, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 12.39 (s, 1H), 9.99 (s, 1H), 9.93 (s, 1H), 8.07 (brs, 1H), 7.99 (brs, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 196.4, 190.0, 166.1, 140.0, 135.4, 133.9, 128.6, 120.4, 35.2, 29.1; ESI-MS: (m/z): 207 $[M+H]^+$.

Analytical Data of Compounds 4a, 4d-g

Ethyl 6-formyl-8-methyl-2-oxo-2H-chromene-3-carboxylate (4a): White solid; yield: 78%; mp: 136-137 ºC; IR (KBr): 3062, 1754, 1625, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.03 (s, 1H), 8.60 (s, 1H), 8.02 (s, 1H), 8.0 (s, 1H), 4.44 (q, J = 7.1 Hz, 2H), 2.55 (s, 3H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.2, 162.8, 157.3, 156.0, 148.4, 135.2, 132.7, 130.0, 128.1, 119.3, 117.9, 62.4, 15.7, 14.4; ESI-MS (m/z): 261 $(M+H)^+$;

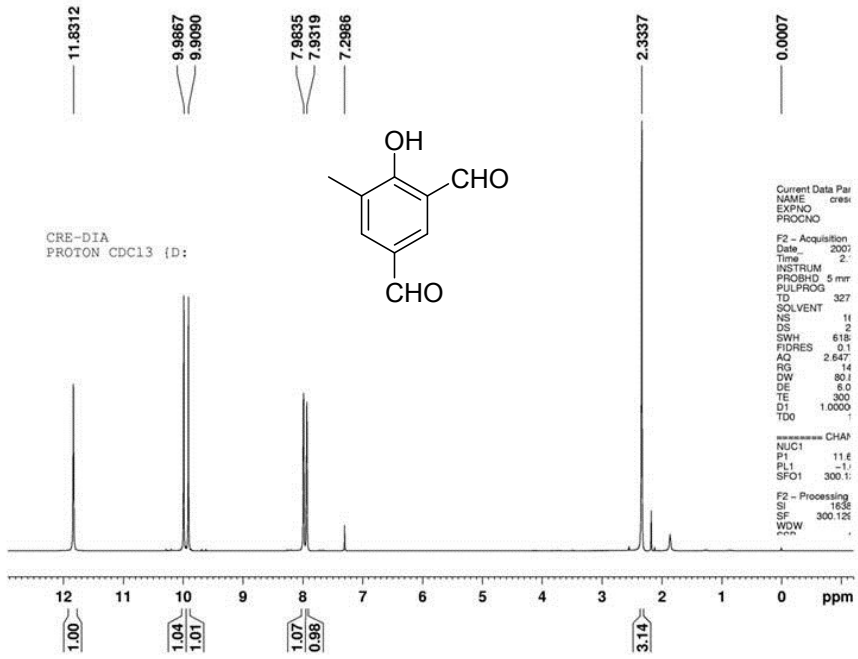
Ethyl 8-tert-Butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (4d): White solid; Yield 71%; mp 91–92 °C; IR (KBr): 3041, 1746, 1615, 1584, 1066 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 10.05 (s, 1H), 8.60 (s, 1H), 8.16 (brs, 1H), 8.02 (brs, 1H), 4.43 (q, 2H, J = 7.1 Hz), 1.55 (brs, 9H), 1.42 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 190.3, 162.7, 157.7, 155.3, 148.8, 139.7, 132.3, 131.6, 130.6, 118.8, 118.6, 62.3, 35.4, 29.6, 14.3. ESI-MS m/z: 303 $(M + H)^+$.

Methyl 8-tert-Butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (4e): White solid; Yield 68%; mp 92–93 °C; IR (KBr): 3048, 1741, 1611, 1584, 1061 cm^{-1}. ^1H NMR ($CDCl_3$, 300

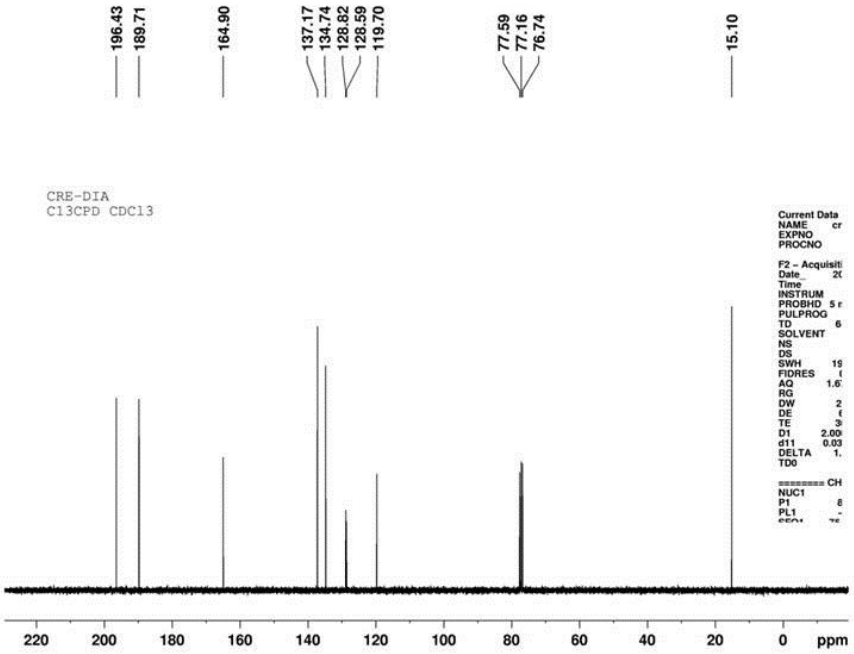
MHz) δ: 10.05 (s, 1H), 8.62 (s, 1H), 8.16 (brs, 1H), 8.02 (brs, 1H), 3.98 (s, 3H), 1.55 (brs, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.2, 163.3, 157.7, 155.3, 149.3, 139.7, 132.5, 131.7, 130.7, 118.6, 118.4, 53.1, 35.4, 29.6. ESI-MS m/z: 289 $(M + H)^+$

Ethyl 8-sec-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (4f): White solid; yield: 65%; mp 89-90 ºC; IR (KBr): 3042, 1747, 1601, 1589, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.05 (s, 1H), 8.60 (s, 1H), 8.05 (brs, 1H), 8.01 (brs, 1H) 4.44 (q, J = 7.1 Hz, 2H), 3.51-3.40 (m, 1H), 1.78-1.69 (m, 2H), 1.43 (t, J = 7.1 Hz, 3H), 1.33 (d, J = 7.0 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.2, 162.7, 156.5, 155.8, 148.5, 137.3, 132.9, 131.7, 129.9, 119.2, 118.0, 62.2, 33.4, 29.6, 20.4, 14.2, 12.0; ESI-MS (m/z): 303 $(M+H)^+$

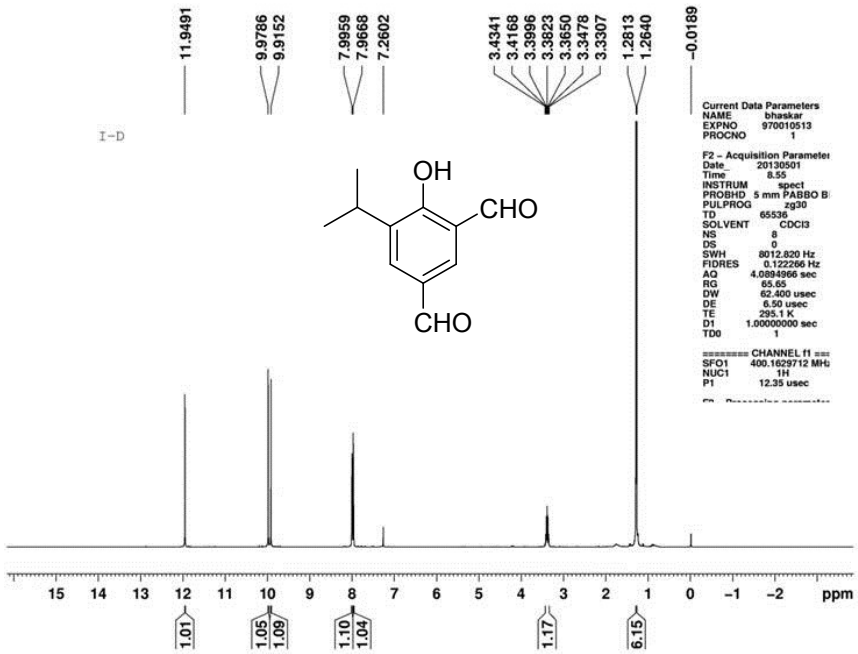
Methyl 8-sec-Butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (4g): White solid; Yield 64%; mp 122−123 °C; IR (KBr): 3062, 1749, 1609, 1576, 1055 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.04 (s, 1H), 8.62 (s, 1H), 8.05 (brs, 1H), 7.98 (brs, 1H), 3.98 (s, 3H), 3.51−3.40 (m, 1H), 1.79−1.69 (m, 2H), 1.32 (d, 3H, J = 7 Hz, 3H), 0.88 (t, 3H, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 190.2, 163.4, 156.6, 155.9, 149.0, 137.5, 133.0, 131.9, 129.9, 118.9, 118.1, 53.2, 33.5, 29.7, 20.5, 12.0. ESI-MS m/z: 289 $(M + H)^+$



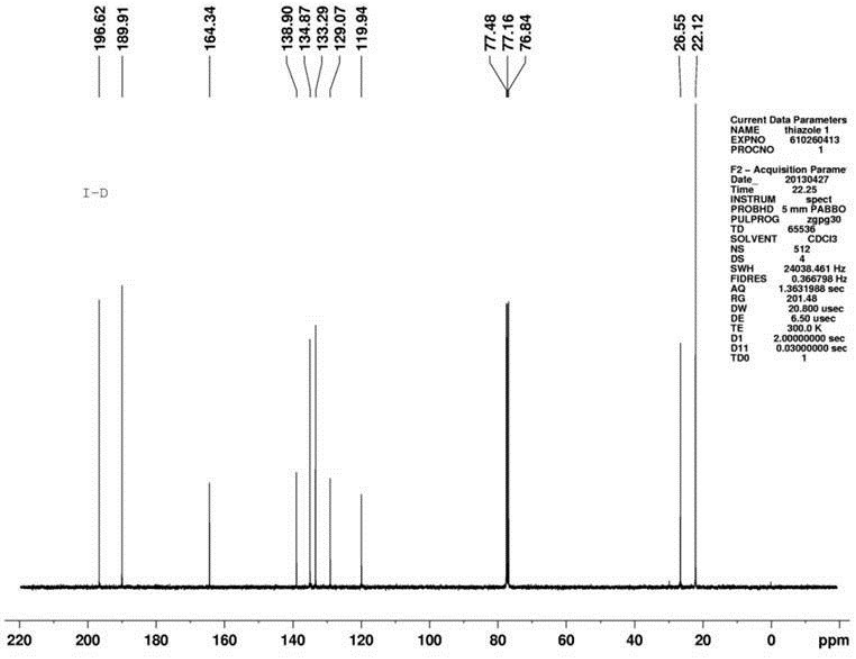
^{1}H NMR of compound **3a** at 300 MHz ($CDCl_3$)



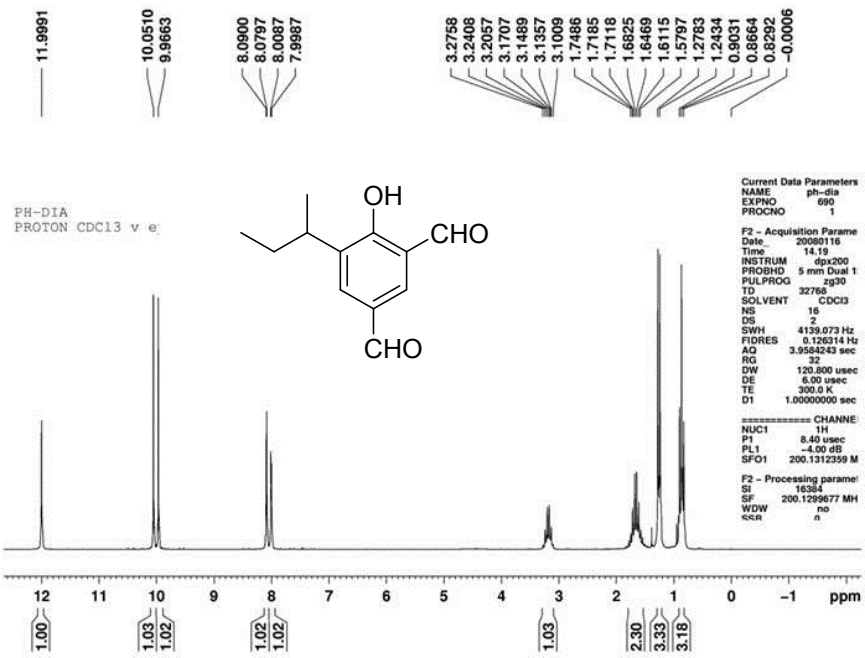
^{13}C NMR of compound **3a** at 100 MHz ($CDCl_3$)



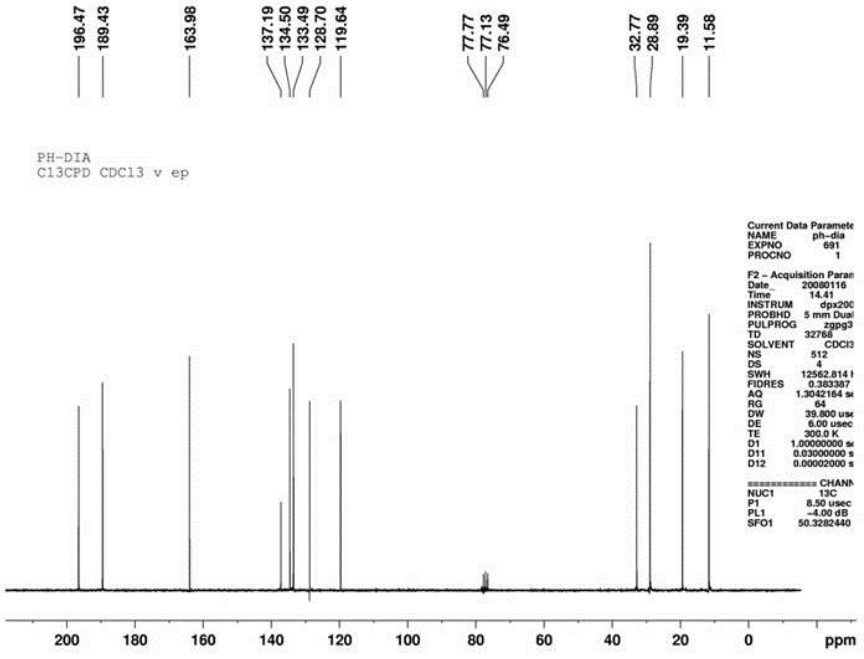
^{1}H NMR of compound **3b** at 400 MHz (CDCl3)



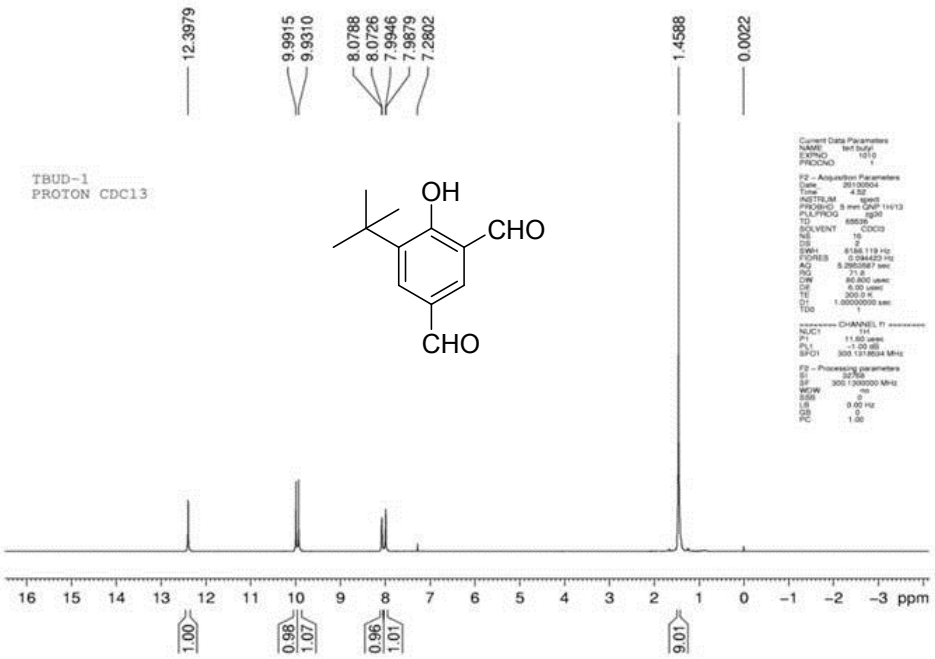
^{13}C NMR of compound **3b** at 100 MHz ($CDCl_3$)



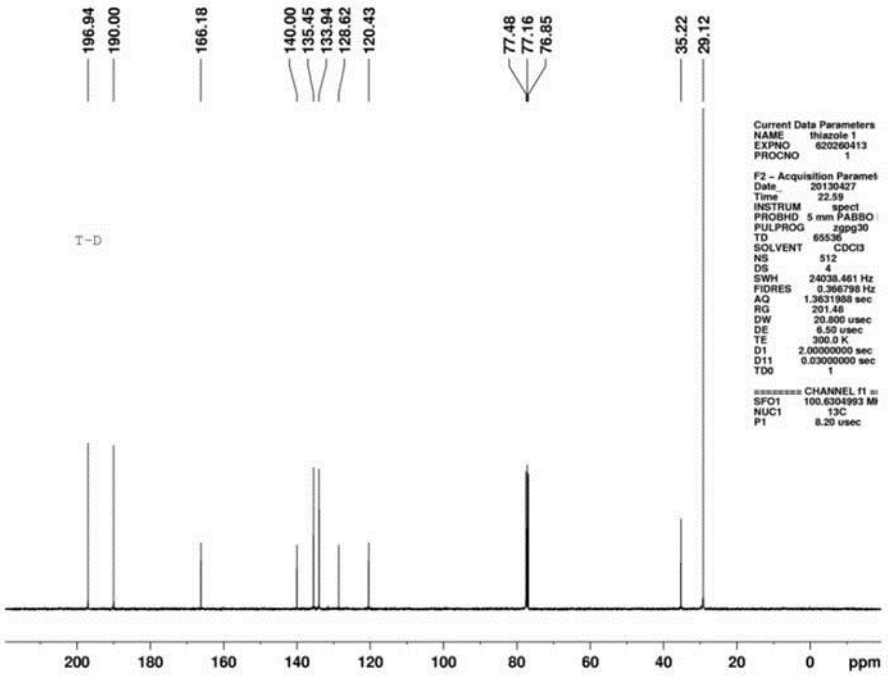
^{1}H NMR of compound **3c** at 200 MHz ($CDCl_3$)



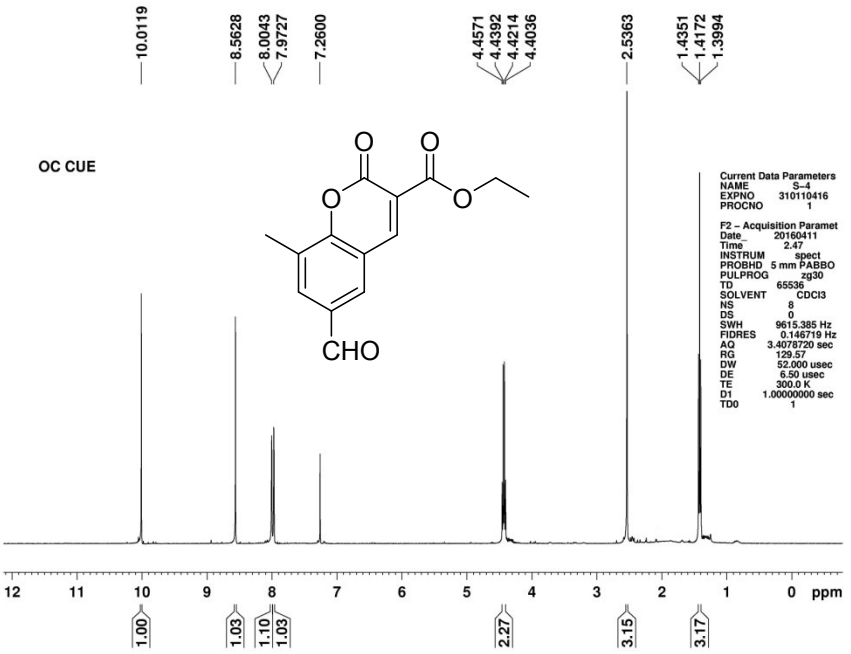
^{13}C NMR of compound **3c** at 50 MHz ($CDCl_3$)



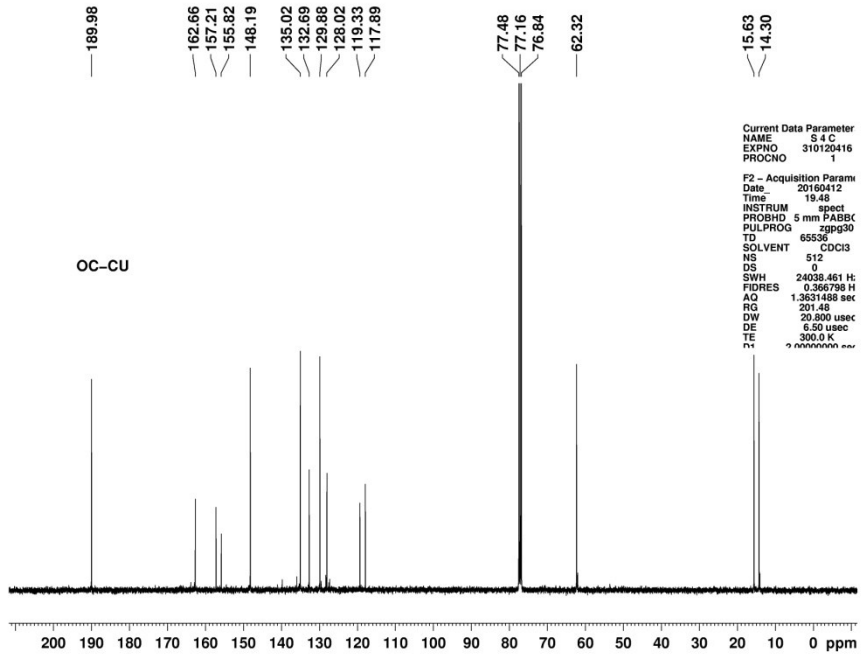
^{1}H NMR of compound **3d** at 300 MHz (CDCl3)



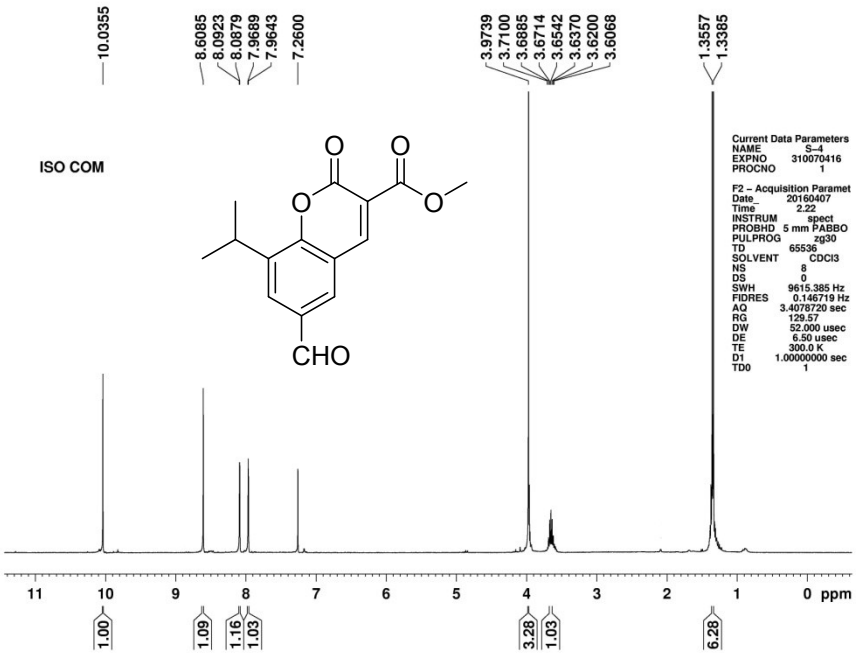
^{13}C NMR of compound **3d** at 100 MHz ($CDCl_3$)



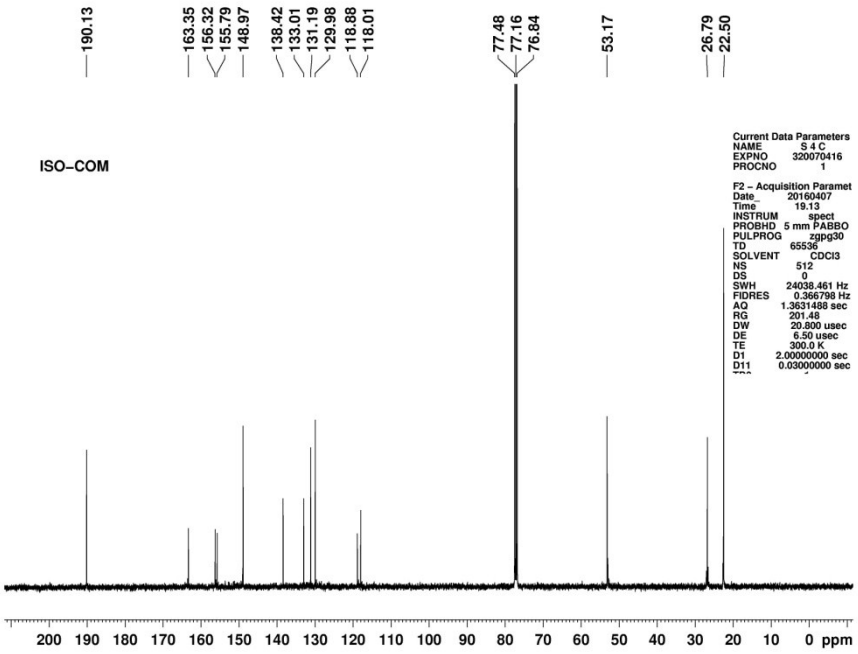
^{1}H NMR of compound **4a** at 400 MHz (CDCl3)



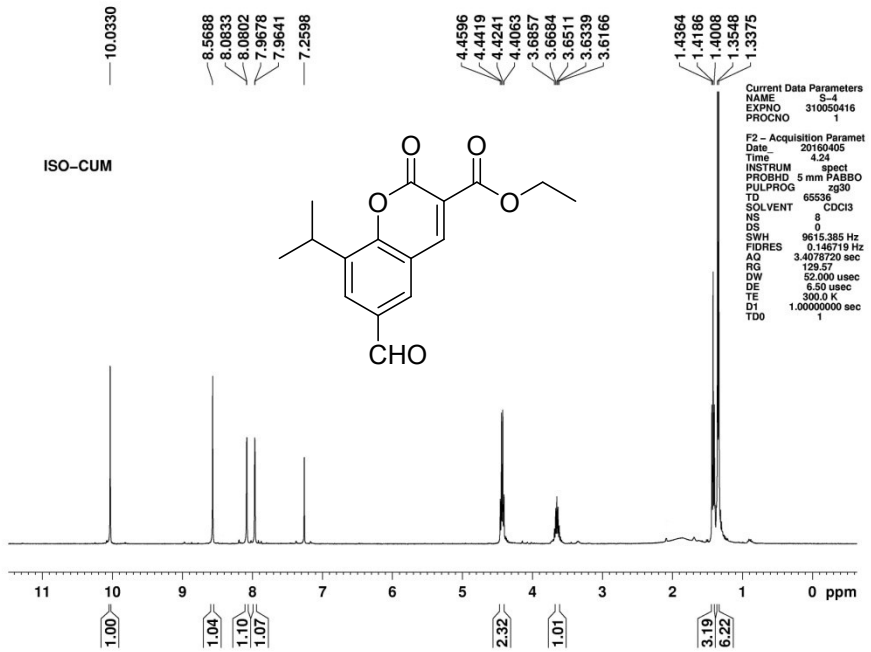
^{13}C NMR of compound **4a** at 100 MHz ($CDCl_3$)



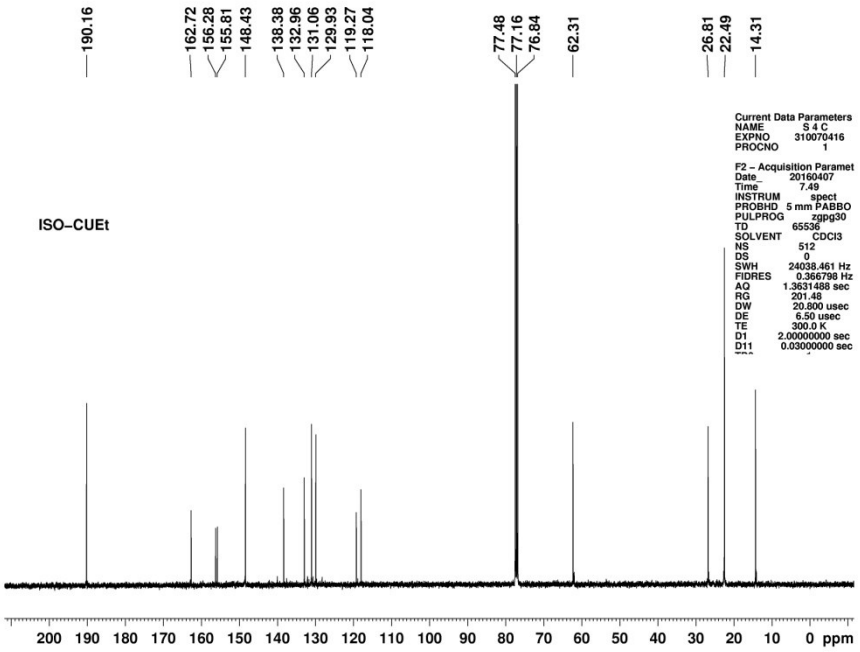
^{1}H NMR of compound **4b** at 400 MHz (CDCl3)



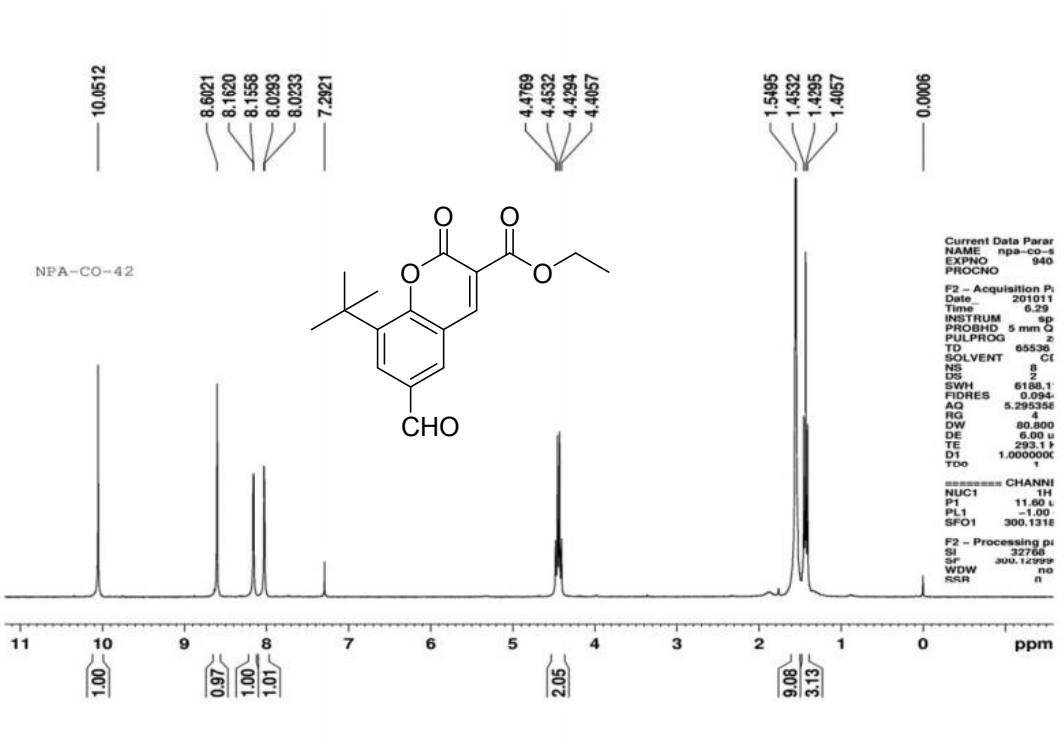
^{13}C NMR of compound **4b** at 100 MHz ($CDCl_3$)



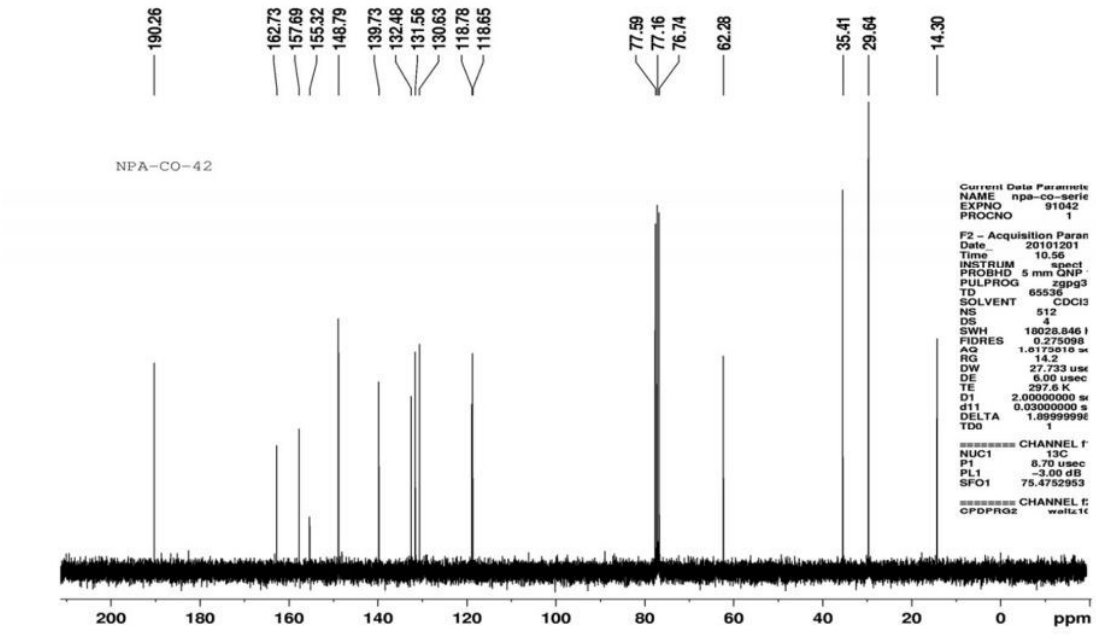
^{1}H NMR of compound **4c** at 400 MHz (CDCl3)



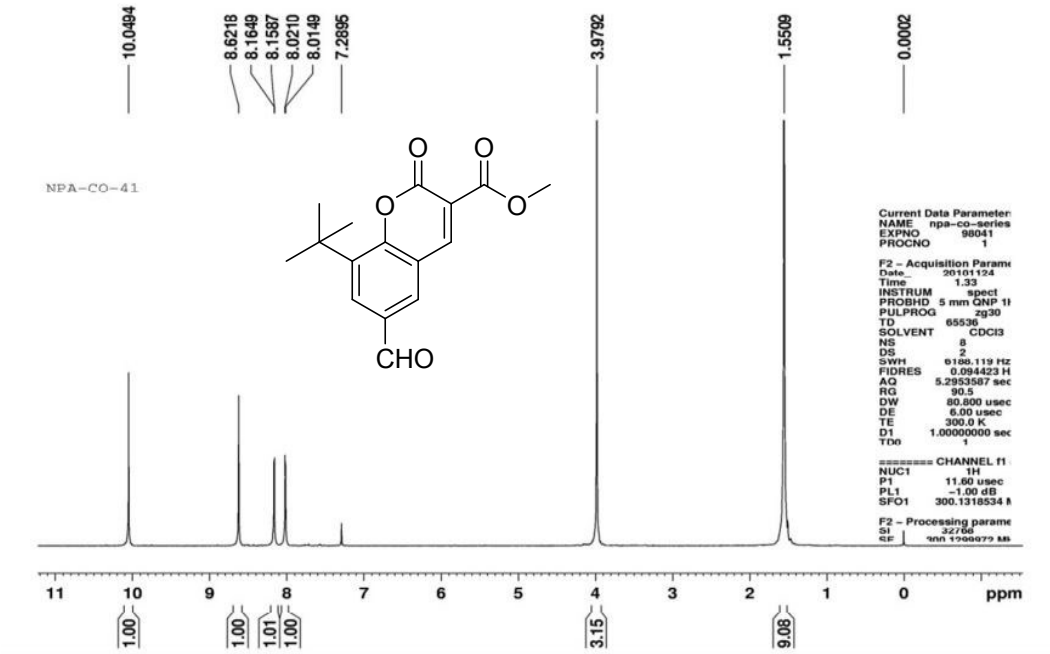
^{13}C NMR of compound **4c** at 100 MHz ($CDCl_3$)



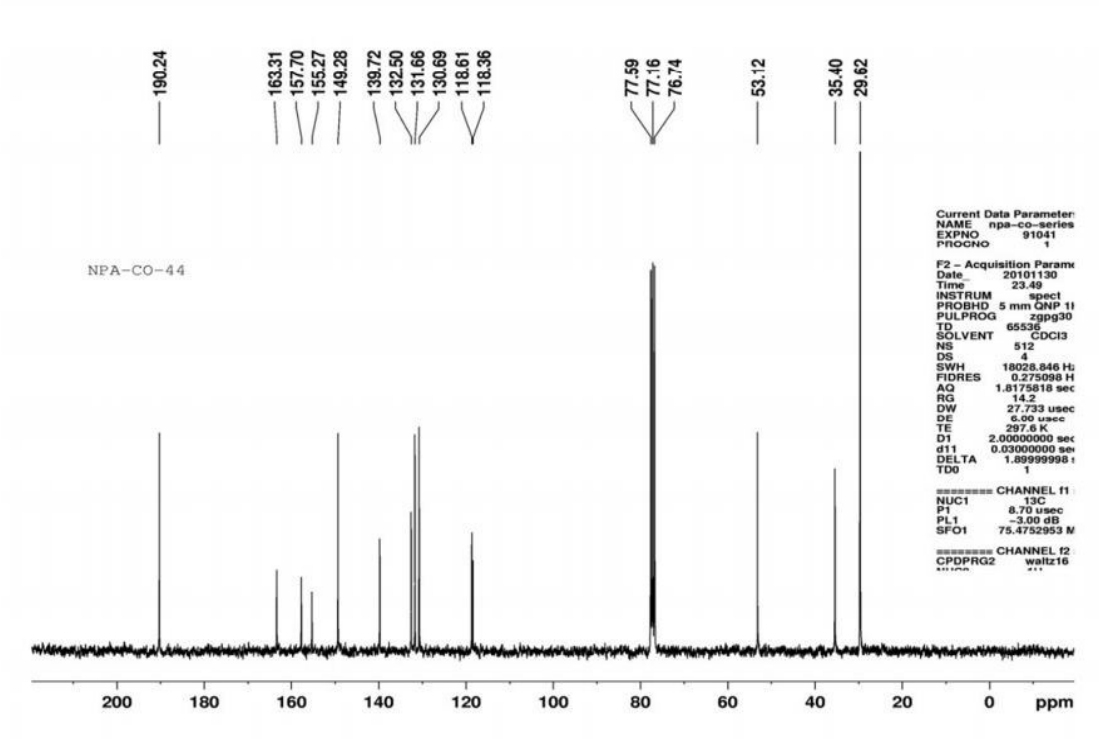
^{1}H NMR of compound **4d** at 300 MHz (CDCl3)



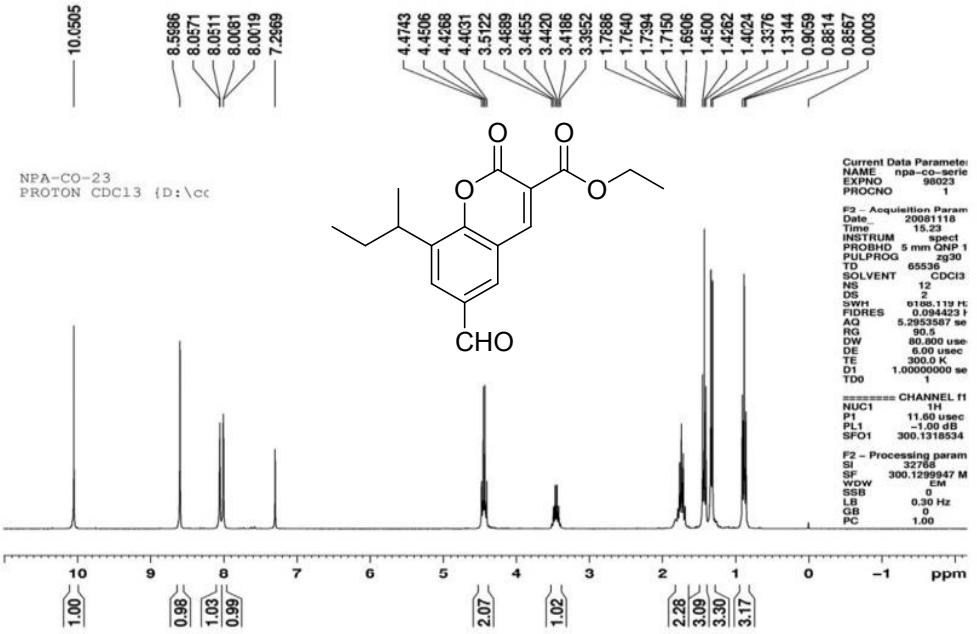
^{13}C NMR of compound **4d** at 75 MHz ($CDCl_3$)



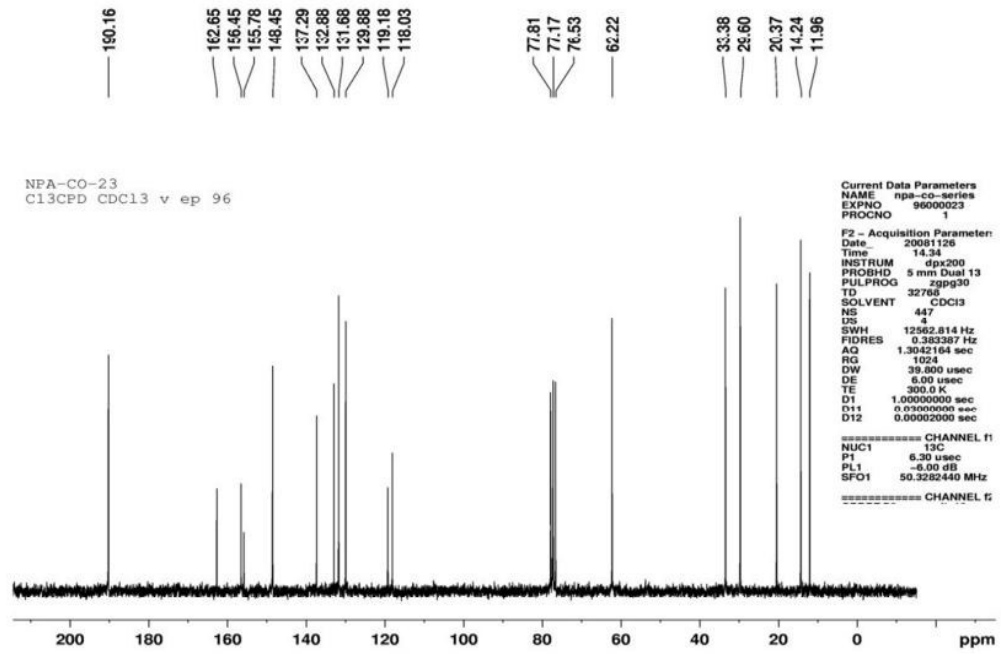
^{1}H NMR of compound **4e** at 300 MHz ($CDCl_3$)



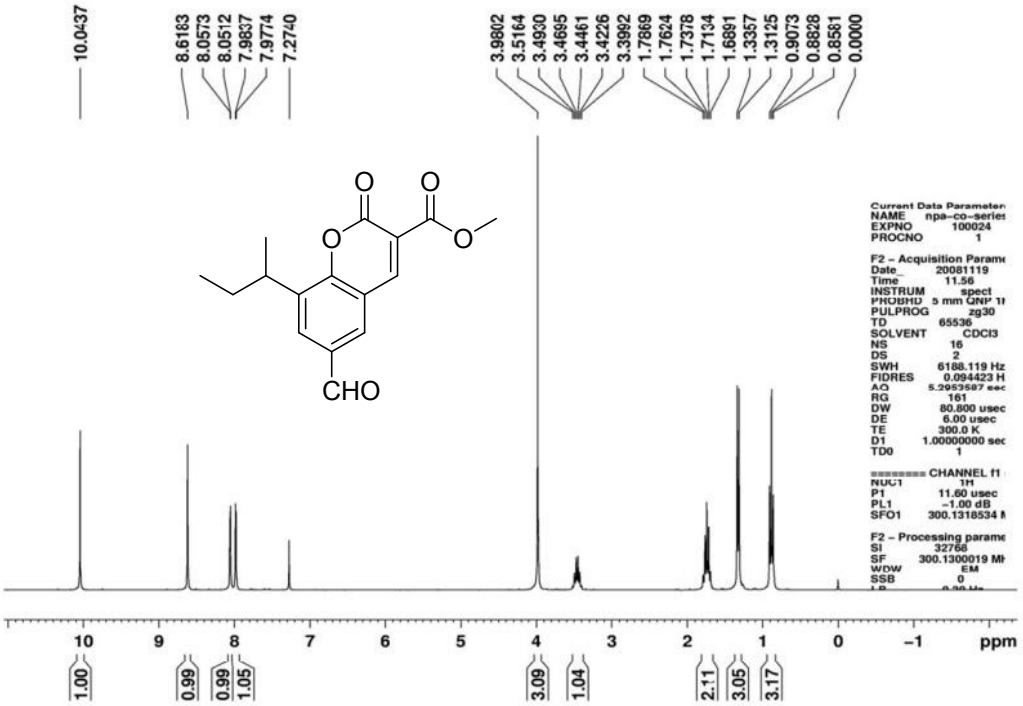
^{13}C NMR of compound **4e** at 75 MHz ($CDCl_3$)



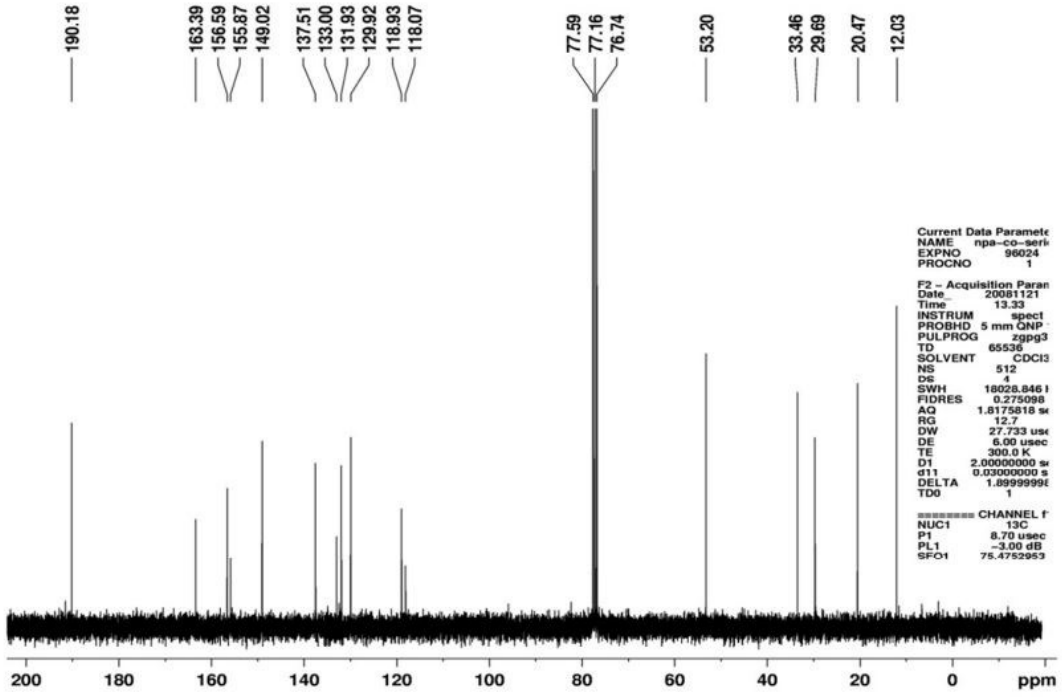
^{1}H NMR of compound **4f** at 300 MHz ($CDCl_3$)



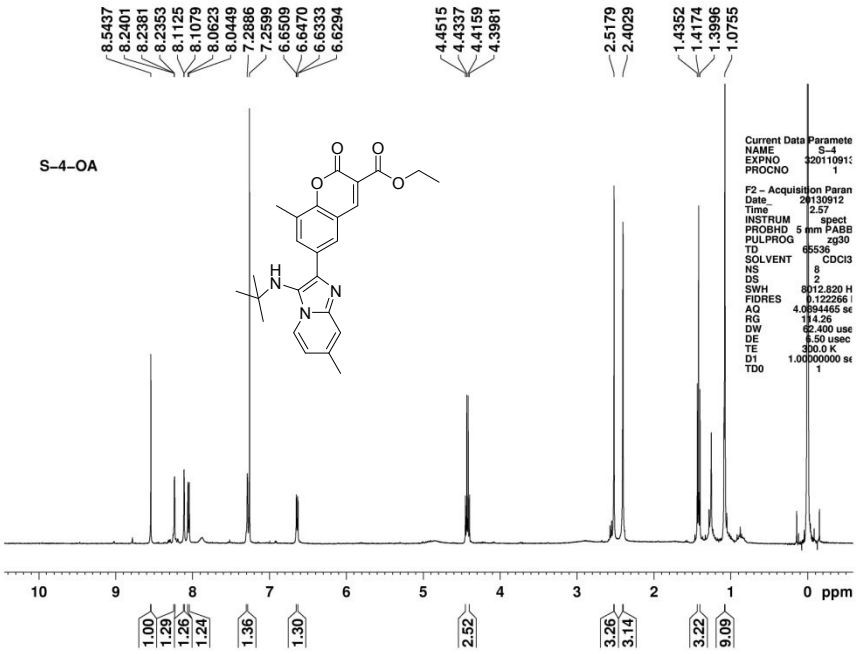
^{13}C NMR of compound **4f** at 50 MHz ($CDCl_3$)



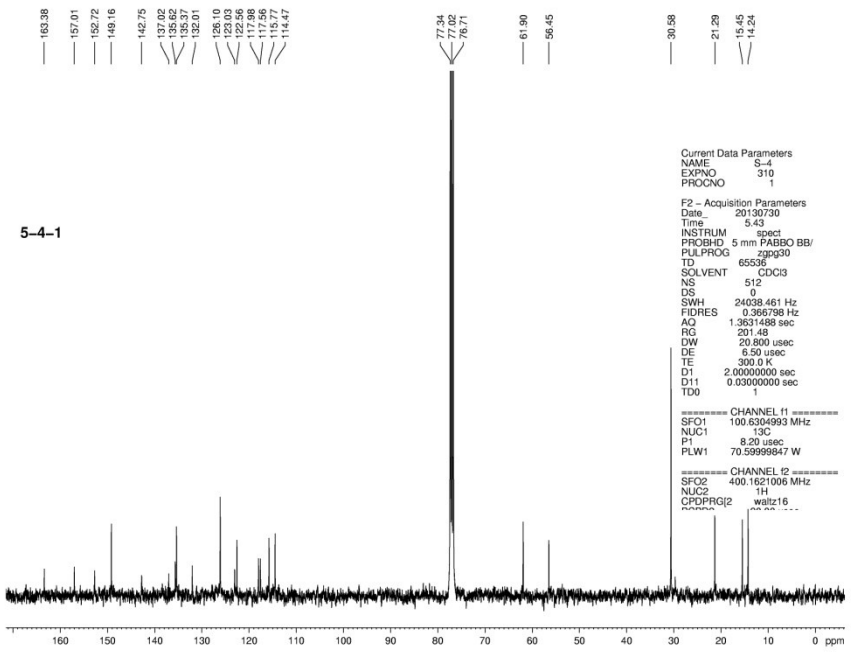
^{1}H NMR of compound **4g** at 300 MHz ($CDCl_3$)



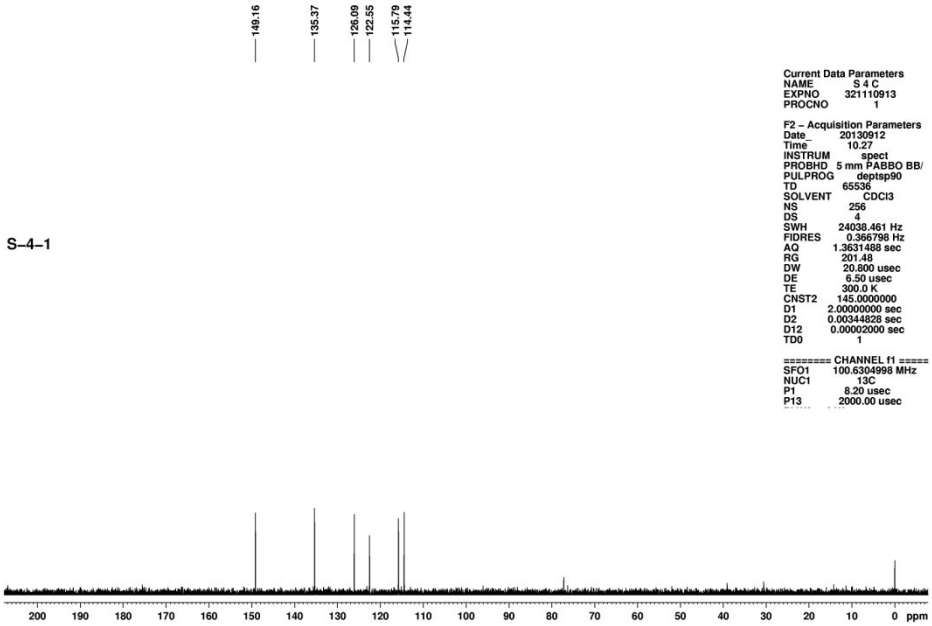
^{13}C NMR of compound **4g** at 50 MHz ($CDCl_3$)



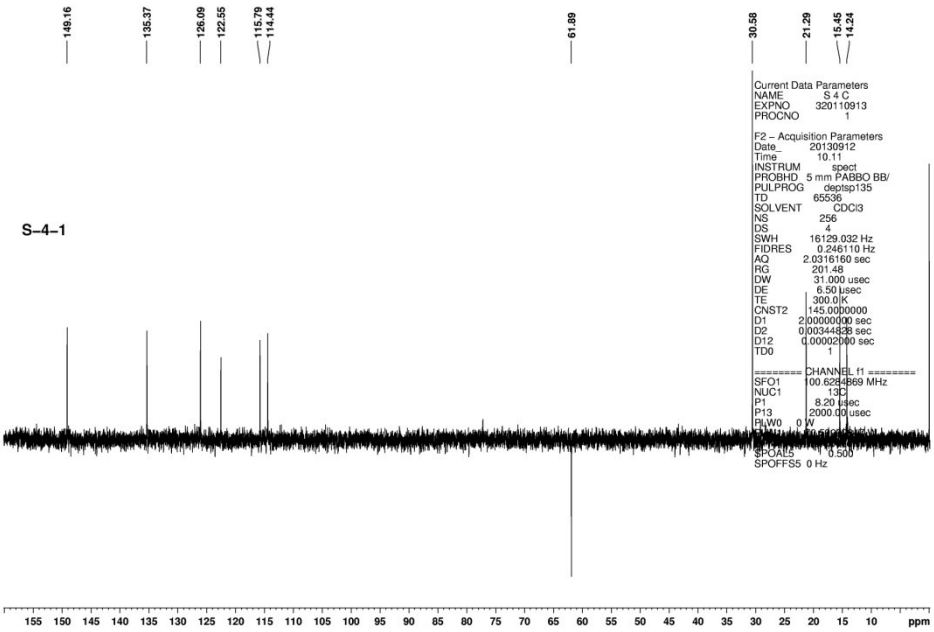
^{1}H NMR of compound **6a** at 400 MHz ($CDCl_3$)



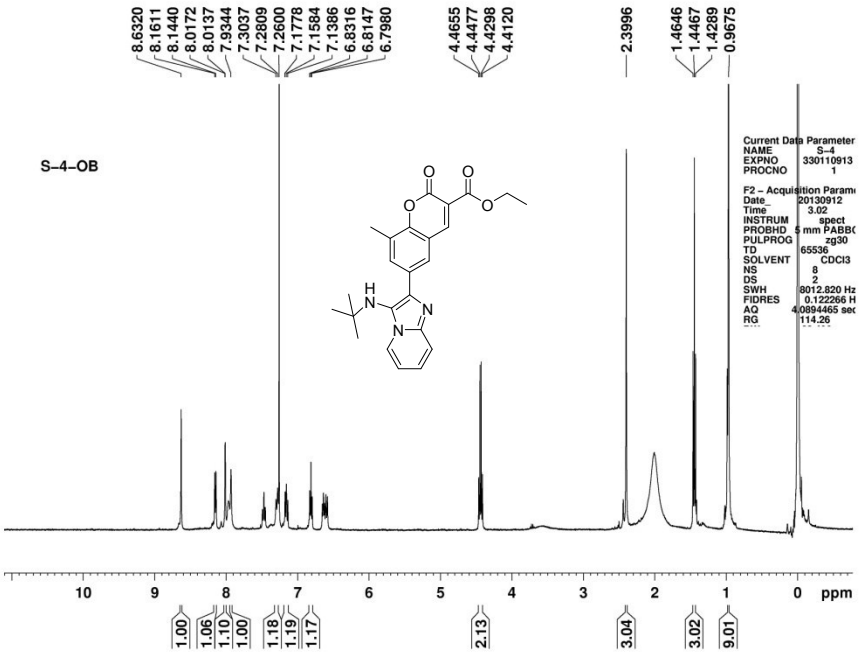
^{13}C NMR of compound **6a** at 100 MHz ($CDCl_3$)



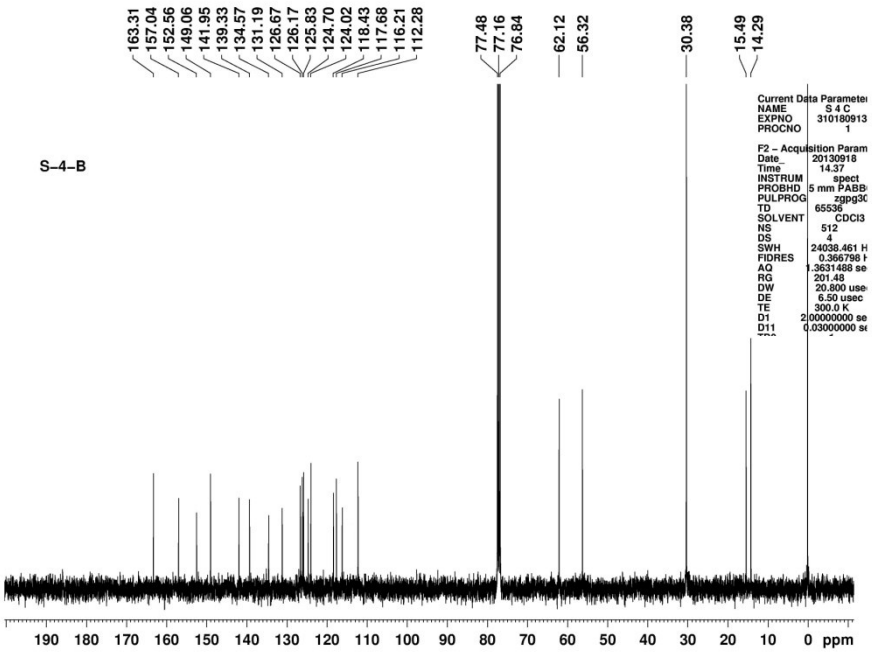
DEPT 90 NMR of compound **6a** at 100 MHz ($CDCl_3$)



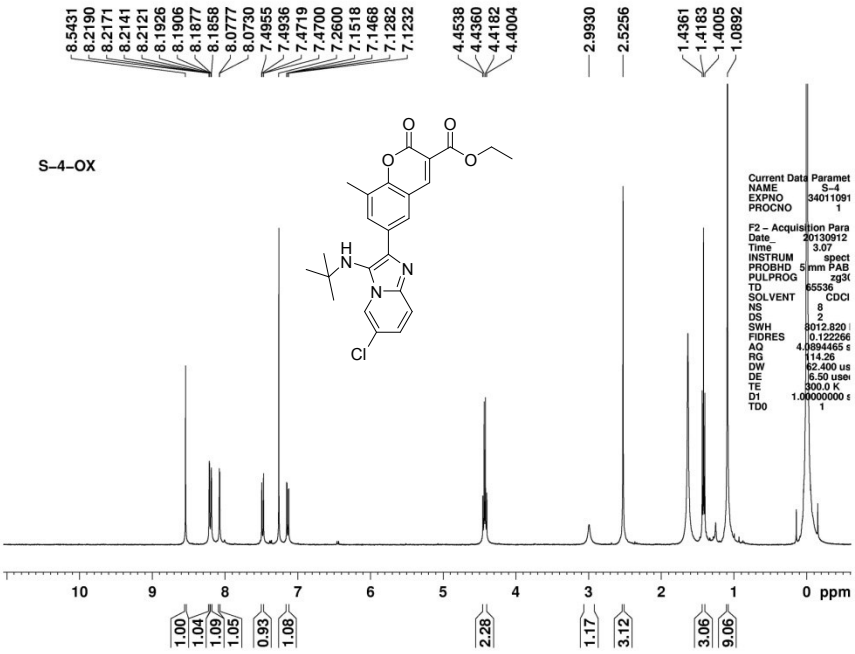
DEPT 135 NMR of compound **6a** at 100 MHz ($CDCl_3$)



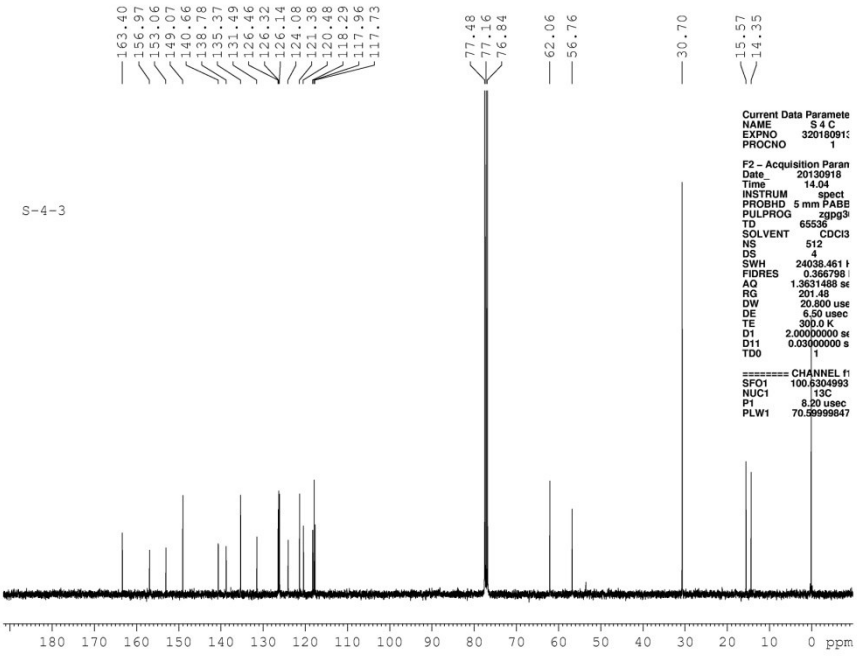
^{1}H NMR of compound **6b** at 400 MHz ($CDCl_3$)



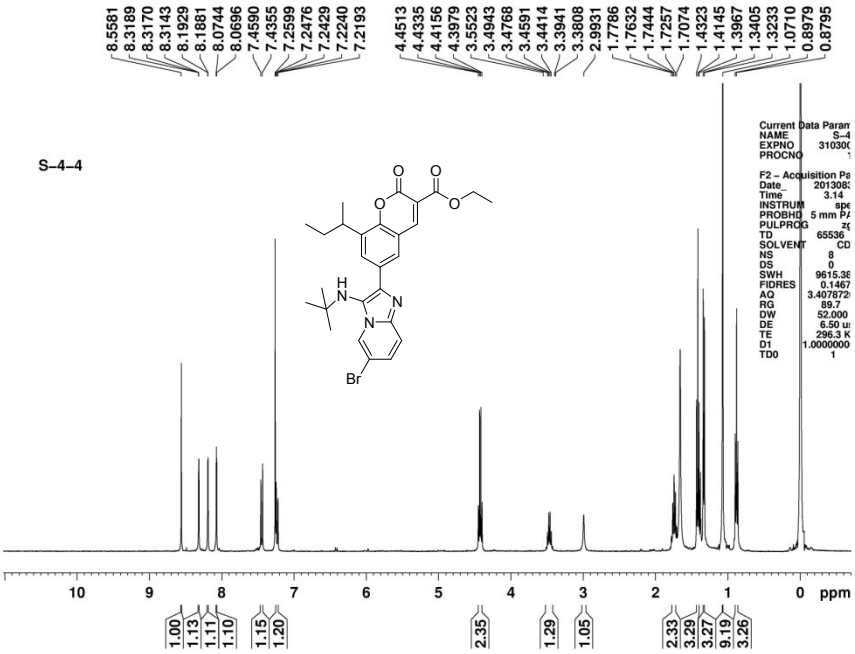
^{13}C NMR of compound **6b** at 100 MHz ($CDCl_3$)



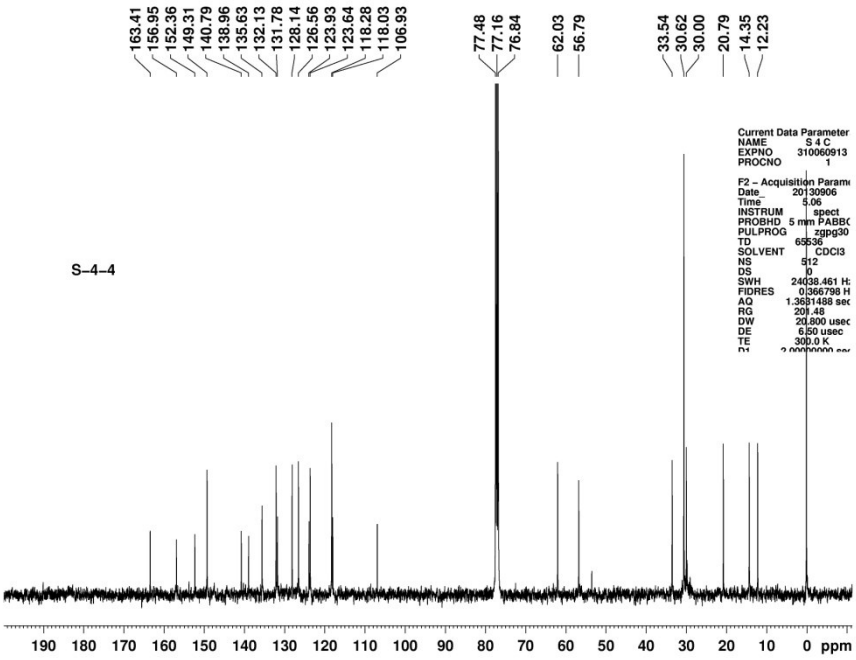
^{1}H NMR of compound **6c** at 400 MHz ($CDCl_3$)



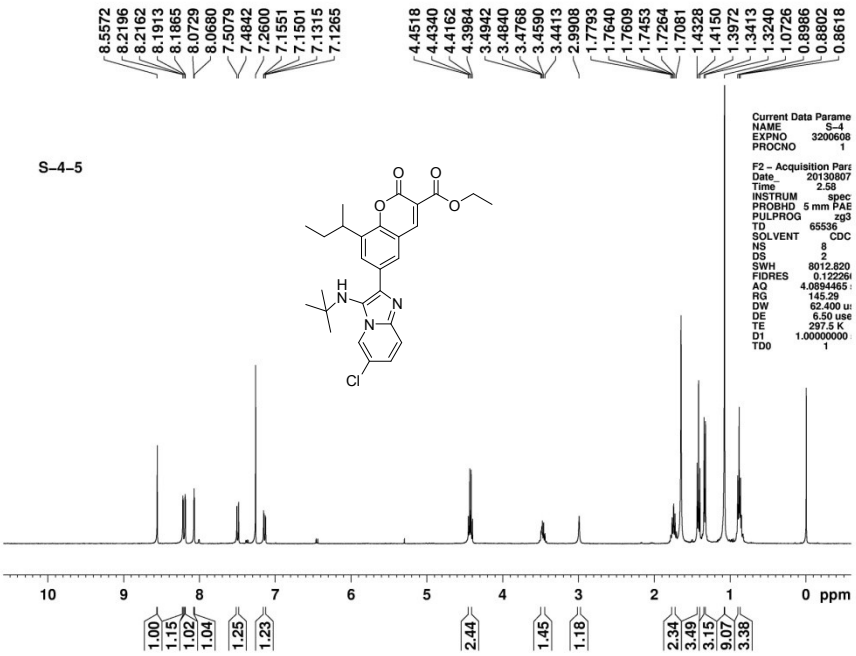
^{13}C NMR of compound **6c** at 100 MHz ($CDCl_3$)



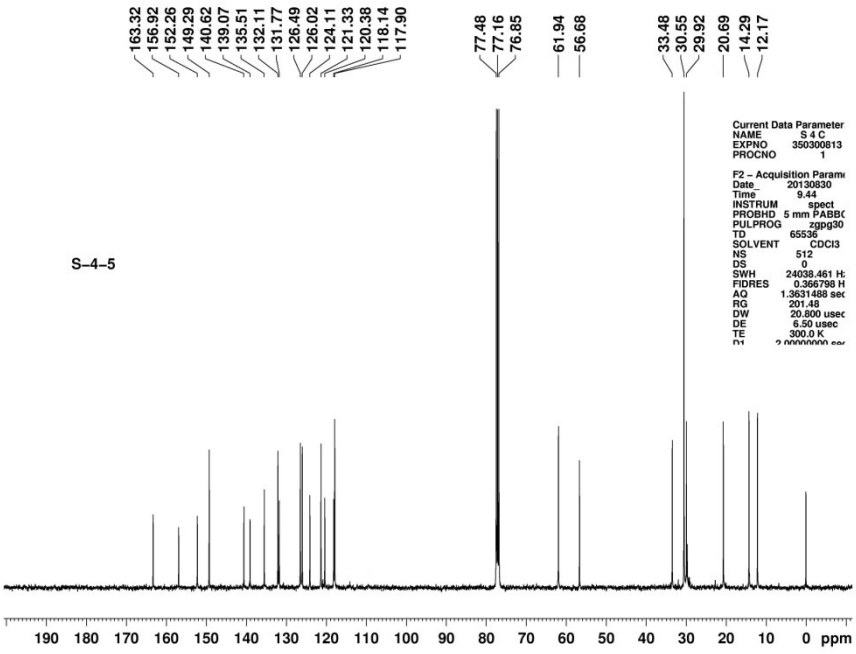
^{1}H NMR of compound **6d** at 400 MHz ($CDCl_3$)



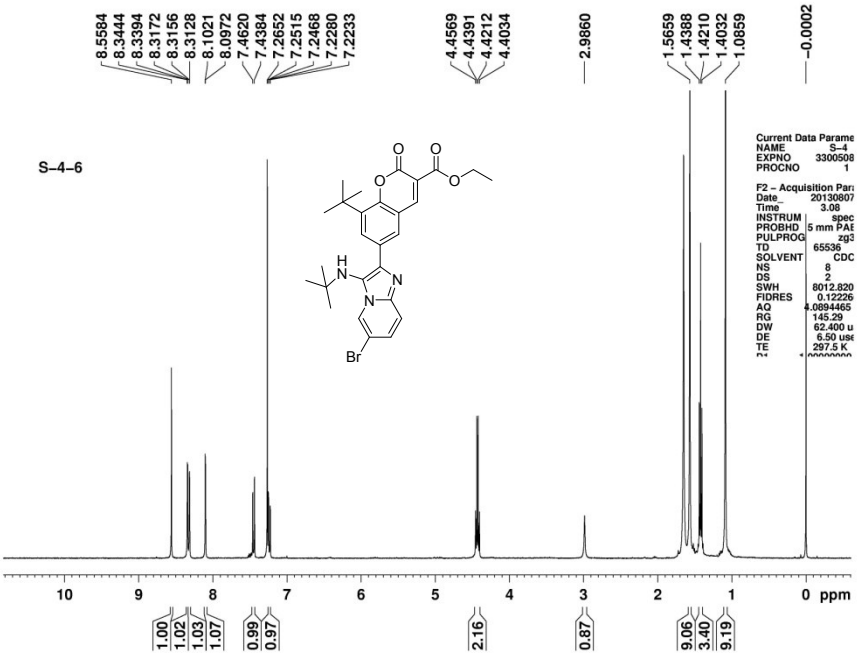
^{13}C NMR of compound **6d** at 100 MHz ($CDCl_3$)



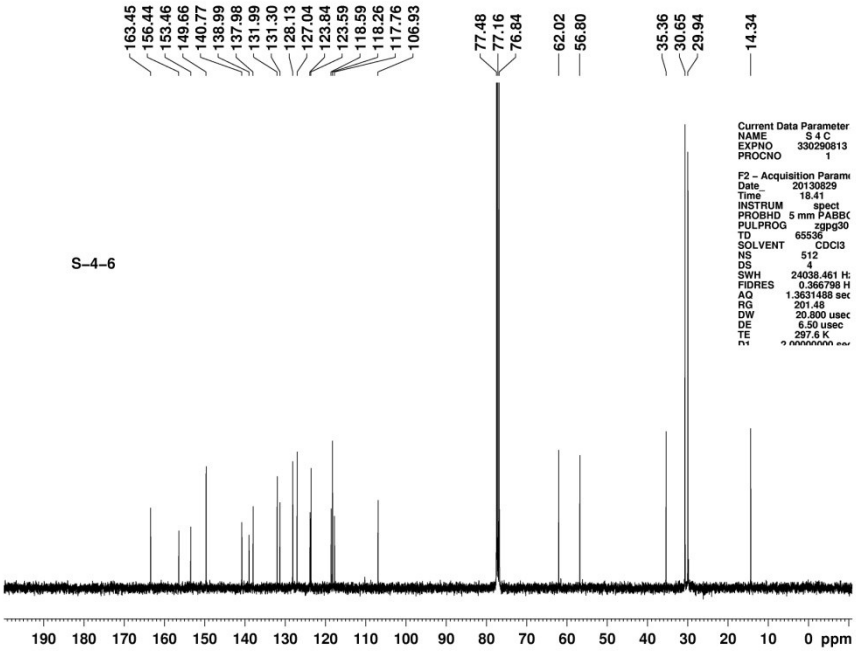
^{1}H NMR of compound **6e** at 400 MHz ($CDCl_3$)



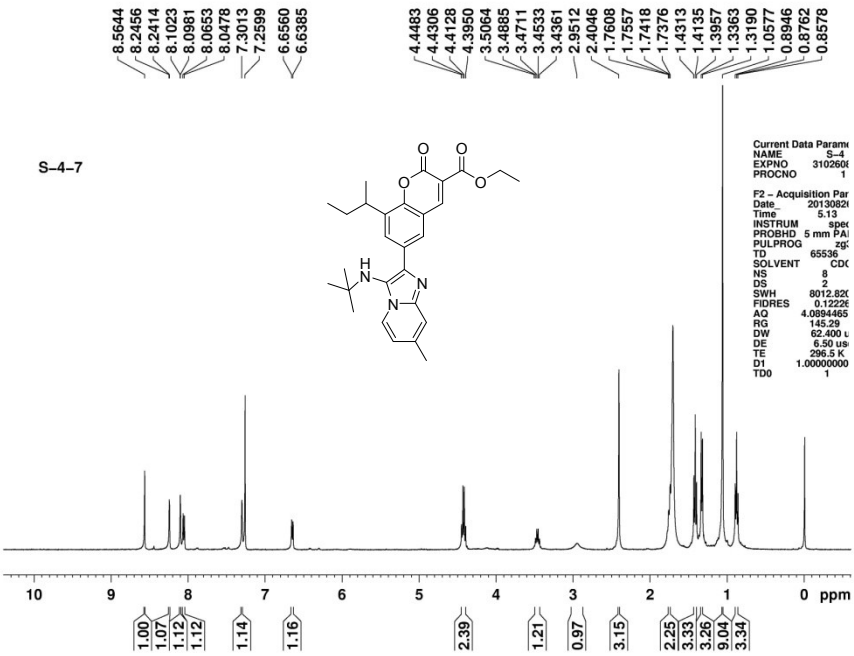
^{13}C NMR of compound **6e** at 100 MHz ($CDCl_3$)



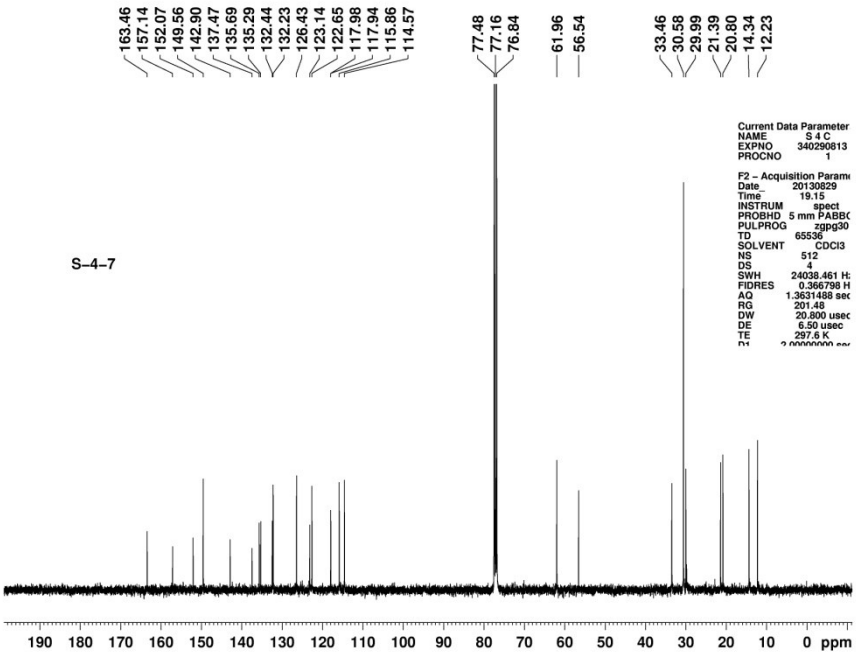
^{1}H NMR of compound **6f** at 400 MHz ($CDCl_3$)



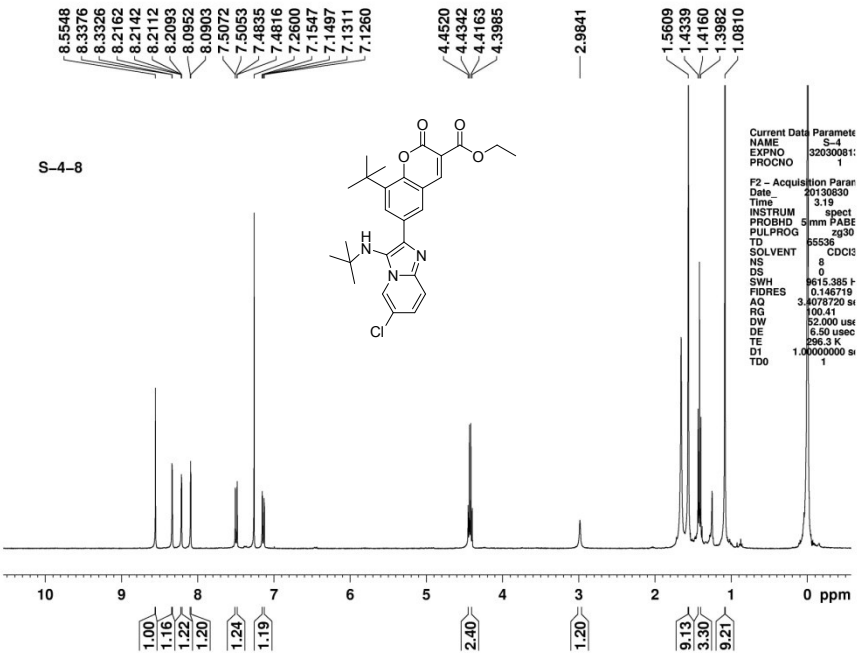
^{13}C NMR of compound **6f** at 100 MHz ($CDCl_3$)



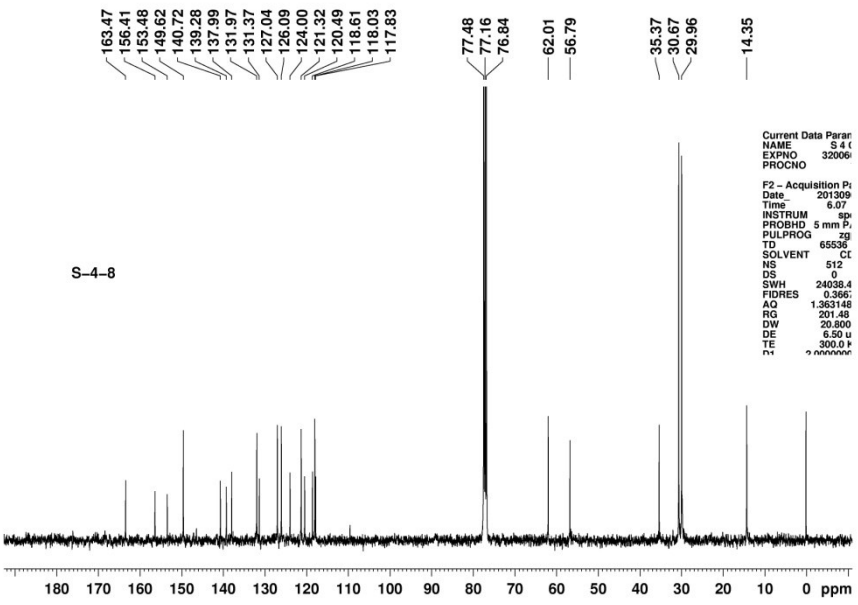
^{1}H NMR of compound **6g** at 400 MHz ($CDCl_3$)



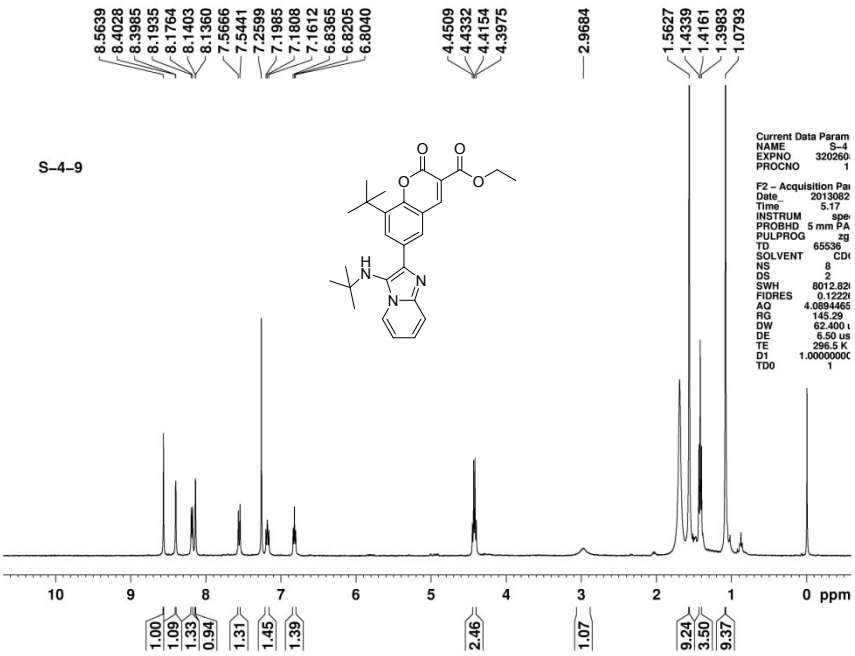
^{13}C NMR of compound **6g** at 100 MHz ($CDCl_3$)



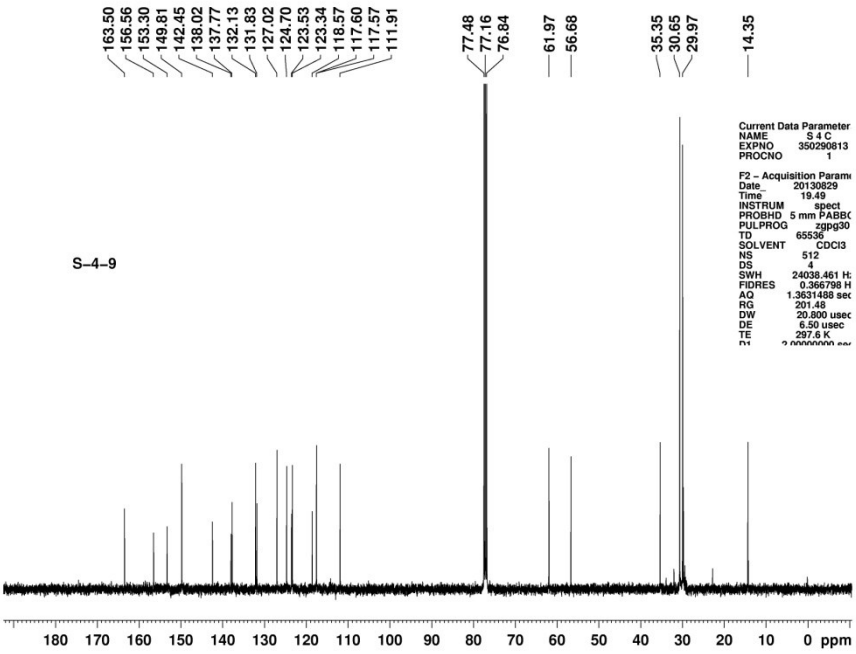
^{1}H NMR of compound **6h** at 400 MHz ($CDCl_3$)



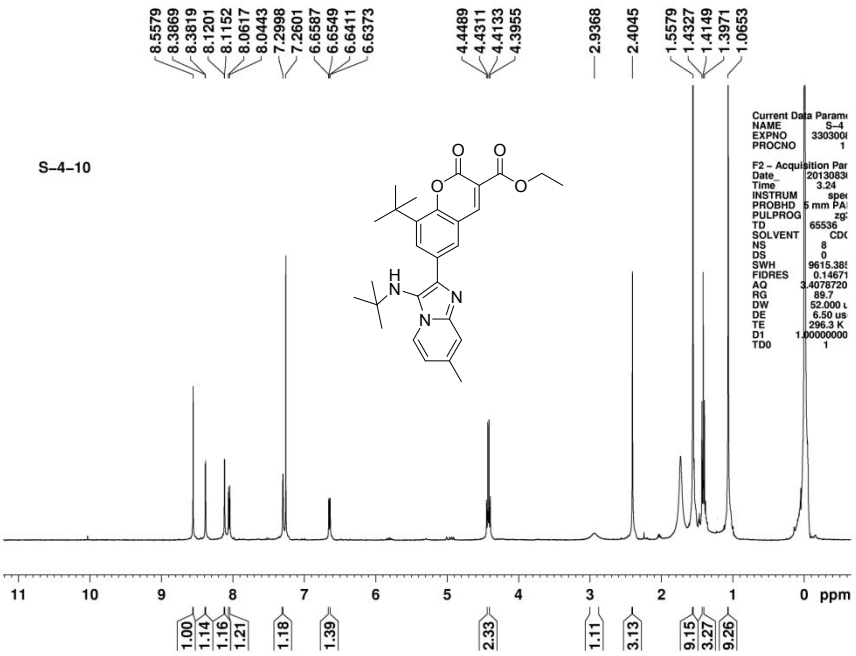
^{13}C NMR of compound **6h** at 100 MHz ($CDCl_3$)



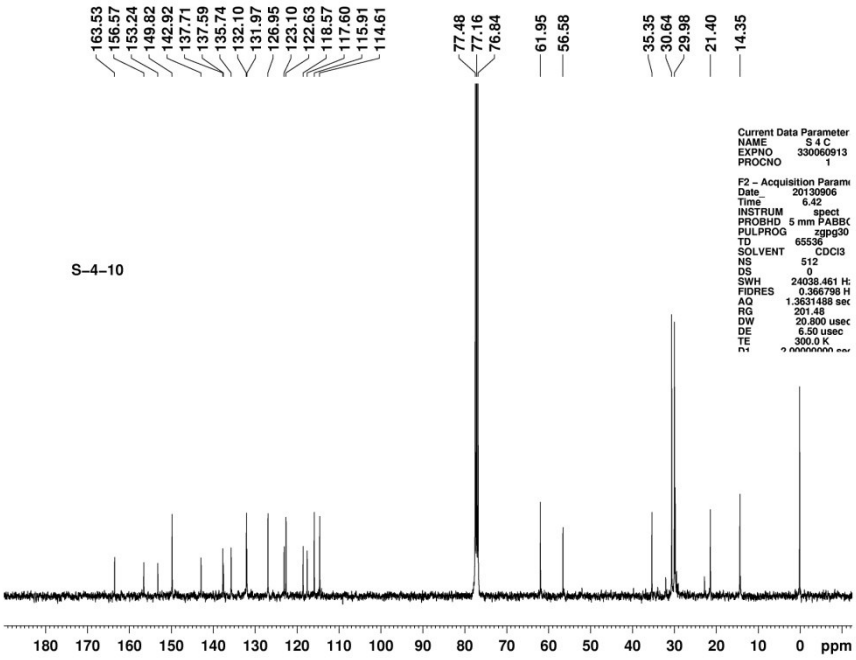
^{1}H NMR of compound **6i** at 400 MHz ($CDCl_3$)



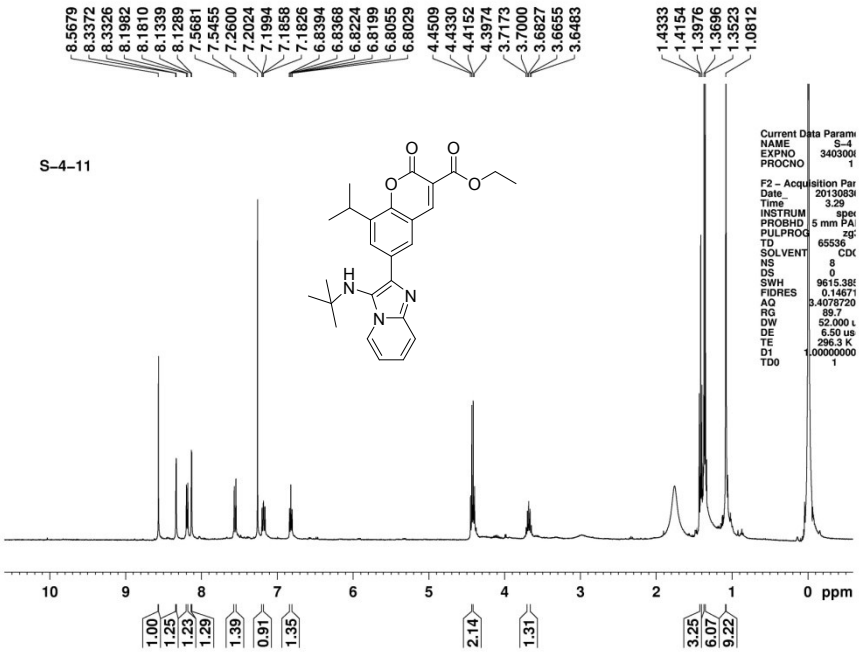
^{13}C NMR of compound **6i** at 100 MHz ($CDCl_3$)



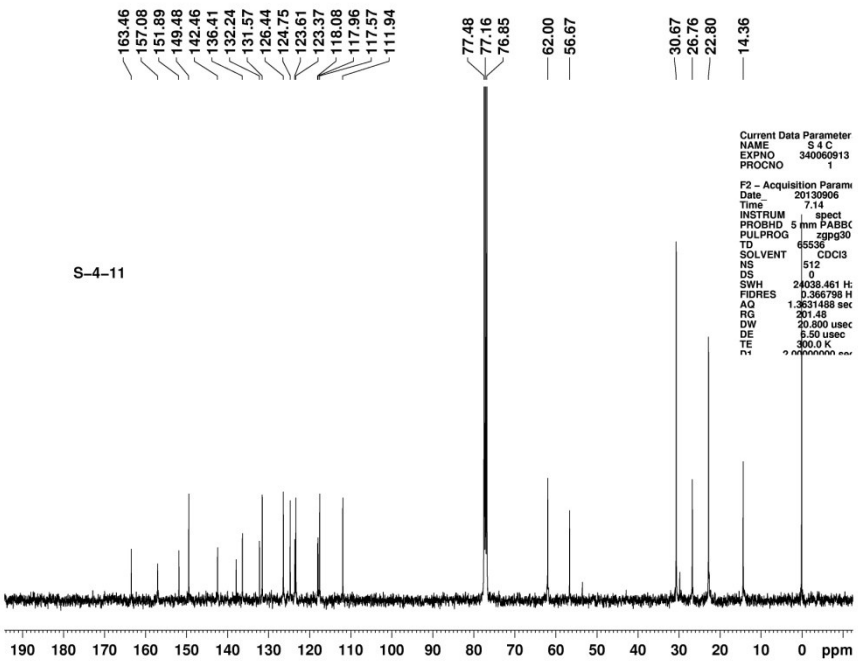
^{1}H NMR of compound **6j** at 400 MHz ($CDCl_3$)



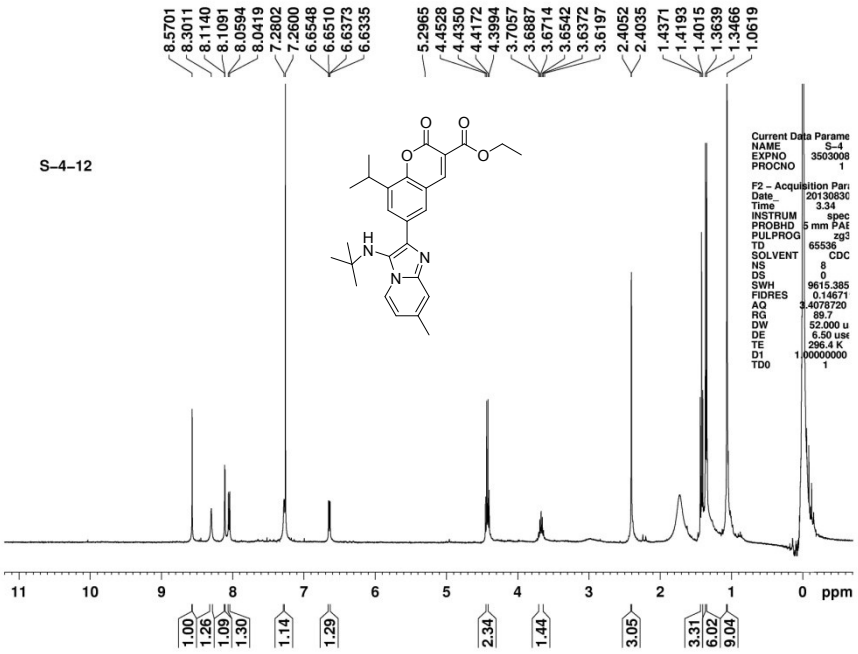
^{13}C NMR of compound **6j** at 100 MHz ($CDCl_3$)



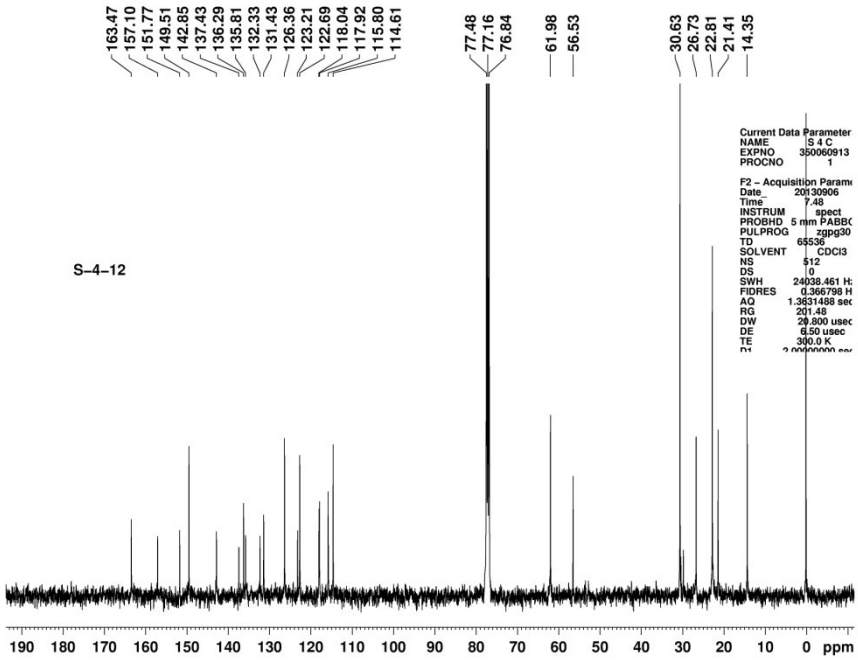
^{1}H NMR of compound **6k** at 400 MHz ($CDCl_3$)



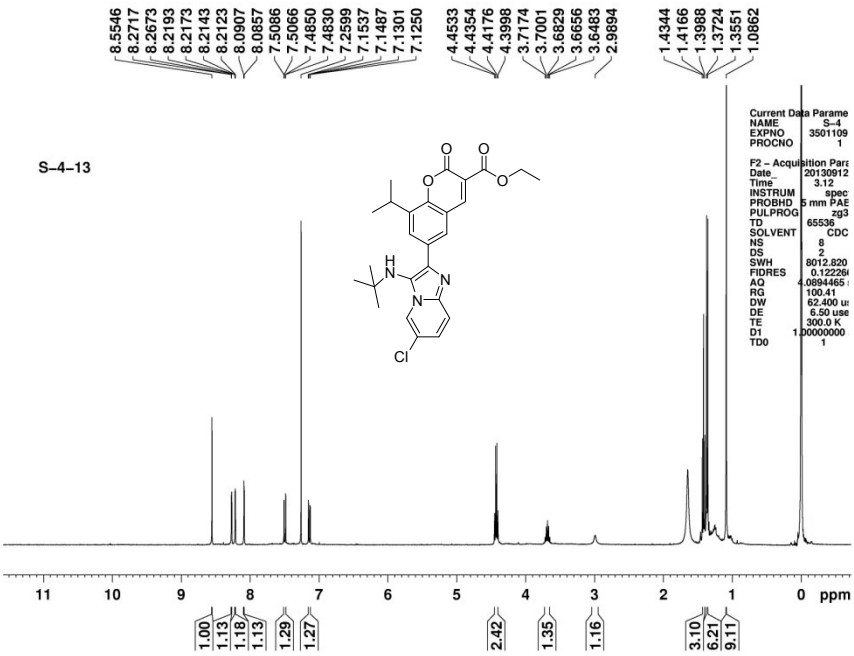
^{13}C NMR of compound **6k** at 100 MHz ($CDCl_3$)



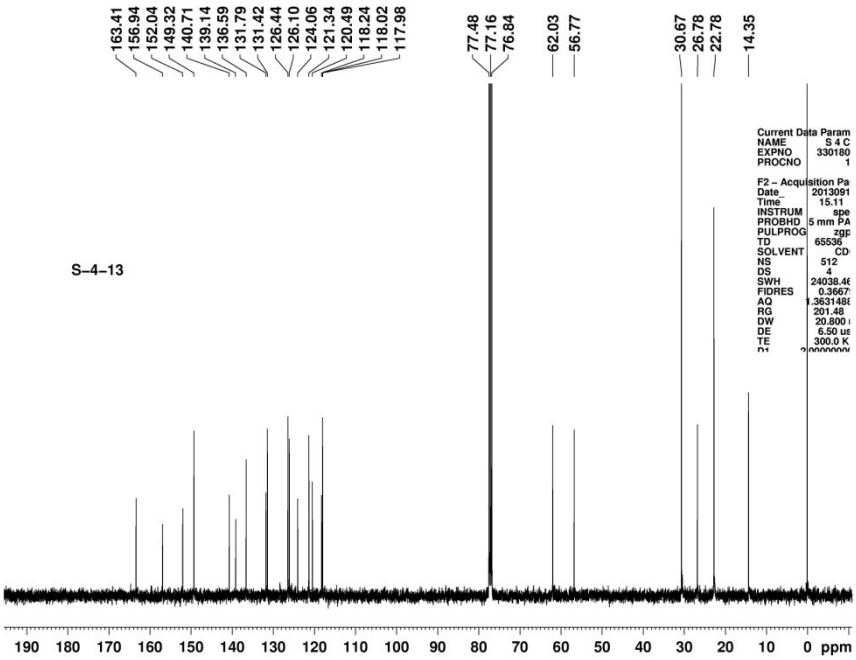
^{1}H NMR of compound **6l** at 400 MHz ($CDCl_3$)



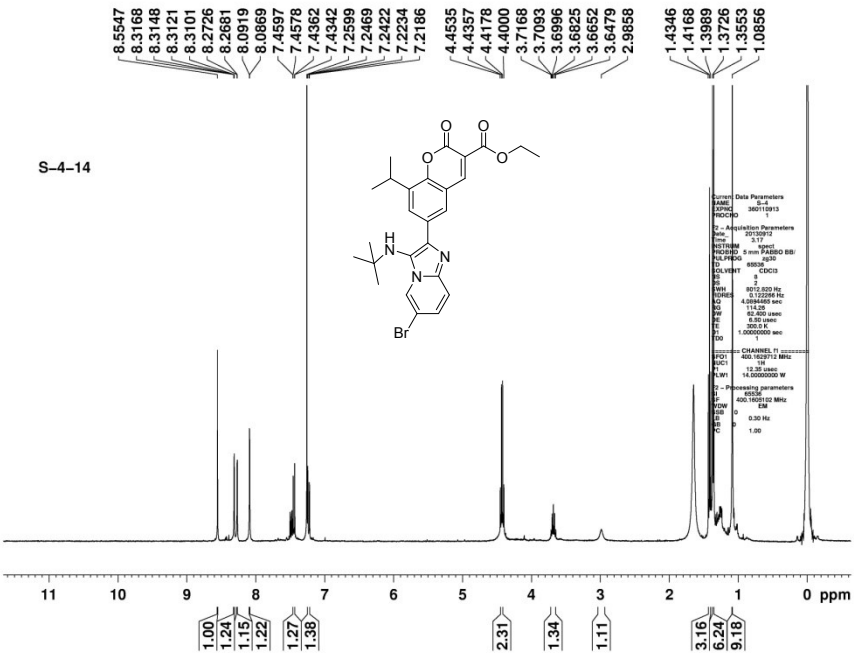
^{13}C NMR of compound **6l** at 100 MHz ($CDCl_3$)



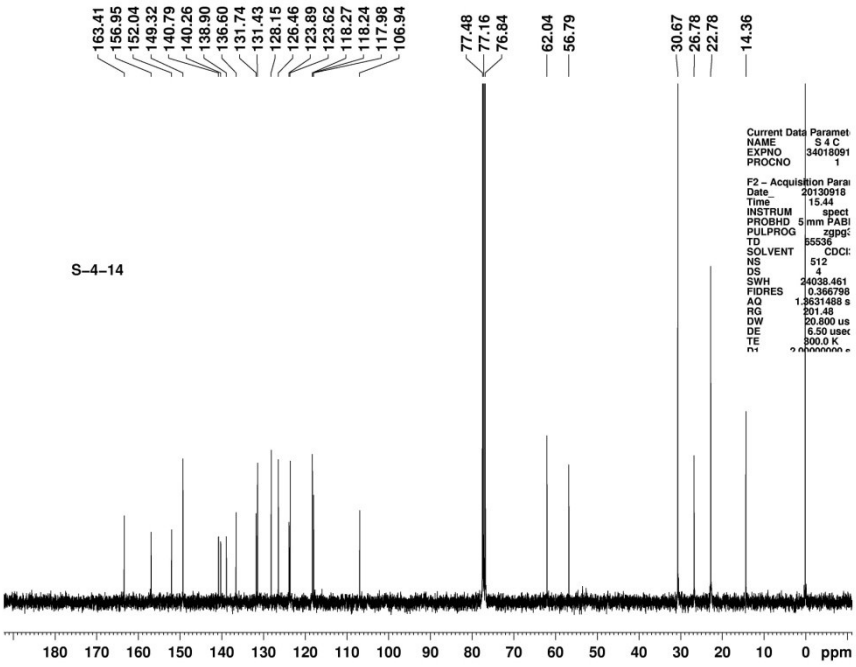
^{1}H NMR of compound **6m** at 400 MHz ($CDCl_3$)



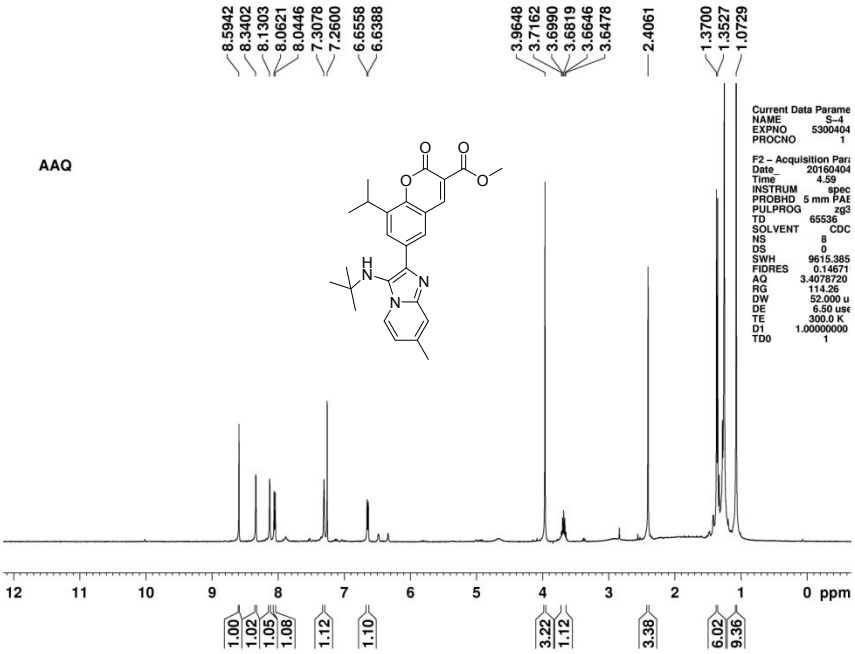
^{13}C NMR of compound **6m** at 100 MHz ($CDCl_3$)



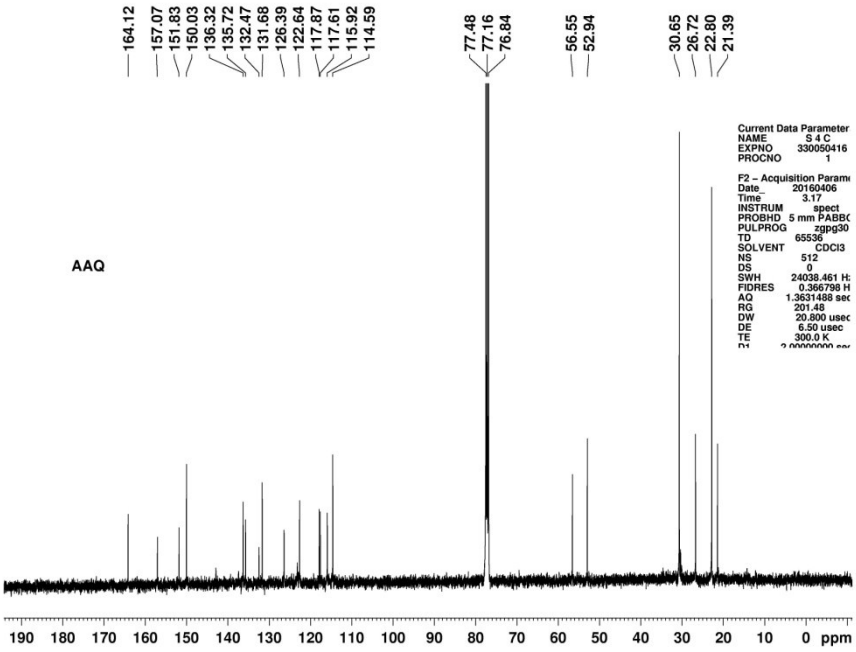
^{1}H NMR of compound **6n** at 400 MHz ($CDCl_3$)



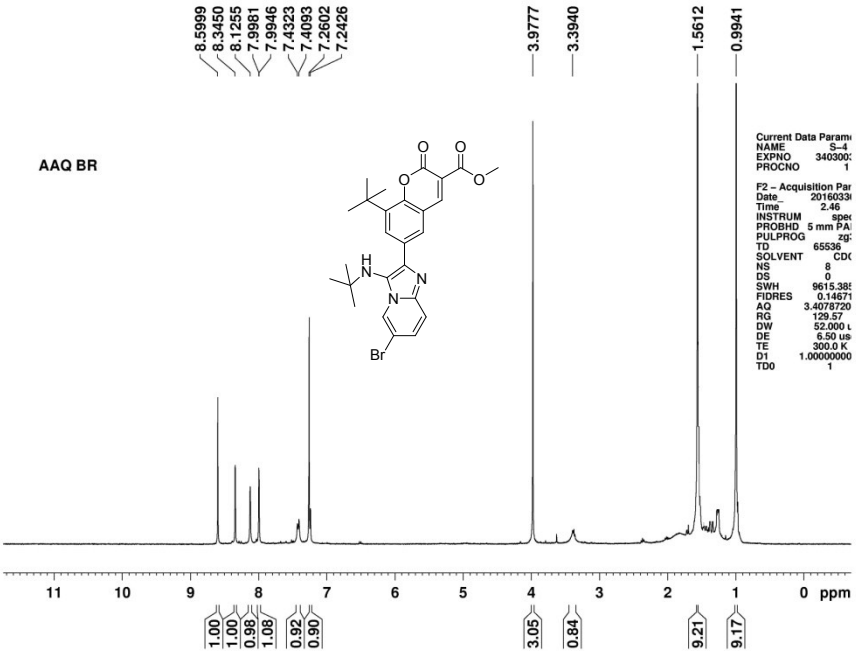
^{13}C NMR of compound **6n** at 100 MHz ($CDCl_3$)



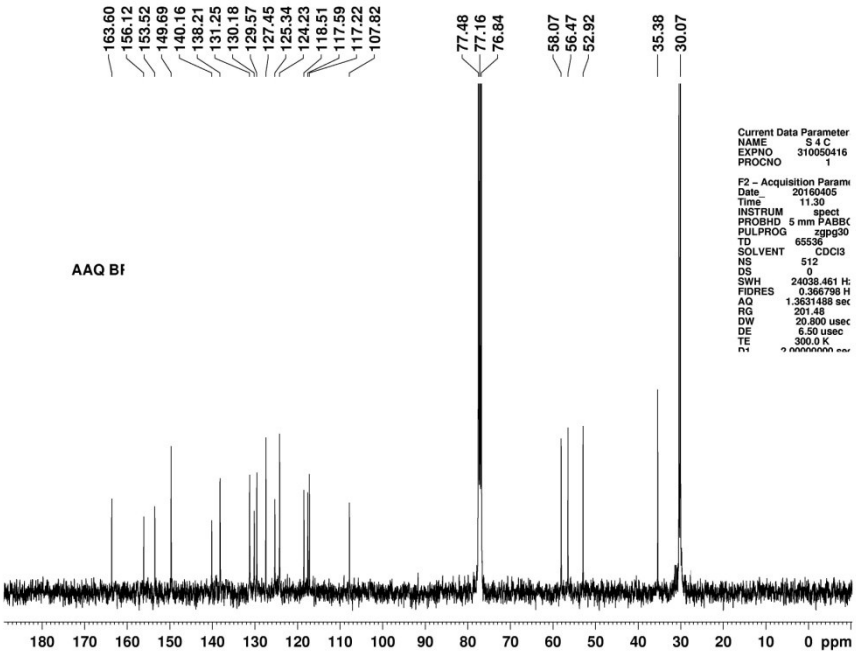
^{1}H NMR of compound **6o** at 400 MHz ($CDCl_3$)



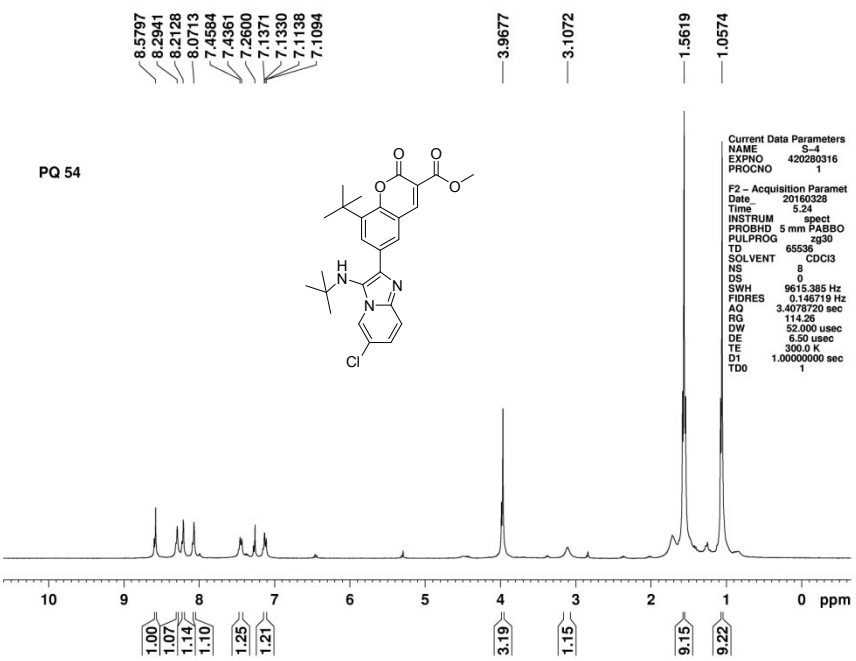
^{13}C NMR of compound **6o** at 100 MHz ($CDCl_3$)



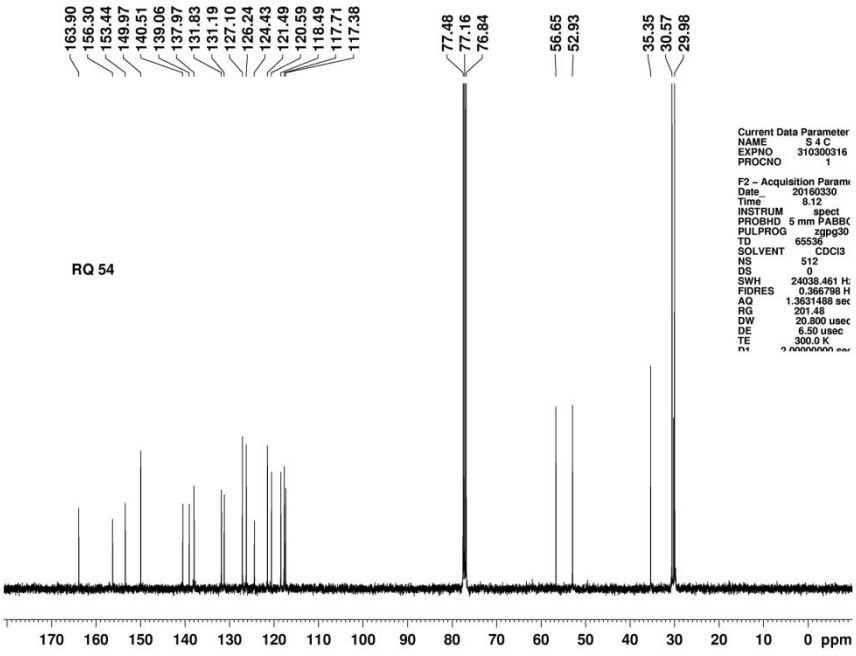
^{1}H NMR of compound **6p** at 400 MHz ($CDCl_3$)



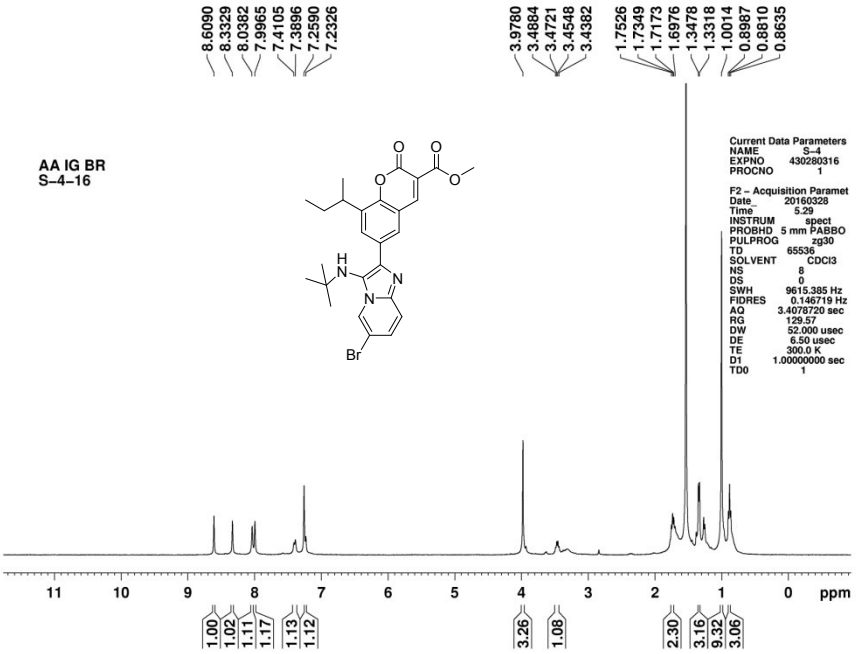
^{13}C NMR of compound **6p** at 100 MHz ($CDCl_3$)



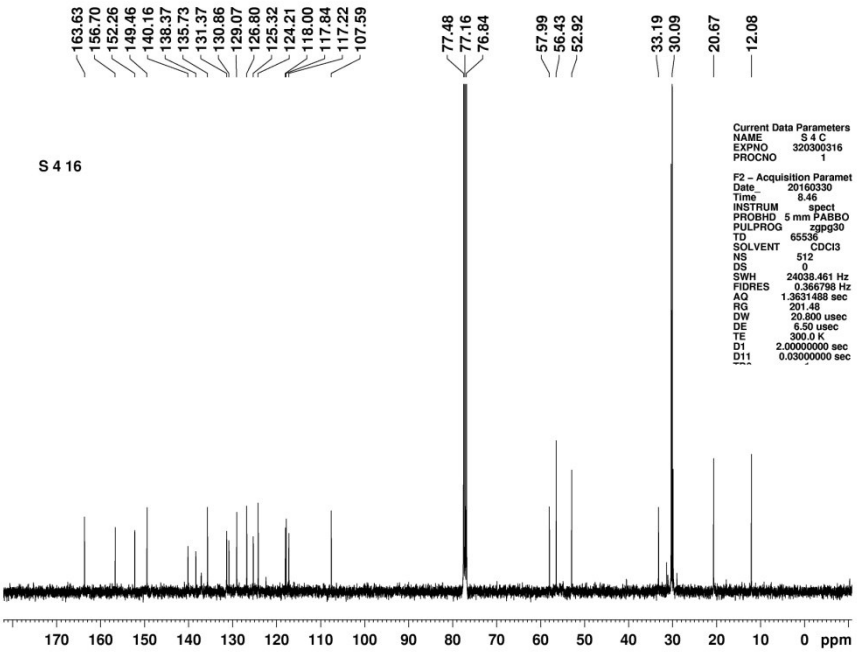
^{1}H NMR of compound **6q** at 400 MHz ($CDCl_3$)



^{13}C NMR of compound **6q** at 100 MHz ($CDCl_3$)



^{1}H NMR of compound **6r** at 400 MHz ($CDCl_3$)



^{13}C NMR of compound **6r** at 100 MHz ($CDCl_3$)

Cytotoxicity assay by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

Cytotoxicity of different compounds was assessed using an MTT assay because live cells have ability to convert soluble MTT into an insoluble purple formazan reaction product. Mice calvarial osteoblast cells were used to test toxicity of synthesized compounds 2 X 10^3 cells/well. Cells were seeded in 96- well plates and treated with or without compounds (6O, H and L) at different concentrations for 24 h. After end of incubation time, the cells were washed with PBS and treated with MTT solution (5 mg/10 mL in α-MEM devoid of Phenol Red) for 4 h. The MTT solution was then aspirated and replaced with 200ml/well dimethyl sulfoxide (DMSO). Formazon crystals formed were dissolved in DMSO and detection was done at OD 540 nm.

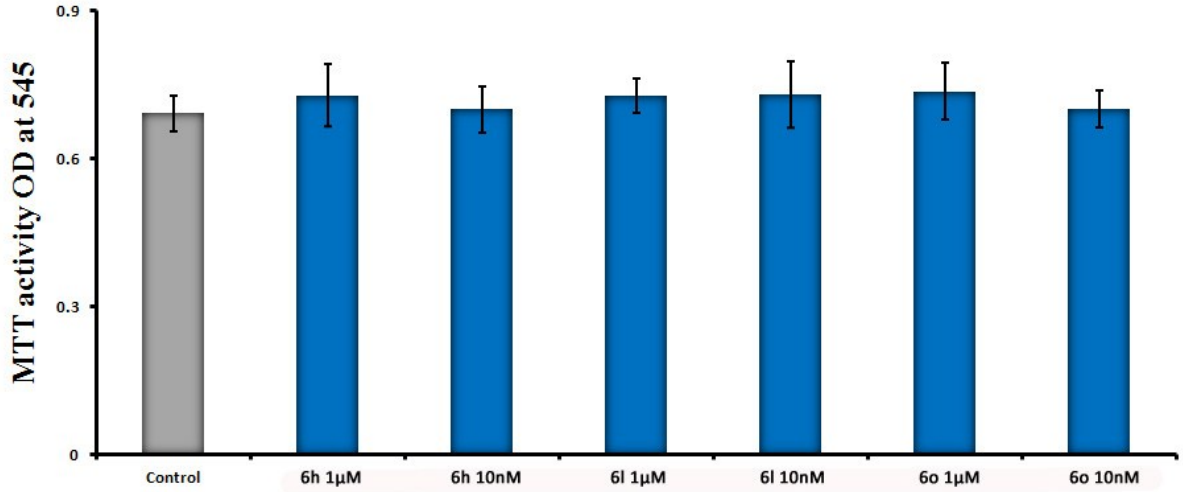


Figure 1. Compounds **6h**, **6l** and **6o** were measured for cell viability and toxicity by MTT after 48 hr of treatment of compounds. MTT was compared to untreated cells (control). All values were expressed as mean values with their standard errors (Mean ± SEM) using (n = 3) of three independent experiments. Comparisons of each parameter among all of the groups were analyzed *via* multiple comparison analysis within the groups using one-way ANOVA (non-parametric) and then a post hoc test. For significance, $P<0.05$ *was* used and in each group, results were reproducible and there were no disagreements amongst the blinded assessors.