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Supporting Information

Synthesis of new chromeno-annulated *cis*-fused pyrano[3,4-*c*]pyran derivatives via domino Knoevenagel-hetero-Diels-Alder reactions and their biological evaluation towards antiproliferative activity

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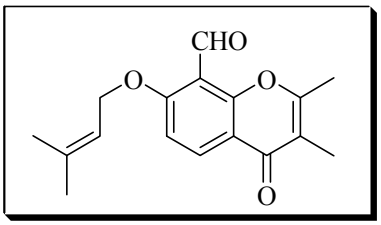
1.1 General Chemical Methods:

All solvents and reagents were used, as received from the suppliers. TLC was performed on Merck Kiesel gel 60, F_{254} plates with the layer thickness of 0.25 mm. Column chromatography was performed on silica gel (100-200 mesh) using a gradient of ethyl acetate and hexane as mobile phase. Melting points were determined on a Fisher John's melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer RX-1 FT-IR system. ^{1}H NMR spectral data were collected at 300, 400, 500 & 600 MHz, while ^{13}C NMR were recorded at 75, 100, 125, 150 MHz. ^{1}H NMR spectral data are given as chemical shifts in ppm followed by multiplicity (s- singlet; d- doublet; t- triplet; q- quartet; m- multiplet), number of protons and coupling constants. ^{13}C NMR chemical shifts are expressed in ppm. HRMS spectral data were collected using ORBITRAP High Resolution Mass Spectrometer. Crystals suitable for X-ray crystallographic studies were obtained by crystallisation from methanol.

1.2 General Procedure for Synthesis of 2,3 disubstituted chromones (2a, 2b, 2c)

1.2.1. 2,3-Dimethyl-7-[(3-methylbut-2-en-1-yl)oxy]-4-oxo-4*H*-chromene-8-carbaldehyde (2a)



A mixture of alcohol (2.18 g, 10 mmol), prenyl bromide (1.76 ml, 15 mmol), potassium carbonate (2.76 g, 20 mmol) and acetone (80 mL) was heated under reflux for 6 hr. After completion of the reaction, K_2CO_3 was filtered and the resulting solution was concentrated in vacuo and the residue was purified residue was purified by column chromatography over silica gel (100–200 mesh, with eluent: 35% EtOAc in hexane) to give 2,3-Dimethyl-7-(3-methylbut-2-enyloxy)-4-oxo-4*H*-chromene-8-carbaldehyde **2a** (2.66 g, 93%).

White solid; mp 169–173 ^{o}C; IR (KBr): ν_{max} 3371, 3079, 2931, 1697, 1641, 1602, 1441, 1410, 1288, 1223, 1181, 1075, 982, 23, 784, 639, 466 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79 (s, 3H), 1.82 (s, 3H), 2.03 (s, 3H), 2.46 (s, 3H), 4.74 (d, J = 6.7 Hz, 2H), 5.49 (t, J = 6.7 Hz, 1H),

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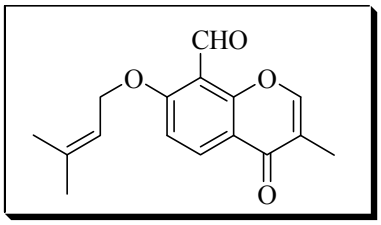
6.98 (d, J = 9.0 Hz, 1H), 8.32 (d, J = 9.0 Hz, 1H), 10.56 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.48, 176.58, 165.03, 162.12, 155.88, 139.72, 133.34, 118.11, 117.22, 116.46, 112.65, 110.10, 66.49, 25.75, 18.43, 18.35, 9.78; MS–ESIMS: *m/z* 287 $[M+H]^+$; HRMS calcd for $C_{17}H_{19}O_4$, 287.1283; found, 287.1285.

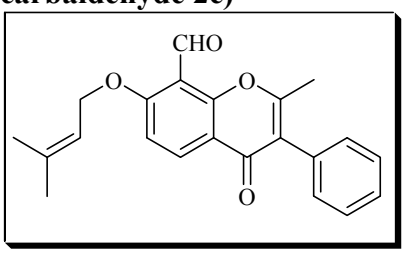
1.2.2. 3-Methyl-7-[(3-methylbut-2-en-1-yl)oxy]-4-oxo-4*H*-chromene-8-carbaldehyde (2b)

A mixture of alcohol (2.80 g, 10 mmol), prenyl bromide (1.76 ml, 15 mmol), potassium carbonate (2.76 g, 20 mmol) and acetone (80 mL) was heated under reflux for 6 hr. After completion of the reaction, K_2CO_3 was filtered and the resulting solution was concentrated in vacuo and the residue was purified by column chromatography over silica gel (100–200 mesh, with eluent: 30 % EtOAc in hexane) to give 3-Methyl-7-(3-methylbut-2-enyloxy)-4-oxo-4*H*-chromene-8-carbaldehyde **2b** (2.50 g, 92%).



White solid; mp: 159–164 ^{o}C ; IR(KBr): ν_{max} 3079, 2967, 2927, 2887, 1692, 1639, 1596, 1412, 1271, 1198, 1155, 1037, 914, 781 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (s, 3H), 1.82 (s, 3H) , 2.02 (s, 3H), 4.78 (d, J = 6.0 Hz, 2H), 5.50 (t, J = 6.0 Hz, 1H), 7.07 (d, J = 9.0 Hz, 1H), 7.83 (s, 1H), 8.41 (d, J = 9.0 Hz, 1H), 10.59 (s, 1H) ; ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.61, 176.91, 165.58, 151.79, 150.86, 139.99, 133.37, 117.95, 117.44, 115.39, 113.26, 110.52, 66.58, 25.76, 18.36, 10.95; MS–ESIMS: *m/z* 273 $[M+H]^+$; HRMS calcd for $C_{16}H_{17}O_4$, 273.1121; found, 273.1123.

1.2.3. 2-Methyl-7-[(3-methylbut-2-en-1-yl)oxy]-4-oxo-3-phenyl-4*H*-chromene-8-carbaldehyde 2c)



A mixture of alcohol (2.8 g, 10 mmol), prenyl bromide (1.76 ml, 15 mmol), potassium carbonate (2.76 g, 20 mmol) and

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acetone (80 mL) was heated under reflux for 6 hr. After completion of the reaction, K_2CO_3 was filtered and the resulting solution was concentrated in vacuo and the residue was purified by column chromatography over silica gel (100–200 mesh, with eluent: 34% EtOAc in hexane) to give 2-Methyl-7-[(3-methylbut-2-en-1-yl)oxy]-4-oxo-3-phenyl-4*H*-chromene-8-carbaldehyde **2c** (3.0 g, 89%).

White solid; mp: 182–185 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.80 (s, 3H), 1.83 (s, 3H), 2.38 (s, 3H), 4.77 (d, J = 6.7 Hz, 2H), 5.50 (t, J = 6.7 Hz, 1H), 7.02 (d, J = 9.0 Hz, 1H), 7.16–7.28 (m, 2H), 7.30–7.48 (m, 3H), 8.37 (d, J = 9.0 Hz, 1H), 10.60 (s, 1H) ;^{13}C NMR (75 MHz, $CDCl_3$): δ 187.29, 175.38, 165.27, 163.55, 155.90, 139.80, 133.69, 132.50, 130.31, 128.41, 127.91, 123.91, 118.16, 117.35, 112.89, 110.33, 66.55, 25.82, 19.45, 18.42; MS–ESIMS: *m/z* 371 $[M+Na]^+$; HRMS calcd for $C_{22}H_{20}O_4Na$, 371.1259, found, 371.1274.

1.3. General Procedure for Synthesis of *cis*-fused pyrano[3,4-*c*]pyrans (4a-o)

A mixture of 7-O-prenyl derivatives of 8- formyl-2,3-disubstituted chromenones **2a-c** (1.0 mmol) and 1,3 diketones **3a-h** (1.0 mmol) in acetonitrile (5 mL) was stirred in the presence EDDA (20 mol %) under reflux condition for the appropriate time. After completion of the reaction as indicated by TLC, the excess acetonitrile was distilled off and the residue was poured into water (20 mL) and extracted with DCM (3 X 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated in vacuo and the residue was purified by column chromatography over silica gel (100–200 mesh) with eluent hexane–ethyl acetate to afford the corresponding pure chromeno *cis*-fused pyrano[3,4-*c*]pyrans (**4a-o)** as solids in (79.9–95.0 %) yields.

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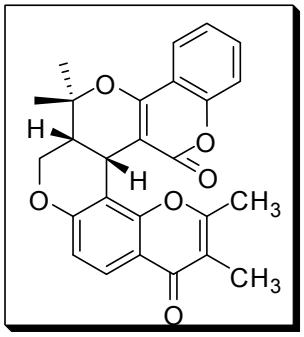
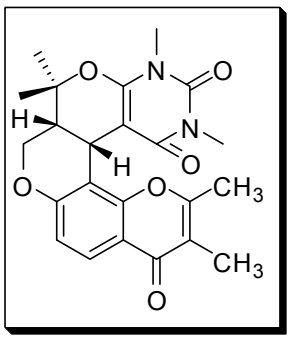
1.3.1. Analytical data for compounds

(1*S*,14*R*)-5,6,15,15-Tetramethyl-4,12,16,24-tetraoxahexacyclo[12.12.0.0^{2,11}.0^{3,8}.0^{17,26}.0^{18,23}]hexacosa-2,5,8,10,17(26),18(23),19,21-octaene-7,25-dione (4a)



Yield 80 %; White solid; mp 220–223 ^{o}C; IR (KBr): ν_{max} 3557, 3450, 2923, 1712, 1608, 1437, 1370, 1326, 1294, 1259, 1192, 1131, 1089, 1021, 832, 756, 604, 455 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.63 (s, 3H), 1.64 (s, 3H), 2.06 (s, 3H), 2.29–2.36 (m, 1H), 2.41 (s, 3H), 4.07 (t, J = 11.7 Hz, 1H), 4.53 (ddd, J = 11.7, 5.3 , 2.1 Hz, 1H), 4.63 (d, J = 4.2 Hz, 1H), 6.77 (d, J = 8.5 Hz, 1H), 7.14–7.30 (m, 2H), 7.50 (td, J = 7.5, 1.1 Hz, 1H), 7.80 (dd, J = 7.5, 1.1 Hz, 1H), 8.02 (d, J = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.92, 160.84, 160.24, 158.29, 156.74, 156.56, 152.65, 131.94, 125.88, 123.56, 122.99, 116.25, 116.15, 115.32, 114.03, 108.49, 100.18, 78.34, 63.35, 36.80, 29.67, 27.10, 25.62, 24.24, 18.41, 10.00; MS–ESIMS: *m/z* 431 [M+H]$^{+}$; HRMS calcd for $C_{26}H_{23}O_6$, 431.1494; found, 431.1498.

(1*S*,14*R*)-5,6,15,15,18,20-Hexamethyl-4,12,16-trioxa-18,20-diazapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,19,21-trione (4b)



Yield 92 %; White solid; mp 169–174 ^{o}C; IR (KBr): ν_{max} 3449, 2924, 1707, 1664, 1611, 1439, 1365, 1295, 1258, 1189, 1128, 1079, 1024, 825, 788, 742, 652, 604, 470 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.57 (s, 3H), 1.61 (s, 3H), 2.05 (s, 3H), 2.15–2.23 (m, 1H), 2.39 (s, 3H), 3.16 (s, 3H), 3.36 (s, 3H), 4.06 (t, J = 11.2 Hz, 1H), 4.48 (ddd, J = 11.0, 4.9 , 1.1 Hz, 1H), 4.53 (d, J = 3.3 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.91, 161.27, 160.77, 156.45, 156.40, 154.40, 150.84,

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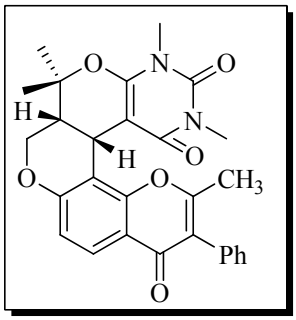
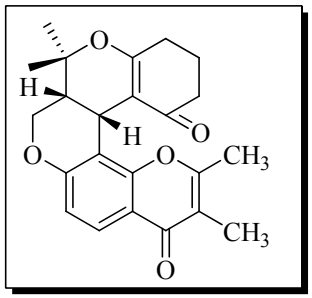
125.67, 116.27, 115.96, 114.05, 109.32, 86.48, 80.50, 62.98, 36.83, 28.87, 28.05, 27.01, 25.53, 23.18, 18.51, 10.10; MS–ESIMS: *m/z* 425 $[M+H]^+$; HRMS calcd for $C_{23}H_{25}N_2O_6$, 425.1712; found, 425.1715.

(1*S*,14*R*)-5,15,15,18,20-Pentamethyl-6-phenyl-4,12,16-trioxa-18,20-diazapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,19,21-trione (4c)



Yield 91 %; White solid; mp 182–185 ^oC; IR (KBr): ν_{max} 3515, 2924, 1702, 1608, 1485, 1439, 1397, 1253, 1135, 1084, 1021, 797, 754, 697, 192, 417 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.58 (s, 3H), 1.62 (s, 3H), 2.22 (dt, *J* = 11.3, 4.5 Hz, 1H), 2.31 (s, 3H), 3.20 (s, 3H), 3.37 (s, 3H), 4.11 (t, *J* = 11.3 Hz, 1H), 4.50 (ddd, *J* = 11.3, 4.5, 1.5 Hz, 1H), 4.57 (dd, *J* = 4.5, 1.5 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 7.30–7.49 (m, 5H), 8.05 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.70, 162.24, 161.38, 156.80, 156.44, 154.47, 150.87, 133.51, 130.64, 128.15, 127.44, 126.11, 122.93, 116.86, 114.35, 109.65, 86.47, 80.59, 63.11, 36.99, 28.89, 28.06, 27.05, 25.54, 23.37, 19.49; MS–ESIMS: *m/z* 487 $[M+H]^+$; HRMS calcd for $C_{28}H_{27}N_2O_6$, 487.1869; found, 487.1865.

(1*S*,14*R*)-5,6,15,15-Tetramethyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4d)



Yield 95 %; Pale yellow solid; mp 223–228 ^oC; IR (KBr): ν_{max} 3443, 2923, 2854, 2297, 1658, 1608, 1582, 1434, 1364, 1325, 1258, 1234, 1191, 1131, 1083, 1023, 989, 936, 834, 788, 755, 638, 609, 537, 442, 419 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 3H), 1.49 (s, 3H), 1.84–1.96 (m, 2H), 2.02-2.13 (m, 4H), 2.21–2.29 (m, 2H), 2.36–2.46 (m, 5H), 4.05 (t, *J* = 11.0 Hz, 1H), 4.30–4.35 (brd, *J* = 2.9 Hz, 1H), 4.42 (dd, *J* =

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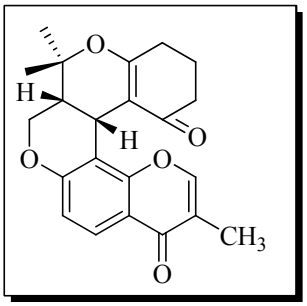
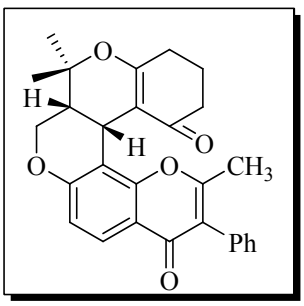
11.0, 5.1 Hz, 1H), 6.74 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.25, 177.91, 169.56, 160.73, 156.45, 156.30, 124.96, 115.92, 115.83, 113.90, 110.75, 110.03, 77.11, 63.54, 37.91, 36.95, 29.65, 26.82, 25.55, 23.13, 20.28, 18.43, 9.94; MS–ESIMS: *m/z* 381 $[M+H]^+$; HRMS calcd for $C_{23}H_{25}O_5$, 381.1701; found, 381.1698.

(1*S*,14*R*)-6,15,15-Trimethyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4e)



Yield 93 %; Pale yellow solid; mp 212–215 ^{o}C; IR (KBr): ν_{max} 3421, 2926, 2858, 1649, 1590, 1432, 1369, 1327, 1288, 1243, 1188, 1065, 1027, 996, 949, 833, 781, 723, 645, 530, 419 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 3H), 1.48 (s, 3H), 1.83–1.95 (m, 2H), 2.02 (s, 3H), 2.04–2.12 (m, 1H), 2.23–2.29 (m, 2H), 2.37–2.45 (m, 2H), 4.04 (t, *J* = 11.6 Hz, 1H), 4.28–4.36 (brd, *J* = 2.1 Hz, 1H), 4.43 (ddd, *J* = 11.6, 5.2, 2.1 Hz, 1H), 6.78 (d, *J* = 9.5 Hz, 1H), 7.81 (s, 1H), 8.01 (d, *J* = 9.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.69, 178.24, 169.77, 157.30, 156.73, 150.85, 125.02, 120.07, 116.89, 114.42, 111.19, 109.93, 77.15, 63.59, 38.00, 36.90, 29.73, 26.82, 25.64, 23.21, 20.37, 11.22; MS–ESIMS: *m/z* 367 $[M+H]^+$; HRMS calcd for $C_{22}H_{23}O_5$, 367.1545; found, 367.1547.

(1*S*,14*R*)-5,15,15-Trimethyl-6-phenyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4f)



Yield 92 %; Pale yellow solid; mp 230–233 ^{o}C; IR (KBr): ν_{max} 3420, 2924, 2854, 2226, 1626, 1588, 1461, 1433, 1371, 1294, 1253, 1175, 1136, 1081, 1020, 921, 786, 727, 641, 485 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 3H), 1.50 (s, 3H), 1.85–1.97 (m, 2H), 2.07–2.12 (m, 1H), 2.27–2.30 (m, 2H), 2.31 (s, 3H), 2.41–2.45 (m, 2H), 4.08 (t,

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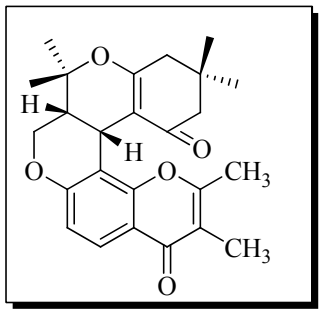
J = 11.2 Hz, 1H), 4.35–4.38 (brd, *J* = 2.2 Hz, 1H), 4.44 (ddd, *J* = 11.2, 5.2, 1.1 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 7.31–7.35 (m, 3H), 7.39–7.42 (m, 2H), 8.01 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.40, 176.78, 169.72, 162.25, 156.89, 156.28, 133.61, 130.62, 128.13, 127.36, 125.51, 122.79, 116.71, 114.29, 111.07, 110.09, 63.68, 37.98, 37.11, 29.71, 29.66, 26.91, 25.63, 23.28, 20.36, 19.50; MS–ESIMS: *m/z* 443 $[M+H]^+$; HRMS calcd for $C_{28}H_{26}O_5Na$, 465.1677; found, 465.1659.

(1*S*,14*R*)-5,6,15,15,19,19-Hexamethyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4g)



Yield 91 %; Pale yellow solid; mp 231–235 ^{o}C; IR (KBr): ν_{max} 3435, 2957, 2925, 1685, 1642, 1599, 1437, 1407, 1367, 1322, 1293, 1260, 1219, 1192, 1136, 1088, 1032, 941, 911, 829, 780, 720, 659, 528, 476, 423 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (s, 3H), 1.06 (s, 3H), 1.43 (s, 3H), 1.48 (s, 3H), 1.99–2.13 (m, 6H), 2.23–2.30 (m, 2H), 2.39 (s, 3H), 3.98 (t, *J* = 11.3 Hz, 1H), 4.22–4.30 (brd, *J* = 3.0 Hz, 1H), 4.39 (ddd, *J* = 11.3, 4.5, 1.5 Hz, 1H), 6.71 (d, *J* = 9.0 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.97, 177.95, 167.86, 160.71, 156.43, 125.09, 116.02, 115.93, 114.00, 110.83, 108.88, 77.42, 63.70, 51.70, 43.42, 37.06, 31.41, 29.65, 28.94, 27.21, 26.80, 25.64, 23.10, 18.48, 9.99; MS–ESIMS: *m/z* 409 $[M+H]^+$; HRMS calcd for $C_{25}H_{29}O_5$, 409.2014; found, 409.2026.

(1*S*,14*R*)-6,15,15,19,19-Pentamethyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4h)

Yield 91 %; White solid; mp 212–215 ^{o}C; IR (KBr): ν_{max} 3337, 2982, 2856, 1730, 1653, 1288, 1261, 1132, 1075, 1053, 1008 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.98 (s, 3H), 1.05 (s, 3H),

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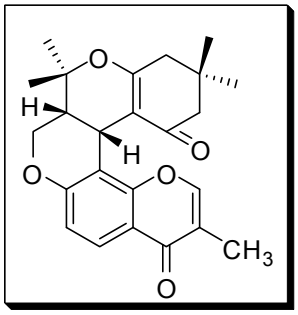
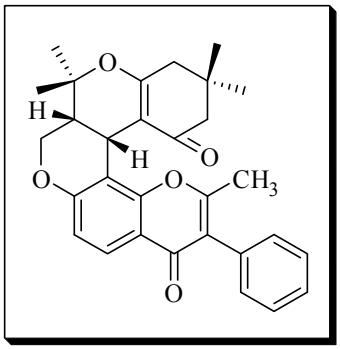


1.44 (s, 3H), 1.48 (s, 3H), 2.02 (s, 3H), 2.05–2.11 (m, 2H), 2.17 (d, *J* = 15.9 Hz, 1H), 2.28 (q, *J* = 17.3 Hz, 2H), 4.01 (t, *J* = 11.2 Hz, 1H), 4.30–4.33 (brd, *J* = 1.9 Hz, 1H), 4.43 (ddd, *J* = 11.0, 5.2, 1.3 Hz, 1H), 6.78 (d, *J* = 9.0 Hz, 1H), 7.82 (s, 1H), 8.01 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 196.41, 178.25, 168.10, 157.29, 156.69, 150.89, 125.03, 120.02, 116.89, 114.45, 111.19, 108.75, 63.71, 51.68, 43.38, 36.87, 31.48, 28.84, 27.35, 26.76, 25.63, 23.09, 11.23.; MS–ESIMS: *m/z* 395 $[M+H]^+$; HRMS calcd for $C_{24}H_{27}O_5$, 395.1858; found, 395.1840.

(1*S*,14*R*)-5,15,15,19,19-Pentamethyl-6-phenyl-4,12,16-trioxapentacyclo[12.8.0.0^{2,11}.0^{3,8}.0^{17,22}]docosa-2,5,8,10,17(22)-pentaene-7,21-dione (4i)



Yield 87 %; Pale brown solid; mp 232–236 ^{o}C; IR (KBr): ν_{max} 3420, 3057, 2957, 2923, 1665, 1634, 1435, 1395, 1364, 1297, 1249, 1214, 1138, 1021, 831, 779, 700, 655, 619, 547, 489 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.00 (s, 3H), 1.06 (s, 3H), 1.45 (s, 3H), 1.50 (s, 3H), 2.07–2.14 (m, 2H), 2.19 (d, *J* = 16.2 Hz, 1H), 2.23–2.37 (m, 5H), 4.04 (t, *J* = 11.0 Hz, 1H), 4.34–4.37 (brs, 1H), 4.44 (dd, *J* = 11.0, 5.2 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 3H), 7.41 (t, *J* = 7.4 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 196.15, 176.77, 168.05, 162.24, 156.80, 156.33, 133.63, 130.62, 128.13, 127.35, 125.52, 122.80, 116.70, 114.32, 111.10, 108.83, 63.77, 51.69, 43.39, 37.07, 31.47, 28.99, 27.19, 26.79, 25.66, 23.16, 19.51; MS–ESIMS: *m/z* 471 $[M+H]^+$; HRMS calcd for $C_{30}H_{31}O_5$, 471.2171; found, 471.2173.

(13*R*,18*S*)-4,5,14,14-Tetramethyl-3,11,15-trioxatetracyclo[8.8.0.0^{2,7}.0^{13,18}]octadeca-1,4,7,9-tetraene-6,16-dione (4j)

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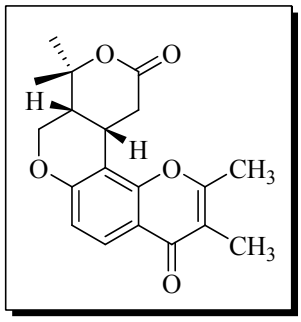
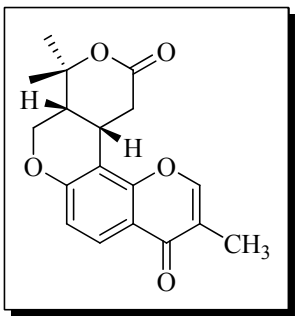


Yield 85%; Violet solid; mp 153–155 ^{o}C; IR (KBr): ν_{max} 3428, 2923, 1726, 1644, 1606, 1467, 1437, 1406, 1357, 1326, 1274, 1250, 1190, 1132, 1092, 1064, 1021, 956, 831, 784, 668 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 1.54 (s, 3H), 1.64 (s, 3H), 2.02 (s, 3H), 2.18–2.24 (m, 1H), 2.43 (s, 3H), 2.49 (dd, J = 18.7, 9.3 Hz, 1H), 3.23 (dd, J = 18.7, 8.5 Hz, 1H), 3.80–3.90 (m, 1H), 4.04 (t, J = 11.6 Hz, 1H), 4.54 (ddd, J = 11.6, 3.8, 1.5 Hz, 1H), 6.82 (d, J = 9.3 Hz, 1H), 7.96 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.59, 177.18, 169.09, 160.61, 156.80, 125.80, 116.98, 116.50, 114.87, 111.56, 81.15, 62.39, 36.82, 32.38, 29.28, 26.27, 24.01, 18.51, 9.95; MS–ESIMS: *m/z* 329 $[M+H]^+$; HRMS calcd for $C_{19}H_{21}O_5$, 329.13835; found, 329.13891

(13*R*,18*S*)-5,14,14-Trimethyl-3,11,15-trioxatetracyclo[8.8.0.0^{2,7}.0^{13,18}]octadeca-1,4,7,9-tetraene-6,16-dione (4k)



Yield 85%; Violet solid; mp 145–149 ^{o}C; IR (KBr): ν_{max} 3310, 3066, 2926, 1729, 1638, 1592, 1437, 1335, 1243, 1178, 1089, 1026, 952, 890, 785, 742, 616 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 1.48 (s, 3H), 1.60 (s, 3H), 1.94 (s, 3H), 2.26–2.38 (m, 1H), 2.46–2.57 (m, 1H), 3.18 (ddd, J = 18.8, 8.0, 0.8 Hz, 1H), 3.82–3.94 (m, 1H), 4.11 (t, J = 11.6 Hz, 1H), 4.58 (dd, J = 11.6, 2.6 Hz, 1H), 6.88 (d, J = 8.9 Hz, 1H), 7.86 (d, J = 8.9 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.63, 169.01, 157.11, 155.37, 150.78, 125.81, 121.02, 117.50, 115.37, 111.98, 81.16, 62.47, 36.72, 32.31, 29.27, 26.30, 23.95, 11.14; MS–ESIMS: *m/z* 315 $[M+H]^+$; HRMS calcd for $C_{18}H_{19}O_5$, 315.12270; found, 315.12349

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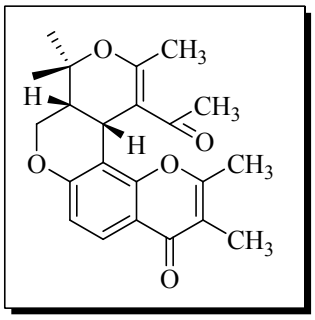
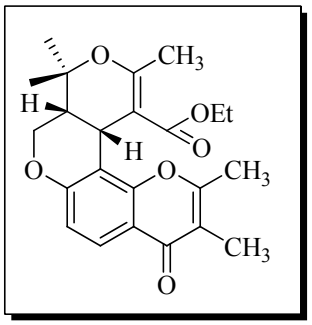
(13*R*,18*S*)-17-Acetyl-4,5,14,14,16-pentamethyl-3,11,15-trioxatetracyclo[8.8.0.0^{2,7}.0^{13,18}]octadeca-1,4,7,9,16-pentaen-6-one (4l)

Yield 85 %; White solid; mp 150–154 ^{o}C; IR (KBr): ν_{max} 3431, 2977, 2921, 2361, 1694, 1662, 1635, 1618, 1598, 1430, 1341, 1258, 1230, 1177, 1088, 1041, 979, 918, 878, 833, 786, 742, 794, 585, 502, 451 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (s, 3H), 1.81 (s, 3H), 2.13 (s, 3H), 2.15 (s, 3H), 2.36 (s, 3H); 2.42 (s, 3H), 4.69 (d, J = 6.42 Hz, 2H), 5.41(t, J = 7.3 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 7.68 (s, 1H), 8.17 (d, J = 8.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.97, 195.83, 176.48, 161.21, 159.73, 154.16, 143.78, 138.79, 131.91, 129.36, 118.77, 116.94, 116.80, 111.17, 109.67, 66.20, 30.10, 27.75, 25.82, 18.38, 18.13, 9.92; MS–ESIMS: *m/z* 391 [M+Na]$^{+}$; HRMS calcd for $C_{22}H_{24}O_5Na$, 391.1521; found, 391.1513.



Ethyl(13*R*,18*S*)-4,5,14,14,16-pentamethyl-6-oxo-3,11,15-trioxatetracyclo[8.8.0.0^{2,7}.0^{13,18}]octadeca-1,4,7,9,16-pentaene-17-carboxylate (4m)



White solid; mp 156–159 ^{o}C; IR (KBr): ν_{max} 3427, 2985, 2927, 2361, 1720, 1641, 1623, 1605, 1583, 1434, 1402, 1358, 1315, 1283, 1242, 1189, 1124, 1066, 1029, 1000, 855, 825, 675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, J = 7.17 Hz, 3H), 1.42 (s, 3H), 1.48 (s, 3H), 1.84 (s, 3H), 1.98–1.99 (m, 1H), 2.01 (s, 3H), 2.38 (s, 3H), 3.45–3.59 (m, 1H), 3.75–3.87 (m, 1H), 3.97 (t, J = 11.1 Hz, 1H), 4.15–4.20 (brs, 1H), 4.40 (dt, J = 10.7, 1.5 Hz, 1H), 6.79 (dd, J = 8.8, 1.7 Hz, 1H), 7.93 (dd, J = 8.8, 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.42, 175.06, 169.47, 160.33, 157.37, 155.78, 151.78, 125.45, 116.53, 115.88, 114.68, 110.06, 103.82, 74.81, 63.53, 60.35, 36.09, 26.56, 26.09, 19.16, 18.13, 13.69, 10.09;

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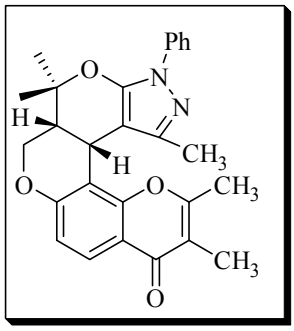
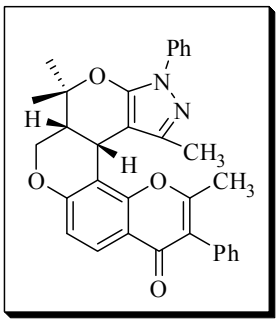
MS–ESIMS: *m/z* 399 $[M+H]^+$, 421 $[M+Na]^+$; HRMS calcd for $C_{23}H_{26}O_6Na$, 421.1627; found, 421.1620.

(2*S*,10*R*)-4,9,9,18,19-Pentamethyl-6-phenyl-8,12,20-trioxa-5,6-diazapentacyclo[11.8.0.0^{2,10}.0^{3,7}.0^{16,21}]henicosa-1(21),3(7),4,13,15,18-hexaen-17-one (4n)



Yield 90 %; White solid; mp 240–243 ^oC; IR (KBr): ν_{max} 3686, 3020, 2400, 2362, 1731, 1643, 1623, 1606, 1519, 1476, 1433, 1374, 1260, 1130, 1087, 1026, 929, 848, 753, 669, 627 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.64 (s, 3H), 1.65 (s, 3H), 1.77 (s, 3H), 2.00–2.17 (m, 4H), 2.47 (s, 3H), 4.05 (t, *J* = 11.3 Hz, 1H), 4.49 (ddd, *J* = 11.3, 4.5, 1.5 Hz, 1H), 4.54 (dd, *J* = 4.5, 1.5 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.67 (d, *J* = 7.5 Hz, 2H), 8.01 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.47, 160.28, 157.51, 155.22, 147.65, 146.93, 138.47, 128.89, 125.83, 125.57, 120.52, 117.06, 115.90, 114.88, 108.59, 95.70, 80.24, 63.10, 36.83, 26.20, 25.85, 23.65, 18.48, 13.38, 10.08.: MS–ESIMS: *m/z* 443 $[M+H]^+$; HRMS calcd for $C_{27}H_{27}N_2O_4$, 443.1970; found, 443.1958.

(2*S*,10*R*)-4,9,9,19-Tetramethyl-6,18-diphenyl-8,12,20-trioxa-5,6-diazapentacyclo[11.8.0.0^{2,10}.0^{3,7}.0^{16,21}]henicosa-1(21),3(7),4,13,15,18-hexaen-17-one (4o)



Yield 92 %; Pale yellow solid; mp 250–253 ^oC; IR (KBr): ν_{max} 3014, 2969, 1739, 1628, 1435, 1368, 1214, 1092, 904,789 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.65 (s, 3H), 1.66 (s, 3H), 1.88 (s, 3H), 2.07–2.11 (m, 1H), 2.40 (s, 3H), 4.10 (t, *J* = 11.5 Hz, 1H), 4.51 (ddd, *J* = 11.5, 4.0, 1.5 Hz, 1H), 4.61 (d, *J* = 4.0 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.29 (d, *J* = 7.0 Hz, 2H), 7.32–7.45 (m, 5H), 7.68 (d, *J* = 8.0 Hz, 2H), 8.06 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.88, 161.50, 157.88, 155.27, 147.59, 146.65, 138.56, 132.98, 130.55, 128.92, 128.38, 127.87, 126.44, 125.63, 123.95,

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120.58, 116.80, 115.21, 108.79, 95.61, 80.13, 63.12, 37.03, 26.35, 26.01, 23.88, 19.61, 13.53; MS–ESIMS: *m/z* 505 $[M+H]^+$; HRMS calcd for $C_{32}H_{29}N_2O_4$, 505.2127; found, 505.2103.

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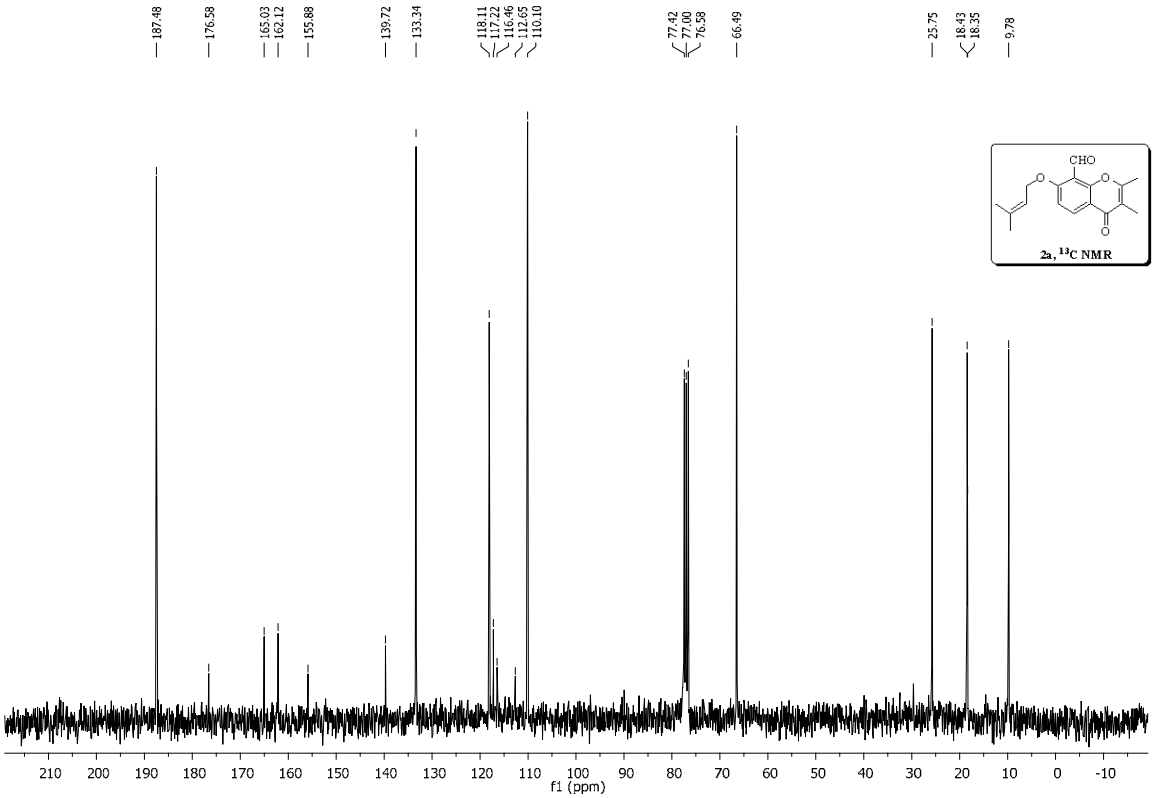

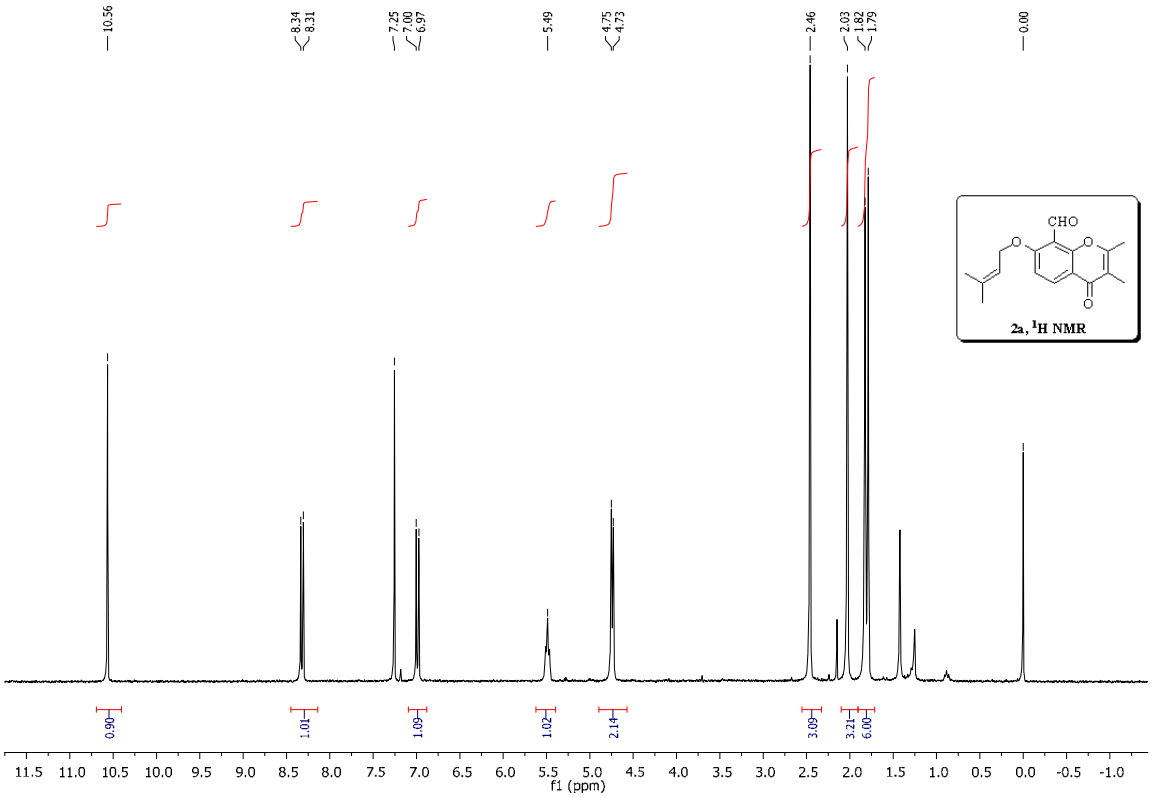

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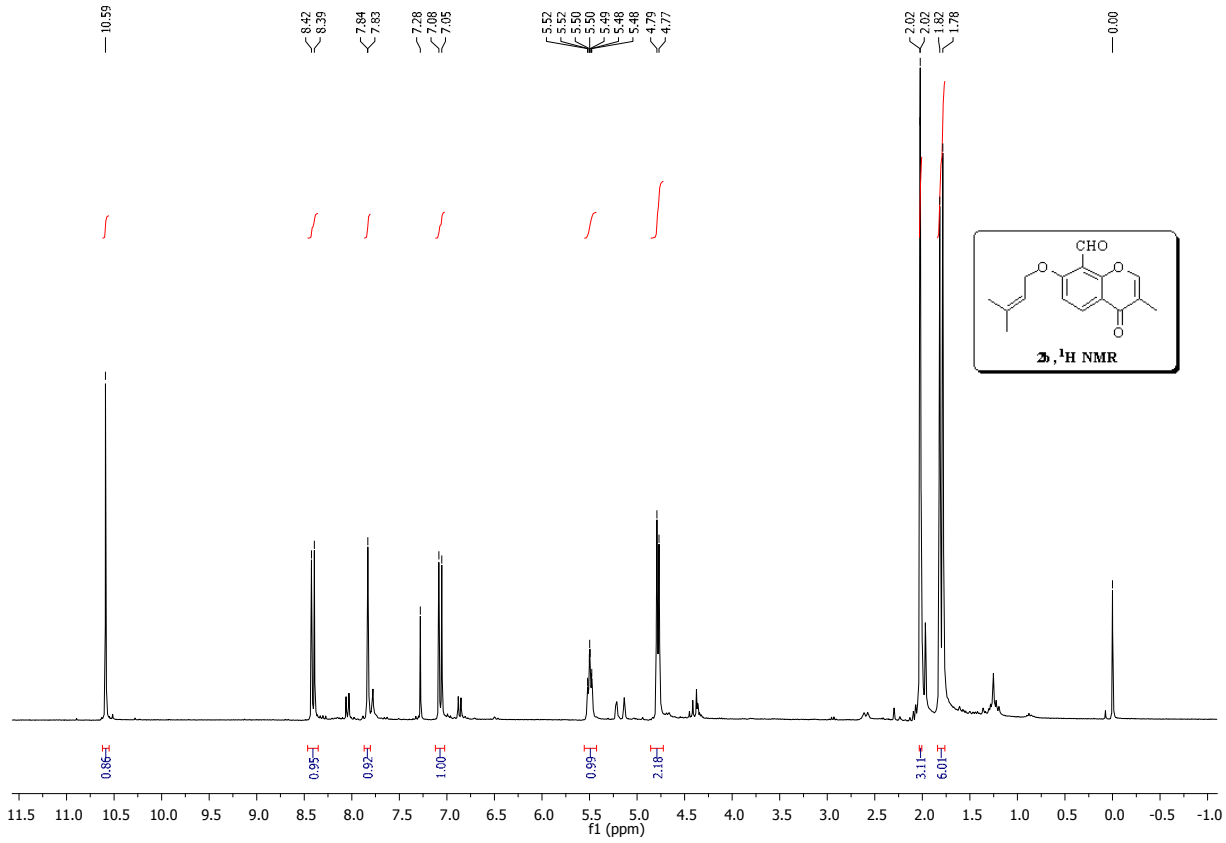
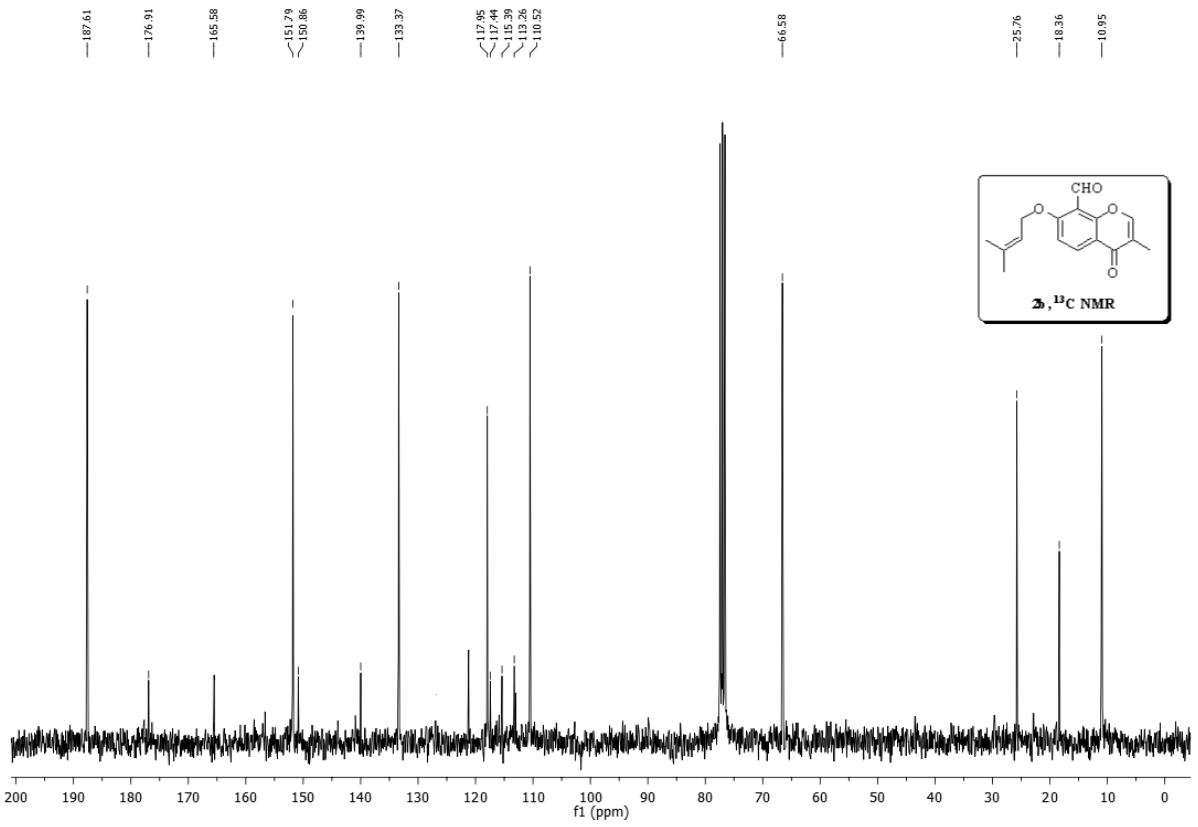


2b, ^{1}H NMR



2b, ^{13}C NMR

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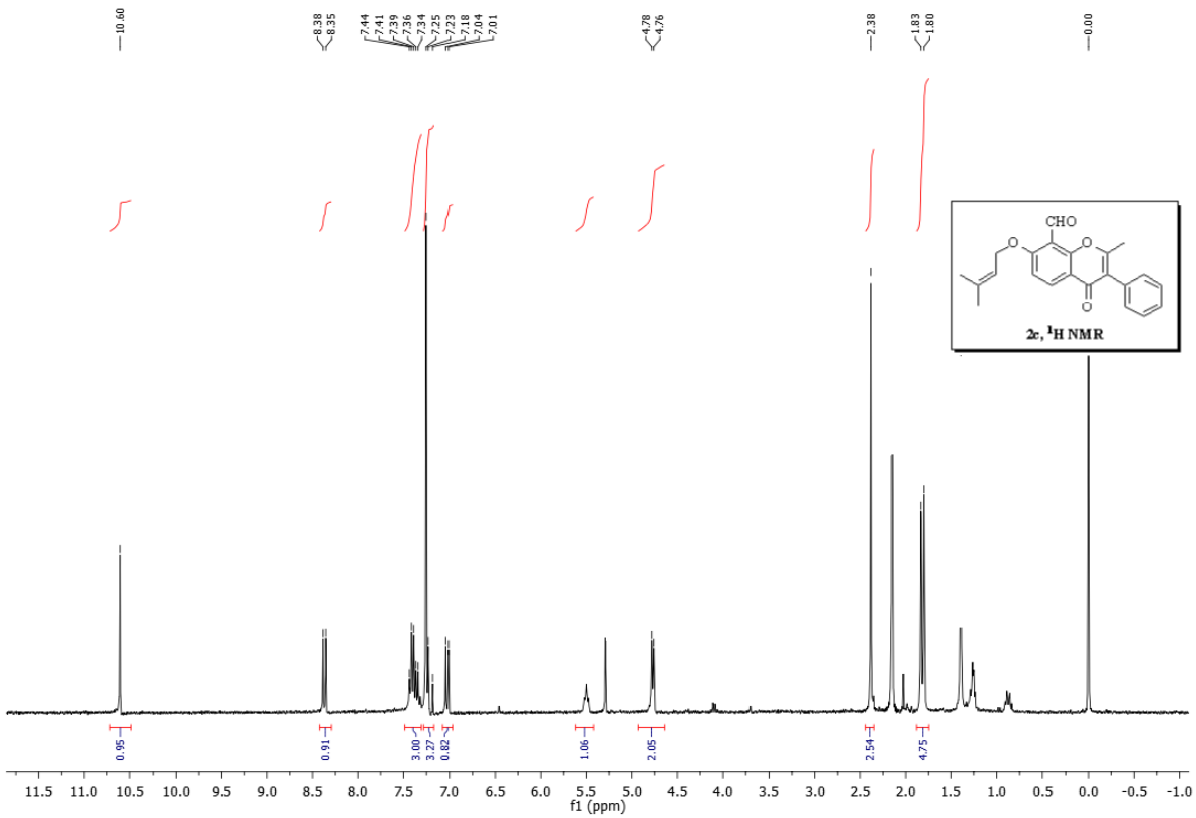

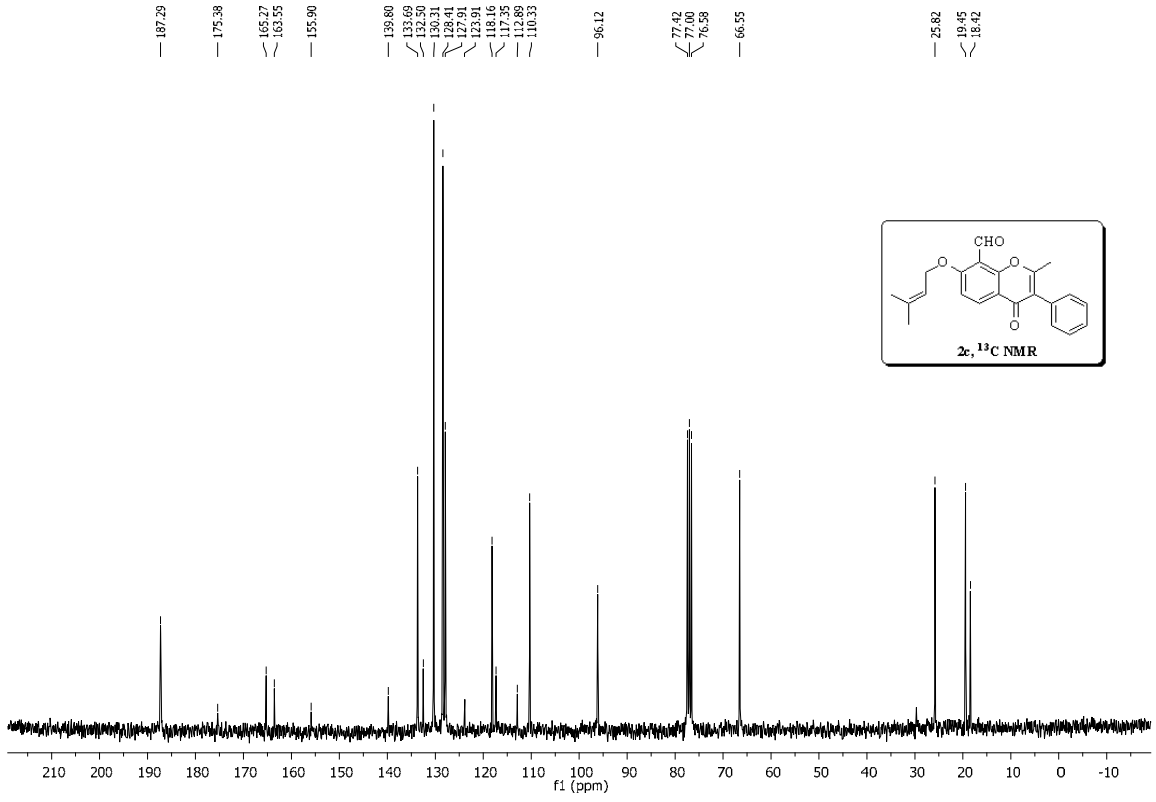

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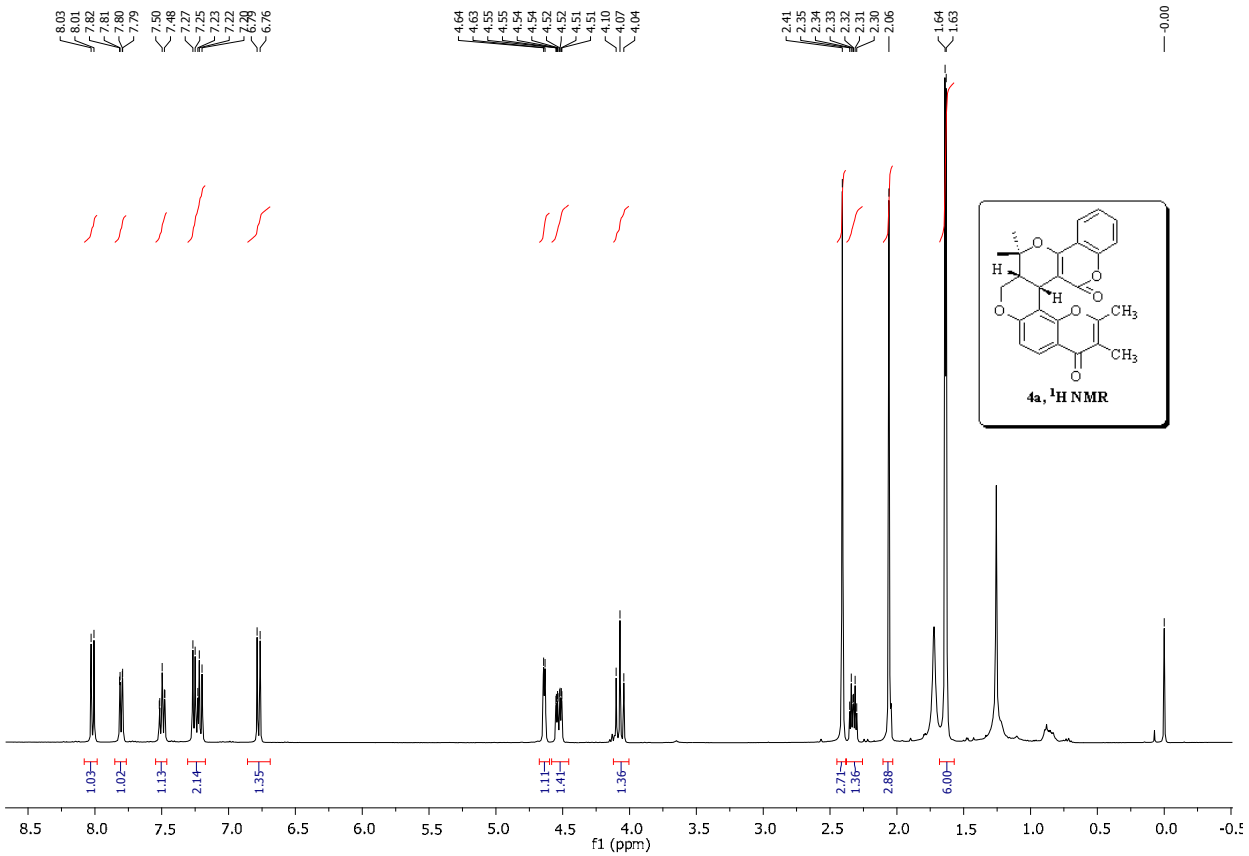

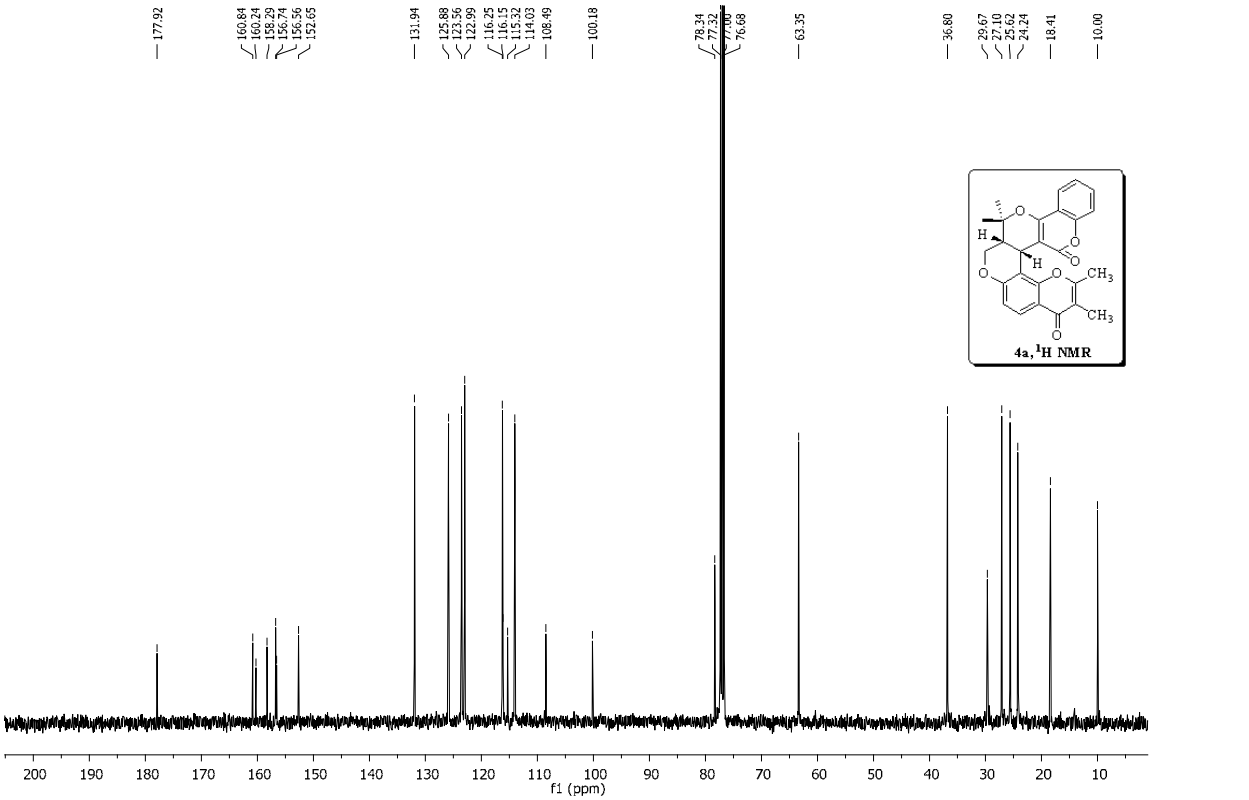

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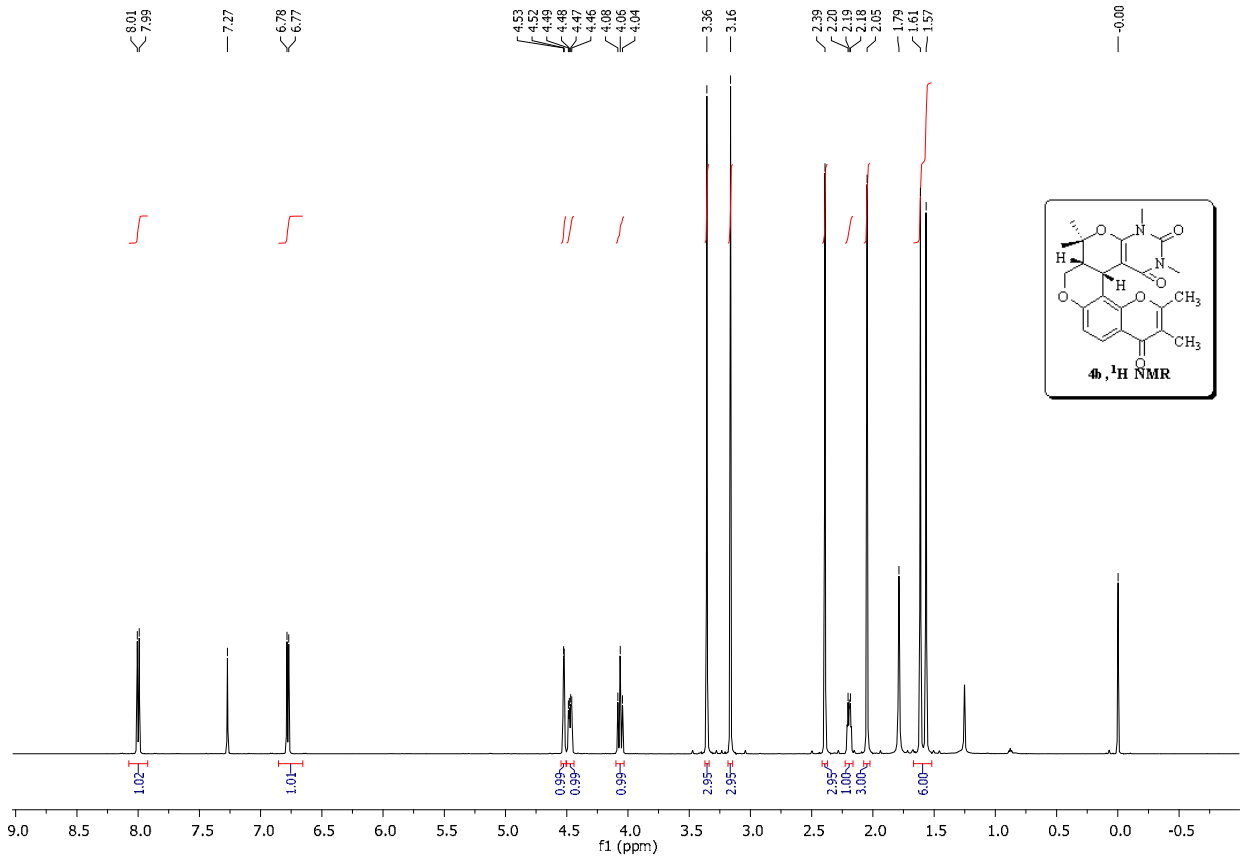

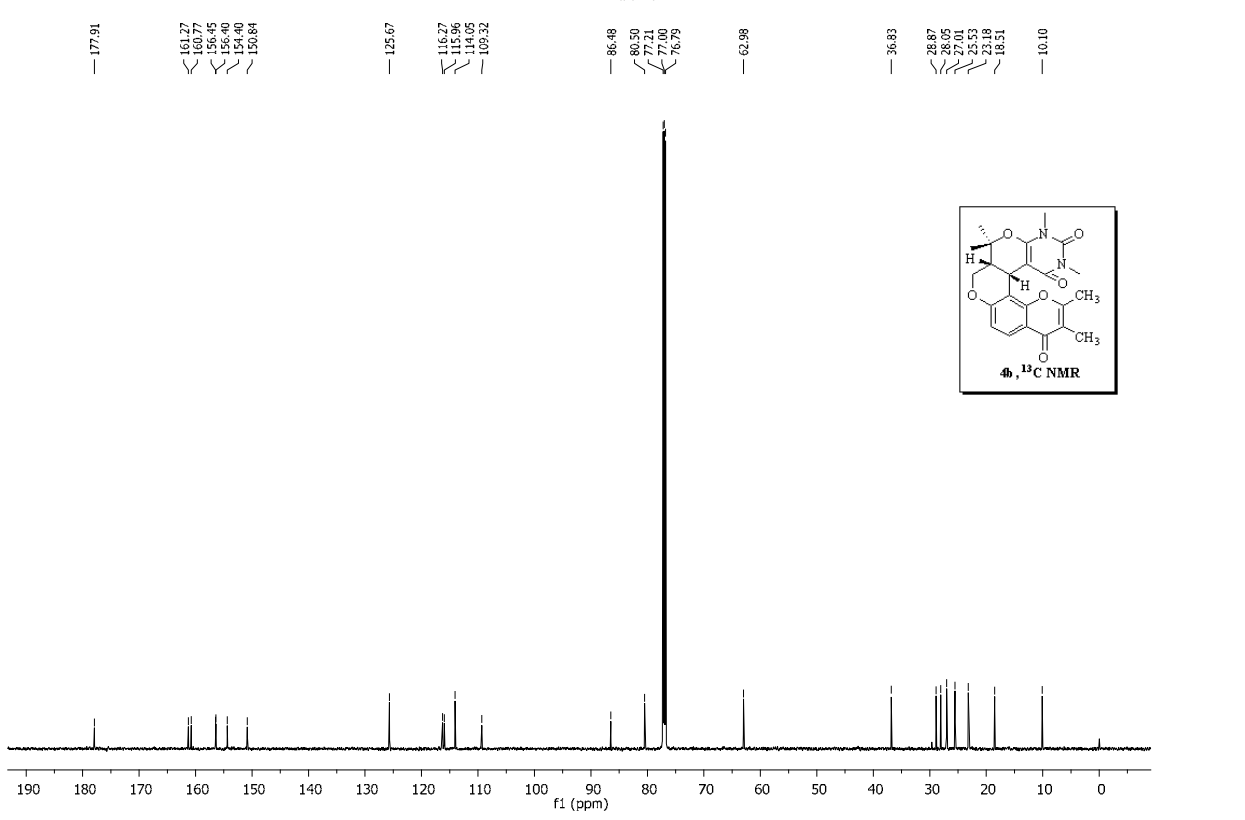

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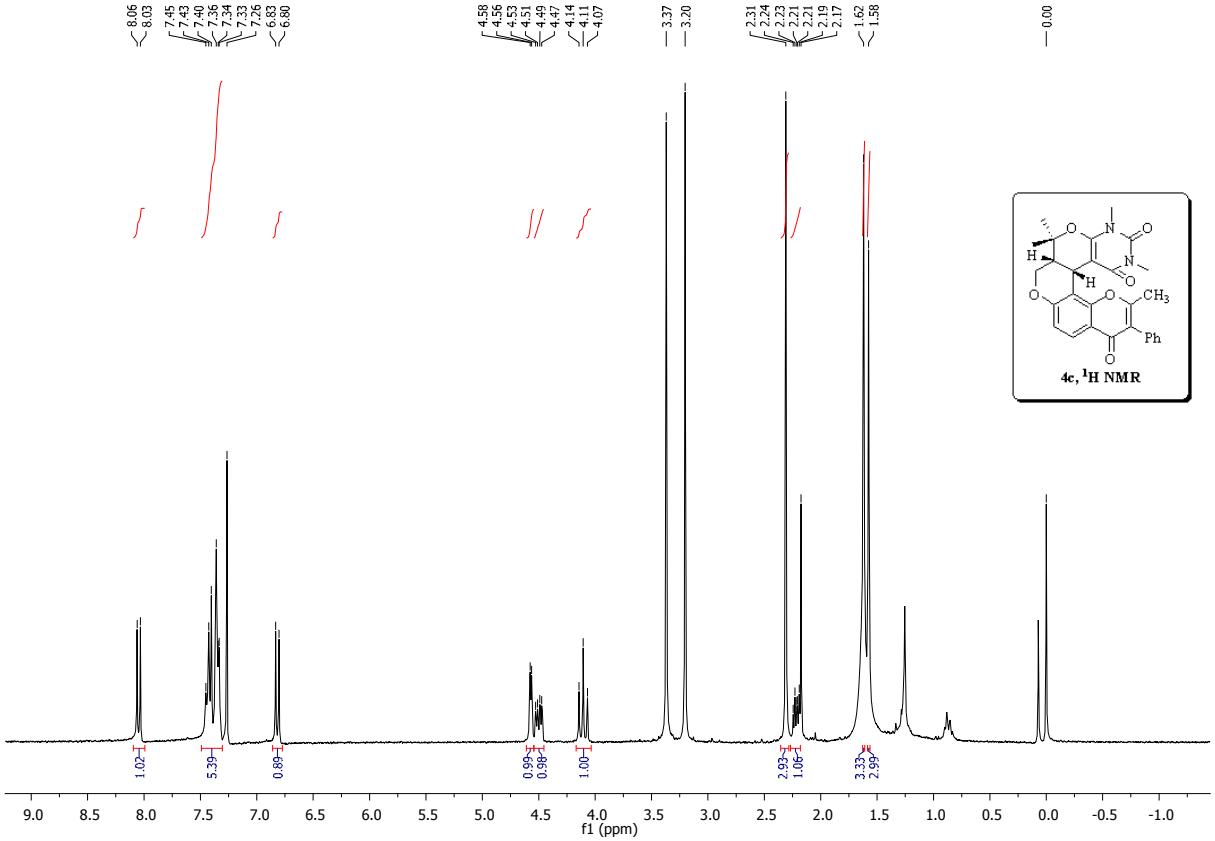

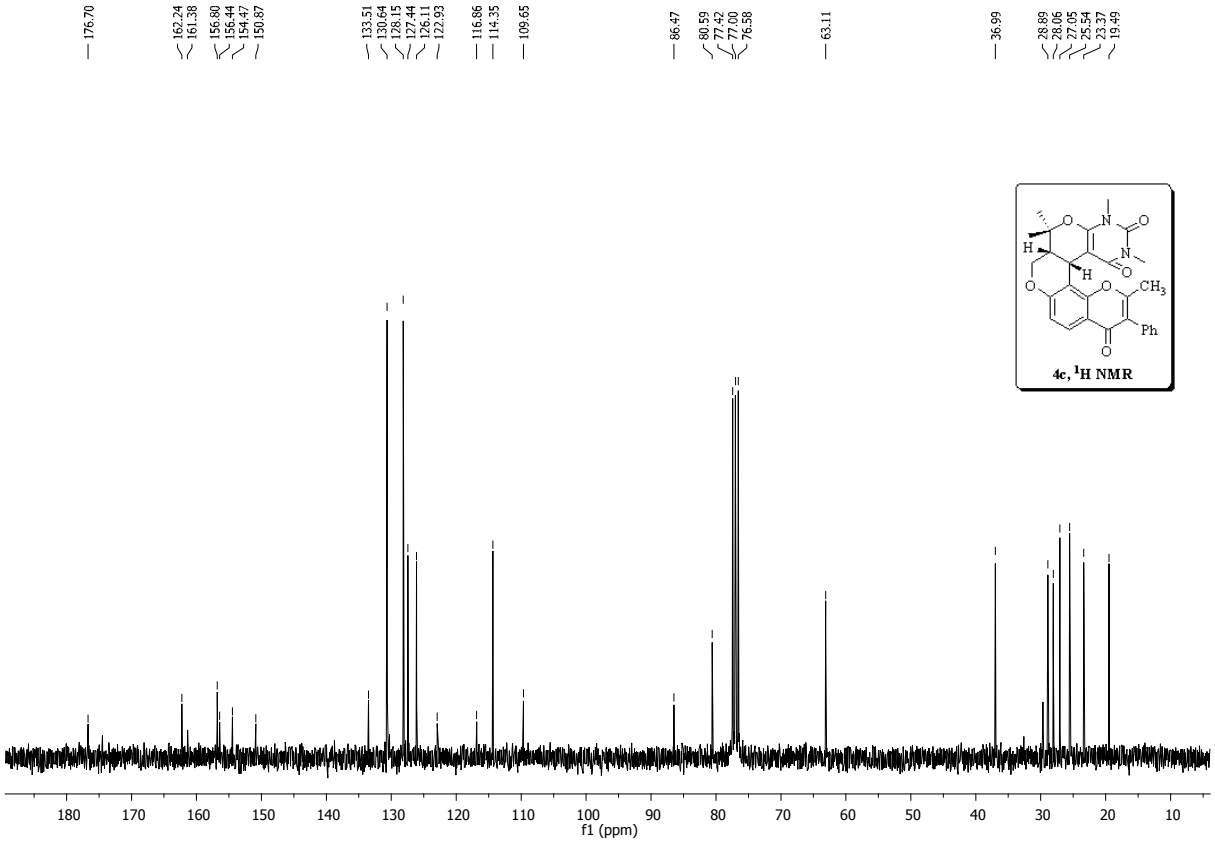

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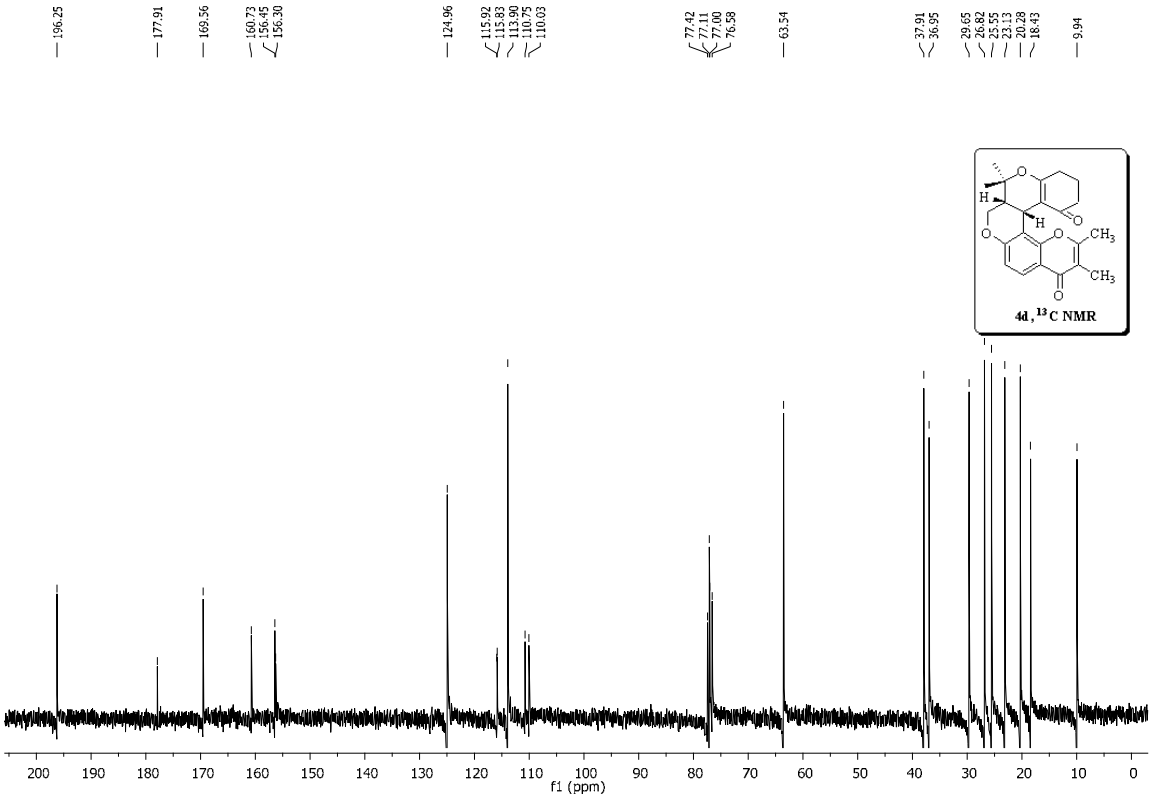

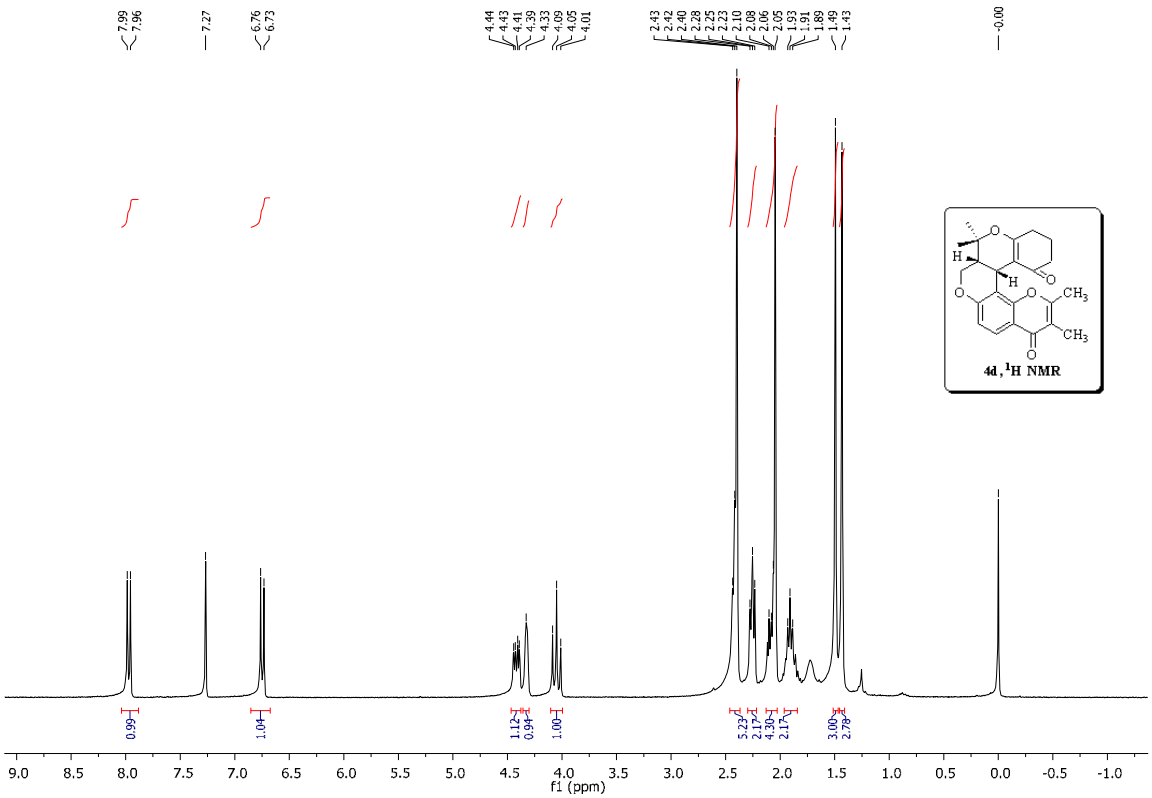

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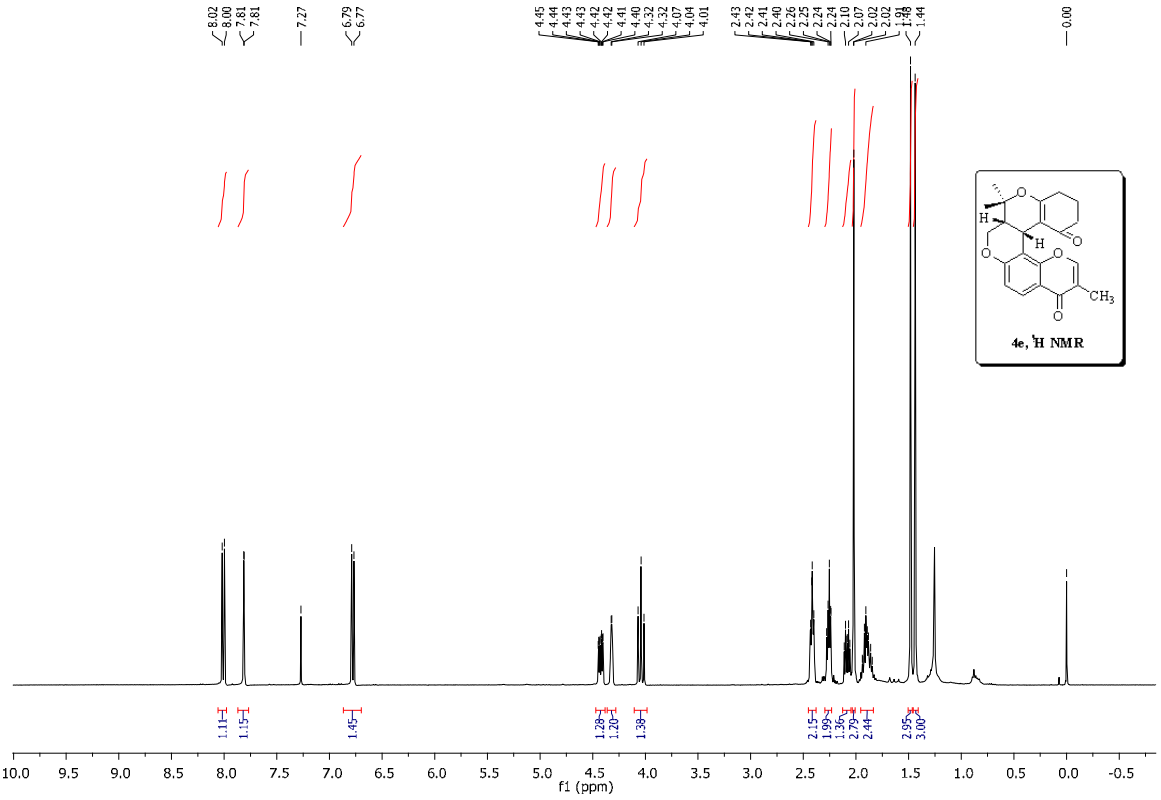

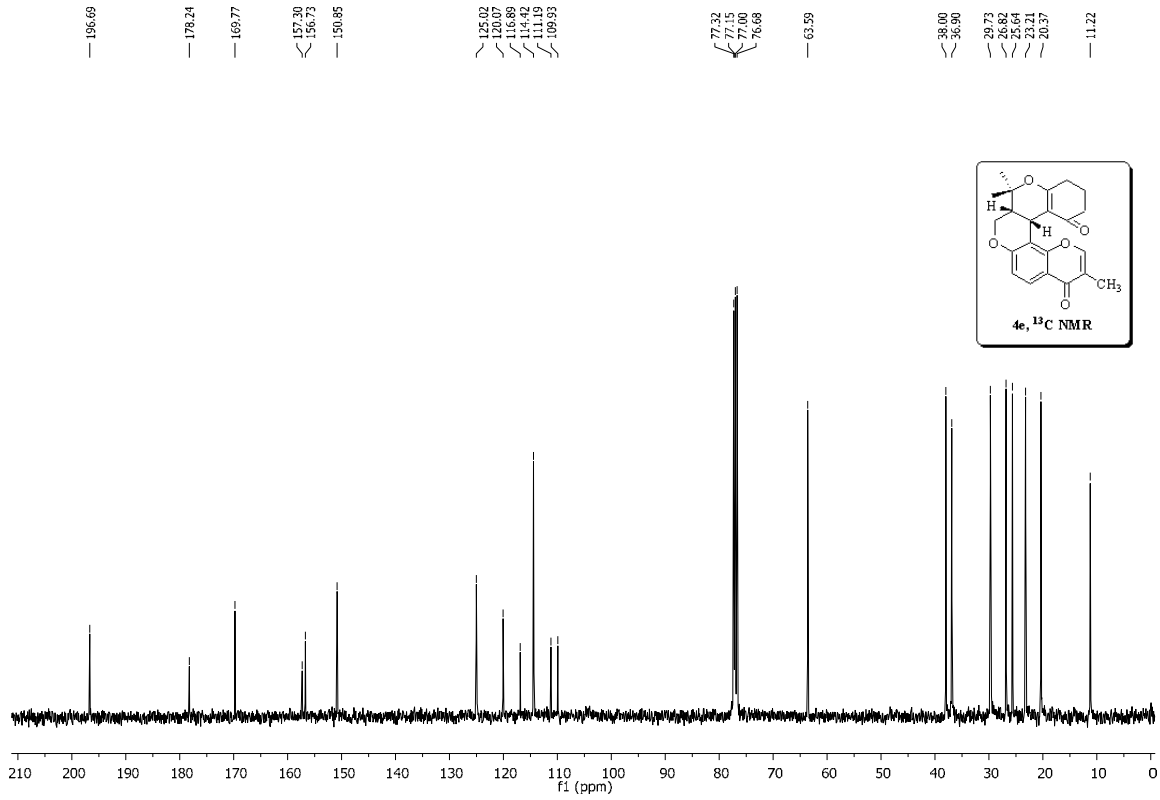

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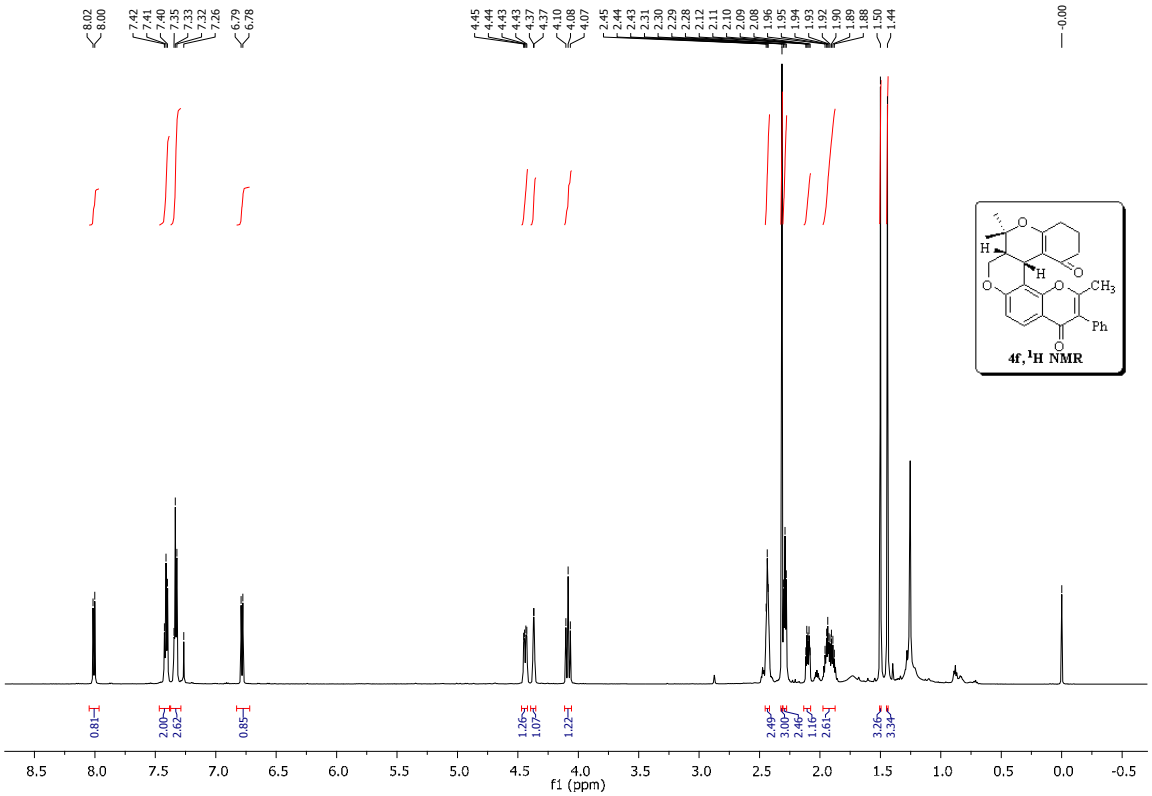

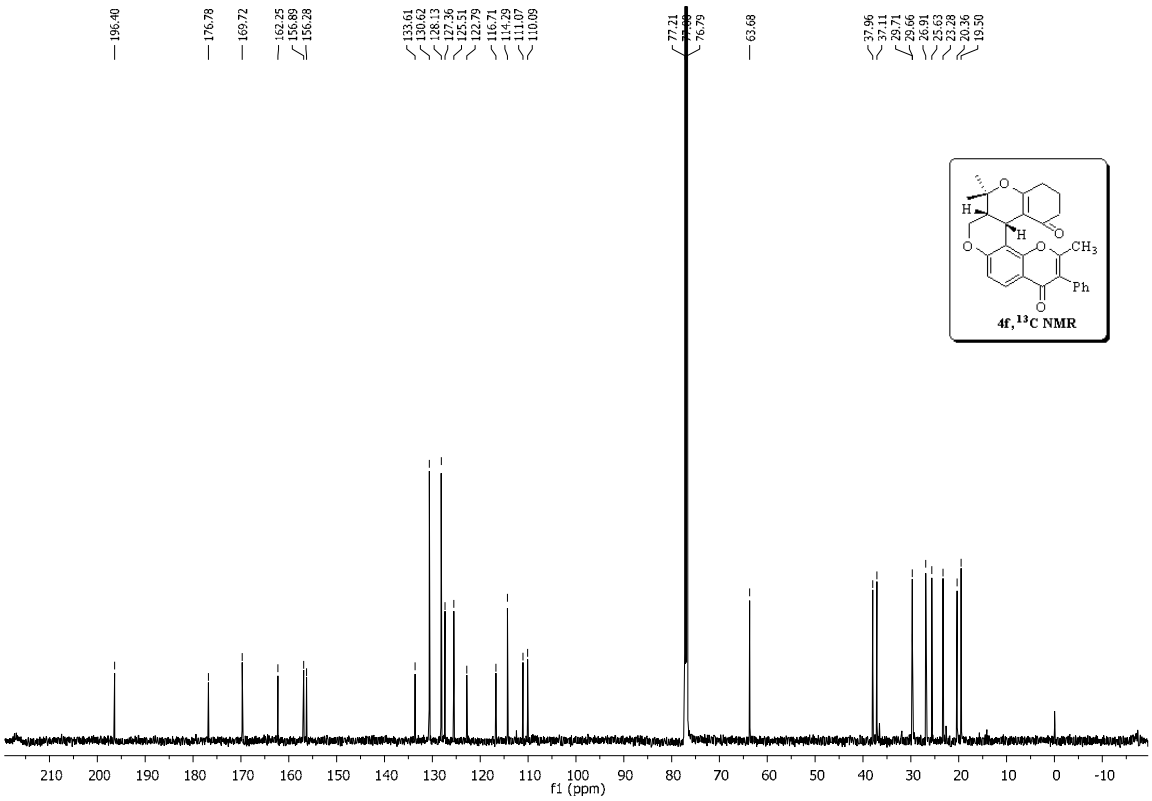

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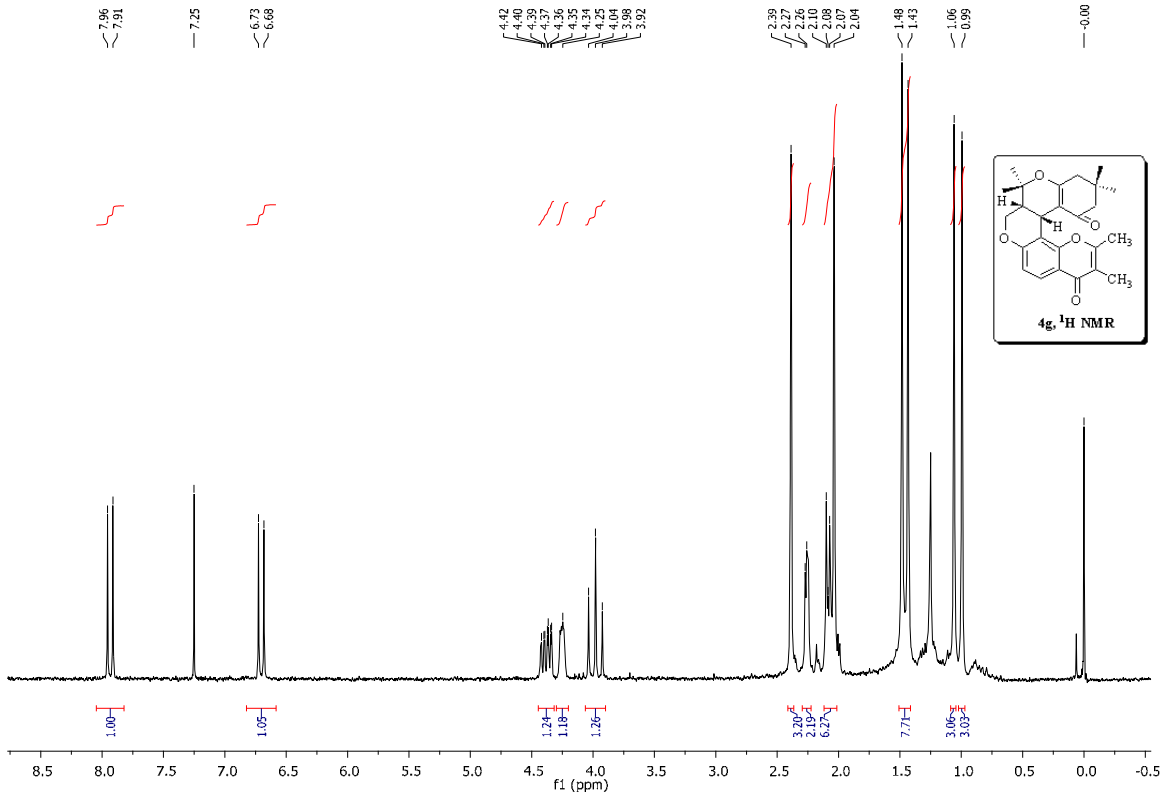

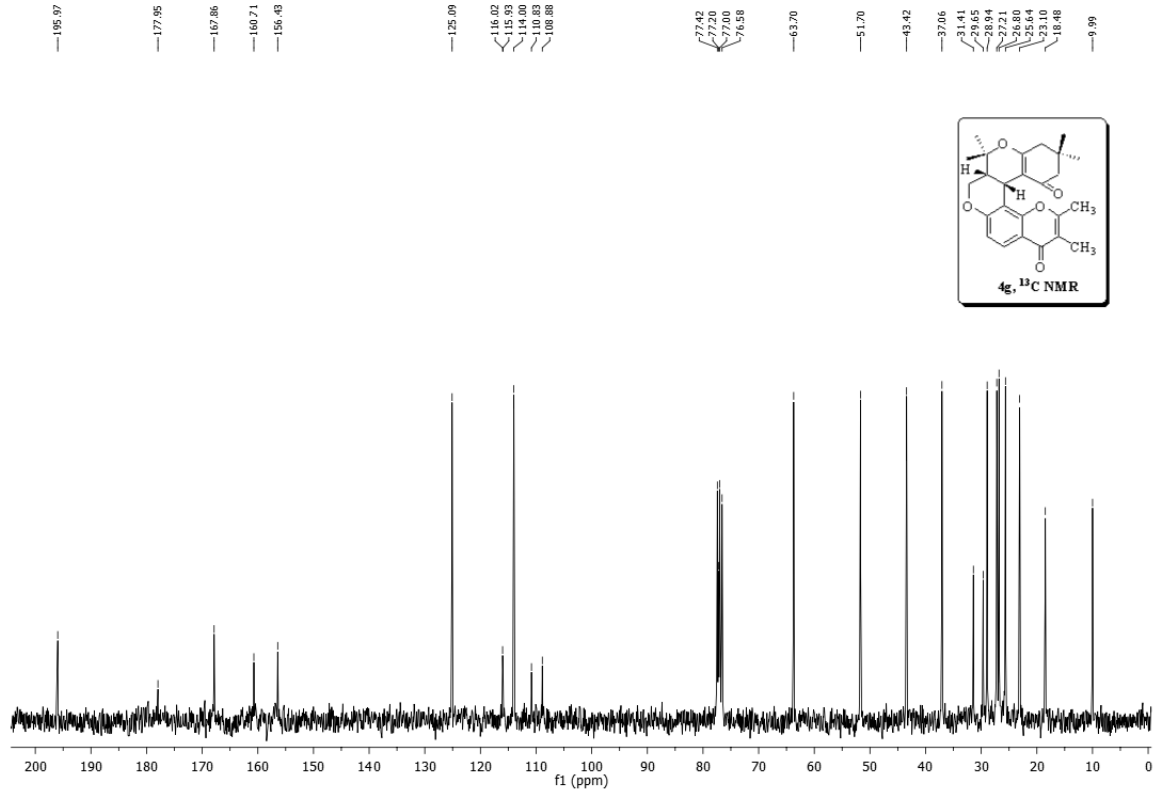

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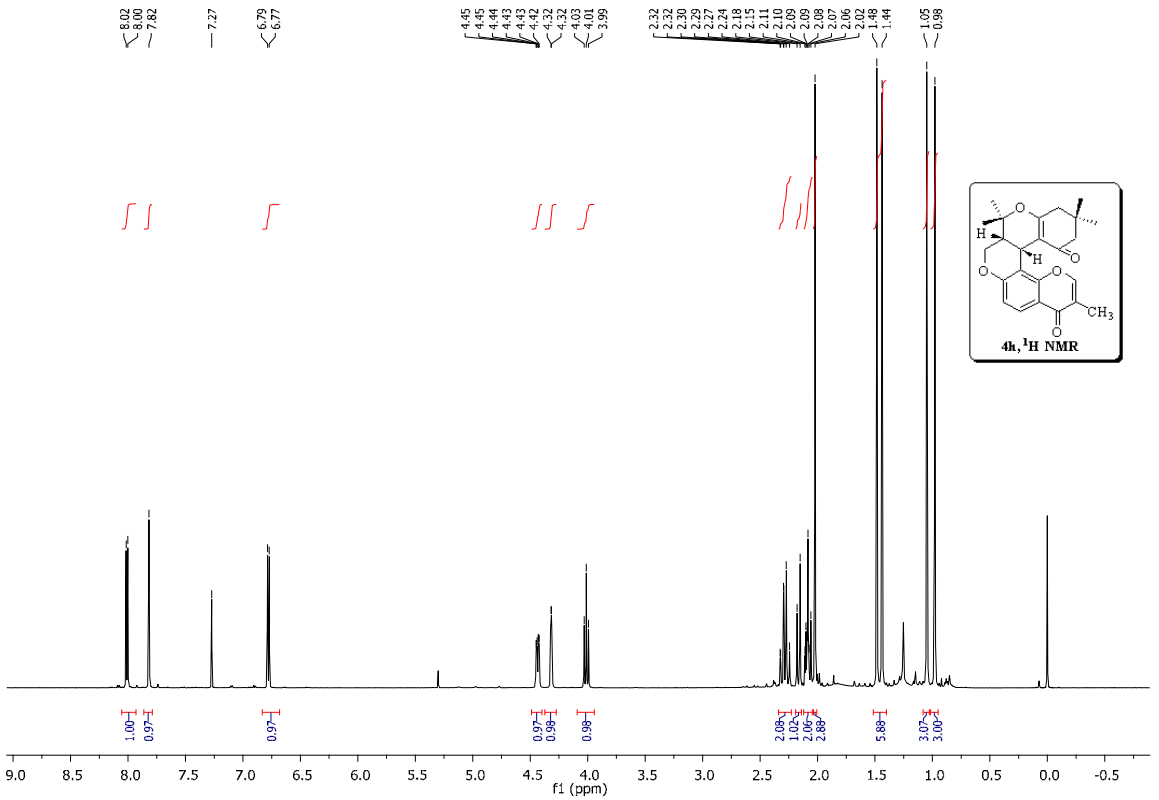

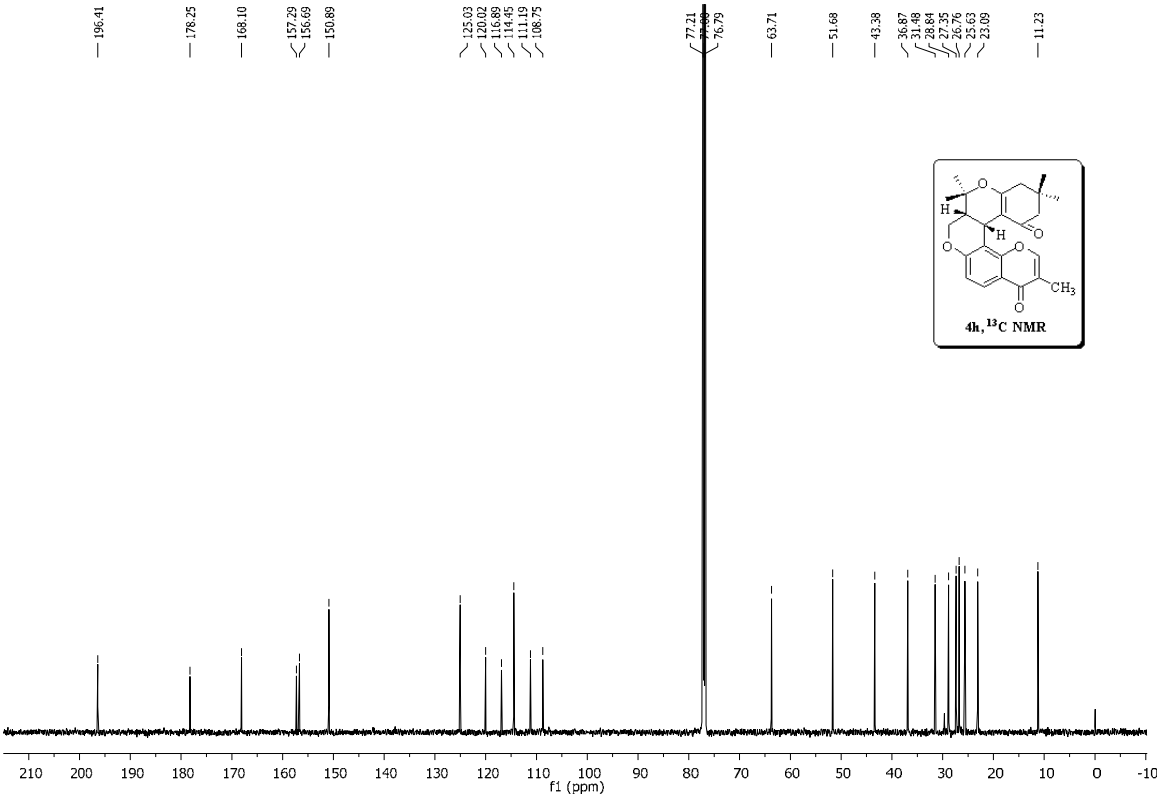

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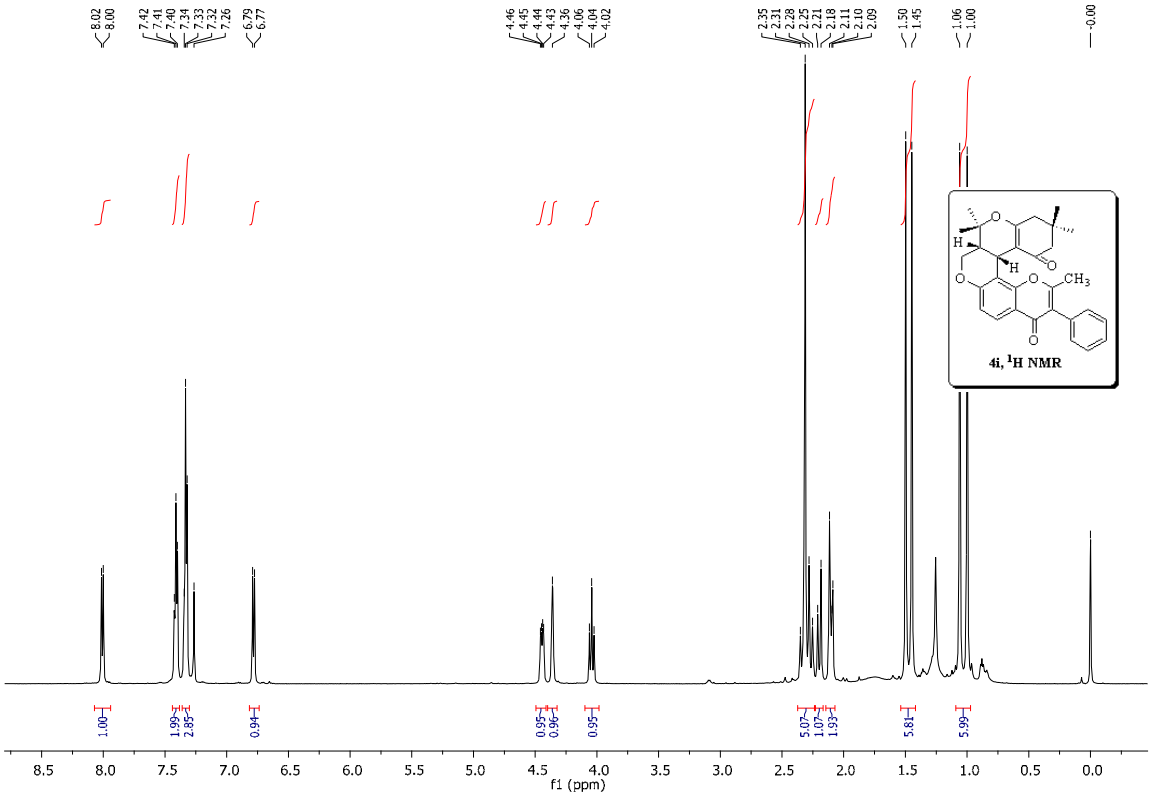

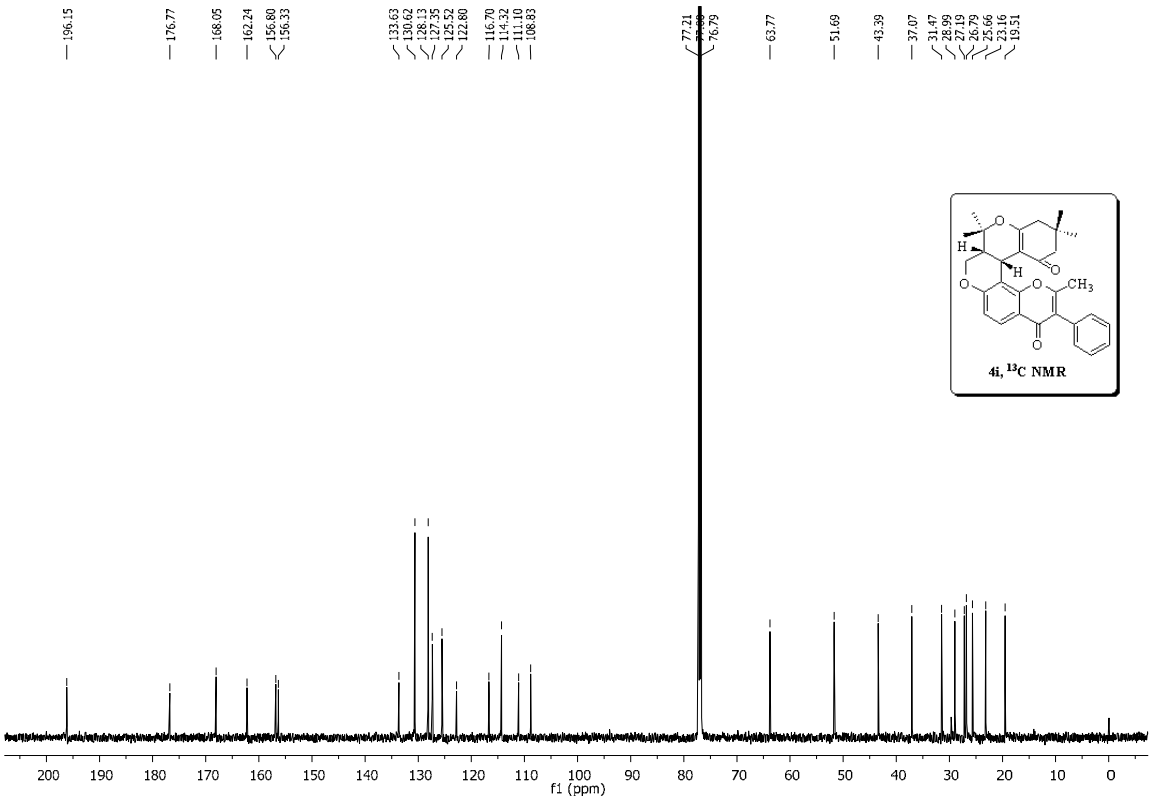

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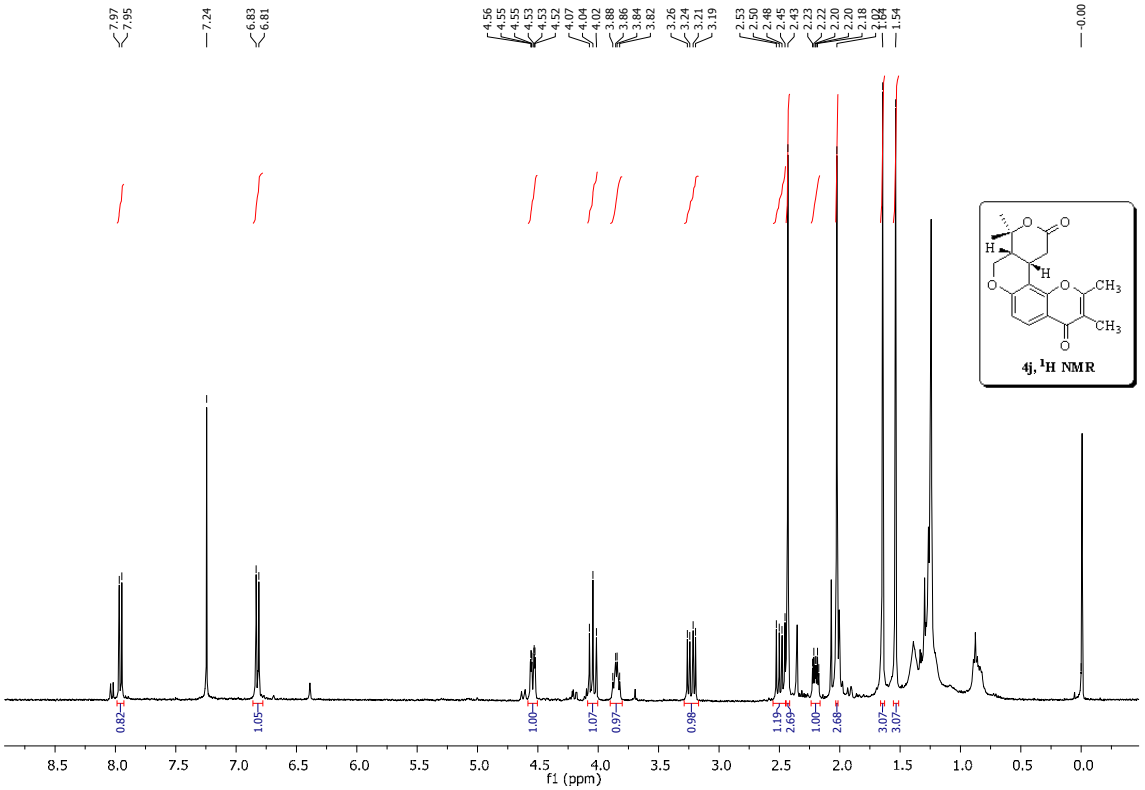

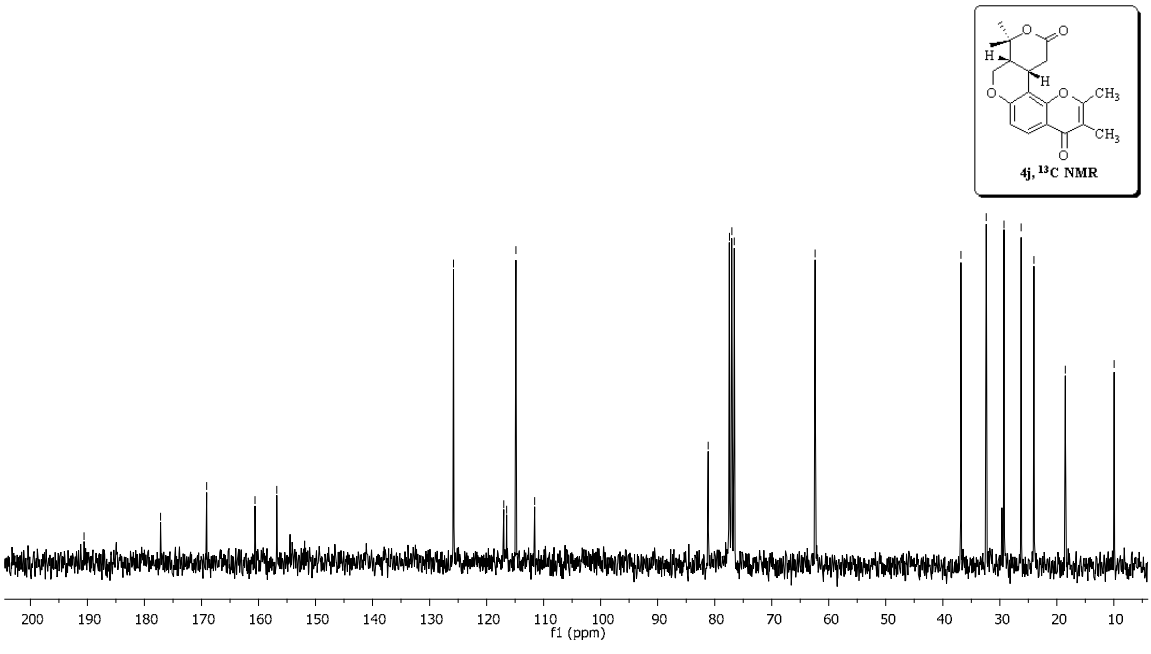

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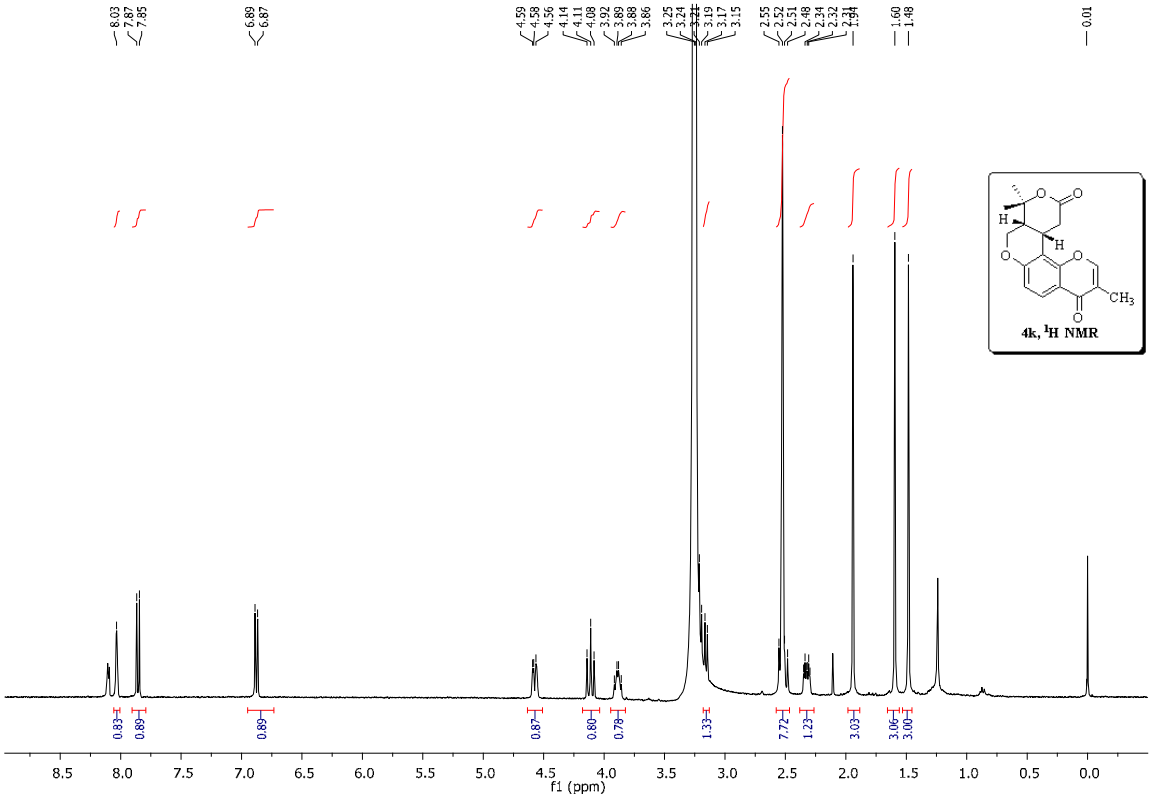

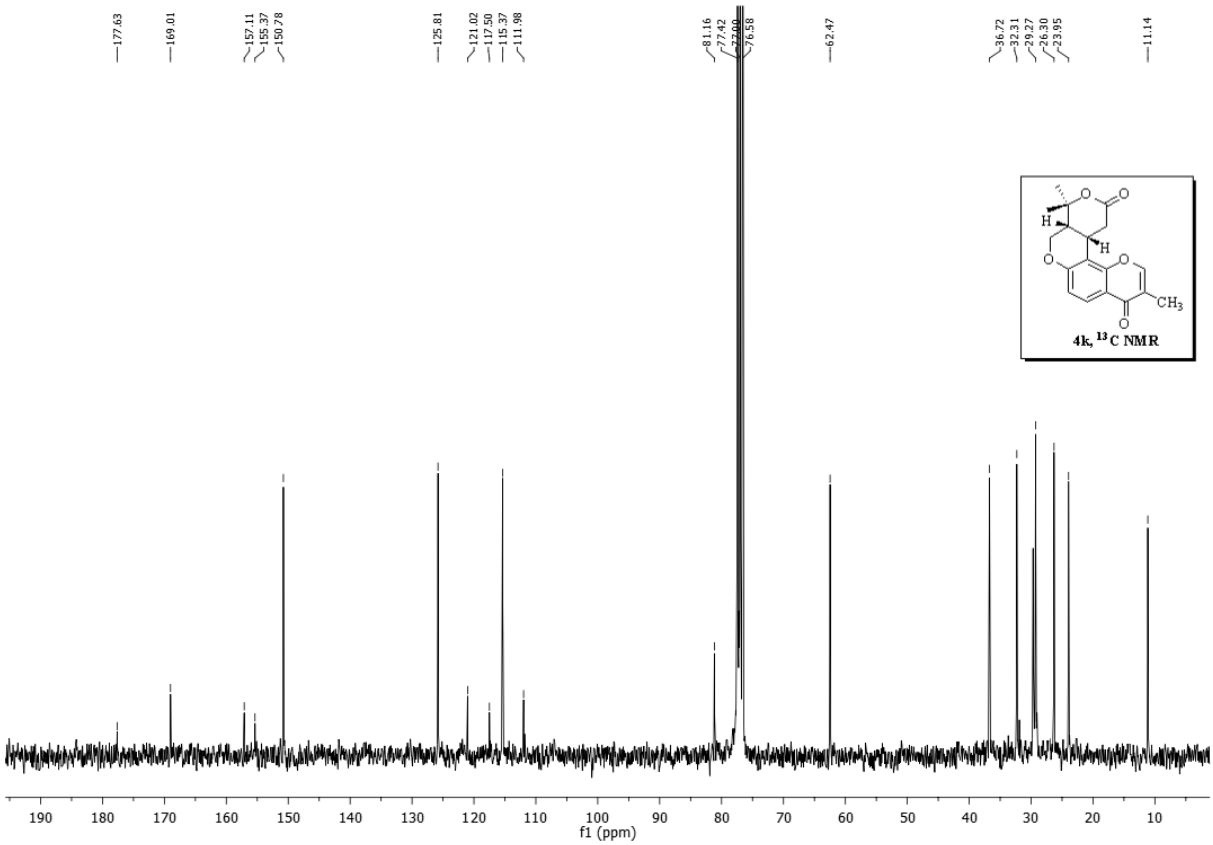

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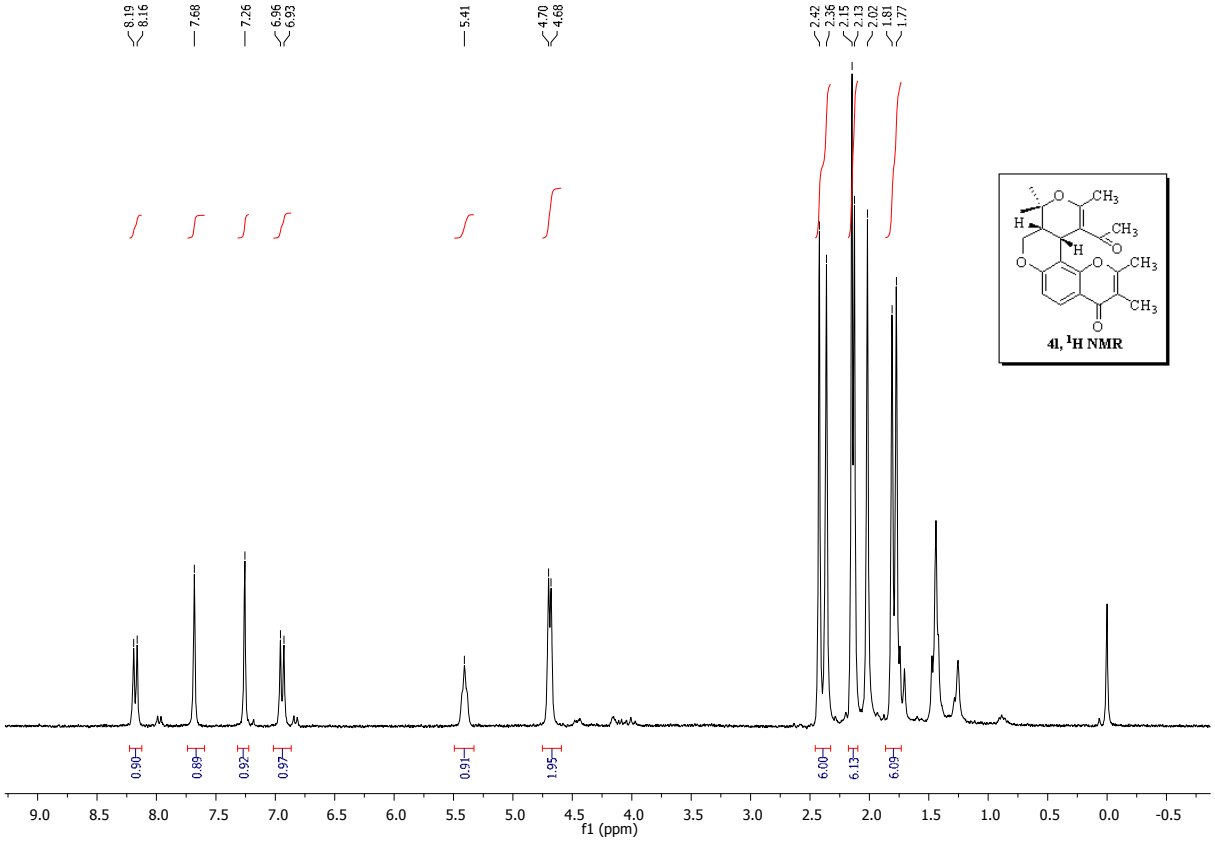

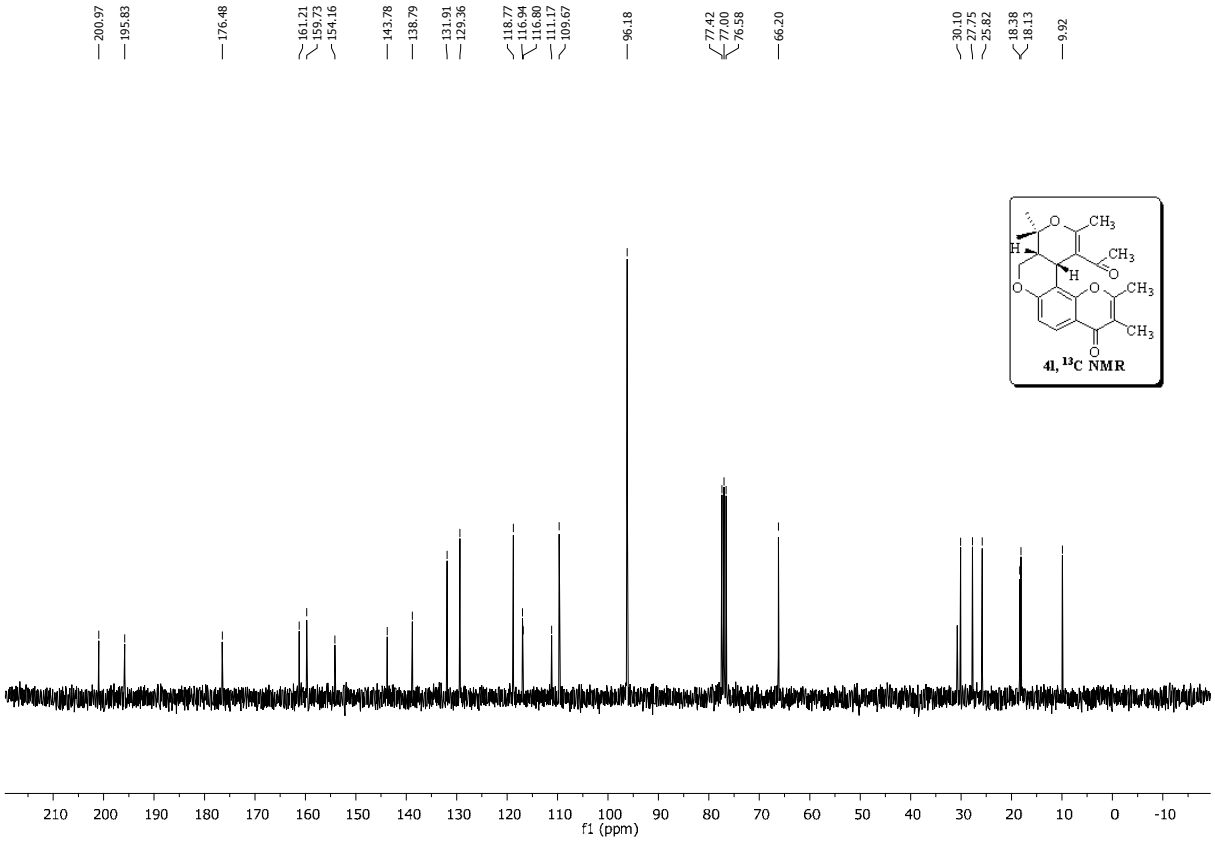

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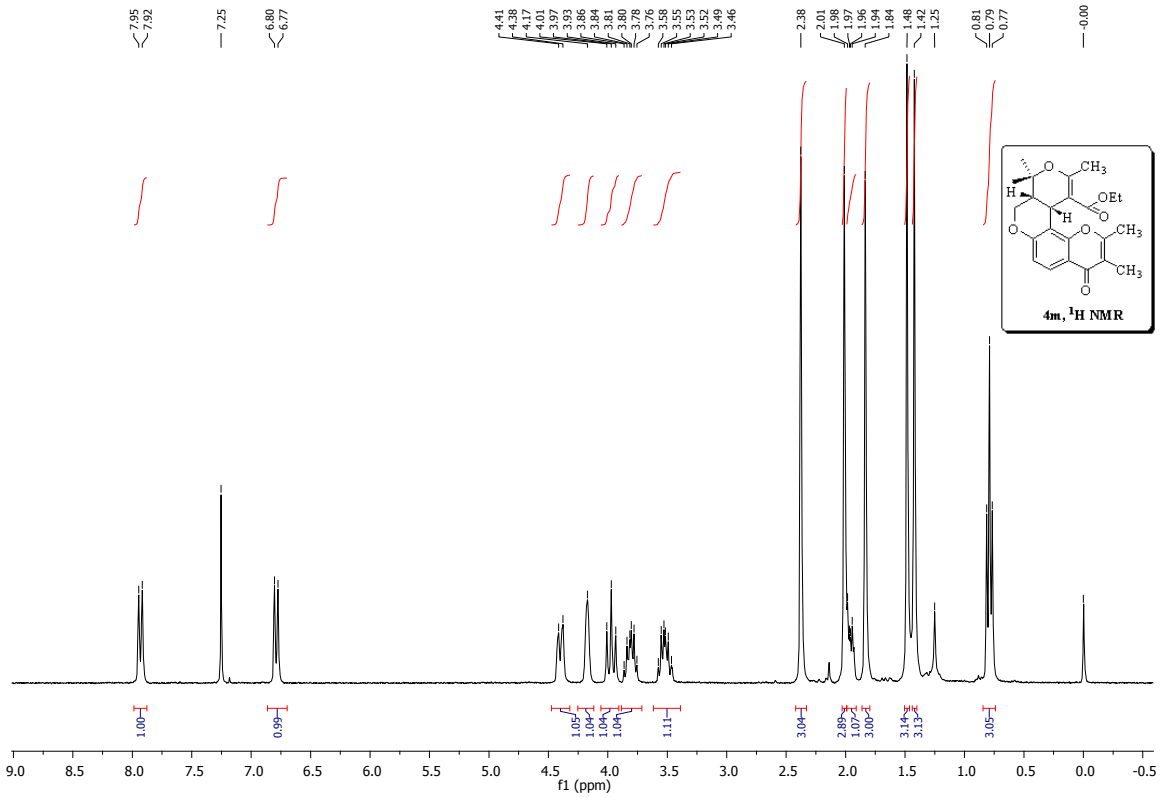

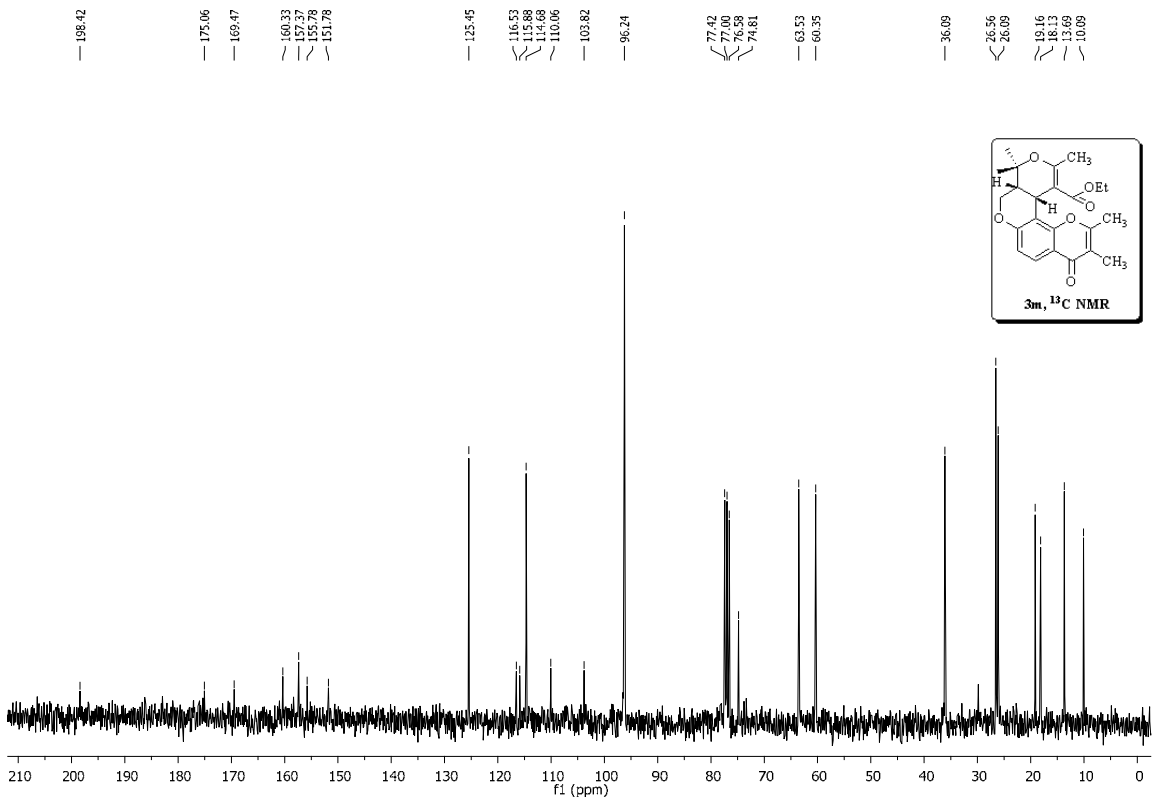

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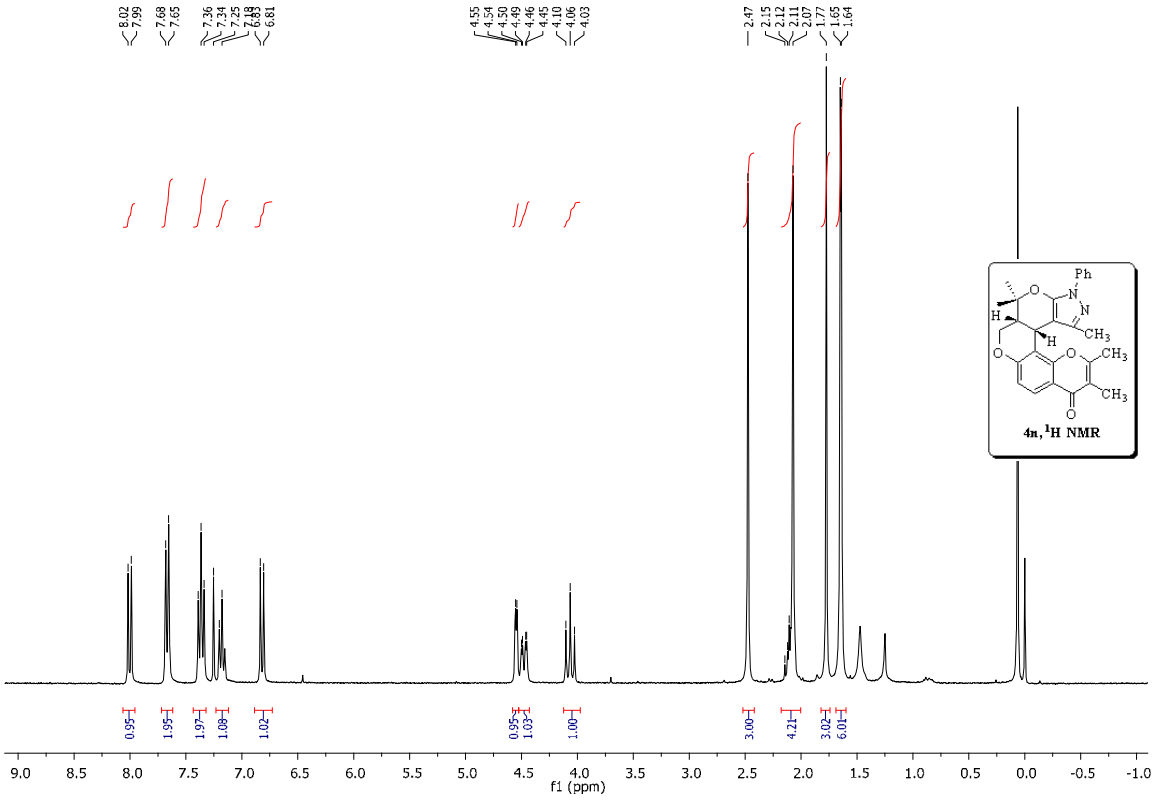

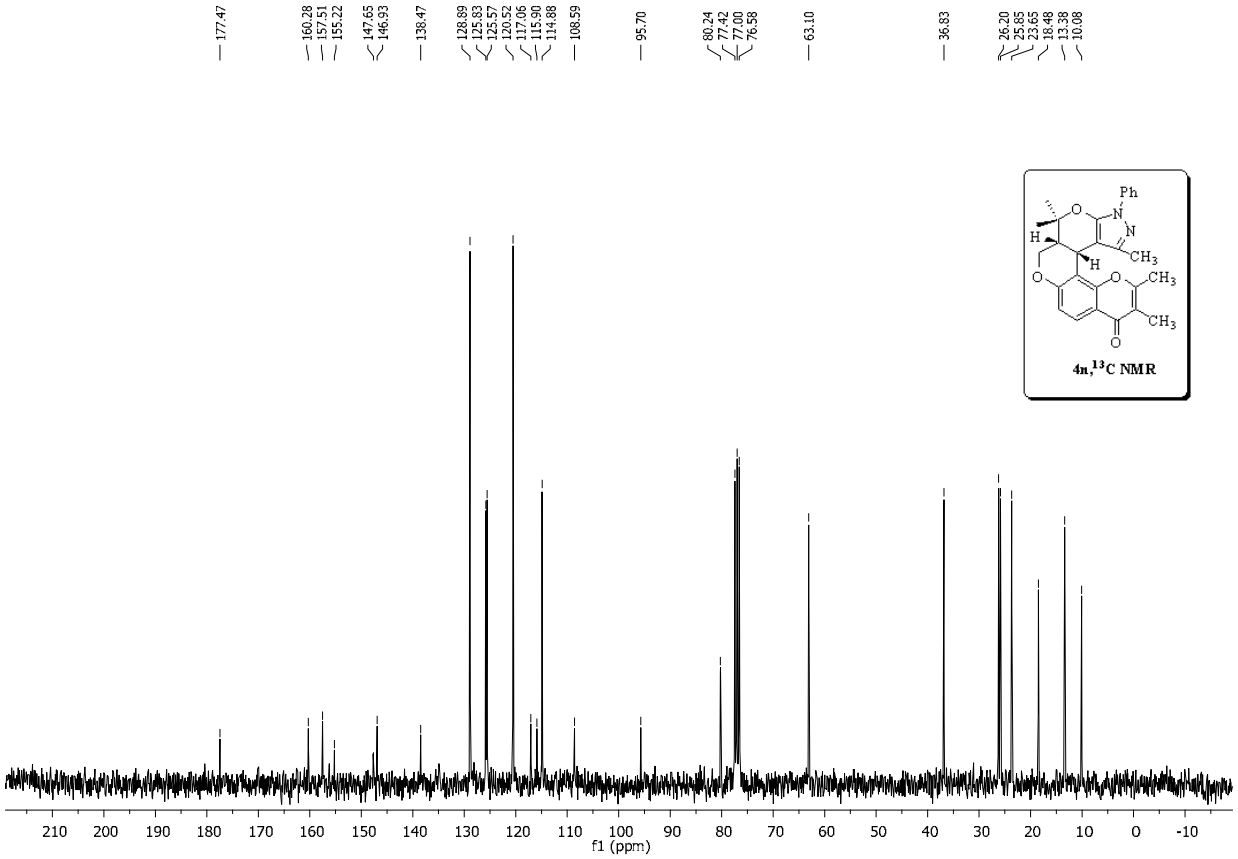

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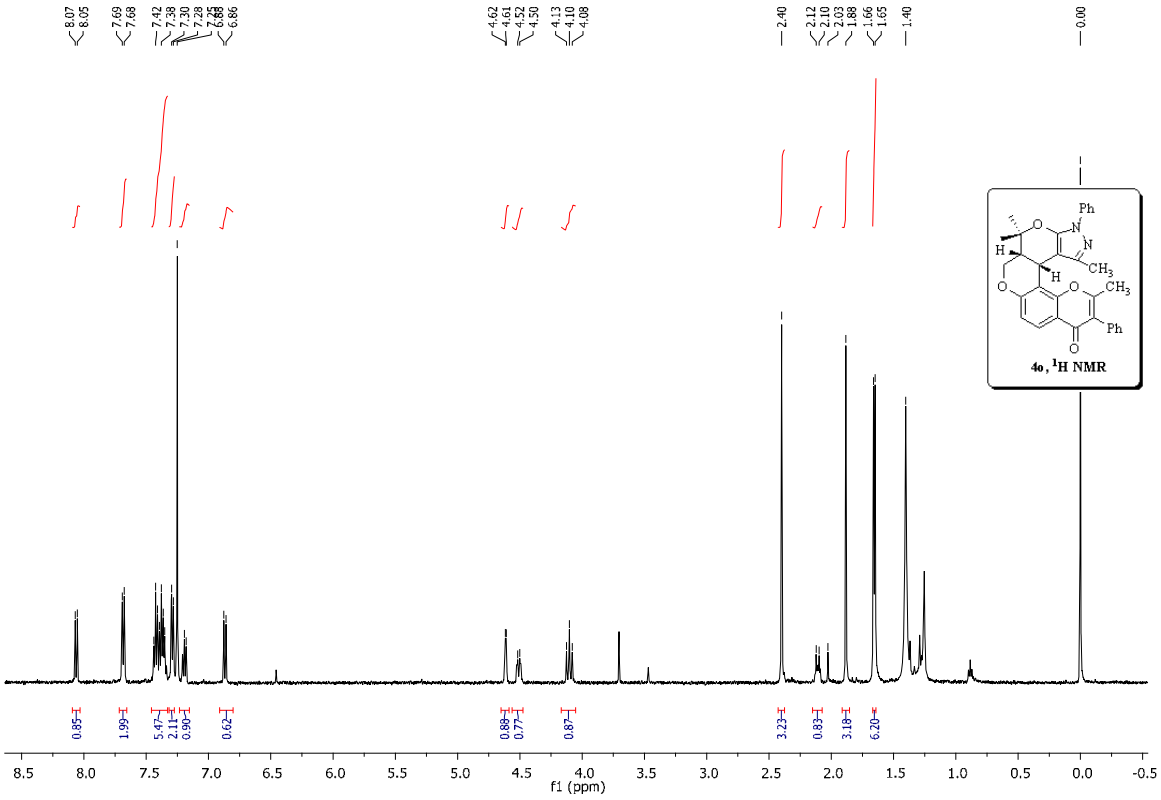

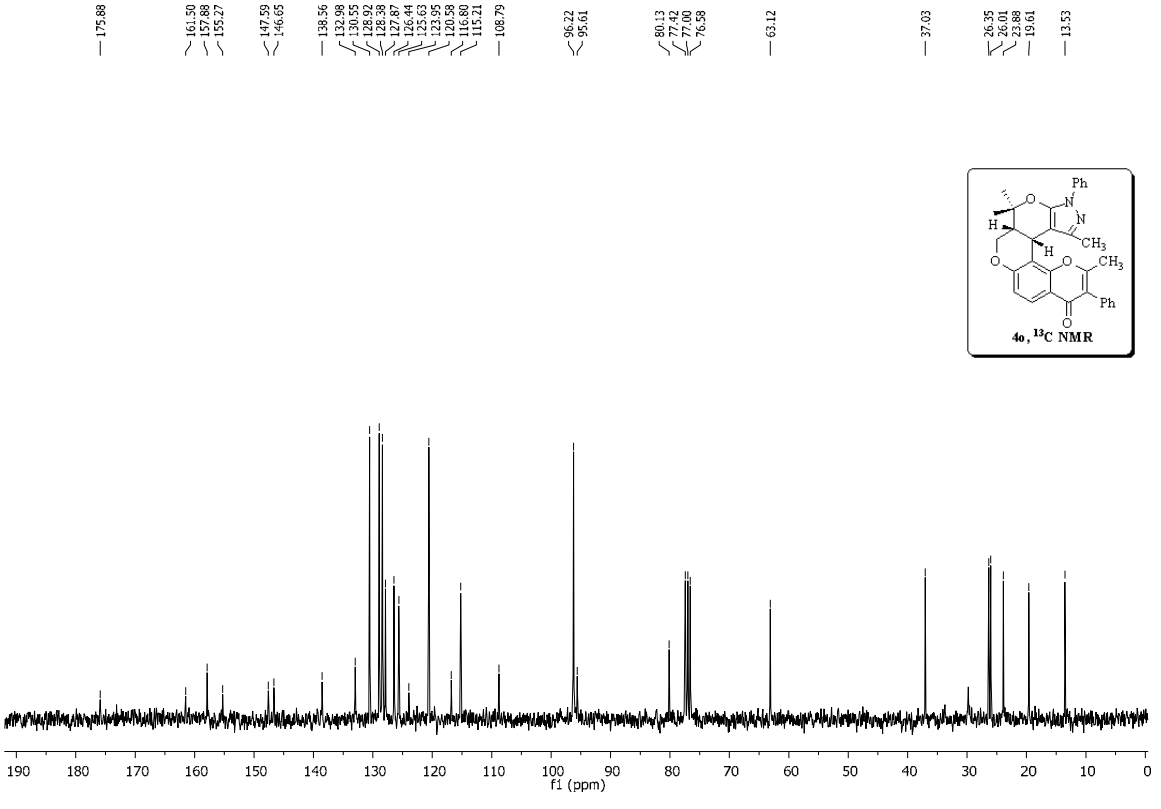

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1.6 Energy minimized structure and 2D NOE for 4a

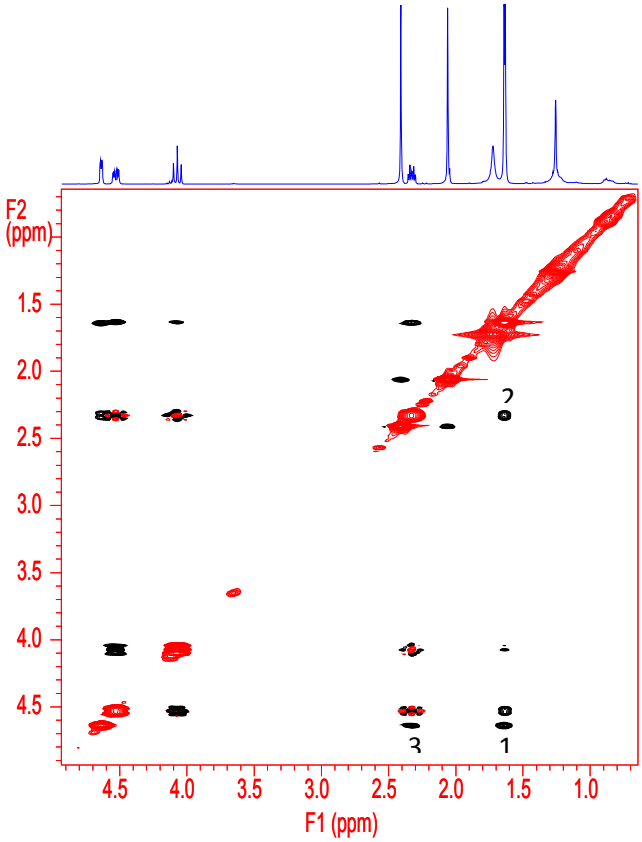

Figure 1. 2D NOE for **4a**

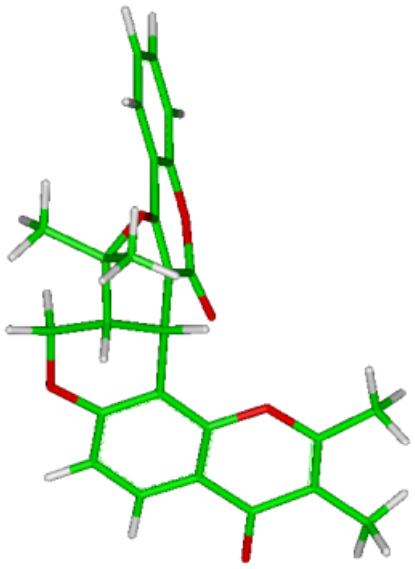

Figure 2. Energy minimized structure for **4a**

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1.7 X-ray crysatallography for 4e and 4g

1.7.1. The CCDC deposition number for **4e** is 848040 Crystal data: $C_{22}H_{22}O_5$, M = 366.40, monoclinic, space group $P2_1/n$, a = 15.4539(11) Å, b = 7.4613(5) Å, c = 16.2148(11) Å, β = 106.214(1) °, V = 1795.3(2) $Å^3$, Z = 4, D_{calcd} = 1.356 mg m^{-3}, T = 294(2) K, μ = 0.096 mm^{-1}, F(000) = 776, λ = 0.71073Å. Data collection yielded 16576 reflection resulting in 3161 unique, averaged reflection, 2889 with I>2σ(I). Full-matrix least-squares refinement led to a final R = 0.0462, wR = 0.1298 and GOF = 1.043. Intensity data were measured on Bruker Smart Apex with CCD area detector

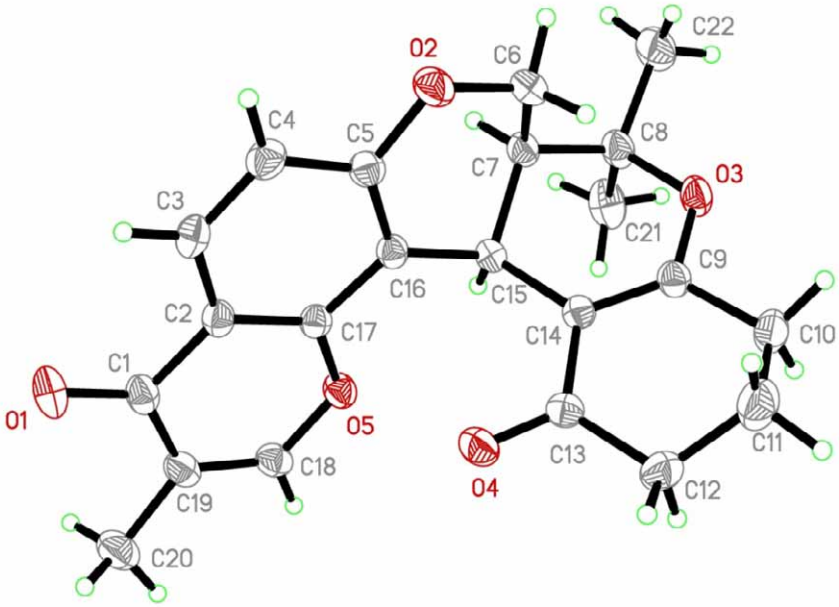

Figure 3. ORTEP diagram for the compound **4e**, with displacement ellipsoids drawn at 30% probability level

1.7.2. The CCDC deposition number for **4g** is 839724. Crystal data: $C_{25}H_{28}O_5$, M = 408.47,monoclinic, space group $P2_1/c$, a = 11.7974(15) Å, b = 13.9420(18) Å, c = 13.4238(17)

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Å, β = 101.254(2) °, V = 2165.5(5) $Å^3$, Z = 4, D_{calcd} = 1.253 mg m^{-3}, T = 294(2) K, μ = 0.086 mm^{-1}, F(000) = 872, λ = 0.71073Å. Data collection yielded 20554 reflection resulting in 3806 unique, averaged reflection, 3113 with I>2σ(I). Full-matrix least-squares refinement led to a final R = 0.0431, wR = 0.1198 and GOF = 1.035. Intensity data were measured on Bruker Smart Apex with CCD area detector.

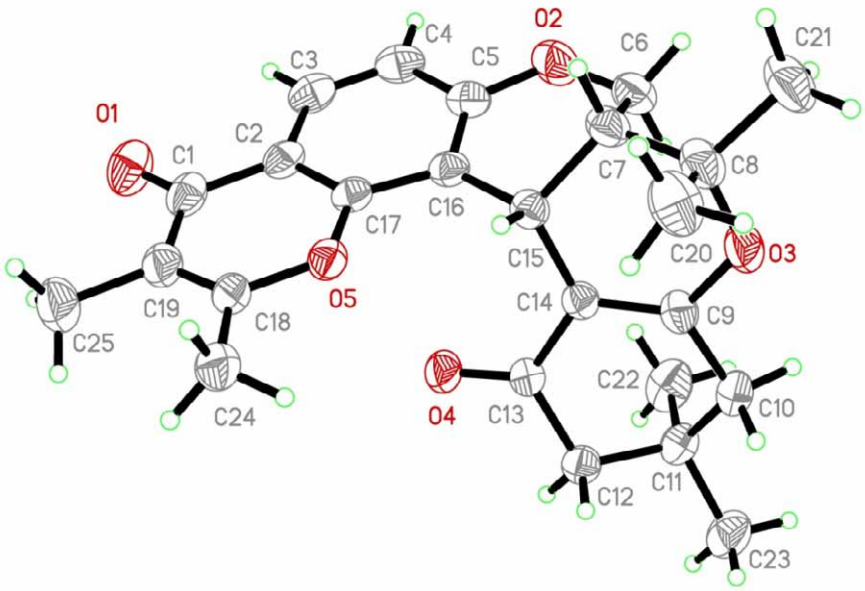

Figure 4. ORTEP diagram for the compound **4g**, with displacement ellipsoids drawn at 30% probability level

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1.8. Biological activity

1.8.1. (a) *General procedure: Biology:* Cellular viability in the presence of test compounds was determined by MTT-microcultured tetrazolium assay following the reported protocol. One human breast cancer cell line, MDA-MB-231 (estrogen receptor-negative); a human neuroblastoma cell line, SK-N-SH; and a human lung cancer cell line, A549; are employed in the current study. Along with these one lung normal cell line, MRC5 was also used. All the four types of cell lines are seeded to flat bottom 96 (10,000 cells/100 ml) well plate and cultured in the medium containing 10% serum. Incubated for 24h in a 5% CO_2 humid chamber so that the cells adhere to the surface. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in PBS at 5 mg/mL and sterile filtered. Different concentrations of the compounds were added to the adhered cells. After 48 h, MTT solution (10ul per well) was added to the culture plate. Cells were further incubated in the CO_2 chamber for 2 h. Following this, media was removed and 100 ml of DMSO was added. Absorbance was measured at 562 nm in a multimode microplate reader (Tecan GENios). The results were represented as percentage of cytotoxity/viability. All the experiments were carried out in triplicates. From the percentage of cytotoxicity the IC_{50} values are calculated and presented in the Table 1; (b) R. S. Upadhyaya, J. K. Vandavasi, V. N. Rao, S. Vivek, S. S. Dixit, J. Chattopadhyaya, *Bioorganic & Medicinal Chemistry,* 2009, **17**, 2830–2841.

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1.8.2. Table 1. In vitro antiproliferative activity of novel chromeno pyrano[3,4-*c*]pyrans against human A549, MDA-MB-231, SK-N-SH cancer cell line and MRC-5 non-cancer cell lines with MTT assay.

S.No	Compound[a]	MDA-MB 231	SK-N-SH	A549	MRC-5
1	**4a**	>100	1.05±0.006	0.1±0.002	0.6±0.001
2	**4b**	>100	1.06±0.02	0.1±0.004	>100
3	**4c**	>100	10.51±0.09	>100	>100
4	**4d**	>100	>100	>100	>100
5	**4e**	>100	>100	>100	>100
6	**4f**	>100	>100	>100	>100
7	**4g**	>100	>100	>100	>100
8	**4h**	>100	>100	>100	>100
9	**4i**	>100	>100	>100	>100
10	**4j**	>100	>100	0.09±0	0.6±0.001
11	**4k**	>100	>100	11.24±0.008	>100
12	**4l**	>100	10.7±0.07	>100	>100
13	**4m**	>100	10.85±0.03	10.94±0.003	0.9±0.001
14	**4n**	>100	10.58±0.06	>100	>100
15	**4o**	>100	>100	>100	>100
16	**Standard(doxo Rubicin)**	8.14±0.14	0.97±0.03	15.07±0.13	14.84±0.25

[a]Results are expressed as IC_{50} values in µM concentrations