Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Information

MCR-Click Synthesis, Molecular docking and Cytotoxicity evaluation of a new series of Indole-Triazole-Coumarin hybrid Peptidomimetics

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Table 1.1. List of alkyne functionalized indole scaffolds synthesized.

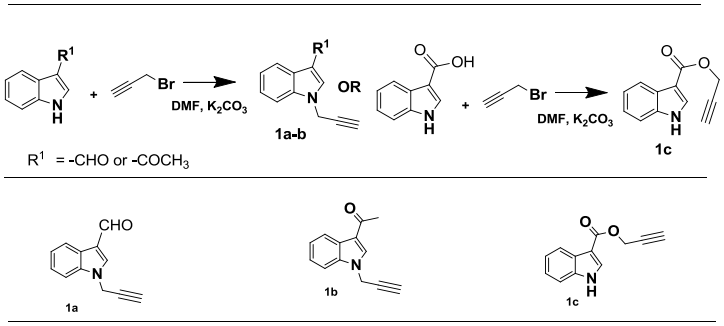
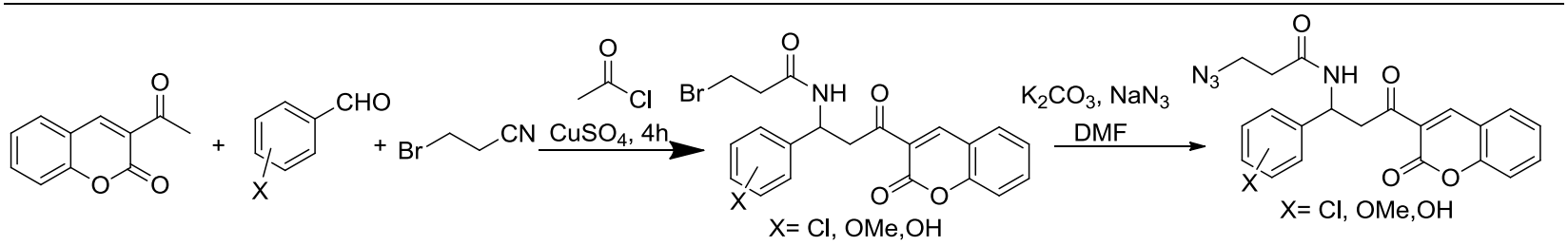
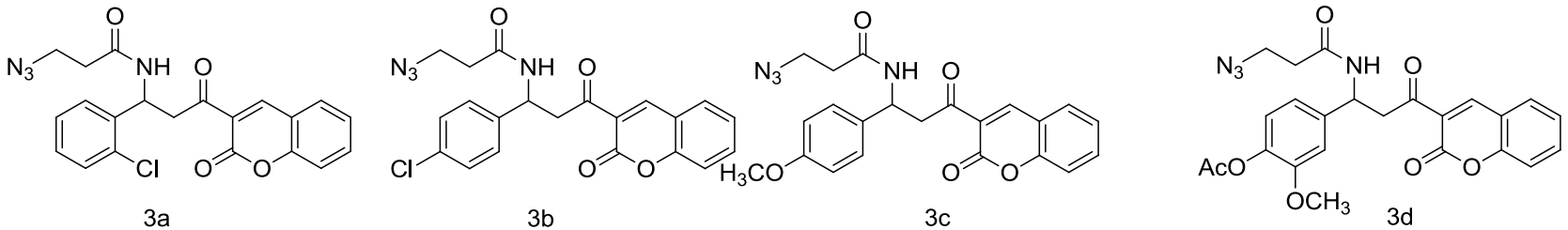


Table 1.2. List of Coumarin azides 3a-3d synthesized via alternate Mannich reaction.





Experimental section

2.1. *General*

All reactions were carried out with oven-dried glassware. Starting materials were purchased from Aldrich and Merck. Alkynes and azides were synthesized based on literature procedure. High resolution mass spectra were measured with DMSO as solvent. IR spectra were recorded on a JASCO-FTIR-4100 spectrometer. 1H and 13C NMR spectra were recorded on a Bruker FT-400 &100MHz, using TMS as an internal standard.

2.2. *General procedure for the synthesis of indole functionalized alkynes**1a-1c***.

Indole derivative (1mmol) and potassium carbonate (414.63mg, 3mmol) were dissolved in minimum amount of DMF, and stirred at 50°C for 10 min. After cooling the reaction mixture to room temperature, propargyl bromide (118.96mg, 1mmol) was added to this and stirred for 4 h and then poured into ice cold water. The solid product predicated was filtered and dried under vacuum to obtain indole functionalized alkynes 1a-1c.

*1-(prop-2-yn-1-yl)-1H indole-3-carbaldehyde (**1a**)*

White solid ; 159 mg (yield 87%); Mp: 90-93°C ;^{1}H NMR (DMSO-d6 , 400 MHz) δH (ppm): 9.94 (s, 1H); 8.37-8.35(d, J=8Hz, 1H); 8.15-8.12 (m, 1H); 7.66-7.64 (m, 1H); 7.38 - 7.28 (m, 2H); 5.24 (s, 2H); 3.43 (s, 1H); ^{13}C NMR (DMSO-d6,100 MHz) δC(ppm): 185.4,140.56,137.05,125.17,124.23, 123.43, 121.78, 118.34,111.85, 78.43,77.20,40.45; ; IR (KBr) υ max :3198, 2121, 1656, 1644, 1615, 1579, 1528, 1471, 1441, 1308, 1244, 1163, 1041, 770, 741, 562 cm^{-1}; MS (ESI): m/z =184 (M +1).

*1-(1-(prop-2-yn-1-yl)-1H-indol-3-yl) ethanone (**1b**)*

White solid ; 167mg (yield 85%);Mp: 103-105°C; ^{1}H NMR (DMSO-d6, 500 MHz) δH(ppm): 8.40(s, 1H), 7.91(s, 1H), 7.35-7.32(m, 1H), 7.26 (s, 2H), 4.92 (s, 2H), 2.17 (s, 1H),1.56(s,3H); ^{13}CNMR(DMSOd6,125MHz);δC(ppm):195.40,140.56,137.05,125.17,124.23,

123.28,121.59,118.05,111.64,78.43,77.20,40.45,25.1; IR (KBr) υ max : 3218, 2125, 1676, 1628, 1578,1523, 1388, 1211, 932, 762, 747 cm-1; MS(HRMS): m/z = 221.0739 (M+Na).

Prop-2-yn-1-yl 1H-indole-3- carboxylate ***(1c)***

White solid ; 163mg (yield 82%);Mp: 80-82°C; 1H NMR (DMSO-d6 , 400 MHz) δH (ppm): 12.00 (S, 1H), 8.05-8.00 (m, 2H), 7.66-7.64 (d, J=8Hz, 1H), 7.35-7.27 (m, 2H), 5.23 (s, 2H), 3.56 (s,1H);^{13}CNMR (DMSO-d6,100MHz) δC(ppm): 163.83,136.87,126.60,122.98,121.91,121.12, 120.78,112.91,111.71,79.69,76.96,51.48 ; IR (KBr) υ max :3298, 3282, 2120,1698, 1698, 1619, 1539, 1271, 1245, 1183, 1149, 1095, 916, 774, 749, 626cm-1;MS(ESI): m/z = 222 (M +Na).

2.3. *General procedure for the synthesis of coumarin functionalized azides****3a-3b***.

A mixture of corresponding benzaldehyde (1 mmol), 3-acetylcoumarin (188 mg, 1mmol), and 3-bromopropionitrile (133 mg, 1 mmol) in acetonitrile (8 ml) were stirred in the presence of catalytic amount of $CuSO_4$ at room temperature for 4 h. Subsequently the reaction mixture was poured into ice cold water and extracted with CH_2Cl_2 (15 mLx2). Evaporation of the solvent followed by purification on a silica gel column (100–200 mesh), by eluting with ethyl acetate/hexane (3:1) afforded the corresponding *β*-amidoketonebromide derivative**2**. **2** (1 mmol), K_2CO_3 (414 mg, 3 mmol), NaN_3 (65 mg, 1 mmol) were dissolved in dimethylacetamide and stirred for 6–8 h and then poured into ice cold water. The precipitate was filtered and dried under vacuum to afford the azides**3a**-**3c**.

3-bromo-N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl) propanamide ***(2b)***

^{1}H NMR (500 MHz, DMSO-d_6): δH (ppm): 8.588 (s, 1H), 8.171 (s, 1H), 7.944-7.929 (d, J = 7.5Hz, 1H), 7.776-7.742 (t, 1H), 7.526-7.482 (m, 4H), 7.302-7.284 (t, 1H), 6.795-6.780 (d, 2H), 5.179-5.138 (t, 1H), 3.891-3.844 (dd, J = 8Hz, J = 16Hz, 1H), 3.452-3.404 (dd, J = 8Hz and J=20Hz, 1H), 3.190-3.176 (t, 2H), 2.364-2.308 (t, 2H);^{13}C NMR (100 MHz, DMSO-d_6) δC(ppm): 199.79, 175.35, 159.92, 153.63, 143.68, 139.07, 134.91, 129.76, 128.92, 125.46, 123.82, 121.88, 116.13, 113.20, 50.16, 47.53, 38.23, 29.45.;FT-IR

(KBr) γ_{max}: 3423.03, 3292.86, 2923.56, 2852.20, 1725.98, 1676.80, 1608.42, 1560.13, 1527.35, 1455.03, 1349.93, 1225.54, 1174.44, 1108.87, 1024.02, 759.82 cm^{-1}

*3-bromo-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl) propanamide**(2c)***

^{1}H NMR (500 MHz, DMSO-d_6): δH (ppm): 8.31 (s, 1H), 8.04 (s, 1H), 7.78-7.76 (d, J = 8Hz, 1H), 7.50-7.49 (t, 1H), 7.44-7.41 (t, 1H), 7.01-7.08 (d, J=8Hz, 2H), 6.79-6.78 (d, J=7.5Hz, 2H), 5.23-5.21 (t, 1H), 3.98 (s, 3H), 3.78-3.74(dd, J = 8Hz, J = 16Hz, 1H) 3.49-3.44 (dd, J = 8Hz and J=20Hz, 1H), 3.41-3.40 (t, 2H), 2.40-2.39 (t, 2H) ;^{13}C NMR (100 MHz, DMSO-d_6) δC(ppm): 200.59, 174.05, 160.86, 156.06, 153.63, 139.77, 134.28, 129.76, 128.98, 125.75, 123.72, 121.88, 118.23, 114.23, 109.20, 56.28, 51.06, 47.69, 39.57, 29.08; FT-IR (KBr) γ_{max} : 3421.10, 3068.19, 2923.56, 2850.27, 1725.98, 1681.62, 1606.41, 1580.38, 1561.09, 1510.95, 1455.03, 1422.24, 1364.39, 1303.64, 1251.58, 1172.51, 1119.48, 1028.84, 922.77, 831.16 cm^{-1}; MS(ESI): m/z =459.3 (M +1).

*3-bromo-N-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl) propanamide **(2d)***

Yellowish solid ; 396.46mg (yield 86%); ^{1}H NMR (500 MHz, DMSO-d_6): δH (ppm): 8.29 (s, 1H), 8.075 (s, 1H), 7.819-7.803 (d, J = 8Hz, 1H), 7.592-7.578 (t, 1H), 7.503-7.488 (t, 1H), 7.156-7.140 (d, J=8Hz, 2H), 6.894-6.879 (d, J=7.5Hz, 2H), 5.125-5.112 (t, 1H), 3.969 (s, 3H), 3.799-3.755 (dd, J = 8Hz, J = 16Hz, 1H) 3.497-3.449 (dd, J = 8Hz and J=20Hz, 1H), 3.273-3.255 (t, 2H), 2.214-2.204 (t, 2H),2.176 (s, 3H) **;**^{13}C NMR (100 MHz, DMSO-d_6) δC(ppm): 199.59, 172.77, 168.06, 159.02, 155.75, 150.82, 142.77, 139.04, 134.91, 129.96, 128.98, 125.05, 122.72, 120.88, 119.23. 114.20, 110.29, 106.22, 56.16, 52.67, 49.61, 47.62, 37.66, 29.96; FT-IR (KBr) γ_{max} : 3429.78, 2923.56, 2853.17, 1760.69, 1724.05, 1677.77, 1607.38, 1560.13, 1509.99,, 1489.74, 1455.99, 1422.24, 1370.18, 1269.9, 1199.51, 1123.33, 1083.8, 1031.73, 914.093, 862.989 cm^{-1}

*3-azido-N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl) propanamide**(3b)***

Brown solid ; 348.37mg (yield 82%); 1H NMR (500 MHz, DMSO-d6): δH (ppm): 8.581 (s, 1H), 8.400 (s, 1H), 7.942-7.926 (d, J = 8Hz, 2H), 7.773-7.744 (t, 2H), 7.521-7.482 (m, 2H), 7.304-7.284 (t, 1H), 6.796-6.780 (d, J=8Hz, 2H), 5.149-5.138 (t, 1H), 3.601-3.554

(dd, J = 8Hz, J = 16Hz, 1H) 3.184-3.136 (dd, J = 8Hz and J=20Hz, 1H), 2.127-2.115 (t, 2H), 1.314-1.300 (t, 2H) ; 13C NMR (100 MHz, DMSO-d6) δC(ppm): 199.75, 175.35, 159.82, 153.66, 143.74, 139.77, 134.91, 129.76, 128.98, 125.75, 123.72, 121.88, 116.23, 113.20, 51.16, 47.69, 45.92, 34.26; FT-IR (KBr) γmax : 3423, 2107, 1720, 1655, 1606, 1489, 1456, 1407,1267, 1213, 1121, 1037, 857, 755,712 cm^{-1}.

3-azido-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl) propanamide ***(3c)***

^{1}H NMR (500 MHz, DMSO-d6): δH (ppm): 8.52 (s, 1H), 8.19 (s, 1H), 7.93-7.92 (d, J = 7Hz, 1H), 7.74-7.71 (t, 1H), 7.52-7.42 (m, 2H), 7.07-7.05 (d, J=8Hz, 2H), 6.49-6.48 (d, J = 8.5Hz, 1H), 5.08-4.94 (t, 1H), 3.91 (s, 3H), 3.62-3.57 (dd, J = 8Hz, J = 16Hz, 1H) 3.26-3.21 (dd, J = 8Hz and J=20Hz, 1H), 2.72-2.70 (t, 2H), 1.98-1.96 (t, 2H) ;^{13}C NMR (100 MHz, DMSO-d6) δC(ppm): 201.21, 172.06, 160.52, 156.86, 153.63, 140.74, 134.11, 131.51, 130.66, 128.25, 125.15, 121.85, 120.91, 116.27, 112.92, 56.33, 51.15, 47.23, 46.11, 33.52; FT-IR (KBr) γmax : 3423.03, 3069.16, 2932.23, 2836.77, 2102.99, 1725.98, 1658.48, 1606.41, 1563.99, 1510.95, 1489.74, 1303.64, 1250.61, 1173.47, 1113.69, 1030.77, 985.447, 923.736, 854.311, 831.169 cm-1; MS(ESI): m/z = 420.3 (M).

3-azido-N-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H-Chromen-3-yl)propyl)propanamide ***(3d)***

Brownish solid ; 382.76mg (yield 80%); ^{1}H NMR (500 MHz, DMSO-d6): δH (ppm): 8.57 (s, 1H), 8.39 (s, 1H), 7.94-7.92 (d, J = 8Hz, 1H), 7.76-7.73 (t, 1H), 7.59-7.49 (m, 2H), 6.97-6.96 (d, J = 7Hz, 1H), 5.14-4.95 (t, 1H), 3.73 (s, 3H), 3.63-3.58 (dd, J = 8Hz, J = 16Hz, 1H), 3.38-3.34 (dd, J = 8Hz and J=20Hz, 1H), 2.46-2.44 (t, 2H), 2.04(s. 3H), 1.68-1.66 (t, 2H) ;^{13}C NMR (100 MHz, DMSO-d6) δC(ppm): 200.59, 173.05, 169.06. 160.82, 156.66, 149.67, 139.77, 137.04, 134.91, 129.76, 128.98, 125.75, 123.72, 121.88, 119.23, 114.20, 110.09, 106.22, 56.16, 52.57,48.69,47.69,35.66,29.26;FT-IR(KBr)γmax:2936,2834,2108,1729,1607, 1510, 1455, 1415,1384,1270,1202, 1122,1030,872,757,524 cm^{-1}

2.4*. General procedure for the Cu (I) 1, 3-dipolar cycloaddition reaction for the formation of indole-triazole-coumarin.*

An equimolar amount 1-(prop-2-yn-1-yl)-1H indole-3-carbaldehyde **1a** (91.5342 mg, 0.5 mmol) and the coumarinazide **3b**(239.0744 mg, 0.5mmol) were dissolved in minimum amount of DMSO. To this, 2 ml of *t*-BuOH, 1 ml of water, $CuSO_4.5H_2O$ (200 mg) and sodium ascorbate (150 mg) were added and stirred in room temperature for 12 h. and then poured in to cold water. The precipitated click product was filtered, washed with water and dried under vacuum to afford **4d** in pure form (555.79 mg, 84%).

*N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3yl)propyl)-3-(4-((3-formyl-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propanamide (**4b**)*

Brownish solid ; 522.92 mg (yield 86%); 1H NMR (400 MHz, DMSO-d6): *δH (ppm):* 9.880(s, 1H), 8.412-8.361(d, J=20 Hz,1H), 8.361-8.341(d, J=8 Hz, 1H), 8.300-8.279(d, J=8 Hz ,1H), 8.216(s, 1H), 8.120(s, 1H), 7.736-7.719(d, J= 7 Hz,1H),7.669(s, 1H), 7.638-7.176(m, 8H),6.365-6.341(m, 1H), 6.223(s, 2H), 5.933-5.871(dd, J= 8Hz and J=16Hz , 1H), 5.483-5.433(dd, J= 8Hz and J= 20Hz, 1H), 5.061-5.018(m,2H), 1.356-1.236(t, 2H); 13C NMR (100 MHz, DMSO-d6) *δC(ppm):* 199.93, 185.42, 177.79, 166.54, 159.39, 141.11,139.87,137.08,131.96,129.58,125.30,124.30,124.16,123.33,123.26,123.11,121.66,121.58,121.55,117.87,112.58,111.59,112.58, 111.66,111.24,90.92,77.21,48.13,46.13,36.50;IR(KBr)υmax:3309,2935,2745,2676,2491,1641,1580,1528,1452,1393,1315,1241,1196, 1030,750,428 cm-1; HRMS m/z ;608.1699 (calc. 608.1701)

*N-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H chromen-3-yl) propyl)-3-(4-((3-formyl-1H-indol-1-yl) methyl)-1H-1,2,3-triazol-1-yl) propanamide (**4d**)*

Brownish solid ; 555.79 mg (yield 84 %); ^{1}H NMR (400 MHz, DMSO-d6): δ 9.937 (s, 1H), 8.908-8.876 (d, J= 12 Hz,1H), 8.408 (s, 1H), 8.408 (t, 1H),8.358(s,1H), 8.213(s, 1H), 8.119-7.118 (9H, m), 7.643(s,1H), 6.378(s, 2H), 6.341-6.223(t, 1H), 5.927-5.905 (d, J= 8 Hz , 1H), 5.753-5.714(d, J= 16Hz, 1H), 5.061-5.014(t,2H), 5.061-5.014(t, 2H), 3.980(s, 3H), 1.234(s, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δC(ppm): 199.93,185.42,177.79,166.54,159.39,156.23,141.11,139.87,137.08,131.96,129.58,125.30,124.30,123.33,123.11, 121.66,117.87,112.58,111.24,90.92,77.21,51.7548.13,46.13,29.46;IR(KBr)mad:2922,1719, 1655, 1608, 1530, 1488, 1465,1401,1384, 1321,1269,1201,1170,1123,1032,752 cm^{-1}; HRMS m/z ; 662.2232 (calc. 662.2251).

*3-(4-((3-acetyl-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl) propanamide (**5b**)*

Brownish solid ; 516.32mg (yield 83%); ^{1}H NMR (400 MHz, DMSO-d6): δH (ppm): 8.582(s, 1H), 8.381(s, 1H), 8.309(s, 1H), 8.215-8.199(d, J= 6Hz ,1H), 8.215(s, 1H), 7.979(s, 1H), 7.628-7.612(d, J= 6Hz,1H),7.481(s, 1H), 7.435(s, 1H), 7.319-7.262(m, 6H), 5.245(s, 2H), 5.199-5.018(m,1H), 4.304-4.199(m, 2H), 3.319-3.262(dd, J=8Hz,16 Hz,1H), 3.199-3.018(dd, J= 10Hz, 1H), 2.460-2.352(m,2H),1.236(s,3H);^{13}CNMR(100MHz,DMSO-d6)δC(ppm): 195.13,185.22,168.60,167.48,160.72,141.07,140.56,139.86,138.65,137.31,137.05,129.48,128.98,125.13,124.22,124.15,124.06,123.32, 123.27,123.09,121.65,121.57,121.54,118.12,118.04,112.57,112.01,111.61,11.58,111.23,60.72,51,69,50.91,48.10,28.54,28.32;IR(KBr) υmax:3218,2923,1716,1680,1627,1609,1525,1488,1455,1388,1340,1275,1207,1090,1013,944, 931,827,753,681,657 cm^{-1}; HRMS m/z ; 622.1854 (calc. 622.1857).

*3-(4-((3-acetyl-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide (**5d**)*

Brownish solid ; 540.55 mg (yield 80%);^{1}H NMR (400 MHz, DMSO-d6): δH (ppm): 9.9321 (s, 1H), 8.380(s, 1H),8.304(s,1H),8.215 (s,1H),7.652-7.633 (d, J=8Hz, 1H), 7.498-7.482 (d, J=6Hz, 1H), 7.416(s, 1H),7.321-7.260(m,7H),6.378(s, 2H), 6.341-6223(t, 1H),5.199-5.042(t, 2H), 4.304(s, 3H), 3.938-3.876(dd, J=10 Hz and J=12 Hz,1H),3.860-3.839(dd, J= 8Hz,1H),2.432-2.414(t, 2H), 1.911(s,3H),1.239(s,3H);^{13}CNMR(100MHz,DMSO-d6)δC(ppm): 195.23,185.40,168.05,162.39,160.72,148.15,147.81,141.07,140.56,139.86,138.65,137.31,129.48,128.98,125.13,124.22,123.32,121.65, 118.12,112.57,111.65,111.23,78.43,77.20,51.73,44.96,31.78,28.32,20.04;IR(KBr)υmax:2927,2851,1718,1681,1608,1513,1488,1455,1 428,1387,1364,1319,1271,1215,1123, 084, 1031, 933, 859, 822, 756, 611, 559cm^{-1}; HRMS m/z; 676.2400 (calc. 676.2407).

*(1-(3-((1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl) propyl)amino)-3-oxopropyl)- 1H-1,2,3-triazol-4-yl)methyl 1H –indole-3-carboxylate (**6b**)*

Brownish solid ; 505.47 mg (yield 81 %); ^{1}H NMR (400 MHz, DMSO-d6): δH (ppm): 9.911(s, 1H),8.296(s,1H),8.240(s, 1H), 8.134(s, 1H), 7.995(s, 2H), 7.769(s, 2H), 7.769-7.636(m, 2H), 7.491-7.045(m, 6H), 5.404(s, 2H), 5.328(t, 1H), 5.068-4.921(t, 2H), 4.83-4.718(dd, J= 20 Hz ,1H), 3.938-3.876(dd, J= 10 Hz and 12 Hz, 1H), 1.296-1.239(m, 2H); ^{13}C NMR (100MHz, DMSO-d6) δC(ppm):194.03,185.17, 168.21,167.69,147.81,141.05,140.05,140.65,140.53,139.87,137.32,137.04,125.13,124.28, 124.23,124.14, 124.05,123.30,123.24,123.08,121.65,121.57,121.54,112.57,112.03,111.65,111.62,111.59, 85.32, 60.92, 51.68, 48.12, 28.54; IR (KBr) υmax: 2974, 2932, 2738, 2676,2490,1748,1655,1591,1528,1456,397,1360,1337, 1314, 1243, 1170,1243,1170,1122, 1089,1036,989,939,821,754 cm^{-1}; HRMS m/z; 624.1650 (calc. 624.1650).

(*1-(3-((1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-4-yl)methyl 1H –indole-3-carboxylate* ***(6d)***

Brownish solid ; 542.13 mg (yield 80%);^{1}H NMR (400 MHz, DMSO-d6): δH (ppm): 9.876(s, 1H), 8.301-8.244(d, J=22 Hz , 1H), 8.135(s, 1H), 8.041(s, 1H), 8.021(s, 1H), 7.716-7.660(d, J=22 Hz , 1H), 7.497(s, 1H),7.458(s, 1H),7.425(s, 1H), 7.335-7.229(m, 6H), 5.224(s, 2H), 5.115-4.876(t, 1H), 4.660-4.643(t, 3H), 4.237(s, 3H), 3.753-3.658(dd, J=16Hz, 1H), 3.458-3.367(dd, J=10 Hz, 1H), 1.474-1.449(t,2H),1.239 (s,3H); ^{13}CNMR (100MHz,DMSO-d6)δC(ppm): 194.37,185.43,167.69,163.00,160.72,148.15, 147.81,140.58, 139.94,137.04,135.38,133.09,130.09,129.20,128.57,128.27,127.49125.23,125.15,124.24,123.29,121.58,118.03,117.00,111.64,78.45,77.19,63.24,44.52,36.50,31.89,29.41;IR(KBr)υmax:2923,1704,1608,1532,1509,1488,1456,1394,1268,1199,1122,1034,1031,861,754,606,518 cm^{-1}; HRMS m/z; 678.2194 (calc. 678.2200).

2.5.Cell culture and treatment

The MCF-7 cells were maintained in RPMI medium 1640 supplemented with 10% fetal bovine serum as well as100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM of Na pyruvate in a humidified atmosphere containing 5% CO_2at 37°C.

2.6.Cytotoxicity assay

Cytotoxicity was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MCF-7cells in the log phase were seeded in 96-well plates at a concentration of 1.0X104cells/well and incubated overnight at 37°Cin5%CO_2 humidified environment. The cells were then treated with different concentrations of the sample **4d**,**5d** and **6d** such as 10, 20, 30, 40, 50,60, 70, 80, 90, and 100 μM/mL (dissolved with RPMI medium1640), respectively. Controls were also cultivated under same conditions without the addition of compound solution. The treated cells were incubated for 48 h and then subjected for MTT assay. The stock concentration (5 mg/mL) of MTT-(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was prepared and 100μL of MTT was added in each compound solution treated wells and incubated for 4 h. Purple colourformazone crystals were observed and these crystalswere dissolved in 100μL of dimethyl sulphoxide (DMSO), and readat 620 nm in a multi well ELISA plate reader (Thermo, Multiskan).

2.7. Cell morphology

To study the morphological changes, MCF-7 cells were grown (1X105cells/cover slip) and incubated with at its IC50 concentration and then they were fixed in a mixture of methanol and acetic acid (3:1, v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Morphological changes of MCF-7 cells were analyzed under a Nikon (Japan) bright field inverted light microscope at 40 magnification. Followed by this, DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) staining was carried out. For this, the MCF-7 cells incubatedwith compound at its IC50 concentration for 48 h and then they were fixed in a mixture of methanol and acetic acid (3:1, v/v) prior to washing with PBS. The washed cells were then stainedwith 1 mg/mL DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) for 20 min. in a dark atmosphere. The stained images were recorded using a fluorescent microscope with appropriate excitation filter.

2.8. Molecular docking study

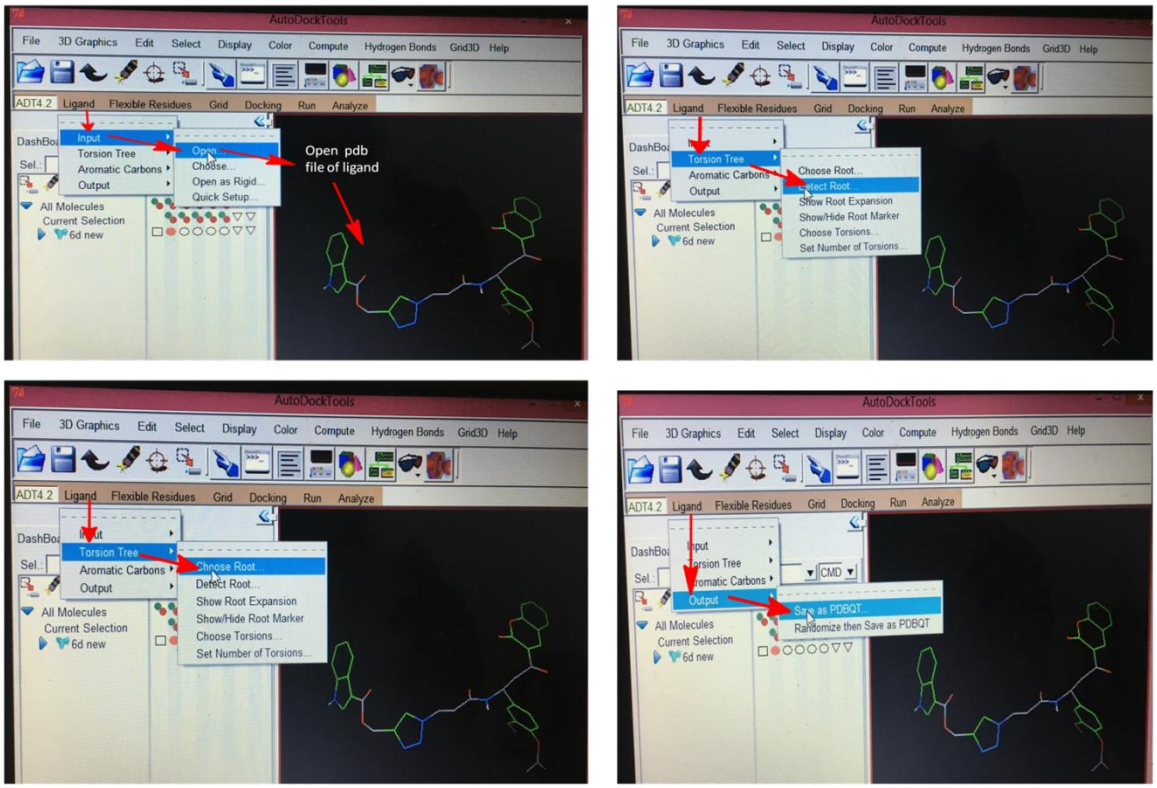
Docking studies were carried out using the Auto Dock Vina 1.1.2 program for the synthesized compounds. AutoDock vina 1.1.2 predicts the bound conformations of a small, flexible ligand to a nonflexible macromolecular target of known structure. The technique combines simulated annealing for conformation searching with a rapid grid-based method of energy

evaluation based on the AMBER force field. For this, the pdb structure of 1GII was retrieved from the Brookhaven protein database (http://www.rcsb.org).

STEP 1: Conversion of pdb file of ligand into pdbqt file using Autodock tool

The 3D structure of the synthesized compounds were provided using Marvine Sketch 5.8.3,2012, ChemAxon(http://www.chemaxon.com) and was converted to pdbqt coordinate by Autodock Tools (ADT; version 1.5.4). While converting the ligand as pdbqt, assign torsion free and detect root and then assign torsion free and choose root then save as pdbqt.

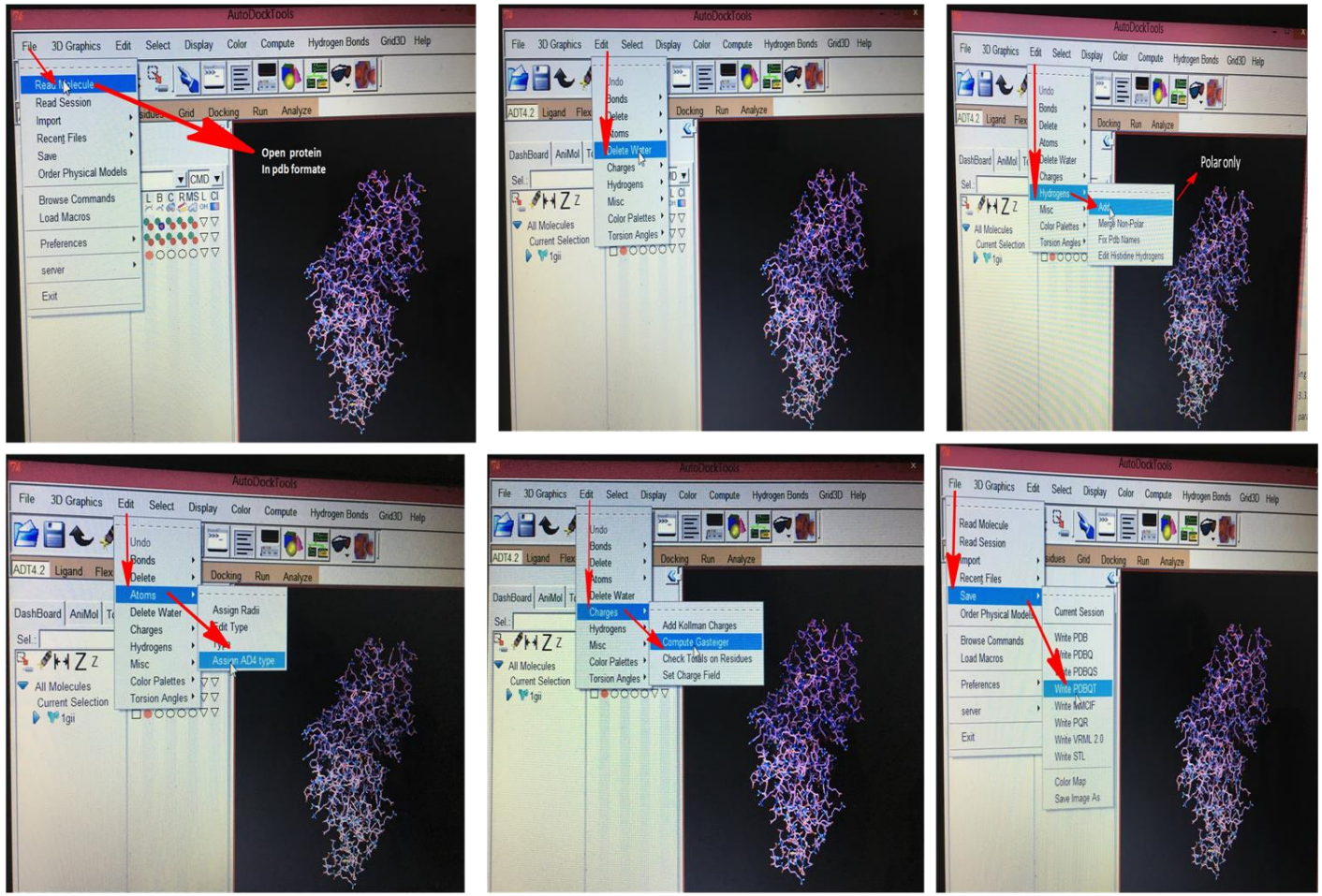
STEP 1



STEP 2: Conversion of pdb file of protien into pdbqt file using Autodock tool

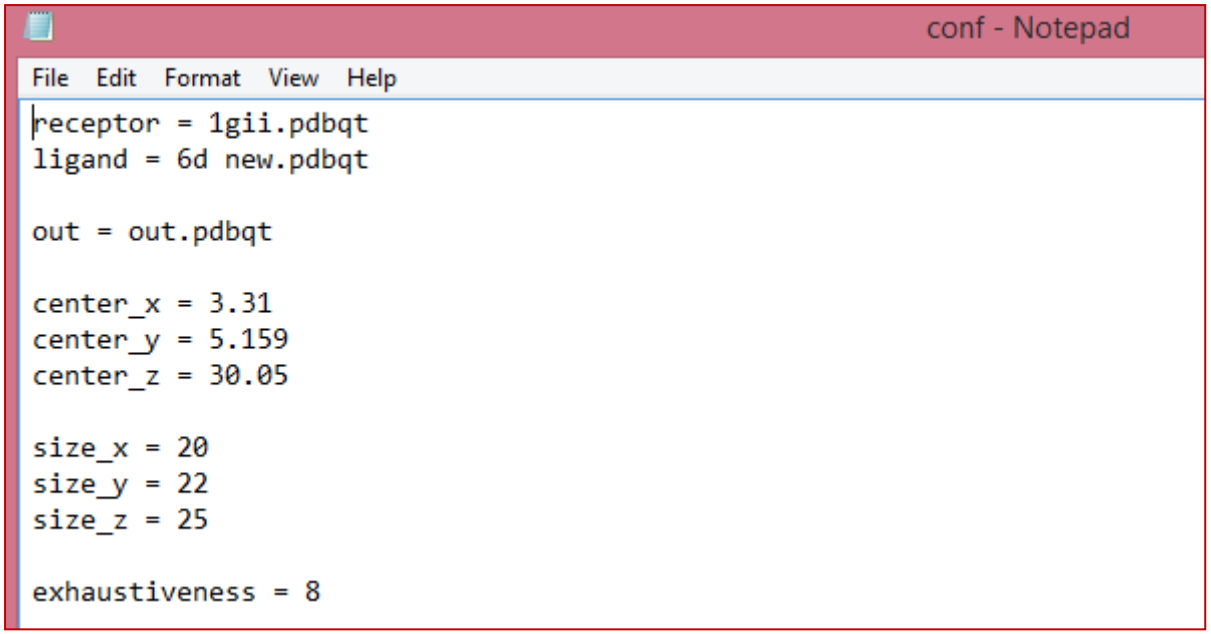
The pdb structure of 1GII (protein) was retrieved from the Brookhaven protein database (http://www.rcsb.org).The Protien was treated in a slightly different way. After removing all water, polar hydrogens were added, and charges assigned using the Kollman united atom library using Autodock Tools (ADT; version 1.5.4).

STEP 2



STEP 3: Note the parameters into conf file

Now to assign the docking site, all maps were calculated with 1 Å spacing between grid points by Auto grid. The center of the grid box was placed at the center of donepezil with coordinates x =3.31, y =5.159, z =30.05 with exhaustiveness 8. The dimensions of the active site box were set at 20 X22 X 25 Å. Note these parameters into conf file.



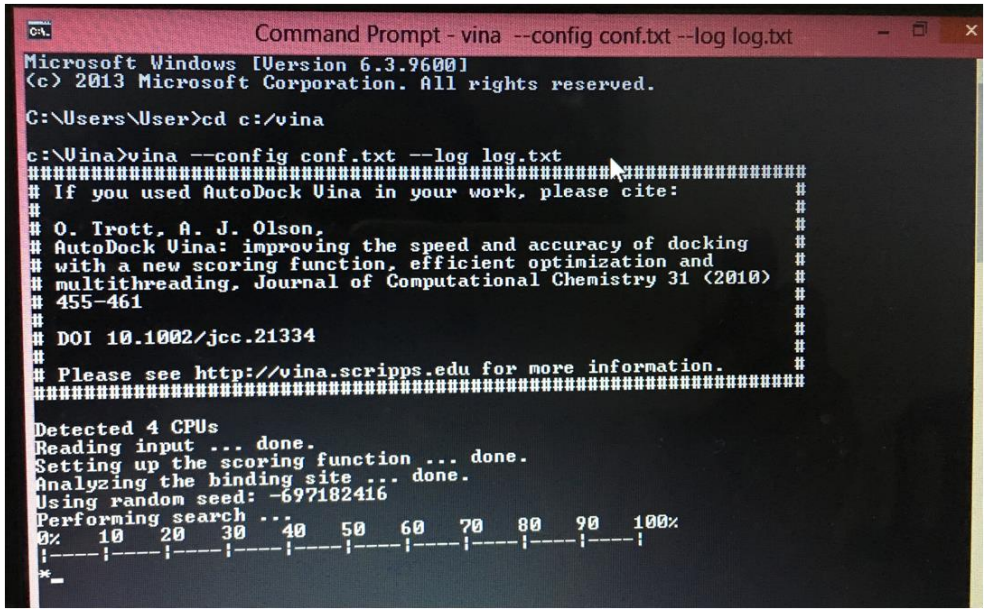
STEP 4: Now Open the command prompt and run the command.

In the fourth step open the command prompt and run the command. The commands given in the box.

C:/vina>vina --config conf.txt --log log.txt

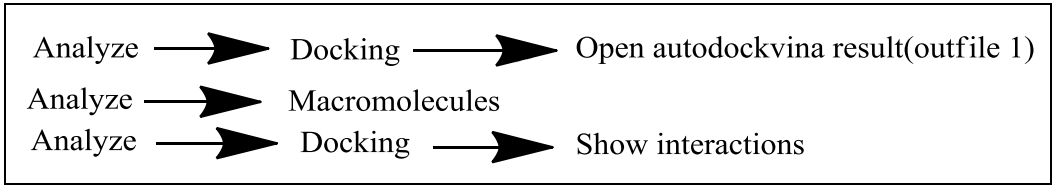
After running this command, put next command to split out file using vinasplit

Vina_split --input out.pdbqt

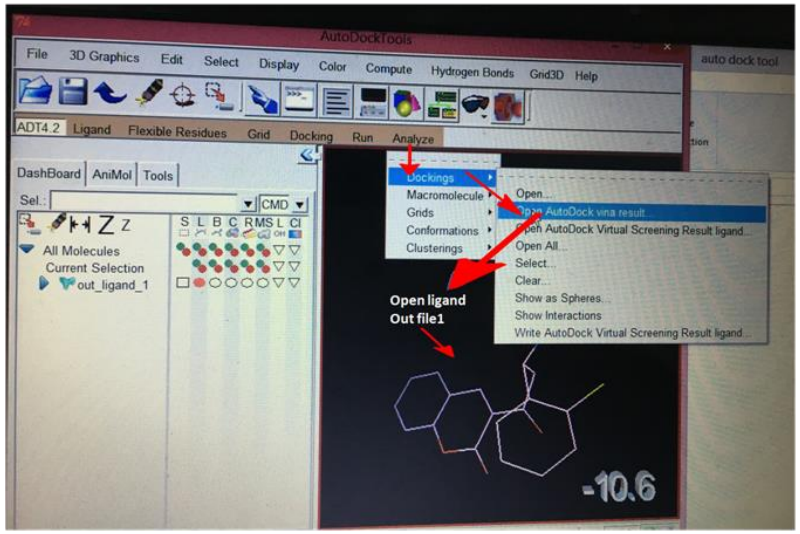


STEP 5: Analyse the docking interaction using autodock tool.

After finishing the vinasplit, analyse the docking interaction using autodock tool.



STEP 5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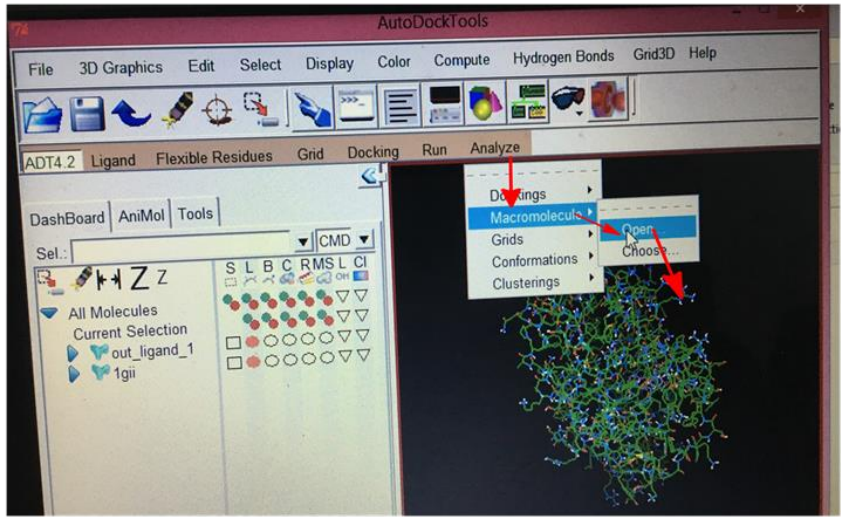
File 3D Graphics Edit Select Display Color Compute Hydrogen Bonds Grid3D Help
ADT4.2 Ligand Flexible Residues Grid Docking Run Analyze
DashBoard AniMol Tools
Sel.:
All Molecules
Current Selection
out_ligand_1
Dockings
Macromolecule
Grids
Conformations
Clusterings
Open...
Open AutoDock vina result...
Open AutoDock Virtual Screening Result ligand...
Open All...
Select...
Clear...
Show as Spheres...
Show Interactions
Write AutoDock Virtual Screening Result ligand...
Open ligand
Out file1
-10.6


AutoDockTools
File 3D Graphics Edit Select Display Color Compute Hydrogen Bonds Grid3D Help
ADT4.2 Ligand Flexible Residues Grid Docking Run Analyze
DashBoard AniMol Tools
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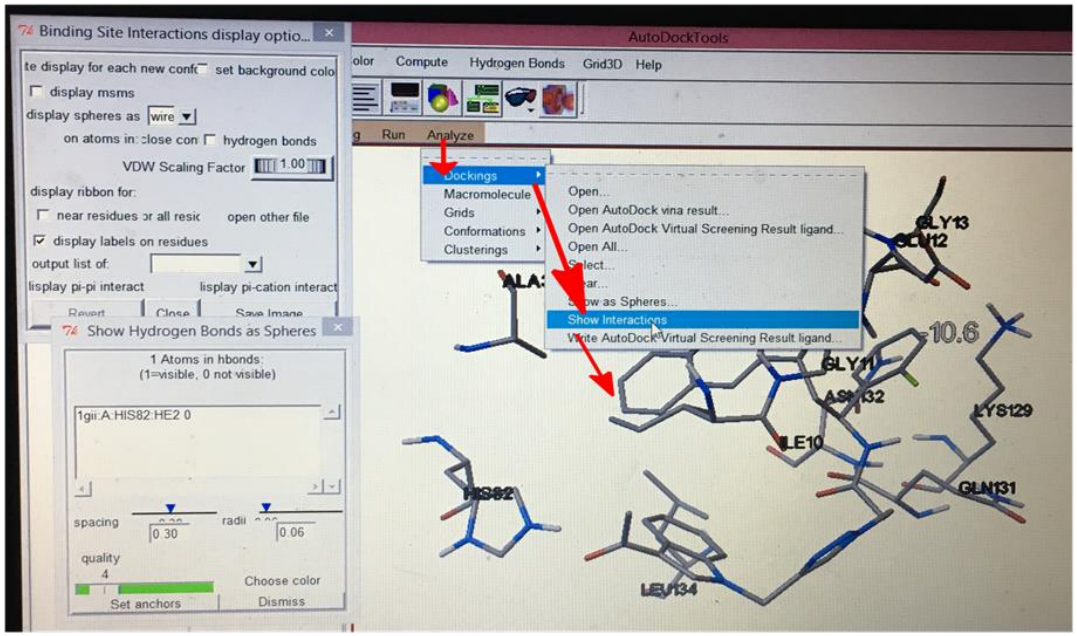
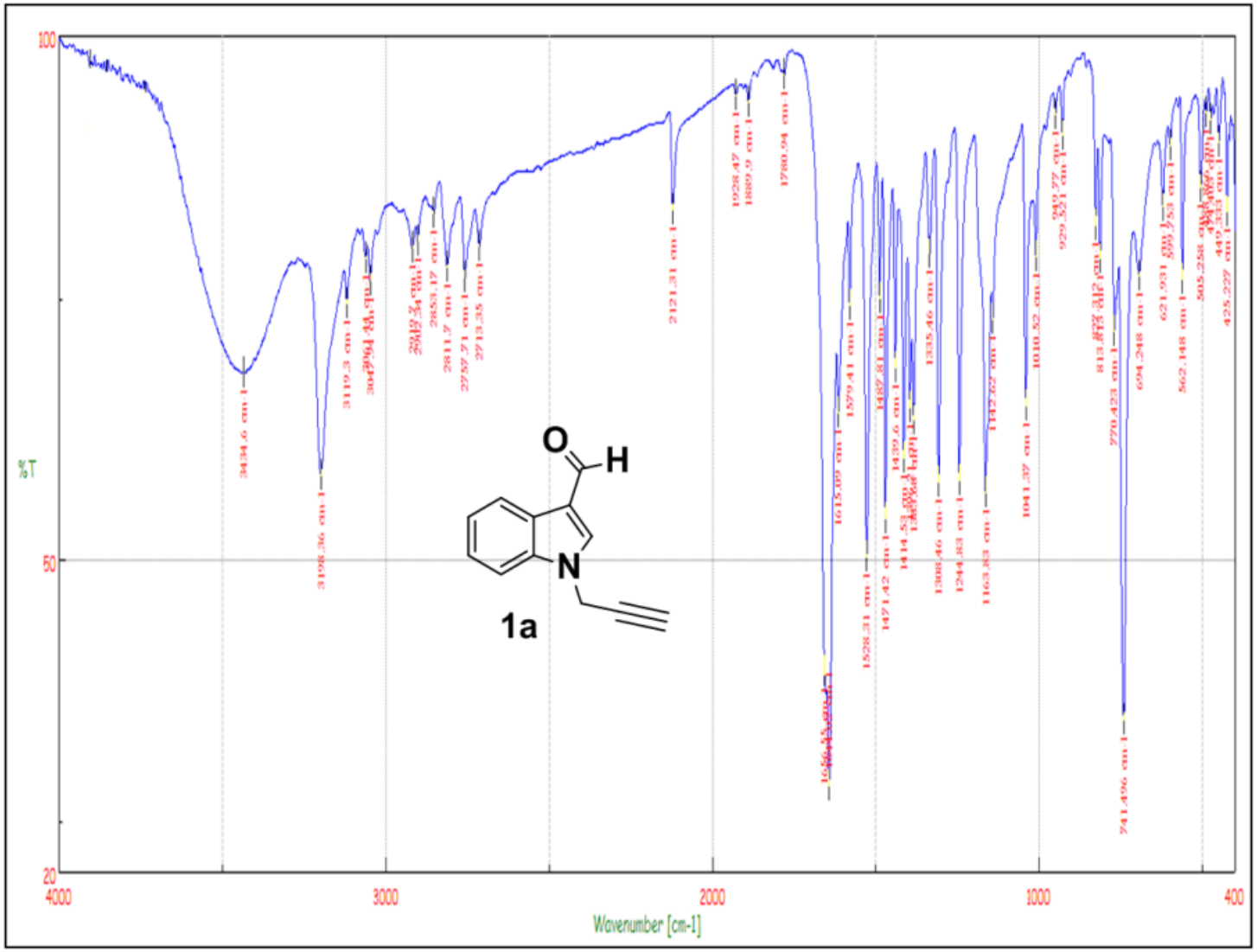
Current Selection
out_ligand_1
1gii
Dockings
Macromolecule
Grids
Conformations
Clusterings
Open...
Choose...


Binding Site Interactions display optio...
display msms
display spheres as wire
hydrogen bonds
VDW Scaling Factor 1.00
display ribbon for:
display labels on residues
output list of:
Show Hydrogen Bonds as Spheres
1 Atoms in hbonds:
(1=visible, 0 not visible)
1gii:A:HIS82:HE2 0
spacing 0.30
radii 0.06
quality 4
Choose color
Set anchors
Dismiss
AutoDockTools
Compute Hydrogen Bonds Grid3D Help
Run Analyze
Dockings
Macromolecule
Grids
Conformations
Clusterings
Open...
Open AutoDock vina result...
Open AutoDock Virtual Screening Result ligand...
Open All...
Show as Spheres...
Show Interactions
Write AutoDock Virtual Screening Result ligand...
GLY13
GLN12
10.6
GLY11
ASN32
LYS129
ILE10
HIS82
GLN131
LEU134

2.9. Western blot Analysis

Western blotting was performed to detect the proteins of CDK2. The MCF-7 cells (1×10^6) were seeded onto 100-mm culture dishes in the presence or absence of **6a** were treated for 48 hrs. Cells were then washed twice with ice-cold PBS and incubated in lysis buffer. The lysates were centrifuged at 10,000 × g for 5 min at 4 ◦C, and were used as the cell protein extracts. Each of the extracts was applied to 12% SDS polyacrylamide gel electrophoresis after which the proteins were transferred onto a nitrocellulose membrane, and then blocked for 1 h using 10% skim milk in water. After washing in a PBS containing 0.1% Tween 20 for 3 times, the primary antibodies were added at a v/v ratio of 1:1000. After overnight incubation at 4 ◦C, the primary antibodies were washed away and the secondary antibodies were added for 1 h incubation at room temperature. Finally, the enhanced chemiluminescence detection reagents were used to develop the signal of the membrane.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%T
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1000
400
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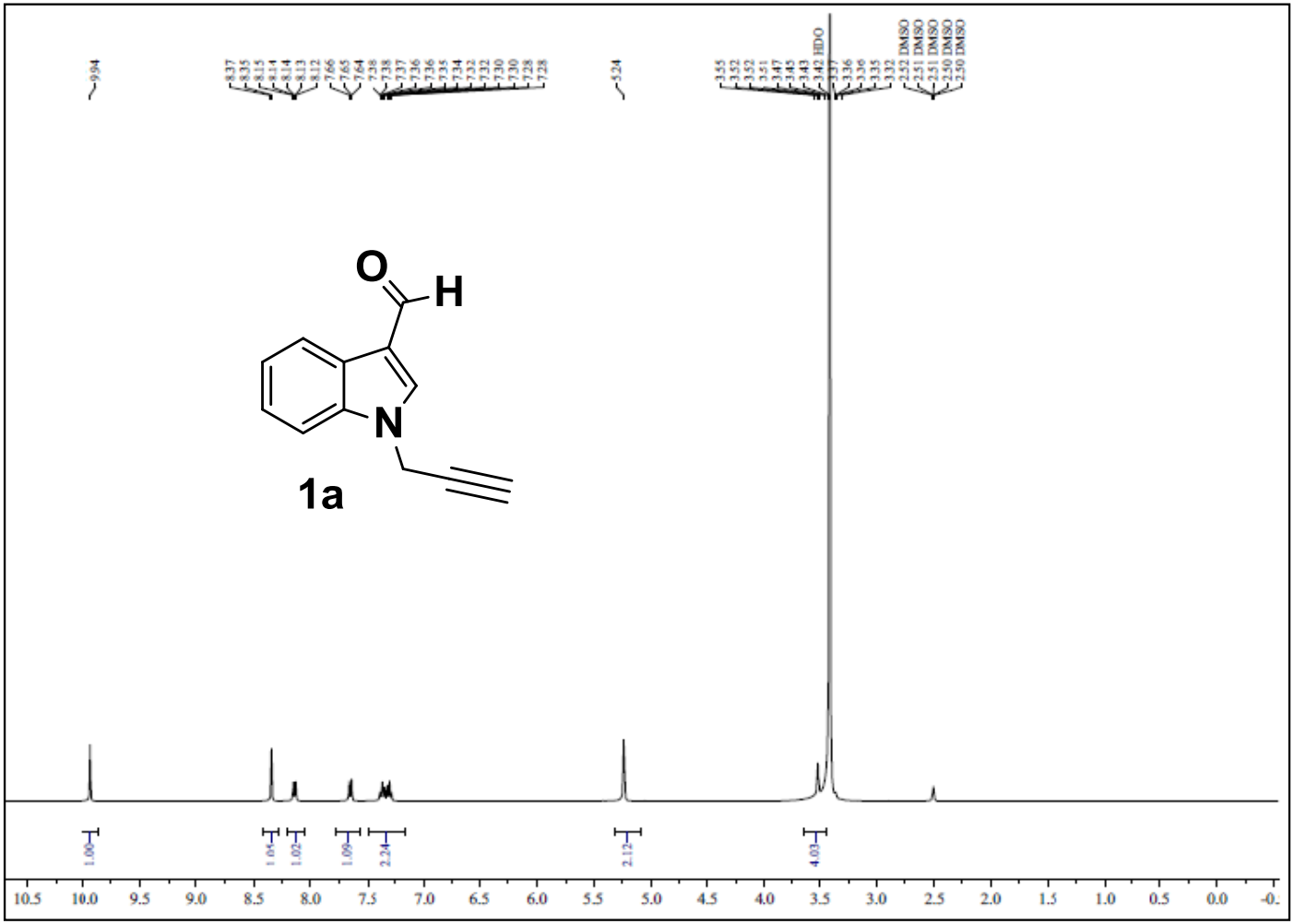
N
1a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N
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7.64
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7.38
7.37
7.36
7.36
7.35
7.34
7.32
7.32
7.30
7.30
7.28
7.28
5.24
3.55
3.52
3.52
3.51
3.47
3.45
3.43
3.42 HDO
3.37
3.36
3.36
3.35
3.32
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
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1.02
1.09
2.24
2.12
4.03
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9.5
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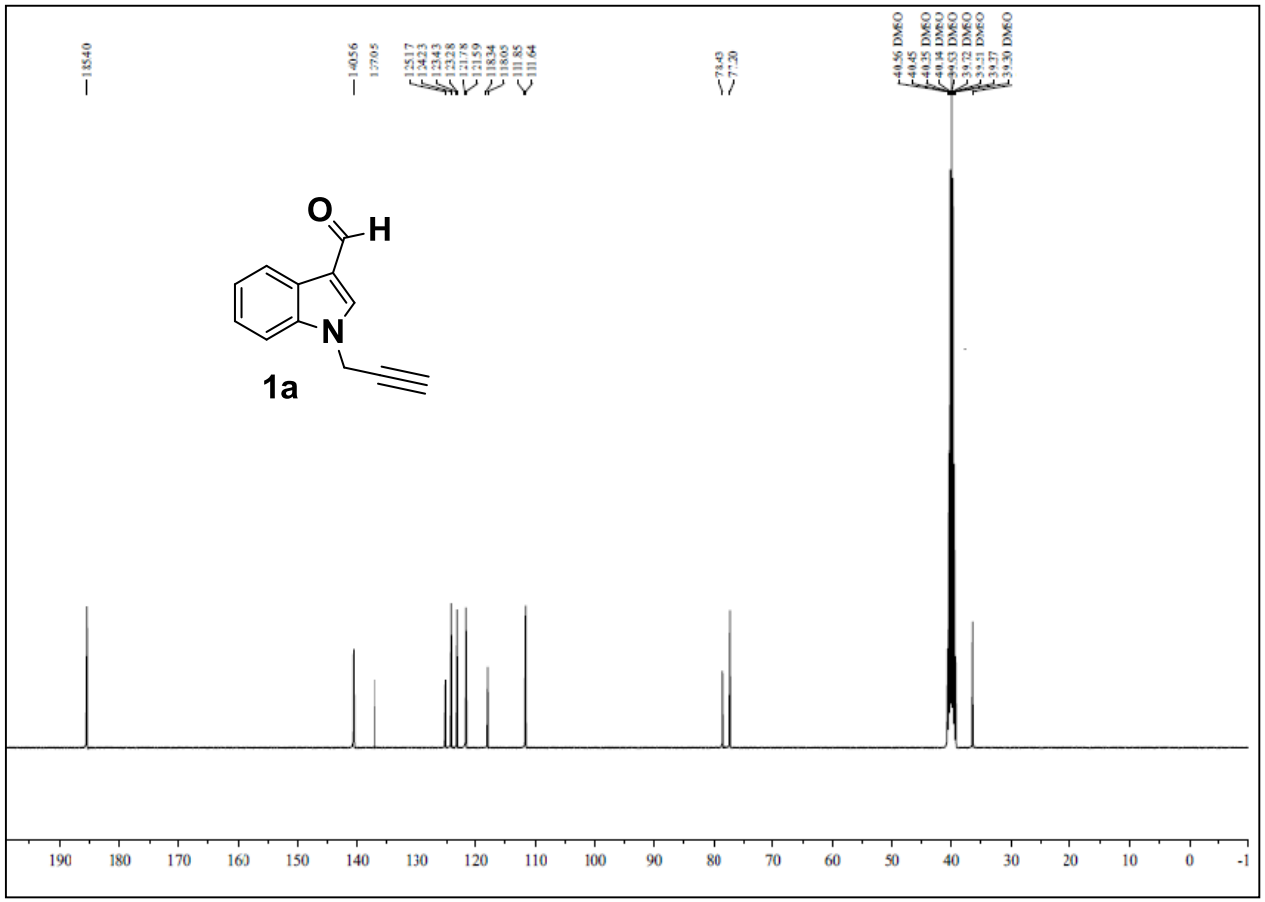
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N
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30
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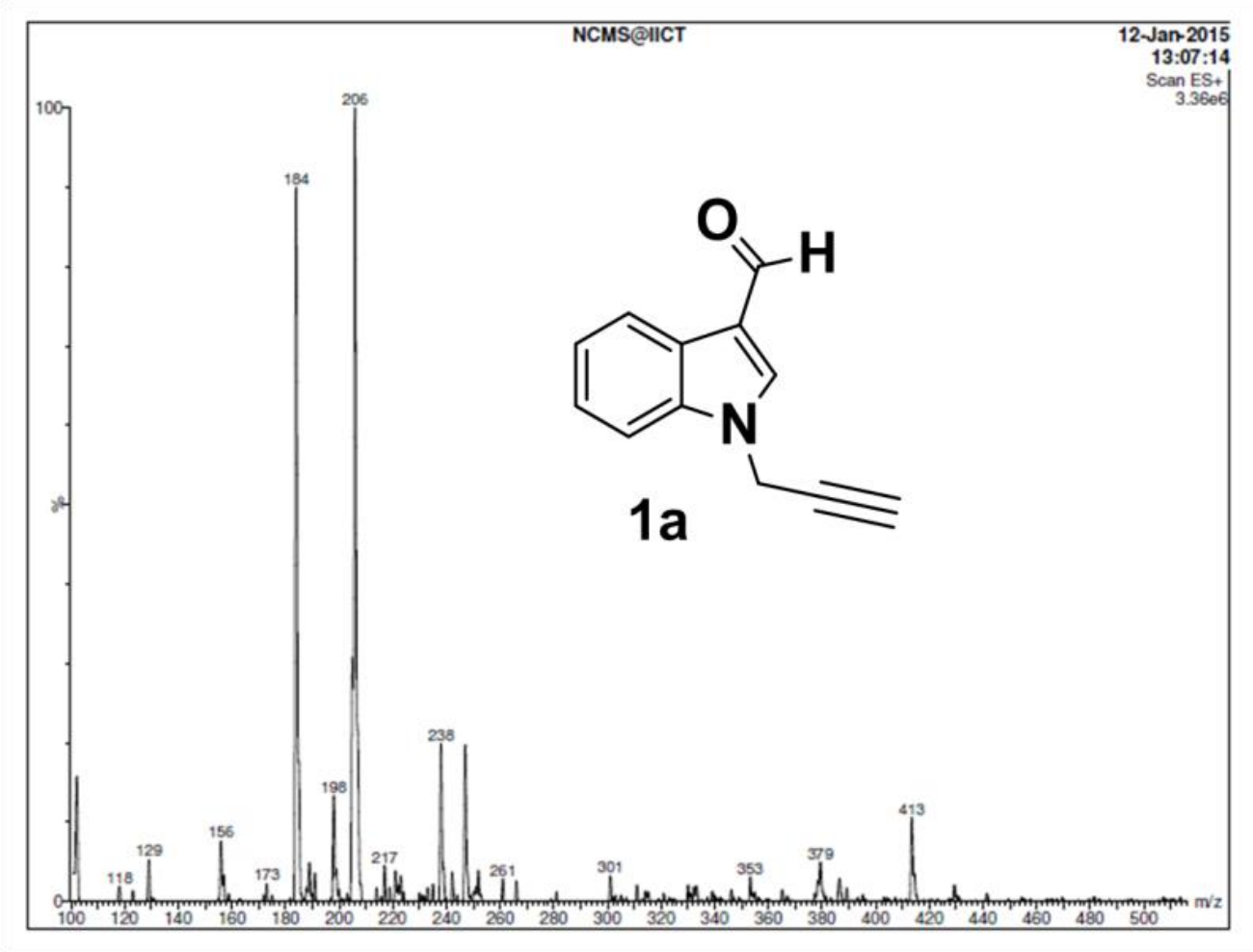
10
0
-1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m/z
O
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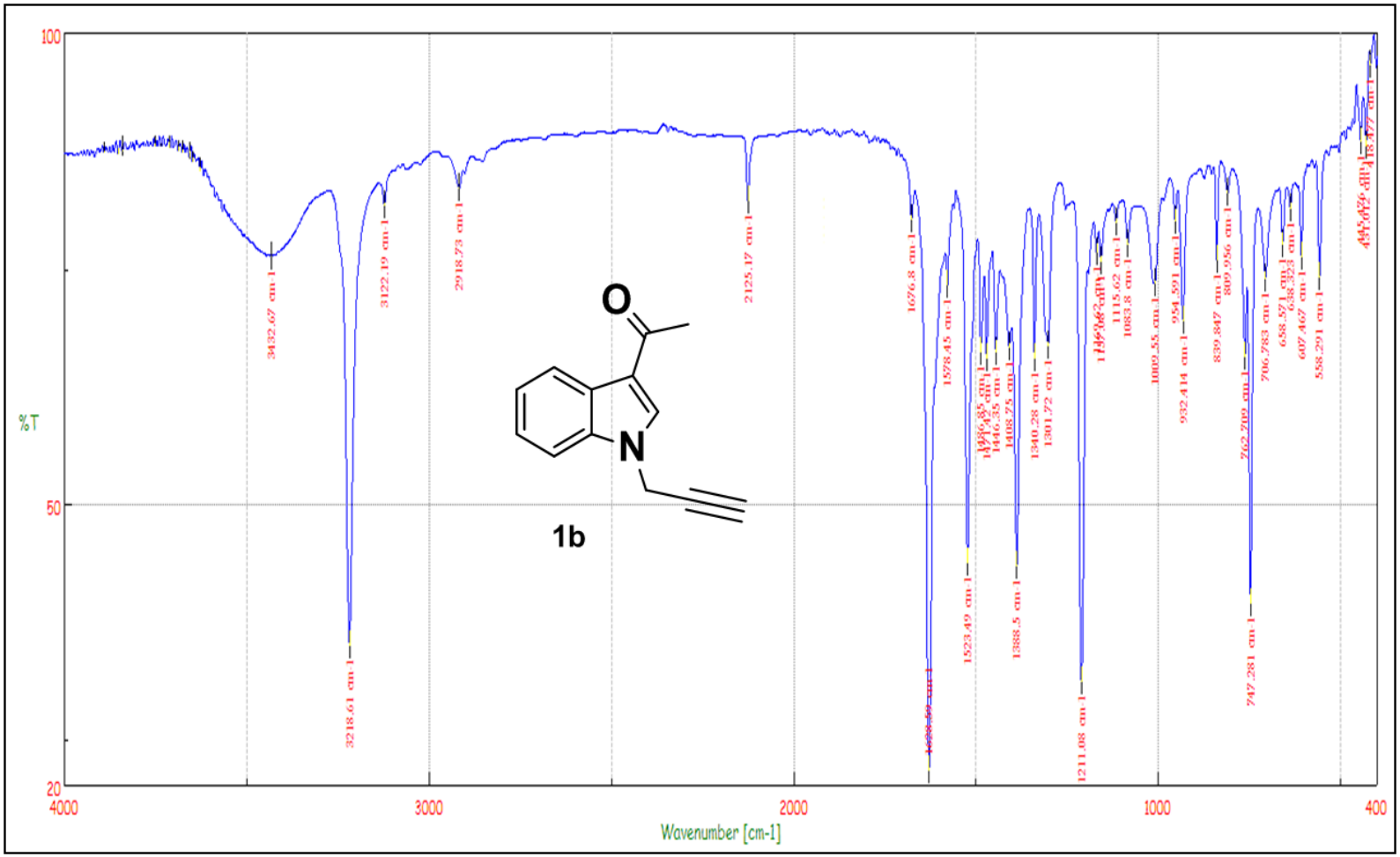
N
1a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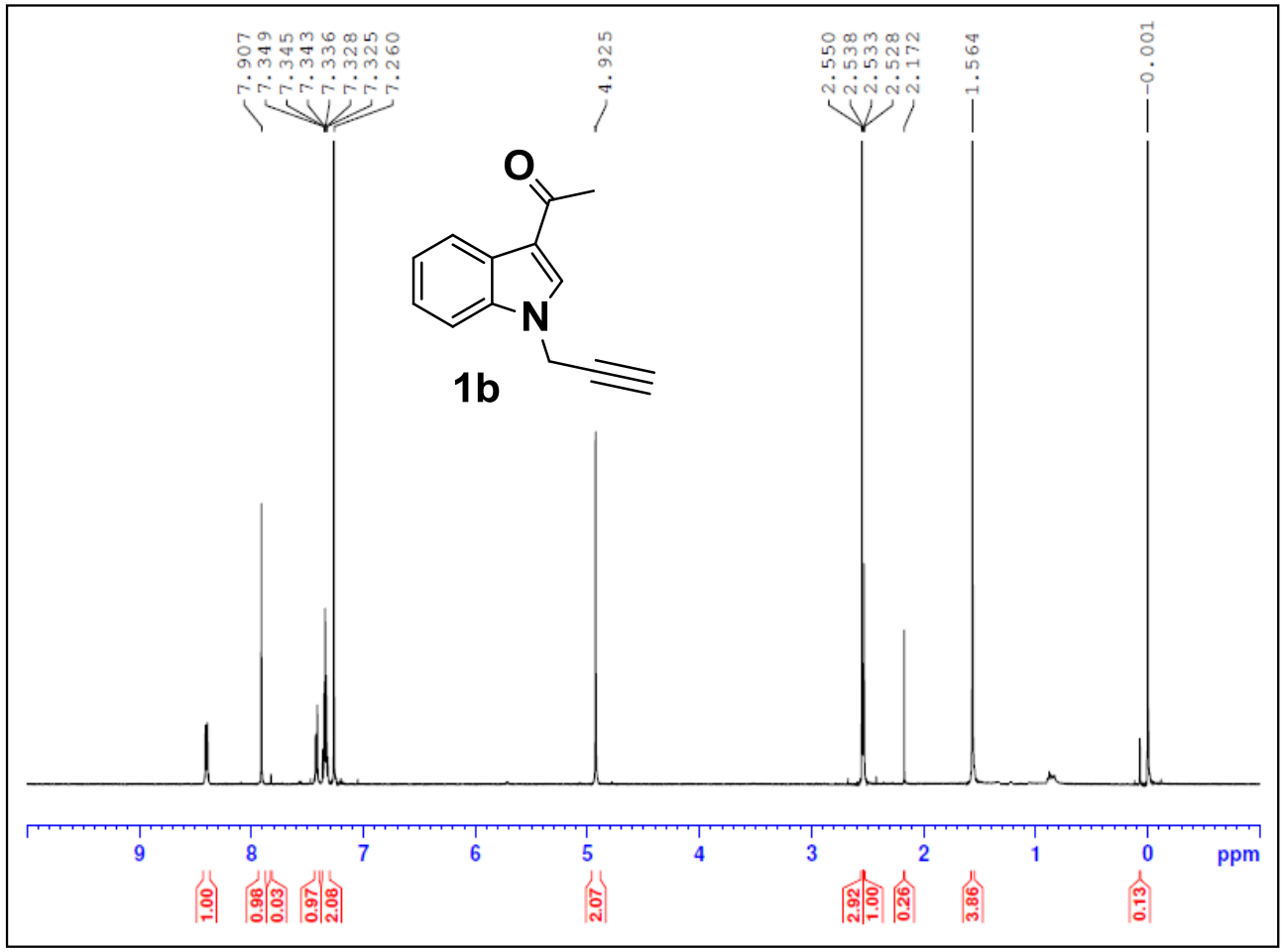
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1b
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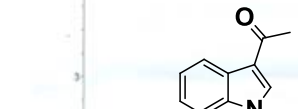
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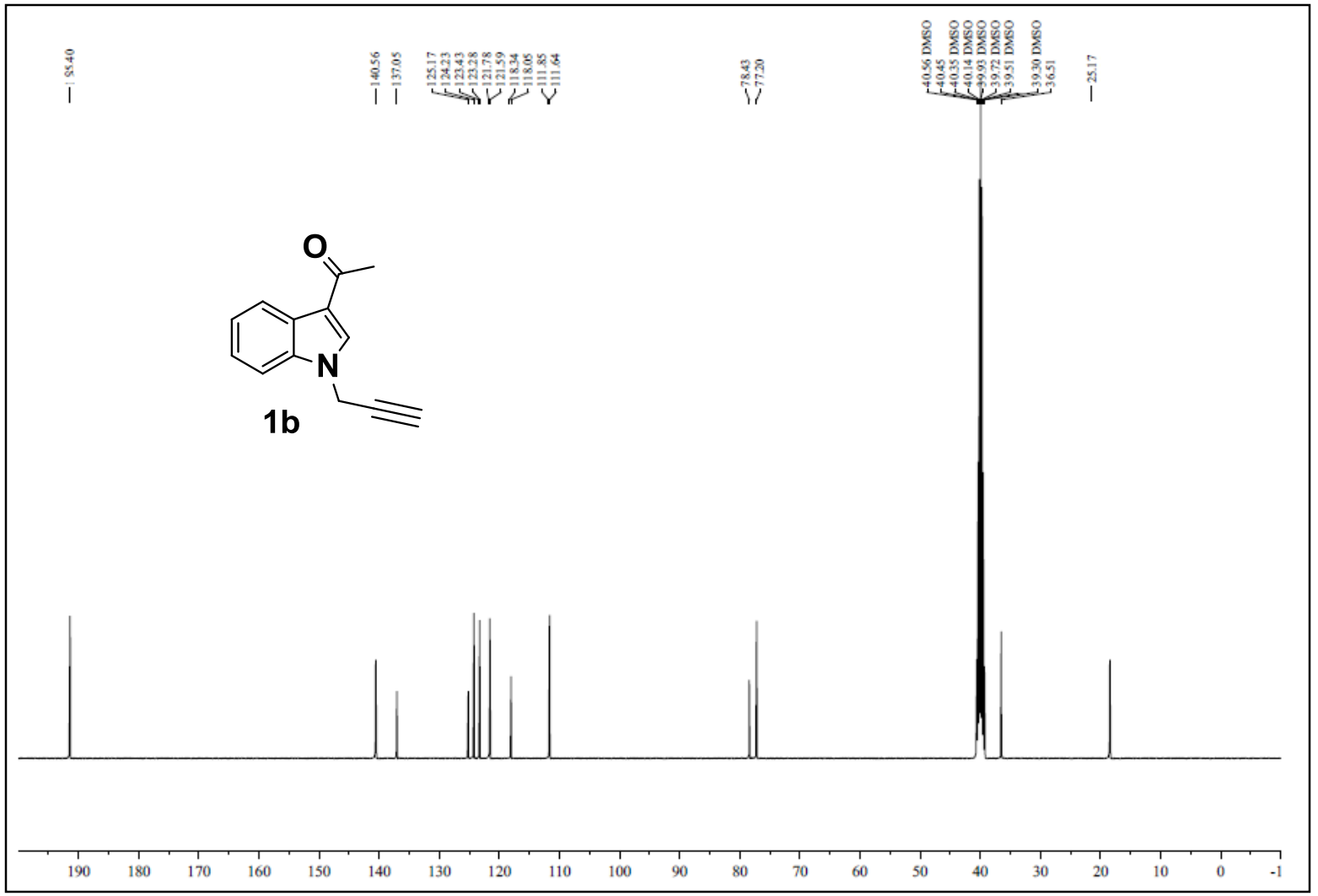
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78.43
77.20
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40.45
40.35 DMSO
40.14 DMSO
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39.72 DMSO
39.51 DMSO
39.30 DMSO
36.51
25.17
O
N
1b
190
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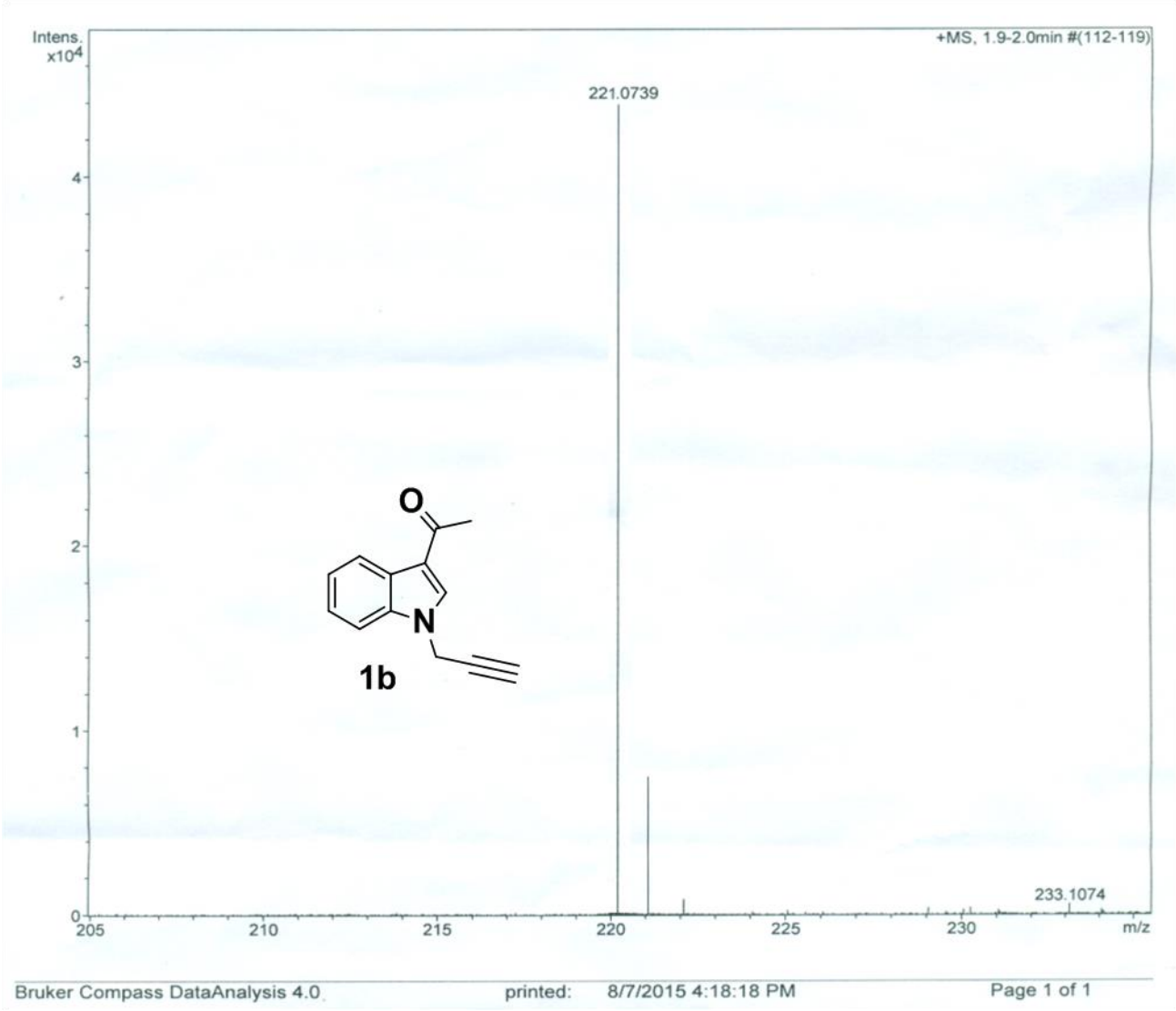
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2
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210
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220
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230
m/z
O
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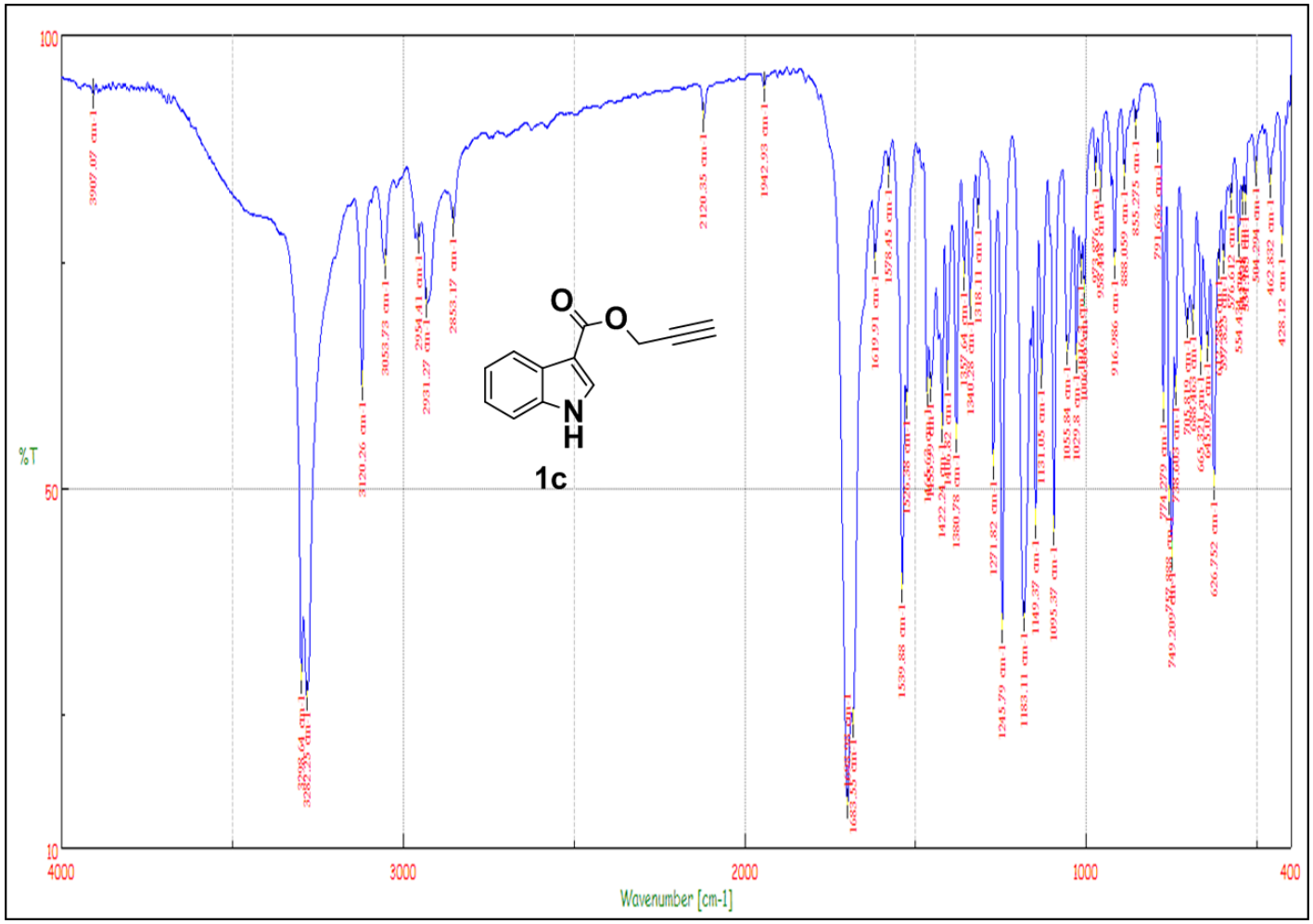
1b
Bruker Compass DataAnalysis 4.0
printed: 8/7/2015 4:18:18 PM
Page 1 of 1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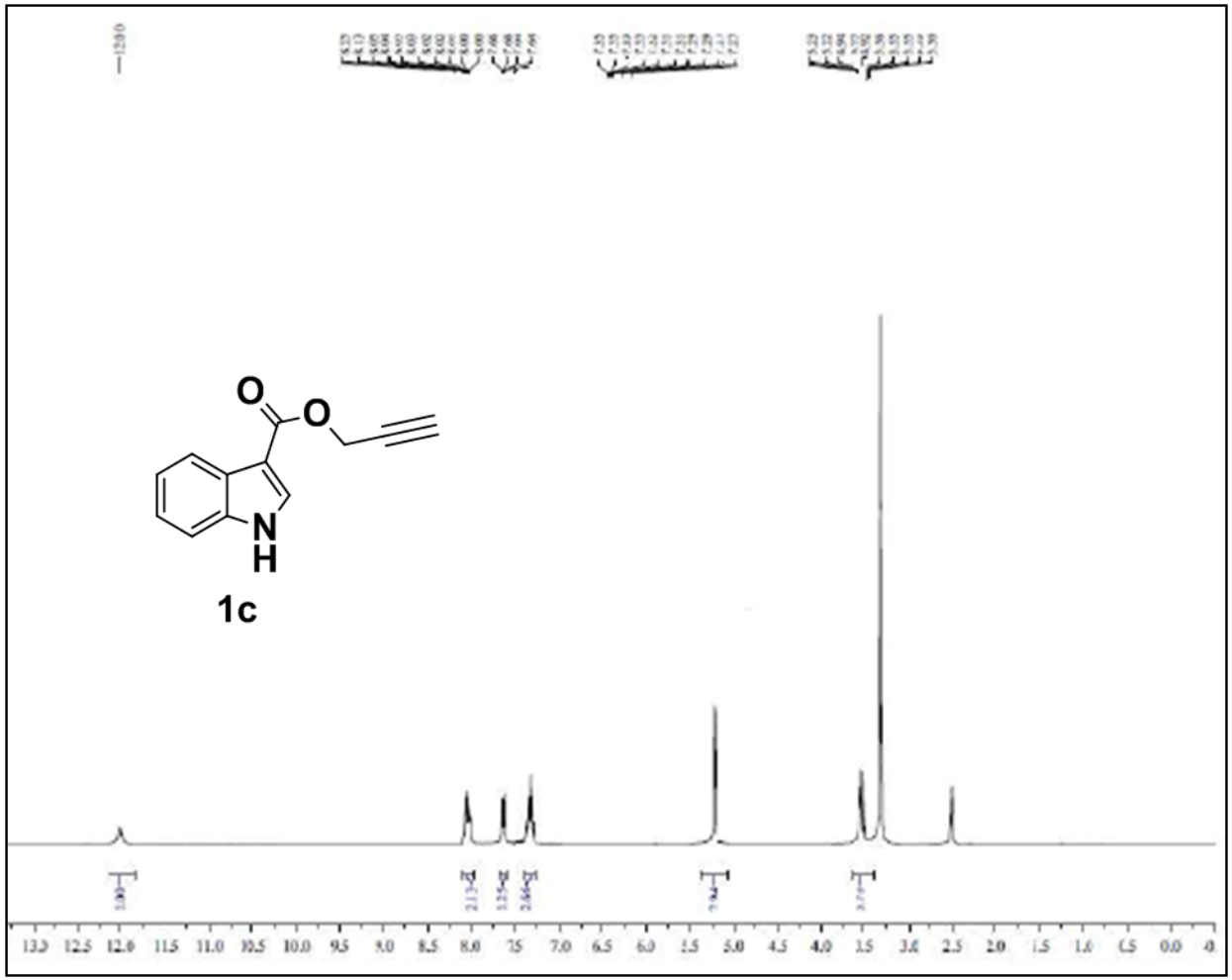
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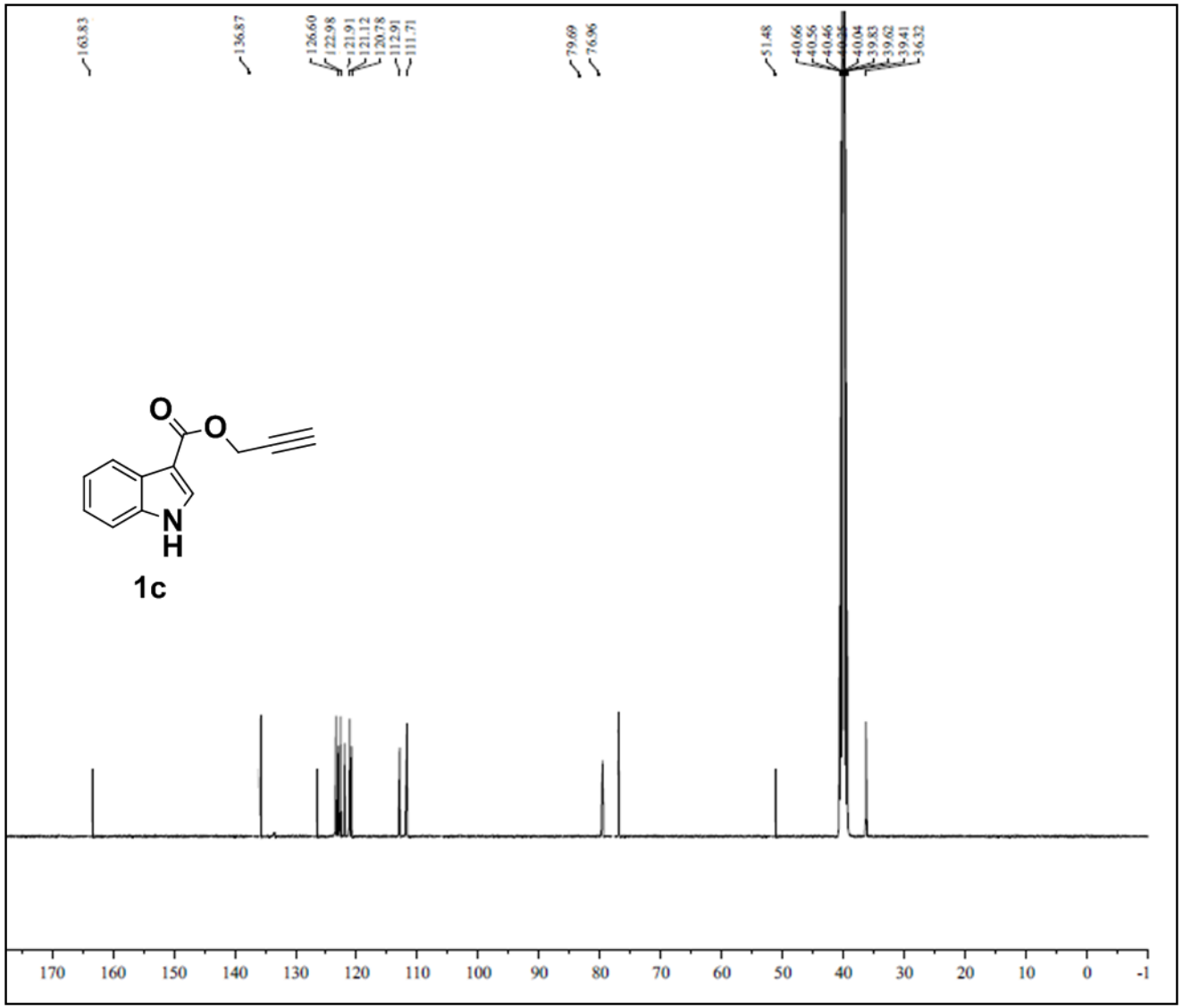
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O
N
H
1c
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0
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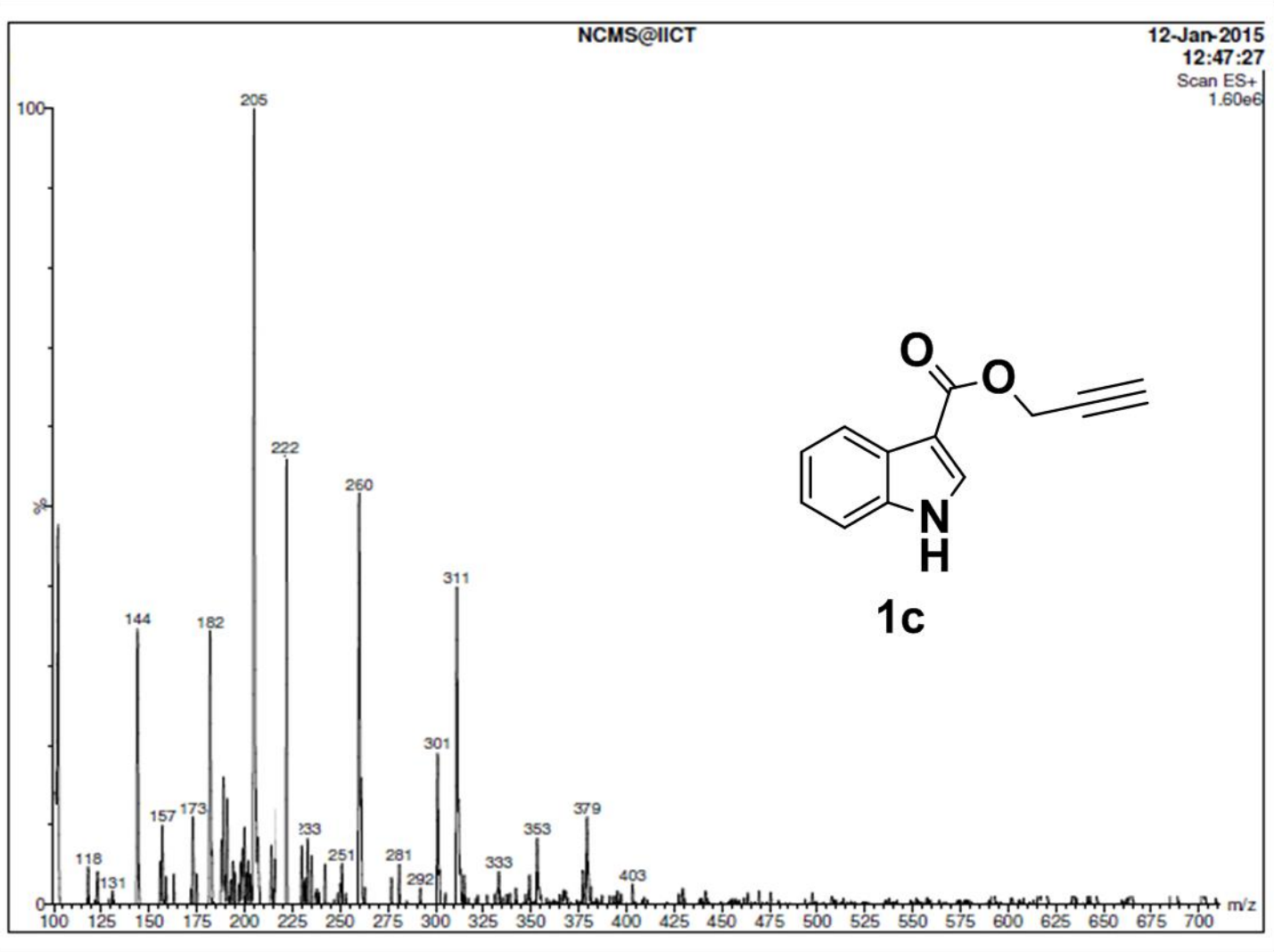
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12-Jan-2015
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%
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301
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173
233
251
281
292
333
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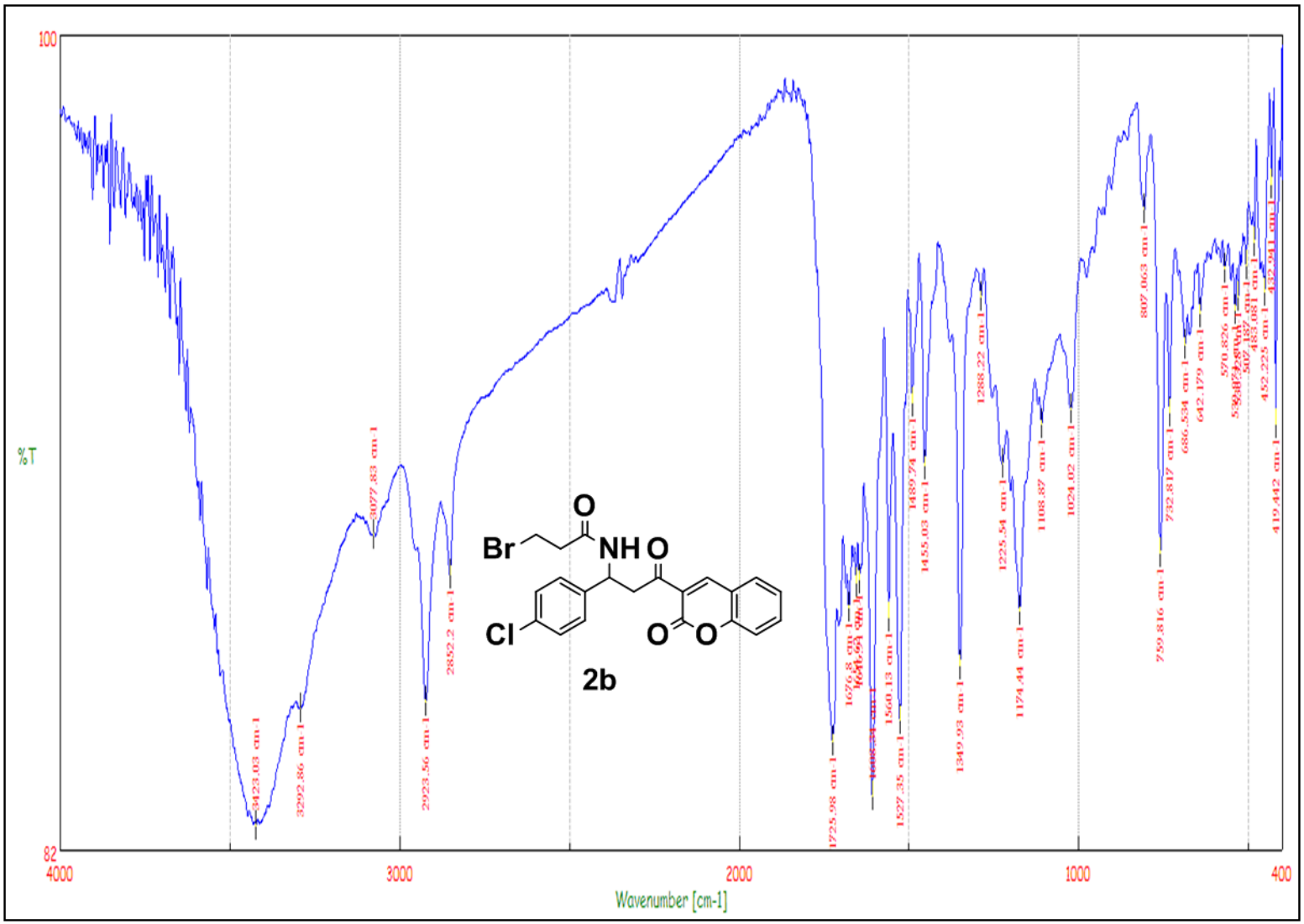
m/z
O
O
N
H
1c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400
Wavenumber [cm-1]
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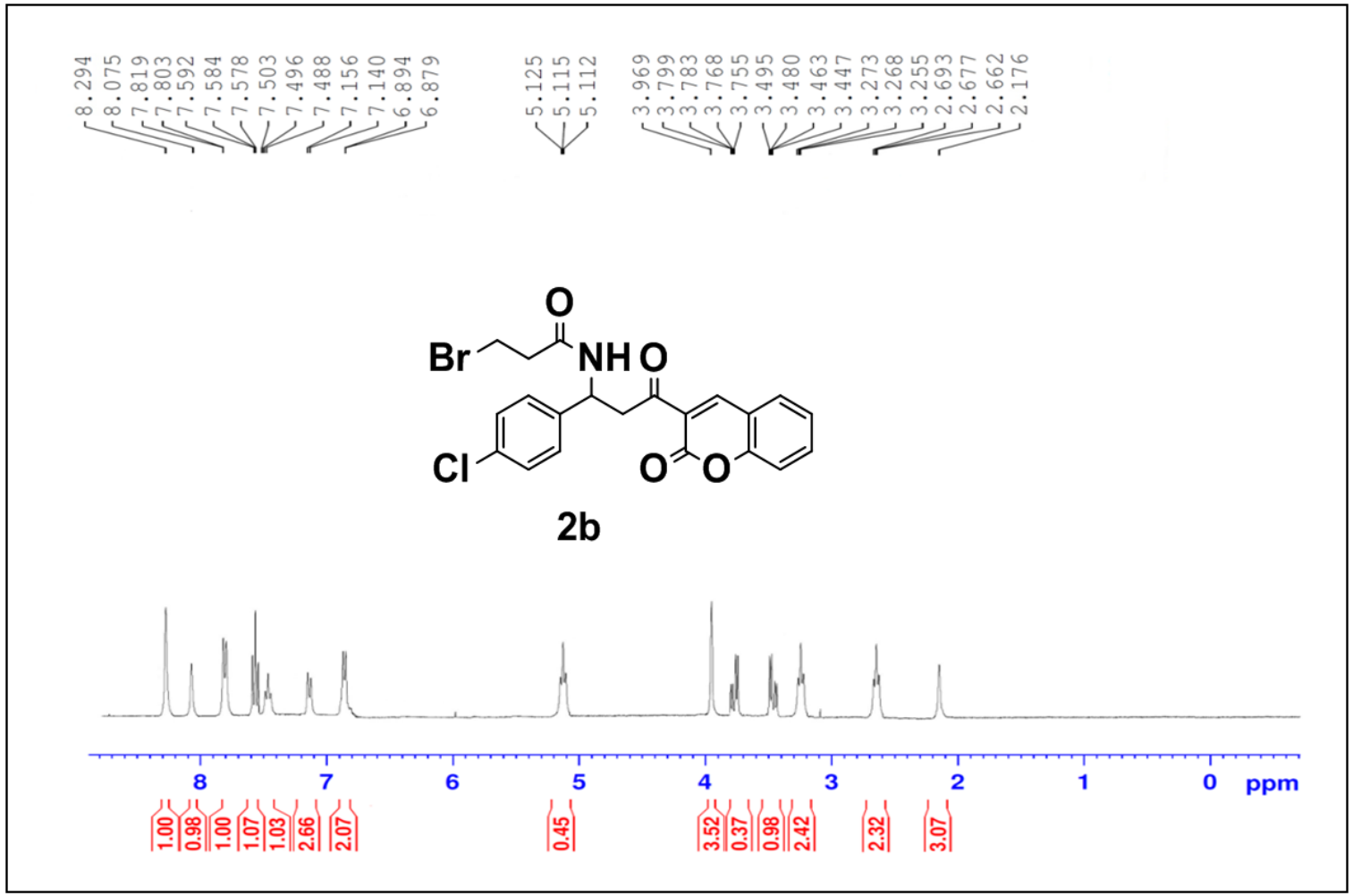
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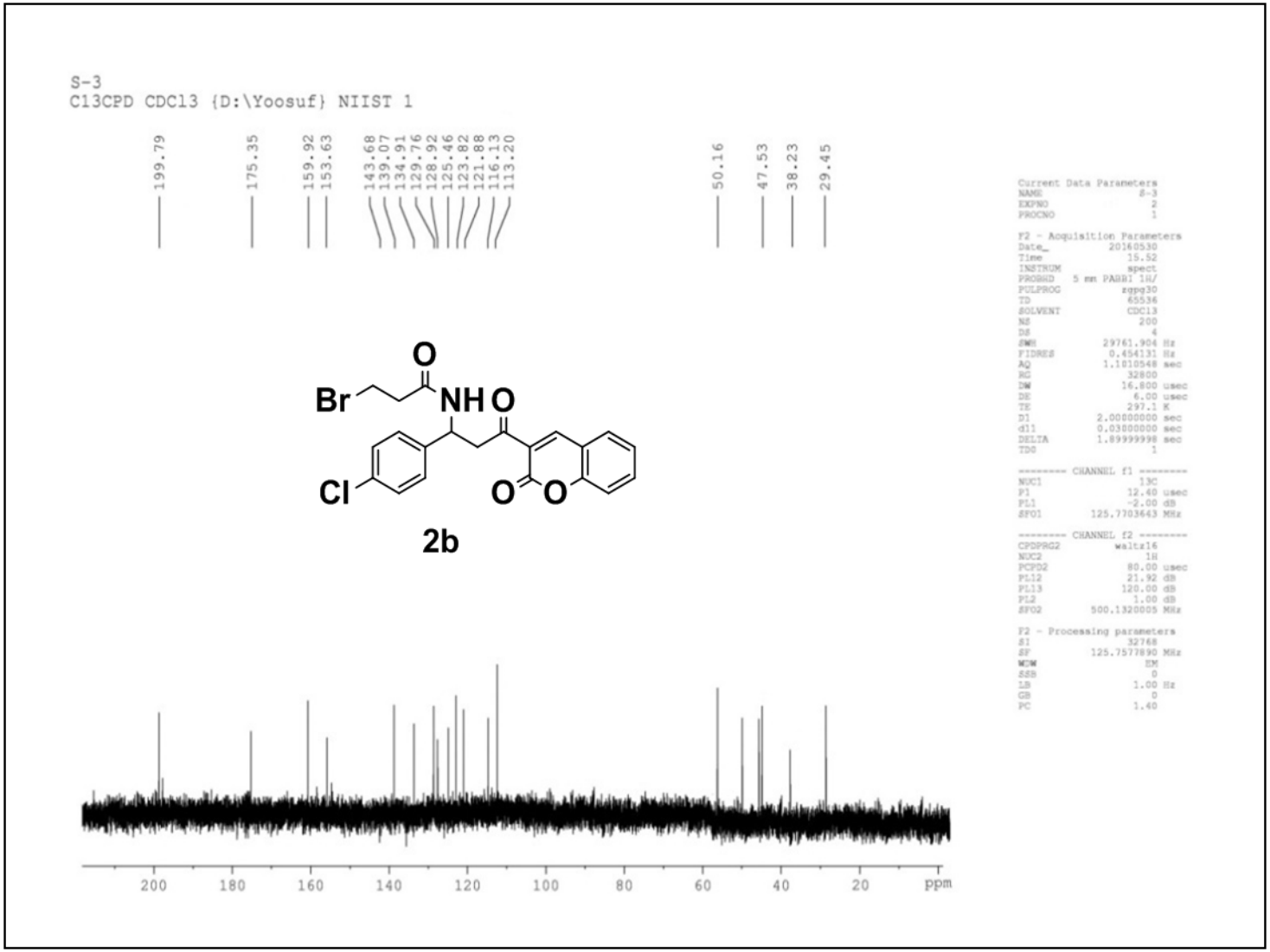
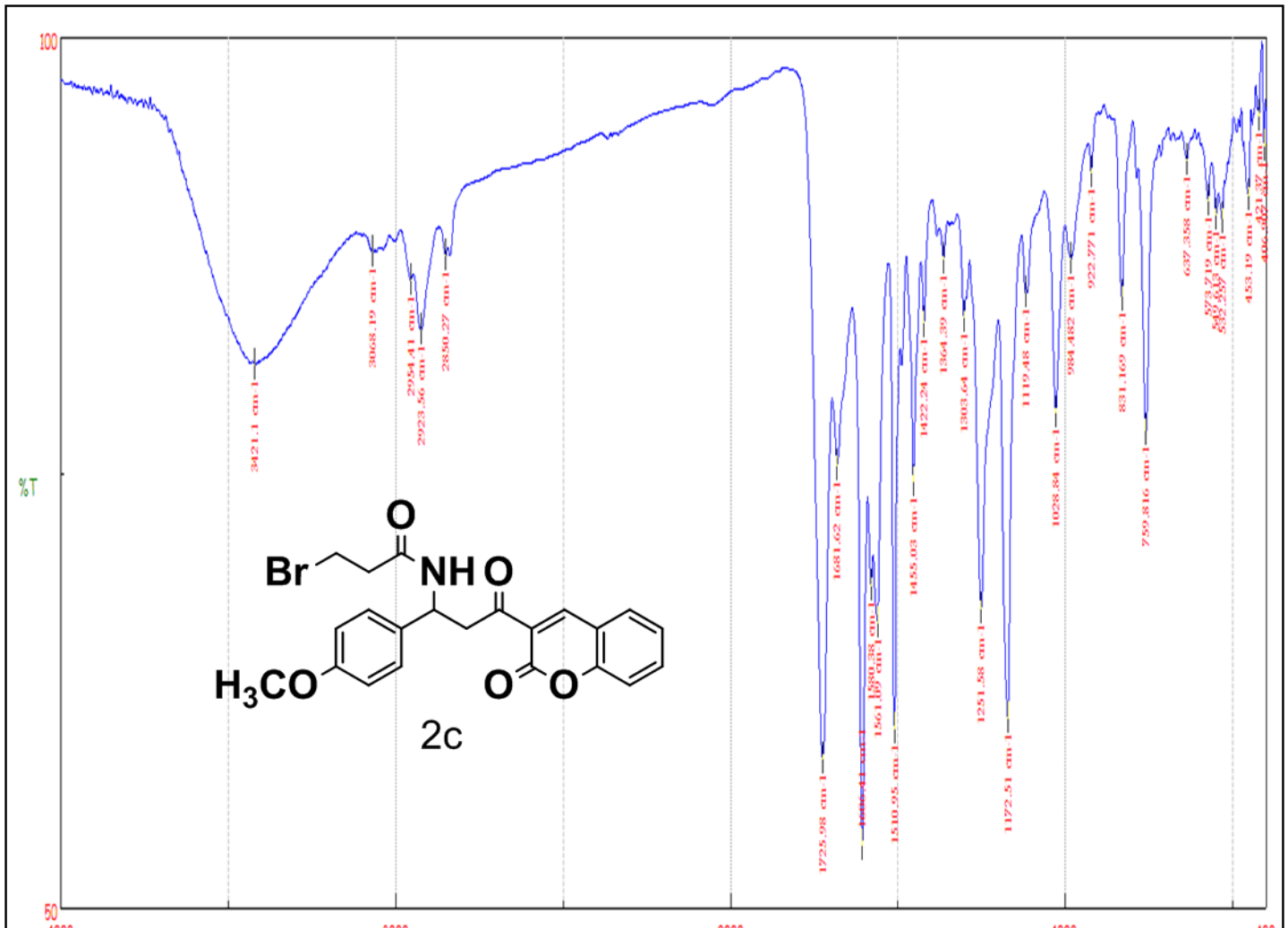
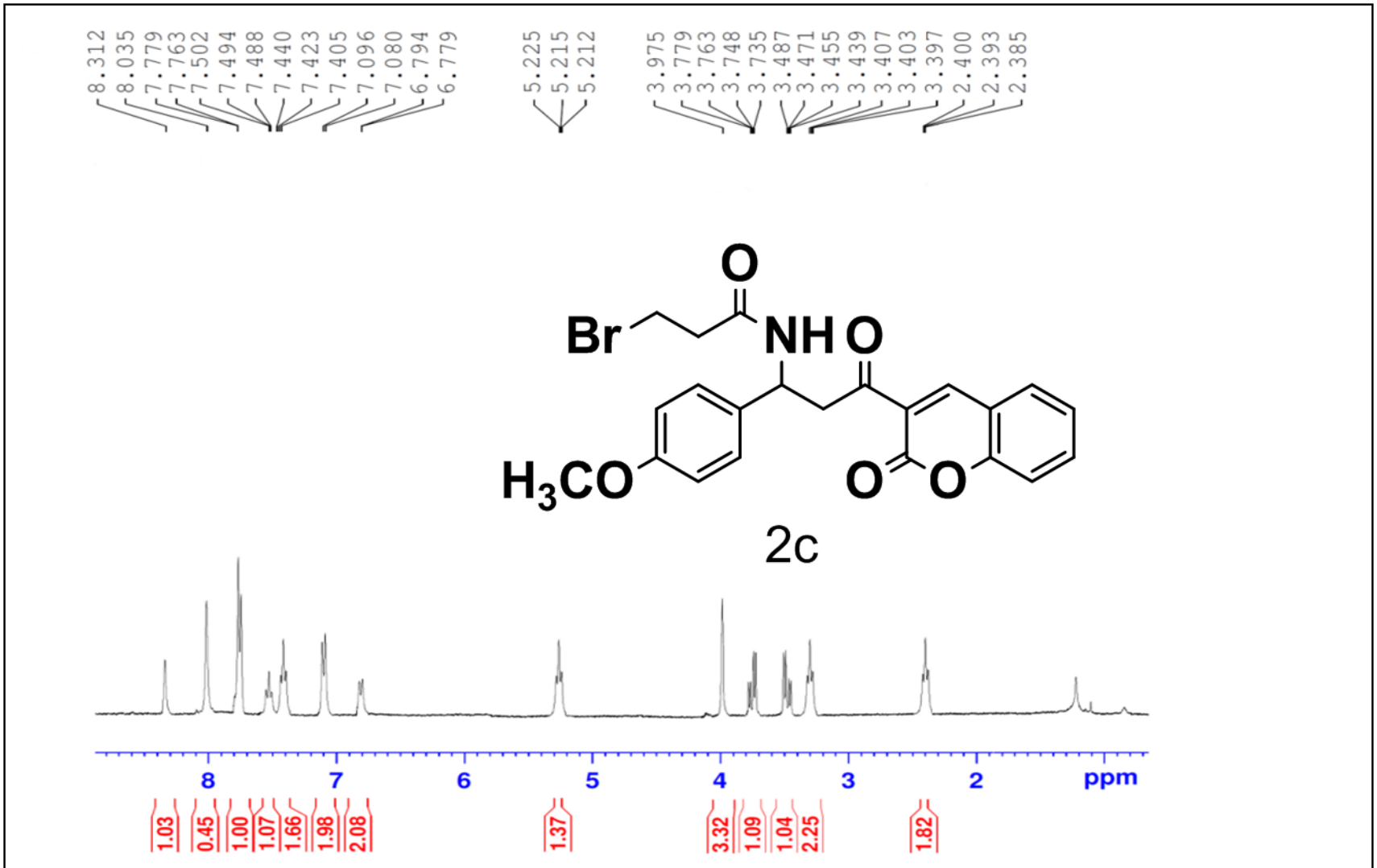
7.819
7.803
7.592
7.584
7.578
7.503
7.496
7.488
7.156
7.140
6.894
6.879
5.125
5.115
5.112
3.969
3.799
3.783
3.768
3.755
3.495
3.480
3.463
3.447
3.273
3.268
3.255
2.693
2.677
2.662
2.176
O
Br
NH
O
Cl
O
O
2b
8
7
6
5
4
3
2
1
0
ppm
1.00
0.98
1.00
1.07
1.03
2.66
2.07
0.45
3.52
0.37
0.98
2.42
2.32
3.07

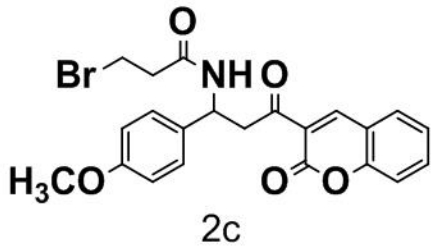

S-3
C13CPD CDCl3 {D:\Yoosuf} NIIST 1
199.79
175.35
159.92
153.63
143.68
139.07
134.91
129.76
128.92
125.46
123.82
121.88
116.13
113.20
50.16
47.53
38.23
29.45
Br
NH
O
Cl
O
O
2b
200
180
160
140
120
100
80
60
40
20
ppm
Current Data Parameters
NAME S-3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160530
Time 15.52
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 200
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 32800
DW 16.800 usec
DE 6.00 usec
TE 297.1 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 12.40 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL12 21.92 dB
PL13 120.00 dB
PL2 1.00 dB
SFO2 500.1320005 MHz
F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


100
%T
50
3421.1 cm-1
3068.19 cm-1
2954.41 cm-1
2923.56 cm-1
2850.27 cm-1
1725.98 cm-1
1681.62 cm-1
1510.95 cm-1
1455.03 cm-1
1422.24 cm-1
1364.39 cm-1
1303.64 cm-1
1251.58 cm-1
1172.51 cm-1
1119.48 cm-1
1028.84 cm-1
984.482 cm-1
922.771 cm-1
831.169 cm-1
759.816 cm-1
637.358 cm-1
573.219 cm-1
532.557 cm-1
453.19 cm-1
Br
NH
O
O
O
O
H3CO
2c

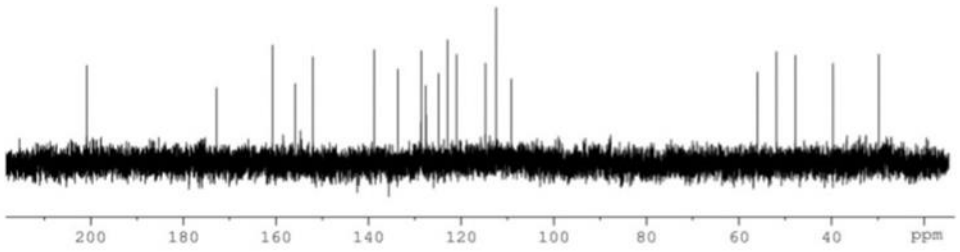

8.312
8.035
7.779
7.763
7.502
7.494
7.488
7.440
7.423
7.405
7.096
7.080
6.794
6.779
5.225
5.215
5.212
3.975
3.779
3.763
3.748
3.735
3.487
3.471
3.455
3.439
3.407
3.403
3.397
2.400
2.393
2.385
O
Br
NH
O
H3CO
O
O
2c
8
7
6
5
4
3
2
ppm
1.03
0.45
1.00
1.07
1.66
1.98
2.08
1.37
3.32
1.09
1.04
2.25
1.82

S-1
C13CPD CDCl3 {D:\Yoosuf} NIIST 7

200.59
172.05
160.86
156.06
153.63
139.77
134.28
129.76
128.98
125.75
123.72
121.88
118.23
114.23
109.20
56.28
51.06
47.69
39.57
29.08



2c



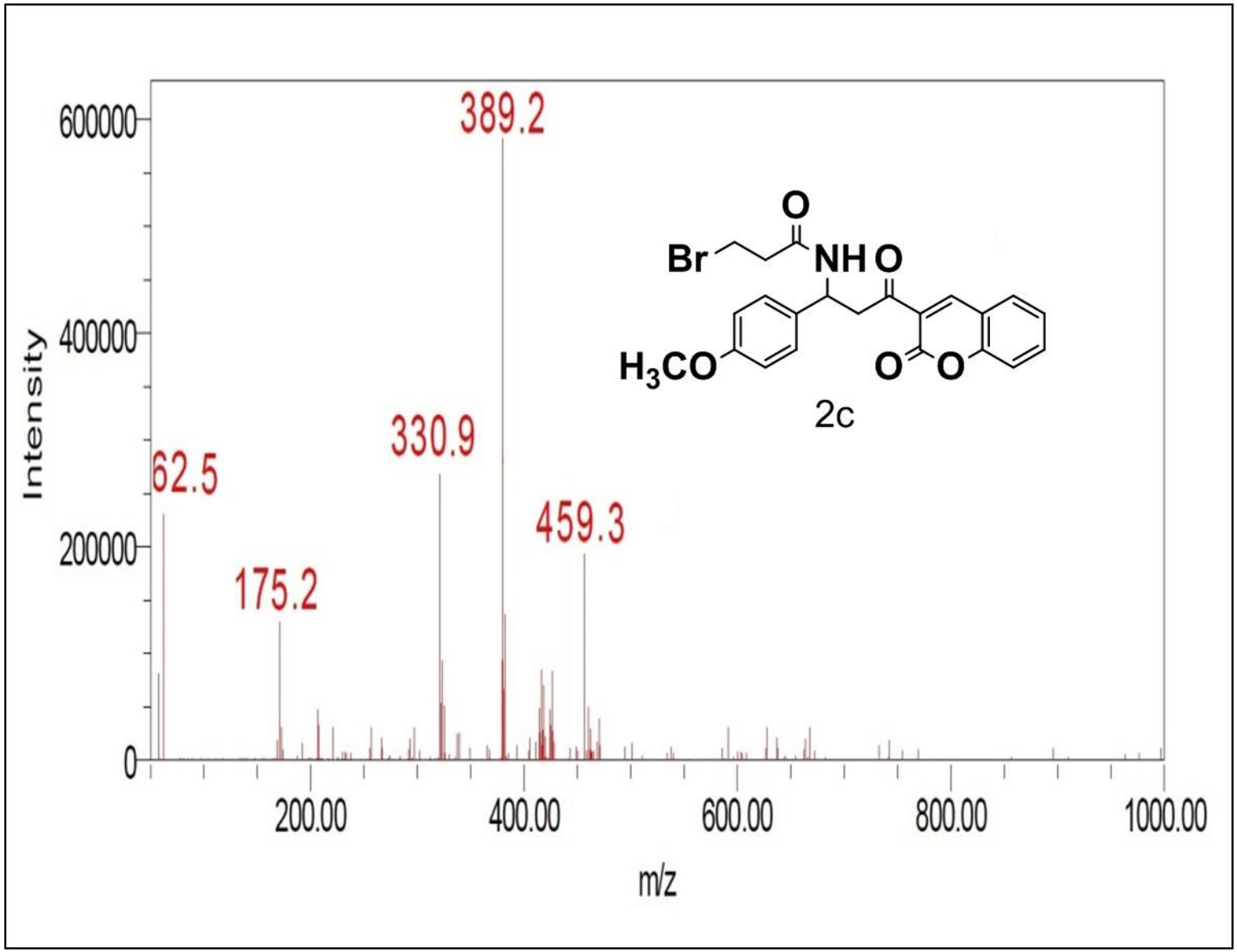
Current Data Parameters
NAME 8-1
EXPNO 2
PROCNO 1

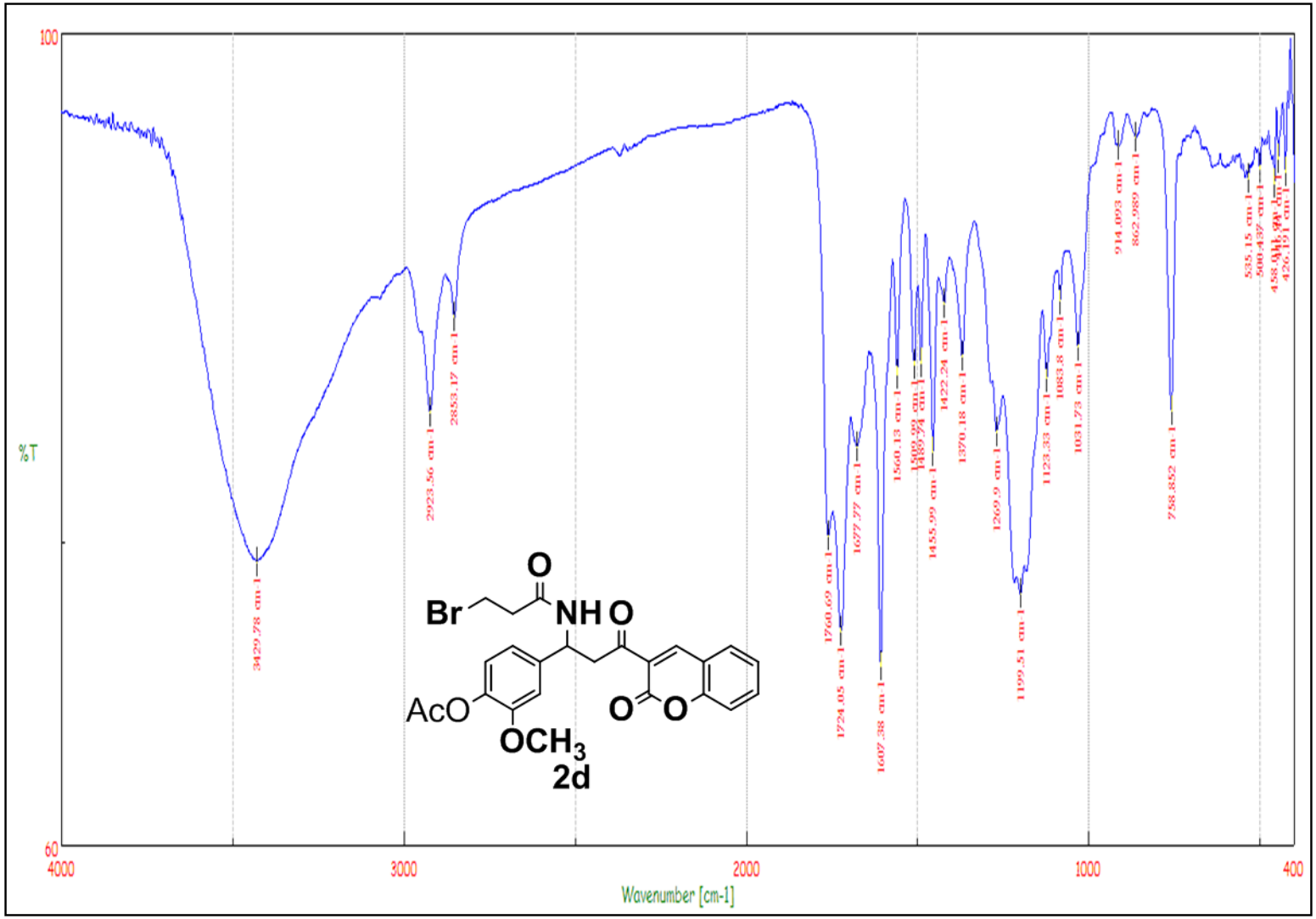
F2 - Acquisition Parameters
Date_ 20160530
Time 15.52
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 200
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 32800
DW 16.800 usec
DE 6.00 usec
TE 297.1 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1

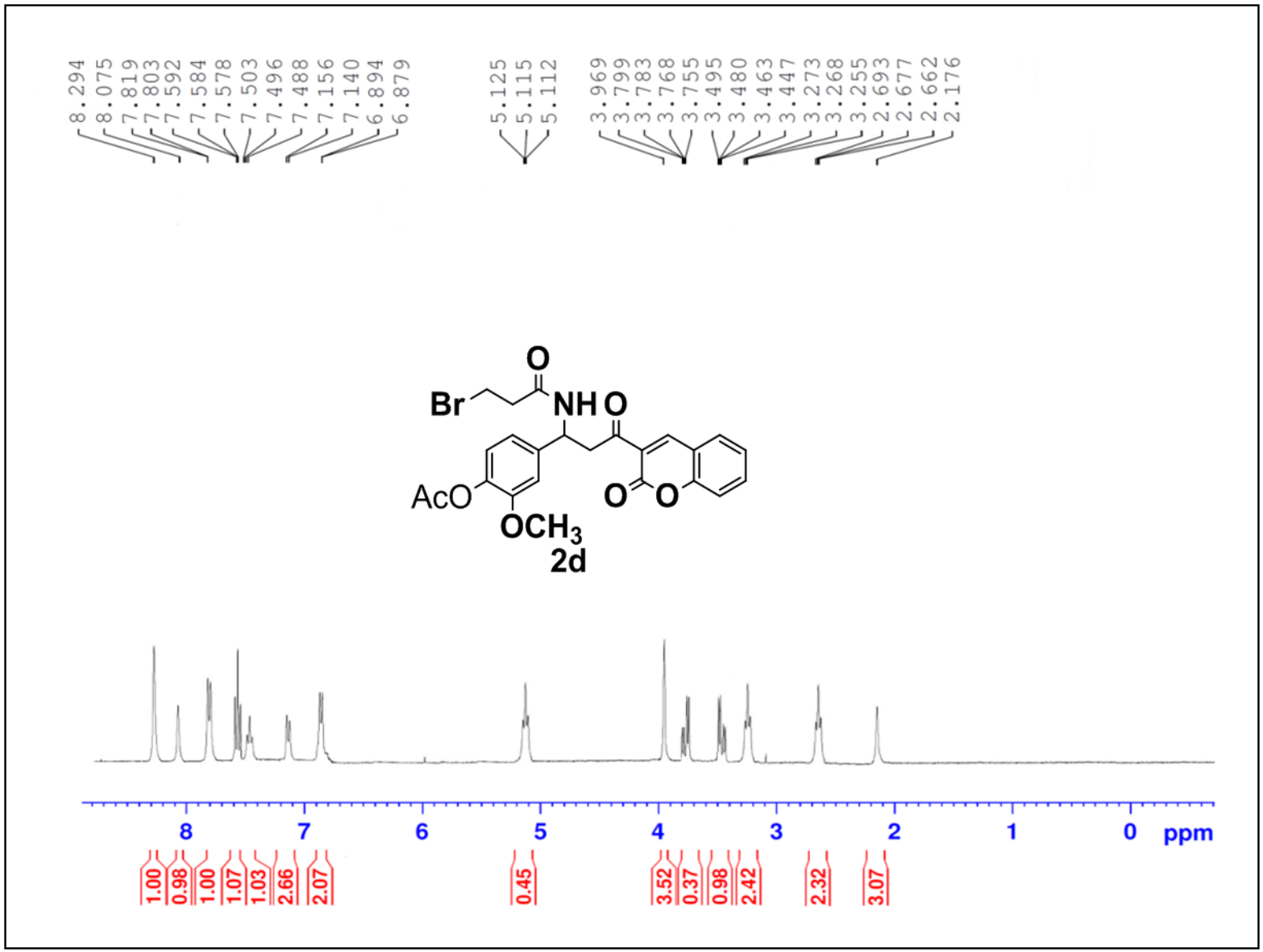
======== CHANNEL f1 ========
NUC1 13C
P1 12.40 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL12 21.92 dB
PL13 120.00 dB
PL2 1.00 dB
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

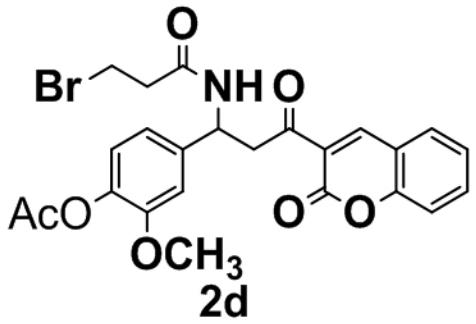

389.2
330.9
62.5
459.3
175.2
Intensity
600000
400000
200000
0
200.00
400.00
600.00
800.00
1000.00
m/z
O
Br
NH
O
H3CO
O
O
2c


100
%T
60
4000
3000
2000
1000
400
Wavenumber [cm-1]
3429.78 cm-1
2923.56 cm-1
2853.17 cm-1
1760.69 cm-1
1724.05 cm-1
1677.77 cm-1
1607.38 cm-1
1560.13 cm-1
1509.99 cm-1
1489.94 cm-1
1455.99 cm-1
1422.24 cm-1
1370.18 cm-1
1269.9 cm-1
1199.51 cm-1
1123.33 cm-1
1083.8 cm-1
1031.73 cm-1
914.093 cm-1
862.989 cm-1
758.852 cm-1
535.15 cm-1
500.437 cm-1
458.9 cm-1
426.191 cm-1
Br
O
NH
O
AcO
O
O
OCH3
2d

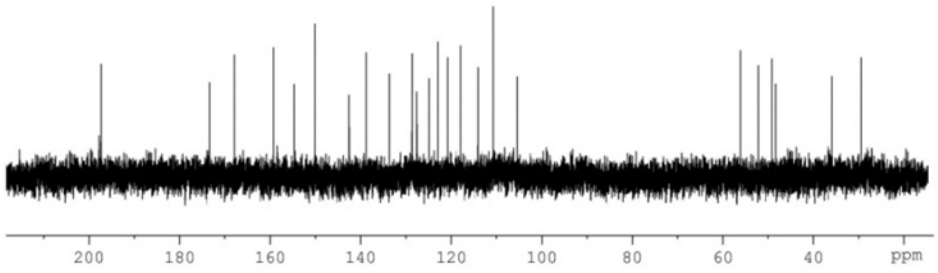

8.294
8.075
7.819
7.803
7.592
7.584
7.578
7.503
7.496
7.488
7.156
7.140
6.894
6.879
5.125
5.115
5.112
3.969
3.799
3.783
3.768
3.755
3.495
3.480
3.463
3.447
3.273
3.268
3.255
2.693
2.677
2.662
2.176
Br
NH
O
AcO
OCH3
2d
8
7
6
5
4
3
2
1
0
ppm
1.00
0.98
1.00
1.07
1.03
2.66
2.07
0.45
3.52
0.37
0.98
2.42
2.32
3.07

S-16
C13CPD CDCl3 {D:\Yoosuf} NIIST 16

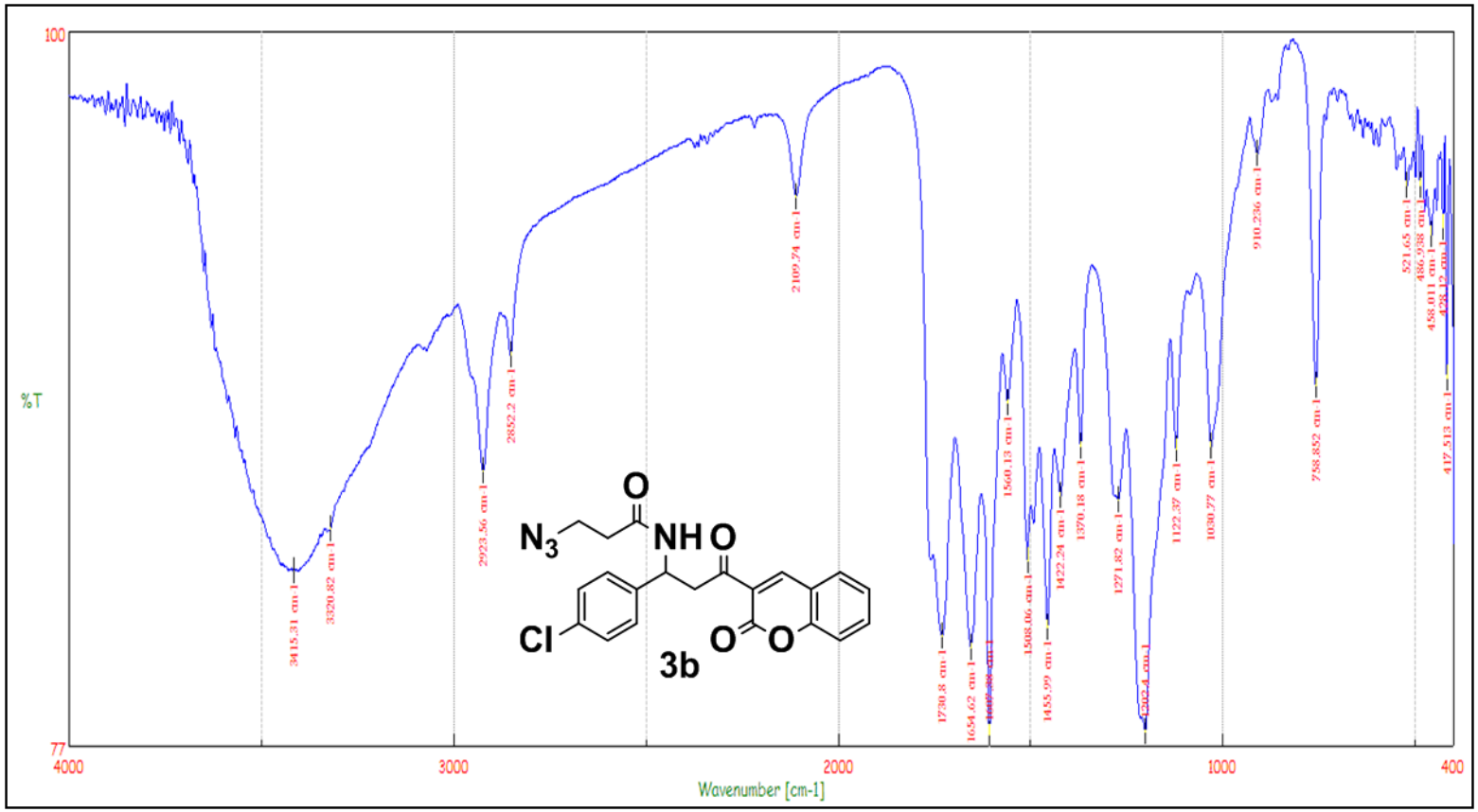
199.59
172.77
168.06
159.02
155.75
150.82
142.77
139.04
134.91
129.96
128.98
125.05
122.72
120.88
119.23
114.20
110.29
106.22
56.16
52.67
49.61
47.62
37.66
29.96

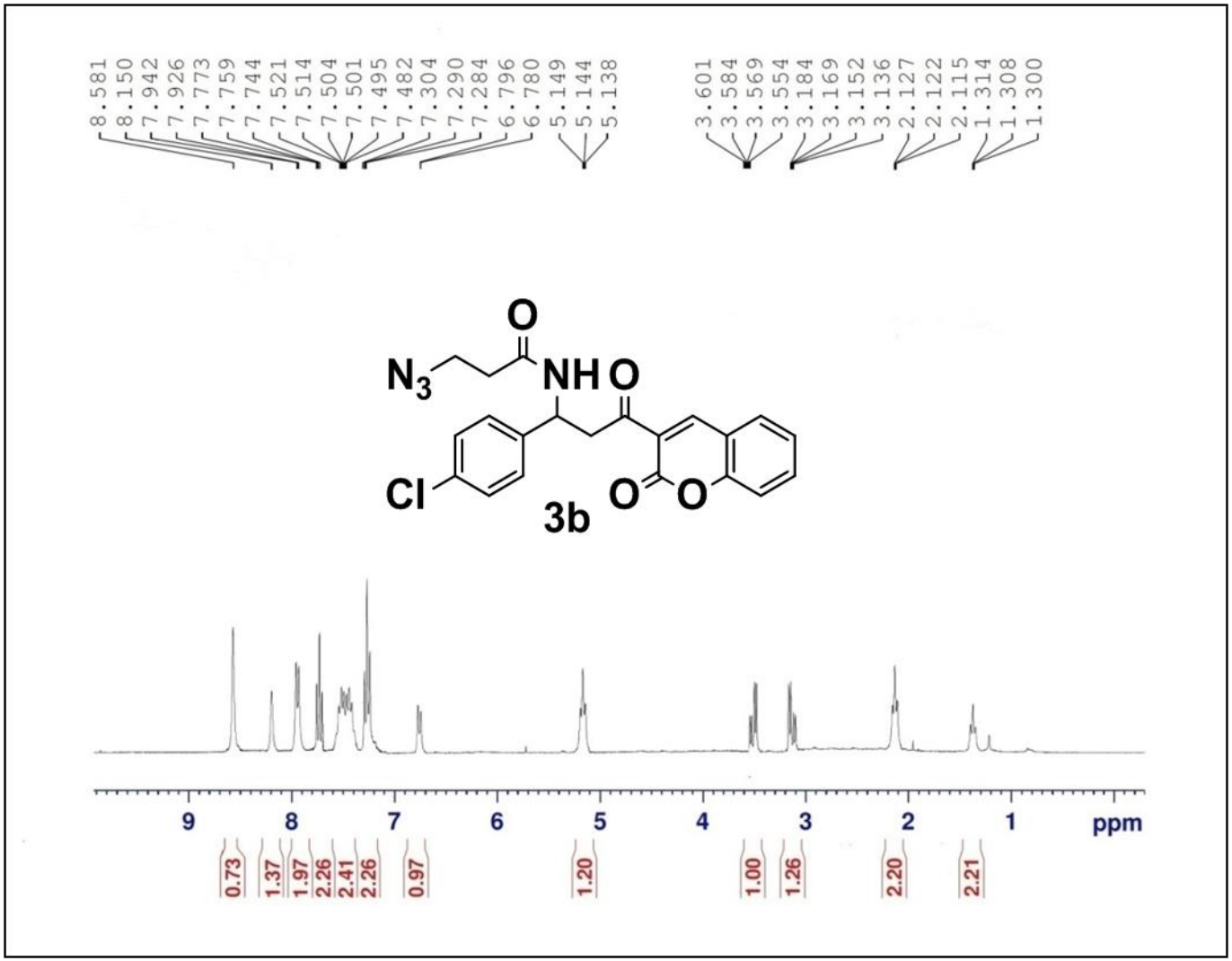


2d



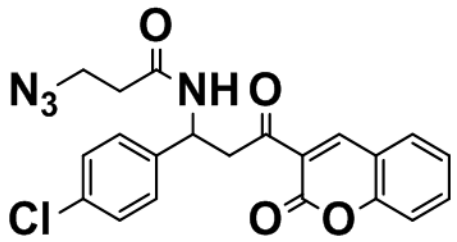

```
Current Data Parameters
NAME                  S-16
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_          20160530
Time               15.52
INSTRUM            spect
PROBHD   5 mm PABBI 1H/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  200
DS                    4
SWH           29761.904 Hz
FIDRES        0.454131 Hz
AQ            1.1010548 sec
RG                32800
DW               16.800 usec
DE                6.00 usec
TE               297.1 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 12.40 usec
PL1                -2.00 dB
SFO1         125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              80.00 usec
PL12               21.92 dB
PL13              120.00 dB
PL2                 1.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                32768
SF           125.7577890 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```


100
77
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3415.31 cm-1
3320.82 cm-1
2923.56 cm-1
2852.2 cm-1
2109.74 cm-1
1730.8 cm-1
1654.62 cm-1
1560.13 cm-1
1508.06 cm-1
1455.99 cm-1
1422.24 cm-1
1370.18 cm-1
1271.82 cm-1
1122.37 cm-1
1030.77 cm-1
910.236 cm-1
758.852 cm-1
521.65 cm-1
486.938 cm-1
458.011 cm-1
428.12 cm-1
417.513 cm-1
O
N3
NH
O
Cl
O
O
3b

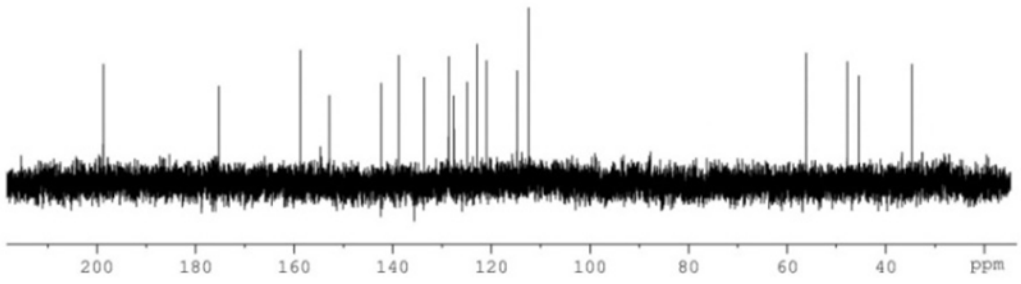

8.581
8.150
7.942
7.926
7.773
7.759
7.744
7.521
7.514
7.504
7.501
7.495
7.482
7.304
7.290
7.284
6.796
6.780
5.149
5.144
5.138
3.601
3.584
3.569
3.554
3.184
3.169
3.152
3.136
2.127
2.122
2.115
1.314
1.308
1.300
O
N3
NH
O
Cl
O
O
3b
9
8
7
6
5
4
3
2
1
ppm
0.73
1.37
1.97
2.26
2.41
2.26
0.97
1.20
1.00
1.26
2.20
2.21

S-73
C13CPD CDCl3 {D:\Yoosuf} NIIST 17

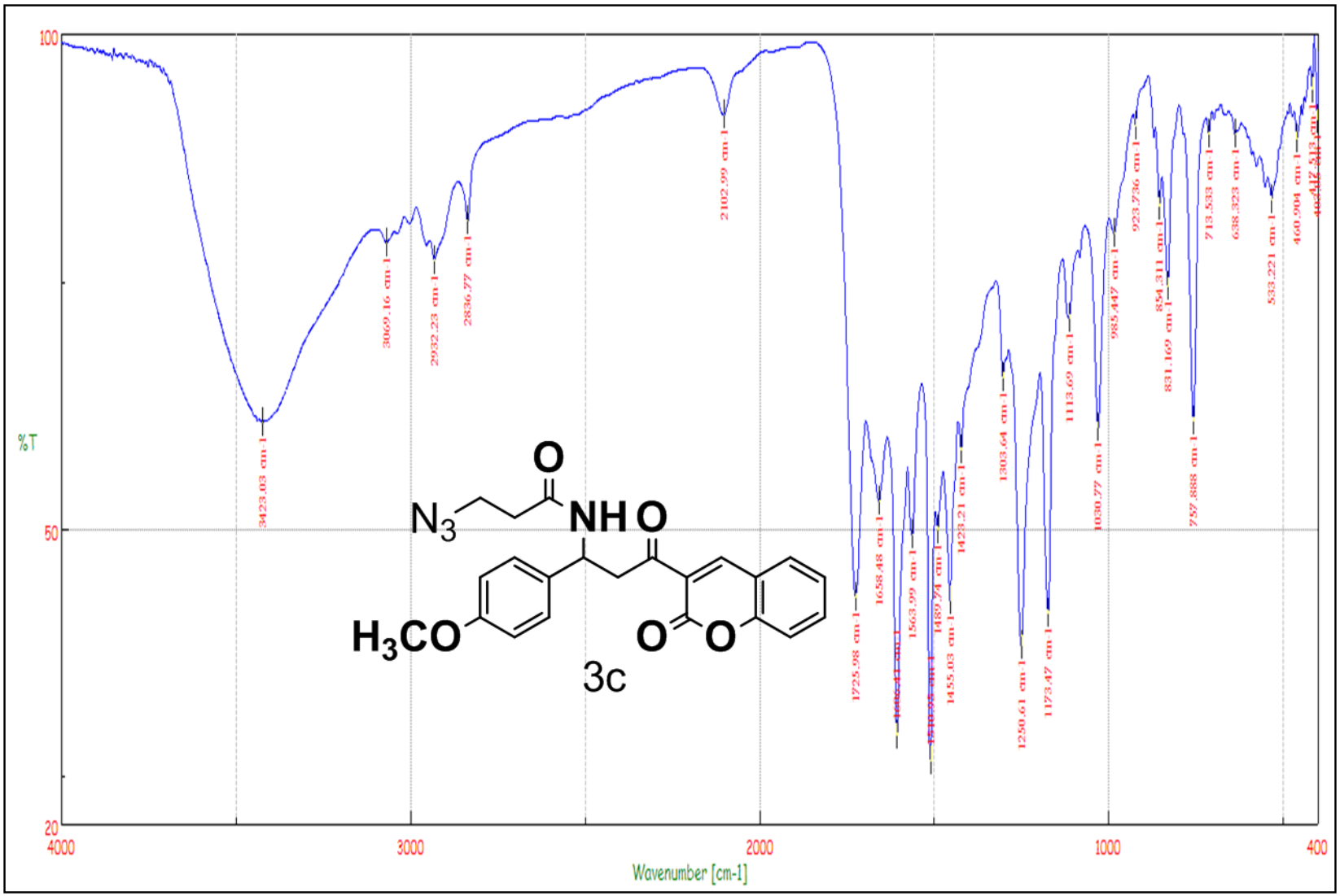
199.75
175.35
159.82
153.66
143.74
139.77
134.91
129.76
128.98
125.75
123.72
121.88
116.23
113.20
51.16
47.69
45.92
34.26

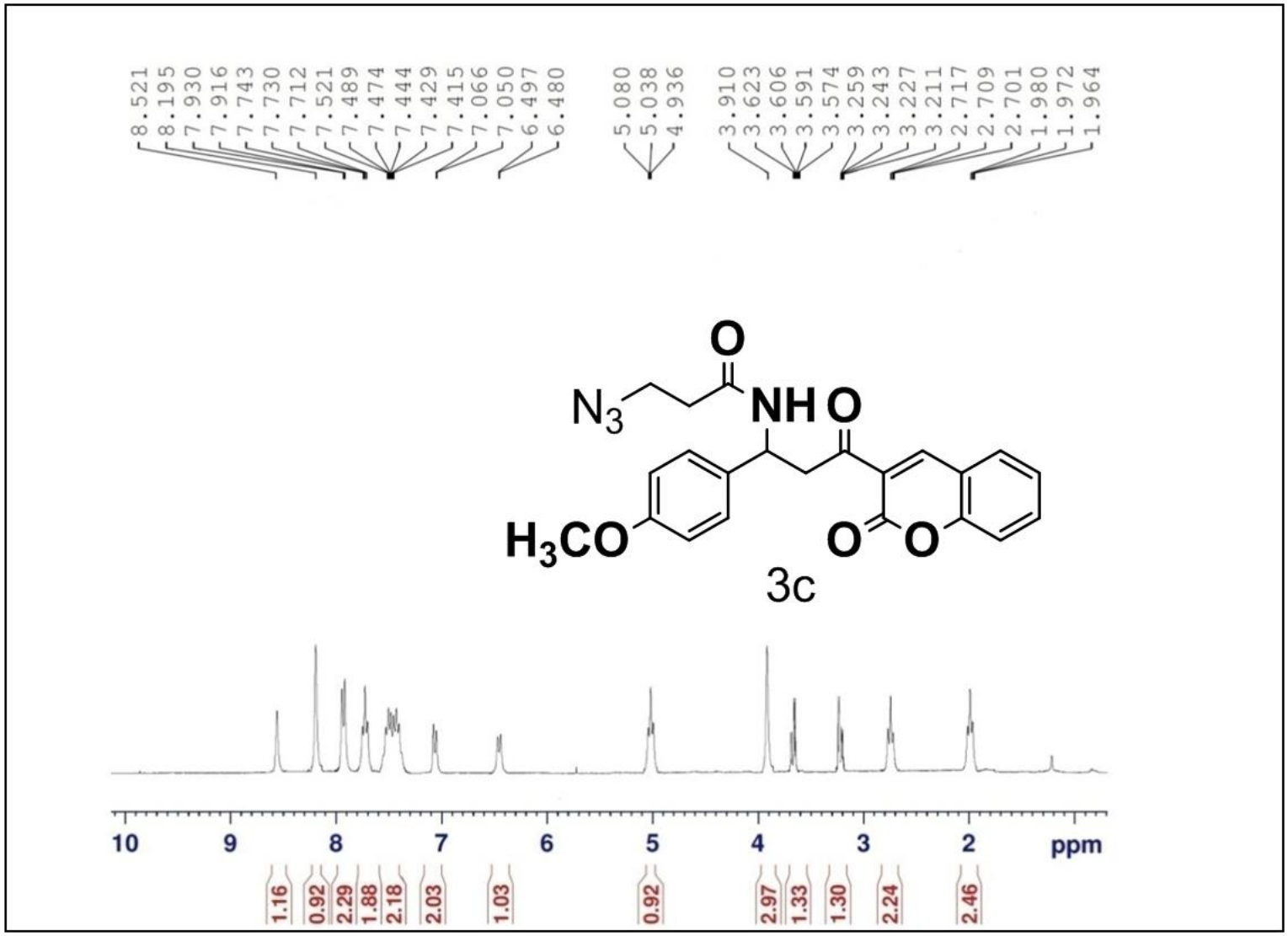


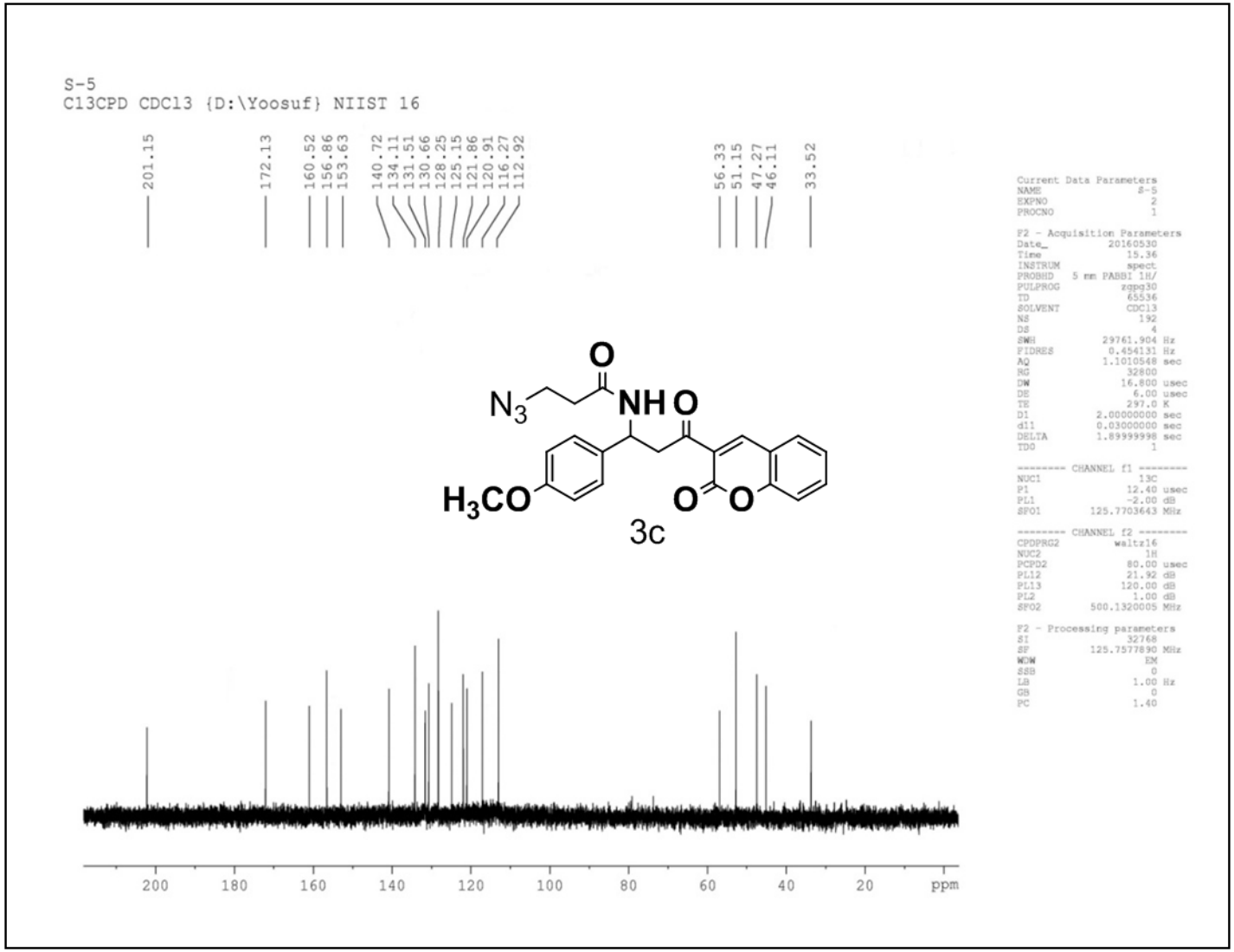
3b

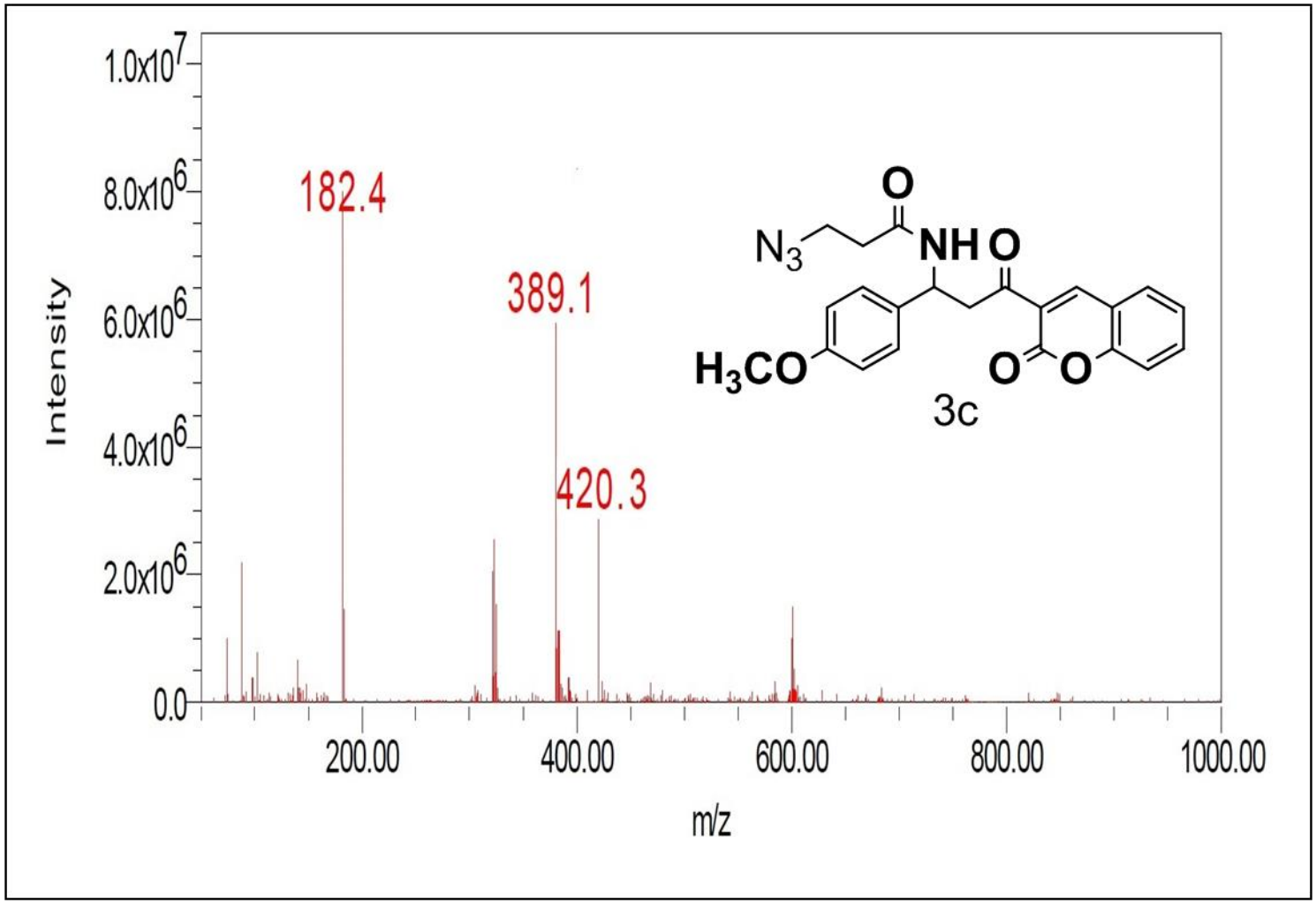


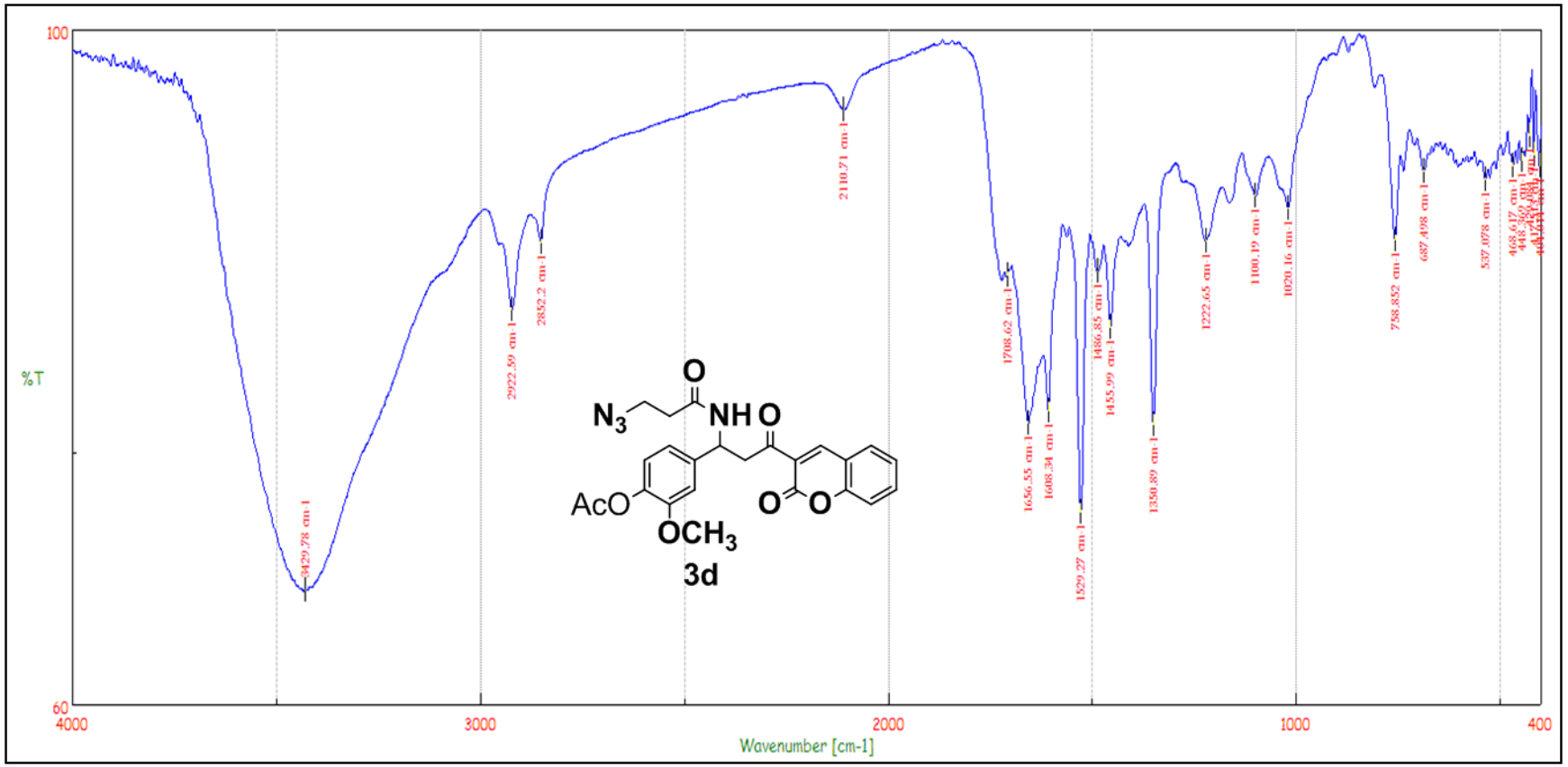

```
Current Data Parameters
NAME                  S-73
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_          20160530
Time                15.52
INSTRUM             spect
PROBHD   5 mm PABBI 1H/
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     200
DS                       4
SWH              29761.904 Hz
FIDRES           0.454131 Hz
AQ               1.1010548 sec
RG                   32800
DW                  16.800 usec
DE                   6.00 usec
TE                  297.1 K
D1              2.00000000 sec
d11             0.03000000 sec
DELTA           1.89999998 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                   12.40 usec
PL1                  -2.00 dB
SFO1          125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                80.00 usec
PL12                 21.92 dB
PL13                120.00 dB
PL2                   1.00 dB
SFO2          500.1320005 MHz

F2 - Processing parameters
SI                   32768
SF            125.7577890 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

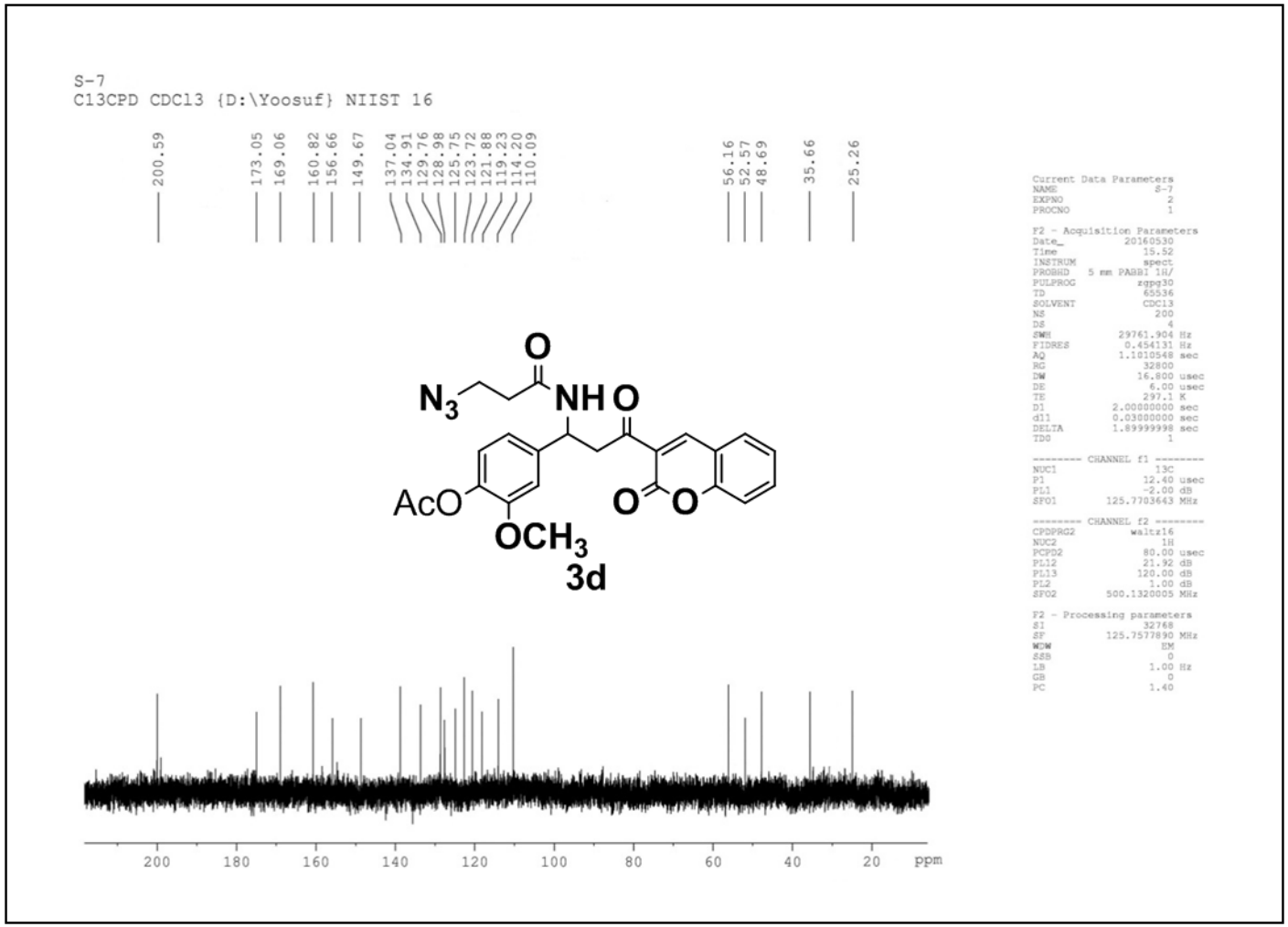

100
50
20
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3423.03 cm-1
3069.16 cm-1
2932.23 cm-1
2836.77 cm-1
2102.99 cm-1
1725.98 cm-1
1658.48 cm-1
1563.99 cm-1
1489.94 cm-1
1455.03 cm-1
1428.21 cm-1
1303.64 cm-1
1250.61 cm-1
1173.47 cm-1
1113.69 cm-1
1030.77 cm-1
985.447 cm-1
923.736 cm-1
854.311 cm-1
831.169 cm-1
757.888 cm-1
713.533 cm-1
638.323 cm-1
533.221 cm-1
460.904 cm-1
O
N3
NH
O
H3CO
O
O
3c

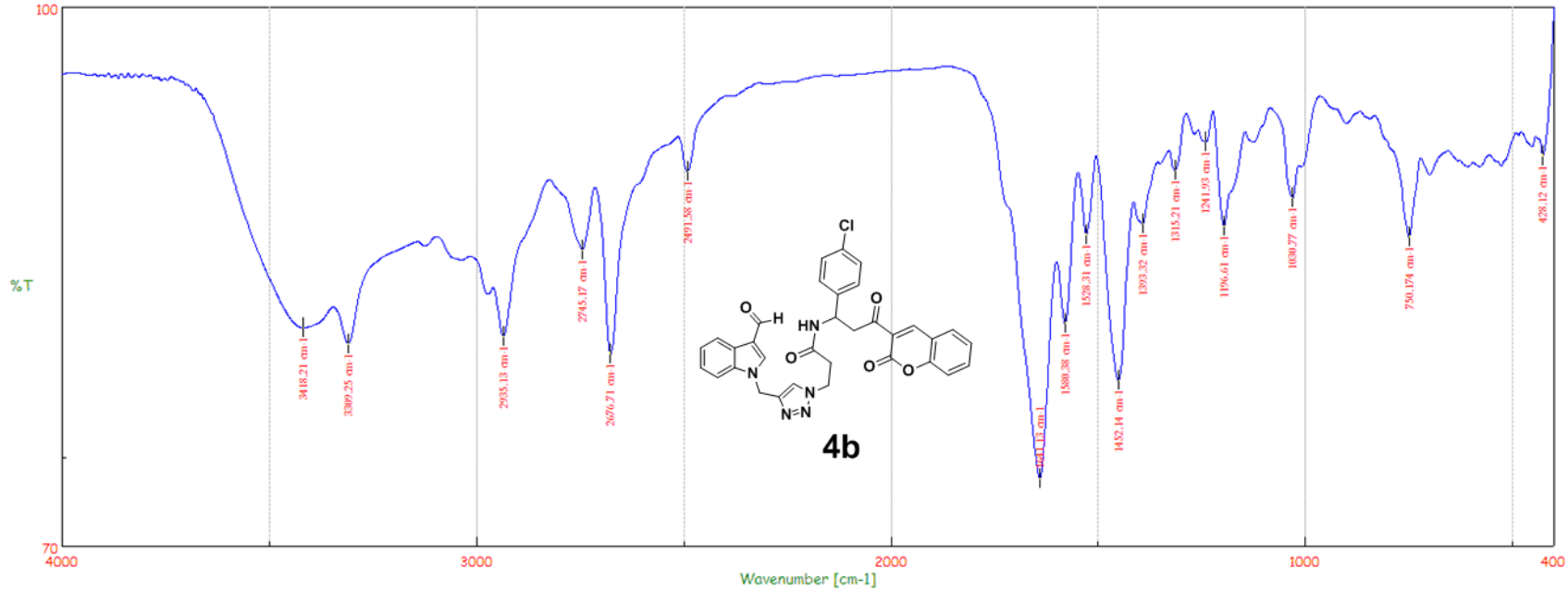

8.521
8.195
7.930
7.916
7.743
7.730
7.712
7.521
7.489
7.474
7.444
7.429
7.415
7.066
7.050
6.497
6.480
5.080
5.038
4.936
3.910
3.623
3.606
3.591
3.574
3.259
3.243
3.227
3.211
2.717
2.709
2.701
1.980
1.972
1.964
O
N3
NH
O
H3CO
O
O
3c
10
9
8
7
6
5
4
3
2
ppm
1.16
0.92
2.29
1.88
2.18
2.03
1.03
0.92
2.97
1.33
1.30
2.24
2.46

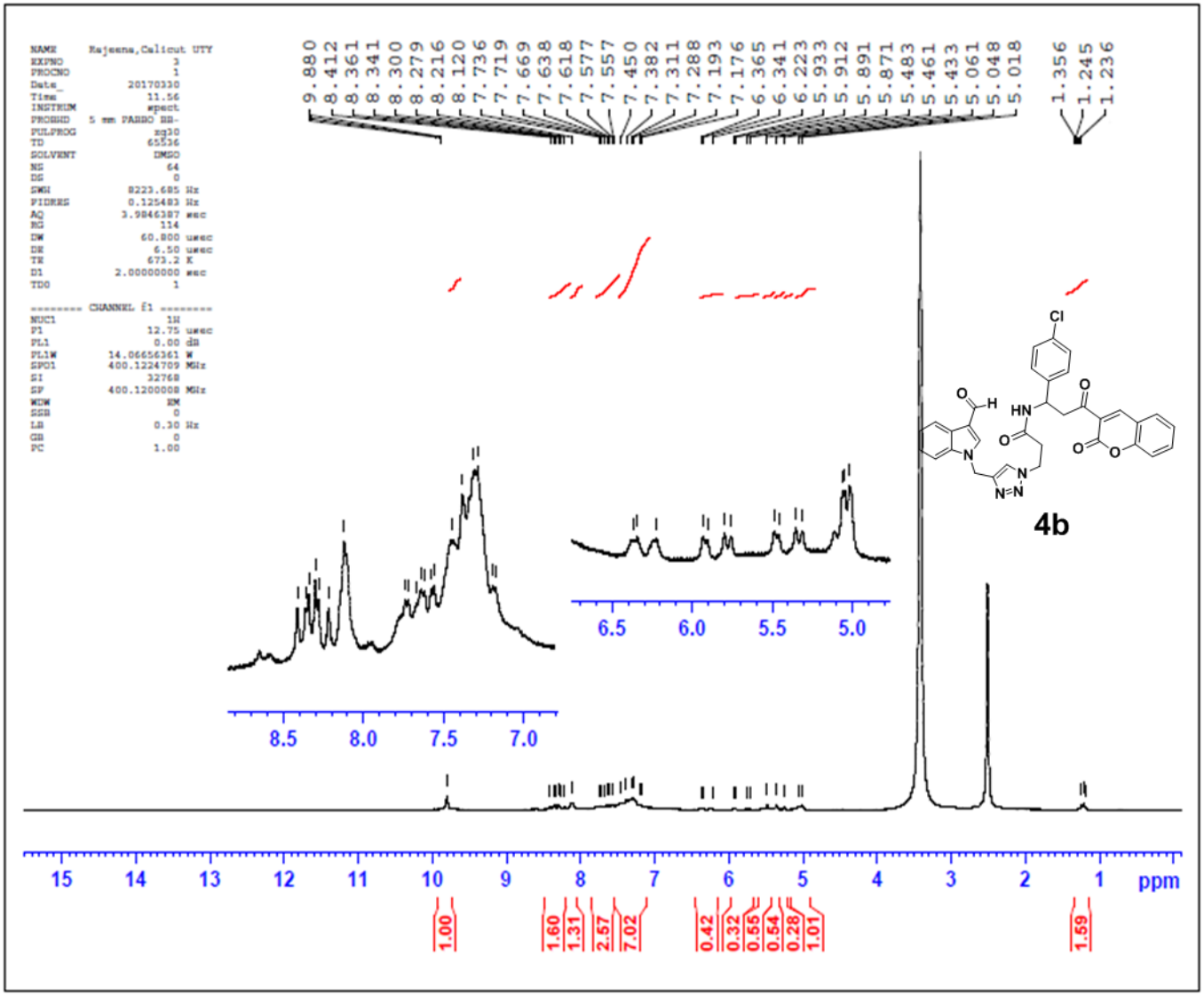

S-5
C13CPD CDCl3 {D:\Yoosuf} NIIST 16
201.15
172.13
160.52
156.86
153.63
140.72
134.11
131.51
130.66
128.25
125.15
121.86
120.91
116.27
112.92
56.33
51.15
47.27
46.11
33.52
O
N3
NH O
H3CO
O O
3c
200
180
160
140
120
100
80
60
40
20
ppm
Current Data Parameters
NAME S-5
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160530
Time 15.36
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 192
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 32800
DW 16.800 usec
DE 6.00 usec
TE 297.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.40 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL12 21.92 dB
PL13 120.00 dB
PL2 1.00 dB
SFO2 500.1320005 MHz
F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
Intensity
182.4
389.1
420.3
200.00
400.00
600.00
800.00
1000.00
m/z
O
N3
NH O
H3CO
O
O
3c


100
60
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3429.78 cm-1
2922.59 cm-1
2852.2 cm-1
2110.71 cm-1
1708.62 cm-1
1656.55 cm-1
1608.34 cm-1
1529.27 cm-1
1486.85 cm-1
1455.99 cm-1
1350.89 cm-1
1222.65 cm-1
1100.19 cm-1
1020.16 cm-1
758.852 cm-1
687.498 cm-1
537.078 cm-1
468.617 cm-1
448.360 cm-1
O
N3
NH O
AcO
OCH3
O O
3d


S-7
C13CPD CDCl3 {D:\Yoosuf} NIIST 16
200.59
173.05
169.06
160.82
156.66
149.67
137.04
134.91
129.76
128.98
125.75
123.72
121.88
119.23
114.20
110.09
56.16
52.57
48.69
35.66
25.26
O
N3
NH O
AcO
O O
OCH3
3d
200
180
160
140
120
100
80
60
40
20
ppm
Current Data Parameters
NAME S-7
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160530
Time 15.52
INSTRUM spect
PROBHD 5 mm PABBI 1H/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 200
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 32800
DW 16.800 usec
DE 6.00 usec
TE 297.1 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 12.40 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL12 21.92 dB
PL13 120.00 dB
PL2 1.00 dB
SFO2 500.1320005 MHz
F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


100
%T
70
4000
3000
2000
1000
400
Wavenumber [cm-1]
3418.21 cm-1
3309.25 cm-1
2935.13 cm-1
2745.17 cm-1
2676.71 cm-1
2491.58 cm-1
1589.38 cm-1
1528.31 cm-1
1452.14 cm-1
1393.32 cm-1
1315.21 cm-1
1241.93 cm-1
1196.61 cm-1
1030.77 cm-1
750.174 cm-1
428.12 cm-1
Cl
O
H
HN
O
O
O
N
N
N=N
4b


9.880
8.412
8.361
8.341
8.300
8.279
8.216
8.120
7.736
7.719
7.669
7.638
7.618
7.577
7.557
7.450
7.382
7.311
7.288
7.193
7.176
6.365
6.341
6.223
5.933
5.912
5.891
5.871
5.483
5.461
5.433
5.061
5.048
5.018
1.356
1.245
1.236
4b
ppm
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2.57
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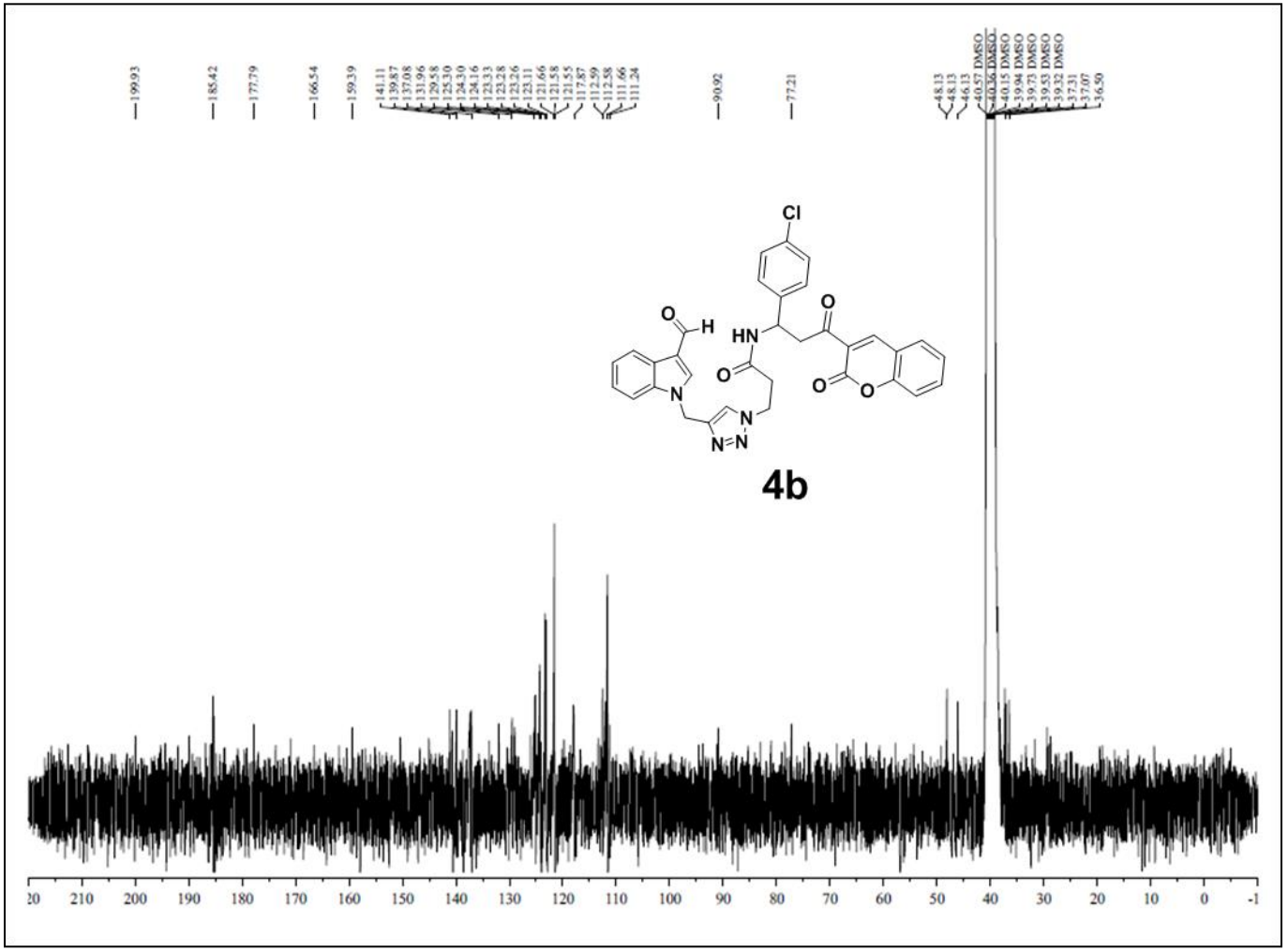
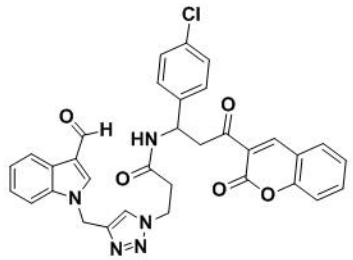
0.66
0.54
0.28
1.01
1.59


199.93
185.42
177.79
166.54
159.39
141.11
139.87
137.08
131.96
129.58
125.30
124.30
124.16
123.33
123.28
123.26
123.11
121.66
121.58
121.55
117.87
112.59
112.58
111.66
111.24
90.92
77.21
48.13
48.13
46.13
40.57 DMSO
40.36 DMSO
40.15 DMSO
39.94 DMSO
39.71 DMSO
39.53 DMSO
39.32 DMSO
37.31
37.07
36.50
Cl
O
H
HN
O
O
O
O
N
N
N=N
4b
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1

Elemental Composition Report

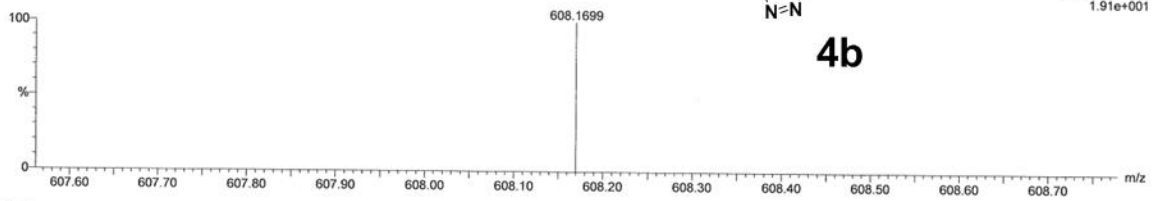
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 33-33 H: 15-28 N: 2-7 O: 2-6 Cl: 1-2
C43H38ClN7O6
CC1 5 (0.119)

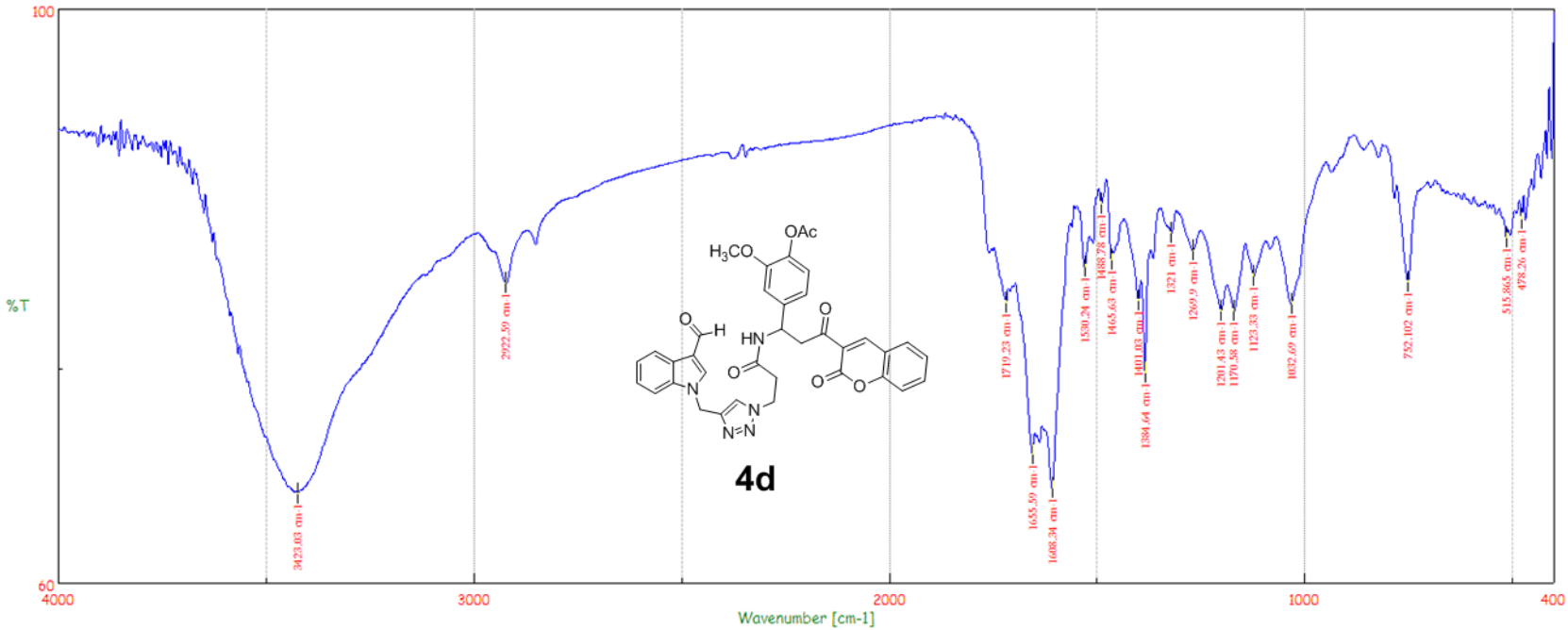


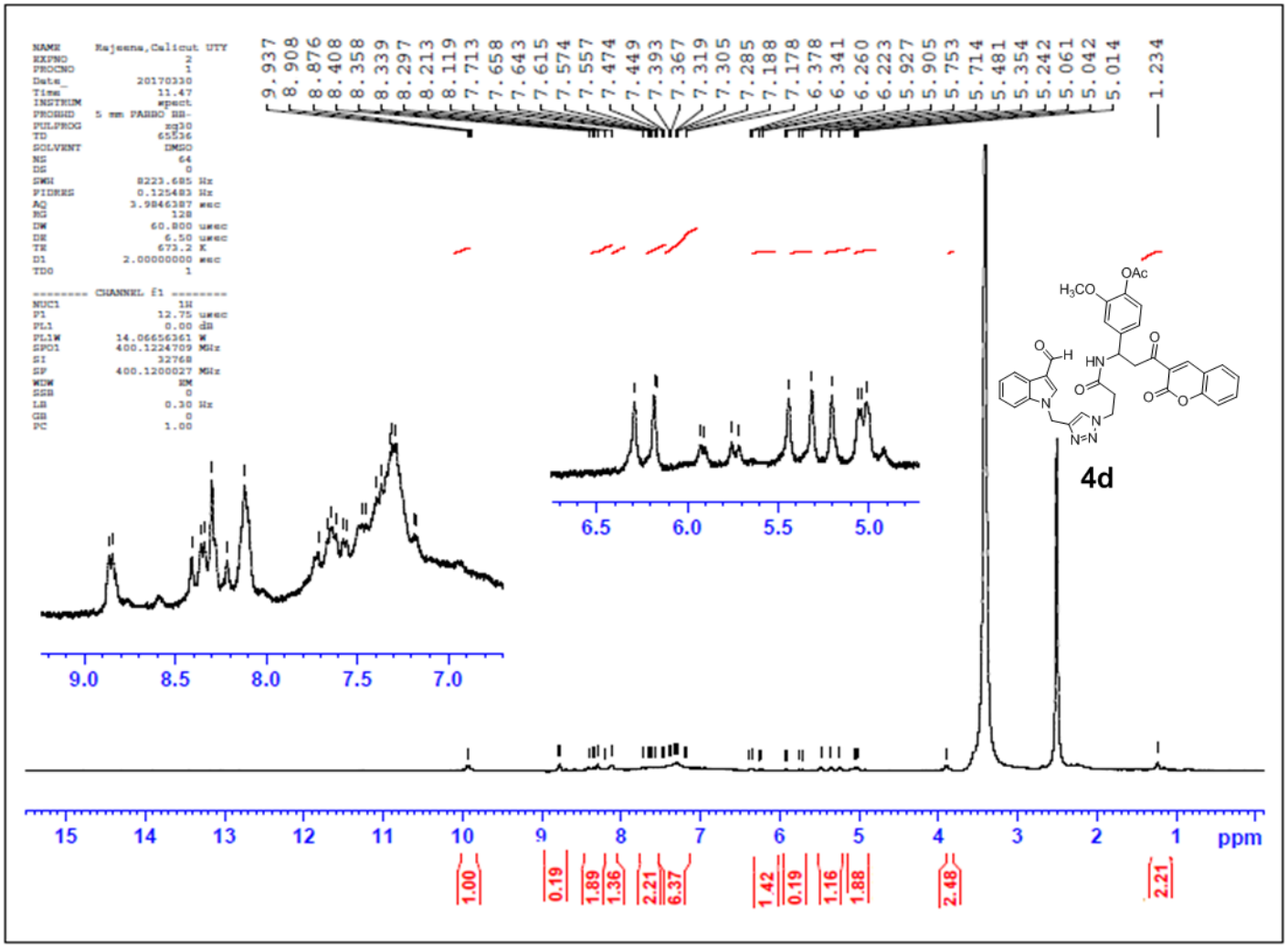
4b

1: TOF MS ES+
1.91e+001



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
608.1699	608.1701	-0.2	-0.3	22.5	14.5	0.0	C33 H27 N5 O5 Cl


100
%T
60
4000
3000
2000
1000
400
Wavenumber [cm-1]
3423.03 cm-1
2922.59 cm-1
1719.23 cm-1
1655.59 cm-1
1608.34 cm-1
1530.24 cm-1
1488.78 cm-1
1465.63 cm-1
1401.83 cm-1
1384.64 cm-1
1321 cm-1
1269.9 cm-1
1201.43 cm-1
1170.58 cm-1
1123.33 cm-1
1032.69 cm-1
752.102 cm-1
515.865 cm-1
478.26 cm-1
H3CO
OAc
HN
4d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8.876
8.408
8.358
8.339
8.297
8.213
8.119
7.713
7.658
7.643
7.615
7.574
7.557
7.474
7.449
7.393
7.367
7.319
7.305
7.285
7.188
7.178
6.378
6.341
6.260
6.223
5.927
5.905
5.753
5.714
5.481
5.354
5.242
5.061
5.042
5.014
1.234
4d
ppm
1.00
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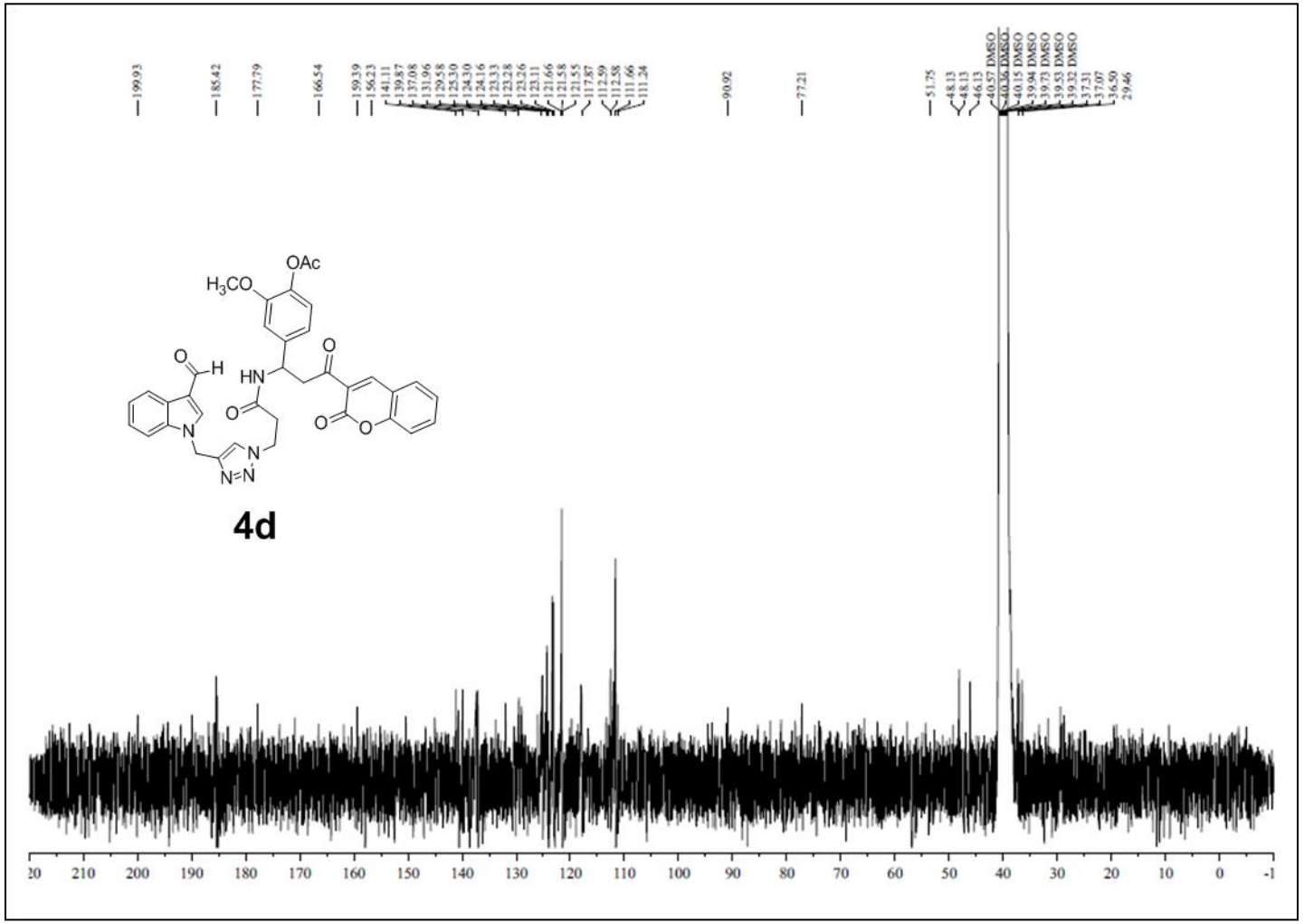
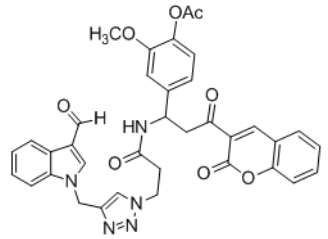
1.36
2.21
6.37
1.42
0.19
1.16
1.88
2.48
2.21


199.93
185.42
177.79
166.54
159.39
156.23
141.11
139.87
137.08
131.96
129.58
125.30
124.30
124.16
123.33
123.28
123.26
123.11
121.66
121.58
121.55
117.87
112.99
112.58
111.66
111.24
90.92
77.21
51.75
48.13
48.13
46.13
40.57 DMSO
40.36 DMSO
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.53 DMSO
39.32 DMSO
37.31
37.07
36.50
29.46
OAc
H3CO
O
H
HN
O
O
O
O
N
N
N=N
4d
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

Elemental Composition Report

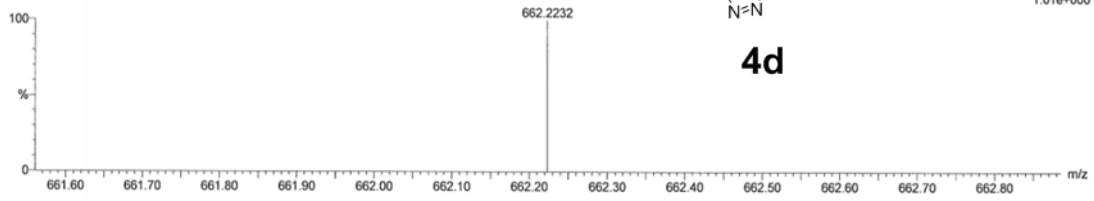
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 36-36 H: 15-34 N: 2-7 O: 2-9
C43H38ClN7O6
CC4 112 (2.447)

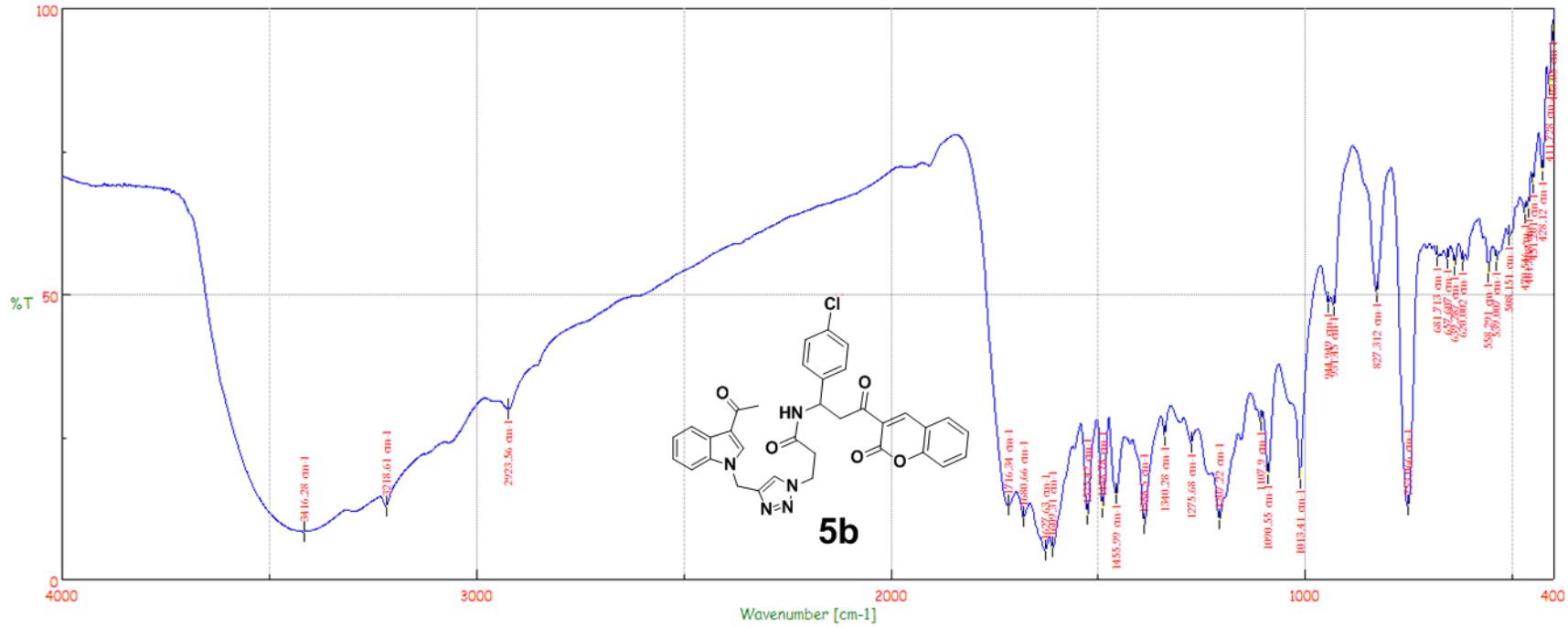


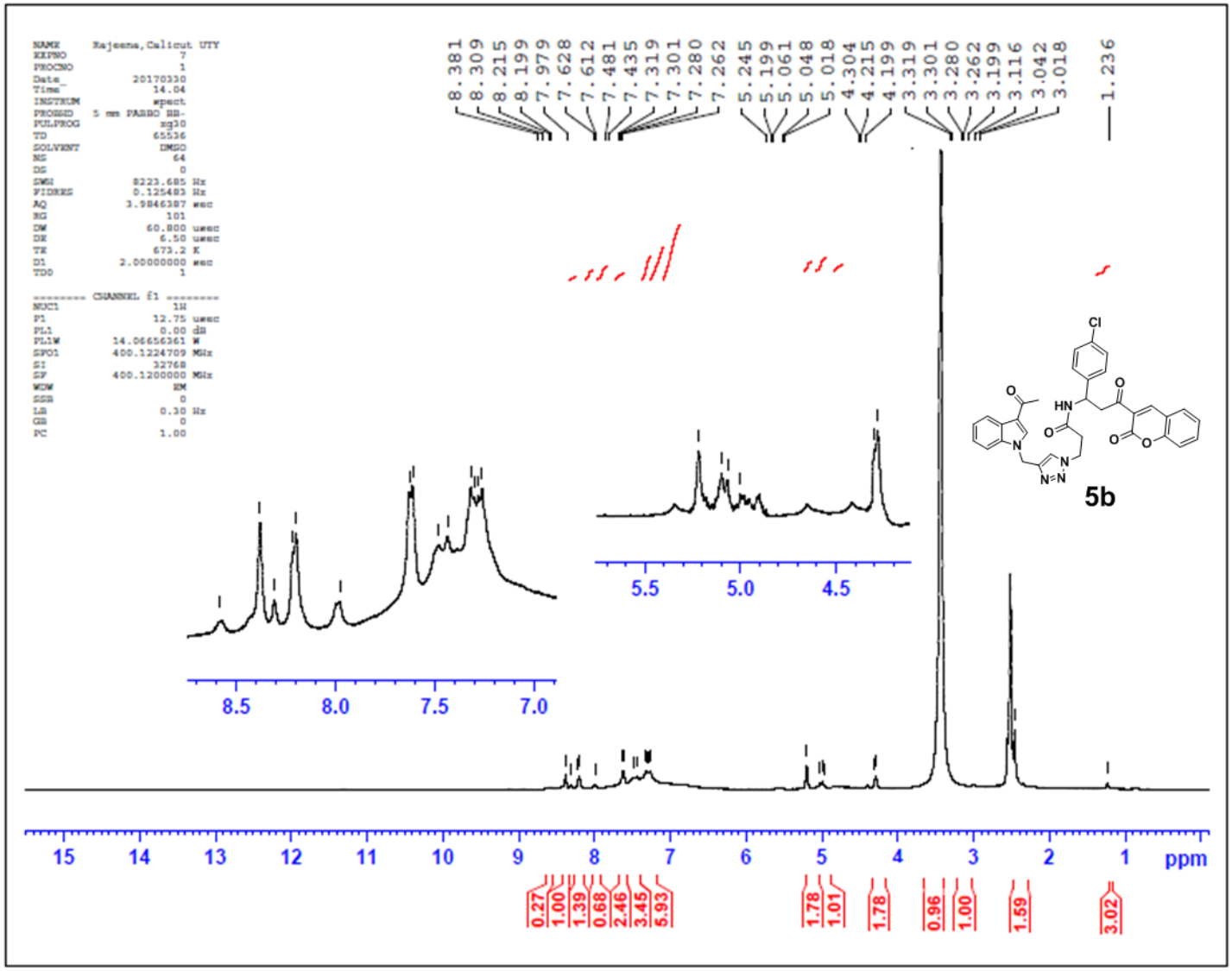
4d

1: TOF MS ES+
1.01e+000



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
662.2232	662.2251	-1.9	-2.9	23.5	12.5	0.0	C36 H32 N5 O8


100
%T
50
0
4000
3000
2000
1000
400
Wavenumber [cm-1]
3416.28 cm-1
3218.61 cm-1
2923.56 cm-1
1716.34 cm-1
1680.66 cm-1
1607.63 cm-1
1580.91 cm-1
1455.99 cm-1
1340.28 cm-1
1275.68 cm-1
1207.22 cm-1
1107.9 cm-1
1090.55 cm-1
1013.41 cm-1
827.312 cm-1
681.718 cm-1
558.291 cm-1
428.12 cm-1
Cl
O
HN
O
O
O
N
N
N=N
5b


8.381
8.309
8.215
8.199
7.979
7.628
7.612
7.481
7.435
7.319
7.301
7.280
7.262
5.245
5.199
5.061
5.048
5.018
4.304
4.215
4.199
3.319
3.301
3.280
3.262
3.199
3.116
3.042
3.018
1.236
Cl
HN
O
N
N=N
5b
8.5
8.0
7.5
7.0
5.5
5.0
4.5
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.27
1.00
1.39
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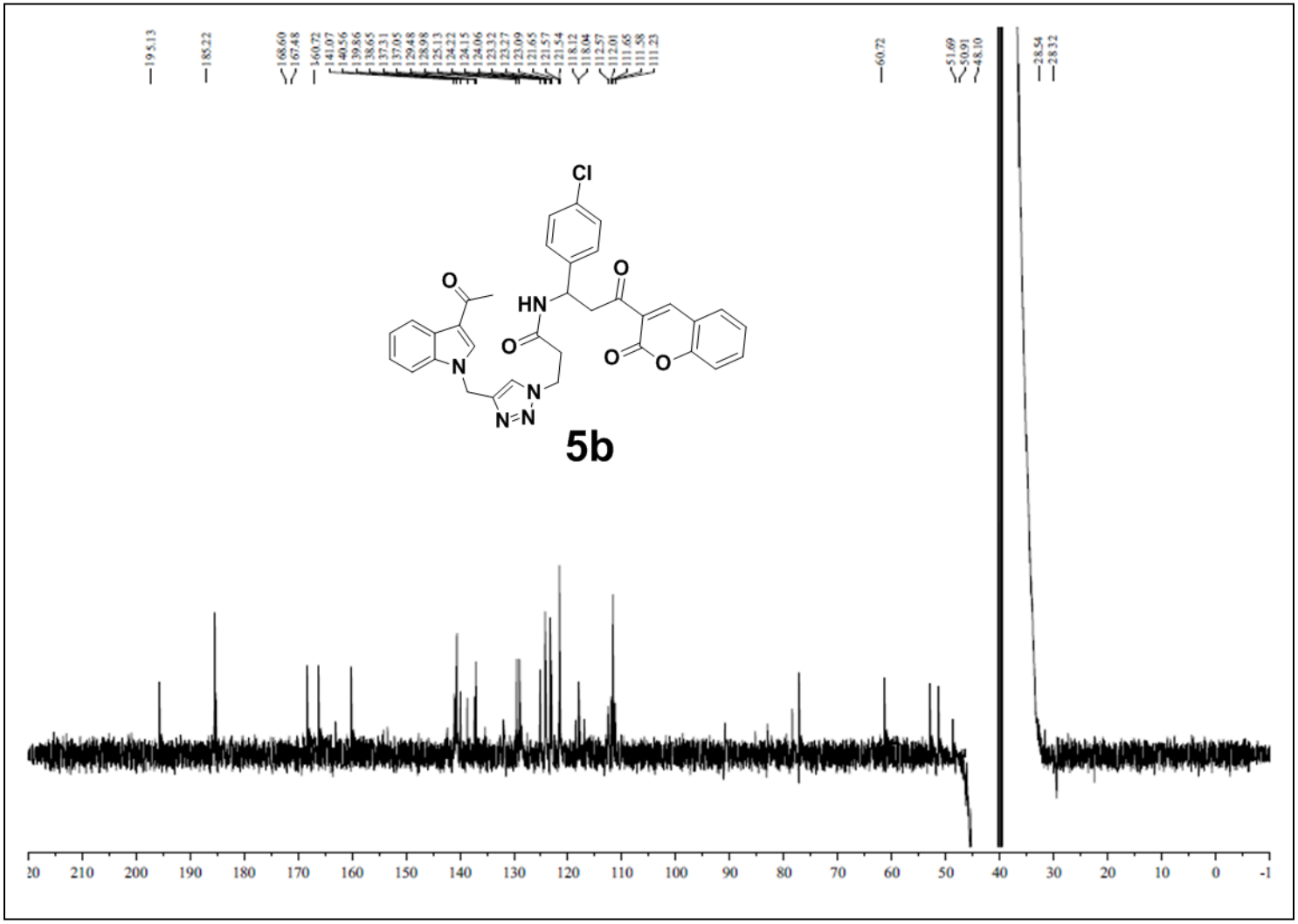
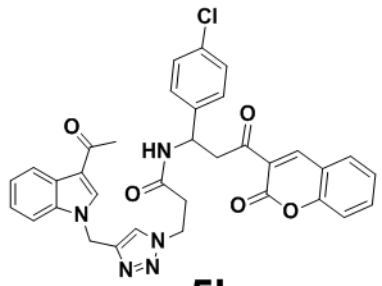
2.46
3.45
5.93
1.78
1.01
1.78
0.96
1.00
1.59
3.02


19.513
185.22
168.60
167.48
160.72
141.07
140.56
139.86
138.65
137.31
137.05
129.48
128.98
125.13
124.22
124.15
124.06
123.32
123.27
123.09
121.65
121.57
121.54
118.12
118.04
112.57
112.01
111.65
111.58
111.23
60.72
51.69
50.91
48.10
28.54
28.32
Cl
O
HN
O
O
O
O
N
N
N
N
5b
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

Elemental Composition Report

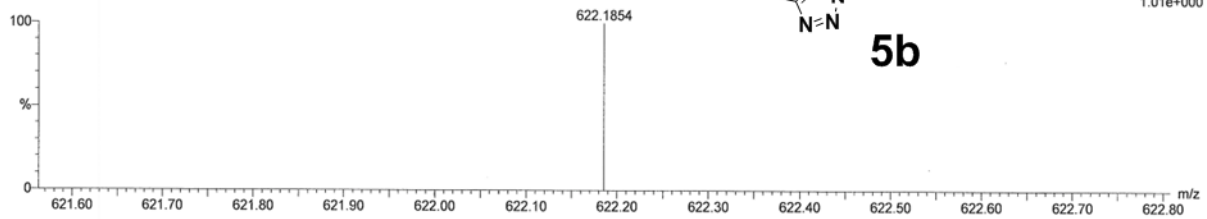
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 34-34 H: 15-30 N: 2-7 O: 2-6 Cl: 1-2
C43H38ClN7O6
CC14 8 (0.175)

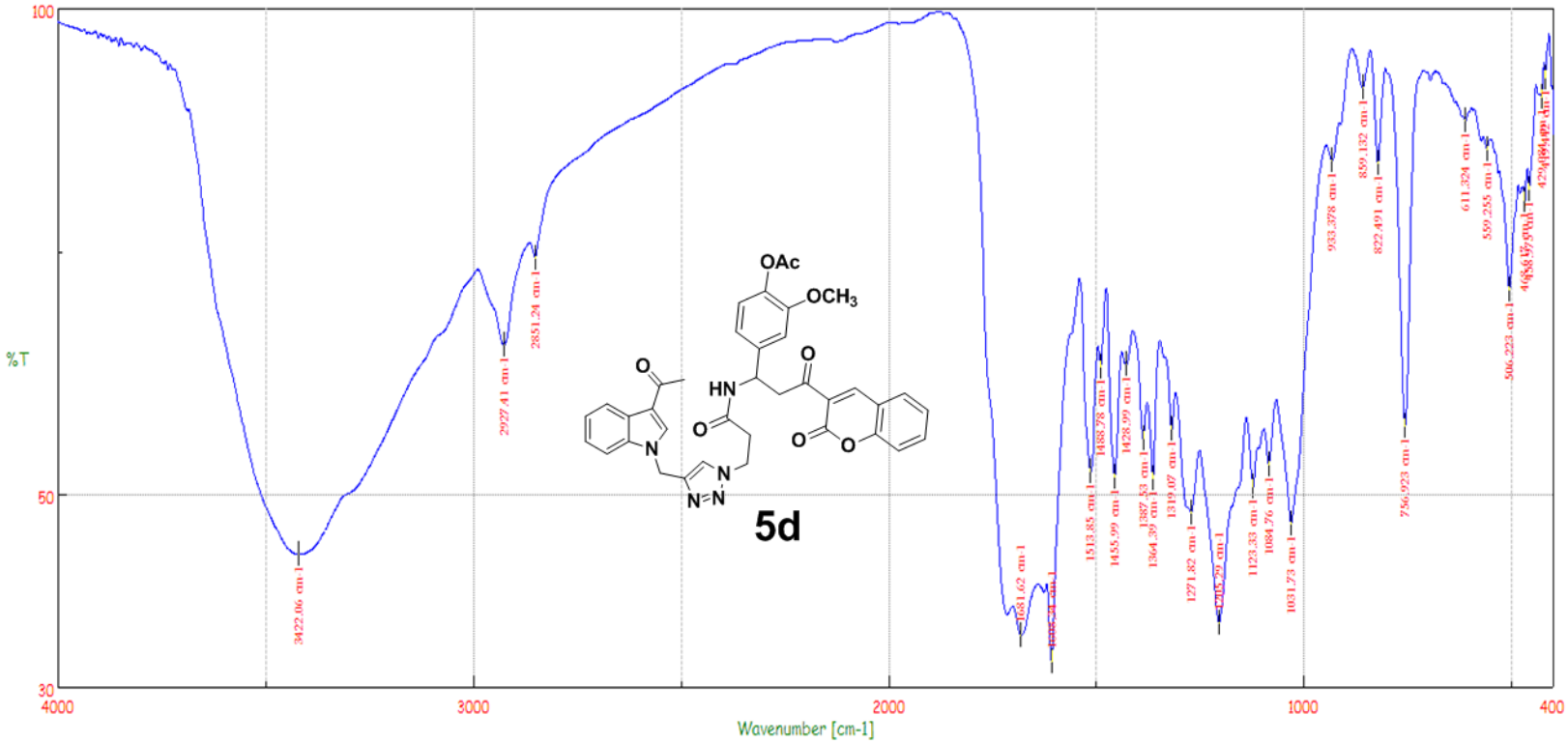


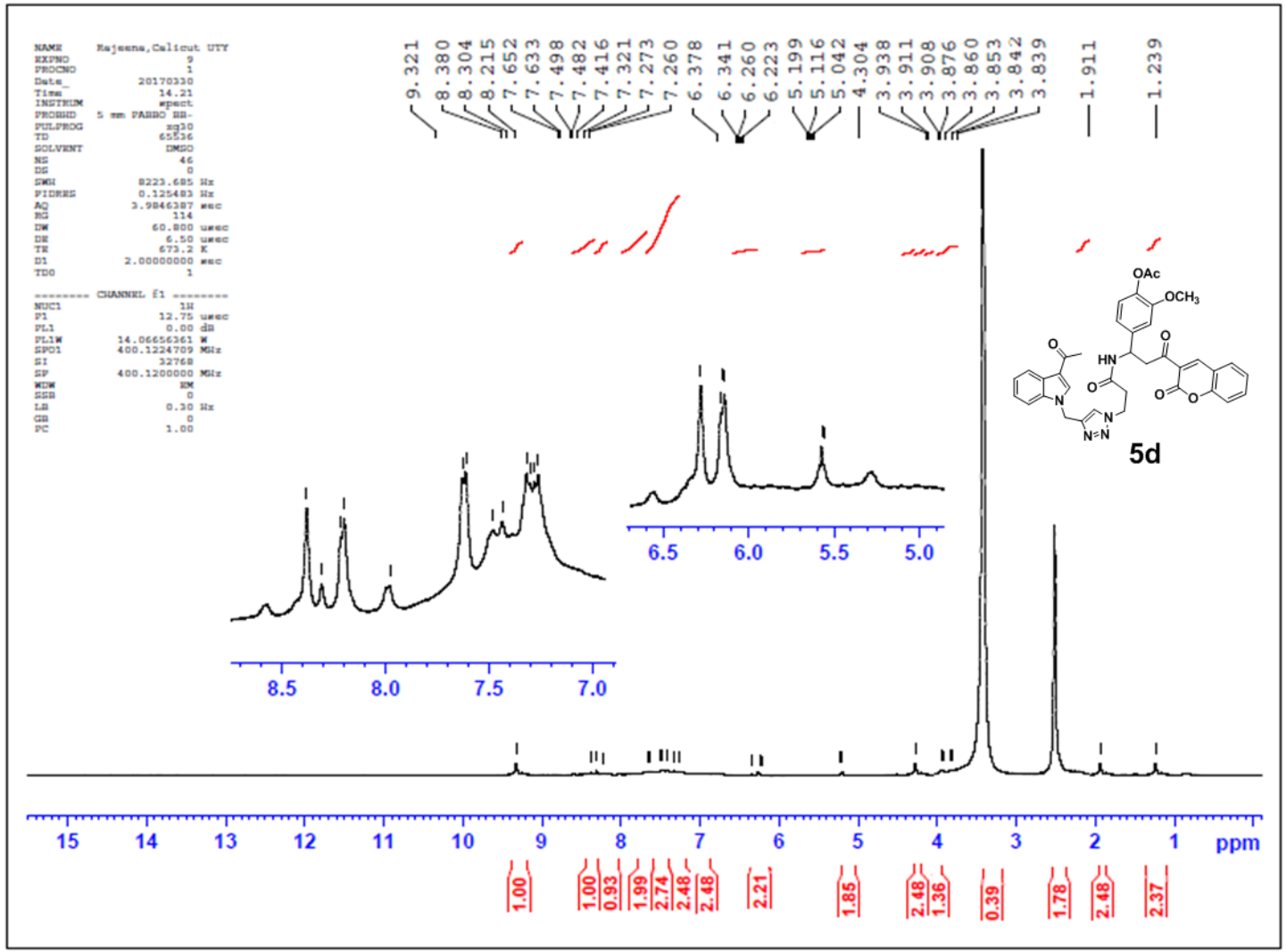
5b

1: TOF MS ES+
1.01e+000



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
622.1854	622.1857	-0.3	-0.5	22.5	12.1	0.0	C34 H29 N5 O5 Cl


100
50
30
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3422.06 cm-1
2927.41 cm-1
2851.24 cm-1
1681.62 cm-1
1513.85 cm-1
1488.78 cm-1
1455.99 cm-1
1428.99 cm-1
1387.53 cm-1
1364.39 cm-1
1319.07 cm-1
1271.82 cm-1
1205.29 cm-1
1123.33 cm-1
1084.76 cm-1
1031.73 cm-1
933.978 cm-1
859.132 cm-1
822.491 cm-1
756.923 cm-1
611.324 cm-1
559.255 cm-1
506.223 cm-1
OAc
OCH3
HN
O
N
N=N
5d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8.215
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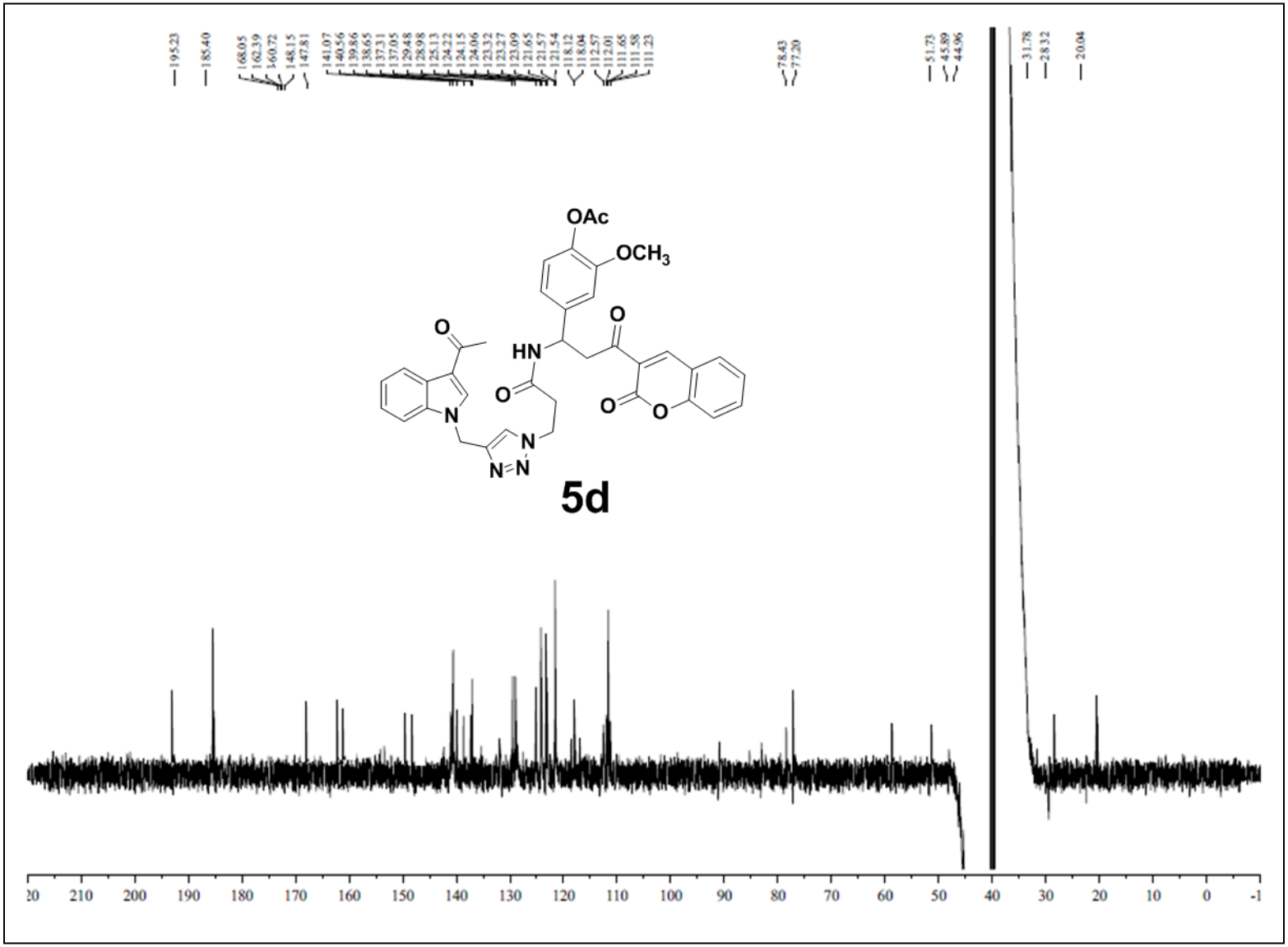
7.633
7.498
7.482
7.416
7.321
7.273
7.260
6.378
6.341
6.260
6.223
5.199
5.116
5.042
4.304
3.938
3.911
3.908
3.876
3.860
3.853
3.842
3.839
1.911
1.239
OAc
OCH3
HN
5d
ppm


195.23
185.40
168.05
162.39
160.72
148.15
147.81
141.07
140.56
139.86
138.65
137.31
137.05
129.48
128.98
125.13
124.22
124.15
124.06
123.32
123.27
123.09
121.65
121.57
121.54
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118.04
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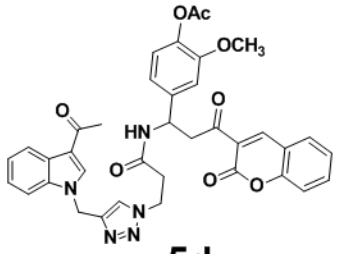
112.01
111.65
111.58
111.23
78.43
77.20
51.73
45.89
44.96
31.78
28.32
20.04
OAc
OCH3
O
HN
O
O
O
O
N
N
N=N
5d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

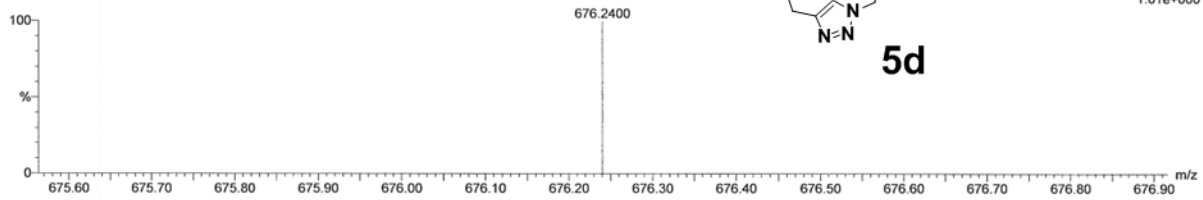
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

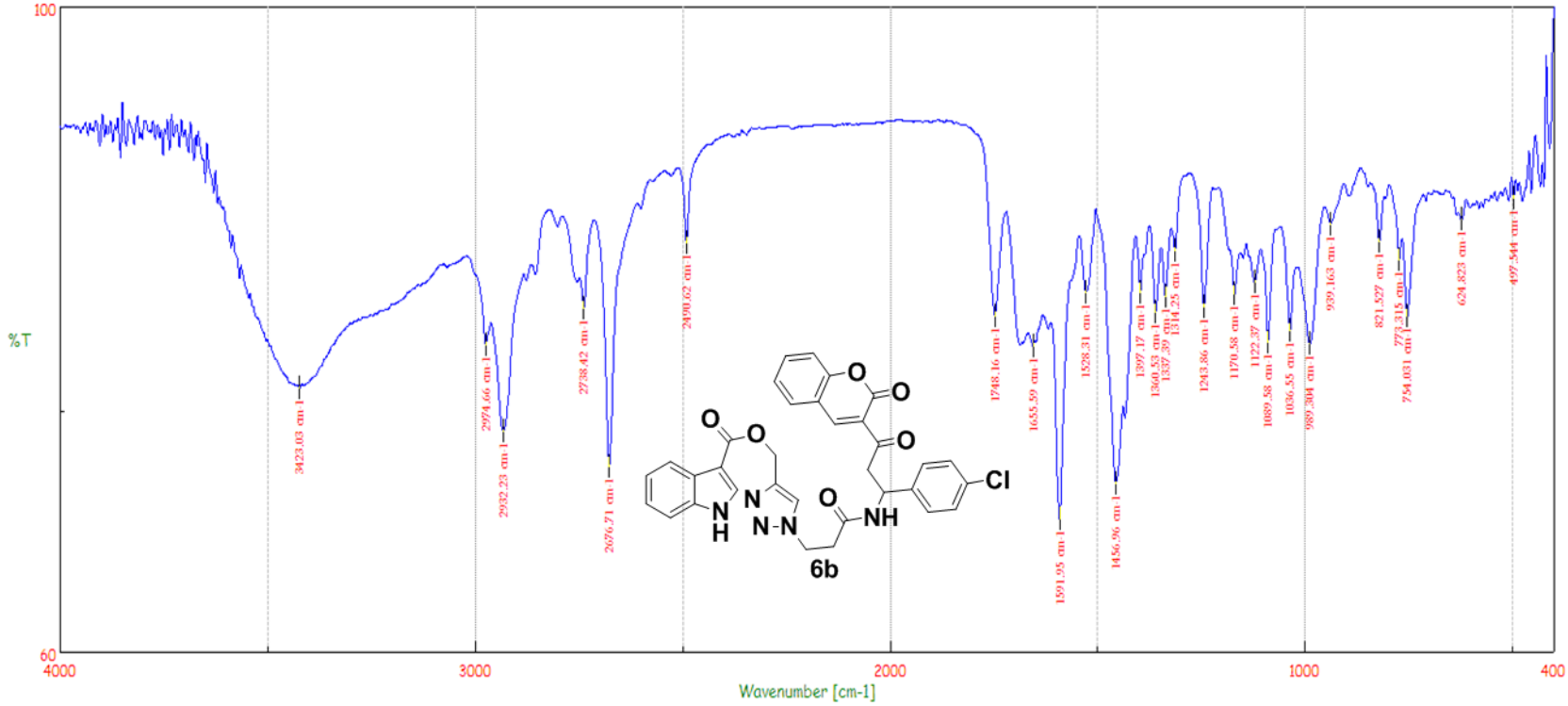
Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 37-37 H: 15-34 N: 2-7 O: 2-9
C43H38ClN7O6
CC16 38 (0.837)

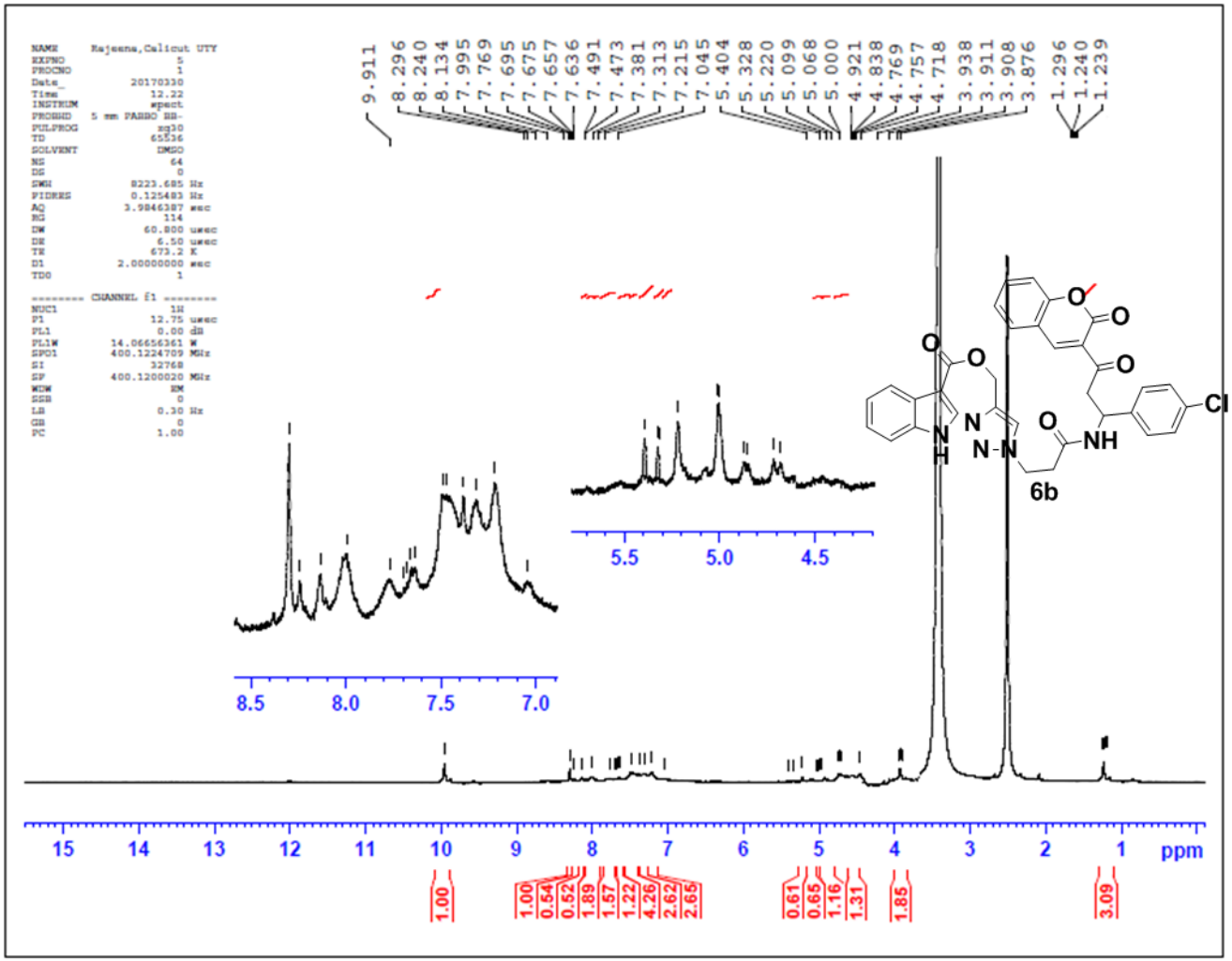


5d



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
676.2400	676.2407	-0.7	-1.0	23.5	12.5	0.0	C37 H34 N5 O8


100
60
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3423.03 cm-1
2974.66 cm-1
2932.23 cm-1
2738.42 cm-1
2676.71 cm-1
2490.62 cm-1
1748.16 cm-1
1655.59 cm-1
1591.95 cm-1
1528.31 cm-1
1456.96 cm-1
1397.17 cm-1
1360.53 cm-1
1337.39 cm-1
1314.25 cm-1
1243.86 cm-1
1170.58 cm-1
1122.37 cm-1
1089.58 cm-1
1036.55 cm-1
989.304 cm-1
939.163 cm-1
821.527 cm-1
773.315 cm-1
754.031 cm-1
624.823 cm-1
497.544 cm-1
O
O
O
O
N
H
N
N-N
O
NH
Cl
6b


9.911
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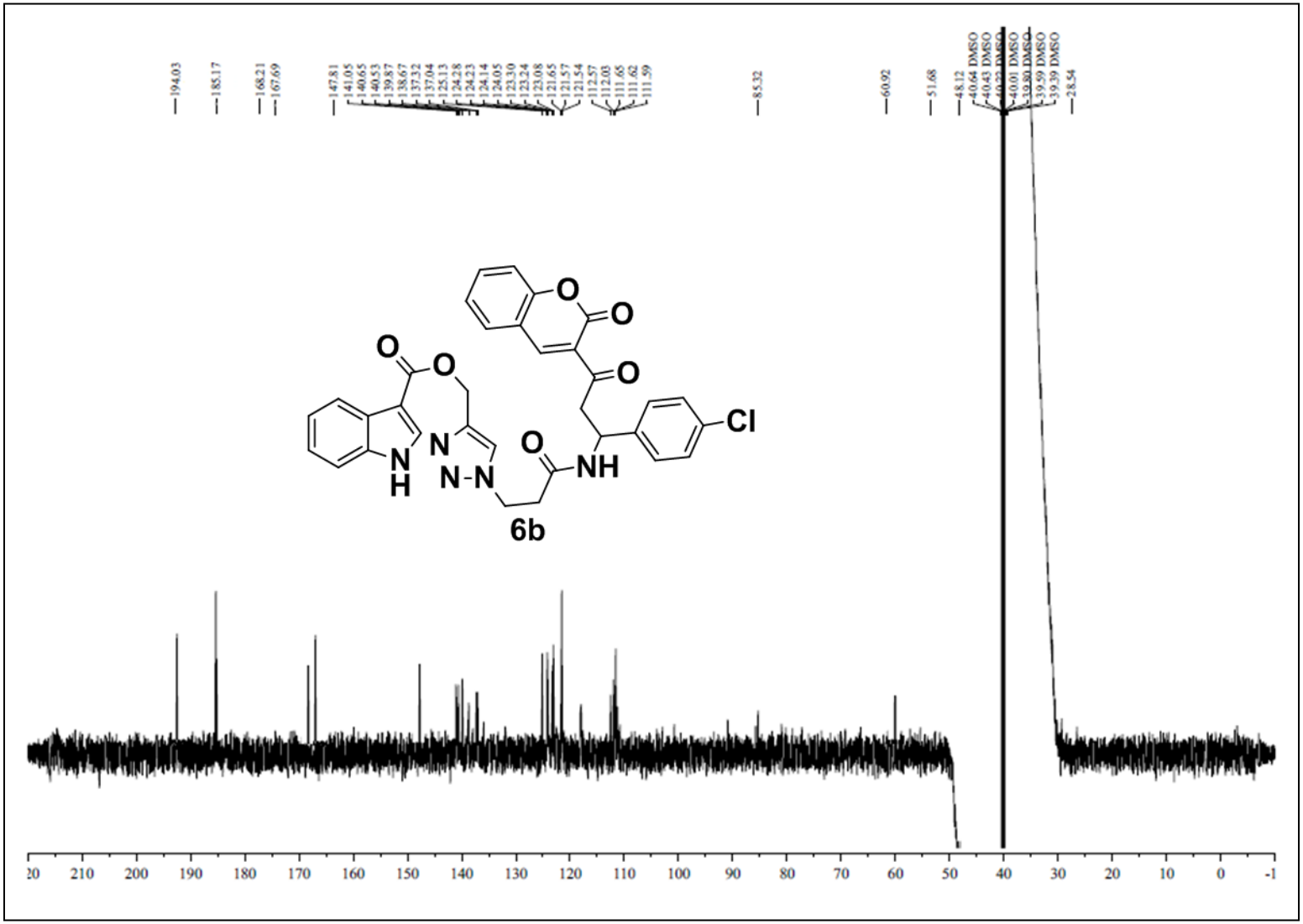
8.240
8.134
7.995
7.769
7.695
7.675
7.657
7.636
7.491
7.473
7.381
7.313
7.215
7.045
5.404
5.328
5.220
5.099
5.068
5.000
4.921
4.838
4.769
4.757
4.718
3.938
3.911
3.908
3.876
1.296
1.240
1.239
6b
ppm
1.00
1.00
0.54
0.52
1.89
1.57
1.22
4.26
2.62
2.65
0.61
0.65
1.16
1.31
1.85
3.09

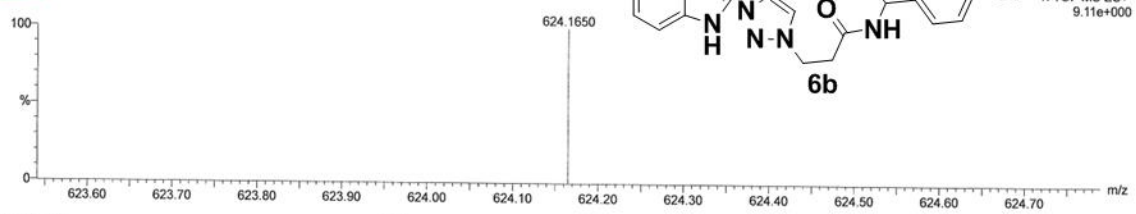

194.03
185.17
168.21
167.69
147.81
141.05
140.65
140.53
139.87
138.67
137.32
137.04
125.13
124.28
124.23
124.14
124.05
123.30
123.24
123.08
121.65
121.57
121.54
112.57
112.03
111.65
111.62
111.59
85.32
60.92
51.68
48.12
40.64 DMSO
40.43 DMSO
40.22 DMSO
40.01 DMSO
39.80 DMSO
39.59 DMSO
39.39 DMSO
28.54
O
O
O
O
O
N
N
H
N-N
O
NH
Cl
6b
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

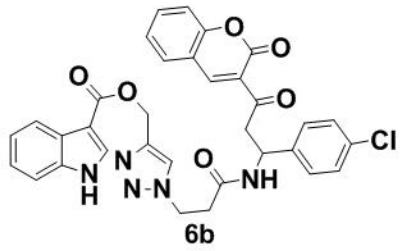
Elemental Composition Report

Page 1

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 33-33 H: 15-27 N: 2-7 O: 2-6 Cl: 1-2
C43H38ClN7O6
CC6 5 (0.119)

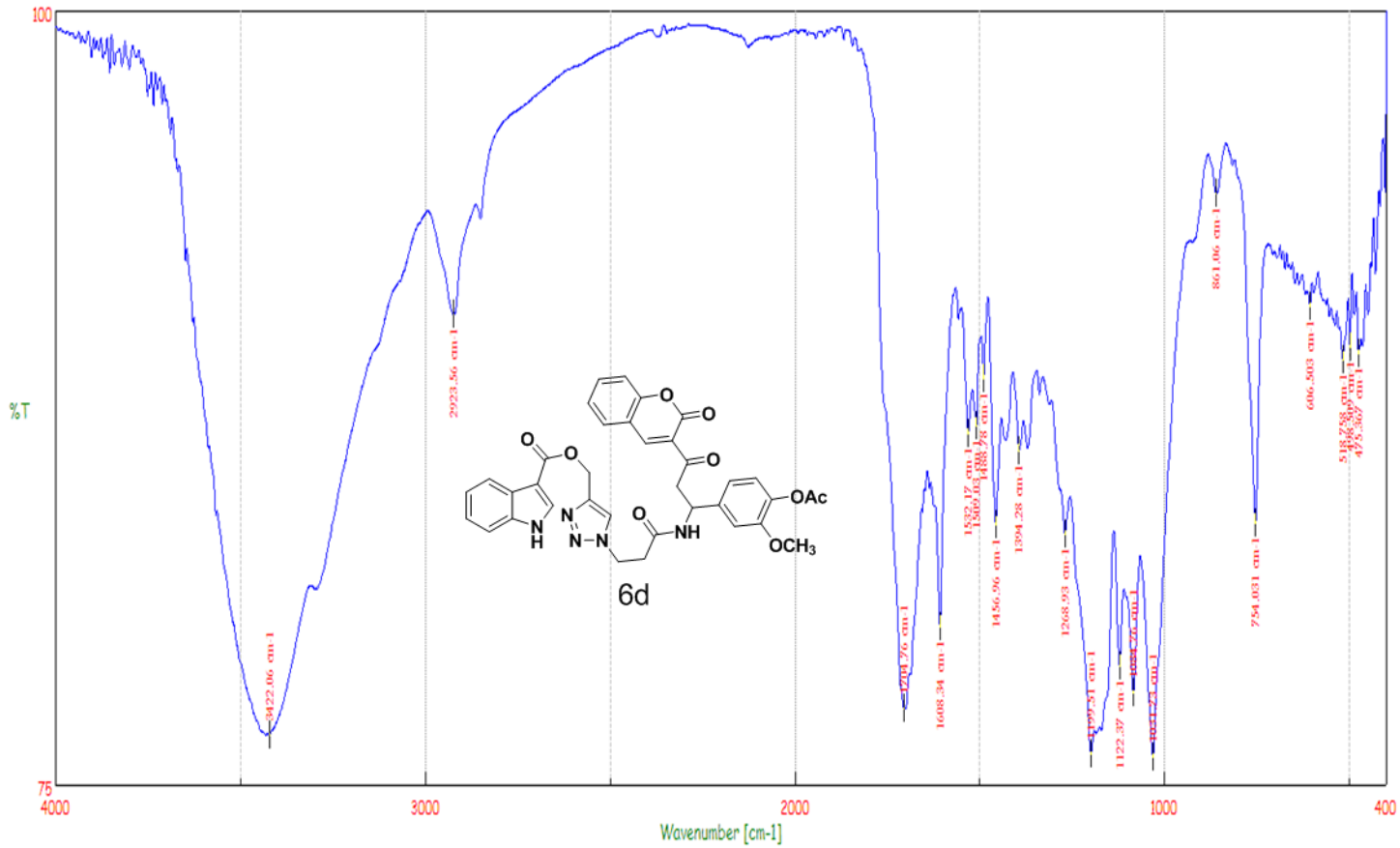


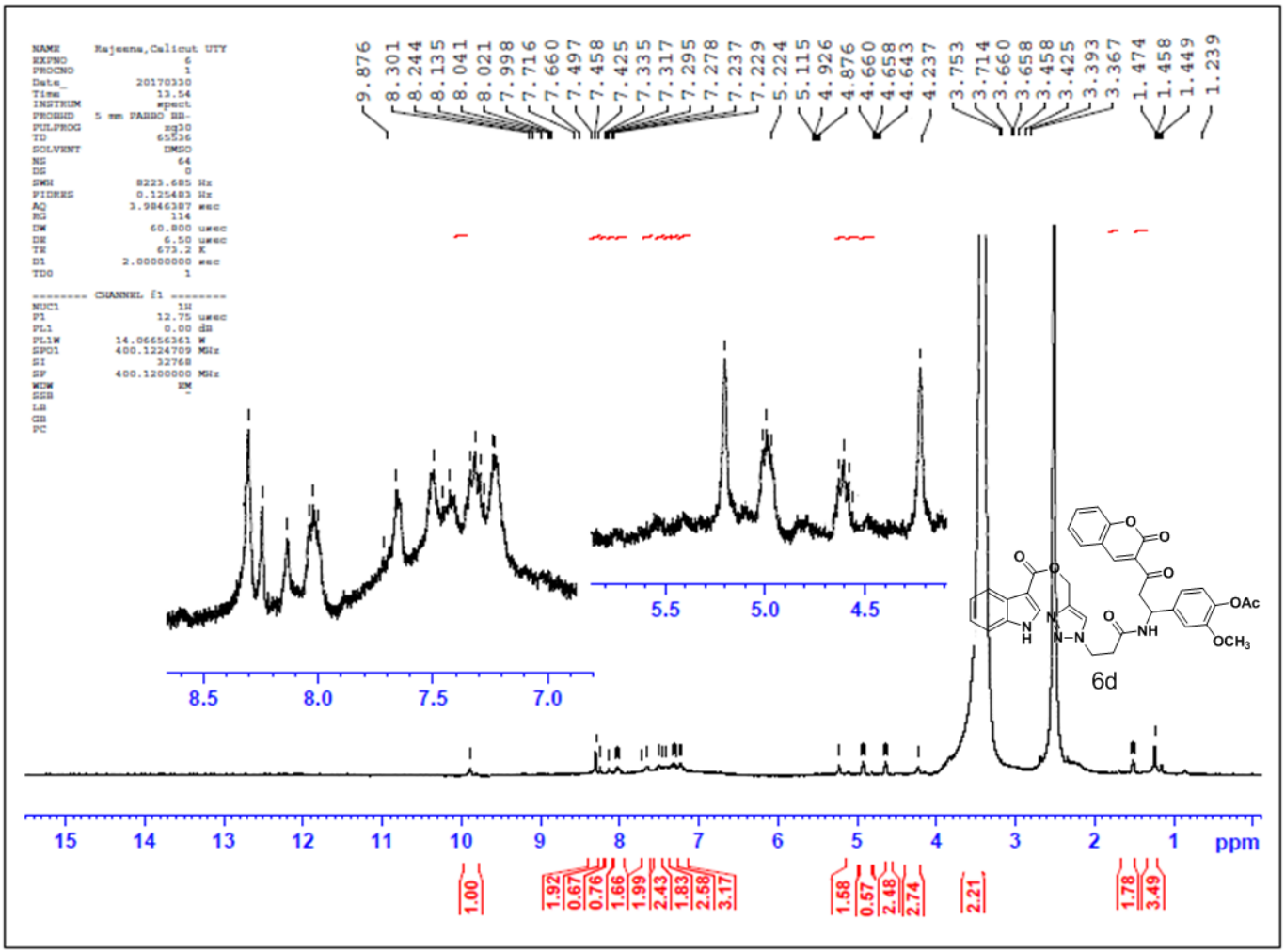


6b

1: TOF MS ES+
9.11e+000

Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
624.1650	624.1650	0.0	0.0	22.5	12.6	0.0	C33 H27 N5 O6 Cl


100
75
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
2923.56 cm-1
3422.06 cm-1
1701.76 cm-1
1608.34 cm-1
1532.17 cm-1
1488.78 cm-1
1456.96 cm-1
1394.28 cm-1
1268.93 cm-1
1179.51 cm-1
1122.37 cm-1
1053.26 cm-1
1023.23 cm-1
861.06 cm-1
754.031 cm-1
606.503 cm-1
518.758 cm-1
475.307 cm-1
O
O
O
O
N
H
N
N
N
O
NH
OAc
OCH3
6d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8.244
8.135
8.041
8.021
7.998
7.716
7.660
7.497
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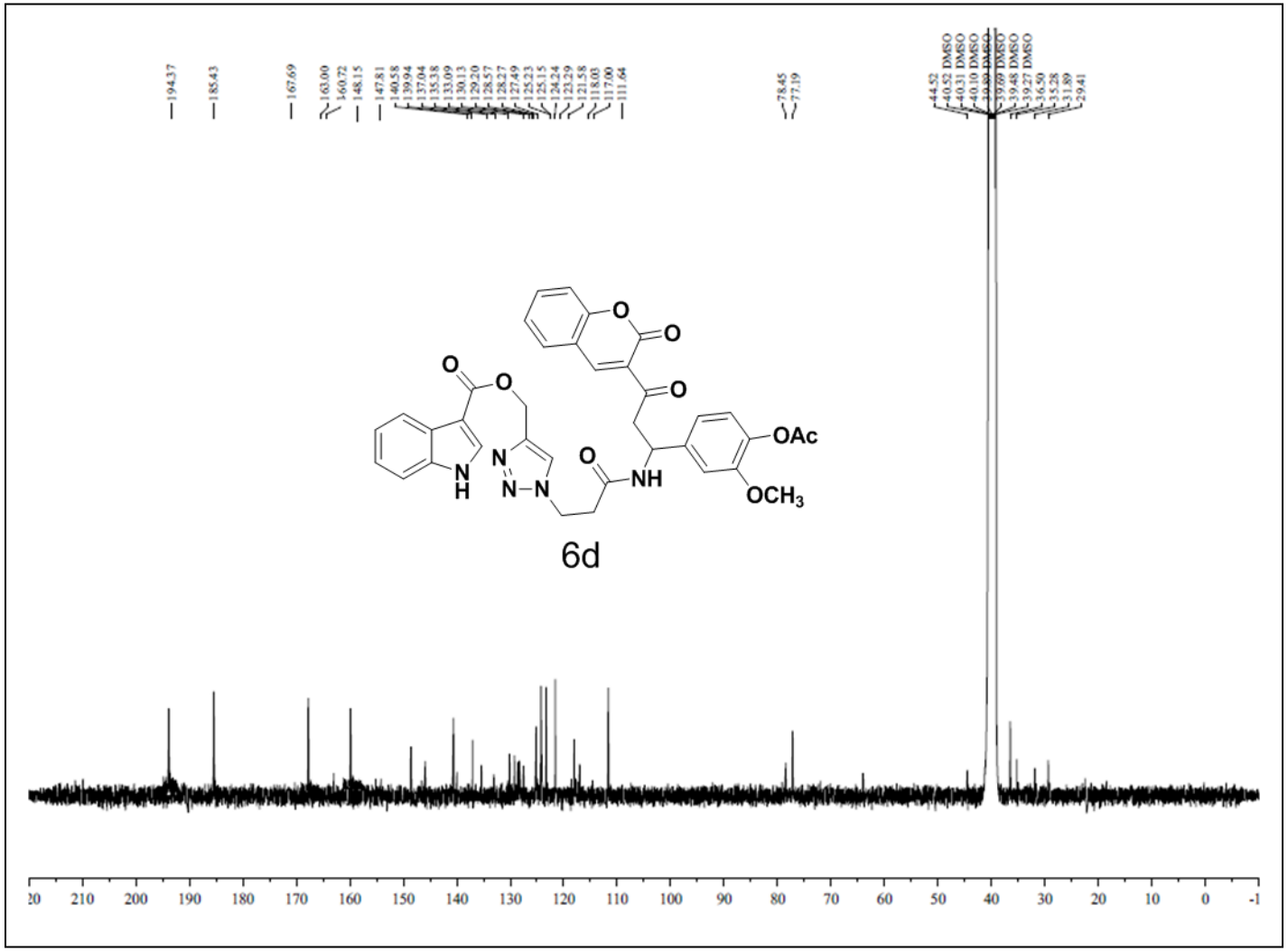
7.425
7.335
7.317
7.295
7.278
7.237
7.229
5.224
5.115
4.926
4.876
4.660
4.658
4.643
4.237
3.753
3.714
3.660
3.658
3.458
3.425
3.393
3.367
1.474
1.458
1.449
1.239
6d
OAc
OCH3
NH
ppm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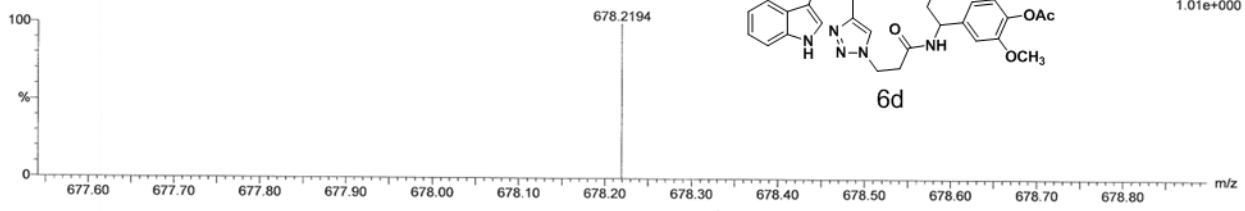
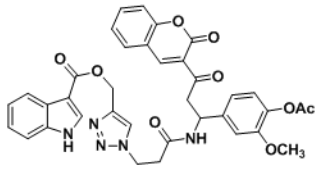
185.43
167.69
163.00
160.72
148.15
147.81
140.58
139.94
137.04
135.38
133.09
130.13
129.20
128.57
128.27
127.49
125.23
125.15
124.24
123.29
121.58
118.03
117.00
111.64
78.45
77.19
44.52
40.52 DMSO
40.31 DMSO
40.10 DMSO
39.89 DMSO
39.69 DMSO
39.48 DMSO
39.27 DMSO
36.50
35.28
31.89
29.41
O
O
O
O
O
N
H
N
N–N
O
NH
OAc
OCH3
6d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

Elemental Composition Report

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 36-36 H: 15-32 N: 2-7 O: 2-9
C43H38ClN7O6
CC9 88 (1.922)





6d

1: TOF MS ES+
1.01e+000

Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
678.2194	678.2200	-0.6	-0.9	23.5	12.5	0.0	C36 H32 N5 O9

Figure S1. The super imposition of the compound 4d, 5d, 6a and 6d with parent ligand in the active site of 1G11.

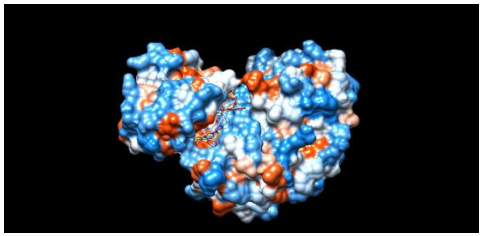


Figure S2. Chemical structure of some Triazole containing potent anticancer compounds

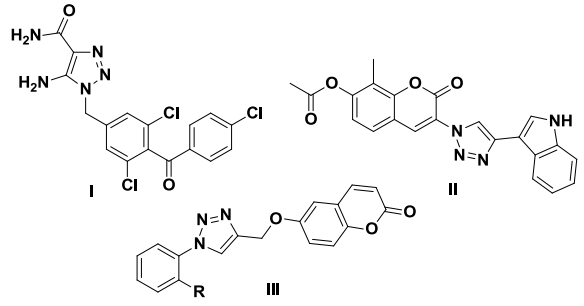
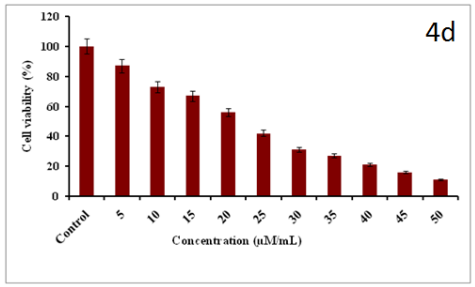
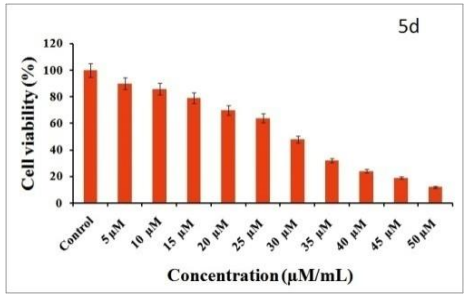


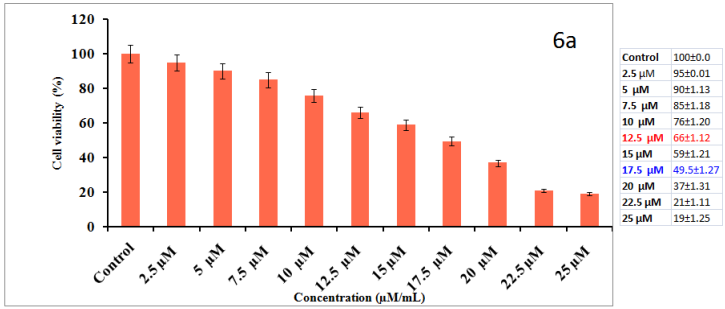
Table S3. Drug property descriptors of the compounds from molinspiration property calculation service

Compound	Molecular weight	miLog p	TPSA	NO. of atoms	n O N	nOHNH
4a	608.05	3.80	129.10	44	10	1
4b	608.05	3.85	129.10	44	10	1
4c	603.63	3.23	138.34	45	11	1
4d	619.63	2.51	158.56	46	12	2
5a	622.08	3.91	129.10	45	10	1
5b	622.08	3.96	129.10	45	10	1
5c	617.66	3.34	138.34	46	11	1
5d	633.66	2.62	158.56	47	12	2
6a	608.05	3.68	139.96	44	10	2
6b	608.05	3.73	139.96	44	10	2
6c	603.63	3.10	149.19	45	11	2
6d	619.63	2.39	169.42	46	12	3

Figure S3. MTT assay results confirming the in vitro cytotoxicity effect of 4d, 5d, 6a and 6d against the MCF-7 cells. The detected IC_{50} concentration was 20,30 and 17.5, 40µM respectively







Control	100±0.0
2.5 µM	95±0.01
5 µM	90±1.13
7.5 µM	85±1.18
10 µM	76±1.20
12.5 µM	66±1.12
15 µM	59±1.21
17.5 µM	49.5±1.27
20 µM	37±1.31
22.5 µM	21±1.11
25 µM	19±1.25

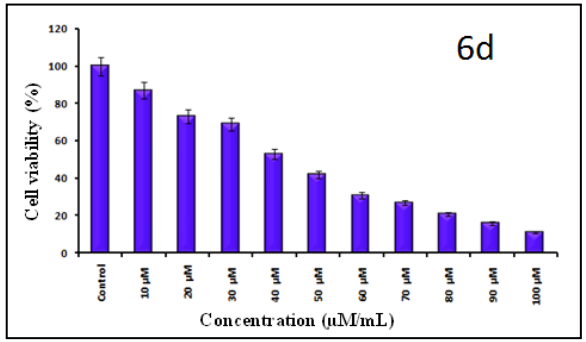
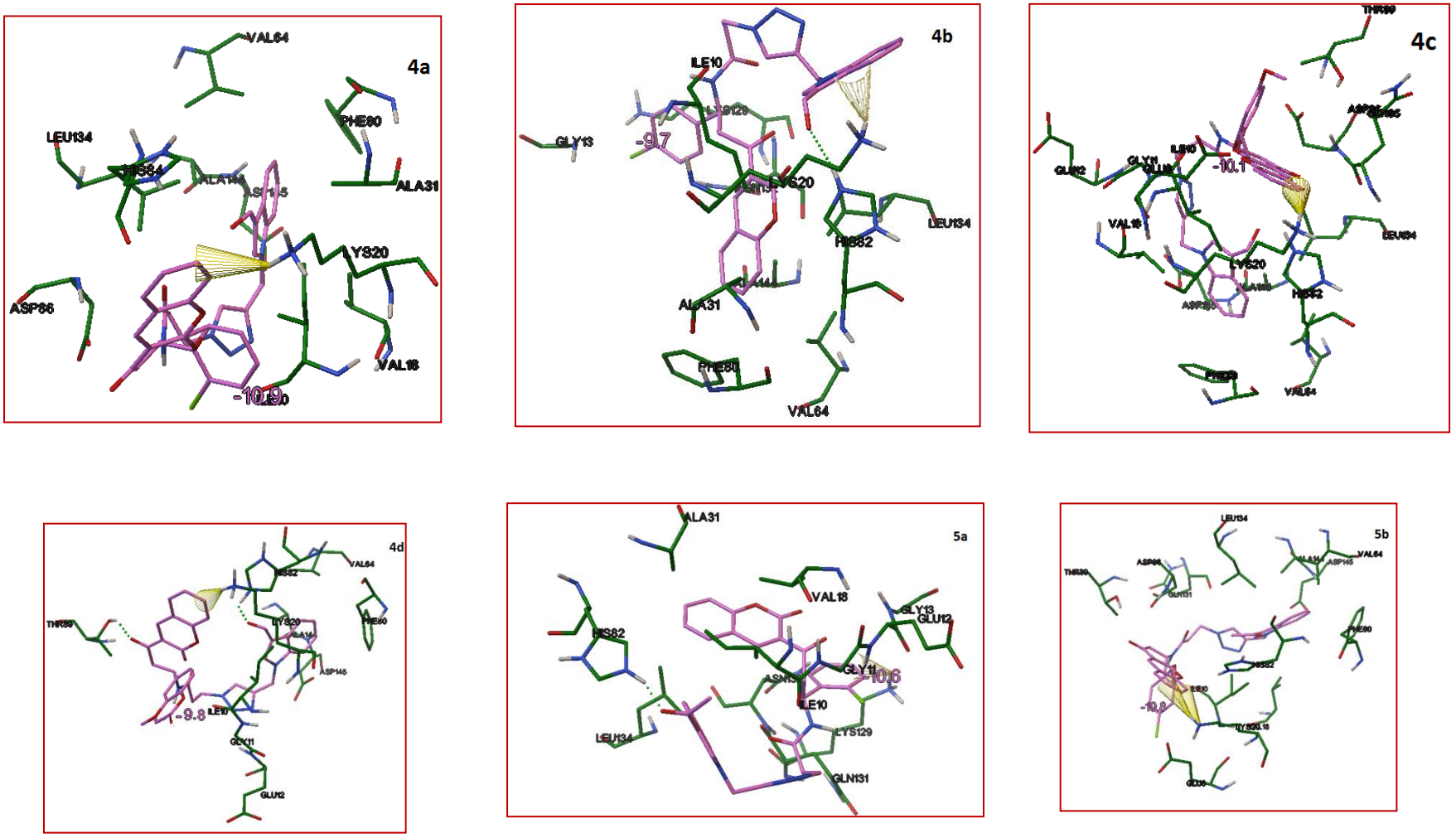
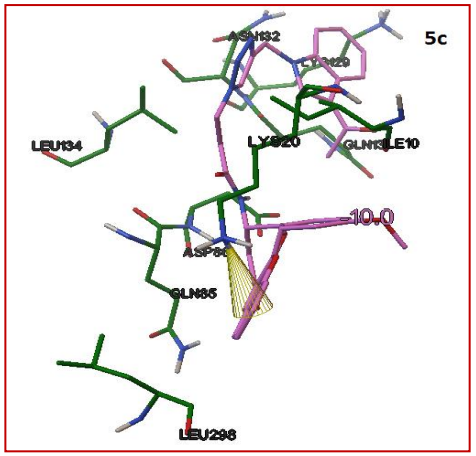
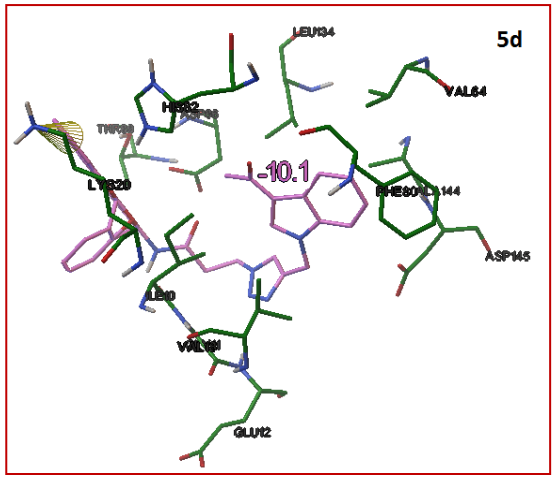
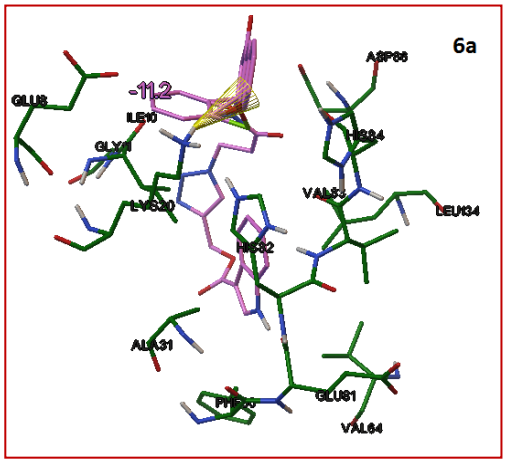


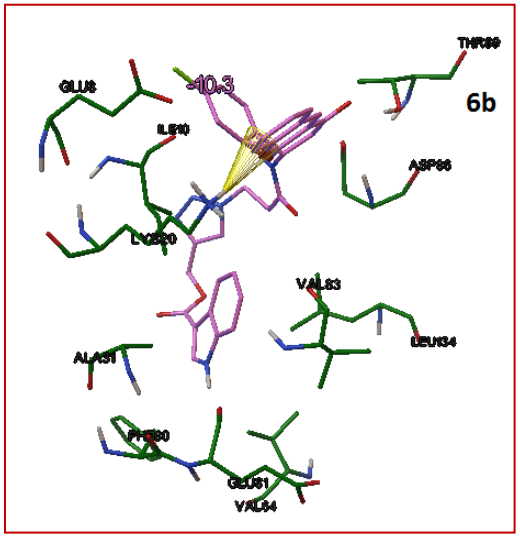
Figure S4. The binding mode of the synthesised compounds in the active site of CDK2-1GII.

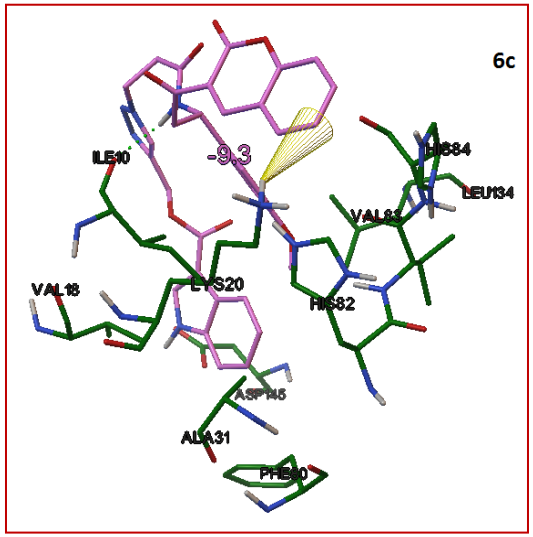



5c
LEU134
LYS20
GLN35
-10.0
LEU298


5d
LEU134
VAL64
-10.1
ASP145
GLU12


6a
-11.2
GLU8
ILE10
LYS20
VAL83
LEU134
ALA31
GLU61
VAL64


6b
-10.3
GLU8
ILE10
LYS20
VAL83
LEU134
ALA31
GLU61
VAL64


6c
-9.3
ILE10
LEU134
VAL18
LYS20
ASP145
ALA31

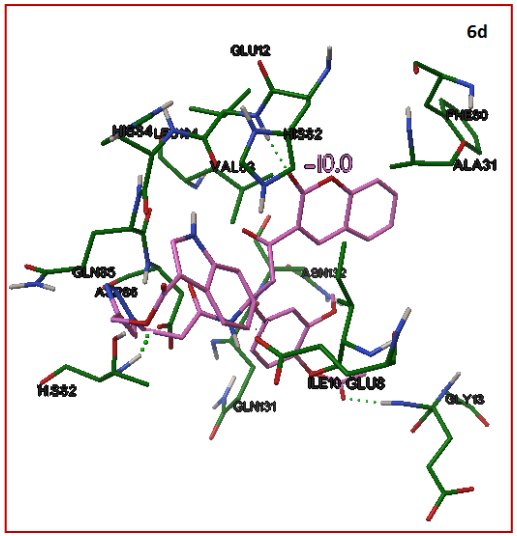

6d
GLU12
-10.0
ALA31
GLN35
ILE10
GLU8
GLY13

Figure S5. The super imposition of the compounds 6d (Red),5d (Green) , 4d(Pink) and (B) 6a (red) with parent ligand(blue) in the active site of CDK2-1GII.

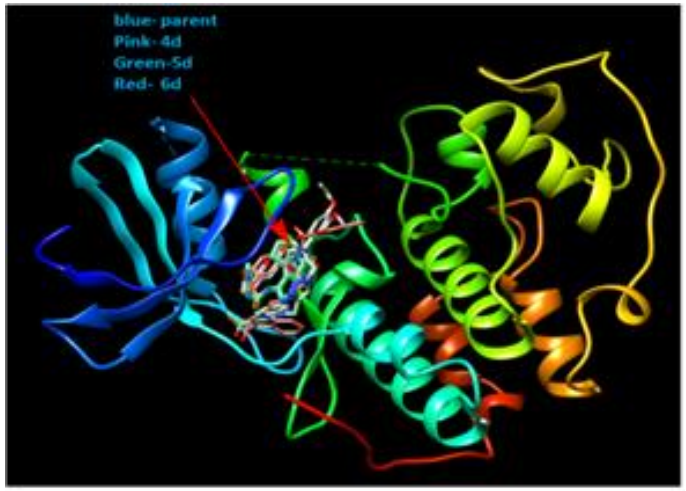


Figure S6. Bright field inverted light microscopy images of MCF-7 treated with 4d, 5d and 6d. (a) controlcells, (b) cells treated with 4d, 5d and 6d at IC 25, (c) cells treated with 4d, 5d and 6d at IC 50 and Fluorescence microscopy images of: (d)control cells, (e)cells treated with 4d, 5d and 6d at IC 25, and (f) cells treated with 4d, 5d and 6dat IC 50