Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Piperazine tethered bergenin-heterocyclic hybrids: Design, Synthesis, anticancer activity and molecular docking studies

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1. Materials and Methods

1.1. Plant materials

The fruits of *Mallotus phillippensis* were collected from Eastern Ghats, AP, India and were authenticated by Dr. K. Madhava Chetty, and a voucher specimen (SVU-MP-0659) was deposited in the herbarium of Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

1.2. Extraction and isolation of Bergenin

The dried fruits of *M. philippensis* were powdered in a pulvarizer (7 kg) and extracted with methanol at room temperature for 72 h. The resulting extract (180 g) was subjected to column chromatography (using 60–120 silica mesh) eluted successively with $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH (4:1), to give four fractions F1-F4. Fraction F2 was further purified using column chromatography eluting with $CHCl_3$/MeOH (92:8) to give bergenin (**5**) as a white amorphous powder which was identified on the basis of its NMR and mass spectral data.

1.2.1. (2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (5):

White amorphous Powder; IR (neat):ν_{max} 3401, 3289, 2968, 2912, 1737, 1656, 1133 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.78 (1H, s), 8.45 (1H, s), 6.99 (1H, s), 5.65 (1H, d, *J* = 3.9 Hz), 5.44 (1H, s), 4.98 (1H, d, *J* = 10.5 Hz), 4.93 (1H, s), 4.00 (1H, t, *J* = 9.9 Hz), 3.83 (1H, dd, *J* = 17.3, 6.7 Hz), 3.77 (3H, s), 3.66 (1H, dd, *J* = 11.9, 5.3 Hz), 3.60-3.52 (1H, m), 3.20 (1H, dd, *J* = 17.1, 8.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.2, 150.9, 150.0, 140.5, 118.0, 115.9, 109.4, 81.7, 79.7, 73.6, 72.0, 70.6, 61.0, 59.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{14}H_{17}O_9$ 329.0873; Found 329.0876.

1.3. General procedure for synthesis of 5a-e

In order to prepare the compound **4a-e**, to the solution of substituted phenacyl bromides (1 equiv) (**1**) and thiourea (1.2 equiv) dissolved in ethanol (15 mL) was heated under reflux conditions. The reaction was continued until the substituted phenacyl bromide was completely consumed. The reaction mixture was cooled to room temperature and the ice-cold water was added to form precipitation which is further neutralized with $NaHCO_3$. The solution was filtered to get a white powder (**2**) and continued until no solid precipitation was obtained. Further, to the stirring solution of TEA (1.5 equiv) and chloroacetyl chloride solution (1.5 equiv in 3 mL of THF), the compound **2** was added and kept at 0 °C for 2-6 h to form 2-chloro-*N*-(benzo[*d*]thiazol-2-yl)acetamide (**3**) [1]. After completion of the reaction, the solution was dissolved in DCM and washed with aqueous 1M NaOH and brine solution. The organic layer was collected and evaporated under reduced pressure, the compound **(3)** was purified by column chromatography and eluted with acetone: hexane (20:80). In the next step, compound **3**(1 equiv) is reacted with piperazine (3 equiv) in the presence of TEA (1 equiv) and 1,4-dioxane at 110 °C for 2-6 h to form compound **4**. Every structural moiety **(4a-e)** can be tailored individually with the aim of expediting systematic enhancement of the search for gradually effective bergenin analogues. In the final step, the bergenin analogues (**5a-5e**) were prepared by electrophilic substitution of various secondary amines at C_7 position of bergenin (**5**) using a Mannich reaction. To the solution of bergenin (1 equiv) dissolved in DMSO in the presence of 37% of formaldehyde (0.5 mL) and arylthiazolylpiperazines (1 equiv) at 50 °C for 12 h. After completion of the reaction, the reaction mixture was allowed to cool at room temperature, filtered and diluted with water. The DMSO in the solution was removed passed through the LH-20 resin bed and was further purified by column chromatography using Sephadex LH-20 eluting

with methanol to get the desired products (**5a-5e**) in pure form. As prepared bergenin analogues were confirmed by the spectral analysis IR, ^{1}H NMR, ^{13}C NMR, Mass and HRMS spectroscopy.

1.3.1. N-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(piperazin-1-yl)acetamide (4a):

White amorphous powder; yield: 42%; IR (neat): ν_{max} 3392, 1633, 1450, 1011, 742, 670 cm^{-1}; ^{1}H NMR (400MHz, CD_3OD): δ 7.79 (2H, dd, *J* = 6.8, 2.1 Hz), 7.23 (1H, s), 6.95 (2H, dd, *J* = 6.7, 2.0 Hz), 3.82 (3H, s), 3.31 (2H, s), 2.97 (4H, t, *J* = 4.9 Hz), 2.62 (4H, t, *J* = 4.3 Hz); ^{13}C NMR (100MHz, CD_3OD): δ 170.5, 161.2, 159.0, 151.0, 128.6, 128.4, 115.1, 107.1, 62.2, 55.8, 54.6 ,46.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{16}H_{21}O_2N_4S$ 333.1374; Found 333.1379.

1.3.2. N-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (5a):

White amorphous powder; yield: 51%; IR (neat): ν_{max} 3319, 2948, 2832, 1754 1652, 1450, 1122, 1021, 782 cm^{-1}; ^{1}H NMR (400MHz, CD_3OD): δ 7.79 (2H, dd, *J* = 6.8, 2.1 Hz), 7.23 (1H, s), 6.95 (2H, dd, *J* =6.8, 2.1 Hz), 4.62 (1H, d, *J* = 9.6 Hz), 4.31 (1H, d, *J* = 14.9 Hz), 4.26 (1H, d, *J* = 14.9 Hz), 4.07-3.99 (2H, m), 3.90 (3H, s), 3.82 (3H, s), 3.79 (1H, t, *J* = 9.0 Hz), 3.70-3.64 (2H, m), 3.41 (1H, t, *J* = 9.0 Hz), 3.36 (2H, d, *J* = 7.7 Hz), 2.83 (4H, s), 2.74 (4H, s); ^{13}C NMR (100MHz, CD_3OD): δ 170.4, 165.0, 161.2, 159.1, 154.5, 151.0, 148.8, 141.4, 128.6, 128.4, 118.7, 117.6, 115.1, 107.6, 82.9, 80.6, 75.6, 74.5, 71.8, 62.7, 61.3, 60.9, 57.1, 55.8, 53.7, 53.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{31}H_{37}O_{11}N_4S$ 673.2192; Found 673.2174.

1.3.3. N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(piperazin-1-yl)acetamide (4b):

White amorphous powder; yield: 47%; IR (neat): ν_{max} 3425, 1672, 1454, 1028, 786, 698 cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.78 (2H, dd, *J* = 6.7, 1.8 Hz), 7.38 (2H, dd, *J* = 6.7, 2.0 Hz), 7.14 (1H, s), 3.26 (2H, s), 3.04 (4H, s), 2.64 (4H, s); ^{13}C NMR (100MHz, $CDCl_3$): δ 168.6, 157.2,

148.9, 133.8, 132.8, 128.9, 127.3, 108.2, 61.6, 54.3, 45.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{15}H_{18}ON_4ClS$ 337.0887; Found 337.0884.

1.3.4. N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (5b):

White amorphous powder; yield: 59%; IR (neat):ν_{max} 3328, 2947, 2836, 1763, 1652, 1454, 1102, 1028, 745 cm^{-1}; ^1H NMR (400MHz, CD_3OD): δ 7.76 (2H, dd, *J* = 6.8, 1.7 Hz), 7.32 (1H, s), 7.29 (2H, dd, *J* = 6.8, 1.8 Hz), 4.79 (1H, s), 4.16 (2H, s), 3.95-3.90 (2H, m), 3.80 (3H, s), 3.69 (1H, t, *J*= 9.0 Hz), 3.59-3.54 (2H, m), 3.31 (1H, t, *J* = 9.0 Hz), 3.27 (2H, s), 2.69 (4H, s), 2.63 (4H, s); ^{13}C NMR (100MHz, CD_3OD): δ 170.5, 165.0, 159.3, 154.5, 149.9, 148.8, 141.4, 134.6, 134.5, 129.8, 128.5, 118.7, 118.2, 117.6, 109.6, 82.9, 80.6, 75.6, 74.5, 71.8, 62.7, 61.3, 60.9, 57.0, 53.6, 53.2; HRMS (ESI) m/z: $[M+2H]^+$ Calcd for $C_{30}H_{35}O_{10}N_4ClS$ 678.1761; Found 678.1757.

1.3.5. N-(4-Phenylthiazol-2-yl)-2-(piperazin-1-yl)acetamide (4c):

White amorphous powder; yield: 48%; IR (neat):ν_{max} 3412, 1631, 1453, 1023, 754, 648 cm^{-1}; ^1H NMR (400MHz, CD_3OD+$CDCl_3$): δ 7.83 (2H, d, *J* = 7.2 Hz), 7.43 (2H, t, *J* = 7.5 Hz), 7.36-7.32 (1H, m), 7.25 (1H, s), 3.32 (2H, s), 3.06 (4H, t, *J* = 4.9 Hz), 2.69 (4H, t, *J* = 4.7 Hz); ^{13}C NMR (100 MHz, CD_3OD+$CDCl_3$): δ 169.6, 158.2, 150.5, 134.7, 129.3, 128.6, 126.5, 108.5, 61.7, 53.9, 45.5; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{15}H_{19}ON_4S$ 303.1266; Found 303.1274.

1.3.6. N-(4-Phenylthiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl) piperazin-1-yl)acetamide (5c):

White amorphous powder; yield: 53%; IR (neat):ν_{max} 3344, 2943, 2830, 1712, 1652, 1452, 1121, 1024 ,754 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.88-7.85 (2H, m), 7.42-7.37 (3H, m), 7.30

(1H, t, *J* = 7.3 Hz), 4.62 (1H, d, *J* = 9.7 Hz), 4.32-4.23 (2H, m), 4.05-3.99 (2H, m), 3.90 (3H, s), 3.79 (1H, t, *J* = 9.0 Hz), 3.70-3.64 (2H, m), 3.41 (1H, t, *J* = 9.0 Hz), 3.36 (2H, d, *J* = 8.2 Hz), 2.82 (4H, s), 2.74 (4H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 170.5, 165.0, 159.2, 154.5, 151.1, 141.4, 135.7, 129.8, 129.1, 127.1, 118.7, 118.3, 117.5, 109.0, 83.0, 80.6, 75.6, 74.5, 71.8, 62.7, 61.3, 60.9, 57.0, 53.7, 53.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{30}H_{35}O_{10}N_4S$ 643.2056; Found 643.2068.

1.3.7. N-(4-(Naphthalen-1-yl)thiazol-2-yl)-2-(piperazin-1-yl)acetamide (4d):

White amorphous powder; yield: 41%; IR (neat): ν_{max} 3385, 1629, 1456, 1121, 1022, 788, 663 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24-8.19 (1H, m), 7.91-7.87 (2H, m), 7.65 (1H, d, *J* = 7.0 Hz), 7.54-7.48 (3H, m), 7.11 (1H, s), 3.22 (2H, s), 2.93 (4H, t, *J* = 4.6 Hz), 2.57 (4H, s); 13C NMR (100 MHz, $CDCl_3$): δ 168.7, 156.8, 149.6, 133.9, 132.8, 131.4, 128.8, 128.4, 127.4, 126.4, 125.9, 125.6, 125.3, 111.8, 61.7, 54.5, 45.7; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{19}H_{21}ON_4S$ 353.1425; Found 353.1430.

1.3.8. N-(4-(Naphthalen-1-yl)thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl) acetamide (5d):

White amorphous powder; yield: 47%; IR (neat): ν_{max} 3356, 2945, 2834, 1791, 1649, 1456, 1101, 1021, 741 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (1H, s), 8.36-8.32 (1H, m), 8.01-7.95 (2H, m), 7.69 (1H, d, *J* = 6.2 Hz), 7.59-7.52 (3H, m), 7.45 (1H, s), 5.67 (1H, s), 5.44 (1H, s), 4.92 (1H, d, *J* = 10.4 Hz), 4.15 (1H, d, *J* = 15.2 Hz), 4.07 (1H, d, *J* = 15.2Hz), 3.93 (1H, t, *J* = 9.3 Hz), 3.85 (1H, d, *J* = 10.5 Hz), 3.77 (3H, s), 3.66-3.55 (4H, m), 3.18 (2H, t, *J* = 9.2 Hz), 2.64 (4H, s), 2.57 (4H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.5, 162.5, 157.0, 153.1, 151.9, 148.7, 146.7, 139.3, 133.4, 132.6, 130.5, 128.3, 128.1, 127.1, 126.1, 125.8, 125.7, 125.3, 117.0,

115.6, 111.8, 81.4, 78.8, 73.5, 72.2, 70.4, 61.0, 59.6, 59.5, 56.3, 52.0, 51.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{34}H_{37}O_{10}N_4S$ 693.2202; Found 693.2225

1.3.9. N-(4-(Naphthalen-2-yl)thiazol-2-yl)-2-(piperazin-1-yl)acetamide (4e):

White amorphous powder; yield: 45%; IR (neat):ν_{max} 3354, 1648, 1434, 1023, 795, 697 cm^{-1}; ^1H NMR (500MHz, CD_3OD+$CDCl_3$): δ 8.36 (1H, s), 7.95 (1H, dd, *J* = 8.5, 1.8 Hz), 7.90-7.87 (1H, m), 7.51-7.45 (3H, m), 3.32 (2H, m), 2.99 (4H, t, *J* = 4.9 Hz), 2.64 (4H, t, *J* = 4.6 Hz); ^{13}C NMR (100Hz, CD_3OD+$CDCl_3$): δ 170.4, 158.9, 150.8, 134.7, 134.3, 132.7, 129.2, 129.1, 128.5, 127.3, 127.0, 125.7, 124.9, 109.3, 62.1, 54.7, 46.1; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{19}H_{21}ON_4S$ 353.1431; found 353.1430.

1.3.10. N-(4-(naphthalen-2-yl)thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (5e):

White amorphous powder; yield: 47%; IR (neat): ν_{max} 3358, 2941, 2831, 1769, 1648, 1450, 1124, 1023, 763 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 8.29 (1H, s), 7.89 (1H, dd, *J*= 8.6, 1.8 Hz), 7.82-7.73 (4H, m), 7.44 (1H, s), 7.41-7.35 (2H, m), 4.80 (1H, s), 4.17 (2H, s), 3.96-3.90 (2H, m), 3.81 (3H, s), 3.69 (1H, t, *J*= 9.0 Hz), 3.59-3.54 (2H, m), 3.34-3.28 (3H, m), 2.71 (4H, s), 2.64 (4H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 170.5, 165.0, 159.3, 154.5, 151.1, 148.8, 141.4, 135.1, 134.6, 133.1, 129.4, 129.3, 128.8, 127.5, 127.2, 125.9, 125.1, 118.7, 118.4, 117.5, 109.6, 82.9, 80.6, 75.7, 71.8, 62.7, 61.3, 60.9, 57.1, 53.7, 53.2; HRMS (ESI) m/z: $[M+]^+$Calcd for $C_{34}H_{36}O_{10}N_4S$ 692.2152; Found 692.2156.

1.4. General experimental procedure for the synthesis of 10a-j

In another reaction, 1 equivalence of different substituted aryl anilines (**6a-j**) are reacted with potassium thiocyanide in the presence of bromine in glacial acetic acid at room temperature

for 3 h [2]. After stirring the reaction mixture for 2-4 h at room temperature, the hydrogen bromide (HBr) salt present in the reaction mixture was separated, filtered, washed with acetic acid. Further, the solution was dried with a rotary evaporator, diluted with hot water and basified with the ammonia solution (pH 11.0). The obtained precipitate was washed with water to afford the 2-amino-4,6-substituted benzothiazoles (**7a-j**) in good yield. As described earlier [2], this amino group of **7a-j** (1 equiv) was reacted with stirring solution of chloroacetyl chloride (1.2 equiv) dissolved in benzene in the presence of K_2CO_3 (1.2 equiv) at room temperature and refluxed for 6-12 h. The resultant was further cool to room temperature and add ice cold water to afford the substituted 2-chloro-*N*-(benzo[*d*]thiazol-2-yl)acetamides (**8a-j**). Further, these substituted chloroacetamides (1 equiv) are reacted with piperazine (3 equiv) in the presence of TEA (1 equiv) and 1,4-dioxane at 110 °C for 2-6 h to form substituted *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamides (**9a-j**)[3]. Finally, the diluted solution of bergenin (1 equiv) in DMSO was treated with substituted benzothiazolylpiperazines (1 equiv) in the presence of 37% of formaldehyde (0.5 mL) at 50 °C for 12 h. The reaction solution was cooled to room temperature, filtered, and diluted with water. The DMSO in the solution was removed passed through the LH-20 resin bed and was further purified by column chromatography using Sephadex LH-20 eluting with methanol to get the desired products (**10a-j**) in pure form. As prepared bergenin analogues were confirmed by the spectral analysis IR, ^{1}H NMR, ^{13}C NMR, Mass and HRMS spectroscopy.

1.4.1. N-(6-methoxybenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide(9a):

White amorphous powder; yield: 48%; IR (neat):ν_{max} 3358, 1639, 1453, 1029, 748, 632 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (1H, d, J= 8.9 Hz), 7.41 (1H, d, *J*= 2.6 Hz), 7.04 (1H, dd, *J*= 8.8, 2.6 Hz), 3.85 (3H, s), 3.34 (2H, s), 2.98 (4H, t, *J*= 4.9 Hz), 2.65 (4H, t, *J*= 4.8 Hz), ^{13}C NMR

(100 MHz, CD_3OD): δ 170.9, 158.6, 157.4, 143.7, 134.5, 122.4, 116.5, 105.3, 62.2, 56.3, 54.4, 46.1; HRMS (ESI) m/z: $[M+]^+$Calcd for $C_{14}H_{18}N_4O_2S$ 306.1150 Found 306.1153.

1.4.2. N-(6-Methoxybenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (10a):

White amorphous powder; yield: 47%; IR (neat): ν_{max} 3358, 2967, 2884, 1718, 1670, 1463, 1166, 1083, 749 cm^{-1}; ^1H NMR (400 MHz, CD_3OD+$CDCl_3$): δ 7.62 (1H, d, *J* = 8.8 Hz), 7.41 (1H, d, *J* = 2.6 Hz), 7.03 (1H, dd, *J* = 8.8, 2.6 Hz), 4.88 (1H, s), 4.26 (2H, s), 4.05-3.99 (2H, m), 3.90 (3H, s), 3.84 (3H, s), 3.79 (1H, t, *J* = 9.1 Hz), 3.70-3.65 (2H, m), 3.43-3.37 (3H, m), 2.80 (4H, s), 2.74 (4H, s); ^{13}C NMR (100 MHz, CD_3OD+$CDCl_3$): δ 168.5, 162.7, 156.3, 155.1, 152.3, 146.6, 141.4, 139.2, 132.3, 120.3, 116.3, 115.2, 114.4, 103.1, 80.7, 78.4, 73.4, 72.4, 69.6, 60.6, 59.3, 58.9, 55.3, 54.3, 51.7, 51.1; HRMS (ESI^+) m/z: $[M+H]^+$Calcd for $C_{29}H_{35}O_{11}N_4S$ 647.1997; Found 647.2017.

1.4.3. N-(Benzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl) piperazin-1-yl)acetamide (10b):

White amorphous powder; yield: 61%; IR (neat): ν_{max} 3312, 2943, 2831, 1751, 1651, 1452, 1136, 1021, 714 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.58 (1H, s), 7.98 (1H, d, *J* = 7.5 Hz), 7.75 (1H, d, *J* = 8.1 Hz), 7.47-7.42 (1H, m), 7.34-7.29 (1H, m), 5.67 (1H, s), 5.42 (1H, s), 4.93 (1H, d, *J* = 10.3 Hz), 4.22-4.08 (2H, m), 3.95 (1H, t, *J* = 9.8 Hz), 3.85 (1H, d, *J* = 10.3 Hz), 3.78 (3H, s), 3.73-3.54 (4H, m), 3.19 (2H, t, *J* = 9.2 Hz), 2.66 (8H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.3, 162.6, 157.4, 153.0, 148.4, 146.7, 139.3, 131.3, 126.1, 123.5, 121.6, 120.4, 117.1,

115.6, 81.4, 78.8, 73.5, 72.2, 70.4, 61.0, 59.7, 59.5, 56.4, 52.0, 51.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{28}H_{33}O_{10}N_4S$ 617.1896; Found 617.1911.

1.4.4. N-(6-Nitrobenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (10c):

Yellow amorphous powder; yield: 49%; IR (neat): ν_{max} 3362, 2976, 2834, 1787, 1655, 1516, 1459, 1298, 1086, 738 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.06 (1H, d, *J* = 2.6 Hz), 8.55 (1H, s), 8.32-8.27 (1H, m), 7.90 (1H, d, *J* = 9.0 Hz), 5.66 (1H, s), 5.42 (1H, s), 4.92 (1H, d, *J* = 10.3 Hz), 4.18-4.07 (2H, m), 3.93 (1H, t, *J* = 9.8 Hz), 3.85 (1H, d, *J* = 10.1 Hz), 3.77 (3H, s), 3.66-3.56 (4H, m), 3.18 (2H, t, *J* = 9.0 Hz), 2.65 (4H, s), 2.60 (4H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 163.1, 162.6, 153.3, 153.0, 146.7, 142.9, 139.3, 132.1, 121.7, 120.5, 119.0, 117.1, 115.6, 81.4, 78.8, 73.5, 72.2, 70.4, 61.0, 59.6, 59.5, 52.0, 51.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{28}H_{32}O_{12}N_5S$ 662.1737; Found 662.1762.

1.4.5. N-(4-chlorobenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (9d):

White amorphous powder; yield: 62%; IR (neat): ν_{max} 3347, 1639, 1453, 1060, 788, 663 cm^{-1}; ^1H NMR (500MHz, CD_3OD+$CDCl_3$): δ 7.78 (1H, d, *J* = 7.9 Hz), 7.49 (1H, d, *J* = 7.8 Hz), 7.29 (1H, t, *J* = 7.8 Hz), 3.35 (2H, s), 3.02 (4H, t, *J* = 4.7 Hz), 2.66 (4H, s); ^{13}C NMR (100 MHz, CD_3OD+$CDCl_3$): δ 170.7, 159.1, 145.8, 133.9, 127.1,126.3, 125.4, 120.7, 61.8, 54.4, 45.6; HRMS (ESI) m/z: $[M+]^+$Calcd for $C_{13}H_{15}ClN_4OS$ 310.0655; Found 310.0648.

1.4.6. N-(4-Chlorobenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl) piperazin-1-yl)acetamide (10d):

White amorphous powder; yield: 37%; IR (neat):ν_{max} 3329, 2967, 2863, 1724, 1682, 1459, 1141, 1060, 793 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (1H, s), 7.97 (1H, d, *J* = 7.9 Hz), 7.31 (1H, d, *J* = 7.9 Hz), 7.54 (1H, d, *J* = 7.7 Hz), 5.68 (1H, s), 5.43 (1H, s), 4,92 (1H, d, *J* = 10.4 Hz), 4.05 (2H, s), 3.94 (1H, t, *J* = 9.8 Hz), 3.85 (1H, d, *J* = 10.3 Hz), 3.77 (3H, s), 3.63 (2H, t, *J* = 9.2 Hz), 3.59-3.55 (2H, m), 2.64 (8H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 162.7, 158.6, 153.0, 147.0, 145.3, 139.4, 133.0, 126.2, 124.5, 124.3, 120.8, 117.2, 115.8, 81.4, 78.8, 73.5, 72.2, 70.5, 61.0, 59.6, 51.7; HRMS (ESI) m/z: $[M+H]^+$ Cal for $C_{28}H_{32}O_{10}N_4ClS$ 651.1541; found 651.1522.

1.4.7. N-(6-Chlorobenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide(9e):

White amorphous powder; yield: 38%; IR (neat): ν_{max} 3381, 1642, 1453, 1025, 761, 643 cm^{-1}. ^{1}H NMR (500MHz, CD_3OD): δ 7.85 (1H, d, *J* = 2.0 Hz), 7.69 (1H, d, *J* = 4.6 Hz), 7.41 (1H, d, *J* = 8.5, 2.1 Hz), 3.35 (2H, s), 2.98 (4H, t, *J* = 4.9 Hz), 2.65 (4H, t, *J* = 4.6 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 170.9, 159.9, 148.6, 134.9, 130.5, 127.9, 122.9, 122.2, 61.2, 51.5, 45.1; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{13}H_{16}ON_4ClS$ 311.0716; Found 311.0727.

1.4.8. N-(6-Chlorobenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide(10e):

White amorphous powder; yield: 43%; IR (neat):ν_{max} 3358, 2994, 2876, 1785, 1659, 1456, 1132, 1029, 762 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (1H, s), 8.12 (1H, d, *J* = 2.2 Hz), 7.73 (1H, d, *J* = 8.8 Hz), 7.46 (1H, dd, *J* = 8.6, 2.2 Hz), 4.91 (1H, d, *J* = 10.5 Hz), 4.14 (1H, d, *J* = 15.2 Hz), 4.07 (1H, d, *J* = 15.2 Hz), 3.93 (1H, t, *J* = 9.8 Hz), 3.84 (1H, d, *J* = 10.1 Hz), 3.76 (3H, s), 3.63 (2H, t, *J* = 9.0 Hz), 3.58-3.55 (2H, m), 3.20-3.16 (2H, m), 2.63 (4H, s), 2.57 (4H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 162.6, 158.3, 153.1, 147.3, 146.8, 139.3, 133.1, 127.6,

126.4, 121.7, 121.4, 117.1, 115.7, 81.4, 78.8, 73.5, 72.3, 70.5, 61.0, 59.7, 59.5, 52.0, 51.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{28}H_{32}O_{10}N_4ClS$ 651.1501; Found 651.1522.

1.4.9. N-(6-Methylbenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (9f):

White amorphous powder; yield: 48%; IR (neat):ν_{max} 3258, 1652, 1453, 1023,798, 699 cm^{-1}; ^1H NMR (400 MHz, $CD_3OD+CDCl_3$): δ 7.64-7.61 (2H, m), 7.26 (1H, dd, *J* = 8.4, 1.5 Hz), 3.34 (2H, s), 2.99 (4H, t, *J* = 4.8 Hz), 2.65 (4H, t, *J* = 4.3 Hz), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CD_3OD+CDCl_3$): δ 170.7, 158.2, 147.1, 135.2, 133.1, 128.7, 122.0, 121.2, 62.1, 54.4, 46.0, 21.5; HRMS (ESI) m/z: $[M+]^+$Calcd for $C_{14}H_{18}N_4OS$ 290.1201; Found 290.1201.

1.4.10. N-(6-Methylbenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide(10f):

White amorphous powder; yield: 56%; IR (neat):ν_{max} 3368, 3331, 2947, 2836, 1771, 1652, 1453, 1022, 769 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.67 (1H, s), 7.63 (1H, d, *J* = 8.3 Hz), 7.27 (1H, d, *J* = 8.3 Hz), 5.0 (1H, d, *J* = 10.5 Hz), 4.76 (1H, d, *J* = 12.7 Hz), 4.48 (1H, d, *J* = 12.7 Hz), 4.15-4.03 (2H, m), 3.97 (3H, s), 3.83 (1H, t, *J* = 9.0 Hz), 3.74-3.66 (3H, m), 3.53-3.41 (6H, m), 3.22-3.11 (2H, m), 2.88-2.75 (2H, m), 2.45 (3H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 170.5, 165.7, 158.6, 152.7, 150.6, 147.6, 135.5, 133.4, 128.9, 122.2, 121.5, 120.2, 119.4, 112.0, 83.2, 80.7, 75.5, 74.3, 71.9, 62.6, 61.2, 60.5, 53.7, 50.6, 21.5; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{29}H_{35}N_4O_{10}S$ 631.2074; Found 631.2093.

1.4.11. N-(4-Methylbenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (9g):

White amorphous powder; yield: 39%; IR (neat):ν_{max} 3269, 1655, 1451, 1023, 861, 697 cm^{-1}. ^1H NMR (500MHz, $CDCl_3$): δ 7.62 (1H, d, *J* = 7.6 Hz), 7.26-7.20 (2H, m), 3.27 (2H, s), 3.01 (4H, t, *J* = 4.8 Hz), 2.67 (3H, s), 2.67 (4H, t, *J* = 4.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.1, 156.1,

147.6, 132.0, 130.8, 126.9, 123.9, 118.8, 61.9, 54.8, 45.9, 18.1; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{14}H_{19}ON_4S$ 291.1269; Found 291.1274.

1.4.12. N-(4-Methylbenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide(10g):

White amorphous powder; yield: 48%; IR (neat):ν_{max} 3364, 2943, 2831, 1747, 1689, 1451, 1109, 1023, 855 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.65 (1H, d, *J* = 7.1 Hz), 7.25-7.16 (2H, m), 4.80 (1H, d, *J* = 10.6 Hz), 4.23 (2H, s), 4.05-3.99 (2H, m), 3.89 (3H, s), 3.78 (1H, t, *J* = 9.0 Hz), 3.71-3.62 (2H, m), 3.41(1H, t, *J* = 8.9 Hz), 3.37 (2H, s), 2.75 (8H, s), 2.58 (3H, s);^{13}C NMR (100 MHz, CD_3OD): δ 171.0, 165.0, 154.3, 149.0, 148.7, 141.4, 133.0, 131.8, 130.2, 128.0, 125.2, 120.0, 118.8, 117.7, 83.0, 80.6, 75.6, 74.5, 71.8, 62.7, 61.3, 60.9, 53.5, 53.3, 18.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{29}H_{35}N_4O_{10}S$ 631.2052.Found 631.2068.

1.4.13. N-(6-Ethoxybenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide(9h):

White amorphous powder; yield: 43%; IR (neat):ν_{max}3382, 1642, 1493, 1119, 810, 699 cm^{-1}; ^1H NMR (500MHz, $CDCl_3$): δ 7.68 (1H, d, *J* = 9.0 Hz), 7.28 (1H, d, *J* = 2.4 Hz), 7.04 (1H, dd, *J* = 9.0, 2.4 Hz), 4.09 (2H, dd, *J* = 14.0, 7.0 Hz), 3.26 (2H, s), 3.0 (4H, s), 2.63 (4H, s), 1.43 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9, 156.2, 155.1, 142.5, 133.4, 121.5, 115.7, 105.0, 64.1, 61.7, 54.6, 45.8, 14.8; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{15}H_{21}N_4O_2S$ 321.1385; Found 321.1394.

1.4.14. N-(6-Ethoxybenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl) methyl)piperazin-1-yl)acetamide (10h):

White amorphous powder; yield: 51%; IR (neat): ν_{max} 3439, 2997, 2896, 1773, 1663, 1439, 1129, 1028, 759 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ 7.59 (1H, dd, *J* = 8.8, 3.3 Hz), 7.35 (1H, d, *J* = 2.3 Hz), 7.0 (1H, dd, *J* = 8.8, 2.4 Hz), 4.81 (1H, s), 4.22 (2H, s), 4.08-3.99 (4H, m), 3.89 (3H, s), 3.78 (1H, t, *J* = 9.0 Hz), 3.70-3.63 (2H, m), 3.41 (1H, t, *J* = 9.0 Hz), 3.37 (2H, s), 2.73 (8H, s), 1.40 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 170.8, 164.9, 157.8, 154.6, 148.6, 143.6, 141.4, 134.5, 122.4, 118.7, 118.5, 117.4, 116.9, 106.0, 82.9, 80.5, 75.6, 74.5, 71.8, 65.2, 62.7, 60.9, 57.3, 53.8, 53.2, 15.2; HRMS (ESI) m/z: $[M+]^+$Calcd for $C_{30}H_{36}N_4O_{11}S$ 660.2101; Found 660.2100.

1.4.15. N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (9i):

White amorphous powder; yield: 36%; IR (neat): ν_{max} 3285, 1672, 1453, 1010, 746, 693 cm^{-1}; ^1H NMR (500MHz, CD_3OD): δ 7.77-7.71 (1H, m), 7.54 (1H, dd, *J* = 16.3, 7.5 Hz), 7.24-7.17 (1H, m), 3.35 (2H, s), 3.01 (4H, s), 2.66 (4H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 171.3, 162.3, 159.4, 146.4, 134.6, 123.0, 122.9, 115.6, 115.4, 108.8, 108.6, 62.3, 54.7, 46.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{13}H_{15}FN_4OS$ 294.0950; Found 294.0952.

1.4.16. N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(4-(((2R,3S,4S)-3,4,8,10-tetrahydroxy-2-(hydroxy methyl)-9-methoxy-6-oxo-2,3,4,4a,6,10b-hexahydropyrano[3,2-c]isochromen-7-yl)methyl)piperazin-1-yl)acetamide (10i):

White amorphous powder; yield: 52%; IR (neat): ν_{max} 3351, 2956, 2896, 1712, 1623, 1456, 1115, 1039, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.55 (1H, s), 7.90 (1H, dd, *J* = 8.8, 2.5 Hz), 7.78-7.72 (1H, m), 7.29 (1H, ddd, *J* = 18.2, 9.4, 2.7 Hz), 5.66 (1H, s), 5.40 (1H, s), 4.92 (1H, d, *J* = 10.2 Hz), 4.19-4.07 (2H, m), 3.93 (1H, t, *J* = 9.8 Hz), 3.85 (1H, d, *J* = 11.0 Hz), 3.77 (3H, s), 3.67-3.54 (4H, m), 3.18 (2H, t, *J* = 9.0 Hz), 2.63 (8H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.4, 162.3, 159.8, 157.4, 145.1, 139.3, 132.7, 121.6, 121.5, 117.1, 115.7, 114.3, 114.1,

108.3, 108.0, 81.4, 78.8, 73.5, 72.2, 70.5, 61.0, 59.6, 52.0, 51.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{28}H_{32}O_{10}N_4FS$ 635.1805; Found 635.1817.

1.5. General experimental procedure for the synthesis of 13a-o

The arylsulfonylpiperazine attached bergenin series was prepared as depicted in the scheme 3. Initially, the anhydrous piperazine (3 equiv) was dissolved in THF and TEA (1.2 equiv) at 0 °C and the different benzenesulfonyl chlorides (1.2 equiv) (**11a-o**) were added dropwise to the solution[4]. Further, the reaction mixture was stirred for 3 h at room temperature until the reaction was completed. After confirmation with TLC, the reaction solution was removed using a rotary evaporator and workup with water and DCM. The organic layer was separated, dried with $NaHSO_4$, evaporated using a rotary evaporator and purified using silica gel (60-120 mesh) column chromatography to afford the different arylsulfonyl substituted piperazine (**12a-o)**. Finally, these arylsulfonyl substituted piperazine (1 equiv) was treated with bergenin (1 equiv) dissolved in DMSO in the presence of 37% of formaldehyde (0.5 mL) at 50 °C for 12 h. After completion of the reaction, the reaction mixture was cool to room temperature and diluted with water. The DMSO in the solution was removed passed through the LH-20 resin bed and was further purified by column chromatography using Sephadex LH-20 eluting with methanol to get the desired products (**13a-o**) in pure form. As prepared arylsulfonylpiperazine attached bergenin analogues were confirmed by the spectral analysis IR, ^{1}H NMR, ^{13}C NMR, Mass and HRMS spectroscopy.

1.5.1. (2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-tosylpiperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13a)

White amorphous powder; yield: 72%; IR (neat):ν_{max} 3395, 3010, 2836, 1697, 1180, 1059, 786 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (2H, d, *J* = 8.3 Hz), 7.43 (2H, d, *J* = 8.1 Hz), 4.85

(1H, s), 4.20 (1H, d, *J* = 14.4 Hz), 4.09 (1H, d, *J* = 14.4 Hz), 4.03-3.95 (2H, m), 3.84 (3H, s), 3.79-3.74 (1H, m), 3.68-3.63 (2H, m), 3.39 (1H, t, *J* = 8.9 Hz), 3.01 (4H, s), 2.65 (4H, s), 2.45 (3H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.5, 148.6, 145.7, 141.2, 133.6, 131.0, 129.1, 118.8, 118.7, 117.9, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.9, 52.8, 47.1, 21.6; HRMS (ESI) m/z: $[M+H]^+$ $C_{26}H_{33}N_2O_{11}S$ 581.1805; Found 581.1829.

1.5.2. (2R,3S,4S)-7-((4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,8,10-tetra hydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13b):

White amorphous powder; yield: 62%; IR (neat):ν_{max} 3348, 2997, 2896, 1682, 1120, 1021, 765 cm^{-1}; ^{1}H NMR (300MHz, CD_3OD): δ 7.84 (2H, d, *J* = 8.5 Hz), 7.73 (2H, d, *J* = 8.5 Hz), 4.98 (1H, s), 4.66 (1H, d, *J* = 12.9 Hz), 4.41 (1H, d, *J* = 12.6 Hz), 4.10-4.01 (2H, m), 3.94 (3H, s), 3.82-3.74 (1H, m), 3.72-3.64 (3H, m), 3.62-3.34 (8H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 165.5, 152.7, 150.5, 141.5, 135.7, 134.0, 130.8, 129.7, 120.0, 119.3, 83.2, 80.6, 75.5, 74.2, 71.8, 62.6, 61.2, 53.7, 52.7, 44.5; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{29}BrN_2O_{11}S$ 644.0675; Found 644.0679.

1.5.3.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-((4-methoxy phenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13c):

White amorphous powder; yield: 57%; IR (neat):ν_{max} 3423, 3012, 2977, 1612, 1190, 1032, 785, cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.61 (2H, dd, *J* = 6.8, 2.1 Hz), 7.03 (2H, dd, *J* = 6.8, 2.1 Hz), 4.83 (1H, s), 4.12 (1H, d, *J* = 14.4 Hz), 4.0 (1H, d, *J* = 14.4 Hz), 3.94-3.86 (2H, m), 3.80 (3H, s), 3.75 (3H, s), 3.70-3.65 (1H, m), 3.59-3.53 (2H, m), 3.29 (1H, t, *J* = 9.0 Hz), 2.92 (4H, s), 2.59-2.55(4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 165.0, 164.8, 153.5, 148.6, 141.2, 131.2,

128.0, 118.9, 118.7, 117.9, 115.6, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 56.3, 55.9, 52.8, 47.1; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{26}H_{33}N_2O_{12}S$ 597.1754; Found 597.1758.

1.5.4.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-(phenylsulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13d):

White amorphous powder; yield: 52%; IR (neat):ν_{max} 3425, 2947, 2886, 1692, 1180, 1022, 775 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.83 (2H, dd, *J* = 7.2, 1.4 Hz), 7.75-7.70 (1H, m), 7.67-7.63 (2H, m), 4.97 (1H, d, *J* = 10.5 Hz), 4.68 (1H, d, *J* = 12.7 Hz), 4.43 (1H, d, *J* = 12.7 Hz), 4.09-4.00 (2H, m), 3.95 (3H, s), 3.81-3.77 (1H, m), 3.73-3.64 (3H, m), 3.56-3.47 (2H, m), 3.44-3.37 (2H, m), 2.83-2.73 (2H, m), 2.66 (2H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 165.5, 152.7, 150.6, 141.5, 136.3, 134.9, 130.7, 129.1, 120.1, 119.4, 111.6, 83.2, 80.7, 75.5, 74.2, 71.8, 62.6, 61.2, 53.0, 52.6, 44.3; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{31}N_2O_{11}S$ 567.1649. Found 567.1653.

1.5.5. (2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-(naphthalen-1-ylsulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(*2H*)-one (13e):

White amorphous powder; yield: 48%; IR (neat): ν_{max} 3401, 2977, 28126, 1658, 1165, 1028, 775 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 8.72 (1H, d, *J* = 8.8 Hz), 8.26-8.22 (2H, m), 8.05 (1H, d, *J* = 8.2 Hz), 7.74-7.69 (1H, m), 7.67-7.63 (2H, m), 4.92 (1H, d, *J* = 10.5 Hz) , 4.55 (1H, d, *J* = 13.1 Hz), 4.32 (1H, d, *J* = 13.2 Hz), 4.07-3.99 (2H, m), 3.91 (3H, s), 3.79-3.75 (1H, m), 3.69-3.63 (2H, m), 3.78 (1H, t, *J* = 8.9 Hz), 3.55-3.32 (4H, m), 3.29-3.20 (4H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 165.3, 153.9, 150.1, 141.5, 136.4, 136.0, 133.0, 132.2, 130.4, 130.1, 129.6, 128.0,

125.9, 125.5, 119.7, 119.0, 113.4, 83.1, 80.6, 75.6, 74.2, 71.8, 62.6, 61.1, 54.0, 52.9, 44.4; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{29}H_{33}N_2O_{11}S$ 617.1805; Found 617.1812.

1.5.6. (2R,3S,4S)-7-((4-((3,4-Dimethoxyphenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,8,10-tetra hydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13f):

White amorphous powder; yield: 69%; IR (neat): ν_{max} 3396, 3094, 3010, 1636, 1145, 1064, 771 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.38 (1H, d, *J* = 8.4, 2.0 Hz), 7.23 (1H, d, *J* = 2.0 Hz), 7.15 (1H, d, *J* = 8.4 Hz), 4.89 (1H, s), 4.24 (1H, d, *J* = 14.3 Hz), 4.12 (1H, d, *J* = 14.3 Hz), 4.06-3.96 (2H, m), 3.92 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.79-3.74 (1H, m), 3.69-3.63 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz), 3.06 (4H, s), 2.71 (4H, s); ^{13}C NMR (100 MHz,CD_3OD): δ 164.8, 153.5, 150.7, 148.7, 141.2, 128.1, 123.1, 118.8, 118.0, 112.4, 111.7, 83.0, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 56.8, 56.7, 55.7, 52.8, 47.0; HRMS (ESI^+) m/z: $[M+H]^+$ Calcd for $C_{27}H_{35}O_{13}N_2S$ 627.1845; Found 627.1854.

1.5.7.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-((2,3,4 trifluorophenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2H)-one (13g):

White amorphous powder; yield: 74%; IR (neat): ν_{max} 3385, 2982, 2867, 1657, 1140, 1034, 779 cm^{-1}. ^1H NMR (300MHz, CD_3OD): δ 7.71-7.762 (1H, m), 7.40-7.30 (1H, m), 4.86 (1H, s), 4.23 (1H, d, *J* = 14.3 Hz), 4.10 (1H, d, *J* = 14.3 Hz), 4.06-3.86 (2H, m), 3.87 (3H, s), 3.81-3.74 (1H, m), 3.70-3.62 (2H, m), 3.39 (1H, t, *J* = 8.7 Hz), 3.23 (4H, s), 2.71-2.64 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.4, 148.6, 141.1, 127.0, 124.1, 124.0, 119.0, 118.8, 118.0, 114.2, 114.0, 83.0, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.7, 52.9, 46.6; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{28}F_3N_2O_{11}S$ 621.1389; Found 621.1360;

1.5.8.(2R,3S,4S)-7-((4-((3,4-Difluorophenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,8,10-tetra hydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13h):

White amorphous powder; yield: 59%; IR (neat): ν_{max} 3281, 2987, 2866, 1633, 1110, 1015, 769 cm^{-1}; ^{1}HNMR (400 MHz, CD_3OD): δ 7.75 (1H, dtd, *J* = 17.0, 9.5, 2.1 Hz), 7.66-7.61 (1H, m), 7.58-7.51 (1H, m), 4.8 (1H, s), 4.20 (1H, d, *J* = 14.3 Hz), 4.09 (1H, d, *J* = 14.3 Hz), 4.06-3.96 (2H, m), 3.85 (3H, s), 3.79-3.74 (1H, m), 3.69-3.62 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz), 3.07, (4H, s), 2.70-2.62 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.4, 148.6, 141.2, 134.1, 126.7, 119.8, 119.6, 119.0, 118.9, 118.7, 118.7 118.0, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.7, 52.8, 47.1; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{29}O_{11}N_2F_2S$ 603.1481; Found 603.1454.

1.5.9.(2R,3S,4S)-7-((4-((3-Chloro-4-fluorophenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13i):

White amorphous powder; yield: 64%; IR (neat):ν_{max} 3325, 3001, 2971, 1662, 1131, 1041, 796 cm^{-1}. ^{1}H NMR (500MHz, CD_3OD): δ 7.93 (1H, dd, *J* = 6.8, 2.3 Hz), 7.78-7.75 (1H, m), 7.51 (1H, ddd, *J* = 17.4, 8.7, 3.1 Hz), 4.88 (1H, s), 4.21 (1H, d, *J* = 14.1 Hz), 4.09 (1H, d, *J* = 14.3 Hz), 4.03-3.96 (2H, m), 3.86 (3H, s), 3.81-3.74 (1H, m), 3.68-3.63 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz) , 3.08 (4H, s), 2.69- 2.63 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.7, 163.4, 160.9, 153.4, 148.6, 141.2, 134.4, 131.7, 130.0, 130.0, 123.5, 123.3, 119.0, 118.8, 118.0, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.7, 52.8, 47.0; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{29}O_{11}N_2ClFS$ 618.1183 ; Found 619.1159;

1.5.10.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-((4-(trifluoro methoxy)phenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13j):

White amorphous powder; yield: 58%; IR (neat): ν_{max} 3311, 3001, 2907, 1629, 1135, 1033, 728 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.90 (2H, dd, *J* = 6.8, 2.1 Hz), 7.55-7.52 (2H, m), 4.89 (1H, s), 4.22 (1H, d, *J* = 14.3 Hz), 4.09 (1H, d, *J* = 14.3 Hz), 4.03-3.96 (2H, m), 3.85 (3H, s), 3.79-3.74 (1H, m), 3.68-3.62 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz), 3.07 (4H, s), 2.70-2.63 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.8, 153.4, 148.6, 141.2, 135.7, 131.5, 122.5, 119.0, 118.7, 118.0, 83.0, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.7, 52.9, 47.1; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{26}H_{30}O_{12}N_2F_3S$ 651.1497; Found 651.1466.

1.5.11.(2R,3S,4S)-7-((4-((2-Chloro-4-fluorophenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13k):

White amorphous powder; yield: 68%; IR (neat):ν_{max} 3311, 3012, 2989, 1671, 1137,1096, 766 cm^{-1}. 1H NMR (400 MHz, CD_3OD): δ 8.09 (1H, dd, *J* = 8.8, 5.9 Hz), 7.50 (1H, dd, *J* = 8.6, 2.5 Hz), 7.28 (1H, dddd, *J* =16.7, 10.3, 7.7, 2.6 Hz), 4.89 (1H, d, *J* = 10.5 Hz), 4.24 (1H, d, *J* = 14.3 Hz), 4.14 (1H, d, *J* = 14.3 Hz), 4.06-3.97 (2H, m), 3.88 (3H, s), 3.81-3.75 (1H, m), 3.69-3.64 (2H, m), 3.39 (1H, t, *J* = 9.1 Hz), 3.37-3.32 (4H, m), 2.70-2.65 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.5, 148.7, 141.2, 135.6, 135.5, 133.6, 121.0, 120.7, 118.8, 118.0 115.8, 115.6, 83.0, 80.6, 75.6, 74.5, 71.8, 62.7, 60.9, 55.8, 53.2, 46.4; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{25}H_{29}O_{11}N_2ClFS$ 619.1188; Found 619.1159;

1.5.12.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-(thiophen-2-yl sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2H)-one (13l):

White amorphous powder; yield: 73%; IR (neat):ν_{max} 3365, 2997, 2876, 1637, 1210, 1075, 715 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.88 (1H, dd, *J* = 5.0, 1.3 Hz), 7.61 (1H, dd, *J* = 3.7, 1.0 Hz), 7.26-7.24 (1H, m), 4.93 (1H, s), 4.23 (1H, d, *J* = 14.2 Hz) , 4.10 (1H, d, *J* = 14.2Hz), 4.03-3.96 (2H, m), 3.86(3H, s), 3.80-3.75 (1H, m), 3.69-3.63 (2H, m), 3.39 (1H, t, *J* = 8.9 Hz), 3.09 (4H, s), 2.73-2.66 (4H, m);^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.5, 148.6, 141.2, 136.7, 134.3, 129.2, 119.0, 118.8, 118.0, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.7, 52.7, 47.2; HRMS (ESI) m/z: $[M+H]^+$Calcd for $C_{23}H_{29}N_2O_{11}S_2$ 573.1213; Found 573.1216.

1.5.13. (2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-((4-(trifluoro methyl)phenyl)sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13m):

White amorphous powder; yield: 61%; IR (neat):ν_{max} 3369, 3083, 2936, 1649, 1137,1019, 767 cm^{-1}; 1H NMR (400 MHz, CD_3OD): δ 7.91 (2H, dd, *J* = 6.8, 2.1 Hz), 7.55-7.52 (2H, m), 4.89 (1H, s), 4.22 (1H, d, *J* = 14.2 Hz), 4.09 (1H, d, *J* = 14.2 Hz), 4.04-3.96 (2H, m), 3.85 (3H, s), 3.79-3.74 (1H, m), 3.69-3.63 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz), 3.07 (4H, s), 2.71-2.63 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.4, 148.6, 141.1, 140.8, 135.8, 135.5, 129.8, 127.6, 123.6, 118.8, 118.0, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.6, 52.8, 47.0; HRMS (ESI) m/z: $[M+H]^+$ $C_{26}H_{30}O_{11}N_2F_3S$ 635.1489; Found 635.1516.

1.5.14.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-7-((4-((4-nitrophenyl) sulfonyl)piperazin-1-yl)methyl)-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2*H*)-one (13n):

Yellow amorphous powder; yield: 56%; IR (neat):ν_{max} 3324, 3001, 2947, 1678, 1486, 1120, 1097, 721 cm^{-1}. ^{1}H NMR (500MHz, CD_3OD): δ 8.45 (2H, dd, *J* = 7.0, 2.0 Hz), 8.02 (2H, dd, *J* = 6.9, 2.0 Hz), 4.88 (1H, s), 4.20 (1H, d, *J* = 14.2 Hz), 4.08 (1H, d, *J* = 14.3 Hz), 4.02-3.95 (2H, m), 3.84 (3H, s), 3.78-3.74 (1H, m), 3.68-3.63 (2H, m), 3.39 (1H, t, *J* = 9.1 Hz), 3.12 (4H, s), 2.69-2.65 (4H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.4, 151.9, 148.6, 142.7, 141.1, 130.4, 125.6, 118.9, 118.7, 118.0, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.6, 52.8 , 47.0; HRMS (ESI) m/z: $[M+H]^+$ $C_{25}H_{30}O_{13}N_3S$ 612.1522; Found 612.1493.

1.5.15.(2R,3S,4S)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-7-((4-((4-iodophenyl)sulfonyl)piperazin-1-yl)methyl)-9-methoxy-3,4,4a,10b-tetrahydropyrano[3,2-c]isochromen-6(2H)-one (13o):

White amorphous powder; yield: 59%; IR (neat):ν_{max} 3310, 2917, 2811, 1629, 1152, 1005, 761 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): 7.91 (2H, dd, *J* = 6.7, 1.8 Hz), 7.42 (2H, dd, *J* = 6.7, 1.8 Hz), 4.21 (1H, d, *J* = 14.3 Hz), 4.09 (1H, d, *J* = 14.3 Hz), 4.03-3.96 (2H, m), 3.85 (3H, s), 3.77 (1H, t, *J* = 9.0 Hz), 3.69-3.62 (2H, m), 3.39 (1H, t, *J* = 9.0 Hz), 3.05 (4H, s), 2.68-2.64 (4H, m); ^{13}C NMR (100 MHz, CD_3OD): δ 164.8, 153.4, 148.6, 141.2, 139.9, 136.5, 130.5, 119.0, 118.7, 118.0, 101.6, 82.9, 80.5, 75.6, 74.5, 71.8, 62.7, 60.9, 55.8, 52.8, 47.1; HRMS (ESI) m/z: [M+H] $C_{25}H_{30}O_{11}N_2IS$ 693.0610; Found 693.0609.

1.6. Anticancer activity

1.6.1. Cell culture

The human cancer cells such as the tongue cancer cell lines CAL27, the oral squamous cell carcinoma cell line SCC09, the colorectal adenocarcinoma cell line HCT-15, the breast carcinoma cell line MCF7, the lung carcinoma cell line A549 and the normal lung epithelial cell lines BEAS-2B were purchased from the American Type Culture Cell lines (ATCC), United

States. These cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium with 1% v/v of penicillin/streptomycin (Gibco) in 75 cm^2 flasks. HCT 116, MCF-7 and A549 cells were cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum and BEAS-2B (human bronchial epithelial cell line-2B) cells were cultured in BEGM media with growth factor supplements (LONZA, USA). The cells were cultured under standard conditions at 37 °C in a humidified atmosphere at 5% CO_2. All cell culture experiments were performed thrice as three biological repetitions.

1.6.2. *In vitro* cytotoxic activity

The *in vitro* cytotoxic activity of bergenin and its derivatives were measured using MTT assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt to formazan product. Cell lines such as HCT, A549 and CAL 27 were seeded 5×10^3cells/wall and A549, SCC09 and BEAS-2B were seeded 8×10^3 cells/well in the 96 well plate and incubated for overnight for attachment. After 16 hrs of incubation, cells were treated with various concentrations of (12.5, 25, 50 and 100 µM) bergenin compounds and incubated for 48 hrs. After the incubation period, 25 µl of MTT solution (5 mg/ml) was added to each well and incubated further for 4 h. After incubation, the medium was discarded and 100 µl of DMSO was added to dissolve formazan crystals and gently mixed well for 10 min. The absorbance was measured at 570 nm using a microplate reader (Bio-rad, USA) and IC_{50} values were calculated using Graph-pad prism.

1.6.3. Cell cycle analysis

The effect of bergenin derivatives on the cell cycle analysis was determined with FxcycleTm PI/RNase staining solution using flow cytometry as per manufacturer's instructions.Cancer cell lines CAL27 and SCC09seeded at 5×10^5 cells/well and were cultured

overnight in 6-well plates and treated with compounds **5a** (16 & 32 μM), **5c** (10&34 μM) **10f** (31.5 & 63 μM) and **13o** (21 & 42 μM) against CAL-27 cells and **5a** (16.2 & 32.4 μM), **5c** (17&34 μM) and **10f** (46 & 92 μM) against SCC09 cells at specified concentration. After 24 h incubation, the cells were trypsinized and counted. Thereafter the cells (5×10^5) were fixed with 75% of cold ethanol for 4 h at 4 °C and then cells were stained withFxcycle^{Tm} PI/RNASE staining solution(0.25 ml/ 5×10^5 cells) for 30 min in the dark at room temperature. After 30 minutes of incubation, cells were analyzed using BD accuri equipped with (Exitation-488 nm and Emission-585/42 nm) filters.

1.6.4. Apoptosis assay

The apoptotic events induced by the compounds 5a & 10f were determined by FITC Annexin V Apoptosis Detection Kit with PI based on the manufacturer protocol (Biolegend, USA). In brief, the CAL27 and SCC09 cells were seeded in 6-well plates at 5×10^5cells/well with DMEM containing 10% FBS medium and incubated overnight at 5% CO_2 and 37 °C. Then,cells were treated with different concentrations of **5a** (16 & 32 μM), **5c** (10 & 34 μM) **10f** (31.5 & 63 μM) and **13o** (21 & 42 μM) against CAL-27 cells and **5a** (16.2 & 32.4 μM), **5c** (17 & 34 μM) and **10f** (46 & 92 μM) against SCC09 cells and DMSO was served as a vehicle control and further incubated for another24 h. After the treatment period, the cells were trypsinized counted and harvested (5×10^5 cells/sample), washed with 1x PBS and then, cells were resuspended in annexin binding buffer with 1 μL of Annexin V and 2 μL of PI and further incubated in the dark for 15 mins. After 15 minutes of incubation, cells were analyzed using BD accuri equipped with (Exitation-488 nm and Emission-585/42 nm) filters.

1.6.5. RNA isolation, cDNA synthesis and qRT-PCR

CAL-27 or SCC09 cells were cultured, and seeded into 6 well plate at a density of 3 lakhs/well and 5 lakhs/well respectively. After 24 hrs of cell seeding, CAL 27 cells were treated with (5a, 10f, and 13p) and SCC09 cells were treated with compounds at specified concentrations and incubated for 48 hrs. Thereafter, cells were washed with 1XPBS and added 0.5 ml of Trizol/well and incubated for 5 minutes. Then cells were scrapped and collected into Eppendorf tubes. Then we followed the RNA isolation as described earlier (Andugulapati et al., 2020). Briefly, 150 ul of chloroform was added to each tube, mixed well and incubated for 10 minutes at room temperature. Then tubes were centrifuged (4 degrees) and supernatant (aquoes layer) was collected and equal amount of isopropanol was added and further incubated for 15 min at room temperature and centrifuged at 4 degrees for 15 minutes. Further supernatant was discarded and RNA pellet was washed with 75% ethanol twice and dried the pellet at room temperature. Thereafter pellet was dissolved in DEPC treated water and integrity and concentration of the RNA was estimated using nano-drop [5]. About 1 µg of RNA was used to synthesis cDNA using script cDNA synthesis kit (Takara bio-India) according to manufacturer's instructions. The specific primers for the genes Vimentin, Oct-4, E-cadtherin, Nanog, Bax and BcL2 and the reference gene β-actin were designed using Primer-3 software and the list of forward and reverse primers were shown in Table 2. RT-qPCR was performed using SYBR green mix and the relative expression of mRNA was measured using the comparative Ct (ΔCt) and the data was expressed as Mean ± SEM. RT-qPCR was performed in triplicates in each biological repeats.

1.7. Molecular docking protocol

The three-dimensional protein templates of BcL2 (ID: 4LVT) [6]are retrieved from the protein data bank (https://www.rcsb.org/) and prepared for docking by protein preparation

wizard. The co-crystallized ligand sites are employed as a druggable target for the current naturally inspired analogues. The chemical structures of ligands are sketched in 3D and prepared by Ligprep and their energies are minimized by macromodel. The standard docking protocol of Glide is employed for current molecular interaction analysis. The result interpretations are made by Academic Maestro 12.2 panel.

1.8.Statistical analysis

Each experiment was carried out at least triplicate and the mean value was calculated as means ± SD. Statistical analysis involved use of the Graphpad Prism version 9 and to determine the significant difference in the anticancer activity of the bergenin derivatives the Duncan's multiple range tests at a level of $p < 0.05$ was used.

Table S1: List of forward and reverse primers used for gene expression studies.

No	Gene	Forward primer Sequence	Reverse Primer Sequence
1	h-BAK	5'GACGACATCAACCGACGCTATG-3'	5'GCTGGTGGCAATCTTGGTGAAG-3'
2	h-BCL-xL/2	5'ACTGTGCGTGGAAAGCGTAGAC-3'	5'GATCCAAGGCTCTAGGTGGTCATTC-3'
3	h-Vim	5'AACAACCGACACTCCTACAAGA-3'	5'TGGTTGGATACTTGCTGGAAA-3'
4	h-Oct-4	5'ACATCAAAGCTCTGCAGAAAGAACT -3'	5'-CTGAATACCTTCCCAAATAGAACCC-3' -3'
5	h-Nanog	5'-CTCCAACATCCTGAACCTCAGC-3'	5'-CGTCACACCATTGCTATTCTTCG-3'
6	h-E-cad	5'AAGGGCTTGGATTTTGAGG	5'AGATGGGGGCTTCATTCAC-3'

Table S2the effect of bergenin derivatives on cell cycle arrest of CAL27 cells

Name of the compound	G0/G1	S	G2/M
Control	76.4±1.5	3.7±0.45	11.9±0.4
Doxorubicin	61.3±2.3	4.1±0.3	34.7±1.2
5a (16μM)	85.2+2.2	2.2±0.4	12.7±0.6
5a (32μM)	86.5±1.5	3.2±0.2	9.6±0.4
5c (10μM)	88.4±2.1	3.0±0.5	10.8±0.5
5c (34μM)	87.5±2.5	4.2±0.6	11.1±0.8
10f (31.5μM)	85.6±1.8	3.8±1.2	11.70.6
10f (63μM)	81.7±1.8	3.3±0.7	15.6±0.3
13o (21 μM)	83.3±0.5	3.7±0.8	12.7±0.9
13o (42 μM)	83.6±0.4	2.01±0.7	11.7±1.3

Table S3the effect of bergenin derivatives on cell cycle arrest of SCC09 cells

Name of the compound	G0/G1	S	G2/M
Control	71.2±1.1	15.2±1.2	14.7±0.65
Doxorubicin	30±2.2	16.6±0.7	56.2±0.6
5a (16μM)	71.5±0.9	16.3±0.7	10.8±0.9
5a (32μM)	78±0.87	12.2±0.5	10.3±0.7
5c (10μM)	71.5±2.1	16.2±0.9	12.1±0.4
5c (34μM)	74±0.6	12.8±0.4	10.5±0.6
10f (31.5μM)	77.1±1.2	11.5±1.2	11.8±0.5
10f (63μM)	86.2±1.7	5.1±-.6	10±0.3

Table S4the effect of bergenin derivatives on apoptosis of CAL27 cells

Name of the compound	Early apoptosis	Late apoptosis	Necrotic
Control	0.1±0.05	0.1±0.07	0
Doxorubicin	0.8±0.1	59±0.67	23±0.9
5a (16μM)	2.7±0.3	2.2±0.4	0.9±0.05
5a (32μM)	2.8±0.4	4.4±0.5	1.8±0.4
5c (10μM)	1.2±0.0.3	2.2±0.6	2.3±0.4
5c (34μM)	2.2±0.4	4.8±0.3	4.4±0.52
10f (31.5μM)	8.3±0.8	2.9±0.4	0.4±0.22
10f (63μM)	11.7±0.3	4.5±0.5	3.2±0.29
13o (21 μM)	1.1±0.4	0.7±0.1	0.9±0.3
13o (42 μM)	1.7±0.1	1.6±0.2	1.8±0.6

Table S5 the effect of bergenin derivatives on apoptosis of SCC09 cells

Name of the compound	Early apoptosis	Late apoptosis	Necrotic
Control	2.7±0.4	1.2±0.2	0.1±0.05
Doxorubicin	0.3±0.07	42±0.8	53.2±1.5
5a (16μM)	6±0.5	40.8±0.32	9.4±0.8
5a (32μM)	6.1±0.4	18.2±0.7	34.3±0.7
5c (10μM)	29.2±0.8	43±0.8	1.5±0.08
5c (34μM)	32.7±1.4	47.2±2.1	1.8±0.2
10f (31.5μM)	10.3±0.3	15.1±0.7	38.2±0.7
10f (63μM)	7.8±0.4	12.6±1.3	27.2±0.8

Figure legends

Fig S1: ^{1}H NMR SPECTRUM OF COMPOUND **5**

Fig S2: ^{13}C NMR SPECTRUM OF COMPOUND **5**

Fig S3: HRESIMS SPECTRUM OF COMPOUND **5**

Fig S4:1H NMR SPECTRUM OF COMPOUND **4a**

Fig S5: ^{13}C NMR SPECTRUM OF COMPOUND **4a**

Fig S6: HRESIMS SPECTRUM OF COMPOUND **4a**

Fig S7: ^{1}H NMR SPECTRUM OF COMPOUND **5a**

Fig S8: ^{13}C NMR SPECTRUM OF COMPOUND **5a**

Fig S9: HRESIMS SPECTRUM OF COMPOUND **5a**

Fig S10: ^{1}H NMR SPECTRUM OF COMPOUND **4b**

Fig S11: ^{13}C NMR SPECTRUM OF COMPOUND **4b**

Fig S12: HRESIMS SPECTRUM OF COMPOUND **4b**

Fig S13: ^{1}H NMR SPECTRUM OF COMPOUND **5b**

Fig S14: ^{13}C NMR SPECTRUM OF COMPOUND **5b**

Fig S15: HRESIMS SPECTRUM OF COMPOUND **5b**

Fig S16: ^{1}H NMR SPECTRUM OF COMPOUND **4c**

Fig S17: ^{13}C NMR SPECTRUM OF COMPOUND **4c**

Fig S18: HRESIMS SPECTRUM OF COMPOUND **4c**

Fig S19: ^{1}H NMR SPECTRUM OF COMPOUND **5c**

Fig S20: ^{13}C NMR SPECTRUM OF COMPOUND **5c**

Fig S21: HRESIMS SPECTRUM OF COMPOUND **5c**

Fig S22: ^{1}H NMR SPECTRUM OF COMPOUND **4d**

Fig S23: ^{13}C NMR SPECTRUM OF COMPOUND **4d**

Fig S24: HRESIMS SPECTRUM OF COMPOUND **4d**

Fig S25: ^{1}H NMR SPECTRUM OF COMPOUND **5d**

Fig S26: ^{13}C NMR SPECTRUM OF COMPOUND **5d**

Fig S27: HRESIMS SPECTRUM OF COMPOUND **5d**

Fig S28: ^{1}H NMR SPECTRUM OF COMPOUND **4e**

Fig S29: NMR SPECTRUM OF COMPOUND **4e**

Fig S30: HRESIMS SPECTRUM OF COMPOUND **4e**

Fig S31: ^{1}H NMR SPECTRUM OF COMPOUND **5e**

Fig S32: ^{13}C NMR SPECTRUM OF COMPOUND **5e**

Fig S33: HRESIMS SPECTRUM OF COMPOUND **5e**

Fig S34: ^{1}H NMR SPECTRUM OF COMPOUND **9a**

Fig S35: ^{13}C NMR SPECTRUM OF COMPOUND **9a**

Fig S36: HRESIMS SPECTRUM OF COMPOUND **9a**

Fig S37: ^{1}H NMR SPECTRUM OF COMPOUND **10a**

Fig S38: ^{13}C NMR SPECTRUM OF COMPOUND **10a**

Fig S39: HRESIMS SPECTRUM OF COMPOUND **10a**

Fig S40:^{1}H NMR SPECTRUM OF COMPOUND **9b**

Fig S41: ^{13}C NMR SPECTRUM OF COMPOUND **9b**

Fig S42: HRESIMS SPECTRUM OF COMPOUND **9b**

Fig S43: ^{1}H NMR SPECTRUM OF COMPOUND **10b**

Fig S44: ^{13}C NMR SPECTRUM OF COMPOUND **10b**

Fig S45: HRESIMS SPECTRUM OF COMPOUND **10b**

Fig S46: ^{1}H NMR SPECTRUM OF COMPOUND **10c**

Fig S47: ^{13}C NMR SPECTRUM OF COMPOUND **10c**

Fig S48: HRESIMS SPECTRUM OF COMPOUND **10c**

Fig S49: ^{1}H NMR SPECTRUM OF COMPOUND **10d**

Fig S50: ^{13}C NMR SPECTRUM OF COMPOUND **10d**

Fig S51: HRESIMS SPECTRUM OF COMPOUND **10d**

Fig S52: ^{1}H NMR SPECTRUM OF COMPOUND **9e**

Fig S53: ^{13}C NMR SPECTRUM OF COMPOUND **9e**

Fig S54: HRESIMS SPECTRUM OF COMPOUND **9e**

Fig S55: ^{1}H NMR SPECTRUM OF COMPOUND **10e**

Fig S56: ^{13}C NMR SPECTRUM OF COMPOUND **10e**

Fig S57: HRESIMS SPECTRUM OF COMPOUND **10e**

Fig S58: ^{1}H NMR SPECTRUM OF COMPOUND **9f**

Fig S59: ^{13}C NMR SPECTRUM OF COMPOUND **9f**

Fig S60: HRESIMS SPECTRUM OF COMPOUND **9f**

Fig S61: ^{1}H NMR SPECTRUM OF COMPOUND **10f**

Fig S62: ^{13}C NMR SPECTRUM OF COMPOUND **10f**

Fig S63: HRESIMS SPECTRUM OF COMPOUND **10f**

Fig S64: ^{1}H NMR SPECTRUM OF COMPOUND **9g**

Fig S65: ^{13}C NMR SPECTRUM OF COMPOUND **9g**

Fig S66: HRESIMS SPECTRUM OF COMPOUND **9g**

Fig S67: ^{1}H NMR SPECTRUM OF COMPOUND **10g**

Fig S68: ^{13}C NMR SPECTRUM OF COMPOUND **10g**

Fig S69: ^{1}HRESIMS SPECTRUM OF COMPOUND **10g**

Fig S70: ^{1}H NMR SPECTRUM OF COMPOUND **9h**

Fig S71: ^{13}C NMR SPECTRUM OF COMPOUND **9h**

Fig S72: HRESIMS SPECTRUM OF COMPOUND **9h**

Fig S73: ^{1}H NMR SPECTRUM OF COMPOUND **10h**

Fig S74: ^{13}C NMR SPECTRUM OF COMPOUND **10h**

Fig S75: HRESIMS SPECTRUM OF COMPOUND **10h**

Fig S76: ^{1}H NMR SPECTRUM OF COMPOUND **9i**

Fig S77: ^{13}C NMR SPECTRUM OF COMPOUND **9i**

Fig S78: HRESIMS SPECTRUM OF COMPOUND **9i**

Fig S79: ^{1}H NMR SPECTRUM OF COMPOUND **10i**

Fig S80: ^{13}C NMR SPECTRUM OF COMPOUND **10i**

Fig S81: HRESIMS SPECTRUM OF COMPOUND **10i**

Fig S82: ^{1}H NMR SPECTRUM OF COMPOUND **13a**

Fig S83: ^{13}C NMR SPECTRUM OF COMPOUND **13a**

Fig S84: RESIMS SPECTRUM OF COMPOUND **13a**

Fig S85: H NMR SPECTRUM OF COMPOUND **13b**

Fig S86: ^{13}C NMR SPECTRUM OF COMPOUND **13b**

Fig S87: HRESIMS SPECTRUM OF COMPOUND **13b**

Fig S88: ^{1}H NMR SPECTRUM OF COMPOUND **13c**

Fig S89: ^{13}C NMR SPECTRUM OF COMPOUND **13c**

Fig S90: HRESIMS SPECTRUM OF COMPOUND **13c**

Fig S91: ^{1}H NMR SPECTRUM OF COMPOUND **13d**

Fig S92: ^{13}C NMR SPECTRUM OF COMPOUND **13d**

Fig S93: HRESIMS SPECTRUM OF COMPOUND **13d**

Fig S94: ^{1}H NMR SPECTRUM OF COMPOUND **13e**

Fig S95: ^{13}C NMR SPECTRUM OF COMPOUND **13e**

Fig S96: HRESIMS SPECTRUM OF COMPOUND **13e**

Fig S97: ^{1}H NMR SPECTRUM OF COMPOUND **13f**

Fig S98: ^{13}C NMR SPECTRUM OF COMPOUND **13f**

Fig S99: HRESIMS SPECTRUM OF COMPOUND **13f**

Fig S100: ^{1}H NMR SPECTRUM OF COMPOUND **13g**

Fig S101: ^{13}C NMR SPECTRUM OF COMPOUND **13g**

Fig S102: HRESIMS SPECTRUM OF COMPOUND **13g**

Fig S103: ^{1}H NMR SPECTRUM OF COMPOUND **13h**

Fig S104: ^{13}C NMR SPECTRUM OF COMPOUND **13h**

Fig S105: HRESIMS SPECTRUM OF COMPOUND **13h**

Fig S106: ^{1}H NMR SPECTRUM OF COMPOUND **13i**

Fig S107: ^{13}C NMR SPECTRUM OF COMPOUND **13i**

Fig S108: HRESIMS SPECTRUM OF COMPOUND **13i**

Fig S109: ^{1}H NMR SPECTRUM OF COMPOUND **13j**

Fig S110: ^{13}C NMR SPECTRUM OF COMPOUND **13j**

Fig S111: HRESIMS SPECTRUM OF COMPOUND **13j**

Fig S112: ^{1}H NMR SPECTRUM OF COMPOUND **13k**

Fig S113: ^{13}C NMR SPECTRUM OF COMPOUND **13k**

Fig S114: HRESIMS SPECTRUM OF COMPOUND **13k**

Fig S115: ^{1}H NMR SPECTRUM OF COMPOUND **13l**

Fig S116: ^{13}C NMR SPECTRUM OF COMPOUND **13l**

Fig S117: HRESIMS SPECTRUM OF COMPOUND **13l**

Fig S118: ^{1}H NMR SPECTRUM OF COMPOUND **13m**

Fig S119: ^{13}C NMR SPECTRUM OF COMPOUND **13m**

Fig S120: HRESIMS SPECTRUM OF COMPOUND **13m**

Fig S121: ^{1}H NMR SPECTRUM OF COMPOUND **13n**

Fig S122: ^{13}C NMR SPECTRUM OF COMPOUND **13n**

Fig S123 HRESIMS SPECTRUM OF COMPOUND **13n**

Fig S124: ^{1}H NMR SPECTRUM OF COMPOUND **13o**

Fig S125: ^{13}C NMR SPECTRUM OF COMPOUND **13o**

Fig S126: HRESIMS SPECTRUM OF COMPOUND **13o**

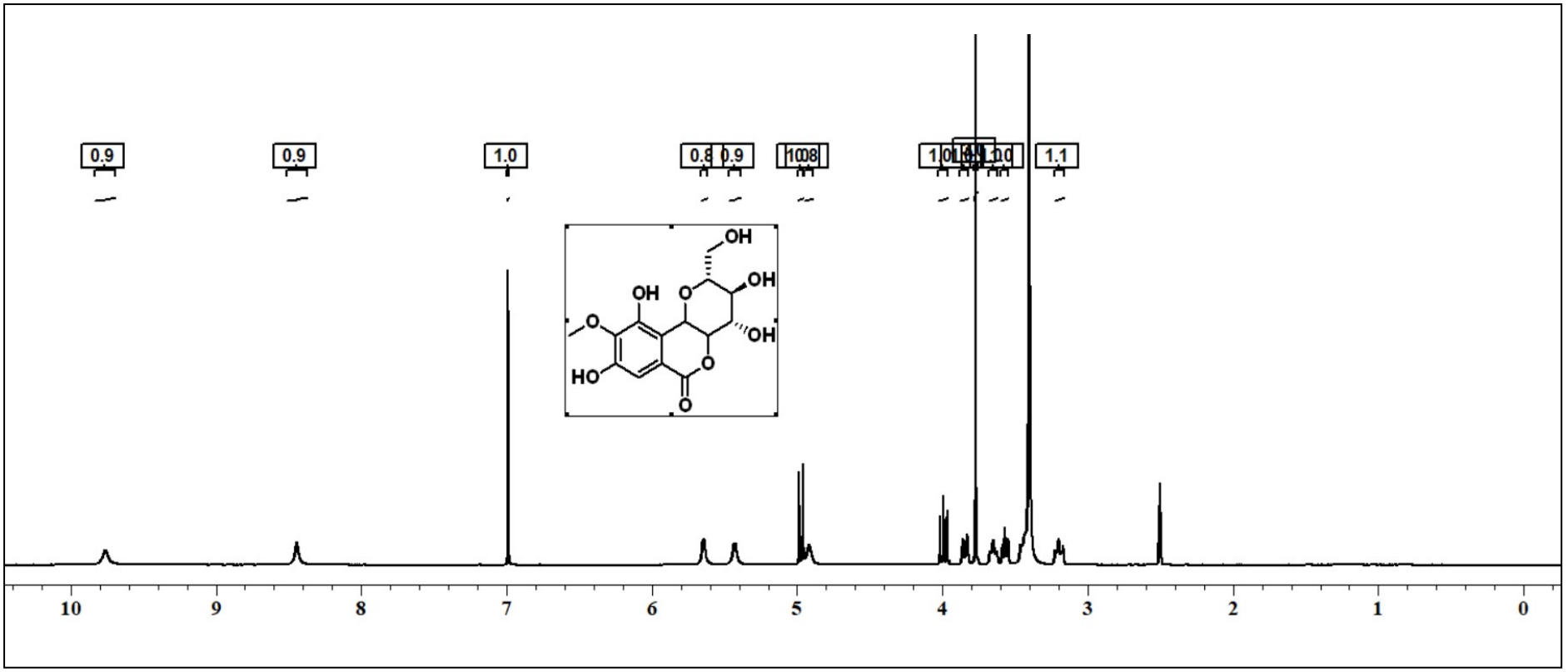


Fig S1: ^{1}H NMR SPECTRUM OF COMPOUND **1**(400 MHz, DMSO-d_6)

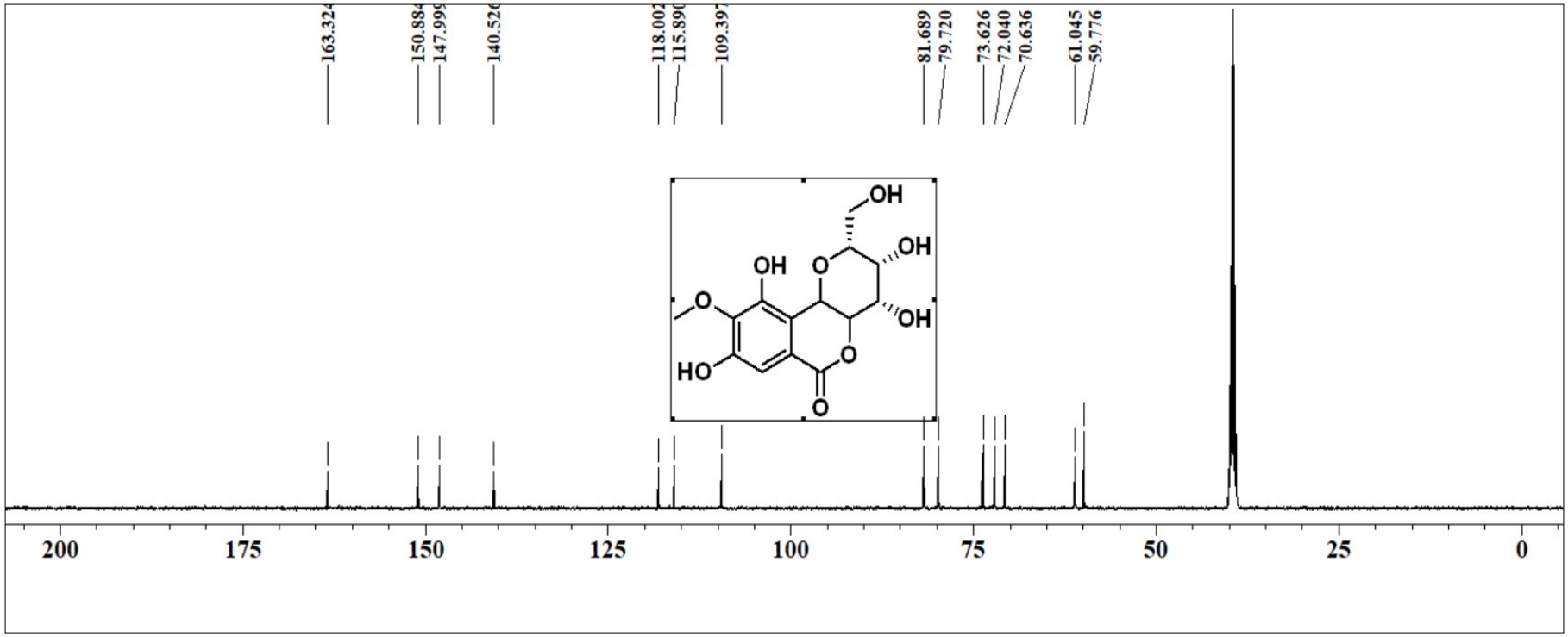
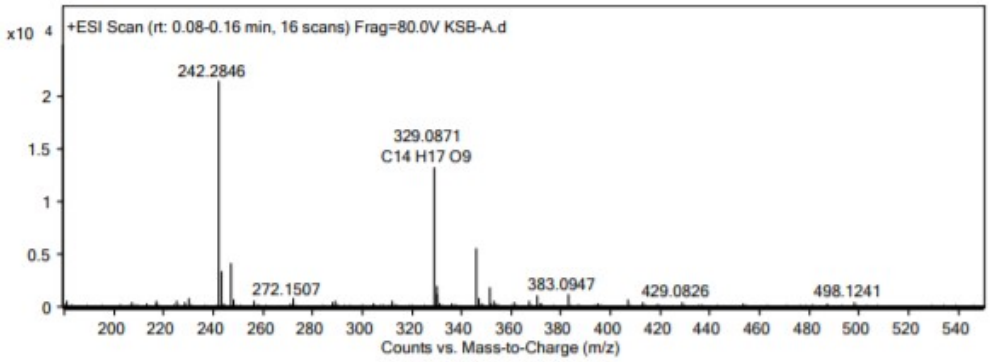


Fig S2: ^{13}C NMR SPECTRUM OF COMPOUND **1**(125 MHz, DMSO-d_6)

Data File	KSB-A.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	CSIR-IICT\Analyst
Acq Method	hrms-pos-method.m	**Acquired Time**	14-06-2021 17:44:46
IRM Calibration Status	Success	**DA Method**	11.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.06.01 (B6172 SP1)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
80	0	ESI



Peak List

m/z	z	Abund
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Formula Calculator Element Limits

Element	Min	Max
C	0	28
H	0	60
O	0	9
N	0	5
P	0	0
F	0	1
S	0	1
Cl	0	0

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C14 H17 O9	True	329.0876	329.0873	-1.05	C14 H17 O9	98.7

Agilent Technologies

Fig S3: HRESIMS SPECTRUM OF COMPOUND **1**

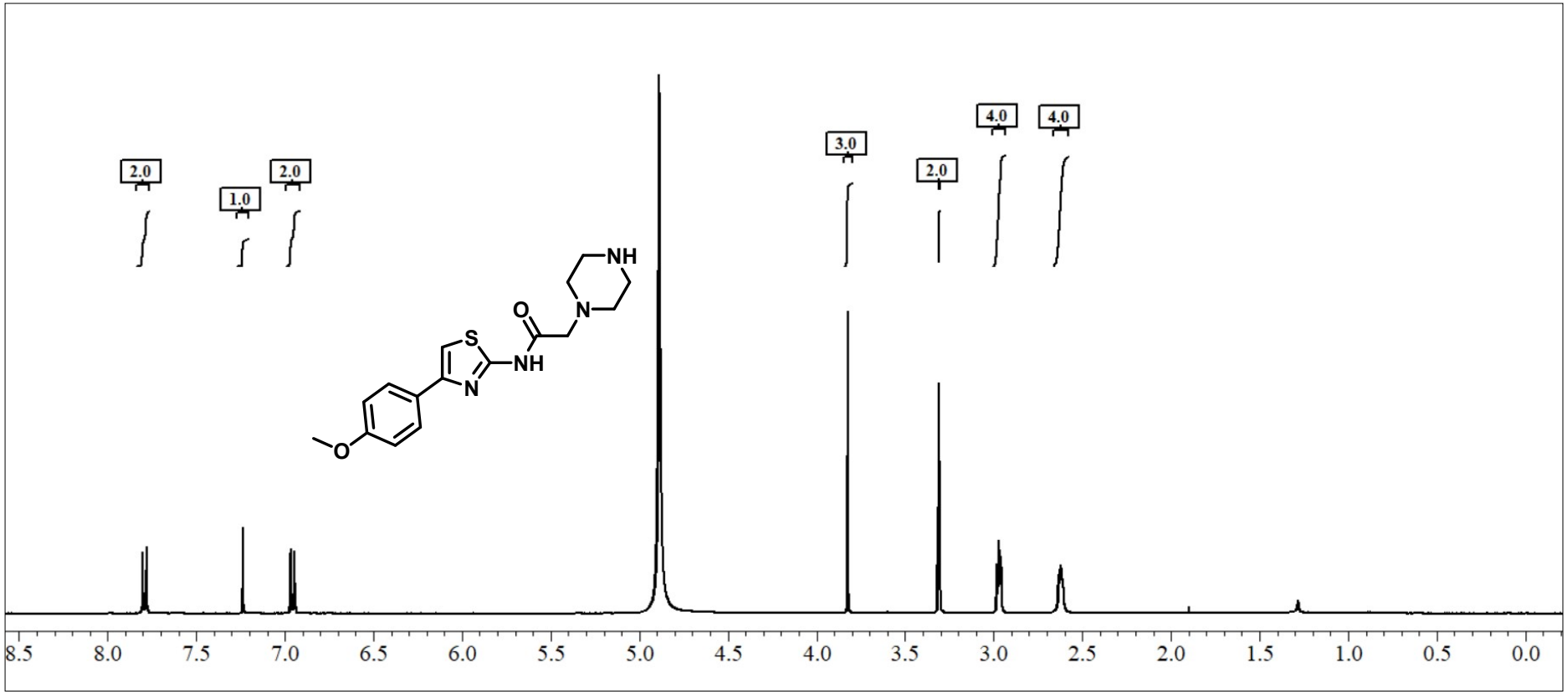


Fig S4: ^{1}H NMR SPECTRUM OF COMPOUND **4a** (400 MHz, CD_3OD)

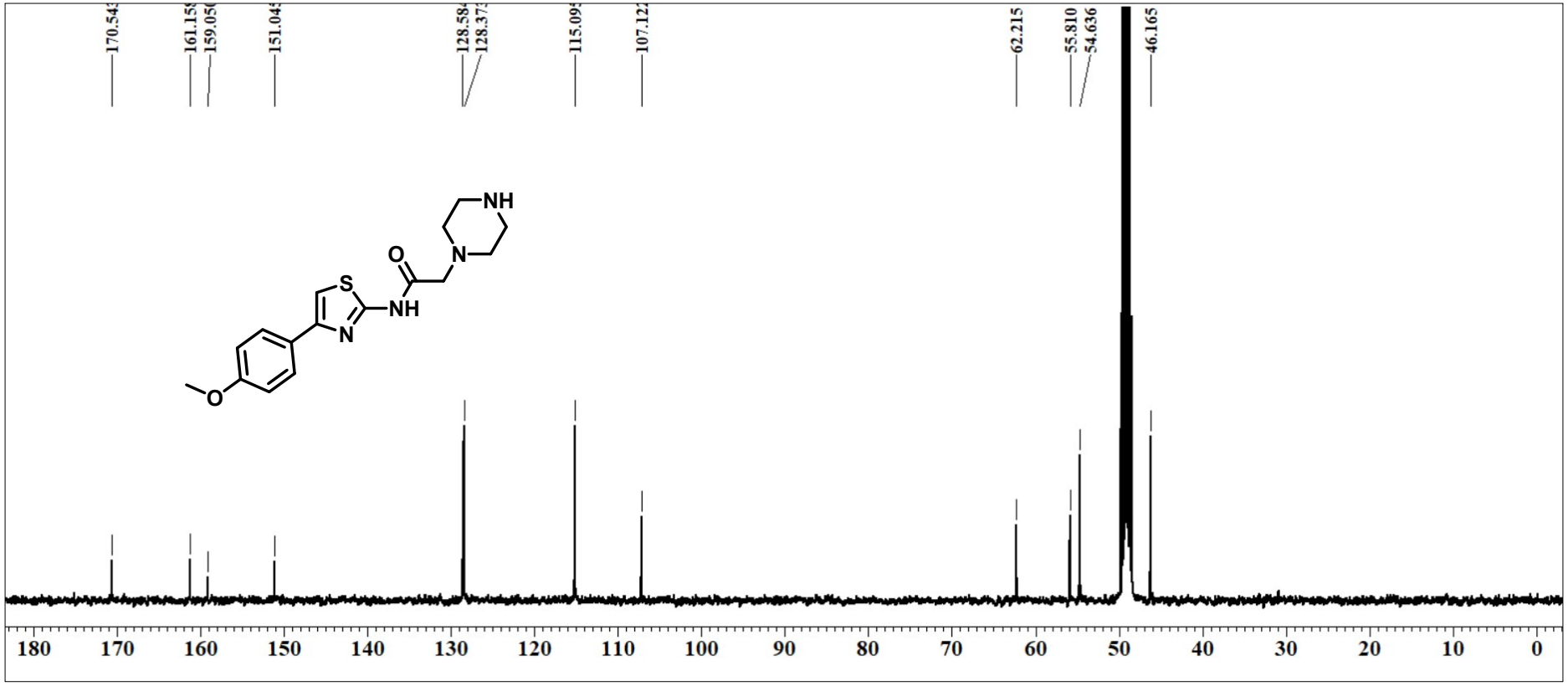


Fig S5: ^{13}C NMR SPECTRUM OF COMPOUND **4a** (100 MHz, CD_3OD)

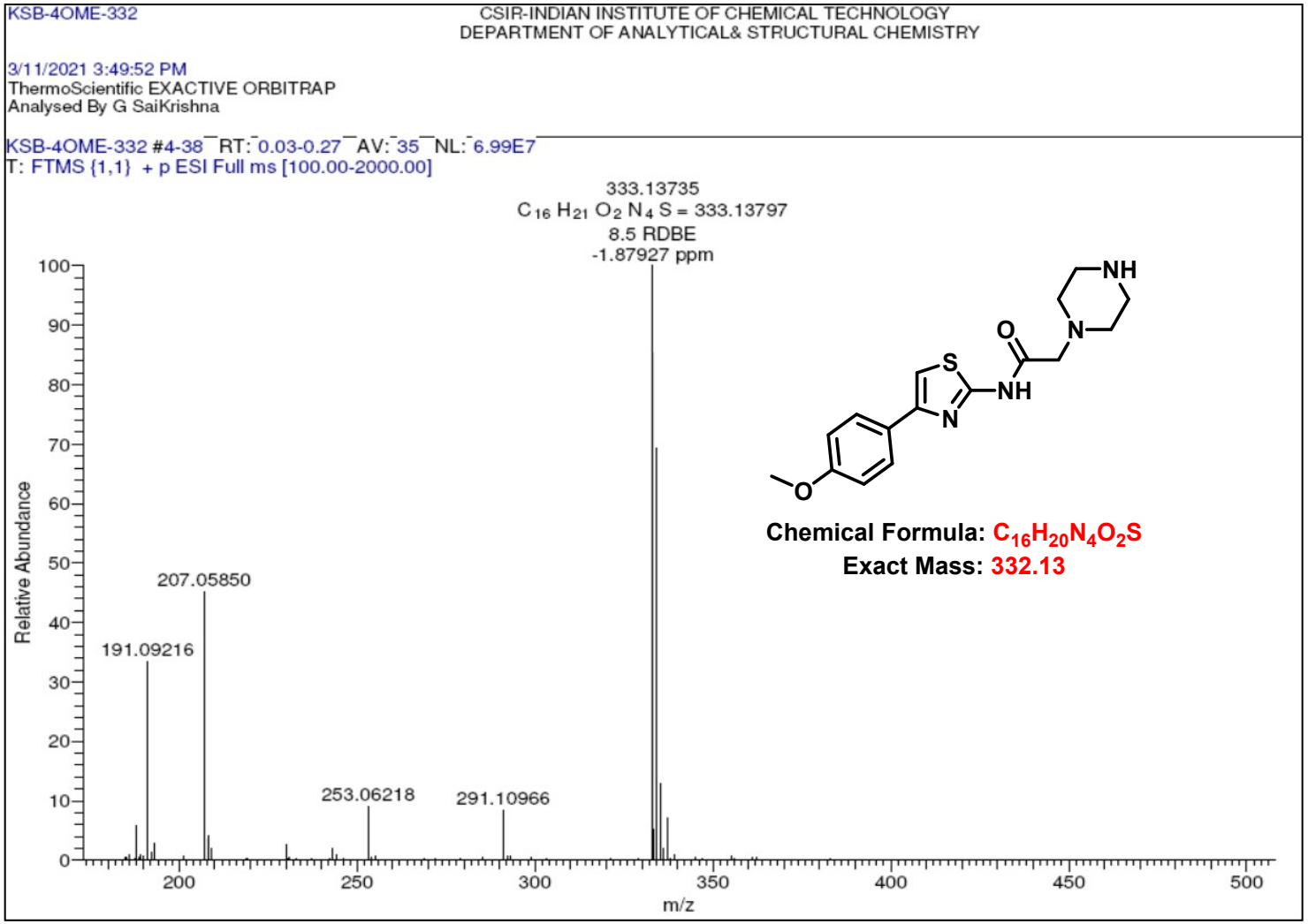


Fig S6:HRESIMS SPECTRUM OF COMPOUND **4a**

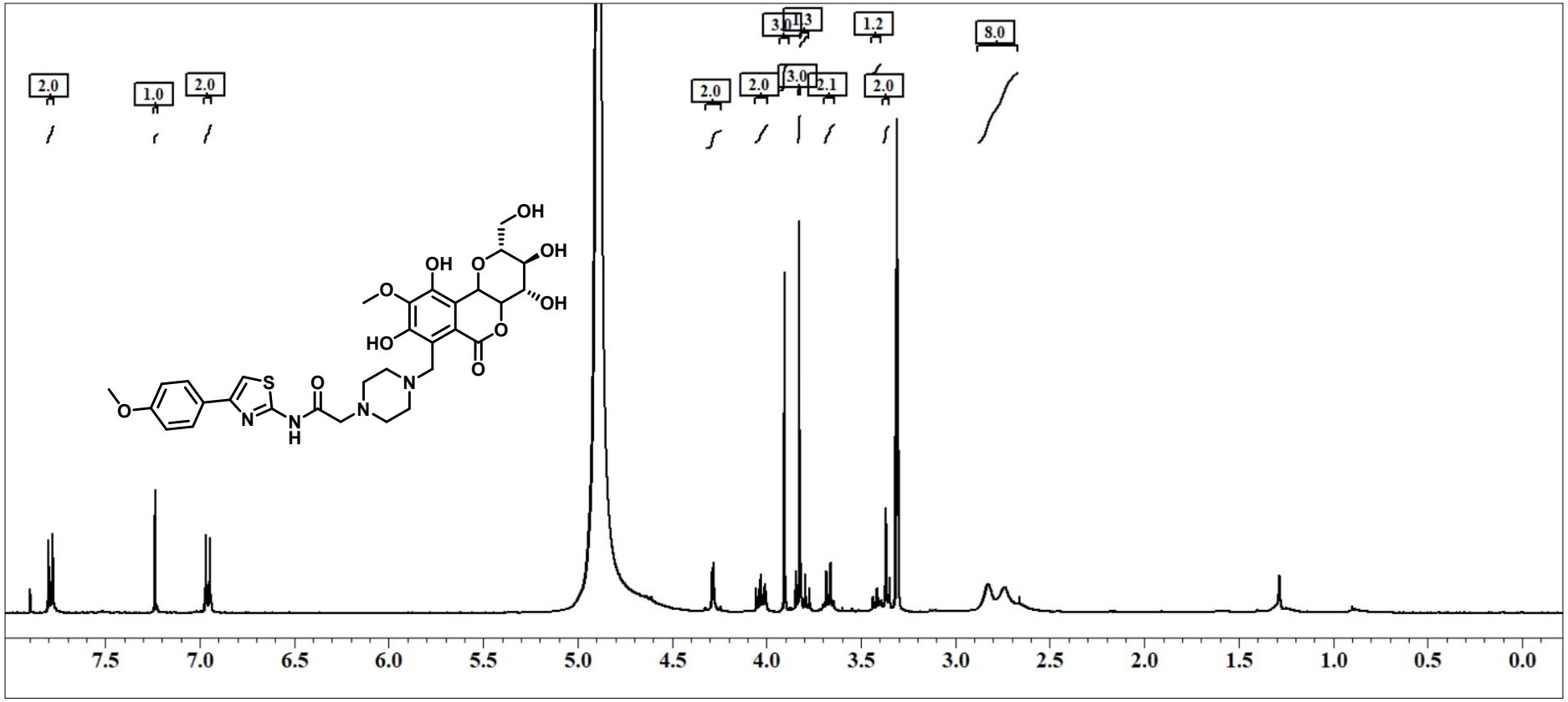


Fig S7: ^{1}H NMR SPECTRUM OF COMPOUND **5a** (400 MHz, CD_3OD)

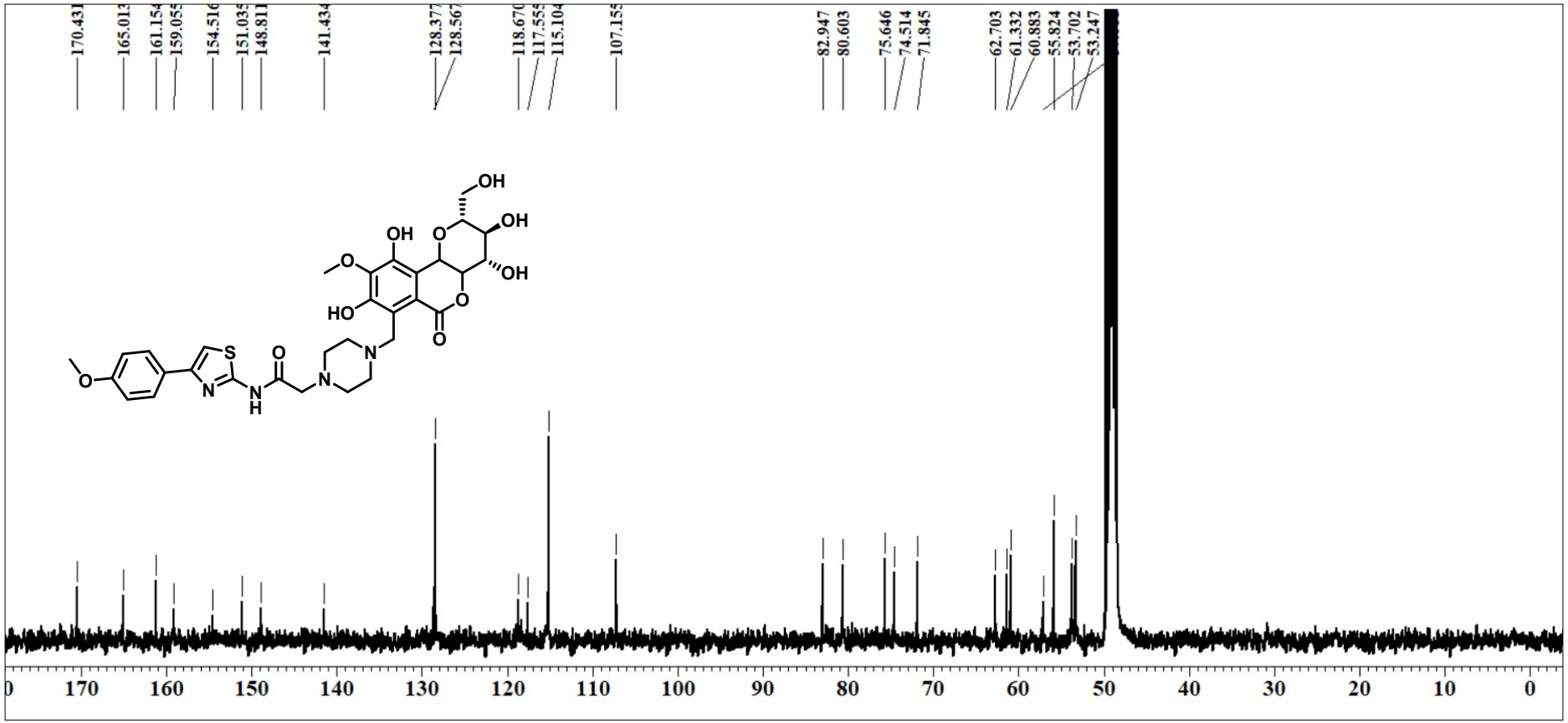


Fig S8: ^{13}C NMR SPECTRUM OF COMPOUND **5a** (100 MHz, CD_3OD)

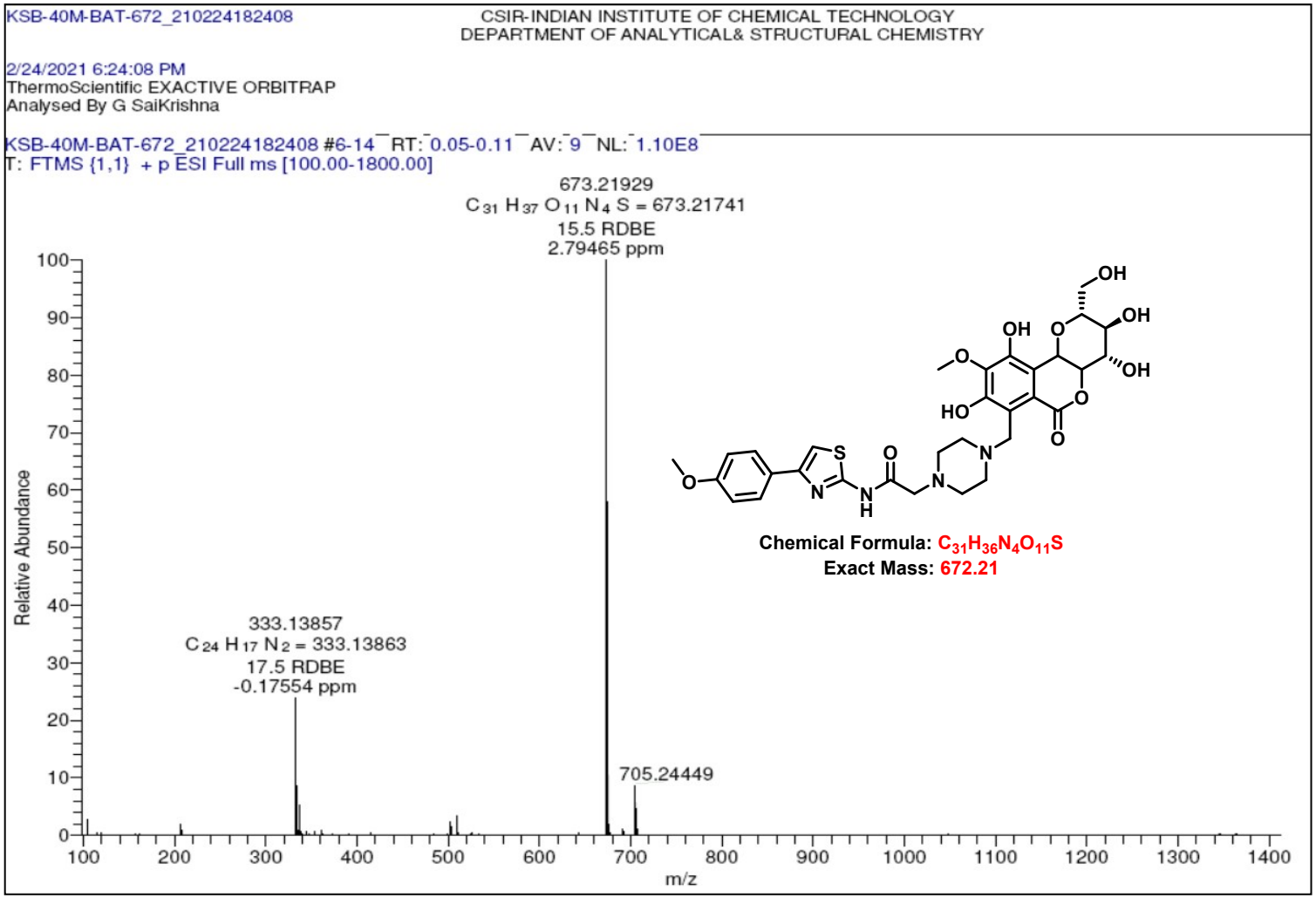


Fig S9: HRESIMS SPECTRUM OF COMPOUND **5a**

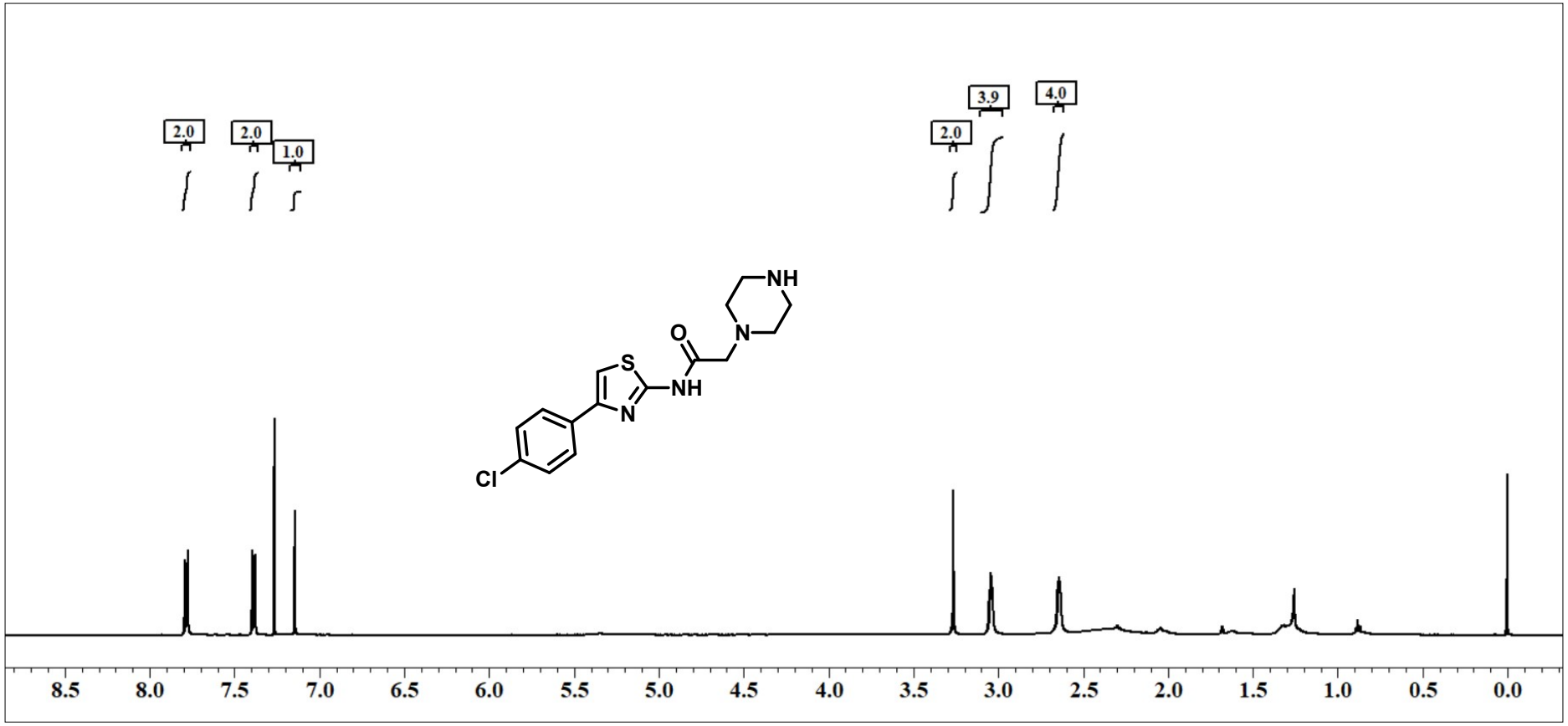


Fig S10: ^{1}H NMR SPECTRUM OF COMPOUND **4b**(400 MHz, $CDCl_3$)

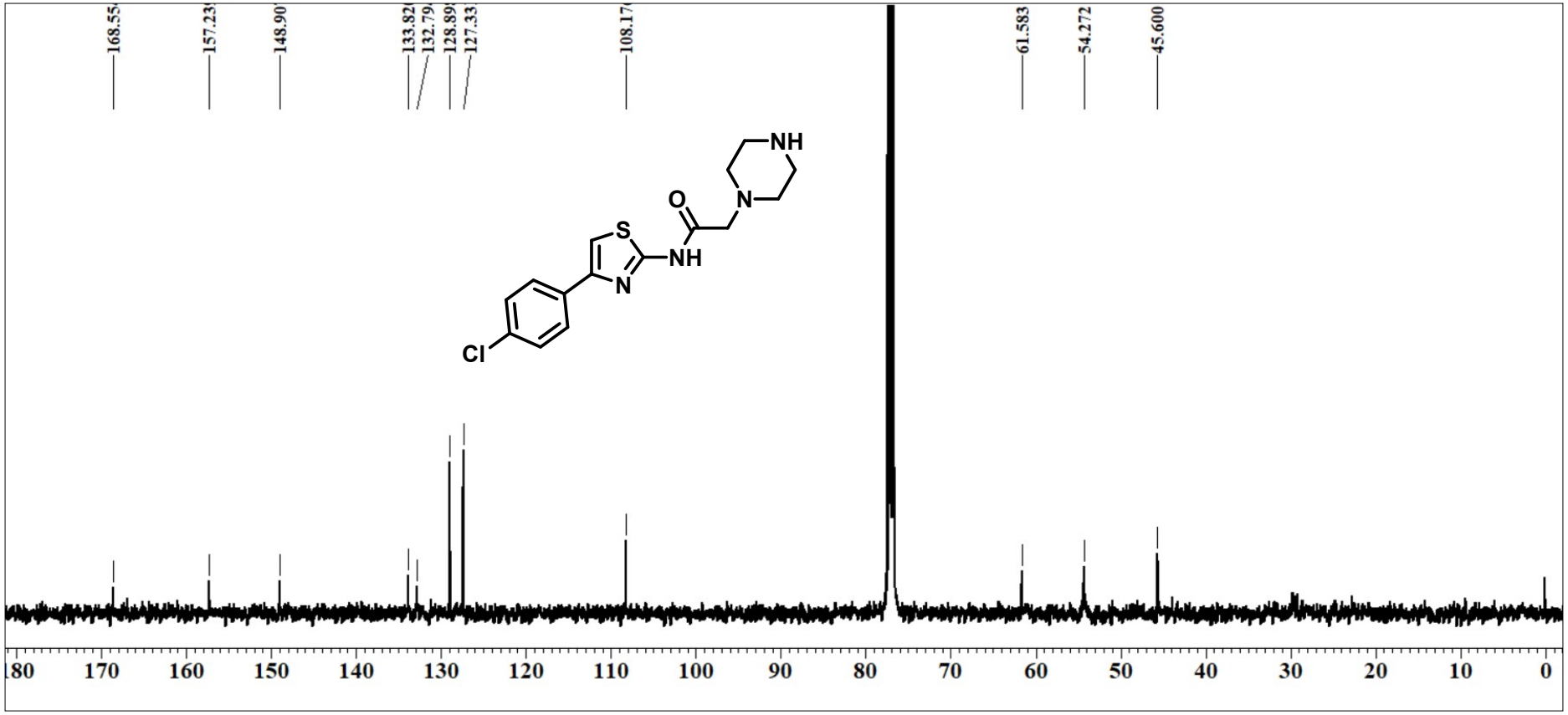


Fig S11: ^{13}C NMR SPECTRUM OF COMPOUND **4b** (100 MHz, $CDCl_3$)

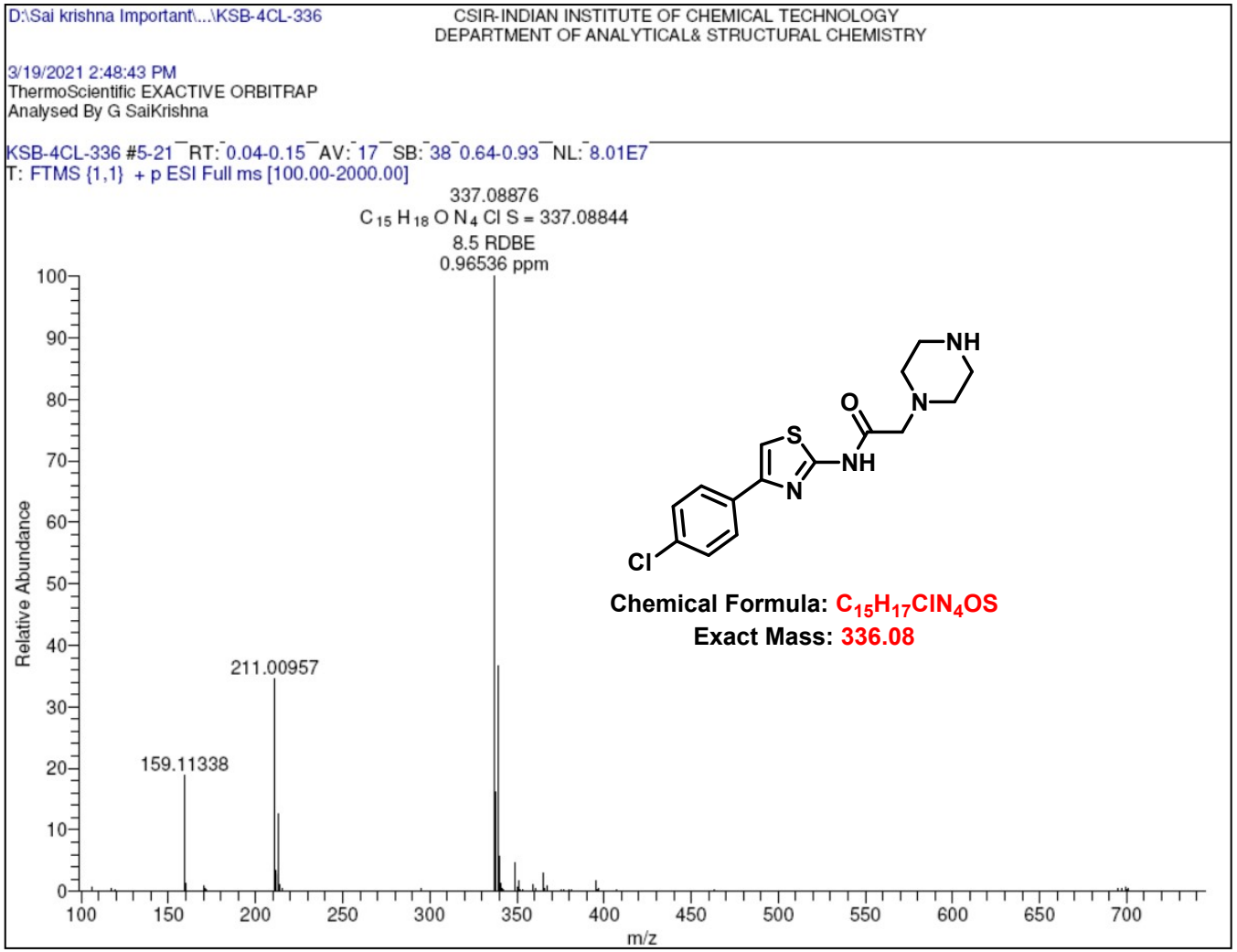


Fig S12: HRESIMS SPECTRUM OF COMPOUND **4b**

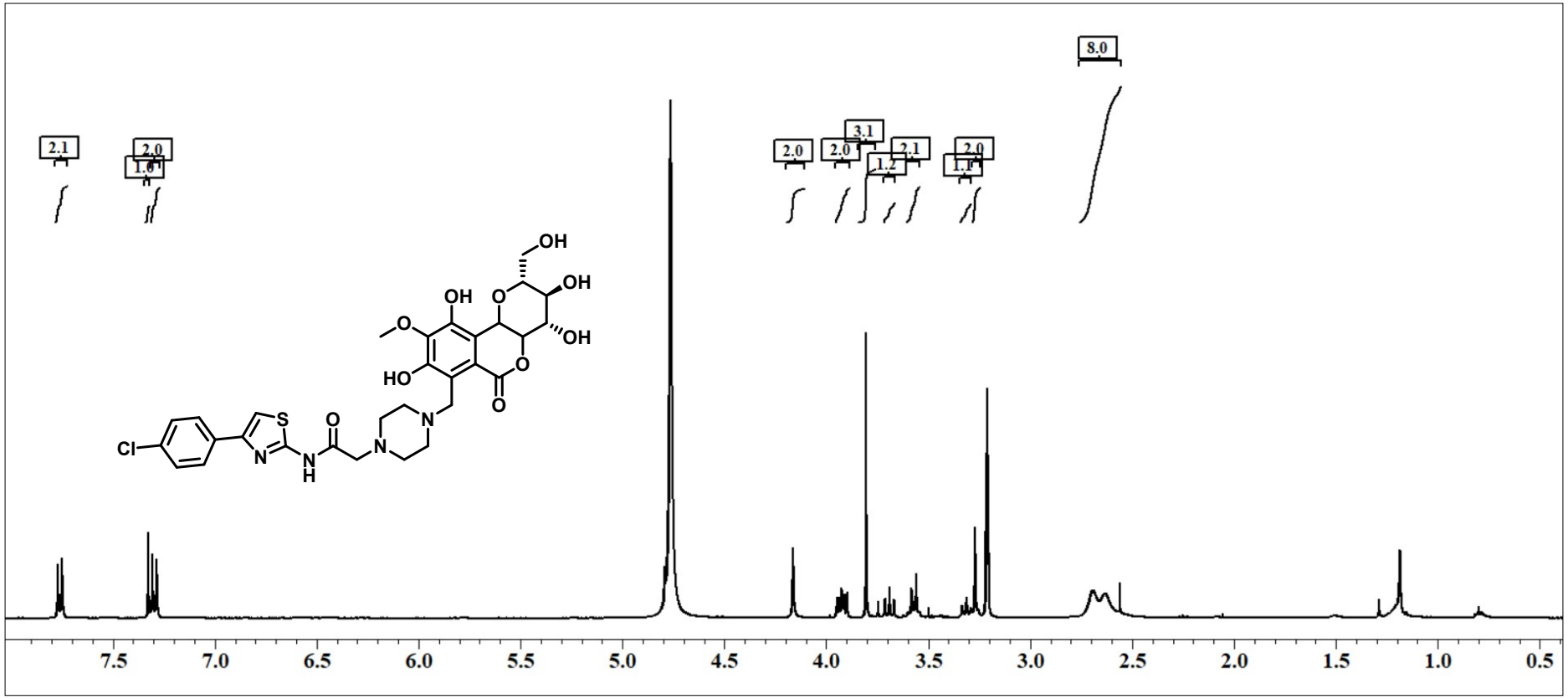


Fig S13: ^{1}H NMR SPECTRUM OF COMPOUND **5b**(400 MHz, CD_3OD)

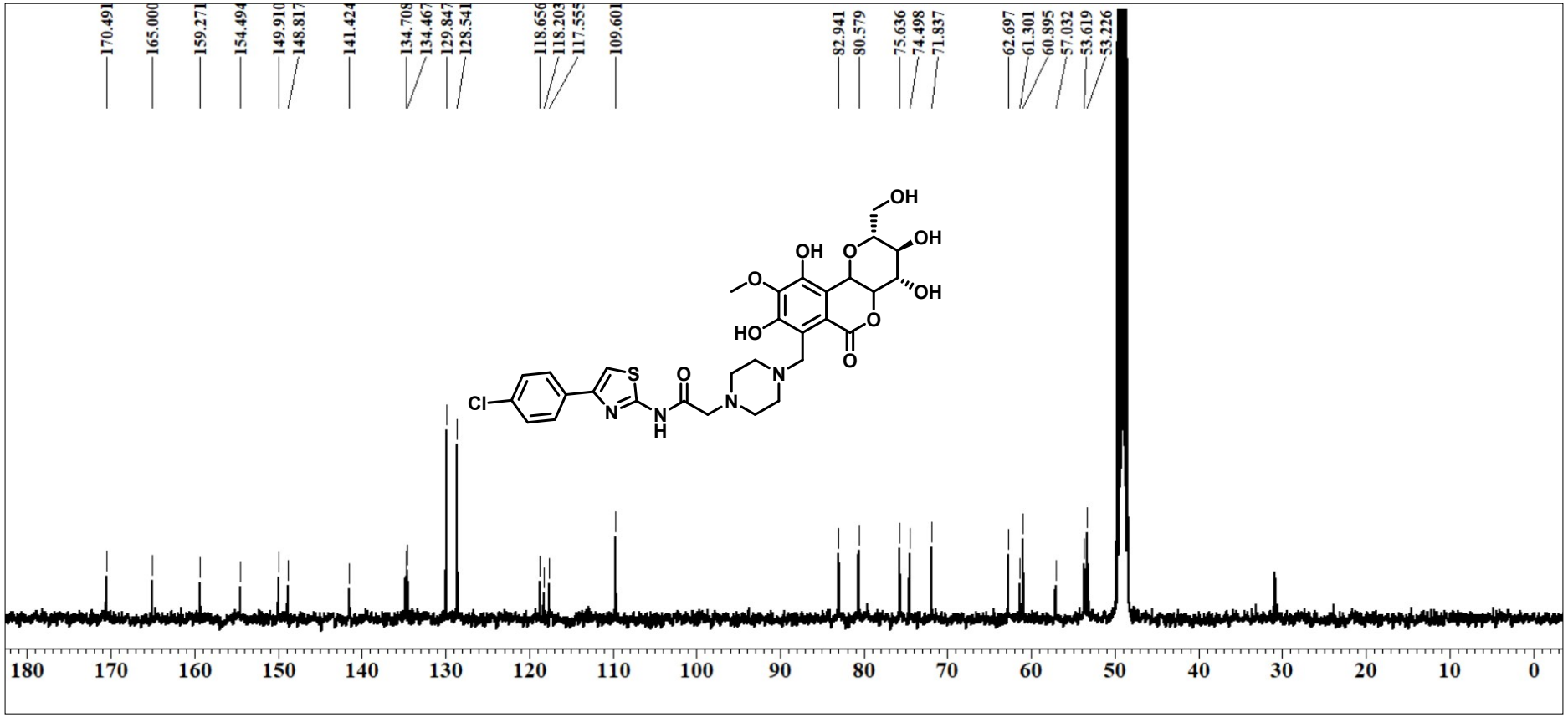


Fig S14: ^{13}C NMR SPECTRUM OF COMPOUND **5b** (100 MHz, CD_3OD)

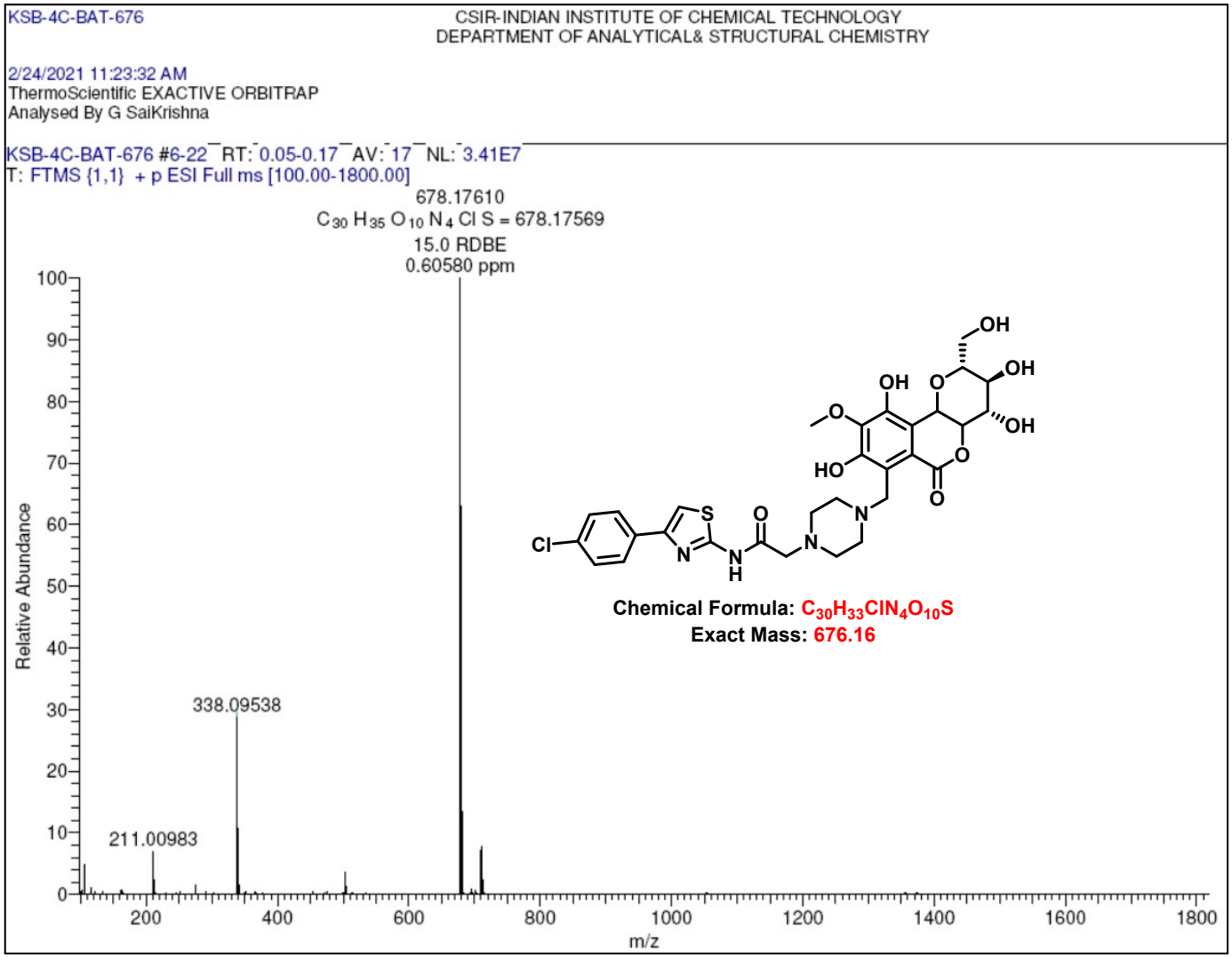


Fig S15: HRESIMS SPECTRUM OF COMPOUND **5b**

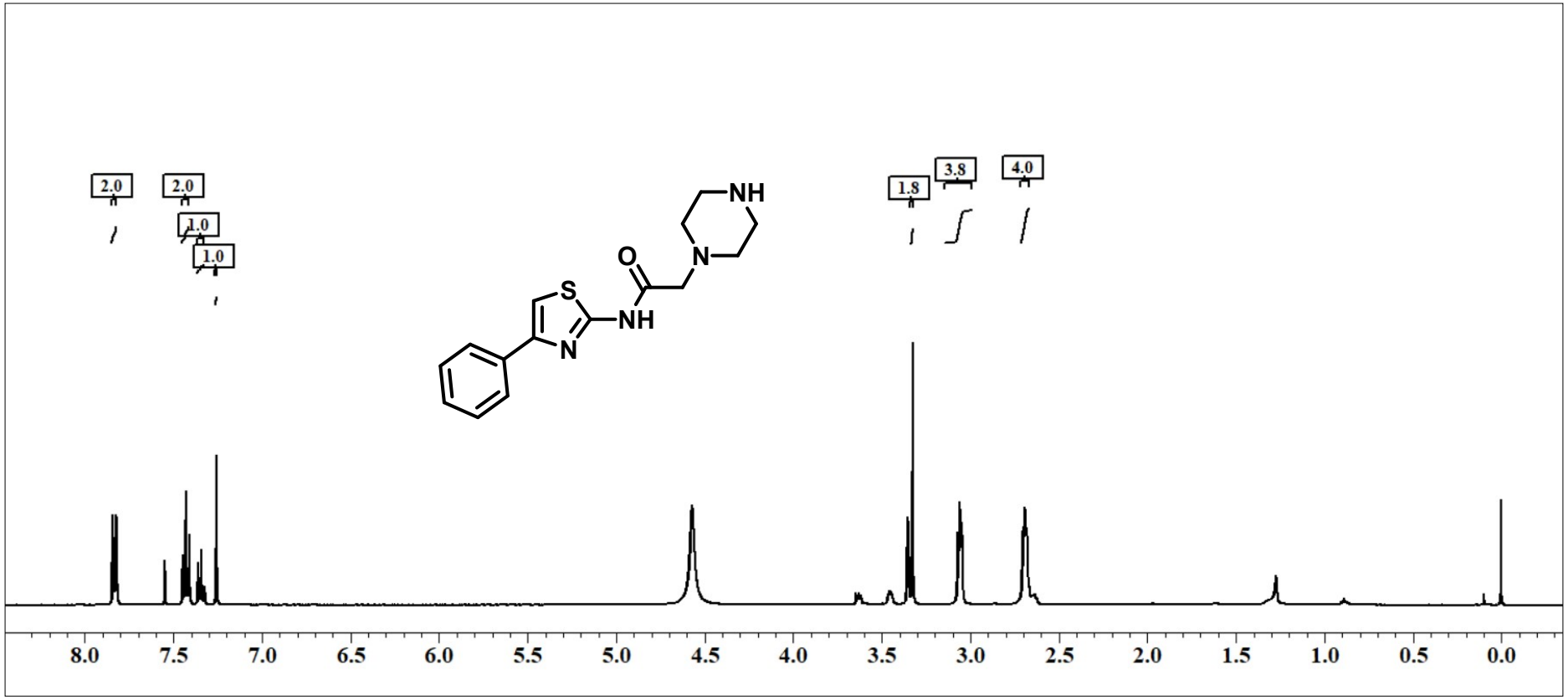


Fig S16: ^{1}H NMR SPECTRUM OF COMPOUND **4c**(400 MHz, CD_3OD+$CDCl_3$)

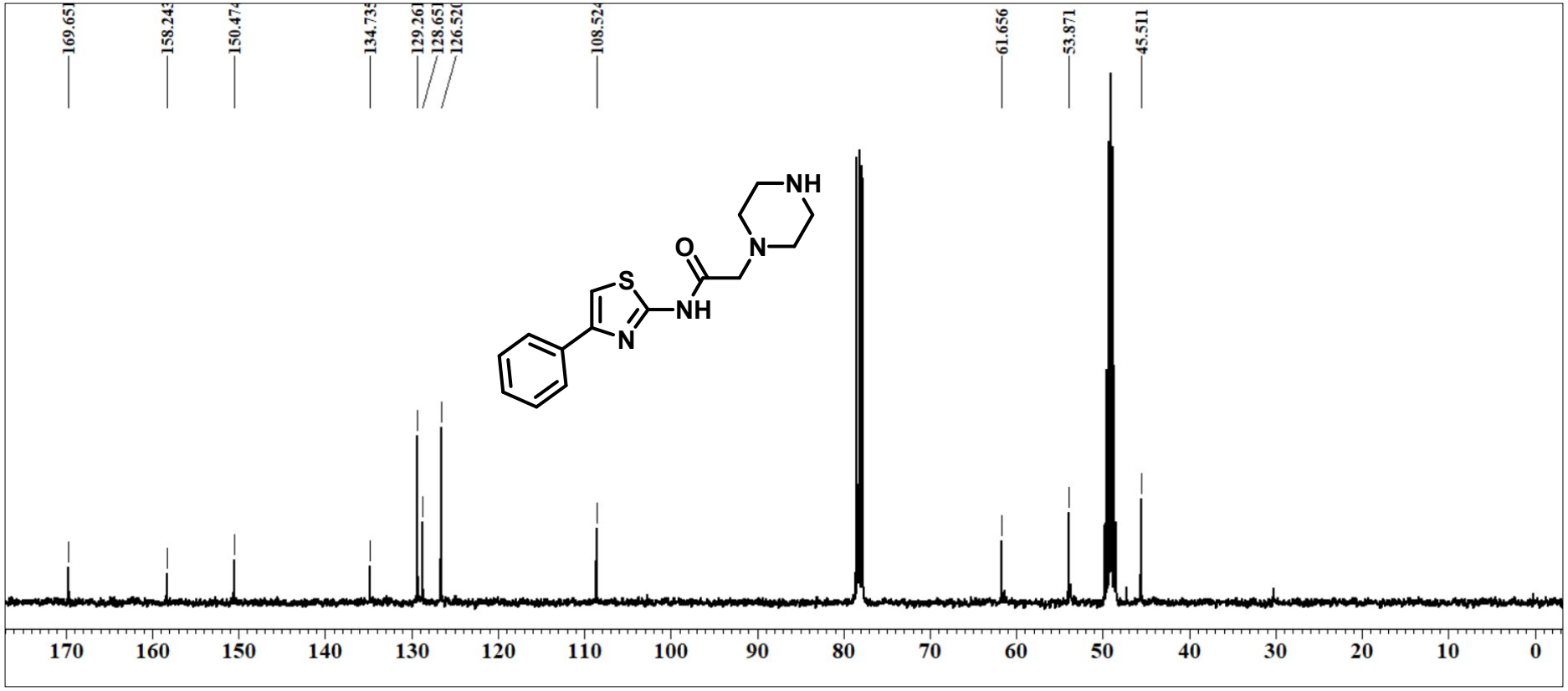


Fig S17: ^{13}C NMR SPECTRUM OF COMPOUND **4c** (100 MHz, $CD_3OD+CDCl_3$)

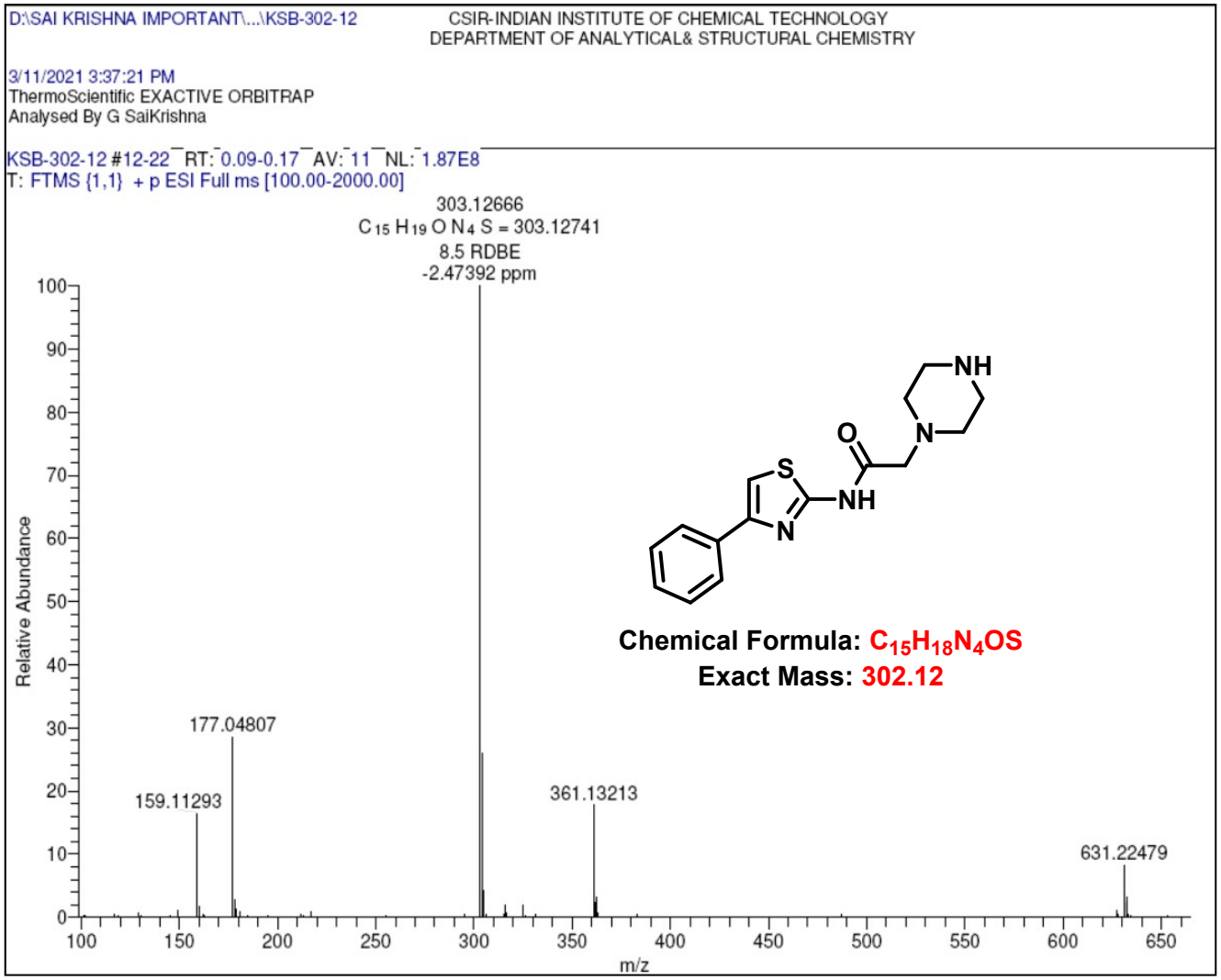


Fig S18: HRESIMS SPECTRUM OF COMPOUND **4c**

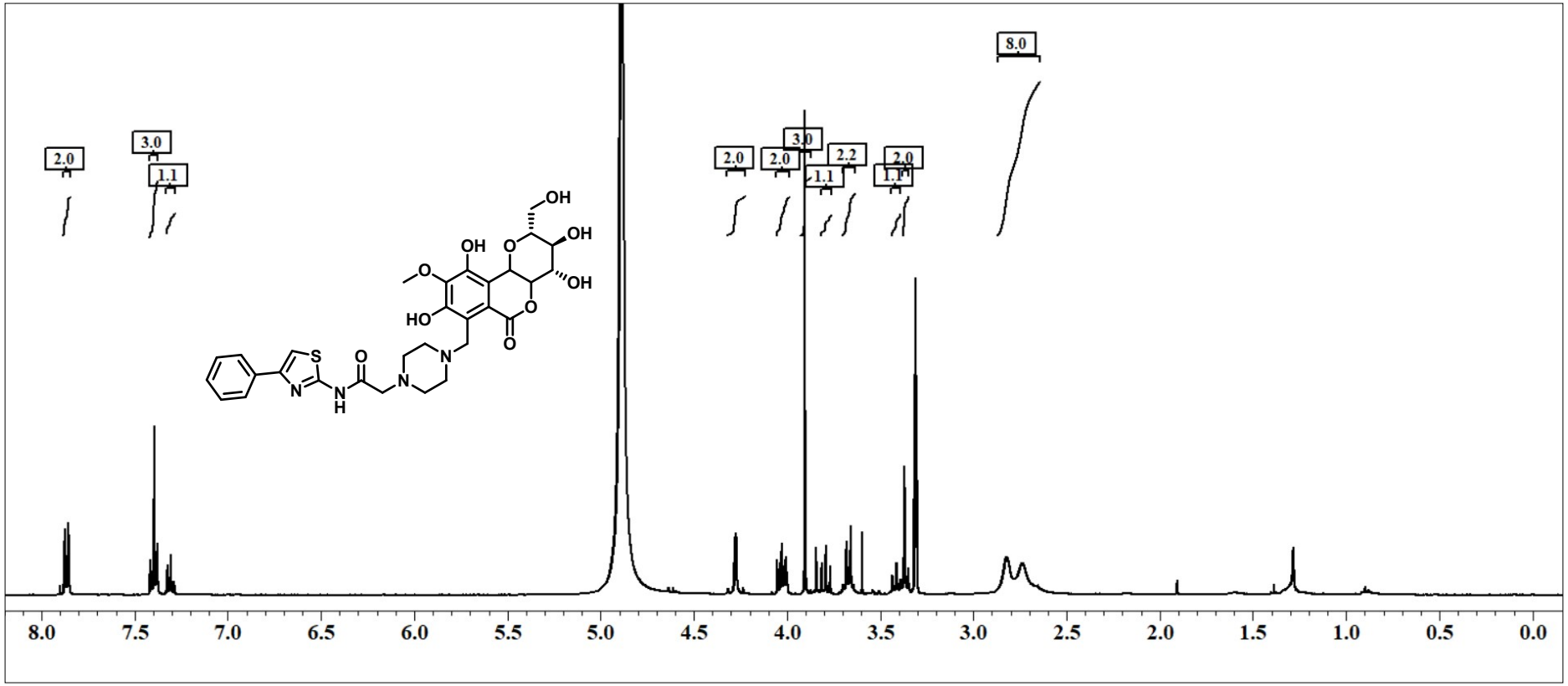


Fig S19: ^{1}H NMR SPECTRUM OF COMPOUND **5c**(400 MHz, CD_3OD)

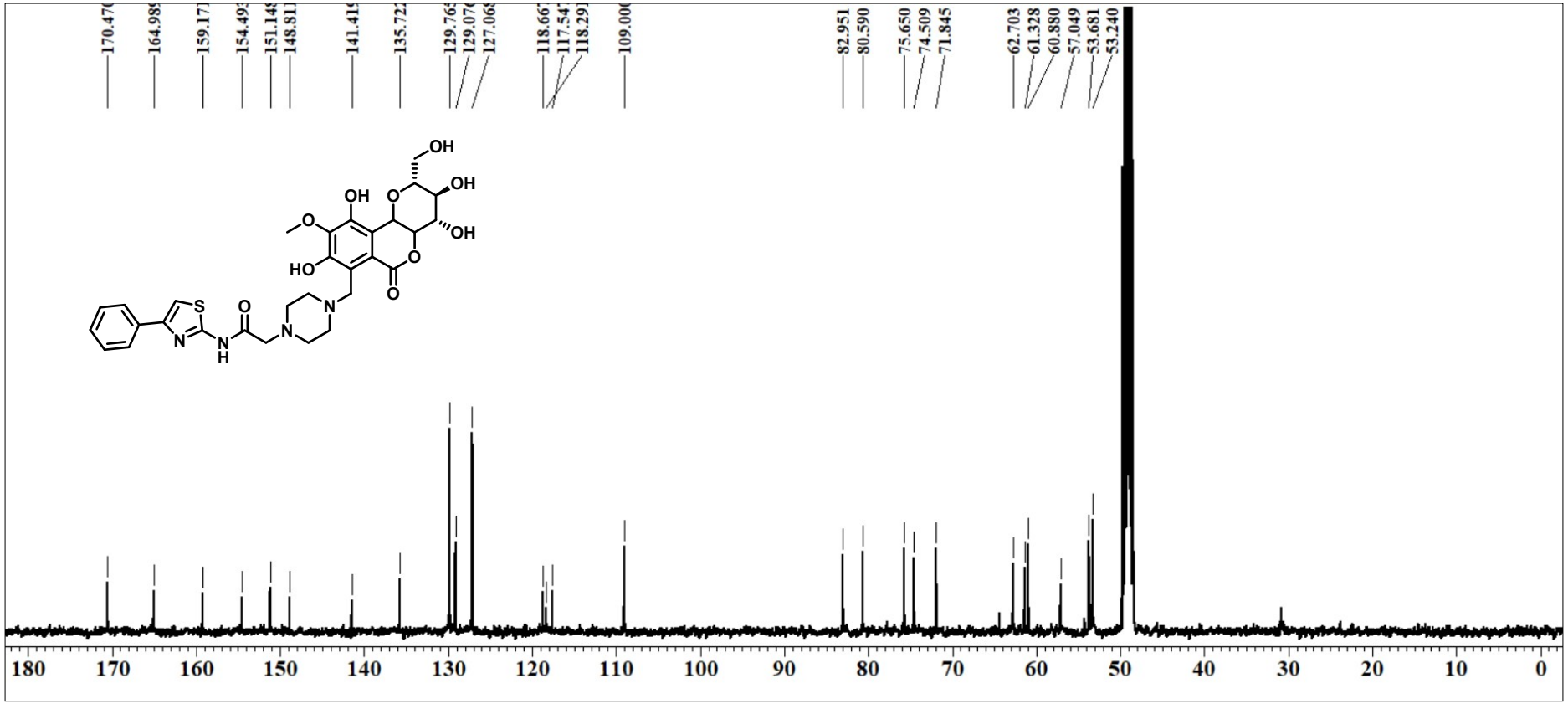


Fig S20: ^{13}C NMR SPECTRUM OF COMPOUND **5c** (100 MHz, CD_3OD)

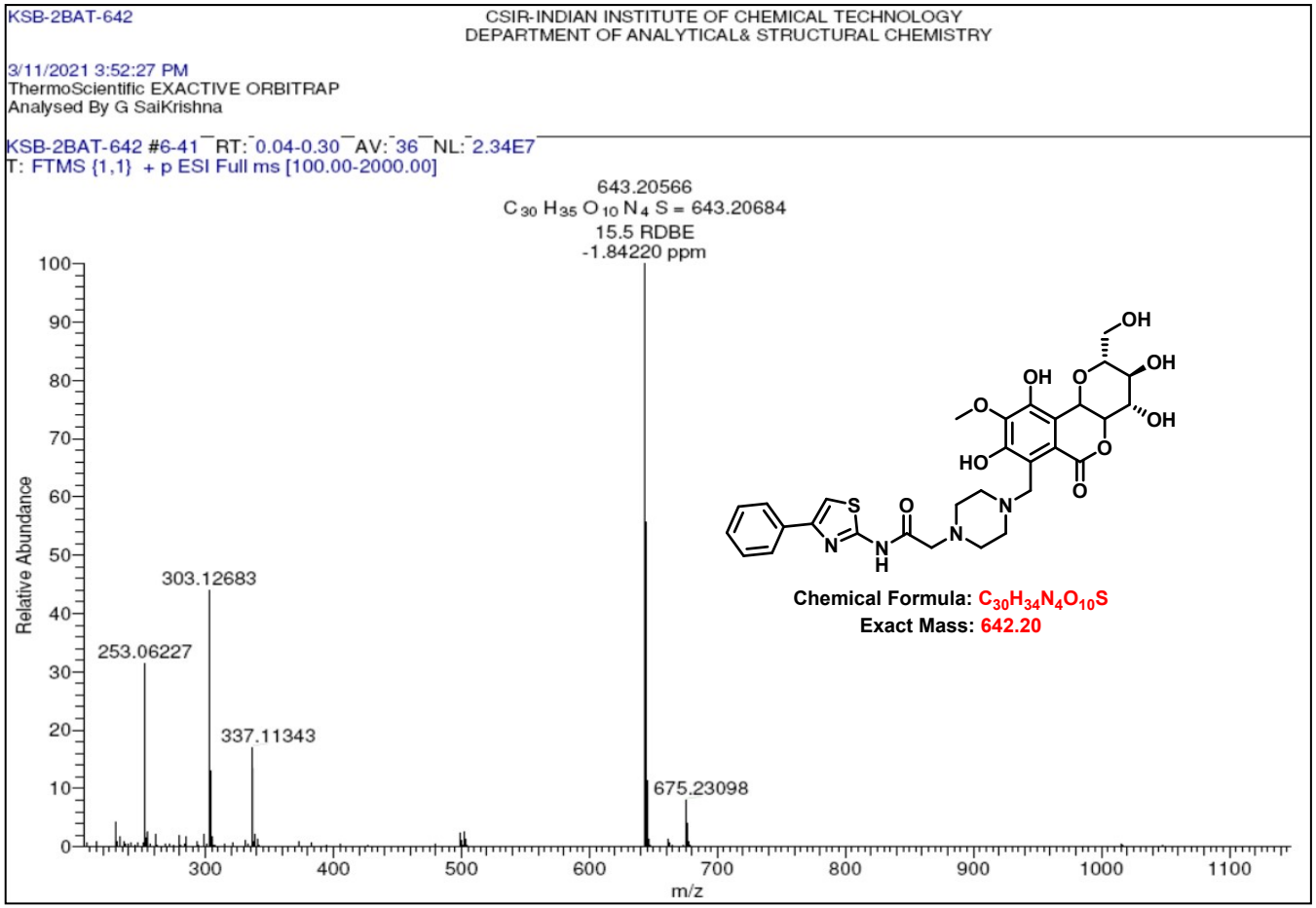


Fig S21: HRESIMS SPECTRUM OF COMPOUND **5c**

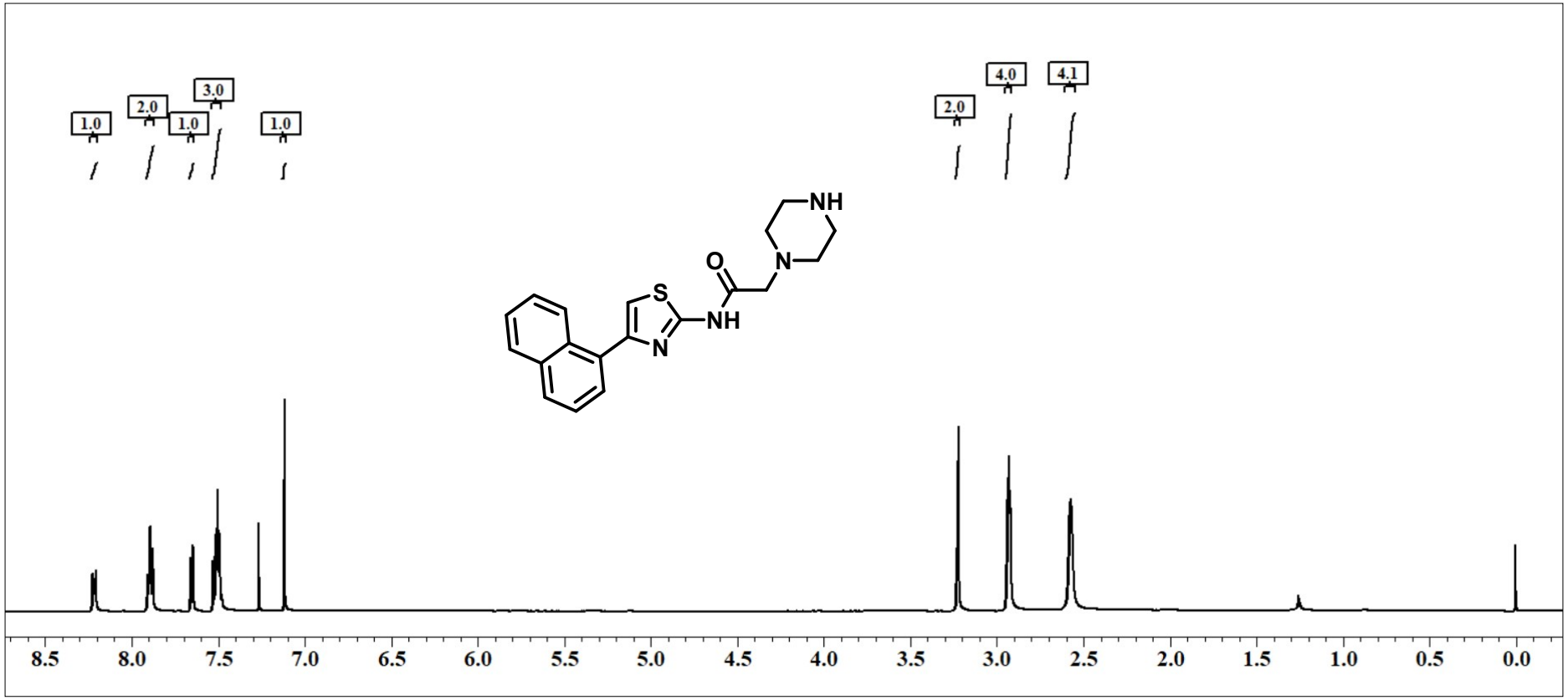


Fig S22: ^{1}H NMR SPECTRUM OF COMPOUND **4d**(500 MHz, CD_3OD)

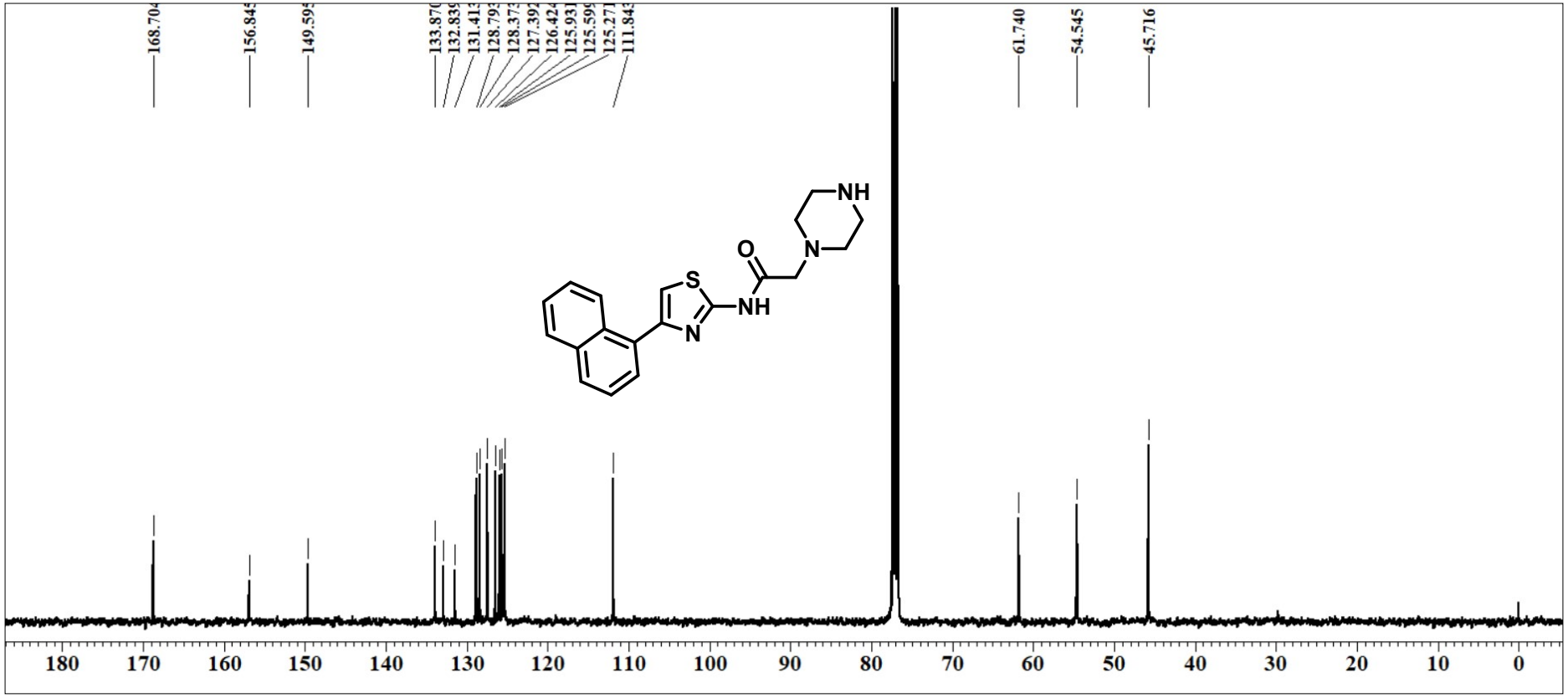


Fig S23: ^{13}C NMR SPECTRUM OF COMPOUND **4d** (100 MHz, $CDCl_3$)

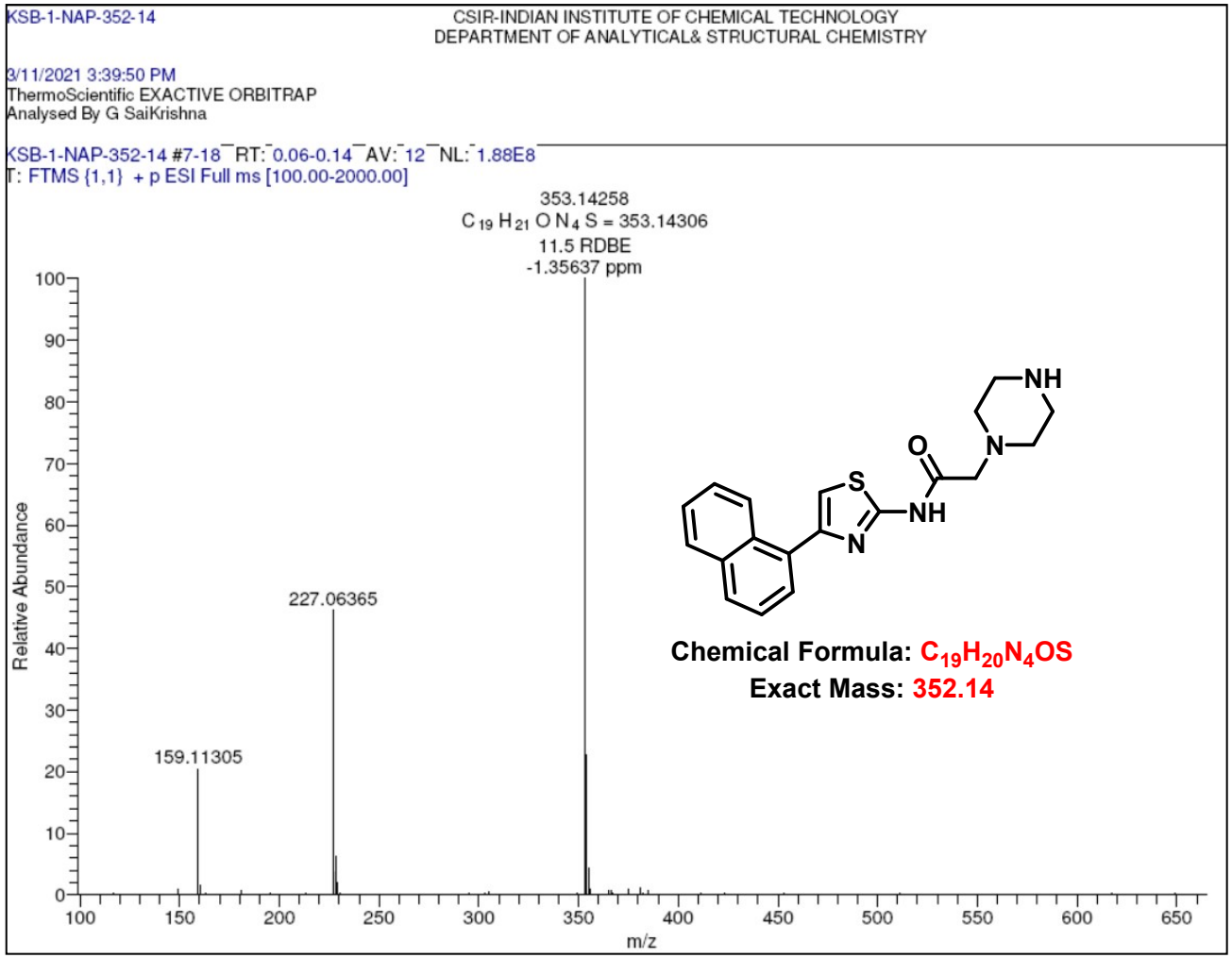


Fig S24: HRESIMS SPECTRUM OF COMPOUND **4d**

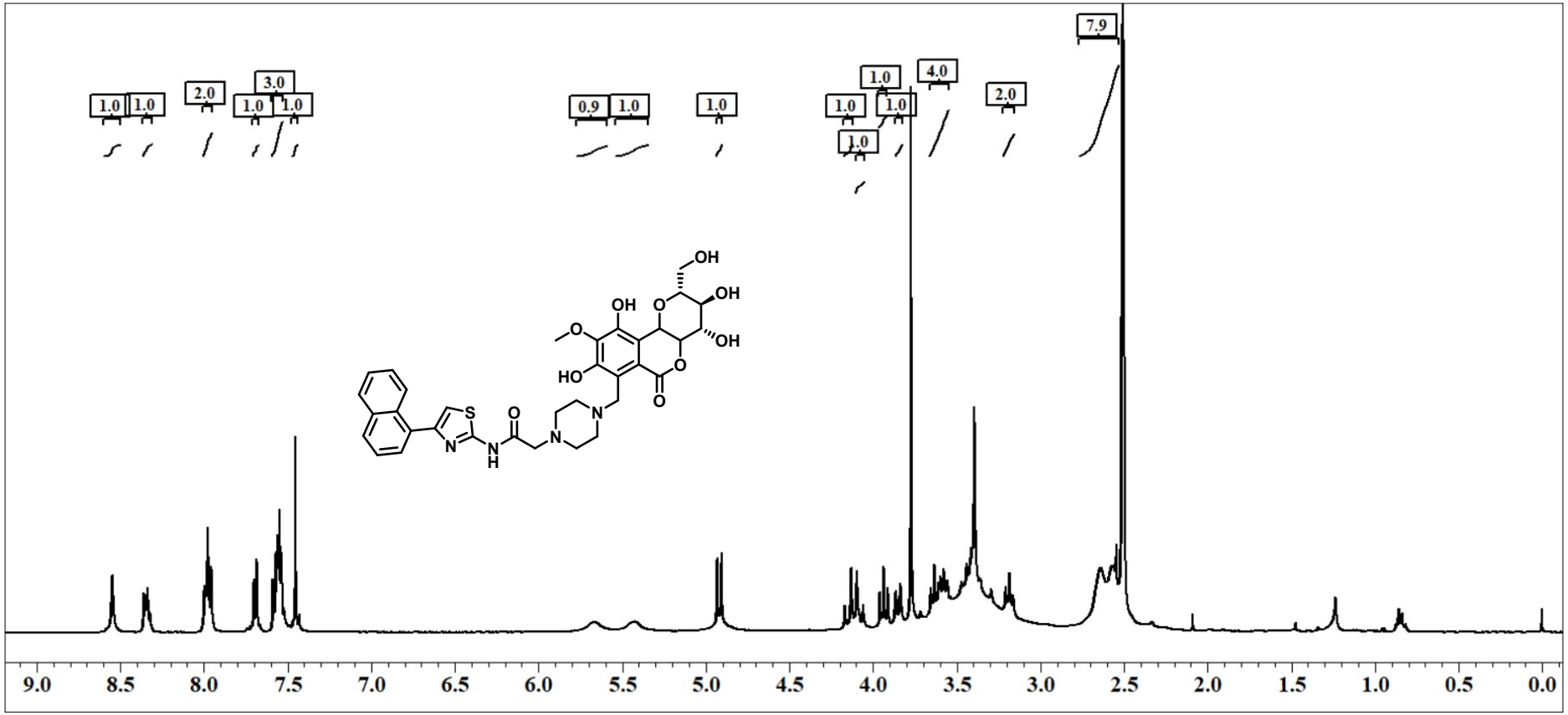


Fig S25: ^{1}H NMR SPECTRUM OF COMPOUND **5d**(400 MHz, DMSO-d_6)

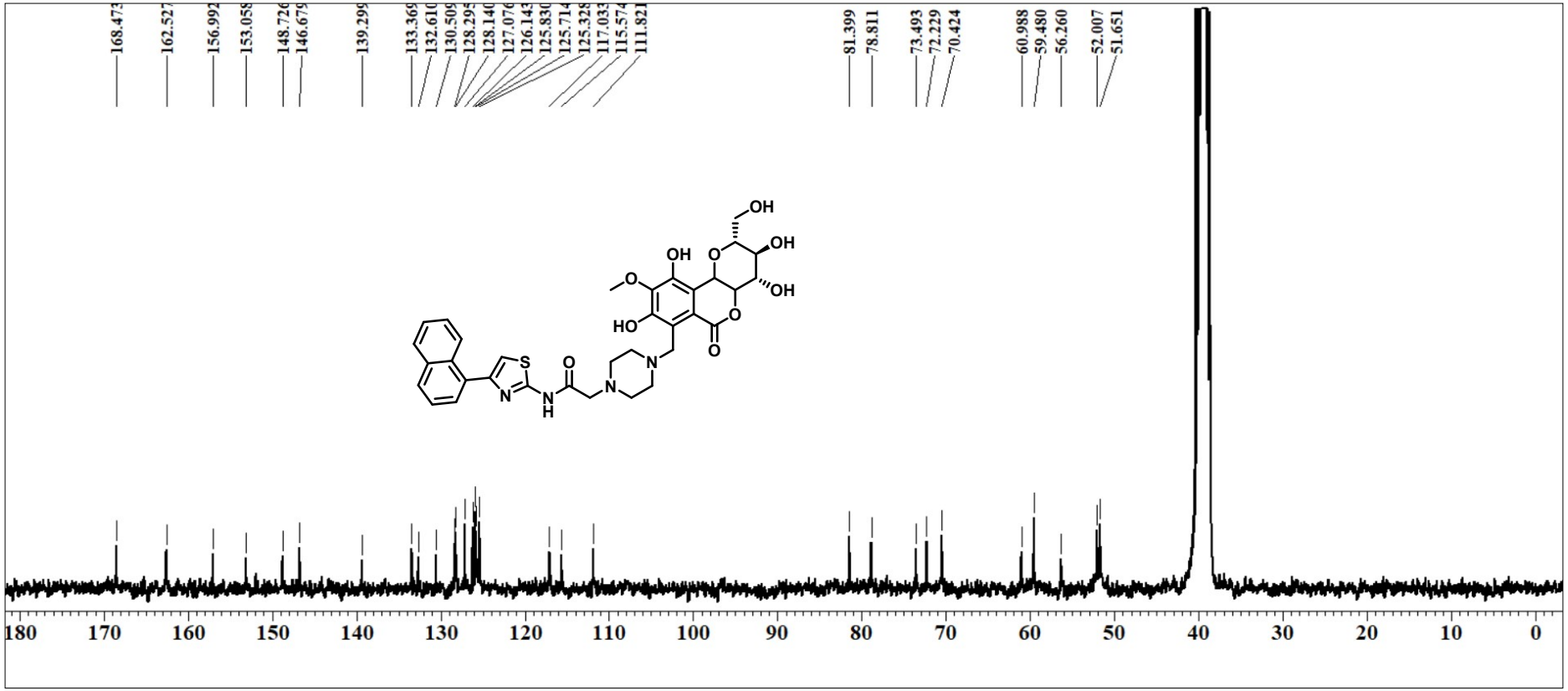


Fig S26: ^{13}C NMR SPECTRUM OF COMPOUND **5d** (100 MHz, DMSO-d_6)

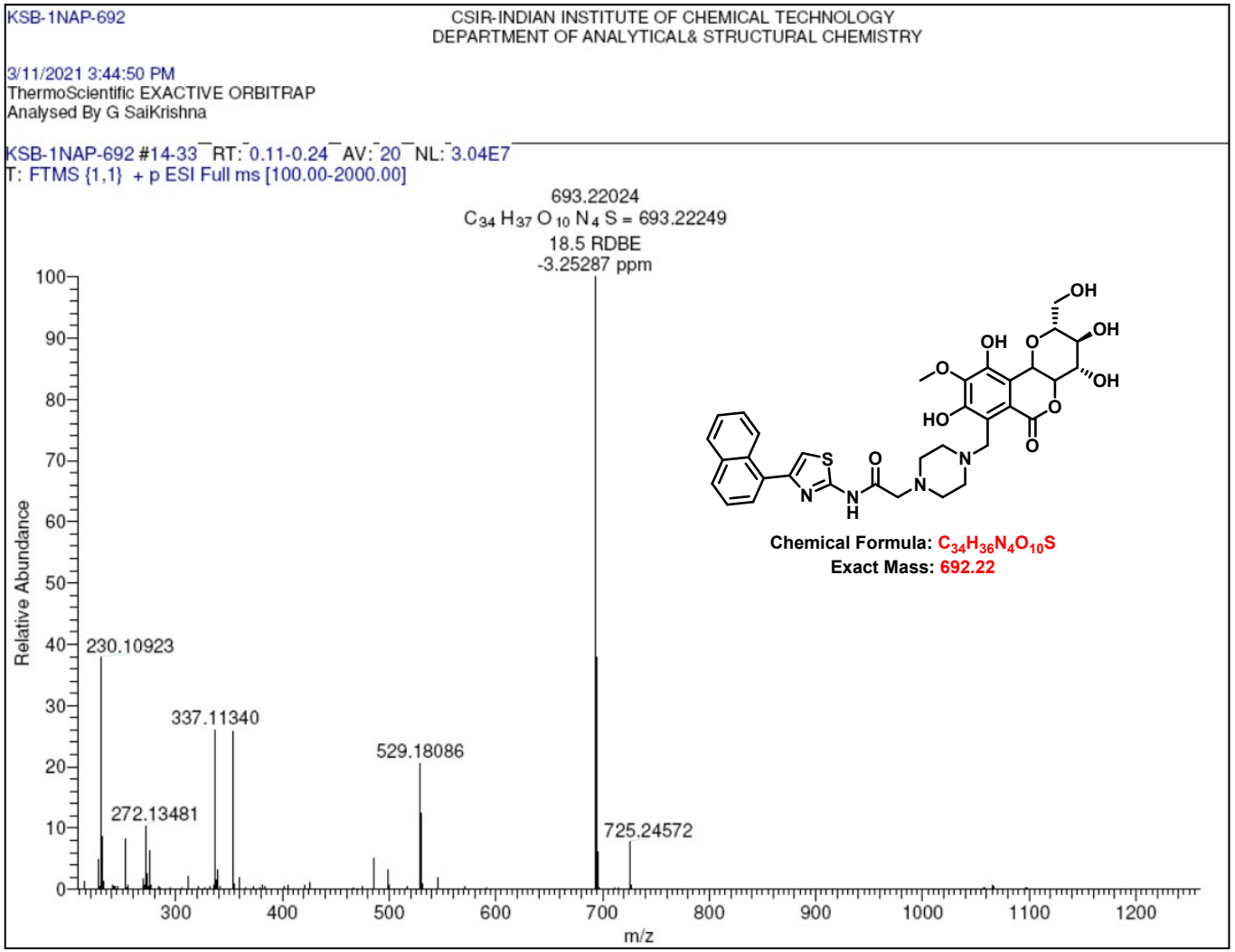


Fig S27: HRESIMS SPECTRUM OF COMPOUND **5d**

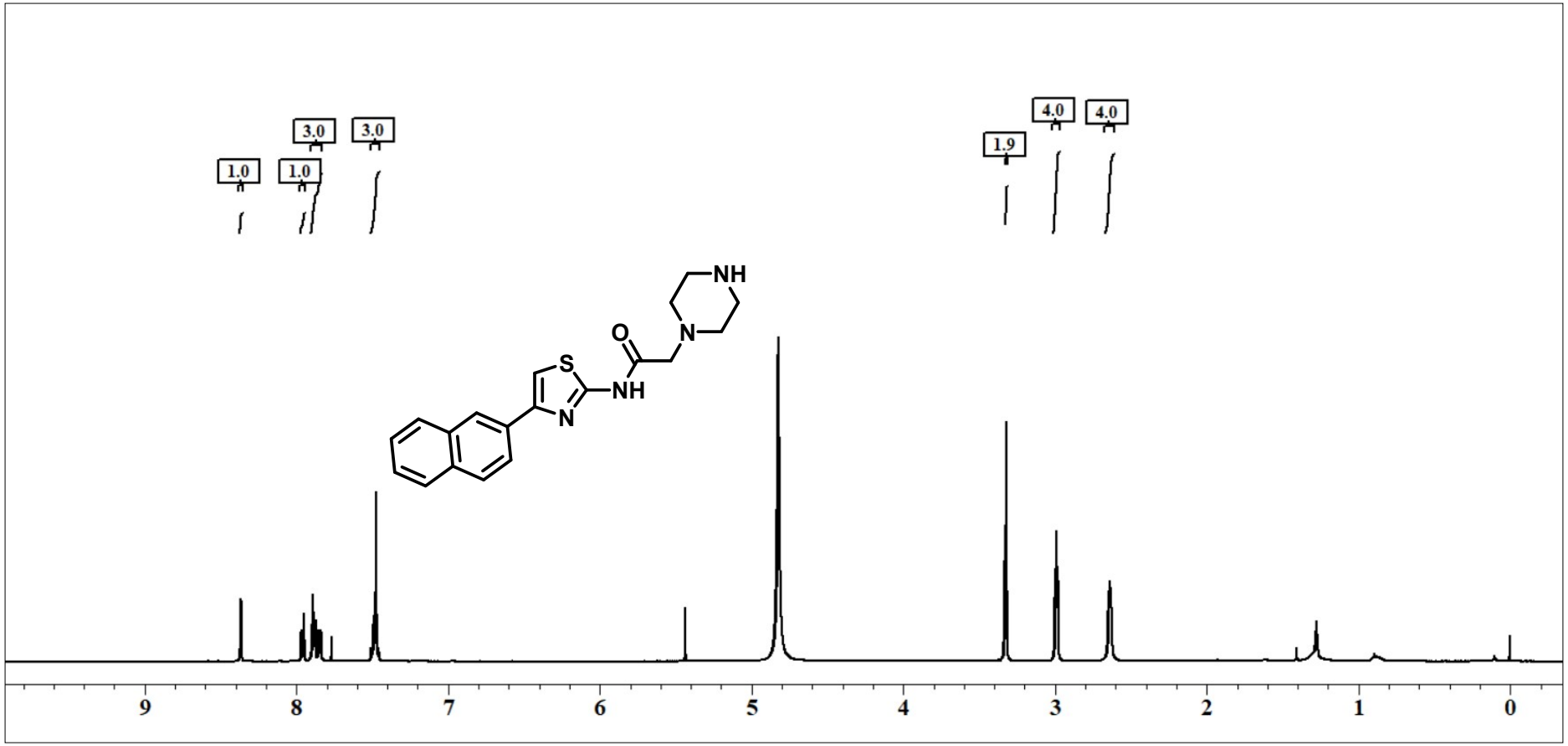


Fig S28: ^{1}H NMR SPECTRUM OF COMPOUND **4e**(500 MHz, CD_3OD+$CDCl_3$)

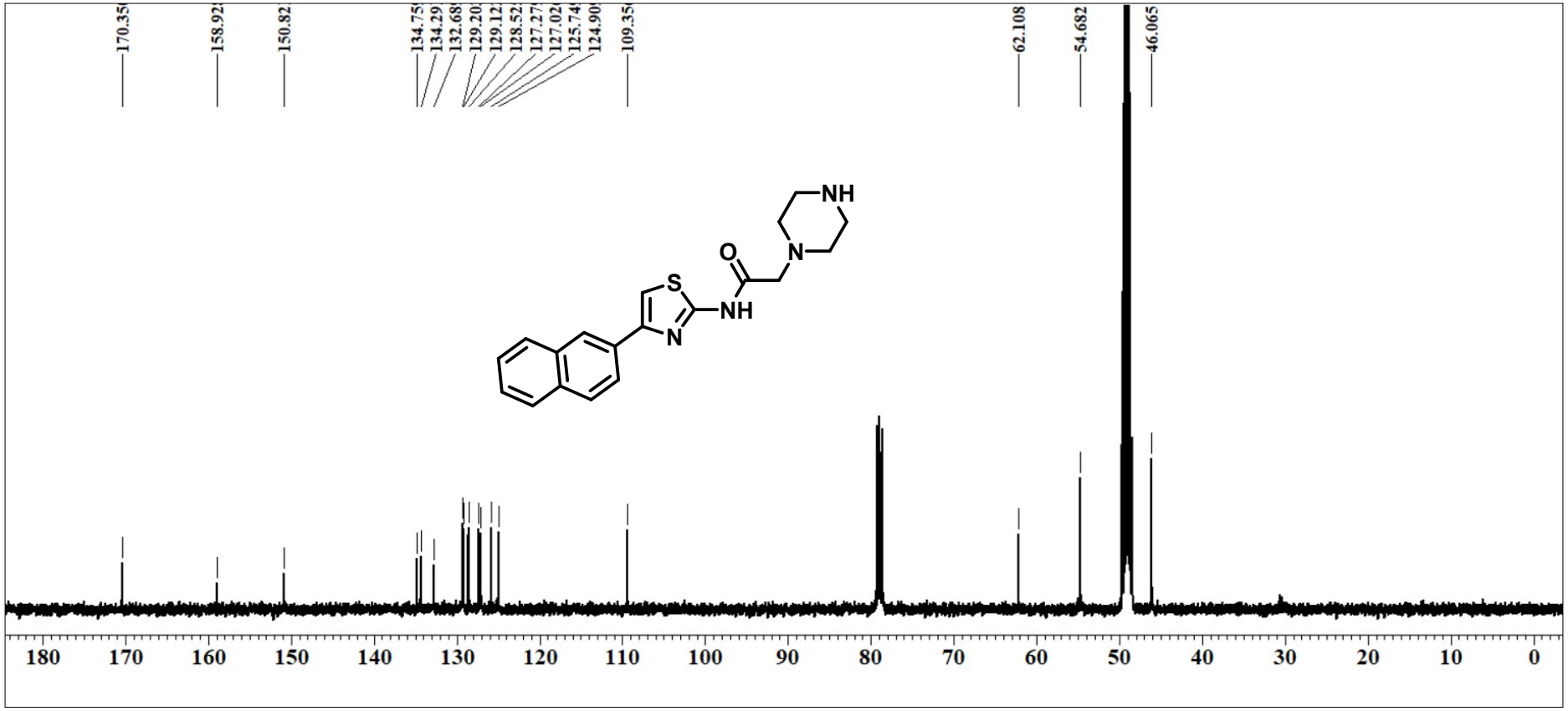


Fig S29: ^{13}C NMR SPECTRUM OF COMPOUND **4e** (100 MHz, $CD_3OD+CDCl_3$)

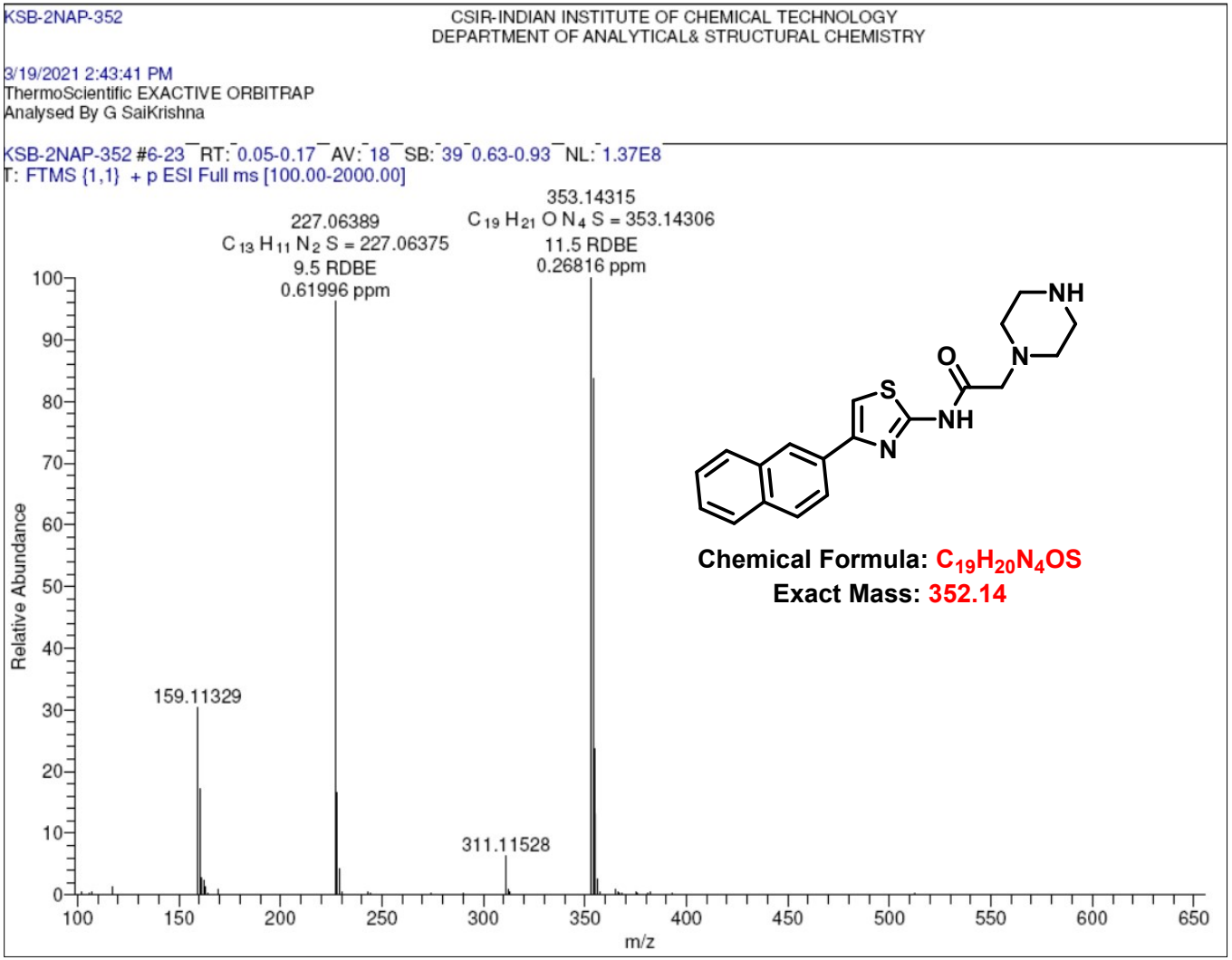


Fig S30: HRESIMS SPECTRUM OF COMPOUND **4e**

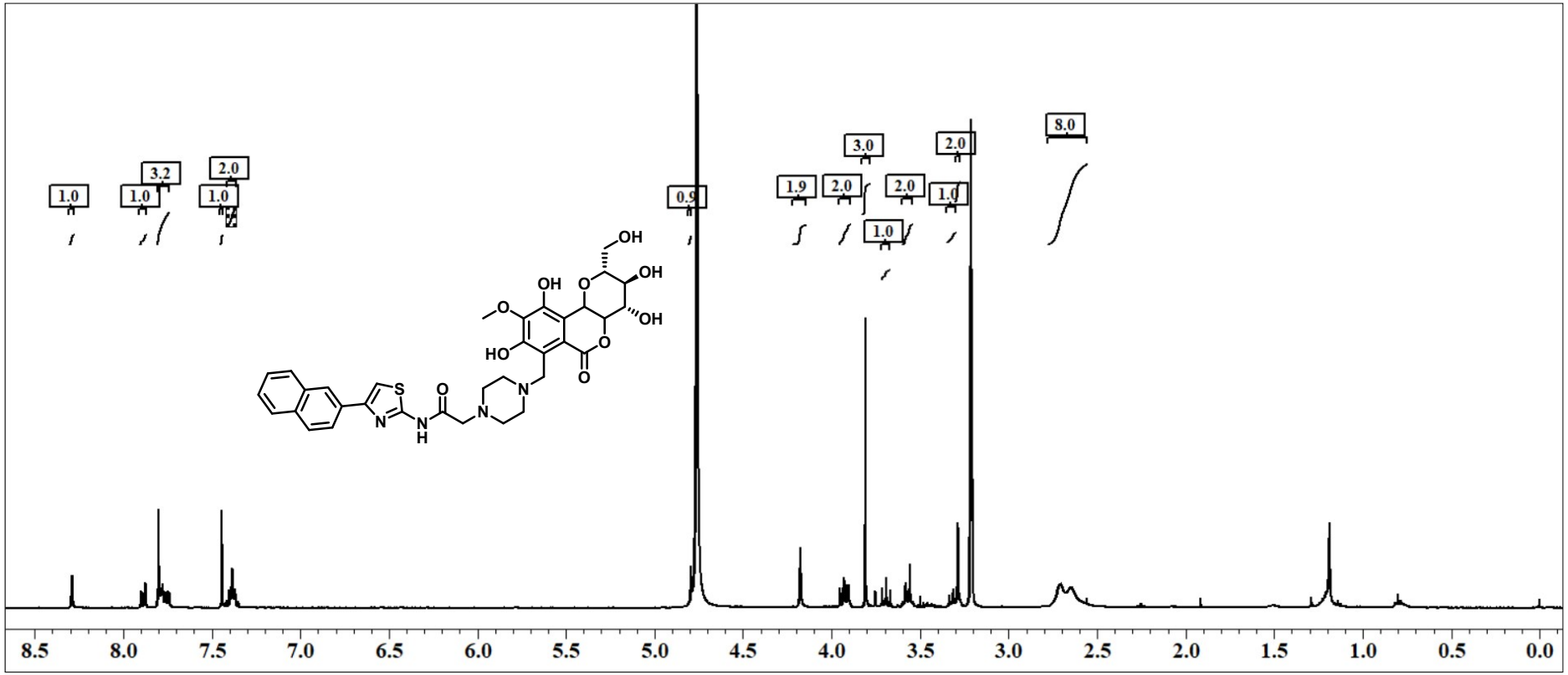


Fig S31: ^{1}H NMR SPECTRUM OF COMPOUND **5e**(400 MHz, CD_3OD)

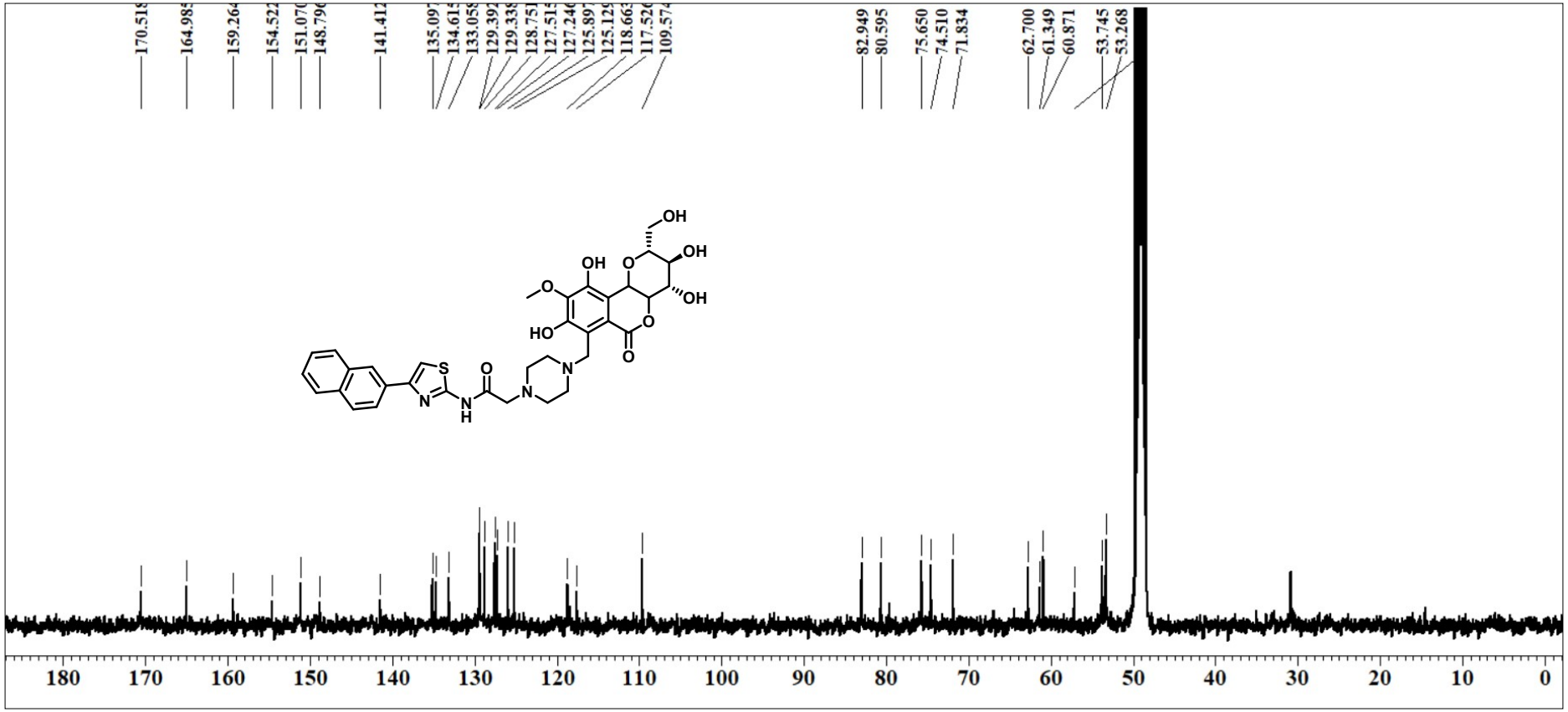


Fig S32: ^{13}C NMR SPECTRUM OF COMPOUND **5e** (100 MHz, CD_3OD)

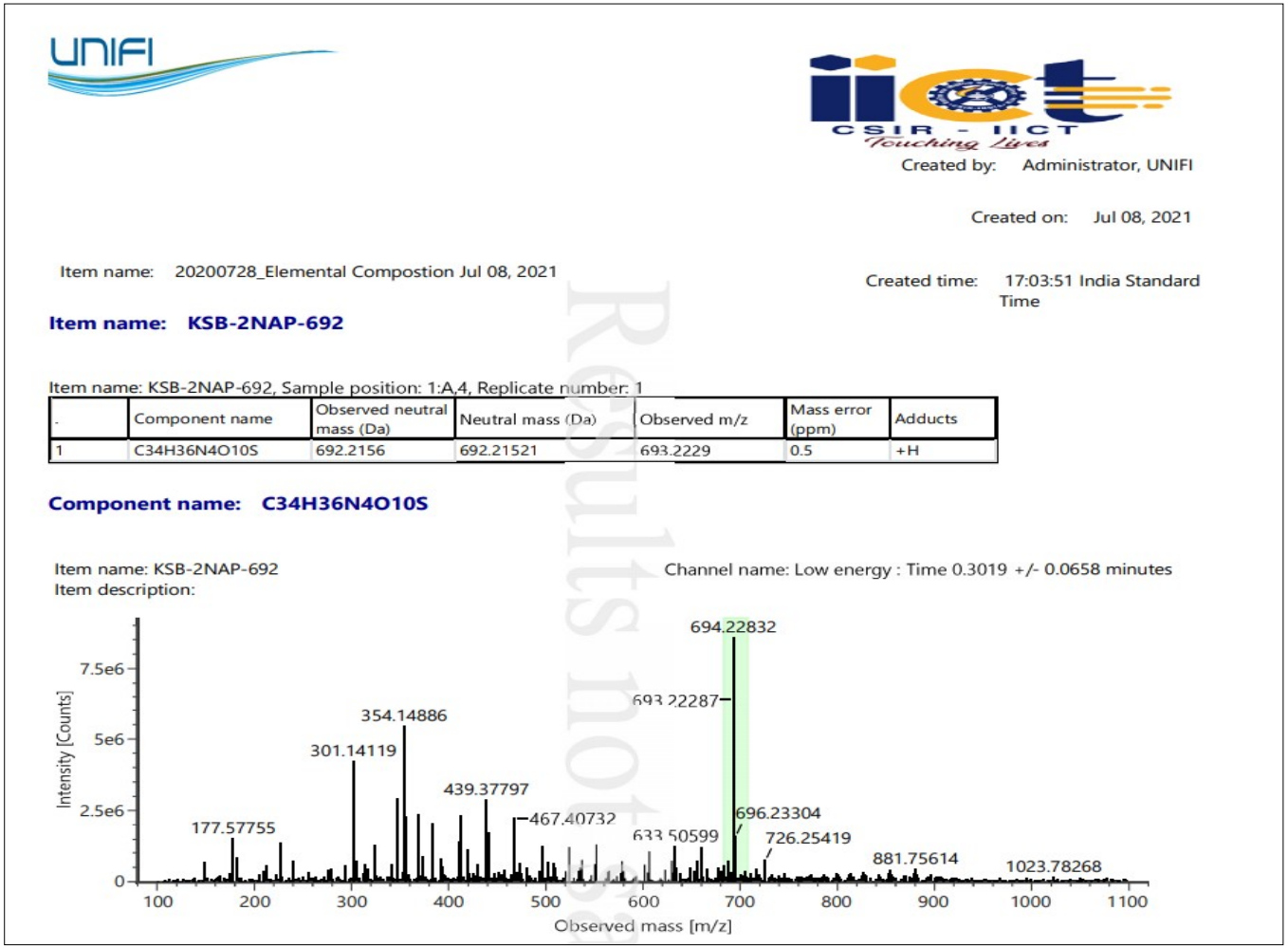


Fig S33: HRESIMS SPECTRUM OF COMPOUND **5e**

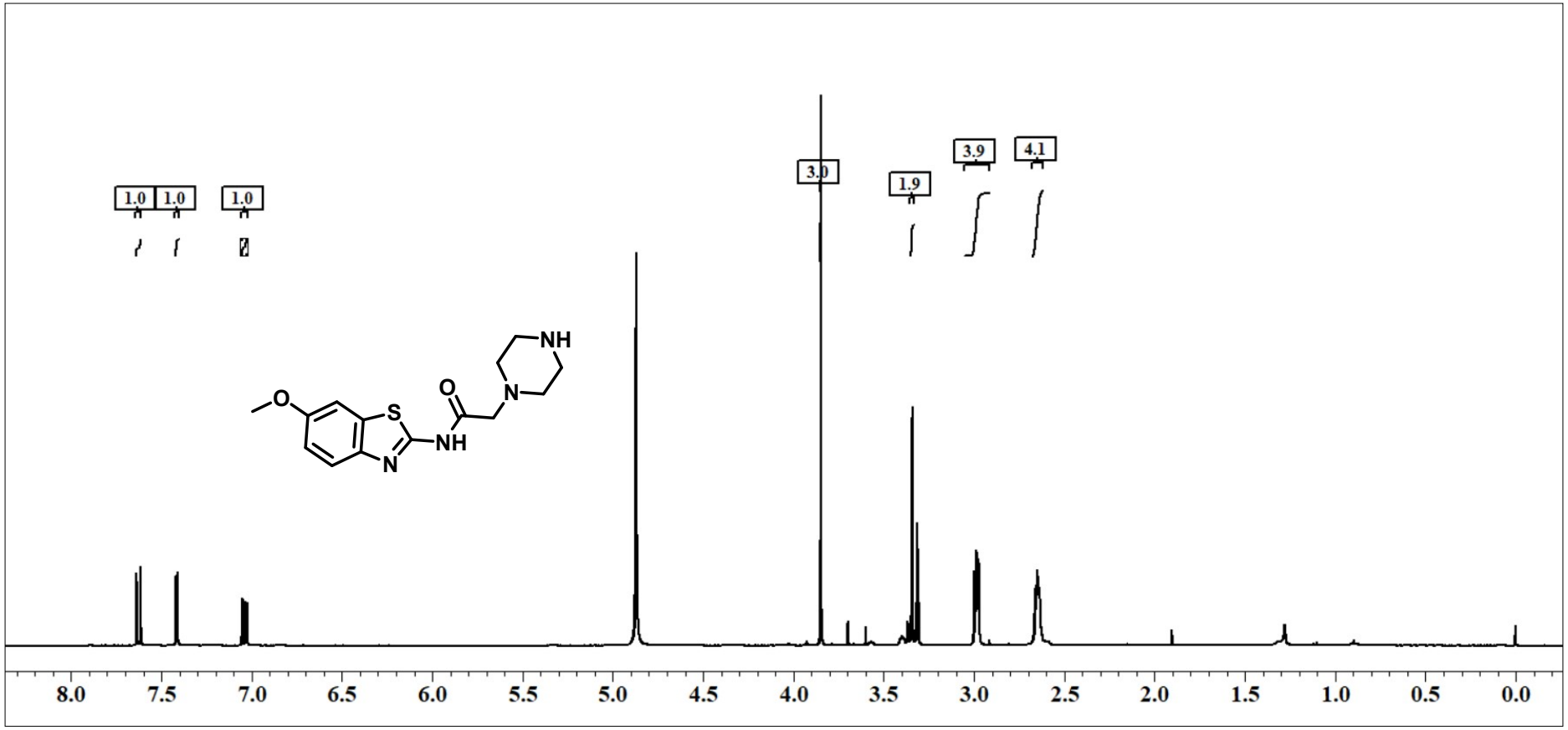


Fig S34: ^{1}H NMR SPECTRUM OF COMPOUND **9a**(400 MHz, CD_3OD)

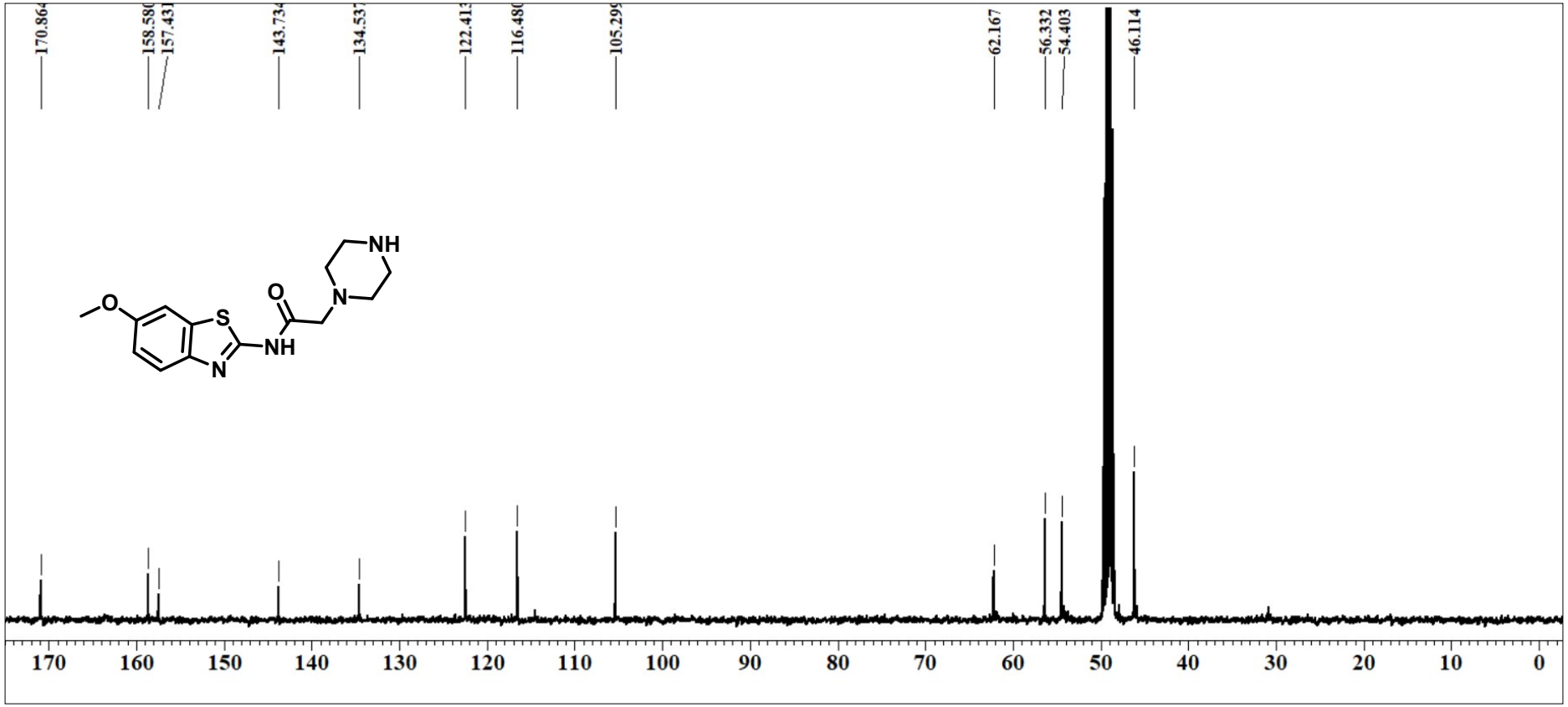


Fig S35: ^{13}C NMR SPECTRUM OF COMPOUND **9a** (100 MHz, CD_3OD)

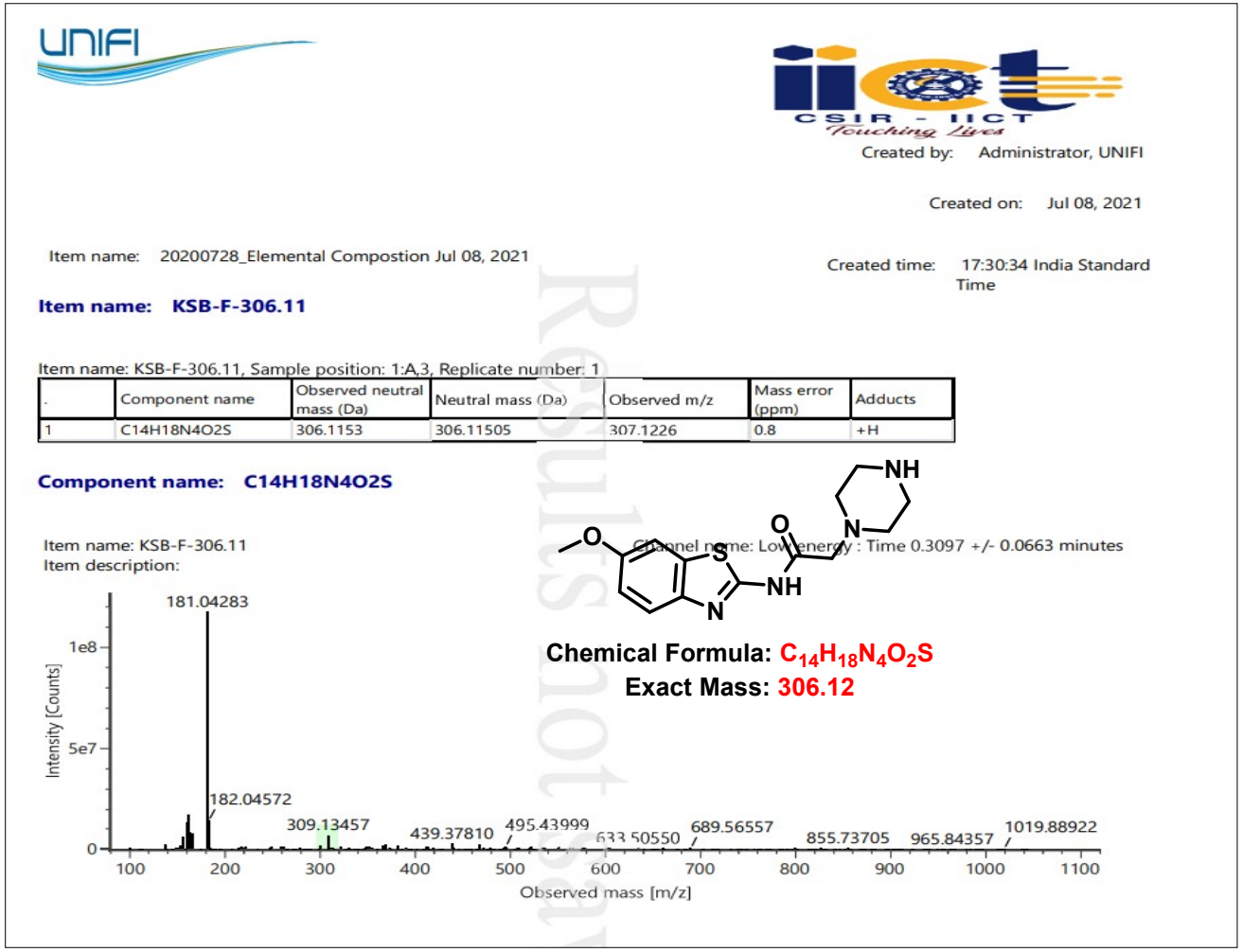


Fig S36: HRESIMS SPECTRUM OF COMPOUND **9a**

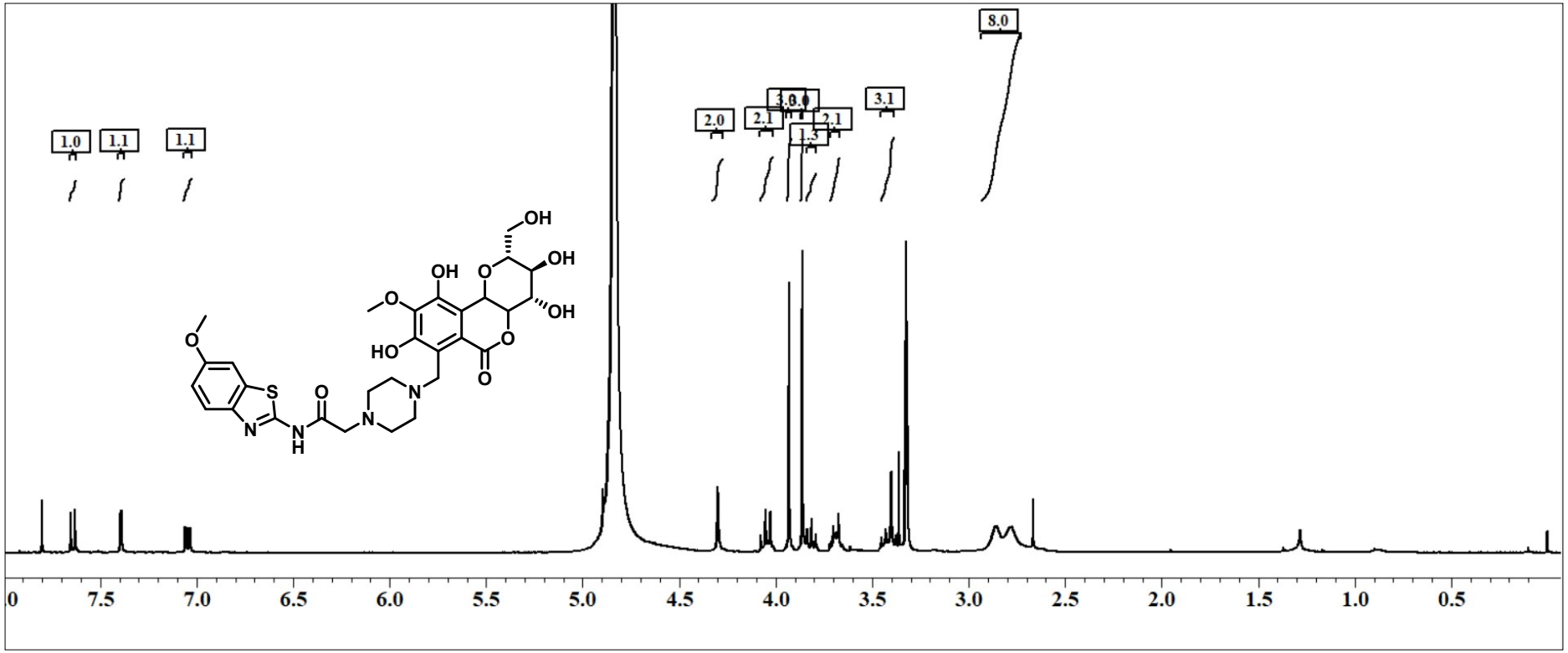


Fig S37: ^{1}H NMR SPECTRUM OF COMPOUND **10a** (400 MHz, CD_3OD+$CDCl_3$)

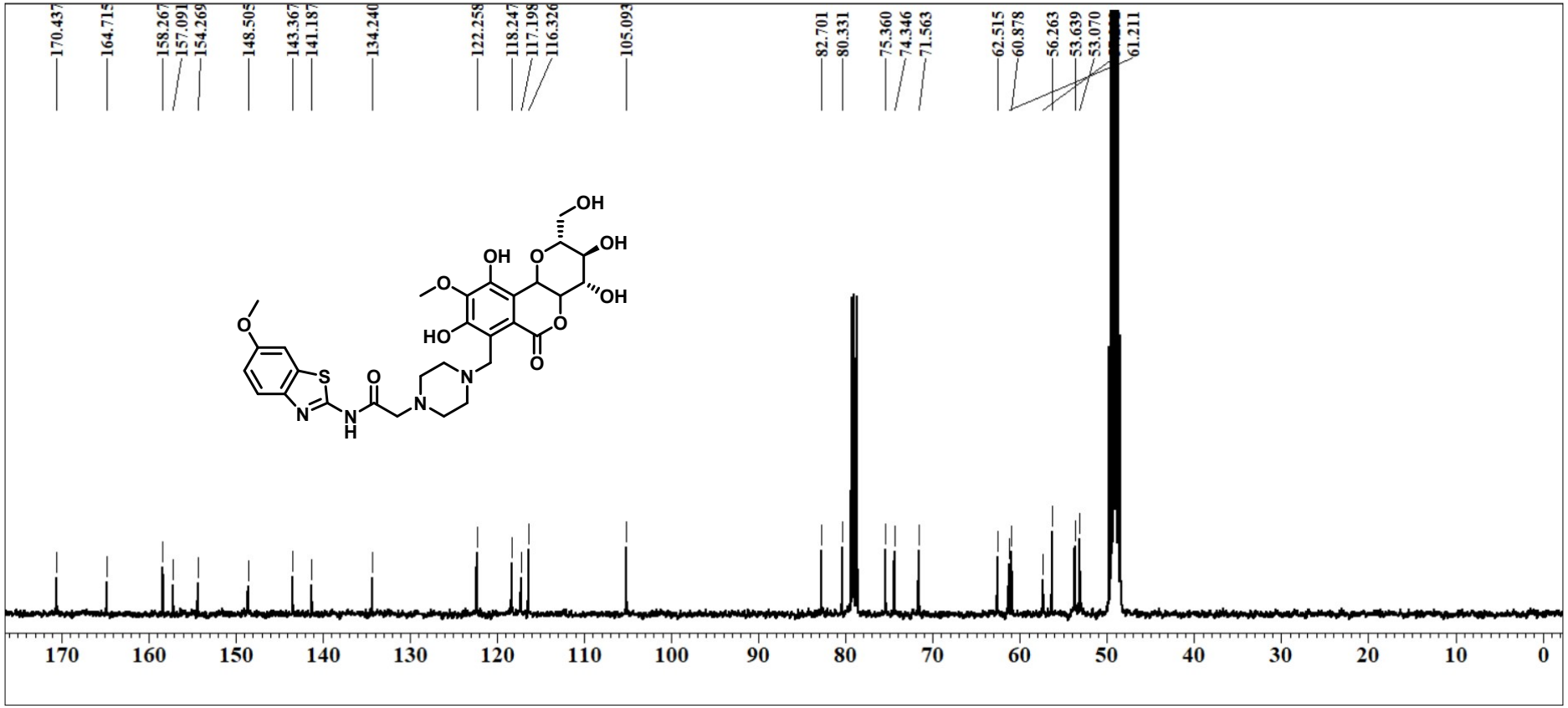


Fig S38: ^{13}C NMR SPECTRUM OF COMPOUND **10a** (100 MHz, $CD_3OD+CDCl_3$)

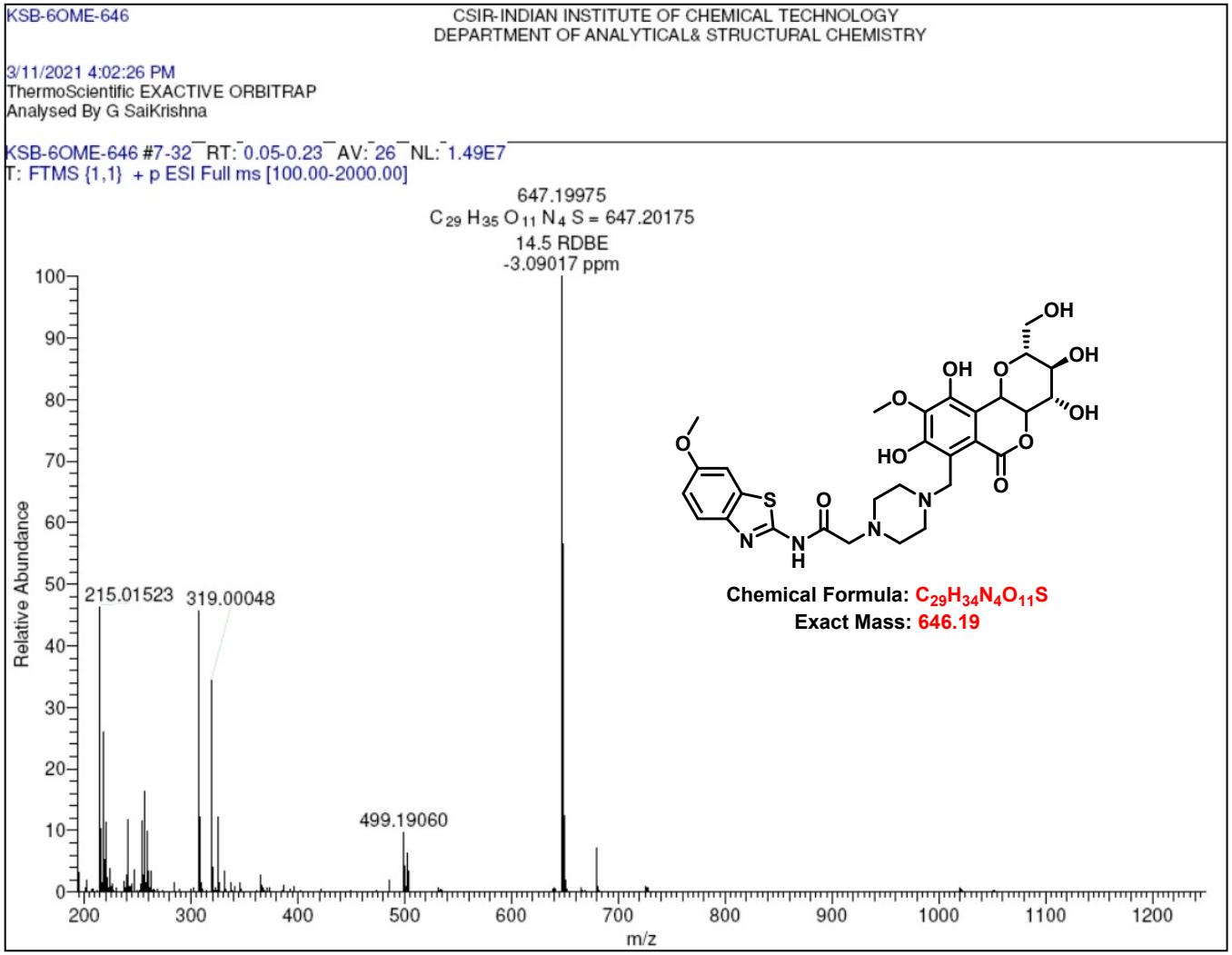


Fig S39: HRESIMS SPECTRUM OF COMPOUND **10a**

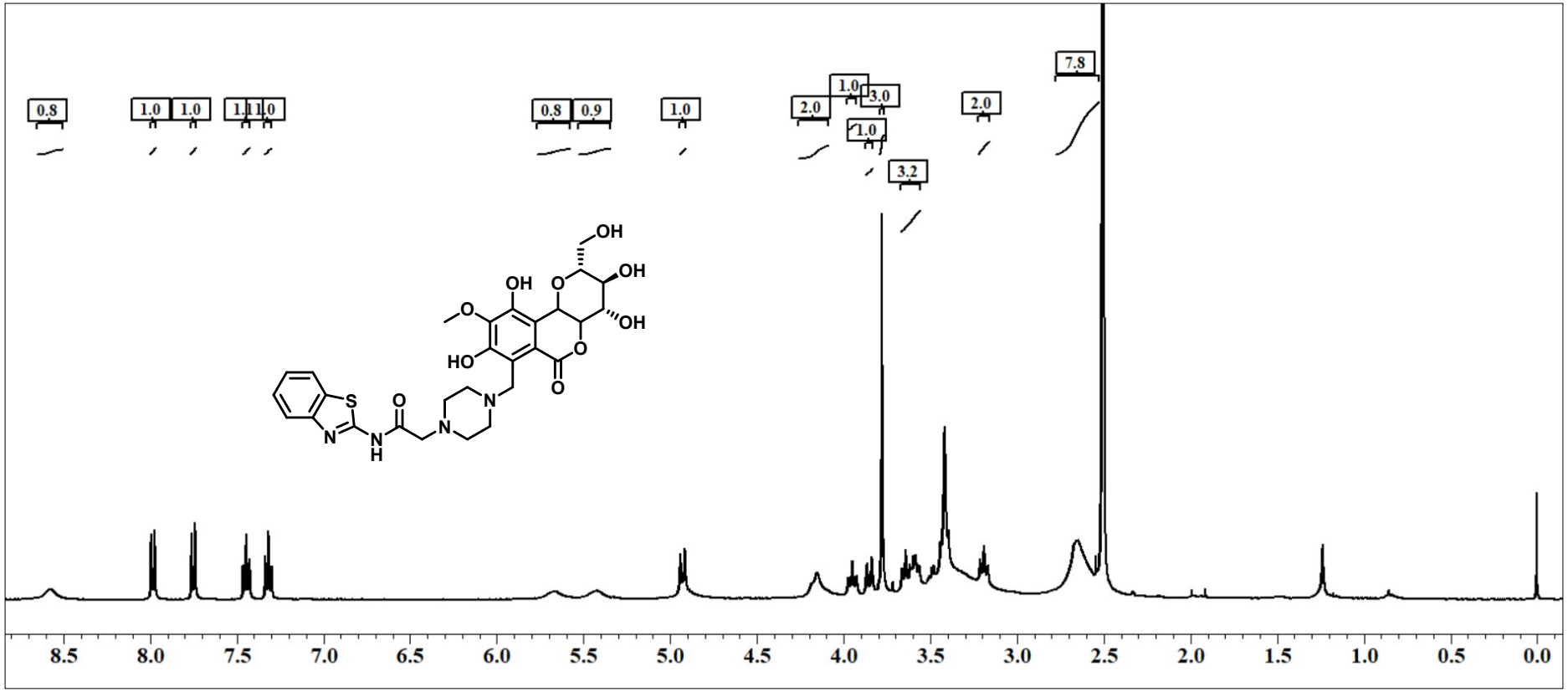


Fig S40: ^{1}H NMR SPECTRUM OF COMPOUND **10b**(400 MHz, DMSO-d_6)

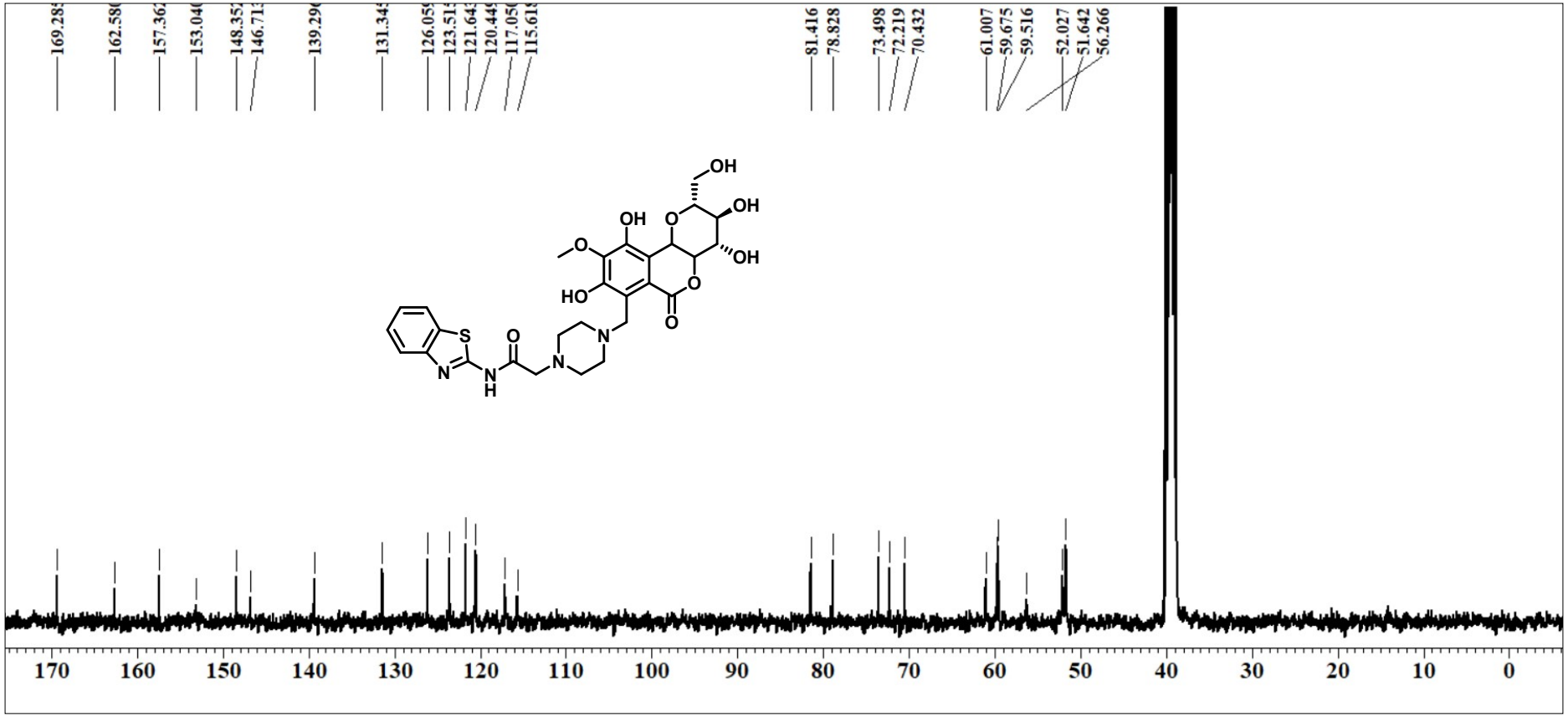


Fig S41: ^{13}C NMR SPECTRUM OF COMPOUND **10b** (100 MHz, DMSO-d_6)

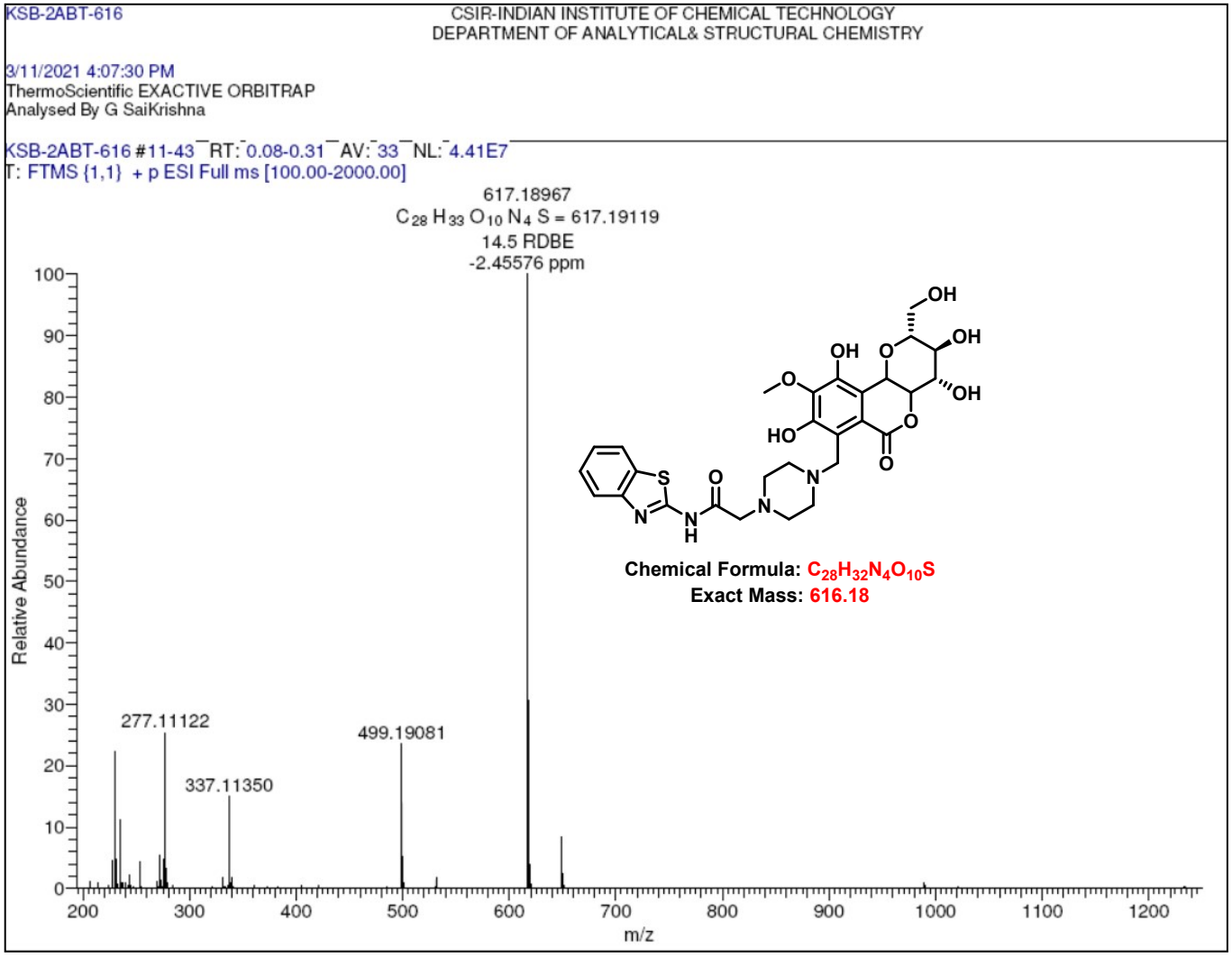


Fig S42: HRESIMS SPECTRUM OF COMPOUND **10b**

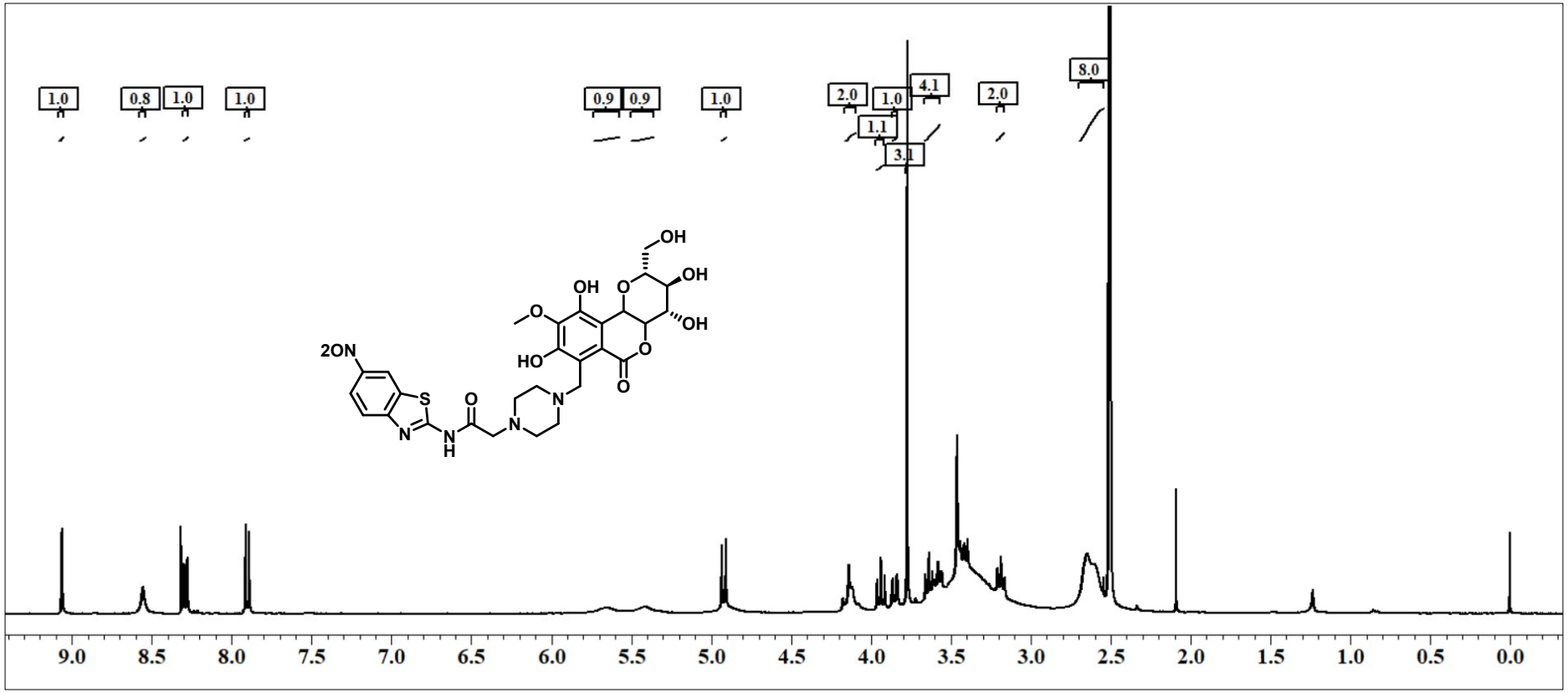


Fig S43: ^{1}H NMR SPECTRUM OF COMPOUND **10c**(400 MHz, DMSO-d_6)

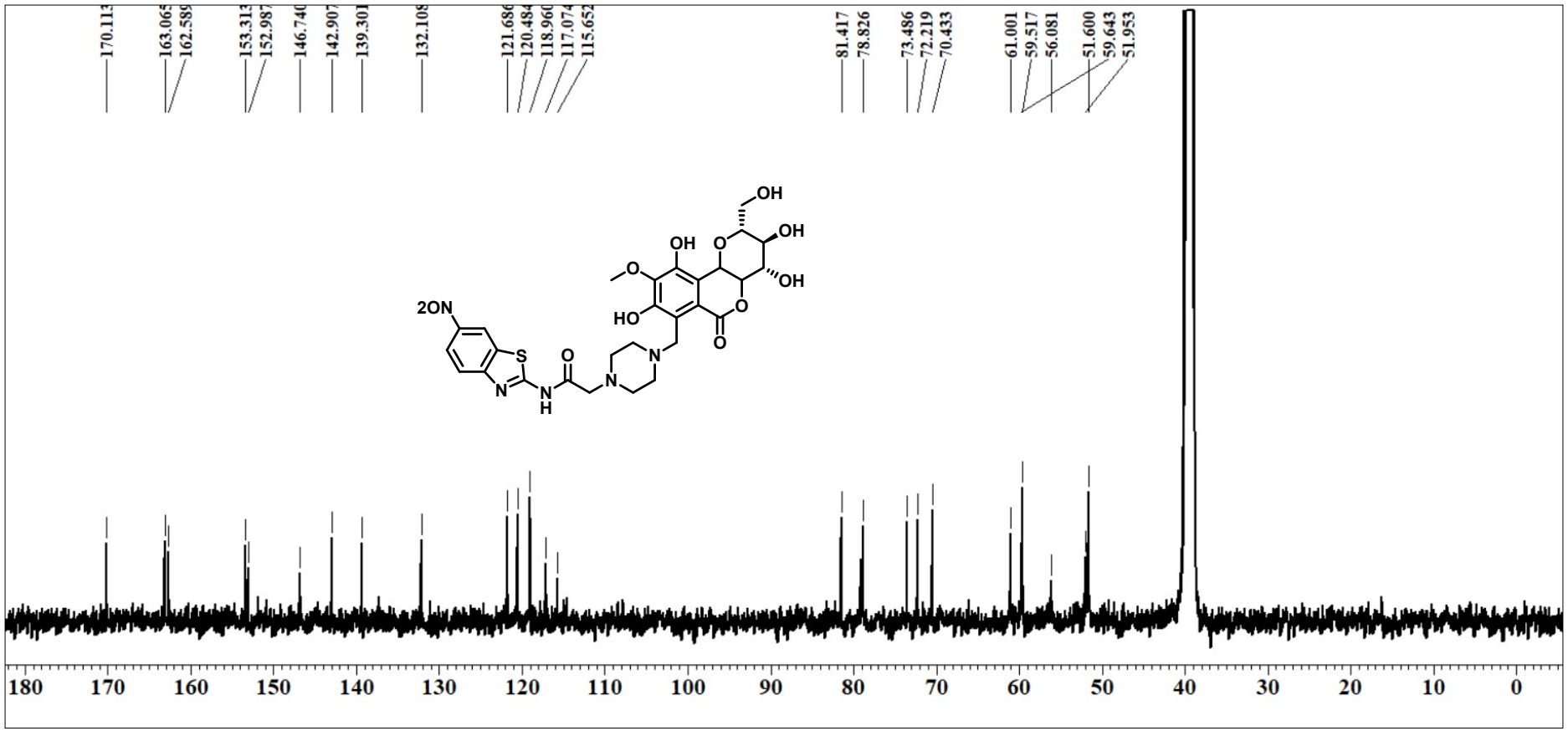


Fig S44: ^{13}C NMR SPECTRUM OF COMPOUND **10c** (100 MHz, DMSO-d_6)

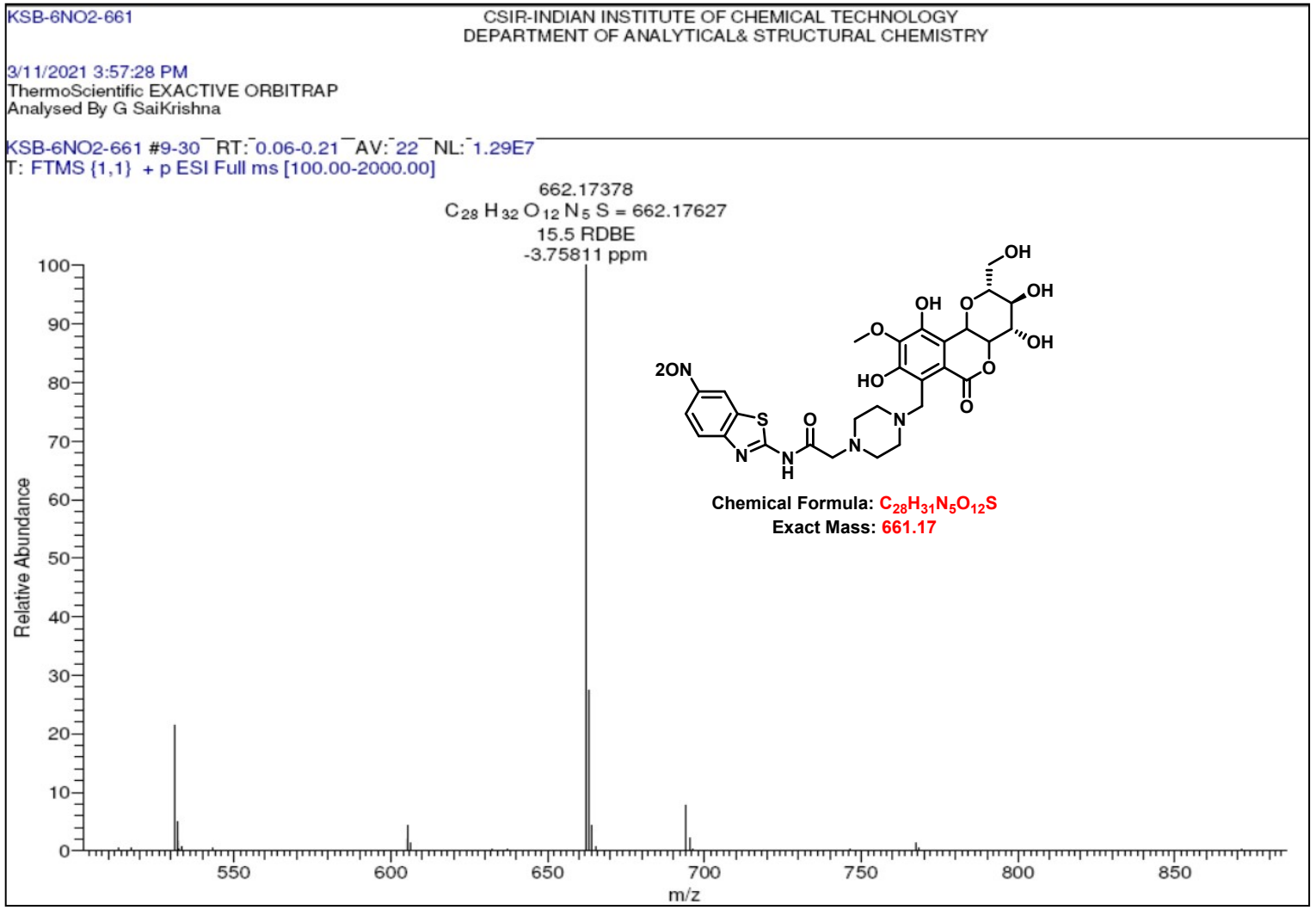


Fig S45: HRESIMS SPECTRUM OF COMPOUND **10c**

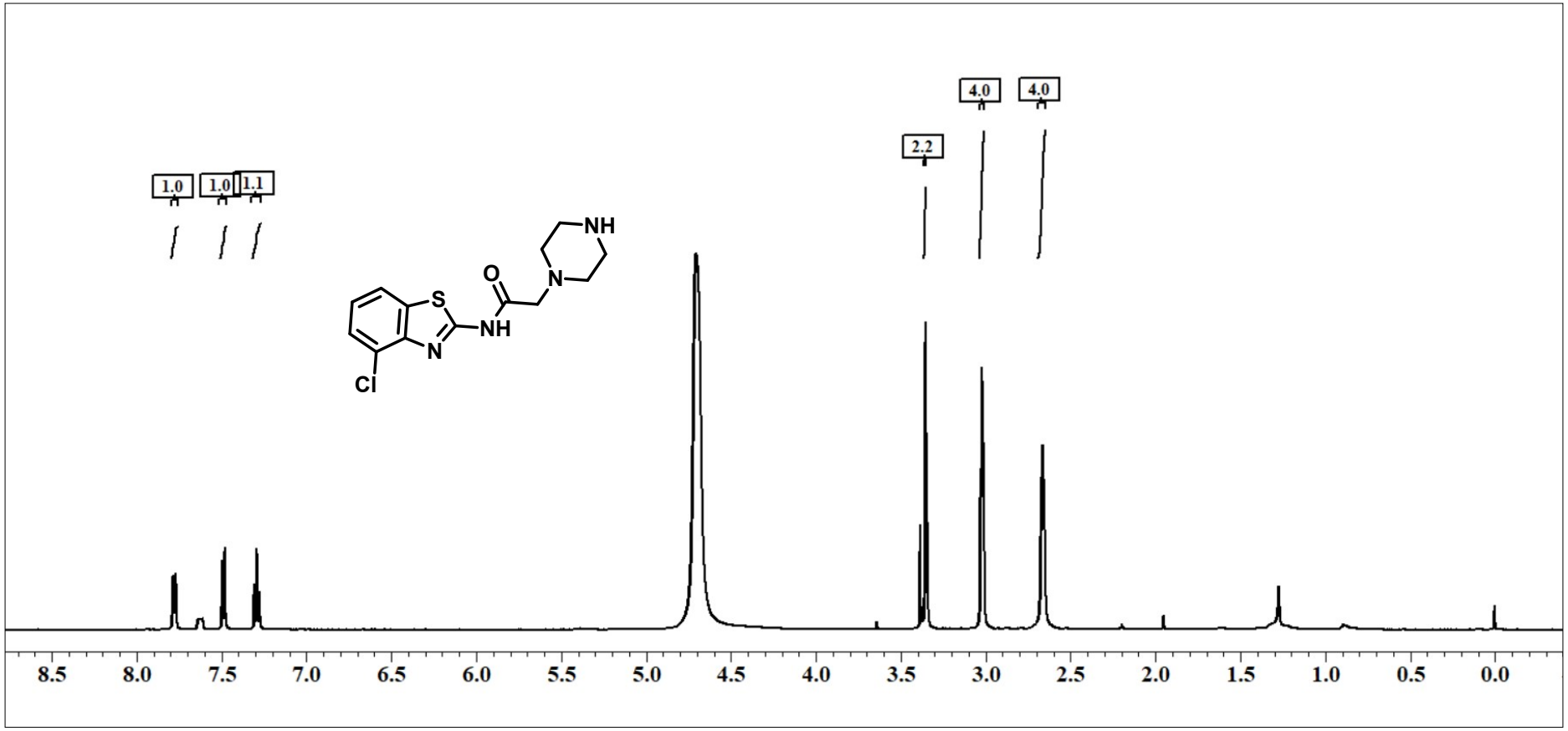


Fig S46: ^{1}H NMR SPECTRUM OF COMPOUND **9d**(500 MHz, CD_3OD+$CDCl_3$)

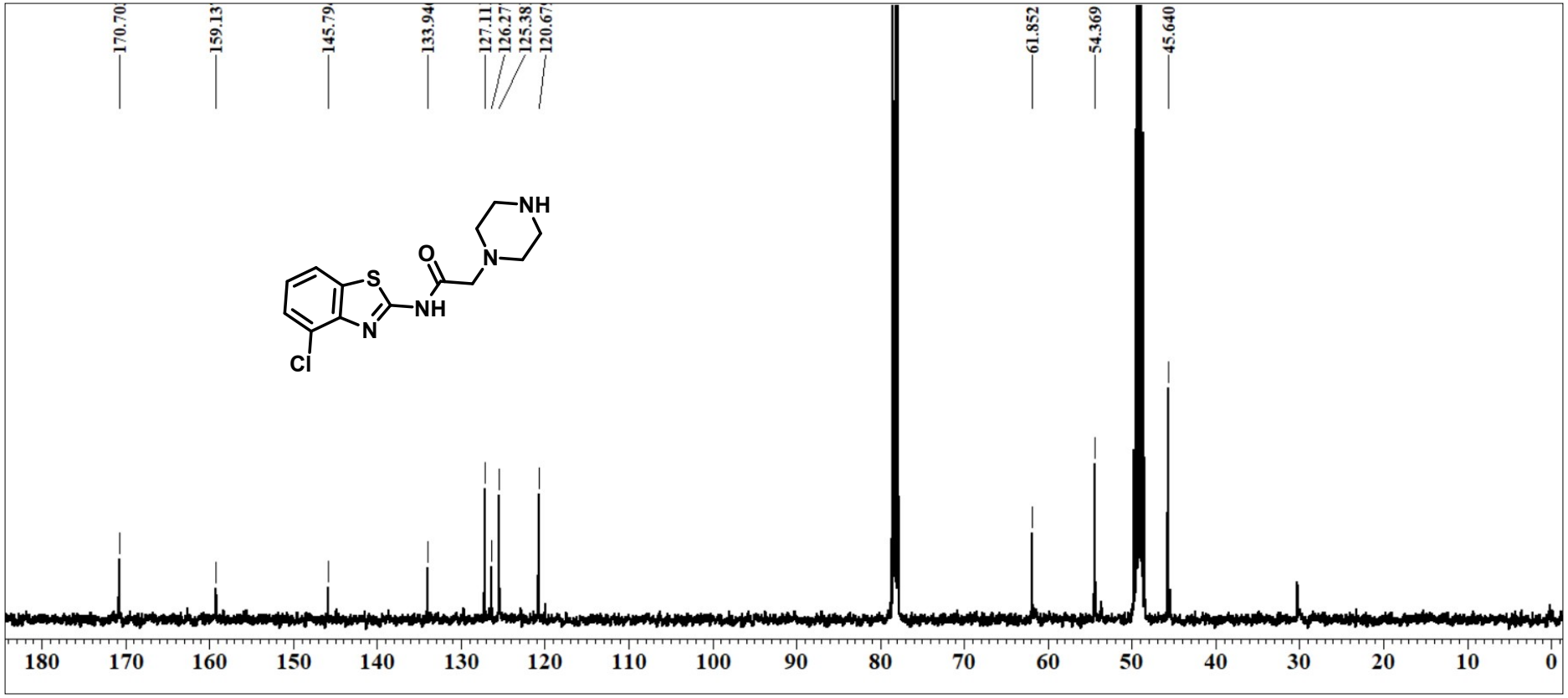


Fig S47: ^{13}C NMR SPECTRUM OF COMPOUND **9d** (100 MHz, $CDCl_3$+$CDCl_3$)

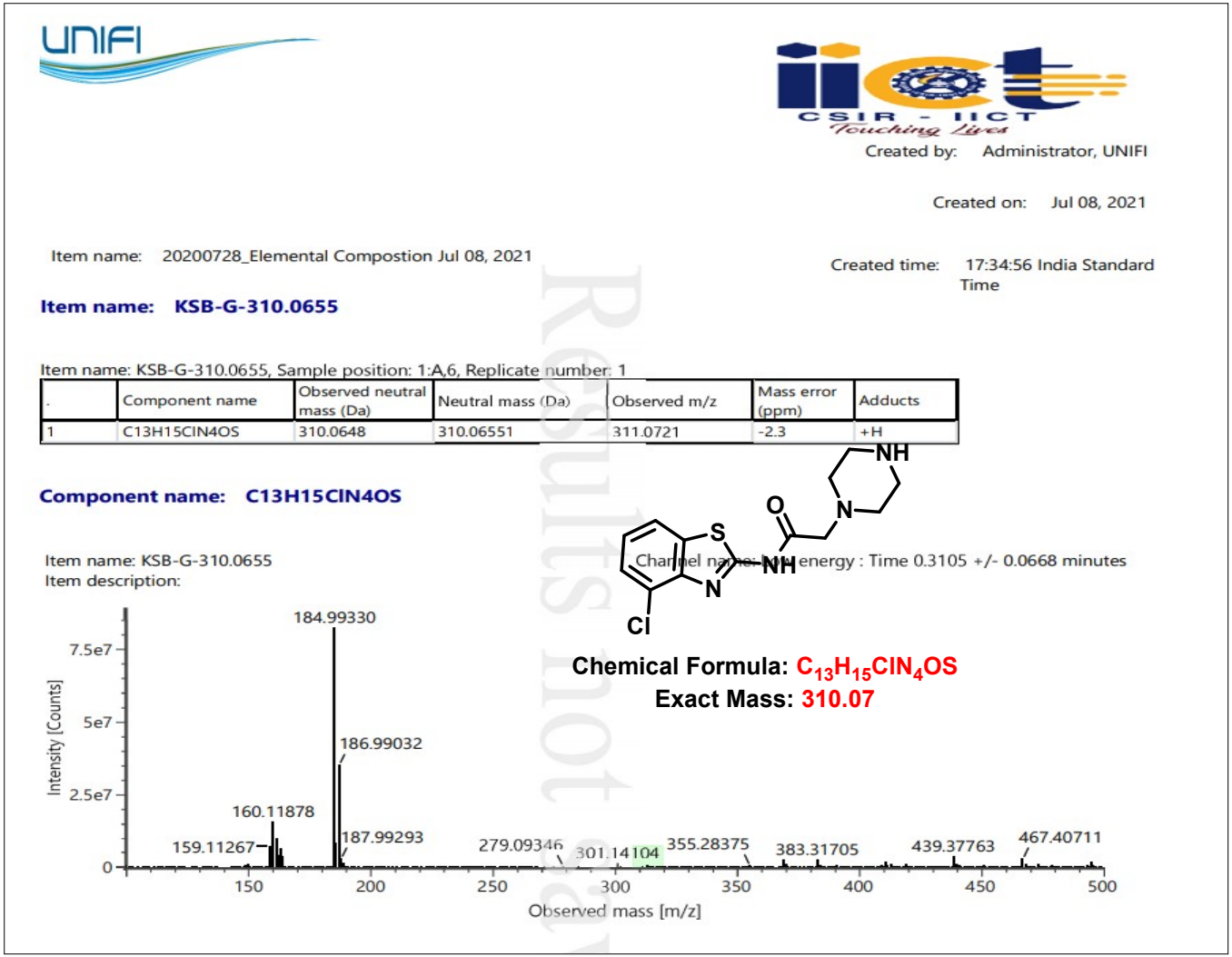


Fig S48: HRESIMS SPECTRUM OF COMPOUND **9d**

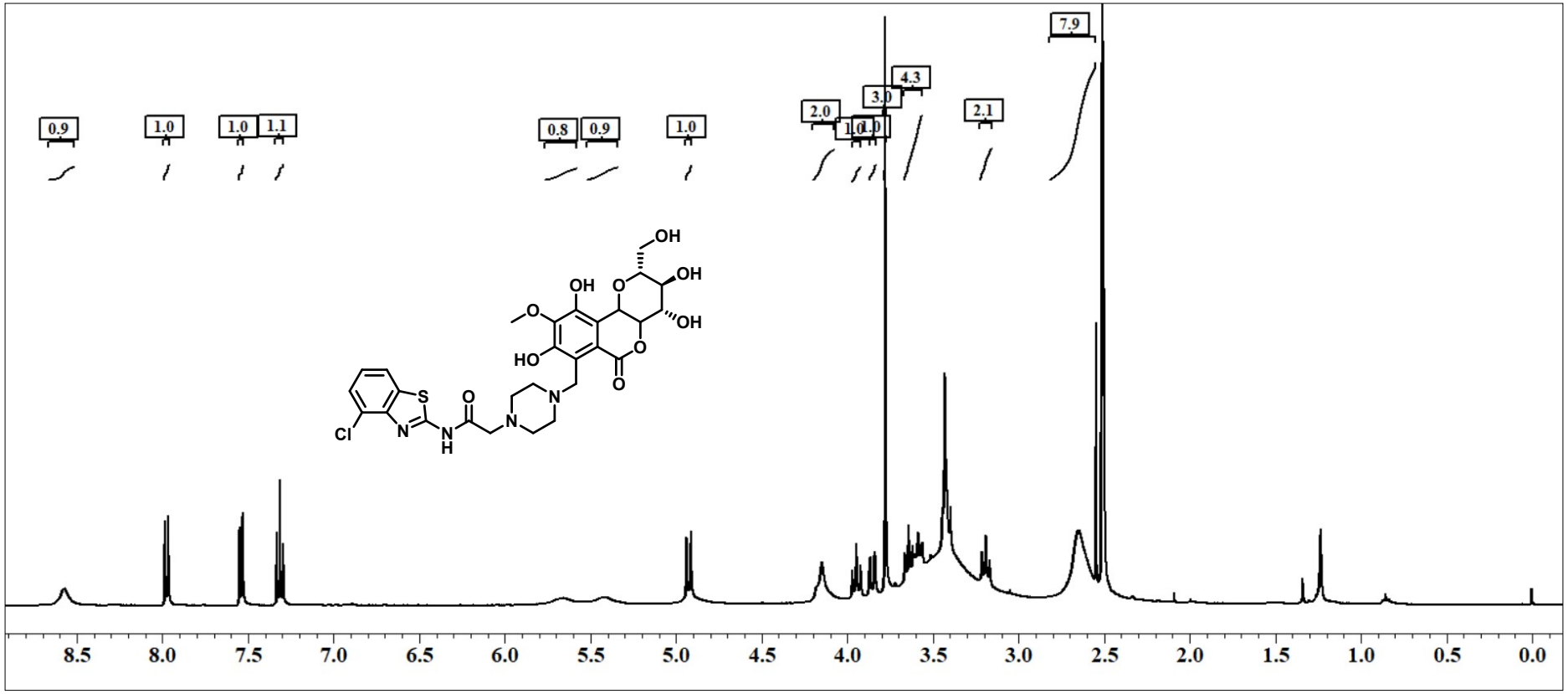


Fig S49: ^{1}H NMR SPECTRUM OF COMPOUND **10d**(400 MHz, DMSO-d_6)

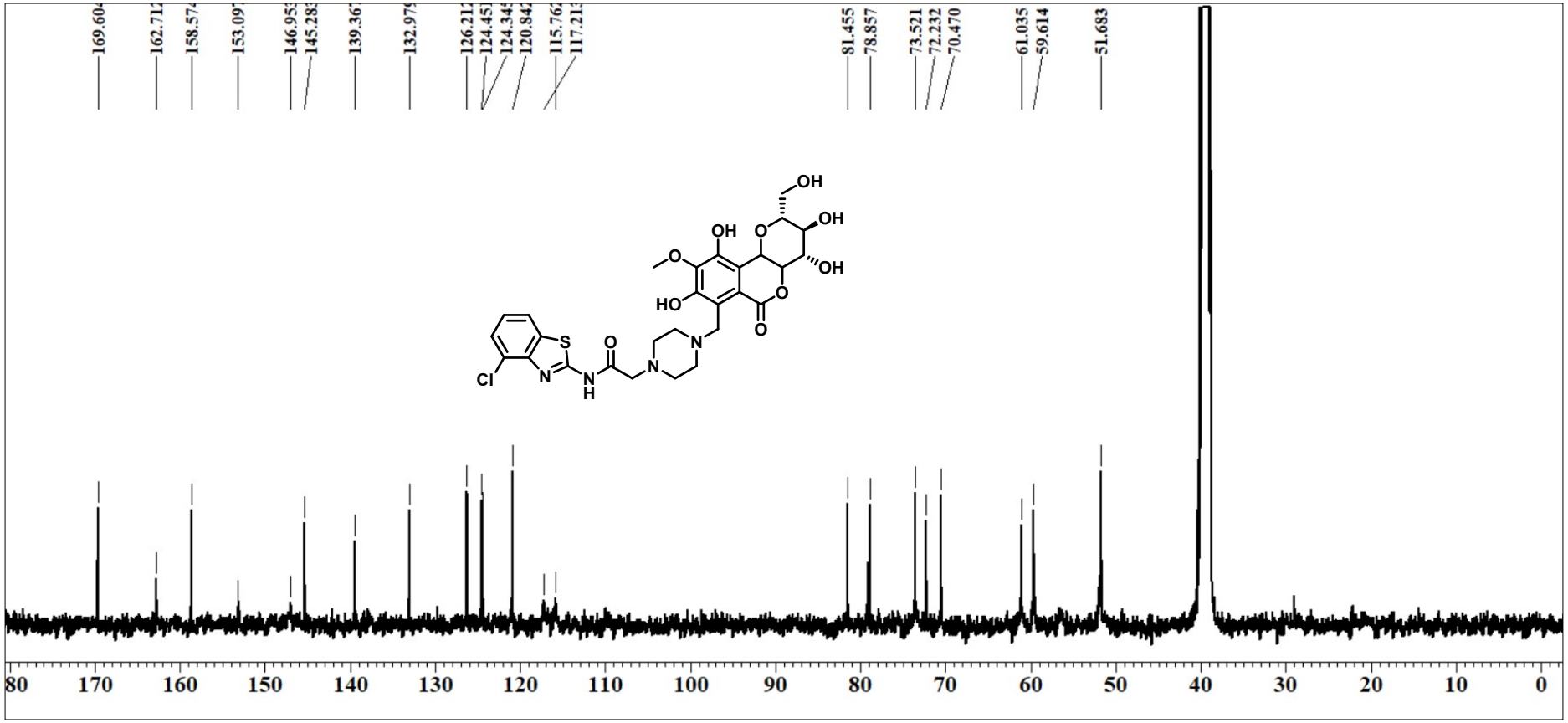


Fig S50: ^{13}C NMR SPECTRUM OF COMPOUND **10d** (100 MHz, DMSO-d_6)

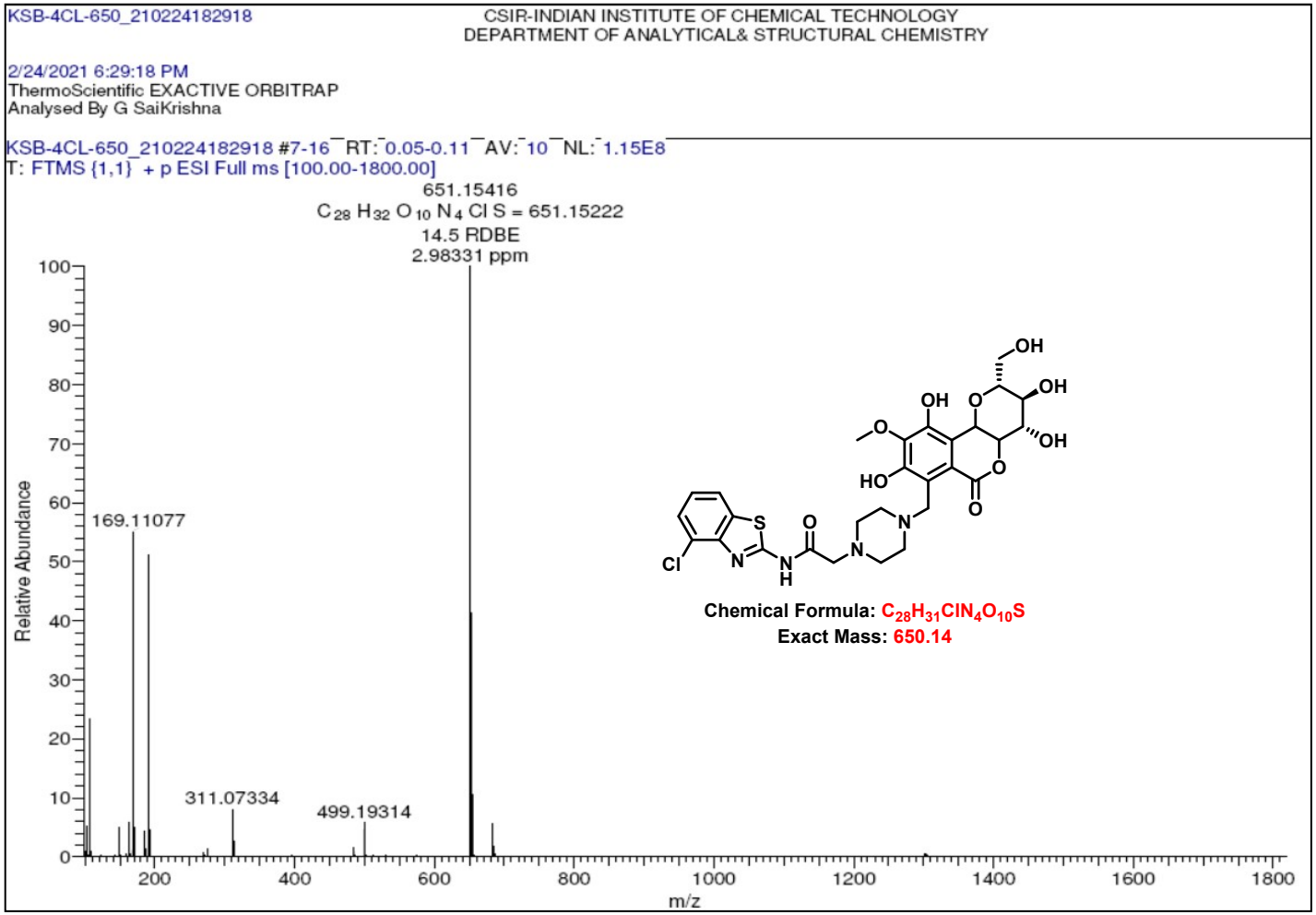


Fig S51: HRESIMS SPECTRUM OF COMPOUND **10d**

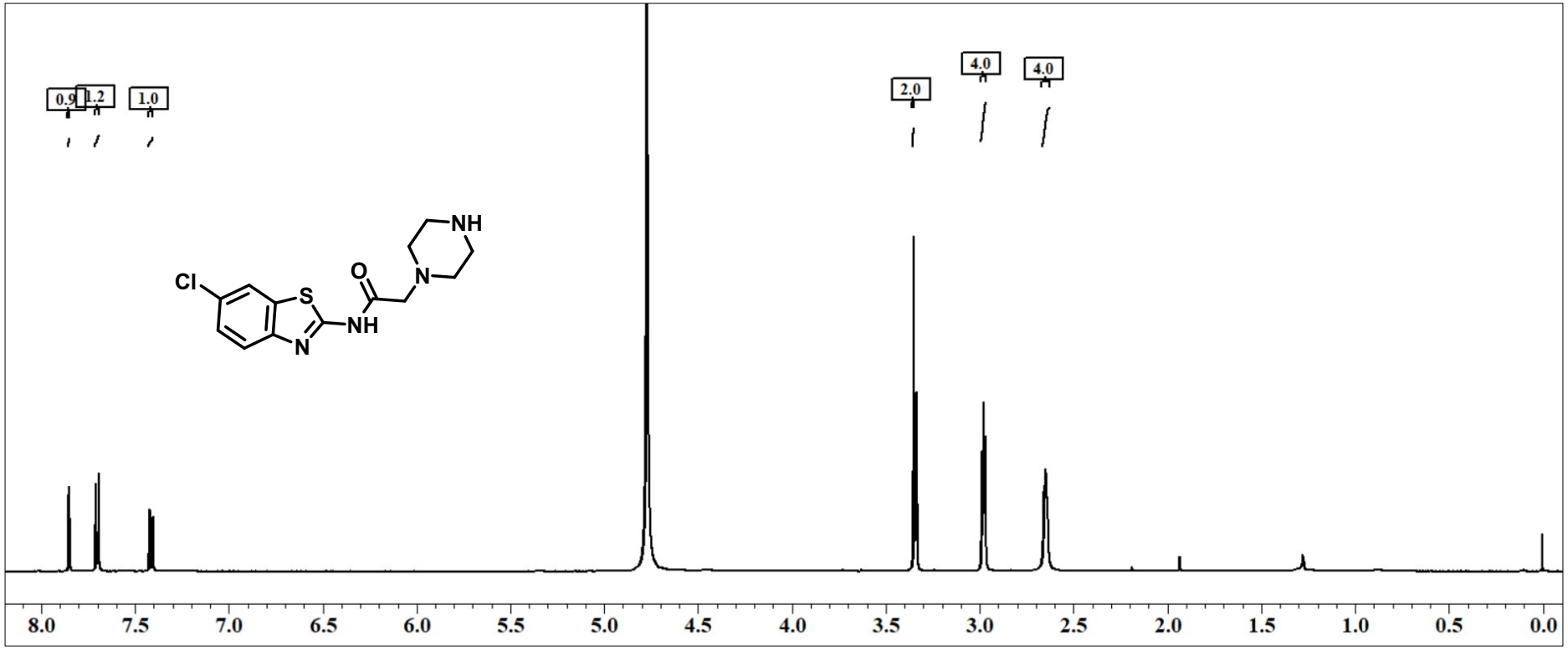


Fig S52: ^{1}H NMR SPECTRUM OF COMPOUND **9e**(500 MHz, CD_3OD)

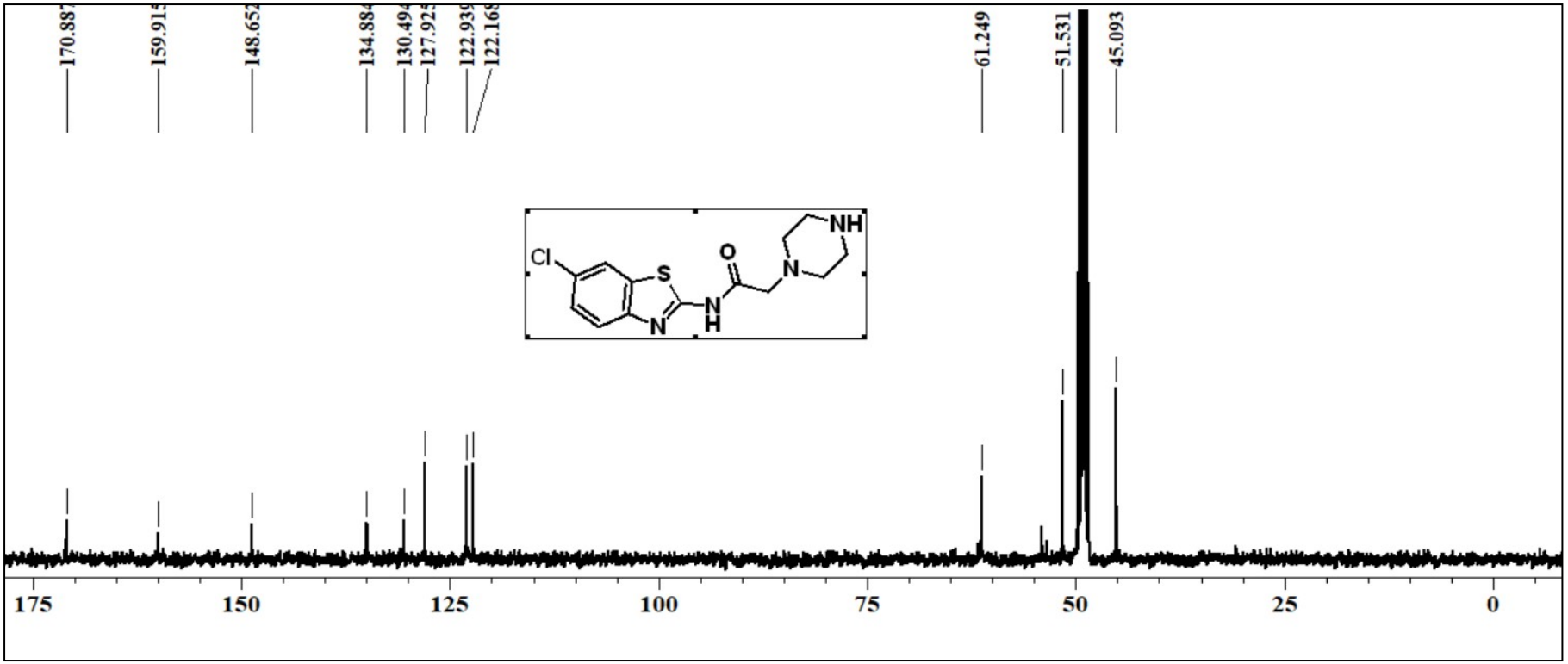


Fig S53: ^{13}C NMR SPECTRUM OF COMPOUND **9e** (100 MHz, CD_3OD)

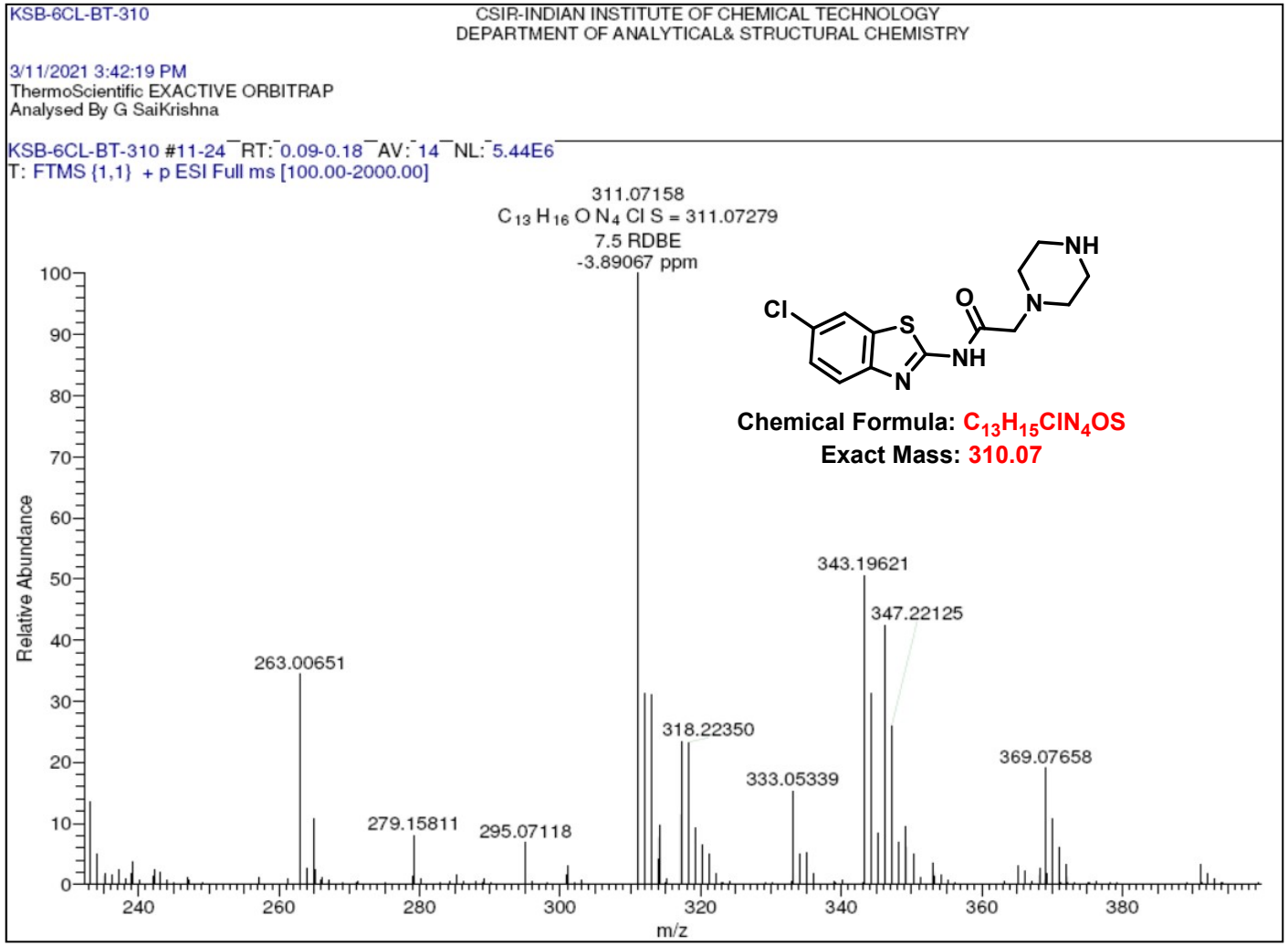


Fig S54: HRESIMS SPECTRUM OF COMPOUND **9e**

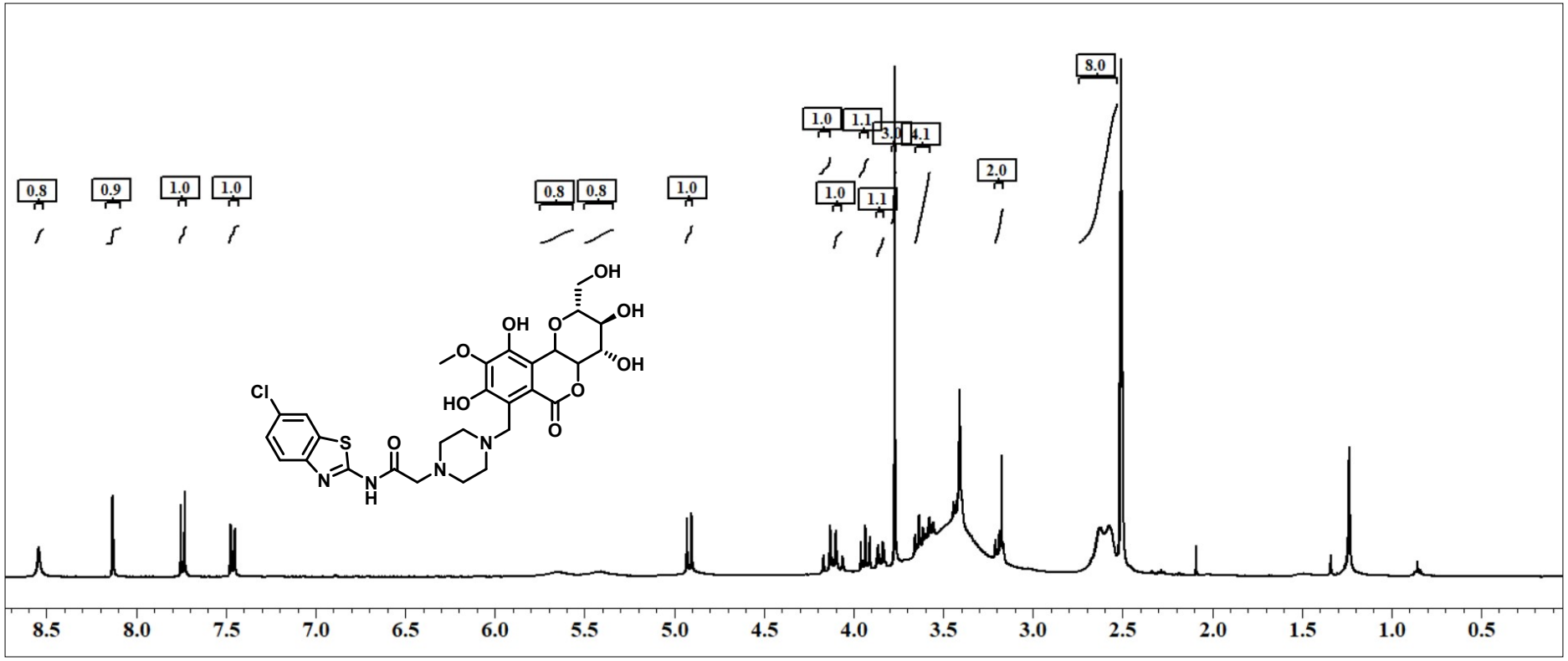


Fig S55: ^{1}H NMR SPECTRUM OF COMPOUND **10e**(300 MHz, DMSO-d_6)

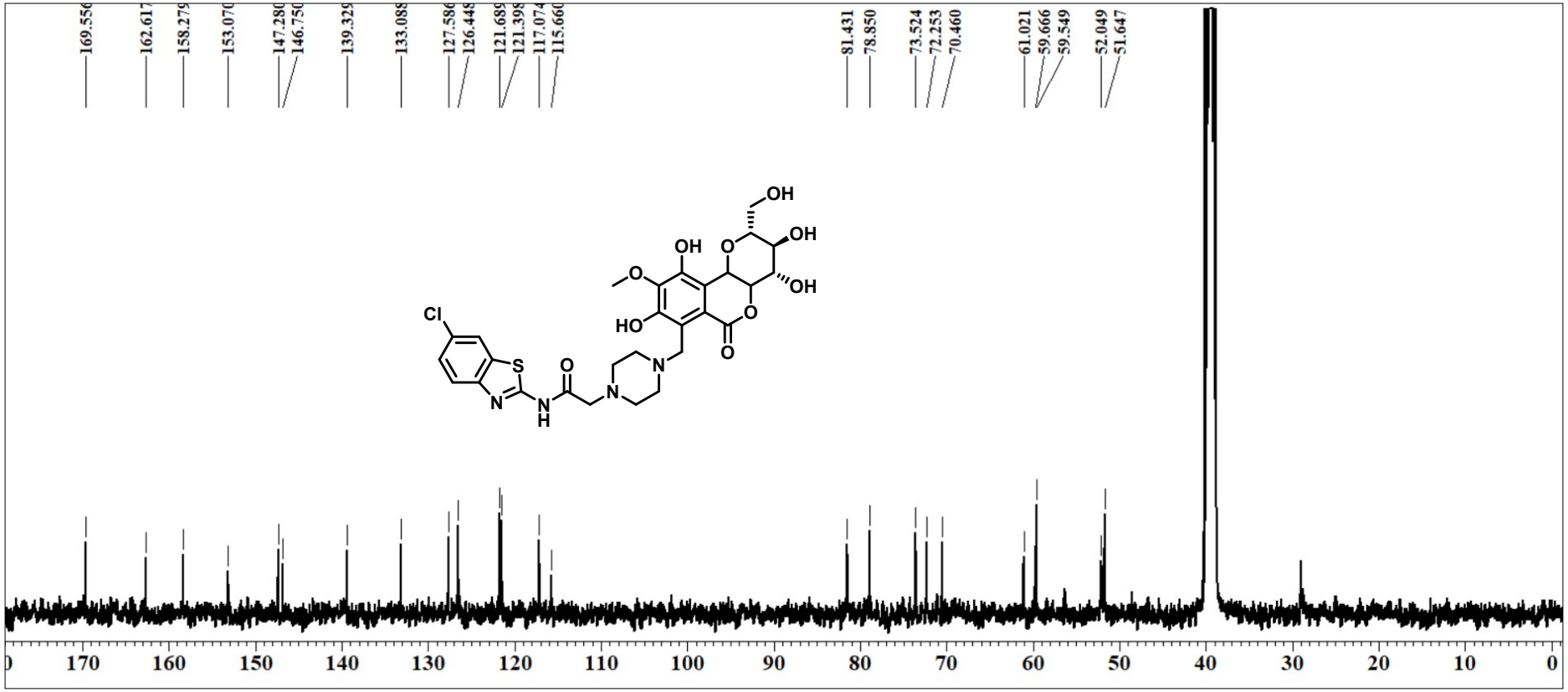


Fig S56: ^{13}C NMR SPECTRUM OF COMPOUND **10e** (100 MHz, DMSO-d_6)

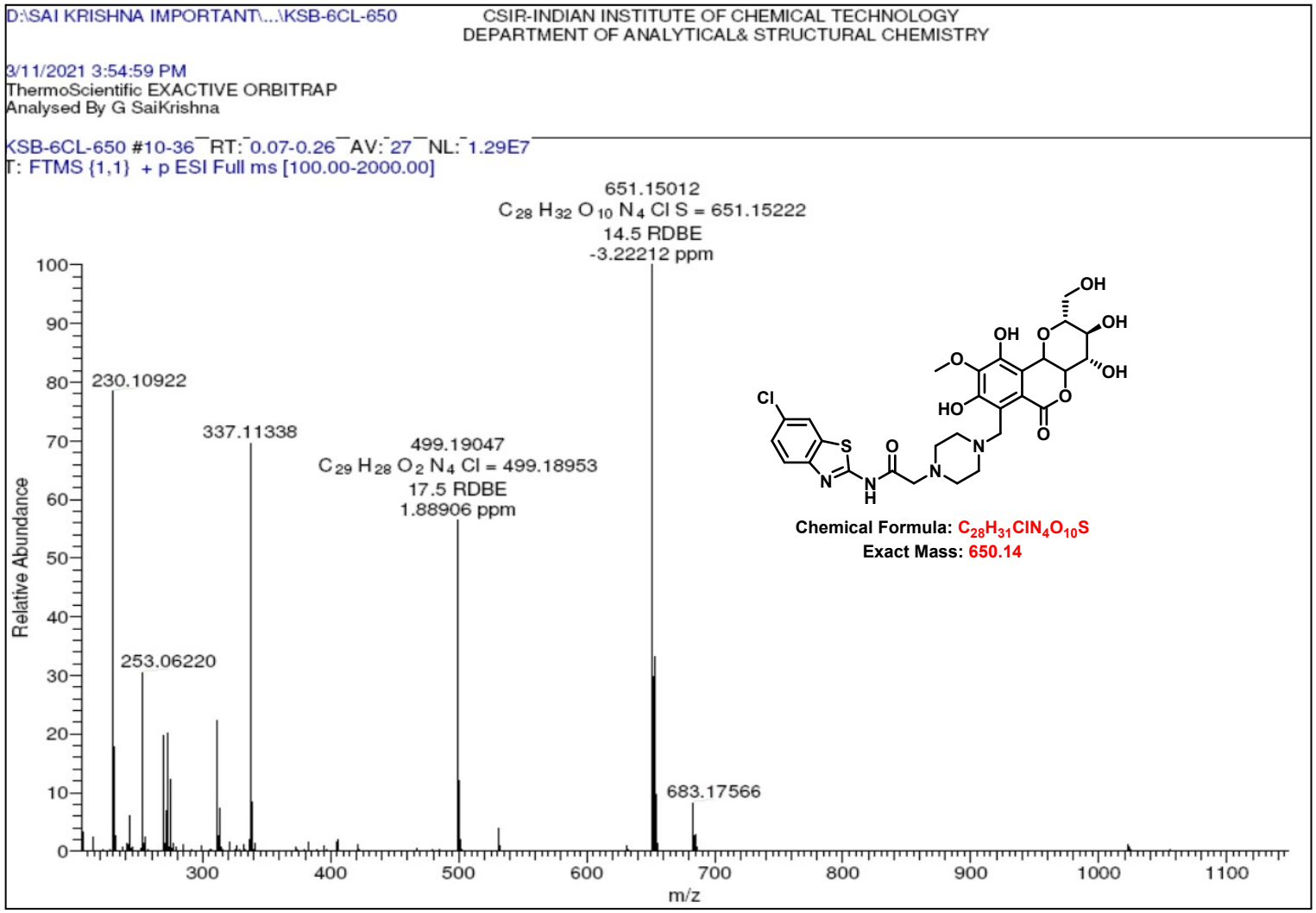
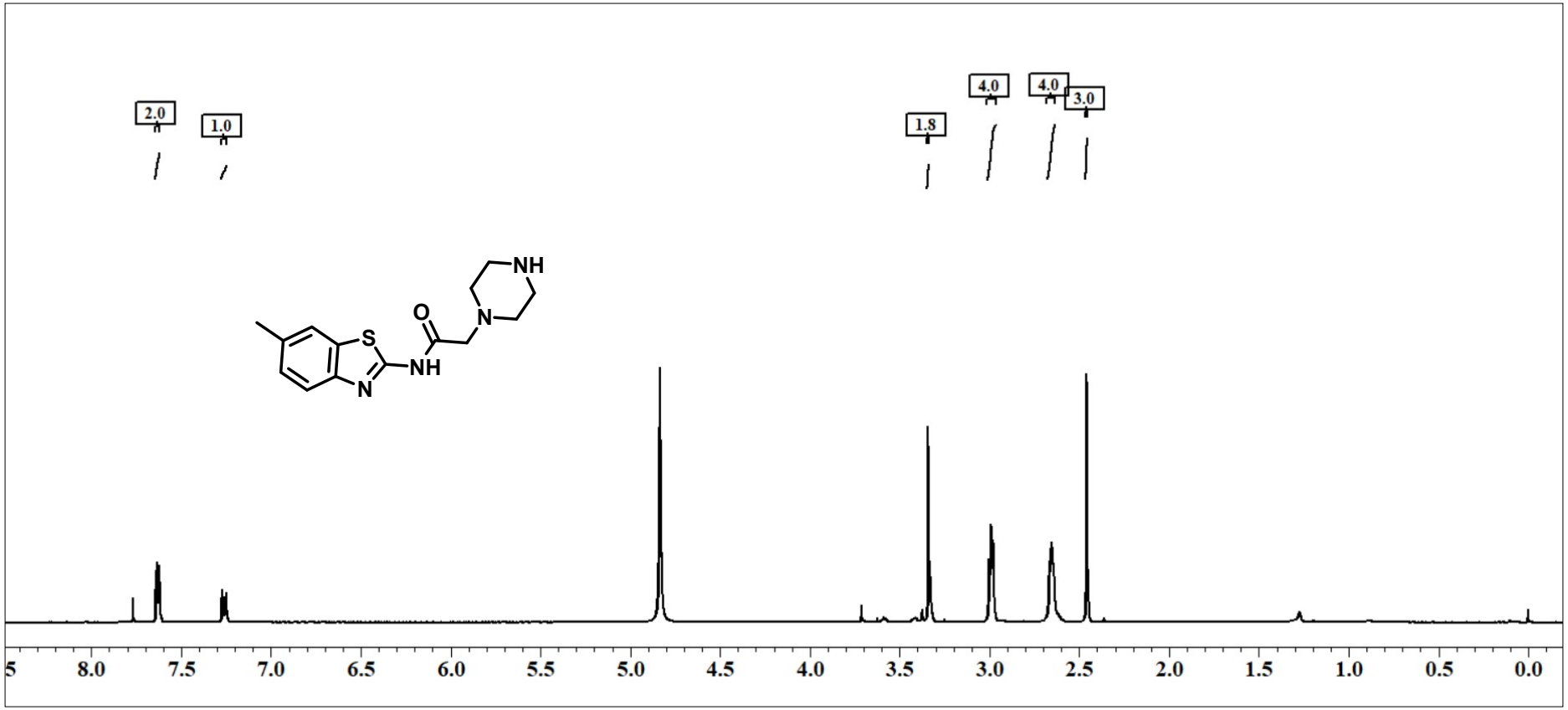


Fig S57: HRESIMS SPECTRUM OF COMPOUND **10e**



6MEFig S58: ^{1}H NMR SPECTRUM OF COMPOUND **9f**(400 MHz, CD_3OD+$CDCl_3$)

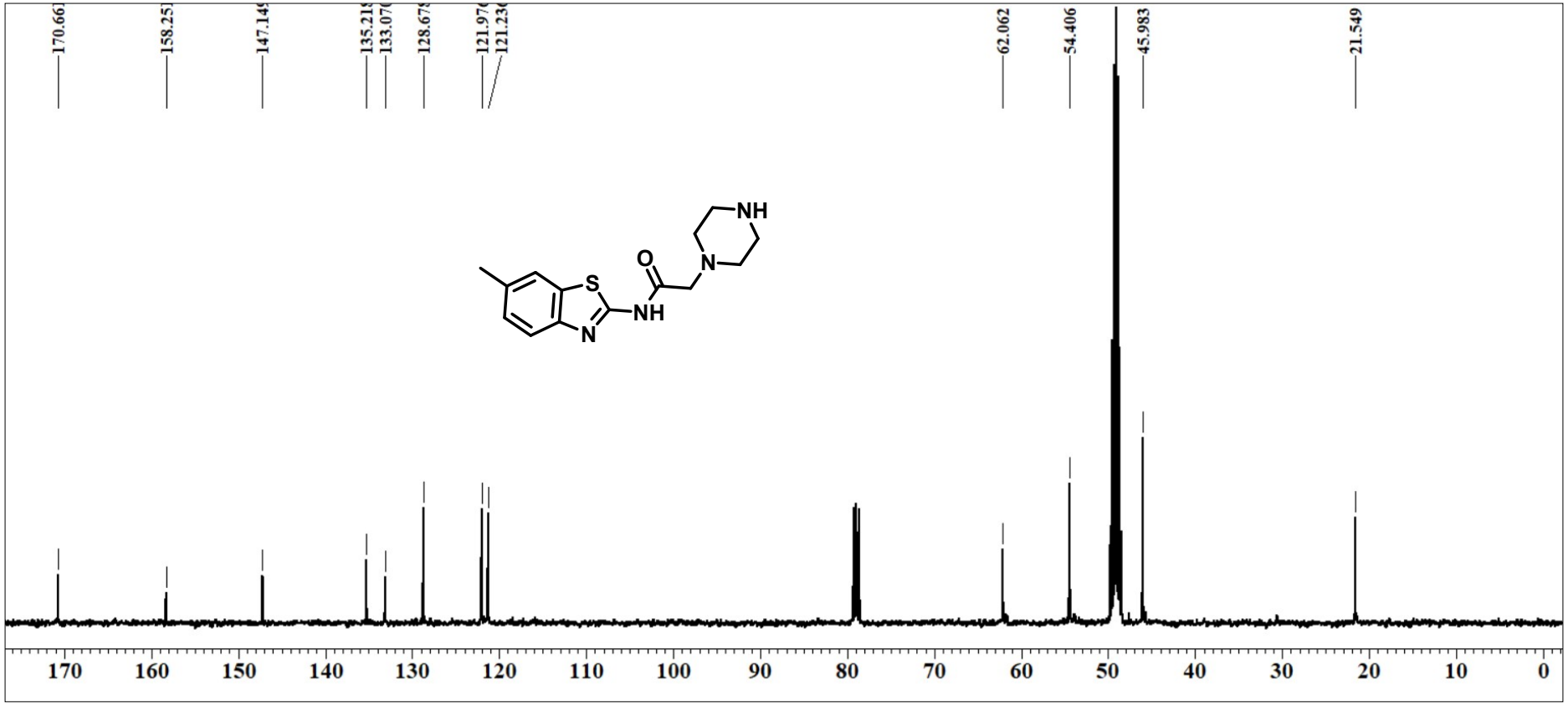


Fig S59: ^{13}C NMR SPECTRUM OF COMPOUND **9f** (100 MHz, $CD_3OD+CDCl_3$)

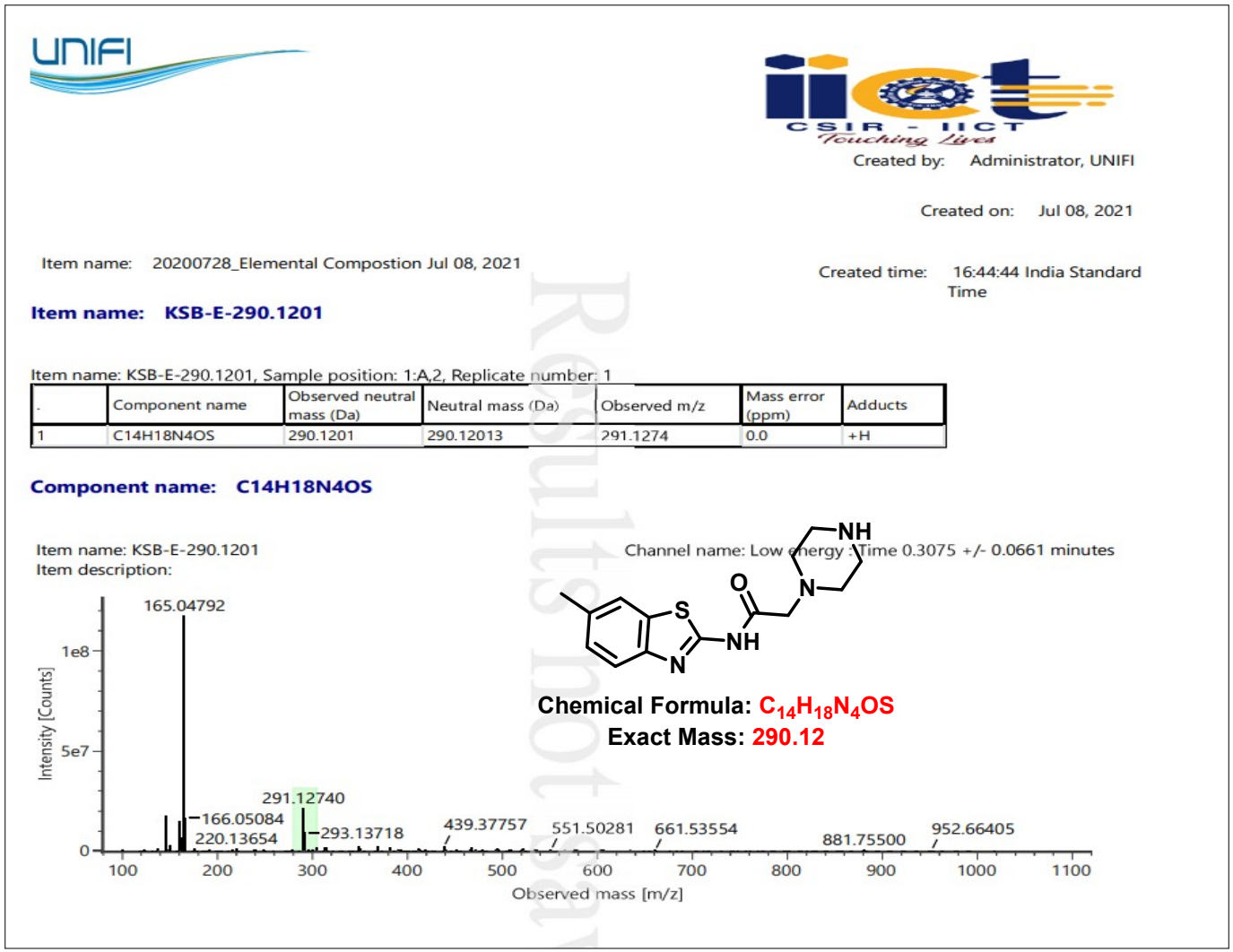


Fig S60: HRESIMS SPECTRUM OF COMPOUND **9f**

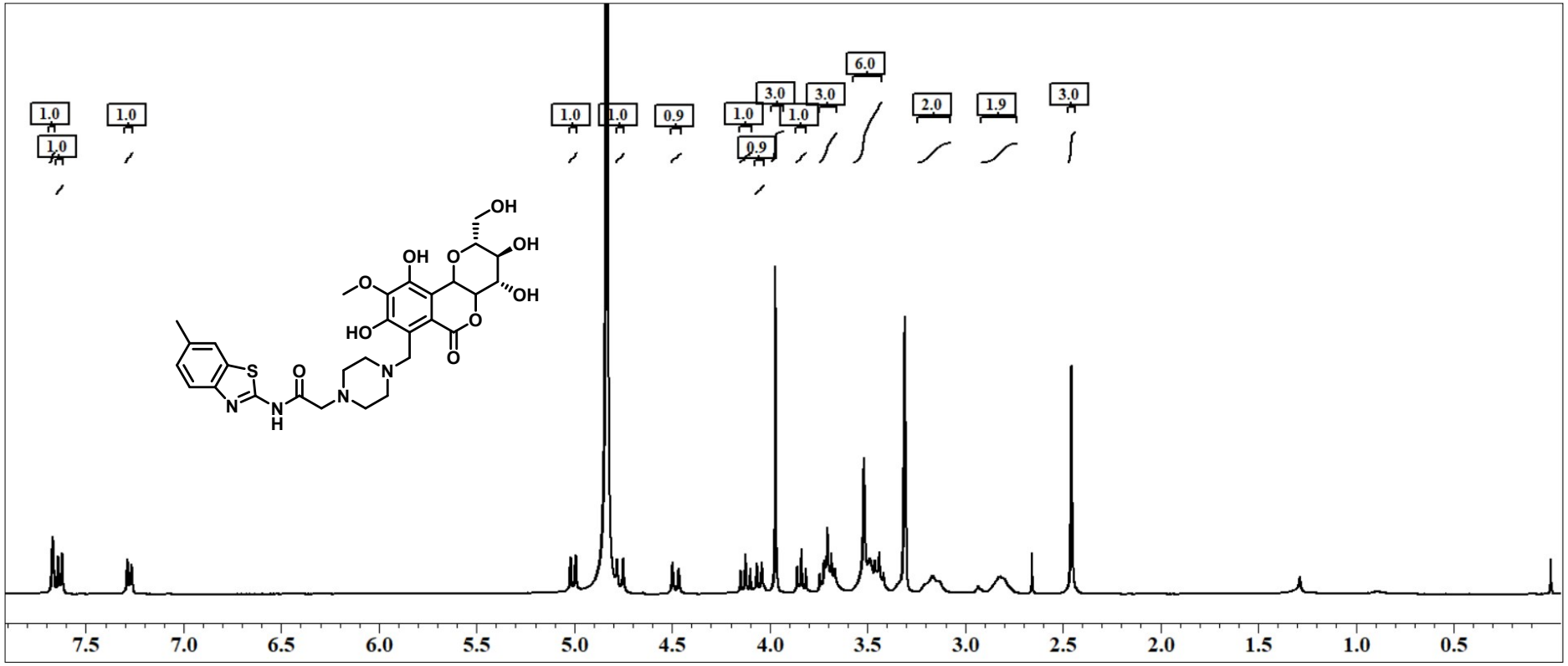


Fig S61: ^{1}H NMR SPECTRUM OF COMPOUND **10f**(400 MHz, CD_3OD)

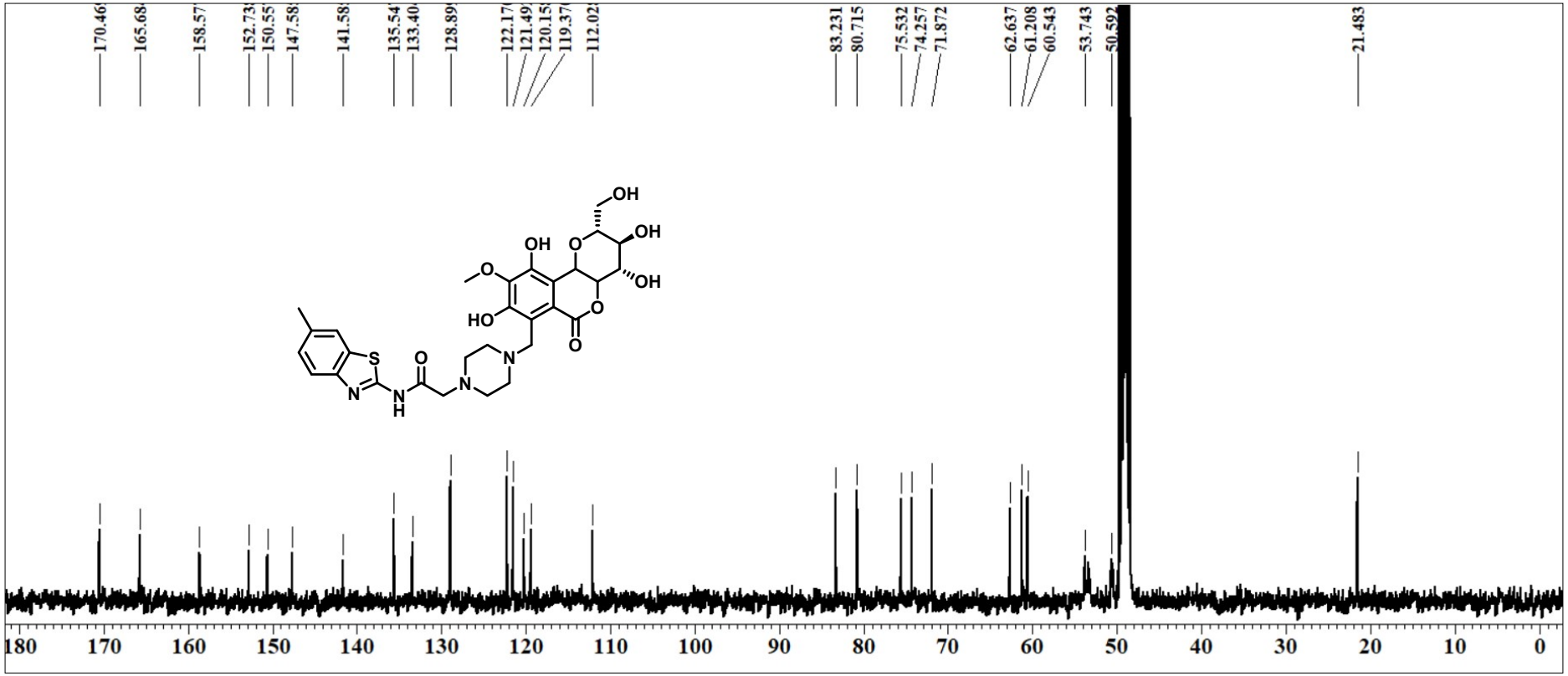
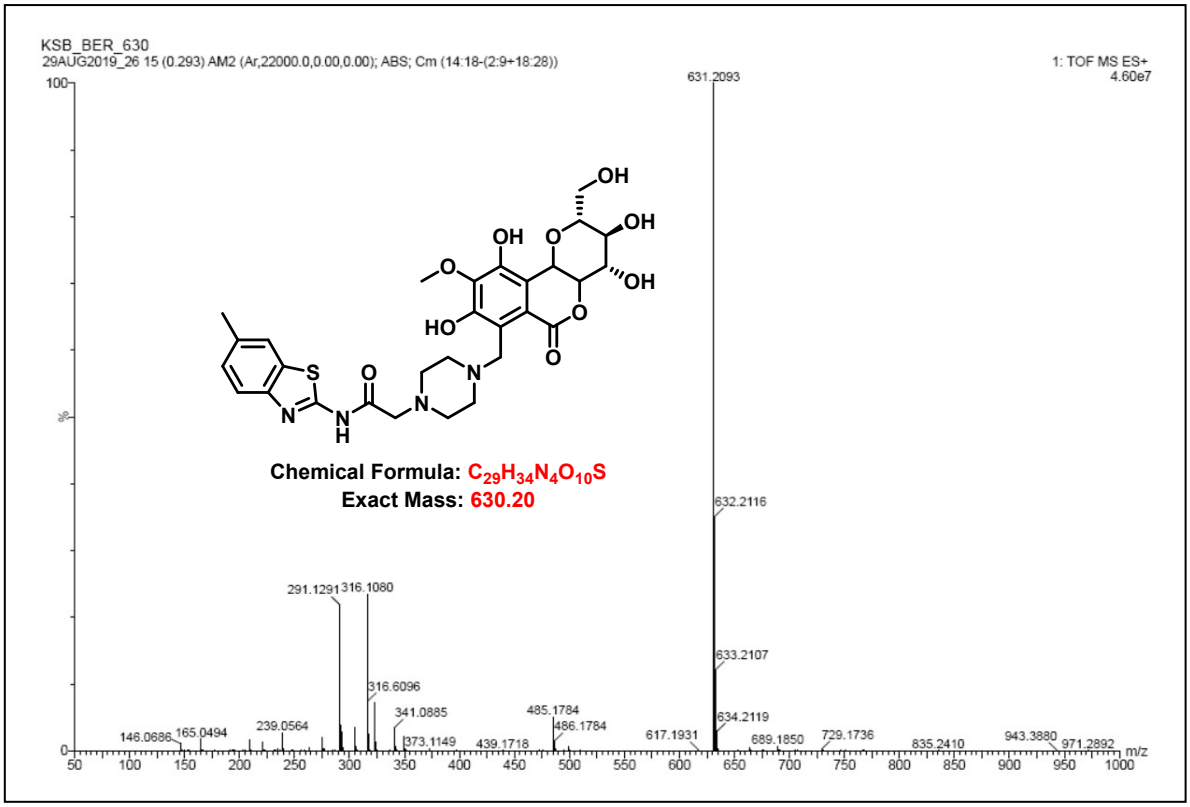


Fig S62: ^{13}C NMR SPECTRUM OF COMPOUND **10f** (100 MHz, CD_3OD)

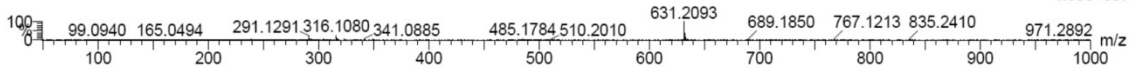


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 100.0 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
282 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-29 H: 0-35 N: 0-4 O: 0-10 S: 0-1 I: 0-1

KSB_BER_630
29AUG2019_26 15 (0.293) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (14:18-(2:9+18:28))
1: TOF MS ES+
4.60e+007



Minimum:				-1.5				
Maximum:		5.0	100.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
631.2093	631.2074	1.9	3.0	14.5	396.6	n/a	n/a	C29 H35 N4 O10 S

Fig S63: HRESIMS SPECTRUM OF COMPOUND **10f**

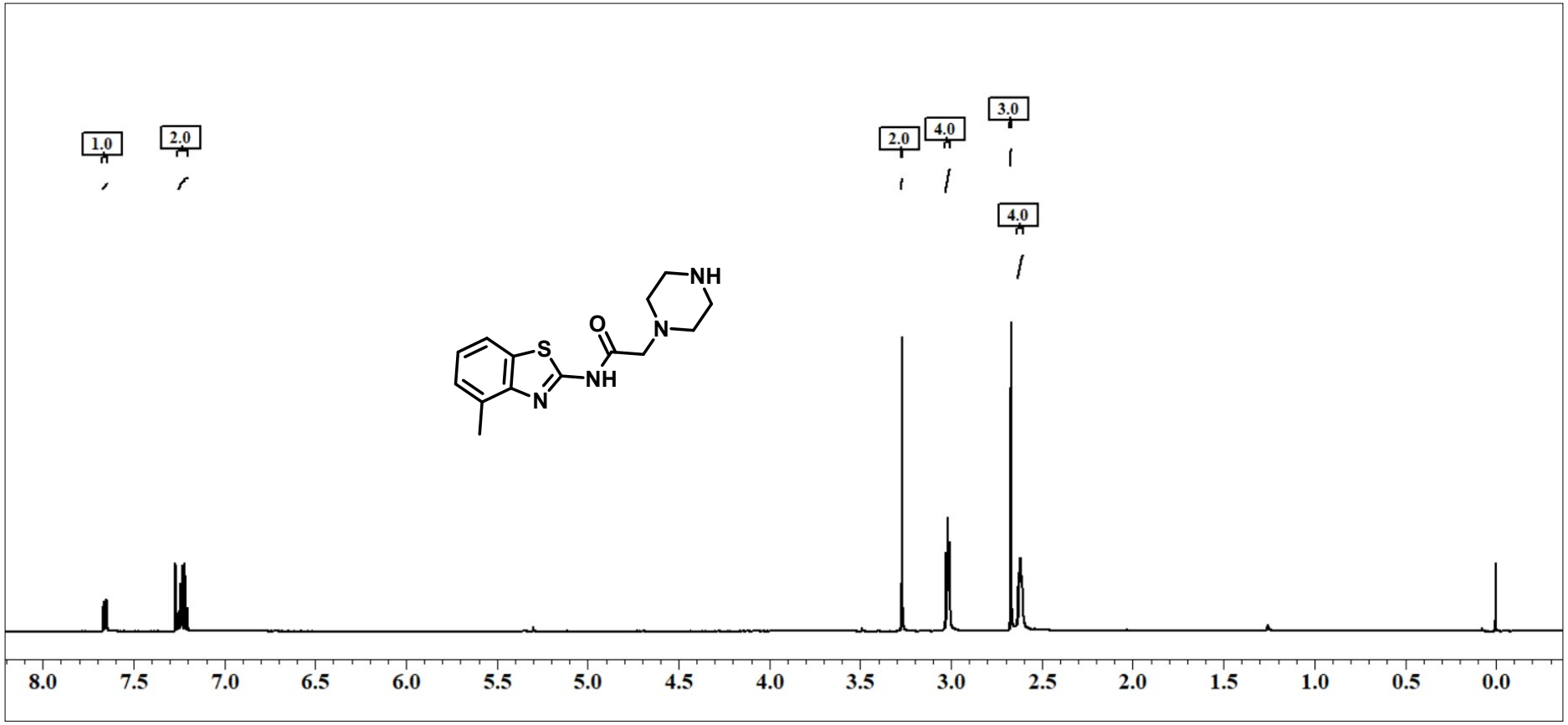


Fig S64: ^{1}H NMR SPECTRUM OF COMPOUND **9g**(400 MHz, CD_3OD)

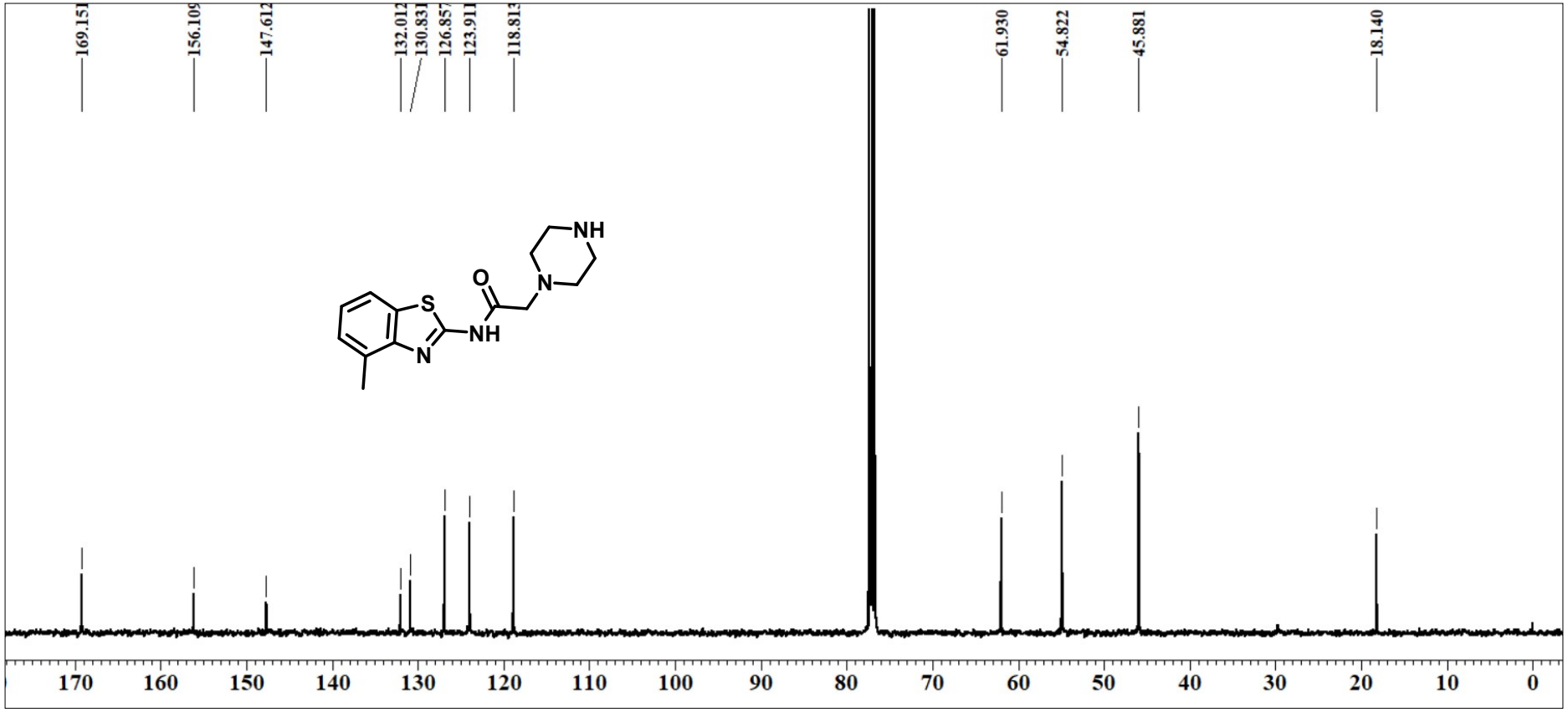


Fig S65: ^{13}C NMR SPECTRUM OF COMPOUND **9g** (100 MHz, $CDCl_3$)

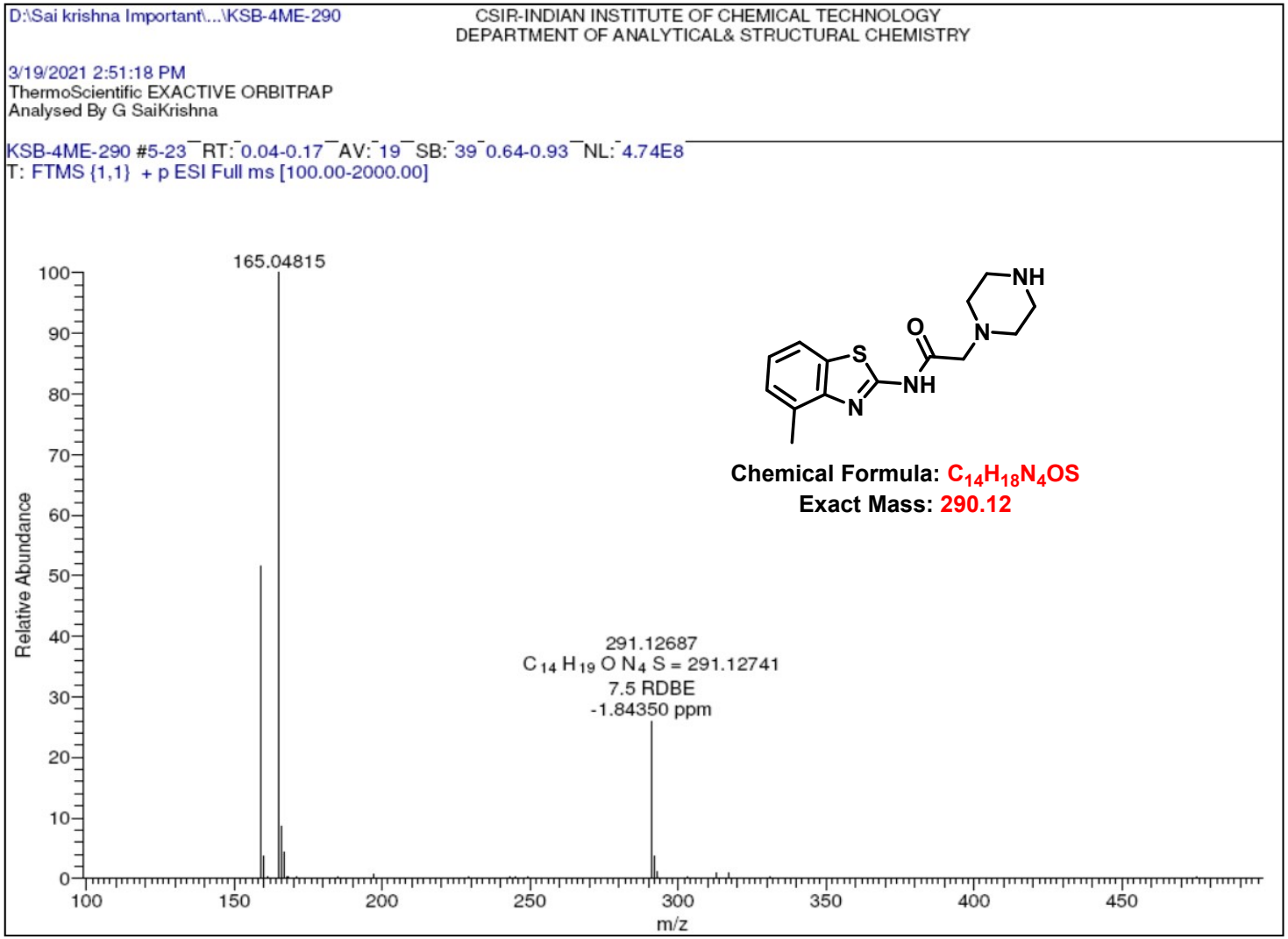


Fig S66: HRESIMS SPECTRUM OF COMPOUND **9g**

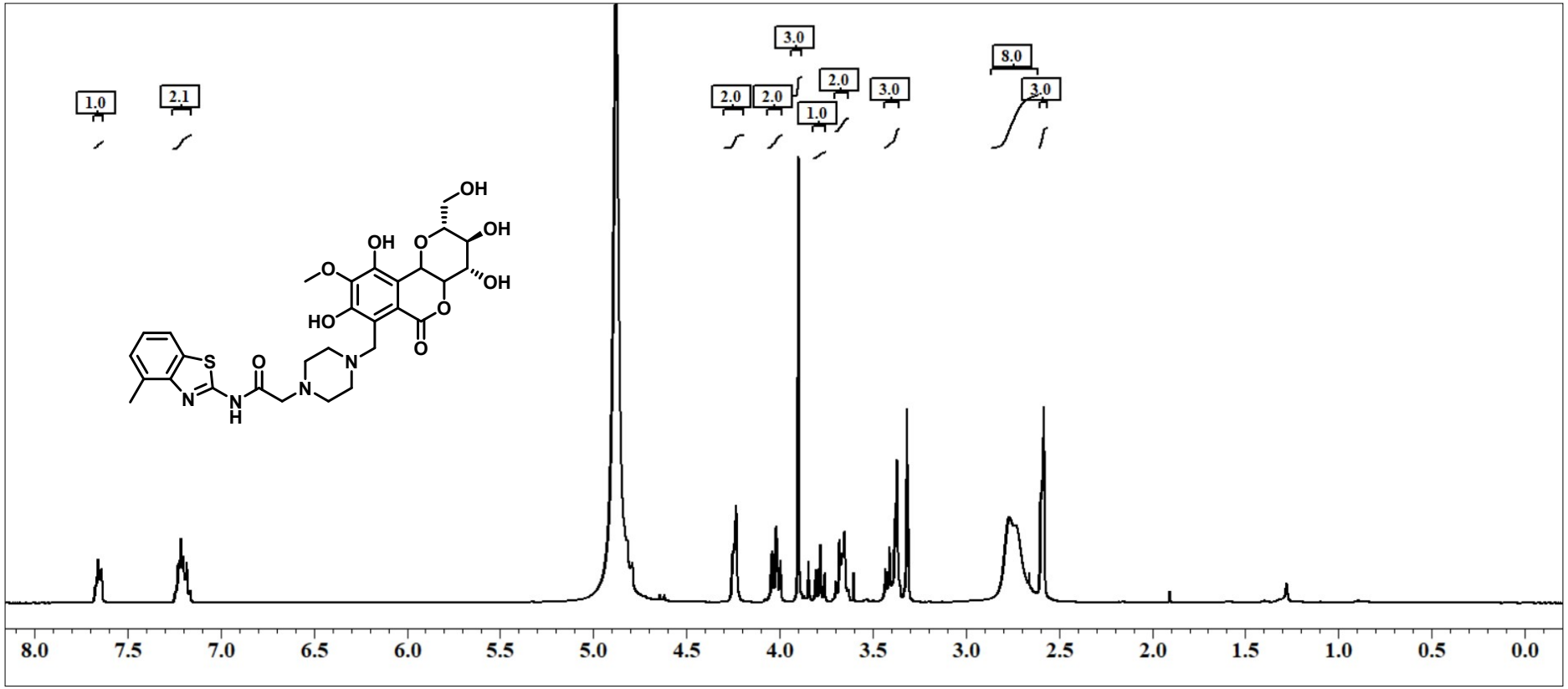


Fig S67: ^{1}H NMR SPECTRUM OF COMPOUND **10g**(500 MHz, CD_3OD)

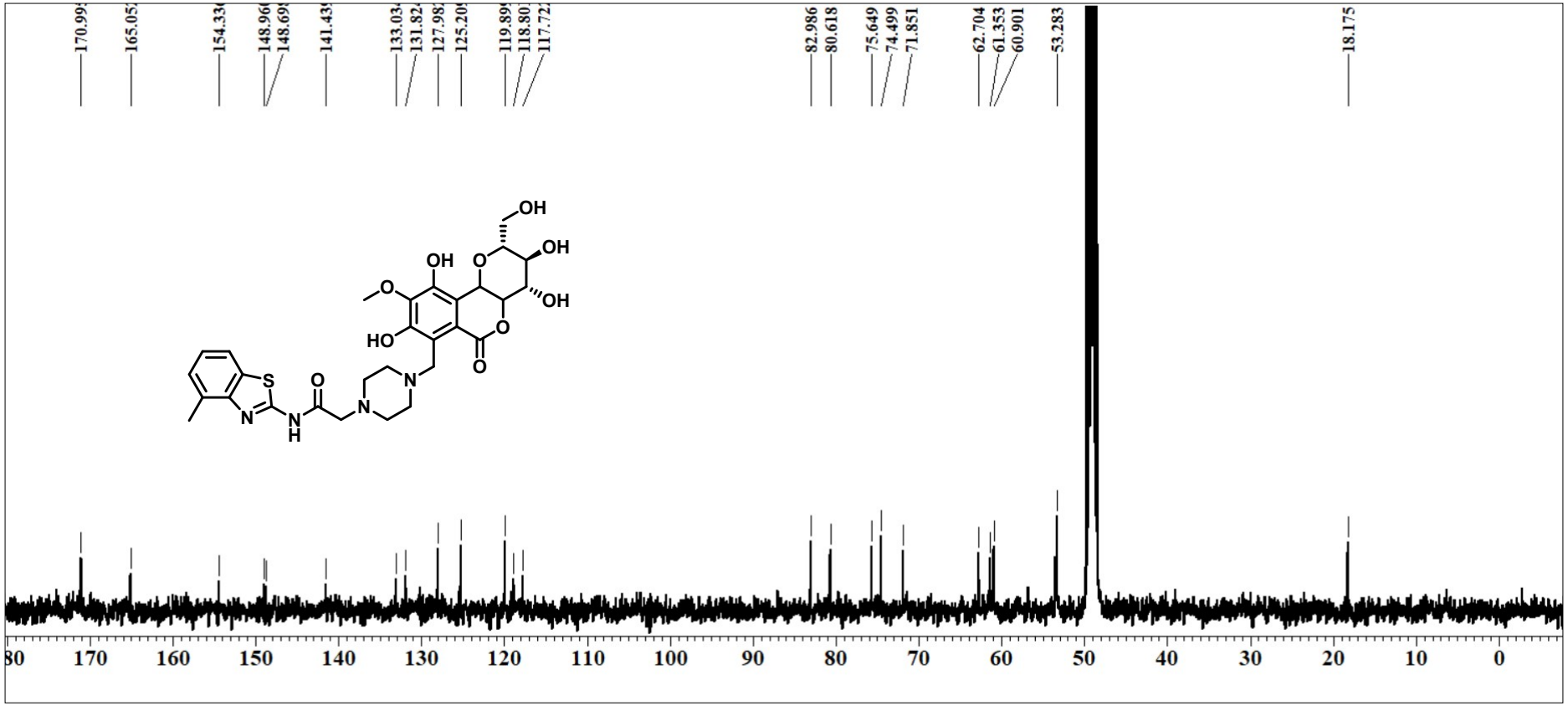


Fig S68: ^{13}C NMR SPECTRUM OF COMPOUND **10g** (100 MHz, CD_3OD)

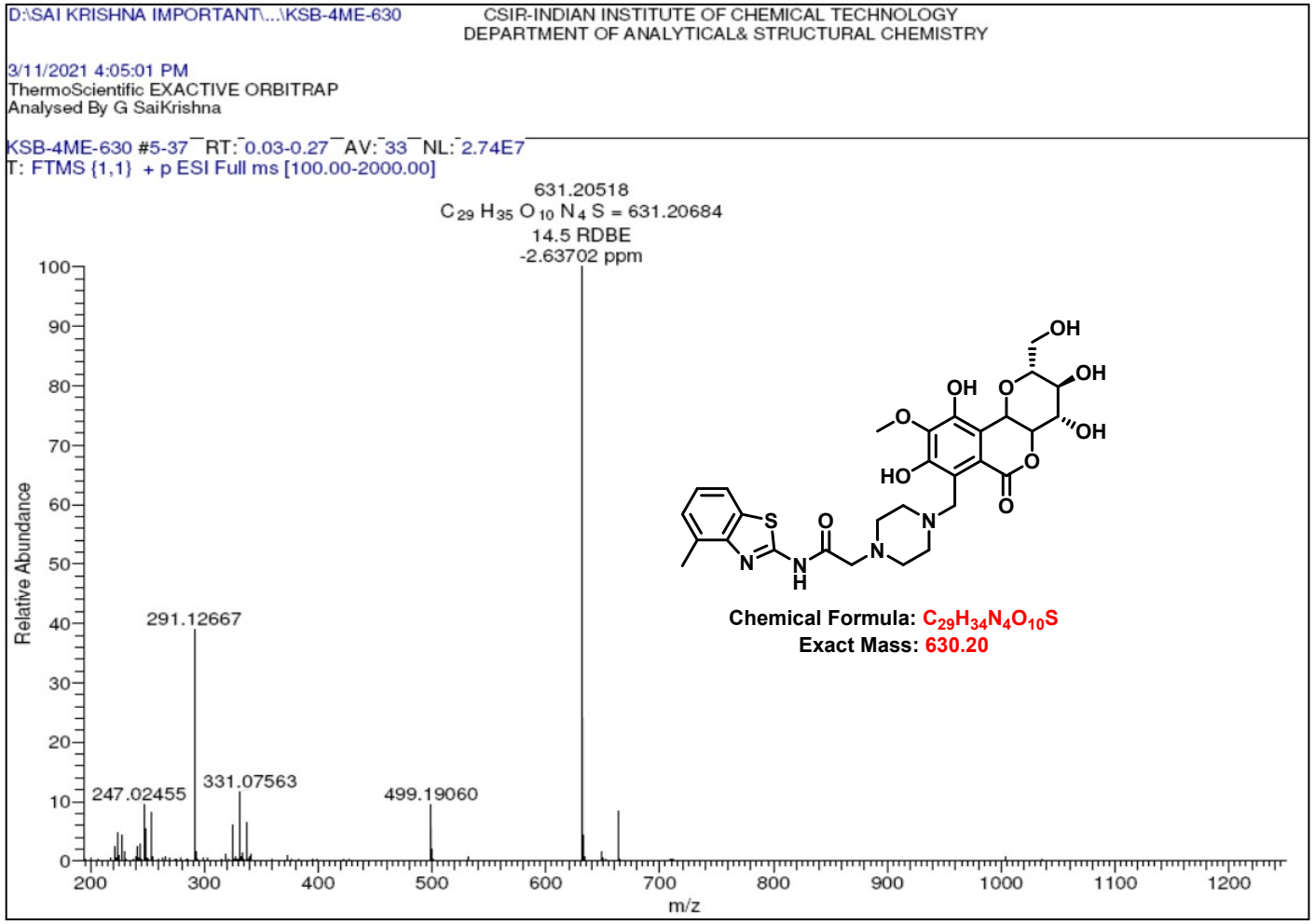


Fig S69: HRESIMS SPECTRUM OF COMPOUND **10g**

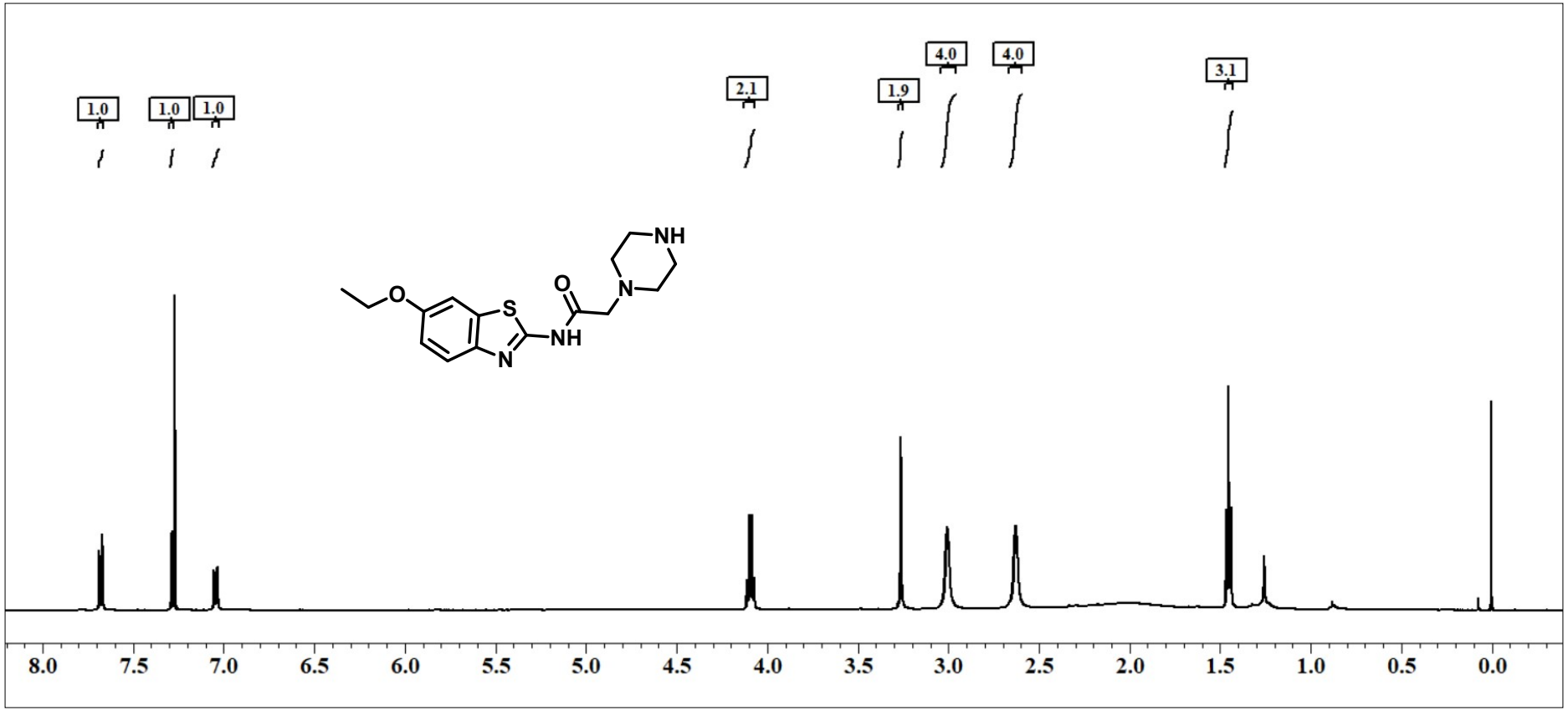


Fig S70: ^{1}H NMR SPECTRUM OF COMPOUND **9h**(500 MHz, CD_3OD)

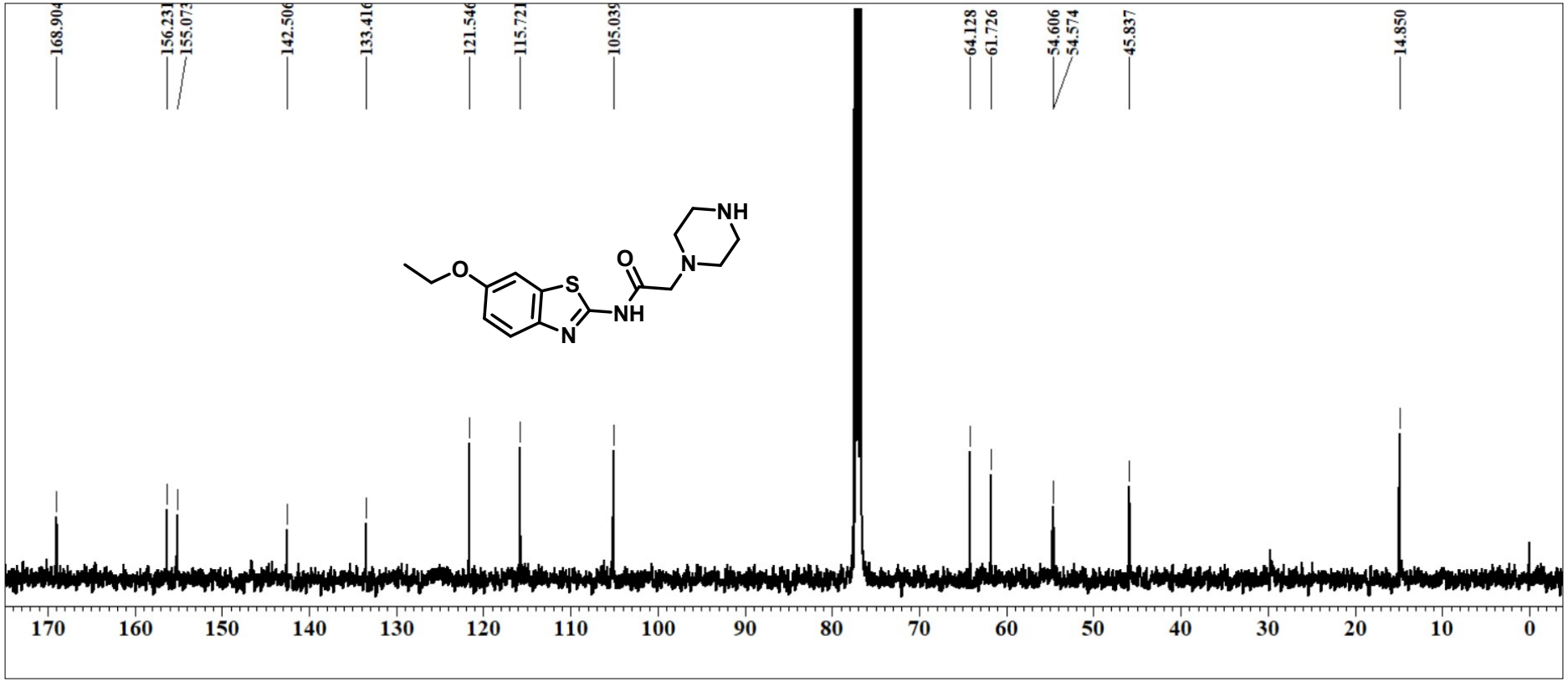
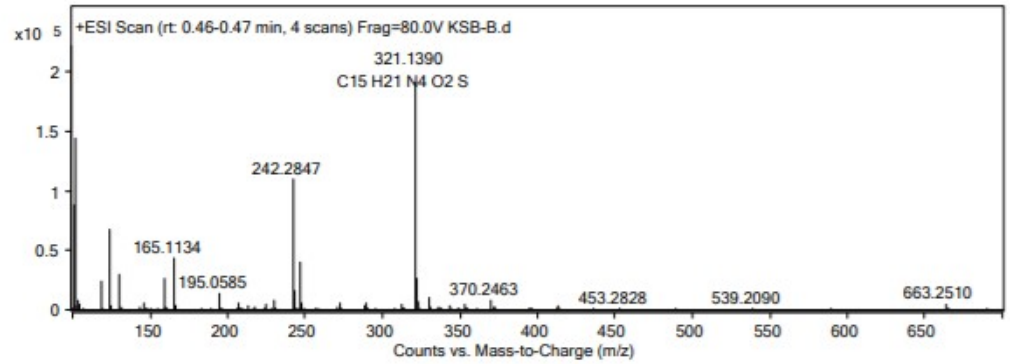


Fig S71: ^{13}C NMR SPECTRUM OF COMPOUND **9h** (100 MHz, $CDCl_3$)

Data File	KSB-B.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A3
Instrument Name	Instrument 1	**User Name**	CSIR-IICT\Analyst
Acq Method	hrms-pos-method.m	**Acquired Time**	14-06-2021 17:47:18
IRM Calibration Status	Success	**DA Method**	11.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.06.01 (B6172 SP1)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
80	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
321.139	1	191584.5	C15 H21 N4 O2 S	M+

Formula Calculator Element Limits

Element	Min	Max
C	0	25
H	0	60
O	0	2
N	0	4
P	0	0
F	0	0
S	0	1
Cl	0	0

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C15 H21 N4 O2 S	True	321.1394	321.1385	2.74	C15 H21 N4 O2 S	90.94

Fig S72: HRESIMS SPECTRUM OF COMPOUND **9h**

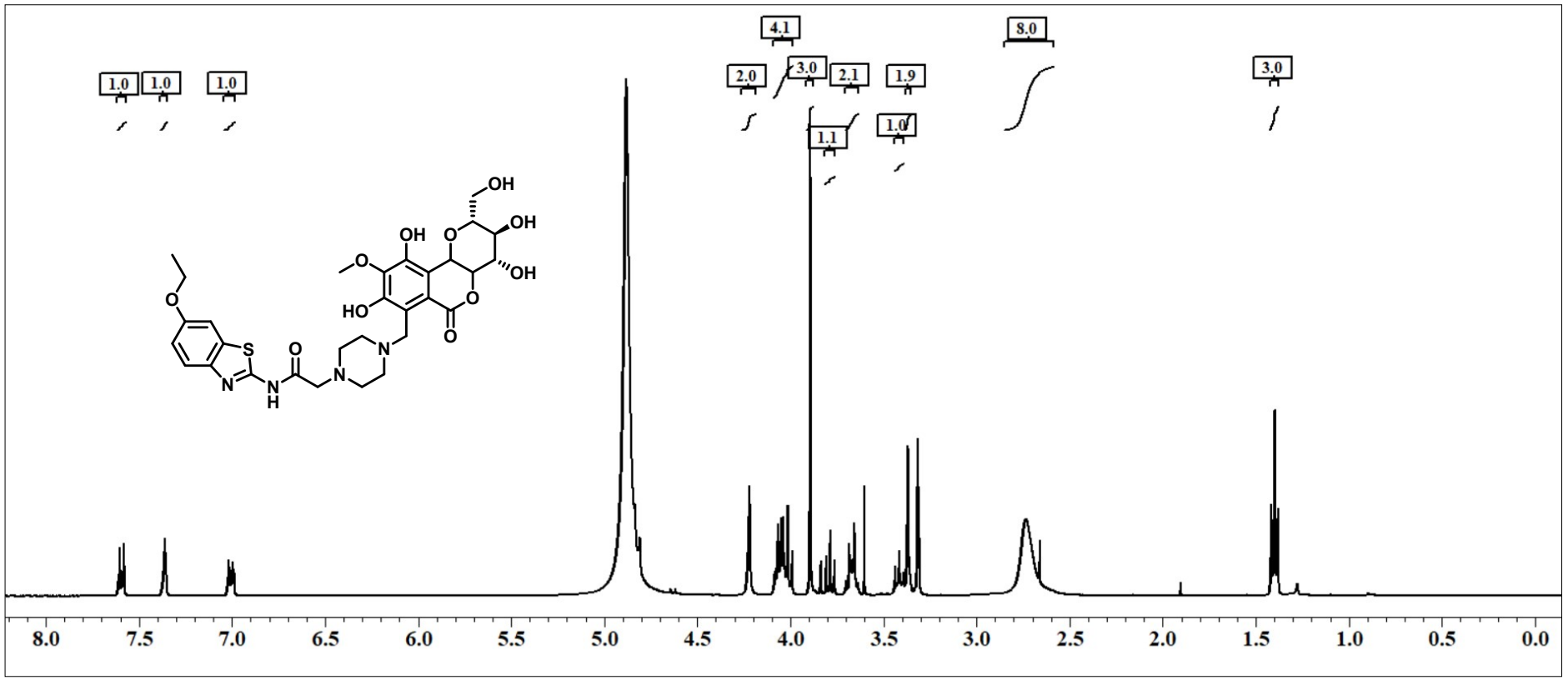


Fig S73: ^{1}H NMR SPECTRUM OF COMPOUND **10h**(400 MHz, CD_3OD)

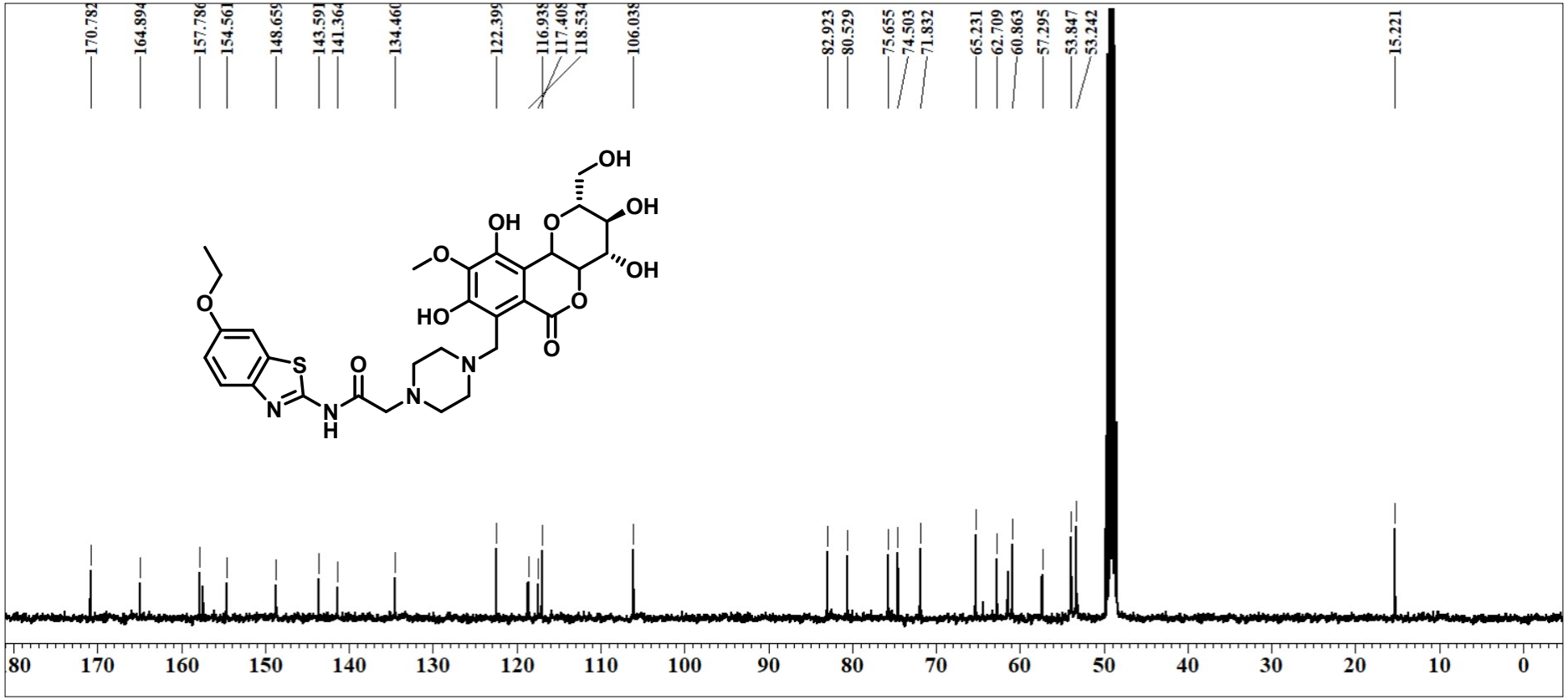


Fig S74: ^{13}C NMR SPECTRUM OF COMPOUND **10h** (100 MHz, CD_3OD)

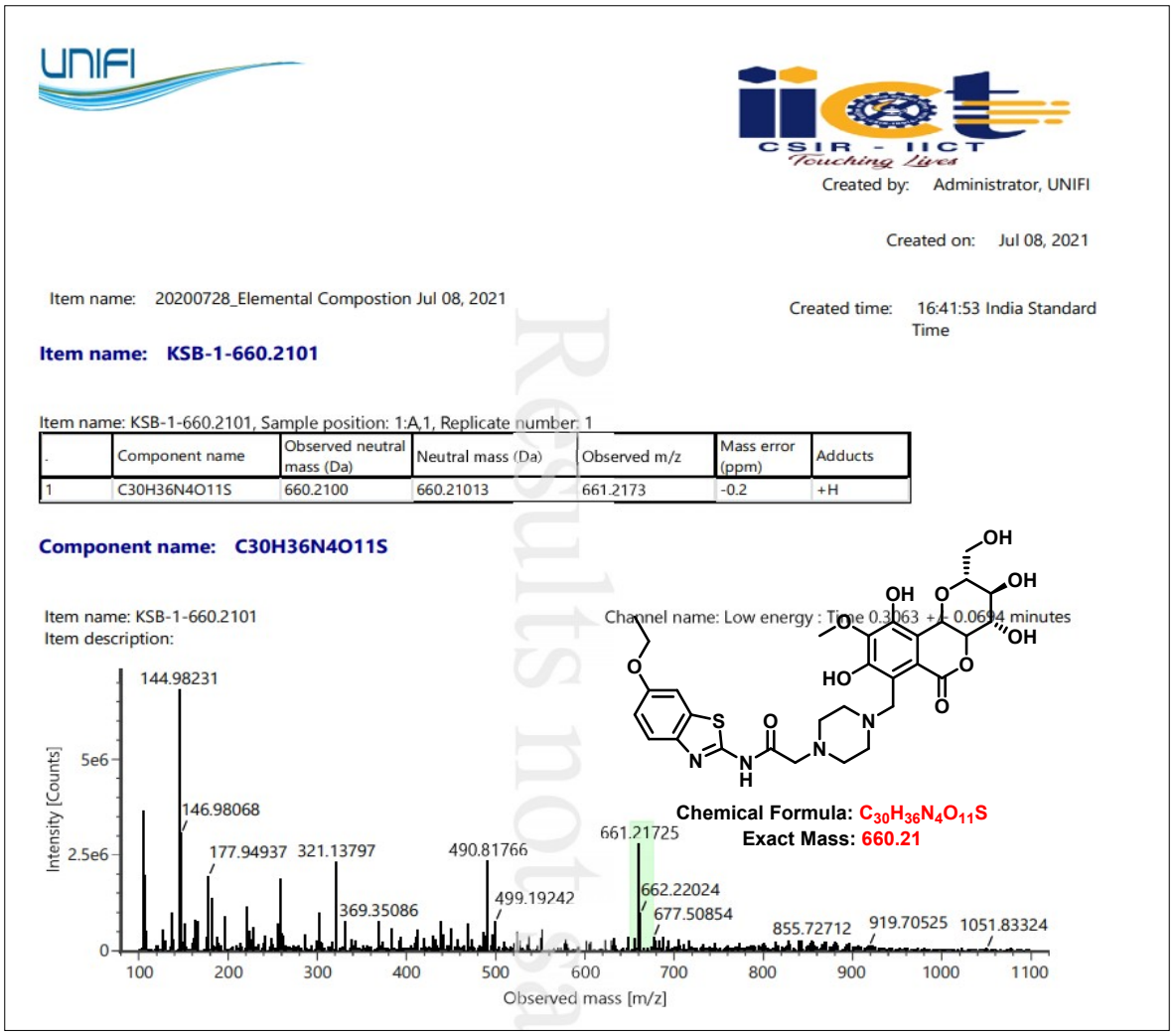


Fig S75: HRESIMS SPECTRUM OF COMPOUND **10h**

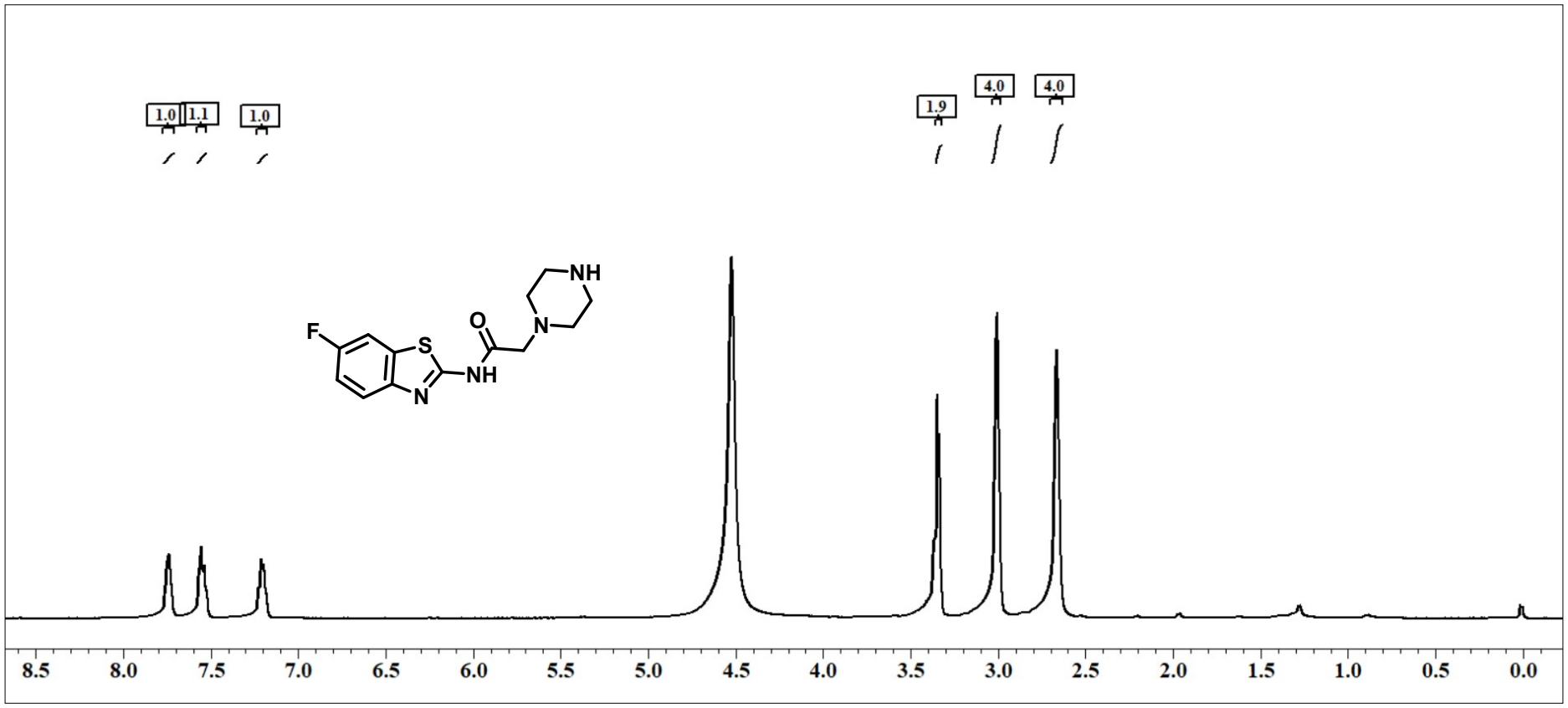


Fig S76: ^{1}H NMR SPECTRUM OF COMPOUND **9i**(500 MHz, CD_3OD)

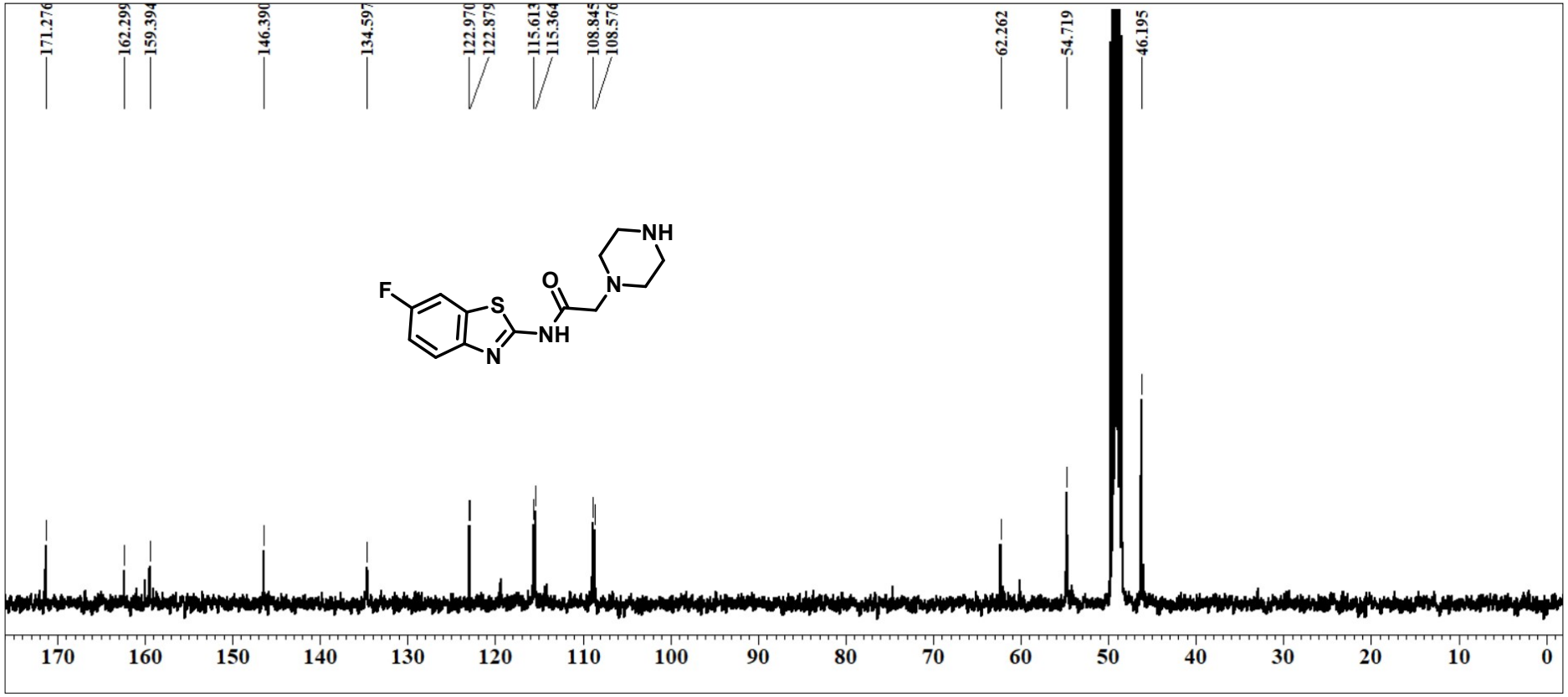


Fig S77: ^{13}C NMR SPECTRUM OF COMPOUND **9i** (100 MHz, CD_3OD)

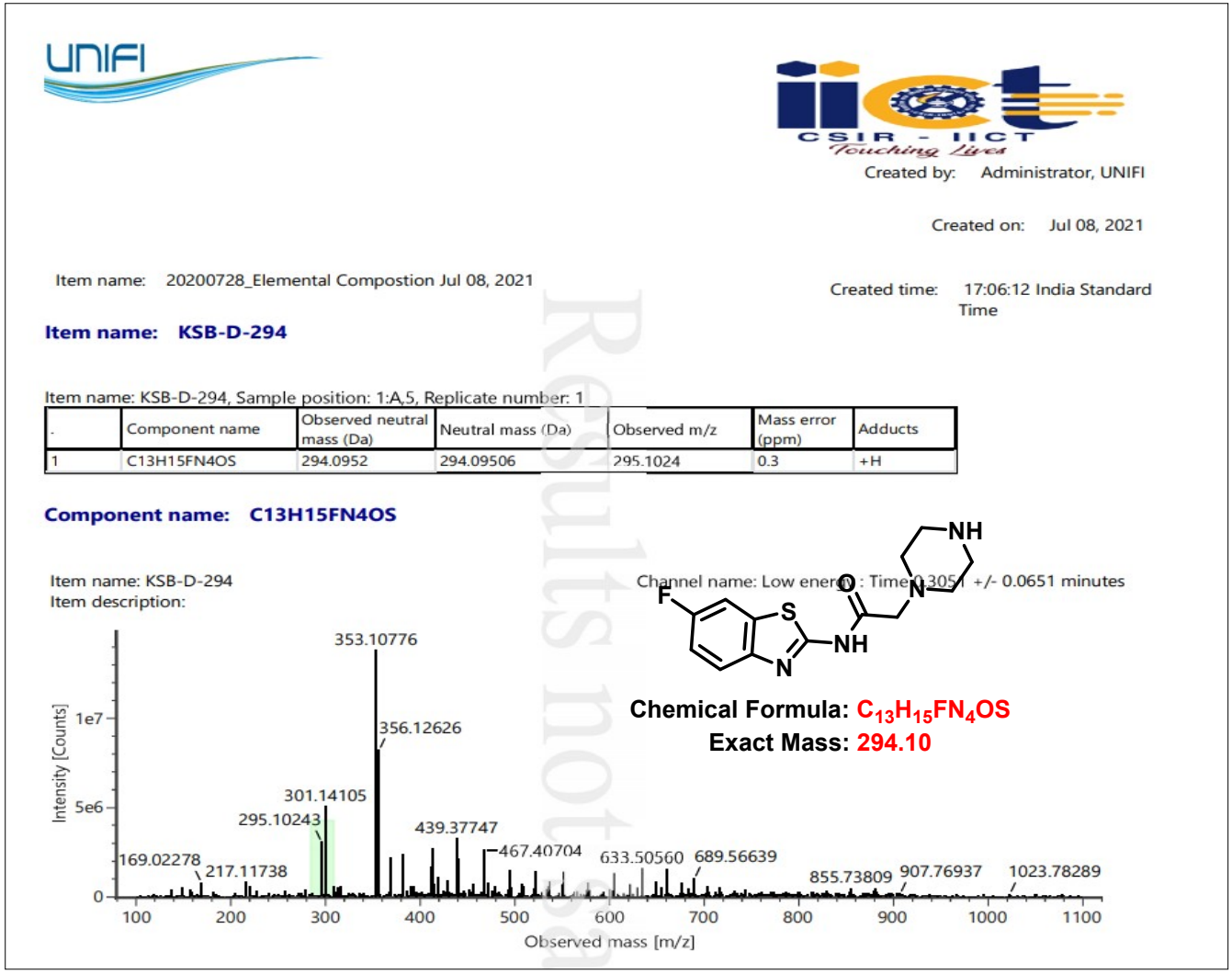


Fig S78: HRESIMS SPECTRUM OF COMPOUND **9**

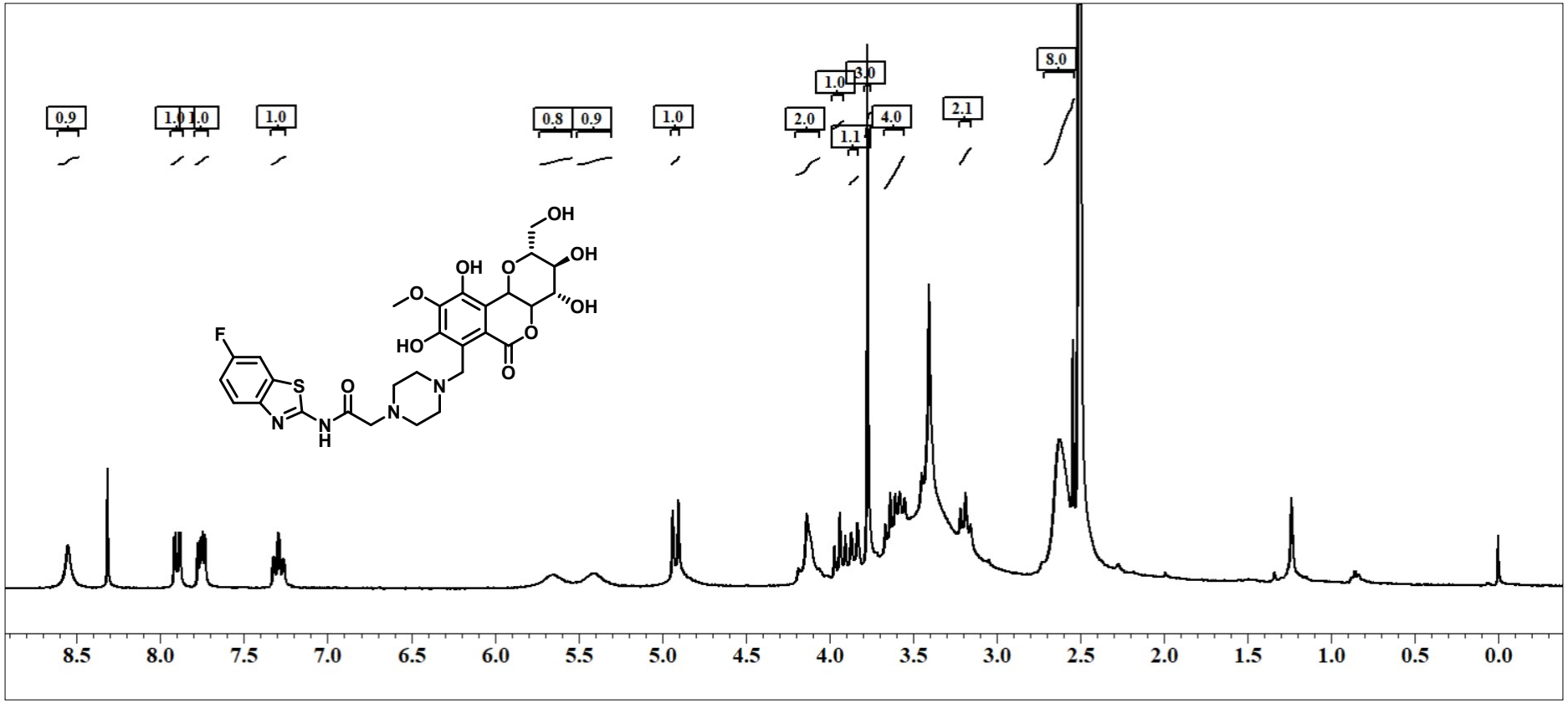


Fig S79: ^{1}H NMR SPECTRUM OF COMPOUND **10i**(400 MHz, DMSO-d_6)

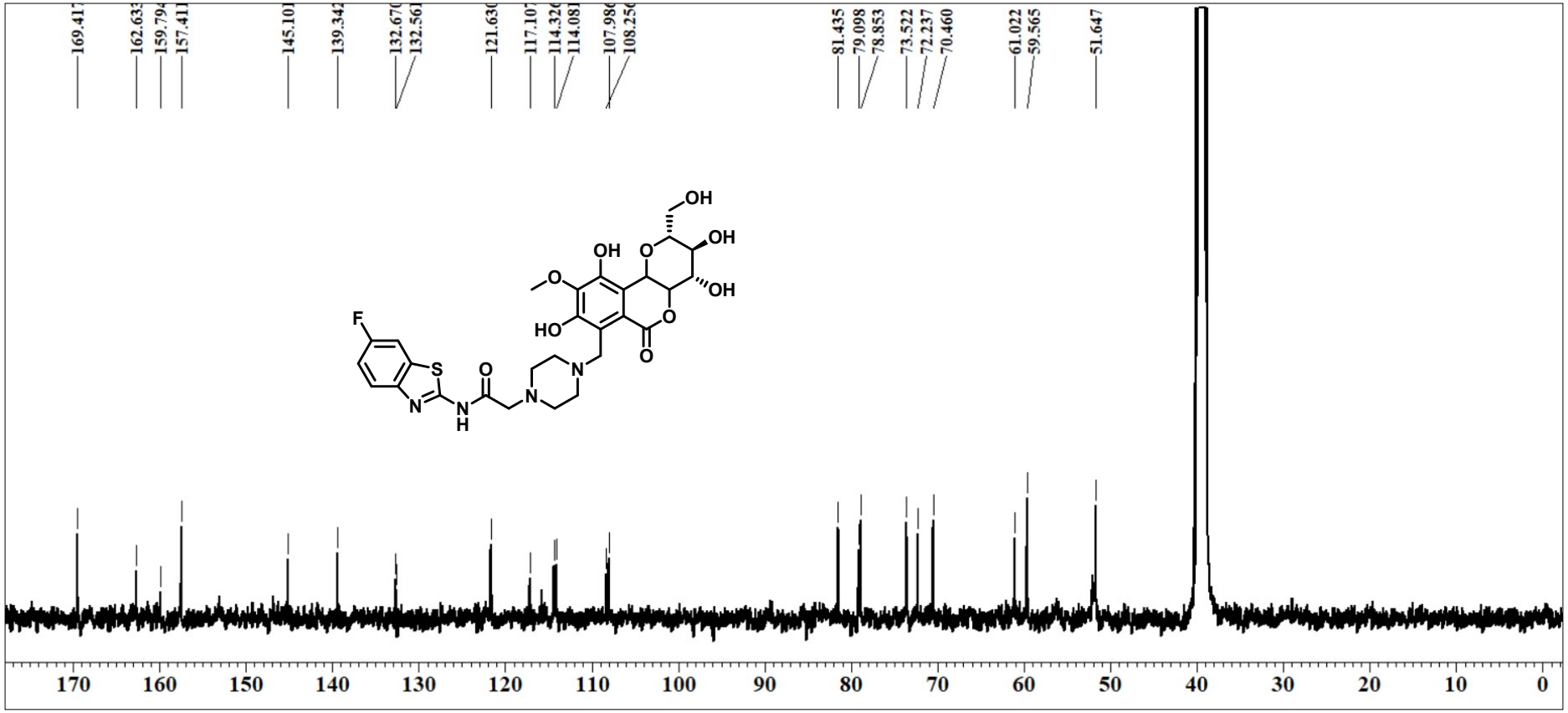


Fig S80: ^{13}C NMR SPECTRUM OF COMPOUND **10i** (100 MHz, DMSO-d_6)

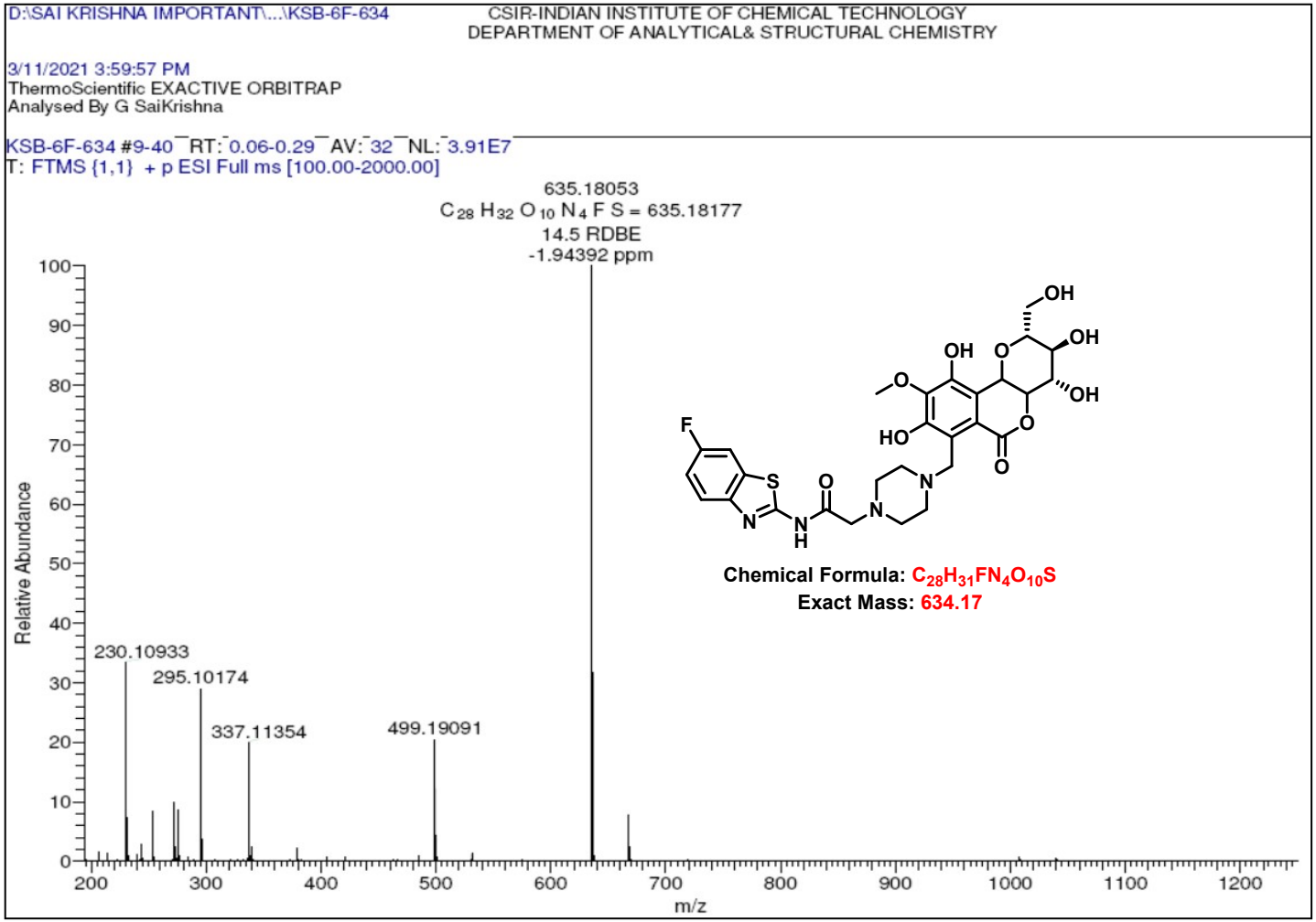


Fig S81: HRESIMS SPECTRUM OF COMPOUND **10i**

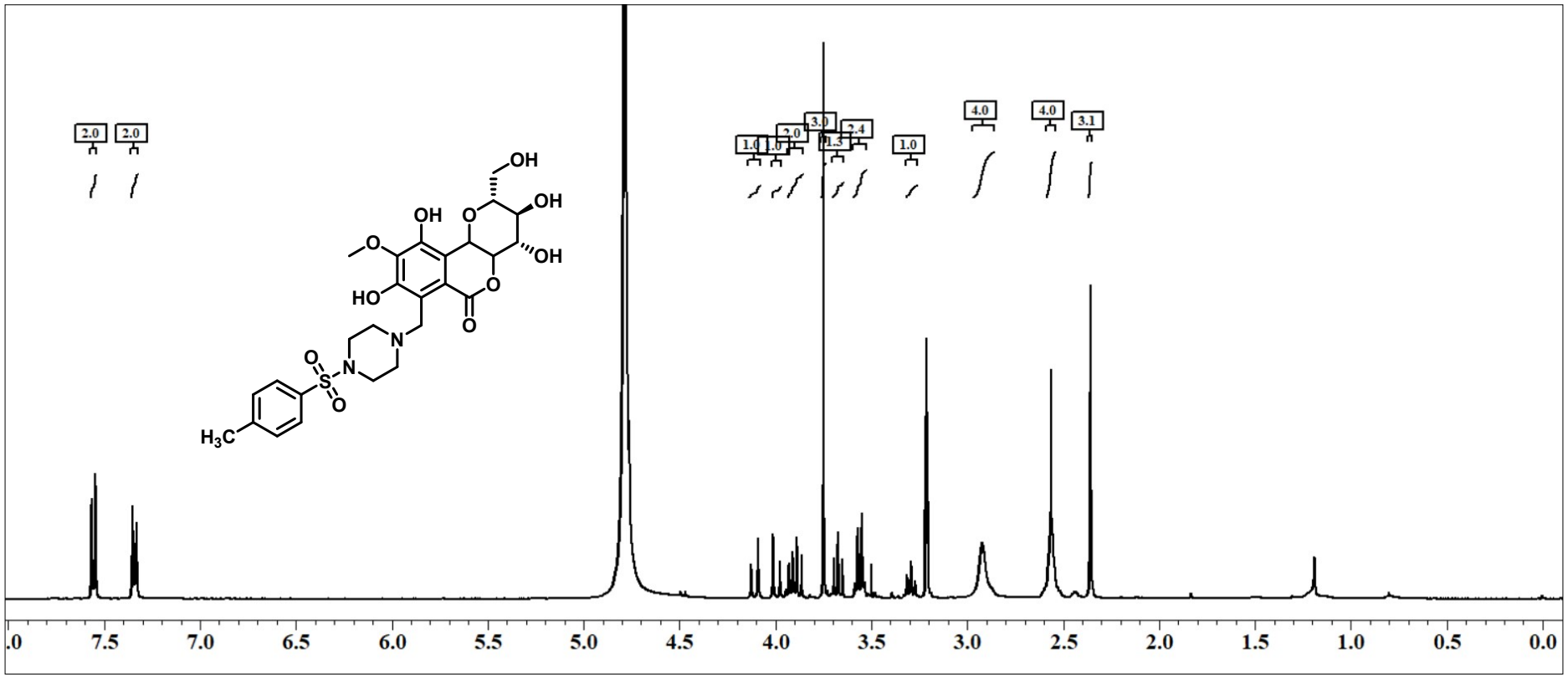


Fig S82: ^{1}H NMR SPECTRUM OF COMPOUND **13a** (400 MHz, CD_3OD)

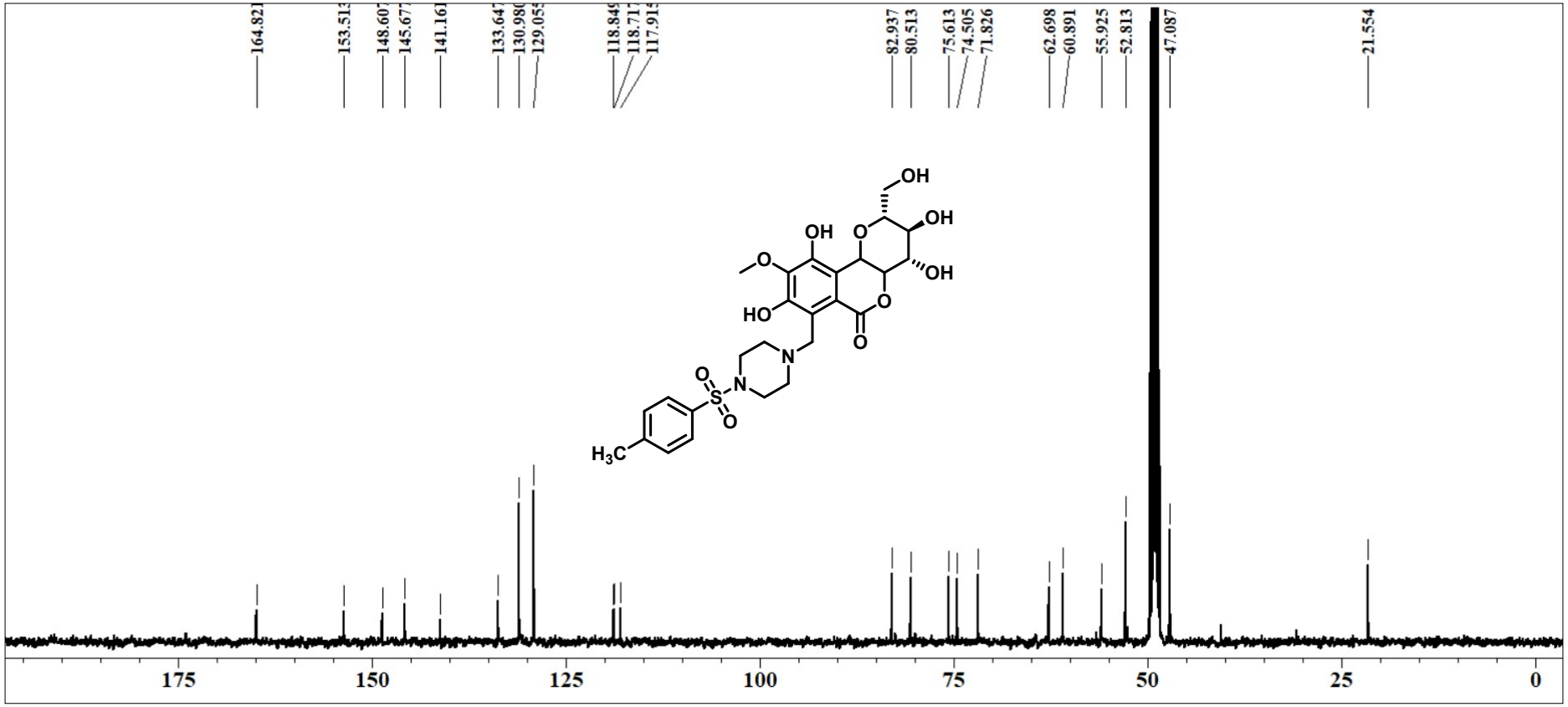
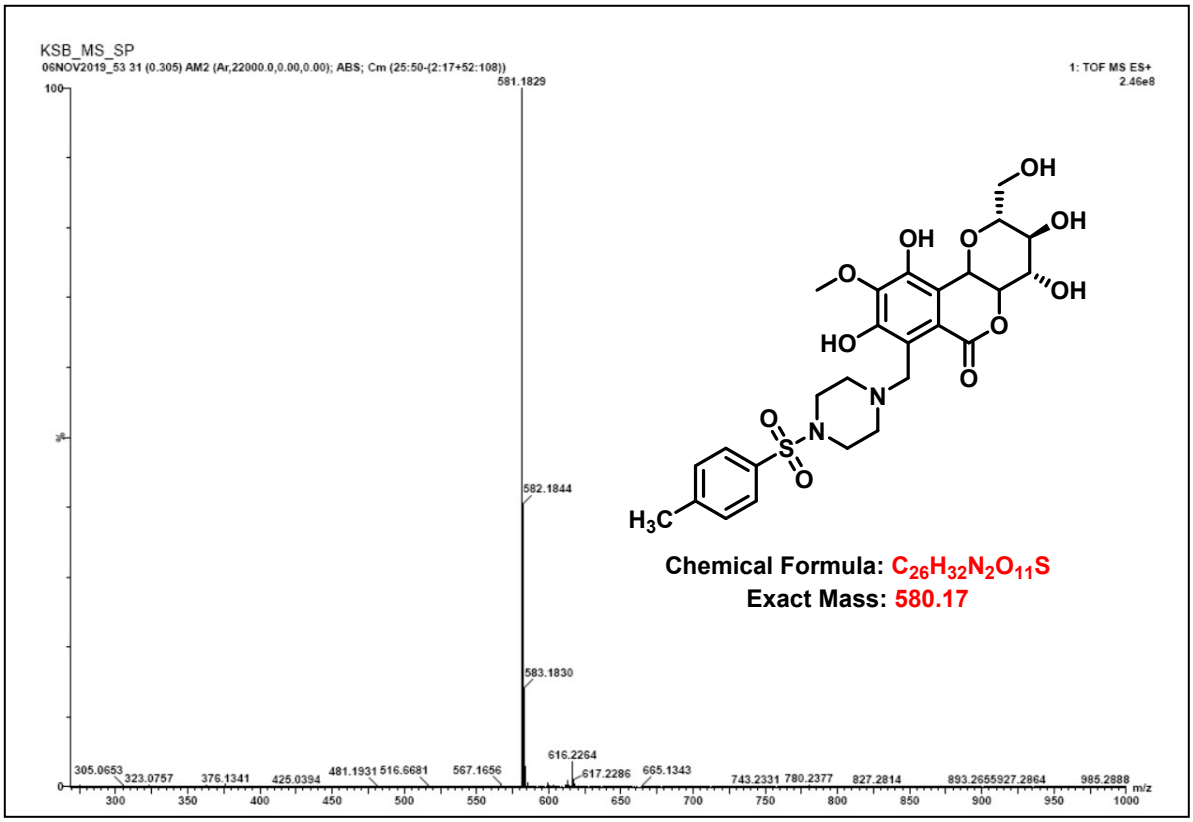


Fig S83: ^{13}C NMR SPECTRUM OF COMPOUND **13a** (100 MHz, CD_3OD)



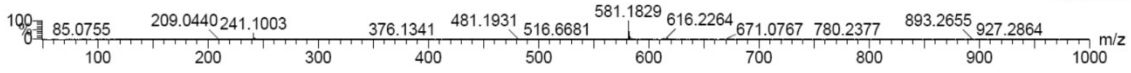
Elemental Composition Report **Page 1**

Single Mass Analysis

Tolerance = 5.0 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
239 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-29 H: 0-35 N: 0-2 O: 0-13 S: 0-3

KSB_MS_SP
06NOV2019_53 31 (0.305) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (25:50-(2:17+52:108))
1: TOF MS ES+
2.46e+008



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
581.1829	581.1805	2.4	4.1	11.5	770.8	n/a	n/a	C26 H33 N2 O11 S

Fig S84: HRESIMS SPECTRUM OF COMPOUND **13g**

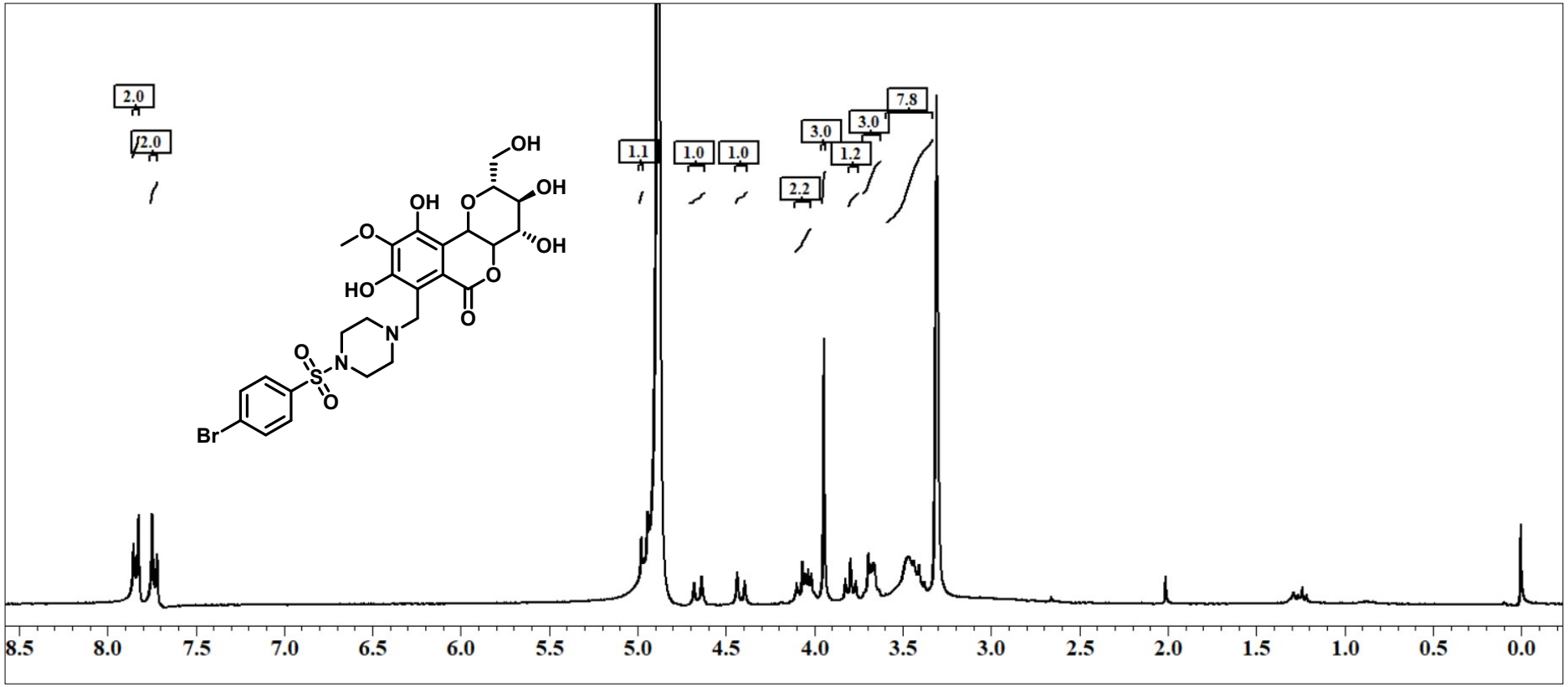


Fig S85: ^{1}H NMR SPECTRUM OF COMPOUND **13b** (300 MHz, CD_3OD)

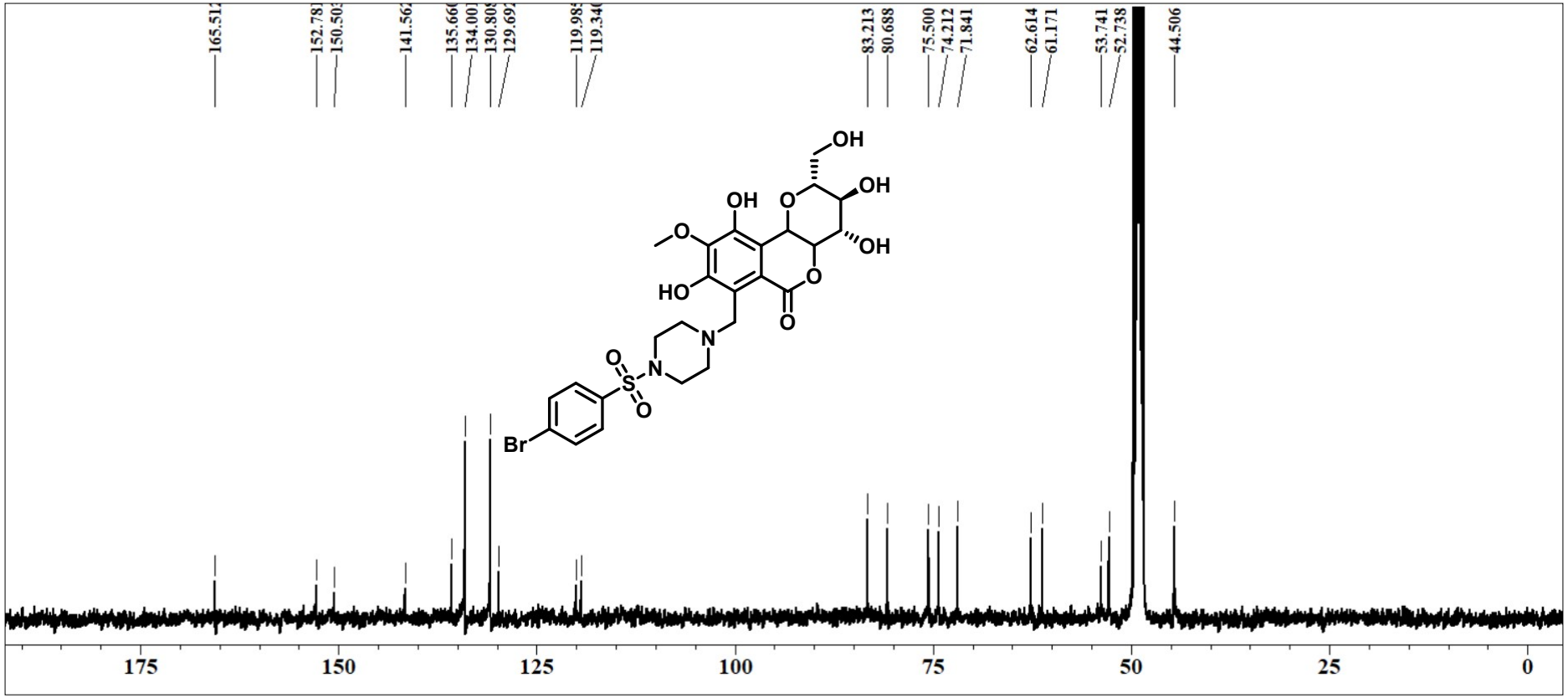


Fig S86: ^{13}C NMR SPECTRUM OF COMPOUND **13b** (100 MHz, CD_3OD)

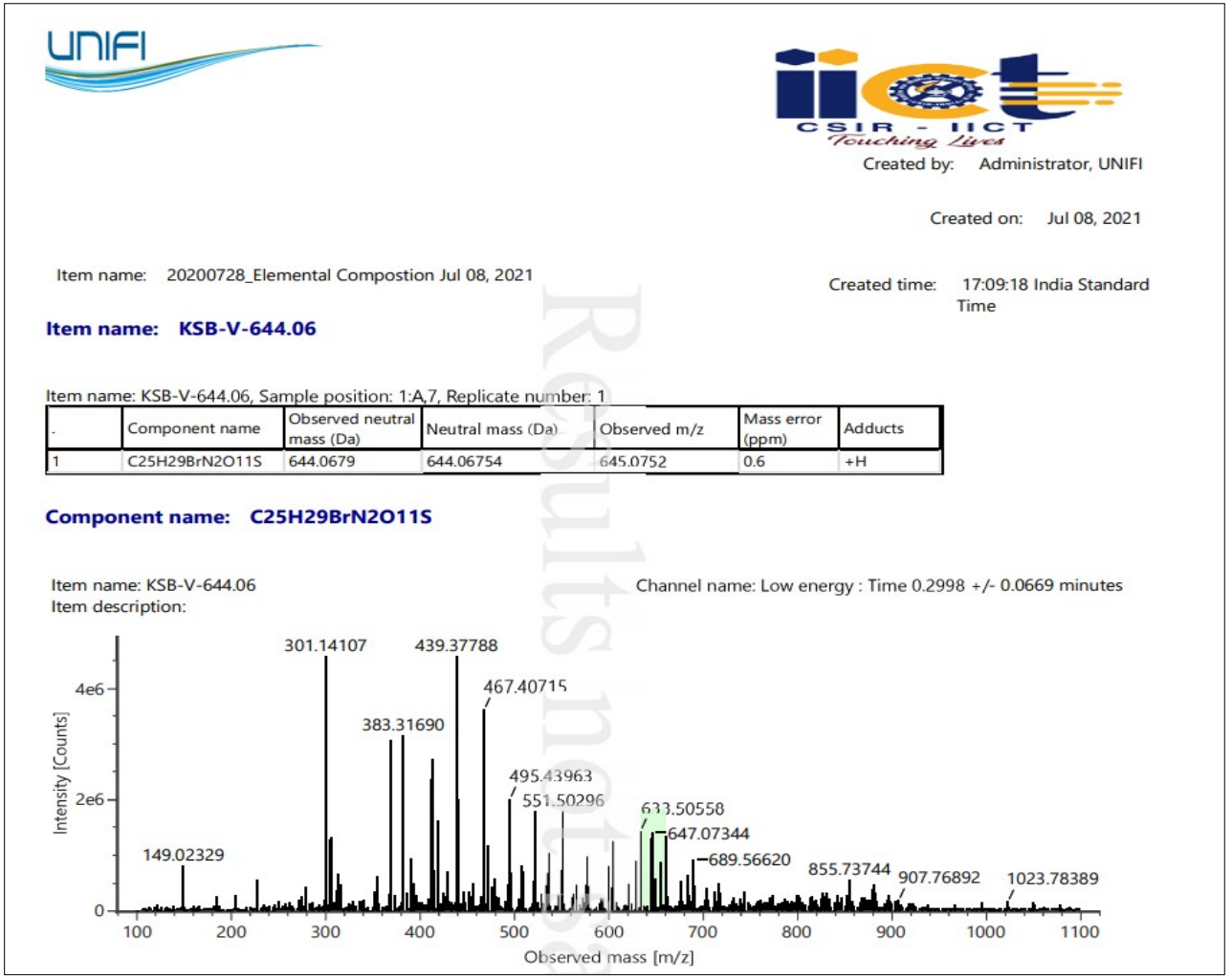


Fig S87: HRESIMS SPECTRUM OF COMPOUND **13b**

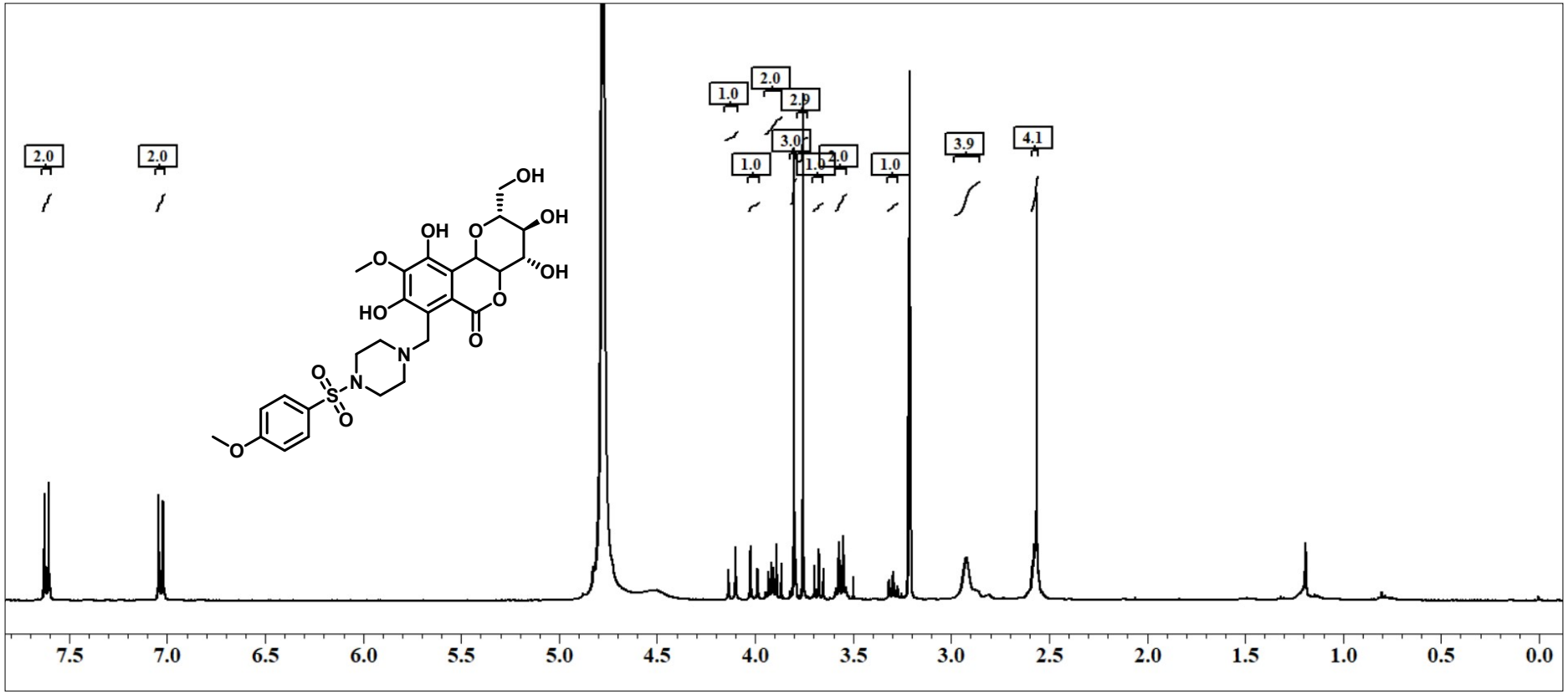


Fig S88: ^{1}H NMR SPECTRUM OF COMPOUND **13c** (400 MHz, CD_3OD)

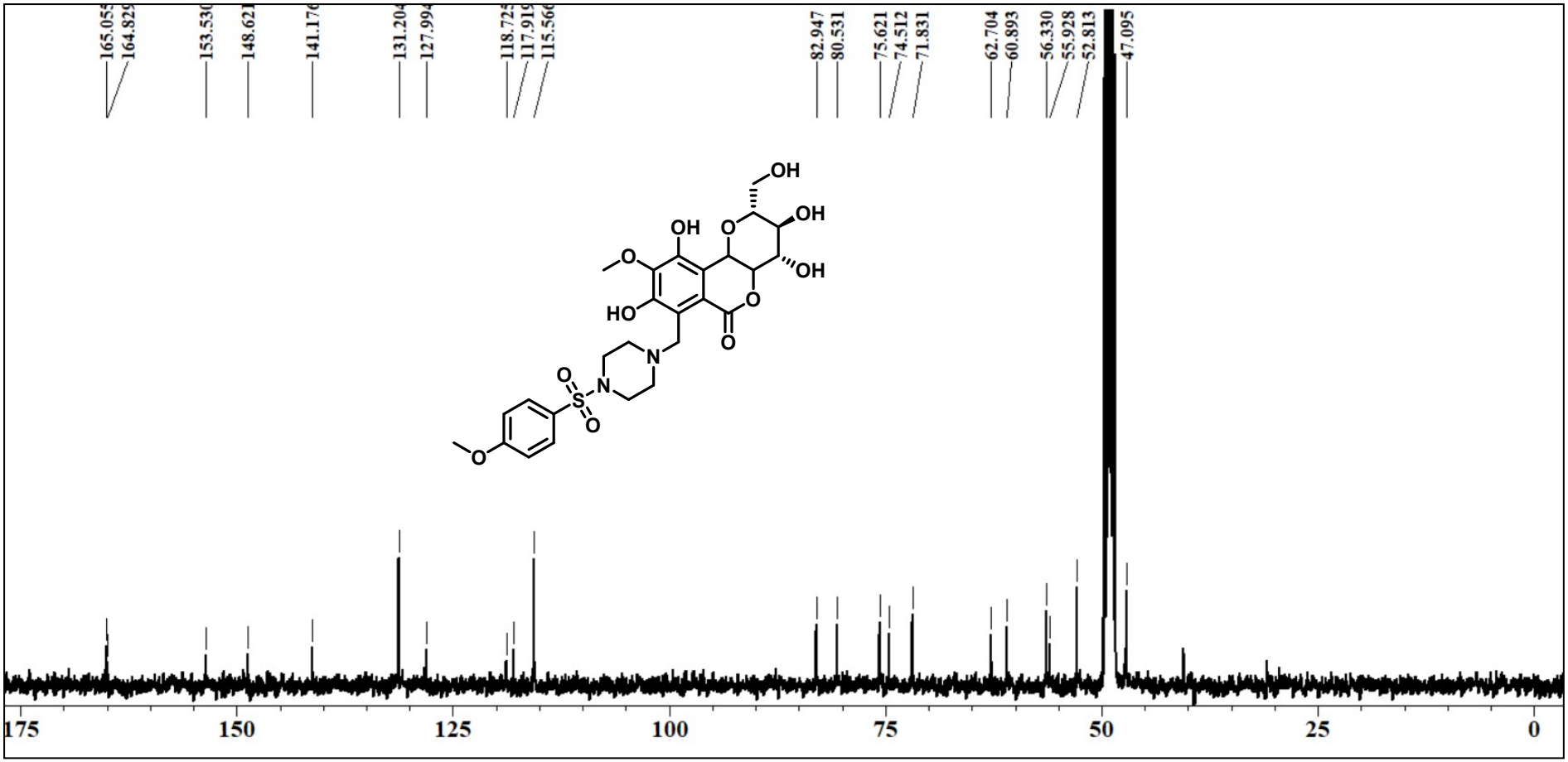
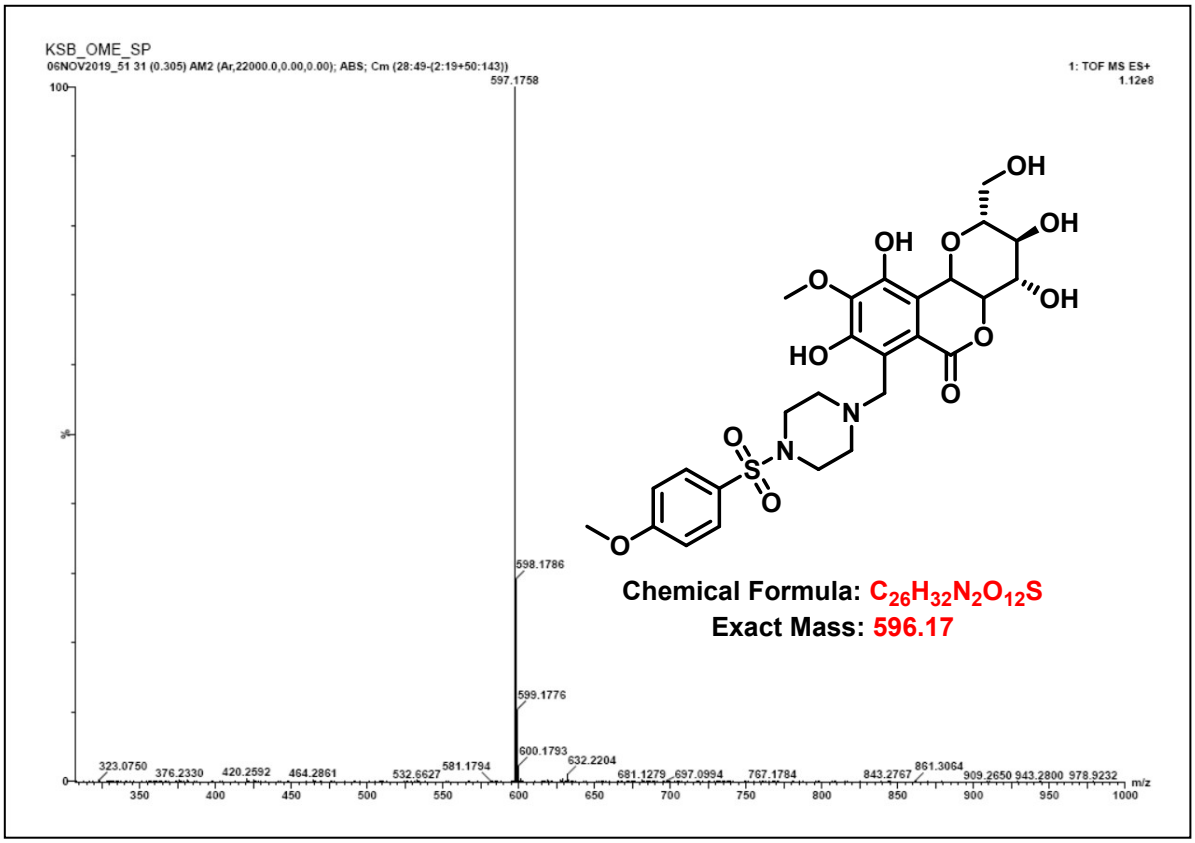


Fig S89: ^{13}C NMR SPECTRUM OF COMPOUND **13c** (100 MHz, CD_3OD)

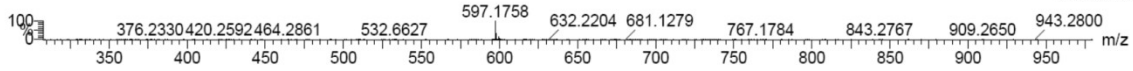


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 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
180 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-26 H: 0-35 N: 0-2 O: 0-13 S: 0-3

KSB_OME_SP
06NOV2019_51 31 (0.305) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (28:49-(2:19+50:143))
1: TOF MS ES+
1.12e+008



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
597.1758	597.1754	0.4	0.7	11.5	584.4	n/a	n/a	C26 H33 N2 O12 S

Fig S90: HRESIMS SPECTRUM OF COMPOUND **13c**

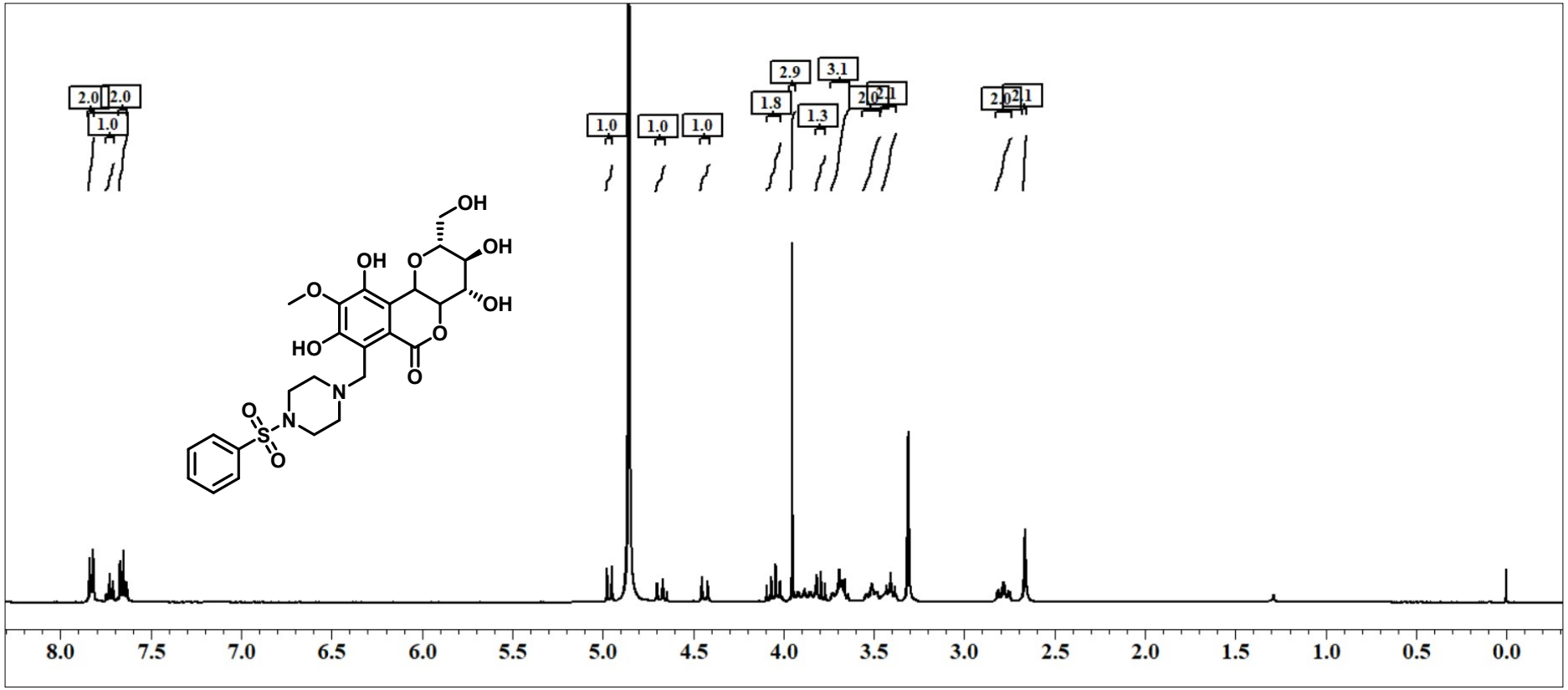


Fig S91: ^{1}H NMR SPECTRUM OF COMPOUND **13d** (400 MHz, CD_3OD)

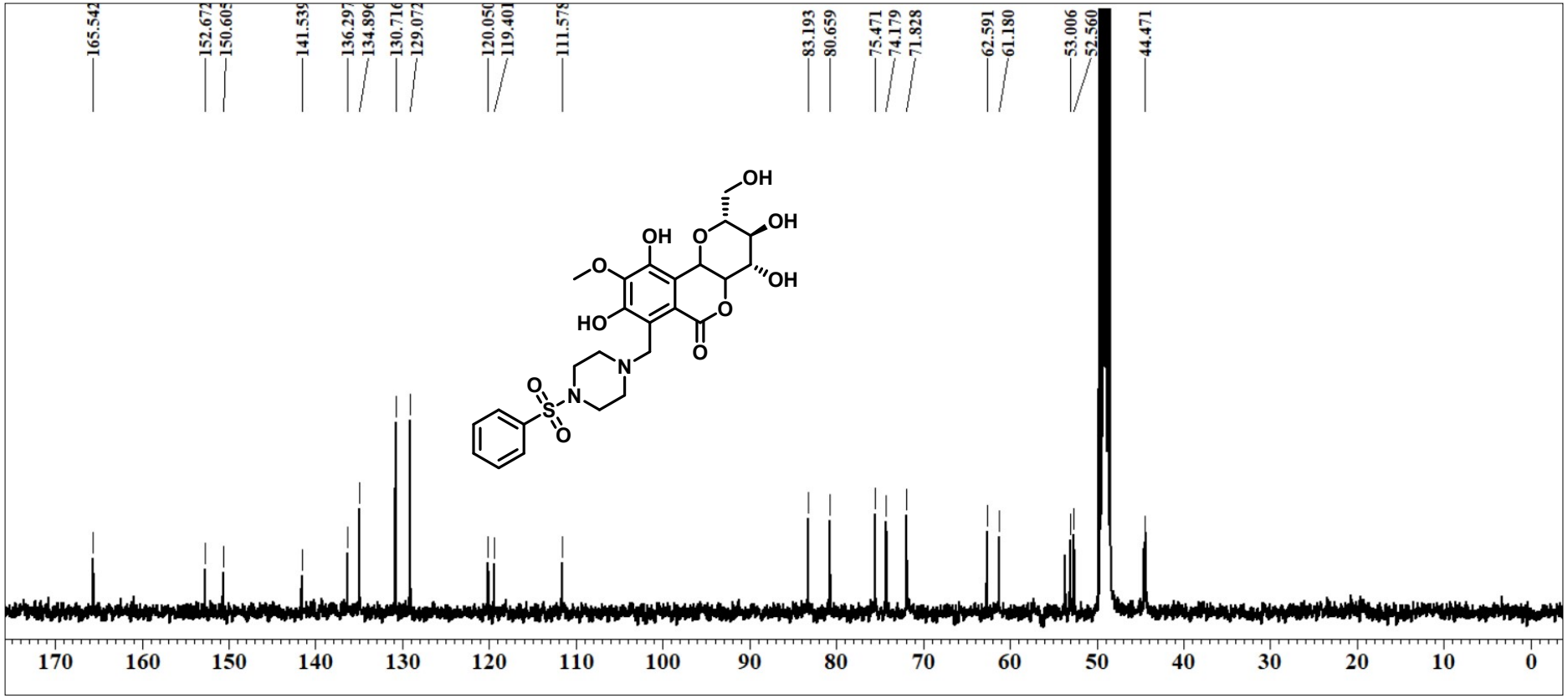
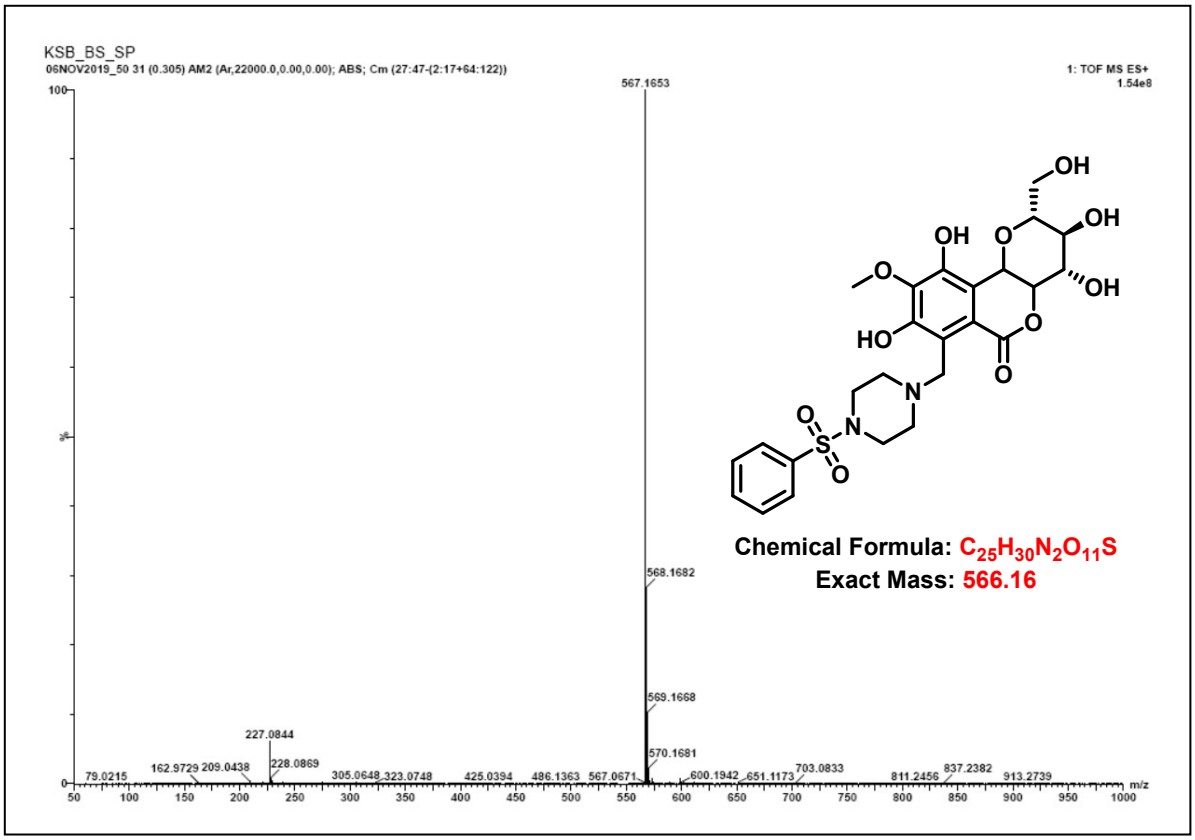


Fig S92: ^{13}C NMR SPECTRUM OF COMPOUND **13d** (100 MHz, CD_3OD)

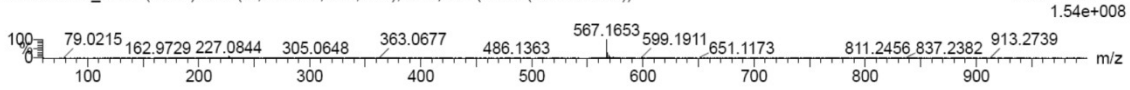


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 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
198 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-25 H: 0-35 N: 0-2 O: 0-13 S: 0-3

KSB_BS_SP
06NOV2019_50 31 (0.305) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (27:47-(2:17+64:122))
1: TOF MS ES+
1.54e+008



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
567.1653	567.1649	0.4	0.7	11.5	678.2	n/a	n/a	C25 H31 N2 O11 S

Fig S93: HRESIMS SPECTRUM OF COMPOUND **13d**

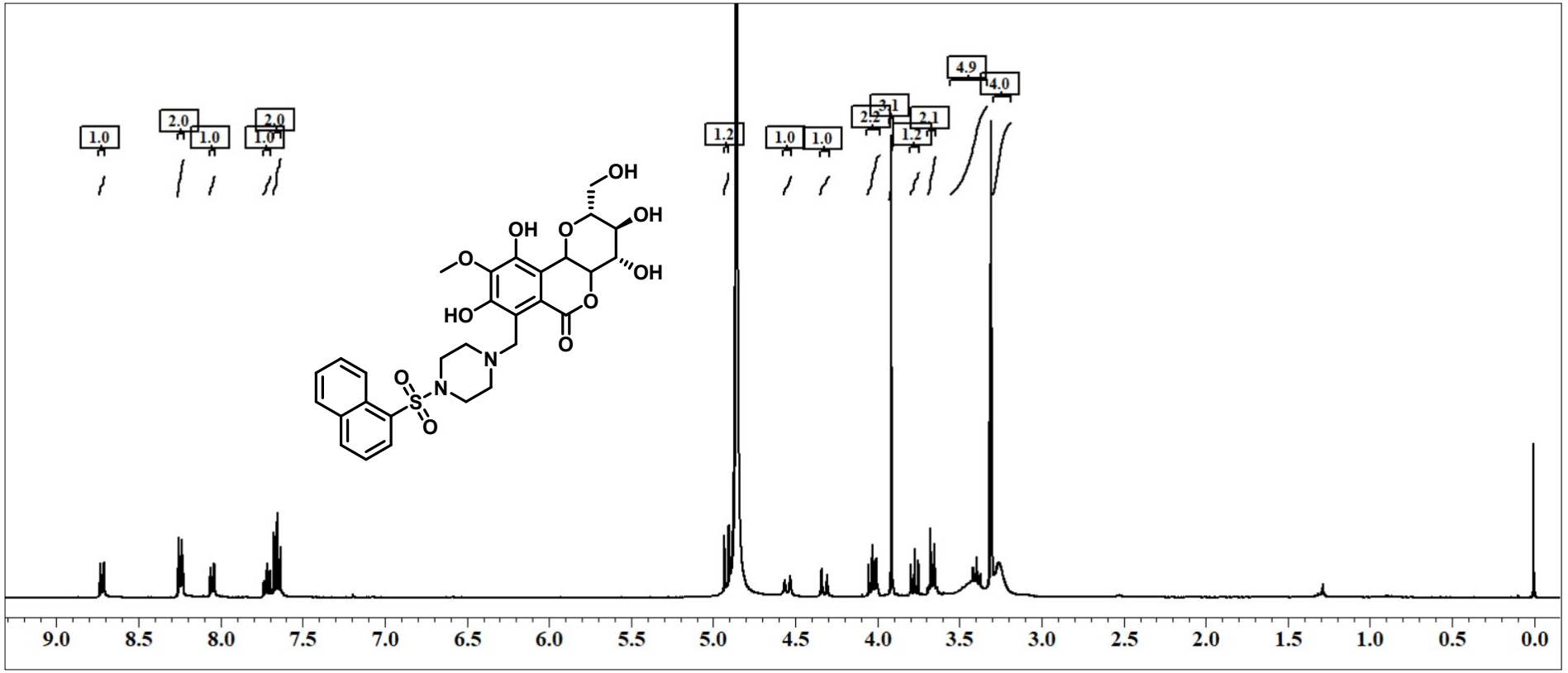


Fig S94: ^{1}H NMR SPECTRUM OF COMPOUND **13e** (400 MHz, CD_3OD)

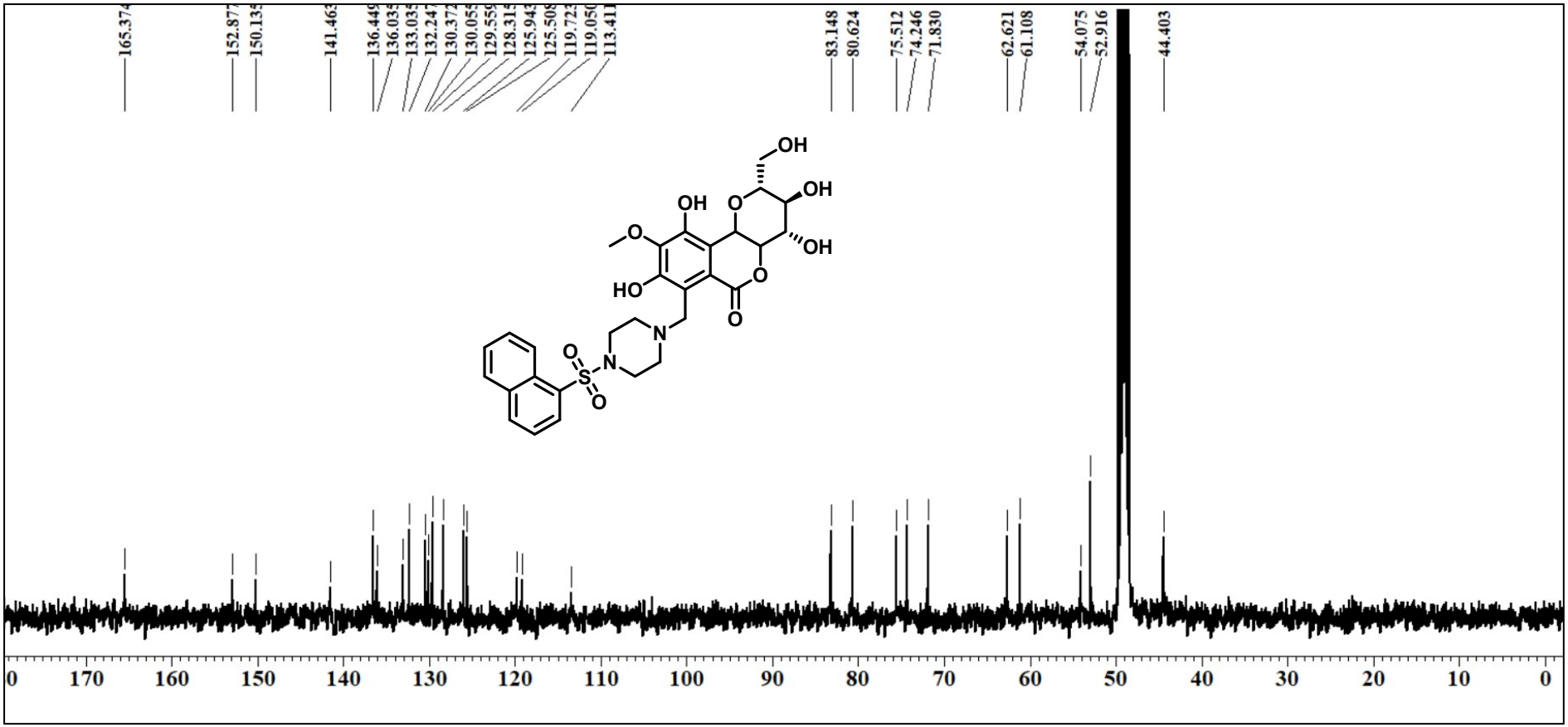
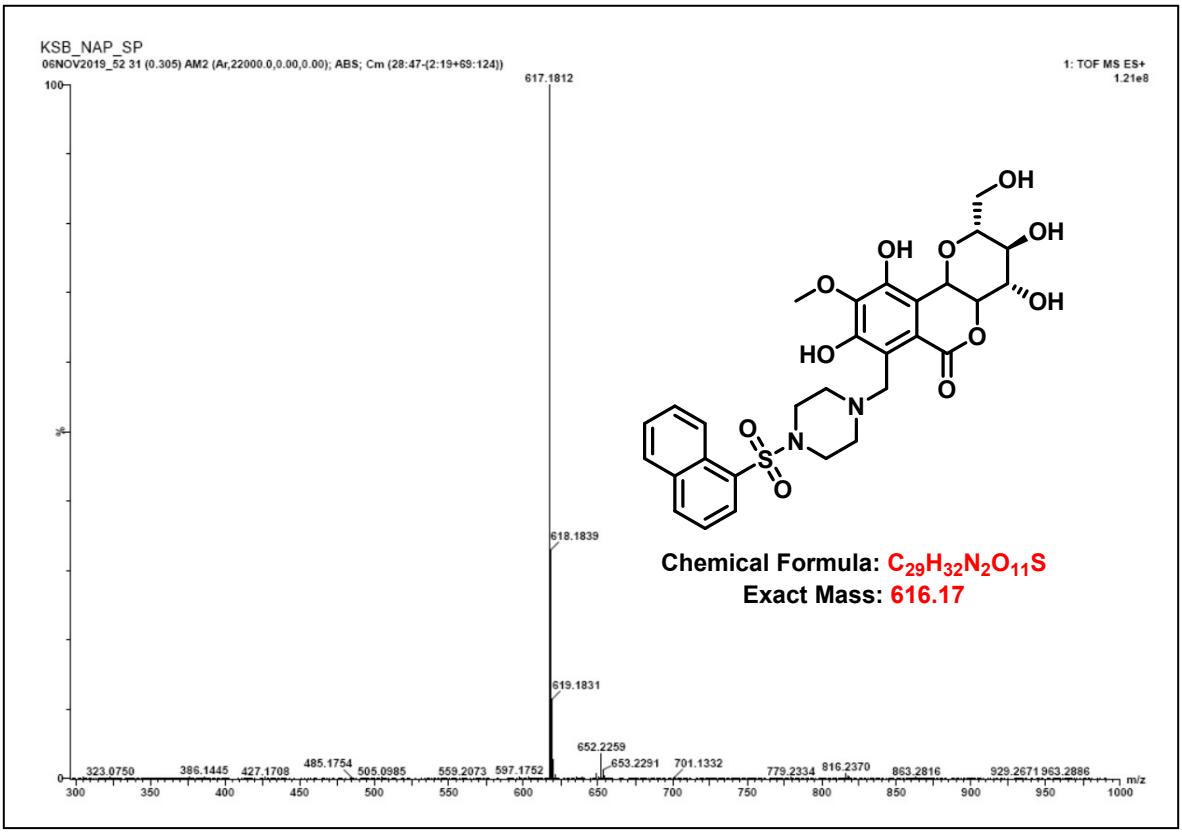


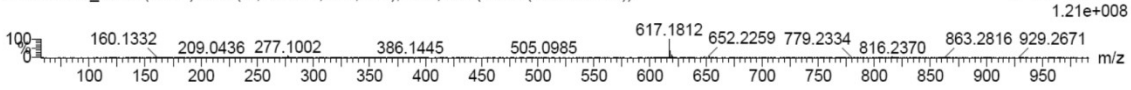
Fig S95: ^{13}C NMR SPECTRUM OF COMPOUND **13e** (100 MHz, CD_3OD)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 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
194 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-29 H: 0-35 N: 0-2 O: 0-13 S: 0-3
KSB_NAP_SP
06NOV2019_52 31 (0.305) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (28:47-(2:19+69:124))
1: TOF MS ES+
1.21e+008



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
617.1812	617.1805	0.7	1.1	14.5	680.8	n/a	n/a	C29 H33 N2 O11 S

Fig S96: HRESIMS SPECTRUM OF COMPOUND **13**

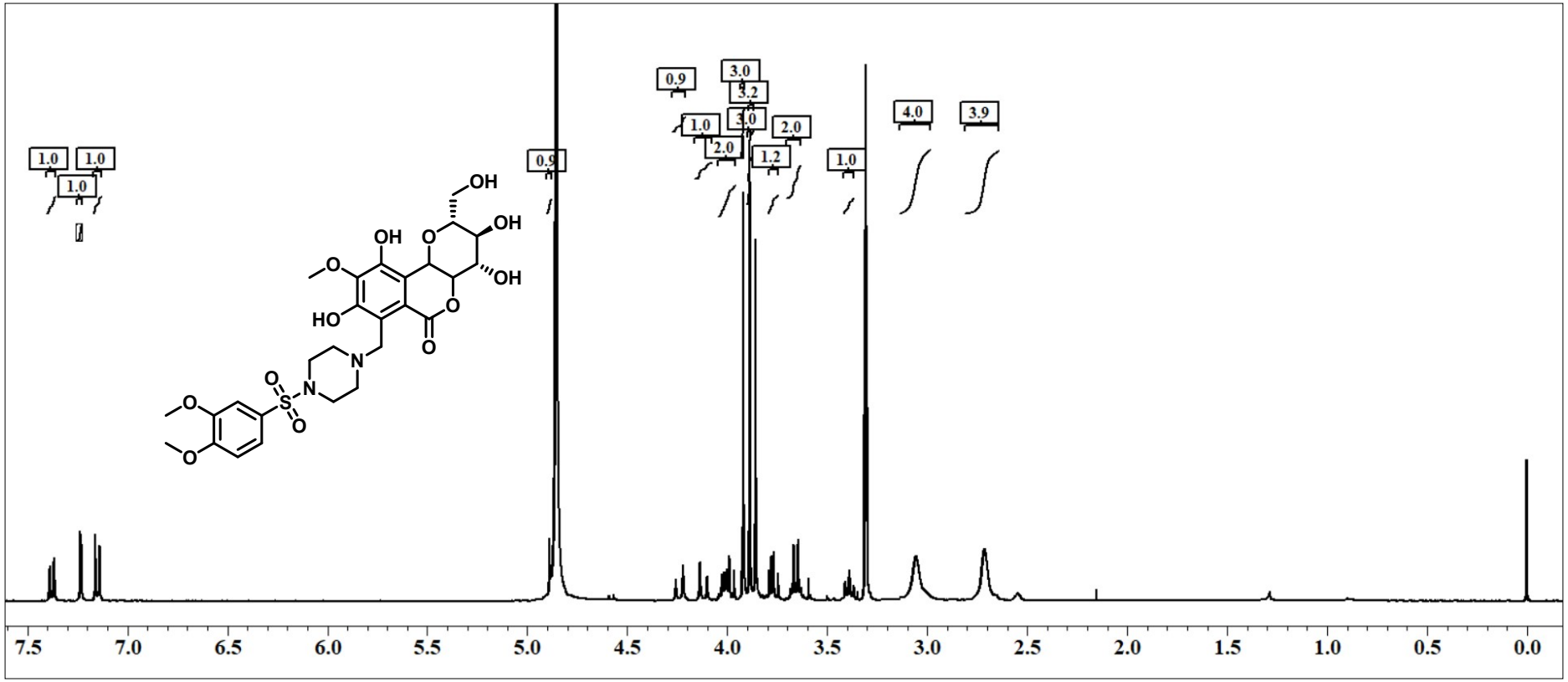


Fig S97: ^{1}H NMR SPECTRUM OF COMPOUND **13f** (400 MHz, CD_3OD)

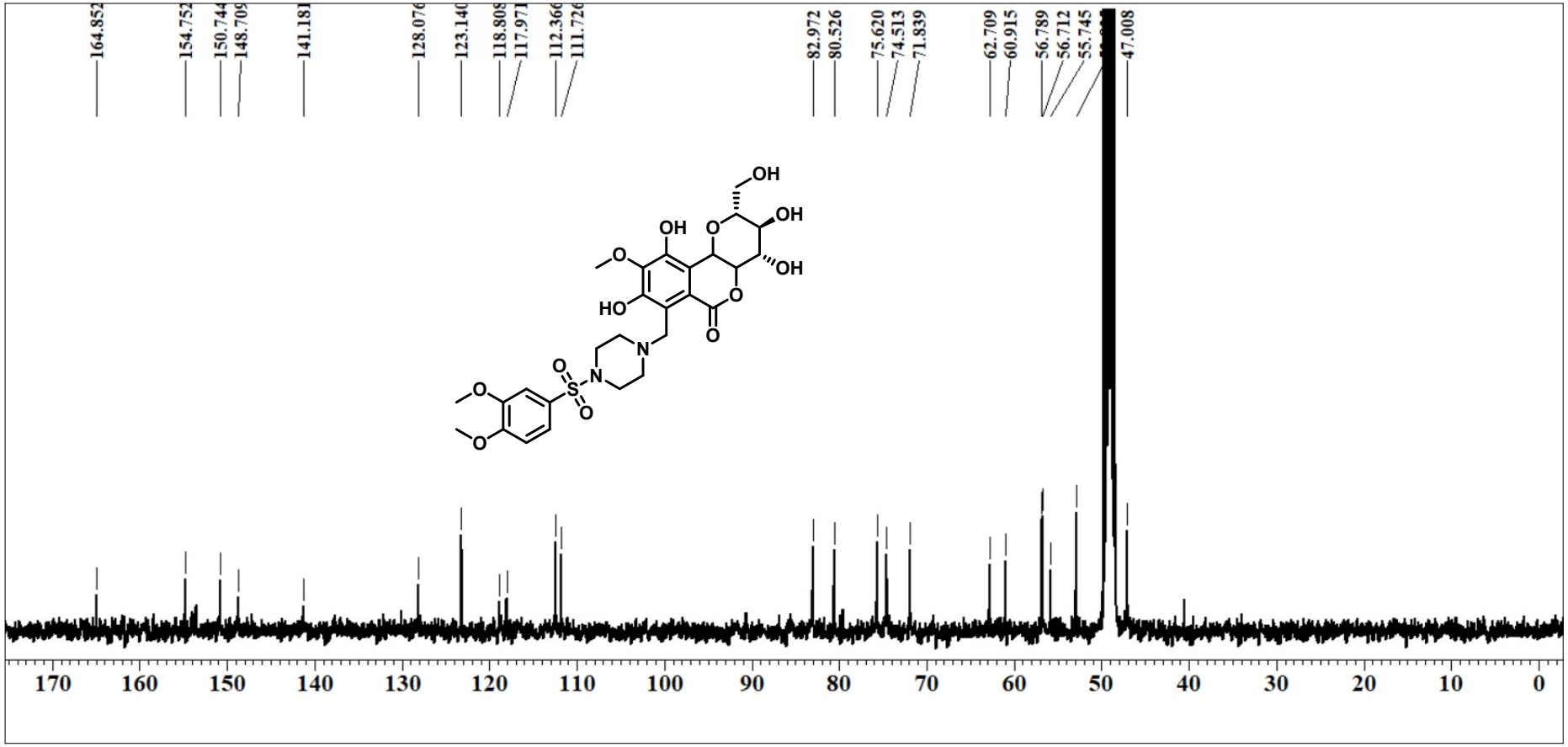


Fig S98: ^{13}C NMR SPECTRUM OF COMPOUND **13f** (100 MHz, CD_3OD)

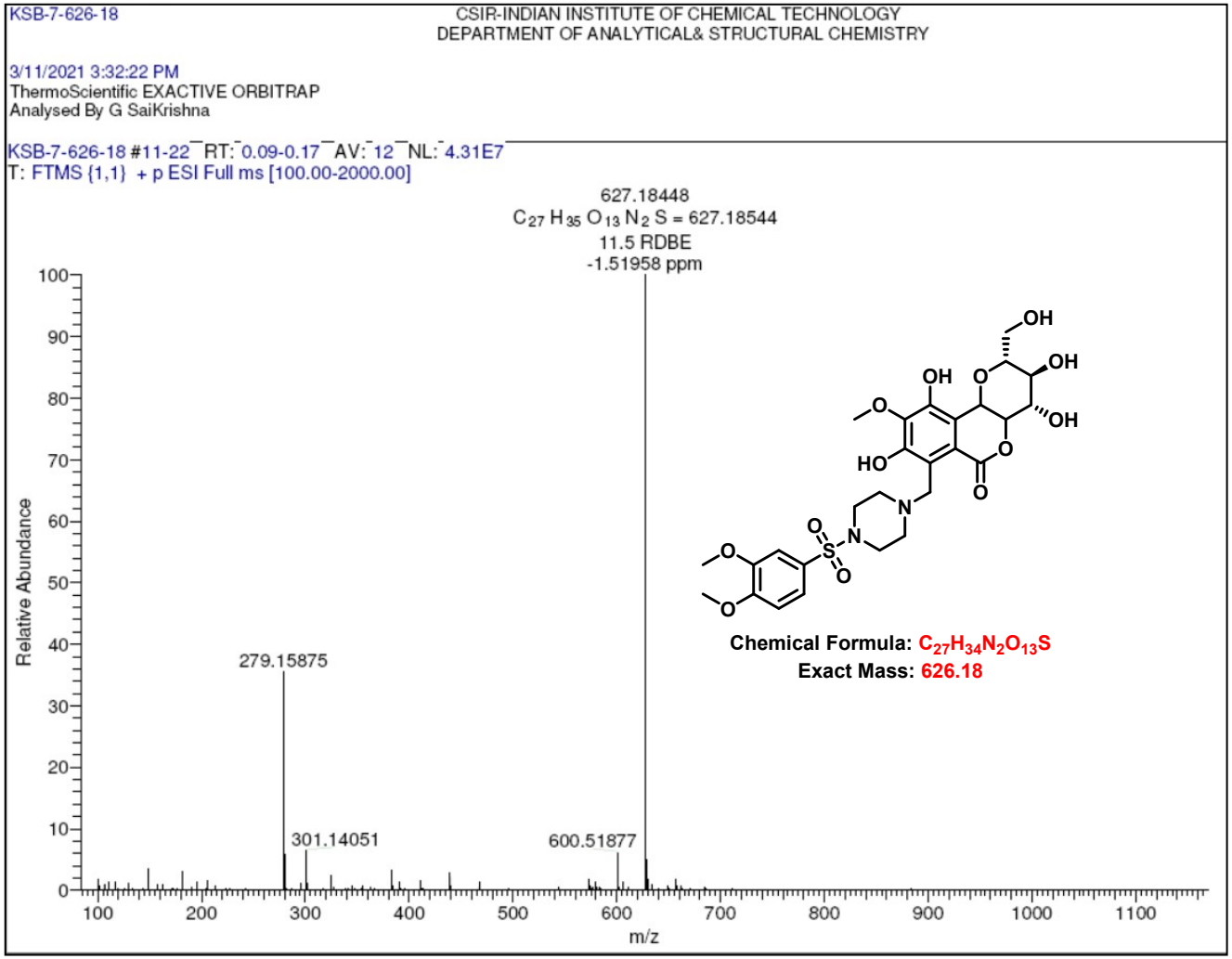


Fig S99: HRESIMS SPECTRUM OF COMPOUND **13f**

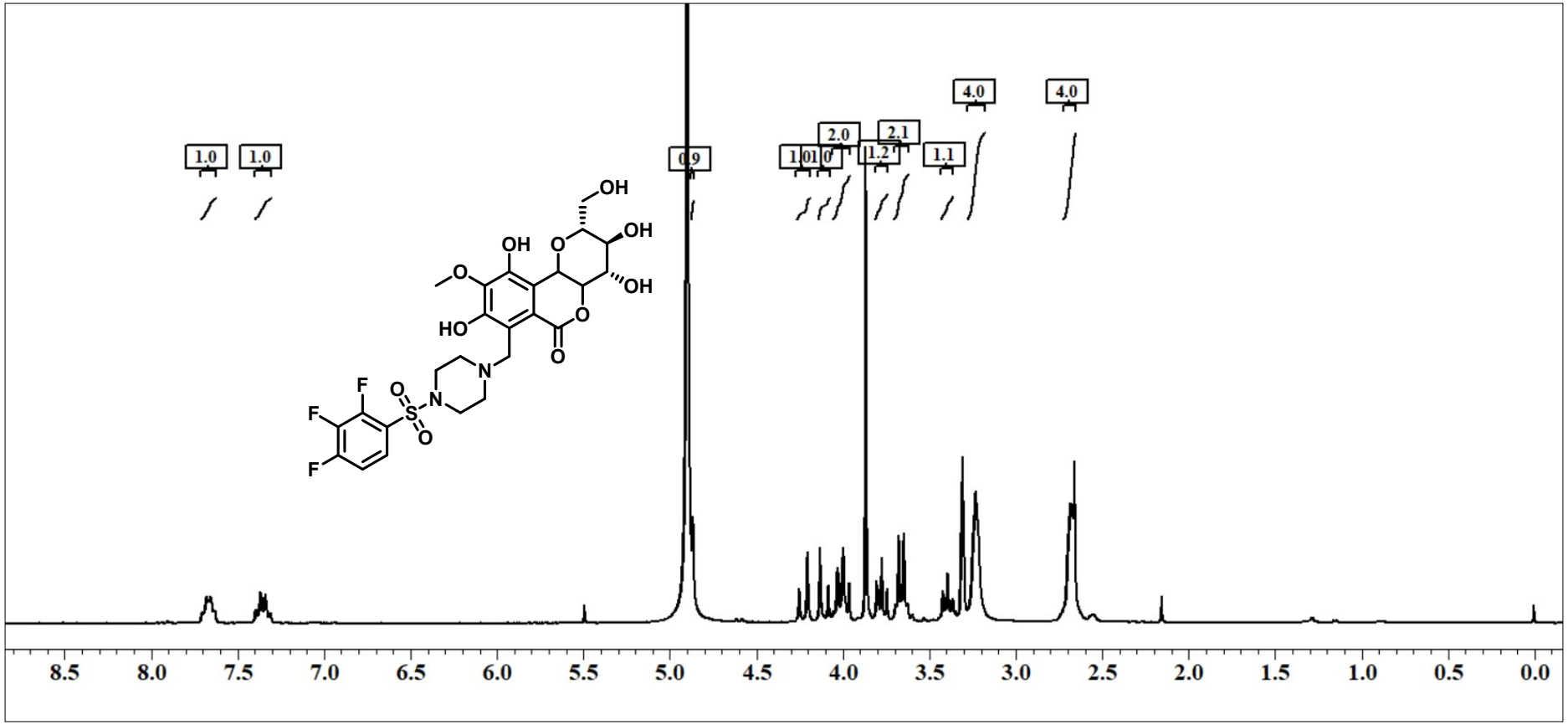


Fig S100: ^{1}H NMR SPECTRUM OF COMPOUND **13g** (300 MHz, CD_3OD)

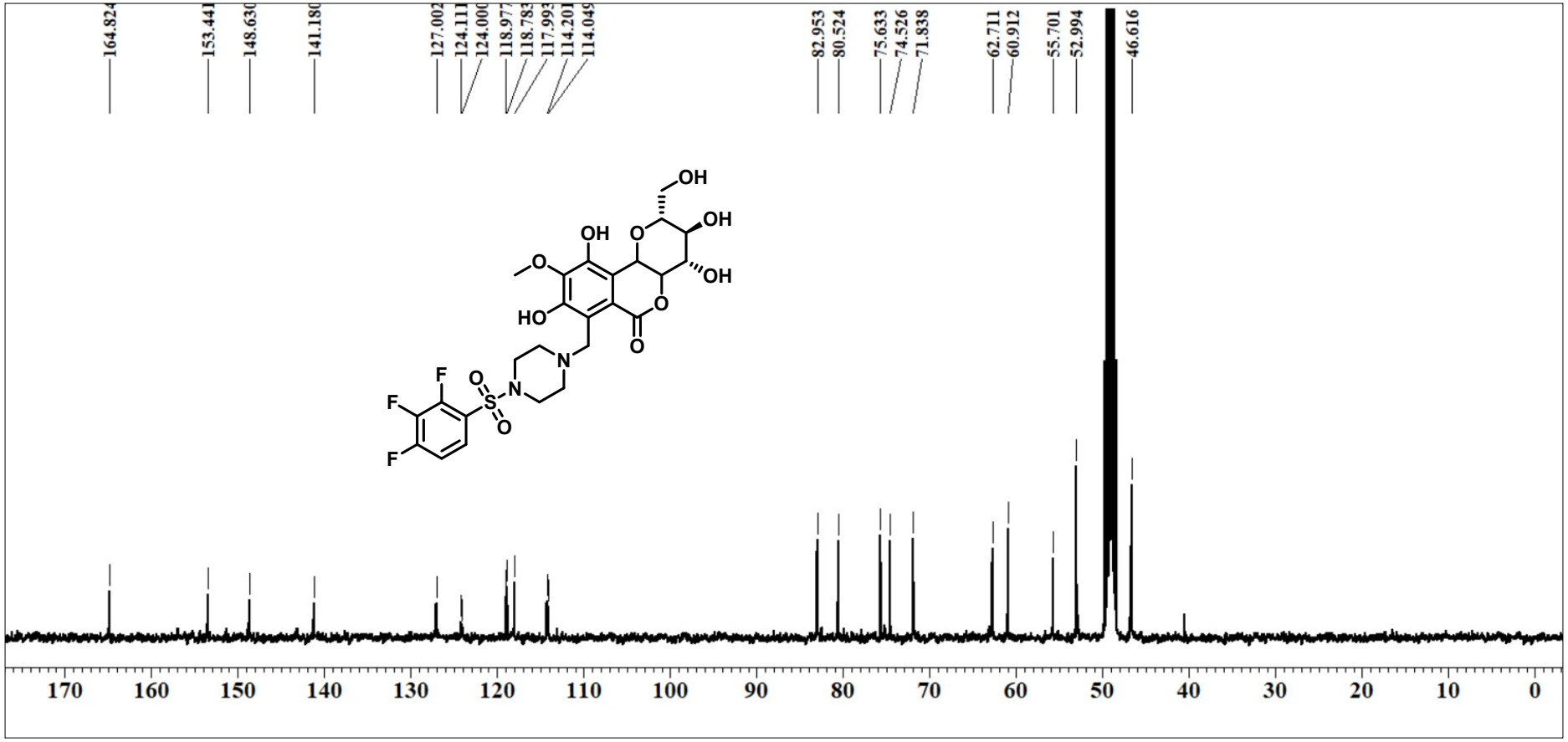


Fig S101: ^{13}C NMR SPECTRUM OF COMPOUND **13g** (100 MHz, CD_3OD)

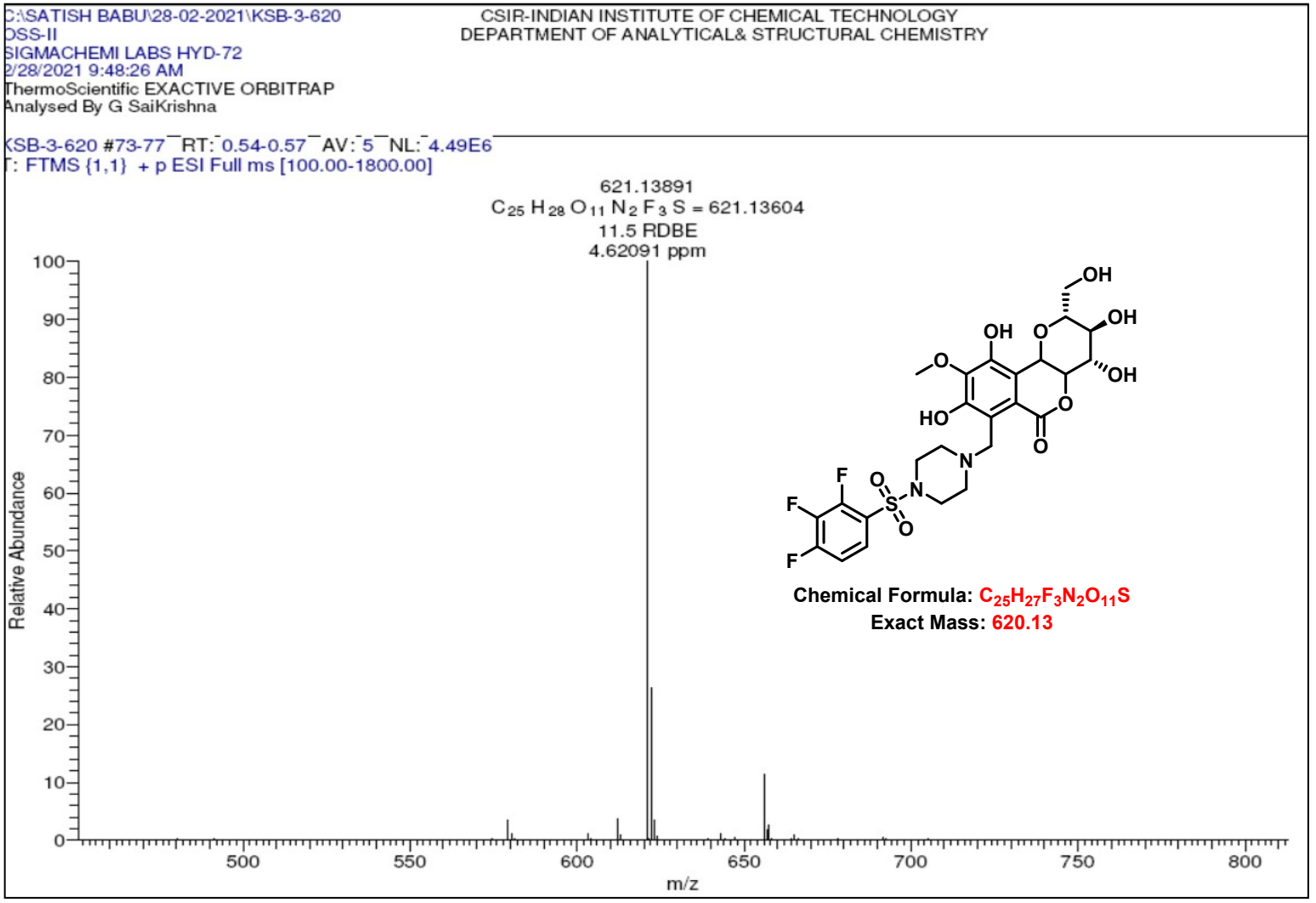


Fig S102: HRESIMS SPECTRUM OF COMPOUND **13g**

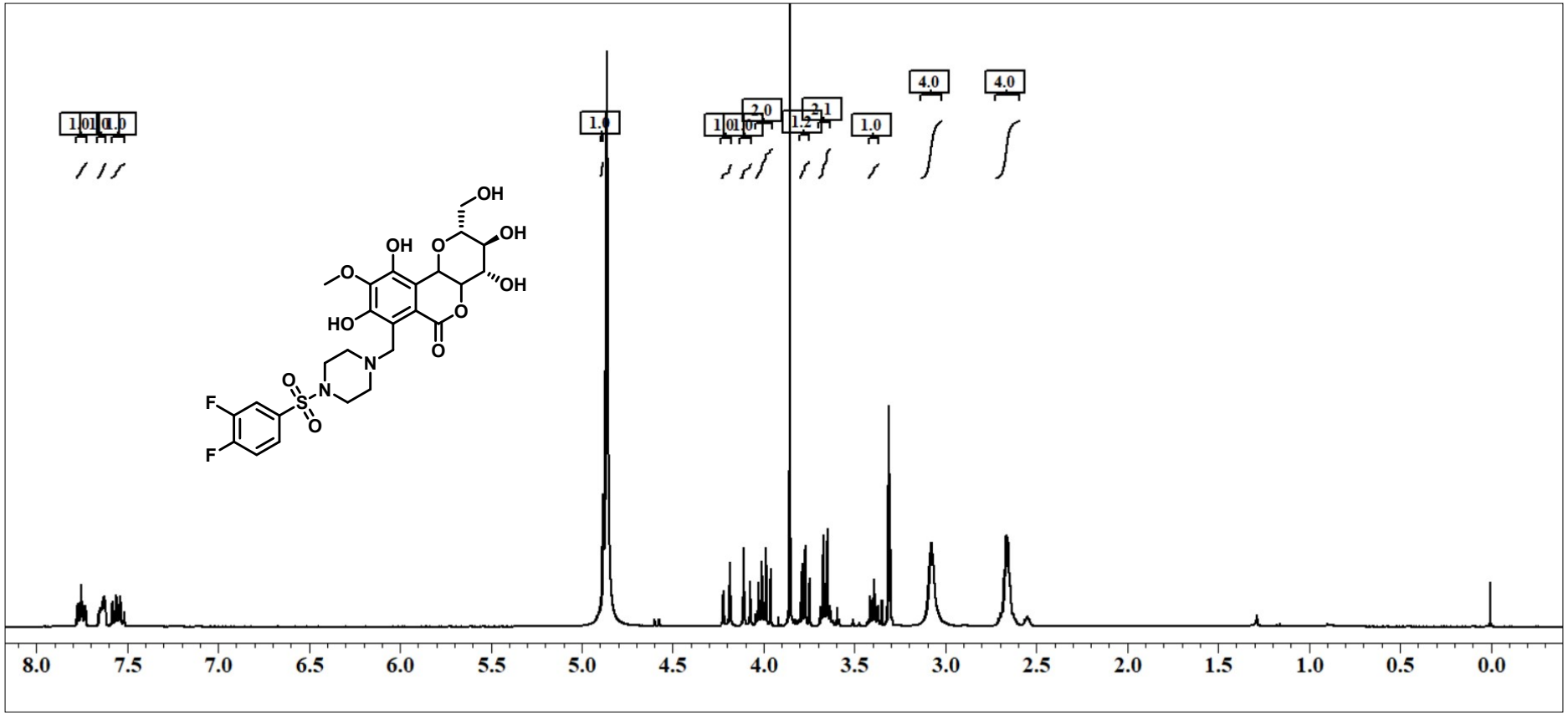


Fig S103: ^{1}H NMR SPECTRUM OF COMPOUND **13h** (400 MHz, CD_3OD)

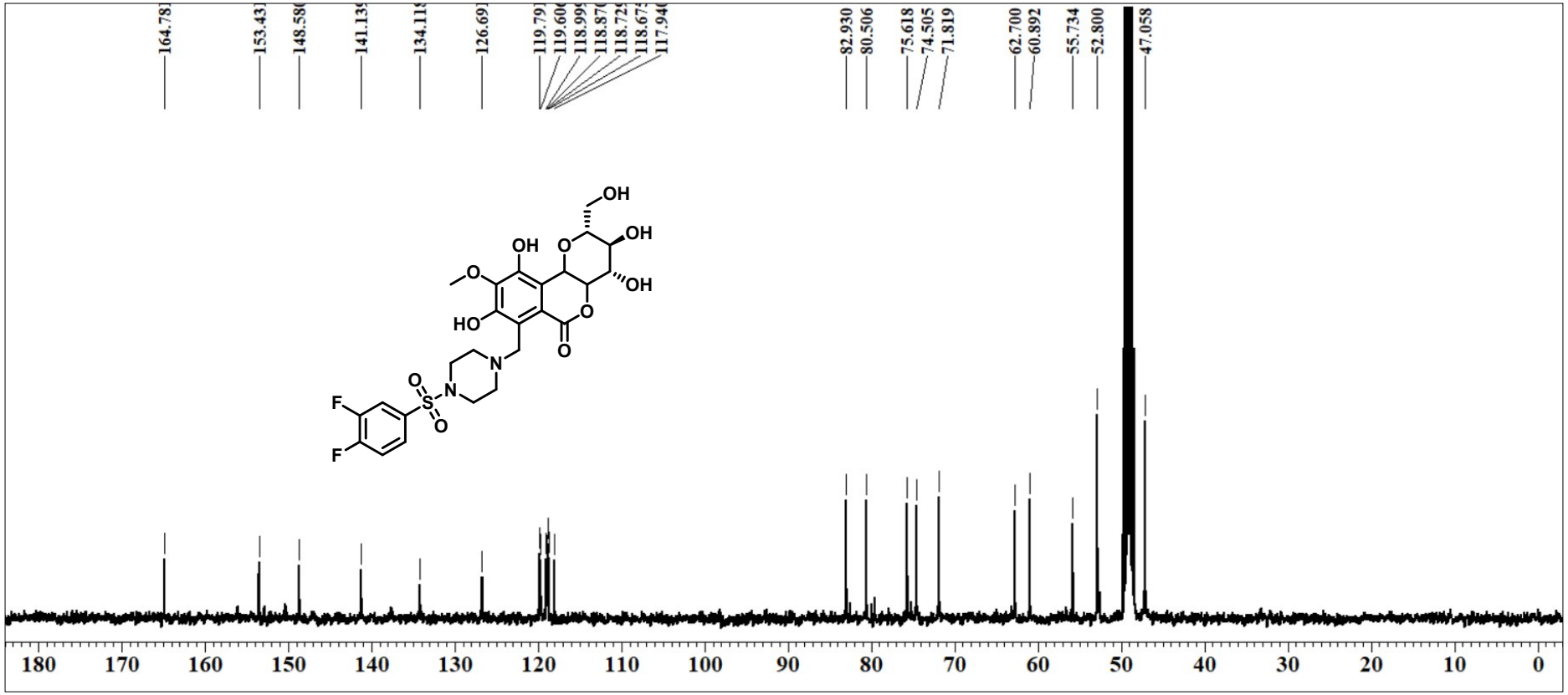


Fig S104: ^{13}C NMR SPECTRUM OF COMPOUND **13h** (100 MHz, CD_3OD)

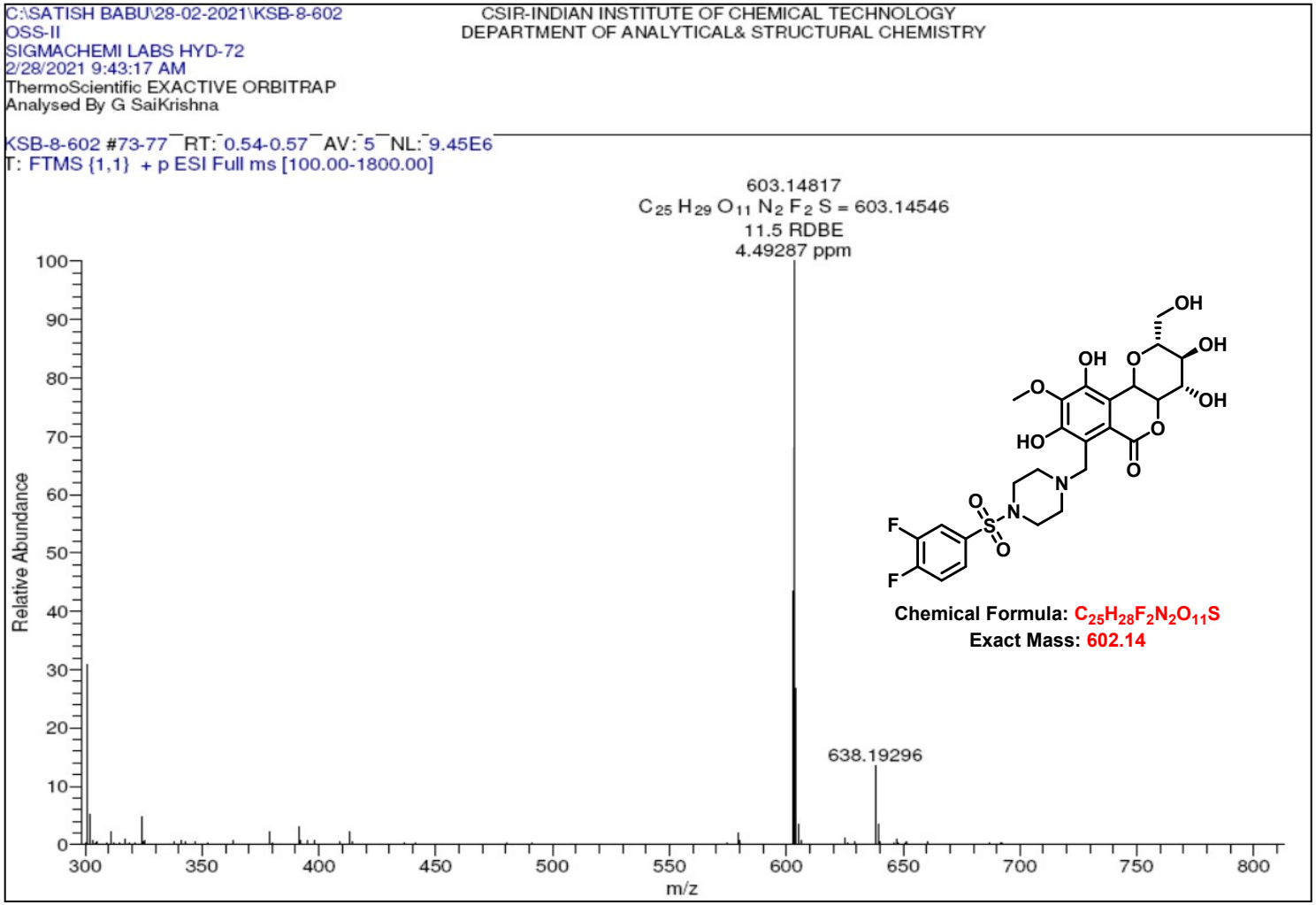


Fig S105: HRESIMS SPECTRUM OF COMPOUND **13h**

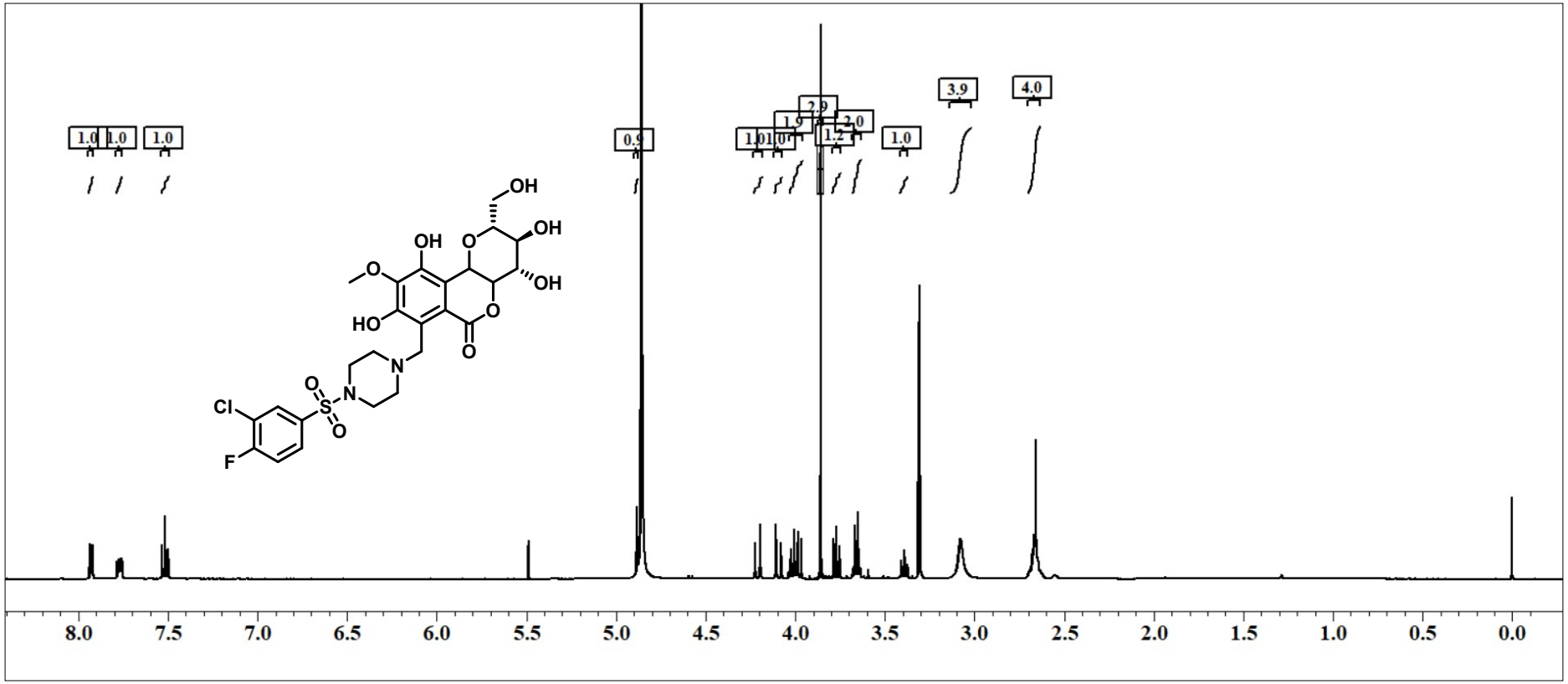


Fig S106: ^{1}H NMR SPECTRUM OF COMPOUND **13i** (500 MHz, CD_3OD)

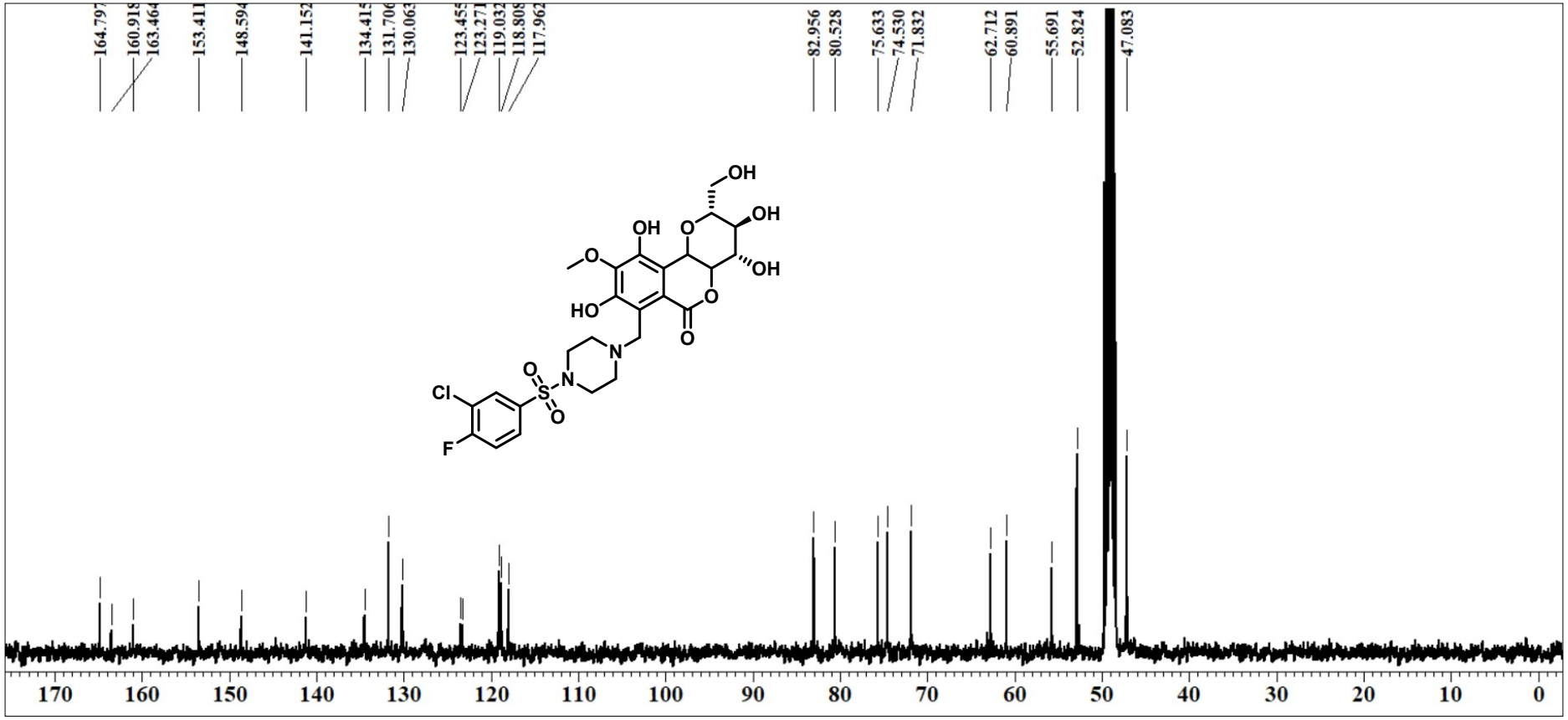


Fig S107: ^{13}C NMR SPECTRUM OF COMPOUND **13i** (100 MHz, CD_3OD)

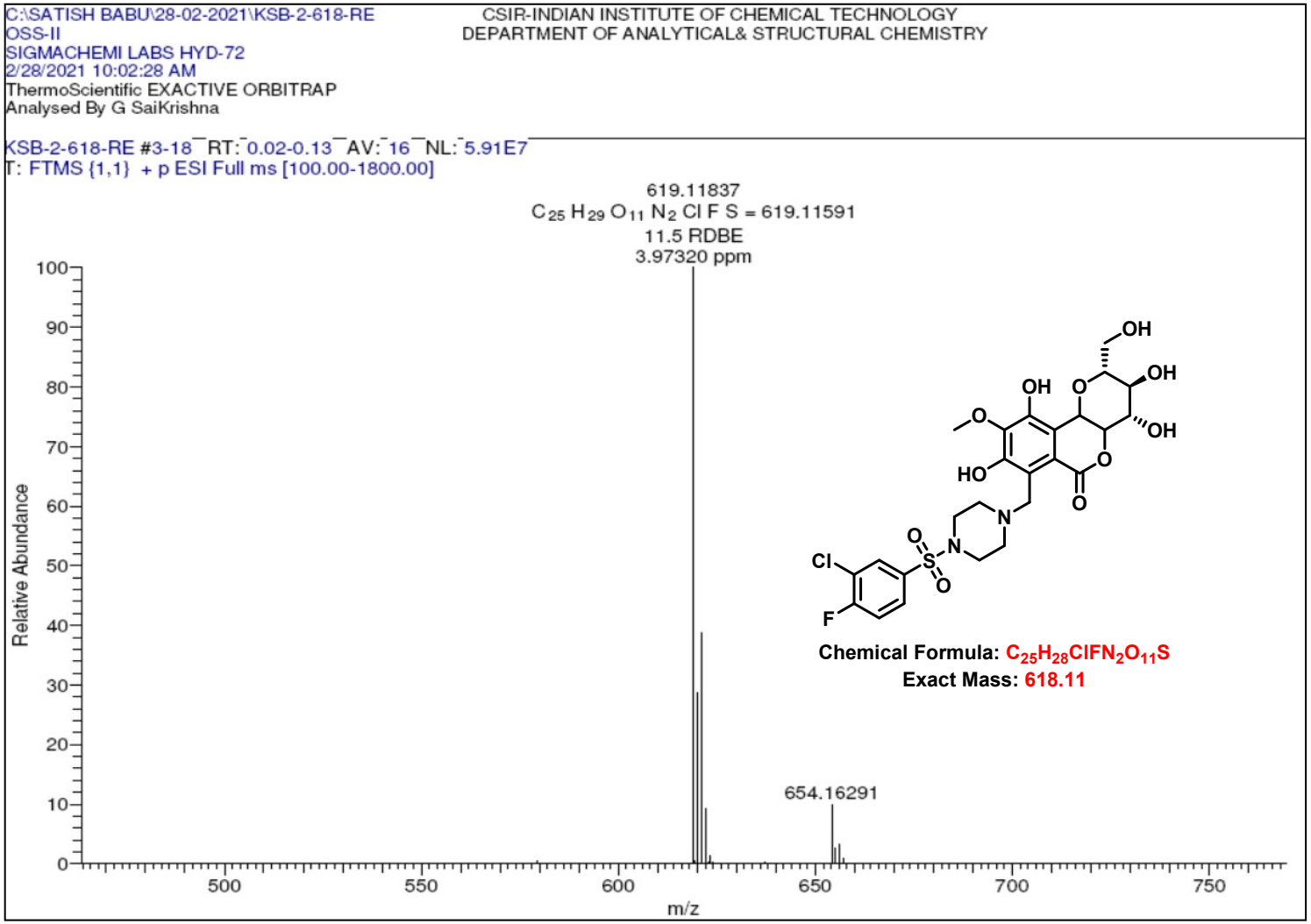


Fig S108: HRESIMS SPECTRUM OF COMPOUND **13i**

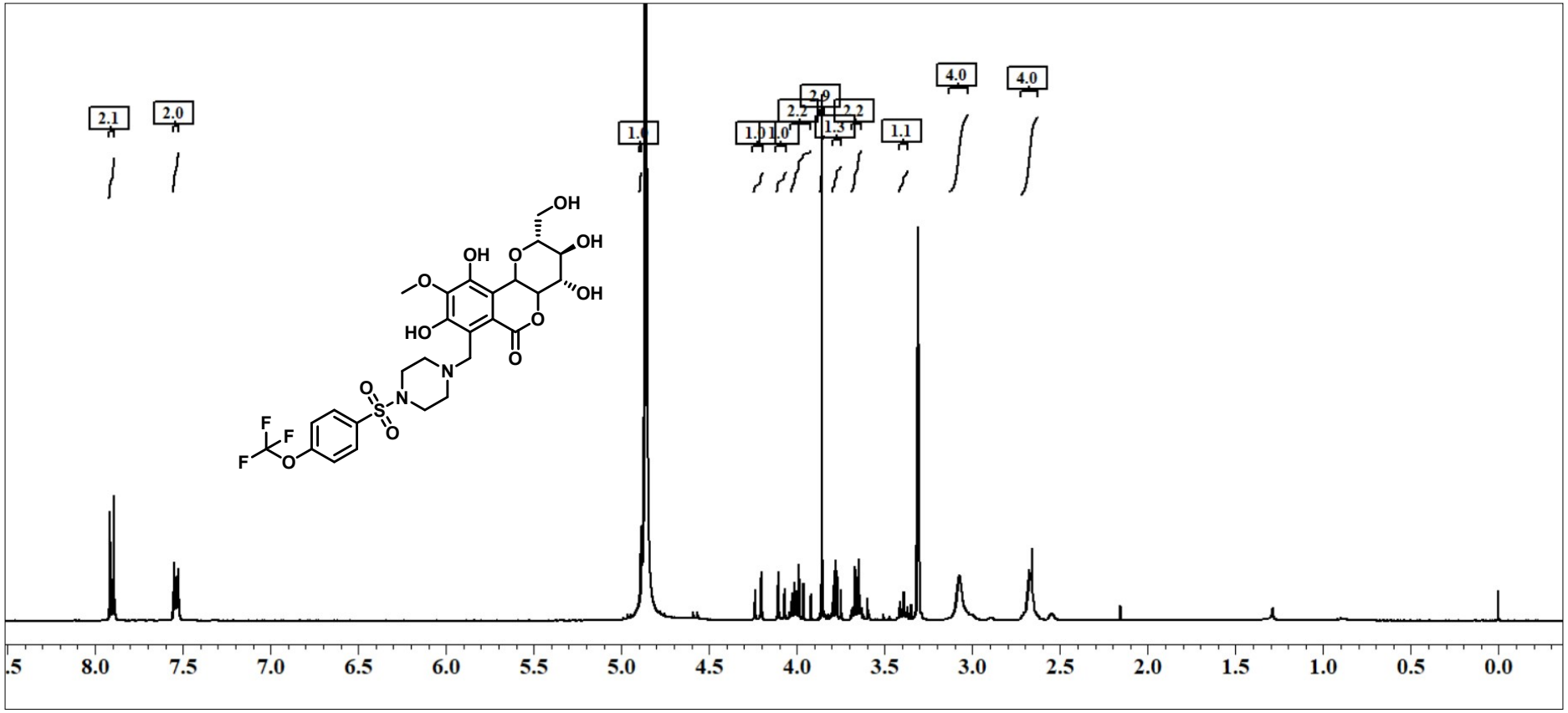


Fig S109: ^{1}H NMR SPECTRUM OF COMPOUND **13j** (400 MHz, CD_3OD)

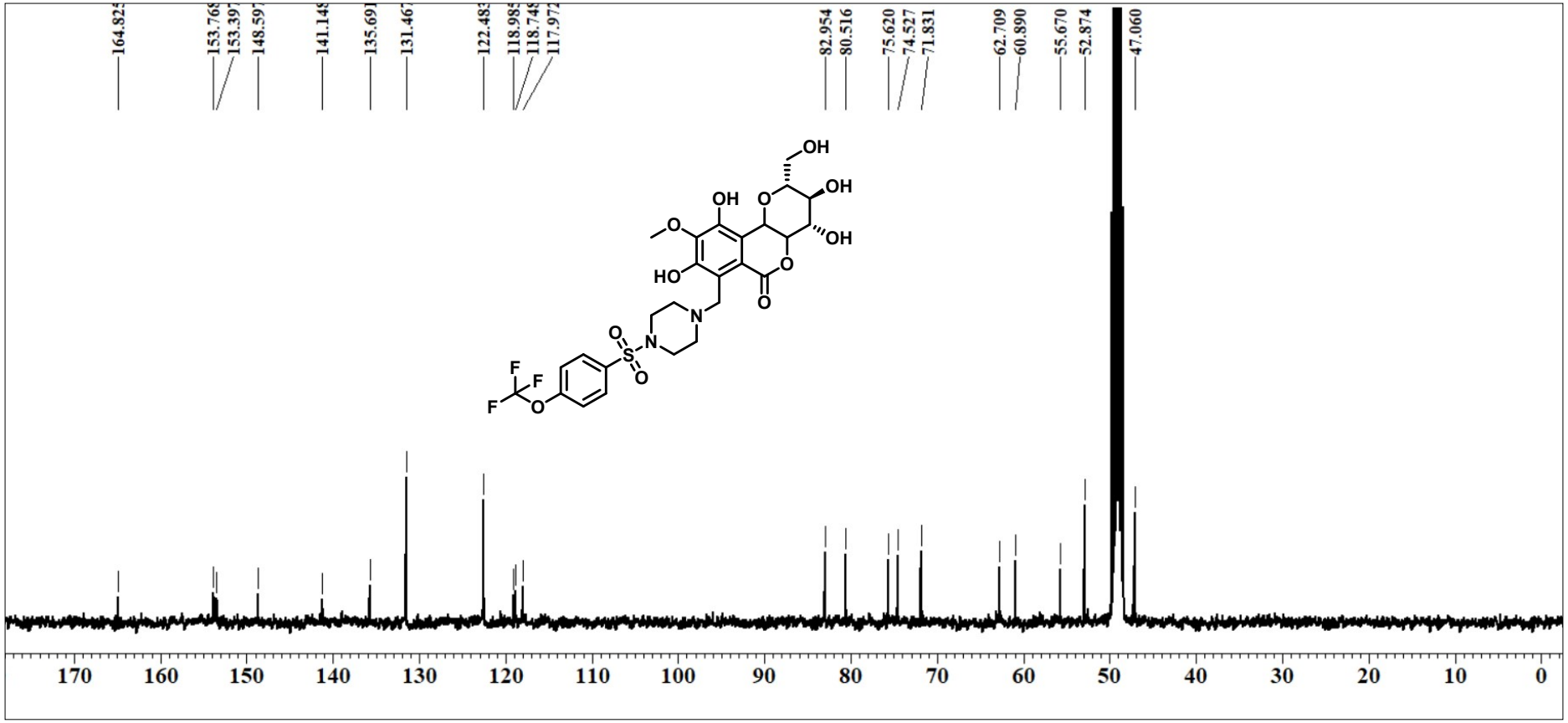


Fig S110: ^{13}C NMR SPECTRUM OF COMPOUND **13j** (100 MHz, CD_3OD)

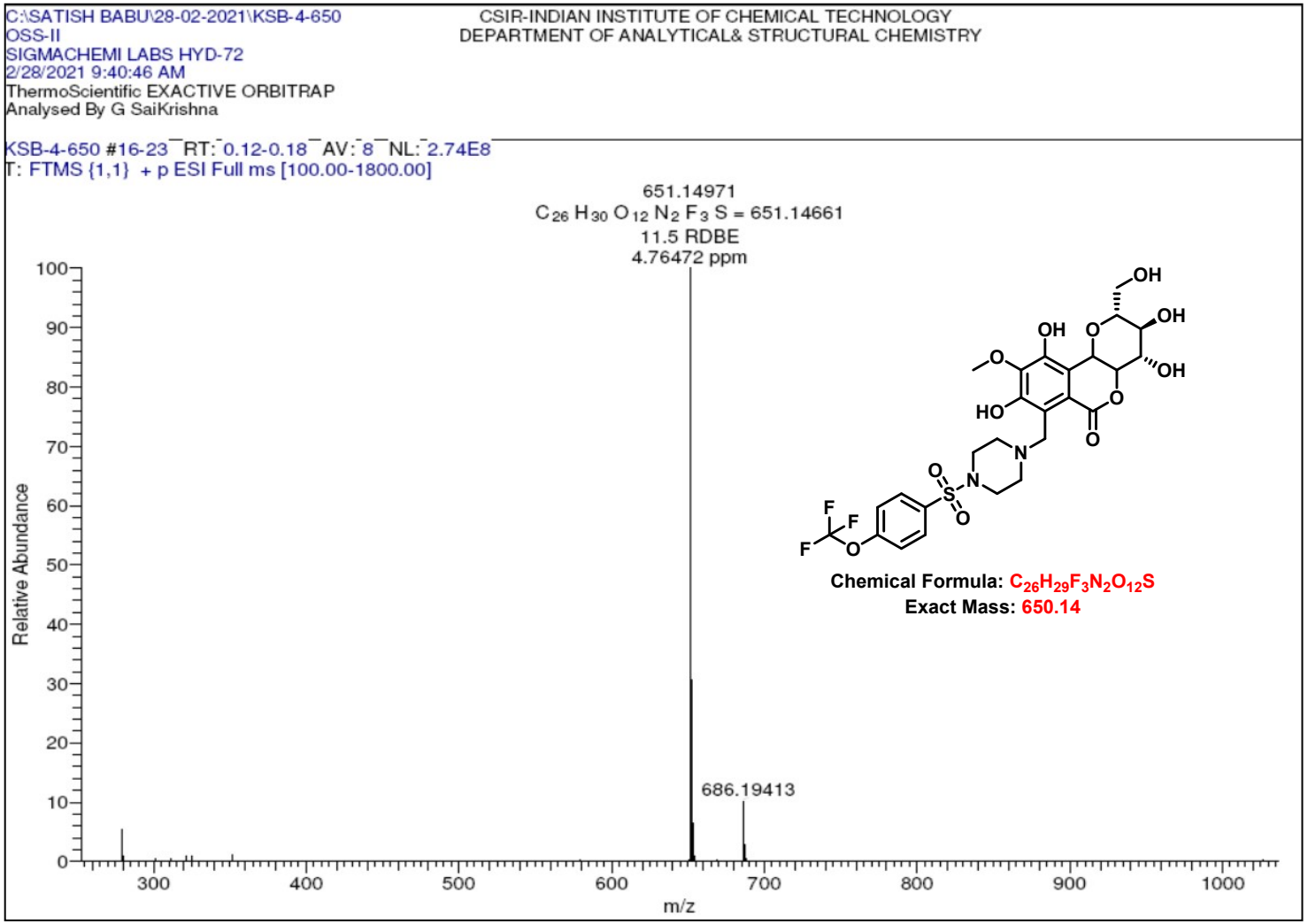


Fig S111: HRESIMS SPECTRUM OF COMPOUND **13j**

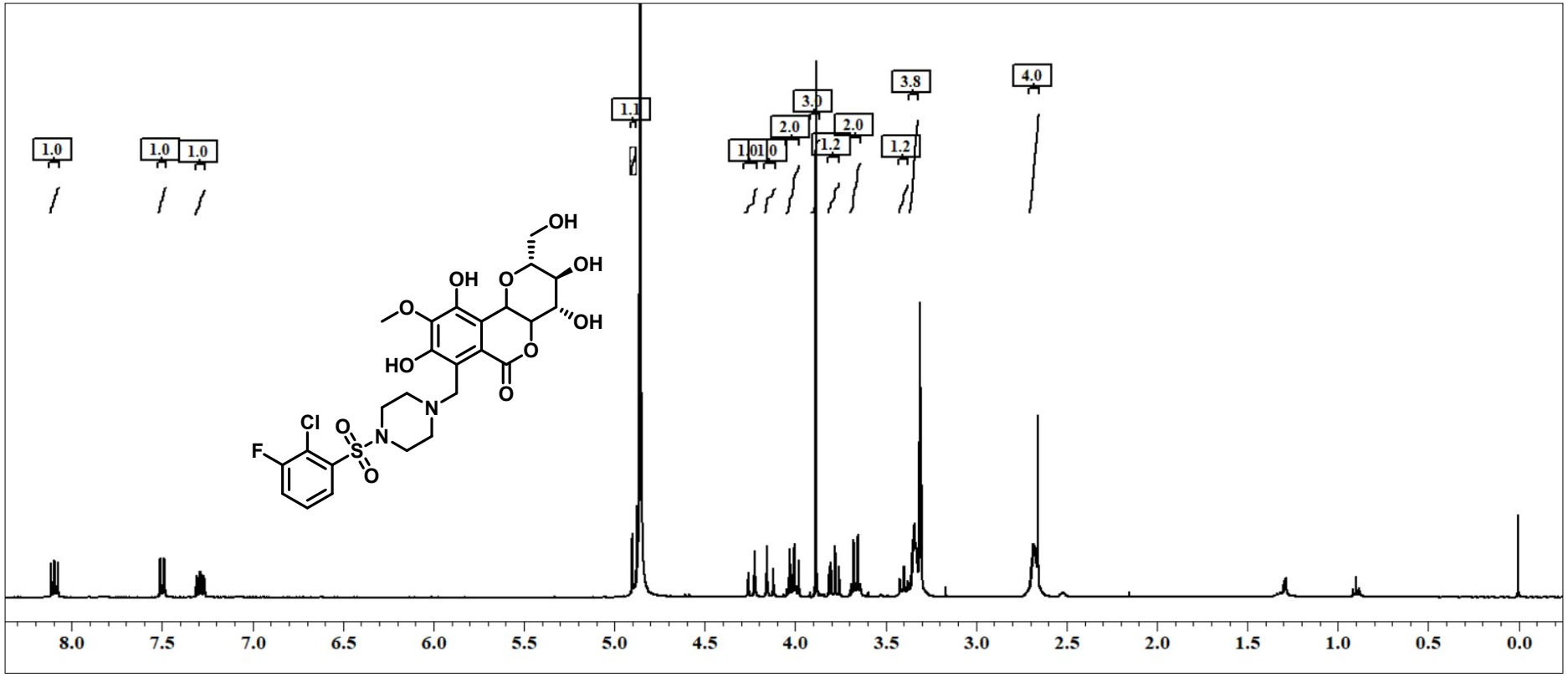


Fig S112: ^{1}H NMR SPECTRUM OF COMPOUND **13k** (400 MHz, CD_3OD)

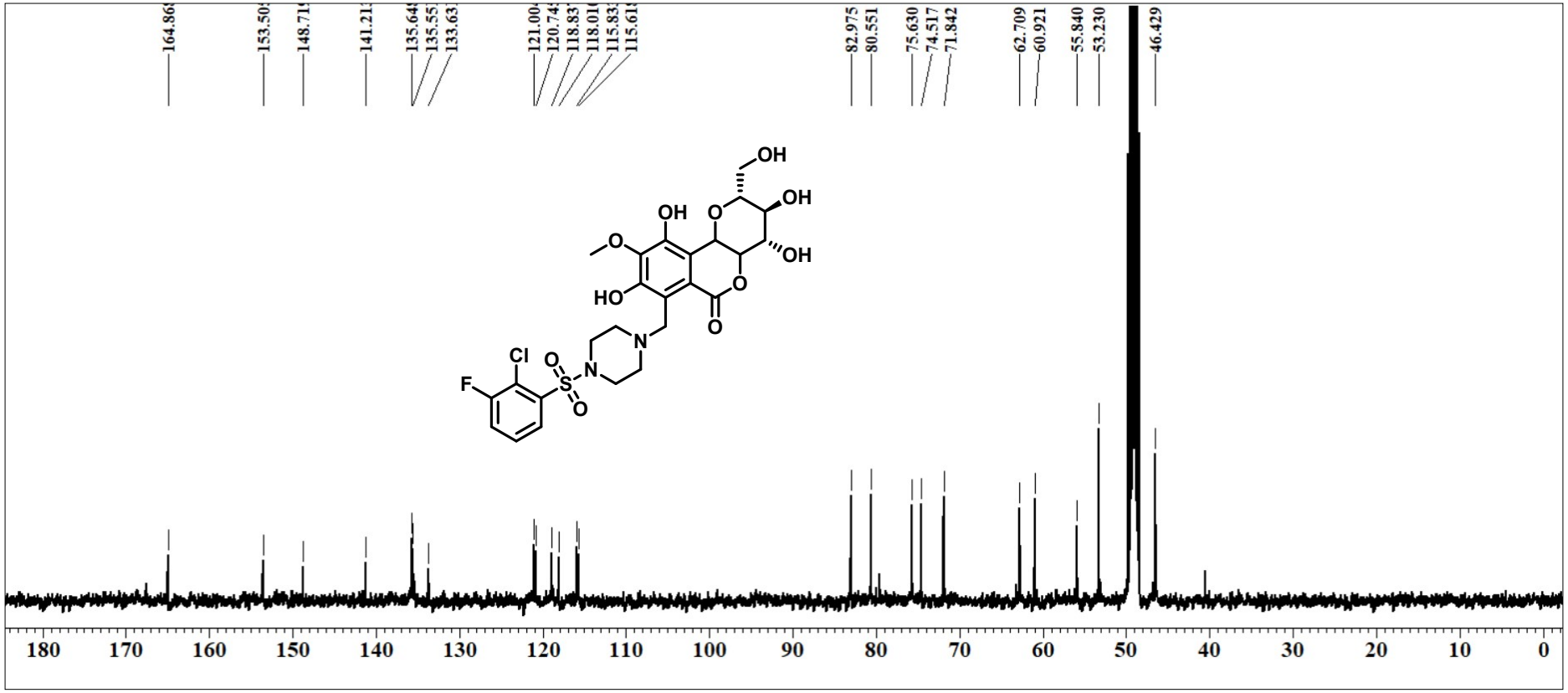


Fig S113: ^{13}C NMR SPECTRUM OF COMPOUND **13k** (100 MHz, CD_3OD)

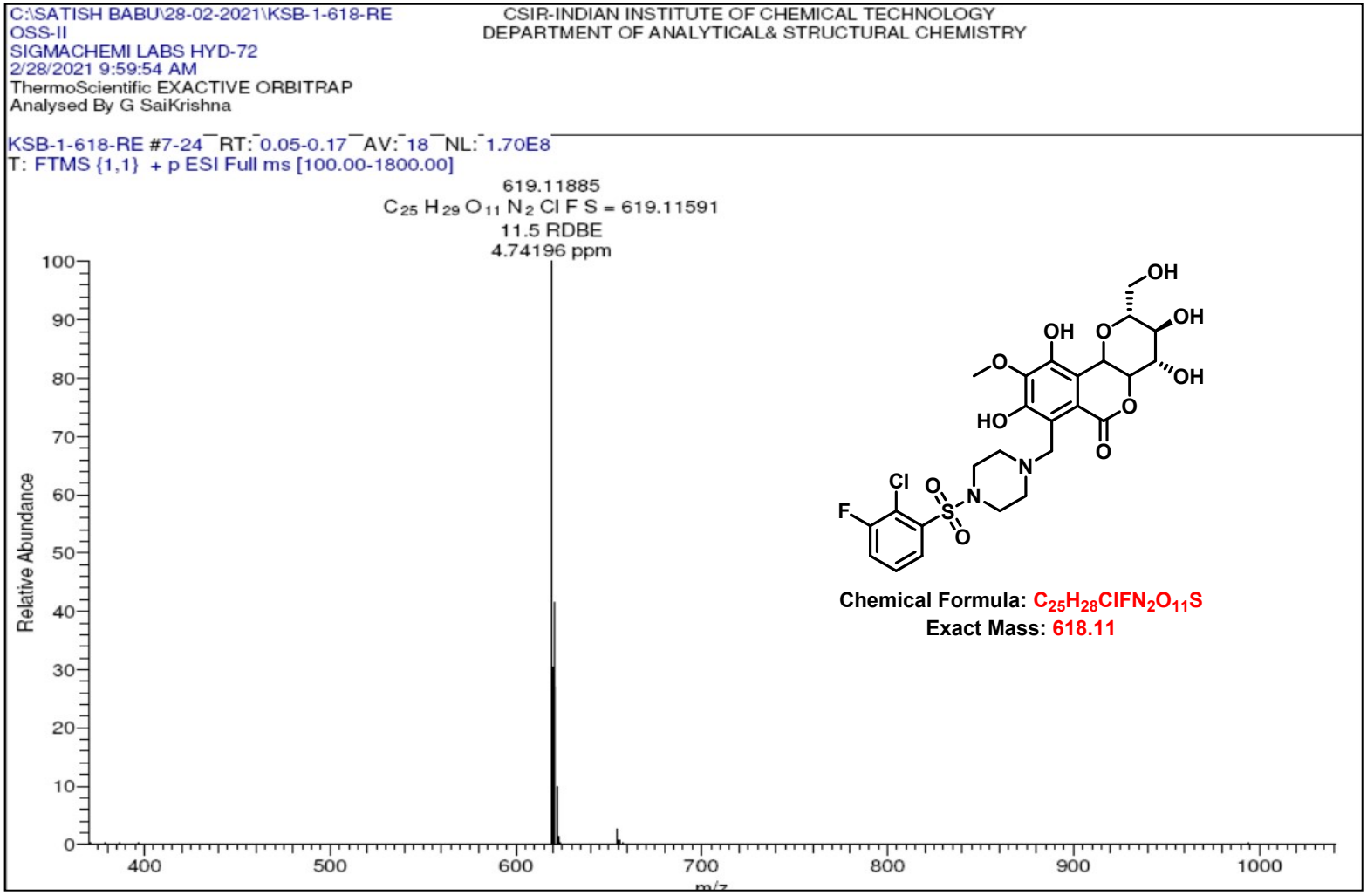


Fig S114: HRESIMS SPECTRUM OF COMPOUND **13k**

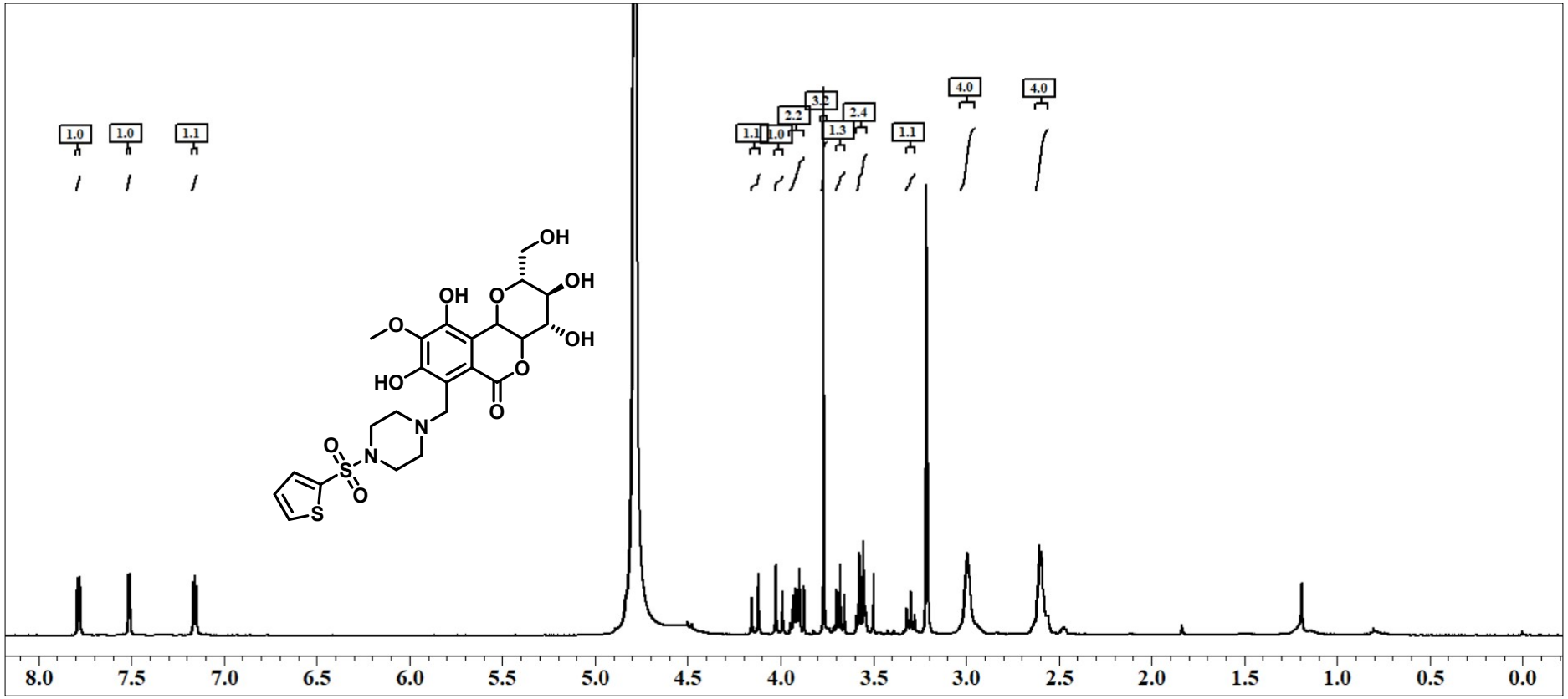


Fig S115: ^{1}H NMR SPECTRUM OF COMPOUND **13l** (400 MHz, CD_3OD)

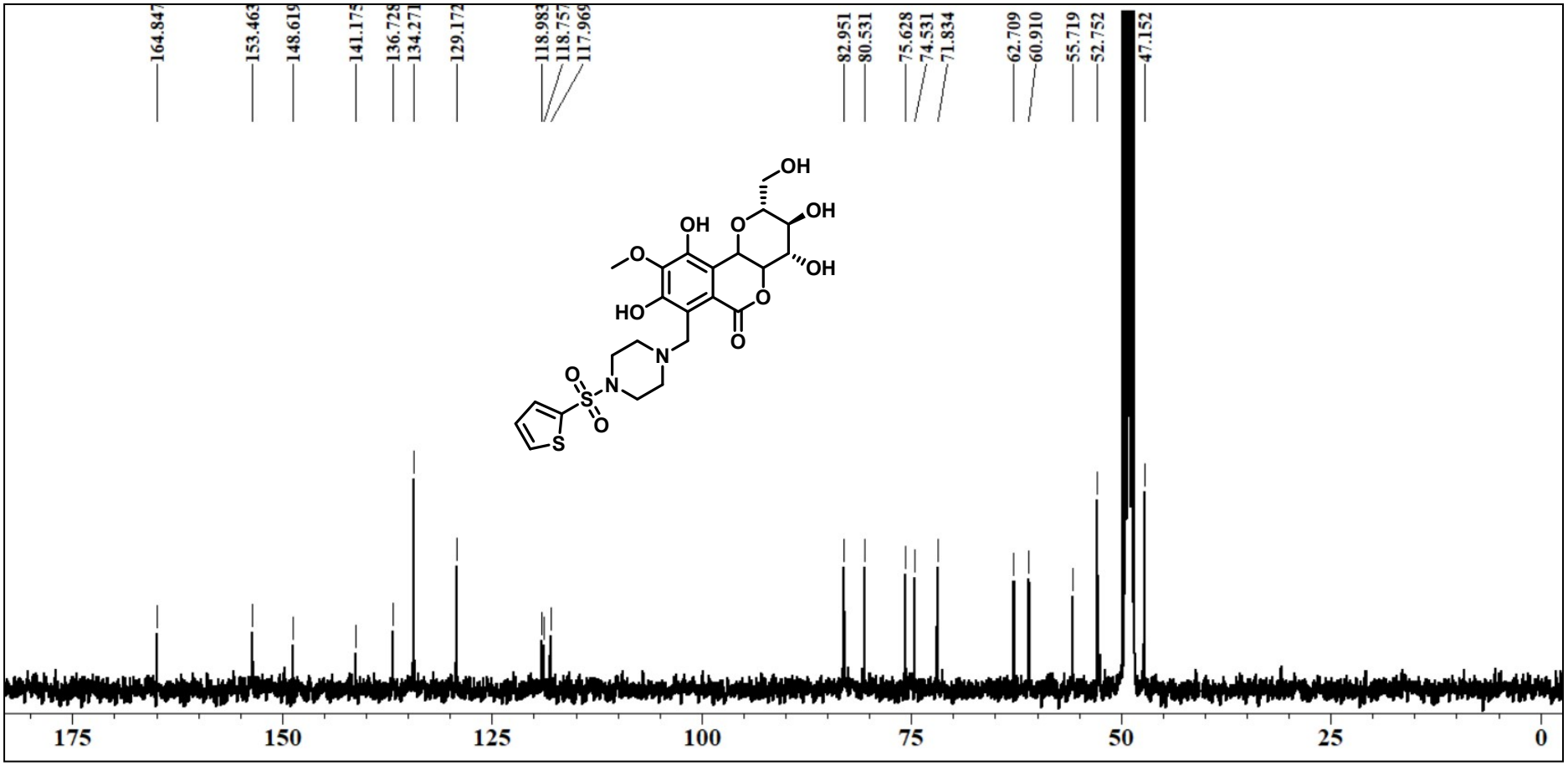
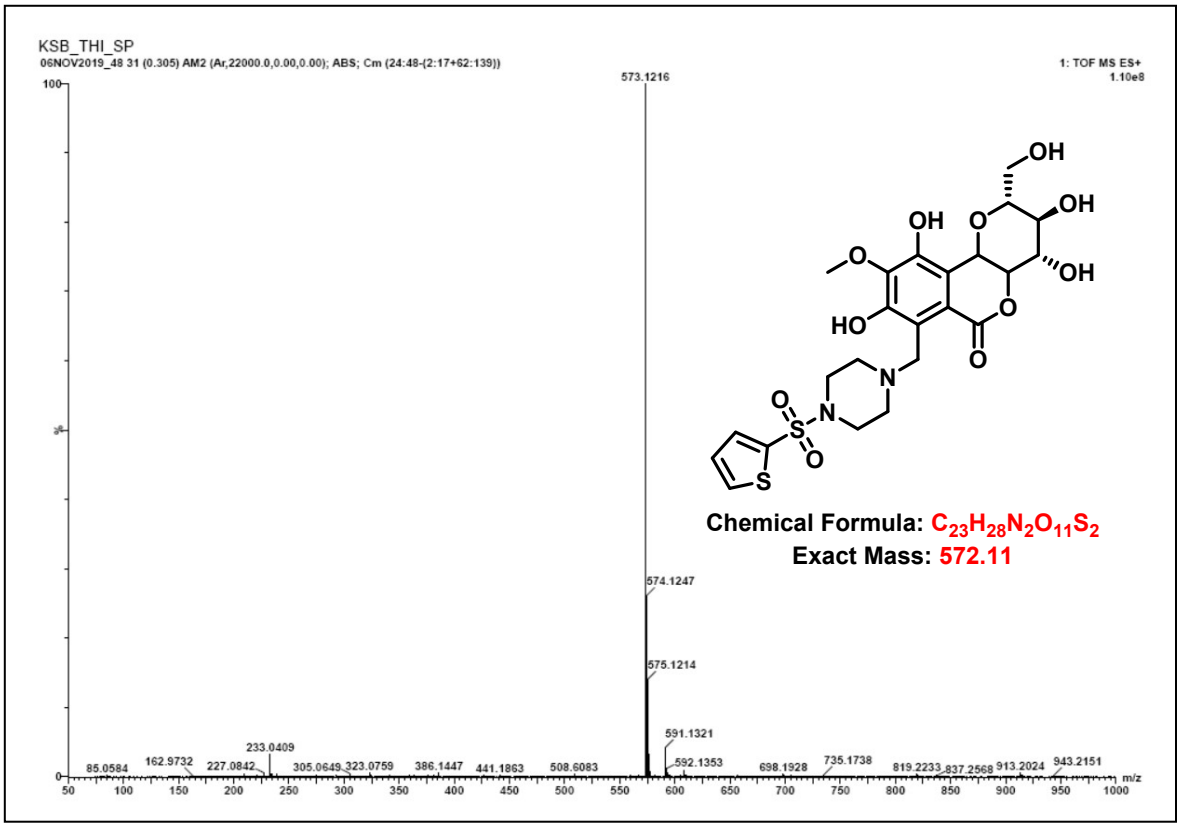


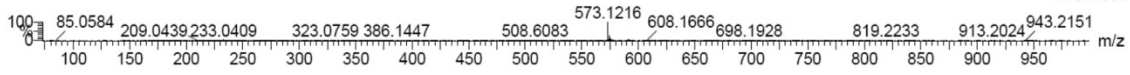
Fig S116: ^{13}C NMR SPECTRUM OF COMPOUND **13l** (100 MHz, CD_3OD)



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 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
203 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-23 H: 0-29 N: 0-2 O: 0-11 S: 0-2 Cl: 0-1
KSB_THI_SP
06NOV2019_48 31 (0.305) AM2 (Ar,22000.0,0.00,0.00); ABS; Cm (24:48-(2:17+62:139))
1: TOF MS ES+
1.10e+008



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
573.1216	573.1213	0.3	0.5	10.5	650.0	n/a	n/a	C23 H29 N2 O11 S2

Fig S117: HRESIMS SPECTRUM OF COMPOUND **13l**

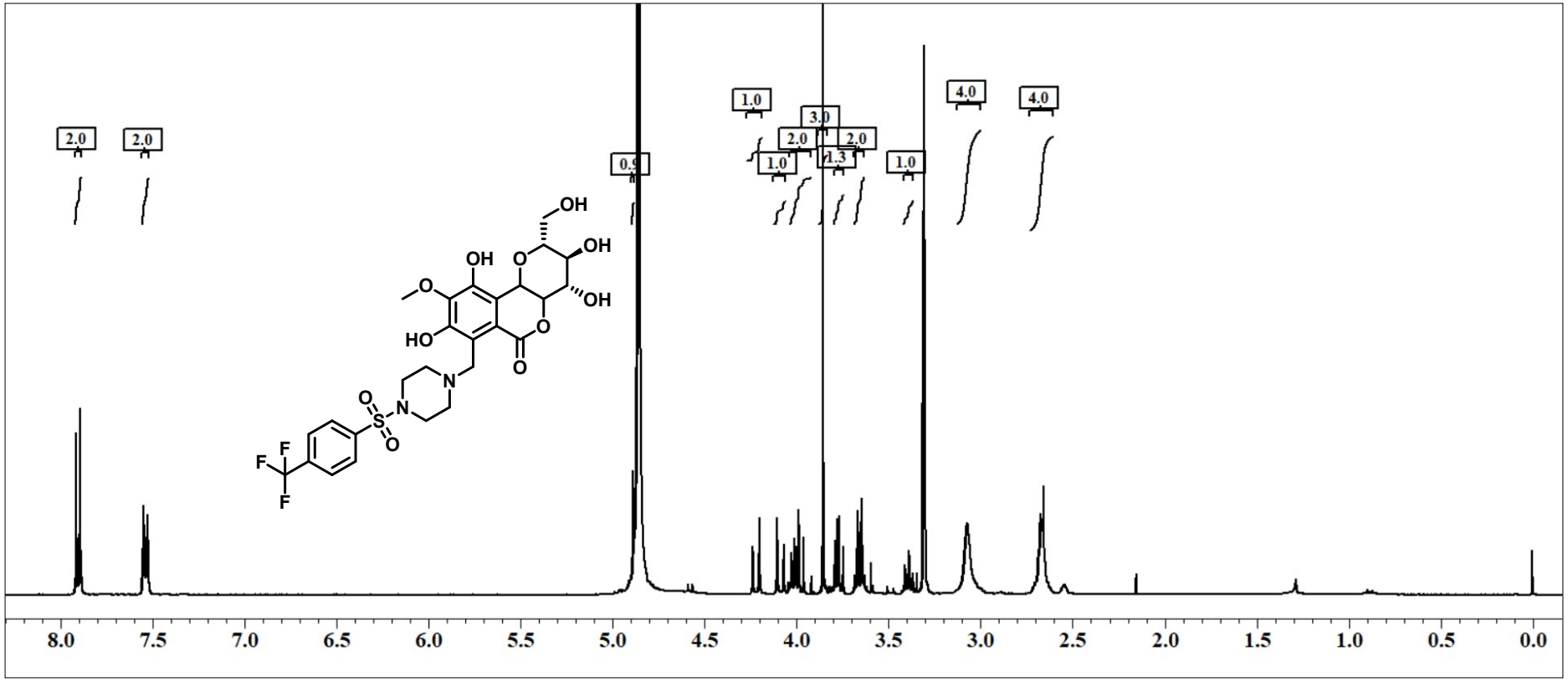


Fig S118: ^{1}H NMR SPECTRUM OF COMPOUND **13m** (400 MHz, CD_3OD)

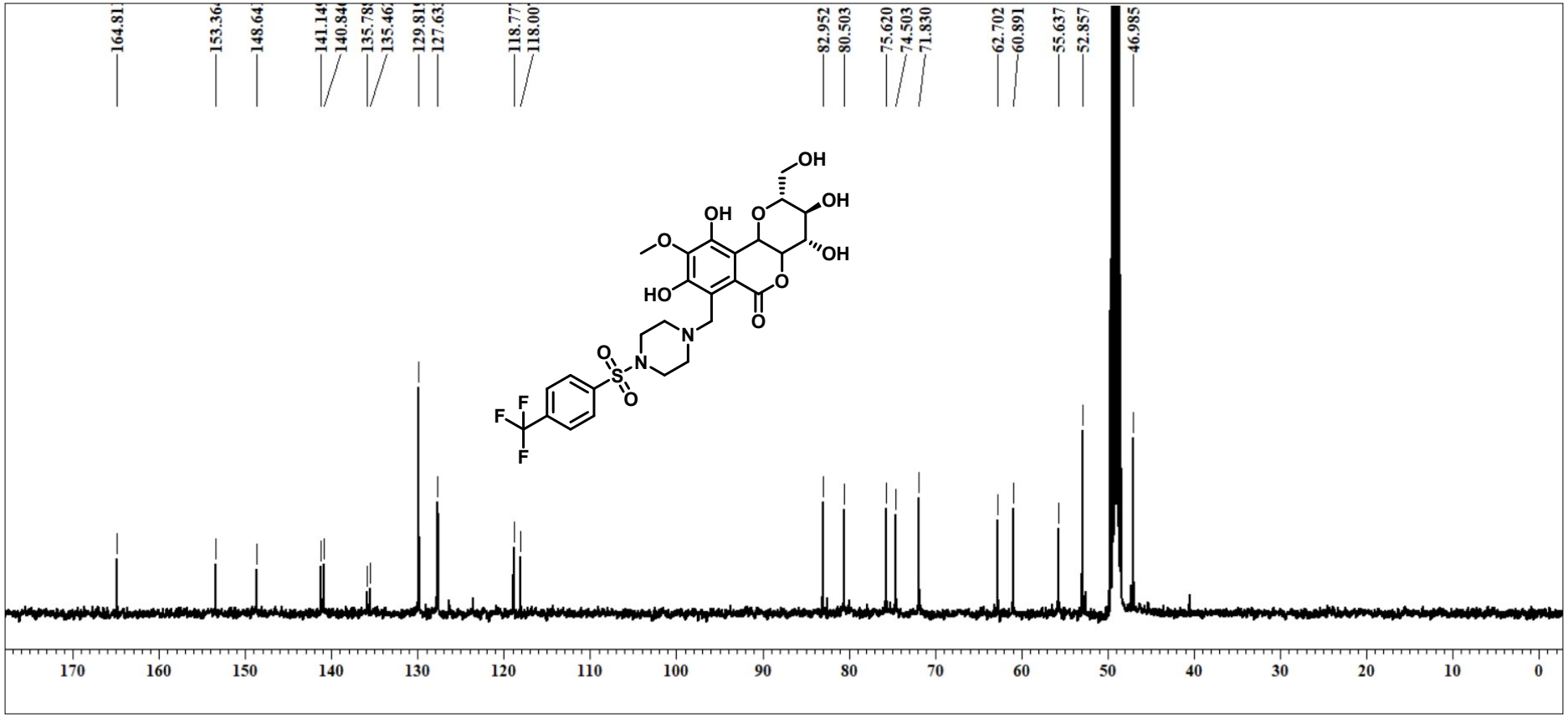


Fig S119: ^{13}C NMR SPECTRUM OF COMPOUND **13m** (100 MHz, CD_3OD)

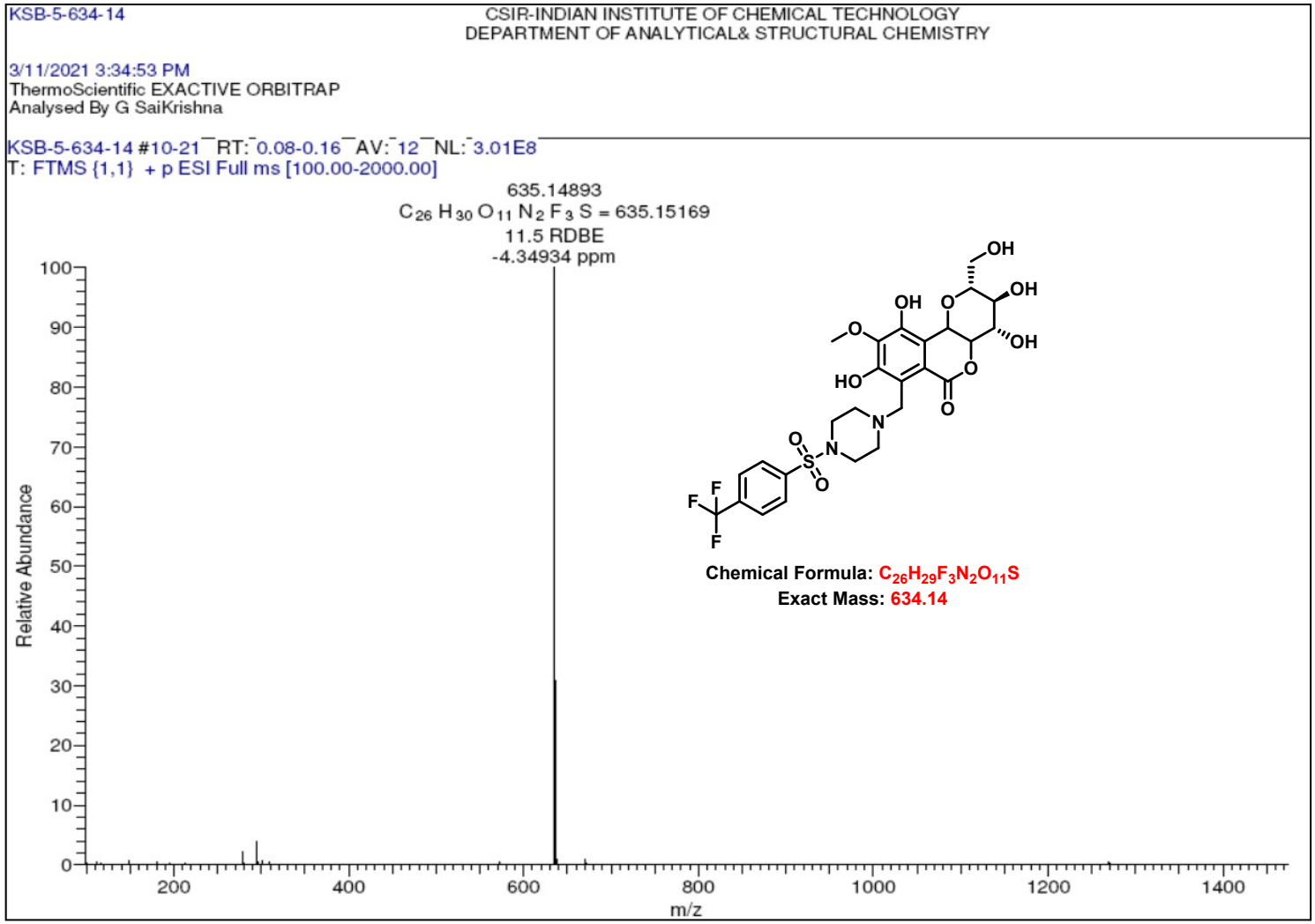


Fig S120: HRESIMS SPECTRUM OF COMPOUND **13**

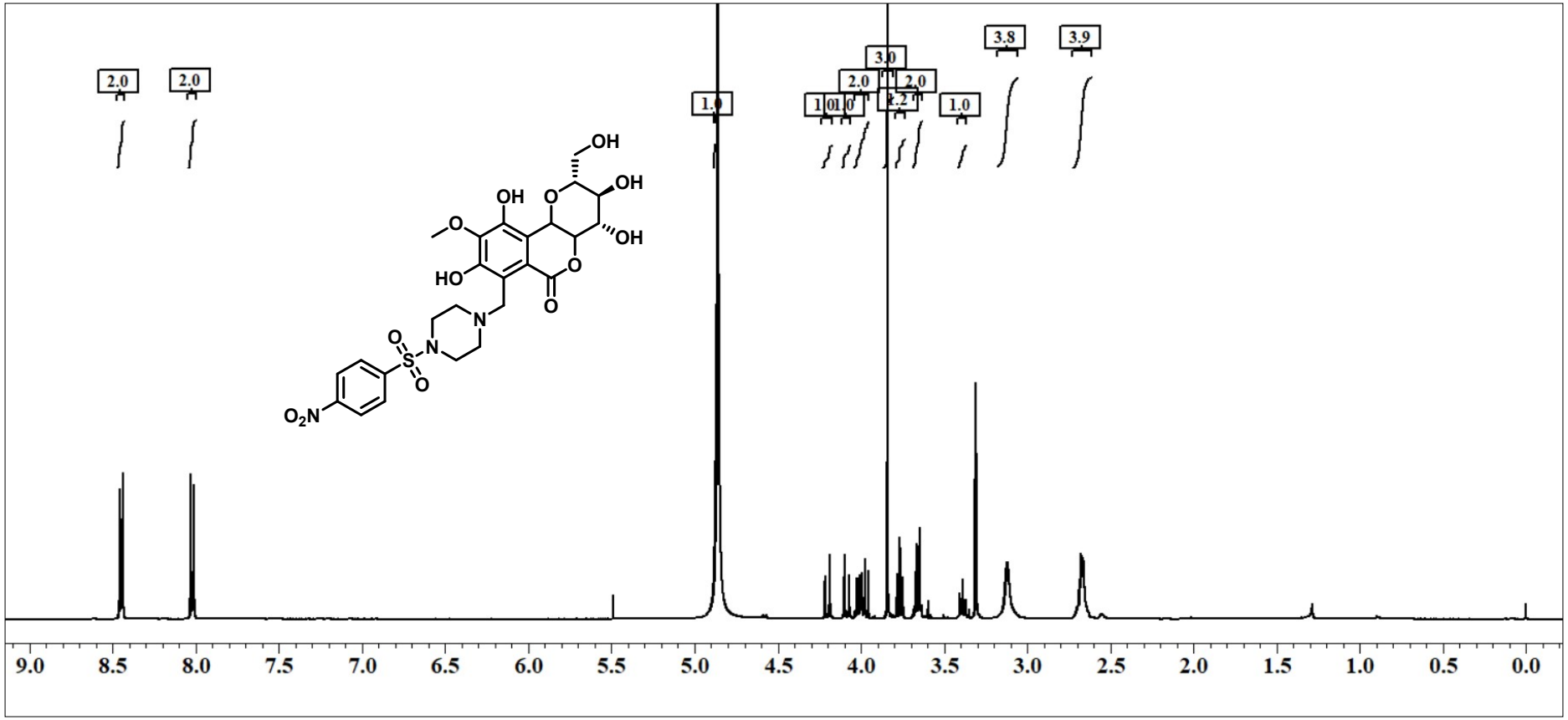


Fig S121: ^{1}H NMR SPECTRUM OF COMPOUND **13n** (500 MHz, CD_3OD)

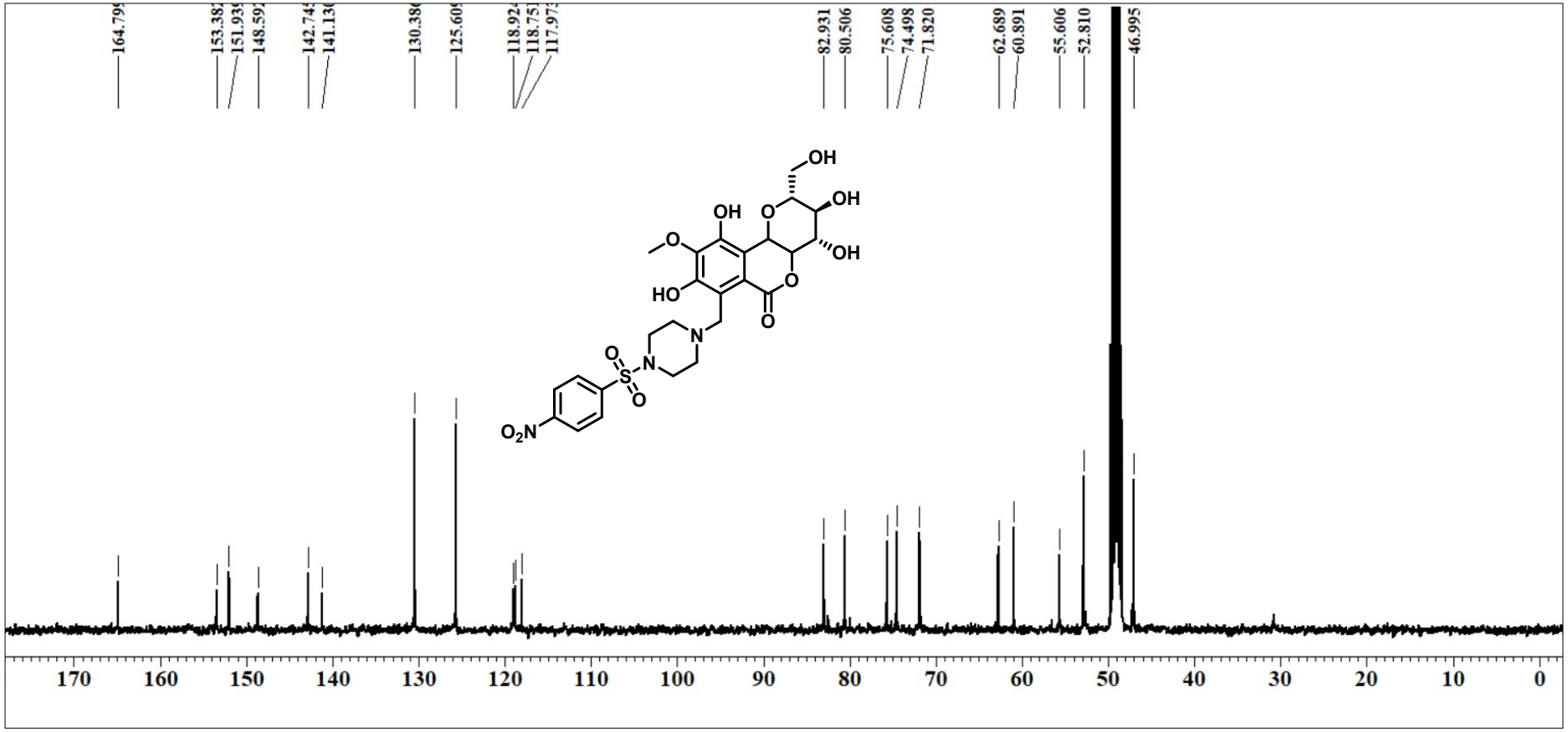


Fig S122: ^{13}C NMR SPECTRUM OF COMPOUND **13n** (100 MHz, CD_3OD)

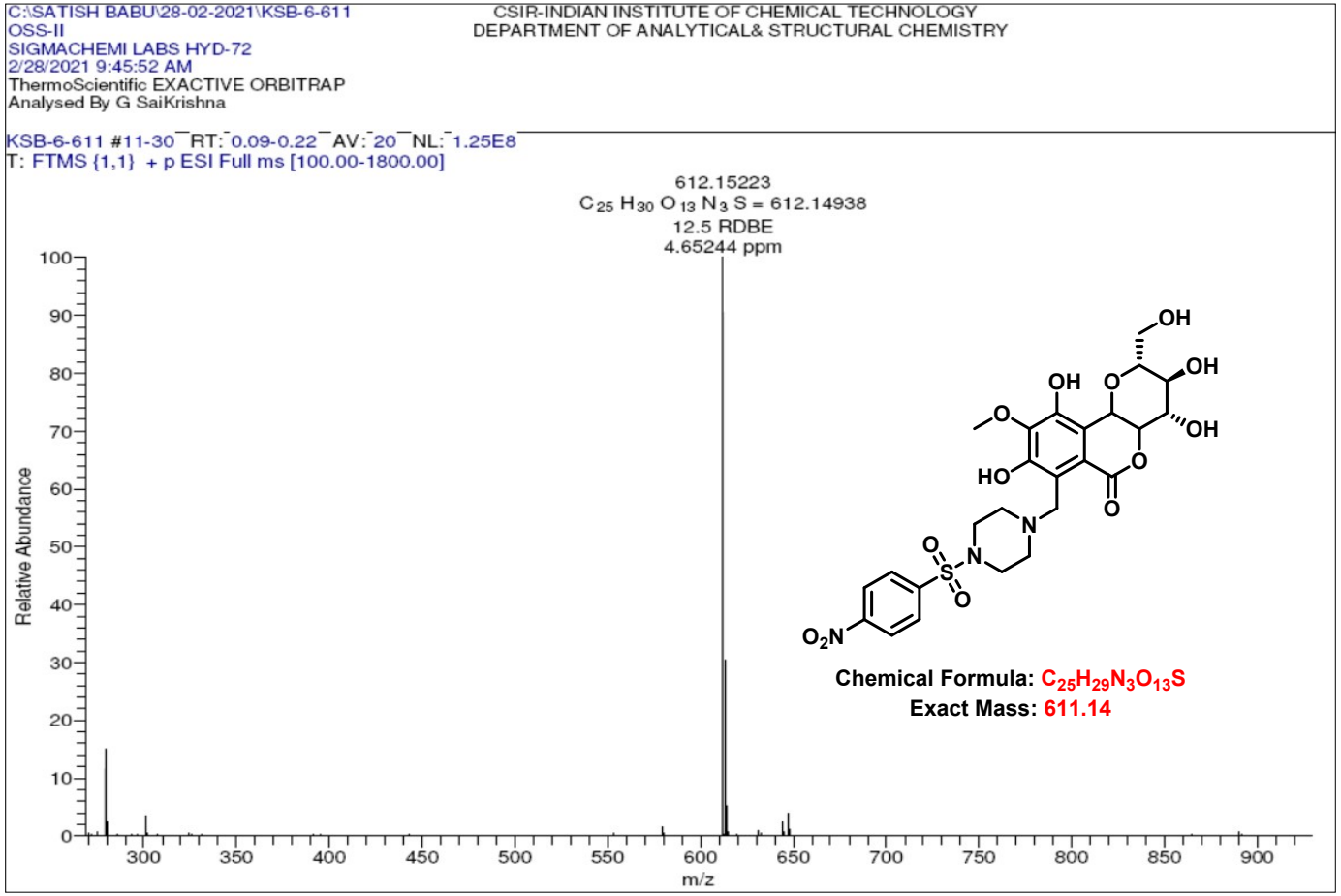


Fig S123: HRESIMS SPECTRUM OF COMPOUND **13n**

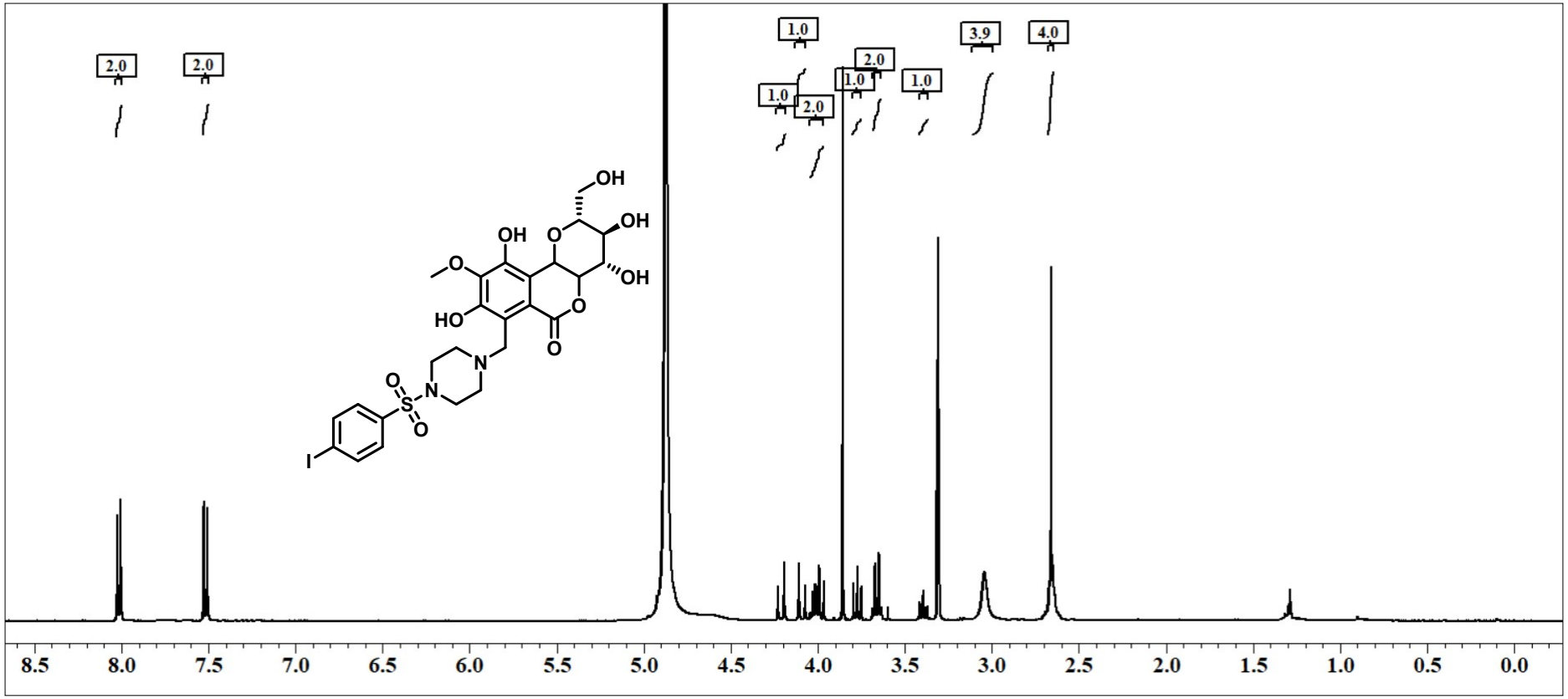


Fig S124: ^{1}H NMR SPECTRUM OF COMPOUND **13o** (400 MHz, CD_3OD)

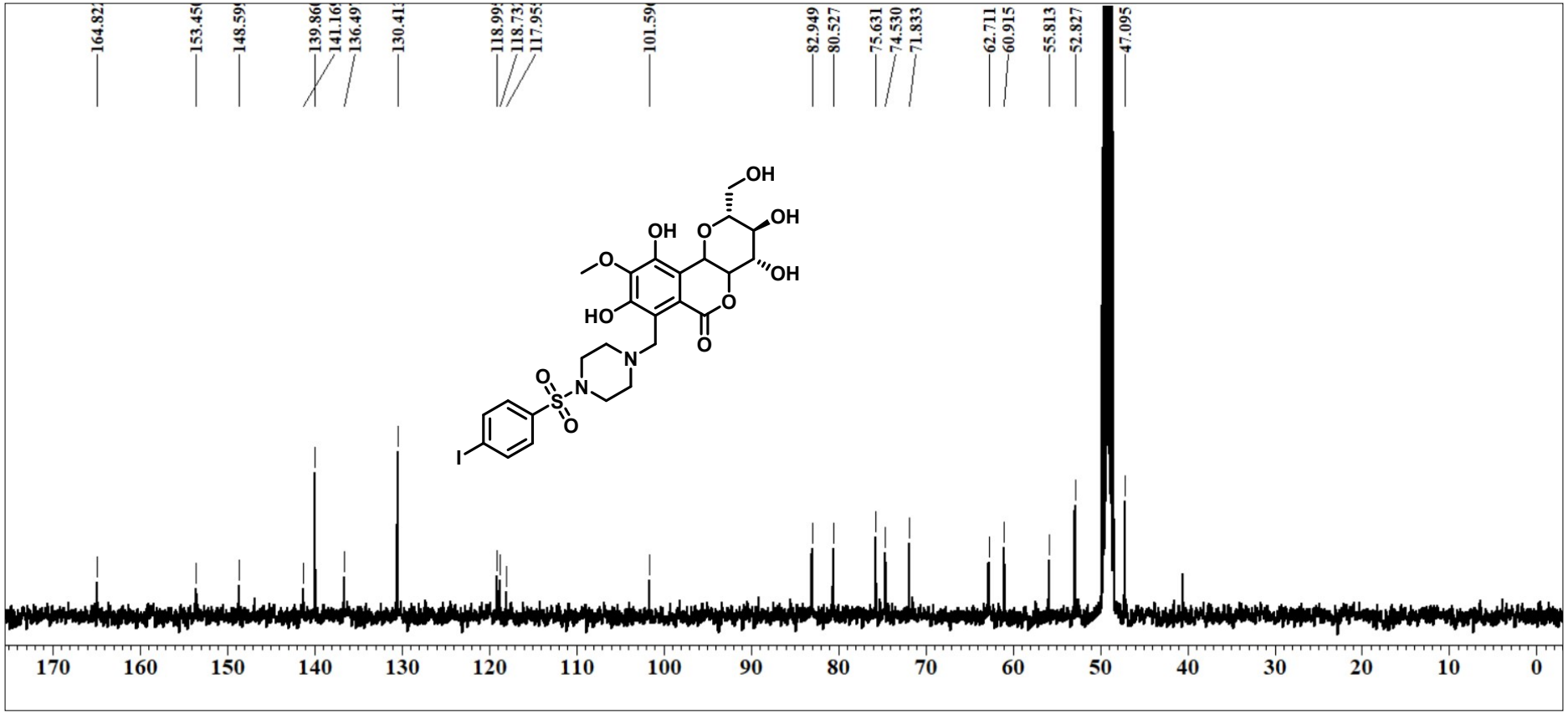


Fig S125: ^{13}C NMR SPECTRUM OF COMPOUND **13o** (100 MHz, CD_3OD)

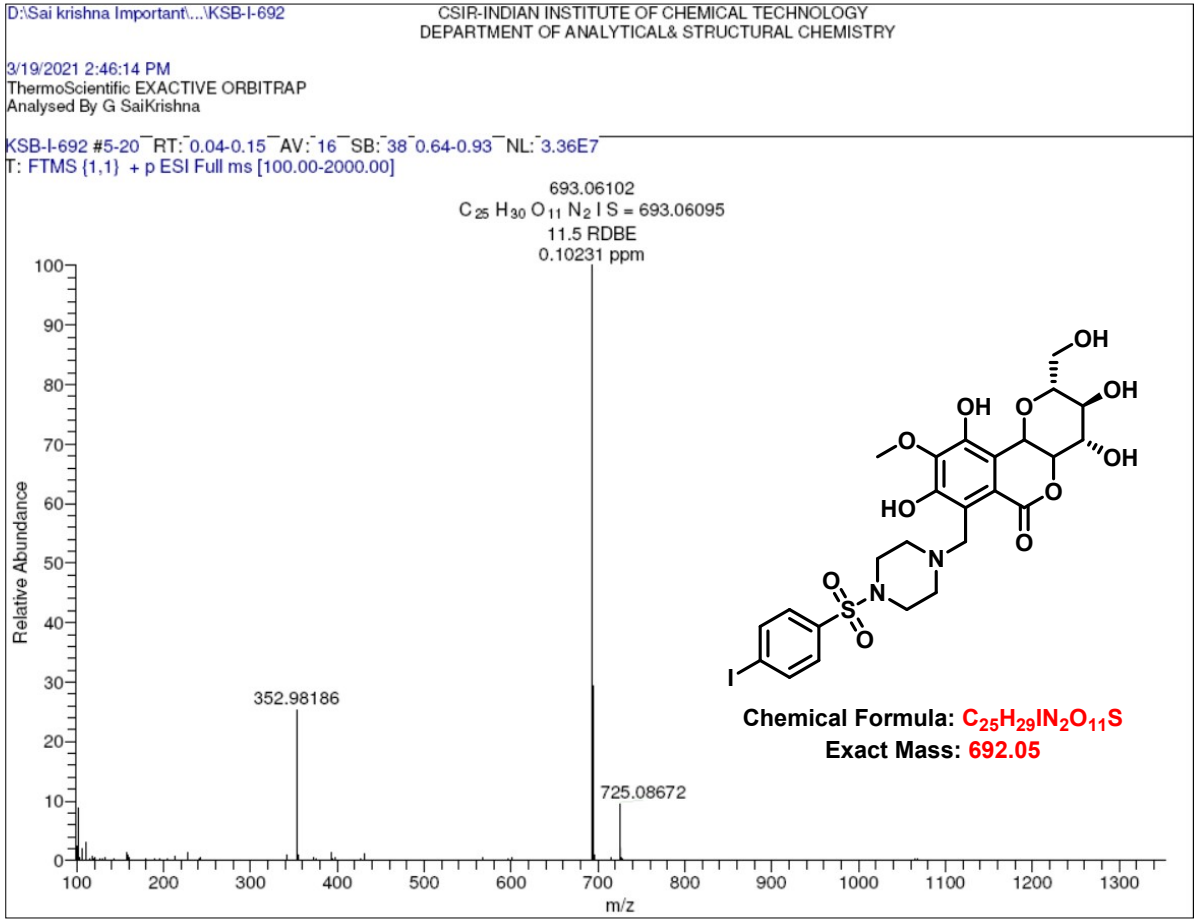


Fig S126: HRESIMS SPECTRUM OF COMPOUND **13o**