SUPPLEMENTARY DATA TO

Antimicrobial and Anti-Tubercular Activities of Isolates and Semi-synthetic Derivatives of Lichen *Ramalina leiodea* (Nyl.) Nyl.

VINAY BHARADWAJ TATIPAMULA and GIRIJA SASTRY VEDULA*

Pharmaceutical Chemistry Department, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530 003, Andhra Pradesh, India.

*Corresponding author. E-mail: vgirijasastry@yahoo.co.in

EXPERIMENTAL

Antimicrobial Activities

Both the antibacterial and antifungal activities for measuring antimicrobial properties were carried out according to standard well plate method[1]. For antibacterial activity all the selected test strains (*Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*) were initially activated and grown in nutrient agar. Whereas for antifungal studies test strain used, *Candida albicans* were grown on the potato dextrose agar medium. All compounds were re-dissolved in DMSO to get a final concentration of 1 mg/mL and used as stock solution. The compounds were used for activity studies and the concentration of each sample is 1 µg/mL along with standard and control. The media, Petri dishes were autoclaved at 121°C for 15 min. After sterilization the agar plates were prepared by pouring 25 mL of agar medium followed by incubation at room temperature for 30 min for solidification under sterile environment. These plates were inoculated with 60 µL of test inoculums using sterile cotton swabs. An 8 mm width size wells were made with sterile cork borer and in each well exactly 100 µL of sample were loaded. Control and standard also placed in separate wells. The plates were initially incubated for 20-30 min at 4°C to allow the compounds to diffuse into the agar, and then subsequently incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Zone diameters were expressed in mm using calibrated scale. Experiments were conducted in triplicate with aliquots to minimize the deviations and the average values were reported.

The compounds having better anti-microbial activity were selected for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) studies against the *Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and

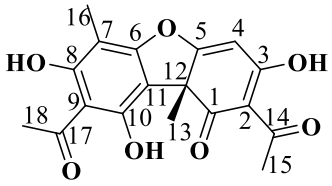
Candida albicans according to reported method[2]. The concentrations of test samples were serially diluted from 1 to 0.001 µg/mL and one tube without drug served as control. All the tubes were inoculated with 1 mL of respective cultures having an OD of 0.2 (~McFarland standard) and the tubes were incubated at 37ºC for 12-16 h. The turbidity of each tube is measured with respect to control tube. MIC values are defined as the lowest concentration of compound at which growth is completely inhibited. After incubation the culture from each tube was plated in nutrient agar to evaluate the MBC concentration. The concentration at which the cells are completely dead was defined as MBC.

Anti-tubercular Activity

The *in vitro* anti-tubercular activity assessment of all compounds have been tested against *Mycobacterium tuberculosis* H37Rv strain ATCC 27294 using microplate alamar blue assay (MABA)[2,3] in three sets (n=3). The Minimum Inhibitory Concentration (MIC) value was determined by a colour change of blue to pink which indicates no bacterial growth and growth, respectively.

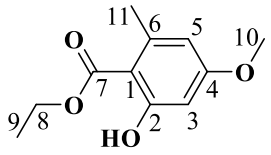
RESULTS

Chemistry



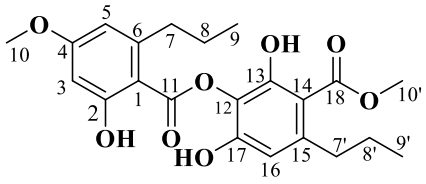
1

Usnic acid (1). Product yield: 236 mg (0.16%), MP: 201-202ºC, Rf: 0.4 (Hexane-DCM, 9:1). IR (KBr): 2923, 2858, 1687, 1626, 1543, 1462, 1368, 1293, 1192, 1138, 1071, 1037, 966, 843 cm^{-1}. UV/Vis λ_{max} (EtOH) nm (log ε): 220. ^{1}H NMR (400 MHz, $CDCl_3$): 1.76 (3H, s, Me-13), 2.11 (3H, s, Me-16), 2.67 (3H, s, Me-15), 2.68 (3H, s, Me-18), 5.98 (1H, s, H-4), 11.03 (1H, s, OH-10), 13.31 (1H, s, OH-8). ^{13}C NMR (400 MHz, $CDCl_3$): 7.6 (CH_3, C-16), 27.9 (CH_3, C-13), 31.3 (CH_3, C-18), 32.2 (CH_3, C-15), 59.1 (C, C-12), 98.4 (CH, C-4), 101.6 (C, C-7), 104.0 (C, C-11), 105.3 (C, C-2), 109.3 (C, C-9), 155.2 (C, C-6), 157.5 (C, C-10), 163.9 (C, C-8), 179.4 (C, C-5), 191.7 (C, C-3), 198.1 (C, C-1), 200.4 (C, C-14), 201.8 (C, C-17). MS (EI, 70 eV): *m/z* (%) = 343 [M-H^+] (100), 345 [M+H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_{18}H_{16}O_7$: 344.09; found: 344. Anal. Calcd for $C_{18}H_{16}O_7$: C, 63.15; H, 6.26. Found C, 63.16; H = 6.26.



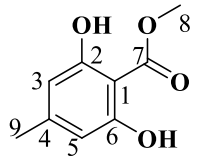
2

Ethyl everninate (2). Product yield: 153 mg (0.10%). MP: 74-76ºC, Rf: 0.4 (Hexane-DCM, 7:3), IR (KBr): 2920, 2855, 1625, 1462, 1371, 1280, 1243, 1164, 757 cm^{-1}. UV/Vis λ_{max} (EtOH) nm (log ε): 219. ^{1}H NMR (400 MHz, Acetone-d_6): 0.95 (3H, t, Me-9), 1.62 (2H, m, CH_2-8), 2.06 (3H, t, Me-11), 2.09 (H, s, OH-2), 3.83 (3H, s, OMe-10), 6.33 (1H, d, H-5), 6.37 (1H, d, H-3). ^{13}C NMR (400 MHz, Acetone-d_6): 14.47 (CH_3, C-9), 25.85 (CH_3, C-11), 54.96 (CH_3, C-10), 55.8 (CH_2, C-8), 99.7 (C, C-1), 111.1 (CH, C-3/5), 149.1 (C, C-6), 165.2 (C, C-2), 167.2 (C, C-4), 174.0 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 209.1 [M-H^+] (100), 211 [M+H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_{11}H_{14}O_4$: 210.09; found: 210. Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found C, 62.87; H = 6.82.



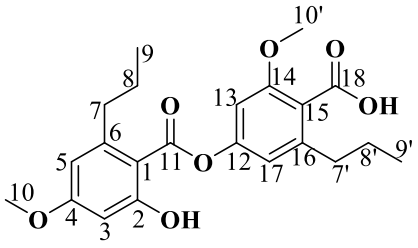
3

Scrobiculin (3). Product yield: 911 mg (0.61%), MP: 134-136ºC, Rf: 0.6 (DCM-EA, 7:3), IR (KBr): 2958, 2926, 2864, 1657, 1619, 1581, 1503, 1455, 1420, 1342, 1239, 1160, 1129, 1088, 1037, 830, 757 cm^{-1}. UV/Vis λ_{max} (EtOH) nm (log ε): 217.5. ^{1}H NMR (400 MHz, DMSO-d_6): 0.88-0.97 (6H, m, Me-9,9'), 1.59-1.65 (4H, dd, CH_2-8, 8'), 2.51 (1H, t, OH-2), 2.82-2.87 (4H, dd, CH_2-7,7'), 3.78 (3H, s, OMe-10), 3.84 (3H, s, OMe-10'), 6.39-6.41 (2H, dd, H-3,5), 6.61 (1H, s, H-16), 10.50 (1H, s, OH-17), 11.85 (1H, s, OH-13). ^{13}C NMR (400 MHz, DMSO-d_6): 14.5 (CH_3, C-9), 14.6 (CH_3, C-9'), 24.8 (CH_2, C-8), 25.2 (CH_2, C-8'), 36.9 (CH, C-7), 37.8 (CH, C-7'), 55.8 (CH_3, C-10), 56.5 (CH_3, C-10'), 99.5 (CH, C-3), 106.2 (CH, C-1), 108.6 (CH, C-16), 108.8 (C, C-14), 109.5 (CH, C-5), 125.2 (C, C-12), 144.1 (C, C-15), 146.0 (C, C-6), 154.3 (C, C-13), 154.7 (C, C-17), 160.6 (C, C-2), 162.9 (C, C-4), 166.7 (C, C-11), 172.7 (C, C-18). MS (EI, 70 eV): *m/z* (%) = 417 [M-H^+] (100), 418.9 [M+H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_{22}H_{26}O_8$: 418.44; found: 418. Anal. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found C, 63.16; H = 6.26.



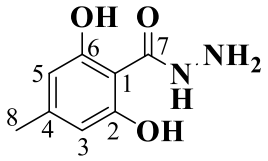
4

Methyl 2,6-dihydroxy-4-methylbenzoate (4). Product yield: 4.05 g (2.70%), MP: 143-144ºC, Rf: 0.6 (Hexane-EA, 1:1), IR (KBr): 3866, 3746, 3370, 3312, 2982, 2955, 2854, 1894, 1642, 1581, 1503, 1447, 1383, 1318, 1265, 1197, 1162, 1114, 1059, 1029, 996, 950, 835, 800, 753, 700, 620, 575 cm^{-1}. UV/Vis λ_{max} (EtOH) nm (log ε): 219.5. ^{1}H NMR (400 MHz, DMSO-d_6): 2.23 (3H, s, Me-9), 3.75 (3H, s, OMe-8), 6.12 (2H, d, H-3, 5), 9.94 (1H, s, OH-6), 10.65 (1H, s, OH-2). ^{13}C NMR (400 MHz, DMSO-d_6): 22.5 (C-9), 52.2 (C-8), 100.8 (C-1), 107.9 (C-5), 110.6 (C-3), 141.2 (C-4), 161.5 (C-2/6), 170.6 (C-7). MS (EI, 70 eV): *m/z* (%) = 183.0 [M + H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_9H_{10}O_4$: 182.18; found: 182. Anal. Calcd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found C, 59.36; H = 5.52.



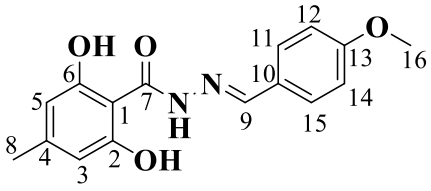
5

2'-O-methyldivaricatic acid (5). Product yield: 11 mg (0.007%), MP: 142-143ºC, Rf: 0.4 (Hexane-EA, 1:1), UV/Vis λ_{max} (EtOH) nm (log ε): 212.5. ^{1}H NMR (400 MHz, $CDCl_3$): 0.93-1.05 (6H, m, Me-9, 9'), 1.66-1.80 (4H, m, CH_2-8, 8'), 2.95-3.04 (4H, m, CH_2-7, 7'), 3.85 (3H, s, OMe-10), 3.91 (3H, s, OMe-10'), 6.40 (2H, s, H-13, 17), 6.46 (2H, s, H-3, 5), 11.18 (1H, s, OH-18), 11.73 (1H, s, OH-2). ^{13}C NMR (400 MHz, $CDCl_3$): 14.3 (CH_3, C-9/9'), 24.81 (CH_2, C-8'), 25.18 (CH_2, C-8), 38.8 (CH, C-7'), 39.1 (CH, C-7), 55.4 (CH_3, C-10), 56.0 (CH_3, C-10'), 99.8 (CH, C-3), 104.5 (C, C-1), 106.2 (C, C-15), 107.1 (CH, C-13), 110.9 (CH, C-5), 146.8 (CH, C-17), 148.7 (C, C-16), 151.6 (C, C-6), 156.1 (C, C-12), 156.9 (C, C-14), 164.4 (C, C-4), 165.5 (C, C-2), 168.9 (C, C-11), 173.7 (C, C-18). MS (EI, 70 eV): *m/z* (%) = 403.53 [M - H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_{22}H_{26}O_7$: 402.17; found: 402.53. Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found C, 65.66; H = 6.62.



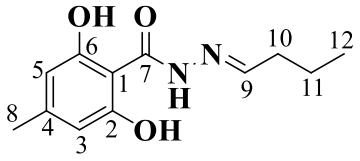
4a

2,6-dihydroxy-4-methylbenzohydrazide (4a). Product yield: 77%, MP: 153-154ºC. IR (KBr): 2917, 2564, 1621, 1498, 1458, 1367, 1325, 1266, 1216, 1173, 1001, 919, 841, 800, 735, 688 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 2.57 (*s*, 3H, Me), 5.07 (*s*, 2H, NH_2), 6.42 (*s*, 2H, Ar-H), 7.41 (*s*, 1H, NH), 10.05 (*s*, 2H, Ar-OH). ^{13}C NMR (100 MHz, $CDCl_3$): 24.4 (CH_3, C-8), 99.7 (C, C-1), 112.2 (CH, C-3/5), 144.7 (C, C-4), 164.0 (C, C-2/6), 166.5 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 183.35 [M + H^+]. HRMS-FAB: *m/z* [M + H^+] calcd for $C_8H_{10}N_2O_3$: 182.18; found: 182.35. Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found C, 52.64; H = 5.52; N = 15.48.



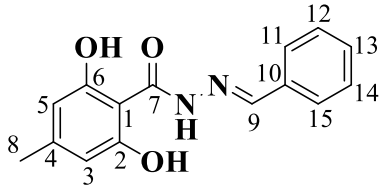
4b

(E)-2,6-dihydroxy-N'-(4-methoxybenzylidene)-4-methylbenzohydrazide (4b). Product yield: 68%, MP: 120-121ºC. IR (KBr): 3205, 3054, 2843, 1655, 1804, 1509, 1462, 1428, 1379, 1340, 1300, 1250, 1171, 1096, 1028, 960, 761, 668 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 2.38 (*s*, 3H, Me), 3.84 (*s*, 3H, OMe), 6.37-6.38 (*d*, 2H, Ar-H), 6.91-6.93 (*m*, 2H, Ar-H), 7.60-7.62 (*m*, 2H, Ar-H), 7.78 (*s*, 1H, =CH), 9.89 (*s*, 2H, Ar-OH). ^{13}C NMR (400 MHz, $CDCl_3$): 20.4 (CH_3, C-8), 55.4 (CH_3, C-16), 111.5 (C, C-1), 114.2 (CH, C-3/5), 126.6 (CH, C-12/14), 128.7 (C, C-10), 138.3 (CH, C-11/15), 143.8 (C, C-4), 147.3 (CH, C-9), 161.2 (C, C-2/6), 164.4 (C, C-13), 174.1 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 301.15 [M + H^+] (100). HRMS-FAB: *m/z* [M + H^+] calcd for $C_{16}H_{16}N_2O_4$: 300.31; found: 300.15. Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37, N, 9.33. Found C, 64.00; H = 5.32; N = 9.37.



4c

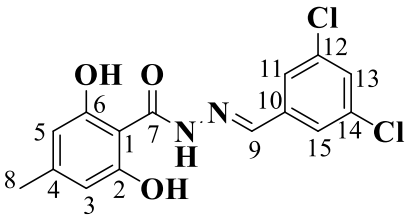
(E)-N'-butylidene-2,6-dihydroxy-4-methylbenzohydrazide (4c). Product yield: 70%, MP: 110-111ºC. IR (KBr): 3814, 3217, 3066, 2973, 2934, 1457, 1379, 1341, 1287, 1167, 1101, 846, 769 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 0.95-1.03 (*m*, 3H, Me), 1.91-1.93 (*m*, 2H, CH_2), 2.15 (*s*, 3H, Me), 2.18-2.20 (*m*, 2H, CH_2), 6.51 (*s*, 2H, Ar-H), 7.19-7.21 (*t*, 1H, =CH), 7.83 (*s*, 1H, NH) 10.14 (*s*, 2H, Ar-OH). ^{13}C NMR (400 MHz, $CDCl_3$): 14.2 (CH_3, C-12), 19.8 (CH_2, C-11), 22.7 (CH_3, C-8), 29.8 (CH_2, C-10), 106.5 (C, C-1), 113.9 (CH, C-3/5), 130.5 (C, C-4), 149.5 (CH, C-9), 155.8 (C, C-2/6), 168.6 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 237.15 [M + H^+]. HRMS-FAB: *m/z* [M + H^+] calcd for $C_{12}H_{16}N_2O_3$: 236.27; found: 236.15. Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83, N, 11.89. Found C, 61.00; H = 6.80; N = 11.89.



4d

(E)-N'-benzylidene-2,6-dihydroxy-4-methylbenzohydrazide (4d). Product yield: 64%, MP: 98-99ºC. IR (KBr): 3183, 3077, 2969, 2858, 1669, 1605, 1500, 1393, 1338, 1224, 1133, 1016, 948, 899, 758, 685 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 2.40 (*s*, 3H, Me), 7.39-7.40 (*m*, 3H, Ar-H), 7.67-7.68 (*m*, 2H, Ar-H), 7.84 (*d*, 1H, =CH), 8.84 (*s*, 1H, NH), 10.02 (*s*, 2H, Ar-OH). ^{13}C

NMR (400 MHz, $CDCl_3$): 20.4 (CH_3, C-8), 105.6 (C, C-1), 112.3 (CH, C-3/5), 127.2 (CH, C-12/14), 128.8 (CH, C-11/13/15), 130.1 (C, C-10), 133.9 (C, C-4), 143.8 (CH, C-9), 165.5 (C, C-2/6), 174.2 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 271.25 [M + H^+]. HRMS-FAB: *m/z* [M + H^+] calcd for $C_{15}H_{14}N_2O_3$: 270.29; found: 470.25. Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.20, N, 10.36. Found C, 66.68; H = 5.20, N = 10.40.



4e

(E)-N'-(3,5-dichlorobenzylidene)-2,6-dihydroxy-4-methylbenzohydrazide (4e). Product yield: 75%, MP: 130-131ºC. IR (KBr): 3082, 2954, 1687, 1595, 1519, 1466, 1385, 1332, 1276, 1217, 1152, 1097, 1020, 927, 860, 816, 769, 665 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): 2.38 (*s*, 3H, Me), 7.27-7.29 (*m*, 2H, Ar-H), 7.41-7.42 (*m*, 1H, Ar-H), 7.91-7.93 (*m*, 1H, =CH), 8.12 (*s*, 1H, NH), 9.37 (*s*, 2H, Ar-OH). ^{13}C NMR (400 MHz, $CDCl_3$): 20.4 (CH_3, C-8), 104.4 (C, C-1), 112.1 (CH, C-3/5), 127.9 (CH, C-11/15), 129.9 (CH, C-13), 134.6 (C, C-12/14), 136.3 (C, C-10), 138.7 (C, C-4), 142.3 (CH, C-9), 159.5 (C, C-2/6), 173.4 (C, C-7). MS (EI, 70 eV): *m/z* (%) = 340.15 [M + H^+]. HRMS-FAB: *m/z* [M + H^+] calcd for $C_{15}H_{12}Cl_2N_2O_3$: 339.17; found: 339.15.

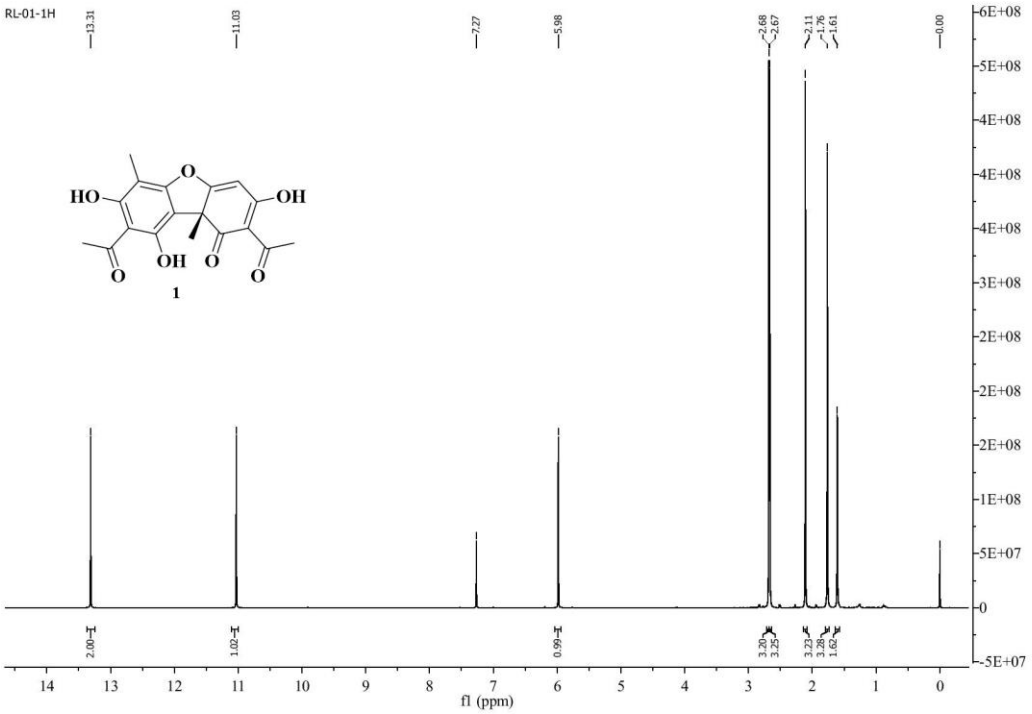


Figure S1: ^1H NMR of **1** (400 MHz, $CDCl_3$)

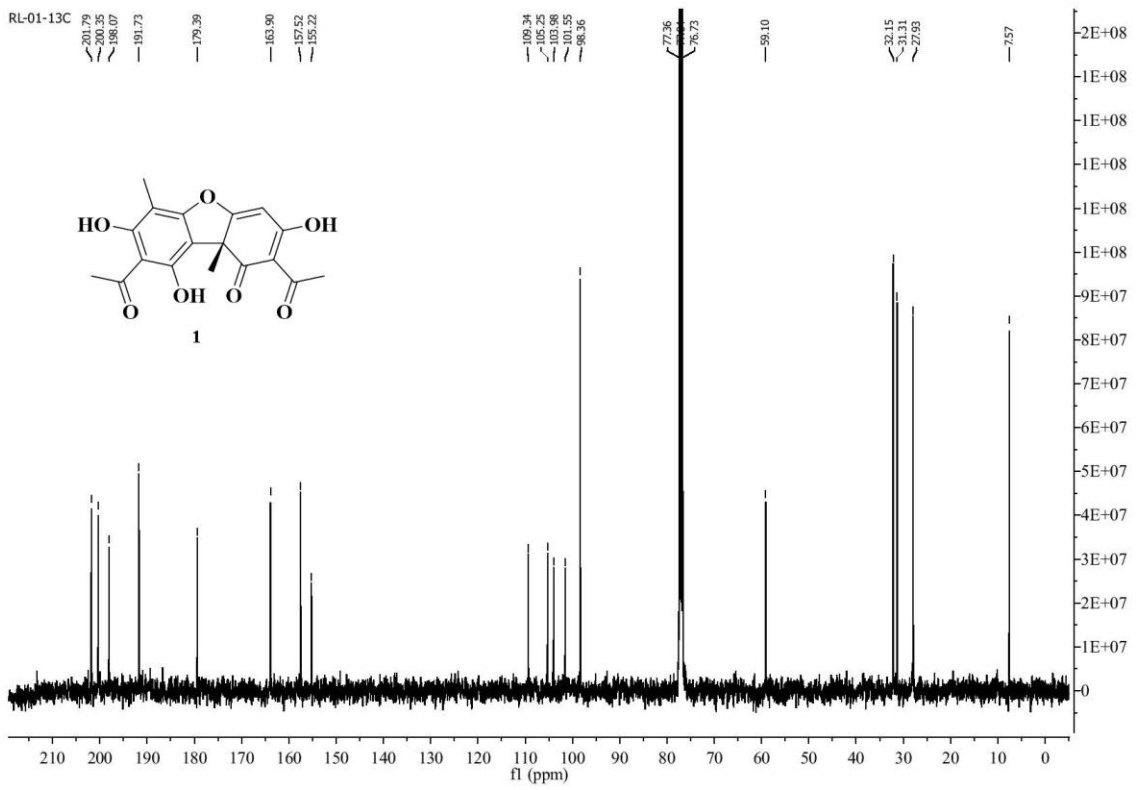


Figure S2: ^{13}C NMR of **1** (400 MHz, $CDCl_3$)

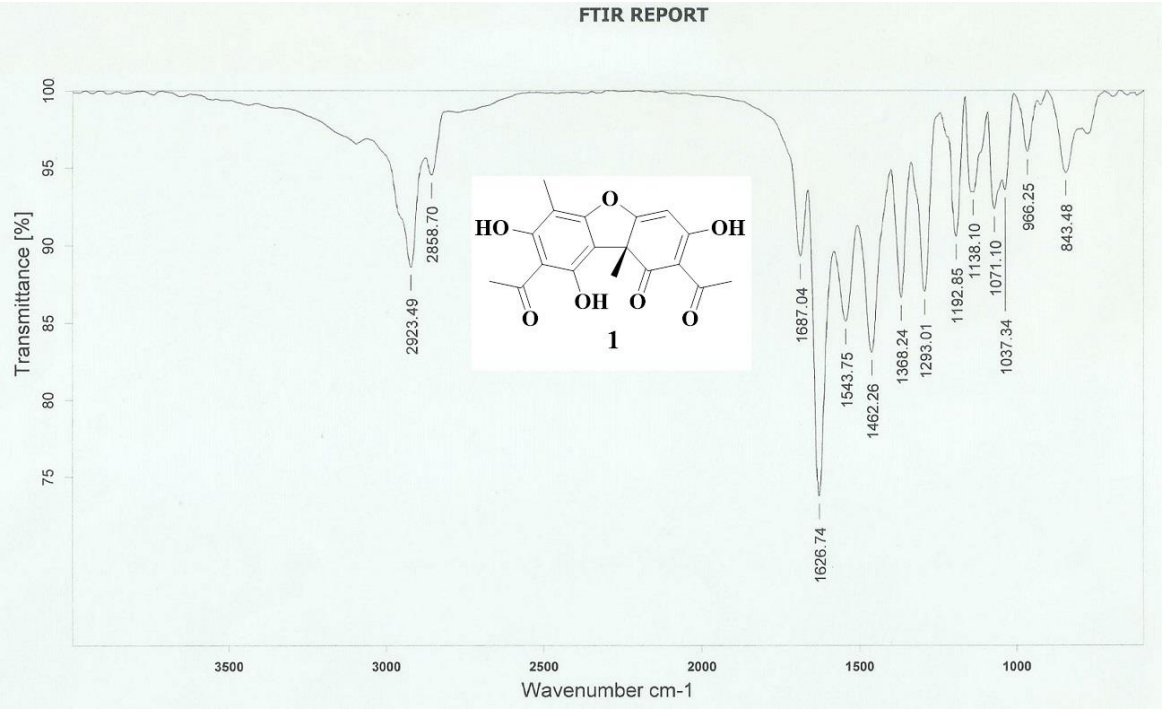


Figure S3: FT-IR of **1** (KBr)

Qualitative Analysis Report

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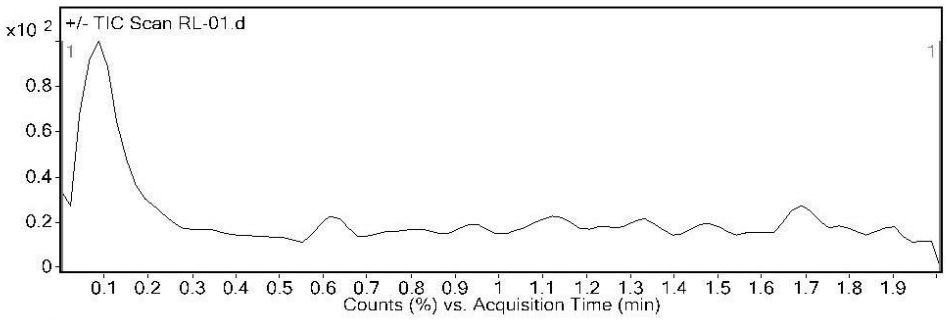
Sample Type	Sample	**Position**	Vial 11
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	7/22/2017 11:56:56 PM
IRM Calibration Status	Not Applicable	**DA Method**	raghu.m
Comment			

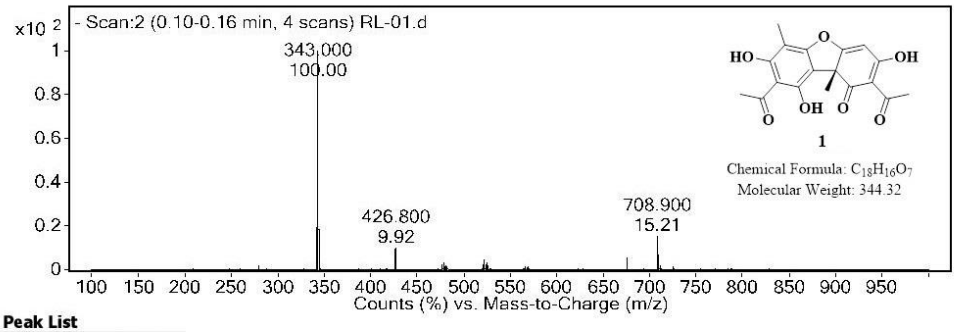
User Chromatograms

Fragmentor Voltage 135 **Collision Energy** 0 **Ionization Mode** ESI



User Spectra

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi



1

Chemical Formula: $C_{18}H_{16}O_7$
Molecular Weight: 344.32

Peak List

m/z	z	Abund
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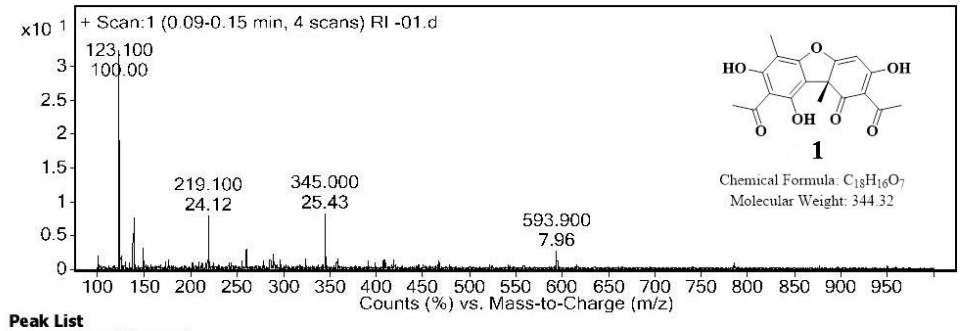
344	1	73057
426.8		39659
675.2		21883
708.9	1	60823
709.9	1	24969

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi

158

159 **Figure S4:** ESI-MS of **1**

Qualitative Analysis Report



Peak List

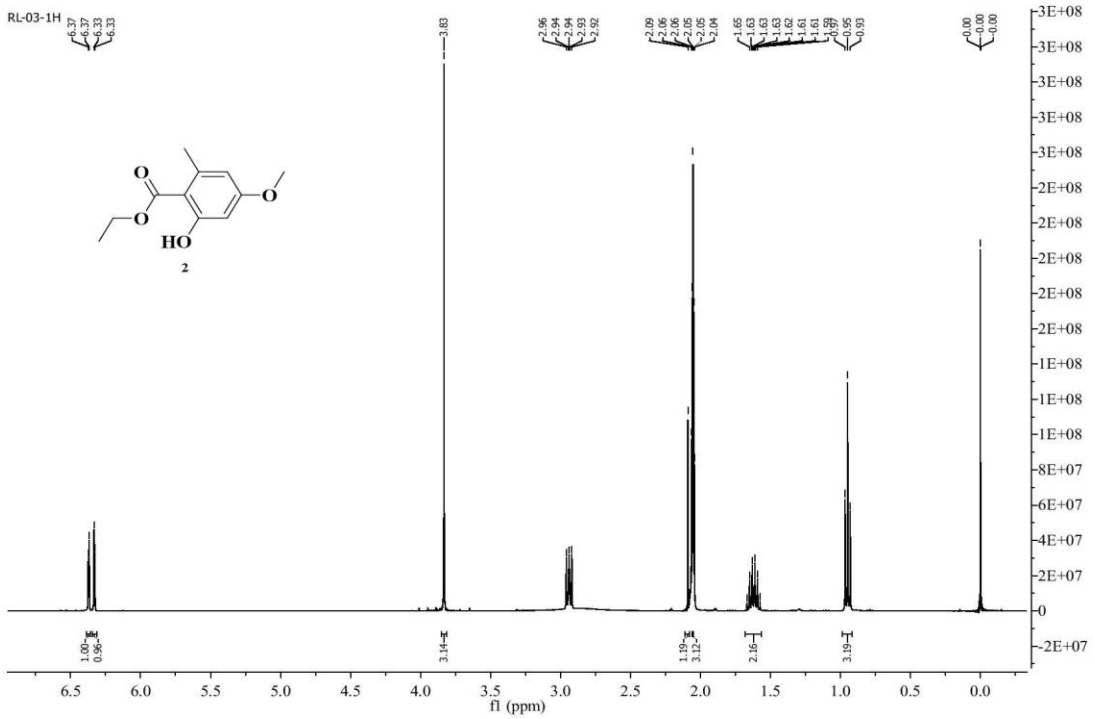
m/z	z	Abund
123.1		129023
124.1	1	75959
125.2	1	7426
139.1		30354
149		12675
219.1		31120
260.1		11611
290		8991
345	1	32807
593.9		10264

--- End Of Report ---

160

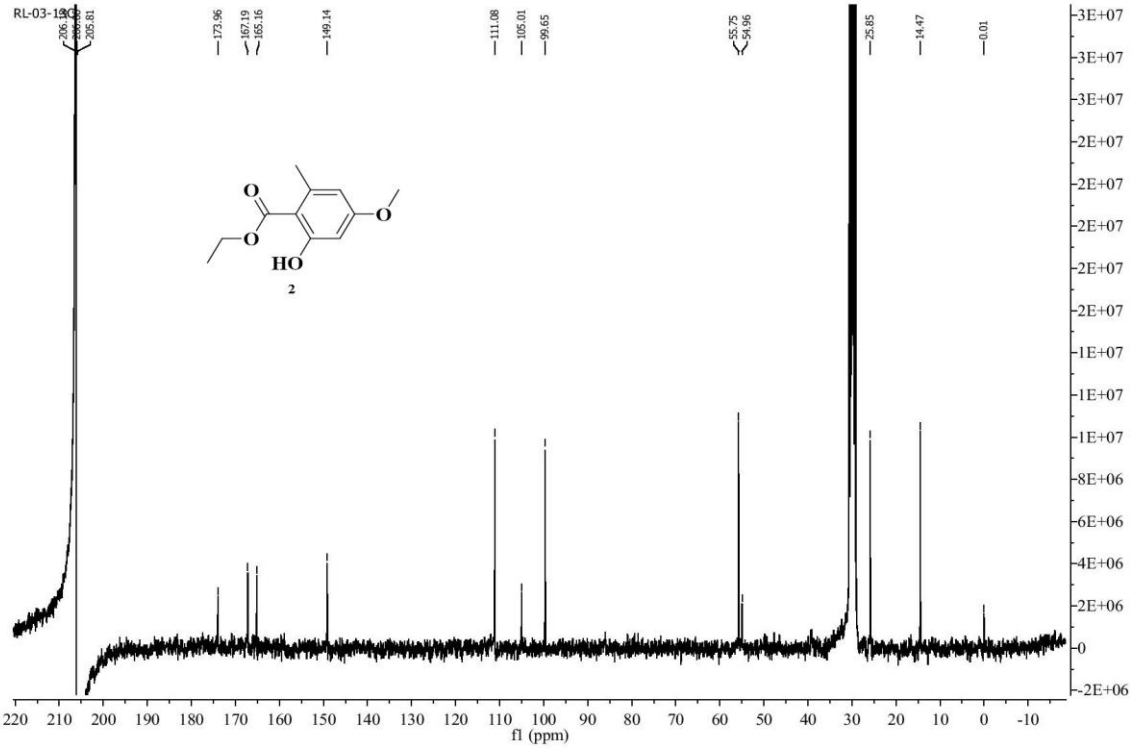
161 **Figure S4a:** ESI-MS of **1**

162



163

164 **Figure S5:** ^{1}H NMR of **2** (400 MHz, Acetone-d_6)



165

166 **Figure S6**: ^{13}C NMR of **2** (400 MHz, Acetone-d_6)

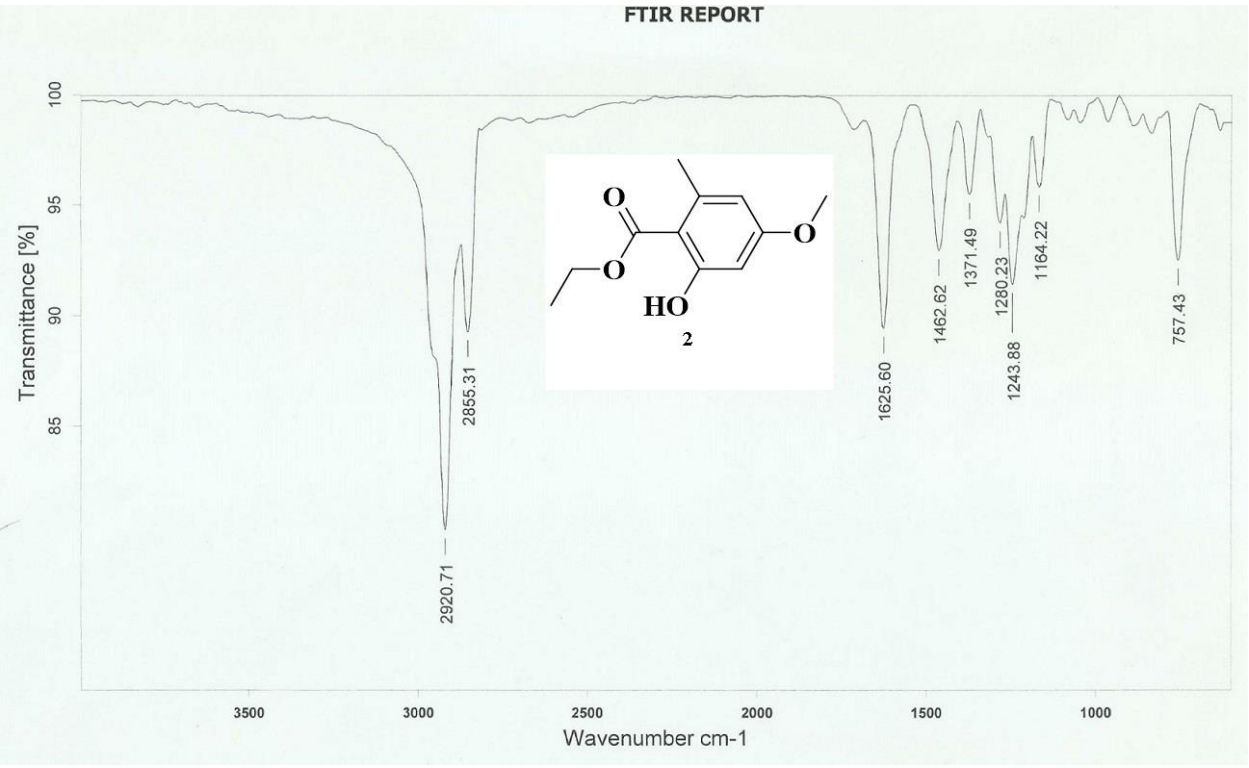


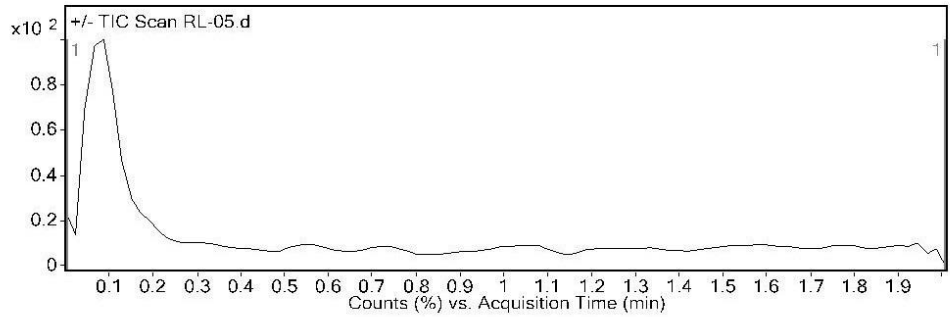
Figure S7: FT-IR of **2** (KBr)

Qualitative Analysis Report

Data Filename	RL-05.d	**Sample Name**	RL-05
Sample Type	Sample	**Position**	Vial 7
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	7/22/2017 11:37:44 PM
IRM Calibration Status	Not Applicable	**DA Method**	raghu.m
Comment			

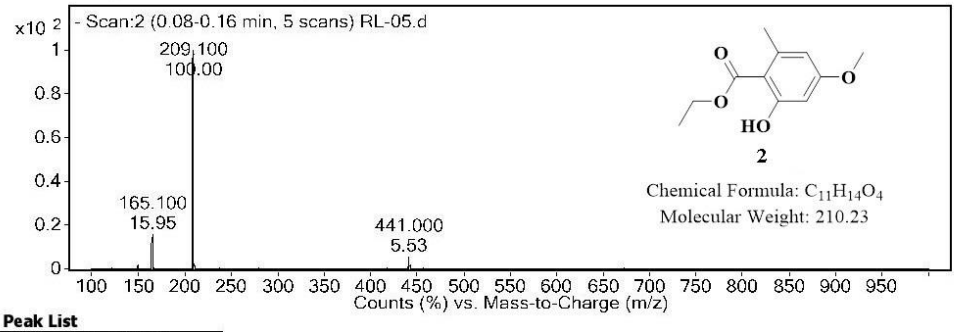
User Chromatograms

Fragmentor Voltage 135 **Collision Energy** 0 **Ionization Mode** ESI



User Spectra

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi



2

Chemical Formula: $C_{11}H_{14}O_4$
Molecular Weight: 210.23

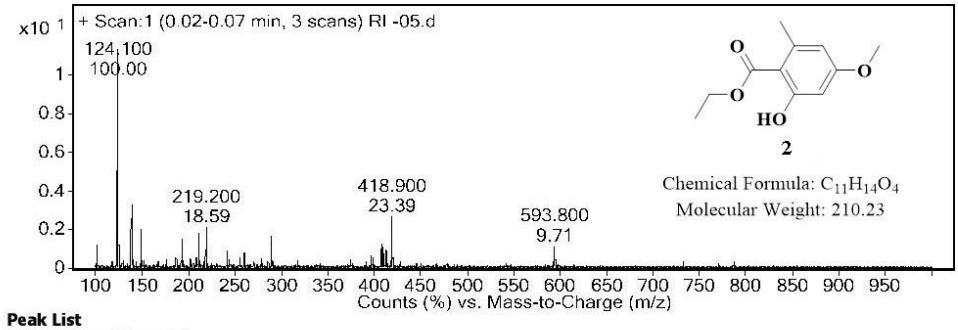
Peak List

m/z	z	Abund
165.1		203259
209.1	1	1274073
210.1	1	127513
441		70451

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi

Figure S8: ESI-MS of **2**

Qualitative Analysis Report

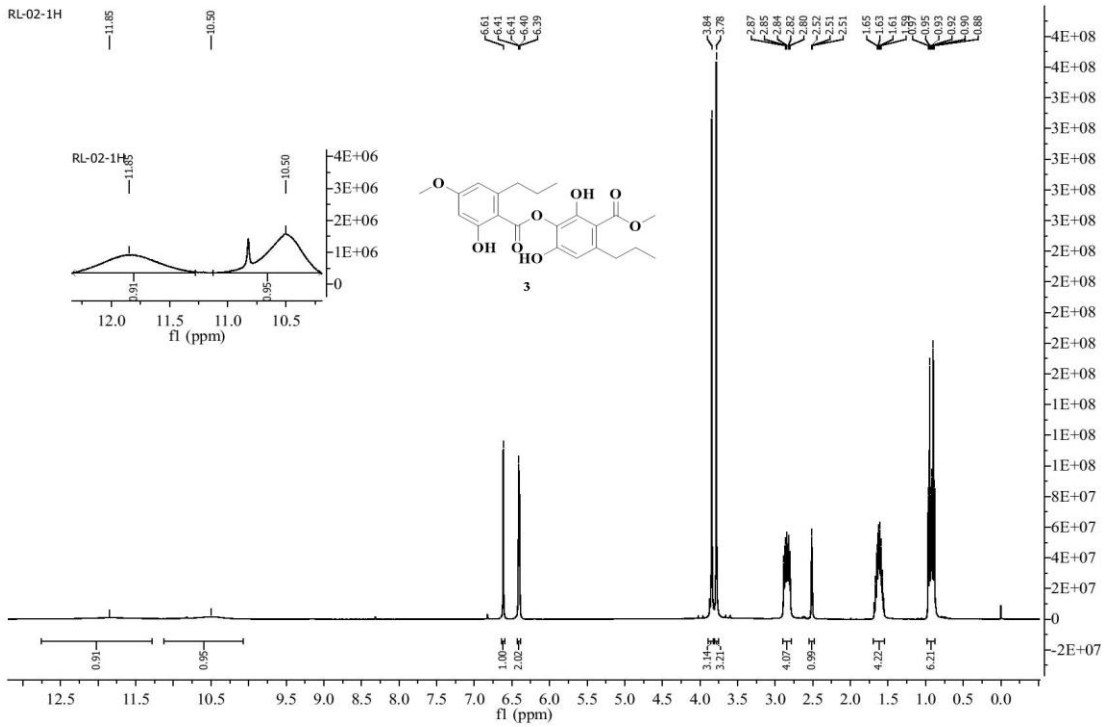


Peak List

m/z	z	Abund
123		63741
124.1	1	143809
139.1		41215
149		25343
193		18383
211		22719
219.2		26732
290		21302
408.7		15573
418.9		33641

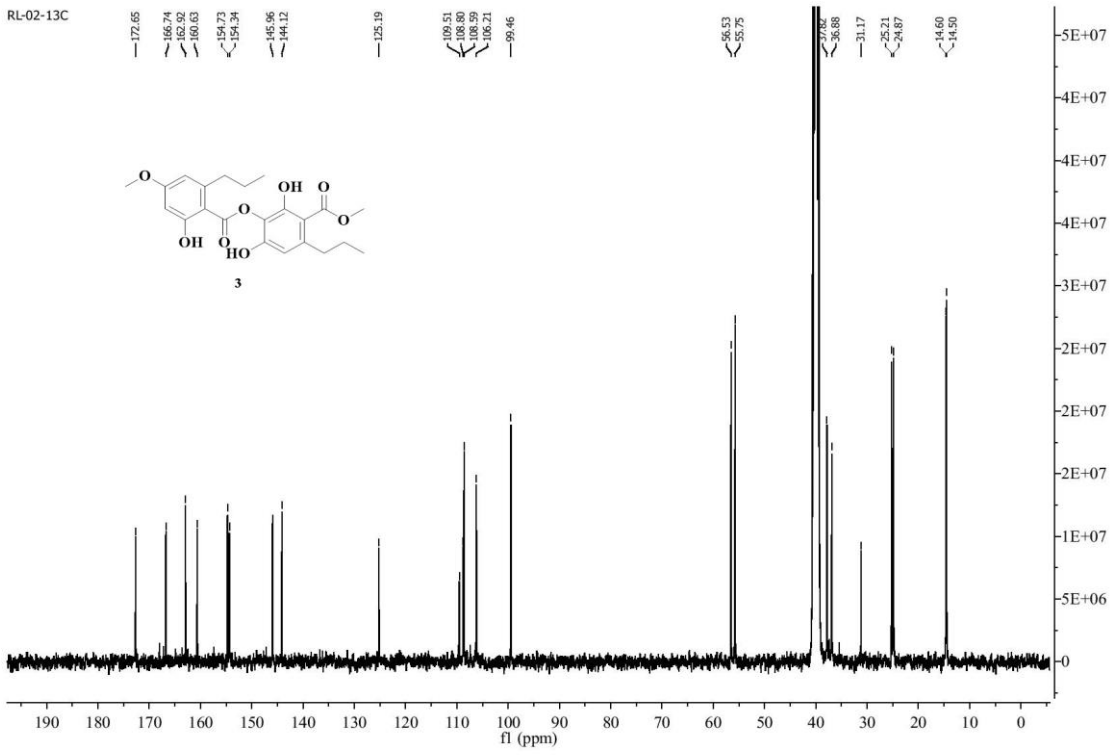
--- End Of Report ---

Figure S8a: ESI-MS of **2**



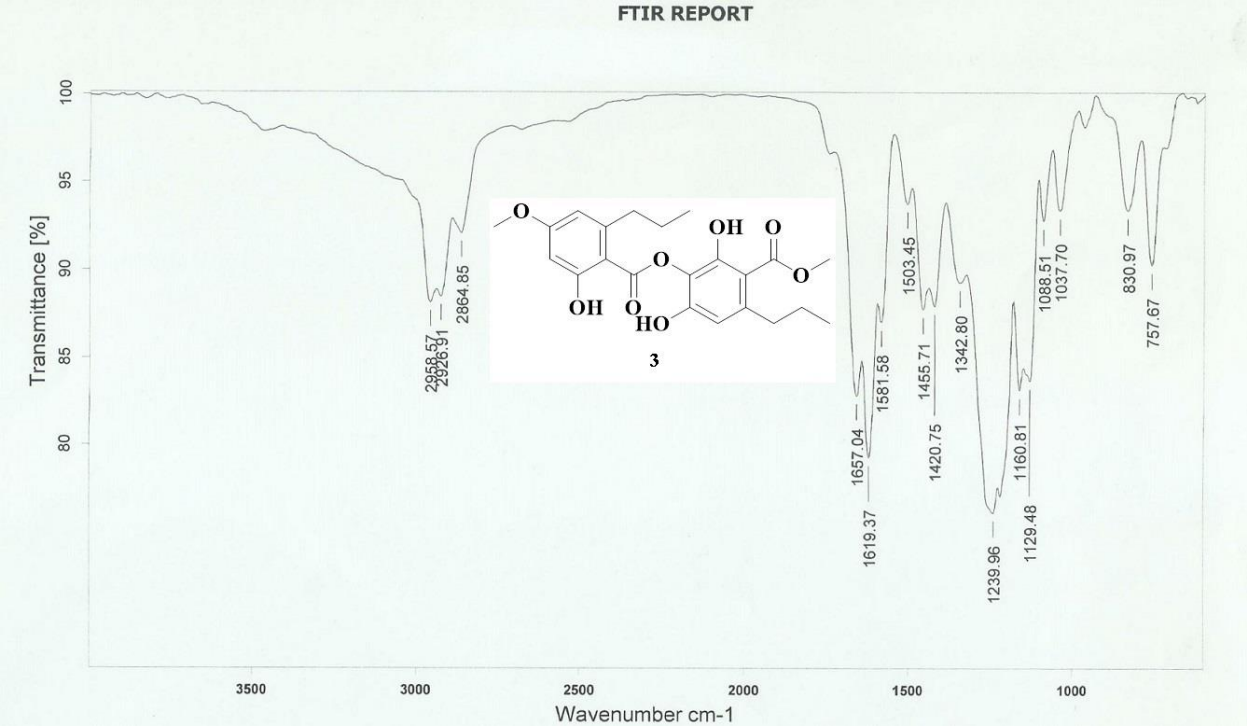
175

176 **Figure S9:** ^{1}H NMR of **3** (400 MHz, DMSO-*d*$_6$)



177

178 **Figure S10:** ^{13}C NMR of **3** (400 MHz, DMSO-*d*$_6$)



179

180 **Figure S11:** FT-IR of **3** (KBr)

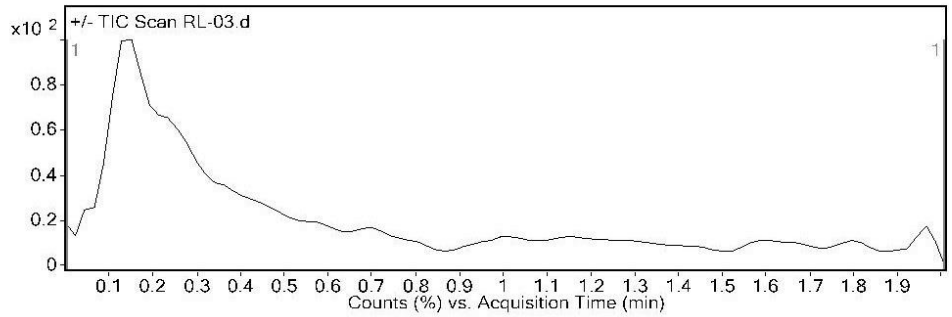
181

Qualitative Analysis Report

Data Filename	RL-03.d	**Sample Name**	RL-03
Sample Type	Sample	**Position**	Vial 8
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	7/22/2017 11:42:29 PM
IRM Calibration Status	Not Applicable	**DA Method**	raghu.m
Comment			

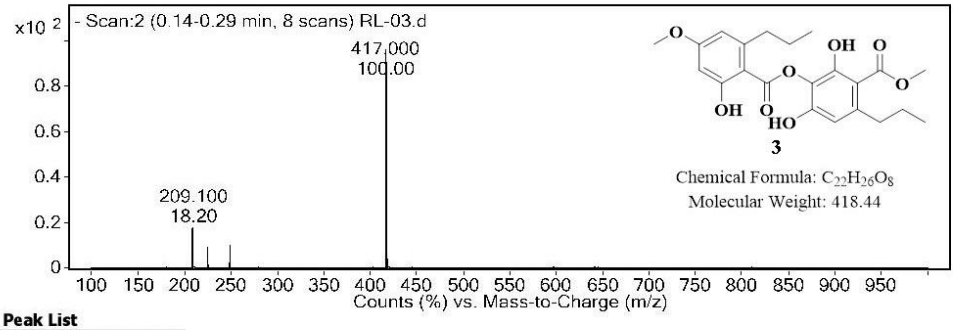
User Chromatograms

Fragmentor Voltage 135 **Collision Energy** 0 **Ionization Mode** ESI



User Spectra

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi



3

Chemical Formula: $C_{22}H_{26}O_8$

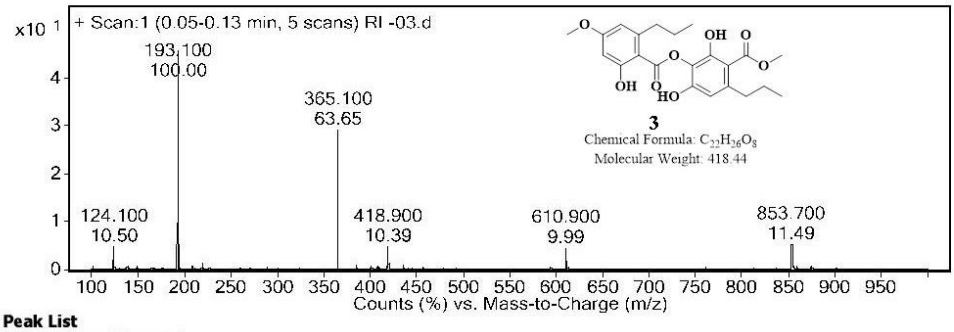
Molecular Weight: 418.44

Peak List

m/z	z	Abund
209.1		147727
225.1		75530
249.3		81514
417	1	811707
418	1	196038

Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
135	0	Esi

Figure S12: ESI-MS of **3**



3

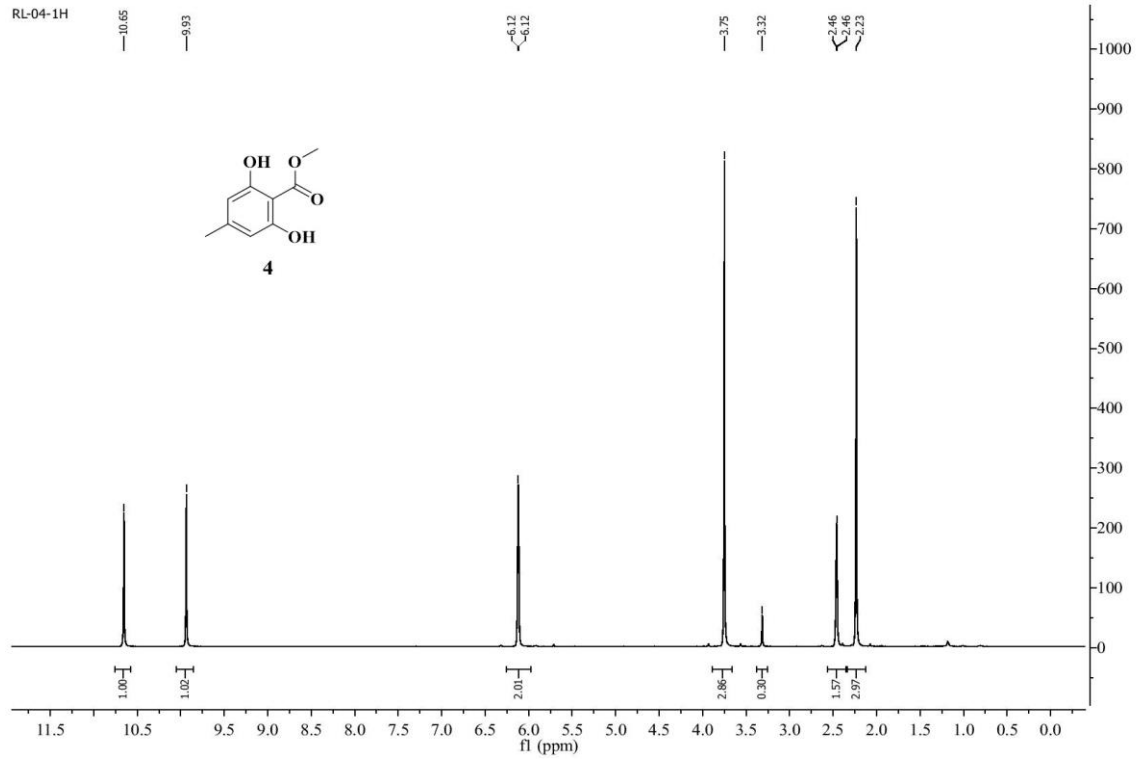
Chemical Formula: $C_{22}H_{26}O_8$
Molecular Weight: 418.44

Peak List

m/z	z	Abund
124.1		40435
193.1	1	385107
194.1	1	43770
365.1		245120
418.9		40017
610.9		38456
853.7	1	44233
854.8	1	24743

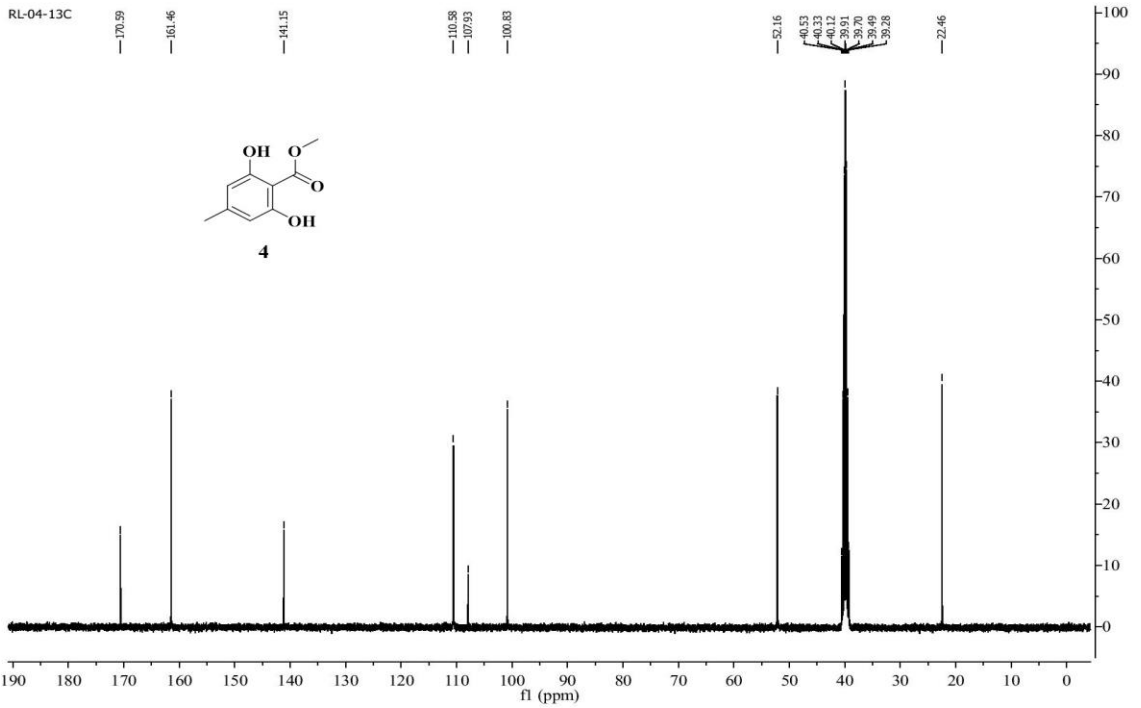
--- End Of Report ---

Figure S12a: ESI-MS of **3**



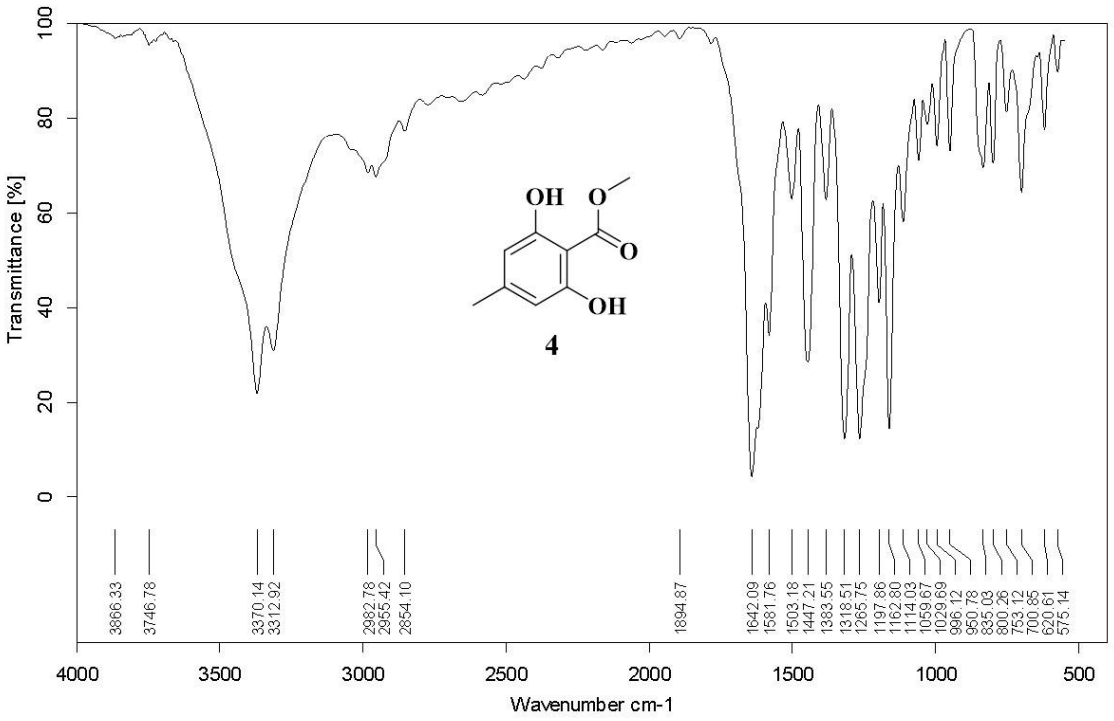
187

188 **Figure S13:** ^{1}H NMR of **4** (400 MHz, DMSO-d_6)



189

190 **Figure S14:** ^{13}C NMR of **4** (400 MHz, DMSO-d_6)



191

192 **Figure S15:** FT-IR of **4** (KBr)

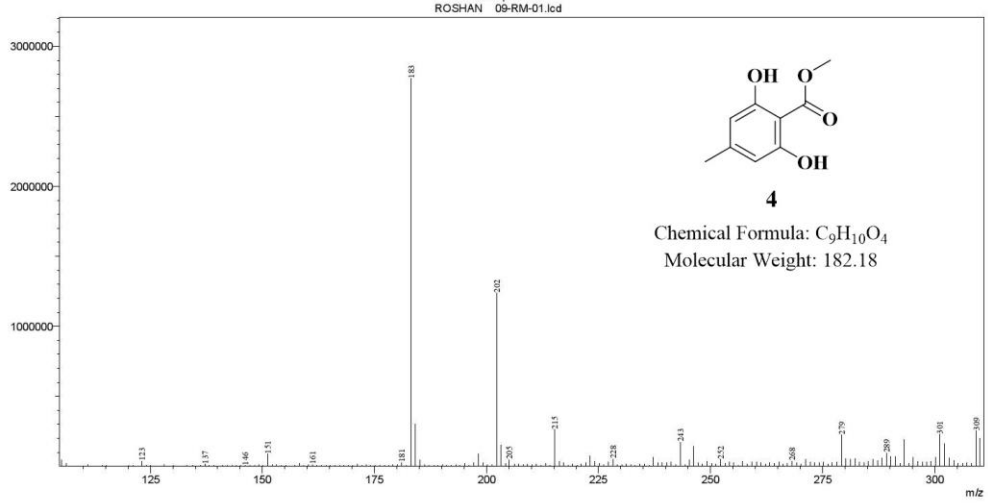
6/10/2016 12:06:31 PM Page 1 / 1

==== Organic and Biomolecular Chemistry Division, IICT ====

Sample Information

Date Acquired : 6/9/2016 3:05:40 PM
Sample Name : ROSHAN
Sample ID : RM-01
Data File : 09-RM-01.lcd
Method File : FCL-+@-.lcm

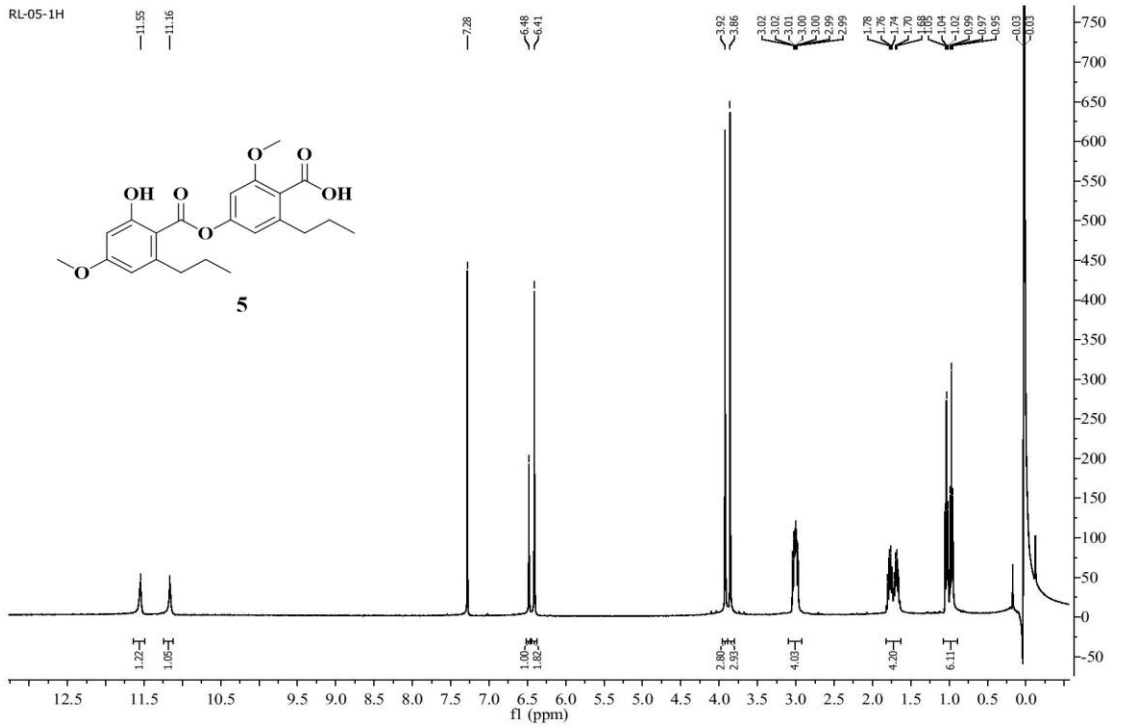
ESI-MS Spectrum
ROSHAN 09-RM-01.lcd



D:¥Data¥MASS¥JUN -2016¥09-RM-01.lcd

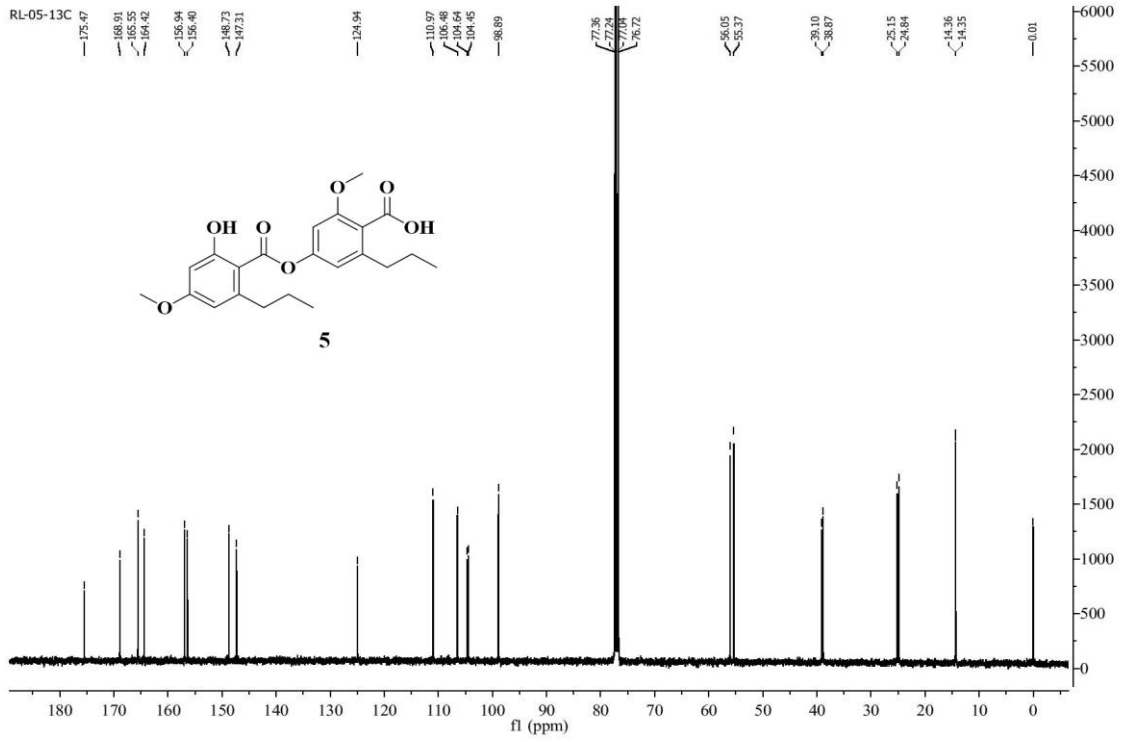
193

194 **Figure S16:** ESI-MS of **4**



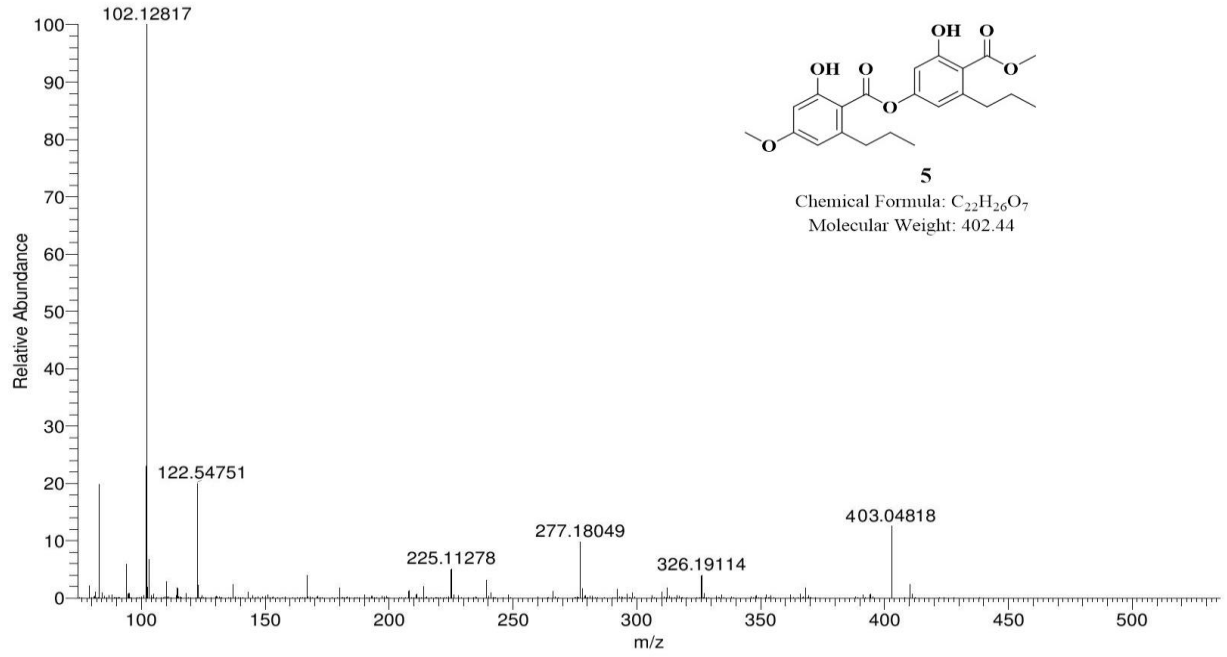
195

196 **Figure S17:** ^{1}H NMR of **5** (400 MHz, $CDCl_3$)



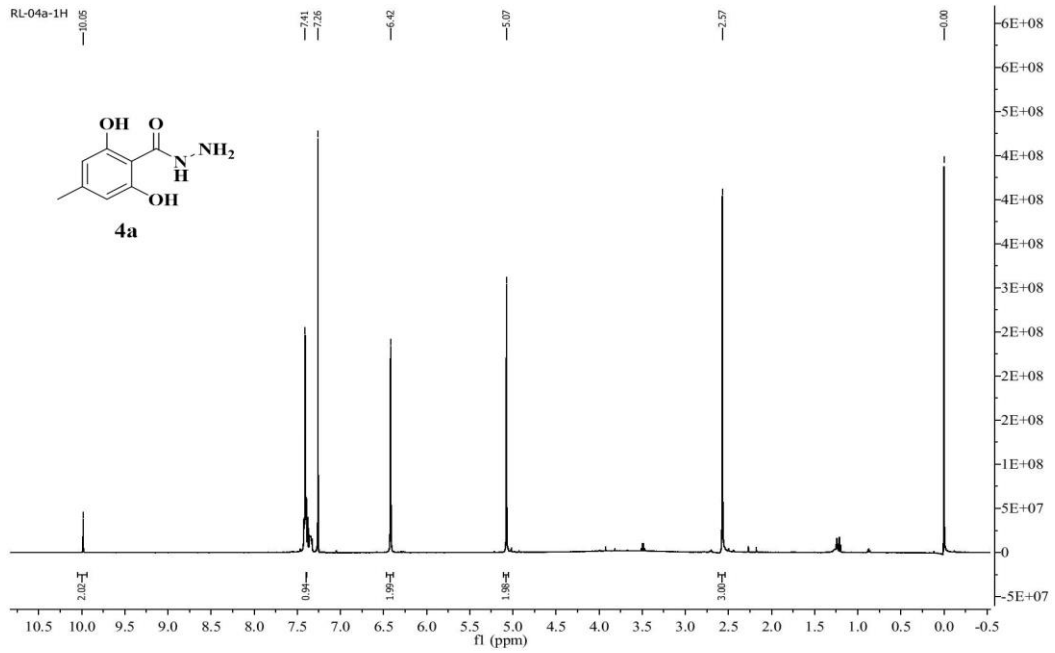
197

198 **Figure S18:** ^{13}C NMR of **5** (400 MHz, $CDCl_3$)



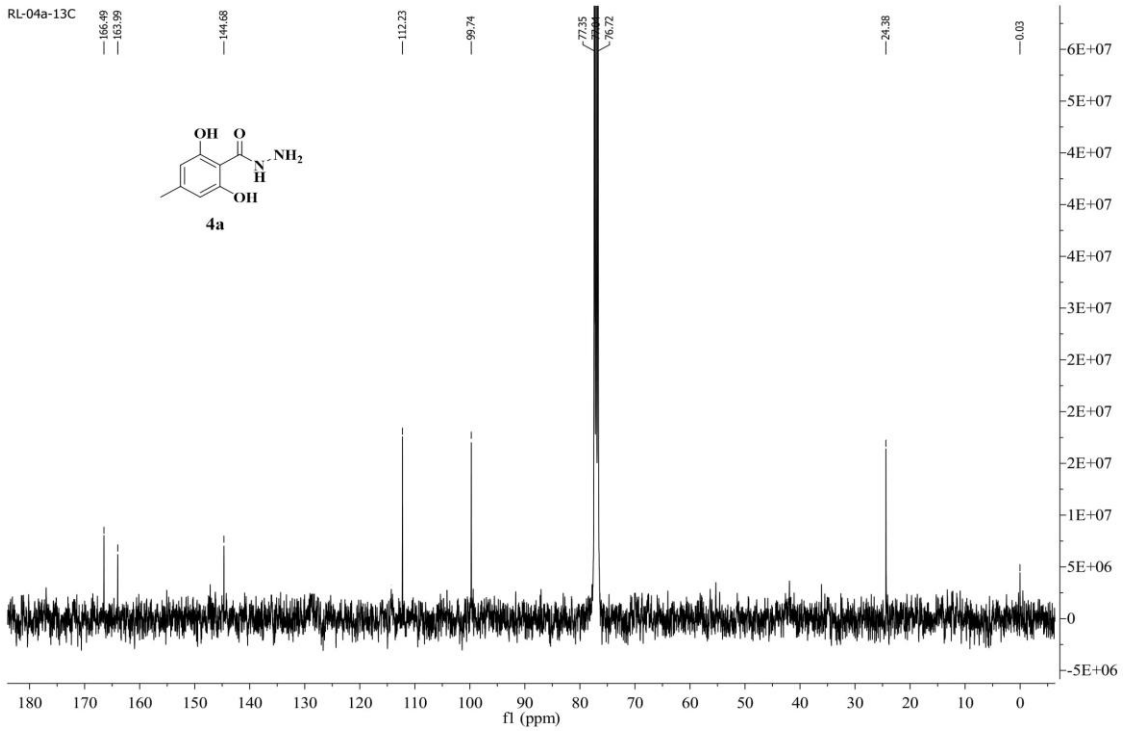
199

200 **Figure S20:** ESI-MS of **5**



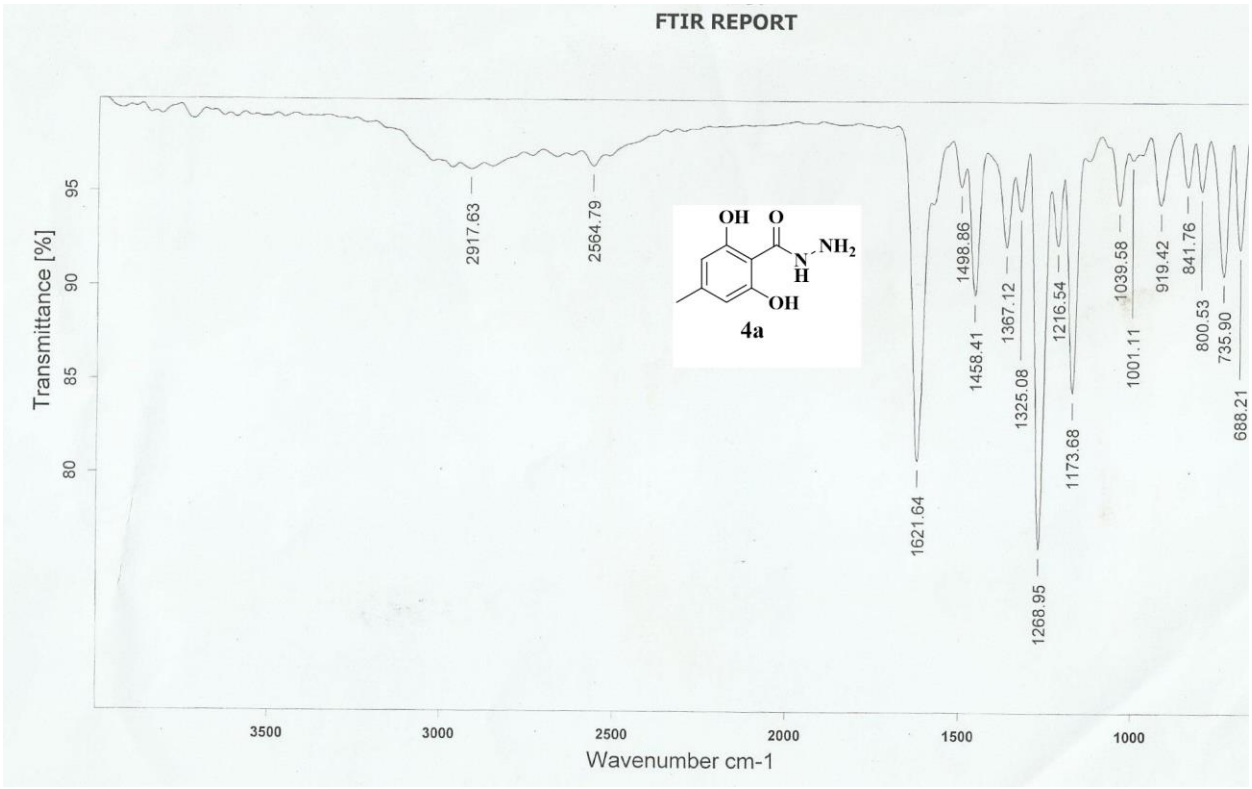
201

202 **Figure S21:** ^{1}H NMR of **4a** (400 MHz, $CDCl_3$)



203

204 **Figure S22:** ^{13}C NMR of **4a** (400 MHz, $CDCl_3$)



205

206 **Figure S23:** FT-IR of **4a** (KBr)

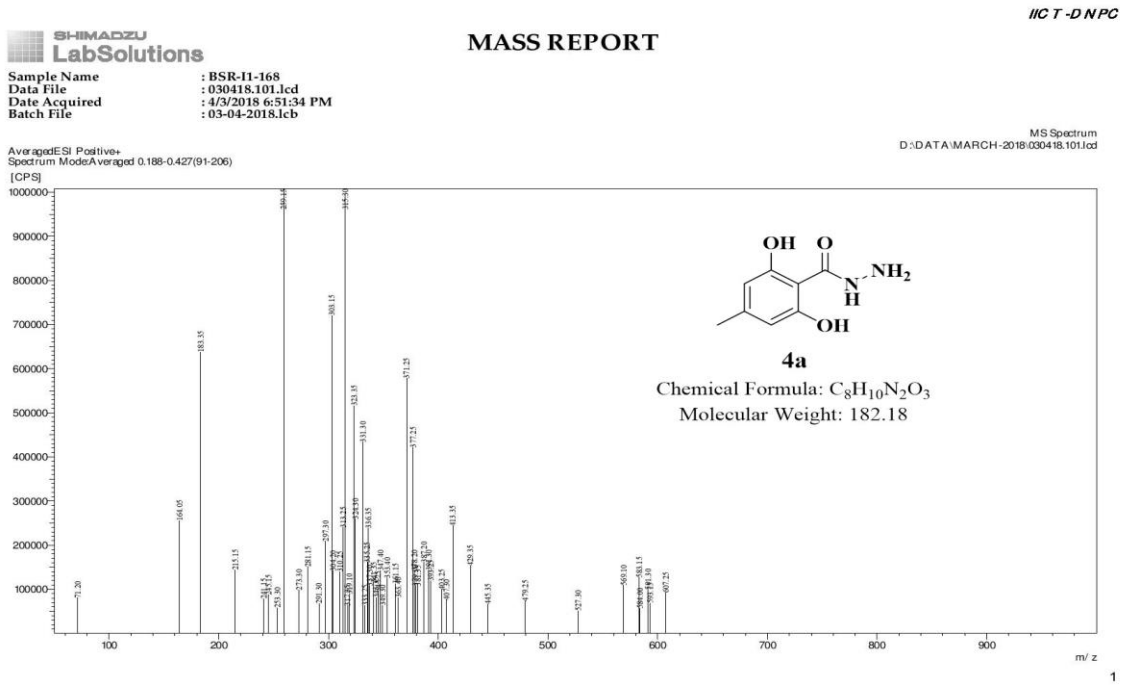


Figure S24: ESI-MS of **4a** (positive mode)

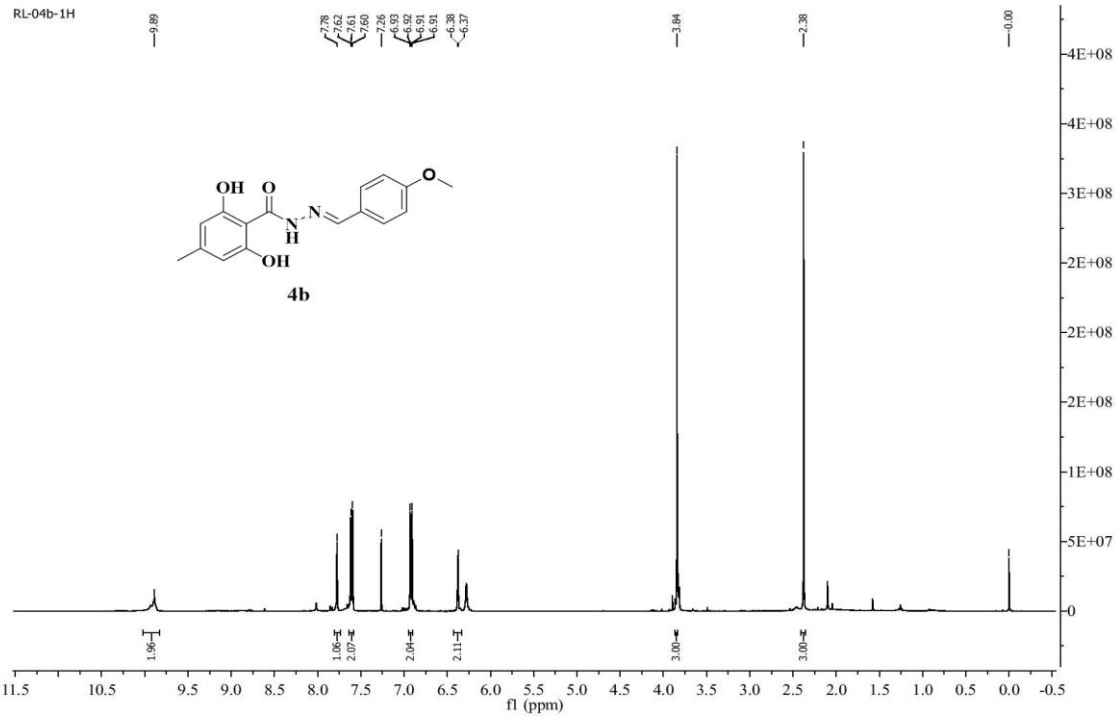
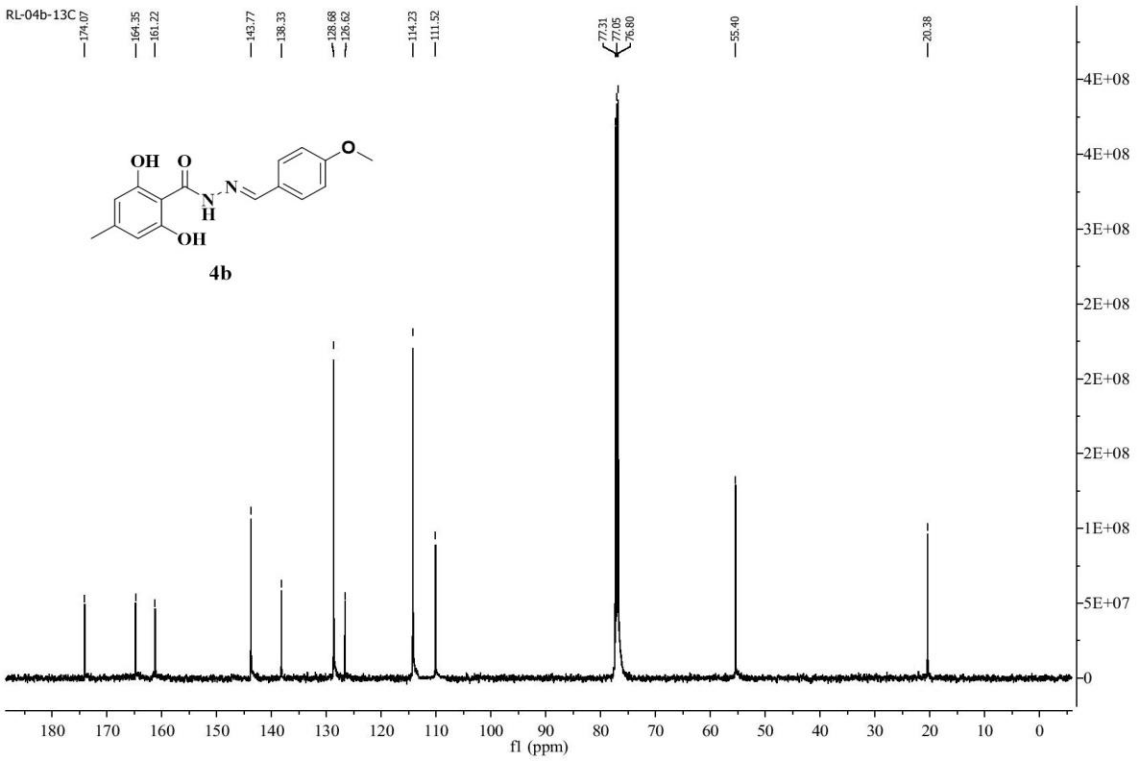
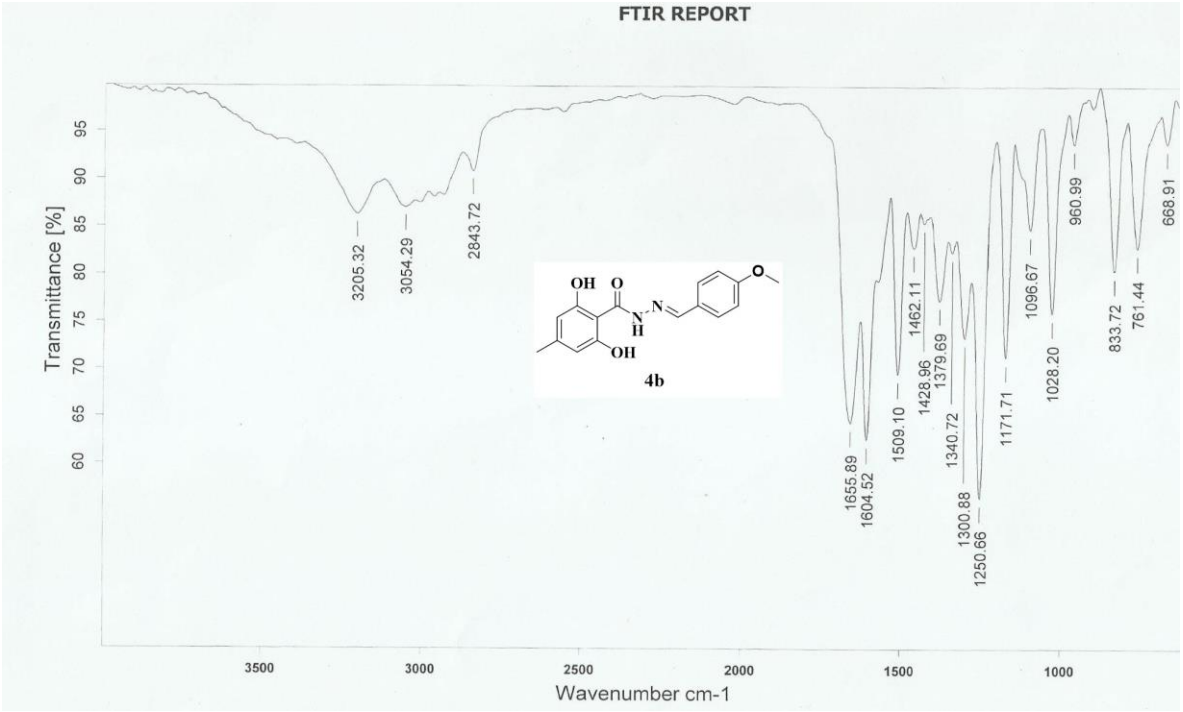


Figure S25: ^{1}H NMR of **4b** (400 MHz, $CDCl_3$)



212

213 **Figure S26:** ^{13}C NMR of **4b** (400 MHz, $CDCl_3$)



214

215 **Figure S27**: FT-IR of **4b** (KBr)

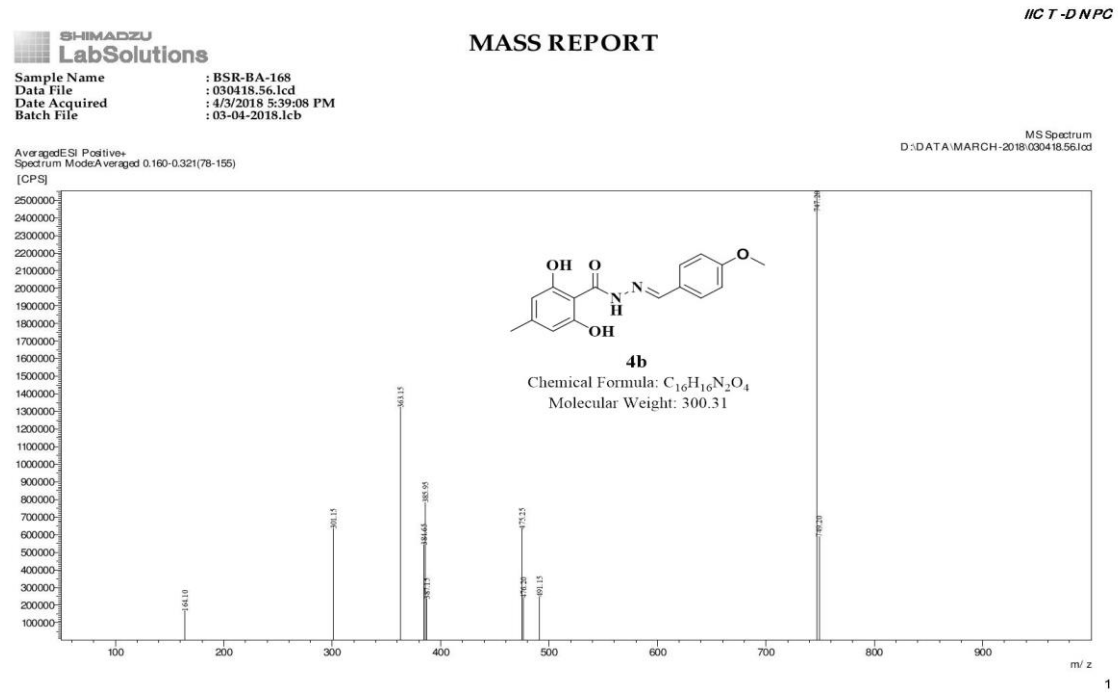


Figure S28: ESI-MS of **4b** (positive mode)

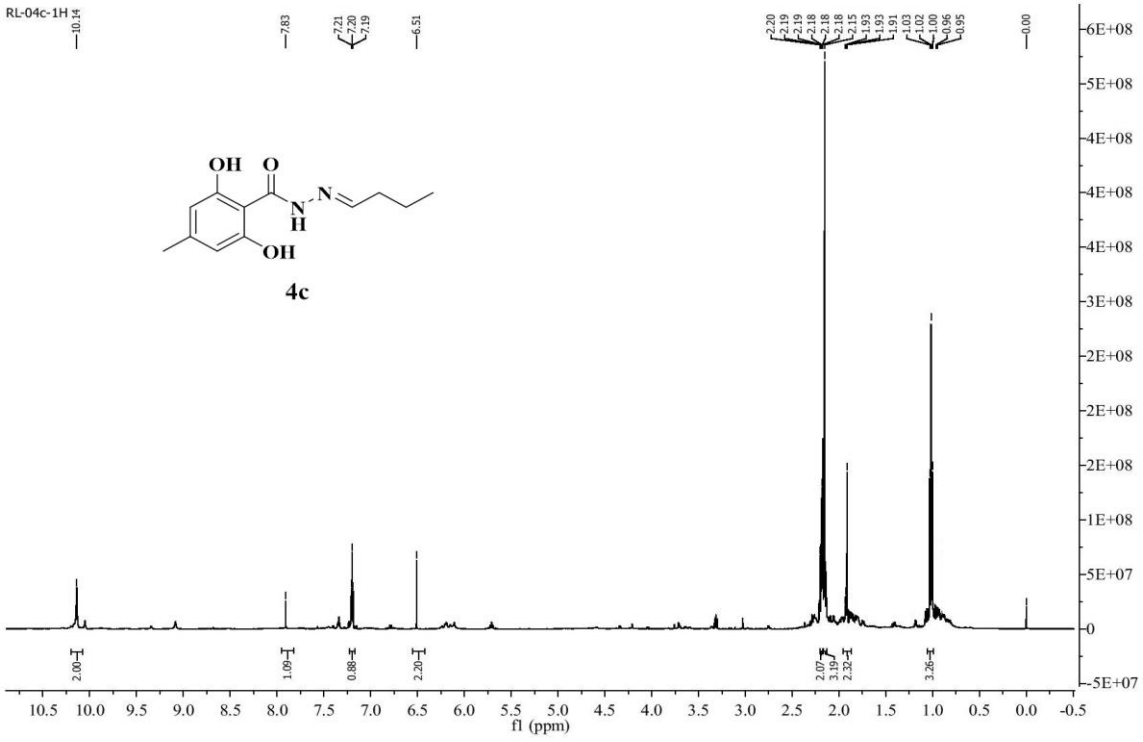


Figure S29: ^{1}H NMR of **4c** (400 MHz, $CDCl_3$)

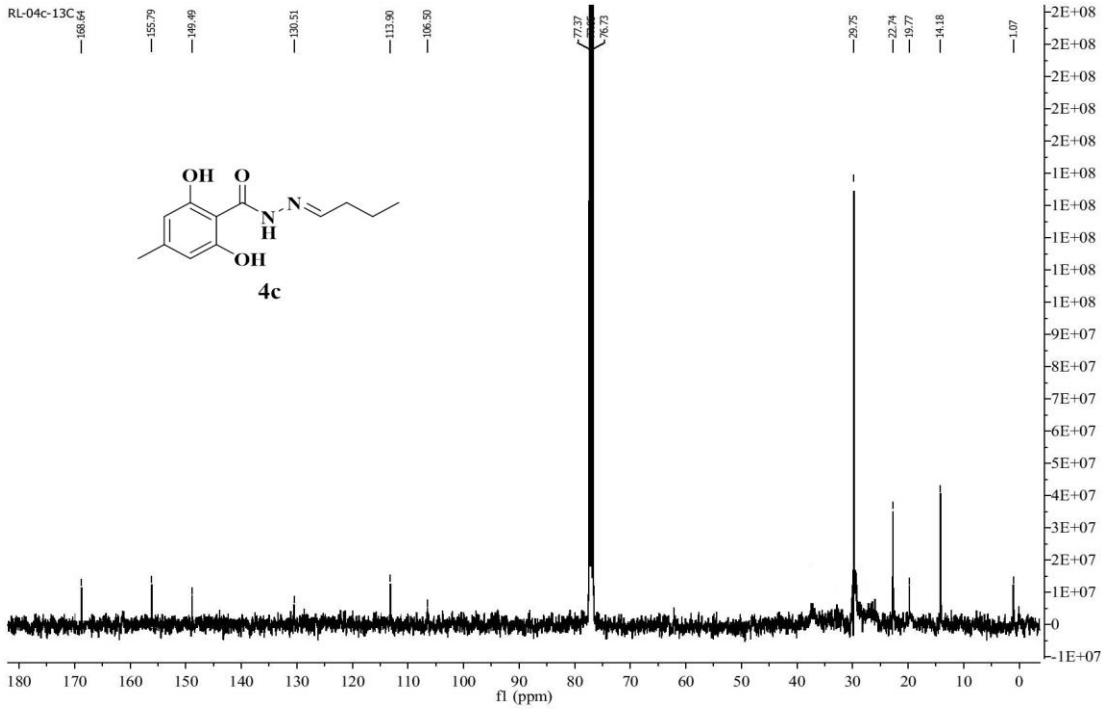


Figure S30: ^{13}C NMR of **4c** (400 MHz, $CDCl_3$)

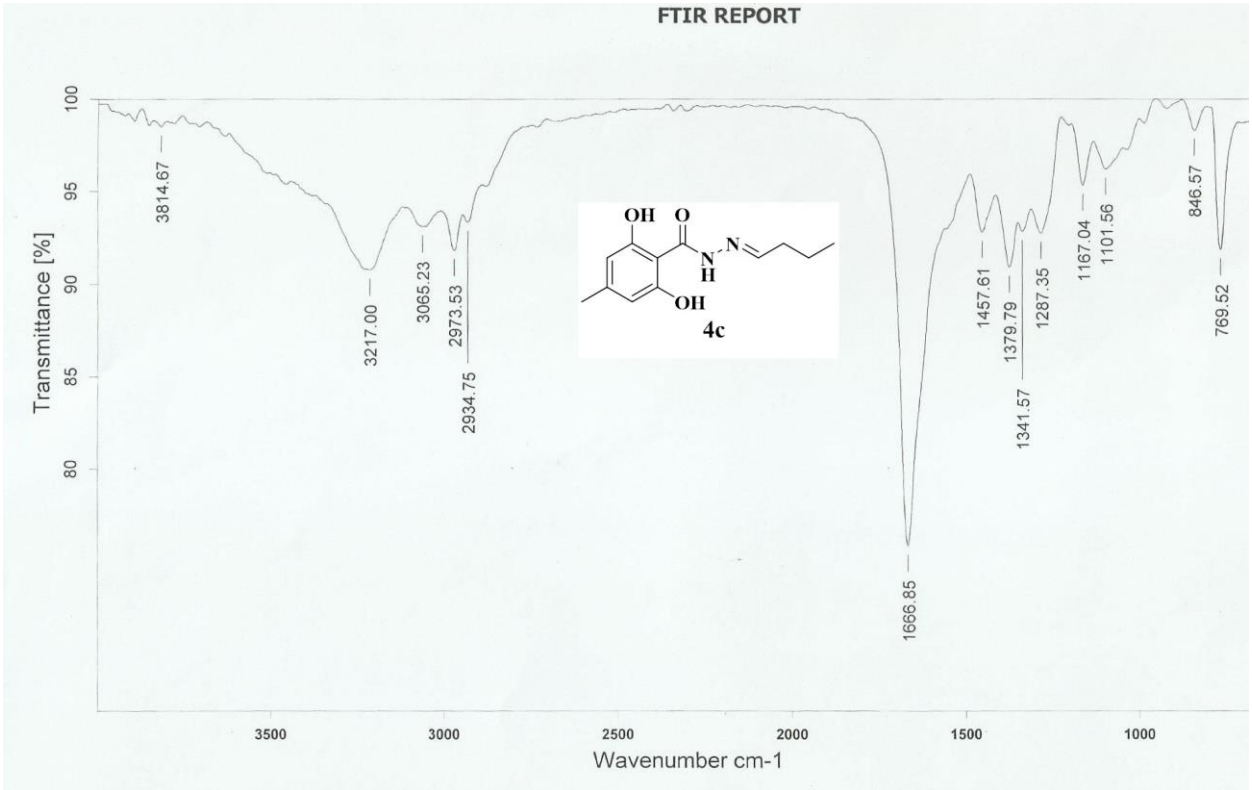
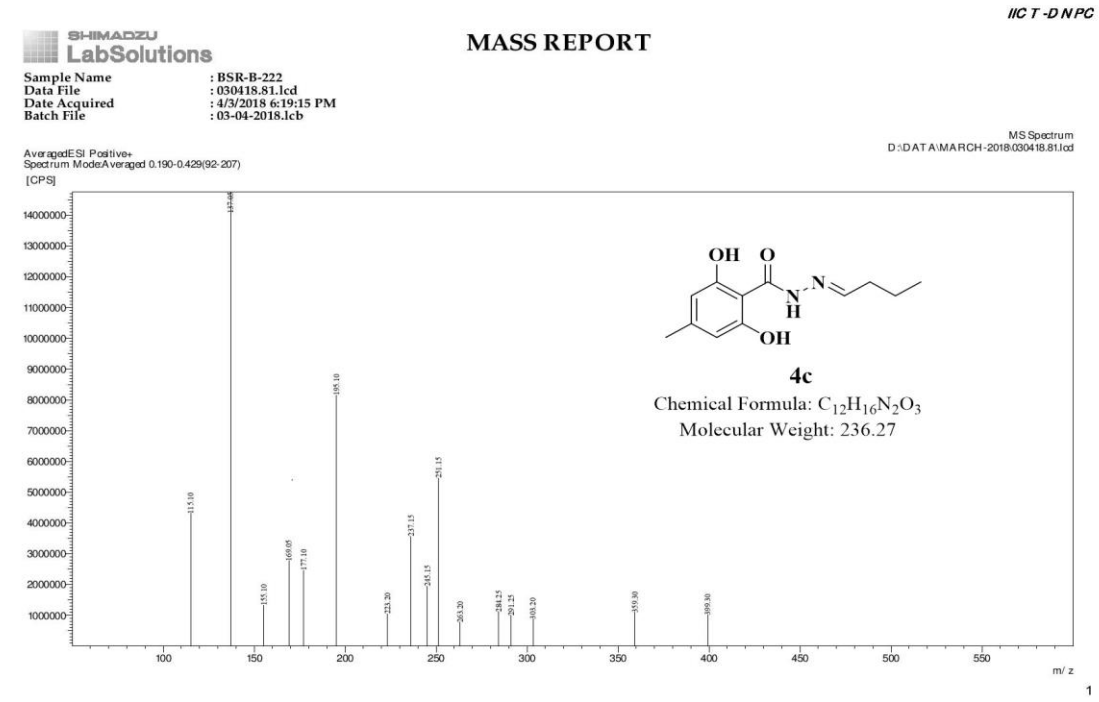
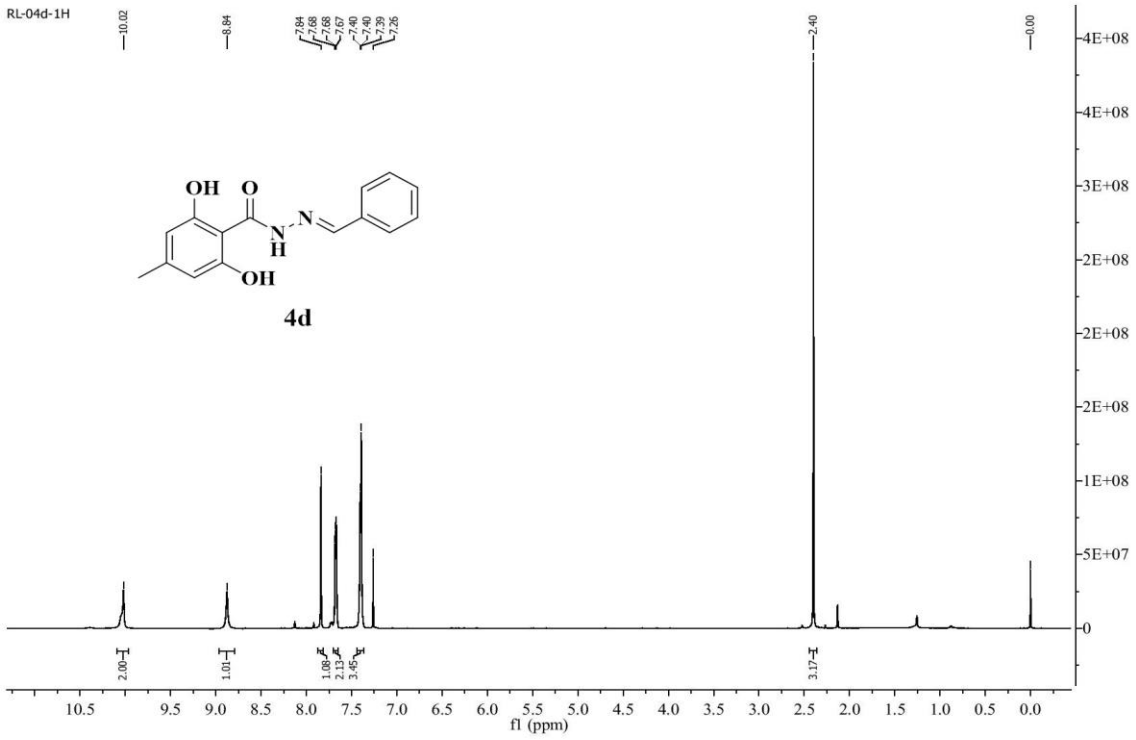


Figure S31: FT-IR of **4c** (KBr)



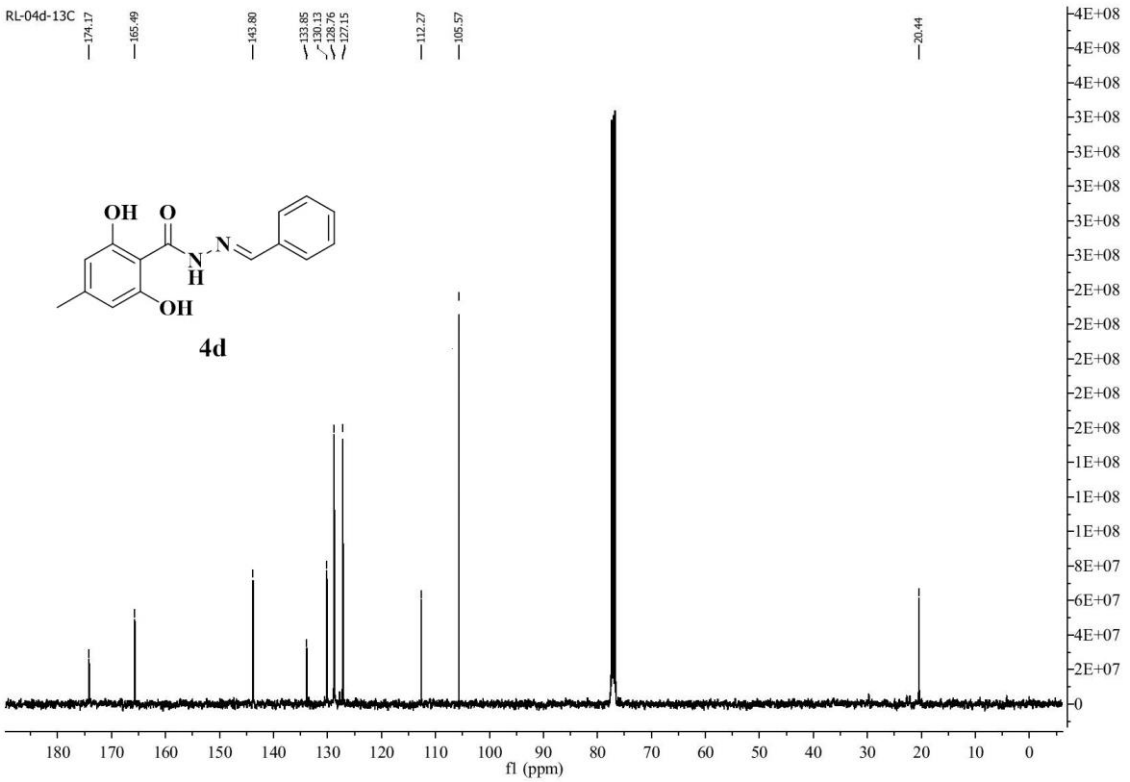
224

225 **Figure S32:** ESI-MS of **4c** (positive mode)



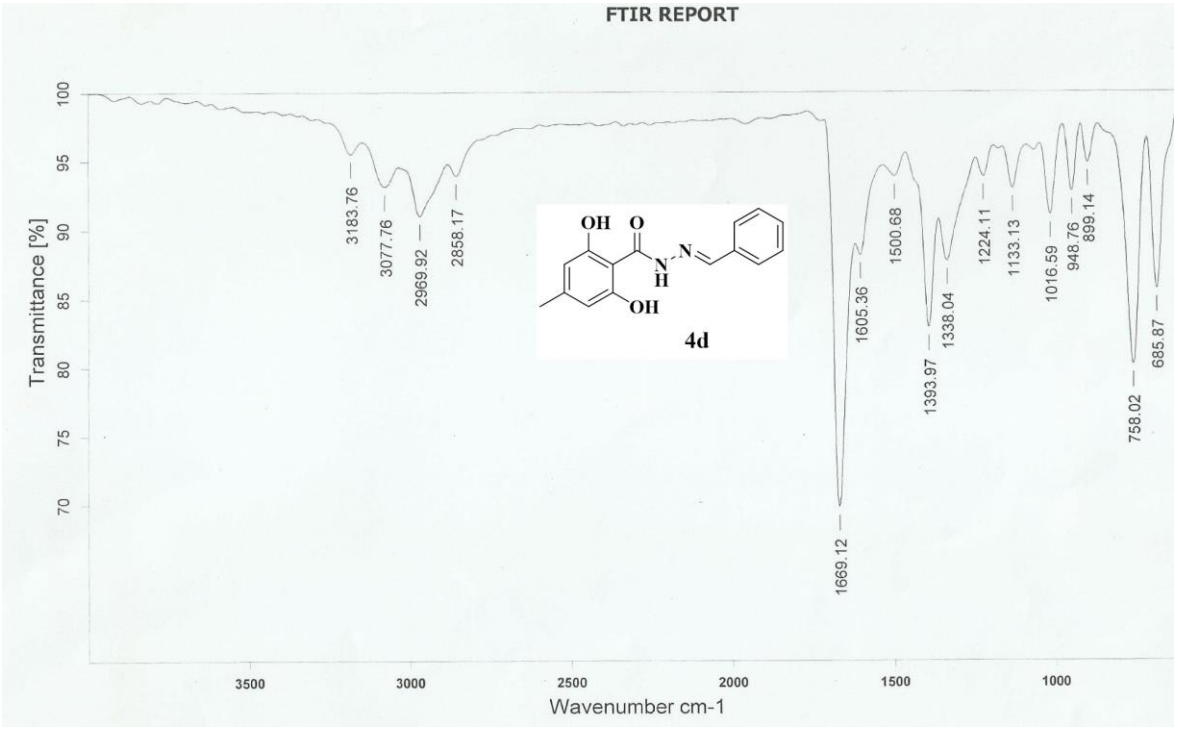
226

227 **Figure S33:** ^{1}H NMR of **4d** (400 MHz, $CDCl_3$)



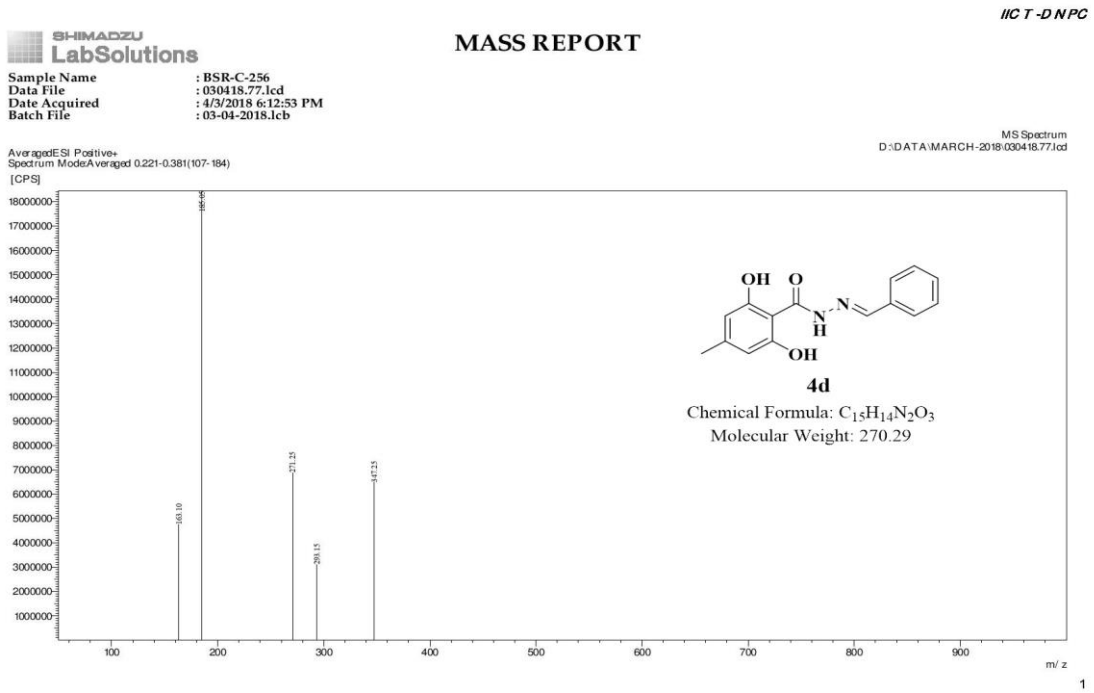
228

229 **Figure S34:** ^{13}C NMR of **4d** (400 MHz, $CDCl_3$)



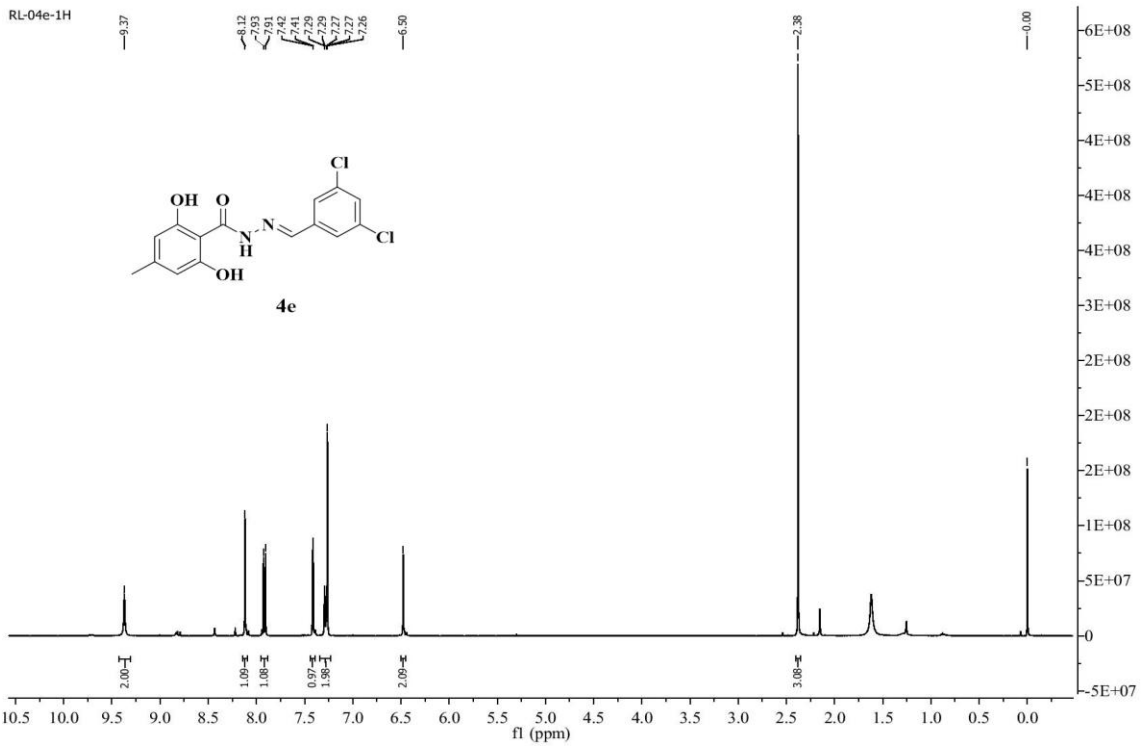
230

231 **Figure S35:** FT-IR of **4d** (KBr)



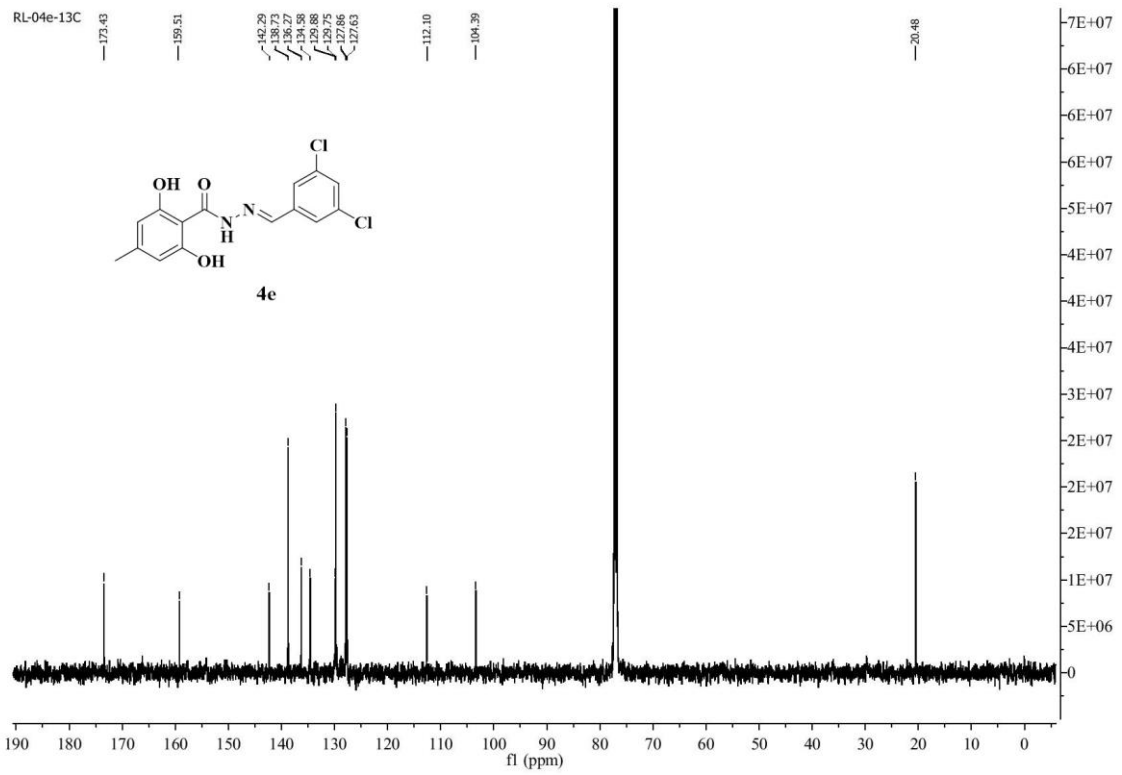
232

233 **Figure S36:** ESI-MS of **4d** (positive mode)



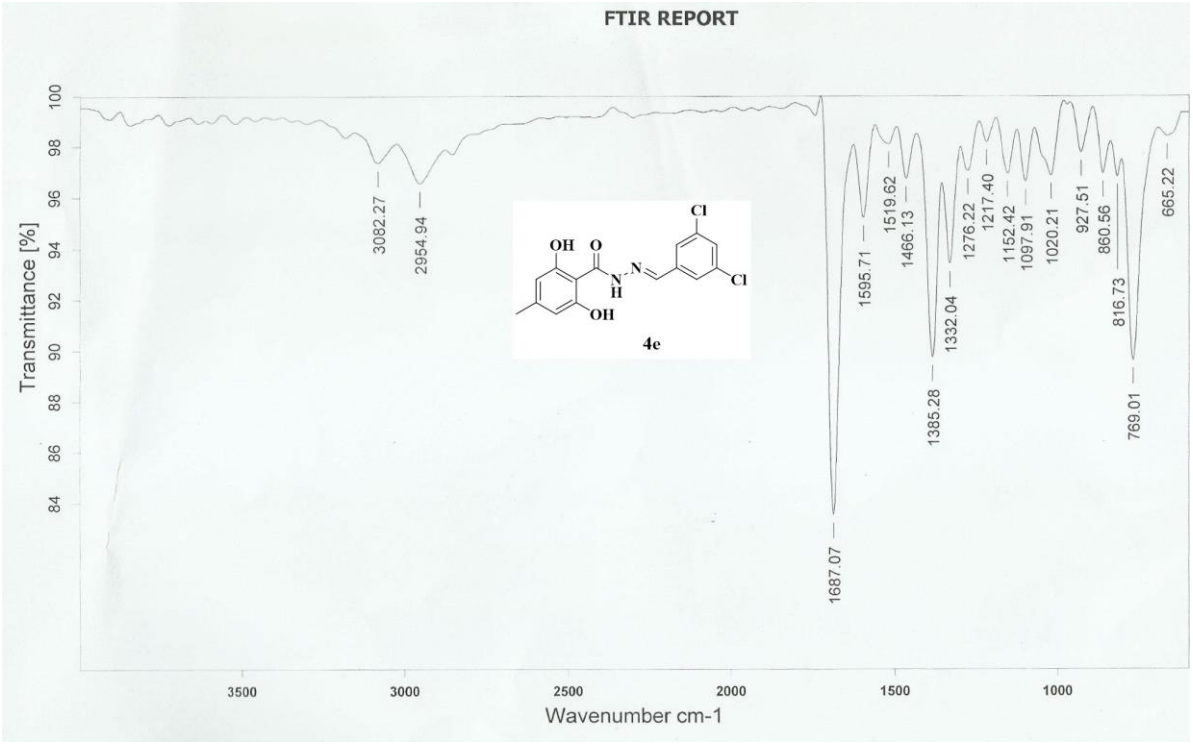
234

235 **Figure S37:** ^{1}H NMR of **4e** (400 MHz, $CDCl_3$)



236

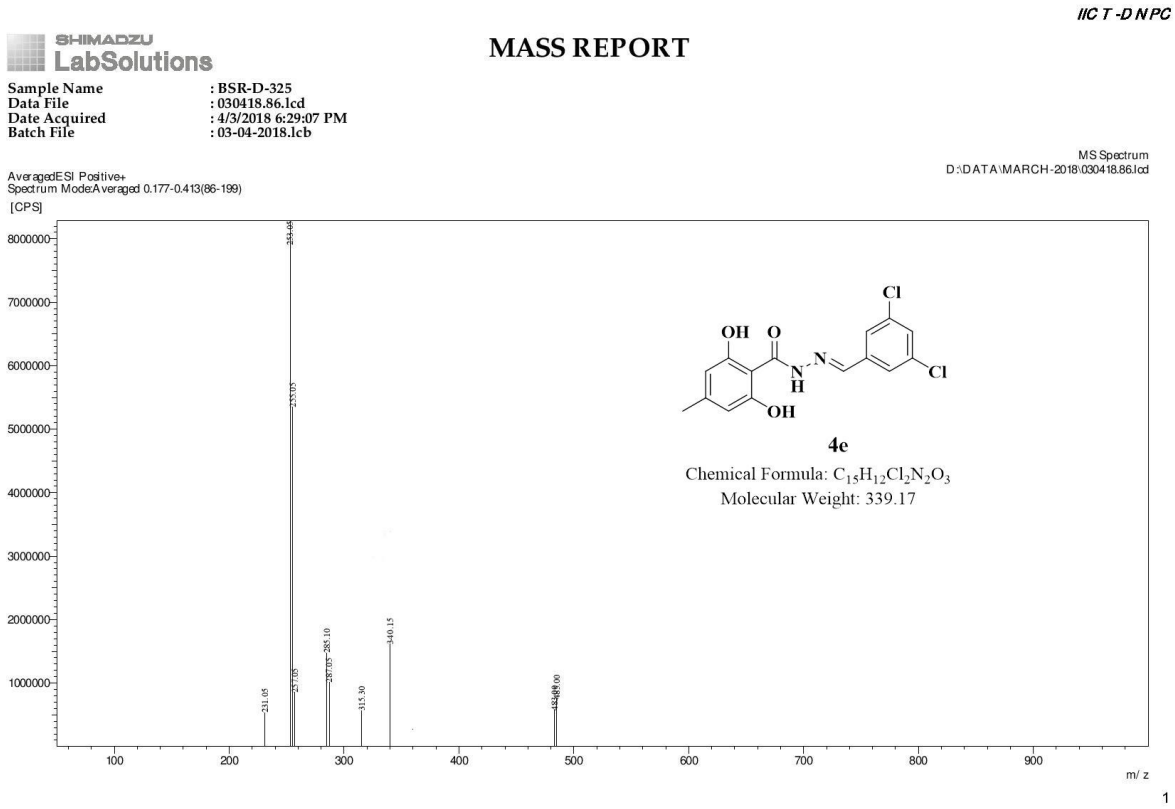
237 **Figure S38:** ^{13}C NMR of **4e** (400 MHz, $CDCl_3$)



238

239 **Figure S39:** FT-IR of **4e** (KBr)

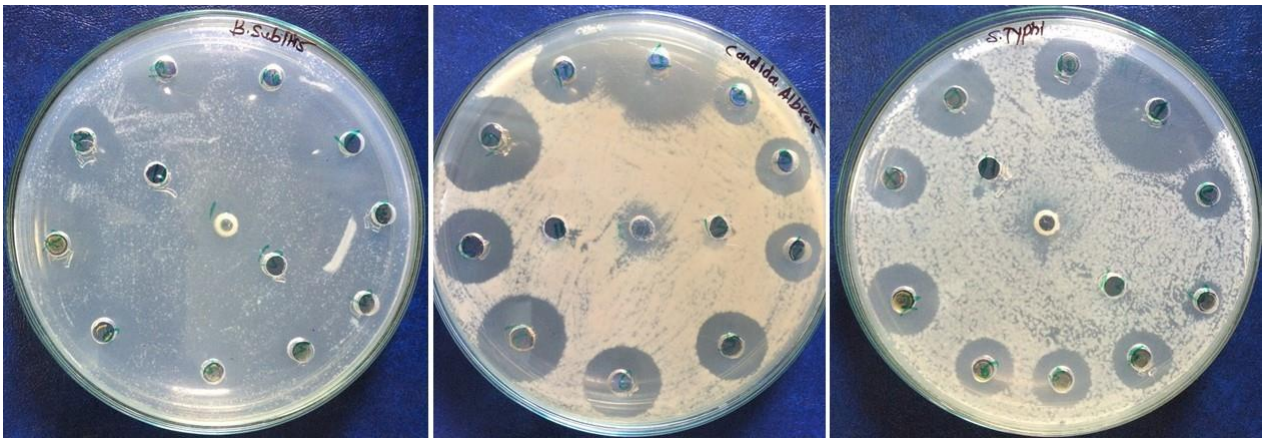
240



241

242 **Figure S40:** ESI-MS of **4e** (positive mode)

243



244

245 **Figure S41:** Diameter of zones of inhibition values for all the isolates and benzohydrazide

246 derivatives of *Ramalina leiodea* against bacterial and fungal stains.

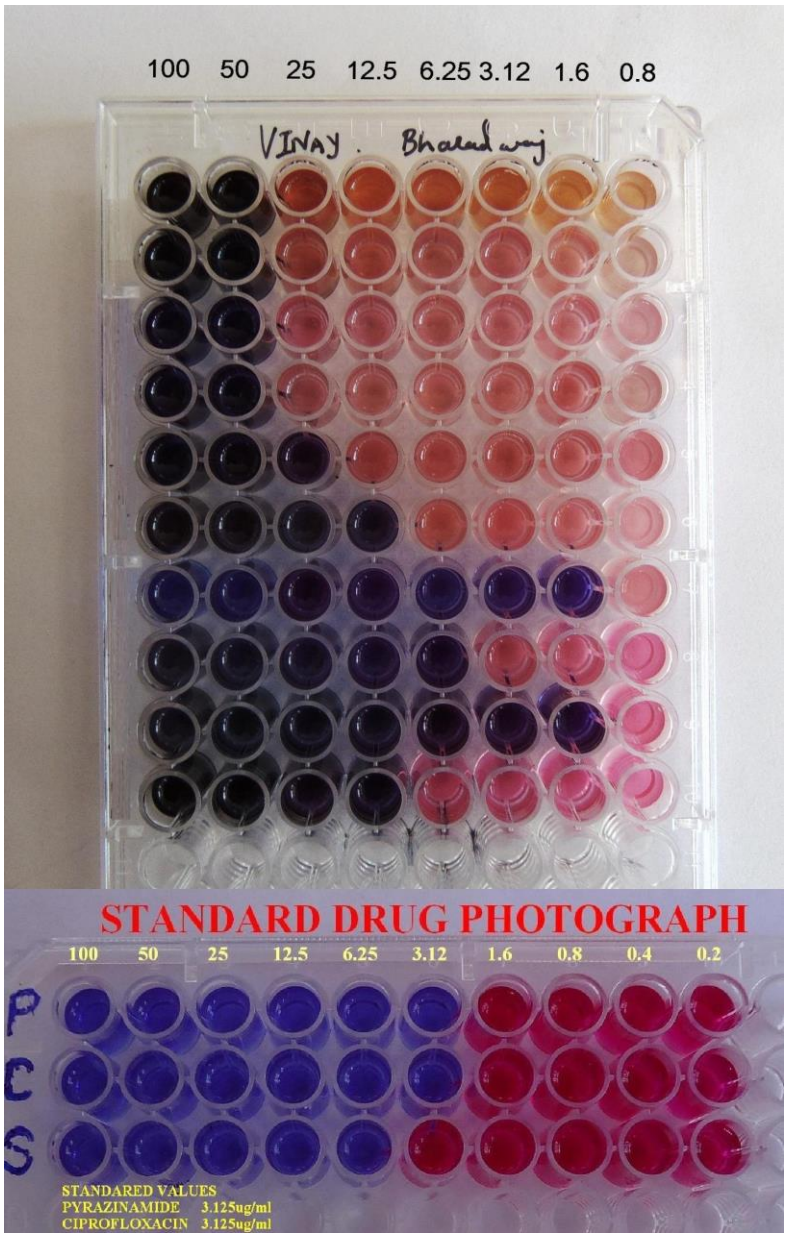


Figure S42: *In vitro* anti-tubercular activity of all the isolates and benzohydrazide derivatives of *Ramalina leiodea* against *Microbacterium tuberculosis* H37Rv strain.

REFERENCES

1. P. R. Shetty, S. K. Buddana, V. B. Tatipamula, Y. V. V. Naga, J. Ahmad, *Braz. J. Microbiol.* **45** (2014) 303-312 (https://doi.org/10.1590/S1517-83822014005000022)

2. V. B. Tatipamula, G. S. Vedula, B. B. Rathod, P. R. Shetty, A. V. S. Sastry, *Inventi Impact: Planta Activa* **2018** (2018) 129 (http://inventi.in/journal/article/impact/61/25058/planta-activa/ci)
3. K. R. Paidi, V. B. Tatipamula, M. K. Kolli, V. R. Pedakotla, *International Journal of Chemical Sciences* **15** (2017), 172 (0972-768X).