Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 84 (6) S140–S168 (2019) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Antimicrobial and anti-tubercular activities of isolates and semi-synthetic derivatives of lichen *Ramalina leiodea* (Nyl.) Nyl.

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J. Serb. Chem. Soc. 84 (6) (2019) 555–562

EXPERIMENTAL

Antimicrobial activities

The determinations of both the antibacterial and antifungal activities for measuring the antimicrobial properties were realized according to the standard well plate method.[1] For the antibacterial activities, all the selected test strains (*Salmonella enterica* ATCC 35664, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 23857 and *Staphylococcus aureus* ATCC 25923) were initially activated and grown in nutrient agar. Whereas, for anti-fungal studies, the test strain used, *Candida albicans*, was grown on potato dextrose agar medium. All compounds were re-dissolved in DMSO to obtain a final concentration of 1 mg mL^{-1}, which were used as stock solutions. The compounds were used for activity studies and the concentration of each sample was 1 μg mL^{-1} 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 a sterile environment. These plates were inoculated with 60 μL of test inoculums using sterile cotton swabs. Wells 8 mm in diameter were made with a sterile cork borer and into each well exactly 100 μL of sample were loaded. The control and standard were 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 the bacteria and 48 h at 28 °C for the fungi. The zone diameters were measured in mm using a calibrated scale. The experiments were conducted in triplicate with aliquots to minimize the deviations and the average values are reported.

The compounds having better anti-microbial activities were selected for the minimum inhibitory concentration (*MIC*) and minimum bactericidal concentration (*MBC*) studies against *Salmonella enterica*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *C. albicans* according to a reported method.[2] The concentrations of test samples were serially diluted from 1 to 0.001 μg mL^{-1} and one tube without drug served as the 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 37 °C for 12–16 h. The turbidity of each tube was measured with respect to the control tube. The *MIC* values are defined as the lowest concentration of a compound at which

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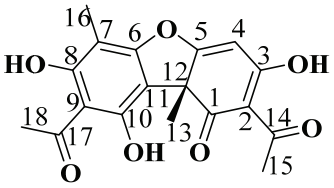
growth was completely inhibited. After incubation, the culture from each tube was plated in nutrient agar to evaluate the *MBC* value. The concentration at which the cells were all dead was defined as the *MBC*.

Anti-tubercular activity

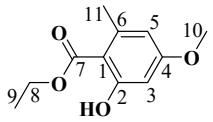
The *in vitro* anti-tubercular activity assessment of all compounds was tested against the *Mycobacterium tuberculosis* H37Rv strain ATCC 25177 using the microplate alamar blue assay (MABA)[2,3] in three sets (n = 3). The minimum inhibitory concentration (*MIC*) was determined by the color change from blue to pink, which indicate no bacterial growth and growth, respectively.

ANALYTICAL AND SPECTRAL DATA FOR THE COMPOUNDS



1

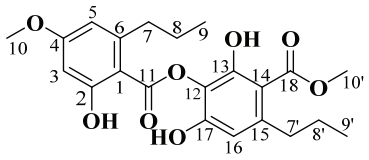
*Usnic acid (**1**)*. Yield: 236 mg (0.16 %, based on total weight of the lichen material); m.p.: 201–202 °C, Anal. Calcd for $C_{18}H_{16}O_7$: C, 63.15; H, 6.26 %. Found C, 63.16; H, 6.26 %; IR (KBr, cm^{-1}): 2923, 2858, 1687, 1626, 1543, 1462, 1368, 1293, 1192, 1138, 1071, 1037, 966, 843; ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 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 (100 MHz, $CDCl_3$, δ / ppm): 7.6 (C-16), 27.9 (C-13), 31.3 (C-18), 32.2 (C-15), 59.1 (C-12), 98.4 (C-4), 101.6 (C-7), 104.0 (C-11), 105.3 (C-2), 109.3 (C-9), 155.2 (C-6), 157.5 (C-10), 163.9 (C-8), 179.4 (C-5), 191.7 (C-3), 198.1 (C-1), 200.4 (C-14), 201.8 (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; UV/Vis (EtOH, λ_{max} / nm: 220. R_f (hexane––DCM, 9:1): 0.4.



2

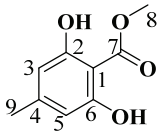
*Ethyl everninate (**2**)*. Yield: 153 mg (0.10 %, based on total weight of the lichen material); m.p.: 74–76 °C, Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71 %. Found: C, 62.87; H, 6.82 %; IR (KBr, cm^{-1}): 2920, 2855, 1625, 1462, 1371, 1280, 1243, 1164, 757; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 0.95 (3H, *t*,

J = 8 Hz, Me-9), 2.22 (3H, *s*, Me-11), 3.02 (H, *s*, OH-2), 3.83 (3H, *s*, OMe-10), 4.37 (2H, *m*, OCH_2), 6.33 (1H, *d*, *J* = 1.2 Hz, H-5), 6.37 (1H, *d*, *J* = 1.2 Hz, H-3);. ^{13}C-NMR (100 MHz, acetone-d_6, δ / ppm): 14.47 (C-9), 25.85 (C-11), 54.96 (C-10), 55.8 (C-8), 99.7 (C-1), 111.1 (C-3/5), 149.1 (C-6), 165.2 (C-2), 167.2 (C-4), 174.0 (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; UV/Vis (EtOH, λ_{max} / nm: 219; R_f (hexane–DCM, 7:3): 0.4.



3

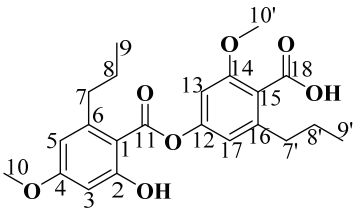
Scrobiculin (***3***). Yield: 911 mg (0.61 %, based on total weight of the lichen material), m.p.: 134–136 °C, Anal. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found C, 63.16; H, 6.26. IR (KBr, cm^{-1}): 2958, 2926, 2864, 1657, 1619, 1581, 1503, 1455, 1420, 1342, 1239, 1160, 1129, 1088, 1037, 830, 757; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 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*, J_1 = 4 Hz, J_2 = 4 Hz CH_2-7, 7′), 3.78 (3H, *s*, OMe-10), 3.84 (3H, *s*, OMe-10′), 6.39–6.41 (2H, *dd*, J_1 = 0.8 Hz, J_2 = 1 Hz H-3, 5), 6.61 (1H, *s*, H-16), 10.50 (1H, *s*, OH-17), 11.85 (1H, *s*, OH-13); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 14.5 (C-9), 14.6 (C-9′), 24.8 (C-8), 25.2 (C-8′), 36.9 (C-7), 37.8 (C-7′), 55.8 (C-10), 56.5 (C-10′), 99.5 (C-3), 106.2 (C-1), 108.6 (C-16), 108.8 (C-14), 109.5 (C-5), 125.2 (C-12), 144.1 (C-15), 146.0 (C-6), 154.3 (C-13), 154.7 (C-17), 160.6 (C-2), 162.9 (C-4), 166.7 (C-11), 172.7 (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, UV/Vis (EtOH, λ_{max} / nm: 217.5; R_f (DCM–EA, 7:3): 0.6.



4

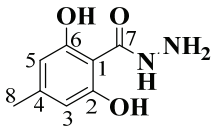
Methyl 2,6-dihydroxy-4-methylbenzoate (***4***). Yield: 4.05 g (2.70 %, based on total weight of the lichen material); m.p.: 143–144 °C, Anal. Calcd for $C_9H_{10}O_4$: C, 59.34; H, 5.53 %. Found C, 59.36, H, 5.52 %; IR (KBr, cm^{-1}): 3866, 3746, 3370, 3312, 2982, 2955, 2854, 1894, 1642, 1581, 1503, 1447, 1383, 1318, 1265, 1197, 1162, 1114, 1059, 1029, 996, 950, 835, 800, 753, 700, 620, 575; ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 2.23 (3H, *s*, Me-9), 3.75 (3H, *s*, OMe-8), 6.12

(2H, *d*, *J*= 2.0 Hz, H-3, 5), 9.94 (1H, *s*, OH-6), 10.65 (1H, *s*, OH-2); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 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; UV/Vis (EtOH, λ_{max} / nm): 219.5; R_f (hexane–EA, 1:1): 0.6.



5

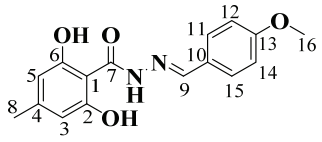
*4-[(2-hydroxy-4-methoxy-6-propylbenzoyl)oxy]-2-methoxy-6-propylbenzoic acid (**5**).* Yield: 11 mg (0.007 %, based on total weight of the lichen material); m.p.: 142–143 °C; Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51 %. Found: C, 65.66; H, 6.62 %; ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 0.93–1.05 (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 (100 MHz, $CDCl_3$, δ / ppm): 14.3 (C-9/9′), 24.81 (C-8′), 25.18 (C-8), 38.8 (C-7′), 39.1 (C-7), 55.4 (C-10), 56.0 (C-10′), 99.8 (C-3), 104.5 (C-1), 106.2 (C-15), 107.1 (C-13), 110.9 (C-5), 146.8 (C-17), 148.7 (C-16), 151.6 (C-6), 156.1 (C-12), 156.9 (C-14), 164.4 (C-4), 165.5 (C-2), 168.9 (C-11), 173.7 (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; UV/Vis (EtOH, λ_{max} / nm: 212.5; R_f (hexane–EA, 1:1): 0.4.



4a

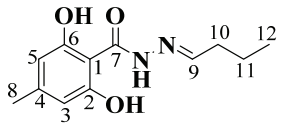
*2,6-Dihydroxy-4-methylbenzohydrazide (**4a**).* Yield: 77 %; m.p.: 153–154 °C; Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38 %. Found C, 52.64; H, 5.52; N, 15.48 %; IR (KBr, cm^{-1}): 2917, 2564, 1621, 1498, 1458, 1367, 1325, 1266, 1216, 1173, 1001, 919, 841, 800, 735, 688; ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.57 (3H, *s*, Me), 5.07 (2H, *s*, NH_2), 6.42 (2H, *s*, Ar-H), 7.41 (1H, *s*, NH), 10.05 (2H, *s*, Ar-OH); ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 24.4 (C-8), 99.7 (C-1), 112.2 (C-3/5), 144.7 (C-4), 164.0 (C-2/6), 166.5 (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.



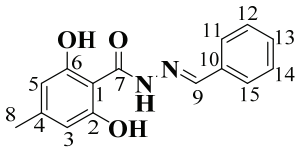
4b

*(*E*)-2,6-Dihydroxy-*N*′-(4-methoxybenzylidene)-4-methylbenzohydrazide (**4b**).* Yield: 68 %, m.p.: 120–121 °C. 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 %; IR (KBr cm^{-1}): 3205, 3054, 2843, 1655, 1804, 1509, 1462, 1428, 1379, 1340, 1300, 1250, 1171, 1096, 1028, 960, 761, 668; ^{1}H-NMR (400 MHz, $CDCl_3$, *δ* / ppm): 2.38 (3H, *s*, Me), 3.84 (3H, *s*, OMe), 6.37–6.38 (2H, *d*, *J* = 4 Hz, Ar-H), 6.91–6.93 (2H, *m*, Ar-H), 7.60–7.62 (2H, *m*, Ar-H), 7.78 (1H, *s*, =CH), 9.89 (2H, *s*, Ar-OH); ^{13}C-NMR (100 MHz, $CDCl_3$, *δ* / ppm): 20.4 (C-8), 55.4 (C-16), 111.5 (C-1), 114.2 (C-3/5), 126.6 (C-12/14), 128.7 (C-10), 138.3 (C-11/15), 143.8 (C-4), 147.3 (C-9), 161.2 (C-2/6), 164.4 (C-13), 174.1 (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.



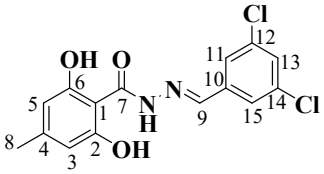
4c

*(*E*)-*N*′-Butylidene-2,6-dihydroxy-4-methylbenzohydrazide (**4c**).* Yield: 70 %, m.p.: 110–111 °C; 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 %; IR (KBr, cm^{-1}): 3814, 3217, 3066, 2973, 2934, 1457, 1379, 1341, 1287, 1167, 1101, 846, 769; ^{1}H-NMR (400 MHz, $CDCl_3$, *δ* / ppm): 0.95–1.03 (3H, *m*, Me), 1.91–1.93 (2H, *m*, CH_2), 2.15 (3H, *s*, Me), 2.18–2.20 (2H, *m*, CH_2), 6.51 (*s*, 2H, Ar-H), 7.19–7.21 (*t*, *J* = 4 Hz, 1H, =CH), 7.83 (*s*, 1H, NH) 10.14 (*s*, 2H, Ar-OH); ^{13}C-NMR (100 MHz, $CDCl_3$, *δ* / ppm): 14.2 (C-12), 19.8 (C-11), 22.7 (C-8), 29.8 (C-10), 106.5 (C-1), 113.9 (C-3/5), 130.5 (C-4), 149.5 (C-9), 155.8 (C-2/6), 168.6 (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.



4d

*(*E*)-*N′*-Benzylidene-2,6-dihydroxy-4-methylbenzohydrazide (**4d**)*. Yield: 64 %, m.p.: 98–99 °C. 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 %; IR (KBr, cm^{-1}): 3183, 3077, 2969, 2858, 1669, 1605, 1500, 1393, 1338, 1224, 1133, 1016, 948, 899, 758, 685; ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.40 (3H, *s*, Me), 7.39–7.40 (3H, *m*, Ar-H), 7.67–7.68 (2H, *m*, Ar-H), 7.84 (1H, *d*, J = 4 Hz, =CH), 8.84 (1H, *s*, NH), 10.02 (2H, *s*, Ar-OH); ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 20.4 (C-8), 105.6 (C-1), 112.3 (C-3/5), 127.2 (C-12/14), 128.8 (C-11/13/15), 130.1 (C-10), 133.9 (C-4), 143.8 (C-9), 165.5 (C-2/6), 174.2 (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: 270.25.



4e

*(*E*)-*N′*-(3,5-Dichlorobenzylidene)-2,6-dihydroxy-4-methylbenzohydrazide (**4e**)*. Yield: 75 %; m.p.: 130–131 °C; IR (KBr, cm^{-1}): 3082, 2954, 1687, 1595, 1519, 1466, 1385, 1332, 1276, 1217, 1152, 1097, 1020, 927, 860, 816, 769, 665; ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.38 (3H, *s*, Me), 7.27–7.29 (2H, *m*, Ar-H), 7.41–7.42 (1H, *m*, Ar-H), 7.91–7.93 (1H, *m*, =CH), 8.12 (1H, *s*, NH), 9.37 (2H, *s*, Ar-OH); ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 20.4 (C-8), 104.4 (C-1), 112.1 (C-3/5), 127.9 (C-11/15), 129.9 (C-13), 134.6 (C-12/14), 136.3 (C-10), 138.7 (C-4), 142.3 (C-9), 159.5 (C-2/6), 173.4 (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.

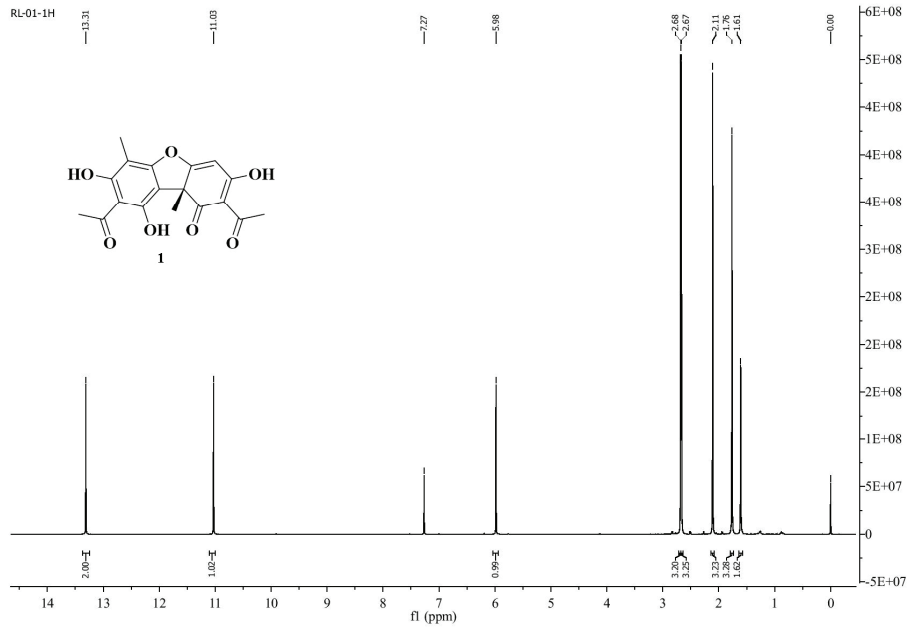


Fig. S-1. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1**.

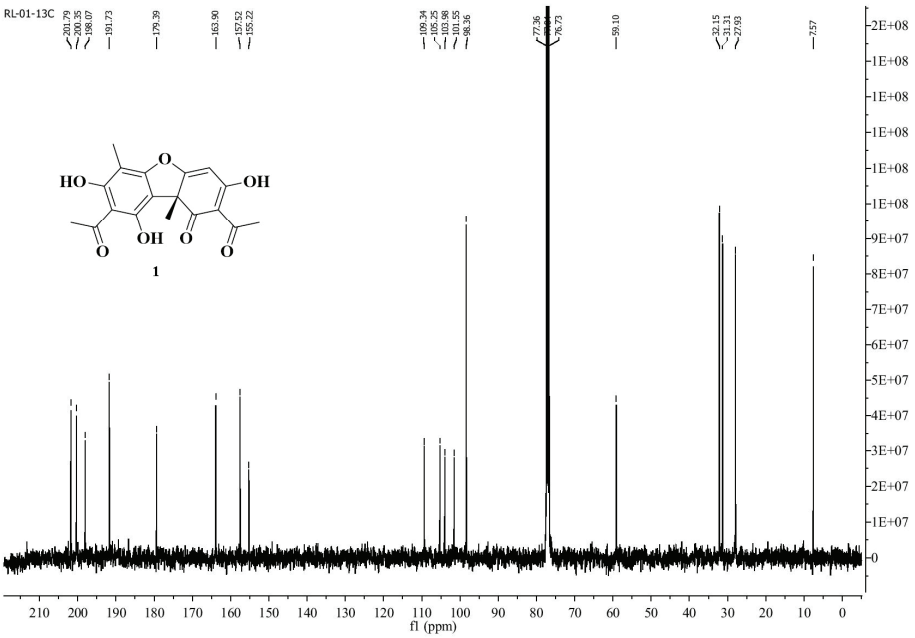


Fig. S-2. ^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1**.

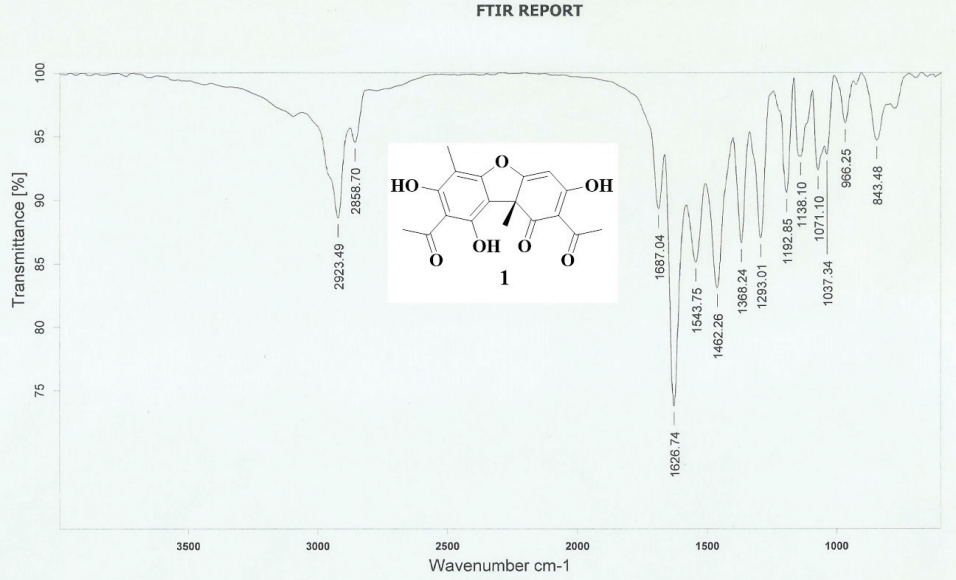
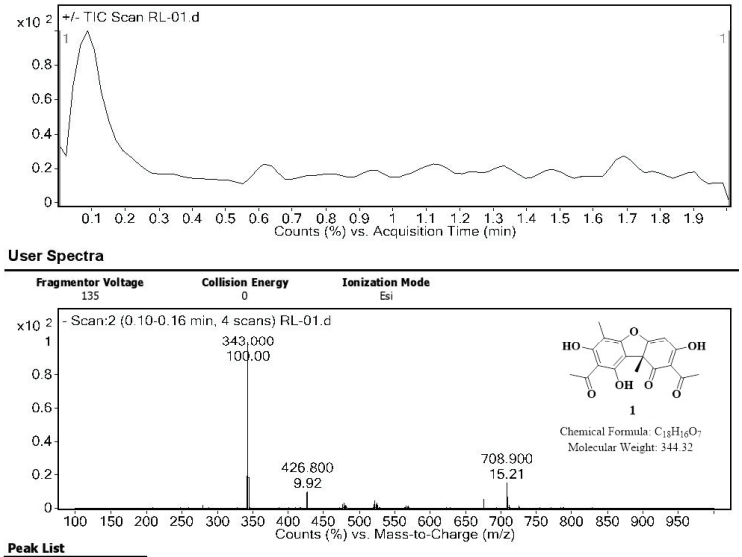


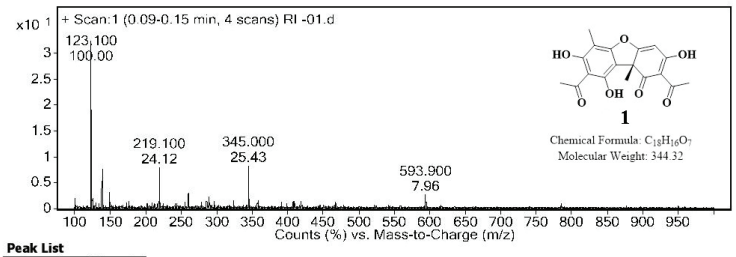
Fig. S-3. FT-IR (KBr) spectrum of **1**.



Peak List

m/z	z	Abund
343	1	399846
344	1	73057
426.8		39659
675.2		21883
708.9	1	60823
709.9	1	24969

Fig. S-4. ESI-MS spectrum of **1**.



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

Fig. S-4a. ESI-MS spectrum of **1**.

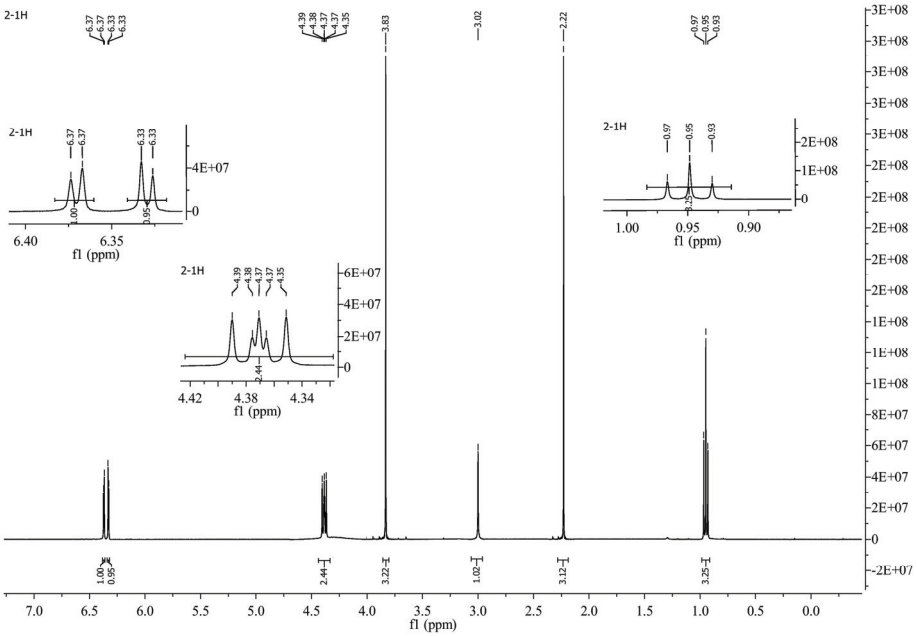


Fig. S-5. ^{1}H-NMR (400 MHz, DMSO-d_6) of **2**.

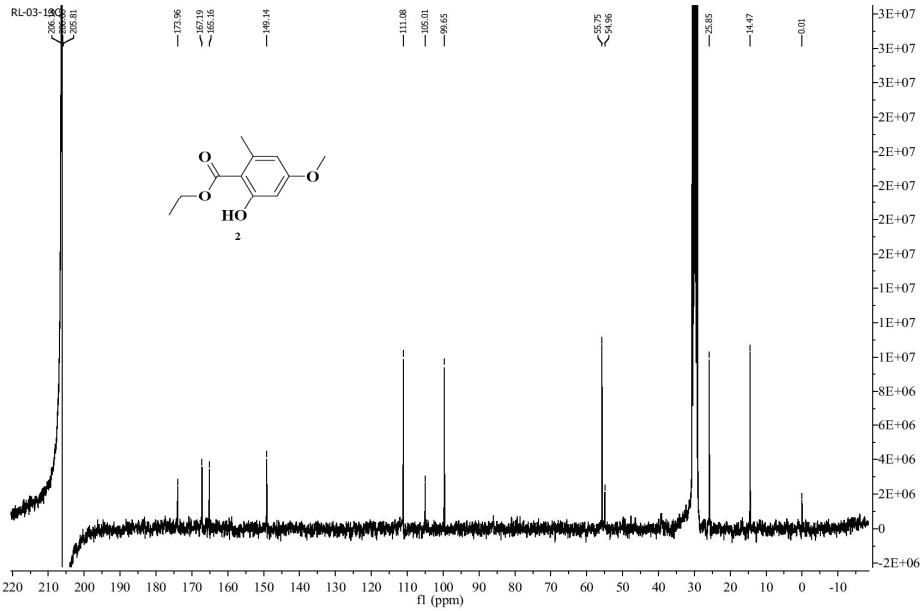


Fig. S-6. ^{13}C-NMR (100 MHz, acetone-d_6) of **2**.

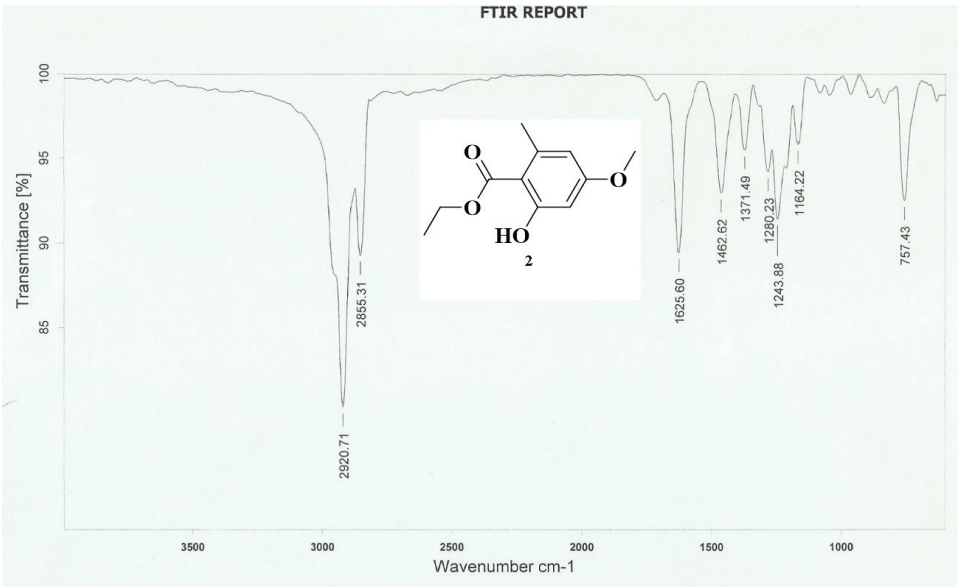
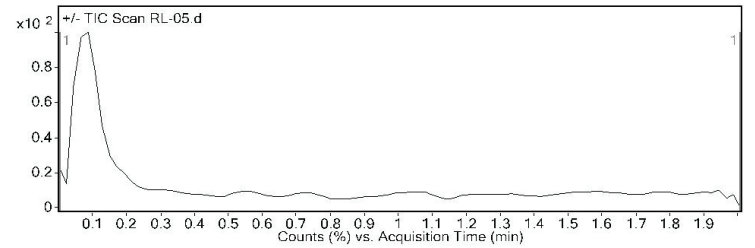
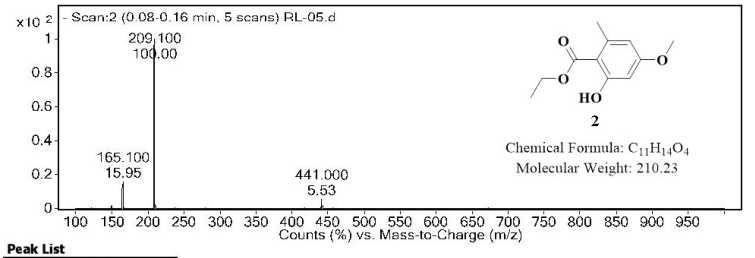


Fig. S-7. FT-IR (KBr) spectrum of **2**.



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



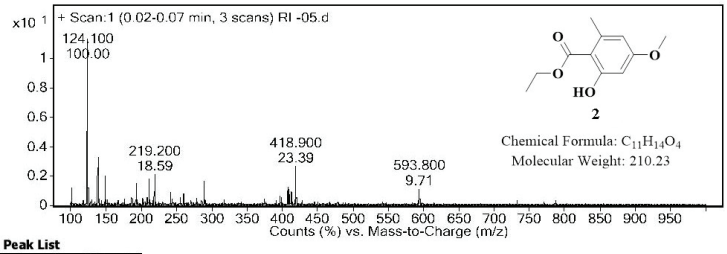
2

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

Fig. S-8. ESI-MS spectrum of **2**.



2

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

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

Fig. S-8a. ESI-MS spectrum of **2**.

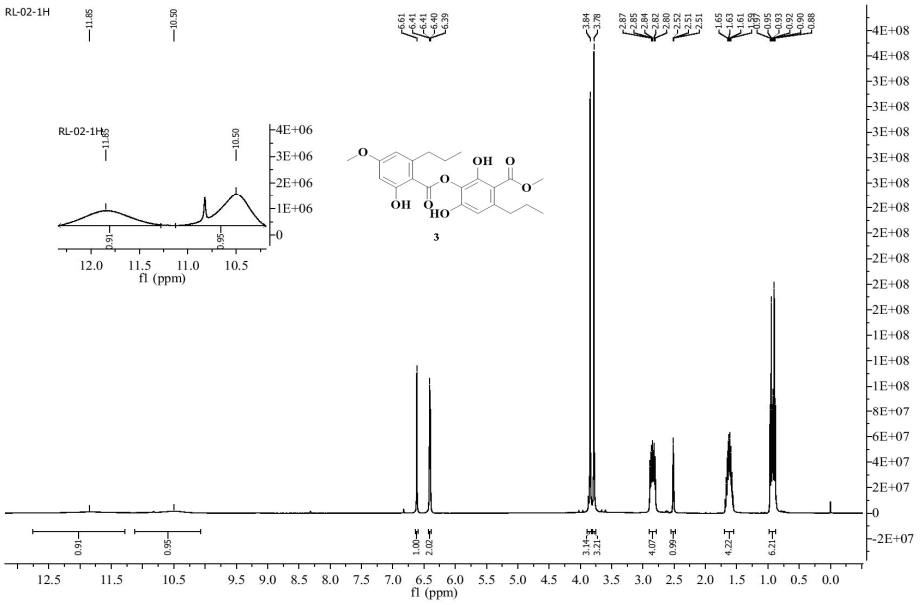


Fig. S-9. ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of **3**.

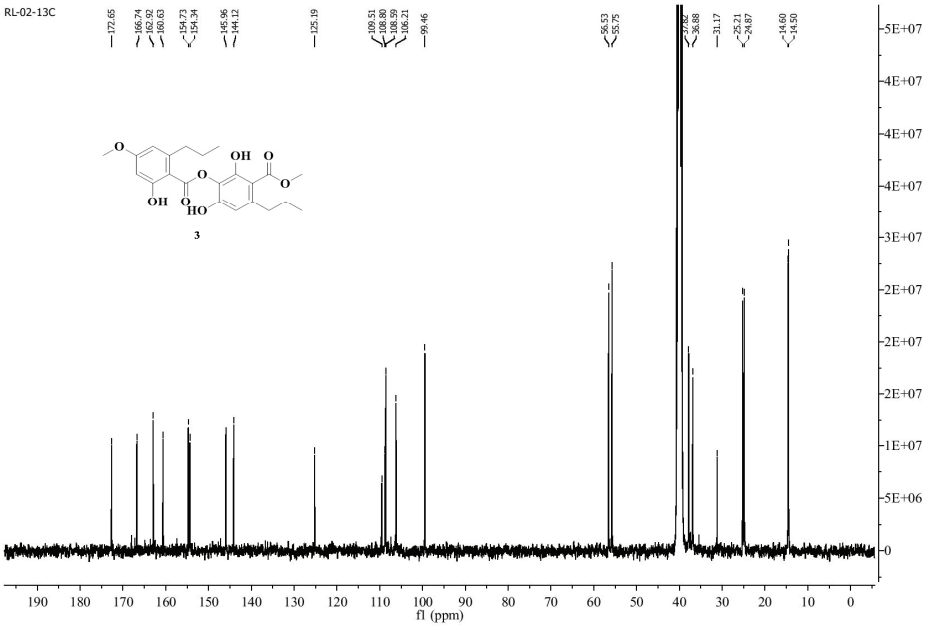


Fig. S-10. ^{13}C-NMR (100 MHz, DMSO-d_6) spectrum of **3**.

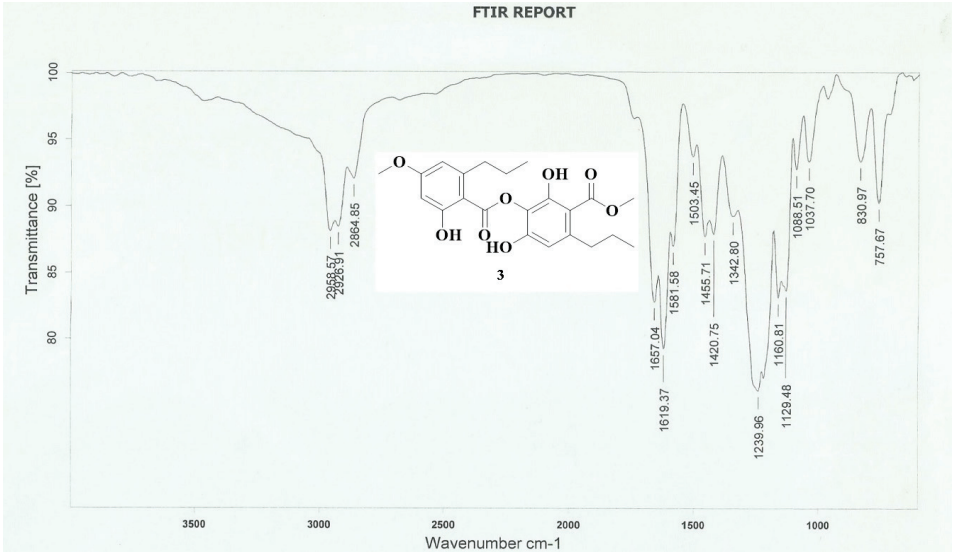


Fig. S-11. FT-IR (KBr) spectrum of **3**.

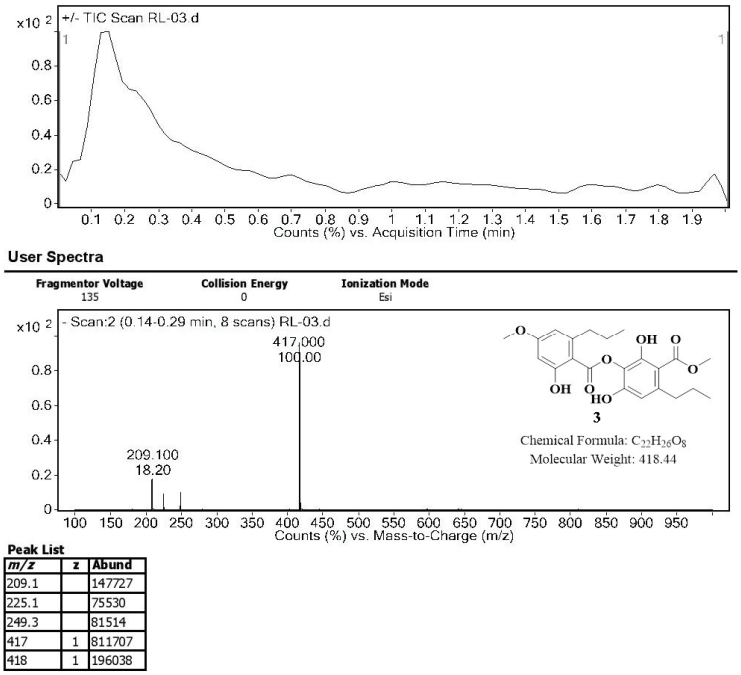
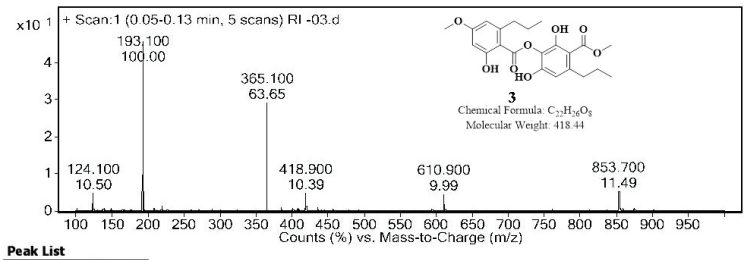


Fig. S-12. ESI-MS spectrum of **3**.



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

Fig. S-12a. ESI-MS spectrum of **3**.

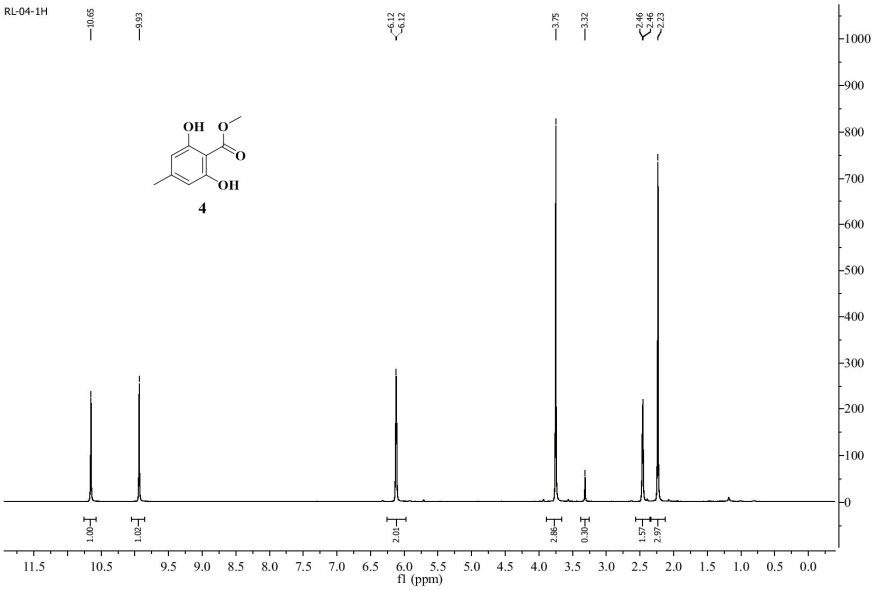


Fig. S-13. ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of **4**.

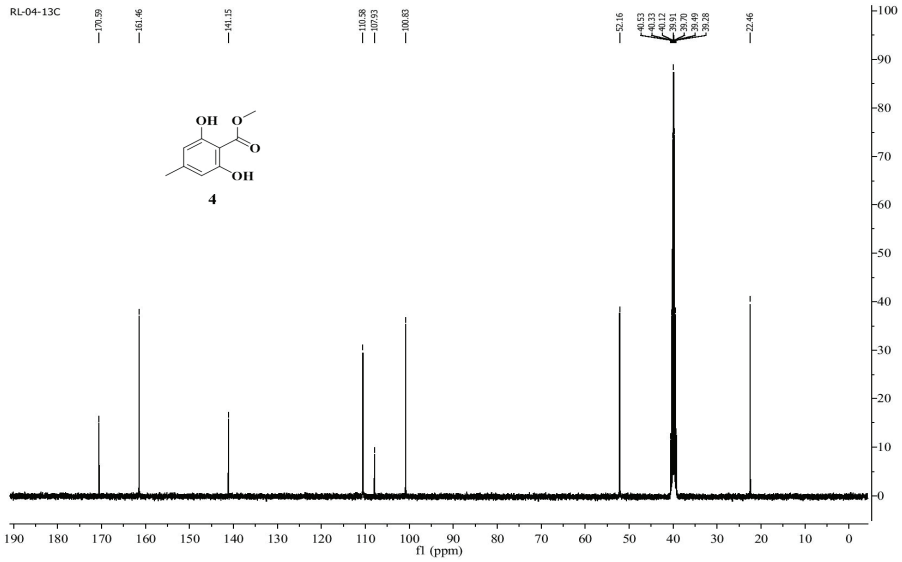


Fig. S-14. ^{13}C-NMR (100 MHz, DMSO-d_6) of **4**.

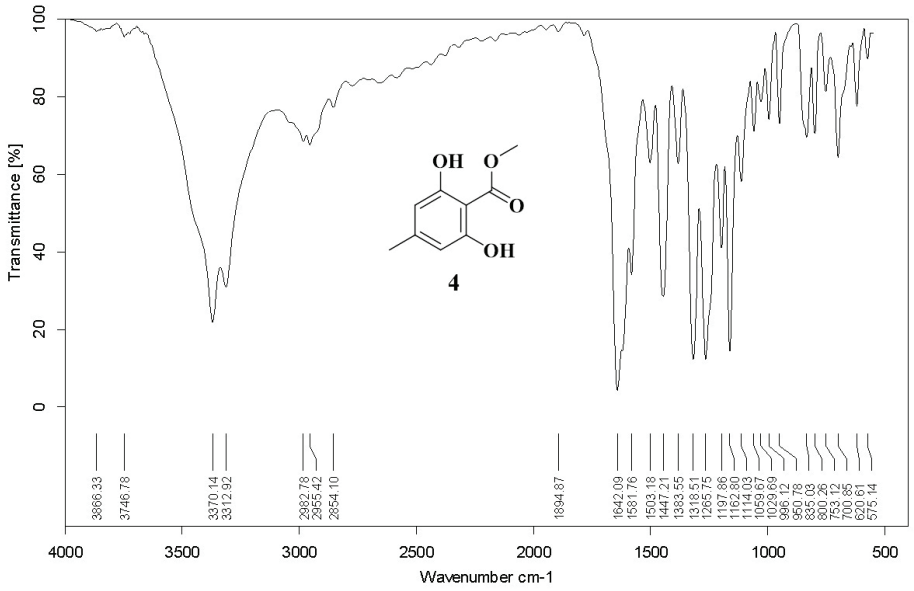


Fig. S-15. FT-IR (KBr) spectrum of **4**.

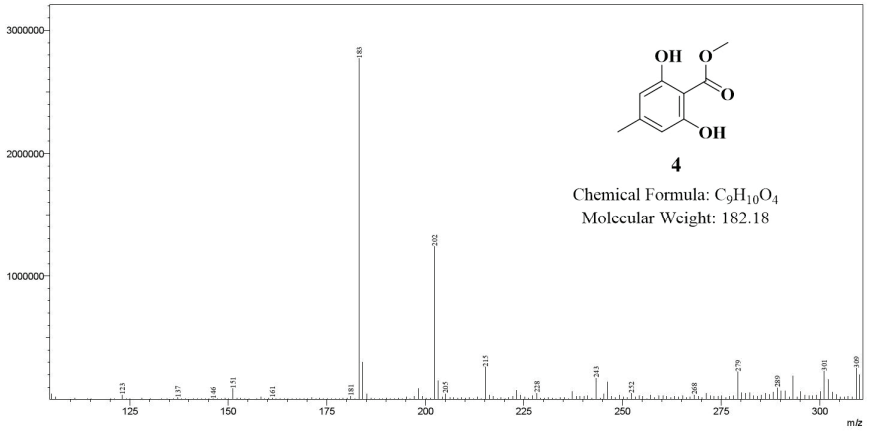


Fig. S-16. ESI-MS spectrum of **4**.

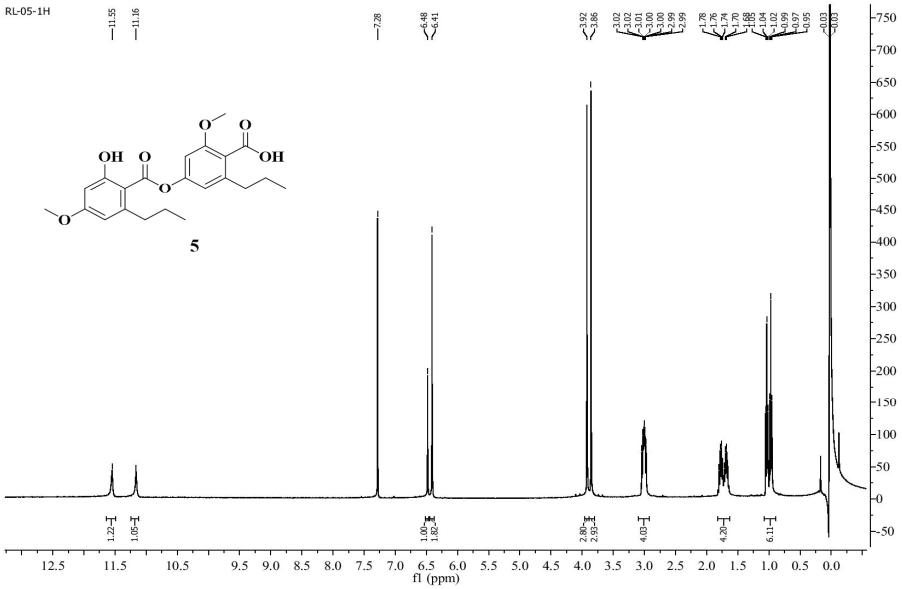


Fig. S-17. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **5**.

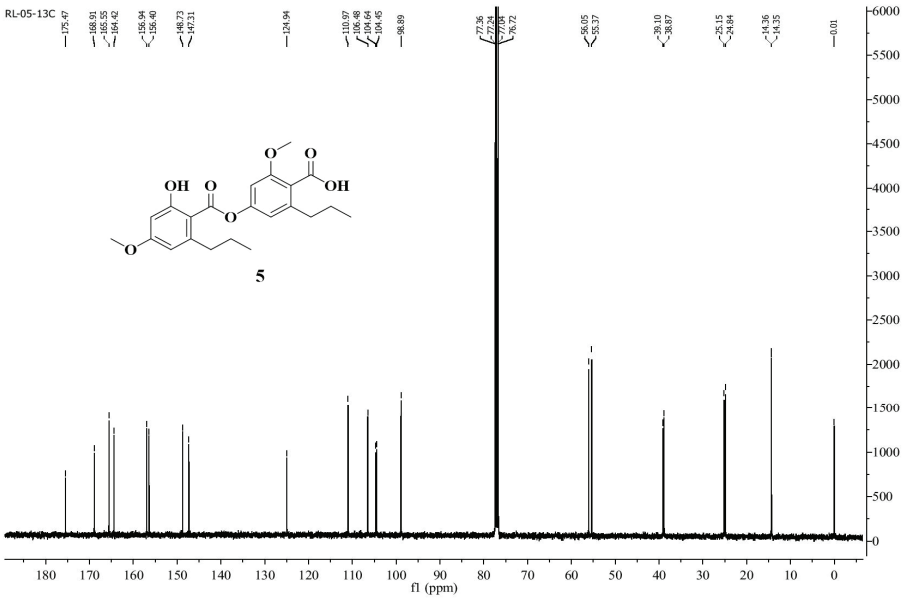


Fig. S-18. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **5**.

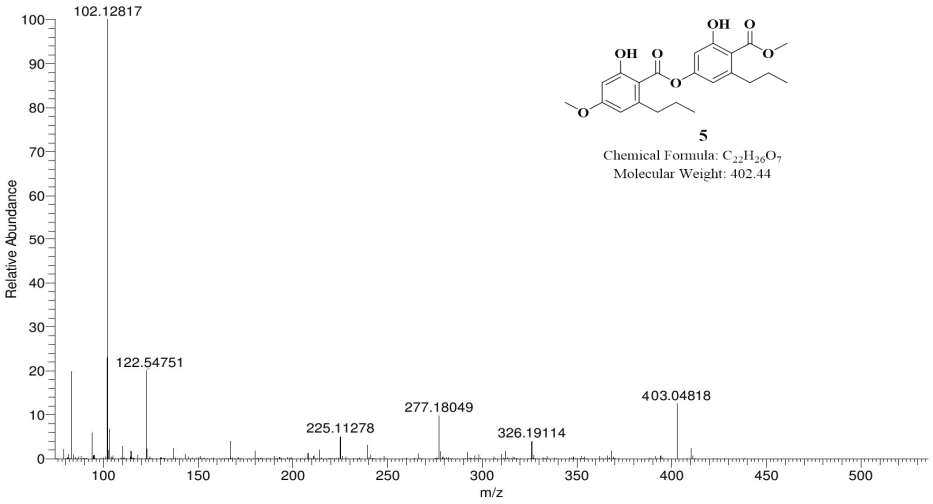


Fig. S-19. ESI-MS spectrum of **5**.

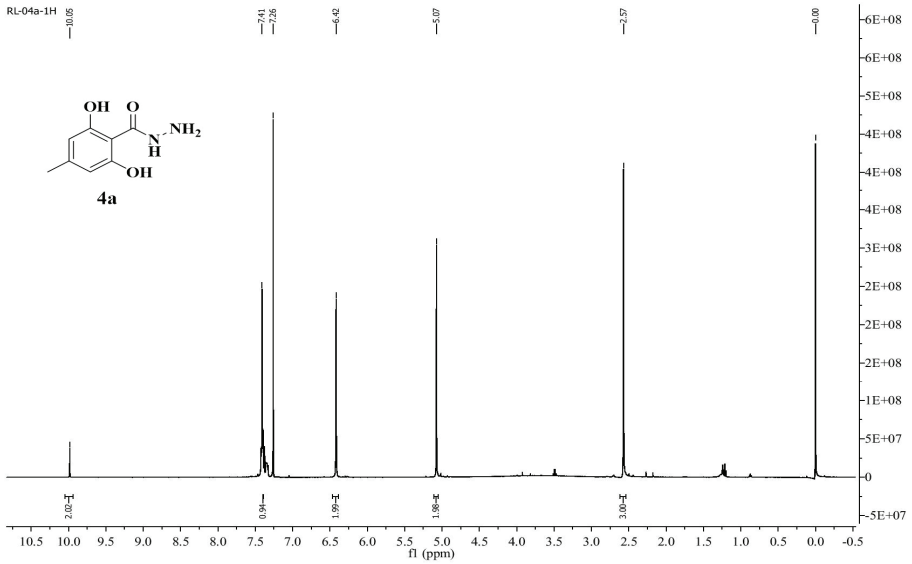


Fig. S-20. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **4a**.

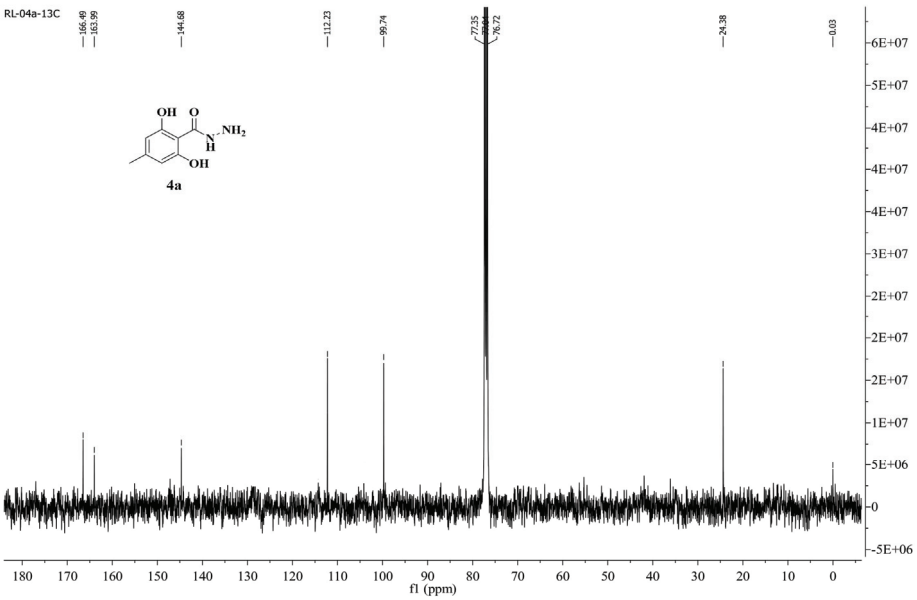


Fig. S-21. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **4a**.

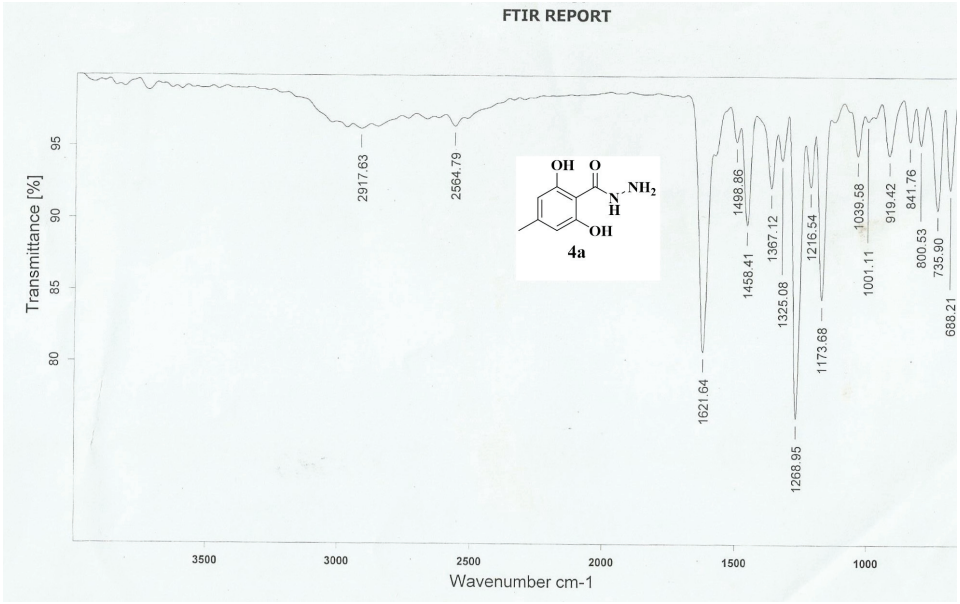


Fig. S-22. FT-IR (KBr) spectrum of **4a**.

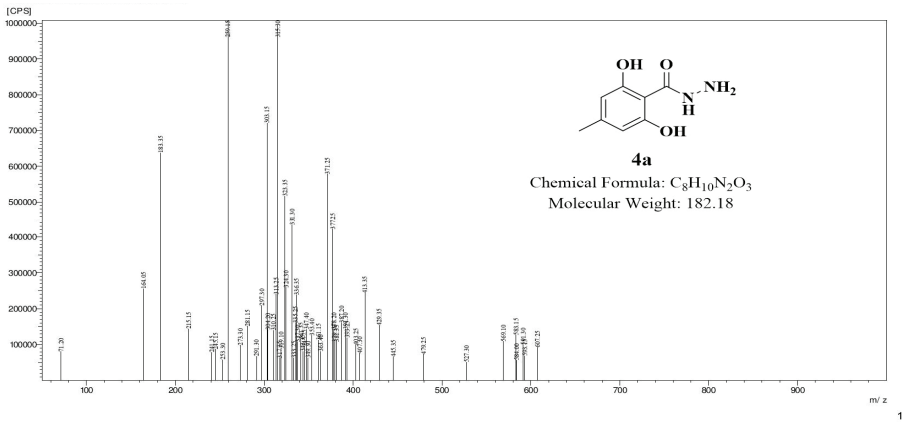


Fig. S-23. ESI-MS (positive mode) spectrum of **4a**.

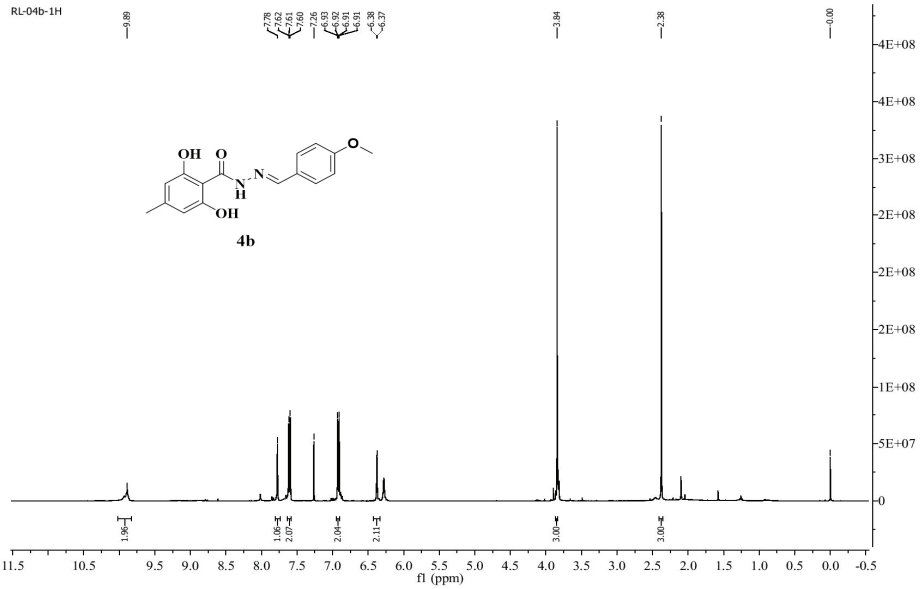


Fig. S-24. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **4b**.

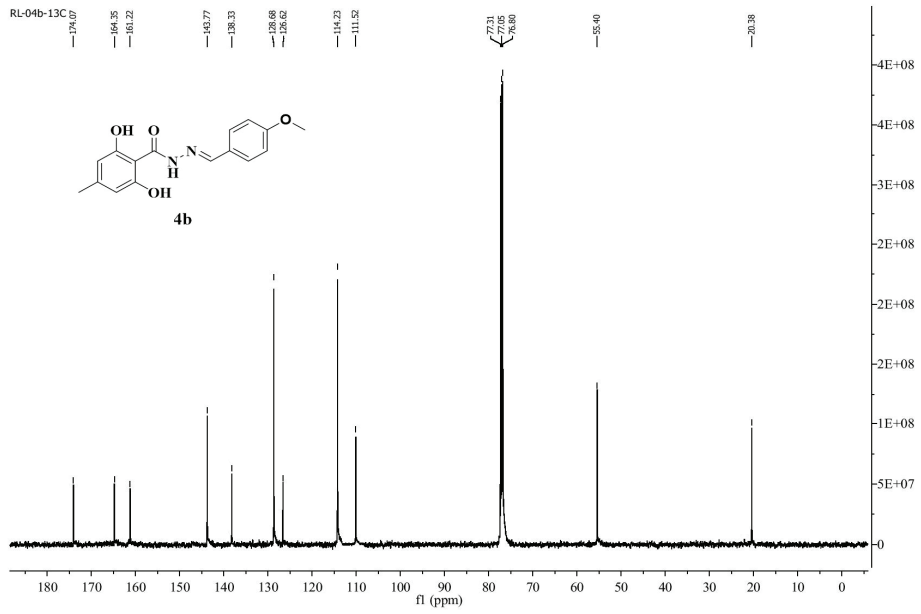


Fig. S-25. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **4b**.

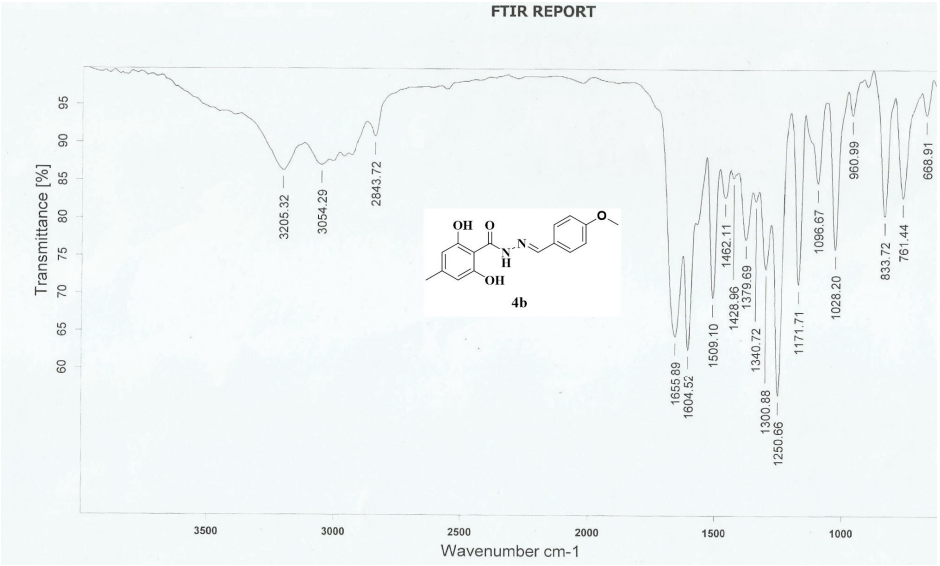


Fig. S-26. FT-IR (KBr) spectrum of **4b**.

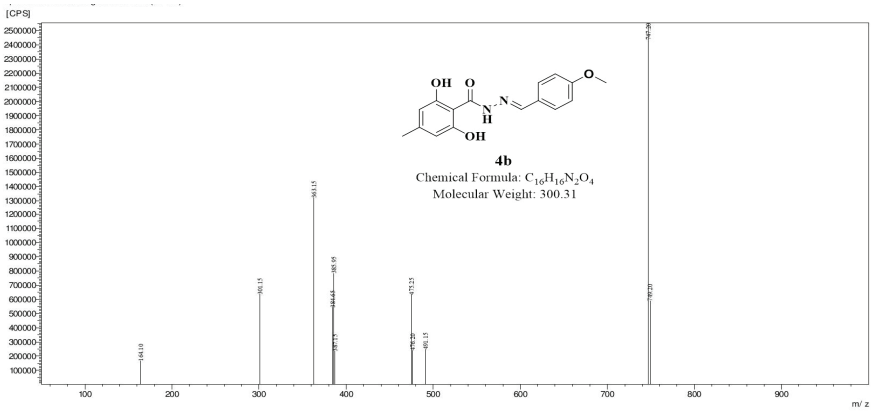


Fig. S-27. ESI-MS (positive mode) spectrum of **4b**.

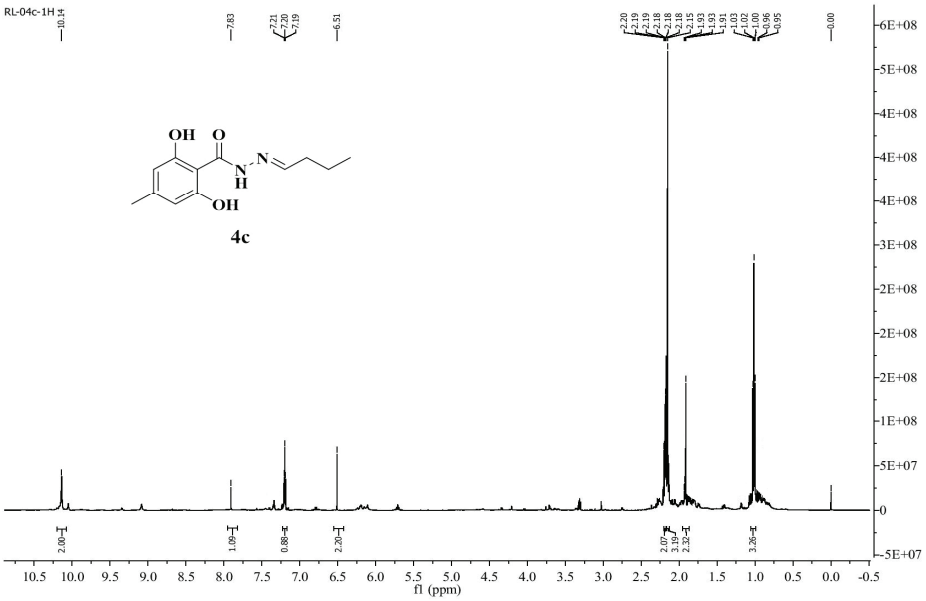


Fig. S-28. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **4c**.

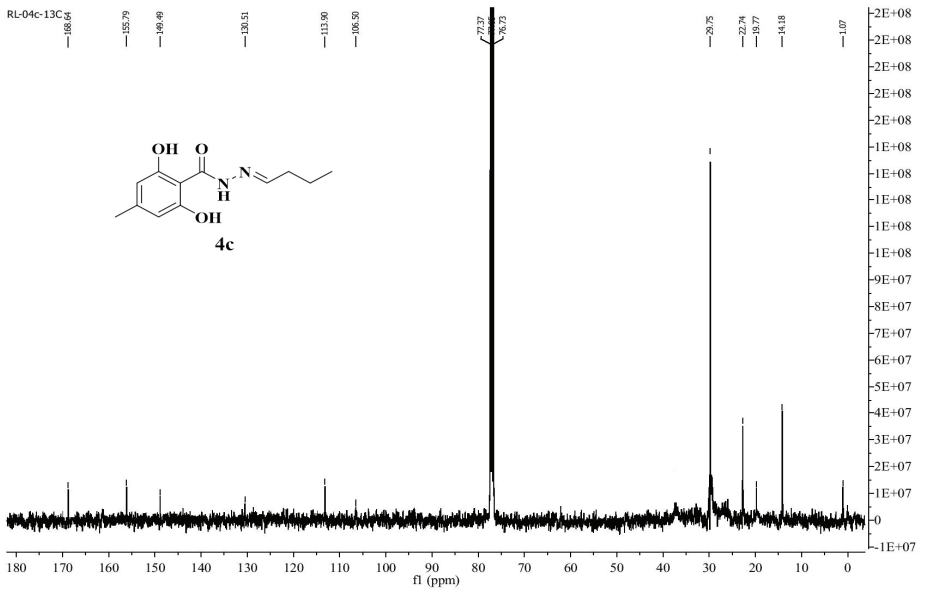


Fig. S-29. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **4c**.

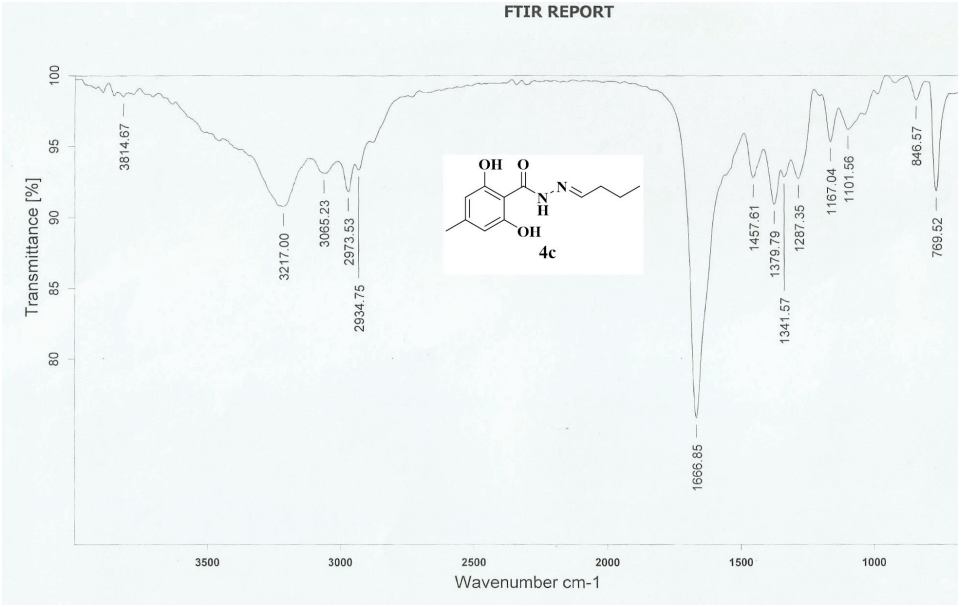


Fig. S-30. FT-IR (KBr) spectrum of **4c**.

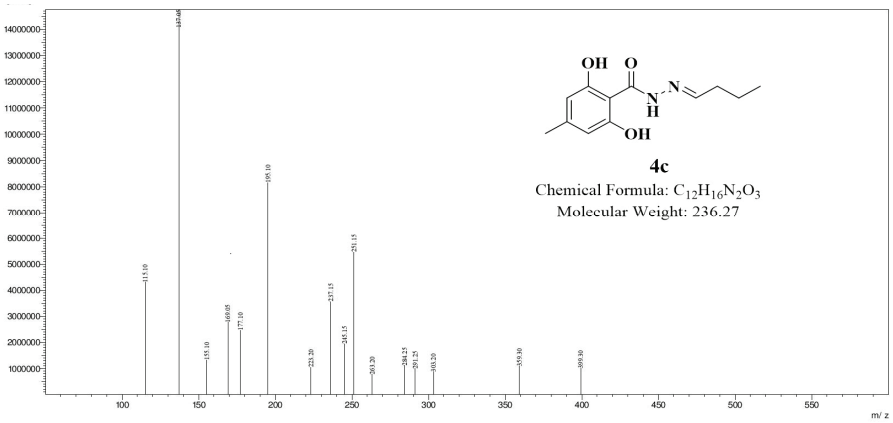


Fig. S-31. ESI-MS (positive mode) spectrum of **4c**.

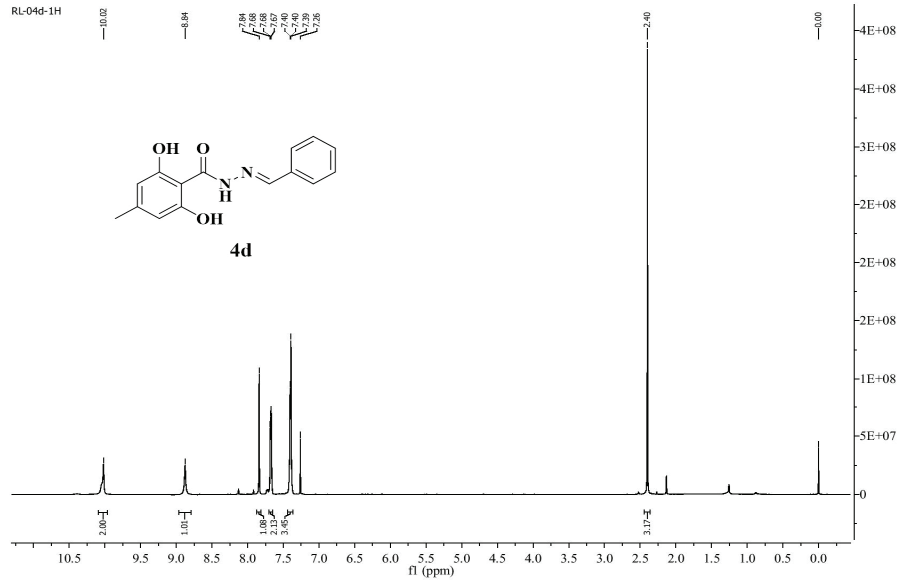


Fig. S-32. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **4d**.

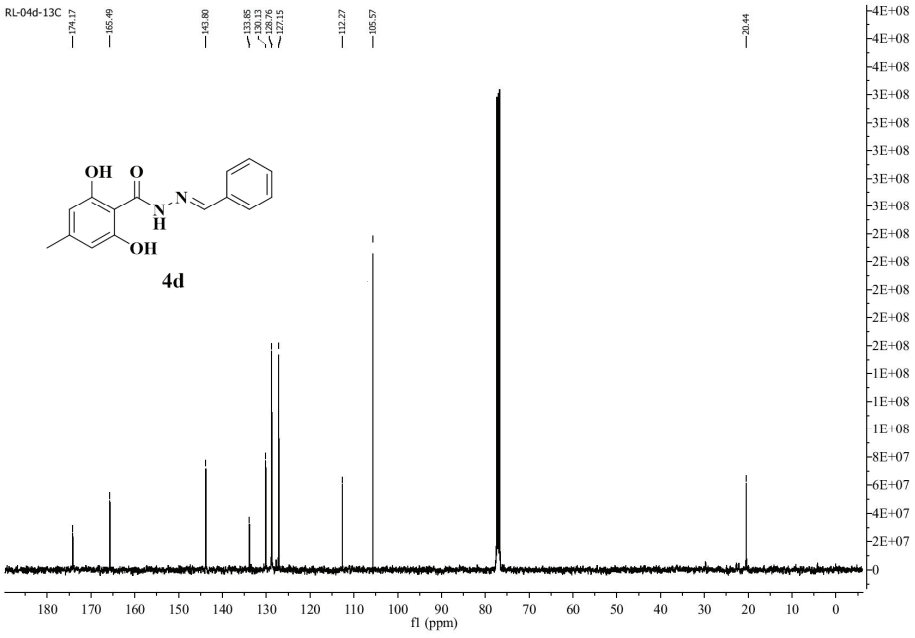


Fig. S-33. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **4d**.

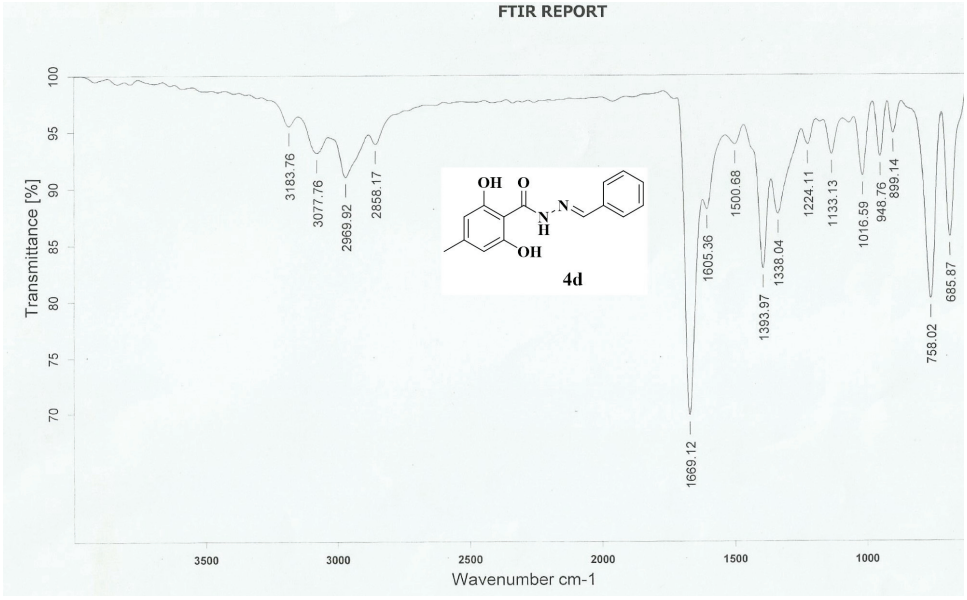


Fig. S-34. FT-IR (KBr) spectrum of **4d**.

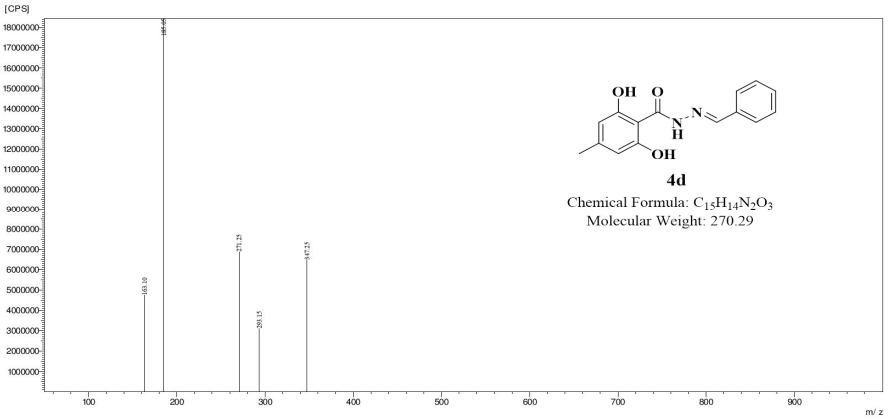


Fig. S-35. ESI-MS (positive mode) spectrum of **4d**.

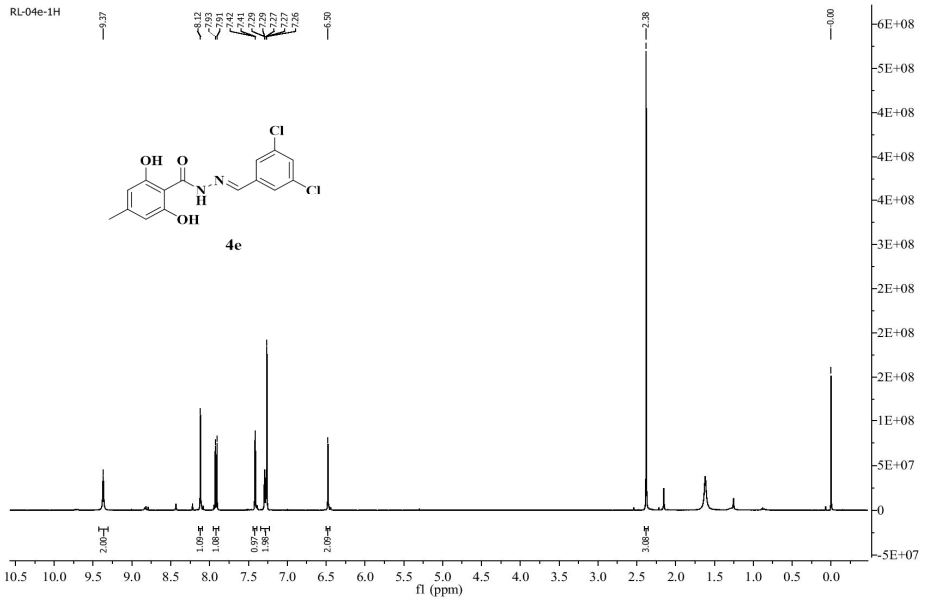


Fig. S-36. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **4e**.

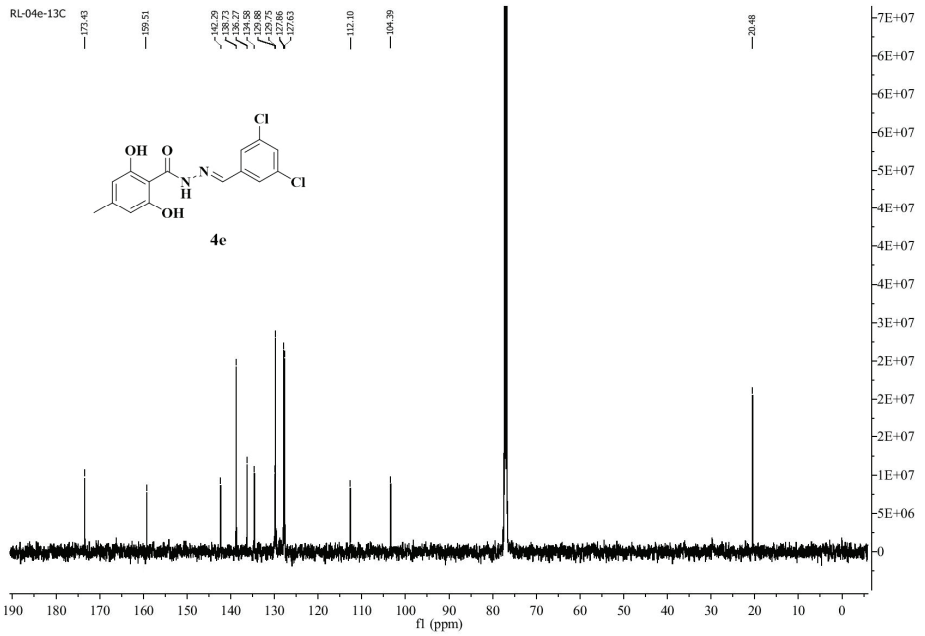


Fig. S-37. ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of **4e**.

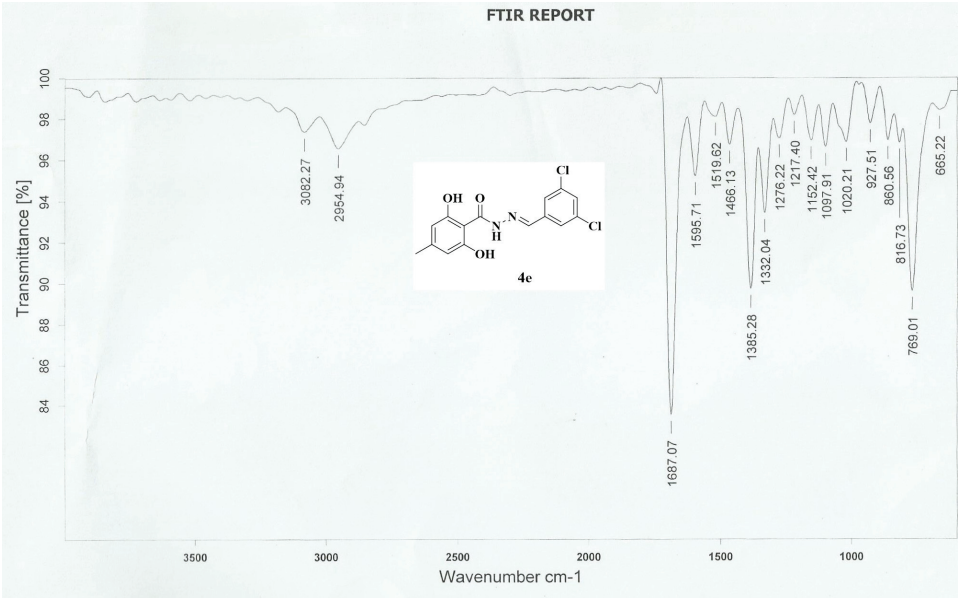


Fig. S-38. FT-IR (KBr) of **4e**.

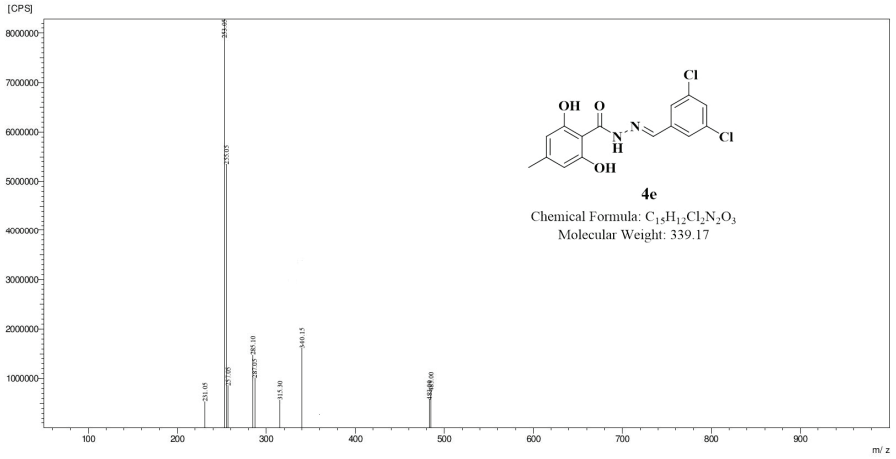


Fig. S-39. ESI-MS (positive mode) spectrum of **4e**.

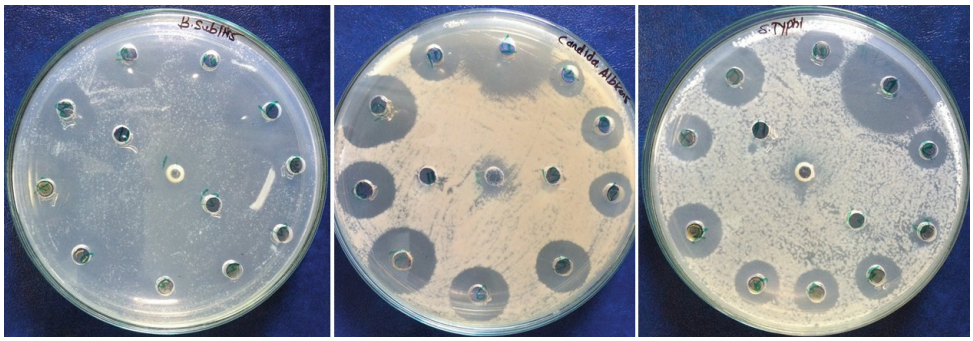


Fig. S-40. Zones of inhibition of **1**–**5** and **4a**–**e** against bacterial and fungal stains.

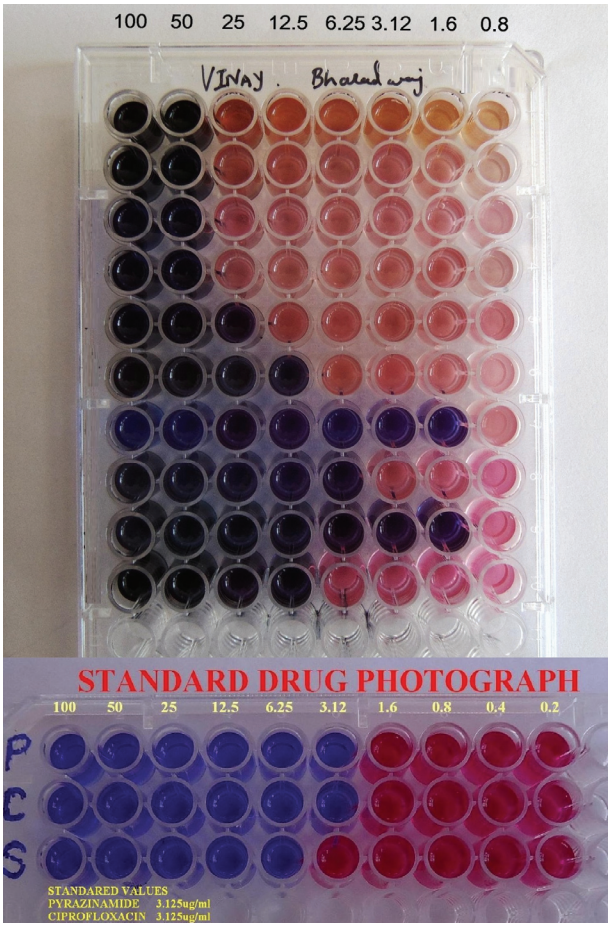


Fig. S-41. *In vitro* anti-tubercular activity of all the isolates and benzohydrazide derivatives of *Ramalina leiodea* against the *Microbacterium tuberculosis* H37Rv strain.

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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, *Int. J. Chem. Sci.* **15** (2017) 172 (https://www.tsijournals.com/articles/benzohydrazide-incorporated-imidazo-12b-pyridazine-synthesis-characterization-and-in-vitro-antitubercular-activity.pdf).