SUPPLEMENTARY MATERIAL

Synthesis and biological evaluation of picroside derivatives as anti-liver injury agents

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Abstract

Picrorhizae Rhizoma as a hepatoprotective herb, has been applied for thousands of years, and picroside was proved to be its active constituent. In this study, twelve derivatives of picroside were synthesized and the hepatoprotective activity of the derivatives was evaluated on SMMC-7721 cells. Six out of the derivatives had shown a better protective effect on H_2O_2-induced SMMC-7221 cells than picroside, and the activity of two derivatives (**2** and **4**) was stronger than that of the reference compound, silybin. Compound **2** shown the strongest protective effect (EC_{50} =6.064 ± 1.295 μM).

Keywords: *Picroside*; Derivatization; Hepatoprotective activity; SMMC-7221

Experimental

General

NMR spectra were performed on Bruker DRX-400 NMR Spectrometer at 400 MHz using CD_3OD as solvent in Zhengzhou University. Mass spectra were recorded on a Triple TOF 5600 instrument. Optical rotation values were measured on a Jasco P-2000 polarimeter. The purity of Picroside and the derivatives were checked on a Waters e2695 liquid chromatograph equipped with 2424 ELS detector and 2998 PDA detector. All the chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Preparation of Picroside

Take the dried rhizome of *Picrorhiza* (about 1-2 cm in length) 5 kg, 6 BV of 95% ethanol reflux extraction for 3 h, repeated three times, combined the extracts, concentration under vacuum, the residue was purified by AB-8 macroporous resin column chromatograph, eluted by 50% ethanol, concentration under vacuum and got the crude picroside 721 g. the further purified was performed with silica gel column chromatograph (CH_2Cl_2-CH_3OH, 10:1, v/v) to yield Picroside I 57 g and Picroside II 204 g.

Synthesis

Synthesis of compound **1**

0.05 g of Picroside Ⅱ was dissolved with 2 mL methanol, and 0.2 mL concentrated nitric acid was added dropwise. The mixture was refluxed for 10 min at 80 ℃. After cooled to room temperature, excess calcium carbonate was added until the solution was neutral, filtered, recovered the solvent under vacuum, the residue was purified by silica gel column chromatography, eluted with ethyl acetate-petroleum ether (1:1) to obtain **1**.

1*α*,3*β*-dimethoxy-3,4-dihydrogen-picroside II (**1**) Yield 37%; yellow powder; $[\alpha]_D^{20}$ - 18.8 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ: 1.85 (1H, m, H-4a), 1.95 (1H, m, H-4b), 2.33 (1H, m, H-5), 2.79 (1H, dd, J = 2.0 Hz, 8.2 Hz, H-9), 3.41

(3H, s, H-OCH_3), 3.45 (3H, s, H-OCH_3), 3.54 (1H, d, J = 12.8 Hz, H-10a), 3.62 (1H, s, H-7), 3.89 (3H, s, H-OCH_3), 4.01 (1H, d, J = 12.8 Hz, H-10b), 4.77 (1H, dd, J = 2.6 Hz, 6.0 Hz, H-3), 4.99 (1H, d, J = 3.7 Hz, H-1), 5.27 (1H, dd, J = 1.0 Hz, 8.2 Hz, H-6), 6.84 (1H, d, J = 8.1 Hz, H-5'), 7.56 (1H, d, J = 1.8 Hz, H-2'), 7.85 (1H, dd, J = 1.8 Hz, 8.1 Hz, H-6'); ^{13}C NMR (100 MHz, CD_3OD) δ: 30.2, 34.0, 41.2, 55.3, 55.9, 56.5, 61.1, 61.4, 81.1, 87.3, 96.3, 99.2, 113.6, 116.0, 122.2, 125.3, 148.8, 153.1, 168.2; ESI-MS *m/z* 395 $[M-H]^-$ 441 $[M+HCOO]^-$ 791 $[2M-H]^-$.

General procedure for synthesis of **2**

0.1 g of Picroside Ⅱ was dissolved with 6 mL $SOCl_2$, and refluxed for 30 min at 50 ℃. The mixture was concentrated under vacuum. The residue was redissolved by methanol, recovered the solvent, the residue was purified by silica gel column chromatography, eluted with ethyl acetate-petroleum ether (1:3) to obtain **2**.

3-ethoxy-3,4-dihydrogen-7*α*-chlorine-8-hydroxy-(1*β*,10)-epoxy-picroside Ⅱ (**2**) Yield 28%; white powder; $[\alpha]_D^{20}$ + 46.5 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.15 (3H, t, J = 7.1 Hz, H-CH3), 1.49 (1H, m, H-4a), 1.93 (1H), 2.53 (1H, dq, J = 2.2 Hz, 14.3 Hz, H-5), 2.59 (1H, dd, J = 4.8 Hz, 4.6 Hz, H-9), 3.55 (1H, m, H-OCH_2a), 3.76 (1H, d, J = 10.4 Hz, H-10a), 3.85 (1H, m, H-OCH_2b), 3.90 (3H, s, H-OCH_3), 4.27 (1H, d, J = 10.4 Hz, H-10b), 4.41 (1H, d, J = 10.3 Hz, H-7), 5.18 (1H, dd, J = 5.7 Hz, 7.6 Hz, H-3), 5.38 (1H, t, J = 9.8 Hz, H-6), 5.51 (1H, d, J = 4.6 Hz, H-1), 6.89 (1H, d, J = 8.3 Hz, H-5''), 7.56 (1H, d, J = 1.8 Hz, H-2''), 7.60 (1H, dd, J = 1.8 Hz, 8.3 Hz, H-6''); ^{13}C NMR (100 MHz, CD_3OD) δ: 15.5, 27.9, 37.2, 52.1, 56.5, 64.7, 69.9, 75.9, 79.3, 86.0, 96.9, 100.2, 113.7, 116.1, 121.6, 125.4, 148.9, 153.5, 167.7; ESI-MS *m/z* 413 $[M-H]^-$ 827 $[2M-H]^-$.

General procedure for synthesis of **3** and **6**

0.05 g of Picroside Ⅱ and I was dissolved with 4 mL acetic anhydride, respectively, and refluxed for 1 h at 80 ℃. Recovered the solvent in vacuum and the

residue was purified by silica gel column chromatography, eluted with ethyl acetate-petroleum ether (1:2) to obtain **3** and **6**, respectively.

Hexaacetyl picroside Ⅱ (**3**) Yield 81%; white powder; $[\alpha]_D^{20}$ – 86.4 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD) *δ*: 1.90 (3H s, H-CH_3), 2.00 (3H, s, H-CH_3), 2.00 (3H, s, H-CH_3), 2.04 (3H, s, H-CH_3), 2.05 (3H, s, H-CH_3), 2.29 (3H, s, H-CH_3), 2.53 (1H, m, H-5), 2.60 (1H, m, H-9), 3.79 (1H, q, J = 12.6 Hz, H-6'a), 3.86 (1H, s, H-7), 3.86 (3H, s, H-OCH_3), 4.09 (1H, m, H-10a), 4.09 (1H, m, H-5'), 4.20 (1H, m, H-10b), 4.76 (1H, q, J = 8.1 Hz, H-6), 4.89 (1H, d, J = 9.7 Hz, H-4), 4.94 (1H, d, J = 8.5 Hz, H-1'), 4.95–5.05 (3H, m, H-3'), 5.17 (1H, d, J = 7.9 Hz, H-1), 5.23 (1H, d, J = 8.0 Hz, H-6'b), 6.46 (1H, dd, J = 1.5 Hz, 6.0 Hz, H-3), 7.29 (1H, d, J = 8.0 Hz, H-6''), 7.64 (1H, s, H-5''), 7.67 (1H, d, J = 1.8 Hz, H-2''); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 20.2, 20.2, 20.3, 20.4, 20.4, 34.8, 40.1, 56.0, 58.4, 60.1, 62.3, 62.6, 67.9, 70.6, 71.7, 72.8, 79.9, 94.3, 96.0, 102.8, 113.1, 122.4, 123.4, 127.8, 142.5, 143.5, 151.0, 165.0, 168.1, 168.9, 169.3, 169.4, 169.9, 170.0; ESI-MS *m/z* 809 $[M+HCOO]^-$.

Pentaacetyl picroside I (**6**) Yield 77%; white powder; $[\alpha]_D^{20}$ – 67.6 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD) *δ*: 2.00 (3H, s, H-CH_3), 2.02 (3H, s, H-CH_3), 2.03 (3H, s, H-CH_3), 2.06 (3H, s, H-CH_3), 2.07 (3H, s, H-CH_3), 2.43 (1H, m, H-5), 2.53 (1H, q, J = 8.1 Hz, H-9), 3.64 (1H, s, H-7), 3.76 (1H, d, J = 12.6 Hz, H-6'a), 3.98 (1H, m, H-5'), 4.36 (1H, dd, J = 2.5 Hz, 12.4 Hz, H-10a), 4.43 (1H, dd, J = 4.6 Hz, 12.4 Hz, H-10b), 4.75 (1H, d, J = 7.8 Hz, H-1'), 4.81 (1H, d, J = 1.0 Hz, H-6), 4.90 (1H, m, H-4), 4.95 (1H, m, H-2'), 4.96 (1H, d, J = 8.1 Hz, H-1), 5.09 (1H, d, J = 12.6 Hz, H-6'b), 5.15 (1H, m, H-4'), 5.31 (1H, t, J = 9.5 Hz, H-3'), 6.33 (1H, dd, J = 1.7 Hz, 6.0 Hz, H-3), 6.56 (1H, d, J = 16.1 Hz, H-α), 7.39 (1H, d, J = 3.2 Hz, H-6''), 7.39 (1H, d, J = 3.2 Hz, H-4''), 7.39 (1H, d, J = 3.2 Hz, H-2''), 7.62 (1H, m, H-5''), 7.62 (1H, m, H-3''), 7.72 (1H, d, J = 16.1 Hz, H-*β*); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 20.6, 20.7, 20.8, 20.8, 20.8, 36.5, 42.7, 60.0, 62.7, 63.7, 64.7, 69.8, 72.3, 73.4, 74.0, 81.1, 95.8, 98.0, 103.1, 118.5, 129.5, 129.5, 129.5, 130.1, 130.1, 131.7, 131.7, 135.7, 142.3, 147.0, 167.9, 170.9, 171.2, 171.6, 172.4, 172.6; ESI-MS *m/z* 747 $[M+HCOO]^-$.

General procedure for synthesis of **4**, **5** and **7**

0.1 g of Picroside Ⅱ and Ⅰ were dissolved with 8 mL DMF, respectively. Then 0.5 g K_2CO_3 (anhydrous) and 0.4 mL CH_3I was added by turn. The mixture was reflux for 8 h at room temperature. Recovered the solvent in vacuum, the residue was purified by silica gel column chromatography, eluted with CH_2Cl_2-CH_3OH (15:1) to obtain **4**, **5** and **7**, respectively.

3*α*-methoxy-3,4-dihydrogen-7-iodine-8*β*-hydroxy-(1*β*,10)-epoxy-picroside Ⅱ (**4**) Yield 42%; yellow powder; $[\alpha]_D^{20}$ + 76.0 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 1.47 (1H, m, H-4a), 2.00 (1H, d, J = 2.5 Hz, 14.7 Hz, H-4b), 2.50 (1H, m, H-5), 2.71 (1H, dd, J = 5.1 Hz, 5.0 Hz, H-9), 3.42 (3H, s, H-OCH_3), 3.80 (1H, d, J = 10.2 Hz, H-10a), 3.90 (3H, s, H-OCH_3), 4.13 (1H, d, J = 10.2 Hz, H-10b), 4.41 (1H, d, J = 11.0 Hz, H-7), 5.06 (1H, t, J = 6.8 Hz, H-3), 5.38 (1H, t, J = 11.0 Hz, H-6), 5.59 (1H, d, J = 5.0 Hz, H-1), 6.89 (1H, d, J = 8.3 Hz, H-5’’), 7.57 (1H, d, J = 2.0 Hz, H-2’’), 7.60 (1H, d, J = 2.0 Hz, H-6’’); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 27.7, 37.1, 39.1, 53.5, 55.8, 56.5, 80.9, 81.2, 83.8, 98.9, 100.0, 113.8, 116.1, 121.5, 125.5, 148.9, 153.6, 167.7; ESI-MS *m/z* 491 $[M-H]^-$ 983 $[2M-H]^-$.

3,4-dihydrogen-7-iodine-8*β*-hydroxy-(3*α*,10*α*)-epoxy-picroside Ⅱ (**5**) Yield 46%; yellow powder; $[\alpha]_D^{20}$ + 18.7 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 2.08 (1H, dd, J = 3.1 Hz, 13.9 Hz, H-4a), 2.39 (1H, q, J = 8.4 Hz, H-4b), 2.50 (1H, dt, J = 2.2 Hz, 7.9 Hz, H-5), 2.68 (1H, d, J = 9.9 Hz, H-9), 3.15 (1H, t, J = 8.1 Hz, H-2’), 3.25 (1H, m, H-5’), 3.25 (1H, m, H-3’), 3.28 (1H, m, H-4’), 3.66 (1H, dd, J = 5.1 Hz, 11.8 Hz, H-6’a), 3.80 (1H, d, J = 1.6 Hz, H-10a), 3.85 (1H, dd, J = 1.6 Hz, 11.8 Hz, H-6’b), 3.89 (3H, s, H-OCH_3), 4.13 (1H, d, J = 12.1 Hz, H-10b), 4.66 (1H, dd, J = 3.5 Hz, 9.0 Hz, H-7), 4.70 (1H, d, J = 8.1 Hz, H-1’), 5.23 (1H, dd, J = 2.2 Hz, 9.0 Hz, H-6), 5.32 (1H, t, J = 2.5 Hz, H-3), 5.68 (1H, d, J = 8.0 Hz, H-1), 6.85 (1H, d, J = 8.3 Hz, H-5’’), 7.53 (1H, d, J = 1.8 Hz, H-2’’), 7.57 (1H, dd, J = 1.8 Hz, 8.3 Hz, H-6’’); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 34.2, 36.9, 41.6, 47.0, 56.5, 62.9, 67.2, 74.7, 78.0,

79.9, 89.8, 93.2, 95.9, 98.9, 113.6, 116.1, 121.9, 125.3, 148.9, 153.4, 167.9; ESI-MS *m/z* 639 $[M-H]^-$.

10-methoxy-picroside I (**7**) Yield 24%; white powder; $[\alpha]_D^{20}$ – 55.7 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD) *δ*: 2.54 (1H, m, H-5), 2.54 (1H, m, H-9), 3.25–3.55 (4H, m, H-3',4',5'), 3.45 (1H, m, H-7), 3.69 (3H, s, H-OCH_3), 3.81 (1H, dd, J = 0.8 Hz, 8.2 Hz, H-6), 4.09 (1H, d, J = 12.4 Hz, H-10a), 4.47 (1H, dd, J = 4.8 Hz, 11.8 Hz, H-6'a), 4.53 (1H, dd, J = 2.3 Hz, 11.8 Hz, H-6'b), 4.75 (1H, d, J = 7.8 Hz, H-1'), 4.85 (1H, d, J = 7.6 Hz, H-1), 4.95 (1H, t, J = 6.5 Hz, H-10b), 5.01 (1H, q, J = 4.8 Hz, H-4), 6.32 (1H, d, J = 6.0 Hz, H-3), 6.56 (1H, d, J = 16.0 Hz, H-α), 7.40–7.60 (4H, m, H-4',5'), 7.72 (1H, d, J = 16.0 Hz, H-*β*); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 39.0, 43.4, 55.4, 62.3, 63.2, 63.8, 67.3, 71.2, 74.8, 75.8, 77.7, 79.5, 95.6, 100.4, 103.7, 118.8, 129.3, 129.3, 130.0, 130.0, 131.6, 135.7, 141.9, 146.5, 168.3; ESI-MS *m/z* 551 $[M+HCOO]^-$.

General procedure for synthesis of **8**

0.1 g of Picroside Ⅱ was dissolved with 8 mL pyridine, 0.104 g Boc-Gly and 0.412 g DCC were added, stirred for 6 h at room temperature, recovered the solvent in vacuum, the residue was purified by silica gel column chromatography, eluted with CH_2Cl_2-CH_3OH (12:1) to obtain **8**.

10-*O*-Boc-glycyl-picroside Ⅱ (**8**) Yield 56%; white powder; $[\alpha]_D^{20}$ – 53.6 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ: 1.44 (9H, s, H-Gly-5,6,7), 2.65 (1H, m, H-5), 2.71 (1H, d, J = 7.8 Hz, H-9), 3.2–3.4 (4H, m, H-3',4',5'), 3.63 (1H, q, J = 7.1 Hz, H-6'a), 3.79 (1H, d, J = 6.0 Hz, H-7), 3.84 (2H, s, H-Gly-2), 3.91 (3H, s, H-OCH3), 3.95 (1H, d, J = 1.8 Hz, H-6'b), 4.09 (2H, q, J = 7.1 Hz, H-10), 4.76 (1H, d, J = 7.8 Hz, H-1'), 4.99 (1H, q, J = 4.6 Hz, H-4), 5.17 (1H, d, J = 8.6 Hz, H-6), 5.21 (1H, d, J = 9.6 Hz, H-1), 6.37 (1H, d, J = 1.5 Hz, 6.0 Hz, H-3), 6.86 (1H, d, J = 8.0 Hz, H-5''), 7.57 (1H, d, J = 1.7 Hz, H-2''), 7.60 (1H, dd, J = 1.7 Hz, 8.0 Hz, H-6''); ^{13}C NMR (100 MHz, CD_3OD) δ: 28.8, 36.6, 43.0, 43.1, 56.5, 60.3, 63.1, 63.9, 65.1, 71.8,

74.6, 77.8, 78.7, 80.8, 81.5, 94.9, 99.7, 102.7, 113.6, 116.0, 122.0, 125.3, 142.0, 148.4, 153.2, 158.6, 167.8, 171.9; ESI-MS *m/z* 668 $[M-H]^-$.

General procedure for synthesis of **9**-**12**

0.2 g of Picroside Ⅱ was dissolved with 16 mL pyridine, 0.224 g Boc-Ala and 0.824 g DCC were added, stirred for 16 h at room temperature, recovered the solvent in vacuum, the residue was purified by silica gel column chromatography, gradient eluted with acetate-mineral ether (1:1) and CH_2Cl_2-CH_3OH (35:1—20:1—15:1) to obtain **9**, **10**, **11** and **12**.

3'-*O*-Boc-alacyl-picroside Ⅱ (**9**) Yield 12%; white powder; $[\alpha]_D^{20}$ – 65 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 1.37 (3H, d, J = 4.0 Hz, H-Ala-3), 1.45 (9H, s, H-Ala-6,7,8), 2.64 (1H, m, H-9), 2.69 (1H, m, H-5), 3.39–3.51 (3H, m, H-2',4',5'), 3.65 (1H, m, H-6'a), 3.75 (1H, s, H-7), 3.84 (1H, m, H-10a), 3.90 (1H, m, H-6'b), 3.90 (3H, s, H-OCH3), 4.16 (1H, d, J = 7.3 Hz, H-10b), 4.21 (1H, d, J = 4.0 Hz, H-Ala-2), 4.80 (1H, d, J = 8.0 Hz, H-1'), 5.01 (1H, m, H-4), 5.13 (1H, d, J = 7.5 Hz, H-6), 5.20 (1H, d, J = 9.1 Hz, H-1), 6.38 (1H, dd, J = 1.2 Hz, 6.0 Hz, H-3), 6.86 (1H, d, J = 8.1 Hz, H-5''), 7.57 (1H, d, J = 1.8 Hz, H-2''), 7.60 (1H, dd, J = 1.8 Hz, 8.2 Hz, H-6''); ^{13}C NMR (100 MHz, CD_3OD) *δ*: 18.0, 28.7, 36.8, 43.2, 50.9, 56.5, 60.3, 61.2, 62.6, 66.9, 69.9, 73.0, 78.4, 79.6, 80.7, 81.7, 95.2, 99.5, 103.0, 113.6, 116.0, 122.0, 125.3, 142.4, 148.4, 153.2, 158.1, 167.9, 174.5; ESI-MS *m/z* 682 $[M-H]^-$.

6',4''-O-2-Boc-alacyl-picroside Ⅱ (**10**) Yield 9%; white powder; $[\alpha]_D^{20}$ – 36.5 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 1.39 (3H, d, J = 7.3 Hz, H-Ala-3), 1.42 (9H, s, H-Ala-6,7,8), 1.46 (9H, s, H- Ala-6,7,8), 1.52 (3H, d, J = 7.3 Hz, H-Ala-3), 2.63 (1H, d, J = 7.5 Hz, H-9), 2.70 (1H, m, H-5), 3.20–3.40 (3H, m, H-3',4'), 3.51 (1H, m, H-5'), 3.81 (1H, m, H-7), 3.82 (1H, m, H-10a), 3.88 (3H, s, H-OCH3), 4.13 (1H, d, J = 13.2 Hz, H-10b), 4.18 (1H, d, J = 7.3 Hz, H-Ala-2), 4.26 (1H, dd, J = 11.0 Hz, 4.1 Hz, H-6'a), 4.40 (1H, d, J = 7.3 Hz, H-Ala-2), 4.61 (1H, d, J

= 11.0 Hz, H-6’b), 4.79 (1H, d, J = 7.9 Hz, H-1’), 5.02 (1H, m, H-4), 5.10 (1H, d, J = 7.5 Hz, H-1), 5.16 (1H, d, J = 7.8 Hz, H-6), 6.38 (1H, dd, J = 1.6 Hz, 6.0 Hz, H-3), 7.20 (1H, d, J = 8.7 Hz, H-6’’), 7.70 (1H s, H-5’’), 7.72 (1H, s, H-2’’); ^{13}C NMR (100 MHz, CD_3OD) δ: 17.6, 17.9, 28.6, 28.7, 36.9, 43.3, 50.6, 50.8, 56.6, 60.0, 61.0, 64.3, 66.9, 71.2, 74.8, 75.9, 77.5, 80.6, 80.7, 82.3, 95.4, 100.0, 103.0, 114.5, 123.8, 124.0, 129.8, 142.5, 145.4, 152.7, 152.7, 157.9, 167.0, 172.8, 174.7; ESI-MS *m/z* 853 $[M-H]^-$ 899 $[M+HCOO]^-$.

6'-O-Boc-alacyl-picroside Ⅱ (**11**) Yield 30%; white powder; $[\alpha]_D^{20}$ – 76.0 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.39 (3H, d, J = 7.3 Hz, H-Ala-3), 1.43 (9H, s, H-Ala-6,7,8), 2.63 (1H, s, H-9), 2.67 (1H, m, H-5), 3.28–3.5 (3H, m, H-3’,4’), 3.51 (1H, m, H-5’), 3.79 (1H, s, H-7), 3.82 (1H, s, H-10a), 3.90 (3H, s, H-OCH3), 4.13 (1H, d, J = 13.2 Hz, H-10b), 4.18 (1H, d, J = 7.3 Hz, H-Ala-2), 4.26 (1H, dd, J = 1.1 Hz, 11.6 Hz, H-6’a), 4.61 (1H, d, J = 11.6 Hz, H-6’b), 4.79 (1H, d, J = 7.9 Hz, H-1’), 5.00 (1H, m, H-4), 5.03 (1H, d, J = 7.5 Hz, H-1), 5.11 (1H, d, J = 7.1 Hz, H-6), 6.37 (1H, dd, J = 1.4 Hz, 6.1 Hz, H-3), 6.86 (1H, d, J = 8.2 Hz, H-5’’), 7.57 (1H, d, J = 1.9 Hz, H-2’’), 7.60 (1H, dd, J = 1.9 Hz, 8.2 Hz, H-6’’); ^{13}C NMR (100 MHz, CD_3OD) δ: 17.9, 28.8, 36.9, 43.3, 50.6, 56.5, 60.1, 61.0, 64.4, 66.7, 71.2, 74.8, 75.8, 77.5, 80.6, 81.7, 95.4, 100.0, 103.1, 113.6, 116.0, 122.0, 125.1, 142.4, 148.8, 153.2, 157.9, 167.8, 174.7; ESI-MS *m/z* 682 $[M-H]^-$ 728 $[M+HCOO]^-$.

4''-*O*-Boc-alacyl-picroside Ⅱ (**12**) Yield 30%; white powder; $[\alpha]_D^{20}$ – 55.0 (*c* 0.10, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.45 (9H, s, H-Ala-6,7,8), 1.52 (3H, d, J = 7.4 Hz, H-Ala-3), 2.66 (1H, m, H-9), 2.70 (1H, dd, J = 1.6 Hz, 4.2 Hz, H-5), 3.25–3.45 (4H, m, H-3’,4’,5’), 3.65 (1H, dd, J = 6.6 Hz, 11.9 Hz, H-6’a), 3.78 (1H, d, J = 0.9 Hz, H-7), 3.84 (1H, d, J = 5.0 Hz, H-10a), 3.89 (3H, s, H-OCH3), 3.93 (1H, dd, J = 1.6 Hz, 11.9 Hz, H-6’b), 4.19 (1H, d, J = 13.2 Hz, H-10b), 4.40 (1H, d, J = 7.4 Hz, H-Ala-2), 4.80 (1H, d, J = 7.9 Hz, H-1’), 5.18 (1H, d, J = 5.3 Hz, H-4), 5.18 (1H, d, J = 5.3 Hz, H-6), 5.20 (1H, d, J = 7.8 Hz, H-1), 6.39 (1H, dd, J = 1.6 Hz, 6.0 Hz, H-3), 7.20 (1H, d, J = 8.7 Hz, H-6’’), 7.70 (1H, s, H-5’’), 7.71 (1H, s, H-2’’); ^{13}C NMR

(100 MHz, CD_3OD) δ: 17.7, 28.7, 36.8, 43.2, 50.8, 56.7, 60.3, 61.2, 62.9, 67.1, 71.8, 74.8, 77.7, 78.6, 80.7, 82.4, 95.1, 99.7, 102.9, 114.5, 123.8, 124.1, 129.8, 142.6, 145.4, 152.7, 157.9, 167.2, 172.9; ESI-MS *m/z* 682 $[M-H]^-$ 728 $[M+HCOO]^-$.

Biological assays

Silybin was purchased from Chengdu Mansite Bio-Technology Co., Ltd (Chengdu, China). SMMC-7721 was obtained from Shanghai Institute of Biochemistry and Cell Biology, SIBS, CAS (Shanghai, China), and was grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin Solution.

The cell viability was evaluated using the MTT assay. The cells were plated in 96-well plate with 100 μL for each well (1×10^4 cells/well) and incubation for 24 h in a humidified atmosphere with 5% CO_2 at 37 ℃. Then the cells were exposed with different concentrations (0.1, 1, 10, 100, and 1000 μM) of the derivatives respectively and incubation for 3 h, Silybin was used as the positive drug. Then the culture media containing derivatives were removed and new medium containing 0.6 mmol/L H_2O_2 was added. After 3 h of treatment, 20 μL MTT regents (5 mg/mL) were added to each well and incubation for 4 h, the medium was removed and 100 μL of DMSO was added to each well and shaken for 10 min. The absorbance was measured by a microplate reader at a wavelength of 490 nm. Control wells received only the medium without the derivatives. Each derivative concentration had four replicates. The cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (A_{Sample} / A_{Blank} - A_{Model} / A_{Blank}) \times 100\%$$

The hepatoprotective effect of each derivative was expressed as EC_{50}. The dose-response curves of the derivatives were showed in Figure S1, and the EC_{50} values were showed in Table S1. All the results were expressed as mean ± SD. Statistical analysis was carried out by SPSS 24 software.**Figure S1**. Dose-response curves of the derivatives

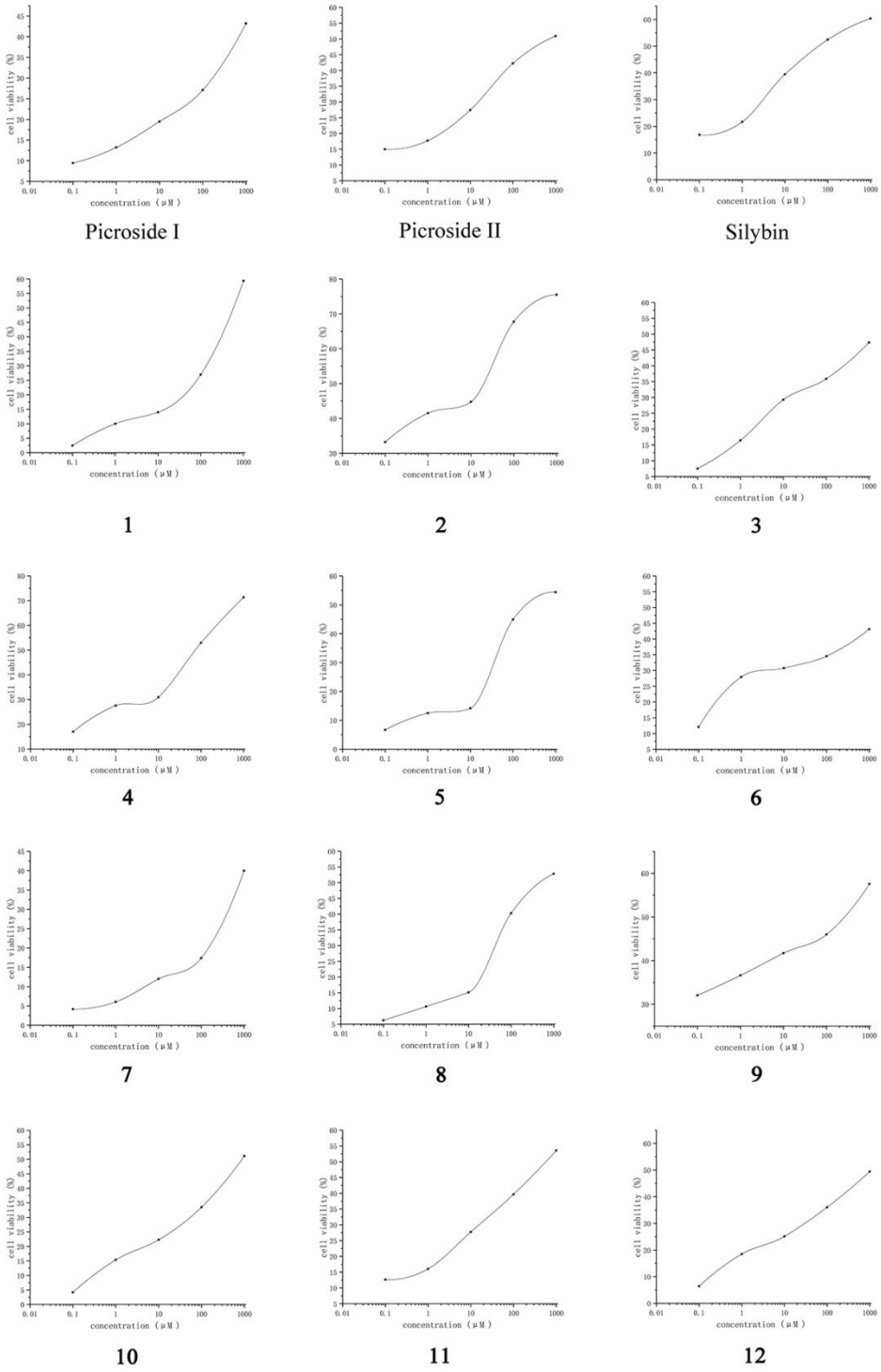

Table S1. EC_{50} of the derivatives

No.	EC_{50}	**No.**	EC_{50}
Picroside I	4409 ± 971.6	6	5906 ± 3845
Picroside II	704.1 ± 123.1	7	3696 ± 809.6
Silybin	116.6 ± 14.68	8	576.6 ± 109.2
1	547.4 ± 70.21	9	157.1 ± 69.96
2	6.064 ± 1.295	10	911.6 ± 140.4
3	1286 ± 314.6	11	568 ± 66.29
4	63.06 ± 11.77	12	1037 ± 240.2
5	794.1 ± 280.4		

Figure S2. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **1**

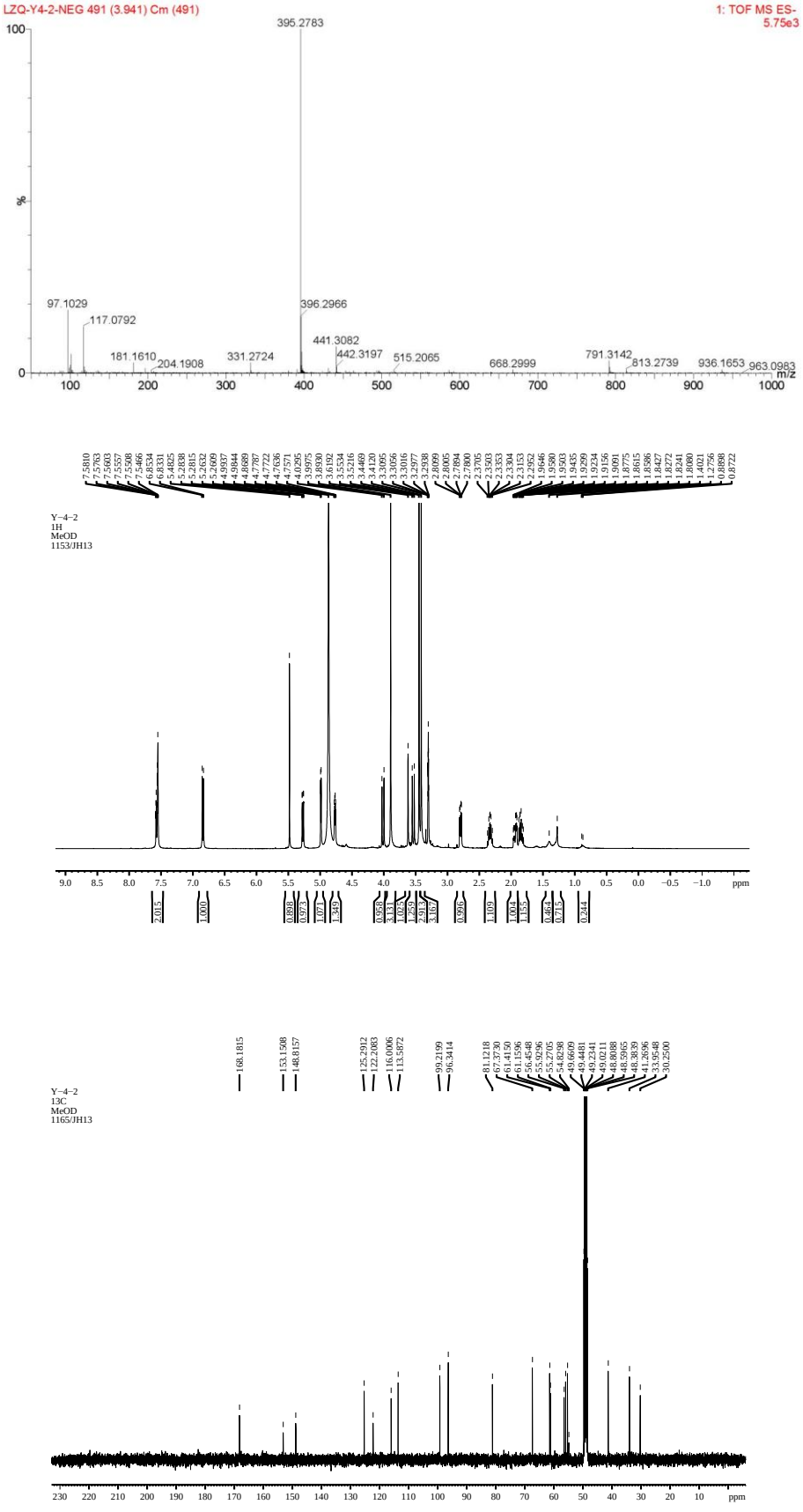

Figure S3. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **2**

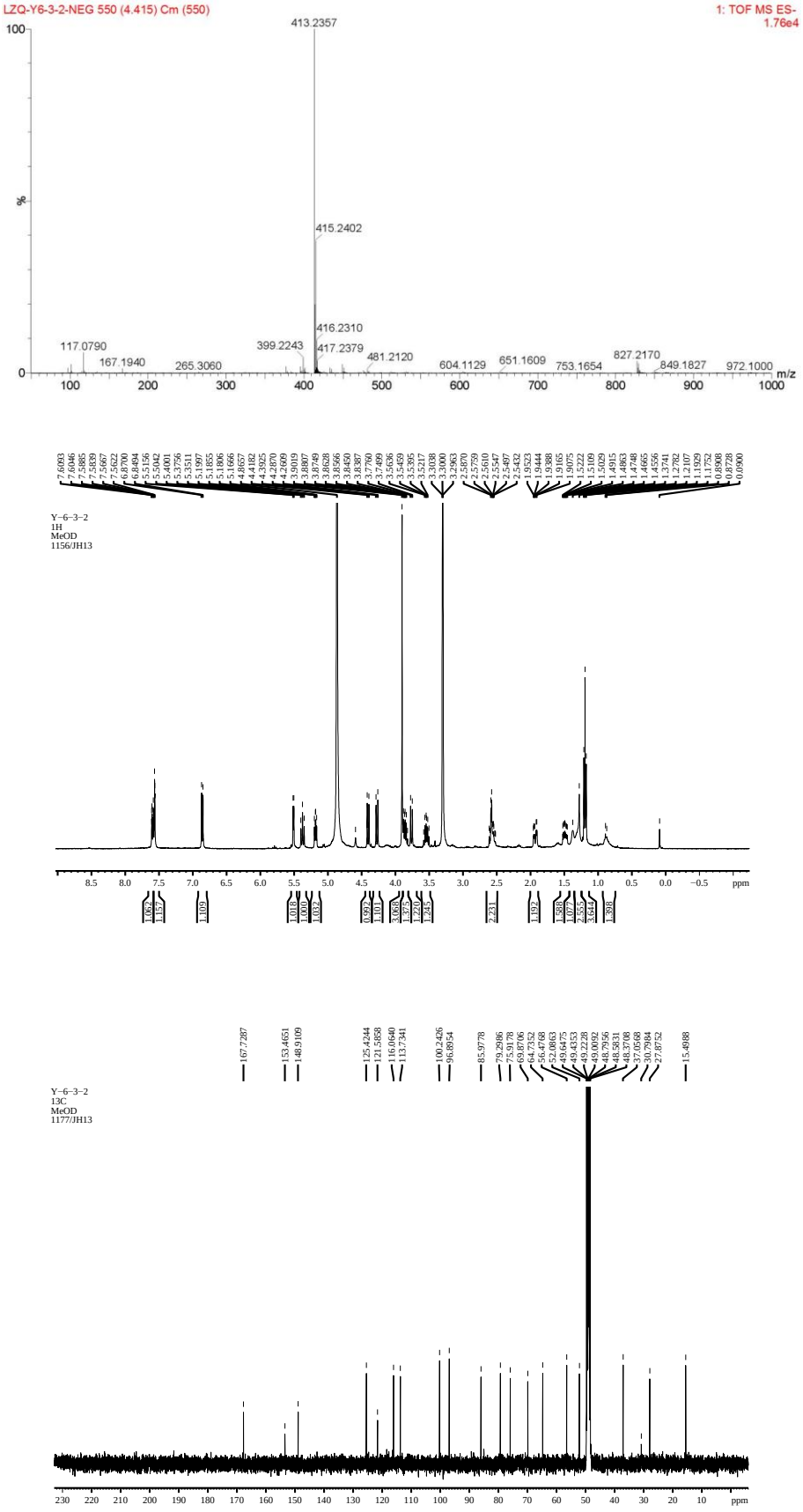

Figure S4. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **3**

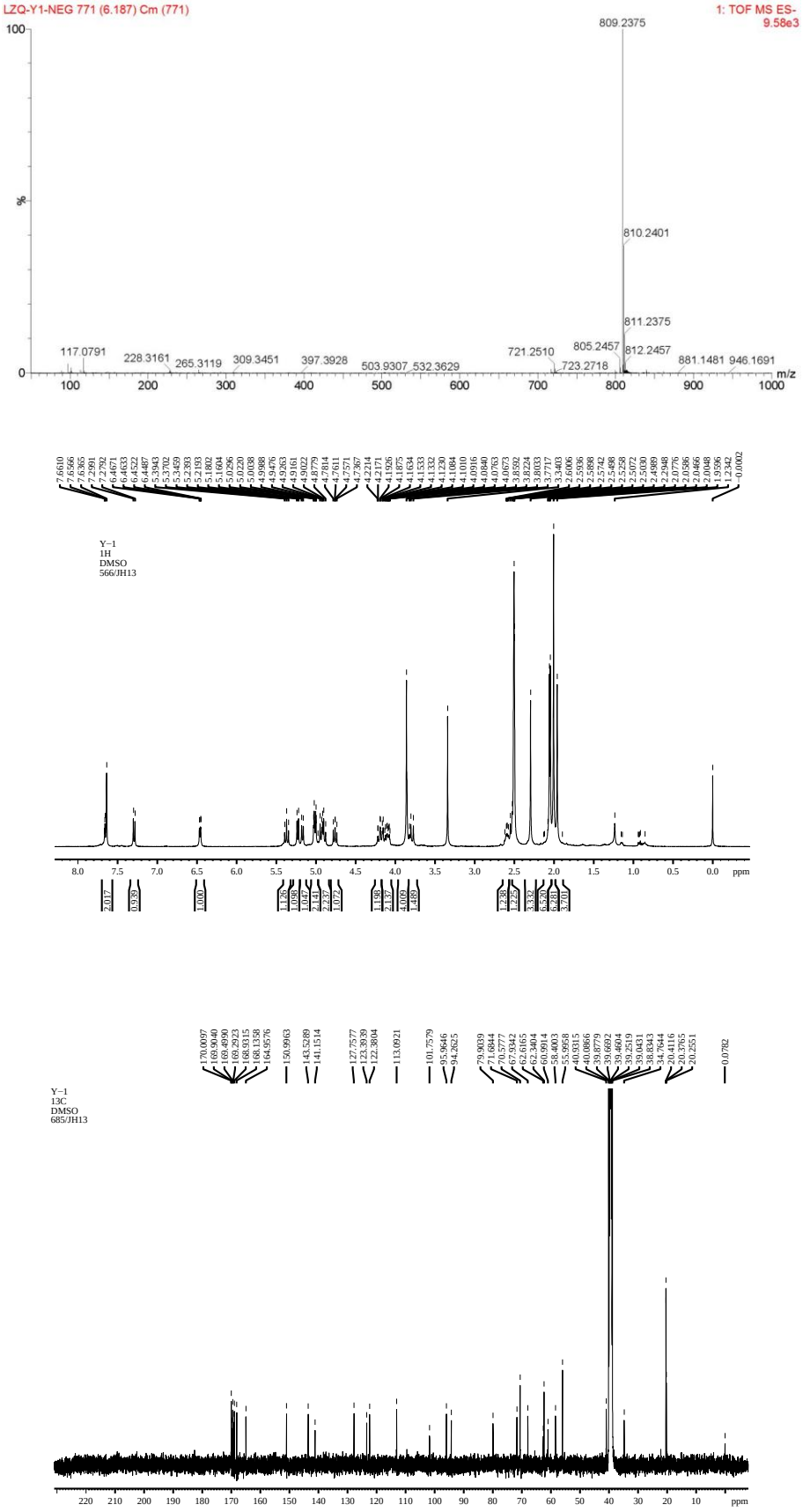

Figure S5. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **4**

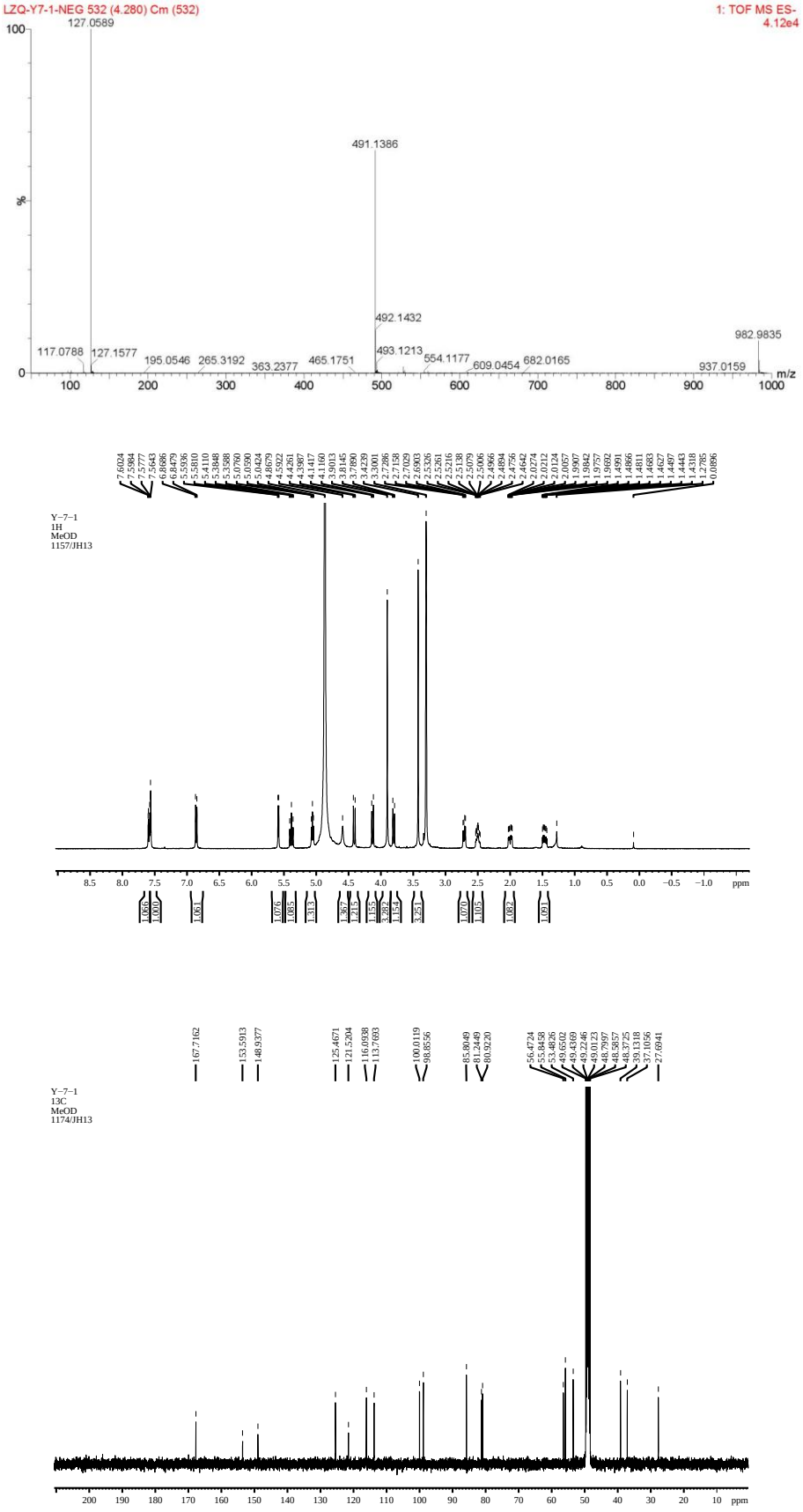

Figure S6. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **5**

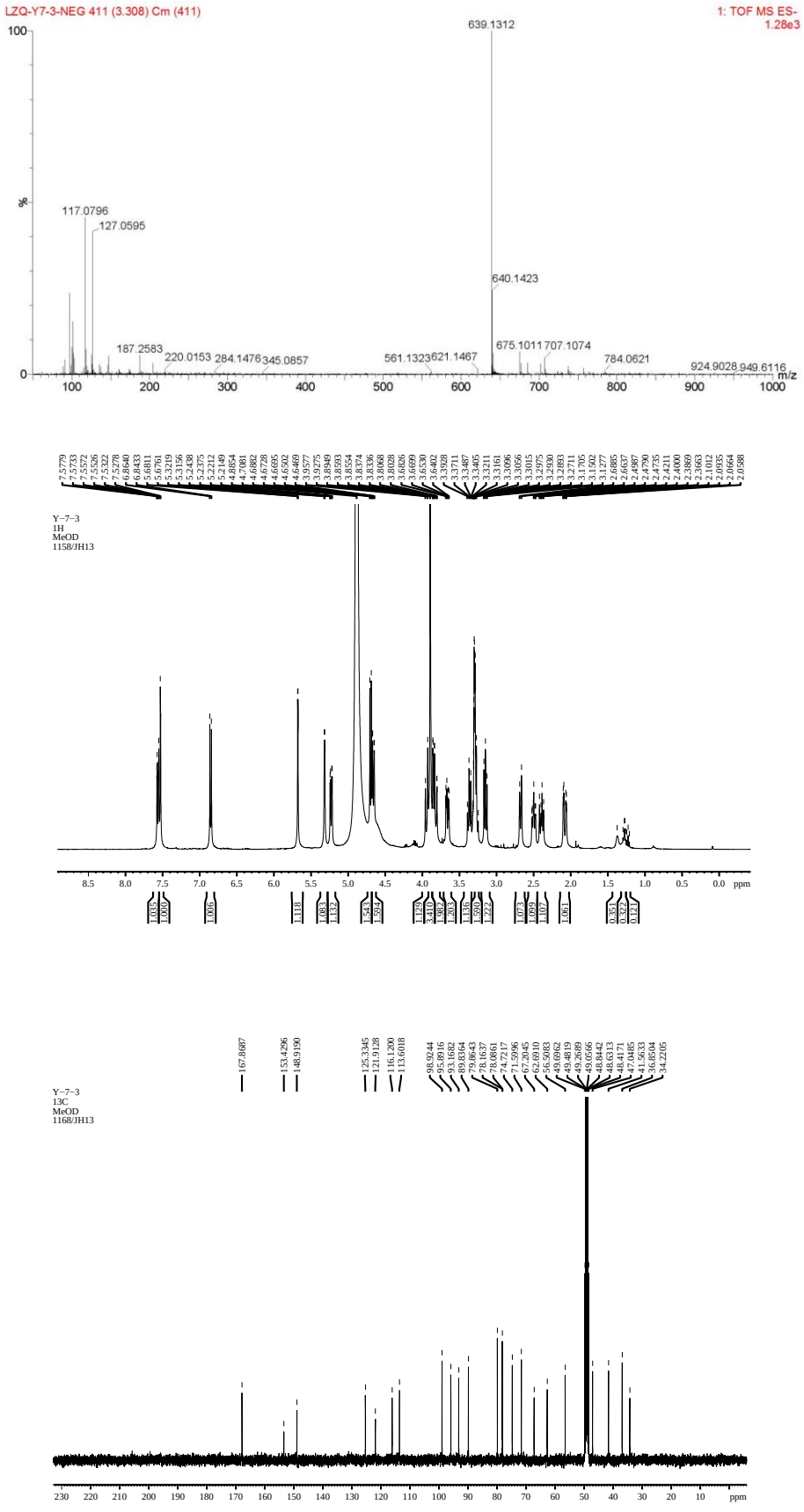

Figure S7. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **6**

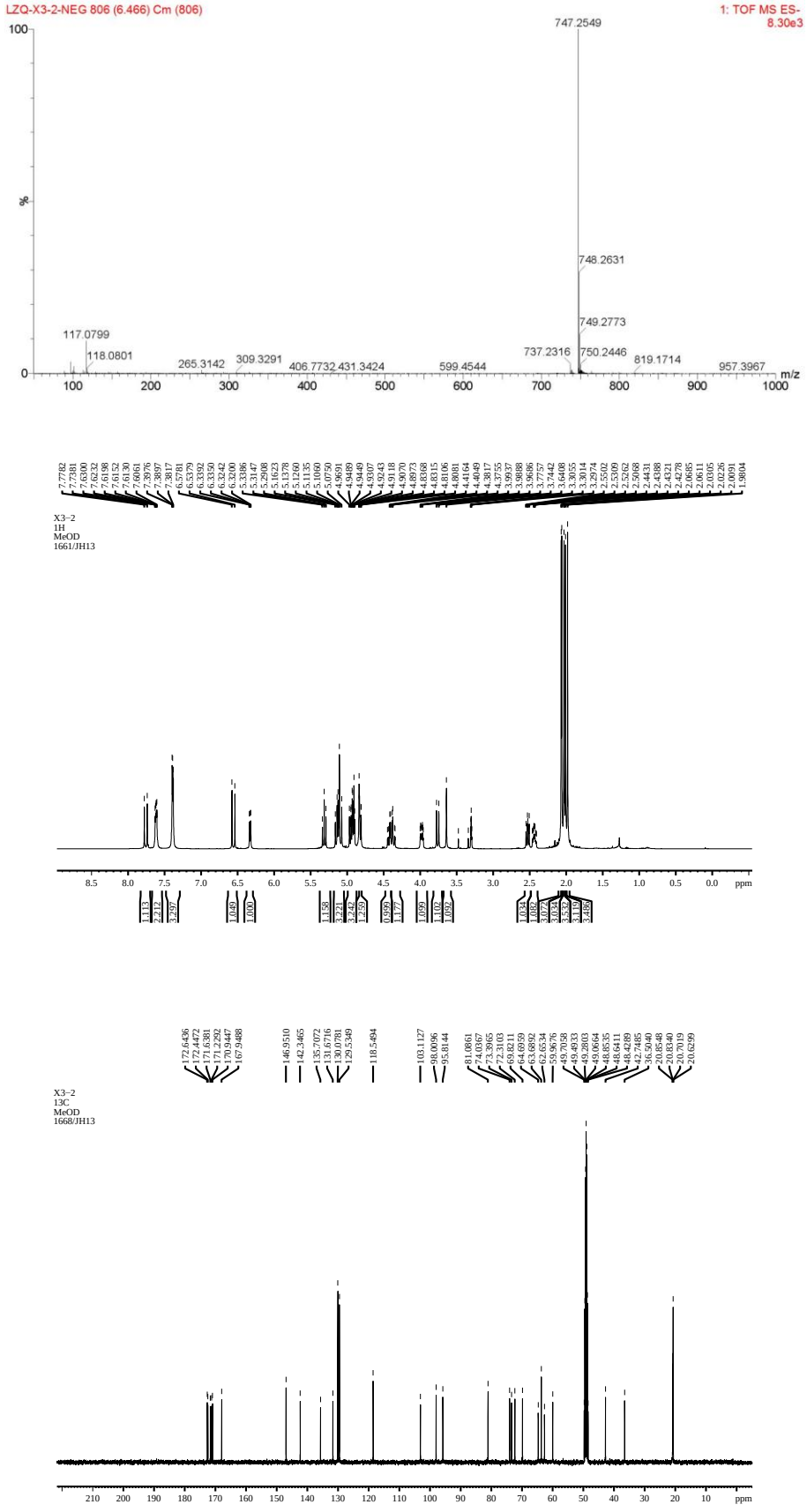

Figure S8. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **7**

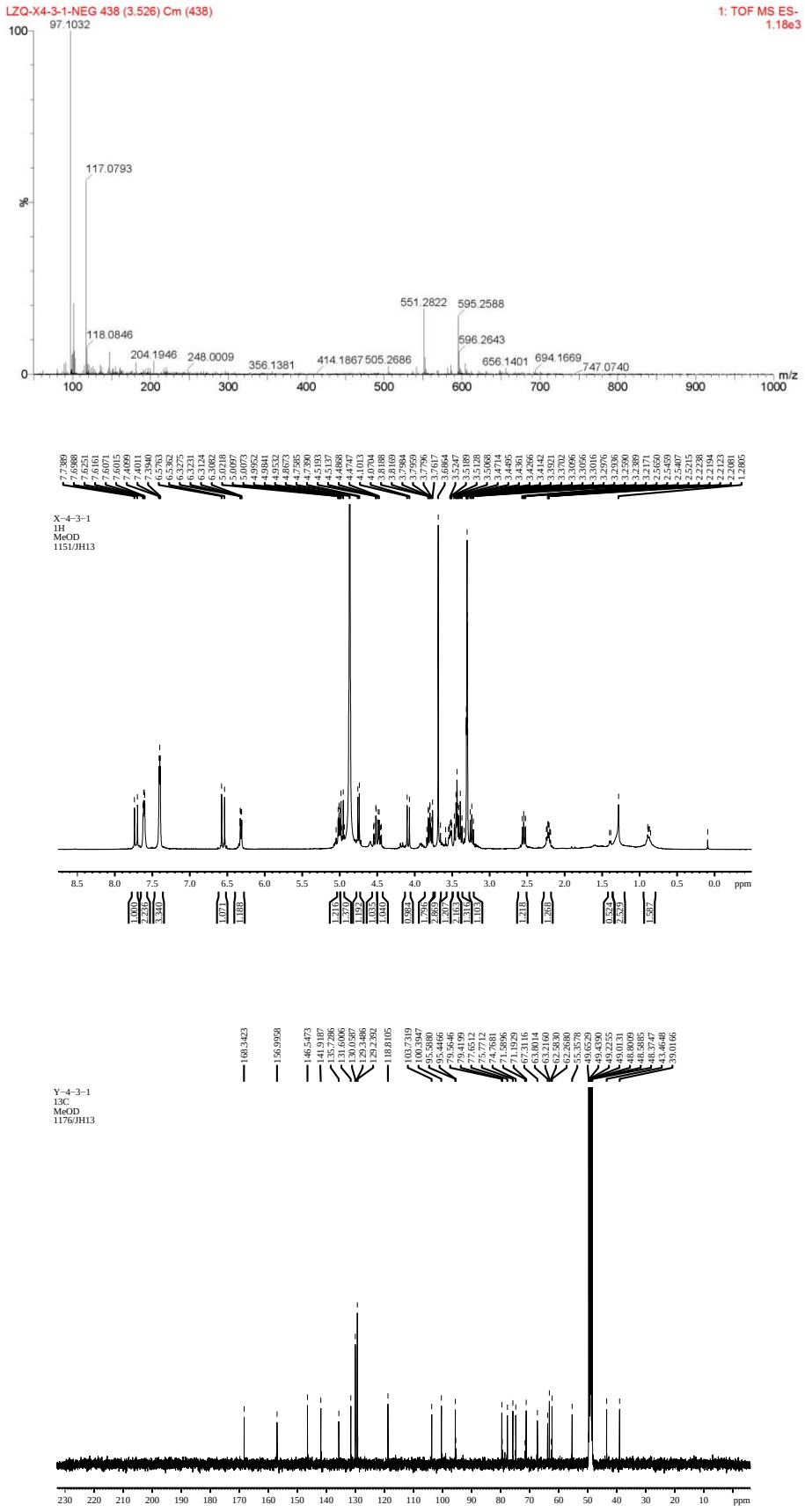

Figure S9. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **8**

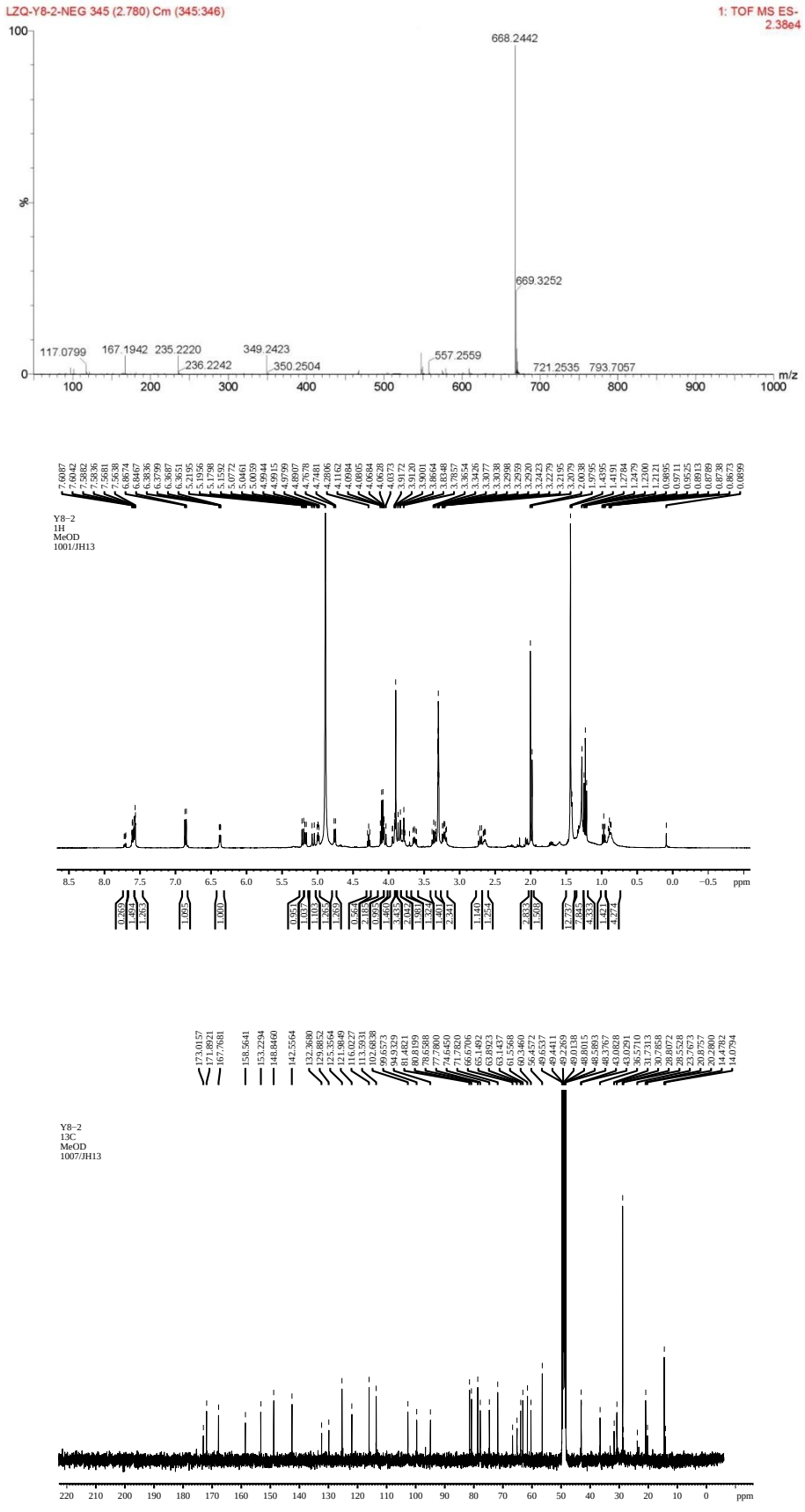

Figure S10. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **9**

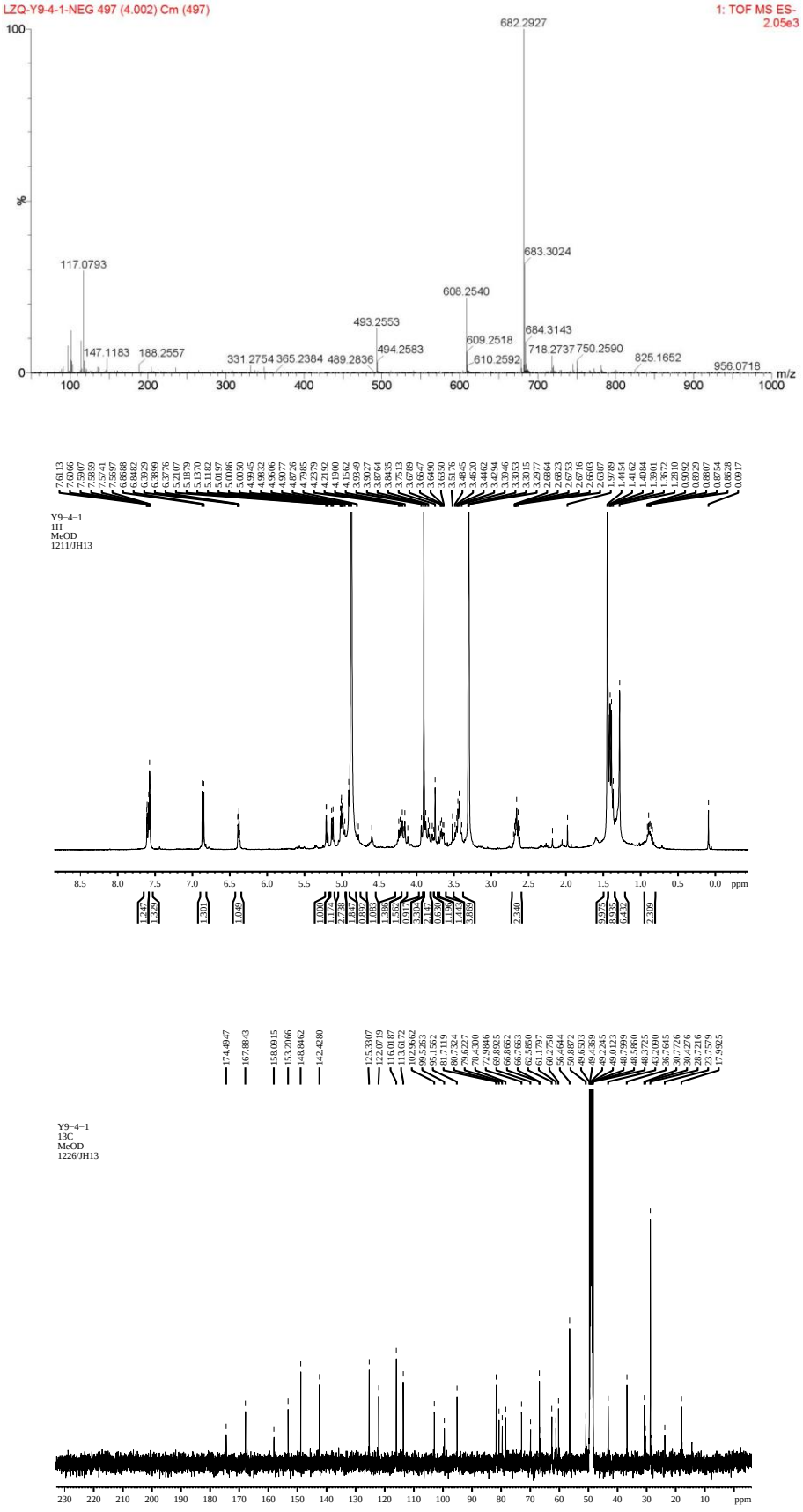

Figure S11. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **10**

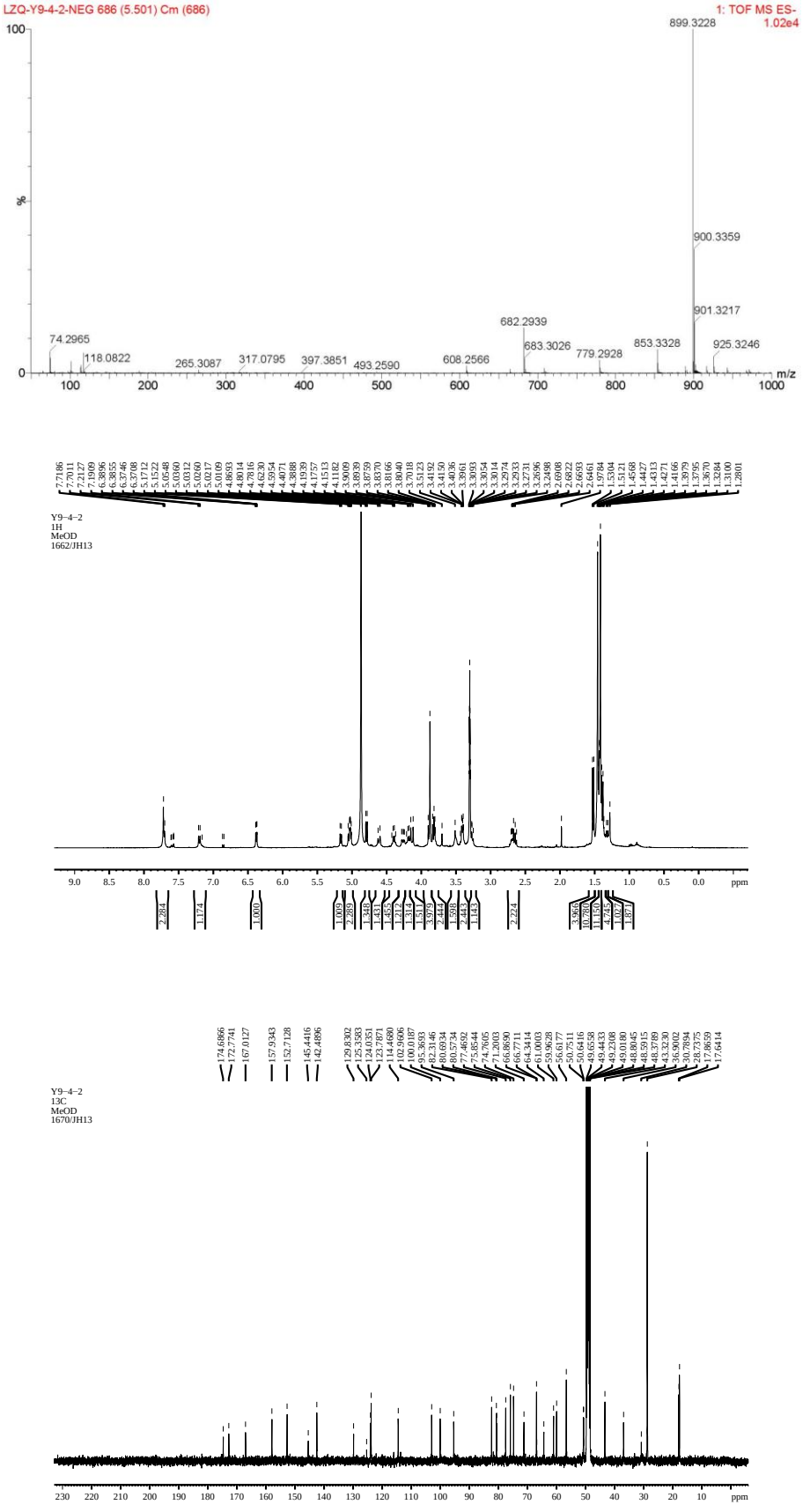

Figure S12. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **11**

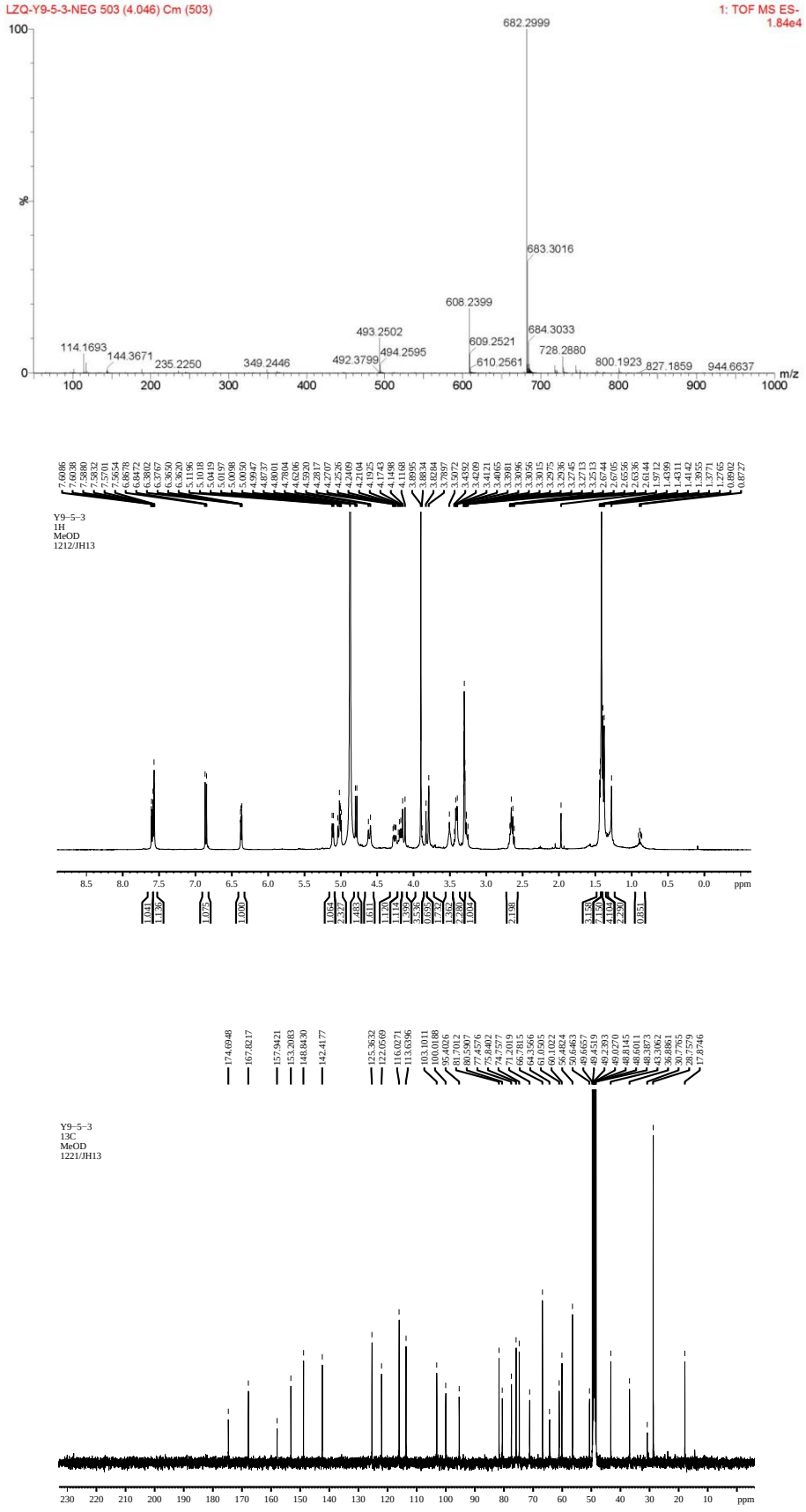

Figure S13. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of compound **12**