Development of lactobionic acid conjugated-copper chelators as anticancer candidates for hepatocellular carcinoma

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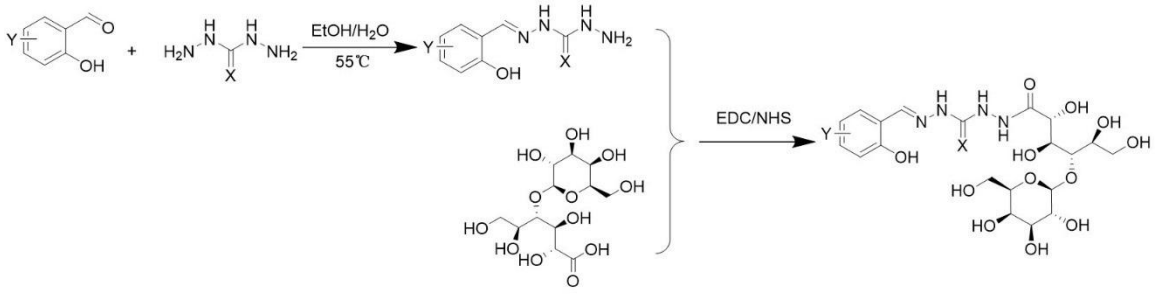
Synthesis:

Scheme S1. Synthesis of the chelators.



Y: -CH_3; -Cl; -OH; -$N(CH_3)_2$; -H.
X: O; S.

General procedure: Synthesis of **TH1**, **TH9** and **TH10**. A water solution of thiocarbohydrazide (10 mmol) was added dropwise to the solution of salicylaldehyde (for **TH1**, 10 mmol) in ethanol at 60-70 ºC. Next, a water solution of carbohydrazide (10 mmol) was added dropwise to the solution of 4-methoxysalicylaldehyde (for **TH9**, 10 mmol) or 5-methsalicylaldehyde (for **TH10**, 10 mmol) in ethanol at 60-70 ºC. Then, the mixture was refluxed for 4 h (monitored by TLC), and a large amount of precipitate was observed. The mixture was cooled to room temperature and filtrated to gain white solids. The solids were washed with methanol and water, and then dried with vacuum to get the intermediate. The crude product ware recrystallized from water/DMF or water/DMSO.

*Data for **TH1:*** yield 1.52 g (72.29%); slight yellow solid; ESI-MS: m/z 210.12 ($M-H^+$), calculated: 210.26; Elem. anal. calcd for $C_8H_{10}N_4OS$: C 45.70, H 4.79, N 26.65, S 15.25; Found: C 45.64, H 4.68, N 26.56, S 15.18. ^1H NMR (600 MHz, DMSO-d_6) δ 11.36 (s, 1H), 9.86 (s, 1H), 9.74 (s, 1H), 8.32 (s, 1H), 7.97 (s, 1H), 7.20 (s, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.81 (s, 1H), 4.84 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 176.15, 156.59, 139.83, 131.73, 127.36, 120.69, 120.11, 116.85. The wave number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **TH1** is 3289, 3258, 3179, 3138, 2955, 1618, 1597, 1551, 1499, 1375, 1333, 1271, 1244, 1213, 1153, 1011, 773, 635, 613, 579.

*Data for **TH9:*** yield 1.73 g (77.15%); white solid; ESI-MS: m/z 224.26 ($M-H^+$), calculated: 224.22; Elem. anal. calcd for $C_9H_{12}N_4O_3$: C 48.21, H 5.39, N 24.99; Found: C 48.16, H 5.28, N 24.88. ^1H NMR (600 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.21 (s, 1H), 7.92 (s, 1H), 7.65 (s, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.92-6.73 (m, 3H), 4.14 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.23, 156.45, 152.31, 130.76, 130.31, 128.14, 120.03, 116.52, 39.94. The wave number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **TH9** is 3344, 3277, 3200, 3088, 2959, 1707, 1670, 1634, 1570, 1510, 1292, 1250, 1236, 1165, 1136, 1032, 806, 683, 638,573.

Data for **TH10**: yield 1.68 g (80.68%); white solid; ESI-MS: m/z 208.31 ($M-H^+$), calculated: 208.22; Elem. anal. calcd for $C_9H_{12}N_4O_2$: C 51.92, H 5.81, N 26.91; Found: C 51.86, H 5.92, N 26.84. ^1H NMR (600 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.83 (s, 1H), 7.51 (s, 1H), 6.43 (dd, J = 12.9, 4.1 Hz, 1H), 4.12 (s, 1H), 3.73 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 145.52, 142.24, 141.35, 136.42, 113.91, 97.40, 90.43, 85.41, 23.79. The wave

number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **TH10** is 3352, 3281, 3242, 3098, 2914, 1724, 1680, 1636, 1558, 1497, 1462, 1367, 1275, 1223, 1180, 957, 814, 687, 606, 573.

General procedure: Synthesis of **GT1**, **GT9** and **GT10**. Lactobionic acid (5 mmol) and NHS (N-Hydroxy succinimide, 6 mmol) were dissolved in DMF (N, N-Dimethylformamide, 30 mL), and stirred in ice water bath for 30 min. Then, EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, 6 mmol) was added, and stirred for 1 h at room temperature. Next, 5 mmol of **TH1** (1.0513 g), **TH9** (1.1205 g), **TH10** (1.0411 g) were dissolved in DMF (10 mL), and added dropwise to the solution for 72 h (monitored by TLC) at room temperature. Finally, we slowly added 3 times the volume of water to precipitate a solid, and the crude products were recrystallized from water/DMF or water/DMSO.

Data for **GT1**: yield 1.706 g (61.97%); white solid; ESI-MS: m/z 549.86 ($M-H^+$), calculated: 550.54; Elem. anal. calcd for $C_{21}H_{32}N_4O_{14}$: C 43.63, H 5.49, N 10.18, S 5.82; Found: C 43.52, H 5.37, N 10.06, S 5.78. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.78 (d, J = 64.7 Hz, 2H), 9.42 (s, 1H), 8.30 (s, 1H), 7.96 (s, 1H), 7.18 (s, 2H), 6.81 (d, J = 27.8 Hz, 2H), 5.60-3.36 (m, 16H), 1.22 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 180.93, 176.39, 161.40, 144.83, 144.55, 136.56, 135.59, 125.52, 124.92, 123.86, 121.68, 110.19, 83.46, 80.69, 78.36, 76.53, 76.33, 73.24, 67.40, 65.55. The wave number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **GT1** is 3169, 3140, 3001, 2982, 1618, 1562, 1535, 1485, 1452, 1396, 1362, 1319, 1285, 1200, 1178, 1034, 955, 903, 795, 746, 642, 582.

Data for **GT9**: yield 1.835g (64.98%); white solid; ESI-MS: m/z 564.86 ($M-H^+$), calculated: 564.50; Elem. anal. calcd for $C_{21}H_{32}N_4O_{14}$: C 44.68, H 5.71, N 9.93; Found: C 44.58, H 5.67, N 9.84. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.65 (s, 2H), 8.30 (s, 2H), 7.56 (s, 1H), 6.63-6.23 (m, 4H), 5.27-4.08 (m, 9H), 3.95 (d, J = 8.1 Hz, 1H), 3.87-3.38 (m, 11H), 1.21 (s, 1H), 1.03 (d, J = 7.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 191.00, 178.15, 176.38, 166.60, 163.45, 157.14, 117.92, 111.86, 111.26, 110.14, 106.63, 106.10, 88.96, 80.71, 78.35, 76.54, 76.33, 73.24, 67.33, 65.48, 60.33. The wave number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **GT9** is 3312, 3235, 3069, 2963, 2932, 2837, 1736, 1676, 1630, 1510, 1441, 1356, 1288, 1221, 1169, 1126, 1082, 1036, 962, 835, 800, 706, 638, 615, 594, 569.

Data for ***GT10***: yield 1.838 g (67.06%); white solid; ESI-MS: m/z 548.15($M-H^+$), calculated: 548.50; Elem. anal. calcd for $C_{21}H_{32}N_4O_{14}$: C 45.99, H 5.88, N 10.21; Found: C 45.92, H 5.78, N 10.18. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.37 (s, 1H), 7.02 (d, J = 8.3 Hz, 1H), 6.77 (d, J = 8.2 Hz, 1H), 5.15 (d, J = 11.3 Hz, 2H), 4.94-3.39 (m, 14H), 2.23 (s, 2H), 1.22 (s, 1H), 1.04 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 178.15, 159.63, 157.13, 136.93, 132.89, 124.45, 121.73, 109.85, 88.91, 88.03, 80.71, 78.37, 76.70, 76.53, 76.32, 73.43, 73.24, 67.45, 65.83, 65.50, 25.23. The wave number of the Fourier Transform Infrared Spectrum (FT-IR, KBr) of compound **GT10** is 3252, 3181, 3101, 3028, 2918, 1649, 1626, 1572, 1491, 1352, 1275, 1225, 1157, 1090, 955, 826, 772, 745, 656, 567.

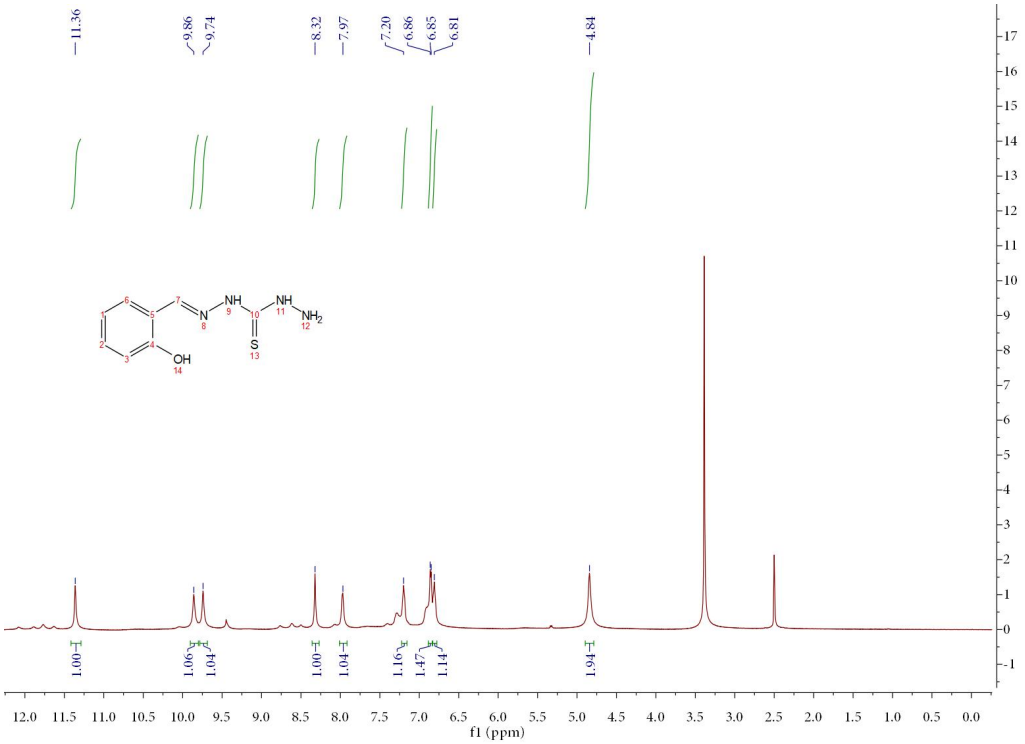


Figure S1.[1]H NMR spectrum (600 MHz) of **TH1** was recorded in DMSO-d_6.

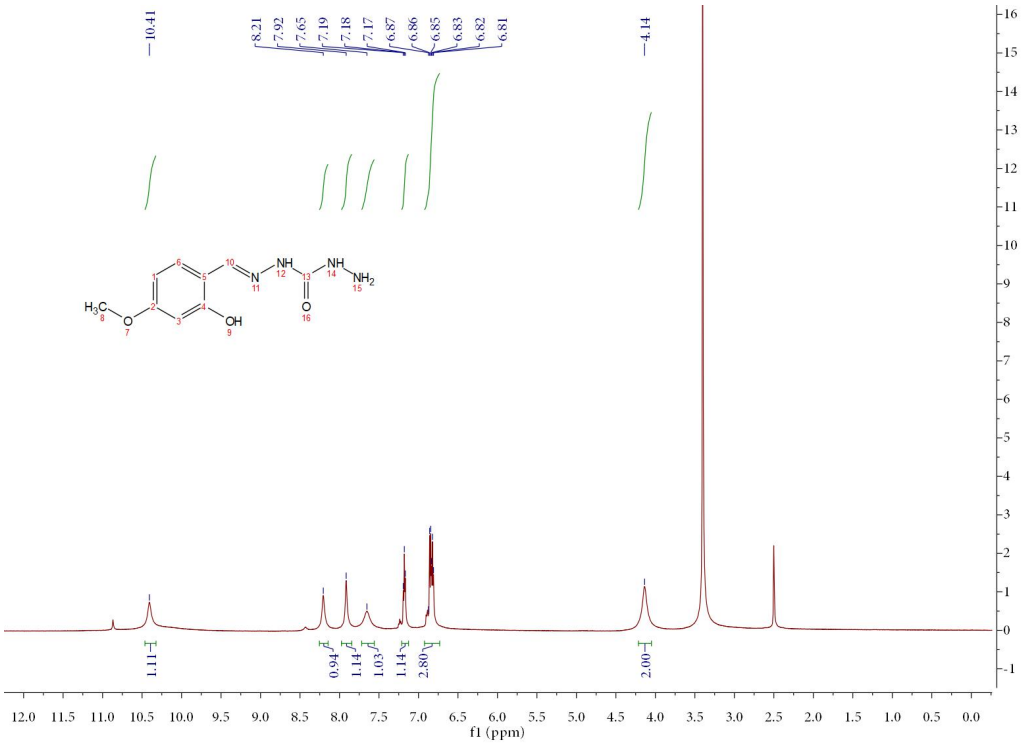


Figure S2. [1]H NMR spectrum (600 MHz) of **TH9** was recorded in DMSO-d_6.

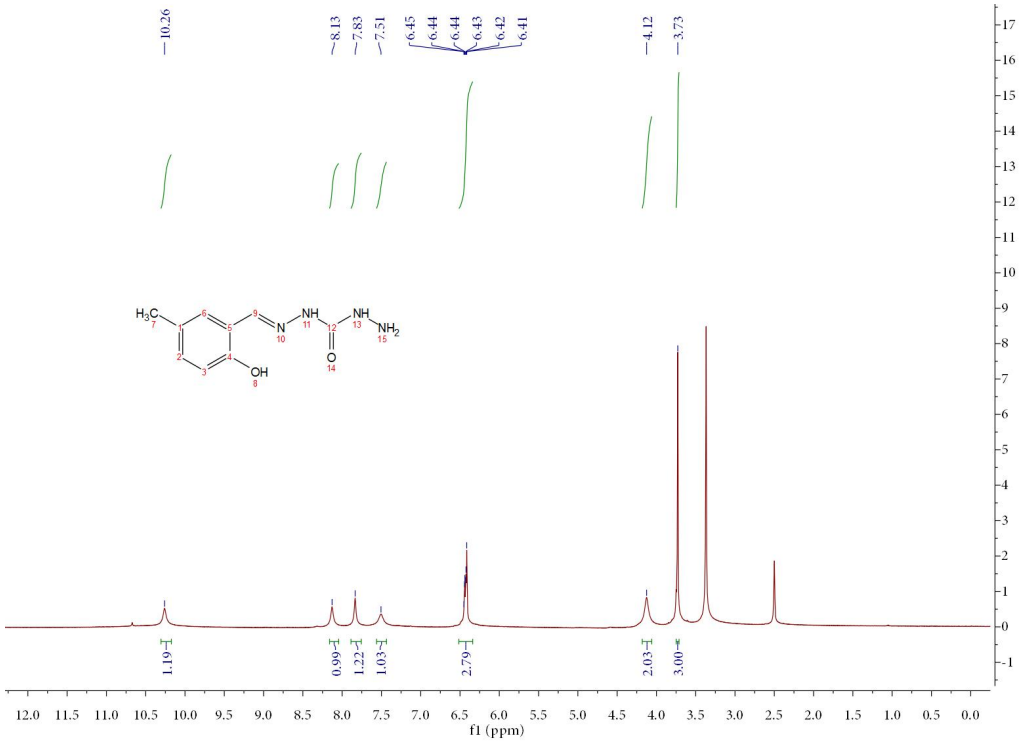


Figure S3. [1]H NMR spectrum (600 MHz) of **TH10** was recorded in DMSO-d_6.

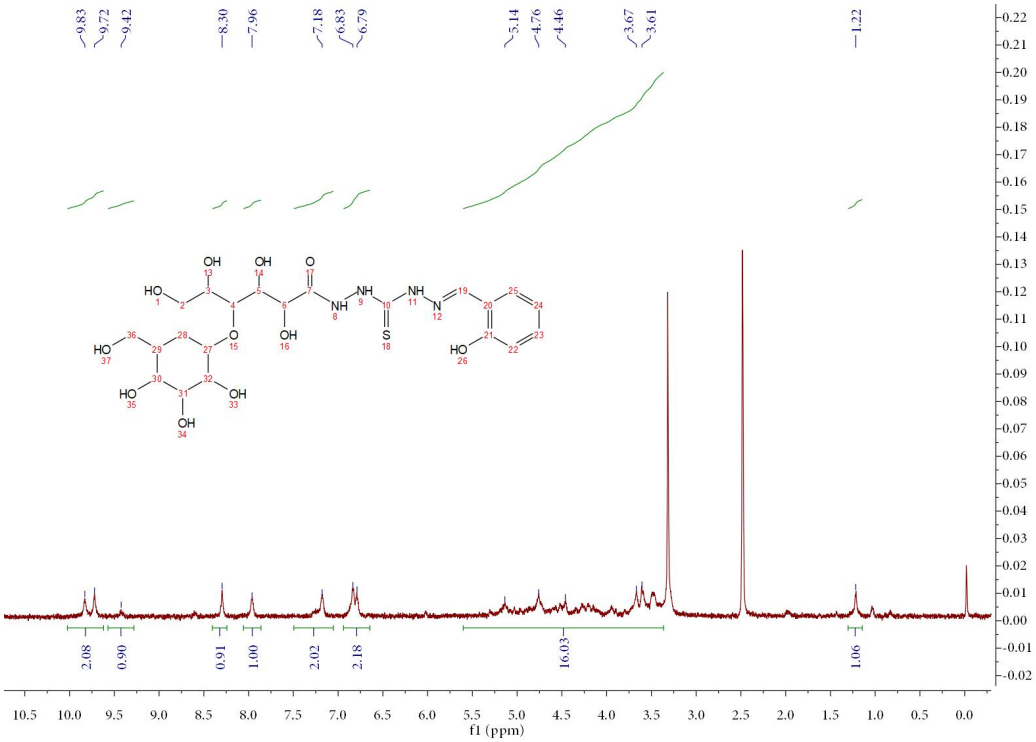


Figure S4. [1]H NMR spectrum (600 MHz) of **GT1** was recorded in DMSO-d_6.

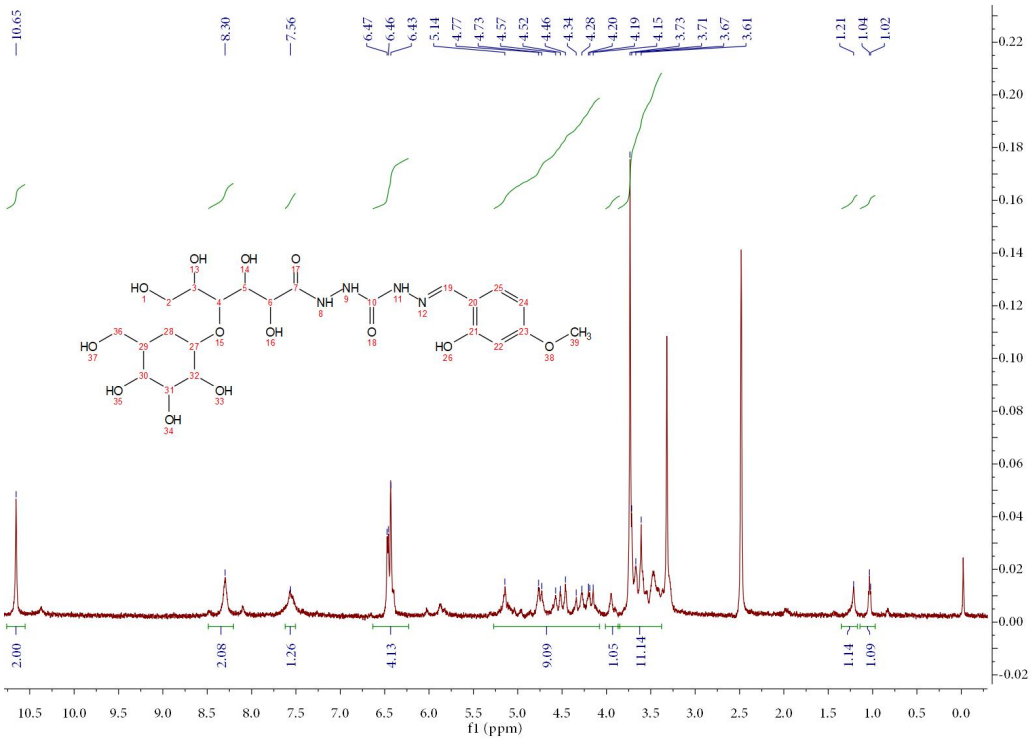


Figure S5. [1]H NMR spectrum (600 MHz) of **GT9** was recorded in DMSO-d_6.

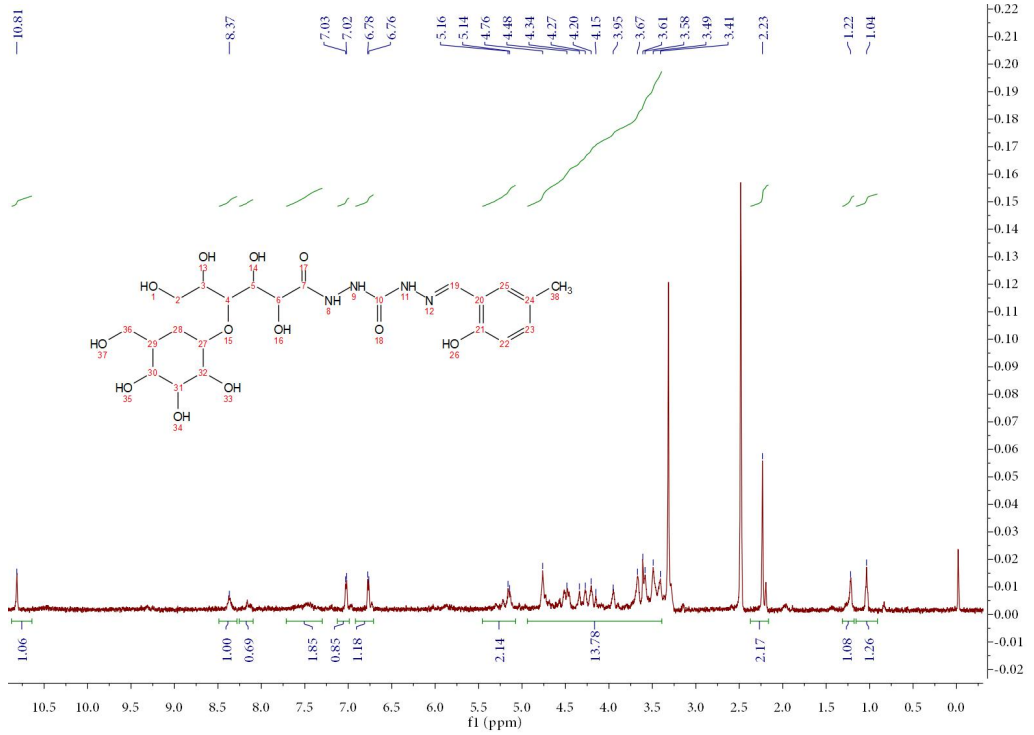


Figure S6. [1]H NMR spectrum (600 MHz) of **GT10** was recorded in DMSO-d_6.

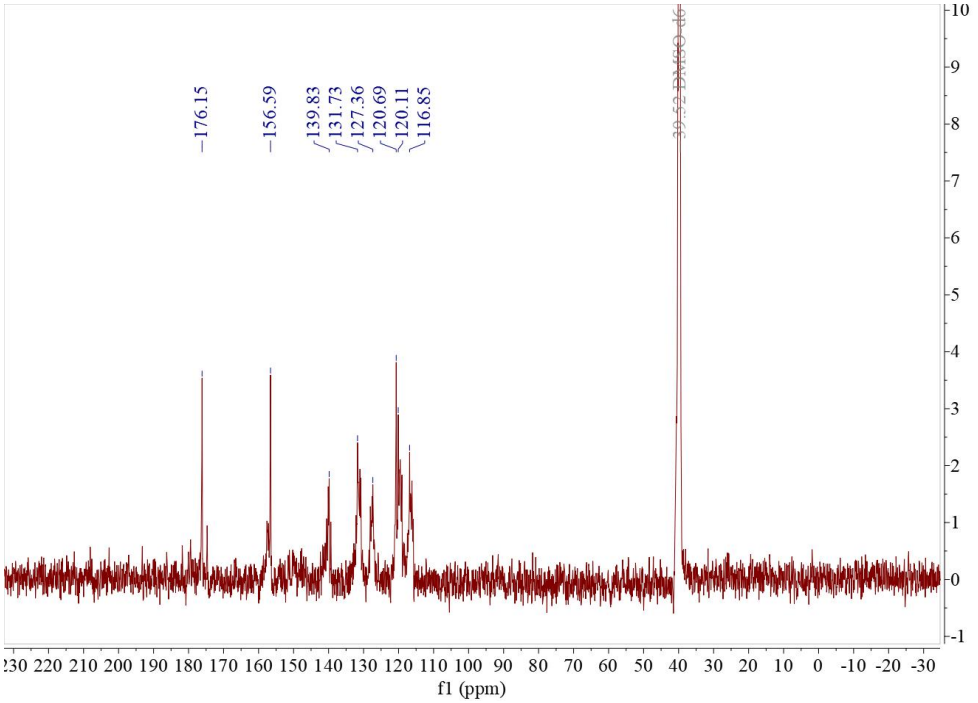


Figure S7. ^{13}C NMR spectrum (150 MHz) of **TH1** was recorded in DMSO-d_6.

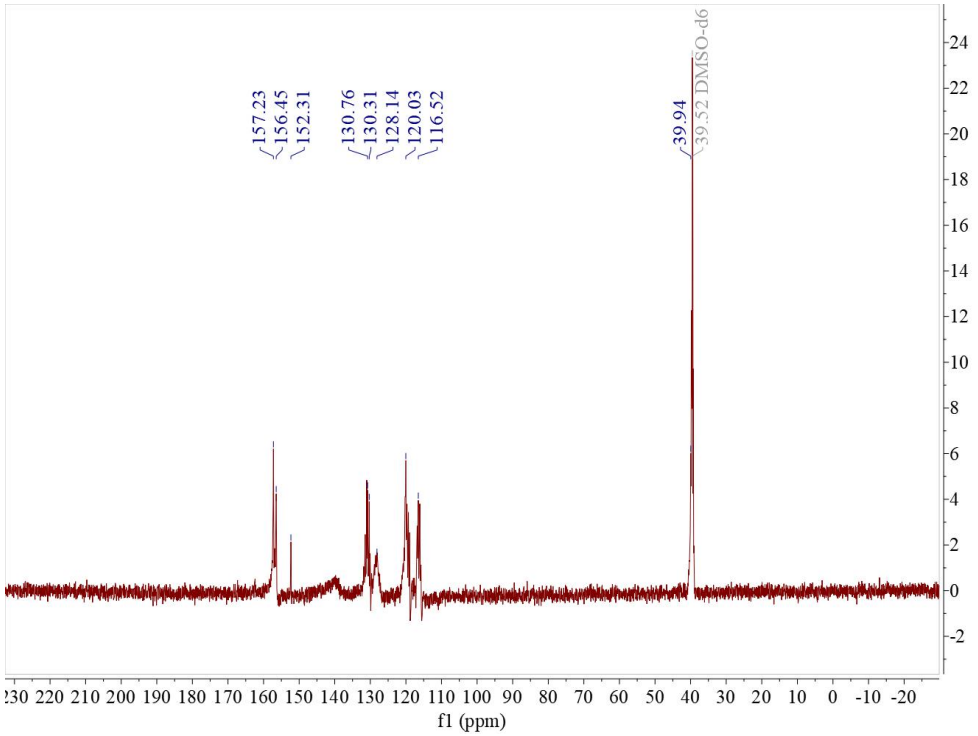


Figure S8. ^{13}C NMR spectrum (150 MHz) of **TH9** was recorded in DMSO-d_6.

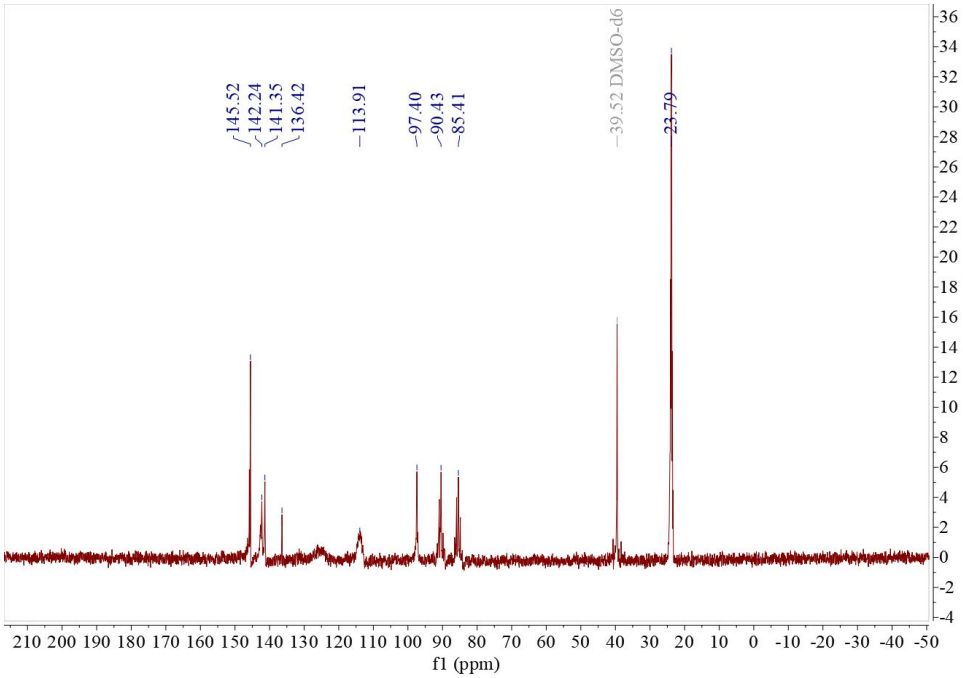


Figure S9. ^{13}C NMR spectrum (150 MHz) of **TH10** was recorded in DMSO-d_6.

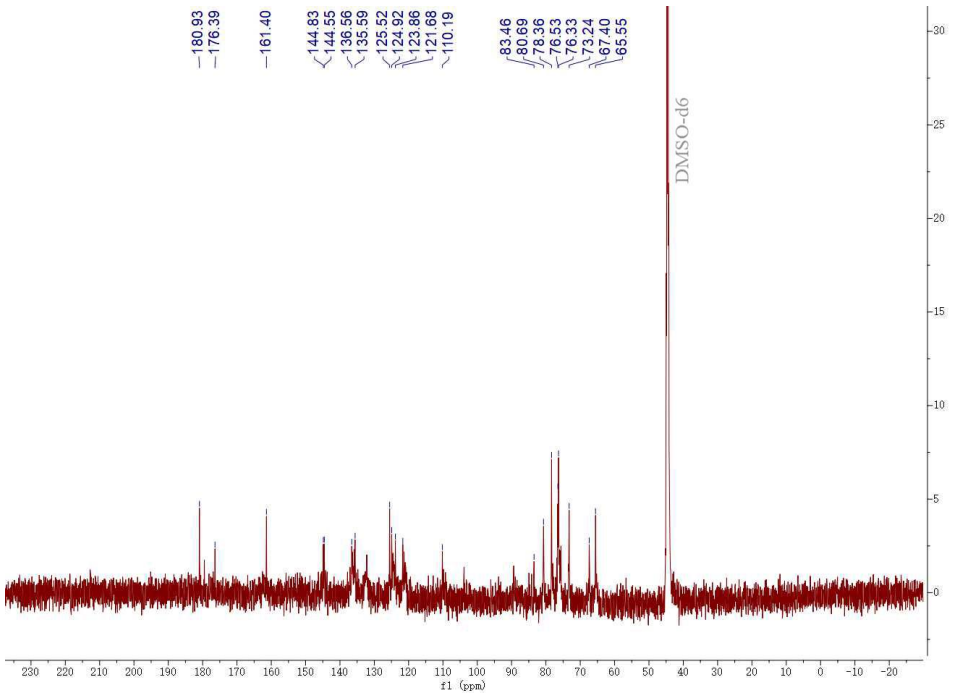


Figure S10. ^{13}C NMR spectrum (150 MHz) of **GT1** was recorded in DMSO-d_6.

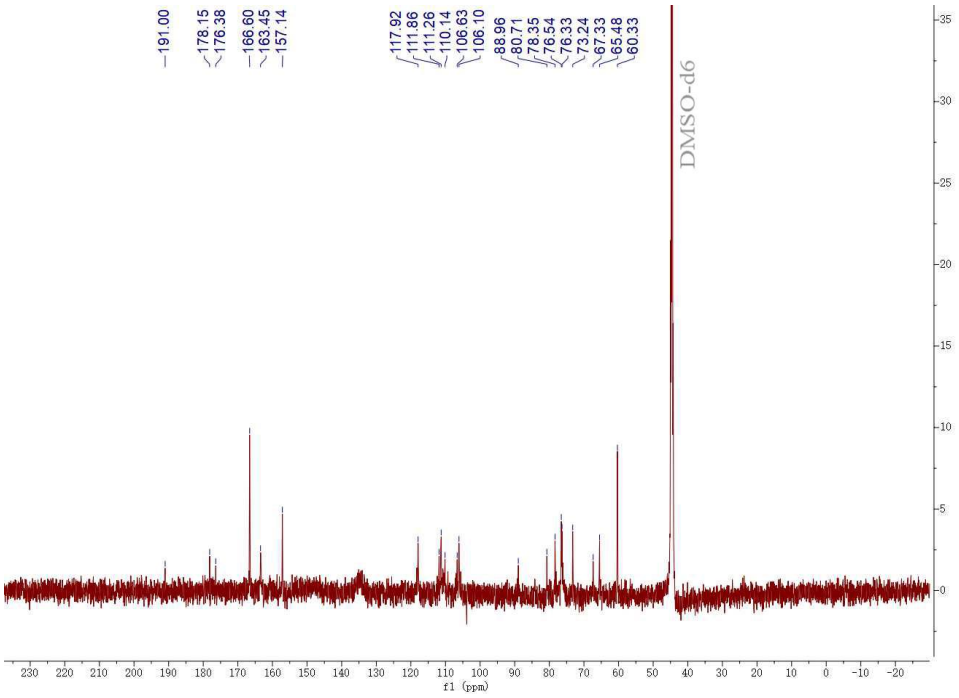


Figure S11. ^{13}C NMR spectrum (150 MHz) of **GT9** was recorded in DMSO-d_6.

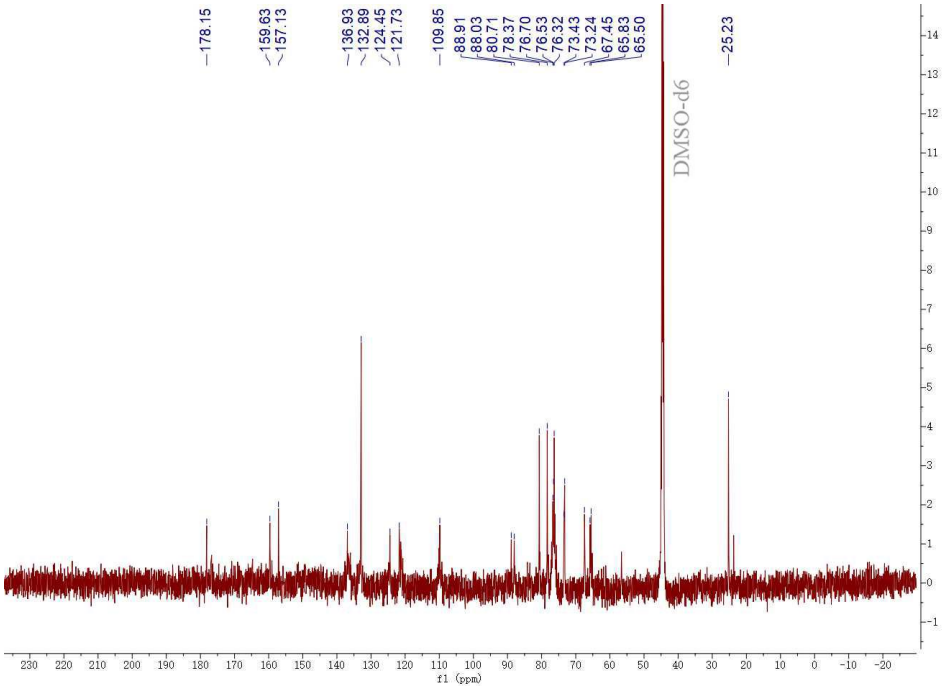


Figure S12. ^{13}C NMR spectrum (150 MHz) of **GT10** was recorded in DMSO-d_6.

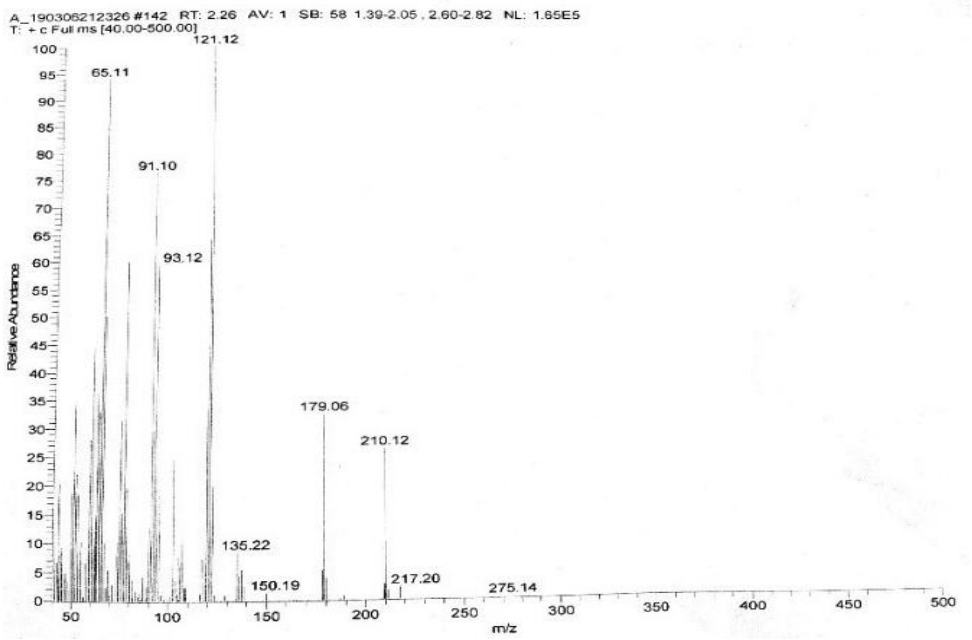


Figure S13. ESI-MS spectrum of **TH1**.

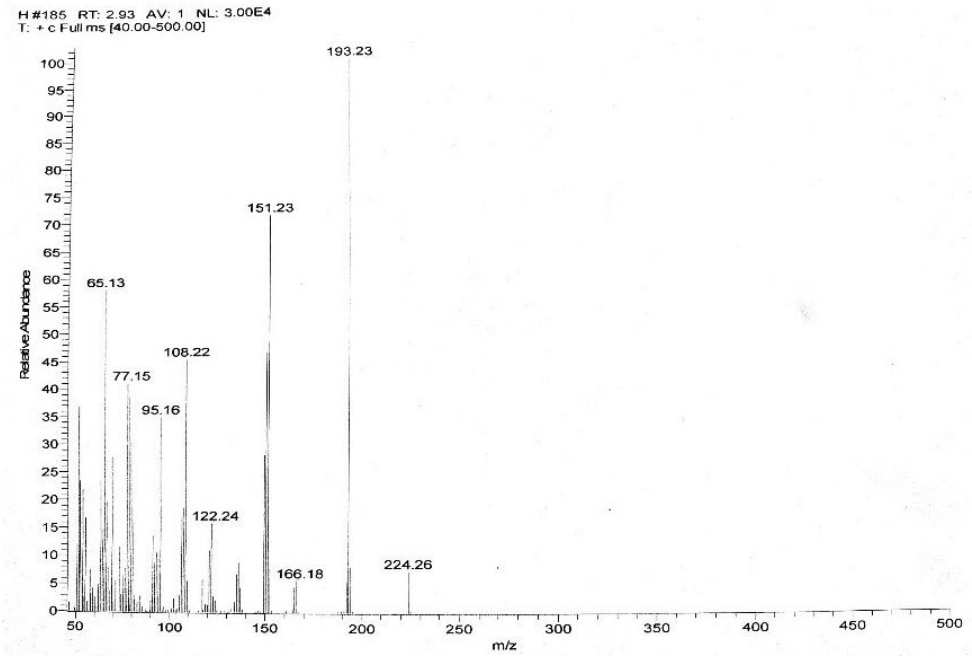


Figure S14. ESI-MS spectrum of **TH9**.

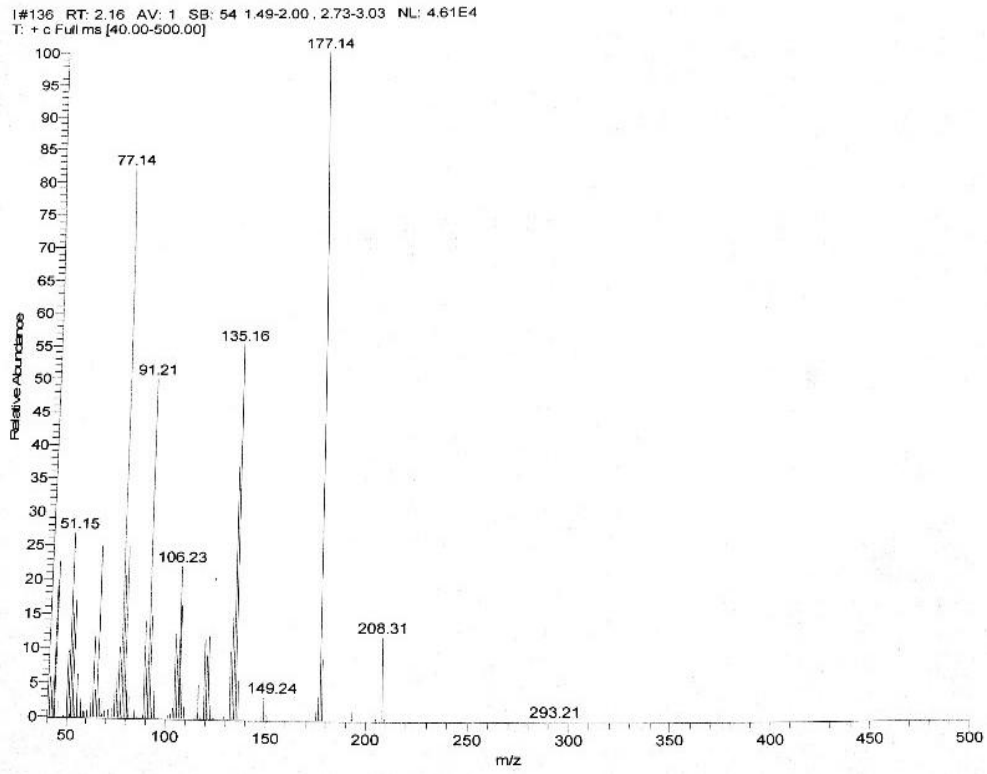


Figure S15. ESI-MS spectrum of **TH10**.

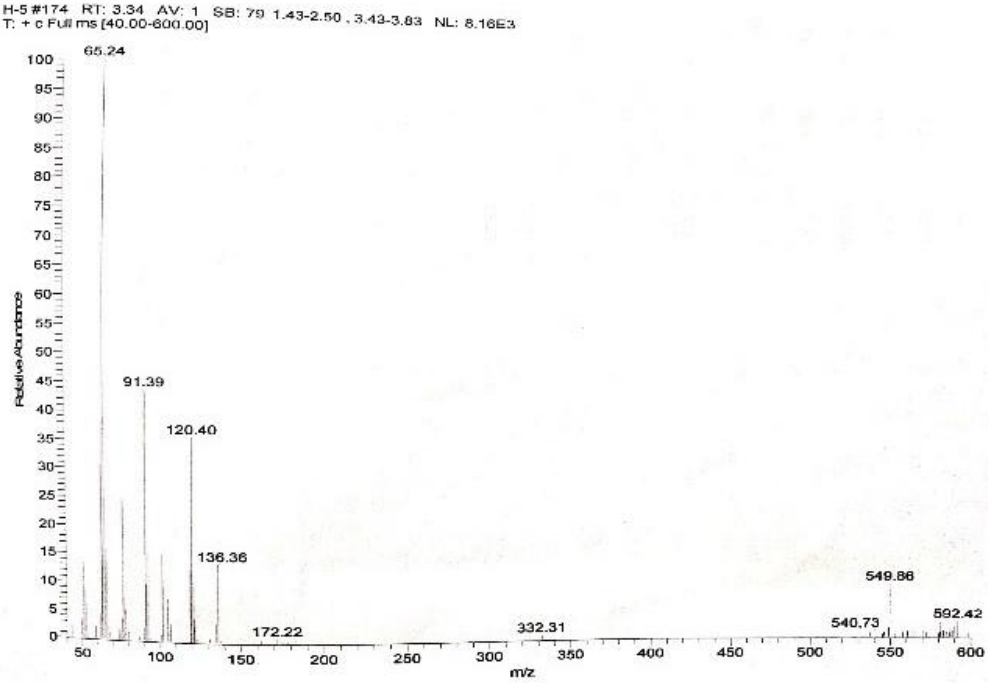


Figure S16. ESI-MS spectrum of **GT1**.

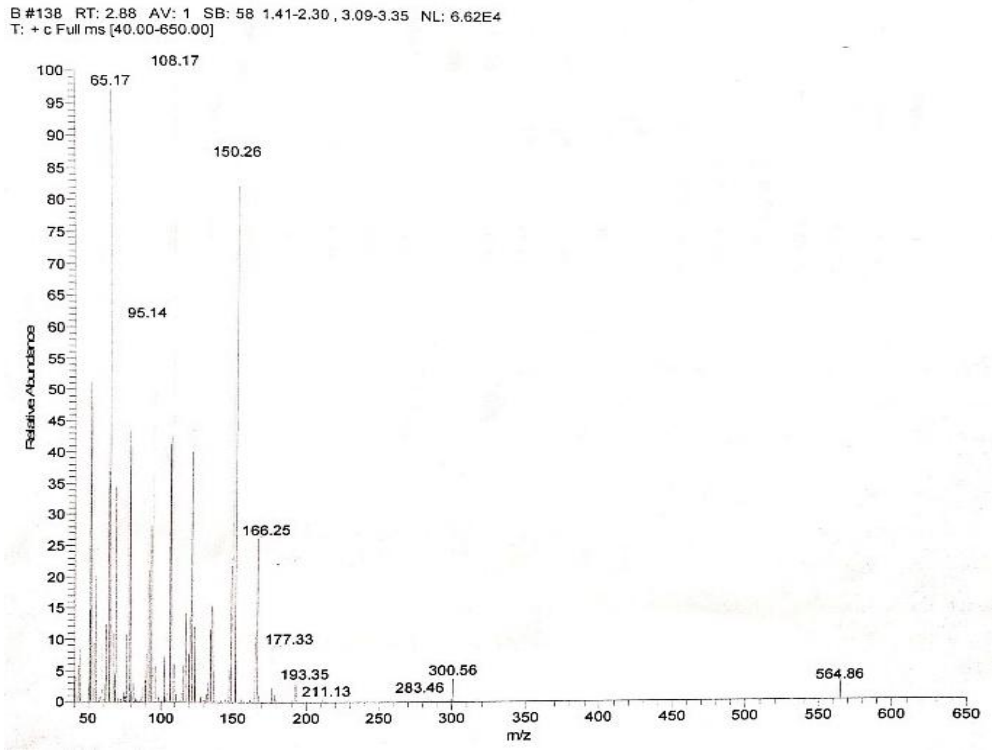


Figure S17. ESI-MS spectrum of **GT9**.

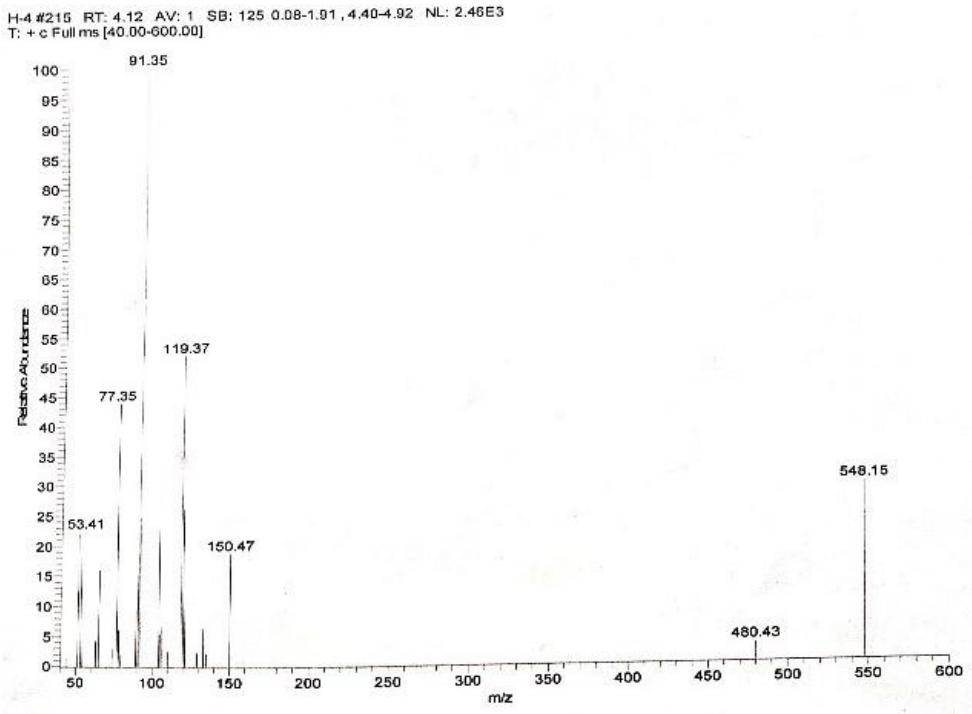
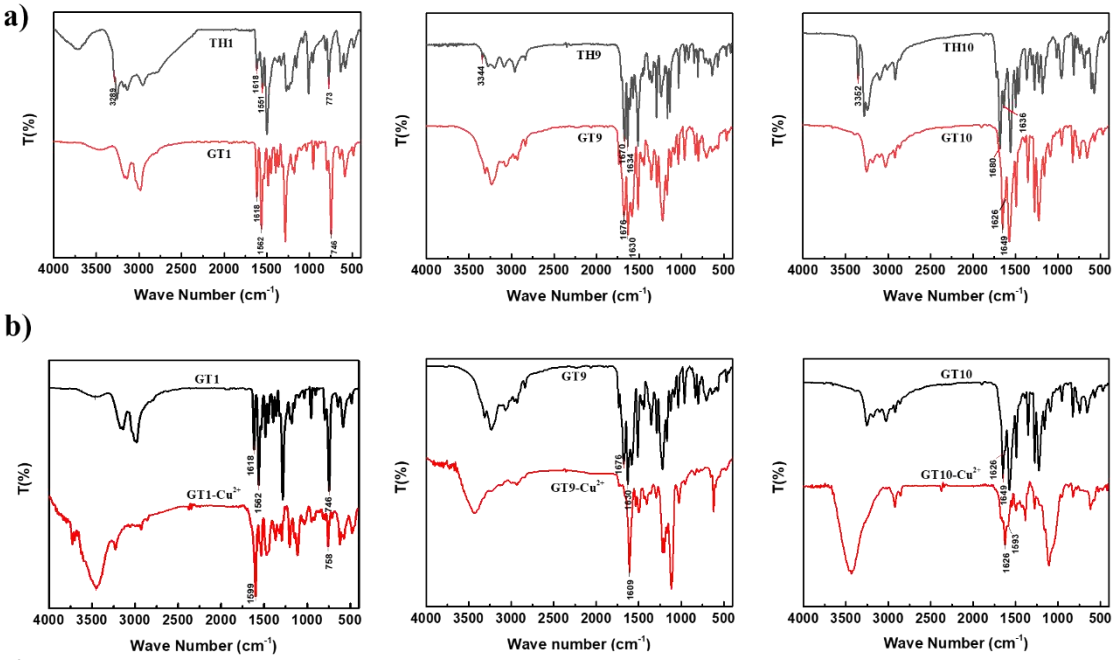


Figure S18. ESI-MS spectrum of **GT10**.



c)

	Key IR spectral bands (cm^{-1})					
Name	**vC=N free**	**vC=N coord.**	**vC=S**	**vC-S**	**vC=O**	**vNH_2**
TH1	1618	—	1551	773	—	3289
TH9	1634	—	—	—	1670	3344
TH10	1636	—	—	—	1680	3352
GT1	1618	—	1562	746	—	—
GT9	1630	—	—	—	1676	—
GT10	1626	—	—	—	1649	—
GT1+Cu^{2+}	—	1599	—	758	—	—
GT9+Cu^{2+}	—	1609	—	—	—	—
GT10+Cu^{2+}	1626	1593	—	—	—	—

Fig. S19 Fourier transform infrared spectrum comparison of the metal-coordinating moiety (**TH1**, **9** and **10**), respective lactobionic acid conjugated-metal chelator (**GT1**, **9** and **10**), and copper complexes. **a**) IR spectra comparison of **TH1**/**GT1**, **TH9**/**GT9**, and **TH10**/**GH10**. **b**) IR spectra comparison of **GT1**/**GT1+Cu^{2+}**, **GT9**/**GT9+Cu^{2+}**, and **GT10**/**GT10+Cu^{2+}**. **c**) Key IR spectral data chelators and copper complexes in (**a**) and (**b**).

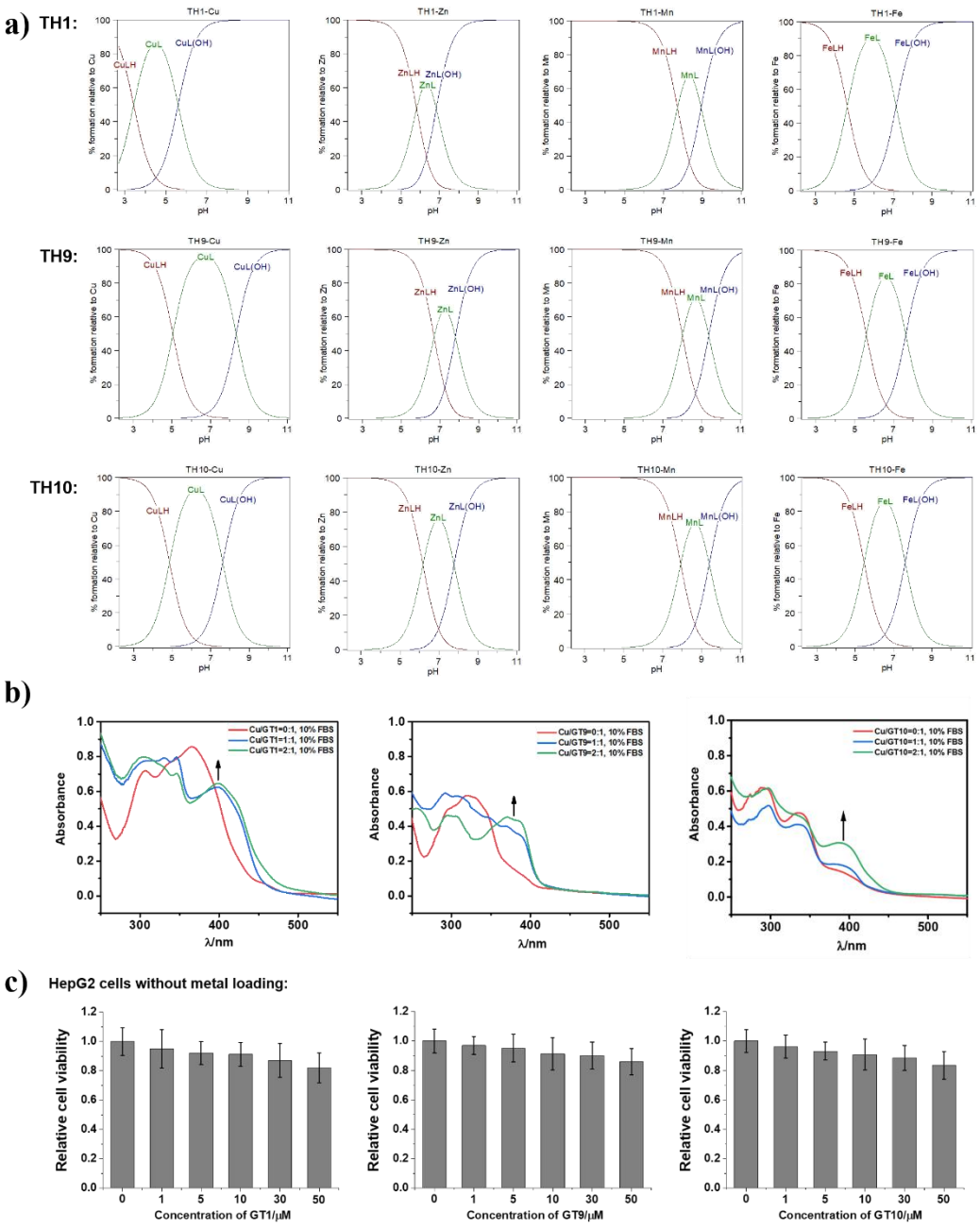


Fig. S20 a) Species distribution plots measured using potentiometric titrations for the Cu^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+}-chelator systems. The sources of Cu^{2+}, Zn^{2+}, Mn^{2+}, and Fe^{2+} are $CuCl_2$, $ZnCl_2$, $MnCl_2$ and $FeCl_2$, respectively. Potentiometric titrations were performed for the solutions containing the chelators and equimolar amounts of metal ions at 25 °C (I = 0.1 M KCl). **b**) Spectrophotometric titrations of chelators (10 mM) with Cu^{2+} (0, 10 and 20 mM) at 25 °C in 10% fetal bovine serum (FBS). **c**) HepG2 cells without metal loading were incubated with 0, 1, 5, 10, 30 and 50 μM chelators (**GT1**, **9** and **10**) for 24 h. Then, the cell viabilities were evaluated using commercial MTS kit. For chelator treatment, the same amount of DMSO (5‰) was added as a control group, because DMSO was used as a co-solvent. All experiments were normalized by respective control data. Data are mean of triplicate samples ± SD (unpaired Student's t test), and all error bars are SD.

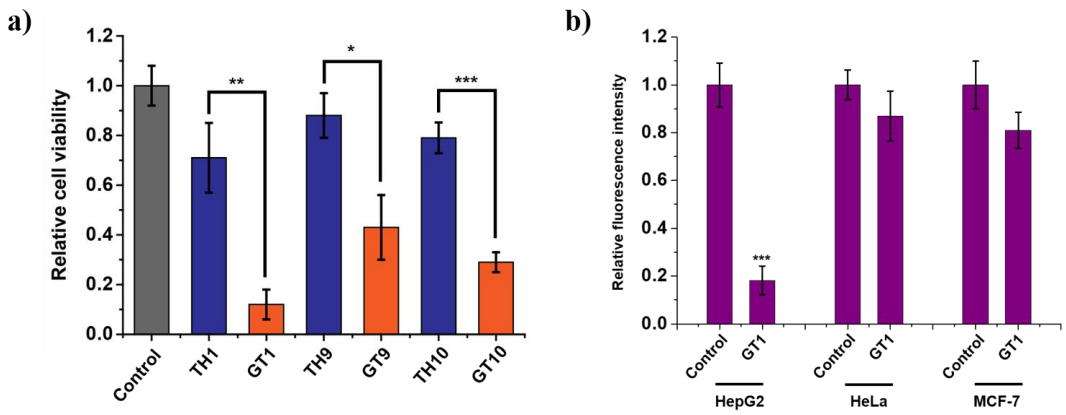


Fig. S21 a) After constructing the high-copper cell model, the **HC HepG2** cells were incubated with or without 50 μM chelators (**TH1**, **TH9**, **TH10**, **GT1**, **GT9** and **GT10**) for another 24 h. Then, the cell viability was evaluated using commercial MTS kit. **b**) After constructing the high-copper cell model, the HepG2, HeLa and MCF-7 cells were incubated with or without 50 μM **GT1** for another 24 h. Then, the cell viability was evaluated using commercial MTS kit. For chelator treatment, the same amount of DMSO (5‰) was added as a control group. All experiments were normalized by respective control data. Data are mean of triplicate samples ± SD (**P* < 0.05, ***P* < 0.01, ****P* < 0.001; unpaired Student's *t* test), and all error bars are SD.

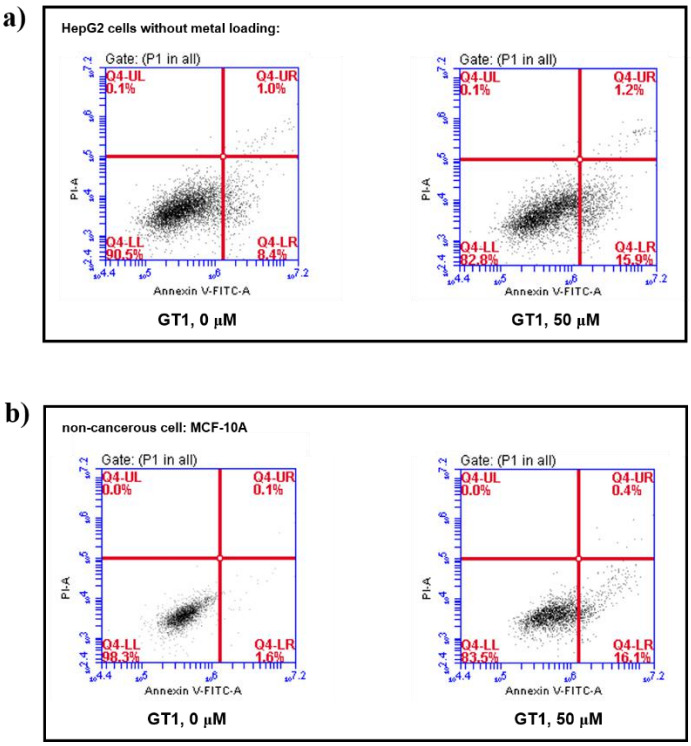


Fig. S22 HepG2 cells without metal loading (**a**) and MCF-10A cells (**b**) were incubated with 0 and 50 μM **GT1** for 24 h. For chelator treatment, the same amount of DMSO (5‰) was added as a control group, because DMSO was used as a co-solvent. Flow cytometric analysis of cell apoptosis was carried out with Annexin V and propidium iodide staining.