Supporting Information

Design, synthesis and biological evaluation of rhein derivatives as anticancer agents

Junkai Huang,[a] Zhuo Zhang,[a] Peng Huang,[a] Liqin He,*[a] Yong Ling*[b]

[a]*Anhui Key Laboratory of Traditional Chinese Medicine, Anhui University of Chinese Medicine, Hefei 230031, China*

[b]*School of Pharmacy, Nantong University, Nantong, 226001, PR China*

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[a] School of Pharmacy, Anhui University of Chinese Medicine, Hefei 230012, PR China
[b] School of Pharmacy, Nantong University, Nantong, 226001, PR China
* Corresponding authors. Tel.: +86 13956022936 (L. He)
E-mail addresses: hlq661125@126.com (L. He). Lyyy111@sina.com (Y. Ling).

Supporting Information

1. Materials and instruments

1. Chemical synthesis materials and instruments

Melting points were determined on a Mel-TEMP II melting point apparatus and uncorrected. Ultraviolet(UV) spectra were taken with a analytikjena SPECORD S600 diode array ultraviolet spectrophotometer. IR spectra were recorded on a Nicolet Avatar370DTGS infrared spectrometer(Therm Electron Corporation). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance spectrometer at 400 K, using $SiMe_4$ as internal standard. The following abbreviations indicate peak multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, br s = broad singlet. MS spectra were recorded on a Mariner Mass Spectrum (ESI). Column chromatography was performed on silica gel (Merck Kieselgel 200-300 mesh ASTM). The progress of the reactions was followed by thin-layer chromatography (TLC) on 5 ×20 cm plates Merck Kieselgel 60 F254, with a layer thickness of 0.20 mm. All chemicals and solvents were purchased from commercial sources and used without further purification in our laboratory. Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. The purity of obtained compounds was determined by HPLC techniques.

2. Synthesis

General procedure for the synthesis of 2a-d.

The solution of rhein **1** (284.0 mg, 1.0 mmol), tetrabutylammonium bromide(322.0 mg, 1.0 mmol), triethylamine (4.0 mmol) in THF (8 mL) was stirred at room temperature for 5 min, then dropwise added dibromoalkane (4.0 mmol). The reaction was stirred at rt until completed (TLC control). Subsequently, the mixture was filtered and poured into H_2O (80 mL), and the resulting products were extracted with CH_2Cl_2 (30mL×3). The collected CH_2Cl_2 layers were washed with saturated NaCl solution, dried over sodium sulfate, and concentrated in vacuo to obtain crude product, which was purified by column chromatography (eluent: PE/EtOAc = 2 : 1, v/v) to give a yellow powder(**2**).

3-bromopropyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (2a)

Yellow powder(354.2mg, 87.7%), mp: 147.2-148.0 °C. ESI-MS (m/z): 404.3[M+H]□; ^{1}H-NMR(400 MHz, $CDCl_3$) δ:11.86 (s, 1H), 11.81 (s, 1H), 8.20 (m, 1H), 7.71 (m, 3H), 7.26 (m, 1H), 4.50 (m, 2H), 3.57 (m, 2H), 2.35 (m, 2H); IR (KBr, cm^{-1}) υ:3061, 2920, 1719, 1630, 1474, 1453, 1387;

4-bromobutyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (2b)

Yellow powder(372.8mg, 89.2%), mp: 134.5-135.4 °C. ESI-MS (m/z): 419.2[M+H]□; ^{1}H-NMR(400 MHz, $CDCl_3$) δ:12.00 (s, 1H), 11.93 (s, 1H), 8.36 (s, 1H), 7.89 (m, 2H), 7.74 (d, *J* = 7.3 Hz, 1H), 7.32 (m, 1H), 4.44 (t, *J* = 10.4Hz, 2H), 3.53 (t, *J* = 10.4, 2H), 2.06 (m, 4H); IR (KBr, cm^{-1}) υ: 3071, 2956, 1727, 1630, 1470, 1454, 1374;

5-bromopentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (2c)

Yellow powder(387.9mg, 89.8%), mp: 130.2-131.6 °C. ESI-MS (m/z): 431.3[M-H]$^{-}$; ^{1}H-NMR(400 MHz, $CDCl_3$) δ:11.95 (s, 1H), 11.90 (s, 1H), 8.33 (s, 1H), 7.94-7.80 (m, 2H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J*=8.5 Hz, 1H), 4.40 (t, *J* = 6.3 Hz, 2H), 3.48 (t, *J* = 6.4 Hz, 2H), 2.03-1.93 (m, 2H), 1.92-1.82 (m, 2H), 1.72-1.60 (m, 2H); IR (KBr, cm^{-1}) υ: 3077, 2936, 1723, 1629, 1471, 1454, 1378;

6-bromohexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (2d)

Yellow powder(387.9mg, 91.6%), mp: 131.4-132.6 °C. ESI-MS (m/z): 445.3[M-H]$^{-}$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.98 (s, 1H), 11.92 (s, 1H), 8.35 (s, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.71 (t, *J* = 7.8Hz, 1H), 7.34 - 7.27 (m, 1H), 4.40 (t, *J* = 6.2Hz, 2H), 3.44 (t ,*J* = 10.8Hz, 2H), 2.00 - 1.74 (m, 8H); IR (KBr, cm^{-1}) υ: 3421, 2947, 1721, 1672, 1630, 1453, 1428, 1399, 1376;

General procedure for the synthesis of 4a-x.

A mixture of 2 (0.5 mmol), K_2CO_3 (276.0 mg, 2.0 mmol), KI (83.0 mg, 0.5 mmol) and amine (2.0 mmol) in

acetonitrile (10 mL) was stirred at 40~50℃ until completed (TLC control). The reaction mixture was cooled to room temperature and poured into H_2O (50 mL), neutralized with dilute hydrochloric acid (pH 7), the resulting products were extracted with CH_2Cl_2 (30mL×3). The collected CH_2Cl_2 layers were washed with saturated NaCl solution, dried over sodium sulfate, and concentrated in vacuo to obtain oil-like materials, which was subsequently purified by column chromatography (eluate: DCM/MeOH= 10:1, v/v) to give reddish brown wax (**3**). Then **3** was dissolved in dichloromethane, dropwise added appropriate saturated solution of hydrogen chloride in isopropanol at 0℃, kept stirring for 12h at 0℃, put it in refrigerator overnight. Subsequently, the mixture was filtered, dry in a vacuum oven to get yellow powder (**4**).

3-(diethylamino)propyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4a)

Yellow powder(147.9 mg, 68.2%), Mp: 250°C dec. without melting.^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.89 - 7.79 (m, 2H), 7.67 (t, J = 7.9 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 4.40 (t, J = 12.4 Hz, 2H), 2.75 (m, 6H), 2.07 (m, 2H), 1.13 (t, J = 14.0 Hz, 6H); IR (KBr, cm^{-1}) υ: 3421.8, 2925.2, 2675.1, 1717.0, 1673.6, 1638.5, 1451.1, 1424.1, 1267.9; ESI-HRMS (m/z): 398.1615$[M+H]^+$;

3-(piperidin-1-yl)propyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4b)

Yellow powder(133.8mg, 60.1%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.79 (s, 1H), 7.74 (d, J = 7.4 Hz, 1H), 7.63 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 8.9 Hz, 1H), 4.32 (t, J = 12.8 Hz, 2H), 2.82 (m, 6H), 1.78 (m, 2H), 1.66 (m, 2H), 1.27 (m, 4H); IR (KBr, cm^{-1}) υ: 3436.3, 3180.1, 2959.0, 2931.0, 2603.5, 2475.9, 2404.3, 1730.1, 1679.7, 1632.1, 1452.4, 1415.2; ESI-HRMS (m/z): 410.1617$[M+H]^+$;

3-morpholinopropyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4c)

Yellow powder(131.3 mg, 58.7%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 12.05 (s, 1H), 11.98 (s, 1H), 8.41 (d, J = 1.1 Hz, 1H), 7.92 (m, 2H), 7.76 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 4.50 (t, J = 12.8Hz, 2H), 3.86 (s, 4H), 2.71 (s, 6H), 2.18 (m, 2H); IR (KBr, cm^{-1}) υ: 3436.1, 2947.0, 2878.2, 2418.1, 1729.3, 1629.9, 1453.6; ESI-HRMS (m/z): 412.1412$[M+H]^+$;

3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4d)

Yellow powder(153.0 mg, 58.1%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.88 (m, 2H), 7.72 (m, 1H), 7.32 (d, J = 8.0 Hz, 1H), 4.46 (t, J=5.2Hz, 2H), 3.65 (t, J=2.8Hz, 2H), 2.59 (m, 12H), 2.02 (m, 2H); IR (KBr, cm^{-1}) υ: 3336.3, 2984.7, 2635.5, 2549.1, 2429.6, 1724.9, 1629.9, 1475.3, 1455.1; ESI-HRMS (m/z): 455.1832$[M+H]^+$;

3-(4-ethylpiperazin-1-yl)propyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4e)

Yellow powder(149.1 mg, 58.3%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 11.99 (s, 1H), 11.92(s, 1H), 8.16 (s, 1H), 7.88 (m, 2H), 7.77 (m, 1H), 7.46 (d, J=7.2Hz, 1H), 4.45 (t, J=12.4Hz, 2H), 2.52 (s, 10H), 2.24 (s, 2H), 1.28 (m, 5H); IR (KBr, cm^{-1}) υ: 3431.0, 3183.2, 2923.8, 2853.0, 2651.6, 2561.9, 2441.9, 1725.9, 1674.7, 1633.1, 1469.7, 1450.5; ESI-HRMS (m/z): 439.1881$[M+H]^+$;

3-(4-methylpiperazin-1-yl)propyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4f)

Yellow powder(146.4 mg, 58.9%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 8.36 (s, 1H), 7.93 - 7.80 (m, 2H), 7.72 (t, J = 7.5 Hz, 1H), 7.31 (m, 1H), 4.45 (t, J=11.6Hz, 2H), 2.77 - 2.55 (m, 10H), 2.41 (s, 3H), 2.03 (m, 2H); IR (KBr, cm^{-1}) υ: 3428.7, 3183.2, 2558.9, 2442.1, 1728, 1631.1, 1469.7, 1448; ESI-HRMS (m/z): 425.1726$[M+H]^+$;

4-(diethylamino)butyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4g)

Yellow powder(146.8 mg, 65.6 %), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 7.94 (d, J =

9.6 Hz, 1H), 7.75 (m, 1H), 7.70 - 7.50 (m, 2H), 7.40 - 7.29 (m, 1H), 4.37 (s, 2H), 3.19 (m, 6H), 1.87 (m, 4H), 1.24 (m, 6H); IR (KBr, cm^{-1}) υ: 3422.8, 2926.3, 2670.1, 1719.0, 1671.6, 1632.5, 1450.1, 1424.1; ESI-HRMS (m/z): 412.1753$[M+H]^+$;

4-(piperidin-1-yl)butyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4h)

Yellow powder(135.8 mg, 59.1%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 11.96(s,1H), 11.92(s, 1H), 8.14 (s, 1H), 7.92 - 7.82 (m, 2H), 7.75 (d, J = 7.5 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 4.39 (t, J = 10.4 Hz, 2H), 3.46 (m, 6H), 1.93 - 1.67 (m, 10H); IR (KBr, cm^{-1}) υ: 3417.2, 2923.2, 2850.7, 1722.2, 1629.4, 1475.6, 1450.6; ESI-HRMS (m/z): 424.1752 $[M+H]^+$;

4-morpholinobutyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4i)

Yellow powder(136.6 mg, 59.2%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.75 (s, 1H), 7.54 (m, 2H), 7.29 (m, 1H), 4.31 (s, 2H), 3.61 (s, 4H), 2.48 (m, 6H), 1.70 (m, 4H); IR (KBr, cm^{-1}) υ: 3433.1, 2943.0, 2876.2, 2412.1, 1727.3, 1626.9, 1451.6, 1296.6; ESI-HRMS (m/z): 426.1645$[M+H]^+$;

4-(4-(2-hydroxyethyl)piperazin-1-yl)butyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4j)

Yellow powder(155.7 mg, 57.6%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 11.91 (s, 2H), 8.10 (s, 1H), 7.76 (m, 3H), 7.43 (s, 1H), 4.35 (s, 2H), 3.80 (s, 2H), 2.52 (s, 12H), 1.44 (s, 4H); IR (KBr, cm^{-1}) υ: 3410.7, 2935.1, 2645.4, 2565.8, 1719.6, 1629.6, 1609.1, 1452.4, 1378.7; ESI-HRMS (m/z): 469.1914 $[M+H]^+$;

4-(4-ethylpiperazin-1-yl)butyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4k)

Yellow powder(152.1 mg, 57.9%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 8.10 (d, J = 9.9 Hz, 1H), 7.88 - 7.69 (m, 3H), 7.42 (d, J = 7.1 Hz, 1H), 4.38 (s, 2H), 3.61 - 2.93 (m, 12H), 1.82 (m, 2H), 1.73 (m, 2H), 1.23 (s, 3H); IR (KBr, cm^{-1}) υ: 3420.9, 2975.5, 2638.6, 2557.8, 1718.8, 1680.8, 1632.9, 1451.3; ESI-HRMS (m/z): 453.1964 $[M+H]^+$;

4-(4-methylpiperazin-1-yl)butyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4l)

Yellow powder(145.1 mg, 56.8%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.61 (s, 2H), 7.34 (d, J = 8.1 Hz, 1H), 4.34 (t, J=8Hz, 2H), 2.80 - 2.39 (m, 15H), 1.94 (m, 2H); IR (KBr, cm^{-1}) υ: 3421.5, 2934.9, 2798.1, 1719.2, 1673.4, 1629.7, 1452.0, 1418.3, 1376.6; ESI-HRMS (m/z): m/z 439.1964$[M+H]^+$;

5-(diethylamino)pentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4m)

Yellow powder(152.1 mg, 65.9%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.90 – 7.76 (m, 2H), 7.70 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 4.1 Hz, 1H), 4.39 (s, 2H), 3.20 (t, J = 12.0 Hz, 4H), 3.08 (t, J = 16.0 Hz, 2H), 1.44 (t, J = 6.9 Hz, 6H), 1.24 (s, 6H); IR (KBr, cm^{-1}) υ: 3421.3, 2922.4, 2650.8, 1723.2, 1630.6, 1476.1, 1451.3, 1268.8; ESI-HRMS (m/z): 426.1900$[M+H]^+$;

5-(piperidin-1-yl)pentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4n)

Yellow powder(152.1 mg, 64.3%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 11.96(s, 1H), 8.39 (s, 1H), 7.96 – 7.85 (m, 2H), 7.75 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 4.42 (t, J=12.0Hz, 2H), 2.66 (m, 6H), 2.30 (m, 2H), 1.91 (s, 6H), 1.27 (s, 4H); IR (KBr, cm^{-1}) υ: 3422.5, 2958.0, 1717.8, 1631.6, 1473.8, 1451.4, 1378.2; ESI-HRMS (m/z): 438.1856 $[M+H]^+$;

5-morpholinopentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4o)

Yellow powder(139.8 mg,58.8%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.87 (s, 1H), 7.82 (d, J = 7.5 Hz, 1H), 7.70 (t, J = 7.9 Hz, 1H), 7.30 (d, J = 8.3 Hz, 1H), 4.39 (t, J = 6.6 Hz, 2H), 3.73 (t, J=8.0Hz, 4H), 2.43 (m, 6H), 1.91 - 1.79 (m, 2H), 1.62 (m, 2H), 1.50 (m, 2H); IR (KBr, cm^{-1}) υ: 3421.7, 2963.8, 2443.6, 1724.2, 1670.4, 1636.5, 1452.6, 1268.6; ESI-HRMS (m/z): 440.1648 $[M+H]^+$;

5-(4-(2-hydroxyethyl)piperazin-1-yl)pentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4p)

Yellow powder(163.6 mg, 58.9%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.89 - 7.75 (m, 2H), 7.68 (t, J = 7.9 Hz, 1H), 7.27 (d, J = 9.2 Hz, 1H), 4.36 (t, J = 6.2 Hz, 2H), 3.63 (t, J = 4.7 Hz, 2H), 2.77 - 2.32 (m, 12H), 1.82 (m, 2H), 1.61 (m, 2H), 1.48 (m, 2H); IR (KBr, cm^{-1}) υ: 3393.1, 2948.2, 1721.6, 1630.9, 1452.1, 1276.6; ESI-HRMS (m/z): 483.2070 $[M+H]^+$;

5-(4-ethylpiperazin-1-yl)pentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4q)

Yellow powder(153.5 mg, 57.0%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.90 - 7.59 (m, 3H), 7.29 (s, 1H), 4.37 (s, 2H), 2.97 - 2.45 (m, 12H), 1.83 (s, 2H), 1.56 (m, 4H), 1.20 (s, 3H); IR (KBr, cm^{-1}) υ: 3420.6, 2974.6, 2441.7, 1721.6, 1673.1, 1626.5, 1452.7, 1380.3; ESI-HRMS (m/z): 467.2161$[M+H]^+$;

5-(4-methylpiperazin-1-yl)pentyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4r)

Yellow powder(158.4 mg, 60.3%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 11.95 (s, 1H), 11.91(s, 1H), 8.12 (d, J = 1.5 Hz, 1H), 7.88 - 7.80 (m, 2H), 7.75 (d, J = 6.6 Hz, 1H), 7.44 (d, J = 7.4 Hz, 1H), 4.38 (t, J = 6.4 Hz, 2H), 3.55 (m, 10H), 3.03 (m, 3H), 1.69 (m, 2H), 1.45 (m, 4H); IR (KBr, cm^{-1}) υ: 3433.1, 2950.6, 2641.9, 2545.9, 1730.2, 1673.7, 1625.3, 1451.3; ESI-HRMS (m/z): 453.2007$[M+H]^+$;

6-(diethylamino)hexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4s)

Yellow powder(160.7 mg, 67.6%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 7.90 (s, 1H), 7.78 (s, 1H), 7.60 (s, 2H), 7.34 (d, J = 4 Hz, 1H), 4.31 (s, 2H), 3.16 - 3.07 (m, 6H), 1.74 (m, 4H), 1.46 (s, 4H), 1.23 (s, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 191.33, 180.90, 164.09, 161.80, 161.40, 138.14, 136.99, 133.90, 133.22, 125.17, 124.35, 119.78, 119.04, 118.62 , 116.21, 66.08, 51.17, 46.74, 28.31, 26.16, 25.47, 23.49, 9.05; IR (KBr, cm^{-1}) υ: 3426.3, 2937.1, 2668.8, 1720.2, 1672.3, 1632.0, 1569.3, 1473.3, 1451.3, 1408.2; ESI-HRMS (m/z): 440.2052$[M+H]^+$; HPLC purity: 99.31%, t_R-20.64 min.

6-(piperidin-1-yl)hexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4t)

Yellow powder(136.4 mg, 56.0%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.78 - 7.67 (m, 2H), 7.62 (t, J = 7.8 Hz, 1H), 7.22 (t, J = 9.3 Hz, 1H), 4.28 (t, J = 6.3 Hz, 2H), 2.93 (m, 6H), 1.88 (m, 2H), 1.75 (m, 2H), 1.42 (m, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 192.54, 180.70, 164.20, 162.70, 162.27, 137.92, 137.71, 133.72, 133.28, 125.16, 124.86, 120.23, 119.96, 118.07 , 115.66, 65.79, 57.33, 53.15, 28.26, 26.53, 25.47, 23.55, 22.71, 22.21; IR (KBr, cm^{-1}) υ: 3421.1, 2920.6, 2843.7, 1722.4, 1626.3, 1472.6, 1456.9; ESI-HRMS (m/z): 452.2052$[M+H]^+$; HPLC purity: 99.31%, t_R-20.64 min. HPLC purity: 98.18%, t_R-20.69 min.

6-morpholinohexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4u)

Yellow powder(147.4 mg, 60.2%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.91 (m, 2H), 7.74 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 4.40 (t, J = 6.5 Hz, 2H), 3.80 (s, 4H), 2.52 (m, 6H), 1.90 - 1.80 (m, 2H), 1.51 (m, 6H); IR (KBr, cm^{-1}) υ: 3433.1, 2935.9, 2866.1, 1727.1, 1626.3, 1473.8, 1455.6; ESI-HRMS (m/z): 454.1844$[M+H]^+$;

6-(4-(2-hydroxyethyl)piperazin-1-yl)hexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4v)

Yellow powder(165.6 mg, 58.2%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.87 (m, 2H), 7.72 (t, J = 7.9 Hz, 1H), 7.31 (m, 1H), 4.38 (t, J = 6.4 Hz, 2H), 3.71 (m, 2H), 2.91 - 2.56 (m, 12H), 1.82 (m, 2H), 1.62 (m, 2H), 1.49 (m, 2H), 1.43 (m, 2H); 13C-NMR (101 MHz, $CDCl_3$) δ 192.73, 180.89, 164.36, 162.78, 162.36, 138.14, 137.73, 133.86, 133.45, 125.24, 124.89, 120.33, 120.14, 118.17, 115.80, 66.02, 59.43, 58.02, 57.55, 52.28, 52.24, 29.66, 28.47, 26.97, 26.01; IR (KBr, cm-1) υ: 3442.7, 3314.7, 2922.7, 2575.2, 1720.8,

1672.8, 1631.2, 1457.8, 1377.0; ESI-HRMS (m/z): 497.2267[M+H]+; HPLC purity: 98.68%, t_R-13.97 min.

6-(4-ethylpiperazin-1-yl)hexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4w)

Yellow powder(167.1 mg, 60.4%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, DMSO) δ 8.34 (s, 1H), 7.91 - 7.78 (m, 2H), 7.70 (t, J = 7.9 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H), 4.37 (t, J = 12.0Hz, 2H), 2.69 - 2.38 (m, 12H), 1.82 (m, 2H), 1.59 - 1.39 (m, 6H), 1.12 (t, J = 7.0 Hz, 3H); IR (KBr, cm^{-1}) υ: 3426.3, 3080.7, 2936.4, 2810.0, 1718.9, 1674.1, 1629.2; ESI-HRMS (m/z): 481.2316[M+H]$^+$;

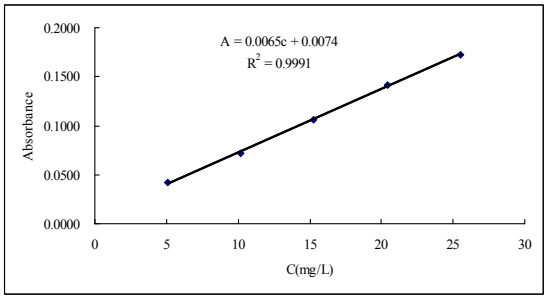
6-(4-methylpiperazin-1-yl)hexyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate hydrochloride (4x)

Yellow powder(157.7 mg, 58.5%), Mp: 250°C dec. without melting. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.90 - 7.76 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 7.29 (d, J = 6.5 Hz, 1H), 4.36 (s, 2H), 3.24 - 2.18 (m, 13H), 1.80 (m, 2H), 1.64 (m, 2H), 1.54 - 1.37 (m, 4H); IR (KBr, cm^{-1}) υ: 3423.1, 2929.4, 2852.8, 2766.7, 2359.6, 1721.0, 1676.5, 1626.7, 1471.7, 1416.1, 1268.1; ESI-HRMS (m/z): 467.2163[M+H]$^+$;

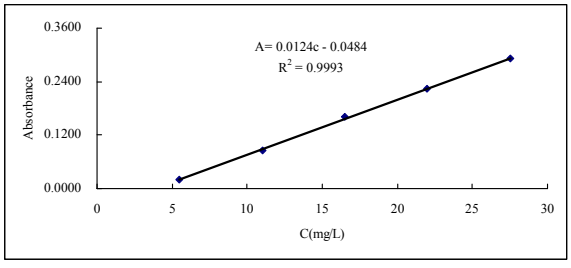
3. Water solubility Assay

As **4v** for example, compound **4v**(13.8mg, 0.02mmol) was dissolved into 100mL standard flasks to get the stock solution. The calibration curve was obtained with **4v** stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, and 6 mL stock solution into separate 10mL standard flasks and diluting to volume with distilled water. The absorbance value of the yellow solution was measured at 440 nm against colorless reagent blanks. Then the regression equation was get: A = 0.0141c - 0.0149, the value of regression coefficient was 0.9991. Saturated aqueous solutions were prepared by adding an excess quantity of compounds **4v** to doubly distilled water in 25-mL flasks with glass stoppers. In general, a saturated solution of the compounds in water was prepared and allowed to reach equilibrium while stirring at 25℃ for at least 48 h in the dark. The saturated aqueous solutions were decanted and filtered through a 5-p Millipore filter to remove suspended particles. The absorbance was measured at 440 nm using a UV spectrophotometer, and the concentration was calculated by reference to a predetermined standard curve. Other compounds were operated according to the method of **4v**.

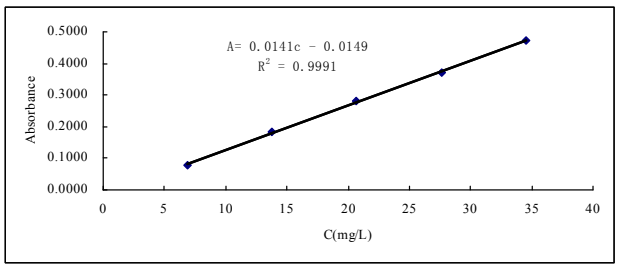
The standard curves and regression equations of 4s



The standard curves and regression equations of 4t



The standard curves and regression equations of 4v



4. Cytotoxicity assay *in vitro*

Cell proliferation was measured with MTT assay. Briefly, human hepatocellular carcinoma cells (HepG2), human colon tumor cells (HCT116), human lung tumor cells (A549), human breast tumor cells (MCF-7), human hepatocellular cancer Bel-7402 and multi-drug resistance of humanepatoma Bel-7402/5-FU cells were cultured in 96-well plates at 4×10^3 cells per well and treated with **4a-x**. After 72h of treatment, the cells were incubated with 100 μl of MTT solution (0.5 mg/ml, Sigma) for 4 h at 37 ℃.After centrifugation, 100 μl of DMSO was added. The absorbance was measured at 490nm using ELISA microplate reader. Data represents the average absorbance of six wells in one experiment. The experiment was repeated thrice with similar results.

5. Flow cytometry assay of cell apoptosis assay

HCT116 cells were cultured overnight and incubated in triplicate with vehicle or the test compound at 0.25 μM、1 μM、4μM for 48 h. The cells were harvested, and stained with Annexin V APC/7-AAD (BioVision) at room temperature for 15 min. The percentage of apoptotic cells was determined by flow cytometry (FACS Calibur Becton-Dickinson) analysis.

6. Cell cycle of cell apoptosis assay

HCT116 cells were cultured overnight and incubated in triplicate with vehicle or the test compound at 0.5μM, 2 μM, 6 μM for 24 h. The cells were harvested, and stained with PI (BioVision) at 4℃ for 30 min. The cells were analyzed by flow cytometry (FACS Calibur Becton-Dickinson) with an argon ion laser at 488 nm. Cell Quest software was applied to analyze the results.

7. Western blot assay

The cells were lysed with RIPA buffer. After centrifugation, the supernatant was collected and quantified. The proteins were then separated by SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% non-fat milk, the membranes were probed with rabbit anti-CDK 1 (Abcam plc., ab131450), rabbit anti-cyclin B (Abcam plc., ab2949), and rabbit-anti-β-actin (Abcam plc., ab8227). Secondary antibodies are HRP-conjugated against rat (Abcam plc., ab6721). The protein levels were first normalized to β-actin, and then normalized to the experimental controls. Densitometry of Western blots was quantified with NIH ImageJ software.

8. HPLC assessment of compound purity

All tested compounds (**4s**, **4t** and **4v**) with a purity of > 98% (HPLC analysis) were used for subsequent experiments. We provided the spectra of HPLC assays as below.

Column: ODS-C18 (150 mm×4.6 mm×3.5μm);

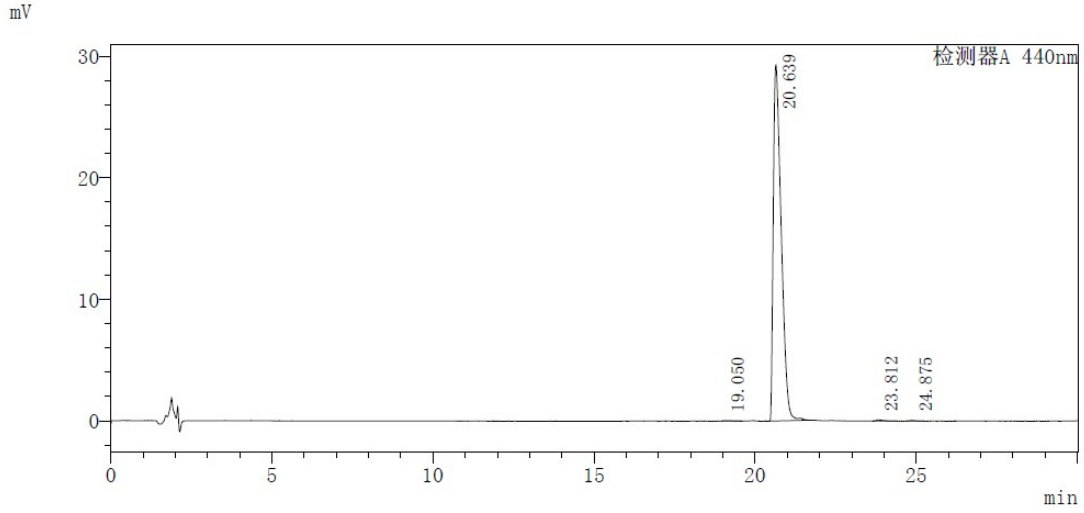
Mobile phase: acetonitrile : water -0.05%H_3PO_4 (20 : 80 to 80 : 20);

Wavelength: 440 nm;

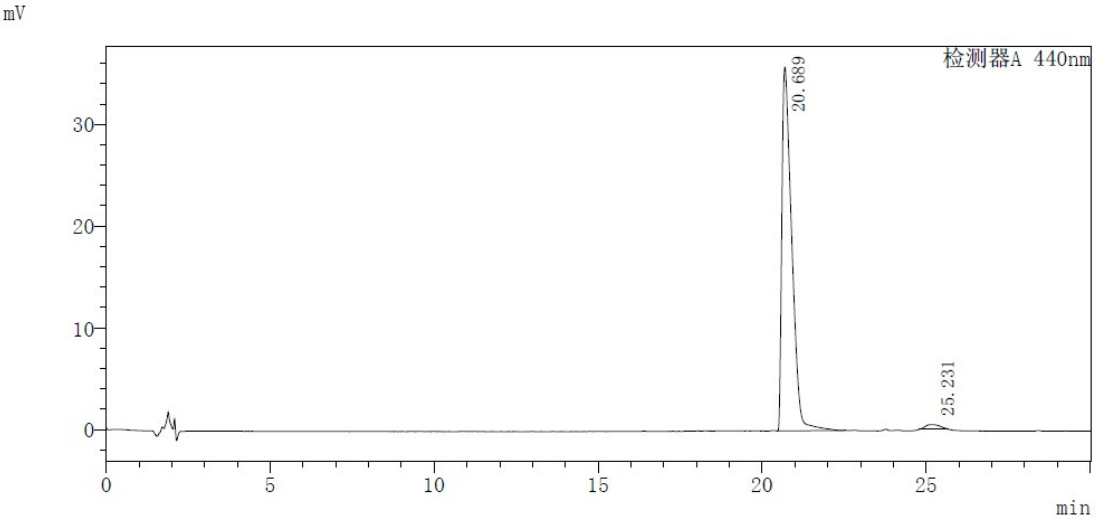
Rate: 1 mL/min;

Temperature: 25 °C

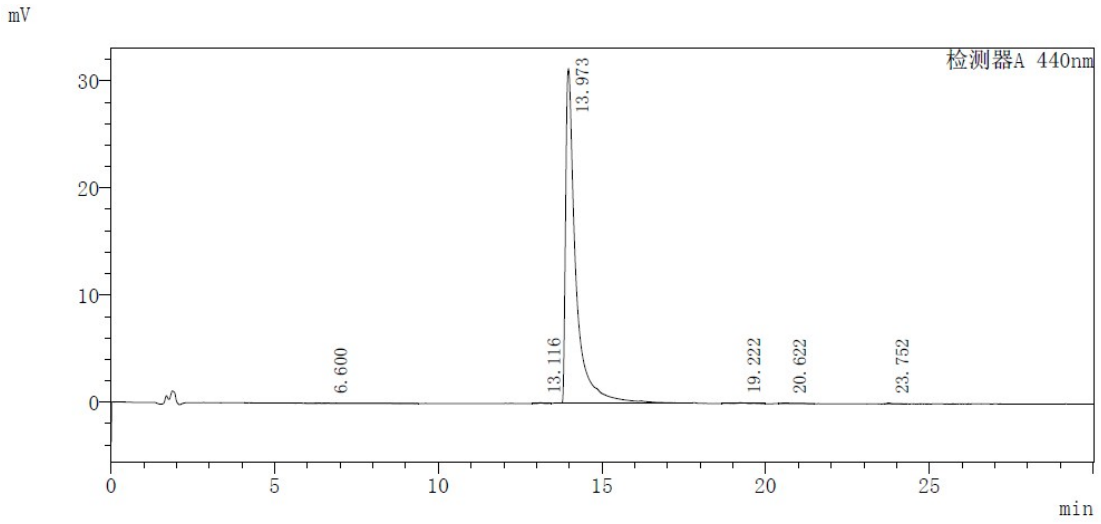
4s, 99.31%



4t, 98.18%

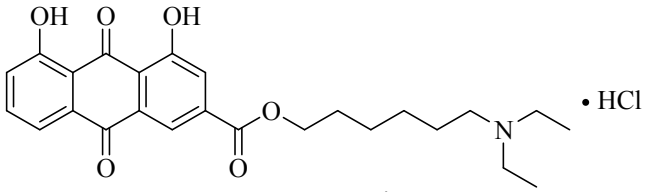


4v, 98.68%



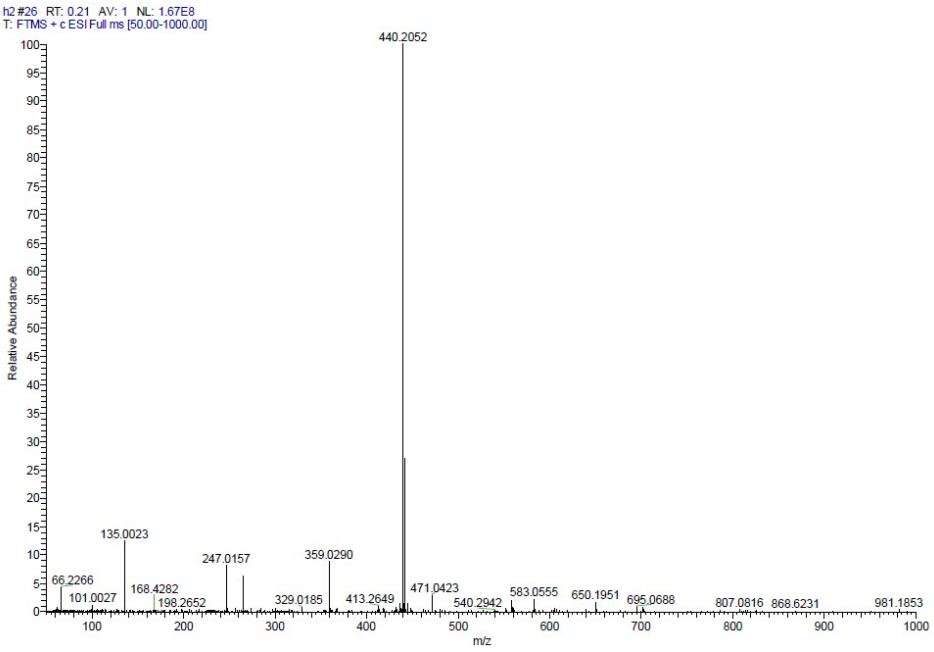
9. HRMS, ^{1}H-NMR and ^{13}C-NMR of compounds

Compound 4s:

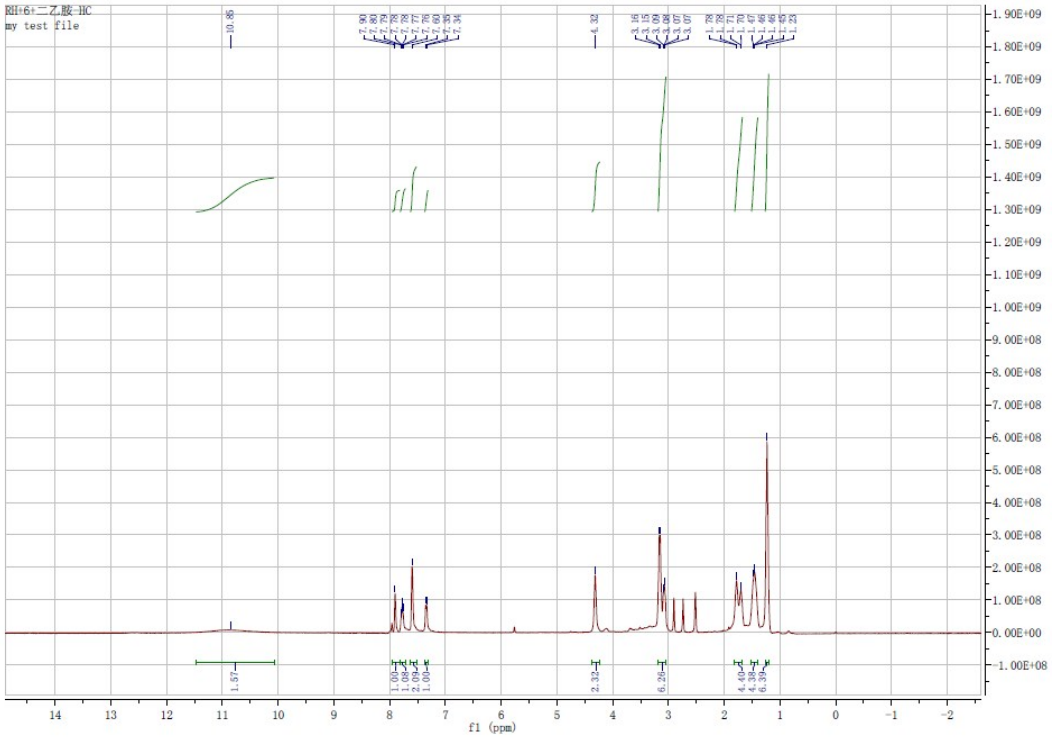


4s

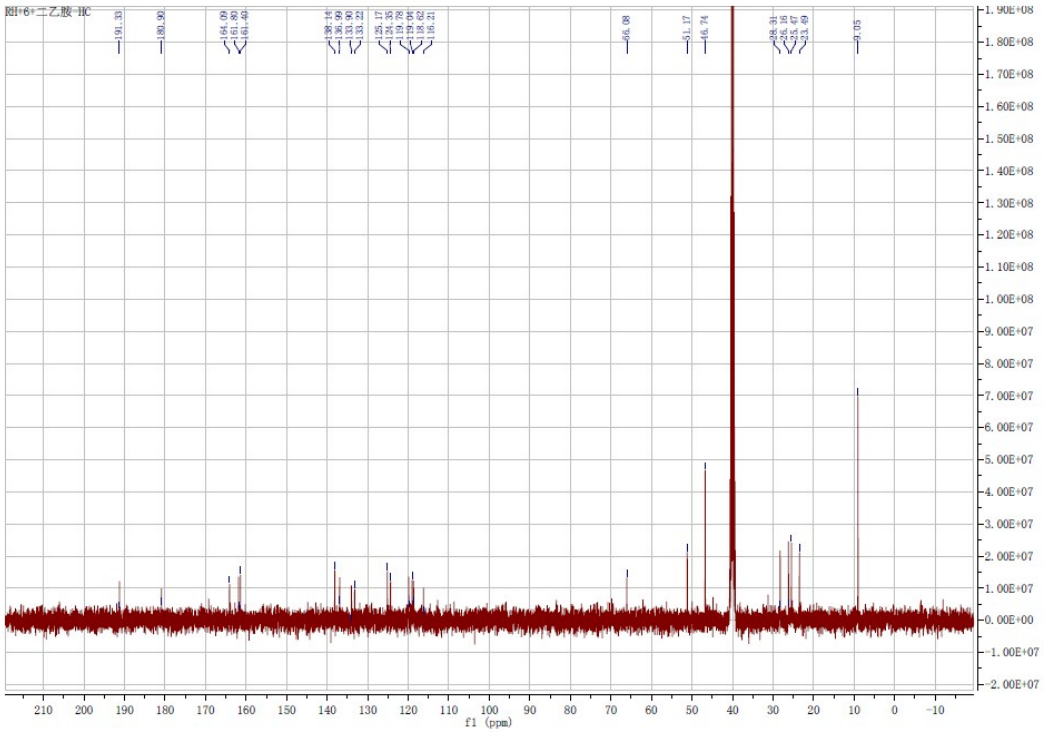
HRMS (4s)



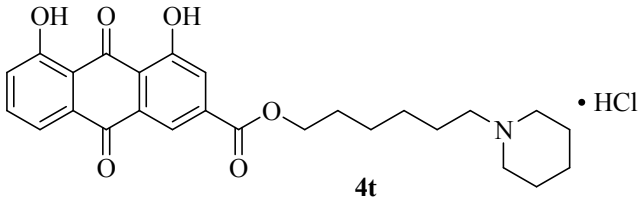
^{1}H-NMR (4s)



^{13}C-NMR (4s)

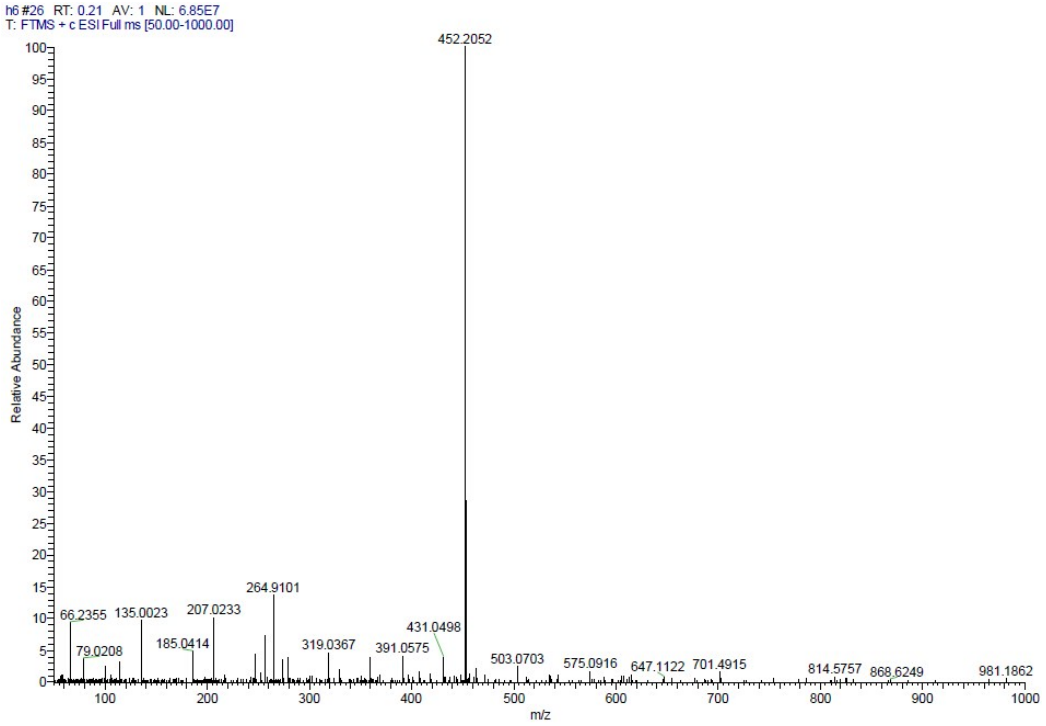


Compound 4t:

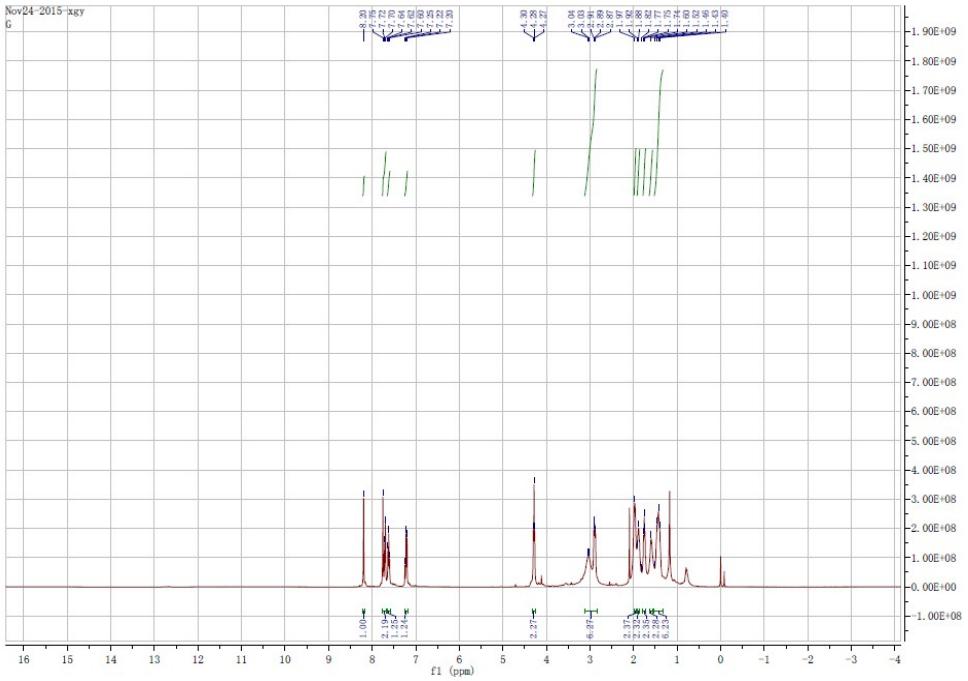


4t

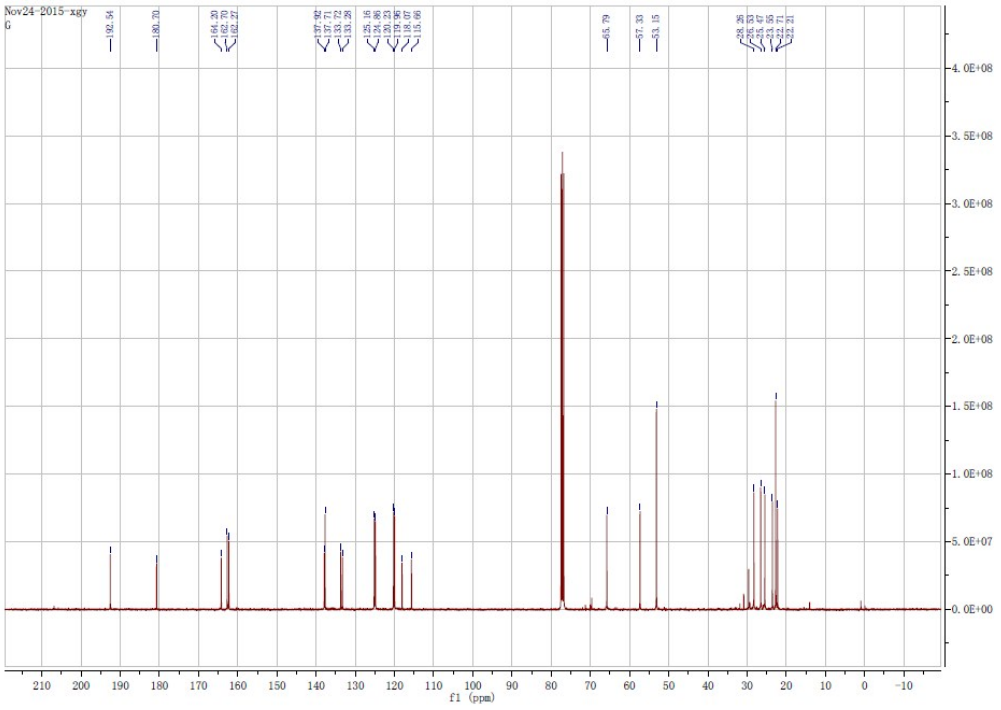
HRMS (4t)



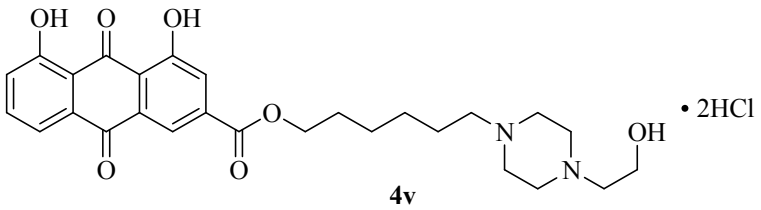
^{1}H-NMR (4t)



^{13}C-NMR (4t)

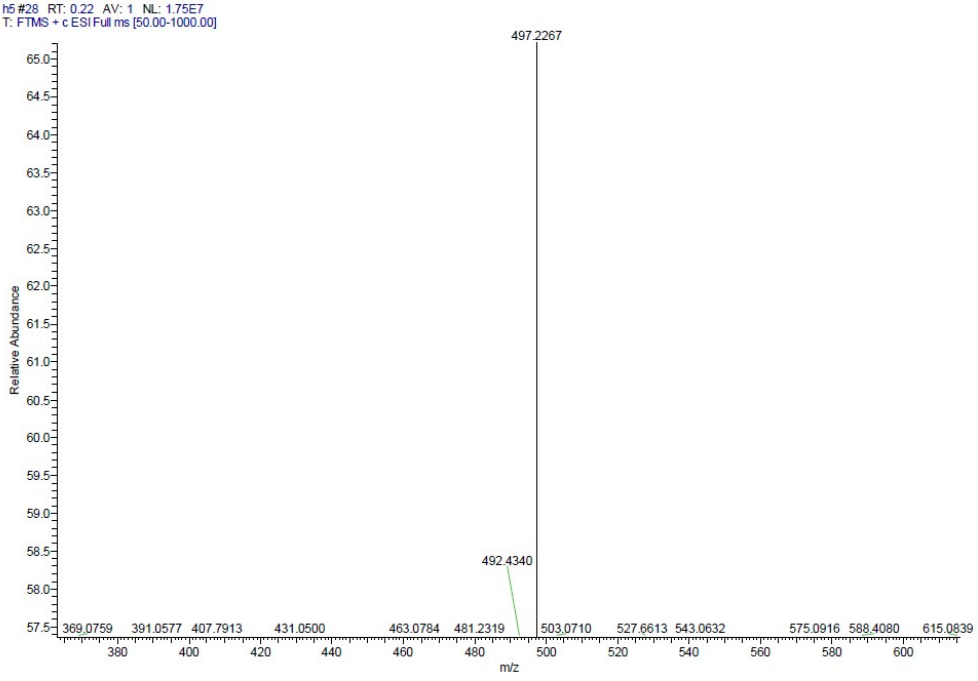


Compound 4v:

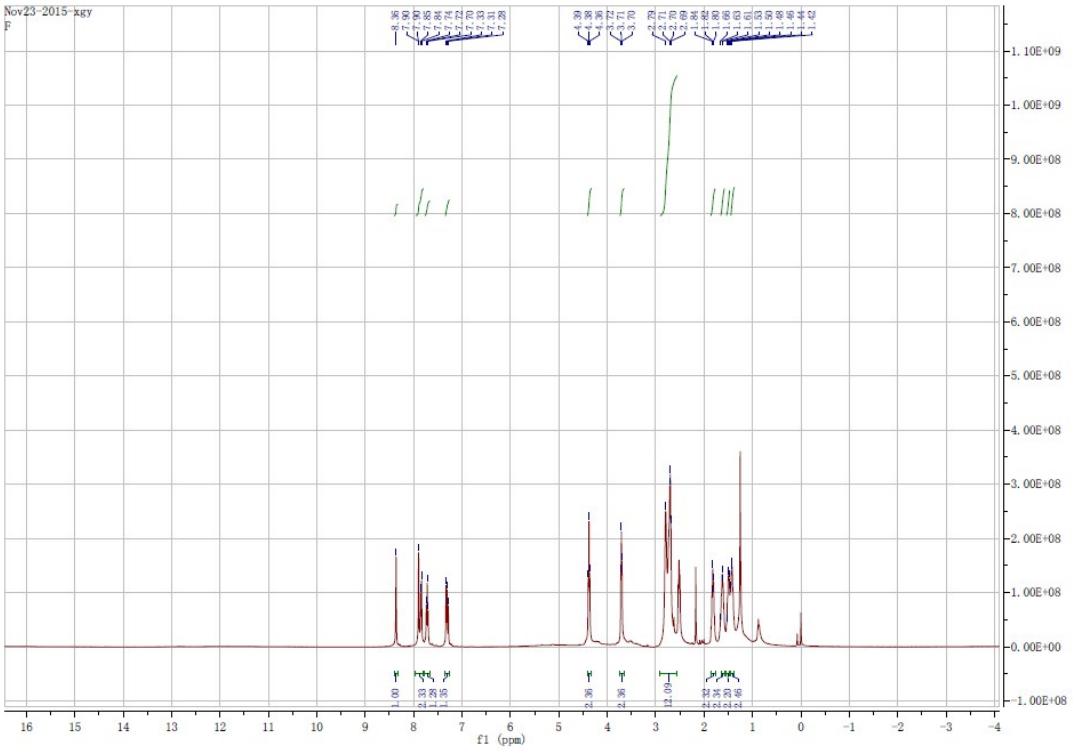


4v

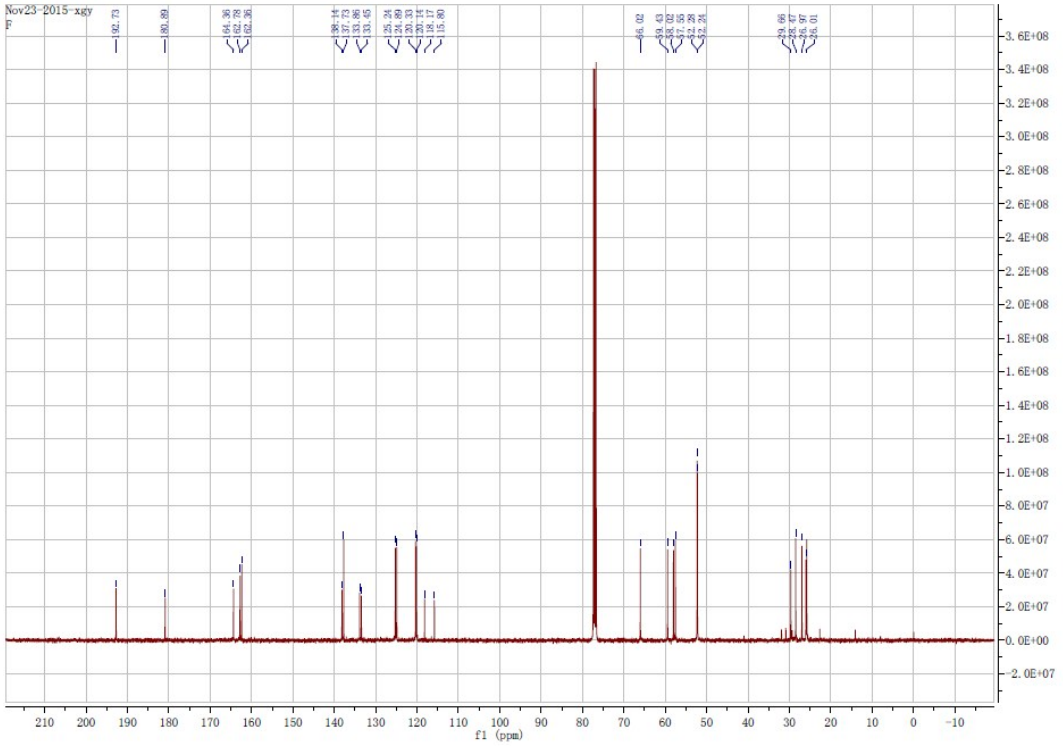
HRMS (4v)



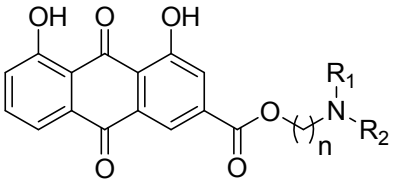
^{1}H-NMR (4v)

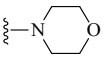
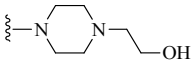
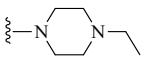
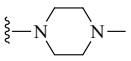
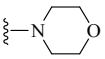
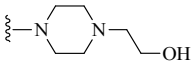
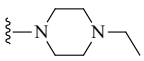
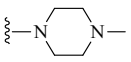
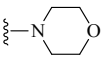
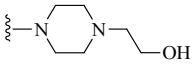
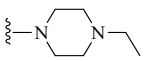
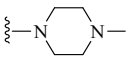


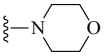
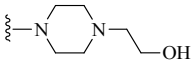
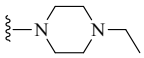
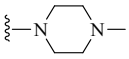
^{13}C-NMR (4v)



10.Date for the aqueous solubility of compounds 3a-x



Compd.	NR_1R_2	n	Solubility (mg/mL)
3a		**3**	0.1718
3b		**3**	0.3329
3c		**3**	0.4143
3d		**3**	0.1583
3e		**3**	0.3877
3f		**3**	0.2284
3g		**4**	0.1624
3h		**4**	0.2336
3i		**4**	0.3526
3j		**4**	0.2572
3k		**4**	0.2462
3l		**4**	0.2135
3m		**5**	0.2346
3n		**5**	0.2003
3o		**5**	0.2955
3p		**5**	0.1606
3q		**5**	0.4848
3r		**5**	0.1712

3s		**6**	0.1547
3t		**6**	0.1214
3u		**6**	0.2903
3v		**6**	0.2145
3w		**6**	0.2124
3x		**6**	0.3314
Rhein			0.0456