Supplementary materials

Design, synthesis, and anticancer activity of natural product hybrids with paclitaxel side chain inducing apoptosis in human colon cancer cells

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Abstract

Based on the strong activity dependence of paclitaxel (Taxol®) or docetaxel (Taxotere®) on the C-13 side chain, a small library of dehydroepiandrosterone (DHEA), cholesterol, vitamin D2, alkaloid talatisamine and songorine-paclitaxel hybrids have been synthesized and evaluated for in vitro anticancer activity by MTT assay against human breast (MCF-7), colon (HCT116), lung carcinoma (A549), renal adenocarcinoma (786-0) cancer cell lines. Most hybrids (11b, 12b, 13b, 15b and 18b) reduced the growth of MCF-7 and 786-0 cells with low paclitaxel sensitivity in vitro. Among the synthesized compounds, hybrid 11b was better in inhibiting the growth of four cells than paclitaxel. A relatively low IC₅₀ value of compound 11b (8.16 ± 0.04 μM) was also examined after exposure for 48 hours. Hybrid 11b showed a pro-apoptotic effect in HCT116 cells evaluated by Annexin V/PI binding assay. The level of hybrid 11b leading to protective cell death in HCT116 cells was detected using Western Blot and not easily observed in our basic examinations.

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1. Experimental

1.1 General Experimental Procedures

Unless otherwise stated, reagents were commercially available and used without further purification. The diterpenoid alkaloids talatisamine (9) and songorine (10) were extracted from *Delphinium delavayi* Franch. HRESIMS data were measured using a Q-TOF micro mass spectrometer (Waters). NMR spectra were recorded on a Bruker AV 600 or 400 spectrometers. Chemical shifts (δ) are reported in units of parts per million (ppm) downfield from tetramethylsilane (TMS).

1.2 General Procedure for the Esterification Reaction of Compounds 6-10 with Protected Side Chain.

A suspension of one of starting compounds 6-10 (0.1 mmol), protected side-chain 4 or 5 (0.12 mmol), (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) EDC (0.12 mmol) and (4-(dimethylamino)pyridine) DMAP (0.1 mmol) in dry dichloromethane (3 mL) was stirred at room temperature for 24 h until TLC showed complete consumption of the starting compounds. Then the reaction mixture was diluted with water (3 mL) and extracted by DCM (5 mL) for three times. The combined organic layer was washed with saturated aqueous solution of NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford the corresponding ester. The yield and spectra data of each compound are given below.

1.3 General Procedure for the Deprotection of the Phenylisoserine Side Chain.

Ester compounds 11a-18a (0.05 mmol) was added to a MeOH solution and treated with p-Toluenesulfonic acid (0.2 mmol). The reaction mixture was stirred at room temperature for 4 h and then diluted with an excess of ethyl acetate. The organic layer was washed with saturated aqueous solution of NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel to afford the desired compound.
Compound 11a:

The reaction was carried out with 57.6 mg (0.2 mmol) of compound 6, 80.6 mg (0.2 mmol) of protected side-chain 4, 45.84 mg (0.12 mmol) EDC and 24.4 mg (0.1 mmol) DMAP in a mixture of toluene (3 mL) to give crude product. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 5:1), white powder, compound 11a (101 mg, 75.8%). 1H NMR (600 MHz, CDCl3) δ 7.50-7.27 (m, 9H), 7.22 (t, J = 7.7 Hz, 3H), 6.85 (s, 2H), 5.44 (d, J = 4.8 Hz, 1H), 5.38 (d, J = 5.1 Hz, 1H), 4.83 (s, 1H), 4.78-4.70 (m, 1H), 3.81 (s, 3H), 2.46 (ddd, J = 19.3, 8.7, 5.2 Hz, 1H), 2.39 (d, J = 7.9 Hz, 2H), 2.17-2.04 (m, 3H), 1.99-1.81 (m, 6H), 1.67 (dd, J = 16.7, 7.4 Hz, 4H), 1.50 (ddd, J = 26.0, 13.2, 5.4 Hz, 3H), 1.31 (dd, J = 14.5, 5.3 Hz, 2H), 1.06 (s, 3H), 0.89 (s, 2H). MS(ESI) m/z : [(M+H)+,674.34].

Compound 12a:

The reaction was carried out with 126.3 mg (0.3 mmol) of compound 7, 120.9 mg (0.3 mmol) of protected side-chain 4, 68.76 mg EDC (0.36 mmol) and 36.6 mg (0.4 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 1:2), compound 12a was obtained as white powder (118 mg, 48.8%). 1H NMR (400 MHz, CDCl3) δ 7.36-7.27 (m, 7H), 7.25-7.18 (m, 4H), 6.87 (d, J = 8.3 Hz, 3H), 4.96 (t, J = 5.0 Hz, 1H), 4.75 (s, 1H), 3.82 (s, 3H), 3.28 (d, J = 9.3 Hz, 6H), 3.18-3.08 (m, 3H), 3.02-2.96 (m, 4H), 2.63-2.58 (m, 1H), 2.56-2.34 (m, 6H), 2.18-2.14 (m, 1H), 2.06 (d, J = 7.8 Hz, 1H), 1.97 (dd, J = 11.6, 5.1 Hz, 2H), 1.90 (d, J = 8.1 Hz, 1H), 1.84 (dd, J = 11.0, 6.3 Hz, 2H), 1.81-1.76 (m, 1H), 1.65 (d, J = 6.9 Hz, 3H), 1.52 (dd, J = 14.3, 7.6 Hz, 1H), 1.42 (t, J = 12.5 Hz, 1H), 1.25 (s, 3H), 1.05 (t, J = 7.1 Hz, 3H). MS(ESI) m/z : [(M+H)+,822.44].

Compound 13a:

The reaction was carried out with 126.3 mg (0.3 mmol) of compound 7, 119.7 mg (0.3 mmol) of protected side-chain 5, 68.76 mg (0.36 mmol) EDC and 36.6 mg (0.4 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 1:1), was obtained compound 13a as white powder (131 mg, 54.4%). 1H NMR (400 MHz, CDCl3) δ 7.44-7.35 (m, 4H), 7.33-7.27 (m, 3H), 6.91 (d, J = 8.6 Hz, 2H), 4.49 (d, J =
1.7 Hz, 2H), 3.82 (s, 3H), 3.30 (s, 3H), 3.24 (s, 3H), 3.11-3.04 (m, 6H), 2.97 (d, J = 9.0 Hz, 1H), 2.89 (s, 1H), 2.52-2.41 (m, 4H), 2.36-2.21 (m, 3H), 2.04 (dd, J = 15.4, 6.7 Hz, 1H), 1.99-1.90 (m, 3H), 1.87-1.79 (m, 1H), 1.72 (d, J = 19.9 Hz, 3H), 1.65-1.58 (m, 2H), 1.55 (d, J = 7.2 Hz, 2H), 1.44 (dd, J = 14.6, 8.8 Hz, 2H), 1.24 (d, J = 5.6 Hz, 1H), 1.10 (s, 9H), 1.01 (t, J = 7.1 Hz, 3H). MS(ESI) m/z : [(M+H)+,802.47].

**Compound 14a:**

The reaction was carried out with 107.1 mg (0.3 mmol) of compound 8, 120.9 mg (0.3 mmol) of protected side-chain 4, 68.76 mg (0.36 mmol) EDC and 36.6 mg (0.4 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product, After purification by column chromatography on silica gel (petroleum ether/EtOAc = 1:2), compound 14a was obtained as white powder (98.7 mg, 44.3%). 1H NMR (400 MHz, CDCl3) δ 7.75 (d, J = 7.3 Hz, 2H), 7.48 (dd, J = 15.0, 7.6 Hz, 3H), 7.41 (t, J = 7.4 Hz, 2H), 7.35 (t, J = 7.5 Hz, 2H), 7.07 (d, J = 8.8 Hz, 1H), 5.66 (d, J = 8.9 Hz, 1H), 5.34-5.10 (m, 3H), 4.58 (t, J = 10.5 Hz, 1H), 4.33 (s, 1H), 3.54 (s, 1H), 3.06 (d, J = 5.1 Hz, 1H), 2.57 (d, J = 11.3 Hz, 1H), 2.18 (d, J = 5.1 Hz, 1H), 2.04-1.90 (m, 2H), 1.73-1.55 (m, 5H), 1.42-1.31 (m, 3H), 1.24 (dd, J = 12.4, 3.8 Hz, 2H), 1.15-1.05 (m, 3H), 0.99 (s, 9H), 0.75 (s, 3H). MS(ESI) m/z : [(M+H)+,789.44].

**Compound 15a:**

The reaction was carried out with 107.1 mg (0.3 mmol) of compound 8, 119.7 mg (0.3 mmol) of protected side-chain 5, 68.76 mg (0.36 mmol) EDC and 36.6 mg (0.4 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product, After purification by column chromatography on silica gel (petroleum ether/EtOAc = 1:2), was obtained compound 15a as white powder (124 mg, 64.3%). 1H NMR (400 MHz, CDCl3) δ 7.44-7.28 (m, 7H), 6.89 (d, J = 8.6 Hz, 2H), 6.25 (s, 1H), 5.24 (s, 1H), 5.17-5.07 (m, 2H), 4.58 (d, J = 6.6 Hz, 1H), 4.25 (d, J = 6.4 Hz, 1H), 3.81 (s, 3H), 3.28 (s, 1H), 2.87 (d, J = 3.0 Hz, 1H), 2.68-2.53 (m, 2H), 2.50-2.41 (m, 3H), 2.27 (d, J = 11.3 Hz, 1H), 2.18 (d, J = 5.1 Hz, 1H), 2.04-1.90 (m, 2H), 1.73-1.55 (m, 5H), 1.42-1.31 (m, 3H), 1.24 (dd, J = 12.4, 3.8 Hz, 2H), 1.15-1.05 (m, 3H), 0.99 (s, 9H), 0.75 (s, 3H). MS(ESI) m/z : [(M+H)+,747.41].
Compound 16a:

The reaction was carried out with 39.6 mg (0.1 mmol) of compound 9, 40.3 mg (0.1 mmol) of protected side-chain 4, 22.9 mg (0.12 mmol) EDC and 12.2 mg (0.1 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 5:1), compound 16a was obtained as white powder (67.8mg, 86.8%). 1H NMR (400 MHz, CDCl3) δ 7.32 (dd, J = 25.5, 7.6 Hz, 8H), 7.25-7.19 (m, 3H), 6.83 (d, J = 7.7 Hz, 3H), 6.27 (d, J = 11.2 Hz, 1H), 6.02 (d, J = 11.2 Hz, 1H), 5.20 (t, J = 5.9 Hz, 2H), 5.13-5.03 (m, 2H), 4.85 (d, J = 2.0 Hz, 2H), 3.81 (s, 3H), 2.89-2.78 (m, 1H), 2.61 (dd, J = 13.4, 3.2 Hz, 1H), 2.44 (dd, J = 13.4, 7.7 Hz, 1H), 2.35-2.26 (m, 1H), 2.23-2.12 (m, 1H), 2.08-1.94 (m, 4H), 1.86 (dd, J = 12.8, 6.5 Hz, 1H), 1.73 (ddd, J = 22.6, 16.3, 8.2 Hz, 5H), 1.58-1.42 (m, 4H), 1.31 (ddd, J = 18.8, 15.0, 9.1 Hz, 4H), 1.03 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 6.4 Hz, 6H), 0.56 (s, 3H). MS(ESI) m/z : [(M+H)+,782.47].

Compound 17a:

The reaction was carried out with 39.6 mg (0.1 mmol) of compound 9, 39.9 mg (0.1 mmol) of protected side-chain 5, 22.9 mg (0.12 mmol) EDC and 12.2 mg (0.1 mmol) DMAP in a mixture of CH2Cl2 (3 mL) to give crude product. After purification by column chromatography on silica gel (petroleum ether/EtOAc = 5:1), compound 17a was obtained as white powder (66.6mg, 85.7%). 1H NMR (400 MHz, CDCl3) δ 7.40-7.35 (m, 6H), 7.32-7.28 (m, 1H), 6.90 (t, J = 8.8 Hz, 2H), 6.13 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 5.18 (dd, J = 17.2, 10.5 Hz, 2H), 5.03 (s, 1H), 4.82 (s, 2H), 4.53 (d, J = 4.3 Hz, 1H), 3.82 (s, 3H), 2.88-2.78 (m, 1H), 2.36-2.28 (m, 1H), 2.16 (dd, J = 11.4, 7.0 Hz, 3H), 2.00 (tt, J = 18.1, 9.0 Hz, 4H), 1.86 (dd, J = 12.9, 6.5 Hz, 1H), 1.69 (t, J = 10.0 Hz, 4H), 1.62 (s, 1H), 1.48 (dd, J = 13.2, 6.8 Hz, 4H), 1.36-1.22 (m, 4H), 1.06 (d, J = 5.9 Hz, 9H), 1.02 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.83 (t, J = 6.5 Hz, 6H), 0.56 (s, 3H). MS(ESI) m/z : [(M+H)+,762.50].

Compound 18a:

The reaction was carried out with 38.6 mg (0.1 mmol) of compound 10, 40.3 mg (0.1 mmol) of protected side-chain 4, 22.9 mg (0.12 mmol) EDC and 12.2 mg (0.1 mmol) DMAP in a mixture of toluene (3 mL) to give crude product, After
purification by column chromatography on silica gel (petroleum ether/EtOAc = 5:1), compound 18a was obtained as white powder (63.5 mg, 82.4%). 1H NMR (400 MHz, CDCl3) δ 7.40-7.27 (m, 8H), 7.22 (t, J = 7.6 Hz, 3H), 6.89 (t, J = 32.2 Hz, 3H), 5.38 (dd, J = 20.5, 4.6 Hz, 2H), 4.83 (s, 1H), 4.78-4.68 (m, 1H), 3.82 (s, 3H), 2.36 (d, J = 7.3 Hz, 2H), 2.06-1.93 (m, 3H), 1.91-1.81 (m, 3H), 1.63-1.54 (m, 4H), 1.53-1.47 (m, 3H), 1.43 (dd, J = 10.6, 4.3 Hz, 1H), 1.34 (d, J = 7.5 Hz, 3H), 1.27-1.22 (m, 3H), 1.19-1.05 (m, 7H), 1.03 (s, 4H), 0.92 (d, J = 6.5 Hz, 3H), 0.87 (dd, J = 6.6, 1.7 Hz, 6H), 0.68 (s, 3H). MS(ESI) m/z : [(M+H)+,772.49].

Compound 11b:

The reaction was carried out with 0.05 mmol (33.6 mg) of compound 11a in MeOH (1.5 mL) and treated with p-toluenesulfonic acid (0.2 mmol, 38.2 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 11b (21 mg, 75.7%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.81-7.71 (m, 2H), 7.54-7.29 (m, 7H), 6.98 (d, J = 9.1 Hz, 1H), 5.77 (dd, J = 9.3, 1.8 Hz, 1H), 5.40 (dd, J = 12.8, 5.0 Hz, 1H), 4.78-4.68 (m, 1H), 4.61 (s, 1H), 2.50-2.23 (m, 4H), 2.07 (dd, J = 18.3, 8.4 Hz, 2H), 1.98-1.80 (m, 5H), 1.67-1.61 (m, 3H), 1.42 (s, 1H), 1.28-1.23 (m, 5H), 1.04 (d, J = 5.6 Hz, 4H), 0.88 (d, J = 2.5 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 166.9, 141.1, 139.7, 138.9, 134.4, 131.8, 128.8, 128.0, 127.1, 127.0, 122.4, 121.0, 73.5, 71.7, 54.8, 51.9, 50.4, 47.6, 42.3, 38.0, 37.3, 36.8, 35.9, 31.5, 30.9, 27.5, 22.0, 20.5, 19.4, 13.6. MS(ESI) m/z : [(M+H)+,556.30].

Compound 12b:

The reaction was carried out with 0.1 mmol (82.2 mg) of compound 12a in MeOH (2 mL) and treated with p-toluenesulfonic acid (0.4 mmol, 76.3 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 12b (54.2 mg, 78.4%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.88-7.81 (m, 2H), 7.53-7.46 (m, 2H), 7.42 (t, J = 7.4 Hz, 4H), 7.32 (t, J = 7.3 Hz, 2H), 5.66 (dd, J = 8.8, 2.4 Hz, 1H), 4.94 (dd, J = 10.7, 5.7 Hz, 1H), 4.59 (d, J = 2.6 Hz, 1H), 3.29 (s, 3H), 3.24 (s, 3H), 3.16 (s, 3H), 3.07 (dd, J = 9.7, 4.3 Hz, 2H), 2.99-2.93 (m, 2H), 2.53-2.34 (m, 6H), 2.19-2.07 (m, 3H), 1.98 (dd, J = 17.3, 9.5 Hz, 4H), 1.88-1.85 (m, 1H), 1.84-1.81 (m, 1H), 1.76 (dd, J = 11.7, 5.6 Hz, 2H), 1.65 (dd, J = 14.7, 7.4 Hz, 1H), 1.56 (d, J = 7.2 Hz, 1H), 1.48-1.36 (m, 2H), 1.26 (d, J = 7.1 Hz,
1H), 1.22 (dd, J = 9.9, 4.0 Hz, 1H), 1.04 (dd, J = 9.2, 5.1 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 171.9, 167.2, 138.8, 134.4, 131.6, 128.6, 127.8, 127.5, 127.1, 85.8, 82.5, 79.6, 74.3, 73.4, 62.5, 59.6, 56.5, 56.3, 55.1, 53.1, 49.5, 48.7, 47.1, 46.0, 45.0, 44.9, 40.4, 38.6, 35.9, 32.7, 29.8, 28.3, 26.2, 24.8, 21.1, 14.3, 13.7. MS(ESI) m/z : [(M+H)+,721.44].

**Compound 13b:**

The reaction was carried out with 0.1 mmol (80.2 mg) of compound 13a in MeOH (2 mL) and treated with p-toluenesulfonic acid (0.4 mmol, 76.3 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 13b (49 mg, 71.6%) was obtained as white powder. 1H NMR (600 MHz, CDCl3) δ 7.38-7.27 (m, 5H), 5.05 (d, J = 17.5 Hz, 1H), 4.44 (s, 1H), 3.34 (d, J = 8.2 Hz, 3H), 3.30 (s, 3H), 3.27 (s, 3H), 3.13-3.08 (m, 2H), 3.00 (d, J = 9.1 Hz, 2H), 2.54-2.43 (m, 4H), 2.30 (s, 1H), 2.21 (dd, J = 17.7, 9.9 Hz, 1H), 2.06 (t, J = 9.5 Hz, 1H), 2.01 (d, J = 7.3 Hz, 3H), 1.85-1.75 (m, 3H), 1.64 (d, J = 6.5 Hz, 4H), 1.53 (dd, J = 14.8, 8.1 Hz, 1H), 1.45 (s, 7H), 1.25 (s, 7H), 1.07 (t, J = 6.8 Hz, 3H), 0.88 (t, J = 6.9 Hz, 1H). 13C NMR (100 MHz, CDCl3) δ 155.5, 128.4, 127.7, 127.1, 85.8, 82.3, 79.6, 75.7, 74.5, 73.4, 72.9, 68.3, 63.1, 62.6, 59.6, 56.9, 56.3, 53.1, 49.5, 48.8, 47.2, 46.1, 45.2, 45.0, 40.5, 38.6, 37.2, 32.9, 32.8, 32.0, 28.5, 28.4, 27.3, 26.3, 25.0, 24.9, 14.2, 13.7. MS(ESI) m/z : [(M+H)+,685.40].

**Compound 14b:**

The reaction was carried out with 0.1 mmol (74.2 mg) of compound 14a in MeOH (2 mL) and treated with p-toluenesulfonic acid (0.4 mmol, 76.3 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 14b (37 mg, 59.3%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.75 (d, J = 7.3 Hz, 2H), 7.48 (dd, J = 15.0, 7.6 Hz, 3H), 7.41 (t, J = 7.4 Hz, 2H), 7.35 (t, J = 7.5 Hz, 2H), 7.07 (d, J = 8.8 Hz, 1H), 5.66 (d, J = 8.9 Hz, 1H), 5.34-5.10 (m, 3H), 4.58 (t, J = 10.5 Hz, 1H), 4.33 (s, 1H), 3.54 (s, 1H), 3.06 (d, J = 3.3 Hz, 1H), 2.57 (ddd, J = 31.7, 19.1, 10.1 Hz, 5H), 2.30 (dd, J = 21.9, 8.4 Hz, 2H), 2.14-2.00 (m, 2H), 1.83-1.73 (m, 2H), 1.59-1.33 (m, 5H), 1.25 (s, 3H), 1.17 (d, J = 7.1 Hz, 3H), 0.91-0.84 (m, 1H), 0.73 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 209.4, 172.6, 166.9, 150.7, 139.3, 134.6, 131.7, 128.8, 128.7, 127.9, 127.2, 127.1, 112.0,
Compound 15b:

The reaction was carried out with 0.1 mmol (73.8 mg) of compound 15a in MeOH (2 mL) and treated with p-toluenesulfonic acid (0.4 mmol, 76.3 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 15b (48.7 mg, 78.5%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.40-7.27 (m, 5H), 5.54 (d, J = 9.4 Hz, 1H), 5.29 (d, J = 1.2 Hz, 1H), 5.18 (d, J = 9.5 Hz, 3H), 4.37 (d, J = 17.5 Hz, 2H), 3.55 (d, J = 13.7 Hz, 1H), 3.09 (d, J = 2.7 Hz, 1H), 2.70 (dd, J = 12.2, 7.2 Hz, 1H), 2.58-2.51 (m, 3H), 2.44-2.33 (m, 2H), 2.30 (d, J = 4.5 Hz, 1H), 2.11 (dd, J = 9.7, 6.1 Hz, 2H), 2.07 (s, 1H), 1.82 (dt, J = 16.5, 8.3 Hz, 3H), 1.51-1.43 (m, 3H), 1.41 (d, J = 7.4 Hz, 9H), 1.26 (d, J = 7.3 Hz, 3H), 1.17 (t, J = 7.1 Hz, 4H), 1.11 (t, J = 7.1 Hz, 1H), 0.77 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 209.0, 172.5, 154.8, 150.7, 139.0, 128.5, 127.7, 126.9, 112.0, 79.8, 77.7, 73.9, 66.3, 57.0, 55.7, 54.0, 50.8, 50.4, 50.2, 49.9, 44.4, 42.9, 40.1, 37.7, 37.6, 35.3, 34.2, 32.0, 29.8, 28.4, 25.8, 23.0, 21.5, 14.2, 13.6, 13.2. MS (ESI) m/z : [(M+H)+,671.40].

Compound 16b:

The reaction was carried out with 0.05 mmol (39.2 mg) of compound 16a in MeOH (1.5 mL) and treated with p-toluenesulfonic acid (0.2 mmol, 38.2 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 16b (24.5 mg, 74.3%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.78-7.73 (m, 2H), 7.53-7.48 (m, 1H), 7.43 (dt, J = 8.7, 4.3 Hz, 4H), 7.36 (dd, J = 10.0, 4.8 Hz, 2H), 7.32-7.26 (m, 1H), 7.00 (d, J = 9.2 Hz, 1H), 6.20 (d, J = 11.2 Hz, 1H), 6.02 (d, J = 11.2 Hz, 1H), 5.74 (dt, J = 9.5, 4.8 Hz, 1H), 5.19 (t, J = 6.0 Hz, 2H), 5.09-5.02 (m, 2H), 4.84 (d, J = 2.1 Hz, 1H), 4.63 (s, 1H), 3.36 (d, J = 14.5 Hz, 1H), 2.80 (dd, J = 11.9, 3.5 Hz, 1H), 2.60 (dd, J = 13.4, 3.8 Hz, 1H), 2.48-2.35 (m, 2H), 2.22-2.14 (m, 1H), 2.08-1.93 (m, 4H), 1.85 (dd, J = 12.7, 6.7 Hz, 1H), 1.74-1.64 (m, 4H), 1.49-1.44 (m, 2H), 1.37-1.24 (m, 5H), 1.02 (t, J = 5.7 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.83 (t, J = 6.5 Hz, 6H), 0.55 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 172.5, 166.9, 144.2, 142.8, 138.8, 135.7, 134.3, 133.7, 132.0, 131.8, 128.8, 128.0, 127.1, 127.0, 122.9, 117.5, 113.2, 74.8, 73.3, 56.5, 54.8, 45.9, 42.9, 42.0, 40.5,
Compound 17b:

The reaction was carried out with 0.1 mmol (74.2 mg) of compound 17a in MeOH (1.5 mL) and treated with p-toluenesulfonic acid (0.2 mmol, 38.2 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 17b (23.4 mg, 71.1%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.37-7.27 (m, 5H), 6.22 (d, J = 11.2 Hz, 1H), 6.04 (d, J = 11.2 Hz, 1H), 5.40 (d, J = 9.4 Hz, 1H), 5.20 (t, J = 5.9 Hz, 2H), 5.07 (s, 2H), 4.86 (s, 1H), 3.19 (d, J = 3.7 Hz, 1H), 2.85-2.77 (m, 1H), 2.61 (dd, J = 13.4, 3.8 Hz, 1H), 2.49-2.37 (m, 2H), 2.21 (ddd, J = 13.4, 8.9, 4.9 Hz, 1H), 2.05-1.94 (m, 4H), 1.84 (dt, J = 9.1, 5.4 Hz, 2H), 1.68 (dd, J = 24.2, 15.8 Hz, 5H), 1.51-1.46 (m, 2H), 1.41 (s, 8H), 1.33 (d, J = 9.5 Hz, 2H), 1.26 (s, 1H), 1.02 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.83 (t, J = 6.5 Hz, 7H), 0.56 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 172.5, 155.1, 144.3, 142.7, 139.4, 135.7, 133.8, 132.1, 128.6, 127.7, 126.9, 122.9, 117.6, 113.1, 79.8, 77.3, 74.5, 73.6, 56.5, 56.0, 45.9, 42.9, 42.0, 40.5, 40.5, 33.2, 32.1, 31.7, 29.8, 29.2, 28.3, 27.9, 23.7, 22.3, 21.2, 21.1, 20.0, 19.9, 19.7, 17.7, 12.4, 11.5. MS (ESI) m/z : [(M+H)+,664.43].

Compound 18b:

The reaction was carried out with 0.1 mmol (39.9 mg) of compound 18a in MeOH (2 mL) and treated with p-toluenesulfonic acid (0.4 mmol, 76.3 mg) for 4h at room temperature. After purification (petroleum ether/EtOAc = 1:1), the expected compound 18b (24.5 mg, 75.2%) was obtained as white powder. 1H NMR (400 MHz, CDCl3) δ 7.76 (dd, J = 5.2, 3.3 Hz, 2H), 7.50 (d, J = 7.4 Hz, 1H), 7.48-7.40 (m, 4H), 7.36 (dd, J = 8.1, 6.7 Hz, 2H), 7.30 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 5.76 (dd, J = 9.2, 2.0 Hz, 1H), 5.38 (d, J = 5.2 Hz, 1H), 4.80-4.67 (m, 1H), 4.60 (s, 1H), 3.35 (s, 1H), 2.47-2.37 (m, 1H), 2.36-2.29 (m, 1H), 2.04-1.93 (m, 2H), 1.87-1.78 (m, 3H), 1.64 (s, 2H), 1.52 (d, J = 6.7 Hz, 1H), 1.50-1.47 (m, 1H), 1.47-1.42 (m, 2H), 1.39-1.29 (m, 4H), 1.26 (s, 4H), 1.09 (ddd, J = 18.4, 14.2, 6.1 Hz, 7H), 1.02 (s, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 1.8 Hz, 3H), 0.85 (d, J = 1.7 Hz, 3H), 0.70-0.64 (m, 3H); 13C NMR (100 MHz, CDCl3) δ 172.5, 166.8, 139.4, 138.9, 134.4, 131.8, 128.8, 128.8, 127.9, 127.1, 127.0, 123.2, 76.9, 73.5, 56.8, 56.2, 54.8, 50.1, 42.4, 39.8,
39.6, 38.0, 36.9, 36.7, 36.3, 35.9, 32.0, 29.8, 28.3, 28.1, 27.6, 24.4, 23.9, 22.9, 22.7, 21.1, 19.4, 18.8, 11.9. MS (ESI) m/z : [(M+H)⁺, 654.45].

2 Bioassays

MCF-7, HTC 116, A549, 786-0 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). MTT (M2128) was purchased from Sigma-Aldrich (St. Louis, Mo, USA). Antibodies used in this study were as follows: Bax (2772, CST), PARP (9532, CST), β-actin (66009-I-Ig, Proteintech, IL, USA).

2.1 Culture of cell lines

Cells were cultured in 175 cm² flasks that were filled with l-glutamine with 10% heat-inactivated fetal bovine serum, 50 U/mL penicillin and 50 mg/L streptomycin in a humidified, 5% CO₂, 37°C incubator. The medium was changed every second day and cells were subcultured at ~80% confluency every 4-5 days using 0.1% trypsin.

2.2 Cytotoxicity assay by MTT assay

MTT assay was employed to this primarily determination as we did in previous report¹. The in-vitro inhibitory activities of eight hybrids on four cells were evaluated. Cells were cultured in 96-well plates with a density of 5×10³ edia for 1 day and then respectively pre-treated at 37°C for 24 h with each compound 11b-18b (50μM, n=3). After that, 10μL/well of MTT of 5 mg/mL (Sigma) was added to all wells. The plates were incubated for an additional 4 h, the supernatants were removed, and then 150μL of dimethyl sulfoxide (DMSO) was added to all the wells to dissolve the dark blue crystals. The plates were then read on a microplate reader (Wellscan MK3, Labsystems Dragon) at a wavelength of 570nm. The inhibition rate was calculated as follows:

\[
\text{Inhibition rate} = \frac{\text{mean OD control} - \text{mean OD drug reated}}{\text{mean OD control} - \text{mean OD vacuity}} \times 100\%.
\]

2.3 Experimental Details for Obtaining IC₅₀ Values.

HCT116 human colon cancer cells were cultured in medium with 10 % fetal bovine serum (FBS), HCT116 human colon cancer cells were added to each well of a 96-well plate. Increasing concentrations of hybrid 11b were added 18 h after plating.
IC$_{50}$ values, the concentration of agent that inhibits cell growth by 50%, were determined after 24, 48 or 72 h at 37 °C, respectively, using the MTT method.

2.4 Determination of apoptosis by annexin V and propidium iodide (PI) staining

Fluorescence intensity was analyzed using a flow cytometer (Exposure of in apoptotic cells was measured by adding Annexin V–FITC (Immunotech Coulter). Additional exposure to PI made it possible to differentiate apoptotic cells (annexin-positive and PI-negative) from necrotic cells (annexin- and PI-positive). The HCT116 human colon cancer cells ($2 \times 10^5$ cells) were seeded on 6-well plates and incubated for 12 h when cells are at 70-80% confluency. Then, cells were treated with hybrid 11b (0μM, 5μM, 10μM, 20μM) in a dose dependent manner for 24 h at 37 °C in a CO$_2$ incubator and dimethyl sulfoxide (DMSO) was used in the control group. Supernatants were removed from culture dishes and adherent cells detached with trypsin-EDTA. The cells were collected by centrifugation, washed with PBS, and resuspended using 1× binding buffer at 1 × 10$^6$ cells/ml. Then, 100 ml of the cell suspension ($1 \times 10^5$ cells) was transferred to a 5 ml culture tube and incubated with 5 ml FITC Annexin V and 5 ml PI for 15 min at room temperature in the dark. Then, 400 ml of 1× binding buffer was added in a 5 ml culture tube.

2.5 Western Blot Analysis

After chemical treatment, cells were washed three times with PBS and lysed with for 30 min on 4°C, followed by centrifugation at 15000 r/min for 10 min on 4°C. Protein concentrations of the supernatants were assayed using the BCA protein assay kit.

According to our method in previous report$^2$, accoraliquots of supernatants were added to SDS denaturing buffer and boiled for 10 min. and transferred electrophoretically onto PVDF membranes. Membranes were blocked with 5% non-fat dry milk in TTBS (Tris-buffered saline containing 0.1% Tween 20) for 1 h at room temperature with shaking and washed four times with TTBS before overnight incubation with the primary antibody at 4 °C. Membranes were washed for 4–5 times, 20 min each, with TTBS and then incubated with the secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature. Proteins were detected with an
enhanced chemiluminescence (ECL) reagent and visualized by ChemiDoc MP imaging system and quantified by densitometry using Quantity One 4.52 software.
3. NMR spectra of the prepared compounds

Figure S2. $^1$H-NMR spectrum in CDCl$_3$ for compound 11a

Figure S3. $^1$H-NMR spectrum in CDCl$_3$ for compound 11b
Figure S4. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 11b

Figure S5. $^1$H-NMR spectrum in CDCl$_3$ for compound 12a
Figure S6. $^1$H-NMR spectrum in CDCl$_3$ for compound 12b

Figure S7. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 12b
Figure S8. $^1$H-NMR spectrum in CDCl$_3$ for compound 13a

Figure S9. $^1$H-NMR spectrum in CDCl$_3$ for compound 13b
Figure S10. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 13b

Figure S11. $^1$H-NMR spectrum in CDCl$_3$ for compound 14a
Figure S12. $^1$H-NMR spectrum in CDCl$_3$ for compound 14b

Figure S13. $^{13}$C -NMR spectrum in CDCl$_3$ for compound 14b
Figure S14. $^1$H-NMR spectrum in CDCl$_3$ for compound 15a

Figure S15. $^1$H-NMR spectrum in CDCl$_3$ for compound 15b
Figure S16. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 15b

Figure S17. $^1$H-NMR spectrum in CDCl$_3$ for compound 16a
Figure S18. $^1$H-NMR spectrum in CDCl$_3$ for compound 16b

Figure S19. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 16b
Figure S20. $^1$H-NMR spectrum in CDCl$_3$ for compound 17a

Figure S21. $^1$H-NMR spectrum in CDCl$_3$ for compound 17b
Figure S22. $^{13}$C-NMR spectrum in CDCl$_3$ for compound 17b

Figure S23. $^1$H-NMR spectrum in CDCl$_3$ for compound 18a
Figure S24. $^1$H-NMR spectrum in CDCl$_3$ for compound 18b

Figure S25. $^1$H-NMR spectrum in CDCl$_3$ for compound 18b
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