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Research Article

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Synthesis and Antitumor Activity of Dehydroepiandrosterone Derivatives on Es-2, A549, and HepG2 Cells *in vitro*

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A series of dehydroepiandrosterone derivatives containing an acid ester was synthesized and evaluated for their antitumor activity on ES-2, A549, and HepG2 cells by the MTT assay. Most compounds showed antitumor activity, while compounds 1c, 2i, and 2o exhibited more potential inhibitory effects compared with dehydroepiandrosterone on ES-2 cells, A549 cells, and HepG2 cells, respectively.

Key words: antitumor activity, dehydroepiandrosterone, synthesis

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Dehydroepiandrosterone (DHEA) a major steroid secreted by the adrenal gland, which decreases with age after adolescence, is available as a nutritional supplement (1) and is the most abundant steroid in humans (2). In addition to serving as a precursor of both androgens and estrogens, DHEA has various other beneficial biological effects. In experimental animals, it decreases body fat without altering food intake, enhances the immune system, suppresses spontaneous decreases in blood glucose in diabetic mice and enhances the memory of old mice (3). In recent years, it has been reported that DHEA possesses an antiproliferative effect on animal tumor models and malignant cell lines (4). Several derivatives of DHEA (3β, 7α, 17β-AET and 3β, 7β, 17β-AET) were evaluated for their antitumor activities, although the mechanisms responsible for the altered tumor growth are not yet established (5).

With an aim to increase the antitumor effects of DHEA, structural modifications were carried out at positions 3 and 17 of DHEA. Two series of derivatives were synthesized through acylation of the hydroxyl group of DHEA and compound **2** that was obtained by reducing the ketone group. The antitumor activities of the derivatives were evaluated against three different human tumor cell lines.

Experimental Section

Material and methods

Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates and developed plates were examined under UV light (254 nm). Column chromatography was performed using Merck 200 mesh silica gel. IR spectra were recorded (in KBr) on a FT-IR1730. ^{1}H NMR spectra were measured on a Bruker AV-300 spectrometer using TMS as an internal standard. Mass spectra were measured on an AXIMA CFR Plus MALDI-TOF (Shimadzu Biotech, Columbia, MD, USA). Elemental analyses for C, H, and N were within ±0.4% of the theoretical values and were performed on a 204Q CHN Rapid Analyzer (Perkin-Elmer, Waltham, MA, USA). The major chemicals were purchased from Aldrich Co. (St. Louis, MO, USA).

DHEA-(3,17)-diol (2)

To a stirred solution of DHEA (2.9 g, 10 mmol) in methanol (50 mL), $NaBH_4$ (0.38 g, 10 mmol) was added in portions. After 2 h, the resulting white solid was collected by filtration under reduced pressure to afford **2** in 90% yield.

General procedure for the synthesis of compounds 1a–1o and 2a–2o

A suspension of DHEA (0.29 g, 1 mmol), acyl acid (1 mmol), DCC (0.41 g, 2 mmol), DMAP (0.12 g, 1 mmol), and KI (0.12 g, 0.72 mmol) in dry dichloromethane (25 mL) was stirred at room temperature for 24 h and filtered (6). The filtrate was washed with 5% $NaHCO_3$ liquor (3 × 15 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 5:1) to afford the corresponding ester. Compounds **2a**–**2o** were prepared by the same method using compound **2** as a reagent. The yield, melting point, and spectra data of each compound are given below.

DHEA-3-yl benzoate (1a)

Yield, 52%; mp 180–182 °C; IR (KBr) per cm: 3267, 2958, 1735, 1712, 1632, 1450, 1378; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86–0.2.51 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.85–4.88 (m, 1H, CH–3), 5.45 (d, J = 3.0 Hz, 1H, CH–6), 7.44 (t, J = 15 Hz, 2H, Ar–H), 7.54 (t, J = 9.0 Hz, 1H, Ar–H), 8.04 (d, J = 6.0 Hz, 2H, Ar–H); MALDI-TOF MS: 393 (M+1); Anal. Calcd for $C_{26}H_{32}O_3$: C, 79.56; H, 8.22. Found: C, 79.44; H, 8.32.

DHEA-3-yl 4-fluorobenzoate (1b)

Yield, 50%; mp 173–175 °C; IR (KBr) per cm: 2947, 1737, 1703, 1649, 1451, 1373;^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.90–2.49 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.85–4.87 (m, 1H, CH–3), 5.46 (d, J = 6.0 Hz, 1H, CH–6), 7.11 (t, J = 7.5 Hz, 2H, Ar–H), 8.05 (t, J = 7.5 Hz, 2H, Ar-H); MALDI-TOF MS: 411 (M+1); Anal. Calcd for $C_{26}H_{31}FO_3$: C, 76.07; H, 7.61. Found: C, 76.21; H, 7.55.

DHEA-3-yl cinnamate (1c)

Yield, 56%; mp 150–150 °C; IR (KBr) per cm: 2941, 1739, 1707, 1635, 1452, 1374; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.90–2.45 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.75–4.77 (m, 1H, CH–3), 5.44 (d, J = 3.0 Hz, 1H, CH–6), 6.44 (d, J = 15 Hz, 1H, CH=C), 7.38–7.54 (m, 5H, Ar–H), 7.69 (d, J = 15 Hz, 1H, C=CH); MALDI-TOF MS: 393 (M+1); Anal. Calcd for $C_{28}H_{34}O_3$: C, 80.35; H, 8.19. Found: C, 80.23; H, 8.22.

DHEA-3-yl 3-(2-chlorophenyl)acrylate (1d)

Yield, 52%; mp 198–200 °C; IR (KBr) per cm: 2946, 1738, 1717, 1590, 1455, 1376; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–2.51 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.71–4.78 (m, 1H, CH–3), 5.44 (d, J = 3.0 Hz, 1H, CH–6), 6.72 (d, J = 15 Hz, 1H, CH=C), 7.28–7.50 (m, 4H, Ar–H), 7.59 (d, J = 15 Hz, 1H, C=CH); MALDI-TOF MS: 453 (M+1); Anal. Calcd for $C_{28}H_{33}ClO_3$: C, 74.24; H, 7.34. Found: C, 74.44; H, 7.22.

DHEA-3-yl acrylate (1e)

Yield, 54%; mp 151–153 °C; IR (KBr) per cm: 2948, 1730, 1717, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.06–2.51 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.68–4.71 (m, 1H, CH–3), 5.42 (d, J = 4.2 Hz, 1H, CH–6), 5.81 (d, J = 10.5 Hz, 1H, C=CH_{2a}), 6.10 (dd, J = 10.5, 10.2 Hz, 1H, C=CH), 6.39 (d, J = 17.4 Hz, 1H, C=CH_{2b}); MALDI-TOF MS: 343 (M+1); Anal. Calcd for $C_{22}H_{30}O_3$: C, 77.16; H, 8.83. Found: C, 77.20; H, 8.81.

DHEA-3-yl but-2-enoate (1f)

Yield, 54%; mp 218–220 °C; IR (KBr) per cm: 2947, 1731, 1714, 1383; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88–2.41 (m, 28H, protons in dehydroepiandrosterone skeleton and CH_3–23), 4.61–4.70 (m, 1H, CH–3), 5.41 (d, J = 4.8 Hz, 1H, CH–6), 5.82 (dd, J = 1.5, 3 Hz, 1H, C=CH), 6.94 (m, 1H, CH=C); MALDI-TOF MS: 357 (M+1); Anal. Calcd for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.40; H, 9.15.

DHEA-3-yl hexa-2,4-dienoate (1g)

Yield, 52%; mp 175–177 °C; IR (KBr) per cm: 2962, 1741, 1712, 1647, 1620, 1375; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88–2.44 (m, 30H, protons in dehydroepiandrosterone skeleton and CH_3–25 and CH_2), 4.64–4.72 (m, 1H, CH–3), 5.41 (d, J = 4.8 Hz, 1H, CH–6), 5.75 (m, 1H, C=CH), 6.15 (m, 2H, CH=CH), 7.24 (m, 1H, C=CH); MALDI-TOF MS: 383 (M+1); Anal. Calcd for $C_{25}H_{34}O_3$: C, 78.49; H, 8.96. Found: C, 78.51; H, 8.86.

DHEA-3-yl pent-4-enoate (1h)

Yield, 55%; mp 168–170 °C; IR (KBr) per cm: 2947, 1737, 1703, 1649, 1375; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–2.48 (m, 29H, protons in dehydroepiandrosterone skeleton and 4H,CH_2–21, 22), 4.61–4.64 (m, 1H, CH–3), 4.98–5.89 (m, 2H, C=CH_2), 5.18 (d, J = 3.2 Hz, 1H, CH–6), 5.83 (m, 1H,CH=C); MALDI-TOF MS: 371 (M+1); Anal. Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 77.85; H, 9.15.

DHEA-3-yl 2-methylbut-2-enoate (1i)

Yield, 52%; mp 165–167 °C; IR (KBr) per cm: 2962, 2945, 1739, 1707, 1649, 1375; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–2.50 (m, 31H, protons in dehydroepiandrosterone skeleton and 6H, CH_3–23, 24), 4.66 (m, 1H, CH–3), 5.40 (d, J = 3.0 Hz, 1H, CH–6), 6.83–6.84 (m, CH=C); MALDI-TOF MS: 371 (M+1); Anal. Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 77.88; H, 9.10.

DHEA-3-yl 2-methylpent-2-enoate (1j)

Yield, 50%; mp 161–162 °C; IR (KBr) per cm: 2959, 2935, 1736, 1710, 1634, 1377; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–2.44 (m, 33H, protons in dehydroepiandrosterone skeleton and 6H, CH_3–24, 25 and 2H, CH_2–23), 4.65–4.68 (m, 1H, CH–3), 5.40 (d, J = 1.5 Hz, 1H, CH–6), 6.70–6.75 (m, CH=C); MALDI-TOF MS: 385 (M+1); Anal. Calcd for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.28; H, 9.50.

DHEA-3-yl hex-2-enoate (1k)

Yield, 56%; mp 143–145 °C; IR (KBr) per cm: 2961, 2955, 1735, 1717, 1639, 1381; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88–2.41 (m, 32H, protons in dehydroepiandrosterone skeleton and 3H, CH_3–25 and 4H, CH_2–23, 24), 4.61–4.65 (m, 1H, CH–3), 5.40 (d, J = 2.4 Hz, 1H, CH–6), 5.78–5.82 (m, C=CH–22), 6.92–6.97 (m, C=CH–21); MALDI-TOF MS: 385 (M+1); Anal. Calcd for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.05; H, 9.40.

DHEA-3-yl undec-10-enoate (1l)

Yield, 54%; mp oil; IR (KBr) per cm: 2964, 2945, 1737, 1718, 1635, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88–2.34 (m, 41H, protons in dehydroepiandrosterone skeleton and 16H, CH_2), 4.58–4.63 (m, 1H, CH–3), 4.91–4.95 (m, 2H, C=CH_2), 5.40 (d, J = 3 Hz, 1H, CH–6), 5.76–5.85 (m, 1H, CH=C); MALDI-TOF MS: 455 (M+1); Anal. Calcd for $C_{30}H_{46}O_3$: C, 79.25; H, 10.20. Found: C, 79.15; H, 10.40.

DHEA-3-yl octadeca-9,12-dienoate (1m)

Yield, 58%; mp oil; IR (KBr) per cm: 2962, 2943, 1735, 1716, 1632, 1381; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86 (s, 3H, CH_3–37), 0.87–2.40 (m, 47H, protons in dehydroepiandrosterone skeleton and 22H, CH_2), 2.74–2.75 (m, 2H, CH_2–30), 4.58–4.64 (m, 1H, CH–3), 5.27–5.57 (m, 5H, CH=C, C–6, 28, 29, 31, 32); MALDI-TOF MS: 551 (M+1); Anal. Calcd for $C_{37}H_{58}O_3$: C, 80.67; H, 10.61. Found: C, 79.89; H, 10.80.

DHEA-3-yl octadec-9-enoate (1n)

Yield, 51%; mp oil; IR (KBr) per cm: 2959, 2941, 1732, 1713, 1630, 1379; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85 (s, 3H, CH_3–37), 0.88–2.77 (m, 53H, protons in dehydroepiandrosterone skeleton and 28H, CH_2), 4.59–4.62 (m, 1H, CH–3), 5.33–5.41 (m, 3H, CH=C, C–6, 28, 29); MALDI-TOF MS: 553 (M+1); Anal. Calcd for $C_{37}H_{58}O_3$: C, 80.38; H, 10.95. Found: C, 80.22; H, 10.96.

DHEA-3-yl henicos-13-enoate (1o)

Yield, 60%; mp iol; IR (KBr) per cm: 2954, 2945, 1729, 1708, 1630, 1378; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86 (s, 3H, CH_3–37), 0.89–2.51 (m, 61H, protons in dehydroepiandrosterone skeleton and 36H, CH_2), 4.60–4.63 (m, 1H, CH–3), 5.33–5.40 (m, 3H, CH=C, C–6, 32, 33); MALDI-TOF MS: 609 (M+1); Anal. Calcd for $C_{41}H_{68}O_3$: C, 80.86; H, 11.25. Found: C, 80.92; H, 11.05.

(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl dibenzoate (2a)

Yield, 52%; mp 135–137 °C; IR (KBr) per cm: 3266, 2959, 1733, 1710, 1631, 1451, 1381; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.96–3.02 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.83–4.89 (m, 2H, CH–3, 17), 5.45 (d, J = 3.0 Hz, 1H, CH–6), 7.44 (m, 4H, Ar–H), 7.54 (m, Hz, 2H, Ar–H), 8.05 (m, 4H, Ar–H). MALDI-TOF MS: 499 (M+1); Anal. Calcd for $C_{33}H_{38}O_4$: C, 79.48; H, 7.68. Found: C, 79.21; H, 7.55.

(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(4-fluorobenzoate) (2b)

Yield, 50%; mp 161–163 °C; IR (KBr) per cm: 2950, 1737, 1703, 1649, 1451, 1373; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.96–2.48 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.85–4.87 (m, 2H, CH–3, 17), 5.44 (d, J = 2.7 Hz, 1H, CH–6), 7.11 (m, 4H, Ar–H), 8.05 (m, 4H, Ar–H); MALDI-TOF MS: 535 (M+1); Anal. Calcd for $C_{33}H_{38}O_4$: C, 74.14; H, 6.79. Found: C, 74.22; H, 6.65.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(3-phenylacrylate) (2c)

Yield, 53%; mp 150–152 °C; IR (KBr) per cm: 2964, 1730, 1713, 1645, 1450, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89–2.44 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.74–4.79 (m, 2H, CH–3, 17), 5.43 (d, J = 3.0 Hz, 1H, CH–6), 6.40–6.48 (dd, J = 6.0, 9 Hz, 2H, CH–21, 30), 7.38–7.70 (m, 10H, Ar–H); MALDI-TOF MS: 551 (M+1); Anal. Calcd for $C_{37}H_{42}O_4$: C, 80.69; H, 7.69. Found: C, 80.72; H, 7.65.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(3-(2-chlorophenyl)acrylate) (2d)

Yield, 52%; mp 198–200 °C; IR (KBr) per cm: 2959, 1732, 1721, 1654, 1451, 1337; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85–2.42 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.72–4.78 (m, 2H, CH–3, 17), 5.41 (d, J = 2.1 Hz, 1H, CH–6), 6.39–6.48 (dd, J = 9.0, 6 Hz, 2H, CH–21, 30), 7.28–7.41 (m, 8H, Ar–H); 7.57–7.62 (m, 2H, CH–22, 31); MALDI-TOF MS: 619 (M+1); Anal. Calcd for $C_{37}H_{40}Cl_2O_4$: C, 71.72; H, 6.51. Found: C, 71.61; H, 6.55.

(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl diacrylate (2e)

Yield, 49%; mp 113–115 °C; IR (KBr) per cm: 2945, 1732, 1721, 1383; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.84–2.39 (m, 25H, protons in dehydroepiandrosterone skeleton), 4.67–4.72 (m, 2H, CH–3, 17), 5.40 (d, J = 2.4 Hz, 1H, CH–6), 5.80 (d, J = 12 Hz, 2H, C=CH–22a, 25b), 6.07–6.16 (m, 2H, C=CH–21, 24), 6.38 (d, J = 15 Hz, 2H, C=CH–22b, 25b); MALDI-TOF MS: 399 (M+1); Anal. Calcd for $C_{37}H_{40}O_4$: C, 75.34; H, 8.60. Found: C, 75.14; H, 8.55.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(but-2-enoate) (2f)

Yield, 57%; mp 132–134 °C; IR (KBr) per cm: 2962, 1735, 1710, 1385; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–2.36 (m, 31H, protons in dehydroepiandrosterone skeleton and 6H, CH_3–23, 27), 4.63–4.69 (m, 2H, CH–3, 17), 5.38 (d, J = 6 Hz, 1H, CH–6), 5.78–5.87 (m, 2H, C=CH–21, 25), 6.91–6.99 (m, 2H, C=CH–23, 26); MALDI-TOF MS: 427 (M+1); Anal. Calcd for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 76.14; H, 8.95.

(2E,2′E,4E,4′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(hexa-2,4-dienoate) (2g)

Yield, 53%; mp 181–183 °C; IR (KBr) per cm: 2964, 1737, 1721, 1647, 1622, 1381; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–2.42 (m, 31H, protons in dehydroepiandrosterone skeleton and 6H, CH_3–23, 27), 4.73–4.76 (m, 2H, CH–3, 17), 5.43 (d, J = 3 Hz, 1H, CH–6), 6.39–6.48 (m, 2H, C=CH–24, 30), 7.26–7.38 (m, 4H, C=CH–21, 23, 27, 29), 7.52–7.63 (m, 2H, C=CH–24, 28); MALDI-TOF MS: 479 (M+1); Anal. Calcd for $C_{31}H_{42}O_4$: C, 77.79; H, 8.84. Found: C, 78.04; H, 8.45.

(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(pent-4-enoate) (2h)

Yield, 50%; mp 143–145 °C; IR (KBr) per cm: 2946, 1735, 1713, 1639, 1382; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80–2.39 (m, 33H, protons in dehydroepiandrosterone skeleton and 8H, CH_2–21, 22, 26, 27), 4.58–4.64 (m, 2H, CH–3, 17), 4.98–5.08 (m, 4H, C=CH–24, 29), 5.37 (d, J = 3.6 Hz, 1H, CH–6), 5.78–5.86 (m, 2H, C=CH–23, 28); MALDI-TOF MS: 455 (M+1); Anal. Calcd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 75.98; H, 9.54.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(2-methylbut-2-enoate) (2i)

Yield, 52%; mp 198–200 °C; IR (KBr) per cm: 2965, 2943, 1738, 1717, 1645, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85–2.37 (m, 37H, protons in dehydroepiandrosterone skeleton and 12H, CH_3–23, 24, 28, 29), 4.61–4.67 (m, 2H, CH–3, 17), 5.37 (d, J = 3.0 Hz, 1H, CH–6), 6.82–6.83 (m, 2H, C=CH–22, 27); MALDI-TOF MS: 455 (M+1); Anal. Calcd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.54; H, 9.32.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(2-methylpent-2-enoate) (2j)

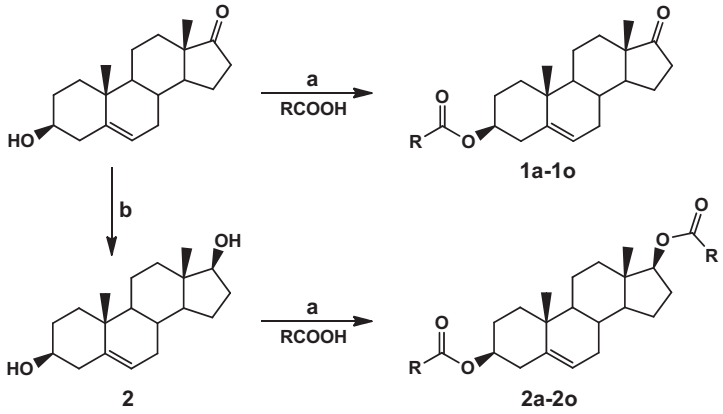
Yield, 57%; mp 123–125 °C; IR (KBr) per cm: 2952, 2939, 1736, 1715, 1630, 1379; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–2.37 (m, 37H, protons in dehydroepiandrosterone skeleton and 12H, CH_3–24, 25, 30, 31), 2.35–2.37 (m, 4H, CH_2–23, 29), 4.61–4.67 (m, 2H, CH–3,17), 5.38 (d, J = 3.0 Hz, 1H, CH–6), 6.72–6.96 (m, 2H, C=CH–22, 28); MALDI-TOF MS: 483 (M+1); Anal. Calcd for $C_{31}H_{46}O_4$: C, 77.14; H, 9.61. Found: C, 77.98; H, 9.23.

(2E,2′E)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl hex-2-enoate (2k)

Yield, 52%; mp 154–156 °C; IR (KBr) per cm: 2965, 2945, 1735, 1718, 1642, 1379; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83–2.37 (m, 39H, protons in dehydroepiandrosterone skeleton and 6H, CH_3–25, 31 and 8H, CH_2–23, 24, 28, 29), 4.62–4.67 (m, 2H, CH–3, 17), 5.39 (d, J = 2.1 Hz, 1H, CH–6), 5.76–5.84 (m, 2H, C=CH–21, 27), 6.92–6.97 (m, 2H, C=CH–22, 28); MALDI-TOF MS: 483 (M+1); Anal. Calcd for $C_{31}H_{46}O_4$: C, 77.14; H, 9.61. Found: C, 76.98; H, 9.65.

(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(undec-10-enoate) (2l)

Yield, 52%; mp oi; IR (KBr) per cm: 2961, 2947, 1738, 1719, 1638, 1382; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81–2.32 (m, 57H, protons in dehydroepiandrosterone skeleton and 16H, CH_2), 4.58–4.63 (m, 2H, CH–3, 17); 4.91–4.95 (m, 4H, C=CH–30, 41), 5.37 (d, J = 4.5 Hz, 1H, CH–6), 5.74–5.85 (m, 2H, C=CH–29, 40). MALDI-TOF MS: 623 (M+1); Anal. Calcd for $C_{41}H_{66}O_4$: C, 79.05; H, 10.68. Found: C, 79.15; H, 10.54.



R
a: C_6H_5-
b: *p-F*-C_6H_4-
c: C_6H_5-CH=CH-
d: *m-Cl*-C_6H_4-CH=CH-
e: CH_2=CH-
f: CH_3CH=CH-
g: CH_3CH=$CHCH$=CH-
h: CH_2=$CHCH_2CH_2$-
i: CH_3CH=$C(CH_3)$-
j: CH_3CH_2CH=$C(CH_3)$-
k: $CH_3CH_2CH_2CH$=CH-
l: CH_2=$CH(CH_2)_7CH_2$-
m: $CH_3(CH_2)_4CH$=$CHCH_2CH$=$CH(CH_2)_6CH_2$-
n: $CH_3(CH_2)_7CH$=$CH(CH_2)_6CH_2$-
o: $CH_3(CH_2)_7CH$=$CH(CH_2)_{10}CH_2$-

Scheme 1: Synthetic scheme for the synthesis of compounds **1a–1o**, **2a–2o**. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2; (b) $NaBH_4$, MeOH.

(9Z,9′Z,12Z,12′Z)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(octadeca-9,12-dienoate) (2m)

Yield, 49%; mp oil; IR (KBr) per cm: 2966, 2948, 1735, 1720, 1636, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.79–2.32 (m, 75H, protons in dehydroepiandrosterone skeleton and 44H, CH_2 and 6H, CH_3–37, 55), 2.74–2.78 (m, 4H, CH_2–30, 48), 4.58–4.64 (m, 2H, CH–3, 17), 5.28–5.36 (m, 9H, C=CH–6, 28, 29, 31, 32, 46, 47, 49, 50); MALDI-TOF MS: 815 (M+1); Anal. Calcd for $C_{55}H_{90}O_4$: C, 81.02; H, 11.13 Found: C, 81.05; H, 11.24.

(9Z,9′Z)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(octadec-9-enoate) (2n)

Yield, 55%; mp oil; IR (KBr) per cm: 2963, 2938, 1731, 1715, 1630, 1378; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80–2.29 (m, 87H, protons in dehydroepiandrosterone skeleton and 56H, CH_2 and 6H, CH_3–37, 55), 4.59–4.63 (m, 2H, CH–3,17), 5.30–5.34 (m, 5H, C=CH–6, 28, 29, 46, 47); MALDI-TOF MS: 819 (M+1); Anal. Calcd for $C_{55}H_{94}O_4$: C, 81.63; H, 11.56 Found: C, 81.75; H, 11.55.

(13Z,13′Z)-(3S,8R,9S,10R,13S,14S,17S)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,17-diyl bis(docos-13-enoate) (2o)

Yield, 56%; mp oil; IR (KBr) per cm: 2965, 2945, 1734, 1710, 1632, 1378; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81–2.31 (m, 103H, protons in dehydroepiandrosterone skeleton and 72H, CH_2 and 6H, CH_3–37, 55), 4.58–4.62 (m, 2H, CH–3, 17), 5.32–5.35 (m, 5H, C=CH–6, 32, 33, 54, 55); MALDI-TOF MS: 932 (M+1); Anal. Calcd for $C_{63}H_{110}O_4$: C, 81.23; H, 11.90 Found: C, 81.35; H, 11.73.

Cell culture

The ovarian cancer ES-2 cells, human hepatocellular carcinoma HepG2 cells, and human lung tumor A-549 cells were routinely maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin and streptomycin antibiotics in a humidified atmosphere containing 5% CO_2 at 37 °C. Cell culture plates were washed to remove non-adherent cells, and adherent cells were over 95% viable as determined with a trypan blue dye exclusion test. The medium was changed every 2 days, and cells were passaged after treatment with a solution of 0.05% trypsin/0.02% EDTA.

Evaluation of antitumor activities in vitro

The antitumor activities of the thirty synthesized compounds were evaluated against the three different human tumor cell lines, ovarian cancer cells (ES-2), human lung tumor cells (A549), and human hepatocellular liver carcinoma cells (HepG2), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (7), which is based on the ability of glycolytic pathway enzymes to cleave MTT to the blue compound formazan. MTT was added during the last 3 h of incubation. The reduction of MTT to formazan was measured using a precision microplate reader at 492 nm. The

Table 1: The growth inhibitory (IC_{50} ± SD, μM, 48 h) of DHEA, **1a**–**1o** and **2a**–**2o** in ES-2, A549 and HepG2 cells. $p < 0.05$

	IC_{50} (μM)		
Compound	ES-2	A549	Hep G2
1a	22.5 ± 0.7	24.6 ± 0.4	>100
1b	43.2 ± 0.2	21.3 ± 0.3	>100
1c	23.6 ± 0.3	8.6 ± 0.6	>100
1d	31.8 ± 0.1	27.1 ± 0.3	>100
1e	11.6 ± 0.4	31.1 ± 0.9	>100
1f	10.7 ± 0.5	18.5 ± 0.4	>100
1g	19.4 ± 0.3	25.5 ± 0.7	>100
1h	21.8 ± 0.5	51.2 ± 0.5	>100
1i	12.2 ± 0.4	37.8 ± 0.4	>100
1j	34.6 ± 0.3	>100	>100
1k	14.7 ± 0.4	12.1 ± 0.3	>100
1l	26.8 ± 0.6	20.2 ± 0.2	>100
1m	22.8 ± 0.8	13.9 ± 0.1	>100
1n	22.9 ± 0.7	17.1 ± 0.2	>100
1o	>100	27.2 ± 0.3	>100
2a	18.3 ± 0.4	22.1 ± 0.4	15.4 ± 0.4
2b	17.0 ± 0.3	21.6 ± 0.5	24.5 ± 0.3
2c	12.3 ± 0.4	>100	21.2 ± 0.2
2d	14.5 ± 0.7	>100	11.1 ± 0.9
2e	22.6 ± 0.1	>100	27.2 ± 0.3
2f	16.1 ± 0.5	21.2 ± 0.1	10.9 ± 0.1
2g	26.5 ± 0.2	>100	15.5 ± 0.3
2h	15.9 ± 0.1	>100	17.6 ± 0.8
2i	6.9 ± 0.2	>100	11.7 ± 0.1
2j	16.4 ± 0.3	>100	19.2 ± 0.5
2k	23.8 ± 0.4	>100	10.6 ± 0.4
2l	26.7 ± 0.2	25.5 ± 0.4	18.0 ± 0.6
2m	18.2 ± 0.8	>100	13.2 ± 0.5
2n	18.9 ± 0.1	17.2 ± 0.2	11.2 ± 0.6
2o	17.7 ± 0.6	>100	9.1 ± 0.3
DHEA	17.5 ± 0.3	18.8 ± 0.4	18.3 ± 0.5

DHEA, Dehydroepiandrosterone.

Table 2: Apoptosis induction was determined by FACS. Es-2 cells were treated with **2i** (6.99 μM), A549 cells were treated with **1c** (8.65 μM) and 2 cells were treated with **2o** (9.17 μM).The compounds were administrated with the indicated concentrations for 48 h. Apoptotic cells were determined after propidium iodide staining

	Ap (%)		
Compounds	Es-2	A549	HepG2
Normal	2.36	0.33	2.47
2i	11.44	–	–
1c	–	17.70	–
2o	–	–	28.81

Ap, apoptotic cells. (–) The experiment did not test.

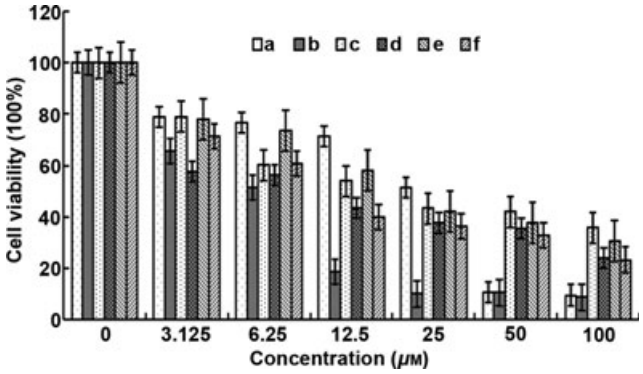


Figure 1: Dose-dependent growth inhibitory of compounds Dehydroepiandrosterone (DHEA), **2i**, **1c** and **2o** on different human tumor cells. Cells were treated with indicated compounds for 48 h. Cell viability was measured by MTT assay and calculated as percent of control untreated cultures. $p < 0.05$. a: Dose-dependent growth inhibitory of compound DHEA on Es-2 cells. b: Dose-dependent growth inhibitory of compound **2i** on Es-2 cells. c: Dose-dependent growth inhibitory of compound DHEA on A549 cells. d: Dose-dependent growth inhibitory of compound **1c** on A549 cells. e: Dose-dependent growth inhibitory of compound DHEA on HepG2 cells. f: Dose-dependent growth inhibitory of compound **2o** on HepG2 cells.

compounds were solubilized in DMSO, and a sample of each solution was added to the medium. The cancer cells were cultured and incubated with test compounds at concentrations ranging from 0 to 100 μM for 48 h. The MTT assay was performed in triplicate for each compound. Data were expressed as mean ± SD. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Dunnett's contrast. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Chemistry

The synthesis of compounds **1a**–**1o** and **2a**–**2o** were presented in Scheme 1. Dehydroepiandrosterone was treated with various unsaturated fatty acids (or arylcarboxylic acids) in dry dichloromethane in the presence of DCC and DMAP at room temperature (24 h) to afford a moderate yields of the corresponding esters **1a**–**1o**. Compound **2** was obtained by reducing compound **1** with $NaBH_4$ in methanol, followed by acylation with unsaturated fatty acids to give the DHEA esters **2a**–**2o**.

Biological results and discussion

As shown in Table 1, most tested compounds showed antitumor effects on the three cell lines. For the ES-2 cells, all the compounds, except **1o** ($IC_{50} > 100$ μM) which has the longest carbon chain acyl group at the 3-position, showed activity and eleven compounds exhibited more potent antitumor effects than DHEA (IC_{50}, 17.56 μM). Compound **2i** showed the most potent activity (IC_{50}, 6.99 μM), near 2.5 times better than DHEA. Among the compounds, which contained a phenyl ring, the bis-acids esters (**2a**–**2d**) exhibited more efficacy than mono-acylated esters (**1a**–**1d**). For the unsaturated fatty acid esters, no significant differences were found between the mono and bis-acids derivatives.

For the A549 cells, all of the mono-acylated compounds displayed antitumor effects. Compound **1c**, a cinnamic acid ester, showed the most potent growth inhibitory effects ($IC_{50} = 8.65$ μM). For the bis-acids derivatives, five compounds showed activity and only **2n** exhibited more potent than DHEA ($IC_{50} = 18.86$ μM).

For the HepG2 cells, most bis-acids derivatives showed more potent growth inhibitory activity than DHEA, while the mono-acylated esters showed no potency. Compound **2o,** bearing the longest carbon chain acyl group, showed the most effective cytotoxic effect ($IC_{50} = 9.17$ μM).

The apoptotic effects of these compounds were further confirmed by subG1 induction, which was determined by flow cytometry (8). As shown in Table 2, the subG1 phase (apoptotic cells) induced by compound **2i** (6.99 μM), **1c** (8.65 μM), and **2o** (9.17 μM) were 11.44%, 17.70% and 28.81%, respectively, after 48 h treatment. The dose-dependent growth inhibition of the most effective compounds **2i**, **1c,** and **2o** are shown in Figure 1. These compounds were more potent inhibitors than DHEA at the same concentration.

Conclusions

In conclusion, we have synthesized two series of DHEA derivatives and investigated their growth inhibitory activity against three different human tumor cell lines. Several derivatives demonstrated stronger potency than DHEA. The most promising compounds in this series were **2i**, **1c,** and **2o**. Further investigations into optimizing the inhibitory activity of these three compounds are in progress.

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