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A Facile and Efficient Synthesis of Some (6E)-Hydroximino-4-En-3-One Steroids, Steroidal Oximes From Cinachyrella spp. Sponges

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A facile and efficient synthesis of some (6E)-hydroximino-4-en-3-one steroids, steroidal oximes from *Cinachyrella* spp. sponges

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Introduction

A variety of steroids with unusual and interesting structures have been isolated from marine sponges, recently [1–3]. Among these steroidal compounds, marine steroids with oxime groups have been reported rarely. In 1997, two steroidal oximes, (6E)-hydroximino-24- ethylcholest-4-en-3-one (**1**) and (6E)-hydroximinocholest-4-en-3-one (**2**), were isolated from *Cinachyrella alloclada* and *C. apion* [4]. In 2005, another steroidal oxime, (3E)-hydroximinocholest-4-en-6-one (**3**), was isolated from *Cinachyrella australiensis* [5]. These steroids exert interesting biological activities [6]. For example, bioassays showed that compound **3** functions against hepatitis virus *in vitro* [5] and compound **2** exhibitted a selective cytotoxic activity against several types of cancer cells such as P-388, A-549, HT-29 (IC$_{50}$: 1.25 µg/mL) and MEL-28 tumor cells (IC$_{50}$: 2.5 µg/mL) [7]. In this paper, a facile and efficient synthetic method for the compound **1**, **2** and **4** is reported.

1   2   3   4
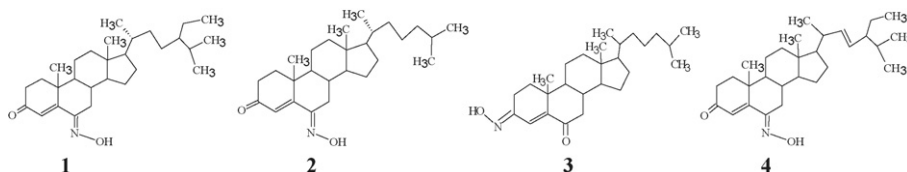
Experimental

Chemistry

The sterol and NaBH$_4$ were purchased from the Merck Co. All chemicals and solvents were analytical grade and solvents were purified by general methods before being used. Melting points were determined on an X$_4$ apparatus and were uncorrected. Infrared spectra were measured with a Nicolet FT-360 Spectrophotometer. The ^{1}H and ^{13}CNMR spectra were recorded in CDCl$_3$ on a Bruker AV-500 spectrometer at working frequencies 500 and 125 MHz, respectively. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. LREIMS were recorded on a Thermo-DSQ instrument, while HREIMS were measured on a Thermo-MAT95XP instrument. The cell proliferation assay was undertaken by a MTS method using 96-well plates on Beckman coulter LD400 AD/LD analysis spectrometer.

24-Ethylcholest-4-en-3,6-dione (6a)

Pyridinium chlorochromate (PCC) (2.564 g, 11.9 mmol) was added to a solution of sitosterol (5a) (0.852 g, 0.50 mmol) in dried CH$_2$Cl$_2$ (40 mL) in one portion at room temperature. The reaction was completed in 26 h. To the mixture was then added 30 mL of CH$_2$Cl$_2$, and the suspension was poured over a silica gel column and eluted with CH$_2$Cl$_2$. The resulting solution was washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the resulting crude product was purified by chromatography on silica gel using petroleum ether (60–90 °C)/EtOAc (5:1) as eluent to give 0.75 g (86%) of 6a as pale yellow crystals, $\theta_{m.p.}$ 172–174 °C. IR (KBr) v: 2959, 1683, 1601, 1581, 1461, 1377, 1246, 1124, 948, 871 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): 0.724 (s, 3H, 18-CH$_3$), 0.816 (d, 3H, J = 7.0, 26- or 27-CH$_3$), 0.841 (d, 3H, J = 7.0, 26- or 27-CH$_3$), 0.848 (t, 3H, J = 8.0, 29-CH$_3$), 0.935 (d, 3H, J = 6.5, 21-CH$_3$), 1.167 (s, 3H, 19-CH$_3$), 2.13–2.17 (m, 1H, C$_2$-αH), 2.44–2.58 (m, 2H, C$_7$-βH and C$_2$-βH), 2.682 (dd, 1H, J = 4.5, 15.5, C$_7$-αH), 6.170 (s, 1H, C$_4$-H).

Cholest-4-en-3,6-dione (6b)

PCC (2.564 g, 11.87 mmol) was added to a solution of cholesterol (0.924 g, 2.2 mmol) in dried CH$_2$Cl$_2$ (40 mL) in one portion at room temperature. The reaction was complete in 28 h. The workup similar to 6a provided 0.795 g (83.5%) of 6b as pale yellow crystals, $\theta_{m.p.}$ 90–91 °C; IR(KBr) v: 2953, 2865, 1693, 1600, 1486, 1249, 1221, 1117, 942 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$): 0.746 (s, 3H, 18-CH$_3$), 0.886 (d, 3H, J = 6.4, 26 or 27-CH$_3$), 0.899 (d, 3H, J = 6.4, 26 or 27-CH$_3$), 0.952 (d, 3H, J = 6.5, 21-CH$_3$), 1.172 (s, 3H, 19-CH$_3$), 2.546 (dd, 1H, J = 5.2, 14.6, C$_2$-βH), 2.706 (dd, 1H, J = 4.0, 16.0, C$_7$-αH), 6.196 (s, 1H, C$_4$-H).

Stigmast-4,22-dien-3,6-dione (6c)

6c was prepared similarly according to the procedure of 6a. PCC (1.30 g, 6.0 mmol) was added to a solution of stigmasterol (0.50 g, 1.2 mmol) in dried CH$_2$Cl$_2$ (10 mL) in one portion at room temperature. The reaction was complete in 27 h. The workup similar to 6a gave 0.42 g (83%) of 6c as pale yellow crystals, $\theta_{m.p.}$ 134–135 °C; IR(KBr)v: 2959, 1714, 1686, 1609, 969, 864 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): 0.743 (s, 3H, 18-CH$_3$), 0.805

(t, 3H, J = 7.0, 29-CH$_3$), 0.798 (d, 3H, J = 6.5, 26- or 27-CH$_3$), 0.849 (d, 3H, J = 6.5, 26- or 27-CH$_3$), 1.036 (d, 3H, J = 7.0, 21-CH$_3$), 1.169 (s, 3H, 19-CH$_3$), 5.040 (dd, 1H, J = 9.0, 15.2, C$_{22}$-H), 5.150 (dd, 1H, J = 8.5, 15.2, C$_{23}$-H), 6.171 (s, 1H, C$_4$-H).

24-Ethylcholest-4-en-3β-ol-6-one (7a)

NaBH$_4$ (30 mg, 0.79 mmol) was added to a solution of 6a (110 mg, 0.25 mmol) and CoCl$_2$.6H$_2$O (61 mg, 0.26 mmol) in CH$_3$OH (15 mL) in the interval of 8 min at room temperature. After 15 min, the reaction was stopped. The solution was neutralized with 1 M HCl. After evaporation of the majority of the MeOH under reduced pressure, ethyl acetate (30 mL) was added to the residue. The resulting solution was washed with cold water and saturated brines. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (2:1) as the eluent. The 7a was obtained as a white solid (98 mg, 87%), $\theta_{m.p.}$ 140–141 °C; IR(KBr) v: 3428, 2962, 2929, 2868, 1716, 1659, 1462, 1377, 1074, 968 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): 0.735 (s, 3H, 18-CH$_3$), 0.839 (d, 3H, J = 7.5, 26-CH$_3$ or 27-CH$_3$), 0.861 (d, 3H, J = 7.5, 26-CH$_3$ or 27-CH$_3$), 0.869 (t, 3H, J = 8.0, 29-CH$_3$), 0.943 (d, 3H, J = 6.0, 21-CH$_3$), 1.202 (s, 3H, 19-CH$_3$), 4.342 (m, 1H, C$_3$-αH), 6.193 (s, 1H, C$_4$-CH).

Cholest-4-en-3β-ol-6-one (7b)

Yield 88.2%, $\theta_{m.p.}$ 125–126 °C; IR(KBr) v: 3391, 2946, 2868, 1708, 1663, 1462, 1373, 1274, 1123, 1070, 960, 878 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): 0.732 (s, 3H, 18-CH$_3$), 0.882 (d, 3H, J = 6.2, 26-CH$_3$ or 27-CH$_3$), 0.895 (d, 3H, J = 6.2, 26-CH$_3$ or 27-CH$_3$), 0.935 (d, 3H, J = 6.5, 21-CH$_3$), 1.184 (s, 3H, 19-CH$_3$), 4.306 (m, 1H, C$_3$-αH), 6.193 (d, J = 1.2, C$_4$-H).

24-Ethylcholest-4,22-dien-3β-ol-6-one (7c)

Yield 90%, $\theta_{m.p.}$ 175–176 °C; IR(KBr) v: 3457, 2954, 2860, 1711, 1659, 1458, 1385, 1279, 1074, 972 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.730 (s, 3H, 18-CH$_3$), 0.797 (d, 3H, J = 6.5, 26-CH$_3$ or 27-CH$_3$), 0.848 (d, 3H, J = 6.5, 26-CH$_3$ or 27-CH$_3$), 0.804 (t, 3H, J = 7.5, 29-CH$_3$), 1.019 (d, 3H, J = 6.5, 21-CH$_3$), 1.184 (s, 3H, 19-CH$_3$), 4.329 (ddd, 1H, J = 1.5, 5.5, 12.0, C$_3$-αH), 5.026 (dd, 1H, J = 8.5, 15.0, C$_{22}$-H), 5.143 (dd, 1H, J = 8.5, 15.0, C$_{23}$-H), 6.167 (d, 1H, J = 1.5, C$_4$-H).

(6E)-Hydroximino-24-ethylcholest-4-en-3β-ol (8a)

7a (150 mg, 0.40 mmol) was dissolved in 15 mL 95% CH$_3$CH$_2$OH. After the mixture was heated to 55 °C, CH$_3$COONa·3H$_2$O (95 mg, 0.70 mmol) and NH$_2$OH• HCl (60.6 mg, 0.87 mmol) were added. The mixture was stirred at the temperature for 1.5 h. Then the reaction was terminated and the majority of solvent was evaporated under reduced pressure. Proper water was added into the reaction mixture, and the product was extracted with ethyl acetate (3 mL $\times$ 20 mL). The combined extracts were washed with saturated brine, dried, and evaporated under reduced pressure. The residue was subjected to chromatography to give 109 mg of 8a (70.3%), $\theta_{m.p.}$ 91–92 °C; IR(KBr) v: 3428, 2958, 2933, 2868, 1634, 1462, 1377 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.722 (s, 3H, 18-CH$_3$), 0.839 (d, 3H, J = 7.0, 26-CH$_3$ or 27-CH$_3$), 0.861 (d, 3H, J = 7.0, 26-CH$_3$ or 27-CH$_3$), 0.870 (t, 3H, J = 8.0, 29-CH$_3$), 0.940 (d, 3H, J = 6.5, 21-CH$_3$), 1.077 (s, 3H, 19-CH$_3$), 3.064 (dd, 1H, J = 3.0, 14.0, C$_7$-βH), 4.284 (ddd, 1H, J = 1.0,

3.5, 11.0, C_3-αH), 6.243 (s, 1H, C_4-H); ^{13}C NMR(CDCl$_3$, 125 MHz): 35.2 (1-C), 29.7 (2-C), 69.1 (3-C), 113.8 (4-C), 156.7 (5-C), 156.9 (6-C), 28.1 (7-C), 33.9 (8-C), 53.6 (9-C), 41.7 (10-C), 21.4 (11-C), 39.7 (12-C), 42.5 (13-C), 56.1 (14-C), 23.1 (15-C), 26.2 (16-C), 55.8 (17-C), 11.9 (18-C), 19.8 (19-C), 36.1 (20-C), 18.7 (21-C), 34.2 (22-C), 24.1 (23-C), 45.9 (24-C), 29.2 (25-C), 18.4 (26-C), 19.0 (27-C), 24.2 (28-C), 12.0 (29-C).

In the reaction, the 6Z-isomer **9a** of **8a** was obtained in 3% yield, $\theta_{m.p.}$ 119–121 °C; IR(KBr) ν: 3412, 2958, 2938, 2872, 1630, 1462, 1377, 1123, 911 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.719 (s, 3H, 18-CH$_3$), 0.839 (d, 3H, $J=7.0$, 26-CH$_3$ or 27-CH$_3$), 0.861 (d, 3H, $J=7.0$, 26-CH$_3$ or 27-CH$_3$), 0.869 (t, 3H, $J=8.0$, 29-CH$_3$), 0.937 (d, 3H, $J=6.0$, 21-CH$_3$), 1.109 (s, 3H, 19-CH$_3$), 2.333-2.370 (m, 2H, C_7-H), 4.322 (dd, 1H, $J=4.5$, 12.0, C_3-αH), 6.922 (s, 1H, C_4-H); ^{13}C NMR(CDCl$_3$, 125 MHz): 36.1 (1-C), 29.2 (2-C), 69.3 (3-C), 113.9 (4-C), 153.7 (5-C), 160.5 (6-C), 29.7 (7-C), 33.9 (8-C), 53.8 (9-C), 39.6 (10-C), 21.2 (11-C), 39.4 (12-C), 42.0 (13-C), 56.0 (14-C), 24.2 (15-C), 26.2 (16-C), 55.7 (17-C), 11.9 (18-C), 19.8 (19-C), 36.8 (20-C), 18.7 (21-C), 34.3 (22-C), 24.5 (23-C), 45.9 (24-C), 28.1 (25-C), 19.0 (26-C), 18.9 (27-C), 23.1 (28-C), 12.0 (29-C).

(6E)-Hydroximinocholest-4-en-3β-ol (8b)
Yield 74.5%, $\theta_{m.p.}$ 155–157 °C; IR(KBr) ν: 3374, 2949, 2864, 1629, 1462, 1376, 1315, 1119, 1070, 968, 890, 727, 674 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.717 (s, 3H, 18-CH$_3$), 0.882 (d, 3H, $J=2.0$, 26-CH$_3$ or 27-CH$_3$), 0.895 (d, 3H, $J=2.0$, 26-CH$_3$ or 27-CH$_3$), 0.931 (d, 3H, $J=6.5$, 21-CH$_3$), 1.070 (s, 3H, 19-CH$_3$), 3.059 (dd, 1H, $J=3.0$, 14.0, C_7-βH), 4.273 (dd, 1H, $J=4.4$, 12.0 Hz, C_3-αH), 6.249 (s, 1H, C_4-H).

In the reaction, the 6Z-isomer **9b** of **8b** was obtained in 4% yield, $\theta_{m.p.}$ 124–126 °C; IR(KBr) ν: 3411, 2958, 2872, 1629, 1462, 1376 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.762 (s, 3H, 18-CH$_3$), 0.894 (d, 3H, $J=2.3$, 26 or 27-CH$_3$), 0.902 (d, 3H, $J=2.3$, 26 or 27-CH$_3$), 0.965 (d, 3H, $J=6.5$, 21-CH$_3$), 1.144 (s, 3H, 19-CH$_3$), 4.277 (m, 1H, C_3-αH), 6.937 (d, 1H, $J=2.1$, C_4-H).

(6E)-Hydroximino-24-ethylcholest-4,22-dien-3β-ol (8c)
Yield 69.1%, $\theta_{m.p.}$ 96–97 °C; IR(KBr) ν: 3399, 2954, 2929, 2868, 1634, 1462, 1381, 1074, 968 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.739 (s, 3H, 18-CH$_3$), 0.822 (d, 3H, $J=7.7$, 26-CH$_3$ or 27-CH$_3$), 0.872 (d, 3H, $J=7.0$, 26-CH$_3$ or 27-CH$_3$), 0.829 (t, 3H, $J=7.5$, 29-CH$_3$), 1.040 (d, 3H, $J=6.0$, 21-CH$_3$), 1.078 (s, 3H, 19-CH$_3$), 3.064 (dt, 1H, $J=3.5$, 17.5, C_7-βH), 4.282 (ddd, 1H, $J=2.0$, 5.5, 12.5, C_3-αH), 5.049 (dd, 1H, $J=9.0$, 15.0, C_{22}-H), 5.172 (dd, 1H, $J=8.5$, 15.0, C_{23}-H), 6.247 (s, 1H, C_4-H); ^{13}C NMR(CDCl$_3$, 125 MHz): 35.2 (1-C), 29.7 (2-C), 69.0 (3-C), 113.9 (4-C), 156.7 (5-C), 156.9 (6-C), 28.8 (7-C), 31.9 (8-C), 51.2 (9-C), 39.5 (10-C), 21.2 (11-C), 38.5 (12-C), 40.4 (13-C), 55.9 (14-C), 21.4 (15-C), 25.4 (16-C), 53.6 (17-C), 12.2 (18-C), 18.7 (19-C), 41.6 (20-C), 19.0 (21-C), 138.1 (22-C), 129.5 (23-C), 42.4 (24-C), 34.2 (25-C), 21.1 (26-C), 18.4 (27-C), 24.3 (28-C), 12.1 (29-C).

In the reaction, the 6Z-isomer **9c** of **8c** was obtained in 3.5% yield, $\theta_{m.p.}$ 125–127 °C; IR(KBr): 3420, 2958, 2868, 1638, 1462, 1381, 1025, 976 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.736 (s, 3H, 18-CH$_3$), 0.820 (d, 3H, $J=6.0$, 26-CH$_3$ or 27-CH$_3$), 0.826 (t, 3H, $J=8.0$, 29-CH$_3$), 0.870 (d, 3H, $J=6.0$, 26-CH$_3$ or 27-CH$_3$), 1.036 (d, 3H, $J=6.5$, 21-CH$_3$), 1.110 (s, 3H, 19-CH$_3$), 4.323 (dd, 1H, $J=4.0$, 11.5, C_3-αH), 5.047 (dd, 1H, $J=9.0$, 14.5, C_{22}-H), 5.168 (dd, 1H, $J=8.5$, 14.5, C_{23}-H), 6.922 (s, 1H, C_4-H).

(6E)-Hydroximino-24-ethylcholest-4-en-3-one (1)
The Jones' reagent of 0.5 mL (0.267 mol/L) was added drop-wise to the solution of **8a** (73 mg, 0.164 mmol) in 10 mL of acetone in 10 min. The reaction mixture was stirred at room temperature for 1 h and then neutralized with 10% K$_2$CO$_3$ solution. The suspension was poured over a silica gel column and eluted with ethyl acetate. The solvent was removed under reduced pressure. The residue was chromatographed on silica gel using petroleum ether (60–90 °C)/EtOAc (3:1) as eluent to give 46 mg (63%) of **1**, $\theta_{m.p.}$ 197–198 °C; IR(KBr) ν: 3383, 2958, 2933, 2868, 1704, 1659, 1585, 1462, 1377, 1250, 984 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.737 (s, 3H, 18-CH$_3$), 0.839 (d, 3H, $J=6.5$, 26-CH$_3$ or 27-CH$_3$), 0.861 (d, 3H, $J=6.5$, 26-CH$_3$ or 27-CH$_3$), 0.868 (t, 3H, $J=8.5$, 29-CH$_3$), 0.956 (d, 3H, $J=6.5$, 21-CH$_3$), 1.064 (s, 3H, 19-CH$_3$), 2.274 (ddd, 1H, $J=5.0$, 14.0, 18.5, C_7-αH), 2.662 (dd, 1H, $J=4.0$, 16.5, C_2-βH), 3.097 (dd, 1H, $J=3.5$, 18.5, C_7-βH), 6.778 (s, 1H, C_4-H), 8.917 (brs, 1H, =N-OH); ^{13}C NMR(CDCl$_3$, 125 MHz): 36.1 (1-C), 33.4 (2-C), 200.9 (3-C), 126.3 (4-C), 155.8 (5-C), 149.2 (6-C), 26.0 (7-C), 33.4 (8-C), 50.0 (9-C), 42.5 (10-C), 21.3 (11-C), 39.3 (12-C), 46.0 (13-C), 56.7 (14-C), 24.0 (15-C), 28.0 (16-C), 55.9 (17-C), 11.9 (18-C), 18.9 (19-C), 38.9 (20-C), 18.7 (21-C), 33.9 (22-C), 26.1 (23-C), 45.9 (24-C), 29.2 (25-C), 19.8 (26-C), 19.0 (27-C), 23.1 (28-C), 12.0 (29-C); LREIMS (70 eV, m/z %): 441 (M$^+$, 26), 426 (M$^+$-CH$_3$, 100), 424 (M$^+$-OH, 35), 152 (38), 127 (89); HREIMS: m/z 441.3604 [M]$^+$ (calcd for C$_{29}$H$_{47}$O$_2$N$_1$, 441.3601).

(6E)-Hydroximinocholest-4-en-3-one (2)
Yield 61%, $\theta_{m.p.}$ 188–191 °C; IR(KBr) ν: 3738, 3281, 2942, 2868, 1716, 1659, 1581, 1471, 1381, 1250, 1283, 1172, 1127, 1078, 1029, 980, 927, 866, 788, 735 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.734 (s, 3H, 18-CH$_3$), 0.884 (d, 3H, $J=6.2$, 26-CH$_3$ or 27-CH$_3$), 0.897 (d, 3H, $J=6.2$, 26-CH$_3$ or 27-CH$_3$), 0.942 (d, 3H, $J=6.5$, 21-CH$_3$), 1.159 (s, 3H, 19-CH$_3$), 2.274 (ddd, 1H, $J=5.0$, 14.0, 18.5, C_7-αH), 2.519 (dd, 1H, $J=5.1$, 14.7, C_2-βH), 3.437 (dd, 1H, $J=4.6$, 15.9, C_7-βH), 6.338 (s, 1H, C_4-H), 9.033 (brs, 1H, N-OH); ^{13}C NMR(CDCl$_3$, 125 MHz): 36.1 (1-C), 33.5 (2-C), 200.9 (3-C), 126.3 (4-C), 155.7 (5-C), 149.2 (6-C), 28.0 (7-C), 33.3 (8-C), 50.0 (9-C), 42.5 (10-C), 21.3 (11-C), 39.5 (12-C), 46.0 (13-C), 56.7 (14-C), 24.0 (15-C), 28.1 (16-C), 56.0 (17-C), 11.9 (18-C), 18.9 (19-C), 35.7 (20-C), 18.7 (21-C), 38.9 (22-C), 23.8 (23-C), 39.3 (24-C), 28.0 (25-C), 22.8 (26-C), 22.5 (27-C); LREIMS (70 eV, m/z %): 413 (M$^+$, 79), 396 (M$^+$-OH, 100), 395 (M$^+$-H$_2$O, 30), 152(93); HREIMS: m/z 413.3289 [M]$^+$ (calcd for C$_{27}$H$_{43}$O$_2$N$_1$, 413.3288).

(6E)-Hydroximino-24-ethylcholest-4,22-dien-3-one (4)
Yield 60%, $\theta_{m.p.}$ 185–187 °C; IR(KBr) ν: 3371, 2958, 2868, 1708, 1679, 1581, 1458, 1377, 1242, 976 cm^{-1}; ^{1}H NMR(CDCl$_3$, 500 MHz): 0.759 (s, 3H, 18-CH$_3$), 0.833 (t, 3H, $J=7.5$, 29-CH$_3$), 0.826 (d, 3H, $J=6.5$, 26-CH$_3$ or 27-CH$_3$), 0.875 (d, 3H, $J=6.5$, 26-CH$_3$ or 27-CH$_3$), 1.061 (d, 3H, $J=8.5$, 21-CH$_3$), 1.069 (s, 3H, 19-CH$_3$), 2.274 (ddd, 1H, $J=5.5$, 14.5, 18.0, C_7-αH), 2.657 (dd, 1H, $J=3.0$, 16.0, C_2-βH), 3.097 (dd, 1H, $J=5.0$, 18.0, C_7-βH), 5.065 (dd, 1H, $J=9.0$, 15.5, C_{22}-H), 5.181 (dd, 1H, $J=8.5$, 15.5, C_{23}-H), 6.779 (s, 1H, C_4-H); ^{13}C NMR(CDCl$_3$, 125 MHz) δ: 38.9 (1-C), 33.5 (2-C), 200.9 (3-C), 126.3 (4-C), 155.8 (5-C), 149.1 (6-C), 28.7 (7-C), 31.9 (8-C), 51.3 (9-C), 42.4 (10-C), 21.3 (11-C), 39.2 (12-C), 46.0 (13-C), 56.8 (14-C), 24.1 (15-C), 31.8 (16-C), 55.9 (17-C), 12.2 (18-C), 18.9 (19-C), 40.4 (20-C), 21.1 (21-C), 137.9 (22-C), 129.7 (23-C), 50.1

(24-C), 33.3 (25-C), 21.2 (26-C), 18.7 (27-C), 25.4 (28-C), 12.1 (29-C); LREIMS (70 eV, m/z %): 439 (M^+, 100), 422 (M^+-OH, 45), 396 (M^+-CH(CH$_3$)$_2$, 89), 298(59), 152(87); HREIMS: m/z 439.3445 [M]$^+$ (calcd for $C_{29}H_{45}O_2N_1$, 439.3445).

The similar method was used for synthesizing the compounds **2** and **4**. Therefore, only experimental details for the synthesis of compound **1** are reported.

Antiproliferative activity

Materials and methods
Stock solutions of compounds **1**, **2** and **4**, were prepared in sterile dimethyl sulfoxide (DMSO) (Sigma) at a concentration of 10 mg/mL and afterwards diluted with complete nutrient medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate.

Cell culture
Sk-Hep-1, H-292, PC-3 (ATCC) and Hey-1B (a gift from Dr. Yan Xu, University of Indiana) cells were cultured in a proper medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO$_2$ at 37 °C.

Treatment of cancer cells
Cancer cells (4 × 10^3 cells/200 μL) were seeded into each well of a 96-well microtiter plate. After incubation for 24 h, the compounds with a series of concentrations (range 20–80 μg/mL) were added to the cells. An equal amount of DMSO was added to the cells used as negative controls. All were treated in triplicate.

Determination of cell viability
MT Stetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (CellTiter 96 AQ$_{ueous}$ Non-Radioactive Cell Proliferation Assay, Cat.# G5421, Promega Corporation) dye reduction assay was used. The assay is dependent on the MTS being reduced to an aqueous, soluble formazan by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture. Briefly, after treatment (see Section2.2.3) for 72 h, the medium was removed and the cells were incubated with 100 μL of fresh medium plus 20 μL of MTS solution according to the instruction for additional 4 h. The absorbance (A) at 490 nm was measured using an Beckman coulter LD400 AD/LD analysis spectrometer. IC$_{50}$ concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared to a control.

Results and discussion

The initial studies for the synthesis of this kind of oxime-steroid system were based on the methodology developed by Holland et al. [8]. Seven steps were needed to synthesize compound **1** as reported in reference [4]. Later Kovganko et al. reported two new synthesis routes for the compound **1**, both were rather complicated and the overall yields were relatively low [9,10]. Here, we introduce a new synthetic method for the steroidal oxime compound **1**, **2** and **4** with higher overall yields and fewer synthetic steps. For example, using β-sitosterol as raw material, four steps are needed for synthesizing the compound **1** with an overall yield of 33%. The synthesis route of the compound **1**, **2** and **4** is as shown in Fig. 1.
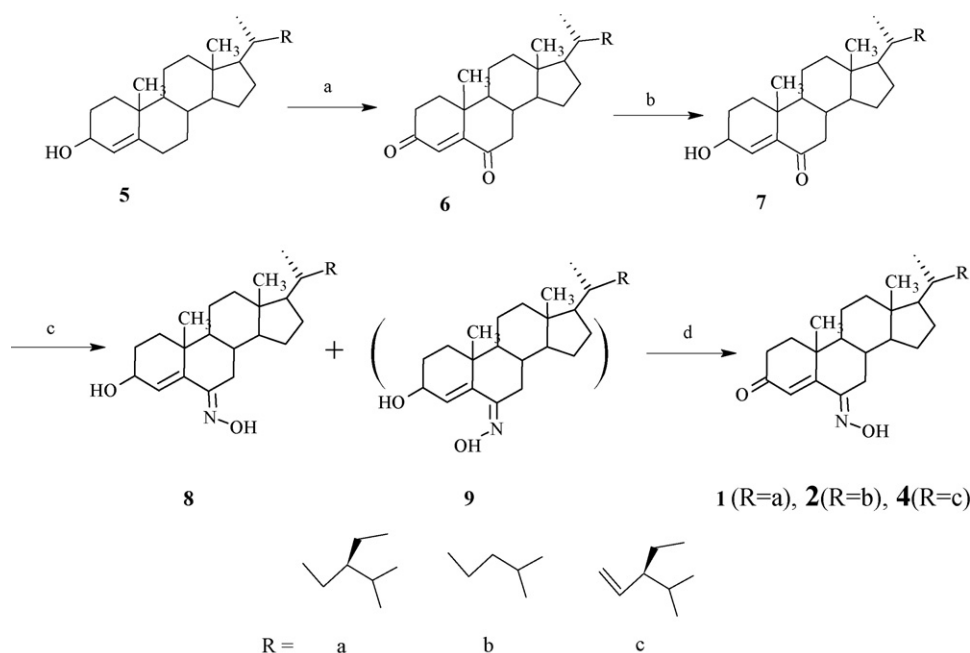
Fig. 1 – The synthesis of some (6E)-hydroximino-4-en-3-one steroids. (a) PCC/C H2Cl2; (b) NaBH4/CH3OH, CoCl2·6H2O; (c) NaAc·3H2O/95% C2H5OH, H2OH·HCl; (d) Jones agent/acetone.

Table 1 – *In vitro* antitumor activities (IC$_{50}$ μg/mL) of 1, 2 and 4[a]				
Compound	Sk-Hep-1	H-292	PC-3	Hey-1B
1	≥80	≥80	≥80	≥80
2	33	32.6	35	54
4	43	59.5	44	37

[a] MTS method was used to assay of antiproliferative activity.

In step1, β-sitosterol (5a) is transformed into the corresponding 24-ethylcholest-4-en-3,6-dione (6a) via oxidation with PCC in CH$_2$Cl$_2$ in 86% yield. Selective reduction of 6a by NaBH$_4$ in the presence of CoCl$_2$ gives 24-ethylcholest-4-en-3β-ol-6-one (7a) in 85% yield according to the synthetic method we developed [11]. The structure of 7a was confirmed by comparing IR and ^{1}H NMR spectra with those of the analogous compound that was synthesized previously in Ref. [12].

Next, the oxime 8a is obtained by the reaction of 7a with hydroxylamine hydrochloride in ethanol in the presence of NaOAc in 75% yield. The structure of 8a was proved by spectral data. At the same time, *cis*-isomer 9a of 8a was obtained in 9.5% yield.

Oxidation of 8a by Jones' reagent in acetone gives the target steroid 1 in 69% yield. The IR and ^{1}H NMR spectra data of 1 are perfectly consistent with those of the natural compound 1.

The compound 2 and 4 were prepared in the similar synthetic method to the compound 1.

To determine the biological activity of these compounds, we investigated their ability against four human tumor cell lines: Sk-Hep-1 (human liver carcinoma), H-292 (human lung carcinoma), PC-3 (human prostate carcinoma) and Hey-1B (human ovarian carcinoma). The results, expressed as IC$_{50}$ values in μg/mL, are reported in Table 1.

Our results showing in the Table 1 revealed that the compound 2 and 4 displayed a modest cytotoxic activity against these cancer cells. Interestingly the structure of side chains on these steroidal oximes plays an important role in their cytotoxicity. The antineoplastic activity of these compounds increases along with the order of the side chain attached: cholesterol-like side chain(2) > stigmasterol-like side chain(4) > sitosterol-like side chain(1). The presence of a cholesterol-type side chain appears to be necessary for the biological activity. This is consist with the conclusion obtained by Rodríguez et al. [7].

Acknowledgments

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