STEROIDS 73 (2008) 252–256

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journal homepage: www.elsevier.com/locate/steroids

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

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ARTICLE INFO

Article history:
Received 15 July 2007
Received in revised form
11 October 2007
Accepted 16 October 2007
Published on line 24 October 2007

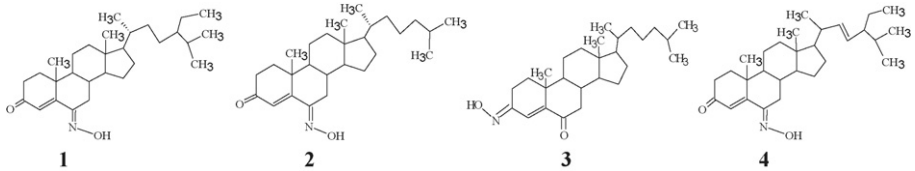
Keywords:
Steroid synthesis
6*E*-Hydroximino-4-ene steroids
Biological activity
Antineoplastic activity

ABSTRACT

Using β-sitosterol as a starting material, (6*E*)-hydroximino-24-ethylcholest-4-en-3-one (**1**), a natural steroidal oxime from *Cinachyrella alloclada* and *C. apion*, was synthesized in four steps with a high overall yield. First, β-sitosterol (**5a**) is transformed into the corresponding 24-ethylcholest-4-en-3,6-dione (**6a**) via oxidation with pyridinium chlorochromate (PCC). Selective reduction of **6a** by $NaBH_4$ in the presence of $CoCl_2$ gives 24-ethylcholest- 4-en-3β-ol-6-one (**7a**). The reaction of **7a** with hydroxylamine hydrochloride offers the oxime **8a** and the oxidation of **8a** by Jones reagent gives the target steroid **1**. (6*E*)-Hydroximinocholest-4-en-3-one (**2**) and (6*E*)-hydroximino-24-ethylcholest-4,22-dien-3-one (**4**) were synthesized by a similar method. The cytotoxicity of the synthesized compounds against sk-Hep-1 (human liver carcinoma cell line), H-292 (human lung carcinoma cell line), PC-3 (human prostate carcinoma cell line) and Hey-1B (human ovarian carcinoma cell line) cells were investigated. The presence of a cholesterol-type side chain appears to be necessary for the biological activity.

1. 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, (6*E*)-hydroximino-24- ethylcholest-4-en-3-one (**1**) and (6*E*)-hydroximinocholest-4-en-3-one (**2**), were isolated from *Cinachyrella alloclada* and *C. apion* [4]. In 2005, another steroidal oxime, (3*E*)-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**

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doi:10.1016/j.steroids.2007.10.007

2. Experimental

2.1. 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 ^1H 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.

2.1.1. 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_2Cl_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_2Cl_2, and the suspension was poured over a silica gel column and eluted with CH_2Cl_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) ν: 2959, 1683, 1601, 1581, 1461, 1377, 1246, 1124, 948, 871 cm^{-1}; ^1H 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).

2.1.2. 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_2Cl_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) ν: 2953, 2865, 1693, 1600, 1486, 1249, 1221, 1117, 942 cm^{-1}. ^1H 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).

2.1.3. 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_2Cl_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)ν: 2959, 1714, 1686, 1609, 969, 864 cm^{-1}; ^1H 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).

2.1.4. 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 $CoCl_2.6H_2O$ (61 mg, 0.26 mmol) in CH_3OH (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) ν: 3428, 2962, 2929, 2868, 1716, 1659, 1462, 1377, 1074, 968 cm^{-1}; ^1H 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).

2.1.5. Cholest-4-en-3β-ol-6-one (7b)

Yield 88.2%, $\theta_{m.p.}$ 125–126 °C; IR(KBr) ν: 3391, 2946, 2868, 1708, 1663, 1462, 1373, 1274, 1123, 1070, 960, 878 cm^{-1}; ^1H 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).

2.1.6. 24-Ethylcholest-4,22-dien-3β-ol-6-one (7c)

Yield 90%, $\theta_{m.p.}$ 175–176 °C; IR(KBr) ν: 3457, 2954, 2860, 1711, 1659, 1458, 1385, 1279, 1074, 972 cm^{-1}; ^1H 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).

2.1.7. (6E)-Hydroximino-24-ethylcholest-4-en-3β-ol (8a)

7a (150 mg, 0.40 mmol) was dissolved in 15 mL 95% CH_3CH_2OH. After the mixture was heated to 55 °C, $CH_3COONa{\cdot}3H_2O$ (95 mg, 0.70 mmol) and $NH_2OH^{\bullet}$ 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 × 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) ν: 3428, 2958, 2933, 2868, 1634, 1462, 1377 cm^{-1}; ^1H 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).

2.1.8. (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).

2.1.9. (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).

2.1.10. (6E)-Hydroximino-24-ethylcholest-4-en-3-one (1)

The Jones' reagent of 0.5 mL (0.267 mol/L) was added dropwise 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_2CO_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 $C_{29}H_{47}O_2N_1$, 441.3601).

2.1.11. (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_2O, 30), 152(93); HREIMS: *m/z* 413.3289 $[M]^+$ (calcd for $C_{27}H_{43}O_2N_1$, 413.3288).

2.1.12. (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.

2.2. *Antiproliferative activity*

2.2.1. *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.

2.2.2. *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.

2.2.3. *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.

2.2.4. *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.

3. 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.

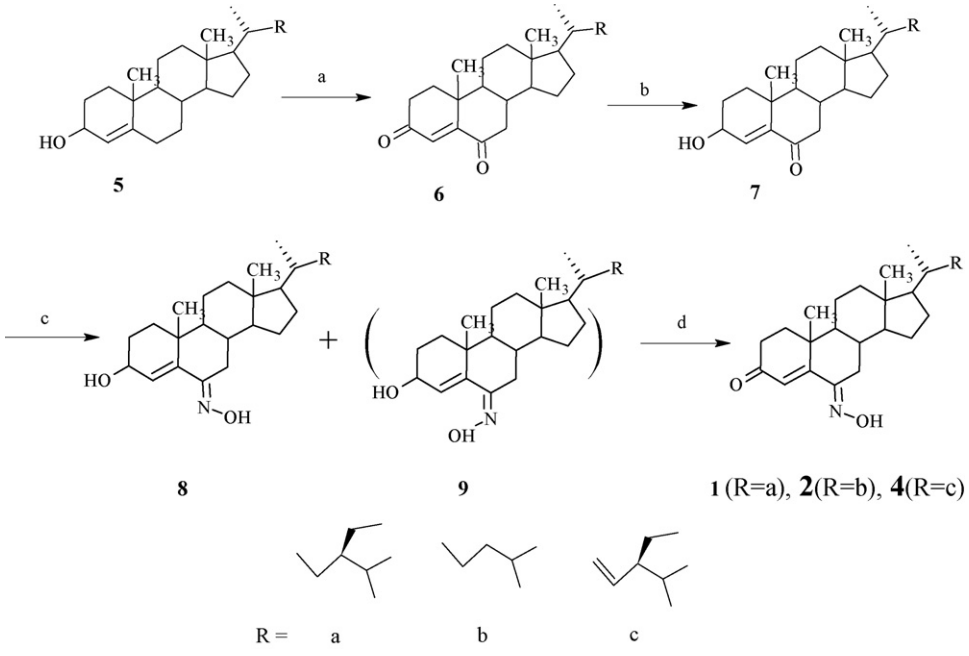

Fig. 1 – The synthesis of some (6E)-hydroximino-4-en-3-one steroids. (a) PCC/C H2Cl2; (b) NaBH4/CH3OH, CoCl2·6H2O; (c) NaAc·3H2O/95% C2H5OH, H2OH·HCl; (d) Jones agent/acetone.

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_2Cl_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 ^1H 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 ^1H 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

The authors acknowledge the financial support of the National Natural Science Foundation of China (Project: 20562001), the Natural Science Foundation of Guangxi Province (Guike: 057554) and the graduate innovation fund of Guangxi Province.

REFERENCES

[1] Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MP. Marine natural products. Nat Prod Rep 2007;24:31–86.
[2] Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MP. Marine natural products. Nat Prod Rep 2005;22:15–61.
[3] Faulkner DJ. Marine natural product. Nat Prod Rep 2001;18:1–49.
[4] Jaime R, Lucia N, Solange P, Carlos J. Isolation and synthesis of the first natural 6-hydroximino- 4-en-3-one steroids from the sponges *Cinachyrella* spp. Tetrahedron Lett 1997;38:1833.
[5] Xiao DJ, Peng XD, Deng SZ. Structure elucidation of (3*E*)-cholest-4-en-3,6-dione-3-oxime in marine sponge *Cinachyrella* australiensis from the south china sea. Chin J of Org Chem 2005;25(12):1606–9.
[6] Zeelen FJ. Medicinal chemistry of steroids. Amsterdam: Elsevier Sci. Publ.; 1990. p. 357.
[7] Deive N, Rodríguez J, Jiménez C. Synthesis of cytotoxic 6*E*-hydroximino-4-ene steroids: structure/activity studies. J Med Chem 2001;44:2612–8.
[8] Holland HL, Kumaresan S, Tan L, Njar VCO. Synthesis of new 6-hydroximino- oxo steroids, a new class of aromatase inhibitor. J Chem Soc Perkin Trans 1992;1:585–7.
[9] Kovganko NB, Chernov YuG. Novel synthesis of (24R, 6E)-24-ethylcholest-6-hydroxyimno-4-en-3-one, a steroidal oxime from *Cinachyrella* spp. sponges. Chem Nat Compd 2000;36(2):189.
[10] Kovganko NV, Chernenko Yu G. Synthesis of oxime of stigmastane 5β-hydroxy-6-ketosteroids. Chem Nat Compd 2001;37(3):256.
[11] Jianguo Cui, Longmei Zeng, Jingyu Su. Synthesis of some polyhydroxysterols and investigation of relationship between their structure and cytotoxicity. Chem J Chin Univ (Chinese) 2000;21(9):1399–404.
[12] Jianguo Cui, Longmei Zeng, Jingyu Su, Cuiwu Lin. Regio- and stereo-selective reductions of steroidal 4-en-3,6-dione. Chem Res Chin Univ 2002;18(4):400–4.