

Synthesis and Biological Evaluation of 3,16,20-Polyoxygenated Steroids of Marine Origin and Their Analogs

Suthinee Boonananwong, Boonsong Kongkathip*
and Ngampong Kongkathip

ABSTRACT

The natural polyoxygenated steroids (20*S*)-20-hydroxycholestane-3,16-dione (**1**), (16*S*, 20*S*)-16,20-dihydroxycholestan-3-one (**2**), (20*S*)-20-hydroxycholest-1-ene-3,16-dione (**3**) and (20*S*)-20-hydroxycholest-4-ene-3,16-dione (**4**), isolated from the gorgonian, *Leptogorgia sarmentosa* and unnatural analogs (**5**) (**6**), (**12**) and (**13**) were synthesized from tigogenin. Antitumor activity against three tumor cell lines (breast cancer, MCF 7, lung cancer NCI and oral cancer KB) was evaluated. Two compounds (**3** and **4**) containing α,β -unsaturated ketone at ring A showed strong activity against NCI-H 187 (IC₅₀ 2.55, 4.35 $\mu\text{g/ml}$) and moderate activity against MCF 7 and KB, the IC₅₀ being in the range 12.69 – 19.55 $\mu\text{g/ml}$ whereas the analog quinone steroid **5** showed moderate activity against all tested cells. Compound **1** containing a keto group at C-3 and a hydroxyl group at C-16 showed moderate activity against NCI (IC₅₀ 17.84 $\mu\text{g/ml}$), but was inactive against MCF 7 and KB, whereas compound **2** showed no activity against all tested cells. Cholestane (**6**) with dihydroxyl groups at C-3 and C-16 showed moderate activity against NCI-H 187 and KB the IC₅₀ being in the range 10.22-11.04 $\mu\text{g/ml}$, but was weakly active against MCF 7 (IC₅₀ 50.0 $\mu\text{g/ml}$). The aromatic cholestane **13**, the analog of **6** was strongly active against KB (IC₅₀ 4.69 $\mu\text{g/ml}$), weakly active against MCF 7 (IC₅₀ 38.2 $\mu\text{g/ml}$) and inactive against NCI-H 187 cell lines. Surprisingly compound **12** containing on unsaturated side chain was inactive with all tested cells.

Key words: steroids, synthesis, marine organisms, anticancer, biological activity

INTRODUCTION

Steroids isolated from various marine organisms (marine steroids) manifest diverse biological activities (Migliuolo, 1991; D'Auria, 1993; Stonik, 2001). Some of them are extremely toxic against tumor cells (Morris, 1998; Reuda, 1998; Aiello, 1999; Kerr, 2000; Anta, 2002) and show anti-inflammatory (He, 1995) and other therapeutic effects (Capon, 1985; Dopeso, 1994).

A series of polyoxygenated steroids that uncommonly present oxidation at both C-16 and C-20 of the cholestane nucleus have been isolated from the gorgonian, *Leptogorgia sarmentosa* (Cimino, 1981; Benvegno, 1982; Cimino, 1984) and similar cholestanes have also been obtained from the anthozoen, *Antipathes subpinnata* (Aiello, 1991; 1992). Recently three new steroids of this type (**1-3**) together with the known steroid (**4**) (Figure 1) were isolated from the gorgonian,

Natural Products and Organic Synthesis Research Unit (NPOS), Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail : fscibsk@ku.ac.th

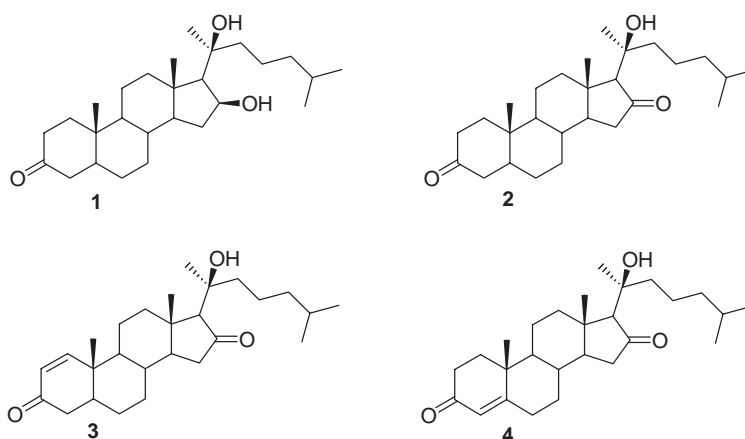


Figure 1 Steroids isolated from *Leptogorgia sarmentosa*.

Leptogorgia sarmentosa (Garrido, 2000). Compounds **1** and **2** and a fraction that contained **3** as the major component have been reported to exhibit cytotoxicity against P-388 of mouse lymphoid neoplasma human lung carcinoma (A 549), human colon carcinoma (HTG) and human melanoma (MEL 28) with an ED_{50} value of $1 \mu\text{g/ml}$ in all cases. In order to determine the anticancer role of the type and degree of unsaturation in ring A and the side chain of polyoxygenated steroids, this study investigated the synthesis of various types of 3,16,20-polyoxygenated steroids with ring A modified as quinone or as an aromatic and unsaturated side chain and evaluated the anticancer activity of these compounds.

MATERIALS AND METHODS

Reactions were monitored by TLC on aluminum sheets SIL G/UV254 from Merck. Chromatographic plates were sprayed with vanillin solution and heated until color developed. Melting points were determined on an electro thermal SMP-10 apparatus and were uncorrected. Infrared spectra were recorded on a Perkin–Elmer 2000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded in CDCl_3 on a Bruker Advance DPX-400 spectrometer operating at 400 MHz (proton) and 100 MHz (carbon-13), with the

chloroform peak used as a standard. Chemical shifts were expressed in parts per million (ppm) and coupling constants (J) in Hz. Mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap; where the first number denotes the m/z value and the ion assignment and abundance are given in parentheses. Tigogenin was isolated from the waste of *Agave sisalana* leaves. All chemicals and solvents were purchased from the Fluka Co. as analytical grade and solvents were purified by general methods before being used. The chemical *m*-Iodoxybenzoic acid (*m*-IBX) was prepared as described by Vogel (Vogel, 1989) and Barton (Barton, 1984).

Synthetic procedures

β -Acetoxy-16 β - γ -acetoxyethylvaleroyloxy-5 α -pregnan-20-one (**8**)

A mixture of tigogenin (**7**) (1 g, 2.4 mmol), acetic anhydride (17 ml), ammonium chloride (256 mg, 4.8 mmol) and pyridine (1.4 ml) was heated to 135°C and kept at that temperature for 16 h. After cooling down the reaction mixture, acetic acid (2 ml), 1,2-dichloroethane (95 ml) and water (0.5 ml) were added and the mixture was cooled to 0°C . A solution of chromium trioxide (424 mg) in water (0.6 ml) and acetic acid (0.2 ml) was added dropwise and the mixture was stirred at room temperature until the reaction was

completed. Then a solution of sodium chloride (480 mg) in water (7.2 ml) and methanol (0.1 ml) was added and the mixture was stirred for 1 h. The reaction mixture was extracted with methylene chloride and the organic phase was washed with water and dried over anhydrous sodium sulphate. The residue from removal of the solvent *in vacuo* was purified by flash column chromatography (7 : 3; ethyl acetate : hexane) to produce **8** (580 mg, 45%) as a white solid, m.p. 97 to 98 °C. **FTIR** (KBr) ν_{\max} , 1749, 1739, 1733, 1717 cm^{-1} . **¹H NMR** (CDCl_3): 5.43 (dt, $J = 7.7, 4.34$ Hz, 1H, H-16), 4.61 (m, 1H, H-3), 3.80 (d, $J = 6.1$ Hz, 2H, H-27), 2.34 (m, 2H, H-15), 2.32 (d, $J = 7.6$ Hz, 1H, H-17), 1.99 (s, 3H, OAc), 2.0 (m, 2H, H-7), 1.98 (s, 3H, OAc), 1.95 (s, 3H, H-21), 1.80 (m, 1H, H-24), 1.70 (m, 1H, H-12), 1.68 (m, 1H, H-25), 1.65 (m, 1H, H-1), 1.62 (m, 2H, H-2, H-23), 1.60 (m, 1H, H-11), 1.56 (m, 1H, H-24), 1.50 (m, 3H, H-2, H-4), 1.40 (m, 1H, H-11), 1.38 (m, 1H, H-23), 1.24 (m, 1H, H-8), 1.22 (m, 2H, H-6), 1.10 (m, 1H, H-5), 0.95 (s, 3H, H-19), 0.94 (m, 1H, H-12), 0.85 (d, $J = 6.7$ Hz, 3H, H-26), 0.84 (m, 2H, H-1, H-9), 0.77 (s, 3H, H-18), 0.62 (m, 1H, H-14). **¹³C NMR** (CDCl_3): 205.5 (C=O), 172.6 (C=O), 171.1 (C=O), 17.7 (C=O), 74.4 (C-16), 73.5 (C-3), 68.7 (C-27), 66.6 (C-17), 54.2 (C-14), 53.9 (C-9), 44.6 (C-5), 42.5 (C-13), 38.1 (C-10), 36.6 (C-12), 35.5 (C-1), 35.0 (C-4), 34.3 (C-8), 33.9 (C-2), 31.9 (C-25), 31.8 (C-15), 31.7 (C-7), 30.6 (C-21), 28.3 (C-23), 28.2 (C-6), 27.4 (C-24), 21.4 ($\text{CH}_3\text{C}=\text{O}$), 20.8 ($\text{CH}_3\text{C}=\text{O}$), 2.5 (C-11), 16.4 (C-26), 13.6 (C-19), 12.2 (C-18).

3,16,20-Trihydroxycholestane (6)

To a mixture of Mg turnings (338 mg), I_2 (catalytic amount) and dry Tetrahydrofuran (30 ml) was added 1-bromo-4-methylpentane (1.4 ml, 9.39 mmol) at room temperature under N_2 . The mixture was stirred at room temperature for 3 h and then a solution of **8** (1 g, 1.87 mmol) in dry Tetrahydrofuran (20 ml) was added dropwise at 0 °C. The reaction was stirred for 20 min and then

quenched with ice water followed by neutralization with 10% aqueous HCl. The mixture was then extracted with methylene chloride, the combined organic layers were washed with water, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product was filtered through a short column (eluting with 3 : 7; ethyl acetate : hexane) to produce (**6**) which was used in the next step without further purification.

(20S)-20-Hydroxycholestane-3,16-dione (2) and (16S, 20S)-16,20-dihydroxy-cholestan-3-one (1)

To a stirred solution of **6** in methylene chloride (50 ml) was added sodium acetate (596 mg, 7.27 mmol) and PCC (2 g, 7.97 mmol) at room temperature. After stirring for 3 h, the mixture was filtered through a celite pad. The filtrate was evaporated and purified by flash column chromatography (15 : 85; ethyl acetate : hexane) to yield **2** (195 mg, 25 % from 2 steps) as a white solid, mp 145 to 146 °C and **1** (1.5 mg, 2.2%) mp. 172 to 173 °C.

Compound **2**. **FTIR** (KBr), ν_{\max} , 3440, 1727, 1717 cm^{-1} . **¹H NMR** (CDCl_3): 2.37 (m, 1H, H-2), 2.32 (m, 1H, H-2), 2.27 (m, 1H, H-4), 2.20 (m, 1H, H-15), 2.13 (s, 1H, H-17), 2.06 (m, 1H, H-4), 2.02 (m, 1H, H-12), 2.04 (m, 1H, H-1), 1.81 (dd, $J = 18.5, 13.5$ Hz, 1H, H-15), 1.59 (m, 1H, H-7), 1.57 (m, 1H, H-5), 1.52 (m, 1H, H-8), 1.47 (m, 2H, H-6), 1.45 (m, 1H, H-25), 1.44 (m, 2H, H-22), 1.43 (m, 1H, H-12), 1.42 (m, 2H, H-11), 1.36 (m, 1H, H-14), 1.32 (m, 1H, H-1), 1.29 (m, 2H, H-23), 1.19 (m, 3H, H-21), 1.08 (m, 4H, H-7, H-9, H-24), 0.98 (s, 3H, H-19), 0.87 (s, 3H, H-18), 0.80 (d, $J = 6.6$ Hz, 6H, H-26, H-27). **¹³C NMR** (CDCl_3): 221.2 (C-16), 211.5 (C-3), 73.9 (C-20), 71.4 (C-17), 53.5 (C-9), 50.7 (C-14), 46.4 (C-5), 44.5 (C-4), 44.3 (C-22), 42.7 (C-13), 39.6 (C-24), 39.4 (C-15), 39.3 (C-12), 38.1 (C-1), 38.0 (C-2), 35.7 (C-10), 33.8 (C-8), 31.6 (C-7), 28.6 (C-6), 27.9 (C-25), 25.4 (C-21), 22.7 (C-27), 22.6 (C-26), 22.4 (C-23), 21.0 (C-11), 14.7 (C-18), 11.4 (C-19). **CIMS**: 417 [(M + H)⁺, 7], 399 [(M + H)⁺-

H₂O, 100)], 381, 331 (M⁺- C₆H₁₃, 5).

Compound **1**. **FTIR** (KBr) ν_{\max} , 3439, 1700 cm⁻¹. **¹H NMR** (CDCl₃): 4.51 (m, 1H, H-16), 2.31 (m, 1H, H-2), 2.20 (m, 1H, H-2), 2.17 (m, 1H, H-15), 2.07 (m, 1H, H-12), 2.00 (m, 2H, H-4), 1.96 (m, 1H, H-1), 1.70 (m, 1H, H-22), 1.64 (m, 1H, H-7), 1.48 (m, 1H, H-25), 1.47 (m, 1H, H-22), 1.46 (m, 1H, H-11), 1.42 (m, 1H, H-5), 1.41 (m, 1H, H-8), 1.37 (m, 1H, H-11), 1.28 (m, 1H, H-1), 1.27 (m, 4H, H-6, H-23), 1.21 (s, 3H, H-21), 1.16 (m, 2H, H-12, H-17), 1.13 (m, 2H, H-24), 1.18 (m, 1H, H-15), 1.09 (s, 3H, H-18), 0.96 (s, 3H, H-19), 0.86 (m, 1H, H-7), 0.80 (d, $J = 6.6$ Hz, 6H, H-26, H-27), 0.77 (m, 1H, H-14), 0.66 (m, 1H, H-9). **¹³C NMR** (CDCl₃): 212.1 (C-3), 76.9 (C-20), 74.0 (C-16), 60.1 (C-17), 54.3 (C-14), 53.5 (C-9), 46.7 (C-5), 44.7 (C-4), 43.1 (C-13), 42.4 (C-22), 40.4 (C-12), 39.5 (C-24), 38.5 (C-1), 38.1 (C-2), 37.3 (C-15), 35.7 (C-10), 34.3 (C-8), 31.5 (C-7), 28.8 (C-6), 28.0 (C-25), 26.8 (C-21), 22.7 (C-27), 22.6 (C-26), 21.1 (C-11), 20.9 (C-23), 15.0 (C-18), 11.5 (C-19). **CIMS**: 419 [(M + H)⁺, 83], 401 (M⁺- H₂O, 73), 383 (100), 333 (M⁺- C₆H₁₃, 5), 316 (M⁺- C₆H₁₄O, 15).

(20S)-20-Hydroxycholest-1-ene-3,16-dione (3), (20S)-20-hydroxycholest-4-ene-3,16-dione (4) and (20S)-20-hydroxycholest-1,4-diene-3,16-dione (5)

A mixture of *m*-iodoxybenzoic acid (76 mg, 0.027 mmol) and diphenyl diselenide (8.0 mg, 0.02 mmol) in toluene (5 ml) was refluxed until the yellow color of diphenyl diselenide disappeared. The solution of **2** (95 mg, 0.23 mmol) in toluene (3 ml) was added to this mixture. The reaction was further heated under reflux for 3 h. The reaction mixture was cooled to room temperature and partitioned with methylene chloride and water. The organic layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (2 : 8; ethyl acetate : hexane) to

yield (20S)-20-hydroxycholest-1-ene-3,16-dione (**3**) (17.8 mg, 18.9%), (20S)-20-hydroxycholest-4-ene-3,16-dione (**4**) (12.1 mg, 12.9%), and (20S)-20-hydroxycholest-1,4-diene-3,16-dione (**5**) (29.2 mg, 31.2%) as a pale yellow wax.

(20S)-20-Hydroxycholest-1-ene-3,16-dione (3).

FTIR (KBr), ν_{\max} , 3510, 1729, 1679. cm⁻¹. **¹H NMR** (CDCl₃): 7.05 (1H, d, $J = 10.2$ Hz, H-1), 5.80 (1H, d, $J = 10.2$ Hz, H-2), 2.18 (m, 2H, H-4), 2.20 (m, 1H, H-15), 2.14 (s, 1H, H-17), 2.08 (m, 1H, H-12), 1.87 (m, 1H, H-5), 1.85 (m, 1H, H-11), 1.83 (dd, $J = 18.5, 14.1$ Hz, 1H, H-15), 1.82 (m, 1H, H-8), 1.59 (m, 2H, H-7), 1.55 (m, 2H, H-22), 1.48 (m, 1H, H-25), 1.47 (m, 2H, H-11, H-12), 1.45 (m, 2H, H-6), 1.40 (m, 1H, H-14), 1.30 (m, 2H, H-23), 1.19 (s, 3H, H-21), 1.10 (m, 1H, H-9), 1.08 (m, 2H, H-24), 1.03 (m, 1H, H-8), 0.98 (s, 3H, H-19), 0.89 (s, 3H, H-18), 0.81 (d, $J = 6.6$ Hz, 3H, H-27), 0.80 (d, $J = 6.6$ Hz, 3H, H-26). **¹³C NMR** (CDCl₃): 220.8 (C-16), 199.9 (C-3), 157.3 (C-1), 127.7 (C-2), 73.9 (C-20), 71.4 (C-17), 50.8 (C-14), 49.7 (C-9), 44.2 (C-5), 43.1 (C-13), 42.4 (C-22), 40.8 (C-4), 39.5 (C-24), 39.3 (C-12), 39.2 (C-15), 39.0 (C-10), 34.81 (C-8), 31.3 (C-7), 28.0 (C-25), 27.3 (C-6), 25.4 (C-21), 22.7 (C-27), 22.6 (C-26), 20.6 (C-23), 21.0 (C-11), 14.8 (C-18), 13.40 (C-19). **CIMS**: 415 [(M + H)⁺, 65], 397 [(M + H)⁺- H₂O, 100], 329 (6), 313 (M⁺- C₆H₁₃, 5), 287 (33).

(20S)-20-Hydroxycholest-4-ene-3,16-dione (4).

FTIR (KBr), ν_{\max} , 3447, 1725, 1676 cm⁻¹. **¹H NMR** (CDCl₃): 5.68 (s, 1H, H-4), 2.37 (m, 1H, H-2), 2.27 (m, 1H, H-2), 2.24 (m, 1H, H-15), 2.22 (m, 2H, H-6), 2.13 (s, 1H, H-17), 2.08 (m, 1H, H-12), 1.96 (m, 1H, H-1), 1.85 (dd, $J = 18.4, 13.4$ Hz, 1H, H-15), 1.68 (m, 1H, H-11), 1.68 (m, 1H, H-11), 1.58 (m, 1H, H-7), 1.48 (m, 2H, H-22), 1.47 (m, 3H, H-8, H-12, H-25), 1.44 (m, 1H, H-11), 1.40 (m, 1H, H-14), 1.30 (m, 2H, H-23), 1.29 (m, 1H, H-1), 1.19 (s, 3H, H-21), 1.19 (s, 3H, H-21), 1.15 (s, 3H, H-19), 1.09 (m, 1H, H-9), 1.08 (m,

2H, H-24), 1.02 (m, 1H, H-7), 0.90 (s, 3H, H-18), 0.80 (d, 6H, $J = 6.6$ Hz, H-26, H-27). $^{13}\text{C NMR}$ (CDCl_3): 220.8 (C-16), 199.7 (C-3), 169.9 (C-5), 124.1 (C-4), 73.9 (C-20), 71.2 (C-17), 50.3 (C-14), 49.7 (C-9), 43.1 (C-13), 42.4 (C-22), 40.9 (C-2), 39.4 (C-24), 39.2 (C-12), 39.1 (C-15), 38.8 (C-1), 38.5 (C-10), 34.0 (C-8), 33.8 (C-6), 32.5 (C-7), 28.0 (C-25), 25.4 (C-21), 22.7 (C-27), 22.6 (C-26), 20.9 (C-23), 21.0 (C-11), 14.8 (C-18), 13.40 (C-19). **CIMS**: 415 [(M + H)⁺, 100], 397 [(M + H)⁺ - H₂O, 93], 345 (45), 313 (M⁺ - C₆H₁₃, 21), 287 (23).

(20S)-20-Hydroxycholest-1,4-diene-3,16-dione

(5). **FTIR** (KBr), ν_{max} , 3455, 1726, 1662 cm^{-1} . $^1\text{H NMR}$ (CDCl_3): 6.97 (d, $J = 10.2$ Hz, 1H, H-1), 6.19 (dd, $J = 10.12, 1.88$ Hz, 1H, H-2), 6.02 (d, m, 1H, H-4), 0.93 (s, 3H, H-18), 0.80 (d, 6H, $J = 6.6$ Hz, H-26, H-27). $^{13}\text{C NMR}$ (CDCl_3): 220.8 (C-16), 186.1 (C-3), 170.7 (C-5), 154.9 (C-1), 127.8 (C-4), 124.3 (C-2), 73.8 (C-20), 71.4 (C-17), 51.9 (C-14), 49.9 (C-9), 43.4 (C-10), 43.1 (C-13), 42.4 (C-22), 39.4 (C-24), 39.3 (C-12), 38.9 (C-15), 33.9 (C-8), 33.4 (C-6), 32.0 (C-7), 28.0 (C-25), 25.3 (C-21), 22.7 (C-27), 22.6 (C-26), 22.2 (C-11), 21.1 (C-23), 18.7 (C-19), 14.6 (C-18). **CIMS**: 413 (62), 412 (13), 395.2 (100), 285.2 (89).

$\Delta^{1,4}\alpha$ -Spirostatriene (9)

A mixture of *m*-iodoxybenzoic acid (43 g, 153.85 mmol) and diphenyl diselenide (972 mg, 3.08 mmol) in toluene (50 ml) was refluxed until the yellow color of diphenyl diselenide disappeared. The solution of tigogenin (**7**) (6.4 g, 15.38 mmol) in toluene (50 ml) was added to this mixture. The reaction was further heated under reflux for 7 h. The reaction mixture was cooled to room temperature and partitioned with methylene chloride and water. The organic layer was separated and dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography to provide **9** as a white solid (4.37 g, 70%), mp 182

– 184 °C. **FTIR** (KBr), ν_{max} , 1664 cm^{-1} . $^1\text{H NMR}$ (CDCl_3): 6.97 (d, $J = 10.14$ Hz, 1H, H-1), 6.2 (dd, $J = 10.2$ Hz, 1.99 Hz, 1H, H-2), 5.99 (m, 1H, H-4), 4.31 (m, 1H, H-16), 3.39 (m, 1H, H-26), 3.28 (m, 1H, H-26), 1.94 (m, 1H, H-15), 1.27 (m, 1H, H-15), 1.18 (s, 3H, H-19), 0.89 (d, $J = 7.0$ Hz, 3H, H-27), 0.78 (s, 3H, H-18), 0.71 (d, $J = 6.36$ Hz, 3H, H-21). $^{13}\text{C NMR}$ (CDCl_3): 186.1 (C-3), 168.8 (C-10), 155.6 (C-1), 127.3 (C-4), 123.7 (C-2), 109.6 (C-5), 80.3 (C-16), 66.7 (C-26), 61.9, 55.1, 52.2, 43.4, 42.5, 40.3 (C-15), 39.3, 34.9, 33.6, 32.6, 31.8, 31.2, 30.1, 28.7, 22.6, 18.6 (C-19), 16.9 (C-21), 16.7 (C-18), 14.3 (C-27). **CIMS**: 410 (18), 351 (14), 289 (10), 181 (28), 139 (100).

19-Nor- $\Delta^{1,3,5(10)}$ alphaostatriene-3-ol (10)

An amount of lithium was cut into small pieces and added to a mixture of biphenyl (17.3 g, 112.1 mmol) in dry tetrahydrofuran (100 ml) under N₂ atmosphere at room temperature. The mixture was stirred under reflux for 0.5 h until the solution had turned blue. The mixture was treated dropwise, under N₂, with the solution of **9** (6.5 g, 16.0 mmol) and diphenyl methane (7.8 ml) in dry tetrahydrofuran. After refluxing for 2 h and cooling down, methanol was added to destroy excess lithium. The solvent was removed and the residue was dissolved in 10% aqueous HCl and extracted with methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulphate and the solvent was removed *in vacuo*. Purification of the crude product by flash column chromatography eluting with ethyl acetate : hexane (1:9) produced the desired product 19-nor- $\Delta^{1,3,5(10)}$ alphaostatriene-3-ol (**10**) as a white solid (2.47 g, 44%), mp 244 – 246 °C. **FTIR** (KBr), ν_{max} , 3402, 3062, 1609 cm^{-1} . $^1\text{H NMR}$ (CDCl_3): 7.06 (d, $J = 8.36$ Hz, 1H, H-1), 6.54 (dd, $J = 8.36, 2.76$ Hz, 1H, CH-2), 6.48 (d, $J = 2.76$ Hz, 1H, CH-4), 4.67 (s, OH), 4.37 (m, 1H, H-16), 3.44 (m, 1H, H-26), 3.32 (t, $J = 10.83$ Hz, 1H, H-26), 2.76 (m, 2H, H-6), 2.01 (m, 1H, H-15), 1.83 (m, 1H, H-17), 1.30 (m, 1H, H-15), 0.92 (d, $J = 6.84$ Hz,

3H, H-27.), 0.75 (s, 3H, H-18), 0.72 (d, $J = 6.32$ Hz, 3H, H-21). $^{13}\text{C NMR}$ (CDCl_3): 153.7 (C-3), 138.3 (C-5), 132.8 (C-10), 126.3 (C-1), 115.2 (C-4), 112.6 (C-2), 109.3 (C-22), 80.9 (C-16), 66.9 (C-26), 62.3 (CH), 55.3 (C-14), 43.7 (C-9), 41.6 (CH), 40.8 (C-13), 39.9 (C-12), 38.3 (C-8), 31.53 (C-15), 31.4, 30.3, 29.5 (C-6), 28.8, 27.8 (C-7), 26.4 (C-11), 17.1 (C-21), 16.4 (C-18), 14.5 (C-21). **CIMS**: 396 (13), 282 (48), 139 (100).

3-Acetoxy-16 β -acetoxyethylvaleroyloxy-17-acetyl- $\Delta^{1,3,5(10)}$ -estratriene (**11**)

A mixture of 19-nor- $\Delta^{1,3,5(10)}$ alphaostatriene-3-ol (**10**) (300 mg, 0.78 mmol), acetic anhydride (5.4 ml), ammonium chloride (83.1 mg, 1.56 mmol) and pyridine (0.06 ml) was heated a temperature between to 125 and 135 °C and kept at that temperature for 16 h. After cooling, the reaction mixture was neutralized with saturated sodium hydrogencarbonate then extracted with methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*.

The crude residue without further purification was dissolved in 1,2-dichloroethane (0.7 ml), water (0.1 ml) and acetic acid (0.25 ml). The mixture was cooled to 0°C. A solution of chromium trioxide (105 mg, 1.05 mmol) in water (2.19 ml) and acetic acid (0.25 ml) was added (the temperature was kept below 7°C). The mixture was allowed to warm to room temperature and stirred for another 2 h. A solution of sodium chloride (124 mg) in water (1.66 ml) and methanol (1.66 ml) was added and the mixture was stirred for 1 h. The reaction mixture was neutralized using sodium hydrogencarbonate and then extracted with methylene chloride, washed with water, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated. Purification of the crude residue by flash column chromatography eluting with ethyl acetate : hexane (1:9) produced 3-acetoxy-16 β -acetoxyethylvaleroyloxy-17-acetyl- $\Delta^{1,3,5(10)}$ -estratriene (**11**) (148 mg, 38%) as

a pale yellow syrup. **FTIR** (neat), ν_{max} , 1756, 1734, 1712 cm^{-1} . $^1\text{H NMR}$ (CDCl_3): 7.19 (d, $J = 8.48$ Hz, 1H, H-1), 6.76 (dd, $J = 8.48, 2.56$ Hz, 1H, H-2), 6.70 (d, $J = 2.48$ Hz, 1H, H-4), 5.49 (m, 1H, H-16), 3.81 (d, $J = 6.12$ Hz, 2H, H-26), 2.78 (m, 2H, H-6), 2.46 (m, 1H, H-15), 2.40 (d, $J = 7.60$ Hz, 1H, H-17), 2.21 (m, 1H, H-9), 2.20 (s, 3H, $\text{CH}_3\text{C}=\text{O}$), 2.19 (m, 1H, H-11), 2.15 (m, 1H, H-7), 2.09 (m, 1H, H-12), 2.01 (s, 3H, H-21), 1.96 (s, 3H, $\text{CH}_3\text{C}=\text{O}$), 1.77 (m, 3H, H-22, H-27), 1.63 (m, 1H, H-23), 1.50 (m, 2H, H-7, H-8), 1.37 (m, 1H, H-15, H-23), 1.35 (m, 1H, H-11),), 1.19 (m, 1H, H-12), 1.05 (m, 1H, H-14), 0.99 (s, 3H, H-18), 0.85 (s, 3H, H-25). $^{13}\text{C NMR}$ (CDCl_3): 260.0 (CO), 172.9 (CO), 171.1 (CO), 169.8 (CO), 148.45 (C-3), 137.9 (C-5), 137.6 (C-10), 126.24 (C-1), 121.5 (C-4), 118.61 (C-2), 74.3 (C-16), 68.7 (C-26), 66.7 (C-17), 53.0 (C-14), 43.9 (C-9), 42.6 (C-13), 37.97 (C-12), 37.1 (C-8), 34.8 (C-15), 32.0 ($\text{CH}_3\text{C}=\text{O}$), 31.9 (C-22), 30.5 (C-24), 29.3 (C-6),), 28.2 (C-23), 27.3 (C-7), 25.7 (C-11), 21.0 (C-21), 20.8 ($\text{CH}_3\text{C}=\text{O}$), 16.3 (C-25), 13.4 (C-18). **CIMS**: 512 (52), 339 (100), 297 (34). **HRMS**: 535.2673 $\text{C}_{30}\text{H}_{40}\text{O}_7\text{Na}$ requires 535.2672.

3, 16S, 20S-Trihydroxy-24-cholestene- $\Delta^{1,3,5(10)}$ -estratriene (**12**)

To a mixture containing Mg turnings (252 mg, 10.5 mmol), I_2 (catalytic amount) and dry tetrahydrofuran (30 ml) was added 5-bromo-2-methyl-2-pentene (0.65 ml, 4.90 mmol) at room temperature under N_2 . The mixture was stirred at room temperature for 3 h and then a solution of **11** (351 g, 0.70 mmol) in dry THF (20 ml) was added dropwise at 0°C. The reaction was stirred at 0°C for 30 min and then quenched with ice-water followed by neutralization with 10% aqueous HCl. The mixture was then extracted with methylene chloride. The combined organic layer was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 3 : 7; ethyl acetate :

hexane to produce 3,16*S*,20*S*-16,20-trihydroxy-24-cholestene- $\Delta^{1,3,5(10)}$ -estratriene (**12**) as a white solid (164.5 mg, 60 %), mp 220 – 222°C. **FTIR** (KBr), ν_{\max} , 3427 cm^{-1} . **¹H NMR** (CDCl_3 and CD_3OD): 7.00 (d, $J = 8.32$ Hz, 1H, H-1), 6.53 (dd, $J = 8.32, 2.56$ Hz, 1H, H-2), 6.47 (d, $J = 2.56$ Hz, 1H, H-4), 5.05 (m, 1H, H-24), 4.54 (m, 1H, H-16), 2.73 (m, 2H, H-6), 2.66 (m, 1H, H-15), 2.16 (m, 2H, H-9, H-12), 1.93 (m, 1H, H-11), 1.91 (m, 2H, H-23), 1.77 (m, 1H, H-22), 1.74 (m, 1H, H-7), 1.66 (s, 3H, H-26), 1.57 (m, 1H, H-22), 1.55 (s, 3H, H-27), 1.43 (m, 1H, H-8), 1.32 (m, 1H, H-17), 1.31 (m, 3H, H-7, H-11, H-15), 1.27 (m, 1H, H-12), 1.25 (s, 3H, H-21), 1.07 (s, 3H, H-18), 0.97 (m, 1H, H-14). **¹³C NMR** (CDCl_3 and CD_3OD): 154.1 (C-3), 137.7 (C-5), 131.7 (C-25), 131.3 (C-10), 126.0 (C-1), 124.4 (C-24), 112.5 (C-2), 115.0 (C-4), overlap with CDCl_3 , C-20, 73.4 (C-16), 59.8 (C-17), 53.3 (C-14), 43.6 (C-13), 43.5 (C-9), 43.1 (C-22), 40.4 (C-12), 37.6 (C-8), 36.5 (C-15), 29.4 (C-6), 27.4 (C-7), 26.3 (C-11), 26.0 (C-27), 25.4 (C-21), 23.2 (C-23), 17.4 (C-26), 14.6 (C-18). **CIMS**: 399 (4), 381 (98), 363 (100) **HRMS**: 421.2721 $\text{C}_{26}\text{H}_{34}\text{O}_4\text{Na}$ requires 421.2719.

3,16*S*,20*S*-Trihydroxycholestane- $\Delta^{1,3,5(10)}$ -estratriene (**13**)

To a stirred suspension of 5% Pd-C (3 mg, 0.0014 mmol) in ethanol (1ml) was added a solution of **12** (11 mg, 0.0276 mmol) in ethyl acetate (1 ml). The reaction mixture was treated with hydrogen (from a hydrogen balloon) and stirred for 16 h. The mixture was filtered through a celite pad and rinsed with ethyl acetate. The filtrate was concentrated by using a rotary evaporator. The crude mixture was purified by flash column chromatography (3:7; ethyl acetate : hexane) to produce 3,16*S*,20*S*-trihydroxycholestan- $\Delta^{1,3,5(10)}$ -estratriene (**13**) (11 mg, 100%) as a white solid, mp 245 to 246°C. **FTIR** (KBr), ν_{\max} , 3389 (OH) cm^{-1} . **¹H NMR** (CDCl_3 and CD_3OD): 7.02 (d, $J = 8.4$ Hz, 1H, H-2), 6.54 (dd, $J = 8.4, 2.7$ Hz, 1H, H-2), 6.47 (d, $J = 2.6$ Hz, 1H,

H-4), 4.52 (m, 1H, H-16), 2.71 (m, 2H, H-6), 2.23 (m, 1H, H-12), 2.15 (m, 1H, H-11), 2.07 (m, 1H, H-9), 1.74 (m, 2H, H-7), 1.49 (m, 1H, H-11), 1.48 (m, 1H, H-), 1.43 (m, 1H, H-25), 1.40 (m, 2H, H-22), 1.29 (m, 2H, H-24), 1.27 (m, 1H, H-12), 1.23 (m, 1H, H-17), 1.22 (s, 3H, H-21), 1.18 (m, 2H, H-23), 1.11 (m, 2H, H-15), 1.07 (s, 3H, H-18), 0.98 (m, 1H, H-14), 0.80 (d, $J = 6.6$ Hz, 6H, H-26, H-27). **¹³C NMR** (CDCl_3 and CD_3OD): 154.0 (C-3), 137.5 (C-5), 131.4 (C-10), 125.8 (C-1), 114.8 (C-4), 112.3 (C-2), 76.7 (C-20), 73.1 (C-16), 59.4 (C-17), 53.1 (C-14), 44.0 (C-13), 43.5 (C-9), 42.9 (C-22), 40.2 (C-12), 39.4 (C-15), 37.5 (C-8), 36.3 (C-24), 29.3 (C-6), 27.6 (C-25), 27.3 (C-7), 26.1 (C-11), 25.7 (C-21), 22.2 (CH_3 X 2), 22.1 (C-23), 14.3 (C-18). **CIMS**: 401 (7), 383 (90), 365 (100).

Biological assays

KB (Human epidermoid carcinoma of the cavity, ATCC CCL-17), MCF 7 (Human breast adenocarcinoma, ATCC HTB-22) and NCI-H 187 (Human small cell lung carcinoma, ATCC CRL-5804) were determined by resazurin microplate assay (REMA) following a modified method of the use of a fluorescent dye for mammalian cell cytotoxicity according to Brien *et al.* (2000) (Brien, 2000). Ellipticine and doxorubicin were used as positive controls. DMSO and sterile distilled water were used as negative controls. Cells at a logarithmic growth phase were harvested and diluted to 10^5 cells/ml in fresh medium and gently mixed. Test compounds were diluted in culture medium in a ratio of 1:2 giving 8 concentrations. Five μl of the test sample and 45 μl of cells were put into 384-well microtiter plates with a total volume of 50 μl /well. Plates were incubated at 37 °C, 5% CO_2 , for 72 h for KB and MCF7 and 5 days for NCI-H187. After the incubation periods, 12.5 μl of resazurin solution was added to each well and the plates were incubated at 37 °C for 4 h. The plates were then processed for optical density absorbance analysis

using a Victor 3 Microplate reader at dual wavelengths of 530 and 590 nm.

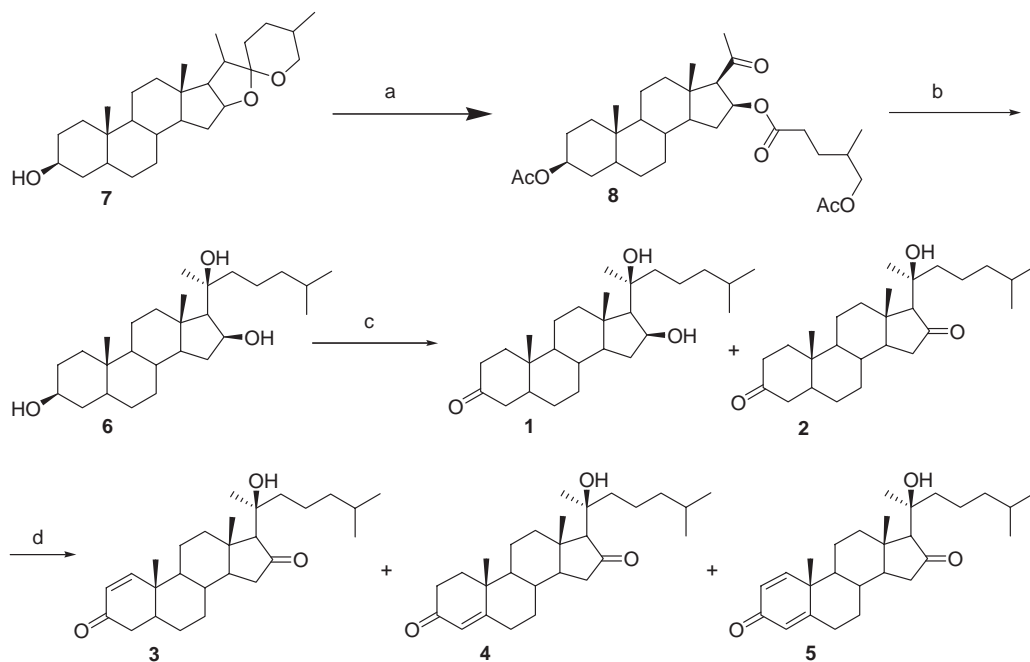
RESULTS AND DISCUSSION

Chemistry

The preparation of 3,16,20-polyoxygenated steroids **1-6** from tigogenin (**7**) is shown in **Scheme 1**. Transformation of the spiro ketal moiety in **7** to keto ester in **8** was performed in one pot preparation by the method developed by Mićović *et al.*, (Micovic *et al.*, 1990) and modified by Fuchs *et al.* (Kim, 1999). Grignard reaction of **8** with 1-bromo-4-methylpentane, Mg in tetrahydrofuran gave 3,16,20-trihydroxy steroid **6**. Oxidation of **6** with PCC gave a mixture of (16*S*,20*S*)-16,20-dihydroxycholestan-3-one (**1**) and (20*S*)-20-hydroxycholestan-3,16-dione (**2**). Dehydrogenation at A ring of **2** using *m*-iodoxybenzoic acid in effecting oxygen atom

transfer to diphenyl diselenide in refluxing toluene smoothly produced a mixture of (20*S*)-20-hydroxycholest-1-ene-3,16-dione (**3**), (20*S*)-20-hydroxycholest-4-ene-3,16-dione (**4**) and (20*S*)-20-hydroxycholest-1,4-diene-3,16-dione (**5**) in a 3:2:5 ratio.

The synthesis of 3,16,20-polyoxygenated steroids containing an aromatic A ring **12, 13** was also started from tigogenin (**7**) (**Scheme 2**). Oxidative dehydrogenation of tigogenin using *m*-iodoxybenzoic acid, diphenyl diselenide in refluxing toluene gave quinone **9** in 70% yield. Aromatization of quinone ring A was accomplished by using lithium, biphenyl in tetrahydrofuran to give aromatic steroid **10** in 44% yield. Transformation of the spiro ketal moiety in **10** to keto ester **11** was performed in one pot preparation by the method developed by Mićović *et al.*, (Micovic *et al.*, 1990) and modified by Fuchs *et al.* (Kim, 1999). A Grignard reaction of **11** with



Scheme-1 Preparation of 3,16,20-polyoxygenated steroids from tigogenin.

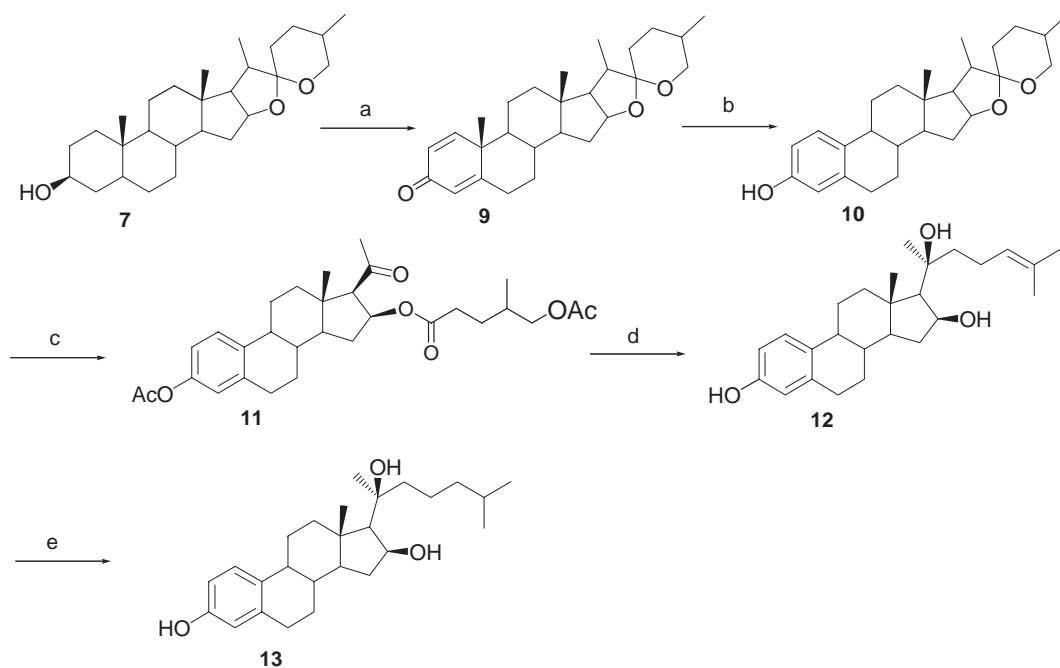
Reagents and conditions: (a) (i). NH_4Cl , py, AcOH, 135°C , 16h; (ii). CrO_3 , AcOH, H_2O , $(\text{CH}_2\text{Cl}_2)_2$, 0°C , 3h, 45%; (b) 4-methylpentylmagnesium bromide, THF, rt, 20 min, 87%; (c) PCC, NaOAc, CH_2Cl_2 , rt, 3h; (d) Ph_2Se_2 , *m*-IBX, toluene, reflux, 3h.

5-bromo-2-methyl-2-pentene, Mg in THF gave 3,16,20-trihydroxy steroid **12**. Hydrogenation of **12** using 5% Pd-C in EtOAc-EtOH at room temperature produced the corresponding **13** in a quantitative yield.

Biological activity

The synthesized polyoxygenated steroids were studied *in vitro* against MCF 7, NCI and KB tumor cell lines (Table 1). Two compounds (**3** and **4**) containing α,β -unsaturated ketone A ring showed strong activity against NCI-H 187 (IC_{50} 2.55, 4.35 $\mu\text{g/ml}$) and moderate activity against MCF 7 and KB, the IC_{50} being in the range 12.69 – 19.55 $\mu\text{g/ml}$ whereas the analog quinone steroid **5** showed moderate activity against all tested cells.

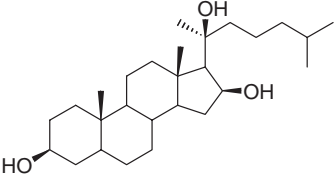
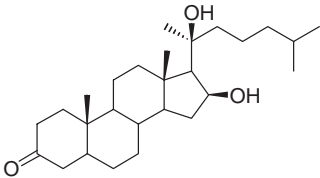
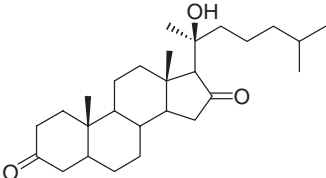
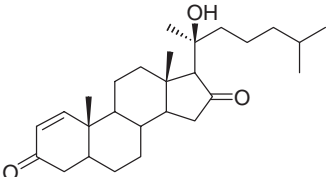
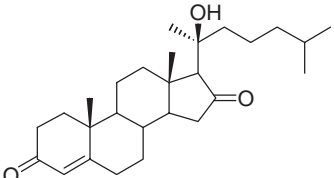
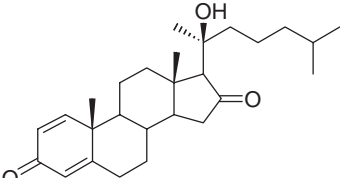
Compound **1** containing keto group at C-3 and hydroxy group at C-16 showed moderate activity against NCI-H187 (IC_{50} 17.84 $\mu\text{g/ml}$) but was inactive against MCF 7 and KB whereas compound **2** showed no activity against all tested cells. Cholestane (**6**) with dihydroxy group at C-3 and C-16 showed moderate activity against NCI-H 187 and KB the IC_{50} being in the range 10.22–11.04 $\mu\text{g/ml}$ but weakly active against MCF 7 (IC_{50} 50.0 $\mu\text{g/ml}$) whereas the analog **13** with an aromatic group at ring A was strongly active against KB (IC_{50} 4.69 $\mu\text{g/ml}$), weakly active against MCF 7 (IC_{50} 38.2 $\mu\text{g/ml}$) and inactive to NCI-H 187 cell lines. Surprisingly, compound **12** containing an unsaturated side chain was inactive with all tested cells.

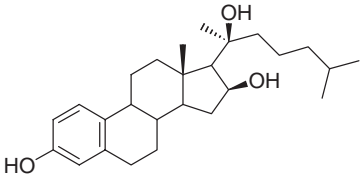
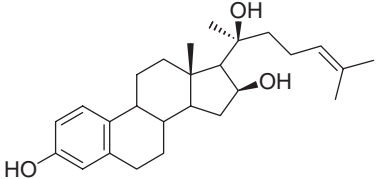


Scheme 2 Synthesis of 3,16,20-polyoxygenated steroids containing an aromatic A ring **12**, **13** from tigogenin (**7**).

Reagents and conditions: (a) *m*-IBX, $(\text{PhSe})_2$, toluene, reflux, 7 h, 70%; (b) Li, Ph_2 , Ph_2CH_2 , THF, reflux, 2h, 44%; (c) (i). NH_4Cl , py, AcOH, 135°C, 16h; (ii). CrO_3 , AcOH, H_2O , $(\text{CH}_2\text{Cl})_2$, 0°C, 2h. 38%, 2 steps; (d) 2-methyl-2-pentenylmagnesium bromide, THF, rt, 30 min, 57%; (e) H_2 , 5% Pd-C, EtOAc, EtOH, rt, 16h, 99%.

Table 1 Cytotoxicity of the synthetic polyoxygenated steroids against KB, MCF 7 and NCI.

Entry	Compound	Cancer cell lines (IC ₅₀ µg/ml)		
		MCF 7	NCI-H 187	KB
1	 6	50.0	11.04	10.22
2	 1	Inactive	17.84	Inactive
3	 2	Inactive	Inactive	Inactive
4	 3	12.69	2.55	19.55
5	 4	13.01	4.35	17.29
6	 5	22.88	22.97	19.94

7		38.20	Inactive	4.69
13				
8		Inactive	Inactive	Inactive
12				

MCF 7, human breast adenocarcinoma; NCI, human small cell lung carcinoma; KB, human epidermoid carcinoma of cavity;

^a Data are typical values from six replicate experiments

^b Inactive = inhibition < 50%

CONCLUSION

This paper has described the chemical synthesis of four polyoxygenated steroids from marine origin and four new steroid analogs. Two of the synthesized compounds, (2*S*)-20-hydroxycholest-1-ene-3,16-dione (**3**) and (2*S*)-20-hydroxycholest-4-ene-3,16-dione (**4**) showed significant cytotoxic activity for NCI-H 187 and moderate activity for MCF 7 and KB cell lines, whereas the analog quinone steroid **5** showed moderate activity against all tested cells. (16*S*, 20*S*)-16,20-Dihydroxycholestan-3-one (**1**) displayed moderate cytotoxic potency for NCI-H 187, but was inactive in MCF 7 and KB cell lines, whereas (2*S*)-20-hydroxycholestan-3,16-dione (**2**) showed no cytotoxicity in all tested cell lines. Cholestan-3-one (**6**) with dihydroxy groups at C-3 and C-16 showed moderate activity against NCI-H 187 and KB, but was weakly active against MCF 7. The aromatic cholestan-3-one (**13**), the analog of **6**, was strongly active against KB, weakly active against MCF 7 and inactive to NCI-H 187 cell lines. Aromatic cholestan-3-one with unsaturated side chain (**12**) was inactive to all tested cells as summarized

in Table 1. These first structure/cytotoxicity investigations demonstrated that one important feature, the presence of a cholesterol-like side chain, appears to play a major role in determining the biological activity. The existence of a hydroxyl functionality at C-16 and an α,β -unsaturated ketone at ring A results in higher bioactivity than that of a ketone group. Further studies on the structure activity relationships of the 3,16,20-polyoxygenated steroids are under investigation.

ACKNOWLEDGEMENTS

The principal author is a PhD student under the Royal Golden Jubilee Program, the Thailand Research Fund (TRF). The authors are grateful for financial support from the TRF, through the Royal Golden Jubilee Program. Financial support from the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and the Kasetsart University Research and Development Institute (KURDI) are gratefully acknowledged.

LITERATURE CITED

- Aiello, A., E. Fattorusso and M. Menna. 1991. Polyoxygenated Steroids of Marine Origin. **Steroids**. 96: 513-517.
- Aiello, A., E. Fattorusso and M.J. Menna. 1992. Marine polar steroids. **Nat. Prod.** 55: 321-325.
- Aiello, A., E. Fattorusso and M. Menna. 1999. Steroids from sponges: Recent reports. **Steroids**. 64: 687-714.
- Anta, C., N. Gonzalez, J. Rodriguez and C. Jimenez. 2002. New Secosterol from the Indonesian Octocoral *Pachyclavularia violacea*. **J. Nat. Prod.** 65: 1357-1359.
- Barton, D.H.R. and W.B. Motherwell. 1984. Dehydrogenation agents based on derivatives of selenium and their use in the dehydrogenation in the 1,4-position of steroids. **U.S. Patent** 4,434,080.
- Benvegnu, R., G. Cimino, S. De Rosa and S. De Stefano. 1982. Guggulsterol-like steroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*. **Experientia**. 38: 1443-1444.
- Brien, J.O., I. Wilson, T. Orton and F. Pognan. 2000. Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. **Eur. J. Biochem.** 267: 5421-5426.
- Capon, R.J., D. Faulkner, J. Herbasterol and Ichthyotoxic. 1985. 9,11-Secosterol from the Sponge *Dysidea herbacea*. **J. Org. Chem.** 50: 4771-4773.
- Cimino, G., S. De Rosa, S. De Stefano, G. Scognamiglio and G. Sodano. 1981. Cholest-4, 14-dien-15,20x-diol-3,16-dione, a novel polyoxygenated marine steroid that easily loses the side chain. **Tetrahedron Lett.** 22: 3013-3016.
- Cimino, G., S. De Rosa, S. De Stefano and G. Sodano. 1984. C-18 Hydroxysteroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*. **Experientia**. 40: 246-8.
- D'Auria, M.V., L. Minale and R. Riccio. 1993. Polyoxygenated steroids of marine origin. **Chem. Rev.** 93: 1839-1895.
- Doposo, J., E. Quinoa, R. Riguera, C. Debitus, P. Bergquist and R. Eryspongiol. 1994. Ten New Highly Hydroxylated 9,11-Secosteroids with Antihistaminic Activity from the Sponge *Euryspongia* sp. **Tetrahedron**. 50: 3813-3828.
- Garrido, L., E. Zubia, M.J. Ortega and J. Salva. 2000. Isolation and structure elucidation of new cytotoxic steroids from the gorgonian *Leptogorgia sarmentosa*. **Steroids** 65: 85-88.
- He, H., P. Kulanthaivel, B.J. Baker, K. Kalter, J. Darges, D. Cofield, L. Wolff and L. Adams. 1995. New Antiproliferative and Antiinflammatory 9,11-Secosterols from the Gorgonian *Pseudopterogorgia* sp. **Tetrahedron**. 51: 51-58.
- Kerr, R.G., S. Naz and R. Narayanan. 2000. New antiproliferative epoxysecosterols from *Pseudopterogorgia americana*. **Tetrahedron Lett.** 41: 6035-6040.
- Kim, S., S.C. Sutton, C. Guo, T.G. LaCour and P.L. Fuchs. 1999. Synthesis of the north 1 cephalostatin family from hecogenin acetate. **J. Am. Chem. Soc.** 121: 2056-2070.
- Mićović V., M.D. Ivanovic and D.M. Piatak. 1990. Simplified preparation of 16-dehydropregnenol-one acetate. **Synthesis**. 7: 591-592.
- Migliuolo, A., V. Piccialli and D. Sica. 1991. Structure Elucidation and Synthesis of 3b,6a-dihydroxy-9-oxo-9,11-seco-5a-cholest-7-en-11-al, a novel 9,11-secosterol from the sponge *Spongia officinalis*. **Tetrahedron**. 47: 7937-7950.
- Morris, L.A., M.E. Christie, M. Jaspars and L.P.A. van Ofwegen. 1998. Secosterol with an Unusual A- and B-Ring Oxygenation Pattern Isolated from an Indonesian Soft Coral *Lobophytum* sp. **J. Nat. Prod.** 61: 538-541.
- Rueda, A., E. Zubia, M.J. Ortega, Carballo and J.L. Salva. 1998. J. New metabolites from the sponge *Spongia agaricina*. **J. Nat. Prod.** 61: 258-261.
- Stonik, V.A. 2001. Marine polar steroids. **Russian Chem. Rev.** 70: 673-715.
- Vogel, A.I. 1989. **Vogel's Textbook of Practical Organic Chemistry**. 5th ed. London; Longman Group UK Ltd. p.871.