SYNTHESIS AND CYTOTOXICITY OF POLYHYDROXYLATED CHOLESTEROL DERIVATIVES

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Abstract. Eight polyhydroxylated cholesterol derivatives (**1-8**) were prepared from cholesterol, using oxidative reagents as SeO_2, OsO_4/NMO, $HCOOH/H_2O_2$ and BH_3/H_2O_2. Their structures were elucidated by using physical methods including NMR 1D and 2D. These compounds were evaluated against two cancer cell lines (Hep-G2, T98). Compounds **2**, **4** and **8** inhibit human hepatocellular carcinoma cell line (Hep-G2) with IC_{50} values of 11.59, 11.89 and 6.87 µM, respectively. In addition, compound **8** exhibited strong cytotoxicity against T98 cell line (glioblastoma) with $IC_{50} = 2.28$ µM.

Keywords: cholesterol, cytotoxicity, polyhydroxylated derivative.

Classification numbers: 1.1.2, 1.4.1.

1. INTRODUCTION

Polyhydroxylsteroids or oxysterols are oxygenated derivatives of steroids and constitute a family of compounds with various biological activities. Especially, oxysterols have been shown to exhibit cytotoxicity in a number of cell lines, including smooth muscle cells, fibroblasts and vascular endothelial cells [1]. This steroidal compound class exhibits very good activities for certain diseases such as muscular dystrophy and cancer [2, 3]. Oxysterols, like steroid hormones, have specific physiological properties and deregulation of their metabolism is associated with several pathologies including cancer. Some oxysterol metabolic pathways represent novel targets for the development of anticancer agents [4].

Herein, we report the preparation of eight polyhydroxysteroids from cholesterol by using one or combination of several oxidative reagents such as SeO_2, OsO_4/NMO, $HCOOH/H_2O_2$ (Figure 1) and BH_3/H_2O_2 and their cytotoxicity evaluation on 2 cancer cell lines as Hep-G2 and T98.

2. MATERIALS AND METHODS

General: All reagents and solvents were purchased from Sigma-Aldrich, Acros and Merck and used without pre-purification. NMR spectra were recorded on a Bruker Avance 500 (Germany) spectrometer using TMS as internal standard. MS spectra and HPLC were recorded on a LC-MS Agilent 1100 (USA). Melting points (m. p) were recorded on a Buchi B-545 apparatus. All reactions were monitored by thin layer chromatography (TLC) using silica gel 60 coated plates F254 (aluminum sheets). Visualization was performed by UV at 254 and 365 nm. Chemical shifts are reported in δ/ppm relative to the external standards and coupling constants J are given in Hz. Abbreviations for the characterization of the signals: s = singlet, d = doublet, t = triplet, quint = quintet, m = multiplet, bs = broad singlet, dd = doublet doublet, dt = doublet triplet.

Bioassays: The *in vitro* cytotoxicity of compounds were tested on the Hepatocellular carcinoma (Hep-G2) cell line at the Institute of Natural Product Chemistry (INPC) and on T98 cell line at Korean Institute of Science and Technology Gangneung (KIST). Cytotoxicity assay was performed based on the method of Skehan *et al.* [5] and Likhiwitayawuid *et al.* [6] using suforhodamine B (SRB). Hep-G2 cell line was cultured in 10 % FBS-DMEM (Fetal Bovine Serum - Dulbecco's Modified Eagle Medium) and incubated in 5 % CO_2 and 95 % air at 37 ^{0}C for 3 days. The fresh cells were treated with trypsin at 37 ^{0}C for 5 min and were resuspended in a fresh medium containing 10 % FBS to a density of $1x10^4$ cells/mL. For activity assay, in triplicate 96-well plates 190 µL of the cell suspension was added in each well which contained 10 µL of test samples with various known concentration prepared in 5 % DMSO. Ellipticine was used as the positive reference. The cultured plates were then incubated for 3 days and cells were fixed with 100 µL of 30 % trichloroacetic acid for 30 min at 4 ^{0}C. Unbound protein was removed gently under tap water and the plates were stained for 30 min with 200 µL of 0.4 % SRB (w/v) in 1% acetic acid. Unbound dye was removed by four washes with 200 µL of 1 % acetic acid. The stained bound protein was then dissolved in 200 µL of 10 mM un buffered Tris base (tris(hydroxymethyl)aminomethane) and the optical density was measured in a computer-interfaced, 96-well microplate reader at A540 nm. The SRB assay results were linear with the number of cells and with values for cellular protein measured at densities ranging from sparse subconfluent to multilayered supraconfluent. The signal-to-noise ratio at 515-564 nm was approximately 1.5 with 1,000 cells per well.

Preparation of 1 and 2: 1M BH_3 in THF (7 mL 7.0 mM) was added slowly to a solution of cholesterol (540 mg, 1.4 mM) in dry THF (8 mL) under inert atmosphere at 0 ^{o}C. The mixture was stirred at room temperature for 4h. Aqueous solution of NaOH (5N, 1.5 mL, 7.5 mM) and 30 % H_2O_2 (0.75 mL, 6.5 mM) were added drop-by-drop at 0°C. The reaction mixture was stirred at room temperature for 1h and then concentrated under reduced pressure. The residue was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed successively with 1N HCl, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by column chromatography of silica gel (DCM/MeOH 100:1) to yield compounds **1** (402 mg, 71 %) and **2** (50 mg, 9 %).

Cholestane-3β,6α-diol (1): white needles; m. p. 191-193 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$-d_4) δ_H (ppm): 3.55 (1H, m, H-3), 3.39 (1H, ddd, J = 4.5, 10.5, 11.0 Hz, H-6), 0.90 (3H, d, J = 6.5 Hz, CH_3-21), 0.86 (6H, d, J = 6.5 Hz, CH_3-26, CH_3-27), 0.80 (3H, s, CH_3-19), 0.65 (3H, s, CH_3-18); ^{13}C NMR (125 MHz, $CDCl_3$-d_4) δ_C (ppm): 71.1 (C-3), 69.4 (C-6), 56.2 (C-14), 56.18 (C-5), 53.8 (C-17), 51.6 (C-9), 42.6 (C-13), 41.5 (C-4), 39.8 (C-12), 39.5 (C-24), 37.3 (C-1), 36.3 (C-10), 36.1 (C-22), 35.7 (C-20), 34.3 (C-8), 32.1 (C-7), 30.8 (C-2), 28.1 (C-16), 28.0 (C-25), 24.2 (C-15), 23.8 (C-23), 22.8 (C-26), 22.5 (C-27), 21.1 (C-11), 18.6 (C-19), 13.4 (C-21), 12.0 (C-18).

Cholestane-3β,6β-diol (2): white needles; m. p. 212-215 $^{\circ}$C; ^{1}H NMR (500 MHz, CDCl$_3$-d_4) $\delta_{\rm H}$ (ppm): 4.09 (1H, br, H-6), 3.70 (1H, br, H-3), 0.68 (3H, s, CH$_3$-18), 0.86 (6H, d, *J* = 6.5 Hz, CH$_3$-26, CH$_3$-27), 0.91 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 1.14 (3H, s, CH$_3$-19); ^{13}C NMR (125 MHz, CDCl$_3$-d_4) $\delta_{\rm C}$ (ppm): 73.3 (C-3), 66.2 (C-6), 56.5 (C-17), 56.4 (C-14), 43.6 (C-9), 42.8 (C-13), 40.2 (C-10), 40.1 (C-12), 39.5 (C-24), 36.2 (C-1), 35.8 (C-20), 34.8 (C-8), 34.4 (C-7), 33.6 (C-5), 30.6 (C-2), 30.1 (C-22), 28.3 (C-16), 28.0 (C-25), 26.1 (C-23), 24.2 (C-15), 23.8 (C-19), 22.8 (C-26), 22.5 (C-27), 20.9 (C-11), 18.7 (C-21), 12.1 (C-18).

Preparation of 3, 4 and 5: SeO$_2$ (0.516 g, 4.64 mM) was added to a solution of cholesterol (1 g, 2.58 mM) in dioxane (20 mL) and water (0.1 mL) at room temperature and the reaction mixture was heated and stirred at 80 $^{\circ}$C for 80 h. After filtering and evaporating the solvent under reduced pressure, the residue was dissolved in DCM and distilled water. The organic layer was separated, dried with Na$_2$SO$_4$ and filtered. After evaporating of solvent, the crude product was purified by flash chromatography (SiO$_2$, *n*-hexane/ethyl acetate 9:1) to yield compounds **3** (500 mg, 50 %), **4** (35 mg, 3.5 %) and **5** (20 mg, 2 %).

Cholestan-5-ene-3β,4β-diol (3): white solid; m. p. 175-178 $^{\circ}$C; ^{1}H NMR (500 MHz, CDCl$_3$-d_4) $\delta_{\rm H}$ (ppm): 5.68 (1H, m, H-6), 4.13 (1H, d, *J* = 3.0 Hz, H-4), 3.56 (1H, dt, *J* = 4.0, 11.5 Hz, H-3), 1.18 (3H, s, CH$_3$-19), 0.92 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.86-0.87 (6H, d, *J* = 7.0 Hz, CH$_3$-26, CH$_3$-27), 0.68 (3H, s, CH$_3$-18); ^{13}C NMR (125 MHz, CDCl$_3$-d_4) $\delta_{\rm C}$ (ppm): 142.8 (C-5), 128.8 (C-6), 77.3 (C-4), 72.3 (C-3), 56.9 (C-17), 56.1 (C-14), 50.2 (C-9), 42.3 (C-13), 39.7 (C-12), 39.5 (C-24), 36.9 (C-1), 36.2 (C-22), 36.0 (C-10), 35.8 (C-20), 32.1 (C-8), 31.8 (C-7), 28.2 (C-15), 28.0 (C-25), 25.4 (C-16), 24.3 (C-2), 23.8 (C-23), 22.8 (C-26), 22.6 (C-27), 21.1 (C-11), 20.6 (C-19), 18.7 (C-21), 11.9 (C-18) .

Cholestan-5-ene-3β,4β,7β-triol (4): white solid; m. p. 190-192 $^{\circ}$C; ^{1}H NMR (500 MHz, CDCl$_3$-d_4) $\delta_{\rm H}$ (ppm): 5.87 (1H, d, *J* = 5.0 Hz, H-6), 4.18 (1H, d, *J* = 3.0 Hz, H-4), 3.94 (1H, d, *J* = 3.5 Hz, H-7), 3.60 (1H, m, H-3), 2.01 (1H, ddd, *J* = 3.0, 3.5, 2.5 Hz, H-12), 1.18 (3H, s, CH$_3$-19), 0.93 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.86-0.87 (6H, d, *J* = 6.5 Hz, CH$_3$-26, CH$_3$-27), 0.69 (3H, s, CH$_3$-18); ^{13}C NMR (125 MHz, CDCl$_3$-d_4) $\delta_{\rm C}$ (ppm): 147.0 (C-5), 129.7 (C-6), 76.9 (C-4), 72.1 (C-3), 65.3 (C-7), 55.8 (C-17), 49.3 (C-14), 42.6 (C-9), 42.1 (C-13), 39.5 (C-24), 39.1 (C-12), 37.6 (C-8), 37.0 (C-10), 36.7 (C-1), 36.2 (C-22), 35.8 (C-20), 28.3 (C-15), 28.0 (C-25), 25.1 (C-16), 24.3 (C-2), 23.7 (C-23), 22.8 (C-26), 22.6 (C-27), 20.1 (C-11), 19.4 (C-19), 18.7 (C-21), 11.6 (C-18).

Cholestan-5-ene-3β,7β-diol (5): white solid; m. p. 178-180 $^{\circ}$C; ^{1}H NMR (500 MHz, CDCl$_3$-d_4) $\delta_{\rm H}$ (ppm): 5.60 (1H, m, H-6), 3.84 (1H, m, H-7), 3.58 (1H, m, H-3), 0.99 (3H, s, CH$_3$-19), 0.92 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.86-0.87 (6H, d, *J* = 6.5 Hz, CH$_3$-26, CH$_3$-27), 0.68 (3H, s, CH$_3$-18); ^{13}C NMR (125 MHz, CDCl$_3$-d_4) $\delta_{\rm C}$ (ppm): 146.3 (C-5), 123.9 (C-6), 71.4 (C-3), 65.4 (C-7), 55.9 (C-17), 49.4 (C-14), 42.3 (C-9), 42.2 (C-13), 42.0 (C-4), 39.5 (C-24), 39.2 (C-12), 37.5 (C-8), 37.4 (C-10), 37.0 (C-1), 36.2 C-22), 35.8 (C-20), 31.4 (C-16), 28.3 (C-15), 28.0 (C-25), 24.3 (C-2), 23.7 (C-23), 22.8 (C-26), 22.6 (C-27), 20.7 (C-11), 18.8 (C-21), 18.3 (C-19), 11.6 (C-18).

Cholestane-3β,5α,6α-triol (6): 4-Methylmorpholine *N*-oxide (150 mg, 1.28 mM) and a 4 % aqueous solution of OsO$_4$ (300 μL, 0.05 mM) were added to a solution of cholesterol (0.258 mM) in a dioxane:H$_2$O (50:1) mixture (5 mL). The reaction mixture was stirred under reflux for 48 h and cooled to room temperature, 20 % NaHSO$_3$ solution (5 mL) was added. The mixture was stirred for more 10 min and concentrated. The residue was extracted with ethyl acetate (5 x 10 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Purification by flash chromatography on silica gel using DCM/MeOH (99:1) as eluent

to give *cis*-dihydroxylated product **6** as a white solid (74%), m. p. 240-241 °C. ^{1}H NMR (500 MHz, CDCl$_3$-d_4) δ_H (ppm): 4.05 (1H, m, H-3), 3.67 (1H, d like, *J* = 10.0 Hz, H-6), 2.14 (2H, dd, *J* = 13.0, 3.5 Hz, H-4), 1.53 (1H, m, H-25), 0.96 (3H, s, CH$_3$-19), 0.90 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.86 (6H, d, *J* = 6.5 Hz, CH$_3$-26, CH$_3$-27), 0.64 (3H, s, CH$_3$-18); ^{13}C-NMR (125 MHz, CDCl$_3$-d_4) δ_C (ppm): 76.7 (C-5), 70.6 (C-6), 67.5 (C-3), 56.2 (C-14), 55.9 (C-17), 44.6 (C-9), 42.7 (C-13), 39.8 (C-12), 39.5 (C-24), 39.1 (C-10), 38.3 (C-4), 36.1 (C-22), 35.8 (C-20), 35.2 (C-7), 33.5 (C-8), 31.0 (C-1), 30.6 (C-2), 28.2 (C-16), 28.0 (C-25), 24.1 (C-15), 23.9 (C-23), 22.8 (C26), 22.5 (C-27), 21.2 (C-11), 18.6 (C-21), 15.5 (C-19), 12.1 (C-18).

Cholestan-3β,4β,5α,6α-tetrol (7): *cis*-dihydroxylation procedure by OsO$_4$/NMO of **3** as above described. Compound **7** was obtained as white needles (78%). ^{1}H NMR (500 MHz, MeOD-d_4 & CDCl$_3$-d_4) δ_H (ppm): 4.11 (1H, dd, *J* = 5.0, 11.5 Hz, H-6), 4.00 (1H, ddd, *J* = 4.0, 4.5, 11.5 Hz, H-3), 3.89 (1H, d, *J* = 3.5 Hz, H-4), 0.92 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.87-0.88 (6H, d, CH$_3$-26, CH$_3$-27), 0.68 (3H, s, CH$_3$-18); ^{13}C NMR (125 MHz, MeOD-d_4 & CDCl$_3$-d_4) δ_C (ppm): 78.0 (C-5), 72.6 (C-4), 69.3 (C-3), 67.8 (C-6), 57.4 (C-14), 57.3 (C-17), 46.5 (C-9), 43.7 (C-13), 41.1 (C-12), 40.5 (C-24), 39.8 (C-10), 37.2 (C-22), 36.9 (C-20), 35.3 (C-7), 34.8 (C-8), 32.3 (C-1), 29.1 (C-16), 28.9 (C-25), 26.3 (C-2), 25.0 (C-15), 24.8 (C-23), 23.1 (C-26), 22.9 (C-27), 21.3 (C-11), 19.1 (C-21), 15.5 (C-19), 12.5 (C-18).

Cholestane-3β,5α,6β-triol (8): Formic acid 88 % (2 mL) was added to a solution of cholesterol (200 mg, 0.516 mM) in dry THF (4 mL) and the mixture was heated at 40-45 °C, 30 % hydrogen peroxide (0.6 mL) was added slowly and the mixture was stirred for 12 h at room temperature. Ethyl acetate (15 mL) and water (10 mL) was added then organic layer was separated. Extraction of aqueous layer with ethyl acetate (3 x 10 mL) and combined organic layers were washed with 10 % NaHCO$_3$, 5 % NaOH, brine, and water, dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was heated under reflux with 3 % KOH solution in MeOH (15 mL) for 15 min, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO$_2$, DCM/MeOH 19:1) to give a white solid (**8**) (160 mg, 75 %), m. p. 231 °C. ^{1}H NMR (500 MHz, CDCl$_3$-d_4) δ_H (ppm): 4.05 (1H, m, H-3), 3.49 (1H, bs, H-6), 0.90 (3H, d, *J* = 6.5 Hz, CH$_3$-21), 0.86 (6H, d, *J* = 6.5 Hz, CH$_3$-26, CH$_3$-27), 0.68 (3H, s, CH$_3$-18). ^{13}C NMR (125 MHz, CDCl$_3$-d_4) δ_C (ppm): 76.0 (C-6), 75.7 (C-5), 67.5 (C-3), 56.3 (C-14), 56.0 (C-17), 45.8 (C-9), 42.8 (C-13), 40.3 (C-4), 40.0 (C-12), 39.5 (C-24), 38.3 (C-10), 36.2 (C-22), 35.8 (C-20), 34.3 (C-7), 32.4 (C-1), 30.6 (C-2), 30.3 (C-8), 28.3 (C-16), 28.0 (C-25), 24.2 (C-15), 23.9 (C-23), 22.8 (C-26), 22.6 (C-27), 21.2 (C-11), 18.7 (C-21), 16.8 (C-19), 12.2 (C-18).

3. RESULTS AND DISCUSSION

The oxidation reaction on cholesterol using BH$_3$.THF/H$_2$O$_2$ agent gave 2 stereoisomers: cholestane-3β,6α-diol (**1**) and cholestane-3β,6β-diol (**2**) with ratio 8:1. The ^{1}H NMR spectrum of **1** and **2** showed the appearance of proton signals at δ_H 3.39 (H-6) in **1** and 4.09 (H-6) in **2** which correspond to oxygenated CH groups. Compound **1** was previously reported being isolated from the starfish *Acanthaster planci* [7].

While treating cholesterol with SeO$_2$ at 80 °C for 18 h using the reported procedure for diosgenin [8, 9] only compound **3** was obtained. However, increasing of heating period to 80 h leads to the formation of others regio-isomers (**4** and **5**) but **3** was still in majority. The isolated yield of **3**, **4** and **5** were 48.0 %, 2.8 % and 1.5 %, respectively. Analytical TLC of the reactional medium after 24 h, 48 h and 80 h showed the presence of compound **3**, the mixture of **3** and **4**, and the mixture of **3**, **4** and **5** respectively. The regioselectivity of this allylic oxidation on

cholesterol may be due to the effect of OH-3 group.

The ^{1}H NMR and ^{13}C NMR data of compound **3** were in agreement with the reported values of 5-cholestene-3β,4β-diol [8]. Indeed, the multiplicity of H-3 signal changing from multiplet to doublet triplet (dt), the chemical shift values of H-4 and C-4 (δ_H 4.13/δ_C 77.3 ppm) and HMBC correlations between H-4 to C-2 and C-3 confirmed that the oxidative reaction occurred at C-4. In the case of compound **4**, the ^{1}H NMR spectrum showed the presence of three protons linked to oxygenated carbons at δ_H 3.60 (H-3), 3.94 (H-7) and 4.18 (H-4). In addition, the signal of H-6 (δ_H 5.87) clearly appeared as a doublet with J = 5.0 Hz. The ^{13}C NMR spectrum showed three signals at δ_C 76.9, 72.1 and 65.3 which are assigned to three oxygenated carbons belonging to C-4, C-3 and C-7, respectively. Thus, by combination of 1D and 2D NMR data, compound **4** was identified as 7-hydroxylated product of **3**. Similarly, compound **5** was determined as 7-hydroxylated product of cholesterol. According to the other reported articles about this reaction on sterols, all products contained hydroxyl groups in 4- or/and 7-β position by the characteristic chemical shift values and multiplicities of oxygenated CH groups [10].
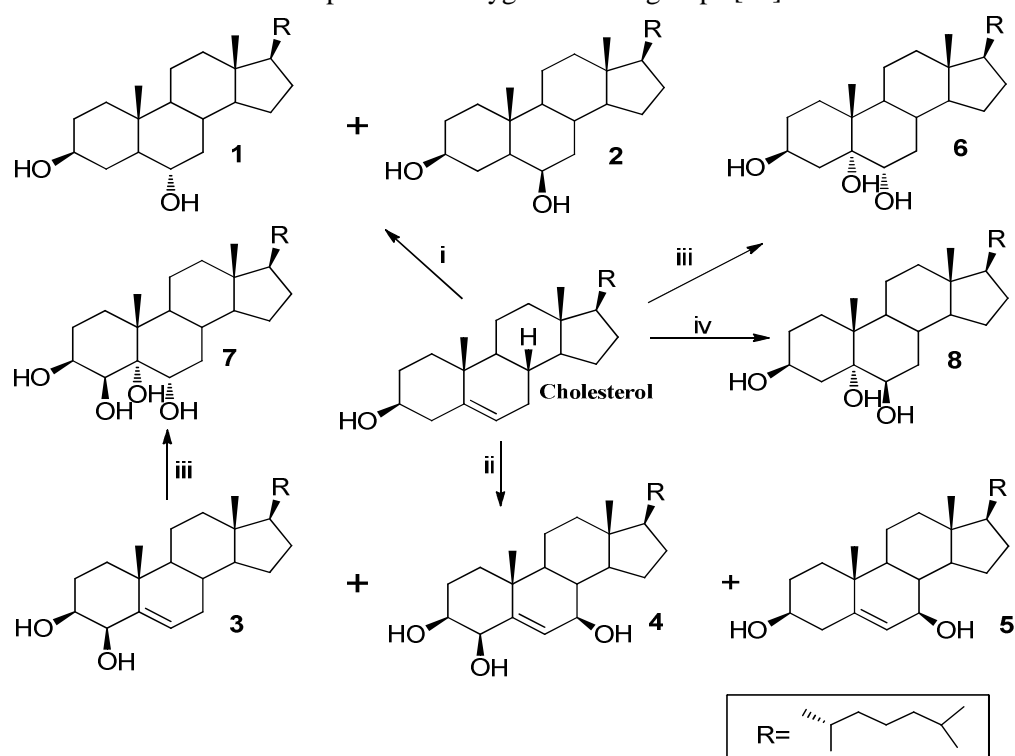

Figure 1. Reagents and conditions: (i): BH$_3$.THF, H$_2$O$_2$, NaOH, 0 ^{o}C then rt, 1h (**1**: 71 %, **2**: 9 %); (ii): SeO$_2$, dioxane, H$_2$O, 80 ^{o}C, 48 h (**3**: 50 %, **4**: 3,5 %, **5**: 2%); (iii): 4 % OsO$_4$/H$_2$O, NMO, reflux, 48 h (**6**: 74 %, **7**: 78 %); (iv): 1. HCOOH 88 %, THF/H$_2$O$_2$, 12h, 2. KOH 3 % in MeOH (**8**: 75 %).

Cis-dihydroxylation on cholesterol using OsO$_4$/NMO yielded **6** which was elucidated as 5,6-*cis*-α-dihydroxyl cholesterol derivative by 1D and 2D NMR spectra. The ^{13}C NMR spectrum showed the presence of two oxygenated carbon signals at δ_C 76.7 (C-5) and 70.6 (C-6). The NOESY spectrum showed the cross-peak between CH$_3$-19 and H-6, indicating the H-6 in β position. Therefore, the OH-6 and thus OH-5 groups were in α position.

While treating compound **3** with OsO$_4$/NMO using the same procedure as above, the tetrahydroxyl product **7** was obtained with 5,6-OH in α position. The NOESY spectrum of **7**

showed correlation between H-6 and CH_3-19, indicating the H-6 in β position. Therefore, the OH-6 and OH-5 groups were in α position.

The formation of only $5\alpha,6\beta$-diol isomer **8** (75 %) was observed when oxidation of cholesterol using performic acid ($HCOOH/H_2O_2$). Although, some previous works [11] reported that a mixture of α,β-diol or β,α-diol can be obtained from other Δ^5 sterols when using this reagent. The $5\beta,6\alpha$-diol isomer with 5-OH group in the same side with CH_3-19 was not favorable owing to steric effect. The ^{1}H and ^{13}C NMR spectra of compound **8** indicated the successful dihydroxylation on the double bond with the presence of an oxygenated methine at δ_H 3.49 (H-6) and two carbons at δ_C 75.7 (C-5) and 76.0 (C-6). The NOESY spectrum showed no interaction between CH_3-19 and H-6, indicating that H-6 and OH-5 group was in α position. Therefore, the OH-6 group was in β position. This compound was previously isolated from marine organisms, but with a trace amount as *Damiriana hawaiiana* sponges, or from the purple coral *Muriceosis flavida* distributed in the East Sea. Compound **8** promotes the apoptosis of A549 lung cancer cells, MG63 malignant bone cancer, and HT-29 human colon cancer [12]. This is the first time compound **8** was synthesized from cholesterol after one step by using performic acid as oxidative agent.

The cytotoxic activity on Hep-G2 cell line (hepatocellular carcinoma) and T98 cell line (glioblastoma) of all compounds were evaluated. Compounds **2**, **4** and **8** exhibited strong cytotoxicity against Hep-G2 cell with IC_{50} values of 11.59, 11.89 and 6.87 µM, respectively. In addition, compound **8** exhibited a quite strongly cytotoxicity against T98 cell line with IC_{50} = 2.28 µM.

4. CONCLUSIONS

Eight polyhydroxyl derivatives of cholesterol with 2-4 hydroxyl groups were prepared after 1-2 steps by using simple and effective procedures. The number, position and stereo configuration of adding hydroxyl groups vary depending on used oxidative agents. This is the first time compound **8** was prepared by the synthetic pathway from cholesterol as starting material. Compounds **2**, **4** and **8** were found potential for cytotoxic activities on Hep-G2 and T98 cancer cell lines.

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