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Steroids 67 (2002) 1015–1019

Steroids

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Synthesis of polyhydroxysterols (III): synthesis and structural elucidation of 24-methylenecholest-4-en-3β,6α-diol

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Received 30 May 2001; received in revised form 8 April 2002; accepted 24 April 2002

Abstract

Using stigmasterol as the starting material, 24-methylenecholest-4-en-3β,6α-diol (**2**) was synthesized in eight steps in 13% overall yield. The introduction of the sterol side-chain was carried out using (3-methyl-2-oxobutyl)-triphenylarsonium bromide (**11**) and K_2CO_3 in a solid–liquid phase-transfer Wittig reaction. Construction of the steroidal nucleus was finished by oxidation of 24-methylenecholest-5-en-3β-ol (**9**) with pyridinium chlorochromate (PCC) in dichloromethane at ambient temperature and by reduction of 24-methylenecholest-4-en-3,6-dione (**10**) with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$.

Keywords: Steroid synthesis; Polyhydroxylated sterol; 24-Methylenecholest-4(5)-en-3β,6α-diol; Solid–liquid phase-transfer Wittig reaction

1. Introduction

In our study on the soft coral, *Alcyonium patagonicum*, a new polyhydroxylated sterol 24-methylenecholest-4-en-3β,6β-diol (**1**) with cytotoxicity to murine leukemia cells ($IC_{50} = 1\,\mu g\,ml^{-1}$) was isolated [1] and synthesized [2]. As a part of our continuing studies on the relationship between configuration and physiological activity of polyhydroxylated sterols, this paper reports the synthesis of the C-6 epimer, 24-methylenecholest-4-en-3β,6α-diol (**2**) (Scheme 1).

2. Experimental

2.1. General methods

Stigmasterol was purchased from the Merck Co. The compounds **11** and **12** were prepared according to [2]. All chemicals and solvents were analytical grade and solvents were purified by general methods before use. Melting points were determined on a X_4 apparatus and are uncorrected. Infrared spectra were measured with Nicolet 205 FT-IR and Nicolet FT-360 spectrophotometers. ^{1}H NMR spectra were recorded on either a JEOL FX-90Q (90 MHz) or a Varian ^{Unity}Inova 500 NB (500 MHz) spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. Mass spectra were measured with a VG-ZAB-HS mass spectrometer using a 70 eV electron impact ion source.

2.2. Stigmast-4,22-dien-3β-(2-tetrahydropyranyl)ether (4)

To a solution of stigmasterol (**3**) (1.23 g; 3 mmol) in 5 ml of dihydropyran, one drop of concentrated HCl was added, and the mixture was stirred at room temperature for 24 h. Ether (15 ml) was added to the mixture to dissolve the precipitate. The organic layer was separated from the aqueous phase, and the aqueous phase was then extracted with ether (3 × 10 ml). The combined organic layer was washed sequentially with water, saturated Na_2CO_3 solution, water and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. EtOAc (1 ml) was added to the residues and the precipitate dissolved again. The solution was put in a refrigerator overnight. The white crystals were filtered, washed with MeOH, and dried under vacuum to yield 1.31 g (88%) of **4**, mp 161–162 °C. IR (KBr) ν: 1651, 1461, 1377, 1207, 1117, 1032, 969, 913, 871, 807 cm^{-1}.

The compounds **5** and **6** were similarly prepared according to the procedure of [2].

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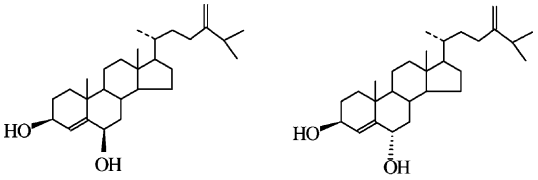
PII: S0039-128X(02)00059-4



1 **2**

Scheme 1.

2.3. 5α,6β-Dibromostigmast-22-en-3β-(2-tetrahydropyranyl)ether (5)

White solid (yield of 76%), mp 117–118 °C. IR (KBr) ν: 1651, 1461, 1377, 1152, 1053, 969, 568 cm^{-1}. ^1H NMR ($CDCl_3$, 90 MHz) δ: 0.70 (s, 3H, 18-CH_3), 0.79 (d, 3H, J = 6.4 Hz, 26-CH_3 or 27-CH_3), 0.81 (t, 3H, J = 7.6 Hz, 29-CH_3), 0.84 (d, 3H, J = 6.4 Hz, 26-CH_3 or 27-CH_3), 1.01 (s, 3H, 19-CH_3), 1.02 (d, 3H, J = 6.7 Hz, 21-CH_3), 3.52 (bm, 2H, THP 6-CH_2), 4.30–4.56 (bm, 1H, 3-CH), 4.84 (bm, 1H, THP 2-CH), 5.08 (t, 1H, J = 5.4 Hz, 6-CH), 5.12 (bm, 1H, 22-CH), 5.35 (bm, 1H, 23-CH).

2.4. Cholest-5-en-3β-ol-22-al (6)

White solid (62%), mp 142–143 °C. IR (KBr) ν: 3409, 3029, 2713, 1721, 1461, 1377, 1060, 955, 842, 800 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.73 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 1.13 (d, 3H, J = 6.5 Hz, 21-CH_3), 2.37 (m, 1H, 20-CH), 3.53 (tt, 1H, J = 11.5 and 5.0 Hz, 3-CH), 5.35 (dd, 1H, J = 2.5 and 3.5 Hz, 6-CH), 9.58 (d, 1H, J = 3.5 Hz, 22-CH).

2.5. Cholest-5,22-dien-3β-ol-24-one (7)

The compounds (3-methyl-2-oxobutyl)-triphenylarsonium bromide (**11**) (140 mg; 0.30 mmol), K_2CO_3 (70 mg; 0.5 mmol) and 0.03 ml of formamide were added to a solution of **6** (73 mg; 0.22 mmol) in 4 ml of MeCN, and the mixture was stirred at room temperature for 9 h under an argon atmosphere. Then the suspension was filtered, and the solid was washed three times with EtOAc. The solvent was removed under vacuum, and the residue was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (4:1) as the eluent. The product was further purified by recrystallization from MeOH to give 61 mg of **7** (69%), mp 114–116 °C. IR (KBr) ν: 3423, 1693, 1665, 1623, 1461, 1053, 990 cm^{-1}. ^1H NMR ($CDCl_3$, 500 Hz) δ: 0.73 (s, 3H, 18-CH_3), 1.01 (s, 3H, 19-CH_3), 1.10 (d, 9H, J = 6.5 Hz, 21-CH_3, 26-CH_3, 27-CH_3), 2.83 (m, 1H, J = 7.0 Hz, 25-CH), 2.21–2.31 (bm, 3H, 7-CH_2, 20-CH), 3.53 (m, 1H, 3-CH), 5.35 (dd, 1H, J = 3 and 2 Hz, 6-CH), 6.07 (d, 1H, J = 15.5 Hz, 23-CH), 6.72 (dd, 1H, J = 15.5 Hz, J = 9 Hz, 22-CH).

2.6. Cholest-5-en-3β-ol-24-one (8)

PtO_2 (7 mg) was added to a solution of **7** (80 mg; 0.18 mmol) in 15 ml of EtOAc. Hydrogen was passed into the stirred mixture at room temperature. After 30 min, the reaction was stopped, and the mixture was filtered. The solvent was removed in vacuum and the resulting white solid was recrystallized from acetone to give 79 mg of **8** as white needles (98%), mp 136–137 °C. IR (KBr) ν: 3416, 1707, 1640, 1581, 1461, 1377, 1243, 1103, 1053, 1025, 955 cm^{-1}. ^1H NMR ($CDCl_3$, 500 Hz) δ: 0.68 (s, 3H, 18-CH_3), 0.92 (d, 3H, J = 7.0 Hz, 21-CH_3), 1.01 (s, 3H, 19-CH_3), 1.09 (d, 6H, J = 7.0 Hz, 26-CH_3 and 27-CH_3), 2.61 (m, 1H, J = 7.0 Hz, 25-CH), 3.53 (tt, 1H, J = 12.0 and 5.5 Hz, 3-CH), 5.35 (dd, 1H, J = 3.0 and 2.5 Hz, 6-CH).

2.7. 24-Methylenecholest-5,6-en-3β-ol (9)

About 42 mg of 80% NaH-paraffin (1.75 mmol of sodium hydride) mixture was placed in a two-neck flask flushed with argon. The paraffin was washed away with petroleum ether (30–60 °C) and 5 ml of anhydrous dimethyl sulfoxide was added into the flask after the sodium hydride had been dried under reduced pressure under argon. The mixture was stirred at 80 °C for about 40 min under the argon atmosphere, and a dark green solution was produced. After cooling to room temperature, a solution of 400 mg (1 mmol) of methyltriphenylphosphonium iodide (**12**) in 4 ml of anhydrous dimethyl sulfoxide was added to the dark green solution, and the solution turned yellow immediately. After 10 min, a solution of 50 mg (0.13 mmol) of **8** in 4 ml of anhydrous dimethyl sulfoxide was added, and the resulting mixture was stirred at 80 °C for 4 h. At the end of this time, the reaction mixture was cooled, diluted with 15 ml of cold water, and extracted thoroughly with ether. The combined organic extracts were washed with water and saturated sodium chloride. 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 (bp 60–90 °C)/EtOAc (5:1) as the eluent to give 43 mg of **9** as white crystals (86%), mp 148–149 °C. IR (KBr) ν: 3416, 3078, 1644, 1461, 1377, 1053, 955, 885, 842, 800 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.95 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.01 (s, 3H, 19-CH_3), 1.03 (dd, 6H, J = 7.0 and 2.5 Hz, 26-CH_3 and 27-CH_3), 3.54 (tt, 1H, J = 11.5 and 5.0 Hz, 3-CH), 4.66 (d, 1H, J = 1.5 Hz, 28-CH), 4.71 (d, 1H, J = 1.5 Hz, 28-CH), 5.35 (dd, 1H, J = 3.0 and 2.5 Hz, 6-CH).

2.8. 24-Methylenecholest-4-en-3,6-dione (10)

Pyridinium chlorochromate (PCC) (240 mg; 1.1 mmol) was added in one portion to a solution of **9** (70 mg; 0.18 mmol) in dried CH_2Cl_2 (4 ml). The reaction mixture was stirred at room temperature for 48 h. Ether (15 ml) was then added to the mixture, and the suspension was poured

over a silica gel column and eluted with ether. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel using petroleum ether (60–90 °C)/EtOAc (5:1) as the eluent to give 43 mg (60%) of **10** as pale yellow crystals, mp 122–124 °C. IR (KBr) ν: 3078, 1686, 1602, 1581, 1454, 1377, 1243, 1124, 1025, 948, 892 cm^{-1}. ^1H NMR ($CDCl_3$, 500 Hz) δ: 0.73 (s, 3H, 18-CH_3), 0.96 (d, 3H, J = 5.5 Hz, 21-CH_3), 1.03 (ddd, 6H, J = 7.0, 3.0 and 1.5 Hz, 26-CH_3 and 27-CH_3), 1.17 (s, 3H, 19-CH_3), 2.44 (brs, 1H, 2β-CH), 2.48 (brs, 1H, 7β-CH), 2.52 (dd, 1H, J = 14.8 and 3.5 Hz, 2α-CH), 2.68 (dd, 1H, J = 15.7 and 3.0 Hz, 7α-CH), 4.66 (s, 1H, 28-CH), 4.72 (s, 1H, 28-CH), 6.17 (s, 1H, 4-CH).

2.9. 24-Methylenecholest-4-en-3β,6α-diol (2)

$NaBH_4$ (16 mg; 0.42 mmol) was added to a solution of **10** (40 mg; 0.10 mmol) and $CeCl_3{\cdot}7H_2O$ (38 mg; 0.10 mmol) in CH_3OH (7 ml) over an interval of 3 min at room temperature. After the mixture was stirred for 15 min, the reaction was stopped. The reaction mixture was neutralized with 1 M HCl. After evaporation of the majority of the MeOH under reduced pressure, H_2O was added to the residue, and the resulting white precipitate was filtered and washed with cold water. The crude product was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/acetone (5:1) as eluent to give 36 mg of **2** as a white solid (89%), mp 170–172 °C. IR (KBr) ν: 3282, 1644, 1581, 1447, 1377, 1271, 1068, 1032, 983, 927, 885 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.94 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.02 and 1.03 (d, 6H, J = 6.5 Hz, 26-CH_3 and 27-CH_3), 1.05 (s, 3H, 19-CH_3), 2.22 (m, 1H, J = 6.5 Hz, 25-CH), 4.17 (m, 1H, 6-CH), 4.21 (brt, 1H, 3-CH), 4.65 (s, 1H, 28-CH), 4.71 (s, 1H, 28-CH), 5.65 (d, 1H, J = 1.5 Hz, 4-CH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.69(C-24), 149.13(C-5), 119.91(C-4), 106.04(C-28), 68.57(C-6), 67.91(C-3), 55.96, 55.80, 54.20, 42.56, 42.05, 39.66, 37.91, 36.00, 35.72(C-20), 34.58(C-22), 34.34, 33.78(C-25), 30.95(C-23), 29.03, 28.15, 24.20, 22.02(C-26), 21.88(C-27), 21.00, 19.69(C-19), 18.66(C-21), 11.99(C-18). MS (FAB) m/z: 414 (M^+), 413 (M − 1), 397, 379, 295, 269, 253, 213, 187, 159, 121, 95, 69, 55.

3. Results and discussion

In this study, stigmasterol (**3**) was chosen as the starting material for the synthesis of 24-methylenecholest-4-en-3β,6α-diol (**2**). First, the 3β-hydroxy group of **3** was protected by forming the tetrahydropyranyl ether (**4**). Then, bromination of **4** was performed by using iodobenzene dibromide, which gave off bromine rapidly at room temperature, but was quite stable at 0 °C, using the method of Fryberg et al. [3]. Because of the greater density of the electron cloud in the 5,6-double bond compared to the 22,23-double bond, bromination of **4** using iodobenzene dibromide gave the 5,6-dibromide (**5**) in a high state of purity, which could be subjected to ozonolysis without further purification. During ozonolysis, the debromination and removal of the tetrahydropyranyl group in the 5,6-dibromide (**5**) took place simultaneously when the ozonide was decomposed with zinc and acetic acid at room temperature.

For the conversion of aldehyde (**6**) to ketone (**7**), a solid–liquid phase-transfer Wittig reaction was used. The reaction was carried out at room temperature using (3-methyl-2-oxobutyl)-triphenylarsonium bromide (**11**) as the reagent and K_2CO_3 as the base in CH_3CN. Addition of a trace of H_2O or $HCONH_2$ to the reaction may greatly increase the speed of the reaction. The use of **11** gave a higher yield and required a shorter reaction time than the reaction using triphenylphosphine isobutanonemethylene as the reagent [3]. From the proton signals of the 22,23-double bond ($^3J_{H-H}$ = 15.5 Hz), we knew that this reaction was stereoselective and produced only the *trans*-isomer.

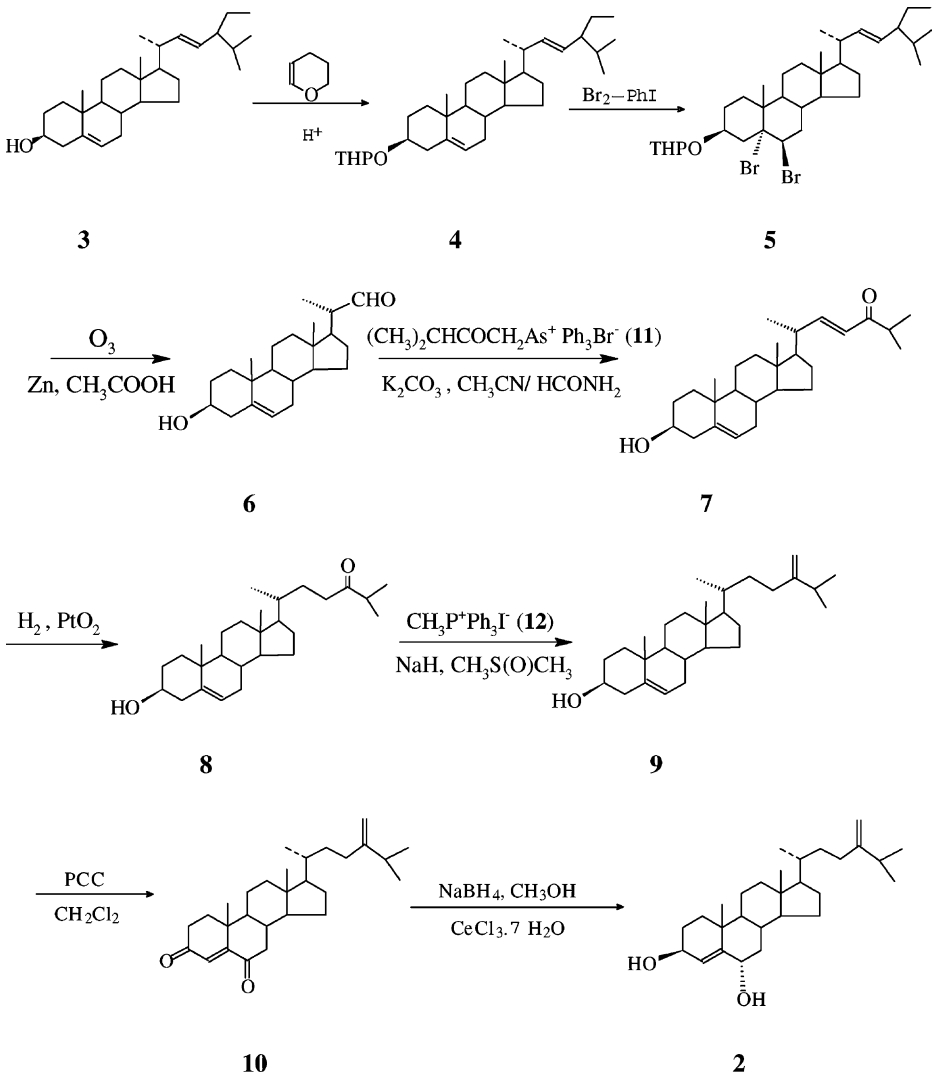
The catalytic hydrogenation of **7** was a regioselective reaction. When PtO_2 was used as catalyzer and the reaction was carried out at room temperature, it nearly quantitatively produced the dihydro-product (**8**) which had a hydrogenated 22,23-double bond and a 5,6-double bond with no change. This occurred because of the greater stereohindrance in the 5,6-double bond than those in the 22,23-double bond (Scheme 2).

Methylenation of **8** was completed via the Wittig reaction with methylenetriphenylphosphorane, prepared from sodium hydride and methyltriphenylphosphonium iodide (**12**) in dimethylsulfoxide. It was necessary to increase the ratio of sodium hydride and phosphonium to steroid in order to insure high yield because the hydroxy group in the steroid consumed some of the base in the reaction. Longer reaction times resulted in the formation of an aryl ether of the steroid. By protecting the hydroxyl group in the steroid, this side reaction was avoided. The spectra data of product **9** was consistent with the spectra of natural fucosterol.

For construction of the steroidal nucleus, compound **9** was oxidized to its corresponding 4-ene-3,6-dione (**10**) by oxidation with PCC in dichloromethane for 48 h at ambient temperature and gave a 60% yield [4]. Shorter reaction times would not improve the formation of **10** since the reaction may proceed by an ionic mechanism [5], and by-products of 4-ene-3-one and 6-hydroxy-4-ene-3-one would be formed. When the reaction was stopped after 24 h, a 53% yield of **10** was obtained.

Before the desired compound **2** was prepared, dione (**13**) was used as a model compound to study the reduction of a similar steroidal 4-en-3,6 dione (Scheme 3). When **13** was reduced with $NaBH_4$ in methanol, stigmast-4,22-dien-3β,6α-diol (**14**) and another isomer, stigmast-4,22-dien-3β,6β-diol, were obtained in a ratio of 9:1, according to HPLC analysis. However, in the presence of $CeCl_3{\cdot}7H_2O$, the reaction afforded **14** as the sole product.

Preparation of the target product **2** was completed by the reduction of **10** with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$

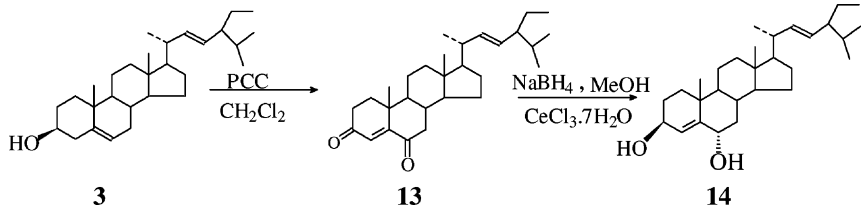


Scheme 2.

in MeOH at room temperature. The ^{1}H NMR and ^{13}C NMR spectra data of product **2** and 24-methylenecholest-4-en-3β,6β-diol (**1**), isolated from the soft coral *A. patagonicum*, are shown in Table 1.

The data in Table 1 reveals some differences between **1** and **2**. For ^{1}H NMR, the difference between the peaks for the 19-CH_3 groups of **1** and **2** is 0.21 ppm. This shows that there is a greater deshielding effect of 6β-OH to 19-CH_3 in sterol **1** than 6α-OH to 19-CH_3 in sterol **2** [6]. On the other hand, the coupling constant of 6β-H in **2** with 7-H was larger so its resonance peak split into lower multi-peaks. Since these resonance peaks overlapped with the resonance peaks of 3α-H, the coupling constant of 6β-H could not be confirmed. For ^{13}C NMR spectra, the chemical shift of 6-C in **1** and **2** showed different values because the configurations of both 6-C were different.

The cytotoxicity of compounds **1** and **2** against human gastric carcinoma cells (MGC) and cervical carcinoma cells (HELA) were studied, and the results are shown in Table 2. Obviously, compound **1** exhibited moderate cytotoxicity



Scheme 3.

Table 1
^{1}H NMR and ^{13}C NMR spectra data of **2** and 24-methylenecholest-4-en-3β,6β-diol (**1**)

Compounds	18-H_3	19-CH_3	21-H_3	3-H_1	4-H_1	6-H_1[a]	25-H_1	28-H_2	26,27-H_3
^{1}H NMR spectra									
1	0.74	1.26	0.93	4.18	5.54	4.23	2.23	4.65, 4.71	1.01, 1.02
2	0.68	1.05	0.94	4.20	5.65	4.17	2.22	4.65, 4.71	1.02, 1.03
	18-C	19-C	21-C	3-C	4-C	6-C	5-C	24-C	28-C
^{13}C NMR spectra									
1	11.82	20.81	18.49	67.66	118.33	73.76	147.36	157.05	105.71
2	11.99	19.69	18.66	67.91	119.91	68.57	149.13	156.69	106.04

[a] 6-H_1: **1**: brt, $J = 2.5$ Hz; **2**: m, determination of J was not possible.

Table 2
Cytoxocity (ED_{50} μg ml^{-1}) of compound **1** and **2** against some human tumor cell lines by the MTT method

Compounds	MGC	HELA
1	10.0	12.0
2	110.0	130.0

toward MGC and HELA cells and compound **2** showed only very weak cytotoxicity towards these two tumor cells.

Acknowledgments

The authors thank the National Natural Science Foundation of China (project no. 29932030) and the Natural Science Foundation of Guangdong Province (project no. 970154) for their support.

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