http://dx.doi.org/10.5012/bkcs.2012.33.7.2378

Notes

Anti-Proliferative Effect of Synthesized Bakuchiol Analogues on Cultured Human Tumor Cell Lines

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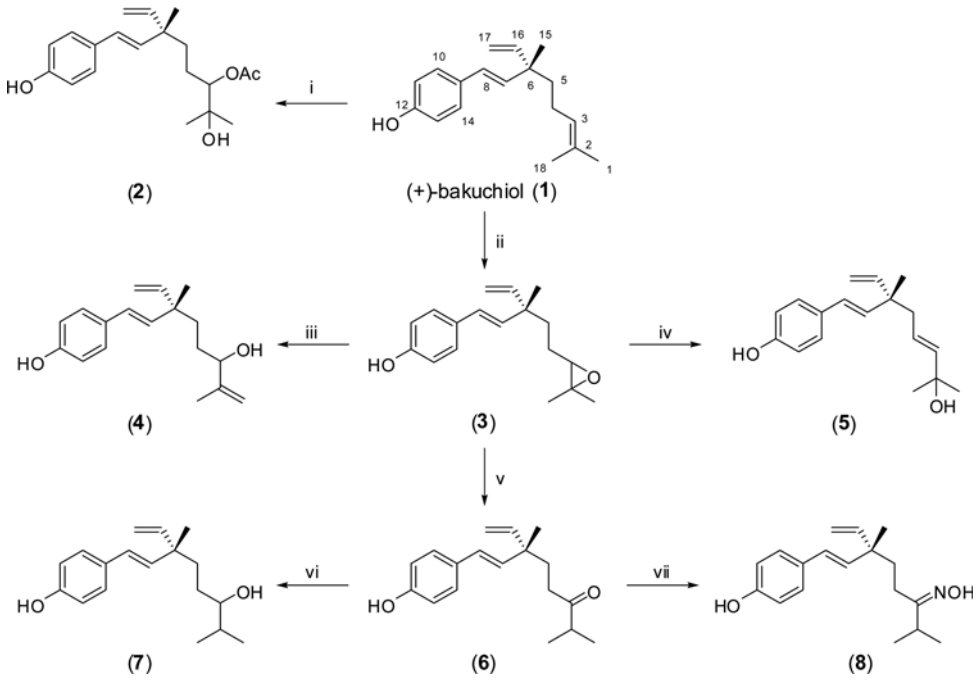
Received March 19, 2012, Accepted April 2, 2012

Key Words : Bakuchiol analogues, *Psoralea corylifolia*, Leguminosae, Anti-proliferation, Tumor cell

Bakuchiol (**1**) is an unique prenylated phenolic meroterpene found exclusively in the seeds of *Psoralea corylifolia* L. *P. corylifolia* is an annual plant of the Leguminosae family and distributed over Southeast Asian countries. Numerous biological actions of the whole extract of the plant or purified constituents have been reported as antioxidant, anti-inflammatory and antibacterial activities.[1-3] Compound **1** has been introduced firstly to be isolated from the species by G. Mehta *et al*. and the chemical structure of **1** was fully determined[3] including the absolute configuration of C-6 as (*S*)-chirality,[4] even the total synthesis was accomplished in 1973.[5] **1** is the main constituent of the species and reported to exert antidiabetic,[6] antitumor effects,[7] BACE-1 inhibitory activity,[8] and hepatoprotective[9] activities. In the previous study,[10] we had reported that the seeds extract of *P. corylifolia* exhibited a marked inhibitory effect on the proliferation of cultured human tumor cell lines and the inhibitory effect was mainly ascribed to **1**, a major component of the extract. As a trial for optimizing the chemical structure of **1** for improved antitumor activity, several analogues (**2**-**8**) of **1** were prepared according to the synthetic routes illustrated in Scheme 1. Briefly, **1** was treated with an equivalent amount of H_2O_2 and $(NH_4)_2Ce(NO_3)_6$ (ammonium cerium nitrate; CAN) in acetic acid to give **2**. It seemed that the $\Delta^{2,3}$ double bond of **1** was oxidized to give a corresponding diol, which was further acetylated to produce **2**.

Compound **3** was prepared by the epoxidation of **1** with *m*-chloroperbenzoic acid in CH_2Cl_2 at 0 °C. The treatment of



Scheme 1. Reagents and reaction conditions: (i) 1 eq. 30% H_2O_2, ammonium cerium nitrate, CH_3COOH, 1 h, 45%; (ii) MCPBA, CH_2Cl_2, rt, (quant.); (iii) $Al(Opr^i)_3$, toluene, 7 h, 110 °C, 85%; (iv) PhSeSePh, $NaBH_4$, EtOH, reflux, 2 h and then 10 eq. 30% H_2O_2, 75%; (v) 0.1 mol % CF_3SO_3H, CH_2Cl_2, 20 min., 65%; (vi) $NaBH_4$, THF-MeOH, 2 h, 85%; (vii) $H_2NOH{\cdot}HCl$, $NaHCO_3$, THF-H_2O, 6 h, 70%.

Table 1. Antitumor activity of (+)-bakuchiol (**1**) and its analogues **2-8** against human cancer cell lines

Components	ED_{50} (μM)[a]		
	A549	SK-OV-3	SK-MEL-2
1	36.21 ± 0.15	41.11 ± 0.25	36.13 ± 0.19
2	> 50	> 50	> 50
3	28.18 ± 0.14	39.45 ± 0.11	39.21 ± 0.14
4	46.32 ± 0.15	> 50	> 50
5	13.14 ± 0.23	22.71 ± 0.42	15.51 ± 0.11
6	43.25 ± 0.11	> 50	45.14 ± 0.21
7	27.36 ± 0.21	43.11 ± 0.14	42.13 ± 0.23
8	23.12 ± 0.05	47.13 ± 0.21	33.23 ± 0.17
Etoposide	2.61 ± 0.13	2.90 ± 0.37	2.73 ± 0.29

[a]ED_{50} value of compound against each cancer cell lines, which was defined as a concentration (μM) that caused 50% inhibition of cell proliferation *in vitro*. Data are expressed as mean ± SEM of three separate experiments.

3 with alumimum isopropoxide and diphenyldiselenide resulted in the opening of epoxide ring and subsequent rearrangement with different manner to give **4** and **5**, respectively. Compounds **3**, **4** and **5** have already been reported to be isolated from the genus *Psoralea.*[11] Another way, the epoxide ring of **3** was opened and converted to a 3-ketone group (**6**) by the treatment of triflormethansulfonic acid $-CH_2Cl_2$ at room temperature. The resultant 3-ketone group of **6** was reduced with $NaBH_4$ to give a corresponding alcohol (**7**) and also converted to oxime (**8**) with NH_2OH, respectively.

Each synthesized analogues of **1** (**2-8**) were evaluated for the inhibitory effect on the proliferation on cultured human cancer cell lines, A549 (non small cell lung), SK-OV-3 (ovary) and SK-MEL-2 (melanoma) cells, when etoposide, a clinically used antitumor agent, was used as a positive reference.

The concentrations of each compounds (**1-8**) required for 50% inhibition of the proliferation of tested tumor cells (ED_{50}) are summarized in Table 1. However, none of each synthesized bakuchiol analogues (**2-8**) except **5** exhibited significantly enhanced anti-proliferative activity on tested tumor cells compared with **1**, respectively. Only **5** exhibited a slightly higher inhibitory activity on the proliferation of tested tumor cells compared with **1**. The epoxidation (**2**) of $\Delta^{2,3}$ double bond of **1** still maintained the anti-proliferative activity of **1**. However, the opening of epoxide ring to diol (**2**) resulted in a profound lessening of activity. Alternatively, the opening of epoxide ring followed by subsequent rearrangement of double bond (**5**) resulted in the increase of activity. Consequently, it is still vague whether the $\Delta^{2,3}$ double bond of **1** was a critical pharmacophore for the inhibitory activity on the proliferation of tumor cells, which needed further investigation with more diverse derivatives of **1**.

Experimental Section

General Procedures. MS spectra were measured on Varian CP3800-1200L (EI-MS) Spectrometer. ^{1}H-NMR (nuclear magnetic resonance), and ^{13}C-NMR spectra were recorded on a Bruker (Rheinstetten, Germany) AM 300 NMR spectrometer using TMS as an internal standard. Column chromatography was performed using a silica gel (230-400 mesh, Merck, Darmstadt, Germany), and Lichroprep RP-18 (40-63 mm, Merck).

Isolation of 1. The dried seeds of *P. corylifolia* (1.2 kg) were soaked in methanol at room temperature for 7 days. Concentration of the solvent gave 180 g of dark syrupy residue, which was suspended in 15 L water and then partitioned with equal volume of dichloromethane to afford 150 g of dichloromethane fraction. The dichloromethane fraction was subjected to column (Ø = 10.0 × 80 cm) chromatography on silica gel eluted by gradient manner with each 5 L of methanol in dichloromethane solution (0 to 20%) to afford eight fractions, Fr. 1 ~ Fr. 8, respectively. Fr. 3 (38 g) was re-chromatographed on silica gel eluted with each 2 L of hexane:EtOAc (10:1) to give three subfractions, Fr. 31 ~ Fr. 33. Fr. 32 yielded 16 g of compound **1** as colorless oil. Compound **1** was identified as (+)-bakuchiol by direct comparison of their spectral data with those in the literature.[11]

***In vitro* Cytotoxicity Assays.** All of the experimental procedures followed the NCI's protocols with some minor changes based on the sulforhodamine B (SRB) method as described previously.[10]

Preparation of 2. To a solution of **1** (0.86 g, 3.4 mmol) in acetic acid (10 mL), 30% H_2O_2 (0.38 g, 3.4 mmol) solution of ammonium cerium nitrate (1.86 g, 3.4 mmol) in water (10 mL) was added over a period of 20 min. After 1 h, the reaction mixture was extracted with CH_2Cl_2 (2 × 10 mL) and dried over Na_2SO_4 and concentrated *in vacuo*. The silica gel column chromatography with 50% ethylacetate in *n*-hexane provided **2** (0.50 g, 45%) as a colorless oil. R_f = 0.52 (hexane/EtOAc 1:1).

MS *m/z* 332 $[M]^+$, ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.21 (2H, ABq, *J* = 8.4 Hz), 6.76 (2H, ABq, *J* = 8.4 Hz), 6.22 (1H, *d*, *J* = 16.2 Hz), 6.08 (1H, *br s*), 5.98 (1H, *d*, *J* = 16.2 Hz), 5.82 (1H, *dd*, *J* = 17.4, 10.7 Hz), 5.06-4.98 (3H, *m*), 4.80-4.78 (1H, *m*), 2.12 (3H, *s*), 1.95 (1H, *br s*), 1.64-1.45 (4H, *m*), 1.18 (6H, *s*), 1.67 (3H, *s*).

Preparation of 3. To a solution of **1** (3.8 g, 14.8 mmol) in CH_2Cl_2 (20 mL) was added *m*-chloroperbenzoic acid (MCPBA, 77%, 3.3 g, 14.8 mmol) at 0 °C. After 6 h, the reaction mixture was purified by silica gel column chromatography with ethyl acetate in *n*-hexane to give **3** (4.03 g, 100%) as a colorless oil. R_f = 0.65 (hexane/EtOAc 5:1).

MS *m/z* 272 $[M]^+$, ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.12 (2H, ABq, *J* = 8.6 Hz), 6.70 (2H, ABq, *J* = 8.6 Hz), 6.20 (1H, *d*, *J* = 16.2 Hz), 5.95 (1H, *d*, *J* = 16.2 Hz), 5.79 (1H, *dd*, *J* = 17.2, 10.8 Hz), 5.00 (1H, *dd*, *J* = 10.8, 1.2 Hz), 4.96 (1H, *dd*, *J* = 17.2, 1.2 Hz), 2.69 (1H, *m*), 1.52 (2H, *m*), 1.48 (2H, *m*), 1.25 (3H, *s*), 1.20 (3H, *s*), 1.12 (3H, *s*).

Preparation of 4. Compound **3** (1.04 g, 3.8 mmol) was dissolved in 50 mL of dry toluene and 10 mL of 0.5 M aluminum isopropoxide in toluene. The solution was

refluxed for 7 h, cooled, washed with 20 mL of 2 M HCl and then with water, followed by dried over $MgSO_4$. The silica gel column chromatography of the reaction mixture with 25% ethylacetate in *n*-hexane afforded **4** (0.88 g, 85%) as a pale yellow oil. R_f = 0.60 (hexane/EtOAc 3:1).

MS *m/z* 272 $[M]^+$, ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.24 (2H, ABq, *J* = 8.6 Hz), 6.75 (2H, ABq, *J* = 8.6 Hz), 6.17 (1H, *d*, *J* = 16.2 Hz), 5.94 (1H, *d*, *J* = 16.2 Hz), 5.84 (1H, *dd*, *J* = 17.2, 10.8 Hz), 5.58 (1H, *br s*), 5.05-4.84 (4H, *m*), 4.04 (1H, *t*, *J* = 4.6 Hz), 1.73 (2H, *m*), 1.70 (3H, *s*), 1.13 (3H, *s*).

Preparation of 5. To a solution of diphenyldiselenide (1.39 g, 4.5 mmol) in 5 mL dry EtOH, sodium borohydride (0.34 g, 8.8 mmol) was added under N_2. The mixture was stirred, and after it turned colorless, 2 mL of EtOH solution of **3** (1.14 g, 4.2 mmol) was added dropwise. The mixture was stirred and heated to reflux for 2 h. The reaction was quenched with 1 M HCl and the aqueous layer was extracted with ethylacetate (3 × 10 mL). The organic extract was washed with a saturated $NaHCO_3$ solution, water, brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in THF (40 mL), and 30% H_2O_2 (4.76 mL, 42.0 mmol) was added dropwise at 0 ºC. The mixture was stirred at room temperature for 2 h. The reaction was quenched with water, and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The organic extract was washed with water, brine, dried over $MgSO_4$, and concentrated *in vacuo*. The silica gel column chromatography with 33% ethyl acetate in *n*-hexane provided **5** (0.86 g, 75%) as a pale yellow oil. R_f = 0.70 (hexane/EtOAc 3:1).

MS *m/z* 272 $[M]^+$, ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.24 (2H, ABq, *J* = 8.6 Hz), 6.92 (1H, *br s*), 6.77 (2H, ABq, *J* = 8.6 Hz), 6.24 (1H, *d*, *J* = 16.3 Hz), 5.91 (1H, *d*, *J* = 16.3 Hz), 5.88 (1H, *dd*, *J* = 17.4, 10.7 Hz), 5.63 (2H, *m*), 5.05-4.98 (4H, *m*), 2.20 (2H, *d*, *J* = 5.8 Hz), 2.11 (1H, *br s*), 1.31 (6H, *s*), 1.16 (3H, *s*).

Preparation of 6. To a solution of **3** (2.72 g, 10.0 mmol) in dry CH_2Cl_2 (20 mL) triflоromethansulfonic acid (0.15 g, 1.0 mmol) was added at 0 °C under N_2 (the mixture turned brown). After stirring at 0 °C for 1 h, Et_3N (460 μL, 3.4 mmol) was added. The mixture was stirred for 10 min. The silica gel column chromatographic purification of the reaction mixture with 16.6% ethylacetate in *n*-hexane yielded **6** (1.77 g, 65%) as a yellow oil. R_f = 0.57 (hexane/EtOAc 5:1).

MS *m/z* 272 $[M]^+$, ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.21 (2H, ABq, *J* = 8.6 Hz), 6.80 (2H, ABq, *J* = 8.6 Hz), 6.61 (1H, *br s*), 6.24 (1H, *d*, *J* = 16.3 Hz), 5.96 (1H, *d*, *J* = 16.3 Hz), 5.82 (1H, *dd*, *J* = 17.4, 10.7 Hz), 5.06-5.00 (3H, *m*), 2.60 (1H, *m*), 2.45 (2H, *m*), 2.79 (2H, *m*), 1.67 (3H, *s*), 1.06 (6H, *d*, *J* = 7.0 Hz).

Preparation of 7. $NaBH_4$ (18.9 mg, 0.5 mmol) was added to a solution of **6** (0.27 g, 1.0 mmol) in THF (5 mL) and methanol (1 mL) at 0 °C. The mixture was stirred for 15 min. After addition of water (0.2 mL), the reaction mixture was stirred for another 2 h. The reaction was quenched with 1 M HCl and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic extract was washed with a saturated $NaHCO_3$ solution, water, brine, dried over $MgSO_4$, and concentrated *in vacuo*. The silica gel column chromatographic purification of the reaction mixture with 33% ethyl acetate in *n*-hexane) provided **7** (0.23 g, 85%) as a pale yellow oil. R_f = 0.68 (hexane/EtOAc 3:1).

MS *m/z* 274 $[M]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (2H, ABq, *J* = 8.4 Hz), 6.73 (2H, ABq, *J* = 8.4 Hz), 6.24 (1H, *d*, *J* = 16.2 Hz), 6.03 (1H, *d*, *J* = 16.2 Hz), 5.83 (1H, *dd*, *J* = 17.4, 10.7 Hz), 5.37 (1H, *br s*), 5.06-4.98 (2H, *m*), 3.35 (1H, *m*), 1.72-1.63 (4H, *m*), 1.42-1.37 (1H, *m*), 1.19 (6H, *s*), 0.90 (6H, *d*, *J* = 7.0 Hz).

Preparation of 8. To a solution of **6** (1.05 g, 3.9 mmol) in THF (15 mL), a solution of hydroxylamine hydrochloride (0.41 g, 5.8 mmol), $NaHCO_3$ (0.49 g, 5.8 mmol) in H_2O (3 mL) was added with stirring. After 24 h, the reaction mixture was extracted with EtOAc (3 × 10 mL). The organic extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The silica gel column chromatographic purification of the reaction mixture with 33% ethyl acetate in *n*-hexane provided **8** (0.78 g, 70%) as a pale yellow oil. R_f = 0.45 (hexane/EtOAc 3:1).

MS *m/z* 287 $[M]^+$, ^{1}H-NMR(300 MHz, $CDCl_3$) δ 7.23 (2H, ABq *J* = 8.4 Hz), 6.74 (2H, ABq, *J* = 8.4 Hz), 6.29 (1H, *d*, *J* = 16.2 Hz), 6.06 (1H, *d*, *J* = 16.2 Hz), 5.88 (1H, *dd*, *J* = 17.4, 10.7 Hz), 5.37 (1H, *br s*), 5.06-5.00 (2H, *m*), 2.49 (1H, *m*), 2.30-2.25 (2H, *m*), 1.75-1.71 (2H, *m*), 1.23 (3H, *s*), 1.09 (6H, *d*, *J* = 7.0Hz).

Acknowledgments. The Authors thanks to the financial support by the expansion of original compounds library and system management (TS113-02) through the Korea Research Institute of Chemical Technology (KRICT), and also by the basic research project (KK-1203-C0) of KRICT funded by the Ministry of Knowledge Economy (MKE), Republic of Korea. The authors also thanks Korean Basic Science Institute (KBSI) for NMR and MS experiments.

References

1. Sun, N. J.; Woo, S. H.; Cassady, J. M.; Snapka, R. M. *J. Nat. Prod.* **1998**, *61*, 362.
2. Lee, S. -J.; Nam, K. -W.; Mar, W. *Arch. Pharm. Res.* **2009**, *32*, 1061.
3. Mehta, G.; Nayak, U. R.; Dev, S. *Tetrahedron* **1973**, *29*, 1119.
4. Prakasa Rao, A. S. C.; Bhakka, V. K.; Nayak, U. R.; Dev, S. *Tetrahedron* **1973**, *29*, 1127.
5. Damodaran, N. P.; Dev, S. *Tetrahedron* **1973**, *29*, 1213.
6. Krenisky, J. M.; Luo, J.; Reed, M. J.; Carney, J. R. *Biol. Pharm. Bull.* **1999**, *22*, 1137.
7. Park, E. -J.; Zhao, Y. -Z.; Kim, Y. -C.; Sohn, D. H. *Eur. J. Pharmacol.* **2007**, *559*, 115.
8. Choi, Y. H.; Yon, G. H.; Hong, K. S.; Yoo, D. S.; Choi, C. W.; Park, W. -K.; Kong, J. Y.; Kim, Y. S.; Ryu, S. Y. *Planta Med.* **2008**, *74*, 1405.
9. Cho, H.; Jun, J. -Y.; Song, E. -K.; Kang, K. -H.; Baek, H. -Y.; Ko, Y. -S.; Kim, Y. -C. *Planta Med.* **2001**, *67*, 750.
10. Ryu, S. Y.; Choi, S. U.; Lee, C. O.; Zee, O. P. *Arch. Pharm. Res.* **1992**, *15*, 356.
11. Labbe, C.; Faini, F.; Coll, J.; Connolly, J. D. *Phytochemistry* **1996**, *45*, 1299.