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Synthesis and Structure-activity Relationship of Cytotoxic 5,2',5'-Trihydroxy-7,8-dimethoxyflavanone Analogues

ByungSun Min, ByungZun Ahn and KiHwan Bae

College of Pharmacy, Chungnam National University, Taejon 305-764, Korea

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Analogues of 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone, a naturally-occurring compound, which had been reported to have potent antitumor activity, were synthesized and examined for the cytotoxicity against three cancer cell lines. Among the intermediate chalcones and synthetic 5-hydroxy-7,8-dimethoxyflavanone analogues, (±)2',5'-dibenzyloxy-5,7,8-trimethoxyflavanone exhibited about 2-8 times stronger activity than 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone against L1210, K562 and A549 cancer cell lines. In the structure-activity relationship, it is suggested that among analogues of 5-hydroxy-7,8-dimethoxyflavanone, the existence of two oxygenated groups of *para*-relation at C-2' and C-5' positions on flavanone B-ring, may be necessary to exhibit effective cytotoxic activity.

Key words : 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone analogues, Substituent effect, Cytotoxic activity, L1210, K562, A549

INTRODUCTION

In cancer therapy, natural product is one of useful tools not only in their isolated form, but also as templates for the formation of analogues with improved activity and as probes for studying biochemical processes at the molecular level (O'Dwyer *et al.*, 1985). Numerous cytotoxic compounds have been isolated from natural sources using a cytotoxicity-based screening system. However, most of these compounds lack selectivity in attacking tumor cells and display minimal therapeutic indices (Jayatilake *et al.*, 1993). New therapeutic approaches of cancer have been directed toward modification of anticancer agents obtained from natural products with greater selectivity (Kingston *et al.*, 1990). Many active compounds have been isolated from plants, marine and microbial source, and vincristine, vinblastine, adriamycin, mitomycin, anthramycin and taxol have been used for clinical treatments (Kingston *et al.*, 1990).

In an effort to investigate natural anticancer compounds (Bae *et al.*, 1992; Bae *et al.*, 1994), it was found that 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone **1**, $[\alpha]_D^{20}$: -90.4° (c 1.0 in pyridine), (Fig. 1) isolated from the roots of *Scutellaria indica* L., displayed a potent cytotoxicity (ED_{50} values; 0.94, 13.7, 0.57 and 1.31 μg/ml against L1210, A549, HL-60 and K562 cancer cell lines) and an antitumor activity in mice bearing Sarcoma 180 cells (T/C value; 142%). And, skullcapflavone II, 5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone had been isolated from *Scutellaria baicalensis* Georgi, showed potent antitumor activity (Ryu *et al.*, 1985). Constituent **1** is found to be similar structurally to skullcapflavone II on A-ring. Among flavonoid compounds, flavone-8-acetic acid exhibited more potent antitumor activity *in vivo* against advanced Lewis Lung carcinoma than *in vitro*. Presently, flavone-8-acetic acid had been tested in a clinical trial (Finlay *et al.*, 1988; Wiltrout *et al.*, 1988). Structurally, flavonoids resemble nucleosides, isoalloxazine and folic acid, and this similarity is the basis of their physiological action (Havsteen, 1983).

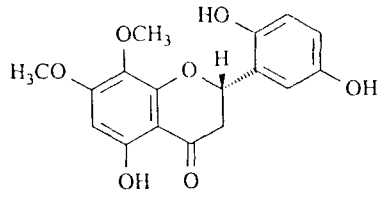
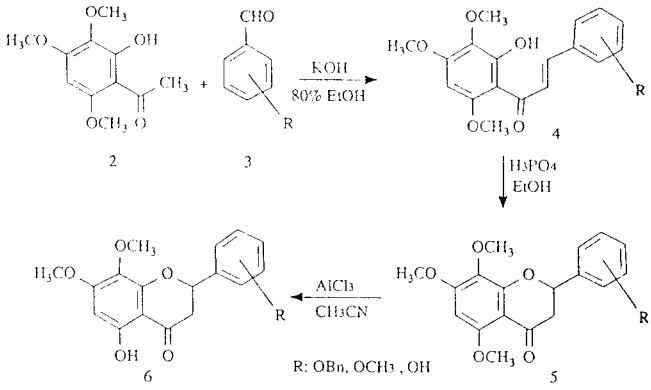


Fig. 1. Structure of 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone

Although, **1** showed a potent cytotoxic index in leukemia tumor systems, it expressed a low potency against human solid cancer cell line A549 (ED_{50} value; 13.7 μg/ml) and also showed a low potency in mice bearing Sarcoma 180. Therefore, in order to develop more potent compounds in the series of **1**,

Correspondence to: KiHwan Bae, College of Pharmacy, Chungnam National University, Taejon 305-764, Korea



Scheme 1. Synthetic method for the preparation of 2(S)-5,2', 5'-trihydroxy-7,8-dimethoxyflavanone analogues

structural modification of **1** was attempted. 5-methoxyl (**5a-m**) and 5-hydroxyl (**6a-m**) analogues were synthesized from chalcones (**4a-m**) as shown in Scheme 1, and their cytotoxic activities were evaluated.

In this paper, the analogues of **1** were synthesized, and their cytotoxic activities against three cancer cell lines (mouse leukemia L1210 cell, human leukemia K 562 cell and human carcinoma A549 cell) were evaluated, and finally structure-activity relationship was discussed.

MATERIALS AND METHODS

Apparatus and methods

All commercial reagents were purchased from Aldrich Co. and all solvents used with purification prior to use (Perrin *et al.*, 1988). Melting points were determined on an Electrothermal melting point apparatus. IR spectra in KBr disk were run on a JASCO Report-100 Infrared spectrophotometer. ^{1}H-NMR spectra were taken on a JEOL LNM-EX90 (90 MHz). Thin layer chromatography (TLC) was carried out on Kiesegel 60 F_{254} (Merck) with following solvent system; *n*-hexane: ethyl acetate (10 : 1, TLC **1**), *n*-hexane:ethyl acetate (6 : 4, TLC **2**), benzene:acetone (5 : 1, TLC **3**). Spots were detected by spraying of 5% $FeCl_3$ in MeOH and 5% H_2SO_4 solution. Flash column chromatography was carried out on silica gel (Merck, 230-400 mesh).

Biological assay

The *in vitro* cytotoxicity assay was carried out according to a National Cancer Institute protocol (Geran *et al.*, 1972) and an SRB assay method (Skehan *et al.*, 1990). ED_{50} value was evaluated by the procedure of Thayer *et al.* (Thayer *et al.*, 1971).

Procedure for the preparation of compound 2

2-Hydroxy-3,4,6-trimethoxyacetophenone **2** was prepared from pyrogallol by the methylation, oxidation, reduction, and acetylation (Baker, 1941).

Procedure for the preparation of compounds 3a-m

2-Benzyloxybenzaldehyde (**3a**), 3-benzyloxybenzaldehyde (**3b**), 4-benzyloxybenzaldehyde (**3c**), 2,5-dibenzyloxybenzaldehyde (**3d**), 3,5-dibenzyloxybenzaldehyde (**3e**), 3,4,5-tribenzyloxybenzaldehyde (**3f**) and 2-benzyloxy-5-methoxy-benzaldehyde (**3g**) were prepared from hydroxybenzaldehydes by benzylation with benzyl chloride and potassium carbonate in dimethylformamide (Freeman, 1990). 2-Methoxybenzaldehyde (**3h**), 3-methoxybenzaldehyde (**3i**), 4-methoxybenzaldehyde (**3j**), 2,5-dimethoxybenzaldehyde (**3k**), 3,5-dimethoxybenzaldehyde (**3l**) and 3,4,5-trimethoxybenzaldehyde (**3m**) were prepared from hydroxybenzaldehydes by methylation with dimethyl sulfate in 2N-sodium hydroxide (Horning, 1955) or potassium carbonate in acetone (Rabjohn, 1963).

Procedure for the preparation of compounds 4a-m

One equivalent of acetophenone **2** and two equivalents of benzaldehyde **3a-m** were dissolved in 80% EtOH containing 4% KOH to be 1% solution (Tanaka *et al.*, 1987). The mixture was stood for overnight at room temperature. The reaction mixture was acidified with d-HCl, then poured into water and extracted with ethyl acetate. The extract was purified by column chromatography.

2-Benzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4a

Yellow needles; m.p. 111-112°; yield: 79%; Rf 0.49 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.95, 8.21 (2H, each d, J=15 Hz, $H_{\alpha,\beta}$), 13.97 (1H, s, 2'-OH), 5.19 (2H, s, -C$\underline{H}_2$Ph), 3.75, 3.83, 3.93 (each 3H, each s, -OCH_3×3), 5.95 (H, s, 6-H).

3-Benzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4b

Yellow needles; m.p. 124.5-125°; yield: 76%; Rf 0.48 (TLC 2); IR (cm^{-1}): (KBr) 1630 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.68, 7.87 (2H, each d, J=15 Hz, $H_{\alpha,\beta}$), 13.87 (1H, s, 2'-OH), 5.10 (2H, s, -C$\underline{H}_2$Ph), 3.83, 3.91, 3.94 (each 3H, each s, -OCH_3×3), 6.00 (H, s, 6-H).

4-Benzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4c

Yellowish red needles; m.p. 142°; yield: 85%; Rf 0.39 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.79 (2H, s, $H_{\alpha,\beta}$), 14.02 (1H, s, 2'-OH), 5.12 (2H, s, -C$\underline{H}_2$Ph), 3.85 (3H, s, -OCH_3), 3.96 (6H, s, -OCH_3×2), 6.02 (H, s, 6-H).

2,5-Dibenzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4d

Yellow powders; m.p. 146.6°; yield: 90%; Rf 0.39 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.93, 8.22 (2H, each d, J=15.7 Hz, $H_{\alpha,\beta}$), 14.04 (1H, s, 2'-OH), 5.09, 5.12 (each 2H, each s, -CH_2Ph×2), 3.77, 3.89, 3.97 (each 3H, each s, -OCH_3×3), 5.99 (H, s, 6-H).

3,5-Dibenzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4e

Yellowish red powders; m.p. 126°; yield: 71%; Rf 0.44 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.66, 7.81 (2H, each d, J=15.5 Hz, $H_{\alpha,\beta}$), 13.89 (1H, s, 2'-OH), 5.08 (4H, s, -CH_2Ph×2), 3.84, 3.89, 3.95 (each 3H, each s, -OCH_3×3), 5.99 (H, s, 6-H).

3,4,5-Tribenzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 4f

Yellowish red powders; m.p. 99-101°; yield: 35%; Rf 0.42 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1580 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.62 (2H, s, $H_{\alpha,\beta}$), 13.94 (1H, s, 2'-OH), 5.12 (2H, s, 4-OCH_2Ph), 5.14 (4H, s, 3,5-OCH_2Ph), 3.81, 3.83, 3.92 (each 3H, each s, -OCH_3×3), 5.96 (H, s, 6-H).

2-Benzyloxy-2'-hydroxy-5,3',4',6'-tetramethoxychalcone 4 g

Yellowish red powders; m.p. 109°; yield: 50%; Rf 0.49 (TLC **2**); IR (cm^{-1}): (KBr) 1630 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.90, 8.16 (2H, each d, J=15.6 Hz, $H_{\alpha,\beta}$), 14.02 (1H, s, 2'-OH), 5.10 (2H, s, -CH_2Ph), 3.74, 3.77, 3.83, 3.91 (each 3H, each s, -OCH_3×4), 5.93 (H, s, 6-H).

2'-Hydroxy-2,3',4',6'-tetramethoxychalcone 4h

Yellowish red powders; m.p. 171°; yield: 87%; Rf 0.36 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.87, 8.17 (2H, each d, J=15.7 Hz, $H_{\alpha,\beta}$), 14.06 (1H, s, 2'-OH), 3.83, 3.88 (each 3H, each s, OCH_3×2), 3.92 (6H, s, OCH_3×2), 5.98 (H, s, 6-H).

2'-Hydroxy-3,3',4',6'-tetramethoxychalcone 4i

Yellowish red needles; m.p. 119°; yield: 85%; Rf 0.34 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.69, 7.89 (2H, each d, J=15.5 Hz, $H_{\alpha,\beta}$), 13.88 (1H, s, 2'-OH), 3.83, 3.94 (each 6H, each s, OCH_3×2×2), 6.00 (H, s, 6-H).

2'-Hydroxy-4,3',4',6'-tetramethoxychalcone 4j

Yellowish red needles; m.p. 140-141°; yield: 86%; Rf 0.26 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.77 (2H, s, $H_{\alpha,\beta}$), 14.07 (1H, s, 2'-OH), 3.83, 3.93 (each 6H, each s, OCH_3×2×2), 6.00 (H, s, 6-H).

2'-Hydroxy-2,5,3',4',6'-pentamethoxychalcone 4k

Yellow powders; m.p. 114.5°; yield: 92%; Rf 0.26 (TLC 2); IR (cm^{-1}): (KBr) 1630 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.86, 8.15 (2H, each d, J=15.6 Hz, $H_{\alpha,\beta}$), 14.03 (1H, s, 2'-OH), 3.79, 3.82, 3.84, 3.91, 3.93 (each 3H, each s, OCH_3×5), 5.98 (H, s, 6-H).

2'-Hydroxy-3,5,3',4',6'-pentamethoxychalcone 4l

Yellowish red powders; m.p. 144°; yield: 96%; Rf 0.24 (TLC **2**); IR (cm^{-1}): (KBr) 1620 (CO), 1580 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) 7.60, 7.81 (2H, each d, J=15.6 Hz, $H_{\alpha,\beta}$), 13.86 (1H, s, 2'-OH), 3.79 (6H, s, OCH_3×2), 3.80, 3.90, 3.91 (each 3H, each s, OCH_3×3), 5.96 (H, s, 6-H).

2'-Hydroxy-3,4,5,3',4',6'-hexamethoxychalcone 4m

Yellow powders; m.p. 157°; yield: 48%; Rf 0.49 (TLC **3**); IR (cm^{-1}): (KBr) 1630 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 7.73 (2H, s, $H_{\alpha,\beta}$), 13.95 (1H, s, 2'-OH), 3.91 (6H, s, OCH_3×2), 3.84, 3.90, 3.94, 3.95 (each 3H, each s, OCH_3×4), 6.00 (H, s, 6-H).

Procedure for the preparation of compound 5a-m

4a-m were dissolved in EtOH containing 4% H_3PO_4 to be 2% solution (Tanaka *et al.*, 1987). The mixture was refluxed for 30 hours, then poured into water and extracted with ethyl acetate. The extract was purified by column chromatography.

(±)-5,7,8-Trimethoxy-2'-benzyloxyflavanone 5a

White powders; m.p. 123-124°; yield: 52%; Rf 0.43 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.90 (1H, d, J=9.9 Hz, 3-H*trans*), 2.92 (1H, d, J=5.9 Hz, 3-H*cis*), 5.87 (1H, dd, J=5.9, 9.9 Hz, 2-H), 5.09 (2H, s, -OCH_2Ph), 6.13 (1H, s, 6-H), 3.79, 3.91, 3.92 (each 3H, each s, -OCH_3×3).

(±)-5,7,8-Trimethoxy-3'-benzyloxyflavanone 5b

White powders; m.p. 130-131°; yield: 53%; Rf 0.35 (TLC **3**); IR (cm^{-1}): (KBr) 1670 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.87 (1H, dd, J=5.3, 16.6 Hz, 3-H*cis*), 3.04 (1H, dd, J=11.1, 16.6 Hz, 3-H*trans*), 5.42 (1H, dd, J=5.3, 11.1 Hz, 2-H), 5.08 (2H, s, -OCH_2Ph), 6.13 (1H, s, 6-H), 3.78, 3.91, 3.94 (each 3H, each s, -OCH3×3).

(±)-5,7,8-Trimethoxy-4'-benzyloxyflavanone 5c

White needles; m.p. 151-152°; yield: 56%; Rf 0.33 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.88 (1H, d, J=4.6 Hz, 3-H*cis*), 2.95 (1H, d, J=10.6 Hz, 3-H*trans*), 5.41 (1H, dd, J=4.6, 10.6 Hz, 2-H), 5.07 (2H, s, -OC$\underline{H}_2$Ph), 6.12 (1H, s, 6-H), 3.77, 3.91, 3.93 (each 3H, each s, -OCH_3×3).

(±)-5,7,8-Trimethoxy-2',5'-dibenzyloxyflavanone 5d

White powders; m.p. 138.5-139°; yield: 43%; Rf 0.46 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1595 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.86 (1H, d, J=10.1 Hz, 3-H*trans*), 2.89 (1H, d, J=5.5 Hz, 3-H*cis*), 5.76 (1H, dd, J=5.5, 10.1 Hz, 2-H), 4.99 (4H, s, 2',5'-OC$\underline{H}_2$Ph), 6.08 (1H, s, 6-H), 3.71, 3.86, 3.88 (each 3H, each s, -OCH_3×3).

(±)-5,7,8-Trimethoxy-3',5'-dibenzyloxyflavanone 5e

White needles; m.p. 174.8-175°; yield: 57%; Rf 0.39 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.88 (1H, d, J=5.1 Hz, 3-H*cis*), 2.92 (1H, d, J=11.7, 3-H*trans*), 5.37 (1H, dd, J=5.1, 11.7 Hz, 2-H), 5.06 (4H, s, 3',5'-OC$\underline{H}_2$Ph), 6.14 (1H, s, 6-H), 3.78, 3.93, 3.95 (each 3H, each s, -OCH_3×3).

(±)-5,7,8-Trimethoxy-3',4',5'-tribenzyloxyflavanone 5f

White needles; m.p. 175.0-175.4°; yield: 38%; Rf 0.39 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.85 (1H, d, J=5.1 Hz, 3-H*cis*), 2.90 (1H, d, J=10.3 Hz, 3-H*trans*), 5.34 (1H, dd, J=5.1, 10.3 Hz, 2-H), 5.08 (2H, s, 4'-OC$\underline{H}_2$Ph), 5.14 (4H, s, 3',5'-OC$\underline{H}_2$Ph), 6.14 (1H, s, 6-H), 3.74, 3.93, 3.95 (each 3H, each s, -OCH_3×3).

(±)-5,7,8,5'-Tetramethoxy-2'-benzyloxyflavanone 5 g

White powders; m.p. 60.1°; yield: 65%; Rf 0.35 (TLC **3**); IR (cm^{-1}): (KBr) 1670 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) 2.88 (1H, d, J=10.1 Hz, 3-H*trans*), δ 2.91 (1H, d, J=5.7 Hz, 3-H*cis*), 5.82 (1H, dd, J=5.7, 10.1 Hz, 2-H), 5.05 (2H, s, 2-OC$\underline{H}_2$Ph), 6.14 (1H, s, 6-H), 3.80, 3.81, 3.92, 3.94 (each 3H, each s, -OCH_3×4).

(±)-5,7,8,2'-Tetramethoxyflavanone 5h

White needles; m.p. 152-153°; yield: 42%; Rf 0.40 (TLC **3**); IR (cm^{-1}): (KBr) 1670 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.86 (1H, d, J=10.6 Hz, 3-H*trans*), 2.89 (1H, d, J=5.5 Hz, 3-H*cis*), 5.78 (1H, dd, J=5.5, 10.6 Hz, 2-H), 6.13 (1H, s, 6-H), 3.80, 3.81, 3.92, 3.93 (each 3H, each s, -OCH_3×4).

(±)-5,7,8,3'-Tetramethoxyflavanone 5i

White powders; m.p. 101-102°; yield: 42%; Rf 0.34 (TLC **3**); IR (cm^{-1}): (KBr) 1670 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.91 (1H, d, J=5.3 Hz, 3-H*cis*), 2.95 (1H, d, J=10.1 Hz, 3-H*trans*), 5.44 (1H, dd, J=5.3, 10.1 Hz, 2-H), 6.14 (1H, s, 6-H), 3.82, 3.83, 3.93, 3.95 (each 3H, each s, -OCH_3×4).

(±)-5,7,8,4'-Tetramethoxyflavanone 5j

White needles; m.p. 112-114°; yield: 43%; Rf 0.2 (TLC **2**); IR (cm^{-1}): (KBr) 1670 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.80 (1H, dd, J=4.8, 16.2 Hz, 3-H*cis*), 3.06 (1H, dd, J=10.7, 16.2 Hz, 3-H*trans*), 5.41 (1H, dd, J=4.8, 10.7 Hz, 2-H), 6.12 (1H, s, 6-H), 3.78, 3.81, 3.91, 3.93 (each 3H, each s, -OCH_3×4).

(±)-5,7,8,2',5'-Pentamethoxyflavanone 5k

White needles; m.p. 118.7-119.4°; yield: 55%; Rf 0.33 (TLC **3**); IR (cm^{-1}): (KBr) 1670 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.85 (1H, d, J=10.7 Hz, 3-H*trans*), 2.89 (1H, d, J=5.1 Hz, 3-H*cis*), 5.75 (1H, dd, J=5.1, 10.7 Hz, 2-H), 6.15 (1H, s, 6-H), 3.78, 3.80, 3.83, 3.93, 3.95 (each 3H, each s, -OCH_3×5).

(±)-5,7,8,3',5'-Pentamethoxyflavanone 5l

White needles; m.p. 155.1-155.6°; yield: 57%; Rf 0.32 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.89 (1H, d, J=5.1 Hz, 3-H*cis*), 2.94 (1H, d, J=10.2 Hz, 3-H*trans*), 5.39 (1H, dd, J=5.1, 10.2 Hz, 2-H), 6.14 (1H, s, 6-H), 3.81 (6H, s, 3',5'-OCH_3×2), 3.82, 3.92, 3.94 (each 3H, each s, -OCH_3×3).

(±)-5,7,8,3',4',5'-Hexamethoxyflavanone 5m

White needles; m.p. 163.3-163.7°; yield: 49%; Rf 0.25 (TLC **3**); IR (cm^{-1}): (KBr) 1680 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.91 (1H, d, J=5.1 Hz, 3-H*cis*), 2.96 (1H, d, J=10.4 Hz, 3-H*trans*), 5.40 (1H, dd, J=5.1, 10.4 Hz, 2-H), 6.15 (1H, s, 6-H), 3.82, 3.86 (each 3H, each s, -OCH_3×2), 3.88 (6H, s, 3',5'-OCH_3×2), 3.92, 3.95 (each 3H, each s, -OCH_3×2).

Procedure for the preparation of compound 6a-m

One equivalent of flavanones 5a-m and four equivalents of $AlCl_3$ were dissolved in acetonitrile to be 1% solution (Farkas *et al.*, 1967). The mixture was refluxed for 3, 6 or 8 hours. After removing the solvent *in vacuo*, the reaction mixture was decomposed by d-HCl on the steam bath. The resulting mixture was filtered off, washed throughly with water, dissolved with ethyl acetate and purified by column chromatography.

(±)-5,2'-Dihydroxy-7,8-dimethoxyflavanone 6a

White needles; m.p. 151-153°; yield: 36%; Rf 0.39 (TLC **2**); IR (cm^{-1}): (KBr) 3300 (OH), 1630 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 3.07 (1H, d, J=4.8 Hz, 3-H*cis*), 3.13 (1H, d, J=9.9 Hz, 3-H*trans*), 5.73 (1H, dd, J=4.8, 9.9 Hz, 2-H), 11.92 (1H, s, 5-OH), 6.49 (1H, s, 2'-OH), 6.13 (1H, s, 6-H), 3.76, 3.90 (each 3H, each s, -OCH_3×2).

(±)-5,3'-Dihydroxy-7,8-dimethoxyflavanone 6b

Colorless needles; m.p. 160°; yield: 50%; Rf 0.35 (TLC **2**); IR (cm^{-1}): (KBr) 3450 (OH), 1640 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.93 (1H, d, J=5.1 Hz, 3-H*cis*), 2.99 (1H, d, J=10.2 Hz, 3-H*trans*), 5.45 (1H, dd, J=5.1, 10.2 Hz, 2-H), 11.94 (1H, s, 5-OH), 6.11 (1H, s, 6-H), 3.79, 3.89 (each 3H, each s, -OCH_3×2).

(±)-5,4'-Dihydroxy-7,8-dimethoxyflavanone 6c

Colorless needles; m.p. 174°; yield: 28%; Rf 0.30 (TLC **2**); IR (cm^{-1}): (KBr) 3350 (OH), 1640 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.77 (1H, dd, J=3.8, 17.2 Hz, 3-H*cis*), 3.13 (1H, dd, J=12.3, 17.2 Hz, 3-H*trans*), 5.36 (1H, dd, J=3.8, 12.3 Hz, 2-H), 11.88 (1H, s, 5-OH), 5.38 (1H, s, 4'-OH), 6.12 (1H, s, 6-H), 3.86, 3.88 (each 3H, each s, -OCH_3×2).

(±)-5,2',5'-Trihydroxy-7,8-dimethoxyflavanone 6d

Colorless needles; m.p. 192°; yield: 30%; Rf 0.26 (TLC **2**); IR (cm^{-1}): (KBr) 3250 (OH), 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: (ACETN) δ 2.97 (1H, d, J=4.4 Hz, 3-H*cis*), 3.00 (1H, d,J=10.5 Hz, 3-H*trans*), 5.77 (1H, dd, J=4.4, 10.5 Hz, 2-H), 12.06 (1H, s, 5-OH), 7.89, 8.11 (each 1H, each s, 5' and 2'-OH), 6.17 (1H, s, 6-H), 3.71, 3.91 (each 3H, each s, -OCH_3×2).

(±)-5,3',5'-Trihydroxy-7,8-dimethoxyflavanone 6e

Colorless needles; m.p. 230.5-230.9°; yield: 48%; Rf 0.28 (TLC **3**); IR (cm^{-1}): (KBr) 3400, 3250 (OH), 1630 (CO), 1600 (arom. C=C); ^{1}H-NMR: (ACETN) δ 2.82 (1H, dd, J=4.6, 17.4 Hz, 3-H*cis*), 3.12 (1H, dd, J=10.6, 17.4 Hz, 3-H*trans*), 5.46 (1H, dd, J=4.6, 10.6 Hz, 2-H), 12.02 (1H, s, 5-OH), 8.35 (2H, s, 3',5'-OH), 6.15 (1H, s, 6-H), 3.70, 3.90 (each 3H, each s, -OCH_3×2).

(±)-5,3',4',5'-Tetrahydroxy-7,8-dimethoxyflavanone 6f

Colorless needles; m.p. 238.2-238.9°; yield: 15%; Rf 0.14 (TLC **3**); IR (cm^{-1}): (KBr) 3250 (OH), 1620 (CO), 1600 (arom. C=C); ^{1}H-NMR: (ACETN) δ 2.80 (1H, dd, J=4.1, 18.8 Hz, 3-H*cis*), 3.14 (1H, dd, J=11.2, 18.8 Hz, 3-H*trans*), 5.39 (1H, dd, J=4.1, 11.2 Hz, 2-H), 12.05 (1H, s, 5-OH), 7.43 (1H, s, 4'-OH), 7.94 (2H, s, 3',5'-OH), 6.15 (1H, s, 6-H), 3.73, 3.90 (each 3H, each s, -OCH_3×2).

(±)-5,2'-Dihydroxy-7,8,5'-trimethoxyflavanone 6g

Faintly yellow powders; m.p. 157-160°; yield: 28%; Rf 0.35 (TLC **3**); IR (cm^{-1}): (KBr) 3400 (OH), 1630 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.94 (1H, dd, J=5.1, 17.3 Hz, 3-H*cis*), 3.18 (1H, dd, J=9.9, 17.3 Hz, 3-H*trans*), 5.69 (1H, dd, J=5.1, 9.9 Hz, 2-H), 11.93 (1H, s, 5-OH), 6.13 (1H, s, 2'-OH), 5.96 (1H, s, 6-H), 3.74, 3.77, 3.89 (each 3H, each s, -OCH_3×3).

(±)-5-Hydroxy-7,8,2'-trimethoxyflavanone 6h

White powders; m.p. 144-145°; yield: 52%; Rf 0.68 (TLC **2**); IR (cm^{-1}): (KBr) 1640 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.93 (1H, d, J=9.2 Hz, 3-H*trans*), 2.95 (1H, d, J=6.4 Hz, 3-H*cis*), 5.80 (1H, dd, J=6.4, 9.2 Hz, 2-H), 12.03 (1H, s, 5-OH), 6.12 (1H, s, 6-H), 3.80, 3.84, 3.90 (each 3H, each s, -OCH_3×3).

(±)-5-Hydroxy-7,8,3'-trimethoxyflavanone 6i

White powders; m.p. 100°; yield: 56%; Rf 0.62 (TLC **2**); IR (cm^{-1}): (KBr) 1640 (CO), 1600 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.94 (1H, d, J=4.8 Hz, 3-H*cis*), 3.01 (1H, d, J=10.7 Hz, 3-H*trans*), 5.45 (1H, dd, J=4.8, 10.7 Hz, 2-H), 11.95 (1H, s, 5-OH), 6.11 (1H, s, 6-H), 3.79, 3.83, 3.89 (each 3H, each s, -OCH_3×3).

(±)-5-Hydroxy-7,8,4'-trimethoxyflavanone 6j

Colorless needles; m.p. 130-131°; yield: 52%; Rf 0.59 (TLC **3**); IR (cm^{-1}): (KBr) 1640 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.83 (1H, dd, J=4.4, 17.9 Hz, 3-H*cis*), 3.12 (1H, dd, J=11.2, 17.9 Hz, 3-H*trans*), 5.42 (1H, dd, J=4.4, 11.2 Hz, 2-H), 11.97 (1H, s, 5-OH), 6.10 (1H, s, 6-H), 3.76, 3.83, 3.89 (each 3H, each s, -OCH_3×3).

(±)-5-Hydroxy-7,8,2',5'-tetramethoxyflavanone 6k

White needles; m.p. 142°; yield: 90%; Rf 0.59 (TLC **2**); IR (cm^{-1}): (KBr) 1640 (CO), 1620 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) 2.91 (1H, d, J=9.7 Hz, 3-H*trans*), 2.93 (1H, d, J=5.9 Hz, 3-H*cis*), 5.77 (1H, dd, J=5.9, 9.7 Hz, 2-H), 12.02 (1H, s, 5-OH), 6.12 (1H, s, 6-H), 3.80 (9H, s, -OCH_3×3), 3.84 (3H, s, -OCH_3).

(±)-5-Hydroxy-7,8,3',5'-tetramethoxyflavanone 6l

White needles; m.p. 153°; yield: 91%; Rf 0.50 (TLC **3**); IR (cm^{-1}): (KBr) 1630 (CO), 1610 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.91 (1H, d, J=4.6 Hz, 3-H*cis*), 2.98 (1H, d, J=10.6 Hz, 3-H*trans*), 5.38 (1H, dd, J=4.6, 10.6 Hz, 2-H), 11.93 (1H, s, 5-OH), 6.10 (1H, s, 6-H), 3.80 (9H, s, -OCH_3×3), 3.88 (3H, s, -OCH_3).

(±)-5-Hydroxy-7,8,3',4',5'-pentamethoxyflavanone 6m

White powders; m.p. 170.5°; yield: 81%; Rf 0.38 (TLC **2**); IR (cm^{-1}): (KBr) 1630 (CO), 1590 (arom. C=C); ^{1}H-NMR: ($CDCl_3$) δ 2.92 (1H, d, J=4.4 Hz, 3-H*cis*), 3.

01 (1H, d, J=10.8 Hz, 3-H*trans*), 5.38 (1H, dd, J=4.4, 10.8 Hz, 2-H), 11.93 (1H, s, 5-OH), 6.10 (1H, s, 6-H), 3.78 (3H, s, -OCH_3), 3.85, 3.87 (each 6H, each s, -OCH_3×4).

RESULTS AND DISCUSSION

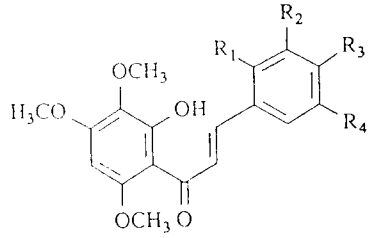
The flavonoids are benzo-γ-pyrone derivatives that serve to protect plants against environmental stress (Ji *et al.*, 1996), their occurrence is therefore widespread in the plant kingdom. The biological pathways with which flavonoids interact are numerous. Active flavonoids tend to inhibit cell activation at various levels. The flavanone analogues **5a-m** were synthesized from appropriate acetophenone **2** and benzaldehydes **3a-m** *via* chalcones **4a-m** according to the reported procedure (Tanaka *et al.*, 1987) as shown in Scheme 1. 5-Hydroxyflavanones **6a-m** were prepared from 5-methoxyl derivatives **5a-m** by the treatment with $AlCl_3$. The partial O-demethylation at C-5 position of **5a-m** is one of well-known methods (Farkas, 1967). Benzyloxyl products **5a-f** were also debenzylated to **6a-f** using the same procedure. Compounds **6a-m** have the same substituents on A and C ring as shown in natural 5,2',5'-trihydroxy-7,8-dimethoxyflavanone **1**. Synthetic flavanones **5a-m** have 5-methoxyl substituent in the place of 5-hydroxyl group in **1**. The synthetic analogues, **5a-m** and **6a-m**, were racemic mixtures at C-2 chiral carbon.

In the cytotoxicity of chalcone series as shown in Table I, it was found that the existence of methoxyl group at C-2 in **4h** and **4k** decreased the activity, in comparison with compound with methoxyl moiety at other position, while benzyloxyl compounds **4a,d,g** exhibited increased cytotoxicity. Among the C-3 mono-substituted chalcones, methoxyl compounds **4i, l,m** showed stronger activities than those of bearing benzyloxyl substituents **4b,e,f**. Introduction of benzyloxyl group at C-4 exhibited a cytotoxicity inferior to that of analogues with methoxyl group. **4i** was the most active among synthetic chalcone series. The compound **4l** substituted at C-3 and C-5 with methoxyl groups also showed a high activity. These results mean that the existence of relatively large or aromatic alkoxyl group at C-2 and small alkoxy substituent at C-3 or C-5 would contribute to increase the toxicity. Especially, oxygenated group at C-5 (that is C-3 oxygen in **4i**), a small substituent, seemed to be an essential factor in eliciting cytotoxicity in chalcone analogues.

In synthetic flavanones **5a-m** and **6a-m**, the structural difference in flavanone A ring is only substituent at C-5; 5-methoxyl *vs.* 5-hydroxyl group as described in Table II and III. 5-Hydroxylated compounds **6a-m**, where hydroxyl group at C-5 can form intramolecular hydrogen bond with carbonyl group at C-4, generally showed lower activity than correspoding 5-methoxyl series **5a-m** as shown in Table II and III. 7-Hydroxylated compounds such as 5,7-dihydroxy-8,2'-dimethoxyflavanone, 5,7,2'-trihydroxy-8-methoxyflavanone, wogonin and 5,7-dihydroxy-8,2'-dimethoxyflavone had been also reported to possess a cytotoxicity lower than **1** (Bae *et al.*, 1994). Synthetic **6d** exhibited the same cytotoxic activity as **1**, suggesting that there was no different potency between stereoisomers.

In mono-substituted flavanone **5** series, the activities of derivatives with benzyloxyl group on B ring were slightly superior to those of methoxyl derivatives, with some exceptions. Introduction of substituents at C-3' in **5b**, **6b** and **6i** was more effective to increase activity than that at other positions (C-2' and C-4'). Among di-substituted flavanones, the compounds **5d** and **5g** which had two *para*-substituents at C-2' and C-5' on B ring exhibited a relatively stronger activity than *meta*-substituent compound **5e**. The importance

Table I. Cytotoxicity[a] of synthesized chalcones



Chalcones					ED$_{50}$ (μg/ml)		
No.	R1	R2	R3	R4	L1210[b]	K562[c]	A549[d]
4a	OBn	H	H	H	2.4[e]	1.1	1.7
4b	H	OBn	H	H	2.6	4.4	1.9
4c	H	H	OBn	H	18.0	28.0	9.5
4d	OBn	H	H	OBn	6.1	3.6	>20.0
4e	H	OBn	H	OBn	7.2	6.2	14.0
4f	H	OBn	OBn	OBn	13.0	9.1	7.5
4g	OBn	H	H	OCH_3	1.1	1.2	2.0
4h	OCH_3	H	H	H	>20.0	>20.0	>20.0
4i	H	OCH_3	H	H	0.86	1.2	2.1
4j	H	H	OCH_3	H	1.6	1.9	2.3
4k	OCH_3	H	H	OCH_3	9.1	19.0	>20.0
4l	H	OCH_3	H	OCH_3	2.1	14.0	2.4
4m	H	OCH_3	OCH_3	OCH_3	2.1	2.3	3.1
5-FU					0.009-0.03	0.11-0.25	0.88-2.3

Bn: Benzyl

[a]Cytotoxicity of these compounds were evaluated by the procedure of Thayer *et al.*.

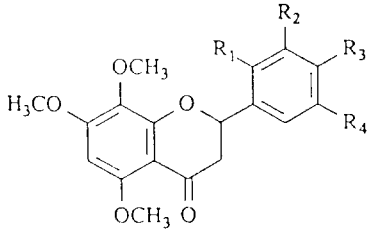
[b]L1210 cell was cultured Fisher's medium with 10% horse serum.

[c]K562 cell was cultured RPMI medium with 10% fetal bovine serum.

[d]A549 cell was cultured RPMI medium with 5% fetal bovine serum.

[e]The values indicate for the range of ED$_{50}$ of each test.

Table II. Cytotoxicity[a] of synthesized 5-methoxyflavanones



Flavanones					ED_{50} (μg/ml)		
No.	R1	R2	R3	R4	L1210[b]	K562[c]	A549[d]
5a	OBn	H	H	H	7.2[e]	3.7	1.3
5b	H	OBn	H	H	5.1	4.9	3.1
5c	H	H	OBn	H	5.5	5.3	3.5
5d	OBn	H	H	OBn	0.45	0.83	1.9
5e	H	OBn	H	OBn	6.5	16.0	4.4
5f	H	OBn	OBn	OBn	8.8	14.0	2.6
5g	OBn	H	H	OCH_3	3.0	2.1	5.7
5h	OCH_3	H	H	H	4.6	8.1	16.0
5i	H	OCH_3	H	H	7.7	4.1	>20.0
5j	H	H	OCH_3	H	7.0	11.0	18.0
5k	OCH_3	H	H	OCH_3	8.7	7.7	7.3
5l	H	OCH_3	H	OCH_3	12.0	4.3	9.6
5m	H	OCH_3	OCH_3	OCH_3	9.0	15.0	12.0
5-FU					0.009-0.03	0.11-0.30	0.83-2.3

Bn: Benzyl

[a]Cytotoxicity of these compounds were evaluated by the procedure of Thayer *et al.*.

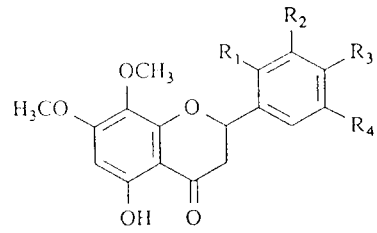
[b]L1210 cell was cultured Fisher's medium with 10% horse serum.

[c]K562 cell was cultured RPMI medium with 10% fetal bovine serum.

[d]A549 cell was cultured RPMI medium with 5% fetal bovine serum.

[e]The values indicate for the range of ED_{50} of each test.

Table III. Cytotoxicity[a] of synthesized 5-hydroxyflavanones



Flavanones					ED_{50} (μg/ml)		
No.	R1	R2	R3	R4	L1210[b]	K562[c]	A549[d]
6a	OH	H	H	H	4.7[e]	5.4	2.7
6b	H	OH	H	H	3.8	10	11
6c	H	H	OH	H	11	10	>20
6d	OH	H	H	OH	0.94	1.3	14
6e	H	OH	H	OH	>20	15	19
6f	H	OH	OH	OH	5.9	4.2	>20
6g	OH	H	H	OCH_3	5.9	5.2	8.7
6h	OCH_3	H	H	H	10	13	>20
6i	H	OCH_3	H	H	3.0	1.8	>20
6j	H	H	OCH_3	H	7.1	10	>20
6k	OCH_3	H	H	OCH_3	10	7.8	>20
6l	H	OCH_3	H	OCH_3	14	20	>20
6m	H	OCH_3	OCH_3	OCH_3	>20	>20	>20
5-FU					0.009-0.03	0.11-0.30	0.88-2.3

Bn:Benzyl

[a]Cytotoxicity of these compounds were evaluated by the procedure of Thayer *et al.*.

[b]L1210 cell was cultured Fisher's medium with 10% horse serum.

[c]K562 cell was cultured RPMI medium with 10% fetal bovine serum.

[d]A549 cell was cultured RPMI medium with 5% fetal bovine serum.

[e]The values indicate for the range of ED_{50} of each test.

of two *para*-oxygen bearing groups also appeared in 5-hydroxyl **6** series as evidenced in the results with compounds of **6d** and **6g**.

Among the **5a-m** and **6a-m** analogues, **d** series such as **5d** or **6d** were the most active against L1210 and K562, while **a** series **5a** and **6a** were most active against A549. Strong activity of **4a**, that possesses the benzyloxyl group at C-2' in flavanone series, was also remarkable for A549 cell.

The cytotoxic activity of synthetic analogues against K562 cell showed a pattern similar to the observation with L1210. However, the cytotoxicity against A549, human lung carcinoma, was considerably different from that of K562, particularly with 5-hydroxylated **6** analogues which showed a very weak activity, These results suggest that analogues of **1** seem to have more effective cytotoxicities against leukemia cell lines such as K562 and L1210.

It is concluded that the two *para*-oxygenated groups (substituted at C-2' and C-5') on B ring in flavanone analogues were a requisite factor to exhibit a cytotoxic activity. Moreover, the existence of oxygenated group at C-2' and C-5' was a fundamental requirement to have a superior cytotoxicity not only among chalcone analogues but also among flavanone analogues of 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone.

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