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Polyoxygenated Flavones; Synthesis, Cytotoxicities and Antitumor Activity against ICR Mice Carrying S-180 Cells

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Fifty two flavones were synthesized from polyoxygenated dibenzoylmethanes which were obtained by a modified Baker-Venkataraman rearrangement of 2-benzoyl oxyacetophenones. The following flavones among them showed good cytotoxic activities against L1210 and HL-60 cells; 2'-benzyloxy-5-methoxyflavone [ED_{50}(L1210)=4.9 μg/ml), ED_{50}(HL-60)=3.1 μg/ml], 2'-benzyloxy-5,7-dimethoxyflavone (8.2 μg/ml, 5.0 μg/ml), 2'-benzyloxy-5,7,8-trimethoxyflavone (5.9 μg/ml, 11.0 μg/ml), 2'-hydroxy-5,7-dimethoxyflavone (8.3 μg/ml, 4.9 μg/ml), 2'-hydroxy-5-methoxyflavone (4.2 μg/ml, 2.7 μg/ml), 2'-hydroxy-5,7,8-trimethoxyflavone (9.8 μg/ml, 6.2 μg/ml), 2'-benzyloxy-5-hydroxyflavone (5.2 μg/ml, 3.6 μg/ml), and 5,2'-dihydroxyflavone (5.1 μg/ml, 4.0 μg/ml). Presence of 5-methoxy group potentiated the cytotoxic activity, while the existence of 7-methoxy group decreased the activity. 5-Hydroxy or methoxy activates 4-carbonyl group, while 7-methoxy group deactivates the carbonyl group. From these observation it was concluded that the activation of carbonyl group at C-4 of a flavone is important for the enhancement of the cytotoxic activity.The presence of both 5-hydroxy and 2'-benzyloxy- or 2'-hydroxy group enhanced the antitumor activity; 2'-benzyloxy-5-hydroxy-7-methoxyflavone (T/C=144%), 5,2'-dihydroxy-7-methoxyflavone (T/C=132%), and 5,2'-dihydroxy-6,7,8,6' tetramethoxyflavone (T/C=172%). 2'-Hexanoylation of 5,2'-dihydroxy-flavones did not improve the antitumor activity; 2'-hexanoyloxy-5-hydroxy-7-methoxyflavone showed T/C=132%, about the same as that of 5,2'-dihydroxy-7-methoxyflavone (T/C=130%)

Key words: Flavone synthesis, Antitumor activity, Structure-activity relationship

INTRODUCTION

Flavonoid compounds belong to a vast class of natural products and are ubiquitously distributed in the plant kingdom. These compounds show a broad spectrum of pharmacological activities (Havsteen., 1983). Recent results from the studies on the flavonoids include the inhibition of protein-tyrosine kinases (Hagiwara *et al.*, 1988; Cusman *et al.*, 1991; Geahlen *et al.*, 1989), retroviral transcriptases (Ono *et al.*, 1989; Nakane *et al.*, 1990), protein kinases C (Ferriola *et al.*, 1989), DNA -topoisomerases II (Markovits *et al.*, 1989; Okura *et al.*, 1989)), and other enzymes(Shoshan *et al.*, 1981; Spanka *et al.*, 1989), and antitumor (Cassady *et al.*, 1990; Atassi *et al.*, 1985) and antimutagenic (Wall *et al.*, 1988) activities.

In a recent study, Cusman (Cusman *et al.*, 1991) found that oxygenation at 7,8 positions and functional variation at 4' position were important for the antitumor activity against five cancer cell lines, and 15 of 55 flavonoids tested showed cytotoxic activity against at least one cell line. Ahn and coworkers (Ahn., 1989) reported that 5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone, isolated from *Scutellaria baicalensis*, showed good antitumor activity against ICR mice carrying S-180 cell (T/C=165%). They modified the ring B of the flavone and evaluated the structure-activity relationship (Ryu *et al.*, 1985, 1987). From this relationship, they concluded that the presence of 2'-oxy or 2,6-dioxy group in ring B and the angle change between rings B and C are important for the antitumor activity.

In addition to the B-ring modification above mentioned, this study has been attempted to modify A of 2'-monoxy, or 2',6'-dioxygenated flavones and assess the influence of the ring A modification on the antitumor activity. New synthetic methods of 2-(2'-monobenzyloxy, or 2',6'-dibenzyloxybenzoyloxy) acetophenones and polyoxygenated dibenzoylmethanes as intermediates for the flavone synthesis was reported elswhere (Song *et al.*, 1994a and 1994b). The polyoxygenated dibenzoylmethanes were heated under acidic condition to give corresponding 2'-

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monoxygenated and 2',6'-dioxygenated flavones with polyoxygenated ring A. The cytotoxic and antitumor effects of these flavones on some tumor cell lines were observed.

MATERIALS AND METHODS

The cancer cells and culture media

The cell line of the mouse Leukemia lymphoblast L 1210 and human promyelocytic leukemia were cultured in auther's laboratory.

Fischer's medium for L1210 and RPMI 1640 medium for HL-60 were purchased from GIBCO Laboratories.

Cell culture and maintainance.

L1210 stock cells were grown in the screw-caped culture flask (100 ml) at 37°C. The *in vitro* culture was transfered twice a week.

Preparation of 1, 000 ml Fischer's medium

i) One package of medium (Gibco) was dissolved in 880 ml of triple distilled water at 15~25°C

ii) Rinse out inside of package to remove all trace of powder

iii) Add 1.125 g of sodium bicarbonate, 100, 000 units/l of penicillin and 0.1 g/l of streptomycin

iv) Adjust pH to 7.2 with 0.1 N HCl

v) Add 100 ml of horse serum under asceptic condition

The same procedure was applied for culture of HL-60 cell

Determination of ED_{50} values

The ED_{50} value, the concentration of a test sample inhibiting the growth of L1210 and HL-60 cell by 50% in reference to the control, was determined by Thayer's method with minor modification:

i) Spinner culture; a culture containing the cells in logarithium phase of growth, was cultured 24 hrs before a test.

ii) Preparation of sample solution; 10 mg/ml ethanol or DMSO.

iii) Dilute the sample solution to 1/10 with fresh medium and put 100, 50 and 20 μl of the diluted solution into individual culture tubes

iv) Dilute spinner culture to 5×10^4 cells/ml

v) Deliver 5 ml of the diluted cell suspension into the culture tubes: the base line count will be 5×10^4 cells /ml

vi) Tubes are stoppered and incubated at 37°C for 48 hrs under 5% CO_2

vii) Count the cells with hemacytometer

viii) Calculate Y(%)=[(T-Co)/(C-Co)], where T=cell count of treated tube after 48 hrs, C=cell count of control tube after 48 hrs and Co= initial cell count

ix)Prepare a regerssion line between Y(%) values and $\log_{10}$ dose and find the $\log_{10}$ value to 50% Y value. Express the value as mg/ml

The animal test

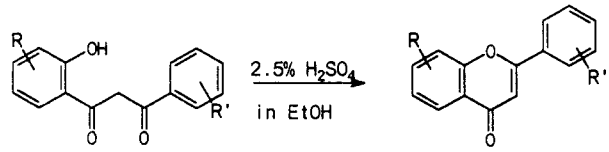
ICR mice were purchased from the Daehan Laboratory animal center. The experimental animals were 3-4 weeks old and weighed 20~24g. They were housed in plastic cage in air-conditioned room and supplied with foods and water without limitation.

For ascite forms of the tumor, 1×10^6 of S-180 cells were inoculated intraperitoneally in ICR mouse. 24 hrs after the inoculation the test substance was administrated intraperitoneally once a day for 7 consecutive days. The mortality of mice was recorded and the T/C values were measured according to following equation.

$$T/C(\%) = \frac{\text{Mean survival days of treated mice}}{\text{Mean survial days of control mice}} \times \%$$

Synthesis of flavones (Scheme 1)

General methods; -0.008 M Polyoxygenated dibenzoylmethanes were dissolved in 20 ml of 2.5% H_2SO_4-EtOH, and refluxed 1 hr. After cooling to room temperature, 50~60 ml of ice water was added to the reaction mixture and extracted with ethylacetate. The



Scheme 1. Synthesis of Flavones R=R'; Hydroxy or methoxygroups.

mass remained after evaporation of ethylacetate was chromatographed on silicagel column (n-hexane/ethylacetate) and recrystallized with methanol.

Flavones synthesized are as follows;

Flavone (1)

Yield 86%, white crystal, mp 96-97°C, IR υ_{max} cm^{-1} 3060, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 6.87 (1H, s, H-3), 7.21-7.78 (6H, m, H-6, 7, 8, 3, '4', 5'), 7.88-8.01 (2H, m, H-2', 6'), 8.25 (1H, dd, J=8.6, 1.7 Hz, H-5).

7-Methoxyflavone (2)

Yield 82%, white crystal, mp 125-127°C , IR υ_{max} cm^{-1} 3050, 2950, 1640, 1605, ^{1}H-NMR ($CDCl_3$)δ(ppm) 3.90 (3H, s, H-OCH_3), 6.78 (1H, s, H-3), 6.90-7.10 (2H, m, H-6, 8), 7.45-7.60 (3H, m, H-3', 4', 5'), 7.88-7.98 (2H, m, H-2', 6'), 8.15 (1H, d, J=8.6 Hz, H-5).

6-Methoxyflavone (3)

ield 78%, white crystal, mp 162.5-163°C, IR υ_{max} cm^{-1} 3100, 2900, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.90 (3H, s, H-OCH_3), 6.78 (1H, s, H-3), 7.20-7.34 (2H, m, H-7, 8), 7.44-7.62 (4H, m, H-5, 3', 4', 5'), 7.88-7.98 (2H, m, H-2', 6').

5-Methoxyflavone (4)

Yield 75%, white crystal, mp 107-108°C, IRυ_{max} cm^{-1} 3100, 2950, 1640, 1610, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.95 (3H, s, H-OCH_3), 6.72 (1H, s, H-3), 6.84 (1H, dd, *J*=8.6, 1.7 Hz, H-6), 7.12 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.42-7.60 (4H, m, H-7, 3', 4', 5'), 7.90-8.00 (2H, m, H-2', 6').

5-Hydroxyflavone (5)

Yield 54%, yellow crystal, mp 107-108°C, IR υ_{max} cm^{-1} 3400, 3100, 2950, 1640, 1610, ^{1}H-NMR ($CDCl_3$) δ (ppm) 6.72 (1H, s, H-3), 6.84 (1H, dd, *J*=8.6, 1.7 Hz, H-6), 7.12 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.42-7.60 (4H, m, H-7, 3', 4', 5'), 7.90-8.00 (2H, m, H-2', 6'), 12.5 (1H, s, H-OH).

5, 7-Dimethoxyflavone (6)

Yield 82%, white crystal, mp 143-145°C, IR υ_{max} cm^{-1} 3070, 2850, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.92 (3H, s, H-OCH_3), 3.98 (3H, s, H-OCH_3), 6.38 (1H, d, *J*=2.2 Hz, H-6), 6.58 (1H, d, *J*=2.2 Hz, H-8), 6.68 (1H, s, H-3), 7.43-7.59 (3H, m, H-3', 4', 5'), 7.79-7.92 (2H, m, H-2', 6').

7,8-Dimethoxyflavone (7)

Yield 79%, white crystal, mp 141-143°C, IR υ_{max} cm^{-1} 3080, 2850, 1648, 1600, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.98 (3H, s, H-OCH_3), 4.04 (3H, s, H-OCH_3), 6.88 (1H, s, H-3), 7.08 (1H, d, *J*=8.6 Hz, H-6), 7.42-7.63 (3H, m, H-3', 4', 5'), 7.98 (1H, d, *J*=8.6 Hz, H-5), 7.88-7.98 (2H, m, H-2', 6').

5,7,8-Trimethoxyflavone (8)

Yield 70%, white crystal, mp 169-171°C, IR υ_{max} cm^{-1} 3090, 2860, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.95 (3H, s, H-OCH_3), 4.02 (3H, s, H-OCH_3), 4.05 (3H, s, H-OCH_3), 6.45 (1H, s, H-6), 6.68 (1H, s, H-3), 7.45-7.59 (3H, m, H-3', 4', 5'), 7.86-8.02 (2H, m, H-2', 6').

2'-Benzyloxyflavone (9)

Yield 71%, white crystal, mp 82-83°C, IR υ_{max} cm^{-1} 3050, 2860, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 5.24 (2H, s, H-**CH_2**-Ph), 7.02 (1H, s, H-3), 7.18-7.72 (11H, m, H-6, 7, 8, 3, '4', 5', -CH_2-**Ph**), 7.82 (1H, dd, *J*=8.4, 1.5 Hz, H-6), 8.22 (1H, dd, *J*=8.6, 1.7 Hz, H-5).

2-Benzyloxy-7-methoxyflavone (10)

Yield 71%, white crystal, mp 138-139.5°C, IR υ_{max} cm^{-1} 3100, 2890, 1648, 1610, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.90 (3H, s, H-OCH_3), 5.20 (2H, s, H-**CH_2**-Ph), 6.84-7.52 (10H , m, H-6, 8, 3, '4', 5', -CH_2-**Ph**), 7.02 (1H, s, H-3), 7.84 (1H, dd, *J*=8.4, 1.5 Hz, H-6), 8.15 (1H, d, *J*=8.4 Hz, H-5).

2'-Benzyloxy-6-methoxyflavone (11)

Yield 78%, white crystal, mp 103-104°C , IR υ_{max} cm^{-1} 3090, 2880, 1645, 1608, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.92 (3H, s, H-OCH_3), 5.22 (2H, s, H-**CH_2**-Ph), 6.98-7.63 (11H , m, H-5, 7.8, 3, '4', 5', -CH_2-**Ph**), 7.04.(1H, s, H-3), 7.82 (1H, dd, *J*=8.4, 1.5 Hz, H-6).

2-Benzyloxy-5-methoxyflavone (12)

Yield 73%, white crystal, mp 119.8-120.4 C, IR υ_{max} cm^{-1} 3100, 2890, 1640, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.90 (3H, s, H-OCH_3), 5.22 (2H, s, H-**CH_2**-Ph), 6.79 (1H, dd, *J*=8.6, 1.7 Hz, H-6), 7.01 (1H, s, H-3), 7.13 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.21-7.63 (9H , m, H-7, 3, '4', 5', -CH2-**Ph**), 7.82 (1H, dd, J=8.4, 1.5 Hz, H-6).

2-Benzyloxy-5-hydroxyflavone (13)

Yield 51%, yellow crystal, mp 190-192 C, IR υ_{max} cm^{-1} 3380, 3090, 2890, 1640, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 5.22 (2H, s, H-**CH_2**-Ph), 6.79 (1H, dd, *J*=8.6, 1.7 Hz , H-6), 7.01 (1H, s, H-3), 7.13 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.21-7.63 (9H , m, H-7, 3, '4' , 5', -CH_2-**Ph**), 7.82 (1H, dd, *J*=8.4, 1.5 Hz, H-6), 12.32 (1H, s, H-OH).

2'-Benzyloxy-5,7-dimethoxyflavone (14)

Yield 74%, white crystal, mp 121-123°C, IR υ_{max} cm^{-1} 3085, 2900, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.88 (3H, s, H-OCH_3), 3.92 (3H, s, H-OCH_3), 5.20 (2H, s, H-**CH_2**-Ph), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.48 (1H, d, *J*= 2.2 Hz, H-8), 6.90 (1H, s, H-3), 6.98-7.48 (8H, m, H-3', 4', 5'-CH_2-**Ph**), 7.80 (1H, dd, *J*=8.4, 1.5 Hz, H-6).

2'-Benzyloxy-5-hydroxy-7-methoxyflavone (15)

Yield 49%, yellow crystal, mp 172-173°C, IR υ_{max} cm^{-1} 3410, 3100, 2900, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.90 (3H, s, H-OCH_3), 5.21 (2H, s, H-**CH_2**-Ph), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.48 (1H, d, *J*=2.2 Hz, H-8), 6.90 (1H, s, H-3), 6.98-7.48 (8H, m, H-3', 4', 5'-CH_2-**Ph**), 7.80 (1H, dd, *J*=8.4, 1.5 Hz, H-6), 12.20 (1H, s, H-OH).

2'-Benzyloxy-7,8-dimethoxyflavone (16)

Yield 73%, white crystal, mp 141-143°C, IR υ_{max} cm^{-1} 3090, 2890, 1645, 1602, ^{1}H-NMR ($CDCl_3$) δ (ppm)

3.98 (3H, s, H-OCH_3), 4.02 (3H, s, H-OCH_3), 5.20 (2H, s, H-**CH_2**-Ph), 7.02 (1H, s, H-3), 6.96-7.50 (9H, m, H-6, 3', 4', 5', -CH_2-**Ph**), 7.96 (1H, d, *J*=8.6 Hz, H-5).

2'-Benzyloxy-5,7,8-trimethoxyflavone (17)

Yield 72%, white crystal, mp 192-193°C, IR υ_{max} cm^{-1} 3060, 2880, 1640, 1600, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3. 90 (3H, s, H-OCH_3), 3.98 (3H, s, H-OCH_3), 4.00 (3H, s, H-OCH_3), 5.22 (2H, s, H-**CH_2**-Ph), 6.42 (1H, s, H-6), 7.00 (1H, s, H-3), 7.08-7.50 (8H, m, H-3', 4', 5', -CH_2-**Ph**), 7.92 (1H, dd, *J*=8.4, 1.5 Hz, H-6').

2'-Benzyloxy-5-hydroxy-7,8-dimethoxyflavone (18)

Yield 45%, yellow crystal, mp 212-214°C, IR υ_{max} cm^{-1} 3390, 3080, 2880, 1640, 1600, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.95 (3H, s, H-OCH_3), 4.00 (3H, s, H-OCH_3), 5.20 (2H, s, H-**CH_2**-Ph), 6.40 (1H, s, H-6), 7.10 (1H, s, H-3), 7.08-7.50 (8H, m, H-3', 4', 5', -CH_2-**Ph**), 7.92 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 12.46 (1H, s, H-OH).

2',6'-dibenzyloxyflavone (19)

Yield 69%, white crystal, mp 129-131°C, IR υ_{max} cm^{-1} 3095, 2850, 1648, 1610, ^{1}H-NMR ($CDCl_3$) δ (ppm) 5. 12 (4H, s, H-**CH_2**-Ph × 2) 6.53 (1H, s, H-3), 6.65 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.10-7.86 (14H, m, H-CH_2-**Ph** × 2, 6, 7, 8, 4'), 8.26 (1H, dd, 8.6, 1.7 Hz, H-5).

2',6'-Dibenzyloxy-7-methoxyflavone (20)

Yield 69%, white crystal, mp 139-140°C, IR υ_{max} cm^{-1} 3080, 2900, 1650, 1610, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.94 (3H, s, H-OCH_3), 5.10 (4H, s, H-**CH_2**-Ph × 2), 6.51 (1H, s, H-3), 6.64 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.90-7.40 (13H, m, H-CH_2-**Ph** × 2, 6, 8, 4'), 7.02 (1H, s, H-3), 8.12 (1H, d, *J*=8.6 Hz, H-5).

2',6'-dibenzyloxy-6-methoxyflavone (21)

Yield 61%, white crystal, mp 116-117.3°C, IR υ_{max} cm^{-1} 3050, 2890, 1645, 1606, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.90 (3H, s, H-OCH_3), 5.12 (4H, s, H-**CH_2**-Ph × 2), 6.50 (1H, s, H-3), 6.62 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.02-7.68 (14H, m, H-CH_2-**Ph** (2, 5, 7, 8, '4').

2',6'-Dibenzyloxy-5-methoxyflavone (22)

Yield 67%, white crystal, mp 129-130°C , IR υ_{max} cm^{-1} 3060, 2890, 1645, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3. 95 (3H, s, H-OCH_3), 5.12 (4H, s, H-**CH_2**-Ph × 2), 6.42 (1H, s, H-3), 6.58 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.80 (1H, dd, *J*=8.6, 1.5 Hz, H-6), 6.92 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.12-7.62 (12H, m, H-CH_2-**Ph** × 2, 7, 4').

2',6'-Dibenzyloxy-5-hydroxyflavone (23)

Yield 46%, yellow crystal, mp 178-180°C, IR υ_{max} cm^{-1} 3390, 3080, 2890, 1645, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 5.14 (4H, s, H-**CH_2**-Ph × 2), 6.47 (1H, s, H-3), 6.58 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.80 (1H, dd, *J*=8.6, 1.5 Hz, H-6), 6.92 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.12-7.62 (12H, m, H-CH_2-**Ph** × 2, 7, 4'), 12.48 (1H, s, H-OH).

2',6'-Dibenzyloxy-5,7-dimethoxyflavone (24)

Yield 69%, white crystal, mp 159-160.2°C, IR υ_{max} cm^{-1} 3040, 2900, 1650, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.73 (3H, s, H-OCH_3), 3.88 (3H, s, H-OCH_3), 5. 02 (4H, s, H-**CH_2**-Ph × 2), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.38 (1H, s, H-3), 6.42 (2H, d, 2.2 Hz, H-8), 6.60 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.01-7.51 (11H, m, H-CH_2-**Ph** × 2, 4').

2',6'-Dibenzyloxy-7,8-dimethoxyflavone (25)

Yield 70%, white crystal, mp 147.2-150.3°C, IR υ_{max} cm^{-1} 3090, 2860, 1650, 1601, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.94 (3H, s, H-OCH_3), 4.02 (3H, s, H-OCH_3), 5.10 (4H, s, H-**CH_2**-Ph × 2), 6.40 (1H, s, H-3), 6.62 (2H, d, 8.6 Hz, H-3', 5'), 7.02 (1H, d, *J*=8.6 Hz, H-6), 7.20-7.54 (11H, m, H-CH_2-**Ph** × 2, 4'), 7.82 (1H, d, *J*=8.6 Hz , H-5).

2',6'-Dibenzyloxy-5,7,8-trimethoxyflavone (26)

Yield 69%, white crystal, mp 154.3-155.1°C, IR υ_{max} cm^{-1} 3100, 2910, 1645, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 3.94 (3H, s, H-OCH_3), 4.01 (3H, s, H-OCH_3), 4.04 (3H, s, H-OCH_3), 5.18 (4H, s, H-**CH_2**-Ph × 2), 6. 40 (1H, s, H-3), 6.42 (1H, s, H-3), 6.60 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.18-7.62 (11H, m, H-CH_2-**Ph** × 2, 4').

2',6'-Dibenzyloxy-5-hydroxy-7,8-dimethoxyflavone (27)

Yield 49%, yellow crystal, mp 198.7-200°C, IR υ_{max} cm^{-1} 3410, 3120, 2910, 1645, 1605, ^{1}H-NMR ($CDCl_3$) δ (ppm) 4.00 (3H, s, H-OCH_3), 4.02 (3H, s, H-OCH_3), 5.18 (4H, s, H-**CH_2**-Ph × 2), 6.40 (1H, s, H-3), 6.42 (1H, s, H-3), 6.60 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.18-7. 62 (11H, m, H-CH_2-**Ph** × 2, 4'), 12.34 (1H, s, H-OH).

2'-hydroxyflavone (28)

Yield 91%, Yellow crystal, mp 101-192°C, IR ϖ_{max} cm^{-1} 3390, 3010, 1650, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 6.92 (1H, s, H-3), 7.03-7.70 (6H, m, H-6, 7, 8, 3, '4', 5), 7.78 (1H, dd, *J*=8, 4, 1.5 Hz, H-6') 8.20 (1H, dd, *J*=8.6 Hz, 1.7 Hz, H-5), 10.61 (1H, s, H-OH).

2'-Hydroxy-7-methoxyflavone (29)

Yield 90%, yellow crystal, mp 154.2-155.6°C, IR υ_{max} cm^{-1} 3400, 3050, 2890, 1654, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 7.00 (1H,

s, H-3), 6.82-7.53 (5H, m, H-6, 8, 3, '4', 5'), 7.82 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 8.14 (1H, d, *J*=8.6 Hz, H-5), 10.56 (1H, s, H-OH).

2'-Hydroxy-6-methoxyflavone (30)

Yield 90%, yelolw crystal, mp 125-126°C, IR υ_{max} cm^{-1} 3400, 3008, 2900, 1650, 1602, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.92 (3H, s, H-OCH_3), 6.94 (1H, s, H-3), 6.98-7.66 (6H, m, H-5, 7.8, 3, '4', 5'), 7.80 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.16 (1H, s, H-OH).

2'-Hydroxy-5-methoxyflavone (31)

Yield 86%, yellow crystal, mp 134-135.1°C, IR υ_{max} cm^{-1} 3390, 3010, 2890, 1640, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 6.78 (1H, dd, *J*=8.6, 1.7 Hz, H-6), 7.00 (1H, s, H-3), 7.10 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.20-7.60 (4H, m, H-7, 3, '4', 5'), 7.80 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.61 (1H, s, H-OH).

2'-Hydroxy-5,7-dimethoxyflavone (32)

Yield 89%, yellow crystal, mp 168.7-169.2°C, IR υ_{max} cm^{-1} 13420, 3006, 2880, 1654, 1600, ^{1}H-NMR ($DMSO_6$) δ (ppm) 3.87 (3H, s, H-OCH_3), 3.92 (3H, s, H-OCH_3), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.48 (1H, d, *J* =2.2 Hz, H-8), 6.84 (1H, s, H-3), 6.97-7.50 (3H, m, H-3', 4', 5'), 7.78 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.44 (1H, s, H-OH).

5,2'-Dihydroxy-7-methoxyflavone (33)

Yield 43%, yellow crystal, mp 168.7-169.2°C, IR υ_{max} cm^{-1} 1340, 3006, 2880, 1654, 1600, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.95 (3H, s, H-OCH_3), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.48 (1H, d, *J*=2.2 Hz, H-8), 6.84 (1H, s, H-3), 6.97-7.50 (3H, m, H-3', 4', 5'), 7.78 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.44 (1H, s, H-OH), 12.21 (1H, s, H-OH).

2'-Hydroxy-7,8-dimethoxyflavone (34)

ield 91%, yellow crystal, mp 169.5-170.1°C, IR υ_{max} cm^{-1} 3400, 3090, 2890, 1650, 1602, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.87 (3H, s, H-OCH_3), 3.92 (3H, s, H-OCH_3), 6.32 (1H, d, *J*=2.2 Hz, H-6), 6.48 (1H, d, *J*=2. 2 Hz, H-8), 6.84 (1H, s, H-3), 6.97-7.50 (3H, m, H-3', 4', 5'), 7.78 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.44 (1H, s, H-OH).

2'-Hydroxy-5,7,8-trimethoxyflavone (35)

Yield 88%, yellow crystal, mp 214-215.2°C, IR υ_{max} cm^{-1} 3390, 3050, 2990, 1657, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 3.96 (3H, s, H-OCH_3), 4.00 (3H, s, H-OCH_3), 6.42 (1H, s, H-6), 6.94 (1H, s, H-3), 7.04-7.26 (3H, m, H-3', 4', 5'), 7.90 (1H, dd, *J*=8.4, 1.5 Hz, H-6'), 10.36 (1H, s, H-OH).

2,6-Dihydroxyflavone (36)

Yield 88%, yellow crystal, mp 178.2-179.5°C, IR υ_{max} cm^{-1} 3450, 3070, 1648, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 6.40 (1H, s, H-3), 6.48 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.08-7.54 (4H, m, H-, 6, 7, 8, 4'), 8.24 (1H, dd, *J*=8.6, 1.7 Hz, H-5), 10.45 (2H, s, H-OH×2).

2,6'-Dihydroxy-7-methoxyflavone (37)

Yield 84%, yellow crystal, mp 164.5-165.2°C, IR υ_{max} cm^{-1} 3460, 3090, 2850, 1645, 1604, ^{1}H-NMR (DMSO-d^6) δ (ppm) 3.90 (3H, s, H-OCH_3), 6.40 (1H, s, H-3), 6.54 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.92-7.54 (3H, m, H-6, 8, 4'), 8.12 (1H, d, *J*=8.6 Hz, H-5), 10.23 (2H, s, H-OH×2).

2,6-Dihydroxy-6-methoxyflavone (38)

Yield 86%, yellow crystal, mp 147.2-148.7°C, IR υ_{max} cm^{-1} 3440, 3050, 2880, 1650, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 6.40 (1H, s, H-3), 6.54 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.98-7.63 (4H, m, H-5, 7.8, 4'), 10.15 (2H, s, H-OH (2).

2',6'-Dihydroxy-5-methoxyflavone (39)

Yield 81%, Yellow crystal, mp 156.8-157.9°C, IR υ_{max} cm^{-1} 3460, 3080, 2880, 1648, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.95 (3H, s, H-OCH_3), 6.38 (1H, s, H-3), 6.56 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.78 (1H, dd, *J*= 8.6, 1.5 Hz, H-6), 6.91 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.12-7.50 (2H, m, H-7, 4'), 10.18 (2H, s, H-OH×2).

2',6'-Dihydroxy-5,7-dimethoxyflavone (40)

Yield 82%, yellow crystal, mp 198.4-199.3°C, IR υ_{max} cm^{-1} 3460, 3080, 2890, 1650, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.73 (3H, s, H-OCH_3), 3.88 (3H, s, H-OCH_3), 6.36 (1H, s, H-3), 6.38 (1H, d, *J*=2.2 Hz, H-6), 6.42 (1H, d, 2.2 Hz, H-8), 6.50 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.08-7.22 (1H, m, H-4'), 10.13 (2H, s, H-OH×2).

2',6'-Dihydroxy-7,8-dimethoxyflavone (41)

Yield 79%, yellow crystal, mp 187-189.1°C, IR υ_{max} cm^{-1} 3440, 3080, 2890, 1650, 1606, ^{1}H-NMR (DMSO-d_6) *d* (ppm) 3.94 (3H, s, H-OCH_3), 4.00 (3H, s, H-OCH_3), 6.48 (1H, s, H-3), 6.53 (2H, d, *J*=8.6 Hz, H-3', 5'), 7.00 (1H, d, *J*=8.6 Hz, H-6), 7.20-7.28 (1H, m, H-4'), 7.82 (1H, d, *J*=8.6 Hz , H-5), 10.10 (2H, s, H-OH×2).

2',6'-Dihydroxy-5,7,8-trimethoxyflavone (42)

Yield 86%, yellow crystal, mp 218.1-219.2°C, IR υ_{max} cm^{-1} 3490, 3080, 2890, 1650, 1602, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.94 (3H, s, H-OCH_3), 4.01 (3H,

s, H-OCH_3), 4.04 (3H, s, H-OCH_3), 6.40 (1H, s, H-6), 6.42 (1H, s, H-3), 6.56 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.12-7.25 (1H, m, H-4'), 10.14 (2H, s, H-OH×2).

5,2',6'-Trihydroxy-6,7,8-trimethoxyflavone (43)

Yield 81%, yellow crystal, mp 229.6-230.7°C, IR υ_{max} cm^{-1} 3450, 3090, 2890, 1640, 1603, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.88 (3H, s, H-OCH_3), 3.94 (3H, s, H-OCH_3), 4.01 (3H, s, H-OCH_3), 6.34 (1H, s, H-3), 6.58 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.20-7.30 (1H, m, H-4'), 10.14 (2H, s, H-OH×2), 12.67 (1H, s, H-OH).

2'-Hydroxy-6'-methoxyflavone (44)

Yield 73%, yellow crystal, mp 158.2-159.1°C, IR υ_{max} cm^{-1} 3400, 3080, 2850, 1650, 1604, ^{1}H-NMR (DMSO-d^6) δ (ppm) 3.94 (3H, s, H-OCH_3), 6.51 (1H, s, H-3), 6.59 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.13-7.56 (4H , m, H-6, 7, 8, 4'), 8.25 (1H, dd, *J*=8.6, 1.7 Hz, H-5), 10.12 (1H, s, H-OH).

2'-Hydroxy-6', 7-dimethoxyflavone (45)

Yield 79%, yellow crystal, mp 157-158°C, IR υ_{max} cm^{-1} 3460, 3090, 2850, 1645, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.92 (3H, s, H-OCH_3), 3.96 (3H, s, H-OCH_3), 6.51 (1H, s, H-3), 6.72 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.95-7.34 (3H, m, H-6, 8, 4'), 8.14 (1H, d, *J*=8.6 Hz, H-5), 10.14 (1H, s, H-OH).

2'-Hydroxy-6,6'-dimethoxyflavone (46)

Yield 86%, yellow crystal, mp 131-132°C, IR υ_{max} cm^{-1} 3470, 3090, 2860, 1650, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 3.94 (3H, s, H-OCH_3), 6.4 8(1H, s, H-3), 6.62 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.04-7.64 (4H, m, H-5, 7.8, 4'), 10.32 (1H, s, H-OH).

2'-Hydroxy-5,6'-dimethoxyflavone (47)

Yield 69%, yellow crystal, mp 147.5-148.2°C, IR υ_{max} cm^{-1} 3500, 3090, 2880, 1650, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 3.94 (3H, s, H-OCH_3), 6.48 (1H, s, H-3), 6.62 (2H, d, *J*=8.7 Hz, H-3', 5'), 6.84 (1H, dd, *J*=8.6, 1.5 Hz, H-6), 6.94 (1H, dd, *J*=8.6, 1.7 Hz, H-8), 7.14-7.56 (2H, m, H-7, 4'), 10.12 (1H, s, H-OH).

2'-Hydroxy-6',5,7-trimethoxyflavone (48)

Yield 69%, yellow crystal, mp 179.1-180.2°C, IR υ_{max} cm^{-1} 3460, 3090, 2880, 1650, 1600, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.73 (3H, s, H-OCH_3), 3.88 (3H, s, H-OCH_3), 3.92 (3H, s, H-OCH_3), 6.40 (1H, s, H-3), 6.46 (2H, d, *J*=2.2 Hz, H-3', 5'), 6.48 (1H, d, *J*=2.2 Hz, H-8), 6.62 (1H, d, *J*=8.7 Hz, H-8), 7.10-7.24 (1H, m, H-4'), 10.25 (1H, s, H-OH).

2'-Hydroxy-6',7,8-trimethoxyflavone (49)

Yield 67%, yellow crystal, mp 154.1-155.4°C , IR υ_{max} cm^{-1} 3460, 3080, 2880, 1654, 1604, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.95 (3H, s, H-OCH_3), 3.96 (3H, s, H-OCH_3), 3.99 (3H, s, H-OCH_3), 6.45 (1H, s, H-3), 6.59 (2H, d, *J*=8.6 Hz, H-3', 5'), 7.03 (1H, d, *J*=8.6 Hz, H-6), 7.20-7.30 (1H, m, H-4'), 7.84 (1H, d, *J*=8.6 Hz, H-5), 10.14 (1H, s, H-OH).

2'-Hydroxy-5,6',7,8-tetramethoxyflavone (50)

Yield 64%, yellow crystal, mp 169.1-170.3°C, IR υ_{max} cm^{-1} 3450, 3090, 2890, 1650, 1602, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.90 (3H, s, H-OCH_3), 3.95 (3H, s, H-OCH_3), 4.00 (3H, s, H-OCH_3), 4.05 (3H, s, H-OCH_3), 6.40 (1H, s, H-6), 6.46 (1H, s, H-3), 6.52 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.12-7.20 (1H, m, H-4'), 10.12 (1H, s, H-OH).

2',5-Dihydroxy-6,6',7,8-tetramethoxyflavone (51)

Yield 62%, yellow crystal, mp 179.2-181.9°C, IR υ_{max} cm^{-1} 3490, 3089, 2890, 1650, 1601, ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.88 (3H, s, H-OCH_3), 3.90 (3H, s, H-OCH_3), 3.96 (3H, s, H-OCH_3), 3.98 (3H, s, H-OCH_3), 6.34 (1H, s, H-3), 6.58 (2H, d, *J*=8.7 Hz, H-3', 5'), 7.20-7.28 (1H, m, H-4'), 10.06 (1H, s, H-OH), 12.70 (1H, s, H-OH).

2'-Hexanoyloxy-5-hydroxy-7-methoxyflavone (52)

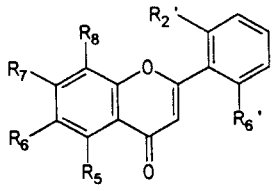
2',5-Dihydroxy-7-methoxyflavone (142 mg) was dissolved in 10 ml dichloromethane, and to the solution were added 0.7 mmol DCC (dicyclohexylcarbodiimide), 0.1 mmol DMAP [4-(dimethylamino)pyridine], and 60 mg hexanoic acid under nitrogen atmosphere in the ice bath. The resulting mixture was stirred for 3 h in the ice bath and then stirred for additional 1 h at room temperature. The reaction mixture was diluted with 30 ml hexane and filtered. The filtrate was concentrated to a dry mass. The mass was chromatographed on silica gel flash column with acetone/benzene (2 : 8). The faster running substance was collected and dried. It gave a pale yellow amorphous mass in methanol. In NMR of the substanc appeared the hexane moiety at 0.7-2.2 ppm and H-5 at 12.3 ppm.

RESULTS AND DISCUSSION

Chemistry

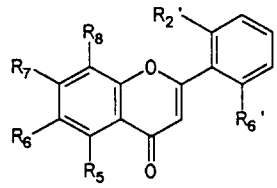
Ring closure of dibenzoylmethanes (Song *et. al.*, 1994a, b) to flavones were accomplished with 2.5% sulfuric acid (Yuchi *et. al;*., 1991). Controled debenzylation, demethylation and remtheylation brought various hydroxy or methoxy groups in the A- and B-rings. Debenzylation was carried out by heat-

Table I. ED_{50} values of Flavones with nonsubstituted B-Ring



No.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
1	H	H	H	H	H	H	11.5	4.2
2	H	H	OCH_3	H	H	H	11.9	5.0
3	H	OCH_3	H	H	H	H	21.1	11.6
4	OCH_3	H	H	H	H	H	11.2	9.2
5	OH	H	H	H	H	H	10.8	9.7
6	OCH_3	H	OCH_3	H	H	H	12.9	19.2
7	H	H	OCH_3	OCH_3	H	H	11.2	11.0
8	OCH_3	H	OCK_3	OCH_3	H	H	4.9	29.0

Table II. ED_{50} values of 2'-benzyloxyflavones



No.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
9	H	H	H	H	OBn	H	10.5	6.15
10	H	H	OCH_3	H	OBn	H	13.4	5.73
11	H	OCH_3	H	H	OBn	H	10.5	7.32
12	OCH_3	H	H	H	OBn	H	4.9	3.1
13	OH	H	H	H	OBn	H	5.2	3.6
14	OCH_3	H	OCH_3	H	OBn	H	8.2	5.0
15	OH	H	OCH_3	H	OBn	H	8.0	5.9
16	H	H	OCH_3	OCH_3	OBn	H	13.1	6.4
17	OCH_3	H	OCH_3	OCH_3	OBn	H	5.9	11.0
18	OH	H	OCH_3	OCH_3	OBn	H	5.4	12.7

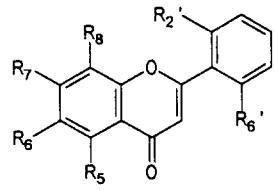
ing the benzyloxyflavones under presence of acetic acid /HCl (Rao *et. al.*, 1947). Methoxy group was demethylated with aluminium chloride. In thise way fifty two flavones were synthesized with good yields.

Cytotoxicity

As seen in Table 1-6, Human leukemic HL-60 cell, on the whole, was more sensitive to the flavones than murine leukemic L1210 cell.

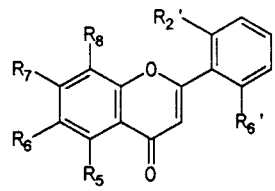
Flavone (1) itself showed a considerable cytotoxic activity, as seen in Table 1. 7-Methoxylation decreased the cytotoxic acitvity of flavone (**1**), while 5-methylation or hydroxylation potentiated the activity of 7-methoxyflavone (**3**). 5,6,7-Trimethoxyflavone (**8**) showed much stronger activity than 6, 7-dimethoxyflavone (**7**). The cytotoxicity-enhancing effect of 5-methoxylation is pronounced in this case. Thus, it is suggested that 5-methylation contributed to enhance the cytotoxicity.

Table III. ED_{50} values of 2', 6'-dibenzyloxyflavone



no.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
19	H	H	H	H	OBn	OBn	10.5	7.3
20	H	H	OCH_3	H	OBn	OBn	21.7	18.7
21	H	OCH_3	H	H	OBn	OBn	27.6	24.2
22	OCH_3	H	H	H	OBn	OBn	18.5	11.6
23	OH	H	H	H	OBn	OBn	20.5	13.6
24	OCH_3	H	OCH_3	H	OBn	OBn	22.4	15.8
25	H	H	OCH_3	OCH_3	OBn	OBn	35.1	29.0
26	OCH_3	H	OCH_3	OCH_3	OBn	OBn	19.4	13.2
27	OH	OCH_3	OCH_3	OCH_3	OBn	OBn	31.6	22.1

Table IV. ED_{50} values of 2'-hydroxyflavones



no.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
28	H	H	H	H	OH	H	9.7	5.9
29	H	H	OCH_3	H	OH	H	10.3	5.0
30	H	OCH_3	H	H	OH	H	9.8	5.4
31	OCH_3	H	H	H	OH	H	4.2	2.7
32	OCH_3	H	OCH_3	H	OH	H	8.3	4.9
33	OH	H	OCH_3	H	OH	H	8.7	5.2
34	H	H	OCH_3	OCH_3	OH	H	12.5	4.8
35	OCH_3	H	OCH_3	OCH_3	OH	H	5.7	10.0

Among 2'-benzyloxyflavones (Table 2), the cytotoxicity-enhancing effects of 5-oxygenation is more pronounced; 2'-benzyloxy-5-methoxyflavone (**12**), 2'-benzyloxy-5,7,8-trimethoxyflavone (**17**) and 2'-benzyloxy-5-hydroxy-7,8-dimethoxyflavone (**18**) showed stronger cytotoxic effects than coresponding 5-nonsubstituted flavones (**9, 16**). In contrast, 7-oxygenation decreased the cytotoxicity activity.

All of 2',6'-dibenzyloxyflavones showed a weak activity. This might be explained by the assumption that was suggested in earlier work(Ahn *et. al.*, 1989); the bulkiness in 2'- and 6'-dibenzyloxy groups increased the angle between B/C rings so much that it is not stucturally optimal for the cytotoxic activity.

Debenzylation of 2'-benzyloxyflavones yield 2'-Hydroxyflavones (Table 4), which exhibited a similar pattern of the cytotoxic activity of 2'-denzyloxyflavones. Similarly debenzylation of 2',6'-dibenzyloxyflavone resulted in 2',6'-dihydroxyflavones. As demonstrated in Table 5, the debenzylation improved the cytotoxicity on the whole. However, their activity was

weaker than that of 2'-monooxyflavones as described in Table 2 and 4.

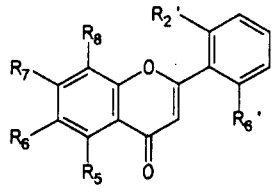
6'-Methylation of 2',6'-dihydroxyflavones gave 2'-hydroxy-2'methoxyflavones (Table 6), which possess the same B-ring pattern as skullcapflavone II, a natural product. As seen in Table 6,2'-methylation increased the activity. The presence of 5-oxy group increased the activity as well, while 7-methoxygenation made the activity weaker. However, all these derivatives were less active than skullcapflavone II (51).

Summarizing the structure-cytotoxicity relationship (Fig. 1), 7-methoxygenation in A-ring moiety reduced the cytotoxic activity, while 5-oxygenation increased the activity for all of the flavones tested. 7-Oxygenation should reduce the chemical activity of the 4-carbonyl group, while 5-hydroxy group or 5-methoxy group increases the electrophilic activity of 4-carbonyl group through hydrogen bond or steric distortion, respectively.

These explanations led to a conclusion that the electrophilic character of the carbonyl group of the flavones is important for the cytotoxic activity.

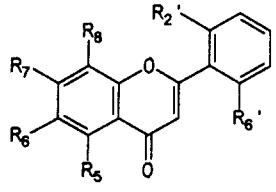
It was also found that the angle change between B/C-rings may play a role for the activity.

Table V. ED_{50} values of 2', 6'-dihydroxyflavones



no.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
36	H	H	H	H	OH	OH	8.7	6.2
37	H	H	OCH_3	H	OH	OH	15.9	9.3
38	H	OCH_3	H	H	OH	OH	13.8	8.5
39	OCH_3	H	H	H	OH	OH	10.4	6.8
40	OCH_3	H	OCH_3	H	OH	OH	13.9	6.9
41	H	H	OCH_3	OCH_3	OH	OH	21.6	15.5
42	OCH_3	H	OCH_3	OCH_3	OH	OH	11.3	8.8
43	OH	OCH_3	OCH_3	OCH_3	OH	OH	22.1	7.9

Table VI. ED_{50} values 2'-hydroxy-6'-methoxyflavones



no.	R5	R6	R7	R8	R2'	R6'	L1210	HL-60
44	H	H	H	H	OH	OCH_3	8.7	4.3
45	H	H	OCH_3	H	OH	OCH_3	11.8	7.9
46	H	OCH_3	H	H	OH	OCH_3	10.5	7.1
47	OCH_3	H	H	H	OH	OCH_3	7.2	5.9
48	OCH_3	H	OCH_3	H	OH	OCH_3	9.8	6.2
49	H	H	OCH_3	OCH_3	OH	OCH_3	17.6	10.7
50	OCH_3	H	OCH_3	OCH_3	OH	OCH_3	8.9	4.8
51	OH	OCH_3	OCH_3	OCH_3	OH	OCH_3	2.2	0.9

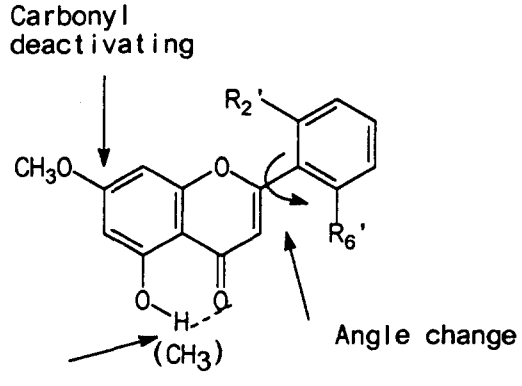


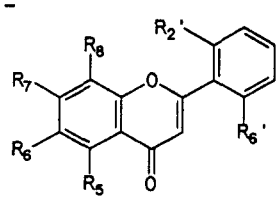
Fig. 1. Structural characteristic of flavones for cytotoxicity.

Antitumor activity against ICR mice bearing S-180 cell

From Table 7 it was found that 2'-benzyloxy-5-hydroxyflavone (13, T/C=118%), 2'-benzyloxy-5-hydroxy-7-methoxyflavones (15, T/C=144%), and 5, 2'-dihydroxy-7-methoxyflavone (33, T/C=132%), all of which showed good cytotoxic activities, exhibited stronger antitumor activities against S-180 tumor than

Table VII. T/C values of flavones against ICR Mice bearing S-180 Cell

Flavones	Dose (mg/kg)	Survival Days	T/C (%)	Survival*
no flavone	solvent	20.5	100	0/8
12	50	19.7		0/8
13	50	24.2	118	0/8
14	50	20.8	104	0/8
15	50	29.5	144	0/8
33	50	27.1	132	0/8
51	50	35.2	171	0/8
1	50	22.7	111	0/8
4	50	20.7	101	0/8
5	50	23.1	112	0/8
52	50	26.7	130	0/8



1; Flavone
4; 5-Methoxyflavone
5; 5-Hydroxyflavone
12; 5-Methoxy-2'-benzyloxyflavone
13; 5-Hydroxy-2'-benzyloxyflavone
14; 5,7-Dimethoxy-2'-benzyloxyflavone
15; 5-Hydroxy-7-methoxy-2'-benzyloxyflavone
33; 2',5-Dihydroxy-7-methoxyflavone
51; Skullkappflavone II
52; 2'-Hexanoyl-5-hydroxy-7-methoxyflavone

*Survival=1/8; one of 8 mice lived more than 50 days.

otherwise substituted flavones. As for the cytotoxic effect, the presence of 5- and 2'-oxy group in the flavone structure may play an important role for the antitumor activity, implying that the presence of 5-oxy group and an optimal angle between B/C rings are essential for the in vivo activity. All of the antitumor flavones have 5-hydroxy group as a common substituent. In contrast, 5-methoxyflavone showed a weak antitumor activity. Thus, it is suggested that the hydrogen bond between 5-OH and 4-carbonyl is more important for in vivo activity.

Edwards(1977) analysed the antitumor activities of about 230 flavonoid compounds and found that there were no flavonoids with a significant antitumor activities. In the contrary, we have discovered here ten cytotoxic flavones, four of which showed significant antitumor activity against S-180 tumor.

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