

# Antitumor Activity of 2(S)-5,2',5'-Trihydroxy-7,8-dimethoxyflavanone and Its Analogues

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In an effort to increase of the antitumor activity of 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone isolated from *Scutellaria indica*, we synthesized its analogues, **II**, **III** and **IV**. They showed potent cytotoxicity *in vitro* against cancer cell lines, L1210, K562 and A549. On the basis of ED<sub>50</sub> values against the cancer cell lines, **III** exhibited about 2-7 times stronger activity than **I** against various cell lines. We tested the antitumor activity of the analogues against Sarcoma 180 cells *in vivo* and evaluated the structure-activity relationship. The antitumor activity appeared to be related to the hydrogen bond between carbonyl group at C-4 and hydroxyl group at C-5, in contrast to cytotoxic action.

**Key words** : 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone, flavanone analogues, cytotoxicity, antitumor activity, L1210, K562, A549, Sarcoma 180

## INTRODUCTION

Many of the new approaches of cancer chemotherapy have been directed toward the modification of antitumor compounds isolated from natural sources (Kingstone *et al.*, 1985). Researchers are showing renewed interest in higher plants as a source for noble lead structures (Hamburger *et al.*, 1991). We isolated four cytotoxic flavonoids and wogonin from *Scutellaria indica* (Bae *et al.*, 1994). Among the isolated compounds, 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone, **I**, exhibited potent cytotoxic activity with ED<sub>50</sub> values of 0.94, 1.3 and 13.7 µg/ml against L1210, K562 and A549 cancer cell lines *in vitro*, respectively.

In search of potent antitumor compounds, we attempted to synthesize the analogues of **I** with stronger activity. In the structure of **I**, dimethoxyl groups at C-7, 8 and a polar part at C-4,5 on flavanone A-ring were found to be important for cytotoxicity. Likewise, skullcapflavone **II** (5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone) isolated from *Scutellaria baicalensis* had similar pattern on flavone A-ring with **I** (Ryu *et al.*, 1985). It showed potent antitumor activity *in vitro* with ED<sub>50</sub> values of 0.9 and 1.5 µg/ml against HL-60 and L1210 cancer cell lines, respectively, and T/C value was 170% against Sarcoma 180 in ICR mice.

Thus, the synthetic compounds were prepared from the A-ring by fixing and the B-ring by different modification of compound **I**. Flavanone analogues were synthesized by cyclization of appropriate chalcones

(Tanaka *et al.*, 1987) and demethylation of 5-methoxyflavanone with the reaction of AlCl<sub>3</sub> (Farkas *et al.*, 1967). The antitumor activity of synthetic compounds was evaluated against various cancer cell lines *in vitro* and *in vivo*.

In this paper, we described the synthetic procedures of **I-IV** and their cytotoxicity against human leukemia K562 cells, human lung carcinoma A549 cells, and murine leukemia L1210 cells *in vitro*. Their effect on the life span of mice bearing Sarcoma 180 cells *in vivo* were also determined.

## MATERIALS AND METHODS

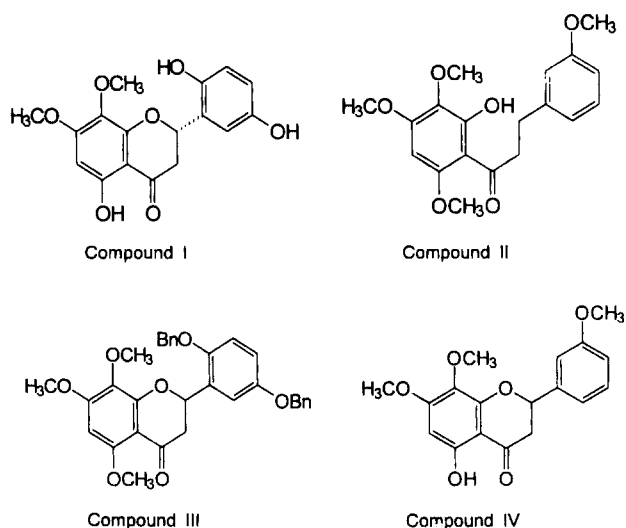
### Apparatus and methods

Melting points were determined by using electrothermal melting point apparatus. IR spectra in KBr disk were run on a JACSCO Report-100 Infrared spectrophotometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were taken on a JEOL LNM-EX90 (90, 22.5 MHz). Thin layer chromatography (TLC) was carried out on Kieselgel 60 F<sub>254</sub> (Merck) with following solvent systems; *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 with 5% FeCl<sub>3</sub> solution in MeOH. Flash column chromatograph was carried out on silica gel (Merck, 230-400 mesh).

### Isolation of 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone (**I**)

The air dried roots (505 g) of *S. indica* was extracted with hot MeOH and concentrated *in vacuo*. The

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**Fig. 1.** Structures of natural compound I, synthetic compound II, III and IV.

MeOH extract (120 g) was suspended in 1000 ml of distilled water. The water suspension was successively fractionated with *n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol. The ether fraction was evaporated *in vacuo* to yield a yellow viscous gum (7.6 g) and was chromatographed on silica gel column gradiently with *n*-hexane:ethyl acetate (3:2 to 1:5) as the eluent to obtain six fractions. The fourth fraction was rechromatographed on silica gel column gradiently with benzene:acetone (20:1 to 5:1) as the eluent to give **I** (215mg). The structure of **I** was confirmed by spectroscopic comparison with those of authentic specimens (Miyachi *et al.*, 1987).

#### Preparation of 2'-hydroxy-3,3',4',6'-tetramethoxychalcone (II)

2'-Hydroxy-3,3',4',6'-tetramethoxyacetophenone (1.66 g, 0.0074 M) and 3-methoxybenzaldehyde (2g, 0.015 M) were dissolved in 80% EtOH containing 4% KOH to be 1% solution (Tanaka *et al.*, 1987). The mixture was reacted overnight at room temperature. The reaction mixture was acidified with *d*-HCl, poured into water and then extracted with ethyl acetate. The extract was purified by flash column chromatography (SiO<sub>2</sub>, *n*-hexane:ethyl acetate=7:3), **II** was obtained as yellowish red needles.

Yellowish red needles; m.p. 119°C; yield: 85%; R<sub>f</sub> 0.34 (TLC 2); IR (cm<sup>-1</sup>): (KBr) 1620 (CO), 1600 (arom. C=C); <sup>1</sup>H-NMR: (CDCl<sub>3</sub>) δ 7.69, 7.89 (2H, each d, J=15.5 Hz, H<sub>α,β</sub>), 13.88 (1H, s, 2'-OH), 3.83, 3.94 (each 6H, each s, OCH<sub>3</sub>×2×2), 6.00 (H, s, 6-H); <sup>13</sup>C-NMR: (CDCl<sub>3</sub>) δ 106.7 (C-1'), 158.5 (C-2'), 130.7 (C-3'), 159.2 (C-4'), 86.9 (C-5'), 158.4 (C-1), 113.6 (C-2), 159.8 (C-3), 115.6 (C-4), 129.7 (C-5), 120.8 (C-6), 127.7 (C-α), 142.3 (C-β), 193.1 (C-β'), 55.1, 55.9, 60.6

(OCH<sub>3</sub>).

#### Preparation of 5,7,8-trimethoxy-2',5'-dibenzyloxyflavanone (III)

2,5-Dibenzyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone 2 g was dissolved in EtOH containing 4% H<sub>3</sub>PO<sub>4</sub> to be 2% solution (Tanaka *et al.*, 1987). The mixture was refluxed for 30 hrs, then poured into water and extracted with ethyl acetate. The extract was purified by flash column chromatography (SiO<sub>2</sub>, *n*-hexane:ethyl acetate=6:4), **III** was obtained as white powders.

White powders; m.p. 138.5-139°C; yield: 43%; R<sub>f</sub> 0.46 (TLC 3); IR (cm<sup>-1</sup>): (KBr) 1680 (CO), 1595 (arom. C=C); <sup>1</sup>H-NMR: (CDCl<sub>3</sub>) δ 2.86 (1H, d, J=10.1 Hz, 3-H<sub>trans</sub>), 2.89 (1H, d, J=5.5 Hz, 3-H<sub>cis</sub>), 5.76 (1H, dd, J=5.5, 10.1 Hz, 2-H), 4.99 (4H, a, s, 2',5'-OCH<sub>2</sub>Ph), 6.08 (1H, s, 6-H), 3.71, 3.86, 3.88 (each 3H, each s, -OCH<sub>3</sub>×3); <sup>13</sup>C-NMR: (CDCl<sub>3</sub>) δ 74.4 (C-2), 44.8 (C-3), 189.7 (C-4), 157.8 (C-5), 89.1 (C-6), 158.5 (C-7), 129.1 (C-8), 156.6 (C-9), 106.4 (C-10), 126.8 (C-1'), 149.2 (C-2'), 114.7 (C-3'), 113.6 (C-4'), 153.2 (C-5'), 113.0 (C-6'), 56.0, 56.1, 61.1, (OCH<sub>3</sub>), 70.4, 70.6 (CH<sub>2</sub>Ph), 127.1, 127.4, 127.9, 128.0, 128.2, 128.5, 128.7, 128.8, 136.8, 137.1 (CH<sub>2</sub>Ph).

#### Preparation of 5-hydroxy-7,8,3'-trimethoxyflavanone (IV)

5,7,8,3'-Tetramethoxyflavanone 0.26 g and aluminium chloride 0.5 g were dissolved in acetonitrile to be 1% solution (Fakas *et al.*, 1967). The mixture was refluxed for 3 hrs. After evaporating the solvent *in vacuo*, the reaction mixture was decomposed by *d*-HCl on a steam bath. The resulting mixture was filtered off. The residue was washed thoroughly with water and dissolved with ethyl acetate and purified by flash column chromatography (SiO<sub>2</sub>, *n*-hexane:ethyl acetate =5:1).

White powders; m.p. 100°C; yield: 56%; R<sub>f</sub> 0.62 (TLC 2); IR (cm<sup>-1</sup>): (KBr) 1640 (CO), 1600 (arom. C=C); <sup>1</sup>H-NMR: (CDCl<sub>3</sub>) δ 2.94 (1H, d, J=4.8 Hz, 3-H<sub>cis</sub>), 3.01 (1H, d, J=10.7 Hz, 3-H<sub>trans</sub>), 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<sub>3</sub>×3) <sup>13</sup>C-NMR: (CDCl<sub>3</sub>) δ 78.9 (C-2), 43.3 (C-3), 195.8 (C-4), 159.8 (C-5), 93.5 (C-6), 161.4 (C-7), 129.8 (C-8), 153.3 (C-9), 102.7 (C-10), 140.0 (C-1'), 111.8 (C-2'), 159.7 (C-4'), 129.7 (C-5'), 118.1 (C-6'), 55.2, 56.1, 61.1 (OCH<sub>3</sub>).

#### *In vitro* cytotoxicity assay

The *in vitro* cytotoxicity assay was carried out according to the National Cancer Institute protocol (Geran *et al.*, 1972) and an SRB assay method (Skehan *et al.*, 1990). The ED<sub>50</sub> value was evaluated by the procedure

of Thayer *et al.* (1971).

### *In vivo* antitumor assay

Each of the female ICR mice weighing 20-25 g as implanted with  $10^6$  of Sarcoma 180 cells into the peritonium and the test compounds were administered i. p. once daily for consecutive 10 days. The mortality of mice was recorded for 60 days and survival rate was calculated by the formular (Geran *et al.*, 1972).

$$\text{Survival rate (T/C\%)} = \frac{\text{mean survival days of treated group}}{\text{mean survival days of control group}} \times 100$$

## RESULTS AND DISCUSSION

### Chemistry

Compound **I**, 2(5)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone, was isolated from the roots of *Scutellaria indica*. Compound **II** (2'-hydroxy-3,3',4',6'-tetramethoxychalcone) was synthesized from 2'-hydroxy-3,3',4',6'-tetramethoxyacetophenone and 3-methoxybenzaldehyde according to the reported method (Tanaka *et al.*, 1987). In the  $^1\text{H-NMR}$  spectrum of **II**, proton peaks of  $\alpha,\beta$ -unsaturated carbonyl group were identified at 7.69, 7.89 ppm (d,  $J=15.5$  Hz), respectively. Compound **III** (5,7,8-trimethoxy-2',5'-dibenzoyloxyflavanone) was obtained from 2,5-dibenzoyloxy-2'-hydroxy-3',4',6'-trimethoxychalcone by cyclization with  $\text{H}_3\text{PO}_4$ . In the  $^{13}\text{C-NMR}$  spectrum of **III**, carbon peak of C-3 was confirmed at 44.8 ppm. Compound **IV** (5-hydroxy-7,8,3'-trimethoxyflavanone) was prepared by partial  $O$ -demethylation at C-5 of 5,7,8,3'-tetramethoxyflavanone. According to reference, we synthesized flavanone analogues, the yield of each step was above 50%. The synthetic flavanones were racemic mixtures at C-2 chiral carbon.

### *In vitro* cytotoxicity

Natural compound **I** showed potent cytotoxicity against murine and human leukemia cells (L1210 and K562 cell lines) and its  $\text{ED}_{50}$  values were less than 2.0  $\mu\text{g/ml}$ . But it showed weak cytotoxicity against human lung carcinoma A549 cells (Table I). Synthetic compounds **II** and **IV** showed intense cytotoxicity against murine and human leukemia cell lines (L1210 and K562 cell lines). **II** also showed potent cytotoxicity against human lung carcinoma A549 cells ( $\text{ED}_{50}$  value, 2.1  $\mu\text{g/ml}$ ), but **IV** was not active against A549 cells. The compound **III** showed more powerful activity than **I**. In testing flavanones, we found that the cytotoxicity was increased when  $\alpha,\beta$ -unsaturated carbonyl groups did not form an intramolecule hydrogen bond next to the hydroxyl group at C-5.

**Table I.** Cytotoxicity of compounds I, II, III and IV against L1210, K562 and A549 cancer cell lines *in vitro*

Compounds	$\text{ED}_{50}$ valuesa ( $\mu\text{g/ml}$ )		
	L1210 <sup>b</sup>	K562 <sup>c</sup>	A549 <sup>d</sup>
I	0.94	1.3	13.7
II	0.86	1.2	2.1
III	0.45	0.83	1.9
IV	3.0	1.8	>20
5-FUe	0.009-0.03	0.11-0.30	0.88-2.3

<sup>a</sup>Cytotoxicity of these compounds were evaluated by the procedure of Thayer *et al.*

<sup>b</sup>L1210 cell was cultured Fisher's medium with 10% horse serum.

<sup>c</sup>K562 cell was cultured RPMI medium with 10% fetal bovine serum.

<sup>d</sup>A549 cell was cultured RPMI medium with 5% fetal bovine serum.

<sup>e</sup>5-FU: 5-Fluorouracil, The values indicate for the range of  $\text{ED}_{50}$  of each test.

### *In vivo* antitumor activity

In the *in vivo* antitumor activity test, the life span effect of natural compound **I** was 142% at 5 mg/kg concentration compared with the control (Table II). Thus, we recognized that **I** might have antitumor activity (Hellmann *et al.*, 1987). On the other hand, the life span effect of synthesized compounds **II** and **III**, even if they showed potent cytotoxicity *in vitro*, were 117% and 89%, respectively, at 10 mg/kg concentration. **III** had free carbonyl group at C-4 which doesn't form intramolecular hydrogen bond and showed toxicity. Moreover, even though synthetic compound **IV** had less cytotoxicity than **II** and **III**, it showed antitumor activity which T/C value was 125% at 10 mg/kg concentration. From these results, the tested flavonoids have toxicity when the carbonyl group exists in a free form at C-4 position.

### Structure-activity relationship

As the carbonyl group at C-4 of **III** dose not form hydrogen bond with the hydroxyl group at C-5 and exists in an independent form, **III** showed potent cytotoxicity *in vitro*. But it had toxicity at 10 mg/kg concentration *in vivo* (T/C value was 89%).

The carbonyl group of **I** and **IV** formed a hydrogen bond with the hydroxyl group of C-5, that is, dose not expose free form. Thus, the toxicity of **I** and **IV** was lower than **III**. And, **I** and **IV** showed antitumor activity against Sarcoma 180 cancer cells. During the *in vivo* test of **I** and **IV**, there was no change of the external shape of mice. But, there were some changes of external shape of mice due to toxicity during the test of that of **III**, with decreased their life spans.

**Table II.** Effects of compounds I, II, III and IV against Sarcoma 180 implanted *i.p.* in ICR mouse

Compounds	Dose (mg/kg)	Survival day	T/C % <sup>a</sup>	60 day (Survival)
Saline	— <sup>b</sup>	21.8	100.0	0/9
I	0.5	24.5	112.4	0/8
	5.0	30.9	141.7	0/8
Saline	—	22.3	100	0/8
II	5.0	24.4	(109)	0/9
	10.0	26.0	(117)	0/9
III	5.0	25.2	(113)	0/8
	10.0	19.8	(89)	0/8
Saline	—	20.3	100	0/8
IV	5.0	21.0	(105)	0/8
	25.0	25.0	125	0/8
Cisplatin	2.0	>37.7	>186	2/10

ICR mice were *i.p.* implanted with  $1 \times 10^6$  cells of Sarcoma 180 and were *i.p.* administered with **I** (0.5, 5.0 mg/kg), **II** (5.0, 10.0 mg/kg), **III** (5.0, 10.0 mg/kg), **IV** (5.0, 25.0 mg/kg) and cisplatin for 10 days after tumor inoculation. Cisplatin was used for positive control. The effects of life span of **I**, **II**, **III**, **IV** and cisplatin on saline solution were determined.

<sup>a</sup>Survival rate (T/C %)=

$$\frac{\text{mean survival days of treated group}}{\text{mean survival days of control group}} \times 100$$

<sup>b</sup>Vehicle only

In antitumor activity of the tested flavanones, we found that the hydroxyl group at C-5 was important to decrease toxicity by forming a hydrogen bond with the carbonyl group at C-4 and to increase antitumor activity.

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