

HETEROCYCLES, Vol. 77, No. 2, 2009, pp. 1379 - 1388. © The Japan Institute of Heterocyclic Chemistry
Received, 30th July, 2008, Accepted, 18th September, 2008, Published online, 22nd September, 2008
DOI: 10.3987/COM-08-S(F)79

FLAVONOIDS FROM *EUPATORIUM ODORATUM* WITH DEATH RECEPTOR 5 PROMOTER ENHANCING ACTIVITY

Takashi Ohtsuki,¹ Takahito Hiraka,¹ Hiroyuki Kikuchi,¹ Takashi Koyano,² Thaworn Kowithyakorn,³ Toshiyuki Sakai,⁴ and Masami Ishibashi^{1,*}

¹Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, ²Temko Corporation, 4-27-4 Honcho, Nakano, Tokyo 164-0012, Japan, and ³Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand, ⁴Department of Molecular-Targeting Cancer Prevention, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

Abstract – Sixteen flavonoids including two new ones (**1** and **2**) were isolated from the leaves of *Eupatorium odoratum* (Compositae) through bioassay-guided isolation. The chemical structures of **1** and **2** were established on the basis of spectroscopic analysis. Compounds **2**, **7**, **9**, and **14** led to more than 2-fold increase in death receptor 5 (DR5) promoter activity at 17.5 or 35 μ M.

INTRODUCTION

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), also known as Apo2L, belongs to the tumor necrosis factor family and is distributed in a wide range of cancer cell types. TRAIL binds to death receptors such as DR5 (death receptor 5 = TRAIL-R2) or DR4 (death receptor 4 = TRAIL-R1), resulting in the induction of apoptosis in many tumor cells, but no apoptotic effect on most normal cells.¹⁻⁵ Thus, TRAIL/ death-receptor signaling pathway has been considered as a promising target for the treatment of cancer. However, recent studies have shown that some tumor cells are resistant to the apoptotic effects of TRAIL.⁶⁻⁷ It is effective to increase the number of death-receptors as one of the method of solving this problem. In fact, it was reported that several chemotherapeutic drugs and natural products, such as PS-341 (bortezomib),⁸ fenretinide,⁹ tunicamycin,¹⁰ α -tocopheryl succinate,¹¹ and curcumin¹² increased expression of DR4 and/or DR5, and caused the activation of TRAIL receptors results in a signal transduction cascade followed by apoptosis, and was implicated in the sensitization of

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

TRAIL-resistant tumor cells to TRAIL-induced apoptosis.^{13,14} Therefore, the compounds that induce an increase in death receptor expression might be a new tool to abrogate the TRAIL resistance. During our studies on search for bioactive natural products, we have examined MeOH extracts of medicinal plants collected in Thailand to search for substances that activate DR5 promoter and have reported the isolation of isoflavonoids from leaves of *Millettia brandisiana* Kurz collected in Thailand, previously.¹⁵ We further screened and found that a MeOH extract of leaves of *Eupatorium odoratum* (Compositae) was potently active. *E. odoratum* (synonym: *Chromolaena odorata*) is a perennial scandent or semi-woody shrub native to South America and now distributed in many tropical countries.¹⁶ It is used as a folk medicine to stop bleeding and to assist wound healing.¹⁷ Other medicinal uses include astringent, antispasmodic, antihypertensive, anti-inflammatory, and diuretic.¹⁸ Previous chemical investigations of the leaves, stems, and flowers of *E. odoratum* by other groups led to the isolation of steroids,¹⁹ triterpenes,²⁰ flavonoids,²⁰⁻²⁵ alkaloids.²⁶

Here, we describe the activity-guided isolation and structure determination of components isolated from the MeOH extract of the leaves of *E. odoratum*. The DR5 promoter enhancement activity was assessed by means of luciferase reporter gene assay using DLD-1/*SacI* cells, a human colon cancer cell line stably transfected with the pDR5/*SacI* plasmids containing the human DR5 promoter sequence and a luciferase gene.²⁷ Sixteen flavonoids containing new compounds (**1** and **2**) were obtained and the DR5 promoter enhancing activity of the isolated compounds are evaluated.

RESULTS AND DISCUSSION

The MeOH extract of the leaves of *E. odoratum*, which showed up-regulation of DR5 promoter activity (5.2-fold at 100 µg/mL), was subjected to Diaion HP-20 CC to remove chlorophylls. The MeOH soluble fraction was then partitioned between *n*-hexane and 10% aqueous MeOH, and the aqueous phase was further extracted with EtOAc and *n*-BuOH to give four fractions. Since the most potent activity was found in the EtOAc soluble fraction (DR5 promoter activity, the EtOAc soluble fraction: 2.5-fold at 12.5 µg/mL; the other fractions: inactive), the EtOAc soluble fraction was subjected to repeated ODS CC, followed by final purification with reversed-phase HPLC on ODS to give 16 flavonoids (**1-16**). Among them, compounds **1** and **2** proved to be new compounds, while 14 ones were known and they were identified as 3,5,7-trihydroxy-4'-methoxyflavanone (**3**),²⁸ (2*R*,3*R*)-3,5,4'-trihydroxy-7-methoxyflavanone (**4**),²⁹ alysiolinone (**5**),³⁰ (2*R*,3*R*)-3,5,3'-trihydroxy-7,4'-dimethoxyflavanone (**6**),³¹ tamarixetin (**7**),³² rhamnetin (**8**),³³ isosakuranetin (**9**),²⁵ 5,6,7,4'-tetramethoxyflavone (**10**),³⁴ 4,2'-dihydroxy-4',5'6'-trimethoxychalcone (**11**),²⁵ 3,5,4'-trihydroxy-7-methoxyflavone (**12**),²⁹ 5,7-dihydroxy-3',4'-dimethoxy flavone (**13**),³⁵ acacetin (**14**),³⁶ kaempferide (**15**),³⁷ and 5,4'-dihydroxy-7-methoxyflavanone (**16**),³⁸ respectively, by comparison of the spectral data with those reported in the literature.

Compound **1**, $[\alpha]_D^{20} +8.6$ (c 0.079, MeOH), was obtained as a pale yellow powder, and its molecular formula was suggested to be $C_{18}H_{18}O_6$ by the HRFABMS data [m/z 331.1191, $(M+H)^+$, Δ +0.9 mmu]. Absorptions at 1600 and 3210 cm^{-1} in the IR spectrum was characteristic of carbonyl and hydroxyl groups, respectively, and the UV absorption maxima at λ_{max} 258 and 307 nm suggested the presence of flavanone nature for **1**.³⁹ The 1H NMR spectrum of **1** displayed signals for three methoxy groups [$(\delta_H$ 3.82 (3H), 3.87 (3H) and 3.95 (3H)], one heteroatom-bearing methine (δ_H 5.33), two aliphatic methylene (δ_H 2.76 and 3.02), and five aromatic hydrogens [δ_H 6.34, 6.90 (2H), and 7.32 (2H)]. Among them, three sets of double doublets at δ_H 5.33 (1H, dd, $J = 13.3, 2.8$ Hz, H-2), 3.02 (1H, dd, $J = 16.8, 13.5$ Hz, H-3_{ax}), and 2.76 (1H, dd, $J = 16.8, 2.8$ Hz, H-3_{eq}) were consistent with typical of C-ring of a flavanone moiety. Additionally, the appearance of four aromatic proton signals observed at δ_H 6.90 (2H, d, $J = 8.7$ Hz, H-3', H-5') and 7.32 (2H, d, $J = 8.7$ Hz, H-2', H-6') with characteristic pattern for an A_2B_2 system confirmed the disubstitution of B-ring at the 1' and 4'-positions. The connectivity of hydrogen and carbon atoms was established from the HMQC spectrum. The ^{13}C NMR and DEPT spectrum also supported this molecular formula. The ^{13}C NMR spectrum of **1** displayed signals due to 18 carbons. Among them, six carbons at δ_C 130.7 (C-1'), 128.0 (2C, C-2' and C-6'), 115.8 (2C, C-3' and C-5'), and 156.4 (C-4') were assigned to B-ring moiety substituted at the 4'-position. Also, three carbons at δ_C 79.3, 45.4, and 190.0 were assigned to C-2, C-3, and C-4 of flavanone skeleton, respectively, and three carbons at δ_C 56.2, 61.5 and 61.7 were assigned to methoxy groups. Remained six carbons, one sp^2 methine (δ_C 96.5) and five sp^2 quaternary carbons (δ_C 109.2, 137.6, 154.7, 159.7, and 160.0) were assigned to that of A-ring moiety in flavanone skeleton. Among them, the carbons at δ_C 109.2 and 159.7 were assigned to C-4a and C-8a, respectively, by the HMBC correlations for H-2/C-8a and H-3_{eq}/C-4a. Also, a signal at δ_H 6.34, which correlated to the carbon at δ_C 96.5 in the HMQC spectrum, was assigned as H-5 on basis of its HMBC correlations with C-4, C-4a, and C-8a. In addition, quaternary carbons at δ_C 160.0 and 137.6 were assigned as C-6 and C-7, respectively, by the HMBC correlations for H-5/C-6, H-5/C-7, 6-OMe/C-6, and 7-OMe/C-7 as well as the NOE correlations for H-5/6-OMe and 6-OMe/7-OMe. Thus, the remaining carbon signal at δ_C 154.3 was assigned to C-8, which bore a hydroxyl group. The remaining methoxy group at δ_H 3.95 was found to be attached to C-4' based on HMBC correlation with a carbon at δ_C 156.4 and the NOE correlations with H-3' and H-5' (δ_H 6.90). To confirm the absence of 5-hydroxyl group in **1**, the UV spectrum of **1** was measured in the presence of $AlCl_3$. Generally, it was known that the UV absorption maximum of 5-hydroxyflavanone shifted to longer wavelength (bathochromic shift) in the presence of $AlCl_3$.⁴⁰ In case of **1**, the addition of $AlCl_3$ caused no bathochromic shift of the UV absorption maximum, indicating that the hydroxyl group was not attached to C-5 position but to C-8. The absolute configuration of C-2 for **1** was deduced from the CD spectrum. It was described in the literature⁴¹ that in the CD spectra of flavanones, a positive Cotton effect at around 330 nm and a negative

Table 1. ^1H , ^{13}C NMR, and HMBC data for compounds **1** and **2** in CDCl_3

| position | compound 1 | | | compound 2 | | |
|----------|----------------------------------|---------------------|---------------------------|----------------------------------|---------------------|---------------------------|
| | δ_{H} (J in Hz) | δ_{C} | HMBC | δ_{H} (J in Hz) | δ_{C} | HMBC |
| 2 | 5.33 (dd, 13.5, 2.8) | 79.3 | C-4, C-1', C-6' | 5.31 (dd, 13.4, 2.5) | 79.1 | C-4, C-1', C-2', C-6' |
| 3 | 3.02 (dd, 16.8, 13.5) | 45.4 | C-2, C-4, C-1' | 3.01 (dd, 16.6, 13.4) | 45.3 | C-2, C-4, C-1' |
| | 2.76 (dd, 16.8, 2.8) | | C-4, C-4a | 2.75 (dd, 16.6, 2.5) | | C-4, C-4a |
| 4 | | 190.0 | | | 189.6 | |
| 4a | | 109.2 | | | 109.1 | |
| 5 | 6.34 (s) | 96.5 | C-4, C-4a, C-6, C-7, C-8a | 6.34 (s) | 96.4 | C-4, C-4a, C-6, C-7, C-8a |
| 6 | | 160.0 | | | 159.8 | |
| 7 | | 137.6 | | | 137.5 | |
| 8 | | 154.3 | | | 154.2 | |
| 8a | | 159.7 | | | 159.5 | |
| 1' | | 130.7 | | | 131.8 | |
| 2' | 7.32 (d, 8.7) | 128.0 | C-2, C-3', C-4', C-6' | 7.06 (d, 1.4) | 112.6 | C-2, C-3', C-4', C-6' |
| 3' | 6.90 (d, 8.7) | 115.8 | C-1', C-2', C-4', C-5' | | 145.9 | |
| 4' | | 156.4 | | | 146.9 | |
| 5' | 6.90 (d, 8.7) | 115.8 | C-1', C-2', C-3', C-4' | 6.88 (d, 8.5) | 110.6 | C-1', C-3' |
| 6' | 7.32 (d, 8.7) | 128.0 | C-2, C-2', C-4', C-5' | 6.93 (dd, 8.5, 1.4) | 118.1 | C-2, C-2', C-4' |
| 6-OMe | 3.87 (s) | 56.2 | C-6 | 3.94 (s) | 61.6 | C-6 |
| 7-OMe | 3.82 (s) | 61.5 | C-7 | 3.82 (s) | 61.4 | C-7 |
| 8-OMe | | | | 3.92 (s) | 56.0 | C-8 |
| 4'-OMe | 3.95 (s) | 61.7 | C-4' | 3.95 (s) | 61.7 | C-4' |
| 3'-OH | | | | 5.70 (br s) | | C-2', C-3' |

The isolated flavonoids (**1-16**) were evaluated for DR5 promoter activity using the luciferase assay using DLD-1/*SacI* cells. As shown in Figure 2, compounds **7** and **9** were found to be the most active and caused 2.3-fold enhancement of DR5 promoter activity at 17.5 μM , while compounds **2** and **14** showed 2.2-fold enhancement at 35 μM . Moreover, these compounds showed similar levels of DR5 promoter activity with that of luteolin at 17.5 μM , used as a positive control.^{42,43}

It was reported that the *p53* tumor-suppressor gene regulated DR5 gene expression.⁴⁴ In this study, we used the DR5 promoter plasmid without a *p53*-binding site for luciferase reporter assay. Accordingly, it was suggested that the isolated active compounds, **2**, **7**, **9**, and **14** induced enhancement of DR5 promoter activity in a *p53*-independent mechanism. In addition, it was also reported that some flavonoids including luteolin,^{42,43} apigenin,⁴⁵ dihydroflavonol (named for BB-1),⁴⁶ and silibinin⁴⁷ increased the expression of DR5 and synergistically enhanced TRAIL-induced apoptosis in various tumor cells. From the present data and the literature, compounds **2**, **7**, **9**, and **14** might be useful in the treatment of various TRAIL-resistant tumor cells with *p53* mutations in combination with TRAIL.

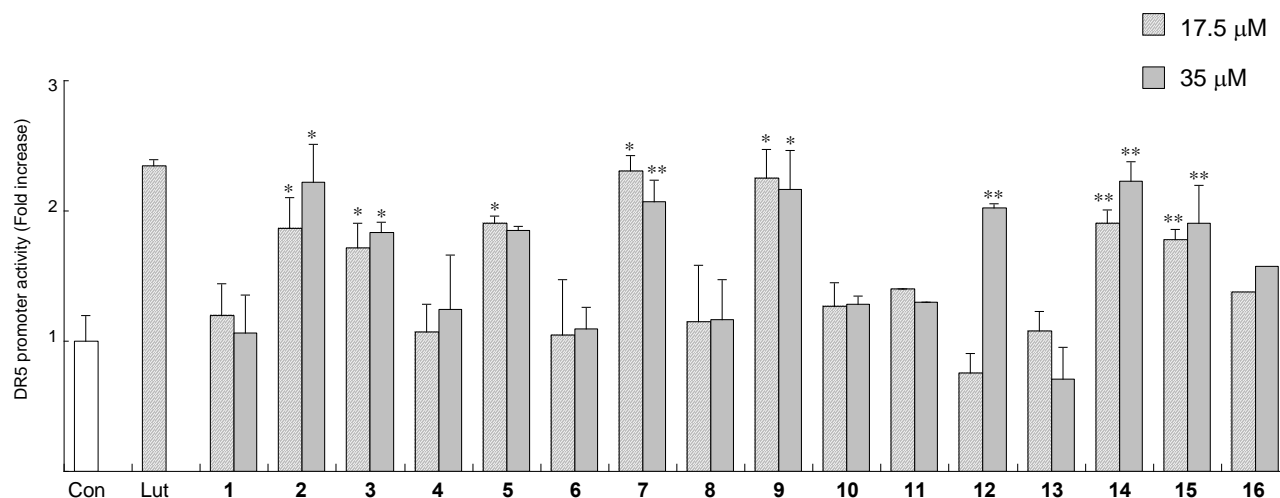


Figure 2. Activation of DR5 promoter activity by compounds **1-16**

All samples were tested at 17.5 and 35 μM . The bar represents the means ($n=3\pm\text{SD}$). Significant differences in activation of DR5 promoter activity were shown at $p<0.05$ (*), $p<0.01$ (**), compare with control (Con). Luteolin (Lut) was used as positive control.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. IR spectra were measured on ATR in a JASCO FT-IR 230 spectrophotometer. UV spectra were measured in a Shimadzu UV mini-1240 spectrometer. CD spectra were obtained in a JASCO J-720WI spectropolarimeter. NMR spectra were recorded on JEOL JNM-A400, JEOL JNM-A500, and JEOL JNM-ECP600 spectrometers with a deuterated solvent, the chemical shift of which was used as an internal standard. EIMS was measured on a JEOL GC-Mate spectrophotometer, and high-resolution fast-atom bombardment mass spectra (HRFABMS) were measured on a JEOL HX-110A spectrometer.

Plant Material. Leaves of *Eupatorium odoratum* were collected in Khon Kaen, Thailand, in April 2001 and were identified by T.K. A voucher specimen (6-365) is maintained in our laboratory.

Extraction and Isolation. The air-dried leaves (167 g) were extracted with MeOH. After removal of chlorophylls from the extract by Diaion HP-20 CC (43 x 400 mm), the fraction (7.8 g) eluted with MeOH was partitioned successively between hexane (180 mL x 3), EtOAc (180 mL x 3), and *n*-BuOH (180 mL x 3), to obtain four fractions (hexane phase, 0.9 g; EtOAc phase, 1.2 g; *n*-BuOH phase, 1.5 g; aqueous phase, 5.2 g). The EtOAc-soluble fraction (1.2 g) was subjected to silica gel CC (30 x 350 mm) eluted

with a stepwise gradient of mixtures (hexane:acetone = 5:1, 2:1, 1:1, 1:2, 1:3, 1:5, 1:9, 0:1 and MeOH) to give ten fractions: 1A (21.8 mg), 1B (4.9 mg), 1C (67.9 mg), 1D (166.0 mg), 1E (224.6 mg), 1F (79.8 mg), 1G (59.3 mg), 1H (45.1 mg), 1I (140.0 mg), and 1J (328.3 mg). Fraction 1E, eluted with hexane:acetone (1:1 and 1:2), was subjected to ODS CC (16 x 500 mm) eluted with a linear gradient of 50-100% MeOH in H₂O to give 13 fractions : 2A (11.3 mg), 2B (3.2 mg), 2C (6.3 mg), 2D (33.7 mg), 2E (75.0 mg), 2F (31.2 mg), 2G (45.0 mg), 2H (12.6 mg), 2I (4.0 mg), 2J (3.6 mg), 2K (5.2 mg), 2L (51.6 mg), and 2M (3.4 mg). Fraction 2E, eluted with 55% MeOH, was purified with HPLC (Develosil ODS-UG-5, 10 x 250 mm; eluent, 52% MeOH; flow rate, 2.0 mL/min) to afford compound **1** (14.8 mg, *t_R* 37 min), compound **2** (3.0 mg, *t_R* 46 min), compound **3** (4.8 mg, *t_R* 49 min), compound **4** (11.3 mg, *t_R* 55 min), and a mixture of compounds **5** and **6** (11.5 mg). The mixture of compounds **5** and **6** was purified with silica gel CC (12 x 450 mm) eluted with CHCl₃ to afford compound **5** (5.0 mg) and compound **6** (4.6 mg). Fraction 2G, eluted with 55% MeOH, was purified with HPLC (Develosil C30-UG-5, 6.0 x 250 mm; eluent, 65% MeOH; flow rate, 2.0 mL/min) to yield compound **7** (4.6 mg, *t_R* 45 min). Fraction 1D, eluted with hexane:acetone (1:1), was subjected to ODS column chromatography (16 x 500 mm) eluted with a linear gradient of 40-100% MeOH in H₂O to give nine fractions : 3A (3.6 mg), 3B (6.9 mg), 3C (4.8 mg), 3D (37.1 mg), 3E (51.1 mg), 3F (39.3 mg), 3G (7.2 mg), 3H (1.0 mg), and 3I (1.5 mg). Fraction 3E, eluted with 80% MeOH, was purified with HPLC (Develosil C30-UG-5, 6.0 x 250 mm; eluent, 68% MeOH; flow rate, 2.0 mL/min) to afford compound **16** (2.3 mg, *t_R* 23 min) and compound **9** (9.5 mg, *t_R* 26 min). Fraction 3F, eluted with 90% MeOH, was purified with HPLC (Develosil C30-UG-5, 6.0 x 250 mm; eluent, 70% MeOH; flow rate, 2.0 mL/min) to yield compound **10** (1.1 mg, *t_R* 31 min), compound **11** (0.7 mg, *t_R* 37 min), compound **13** (0.3 mg, *t_R* 36 min), compound **12** (1.6 mg, *t_R* 46 min), compound **14** (1.0 mg, *t_R* 47 min), and compound **15** (7.5 mg, *t_R* 50 min). HPLC (Develosil C30-UG-5, 6.0 x 250 mm; eluent, 65% MeOH; flow rate, 2.0 mL/min) to yield compound **7** (4.6 mg, *t_R* 47 min). Fraction 2H, eluted with 55% MeOH, was purified with HPLC (Develosil ODS-MG-5, 10 x 250 mm; eluent, 63% MeOH; flow rate, 2.0 mL/min) to yield compound **8** (2.2 mg, *t_R* 47 min).

(2S)-8-hydroxy-6,7,4'-trimethoxyflavanone (1): pale yellow powder, $[\alpha]_D^{20} +8.6$ (*c* 0.079, MeOH); IR ν_{\max} (ATR) 3210 and 1600 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ) 258 (3.7) and 307 (3.3) nm; CD (MeOH) λ_{\max} ([θ]) 346 (3230), 313 (-4180) nm; ¹H and ¹³C NMR data in Table 1; EIMS *m/z* 330 (M⁺); HRFABMS 331.1191, calcd for C₁₈H₁₉O₆, 331.1182.

(2S)-3'-hydroxy-6,7,8,4'-tetramethoxyflavanone (2): pale yellow powder, $[\alpha]_D^{20} +11.7$ (*c* 0.023, MeOH); IR ν_{\max} (ATR) 3380 and 1600 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ) 279 (3.8) nm; CD (MeOH) λ_{\max} ([θ]) 347 (15000), 313 (-19000) nm; ¹H and ¹³C NMR data in Table 1; EIMS *m/z* 360 (M⁺); HRFABMS 399.0865, calcd for C₁₉H₂₀O₇, 399.0890.

Luciferase Assay to Assess the Enhancement of DR5 Promoter Activation. The procedure of assay was the same as previously described.¹⁵ Briefly, DLD-1/*SacI* cells (2×10^5 cells per well), a human colon cancer cell line stably transfected with the DR5 promoter-luciferase reporter plasmids, pDR5/*SacI*²⁷ were treated with different concentrations of each isolated compound for 24 h at 37 °C. After the medium containing the isolated compounds was removed, cells were lysed in a Cell Culture Lysis Reagent (Promega). The lysate was measured for 10 sec as relative light units by a luminometer and the DR5 promoter activity was evaluated by relative light unit of sample compared with that of the control (cells treated with EtOH).

ACKNOWLEDGEMENTS

This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by a Grant-in-Aid to TO from Cosmetology Research Foundation and from Astellas Foundation for Research on Metabolic Disorders.

REFERENCES

1. M. Ishibashi and T. Ohtsuki, *Med. Res. Rev.*, 2008, **28**, 688.
2. H. Walczak, M. A. Degli-Esposti, R. S. Johnson, P. J. Smolak, J. Y. Waugh, N. Boiani, M. S. Timour, M. J. Gerhart, K. A. Schooley, C. A. Smith, R. G. Goodwin, and C. T. Rauch, *EMBO J.*, 1997, **16**, 5386.
3. A. Ashkenazi and V. M. Dixit, *Curr. Opin. Cell Biol.*, 1999, **11**, 255.
4. X. Chen, H. Thakkar, F. Tyan, S. Gim, H. Robinson, C. Lee, S. K. Pandey, C. Nwokorie, N. Onwudiwe, and R. K. Srivastava, *Oncogene*, 2001, **20**, 6073.
5. T. R. Singh, S. Shankar, X. Chen, M. Asim, and R. K. Srivastava, *Cancer Res.*, 2003, **63**, 5390.
6. A. Hernandez, F. Smith, Q. Wang, X. Wang, and B. M. Evers, *Ann. Surg.*, 2000, **232**, 576.
7. L. Pasquini, E. Petrucci, R. Riccioni, A. Petronelli, and U. Testa, *Cancer Ther.*, 2006, **4**, 47.
8. S. Chen, L. Hu, S. Lonial, F. R. Khuri, and S. Y. Sun, *Cancer Res.*, 2007, **67**, 4981.
9. J. Kouhara, T. Yoshida, S. Nakata, M. Horinaka, M. Wakada, Y. Ueda, H. Yamagishi, and T. Sakai, *Int.J. Oncol.*, 2007, **30**, 679.
10. T. Shiraishi, T. Yoshida, S. Nakata, M. Horinaka, M. Wakada, Y. Mizutani, T. Miki, and T. Sakai, *Cancer Res.*, 2005, **65**, 6364.
11. M. Tomasetti, L. Andera, R. Alleva, B. Borghi, J. Neuzil, and A. Procopio, *FEBS lett.*, 2006, **580**, 1925.
12. E. M. Jung, J. Park, K. S. Choi, J. Park, H. I. L. Lee, K. S. Lee, and T. K. Kwon, *Carcinogenesis*, 2006, **27**, 2008.

13. H. Zhu, W. Guo, L. Zhang, S. Wu, F. Teraishi, J. J. Davis, F. Dong, and B. Fang, [*Cancer Biol. Ther.*, 2005, 4, 787.](#)
14. S. Nakata, T. Yoshida, T. Shiraishi, M. Horinaka, J. Kouhara, M. Wakada, and T. Sakai, [*Mol. Cancer Ther.*, 2006, 5, 1827.](#)
15. H. Kikuchi, T. Ohtsuki, T. Koyano, T. Kowithayakorn, T. Sakai, and M. Ishibashi, [*J. Nat. Prod.*, 2007, 70, 1910.](#)
16. N. Pisutthanan, B. Liawruangrath, S. Liawruangrath, A. Baramée, A. Apisariyakul, J. Korth, and J. B. Bremner, [*Nat. Prod. Res.*, 2006, 20, 636.](#)
17. N. Bunyaphatsara, 'Medicinal Plants Indigenous to Thailand,' Vol.4, Prachachon, Bangkok, 622.
18. M. M. Iwu, 'Handbook of African medicinal plants', CRC Press, Ann Arbor, p.181.
19. M. Ahmad and M. N. Nabi, *Sci. Res. Dacca Pakistan*, 1967, 4, 154.
20. S. K. Talapatra, D. S. Bhar, and B. Talapatra, [*Phytochemistry*, 1974, 13, 284.](#)
21. B. K. Bose, P. Chakrabarti, S. Chakrabarti, S. P. Dutta, and A. K. Baura, [*Phytochemistry*, 1973, 12, 667.](#)
22. A. M. Metwally and E. C. Ekejiuba, [*Planta Med.*, 1981, 42, 403.](#)
23. M. A. Hai, P. K. Biswas, K. C. Shil, and M. U. Ahmad, *J. Bangladesh Chem. Soc.*, 1991, 4, 47.
24. M. A. Hai, K. saha, and M. U. Ahmad, *J. Bangladesh Chem. Soc.*, 1995, 8, 139.
25. A. Suksamrarn, A. Chotipong, T. Suavavrsri, S. Boongird, P. Timsuksai, S. Vimuttipong, and A. Chuaynugul, [*Arch. Pharm. Res.*, 2004, 27, 507.](#)
26. A. Biller, M. Boppre, L. Witte, and T. Hartmann, [*Phytochemistry*, 1994, 35, 615.](#)
27. T. Yoshida, A. Maeda, N. Tani, and T. Sakai, [*FEBS Lett.*, 2001, 507, 381.](#)
28. E. K. Malterud, E. T. Bremnes, A. Faegri, T. Moe, and D. K. E. Sandanger, [*J. Nat. Prod.*, 1985, 48, 559.](#)
29. M. Grande, F. Piera, A. Cuerca, P. Tornes, and I. S. Bellido, [*Planta Med.*, 1985, 51, 414.](#)
30. G. Topcu and C. Eris, [*Turkish J. Chem.*, 1996, 20, 265.](#)
31. M. T. Islam and S. Tahara, [*Phytochemistry*, 2000, 54, 901.](#)
32. N. Ruangrungsri, P. Tappayuthpijarn, and P. Tantivatana, [*J. Nat. Prod.*, 1981, 44, 541.](#)
33. H. Wagner and M. V. Chari, [*Tetrahedron Lett.*, 1976, 17, 1799.](#)
34. S. Li, C. Lo, and C. Ho, [*J. Agric. Food Chem.*, 2006, 54, 4176.](#)
35. J. F. Stevens, E. Wollenweber, M. Ivancic, V. L. Hsu, S. Sundberg, and M. L. Deinzer, [*Phytochemistry*, 1999, 51, 771.](#)
36. J. B. Harborne, 'The Flavonoids: Advances in Research Since 1986', Chapman & Hall, London, pp. 448-449.
37. A. E. Star, H. Rosler, T. J. Mabry, and D. M. Smith, [*Phytochemistry*, 1975, 14, 2275.](#)

38. Y. Liu, D. K. Ho, and J. M. Cassady, [*J. Nat. Prod.*, 1992, **55**, 357.](#)
39. A. Marston and K. Hostettmann, 'Flavonoids: chemistry, biochemistry and applications', ed. by O. M. Andersen and K. R. Markham, Taylor & Francis, London, 2005, pp.1-36.
40. L. Jurd, [*Phytochemistry*, 1969, **8**, 445.](#)
41. W. Gaffield, [*Tetrahedron*, 1970, **26**, 4093.](#)
42. M. Horinaka, T. Yoshida, T. Shiraishi, S. Nakata, M. Wakada, R. Nakanishi, H. Nishino, H. Matsui, and T. Sakai, [*Oncogene*, 2005, **24**, 7180.](#)
43. M. Horinaka, T. Yoshida, T. Shiraishi, S. Nakata, M. Wakada, R. Nakanishi, H. Nishino, and T. Sakai, [*Biochem. Biophys. Res. Commun.*, 2005, **333**, 833.](#)
44. R. Takimoto and W. S. El-Deiry, [*Oncogene*, 2000, **19**, 1735.](#)
45. M. Horinaka, T. Yoshida, T. Shiraishi, S. Nakata, M. Wakada, and T. Sakai, [*Mol. Cancer Ther.*, 2006, **5**, 945.](#)
46. H. Hasegawa, Y. Yamada, K. Komiyama, M. Hayashi, M. Ishibashi, T. Yoshida, T. Sakai, T. Koyano, T.-S. Kam, K. Murata, K. Sugahara, K. Tsuruda, N. Akamatsu, K. Tsukasaki, M. Masuda, N. Takasu, and S. Kamihira, [*Blood*, 2006, **107**, 679.](#)
47. Y. G. Son, E. H. Kim, J. Y. Kim, S. U. Kim, T. K. Kwon, A. R. Yoon, C. O. Yun, and K. S. Choi, [*Cancer Res.*, 2007, **67**, 8274.](#)