Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4738–4742

# Fatty acid synthase inhibitory activity of acylphloroglucinols isolated from *Dryopteris crassirhizoma*

MinKyun Na,[a] JunPil Jang,[a] Byung Sun Min,[b] Sang Jun Lee,[c] Myung Sun Lee,[a] Bo Yeon Kim,[a] Won Keun Oh[a,*] and Jong Seog Ahn[a]

[a]*Korea Research Institute of Bioscience and Biotechnology (KRIBB), 52 Eoun-dong, Yuseong-gu, Daejeon 305-333, Republic of Korea*
[b]*College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea*
[c]*AmorePacific Co. Ltd, Bora-ri, Yongin, Gyeonggi-do 449-729, Republic of Korea*

Received 3 April 2006; revised 15 June 2006; accepted 5 July 2006
Available online 25 July 2006

**Abstract**—Fatty acid synthase (FAS) is emerging as a potential therapeutic target to treat cancer and obesity. Bioassay-guided fractionation of a MeOH extract of the rhizomes of *Dryopteris crassirhizoma* (Dryopteridaceae), using an in vitro FAS inhibitory assay, resulted in the isolation of a series of acylphloroglucinols, as the active principles. The isolates **1**–**10** inhibited FAS with $IC_{50}$ values ranging from 23.1 ± 1.4 to 71.7 ± 3.9 μM. The results of the present study indicate that the acylphloroglucinol derivatives could be considered to be a promising class of FAS inhibitors.


Mammalian fatty acid synthase (FAS) is a multi-enzyme complex that catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent condensation of acetyl-CoA and malonyl-CoA to produce the saturated 16-carbon fatty acid, palmitate.[1] This enzyme is composed of two identical subunits, each containing seven catalytic domains and an acyl carrier protein.[1] Recently, a number of studies have suggested that FAS is a potential target for drug discovery.[2–11] In contrast to normal tissues, high levels of FAS expression have been found in many human cancers, including carcinoma of the breast, prostate, colon, and ovary.[2–6] This difference in expression of FAS between normal and cancer cells has raised the possibility that inhibition of FAS might offer a therapeutic approach in cancer treatment.[2–6] Actually, administration of FAS inhibitors such as cerulenin and C75 to various cancer cell lines resulted in the induction of apoptosis in vitro and in vivo.[2–6] Furthermore, specific blockage of FAS using RNA interference technology resulted in growth arrest and cell death of cancer cells.[7] FAS is also expressed in hypothalamic neurons and possibly involved in the regulation of food intake.[8,9] Recent studies have shown that the FAS inhibitors, cerulenin and C75, inhibit food intake, and cause significant body weight loss in both lean and *ob*/*ob* mice.[8,9] These results suggest that compounds that reduce FAS activity or expression levels may be useful for the treatment of cancer and obesity. Although there have been a number of reports on the designing and development of FAS inhibitors,[5,10–12] new types of FAS inhibitors with suitable pharmacological properties remain to be discovered. Because plants could be a promising source for the development of new FAS inhibitors,[11,12] we have undertaken a screen of hundreds of plant extracts against FAS.

During this screening effort we found that a MeOH extract of the rhizomes of *Dryopteris crassirhizoma* inhibited FAS activity (>70% inhibition at 50 μg/ml). *D. crassirhizoma* belongs to Dryopteridaceae and is a fern growing in wet, shaded forests, open grassy areas, or on rocks and along streams primarily in mountains.[13] The rhizomes of this species have been used as a vermifuge, astringent, vulnerary, antibacterial, and anti-inflammatory agent, and used internally in the treatment of hemorrhage, uterine bleeding, and mumps.[13] Characteristic phloroglucinol derivatives, such as albaspidins, norflavaspidic acids, flavaspidic acids, deaspidins, *para*-aspidins, and filixic acids, have been reported as constituents of the genus *Dryopteris*,[14] and have been found to possess antioxidant, antibacterial, and antitumor promoting activities.[15–17] Previous study has

*Keywords*: Fatty acid synthase (FAS); *Dryopteris crassirhizoma*; Dryopteridaceae; Acylphloroglucinols; FAS inhibitors.
* Corresponding author. Tel.: +82 42 860 4295; fax: +82 42 860 4595; e-mail: wkoh@kribb.re.kr

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.018

demonstrated that kaempferol glycosides isolated from *D. crassirhizoma* inhibited human immunodeficiency virus reverse transcriptase-associated DNA polymerase and ribonuclease H activities.[18] Despite a number of studies on the chemical constituents and biological activities of the genus *Dryopteris*, there have been no investigations with regard to its FAS inhibitory activity. This prompted us to investigate the FAS inhibitory compounds from the species. Bioassay-guided fractionation of a MeOH extract of the rhizomes of this species led to the isolation of a series of phloroglucinol derivatives, as the active principles (Fig. 1). In this paper, we report on the isolation and structure identification of these compounds, and their FAS inhibitory activity.

The rhizomes of *D. crassirhizoma* were purchased from a local market in Daejeon, Korea, and identified by Professor Byung Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (KRIBB No. 018–012) has been deposited in authors' laboratory. The dried rhizomes (4 kg) were extracted with MeOH at room temperature for 2 months. The MeOH extract (420 g) was suspended in $H_2O$ (4 l) and partitioned with hexane, EtOAc, and BuOH, sequentially. The FAS inhibitory activity was concentrated in the hexane- and EtOAc-soluble fraction (90% and 92% inhibition at 50 μg/ml). The hexane-soluble fraction (129 g) was separated by silica gel column chromatography (10 × 30 cm; 63–200 μm particle size) using a gradient of hexane–$CHCl_3$ (from 9:1 to 0:1), to yield eight fractions (Hfr. 1–Hfr. 8) according to their TLC profile. Although most of the fractions except for Hfr. 1 showed strong FAS inhibitory activity (>90% inhibition at 50 μg/ml), we were interested in

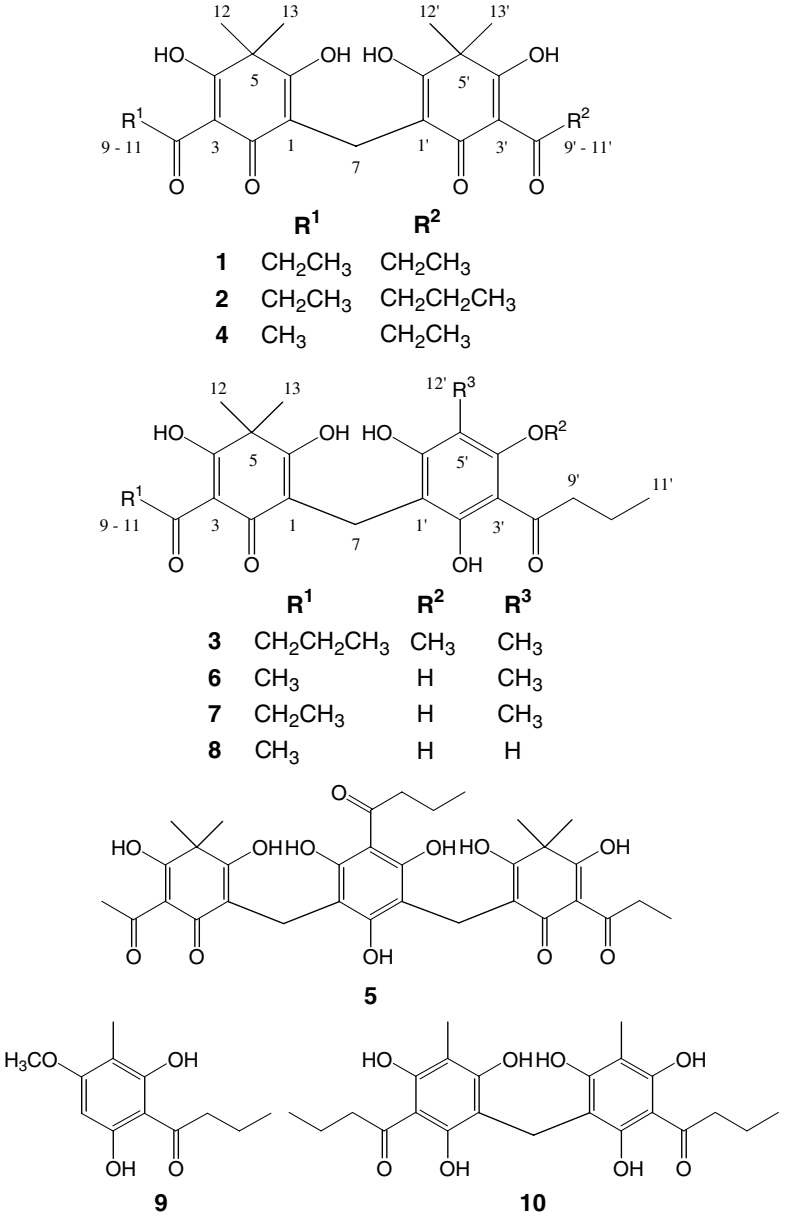


**Figure 1.** Structures of compounds **1**–**10** isolated from *D. crassirhizoma*.

Hfr. 2 and Hfr. 3 because their $^1$H NMR spectra indicated the characteristic signals for phloroglucinols different from those in other fractions. Hfr. 2 [eluted with hexane–$CHCl_3$ (8:2), 16.7 g] was chromatographed over silica gel (6.5 × 35 cm; 63–200 μm particle size) using a mixture of hexane–$CH_2Cl_2$–MeOH (30:10:0.5), to yield four subfractions (Hfr. 2-1–Hfr. 2-4). The activity was found to be highest in Hfr. 2-1 (2.4 g), which was further separated by reversed phase column chromatography (3.5 × 30 cm; LiChroprep® RP-18 40–63 μm particle size) using a stepwise gradient of AcCN–$H_2O$ (from 60:40, 70:30, 80:20 to 100:0; 2 l for each step), to yield four subfractions, Hfr. 2-1-1 to Hfr. 2-1-4. Further purification of Hfr. 2-1-1 (753 mg) by preparative reversed phase HPLC (YMC J-sphere ODS-H80® column; 20 × 150 mm; 4 μm particle size; 5 ml/min; UV detection at 254 nm) with an isocratic solvent system of AcCN–$H_2O$ (94:6) resulted in the isolation of compounds **1** (35 mg; $t_R$ = 36.5–39.8 min), **2** (23 mg; $t_R$ = 42.0–46.0 min), and **3** (30 mg; $t_R$ = 46.5–52.0 min). Hfr. 2-1-2 (917 mg) was also purified by additional HPLC using an isocratic solvent system of AcCN–$H_2O$ (90:10) to isolate 23 mg of **4** ($t_R$ = 35–40 min). Another active fraction, Hfr. 3 [eluted with hexane–$CHCl_3$ (7:3), 7.4 g], was subjected to silica gel column chromatography using a gradient of hexane–EtOAc (from 10:0 to 9:1) to yield 230 mg of crude compound **5**, which was recrystallized in EtOAc to give pure compound **5**. The EtOAc-soluble fraction (91 g) was chromatographed on a silica gel column (10 × 30 cm; 63–200 μm particle size) using a gradient of hexane–$CHCl_3$–EtOH (from 10:10:0.1 to 9:9:2), to yield six fractions (EAfr. 1–EAfr. 6) according to their TLC pattern. Since the EAfr. 1 [eluted with hexane–$CHCl_3$–MeOH (from 10:10:0.1 to 10:10:0.5)] exhibited the strongest inhibitory activity (91% inhibition at 30 μg/ml), this fraction (30 g) was fractionated by silica gel column chromatography (9.5 × 17 cm; 63–200 μm particle size) using a gradient of $CHCl_3$–MeOH (from 40:1 to 1:10), to obtain four subfractions (EAfr. 1-1–EAfr. 1-4). A portion of EAfr. 1-1 was purified using preparative TLC [Silica gel 60 F254, 0.5 mm; mobile phase: hexane–acetone–acetic acid (6:4:0.1); UV detection at 254 nm] to obtain compounds **6** (1.5 mg; $R_f$ value 0.32) and **7** (1.7 mg; $R_f$ value 0.35). EAfr. 1-2 [eluted with $CHCl_3$–MeOH (from 10:1 to 9:1), 4.1 g] was isolated by reversed phase column chromatography (3.5 × 30 cm; LiChroprep® RP-18 40–63 μm particle size) using a stepwise gradient of AcCN–$H_2O$ (from 35:65, 40:60, 50:50, 60:40 to 70:30; 2 l for each step), to afford compounds **8** (27 mg) and **9** (33 mg). Purification of EAfr. 1-3 [eluted with $CHCl_3$–MeOH (from 8:2 to 7:3), 2.2 g] by preparative reversed phase HPLC with an isocratic solvent of 33% AcCN in $H_2O$ resulted in the isolation of compound **10** (38 mg; $t_R$ = 25–35 min). The structures of the isolated compounds **1**–**10** were identified as albaspidin-PP (**1**), albaspidin-PB (**2**), aspidin-BB (**3**), albaspidin-AP (**4**), filixic acid-ABP (**5**), flavaspidic acid-AB (**6**), flavaspidic acid-PB (**7**), norflavaspidic acid-AB (**8**), aspidinol-B (**9**), and methylene-bis-methylphlorobutyrophenone (**10**), respectively, by analyses of MS and NMR data,[19] and comparison with those in the literature (Fig. 1).[19–24]

FAS was purified from chicken liver by using stepwise ammonium sulfate precipitations, gel filtration, and anion exchange chromatography as previously described.[25] FAS was 95% pure as estimated from SDS/PAGE with Coomassie blue staining. FAS activity was measured by analysis of the incorporation of [$^3$H] acetyl CoA into palmitate.[25,26] All the isolates were assayed for their inhibitory activity against FAS, and the results are presented in Table 1. The known FAS inhibitors, cerulenin ($IC_{50}$ = 13.0 ± 0.9 μM) and luteolin[11,12] ($IC_{50}$ = 62.5 ± 4.0 μM), were used as positive controls in this assay. Most of the isolates inhibited FAS in dose-dependent manners and several of these were more potent than luteolin. Of the compounds tested, compounds **7** and **10** exhibited the strongest inhibitory activity with $IC_{50}$ values of 23.0 ± 1.0 and 25.0 ± 1.1 μM, respectively. In general, compounds **3**, **6**, **7**, and **8** in which an acylphloroglucinol moiety is linked with an acylfilicinic acid moiety (3,5-dihydroxy-4,4-dimethyl-2,5-cyclohexadien-1-one) were more effective than albaspidin derivatives **1**, **2**, and **4** bearing two acylfilicinic acid analogues. Clearly, flavaspidic acid-PB (**7**) was twofold more active than albaspidin-PB (**2**). Compound **10** containing two acylphloroglucinol units retained the activity. These results indicate that the acylphloroglucinol moiety is required for the activity. However, the loss of FAS inhibition by aspidinol-B (**9**) suggests that the presence of the acylphloroglucinol alone does not confer high activity. Another trend was recognized from our data in Table 1. As the length of acyl side chain is increased, the FAS inhibitory activity appeared to be slightly increased. Compound **2** displayed slightly higher activity than compounds **1** and **4** both with shorter acyl side chains. A similar case was observed between compounds **6** and **7**, indicating that the acyl side chains may affect the FAS inhibitory activity. Despite the presence of butyryl side chains, compound **3** appeared not to

**Table 1.** The inhibitory activity of the compounds **1**–**10** isolated from *D. crassirhizoma* against FAS

| Compounds | FAS inhibitory activity $IC_{50}$ (μM)[a] |
|---|---|
| Albaspidin-PP (**1**) | 60.2 ± 3.5 |
| Albaspidin-PB (**2**) | 56.1 ± 2.6 |
| Aspidin-BB (**3**) | 32.6 ± 3.0 |
| Albaspidin-AP (**4**) | 71.7 ± 3.9 |
| Filixic acid-ABP (**5**) | 31.0 ± 2.7 |
| Flavaspidic acid-AB (**6**) | 28.7 ± 1.4 |
| Flavaspidic acid-PB (**7**) | 23.1 ± 1.4 |
| Norflavaspidic acid-AB (**8**) | 29.7 ± 1.1 |
| Aspidinol-B (**9**) | 49.1 ± 2.7 |
| Methylene-bis-methylphlorobutyrophenone (**10**) | 25.4 ± 1.4 |
| 3-Hydroxyflavone[b] | >100 (6.5%)[c] |
| (−)-Epicatechin[b] | >100 (10.2%)[c] |
| Luteolin[d] | 62.5 ± 4.0 |
| Cerulenin[d] | 13.0 ± 0.9 |

[a] $IC_{50}$ values were determined by regression analyses and expressed as means ± SD of three replicates.
[b] Negative controls.[11,12]
[c] The number in parentheses represents a percentage of inhibition at a level of 100 μM.
[d] Positive controls.

be as effective as **7** and **10**, suggesting that the substitution pattern of acylphloroglucinol moiety is more important for the activity and replacement of a phenolic hydroxyl group with a methyl ether might be responsible for the loss of activity. Compounds **6** and **8** demonstrated similar activity against FAS, suggesting that substitution of the C-5′ hydrogen on the acylphloroglucinol with a methyl group may not affect the activity. The trimeric compound, filixic acid ABP (**5**), was also an effective inhibitor of FAS ($IC_{50} = 31.0 \pm 2.7$ μM). Although the structure–activity relationships of phloroglucinol derivatives were not thoroughly investigated, our data indicate that the acylphloroglucinol moiety linked with either acylfilicinic acid or another phloroglucinol, and appropriate length of acyl side chain are required for the activity. These results indicate that the acylphloroglucinol skeleton is worthy of further investigation as a lead for novel FAS inhibitors.

Acylphloroglucinol derivatives have been reported to have a wide range of biological activities that include antioxidant, antibacterial, and antitumor activities.[15–17,21] However, to our knowledge, the FAS inhibitory activity of the acylphloroglucinols is now being reported for the first time in this study. Several in vitro studies have shown the cytotoxic effect of acylphloroglucinol derivatives on the human cancer cells such as carcinoma of the lung, prostate, breast, skin, and epidermoid in the mouth.[27–29] Kapadia et al. also demonstrated that the acylphloroglucinols isolated from the genus *Dryopteris* had in vitro antitumor activity and, in particular, the dimeric phloroglucinols, aspidin, and deaspidin, inhibited tumorigenesis in animal model.[17] Despite these findings, the mechanisms by which these compounds exert the antitumor effects are poorly understood. Since high levels of FAS expression have been found in many human cancers including breast, prostate, colon, and ovary, this enzyme has emerged as a selective route to treat cancer.[2–7] Recent studies have demonstrated that suppression of FAS by RNA interference results in induction of apoptosis and inhibition of FAS by small molecules such as cerulenin and C75 leads to selective cytotoxicity against various cancer cell lines in vitro and in vivo.[4–7,10,12] As shown in the present study, the acylphloroglucinol derivatives could be considered as a promising class of FAS inhibitors. This may provide a new insight into the mechanism underlying their antitumor activity. Further investigation and optimization of acylphloroglucinols might enable the preparation of new FAS inhibitors potentially useful in the treatment of cancers and obesity.

## Acknowledgments

This research was supported in part by the grants from the Plant Diversity Research Center of 21st Frontier Research Program (PF-0320903-00) of the Ministry of Science and Technology of Korea, the Technology Development Program for Agriculture and Forestry (AGM0800611) of the Ministry of Agriculture and Forestry of Korea, and from the KRIBB Research Initiative Program.

## References and notes

1. Wakil, S. J.; Stoops, J. K.; Joshi, V. C. *Ann. Rev. Biochem.* **1983**, *52*, 537.
2. Kuhajda, F. P.; Jenner, K.; Wood, F. D.; Hennigar, R. A.; Jacobs, L. B.; Dick, J. D.; Pasternack, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6379.
3. Kuhajda, F. P. *Nutrition* **2000**, *16*, 202.
4. Pizer, E. S.; Thupari, J.; Han, W. F.; Pinn, M. L.; Chrest, F. J.; Frehywot, G. L.; Townsend, C. A.; Kuhajda, F. P. *Cancer Res.* **2000**, *60*, 213.
5. Kuhajda, F. P.; Pizer, E. S.; Li, J. N.; Mani, N. S.; Frehywot, G. L.; Townsend, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3450.
6. Lu, S.; Archer, M. C. *Carcinogenesis* **2005**, *26*, 153.
7. De Schrijiver, E.; Brusselmans, K.; Heyns, W.; Verhoeven, G.; Swinnen, J. V. *Cancer Res.* **2003**, *63*, 3799.
8. Loftus, T. M.; Jaworsky, D. E.; Frehywot, G. L.; Townsend, C. A.; Ronnett, G. V.; Lane, M. D.; Kuhajda, F. P. *Science* **2000**, *288*, 2379.
9. Dowell, P.; Hu, Z.; Lane, M. D. *Ann. Rev. Biochem.* **2005**, *74*, 515.
10. McFadden, J. M.; Medghalchi, S. M.; Thupari, J. N.; Pinn, M. L.; Vadlamudi, A.; Miller, K. I.; Kuhajda, F. P.; Townsend, C. A. *J. Med. Chem.* **2005**, *48*, 946.
11. Li, B. H.; Tian, W. X. *J. Biochem.* **2004**, *135*, 85.
12. Brusselmans, K.; Vrolix, R.; Verhoeven, G.; Swinnen, J. V. *J. Biol. Chem.* **2005**, *280*, 5636.
13. Bae, K. *The Medicinal Plants of Korea*; Kyo-Hak Publishing: Seoul, 2000, p 25.
14. Widén, C. J.; Fraser-Jenkins, C.; Reichstein, T.; Gibby, M.; Sarvela, J. *Ann. Bot. Fennici* **1996**, *33*, 69.
15. Lee, S. M.; Na, M.; An, R. B.; Min, B. S.; Lee, H. K. *Biol. Pharm. Bull.* **2003**, *26*, 1354.
16. Do, D. S.; Min, B. S.; Bae, K. *Yakhak Hoeji* **1996**, *40*, 478.
17. Kapadia, G. J.; Tokuda, H.; Konoshima, T.; Takasaki, M.; Takayasu, J.; Nishino, H. *Cancer Lett.* **1996**, *105*, 161.
18. Min, B. S.; Tomiyama, M.; Ma, C. M.; Nakamura, N.; Hattori, M. *Chem. Pharm. Bull.* **2001**, *49*, 546.
19. (a) Albaspidin-PP (**1**): ESI-MS *m/z*: 431 $[M-H]^+$, 455 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 1.18 (6H, t, $J = 7.2$ Hz, H-10, -10′), 1.48 (6H, s, H-12, -12′), 1.55 (6H, s, H-13, -13′), 3.22 (4H, m, H-9, -9′), 3.32 (2H, br s, H-7); (b) albaspidin-PB (**2**): ESI-MS *m/z*: 445 $[M-H]^+$, 469 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 1.02 (3H, t, $J = 7.2$ Hz, H-11′), 1.18 (3H, t, $J = 7.2$ Hz, H-10), 1.48 and 1.49 (each 3H, s, H-12, -12′), 1.55 and 1.56 (each 3H, s, H-13, -13′), 1.70 (2H, m, H-10′), 3.17 (2H, m, H-9), 3.23 (2H, m, H-9′), 3.32 (2H, br s, H-7); (c) aspidin-BB (**3**): ESI-MS *m/z*: 459 $[M-H]^+$, 483 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 1.00 (3H, t, $J = 8.0$ Hz, H-11), 1.02 (3H, t, $J = 7.6$ Hz, H-11′), 1.45 (3H, br s, H-12), 1.57 (3H, br s, H-13), 1.73 (4H, m, H-10, -10′), 2.15 (3H, s, H-12′), 3.09 (4H, m, H-9, -9′), 3.58 (2H, br s, H-7), 3.74 (3H, s, $OCH_3$, H-13′); (d) albaspidin-AP (**4**): ESI-MS *m/z*: 417 $[M-H]^+$, 441 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 1.17 (3H, t, $J = 7.3$ Hz, H-10′), 1.47 (6H, s, H-12, -12′), 1.54 (6H, s, H-13, -13′), 2.72 (3H, s, H-9), 3.22 (2H, m, H-9′), 3.31 (2H, br s, H-7); (e) filixic acid-ABP (**5**): ESI-MS *m/z*: 625 $[M-H]^+$, 649 $[M+Na]^+$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 1.03 (3H, t, $J = 7.2$ Hz, H-11′), 1.20 (3H, t, $J = 7.4$ Hz, H-10″), 1.46 (6H, br s, H-12, -12″), 1.56 (6H, br s, H-13, -13″), 1.74 (2H, m, H-10′), 2.75 (3H, br s, H-9), 3.22 (4H, m, H-9′, -9″), 3.53 and 3.59 (each 2H, br s, H-7, -7′); (f) flavaspidic acid-AB (**6**): ESI-MS *m/z*: 417 $[M-H]^+$, 441 $[M+H]^+$; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 0.91 (3H, t, $J = 7.2$ Hz, H-11′), 1.16 (6H, br s, H-12, -13), 1.60 (2H, m, H-10′), 1.87 (3H, s, H-12′), 2.38 (3H, s, H-9), 3.04 (2H, m, H-9′), 3.33 (2H, br s, H-7); (g) flavaspidic acid-PB (**7**):

ESI-MS *m/z*: 431 $[M-H]^+$, 455 $[M+Na]^+$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 0.99 (3H, t, $J$ = 7.5 Hz, H-11′), 1.10 (3H, t, $J$ = 7.5 Hz, H-10), 1.40 (6H, br s, H-12, -13), 1.66 (2H, m, H-10′), 2.05 (3H, s, H-12′), 3.05 (2H, m, H-9′), 3.10 (2H, m, H-9), 3.55 (2H, br s, H-7); (h) norflavaspidic acid-AB (**8**): ESI-MS *m/z*: 403 $[M-H]^+$, 427 $[M+Na]^+$; $^1H$ NMR(400 MHz, $CD_3OD$) $\delta$: 0.99 (3H, t, $J$ = 7.2 Hz, H-11′), 1.25 (6H, br s, H-12, -13), 1.68 (2H, m, H-10′), 2.43 (3H, s, H-9), 3.10 (2H, m, H-9′), 3.45 (2H, br s, H-7), 5.81 (1H, s, H-5′); (i) aspidinol-B (**9**): ESI-MS *m/z*: 223 $[M-H]^+$, 247 $[M+Na]^+$; $^1H$ NMR (400 MHz, acetone-$d_6$) $\delta$: 0.96 (3H, t, $J$ = 7.2 Hz, H-11), 1.69 (2H, m, H-10), 1.92 (3H, s, H-3), 3.07 (2H, m, H-9), 3.81 (3H, br s, $OCH_3$), 6.01 (1H, br s, H-6′); (j) methylene-bis-methylphlorobutyrophenone (**10**): ESI-MS *m/z*: 431 $[M-H]^+$, 455 $[M+Na]^+$; $^1H$ NMR (400 MHz, acetone-$d_6$) $\delta$: 0.95 (6H, t, $J$ = 7.2 Hz, H-11, -11′), 1.67 (4H, m, H-10, -10′), 1.92 (6H, s, H-12, -12′), 3.10 (4H, m, H-9, -9′), 3.46 (2H, br s, H-7).

20. Wollenweber, E.; Stevens, J. F.; Ivanic, M.; Deinzer, M. L. *Phytochemistry* **1998**, *48*, 931.
21. Ito, H.; Muranaka, T.; Mori, K.; Jin, Z. X.; Tokuda, H.; Nishino, H.; Yoshida, T. *Chem. Pharm. Bull.* **2000**, *48*, 1190.
22. Patama, T. T.; Widén, C. J. *Phytochemistry* **1991**, *30*, 3305.
23. Coskun, M.; Sakushima, A.; Nishibe, S.; Hisada, S.; Tanker, N. *Phytochemistry* **1982**, *21*, 1453.
24. Lounasmaa, M. *Planta Med.* **1978**, *33*, 173.
25. Arslanian, M. J.; Wakil, S. J. *Methods Enzymol.* **1975**, *35*, 59.
26. To each microtube (final volume: 100 μl) was added FAS (20–30 μg protein) in a buffer containing 100 mM potassium phosphate (pH 7.0), 2.5 mM dithiothreitol (DTT), and 2.0 mM EDTA with or without test compounds. The mixtures were preincubated at 37 °C for 60 min, and reaction was started by the addition of a substrate mixture containing 0.25 mM NADPH, 0.4 nmol of malonyl CoA, and 0.02 μCi of [$^3$H] acetyl CoA. Following incubation at 37 °C for 10 min, the reaction was terminated with 60% $HClO_4$. Fatty acids were extracted with 1 ml of hexane and incorporation of radioactivity into the fatty acids was assessed by scintillation counting.
27. Arisawa, M.; Fujita, A.; Morita, N.; Koshimura, S. *Planta Med.* **1990**, *56*, 377.
28. Arisawa, M.; Fujita, A.; Morita, N. *J. Nat. Prod* **1991**, *54*, 1409.
29. Lobo-Echeverri, T.; Rivero-Cruz, J. F.; Su, B. N.; Chai, H. B.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Soejarto, D. D.; Kinghorn, A. D. *J. Nat. Prod.* **2005**, *68*, 577.