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Inhibition of protein tyrosine phosphatase 1B by diterpenoids isolated from *Acanthopanax koreanum*

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Abstract—Inhibition of protein tyrosine phosphatase 1B (PTP1B) has been proposed as a therapy to treat type 2 diabetes and obesity. In our preliminary screening study on the PTP1B inhibitory activity, a CH_2Cl_2-soluble extract of the roots of *Acanthopanax koreanum* (Araliaceae) was found to inhibit PTP1B activity at 30 μg/ml. Eight diterpenoids were isolated from the active fraction and were evaluated for their inhibitory effect on PTP1B. A kaurane-type diterpene, 16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**), inhibited PTP1B with an IC_{50} value of 7.1 ± 0.9 μM in a non-competitive manner. Acanthoic acid (**2**) and *ent*-kaur-16-en-19-oic acid (**5**) also inhibited PTP1B in dose-dependent manners. Either introduction of a hydroxyl group or reduction of a carboxyl group at C-19 in pimarane-type to alcohol abolished the inhibitory effects toward PTP1B.

Protein tyrosine phosphatases (PTPs), which dephosphorylate the phosphotyrosine residues of proteins, have an important role in intracellular signaling and metabolism. Although several PTPs such as PTP-α, leukocyte antigen-related tyrosine phosphatase (LAR), SH2-domain-containing phosphotyrosine phosphatase (SHP2) have been implicated in the regulation of insulin signaling, there are substantial evidences supporting PTP1B as the critical PTP controlling insulin signaling pathway.[1,2] PTP1B can interact with and dephosphorylate the activated insulin receptor (IR) as well as insulin receptor substrate (IRS) proteins.[1,2] Its overexpression has been shown to inhibit the IR signaling cascade and increased expression of PTP1B occurs in insulin-resistant states.[3] Furthermore, recent genetic evidence has shown that PTP1B gene variants are associated with changes in insulin sensitivity.[4] As with the insulin signaling pathway, the leptin signaling pathway can be attenuated by PTPs and there is compelling evidence that PTP1B is also involved in this process.[1,2] Therefore, it has been suggested that compounds that reduce PTP1B activity or expression levels could not only be used for treating type 2 diabetes but also obesity. Although there have been a number of reports on the designing and development of synthetic PTP1B inhibitors,[1,5] only a few studies have been reported as PTP1B inhibitors derived from plants.[6]

In our screening program for search of PTP1B inhibitors from plants, a CH_2Cl_2-soluble extract of the roots of *Acanthopanax koreanum* (Araliaceae) was found to inhibit PTP1B activity (72% inhibition at 30 μg/ml). *A. koreanum* is a medicinal plant indigenous to Korea.[7] The roots and stem barks of *A. koreanum* have been traditionally used as a tonic and to treat rheumatism, hepatitis, and diabetes.[7] Previous phytochemical investigations on this plant have resulted in the isolation of triterpenes, lignans, and diterpenes.[8–12] Although *A. koreanum* has been traditionally used to treat diabetes, there has been no study with regard to its anti-diabetic effect. Because the CH_2Cl_2-soluble extract of the roots of this plant was found to inhibit PTP1B considered as a target for the treatment of type 2 diabetes, in this study, we investigated the PTP1B inhibitory compounds from this active fraction. Bioassay-guided fractionation of the CH_2Cl_2-soluble fraction led to the isolation of three PTP1B inhibitory diterpenoids, acanthoic acid (**2**), *ent*-kaur-16-en-19-oic acid (**5**), and 16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**), along with their five derivatives. All the isolated diterpenoids **1**–**8** (Fig. 1) were evaluated for their inhibitory effect on PTP1B.

Keywords: Protein tyrosine phosphatase 1B (PTP1B); *Acanthopanax koreanum*; Araliaceae; Diterpenoids; Non-competitive inhibitor.
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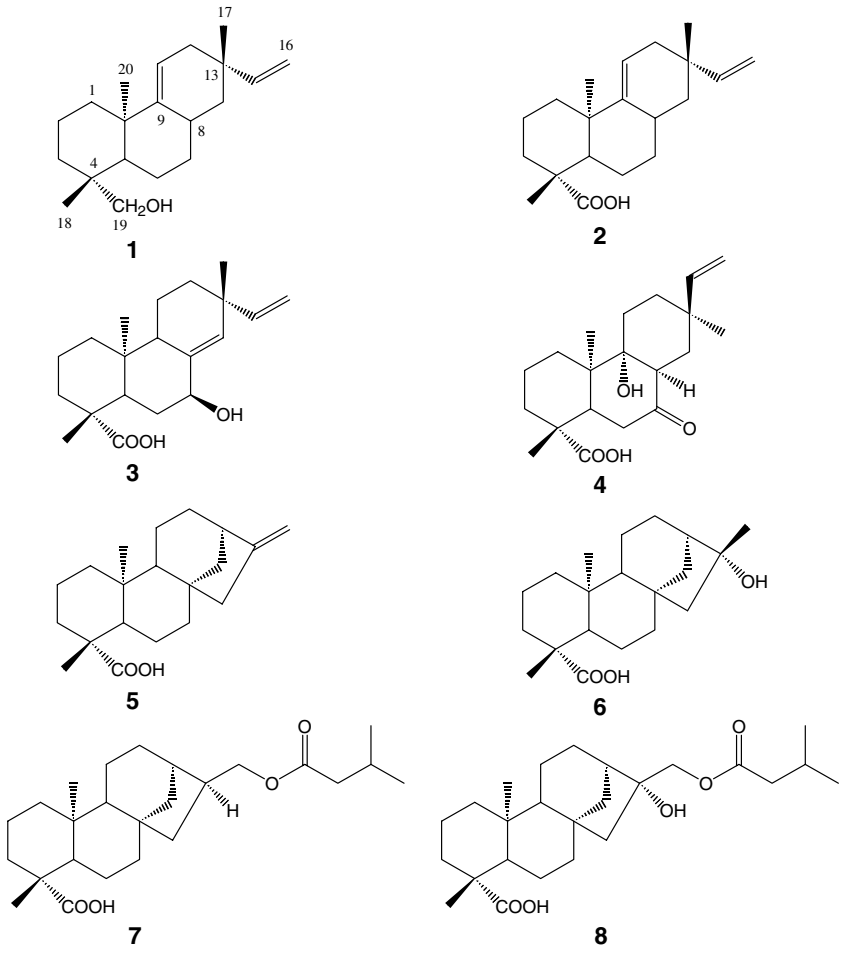

Figure 1. Structures of diterpenoids **1**–**8** isolated from *Acanthopanax koreanum*.

The roots of *A. koreanum* were obtained from Susin Ogapi Co., Korea, and identified by Prof. Young Ho Kim, College of Pharmacy, Chungnam National University. A voucher specimen (CNU96076) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University (Korea). The dried roots (1.2 kg) were extracted with MeOH at 50 °C for 72 h. The MeOH extract (98 g) was suspended in H_2O (1.5 L) and partitioned with CH_2Cl_2 (1.5 L × 3) and BuOH (1.5 L × 3), sequentially. Since the CH_2Cl_2-soluble fraction showed PTP1B inhibitory activity (72% inhibition at 30 μg/ml), this fraction (47 g) was further purified by silica gel column chromatography using a stepwise gradient of hexane–EtOAc (from 20:1, 10:1, 5:1, 3:1, 1:1 to 0:1; 2 L for each step), to yield five fractions (Fr. 1–Fr. 5). Of these, Fr. 1 and Fr. 3 showed the most potent PTP1B inhibitory activity (75 and 74% inhibition at 10 μg/ml). Fr. 1 [eluted with hexane–EtOAc (from 20:1 to 10:1), 10.7 g] was purified by preparative reversed-phase HPLC using a gradient from 80% to 100% MeOH over 30 min, then 100% MeOH for 20 min (Shiseido Capcell Pak C_{18} column; 10 × 250 mm; 5 μm particle size; 2 ml/min; UV detection at 210 nm), to afford compounds **2** (320 mg) and **5** (1.7 mg). The active fraction, Fr. 3 [eluted with hexane–EtOAc (from 20:1 to 10:1), 1.2 g], was subjected to silica gel column chromatography using a stepwise gradient of hexane–EtOAc (from 9:1, 5:1, 3:1, 1:1 to 0:1; 1 L for each step), to yield four subfractions (Fr. 3–1–Fr. 3–4). Compound **7** (2.9 mg) was obtained by recrystallization in MeOH from Fr. 3-4 [eluted with hexane–EtOAc (1:1)]. Fr. 5 which showed moderate PTP1B inhibitory activity (45% inhibition at 30 μg/ml) was also separated by repeated silica gel column chromatography and reversed-phase MPLC on LiChroprep® RP-18 column [25 × 310 mm; 40–63 μm particle size; 5 ml/min; eluted with MeOH–H_2O (2:1)] as described previously,[8,9,12] to afford compounds **1** (1.2 mg), **3** (4.0 mg), **4** (3.0 mg), **6** (3.5 mg), and **8** (10.3 mg). These compounds isolated were determined by HPLC to be >95% pure. Eight diterpenoids were identified as acanthol (**1**), acanthoic acid (**2**), 7β-hydroxy-*ent*-pimara-8(14), 15-dien-19-oic acid (**3**), acanthokoreoic acid A (**4**), *ent*-kaur-16-en-19-oic acid (**5**), 16α-hydroxy-*ent*-kauran-19-oic acid (**6**), 16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**), and 16α-hydroxy-17-isovaleryloxy-*ent*-kauran-19-oic acid (**8**) by analyses of MS and NMR data, and comparison with those in the literature.[8,9,12,13]

PTP1B (human, recombinant) was purchased from BIOMOL® International LP (USA) and the enzyme activity was measured using *p*-nitrophenyl phosphate (*p*NPP) as described previously.[14,15] All the isolated diterpenoids were dissolved in DMSO to obtain a stock

solution of 5 mM, and appropriate dilutions were made before the enzyme assay (final DMSO concentration <3%, and control activity was not affected by this concentration). The PTP1B inhibitory activity of the isolates was tested in vitro, and the results are presented in Table 1. Of the compounds tested, 16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**) which possesses an isovaleryloxy group at C-17 of kaurane-type exhibited the most potent inhibitory activity (IC_{50} = 7.1 ± 0.9 μM). However, compound **8** (IC_{50} > 30 μM) substituted a hydroxyl group at C-16 of **7** exhibited significantly lower activity than **7**. A similar case was observed between compounds **5** and **6**. *ent*-Kaur-16-en-19-oic acid (**5**) without a hydroxyl group at C-16 was much more effective than **6** with a hydroxyl group. These results indicate that substitution of a hydroxyl group at C-16 of kaurane-type decreases the inhibitory activity of PTP1B. A pimarane-type diterpene, acanthoic acid (**2**), inhibited PTP1B in a dose-dependent manner (IC_{50} = 23.5 ± 1.8 μM), whereas compound **1** converted a carboxyl group at C-19 to a primary alcohol did not exhibit PTP1B inhibitory activity up to 30 μM. This suggests that a carboxyl group at C-19 of pimarane-type is essential for the activity. Other pimarane-type diterpenes, compounds **3** and **4** bearing a hydroxyl group at C-7 and C-9, respectively, had no activity at levels up to 30 μM. A known phosphatase inhibitor, RK-682 (3-hexadecanoyl-5-hydroxymethyl tetronic acid, IC_{50} = 4.5 ± 0.5 μM) isolated from *Streptomyces* sp. 88-682,[16] was used as a positive control in this assay. Although the structure–activity relationships of these compounds were not thoroughly investigated, substitution of a hydroxyl group and reduction of a carboxyl group appeared to reduce the inhibitory activity of PTP1B. To elucidate the inhibition mode of the most active diterpene **7** on the activity of PTP1B, kinetic analysis was performed with different concentrations of substrate.[15] As shown in Figure 2, the mechanism of inhibition by the compound was determined using a Lineweaver–Burk plot. When *p*-nitrophenyl phosphate (*p*NPP) was used as substrate, **7** decreased the V_{max} value, but did not alter the K_m value of PTP1B (Fig. 2). Thus, **7** was determined as a non-competitive inhibitor with a K_i value of 10.4 μM, indicating that it may bind to the enzyme–substrate complex or interact with an allosteric site distinct from the active site of PTP1B.[17]

Table 1. The inhibitory activity of the compounds **1**–**8** isolated from *Acanthopanax koreanum* against PTP1B

Compounds	PTP1B inhibitory activity IC_{50}[a] (μM)
Acanthol (**1**)	>30
Acanthoic acid (**2**)	23.5 ± 1.8
7β-Hydroxy-*ent*-pimara-8(14),15-dien-19-oic acid (**3**)	>30
Acanthokoreoic acid A (**4**)	>30
ent-Kaur-16-en-19-oic acid (**5**)	20.2 ± 1.3
16α-Hydroxy-*ent*-kauran-19-oic acid (**6**)	>30
16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**)	7.1 ± 0.9
16α-Hydroxy-17-isovaleryloxy-*ent*-kauran-19-oic acid (**8**)	>30
RK-682[b]	4.5 ± 0.5

[a] IC_{50} values were determined by regression analyses and expressed as means ± SD of three replicates.
[b] Positive control.[16]

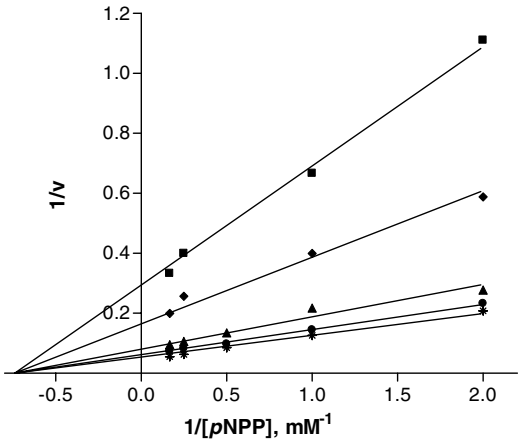

Figure 2. A Lineweaver–Burk plot of the inhibitory effect of compound **7** on PTP1B-catalyzed hydrolysis of *p*NPP. Data are expressed as mean initial velocity for n = 3 replicates at each substrate concentration. Symbols: (∗) 0 μM, (●) 4 μM, (▲) 6 μM, (♦) 10 μM, and (■) 12 μM compound **7**.

Pimarane-type and kaurane-type diterpenes have been reported to reduce nitric oxide and prostaglandin E_2 production,[18] to suppress IL-1, IL-8, and TNF-α production,[8,19] and to inhibit NFAT transcription factor.[12] Besides, these diterpenes have been known to possess a wide range of biological activities that include analgesic, anti-inflammatory, antibacterial, cytotoxic, and hepatoprotective activities.[20,21] However, to our knowledge, PTP1B inhibitory activity of diterpenoids is now being reported for the first time in this study. Binding of insulin to the extracellular α-subunit of IR triggers a conformational change that activates the intrinsic tyrosine kinase activity of the β-subunit via autophosphorylation of specific tyrosine residues. This results in the phosphorylation of IRS 1–4, which then activates several signaling cascade that biological response, such as glucose transport into the cell and glycogen synthesis.[1,2] Since PTP1B acts as a negative regulator by dephosphorylating the IR as well as IRS proteins, its inhibitors could be potential agents for the treatment of type 2 diabetes. Interestingly, the genus *Acanthopanax* has been used to treat diabetes, and a recent study has demonstrated that this genus has the ability to improve insulin sensitivity in rats induced by fructose-rich chow feeding and to reduce plasma glucose levels in animal model.[22] Although the antihyperglycemic activity of isolated diterpenoids in diabetes-related animal models has not been directly evaluated yet, our results suggest that the PTP1B inhibitory activity of diterpene constituents might be related with the anti-diabetic effect of this plant.

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References and notes

1. Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discov.* **2002**, *1*, 696.
2. Asante-Appiah, E.; Kennedy, B. P. *Am. J. Physiol.* **2003**, *284*, E663.
3. Ahmad, F.; Azevedo, J. J.; Cortright, R.; Dohm, G.; Goldstein, B. *J. Clin. Invest.* **1997**, *100*, 449.
4. Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544.
5. Taylor, S. D.; Hill, B. *Expert Opin. Investig. Drugs* **2004**, *13*, 199.
6. Kim, Y. C.; Oh, H.; Kim, B. S.; Kang, T. H.; Ko, E. K.; Han, Y. M.; Kim, B. Y.; Ahn, J. S. *Planta Med.* **2005**, *71*, 87.
7. Bae, K. *The Medicinal Plants of Korea*; Kyo-Hak Publishing: Seoul, 2000, pp 361.
8. Cai, X. F.; Shen, G.; Dat, N. T.; Kang, O. H.; Kim, J. A.; Lee, Y. M.; Lee, J. J.; Kim, Y. H. *Chem. Pharm. Bull.* **2003**, *51*, 605.
9. Cai, X. F.; Shen, G.; Dat, N. T.; Kang, O. H.; Lee, Y. M.; Lee, J. J.; Kim, Y. H. *Arch. Pharm. Res.* **2003**, *26*, 731.
10. Cai, X. F.; Lee, I. S.; Dat, N. T.; Shen, G.; Kang, J. S.; Kim, D. H.; Kim, Y. H. *Arch. Pharm. Res.* **2004**, *27*, 738.
11. Cai, X. F.; Lee, I. S.; Shen, G.; Dat, N. T.; Lee, J. J.; Kim, Y. H. *Arch. Pharm. Res.* **2004**, *27*, 825.
12. Cai, X. F.; Lee, I. S.; Dat, N. T.; Shen, G.; Kim, Y. H. *Phytother. Res.* **2004**, *18*, 677.
13. (a) Acanthol (**1**): white needle; mp 73–74 °C; $[\alpha]_D$ −15° (*c* 0.2, $CHCl_3$); EIMS *m/z* (%): 288 $[M]^+$ (1.4), 273 $[M-CH_3]^+$ (5), 257 $[M-CH_2OH]^+$ (10), 241 (17), 220 $[M-C_5H_8]^+$ (26), 189 $[M-C_5H_8-CH_2OH]^+$ (42), 173 (18), 119 (58); ^1H NMR (300 MHz, $CDCl_3$) δ: 0.90 (3H, s, H-18), 0.91 (3H, s, H-17), 0.97 (3H, s, H-20), 4.79 (1H, dd, J = 10.6, 1.3 Hz, H-16), 4.86 (1H, dd, J = 17.5, 1.3 Hz, H-16), 5.29 (1H, m, H-11), 5.74 (1H, dd, J = 17.5, 10.6 Hz, H-15); (b) acanthoic acid (**2**): white powder; mp 135–136 °C; $[\alpha]_D$ −55° (*c* 1.0, MeOH); EIMS *m/z* (%): 302 $[M]^+$ (17), 287 $[M-CH_3]^+$ (32), 241 (40), 234 (34), 219 (12), 201 (10), 189 (50), 173 (44); ^1H NMR (300 MHz, CD_3OD) δ: 0.98 (3H, s, H-20), 1.06 (3H, s, H-17), 1.20 (3H, s, H-18), 4.86 (1H, dd, J = 10.8, 1.5 Hz, H-16), 4.94 (1H, dd, J = 17.4, 1.5 Hz, H-16), 5.43 (1H, m, H-11), 5.83 (1H, dd, J = 17.4, 10.8 Hz, H-15); (c) 7β-hydroxy-*ent*-pimara-8(14),15-dien-19-oic acid (**3**): white powder; mp 214–216 °C; FABMS *m/z*: 341 $[M+Na]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.64 (3H, s, H-20), 1.05 (3H, s, H-17), 1.28 (3H, s, H-18), 4.28 (1H, s, H-7), 4.80 (1H, d, J = 17.3 Hz, H-16), 4.97 (1H, d, J = 10.3 Hz, H-16), 5.46 (1H, s, H-14), 5.69 (1H, dd, J = 17.3, 10.3 Hz, H-15); (d) acanthokoreoic acid A (**4**): white powder; mp 60–62 °C; $[\alpha]_D$ +3.5° (*c* 1.0, MeOH); FABMS *m/z*: 335 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.12 (3H, s, H-20), 1.22 (3H, s, H-18), 1.24 (3H, s, H-17), 4.38 (1H, br d, J = 3.1 Hz, H-8), 4.82 (1H, dd, J = 10.6, 1.1 Hz, H-16), 4.93 (1H, dd, J = 17.3, 1.1 Hz, H-16), 5.95 (1H, dd, J = 17.3, 10.6 Hz, H-15); (e) *ent*-kaur-16-en-19-oic acid (**5**): white powder; mp 162–163 °C; $[\alpha]_D$ −104° (*c* 1.0, MeOH); EIMS *m/z* (%): 302 $[M]^+$ (77), 287 $[M-CH_3]^+$ (35), 259 (43); ^1H NMR (300 MHz, CD_3OD) δ: 1.00 (3H, s, H-20), 1.19 (3H, s, H-18), 2.62 (1H, m, H-13), 4.78 (2H, m, H-17); (f) 16α-hydroxy-*ent*-kauran-19-oic acid (**6**): white powder; mp 254–255 °C; FABMS *m/z*: 343 $[M+Na]^+$; ^1H NMR (300 MHz, CD_3OD)δ: 0.97 (3H, s, H-20), 1.16 (3H, s, H-17), 1.32 (3H, s, H-18); (g) 16αH,17-isovaleryloxy-*ent*-kauran-19-oic acid (**7**): white powder; mp 148–150 °C; FABMS *m/z*: 405 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, s, H-20), 0.88 (6H, d, J = 6.6 Hz, H-4′, 5′), 1.16 (3H, s, H-18), 2.09 (1H, m, H-16), 3.84 (2H, m, H-17); (h) 16α-hydroxy-17-isovaleryloxy-*ent*-kauran-19-oic acid (**8**): white powder; mp 185–187 °C; $[\alpha]_D$ −70° (*c* 1.0, MeOH); FABMS *m/z*: 443 $[M+Na]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.94 (3H, s, H-20), 0.96 (6H, d, J = 6.6 Hz, H-4′, 5′), 1.22 (3H, s, H-18), 4.23 (2H, dd, J = 11.4, 15.3 Hz, H-17).
14. Cui, L.; Na, M.; Oh, H.; Bae, E. Y.; Jeong, D. G.; Ryu, S. E.; Kim, S.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1426.
15. To each 96 well (final volume: 100 μl) were added five different concentrations of *p*NPP (0.5, 1.0, 2.0, 4.0, and 8.0 mM) and PTP1B (0.05–0.1 μg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without test compound. Following incubation at 30 °C for 20 min, the reaction was terminated with 10 N NaOH. The amount of produced *p*-nitro phenol was estimated by measuring the absorbance at 405 nm. The non-enzymatic hydrolysis of *p*NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. The Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) of PTP1B were determined by the Lineweaver–Burk plot using a GraphPad Prism® 4 program (GraphPad Software Inc., USA).
16. Hamaguchi, T.; Sudo, T.; Osada, H. *FEBS Lett.* **1995**, *372*, 54.
17. Wiesmann, C.; Barr, K. J.; Kung, J.; Zhu, J.; Erlanson, D. A.; Shen, W.; Fahr, B. J.; Zhong, M.; Taylor, L.; Randal, M.; McDowell, R. S.; Hansen, S. K. *Nat. Struc. Mol. Biol.* **2004**, *11*, 730.
18. Hwang, B. Y.; Lee, J. H.; Koo, T. H.; Kim, H. S.; Hong, Y. S.; Ro, J. S.; Lee, K. S.; Lee, J. J. *Planta Med.* **2001**, *67*, 406.
19. Kang, H. S.; Kim, Y. H.; Lee, C. S.; Lee, J. J.; Choi, I.; Pyun, K. H. *Cell Immunol.* **1996**, *170*, 212.
20. Hanson, J. R. *Nat. Prod. Rep.* **2003**, *20*, 70.
21. Park, E. J.; Zhao, Y. Z.; Kim, Y. H.; Lee, J. J.; Sohn, D. H. *Planta Med.* **2004**, *70*, 321.
22. Liu, T. P.; Lee, C. S.; Liou, S. S.; Liu, I. M.; Cheng, J. T. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32*, 649.