Taxane Diterpenoids from Taiwanese Yew *Taxus sumatrana*

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Thirty seven taxanes were characterized from the leaves and twigs of Taiwanese yew (*Taxus sumatrana*, Taxaceae). Four of these metabolites are new and designated as sumataxins A – D (**1** – **4**). Compound **1** possesses an 11(15 → 1),11(10 → 9)-di-*abeo*-taxane skeleton with an unusual spiro-connected 2,2-dimethyl-1,3-dioxolane ring at C(4), whereas compound **2** has a rare *β*-OH orientation at C(13) of taxane diterpene ester. In addition, sumataxin C (**3**) is formulated as an 11(15 → 1)-*abeo*-taxane with a 4,5-acetonide ring skeleton. Compound **4** is the first metabolite with a 4,20-epoxy-taxane structure. The structures of all the taxanes were established by spectroscopic methods. All compounds were evaluated for anti-HSV-1 and PBMC activities. Compound **9** exhibited significant enhancement of cell proliferation on peripheral blood mononuclear cells.

Introduction. – The discovery of the potent anticancer drug paclitaxel demonstrated once more that nature was the primary source for most of the drugs presently on the market [1]. It has been approved for the treatment of advanced ovarian and breast cancers and is currently in clinical trials for the treatment of lung, skin, head, and neck cancers with encouraging results [2]. Its unique chemical structure and novel mechanism of action led to extensive research from different fields [3]. Taxonomically, various plant species, representing a large variety of decorative and evergreen bushes, constitute the genus *Taxus* that belongs to the Taxaceae family. Yew trees are widely distributed in the northern hemisphere, occurring in Europe, North America, and Asia [4]. In the past two decades, species of the genus *Taxus* have previously been under phytochemical investigation due to novel structures and interesting biological activities. As a consequence, more than 500 taxoid metabolites have been discovered from various *Taxus* plants [5]. The isolation of new taxanes might provide important clues in the biosynthesis of paclitaxel, especially for those compounds related to the intermediates of the verticillene pathway. In view of investigations of Formosan yew taxoids from *Taxus mairei*, *T. chinensis* [6], and *T. sumatrana* [7], several bioactive taxoids have been isolated.

Taxus sumatrana (MIQ.) de LAUB. (Taxaceae) is a rare plant growing at a high altitude (2600 m) in central parts of Taiwan [8]. In our continuing search for taxoid

diterpenoids with novel skeletal constitution and bioactivity, we now report the four new taxoids **1**–**4**. Sumataxin A[1]) (**1**) possesses an 11(15→1),11(10→9)-di-*abeo*-taxane skeleton with an unusual spiro-connected 2,2-dimethyl-1,3-dioxolane ring at C(4), whereas sumataxin B[1]) (**2**) has a rare β-OH orientation at C(13) of a taxane diterpene ester (*Fig. 1*). In addition, sumataxin C[1]) (**3**) was formulated as an 11(15→1)-*abeo*-taxane with a 4,5-acetonide ring skeleton. Sumataxin D[1]) (**4**) is the first metabolite with a 4,20-epoxy tricyclic taxane skeleton. Thirty three known taxoids (names as given in the corresponding ref., no formulas shown) were also isolated and identified as taxuyunnanine C (**5**) [5], 5α,7β,9α,10β,13α-pentaacetoxy-4(20),11-taxadiene (**6**) [5], 2α,5α,9α,10β,14β-pentaacetoxytaxa-4(20),11-diene (**7**) [9], 14β-hydroxytaxusin (**8**) [5], 2α-deacetoxytaxinine J (**9**) [5], taxa-4(20),11-diene-2α,5α,7β,9α,10β,13α-hexaol hexaacetate (**10**) [10], 1β-dehydroxy baccatin VI (**11**) [5], 7β,9α,10β,13α,20-pentaacetoxy-2α-benzoyloxy-4α,5α-dihydroxytax-11-ene (**12**) [10], taxacin (**13**) [9], baccatin VI (**14**) [5], taxuspinanane J (**15**) [5], 2-deacetoxy-5-decinnamoyltaxinine J (**16**) [10], *N*-methyl taxol C (**17**) [10], 10-deacetyl yunnanaxane (**18**) [10], taxumairol B (**19**) [5], taxinine M (**20**) [5], baccatin III (**21**) [5], taxuspinanane I (**22**) [5], taxumairol K (**23**) [5], wallifoliol (**24**) [10], 13-oxobaccatin III (**25**) [11], taxol (**26**) [5], 7-*epi*-10-deacetyl taxol (**27**) [11], 10-deacetyl-13-oxo-baccatin III (**28**) [11], 19-hydroxybaccatin III (**29**) [10], 10-deacetyl taxol (**30**) [11], 10-deacetyl-baccatin III (**31**) [10], 13-acetyl-13-decinamoyltaxachinin B (**32**) [12], 5-deacetyltaxachitriene B (**33**) [10], 5-*epi*-canadensene (**34**) [11], taxezopidine F (**35**) [13], 3α,7β-diacetoxy-2α,5α,10β-trihydroxy-9-keto-2(3→20)*abeo*taxane (**36**) [14], and 2-deacetyl taxine B (**37**) [15]. We have rigorously elucidated their chemical structures by 1D- (^{1}H- and ^{13}C-NMR) and 2D-NMR (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY) methods and confirmed them by HR-ESI-MS. The biological activities of

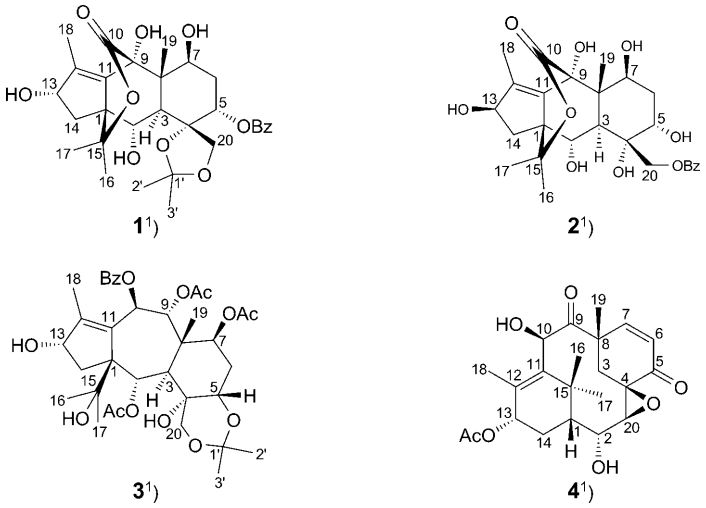

Fig. 1. *Taxanes* **1**–**4** *isolated from Taiwanese yew* Taxus sumatrana

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

compounds **1**–**37** were tested against *Herpes simplex* virus type 1 (HSV-1) and evaluated with peripheral blood mononuclear cell (PBMC) proliferation induced by phytohemagglutinin (PHA).

Results and Discussion. – 1. *Isolation and Structure Elucidation.* An acetone extract of the leaves and young twigs was partitioned between AcOEt and H_2O. The AcOEt-soluble portion was purified by multiple chromatographic separation over normal-phase and reversed-phase silica gel, followed by *Sephadex LH-20*, to afford the four new metabolites **1**–**4** (*Fig. 1*) in addition to the 33 known taxoids.

Sumataxin A (**1**) was obtained as an optically active, amorphous powder and had the molecular formula of $C_{30}H_{38}O_{10}$ based on the HR-ESI-MS (*m/z* 581.2360 ($[M+Na]^+$), suggesting the presence of twelve degrees of unsaturation. The IR spectrum of **1** showed absorption bands diagnostic of OH (3443 cm^{-1}) and ester C=O (1715 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (*Table 1*) indicated the presence

Table 1. *^{1}H-NMR Data* (500 MHz, (D_6)acetone) *of Compounds* **1**–**4**[1]). δ in ppm, *J* in Hz.

	1	**2**	**3**	**4**
H–C(1)				1.73 (*d*, *J* = 8.1)
H–C(2)	4.41 (*dd*, *J* = 4.5, 11.0)	4.43 (*d*, *J* = 11.9)	5.93 (*d*, *J* = 6.5)	3.67 (*d*, *J* = 8.1)
H–C(3) or CH_2(3)	2.98–3.02 (*m*)	2.45 (*d*, *J* = 11.9)	2.99 (*d*, *J* = 6.5)	2.52 (*d*, *J* = 8.1)
H–C(5)	5.32 (br. *s*)	3.78 (br. *s*)	3.72 (*t*, *J* = 2.5)	
CH_2(6) or H–C(6)	2.05–2.10 (*m*)	2.01–2.07 (*m*)	1.64–1.70 (*m*, H_α), 2.17 (*d*, *J* = 3.0, H_β)	6.42 (*d*, *J* = 10.1)
H–C(7)	4.25 (*dd*, *J* = 6.5, 3.5)	4.25 (*dd*, *J* = 10.4, 4.1)	5.55 (*dd*, *J* = 11.5, 4.0)	6.63 (*d*, *J* = 10.1)
H–C(9)			6.33 (*d*, *J* = 11.0)	
H–C(10)			6.54 (*d*, *J* = 11.0)	5.18 (*d*, *J* = 1.1)
H–C(13)	4.65 (*t*, *J* = 6.5)	4.35 (*t*, *J* = 3.3)	4.38–4.43 (*m*)	5.33 (*d*, *J* = 10.5)
CH_2(14)	2.04–2.10 (*m*)	1.96–2.02 (*m*)		
–C(14)			1.89 (*dd*, *J* = 8.5, 6.5, H_α), 2.43 (*dd*, *J* = 15.5, 8.5, H_β)	2.00–2.06 (*m*, H_α), 2.65–2.70 (*m*, H_β)
Me(16)	1.43 (*s*)	1.41 (*s*)	1.22 (*s*)	1.09 (*s*)
Me(17)	1.20 (*s*)	1.12 (*s*)	1.09 (*s*)	1.24 (*s*)
Me(18)	2.09 (*s*)	1.97 (*s*)	2.06 (*s*)	1.59 (*s*)
Me(19)	1.08 (*s*)	1.06 (*s*)	1.52 (*s*)	1.51 (*s*)
H_α–C(20)	4.36 (*d*, *J* = 9.5)	4.50 (*d*, *J* = 12.3)	3.46 (*d*, *J* = 12.5)	3.55 (*d*, *J* = 9.4)
H_β–C(20)	4.17 (*d*, *J* = 9.5)	4.72 (*d*, *J* = 12.3)	4.34 (*d*, *J* = 12.5)	
OH–C(2)	4.89 (*d*, *J* = 4.5)	6.26 (*d*, *J* = 4.5)		
OH–C(10)				4.07 (br. *s*)
H_o (Ph)	8.14 (*d*, *J* = 7.5)	8.13 (*d*, *J* = 7.5)	7.85 (*d*, *J* = 7.5)	
H_m (Ph)	7.56 (*d*, *J* = 7.5)	7.51 (*d*, *J* = 7.5)	7.42 (*d*, *J* = 7.5)	
H_p (Ph)	7.64 (*t*, *J* = 7.5)	7.63 (*t*, *J* = 7.5)	7.55 (*t*, *J* = 7.5)	
Me(2′)	1.38 (*s*)		1.44 (*s*)	
Me(3′)	1.32 (*s*)		1.43 (*s*)	
AcO–C(2)			2.08 (*s*)	
AcO–C(7)			2.02 (*s*)	
AcO–C(9)			1.69 (*s*)	
AcO–C(13)				2.08 (*s*)

of a benzoate moiety at δ(H) 8.14, 7.56 and 7.64, along with four quaternary Me groups with signals at δ(H) 1.43 (Me(16)), 1.20 (Me(17)), 2.09 (Me(18)), and 1.08 (Me(19)). Analysis of the ^{13}C-NMR (*Table 2*), DEPT, and HMQC spectra revealed that **1** contains 30 C-atom signals corresponding to five sp^3 CH, five sp^2 CH, two sp^3 CH_2, one CH_2–O at δ(C) 86.4 (C(20)), six Me, and eleven quaternary C-atoms, including five sp^2, two C-atoms of a tetrasubstituted C=C bond, two sp^3 and four O-bearing C-atoms (δ(C) 86.4, 84.6, 89.7, and 109.3). The signals at δ(C) 86.4 and 109.3 arise from the spiro atom C(4) and the dimethyl-substituted C-atom of the 1,3-dioxolane ring at C(4). Considering the twelve degrees of unsaturation, seven are left after subtracting the benzoate moiety, compatible with a tetracyclic diterpene a C=C bond (δ(C) 131.0 and 133.0 (2*s*)), and a C=O (δ(C) 173.7); the remaining one should arise from the 1,3-

Table 2. *^{13}C-NMR Data* (125 MHz, (D_6)acetone) *of Compounds* **1**–**4**[a]). δ in ppm.

	1	**2**	**3**	**4**
C(1)	62.6 (*s*)	61.1 (*s*)	67.2 (*s*)	48.0 (*d*)
C(2)	68.7 (*d*)	68.2 (*d*)	71.5 (*d*)	69.1 (*d*)
C(3)	42.5 (*d*)	44.2 (d)	40.6 (*d*)	34.4 (*t*)
C(4)	86.4 (*s*)	76.4 (*s*)	68.6 (*s*)	60.9 (*s*)
C(5)	73.0 (*d*)	68.2 (*d*)	73.8 (*d*)	192.2 (*s*)
C(6)	33.9 (*t*)	35.9 (*t*)	29.8 (*t*)	130.8 (*d*)
C(7)	68.9 (*d*)	68.0 (*d*)	68.4 (*d*)	149.3 (*d*)
C(8)	49.7 (*s*)	47.4 (*s*)	44.2 (*d*)	49.5 (*s*)
C(9)	84.6 (*s*)	84.4 (*s*)	76.6 (*d*)	211.0 (*s*)
C(10)	173.7 (*s*)	173.3 (*s*)	69.0 (*d*)	78.9 (*d*)
C(11)	131.0 (*s*)	130.2 (*s*)	134.9 (*s*)	136.0 (*s*)
C(12)	133.0 (*s*)	132.1 (*s*)	150.6 (*s*)	136.2 (*s*)
C(13)	79.4 (*d*)	78.7 (*d*)	77.5 (*d*)	68.1 (*d*)
C(14)	36.6 (*t*)	35.0 (*t*)	41.1 (*t*)	26.0 (*t*)
C(15)	89.7 (*s*)	88.6 (*s*)	75.9 (*s*)	37.2 (*s*)
C(16)	23.4 (*q*)	22.8 (*q*)	25.5 (*q*)	24.9 (*q*)
C(17)	25.3 (*q*)	24.9 (*q*)	27.4 (*q*)	33.8 (*q*)
C(18)	11.2 (*q*)	11.0 (*q*)	12.1 (*q*)	17.4 (*q*)
C(19)	11.4 (*q*)	11.6 (*q*)	14.2 (*q*)	29.1 (*q*)
C(20)	86.4 (*t*)	67.2 (*t*)	67.1 (*s*)	69.0 (*d*)
Ph*C*O	165.6 (*s*)	166.2 (*s*)	163.9 (*s*)	
C_{ipso}	139.3 (*s*)	138.5 (*s*)	129.1 (*s*)	
C_o	130.8 (*d*)	129.7 (*d*)	129.4 (*d*)	
C_m	129.7 (*d*)	128.8 (*d*)	128.7 (*d*)	
C_p	134.1 (*d*)	133.4 (*d*)	133.3 (*d*)	
*Ac*O–C(2)			172.6 (*s*), 21.9 (*q*)	
*Ac*O–C(7)			169.2 (*s*), 21.5 (*q*)	
*Ac*O–C(9)			169.9 (*s*), 20.6 (*s*)	
*Ac*O–C(13)				170.2 (*s*), 21.0 (*q*)
C(1′)	109.3 (*s*)		98.8 (*s*)	
C(2′)	26.3 (*q*)		18.4 (*q*)	
C(3′)	28.5 (*q*)		29.4 (*q*)	

[a]) Assignments were supported by HMBC and DEPT techniques.

dioxolane ring at C(4). The above mentioned data were in agreement with a 11(15 → 1),11(10 → 9)-bis-*abeo*-taxane skeleton [16]. The gross structure of **1** was deduced from extensive analyses of the 2D-NMR data, including the ^{1}H,^{1}H-COSY, HMQC, and HMBC. Detailed analysis of the COSY allowed us to distinguish the coupling spin systems of the primary CH_2O and secondary CH−O. The H-atom at δ(H) 4.41 (*dd*, J = 11.0, 4.5 Hz, H−C(2)) coupled to the signal at δ(H) 2.98–3.02 (*m*, H−C(3)) and the OH group appeared as *d* at δ(H) 4.89 (*d*, J = 4.5 Hz, OH−C(2)). The signal at δ(H) 4.65 (*t*, J = 6.5 Hz, H−C(13)) coupled to that at δ(H) 2.04–2.10 (*m*, CH_2(14)). In the COSY plot, a coupling between the geminal H-atoms of the CH_2−O group was observed (δ(H) 4.36 (*d*, J = 9.5 Hz, H_α−C(20)) and 4.17 (*d*, J = 9.5 Hz, H_β−C(20))). In addition, the *dd* at 4.25 (H−C(7)) showed an ^{1}H,^{1}H-COSY with the *m* at δ(H) 2.05–2.10 (CH_2(6)). A complete assignment of the H- and C-atoms was supported by a HMBC experiment. The low-field signal of C(4) (δ(C) 86.4) as well as the relatively small coupling constant between the geminal H-atoms of CH_2(20) (9.5 Hz) in comparison with related compounds (>11 Hz) suggested a different substitution pattern at C(4). The HMBC spectrum revealed that both H-atoms of CH_2(20) (δ(H) 4.36 and 4.17) showed ^3J-correlations with an extremely downfield-shifted aliphatic C-atom at δ(C) 109.3 (C(1′)) and with C(3) at δ(C) 42.5 and C(5) at δ(C) 73.0, suggesting the presence of an unusual heterocyclic ring. The downfield shift of C(1′) was ascribed to its attachment to two O-atoms [16]. The proposed 2,2-dimethyl-1,3-dioxolane ring at C(4) was further supported by its ^2J correlation of both downfield Me at δ(H) 1.38 (Me(2′)) and 1.32 (Me(3′)) with the extremely downfield-shifted C(1′). The absence of signals assignable to H−C(9) and H−C(10), and the downfield shift of the quaternary C(15) (δ(C) 89.7) suggested its unusual oxygenation and its correlations in the HMBC spectrum to Me(16), Me(17), H_β−C(2), and CH_2(14), along with a ^2J-correlation of Me(16) and Me(17) with C(15) confirmed the presence of an 11(15 → 1),11(10 → 9)-di-*abeo*-taxane skeleton. Another quaternary O-bearing C-atom at δ(C) 84.6 was assigned to C(9) on the basis of its ^3J-correlation with Me(19). To account for the twelve degrees of unsaturation and the unusual deshielding of C(9) and C(15), we propose that C(15), C(1), C(11), C(9), and C(10) are involved in a 10,15-lactone ring allowing the possible assignment of the carboxy C=O at δ(C) 173.7 to C(10). This was supported by the absence of HMBCs with the C=O implying its attachment to quaternary C-atoms at both ends, as well as by comparison of the data of **1** with the corresponding data of wallifoliol and its analogues [16]. The relative configuration of **1** is proposed on a biogenetic basis, by comparison with tasumatrol S [16] and by inspection of the NOESY data (*Fig. 2*), which showed the correlations Me(19)/H−C(2), H_β−C(6), and CH_2(20), CH_2(20)/H−C(2), H−C(5), Me(19), Me(16)/H−C(2) and Me(16), and

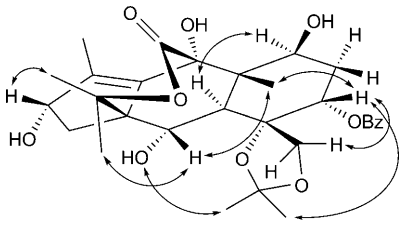

Fig. 2. *Key NOESY correlations of* **1**

H–C(3)/H–C(7). These data are in accordance with the β-orientation of H–C(2), H–C(5), and CH_2(20) and the α-orientation of H–C(7). Thus the structure of sumataxin A was established as **1**.

Sumataxin B (**2**) has the molecular formula $C_{27}H_{34}O_{10}$ as deduced from its HR-ESI-MS (*m/z* 541.2053 ($[M+Na]^+$)). The MS, together with the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) established the presence of an 11(15 → 1),11(10 → 9)di-*abeo*-taxane skeleton in **2** as in **1** and tasumatrol T [16], with a six-membered lactone and a PhCOO moiety but with significant differences in the chemical shift values of C(4), C(5), and C(13) compared to **1** and those of C(2), C(13), and C(14) compared to tasumatrol T [16]. The former were attributed to the absence in **2** of a 2,2-dimethyl-1,3-dioxolane ring at C(4) and a different orientation of OH–C(13) in comparison with **1**. The DEPT experiment indicated the presence of two non-O-bearing CH_2 groups instead of three in case of tasumatrol T, which was associated with the appearance of the correlated ^{1}H- and ^{13}C-NMR signals of one CH–O group at δ(H) 4.43 (H–C(2)) and δ(C) 68.2 (C(2)). ^3J-Correlations between H–C(2) and the two quaternary C–O groups at δ(C) 76.4 (C(4)) and 88.6 (C(15)) confirmed oxygenation of C(2). The large J(2,3) value (11.9 Hz) indicated the a *trans*-diaxial arrangement of H–C(2) and H–C(3). In the ^{1}H-NMR spectrum of **2**, the *t* characteristic of H–C(13) at δ(H) 4.35 was shifted upfield by comparison to **1** (resonating at δ(H) 4.65), although there was no significant change in the respective ^{13}C-NMR spectra. H–C(13) showed a NOESY (*Fig. 3*) correlation with OH–C(2) which was resonating at δ(H) 6.26 (*d*, $J=4.5$). To the best of our knowledge, the only example with a β-substitution at C(13) reported so far is 13-*epi*-10-deacetylbaccatin III, which was isolated from the needles of *T. baccata*. Recently, it has been reported that (2α,5α,7β,10β,13β)-2,7-bis(acetyloxy)-5,10,13-trihydroxy-2(3 → 20)-*abeo*-taxa-4(20),11-dien-9-one is a new taxane with a 6/8/6-ring system with OH–C(13) in β-orientation [17]. Sumataxin B is the first example of a taxane with the 5/6/6-ring system with OH–C(13) in β-orientation.

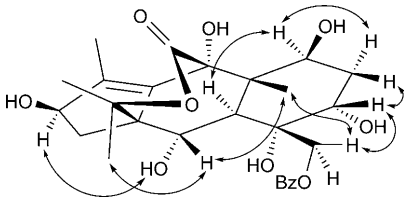

Fig. 3. *Key NOESY correlations of* **2**

Sumataxin C (**3**) had the composition $C_{36}H_{48}O_{13}$ as derived from a combination of HR-ESI-MS and DEPT spectroscopy. Its UV and IR bands indicated the presence of PhCO (228.8 nm), OH (3443 cm^{-1}), and AcO groups (1730 cm^{-1}). The presence of one PhCO and three AcO groups as well as of three OH groups were confirmed by the ^{1}H- and ^{13}C-NMR data of **3** (*Tables 1* and *2*). A taxene skeleton was inferred from the observation of characteristic resonances, such as four Me *s* (δ(H) 1.09 (Me(17)), 1.22 (Me(16)), 1.52 (Me(19)), and 2.06 (Me(18))) and the corresponding ^{13}C-NMR signals [18]. Both the ^{1}H- and ^{13}C-NMR data indicated the presence of three AcO units at δ(H) 1.69, 2.02, and 2.08 (3*s*), δ(C) 20.6, 21.5, and 21.9, and δ(C) 169.9, 169.2, and 172.6. A benzoyl ester was evident from signals resonating at δ(C) 163.9, 129.1, 129.4,

128.7, and 133.3 and δ(H) 7.85 (*d*, $J=7.5$ Hz), 7.42 (*t*, $J=7.5$ Hz), and 7.55 (*d*, $J=7.5$ Hz). The ^{1}H-NMR spectrum revealed six CH−O groups at δ(H) 3.72 (*t*, $J=2.5$ Hz, H−C(5)), 6.54 (*d*, $J=11.0$ Hz, H−C(10)), 4.38–4.43 (*m*, H−C(13)), 5.93 (*d*, $J=6.5$ Hz, H−C(2)), 5.55 (*dd*, $J=11.5$, 4.0 Hz, H−C(7)), and 6.33 (*d*, $J=11.0$ Hz, H−C(9)). The relatively low-field chemical shifts of the latter three H-atoms, (H−C(2)), H−C(7)), and H−C(9), implied their acylation, corroborated by the expected HMBCs. The COSY experiment displayed the connectivities H−C(13)/CH_2(14), H−C(2)/H−C(3), H−C(5)/CH_2(6)/H−C(7), and H−C(9)/H−C(10). The H-atom at δ(H) 3.72 (*t*, $J=2.5$ Hz), assigned to H−C(5), was attached to a C-atom resonating at δ(C) 73.8 (C(5)) which correlated with CH_2(20), confirming oxygenation at C(5). The ^2J-correlations between CH_2(20) and the quaternary C-atom at δ(C) 68.6 (C(4)) established the oxygenation at C(4). On the other hand, the Me group at δ(H) 1.43 (Me(3′)) showed correlations with an acetal C-atom at δ(C) 98.8 (C(1′)) and a Me group at δ(C) 29.4 (C(2′)). This suggested that the three C-atoms at δ(C) 18.4, 29.4, and 98.8 are involved in an acetonide moiety at C(5)−C(20) which was confirmed by the HR-ESI-MS (*m*/*z* 711.2996), and by comparison with published data [16]. The two quaternary C-atoms at δ(C) 67.2 (C(1)) and 75.9 (C(15)) are characteristic of an 11(15 → 1)-*abeo*-taxane skeleton [19]. The relative configuration of **3** was determined by comparing ^{1}H-NMR data as well as by its NOESY experiment (*Fig. 4*). The NOESY correlations Me(19)/H−C(2), H−C(5), Me(16), and CH_2(20), H−C(2)/Me(16), Me(16)/H−C(13), and H_α−C(3)/H−C(7) and Me(2′), together with the coupling constants of H−C(2), H−C(3), and H−C(5) confirmed the relative configuration of **3**. Comparison of the optical rotation of **3** with related taxanes [18] is suggestive of the absolute configuration shown by the formula. Hence, the structure of **3** was established as sumataxin C (**3**).

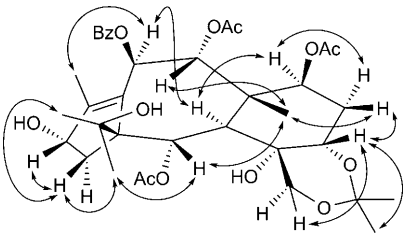

Fig. 4. *Key NOESY correlations of* **3**

Sumataxin D (**4**) was obtained as a colorless amorphous powder. Its IR spectrum showed the presence of OH (3466 cm^{-1}), ester (1733 cm^{-1}), C=O (1720 cm^{-1}), and α,β-unsaturated C=O groups (1685 cm^{-1}). Its molecular formula was established as $C_{22}H_{28}O_7$ from HR-ESI-MS (*m*/*z* 427.1730) and DEPT spectra. The ^{1}H-NMR spectrum of **4** revealed four distinctive quaternary Me *s* of a taxane skeleton, detected in the upfield region (δ(H) 1.51 Me(19), 1.24 Me(17), 1.09 Me(16)), an Ac group δ(H) 2.08 (AcO−C(13)), along with four CH−O groups (δ(H) 3.67, 5.18, 5.33, and 3.55), and two *AB*-system olefinic H-atoms (δ(H) 6.42 and 6.63). The ^{13}C-NMR and DEPT data indicated the presence of a ketone C=O (δ(C) 211.0 (C(9))), an α,β-unsaturated 6-membered-ring ketone (δ(C) 192.2 (C(5))), an acetate moiety (δ(C) 170.2 and 21.0

(AcO–C(13))), a tetrasubstituted olefin (δ(C) 136.0 (C(11)) and 136.2 (C(12))), a disubstituted olefin (δ(C) 130.8 (C(6)) and 149.3 (C(7))), CH–O moieties C-atoms (δ(C) 69.1 (C(2)), 78.9 (C(10)), 68.1 (C(13)), and 69.0 (C(20))), as well as an aliphatic CH (δ(C) 48.0 (C(1))), a quaternary C–O (δ(C) 60.9 (C(4))), an aliphatic quaternary C-atom (δ(C) 37.2 (C(15))), along with two CH_2 (δ(C) 34.4 (C(3)) and 26.0 (C(14))), and four Me (δ(C) 24.9 (C(16)), 33.8 (C(17)), 17.4 (C(18)), and 29.1 (C(19))), which were supportive of a tricyclic taxane structure. The NMR data suggested the presence of a 2(3 → 20)-*abeo*-taxane having a 6/10/6-ring system [7], which was supported by the COSY data. Analysis of the COSY plot revealed connectivities of C(14) to C(1), C(13), C(1) to C(2), C(2) to C(20), and C(6) to C(7), based on their geminal coupling of H_α–C(14) and H_β–C(14), their coupling to H_β–C(13), and the coupling of the latter with H–C(1) at δ(H) 1.73. Careful inspection of the ^{1}H- and ^{13}C-NMR spectra revealed the absence of a H–C(9), but a narrow *d* was detected at δ(H) 5.18 for H–C(10), suggesting the presence of a C(9)=O and an OH–C(10). This was supported by the ^2J-HMBC correlation H–C(10)/C(9)=O (δ(C) 211.0) and the tetrasubstituted olefinic C(11) (δ(C) 136.0). Moreover, no *AX* system of an endocyclic C=C bond (expected at δ(H) 4.8–6.5) was observed in the ^{1}H-NMR specrum, nor a trisubstitued C=C bond (expected at δ(C) 128–137) in the ^{13}C-NMR spectrum. Instead, an isolated *AX* spin system at δ(H) 3.67 (H–C(2)) with a coupling constant J = 8.1 Hz and C-atom signals at δ(C) 60.9 (C(4)) and 69.0 (C(20)) were observed. This implied that a potential C(20)=C(4) bond was converted into a 20,4 epoxy function. This assumption was supported by correlations of CH_2(3) with C(4) and C(20), as well as the ^3J-correlation of H–C(6) with C(4). Comparison the above data with (2α,10β,13α)-2,13-bis(acetyloxy)-10-hydroxy-2(3 → 20)-*abeo*-taxa-4(20),6,11-triene-5,9-dione previously isolated from *Taxus mairei* [19], suggested that compound **4** has a 4,20-epoxy moiety. The coupling constants and the NOESY data (*Fig. 5*) enabled us to establish the configuration of the H-atoms of taxane **4** as H_β–C(2), H_α–C(10), H_β–C(13), and H_α–C(20), and implied that rings *A* and *B* adopt boat conformations. This was in accordance with the conformation of taxines. Compound **4** is the first metabolite with a 4,20-epoxy moiety in a 6/10/6-ring taxane.

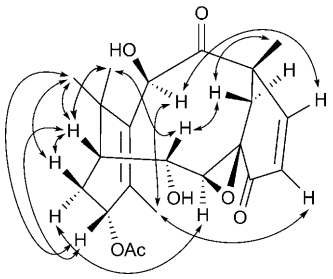

Fig. 5. *Key NOESY correlations of* **4**

In this investigation, the four new taxanes **1**–**4** were isolated from the Taiwanese *T. sumatrana*. Sumataxin A (**1**) and B (**2**) belong to a 11(15 → 1),11(10 → 9)-di-*abeo*-taxane series with a spiro-connected 2,2-dimethyl-1,3-dioxolane ring at C(4) in the case of **1**. Sumataxin C (**3**) is a 11(15 → 1)-*abeo*-taxane with a 5,20-acetonide moiety.

Compound **2** has a rare β-orientation of OH–C(13) and is a taxane diterpene ester, while sumataxin D (**4**) is a 6/10/6-ring taxane with a 4,20-epoxy moiety. Compounds **1** and **3** might be artifacts of isolation, arising from the corresponding diols and the acetone used for the extraction.

The isolated diterpenes **1**, **3**, **4**, **11**, **13**, **16**, **17**, **21**, **23**, **25**, **27**, **28**, **30**–**33**, and **35**–**37** were tested *in vitro* against HSV-1 virus (*Table 3*); compounds **1**, **3**, **4**, **11**, **13**, and **27** exhibited weak activity when compared with acyclovia. A preliminary study on resting cells and cells activated with phytohemagglutinin (PHA) was performed with compounds **1**–**37** at 100 μM (*Table 4*). The inhibition or enhancement of cell proliferation were determined by tritiated thymidine uptake. Compound **9** showed significant enhancement of cell proliferation on peripheral blood mononuclear cells, while compounds **5**, **7**, **12**, **13**, **18**, **26**, and **27** exhibited inhibition of peripheral blood mononuclear cells (PBMC) proliferation induced by phytohemagglutinin (PHA).

Table 3. *Anti-HSV-1 Activity of the Isolated Taxoids*[a])

Drug [100 μM]	Inhibitory activity [%]	Drug [100 μM]	Inhibitory activity [%]
1	53.9 ± 1.1	**25**	38.0 ± 2.1 (light cell lysis)
3	54.9 ± 6.1 (light cell lysis)	**27**	57.4 ± 3.2 (medium cell lysis)
4	55.7 ± 11.4	**28**	37.0 ± 0
11	100 ± 0 (heavy cell lysis)	**30**	31.2 ± 2.5
13	100 ± 0 (heavy cell lysis)	**31**	40.1 ± 5.7 (medium cell lysis)
16	28.5 ± 20.0 (light cell lysis)	**32**	34.5 ± 2.9
17	10.6 ± 1.8 (medium cell lysis)	**33**	36.8 ± 1.1
21	16.6 ± 3.2	**35**	21.2 ± 1.1
23	20.4 ± 2.5 (light cell lysis)	**36**	42.6 ± 0.7
		37	34.3 ± 2.9 (medium cell lysis)
		Acyclovir [2.5 μM]	100 ± 0

[a]) HSV-1: Herpes simplex virus type 1.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; *Merck*) and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Uppsala, Sweden); flash chromatography (= FC). Prep. TLC: precoated SiO_2 plates (SiO_2 *60 F-254*, 1 mm; *Merck*). Optical rotations: *Jasco-DIP-1000* polarimeter. UV Spectra: *Hitachi-U-3210* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi-T-2001* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY Experiments: *Bruker-FT-300* spectrometer or *Varian-Unity-Inova-500* FT-NMR spectrometers; at 500 (^1H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, J in Hz. Low-resolution ESI-MS and FAB-MS: *VG-Quattro-5022* mass spectrometer; in m/z (rel. %). HR-ESI-MS: *Jeol-HX-110* mass spectrometer; in m/z (rel. %).

Plant Material. *Taxus sumatrana* (MIQ.) de LAUB. was collected from Kaohsiung county, Taiwan, at an altitude of 1000 m in March 2002. A voucher specimen (TPG 8-7) has been deposited with the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The dried leaves and twigs (15.5 kg) were ground and extracted three times with acetone at r.t. The combined extracts were filtered and concentrated to obtain a crude extract

Table 4. *Anti-PBMC Activity of the Isolated Taxoids*[a])

Drug [100 μM]	Enhancement activity [%]		
	Resting	PHA [0.5 μg/ml]	PHA [5 μg/ml]
1	– 15.3 ± 2.3	– 74.9 ± 7.3	– 39.7 ± 6.4
2	11.38 ± 7.3	14.2 ± 14.6	5.5 ± 2.8
3	– 34.3 ± 6.1	– 86.9 ± 2.4	– 63.5 ± 3.2
4	9.6 ± 5.5	– 72.4 ± 7.3	– 41.1 ± 2.6
5	– 53.4 ± 6.2	– 75.7 ± 8.5	– 92.0 ± 1.8
6	– 43.3 ± 14.0	– 74.7 ± 1.6	– 76.4 ± 4.4
7	– 77.7 ± 3.2	– 90.7 ± 4.5	– 93.4 ± 1.7
8	– 35.6 ± 2.0	– 54.7 ± 4.1	– 13.4 ± 3.8
9	165.7 ± 22.7	36.8 ± 7.3	107.5 ± 11.6
10	50.7 ± 6.6	– 48.4 ± 7.6	– 41.1 ± 3.3
11	– 8.9 ± 2.2	– 21.5 ± 3.5	– 16.5 ± 1.8
12	– 48.6 ± 8.7	– 88.6 ± 5.7	– 83.2 ± 2.7
13	– 66.7 ± 3.4	– 85.2 ± 8.5	– 91.7 ± 1.2
14	– 33.5 ± 4.2	– 47.8 ± 5.3	– 33.6 ± 2.7
15	125.3 ± 12.6	14.2 ± 4.1	– 12.0 ± 0.9
16	– 29.6 ± 5.1	– 22.2 ± 3.8	– 14.3 ± 3.6
17	– 44.6 ± 3.0	– 87.9 ± 5.1	– 73.1 ± 1.3
18	– 70.2 ± 1.4	– 82.2 ± 1.0	– 81.4 ± 3.5
19	29.8 ± 1.5	– 37.7 ± 3.9	– 15.2 ± 3.3
20	– 7.9 ± 4.8	– 13.8 ± 1.5	– 17.1 ± 0.6
21	50.7 ± 6.3	– 48.4 ± 7.6	– 41.1 ± 3.3
22	– 14.0 ± 1.1	– 38.5 ± 0.2	– 28.8 ± 3.5
23	9.5 ± 4.8	– 8.5 ± 6.6	– 18.1 ± 5.8
24	6.7 ± 7.6	5.9 ± 2.7	– 23.4 ± 1.4
25	– 32.6 ± 3.9	– 53.8 ± 3.3	– 36.1 ± 2.7
26	– 53.5 ± 3.9	– 93.8 ± 3.5	– 93.9 ± 0.7
27	– 61.5 ± 6.6	– 80.0 ± 3.6	– 90.4 ± 0.6
28	– 24.6 ± 0.3	– 61.4 ± 4.7	6.5 ± 2.6
29	– 36.8 ± 2.6	– 61.5 ± 5.4	– 35.6 ± 3.2
30	– 11.8 ± 7.6	– 70.9 ± 4.4	– 38.3 ± 5.6
31	– 4.8 ± 4.2	– 1.0 ± 0.93	– 2.2 ± 3.4
32	16.4 ± 2.7	– 38.4 ± 11.1	– 37.3 ± 1.4
33	12.8 ± 12.9	– 43.0 ± 2.1	– 39.9 ± 2.7
34	– 6.3 ± 2.1	57.3 ± 9.4	20.6 ± 2.6
35	116.6 ± 13.4	– 38.3 ± 13.0	– 1.9 ± 1.9
36	– 79.4 ± 6.2	– 93.0 ± 2.7	– 86.5 ± 4.4
37	– 78.6 ± 2.5	– 82.7 ± 11.1	– 65.7 ± 7.3
IL-2 [10 U/ml]	95.3 ± 10.1		208 ± 25.7
Cyclosporine A [2.5 μg/ml]	– 15.9 ± 4.4		– 92.2 ± 6.8

[a]) Negative represents inhibitory activity; positive represents enhancement of proliferation.

(3 kg). The extract was stirred twice with H_2O (2 × 3.5 l), and the resulting emulsion was separated from the residue and partitioned between AcOEt/H_2O 1:1 to afford an AcOEt extract (173 g). The AcOEt extract was separated by CC (*Sephadex LH-20*, MeOH) into *Fractions T1* and *T2*. *Fr. T2* (60 g) was subjected to CC (SiO_2, hexane/AcOEt gradient): *Frs. T2-1* to *T2-18*. *Fr. T2-8* (7.58 g) was further separated by CC (*Sephadex LH-20*, MeOH) followed by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1): **1**

(2 mg) and **2** (64 mg). Part of *Fr. T2-9* (80 g) was subjected to CC (SiO_2, hexane/AcOEt gradient) followed by CC (*Sephadex LH-20*, MeOH): *Frs. T2-9-1* to *T2-9-9*. *Fr. T2-9-1* was further separated by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) followed by reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **5** (73 mg), **6** (31 mg), **7** (2 mg), **8** (12 mg), **9** (12 mg), **10** (5 mg), and **11** (49 mg). *Fr. T2-9-2* was purified by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and on reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **15** (6 mg) and **16** (9 mg). *Fr. T2-9-5* was separated by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **17** (20 mg), **18** (5 mg), and **19** (2 mg). *Fr. T2-9-6* was purified by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **20** (1.6 g), **21** (102 mg), and **22** (10 mg). *Fr. T2-9-7* was separated by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **23** (43 mg) and **24** (18 mg). *Fr. T2-9-8* was separated by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **11** (49 mg), **12** (2 mg), **13** (11 mg), and **14** (15 mg). *Fr. T2-9-9* was fractionated by CC (SiO_2, hexane/AcOEt gradient) and CC (*Sephadex LH-20*, MeOH) followed by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **23** (43 mg) and **24** (18 mg). *Fr. T2-10* was subjected to CC (SiO_2, hexane/AcOEt gradient) and CC (*Sephadex LH-20*, MeOH) followed by further separation by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **25** (18 mg), **26** (13 mg), **27** (2 mg), **28** (28 mg), **29** (62 mg), **30** (10 mg), and **31** (102 mg). *Fr. T2-10* was purified by FC (hexane/AcOEt gradient) and CC (*Sephadex LH-20*, MeOH) followed by further separation by HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5): **3** (2 mg), **4** (2 mg), **32** (4 mg), **33** (16 mg), **34** (16 mg), **35** (2 mg), **36** (1 mg), and **37** (2 mg).

Sumataxin A (= rel-*(2′*R,*3′a*R,*4*R,*4′*R,*4′a*S,*6′*R,*8′*R,*8′a*R,*9′*R*)-6′-(Benzoyloxy)-2′,4′,4′a,6′,7′,8′,8′a,9′-octahydro-2′,4′,8′,9′-tetrahydroxy-1′,2,2,8′a,12′,12′-hexamethylspiro[1,3-dioxolane-4,5′(3′*H*)-[3a,9](methanoxymethano)[3a*H*]benz[f]inden-10′-one*; **1**): Colorless amorphous powder. $[\alpha]_D^{27} = -84$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 229 (4.30). IR (neat): 3443 (OH), 2931, 2875 (C–H), 1715 (C=O, γ-lactone, Bz). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 581 ($[M+Na]^+$). HR-ESI-MS: 581.2360 ($[M+Na]^+$, $C_{30}H_{38}NaO_{10}^+$; calc. 581.2360).

Sumataxin B (= rel-*(2*R,*3a*S,*4*S,*4a*R,*5*S,*6*S,*8*S,*8a*S,*9*S*)-5-[(Benzoyloxy)methyl]-2,3,4,4a,5,6,7,8,8a,9-decahydro-2,4,5,6,8,9-hexahydroxy-1,8a,12,12-tetramethyl-3a,9-(methanoxymethano)-3a*H*-benz[f]inden-10-one*; **2**): Colorless amorphous powder. $[\alpha]_D^{27} = -76$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 229 (3.70). IR (neat): 3383 (OH), 2932 (C–H), 1717 (C=O, γ-lactone, Bz). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 541 ($[M+Na]^+$). HR-ESI-MS: 541.2053 ($C_{27}H_{34}NaO_{10}^+$; calc. 541.2050).

Sumataxin C (= rel-*(4a*R,*6*R,*6a*R,*7*S,*8*S,*10*R,*11a*R,*12*R,*12a*S,*12b*R*)-5,6,6a,7,8,10,11,11a,12,12a-Decahydro-11a-(1-hydroxy-1-methylethyl)-3,3,6a,9-tetramethyl-1*H*-azuleno[5,6-f][1,3]benzodioxin-6,7,8,10,12,12b(4a*H*)-hexol 6,7,12-Triacetate 8-Benzoate*; **3**): Colorless amorphous powder. $[\alpha]_D^{27} = -24$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 228.8 (4.00). IR (neat): 3443 (OH), 2924, 2854 (C–H), 1730 (C=O, Ac, Bz). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 711 ($[M+Na]^+$). HR-ESI-MS: 711.2996 ($[M+Na]^+$, $C_{36}H_{48}NaO_{13}^+$; calc. 711.2992).

Sumataxin D (= rel-*(1*R,*3*S,*4*R,*5*R,*7*S,*10*R,*12*S*)-7-(Acetyloxy)-4,10-dihydroxy-8,12,17,17-tetramethyl-2-oxatetracyclo[10.3.1.1^{5,9}.0^{1,3}]heptadeca-8,13-diene-11,15-dione*; **4**): Colorless amorphous powder. $[\alpha]_D^{27} = -58$ ($c = 0.2$, $CHCl_3$). IR (neat): 3446 (OH), 2922, 2852 (C–H), 1733, 1720, 1685 (C=O, α,β-unsaturated γ-keto, Ac). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 427 ($[M+Na]^+$). HR-ESI-MS: 427.1730 ($[M+Na]^+$, $C_{22}H_{28}NaO_7^+$; calc. 427.1733).

Cell Culture and Viruses. Vero cells were cultured in minimal essential medium (MEM; *Gibco*, Grand Island, NY) supplemented with 10% fetal calf serum (FCS; *Hyclone*, Logan, UT), 100 U/ml of penicillin, and 100 μg/ml of streptomycin and incubated at 37° in a 5% CO_2 incubator. To prepare HSV-1 (KOS strain, VR-1493, ATCC) stocks, Vero cells were infected by HSV-1 at a multiplicity of infection of 3 plaque forming units (PFU)/cell and harvested 24 h post infection and centrifuged at 1500 *g* (centrifuge *5810 R*, *Eppendrof*) at 4° for 20 min. The supernatant was collected and stored at −70° for use.

Plaque Reduction Assay. The assay followed procedures described previously [20]. Acyclovir was used as a positive control. Vero cells ($3.5 \cdot 10^5$/dish) were incubated with 100 PFU of HSV-1, and various compounds (100 μM) or acyclovir (2.5 μM) were added to the cells. The viruses were adsorbed for 1 h at 37°, and 1% methylcellulose was added to each well. After 5 d, the virus plaques formed in HeLa cells

were counted by crystal-violet staining. The activities of various compounds and acyclovir for inhibition of plaque formation were calculated.

Lymphoproliferation Test. The lymphoproliferation test was modified from previously described methods [21][22]. The density of PBMC was adjusted to $2 \cdot 10^6$ cells/ml before use. A 100 µl of cell suspension was applied into each well of a 96-well flat-bottomed plate (*Nunc 167008*; *Nunclon*, Raskilde, Denmark) with or without PHA (*Sigma*). Various compounds were added to the cells at 100 µM. The plates were incubated in 5% CO_2/air-humidified atmosphere at 37° for 3 d. Subsequently, tritiated thymidine (1 µCi/well, *NEN*) was added into each well. After 16 h incubation, the cells were harvested on glass-fiber filters by an automatic harvester (*Multimash 2000*; *Dynatech*, Billingshurst, U.K.). Radioactivity in the filters was measured by a scintillation counter. IL-2 (interleukin 2) and cyclosporine A were used as positive and negative standard compounds, resp.

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