Verruculides A and B, two new protein tyrosine phosphatase 1B inhibitors from an Indonesian ascidian-derived Penicillium verruculosum

by Deiske Sumilat 19

Submission date: 20-Aug-2019 12:04PM (UTC+0700)
Submission ID: 1161638585
File name: 2015_DASumilat_Verruculides_A_and_B.pdf (544.53K)
Word count: 3932
Character count: 16465

Bioorganic & Medicinal Chemistry Letters 25 (2015) 3087–3090

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Verruculides A and B, two new protein tyrosine phosphatase 1B inhibitors from an Indonesian ascidian-derived *Penicillium verruculosum*

Hiroyuki Yamazaki [a,*], Wataru Nakayama [a], Ohgi Takahashi [a], Ryota Kirikoshi [a], Yuta Izumikawa [a], Kohei Iwasaki [a], Kengo Toraiwa [a], Kazuyo Ukai [a], Henki Rotinsulu [b], Defny S. Wewengkang [c], Deiske A. Sumilat [d], Remy E. P. Mangindaan [d], Michio Namikoshi [a]

[a] Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, Sendai 981-8558, Japan
[b] Research Institute, University of Pembangunan Indonesia, Bahu, Manado 95115, Indonesia
[c] Faculty of Mathematic and Natural Sciences and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, Indonesia
[d] Faculty of Fisheries and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, Indonesia

ARTICLE INFO

Article history:
Received 6 April 2015
Revised 3 June 2015
Accepted 6 June 2015
Available online 14 June 2015

Keywords:
Verruculide
Ascidian-derived fungus
Penicillium verruculosum
Protein tyrosine phosphatase 1B
Inhibitor

ABSTRACT

Two new merosesquiterpenes, verruculides A (**1**) and B (**2**), were isolated from a culture broth of the Indonesian ascidian-derived *Penicillium verruculosum* TPU1311, together with three known congeners, chrodrimanins A (**3**), B (**4**), and H (**5**). The structures of **1** and **2** were assigned on the basis of their spectroscopic data (1D and 2D NMR, HRMS, UV, CD, and IR). Compound **2** had a linear sesquiterpene moiety and was considered to be the derivative of the biosynthetic precursor for **1** and **3**–**5**. Compounds **1**, **3**, and **5** inhibited the activity of protein tyrosine phosphatase 1B (PTP1B) with IC_{50} values of 8.4, 8.5, and 14.9 μM, respectively. Compound **2** showed 40% inhibition at 23.1 μM, while **4** was not active at 20.7 μM.

Protein tyrosine phosphatase 1B (PTP1B) dephosphorylates the insulin receptors (IRs), insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) and, thus, is crucially involved in the negative regulation of the insulin signaling pathway.[1] Recent studies have also implicated PTP1B in the inhibition of the leptin signaling pathway. PTP1B is now considered to be a promising therapeutic target for insulin-resistant type 2 diabetes mellitus and obesity.[1] Although a number of studies have been conducted on natural and synthetic PTP1B inhibitors in the last decade,[2] a clinical application has not yet been achieved because of their low selectivities and activities against PTP1B. Therefore, the search for a new type of PTP1B inhibitor with more prominent properties is an important and interesting subject in natural product chemistry.

In the course of our research on PTP1B inhibitors from marine organisms such as ascidians, sponges, and microorganisms, we found that polybromodiphenyl ethers, dehydroeuryspongin, hyattellactones, and trichoketides markedly inhibited PTP1B.[3] Further investigations on the culture broth of the Indonesian ascidian-derived *Penicillium verruculosum* strain TPU1311, which exhibited strong inhibitory activity against PTP1B, led to the isolation of two new merosesquiterpenes, named verruculides A (**1**) and B (**2**) (Fig. 1), together with three known analogs, chrodrimanins A (**3**), B (**4**), and H (**5**).[4] We herein described the fermentation, isolation, structure elucidation including absolute configurations, and biological properties of compounds **1** and **2**.

The fungal strain TPU1311 was isolated from an ascidian *Polycarpa aurata* collected in Indonesia.[5] The 233 bp of the ITS1 rDNA sequence were identical with those of *Penicillium verruculosum*.

The strain TPU1311 was cultured in a seawater-based medium under agitation for 30 days.[6] The culture broth was treated with acetone and filtered. The filtrate was evaporated to remove acetone and the aqueous residue was extracted with EtOAc. The EtOAc extract was subjected to ODS column chromatography followed by preparative HPLC to give compounds **1**–**5**.[7] Compounds **3**–**5** were identified as chrodrimanins A (**3**), B (**4**), and H (**5**), respectively, by comparing their spectroscopic data[8–10] with those of the reported values.[4]

The molecular formula of compound **1**[11] was deduced as $C_{25}H_{30}O_5$ from HREIMS (*m*/*z* 410.2087 $[M]^+$, Δ −0.6 mmu) and

* Corresponding author. Tel./fax: +81 22 727 0218.
E-mail address: yamazaki@tohoku-pharm.ac.jp (H. Yamazaki).

http://dx.doi.org/10.1016/j.bmcl.2015.06.026
0960-894X/

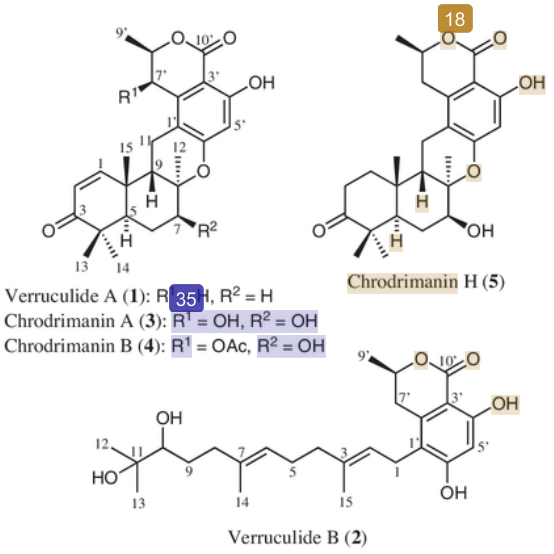

Figure 1. Structures of compounds **1–5** produced by *Penicillium verruculosum* TPU1311.

NMR data (Table 1). The ^{1}H NMR and UV spectra of **1** resembled those of chrodrimanin A (**3**). The difference in the molecular formulae of **1** and **3** was O_2 (32). Two oxygenated sp^3 methine signals at δ 4.64 and 4.19 in the ^{1}H NMR spectrum of **3** were not detected in that of **1**. Therefore, we speculated that compound **1** was a dideoxy derivative of **3**. The ^{1}H–^{1}H COSY spectrum of **1** revealed the presence of four partial structures, as indicated by the bold blue lines in Figure 2a. These partial structures and missing links were connected by an analysis of the HMBC data for **1**, as shown in Figure 2a. Thus, verruculide A (**1**) was assigned as the 7,7′-dideoxy derivative of chrodrimanin (**3**).

Table 1
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **1** and **2** ($CDCl_3$)

Position	1		2	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	155.4	7.21 d (10.5)	24.4	3.22 d (6.3)
2	127.7	5.98 d (10.5)	122.8	4.98 t (6.3)
3	204.3		135.9	
4	44.9		38.9	2.07 m
5	44.5	2.09 m	25.2	2.13 m
6	17.1	1.79 m	124.4	5.11 t (6.4)
7	35.9	2.11 m	134.8	
8	78.0		36.4	2.03 m, 2.21 m
9	47.8	1.93 dd (14.0, 5.1)	29.2	1.42 m, 1.64 m
10	38.7		78.1	3.39 d (10.1)
11	22.2	(a) 2.51 dd (15.0, 14.0) (b) 2.61 dd (15.0, 5,1)	73.7	
12	23.4	1.27 s	23.5	1.20 s
13	21.2	1.15 s	26.5	1.24 s
14	27.5	1.17 s	16.1	1.58 s
15	28.1	1.30 s	15.9	1.71 s
1′	110.7		117.3	
2′	139.1		138.6	
3′	101.8		101.9	
4′	162.5		162.8	
5′	103.4	6.30 s	101.9	6.31 s
6′	160.0		161.9	
7′	31.9	(a) 2.89 dd (16.0, 3.4) (b) 2.75 dd (16.0, 14.0)	32.1	(a) 2.72 dd (16.5, 11.3) (b) 2.97 dd (16.5, 3.1)
8′	74.6	4.65 m	74.8	4.61 m
9′	21.0	1.57 d (6.3)	20.9	1.52 d (6.4)
10′	170.0		170.4	
4′-OH		11.1 s		11.3 s

The absolute configurations at the C-5, C-8 (C-12), C-9, C-10 (C-15), and C-8′ (C-9′) positions of **1** may be identical to those of chrodrimanins[4] because compounds **1–5** were produced by the strain TPU1311 via the same biosynthetic pathway and compounds **1** and **4** had very similar Circular Dichroism (CD) spectra.[9,11] The configurations of the sesquiterpene moiety (C-1–C-15) were confirmed by the 1D NOE difference experiments. Irradiation at δ 1.27 (H_3-12), 1.15 (H_3-13), 1.30 (H_3-15), 1.93 (H-9), 2.51 (H-11a), and 2.61 (H-11b) gave NOE enhancements to H-5 (δ 2.09)/H-11a, H_3-15, H-9/H_3-13, H-11b/H_3-15, H_3-12, and H-9, respectively (Fig. 2b). A systematic conformational analysis was performed with an MMFF94 force field utilizing Spartan'14[12] based on the NOE data for **1** (Fig. 2b).[13]

Thus, the stereostructure of verruculide A (**1**) was assigned as shown in Figures 1 and 2.

Compound **2**[14] was assigned the molecular formula, $C_{25}H_{36}O_6$, from HRFABMS *m/z* 431.2443 [M–H]$^-$, Δ + 1.0 mmu] and NMR data (Table 1). The ^{1}H and ^{13}C NMR spectra ($CDCl_3$) of **2** showed 33 proton and 25 carbon signals, which were classified into five methyl, six sp^3 methylene, two sp^3 oxygenated methine, one sp^3 oxygenated quaternary, three sp^2 methine, five sp^2 quaternary, two sp^2 oxygenated quaternary, and one carbonyl carbons by the analysis of HMQC and DEPT spectra. The ^{1}H and ^{13}C NMR signals due to the 3,4-dihydroisocoumarin moieties (C-1′–C-10′) in **1** and **2** were very similar to each other (Table 1). The presence of this moiety in **2** was confirmed by ^{1}H–^{1}H COSY data (C-7′–C-9′) and HMBC correlations from H-5′ (δ 6.31) to C-1′ (δ 117.3), C-3′ (δ 101.9), C-4′ (δ 162.8), and C-6′ (δ 161.9), H_2-7′ (δ 2.72, 2.97) to C-1′, C-2′ (δ 138.6), and C-3′, and from 4′-OH (δ 11.3) to C-3′, C-4′, and C-5′ (δ 101.9), as shown in Figure 3. 3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin is known as 6-hydroxymelein and was isolated from fungi,[15] and CD data ascribed to the isocoumarin moiety in **2** ($\Delta\varepsilon$ +0.73 at 308 nm and $\Delta\varepsilon$ −3.46 at 270 nm) were very similar to those for (*R*)-6-hydroxymellein.[15c] Therefore, the absolute configuration at the C-8′ position in **2** was assigned as *R*. The planar structure of the sesquiterpene moiety in **2** was revealed from ^{1}H–^{1}H COSY and HMBC data, as shown in Figure 3. The NOESY correlations between H-1 (δ 3.22)/H_3-15 (1.71), H-2 (4.98)/H_2-4 (2.07), H-5 (2.13)/H_3-14 (1.58), and H-6 (5.11)/H_2-8 (2.03 and 2.21) assigned the orientations of the two double bonds as 2*E* and 6*E*. The connection between the sesquiterpene and isocoumarin moieties was established by the HMBC correlations from H_2-1 (δ 3.22) to C-1′, C-2′, and C-6′ and from H-2 to C-1′.

The absolute configuration at the C-10 position of **2** currently remains unknown because an application of the modified Mosher's method[16] has not yet been successful. The reactions of **2** with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chlorides only gave di-MTPA esters at C-4′ and C-6′. More chemical transformations are needed to elucidate the absolute configuration at the C-10 position in **2**.

Verruculide B (**2**) possessed a linear sesquiterpene moiety, and, therefore, compounds **1** and **3–5** would be biosynthesized via the 10,11-epoxy derivative of compound **2**.

PTP1B has received a lot of attention as a target molecule for the treatment of type 2 diabetes and obesity because of its negative regulatory activity against insulin and leptin signaling cascades.[1] Compounds **1–5** were evaluated for their inhibitory activities against PTP1B by the bioassay method described previously.[3] Compounds **1**, **3**, and **5** inhibited PTP1B activity with IC_{50} values of 8.4, 8.5, and 14.9 μM, respectively. On the other hand, compound **2** showed 40% inhibition at 23.1 μM, while **4** did not show apparent activity at 20.7 μM. The IC_{50} value of oleanolic acid,[17] a positive control, was 0.7 μM in the same experiment. A comparison

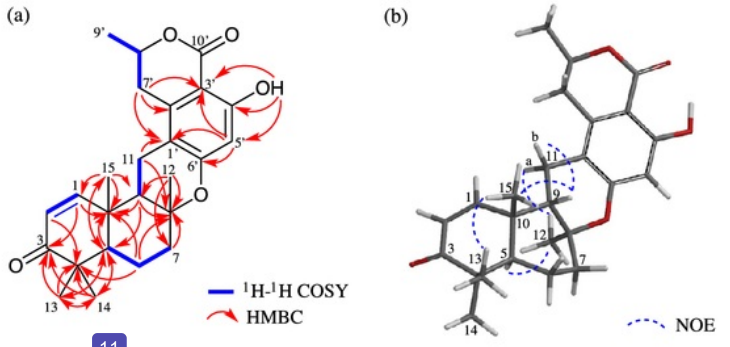

Figure 2. (a) ^{1}H–^{1}H COSY and HMBC correlations and (b) Key NOE correlations for verruculide A (**1**).

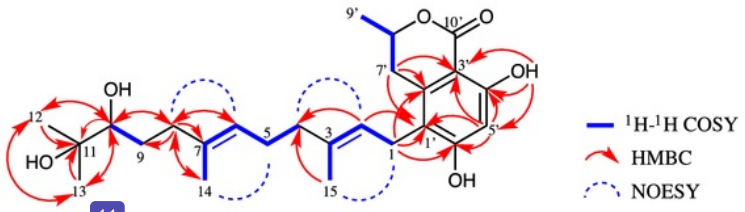

Figure 3. ^{1}H–^{1}H COSY, HMBC, and key NOE correlations for verruculide B (**2**).

of their activities revealed that two OH groups at the C-7 and C-7′ positions did not affect PTP1B activity, whereas the acetylation of the 4′-OH group significantly reduced it.

This is the first study to demonstrate that compounds in the chrodrimanin family exhibited inhibitory activities against PTP1B. Therefore, this study added a new type of compound to PTP1B inhibitors. The mechanism of action and effect to animal models will be the interesting future studies.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (25870660) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan to H.Y. and by the Foundation for Japanese Chemical Research to H.Y. We express our thanks to Mr. T. Matsuki and S. Sato of Tohoku Pharmaceutical University for the measurements of mass and NMR spectra, and Mr. K. Endo, T. Ogasawara, Y. Watanabe, and Ms. M. Takahashi of Tohoku Pharmaceutical University for their technical assistance.

References and notes

1. (a) Barr, A. J. *Future Med. Chem.* **2010**, *2*, 1563; (b) Zhang, S.; Zhang, Z. Y. *Drug Discovery Today* **2007**, *12*, 373; (c) Lee, S.; Wang, Q. *Med. Res. Rev.* **2007**, *27*, 553.
2. Jiang, C. S.; Liang, L. F.; Guo, Y. W. *Acta Pharmacol. Sin.* **2012**, *33*, 1217.
3. (a) Yamazaki, H.; Sumilat, D. A.; Kanno, S.; Ukai, K.; Rotinsulu, H.; Wewengkang, D. S.; Ishikawa, M.; Mangindaan, R. E.; Namikoshi, M. *J. Nat. Med.* **2013**, *67*, 730; (b) Yamazaki, H.; Nakazawa, T.; Sumilat, D. A.; Takahashi, O.; Ukai, K.; Takahashi, S.; Namikoshi, M. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2151; (c) Abdjul, D. B.; Yamazaki, H.; Takahashi, O.; Kirikoshi, R.; Mangindaan, R. E. P.; Namikoshi, M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 905; (d) Yamazaki, H.; Takahashi, O.; Kanno, S.; Nakazawa, T.; Takahashi, S.; Ukai, K.; Sumilat, D. A.; Ishikawa, M.; Namikoshi, M. *Bioorg. Med. Chem.* **2015**, *23*, 797; (e) Yamazaki, H.; Saito, R.; Takahashi, O.; Kirikoshi, R.; Toraiwa, K.; Iwasaki, K.; Izumikawa, Y.; Nakayama, W.; Namikoshi, M. *J. Antibiot.* **2015**. http://dx.doi.org/10.1038/ja.2015.44. in press.
4. (a) Nakajima, M.; Takahashi, H.; Furuya, K.; Sato, S.; Itoi, K.; Kagasaki, T.; Hara, M. Japan Kokai Tokkyo Koho, 02221277 (Sep. 4, 1990).; (b) Wei, M.-Y.; Chen, G.-Y.; Wang, Y.; Zhang, X.-L.; Wang, C.-Y.; Shao, C.-L. *Chem. Nat. Compd.* **2011**, *47*, 571; (b) Hayashi, H.; Oka, Y.; Kai, K.; Akiyama, K. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 745; (c) Hayashi, H.; Oka, Y.; Kai, K.; Akiyama, K. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1765.
5. The strain TPU1311 was isolated from an ascidian *Polycarpa aurata* collected in Manado, Indonesia, in December 2013. A small piece of the ascidian was minced with a mortar and pestle in approximately 1 mL of sterilized seawater, and the liquid was spread on an agar plate [PDA (BD, Franklin Lakes, NJ, USA) containing 0.005% rose bengal and 0.01% kanamycin]. The plate was incubated at 25 °C for a week, and the strain TPU1311 grown on the plate was isolated and inoculated on a PDA plate. The strain TPU1311 was identified as *Penicillium verruculosum* by a comparison of the 233-bp ITS1 rDNA sequence (100% match).
6. The strain TPU1311 was inoculated into a 100-mL Erlenmeyer flask containing 50 mL of the seed medium (2.0% glucose, 0.50% polypeptone, 0.050% $MgSO_4{\cdot}7H_2O$, 0.20% yeast extract, 0.10% KH_2PO_4 and 0.10% agar in natural seawater; adjusted to pH 6.0 before sterilization). The flask was shaken reciprocally for 3 days at 25 °C and then transferred to the production medium (3.0% sucrose, 3.0% soluble starch, 1.0% malt extract, 0.30% Ebios (Asahi Food & Healthcare Co. Ltd., Tokyo, Japan), 0.50% KH_2PO_4, and 0.050% $MgSO_4{\cdot}7H_2O$ in natural seawater; adjusted to pH 6.0 before sterilization). The production culture was carried out at 25 °C for 7 days under agitation.
7. The culture broth (2.4 L) was treated with 2.4 L of acetone after 7 days and filtered. The filtrate, after the evaporation of acetone, was extracted with EtOAc, and the extract was concentrated to yield a red brown oil (1.37 g). The extract was suspended in 30% CH_3OH and adsorbed on an ODS column (100 g), and the column was eluted stepwise with 400 mL each of 30, 50, 70, 85, and 100% CH_3OH in water into five fractions (Fr. 1–Fr. 5). A portion (140 mg) of the active Fr. 4 (85% CH_3OH eluate, 366.3 mg) was separated by preparative HPLC [column; PEGASIL ODS SP100 (Senshu Scientific. Co. Ltd. Tokyo, Japan), 10 × 250 mm; solvent, 60% CH_3CN; detection, UV at 210 nm; flow rate, 2.0 mL/min] to give compounds **1** (1.0 mg) and Fr. 4-1 (36.1 mg), which was purified by preparative HPLC [column; PEGASIL ODS SP100, 10 × 250 mm; solvent, 40% CH_3CN; detection, UV at 210 nm; flow rate, 2.0 mL/min] to yield compounds **2** (2.2 mg), **3** (0.8 mg), **4** (6.1 mg), and **5** (0.7 mg).
8. *Chrodrimanin A* (**3**): a colorless oil; $[\alpha]_D^{20}$ +3.9 (*c* 0.10, $CH_3OH/CHCl_3$ = 1:1); UV (CH_3OH) λ_{max} nm (log ε) 220 (4.60), 271 (4.22), 313 (3.92); EIMS *m*/*z* 442 $[M]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.1 (s, 1H), 7.38 (d, 1H, *J* = 10.2), 6.28 (s, 1H), 5.89 (d, 1H, *J* = 10.2), 5.67 (d, 1H, *J* = 6.3), 5.06 (d, 1H, *J* = 2.9), 4.63 (m, 2H), 4.02 (m, 1H), 3.13 (dd, 1H, *J* = 15.0, 5.1), 2.57 (t, 1H, *J* = 15.0), 2.16 (m, 3H), 1.67 (m, 1H), 1.43 (d, 3H, *J* = 6.3), 1.34 (s, 3H), 1.17 (s, 3H), 1.05 (s, 3H), 1.03 (s, 3H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.1 (s, 1H), 7.22 (d, 1H, *J* = 10.2), 6.47 (s, 1H), 5.98 (d, 1H, *J* = 10.2), 4.64 (s, 1H), 4.62 (dq, 1H, *J* = 6.8, 1.8), 4.19 (dd, 1H, *J* = 9.0, 3.0), 3.16 (dd, 1H, *J* = 15.0, 5.0), 2.63 (t, 1H, *J* = 15.0), 2.34 (dd, 1H, *J* = 15.0, 5.0), 2.27 (m, 1H), 2.19 (dd, 1H, *J* = 14.0, 4.6), 1.88 (dt, 1H, *J* = 14.0, 3.0), 1.64 (d, 3H, *J* = 6.8), 1.42 (s, 3H), 1.28 (s, 3H), 1.17 (s, 3H), 1.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.0, 169.4, 160.7, 159.8, 157.9, 141.7, 125.9, 112.1, 103.4, 100.6, 79.38, 79.38, 77.6, 61.7, 44.0, 41.9, 41.7, 38.2, 27.5, 26.89, 26.89, 21.1, 21.0, 20.5, 16.0.
9. *Chrodrimanin B* (**4**): a colorless oil; $[\alpha]_D^{20}$ −45.2 (*c* 0.10, $CH_3OH/CHCl_3$ = 1:1); UV (CH_3OH) λ_{max} nm (log ε) 221 (4.38), 272 (3.99), 314 (3.67); CD (CH_3CN) $\lambda_{extermum}$ nm 309 (Δε +1.00), 271 (Δε −12.7), 227 (Δε +23.9), 212 (Δε −22.2); EIMS *m*/*z* 484 $[M]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.1 (s, 1H), 7.15 (d, 1H, *J* = 10.2), 6.50

(s, 1H), 6.16 (d, 1H, J = 2.0), 5.99 (d, 1H, J = 10.2), 4.71 (dq, 1H, J = 6.7, 2.0), 4.17 (dd, 1H, J = 8.5, 2.8), 2.95 (dd, 1H, J = 15.6, 5.0), 2.60 (dd, 1H, J = 15.6, 15.0), 2.26 (dd, 1H, J = 15.0, 5.0), 2.24 (m, 1H), 2.17 (dd, 1H, J = 14.0, 4.4), 2.16 (s, 3H), 1.85 (td, 1H, J = 14.0, 2.8), 1.48 (d, 3H, J = 6.7), 1.35 (s, 3H), 1.27 (s, 3H), 1.16 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.7, 170.6, 168.8, 162.4, 159.6, 156.1, 135.9, 127.6, 112.3, 106.3, 102.4, 79.7, 76.1, 72.0, 64.1, 44.7, 42.5, 42.0, 38.5, 27.6, 27.5, 26.6, 21.50, 21.45, 21.3, 20.7, 16.4.

10. *Chrodrimanin H* (***5***): a colorless oil; $[\alpha]_D^{20}$ −6.0 (c 0.10, $CH_3OH/CHCl_3$ = 1:1); UV (CH_3OH) λ_{max} nm (log ε) 220 (4.68), 273 (4.39), 311 (4.03); EIMS m/z 428 $[M]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.1 (s, 1H), 6.35 (s, 1H), 4.64 (m, 1H), 4.03 (t, 1H, J = 7.6), 2.85 (dd, 1H, J = 16.3, 3.2), 2.71 (dd, 1H, J = 16.3, 12.0), 2.65 (m, 1H), 2.40-2.50 (m, 3H), 2.17 (m, 1H), 2.03 (m, 2H), 1.95 (dd, 1H, J = 14.1, 2.4), 1.71 (m, 1H), 1.59 (m, 1H), 1.56 (d, 3H, J = 6.3), 1.37 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.2, 170.0, 162.4, 158.7, 139.1, 109.9, 103.2, 102.3, 78.3, 74.7, 73.1, 46.9, 43.7, 40.9, 35.8, 33.6, 32.2, 31.8, 28.6, 28.2, 23.1, 22.8, 21.0, 20.0, 19.6.
11. *Verruculide A* (***1***): a colorless oil; $[\alpha]_D^{20}$ −86.0 (c 0.12, $CH_3OH/CHCl_3$ = 1:1); IR (KBr) ν_{max} 3437, 1668, 1651, 1475, 1384, 1260 cm^{-1}; UV (CH_3OH) λ_{max} nm (log ε) 219 (4.41), 273 (4.08), 309 (3.64); CD (CH_3CN) $\lambda_{extermum}$ nm 308 ($\Delta\varepsilon$ +0.78), 272 ($\Delta\varepsilon$ −4.26), 228 ($\Delta\varepsilon$ +9.72), 210 ($\Delta\varepsilon$ −17.4); EIMS m/z 410 $[M]^+$; HREIMS m/z 410.2087 ($[M]^+$; calcd for $C_{25}H_{30}O_5$, 410.2093); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1.
12. Spartan'14; Wavefunction Inc: Irvine, CA, USA, 2014.
13. The most stable conformer of verruculide A (**1**) was predicted using Spartan'14 by a preliminary conformational analysis with the MMFF94 force field followed by geometry optimization using the density functional theory (DFT) with the B3LYP functional and 6-31G(d) basis set.
14. *Verruculide B* (***2***): a colorless oil; $[\alpha]_D^{20}$ −45.2 (c 0.10, $CH_3OH/CHCl_3$ = 1:1); IR (KBr) ν_{max} 3437, 1651, 1545, 1465, 1380, 1262 cm^{-1}; UV (CH_3OH) λ_{max} nm (log ε) 219 (ε 4.49), 271 (4.16), 313 (3.86); CD (CH_3CN) $\lambda_{extermum}$ nm 308 ($\Delta\varepsilon$ +0.73), 270 ($\Delta\varepsilon$ −3.46), 227 ($\Delta\varepsilon$ +5.97), 213 ($\Delta\varepsilon$ −8.33); FABMS m/z 431 $[M-H]^-$; HRFABMS m/z 431.2443 ($[M-H]^-$; calcd for $C_{25}H_{36}O_6$, 431.2433); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1.
15. (a) Ayer, W. A.; Attah-Poku, S. K.; Browne, L. M.; Orszanska, H. *Can. J. Chem.* **1987**, *65*, 765; (b) Krohn, K.; Bahramsari, R.; Flörke, U.; Ludewig, K.; Kliche-Spory, C.; Michel, A.; Aust, H. J.; Draeger, S.; Schulz, B.; Antus, S. *Phytochemistry* **1997**, *45*, 313; (c) Schlingmann, G.; Roll, D. M. *Chirality* **2005**, *17*, 48.
16. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
17. Liu, J. *J. Ethnopharmacol.* **1995**, *49*, 57.

Verruculides A and B, two new protein tyrosine phosphatase 1B inhibitors from an Indonesian ascidian-derived Penicillium verruculosum

ORIGINALITY REPORT

23%	13%	18%	11%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Graphical contents list", Bioorganic & Medicinal Chemistry Letters, 2015. Publication	2%
2	www.foodbiomedicalscience.com Internet Source	2%
3	www.mdpi.org Internet Source	2%
4	Bing Tian Zhao, Duc Hung Nguyen, Duc Dat Le, Jae Sue Choi, Byung Sun Min, Mi Hee Woo. "Protein tyrosine phosphatase 1B inhibitors from natural sources", Archives of Pharmacal Research, 2017 Publication	1%
5	www.ncbi.nlm.nih.gov Internet Source	1%
6	Ishii, Takahiro, Kenichi Nonaka, Akihiro Sugawara, Masato Iwatsuki, Rokuro Masuma, Tomoyasu Hirose, Toshiaki Sunazuka, Satoshi	1%

Ōmura, and Kazuro Shiomi. "Cinatrins D and E, and virgaricin B, three novel compounds produced by a fungus, Virgaria boninensis FKI-4958", The Journal of Antibiotics, 2015.
Publication

7	www.j3.jstage.jst.go.jp Internet Source	1%
8	www.jesc.ac.cn Internet Source	1%
9	Sun, Liang-Peng, Zhe Jiang, Li-Xin Gao, Li Sheng, Ying-Chun Quan, Jia Li, and Hu-Ri Piao. "Synthesis and Biological Evaluation of Furan-chalcone Derivatives as Protein Tyrosine Phosphatase Inhibitors", Bulletin of the Korean Chemical Society, 2013. Publication	1%
10	Submitted to University of Liverpool Student Paper	1%
11	Meffo Yemele Bouberte, Karsten Krohn, Hidayat Hussain, Etienne Dongo, Barbara Schulz, Qunxiu Hu. " Tithoniamarin and tithoniamide: a structurally unique isocoumarin dimer and a new ceramide from ", Natural Product Research, 2006 Publication	1%
12	carbsanity.blogspot.com Internet Source	1%

13	ir.uiowa.edu Internet Source	1%
14	tel.archives-ouvertes.fr Internet Source	<1%
15	Gerhard Schlingmann. "Absolute stereochemistry of unusual biopolymers fromAscomycete culture LL-W1278: Examples that derivatives of (S)-6-hydroxymellein are also natural fungal metabolites", Chirality, 2005 Publication	<1%
16	Lin, Sheng, Yanling Zhang, Mingtao Liu, Sen Yang, Maoluo Gan, Jiachen Zi, Weixia Song, Xiaona Fan, Sujuan Wang, Yang Liu, Yongchun Yang, Xiaoguang Chen, Ying Guo, Wenjie Wang, and Jiangong Shi. "Abietane and C_{20}-Norabietane Diterpenes from the Stem Bark of *Fraxinus sieboldiana* and Their Biological Activities", Journal of Natural Products, 2010. Publication	<1%
17	Submitted to Yeungnam University Student Paper	<1%
18	HAYASHI, Hideo, Yuki OKA, Kenji KAI, and Kohki AKIYAMA. "New Chrodrimanin Congeners, Chrodrimanins D^|^#8211;H, from YO-2 of Talaromyces sp.", Bioscience Biotechnology and Biochemistry, 2012.	<1%

Publication

19	Linfu Liang, Jinan Wang, Xiaoxuan Shi, Yinghua Zhu, Jia Li, Weiliang Zhu, Heyao Wang, Yuewei Guo. " A Novel Sarsolenane Diterpene as a PTP1B Inhibitor from Hainan Soft Coral Marenzeller ", Chinese Journal of Chemistry, 2017 Publication	<1%
20	Catherine Koukoulitsa, Dimitra Hadjipavlou–Litina, George D. Geromichalos, Helen Skaltsa. " Inhibitory effect on soybean lipoxygenase and docking studies of some secondary metabolites, isolated from L. ssp. ", Journal of Enzyme Inhibition and Medicinal Chemistry, 2008 Publication	<1%
21	Jyh-Horng Sheu, Guey-Horng Wang, Ping-Jyun Sung, Chang-Yih Duh, Michael Y. Chiang. "Pachyclavulariolides G–L and secopachyclavulariaenone A, seven novel diterpenoids from the soft coral Pachyclavularia violacea", Tetrahedron, 2001 Publication	<1%
22	Submitted to Chulalongkorn University Student Paper	<1%
23	Submitted to China Medical University, Taiwan Student Paper	<1%

24	Junji Inokoshi. "Production of a new type of amidepsine with a sugar moiety by static fermentation of Humicola sp. FO-2942", The Journal of Antibiotics, 11/27/2009 Publication	<1%
25	www.tandfonline.com Internet Source	<1%
26	www.ciq.uaem.mx Internet Source	<1%
27	Submitted to Panjab University Student Paper	<1%
28	Ohta, T.. "Paradisin C: a new CYP3A4 inhibitor from grapefruit juice", Tetrahedron, 20020812 Publication	<1%
29	Warashina, Tsutomu, Yoshimi Nagatani, and Tadataka Noro. "Constituents from the Bark of Tabebuia impetiginosa", CHEMICAL & PHARMACEUTICAL BULLETIN, 2006. Publication	<1%
30	patents.google.com Internet Source	<1%
31	www.osti.gov Internet Source	<1%
32	Ondrus, A.E.. "Dimerization of (+)-myrmicarin 215B. A potential biomimetic approach to	<1%

complex myrmicarin alkaloids", Tetrahedron, 20060529
Publication

---

33 Sang Hee Shim, James B. Gloer, Donald T. Wicklow. " Penifulvins B−E and a Silphinene Analogue: Sesquiterpenoids from a Fungicolous Isolate of ", Journal of Natural Products, 2006 <1%
Publication

---

34 Submitted to University of Hong Kong <1%
Student Paper

---

35 Submitted to 7996 <1%
Student Paper

---

36 Kai Li, Shuai Ji, Wei Song, Yi Kuang, Yan Lin, Shunan Tang, Zexu Cui, Xue Qiao, Siwang Yu, Min Ye. " Glycybridins A–K, Bioactive Phenolic Compounds from ", Journal of Natural Products, 2017 <1%
Publication

---

37 Wu, J.. "Xyloccensins Q-V, six new 8,9,30-phragmalin ortho ester antifeedants from the Chinese mangrove Xylocarpus granatum", Tetrahedron, 20050829 <1%
Publication

---

38 Su, J.H.. "Novel steroids from the soft coral Nephthea chabrolii", Tetrahedron, 20070115 <1%
Publication

39	Submitted to Mahidol University Student Paper	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		