Usimines A−C, Bioactive Usnic Acid Derivatives from the Antarctic Lichen *Stereocaulon alpinum*

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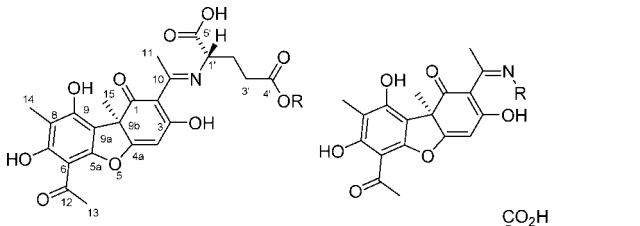
Usimines A−C (**1**–**3**), three new usnic acid derivatives, have been isolated from a MeOH extract of the Antarctic lichen *Stereocaulon alpinum* by various chromatographic methods. The structures of **1**–**3** were determined by analysis of their spectroscopic data (NMR, UV, MS) and by chemical methods. The known compound usnic acid (**4**) was also obtained. Compounds **1**–**4** showed moderate inhibitory activity against therapeutically targeted protein tyrosine phosphatase 1B (PTP1B).

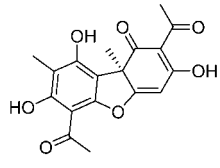
Lichens, comprising about 17 000 species, are symbiotic associations formed between fungi and algae and/or cyanobacteria.[1] They produce secondary metabolites that have been suggested to serve as antimicrobial or antiherbivore agents.[1,2] The slow growth of lichens and harsh conditions they encounter during growth are presumed to make production of protective metabolites a necessity for them.[2,3] Several lichen extracts have been used in folk medicine, and some lichen metabolites have been shown to have antibiotic, antimycobacterial, antiviral, analgesic, or antipyretic properties.[1,4] Thus, there is considerable interest in lichen metabolites as potential sources of pharmacological agents.[5]

During the course of chemical studies of lichens from Antarctica as potential sources of new bioactive secondary metabolites, three new metabolites named usimines A−C (**1**–**3**), along with the well-known lichen metabolite usnic acid (**4**), were isolated from a MeOH extract of *Stereocaulon alpinum*. This report describes the isolation, structure elucidation, and biological activities of compounds **1**–**4**.



1 R = CH_3
2 R = H

3 R = $-C(CO_2H)(H)-CH_2CH_2CO_2H$



4

Usnic acid (**4**)[2,6] was isolated from the *S. alpinum* extract as a major component. The structure of **4** was readily established by comparison of NMR and MS data with literature values.[6] Compounds **1**–**3** were recognized as derivatives of usnic acid by analysis of their NMR data. The general spectroscopic characteristics of usnic acid include the presence of ^{1}H and/or ^{13}C NMR signals corresponding to a 1,3-keto-enolic moiety, four isolated methyl groups, two phenolic OH groups, and an enolic OH group. The NMR signals for the basic skeleton of **4** (i.e., the 9b*H*-dibenzofurandione moiety) and its substitution pattern, except for the absence of one ketone functionality, were apparent in the NMR spectra of **1**–**3**. Therefore, the structure elucidation of these compounds focused mainly on identification of the remaining parts of the molecules and assignments of their absolute configurations.

Usimine A (**1**) had the molecular formula $C_{24}H_{25}NO_{10}$, as deduced from ^{13}C NMR and HRESIMS [*m*/*z* 488.1552 $(M + H)^+$; Δ + 0.5 mmu] data. This formula indicated 13 degrees of unsaturation. Analysis of ^{1}H and ^{13}C NMR data, and comparison with those of **4**, enabled identification of the signals corresponding to the 9b*H*-dibenzofurandione moiety. The numbering system shown for **1** was chosen by analogy to that used previously for **4**. ^{1}H and ^{13}C NMR assignments for this portion of the molecule were confirmed by analysis of HMQC and HMBC data. The most notable differences relative to the data for **4** were the absence of a ^{13}C NMR signal for the C-10 ketone functionality and chemical shift changes for the methyl group α to the ketone moiety. This observation suggested that the difference of **1** relative to **4** occurred at C-10. Aside from the aforementioned NMR signals, the ^{1}H and ^{13}C NMR spectra showed signals for three carbonyl carbons (one of which was later identified to be involved in an imine group), a methoxy group, and a $-CH-CH_2-CH_2-$ unit. These structural features accounted for 13 unsaturation equivalents, requiring linear connection of the remaining structural unit to C-10. Analysis of the COSY data revealed the spin system ranging from C-1′ to C-3′ in **1**. The carbonyl carbon at δ_C 173.0 was connected to C-1′ on the basis of HMBC correlations of H-1′/C-5′ and H_2-2′/C-5′. The HMQC data showed a correlation of the methyl group protons at δ_H 3.59 to δ_C 51.7, and an HMBC correlation of this signal to the carbonyl carbon at δ_C 172.9 indicated the presence of a methyl ester group. The methyl ester group was connected to C-3′ by HMBC correlation of H_2-3′ with C-4′. Connection of the methyl carbon (C-11) to the carbon resonating at δ_C 175.1 (C-10) and to C-2 was established on the basis of HMBC correlations of H_3-11 to C-2 and C-10. An HMBC correlation of H-1′ with C-10, together with the presence of nitrogen in the molecule, and the chemical shifts for C-10, C-1′, and C-5′ enabled the assignment of C-10 as the carbon (C=N) of an imine group. The remaining OH group was located at C-5′, thereby completing the gross structure of **1** as shown.

It is noteworthy that the ^{13}C NMR spectrum of **1** measured in $CDCl_3$ revealed only 19 distinctive carbon signals. Detailed analysis of the NMR data (see Experimental Section) indicated that signals corresponding to C-2, C-3, and C-10 were missing. These carbons were presumed to be involved in imine−enamine tautomerism

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involving the OH group at C-3. Similar spectroscopic behavior due to imine−enamine tautomerism has been observed in the NMR spectra of gymnodimine, a cyclic imine containing marine toxin from *Gymnodinium* sp.[7] The imine configuration of **1** was proposed on the basis of NOESY data. A NOESY correlation of H_3-11 to H-1′ required C-11 and C-1′ to be *cis* with respect to the imine group, and thus the *E*-geometry was assigned to the imine.

The molecular formula of usimine B (**2**) was established as $C_{23}H_{23}NO_{10}$ by analysis of its HRESIMS [*m/z* 474.1392 $(M + H)^+$; Δ +0.8 mmu] and NMR data, which reflected a 14 mass unit (CH_2) decrease compared with that of **1**. The ^1H and ^{13}C NMR data of **2** were almost identical with those of **1**, except for the absence of the methoxy group signals. Therefore, the methoxy group in **1** was presumed to be replaced by an OH group in **2**. Analysis of COSY, HMQC, and HMBC data was consistent with the structure as shown. The imine configuration of **2** was again proposed to have the *E*-geometry on the basis of a NOESY correlation of H_3-11 to H-1′.

The isomeric compound usimine B (**3**) was assigned the same gross structure as **2** by analysis of its molecular formula, $C_{23}H_{23}NO_{10}$ [*m/z* 474.1393 $(M + H)^+$; Δ +0.7 mmu], and NMR data. The ^1H and ^{13}C NMR data for **3** were almost identical with those of **2**. The only noticeable differences were slight changes in the chemical shifts of the signals for C-2′, C-3′, C-4′, and C-5′. Thus, **3** was suggested to be a geometrical isomer of **2**, with the imine group in **3** having a *Z*-geometry. This assignment was consistent with the NOESY data, which showed no correlation between H_3-11 and H-1′.

Usimines A−C are related to usnic acid (**4**), a well-known lichen metabolite that is derived biosynthetically from oxidative phenolic coupling.[2,8] However, usimines **1**–**3** are unique usnic acid derivatives possessing a nitrogen-bearing side chain that is presumably derived from glutamic acid. Encountering secondary metabolites with an imine group is interesting since this functional group is generally relatively reactive and sensitive to hydrolysis.[9] It is unlikely that compounds **1**–**3** are produced artificially from usnic acid and glutamic acid during the isolation process because HPLC analysis of mixtures of usnic acid and glutamic acid that were dissolved in MeOH or MeOH containing 0.1% formic acid, and stirred at room temperature for 15 days, showed no chromatographic changes.

The absolute configurations of the chiral centers in **1**–**3** were determined by comparison of optical rotations and Marfey's derivatization of the mixture from acid hydrolysis. Treatment of **1** with 3 N HCl in THF resulted in conversion of **1** to **4**, as confirmed by HPLC and ^1H NMR analysis. The specific rotation of the resulting sample of **4** (+125) was comparable with that of (+)-usnic acid.[10] Thus the absolute configuration of C-9b in **1** was determined as *R*. The absolute configuration of the glutamic acid residue was determined by acid hydrolysis (6 N HCl, 120 °C, 24 h) of **1**, followed by derivatization with Marfey's reagent (1-fluoro-2,4-dinitrophnyl-5- L-alanine amide)[11] and subsequent HPLC analysis. By comparing the retention times of authentic standards of L- and D-Glu, the hydrolysate was identified to contain L-Glu. Thus, the absolute configuration of C-1′ in **1** was determined as *S*. The absolute configurations of compounds **2** and **3** were presumed to be analogous to that of **1**.

Compounds **1**–**4** moderately inhibited the activity of PTP1B in a dose-dependent manner, and their IC_{50} values were determined as 15.0 ± 0.1, 27.7 ± 2.1, 23.2 ± 3.2, and 16.4 ± 0.4 μM, respectively. A known phosphatase inhibitor, RK-682 (IC_{50} = 4.5 ± 0.5 μM), was employed as a positive control in the assay.[12,13] Inhibitors of PTP1B, a major nontransmembrane phosphotyrosine phosphatase in human tissues and a negative regulator of the insulin-stimulated signal transduction pathway,[14] are considered as potential agents in efforts to develop new treatments for type 2 diabetes and related metabolic syndromes.[15–17]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter. UV spectra were recorded on a Biochrom 1300 UV/visible spectrophotometer. ESIMS data were obtained using a Mariner ESI-MS instrument (Perseptive Biosystem, USA) at the Korean Basic Science Institute, Daejeon, Korea. NMR spectra (1D and 2D) were recorded in pyridine-d_5 and $CDCl_3$ using a JEOL JNM ECP-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), and chemical shifts were referenced relative to tetramethylsilane ($\delta_H/\delta_C = 0$). HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8 Hz, respectively. Solvents for extractions and open-column chromatography were reagent grade and used without further purification. Solvents used for HPLC were analytical grade. Flash column chromatography was carried out using Aldrich octadecyl-functionalized silica gel (C_{18}). HPLC separations were performed on a Shiseido Capcell Pak C_{18} column (10 × 250 mm; 5 μm particle size) with a flow rate of 2 mL/min. Compounds were detected by UV absorption at 254 nm.

Specimen Collection and Identification. *Stereocaulon alpinum* was collected and identified by one of us (J.H.Y.) from Barton Peninsula around King Sejong Station (S 62°13.3′, W 58°47.0′) on King George Island, Antarctica, in January 2003. Voucher specimens (reference L-5) have been deposited in the Korea Polar Research Institute.

Isolation of Compounds 1−4. A dried sample of *S. alpinum* (50 g) was extracted with MeOH (1 L × 2) for 24 h. The resulting crude MeOH extract (5.9 g) was subjected to C_{18}-functionalized silica gel flash column chromatography (3 × 15 cm), eluting with a stepwise gradient of 20%, 40%, 60%, 70%, 80%, 90%, and 100% (v/v) MeOH in H_2O (400 mL each). The fraction eluted with 70% MeOH (76 mg) was then subjected to semipreparative reversed-phase HPLC using a gradient from 50 to 91% CH_3CN in H_2O (0.1% formic acid) over 49 min to yield **1** (7.8 mg; t_R = 29.2 min). The fraction (56 mg) eluted with 60% MeOH in H_2O was subjected to semipreparative reversed-phase HPLC using a gradient from 40 to 69% CH_3CN in H_2O (0.1% formic acid) over 29 min to yield **2** (3.6 mg; t_R = 22.2 min) and **3** (2.0 mg; t_R = 27.2 min). Application of open silica gel column (2.5 × 50 cm) chromatography utilizing a stepwise gradient solution (hexane−CH_2Cl_2−MeOH) on the fraction eluted at 100% MeOH from the C_{18}-functionalized silica gel flash column chromatography yielded compound **4** (225.9 mg, eluted between 90% hexane−CH_2Cl_2 and 100% CH_2Cl_2).

Usimine A (1): yellow gum; $[\alpha]^{25}_D$ +39 (*c* 0.77, CH_2Cl_2); UV (CH_3OH) λ_{max} (log ϵ) 297 (4.1), 216 (4.0); ^1H NMR (pyridine-d_5, 400 MHz) δ 12.65 (OH-9), 6.15 (1H, s, H-4), 5.16 (1H, dd, *J* = 13.6, 5.9 Hz, H-1′), 3.59 (3H, s, 4′-OCH_3), 2.84 (3H, s, H_3-11), 2.79 (2H, m, H_2-3′), 2.66 (3H, s, H_3-13), 2.59 (2H, m, H_2-2′), 2.40 (3H, s, H_3-14), 1.72 (3H, s, H_3-15); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 201.2 (C-12), 198.6 (C-1), 190.3 (C-3), 175.1 (C-10), 174.1 (C-4a), 173.0 (C-5′), 172.9 (C-4′), 163.9 (C-7), 158.9 (C-9), 156.5 (C-5a), 107.8 (C-8), 106.0 (C-9a), 103.1 (C-2, C-4), 101.8 (C-6), 57.3 (C-9b), 57.0 (C-1′), 51.7 (4′-OCH_3), 31.8 (C-15), 31.2 (C-13), 30.0 (C-3′), 28.7 (C-2′), 19.2 (C-11), 8.1 (C-14); HMBC data (pyridine-d_5, 400 MHz) H-4 → C-2, C-4a, C-9b, C-15; H_3-11 → C-2, C-10; H_3-13 → C-6, C-12; H_3-14 → C-7, C-8, C-9; H_3-15 → C-1, C-4a, C-9a, C-9b; H-1′ → C-10, C-2′, C-3′, C-5′; H-2′ → C-1′, C-3′, C-5′; H-3′ → C-1′, C-2′, C-4′; 9-OH → C-8, C-9, C-9a; 4′-OCH_3 → C-4′; ^1H NMR ($CDCl_3$, 400 MHz) δ 13.33 (OH-7), 11.67 (OH-9), 5.84 (1H, s, H-4), 4.69 (1H, m, H-1′), 2.67 (3H, s, H_3-13), 2.65 (3H, s, H_3-11), 2.57 (2H, m, H_2–3′), 2.30 (2H, m, H_2-2′), 2.09 (3H, s, H_3-14), 1.71(3H, s, H_3-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.8 (C-12), 198.9 (C-1), 175.1 (C-4a), 172.6 (C-4′), 172.0 (C-5′), 163.7 (C-7), 158.2 (C-9), 155.9 (C-5a), 108.4 (C-8), 104.9 (C-9a), 102.3 (C-4), 101.5 (C-6), 57.6 (C-9b), 55.3 (C-1′), 52.2 (OCH_3), 32.0 (C-15), 31.4 (C-13), 29.3 (C-3′), 27.9 (C-2′), 18.9 (C-11), 7.6 (C-14); HMBC data ($CDCl_3$, 400 MHz) H-4 → C-1, C-4a, C-9b, C-15; H_3-13 → C-6, C-12; H_3-14 → C-7, C-8, C-9; H_3-15 → C-1, C-4a, C-9a, C-9b; H-1′ → C-2′, C-5′; H-2′ → C-1′, C-3′, C-4′, C-5′; H-3′ → C-1′, C-2′, C-4′; 7-OH → C-6, C-7, C-8; 9-OH → C-8, C-9; OCH_3 → C-4′; HRESIMS *m/z* 488.1552 $(M + H)^+$ (calc for $C_{24}H_{26}NO_{10}$, 488.1557).

Usimine B (2): yellow gum; $[\alpha]^{25}_D$ +159 (*c* 0.46, MeOH); UV (CH_3OH) λ_{max} (log ϵ) 297 (4.2), 216 (4.3); ^1H NMR (pyridine-d_5, 400 MHz) δ 6.08 (1H, s, H-4), 5.17 (1H, br dd, H-1′), 2.85 (2H, m, H_2-3′), 2.83 (3H, s, H_3-11), 2.73 (2H, m, H_2-2′), 2.66 (3H, s, H_3-13), 2.37 (3H, s, H_3-14), 1.70 (3H, s, H_3-15); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 201.2 (C-12), 198.4 (C-1), 190.1 (C-3), 174.9 (C-10), 174.3 (C-4′),

173.9 (C-4a), 173.6 (C-5′), 164.1 (C-7), 158.9 (C-9), 156.4 (C-5a), 107.7 (C-8), 106.0 (C-9a), 103.1 (C-4), 102.9 (C-2), 101.7 (C-6), 57.6 (C-1′), 57.2 (C-9b), 31.8 (C-15), 31.4 (C-3′), 31.2 (C-13), 29.5 (C-2′), 19.3 (C-11), 8.0 (C-14); HMBC data (pyridine-d_5, 400 MHz) H-4 → C-2, C-4a, C-9b, C-15; H_3-11 → C-2, C-10; H_3-13 → C-6, C-12; H_3-14 → C-7, C-8, C-9; H_3-15 → C-1, C-4a, C-9a, C-9b; HRESIMS *m*/*z* 473.1392 $(M + H)^+$ (calc for $C_{23}H_{24}NO_{10}$, 474.1400).

Usimine C (3): yellow gum; $[\alpha]^{25}_D$ +162 (*c* 0.43, MeOH); UV (CH_3OH) λ_{max} (log ϵ) 297 (4.2), 216 (4.3); ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.07 (1H, s, H-4), 5.16 (1H, br dd, H-1′), 2.93 (2H, m, H_2-3′), 2.86 (3H, s, H_3-11), 2.68 (2H, m, H_2-2′), 2.66 (3H, s, H_3-13), 2.37 (3H, s, H_3-14), 1.71 (3H, s, H_3-15); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 201.1 (C-12), 198.4 (C-1), 190.1 (C-3), 175.1 (C-5′), 174.8 (C-10), 173.8 (C-4a), 173.5 (C-4′), 164.1 (C-7), 159.0 (C-9), 156.5 (C-5a), 107.7 (C-8), 106.0 (C-9a), 103.1 (C-4), 103.0 (C-2), 101.7 (C-6), 57.6 (C-1′), 57.2 (C-9b), 31.8 (C-15), 31.2 (C-13), 30.7 (C-3′), 29.3 (C-2′), 19.3 (C-11), 8.0 (C-14); HRESIMS *m*/*z* 473.1393 $(M + H)^+$ (calc for $C_{23}H_{24}NO_{10}$, 474.1400).

(+)-Usnic acid (4): yellow needles; $[\alpha]^{25}_D$ +300 (*c* 0.54, CH_2Cl_2); ^{1}H, ^{13}C NMR data ($CDCl_3$) comparable to published data;[6] ESIMS *m*/*z* 345 $(M + H)^+$.

Acid Hydrolysis of Usimine A (1): Compound **1** (1.5 mg) was dissolved in a mixture of THF (1 mL) and 3 N HCl (1.5 mL) and heated (40 °C) for 12 h. After confirming the presence of **4** in the reaction mixture by HPLC analysis, the reaction mixture was concentrated and partitioned betwen CH_2Cl_2 and aqueous acid (pH = 2). The organic phase was dried and separated using a C_{18} cartridge [900 mg; elution with 10% and 100% (v/v) MeOH in H_2O] to afford 0.4 mg (eluted with 100% MeOH) of **4**, $[\alpha]^{25}_D$ +125 (*c* 0.04, CH_2Cl_2).

Preparation and Analysis of Marfey Derivatives. Compound **1** (1.5 mg) was hydrolyzed by heating in 6 N HCl (1 mL) at 110 °C for 24 h. After cooling, the solution was evaporated to dryness and redissolved in H_2O (50 μL). A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, 1-fluoro-2,4-dinitrophnyl-5-L-alanine amide)[11] in acetone was added to the mixture from acid hydrolysis. After addition of $NaHCO_3$ solution (1 M, 20 μL), the mixture was incubated for 1 h at 40 °C. The reaction was stopped by the addition of HCl (2 N, 10 μL), the solvents were evaporated to dryness, and the residue was dissolved in MeOH−H_2O (1:1; 1 mL). An aliquot of this solution (20 μL for **1** and 10 μL for the standards) was analyzed by HPLC [CapcellPak C_{18} column, linear gradient from 30 to 60% CH_3CN in H_2O (0.1% formic acid) over 60 min; 1 mL/min; 25 °C; 340 nm]. L-Glu, and D-Glu were derivatized with FDAA in the same manner as that of **1**. Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: L-Glu (32.7), D-Glu (33.9). The retention time of the peak in the HPLC trace of the FDAA-derivatized hydrolysis product of **1** was 32.4.

Assay Procedures. PTP1B (human, recombinant) was purchased from BIOMOL Research Laboratories, Inc. The enzyme activity was measured in a reaction mixture containing 2 mM *p*-nitrophenyl phosphate (*p*NPP) in 50 mM citrate, pH 6.0, 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT). The reaction mixture was placed in a 30 °C incubator for 30 min, and the reaction was terminated by addition of 1 N NaOH. The amount of *p*-nitrophenol produced was estimated by measuring the increase in absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM pNPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.[12,13]

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Supporting Information Available: 1D and 2D NMR spectra for compound **1**; ^{1}H NMR and ^{13}C NMR spectra for compounds **2** and **3**. This material is available free of charge on the Internet at http://pubs.acs.org.

References and Notes

(1) Huneck, S. *Naturwissenschaften* **1999**, *86*, 559–570.
(2) Ingólfsdóttir, K. *Phytochemistry* **2002**, *61*, 729–736.
(3) Lohezic-Le Devehat, F.; Tomasi, S.; Elix, J. A.; Bernard, A.; Rouaud, I.; Uriac, P.; Boustie, J. *J. Nat. Prod.* **2007**, *70*, 1218–1220.
(4) Kumar, K. C. S.; Müller, K. *J. Nat. Prod.* **1999**, *62*, 817–820.
(5) Ernst-Russell, M. A.; Chai, C. L. L.; Wardlaw, J. H.; Elix, J. A. *J. Nat. Prod.* **2000**, *63*, 129–131.
(6) Rashid, M. A.; Majid, M. A.; Quader, M. A. *Fitoterapia* **1999**, *70*, 113–115.
(7) Seki, T.; Satake, M.; Mackenzie, L.; Kaspar, H.; Yasumoto, T. *Tetrahdron Lett.* **1995**, *36*, 7093–7096.
(8) Sugawara, F.; Strobel, S.; Strobel, G.; Larsen, R. D.; Berglund, D. L.; Gray, G.; Takahashi, N.; Coval, S. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 909–910.
(9) Pelc, M. J.; Zakarian, A. *Org. Lett.* **2005**, *7*, 1629–1631.
(10) Conover, M. A.; Mierzwa, R.; King, A.; Loebenberg, D.; Bishop, R.; Puar, M.; Patel, M.; Coval, S. J.; Hershenhorn, J.; Strobel, G. A. *Phytochemistry* **1992**, *31*, 2999–3001.
(11) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(12) Hamaguchi, T.; Sudo, T.; Osada, H. *FEBS Lett.* **1995**, *372*, 54–58.
(13) Oh, H.; Kim, B. S.; Bae, E. Y.; Kim, M. S.; Kim, B. Y.; Lee, H. B.; Kim, C. J.; Ahn, J. S. *J. Antibiot.* **2004**, *57*, 528–531.
(14) Saltiel, A. R.; Kahn, C. R. *Nature* **2001**, *414*, 799–806.
(15) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug. Discovery* **2002**, *1*, 696–709.
(16) Dadke, S.; Chernoff, J. *Curr. Drug Targets-Immune, Endocr. Metabol. Disord.* **2003**, *3*, 299–304.
(17) Tonks, N. K. *FEBS Lett.* **2003**, *546*, 140–148.

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