Organic LETTERS

Letter

pubs.acs.org/OrgLett

Urupocidin A: A New, Inducing iNOS Expression Bicyclic Guanidine Alkaloid from the Marine Sponge *Monanchora pulchra*

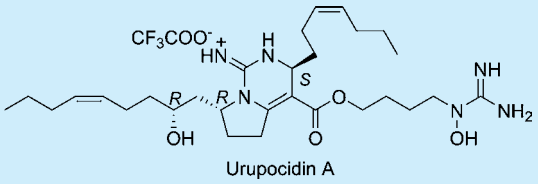
Tatyana N. Makarieva,*[,†] Ekaterina K. Ogurtsova,[†] Vladimir A. Denisenko,[†] Pavel S. Dmitrenok,[†] Ksenya M. Tabakmakher,[†] Alla G. Guzii,[†] Evgeny A. Pislyagin,[†] Andrey A. Es'kov,[†] Valery B. Kozhemyako,[†] Dmitry L. Aminin,[†] Yun-Ming Wang,[‡] and Valentin A. Stonik[†]

[†]G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-East Branch of the Russian Academy of Sciences, Vladivostok-22, Prospect 100-let, Vladivostok 159, Russia

[‡]Department of Biological Science and Technology, Institute of Molecular Medicine and Bioengineering, National Chiao Tung University, 75 Bo-Ai Street, HsinChu 300, Taiwan

Supporting Information

ABSTRACT: Urupocidins A and B (**1** and **2**), bisguanidine alkaloids with an unprecedented skeleton system, derived from polyketide precursors and containing an unusual *N*-alkyl-*N*-hydroxyguanidine moiety, have been isolated from the sponge *Monanhora pulchra*. The structures of **1** and **2**, including absolute configuration, were established using the detailed analysis of 1D and 2D NMR, CD, and mass spectra as well as chemical transformations. Compound **1** increases nitric oxide production in murine macrophages via inducing iNOS expression.

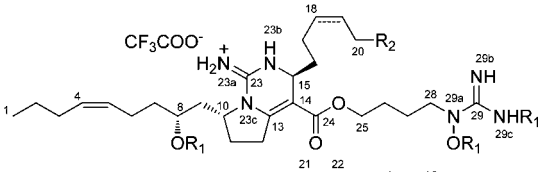


Urupocidin A

The discovery that NO is responsible for an astonishing range of physiological processes in humans presents one of the most exciting findings in biological chemistry.[1−4] The biosynthesis of NO is catalyzed by nitric oxide synthase (NOS), which is classified into three isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Both the endothelial NOS (eNOS) and the neuronal NOS (nNOS) are constitutive enzymes; however, the third isoform is inducible, produced by macrophages as part of the immune response. Therefore, the search for new natural modulators of iNOS may have therapeutic significance at the search for immunoactive compounds.[5]

Guanidine alkaloids are known to be characteristic metabolites of some marine sponges.[6,7] These alkaloids demonstrate a broad spectrum of biological activities, including potent cytotoxic effects, induction of cellular apoptosis, and inhibition of the TRPV1 receptor.[6−13] During our search for bioactive substances from marine organisms, we have previously isolated some guanidine alkaloids with unusual cyclic skeletons[8−10,13] and rare acyclic alkaloids[11,12] from sponges of the genus *Monanchora*. Herein, we describe the isolation and structure elucidation of urupocidins A (**1**) and B (**2**), possessing a new trisubstituted bicyclic skeleton system with an unprecedented *N*-alkyl-*N*-hydroxyguanidine fragment in one of the side chains, from a new collection of the sponge *Monanchora pulchra* (Figure 1). The induction of endogenous NO in Raw 264.7 cells and iNOS expression in macrophages by **1** is shown (Supporting Information). Urupocidins A and B (**1** and **2**) were named after one of the biggest Kuril Islands known as Urup Island, near which the sponge was collected.

The alkaloids were isolated from the frozen sponge (0.09% and 0.0024% of dry weight, respectively) by extraction with EtOH, evaporation, and partition between H_2O and *n*-BuOH, followed by the partition of the BuOH-soluble materials between aqueous EtOH and hexane, repeated column chromatography of the ethanol-soluble materials on Sephadex LH-20 (EtOH) and HPLC on an YMC-ODS-A column (65% EtOH/0.1% aqueous TFA).



1 R_1 = H; R_2 = CH_2CH_3; Δ^4Z; $\Delta^{18}Z$
1a R_1 = Ac; R_2 = CH_2CH_3; Δ^4Z; $\Delta^{18}Z$
2 R_1 = R_2 = H; Δ^4Z

Figure 1. Structures of urupocidins A and B (**1** and **2**) and urupocidin A triacetate (**1a**).

Urupocidin A (**1**), a colorless glass, has molecular formula $C_{29}H_{50}N_6O_4$ established by HRESIMS measurement of the $[M + H]^+$ ion peak at *m*/*z* 547.3956. The peak at *m*/*z* 274.2015 (calcd for $C_{29}H_{50}N_6O_4$ 274.2020) in the HRESIMS of **1** corresponds to a doubly charged $[M + 2H]^{2+}$ ion like similar peaks in MS of the earlier reported monanchomycalins A−C[10,13] and two-headed sphingolipids.[14]

NMR data (DMSO-d_6, Table 1) of **1** revealed the presence of two guanidine groups (δ_H 8.35 (2H), 9.39 (1H) and δ_C 150.6; δ_H 7.55 (3H) and δ_C 157.8), two methyl groups (δ_H 0.86, 0.85 and δ_C 13.57, 13.78), two hydroxy groups (δ_H 10.61,

Received: July 10, 2014
Published: August 5, 2014

dx.doi.org/10.1021/ol502013f | *Org. Lett.* 2014, 16, 4292−4295

Table 1. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR Data of Urupocidins A (1) and B (2)

	urupocidin A (DMSO-d_6)[a]		urupocidin B (DMSO-d_6)[a]			urupocidin A (DMSO-d_6)[a]		urupocidin B (DMSO-d_6)[a]	
position	δ_C	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)	position	δ_C	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)
1	13.64*	0.86*, t (7.3)	13.63*	0.86,* t (7.3)	16b		1.50, m		1.47, m
2	22.3*	1.33, sext (7.3)	22.3*	1.33, sext (7.3)	17a	21.5	1.93, m	29.1	1.25, m
3	28.74*	1.97, m	28.7	1.98, m	17b		2.07, m		
4	129.5	5.33, m	129.5	5.33, m	18	128.5	5.36, m	30.8	1.24, m
5	129.6	5.33, m	129.6	5.33, m	19	130.2	5.36, m	22.0	1.27, m
6a	23.0	2.00, m	23.0	2.00, m	20	28.68*	1.95, m	13.77*	0.85*, t (7.3)
6b		2.09, m		2.08, m	21	22.2*	1.31*, sext (7.3)		
7	38.2	1.39, m	38.1	1.40, m	22	13.57*	0.85*, t (7.3)		
8	66.8	3.51, m	66.8	3.51, m	23	150.6		150.5	
8-O*H*		4.70, brs		4.70, brs	23a-NH_2		8.35, brs		8.21, brs
9a	39.5	1.62, m	39.7	1.63, m	23b-N*H*		9.39, d (3.7)		9.17, d (3.9)
9b		1.68, m		1.66, m	24	164.2		164.2	
10	57.5	4.51, m	57.5	4.51, m	25a	63.8	4.11, m	63.7	4.10, m
11a	26.4	1.96, m	26.4	1.98, m	25b		4.14, m		4.15, m
11b		2.16, td (8.4; 4.0; 18.6)		2.15, m	26	25.2	1.65, m	25.2	1.63, m
12a	29.2	3.05, m	29.1	3.04, m	27	22.7	1.67, m	22.6	1.66, m
12b		3.13, ddd (8.4; 4.0)		3.13, m	28	50.4	3.55, brt	50.4	3.55, brt (6.6)
13	151.5		151.3		29	157.8		157.7	
14	101.0		101.4		29b-N*H*		7.55, brs		7.52, brs
15	48.9	4.30, dd (4.1; 3.8)	49.0	4.30, dd (4.1; 3.8)	29c-NH_2		7.55, brs		7.52, brs
16a	36.1	1.47, m	35.7	1.42, m	29a-N-O*H*		10.61, s		10.58, s

[a] ^{13}C NMR assignments supported by HSQC and HMBC data. *Overlapping signals.

4.70), two disubstituted double bonds (δ_H 5.33 (2H) and δ_C 129.5, 129.6; δ_H 5.36 (2H) and δ_C 128.5, 130.2), one tetrasubstituted double bond (δ_C 151.5, 101.0), two *N*-substituted CH groups (δ_H 4.51, 4.30 and δ_C 57.5, 48.9), one *N*-substituted CH_2 group (δ_H 3.55 and δ_C 50.4), one oxymethine (δ_H 3.51 and δ_C 66.8), one carbonyl-linked oxymethylene group (δ_H 4.11, 4.14 and δ_C 63.8), and 13 other methylene groups (Table 1). A quaternary carbon signal at δ_C 164.2 was assigned to the carbonyl group of an ester, which should be conjugated with a double bond according to the IR spectrum data.

Substructures **a**–**d** were established by COSY, HSQC, and HMBC experiments (Figure 2). Fragment **a** had never been previously seen in guanidine alkaloids[6,7] isolated from marine invertebrates. It was revealed starting from the signals of CH_2-12 group (δ_H 3.05, 3.13 and δ_C 29.2) and sequentially extended

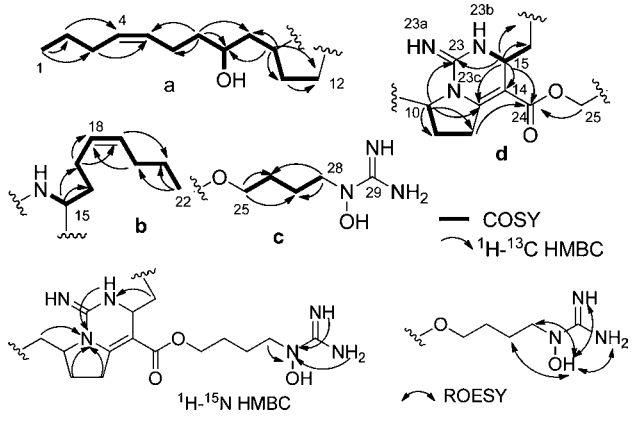


Figure 2. Partial structures of **1** with selected COSY, HMBC, and key ROESY correlations.

up to end methyl group that showed a higher field methyl triplet CH_3-1 in the ^{1}H NMR spectrum (δ_H 0.86 and δ_C 13.64).

Interpretation NMR data, starting from the low-field NH-23b doublet (δ_H 9.39) cross-over signals of Δ^{18}-olefin (δ_H 5.36 (2H) and δ_C 128.5, 130.2) and extending until to the high field CH_3-22 triplet (δ_H 0.85 and δ_C 13.57), indicated the substructure **b** (Figure 2).

Detailed analysis of NMR data concerning the substructure "**c**" led to the identification of four methylene groups of an open hydrocarbon chain, linked by one side to an oxygen atom (δ_H 4.11, 4.14 and δ_C 63.8) and by another side to a tertiary nitrogen atom of guanidine group (δ_H 3.55 and δ_C 50.4). Diagnostic ROESY correlations between the resonances of 29a-N-O*H* (δ_H 10.61) and 2H-27 (δ_H 1.67), 2H-28 (δ_H 3.55), 29b-N*H*, 29c-NH_2, as well as the $^{1}H-^{15}N$ HMBC correlations between 29b-N*H*, 29c-NH_2, 2H-28, and 29a-N indicated the position of N-OH group in substructure **c**. Moreover, it was confirmed by a downfield shift of proton and carbon signals of CH_2-28 group when compared with the corresponding signals in relative guanidine compounds.[15,16]

Substructure **d** and its connectivity with **a**–**c** was assigned by HMBC experiments, which indicated that the H-10 proton at δ_H 4.51 correlated to C-11 (δ_C 26.4), C-12 (δ_C 29.2), C-14 (δ_C 101.0), and C-23 (δ_C 150.6) signals. In addition, the H-15 proton at δ_H 4.30 correlated to C-23 (δ_C 150.6), C-13 (δ_C 151.5), C-14 (δ_C 101.0), C-24 (δ_C 164.2), C-16 (δ_C 36.1), and C-17 (δ_C 21.5), and protons CH_2–25 group was also correlated to C-24 (δ_C 164.2). Moreover, the $^{1}H-^{15}N$ HMBC correlations between 23b-N*H*, 16a,b-2H and 23b-N, 23b-N*H*, 11a,b-2H, 9a,b-2H, and 23c-N indicated that a bicyclyc moiety with two N atoms at positions 23b, 23c and three substitutions at position C-10, C-15, and C-14 presented in the compound **1** (Figure 1). The *Z*-geometry of double bonds was assigned using the NMR signals of allylic carbons (Table 1).[17,18]

dx.doi.org/10.1021/ol502013f | *Org. Lett.* 2014, 16, 4292–4295

When reacted with Ac_2O, urupocidin A gave the triacetate **1a**, the NMR spectrum of which showed, along with the expected signals of the urupocidin skeleton system, the characteristic singlets at δ_H 2.07 (3H) (δ_C 171.0), δ_H 2.17 (3H) (δ_C 181.5), and δ_H 2.20 (3H) (δ_C 168.7) attributable to two *O*-acetyl and one acetamide groups, respectively (Supporting Information, Table S2).

A determination of a relative stereochemistry of C-10 and C-15 by NOE experiments using **1** was unsuccessful as a consequence of the overlapping of 2H-9, 2H-26, and 2H-27 signals in the ^{1}H NMR spectrum (DMSO-d_6, Table 1) as well as of H-10 with H-15 and allylic signals with the H-11a signal in the ^{1}H NMR spectrum in CD_3OD (Supporting Information, Table S2). In order to determine their relative stereochemistry, the NOESY analysis in CD_3OD was applied to a tetrahydro derivative **1b** prepared from **1** (Figure 3). Diagnostic NOEs

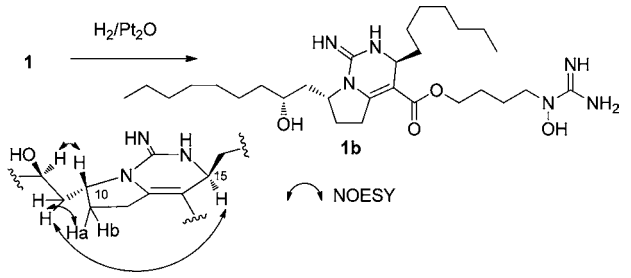


Figure 3. Hydrogenation of **1** and key NOEs correlations in **1b**.

correlations between the resonances of H-9a (δ_H 1.68, ddd, 2.4, 9.4, 12.4), H-15 (δ_H 4.41, dd, 4.1, 7.1), and H-11a (δ_H 2.04, dddd, 3.8, 5.1, 8.2, 13.0) were indicative of the 10*R** and 15*S** relative configurations in **1** (Figure 3). Absolute 15*S* configuration were proposed using CD (n → π* transition of the enone) and comparison with crambescin A2[19] (see the Supporting Information).

In order to determine the absolute configuration of C-8 asymmetric center in **1**, the modified Mosher's method was applied to the compound **3** prepared from **1** (Figure 4). Urupocidin A (**1**) was treated with NaOMe in methanol, and subsequent purification of the obtained products using reversed-phase HPLC gave the methyl ester (**3**). Esterification of **3** with (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)-

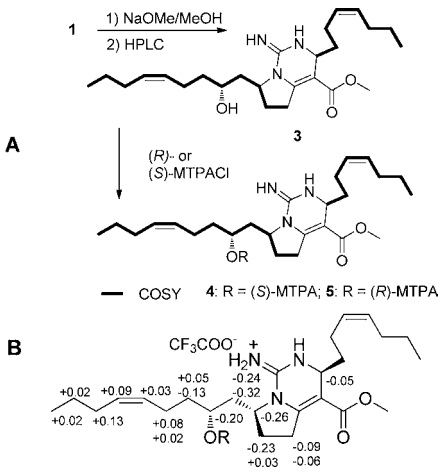


Figure 4. (A) Hydrolysis and derivatization of **1**. (B) $\Delta\delta_H^{SR}$ ($\delta_H^S - \delta_H^R$) values of derivatized products **3**.

phenylacetyl chlorides (MTPA-Cl) in an NMR tube[20,21] gave (*S*)- and (*R*)-MTPA esters **4** and **5**, respectively. Positive $\Delta\delta_H^{SR}$ values for the left-hand protons and negative $\Delta\delta_H^{SR}$ values for the most right-hand protons were observed in the ^{1}H NMR spectra of **4** and **5** (Figure 4). These data established the absolute configuration at C-8 as *R*. Therefore, the 8*R*,10*R*,15*S* absolute configuration in **1** was assigned.

Urupocidin B (**2**), as a colorless glass, has the molecular formula of $C_{27}H_{48}N_6O_4$ established by HRESIMS measurement of the $[M + H]^+$ ion peak at m/z 521.3816.

The ^{1}H NMR data (Table 1) of **2** closely coincided with those of urupocidin A (**1**), maintaining the Δ^{18}-olefin signals. In addition, MS data showed the molecular mass of **2** to be 26 amu less than that of **1**, suggesting that the hydrocarbon chain at C-15 is shortened by two CH_2 group in **2**. Comparison of the ^{1}H and ^{13}C NMR, and CD spectra and optical rotation data of urupocidin B (**2**) with those of urupocidin A (**1**) and their complete coincidence suggest the same absolute configurations of **2** as for **1**.

The structures of urupocidins A and B (**1** and **2**) possess uncommon structural features, including a trisubstituted bicyclic system and an *N*-alkyl-*N*-hydroxyguanidine moiety unprecedented in comparison with other marine guanidine alkaloids. So far, natural *N*-alkyl-*N*-hydroxyguanidines have been detected in some terrestrial microorganisms only.[22−27]

Compound **1** induced an expression of iNOS with increase in NO production in macrophages at 10.0 and 1.0 μM concentrations, respectively (see the Supporting Information).

Urupocidin A is the first marine alkaloid stimulator of NO production. To date, a few natural products have been reported to up-regulate NO level in cells; examples include polysaccharides from green alga *Capsosiphon fulvescens*[28] and *Strongylocentrotus nudus* eggs[29] and triterpene glycosides from *Panax ginseng*.[30]

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, copies of 1D and 2D NMR, CD spectra for compounds **1** and **2**, tabulated NMR data for compounds **1a**, **1b**, **3**, **4**, and **5**, and bioassay results. This material is available free via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: makarieva@piboc.dvo.ru.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research described in this publication was supported by Grant No. SSch 148.2014.4 from the President of RF, the Program of FEB RAS "Far East", and Grant No. 12-04-00749-a from RFBR.

■ REFERENCES

(1) Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science* **1992**, *258*, 1898−1902.

(2) Feldman, P. L.; Griffith, O. W.; Stuehr, D. J. *Chem. Eng. News* **1993**, *71*, 26−38.

(3) Butler, A. R.; Lyn, D.; Williams, H. *Chem. Soc. Rev.* **1993**, *22*, 233−241.

(4) Henry, Y.; Ducrocq, C.; Drapier, J. C.; Servent, D.; Pellat, C.; Guissani, A. *Eur. Biophys. J.* **1991**, *20*, 1−15.
(5) Kilbourn, R. G.; Griffith, O. W. *J. Natl. Cancer Inst.* **1992**, *84*, 827−831.
(6) Berlinck, R. G. S.; Trindade-Silva, A. E.; Santos, M. F. C. *Nat. Prod. Rep.* **2012**, *29*, 1382−1406.
(7) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160−258.
(8) Guzii, A. G.; Makarieva, T. N.; Denisenko, V. A.; Dmitrenok, P. S.; Kuzmich, A. S.; Dyshlovoy, S. A.; Krasokhin, V. B.; Stonik, V. A. *Org. Lett.* **2010**, *12*, 4292−4295.
(9) Makarieva, T. N.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Shubina, L. K.; Kuzmich, A. S.; Lee, H. S.; Stonik, V. A. *J. Nat. Prod.* **2011**, *74*, 1952−1958.
(10) Makarieva, T. N.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Kuzmich, A. S.; Lee, H. S.; Stonik, V. A. *Tetrahedron Lett.* **2012**, *53*, 4228−4231.
(11) Guzii, A. G.; Makarieva, T. N.; Korolkova, Y. V.; Andreev, Y. A.; Mosharova, I. V.; Tabakmaher, K. M.; Denisenko, V. A.; Dmitrenok, P. S.; Ogurtsova, E. K.; Antonov, A. S.; Lee, H. S.; Grishin, E. V. *Tetrahedron Lett.* **2013**, *54*, 1247−1250.
(12) Makarieva, T. N.; Ogurtsova, E. K.; Korolkova, Y. V.; Andreev, Y. A.; Mosharova, I. V.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Grishin, E. V.; Stonik, V. A. *Nat. Prod. Commun.* **2013**, *8*, 1229−1232.
(13) Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Lee, H. S.; Makarieva, T. N. *Nat. Prod. Commun.* **2013**, *8*, 1399−1402.
(14) Makarieva, T. N.; Dmitrenok, P. S.; Zakharenko, A. M.; Denisenko, V. A.; Guzii, A. G.; Li, R.; Skepper, C. K.; Molinski, T. F.; Stonik, V. A. *J. Nat. Prod.* **2007**, *70*, 1991−1998.
(15) Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Hallenga, K.; Ottinger, R.; Bruno, I.; Riccio, R. *Terahedron Lett.* **1990**, *31*, 6531−6534.
(16) Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Rogea, U. D.; Amade, P. *J. Nat. Prod.* **1992**, *55*, 528−532.
(17) Makarieva, T. N.; Santalova, E. A.; Gorshkova, I. A.; Dmitrenok, A. S.; Guzii, A. G.; Gorbach, V. I.; Svetashev, V. I.; Stonik, V. A. *Lipids* **2002**, *37*, 75−80.
(18) Snider, B. B.; Chen, J. *Tetrahedron Lett.* **1998**, *39*, 5697−5700.
(19) Bondu, S.; Genta-Jouve, G.; Leiros, M.; Vale, C.; Guigonis, J.-M.; Botana, L. M.; Thomas, O. P. *RSC Adv.* **2012**, *2*, 2828−2835.
(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(21) Su, B. N.; Park, E. J.; Mbwanbo, Z. H.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. R. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 1278−1282.
(22) Maehr, H.; Blount, J. F.; Pruess, D. L.; Yarmchuk, L.; Kellett, M. *J. Antibiot.* **1973**, *26*, 284−288.
(23) Perlman, D.; Vlietinck, A. J.; Matthews, H. W.; Lo, F. F. *J. Antibiot.* **1974**, *27*, 826−832.
(24) Seto, H.; Koyama, M.; Ogino, H.; Tsuruoka, T. *Tetrahedron Lett.* **1983**, *24*, 1805−1808.
(25) Dobashi, K.; Matsuda, N.; Hamada, M.; Naganawa, H.; Takita, T.; Takeuchi, T. *J. Antibiot.* **1988**, *41*, 1525−1532.
(26) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Lenoy, E.; Lotvin, J.; Petersen, P. J.; Siegel, M. M.; Singh, G.; Williamson, R. T. *J. Am. Chem. Soc.* **2002**, *124*, 10260−10261.
(27) Nemoto, A.; Hoshino, Y.; Yazawa, K.; Ando, A.; Mikami, Y. *J. Antibiot.* **2002**, *55*, 593−597.
(28) Na, Y. S.; Kim, W. J.; Kim, S. M.; Park, J. K.; Lee, S. M.; Kim, S. O.; Synytsya, A.; Park, Y. I. *Int. Immunopharm.* **2010**, *10*, 364−370.
(29) Liu, C.; Xi, T.; Lin, Q.; Xing, Y.; Ye, L.; Luo, X.; Wang, F. *Int. Immunopharm.* **2008**, *8*, 1835−1841.
(30) Kim, N. D.; Kim, E. M.; Kang, K. W.; Cho, M. K.; Choi, S. Y.; Kim, S. G. *Br. J. Pharmacol.* **2003**, *140*, 661−670.