Bunodosine 391: An Analgesic Acylamino Acid from the Venom of the Sea Anemone Bunodosoma cangicum

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · FEBRUARY 2011

Impact Factor: 3.8 · DOI: 10.1021/np100738m · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Bunodosine 391: An Analgesic Acylamino Acid from the Venom of the Sea Anemone *Bunodosoma cangicum*

André J. Zaharenko,[†,⊥,‡] Gisele Picolo,[∥,‡] Wilson A. Ferreira, Jr.,[∥,▽] Takanori Murakami,[o,§] Kohei Kazuma,[△] Masaru Hashimoto,[o,§] Yara Cury,[∥] José C. de Freitas,[▽] Motoyoshi Satake,[△] and Katsuhiro Konno*[,△]

[†]Biotechnology Center, Nuclear and Energy Research Institute, São Paulo 05508-000, Brazil

[⊥]Laboratory of Genetics, Butantan Institute, São Paulo 05503-900, Brazil

[∥]Laboratory of Pain and Signaling, Butantan Institute, São Paulo, 05503-900, Brazil

[▽]Institute of Biosciences, University of São Paulo, São Paulo 05508-900, Brazil

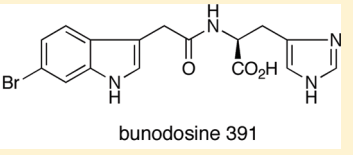
[o]Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan

[§]The United Graduate School of Agricultural Science, Iwate University, Hirosaki 036-8561, Japan

[△]Institute of Natural Medicine, University of Toyama, Toyama 930-0194, Japan

Supporting Information

ABSTRACT: A new acylamino acid, bunodosine 391 (BDS 391), was isolated from the venom of the sea anemone *Bunodosoma cangicum*. The structure was elucidated by spectroscopic analyses (2D NMR, ESIMS/MS) and verified by its synthesis. Intraplantar injection of BDS 391 into the hind paw of a rat induced a potent analgesic effect. This effect was not altered by naloxone (an opioid receptor antagonist), but was completely reversed by methysergide (a serotonin receptor antagonist), indicating that the effect is mediated by activation of serotonin receptors.

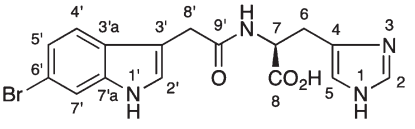


bunodosine 391

Sea anemones are known as a rich source of protein and peptide toxins.[1] In particular, peptide neurotoxins acting on the sodium and potassium channels have been well documented, and some of them are useful tools for pharmacological research of such ion channels.[2] In contrast, little is known about the non-peptidic, small molecules in sea anemones.[3] One example is caissarone, a purine derivative isolated from the Brazilian sea anemone *Bunodosoma caissarum*, which is an adenosine receptor antagonist.[4] Another is amphikuemin, a compound that induces symbiosis between the sea anemone and anemone fish.[5]

Sea anemones have characteristic stinging organelles, nematocysts, that are used for prey capture and defense.[6] The nematocysts discharge venom containing a high concentration of polypeptides and proteins that act as neurotoxins, hemolysins, and phospholipase A2 (PLA2) enzymes, which are responsible for a variety of pathological responses (cardiotoxicity, dermatitis, local itching, swelling, erythema, paralysis, pain, and necrosis). Nevertheless, the toxins have usually been purified from whole body extracts. In contrast, our group has developed a technique to obtain the sea anemone venom induced by electrical stimulation, which simplifies the purification process due to less contamination by other compounds.[7]

With this technique, we searched for new toxins in the venoms of the Brazilian sea anemones, *Bunodosoma cangicum* and *Bunodosoma caissarum*, which led to the isolation of new peptide neurotoxins and hemolysins.[8] Besides the protein and peptide fractions, we also investigated the small-molecule fractions based on a study reporting the antagonism of glutamate receptors by a low molecular weight fraction from the venom of the Caribbean sea anemone *Phyllactis flosculifera*.[9] As a consequence, we found a new acylamino acid, called bunodosine 391 (BDS 391, **1**). We now report the isolation, structure elucidation, and synthesis of **1** as well as the results of its biological evaluation, showing a potent analgesic activity mediated by serotonin receptors.



Bunodosine 391 (**1**)

RESULTS AND DISCUSSION

Electrical stimulation of the *B. cangicum* specimens in artificial seawater gave a cloudy solution of the venom, which was subjected to gel filtration chromatography on Sephadex G-50.

Special Issue: Special Issue in Honor of Koji Nakanishi

Received: October 15, 2010
Published: February 10, 2011

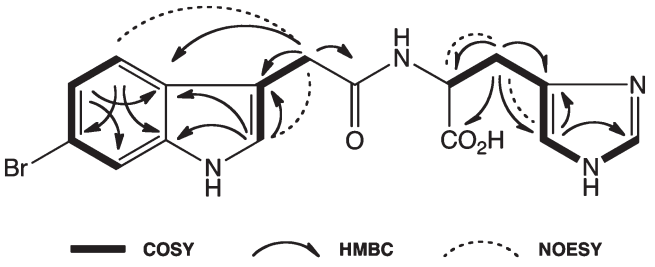


Figure 1. 2D NMR data for bunodosine 391 (**1**).

A small-molecule fraction that eluted as the final peak was then purified by reversed-phase HPLC to give pure BDS 391 (**1**).

ESIMS indicated that this compound has a bromine atom due to the peaks at m/z 391.03 and 393.04 $[M + H]^+$ at an approximate 1:1 ratio. FAB-HRMS revealed the molecular formula as $C_{16}H_{15}BrN_4O_3$ on the basis of the peak at m/z 391.0410 $[M + H]^+$, which confirmed the existence of a bromine in the molecule. The UV spectrum gave the typical absorption of indole at λ_{max} 285 nm with a shoulder at 294 nm. The presence of an indoleacetic acid moiety was indicated by the 2D NMR analysis (Figure 1). Three aromatic protons at δ_H 7.14 (dd, 2.0, 8.3 Hz, H-5′), 7.21 (d, 8.3 Hz, H-4′), and 7.63 (d, 2.0 Hz, H-7′) clearly indicated a 1,3,4-trisubstituted benzene substructure based on the correlation pattern demonstrated by COSY. The HMBC showed correlations from δ_H 7.21 (H-4′) to δ_C 137.1 (C-7′a) and from δ_H 7.14 (H-5′) to δ_C 126.5 (C-3′a), and these two carbon signals were further correlated with δ_H 7.18 (brs, H-2′), which disclosed a 3,6-disubstituted indole ring as shown in Figure 1. Furthermore the chemical shifts of the methylene signals at δ_H 3.66 and 3.56 (H-8′a/H-8′b) and their large coupling constant (15.6 Hz) indicated that these are located at a benzylic position and α to a carbonyl group. In fact, these protons were correlated to the two quaternary carbons at δ_C 108.0 (C-3′) and 126.5 (C-3′a) and further to the carbonyl carbon at δ_C 174.8 (C-9′), suggesting the presence of an indoleacetic acid moiety. It was further confirmed by the NOESY correlations between H_2-8′/H-2′ (δ_H 7.18, s) and H_2-8′/H-4′. The position of the bromine at C-6′ was deduced by HMBC correlations and its chemical shift value. The H-4′ resonance (δ_H 7.21) showed a strong HMBC signal with δ_C 114.9 (C-6′), which should be linked to a bromine atom based on its chemical shift. The rest of the signals were ascribable to a histidine moiety as described below. There was an acylated α-proton at δ_H 4.65 (H-7), which was coupled with a β-methylene at δ_H 3.21/2.97 (H-6a/H-6b). These methylene signals showed HMBC correlations with the carbon resonances at δ_C 128.7 (C-4) and 116.8 (C-5). Both of the aromatic proton signals at δ_H 6.76 (H-5) and 8.15 (H-2) gave HMBC correlations with carbons at δ_C 133.0 (C-2) and 128.7 (C-4), indicating the presence of an imidazole ring moiety. The two subunits were connected from C-7 to C-9′ through an amide linkage based on the chemical shift of the α-proton (δ_H 4.65) in the histidine, which was confirmed by an HMBC correlation between H-7 and C-9′. Thus, the complete structure of bunodosine 391 was deduced to be **1**.

The ESIMS/MS analysis supported this structure as shown in Figure 2. Notably, the product ions containing the bromine atom appeared as a doublet with a two m/z unit difference. Additionally, m/z 110 is a characteristic immonium ion arising from histidine.

The compound represented by **1** was synthesized in order to confirm the structure and determine the absolute configuration.

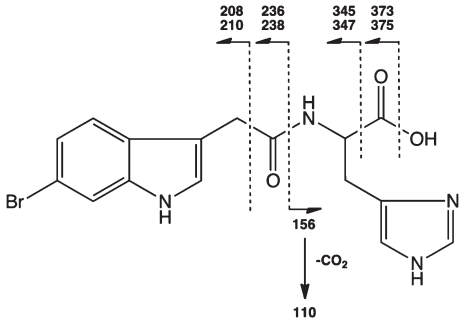


Figure 2. ESIMS/MS data for bunodosine 391 (**1**).

This was accomplished by following the procedure of Katritzky and co-workers for synthesizing indoleacetic acid−amino acid conjugates[10] as shown in Scheme 1. The starting material 6-bromoindoleacetic acid[11] was activated as a 1*H*-benzotriazol-1-yl amide,[10a] and the resulting active amide was then coupled with L-histidine in aqueous media in the presence of Et_3N to give the desired compound **1**. The synthetic specimen was identical to the natural product in all respects (HPLC, NMR, MS) including its specific rotation. Thus, the absolute configuration was determined to be *S*, in other words, the L-form, and structure **1** was unambiguously determined.

The structural similarity of the 6-bromoindole-3-acetic acid moiety in **1** to serotonin[12] prompted us to evaluate the possible effects of **1** on the nociceptive system because serotonin is a known mediator of pain and inflammation.[13] BDS 391 (**1**) induced an increased pain threshold in naive rats in a dose-dependent manner (Figure 3) and also inhibited the hyperalgesia induced by carrageenan (Figure 4), a standard model of inflammatory pain. These results suggested that BDS 391 displays an antinociceptive effect. This effect was not due to anti-inflammatory action, as BDS 391 did not interfere with the genesis or development of the edematogenic response induced by carrageenan (Figure 5). Furthermore, this effect was not altered by naloxone, a non-selective opioid receptor antagonist[14] (Figure 6A), but was completely reversed by methysergide, a nonselective serotonin receptor antagonist, suggesting the involvement of serotonin receptors in this effect (Figure 6B). The characterization of the type of serotonin receptors responsible for this effect is now in progress.

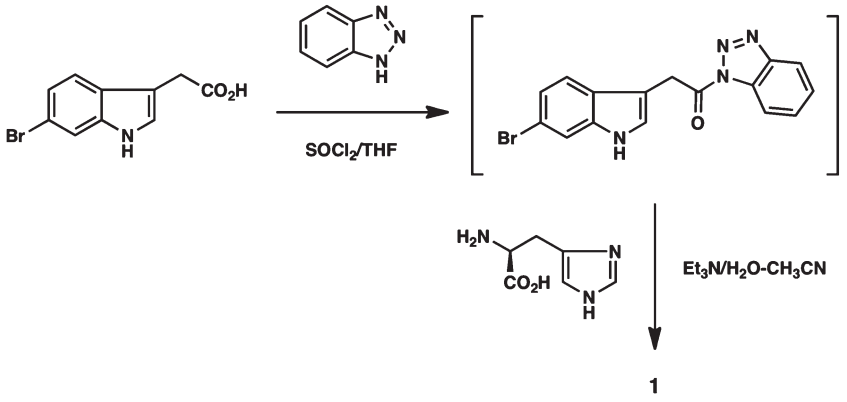
EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-320 digital polarimeter. The UV spectra were recorded on a Hitachi U-2010 spectrophotometer. The IR spectra were recorded on a Perkin-Elmer Spectrum 100. ^1H NMR and ^{13}C NMR spectra were measured on a JEOL ALPHA 400 (^1H 400 MHz, ^{13}C 100 MHz). Chemical shifts (δ) in ppm were determined relative to the solvent signals, δ_H (HDO) = 4.65 ppm and δ_C (CD_3OD) = 49.0 ppm. ESIMS and ESIMS/MS were measured on a Q-TOF Ultima API (Micromass). High-resolution FAB mass spectrum (FAB-HRMS) was measured on a JEOL JMS-SX102A. Reversed-phase HPLC analysis and purification were performed with a Shimadzu LC-20AB (analytical), a Shimadzu LC-10A (semipreparative), and a TOSOH CCPM (preparative).

Animal Material. Twenty *Bunodosoma cangicum* specimens were collected during low tides in the coastline of the city of São Sebastião, northern coast of São Paulo state, Brazil, in the coordinates of latitude 23°43′33″ S and longitude 45°24′41″ W. Identification was conducted

dx.doi.org/10.1021/np100738m |*J. Nat. Prod.* 2011, 74, 378–382

Scheme 1. Synthesis of Bunodosine 391 (1)



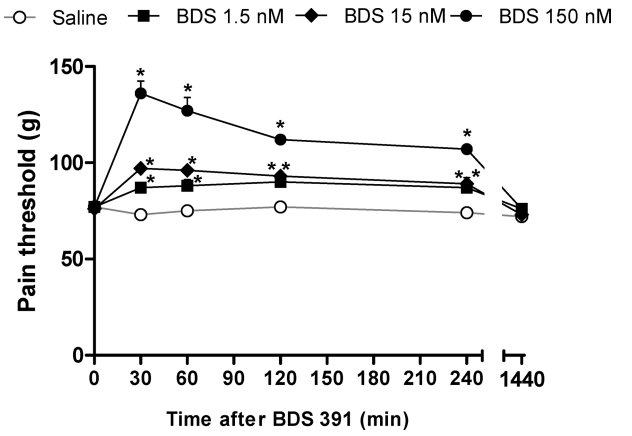


Figure 3. Increase in the pain threshold induced by BDS 391 (**1**). The paw pressure test was applied before and at different times after intraplantar (i.pl.) injection of BDS 391 (1.5, 15, and 150 nM/paw) in the naive rats. *Significantly different from values from control group (saline) ($p < 0.05$).

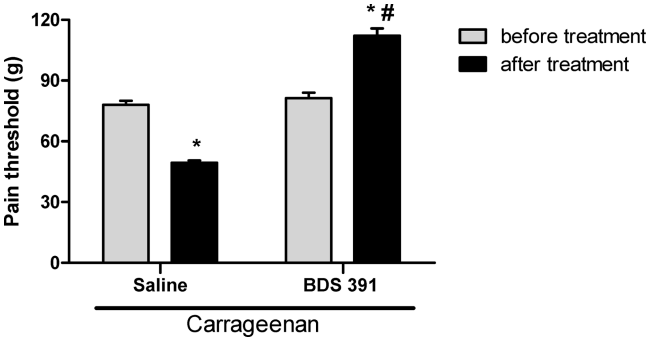


Figure 4. Antinociceptive effect of BDS 391 on hyperalgesia induced by carrageenan. The rat paw pressure test was applied before and 180 min after intraplantar (i.pl.) injection of carrageenan (200 μg/paw). BDS 391 (150 nM/paw) was administered 150 min after injection of carrageenan. #Significantly different from mean values before carrageenan treatment and *significantly different from mean values from control group (saline) ($p < 0.05$).

by two of the authors (A.J.Z. and J.C.F.). The voucher specimens (Code No. Bcg0001) are held at the University of São Paulo.

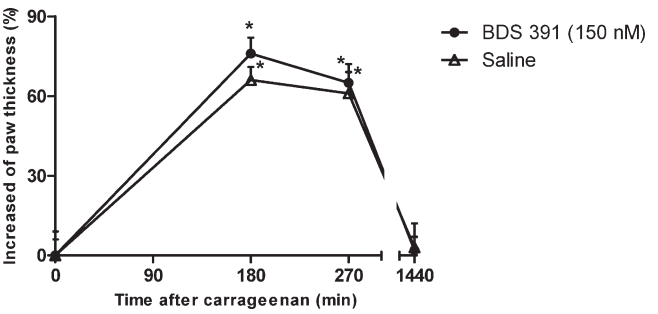


Figure 5. Effect of BDS 391 on edema induced by carrageenan. Paw edema was determined by measuring paw thickness using a digital caliper. BDS 391 (150 nM/paw) was injected 30 min before carrageenan (200 μg/paw). *Significantly different from mean values before treatments ($p < 0.05$).

Extraction and Isolation. Venom was obtained by electrical stimulation of animals as previously described.[7] The *B. cangicum* venom (approximately 70 mg) was fractionated by gel filtration chromatography using a Sephadex G-50 column (1.9 × 131 cm, Amersham Biosciences), according to the procedure as previously reported.[8b] The low molecular weight fraction (10 mg), which eluted in the final peak (Fr V), was submitted to reversed-phase HPLC using a semipreparative CAPCELL PAK C-18, 10 × 250 mm (Shiseido Corp.) column with a linear gradient of 15−50% MeCN/H_2O/0.1% TFA over 55 min at a flow rate of 2.5 mL/min, monitored at UV 214 nm. The fraction that eluted at 27 min gave bunodosine 391 as a pale brown syrup (5.5 mg): $[\alpha]^{28}_D$ +12.2 (*c* 0.027, H_2O); UV (H_2O) λ_{max} (log ε) 224 (4.51), 285 (3.75) nm; IR ν_{max} (neat) 3238, 3148, 3034, 2867, 2638, 1671, 1626, 1532, 1434, 1402, 1337, 1203, 1135, 1068, 1050, 896, 836, 801, 723 cm^{-1}; ^1H NMR (400 MHz, D_2O) and ^{13}C NMR data, see Table 1; ESIMS *m/z* 391.03/393.04 $[M + H]^+$; FAB-HRMS *m/z* 391.0410 $[M + H]^+$ calcd for $C_{16}H_{16}{}^{79}Br\ N_4O_3$ 391.0406.

Synthesis of Bunodosine 391. Thionyl chloride (9 μL, 0.13 mmol) was added to a solution of 1*H*-benzotriazole (59.0 mg, 0.50 mmol) in THF (380 μL), and the mixture was stirred at room temperature for 20 min. To the mixture was added 6-bromoindole-3-acetic acid (6BrIAA)[11] (33.0 mg, 0.13 mmol) in THF (125 μL). After the resulting reaction mixture was stirred at room temperature for 2 h, it was poured into 4 N HCl and extracted three times with EtOAc. The organic solution was washed with saturated NaCl solution, and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give crude 6BrIAA-Bt as a pale brown, amorphous powder (not weighed), which was dissolved in MeCN (1 mL). This solution was

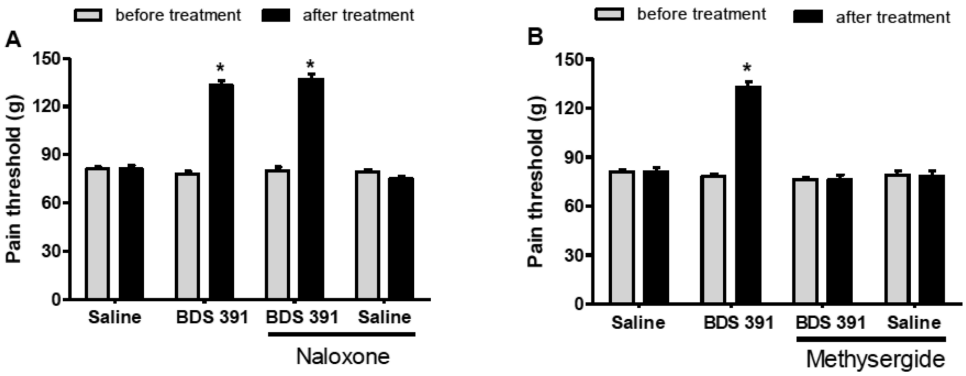


Figure 6. Effect of opioid and serotonin receptor antagonists on BDS 391-induced antinociception. The rat paw pressure test was applied before and 30 min after intraplantar (i.pl.) injection of BDS 391. Naloxone (1 μg/paw, panel A) and methysergide (5 mg/kg, i.p., panel B), opioid and serotonin antagonist receptors, respectively, were administered 30 min before BDS 391 (150nM/paw). *Significantly different from mean values from control group (saline) ($p < 0.05$).

Table 1. NMR Data for Bunodosine 391 in D_2O (^1H 400 MHz; ^{13}C 100 MHz)

position	δ_C, multi.	δ_H (m, J in Hz)
2	133.0, CH	8.15, d (1.9)
4	128.7, qC	
5	116.8, CH	6.76, brs
6	26.2, CH_2	a: 3.21, ddd, 1.0, 4.9, 15.6)
		b: 2.97, dd (10.3, 15.6)
7	51.6, CH	4.65, dd (4.9, 10.3)
8	173.9, CO	
2′	125.7, CH	7.18, brs
3′	108.0, C	
3′a	126.5, C	
4′	119.7, CH	7.21, d (8.3)
5′	122.4, CH	7.14, dd (2.0, 8.3)
6′	114.9, C	
7′	114.7, C	7.63, d (2.0)
7′a	137.1, C	
8′	32.4, CH_2	a: 3.66, d (15.6)
		b: 3.56, dd, 0.7, 15.6)
9′	174.8, CO	

added to a solution of L-histidine (22.4 mg, 0.13 mmol) in a 1:1 mixture of MeCN and H_2O (1.5 mL) in the presence of Et_3N (18 μL, 0.28 mmol). The reaction mixture was stirred at room temperature for 30 min and then allowed to heat at 40−50 °C for 10 min. After 6 N HCl was added, MeCN was removed under reduced pressure to give an aqueous solution. This solution was washed with EtOAc. The aqueous phase was loaded on a pre-equilibrated Sep-Pak C_{18} Plus cartridge (1 g, Waters) with aqueous 0.05 M TFA, then eluted with MeCN/H_2O (6/4, v/v, 3 mL). The eluted solution was concentrated under the reduced pressure, then purified by repeated preparative HPLC (solvent A, aqueous 0.05 M TFA/MeCN with 0.05 M TFA (19/1, v/v); solvent B, aqueous 0.05 M TFA/MeCN with 0.05 M TFA (1/1, v/v); elution, 0−50% B gradient in A for 120 min; column, Develosil C30-UG-5 (20 mm i.d. × 250 mm, Nomura Chemical Co.); flow, 5 mL/min; temp., 30 °C) to give pure bunodosine 391 (8 mg, 0.02 mmol; yield, 15%): $[\alpha]^{28}_D$ +9.4 (c 0.05, H_2O); IR ν_{max} (neat) 3261, 3150, 3028, 2860, 2631, 1655, 1623, 1529, 1434, 1409, 1336, 1188, 1133, 1067, 1050, 896, 832, 799, 721 cm^{-1}; see Supporting Information for a comparison of ^1H NMR spectra; ESI-HRMS m/z 391.0413 $[M + H]^+$ calcd for $C_{16}H_{16}{}^{79}BrN_4O_3$ 391.0406.

Evaluation of Pain Threshold. Male Wistar rats (170−190 g) were used throughout this study. Rats were housed in a temperature-controlled (21 ± 2 °C) and light-controlled (12/12 h light/dark cycle) room. All behavioral tests were performed between 9:00 A.M. and 4:00 P.M. Standard food and water were available ad libitum until 3 h before BDS 391 administration. After this period, only H_2O was available. All procedures were in accordance with the guidelines for the ethical use of conscious animals in pain research published by the International Association for the Study of Pain[15] and were approved by the Institutional Animal Care Committee at the Butantan Institute protocol no. 494/08 CEUAIB.

The pain threshold of animals was determined in the rat paw pressure test, in the presence or absence of hyperalgesia induced by intraplantar injection of 0.1 mL of sterile saline (0.85% NaCl solution) containing carrageenan (200 μg/paw, Marine Colloids) into one of the hind paws, as previously described.[16] The rat paw pressure test (Ugo-Basile) was applied as described by Randall and Selitto.[17] Testing was blind with regard to group designation. Briefly, increasing force (in g) was applied to the right hind paw. The force needed to induce paw withdrawal was recorded as the pain threshold. To reduce stress, rats were habituated to the testing procedure the day before the experiment. The test was applied before and at different times after BDS 391 administration.

Evaluation of Rat Paw Edema. Paw edema was induced by injection of carrageenan (200 μg/paw) into the subplantar tissue of rats' right hind paw. BDS 391 or sterile saline (control) were administered 30 min before carrageenan. The contralateral paw received the same volume of vehicle. Paw edema was determined by measuring paw thickness using a digital caliper (Mitutoyo Absolute Digimatic) at 0 (time before treatments), 180, 270, and 1440 min after carrageenan injection. Results were calculated as the difference of thickness of both paws, and edema was expressed as the percentage increase in paw thickness.

Pharmacological Treatments. BDS 391 was dissolved in sterile saline and administrated by intraplantar (i.pl.) route into the rat hind paw. In order to characterize some of the mechanisms involved in the action of BDS 391, naloxone (1 μg/paw), a nonselective opioid receptor antagonist, or methysergide (5 mg/kg, administered by intraperitoneal route), a nonselective serotonin receptor antagonist, was used to evaluate the involvement of the opioid and serotonin receptors, respectively. It is important to point out that these doses were based on the previous works from our group.[16]

■ ASSOCIATED CONTENT

Supporting Information. HPLC profile and spectroscopic data (UV, MS, ^1H and ^{13}C NMR) of bunodosine 391

dx.doi.org/10.1021/np100738m |J. Nat. Prod. 2011, 74, 378–382

and comparison of ^{1}H NMR spectra for natural and synthetic material. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +81-76-434-7605. Fax: +81-76-434-5055. E-mail: kkgon@inm.u-toyama.ac.jp.

Author Contributions

‡These authors contributed equally.

ACKNOWLEDGMENT

We thank Mr. K. Watanabe (GC-MS & NMR Laboratory, Graduate School of Agriculture, Hokkaido University) for FAB-HRMS measurements. Thanks are also due to Prof. Y. Igarashi (Toyama Prefectural University) for IR measurements. The grants from the Foundation for Research Support of the State of São Paulo, or FAPESP (01/14243-5 and 07/56525-3 to A.J.Z., 06/02892-2 and 08/01988-1 to W.A.F.J., 09/08089-5 to Y.C.), and the National Counsel of Technological and Scientific Development, or CNPq (563874/2005-8 to J.C.F.), are acknowledged.

DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

REFERENCES

(1) (a) Anderluh, G.; Macek, P. *Toxicon* **2002**, *40*, 111–124. (b) Turk, T.; Kem, W. R. *Toxicon* **2009**, *54*, 1031–1037. (c) Kristan, K. M.; Viero, G.; Serra, M. D.; Macek, P.; Anderluh, G. *Toxicon* **2009**, *54*, 1125–1134. (d) Suput, D. *Toxicon* **2009**, *54*, 1190–1200.

(2) (a) Honma, T.; Shiomi, K. *Mar. Biotechnol.* **2006**, *8*, 1–10. (b) Norton, R. S. *Toxicon* **2009**, *54*, 1075–1088. (c) Moran, Y.; Gordon, D.; Gurevitz, M. *Toxicon* **2009**, *54*, 1089–1101. (d) Wanke, E.; Zaharenko, A. J.; Redaelli, E.; Shiavon, E. *Toxicon* **2009**, *54*, 1102–1111. (e) Shiomi, K. *Toxicon* **2009**, *54*, 1112–1118. (f) Castañeda, O.; Harvey, A. L. *Toxicon* **2009**, *54*, 1119–1124.

(3) (a) Ackermann, D.; Janka, R. *Hoppe Seylers Z. Physiol. Chem.* **1953**, *294*, 93–97. (b) Howe, N. R.; Sheikh, Y. M. *Science* **1975**, *189*, 386–388. (c) Cimino, G.; Crispino, A.; De Rosa, S.; De Stefano, S.; Gavagnin, M.; Sodano, G. *Tetrahedron* **1987**, *43*, 4023–4030. (d) Zheng, G.-C.; Hatano, M.; Ishitsuka, M. O.; Kusumi, T.; Kakisawa, H. *Tetrahedron Lett.* **1990**, *31*, 2617–2618. (e) Zheng, G.-C.; Ichikawa, A.; Ishitsuka, M.; Kusumi, T.; Yamamoto, H.; Kakisawa, H. *J. Org. Chem.* **1990**, *55*, 3677–3679. (f) Stochaj, W. R.; Dunlap, W. C.; Shick, J. M. *Mar. Biol.* **1994**, *118*, 149–156. (g) For a review, see: Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcotec, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237.

(4) (a) Zelnik, R.; Haragushi, M.; Matida, A. K.; Lavie, D.; Frolow, F.; Weis, A. L. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2051–2053. (b) Freitas, J. C.; Sawaya, M. I. *Toxicon* **1986**, *24*, 751–755. (c) Freitas, J. C.; Sawaya, M. I. *Toxicon* **1990**, *28*, 1029–1037. (d) Cooper, R. A.; Freitas, J. C.; Porreca, F.; Eisenhour, C. M.; Lukas, R.; Huxtable, R. J. *Toxicon* **1995**, *33*, 1025–1031.

(5) (a) Murata, M.; Miyagawa-Kohshima, K.; Nakanishi, K.; Naya, Y. *Science* **1986**, *234*, 585–587. (b) Konno, K.; Qin, G.-W.; Nakanishi, K.; Murata, M.; Naya, Y. *Heterocycles* **1990**, *30*, 247–251.

(6) Özbek, S.; Balasubramanian, P. G.; Holstein, T. W. *Toxicon* **2009**, *54*, 1038–1045.

(7) Malpezzi, E. L. A.; Freitas, J. C.; Muramoto, K.; Kamiya, H. *Toxicon* **1993**, *31*, 853–864.

(8) (a) Oliveira, J. S.; Redaelli, E.; Zaharenko, A. J.; Cassulini, R. R.; Konno, K.; Pimenta, D. C.; Freitas, J. C.; Clare, J. J.; Wanke, E. *J. Biol. Chem.* **2004**, *279*, 33323–33335. (b) Oliveira, J. S.; Zaharenko, A. J.; Freitas, J. C.; Konno, K.; Andrade, S. A.; Portaro, F. C. V.; Richardson, M.; Sant'Anna, O. A.; Tambourgi, D. V. *Biochim. Biophys. Acta* **2006**, *1760*, 453–461. (c) Oliveira, J. S.; Zaharenko, A. J.; Ferreira, W. A., Jr.; Konno, K.; Shida, C. S.; Richardson, M.; Lúcio, A. D.; Beirão, P. S. L.; Freitas, J. C. *Biochim. Biophys. Acta* **2006**, *1764*, 1592–1600. (d) Zaharenko, A. J.; Ferreira, W. A., Jr.; Oliveira, J. S.; Konno, K.; Richardson, M.; Schiavon, E.; Wanke, E.; Freitas, J. C. *Toxicon* **2008**, *51*, 1303–1307. (e) Zaharenko, A. J.; Ferreira, W. A., Jr.; Oliveira, J. S.; Richardson, M.; Pimenta, D. C.; Konno, K.; Freitas, J. C. *Comp. Biochem. Physiol. D* **2008**, *3*, 219–225.

(9) Garateix, A.; Flores, A.; Garcia-Andrade, J. M.; Palmero, A.; Aneiros, A.; Vega, R.; Soto, E. *Toxicon* **1996**, *34*, 443–450.

(10) (a) Katritzky, A. R.; Khelashvili, L.; Munawar, M. A. *J. Org. Chem.* **2008**, *73*, 9171–9173. (b) Katritzky, A. R.; Angrish, P.; Todadze, E. *Synlett* **2009**, 2392–2411.

(11) Rasmussen, T.; Jensen, J.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1993**, *56*, 1553–1558.

(12) Hedner, H.; Sjögren, M.; Fraöndberg, P.-A.; Johansson, T.; Göransson, U.; Dahlström, M.; Jonsson, P.; Nyberg, F.; Bohlin, L. *J. Nat. Prod.* **2006**, *69*, 1421–1424.

(13) (a) Owen, D. A. A. *Br. Med. Bull.* **1987**, *43*, 256–269. (b) Sommer, C. *Mol. Neurobiol.* **2004**, *30*, 117–125. (c) Nakajima, K.; Obata, H.; Ito, N.; Goto, F.; Saito, S. *Eur. J. Pain* **2009**, *13*, 441–447.

(14) Cury, Y.; Picolo, G. *Drug News Perspect.* **2006**, *19*, 381–392.

(15) Zimmermann, M. *Pain* **1983**, *16*, 109–110.

(16) (a) Picolo, G.; Giorgi, R.; Cury, Y. *Eur. J. Pharmacol.* **2000**, *391*, 55–62. (b) Chacur, M.; Longo, I.; Picolo, G.; Gutiérrez, J. M.; Lomonte, B.; Guerra, J. L.; Teixeira, C. F. P.; Cury, Y. *Toxicon* **2003**, *41*, 667–678.

(17) Randall, L. O.; Selitto, J. J. *Arch. Inst. Pharmacodyn.* **1957**, *111*, 209–219.