Organic LETTERS

Letter

pubs.acs.org/OrgLett

Small Molecules in the Cone Snail Arsenal

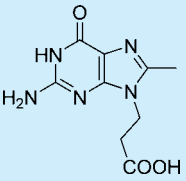
Jorge L. B. Neves,[†,∥] Zhenjian Lin,[‡,∥] Julita S. Imperial,[§] Agostinho Antunes,[†] Vitor Vasconcelos,[†] Baldomero M. Olivera,[*,§] and Eric W. Schmidt[*,§,‡]

[†]Interdisciplinary Centre of Marine and Environmental Research, CIIMAR/CIMAR, and Faculty of Sciences, University of Porto, 40150-123 Porto, Portugal

[‡]Departments of Medicinal Chemistry and [§]Biology, University of Utah, Salt Lake City, Utah 84112, United States

Supporting Information

ABSTRACT: Cone snails are renowned for producing peptide-based venom, containing conopeptides and conotoxins, to capture their prey. A novel small-molecule guanine derivative with unprecedented features, genuanine, was isolated from the venom of two cone snail species. Genuanine causes paralysis in mice, indicating that small molecules and not just polypeptides may contribute to the activity of cone snail venom.



Marine mollusks of the genus Conus produce bioactive peptides that are used in medicine.[1] Each snail synthesizes an array of different peptides, each of which targets a specific receptor or ion channel subtype, often with exquisite selectivity. The combination of these peptides, referred to as a "cabal", creates a unique response in prey animals, incapacitating prey and enabling consumption by the cone snail.[2] Although cone snails eat fish, polychaete worms, or other mollusks, because of the striking conservation of many ion channels and receptors across higher animals, the peptides are often highly selective to human proteins as well.

Because of these properties, cone snail research has focused on venom peptides, with good reason. Recently, we found potent neuroactivity in a venom fraction containing small molecules. Here, we describe the discovery of a novel guanine derivative, genuanine, which induces paralysis in mice. Given the extensive previous chemical characterization in cones, even in one of the species described below, it is remarkable that this bioactive guanine derivative with novel modifications of the purine ring was never characterized prior to the present study. Natural small molecule derivatives of nucleic acids, such as cytosine arabinoside, often have novel biological activity, sometimes with important biomedical applications.

Conus genuanus was collected in São Vicente, Cape Verde. *C. genuanus* belongs to a small number of cones with a pigmented venom duct, prompting us to search for small molecule pigments, which have not yet been characterized in cones. The red pigment is asymmetrically distributed when the venom is *in situ* in the venom duct of the snail. Dry venom collected from the red portion of the venom duct was extracted, providing a mixture of small molecules that was neuroactive when injected into mice. Careful examination of the venom components indicated several compounds, including unstable pigments that are under investigation. However, one of these, genuanine, was stable and exhibited a very similar paralytic activity in mice to what was seen in the crude small-molecule fraction.

Genuanine was isolated as a colorless solid (1 mg). Solubility issues and the small amount of available material limited the NMR analysis. The ^{1}H NMR spectrum showed only two methylene triplets (∂ 3.00, 4.43 ppm, t, J = 7.5 Hz) and one methyl singlet (∂ 2.74 ppm, s). An HSQC spectrum indicated chemical shifts consistent with adjacency of the methylenes to nitrogen and the methyl to an aromatic carbon. Indeed, a ^{15}N-HMBC spectrum revealed connectivity to two nitrogen units, as well as the proximity of the methylene and methyl groups. A ^{13}C-HMBC experiment indicated the substructure as shown in Figure 1a.

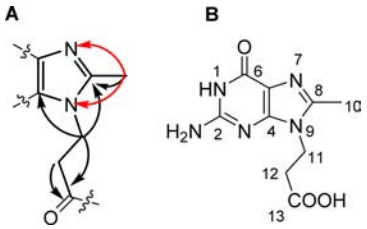


Figure 1. Structure of genuanine. (A) Key HMBC data used to determine the right ring substructure. Red arrows, ^{15}N-HMBC; black arrows, ^{13}C-HMBC. (B) Structure of genuanine.

Despite insufficient NMR data, the molecular mass (m/z = 238.0935) and a characteristic UV signature led us to speculate that the genuanine was a derivative of guanine (Figure 1b). Genuanine was synthesized from guanine in two steps. The synthetic material initially appeared on the basis of ^{1}H NMR spectroscopy to be different from the natural genuanine. However, upon mixing the natural and synthetic material together, it became apparent that the two are identical (Figure S1, 3.11 and 3.12), but that chemical shifts are extremely sensitive to slight changes in conditions (Figure S2). Combination of synthetic and natural genuanine in 35% DCl enabled measurement of a ^{13}C spectrum, providing further

Received: August 18, 2015
Published: September 30, 2015

DOI: 10.1021/acs.orglett.5b02389
Org. Lett. 2015, 17, 4933−4935

evidence of the structure as shown. Thus, the structure of genuanine was simultaneously elucidated and confirmed.

Compound **1** exhibited profound activity at doses as low as 40 nmol per mouse, in which the mouse was paralyzed in all four limbs for >2 h. However, best activity was obtained with fresh samples, and activity decreased with storage time (see Supporting Information for a complete table of all conditions and doses tested). This activity mimicked the potent paralytic activity found in the venom extract. The reason for this loss of activity over time has yet to be elucidated, but it was reproducible with both natural and synthetic **1**.

Initial attempts to improve synthetic yield also led to an array of regioisomers of genuanine, compounds **2**–**4** (Figure 2), for

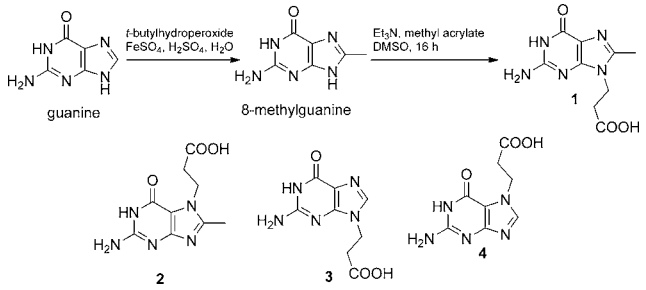


Figure 2. Synthesis of genuanine (top)[3] and synthetic isomers used in biological assays (bottom).

which no activity was detected (Table S1), suggesting a possible structure–activity relationship underlying neuroactivity. Other nucleic acid analogues with neuroactivity have previously been described. For example, a series of 8-oxoisoguanines from sponges inhibit GABAergic transmission in mice, while a sea anemone adenosine derivative caissarone is an adenosine receptor antagonist.[4] In the synthetic arena, guanine derivatives have been synthesized as purinergic receptor agonists and antagonists that act centrally,[5] among many other activities. It is highly speculative to connect any of these previous results to our observed activity here, but they provide further confidence that nucleic acids can affect paralysis and the CNS. It is noteworthy that genuanine is present in sufficient quantity to exert the same effect in the crude venom. Thus, we propose that **1** is an active constituent in the cone snail prey-capturing arsenal and not just a passive ingredient in the venom duct.

Venom peptides vary greatly between cone snail species,[1b] so we sought to determine whether genuanine was widespread or restricted to *C. genuanus*. At random, we selected nine additional cone snails collected from various locations in the Pacific Ocean for chemical analysis. We also selected *C. imperialis* from Oahu, Hawaii. While *C. imperialis* is not closely related to *C. genuanus*, it shares in common a pigmented venom duct (Figure 3) and, to the best of our knowledge, is the only other cone with a similar venom duct, having two regions of different colors. Only *C. genuanus* and *C. imperialis* contained genuanine. Other venom duct extracts were rich in nucleic acids, but these were the primary metabolites guanine and hypoxanthine.

Recently, it has been shown that *Conus geographus* venom peptides vary in different regions of the venom duct.[6] Similarly, in *C. genuanus*, the red (distal) and yellow (proximal) venom duct products are very different (Figures 4 and S6). While the proximal venom duct contains largely peptidic, high-molecular weight components, the distal venom duct is dominated by

Figure 3. Out of the ~800 known species of cone snails, *C. imperialis* (left) and *C. genuanus* (center) have the rare property of venom ducts containing red pigment at the distal end (right).

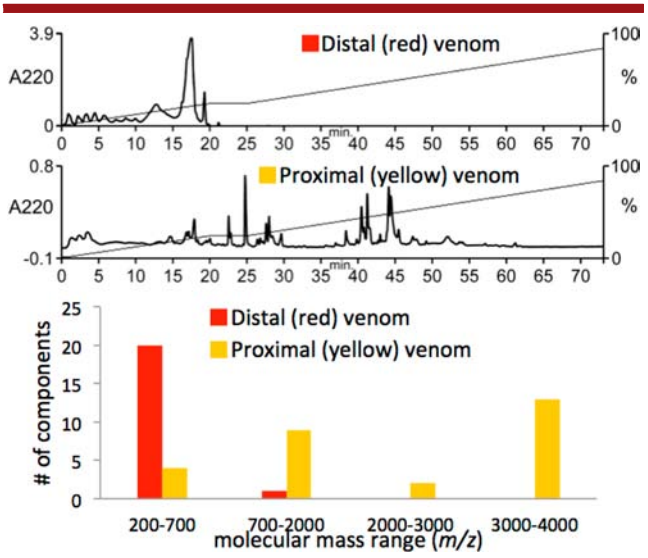


Figure 4. Venom components from *C. genuanus*. HPLC-UV traces show that the distal and proximal venom ducts contain quite different components (top). Enumeration of major peaks in HPLC-MS spectra show that, in contrast to the proximal duct, the distal duct is dominated by small molecules (bottom).

low-molecular weight materials, of which genuanine **1** is the major stable metabolite.

Genuanine belongs to a rare family of C-8 methylated nucleic acid derivatives. Nucleosides are methylated at C-8 by radical alkylators, such as procarbazine.[7] In nature, a C-8 methylated adenosine derivative is known as a component of bacteria rRNA;[8] its biosynthesis utilizes radical SAM enzymes.[9] To the best of our knowledge, these compounds were not known from eukaryotes nor was the methylated guanine or guanosine known in nature. Genuanine is also modified by propionate on N-9, which is a previously unknown modification of nucleic acids. Speculatively, this group could possibly also be added from methionine via a radical SAM enzyme, among other possible routes, possibly based upon tRNA metabolism. Alternatively, in other mollusks pigmented organs are associated with oxidative/radical metabolism[10] consistent with C-8 modification. A large family of nucleic acid analogues from marine animals. Most recently, several nucleosides from a sponge were produced by bacteria cultivated from the same sponge.[11] Both cone snails and their associated bacteria are known to produce compounds,[12] but determination of the ultimate source of genuanine awaits experiment.

DOI: 10.1021/acs.orglett.5b02389
Org. Lett. 2015, 17, 4933–4935

The finding of paralytic small molecules as venom components in cone snails was unanticipated. To the best of our knowledge, only one previous report documents a small molecule: serotonin was found in small amounts in the venom duct of *C. imperialis*.[13] Thus, it appears that small molecules are important and previously unrecognized contributors to the toxicity of cone snail venom.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02389.

Materials and methods, NMR, and MS spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: olivera@biology.utah.edu.
*E-mail: ews1@utah.edu.

Author Contributions

‖These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was funded by NIH R01GM107557 to E.W.S., NIGMS P01GM048677 to B.M.O., NIH ICBG U01TW008163 to E.W.S. and B.M.O., and UID/Multi/04423/2013, MARBIOTECH, and NOVOMAR. We thank J. Cox of the Utah Mass Spectrometry and Proteomics Core for mass measurements reported in this study; and the Foundation for Science and Technology (FCT-SFRH/BD/51477/2011), Fundação Calouste Gulbenkian, and University of Cape Verde (UniCV) for sample collection support.

■ REFERENCES

(1) (a) Brady, R. M.; Baell, J. B.; Norton, R. S. *Mar. Drugs* **2013**, *11*, 2293. (b) Robinson, S. D.; Norton, R. S. *Mar. Drugs* **2014**, *12*, 6058.

(2) Jimenez, E. C.; Shetty, R. P.; Lirazan, M.; Rivier, J.; Walker, C.; Abogadie, F. C.; Yoshikami, D.; Cruz, L. J.; Olivera, B. M. *J. Neurochem.* **2003**, *85*, 610.

(3) (a) Maeda, M.; Nushi, K.; Kawazoe, Y. *Tetrahedron* **1974**, *30*, 2677−2682. (b) Vo, D. D.; Staedel, C.; Zehnacker, L.; Benhida, R.; Darfeuille, F.; Duca, M. *ACS Chem. Biol.* **2014**, *9*, 711. (c) Kim, J. C.; Kim, M.; Jung, J.; Lee, J.; Ree, B. J.; Kim, H.; Kim, Ik J.; Kim, J. R.; Ree, M. *J. Polym. Sci., Part A: Polym. Chem.* **2015**, *53*, 1151. (d) Cheng, G.; Wang, M.; Villalta, P. W.; Hecht, S. S. *Chem. Res. Toxicol.* **2010**, *23*, 1089.

(4) (a) Sakurada, T.; Gill, M. B.; Frausto, S.; Noguchi, K.; Shimamoto, K.; Swanson, G. T.; Sakai, R.; Copits, B. *J. Med. Chem.* **2010**, *53*, 6089. (b) Cooper, R. A.; de Freitas, J. C.; Porreca, F.; Eisenhour, C. M.; Lukas, R.; Huxtable, R. J. *Toxicon* **1995**, *33*, 1025.

(5) Matasi, J. J.; Brumfield, S.; Tulshian, D.; Czarnecki, M.; Greenlee, W.; Garlisi, C. G.; Qiu, H.; Devito, K.; Chen, S. C.; Sun, Y.; Bertorelli, R.; Geiss, W.; Le, V. D.; Martn, G. S.; Vellekoop, S. A.; Haber, J.; Allard, M. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3805.

(6) Safavi-Hemami, H.; Hu, H.; Gorasia, D. G.; Bandyopadhyay, P. K.; Veith, P. D.; Young, N. D.; Reynolds, E. C.; Yandell, M.; Olivera, B. M.; Purcell, A. W. *Mol. Cell. Proteomics* **2014**, *13*, 938.

(7) Lemke, T. L.; Williams, D. A., Eds. *Foye's Principles of Medicinal Chemistry*; Lippincott Williams & Wilkins: Baltimore, MD, 2012.

(8) Giessing, A. M.; Jensen, S. S.; Rasmussen, A.; Hansen, L. H.; Gondela, A.; Long, K.; Vester, B.; Kirpekar, F. *RNA* **2009**, *15*, 327.

(9) Grove, T. L.; Benner, J. S.; Radle, M. I.; Ahlum, J. H.; Landgraf, B. J.; Krebs, C.; Booker, S. J. *Science* **2011**, *332*, 604.

(10) Derby, C. D. *Biol. Bull.* **2007**, *213*, 274.

(11) Bertin, M. J.; Schwartz, S. L.; Lee, J.; Korobeynikov, A.; Dorrestein, P. C.; Gerwick, L.; Gerwick, W. H. *J. Nat. Prod.* **2015**, *78*, 493.

(12) (a) Hillyard, D. R.; Olivera, B. M.; Woodward, S.; Corpuz, G. P.; Gray, W. R.; Ramilo, C. A.; Cruz, L. J. *Biochemistry* **1989**, *38*, 358. (b) Lin, Z.; Torres, J. P.; Ammon, M. A.; Marett, L.; Teichert, R. W.; Reilly, C. A.; Kwan, J. C.; Hughen, R. W.; Flores, M.; Tianero, M. D.; Peraud, O.; Cox, J. E.; Light, A. R.; Villaraza, A. J.; Haygood, M. G.; Concepcion, G. P.; Olivera, B. M.; Schmidt, E. W. *Chem. Biol.* **2013**, *20*, 73.

(13) McIntosh, J. M.; Foderaro, T. A.; Li, W.; Ireland, C. M.; Olivera, B. M. *Toxicon* **1993**, *31*, 1561.

DOI: 10.1021/acs.orglett.5b02389
Org. Lett. 2015, 17, 4933−4935