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Small molecules in the cone snail arsenal

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Abstract

Cone snails are renowned for producing peptide-based venoms, the conopeptides and conotoxins, to capture their prey animals. Here, we show that small molecules also contribute to the activity of the venoms. A novel guanine derivative, genuanine, causes paralysis in mice and is found in the venoms of at least two cone snail species. Genuanine contains unprecedented features for a natural nucleic acid.

Graphical abstract

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

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ASSOCIATED CONTENT

Supporting Information

Supporting Information. Materials and methods, NMR and MS spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI:.

Author Contributions

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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 was solely responsible for the paralytic activity in mice observed in the 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 ¹H 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 ¹⁵N-HMBC spectrum revealed connectivity to two nitrogen units, as well as the proximity of the methylene and methyl groups. A ¹³C-HMBC experiment indicated the substructure as shown in Figure 1a.

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 ¹H 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 ¹³C spectrum, providing further 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 4 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 guanine, compounds **2–4** (Figure 2), for which no activity was detected (Table S1), suggesting a possible structure-activity relationship underlying neuroactivity. Other nucleic acid analogs 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 guanine is present in sufficient quantity to exert the same effect in the crude venom. Thus, we propose that **1** is an active constituent 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 guanine was widespread or restricted to *C. gualanensis*. At random, we selected 9 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. gualanensis*, it shares in common a pigmented venom duct and to the best of our knowledge is the only other cone with a similar venom duct, having 2 regions of different colors. Only *C. gualanensis* and *C. imperialis* contained guanine. 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. gualanensis*, the red (distal) and yellow (proximal) venom duct products are very different (Figures 3 and S6). While the proximal venom duct contains largely peptidic, high-molecular weight components, the distal venom duct is dominated by low-molecular weight materials, of which guanine **1** is the major stable metabolite.

Guanine belongs to a rare family of C-8 methylated nucleic acid derivatives. Nucleosides are methylated at C-8 by radical alkylators, such as procabazine.⁷ In nature, a C-8 methylated adenosine derivative is known as a component of bacteria ribosomal RNA;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. Guanine 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 metabolism¹⁰ consistent with C-8 modification. A large family of nucleic acid analogs 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 genganine awaits experiment.

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 venoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

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

Figure 3.

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).