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Structure, synthesis and biological properties of the pentacyclic guanidinium alkaloids

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Abstract

The pentacyclic guanidinium alkaloids (PGAs) are a family of marine natural products that possess a poly-cyclic guanidine-containing core and a long alkyl chain tethered spermidine-derived tail that is rarely observed in other natural products. These natural products exhibit potent activities on a wide range of organisms and therefore have attracted the attention of many synthetic chemists; however, the structure-activity relationships and mechanisms of action of PGAs remain largely elusive. Herein we summarize the structure, synthesis, toxicity and mechanisms of action of PGAs and highlight their potential as chemical probes and/or therapeutic leads.

Keywords

Guanidinium alkaloids; Marine natural products; Chemical probe; Structure-activity relationship; Mechanism of action

1. Introduction

For many years the diverse molecular architectures of natural products have been a major source of inspiration for both novel reaction development and therapeutic lead molecules. The majority of the natural products utilized as leads in drug discovery and development research are of terrestrial origin; however, over the past few decades the marine environment has been demonstrated to be a prolific source of chemical and biological diversity.¹

Despite the enormous diversity offered by marine life, this source of novel small molecules remains largely unexplored, partially due to the lack of necessary technologies required for sample collection and the difficulties in the isolation and purification of the many metabolites obtained in a single sample.² Nevertheless, in a matter of only a few decades of exploration, seven marine natural products, cytarabine (Cytosar-U[®], Depocyt[®]), vidarabine (Vira-A[®]), ziconotide (Prialt[®]), omega-3-acid ethyl esters (Lovaza[®]), eribulin mesylate (Halaven[®]), brentuximab vedotin (Adcetris[®]) and trabectedin (Yondelis[®]) have become approved by the Food and Drug Administration (FDA) in the United States, along with a plethora of other molecules in different phases of preclinical and clinical development.³ Through the emergence of recent technological advancements such as robotic collection,

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aquaculture, “smart screening” along with advances in synthetic chemistry and biology,⁴ more attention has been directed to exploit the biodiversity that is offered by the marine environment.⁵

The guanidine motif is abundant in nature and is observed in the amino acid arginine (**1**) and the nucleobase guanine (**2**) (Fig. 1). Because of the basicity of guanidine ($pK_a = 13.6$), its conjugate acid, guanidinium cation (**3**), binds with anionic substrates such as carboxylates or phosphates.⁶ Marine natural products containing the guanidine functionality exhibit a broad spectrum of biological activities, attributed to the multiple ways that the guanidinium cation engages in non-covalent interactions.⁷

Among the guanidine containing scaffolds, pentacyclic guanidinium alkaloids (PGAs) are a class of marine natural products that bear a (5,6,8b)-triazaperhydroacenaphthalene skeleton (**4**) and two hemiaminal rings as their core structure.^{8–10} The first member of this class to be reported, ptilomycin A (**6**, Fig. 2), was isolated from the Caribbean sponge *Ptilocaulis spiculifer* and from the Red Sea sponge *Hemimyscale* sp. in 1989 by Kashman and co-workers.¹¹ In the following years, several PGA families, namely the crambescidins^{12–15}, neofolitispates¹⁶, fromiamycalin¹⁷, celeromycalin¹⁷, monanchocidins^{18,19}, monanchomycalins^{20,21} and normonanchocidins²² have been isolated, primarily from marine sponges and other aquatic organisms.

Most members of the PGAs possess similar structural features, including a densely functionalized and rigid pentacyclic guanidinium core (the “vessel unit”, such as **5**, **19** or **23**) tethered via a long hydrocarbon fatty acid chain at the C-14 ester linkage to a spermidine or a spermidine-derived moiety (the “anchor unit”).^{8–10} The guanidinium core **5** is shared among the majority of the members of this class with a few exceptions.

13,14,15-Isocrambescidin 800 (**20**) possessing a *trans*-ring juncture is the diastereomer of crambescidin 800 (**13**) with three alternate stereocenters in its pentacyclic core.²³ The variation of stereochemical configuration in PGAs is generally limited to the pentacyclic guanidinium core, specifically in the pyrrolidine sub-unit. In addition to the observed stereochemical diversity, members such as monanchocidin A (**24**), D (**25**) and E (**26**) and monanchomycalin A (**21**) feature a 5-membered spiro-ring versus the more frequently observed 7-membered spiro-ring. As highlighted in Fig. 2, the majority of the structural diversity in this class of natural products is contained in the pendent ester linkage and the anchor unit. Members of this class feature ester linkages and anchor units of varying chain length, substitution pattern, and oxidation states. Other structural variations include the unusual and heavily oxygenated morpholinone fragment, which is a unique feature only observed in the monanchocidin family, and to date is the most complex “anchor unit” observed in this class of natural products.^{18,19} Moreover, crambescidin 359 (**11**) isolated from the marine sponge *Monanchom unguiculata* in 2000, is the first member of this class devoid of the C-14 ester linkage.¹⁴

Overall, the pentacyclic guanidinium natural products represent some of the most complex guanidinium alkaloids isolated to date. The intricacy and novelty observed in the structure of the PGAs, coupled with the wide range of biological activities exhibited by these molecules

have attracted significant attention from the scientific community. Indeed, PGAs have been the subject of intense study by numerous research groups, and significant synthetic contributions have been made in this area. A brief summary of those synthetic efforts is highlighted in the next section. Despite the elegant approaches to these natural products, surprisingly little information regarding the true interaction of these molecules with biological targets is known. Herein, we offer a summary of the important biological activities exhibited by the vessel and the anchor units of the PGAs, and highlight a few of the many unanswered questions that remain.

2. An overview of synthetic approaches

As mentioned earlier, the structural complexity, low natural abundance, and impressive biological activities exhibited by the PGAs have made these natural products exciting targets for total synthesis. Several members of the batzelladine, crambescidin, and ptilomycalin families have been accessed through total synthesis.^{8,10} The challenges faced in all of the approaches to this class of natural products have highlighted the many difficulties associated with the synthesis of charged and densely functionalized structures in an asymmetric fashion. These efforts have provided the first synthetic access to this class of natural products and in some limited studies the material obtained through synthesis has provided insight into the biological potential offered by these alkaloids.

The Snider group developed a synthetic route to the methyl ester of the guanidinium core of ptilomycalin A (**6**), based on a cascade approach (Scheme 1).²⁴ The use of a cascade approach was of particular interest to them based on the hypothesis that the biogenesis of the natural product follows a similar path. Their synthesis relied on the construction of bis-enone intermediate **31**, followed by the Michael addition of *O*-methylisourea **32** to generate intermediate **33** as a mixture of diastereomers. Ammonolysis of intermediate **33**, followed by removal of silyl ether protecting groups, and the subsequent imine and hemiaminal formation resulted in the desired pentacyclic guanidinium core and the corresponding diastereomer. Overall, this synthesis was accomplished in 14 steps with a 2.7% yield starting from commercially available materials. Most notably, their synthesis demonstrated the feasibility of a cascade approach in complex guanidinium natural product synthesis. This approach was also utilized for the construction of the tricyclic guanidinium core of other structurally related natural products such as the batzelladine family.²⁵ A conceptually similar strategy, also utilizing a linear precursor cyclization cascade approach was employed by the Murphy group in the synthesis of crambescidin 359 (**11**), and the core of the tricyclic guanidinium alkaloids such as the batzelladine family.^{10,26–32} Their synthetic approach utilized guanidine directly in place of a guanidine surrogate in the double Michael addition to the requisite bis-enone intermediate.

The first significant step toward the asymmetric total synthesis of the PGAs was achieved through the work of the Overman group, who has been one of the pioneers in this research area. The first total synthesis of ptilomycalin A (**6**) was accomplished by the Overman group by employing a tethered Biginelli reaction.^{33,34} To this end, β -keto ester **34** and compound **35** served as the urea and the masked aldehyde units of this reaction to furnish intermediate **36**. Silyl deprotection of **36**, and further functional group manipulations resulted in

formation of a single pentacyclic guanidinium core in 7% yield over 13 linear steps. Overall, this synthesis was a landmark achievement which allowed for the first enantioselective preparation of this core. The versatility of the tethered Biginelli condensation was further extended to the synthesis of several other tricyclic guanidinium alkaloids as well. More notably, their synthetic strategy not only allowed for the first enantioselective total synthesis of a pentacyclic guanidinium natural product, but the sequence also controlled the stereochemical configuration of either *cis* or *trans* pyrrolidine via the proper choice of reaction conditions, which ultimately allowed for the synthesis of other polycyclic guanidinium alkaloids such as isocrambescidin 800 (**20**).^{35–37}

The Nagasawa group has also reported a total synthesis of crambescidin 359 (**11**), the PGA lacking the ester linkage at the C-14 position.³⁸ Their synthesis was accomplished using a completely distinct synthetic strategy toward the guanidinium core, relying on successive 1,3-dipolar cycloaddition reactions of an optically active nitron **37** and an olefin **38** to generate the isoxazolidine **39**. Notably, both cycloaddition reactions proceeded with complete regio- and stereocontrol. Subsequent cleavage of the isoxazolidine **39** using *m*-CPBA, followed by reduction of the corresponding nitron resulted in the targeted 2,5-*cis* pyrrolidine intermediate, which after further functionalization through oxidation, installation of the guanidine moiety, and acid-mediated cyclization resulted in the natural product. This synthetic route was initially developed for the synthesis of crambescidin 359 (**11**), but was later extended to the synthesis of the tricyclic guanidinium core of batzelladine A, which possess the *anti*-configuration of the pyrrolidine ring and the ester linkage at C-14. Using their synthetic strategy, they also designed and synthesized a small collection of C₂-symmetric pentacyclic guanidinium derivatives for their application as phase-transfer catalysts in asymmetric alkylation of *N*-glycinate Schiff's base using various alkylating reagents.⁹ This design was based on the charged and cage-like structure of the PGAs, which could serve as a chiral cavity to interact with a guest anion molecule through ionic and hydrogen binding interactions.

As part of efforts to further investigate the chemistry and biology of PGAs, the Pierce group developed a rapid synthesis of the unusual and heavily oxygenated morpholinone fragment (**43**) of the monanchocidins.³⁹ The key reaction of this approach features an acid promoted hemiketalization/hemiaminalization of α -hydroxyamide **40** and α -ketoaldehyde **41** that proceeds with exclusive regioselectivity and high diastereoselectivity (up to 9:1 dr) to form the morpholinone **42** in moderate yield.

3. Toxicity and general SAR picture

Cytotoxic, antibacterial, antiviral, antifungal and antiprotozoal activities of PGAs have been reported (Table 1).

In general, these toxic effects appear to be non-selective for various organisms. For example, pitilomycalin A (**6**) displays anti-HIV-1 activity in human PBM cells with EC₅₀ and EC₉₀ of 11 nM and 46 nM, respectively, antibacterial activity against both *S. aureus* and methicillin-resistant *S. aureus* with an MIC of 0.63 μ g/mL, activity against protozoan parasites *P. falciparum* D6 and W2 (IC₅₀ = 0.11–0.12 μ g/mL), and cytotoxicity against multiple human

cancer cell lines with GI₅₀ values ranging from 0.03 to 0.08 µg/mL.⁴⁰ However, Dyshlovoy et al. reported that monanchocidin A (**24**) displayed up to 4-fold higher potency against several cancer cell lines than non-malignant cell lines.⁴⁷ Monanchocidin A (**24**) was also equally active in cisplatin-sensitive and -resistant germ cell tumor (GCT) cell lines. Strong synergistic effects of combined treatment of monanchocidin A (**24**) and cisplatin were observed as well⁴⁷

Although there are no systematic studies on the structure-activity relationship (SAR) of PGAs across organisms or therapeutic areas, some generalizations can be made based on previous reports (Fig. 3). Most biologically active PGAs are fully functionalized with all three structural units (guanidinium core, aliphatic linker and spermidine tail), and the absence of the spermidine unit and/or the linker (for example: crambescidic acid (**14**)⁴² and crambescidin 359 (**11**)^{31,50}) results in significant loss of activity.

There are five stereocenters in the guanidine-containing tricycle which frames the skeleton of the guanidinium core. A diastereomer of crambescidin 800 (**13**), 13,14,15-isocrambescidin 800 (**20**) which possesses a completely different conformation has been shown to have diminished antiviral and cytotoxic activities.⁴⁵ On the other hand, the ring size of the aminated spiro-rings appears to have little impact on the activity of PGAs, as two types of spiro-rings possess similar biological activities in the monanchocidin family.^{18,19} Additionally, the Murphy group synthesized an analog (**45**) of ptilomycalin A (**6**) featuring two simplified six-membered spiro-rings (Fig. 5), and obtained comparable levels of activity to that of the natural product.²⁹

The Overman group developed a series of side chain analogs of crambescidin 800 (**13**) and discovered a trend of increased activity (as determined by GI₅₀ values) with an increase in the length of the linker (6 to 16 carbons, the natural product contains 16 carbons).⁵⁰ Interestingly, replacing the linker and spermidine unit with non-polar groups such as in the cinnamyl analog **46** (Fig. 5), also generated a potent cytotoxic compound, which further complicates the side chain requirement for an active PGA.⁵⁰

The spermidine unit in PGAs are often oxidized to varying degrees, from a hydroxylspermidine (e.g. crambescidin 800), to a heavily oxidized morpholinone ring (e.g. monanchocidins). Normonanchocidins A, B and D (**27–29**) lose an aminobutyl group in their spermidine unit. PGAs with a truncated or modified spermidine unit appear to be slightly less active;⁴¹ however, the SAR profile of the spermidine unit has yet to be directly studied.

4. Mechanism of action

Although there has been significant synthetic study and biological screening of PGAs since their first isolation in 1989, the mechanisms behind their striking biological activities are less well studied. In the past decade, the isolation of several potent PGA families from the marine sponge *Monanchora pulchra* has reignited the desire to explore the treasure trove of biology surrounding these exciting natural products.^{18–22} In particular, cellular mechanisms regarding how PGAs affect cancer cells have been the subject of study recently due to their

potential exploitation in antitumor therapy; however, the detailed function at a molecular level remains largely unknown, with several hypotheses and limited model studies. Herein, we wish to summarize the previous studies on the mechanism of action of PGAs.

4.1. PGAs as potential anion hosts

Structurally, the pentacyclic guanidinium core is a shell-like molecule with a well-defined 3-dimensional architecture. Electronically, the positively-charged guanidine moiety is known for its ability to engage in anion binding.^{6,51} Together, these properties make this core a promising candidate as an anion host molecule (Fig. 4, left). Several observations support the anion hosting properties of the PGAs: 1) 13,14,15-Isocrambescidin 800 (**20**), an isomer which lacks the cage-like shape, displayed reduced biological activity compared to crambescidin 800 (**13**).⁴⁵ 2) A crystal structure highlighted bidentate hydrogen bonding between synthetic analogs of a pentacyclic guanidinium core and tetrafluoroborate anion.⁵² 3) Ptilomycalin A (**6**) is a relatively non-polar compound which is readily dissolved in chloroform, indicating its polar functional groups, such as the guanidine and spermidine moiety, are likely buried inside the pocket.⁵³ 4) A constant difference in the ¹H NMR chemical shift of two methyl groups was observed when mixing 2-methylpropionate anion Me₂CHCO₂⁻ and a ptilomycalin A-TFA derivative at various concentrations (0.3–6.0 mM), indicating that the anion is tightly bound in a chiral environment.⁵³ 5) A similar guanidine-containing structure that enantioselectively recognizes carboxylate anions has been reported.⁵⁴ Although the guest molecule which gives rise to the biological activities of PGAs is yet to be identified, a derivative of ptilomycalin A (**6**) showed selective binding capability toward different *N*-acetylamino carboxylates.⁵³

4.2. Acceptor for biological nucleophiles

Covalent modification of protein targets may also be responsible for the demonstrated biological activities of PGAs. A study regarding crambescidin 359 (**11**) has revealed a base-promoted spiro-ring opening to generate an imine electrophile (**44**) which could covalently bind to protein targets (Fig. 4, right); however, this mechanism was only observed when employing ethanethiol as a nucleophile and has not been observed in a biological setting to date.³⁶ The lack of detailed structure-function studies of the guanidine core presents challenges in evaluating the true potential of this scaffold as a selective chemical probe. It therefore remains an open question as to whether or not these reactive chemical entities can be useful as therapeutic lead molecules, but it is certain that their unique properties and potent biological activities warrant further investigation.

4.3. Ability to block Na⁺, K⁺ and Ca²⁺ transport

Some guanidine-containing natural toxins (such as saxitoxin and tetrodotoxin) block the voltage-gated ion channels and as a result interfere with normal cellular function.⁵⁵ Crambescidin 816 (**8**) was found to exhibit a strong but reversible Ca²⁺ antagonist activity (IC₅₀ = 0.15 nM) in neuroblastoma hybrid NG 108-15 cells, as well as inhibition of the acetylcholine-induced contraction of guinea pig ileum at very low concentrations.²³ The Botana group also reported that crambescidin 816 (**8**) partially blocked Na⁺ and Ca²⁺, but

not K⁺ current in cortical neurons from embryonic mice. They further identified the L-type calcium channels as the main targets.⁵⁶

Ptilomycalin A (**6**) inhibited brain Na⁺, K⁺-ATPase and Ca²⁺-ATPase from skeletal sarcoplasmic reticulum in a dose-dependent manner (IC₅₀ = 2 μM and 10 μM, respectively). Kinetic studies suggested it acted on the ATP binding site in a competitive manner.⁵⁷ The Nagasawa group synthesized two analogs of ptilomycalin A (**47** and **48**), with one analog (**47**) featuring a “twisted” guanidinium core similar to isocrambescidin 800 (**18**). Both analogs (**47** and **48**) displayed a strong inhibitory effect on Ca²⁺-ATPase (Fig. 5, IC₅₀ = 1–3 μM). Interestingly, the guanidinium core alone did not show such activity.⁹ It is unclear if the blocking of ATPases is linked to toxicity, since cytotoxicity data was not reported on these analogs.

Nicotinic acetylcholine receptors (nAChRs) are ligand-based, non-selective ion channels which upon acetylcholine binding become permeable to Na⁺, K⁺ and sometimes Ca²⁺.⁵⁸ The Kasheverov group conducted an *in silico* docking-guided screening of 13 marine natural products as nAChR inhibitors. Among these candidates, PGAs crambescidin 359 (**29**) and monanchocidin A (**22**) displayed moderate affinity with *T. californica* and human α7 nAChR with K_i values ranging from 8.0–310 μM. At 10 μM concentration, they also efficiently blocked murine muscle-type and human α7 nAChR expressed in *Xenopus laevis* oocytes.⁵⁹

4.4. Mode of anti-HIV action

Approved anti-HIV drugs exert their effects on various targets by blocking viral entry or fusion, or by inhibiting key enzymes such as integrase, reverse transcriptase or protease.⁶⁰ Novel anti-HIV agents are in constant demand due to the emergence of drug resistance and side effects associated with long-term treatment. Several reports suggested that members of the PGA family are promising as anti-HIV lead compounds. For instance, ptilomycalin A (**6**) and crambescidin 800 (**13**) were highly effective in HIV-1_{LAV} infected human PBM cells (EC₅₀ = 11 nM and 40 nM, EC₉₀ = 46 nM and 120 nM, respectively).⁴⁰ It appears PGAs battle HIV-1 via multiple modes of action: crambescidin 800 (**13**), 826 (**12**) and fromiamycalin (**17**) all efficiently inhibited HIV-1 envelope-mediated fusion (IC₅₀ = 1–3 μM) against a *T*-cell tropic strain and a macrophage tropic strain.¹³ In another study, the Overman group focused on the HIV-1 Nef protein, which is required for the replication of HIV-1 and the progression to AIDS. Several tricyclic (batzelladine-based) and pentacyclic (crambescidin-based) guanidinium alkaloid analogs inhibited Nef interactions with ligands including p53, actin, and p56^{lck}. The most potent compounds possessed IC₅₀ values in the low micromolar range; however, those compounds possessed high levels of cytotoxicity, preventing analysis in cell culture.⁶¹ The Murphy group reported that ptilomycalin A (**6**) and two simplified analogs inhibited HIV-1 reverse transcriptase (HIV-1 RT) by 55–64% at 10 μM concentration.³⁰

4.5. Cellular mechanisms of cytotoxic action

The Kobayashi group reported that crambescidin 800 (**13**) caused cell cycle arrest of K562 chronic myelogenous leukemia cells at S-phase. Additionally, an increased expression of p21 was observed, often indicating the induction of differentiation. Meanwhile, treatment

with crambescidin 800 (**13**) led to changes in Neuro 2A cell morphology at bipolar orientation.⁶²

The Botana group reported that crambescidin 816 (**8**) was cytotoxic against hepatocellular carcinoma HepG2 cells and several other cancer cell lines at sub-micromolar concentrations. Microarray results revealed approximately 5% altered gene targets upon short treatment at low concentration (6 h, 150 nM). Among the down-regulated targets, genes involving cell migration, cell-cell/cell-matrix adhesion and regulation of cell cycle were found. These results were further validated: cell cycle analysis showed an arrest at the G0/G1 phase of HepG2 cells; Western blot analysis and microscopic images provided evidence on the disruption of cell-cell and cell-matrix adhesion; finally, a dose-dependent inhibition of cell migration was directly observed in a wound healing assay.⁴⁴

In a follow-up study, the Botana group investigated three crambescidin-type natural products (crambescidin 800 (**13**), 816 (**8**), 830 (**9**)). They displayed different potency levels against several tumor cell lines. Generally, the order of cytotoxicity was crambescidin 816 (**8**) > 830 (**9**) > 800 (**13**). They also decreased cell-cell and cell-matrix adhesion, halted the cell cycle in the G0/G1 phase and induced p53-dependent apoptosis. Again, crambescidin 800 (**13**) was the least potent compound which required relatively higher concentrations to exhibit the observed activities. The authors suggested a potential dehydration of the activated C-13 hydroxyl group in crambescidin 816 (**8**) and 830 (**9**), revealing an electrophilic imine for covalent binding that contributes to their biological activity. Furthermore, crambescidin 816 (**8**) was tested in a zebrafish xenograft model, showing *in vivo* activity against human colorectal carcinoma HCT-116 cells.⁴³

4.6. Novel targets and mechanisms

A study by the Dyshlovoy group revealed a dual mode of cytotoxic action by monanchocidin A (**24**): in addition to traditional apoptosis hallmarks including the cleavage of PARP and caspase-3, at low concentration (<2 μ M), monanchocidin A (**24**) was observed to induce autophagy and cell cycle arrest at the S-phase; at high concentration (>2 μ M), it caused permeabilization of the lysosome membranes and cell cycle arrest at the G1-phase. These mechanisms eventually led to the non-classical cell death of cis-platin-resistant NCCIT-R cells.⁴⁷

The Dyshlovoy group also reported four PGAs (ptilomycalin A (**6**), monanchomycalin C (**22**), monanchocidin A (**24**) and B (**25**)) that inhibit the tumor promoter EGF-induced colony formation of murine epithelial JB6 P⁺ C141 cells at non-toxic concentrations. Evidence suggested that these compounds did not use p53-dependent pathways, and instead activation of the MAPK/AP-1 signaling pathway⁶³ was likely the cause of apoptosis. These compounds and two other PGAs (monanchomycalin B (**30**) and normonanchocidin D (**29**)) displayed similar activities against human cervix cancer HeLa cells (IC₅₀ = 0.58–2.1 μ M). At their corresponding IC₅₀ values, all compounds induced cell cycle arrest at S-phase, DNA fragmentation, and elevated caspase-3/7 activity; however, comparing to traditional antitumor agent cisplatin, significantly weaker activations of caspase-3/7 were observed for these PGAs, indicating that underlying non-apoptotic mechanisms may exist.⁴¹

To determine the molecular targets of monanchocidin A (**24**), a proteomics study was conducted to identify altered proteins.⁶⁴ When NCCIT-R cells were treated with monanchocidin A (**24**) for 48 h, 11 (0.5 μ M) and 73 (1 μ M) instances of differential protein expression with greater than 2-fold changes were observed. These targets are associated with cell migration, growth and proliferation, cell death and survival, metastasis formation and cell cycle progression. Three of them (vimentin, apoE and eIF5A) were further analyzed. Vimentin is an intermediate filament protein and one of the major components that forms the cytoskeleton. Overexpression of vimentin in cancer cells is associated with enhanced growth rate and aggressiveness.⁶⁵ Monanchocidin A (**24**) did not affect the total level of vimentin, but instead regulated the distribution of its isoforms which likely have different phosphorylation states. Consequently, the altered filament structure could give rise to the observed activities of monanchocidin A (**24**) in cell migration and colony formation assays. Monanchocidin A (**24**) caused an upregulation of apoE, an apolipoprotein protein with anti-adhesive activity.⁶⁶ This event could also contribute to its anti-migratory activity. The third protein of interest, eIF5A, is the only known protein with a hypusine residue. This unnatural amino acid residue is installed via a 2-step post-translational modification⁶⁷ on the eIF5A precursor protein: first, deoxyhypusine synthase (DHS) functionalizes the Lys50 residue by transferring an aminobutyl moiety from spermidine, followed by installation of a hydroxyl group by deoxyhypusine hydroxylase (DOHH) (Fig. 6). Due to its involvement in many key cellular processes, eIF5A is critical to cell viability and proliferation.⁶⁸ The predominant isoform, eIF5A-1, plays important roles in regulating apoptosis.⁶⁹ Interestingly, accumulation of the eIF5A-1 precursor (lacking the hypusine residue) leads to apoptotic cell death.⁶⁹ Upon treatment with 1 μ M monanchocidin A (**24**), the hypusine-containing form was suppressed by 50%, while the total eIF5A level was not affected. Two questions remain to be addressed: 1) is the shift toward eIF5A precursor the cause of apoptosis induced by monanchocidin A (**24**), and 2) how does monanchocidin A (**24**) affect the hypusine synthesis? The morpholinone unit in monanchocidin A (**24**) has been suggested as a potential inhibitor of DHS, as it resembles the enzyme substrate (spermidine); however, this hypothesis has been challenged because PGAs with different spermidine-derived units did not exhibit significant differences in cytotoxic activity.⁴¹

5. Conclusions

In this review, we summarized the structures, syntheses, biological activities and mechanisms of action of PGAs. Many elegant synthetic approaches toward the guanidinium alkaloids have been reported, while more recent studies on PGAs have been aimed at unravelling their biological mechanisms (Fig. 7). Given the broad range of biological activity displayed by PGAs and the lack of understanding of their mechanism of action at a molecular level, many questions remain regarding the potential of these scaffolds as chemical probes and/or therapeutic leads. The application of modern chemical biology approaches, in combination with chemical synthesis efforts, as well as advances in molecular biology and genetics, will no doubt reveal important insights into PGAs mechanism and therapeutic potential.

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Abbreviations

PGA pentacyclic guanidinium alkaloid

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Fig. 1.
Natural occurrences of the guanidine motif.

Fig. 2.
Representative structures of pentacyclic guanidinium alkaloids (PGAs).

Fig. 3.
General SAR picture of PGAs (ptilomycalin A (**6**) shown).

Fig. 4.
Potential ionic and covalent interactions of PGAs.

Fig. 6.
Post-translational modification of eIF5A.

Fig. 7. Summary of recent mechanistic studies of PGAs (note: not all PGAs exert these functions, and some are evidence-supported hypotheses).

Scheme 1.

Representative synthetic approaches toward pentacyclic guanidinium alkaloids (PGAs).

Table 1Literature reports of the in vitro toxicity of pentacyclic guanidinium alkaloids (PGAs).^a

Natural Product	Cytotoxic	Antibacterial	Antiviral ^b	Antifungal	Antiprotozoal
Ptilomycalin A	11, 17, 21, 41, 42	11, 41	11, 17, 41	11	41
Ptilomycalin D ^c	49				
Crambescidin 800	12, 17, 41, 43, 44, 46	41	12, 13, 17, 41	47	41
Crambescidin 816	12, 44, 45, 46, 50		12, 46	47	
Crambescidin 830	12, 44		12	47	
Crambescidin 844	12, 46		12		
Crambescidin 826	43		13		
Crambescidin 814	43				
Crambescidin 786	43				
Crambescidic acid ^c	43				
Celeromycalin	17		17		
Fromiamycalin	17		13, 17		
Monanchocidin A	18, 42, 48				
Monanchocidin B	19, 42				
Monanchocidin C-E	19				
Monanchomycalin A	20				
Monanchomycalin B	20, 42				
Monanchomycalin C	21, 42				
Normonanchocidin A-B	22				
Normonanchocidin D	22, 42				

^a Only including toxicity data of naturally occurring PGAs. Synthetic and natural product analogs are not listed.^b Including those with potency against HIV-infected cells.^c These compounds lack the spermidine-derived tail and possess reduced cytotoxicity relative to other reported compounds.