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Articles

Synthetic Natural Product Inspired Cyclic Peptides

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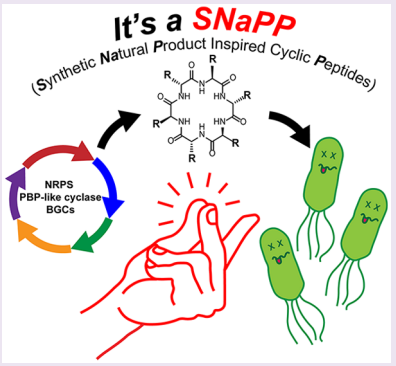
ABSTRACT: Natural products are a bountiful source of bioactive molecules. Unfortunately, discovery of novel bioactive natural products is challenging due to cryptic biosynthetic gene clusters, low titers, and arduous purifications. Herein, we describe SNaPP (Synthetic Natural Product Inspired Cyclic Peptides), a method for identifying NP-inspired bioactive peptides. SNaPP expedites bioactive molecule discovery by combining bioinformatics predictions of nonribosomal peptide synthetases with chemical synthesis of the predicted natural products (pNPs). SNaPP utilizes a recently discovered cyclase, the penicillin binding protein-like cyclase, as the lynchpin for the development of a library of head-to-tail cyclic peptide pNPs. Analysis of 500 biosynthetic gene clusters allowed for identification of 131 novel pNPs. Fifty-one diverse pNPs were synthesized using solid phase peptide synthesis and solution-phase cyclization. Antibacterial testing revealed 14 pNPs with antibiotic activity, including activity against multidrug-resistant Gram-negative bacteria. Overall, SNaPP demonstrates the power of combining bioinformatics predictions with chemical synthesis to accelerate the discovery of bioactive molecules.



Natural products (NPs) have been a bountiful source of medicines, including antimicrobials, anticancer agents, antiparasitics, immunosuppressants, as well as many others.[1] Historically, bacteria have been one of nature's most prolific producers of biologically active NPs.[2] One important class of biologically active bacterial NPs are nonribosomal peptides (NRPs). These peptides are synthesized by modular enzyme complexes known as nonribosomal peptide synthetases (NRPS) and comprise a rich set of structurally diverse NPs, including many clinically used antibiotics such as daptomycin, bacitracin, polymyxin B, and colistin.[3] Cyclic peptides are an especially important class of NRPs, possessing many favorable pharmacological properties over their linear counterparts.[4−6] Their relatively large size and structural rigidity allow them to engage challenging targets, including protein−protein interactions.[4,7−9] Cyclic NRPs are also generally more cell permeable and resistant to proteases compared to linear peptides.[5,10,11] For these reasons, there is great interest in the discovery of additional cyclic NRPs as biological tools and drug leads.

Traditionally, novel NRPs have been discovered by a classical fermentation approach[12] whereby crude bacterial extracts are screened for biological activity. While this approach has been extremely successful, it is very time-consuming. The process of going from a bioactive extract to a completely elucidated structure takes minimally several months and oftentimes over a year. Additionally, each new NP requires optimization of fermentation conditions and purification sequences, thus preventing easy automation of the process. Rediscovery of known NPs is also a major limitation.[13] Recent advances in whole-genome sequencing and bioinformatics have revealed a vast number of NRPS biosynthetic gene clusters (BGCs) for which no known NP can be attributed.[14] Harnessing the full biosynthetic potential of these organisms is complicated by the fact that a small fraction (∼2%) of bacteria are culturable in the laboratory,[2,15] and many BGCs are transcriptionally inactive (cryptic) under standard laboratory conditions.[14] Access to the NPs produced via these BGCs often requires heterologous expression or promoter optimization, both of which are very time-consuming and frequently unsuccessful.

We hypothesized that we could overcome these difficulties by developing SNaPP (Synthetic Natural Product Inspired Cyclic Peptides, Figure 1), a method that combines bioinformatics with chemical synthesis. Specifically, the method utilizes (1) bioinformatics tools such as antiSMASH[16] and PRISM[17] to predict peptide products formed by NRPS BGCs identified in bacterial genomes and (2) chemical synthesis to access the predicted peptides. This synthesis-first approach has many advantages over traditional fermentation approaches: (1) This approach skips bacterial culture and the need for fermentation optimization, (2) it avoids rediscovery of known NPs by comparison with known BGCs, (3) products

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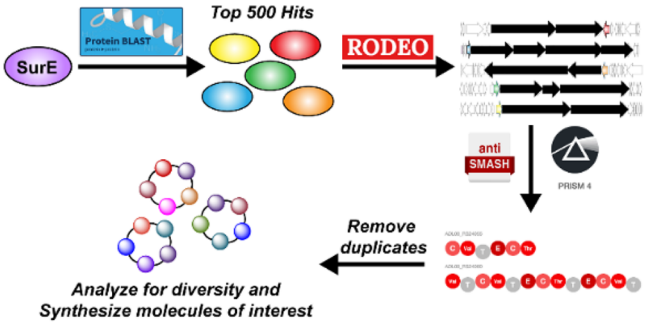

Figure 1. Outline of the SNaPP method.

from cryptic BGCs or currently unculturable bacteria can easily be accessed, and (4) each part of SNaPP from the identification of the BGCs to NP predictions to chemical synthesis is scalable and easily automated, greatly expediting the process.

Others have previously prepared predicted NRPs by solid-phase peptide synthesis and were successful in the discovery of several biologically active compounds.[18−22] However, few of these reports have explored the synthesis of predicted cyclic NRPs,[22,23] despite the fact that nearly 67% of known NRPs possess a cyclic motif.[24,25] One reason for this observation may be the limited ability of bioinformatics programs to predict how NRPs cyclize. The thioesterase (TE) domain is typically the terminal module of an NRPS and is often responsible for peptide cyclization.[26] However, TE domains catalyze the production of multiple cyclic motifs including lactams and lactones in head-to-tail or side chain-to-tail form.[27,28] Others have overcome this by synthesizing all the potential cyclic structures.[22,23] This comprehensive approach is impressive and resulted in a very good antibiotic hit rate (15/157, ∼10%).[23] However, it requires synthesis of multiple compounds per BGC, greatly increasing the time and reagents necessary make these molecules. Additionally, the approach significantly increases the number of compounds needed to be screened. One of the major advantages of prioritizing NRPs is their increased likelihood of having bioactivity compared to a random cyclic peptide.[29] It is highly unlikely that the incorrectly cyclized structures will have activity due to the large effect the cyclization site has on three-dimensional shape of molecules. Therefore, a strategy that does not prioritize the correct cyclization site is hypothesized to be less efficient than one that targets only the molecules with the natural cyclization site.

Interestingly, numerous NRPS BGCs do not contain a thioesterase domain and instead are thought to be released from the NRPS via stand-alone enzymes. Recently, the penicillin binding protein (PBP)-like cyclases have been identified as a novel class of stand-alone NRPS cyclases.[30−32] PBP-like cyclases have thus far only been found to catalyze cyclization of the C-terminus with the N-terminus to furnish head-to-tail cyclic lactams. Herein, we describe a new method SNaPP, which expedites discovery of novel bioactive cyclic peptides via the synthesis of predicted NPs (pNPs). SNaPP prioritizes head-to-tail cyclic peptides by focusing on NRPS BGCs containing PBP-like cyclases. While these peptides are not intended to be the true NPs, we expect to bias ourselves toward head-to-tail cyclic peptides with very similar structures and bioactivities to the true NPs.

RESULTS AND DISCUSSION

Identification of pNPs. SurE, the PBP-like cyclase that catalyzes the cyclization of the surugamides, is one of the most well studied PBP-like cyclases.[30−33] *surE* along with the genes encoding the PBP-like cyclases for the head-to-tail cyclized peptide NPs ulleungmycin (*ulm16*), desotamide B (*dsaJ*), the mannopeptimycins (*mppK*), the pentaminomycins (*penA*), the noursamycins (*nsm16*), and the curacomycins (KUM80512.1) are all found in close proximity to the NRPS that produces the peptide NP.[31,34−36] This colocalization suggests that the genes for these cyclases could be used as a genetic handle for identifying other cyclic head-to-tail NRPs. Our strategy is outlined in Figure 1. We have chosen to focus exclusively on head-to-tail cyclic peptides because all PBP-cyclase containing BGCs analyzed to date encode for the production of head-to-tail cyclic peptides. However, a limitation of this strategy is that the PBP-like cyclases are a relatively new class of enzymes. It is possible that some PBP-like cyclases perform alternative cyclizations (*e.g.* side chain-to-head) and remain undiscovered at this time.

First, a BlastP[37] search for SurE was performed, and the top 500 hits were analyzed further. The genetic neighborhood for these hits was identified using RODEO.[38] Three hundred and ninety-six (79%) of the BGCs had NRPS genes 10 genes or less away. Clusters at the end of a contig or with incomplete records in NCBI (80, ∼20%) were removed prior to further analysis. The remaining 316 NRPS containing BGCs were then analyzed using bioinformatics softwares including PRISM 4[17] and antiSMASH 5.0[16] to predict the structure of the NRPs (Supplementary Excel File). Generally, predictions between the two programs agreed well. Tanimoto analysis of the predictions from PRISM 4 or antiSMASH 5.0 for the 5 known molecules within our data set compared to their actual structures suggested similar accuracies (Figure S1A). Additionally, their predictions for uncharacterized BGCs also was similar (Figure S1B). We ultimately chose to use the PRISM predictions as the basis for our studies for two major reasons. First, and most importantly, other studies have found that PRISM is better at predicting known NPs compared to antiSMASH when the data set is larger than the knowns that we have in our data set.[39] Specifically, the structures predicted by PRISM 4 and antiSMASH 5.0 for 753 BGCs that encoded known NPs were previously analyzed for their similarity to the known structure. PRISM 4 significantly outperformed antiSMASH 5.0.[39] Second, PRISM is more likely to give a structural prediction.[39] When 3759 bacterial genomes were analyzed, PRISM was able to predict structures for 3078 NRPS, while antiSMASH 5.0 was able to predict structures for 2779 NRPS.[39] Using PRISM, 140 unique cyclic peptides were identified. Nine of the peptides were previously known NRPs (mannopeptimycin, desotamide B, ulleungmycin, and 6 copies of the surugamide cluster), leaving 131 unique and novel cyclic peptides of varying sizes to explore further (Figure 2A and Supplementary Excel File).

Previously, Jacques and coworkers found that NRPs vary in size between 2 and 23 amino acids with the most frequent sizes of NRPs being between 7 and 9 amino acids.[40] While we see many peptides with 7 and 9 amino acids, we see very few with 8 amino acids and instead see a large number of 6 and 10. Additionally, the unnatural amino acid ornithine is predicted much more often than expected. Based on the number of occurrences in the Norine database,[24] we would expect ∼8% of

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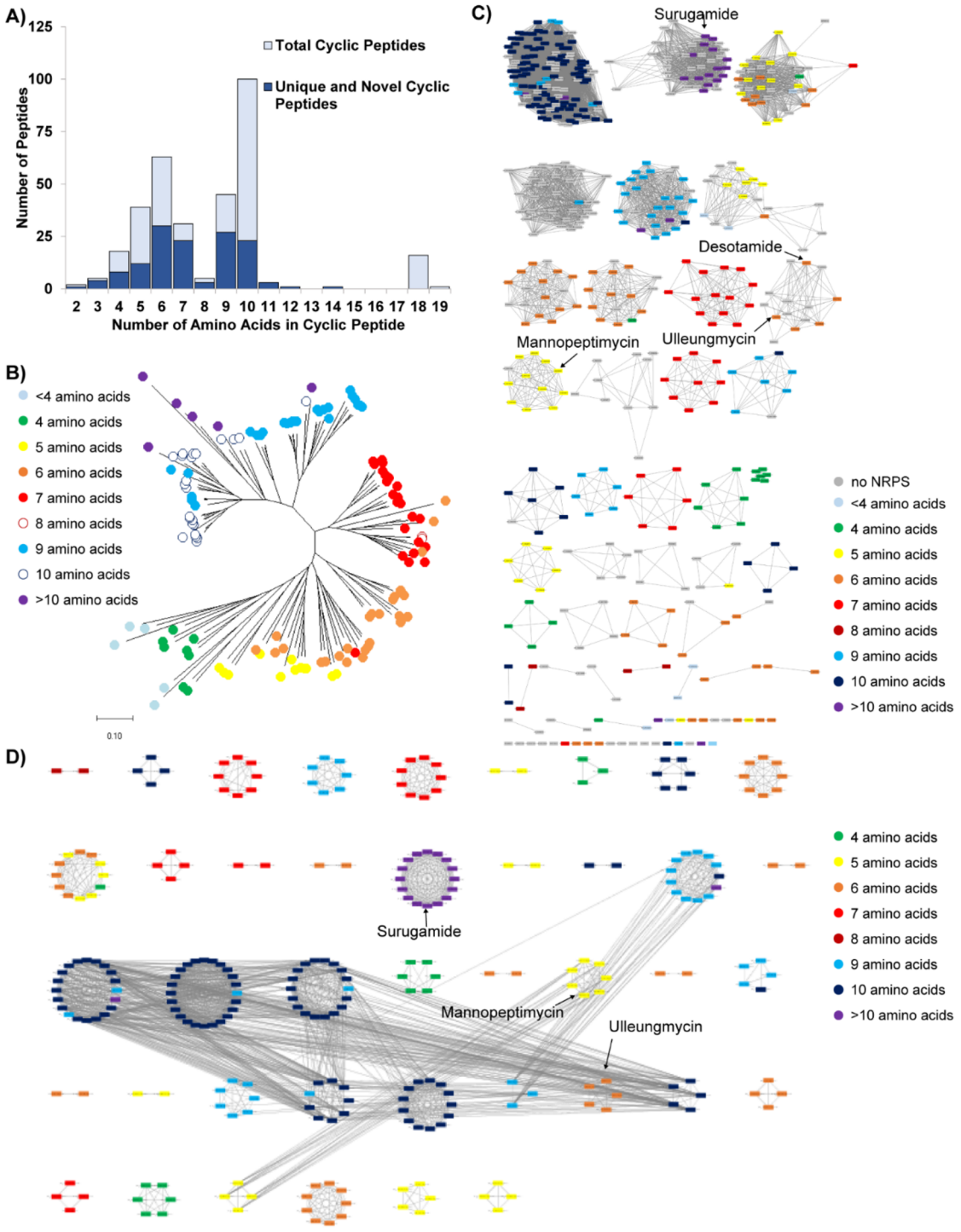

Figure 2. Diversity of pNPs. (A) pNPs distribution with total number of cyclic peptides noted in light blue, and the number of unique and novel cyclic peptides noted in dark blue. (B) Tanimoto similarity data represented in tree form. Details of strains and molecules synthesized can be found in Figure S2A. (C) Sequence similarity network for PBP-like cyclases. The size (number of amino acids) of the predicted cyclic peptide product is indicated by the color of the nodes. (D) BiG-SCAPE network of PBP-like cyclase and NRPS containing BGCs. Each circle represents a family (closely related) of BGCs. Branches to other circles indicate clans (more distantly related BGCs). The size (number of amino acids) of the predicted cyclic peptide product is indicated by the color.

NRPs to contain ornithine. We found that ~70% of our pNPs contain ornithine. It is unclear whether this is due to the prediction software or if ornithine is truly overrepresented in this set of peptides. Interestingly, antiSMASH often predicted glutamine when PRISM predicted ornithine. Another common difference was that antiSMASH would often predict tyrosine when PRISM predicted tryptophan. Given the structural similarity of these amino acids, we were not surprised by these differences.

Diversity of pNPs. Because the structures of molecules determine their functions, structural diversity is essential for any compound library that will be used for bioactivity screening.[41] To assess the diversity of the pNPs and determine the best molecules to synthesize for testing, we first used

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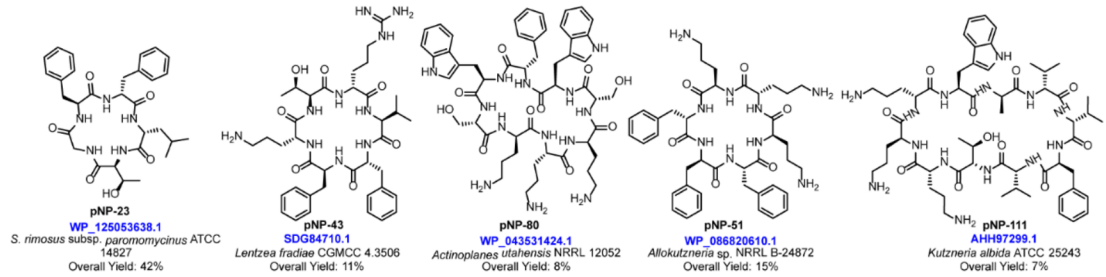

	E. coli		*K. pneumoniae*		*A. baumannii*		*P. aeruginosa*		Hemolysis	A549 toxicity
Compound	WT	R	WT	R	WT	R	WT	R	53 μg/mL	16 μg/mL
pNP-23	16 (28)	>32 (>57)	>32 (>57)	>32 (>57)	>32 (>57)	>32 (>57)	>32 (>57)	>32 (>57)	<10%	<50% death
pNP-43	32 (42)	32 (42)	32 (42)	>32 (>42)	16 (21)	32 (42)	>32 (>42)	>32 (>42)	<10%	<50% death
pNP-80	32 (31)	32 (31)	32 (31)	>32 (>31)	16 (15)	16-32 (15-31)	>32 (>31)	>32 (>31)	>10%	<50% death
pNP-51	>32 (>41)	>32 (>41)	>32 (>41)	>32 (>41)	>32 (>41)	>32 (>41)	32 (41)	16 (20)	<10%	<50% death
pNP-111	32 (28)	32 (28)	>32 (>28)	32 (28)	>32 (>28)	32 (28)	>32 (>28)	>32 (>28)	<10%	<50% death
Cipro	<0.1 (<1.4)	>32 (>87)	<0.1 (<1.4)	>32 (>87)	1 (2.7)	>32 (>87)	0.5-1.0 (1.4-2.7)	>32	ND	ND

Figure 3. Structures of pNPs that hit against Gram-negative bacteria. Gram-negative antibiotic peptides and a table describing their activities. The strains analyzed are described in the Materials and Methods section. Potencies of hits are given in μg mL^{-1} and in parentheses are the potency in μM. WT: wild type; R: antibiotic resistant; Cipro: ciprofloxacin; WT *E. coli*: ATCC 25922; R *E. coli*: ATCC BAA-2469; WT *K. pneumoniae*: ATCC 27736; R *K. pneumoniae*: ATCC BAA-21469; WT *A. baumannii*: ATCC 19606; R *A. baumannii*: KB349; WT *P. aeruginosa*: PAO1; R *P. aeruginosa*: PA1000. Hemolysis of human red blood cells and toxicity to the human cancer cell line A549 are also reported. ND = not determined.

ChemMine Tools[42] to calculate the Tanimoto coefficients for the novel molecules identified. The Tanimoto coefficients were then used to generate both a heat map (Supplementary Excel File) as well as a tree (Figure 2B and Figure S2A). Peptides of the same size generally cluster together while still having noticeable structural differences.

Bioinformatics methods were also employed to analyze the diversity of the library. A sequence similarity network (SSN)[43] of the PBP-like cyclases was generated. The PBP-like cyclases tend to cluster based on the size of their corresponding NRPs, suggesting that PBP-like cyclases might be specific for certain ring sizes (Figure 2C and Figure S3). Interestingly, occasionally different sizes are predicted within the same cluster, suggesting that either these cyclases are more flexible or potentially that the NRPS next to the aberrant cyclase may act in an iterative fashion. We also performed BiGSCAPE analysis[44] on the BGCs containing the PBP-like cyclases and NRPS genes (Figure 2D and Figure S4). This analysis revealed 86 NRPS families with an average of 4 BGCs per family. These data, in agreement with the Tanimoto data, confirmed a varied set of structures and helped us to design a diverse library.

Synthesis of a Diverse pNP Library. Fifty-one chemically diverse pNPs were chosen for synthesis (see Figures S2−4 and Table S1). Specifically, molecules from distinct branches on the Tanimoto tree were chosen. These were further narrowed down by selecting molecules for synthesis from a variety SSN clusters and BigSCAPE families with a particular emphasis on molecules not from clusters or families with previously known NPs. Challenging to access amino acids such as protected enduracididine and hydroxyphenylglycine were replaced with the structurally similar amino acids arginine and phenylglycine, respectively. Linear sequences were prepared using standard solid-phase peptide synthesis (SPPS) followed by solution-phase cyclization, deprotection, and purification (Figure S5).[45,46] The entire sequence from pNP prediction through purification can be completed in seven days and is straightforward enough to be completed by an undergraduate. Additionally, all steps except HPLC purification can easily be accomplished in parallel. Growth of a NP-producing organism often takes longer than this, with fermentation optimization, purification, and structure validation regularly exceeding a year. Thus, the SNaPP process significantly expedites the process compared to traditional fermentation.

Bioactivity Testing. Initial compounds were tested for activity against antibiotic sensitive and antibiotic resistant ESKAPE pathogens at concentrations varying between 0.5 and 32 μg mL^{-1} using the CLSI microbroth dilution assay.[47] Any well with greater than 90% death was considered a hit. Overall, 14 hits (MIC $\leq$ 32 μg mL^{-1}) were observed with 4 against Gram-negative organisms (Figure 3), 9 of them being against Gram-positive organisms (Figure S6), and 1 hit against both. This is a very promising hit rate (~30%), particularly when compared to other antibiotic discovery programs, which have struggled to find any hits, especially against Gram-negative organisms.[48,49] It also is approximately 3-fold more efficient compared to previous syn-BNP approaches that did not prioritize correctly cyclized structures.[23] An Alamar blue viability assay revealed that these molecules are nontoxic to the A549 nonsmall cell lung cancer cell line, suggesting they likely have good selectivity for bacterial cells over mammalian cells. (Figure 3 and S6) Additionally, hemolysis assays with human red blood cells revealed that many also had no hemolytic effects at concentrations up to 53 μg mL^{-1} (Figure 3 and S6), providing strong evidence that they are promising antibiotic leads.

Derivative Development and Mechanism of Action Studies. Based on the results described above, we chose to explore derivatives of **pNP-43**, a compound with activity against several Gram-negative bacteria and no observed hemolytic activity or mammalian cell toxicity. **pNP-43** is predicted to be produced by *Lechevalieria fradiae* CGMCC 4.3506, a strain originally isolated from the Wutaishan Mountain in the Shanxi province of China. In addition to the PBP-like cyclase and NRPS genes, the BGC contains genes with high similarity to the enduracididine biosynthetic genes, providing strong support that enduracididine is incorporated into this cyclic peptide (Figure 4 and Table S2). Structure predictions by PRISM further support this with adenylation domain 6 predicted to load enduracididine. Due to challenges in obtaining enduracididine, we chose to substitute endur-

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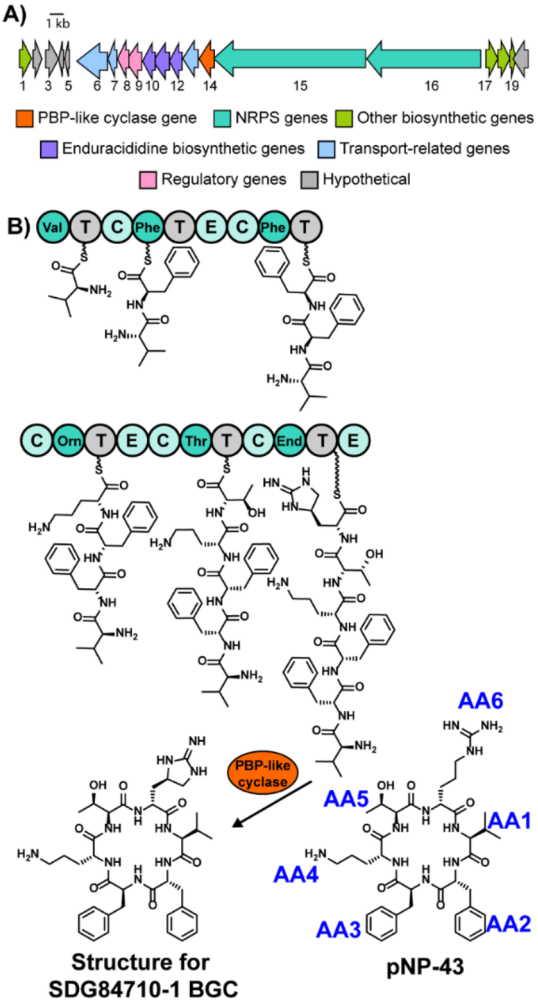

Figure 4. BGC for **pNP-43**. (A) NRPS BGC including the PBP-like cyclase SDG84710.1. (B) NRPS modules and amino acid predictions by PRISM. AA# refer to the amino acid position of pNP-43.

acididine for the next highest prediction, arginine. While enduracididine is often important for the bioactivity of natural products (*e.g.* teixobactin), others have shown that replacement of enduracididine with arginine often results in a molecule that retains bioactivity.[50−52] However, at least in the case of teixobactin, this substitution does result in an approximate 10-fold decrease in potency. When developing derivatives, the arginine was exchanged with amino acids having similar chemical structures including lysine, ornithine, and 2,4-diaminobutyric acid (**pNP-43a−c**, Figure S7). However, the parent molecule was the most active (Figure S7). After further examination of the predictions by antiSMASH[16] and PRISM[17] (Table S3), we chose to develop other derivatives by modifying the amino acid at position 4. While ornithine is the number one prediction for amino acid 4, arginine and lysine also scored well thus we chose to incorporate these residues into our derivatives (**pNP-43d**, **e** in Figure S7). Substituting lysine in place of ornithine at position 4 (**pNP-43d**) resulted in biological activity that was twofold more potent against antibiotic resistant *A. baumannii* compared to the initial molecule. We then performed an alanine scan on **pNP-43d** to determine the amino acids that were necessary for activity. Substitution of each amino acid except for threonine resulted in inactive molecules, suggesting that all amino acids except amino acid 5 are essential for activity. Finally, we explored other substitutions at position 6. Derivatives that substituted this position with histidine, tryptophan, asparagine, or glutamine were all inactive, suggesting that amino acid position 6 must be a basic amino acid. Further derivatives helped us to establish a structure activity relationship (Figure 5A and S7). Additionally, the linear version of **pNP-43d** (**pNP-43r**) was completely inactive (MIC > 128 μg mL^{-1}), confirming the importance of cyclizing the peptides.

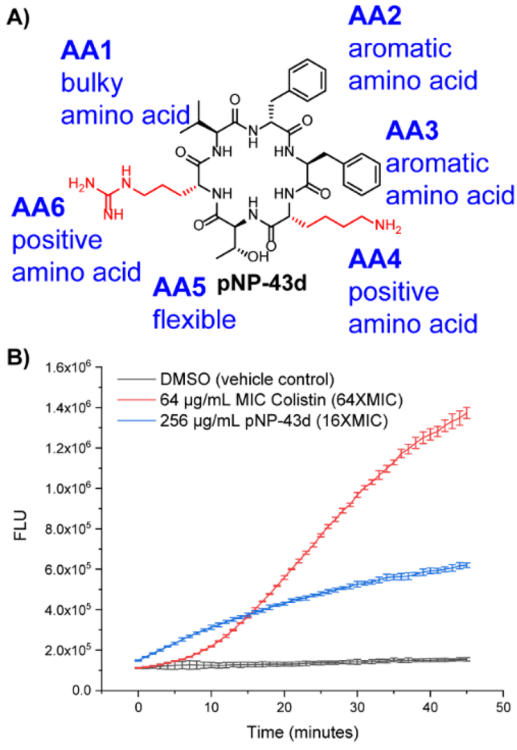

Figure 5. Mechanism of action studies. (A) Chemical structure of **pNP-43d** with basic residues indicated in red. (B) Representative data from Sytox Green lysis assay with *A. baumannii* 19606. Error bars are standard deviation from three technical replicates. $N = 3$

Due to the improved activity of **pNP-43d** against the antibiotic resistant *A. baumannii*, we chose to study its mechanism of action. Many cyclic peptides are known to cause bacterial cell lysis. This is particularly true of cationic peptides such as the polymixins.[53] Specifically, colistin (*i.e.* polymixin E) is known to interact with Lipid A via its five positively charged amino acids, displace divalent cations, and weaken the bacterial outer membrane of Gram-negative bacteria.[54] This ultimately allows the peptide to enter the cell, where its additional activities have been postulated to cause cell death. The success of cationic peptides as Gram-negative antibiotics is so well precedented that others have even used it as a strategy to find novel antibiotics such as NRPs asbrevicidine and laterocidine, each of which has three basic residues.[53] Because pNP-43 requires basic amino acids at positions 4 and 6 for activity and because they only show activity against Gram-negative bacteria, it is possible that it acts similarly to colisitin and other cationic peptides. Specifically, it may utilize its positively charged amino acids to interact with the outer membrane and then induce bacterial cell lysis. Colistin-resistant bacteria are also resistant to **pNP-43** and

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pNP-43d (Table S4). The fact that these molecules are active against antibiotic resistant strains that are sensitive to colistin but not those that are colistin-resistant suggests that it may be acting similarly. To further explore this hypothesis, we tested **pNP-43d** for its ability to lyse bacterial cells using a previously reported Sytox green assay.[55] **pNP-43d** clearly resulted in bacterial cell lysis at concentrations varying from 2 to 16 times the MIC for both wild type and antibiotic resistant *A. baumannii* (Figure 5B and Figure S8). Based on these combined results, **pNP-43d** appears to have a similar mechanism of action to colistin.

CONCLUSIONS

Described herein is the development of SNaPP, a method to greatly expedite the discovery of bioactive molecules inspired by NPs. Cyclic peptides were chosen as an initial target due to their history as important sources of medicines along with the established bioinformatics approaches for predicting the peptide sequences. Head-to-tail peptides were targeted by identifying NRPS BGCs that co-occur with genes from a recently discovered family of stand-alone cyclases, the PBP-like cyclases. To date, PBP-like cyclases have only been found in BGCs that produce head-to-tail cyclic peptides. This approach allowed for identification of 131 unique and novel cyclic peptides. Fifty-one diverse pNPs were chemically synthesized and tested for antibiotic activity. Approximately 30% of pNPs had activity with several showing very promising activity against difficult-to-treat Gram-negative bacteria. As prediction software for NP BGCs improves, this strategy will only increase in its utility. Overall, SNaPP is a powerful method for the rapid identification of biologically inspired lead molecules.

MATERIALS AND METHODS

General Information. Solvents were purchased from Fisher Scientific and used without further purification. Fmoc-protected amino acids and coupling reagents were purchased from Chem-Impex International. 2-CTC resin was purchased from ChemPep Incorporated. All other reagents were purchased from commercially available sources (Sigma-Aldrich, Acros Organics, Oakwood Chemical, TCI Chemicals) and used without further purification.

Bacterial Strains. All strains used in this study except the *Bacillus* strain and the colistin resistant *E. coli* strains were obtained from P.J. Hergenrother (UIUC). The *Bacillus* strain was obtained from W.W. Metcalf (UIUC). The colistin resistant *E. coli* strains (AR Bank Number 0346, 0349, and 0350) were obtained from the CDC AR Isolate bank. *E. coli* ATCC 25922 (wild type, WT) BAA-2469 (resistant, R), colistin resistant *E. coli*, *K. pneumonia* ATCC 27736 (WT) and BAA-2146 (R), *A. baumannii* ATCC 19606 (WT) and KB349 (R), and *P. aeruginosa* PAO1 (WT) and PA1000 (R) were grown on Mueller Hinton Broth 2 (Sigma-Aldrich). *S. aureus* ATCC 29213 (WT) and NRS3 (R), *Enterococcus* species ATCC 19433 (WT) and S235 (R), and *B. subtilis* 6633 (WT) were maintained on Bacto Brain Heart Infusion.

Prediction of Cyclic Peptide Structure. The accession numbers for the top 500 hits from the SurE BlastP were downloaded and used as the input for RODEO.[38] Biosynthetic gene clusters were then manually analyzed for the presence of nonribosomal peptide synthetase (NRPS) genes. If an NRPS was at the end of a contig, the cluster was not considered further. If the NRPS was not at the end of the contig, the FASTA file for the cluster was then analyzed using both PRISM 4.0[17] and antiSMASH 5.0.[16] Generally, both programs agreed well. Initial structures were assigned based on the PRISM results (see Supplementary Excel Document). Derivatives were designed based on results from both programs.

Tanimoto Similarity Analysis. Tanimoto similarity analysis was accomplished with ChemMine Tools[56] using the following parameters for hierarchical clustering: Display values: Z-scores; Linkage method: single; Heat map: distance matrix.

Sequence Similarity Analysis. Sequence similarity analysis of the PBP-like cyclases was accomplished using the EFI-Enzyme Similarity Tool[43] and visualized using Cytoscape 3.6.1.[57] An alignment score of 120 was used for generating the networks in this paper.

BiG-SCAPE Analysis. BiG-SCAPE analysis[44] was performed on the 316 BGCs containing both a PBP-like cyclase and an NRPS. The antiSMASH outputs from the prediction of the cyclic peptide structure were used an inputs for BiG-SCAPE. The output was visualized using Cytoscape 3.6.1.

Mass Spectrometry. Mass spectra (MS) were recorded on an Advion Expression CMS single quadrupole mass spectrometer using electrospray ionization (ESI).

Antibacterial Activity Analysis. Antibacterial activity analysis for all bacteria was performed using the microdilution broth method as outlined by the Clinical and Laboratory Standards Institute (CLSI).[47] Mueller Hinton Broth 2 (MH, Sigma-Aldrich, 90922) was used for all testing. Testing was performed as previously described.[58] Turbidity (OD600) of the wells was determined using a SpectraMax iD3 platereader (Molecular Devices). For the compounds that hit during initial screens, minimum inhibitory concentrations were determined. A minimum of three biological replicates were performed. Ciprofloxacin was used as a control in these assays. Colistin was also used as a control in the colistin resistant strains.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.1c00641.

Supporting figures, experimental protocols, and spectral data (PDF)

PRISM predictions for the structures of the peptides and the Tanimoto similarity heatmap (XLSX)

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M.A.H. and E.I.P. designed the experiments. M.A.H., C.S., I.W., A.F., J.G., and E.I.P. performed the bioinformatics predictions. M.A.H., C.S., S.N., Z.B., and B.B. synthesized the pNPs. E.I.P. and S.N. performed the antibiotic and hemolysis screening. R.M. performed the mammalian cell screening. M.A.H. and E.I.P. wrote the paper.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NP, natural product; pNP, predicted natural product; BGC, biosynthetic gene cluster; SNaPP, Synthetic Natural Product Inspired Cyclic Peptides; NRPS, nonribosomal peptide synthetase; NRP, nonribosomal peptide; PBP, penicillin-binding protein; SSN, sequence similarity network

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