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An Efficient Synthesis of 3-Alkylpyridine Alkaloids Enables Their Biological Evaluation

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3-Alkylpyridine alkaloids (3-APAs) isolated from the arctic sponge *Haliclona viscosa* are a promising group of bioactive marine alkaloids. However, due to limited bioavailability, investigations of their bioactivity have been hampered. Additionally, synthesis of a common intermediate requires the use of protecting groups and harsh conditions. In this work, we developed a simple and concise two-step route to nine different natural and synthetic haliclocyclins. These compounds displayed modest antibiotic activity against several Gram-positive bacterial strains.

Introduction

Alkaloids are a class of molecules with a broad range of biological activity often isolated from marine sponges.[1] The *Haliclona* sp. sponges in particular are an abundant source of 3-alkylpyridine alkaloids (3-APAs). These molecules contain at least one pyridinium or tetrahydropyridine moiety most commonly connected at the 1- and/or 3-position to an alkyl chain.[2] Examples include methylaruguspongine C, haliclamine A, manzamine A, and sarain-1 (Figure 1A).[3] The biosynthetic diversity within this genus has been more extensively studied in tropical waters due to increased chemical defenses produced to combat higher levels of predation,[4] in comparison to species growing in more temperate climates such as the arctic, which remain under explored.[2] The Arctic sponge *Haliclona viscosa* has been found to produce five structural classes of 3-APAs: haliclocyclins, cyclostelletamines, viscosamines, haliclamines, and viscosalines, differing between classes by their pyridine content and oxidation states and within each class by the length of the alkyl chain (Figure 1B).[5] Interestingly, cyclostellettamines and haliclamines closely resemble a common intermediate in the synthesis of the more complex 3-APAs such as manzamine A and halicyclamine A.[6]

Despite the structural simplicity of *H. viscosa* 3-APAs relative to those in Figure 1A, they have demonstrated a broad range of preliminary biological activity, including epidermal growth factor inhibition, muscarinic acetylcholine receptor antagonistic activity, and mouse embryonic fibroblast cytotoxicity.[2,5g,7] The extent of these biological studies are unfortunately quite limited. This is largely due to challenges with isolation of sufficient material from the sponges, which are under far less pressure to produce large quantities of chemical defense molecules in far less densely populated arctic waters.[4] Further, synthetic studies by other groups have been conducted primarily for the purpose of structural validation as these often symmetrical structures are challenging to deduce by NMR exclusively.[8] The syntheses of each class of these 3-APAs have previously all hinged on the use of a common intermediate, **I**. However, the synthetic route to this seemingly simple intermediate required six synthetic transformations, from the diacid shown, and necessitated harsh conditions in several of those steps as well as protection of the primary alcohol for the alkylation of picoline (Figure 2A).[5e,g,7–9]

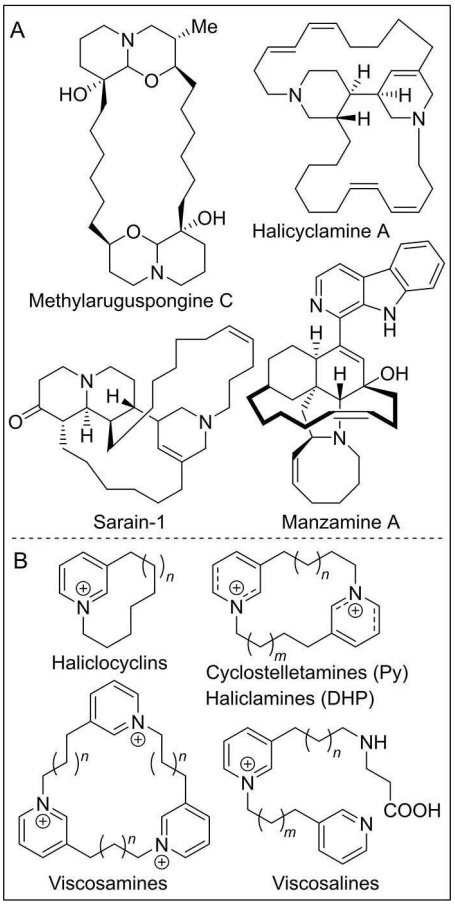

Figure 1. A) Structures of 3-APAs isolated from *Haliclona* sp. B) Structures of the five classes of 3-APAs isolated from *Haliclona viscosa*. Py = pyridinium, DHP = dihydropyridine.

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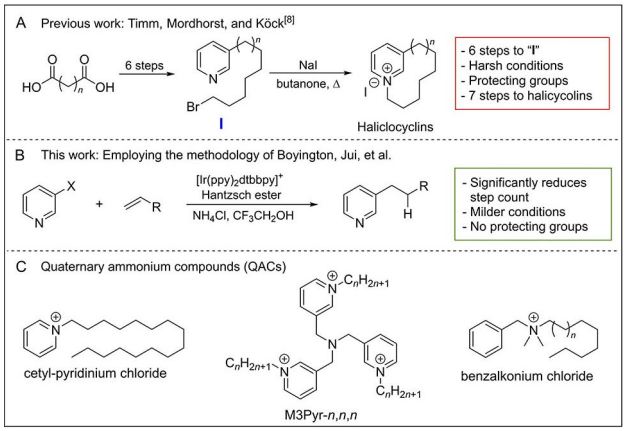


Figure 2. A) Example of a synthetic route by Köck, et al. previously employed to access common intermediate **A** and finally haliclocyclins. B) Hydroarylation reaction developed by the Jui group enables construction of **A** in a single step. C) Structures of commercial quaternary ammonium compounds.

To improve upon the synthetic strategy of **I**, we postulated that the methodology recently developed by the Jui Lab at Emory University would enable rapid access to this 3-alkylated pyridine through the use of mild photocatalytic conditions (Figure 2B).[10] It has been shown that this regioselective process is tolerant of a wide range of functional groups, as indicated by the broad scope of both halopyridines and alkenes as well as its application to the synthesis of the fungicide fluopyram produced by Bayer.[10c] By employing this methodology, access to intermediate **I** would be achieved in a single step, ablating any harsh conditions or protecting groups. A more streamlined approach to this intermediate enables the rapid construction of libraries of 3-APAs and derivatives for biological analysis.

While *H. viscosa* 3-APAs represent a promising opportunity for synthetic improvements, their antibiotic activity also sparks interest due to their quaternary ammonium structure, classifying them as quaternary ammonium compounds (QACs). Notably, the 3-APAs are structurally similar to a well-known QAC, cetylpyrdinium chloride (CPC), which is an active ingredient in most mouthwash and a linear variant to the haliclocyclins (Figure 2C). QACs are the leading molecular scaffold found in disinfectant products, with use in commercial antiseptics such as Lysol® and Clorox® for over 70 years.[11] They kill bacteria by lysing bacterial membranes, leading to the idea that resistance to these therapeutics should be near impossible, although some instances of resistances have been identified and evaluated.[12] Further, despite being ubiquitous in both house cleaning products and cosmetics, research into their structural development and optimization has been quite limited.[13] Therefore, further study into this class of molecules is needed. Towards this end, the Wuest and Minbiole labs have extensively studied the bioactivity and therapeutic potential of several classes of both natural and synthetic QACs, and therefore have a wealth of experience in both their synthesis and biological evaluation.[14] Thus, we were excited by the opportunity to apply novel methodology to synthesize these compounds and study their antimicrobial activity.

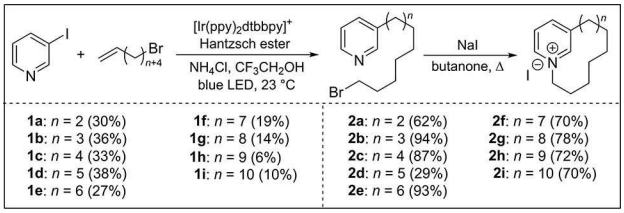
Rapid access to the 3-APA monomer class, haliclocyclins will enable the full breadth of their biological activity to be evaluated. Previous studies used disc-diffusion assays to assess the antibacterial activity of a small subset of these compounds. While this technique is useful in determining susceptibility of bacteria to most antibiotics, the cationic nature of 3-APAs causes a slower rate of diffusion through the agar, thereby limiting the accuracy of the assay in this circumstance.[15] A more streamlined approach will give a more accurate picture of the bioactivity of these molecules, which will be further detailed in the next section.

In this work, we developed a concise synthetic route to the key intermediate, **I**, enabling us to access a nine-compound library comprised of 2 natural and 7 unnatural haliclocyclins (Figure 2D). This library was assessed for antibacterial and hemolytic activity.

Results and Discussion

Synthesis of the above-mentioned nine-compound series commenced with hydroarylation of 3-iodopyridine with terminal-bromo alkenes containing 8–16 carbon atoms giving the desired 3-alkylated adducts **1a**–**i** (Scheme 1). We noticed a decrease in the yield of this reaction correlating to the increase in alkene chain length, which we believe is likely due to a decrease in solubility as chain length of these highly nonpolar alkenes in the reaction solvent increases. While the addition of small amounts of toluene was found to slightly increase these yields in certain instances, alternative solvents were not evaluated as this has been studied extensively and shown to be critical to the outcome of the reaction.[10a,b] It should be noted that alkenes containing 13–16 carbons were difficult to obtain through commercial sources and were thus synthesized via a Grignard reaction between 4-pentenylmagnesium bromide and the necessary dibromoalkane (see Supporting Information for full details). All nine compounds were then cyclized, giving the desired 3-APA molecules **2a**–**i** in modest to good yields. This highly efficient synthetic route accesses nine monomeric cyclic 3-APAs in just two to three steps. Not only did this hydroarylation methodology reduce the step count by four steps in comparison to previous work presented by Köck et al., it also employed milder conditions overall.

Upon completion of the library synthesis, compounds were assessed for their antimicrobial activity against seven different strains of Gram-negative and Gram-positive bacteria, including



Scheme 1. Synthesis of haliclocyclins and analogs and corresponding yields.

the highly prevalent oral pathogen, *Streptococcus mutans* (Table 1). As mentioned previously, some of these compounds were evaluated using a disc-diffusion assay, which is not necessarily compatible with this class of compounds. To gain a clearer understanding of their potency, we performed minimum inhibitory concentration (MIC) assays. In these studies, decreasing amounts of compound are incubated with the bacteria, and the MIC is determined as the minimum concentration of compound required to fully inhibit bacterial growth.[16] Hence, diffusion of the cationic compound through agar medium is no longer required to inhibit bacterial growth. Overall, they exerted more potent activity against Gram-positive species than Gram-negative species as is typical for most QACs. These results support the notion that these compounds act by perturbing cellular membranes, additionally coinciding with previous QAC bioactivity data. In addition, several compounds displayed modest activity against *S. mutans*. As seen previously MIC values varied slightly across the 9-compound library, with increased potency correlating to longer chain length. However, it should be noted that previous work has shown that there is an optimized amount of alkyl character in a QAC with respect to its bioactivity, and therefore it is likely that this trend also applies to haliclocyclins.[14]

To further assess therapeutic potential, the toxicity was analyzed in a red blood cell (RBC) lysis assay using mechanically defibrillated sheep's blood. These values are indicated as $Lysis_{20}$ and are measured as the concentration that lyse 20% or less of RBC. Overall, the toxicity of these compounds was quite high, indicated by the low $Lysis_{20}$ values. This is not surprising, however, as these compounds previously showed cytotoxicity against mouse embryonic fibroblasts.[5g]

Conclusion

In conclusion, we developed a concise and straight-forward route to the monomeric 3-APAs, haliclocyclins, and structural analogs. Through this route, we synthesized a library of nine haliclocyclins in two to three steps with moderate to good yields and mild photocatalytic conditions. We also investigated their antibiotic and cytotoxic activity using MIC and RBC lysis assays, respectively. While this activity was rather modest, it did contribute to our growing database of QACs and their SAR information, thereby furthering our knowledge of these compounds and their bioactivity. In addition to their antibacterial activity, 3-APAs in general, have other reported bioactivity as previously mentioned: epidermal growth factor inhibition, muscarinic acetylcholine receptor antagonistic activity, and mouse embryonic fibroblast cytotoxicity.[2,5g,7] Thus, the new route we have developed provides a platform for accessing these types of compounds in a more efficient manner, so their bioactivity can be further evaluated. Additionally, this route can be applied towards the synthesis of more complex alkaloids such as the previously mentioned manzamine A and halicyclamine A for which these simpler 3-APAs function as a common intermediate. This work has displayed a simplified synthetic route and initial antibacterial testing of haliclocyclins and analogs, paving the way for further synthetic and biological investigations of 3-APAs.

Experimental Section

See the Supporting Information.

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Table 1. MIC and red blood cell hemolysis values for compounds **2a**–**2i**. MIC values are reported as the highest concentration of compound required to inhibit growth completely across three replicate trials. Cell lysis values are reported as the lowest concentration required to lyse red blood cells. Both assays were performed in triplicate. CA (community acquired); HA (hospital acquired).

Compound 	Minimum inhibitory concentration [μM]							$Lysis_{20}$ [μM]
	S. aureus	CA-MRSA	HA-MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*S. mutans*	
2a ($n=2$)	32	32	32	64	32	64	64	2
2b ($n=3$)	32	32	16	64	32	64	125	0.25
2c ($n=4$)	32	32	16	64	32	64	32	0.5
2d ($n=5$)	32	32	32	64	64	64	125	0.125
2e ($n=6$)	32	32	16	125	125	125	64	8
2f ($n=7$)	16	16	8	125	32	125	16	4
2g ($n=8$)	32	32	16	250	64	250	32	16
2h ($n=9$)	32	16	16	250	64	250	32	16
2i ($n=10$)	8	8	8	125	64	125	16	8
CPC[a]	0.5	1	1	250	32	250	1	16
BAC[a]	2	4	4	125	64	125	1	8

Conflict of Interest

The authors declare no conflict of interest.

Keywords: antimicrobial · haliclocyclins · hydroarylation · MRSA · quaternary ammonium compounds

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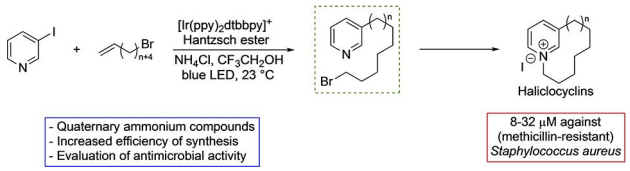

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