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Biomimetic Synthesis and Biological Evaluation of Aplidiopsamine A

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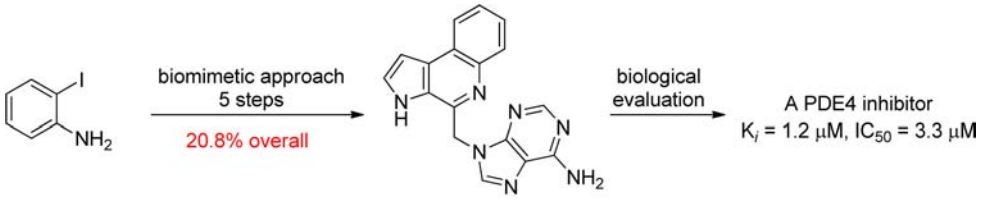
ABSTRACT



The first total synthesis of Aplidiopsamine A, a rare 3*H*-pyrrolo[2,3-*c*]quinoline alkaloid from the *Aplidiopsis confluata*, has been achieved following the proposed biosynthesis. This biomimetic synthesis requires only five steps and proceeds in 20.8% overall yield. Biological evaluation across large panels of discrete molecular targets identified that Aplidiopsamine A is a highly selective PDE4 inhibitor, a target for numerous CNS disorders.

The tricyclic 3*H*-pyrrolo[2,3-*c*]quinoline ring system is a rare moiety found only in a few marine natural products.[1,2] The first members of this structural class were the marinoquinolines A–F (**1**–**5**), isolated from multiple species of bacteria and found to possess antibacterial, antifungal, and acetylcholinesterase activities (Figure 1).[1,2] Several synthetic approaches to marinoquinolines A–C and E (**1**–**3**, **5**) have been reported, relying on either a key Morgen–Walls or Pictet–Spengler reaction.[3,4] Recently, aplidiopsamine A (**6**) was isolated from the Australian ascidian, *Aplidiopsis confluata*, and represents the first example of the 3*H*-pyrrolo[2,3-*c*]quinoline moiety linked to an adenine via a methylene bridge, a rare nonglycoside adenine conjugate. Thus, **6** represents only the second report of a marine organism producing the 3*H*-pyrrolo-[2,3-*c*]quinoline ring system; importantly, **6** was found to be a potent antimalarial agent, without toxicity to healthy cells, further providing support for synthesis.[5]

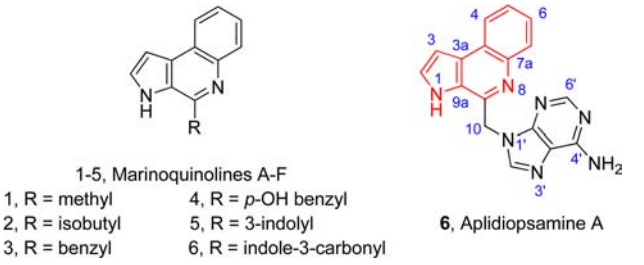


Figure 1. Structures of the marinoquinolines A–F (**1**–**5**) and aplidiopsamine A (**6**), marine natural products possessing the rare 3*H*-pyrrolo[2,3-*c*]quinoline ring system (shown in red).

Carroll and co-workers developed a convergent biosynthetic proposal for **6** from adenine **7** and tryptophan **10** (Scheme 1). Here, **9** is generated from the reaction of adenine and hydroxyacetic acid. Pyrrole **11**, itself a natural product isolated from *R. thailandica*, was postulated to be a dead-end product in the biosynthesis of pyrrolnitrin from tryptophan. Condensation of **9** and **11** would ultimately

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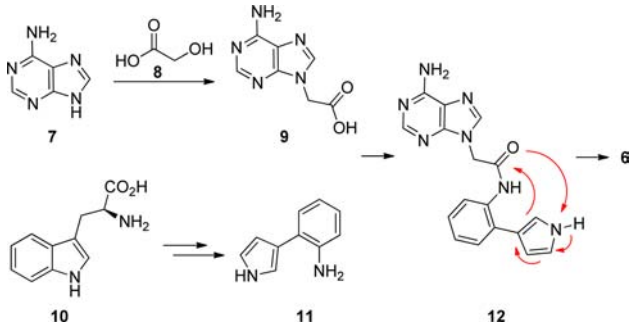
lead to aplidiopsamine A, via a Bischler–Napieralski-like cyclization and dehydration sequence.[5]

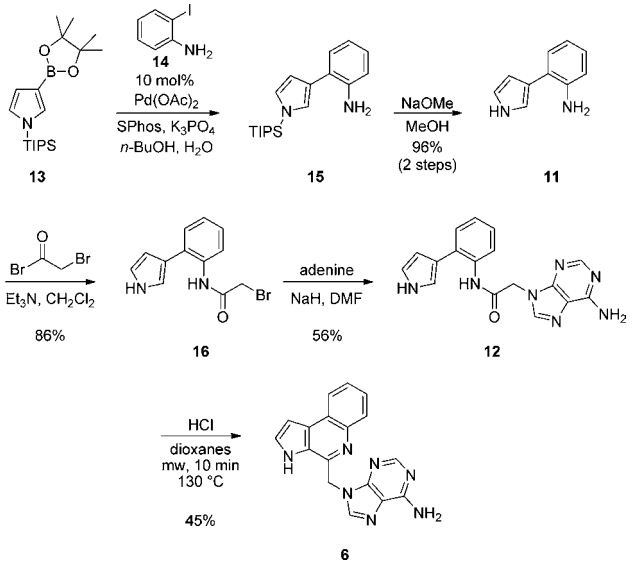
Scheme 1. Proposed Biosynthesis of Aplidiopsamine (**6**)



Therefore, we elected to pursue a biomimetic approach for the first total synthesis of aplidiopsamine A and attempt the condensation of a suitable synthetic surrogate of **9** and pyrrole **11** to access **12**. Interestingly, Correia synthesized **11**, en route to marinoquinolines A–C and E, from advanced materials in five synthetic steps in 47.5% overall yield.[4] We envisioned a more expedited route and were gratified that a two-step sequence from commercial reagents produced **11**. In the event, aniline **14** smoothly underwent a Suzuki coupling with boronate ester **13** to deliver TIPS protected pyrrole **15**, which upon basic hydrolysis generated **11** in 96% yield over the two steps (Scheme 2).[6] Acylation with α-bromo acetyl bromide provided **16** in 86% yield. Selective *N*-alkylation at the 9- versus 7-position of adenine to afford **12** was achieved via a hard deprotonation with NaH in 56% yield. Softer approaches with cesium carbonate resulted in 2:1 regiosiomeric mixtures and poor conversion. With **12** in hand, we were now posied to perform the biomimetic condensation via a Bischler–Napieralski-type cyclization. Classical variants of this cyclization/dehydration sequence[7] employ TFA, $POCl_3$, or other Lewis acids; however, in the presence of the adenine moiety, these conditions either failed or led to intractable gums or poor conversion (less than 10% yields). Ultimately, we found that 4 M HCl/dioxanes under microwave irradiation (130 °C, 10 min) facilitated the reaction sequence to deliver, for the first time, aplidiopsamine A in 45% yield.[6] Overall, the biomimetic synthesis of **6** required five steps and proceeded in 20.8% overall yield, an ideal route to prepare unnatural analogs. The synthetic **6** exhibited physical and spectroscopic data identical to those of the natural aplidiopsamine A.[5,6]

With large quantities of **6** in hand, we elected to further profile **6** against a larger panel of discrete molecular targets of therapeutic significance beyond antimalarial activity, as **6** was shown to not be cytotoxic. Indeed, we have previously elucidated intriguing activities for a number of

Scheme 2. Biomimetic Total Synthesis of Aplidiopsamine (**6**)



marine alkloids at CNS targets with unprecedented selectivities among highly conserved receptor families.[8–11] As **6** possesses the basic pharmacophore (H-bond donor/acceptor triad) of many known ATP-competitive kinase inhibitors,[12] we profiled synthetic **6** in a KINOMEscan panel against 97 kinases (both wild-type and mutants) at a 10 μM concentration.[6,13] Despite the presence of a known pharmacophore,[12] **6** was uniformly inactive; however, this can also be viewed as possessing very clean ancillary pharmacology. In parallel, we also profiled **6** in a Lead Profiling screen (a radio ligand binding panel at a 10 μM concentration) against 68 GPCRs, ion channels, and transporters.[14] Once again, **6** displayed very clean ancillary pharmacology, possessing activity at only two targets: 5-HT_{2B} (62% @ 10 μM) and PDE4/Rolipram (74% @ 10 μM). When full concentration–response curves (CRCs) were obtained for **6**, the 5-HT_{2B} K_i was weak at ~10 μM, but **6** proved to be a moderately potent PDE4 inhibitor (K_i = 1.2 μM, IC_{50} = 3.3 μM).[6,14] PDE4 is a high profile target for antidepressant, antipsychotic, and neuroprotective drug

(6) See Supporting Information for full details.

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(15) Pages, L.; Gavalda, A.; Lehner, M. D. *Expert Opin. Ther. Pat.* **2009**, *19*, 1501–1519.

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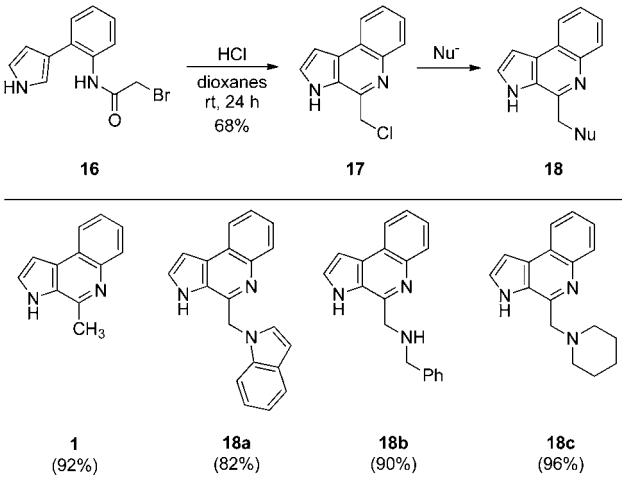
development; moreover, **6** represents a fundamentally new PDE4 inhibitor chemotype.[15,16]

With the identification of a therapeutically relevant target for **6**, the need emerged to develop rapid chemistry to enable the synthesis of unnatural analogs, and ideally, from a common intermediate. Application of our optimized cyclization/dehydration conditions with the advanced α-bromo intermediate **16** led directly to the 3*H*-pyrrolo[2,3-*c*]quinoline derivative **17**, wherein the reaction conditions with HCl generated the corresponding chloromethyl congener (Scheme 3). Notably, high reaction temperatures typically employed in Bichler–Napieralski reactions were avoided as the condensation was found to proceed at room temperature. Compound **17** proved to be an ideal common intermediate for not only the synthesis of unnatural analogs of **6**, compounds **18a**–**18c**, in high yields (82–96%) but also the synthesis of marinoquinoline A (**1**) when the nucleophile was a hydride source, $LiAlH_4$. This approach affords the natural product **1** in only five steps and in 52% overall yield, representing a highly expeditious route.[3,4]

Scheme 3. Synthesis of Marinoquinoline A (**1**) and Unnatural Analogs of **6** from a Common Intermediate



With unnatural analogs of **6** in hand, we evaluated them against both rat PDE4 (Rolipram) and human PDE4 to discern structure–activity relationships (SAR). As shown in Table 1, several of the unnatural analogs possessed PDE4 actvity but were weaker than **6** (rat K_i's of 4.6 to 25 μM and IC_{50}'s of 13 to 71 μM). All proved to be weaker functional antagonists on human PDE4 than rat. Interestingly, marinoquinoline A (**1**), lacking the amino methyl moiety at C9, was inactive as was the Bischler–Napieralski acyclic substrate **12**, devoid of the 3*H*-pyrrolo[2,3-*c*]-quinoline ring system. Overall, this suggests that the tricyclic 3*H*-pyrrolo[2,3-*c*]quinoline ring system with a C9 amino methyl moiety is essential for PDE4 activity, and this chemotype represents a fundamentally new PDE4 pharmacophore.

Table 1. Phosphodiesterase PDE4 Activities of **6** and Unnatural Analogs

	rat Rolipram[a]		human PDE4[b]
compd	K_i (μM)	IC_{50} (μM)	IC_{50} (μM)
6	1.2	3.3	6.1
1	>100	>100	>100
18a	25.6	71.7	31.8
18b	4.7	13.1	72.9
18c	5.0	14.0	>100
12	>100	>100	>100

[a] Wistar rat brain, 1.8 nM [^{3}H] rolipram, 1% DMSO, incubation for 60 min at 4 °C in 50 mM Tris-HCl, pH 7.4. [b] Human U937 cells, 1.10 μM [^{3}H]cAMP+cAMP, incubation for 20 min at 25 °C, 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, quantitaion of [^{3}H]adenosine.[14]

In summary, we have completed the first total synthesis of aplidiopsamine A (**6**) following the proposed biosynthetic pathway in five steps and with a 20.8% overall yield. Extensive biological profiling identified **6** as a moderately potent ligand for, and inhibitor of, PDE4, an important target for CNS drug discovery. These data further argue for natural products as a source of novel chemotypes to initiate drug discovery efforts. Furthermore, we developed chemistry to rapidly access unnatural analogs of **6** and also synthesized marinoquinoline A (**1**) in only five steps and in 52% overall yield. The SAR within this novel class of PDE4 ligands demonstrated that both the tricyclic 3*H*-pyrrolo[2,3-*c*]quinoline ring system and a C9 amino methyl moiety are required for PDE4 activity. Further refinements, DMPK analysis, and additional unnatural analog synthesis are underway and will be reported in due course.

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Supporting Information Available. Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.