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Synthesis of a *Microcystis aeruginosa* predicted metabolite with antimalarial activity

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ABSTRACT

The synthesis of a *Microcystis aeruginosa* predicted metabolite analog of aerucyclamide B was performed. This hexacyclopeptide was obtained from three heterocyclic building blocks by a convergent macrocycle-assembly methodology. The compound exhibited good in vitro antiplasmodial activity (IC_{50}: 0.18 μM, K1, cholorquine resistant strain).

Marine natural products[1] play an important role in drug development particularly in anticancer, antibiotics and antiparasitics drugs.[2] Various bioactive cyanobacterial compounds exhibit unique structural features like cyclic peptides containing azole heterocycles.[3] It is well known that macrocyclic peptides can demonstrate drug-like physicochemical and pharmacokinetic properties such as good metabolic stability, solubility, lipophilicity and bioavailability.[4] In addition, asymmetrical cyclopeptides are considered as new scaffolds for supramolecular chemistry and provides an important method for the development and preparation of nanoscale objects.[5]

Examples of azole cyclopeptides are aerucyclamide B (**1**),[6] dendroamide A (**2**),[7] and venturamide A (**3**),[8] Figure 1. Aerucyclamide B was isolated in 2008 by Gademann and co-workers from the toxic freshwater cyanobacterium *Microcystis aeruginosa* PCC 7806 and displays potent and selective antiplasmodial activity against *Plasmodium falciparum* K1. This species is the most virulent of the genus *Plasmodium* and causes more than 95% of malaria-related morbidity and mortality. According to the World Health Organization 2011 report, there were an estimated 216 million episodes and 655000 deaths of malaria in 2010.[9] Various antimalarial drugs are presently used for the treatment of this tropical disease, but the rapid spread of resistance seriously compromises their efficacy.[10]

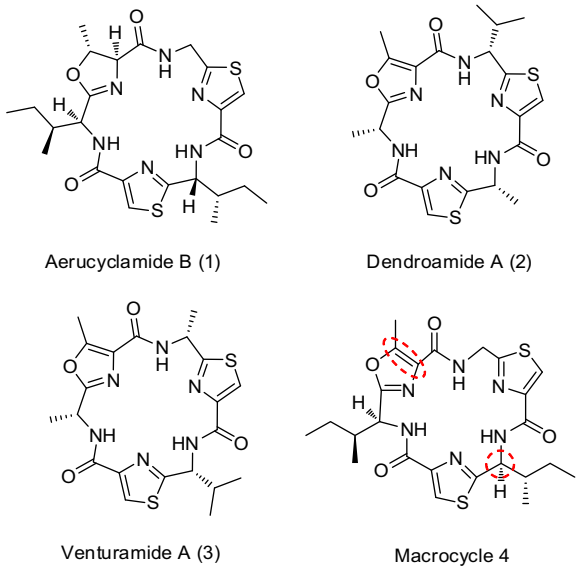

Figure 1. Natural cyclopeptides and predicted macrocycle **4**.

In spite of the potent antimalarial activity displayed by aerucyclamide B, its total synthesis has not been described in the literature to date. However, aerucyclamide B was obtained through oxidation of the natural aerucyclamide A using MnO_2/benzene.[6]

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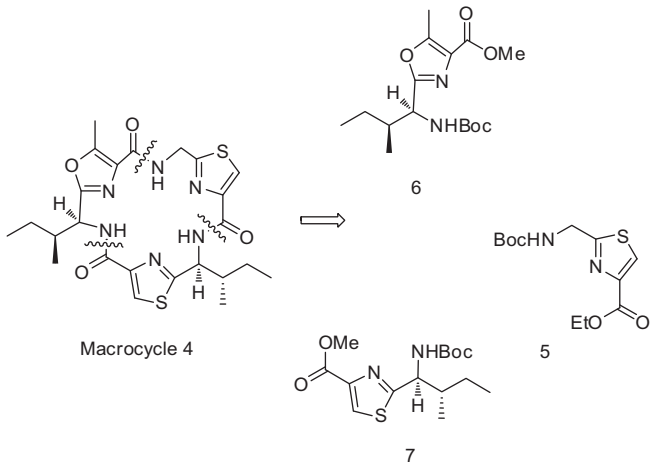


Scheme 1. Retrosynthetic analysis for macrocycle **4**.

From the analyses of genomic data of *M. aeruginosa* PCC 7806, Dittmann and co-workers predicted three hexapeptides containing thiazole and oxazole rings.[11] One of these predicted compounds is the structural analog of aerucyclamide B, macrocycle **4**, containing an oxazole and L-Ile instead of an oxazoline ring and D-*allo*-Ile, respectively, Figure 1. Based on the well-known oxazolines instability, we hypothesize that macrocycle **4** is a more stable compound than aerucyclamide B.[12]

As part of our search for candidates of antiparasitic new drugs,[13] we embarked in the synthesis of cyclohexapeptides that alternate oxa/thiazole rings, analogs of marine natural products.

In this Letter, we present the synthesis and antimalarial activity of macrocycle **4**, a predicted metabolite that could be isolated from *Microcystis aeruginosa* PCC 7806, as a more stable analog of the antimalarial aerucyclamide B.

Considering that macrocyclizations of linear peptides are often slow, low yielding procedures accompanied by side reactions, we planned our synthesis from three heterocyclic building blocks **5**,[14] **6**,[15] and **7**,[15] Scheme 1. The presence of these heterocycles in the open precursor of **4**, could rigidized the hexapeptide and facilitate the macrocycle formation.[16]

Different methodologies were employed in order to obtain azoles **5**, **6** and **7** using aminoacids as starting materials.

Our synthesis of thiazole **5**, utilized conventional Hantszch's methodology, Scheme 2.[17] Amide formation from Boc-Gly-OH employing 2,2,2-trichloroethyl chloroformiate/aqueous ammonia and further reaction with Lawesson's reagent allowed us to obtain thioamide **8.** Hantszch synthesis using ethyl bromopyruvate in Py/EtOH at reflux, afforded thiazole **5**. This compound was obtained from Boc-Gly-OH in 40% overall yield.

The cyclodehydration of β-hydroxyamides or β-hydroxythioamides and further oxidation protocol was selected for the preparation of oxazole **6** and thiazole **7**, respectively.

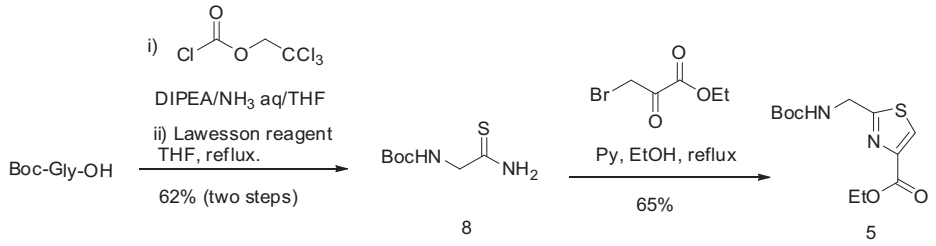
First, dipeptide Boc-L-Ile-L-Thr-OMe (**9**) was synthesized in very good yield from the corresponding protected aminoacids employing HBTU as coupling reagent, Scheme 3. Then, the smooth cyclodehydration reaction with DAST and further oxidation with $BrCCl_3$/DBU allowed us to obtain oxazole **6**.[18]

High yielding preparation of dipeptide **10** was achieved by using HBTU, Scheme 4. Attempts to prepare the thioamide **11** from the TBS ether derivative of **10**,[19] and Lawesson's reagent failed.[20] In contrast, **11** was obtained in good yield using Wipf's procedure by cyclodehydration of **10** with DAST, giving oxazoline **12** (68%) and then thiolysis using H_2S/MeOH/Et_3N (85%).[21] This reaction is high-yielding and offers an alternative to the thionation of peptides using Lawessońs reagent. To produce the desired thiazole **7** from thioamide **11**, we investigated the one-pot procedure using DAST and then $BrCCl_3$/DBU. Following this protocol, compound **11** was obtained in excellent yield (98%).

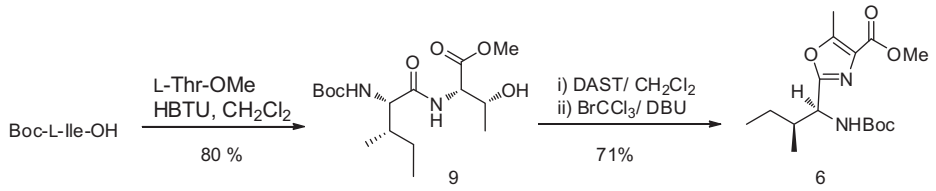
The next steps were the assembling of the key fragments into the open intermediate of macrocycle **4**. With the purpose of obtaining a suitable macrocyclization reaction, the less hindered amide bond formation was selected as the last reaction.

Compound **15**,[22] was prepared by ethyl ester hydrolysis of **5**, followed by coupling with the N-deprotected thiazole **14** using HBTU, Scheme 5. Methyl ester hydrolysis of **15**, and coupling with the N-deprotected oxazole **16**, rendered the tris-azole compound **17**.[23] C- and N- deprotection of the linear precursor **17** was achieved using aqueous KOH and then HCl/dioxane. Macrocyclization reaction was performed in diluted conditions (0.001 M) using HBTU. The desired macrocycle **4**,[24] which resulted in a stable compound, was obtained in 40%.[25]

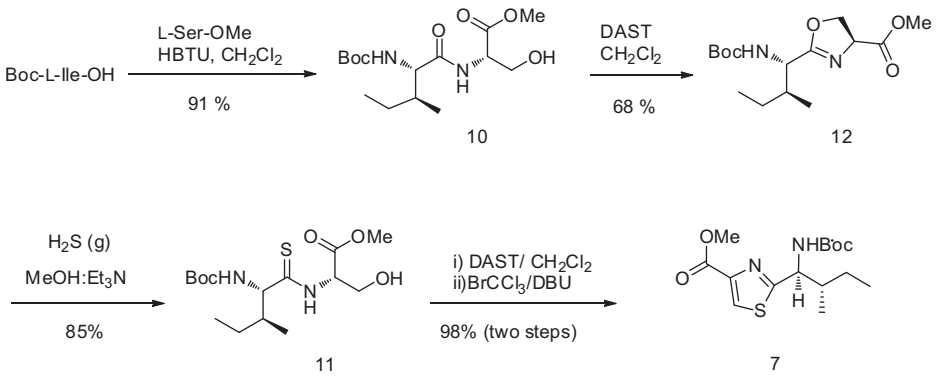
The antimalarial activity of the macrocycle **4** was determined in vitro against the chloroquine-resistant K1 strain of *P. falciparum* by using the [3H]hypoxanthine incorporation method reported by Desjardins et al.[26] This compound displays an IC_{50} value of 0.18 μM



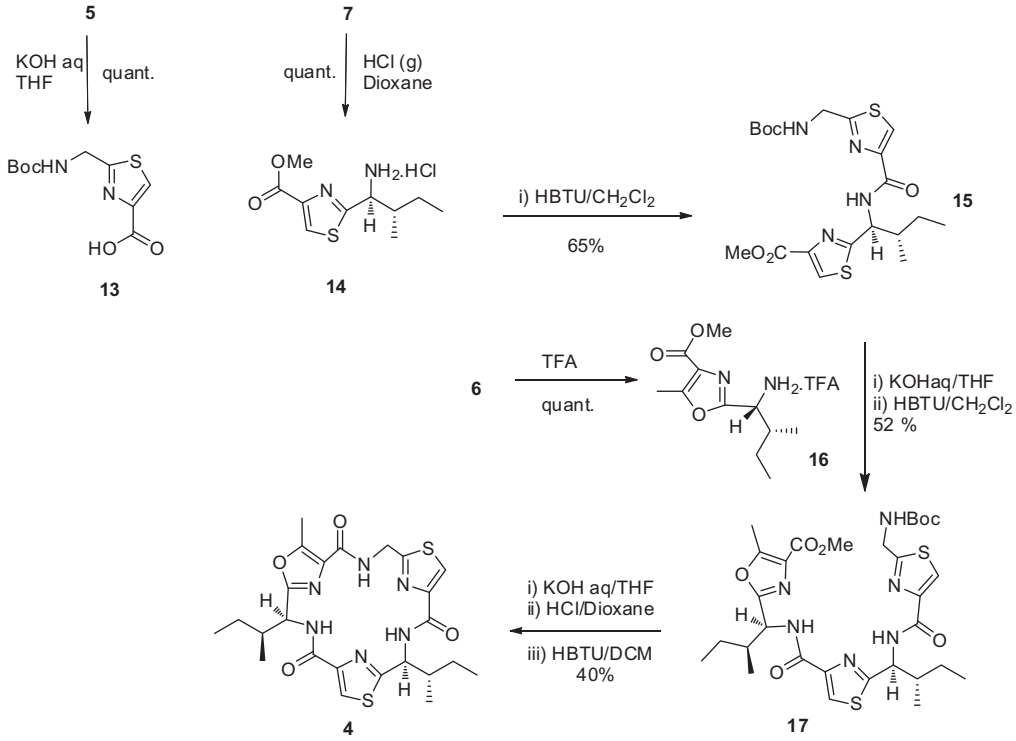
Scheme 2. Synthesis of building block **5**.



Scheme 3. Synthesis of building block **6**.



Scheme 4. Synthesis of building block **7**.



Scheme 5. Synthesis of macrocycle **4**.

showing enhanced activity when compared with its analog aerucyclamide B (IC_{50} = 0.7 μM).

In summary, the total synthesis of the previously predicted analog of aerucyclamide B, compound **4**, was accomplished through a convergent macrocycle-assembly strategy from oxa/thiazole fragments in good yield. The experimental data of the synthesized macrocycle should assist in identification of this metabolite from *Mycrocycstis aeruginosa*.

This stable compound shows 4 times more in vitro antimalarial activity than aerucyclamide B.

We are currently investigating the synthesis and biological evaluation of new analogs of macrocycle 4.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.028. These data include MOL files and InChiKeys of the most important compounds described in this article.

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22. *Experimental data for compound* ***15***: R_f = 0.3 (EtOAc:hexane, 1:1); $[\alpha]_D$ = −2.8 (*c* 2.45, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, 3H, *J* = 7.4 Hz), 0.97 (d, 3H, *J* = 6.8 Hz), 1.22–1.33 (m, 1H), 1.47 (s, 9H), 1.56–1.67 (m, 1H), 2.32–2.42 (m, 1H), 3.94 (s, 3H), 4.63 (d, 2H, *J* = 5.9 Hz), 5.32–5.40 (m, 2H), 7.97 (d, 1H, *J* = 9.3 Hz), 8.05 (s, 1H), 8.11 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 16.0, 24.8, 28.3 (**3C**), 39.4, 42.3, 52.5, 55.6, 80.6, 124.3, 127.3, 147.1, 149.1, 155.6, 160.7, 161.8, 169.6, 171.8. HRMS *m*/*z* calcd for $C_{20}H_{28}N_4NaO_5S_2$ (M+Na) 491.1399, found 491.1414. IR ν_{max} (liquid film) 2968, 2933, 1718, 1701, 1670, 1481, 1276, 1248, 1217, 1167 cm^{-1}.
23. *Experimental data for compound* ***17***: R_f = 0.38 (EtOAc:hexane, 3:2); $[\alpha]_D$ = −5.0 (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92–1.02 (m, 12H), 1.24–1.33 (m, 2H), 1.46 (s, 9H), 1.58–1.66 (m, 2H), 2.11–2.18 (m, 1H), 2.24–2.34 (m, 1H), 2.62 (s, 3H), 3.89 (s, 3H), 4.62–4.67 (m, 1H), 5.26 (dd, 1H, *J* = 7.6 Hz, *J* = 9.3 Hz), 5.40 (dd, 1H, *J* = 6.2 Hz, *J* = 9.1 Hz) 5.54 (dd, $J_1 = J_2$ = 5.8 Hz, 1H), 7.91 (dd, $J_1 = J_2$ = 9.6 Hz, 1H), 8.03 (s, 1H), 8.09 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 11.5, 12.1, 15.5, 15.9, 24.8, 25.3, 28.3 (3C), 39.1, 39.5, 42.4, 51.5, 52.0, 55.5, 80.5, 123.6, 124.5, 127.5, 149.0, 149.4, 155.7, 156.2, 160.6, 160.7, 161.8, 162.7, 170.1, 171.1. HRMS *m*/*z* calcd for $C_{30}H_{42}N_6NaO_7S_2$ [M+Na]+ 685,2454 found 685.2447. IR ν_{max} (liquid film) 2959, 2928, 2872, 2857, 1719, 1654, 1544, 1182, 1130, 1101 cm^{-1}.
24. *Experimental data for macrocycle* ***4***: R_f = 0.37 (EtOAc:hexane, 3:2); $[\alpha]_D$ = −56.0 (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93–1.04 (m, 12H), 1.26–1.37 (m, 2H), 1.50–1.59 (m, 1H), 1.63- 1.74 (m, 1H), 2.10–2.18 (m, 2H), 2.68 (s, 3H), 4.71 (dd, 1H, J_1 = 2.6 Hz, J_2 = 18.0 Hz), 5.07 (dd, 1H, J_1 = 5.6 Hz, J_2 = 18.0 Hz), 5.31 (dd, 1H, J_1 = 3.8 Hz, J_2 = 8.5 Hz), 5.44 (dd, 1H, J_1 = 6.0 Hz, J_2 = 8.5 Hz), 8.08 (s, 1H), 8.16 (s, 1H), 8.42–8.50 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 11.7, 11.8, 14.8, 15.2, 25.1, 26.0, 39.9, 40.8, 41.0, 52.1, 55.0, 123.1, 124.3, 128.3, 148.7, 149.5, 153.8, 159.5, 160.0, 160.6, 161.1, 165.2, 167.7. HRMS *m*/*z* calcd for $C_{24}H_{30}N_6NaO_4S_2$ (M+Na) 553.1668, found 553.1649. IR ν_{max} (liquid film) 3400, 3123, 2965, 2930, 2860, 1670, 1539, 1491 cm^{-1}.
25. Macrocycle 4 was stable even at room temperature.
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