NOTE

Adipostatins A–D from *Streptomyces* sp. 4875 inhibiting *Brugia malayi* asparaginyl-tRNA synthetase and killing adult *Brugia malayi* parasites

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Lymphatic filariasis (LF), also known as elephantiasis, is one of the World Health Organization's (WHO's) top 10 neglected tropical diseases.¹ LF is caused primarily by two related parasites, *Brugia malayi* and *Wuchereria bancrofti*, and represents a worldwide health crisis with over 200 million people infected and another 20% of the global population at risk for infection.^{1,2} Aminoacyl-tRNA synthetases (AARSs) were the first filarial targets for antiparasite drug discovery embraced by the WHO and are generally regarded as excellent therapeutic targets because AARSs have a key role in protein synthesis.³ Among the AARSs, in particular, asparaginyl-tRNA synthetase (AsnRS) in *B. malayi* is considered an excellent antifilarial target because (i) it is highly expressed in both sexes, adults and larvae of *B. malayi*; (ii) it is well characterized biochemically and structurally in *B. malayi*; (iii) it shows significant structural differences in comparison with human AARSs; and (iv) a high-throughput screening (HTS) platform with recombinant *B. malayi* AsnRS (*BmAsnRS*) for inhibitors has been developed.^{2,4–6}

Natural products remain a valuable source of new drug leads and have demonstrated almost limitless potential in showcasing new molecular scaffolds with clinically relevant biological activities.⁷ In an effort to discover drug leads targeting *BmAsnRS*, we recently completed a HTS campaign of ~73 000 extracts from a collection of 36 720 microbial strains. Of the extracts screened, 177 active strains were identified. Subsequent bioassay-guided dereplication of two of the active strains resulted in the discovery of two natural product scaffolds as promising antifilarial drug leads, represented by tirandamycin B (TAM B) from *Streptomyces* sp. 17944⁸ and the depsipeptide WS9326D from *Streptomyces* sp. 9078.⁹ TAM B and WS9326D kill the adult *B. malayi* parasites at low nanomolar concentrations yet do not exhibit significant general cytotoxicity to human hepatic cells.^{8,9}

Here we now report the bioassay-guided dereplication of another active strain, *Streptomyces* sp. 4875, leading to the discovery of four alkylresorcinols, named adipostatin A (1), adipostatin B (2), adipostatin C (3) and adipostatin D (4; Figure 1). All four adipostatins

inhibit *BmAsnRS* and efficiently kill the adult *B. malayi* parasite. The adipostatins, which have not been recognized previously for use in the treatment of LF, represent another lead scaffold for antifilarial drug discovery.^{8,9}

Streptomyces sp. 4875 was identified as a *Streptomyces* species on the basis of its 16S ribosomal RNA (Supplementary Figure S1). A two-stage fermentation of *Streptomyces* sp. 4875 was performed as reported before (Supplementary Information).^{8,9} The production culture (8 l) was extracted with 3% Amberlite XAD-16 (Sigma-Aldrich, St Louis, MO, USA) resin to afford the crude extract. The crude extract was subjected to multiple steps of SiO₂, Sephadex LH-20 and preparative C-18 chromatography. Dereplication and natural product isolation were guided by bioassay for inhibitory activity against the recombinant *BmAsnRS*, affording pure 1 (3 mg), 2 (4.2 mg), 3 (7 mg) and 4 (5 mg) as white powders (Supplementary Information). The ¹H and ¹³C NMR spectra of 1–4 were obtained in CD₃OD (Table 1 and Supplementary Figures S2–S15).

Analysis of the HRESIMS data, the ¹H and ¹³C NMR spectra and the 2D NMR data (Supplementary Figures S2–S11) of 1 and 2 suggested them to be adipostatin A and B, respectively, two alkylresorcinol regioisomers that have been previously isolated from *Streptomyces cyaneus* 2299-SV1¹⁰ and were patented as potent glyceryl-3-phosphate dehydrogenase inhibitors.¹¹ The identity of 1 and 2 was subsequently confirmed by NMR analysis (Supplementary Figures S2–S11), as well as comparison with the spectroscopic data reported previously (Table 1).¹⁰ HRESIMS analysis of 3 and 4 yielded the [M+H]⁺ ions at *m/z* 335.2946 and *m/z* 335.2944, respectively, indicative that 3 and 4 were also regioisomers with the same molecular formula of C₂₂H₃₈O₂ (calculated [M+H]⁺ ion for C₂₂H₃₈O₂ at *m/z* 335.2944) but differed from 1 and 2 (C₂₁H₃₆O₂) by an additional CH₂ unit. On the basis of the ¹H and ¹³C NMR data of 3 and 4 (Supplementary Figures S12–S15), as well as in comparison with those of 1 and 2 (Supplementary Figures S2–S11), 3 and 4 were established as adipostatins C (3) and D (4) (Figure 1). Although 3 and

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4 had been reported previously in the patent literature,¹¹ their spectral data were not available. The ¹H and ¹³C NMR data for 3 and 4 are therefore reported here, in comparison with 1 and 2 reported previously¹⁰ (Table 1).

Each of the four purified adipostatins was re-evaluated for their inhibitory activity against *BmAsnRS*, using the recently reported pretransfer editing assay.^{6,8,9} Inhibition against *BmAsnRS* was observed for all the four adipostatins with apparent IC₅₀s estimated to be 15 μM (for 1), 15 μM (for 2), 15 μM (for 3) and 30 μM (for 4) (Supplementary Figure S16). We next tested the four adipostatins for their ability to kill adult *B. malayi* worms *in vitro* following the published procedure.^{8,9} Live adult *B. malayi* worms were maintained in six-well plates (three worms per well), and each of the four adipostatins, varying from 100 nm to 100 μM, was added to selected

wells to test their parasite-killing activity, with TAM B as positive controls (Supplementary Figures S17–S20). At 100 μM concentration, 2 and 3 kill all the adult *B. malayi* worms rapidly, that is, within 24 h, whereas at that concentration, 1 and 4 require 48 h for complete killing, with 3 and 1 efficiently kill the adult worms within 8 days and 9 days, respectively, at concentrations as low as 100 nm (Table 2). Worm death was unambiguously differentiated from simple paralysis by the modified MTT assay.^{8,9} Although 2 and 3 seemed to be more potent than 1 and 4 at higher concentrations (for example, at 100 μM or 50 μM), 1 killed the worms sooner than 2 and 3 at lower concentrations (for example, at 1 μM). Finally, the four adipostatins were evaluated for general cytotoxicity by using human HepG2 cells. Cytotoxicity was defined as >50% cell death at 24 h as measured by the MTT assay for mitochondrial activity,¹² and all four adipostatins were found to be nontoxic at concentration as high as 100 μM (Supplementary Figure S21). This finding, in combination with the ability of 1–4 to selectively inhibit the *BmAsnRS* and efficiently kill the adult *B. malayi* worms, constitutes the basis for continued chemical and biological efforts to understand the mode of action and the structure-activity relationship of the alkylresorcinols, as exemplified by the adipostatins, as a new lead scaffold for antifilarial drug discovery.

Figure 1 Structures of adipostatins A–D (1–4) from *Streptomyces* sp. 4875.

Table 2 Complete killing time of adult *B. malayi* worms by adipostatins A–D (1–4) at varying concentrations^a

	100 μM	50 μM	25 μM	10 μM	1 μM	100 nm
Adipostatin A (1)	2	5	5	5	6	9
Adipostatin B (2)	1	3	8	8	9	– ^b
Adipostatin C (3)	1	2	3	4	8	8
Adipostatin D (4)	2	5	7	8	9	– ^b

^aUnit of killing time: day.

^bAt least one of the three *B. malayi* worms in each of the wells was still alive after 10 days of treatment.

Table 1 ¹H (700 MHz) and ¹³C (175 MHz) NMR data of adipostatins A–D (1–4) in CD₃OD^a

Position	Adipostatin A (1)		Adipostatin B (2)		Adipostatin C (3)		Adipostatin D (4)	
	δ _C	δ _H (mult, J in Hz)	δ _C	δ _H (mult, J in Hz)	δ _C	δ _H (mult, J in Hz)	δ _C	δ _H (mult, J in Hz)
1, 3	159.3, C		159.2, C		159.2, C		159.3, C	
2	100.9, CH	6.08 (t, 2.1)	100.9, CH	6.08 (t, 2.0)	100.9, CH	6.08 (t, 2.1)	100.9, CH	6.08 (t, 2.1)
4, 6	107.9, CH	6.12 (d, 2.1)	107.9, CH	6.12 (d, 2.0)	107.9, CH	6.12 (d, 2.1)	107.9, CH	6.12 (d, 2.1)
5	146.3, C		146.3, C		146.3, C		146.3, C	
7	37.0, CH ₂	2.43 (t, 7.3)	37.0, CH ₂	2.43 (t, 7.4)	37.0, CH ₂	2.43 (t, 7.2)	37.0, CH ₂	2.43 (t, 7.4)
8	32.4, CH ₂	1.56, m	32.4, CH ₂	1.56, m	32.4, CH ₂	1.56, m	32.4, CH ₂	1.55, m
9	30.8, ^b CH ₂	1.27–1.33	31.1, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	31.0, ^b CH ₂	1.27–1.33
10	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33
11	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33
12	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33
13	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33
14	30.7, ^b CH ₂	1.27–1.33	30.7, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33	30.8, ^b CH ₂	1.27–1.33
15	30.7, ^b CH ₂	1.27–1.33	30.6, ^b CH ₂	1.27–1.33	30.7, ^b CH ₂	1.27–1.33	30.7, ^b CH ₂	1.27–1.33
16	30.6, ^b CH ₂	1.27–1.33	30.4, ^b CH ₂	1.27–1.33	30.6, ^b CH ₂	1.27–1.33	30.6, ^b CH ₂	1.27–1.33
17	30.5, CH ₂	1.27–1.33	28.5, CH ₂	1.27–1.33	30.6, ^b CH ₂	1.27–1.33	30.4, ^b CH ₂	1.27–1.33
18	30.4, CH ₂	1.27–1.33	40.2, CH ₂	1.18, m	30.4, ^b CH ₂	1.27–1.33	28.5, ^b CH ₂	1.27–1.33
19	33.1, CH ₂	1.27–1.33	29.1, CH	1.52, m	31.0, ^b CH ₂	1.27–1.33	40.2, CH ₂	1.18, m
20	23.7, CH ₂	1.27–1.33	23.1, CH ₃	0.87 (d, 6.5)	31.1, ^b CH ₂	1.27–1.33	29.2, CH	1.52, m
21	14.4, CH ₃	0.90 (t, 7.0)	23.1, CH ₃	0.87 (d, 6.5)	23.1, CH ₂	1.27–1.33	23.0, CH ₃	0.88 (d, 6.5)
22					11.8, CH ₃	0.88 (t, 6.9)	23.0, CH ₃	0.88 (d, 6.5)

^aThe ¹H and ¹³C NMR data for 1 and 2, reported previously in CDCl₃,¹⁰ are included here in CD₃OD for comparison with those of 3 and 4 also recorded in CD₃OD.

^bInterchangeable.

In summary, **1** and **2** were first isolated as glycerol-3-phosphate dehydrogenase inhibitors from *S. cyaneus* 2299-SV1 two decades ago.¹⁰ Guided by the innovative HTS targeting *BmAsnRS*, we now report that **1** and **2**, together with two additional congeners, **3** and **4**, are novel *BmAsnRS* inhibitors that efficiently kill the adult *B. malayi* parasites, but do not exhibit significant general cytotoxicity to human hepatic cells. The adipostatins therefore represent another natural product lead scaffold that could be exploited to combat the global health crisis of LF. These findings, together with our early reports of TAM B and WS9326D as promising antifilarial drug leads,^{8,9} underscore the great promise of our strategy in screening microbial natural products as *BmAsnRS* inhibitors for antifilarial drug discovery. The microbial origin of the natural product leads, thereby their availability by scale-up fermentation, should greatly facilitate the follow-up mechanistic and preclinical studies needed to advance the most promising leads into clinical drugs.

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