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Adipostatins E-J, New Potent Antimicrobials Identified as Inhibitors of Coenzyme-A Biosynthesis

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Abstract

Phosphopantetheine is a key structural element in biological acyl transfer reactions found embedded within coenzyme A (CoA). Phosphopantothenoylcysteine synthetase (PPCS) is responsible for installing a cysteamine group within phosphopantetheine. Therefore, it holds considerable potential as a drug target for developing new antimicrobials. In this study, we adapted a biochemical assay specific for bacterial PPCS to screen for inhibitors of CoA biosynthesis against a library of marine microbial derived natural product extracts (NPEs). Analysis of the NPE derived from *Streptomyces blancoensis* led to the isolation of novel antibiotics (10–12, and 14) from the adipostatin class of molecules. The most potent molecule (10) displayed in vitro activity with IC₅₀= 0.93 μM, against *S. pneumoniae* PPCS. The whole cell antimicrobial assay against isolated molecules demonstrated their ability to penetrate bacterial cells and inhibit clinically relevant pathogenic strains. This establishes the validity of PPCS as a pertinent drug target, and the value of NPEs to provide new antibiotics

Graphical Abstract

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Supplementary Material

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures for assays, including statistical analyses; and the methods used for isolation, purification, and analytic characterization of **6–7** and **10–15** (PDF)

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Keywords

Natural Products; Antibiotics; Coenzyme A Biosynthesis; Phosphopantothenoylcysteine synthetase

1. Introduction

The emergence and rapid spread of antibiotic resistance presents a multifaceted challenge to health management through serious pathogenic infections. Currently, the extent of these multidrug resistance pathogens is epitomized by ‘ESKAPE’ organisms (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.).¹ Although not generally appreciated, the genesis of the current antibiotic crisis can be directly attributed to the lack of discovery platforms for novel antimicrobials.² Efforts that began in the past few decades by enhancing the established core scaffolds by synthetic modifications are continuing, but only one new antibiotic class (daptomycin) was introduced between 1962–2015.^{3, 4} Therefore, a reliable platform to combat antibiotic resistance needs to be developed through novel target based approaches for the discovery of new chemical entities.^{2, 5} Coenzyme A (CoA) biosynthesis in particular may represent an effective antimicrobial target due to the substantial hurdles it presents to bacterial antibiotic resistance (Fig 1).⁶ CoA is an indispensable enzyme cofactor for all living organisms involved in metabolic reactions such as fatty acid metabolism. Deprivation of CoA leads to cell stasis or improper cell development, while knockouts of CoA biosynthetic genes are lethal.^{6, 7}

CoA biosynthesis occurs as part of a five-step process; phosphopantothenoylcysteine synthetase (PPCS) is involved in the second step by catalyzing the installation of a cysteine moiety to 4'-phosphopantothenate (Fig 1–2).^{8, 9} PPCS can be broadly classified into three groups: Type I PPCSs, primarily found in bacteria and archaea, are manifested in nature as the C-terminal domain of a bifunctional protein fusion in conjunction with

phosphopantothenoylcysteine decarboxylase (PPCDC) and require CTP to function.^{8, 9} Type II PPCSs, expressed as monofunctional enzymes, utilize either ATP or CTP and can be found mainly in eukaryotes.^{10, 11} Type III PPCSs, also expressed monofunctionally, have been shown to be CTP specific and are found in a smaller subset of bacteria.¹¹ Although the peculiar nucleotide selectivity could be an attractive target for the design of bacterial inhibitors validated by a series of intermediate-mimicking inhibitors,¹¹ no novel chemical structures have been identified.

In our ongoing quest to discover new structural classes of antibiotics,^{5, 12–14} we developed an *in vitro* high throughput assay against bacterial PPCS to investigate our unique natural product extract (NPE) library in the search for new microbial inhibitors.¹⁵ Herein, we describe the discovery of six new congeners belonging to the adipostatin class of metabolites, isolated and characterized through an HTS campaign. We have also established the efficacy of newly discovered molecules through an *in vitro* study against a panel of 11 clinically relevant pathogenic microbes.

Results and Discussion

High-Throughput Screening for Inhibitors of Coenzyme A Biosynthesis Derived from Natural Product Extracts

Motivated by the urgent requirement of new class of antimicrobials, we selected a UM Center for Chemical Genomics NPE library to identify inhibitors of bacterial PPCS. According to a recent study, natural products account for the majority of currently marketed drugs⁴ and the marine environment represents a potentially unexplored source of novel chemical entities.¹⁶ We optimized the malachite green reporter based enzymatic HTS assay (Fig 2) specific to *Streptococcus pneumoniae* PPCS,¹⁷ and conducted a high throughput campaign against a library of 11,000 marine microbial-derived NPEs (Fig 3; Fig SI-2). The malachite based HTS assay and the inorganic pyrophosphatase based counterscreen when selected against the threshold of 90% and 10% respectively, yielded active extracts from 22 strains (Fig 3). The lowest concentration of PPCS used for the HTS assay was 12.5 nM, which produced a response fit for screening (SI-1). Low enzyme concentrations were preferred to capitalize on the opportunity for detecting extracts that contain low abundance bioactive constituents.

Among the active extracts, *Streptomyces blancoensis* (strain 20733–1; Fig SI-1–6), isolated from marine sediments in our laboratory, was of particular interest owing to the observed Hill slope of 2.6 with an IC₅₀ value of 0.03 mg/mL (Fig SI-5). The strain was originally isolated from marine sediments collected in San Miguel, Costa Rica (–85° 8' 33.5", 9° 34' 25.5") in the Cabo Blanco Nature Reserve.

Isolation and Structural Elucidation of the Adipostatins (6–7, 10–15)

A recurring bioactivity guided preparative C18 fractionation (Fig SI-7) and ensuing RP-18 HPLC purification (Fig SI-8) yielded two known adipostatins A-B (6–7), which were also reported to be isolated from *Streptomyces sp.*,¹⁸ and six new congeners, adipostatins E-J (10–15) with structures likely derived from a type I polyketide synthase pathway.

The HR-APCI-MS data and the analysis of 1D and 2D NMR data confirmed the identity of **6** and **7** to be adipostatins A and B; previously reported as glycerol-3-phosphate dehydrogenase inhibitors.¹⁸ Furthermore, **6** and **7** in addition to two more congeners adipostatins C-D (**8–9**) have been recently reported to possess anti-parasitic activity,¹⁹ however **8** and **9** were not observed in our study. Adipostatin E (**10**) was purified as light yellow amorphous solid possessing the molecular formula of C₂₂H₃₈O₂ based on [M+H]⁺ ion peak *m/z* 335.2899 (Figure SI-9). The ¹H and ¹³C NMR data, recorded in CD₃OD, indicated the polyketidic nature of **10** with the tell-tale presence of ¹H NMR peak at δ 1.31 suggesting an aliphatic chain consisting primarily of methylene groups. The presence of triplet at δ_H 0.86 (δ_C 11.2) suggested a terminal methyl group, which in turn showed a COSY relay with δ_H 1.32 (δ_C 30.1) methylene connected to the tertiary carbon (δ_C 40.3) through δ_H 1.18. Further analysis of the ¹H and gCOSY spectrum suggested two more methyl doublets at δ_H 0.88 and 0.89 connected to δ_H 1.52 (δ_C 27.3), completing the terminal dimethyl moiety (Fig SI-9–14).

Furthermore, the ¹³C and HSQC NMR spectra revealed the presence of at least three quaternary carbons at δ_C 145.3 (an aromatic carbon) and δ_C 159.2 (two aromatic carbons). Moreover, the COSY correlations between two equivalent aromatic protons with signals at δ_H 6.08, 6.12 along with their gHMBCAD correlation with carbons at δ_C 100.9, 107.9, 145.3 and 159.2 unambiguously demonstrated the presence of a resorcinol moiety (Table I; Figure SI-9–14). The rest of the molecule consisted of a methylene aliphatic chain en suite to complete the planar structure of **10** (Fig 4).

The adipostatin E (**10**) carries a single stereocenter at carbon-17. However, upon analyzing NMR data (Fig SI-16–17) closely, it became evident that the defined difference of proton chemical shifts at δ_H-19 and δ_H-20 does not provide robust evidence to suggest a NMR based conformation. There are some recent developments using atomic force microscopy (AFM) for absolute stereochemistry of single stereo center in aliphatic system.²⁰ Or relative stereochemistry using *in silico* analysis of biosynthetic cluster involved in production of compound.²¹ Unfortunately, both technologies were either unavailable or out of the scope of this study.

Adipostatin F (**11**) was isolated by RP-HPLC from the same C18 fraction containing compound **10**. The HR-APCI-MS of the molecule provided the same molecular formula C₂₂H₃₈O₂ as **10** based on similar [M+H]⁺ ion peak *m/z* 335.2920 indicating **10** and **11** to be regioisomers (Fig SI-18). As expected, the basic 1D and 2D NMR spectra showed comparable chemical shifts with a similar carbon backbone except towards the terminal end. The presence of merged doublets at δ_H 0.88–0.90 with distinct carbon shifts (δ_C 22.6, 22.61 and 16.4, respectively) together with COSY relay from δ_H 0.88 to δ_H 0.90 through δ_H 1.35 and δ_H 1.30, respectively suggested the tri-methyl containing terminal moiety completing the planar structure of **11** (Table I; Fig 4; Fig SI-19–21).

Furthermore, HR-APCI-MS analysis of **12** and **13** yielded the [M+H]⁺ ions at *m/z* 349.3090 and 349.3087, respectively (Fig SI-20, 26), indicative of their structural relationship as positional isomers established through the same predicted molecular formula of C₂₃H₄₀O₂. The extensive 1D and 2D NMR spectra revealed equivalent chemical shifts compared to the

carbon skeleton of **10** and **11** with the only apparent difference appearing as an additional CH₂ component (Fig SI-21–29). Further characterization revealed a clear difference at the terminus of the aliphatic chain; where the presence of a clear doublet at δ_{H} 0.90 (δ_{C} 23.1) in **12** and a triplet at δ_{H} 0.88 (δ_{C} 22.7) in **13** established them to be adipostatins G (**12**) and H (**13**), respectively (Table I; Fig 4).

The same C18 fraction also led to the isolation of **14** and **15** with HR-APCI-MS providing the molecular formula of C₂₄H₄₂O₂ and C₂₆H₄₆O₂ based on [M+H]⁺ ion peak at m/z 363.3180 and m/z 391.2852, respectively (Fig SI-30, 34). The in-depth analysis and comparison of ¹H, HSQCAD and gCOSY spectra obtained for **14**, clearly indicated the extension in aliphatic framework with terminal branching into a di-methyl unit for completing the structure of adipostatin I (**14**). We didn't acquire HMBC spectra as it was of limited use and would have just provided the linkages between terminal methyl groups with associated methylene groups. This information was successfully extracted through COSY. Similarly, the NMR spectra acquired for compound **15** suggested further elongation of the aliphatic chain by two CH₂ units ending with a terminal triplet at δ_{H} 0.92 (δ_{C} 22.9) affirming the structure of adipostatin J (**15**) (Table I; Fig 4; Fig SI-31–37). Although both **13** and **15** had been recently putatively identified in a metabolic profiling study of wheat,²² their spectral data and the antimicrobial activity are not available.

Assessment of the Biological Activity of Adipostatins against Purified PPCS Enzyme and Microbial Cultures

The malachite green based bioassay was next employed to assess the dose response of known adipostatins A, B, H and J (**6–7**, **13** and **15**) and new congeners adipostatins E-G and I (**10–12** and **14**) against purified *S. pneumoniae* PPCS. The *in vitro* enzymatic assay revealed an intriguing trend where **6–7**, **13** and **15** were observed to possess minimal inhibitory activity against the phosphopantothenoylcysteine synthetase tested up to a concentration of 300 μM . However, adipostatins E-G and I (**10–12**, **14**) exhibited significant *in vitro* inhibition against PPCS with IC₅₀ values of 0.93 μM , 7.1 μM , 4.3 μM and 6.62 μM , respectively (Table II, SI 38–39). The differential *in vitro* activity of molecules seems to suggest a structure activity relationship, where a critical chain length is required for bioactivity that increases with simple functional group branching at the chain terminus (Table SI-1). Unfortunately, yield of isolated molecules were not enough to compute thorough MIC values in current setup.

Coenzyme A biosynthesis plays a vital role in the growth and survival of pathogenic microbes. Also, this class of metabolites are known to be antibacterial against Gram-positive bacteria.²³ Therefore, we envisaged a live microbial culture study to demonstrate that the new adipostatin natural products can penetrate clinically relevant bacteria in order to access the enzyme target, a recognized asset of secondary metabolites over some synthetic chemical scaffolds.²⁴ The *in vitro* study included all the isolated adipostatins against a panel of eleven pathogenic microbial isolates (SI-I; Table SI-1). All the pathogens for bioassay were selected. Microbes were grown to mid log phase ($0.4 < \text{OD}_{600} < 0.6$) and 48 μL of culture was inoculated in 384 well plates pre-spotted with positive controls (kanamycin, vancomycin) and adipostatins A-B & E-J over the concentration range of 200 $\mu\text{g/mL}$ to 0.8

ng/mL (Table II; Fig SI-38–39; SI 1). The *in vitro* assay unambiguously established adipostatin E (**10**) to be the most potent molecule within the class with an IC₅₀ value against *Enterococcus faecalis*, *Bacillus subtilis*, *Listeria monocytogenes*, *Bacillus anthracis* and *S. pneumoniae* observed to be 9.23 µM, 3.44 µM, 5.98 µM, 15.6 µM, and 10.1 µM, respectively (Table II; Fig SI-38; Table SI-1). Interestingly, none of the adipostatins were observed to possess noticeable inhibition against Gram-negative microbes (*viz.*, *E. coli*, *Salmonella enterica* and *Shigella flexneri*; Fig SI-39; Fig SI-38; Table II). This might indicate that the molecule could also be affecting peptidoglycan synthesis and fatty acid synthesis through inhibition of CoA biosynthesis and, therefore, are more potent towards Gram-positive bacteria. Furthermore, adipostatins A–B (**6–7**), which were earlier observed to possess modest PPCS inhibitory activity *in vitro* (Table II), showed modest *in vitro* antimicrobial activity with IC₅₀ values at least 10 orders of magnitude higher compared to **10** (Table II). This observation indicates an additional cellular target, which could be glycerol-3-phosphate dehydrogenase given their earlier reported inhibitory mechanism.¹⁸ In the future, further chemical diversification through synthesis or biosynthetic pathway engineering could enable extensive analysis of the selectivity (Gram-positive only) and the preliminary SAR for further optimizing the antibiotic activity of adipostatins.

The compounds reported herein represent the first known natural product inhibitors of PPCS isolated from a marine microbial-derived NPE library. Adipostatins A (**6**), B (**7**) were earlier reported to possess glycerol-3-phosphate dehydrogenase and anti-parasitic activity, but intriguingly both **6** and **7** did not exhibit any appreciable PPCS inhibitory activity. Interestingly, same is the case with adipostatin H (**13**) and J (**15**) which have been previously described to be isolated from Cameroonian propolis and cereal bran, respectively.^{25, 26} This class of molecules has been traditionally isolated with varying biological activity profiles, and recent studies have also shown their participation in physiological and metabolic regulations. For e.g., alkyl-resorcinols produced by cereal plants were reported to decrease grazing by livestock due to its supposed antifeedant properties.²⁷ Moreover, there are multiple reports where the ability of resorcinol lipids to bind with phospholipid proteins are described at great length indicating their potential to effectively interact with protein drug targets.²⁷

Earlier reports have validated PPCS as an effective drug target,^{11, 28, 29} one of which describes the fungal metabolite CJ-15,801 acting as an antimetabolite of pantothenic acid, and processed by the PanK enzyme. The subsequent product is then accepted by *E. coli* PPCS as a tight binding inhibitor that impedes CoA biosynthesis (Figure 1).^{28–30} In contrast, the current study establishes direct competitive inhibition of PPCS with respect to CTP and is devoid of antimetabolite activity due to the unique structural characteristics of **10–15** compared to reported counterparts.^{28–31} Therefore, our study further supports the therapeutic utility of PPCS and demonstrates its potential for uncovering natural product chemical diversity *viz.*, adipostatins E–J (**10–15**). Furthermore, the differential *in vitro* activity suggests an apparent structure activity relationship relating to aliphatic chain length and terminal branching. This information provides important insights for future (bio) synthetic manipulation to improve both potency and target selectivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Antibiotic resistance presents a multifaceted health challenge to entire world.
- Newer approaches and more effective antibacterial targets are required to combat this threat.
- Coenzyme A (CoA) biosynthesis in particular may represent an effective antimicrobial target.
- Natural products have been at the forefront of antibacterial drug discovery.
- Alkyl-resorcinol class of molecules could be an attractive class to develop as antibacterial.

Figure 1.

Schematic depicting pantothenate uptake and transformation into CoA through the CoA biosynthetic pathway. Most organisms rely on the uptake of exogenous pantothenate and virtually all organisms have pantothenate uptake capabilities.

Figure 2.
Reactions catalyzed by phosphopantothenoilcysteine synthetase.

Figure 3.
Triage of hits obtained during HTS of PPCS against the microbial natural product extracts (NPEs) library.

Figure 4.
Planar structure of adipostatins (A-B, E-J) elucidated using extensive 1D and 2D NMR spectroscopy.

Table I:

NMR spectroscopic data for adipostatins E-J (10–15) in CD₃OD at 600 MHz

Position	Adipostatin E (10)				Adipostatin F (11)				Adipostatin G (12)				Adipostatin H (13)				Adipostatin I (14)				Adipostatin J (15)			
	δ_C	δ_H , multi (<i>J</i> in Hz)	COSY	HMBC	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)	δ_C	δ_H , multi (<i>J</i> in Hz)
1, 3	159.2				159.1		159.2		159.2		159.2		158.9		159.1		158.9		159.1		159.1		159.1	
2	100.9	6.08, t (1.9)	4, 6	1, 3, 4, 5, 6	101.1	6.08, t (2.0)	100	6.10, t (2.0)	100.8	6.10, t (2.0)	100.8	6.10, t (2.0)	100.9	6.12, t (1.9)	100.9	6.11, t (2.0)	100.9	6.12, t (1.9)	100.9	6.11, t (2.0)	100.9	6.11, t (2.0)	100.9	6.11, t (2.0)
4, 6	107.9	6.12, d (1.9)	2	1, 2, 3, 5	107.9	6.13, d (2.0)	107.6	6.13, d (2.0)	107.6	6.13, d (2.0)	107.6	6.13, d (2.0)	107.8	6.15, d (1.9)	107.9	6.14, d (2.0)	107.8	6.15, d (1.9)	107.9	6.14, d (2.0)	107.9	6.14, d (2.0)	107.9	6.14, d (2.0)
5	145.3				145.4		145.4		145.4		145.4		145.1		145.4		145.1		145.4		145.4		145.4	
7	36.9	2.43, t (7.7)	8	4, 5, 6, 8	36.8	2.40, t (7.5)	36.9	2.42, t (7.5)	36.9	2.42, t (7.5)	36.9	2.42, t (7.5)	36.7	2.46, t (7.5)	36.8	2.43, t (7.5)	36.7	2.46, t (7.5)	36.8	2.43, t (7.5)	36.8	2.43, t (7.5)	36.8	2.43, t (7.5)
8	32.4	1.52, m	7, 6	5, 9	31.8	1.52, m	32.3	1.53, m	32.3	1.53, m	32.3	1.53, m	32.7	1.55, m	32.6	1.52, m	32.7	1.55, m	32.6	1.52, m	32.6	1.52, m	32.6	1.52, m
9 – 15	30.6	1.27–1.30, m			30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
16	30.4	1.14, m	15, 17	18, 21	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
17	40.3	1.18, m (2.9, 6.1, 6.7)	16, 18, 21		30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
18	27.3	1.52, m (6.1, 7.1)	17, 19, 20		40.2	1.18, m	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
19	22.7	0.88, d (7.1)	18	17, 18	27.9	1.52, m	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
20	19.8	0.89, d (7.1)	18	17	22.8	0.87, d (7.0)	40.2	1.18, m	30.8	1.28–1.31	30.8	1.28–1.31	32.7	1.28–1.31	30.8	1.28–1.31	32.7	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
21	30.1	1.32, m (3.1, 6.7)	17, 22		22.7	0.86, d (7.0)	29.1	1.56, m	32.2	1.28–1.31	32.2	1.28–1.31	40.5	1.20, m	30.8	1.28–1.31	40.5	1.20, m	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
22	11.2	0.86, t (3.1)	21	17, 18	19.5	0.89, d (6.7)	23.1	0.90, d (7.0)	29.3	1.28–1.31	29.3	1.28–1.31	29.5	1.56, m	30.8	1.28–1.31	29.5	1.56, m	30.8	1.28–1.31	30.8	1.28–1.31	30.8	1.28–1.31
23							23.1	0.91, d (7.0)	22.7	0.88, t (6.8)	22.7	0.88, t (6.8)	22.9	0.90, d (6.9)	33.1	1.28–1.31	22.9	0.90, d (6.9)	33.1	1.28–1.31	33.1	1.28–1.31	33.1	1.28–1.31
24													22.9	0.91, d (6.9)	31.9	1.28–1.31	22.9	0.91, d (6.9)	31.9	1.28–1.31	31.9	1.28–1.31	31.9	1.28–1.31
25															28.6	1.28–1.31			28.6	1.28–1.31	28.6	1.28–1.31	28.6	1.28–1.31
26															22.9	0.92, t (6.9)			22.9	0.92, t (6.9)	22.9	0.92, t (6.9)	22.9	0.92, t (6.9)

Table II.

In vitro bioactivity against purified *S. pneumoniae* PPCS enzyme and anti-microbial assay against five Gram-positive pathogens.

	<i>In Vitro</i> Assay IC ₅₀ (μM)	<i>In Vivo</i> Microbial Assay IC ₅₀ (μM)										
	PPCS	<i>E.coli</i> (MC1061)	<i>E. faecalis</i> (ATCC29212)	<i>B.subtilis</i> (168)	MRSA (USA300)	<i>L.monocytogenes</i> (ATCC19115)	<i>S.aureus</i> (Newman)	<i>B.anthraxis</i> (34f2)	<i>S.enterica</i> (ATCC14028)	<i>S.flexneri</i> (ATCC14028)	<i>K.pneumoniae</i> (ATCC29665)	<i>S.pneumoniae</i> (D39)
Adipostatin A (6)	>300	N/A	>200	26.7	N/A	>200	N/A	46.8	N/A	N/A	N/A	>200
Adipostatin B (7)	>300	78.1	81.1	54.6	N/A	N/A	N/A	32.5	N/A	>200	>200	77.3
Adipostatin E (10)	0.93	34.3	9.2	3.4	>200	5.9	N/A	15.6	N/A	>200	>200	30.2
Adipostatin F (11)	7.1	50.2	13.2	11.4	N/A	34.2	N/A	26.8	N/A	N/A	>200	47.5
Adipostatin G (12)	4.3	47.3	16.9	10.2	>200	20.3	N/A	20.5	N/A	N/A	>200	46.3
Adipostatin H (13)	>300	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	>200
Adipostatin I (14)	6.62	70.2	15.3	14.5	>200	N/A	N/A	>200	N/A	N/A	>200	70
Adipostatin J (15)	>300	>200	N/A	>200	N/A	N/A	N/A	N/A	N/A	N/A	N/A	>200

N/A Not active within the tested range.

> Showing inhibition curve at/around highest tested range.