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Array Synthesis of Novel Lipodepsipeptide

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Abstract—Synthetic array technology was utilized to rapidly synthesize and analyze a diverse set of reductive alkylation analogues of daptomycin. Analysis of the array suggested the use of polar functionality such as sulfonamides or amide or polar spaces such as piperazine would beneficially affect activity.

The need to develop new antibacterial agents is growing due to the continued emergence of drug-resistant bacteria.[1–7] A number of lipodepsipeptides of microbial origin are known to have antibacterial activity. Among them are a number of cyclic lipodepsipeptide natural products produced by *Streptomyces roseosporus*.[8] One such product, daptomycin, is a novel 13-member amino acid cyclic lipodepsipeptide (Fig. 1, R = H). Daptomycin has a demonstrated potent bactericidal activity against a wide variety of Gram-positive pathogens, in particular methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA, respectively) and vancomycin-resistant *Enterococcus* (VRE).[9–13] In developing new bactericidal agents, we have focused on modifications of lipodepsipeptide natural products. Increasing the length of the acyl tail yields improved in vitro potency against a number of bacterial strains. This is consistent with insertion of the lipophylic tail into the cytoplasmic membrane making a contribution to the lethal event.[14,15]

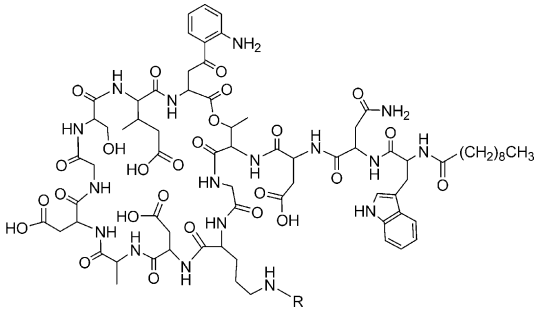
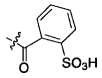
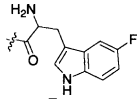


Figure 1. Daptomycin (R = H) and ornithine analogues.

Conversion of the free ornithine amine to an amide results in an increased MIC (compounds **1** and **2**, Table 1).[16–18] Activity can be recovered, compounds **3**–**5**, Table 1, by

Table 1. Amide analogues at the ornithine site of daptomycin

Compd	Figure 1, R =	MIC[a]			
		MRSA	MSSA	*E. faecalis*	*E. faecium*
Daptomycin	H	0.78	0.78	1.56	1.56
1		100	100	100	100
2		6.25	12.5	25	25
3		0.78	1.56	3.1	12.5
4		1.56	1.56	3.1	12.5
5		0.78	0.78	3.1	3.1

[a]Strains used for MIC determinations: MRSA: *S. aureus* ATCC 43300, MSSA: *S. aureus* ATCC 29213, *E. faecium* ATCC 6569, *E. faecalis* ATCC 49452.

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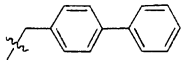
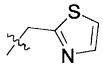
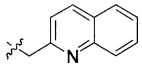
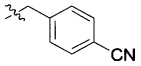
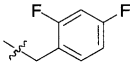
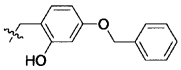
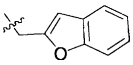
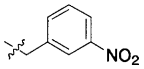
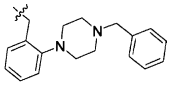
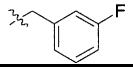
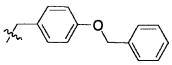
Table 2. Pharmacokinetics of compound **6** versus daptomycin[a]

Compd[b]	Figure 1, R =	Percent serum	MIC[c] μg/mL)				PD_{50} MRSA	$T_{1/2}$	Cl_{total}	V_d
			MSRA	MSSA	*E. faecalis*	*E. faecium*	(mg/kg)	(h)	(mL h^{-1} kg^{-1})	(mL/kg)
Daptomycin	H	0	0.78	0.78	1.56	1.56	0.22	1.7	102	251
		10	0.78	0.78	3.13	1.56				
6		0	0.20	0.39	1.56	1.56	1.04	7.9	9.4	107
		10	1.56	3.13	12.50	25.00				

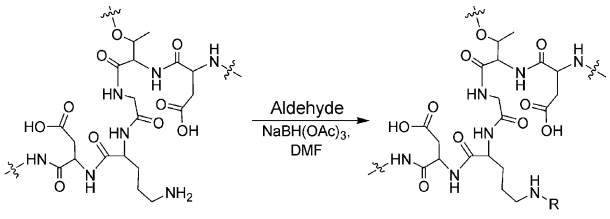
[a]Mice were injected intraperitoneally (ip) with a lethal dose of the pathogen (1×10^8 cfu/mouse) then injected subcutaneously (SC) with the test compound immediately and 4 h post infection.
[b]Daptomycin dosed SC at 50 mg/kg; Compound **6** dosed IV at 50 mg/kg.
[c]Strains used for MIC determinations: MRSA: *S.aureas* ATCC 43300, MSSA *S. aureus* ATCC 29213, *E. faecium* ATCC 6569, *E. faecalis* ATCC 49452.

Table 3. MIC's of derivative from array

Compd	Figure 1, R =	Percent serum	MIC[a] (μg/mL)				Compd	Figure 1, R =	Percent serum	MIC[a] (μg/ML)			
			MRSA	MSSA	*E. faecalis*	*E. faecium*				MRSA	MSSA	*E. faecalis*	*E. faecium*
Daptomycin	H	0	0.78	0.78	1.56	1.56	**51**		0	0.78	0.78	6.25	6.25
		10	0.78	0.78	3.13	1.56			10	1.56	3.13	25.00	6.25
9		0	0.78	0.78	6.25	6.24	**54**		0	0.78	0.78	6.25	6.25
		10	0.78	1.56	12.50	6.25			10	0.78	1.56	12.50	25.00
10		0	0.78	0.78	6.25	6.25	**55**		0	0.78	0.78	12.50	12.50
		10	0.78	0.78	6.25	12.50			10	0.78	1.56	25.00	25.00
17		0	0.78	1.56	6.25	6.25	**78**		0	1.56	3.13	1.56	6.25
		10	0.78	3.13	12.50	25.0			10	12.50	25.00	50.00	100.00
45		0	0.78	0.78	3.13	3.13	**79**		0	0.78	0.78	6.25	6.25
		10	0.78	1.56	6.25	12.50			10	1.56	1.56	12.50	12.50
48		0	0.78	0.78	6.25	6.25	**81**		0	0.78	0.78	6.25	12.50
		10	0.78	1.56	12.50	12.50			10	0.78	0.78	12.50	25.00
49		0	0.78	0.78	6.25	12.50	**82**		0	0.78	0.78	1.56	3.13
		10	0.56	1.56	12.50	12.50			10	6.25	6.25	12.50	25.00

[a]Strains used for MIC determinations: MRSA: *S. aureus* ATCC 43300, MSSA: *S. aureus* ATCC 29213, *E. faecium* ATCC 6569, *E. faecalis* ATCC 49452.

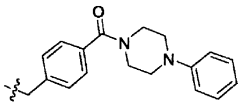
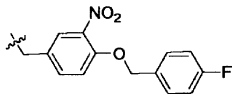
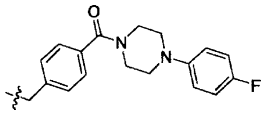
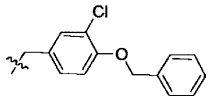
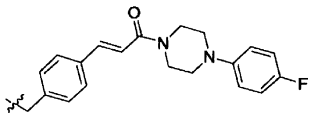
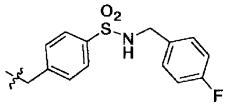
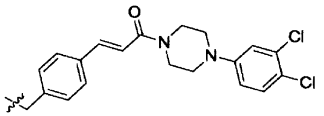
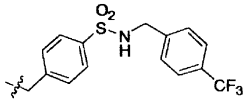
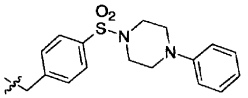
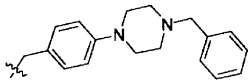
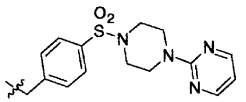
the reintroduction of a non-amide amine. Hence, we elected to modify the ornithine amino acid residue by selective reductive amination to afford a series of benzylic substitutions. This reaction allowed easy exploration of this region while maintaining the non-amide amine, which appears to be required for activity. A number of conditions and reagents were examined to carry out the in situ reductive alkylation; however, only use of sodium triacetoxyborohydride in dimethylformamide was successful for effecting the desired reaction (Scheme 1).



Scheme 1. Reductive alkylation of daptomycin.

An initial lead, compound **6** [Fig. 1, R = 4-(phenyl)benzyl], showed promising antibacterial activity; however, this compound has a significantly longer half-life and a higher PD_{50} than daptomycin (Table 2). It is believed that the longer half-life and increased PD_{50} is a result of increased protein binding. The increased protein binding is consistent with the increase in MIC in the presence of 10% serum. The increase in PD_{50} does not seem so proportionally affected and is possibly due to the increased half life maintaining the drug at above effective levels (albeit lower levels) for a greater period of time versus daptomycin. Removal of the tail on derivative **6** by enzymatic hydrolysis, produced a compound with lower but distinct antibacterial activity.[19] This finding suggests that the ornithine amine might be a secondary location for membrane interaction. To determine whether a second hydrophobic group could improve potency in a manner consistent with this model, we generated daptomycin analogues with additional hydrophobic substituents at the ornithine.

Table 4. MICs of lead optimization analogues

Compd	Figure 1, R =	Percent serum	MIC[a] (μg/mL)				Compd	Figure 1, R =	Percent serum	MIC[a] (μg/ML)			
			MRSA	MSSA	*E. faecalis*	*E. faecium*				MRSA	MSSA	*E. faecalis*	*E. faecium*
Daptomycin	H	0	0.78	0.78	1.56	1.56	**102**		0	0.78	0.78	3.13	6.25
		10	0.78	0.78	3.13	1.56			10	1.56	1.56	6.25	6.25
97		0	0.39	0.78	0.78	1.56	**103**		0	0.78	0.78	3.13	6.25
		10	3.13	6.25	12.50	25.00			10	0.78	1.56	6.25	6.25
98		0	0.78	0.78	0.78	0.39	**104**		0	0.78	0.39	1.56	3.13
		10	3.13	3.13	12.50	25.00			10	3.13	6.25	12.5	12.5
99		0	0.39	0.39	1.56	1.56	**105**		0	0.78	0.78	1.56	1.56
		10	0.78	1.56	3.13	6.25			10	12.50	12.50	50.00	50.00
100		0	0.39	0.78	0.78	0.39	**106**		0	0.78	0.39	0.78	1.56
		10	0.78	3.13	6.25	6.25			10	0.78	3.13	6.25	12.50
101		0	0.39	0.78	6.25	3.13	**107**		0	0.39	0.78	3.13	3.13
		10	0.39	0.78	6.25	6.25			10	1.56	1.56	6.25	12.5

[a]Strains used for MIC determinations: MRSA: *S. aureus* ATCC 43300, MSSA: *S. aureus* ATCC 29213, *E. faecium* ATCC 6569, *E. faecalis* ATCC 49452.

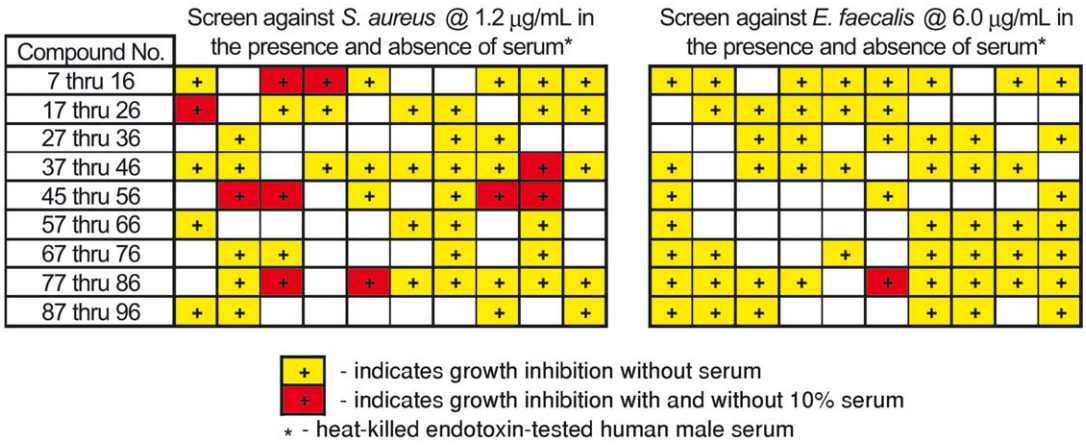


Figure 2. Analysis of synthetic array.

Using our synthetic array approach to probe new analogues, the reductive alkylation reaction was utilized and a diverse set of analogues was generated (Fig. 2). Ninety analogues were synthesized using the reductive alkylation. To characterize these analogues all compounds were screened initially using a one-point assay of 1.2 μg/mL against wild type *S. aureus* (MSSA) and 6.0 μg/mL against wild type *Enterococcus faecalis* both in the absence, and presence of serum. A significant number of analogues were found to inhibit growth of the pathogen at the respective test concentration. In the presence of serum however, it was found that a significant number of compounds no longer inhibited the pathogen at these respective concentrations. We believe the 'serum effect' demonstrated by these compounds is indicative of the protein binding these compounds would have. The 11 analogues that were active in the presence of serum were then further evaluated against MRSA and *Enterococcus faecium* and retested against MSSA and *E. faecalis* to obtain precise MIC values (Table 3).

These analogues suggest that, increased electron deficiency on the aryl ring attached to the ornithine (**10**, **17**, **48**, and **49**), the use of heterocyclic spacers (**81**) or more polar functionality (**9** and **54**) helps maintain drug-like properties and is beneficial for *S. aureas* activity; however, an extended lypophylic moiety with a second ring (**78** and **82**) is better than a single ring (**10**, **49**, and **55**) for enterococcal activity. A significant serum effect was noted for those analogues that lack a polar functionality in enterococci.

Use of the polar functionality of a sulfonamide or amide does assist in maintaining activity in serum (Compound **99**) (Table 4) as compared to compound **6**. In addition, the piperazine as a polar spacer by itself (compound **101**) or in conjunction with the amide (compound **103**) has also been found to be active in the presence of serum. However, these substitutions do not overcome significantly larger hydrophobic groups like dihalogenated aromatic rings (compound **105**).

In summary, addition of a lypophylic moiety to daptomycin (**6**) does increase antibacterial potency in the absence of serum; however, this change results in a longer $T1/2$ and increased PD50. This effect is believed to be due to increased protein binding in vivo. The increased binding can be noted in vitro by a significant increase in MIC in the presence of serum. The presence of activity after removing the acyl tail for compound **6**[19] suggests that the ornithine amine may be a site where modifications can be made for a secondary interaction with the bacterial cytoplasmic membrane, and is consistent with the proposal that insertion of the acyl tail into the bacterial membrane makes a contribution to the lethal event in the mechanism of action.[14,15] Using synthetic array technology has aided in the rapid preparation and analysis of a diverse set of analogues. A number of active analogues of daptomycin have been synthesized by selective reductive alkylation of aryl aldehydes. Using the reductive alkylation reaction, the requisite non-amide amine was maintained while simultaneously exploring this secondary interaction and the properties of many analogues. SAR of the array focused our efforts on the use of the sulfonamide and amides as polar functionalities and attempts to increase the electron deficiency of the internal aryl ring. In addition, use of piperazine as a polar spacer to extend the aryl moiety was utilized. Incorporation of a polar functionality and spacer resulted in improved antibacterial activity in serum.

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