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Synthesis and Biological Activity of *N*-Acylated Ornithine Analogues of Daptomycin

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Abstract—*N*-Acylated ornithine analogues of daptomycin were synthesized and tested for their antibacterial efficacy.

The need to develop new agents that combat Gram-positive bacteria, including those resistant to currently available therapies, is continuing to grow as newly resistant strains such as vancomycin-resistant *Staphylococcus aureus* (VRSA) emerge.[1] Antibacterial agents that also rapidly kill bacteria (bactericidal agents) offer advantages over ones that merely inhibit growth (bacteristatic agents) particularly in the treatment of immunocompromized patients.[2] In developing new bactericidal agents, we have focused on the lipodepsipeptide family of natural products that includes daptomycin (Fig. 1). Daptomycin, is a 13-member amino acid cyclic lipodepsipeptide natural product isolated from *Streptomyces roseosporus*.[3] It demonstrates excellent bactericidal activity against a wide variety of Gram-positive bacteria, in particular MRSA (methicillin-resistant *S. aureus*), VRE (vancomycin-resistant *Enterococcus*) and VRSA.[4] As such, daptomycin provides an excellent scaffold with good physical properties from which to develop new and more potent lipopeptides. In an effort to enhance the potency or pharmacological properties of daptomycin, we examine the formation of a variety of *N*-acylated ornithine analogues.

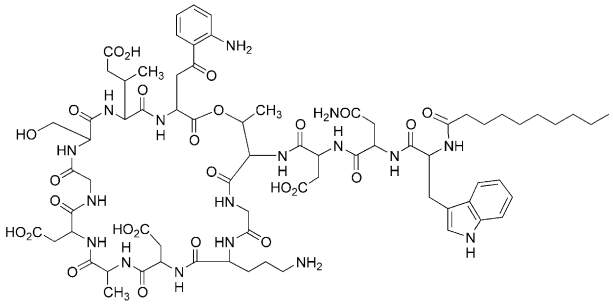

Figure 1. Daptomycin.

The synthesis of *N*-acylated ornithine analogues of daptomycin was achieved by the selective acylation of the ornithine amino group with activated esters, anhydrides, or guanidinylating reagents (Scheme 1). Protecting groups on daptomycin were not required, as the ornithine amino group is the most nucleophilic site in the molecule. When a *tert*-butylcarbamate was used as a protecting group on the acylating agent, it was necessary to use cation scavengers in the deprotection step to prevent side reactions at the indole nitrogen of the tryptophan residue. Yields for the condensation reaction ranged from 50 to 90% depending on the method and substrate used. All products were purified by reverse phase HPLC (Table 1).[5]

Compounds were then screened for their antibacterial activity against a panel of Gram-positive bacteria, and their minimum inhibitory concentration (MIC) determined.[6] Based on the MIC data, it was clear that the ornithine amino group was not essential for whole cell activity as demonstrated by compounds **21** and **22**, which only contain non-polar groups. This also meant that the overall charge of the molecule can be varied without seriously impacting activity. However, conversion of the

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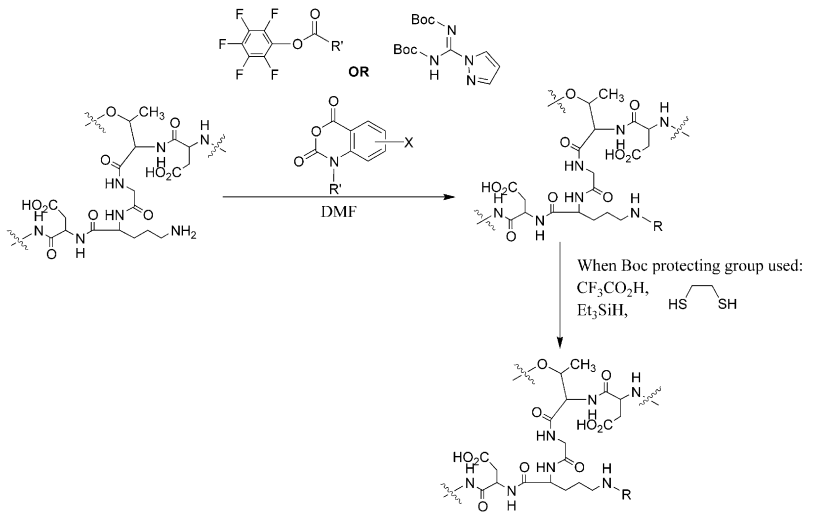
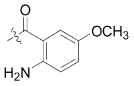
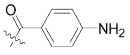
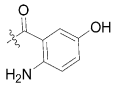
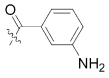
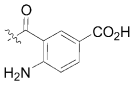
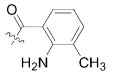
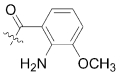
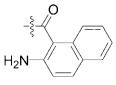
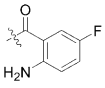
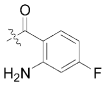
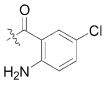
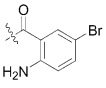
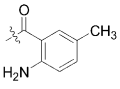
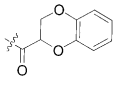


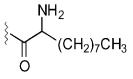
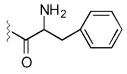
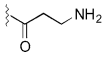
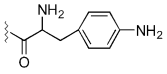
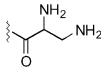
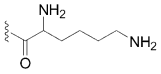
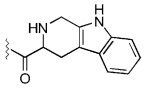
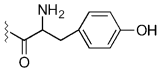
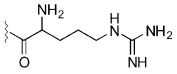
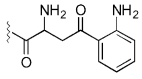
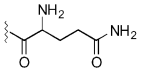
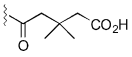
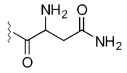
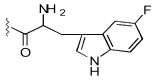
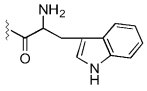
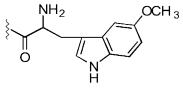
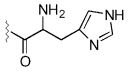
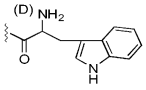
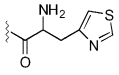
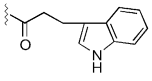
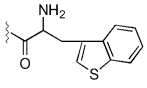
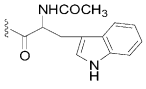
Scheme 1. Synthetic route used to make *N*-acylated ornithine analogues.

Table 1. MIC's resulting from isatoic anhydrides and activated esters

Compd	R	MIC (μg/mL)[a]				Compd	R	MIC (μg/mL)[a]			
		MSSA	MRSA	*E. faecium*	*E. faecalis*			MSSA	MRSA	*E. faecium*	*E. faecalis*
Dapto		0.78	0.39	1.56	1.56	**12**		2.5	ND	10	10
1		3.1	3.1	25	25	**13**		1.25	ND	10	10
2		3.1	1.56	12.5	25	**14**		1.25	3.1	10	12.5
3		0.78	1.56	6.25	12.5	**15**		0.63	ND	10	10
4		0.78	0.39	6.25	6.25	**16**		0.63	ND	10	10
5		1.56	3.1	12.5	50	**17**		0.63	ND	10	10
6		0.78	0.78	3.1	3.1	**18**		0.78	0.78	6.25	6.25
7		0.78	1.56	6.25	6.25	**19**		1.25	3.1	6.25	25
8		1.56	1.56	12.5	12.5	**20**		3.1	1.56	6.25	25
9		1.56	ND	6.25	12.5	**21**		1.56	1.56	3.1	12.5
10		0.78	0.78	3.1	6.25	**22**		6.25	12.5	25	25
11		1.25	ND	10	10	**23**		1.56	1.56	25	25

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

Table 2. MIC's of amino acid derivatives[a]

Compd	R	MIC (μg/mL)[b]				Compd	R	MIC (μg/mL)[b]			
		MSSA	MRSA	*E. faecium*	*E. faecalis*			*MSSA*	MRSA	*E. faecium*	*E. faecalis*
Dapto		0.78	0.39	1.56	1.56	**35**		>100	>100	>100	>100
24		3.1	3.1	6.25	25	**36**		6.25	6.25	25	100
25		3.1	1.56	25	12.5	**37**		3.1	3.1	25	50
26		1.56	0.78	ND	6.25	**38**		0.78	0.78	3.1	12.5
27		0.78	1.56	3.1	6.25	**39**		0.78	0.78	6.25	12.5
28		6.25	6.25	25	50	**40**		1.56	1.56	6.25	12.5
29		1.25	ND	5	10	**41**		12.5	12.5	50	50
30		0.63	ND	5	10	**42**		1.56	1.56	3.1	12.5
31		0.78	0.78	1.56	3.1	**43**		1.56	1.56	12.5	25
32		1.56	1.56	12.5	50	**44**		1.56	1.56	6.25	12.5
33		1.56	0.78	6.25	25	**45**		1.56	1.56	12.5	25
34		12.5	6.25	50	100	**46**		3.1	3.1	12.5	25

[a]All amino acids L unless indicated otherwise.
[b]Strains used for MIC determinations: MRSA ATCC 43300, *S. aureus* ATCC 29213, *E. Faecium* ATCC 6569, *E. faecalis* ATCC 49452.

Table 3. In vivo activity of selective derivatives

Compd	PD_{50} MRSA[a] (mg/kg)	C_{max}[b] (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC (μg h mL^{-1})	Cl_{total} (mL h^{-1} kg^{-1})	V_d (mL/kg)
Dapto	0.22	214	0.5	1.7	489	102	251
3	1.1	464	0.5	2.4	1505	66	230
18	2.1	230	4	5.6	2818	18	143
26	1.1	297	0.5	1.2	589	85	147
31	0.15	258	1	2.8	936	53	216
38	1.75	418	6	>6	ND	ND	ND
39	0.17	254	0.5	2.1	766	65	198

[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]For the PK studies, compound **3** was dosed at 100 mg/kg, The remaining compounds and daptomycin were dosed at 50 mg/kg.

amino group to a simple aromatic amide compound **22** does result in an 8-fold loss in potency compared to daptomycin. Re-introduction of an amine on the aromatic ring in compounds **6**, **7** and **18** show it is possible to restore most, if not all, of the potency seen in daptomycin.

For the aniline series, compounds **1**–**3**, there is a preference for the amino group to be in the *ortho* position. This is shown by compound **3**, which is more potent than the other isomers. Retention of at least one NH proton also imparts an advantage as can be seen when

comparing compound **5** to compounds **3** and **4**. Addition of electron withdrawing groups on the aromatic ring has only a slight positive effect on potency, whereas polar or electron donating substituents have a negative impact.

Provided a 'free' amine was present, increasing the size of the aromatic ring system compound **18**, or including heteroatoms, compound **17**, had little effect on potency compared to compound **3**.

Another means of maintaining a 'free' amine in the ornithine region and retaining the good pharmacological properties of daptomycin was achieved through the addition of an additional amino acid. A series of amino acid coupled analogues were synthesized (Table 2). It became readily apparent that the ornithine region did not tolerate incorporation of long alkyl chains or acidic groups, such as those in compounds **35** or **41**, respectively. A significant reduction in potency was seen when these groups were placed in this region of the molecule.

One of the most interesting compounds turned out to be compound **31**; it displayed activity equivalent to daptomycin both in vitro and in vivo. The stereochemistry of the additional tryptophan was shown to be important as the D-tryptophan isomer compound **44** was 2- to 4-fold less potent than the L-isomer **31**. The importance of maintaining a 'free' amine was again demonstrated by the removal of the α-amino group in compound **31** to create compound **45**. An 8-fold reduction in potency against the enterococci was observed for compound **45**. Acylation of the α-amino group, as in compound **46**, also had the same effect as removing it entirely. Interestingly, removal of the indole NH in compound **34** had a much greater effect. Potency against both the enterococci and staphylococci was greatly diminished, again suggesting that a NH group, not the positive charge, is important for whole cell activity.

Conversion of ornithine into arginine in compound 39 was readily achieved using *N*,*N*′-bis-boc-1-guanylpyrazole and resulted in a loss of potency against the enterococci. This result along with those of compounds **26**, **28**, **36**, and **37** would suggest that the location and number of 'free' amino group(s) is critical for activity against the enterococci. As the charge is moved farther away from the ornithine amide, or additional amines are added the activity in the enterococci is further reduced. The activity in *S. aureus* is also affected, as the 'free' amine is placed farther out; however, the presence of a 'free' amino group alpha to the amide appears to be the major requirement for activity.

Several of the compounds were subsequently examined for activity in murine septicemia models and for pharmacokinetic parameters at doses of 50 mg/kg and in the case of compound **3**, 100 mg/kg. In vivo studies against MRSA have shown that compounds **3**, **18**, and **38** have PD_{50} values of 1.1, 2.1, and 1.75 mg/kg, respectively (Table 3). While slightly less potent than daptomycin, their pharmacokinetic parameters show significant differences. For example, compound **18** has a half-life 3-fold greater than that of daptomycin, and compound **38** did not even reach peak concentration until 6 h after dosing. Generally the compounds with the more lipophilic groups tended to have longer half-lives than daptomycin, and those with more polar groups, such as compound **26**, had shorter half-lives. This effect may relate to the extent of protein binding of the individual compounds. The in vivo studies of compounds **31** and **39** demonstrated that their activity was similar to, or slightly better than, daptomycin against MRSA. While the C_{max} for these compounds are similar to daptomycin, their half-lives are longer, which is reflected in their AUC values. It is this greater overall exposure without the increase of initial exposure that may be reflective in the slightly enhanced protection noted. In addition, they do not suffer the increased protein binding noted for compound **18**. These compounds are currently being further evaluated against enterococci and in deep-seated tissue infection models. All of the compounds tested in vivo were well tolerated by the animals, and no adverse clinical signs were observed during the experiments.

In summary, we have generated a number of highly active, *N*-acylated ornithine analogues of daptomycin in an efficient manner using activated esters, anhydrides, and guanidinylating reagents without the use of protecting group chemistry. We have been able to demonstrate that the ornithine amino group of daptomycin is not essential for whole-cell activity, and the overall charge of the molecule can be varied. These compounds demonstrate excellent in vitro and in vivo antibacterial activity against a variety of Gram-positive bacteria and have significantly different pharmacokinetic profiles than that of daptomycin. The compounds are well tolerated in animal studies and are being further evaluated for their potential as new therapies against drug-resistant Gram-positive bacteria.

References and Notes

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5. Preparative HPLC done with a 250×21.2 mm IBSIL 5 μ C8 column using a gradient of 20–60% acetonitrile in 0.5% ammonium hydrogenphosphate buffer as eluent. Single peak products were desalted by elution with water onto Bondesil 40 μM C8 resin, and isolated from the resin by elution with methanol. Purity and structural confirmation obtained by

LCMS with a Luna 3 μ C18 column eluting with a gradient of 5–95% acetonitrile: 0.1% formic acid in water 0.1% formic acid monitoring at 214 and 254 nm. All isolated products showed single peaks in both wavelengths and correct molecular ions.

6. Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to NCCLS guidelines[7] except that Mueller–Hinton Broth was supplemented to 50 mg/L $Ca2+$ and all assays were performed at 37 °C as previously described.[8] Heat-inactivated pooled human serum was included at 10% where indicated.

7. NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 6th ed.; Document M7-A6; NCCLS: Wayne, PA.

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