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N′-(α-Aminoacyl)- and *N′*-α-(*N*-Alkylamino)acyl Derivatives of Vancomycin and Eremomycin

II. Antibacterial Activity of *N′*-(α-Aminoacyl)- and *N′*-α-(*N*-Alkylamino)acyl Derivatives of Vancomycin and Eremomycin

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Abstract The antibacterial activities of the series of novel *N′*-(α-aminoacyl)- and *N′*-α-(*N*-akylamino)acyl derivatives of eremomycin and vancomycin containing hydrophobic moieties have been investigated. The *N′*-(*N*-alkylglycyl) derivatives of vancomycin are more active against vancomycin-susceptible staphylococci and enterococci and glycopeptide intermediate-resistant *Staphylococcus aureus* (GISA) than the corresponding eremomycin derivatives, but except for *N′*-[*N*-(*p*-octyloxybenzyl)glycyl-vancomycin] (**28**) and *N′*-[*N*-(*p*-octyloxybenzyl)-L-alanyl-vancomycin (**33**)—they are less active against glycopeptide-resistant enterococci (GRE). Derivatives **28** and **33** are the most active compounds (MIC's for glycopeptide-sensitive staphylococci and enterococci are 0.25∼1 μg/ml, for GISA 1∼2 μg/ml, for GRE 2∼6 μg/ml). In *in vivo* studies, derivative **28** was active against *S. aureus* infections in mice with ED_{50} 1 mg/kg *versus* 2 mg/kg for vancomycin (*iv*). In general *N′*-(*N*-alkylglycyl)-derivatives of vancomycin and eremomycin were more active than the corresponding *N′*-aminoacylated derivatives of these antibiotics containing other than glycin amino acids (L-Lys, L-Met, L-Orn, L- and D-Ala) and also L- and D-Phe or benzyl-*O*-L-Tyr.

Keywords vancomycin, eremomycin, semisynthetic derivatives of antibacterial glycopeptides, SAR, antibacterial activity

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Introduction

The prevalence of glycopeptide-resistant enterococci (GRE), the emergence of *Staphylococcus aureus* with intermediate resistance to glycopeptides (GISA) and *S. aureus* strains with vancomycin-resistance (VRSA) emphasizes the need for new compounds with activity against bacteria that are resistant to vancomycin and teicoplanin, glycopeptide antibiotics used in clinical practice for the treatment of infections caused by multi-drug-resistant Gram-positive bacteria [1]. The introduction of a hydrophobic substituent into a glycopeptide molecule is a way to obtain derivatives active against glycopeptide-resistant enterococci [2, 3]. However the introduction of a hydrophobic substituent into a glycopeptide molecule usually leads to compounds, which are less soluble than the parent antibiotics and due to increased binding with blood serum albumin hydrophobic substituents decrease the activities *in vivo*. In addition, the hydrophobic substituents increase serum half life, reduce urinary clearance and increase liver and kidney deposition when compared with the the parent antibiotics [4]. In order to reduce these negative pharmacological consequences of the introduction of a hydrophobic substituent into a

glycopeptide, we explored the introduction of both polar and hydrophobic substituent in the same molecule. The compounds of this type often demonstrate improved tissue distribution and clearance in comparison with those of the parent derivative containing only a single hydrophobic substituent while keeping good antibacterial properties at least against glycopeptide-sensitive microorganisms. In the paper, we report the antibacterial activities of the series of *N′*-(*α*-aminoacyl)- and *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin and vancomycin and their amides [3, 5] against vancomycin-sensitive and resistant bacteria. The lead compounds were active against vancomycin sensitive and resistant microorganisms *in vitro*, demonstrate antibacterial activity *in vivo* as well as good pharmacokinetic properties.

Experimental

Materials and Methods

Eremomycin sulfate was produced at the pilot plant of the Gause Institute of New Antibiotics, Moscow. The synthesis of the series of *N′*-(*α*-aminoacyl)- and *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin and vancomycin and their amides is described in the previous paper [5].

Antibacterial Activity

Minimum inhibitory concentrations (MICs) were determined by broth microdelution method in Mueller-Hinton medium as described by National Committee for Clinical Laboratory Standards. Resistant strains with the confirmed genotype for vancomycin-resistant enterococci are the same as used in the previously published paper [6]. 533 *Staphylococcus epidermidis* and 602 *S. haemolyticus* are clinical isolates. Glycopeptide intermediate strains are 3797 *S. aureus* (GISA HIP-5836 New Jersey) and 3798 *S. aureus* (GISA HIP-5827 Michigan).

ED_{50} Systemic model of mouse infection. Strain/Species: CF-1 mice; source: Charles River. Treated group: A single 100X LD_{50} dose were delivered by intraperitoneal injection (typically 0.5 ml of bacterial broth culture), mice were monitored for 3 days and LD_{50} was calculated based on concentration of inoculum which causes approximately 50% percent mortalities). Five animals per group were treated over a five-dose range for compound **28** at one and five hours post-infection and observed daily for five days post-dosing. Mice were separated into groups: 5 mice in negative untreated control group (to confirm lethal dose), 25 mice per each drug being evaluated representing 5 mice at 5 concentrations (typically covering a 0.5 to 1 log concentration range (1～50 mg/kg), 25 mice in a positive control group treated with vancomycin. The ED_{50} dose was then calculated from by using a Trimmed Logit Method (log based non-linear regression) where approximately 50% of the animals survive infection, this was called the ED_{50} dose for the drug **28**.

Results and Discussion

The structure of the series of *N′*-(*α*-aminoacyl)- and *N′*-*α*-(*N*-alkylamino)acyl derivatives of eremomycin and vancomycin is shown in Figure 1. Antibacterial activity of eremomycin and vancomycin derivatives were compared of the parent compounds (Table 1). In the most cases

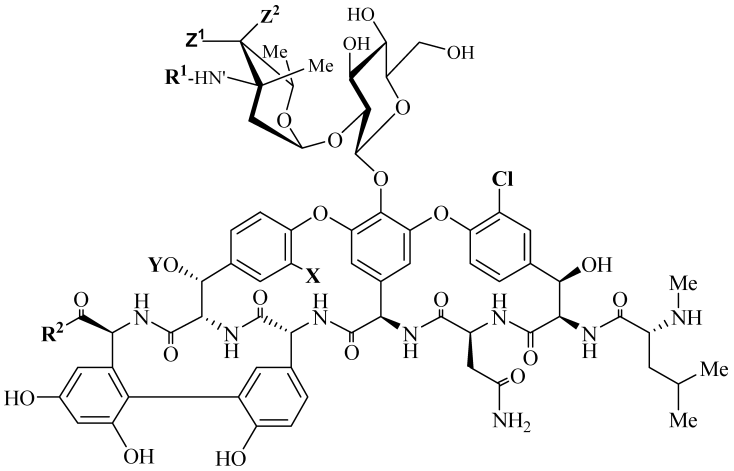

Fig. 1 The structures for eremomycin (R^1=H, R^2=OH; X=H; Y=eremosaminyl-*α*; Z^1=H; Z^2=OH) or vancomycin (R^1=H; R^2=OH; X=Cl; Y=H; Z^1=OH; Z^2=H), their *N′*-(*α*-aminoacyl)- or *N′*-*α*-(*N*-alkylamino)-acyl derivatives (**1**～**35**), and for some of their carboxamides (**36**～**44**) (R^1 and R^2 see in Table 1, 4 and 5).

Table 1 Antibacterial activity of *N′*-(*N*-alkyl-glycyl) derivatives of eremomycin* and vancomycin** in comparison with activity of the parent antibiotics (R^1 and R^2 for Fig. 1)

Compound	*N′*-Substituent: (R^2=OH) R^1:	MIC, μg/ml							
		533 *S. epidermidis*	602 *S. haemo-lyticus*	3797 *S. aureus* (GISA)	3797 *S. aureus* (GISA)	568 *E. faecium* (GSE)	559 *E. faecalis* (GSE)	569 *E. faecium* (GRE)	560 *E. faecalis* (GRE)
Eremomycin/	H/	0.25	0.25	8	8	0.25	0.25	>128	>128
Vancomycin	H	1	1	16	8	1	1	>128	>128
1*	Fmoc-$NHCH_2CO$[a]	ND	ND	ND	ND	0.5	0.5	64	>64
2*	Adoc-$NHCH_2CO$[b]	ND	ND	ND	ND	1	2	>64	>64
3*	*p*-Cl-$PhPhCH_2NHCH_2CO$	0.5	2	4	4	1	1	8	8
25**	*p*-Cl-$PhPhCH_2NHCH_2CO$	0.13	0.25	1	1	0.25	0.5	16	16
4*	*p*-$BuPhCH_2NHCH_2CO$	0.5	2	4	4	0.5	0.5	4	4
26**	*p*-$BuPhCH_2NHCH_2CO$	0.25	0.5	2	2	0.5	1	32	32
5*	$C_9H_{19}CONHCH_2CO$	2	2	16	16	1	2	>64	>64
6*	Bu_2N $PhCH_2NHCH_2CO$	1	1	8	8	1	2	32	32
7*	*p*-$PhCH{=}CHPhCH_2NHCH_2CO$	0.5	0.5	8	8	0.5	1	4	8
8*	*p*-$BuOPhCH_2NHCH_2CO$	0.5	1	4	4	1	2	64	64
27**	*p*-$BuOPhCH_2NHCH_2CO$	0.13	0.13	0.5	1	0.5	1	>64	>64
9*	$(C_{10}H_{21})_2NHCH_2CO$	2	2	8	8	2	4	8	16
10*	*p*-octyl-$OPhCH_2NHCH_2CO$	1	1	4	4	0.5	1	8	8
28**	*p*-octyl-$OPhCH_2NHCH_2CO$	0.5	1	2	2	0.25	0.25	2	4
11*	*p*-$PhCH_2OPhCH_2NHCH_2CO$	2	4	8	8	2	2	16	64
12*	(5-$PhCH_2O$-indol-3-yl)-CH_2NHCH_2CO	2	2	8	8	1	2	16	>64
13*	(1-$PhCH_2$-indol-3-yl)-CH_2NHCH_2CO	1	2	4	4	4	2	16	>64
14*	(Phenanthten-9-yl)-CH_2NHCH_2CO	2	2	16	16	2	2	>64	>64
15*	(Fluoren-2-yl)-CH_2NHCH_2CO	4	4	8	8	2	2	32	32
16*	*p*-F-$PhCH_2NHCH_2CO$	4	4	8	8	4	4	16	16
29**	*p*-F-$PhCH_2NHCH_2CO$	4	4	4	4	1	1	8	8
17*	*p*-$CF_3PhCH_2NHCH_2CO$	1	1	4	4	0.5	0.5	>64	>64
30**	*p*-$CF_3PhCH_2NHCH_2CO$	4	4	4	4	4	4	>64	>64
18*	(Quinolin-2-yl)-$NHCH_2CO$	4	4	>32	>32	2	2	>64	>64
31**	(Quinolin-2-yl)-$NHCH_2CO$	8	8	8	8	4	2	>64	>64

* Eremomycin derivative; ** Vancomycin derivative; [a] Fmoc: 9-fluorenylmethoxycarbonyl; [b] Adoc: 1-adamantyloxycarbonyl.

N′-(*N*-alkylglycyl) derivatives of vancomycin are more active against vancomycin-susceptible staphylococci and enterococci and GISA than the corresponding eremomycin derivatives, but except for derivatives **28** and **29**, they were less active against Van A. *N′*-(*N*-*p*-octyloxybenzylglycyl)-vancomycin) (**28**) was the most active compound (MIC's for glycopeptide-sensitive staphylococci and enterococci are 0.25～1 μg/ml, for GISA 2 μg/ml, for GRE 2～4 μg/ml). In Table 2 the *in vitro* antibacterial activity against various strains of staphylococci, enterococci and streptococci for **28** and vancomycin are compared. The data demonstrate that compound **28** is superior to vancomycin against all the strains tested. Derivative **28** was also active in mice against a systemic infection with *S. aureus* at mice. The ED_{50} for derivative **28** was 1 mg/kg *versus* 2 mg/kg for vancomycin (*iv*). The half-life time in mice for **28** was 273 minutes (after a single 25 mg/kg dose). The compound **28** was shown to be substantially more effective than vancomycin in the mouse thigh infection model against *S. aureus* (Table 3). It achieved more than 6 logs bacterial count reduction at 10 mg/kg compared to 50 mg/kg for vancomycin.

Derivatives of eremomycin and vancomycin with other than glycin *N*-substituted amino acids are presented in Table 4. In most cases *N′*-(*N*-alkyl-glycyl) derivatives of vancomycin are more active against vancomycin-susceptible staphylococci and enterococci and GISA than the corresponding eremomycin derivatives, but they are less active against Van A. *N′*-(*N*-*p*-octyloxybenzyl-L-alanyl)-vancomycin) (**33**) is the most active compound in this series, MIC's for glycopeptide-sensitive staphylococci and enterococci are 0.25～1 μg/ml, for GISA 1～2 μg/ml, for GRE 2～4 μg/ml.

Double modified derivatives-carboxamides of *N′*-α-(N^{α}-alkylamino)acyl derivatives of eremomycin and vancomycin are presented in Table 5. Compound **44** [3-(dimethylamino)propyl amide of **28**] was the most active against vancomycin sensitive and resistant strains, however

Table 2 Antibacterial activity *in vitro* (MIC's, μg/ml) for **28** and vancomycin

Strain	**28**	Vancomycin
Staphylococcus aureus Stau_29213	0.4	1
Staphylococcus aureus Stau_33591	0.4	1
Staphylococcus aureus Stau_b11386	0.2	1
Staphylococcus aureus Stau_Mu50-HIP5406	0.4	2
Staphylococcus aureus Stau_HIP5827	0.78	8
Enterococcus faecalis Enta-29212	0.2	2
Enterococcus faecium Enta-t29882	1.56	>64
Enterococcus faecium Enta-vre2	6.25	>64
Streptococcus pyogenes Stpy_8888	≦0.05	0.5
Escherichia coli Esco_25992	>50	>64

Table 3 Activity of derivative **28** superior than vancomycin in mouse thigh infection model against *Staphylococcus aureus*

	Dose			
	Control	10 mg/kg	15 mg/kg	50 mg/kg
Avg. CFU/ml	Results for Vancomycin (MIC 1.0 μg/ml)			
	1.69×10^{7}	1.39×10^{4}	6.0×10^{4}	0.0
Avg. CFU/ml	Results for **28** (MIC 0.4 μg/ml)			
	1.69×10^{7}	0.0	ND	ND

Table 4 *N′*-*α*-Aminoacyl- and *N′*-*α*-(*N*$^{\alpha}$-alkylamino)acyl derivatives of eremomycin and vancomycin with other than glycine amino acids (R^1 and R^2 for Fig. 1)

Compound	*N′*-Substituent: (R^2=OH) R^1:	MIC, μg/ml							
		533 *S. epidermidis*	602 *S. haemo-lyticus*	3797 *S. aureus* (GISA)	3798 *S. aureus* (GISA)	568 *E. faecium* (GSE)	559 *E. faecalis* (GSE)	569 *E. faecium* (GRE)	560 *E. faecalis* (GRE)
Eremomycin Derivatives									
19	D-Phe	1	1	>32	>32	0.25	0.5	>64	>64
20	L-Phe	4	4	>32	>32	2	2	>64	>64
21	$PhCH_2$-*O*-L-Tyr	4	4	16	16	2	2	>64	>64
22	L-Lys	0.13	0.13	4	8	0.25	0.25	16	>64
23	*N*$^{\alpha}$-*p*-$BuPhCH_2$-L-Orn	0.25	1	4	4	0.5	1	8	8
24	*N*$^{\alpha}$-*p*-C_8H_{17}-$OPhCH_2$-L-Ala	4	4	8	8	4	8	8	8
Vancomycin derivatives									
32	*N*$^{\alpha}$-*p*-C_8H_{17}-$OPhCH_2$-L-Orn	0.5	1	2	2	1	1	16	16
33	*N*$^{\alpha}$-*p*-octyl-$OPhCH_2$-L-Ala	0.13	1	1	1	0.25	0.25	4	4
34	*N*$^{\alpha}$-*p*-octyl-$OPhCH_2$-D-Ala	0.25	2	4	4	0.5	0.5	8	8
35	*N*$^{\alpha}$-*p*-octyl-$OPhCH_2$-L-Met	0.5	2	4	2	1	0.5	16	16

Table 5 Carboxamides of *N′*-(*N*-alkylglycyl) derivatives of eremomycin and vancomycin (R^1 and R^2 for Fig. 1) Adam=adamantyl-.

Compound	R^1	R^2	MIC, μg/ml							
			533 *S. epidermidis lyticus*	602 *S. haemo-lyticus*	3797 *S. aureus* (GISA)	3798 *S. aureus* (GISA)	568 *E. faecium* (GSE)	559 *E. faecalis* (GSE)	569 *E. faecium* (GRE)	560 *E. faecalis* (GRE)
Eremomycin derivatives										
36	*p*-$BuPhCH_2NHCH_2CO$	MeNH	1	2	2	2	2	8	8	8
37	*p*-$BuPhCH_2NHCH_2CO$	(Adam-2)-NH	8	8	16	16	8	8	8	8
38	*p*-$BuPhCH_2NHCH_2CO$	(Adam-1)-CH-(Me)N	4	8	16	4	4	8	8	8
39	*p*-C_8H_{17}-O-$PhCH_2$-$NHCH_2CO$	(Adam-2)-NH	32	32	>32	>32	ND	ND	ND	ND
40	*p*-C_8H_{17}-O-$PhCH_2$-$NHCH_2CO$	(Adam-1)-CH(Me)N	16	32	>32	>32	ND	ND	ND	ND
41	*p*-(*p*-Cl-Ph)$PhCH_2$-$NHCH_2CO$	*p*-F-$PhCH_2NH$	4	8	8	8	ND	ND	ND	ND
Vancomycin derivatives										
42	*p*-$BuPhCH_2NHCH_2CO$	*p*-F-$PhCH_2NH$	0.5	2	2	2	2	4	16	16
43	*p*-C_8H_{17}-O-$PhCH_2$-$NHCH_2CO$	*p*-F-$PhCH_2NH$	4	8	8	4	4	4	8	8
44	*p*-C_8H_{17}-O-$PhCH_2$-$NHCH_2CO$	$Me_2CH(CH_2)_3NH$	1	2	4	4	4	2	4	4

it was less active than compound **28** with the unsubstituted *C*-terminal carboxyl group. All amides investigated have decreased antibacterial activities *in vitro* in comparison with the parent carboxyl compounds. It is in accordance with the previously published data, that the introduction of two hydrophobic substituents into a molecule of vancomycin-type antibiotic results in decrease of antibacterial activity in comparison with mono-substituted antibiotic [7].

Conclusions

N′-(*N*-*p*-Octyloxybenzylglycyl)-vancomycin (**28**) and *N*′-(*N*$^{\alpha}$-*p*-octyloxybenzyl-L-alanyl)-vancomycin (**33**) were selected as the most active derivatives of vancomycin and eremomycin. Derivative **28** was active against *S. aureus* in mice with ED_{50} 1 mg/kg *versus* 2 mg/kg of vancomycin (*iv*) and was shown to be substantially more effective than vancomycin in the mouse thigh infection model against *S. aureus*.

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