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Structure-Activity Relationships in the Series of Eremomycin Carboxamides

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A series of new carboxamides of the glycopeptide antibiotic eremomycin was synthesized and investigated *in vitro*. The goal of the study was the comparison of the influence of the substituents introduced onto the eremomycin skeleton on the activity of these compounds against vancomycin susceptible and resistant bacterial strains. Eremomycin amides derived from amines with small substituents (C_0~C_4) demonstrated antibacterial activity against vancomycin susceptible strains similar to that of the parent antibiotic and were inactive against vancomycin resistant strains. The derivatives of alkylamines with linear lipophilic substituents (like $C_{10}H_{21}$) were active against *Van*A and *Van*B enterococci strains with the scope of activity similar to that of *N'*-decyl or 7d-CH_2NH-decyl eremomycins described earlier. Eremomycin amides of 5-methoxy- and 5-benzyloxytryptamine were active both against vancomycin susceptible and resistant strains. The introduction of a spacer (lysine or piperazine) between the decyl and antibiotic moieties did not seriously influence antibacterial properties of the compounds in comparison with the corresponding derivatives without a spacer. The most active carboxamides are of interest for secondary modifications of the antibiotic.

Glycopeptide antibiotics display high activity against Gram-positive bacteria including pathogens resistant to the β-lactams, tetracyclines, and fluoroquinolones. In the last years widespread use of glycopeptide antibiotics such as vancomycin and teicoplanin in clinical practice and usage of avoparcin in agriculture has given rise to bacterial strains resistant to these antibiotics. This requires the design of new semisynthetic glycopeptides that would be active against highly resistant clinical strains, especially, against vancomycin-resistant enterococci.[1,2] Study of glycopeptide antibiotics derivatives have demonstrated that some of the compounds containing lipophilic substituents at the NH_2 group of disaccharide moiety of eremomycin or chloreremomycin (*N'*-derivatives)[3] or in the nucleus of the amino acid # 7 of eremomycin or teicoplanine aglycone (Mannich 7d-compounds at the amino acid 7)[4,5] are active against vancomycin resistant enterococci, and that among 7d-CH_2NH-alkyl eremomycin derivatives *N*-decyl compound is the most active.[4] In the last years, the study of chloreremomycin derivatives was directed mainly to the preparation of *N'*-derivatives with lipophilic substituents, from which LY-333328 [*N'*-*p*-(*p*-chlorophenyl)benzyl-chloreremomycin], that is highly active against vancomycin susceptible and vancomycin resistant strains, was selected.[6] The role of a lipophilic substituent in a glycopeptide antibiotic was explained as an anchoring into bacterial cell membrane.[7] Recently it was demonstrated that carbohydrate-modified vancomycin compounds effective against resistant bacteria operate by a different mechanism than vancomycin without binding the target D-Ala-D-Ala.[8] Antibiotic derivatives containing lipophilic substituents in various positions of the molecule (not only at the disaccharide moiety) may also have mechanism of antibacterial activity different from that of vancomycin.

In this paper we study the influence of the substituents introduced onto the amide group of eremomycin amide on the biological properties. Earlier we have described the synthesis of several eremomycin carboxamides and have shown that the antibacterial properties of eremomycin amide and methylamide are similar to those of the parent antibiotic.[9] These compounds and also other eremomycin derivatives with a substituted carboxyl group were devoid

Table 1. Yields and properties of the eremomycin carboxamides obtained.

Compound	Yield (%) (Method)	TLC		Molecular formula	ESI MS	
		A1	A2		Calculated MW	Found $[M+H]^+$
2	58 (A)	0.07	0.27	$C_{73}H_{90}N_{11}O_{26}Cl$	1571.5	1572.4
3	70 (A)	0.08	0.31	$C_{74}H_{92}N_{11}O_{26}Cl$	1585.5	1586.4
4	50 (B)	0.16	0.55	$C_{75}H_{94}N_{11}O_{25}Cl$	1583.6	1585
5	48 (B)	0.38	0.66	$C_{75}H_{91}F_3N_{11}O_{25}Cl$	1637.6	1638
6	51 (B)	0.09	0.45	$C_{75}H_{94}N_{11}O_{26}Cl$	1599.6	1622.5[a]
7	49 (B)	0.10	0.55	$C_{76}H_{96}N_{11}O_{26}Cl$	1613.6	1635[a]
8	39 (B)	0.22	0.62	$C_{76}H_{94}N_{11}O_{25}Cl$	1595.6	1596
9	50 (B)	0.19	0.62	$C_{76}H_{92}N_{11}O_{25}Cl$	1593.6	1594
10	80 (A)	0.15	0.45	$C_{75}H_{94}N_{11}O_{25}Cl$	1583.5	1584.5
11	52 (B)	0.24	0.66	$C_{76}H_{96}N_{11}O_{25}Cl$	1597.6	1620.1[a]
12	86 (B)	0.18	0.62	$C_{76}H_{94}N_{11}O_{25}Cl$	1595.5	1596
13	51 (B)	0.35	0.68	$C_{77}H_{98}N_{11}O_{25}Cl$	1611.6	1613
14	47 (B)	0.28	0.66	$C_{77}H_{96}N_{11}O_{25}Cl$	1609.6	1611
15	65 (C)	0.50	0.69	$C_{83}H_{106}N_{11}O_{25}Cl$	1692.8	1693
16	75 (C)	0.45	0.68	$C_{82}H_{108}N_{11}O_{25}Cl$	1681.5	1722.1[b]
17	60 (C)	0.45	0.68	$C_{83}H_{110}N_{11}O_{25}Cl$	1695.6	1697.1
18	48 (D)	0.41	0.67	$C_{89}H_{122}N_{13}O_{26}Cl$	1823.8	1825
19	60 (C)	0.46	0.69	$C_{87}H_{117}N_{12}O_{25}Cl$	1764.8	1765
20	63 (C)	0.47	0.69	$C_{88}H_{119}N_{12}O_{25}Cl$	1778.8	1780
21	66 (C)	0.30	0.64	$C_{84}H_{101}N_{13}O_{25}Cl_2$	1761.5	1762.5
22	62 (C)	0.50	0.69	$C_{90}H_{106}N_{12}O_{26}Cl$	1790.9	1791
23	48 (C)	0.45	0.68	$C_{82}H_{107}N_{12}O_{25}Cl$	1694.7	1695
24	68 (C)	0.42	0.67	$C_{84}H_{101}N_{12}O_{25}Cl$	1712.6	1713
25	62 (C)	0.43	0.68	$C_{84}H_{101}N_{12}O_{26}Cl$	1728.6	1729
26	35 (D)	0.44	0.68	$C_{90}H_{105}N_{12}O_{26}Cl$	1804.7	1663[c]
27	60 (C)	0.48	0.61	$C_{87}H_{103}N_{11}O_{25}Cl$	1735.8	1736

[a] Determined by MALDI method; corresponds to the ion $[M+Na]^+$

[b] Determined by MALDI method; corresponds to the ion $[M+K]^+$

[c] Corresponds to the ion $[[M-Me]+2H]^+$; where Me (144.1) -corresponds to the ion of the eremoseamine moiety lost in the process of mass-spectrometry; the presence of the eremosamine fragment in disaccharide branch was shown by the method of hydrolysis as described in ref. 10.

of histamine-releasing properties which is the reason of undesirable side-effects of vancomycin type antibiotics. This makes eremomycin amides the suitable compounds for secondary modifications. We describe here twenty six new eremomycin carboxamides, which were obtained from eremomycin and corresponding amines with the use of (benzotriazol-1-yloxy)-tris-(pyrrolidino) phosphonium-hexafluorophosphate (PyBOP) or *O*-(benzotriazol-1-yloxy)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate (HBPyU) condensing agents without preliminary protection of other functional groups of the antibiotic (Table 1). *N*-*n*-Decylpiperazine, *N*-*n*-undecylpiperazine, *N*-*p*-phenylbenzylpiperazine, *N*-(3-aminopropyl)-α-pipecoline, decylamide of L-lysine and imine of *p*-chlorobenzaldehyde and *N*-aminopiperazine were used for the preparation of eremomycin amides in which lipophilic substituent was connected with the amino group through a spacer.

Homogenity of the compounds obtained was demonstrated by TLC and HPLC methods. In paper electrophoresis R_e values of amides were close to R_e for eremomycin though highly lipophilic derivatives due to the strong adsorption on paper did not move in electrophoresis. For all the compounds obtained except 5-benzyloxytryptamine derivative **26** peaks corresponding to molecular ions were observed in ESI or MALDI mass-spectra. In the ESI mass-spectrum of **26** the heaviest peak corresponded to the ion obtained after splitting off the eremosamine moiety from the diprotonated molecular ion. The presence of

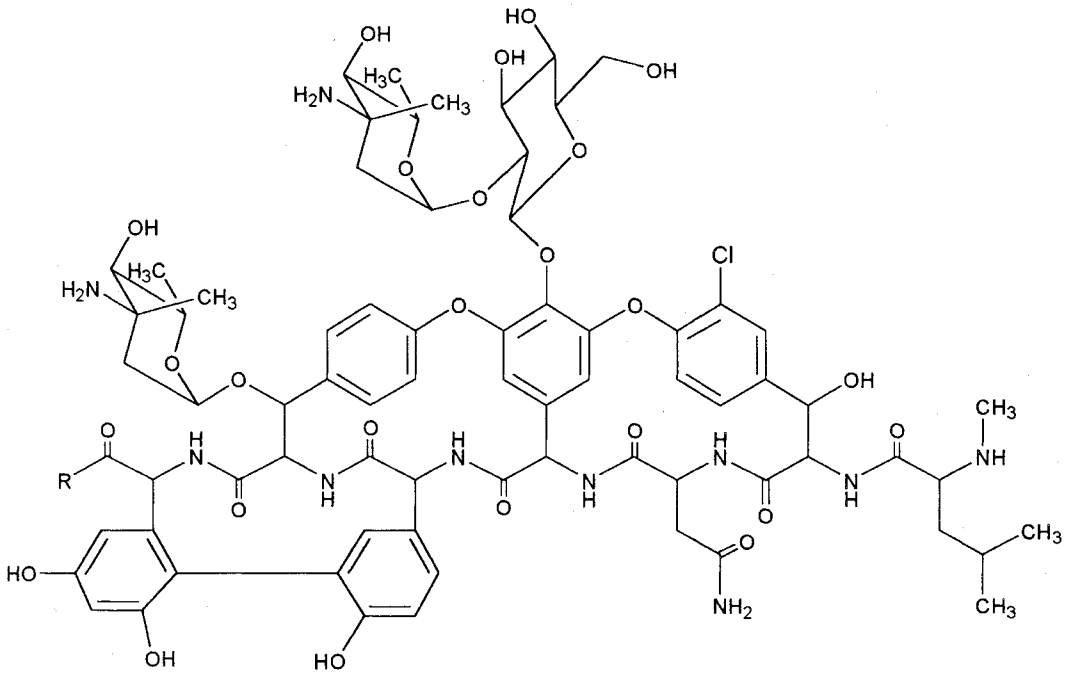
Table 2. Minimum inhibitory concentrations (μg/ml) of the eremomycin carboxamides in comparison with natural glycopepeptide antibiotics.

Strain	Vancomycin	Teicoplanine	Eremomycin (**1**)	**2**	**3**	**4**	**5**	**6**	**7**
S. aureus ATCC 29213 (MSSA)	1	0.12	0.12	≤0.12	≤0.12	0.12	0.12	0.12	0.5
S. aureus 6538P (MSSA)	0.5	0.12	≤0.06	≤0.12	≤0.12	0.03	0.03	≤0.06	≤0.06
S. aureus NCTC 10649 (MSSA)	1	0.25	≤0.06	≤0.12	≤0.12	0.25	0.25	≤0.06	≤0.06
S. aureus CMX 553 (MSSA)	1	0.25	≤0.06	≤0.12	≤0.12	0.5	0.12	0.12	0.25
S. aureus 1664 (MRSA)	1	0.25	0.12	≤0.12	≤0.12	0.5	0.12	0.12	0.25
S. aureus 1690 (MRSA)	1	0.12	0.12	≤0.12	≤0.12	0.25	0.12	0.12	0.12
S. aureus 3384 (MRSA)	1	≤0.06	≤0.06	≤0.12	≤0.12	0.25	0.25	0.12	0.5
S. aureus 3480 (MRSA)	1	1	0.12	≤0.12	≤0.12	0.25	0.25	0.12	0.5
E. faecium ATCC 8043 (Van^S)	1	≤0.06	0.25	≤0.12	≤0.12	0.25	0.25	0.12	0.12
E. faecium 7096 (Van^S)	0.5	0.12	0.12	≤0.12	≤0.12	1	0.5	0.12	0.12
E. faecalis ATCC 29212 (Van^S)	1	≤0.06	0.25	≤0.12	≤0.12	0.5	0.25	0.25	0.25
E. faecalis 7074 (Van^S)	0.5	≤0.06	0.25	≤0.12	≤0.12	0.5	0.5	0.25	0.25
E. faecium 5205 (*Van*A)	>128	>64	>64	>128	>128	>128	>128	>64	>64
E. faecium 6253 (*Van*B)	>128	>64	>64	128	>128	>128	>128	>64	>64
E. faecalis 5206 (*Van*B)	32	0.5	16	≤0.12	≤0.12	2	1	64	32
E. faecalis 7065 (*Van*B)	>128	0.25	>64	16	64	>128	>128	>64	>64

Strain	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
S. aureus ATCC 29213 (MSSA)	0.12	0.5	≤0.12	1	0.12	0.25	0.12	2	2	1
S. aureus 6538P (MSSA)	0.03	0.5	≤0.12	≤0.06	≤0.06	0.5	0.03	0.5	–	0.25
S. aureus NCTC 10649 (MSSA)	0.5	1	≤0.12	0.12	≤0.06	0.25	0.25	0.5	1	0.5
S. aureus CMX 553 (MSSA)	0.12	1	≤0.12	0.25	0.12	0.25	0.12	0.5	1	1
S. aureus 1664 (MRSA)	0.5	0.5	0.25	0.5	0.12	0.25	0.12	2	1	0.5
S. aureus 1690 (MRSA)	0.12	1	–	0.5	0.12	0.25	0.12	1	2	0.5
S. aureus 3384 (MRSA)	0.12	2	≤0.12	0.5	0.12	0.25	0.12	1	1	0.5
S. aureus 3480 (MRSA)	0.12	2	≤0.12	0.5	0.12	0.25	0.25	1	0.5	0.5
E. faecium ATCC 8043 (Van^S)	0.25	1	≤0.12	0.12	≤0.06	0.25	0.25	0.5	0.5	0.5
E. faecium 7096 (Van^S)	0.5	1	≤0.12	0.12	≤0.06	0.5	0.5	0.5	0.5	0.5
E. faecalis ATCC 29212 (Van^S)	0.5	1	≤0.12	0.25	0.12	0.5	0.25	0.5	0.5	0.25
E. faecalis 7074 (Van^S)	0.5	1	≤0.12	0.25	0.12	0.5	0.25	0.5	0.5	0.5
E. faecium 5205 (*Van*A)	>128	>128	>128	>64	>64	>128	>128	32	4	1
E. faecium 6253 (*Van*B)	>128	>128	>128	>64	>64	>128	>128	4	4	1
E. faecalis 5206 (*Van*B)	2	64	≤0.12	2	16	1	1	0.5	4	1
E. faecalis 7065 (*Van*B)	>128	>128	16	2	>64	>128	>128	32	8	2

Strain	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
S. aureus ATCC 29213 (MSSA)	1	1	2	0.5	0.5	≤0.12	0.125	≤0.12	2	2
S. aureus 6538P (MSSA)	0.25	0.5	0.5	≤0.12	0.25	≤0.12	0.125	≤0.12	≤0.12	0.25
S. aureus NCTC 10649 (MSSA)	1	2	0.25	0.5	1	≤0.12	0.125	≤0.12	2	0.5
S. aureus CMX 553 (MSSA)	1	1	1	0.5	1	≤0.12	0.125	≤0.12	2	1
S. aureus 1664 (MRSA)	1	0.5	0.5	0.5	1	≤0.12	0.25	≤0.12	2	2
S. aureus 1690 (MRSA)	1	1	1	0.5	0.25	≤0.12	0.125	0.25	1	1
S. aureus 3384 (MRSA)	1	2	1	0.5	0.25	≤0.12	0.25	≤0.12	0.5	1
S. aureus 3480 (MRSA)	1	1	1	0.25	0.5	≤0.12	0.25	≤0.12	1	2
E. faecium ATCC 8043 (Van^S)	0.5	1	1	0.25	0.5	≤0.12	0.125	≤0.12	0.5	0.25
E. faecium 7096 (Van^S)	0.5	1	1	0.25	1	≤0.12	0.125	≤0.12	0.5	0.25
E. faecalis ATCC 29212 (Van^S)	2	1	1	≤0.12	0.5	≤0.12	0.125	≤0.12	0.25	0.5
E. faecalis 7074 (Van^S)	1	0.5	0.5	≤0.12	1	≤0.12	0.25	≤0.12	0.25	0.5
E. faecium 5205 (*Van*A)	1	4	4	32	>128	64	>128	16	4	8
E. faecium 6253 (*Van*B)	1	1	1	8	2	64	>128	4	1	4
E. faecalis 5206 (*Van*B)	2	1	1	0.25	4	≤0.12	64	≤0.12	0.25	1
E. faecalis 7065 (*Van*B)	4	16	16	1	4	≤0.12	>128	0.5	2	1

Fig. 1. Eremomycin and its carboxamides.



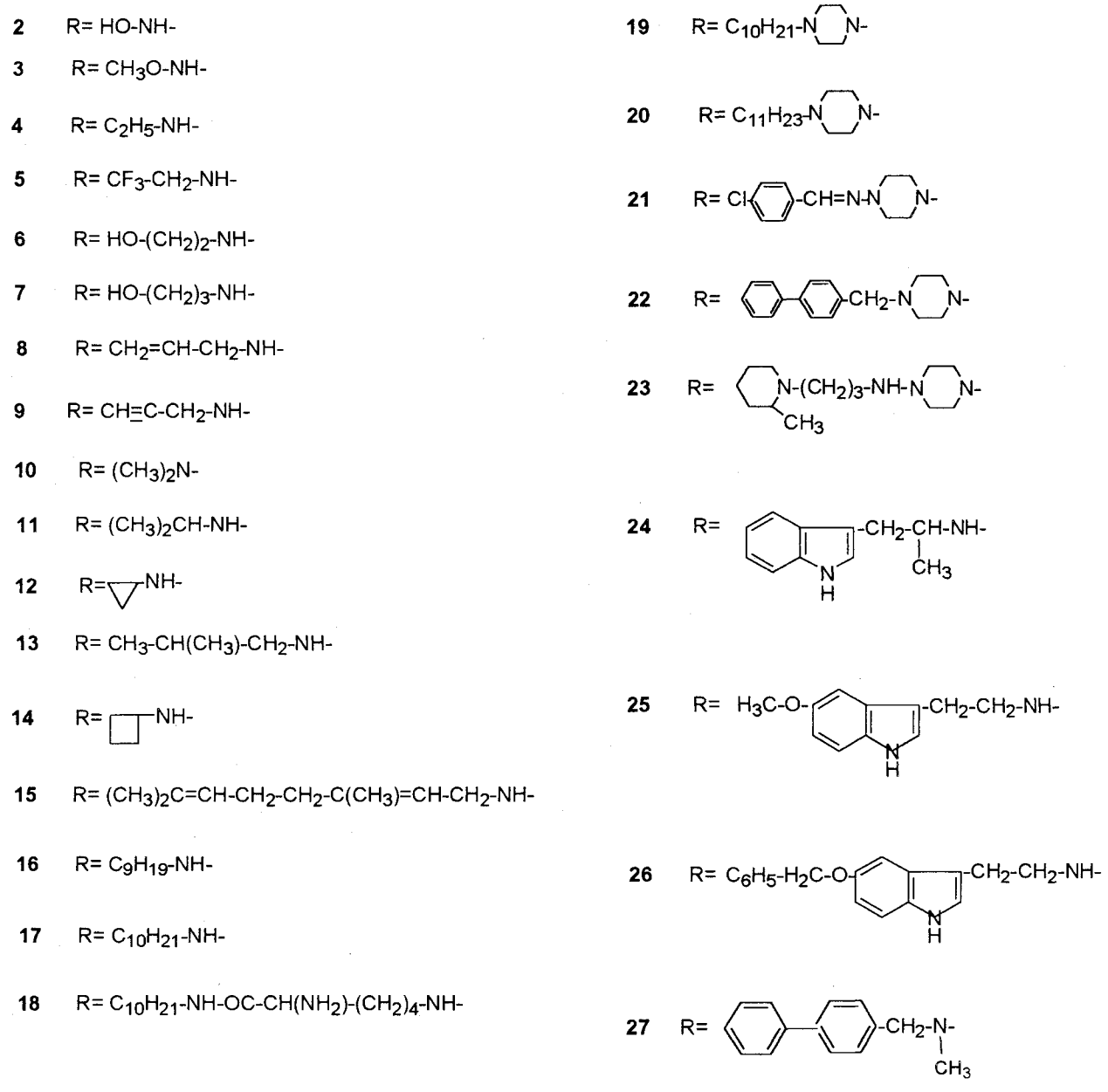
the eremosamine moiety in the disaccharide in this case was demonstrated by the presence of the unsubstituted eremosamine after mild hydrolysis of eremosamine-glucose bond of the eremomycin amide by the method described.[10]

All carboxamides investigated were highly active against *S. aureus*, four strains were methicillin susceptible and four strains were methicillin resistant (Table 2). The MICs for amides **2**~**14** ranged from 0.06 to 0.5 mcg/ml. The activities were similar to teicoplanine and eremomycin and were better than that of vancomycin for which the MICs were about 1 mcg/ml. The amides **2**~**14**, which have small substituents (C_0~C_4) were active against the vancomycin susceptible strains of *E. faecium* and *E. faecalis*, with MICs comparable to vancomycin, teicoplanine, and eremomycin; however, they had variable activity against vancomycin resistant *E. faecalis*. The isobutylamide **11** was almost as active as teicoplanine against both strains of resistant *E. faecalis*. Compounds **6**, **7**, **9** and **12** were poorly active against both resistant strains of *E. faecalis*, as were vancomycin and eremomycin. The other eight compounds (**2**, **3**, **4**, **5**, **8**, **10**, **13**, **14**) had improved activity against *E. faecalis* 5206 comparable with teicoplanine, although their activity was only comparable to vancomycin against the second resistant strain, *E. faecalis* 7065. None of the amides had detectable activity against vancomycin resistant *E. faecium*.

The amides with a linear lipophilic substituent (**15**, **16**, **17**) were less active than teicoplanin or eremomycin against staphylococci and vancomycin susceptible enterococci, although the activity was comparable to vancomycin. They demonstrated activity against vancomycin resistant enterococci (*Van*A and *Van*B). *N*-Decylamide (**17**) was the most active in this series overall. In particular, it was significantly more active than teicoplanine against the vancomycin resistant strains of *E. faecium* and was nearly as active as teicoplanine against the vancomycin resistant strains of *E. faecalis*; it inhibited growth of *Van*A *E. faecium* 5205, *Van*B *E. faecium* 6253, and *Van*B *E. faecalis* 5206 at the concentrations 1 mcg/ml and growth of *Van*B *E. faecalis* 7065 at 2 mcg/ml. Unlike chloreremomycin derivatives[3], the *p*-phenylbenzyl derivative **27** was slightly less active than decyl derivative (**17**) against the *Van*A strain of *E. faecium* (MIC 8 mcg/ml), though both compounds demonstrated good activity against staphylococci and *Van*B enterococci.

It is interesting to compare amides of α-methyltryptamine (**24**), 5-methoxytryptamine (**25**) and 5-benzyloxytryptamine (**26**). α-Methyltryptamine derivative (**24**) was highly active against staphylococci and vancomycin susceptible enterococci, but had little to no detectable

Fig. 2. *N*-Substituents in eremomycin carboxamides.



activity against *Van*A and *Van*B enterococci. 5-Methoxytryptamine derivative (**25**) had comparable activity to **24** against all staphylococci and vancomycin susceptible strains; but, at the same time, demonstrated potent activity against the vancomycin resistant enterococci ranging from 0.12 to 16 mcg/ml. The activity of **25** was similar to teicoplanine against *Van*B *E. faecalis*. 5-Benzyloxytryptamine (**26**) was less potent than **24** and **25** against the staphylococci and vancomycin susceptible enterococci, but was highly potent against the vancomycin resistant enterococci.

To increase the solubility of the amides we condensed eremomycin with the hydrophobic compounds through spacers containing additional amino groups. The compounds in which a L-lysine (**18**) or a piperazine (**19**) moiety is inserted between decyl and eremomycin parts of molecule demonstrated antibacterial properties similar to *n*-decylamide **17**. When a spacer was inserted between phenylbenzyl moiety and eremomycin (**22**) the compound had showed lower activity against *Van*A *E. faecium* strain in

Table 3. Comparison of antibacterial activity *in vitro* of decyl-derivatives of eremomycin.

Type of compound	Compound	MIC range values (μg/ml)		
		Meth.-R *Staph. aureus*	Enterococci sensitive to vancomycin	*Van*A enterococci
Amide	AA7-CONHC$_{10}$H$_{21}$	0.5~1	0.5	2~8
Mannich derivative at of AA7	7d-CH$_2$NHC$_{10}$H$_{21}$	0.25~0.5	0.25~0.5	8
Derivative of N′H$_2$-group of disaccharide branch	N′H-C$_{10}$H$_{21}$	0.5~1	0.25~0.5	4~8
Decyl derivatives connected with eremomycin through a spacer (piperazine or lysine moiety)				
Amide (spacer piperazine)	AA7-CON(CH$_2$CH$_2$)$_2$NC$_{10}$H$_{21}$	0.5~1	0.5~1	4~8
Amide (spacer lysine)	AA7-CONH-CH(COOH)-(CH$_2$)$_4$NH-C$_{10}$H$_{21}$	1	0.5~1	2~8
Mannich Derivative at AA7 (spacer piperazine)	7d-CH$_2$N(CH$_2$CH$_2$)$_2$N-C$_{10}$H$_{21}$	0.25~0.5	0.5	8

comparison with **27**, though retaining high activity against all investigated stains of staphylococci. It suggests that the addition of a spacer made this lipophilic substituent too distant for the interaction (anchoring) with a bacterial cell target. Using *N*-aminopropylpipecoline led to the compound (**23**), which was highly active against staphylococci and *Van*B *E. faecalis* strains, but with low activity (64 mcg/ml) against *Van*A and *Van*B *E. faecium* strains.

Comparison of the antibacterial activities of eremomycin amides with other antibiotic derivatives obtained previously shows that the activity of new semisynthetic eremomycins against *Van*A *E. faecium* depends strongly on the size of the lipophilic substituent introduced and is less dependent on the position of the substituent. In Table 3 it is shown that decyl containing amide, Mannich derivative (7d-CH$_2$NHR) and *N′*-decyl eremomycin have equal MIC values against methicillin susceptible and resistant staphylococci and vancomycin susceptible and resistant enterococci. Recently it was demonstrated that some of carbohydrate-modified vancomycin compounds with activity against resistant bacteria operate by a different mechanism than vancomycin without binding D-Ala-D-Ala.[8] Similar level of activity for the isomeric modified eremomycins (*N′*-, amide or 7d substituted) suggests these derivatives containing substituents in various positions of the molecule may also have mechanism of antibacterial activity which is different from that of vancomycin.

Experimental

General

Eremomycin sulfate was produced at the pilot plant of Institute of New Antibiotics of the Russian Academy of Medical Sciences. Amines were purchased from Aldrich and Fluka, PyBop- and HBPyU-reagents from Aldrich, CH_3CN and DMSO from Merck. Decylpiperazine, undecylpiperazine, and *p*-phenylbenzylpiperazine were obtained by the reductive alkylation of piperazine with nonylaldehyde, decylaldehyde or *p*-phenylbenzaldehyde and $NaBH_3CN$ in MeOH.[5] N^{α}-Boc-L-Lys-decylamide was obtained from N^{α}-Boc-L-Lys-OPfp and decylamine[5]. (*p*-Phenylbenzyl) (methyl) amine was obtained from *p*-phenylbenzaldehyde and methylamine with the use of $NaBH_3CN$.

The samples were analyzed by TLC on the Merck Silica Gel 60F$_{254}$ plates in systems EtOAc - PrOH - 25% NH_4OH 3 : 2 : 2 (A1), and (7 : 7 : 9) (A2). Additionally the individuality of samples was controlled by HPLC method as in the reference[10], which showed that the concentration of eremomycin in each samples was less than 1%. Paper electrophoresis was performed in 0.05 M AcOH-pyridine buffer (pH 5.6) at 900 V for 3 hours or in 2 N AcOH (pH 2.4) at 700 V for 3 hours on Filtrak FN-12 paper (Germany). Electrophoretic mobility of the samples of amides relative to eremomycin was between 1.24~1.05. Reaction products were purified by reversed-phase column chromatography on silanized silica gel (0.063~0.2 mm) or CM-cellulose (CM-32 Whatman). Ion-exchange resin

Dowex 50×2 (H^+-form) or Dowex 50×16 (H^+-form) were used for desalting.

MALDI mass-spectra were recorded on MALDI-MS Vision 2000 instrument (UK). Mass spectra were also determined by Electrospray Ionization (ESI) on a Finnigan SSQ7000 single quadrupole mass spectrometer.

General Synthetic Procedure

To a mixture of eremomycin sulfate (165 mg, 0.1 mmol) and 1 mmol of an amine hydrochloride dissolved in 5 ml of DMSO were added portion-wise Et_3N to adjust pH 8.5~9 and afterwards during 1 hour 0.2 mmol of PyBOP- or HBPyU-reagent. The reaction mixture was stirred at room temperature for 3 hours.

Method A. Purification of Eremomycin Hydroxylamide (**2**), Methoxylamide (**3**), Dimethylamide (**10**) and Cyclopropylamide (**12**)

Addition of ether (~150 ml) to the reaction mixture led to an oily residue, which was shaken successively with ether (15 ml×2) and acetone (~15 ml). After addition of 100 ml of acetone a precipitate of crude amide was collected, dissolved in 150 ml of water and loaded on Dowex 50×16 (H^+-form) column. The eluate was concentrated *in vacuo* to a minimal volume, and 70 ml of acetone was added to form the precipitate, which was collected to give a pure amide.

Method B. Purification of Eremomycin Ethylamide (**4**), Trifluorethylamide (**5**), Ethanolamide (**6**), Propanolamide (**7**), Allylamide (**8**), Propargylamide (**9**), Isopropylamide (**11**), Isobutylamide (**13**), Cyclobutylamide (**14**) and Decylamide (**17**)

Adding ether (~150 ml) to the reaction mixture led to an oily residue, which was shaken with ether (35 ml×2), acidified to pH 5 with 0.05 N HCl and after addition of 70 ml of acetone formed a precipitate of crude amide, which was collected, washed by acetone and dissolved in 4 ml of 0.2 M $AcONH_4$ - EtOH 9 : 1 mixture (pH 6.7) and applied to a chromatographic column with CM cellulose (45 cm× 1 cm) preequilibrated with 0.2 M $AcONH_4$ - EtOH 9 : 1 mixture (pH 6.7). The column chromatography was carried out with 0.2 M $AcONH_4$ with linear pH gradient (6.7→8). The fractions containing unreacted eremomycin were combined and used for regeneration. The fractions containing amide were combined, acidified with 6 N H_2SO_4 to pH 3 and passed through column (2×10 cm) of Dowex 50×2 resin (H^+-form), which was washed with water and eluted with 250 ml of 0.25 N NH_4OH. The eluates were concentrated under reduced pressure to minimal volume, acidified with 0.05 N HCl to pH 5 and precipitated with 50 ml acetone. The precipitate was collected, rinsed successively with acetone and dried *in vacuo* to give an amide.

Method D. Purification of Eremomycin Amide of α-Methyltryptamine (**24**) and Amide of 5-Methoxytryptamine (**25**)

Addition of ether (~150 ml) to the reaction mixture led to an oily residue, which was shaken with ether (35 ml×2) and 100 ml acetone. The precipitate of crude amide was collected, washed by acetone and dissolved in 70 ml of water, extracted by *n*-BuOH (70 ml×3). The water solution containing amides was evaporated *in vacuo* to 4 ml and applied to a chromatographic column with silanized silica gel (2×100 cm), preequilibrated with 0.01 M AcOH. The column chromatography was carried out with 0.1 M AcOH. The fractions with pure amide were collected, evaporated to the minimal volume, acidified with 0.05 N HCl to pH 5 and the amide was precipitated with the mixture Et_2O : acetone (1 : 1). The precipitate was filtrated, washed by acetone and dried *in vacuo*.

Method E. Purification of Eremomycin ε-Amide of L-Lysyldecylamide (**18**) and Eremomycin Amide of 5-Benzyloxytryptamine (**26**)

Amides **18** and **26** were purified as described above but the column chromatography was carried out with liner gradient MeOH in 0.01 M AcOH (0→70%).

Determination of Antibacterial Activity

Minimum inhibitory concentrations (MICs) were determined by broth microdilution in cation-adjusted Meuller-Hinton broth (Becton Dickinson and Company, Cockeysville, MD) as described by the National Committee for Clinical Laboratory Standards[11]. The strains tested were either clinical isolates from the Abbott Laboratories culture collection or were reference strains obtained from the American Type Culture Collection, Rockville, MD. The genotypes of vancomycin-resistant enterococci were identified by PCR-based techniques[12].

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