Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 71 (2006) 227–236
Antiviral Research
www.elsevier.com/locate/antiviral

Mini-review

Polycyclic peptide and glycopeptide antibiotics and their derivatives as inhibitors of HIV entry

Maria N. Preobrazhenskaya *, Eugenia N. Olsufyeva [1]

Gause Institute of New Antibiotics, B. Pirogovskaya 11, Moscow 119021, Russia

Received 24 January 2006; accepted 18 April 2006

Dedicated to Professor Erik DeClercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Antiviral activity and other biological properties of two groups of polycyclic peptides are discussed. Antibiotics of the complestatin–kistamycin group have a structural motif similar to that of the peptide core of antibacterial antibiotics of the vancomycin–teicoplanin group though no amino acid component in the chloropeptin–kistamicin antibiotics is identical to an amino acid incorporated in the peptide core of the antibiotics of the vancomycin–teicoplanin group. Chloropeptins and the hydrophobic several derivatives of antibacterial antibiotics are inhibitors of HIV and some other viruses. They interfere with the viral (i.e. HIV) entry process. Chemical modifications of natural glycopeptide antibiotics led to the compounds with antiviral properties whereas antibacterial properties were lost. These glycopeptide aglycons derivatives can be envisaged as potential lead compounds for application as microbicides against sexual HIV transmission.

Keywords: Complestatin; Kistamicin; Vancomycin; Eremomycin; Hydrophobic derivatives; Viral entry

Contents

1. Introduction

Inhibitors targeted to the HIV entry and fusion process may add a further treatment for HIV-1 infection along with protease inhibitors and reverse transcription inhibitors (DeClercq,

* Corresponding author. Tel.: +7 495 2453753; fax: +7 495 2450295.
E-mail address: mnp@space.ru (M.N. Preobrazhenskaya).
[1] Tel.: +7 495 2453753; fax: +7 495 2450295.

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.04.008

2005a,b). Peptide antibiotics and their derivatives or analogs represent a special group of membrane-active peptides. They act by disrupting the structural integrity of the microbial (bacterial, viral or fungal) membranes. The most important peptides, which showed high or moderate anti-HIV-activity in "in vitro" models include *linear peptides*: peptide T and DAPTA (Polianova et al., 2003), fuzeon (T-20, enfuvirtide), tifuvirtide (T-1249), cyanovirin-N (CV-N) (Reeves and Piefer, 2005; DeClercq, 2005a,b), ALX 40-4C, the antibiotics polyphemusins and their synthetic analogs (T-22, T-134, T-140) (Rusconi et al., 2004) peptides V1 and enantiomer DV1 and their analogs (Zhou et al., 2002), peptoid CGP 64222 (DeClercq, 2001); and *cyclic peptides*: antibiotics siamycins I and II, FC131 (synthetic polyphemusin analog), the antibiotics cyclosporins (Tandon and Chhor, 2005) and gramicidins (Bourinbaiar and Jirathitikal, 2003); and *polycyclic peptides*: antibiotics of the chloropeptin–kistamicin group (Tandon and Chhor, 2005) and the aglycons of glycopeptides of the vancomycin–teicoplanin group (Balzarini et al., 2003; Printsevskaya et al., 2005; DeClercq, 2005b). Several of these peptides (fuzeon, tifuvirtide, cyanovirin-N) demonstrate efficacy in vivo and are currently being used in clinical trials or in preclinical investigations, other peptides are now in development as the potential drugs for cure of AIDS (DeClercq, 2005a; Rusconi et al., 2004; Tandon and Chhor, 2005). Some compounds such as Alx-40-4C, T-1249, T-22 and analogs, CGP64222 are no longer being considered as potential drugs. Some (e.g. Alx-40-4C) were tested in clinic and failed, some (e.g. T-1249) showed activity in clinic but were discontinued for other technical reasons.

This review is focused on the structural and functional features of one of the groups of the naturally occurring antiviral peptides–polycyclic peptides or glycopeptides and their deglycosylated (aglycon) derivatives as inhibitors of HIV entry.

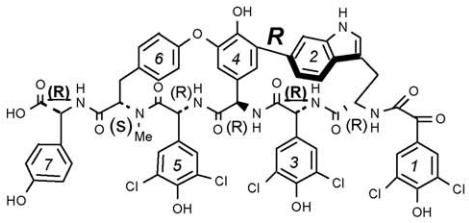
2. Hexa- and heptapeptide antibiotics of chloropeptin and kistamicin group

2.1. Comparison of structures of antibiotics of the chloropeptin–kistamicin group and antibacterial glycopeptides

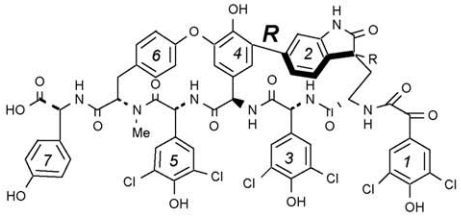
A specific group of polycyclic peptide antibiotics-*N*-acylhexapeptides (chloropeptins, complestatins) and heptapeptides (kistamicins) (shown in the left column in Fig. 1) has a common structural motif similar to that of the antibacterial antibiotics of the vancomycin or teicoplanin (ristocetin) groups (shown in the right column, Fig. 1), although the size of their macrocycles, i.e. the framework of these antibiotics, is different. Indeed, the structures of these hexa- or heptapeptide antibiotics and the structures of antibacterial glycopeptide antibiotics and their aglycons show profound differences in amino acid sequence, composition and stereochemistry. The cycle formed with the participation of the amino acids 5–7 present in glycopeptide antibiotics, is absent in chloropeptins (**1**, **2a**) and complestatins (**1a**, **1b**) and is of a different type and size in kistamicins (**3a**, **3b**). In this review the numeration of the amino acids in antibiotics of the chloropeptin group starts from the N-terminal amino acid similarly to numeration of amino acids in antibacterial glycopeptide antibiotics. No amino acid, presented in the structures in the left column, is identical with an amino acid incorporated in the peptide core of the antibiotic presented in the right column. Kistamicins (**3a**, **3b**), complestatins (**1**, **1a**, **1b**) contain a tryptophan moiety linked to the central amino acid number 4, connected with the amino acid 4 through the atom in the indole position 6 (**1**, **1a**, **1b** and **3a**, **3b**) or 7 (**2a**, **2b**), whereas a substituted phenylalanine moiety is present in the antibacterially active glycopeptides (**4**–**8**) (Fig. 1). Both groups have a central triheaded triamino-tricarboxy acid 2-4-6, in which aromatic residues are connected through two C–O–C (vancomycin–teicoplanin series) or through C–O–C (4-6) and C–C (2-4) (chloropeptin–kistamycin group) bonds (Hedge et al., 2003). Teicoplanin and relative antibiotics have also a cycle formed by amino acids 1–3. Both groups differ in the stereochemistry of the amino acids incorporated: 1*R*-2*R*-3*S*-4*R*-5*R*-6*S*-7*S* for the vancomycin–teicoplanin group and (1*R*)-2*R*-3*R*-4*R*-5*R*-6*S*-7*R* for the kistamicin and complestatin group of antibiotics. Complestatin (**1a**) has *R* configuration of the C–C bond between amino acids 2 and 4. Besides an *S* atropoisomer of **1a** called isocomplestatin (**9**) was obtained synthetically (Fig. 2) (Shinohara et al., 2005). In the chloropeptin group amino acid 1 is substituted by an acyl group (Deng et al., 2003). Last but not least, the peptides in the left column are not glycosylated. Complestatin (**1**) rearranges into **2a** under acidic conditions (Jayasurriya et al., 1998). **1** and related compounds were isolated from various soil *Streptomyces* species. Kistamycins were isolated from the fermentation broth of *Microtetraspora parvosata* subsp. + *kistnae* (Naruse et al., 1993).

2.2. Biological properties of chloropeptins

Chloropeptins **1** and **2a** are equipotent inhibitors of HIV-1 gp120 glycoprotein (Kaneko et al., 1989; Seto et al., 1989). **1** inhibited HIV-1 induced cytopathicity and HIV-1 antigen expression in MT-4 cells; the 50% effective doses for these effects were 2.2 and 1.5 μM, respectively. No toxicity for MT-4 cells was observed at concentrations up to 400 μM. The agent inhibited the focus formation in HT4-6C cells (CD4-positive HeLa cells); the concentration for 50% focus reduction was 0.9 μg/mL HIV-1 induced cell fusion in co-cultures of MOLT-4 cells and MOLT-4/HTLV-III$_B$ were also blocked by **1** (the concentration for 50% fusion inhibition was 0.9 μg/mL). It had no ability to inhibit HIV-1 reverse transcriptase activity. When MT-4 cells were pretreated with **1** for 2 h prior to exposure to HIV-1, the HIV-1 induced cytopathicity was markedly inhibited, while pretreatment of HIV-1 with the agent did not affect the infection. The results obtained in vitro suggest that complestatin (**1**) primarily interacts with cells and inhibits viral adsorption to the cell surface as well as adsorption of infected cells to adjacent cells (Memota et al., 1999). Compounds **1** and **2a** inhibited gp120 to CD4 binding (IC_{50} 1.3 and 2.0 μM, respectively), the cytopathic effect of HIV-1 in MT-4 cells (EC_{50} 1.6 and 1.7 μM, respectively) and syncytium formation in co-cultured HIV-1 infected and uninfected MOLT-4 cells (IC_{50} 0.5 and 1.1 μM, respectively). It was demonstrated that **2a** was synergistic in the

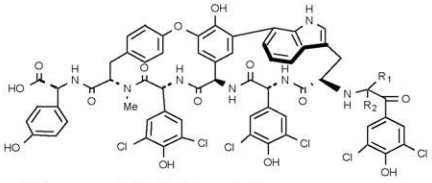


Complestatin (Chloropeptin II) **1**



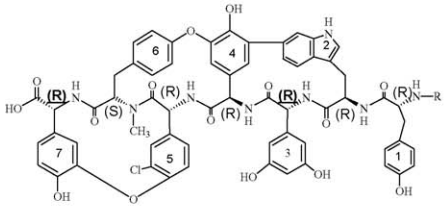
Complestatin A. R = H **1a**;

Complestatin B R = OH **1b**



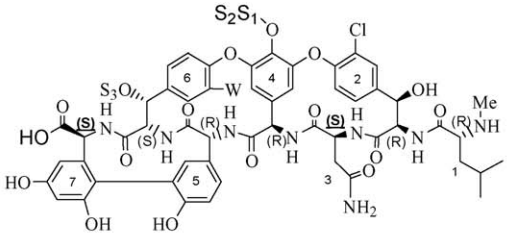
Chloropeptin I: R_1,R_2 = O **2a**;

SCH204698: R_1=OH, R_2=CH_2COCH_3 **2b**



Kistamicin A: R = H **3a**;

Kistamicin B: R = $CONHCH_2CH_2Ph$ **3b**



Vancomycin: W=Cl, S_1=Glc,

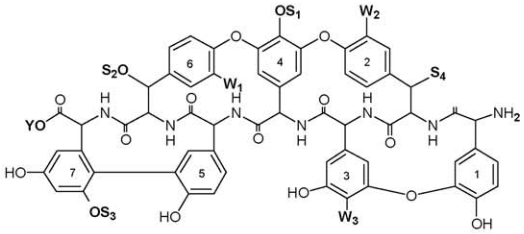
S_2=vancosamine, S_3=H **4**;

Chloroeremomycin: W=Cl, S_1=Glc,

S_2=S_3=eremosamine **5**;

Eremomycin: W=H, S_1=Glc,

S_2=S_3=eremosamine **6**



Ristocetin A: W_1=W_2=H, Y= W_3=Me,

S_1=tetrasaccharide, S_2=Ristosamine,

S_3=Man, S_4=OH **7**;

Teicoplanin: W_1=W_2=Cl,

Y=W_3=H, S_1=GlcNAcyl, S_2=GlcNAc,

S_3=Man, S_4 =H **8**

Fig. 1. Antibiotics of the complestatin, chloropeptins and kistamycin group (left column) and antibacterial antibiotics of the vancomycin and teicoplanin group (right column).

inhibition of the cytopathic effect when combined with other anti-HIV drugs such as zidovudune (AZT), ddI, ddC and nevirapine (Tanaka et al., 1997). **1** inhibited also recombinant feline immunodeficiency virus with an IC_{50} value of 0.5 μM and was a more potent inhibitor of recombinant simian immunodeficiency virus, with an IC_{50} value of 0.1 μM (Singh et al., 2001). **1** and **2a** exhibit no antimicrobial activity against various bacteria and fungi at 1 mg/mL and no cytotoxicity at 20 μM for B16 melanoma (Matsuzaki et al., 1994). They are also inhibitors of the integrase from several related retroviruses (in vitro). **1** was first discovered as a very strong inhibitor of protease activity of complement in the human complement system—hence is the name "complestatin" (Seto et al., 1989). **2a** enhances plasminogen binding and fibrinolysis (Endo, 1997). Some other bio-

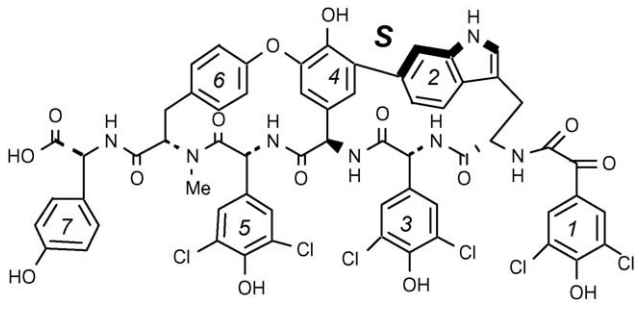


Fig. 2. Isocomplestatin **9**.

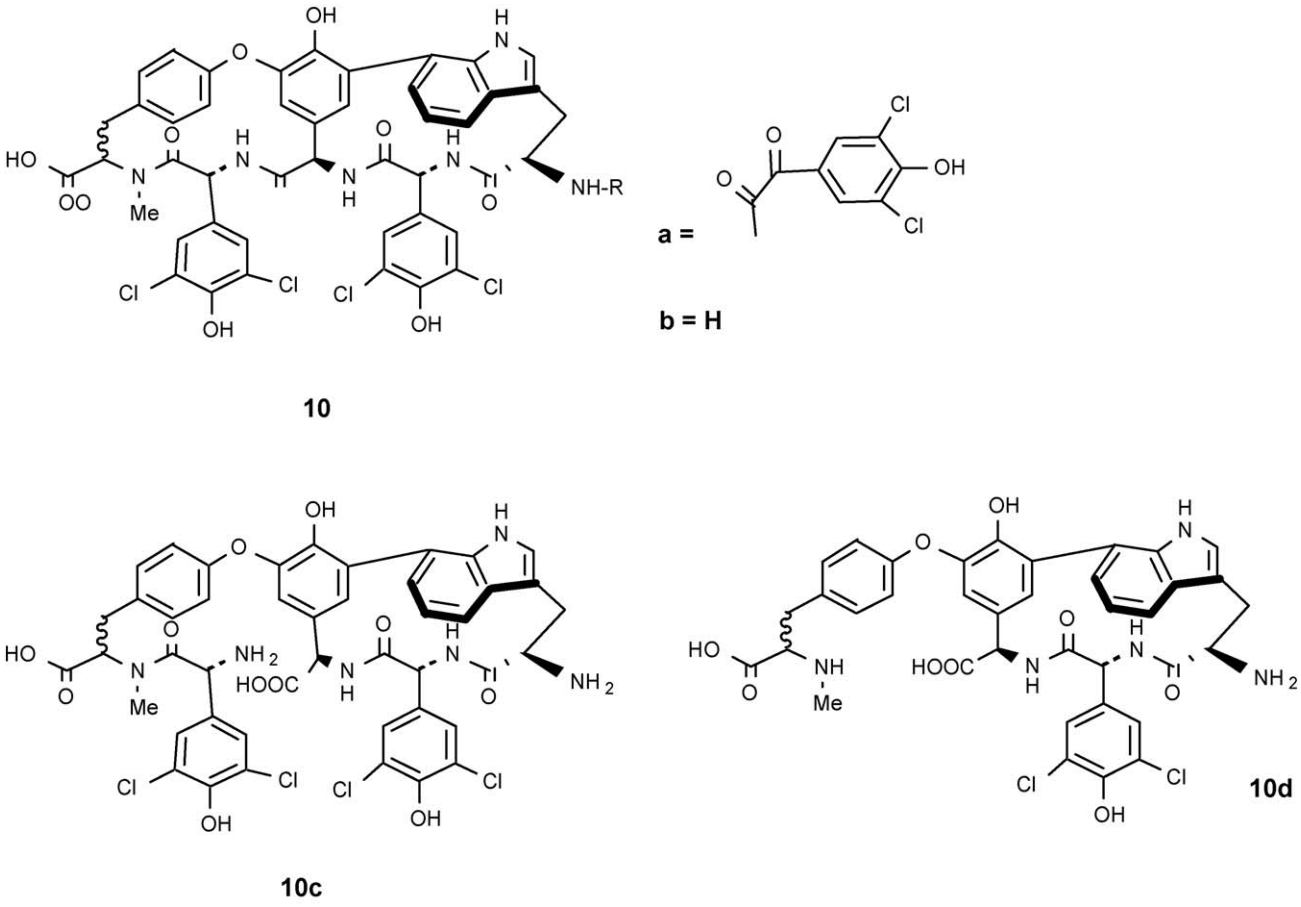


Fig. 3. The products of chloropeptin I degradation (**2a**).

logical properties of these antibiotics were demonstrated (Kim et al., 2004; Hedge et al., 2003; Seo et al., 2001). It supports the previously made conclusion that some peptides are multivalent compounds able to interact with several natural targets (DeClercq, 2001).

2.3. Biological properties of kistamicins

Kistamycins A and B (**3a** and **3b**) demonstrated in vitro stronger anti-influenza virus activity than ribavirin (virazole) (Naruse et al., 1993). **3b** exhibited about a two-fold greater antiviral activity than **3a**. Both compounds showed low or no antiviral activities against either HSV or HIV. They were also inactive in the syncytium formation inhibition assay in two cell lines: HeLa-T4 cells expressing CD4 antigen and BSL-1 cells expressing gp-120. They exhibited very weak cytotoxicities against human colon carcinoma (HCT-116) and murine melanoma (B16-F10) cells with $IC_{50} > 200$ μg/mL. Kistamycin A showed moderate activity against Gram-positive bacteria but no activity against Gram-negative bacteria. MICs for seven strains of *Staphylococcus aureus* are 6.3–25 μg/mL and for *Enterococcus faecalis* A9612 50 μg/mL. These findings clearly demonstrated the differences in the biological activities between kistamicin and complestatins in spite of the structural similarity of amino acids 2, 4, 6.

2.4. Structure–activity relationship study of chloropeptin I

To define the minimal pharmacophore required for antiviral inhibitory activities of **2a** the selective hydrolysis of **2a** was investigated. Cleavage of the amino acid 7 led to pentapeptides with *N*-acyl or without *N*-acyl each of which represents interconvertible isomers (~1:1 mixture) deriving from the 6th amino acid racemization (Fig. 3, **10a** or **10b**). The pentapeptides show IC_{95} values of 5 and 7.5 μM, respectively (in HIV-1 viral spread model), which were comparable to chloropeptin **2a** ($IC_{95} = 5$ μM). Thus the pharamacophore unit was determined as the pentapeptide **10a** or its *N*-deacylated product **10b** (Singh et al., 1998).

Model of chloropeptin I docking to CD4 demonstrated that the antibiotic exhibits a high degree of structural and electrostatic homology to the CD4-binding domain of gp120. It was supposed that the macrocycle [amino acids 4–6 in chloropeptin I (**2a**)] is the key determinant fragment of antiviral activities, and the structural homology suggests that **2a** may compete for a common binding site on CD4 (Smith et al., 2004). The derivatives **10a** and **10b** also have the key determinant fragment of antiviral activities as they contain in their structure undestroyed macrocycle of the amino acids 4–6.

Ring opening of the pentapeptide or extraction of amino acid 5 from the pentapeptide **10b** led to the peptides (Fig. 3, **10c**,

10d), which were significantly less potent than the **2a** or **10a**, **10b** in others models (HIV-1 integrase coupled and strand transfer assays). It was mentioned that some peptides are multivalent compounds and are able to interact with several natural targets (DeClercq, 2001).

3. Antibacterial glycopeptide antibiotics

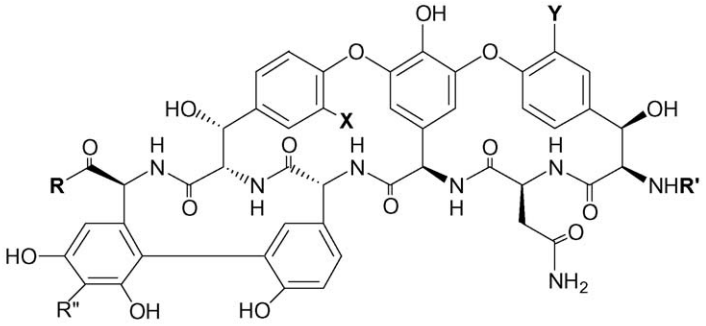
3.1. Structure and antibacterial properties of glycopeptide antibiotics of the vancomycin and teicoplanin groups and their derivatives

Glycopeptide antibiotics (Vancomycin **4**, Teicoplanin **8**) (Fig. 1) are vital therapeutic agents used for the treatment of life-threatening infections caused by Gram-positive bacteria. They are classified according to a variation at amino acids 1 and 3. In the vancomycin type, they are aliphatic amino acids D-MeLeu and L-Asn, respectively, in the ristocetin (teicoplanin)-type antibiotics, the amino acids 1 and 3 contain aromatic residues with an ether linkage joining these residues. Several natural glycopeptide antibiotics of this group, such as eremomycin (**6**), chloroeremomycin (**5**), ristocetin A (**7**) (Fig. 1) and others, were not introduced as chemotherapeutic agents but are intensively used in second generation drug design. The rapid increase in the incidence of infections resistant to vancomycin accelerated the search for glycopeptide derivatives active against vancomycin-resistant strains of bacteria. This resulted in discovery of the anti-GRE (glycopeptide resistant enterococci) activity of semisynthetic derivatives of natural glycopeptides containing various hydrophobic moieties. The antibacterial activity of these derivatives depends on the size of the introduced hydrophobic moiety; the optimal size is C_9–C_{14} as linear alkyl type or *p*-Ar–Bn or the combination of the two types. The most frequently modified positions in the antibiotic molecule are the amino groups of the disaccharide branch, carboxyl group and the resorcinol ring of the amino acid 7 (Preobrazhenskaya and Olsufyeva, 2004).

3.2. Mechanism of antibacterial activity

Glycopeptides exert their antibacterial activity by inhibiting one or both sequential enzymatic reactions involved in the synthesis of cell wall, namely peptidoglycan, elongation (transglycosylation) and cross-linking (transpeptidation). The antibiotics bind to a peptidoglycan precursor in a highly selective manner, the lipid-bound *N*-acetylglucosaminyl-*N*-acetyl-muramyl-pentapeptide interacting with the terminal D-Ala-D-Ala (Walsh, 2003).

Resistance to these antibiotics occurs when the bacterium acquires the resistance genes, in which expression leads to cell wall precursors terminating in acyl-D-Ala-D-Lac rather than D-Ala-D-Ala (Bugg et al., 1991). The complex of vancomycin with acyl-D-Ala-D-Lac lacks the central H-bond and also suffers from a repulsive lone pair interaction between the vancomycin amino acid 4 carbonyl group and the D-Ala-D-Lac oxygen. The derivatives of natural antibacterial glycopeptides containing hydrophobic moieties (size C_9–C_{14}) demonstrate antibacterial activity against GRE (Cooper et al., 1996) and act in a different manner

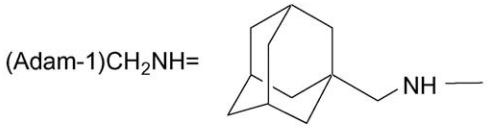


11. R=OH; X=Y=Cl; R'= D-MeLeu; R"=H. Vancomycin aglycon. **11a**. R= (Adam-1)CH_2NH.

12. R=OH; X=H; Y=Cl; R'= D-MeLeu; R"=H. Eremomycin aglycon. **12a**. R= (Adam-1)CH_2NH.

13. R=OH; X=Y=Cl; R'=H; R"=H; Des-(D-MeLeu)-vancomycin aglycon, **13a**. R= (Adam-1)CH_2NH.

14. R=OH; X=H; Y=Cl; R'=H; R"=H; Des-(D-MeLeu)-eremomycin aglycon. **14a**. R= (Adam-1)CH_2NH.



15. R=OH; X=H; Y=Cl; R'= D-MeLeu; R"= $CH_2N[CH_2CH_2]_2N$-BnPh-p.

Fig. 4. Derivatives of vancomycin and eremomycin aglycons.

Table 1
Antibacterial and antiviral activities of glycopeptide aglycons and their derivatives (Printsevskaya et al., 2005) (See Fig. 4)

Compound	MIC[a] (μg/ml)			IC_{50}[b] (μM)			EC_{50}[c] (μM)	
	533 *S. epider-midis*	559 *E. faecalis* (GSE)	560 *E. faecalis* (GRE)	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2
Vancomycin aglycon and its amide								
11	4	2	>64	>500	>500	>500	65	≥250
11a	2	1	16	≥250	72	≥250	3.0	9.5
Eremomycin aglycon and its amide								
12	32	16	>128	>500	>500	>500	50.6	≥250
12a	8	4	8	94	126	148	1.6	7
Des-(*N*-D-MeLeu)-vancomycin aglycon and its amide								
13	>128	>128	>128	≥250	≥250	>250	≥125	≥125
13a	>32	>64	>64	≥250	178	≥250	20	30
Des-(*N*-D-MeLeu)-eremomycin aglycon and its amide								
14	>128	>128	>128	>250	>250	>250	115.2	>250
14a	>32	>64	>64	175	>250	>250	5.5	3.5

[a] Minimum inhibitory concentration of the compound. As MWs of all compounds are in the interval ~1000–1400 Da and MICs values given in "μg/ml" are very close to those calculated in "μM".
[b] Inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.
[c] Effective concentration or concentration required to protect cells against the cytopathicity of the different HIV strains by 50%.

from that of parent antibiotics. They inhibit the transglycosylase stage of peptidoglycan biosynthesis even in the absence of D-Ala-D-Ala or D-Ala-D-Lac binding (Printsevskaya et al., 2002). So it was shown that the introduction of the hydrophobic moiety of definite size (~C_{10}) into the molecule of glycopeptide antibiotic may impart it activity against VRE strains and change the mechanism of action of the antibiotic (Ge et al., 1999).

3.3. Antiviral properties of glycopeptide derivatives

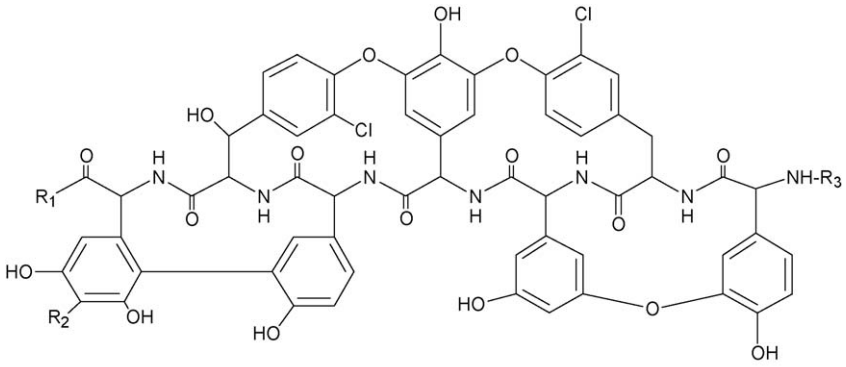
The natural antibiotics vancomycin (**4**), eremomycin (**6**) and ristocetin A (**7**) are neither toxic to the human CEM and MOLT-4/C8 and murine embryo fibroblast cells nor inhibitory to HIV-1, HIV-2, and MSV. In contrast teicoplanin (**8**) is moderately active against HIV-1 (EC_{50} = 17 μM) (Balzarini et al., 2003). The introduction of hydrophobic substituents led to the antibiotic derivatives with marked anti-HIV-1 activities, though some of the compounds were toxic for the cells at rather low drug concentrations (Balzarini et al., 2003). Removing all carbohydrates from vancomycin (**4**) and eremomycin (**6**) led to the, respectively, aglycons (**11**, **12**, Fig. 4), which were not cytotoxic (IC_{50} > 500 μM), but active against HIV-1 at rather high concentrations (EC_{50} ~ 50–65 μM), whereas it resulted in a significant decrease or loss of antibacterial activity (Printsevskaya et al., 2005). Some of the hydrophobic derivatives of eremomycin aglycon and des- (D-MeLeu)-eremomycin aglycon (**11a**–**14a**, **15**, Fig. 4) (Table 1) inhibited both HIV-1 and HIV-2 at comparable extent at EC_{50} values ranging between 3.0 and 30 μM. The aglycon antibiotic derivatives were invariably non-toxic for CEM cells (IC_{50} > 100–500 μM). The aglycons of ristocetin, teicoplanin, and some other natural glycopeptides were predominantly endowed with moderate anti-HIV-1 activity, while not being toxic for mammalian cells.

A large variety of teicoplanin aglycon semisynthetic derivatives with hydrophobic substituents (**16**–**21**, Fig. 5) were explored for their anti-HIV and Maloney sarcoma virus (MSV) activity. They showed very pronounced anti-HIV-1 and anti-HIV-2 activity in cell culture (Table 3), often with a tendency of being slightly more active against HIV-1 than HIV-2 (~two- to five-fold). The most active congeners were inhibitory against HIV-1 in the range of 0.75–2.5 μM (Balzarini et al., 2003). For example perhydroisoquinolinylamide of teicoplanin aglycon (**21**, Fig. 5) was active against HIV-1 and HIV-2 with EC_{50} 0.75 and 4.5 μM, respectively, being not toxic against L-1210, MOLT-4/C8 and CEM cells at concentrations up to 250 μM. Several teicoplanin aglycon derivatives reached a selectivity index (ratio IC_{50}/EC_{50}) that was ≥100. Many of the compounds active against HIV-1, were also potent inhibitors of Maloney sarcoma virus-induced transformation of murine fibroblast cell cultures at comparable drug concentrations.

3.4. Comparison of structure–activity relationships for antibacterial and antiviral activities

Degradation of the peptide core of teicoplanin aglycon was performed with the aim to define the minimal pharmacophore requested for antiviral activity. Modifications of the aglycon resulting in the disruption of the macro-cycles or deleting amino acid number 1 leads to the compounds (**22**, **23**) with anti-HIV activity (Figs. 6 and 7) (Balzarini et al., 2003). The SAR for degraded aglycons are similar to those described for complestatin degraded derivatives (Singh et al., 1998). It seems that the peptide framework formed by amino acids 4–6 with a hydrophobic substituent is a prerequisite for the antiretroviral activity.

The use of antiretroviral agents possessing concomitant antibacterial activity might be dangerous as it can lead to shifts in bacterial populations and induction of bacterial resistance to glycopeptide antibiotics. It means that for this class of compounds the absence of antibacterial activity is preferable. A study was directed to the preparation of the compounds with the highest antiviral activity and no antibacterial properties



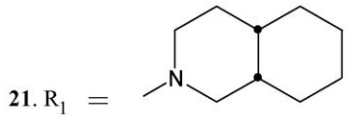
16. R_1 = -NH$(CH_2)_3N^+Me_2C_{10}H_{21}$, R_2 = -CH_2NH$(CH_2)_3N^+Me_2C_{10}H_{21}$, R_3 = H;

17. R_1 = -NH$(CH_2)_3$NMe_2, R_2 = -CH_2NHC_9H_{19}, R_3 = H;

18. R_1 = -NH$(CH_2)_3$NMe_2, R_2 = H, R_3 = $C_{11}H_{23}$;

19. R_1 = OH, R_2 = -CH_2NH$(CH_2)_3N^+Me_2C_{10}H_{21}$, R_3 = H;

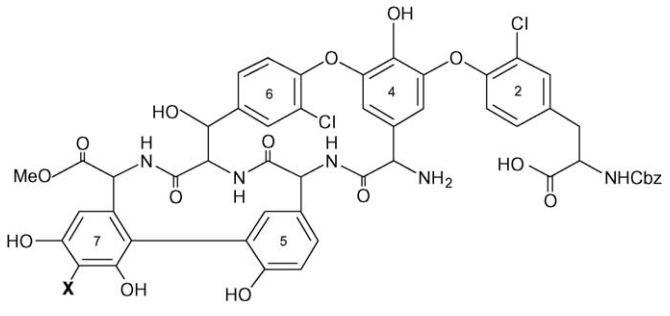
20. R_1 = -NH$(CH_2)_3$NMe_2, R_2 = -CH_2NH(Adam-2), R_3 =H.



21. R_1 = R_2 = R_3 = H

Fig. 5. Derivatives of teicoplanin aglycon.

(Printsevskaya et al., 2005). The investigation of antiretroviral activity of aglycon derivatives demonstrated that amides of vancomycin and eremomycin aglycons (**11a**, **12a**) have good anti-retroviral properties (Table 1). (Adamantyl-1) methylamine of eremomycin aglycon (**12a**) was the most active compound within this series. However these amides (**11a**, **12a**) also retained activity against vancomycin-sensitive and vancomycin-resistant bacteria (MIC: 1–16 μg/mL). But amides of vancomycin or eremomycin aglycons from which the first amino acid was split off (**13a**, **14a**) had no antibacterial activity at the MICs > 32–64 μM, and they demonstrated good anti-retroviral activities (Table 2). (Adamantyl-1) methyl amide of des-(D-MeLeu) eremomycin aglycon (**14a**) (EC_{50} = 5.5 μM, HIV-1 and 3.5 μM, HIV-2) is of special interest as it is devoid of any antibacterial activity, keeping its pronounced anti-HIV activity. The antibiotic aglycon derivatives should be considered as selective anti-HIV agents.



X = CH_2NHAdam-2: EC_{50} = 17 (HIV-1), 11 (HIV-2), 26 μM (MSV).
IC_{50} = 181 (L1210), >250 μM (MOLT4/C8 or CEM)

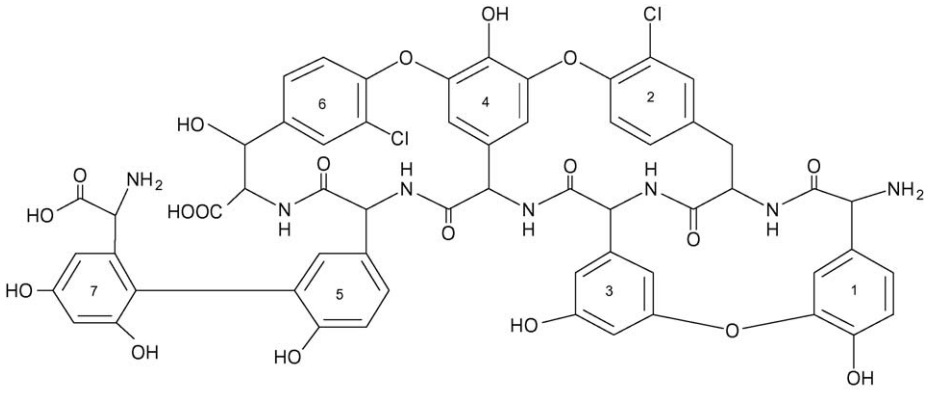
Fig. 6. Teicoplanin aglycon with the eliminated amino acids 1 and 2 (**22**).

The series of teicoplanin aglycon derivatives (**15–19**) (Figs. 4 and 5) were active against different types of wild HIV-1 and HIV-2 viruses (Table 2) and against the resistant strains of HIV-1 (Table 3) (Anon, 2002).

Changes, which do not disturb the binding pocket of the antibiotics of the vancomycin group (e.g. amidation of the carboxylic group), may lead to principal changes in the biological activity: introduction of a hydrophobic substituent makes them active against GRE and deglycosylation and introduction of a hydrophobic substituent impart them antiviral activity. Modification of the glycopeptide aglycon leads to decreased antibacterial activity, however, interestingly, it influences the antiretroviral properties to a much lesser extent.

3.5. Mechanism of antiretroviral action

Preliminary studies of mechanism of action studies of modified antibiotic derivatives against HIV have been performed in "time-of-addition" experiments carried out with the teicoplanin aglycon derivative **20** with anti-HIV activity (EC_{50} = 2.5, HIV-1 and 8.0 μM, HIV-2) (Fig. 5) (Balzarini et al., 2003). The drug was added to the virus-infected cell cultures at different time points after infection. The compound lost its antiviral



EC_{50} = 22 (HIV-1), 32 (HIV-2), 14 μM (MSV).

IC_{50} = >250 μM (CEM)

Fig. 7. Teicoplanin aglycon with the disrupted bond between amino acids 6 and 7 (**23**).

Table 2
Cytostatic and anti-HIV-1 and -HIV-2 activities of eremomycin aglycon and teicoplanin aglycon derivatives (Figs. 4 and 5) against different HIV-1 and HIV-2 strains and in different cell lines (Anon, 2002)

Compound	IC_{50}[a]			ED_{50}[b]						
	L1210	MOLT-4/C8	CEM	C8166	MOLT-4/8	CEM/0				
				HIV-1[c] (III_B)	HIV-1[c] (HE)	HIV-1[c] (III_B)	HIV-1[c] (HE)	HIV-2[c] (EHO)	HIV-1[c] (MN)	HIV-2 (RF)[c]
15	250	>500	>500	ND	22	5.5	40	50	20	16
16	12	19	9.4	9.0	7.5	7.5	17	11	12	8.5
17	43	136	179	9.5	9.0	6.0	12	15	13	9.5
18	23	35	90	≥5	4.5	2.8	5.5	11	7.0	3.7
19	51	65	74	6.6	3.7	2.8	9.5	6.8	6.5	3.7

[a] Inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.
[b] Effective concentration or concentration required to protect C8166, MOLT-4/C8 or CEM cells against the cytopathicity of the different HIV strains by 50%.
[c] Different types of the HIV strains.

activity when added at 1–2 h post-infection. This is also in agreement with the report that chloropeptins I and II interfere with gp120-CD4 binding. Also, the inhibitory activity of **20** against fusion between uninfected MOLT-4/C8 and persistently HIV-1-infected HUT-78 cells (resulting in syncytium formation between both cell types) (EC_{50} = 20 and 11 μM, respectively) is in agreement with the suggestion that inhibition of viral entry is the most likely molecular event for the anti-HIV activity of this type of compounds.

The viral entry process is the result of a specific interplay between viral glycoproteins (gp120, gp41) and cellular (co)receptors (CD4, CCR5, CXCR4). The preliminary findings show that viral entry is a target for the anti-HIV activity of the glycopeptide antibiotics aglycons. It is not so surprising that there is no close correlation between the anti-HIV activity and anti-MSV activity of the derivatives in cell culture. In agreement with these observations, it is important noting that the compounds kept their antiviral efficacy against HIV-1 strains that contain mutations in the reverse transcriptase (Leu-100-Ile RT, Lys-103-Asn, RT, Tyr-181-Cys RT, or Tyr-188-His RT) that resulted in resistance to non-nucleoside reverse transcriptase inhibitors. It was demonstrated for the eremomycin aglycon

Table 3
Anti-HIV-1 activity of the test compounds (Figs. 4 and 5) against mutant HIV-1 strains in CEM cell cultures (Anon, 2002)

Compo-und	EC_{50}[a] (μM)				
	HIV-1 (III_B)[b]	Leu-100-Ile[b]	Lys-103-Asn[b]	Tyr-181-Cys[b]	Tyr-188-His[b]
15	5.5	ND	11	10	ND
16	7.5	12.5	9.0	10	12.5
17	6.0	11.5	8.5	7.5	11.0
18	2.8	6.0	7.0	10	7.5
19	2.8	5.3	6.0	8.5	6.0

[a] Effective concentration or concentration required to protect CEM cells against the cytopathicity of resistant HIV-1 strains by 50%.
[b] Different types of the resistant HIV-1 strains.

(**15**) and teicoplanin aglycon derivatives (**16–19**) (Figs. 4 and 5; Table 3). Also, flow cytometric analysis and monoclonal antibody binding studies, and a PCR-based assay revealed that the drugs likely interrupt the viral entry process. The drugs did not interfere with monoclonal antibody binding to the CD4 receptor and CXCR4 co-receptors of HIV.

Extensive attempts to select resistant virus strains against several teicoplanin derivatives failed under experimental conditions that easily resulted in the emergence of nucleoside RT inhibitors (NRTI) (i.e. lamivudine) or NNRTI (i.e. nevirapine) resistant virus strains.

3.6. Activity against other viruses belonging to Retroviridae, Herpes viridae, Flaviviridae and Coronaviridae

A variety of semisynthetic derivatives of glycopeptide antibiotics including vancomycin, eremomycin, teicoplanin, ristocetin A and DA-40926 have been evaluated on their inhibitory activity against feline (Fe-CoV, FIPV strain) and human (SARS-CoV, Frankfurt-1 strain) corona viruses in cell culture. Several glycopeptide derivatives modified at several places with hydrophobic substituents showed selective antiviral activity at 50% effective concentrations in the lower micromolar range, being not cytostatic at 100-fold higher concentrations against Crandel feline kidney (CrFK), simian kidney (Vero) or human lymphocyte (CEM) cells. Removal of the carbohydrate parts of the molecules usually did not markedly affect the antiviral activity of the compounds. Besides compounds that showed inhibitory activity against both viruses, there were also compounds that proved solely inhibitory to either Fe-CoV or SARS-CoV. Therefore, there was no close correlation between the EC_{50} values of the modified glycopeptide antibiotics for Fe-CoV and SARS-CoV. Inhibition of Fe-CoV entry into the target (CrFK) cells proved to be the most likely mechanism of anti-coronavirus action of the modified glycopeptide antibiotics.

There are a few common structural features of glycopeptide antibiotics to be active against Fe-CoV and/or SARS-CoV. The introduction of a hydrophobic substituent on the molecules is required though not sufficient to exert antiviral activity. While several active compounds ($EC_{50} < 10\ \mu M$) against Fe-CoV have been found among the antibiotics bearing intact sugar moieties, the most active compounds against both viruses belonged to the aglycon derivatives of vancomycin, teicoplanin and eremomycin. However, there was not much of a correlation between the anti-HIV activity of the test compounds on the one hand and the antiviral activity against the coronaviruses on the other. Several potent anti-HIV compounds were barely active against the coronaviruses whereas several compounds active against the coronaviruses were poorly active against HIV. Moreover, there was also no marked correlation between the EC_{50} values of the compounds against both coronaviruses.

It may be not so surprising that no close correlation between the anti-HIV and anti-coronavirus activities of the glycopeptide antibiotics has been found. Previous investigations are indeed strongly suggestive for the inhibition of the gp120–CD4 interaction during HIV entry in its target cells as the molecular mechanism of anti-HIV action (Balzarini et al., 2003). These observations may point to a rather specific interaction of the compounds with a viral (HIV) factor that is absent in the coronavirus entry process. Moreover, the glycopeptide antibiotics, most likely interfere with the coronavirus entry process, as observed with HIV, it is known that both human and feline coronaviruses recognize a different cellular receptor to enter their target cells (i.e. ACE-2 for Hu-CoV and amino peptidase for Fe-CoV) (Anon, 2002). Therefore, both viruses may obviously have different structural requirements for optimal interaction with the glycopeptide antibiotic derivatives.

Some of the eremomycin aglycon derivatives were found to be active against HSV or VZV with $EC_{50} < 1\ \mu M$ (Anon, 2002). No further mechanistic information is currently available to explain the antiherpetic activity.

4. Conclusion

Both groups of above presented antibiotics inhibit the viral entry into cells. Complestatins and antiviral glycopeptides have important structural differences but the macrocycle formed by amino acids 4–6 is present in both groups, though it has different hydrophobic substituents. They are aryl nuclei of amino acids 5 and 7 in chloropeptins and the introduced hydrophobic substituents in semisynthetic glycopeptides. No semisynthetic complestatin derivatives were studied as inhibitors of HIV entry, whereas more than 700 semisynthetic derivatives of vancomycin, eremomycin, teicoplanin and other antibiotics of this class have been synthesized and their antibacterial and antiviral activities were investigated. Deglycosylation of these antibiotics and the degradation of the peptide core led to compounds which have lost antibacterial properties due to the degradation of the binding pocket interacting with bacterial target; however some of them retained anti-HIV activity. The compounds kept their antiviral efficacy against HIV-1 strains that contain mutations in the reverse transcriptase. As these compounds cannot interact with the molecular target of the antibacterial glycopeptides (D-Ala-D-Ala of the bacterial peptidoglycan), their ability to induce resistance to glycopeptides during prolonged administration may be expected to be very low or even absent. Therefore, these novel glycopeptide aglycon derivatives (*but not any glycopeptide antibiotic or the derivative with high antibacterial activity*) should become promising candidate drugs to be further explored for the treatment and/or prophylaxis of HIV infections. The glycopeptide antibiotics (vancomycin, eremomycin, etc.) may be used as the starting material for preparation their modified deglycosylated derivatives (aglycons), which possess definite or high anti-HIV activity and simultaneously low toxicity and no antibacterial activity. So they can be envisaged as potential lead compounds for application as microbicides against sexual HIV transmission (Balzarini et al., 2003).

Acknowledgment

We are grateful to Prof. Jan Balzarini (K.U. Leuven-Rega Institute, Belgium) for critical reading the manuscript.

References

Anon, 2002. Patent WO 2004/019970. Priority date August 30, 2002.

Balzarini, J., Pannecouque, C., DeClercq, E., Pavlov, A., Printsevskaya, S., Miroshnikova, O., Reznikova, M., Preobrazhenskaya, M.N., 2003. Antiretroviral activity of semisynthetic derivatives of glycopeptide antibiotics. J. Med. Chem. 46 (13), 2755–2764.

Bourinbaiar, A.S., Jirathitikal, V., 2003. Low-cost anti-HIV compounds: potential application for AIDS therapy in developing countries. Curr. Pharm. Des. 9, 1419–1431.

Bugg, T.D.H., Wright, G.D., Dutka-Malen, S., Arthur, M., Courvalin, P., Walsh, C.T., 1991. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry 30, 10408–10415.

Cooper, R.D.G., Snyder, N.J., Zweifel, M.J., Staszak, M.A., Rodrigues, M.J., Huff, B.E., Thomphson, R.C., 1996. Reductive alkylation of glycopeptide antibiotics: synthesis and antibacterial activity. J. Antibiot. 49, 575–581.

DeClercq, E., 2001. New developments in anti-HIV chemotherapy. Curr. Med. Chem. 8 (13), 1543–1572.

DeClercq, E., 2005a. Emerging anti-HIV drugs. Expert Opin. Emerg. Drugs 10, 241–274.

DeClercq, E., 2005b. New approaches towards anti-HIV chemotherapy. J. Med. Chem. 48, 1297–1313.

Deng, H., Jung, J.-K., Liu, T., Kuntz, K.W., Snapper, M.L., Hoveyda, A.H., 2003. Total synthesis of anti-HIV agent chloropeptin I. JACS 125, 9032–9034.

Endo, A., 1997. Enhancement of plasminogen binding and fibrinolysis by chloropeptin I. Thromb. Res. 87, 571–576.

Ge, M., Chen, Z., Onishi, H.R., Kohler, J., Silver, L.L., Kerns, R., Fukuzawa, S., Thompson, C., Kahne, D., 1999. Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. Science 284, 507–511.

Hedge, V.R., Puar, M.S., Dai, P., Patel, M., Gullo, V.P., Chan, T.-M., Praamanik, B.N., Jenh, Ch.-H., 2003. Condensed aromatic peptide family of microbial metabolites. Inhibitors of CD28–CD80 interactions. Bioorg. Med. Chem. Lett. 44, 573–575.

Jayasurriya, H., Salituro, G.M., Smith, S.K., Heck, J.V., Gould, S.J., Singh, S.B., 1998. Complestatin to chloropeptin I via a quantitative acid catalyzed rearrangement. Absolute stereochemical determination of complestatin. Tetrahedron Lett. 39, 2247–2248.

Kaneko, I., Kamoshida, K., Takahashi, S., 1989. Complestatin, a potent anticomplement substance produced by *Streptomyces lavendulae*. I. Fermentation, isolation and biological characterization. J. Antibiot. 42, 236–241.

Kim, E.-Ch., Yun, B.-S., Ryoo, I.-J., Min, J.-K., Won, M.H., Lee, K.S., Kim, Y.-M., Yoo, I.-D., Kwoon, Y.-G., 2004. Complestatin prevents apoptic cell death: inhibition of a mitochondrial caspase pathway through AKT/PKB activation. Biochem. Biophys. Res. Commun. 313, 193–204.

Matsuzaki, K., Ikeda, H., Ogino, T., Matsumoto, A., Woodruff, H.B., Tanaka, H., Omura, S., 1994. Chloropeptins I and II novel inhibitors against gp120–CD4 binding from *Streptomyces* sp. J. Antibiot. 47, 1173–1174.

Memota, K., Kaneko, I., Kimura, S. Mitamura, K., Shimada, K, 1999. Inhibition of human immunodeficiency virus type-1-induced syncytium formation and cytopathicity by complestatin. Biochem. Biophys. Res. Commun. 179, 243–250.

Naruse, N., Tomita, K., Yamamoto, S., Tenmyo, O., Oki, T., 1993. New antiviral antibiotics, kistamicins A and B. I. Taxonomy, production, isolation, physico-chemical properties and biological activities. J. Antibiot. 46, 1804–1811.

Polianova, M.T., Ruscetti, F.W., Pert, C.B., Tractenberg, R.E., Leoung, G., Strang, S., Ruff, M.R., 2003. Antiviral and immunological benefits in HIV patients receiving intranasal peptide T (DAPTA). Peptides 24, 1093–1098.

Preobrazhenskaya, M.N., Olsufyeva, E.N., 2004. Patents on glycopeptides of the vancomycin family and their derivatives as antimicrobials: January 1999–June 2003. Expert Opin. Ther. Patents 14, 141–173.

Printsevskaya, S.S., Pavlov, A.Y., Olsufyeva, E.N., Mirchink, E.P., Isakova, E.B., Reznikova, M.I., Goldman, R.C., Brandstrom, A.A., Baizman, E.R., Longley, C.B., Sztaricskai, F., Batta, G., Preobrazhenskaya, M.N., 2002. Synthesis and mode of action of hydrophobic derivatives of glycopeptide antibiotic eremomycin and des-(*N*-methyl-D-leucyl) eremomycin against glycopeptide-sensitive and -resistant bacteria. J. Med. Chem. 45, 1340–1345.

Printsevskaya, S.S., Solovieva, S.E., Olsufyeva, E.N., Mirchink, E.P., Isakova, E.B., De Clercq, E., Balzarini, J., Preobrazhenskaya, M.N., 2005. Structure–activity relationships studies on a series of antiviral and antibacterial aglycon derivatives of the glycopeptide antibiotics vancomycin, eremomycin and dechloroeremomycin. J. Med. Chem. 45, 3885–3889.

Reeves, J.D., Piefer, A.J., 2005. Emerging drugs targets for antiretroviral therapy. Drugs 65, 1747–1766.

Rusconi, S., Bulgheroni, E., Citterio, P., 2004. Entry and fusion inhibitors of HIV. Expert Opin. Ther. Patents 14, 733–748.

Shinohara, T., Deng, H., Snapper, M.L., Hoveyda, A.H., 2005. Isocompklestatin: total synthesis and stereochemical revision. JACS 127, 7334–7336.

Seo, S.Y., Yun, B.S., Ryoo, I.-J., Choi, J.S., Joo, C.K., Chang, S.Y., Chung, J.M., Oh, S., Gwag, B.J., Yoo, I.-D., 2001. Complestatin is a noncompetitive peptide antagonist of *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptors: secure blockade of ischemic neuronal death. J. Pharmacol. Exp. Ther. 299, 377–384.

Seto, H., Fujioka, T., Furihata, K., Kaneko, I., Takahashi, S., 1989. Structure of complestatin, a very strong inhibitor of protease activity of complement in the human complement system. Tetrahedron Lett. 30, 4987–4990.

Singh, S.B., Jayasuriya, H., Hazuda, D.L., Felock, P., Homnick, C.F., Sardans, M., Patane, M.A., 1998. Selective and controlled hydrolysis of chloropeptin I HIV-1 integrase activity of fragments. Tetrahedron Lett. 39, 8769–8770.

Singh, S.B., Jayasuriya, H., Salituro, G.M., Zink, D.L., Shafiee, A., Heimbuch, B., Silverman, K.C., Lingham, R.B., Genilloud, O., Teran, A., Vilella, D., Felock, P., Hazuda, D., 2001. The complestatins as HIV-1 integrase inhibitors. Efficient isolation, structure elucidation, and inhibitory activities of complestatin, chloropeptin I, new complestatins, A and B, and acid-hydrolyses products of chloropeptin I. J. Nat. Prod. 64, 874–882.

Smith III, A.B., Chruma, J.J., Han, Q., Barbosa, J., 2004. Complestatin synthetic studies: the effect of amino acid configuration on peptide backbone conformation in the common western BCD macrocycle. Bioorg. Med. Chem. Lett. 14, 1697–1702.

Tanaka, H., Matsuzaki, K., Nakashima, H., Ogino, T., Matsumoto, A., Ikeda, H., Woodruff, H.B., Omura, S., 1997. Chloropeptins, new anti-HIV antibiotics inhibiting gp120–CD4 binding from *Streptomyces* sp. J. Antibiot. 50, 58–65.

Tandon, V.K., Chhor, R.B., 2005. Current status of anti-HIV agents. Curr. Med. Chem. Anti-Infective Agents 4, 3–28.

Walsh, C., 2003. Antibiotics: Actions, Origins, Resistance. ASM Press, Washington, DC, p. 47.

Zhou, N., Luo, Z., Luo, J., Fan, X., Pesavento, J., Dong, C.-Z., Wang, Y., An, J., Kaji, H., Sodroski, J.G., Huang, Z., 2002. Exploring the stereochemistry of CXCR4-peptide recognition and inhibiting HIV-1 with D-peptides derived from chemokins. J. Biol. Chem. 277, 17476–17485.