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Inhibition of feline (FIPV) and human (SARS) coronavirus by semisynthetic derivatives of glycopeptide antibiotics

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Abstract

Various semisynthetic derivatives of glycopeptide antibiotics including vancomycin, eremomycin, teicoplanin, ristocetin A and DA-40926 have been evaluated for their inhibitory activity against feline infectious peritonitis virus (FIPV) and human (SARS-CoV, Frankfurt-1 strain) coronavirus in cell culture in comparison with their activity against human immunodeficiency virus (HIV). Several glycopeptide derivatives modified with hydrophobic substituents showed selective antiviral activity. For the most active compounds, the 50% effective concentrations (EC_{50}) were in the lower micromolar range. In general, removal of the carbohydrate parts of the molecules did not affect the antiviral activity of the compounds. Some compounds showed inhibitory activity against both, whereas other compounds proved inhibitory to either, FIPV or SARS-CoV. There was no close correlation between the EC_{50} values of the glycopeptide derivatives for FIPV or SARS-CoV.

Keywords: Coronaviruses; FIPV; SARS; Glycopeptide antibiotics; Vancomycin; Teicoplanin; Eremomycin

1. Introduction

In 2003, a new member of the coronavirus family was identified as the causative agent of the previously unknown disease severe acute respiratory syndrome (SARS) (Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003). This highly contagious human disease originated in Southern China, but was quickly and efficiently spread to other places in the world. At least three other human coronaviruses OC43, 229E and NL63 are known to cause upper respiratory tract illnesses. They account for approximately one-third of the common colds that appear in the late fall and winter (Holmes, 2004). Sequence analysis of the RNA genome of the SARS-associated coronavirus (SARS-CoV) indicated that this virus is genetically distinct from the other human coronaviruses (Rota et al., 2003; Marra et al., 2003). SARS-CoV-like virus was isolated from a few Himalayan palm civets (*Paguma larvata*) and a raccoon dog (*Nyctereutes procyonoides*) during the SARS epidemic of 2002–2003, whose genomic sequence displayed 99.8% identity with that of the human SARS-CoV (Guan et al., 2003). Also, Song et al. (2005) reported that the genomic sequence of SARS coronaviruses from human and palm civet of the 2003/2004 outbreak in the city of Guangzhou, China, were nearly identical. Very recently, Lau et al. (2005) reported the isolation of a CoV closely related to SARS-CoV of humans and CoV of civets from wild Chinese horseshoe bats. Coronaviruses seem to exist in a wide variety of other animals including bovine, murine, porcine, avian, canine and feline species (Holmes, 2004).

In cats, the coronavirus feline infectious peritonitis virus (FIPV) causes a severe disease characterized by a vasculitis and disseminated pyogranulomatous lesions in various tissues and organs. Type II strains of FIPV can be easily cultured in Crandell-Reese feline kidney (CRFK) cells and are harmless to humans. We have now evaluated a wide variety of semisynthetic-modified glycopeptide antibiotics that were previously found to inhibit HIV (Balzarini et al., 2003; Printsevskaya et al., 2005), for their side-by-side activity against both SARS-CoV and FIPV. These studies were aimed to determine (i) whether these gly-

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copeptide antibiotic derivatives would also be active against SARS-CoV and FIPV, and, if so, (ii) whether there would be a correlation in their structure-activity relationship for both coronaviruses. These studies should also reveal whether FIPV could be used as a surrogate virus to discover active compounds against SARS-CoV. The antiviral activity values found for FIPV and SARS-CoV in this study were compared with the previously reported anti-HIV data (Balzarini et al., 2003).

While we could demonstrate that several lipophylic derivatives of the glycopeptide antibiotics, including a variety of aglycon derivatives, showed anti-coronavirus activity in the lower micromolar range, there was not a close structure-activity relationship for the glycopeptide derivatives against both viruses, suggesting that at least for this particular class of compounds, the FIPV cell culture model cannot be regarded as a reliable surrogate model to screen for efficient anti-SARS-CoV inhibitors.

2. Materials and methods

2.1. Cell culture and viruses

The SARS-CoV (Frankfurt 1 strain) was kindly provided by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated in minimal essential medium (MEM; Gibco, Life Technologies, Rockville, MD) supplemented with 10% fetal calf serum (FCS; Integro, Zaandam, The Netherlands), 2 mM L-glutamine (Gibco), and 1.4% sodium bicarbonate (Gibco). Virus-infected cells were maintained at 37 °C in a 5% CO_2 atmosphere in MEM supplemented with 2% FCS. The isolation of FIPV strain 79-1146 was described by McKeirnan et al. (1981). Crandell-Reese feline kidney (CRFK) cells were maintained in RPMI-1640 medium (Gibco) supplemented with 10% foetal calf serum (Harlan Sera-Lab Ltd., Loughborough, UK), 2 mM L-glutamine (Gibco), and 0.075% sodium bicarbonate (Gibco). Virus-infected cells were maintained at 37 °C in RPMI-1640 medium supplemented with 2% FCS.

2.2. Compounds

The structures of the different classes of the semisynthetic glycopeptide antibiotic derivatives are presented in the different tables. Each table contains the manuscript code number of the individual compounds in bold and a LCTA number (laboratory code number that corresponds to the synthesis of the individual compounds).Their chemical synthesis has been described in earlier work, or is presented in this report.

2.3. Chemistry

The methods for chemical modifications in the sugar moieties, at the amide part, at the resorcinol fragment and at the N-end of the antibacterial glycopeptide antibiotics were elaborated earlier, and used for the preparation of a variety of semisynthetic glycopeptides. The novel compounds were obtained by the methods (e.g. Mannich reaction, amidation, *N*-acylation, alkylation) previously described for the synthesis of analogous glycopeptide derivatives. The references on the methods of the preparation of the compounds previously described are as follows:

- *Method A*: 7d-Aminomethylated derivatives **5**, **12**, **13**, **14**, **15**, **18**, **21**, **22**, **26**, **29**, **43**, **63**, **99**, **100**, **104**, **106**, **127**, **139**, **142**, **144**, **161** were obtained by the method described by Pavlov et al. (1997).
- *Method B*: Carboxamides **3**, **4**, **17**, **23**, **30**, **38**, **32**, **33**, **34**, **35**, **36**, **37**, **40**, **41**, **42**, **44**, **45**, **53**, **72**, **78**, **79**, **80**, **88**, **114**, **115**, **116**, **117**, **118**, **119**, **120**, **122**, **131**, **135**, **141**, **145**, **167**, **170**, **171** were obtained by the method described by Miroshnikova et al. (2000).
- *Method C*: Carboxamides of aminomethylated derivatives **25**, **64**, **98**, **101**, **102**, **105**, **107**, **109**, **121**, **128**, **129**, **130**, **134**, **143**, **146**, **147**, **162** were obtained by the method B starting from 7d-aminomethylated derivatives obtained by the method A.
- *Method D*: *N*-Carbamoylated derivative of carboxamide of aminomethylated derivative **155** was obtained by the method described by Pavlov et al. (1993).
- *Method E*: Ester **28** was obtained analogously to compounds **8** and **9** by Pavlov et al. (1994).
- *Method F*: Diamide of DA40926 (**56**). To a mixture of 0.5 mmol of DA40926 and 5 mmol of an appropriative amine hydrochloride dissolved in 5 ml of DMSO were added portion-wise Et_3N to adjust pH 8–8.5 and afterwards during 1 h and 2.4 mmol of PyBOP-reagent (benzotriazol-1-yloxy)-tris-(pyrrolidino) phosphonium-hexafluorophosphate). The reaction mixture was stirred at room temperature for 6 h. Addition of ether (∼100 ml) to the reaction mixture led to an oily residue, which was shaken successively with ether (2× 15 ml) and acetone (∼15 ml). After addition of 100 ml of acetone a precipitate was collected and dried in vacuum.
- *Method G*: Diamide of 7d-aminomethylated derivative of DMDA 40926 (**64**) was obtained by the method F starting from 7d-aminomethylated derivative obtained by the method A.
- *Method H*: 1,3-Dicycloureides **80** and **171** were obtained by the treatment of solution of antibiotic aglycon in DMSO by 4 equiv. of DCC (dicyclohexylcarbodiimide) at the room temperature for 24–48 h. Addition of aceton to the reaction mixture led to precipitate which was collected and dried in vacuum.
- *Method I*: Carboxamide of 7d-aminomethylated derivative of *N*-L-lyzyl derivative of teicoplanin aglycon (octapeptide) **111** was obtained by the method C starting from *N*-lyzyl-teicoplanin aglycon synthesized by the method of Barna et al. (1985).
- *Method K*: *N*-Alkylated derivative of teicoplanin aglycon **112** was obtained by the method described by Malabarba et al. (1990).
- *Method L*: Carboxamide of 7d-aminomethylated derivative of *N*-alkylated derivative of teicoplanin aglycon **113** was obtained by the method C starting from *N*-alkylated derivative synthesized by the method K.
- *Method M*: Carboxamide of *N*-alkylated derivative of teicoplanin aglycon **123** was obtained by the method B start-

ing from *N*-alkylated derivative synthesized by the method K.

- *Method N*: 4d-*O*-Alkyl-derivative of teicoplanin aglycon **132** was obtained by the method described by a patent of Smithkline Beecham Corp.: EP-0273727A2 (1987).
- *Method O*: Carboxamide of 4d-*O*-alkyl-derivative of teicoplanin aglycon **124** was obtained by the method B starting from 4d-*O*-alkyl-derivative of teicoplanin aglycon synthesized by the method N.
- *Method P*: *N*-Alkylated derivative of 4d-*O*-alkyl-derivative of teicoplanin aglycon **133** was obtained by reductive alkylation of 4d-*O*-alkyl-derivative of teicoplanin aglycon **132**.

The synthesis of compound **2** and **103** has been described in Printsevskaya et al. (2003); compound **6** in Nagarajan et al. (1989) and Nagarajan (1993); compounds **8**, **9**, **47** and **48** in Pavlov et al. (1994); compound **10** in Pavlov et al. (1996); compound **11** in Pavlov et al. (1997); compound **14** in Olsufyeva et al. (1999); compounds **16** and **38** in Miroshnikova et al. (2000); compounds **19**, **20** and **24** in Pavlov et al. (2001); compound **24** also in Printsevskaya et al. (2002); compounds **39**, **55**, **73–76**, **85**, **125**, **126**, **137**, **138**, **140**, **148–150**, **153**, **158–160**, **163–166**, **168**, **169**, and **174–177** in Balzarini et al. (2003); compounds **46** and **49** in Pavlov et al. (1993); compounds **50** and **51** in Gerhard et al. (1993); compounds **54** and **110** in Malabarba et al. (1989); compounds **57** and **96** in Hermann et al. (1996); compounds **58–62** in Maffiolli et al. (2005); compound **65** in Kannan et al. (1988); compounds **66–70**, **77**, **81**, **82**, **86**, **87** and **89–94** in Printsevskaya et al., 2005); compound **71** in Berdnikova et al. (1991); compounds **83** and **84** in Miroshnikova et al. (1996); compound **95** in Bognar et al. (1974); compound **97** in Malabarba et al. (1986); compound **108** in Pavlov et al. (1998); compound **136** in Malabarba et al. (1987); compounds **151** and **154** in Malabarba et al. (1992); compound **157** in Trani et al. (1989); compounds **172** and **173** in Malabarba et al., 1996); and compound **178** in Cavalleri et al. (1987).

2.4. Antiviral and cytostatic activity assays

Antiviral activity and cytotoxicity measurements were based on the viability of Vero cells that had been infected (or mock-infected) with 100 $CCID_{50}$ (50% cell culture infective dose) of SARS-CoV (Keyaerts et al., 2004), and CRFK cells that had been infected (or mock-infected) with 100 $CCID_{50}$ of FIPV in the presence of various concentrations (five-fold dilutions) of the test compounds. The Vero and CRFK cells were seeded in 200 μl-wells of 96-well-microtiter plates and grown to nearly confluency. The drugs were then added to the cell cultures before virus was administered. This allows the compounds to block any of the different steps in the virus-infected process, including virus adsorption. Three days (SARS-CoV) or 4 days (FIPV) after infection, the number of viable cells was quantified by a tetrazolium (MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Chemical Co., St. Louis, MO))-based colorimetric method as previously described for HIV by Pauwels et al. (1988). The cytotoxic concentration was determined as the concentration of the compound that reduced cell viability by 50% (50% cytotoxic concentration (CC_{50})) and the antivirally effective concentration was determined as the compound concentration that suppressed the viral cytopathic effect by 50% (50% effective concentration (EC_{50})). An EC_{50} value preceeded by the sign ">" means that the indicated compound concentration does not afford antiviral activity. Higher (five-fold) concentrations were either not evaluated, or, were cytotoxic to the cell cultures. A CC_{50} value preceeded by the sign ">" means that at the indicated compound concentration no significant cytotoxicity was observed.

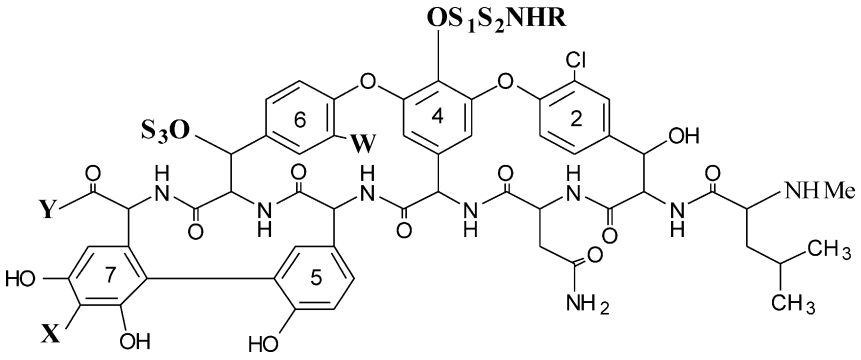
3. Results

3.1. Antiviral activity of different classes of glycopeptide antibiotic derivatives

A wide variety of ~180 semisynthetic lipophylic derivatives of the vancomycin, eremomycin, teicoplanin, *N*-deacyl-A40926 (DA40) and demannosyl-*N*-deacyl A 40926 (DMDA40) antibiotics, aglycon derivatives derived thereof, and glycopeptide antibiotics with a modified or partially destroyed peptide core, were evaluated against SARS-CoV and FIPV in cell culture. Many of these compounds were reported previously to be endowed with selective anti-HIV activity in the lower micromolar range (Balzarini et al., 2003; Printsevskaya et al., 2005). The anti-HIV-1 (III_B) activity of the test compounds is indicated in the tables for comparative reasons. The general structures of the investigated compounds are depicted at the top of each table. The compound identification numbers are shown in bold and correspond to the code number in the second column of the tables. The antiviral activities are represented by their 50% effective concentrations (EC_{50}). The cytotoxic activities in simian kidney Vero and feline kidney CRFK cell cultures and the cytostatic activities in human lymphocyte CEM cell cultures are represented by their 50% cytotoxic concentrations (CC_{50}) and 50% cytostatic concentrations (IC_{50}), respectively. EC_{50} values for FIPV and SARS-CoV that were $\leq$10 μM are printed in bold. The compounds endowed with a selectivity index (ratio CC_{50}/EC_{50}) >10 have an asterix after their code number in the tables.

Vancomycin (**1**), eremomycin (**7**), ristomycin (**50**), teicoplanin (**52**), DA40 (**55**) and DMDA40 (**57**) were neither toxic to human CEM, simian Vero and feline CRFK cells, nor inhibitory to SARS-CoV and FIPV ($EC_{50} > 80$ μM). However, the introduction of a hydrophobic substituent in vancomycin and eremomycin molecules resulted in new glycopeptide derivatives endowed with anti-coronavirus activity (Tables 1 and 2). In particular, compounds **5**, **6**, **42** and **43** showed comparable EC_{50} values (ranging between 20 and 45 μM) for both viruses, whereas **13** and **15** showed more pronounced activity against FIPV (EC_{50}: 3.4–8.9 μM) but lesser activity against SARS-CoV (EC_{50}: 31–65 μM) (Table 1). Compounds **39** and **27** had the highest activity against both viruses (EC_{50}: 12–22 and 5.4–14 μM, respectively). However, in a few cases, the compounds were solely active against FIPV (i.e. **29**, **34**) or solely active against SARS-CoV (i.e. **9**, **22**, **37**, **38**, **44**). It is clear from a structure-activity relationship (SAR) viewpoint

Table 1
Vancomycin and eremomycin type glycopeptides and their derivatives substituted at the X, Y and R positions



LCTA	Code no.	X	Y	R	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
					EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
Vancomycin (Van) and its derivatives W = Cl, S_1 = Glc, S_2 = vancosamine, S_3 = H										
878	**1**	H	OH	H	>250	>500	>100	>100	>100	>100
854	**2**	H	$NHC_{10}H_{21}$	H	>10	30 ± 2	>50	50 ± 1	>80	>80
892	**3**	H	$NH(CH_2)_3N^+Me_2C_{10}H_{21}$	H	5.5 ± 0.7	172 ± 15	>80	>80	57 ± 14	>80
893	**4**	H	NHMe	H	>250	>500	>80	>80	>80	>80
941	**5**	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	OH	H	12 ± 3.5	>100	30 ± 12	>50	37 ± 2	>100
1002	**6**	H	OH	BnPhCI-*p*	NT[a]	NT	20	61	22 ± 14	>100
Eremomycin (Ere) and its derivatives W = H, S_1 = Glc, S_2 = S_3 = eremosamine										
516	**7**	H	OH	H	>250	>500	>100	>100	>100	>100
177	**8**	H	$CH_3(CH_2)_2O$	H	NT	NT	>80	>80	>80	>80
200	**9**	H	$CH_3(CH_2)_{11}O$	H	NT	NT	>16	18 ± 4	27 ± 4	>80
261	**10**	H	NHMe	H	>250	>500	>80	>80	>80	>80
284	**11**	$CH_2NHC_{10}H_{21}$	OH	H	>20	24 ± 13	>16	44 ± 3	>40	54 ± 22
288	**12**	$CH_2NMeCH_2(CHOH)_4CH_2OH$	OH	H	>250	>250	>80	>80	>80	>80
289	**13**[c]	$CH_2NHC_{18}H_{37}$	OH	H	>10	15 ± 2	*3.4* ± 1.4	15 ± 2	65 ± 48	>80
302[b]	**14**	H	OH	H	NT	NT	>80	>80	>80	>80
298	**15**[c]	$CH_2NHC_{12}H_{25}$	OH	H	>10	94 ± 4	*8.9* ± 1	69 ± 8	31 ± 2	>80
340	**16**	H	$NHC_{10}H_{21}$	H	>10	9.4 ± 4.7	>16	19.5 ± 5	>40	53 ± 15
353	**17**	H	NHBnCI-*p*	H	>250	>250	>80	>80	>80	>80
356	**18**	$CH_2NHBnPh$-*p*	OH	H	17.5 ± 11	>500	>80	>80	51 ± 17	>100
368	**19**	H	OH	$C_{10}H_{21}$	>20	44 ± 2	>16	51 ± 0	43 ± 26	>80
375	**20**	H	OH	BnCI-*p*	>250	>250	>80	>80	>80	>80
512	**21**	$CH_2N[CH_2CH_2]_2NC_{10}H_{21}$	OH	H	>2	18 ± 11	>10	16 ± 1	>10	45 ± 8
518	**22**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	OH	H	>10	53 ± 15	>50	52 ± 6	14 ± 0	>80
670	**23**	H	$NH(CH_2)_4CH(CONH(CH_2)_3NMe_2)NHBnOBu$-*p*	H	>50	117 ± 18	>80	>80	>80	>80
717	**24**	H	OH	Bn(PhCI-*p*)-*p*	>10	44 ± 1	>50	60 ± 5	33 ± 11	>100
728	**25**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	7.0 ± 0	27 ± 7	>60	61 ± 0	>40	45 ± 1
766	**26**	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	OH	H	1.4 ± 0.6	96 ± 13	14 ± 4	58 ± 9	33 ± 1	>80
768	**27**[c]	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	NHMe	H	0.43 ± .3	40 ± 4	*5.4* ± 0.2	28 ± 3	14 ± 2	50 ± 6
770	**28**	H	$OC_{11}H_{23}$	H	>10	7.6 ± 1	>16	35 ± 4	44 ± 9	>80
784	**29**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	OH	H	>20	44 ± 3	>80	>80	>80	>80
826	**30**[c]	H	$NH(CH_2)_3N^+Me_2\ C_{10}H_{21}$	H	>20	38 ± 1	*6.9* ± 0.4	48 ± 1	>40	56 ± 21

Table 1 (*Continued*)

LCTA	Code no.	X	Y	R	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
					EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
827	**31**	H	$NH(CH_2)_6NH_2$	H	>100	>100	>80	>80	>80	>80
828	**32**	H	$NH(CH_2)_{10}NH_2$	H	>20	>100	>80	>80	>80	.80
829	**33**	H	$NHBnCH_2NH_2$-*p*	H	>100	>100	>80	>80	>80	>80
832	**34**	H	$NHBnNHC_{10}H_{21}$-*p*	H	>4	5 ± 0.5	>16	21 ± 8	22 ± 1	>80
833	**35**	H	$NHBnN^+Me_2C_{10}H_{21}$-*p*	H	>20	19 ± 8	14 ± 7	41 ± 5	>40	46 ± 0
834	**36**	H	$NHCH_2C_5H_4N^+C_{10}H_{21}$	H	>20	35 ± 2	>16	72 ± 9	>80	>80
837	**37**	H	NHBnPhCI-*p*	H	>10	8.6 ± 0.2	>20	27 ± 8	30 ± 24	>80
846	**38**	H	NHBnPh-*p*	H	>10	35 ± 1	>50	53 ± 4	31 ± 8	>100
847	**39**	CH_2NHBnPhCI-*p*	OH	H	22.5 ± 3.5	106 ± 65	12 ± 3	54 ± 8	22 ± 12	>100
848	**40**	H	NHBnBu-*p*	H	>50	29 ± 12	>50	52 ± 3	50 ± 3	>100
864	**41**	H	NHC_7H_{15}	H	≫50	182 ± 013	>80	>80	60 ± 19	>100
869	**42**	H	$NHBnNBu_2$	H	≫10	30 ± 2	37 ± 16	49 ± 8	35 ± 19	>100
921	**43**	$CH_2N[CH_2CH_2]_2$ $NCOCH_2NHBnBu$-*p*	OH	H	>10	63 ± 29	42	>80	45 ± 11	>80
923	**44**	H	$N[CH_2CH_2]_2NCH\ CH_2$ NHBnBu-*p*	H	>50	>100	>60	66 ± 12	31 ± 7	>100
972	**45**	H	NHCH($(CH_2)_4$ NH_2)CONHBnBu-*p*	H	>50	104 ± 11	>16	73 ± 7	>80	>80

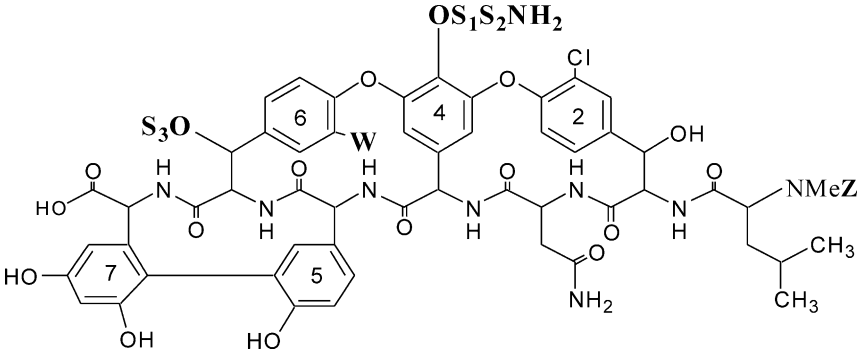
[a] NT, not tested.
[b] Carboxyeremomycin.
[c] Antiviral values in italics denotes EC_{50} values equal or lower than 10 μg/ml. An asterix after the compound code no. indicates a selectivity (CC_{50}/EC_{50}) of >10 for the compound against either FIPV and/or SARS-CoV.

that only among those compounds bearing an alkyl substituent with more than nine carbon atoms or a substituted phenyl or biphenyl moiety, antivirally active antibiotic derivatives were found.

Whereas none of the evaluated teicoplanin derivatives showed antiviral activity (Table 3), three compounds among the DMDA40 derivatives were found markedly active against FIPV (EC_{50}: 4.5–7.5 μM for **60**, **61** and **62**) but lesser active against SARS-CoV (EC_{50}: 21–43 μM) (Table 4). These compounds, as also noted for the vancomycin/eremomycin derivatives, contained a hydrophobic group at ring 7. Compound **62** was virtually not cytotoxic against either Vero or CRFK cells (CC_{50}: >80 μM) or against CEM cells (IC_{50}: 106 μM) (Table 4).

When the carbohydrate moieties were removed from the glycopeptide antibiotics, several active vancomycin and ere-

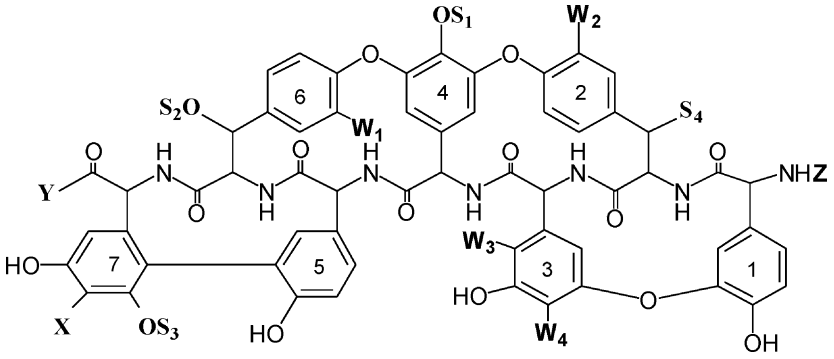
Table 2
Vancomycin and eremomycin type glycopeptides and their derivatives substituted in the Z position



LCTA	Code no.	Z	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
			EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
Vancomycin (Van) and its derivatives W = Cl, S_1 = Glc, S_2 = vancosamine, S_3 = H								
222	**46**	NO	NT[a]	NT	>80	>80	>80	>80
Eremomycin (Ere) and its derivatives W = H, S_1 = Glc, S_2 = S_3 = eremosamine								
147	**47**	$(CH_3)_2$	NT	NT	>80	>80	>80	>80
182	**48**	$(CH_2CHCH_2)_2$	NT	NT	>80	>80	>80	>80
246	**49**	Cbz	NT	NT	>80	>80	>80	>80

[a] NT, not tested.

Table 3
Teicoplanin type glycopeptides and their derivatives



LCTA	Code no.	X	Y	Z	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
					EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
Ristomycin (Risto) $W_1 = W_2 = W_3 = H$, $W_4 = Me$, S_1 = tetrasaccharide, S_2 = ristosamine, S_3 = Man, $S_4 = OH$										
903	**50**	H	OMe	H	>250	>500	>80	>80	>80	>80
Ristosaminylaglycon of ristomycin $W_1 = W_2 = W_3 = S_1 = S_3 = H$, $W_4 = Me$, S_2 = ristosamine, $S_4 = OH$										
929	**51**	H	OMe	H	>50	>100	>80	>80	>80	>80
Teicoplanin (Teico) and its derivatives $W_1 = W_2 = Cl$, $W_3 = W_4 = H$, S_1 = GlcNAcyl, S_2 = GlcNAc, S_3 = Man, $S_4 = H$										
879	**52**	H	OH	H	18	>500	>80	>80	>100	>100
894	**53**	H	$NH(CH_2)_3N^+Me_2C_{10}H_{21}$	H	>10	54 ± 9	>80	>80	>80	>80
895	**54**	H	NHMe	H	80 ± 28	>500	>80	>80	>80	>80

momycin aglycon derivatives emerged (Table 5). The most active compound in this series against FIPV was the eremomycin aglycon derivative **75** (EC_{50}: 3.6 μM) being nontoxic at 100 μM. It was 15-fold less potent (EC_{50}: 52 μM) against SARS-CoV. Only a few other derivatives showed activity between 14 and 48 μM for FIPV and between 32 and 59 μM for SARS-CoV (i.e. **67**, **73**, **74**, **77**, **81**). Dechlorination of some of the derivatives did not result in a better antiviral activity profile (i.e. **89–94**) (Table 5).

The highest number of derivatives were made within the substituted teicoplanin aglycon derivatives. Among them, several compounds showed pronounced anti-FIPV activity with EC_{50} values < 10 μM (Table 6) (i.e. **141**, **144**, **157**, **158**, **166–168**, **170** and **171**). Although in most cases, anti-FIPV activity was more pronounced than anti-SARS-CoV activity, **156** was equally active (8–8.5 μM) against both viruses. Interestingly, a few compounds were solely active against SARS-CoV (i.e. **116**, **138**, **153–155**, **161**, **163**). However, it is not clear whether a potential activity of these compounds against FIPV was masked by their more pronounced cytotoxicity against CRFK cells than Vero cells (Table 6).

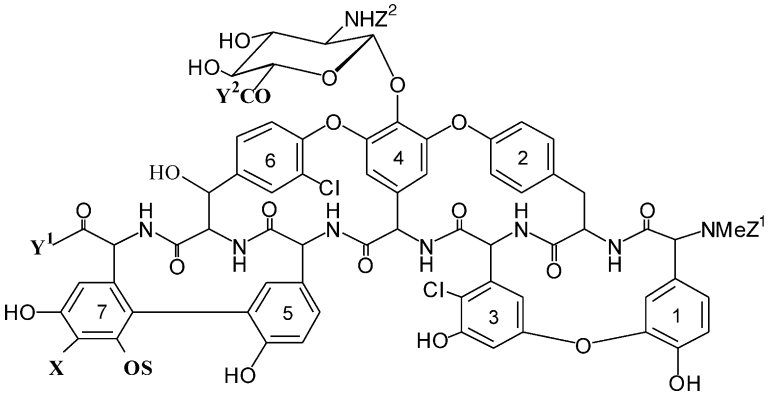
Among the teicoplanin aglycon derivatives in which the amino acids 1 and 3 were eliminated (Table 7), or had a disrupted bond between amino acids 1 and 2 (Table 8) or 6 and 7 (Table 9), several compounds (i.e. **173**, **177**) were moderately active against both viruses (EC_{50}: 19–48 μM), and no visible cytotoxicity was noted at 80 μM. However, given the relatively high EC_{50} values, it cannot be excluded that the virus inhibition is rather due to underlying toxicity of the compounds in the cell culture.

4. Discussion

There are a few common structural features of glycopeptide antibiotics to be active against FIPV or SARS-CoV. The introduction of a hydrophobic substituent on the molecules is required, although not sufficient to exert antiviral activity. While several active compounds ($EC_{50} < 10$ μM) against FIPV have been found among the antibiotics bearing intact sugar moieties, the most active compounds against both FIPV and SARS-CoV belong to the aglycon derivatives of vancomycin, teicoplanin and eremomycin. Such increased antiviral activity upon substitution with hydrophobic entities and removal of the carbohydrate part of the molecules was also noted and even more pronounced for HIV (compare data in Tables 1–4 with those in Tables 5–9). 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 that were markedly active against the coronaviruses were poorly active against HIV.

When the correlation coefficient was calculated between the anti-HIV activity of the antibiotic derivatives on the one hand, and their anti-FIPV or anti-SARS-CoV activity on the other hand, *r*-values of −0.23 and 0.49, respectively, were found. Moreover, no marked correlation was found between the EC_{50} values of the compounds against both coronaviruses (Fig. 1). Indeed, when all compounds for which a correct EC_{50} value could be determined were taken into account, a *r*-value of 0.51 was calculated for the EC_{50} values of the glycopeptide antibiotics against FIPV and SARS-CoV. When the *r*-values were

Table 4
N-Deacyl-A40926 (DA40), demannosyl-*N*-deacylA40926 (DMDA40) and their derivatives



LCTA	Code no.	X	$Y^1 = Y^2$	Z^1	Z^2	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
						EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
DA40926 and its derivatives S = Man											
519	**55**	H	OH	H	H	>250	>500	>80	>80	>80	>80
700	**56**	H	$NH(CH_2)_3N^+Me_2BnPh$-*p*	H	H	4.0 ± 1.4	32 ± 5	>50	63 ± 3	>80	>80
DMDA40926 and its derivatives S = H											
599	**57**	H	OH	H	H	115 ± 21	>500	>80	>80	>80	>80
604	**58**	H	$NH(CH_2)_3NMe_2$	*p*-BuOBn	*p*-BuOBn	5.0 ± 0.7	80 ± 6	>50	53 ± 7	>80	>80
605	**59**	H	$NH(CH_2)_3NMe_2$	H	*p*-BuBn	12 ± 3.5	>250	>60	63 ± 3	58 ± 2	>80
613	**60***[a]	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	OH	H	*p*-BuBn	3.5	81	*4.5* ± 0.4[a]	50 ± 0	21 ± 7	>80
614	**61**[a]	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	$NH(CH_2)_3NMe_2$	H	*p*-BuBn	3.5 ± 2.1	212 ± 54	*5.9* ± 1.5	30 ± 9	21 ± 5	>80
737	**62***[a]	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	OH	H	H	20 ± 7	106 ± 2	*7.5* ± 1.8	>80	43 ± 6	>80
738	**63**	$CH_2NH(CH_2)_3N^+C_{10}H_{21}Me_2$	OH	H	H	3.5	92	23 ± 17	>80	>80	>80
740	**64**	$CH_2NH(CH_2)_3N^+C_{10}H_{21}Me_2$	$NH(CH_2)_3NMe_2$	H	H	3.5 ± 0.7	92 ± 5	>50	55 ± 2	52 ± 21	>80

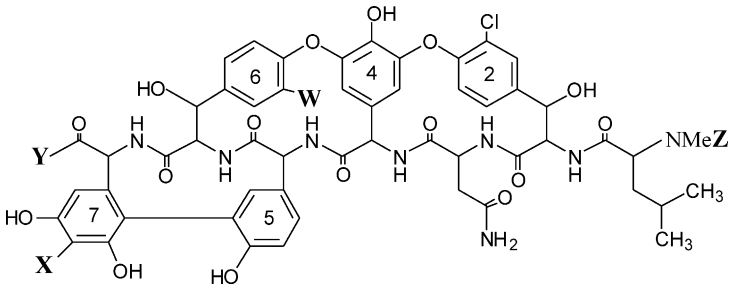
[a] Antiviral values in italics denotes EC_{50} values equal or lower than 10 μg/ml. An asterix after the compound code no. indicates a selectivity (CC_{50}/EC_{50}) of >10 for the compound against either FIPV and/or SARS-CoV.

separately calculated for the carbohydrate-containing antibiotics (Tables 1–4) and the aglycon antibiotics (Tables 5–9), *r*-values of 0.191 and 0.616, respectively, were obtained. Thus, the correlation was somewhat better when solely the lipophylic aglycon antibiotic derivatives were considered, but was still too low to consider the feline coronavirus as a reliable surrogate model to replace the hazardous SARS-CoV cell culture model in the design or discovery of novel active SARS-CoV compounds, at least within the structural class of glycopeptide antibiotics.

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. 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. Although we assume that the glycopeptide antibiotics, akin to their action against HIV, most likely interfere with the coronavirus entry process, it is known that both human and type II feline coronaviruses recognize a different cellular receptor to enter their target cells (i.e. angiotensin converting enzyme-2 (ACE-2) for SARS-CoV and feline aminopeptidase N for FIPV) (Li et al., 2003; Tresnan et al., 1996). Therefore, both viruses may obviously have different structural requirements for optimal interaction with the glycopeptide antibiotic derivatives.

The often rather narrow selectivity index (ratio CC_{50}/EC_{50}) of the glycopeptide antibiotics for SARS-CoV and FIPV, in contrast with HIV, does not exclude a cellular target rather than a specific antiviral target for these compounds. Indeed, the observation that the CRFK cells used in the FIPV assay are generally more sensitive to the toxic effects of the compounds than the Vero cells used in the SARS-CoV assay, and that the compounds were generally also endowed with lower EC_{50} values (more potent antiviral activity) against FIPV than SARS-CoV, may be in agreement of the latter hypothesis. The elucidation of the molecular basis of the interaction of the lipophylic glycopeptide antibiotics with their cellular or viral target is currently subject of further investigations in our laboratory and may lead to the rational design of more potent and specific anti-coronavirus

Table 5
Vancomycin type aglycons and their derivatives

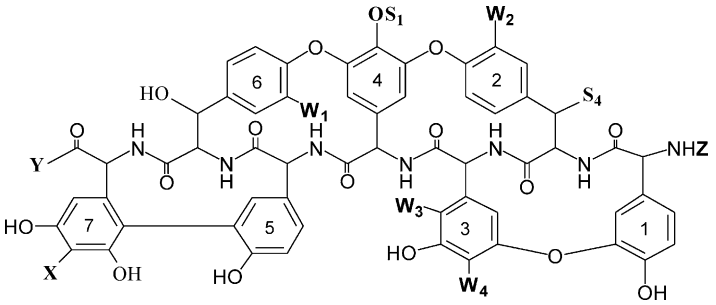


LCTA	Code no.	X	Y	Z	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
					EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
Vancomycin aglycon (VA) and its derivatives W = Cl										
890	**65**	H	OH	H	65	>500	>80	>80	>80	>80
1130	**66**	H	(1-Adam)CH_2NH	H	3.0 ± 0	NT	>20	68 ± 16	57 ± 12	>100
1131	**67**	H	(2-Adam)NH	H	3.0 ± 0	NT	24 ± 21	83 ± 24	51 ± 8	>100
1132	**68**	H	$H_2N(CH_2)_{10}NH$	H	2.5 ± 0.7	NT	>20	73 ± 29	26 ± 13	>100
Vancomycin aglycon hexapeptide (VAH) and its derivatives W = Cl, first amino acid (*N*-Me-D-Leu) is absent (=H)										
1147	**69**	H	OH	–	≥125	NT	>100	>100	>100	>100
1136	**70**	H	(1-Adam)CH_2NH	–	20.0 ± 7.1	NT	>20	≥100	>100	>100
Eremomycin aglycon (EA) and its derivatives W = H										
312	**71**	H	OH	H	50	>500	>80	>80	>80	>80
891	**72**	H	$NH(CH_2)_3N^+Me_2C_{10}H_{21}$	H	3.5 ± 0.7	57 ± 4	>16	52 ± 1	26 ± 6	>80
902	**73**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	OH	H	5.5 ± 0.7	>500	29 ± 2	>80	35 ± 2	>80
930	**74**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	OH	Boc	4.5 ± 0.7	>100	14 ± 12	>80	34 ± 8	>80
935	**75***[a]	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	NHMe	Boc	4.0 ± 0	>100	*3.6* ± 1.6[a]	>50	52 ± 20	>100
936	**76**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	NHMe	H	4.0 ± 1.7	>100	>80	>80	15 ± 2	>100
1060	**77**	H	(1-Adam)CH_2NH	H	1.6 ± 0.36	148 ± 3	23 ± 9	>100	32 ± 2	>100
1061	**78**	H	*p*-FBnNH	H	41.7 ± 20.2	>250	>80	>100	>100	>100
1062	**79**	H	(Perhydroiso-quinolin-1-yl)NH	H	63.3 ± 53.5	>250	46 ± 23	>100	>100	>100
1063	**80**	H	1,3-dicyclohexylureide	H	7.5 ± 4.8	>250	>80	>100	55 ± 9	>100
1133	**81**	H	(2-Adam)NH	H	8.5 ± 2.1	NT	48 ± 13	>100	59 ± 9	>100
1134	**82**	H	$H_2N(CH_2)_{10}NH$	H	8.5 ± 2.1	NT	>20	76 ± 25	29 ± 2	>100
Eremomycin aglycon hexapeptide (EAH) and its derivatives W = H, first amino acid (*N*-Me-D-Leu) is absent (=H)										
311	**83**	H	OH	D-Trp	7.3 ± 0.58	>250	28 ± 1	>100	>80	>100
964	**84**	H	OH	–	115 ± 21.2	>250	>80	>80	>80	>80
966	**85**	CH_2NHAdam-2	NHMe	–	13 ± 9.9	>250	25 ± 15	>50	>80	>80
1135	**86**	H	(2-Adam)NH	–	50.0 ± 0	NT	>100	>100	>100	>100
1138	**87**	H	$H_2N(CH_2)_{10}NH$	–	≥25	NT	>100	>100	72 ± 24	>100
1140	**88**	H	*p*-F-Ph-N$[CH_2CH_2]_2N$	–	12	NT	>20	≥100	55 ± 2	>100
De-Cl-eremomycin aglycon (De-Cl-EA) and its derivatives, W = H										
1139	**89**	H	OH	H	>125	NT	>100	>100	>100	>100
1141	**90**	H	(1-Adam)CH_2NH	H	8.5 ± 2.1	NT	>4	53 ± 33	46 ± 11	>100
1142	**91**	H	(2-Adam)NH	H	8.5 ± 2.1	NT	>20	79 ± 30	48 ± 0	>100
1143	**92**	H	$H_2N(CH_2)_{10}NH$	H	15.0 ± 0	NT	>20	≥100	60 ± 17	>100
De-Cl-eremomycin aglycon hexapeptide (De-Cl-EAH) and its derivatives W = H, first amino acid (*N*-Me-D-Leu) is absent (=H)										
1148	**93**	H	(1-Adam)CH_2NH	–	30.0 ± 7.1	NT	52 ± 9	≥100	>100	>100
1149	**94**	H	$H_2N(CH_2)_{10}NH$	–	>25	NT	>100	>100	78 ± 10	>100

[a] Antiviral values in italics denotes EC_{50} values equal or lower than 10 μg/ml. An asterix after the compound code no. indicates a selectivity (CC_{50}/EC_{50}) of >10 for the compound against either FIPV and/or SARS-CoV.

glycopeptide antibiotic derivatives. In a preliminary experiment, the teicoplanin glycopeptide antibiotic has been included in a "time-of-addition" experiment, in which the administration of the compound was delayed for several time periods after virus infection. A reference pyridine N-oxide compound known to inhibit the transcription process (Balzarini et al., 2006) was added as a control compound. Clearly, the addition of the glycopeptide antibiotic to the virus-infected cell cultures could be markedly less delayed after FIPV infection than the pyridine N-oxide compound (data not shown) to ascertain full antiviral

Table 6
Teicoplanin type aglycons and their derivatives



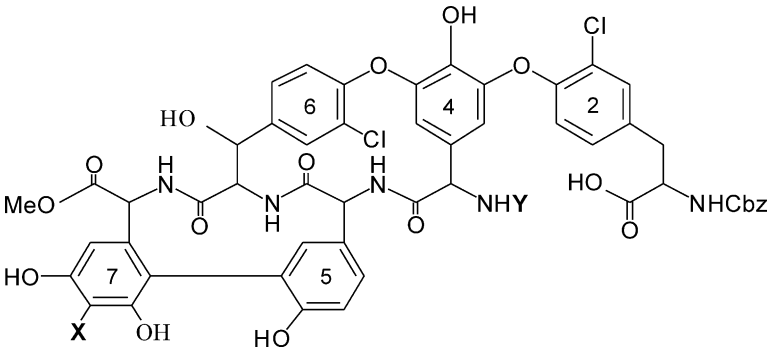
LCTA	Code no.	X	Y	Z	S_1	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
						EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
Ristomycin aglycon $W_1 = W_2 = W_3 = H$, $W_4 = Me$, $S_4 = OH$											
928	**95**	H	OMe	H	H	25 ± 7	>100	NT	NT	NT	NT
Aglycon DA40926 $W_1 = W_3 = Cl$, $W_4 = W_4 = H$, $S_4 = H$											
896	**96**	H	OH	Me	H	40 ± 14	>500	>80	>80	>80	>80
Teicoplanin aglycon (TD) and its derivatives $W_1 = W_2 = Cl$, $W_3 = W_4 = S_4 = H$											
874	**97**	H	OH	H	H	17	>500	37 ± 3	>80	47 ± 4	>100
330	**98**	$CH_2NH(CH_2)_4CH(NH_2)CONHC_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	H	3.5 ± 0.7	389 ± 99	>50	57 ± 7	>60	67 ± 4
335	**99**	$CH_2N[CH_2CH_2]_2N{=}NCHPhCl$-*p*	OH	H	H	8.0 ± 2.8	>100	>16	49 ± 3	34 ± 4	>80
345	**100**	$CH_2N(COLys)C_{10}H_{21}$	OH	H	H	3 ± 1.4	49 ± 10	>50	48 ± 7	26 ± 7	>100
346	**101**	$CH_2N(COLys)C_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	H	3.0 ± 1.4	140 ± 26	>50	52 ± 9	>70	70 ± 9
347	**102**	$CH_2NHC_{10}H_{21}(CH_2)_3NH_2$	$NH(CH_2)_3NH(CH_2)_3NH$	H	H	4 ± 0	57 ± 13	>20	18 ± 3	>50	52 ± 12
349	**103**	H	$NHC_{10}H_{21}$	H	H	2.6 ± 2	21 ± 0.2	>3.2	10 ± 0	11 ± 3	>80
350	**104**	$CH_2NMeBnPh$-*p*	OH	H	H	17	>100	32 ± 2	>80	>80	>80
354	**105**	$CH_2NH(CH_2)_4CH(NH_2)CONH(CH_2)_3NMe_2$	$NH(CH_2)_3NMe_2$	H	H	20	>100	>80	>80	34 ± 12	>80
355	**106**	$CH_2N[CH_2CH_2]_2NBnCl$-*p*	OH	H	H	17	>100	43 ± 18	>80	32 ± 1	>80
358	**107**	$CH_2NH(CH_2)_3NMe_2$	$NHC_{10}H_{21}$	H	H	4.5 ± 0.7	53 ± 11	28 ± 14	46 ± 6	31 ± 7	>100
360	**108**	CH_2NHAdam-2	$NH(CH_2)_3NMe_2$	H	H	2.5 ± 0.7	>500	11 ± 7	>50	18 ± 7	>80
394	**109**	$CH_2NHC_9H_{19}$	$NH(CH_2)_3NMe_2$	H	H	2.2 ± 0	179 ± 1	>70	72 ± 5	7.0 ± 0.2	>100
415	**110**	H	NHMe	H	H	15 ± 7	>500	>80	>80	64 ± 10	>100
433	**111**	$CH_2NHC_{10}H_{21}$	$NH(CH_2)_3NMe_2$	COLys	H	2	67 ± 30	>40	48 ± 14	>40	>40
563	**112**[a]	H	OH	p-PhBn	H	7.3 ± 2	44 ± 4	12 ± 3	29 ± 0	*8.2* ± 1.9[a]	47 ± 4
610	**113**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	$N[CH_2CH_2]_2NBnPh$-*p*	p-BuBn	H	>10	>100	>80	>80	>80	>80
621	**114**	H	$N[CH_2CH_2]_2NBnBu$-*p*	H	H	0.5 ± 0.2	11 ± 0.5	>20	29 ± 17	11 ± 4	>100
622	**115**[*a]	H	$N[CH_2CH_2]_2NBnOBu$-*p*	H	H	0.7	2.6 ± 0.2	*1.6* ± 0.3	14 ± 0	*8.0* ± 2.6	>100
636	**116**[*a]	H	$N[CH_2CH_2]_2NC_{10}H_2$	H	H	1.8 ± 0.5	8.1 ± 0.1	>3.2	8.6 ± 0.5	*7.0* ± 0.3	>80
645	**117**	H	$N[CH_2CH_2]_2NBnCH{=}CHPh$-p	H	H	1.5 ± 0.7	8.6 ± 0.6	>10	13 ± 2	16 ± 9	59 ± 9
646	**118**	H	$N[CH_2CH_2]_2N$-2-naphtyl	H	H	2.1 ± 1.3	113 ± 28	>50	50 ± 3	24 ± 3	>80
669	**119**	H	$NH(CH_2)_4CH(NHBnOBu$-*p*$)CONH(CH_2)_3NMe_2$	H	H	5.0 ± 1.4	228 ± 91	>50	59 ± 5	>100	>100
689	**120**[a]	H	$NH(CH_2)_3N^+Me_2C_{10}H_{21}$	H	H	1.5 ± 0.4	18 ± 3	23 ± 2.	52 ± 4	>80	>80
693	**121**	$CH_2N[CH_2CH_2]_2NBnPh$-*p*	$NH(CH_2)_3NMe_2$	H	H	3.5 ± 0.7	>500	>20	21 ± 17	11 ± 8	>100
694	**122**		$NH(CH_2)_3N^+Me_3$	H	H	4.5 ± 0.7	>250	>80	>80	51 ± 9	>80
715	**123**	H	$NH(CH_2)_3NMe_2$	$C_{11}H_{23}$	H	5.5 ± 2.1	90 ± 27	>40	53 ± 8	50 ± 5	77 ± 12
716	**124**	H	$NH(CH_2)_3NMe_2$	H	$C_{11}H_{23}$	>10	33 ± 7	>50	61 ± 4	>100	>100

Table 6 (*Continued*)

LCTA	Code no.	X	Y	Z	S_1	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
						EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
719	**125**[a]	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	$NH(CH_2)_3NMe_2$	H	H	3.0 ± 0	>500	9.2 ± 4.8	>50	22 ± 15	>80
720	**126**	$CH_2N[CH_2CH_2]_2NBnBu$-*p*	NHMe	H	H	1.7 ± 0.4	>500	62 ± 29	>80	19 ± 2/46 ± 7	>80
721	**127**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	OH	H	H	2.2 ± 0	74 ± 5	>16	58 = 1	48 ± 3	>80
722	**128**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	H	2.7 ± 1.8	50 ± 8	>16	49 ± 4	24 ± 7	>80
724	**129**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_2OH$	H	H	2.1 ± 0.1	100	>16	58 ± 1	>80	>80
725	**130**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_3N^+Me_2C_{10}H_{21}$	H	H	1.6 ± 0.6	9.4 ± 1.9	>80	78 ± 3	>80	>80
727	**131**	H	$NH(CH_2)_6NHBnBu$-*p*	H	H	12.5 ± 10	>250	>80	>80	>80	>80
796	**132**	H	OH	H	CH_2CH_2 NH_2	50 ± 28	>100	>80	>80	>80	>80
797	**133**	H	OH	p-BuOBn	CH_2CH_2 NHBnO Bu-*p*	6	14.3 ± 0.42	>50	50 ± 6	41 ± 4	>80
799	**134**	$CH_2N[CH_2CH_2]_2N^+C_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	H	2.1 ± 0.9	>100	>16	51 ± 1	37 ± 2	>80
817	**135**	H	$N[CH_2CH_2]_2NCOC_9H_{19}$	H	H	1.5 ± 0.7	44 ± 0.4	18 ± 9	46 ± 2	26 ± 7	>80
818	**136**	H	OMe	H	H	9.5 ± 7.8	248 ± 1	40 ± 27	>80	38 ± 1	>100
819	**137**	H	$NH(CH_2)_6NH_2$	H	H	15 ± 0	>500	21 ± 6	>50	32 ± 20	>100
820	**138**	$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_6NH_2$	H	H	1.8 ± 0.5	66 ± 2	>16	52 ± 1	11 ± 2	>80
853	**139**	$CH_2NHBnBu$-*p*	OH	H	H	17.5	>100	>40	46 ± 4	36 ± 2	>80
876	**140**	H	$NH(CH_2)_{10}NH_2$	H	H	6.5 ± 0.7	402 ± 138	>80	>80	54 ± 16	>100
877	**141**[a]	H‘	$NHBnNBu_2$-*p*	H	H	5	37.6	9.2 ± 1.1	32 ± 15	21 ± 10	>100
899	**142**	CH_2NHMe	OH	H	H	50 ± 28	>500	>80	>80	>80	>80
901	**143**	CH_2NHMe	NHMe	H	H	15 ± 7.1	>500	>80	>80	35 ± 7	>80
914	**144**[a]	$CH_2N[CH_2CH_2]_2NCOC_9H_{19}$	OH	H	H	6	30.9 ± 1.1	6.1 ± 0	47 ± 2	35 ± 2	>80
916	**145**	H	$N[CH_2CH_2]_2NCOCH_2$ NHBnBu-*p*	H	H	4 ± 0	>100	40 ± 4	>80	>80	>80
917	**146**	$CH_2N[CH_2CH_2]_2NCOCH_2$ NHBnBu-*p*	NHMe	H	H	15 ± 7	>100	>70	71 ± 4	38 ± 1	>80
918	**147**	$CH_2N[CH_2CH_2]_2NCOC_9H_{19}$	NHMe	H	H	15 ± 7	>100	38 ± 10	>80	>80	>80
932	**148**	$CH_2NHBnBu$-*p*	OH	Boc	H	20	59.7	>40	43 ± 3	42 ± 10	>80
933	**149**	$CH_2NHBnBu$-*p*	NHMe	Boc	H	6 ± 1	>100	22 ± 13	>80	>80	>80
934	**150**	$CH_2NHBnBu$-*p*	NHMe	H	H	9.7 ± 9	>100	44 ± 15	>80	>80	>80
945	**151**	H	OH	Boc	H	13 ± 10	>250	>16	51 ± 7	35 ± 7	>80
946	**152**	H	OH	Fmoc	H	17.5 ± 3	114 ± 1	>60	74 ± 8	32 ± 5	>80
947	**153**[*]	H	OH	Adoc	H	13 ± 9.9	104	>10	11 ± 1	7.3 ± 1	>80
948	**154**	H	OH	Cbz	H	12.5 ± 3	>250	>16	54 ± 4	18 ± 12	>80
949	**155**	H	NHAdam-2	Boc	H	>10	72 ± 6	>3.2	11 ± 1	18 ± 11	>80
950	**156**	H	NHMe	Boc	H	13.5 ± 9	229 ± 30	25 ± 4	48 ± 6	33 ± 6	>80
952	**157**[*a]	H	OH	C(S)NHPh	H	6 ± 1.4	220 ± 43	8.5 ± 1.4	47 ± 7	8.0 ± 0.3	>80
953	**158**[a]	H	NHAdam-2	H	H	7.0 ± 4.2	123	5.2 ± 1.2	44 ± 6	20 ± 7	>80
954	**159**	$CH_2NHAdam$-2	OH	H	H	25	>250	53 ± 9	>80	>80	>80
955	**160**	$CH_2NHAdam$-2	NHMe	H	H	5.0 ± 1.4	>250	15 ± 1	93 ± 10	34 ± 7	>80
956	**161**	$CH_2NHC_{12}H_{25}$	OH	H	H	>2	5.6 ± 1	>10	9.7 ± 1.6	12 ± 1	37 ± 13
957	**162**	$CH_2NHC_{12}H_{25}$	NHMe	H	H	>10	12 ± 2	>10	11 ± 0	>30	37 ± 3
958	**163**	$CH_2NHC_{18}H_{37}$	OH	H	H	4.7 ± 0.5	22 ± 1	>3.2	10 ± 1	5.4 ± 3.1	37 ± 13
959	**164**	$CH_2NHC_{18}H_{37}$	NHMe	H	H	4.5 ± 0.7	60 ± 1	>16	36 ± 22	20 ± 0	>80
960	**165**	$CH_2NHAdam$-2	NHAdam-2	H	H	2.5 ± 0.7	>250	14 ± 6	>50	>80	>80
1011	**166**[*a]	H	(Perhydroiso-quinolin-1-yl) NH	H	H	1.8	>250	9.4 ± 0.7	>100	16 ± 6	>100
1012	**167**[*a]	H	(2-exo-norbornyl)NH	H	H	4.5	>250	4.7 ± 0	94 ± 8	21 ± 5	>100
1013	**168**[*a]	H	OH	(glyoxalyl-indol-3-yl)	H	10	108	2.2 ± 1.4	63 ± 0	17 ± 13	>100
1014	**169**[*a]	H	OH	1-adamantoyl	H	10	83	>10	15 ± 5	7.4 ± 0.2	>100
1051	**170**[a]	H	(1-Adam)CH_2NH	H	H	1.8 ± 0.6	125	8.5 ± 2.1	49 ± 12	24 ± 9	>100
1064	**171**[a]	H	1,3-dicyclohexylureide	H	H	6.0 ± 2.6	165	7.7 ± 0.05	62 ± 3	39 ± 14	>100

[a] Antiviral values in italics denotes EC_{50} values equal or lower than 10 μg/ml. An asterix after the compound code no. indicates a selectivity (CC_{50}/EC_{50}) of >10 for the compound against either FIPV and/or SARS-CoV.

Table 7
Teicoplanin aglycon derivatives with eliminated amino acids 1 and 3

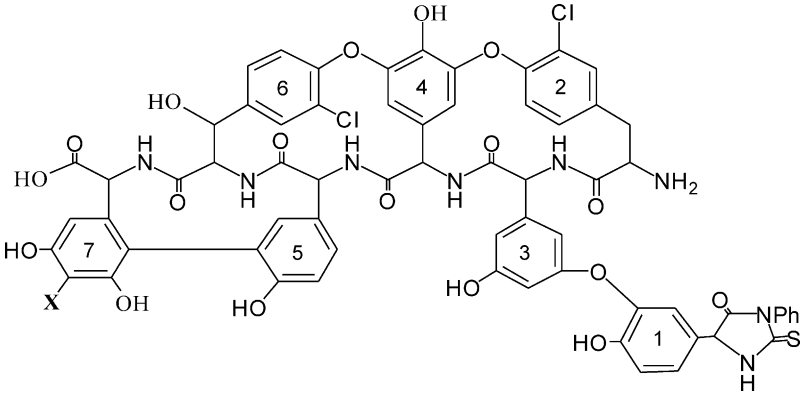


LCTA	Code no.	X	Y	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
				EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
913	**172**	H	H	25	>500	>80	>80	>80	>80
961	**173**	H	Boc	17.5	>250	23 ± 8	>80	43 ± 5	>80
962	**174**	CH_2NHAdam-2	Boc	17 ± 3	240 ± 13	35 ± 30	>80	61 ± 45	>80
963	**175**	CH_2NHAdam-2	H	17 ± 3	>250	19 ± 0	>50	>80	>80

activity of the compound. These data point to inhibition of a much earlier event in the infection cycle of the virus than targeted by the pyridine N-oxide derivative, and may be suggestive for inhibition of the viral entry process as also shown to be the target of inhibition of HIV infection (Balzarini et al., 2003).

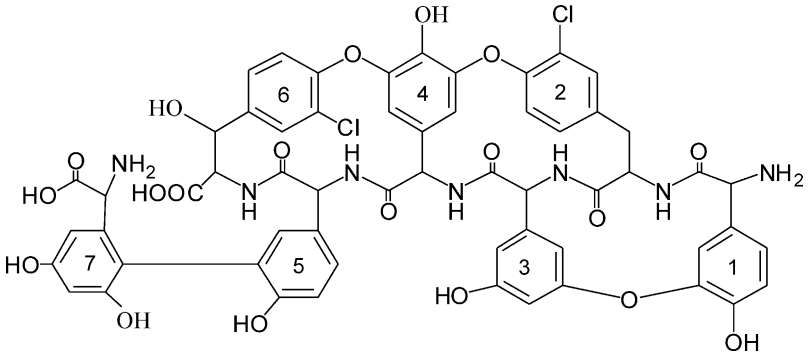
The most antivirally active lipophylic glycopeptide analogues have EC_{50} values between 3 and 5 μM against coronaviruses. This is in the same range as the minimum inhibitory concentration (MIC) at which vancomycin and teicoplanin are inhibitory to *Staphylococcus aureus*. Several lipophilic glycopeptides have been given to humans (oritavancin, telavancin) without acute side effects, and it is therefore possible that a therapeutic agent based on the lipophilic glycopeptide structure described in this study could become a useful therapeutic agent. Recently it was demonstrated that adamantyl-2 amide of eremomycin (AN0900) is effective in a vegetative anthrax intravascular infection in BALB/c mouse model, and implies excellent deep tissue penetration. Pharmacokinetic parameters of AN0900 obtained after single dose intravenous administration to mice showed that AN0900 had long half-life (185 min), high tissue levels (Vss 26285 ml/kg) and deep tissue penetration (lung, spleen) in comparison with vancomycin. AN0900 completely protects mice in a mouse model of inhalational anthrax at doses as low as 10 mg/kg when given subcutaneously (Maples et al., 2005). However, given the limited selectivity index seen for most of the glycopeptide antibiotics included in this study, it is felt that further improvement of potency and/or selectivity needs to be made before a clinical candidate lead compound can be put for-

Table 8
Teicoplanin aglycon derivatives with the disrupted bond between amino acids 1 and 2



LCTA	Code no.	X	HIV-1 (CEM)		FIPV (CRFK)		SARS (Vero)	
			IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)
968	**176**	H	15	>250	48 ± 46	>80	40 ± 5	>80
969	**177**	CH_2NHAdam-2	13 ± 9.9	242 ± 11	22 ± 3	>80	45 ± 13	>80

Table 9
Teicoplanin aglycon with the disrupted bond between amino acids 6 and 7



LCTA	Code no.	HIV-1 (CEM)		FIPV (CRFK)		SARS-CoV (Vero)	
		IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)
970	**178**	22.5	>250	57 ± 37	>80	>80	>80

Adam-1 = adamant-1-yl, Adam-2 = adamant-2-yl.

ward. Our structure-activity relationship study may be helpful to design such novel compounds.

It is important to discover new glycopeptide derivatives that are endowed with potent and selective antiviral activity but lack antibacterial activity. In fact, whereas the introduction of a hydrophobic substituent is beneficial for both antiviral and antibacterial activities, the lack of the sugar moieties in the glycopeptide molecules is often detrimental for antibacterial activity, although several hydrophobic derivatives of eremomycin and teicoplanin aglycons are known to exhibit good antibacterial activity (Printsevskaya et al., 2003). However, the fact that the antibacterial activity of the glycopeptide derivatives evaluated in this study is mainly based on their ability to inhibit the bacterial cell wall biosynthesis by a reversible non-covalent binding of the drugs to the D-Ala-D-Ala fragment of the prokaryotic peptidoglycan cell wall precursor (Walsh, 1993), an efficient dissection must be able between antiviral and antibacterial activity, since the molecular target (peptidoglycan synthesis) for antibacterial activity is entirely absent in viruses and mammalian cells.

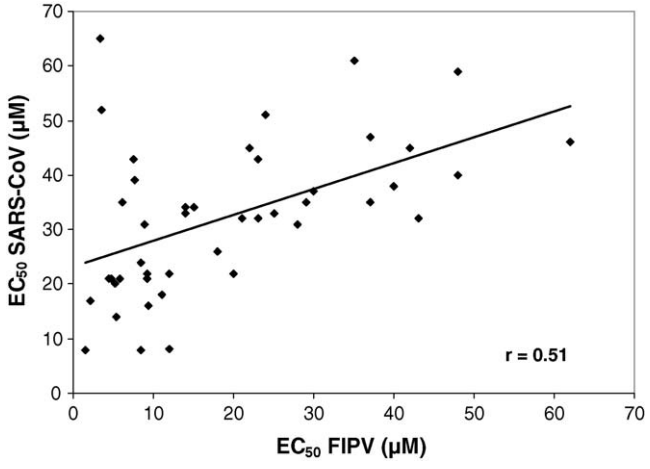


Fig. 1. Correlation between the 50% effective concentrations (EC_{50}) of glycopeptide antibiotic derivatives against FIPV in CRFK cell cultures and SARS-CoV in Vero cell cultures. Only those compounds have been taken into account for which exact EC_{50} values against both viruses could be determined. Data were taken from Tables 1–9.

5. Conclusion

Several semisynthetic, lipophylic glycon and aglycon derivatives of glycopeptide antibiotics with selective anti-coronavirus activity in cell culture have been described in this study. Some of the compounds inhibited virus infection in the lower micromolar range without measurable cytotoxicity at 80–100 μM. Although the molecular mechanism of anti-HIV and anti-FIPV action is likely to be the viral entry process, no close correlation could be established between the activity of the compounds against HIV-1 and both coronaviruses, or between their activity against SARS-CoV and the FIPV. It would appear, therefore, that the FIPV model is not an adequate surrogate model for detecting specific anti-SARS coronavirus inhibitors within the structural class of glycopeptide antibiotics.

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