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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\textsubscript{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\textsubscript{50} values of the glycopeptide derivatives for FIPV or SARS-CoV.

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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-
glycopeptide 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 by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankfurt, Germany. Vero E6 cells were propagated by Prof. Dr. H.F. Rabenau from the Johann Wolfgang Goethe University, Frankie...
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 Maffioli 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 CCID50 (50% cell culture infective dose) of SARS-CoV (Keyaerts et al., 2004), and CRFK cells that had been infected (or mock-infected) with 100 CCID50 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 (CC50)) and the antivirally effective concentration was determined as the compound concentration that suppressed the viral cytopathic effect by 50% (50% effective concentration (EC50)). An EC50 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 CC50 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-deacetyl 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 (IIIb) 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 (EC50). 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 (CC50) and 50% cytostatic concentrations (IC50), respectively. EC50 values for FIPV and SARS-CoV that were ≤10 μM are printed in bold. The compounds endowed with a selectivity index (ratio CC50/EC50) >10 have an asterisk 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 cell cultures nor inhibitory to SARS-CoV and FIPV (EC50 >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 EC50 values (ranging between 20 and 45 μM) for both viruses, whereas 13 and 15 showed more pronounced activity against FIPV (EC50: 3.4–8.9 μM) but lesser activity against SARS-CoV (EC50: 31–65 μM) (Table 1). Compounds 39 and 27 had the highest activity against both viruses (EC50: 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) EC₅₀ (µM) | HIV-1 (CEM) IC₅₀ (µM) | FIPV (CRFK) EC₅₀ (µM) | FIPV (CRFK) IC₅₀ (µM) | SARS-CoV (Vero) EC₅₀ (µM) | SARS-CoV (Vero) CC₅₀ (µM) |
|---------------|-------|---------|-----------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Vancomycin (Van) and its derivatives W = Cl, S₁ = Glc, S₂ = vancosamine, S₃ = H |
| 878           | 1     | H       | OH              | >250                   | >500                   | >100                   | >100                   | >100                   | >100                   |
| 854           | 2     | H       | NHCl₀H₂        | >10                    | 30 ± 2                 | >50                    | 50 ± 1                 | >80                    | >80                    |
| 892           | 3     | H       | NH(CHOH)CH₂Me₂Cl₁₀H₂1 | 5.5 ± 0.7      | 172 ± 15               | >80                    | >80                    | 57 ± 14                | >80                    |
| 893           | 4     | H       | NHMe            | >250                   | >500                   | >80                    | >80                    | >80                    | >80                    |
| 941           | 5     | CH₂N(CHOH)₂[NHMe] | OH              | 12 ± 3.5               | >100                   | 30 ± 12                | ≥50                    | 37 ± 2                 | >100                   |
| 1002          | 6     | H       | OH              | BnPhCl₃-p               | NTₚ                     | NT                     | 20                     | 61                     | 22 ± 4                 | >100                   |
| Eremomycin (Ere) and its derivatives W = H, S₁ = Glc, S₂ = S₃ = eremosamine |
| 516           | 7     | H       | OH              | >250                   | >500                   | >100                   | >100                   | >100                   | >100                   |
| 177           | 8     | H       | CH₃(CH₂)₃O     | NT                     | NT                     | NT                     | >80                    | >80                    | >80                    |
| 200           | 9     | H       | CH₃(CH₂)₃(OH) | NT                     | NT                     | 16 ± 4                 | >80                    | 27 ± 4                 | >80                    |
| 261           | 10    | H       | NHMe            | >250                   | >500                   | >80                    | >80                    | >80                    | >80                    |
| 284           | 11    | CH₂NHCl₁₀H₂1 | OH              | >20                    | 24 ± 13                | >16                    | 44 ± 3                 | >40                    | 54 ± 22                |
| 288           | 12    | CH₂NMεCH₂ | OH              | >250                   | >250                   | >80                    | >80                    | >80                    | >80                    |

Footnotes:
- NTₚ: Not tested
- ε: 5 µM
Table 1 (Continued)

| LCTA Code no. | Z     | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|---------------|-------|-------------|-------------|-----------------|
|               |       | EC$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) | EC$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) | EC$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) |
| 827           | 31    | H           | NH(CH$_2$)$_6$NH$_2$ | 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           | NHBNH(CH$_2$)$_2$-p | H | >100 | >100 | >80 | >80 | >80 | >80 |
| 832           | 34    | H           | NHBNHNC$_6$H$_{12}$-p | H | >4   | 5 ± 0.5 | >16 | 21 ± 8 | 22 ± 1 | >80 |
| 833           | 35    | H           | NHBNN'NMe$_2$C$_6$H$_{12}$-p | H | >20  | 19 ± 8 | 14 ± 7 | 41 ± 5 | >40 | 46 ± 0 |
| 834           | 36    | H           | NHCH$_2$CH$_3$N'NMe$_2$C$_6$H$_{12}$-p | H | >20  | 35 ± 2 | >16 | 72 ± 9 | >80 | >80 |
| 837           | 37    | H           | NHBNPhCl-p           | H | >10  | 8.6 ± 0.2 | >20 | 27 ± 8 | >30 | 24 ± 8 |
| 838           | 38    | H           | NHBNPh-p             | H | >10  | 35 ± 1 | >50 | 53 ± 4 | 31 ± 8 | >100 |
| 847           | 39    | CH$_2$NHBNPhCl-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           | NHNC$_7$H$_{15}$     | H | >50  | 182 ± 013 | >80 | >80 | 60 ± 19 | >100 |
| 869           | 42    | H           | NHBNBu$_2$           | H | >50  | 30 ± 2 | >40 | 49 ± 8 | 35 ± 19 | >100 |
| 921           | 43    | CH$_2$N[(CH$_2$)$_2$]$_2$ | OH | H | >10  | 63 ± 29 | 42 | >80 | 45 ± 11 | >80 |
| 923           | 44    | H           | NH[(CH$_2$)$_2$]$_2$NCH$_2$OH | H | >50  | >100 | >60 | 66 ± 12 | 31 ± 7 | >100 |
| 972           | 45    | H           | NHCH$_2$[(CH$_2$)$_4$NH$_2$]CONHBnBu-p | H | >50  | 104 ± 11 | >16 | 73 ± 7 | >80 | >80 |

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$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) | EC$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) | EC$_{50}$ ($\mu$M) | IC$_{50}$ ($\mu$M) |
| Vancomycin (Van) and its derivatives W = Cl, S$_1$ = Glc, S$_2$ = vancosamine, S$_3$ = H | 222   | 46 NO | NT* | 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$_3$CH$_2$)$_2$ | NT | NT | >80 | >80 | >80 | >80 |
|                                                  | 246   | 49 Chz | NT | NT | >80 | >80 | >80 | >80 |

* NT, not tested.
momycin aglycon derivatives emerged (Table 5). The most active compound in this series against FIPV was the eremomycin aglycon derivative 75 (EC50: 3.6 μM) being non-toxic at 100 μM. It was 15-fold less potent (EC50: 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 EC50 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 (EC50: 19–48 μM), and no visible cytotoxicity was noted at 80 μM. However, given the relatively high EC50 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 (EC50 <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 EC50 values of the compounds against both coronaviruses (Fig. 1). Indeed, when all compounds for which a correct EC50 value could be determined were taken into account, a r-value of 0.51 was calculated for the EC50 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 \, Y¹ = Y² | Z \, Z¹ | Z²     | HIV-1 (CEM) EC₅₀ (µM) | HIV-1 (CEM) IC₅₀ (µM) | FIPV (CRFK) EC₅₀ (µM) | FIPV (CRFK) IC₅₀ (µM) | SARS-CoV (Vero) EC₅₀ (µM) | SARS-CoV (Vero) IC₅₀ (µM) |
|---------------|-------|-------------|--------|--------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| DA40926 and its derivatives S = Man |
| 519           | 55    | H           | OH     | H      | >250                 | >500                  | >80                  | >80                  | >80                  | >80                  |
| 700           | 56    | H           | NH(CH₂)₃N⁺ | H      | 4.0 ± 1.4            | 32 ± 5                | >50                  | 63 ± 3               | >80                  | >80                  |
| DMDA40926 and its derivatives S = H |
| 599           | 57    | H           | OH     | H      | 115 ± 21             | >500                  | >80                  | >80                  | >80                  | >80                  |
| 604           | 58    | H           | NH(CH₂)₃NMe₂ | p-BuOBn | 5.0 ± 0.7            | 80 ± 6                | >50                  | 53 ± 7               | >80                  | >80                  |
| 605           | 59    | H           | NH(CH₂)₃NMe₂ | H      | 12 ± 3.5             | >250                  | >60                  | 63 ± 3               | 58 ± 2               | >80                  |
| 613           | 60*   | CH₂[N(CH₂)₂]₂NMe₂ | OH     | H      | 3.5                  | 81                    | 4.5 ± 0.4₃           | 50 ± 0               | 21 ± 7               | >80                  |
| 614           | 61*   | CH₂[N(CH₂)₂]₂NMe₂ | NH(CH₂)₃NMe₂ | H      | 3.5 ± 2.1            | 212 ± 54              | 5.9 ± 1.5            | 30 ± 9               | 21 ± 5               | >80                  |
| 737           | 62*   | CH₂[N(CH₂)₂]₂NMe₂ | OH     | H      | 20 ± 7               | 106 ± 2               | 7.5 ± 1.8            | >80                  | 43 ± 6               | >80                  |
| 738           | 63    | CH₂[N(CH₂)₃]₂NMe₂ | OH     | H      | 3.5                  | 92                    | 23 ± 17              | >80                  | >80                  | >80                  |
| 740           | 64    | CH₂[N(CH₂)₃]₂NMe₂ | NH(CH₂)₃NMe₂ | H      | 3.5 ± 0.7            | 92 ± 5                | >50                  | 55 ± 2               | 52 ± 2               | >80                  |

*Antiviral values in italics denote EC₅₀ values equal or lower than 10 µg/ml. An asterix after the compound code no. indicates a selectivity (CC₅₀/EC₅₀) 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 CC₅₀/EC₅₀) 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 EC₅₀ 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...
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 activity. 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 activity.

Table 5
Vancomycin type aglycons and their derivatives

| LCTA | Code no. | X | Y | Z | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|------|----------|---|---|---|-------------|-------------|----------------|
|      |          | EC50 (µM) | IC50 (µM) | EC50 (µM) | CC50 (µM) | EC50 (µM) | CC50 (µM) |
| 890  | 65       | H | OH | H | 65 >500 | >80 >80 >80 >80 |
| 1130 | 66       | H | (1-Adam)CH2NH | 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 | H2N(CH2)10NH | H | 2.5 ± 0.7 NT | >20 73 ± 29 26 ± 13 >100 |
| 1114 | 69       | H | OH | – | ≥125 NT | >100 >100 >100 >100 |
| 1136 | 70       | H | (1-Adam)CH2NH | – | 20.0 ± 7.1 NT | >20 >100 >100 >100 |

Vancomycin aglycon hexapeptide (VAH) and its derivatives W = Cl, first amino acid (N-Me-n-Leu) is absent (=H)

| LCTA | Code no. | X | Y | Z | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|------|----------|---|---|---|-------------|-------------|----------------|
|      |          | EC50 (µM) | IC50 (µM) | EC50 (µM) | CC50 (µM) | EC50 (µM) | CC50 (µM) |
| 1060 | 77       | H | (1-Adam)CH2NH | 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 | H2N(CH2)10NH | 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-n-Leu) is absent (=H)

| LCTA | Code no. | X | Y | Z | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|------|----------|---|---|---|-------------|-------------|----------------|
|      |          | EC50 (µM) | IC50 (µM) | EC50 (µM) | CC50 (µM) | EC50 (µM) | CC50 (µM) |
| 311  | 83       | H | OH | – | n-Trp 7.3 ± 0.58 >250 | 28 ± 1 >100 >80 >100 |
| 964  | 84       | H | OH | – | 115 ± 21.2 >250 | >80 >100 >80 >100 |
| 965  | 85       | CH2NHAdam-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 | H2N(CH2)10NH | – | ≥25 NT | >100 >100 72 ± 24 >100 |
| 1140 | 88       | H | p-F-Ph-N[CH2CH2]2N | – | 12 NT | >20 >100 55 ± 2 >100 |

De-Cl-eremomycin aglycon (De-Cl-EA) and its derivatives, W = H

| LCTA | Code no. | X | Y | Z | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|------|----------|---|---|---|-------------|-------------|----------------|
|      |          | EC50 (µM) | IC50 (µM) | EC50 (µM) | CC50 (µM) | EC50 (µM) | CC50 (µM) |
| 1139 | 89       | H | OH | H | ≥125 NT | >100 >100 >100 >100 |
| 1141 | 90       | H | (1-Adam)CH2NH | 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 | H2N(CH2)10NH | 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-n-Leu) is absent (=H)

| LCTA | Code no. | X | Y | Z | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|------|----------|---|---|---|-------------|-------------|----------------|
|      |          | EC50 (µM) | IC50 (µM) | EC50 (µM) | CC50 (µM) | EC50 (µM) | CC50 (µM) |
| 1148 | 93       | H | (1-Adam)CH2NH | – | 30.0 ± 7.1 NT | ≥100 >100 >100 >100 |
| 1149 | 94       | H | H2N(CH2)10NH | – | ≥25 NT | >100 >100 78 ± 10 >100 |

a Antiviral values in italics denotes EC50 values equal or lower than 10 µg/ml. An asterix after the compound code no. indicates a selectivity (CC50/EC50) of >10 for the compound against either FIPV and/or SARS-CoV.
Table 6
Teicoplanin type aglycons and their derivatives

| LCTA Code no. | X    | Y    | Z    | S1   | HIV-1 (CEM) EC50 (µM) IC50 (µM) | FIPV (CRFK) EC50 (µM) CC50 (µM) | SARS-CoV (Vero) EC50 (µM) CC50 (µM) |
|--------------|------|------|------|------|---------------------------------|---------------------------------|---------------------------------|
| 928          | 95   | H    | H    | H    | 25 ± 7                          | >100                            | NT                              | NT                              |
| 896          | 96   | H    | OH   | Me   | 40 ± 14                         | >500                            | 80 >80                          | >80 >80                          |
| 874          | 97   | H    | OH   | H    | 17 >500                         | 37 ± 3                          | >80                             | 47 ± 4                           |
| 330          | 98   | CH₂NH(CH₂)₂CH(NH₂) | NH(CH₂)₂NMe₂ | H    | 3.5 ± 0.7                      | 389 ± 99                       | >50                             | 57 ± 7                           |
| 335          | 99   | CH₂N(COLys)C₆H₁₂ | OH   | H    | 8.2 ± 2.8                      | >100                            | 16 >50                          | 49 ± 3                           |
| 345          | 100  | CH₂N(COLys)C₆H₁₂ | OH   | H    | 3 ± 1.4                        | 49 ± 10                         | >50                             | 48 ± 7                           |
| 346          | 103  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 3.0 ± 1.4                      | 140 ± 26                       | >50                             | 52 ± 9                           |
| 347          | 92   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | NH   | 4 ± 0.0                        | 57 ± 13                         | >20                             | 18 ± 3                           |
| 348          | 93   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 2.6 ± 2                        | 21 ± 0.2                       | >3.2                            | 10 ± 0                           |
| 350          | 94   | CH₂NMeBnCl-p | OH   | H    | 17 >500                         | 32 ± 2                          | >80                             | >80 >80                          |
| 354          | 95   | CH₂NMeBnCl-p | NH(CH₂)₂NMe₂ | H    | 20 >100                         | >80                             | >80 >80                          |
| 355          | 96   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 17 >500                         | 43 ± 18                         | >80                             | 32 ± 1                           |
| 358          | 97   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 4.5 ± 0.7                      | 53 ± 11                         | 28 ± 14                         | 46 ± 6                           |
| 360          | 98   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 2.5 ± 0.7                      | 500                            | 11 ± 7                          | 50 ± 8                           |
| 368          | 99   | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 2.2 ± 0.0                      | 179 ± 20                       | >70                             | 72 ± 5                           |
| 363          | 100  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 15 ± 7                         | >500                            | >80                             | 64 ± 10                          |
| 343          | 101  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | COLys | 2                            | 67 ± 30                         | 40                             | 48 ± 14                          |
| 563          | 102  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 7.3 ± 2                        | 44 ± 4                          | 12 ± 3                          | 29 ± 0                           |
| 610          | 103  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | p-PhBn | >10                            | >100                            | >80                             | >80 >80                          |
| 621          | 104  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 0.5 ± 0.2                      | 11 ± 0.5                        | >20                             | 29 ± 17                          |
| 622          | 105  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 0.2 ± 0.2                      | 2.6 ± 0.2                       | 1.6 ± 0.3                       | 14 ± 0                           |
| 636          | 106  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 1.8 ± 0.5                      | 8.1 ± 0.1                       | >3.2                            | 8.6 ± 0.5                        |
| 645          | 107  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 1.5 ± 0.7                      | 8.6 ± 0.6                       | >10                            | 13 ± 2                           |
| 646          | 108  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 2.1 ± 1.3                      | 113 ± 28                       | >50                             | 50 ± 3                           |
| 669          | 109  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 5.0 ± 1.4                      | 228 ± 91                       | >50                             | 59 ± 5                           |
| 689          | 110  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 0.5 ± 0.2                      | 2.6 ± 0.2                       | 1.6 ± 0.3                       | 14 ± 0                           |
| 715          | 111  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 1.5 ± 0.4                      | 18 ± 3                          | 23 ± 2                          | 52 ± 4                           |
| 716          | 112  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 3.5 ± 0.7                      | >500                            | 20                             | 21 ± 17                          |
| 717          | 113  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 4.5 ± 0.7                      | >250                            | >80                             | 51 ± 9                           |
| 718          | 114  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 5.5 ± 2.1                      | 90 ± 27                         | >40                             | 53 ± 8                           |
| 719          | 115  | CH₂N(COLys)C₆H₁₂ | NH(CH₂)₂NMe₂ | H    | 10 >100                        | 33 ± 7                          | >50                             | 61 ± 4                           |
| LCCTA | Code no. | X | Y | Z | $S_1$ | HIV-1 (CEM) | FIPV (CRFK) | SARS-CoV (Vero) |
|-------|----------|---|---|---|------|-------------|-------------|----------------|
|       |          |   | NH(CH$_2$)$_3$NM$e_2$ | H | H | 3.0 ± 0.0 | ≥500 | 9.2 ± 4.8 | ≥15 | >80 |
|       |          |   | NHMe | H | H | 1.7 ± 0.4 | ≥500 | 62 ± 29 | ≥80 | 19 ± 2.4 | 7 ± 6 | >80 |
|       |          |   | CH$_2$NH(CH$_2$)$_3$NM$e_2$C$_{10}$H$_{21}$ | OH | H | 2.2 ± 0.0 | 74 ± 5 | >16 | 58 ± 1 | 48 ± 3 | >80 |
|       |          |   | NH(CH$_2$)$_3$NM$e_2$C$_{10}$H$_{21}$ | H | H | 2.7 ± 1.8 | 50 ± 8 | >16 | 49 ± 4 | 24 ± 7 | >80 |
|       |          |   | NH(CH$_2$)$_3$OH | H | H | 2.1 ± 0.1 | 100 | >16 | 58 ± 1 | >80 | >80 |
|       |          |   | NH(CH$_2$)$_3$NM$e_2$C$_{10}$H$_{21}$ | H | H | 1.6 ± 0.6 | 9.4 ± 1.9 | >80 | 78 ± 3 | >80 | >80 |
|       |          |   | NH(CH$_2$)$_3$NM$e_2$C$_{10}$H$_{21}$ | H | H | 12.5 ± 10 | >250 | >80 | >80 | >80 | >80 |
|       |          |   | CH$_2$NHMe | H | H | 50 ± 28 | >100 | >80 | >80 | >80 | >80 |
|       |          |   | OH | p-BuOBn | H | 6 | 14.3 ± 0.42 | >50 | 50 ± 6 | 41 ± 4 | >80 |
|       |          |   | CH$_2$NHMe | NHMe | H | 2.1 ± 0.9 | >100 | >16 | 51 ± 1 | 37 ± 2 | >80 |
|       |          |   | CH$_2$NHMe | NHMe | H | 1.5 ± 0.7 | 44 ± 0.4 | 18 ± 9 | 46 ± 2 | 26 ± 7 | >80 |
|       |          |   | NH($CH_2$)$_3$NCO$_{2}$C$_{10}$H$_{19}$ | H | H | 9.5 ± 7.8 | 248 ± 1 | 40 ± 27 | >80 | 38 ± 1 | >100 |
|       |          |   | NH($CH_2$)$_3$NM$e_2$ | H | H | 15 ± 0.0 | >500 | 21 ± 6 | >50 | 32 ± 20 | >100 |
|       |          |   | CH$_2$NHMe | OH | H | 1.8 ± 0.5 | 66 ± 2 | >16 | 52 ± 1 | 11 ± 2 | >80 |
|       |          |   | CH$_2$NHMe | OH | H | 17.5 | >100 | >40 | 46 ± 4 | 36 ± 2 | >80 |
|       |          |   | CH$_2$NHMe | OH | H | 6.5 ± 0.7 | 402 ± 138 | >80 | >80 | 54 ± 16 | >100 |
|       |          |   | H$^+$ | NH$_2$N$Bu_2$-p | H | H | 5 | 37.6 | 9.2 ± 1.1 | 32 ± 15 | 21 ± 10 | >100 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 50 ± 28 | >500 | >80 | >80 | >80 | >80 |
|       |          |   | CH$_2$NHMe | OH | H | 15 ± 7.1 | >500 | >80 | >80 | >80 | >80 |
|       |          |   | CH$_2$NHMe | OH | H | 5 | 30.9 ± 1.1 | 6.1 ± 0 | 47 ± 2 | 35 ± 2 | >80 |
|       |          |   | H | NH$_2$N$Bu_2$-p | H | H | 4 ± 0 | >100 | 40 ± 4 | >80 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 15 ± 7 | >100 | >70 | 71 ± 4 | 38 ± 1 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 20 ± 5 | 59.7 | >40 | 43 ± 3 | 42 ± 10 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 6 ± 1 | >100 | 22 ± 13 | >80 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 9.7 ± 9 | >100 | 44 ± 15 | >80 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 13 ± 10 | >250 | >16 | 51 ± 7 | 35 ± 7 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 17.5 ± 3 | 114 ± 1 | >60 | 74 ± 8 | 32 ± 5 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 13 ± 9.9 | 104 | >10 | 11 ± 1 | 7.3 ± 1 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 12.5 ± 3 | >250 | >16 | 54 ± 4 | 18 ± 12 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 72 ± 6 | >3.2 | 11 ± 1 | 18 ± 11 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 13.5 ± 9 | 229 ± 30 | 25 ± 4 | 48 ± 6 | 33 ± 6 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 6 ± 1.4 | 220 ± 43 | 8.5 ± 4 | 47 ± 7 | 8.0 ± 0.3 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 7.0 ± 4.2 | 123 | 5.2 ± 2 | 44 ± 6 | 20 ± 7 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 25 ± 8 | >250 | 53 ± 9 | >80 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 5.0 ± 1.4 | >250 | 15 ± 1 | 93 ± 10 | 34 ± 7 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | >2 | 5.6 ± 1 | >10 | 9.7 ± 1.6 | 12 ± 1 | 37 ± 13 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | >10 | 12 ± 2 | >10 | 11 ± 0 | >30 | 37 ± 3 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 4.7 ± 0.5 | 22 ± 1 | >3.2 | 10 ± 1 | 5.4 ± 3.1 | 37 ± 13 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 4.5 ± 0.7 | 60 ± 1 | >16 | 36 ± 22 | 20 ± 0 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 2.5 ± 0.7 | >250 | 14 ± 6 | >80 | >80 | >80 |
|       |          |   | NH$_2$N$Bu_2$-p | H | H | 1.8 | >250 | 9.4 ± 0.7 | >100 | 16 ± 6 | >100 | >80 |

* Antiviral values in italics denotes EC$_{50}$ values equal or lower than 10 μg/ml. An asterisk after the compound code no. indicates a selectivity (CC$_{50}$/EC$_{50}$) of >10 for the compound against either FIPV and/or SARS-CoV.

I. Balzarini et al. / Antiviral Research 72 (2006) 20–33
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 EC₅₀ 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-

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

| LCTA | Code no. | X | Y | HIV-1 (CEM) EC₅₀ (μM) IC₅₀ (μM) | FIPV (CRFK) EC₅₀ (μM) IC₅₀ (μM) | SARS-CoV (Vero) EC₅₀ (μM) IC₅₀ (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     | CH₂NHAdam-2 | Boc | 17 ± 3 | 240 ± 13 | 35 ± 30 | >80 | 61 ± 45 | >80 |
| 963  | 175     | CH₂NAdam-2 | H | 17 ± 3 | >250 | 19 ± 0 | >50 | >80 | >80 |

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

| LCTA | Code no. | X | Y | HIV-1 (CEM) IC₅₀ (μM) | FIPV (CRFK) IC₅₀ (μM) | SARS (Vero) IC₅₀ (μM) |
|------|---------|---|---|------------------|-------------------|-------------------|
| 968  | 176     | H | 15 | >250 | 48 ± 46 | 40 ± 5 |
| 969  | 177     | CH₂NHAdam-2 | 13 ± 9.9 | 242 ± 11 | 22 ± 3 | 45 ± 13 |
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\textsubscript{50} (\textmu M) | IC\textsubscript{50} (\textmu M) | IC\textsubscript{50} (\textmu M) | IC\textsubscript{50} (\textmu M) |
| 970 178       | 22.5        | >250        | 57 ± 37         | >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.

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 \textmu M. 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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Fig. 1. Correlation between the 50% effective concentrations (EC\textsubscript{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\textsubscript{50} values against both viruses could be determined. Data were taken from Tables 1–9.

\[ r = 0.51 \]
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