Supporting Information

Synthesis of Antiviral Perfluoroalkyl Derivatives of Teicoplanin and Vancomycin

Ilona Bereczki, Magdolna Csávás, Zsolt Szűcs, Erzsébet Rőth, Gyula Batta, Eszter Ostorházi, Lieve Naesens, Anikó Borbás,* and Pál Herczegh*
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Table S1. Cytotoxicity and antiviral activity in HeLa cell cultures

| Compound | Cytotoxicity | Antiviral EC₅₀<sup>c</sup> Vesicular stomatitis virus | Antiviral EC₅₀<sup>c</sup> Coxsackie virus B4 | Antiviral EC₅₀<sup>c</sup> Respiratory syncytial virus |
|----------|-------------|-------------------------------------------------|---------------------------------|-----------------------------------------------------|
|          | CC₅₀<sup>a</sup> | MCC<sup>b</sup> | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS |
| 12       | >100        | >100             | -                  | -  | -                  | 20  | 10                |
| 13       | >100        | ≥100             | -                  | -  | -                  | 9.2 | 10                |
| 15       | 43          | -                | >100              | -  | -                  | 9.9 | -                 |
| 16       | >100        | >100             | >100              | >100| >100              | 12  | 8.9               |
| 17       | >100        | >100             | >100              | >100| >100              | 7.5 | 6.8               |
| 18       | 41          | -                | >100              | -  | -                  | >100| -                 |
| DS-10,000| >100        | -                | 2.5               | -  | >100              | 0.1 | -                 |
| Ribavirin| >250        | -                | 87                | -  | 126               | 5   | -                 |

<sup>a</sup>50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

<sup>b</sup>Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

<sup>c</sup>50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table S2. Cytotoxicity and antiviral activity in Vero cell cultures

| Compound | Cytotoxicity | Antiviral EC₅₀<sup>c</sup> Reovirus-1 | Antiviral EC₅₀<sup>c</sup> Sindbis virus | Antiviral EC₅₀<sup>c</sup> Coxsackie virus B4 | Antiviral EC₅₀<sup>c</sup> Punta Toro virus | Antiviral EC₅₀<sup>c</sup> Yellow Fever virus | Antiviral EC₅₀<sup>c</sup> Zika virus |
|----------|-------------|----------------------------------------|------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|          | CC₅₀<sup>a</sup> | MCC<sup>b</sup> | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS |
| 12       | -           | -                         | -                        | -  | -                  | -  | -                  | -  | -                  | -  | -                  | -  |
| 13       | -           | -                         | -                        | -  | -                  | -  | -                  | -  | -                  | -  | -                  | -  |
| 15       | >100        | >100                      | >100                     | 82  | 100               | >100| >100              | >100| >100              | >100| 9.4                | 45  |
| 16       | >100        | -                         | >100                     | -  | >100              | >100| >100              | >100| >100              | >100| -                  | -  |
| 17       | >100        | -                         | >100                     | -  | >100              | >100| >100              | >100| >100              | >100| -                  | -  |
| 18       | >100        | -                         | >100                     | -  | >100              | >100| >100              | >100| >100              | >100| -                  | -  |
| DS-10,000| >100        | >100                      | 20                       | 28.3| 20                | 40.6| 44.7              | 29.2| 58.5              | 7.2 | 20                 | 95.5|
| Mycophenolic acid | >100 | >100                     | 3.3                     | 4   | 5.7               | 4   | >100              | >100| >100              | >100| 0.8               | 1.5 |

<sup>a</sup>50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

<sup>b</sup>Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

<sup>c</sup>50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
Antiviral procedures

Inhibitory effect against human coronavirus 229E was determined using a CPE reduction assay in human embryonic lung fibroblast (HEL) 299 cells, described in full detail elsewhere.1 The other virus/cell assays were: in HEL cells: herpes simplex virus type 1 (HSV-1, including a thymidine kinase deficient strain HSV-1/TK-), herpes simplex virus type 2 (HSV-2), vaccinia virus and adenovirus; in human cervix carcinoma HeLa cells: respiratory syncytial virus; and in African green monkey kidney Vero cells: yellow fever virus (17D) and Zika virus (strain MR766). Semiconfluent cell cultures in 96-well plates were inoculated with the virus at a multiplicity of infection of 100 CCID50 (50% cell culture infective dose) per well. Together with the virus, serial dilutions of the compounds were added. The plates were incubated at 37 °C during 3 to 6 days until clear CPE was reached. Microscopic scoring was then performed to determine the antiviral activity [expressed as 50% effective concentration (EC50)] and cytotoxicity [expressed as minimum cytotoxic concentration (MCC)], calculated as reported.2

References

1. Ç. B. Apaydin, N. Cesur, A. Stevaert, L. Naesens, Z. Cesur, Synthesis and anti-coronavirus activity of a series of 1-thia-4-azaspiro[4.5]decan-3-one derivatives. Arch. Pharm. (Weinheim) 2019, 352:e1800330.

2. P. Vrijens, S. Noppen, T. Boogaerts, E. Vansstreels, R. Ronca, P. Chiodelli, M. Laporte, E. Vanderlinden, S. Liekens, A. Stevaert, L. Naesens, Influenza virus entry via the GM3 ganglioside-mediated platelet-derived growth factor receptor beta signalling pathway. J. Gen. Virol. 2019, 100, 583-601.
$^1$H-DOSY spectra of compound 17

**Figure S1.** Detection of possible oligomerisation of 17 by DOSY (Diffusion Ordered Spectroscopy) NMR experiments with TMS as internal reference. On this scale 0.1 unit accords to double mass ratio. The vertical scale represent diffusion constant on a log10 scale. This experiment yielded MW between 7-8 kDa

**Figure S2.** DOSY NMR experiment of 17 in MeOD with β-CD as internal reference, the vertical scale is a log10 based diffusion constant. On this scale 0.1 unit is a mass factor of two. β-CD (anomeric doublet in 1D spectrum, bottom at 5 ppm) appears at -9.15 while the 17 front at -9.26. This results in mass 2400 (instead of nominal 2030), according to rather a monomeric mass
NMR Spectra of the compounds
$^1$H NMR and $^{13}$C NMR spectra of compound 2a
$^1$H NMR and $^{13}$C NMR spectra of compound 2b
$^1$H NMR and $^{13}$C NMR spectra of compound 3a
$^1$H NMR and $^{13}$C NMR spectra of compound 3b
$^1$H NMR and $^{13}$C NMR spectra of compound 4a
$^1$H NMR and $^{13}$C NMR spectra of compound 4b
$^1$H NMR and $^{13}$C NMR spectra of compound 5a
$^1$H NMR and $^{13}$C NMR spectra of compound 5b
$^1$H NMR and $^{13}$C NMR spectra of compound 6a
$^1$H NMR and $^{13}$C NMR spectra of compound 6b
$^1$H NMR and $^{13}$C NMR spectra of compound 7a
$^1$H NMR and $^{13}$C NMR spectra of compound 10
JMOD, HSQC and HMBC spectra of compound 11, (125, 500 MHz) DMSO
$^{13}$C, HSQC and HMBC spectra of compound 12, (125, 500 MHz) DMSO
\(^{19}\text{F},\) JMOD and HSQC spectra of compound 13, \((470, 125, 500 \text{ MHz})\) DMSO
JMOD, HSQC and HMBC spectra of compound 15, (125, 500 MHz) DMSO
$^{19}$F, $^{13}$C, HSQC and HMBC spectra of compound 16, (470, 125, 500 MHz) DMSO
JMOD, COSY, HSQC spectra of compound 17, (125, 500 MHz) DMSO
$^{13}$C, HSQC, HMBC spectra of compound 18, (125, 500 MHz) DMSO
