Original article

Phenylboronic-acid-based carbohydrate binders as antiviral therapeutics: bisphenylboronic acids

Paul C Trippier¹, Jan Balzarini², Christopher McGuigan^{1*}

¹Welsh School of Pharmacy, Cardiff University, Cardiff, UK

²Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium

*Corresponding author e-mail: mcguigan@cardiff.ac.uk

Background: Carbohydrate-binding agents are considered as potential therapeutic agents for the inhibition of highly glycosylated enveloped viruses such as HIV type-1. Phenylboronic acids are well-known to bind the *cis*-diol functionality of carbohydrate structures, thereby identifying themselves as potential lead structures.

Methods: Bisphenylboronic acids connected via a functionalized linker at variable length (1–13 atoms) bearing the binding boronic acid functionality at the three possible ring geometries relative to the linker have been investigated as probes for selective and non-selective saccharide sensors. Herein, we describe the compilation of a 'linker-diverse' compound library of bisphenylboronic

acids and the determination of the structure–activity relationship versus a variety of enveloped viruses. Molecular modelling of the gp120 glycans of simian immunodeficiency virus was undertaken to ascertain a theoretical minimum length of the linker unit.

Results: The compounds demonstrated no pronounced antiviral activity. The general low toxicity of the boronic acids became evident in this study, thereby justifying further studies.

Conclusions: A higher concentration of phenylboronate functional groups per molecule, resulting in multivalency, might be necessary to bind with sufficient potency to HIV type-1 gp120 and to elicit an antiviral action.

Introduction

The development of carbohydrate-binding agents (CBAs) as potential antiviral therapeutics has recently been advanced [1,2]. Uniquely, these compounds exhibit two potential modes of action: the binding of the *cis*-diol functionality of the carbohydrate moieties displayed on the glycans of the viral envelope, thereby preventing viral entry and inducement of mutation in the virus (specifically the gp120 glycan) to allow for the escape of CBA pressure (via progressive deletion of the carbohydrate surface of the viral envelope) exposing the mutant virus to an immune response by the host organism [2].

Previous investigations on monophenylboronic acids [3] have shown a general lack of activity of this class of compounds in the inhibition of enveloped virus replication. Evidence is provided for the need for a larger concentration of phenylboronic acids in one molecule to bind to the high number of *cis*-diols displayed on highly glycosylated enveloped virus surfaces to prevent efficient interaction of such viruses with the host cells. Use of a molecule that possesses multiple binding sites might thus alleviate the need for high concentrations of monomeric compound.

The structure of HIV type-1 (HIV-1) gp120 [4] was considered in this project as a target model. The glycans of the protein are assembled in such a way that a highly diverse array of oligosaccharides comprising the terminal carbohydrate residues of the glycan surface are displayed upon the cell surface of the virus. Such oligosaccharides branch from a peptide backbone and, as a direct result, distances between the extruding oligosaccharide chains, and hence the terminal mannose carbohydrate residues, might vary widely [5]. Thus, any linker unit connecting ≥ 2 CBA functionalities must be of sufficient length to allow bridging from one functionality of a mannose *cis*-diol to a second mannose residue. Although the post-attachment structure of HIV-1 gp120, bound to the CD4 receptor, has been known for some time [4], the unattached gp120 structure has yet to be clarified. Because of this situation, it was necessary to utilize simian immunodeficiency virus gp120, whose pre-attachment structure has been crystallized and fully characterized [6]; thus, molecular modelling (Molecular operating environment software; Chemical Computing Group, Montreal, QC, Canada; Figure 1)

of the terminal oligosaccharide chains allows for determination of mean distances between mannose *cis*-diol functionalities. The calculated distances can then be ‘engineered’ into synthesized linkers.

Herein, we report the compilation of a known (Figures 2 and 3) and synthesized (Figures 4 and 5) bisphenylboronic acid library, each constructed around a central linker skeleton of variable length and functionality, ranging from 1 to 13 atom chains. Compounds that contained incremental increases in length of the linker unit were sought. The compound library was screened against HIV-1 and a variety of enveloped viruses to determine antiviral potency.

Bisphenylboronic acids comprising boronic acid functionalities at the *ortho*-, *meta*- and *para*- positions relative to the linker substituent were synthesized. The ring geometry of the boronic acid further elongates or contracts the spacing of the binding functionalities, allowing for additional structure–activity relationship investigations upon binding and antiviral activity determination.

The linkers display a variety of constituent atoms (C, N and O) and functionalities (amines, ketones, olefin, furan and amide bonds) to allow for engineered rigidity and synthetic simplicity. Rigidity is introduced through carbonyl groups and amide bonds to reduce

Figure 1. Mean distance approximation between mannose *cis*-diols

Figure 2. Known and commercially available bisphenylboronic acid library members **Figure 3.** Bisphenylboronic acids from known dibromides

Figure 4. Synthesis of bisphenylboronic acids with linkers comprising 2–6 atoms

1) $^t\text{BuLi}$, THF, B(OPr)_3 , HCl; 2) AlCl_3 , MeOH, $n=1$ malonyl chloride or $n=2$ succinyl chloride or $n=3$ glutaryl chloride or $n=4$ adipoyl chloride; 3) H_2O :MeOH, 100°C .

conformational freedom and to enable the bridging of ≥ 2 carbohydrate residues. In particular, carbon chains might adopt many minimum energy configurations, which would be expected to include ‘folded’ conformations with a reduced distance between boronic acid functionalities.

Methods

Chemistry

¹H NMR spectra were recorded on a Bruker AMX500 (500 MHz) spectrometer (Bruker Instruments, Inc., Billerica, MA, USA). Chemical shifts (δ_{H}) are reported in parts per million (ppm) and are referenced to the residual protonated solvent peak. Abbreviations used in the description of multiplicities are singlet (s), doublet (d), double doublet (dd), double triplet (dt),

triplet (t), quartet (q), multiplet (m) and broad (br). Coupling constants (J) are quoted in Hertz (Hz) to the nearest 0.1 Hz.

¹³C NMR spectra were recorded on a Bruker AMX500 (125.8 MHz) spectrometer. Chemical shifts are quoted in ppm and referenced to the residual protonated solvent peak with proton decoupling, using DEPT, HMQC and APT editing to aid assignment.

¹¹B NMR spectra were recorded on a Bruker AMX500 (160.5 MHz) spectrometer. Chemical shifts are quoted in ppm and referenced to the residual protonated solvent peak with proton decoupling. Samples were performed in a pure quartz 5 mm NMR tube.

Low-resolution and high-resolution mass spectra (m/z) were recorded on a Waters GCT Premier spectrometer (Waters, Elstree, UK) using an electron ionization (EI) source. m/z values of major peaks are

Figure 5. Synthesis of 7-atom-containing linked bisphenylboronic acids

reported in Daltons, with intensities quoted as percentages of the base peak.

Thin-layer chromatography (TLC) was performed on Merck and Co. (Darmsted, Germany) aluminum-foil-backed sheets precoated with 0.2 mm Kieselgel 60 F-54. Development of TLC plates was performed by the ascending method. Product spots were visualized by the quenching of ultraviolet fluorescence (λ_{max} 254 nm), or by staining with a solution of 5% (w/v) dodecamolybdophosphoric acid in ethanol.

Flash column chromatography was performed under pressure on a silica gel, 60 Å pore size, 40–60 µm (Phase Separations Ltd, Deeside, UK). Samples were applied as a concentrated solution in the same eluent or preadsorbed on silica gel. Fractions containing the product were identified by TLC, pooled and the solvent removed *in vacuo*.

All solvents were used, as obtained from Sigma–Aldrich Co. (St Louis, MO, USA). All reactions were performed under an inert argon atmosphere unless otherwise stated.

Compounds 1 [7], 2 [8], 3 and 4 [9] (Figure 2) were synthesized as described in the literature; obtained spectroscopic data matched that reported. Compounds 5 and 6 were obtained from Sigma–Aldrich Co. Compound 7 was obtained from Alfa Aesar Co. (Heysham, UK).

It is well-documented [10–12] that boronic acids poorly fragment in many ionization techniques used in mass spectral analysis because of their low volatility. This is amplified by the facile occurrence of gas-phase dehydration and anhydride formation in the ion source. Derivatization as the ester with ethylene glycol can be beneficial in aiding identification of the molecular species as illustrated in the following experimental section. Many ionization methods were applied to the compounds reported herein, including derivatization with ethylene glycol. Several compounds failed to provide molecular species, those that did are reported below.

General procedure A for boronation of bromides

The corresponding bromide (1.0 eq.) was dissolved in THF or Et₂O (40 ml) and cooled to -78°C. *n*-BuLi (1.5 eq. of a 2.5 M solution in hexanes) was added dropwise and stirring continued at -78°C for 1 h. Trimethyl borate (1.5 eq.) was added dropwise and the mixture was allowed to warm gradually to room temperature (RT) and stirred overnight. Water (20 ml) was added and the mixture stirred for 1 h. Brine (25 ml) was added and the product extracted with EtOAc (2×30 ml), dried (MgSO₄), filtered and concentrated *in vacuo* to provide the desired compound.

General procedure B for boronation of bromides

To a stirred suspension of magnesium turnings (2.5 eq.) in Et₂O or THF (100 ml), 1,2-dibromoethane

(catalyst) and the corresponding bromide precursor (1.0 eq.) was added and stirring was continued at RT overnight. The mixture was cooled to -78°C and trimethyl borate (1.0 eq.) in dry Et₂O (1 ml) was added dropwise. The reaction was stirred for a further 1 h at -78°C and allowed to warm to RT overnight. The reaction was cooled to 0°C and 8% HCl (10 ml) was added slowly, the reaction was diluted with water (10 ml) and stirred for 10 min at 0°C. The organic layer was extracted with Et₂O (2×25 ml). The organic layers were then combined, dried (MgSO₄), filtered and concentrated *in vacuo* to yield the desired compound.

1,2-Bis(4-phenylboronic acid)ethane-1,2-dione (9)

Dibromide dione 8 underwent general procedure A to provide the title compound in 76% yield as a yellow powder. ¹H NMR (500 MHz; CD₃OD) δ_{H} : 7.45–7.60 (8H, m, ArH), 7.92 (4H, d, B(OH)₂). ¹³C NMR (125.8 MHz; CDCl₃) δ_{C} : 128.61, 130.54, 131.21 (all ArCH), 134.44 and 134.86 (both C_{ipso}), 194.22 (CO). ¹¹B NMR (160.5 MHz; CD₃OD) δ_{B} : 28.39 (B(OH)₂).

1,2-Bis(4-phenylboronic acid)propane-1,3-dione (10)

Phenylboronic acid (750 mg, 6.15 mmol) was dissolved in methanol (30 ml), the solution was cooled to 0°C and AlCl₃ (885 mg, 6.65 mmol) was added; stirring was continued until fully solvated. A solution of malonyl chloride (434 mg, 3.08 mmol) in methanol (2 ml) was carefully added dropwise over 1 h at 0°C, stirring was continued for a further 1 h and then allowed to warm to RT overnight. Ice (1.0 g) was added to the resultant orange solution and diluted with methanol (20 ml). The organic layer was washed with 2.5 M NaOH (20 ml) and brine (20 ml), extracted with EtOAc (100 ml), dried (MgSO₄), filtered and evaporated *in vacuo* to yield 10 as a white powder (200 mg, 0.64 mmol, 21%). ¹H NMR (500 MHz; CD₃OD) δ_{H} : 1.96 (2H, s, CH₂), 7.32 (4H, d, *J* 6.5 Hz, ArH), 7.59 (4H, d, *J* 6.5 Hz, ArH). ¹³C NMR (125.8 MHz; CDCl₃) δ_{C} : 85.52 (CH₂), 122.70 (C_{ipso}-B(OH)₂), 129.98, 132.08, 132.48, 132.83 (all ArCH), 143.21 (C_{ipso}), 179.38 (C(O)). ¹¹B NMR (160.5 MHz; CD₃OD) δ_{B} : 18.52 (B(OH)₂). *m/z* (TOF-EI): 312.10 (100%) [MH⁺], 208.09 (38), 164.08 (75), 104.04 (80), 78.04 (55). HRMS: found: 312.0992; C₁₅H₁₄B₂O₆ requires: 312.0976.

1,2-Bis(4-phenylboronic acid)butane-1,4-dione (11)

The procedure for compound 10 was followed using succinyl chloride to create the linker providing 11 as an orange powder in 63% yield. ¹H NMR (500 MHz; CD₃OD) δ_{H} : 2.69 (2H, t, *J* 6.5 Hz CH₂), 3.26 (2H, t, *J* 6.5 Hz, CH₂), 7.64 (4H, d, *J* 8.7 Hz, ArH), 7.89 (2H, d, *J* 8.6 Hz, ArH). ¹³C NMR (125.8 MHz; CDCl₃) δ_{C} : 23.77 (CH₂), 128.50 and 131.20 (ArCH), 134.81 (C_{ipso}),

180.73 (C(O)). ^{11}B NMR (160.5 MHz; CD_3OD) δ_{B} : 17.10 ($\text{B}(\text{OH})_2$). m/z (TOF-ES $^+$): 327.12 (100%) [MH^+].

1,2-Bis(4-phenylboronic acid)pentane-1,5-dione (12)

The procedure for compound **10** was followed using glutaryl chloride to create the linker providing **12** as a white powder in 44% yield. ^1H NMR (500 MHz; CDCl_3) δ_{H} : 2.28 (2H, m, CH_2), 3.86 (4H, m, $2\times\text{C}(\text{O})\text{CH}_2$), 6.34 (4H, br. s, ArH), 7.25 (4H, br. s, ArH). ^{13}C NMR (125.8 MHz; CDCl_3) δ_{C} : 23.65 (CH_2), 37.73 (CH_2), 128.36, 128.63, 128.83, 128.96, 131.36, 134.90, 135.22 (ArCH), 182.62 (C(O)). ^{11}B NMR (160.5 MHz; CD_3OD) δ_{B} : 28.30 ($\text{B}(\text{OH})_2$). m/z (TOF-ES): 393.24 (50%) [$\text{MH}^+ + 2$ ethylene glycol], 349.21 (95%) [$\text{MH}^+ +$ ethylene glycol], 305.16 (100%) [$\text{MH}^+ - 4\text{H}$].

1,2-Bis(4-bromophenyl)hexane-1,6-dione (13)

The procedure for compound **10** was followed using 4-bromobenzene (2 eq.) and adipoyl chloride (1 eq.) to provide **13** as a yellow oil in 50% yield. ^1H NMR (500 MHz; CDCl_3) δ_{H} : 1.81 (4H, dt, J 6.8 Hz, $2\times\text{CH}_2$), 2.97 (4H, dt, J 6.7 Hz, $2\times\text{CH}_2$), 7.33 (4H, d, ArH), 7.52 (4H, d, ArH). ^{13}C NMR (125.8 MHz; CDCl_3) δ_{C} : 23.79 (CH_2), 46.40 (CH_2), 122.52 ($\text{ArC}-\text{Br}$), 126.88 and 130.04 (ArCH), 131.55 (ArC_{ipso}), 173.25 (C(O)).

1,2-Bis(4-phenylboronic acid)hexane-1,6-dione (14)

Compound **13** underwent general procedure B to provide **14** as a white powder in 32% yield. ^1H NMR (500 MHz; CD_3OD) δ_{H} : 1.64 (4H, dt, J 7.1 Hz, $2\times\text{CH}_2$), 2.29 (4H, dt, J 7.0 Hz, $2\times\text{CH}_2$), 7.26 (4H, d, J 7.8 Hz, ArH), 7.50 (4H, d, J 7.9 Hz, ArH). ^{13}C NMR (125.8 MHz; CD_3OD) δ_{C} : 23.85 (CH_2), 49.26 (CH_2), 128.68 and 134.76 (ArCH), 135.09 (ArC_{ipso}), 180.72 (C(O)). ^{11}B NMR (160.5 MHz; CD_3OD) δ_{B} : 17.04 ($\text{B}(\text{OH})_2$).

9H-fluorene-2,7-diboronic acid (15)

Commercially (Sigma-Aldrich Co.) available 9H-fluorene-2,7-dibromide underwent general procedure A to provide **15** as an orange powder in 55% yield. ^1H NMR (500 MHz; CDCl_3) δ_{H} : 3.86 (2H, s, CH_2), 7.59 (2H, d, J 8.2 Hz, ArH), 7.62 (2H, d, J 8.2 Hz, ArH), 7.69 (2H, s, ArH). ^{13}C NMR (125.8 MHz; CDCl_3) δ_{C} : 36.58 (CH_2), 121.18 ($\text{ArC}_{\text{ipso}}-\text{B}(\text{OH})_2$), 121.22, 128.33 and 130.25 (ArCH), 141.11 and 144.81 (ArC_{ipso}). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 19.94 ($\text{B}(\text{OH})_2$).

1,1'-Binaphthyl-2,2'-diboronic acid (16)

2,2'-Dibromo-1,1'-binaphthyl underwent general procedure A to provide **16** as a yellow oil in 92% yield [13]. ^1H NMR (500 MHz; CD_3CN) δ_{H} : 7.00–8.12 (12H, m, ArH). ^{13}C NMR (125.8 MHz; CDCl_3) δ_{C} : 116.80 ($\text{C}_{\text{ipso}}-\text{B}(\text{OH})_2$), 125.94, 125.97, 126.00,

126.07, 126.24, 126.36, 126.78, 126.91, 127.25, 127.47, 127.91, 127.98 (ArCH). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 28.52 ($\text{B}(\text{OH})_2$).

3,3'-Azanediylbis(methylene)bis(3,1-phenylene)diboronic acid (17)

The corresponding known [14] dibromide underwent general procedure A to provide **17** as a dark yellow oil in 98% yield. ^1H NMR (500 MHz; CD_3CN) δ_{H} : 4.66 (4H, s, CH_2), 7.25 (2H, d, J 7.8 Hz, ArH), 7.29 (2H, t, J 7.8 Hz, ArH), 7.42 (2H, d, J 7.9 Hz, ArH), 7.53 (2H, s, ArH). ^{13}C NMR (125.8 MHz; CD_3CN) δ_{C} : 64.46 (CH_2), 122.66 ($\text{ArC}-\text{B}(\text{OH})_2$), 125.22, 129.90, 130.11 and 130.64 (ArCH), 143.15 (C_{ipso}). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 18.56 ($\text{B}(\text{OH})_2$).

4,4'-Azanediylbis(methylene)bis(4,1-phenylene)diboronic acid (18)

The corresponding known [14] dibromide underwent general procedure A to provide **18** as a white powder in 99% yield. ^1H NMR (500 MHz; CD_3CN) δ_{H} : 2.53 (4H, br. s, $\text{B}(\text{OH})_2$), 4.53 (4H, s, CH_2), 7.26 (4H, d, J 8.6 Hz, ArH), 7.49 (4H, d, J 8.5 Hz, ArH). ^{13}C NMR (125.8 MHz; CD_3CN) δ_{C} : 62.61 (CH_2), 116.98 ($\text{ArC}-\text{B}(\text{OH})_2$), 128.29 and 130.86 (ArCH), 141.23 (C_{ipso}). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 19.91 ($\text{B}(\text{OH})_2$).

3,3'-(1,3-Phenylenebis(methylazanediyl))bis(methylene)bis(3,1-phenylene)diboronic acid (19)

To a solution of 1,3-dibromobenzene (100 mg, 0.42 mmol) and K_2CO_3 (580 mg, 4.2 mmol) in anhydrous methanol (20 ml) 3-methylaminomethylphenylboronic acid [15] was added (140 mg, 0.85 mmol) dropwise. The reaction mixture was stirred at RT for 48 h and the solvent evaporated *in vacuo* to provide a white precipitate contaminated with a red oil. The oil was dissolved in MeOH (4 ml), removed by pipette, concentrated and dried under high vacuum to yield **19** as a sticky red gum (324 mg, 0.80 mmol, 80%). ^1H NMR (500 MHz; CD_3OD) δ_{H} : 3.32 (6H, s, $2\times\text{CH}_3$), 4.52 (4H, s, $2\times\text{CH}_2$), 6.45 (1H, s, ArH), 6.54 (2H, m, ArH), 6.98 (1H, t, J 7.8 Hz, ArH), 7.04 (1H, d, J 7.5 Hz, ArH), 7.09 (1H, d, J 7.6 Hz, ArH), 7.15 (2H, dd, J 6.9 and 7.3 Hz, ArH), 7.43 (4H, m, ArH), 7.48 (2H, s, ArH). ^{13}C NMR (125.8 MHz; CD_3OD) δ_{C} : 35.44 (CH_3), 66.61 (CH_2), 113.94, 122.20, 124.96, 127.12, 127.54, 129.95, 133.22, 133.47, 134.39, 134.67 (ArCH), 137.61 (ArC_{ipso}), 139.80 ($\text{ArC}-\text{B}(\text{OH})_2$). ^{11}B NMR (160.5 MHz; CD_3OD) δ_{B} : 4.76 ($\text{B}(\text{OH})_3$).

3,3'-(1,3-Phenylenebis(methylazanediyl))bis(methylene)bis(4-methoxy-3,1-phenylene)diboronic acid (20)

The procedure described previously for compound **19** was followed using commercially available 4-methoxy-3((methylamino)methyl)phenylboronic acid to

provide **20** as a dark orange oil in 88% yield. ^1H NMR (500 MHz, CD_3OD) δ_{H} : 3.35 (6H, s, $2\times\text{NCH}_3$), 3.84 (6H, s, $2\times\text{OCH}_3$), 4.61 (4H, s, CH_2), 6.77–6.83 (4H, m, ArH), 7.31–7.44 (6H, m, ArH). ^{13}C NMR (125.8 MHz; CD_3OD) δ_{C} : 49.86 (NCH_3), 55.66 (OCH_3), 62.05 (CH_2), 109.97, 134.62, 134.87, 135.39, 136.59 (ArCH). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 6.40, 10.60 ($\text{B}(\text{OH})_3$).

4,4'-(1,3-Phenylenebis(methylazanediyl))bis(methylene)bis(4,1-phenylene)diboronic acid (21**)**

The procedure described previously for compound **19** was followed using known 4-methylaminomethylphenylboronic acid [15] to provide **21** as a white gel in 99% yield. ^1H NMR (500 MHz, CD_3OD) δ_{H} : 3.32 (6H, s, $2\times\text{CH}_3$), 4.55 (4H, s, $2\times\text{CH}_2$), 7.13–7.28 (5H, m, ArH), 7.29–7.35 (3H, m, ArH), 7.47–7.60 (4H, m, ArH). ^{13}C NMR (125.8 MHz; CD_3OD) δ_{C} : 49.01 (CH_3), 65.25 (CH_2), 126.75, 128.00, 128.26, 128.34, 128.54, 129.35, 129.57, 129.73, 129.81, 132.42, 134.37, 134.84 (ArCH). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 6.53, 10.62 ($\text{B}(\text{OH})_3$).

4,4'-(3,3'-(Ethane-1,2-diylbis(azanediyl))bis(3-oxopropane-3,1-diyl))bis(4,1-phenylene)diboronic acid (22**)**

To a solution of known boronic acid [3] (1.0 g, 5.46 mmol), dicyclohexylcarbodiimide (1.2 g, 6.0 mmol) and 4-dimethylaminopyridine (catalyst) in DCM:MeOH (10:1; 80 ml) ethyldiamine was added (0.19 ml, 2.73 mmol) dropwise. The mixture was stirred at RT for 48 h, filtered through a cotton plug to remove precipitated dicyclohexyl urea and evaporated *in vacuo* to provide **22** as a pale yellow powder (1.47 g, 3.56 mmol, 65%). ^1H NMR (500 MHz; $\text{DMSO}-d_6$) δ_{H} : 2.32 (4H, m, $2\times\text{CH}_2$), 2.81 (4H, m, $2\times\text{CH}_2$), 5.58 (4H, d, J 7.8 Hz, $2\times\text{NHCH}_2$), 7.13 (4H, d, J 7.8 Hz, ArH), 7.42 (4H, d, J 7.8 Hz, ArH), 7.85 (1H, br. s, NH), 8.10 (1H, d, J 6.3 Hz, NH). ^{13}C NMR (125.8 MHz; $\text{DMSO}-d_6$) δ_{C} : 30.31, 31.64, 32.31 and 35.28 (CH_2), 38.29 and 38.55 (NHCH_2), 118.33 (ArC-B(OH) $_2$), 130.46, 130.48, 131.02, 131.06 (ArCH), 140.57 and 140.77 (C_{ipso}), 168.59 ($\text{C}(\text{O})$), 171.19 ($\text{C}(\text{O})$). ^{11}B NMR (160.5 MHz; $\text{DMSO}-d_6$) δ_{B} : -13.48 ($\text{B}(\text{OH})_3$).

4,4'-(3,3'-(Tetrahydrofuran-2,5-diyl))bis(methylene)bis(azanediyl)bis(3-oxopropane-3,1-diyl)bis(4,1-phenylene)diboronic acid (23**)**

A solution of known boronic acid [3] (833 mg, 4.3 mmol), dicyclohexylcarbodiimide (976 mg, 4.73 mmol) and 4-dimethylaminopyridine (catalyst) in DCM (40 ml) was cooled to 0°C and a solution of known [16] carboxylic acid (280 mg, 2.15 mmol) in DCM:MeOH (1:1; 6 ml) was added dropwise. The mixture was stirred at 0°C for 15 min and at RT for

12 h (a colour change from opaque to transparent green occurred), was filtered through a cotton plug to remove precipitated dicyclohexyl urea and evaporated *in vacuo* to provide **23** as a green powder (1.79 g, 3.70 mmol, 86%). ^1H NMR (500 MHz; CD_3CN) δ_{H} : 1.27–1.38 (4H, m, $2\times\text{CH}_2\text{CH}$), 2.62 (4H, t, J 7.5 Hz, CH_2), 2.88 (4H, t, J 7.5 Hz, $\text{CH}_2\text{C}(\text{O})$), 3.12 (2H, m, CH), 3.63 (4H, m, $2\times\text{CH}_2\text{NH}$), 7.18 (4H, d, J 8.3 Hz, ArH), 7.48 (2H, d, J 8.3 Hz, ArH), 8.03 (2H, br. s, NH). ^{13}C NMR (125.8 MHz; CD_3CN) δ_{C} : 29.53 (CH_2), 34.52 (CH_2), 45.30 (CH_2NH), 50.73 (CH), 130.14, 130.92, 130.99 (ArCH), 140.03 (ArC_{ipso}), 172.51 ($\text{C}(\text{O})$). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 20.65 ($\text{B}(\text{OH})_2$). *m/z* (TOF-ES $^+$): 483.39 [MH^+] (15%), 448.40 (15), 294.29 (55), 280.27 (60), 224.21 (100), 137.11 (25).

(Z)-stillbenediboronic acid (24**)**

A solution of (Z)-stillbenediboronic acid bis(pinacol) ester (250 mg, 0.58 mmol) in water:MeOH (1:1; 10 ml) was heated at 80°C for 30 min. The mixture was allowed to cool, evaporated *in vacuo* to approximately 2 ml volume, water was added and allowed to stand at RT overnight. The resultant orange precipitate was filtered to provide **24** as an orange powder (135 mg, 5.0 mmol, 86%). ^1H NMR (500 MHz; CD_3CN) δ_{H} : 7.54–7.65 (6H, m, ArH), 7.97 (4H, d, J 7.2 Hz, ArH). ^{13}C NMR (125.8 MHz; CD_3CN) δ_{C} : 116.95 ($\text{C}_{\text{ipso}}\text{B}(\text{OH})_2$), 126.47, 127.98, 128.58 (all ArCH), 134.91 (C_{ipso}). ^{11}B NMR (160.5 MHz; CD_3CN) δ_{B} : 19.97 ($\text{B}(\text{OH})_2$).

Virological assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathic effect in human lung fibroblast (herpes simplex virus type-1 [HSV-1; strain KOS], herpes simplex virus type-2 [HSV-2; strain G], vaccinia virus and vesicular stomatitis virus [VSV]), African green monkey kidney (Vero, parainfluenza-3, reovirus-1, Sindbis, Coxsackie virus B4 and Punta Toro virus), human cervix carcinoma (VSV, Coxsackie virus B4 and respiratory syncytial virus) or Madin–Darby canine kidney (influenza A [H1N1 and H3N2] and influenza B) cell cultures. Confluent cell cultures in microtitre 96-well plates were inoculated with 100 50% cell culture infective doses (CCID_{50}) of virus (1 CCID_{50} being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (100, 40, 8, 1.6 and 0.32 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. For the human cytomegalovirus assays, confluent human embryonic lung (HEL) fibroblasts were grown in 96-well microtitre plates and infected with the human cytomegalovirus strains Davis and AD-169 at 100 plaque-forming units

per well. After a 2 h incubation period, residual virus was removed and the infected cells were further incubated with medium containing different concentrations of the test compounds (in duplicate). After incubation for 7 days at 37°C, virus-induced cytopathic effect was monitored microscopically after ethanol fixation and staining with Giemsa. Varicella zoster virus (VZV) drug susceptibility tests were performed on confluent HEL cells in 96-well microtitre plates by the plaque reduction assay. Monolayers were infected with 20 plaque-forming units of cell-associated virus per well. For each assay, virus controls (infected untreated cells) were included. After a 2 h incubation period, the virus inoculum was removed and the media replaced by the different dilutions (in duplicate) of the tested molecules. Serial dilutions of test compounds were incubated with the infected monolayers for 5 days. After a 5-day incubation period, the cells were fixed and stained with Giemsa, and the level of virus-induced cytopathic effect was determined by counting the number of plaques for each dilution. The methodology of the anti-HIV assays was as follows: human T-lymphocyte CEM (approximately 3×10^5 cells/cm³) cells were exposed to 100 CCID₅₀ of HIV-1(III_B) or HIV type-2(ROD)/ml and 200 µl/well was seeded into a microtitre plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37°C, HIV-induced CEM giant cell formation was examined microscopically.

Determination of binding of the test compounds to HIV type-1 gp120 through surface plasmon resonance analysis
Recombinant gp120 protein from HIV-1 strain III_B (ImmunoDiagnostics Inc., Woburn, MA, USA; produced by Chinese hamster ovary cell cultures) was covalently immobilized on the carboxymethylated dextran matrix of a CM5 sensor chip (GE Healthcare, Uppsala, Sweden) in 10 mM sodium acetate (pH 4.0) using standard amine coupling chemistry to a final density of 122 RU (approximately 1 fmol of gp120). A reference flow cell was used as a control for non-specific binding and refractive index changes. All interaction studies were performed at 25°C on a Biacore T100 instrument (GE Healthcare). The compounds were diluted in HBS-P (10 mM HEPES, 150 mM NaCl and 0.05% surfactant P20; pH 7.4) and with 10 mM CaCl₂ (final concentration 50 µM). Samples were injected for 2 min at a flow rate of 45 µl/min and the dissociation was followed for 6 min. Several buffer blanks were used as double referencing. The CM5 sensor chip surface was regenerated with one injection of 50 mM NaOH.

Results

Synthesis of bisphenylboronic acids was based upon three general strategies. Two methods involved the

generation of an organolithium (by halogen–lithium exchange) or Grignard intermediate, which was not isolated, from a corresponding dibromide starting material. Subsequent trapping of the pseudo-anion via a boronate species provided access to the desired corresponding bisphenylboronic acid in moderate-to-good yield. A third method was the relatively simple deprotection of commercially available boronic esters, which, upon hydrolysis, yielded the desired boronic acids.

Figure 3 illustrates the compounds accessed through application of either of the organometallic intermediate methods described earlier. The respective dibromide of each bisphenylboronic acid was commercially available or known in the literature.

The family of diones ($n=1-4$ methylene groups) synthesized as described in *Methods* (Figure 4) relied upon Friedel–Crafts acylation of either phenylboronic acid or bromobenzene with a suitable length, commercially available acid chloride in moderate (21–76%) yield. Of note was the failure to prepare compound **14** by direct Friedel–Crafts acylation of phenylboronic acid in contrast to the synthesis of the other members of this subfamily. Instead, the route was required to proceed via the dibromide intermediate **13**. Figure 4 also illustrates access to stillbene-based bisboronic acid **24** via hydrolysis of protected diboronic ester **7** in 86% yield.

The synthesis of 7-atom length bisphenylboronic acids based around a central 1,3-disubstituted benzene moiety (Figure 5) proceeded from known boronic acids with methylamine functionality at various ring geometries. Deprotonation followed by nucleophilic attack of the amine anion resulted in compounds **19–21** in good-to-excellent yield.

To complete the compound library, 10- and 13-atom length bisphenylboronic acids were synthesized (Figure 6). Dicyclohexylcarbodiimide-enabled peptide bond coupling between the known carboxylic-acid-bearing phenylboronic acid and ethyldiamine or known diaminomethylfuran resulted in amide-bond-linked bisphenylboronic acids **22** and **23**, respectively.

Discussion

All compounds were tested for their inhibitory activity against HIV-1 and HIV type-2 (Table 1) in human T-lymphocyte (CEM) cell cultures. Selected compounds were also tested against a variety of enveloped viruses including VZV (Table 2), HSV-1 (KOS strain), acyclovir-resistant thymidine-kinase-deficient HSV-1 KOS strain, HSV-2 (G strain), vaccinia virus and VSV (Table 3) in HEL cells and against influenza A virus (H1N1 and H3N2 subtypes) and influenza B virus (Table 4) in Madin–Darby canine kidney cells. Included in Tables 3 and 4 are data for the current clinical drugs commonly used to treat the virus.

Figure 6. Synthesis of 10- and 13-atom length linked bisphenylboronic acids

Table 1. Anti-HIV-1 activity, anti-HIV-2 activity and cytostatic activity in human T-lymphocyte cell cultures

Compound	HIV-1 EC_{50}^a μM	HIV-2 EC_{50}^a μM	CC_{50}^a μM
1	>250	>250	>250
2	>10	>10	ND
3	>50	>50	ND
4	>50	>50	ND
5	>250	>250	>250
6	>50 ^a	>50 ^a	ND
7	>50	>50	ND
9	>50	>50	155 $\pm$ 2.8 ^b
10	>250	>250	ND
11	>250	>250	ND
12	>250	>250	ND
14	–	–	ND
15	>50	>50	107 $\pm$ 3.6 ^b
16	>10	>10	ND
17	>50	>50	ND
18	>50	>50	ND
19	>250	>250	>250
20	>250	>250	>250
21	>250	>250	>250
22	>10	>10	ND
23	>50	>50	>50
24	>50	>50	ND

^aPrecipitation occurred at higher concentrations. ^bValues presented as $\pm$ standard deviations. CC_{50} , compound concentration required to inhibit CEM cell proliferation by 50%; EC_{50} , 50% effective concentration; HIV-1, HIV type-1; HIV-2, HIV type-2; ND, not determined.

Table 2. Anti-VZV activity and cytotoxic/cytostatic activity in human embryonic lung cells

Compound	Antiviral activity		Cytotoxicity	
	TK ⁺ VZV EC ₅₀ ^a μM	TK ⁻ VZV EC ₅₀ ^a μM	Cell morphology ^a , μM	Cell growth CC ₅₀ ^a μM
2	>50	>50	>50	>100
3	>50	>40	>50	>100
4	>50	>50	>50	>100
7	>50	>50	>50	>100
10	35	41	>50	>100
11	>50	>50	>50	>100
12	>50	>50	>50	>100
16	>5	>5	20	39
17	>50	>50	>50	>100
18	>50	>50	>50	>100
22	>20	>20	50	>100
23	>20	>50	>50	>100
24	>50	>50	>50	>100
Acyclovir	2.4	104	>200	355
Brivudin	0.011	163	>300	351

^aMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. CC₅₀, compound concentration required to inhibit CEM cell proliferation by 50%; EC₅₀, 50% effective concentration required to reduce virus plaque formation by 50% for thymidine kinase (TK) containing (+) and TK deficient (-) varicella zoster virus (VZV). Virus input was 100 plaque-forming units.

Table 3. Antiviral activity and cytotoxicity of compounds in human embryonic lung cell cultures

Compound	MCC, μM	EC ₅₀ ^a μM				
		Herpes simplex virus-1 ^a	Herpes simplex virus-2 ^b	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK ⁻ KOS ACV ^c
1	>100	>100	>100	>100	>100	>100
2	20	>4	>4	>4	>4	>4
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100
15	100	>20	>20	>20	>20	>20
16	20	>4	>4	>4	4	>4
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.06	50	2	>250	10
Ribavirin	>250	250	>250	112	250	>250
Cidofovir	>250	0.8	0.8	10	>250	0.8

^aKOS strain. ^bG strain. ACV^c, acyclovir-resistant; EC₅₀, 50% effective concentration; MCC, minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology; TK⁻, thymidine kinase deficient.

Table 1 shows that none of the compounds evaluated against HIV showed significant inhibitory activity at subtoxic concentrations. Similar inactivity of the test compounds at the evaluated concentrations was observed for a variety of other enveloped (Tables 2, 3 and 4) and non-enveloped (JB, data not shown) viruses in cell culture. Also, surface plasmon resonance (SPR) studies revealed that the bisphenylboronic acid derivatives do not efficiently bind to HIV-1 gp120 (JB, data not shown) at 50 μ M.

To ensure that the bisphenylboronic acids can indeed bind to the *cis*-diols of carbohydrate residues, high-field (11.74 Tesla) ^{11}B NMR studies were conducted upon the ability of compounds 5 (Figure 7) and 6 to bind D-mannose. This study clearly showed a reduction of intensity in the free boronic acid signal at δ 28.50, indicative of the boronic acid converting to the putative corresponding cyclic ether upon binding. This suggests that the compounds herein reported might bind to mannose units; however, as observed in the monophenylboronic acid series, the concentrations are not high enough and/or the number of CBA functionalities might not be sufficient to elicit a biological response at the compound concentrations indicated. CBAs might, indeed, act as antiviral agents and

there is clear evidence that they bind to glycans on the viral envelope, presumably acting as cross-linking agents [17]. Multivalency is, therefore, an important property of such CBAs and the 'linear' bivalency of the borane derivatives described in this study might not be sufficient to display eventual antiviral activity. It is interesting to note that recently, Jay *et al.* [18] reported a high molecular weight borane derivative as a selective and potent inhibitor of HIV-1 in cell culture and an efficient binder of HIV-1 gp120 as shown by SPR technology. These findings motivate us to further explore the borane concept and synthesize higher molecular weight multifunctional borane derivatives.

Binaphthylene-based compound 2 appears to show a more pronounced cytostatic/toxic activity than the other derivatives. Clearly, by comparison with other bisphenylboronic acids, this toxicity cannot be ascribed to the boronic acid functionalities. The binaphthyl structure of the compound must be responsible for the observed toxicity and thus cautions against reproducing this structure in further studies.

In conclusion, this study demonstrates that bisphenylboronic acids do not inhibit glycosylated enveloped viruses. High-field NMR confirms that binding is possible and suggests it is taking place; however,

Table 4. Anti-influenza-virus activity and cytostatic activity and cytotoxicity in Madin–Darby canine kidney cell cultures

Compound	Cytotoxicity		Antiviral EC ₅₀					
			Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
	CC ₅₀	MCC	Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
1	>100	>100	>100	>100	>100	>100	>100	>100
2	2.1	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
3	>100	>100	>100	>100	>100	>100	>100	>100
4	61	100	>20	>20	>20	>20	>20	>20
5	>100	>100	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100	>100	>100
9	42	20	>4	>4	>4	>4	>4	>4
10	>100	>100	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100	>100	>100
15	14	20	>4	>4	>4	>4	>4	>4
19	>100	>100	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100	>100	>100
22	13.7	20	>4	>4	>4	>4	>4	>4
23	>100	>100	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100	>100	>100
Oseltamivir carboxylate	>100	>100	4	3.7	4	3.1	9	7.9
Ribavirin	>100	≥100	9	9.5	9	10.2	4	1.2

CC₅₀, 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) assay; EC₅₀, 50% effective concentration, or compound concentration required to afford 50% inhibition of virus-induced cytopathic effect (CPE), as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay; MCC, minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

the data presented herein indicate the binding is not of sufficient strength to inhibit viral entry, as was also observed in the SPR binding studies using HIV-1 gp120 as the ligand (50 μ M used as the highest concentration). The low toxicity of almost all compounds presented shows possibilities for further modifications. The synthesis of larger aggregate molecules containing increased numbers of boronic acid

functional groups is currently underway in our laboratories (Cardiff University, Cardiff, UK).

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Disclosure statement

The authors declare no competing interests.

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Figure 7. ^{11}B NMR, 160.5 MHz, D_2O , binding of 5 with *D*-mannose

(A) Spectrum of free boronic acid compound 5. (B) Loss of peak corresponding to the free boronic acid upon addition of 1 mol% *D*-mannose.

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