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α-Amino boronates as cyanoborane complexes: crystal structure and inhibition properties for the serine proteases: α-chymotrypsin and trypsin

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The first examples of α-amino boronate complexes stabilized by amino cyanoborane complexation were tested as trypsin and chymotrypsin inhibitors, and they showed moderate inhibition. The structure of compound 1 that contains two different boron atoms reveals that the geometry around the boron atom in the cyano group is tetrahedral, whereas a trigonal planar geometry exists around the boron atom attached to two oxygen atoms and a carbon atom. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: crystal structure; α-amino boronic acid; amine cyanoborane; serine proteases; biological properties; trypsin; chymotrypsin

INTRODUCTION

During the last 30 years, a considerable effort on part of the area of pharmaceutical chemistry has been focused on the synthesis and biological activity studies of boron analogues of α-amino boronic acids. Boronic acid analogues of α-amino acids represent an interesting class of enzyme inhibitors. They are often very potent transition-state[1–4] inhibitors of serine proteases[5,6] and form tetrahedral adducts with the active site serine that are highly stabilized by oxyanion–hole interaction. α-Amino boronic acids have been shown to possess various biological activities including inhibition of proteasomes, arginase, NOS[7] and cysteine enzymes.[8] It is known that α-amino boronic acids are not stable in their neutral form. However, they can be stabilized either by forming hydrochloride salts or by conversion to amide derivatives.[9–11]

Amine cyanoboranes have been shown to be antihyperlipidemic and induced considerable reduction in serum cholesterol levels.[12] They have also been shown to possess anticancer,[13–15] antiosteoporotic,[16] anti-inflammatory[17,18] and hypolipidemic[19] activities. These molecules have also been mentioned as possible boron carriers to tumor cells for boron neutron capture therapy (BNCT).

In this paper, we report for the first time a study to examine α-amino boronates stabilized by complexation with cyanoborane moiety, as inhibitors of trypsin and α-chymotrypsin. Since no structural data exist for this type of compounds, we also undertook an X-ray diffraction study of compound **1**.

EXPERIMENTAL

Materials and instrumentation

Solvents were dried over sodium/benzophenone and freshly distilled before use. All reactions were carried out under a dry nitrogen atmosphere in oven-dried glassware. Infrared spectra were run for samples as neat films for liquids and in KBr disks for solids on a Bruker Vector 22 FT-IR spectrophotometer. ^{1}H, ^{13}C and ^{11}B NMR spectra were recorded on a Varian Unity spectrometer (300, 75 and 96 MHz), respectively. Elemental analysis was performed in house at the Hebrew University Microanalysis laboratory. Chemical shifts were recorded relative to an internal standard Me_4Si for ^{1}H and ^{13}C NMR and $Et_2O{\cdot}BF_3$ as an external standard for ^{11}B

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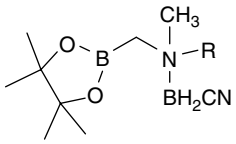

R= CH_3 (**1**), C_5H_{11} (**2**), C_9H_{19} (**3**), $C_{12}H_{25}$ (**4**)

Scheme 1. The structures of **1**–**4**.

NMR. Liquid chromatography was performed using column chromatography of the indicated solvent system on Merck silica gel 60 (0.040–0.063 mm). Compounds **1**–**4** (Scheme 1) were prepared from suitable trialkyl amine cyanoboranes that were α-deprotonated selectively and then reacted with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane as an electrophile as described elsewhere[20] and detailed herein.

General procedure

A solution of *s*-BuLi in cyclohexane (1.3 M, 2 mmol) was added drop-wise to a solution of trimethylamine cyanoborane (0.098 g, 1 mmol) in THF (2.5 ml) at −78 °C. The solution was stirred for 30 min at −78 °C, then it was allowed to warm up to room temperature for 30 min, before recooling down again to −78 °C and addition of 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.186 g, 1 mmol) in one portion. After 2 min the cooling bath was removed, and the reaction mixture was stirred for 30 min at room temperature. A saturated $NaHCO_3$ solution (10 ml) was added and the layers were separated, the aqueous layer was extracted with ether (2 × 10 ml); the combined organic layers were after dried over sodium sulfate. After evaporation of all the volatile chemicals the residue was purified. The crude was dissolved in 5 ml of benzene and washed with distilled water (7 × 5 ml). The benzene layer was dried over sodium sulfate, filtered, and concentrated under vacuum to give *N*,*N*-dimethyl (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methanamine cyanoborane (**1**) as yellow oil (0.186 g, yield 83%). ^{1}H NMR ($CDCl_3$, 25 °C): δ 1.27 {s, 12 H, $[C(CH_3)_2]_2$}, 2.68 (s, 2 H, NCH_2B), 2.79 [s, 6 H, $N(CH_3)_2$], BH can not be detected. ^{13}C {^{1}H} NMR ($CDCl_3$, 25 °C): δ 24.6, 52.0, 84.5, BC cannot be detected. ^{11}B NMR ($CDCl_3$, 25 °C): δ −14.1 (t, J = 103.12 Hz), 29.6 (s). IR (neat, cm^{-1}): $\acute{\upsilon}$ = 2354 (B–H), 2330 (B–H), 2199 (C≡N), 1470 (C–N), 752 (B–N). MS (EI) m/z: 224 (M^+), 209, 194, 184, 170, 155, 127. $C_{10}H_{22}N_2O_2B_2$ (224): calcd, C 53.57, H 9.82, N 12.50; found, C 54.01, H 9.69, N 12.70.

Preparation of *N*-methyl-*N*-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl]butan-1-amine cyanoborane (2)

The crude product was purified on column chromatography using 20% of EtOAc in petroleum ether to give yellow oil (0.186 g, yield 70%). ^{1}H NMR ($CDCl_3$, 25 °C): δ 0.95 (t, J_{H-H} = 15 Hz, 3 H, CH_2CH_3), 1.26 {s, 12 H, $[C(CH_3)_2]_2$}, 1.68 [m, 4 H, $CH_3(CH_2)_2$], 2.60 (s, 2 H, NCH_2B), 2.74 (s, 3 H, NCH_3), 2.99 (m, 2 H, NCH_2CH_2), BH cannot be detected. ^{13}C {^{1}H} NMR ($CDCl_3$, 25 °C): δ 13.7, 20.1, 24.6, 25.4, 49.9, 62.6, 84.4, BC cannot be detected. ^{11}B NMR ($CDCl_3$, 25 °C): δ −15.5 (t, J = 103.5 Hz), 29.5 (s). IR (neat, cm^{-1}): $\acute{\upsilon}$ = 2407 (B–H), 2338 (B–H), 2197 (C ≡ N), 1467 (C–N), 653 (B–N). MS (EI) m/z: 266 (M^+), 251, 227, 212, 198, 184, 169, 154. $C_{13}H_{28}N_2O_2B_2$ (266): C 58.64, H 10.52, N 10.52. Found: C 58.75, H 10.33, N 10.32.

Preparation of *N*-methyl-*N*-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl]nonan-1-amine cyanoborane (3)

The crude product was purified on column chromatography using 20% EtOAc in petroleum ether to give yellow oil (0.269 g, yield 80%). ^{1}H NMR ($CDCl_3$, 25 °C): δ 0.87 (t, J_{H-H} = 13.2 Hz, 3 H, CH_2CH_3), 1.26 {bs, 22 H, $[C(CH_3)_2]_2 + (CH_2)_5$}, 1.66 (m, 4 H, $NCH_2CH_2CH_2$), 2.59 (s, 2 H, NCH_2B), 2.74 (s, 3 H, NCH_3), 2.98 (m, 2 H, NCH_2CH_2), BH cannot be detected. ^{13}C {^{1}H} NMR ($CDCl_3$, 25 °C): δ 14.0, 22.6, 23.3, 24.6, 26.8, 29.12, 29.17, 29.3, 31.7, 49.8, 62.9, 84.4, BC cannot be detected. ^{11}B NMR ($CDCl_3$, 25 °C): δ −14.6 (t, J = 104.0 Hz), 30.2 (s). IR (neat, cm^{-1}): $\acute{\upsilon}$ = 2337 (B–H), 2236 (B–H), 2197 (C ≡ N), 1466 (C–N), 663 (B–N). MS (EI) m/z: 336 (M^+), 297, 282, 254, 240, 185, 170, 156, 142, 128, 114, 100, 84. $C_{18}H_{38}N_2O_2B_2$ (336): calcd. C 64.28, H 11.30, N 8.33. Found: C 63.55, H 11.21, N 8.37.

Preparation of *N*-methyl-*N*-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl]dodecan-1-amine cyanoborane (4)

The crude product was purified on column chromatography using 20% of EtOAc in Petroleum ether to give yellow oil (0.255 g, yield 70%). ^{1}H NMR ($CDCl_3$, 25 °C): δ 0.87 (t, J_{H-H} = 13.2 Hz, 3 H, CH_2CH_3), 1.26 {bs, 28 H, $[C(CH_3)_2]_2 + (CH_2)_8$}, 1.72 (m, 4 H, $CH_3CH_2CH_2$), 2.59 (s, 2 H, NCH_2B), 2.74 (s, 3 H, NCH_3), 3.00 (m, 2 H, NCH_2CH_2), BH can not be detected. ^{13}C {^{1}H} NMR ($CDCl_3$, 25 °C): δ 14.0, 22.6, 23.3, 24.6, 26.8, 29.1, 29.2, 29.42, 29.47, 29.5, 31.8, 43.3, 49.8, 62.9, 84.4, BC cannot be detected. ^{11}B NMR ($CDCl_3$, 25 °C): δ −15.3 (t, J = 104.23 Hz), 29.6 (s). IR (neat, cm^{-1}): $\acute{\upsilon}$ = 2362 (B–H), 2339 (B–H), 2196 (C ≡ N), 1465 (C–N), 667 (B–N). MS (EI) m/z: 378 (M^+), 339, 324, 310, 296, 282, 268, 254, 240, 226, 212, 198, 184, 170, 156, 141, 126, 111, 96. $C_{21}H_{44}N_2O_2B_2$ (378): calcd C 66.66, H 11.64, N 7.40; found, C 65.77, H 11.48, N 7.54.

X-ray crystallography

Colorless single crystals for compound **1** which are suitable for X-ray diffraction analysis were grown by slow evaporation of a benzene–ether solution at room temperature. Measurements were made at 110(2) K on a Nonius Kappa CCD diffractometer using M_oK_α radiation. Crystal data for: $C_{10}H_{22}B_2O_2N_2$, M = 223.92, monoclinic, space group $P2_1/c$, a = 6.2658(1), b = 11.4851(2), c = 18.7034(5) Å, β = 93.2163(9)°, V = 1343.84(5)Å^3, Z = 4, D_c = 1.107 g cm^{-1}, θ_{max} = 27.4°, μ = 0.073 mm^{-1}, 3050 reflection collected, 2492 reflections with $I \geq 2\sigma(I)$. R = 0.041 (observed data), wR^2 =

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0.100 (all data). Programs used: SHELXL-97, Denzo, SIR-97. CCDC deposition number = 601488.

Biochemical assays

All hydrolytic reactions were performed twice. K_i and IC_{50} determination is described.

Determination of dissociation constant (K_i) to chymotrypsin for compound 2

α-Chymotrypsin, type II, from bovine pancreas was purchased from Sigma-Aldrich Company (M_w 25 000). The inhibitor was tested by competitive assay against benzoyl-tyrosine-*p*-nitroanilide as a substrate. The hydrolytic reaction was carried out at 37 °C at pH 8.00, with sodium phosphate buffer and 0.25 mM NaCl. Inhibitor stock solutions in DMSO were prepared with concentrations ranging from 0.06 to 0.8 mM. A stock enzyme solution of 0.2 mg ml^{-1} was made in 1 mM HCl solution. A series of substrate concentrations (dissolved in 36.6% DMSO and 63.4% methanol) ranging from 0.01 to 0.3 mM was prepared. The assay was performed by adding 750 µl sodium phosphate buffer to a 1.5 ml Eppendorf, followed by 100 µl enzyme and 50 µl inhibitor. The mixture was incubated for 10 min at 37 °C. Then, 100 µl of substrate were added to the reaction followed by incubation for 20 min at 37 °C. The reaction was stopped by adding 200 µl of 30% acetic acid solution. The progress of the reaction was followed by monitoring the appearance of the absorption band of *p*-nitroanilide at 410 nm. The K_i value for this reversible competitive inhibitor was estimated by the method of Lineweaver and Burk. Data were fitted to the best straight line by the least-squares procedures.

Determination of IC_{50} values to chymotrypsin

The hydrolytic reaction was carried out exactly as was done for the determination of the K_i, except for using one substrate solution of 1 mM (dissolved in 36.6% DMSO and 63.4% methanol) instead of a series of substrate concentrations.

Determination of IC_{50} values to trypsin

Trypsin, type IIs, from bovine pancreas was purchased from Sigma-Aldrich Company. The IC_{50} was determined exactly as was done for chymotrypsin, except that the reaction was carried out with a 50 mM Tris buffer and 0.02 M $CaCl_2$ at pH 7.5. A 1 mM solution in DMF of benzoyl-arginine-*p*-nitroanilide was used as a substrate.

RESULTS AND DISCUSSION

Compounds **1**–**4** were prepared in one-pot reaction that we published recently.[20] All compounds were stable as a result of cyanoborane complexation. Thus, they could be purified using column chromatography; all complexes were soluble in benzene and in organic solvents such as chloroform, DMSO, ethanol, ether and dichloromethane, but insoluble in water and in saturated aliphatic hydrocarbons.

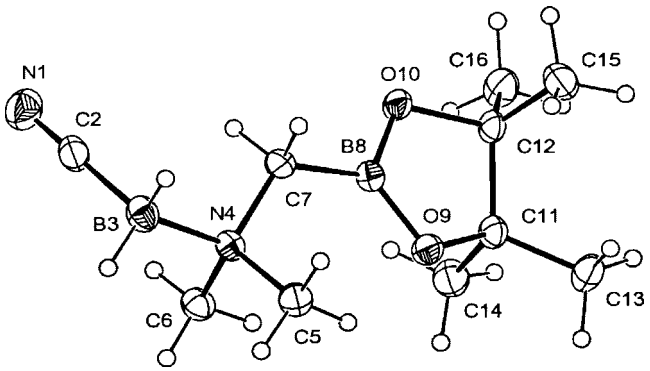

Figure 1. Molecular structure of $C_{10}H_{22}B_2O_2N_2$ **1**. The selected bond distances (Å) and angels (°) are: C2 ≡ N1 1.1514(18), B3–C2 1.5897(19), B3–N4 1.6129(16), N4–C5 1.4968(14), N4–C6 1.4963(14), N4–C7 1.5008(15), C7–B8 1.5777(17), B8–O9 1.3603(16), B8–O10 1.3671(15), B3–C2 ≡ N1 179.31(14), C2–B3–N4 108.75(10), C5–N4–C6 108.53(9), C6–N4–C7 109.98(9), B3–N4–C5 107.87(9), B3–N4–C6 110.89(9), B3–N4–C7 109.56(9), C7–B8–O9 128.08(11), C7–B8–O10 117.93(10), O9–B8–O10 113.97(10).

Crystal structure of 1

The crystal structure of **1** is shown in Fig. 1. Selected geometric parameters are given in the figure caption. The B–CN moiety has the bond angle at C2 being 179.31(14)°. The N1–C2 [1.1514(18) Å], C2–B3 [1.5897(19) Å] and B3–N4 [1.6129(16)Å] distances in the BH_2CN group of **1** are in accord with those found in other cyanoboranes.[21] The coordination geometry of the two boron atoms present in **1** is different. The boron atom with four substituents has a tetrahedral geometry, while the boron atom coordinated to two oxygen atoms and one carbon atom has a trigonal planar geometry.

Biological activity

The α-amino boronate complexes **1**–**4** were all tested for their ability to inhibit trypsin and α-chymotrypsin, which were selected as representative serine proteases. The percentage inhibition was followed by monitoring the appearance of the absorption band at 410 nm (*p*-nitroanilide). The IC_{50} values are listed in Table 1.

As can be seen in Table 1, complexes **1** and **4** had moderate inhibition for chymotrypsin with IC_{50} values of 6.4 and 4.7 mM, respectively. On the other hand, complexes **2** and **3** had better inhibition properties with a K_i of 0.56 mM and an IC_{50} of 0.95 mM, respectively. It is known that aryl and arylalkylboronic acids bind strongly to the serine proteases chymotrypsin.[3,22,23] The reason for this affinity is that the boronic group reversibly forms a tetrahedral adduct with the active site serine hydroxyl group which crudely resembles the transition state for ester or amide

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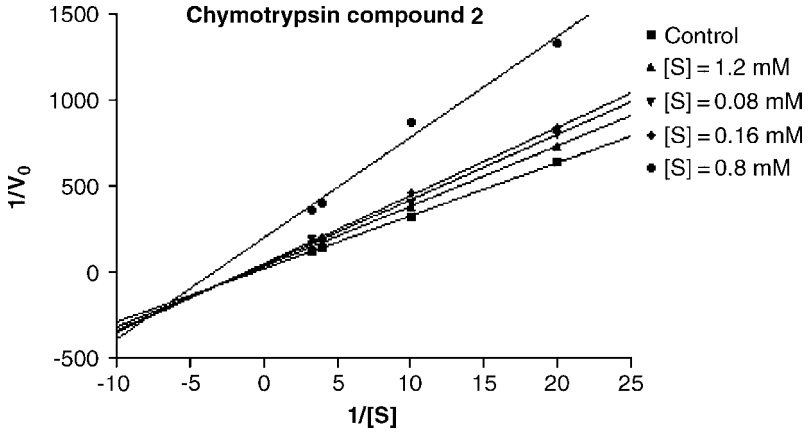

Figure 2. Lineweaver–Burk plot for the inhibition of chymotrypsin without and with complex **2** as inhibitor.

Table 1. Inhibition of α-chymotrypsin and trypsin by α-amino boronate complexes **1**–**4**

α-Amino boronate complexes	Trypsin IC_{50} (mM)	Chymotrypsin IC_{50} (mM)
1	0.08	6.4
2	6.8	0.56[a]
3	2.4	0.95
4	0.53	4.7

[a] K_i value.

hydrolysis.[23–25] Even though there is no aryl group present in the studied complexes, they surprisingly showed moderate inhibition activity. In this context, a study of a K_i for complex **2** that showed the best inhibition properties was performed in order to understand the mechanism of inhibition. The Lineweaver–Burk plot for complex **2** is shown in Fig. 2. The inhibition was competitive and reversible meaning that these complexes bind to the active site of the chymotrypsin. The boron atom is coordinated to two oxygen atoms and one carbon atom interacts with the serine or histidine in the active site of the enzyme, leading to inhibition. More potent inhibitors could be synthesized by introducing functional groups that fit the S subsite of the enzyme. The serine protease, trypsin favors positively charged groups (Arg and Lys). Although the studied complexes herein do not contain charged groups, they were able to inhibit trypsin moderately with IC_{50} values ranging from 0.08 to 6.8 mM. One possible explanation for this behavior is that these complexes may be able to fit into the active site and interact with histidine or serine.

Acknowledgements

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