Research Article

Drug Testing and Analysis

Received: 9 March 2009 Revised: 25 March 2009 Accepted: 26 March 2009 Published online in Wiley Interscience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.24

Synthesis of labeled BCX-4208, a potent inhibitor of purine nucleoside phosphorylase

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BCX-4208, a novel inhibitor of the enzyme purine nucleoside phosphorylase, mimics the charged ribosyl oxocarbenium ion formed during the transition state of the enzyme-catalyzed C-N bond cleavage of nucleosides. A slow-onset, tight-binding inhibitor with a K_i^* of 16 ± 1.4 *p*M, BCX-4208 is one of the most potent inhibitors known for the enzyme. In support of our BCX-4208 clinical program, a mass spectrometric assay has been developed that required labeled BCX-4208 as an internal standard. The synthesis of $[^2H]_2$-BCX-4208 and [^{13}C]-BCX-4208 is described in this report. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: BCX-4208; purine nucleoside phosphorylase; T-cell

Introduction

The enzyme purine nucleoside phosphorylase (PNP, EC 2.4.2.1) catalyzes the reversible cleavage of purine nucleosides to the corresponding purine base and sugar phosphate in the purine salvage pathway as shown in Figure 1.[1]

In the absence of PNP, nucleoside substrates such as 2′-deoxyguanosine (dGuo) accumulate. dGuo accumulation has been observed in children with inherited PNP deficiency and, as a consequence, these children exhibit severe T-cell immunodeficiency but retain normal or exaggerated B-cell function.[2] T-cell cytotoxicity is due to phosphorylation of dGuo (*via* 2′-deoxycytidine kinase, dCK, (EC 2.7.1.74)) to 2′-deoxyguanosine triphosphate (dGTP). Increased dGTP concentration causes an imbalance of the natural nucleotide pool leading to T-cell apoptosis.[3] The relatively unique sensitivity of T-cells is attributed to their relatively high level of dCK compared with other cells. This observation has led to the development of PNP inhibitors for the treatment of T-cell cancers and T-cell autoimmune indications. The biochemical basis for the use of PNP inhibitors as well as the various classes of inhibitors developed has been reviewed.[4]

A new class of PNP inhibitors has been developed using transition state analysis.[5] One PNP inhibitor called BCX-4208 is currently in human clinical trials for the treatment of psoriasis.[6,7] In support of our clinical program we needed to develop a rapid and sensitive method of determining drug levels in biological matrices such as plasma and urine. One method evaluated for this purpose was an LC-MS-MS approach using an isotopically labeled analog as an internal standard. In this paper, we present the synthesis of [^{13}C]-BCX-4208 (**2**) and a $[^2H]_2$-BCX-4208 (**3**) analog, which have been used in these assays (see Figure 2).

Results and Discussion

The synthesis of 9-aminomethyl-9-deazahypoxanthines via a Mannich condensation has been reported.[8] The synthesis of compound **1** was reported from the Mannich condensation of 9-deazahypoxanthine **4**,[9] the iminoribitol derivative **5**,[10,11,12] and formaldehyde (see Figure 3). Using this approach we envisioned that the required labeled BCX-4208 samples could be prepared using HCHO containing the appropriate label. Compounds **2** and **3** were synthesized accordingly, using the Mannich conditions, with yields of 32% and 30% respectively, with isotopic purities of about 98%. The position of the [^{2}H]-label was confirmed at the 1′-CH_2 linker through comparison of the 1′-CH_2 resonances of compounds **1** and**3** (as determined by DEPT 135 experiment to be δ 48.45 for **1**). In the ^{13}C NMR experiment for compound **3**, this signal was greatly diminished in intensity and very broad, possibly due to C-[^{2}H] coupling. The ^{13}C NMR experiment for **2** showed that the 1′-CH_2 signal was greatly enhanced as a result of the ^{13}C enrichment. Further examination of the spectral analysis was outside the scope of this study.

Experimental

General

The ^{1}H NMR spectra were recorded on a Bruker Avance spectrometer at 300 MHz. The ^{2}H NMR spectra were recorded on a Bruker AMX-360 spectrometer at 55.28 MHz. The ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 75.5 MHz. Spectra were recorded at ambient temperature unless otherwise noted. Infrared spectra were obtained on a Bio-Rad FTS-7 FT-IR. Mass spectra were recorded on a Micromass ZMD in the positive electrospray mode with a scan range of 0–1000 m/z and cone voltage setting of 20 V. A solution of the sample (≅100 μg/mL) in methanol (100%) was introduced into the source via a Waters 2690 autosampler. Thin-layer chromatography (TLC) was performed using silica gel 60 plates from E. Merck. Analytical high-performance liquid chromatography (HPLC) was run on a Hewlett-Packard system, Model no. HP-1100, with a photodiode array detector. Ultraviolet detection was at 220 or 256 nm. Column

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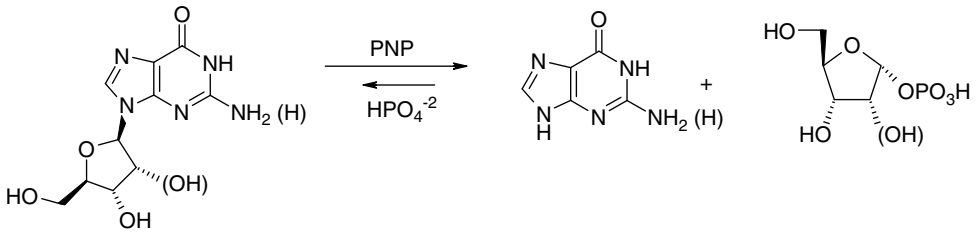

Figure 1. Purine nucleoside phosphorylase (PNP) enzyme catalyzed reaction.

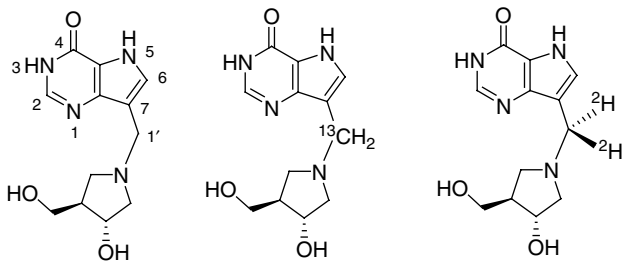

Figure 2. Synthesis of [^{13}C] BCX-4208 (2) and [^{2}H]$_2$-BCX-4208 (3).

chromatographic separations were effected with CMA-80 ($CHCl_3$-MeOH- NH_4OH; 80 : 18 : 2 v/v) and CMA-50 ($CHCl_3$-MeOH-NH_4OH; 50 : 40 : 10 v/v) as eluent. [^{2}H]$_2$-formaldehyde (20% in D_2O, 98 atom % ^{2}H) and ^{13}C-formaldehyde (20% in H_2O, 99 atom % ^{13}C) were obtained from Aldrich Chemical Company, St Louis, Missouri.

7-[-(3R-Hydroxy-4R-hydroxymethyl-pyrrolidin-1-yl)-^{13}C-methyl]-3,5-dihydro-pyrrolo[3,2-d]-pyrimidin-4-one monohydrochloride (2)

To a 100 mL three-neck flask was added 25 mL of deionized water, which was heated to 85°C. To this hot solution were added deazahypoxanthine **4** (0.987 g, 7.31 mmol), sodium acetate (0.705 g, 8.68 mmol), aza sugar **5** (1.32 g, 8.68 mmol) and 20% aq ^{13}C-HCHO (2 g, 12.9 mmol). The reaction mixture was heated further for 20 h while maintaining the same temperature. The completion of the reaction was determined by TLC ($CHCl_3$-MeOH-NH_4OH; CMA80/50 1 : 1). The mixture was cooled to ambient temperature and this was followed by the addition of charcoal (1 g). The reaction mixture was stirred for 30 min and then filtered through a Celite pad. The filtrate was evaporated to dryness under vacuum to furnish a syrupy residue. The crude was purified by column chromatography using CMA-80/CMA-50 (1 : 1) mixture as eluent. The appropriate fractions were pooled together and evaporated to furnish a solid residue. This residue was dissolved in a sufficient amount of 10% EtOH followed by addition of 1.1 eq of concentrated HCl. The reaction mixture was refluxed for 5 min and then cooled to ambient temperature. Evaporation of the solvent gave a solid. The solid residue was dissolved in 5% aq EtOH, refluxed for 0.5 h and cooled to ambient temperature overnight. The crystalline product obtained was collected by vacuum filtration to furnish the desired product **2** (0.818 g, 32%). High-performance liquid chromatography analysis: 98.1% pure. Analytical HPLC conditions: Zorbax SBC3 150 × 4.6 mm and Zorbax SBC3 250 × 4.6 mm plumbed in series. A: 0.05M formic acid, B: acetonitrile, gradient from 0 to 13% B 12 min; 13 to 50% B 6 min; hold 50% B 4 min; 0% B 2 min; hold 100% A 2 min; post time 10 min; flow rate: 1.3 ml/min; temperature 25°C. Retention time for [^{13}C]–**2**: 9.45 min IR (KBr) 3359, 3133, 3041, 1678, 1417 cm^{-1}; MS [ES^+, $(M + H)^+$]: 266.18 (100%); HRMS calculated for $^{13}C_1C_{11}H_{16}N_4O_3$: 266.13228, found: 266.13329, Δ 3.80 ppm; ^{1}H-NMR (ND_4OD): δ 1.37 (m, 1 H), 1.60 (t, 1 H), 1.75 (dd, 1 H), 2.10 (m, 2 H), 2.60–2.85 (m, 3 H), 3.21 (m, 2 H), 6.60 (s, 1 H), 7.38 (s, 1 H); ^{13}C-NMR (ND_4OD): δ 48.45 (^{13}C-1′), 50.05, 55.78, 61.25, 63.49, 73.39, 111.40, 119.76, 128.46, 146.20, 151.39, 163.37. Anal. calculated for: $^{13}C_1C_{11}H_{16}N_4O_3$. HCl: C, 47.41; H, 5.67; N, 18.56; Cl, 11.74. Found: C, 47.17; H, 5.75; N, 18.26; Cl, 11.72.

7-[Dideuterio-(3R-Hydroxy-4R-hydroxymethyl-pyrrolidin-1-yl)-methyl]-3,5-dihydro-pyrrolo[3,2-d]-pyrimidin-4-one monohydrochloride (3)

To a 500 mL three-neck flask was added 250 mL of deionized water and this was heated to 85 °C. To this was added the deazahypoxanthine **4** (9.62 g, 71.3 mmol), sodium acetate (6.80 g, 83.6 mmol), aza sugar **5** (12.8 g, 83.6 mmol) and 20% aq [^{2}H]$_2$-HCHO (20 mL, 125 mmol). The reaction was carried out in the same manner as described earlier for compound **2** followed by crystallization to furnish the desired product **3** (7.12 g, 30%). High-performance liquid chromatography analysis: 97.6% pure. Analytical HPLC conditions: Zorbax SBC3 150 × 4.6 mm and Zorbax

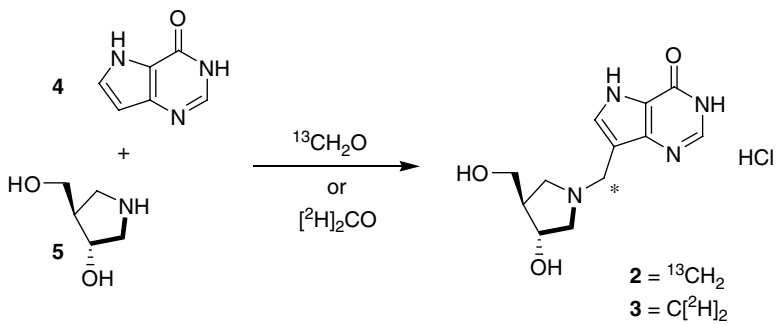

Figure 3. Synthesis of isotopically labelled analogs of BCX-4208 via Mannich condensation.

SBC3 250 × 4.6 mm plumbed in series. A: 0.05M formic acid, B: acetonitrile, gradient from 0 to 13% B 12 min; 13 to 50% B 6 min; hold 50% B 4 min; 0% B 2 min; hold 100% A 2 min; post time 10 min; flow rate: 1.3 ml/min; temperature 25 °C. Retention time for $[^2H]_2$-**3**: 8.99 min IR (KBr) 3359, 3133, 3041, 1678, 1417 cm^{-1}; MS [ES^+, $(M + H)^+$]: 267.17 (100%); HRMS calculated for $C_{12}H_{14}D_2N_4O_3$: 267.14207, found: 267.14247, Δ 1.50 ppm; ^{1}H-NMR (ND_4OD): δ 1.37 (m, 1 H), 1.60 (dd, 1 H), 1.75 (dd, 1 H), 2.10 (m, 2 H), 2.60–2.85 (m, 2 H), 3.21 (m, 1 H), 6.60 (s, 1 H), 7.38 (s, 1 H); ^{13}C-NMR (ND_4OD): δ 48.43 ($^2H_2$C-1′), 50.02, 55.65, 61.12, 63.48, 73.41, 110.96, 119.71, 128.50, 146.20, 151.21, 163.26. Anal. calculated for: $C_{12}H_{14}D_2N_4O_3$. HCl. 0.15 H_2O: C, 47.50; H, 5.96; N, 18.46; Cl, 11.68. Found: C, 47.16; H, 5.77; N, 18.44; Cl, 11.80.

Acknowledgements

We thank Dr Mike Kilpatrick of BioCryst Pharmaceuticals for the isotopic purity determination, Ms Lonna Tucker for checking the manuscript and Dr Ken Belmore of the University of Alabama, Tuscaloosa for carrying out the high-field NMR spectral analysis.

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