

Supplementary Information

Computationally motivated synthesis and enzyme kinetic evaluation of *N*-(β -D-glucoopyranosyl)-1,2,4-triazolecarboxamides as glycogen phosphorylase inhibitors

Jaida Begum,^a Gergely Varga,^b Tibor Docsa,^c Pál Gergely,^c Joseph M. Hayes,^{*a} László Juhász,^{*b} László Somsák^{1b}

^a*Centre for Materials Science, Division of Chemistry, University of Central Lancashire, Preston PR1 2HE, United Kingdom*

^b*Department of Organic Chemistry, University of Debrecen, POB 20, H-4010 Debrecen, Hungary*

^c*Department of Medical Chemistry, Medical and Health Science Centre, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*

General synthetic methods

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter at room temperature. NMR spectra were recorded with Bruker 360 (360/90 MHz for ¹H/¹³C) spectrometer. Chemical shifts are referenced to TMS as the internal reference (¹H), or to the

¹ Corresponding authors

tel: +3652512900 ext 22474 (LJ), 22348 (LS); fax: +3652512744; e-mail: juhasz.laszlo@science.unideb.hu, somsak.laszlo@science.unideb.hu

tel: +44172894334 (JMH), fax: +44172894981; email: jhayes@uclan.ac.uk

residual solvent signals. Microanalyses were performed on an Elementar vario Micro cube. TLC was performed on DC-Alurolle Kieselgel 60 F₂₅₄ (Merck). TLC plates were visualized under UV light, and by gentle heating. For column chromatography Kieselgel 60 (Merck, particle size (0.063-0.200mm) was applied.

General procedure for the preparation of 4-benzyl-*N*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-5-aryl-1,2,4-triazole-3-carboxamides

Method A: A solution of *N*-benzyl-arylcaboxamide (4.7 mmol) in thionyl-chloride (20 mL) was refluxed for three hours and then the excess of SOCl₂, and dry toluene (2 x 20 mL) were evaporated by rotatory evaporator and the residue was used without any purification. Subsequently the crude imidoyl chloride and tetrazole (1.58 mmol) were dissolved in dry toluene (15 mL) and the mixture was stirred at 80 °C. The progress of the reaction was detected by TLC (hexane : ethyl-acetate = 1:1) as a eluent, and when the starting material was transformed completely the solvent was evaporated in vacuum and the residue was purified by column chromatography (eluent: hexane: ethyl-acetate = 3:1)

General procedure for the catalytic hydrogenation.

Method B: A suspension of Pd(C) (10 m/m%; 20 mg) in dry methanol was stirred for 15 minutes under hydrogen atmosphere and then a solution of 4-benzyl-*N*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-5-aryl-1,2,4-triazole-3-carboxamide (0.6 mmol) in dry methanol was added (30 mL) and stirred at 50 °C until the reaction was completed (TLC; eluent:

chloroform-methanol = 1:1). Subsequently the catalyst was filtered off, concentrated in vacuum and used without any purification.

General procedure for the removal of *O*-acetyl protecting groups

Method C: To the solution of a protected sugar derivative in dry methanol (or in dry methanol and dry chloroform) a catalytic amount of NaOMe (1M solution in methanol) was added and stirred at room temperature. The progress of the reaction was monitored by TLC (chloroform: methanol = 9:1). When the starting material was consumed the mixture was neutralized with a cation exchange resin Amberlyst 15 (H⁺ form) or with acetic acid, then the resin was filtered off and the solvent removed. The precipitated product was filtered off, washed with ether and dried.

4-Benzyl-*N*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-5-phenyl-1,2,4-triazole-3-carboxamide (**11a**)

By method A., starting from *N*-benzylbenzamide¹ (**9a**) (1.2 g; 5.6 mmol) and tetrazole **6** (839 mg 1.89 mmol) to give **11a** as white crystals [437 mg, 38%, mp.: 74-76 °C, [α]_D = -5.632, c : 0.4, DMSO] ¹H NMR (CDCl₃) δ (ppm): 1.96 (3H, s, CH₃); 2.02 (3H, s, CH₃); 2.03 (3H, s, CH₃); 2.06 (3H, s, CH₃); 3.82 (1H, ddd, *J* = 9.8, 4.2, <1 Hz, H-5); 4.10 (1H, dd, *J* = 12.6, <1 Hz, H-6[?]); 4.27 (1H, dd, *J* = 12.6, 4.6 Hz, H-6); 5.10, 5.15, 5.30, 5.36 (4 x 1H, 4 x pt, *J* = 9.8, 9.1, 9.1, 9.1 Hz, H-1, H-2, H-3, H-4); 5.63 (1H, d, *J* = 15.4 Hz, CH_{2A}); 5.72 (1H, d, *J* = 15.4 Hz, CH_{2B}) 6.91 - 7.51 (10H, m, aromatic); 8.20 (1H, d, *J* = 9.1 Hz, NH); ¹³C NMR (CDCl₃) δ (ppm): 20.5, 20.6, 20.8, 20.9 (4 x CH₃); 48.9 (CH₂); 61.7 (C-6); 68.2, 70.5, 73.2, 73.9 (C-2, C-3, C-4, C-5); 78.1 (C-1); 126.2 – 136.1 (aromatic); 146.5, 157.8 (C-3, C-5

triazole); 158.26 (NHCO); 169.5, 170.0, 170.1, 170.7 (4 x CO); Anal. Calcd for: C₃₀H₃₂N₄O₁₀ (608.21): C: 59.21, H: 5.30, N: 9.21. Found: C: 59.25, H: 5.32, N: 9.27.

4-Benzyl-*N*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-5-(naphth-2-yl)-1,2,4-triazole-3-carboxamide (11b)

By method A, starting from *N*-benzyl-(naphth-2-yl)-carboxamide² (**9b**) (1.2 g; 4.7 mmol) and tetrazole **6** (700 mg 1.59 mmol) to give **11b** as white crystals [506 mg; 50 %, mp.: 129-131 °C, $[\alpha]_D = -4.323$, $c : 0.5$, DMSO]. ¹H NMR (CDCl₃) δ (ppm): 1.98 (3H, s, CH₃); 2.04 (6H, s, 2 x CH₃); 2.08 (3H, s, CH₃); 3.83 (1H, ddd, $J = 9.8, 4.2, <1$ Hz H-5); 4.12 (1H, dd, $J = 12.6, <1$ Hz, H-6_A); 4.29 (1H, dd, $J = 12.6, <1$ Hz, H-6_B); 5.15, 5.19, 5.34, 5.42 (4 x 1H, 4 x *pseudo t*, $J = 9.8, 9.1$, Hz, H-1, H-2, H-3, H-4); 5.71 (1H, d, $J = 15.4$, CH_{2A}); 5.7 (1H, dd, $J = 15.4$ Hz, CH_{2B}); 6.96 - 7.97 (12H, m, aromatic); 8.35 (1H, d, $J = 9.1$ Hz, NH). ¹³C NMR (CDCl₃) δ (ppm): 20.5, 20.6, 20.7, 21.1 (4 x CH₃); 49.1 (CH₂); 61.7 (C-6); 68.1, 70.4; 73.1, 73.8 (C-2, C-3, C-4, C-5); 77.9 (C-1); 123.2 – 136.1 (aromatic); 146.5, 157.7 (C-3, C-5 triazole); 158.2 (NHCO); 169.6, 170.0, 170.1, 170.7 (CO); Anal. Calcd for: C₃₄H₃₄N₄O₁₀ (658.23): C: 62.00, H: 5.20, N: 8.51. Found: C: 62.10, H: 5.29, N: 8.60.

***N*-(2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranosyl)-5-phenyl-1,2,4-triazole-3-carboxamide (12a)**

By method B, starting from **11a** (0.48 g, 0.78 mmol) and 26 mg 10 m/m% Pd(C) to give **12a** as white crystals (0.384 g, 94 %, mp.: 115-118 °C; $[\alpha]_D = -20.027$, $c : 0.4$; DMSO). ¹H NMR (CDCl₃) δ (ppm): 2.06 (3H, s, CH₃) 2.10 (3H, s, CH₃); 2.11 (3H, s, CH₃); 2.12 (3H,

s, CH₃); 3.93 (1H, ddd, $J = 8.5, 4.0, <1$ Hz, H-5); 4.15 (1H, dd, $J = 12.3, <1$ Hz, H-6_A); 4.34 (1H, dd, $J = 12.3, 4.0$ Hz, H-6_B); 5.18, 5.28, 5.33, 5.59 (4 x 1H, 4 x t, $J = 9.4, 9.4, 9.4, 9.4$ Hz, H-1, H-2, H-3, H-4); 7.53 (3H, m, aromatic); 8.12 (3H, m, aromatic and NH). ¹³C NMR (CDCl₃) δ (ppm): 20.5 (4 x CH₃); 61.7 (C-6); 68.2, 70.5, 73.0, 73.9 (C-2, C-3, C-4, C-5); 78.0 (C-1); 123.4 – 134.1 (aromatic); 146.5, 155.7 (C-3, C-5 triazole); 159.4 (NHCO); 170.0, 170.2, 170.5, 170.7 (4 x CO); Anal. Calcd for: C₂₃H₂₆N₄O₁₀ (518.16): C: 53.28, H: 5.05, N: 10.81. Found: C: 53.34, H: 5.15, N: 10.88

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl)-5-(naphth-2-yl)-1,2,4-triazole-3-carboxamide (12b)**

By method B, starting from **11b** (0.4 g, 0.6 mmol) 20 mg 10 m/m% Pd(C) to give **12b** white crystals [0.296 g, 85 %, op.: 124-126 °C; [α]_D = -22.118, c : 0.3; DMSO]. ¹H NMR (CDCl₃) δ (ppm): 2.03 (3H, s, CH₃), 2.04 (3H, s, CH₃); 2.06 (6H, s, CH₃); 3.91 (1H, d, $J = 8.6$ Hz, H-5); 4.13 (1H, d, $J = 12$ Hz, H-6_A); 4.30 (1H, dd, $J = 12, 4$ Hz, H-6_B); 5.18, 5.27, 5.41, 5.58 (4 x 1H, 4 x t, $J = 9.4$ Hz, H-1, H-2, H-3, H-4); 7.40 - 7.50 (2H, m, aromatic); 7.70 – 7.85 (3H, m, aromatic); 8.04 (1H, d, $J = 8.4$ Hz, aromatic); 8.24 (1H, d, $J = 8.4$ Hz, aromatic); 8.51 (1H, s, NH). ¹³C NMR (CDCl₃) δ (ppm): 20.6 (4 x CH₃); 61.8 (C-6); 68.2, 70.6, 73.0, 73.8 (C-2, C-3, C-4, C-5); 78.0 (C-1); 123.5 – 134.1 (aromatic); 146.5, 155.7 (C-3, C-5 triazole); 165.6 (NHCO); 169.6, 170.1, 170.7 (4 x CO); Calc. m/z : 568.53, Found: (M+Na)⁺: 591.191, Anal. Calcd for: C₂₇H₂₈N₄O₁₀ (568.18): C: 57.04, H: 4.96, N: 9.85. Found: C: 57.11, H: 4.99, N: 9.90.

***N*-(β-*D*-Glucopyranosyl)-5-phenyl-1,2,4-triazole-3-carboxamide (5a)**

By the method C, starting from **12a** (0.3 g; 0.57 mmol) to give **5a** as white crystals [0.175 g, 86%, mp.: 169-171 °C, $[\alpha]_D = 9.34$, $c = 0.54$; DMSO]. ^1H NMR (DMSO- d_6): δ (ppm): 3.18 – 3.24 (1H, m, H-5); 3.31 - 3.38 (2H, m, H-2, H-3); 3.41 – 3.52 (2H, m, H-4, H-6_A); 5.08 (1H, m, H-1); 7.69 - 7.81 (3H, m, aromatic); 8.20 – 8.32 (2H, m, aromatic); 8.69 (1H, s, NH); ^{13}C NMR (DMSO- d_6): δ (ppm): 61.9 (C-6); 69.1, 72.5, 76.91, 78.12, 79.69 (C-1; C-2; C-3; C-4; C-5); 122.91, 123.03, 125.60, 126.17, 126.99, 127.23, 127.72, 128.01, 128.97, 129.58 (aromatic); 148.55, 157.35 (C-3, C-5 triazole); 162.22 (NHCO); Anal. Calcd for: $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_6$ (350.12): C: 51.43; H: 5.18; N: 15.99. Found: C: 51.49; H: 5.21; N: 15.99.

***N*-(β -D-Glucopyranosyl)-5-(naphth-2-yl)-1,2,4-triazole-3-carboxamide (**5b**)**

By method C, starting from **12b** (0.138 g, 0.24 mmol) to give **5b** as white crystals [39 mg, 40%, mp.: 171-173 °C, $[\alpha]_D = 8.14$, $c = 0.64$; DMSO]. ^1H NMR (DMSO- d_6): δ (ppm): 3.15 – 3.26 (1H, m, H-5); 3.29 - 3.40 (2H, m, H-2, H-3); 3.40 – 3.51 (2H, m, H-4, H-6_A); 4.98 (1H, m, H-1); 7.61-7.77 (4H, m, aromatic); 8.10 – 8.21 (3H, m, aromatic); 8.61 (1H, s, NH). ^{13}C NMR (DMSO- d_6): δ (ppm): 60.9 (C-6); 69.9, 71.9, 77.31, 78.81, 79.69 (C-1; C-2; C-3; C-4; C-5); 123.21, 123.43, 125.60, 126.67, 126.83, 127.06, 127.72, 128.32, 128.57, 129.45 (aromatic); 152.41, 156.8 (C-3, C-5 triazole); 157.2 (NHCO); Anal. Calcd for: $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_6$ (400.14): C: 57.00; H: 5.03; N: 13.99. Found: C: 57.07; H: 5.10; N: 14.03.

References

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