Supplementary Information

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

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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 $^{1}H/^{13}C$) spectrometer. Chemical shifts are referenced to TMS as the internal reference (^{1}H), or to the

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residual solvent signals. Microanalyses were performed on an Elementar vario Micro cube. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (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 $SOCl_2$, 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 ºC. 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*-benzylbenzamide[1] (**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, $[\alpha]_D$ = -5.632, c : 0.4, DMSO] ^{1}H NMR ($CDCl_3$) δ (ppm): 1.96 (3H, s, CH_3); 2.02 (3H, s, CH_3); 2.03 (3H, s, CH_3); 2.06 (3H, s, CH_3); 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); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 20.6, 20.8, 20.9 (4 x CH_3); 48.9 (CH_2); 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: $C_{30}H_{32}N_4O_{10}$ (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)-carboxamide[2] (**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]. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.98 (3H, s, CH_3); 2.04 (6H, s, 2 x CH_3); 2.08 (3H, s, CH_3); 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). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 20.6, 20.7, 21.1 (4 x CH_3); 49.1 (CH_2); 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: $C_{34}H_{34}N_4O_{10}$ (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]. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.06 (3H, s, CH_3) 2.10 (3H, s, CH_3); 2.11 (3H, s, CH_3); 2.12 (3H,

s, CH_3); 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). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5 (4 x CH_3); 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: $C_{23}H_{26}N_4O_{10}$ (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; $[\alpha]_D$ = -22.118, c : 0.3; DMSO]. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.03 (3H, s, CH_3), 2.04 (3H, s, CH_3); 2.06 (6H, s, CH_3); 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). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.6 (4 x CH_3); 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: $C_{27}H_{28}N_4O_{10}$ (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]. ^{1}H NMR (DMSO-d6): 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-d6): 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: $C_{15}H_{18}N_4O_6$ (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%, op.: 171-173 °C, $[\alpha]_D$= 8.14, c = 0.64; DMSO]. ^{1}H NMR (DMSO-d6): 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-d6): 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: $C_{19}H_{20}N_4O_6$ (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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