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Novel design principle validated: Glucopyranosylidene-spiro-oxathiazole as new nanomolar inhibitor of glycogen phosphorylase, potential antidiabetic agent

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ABSTRACT

2-Naphthyl-substituted glucopyranosylidene-spiro-oxathiazole prepared following a novel design principle was found to be the best known glucose analogue inhibitor of rabbit muscle glycogen phosphorylase b (K_i 160 nM).

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Glycogen phosphorylase (GP) is a peculiar glycoenzyme involved in the breakdown of the storage polysaccharide glycogen.¹ The liver isoform of GP is the rate limiting enzyme of glycogen metabolism and is, therefore, directly responsible for regulating blood sugar levels. Evidence are accumulating that in the non-insulin dependent (type 2) form of *diabetes mellitus*,² comprising more than 90% of all diagnosed cases,^{3,4} elevated hepatic glucose output is the most important cause of hyperglycemia.^{5–8} Thus, GP is a validated target for the treatment of type 2 diabetes and its inhibition appears of great interest in both academia and industry.^{9–11} GP has six known binding sites for which several inhibitors have been identified.^{12,13} Among these, glucose-based compounds represent a large family with the capability of binding at the catalytic site of the enzyme in most cases with very high selectivity.^{14–16}

N-Acetyl- β -D-glucopyranosylamine¹⁷ (**1**, Chart 1) was among the first efficient glucose analogue inhibitors of rabbit muscle

GPb (RMGPb). Analogous compounds with aromatic substituents as **2**^{17,18} and **3**¹⁹ showed weaker binding properties, and **4**¹⁹ was only slightly better than **1**. As a corollary of early inhibitor design based on glucose derivatives, spiro-hydantoin **5**^{18,20} reached the low micromolar range and its thiohydantoin analogue **6**¹⁸ proved similarly efficient. Extensive protein crystallographic investigations of the corresponding GP-inhibitor complexes showed that a H-bridge between the β -anomeric NH and His 377 main chain carbonyl of the enzyme exists for both *N*-acyl- β -D-glucopyranosylamines^{17,21} **1–4** and spiro-hydantoins^{22–24} **5** and **6** (see outline **A** in Chart 1). This feature was then considered as a main contribution to the strong binding of glucose analogues at the catalytic site of GP, and became almost a dogma for further inhibitor design. In the cases of the spiro-hydantoins, the rigid structure of the planar (thio)hydantoin rings, and that of the bicyclic ring system as a whole was also accounted for the tight binding.^{22,23}

In recent years, we have introduced several new classes of glucose-based compounds as inhibitors of GP displaying inhibition constants in the low micromolar range, among others

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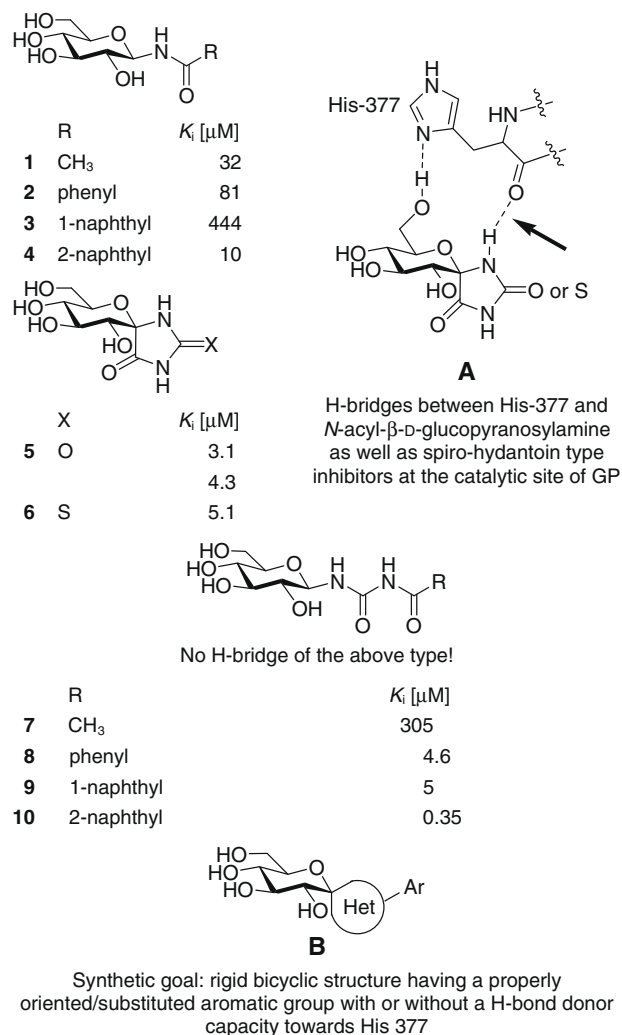


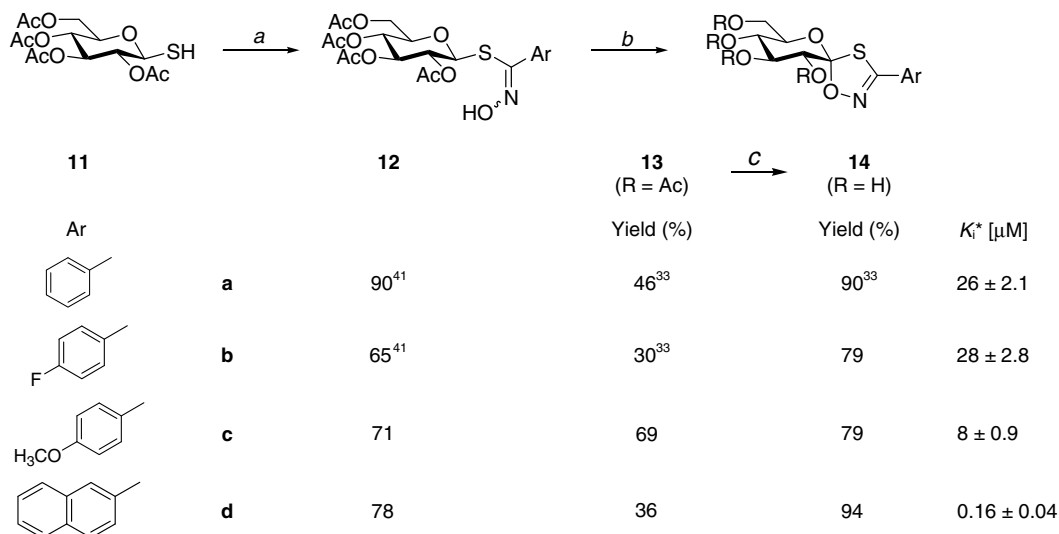
Chart 1. Some glucose-based GP inhibitors (**1–10**) with their inhibition constants (K_i against RMGPb); characteristic binding mode of some inhibitors (A); and general structure of the spiro-bicyclic inhibitors (B).

N -acyl- N' - β -D-glucopyranosyl ureas^{13,16,25–28} **7–10** and C-(β -D-glucopyranosyl) derivatives of aromatic heterocycles such as 1,3,4-oxadiazole, benzothiazole and benzimidazole,^{29,30} as well as 1,2,4-oxadiazoles.^{31,32} The latter heterocyclic derivatives (apart from the benzimidazole) cannot form H-bridges similar to those discussed above because of the lack of suitable hydrogens. On the other hand and rather surprisingly, the absence of this particular H-bond to His 377 was demonstrated by crystallography for **8–10**^{25,27,28} (note that **7** binds to the enzyme in a different conformation²⁵ in which the β -anomeric NH is involved in an intramolecular H-bridge with the acetyl CO). Comparison of the efficiency of inhibition for the pairs **2** and **8**, **3** and **9** and **4** and **10** shows a significant increase in affinity to the enzyme in favour of the acyl ureas by a factor of ~ 18 , ~ 89 and ~ 29 , respectively. Thus, even in the absence of that most important H-bond, very strong binding is possible which must be ascribed to the interactions of the inhibitor with the so-called β -channel^{9,16} (an empty space in the direction of the β -anomeric substituent of bound D-glucose surrounded by amino acid side chains of mixed character) of the enzyme next to the catalytic site. This is further corroborated by the comparison of inhibition by **9** and **10** (~ 14 -fold increase) showing that the orientation of the aromatic part of the molecule is extremely important in order to properly fit into the β -channel as it was also demonstrated by X-ray crystallography.²⁸

Based on these findings, we envisaged that a novel design principle for efficient glucose-based inhibitors of GP can be set up which unifies the properties of the best inhibitors (see generalized formula **B** in Chart 1): (i) such molecules should have a rigid spiro-bicyclic scaffold in which a (preferably five-membered hetero)cycle is attached to the anomeric carbon of β -D-glucopyranose, (ii) this cycle, although it may, should not necessarily be a H-bond donor towards His 377 and (iii) a suitably oriented, large aromatic appendage must be present on this cycle to fit into the β -channel.

In this letter we present some glucopyranosylidene-spiro-oxathiazoles as the first compounds which meet the above design principle and one of them is the most efficient glucose analogue inhibitor of GP known to date.

The synthesis of glucopyranosylidene-spiro-oxathiazoles in a minimum number of steps was previously reported.^{33,34} Besides the known **14a**,³³ we synthesized substituted phenyl derivatives **14b** and **14c** as well as 2-naphthyl derivative **14d** (Scheme 1).



Scheme 1. Synthetic route to the glucopyranosylidene-spiro-oxathiazoles **14a–d**. Reagents and conditions: (a) ArC(=O)NOH , Et_3N , CH_2Cl_2 , rt; (b) NBS, hv, CCl_4 , reflux, 45 min; (c) NaOMe, MeOH, rt. Inhibition constants (K_i) measured for **14** against RMGPb.

Reaction of nitrile oxides, obtained in situ by base treatment of hydroximoyl chlorides,³⁵ with the readily available 2,3,4,6-tetra-*O*-acetyl-1-thio- β -D-glucopyranose³⁶ (**11**) afforded the corresponding thiohydroximates **12a–d**³⁷ in good yields. The spiro-cyclization under photochemical conditions afforded the acetylated glucopyranosylidene-spiro-oxathiazoles **13a–d**³⁸ and subsequent deacetylation under basic transesterification conditions provided the *O*-unprotected target molecules **14a–d**.³⁹ The spiro-cyclization process was stereoselective with the oxygen atom adopting predominantly the axial position as previously demonstrated and the main product was isolated by column chromatography.

The GP inhibitor candidates were evaluated for their inhibition against RMGPb enzyme as previously described⁴⁰ and the obtained inhibitor constants (K_i) are shown in Scheme 1. The phenyl derivative **14a** proved ~5 times weaker inhibitor than the corresponding acyl urea **8**. Substitution of the phenyl ring in the 4-position by a fluorine (**14b**) brought about no change probably due to the similar size of the H and F atoms. Introduction of a methoxy group into the same position (**14c**) made a ~3 times better inhibitor than **14a** suggesting that a bulky substituent on the phenyl ring can be beneficial. Finally, the 2-naphthyl derivative (**14d**) inhibited the enzyme ~2 times stronger than **10**. These preliminary results demonstrate that the combination of a rigid spiro-bicyclic structure with the introduction of a large hydrophobic aromatic moiety in a proper orientation for an optimal interaction with the enzyme significantly improves the biological activity of the molecules.

The 2-naphthyl substituted derivative **14d** is the most potent glucose-based inhibitor of GP to date with an inhibition in the nanomolar range. Based on these preliminary results, we are now synthesizing a more populated family of glucopyranosylidene-spiro-oxathiazoles for their biological evaluation as GP inhibitors. These molecules are expected to bind at the catalytic site of GP and further enzymatic and crystallographic investigations will be reported elsewhere.

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- Typical procedure I*: 2,3,4,6-Tetra-*O*-acetyl-1-thio- β -D-glucopyranose (**11**, 0.363 g, 1 mmol) dissolved in CH₂Cl₂ (5 mL) and Et₃N (0.42 mL, 3 mmol) were added under an Ar atmosphere with continuous stirring to a solution of a hydroximoyl chloride (1.2 mmol) in Et₂O or CH₂Cl₂ (5 mL). After immediate precipitation of Et₃N-HCl, the mixture was stirred further at rt. When TLC indicated completion of the transformation (~1 h), 0.5 M H₂SO₄ (20 mL) was added, and the organic phase was separated, washed by water (2 × 20 mL), and dried (MgSO₄). After removal of the solvent under diminished pressure, the residue was purified by crystallization or column chromatography to afford the desired acetylated hydroximothioates **12a–d**. Characterization for **12d**: R_f = 0.25 (PE/EtOAc, 2:1); $[\alpha]_D^{25} + 4.5$ (c 0.29, MeOH); mp = 154–156 °C; ¹H NMR (360 MHz, CDCl₃) δ 1.90, 1.95, 2.05, 2.07 (4s, 12H, 4 × CH₃), 2.96 (ddd, 1H, H-5), 3.92 (dd, 1H, J_{5,6} = 2.6 Hz, H-6'), 4.05 (dd, 1H, J_{5,6} = 3.9 Hz, J_{6,6'} = 11.9 Hz, H-6), 4.55 (d, 1H, J_{1,2} = 9.2 Hz, H-1), 5.09, 5.04, 4.97 (3t, 3H, J = 9.2 Hz, H-2, H-3, H-4), 7.50–8.10 (m, 7H, H-ar), 10.07 (s, 1H, OH); ¹³C NMR (90 MHz, CDCl₃) δ 20.6, 20.5, 20.4, 20.3 (CH₃), 61.4 (C-6), 75.4, 73.6, 69.8, 67.6 (C-2, C-3, C-4, C-5), 88.3 (C-1), 125.6, 126.8, 127.3, 127.7, 127.9, 128.3, 129.8, 132.6, 133.5 (aromatics), 151.9 (C=N), 170.6, 170.2, 169.2 (C=O); Anal. Calcd for C₂₅H₂₇NO₁₀S (533.56): C, 56.28; H, 5.10; N, 2.63; S, 6.01. Found: C, 56.41; H, 5.06; N, 2.44; S, 5.88.
- Typical procedure II*: A solution of hydroximothioate **12a–d** (1 mmol) and *N*-bromosuccinimide (2 mmol) in CCl₄ (20 mL) was boiled and illuminated by a 60 W heat lamp for 45 min. The reaction was diluted with EtOAc (150 mL) and the organic layer was washed with 5% aqueous Na₂SO₃ (2 × 100 mL) and water (2 × 100 mL), dried (Na₂SO₄), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (PE then PE/EtOAc 65:35) to afford the desired acetylated spiro-oxathiazoles **13a–d**. Characterization for **13d**: R_f = 0.59 (PE/EtOAc, 3:2); $[\alpha]_D^{20} + 44$ (c 1, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 2.04, 2.06, 2.09 (3s, 12H, 4 × CH₃), 4.09 (dd, 1H, J_{6,5} = 2.1 Hz, J_{6,6'} = 12.7 Hz, H-6), 4.35 (dd, 1H, J_{6,5} = 3.7 Hz, J_{6,6'} = 12.7 Hz, H-6'), 4.45 (ddd, 1H, J_{5,6} = 2.1 Hz, J_{5,6'} = 3.7 Hz, J_{5,4} = 10.3 Hz, H-5), 5.28 (m, 1H, H-4), 5.66 (m, 2H, H-2 H-3), 7.51–7.62 (m, 2H, H-ar), 7.84–7.90 (m, 4H, H-ar), 8.01 (s, 1H, H-ar); ¹³C NMR (75 MHz, CDCl₃) δ 20.4, 20.6 (CH₃), 61.1 (C-6), 67.5 (C-4), 68.0 (C-2 or C-3), 70.7 (C-5), 71.1 (C-2 or C-3), 122.4 (C-1), 123.5, 124.4, 127.1, 127.8, 128.0, 128.6, 128.8, 129.4, 132.7, 134.6 (aromatics), 156.5 (C=N), 169.4, 169.5, 169.7, 170.5 (C=O); MS (ESI) m/z = 531.7 [M+H]⁺, 554.0 [M+Na]⁺, 1062.6 [2M+H]⁺, 1084.7 [2M+Na]⁺; HRMS (ESI) m/z = C₂₅H₂₅NNaO₁₀S [M+Na]⁺ Calcd 554.1097. Found 554.1097.
- Typical procedure III*: A solution of acetylated spiro-oxathiazoles **13a–d** (150 mg) and NaOMe (5 mg) in MeOH (15 mL) was stirred at rt for 3 h. The

reaction was neutralized to pH 5–6 with Amberlite IR-120 resin (H⁺ form) and the resin washed with MeOH (2 × 10 mL). The solvent was evaporated under reduced pressure to afford the desired hydroxylated spiro-oxathiazoles **14a–d**. Characterization for **14d**: *R*_f = 0.34 (EtOAc/MeOH, 85:15); [α]_D²⁰ +53.2 (c 1, DMSO); mp = 177–179 °C; ¹H NMR (300 MHz, CD₃OD) δ 3.54 (t, 1H, *J* = 9.4 Hz), 3.72–3.98 (m, 5H), 7.54–7.60 (m, 2H, H-ar), 7.83–7.96 (m, 4H, H-ar), 8.06–8.08 (m, 1H, H-ar); ¹³C NMR (75 MHz, CD₃OD)

δ 62.0 (C-6), 70.7, 72.7, 76.2, 77.5 (C-2 to C-5), 124.4, 126.8, 127.8, 128.2, 128.9, 129.6, 129.8, 129.9, 134.4, 136.0 (aromatics), 157.4 (C=N); MS (ESI < 0) *m/z* = 397.9 [M+Cl][−]; HRMS (ESI < 0) *m/z* = C₁₇H₁₇ClNO₆S [M+Cl][−] Calcd 398.0465. Found 398.0467.

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