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van der Waals interactions govern *C*-β-D-glucopyranosyl triazoles' nM inhibitory potency in human liver glycogen phosphorylase

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ABSTRACT

3-(*C*-Glucopyranosyl)-5aryl-1,2,4-triazoles with an aryl moiety larger than phenyl have been shown to have strong inhibitory potency (K_i values in the range of upper nM) for human liver glycogen phosphorylase (hlGP), a pharmacologically relevant target for diabetes type 2. In this study we investigate in a comparative manner the inhibitory effect of the above triazoles and their respective imidazoles on hlGPa. Kinetic studies show that the imidazole derivatives are 6–8 times more potent than their corresponding triazoles. We also seek to answer how the type of the aryl moiety affects the potency in hlGPa, and by determination of the crystal structure of rmGPb in complex with the triazole derivatives the structural basis of their inhibitory efficacy is also elucidated. Our studies revealed that the van der Waals interactions between the aryl moiety and residues in a hydrophobic pocket within the active site are mainly responsible for the variations in the potency of these inhibitors.

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1. Introduction

Type 2 diabetes (T2D), the most common metabolic disorder, is characterized by abnormal insulin secretion caused by impaired β-cell function and insulin resistance in target tissues and represents more than 90% of all diabetic cases (Alberti et al., 2004). It has emerged as one of the main threats to human health in the 21st century and its prevalence is reaching epidemic proportions, while in 2015 415 million people were living with this disease (approx. half of them may be undiagnosed), the prediction for 2040 is 642 million (International et al., 2015). At present, therapy relies on diet, exercise and several orally administrated hypoglycemic drugs (metformin, sulfonylureas, biguanides, thiazolidinediones, and α-glucosidase inhibitors) (Cheng and Fantus, 2005; Israili, 2011; Krentz and Bailey, 2005; van de Laar et al., 2005) intended to reduce the hyperglycemia. However, these treatments have many adverse side effects, the risk of occasionally severe hypoglycemia, and can be inadequate for large proportions of patients (Moller, 2001; Murata et al., 2004; Oikonomakos and Somsak, 2008; Pasupuleti and Anderson, 2008; Wagman and Nuss, 2001). Accordingly, there is a continued interest for compounds that can improve therapy to combat T2D.

Glycogen phosphorylase (GP; EC 2.4.1.1) is a key enzyme of the human carbohydrate metabolism, catalyzing the first step in glycogen degradation to yield glucose-1-phosphate (Glc-1-P) (Oikonomakos, 2002). Because of this central role, GP is considered an attractive target for the design of hypoglycemic agents (Hayes et al., 2014; Somsak, 2011; Somsak et al., 2008) and many of them have been patented (Donnier-Marechal and Vidal, 2016). The efficacy of GP inhibitors on blood glucose control and hepatic glycogen balance has been confirmed in animal models (Docsa et al., 2011, 2015; Furukawa et al., 2005; Goyard et al., 2016; Hoover et al., 1998; Martin et al., 1998; Nagy et al., 2013). The biologically active GP (MW ~97,500 Da; 842 residues) is a dimer that has pyridoxal 5′-phosphate (PLP) as a cofactor. There are three isoforms of GP designated liver, muscle and brain after the tissues in which they are expressed, that share ~80% sequence homology. The tissue-specific functional differences between liver and muscle phosphorylase result from differences in the subunit interface and not from alterations in residues that bind ligands. There are no insertions or deletions in the sequence of the liver enzyme as compared to the

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muscle and out of the 171 amino acid differences between the two enzymes, 49% (85 amino-acids) are conservative and most of the amino acid changes are functionally neutral, and the active site is conserved in both amino acid sequence and structural architecture (Rath et al., 2000a).

The enzyme activity is regulated allosterically by binding of several metabolite effectors and by reversible phosphorylation of serine-14 and follows the MWC model (Monod et al., 1965). Thus, it exists in two intercovertible forms: a phosphorylated form (GPa, R state; high activity and substrate specificity) and an unphosphorylated (GPb, T state; low activity and substrate specificity), which are in equilibrium. The molecular basis of the allosteric transition from the T to R state lies with a flexible loop (280s, residues 282–286) which in the T state partially blocks access to the catalytic site. On transition from T state to R state (activation of the enzyme), the 280s loop becomes disordered and displaced, opening a channel that allows a crucial residue, Arg569, to enter the catalytic and create the recognition site for the substrate phosphate; that also allows access of the substrate (glycogen) to reach the catalytic site and promotes a favorable electrostatic environment for the 5′-phosphate of the essential cofactor PLP (Barford and Johnson, 1989; Barford et al., 1991; Johnson et al., 1990; Oikonomakos et al., 2006). Apart from the catalytic site at the center of the GP structure, X-ray crystallography studies have revealed the existence of another six regulatory peripheral sites: the allosteric, the inhibitor, the new allosteric, the quercetin, the benzimidazole and the glycogen storage site (Hayes et al., 2014).

Although the binding of a large number of ligands to the various GP sites has been investigated by biochemical and structural studies, the vast majority refers to ligands that bind at the catalytic site and are glucose derivatives (Kantsadi et al., 2012b; Oikonomakos and Somsak, 2008; Praly and Vidal, 2010; Somsak, 2011). α-D-Glucose is the physiological inhibitor of GP, albeit weak, with an inhibition constant (K_i) of 1.7 mM (Martin et al., 1991) for GPb and 4.9 mM (Papageorgiou et al., 1989) for GPa, respectively. Glucose, in a self-regulated system, inhibits glycogen degradation and induces glycogen synthesis by acting directly on GP and as a result lowers blood glucose levels. Furthermore, the GPa-glucose complex is a better substrate for inactivation by dephosphorylation with phosphorylase phosphatase than free GPa (Oikonomakos, 2002). Inhibition of the catalytic site of the enzyme has therefore been probed, during the last decade, with more than 200 glucose derivatives (Hayes et al., 2014; Oikonomakos, 2002; Somsak, 2011; Somsak et al., 2008; Somsák et al., 2005). The efficiency of glucose analogues has been attributed to the exploitation of an extensive network of polar and non-polar interactions with residues that form the β-cavity within the catalytic site, so called because only β-substituents at the anomeric *C*1 atom of glucose can bind there (Oikonomakos, 2002). From the variety of the investigated glucose analogue GPIs glucopyranosylidene-spiro-hetero cycles, *N*-acyl-*N*′-β-D-glucopyranosyl ureas, and C-glucopyranosyl heterocycles (Praly and Vidal, 2010; Somsak, 2011), emerged as submicromolar inhibitors against rabbit muscle glycogen phosphorylase b (rmGPb) the prototype of GPs (Chrysina, 2010). From the latter class *C*-β-D-glucopyranosyl 1,2,4 triazoles (Kun et al., 2014) and imidazoles (Bokor et al., 2015) have shown the highest potency against rabbit muscle GPb (rmGPb). Recently, it has been demonstrated that 3-(β-D-glucopyranosyl)-5-substituted-1,2,4-imidazoles are the best known glucose derived inhibitors for the pharmacologically relevant hlGPa (Kantsadi et al., 2016). X-ray crystallography revealed the structural features of the strong binding, and also offered an explanation for the absence of inhibition for the pyrrole derivatives (Kantsadi et al., 2016).

In this study we investigate in a comparative manner the inhibitory effect of the above imidazoles and triazoles (Table 1) on hlGPa. We also seek answer to the question how the number of hydrogen bond acceptor atoms in the heterocycle affects the potency in hlGPa, and by determination of the crystal structure of rmGPb in complex with the triazole derivatives the structural basis of their inhibitory efficacy is also elucidated.

2. Results and discussion

2.1. Synthesis

5-Aryl-3-(β-D-glucopyranosyl)-1,2,4-triazoles **2a**, **2b**, and **2d** have been described earlier (Kun et al., 2014). Compound **2c** with a partially saturated aromatic group was obtained when **3** (Kun et al., 2014) was subjected to catalytic hydrogenation (Scheme 1) to remove the *N*-benzyl protecting group. Under the applied conditions an inseparable mixture of **2b** and **2c** was obtained. Therefore, the mixture was acetylated by Ac_2O in pyridine and the so obtained compounds **4b** and **4c** could be separated by column chromatography. *O*-Deacetylation of these derivatives under Zemplén conditions gave pure **2b** and **2c**, respectively.

2.2. Kinetic studies

To validate our 1,2,4-triazole series we tested their inhibitory potency via kinetics experiments with hlGPa in the direction of glycogen synthesis and for comparison purposes we also determined their potency with rmGPa. The inhibition constants are shown in Table 1 together with those of imidazoles. All compounds displayed competitive inhibition with respect to the substrate Glc-1-P as revealed by the Lineweaver-Burk plots that intersect at the same point on the y-axis for hlGPa and rmGPa. The K_i values derived from the Dixon plots (1/v vs [I]). The inhibitors display K_i values lower (**2a**, 4-fold; **2b**, 2.4-fold; **2c**, 6.8-fold; **2d**, 6-fold) for rmGPa (Table 1) (Kun et al., 2014) than for rmGPb indicating a small preference for rmGPa over rmGPb. The most potent compounds of this group are **2b** (K_i = 0.172 μM) and **2c** (K_i = 0.216 μM) with a 2-naphthyl and a 5,6,7,8 tetrahydronaphthalen-2-yl group as a substituent, respectively. It seems that the presence of a longer *R* group leads to increased inhibitory potency. Furthermore, it seems that the compounds do not display any preference for the two isoenzymes (hlGPa and rmGPa) since they inhibit both with similar potency. Interestingly the substitution of imidazole by a triazole does not lead to an increase of the potency since **2a** and **2b** are approximately 10 times less potent than their imidazole counterparts **1a** and **1b** (Table 1).

2.3. Structural studies

Towards elucidating the structural basis of inhibition and most importantly the differences in the inhibition constants, we have determined the crystal structures of the inhibitors in complex with rmGPb. The $2F_o–F_c$ and $F_o–F_c$ electron density maps clearly defined the position of each atom of the inhibitors and specifically showed that all the studied compounds were bound at the catalytic site of rmGPb (Fig. 1). For **2b** and **2c** additional electron density (Fig. 1) was located at the new allosteric site indicating that these inhibitors were also bound at this site.

The superposition of the structures of native rmGPb and the rmGPb – inhibitor complexes over well-defined residues (18–249, 262–312, 326–829) gave r.m.s.d. values of 0.16, 0.14, 0.14, 0.15 Å (Cα positions), for the **2a**, **2b**, **2c**, and **2d** complexes respectively, indicating that the binding of the inhibitors did not trigger any major conformational change of the overall protein structure. The anchor point for each of the inhibitors at the catalytic site appears to be the glucose moiety which is engaged in hydrogen bond and van der Waals interactions (Table 2) almost identical to

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