The Prototype of Glycogen Phosphorylase

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Abstract: The quest for the discovery of new antihyperglycaemic agents has been more intense the last years due to the rapid increase of mortality associated with type 2 diabetes. Glycogen metabolism has been one of the major causes of the elevated blood glucose levels; hence, special attention has been drawn to the control of the enzymes implicated in the relevant pathway. To this end, the allosteric enzyme of glycogen phosphorylase, has been proposed as molecular target for the design of potential new antidiabetic agents by an interdisciplinary approach comprising organic synthesis, kinetic and X-ray crystallographic studies and physiological experiments. The results derived from the thorough investigation of the catalytic site of the enzyme with the structure-based inhibitor design approach are summarized with emphasis on the most potent inhibitors identified for different classes of compounds.

Keywords: Glycogen phosphorylase, structure-based drug design, type 2 diabetes, X-ray crystallography, β-D-glucopyranosyl analogues.

Dedicated to the memory of Dr. Nikos G. Oikonomakos for his devotion in the research of glycogen phosphorylase inhibitor design with biochemical and structure-based approaches.

INTRODUCTION

Type 2 diabetes (T2D) is among the most prominent diseases because of both its increasing prevalence and the concomitant socioeconomic impact according to the World Health Organization. There is growing evidence that it is also interconnected with other major diseases such as cancer and micro- and macrovascular disease (including cardiovascular disease) by stimulating insulin resistance pathways involved in inflammation, cell proliferation and atherosclerosis. Therefore, researchers make significant efforts to investigate the pathophysiology of T2D from the prediabetic state to post diagnosis treatment in order to prevent the effects of this disease at gene or protein level [1-4].

Different interventions have been suggested for the treatment of T2D based on the ability of potential antihyperglycemic agents to lower the levels of glycated hemoglobin (HbA1c less than 7.0 %). These are metformin (biguanide, decreases hepatic glucose output), sulfonylureas (enhance insulin secretion), glinides (stimulate insulin secretion), α-glucosidase inhibitors (reduce the polysaccharide digestion rate in the proximal small intestine), thiazolinidinediones (glitazones, peroxisome proliferator-activated receptor γ-modulators that increase the sensitivity of muscle, fat and liver to endogenous and exogenous insulin), insulin, glucagon-like peptide-1 agonists (exenatide, prevents glucose-mediated insulin secretion), amylin agonists (pramlintides, inhibits glucagon production) and dipeptidyl peptidase-4 inhibitors (increase glucose-mediated insulin secretion and suppress glucagon secretion) [3, 5] and are prescribed either as mono- or combination therapy. Hence, the need to develop a consensus algorithm for the medical management of hyperglycemia in type 2 diabetes became apparent to help medical practitioners (physicians, endocrinologists and others) choose the most appropriate therapeutic interventions for T2D with the minimum risk of hypoglycemia [5, 6]. In addition, drug interactions in the case of combination treatments is a major issue that both medical doctors and researchers need to consider minimizing unwanted clinical implications [7]. Thus, the quest for the discovery of novel or improved antihyperglycaemic agents has become more intense.

Long-term studies on glycogen metabolism, one of the major causes of the elevated blood glucose levels, revealed that the enzymes implicated in glycogenolysis / gluconeogenesis metabolic pathway could serve as molecular targets for the treatment of T2D. In particular, glycogen phosphorylase (GP), discovered by Carl and Gerti Cori in the late 1930s, is an allosteric enzyme directly involved in glycogen degradation through phosphorolytic cleavage of α-1,4-glucosidic bonds to form glucose-1-phospate that is finally released as glucose in the blood stream. Hence, it can be classified among the targets for the development of new hypoglycaemic agents as proposed also by previous work performed in isolated cells and in physiological experiments with animal models [8].

GP is a dimer and is allosterically activated by phosphorylation through its specific kinase, glycogen phosphorylase (PhK) (discovered by Fisher and Krebs, 1955). In fact, it was the first enzyme for which control by reversible phosphorylation was established, and its biochemical and structural properties have been under investigation since then. It is expressed in three different tissues in human body; liver,

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muscle and brain. The three isoforms share high identity (overall 80-83 % and at the catalytic site 100%) offering a challenge to design inhibitors with high specificity. In all tissues, GP along with the bifunctional glycogen debranching enzyme, catalyse glycogen degradation to glucose-1-phosphate (Glc-1-P). The liver is mainly responsible for glucose homeostasis by rapidly switching from glucose uptake in the fed state to glucose production during fasting. It exists in at least two inter-convertible forms, a low affinity T-state and a high affinity R-state, which are in equilibrium. In the presence of various allosteric inhibitors (ATP, glucose-6-phosphate, glucose and caffeine), transition from the T to R state promotes the inactive form GP*b* of the enzyme, which is better substrate for protein phosphatase 1 and is the prevalent form in the resting muscle [9]. Similarly, allosteric activators (AMP (or IMP), substrates or certain substrate analogues) promote the active form, GPa.

The crystal structures of T and R states of the rabbit muscle enzyme (RMGP) in the presence of various effectors showed that the most significant changes in the tertiary structure occur at the intersubunit interface and are mainly reflected to the flexible 280s loop (comprising residues 282 to 285) that blocks access to the catalytic site of the enzyme (in the T-state) [10-19]. Structural studies have revealed five distinct binding sites; the catalytic site, the glycogen storage site, the Ser14-phosphate recognition site, the allosteric site, the inhibitor and the new allosteric site [13, 20] as well as a new benzimidazole site [21] the significance of which has yet to be elucidated. Most of these binding sites have been thoroughly investigated for the design of potent inhibitors and the discovery of new antidiabetic agents. Various classes of compounds including glucose and non-glucose analogues have been kinetically and structurally characterized and physiological experiments in hepatocytes have been reported for several potent candidates emerging from studies with either the liver or muscle enzyme [8, 18, 22-27].

Previous reviews provide a detailed analysis of the binding mode of different classes of compounds to GP as shown by their complex crystal structures determined by X-ray crystallography [17-18, 28 and references therein]. This review will give special emphasis on the catalytic site of RMGP*b* (shares 97% identify with the human muscle enzyme and 100% identify with the liver isoenzyme) and highlight the most potent glucose analogues identified until present for this site. The new directions in structure-based inhibitor design for this site will also be discussed.

CATALYTIC SITE

The catalytic site of GP is located at the core of the molecule and is lined by both polar and non-polar residues. The essential cofactor for catalysis, pyridoxal 5'-phosphate (PLP) is also located at the same site at a distance of some 15 Å from the surface. To elucidate the role of PLP in GP catalysis, systematic studies were performed. The results obtained converged that PLP has a catalytic role with the 5'-phosphate group being characterized as a 'proton shuttle'. The catalytic reaction in the direction of synthesis is initiated by activation of the substrate by protonation and the same mechanism applies in the reverse reaction [29-32]. Entrance to catalytic site is possible *via* a narrow channel (~12 Å long and ~5 Å wide), which is flooded with water molecules that participate in an extensive network of water-mediated interactions as indicated by the crystal structure of both the native enzyme and its complexes with various compounds determined at high resolution. The overall shape of the interior resembles a lagoon bisected into two sub-sites; one adjacent to the 280s loop (comprising residues 282-287) that prevent access to the catalytic site in the T state of RMGP*b*, the so-called β–pocket with no access to the bulk solvent and a smaller water-filled side channel accessible to α–C–1-substituents (Fig. (**1**)). The water structure at both sites is rather conserved forming interactions with the residues lining the site. In particular, the complex structure of RMGP*b* with α-D-glucose reveals that the glucopyranose peripheral hydroxyl groups form strong either direct or water-mediated hydrogen bonds with the environment determining the properties of this site. To this end, both the water structure and the interactions formed with residues lying at both sub-sites of the catalytic channel have been considered for incorporating additional groups to obtain more potent inhibitors compared to glucose [21, 33-36].

GLUCOSE AS A LEAD FOR ANTIDIABETICS

A number of different classes of compounds have been studied in complex with RMGP*b* all emerging from either α- or β-D-glucose as a lead molecule [17-18, 27-28, 37 and references therein]. The collection includes among others glucopyranose amides [34, 38-46], acetyl and benzoyl urea [42], phosphoramidate [36] and oxadiazole derivatives [47]. Out of the various scaffolds of those analogues tested for their potency to inhibit RMGP*b* activity, the best compounds identified for each class as competitive inhibitors of the substrate glucose-1-phosphate are summarized in Table **1**.

To elucidate the binding mode of the most interesting glucose analogues, structural studies with all compounds have been performed in complex with RMGP*b*. Some of the fundamental design issues for the new derivatives aspiring to trace more potent inhibitors than α-D-glucose (K_i of 1.7 mM) at this stage, have been i) to comprise a glucopyranose ring, ii) to make the most of the position of the water structure at the catalytic site by either forming energetically favorable interactions with the residues in the vicinity or introducing new groups to the lead molecules to exploit the positions previously occupied by solvent molecules and iii) to stabilize the closed conformation of the 280s loop that promotes the T-state (inactive form) of the enzyme. These requirements are only at the level of design, synthesis and *in vitro* binding studies and no additional criteria to nominate them as eligible for further studies (e.g. physiological experiments) have been considered.

N-Acetyl-β-D-glucopyranosylamine (**1**) has been reported as "one of the first successes" with a K_i of 32 μM, ~50 times better compared to that of α-D-glucose [34]. Its three dimensional structure in complex with RMGP*b* highlighted a hydrogen bond interaction formed between the amide nitrogen and the backbone carbonyl oxygen of His377. In fact, structural studies of more analogues with an amide nitrogen at C1 position in β-configuration showed that binding is enhanced in the presence of this favorable hydrogen bond that has been designated as 'characteristic' [44-45].

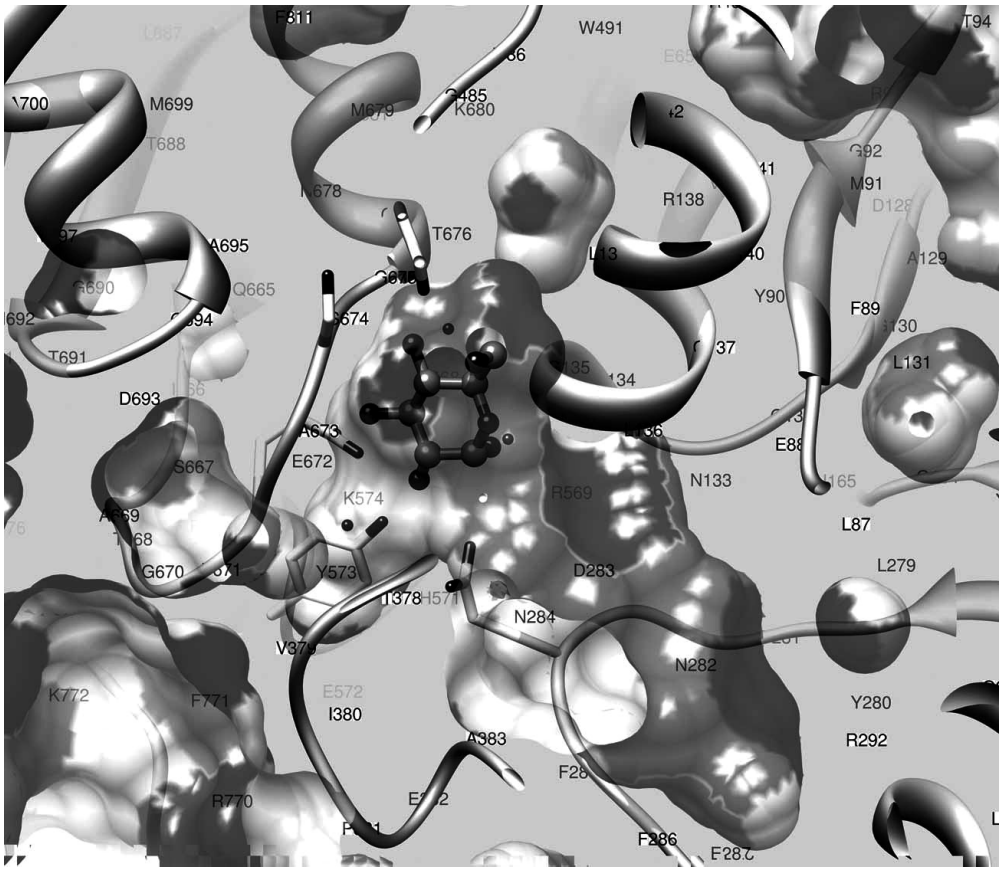


Fig. (1). Schematic representation of the hydrophobic molecular surface of RMGP*b* at the catalytic site (light grey: hydrophilic, dark grey: hydrophobic). A clipping plane perpendicular to the view axis has been applied to reveal the binding pocket of glucopyranose moiety. The secondary structure of the enzyme and the position of the residues lining the site are shown. The figure was prepared with *UCSF Chimera* [65].

The structure of N-acetyl-β-D-glucopyranosylamine was used as a lead for the design of new inhibitors [17-18, 28, 37] exploring also substituents such as phosphoramidate [36] azidoacetamido group [40], 1,4-benzodioxane derivatives [46], C-glycosylated oxadiazoles [47], and 1-(β-D-glucopyranosyl)biurets [48]. Although several similar studies highlight the pivotal role of this interaction in the new analogues' affinity, there is evidence that when present it does promote binding but is not a prerequisite. This finding was supported by kinetic and structural studies of a new compound, a substituted urea of β-D-glucose, the N-benzoyl-N'-β-D-glucopyranosyl urea [42]. The rationale behind the design of this molecule was to improve potency coupling the amide nitrogen aptitude with the knowledge derived from the solvent structure at the catalytic site. The new glucose analogue was shown to be more potent inhibitor of RMGP*b* (K_i value of 4.6 μM) than N-acetyl-β-D-glucopyranosylamine, although the aforementioned direct hydrogen bond between the amide nitrogen and the carboxyl oxygen of His377 is missing and a remarkable shift of the 280s loop is documented (Fig. (**2**)) [42]. Comparable were the results obtained by more N-β-D-glucopyranosyl urea derivatives [(Nagy, V.; Felföldi, N.; Praly, J.-P.; Docsa, T.; Gergely, P.; Chrysina, E.D.; Tiraidis, C.; Alexacou, K.M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Somsák, L. in preparation] except one analogue where the phenyl ring was substituted by a naphthyl group resulting in a refined K_i to nM range [Chrysina, E.D.; Nagy, V.; Felföldi, N.; Telepó, K., Praly, J.-P.; Docsa, T.; Gergely, P.; Alexacou, K.M.; Hayes, J.M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Somsák, L. in preparation] (Table **1**-IV, compound **7**).

The significance of the naphthyl group for this site is illustrated by kinetic and structural studies of different classes of glucose analogues where the naphthyl moiety is present at a distance from C1 of the sugar ring similar to the one of the aforementioned naphthyl urea analogues (Table **1**-V,VI). The results obtained showed that the presence of this group promotes the inactive state of RMGP*b* with potency also in the μM range [49, 50] indicating that RMGP*b* is prone to binding aromatic, rigid structures at the β-pocket of the catalytic site.

The α-configuration of glucopyranose analogues was also explored to utilize the structural features of the catalytic channel (i.e. the pocket filled with water molecules in the vicinity of hydroxyl group in α-D-glucose) without any exceptional results though [51, 52] (Table **1**-IX). To this end compounds such as spirohydantoin of glucopyranose exhibited satisfactory inhibition, however, it was only through the glucopyranosylidene spiro compounds that the importance of

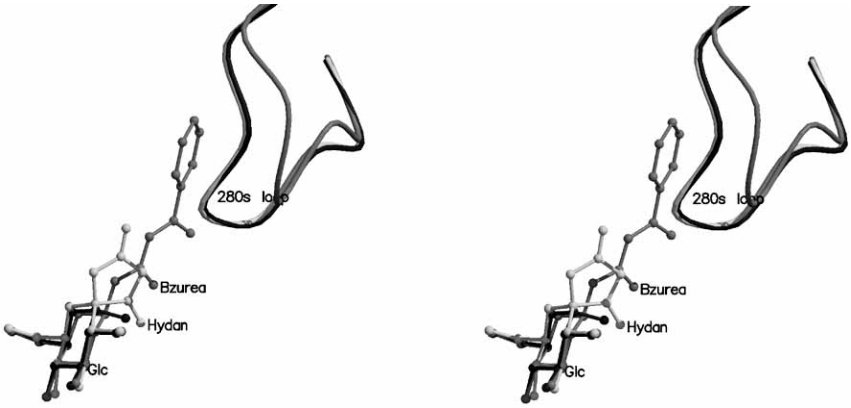


Fig. (2). Stereo representation of the superimposed β-D-glucopyranose analogues in complex with RMGP*b* bound at the catalytic site. The relevant position of the 280s loop in each complex structure is shown focusing on the shift of the loop Ca atoms upon binding of N'-acetyl-N-β-D-glucopyranosyl urea. Different shades of grey colour have been used. Dark grey, RMGP*b*:α-D-glucose (Glc); grey, RMGP*b*: N'-acetyl-N-β-D-glucopyranosyl urea (Bzurea); light grey,: RMGP*b*: spirohydantoin of glucopyranose (Hydan). The figure was prepared with the program *MolScript* [66] and rendered with *Raster3D* [67].

spiro moiety along with the naphthyl group was highlighted, coupling the information suggested by previous studies on the glucopyranose urea derivatives. Apparently, the rigidity of the spiro- and 2-naphthyl groups that undergo little conformational entropy are considered favourable as reflected in the *K*i obtained from the corresponding compounds. (Table **1**-X).

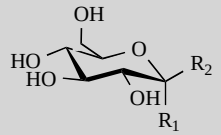
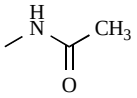
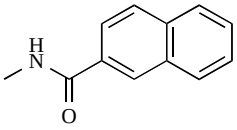
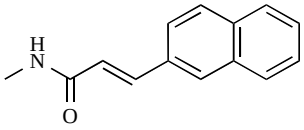
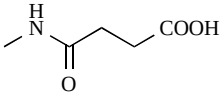
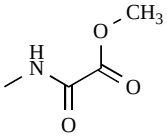
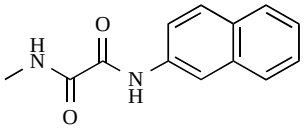
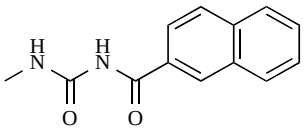
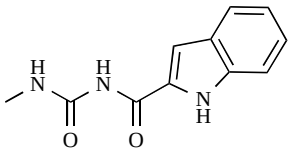
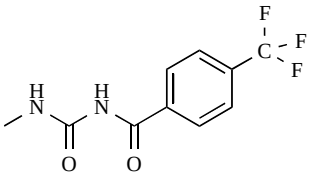
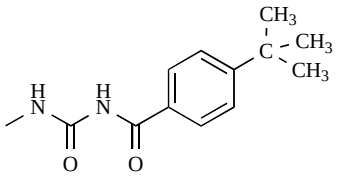
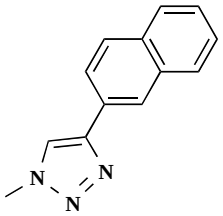
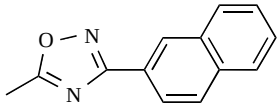
In an attempt to further investigate the preference shown on rigid structures new compounds were designed utilizing natural substituents (pyrimidines, purines, aminoacids etc.). These were synthesized by Gimisis and coworkers [Cismas, C.; Pantzou, A.; Gimisis, T. in preparation] and studied with kinetic and X-ray crystallographic experiments [Chegkazi, M.; Stathis, D.; Sovantzis, D.; Mamais, M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Gimisis, T.; Chrysina, E.D., in preparation]. Docking calculations taking into account ionization/tautomeric states of the nucleic acid functionalized β-D-glucose derivatives which in combination with the X-ray data provided a clearer picture of the binding modes of each analogue. Compound **15** (Table **1**, VII) is the result of computer-aided inhibitor design and was found to exhibit improved potency against RMGP*b* with the phenyl ring pointing into the β-subsite of the catalytic channel. The knowledge derived by both *in silico* and *in vitro* studies with this compound direct the synthetic approaches towards incorporating extended aromatic substituents targeting the β-cavity [Chegkazi, M.; Stathis, D., Sovantzis, D., Mamais, M., Leonidas, D.D., Zographos, S.E., Oikonomakos, N.G., Gimisis, T., Chrysina, E.D. in preparation].

In general, the studies performed on the selection of analogues described in Table **1** give insights in the structure-activity relationship of these compounds upon binding to RMGP*b*. The major determinant of the inhibition observed appears to be the less rigid, mostly hydrophobic part of the compounds investigated positioned at the β-channel. This resulted in tight binding as reflected in the enzyme inhibition observed, although significant changes i.e. in the backbone atoms of the 280s loop occur. Apparently, stabilization of the loop residues in the new position is overall energetically favourable. The rigidity of the glucopyranosylidene analogues in the α-configuration coupling the previously recorded preference for spiro and naphthyl groups, also promotes binding. This suggests that introduction of new functional groups applying a similar rationale at the α-subsite could also be explored even though accommodation of substituents at this position might need to induce larger changes compared to those observed in the residues lining the β-channel.

FUTURE CHALLENGES

Overall, the catalytic site of RMGP*b* has been probed with different classes of compounds and its mixed character (polar and non-polar) was revealed. The importance of 280s loop has been elucidated; however, further investigation is required to achieve both tight binding of an enzyme inhibitor and energetically favourable conformation of the residues lining the loop towards promoting the T-state of RMGP*b*. In particular, detailed study of Asn282, Asp283 and Asn284, the side chains of which are involved in the binding mode of different glucose analogues studied until present, might give new insights in this direction. Therefore, the flexibility of the 280s loop should be taken into account in docking calculations to ensure that more accurate results will be produced. The significance of the enzyme environment on the tautomeric equilibrium, (when applicable) has also been highlighted and it is an extra variable that should be taken into consideration in the process of ligand design [Cismas, C.; Hayes, J.M.; Sovantzis, D.; Hadjiloi, T.; Mamais, M.; Lazoura, E.; Grammatopoulos, P.; Panagopoulos, P.; Stathis, D.; Zographos, S.E.; Leonidas, D.D.; Oikonomakos, N.G.; Gimisis, T.; Chrysina, E.D. in preparation]. Although mapping of the direct or water-mediated interactions formed upon binding of these compounds alone does not give sufficient information to assess the binding mode of a new analogue, the solvent structure has served as a good probe and

Table 1. Selection of Glucose Derivatives that Exhibited Increased Potency to Inhibit RMGP*b* Activity and Might be Exploited as Leads for New Antidiabetic Agents

<table>
<tr><td> 
R1 = H
R2 =</td><td colspan="2">I. N-acyl-β-D-glucopyranosylamines
 
1. K_i 32 μM [30]
 
2. K_i 4 (10) μM [35]
 
3. K_i 3.5 μM [35]</td></tr>
<tr><td>II. N-β-D-glucopyranosyl monoamides of dicarboxylic acids
 
4. K_i 20μM [37]</td><td colspan="2"></td></tr>
<tr><td colspan="3">III. N-β-D-glucopyranosyl oxamic acid derivatives
 
5. K_i 210 μM [39]
 
6. K_i 56μM [#]</td></tr>
<tr><td colspan="3">IV. N-acyl-N'-β-D-glucopyranosyl ureas
 
7. K_i 0.35μM [=]
 
8. K_i 4μM [=]
 
9. K_i 1.8 μM [=]
 
10. K_i 0.7 μM [=]</td></tr>
<tr><td>V. 1(-β-D-glucopyranosyl)-4-substituted-1,2,3-triazoles
 
11. K_i 16.1 μM [46]</td><td>VI. C-β-D-glycopyranosyl derivatives
 
12. K_i 2.4 μM [45]</td></tr>
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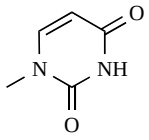
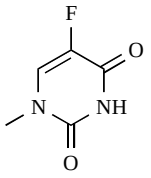
(Table 1). Contd…..

VII. β-D-glucopyranosyl nucleosides

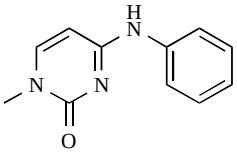
i) N-β-D-glucopyranosyl pyrimidines *



13. K_i 6.1 μM

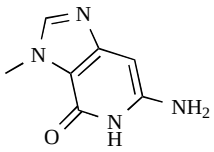


14. K_i 5.5 μM



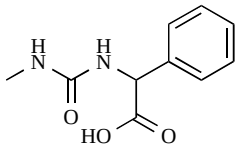
15. K_i 1.5 μM

ii) N-β-D-glucopyranosyl purines

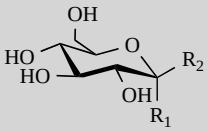


16. K_i 0.63 μM +, ++

VIII. *N*-(β-D-glucopyranosylaminocarbonyl)-L-amino acids



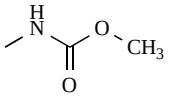
17. K_i 350 μM +, ++



R1 = –C(=O)–NH_2

R2 =

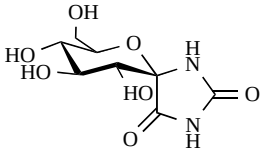
IX. N-glycosides of β-D-gluco-hept-2-ulopyranosonic acid derivatives



18. K_i 16 μM [51, 53]

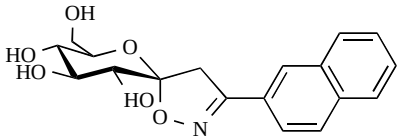
X. Glucopyranosylidene analogues

i). spirohydantoin



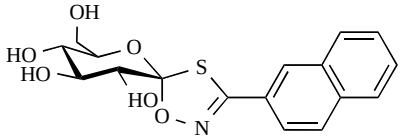
19. K_i 3.1 μM [54]

ii). spiro-isoxazolines



20. K_i 0.63 μM [51]

iii). spiro-oxathiazoles



21. K_i 0.25 (0.16) μM [52] ≈

#Czifrák, K.; Felföldi, N.; Docsa, T.; Gergely, P.; Chrysina, E.D.; Kyritsi, C.; Siafaka-Kapadai, A.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Somsák, L. in preparation

≈Chrysina, E.D.; Nagy, V.; Felföldi, N.; Telepó, K.; Praly, J.-P.; Docsa, T.; Gergely, P.; Alexacou, K.M.; Hayes, J.M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Somsák, L. in preparation.

*Cismas, C.; Hayes, J.M.; Sovantzis, D.; Hadjiloi, T.; Mamais, M.; Lazoura, E.; Grammatopoulos, P.; Panagopoulos, P.; Stathis, D.; Zographos, S.E.; Leonidas, D.D.; Oikonomakos, N.G.; Gimisis, T.; Chrysina, E.D. in preparation.

+Cismas, C.; Pantzou, A.; Gimisis, T. unpublished results.

++Chegkazi, M; Stathis, D.; Sovantis, D.; Mamais, M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Gimisis, T.; Chrysina, E.D. in preparation.

≈Skourti, P.V.; Alexacou, K.M.; Vidal, S.; Czifrák, K.; Nagy, V.; Berzsényi, E.; Praly, J.-P.; Somsák, L.; Siafaka-Kapadai, A.; Chrysina, E.D. in preparation.

has been the starting point for the design of rather potent inhibitors.

Out of all the analogues studied until present for the catalytic site of RMGP*b* it is only N-β-D-glucopyranosyl-N-2-naphthoyl urea (compound **7**) [Chrysina, E.D.; Nagy, V.; Felföldi, N.; Telepó, K.; Praly, J.-P.; Docsa, T.; Gergely, P.; Alexacou, K.M.; Hayes, J.M.; Leonidas, D.D.; Zographos, S.E.; Oikonomakos, N.G.; Somsák, L. in preparation], D-glucopyranosylidene-spiro-isoxazoline (compound **20**) [55], and D-glucopyranosylidene-spiro-oxathiazole (compound **21**) [56], that achieved inhibition to nM range (350, 630, and 160 nM, respectively) (Table 1). The potential of nucleosides has also been illustrated with the aid of both docking calculations and kinetic/X-ray crystallographic approaches [in collaboration with T. Gimisis and co-workers].

The structure-based inhibitor design approach has been successfully applied until present giving particular emphasis on the catalytic site of RMGP*b*; yet research efforts have been directed in other binding sites of the enzyme to increase specificity [12, 21-22, 31]. To achieve concerted controlled action of RMGP*b* and other enzymes implicated in glycogen metabolism (i.e. glycogen synthase [57]) it might be worth pursuing glucose-based analogues as cell-friendly antidiabetic drug candidates. Previous work has shown that promising potent inhibitors targeting different sites of RMGP*b* have not been successful in the next evaluation steps [12, 58]. In addition, sugar-based compounds could potentially modulate more than one molecular targets implicated not only in type 2 diabetes but also other diseases such as cancer or micro- and macrovascular disease with decreased toxicity considering that sugars and carbohydrates in general play pivotal role in cell function and glucose, in particular, is a vital component. The case of pyrimidines would provide such an example, where fluorouracil is prescribed as a chemotherapeutic drug but further research is required in long term to explore such a combined therapeutic approach.

For the near future it is absolutely essential to make the most of the knowledge derived by the detailed studies performed until present and utilize the new scaffolds identified taking advantage of new methodology tools in drug design developed the last decade. Rational and/or fragment-based screening of compounds targeting the catalytic and/or other sites will reveal the type of preferred scaffolds for each site and shed light on the features identified as important for binding.

In addition, use of modified mono- or oligosaccharides other than glucose might be worth exploring (currently under investigation in collaboration with J.P. Praly and coworkers) as reported previously in the case of cyclodextrins [59]. These might initially exhibit poor affinity for the catalytic site in particular compared to known leads; however, their binding mode might be worth investigating in depth for optimization purposes. X-ray crystallography as well as screening with NMR [60] or mass spectrometry [61] would ideally form an integrated approach for advancing our work towards developing new hypoglycaemic agents.

A number of available or newly developed geometric algorithms that would enable detailed mapping of the catalytic site of the enzyme [62, 63] will also give new insights. Structure-based inhibitor design is a multidisciplinary approach of sciences and it is only through coordinated actions that would lead to a satisfactory result.

Research on glycogen phosphorylase enzyme while living in the genomics and proteomics era could be easily justified only by quoting what Johnson & coworkers, the pioneers in this field, wrote in 1989: "*Perhaps the main driving force in working on phosphorylase has been the fact that it comprises an excellent model for the study of almost any aspect of enzyme action and regulation. After half a century of extensive work, it still can produce many "firsts" and surprises….*" [64]. Twenty one years later this statement is still true.

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ABBREVIATIONS

T2D = type 2 diabetes

HbA1c = glycated hemoglobin

GP = glycogen phosphorylase

RMGP*b* = rabbit muscle glycogen phosphorylase *b*

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