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Sugar-derived Heterocycles and Their Precursors as Inhibitors Against Glycogen Phosphorylases (GP)

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Abstract Non-insulin-dependent diabetes mellitus (NIDDM or Type II diabetes) is a multifactorial metabolic disorder in which hepatic glucose production is increased. Glycogenolysis and the main regulatory enzyme glycogen phosphorylase (GP) are responsible for the release of mono-glucose from poly-glucose (glycogen, as stored form in the liver). This protein possesses several binding pockets or cavities that regulate the catalytic functions of GP. So obviously, the inhibitors of GP will stop or slow down glycogenolysis as well as glucose production and ultimately the whole process will result in the recovery of diabetes in NIDDM patients. Glucose is one of most important regulators of GP, and glucose analog inhibitors (GAIs) have shown very promising activity for the inhibition of GP. There have been a large number of GAIs reported in last few decades that are promising for the control of NIDDM. This review briefly describes some aspects of GP and its relation with GAIs, mostly containing heterocyclic building blocks.

Keywords Glycogen phosphorylase · Glucose · Glucose analog inhibitors · NIDDM · Sugar-derived heterocycles

Abbreviations

AMP	Adenosine mono-phosphate
ATP	Adenosine tri-phosphate
CNS	Central nervous system
G1P	Glucose-1-phosphate
G6P	Glucose-6-phosphatase
GA	Glucose analogs
GAI	Glucose analog inhibitor
GK	Glucokinase
GlcNAc	*N*-Acetylglucosamine
GLP-1	Glucagon-like polypeptide 1
GP	Glycogen phosphorylase
GS	Glycogen synthase
NIDDM	Non-insulin-dependent diabetes mellitus
PDB	Protein databank
Pi	*o*-Phosphate
PK	Phosphorylase kinase
PLP	Pyridoxal 5′-phosphate
QSAR	Quantitative structure–activity relationship

1 Introduction

Type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM) is a multifaceted metabolic disease with hyperglycemia as its recognizable feature. The liver is a key tissue in overall metabolic regulation and the hepatic glucose output is elevated in NIDDM patients. Experimental data recommends drugs that inhibit or lower the hepatic glucose production as efficient antihyperglycemic agents [1, 2]. Current treatments for NIDDM rely on diet, exercise, hypoglycemic drugs intended to reduce hyperglycemia, etc., and if these fail insulin itself, which ultimately suppresses glucose production [3, 4] in liver. The present medications have inadequate efficiency and acceptability and noteworthy mechanism-based side effects [4, 5]. As a consequence there is a sustained exploration for molecules that could improve treatment and give a better life to diabetic patients.

Glycogenolysis, which is the release of monomeric glucose from its polymeric storage form called glycogen, is a key contributor to hepatic glucose output. Glycogen phosphorylase (GP) is the key enzyme catalyzing this procedure [1, 2]. A molecular target intended at reducing unnecessary glucose assembly from liver engages the inhibition of GP of human carbohydrate metabolism, which is of particular significance for the mobilization of glycogen deposits [4].

The most modern technologies have been utilized to discover a way of exploiting the possibility of GLP-1 (glucagon-like polypeptide 1) for the treatment of NIDDM. This demonstrates how discoveries of novel binding pockets

on GP and GK as the result of drug discovery programs have led to increased understanding of these key metabolic enzymes, and also to potential new therapies for NIDDM [1].

The recent advances, especially during last few years, in different heterocyclic glucose analog inhibitors (GAIs) and their precursors are briefly discussed in this chapter, including their biological activities against this very important enzyme GP, from different perspectives.

2 Glycogen Metabolism

Liver is the major source of blood glucose. Hepatic glucose is produced from two pathways [6, 7]:

Glycogenolysis – the breakdown of glycogen
Gluconeogenesis – de novo glucose synthesis

Glycogenolysis can explain why more than 70% of the hepatic glucose created by gluconeogenesis is cycled during the collection of glycogen preceding the start of efflux in the liver cells [6, 7]. Hepatic glucose productivity is synchronized by an intricate coordination of enzymes. The most important dogmatic enzyme of this coordination is glycogen phosphorylase (GP), and only the phosphorylated enzyme (known as GP*a*) has noteworthy biochemical activities in living systems [7]. Figure 1 show the pathway of gluconeogenesis in liver.

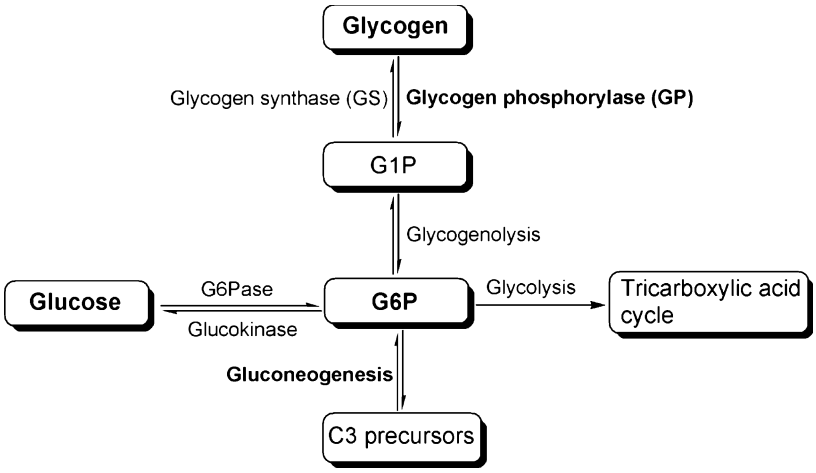

Fig. 1 Flowchart of gluconeogenesis in liver cells, which explains the role of GP and GS in the interconversion between glucose and glycogen (modified from Somsak et al. 2003) [7]

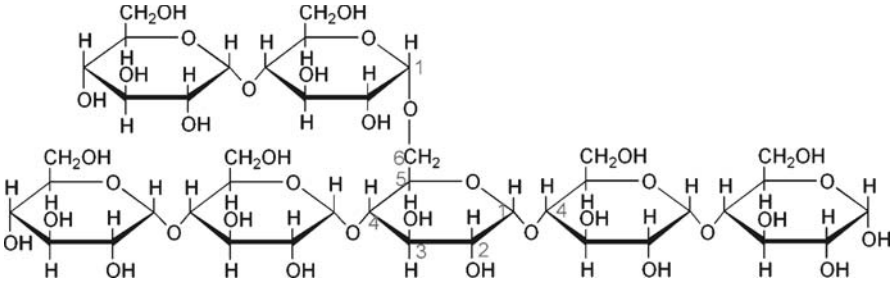

Fig. 2 Typical example of glycogen (*numbers in gray* show the numbering system)

Glycogens are polymers of glucose residues linked by α(1 → 4) glycosidic bonds, mainly, and α(1 → 6) glycosidic bonds at branch points. Figure 2 shows a typical example of glycogen, where the glycogen chains and branches are longer than shown. This is stored as glycogen predominantly in liver and muscle cells.

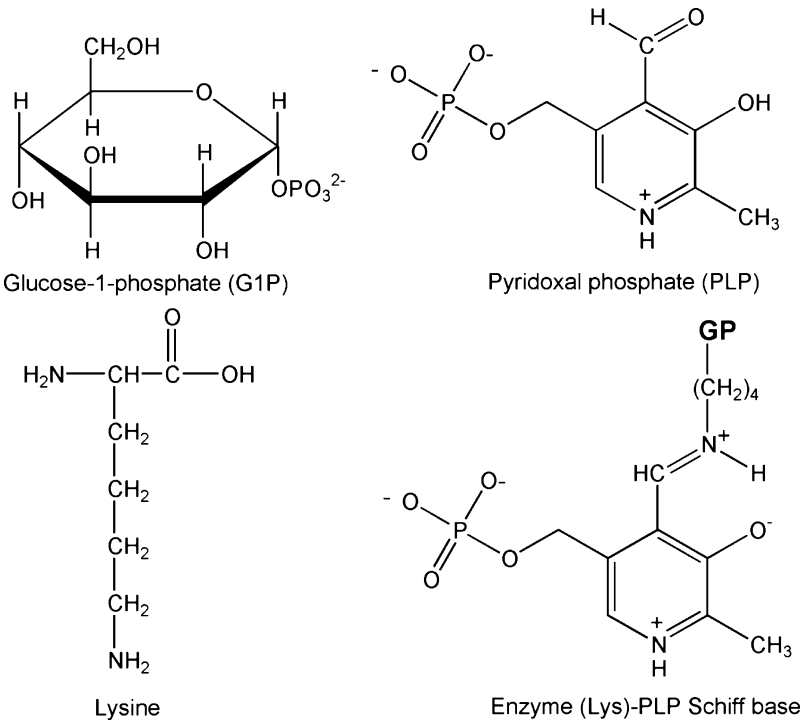

Fig. 3 Structures of the molecules involved in the glycogenolysis process. Here PLP is a derivative of vitamin B6 and serves as prosthetic group for GP; PLP is apprehended at the active site by a Schiff base linkage, formed by reaction of the aldehyde of PLP with the ε-amino group of a Lys residue

GP catalyzes phosphorolytic cleavage of the $\alpha(1 \rightarrow 4)$ glycosidic linkages of glycogen, releasing G1P as reaction product:

$$\text{Glycogen}(n\text{residues}) + P_i \rightarrow \text{glycogen}(n - - 1\text{residues}) + \text{G1P}$$

Figure 3 shows the structures of the molecules that are involved in the glycogenolysis process.

3
Glycogen Phosphorylase (GP) as a Novel Target and its Role

The carbohydrate reserve of most metabolically active cells in the animal kingdom is glycogen, a glucose polymer. The cellular demands to convert glycogen and *o*-phosphate (P_i) to G1P are met by GP, one of the most complex and finely regulated enzymes yet encountered [8]. In the liver, G1P is mostly converted by phosphoglucomutase and glucose-6-phosphatase (G6P), which is released for the benefit of other tissues, the CNS in particular relies on glucose as its major source of energy [4, 9].

GP is an allosteric enzyme that exists in two interchangeable forms [10]:
GP*a*High activity, high substrate affinity, and predominantly "R" state
GP*b*Low activity, low affinity toward the substrate, and predominantly "T" state [10].

The allosteric activators (like AMP) or inhibitors (like ATP, G6P, glucose or caffeine) can alter the equilibrium between a lower active "T" state to a more active "R" state or vice versa [4, 10]. The molecular structures of T and R states of GP have been illustrated through the X-ray diffraction studies, which have shown that the conformational transformations take place following the commencement of the muscle enzyme and its translation from the T to R state by phosphorylation or AMP [4, 11–15].

There are three mammalian GP isoenzymes, termed "muscle", "brain", and "liver" according to where they are expressed [7]. All are encoded by different genes, located on human chromosomes 11, 20, and 14, respectively [7, 16]. All the isoenzymes can be converted from the inactive (GP*b*) form to the active (GP*a*) form through the phosphorylation of Ser14 by phosphorylase kinase (PK) [7].
Several inhibitor binding sites have been identified in GP [4, 17–19]:

Ser14-phosphate recognition site
Allosteric site, which binds activator AMP and the inhibitor G6P,
Catalytic site, which binds substrates G1P and glycogen, and the inhibitor glucose and glucose analogs (GA)
Inhibitor site, which binds caffeine and related molecules
Glycogen storage site
Novel allosteric inhibitor site, which was discovered recently [4, 17–19]

In this chapter the main topic for discussion are the GAIs of GP, so of the above mentioned binding sites, the main concern is the *catalytic site*. This catalytic site is a deep cavity located at the center of the whole protein, 15 Å from the protein surface, and close to the essential cofactor pyridoxal 5′-phosphate (PLP) It has been probed with glucose and GAIs [4].

In 1976, Fletterick et al. [20] first reported a model of the polypeptide backbone of the dimer of GP*a*, which was built from a 3 Å resolution electron density map resulting from the X-ray diffraction analysis of native tetragonal crystals and two heavy atom isomorphous substitution derivatives [20]. The active site, of which there are two/dimer, is shared between the two subunits at their interface and comprises a pocket-like region within a "V"-shaped framework of two α-helices [20]. Within this region are found the binding sites for the substrates, G1P and arsenate, a competitive inhibitor UDP-glucose, and the allosteric effector AMP [20]. The site of metabolic control, Ser-14 phosphate, is hydrogen-bonded to a side chain on the outside of one of the α-helices forming the active site and is 15 Å from the AMP binding site [20].

Maltoheptaose, a glycogen analog and substrate for these enzymatically active crystals, binds in a second region of interest. Fletterick et al. suggested that this polysaccharide binding site may represent a storage site where phosphorylase is bound to the glycogen particle in the muscle cell. The polypeptide chain in a third region has the same topological structure as has been observed for the nucleotide binding domains in the dehydrogenases [20].

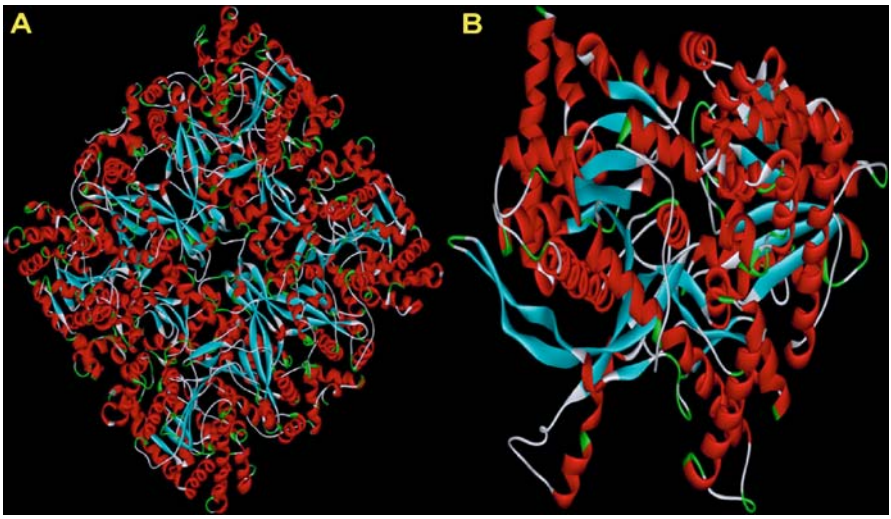

Fig. 4 Crystallographic structures of *A* GP*a* (PDB code 1gpa) [13], and *B* GP*b* (PDB code 1gpb) in 2.9 and 1.9 Å resolutions, respectively. Here, GP*a* contains four chains and GP*b* contains one [21]. The figure was created using the Accelrys DS Visualizer, version 1.6

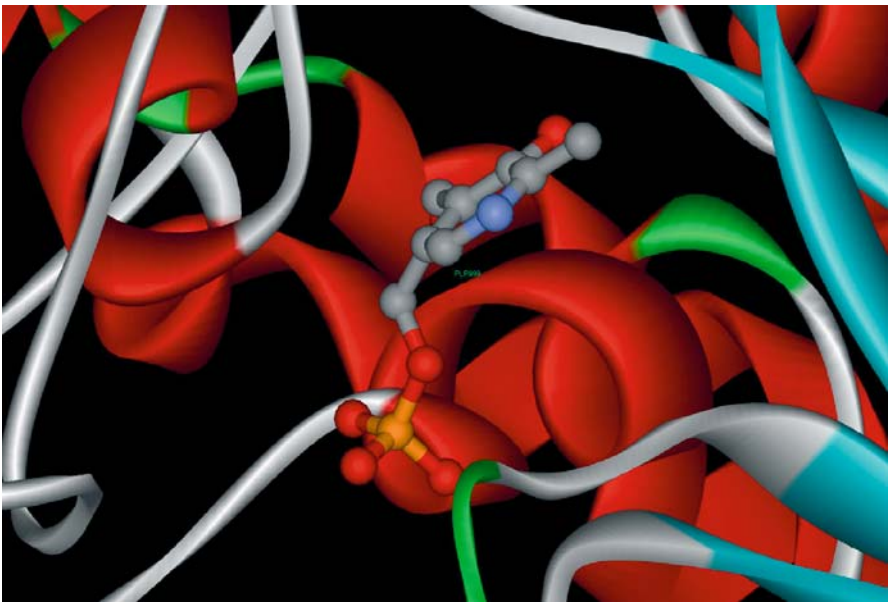

Fig. 5 A PLP molecule is bound in a catalytic domain of GP*a* (1pga) [13]. Here, the protein structure of GP*a* is shown in *solid ribbon model* and the GP*a*-bound PLP is shown in *ball and stick model*. The figure was created using the Accelrys DS Visualizer, version 1.6

Adenine or adenosine (but not the AMP) bind here in a position similar to the adenine ring of NAD in the dehydrogenases, while glucose binds 17 Å away in an interior crevice near the center of the monomer [20].

Figure 4 shows the 3D structures (X-ray crystallographic) of GP*a* (1gpa) [13] and *b* (1gpb) [21]. GP*a* contains four chains and is bound with PLP (shown in Fig. 5) at the catalytic domain [13].

4 The Role of GP Inhibitors

The isozymes of GP from muscle and liver are well characterized [22], but very less information is available about the brain-specific isozyme [23]. The crystal structures of human liver GP*a* and *b* are also known, which aids in the recognition of the binding modes of effector molecules [24, 25]. For convenience, most research has been accomplished with rabbit muscle GP; however, cloning and expression of human liver GP [26] has paved the way for investigations with the real target [7]. Figure 6 shows the crystal structure (3D) of human liver GP*a*, which contains several active sites, including the active site for the GP inhibitors. Figure 7 shows some early developed GP inhibitors [7].

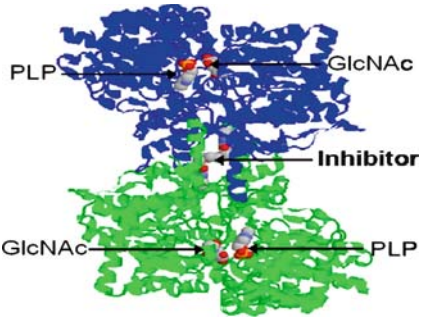

Fig. 6 Flat ribbon structure of human liver GP*a* (PDB code 1EM6). GP is a homodimeric enzyme, subject to allosteric control. It changes between "relaxed" (active) and "tense" (inhibited) conformations. *N*-Acetylglucosamine (GlcNAc, in space-filling CPK model), a glucose analog, is contiguous to PLP (in space-filling CPK model) at the active site in the 3D structure of the GP*a*. A class of molecules (inhibitors, in space-filling CPK model) developed for treating NIDDM inhibit the liver phosphorylase allosterically. These inhibitors bind at the interface of the dimer, stabilizing the inactive ("tense") conformation [20]

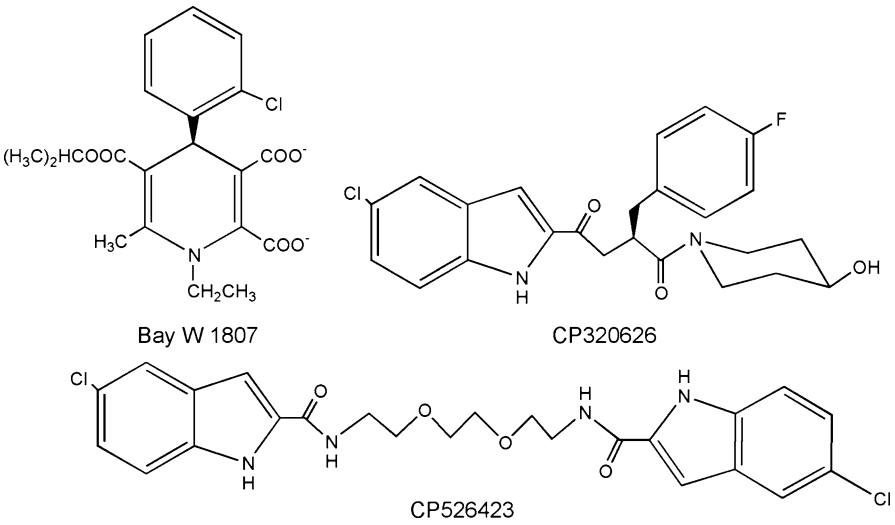

Fig. 7 Some potential GP inhibitors against GP*a*. Bay W 1807, CP320626, and CP526423 exhibited potent inhibition against GP*a* with IC_{50} values of 10.8 nM (against rabbit muscle GP*a*) [27], 205 nM (human liver GP*a*) [28], and 6 nM (human liver GP*a*) [24]

A large number of reviews have described the inhibition of GP, the role of GP inhibitors as oral antihyperglycemics (or oral hypoglycemics), as well as the GAIs [4, 7, 29–35]. Prof. Nikos G. Oikonomakos and his research team (National Hellenic Research Foundation, Athens, Greece) have reported huge numbers of discoveries of GP inhibitors over the last decades [27, 36–93].

4.1
Glucose

In certain physiological circumstances, glucose performs as a controller of GP by alleviating the less active T state of the enzyme through binding to the catalytic center [94]. It is an effective allosteric inhibitor for both the GP*a* and GP*b* with K_i values in the low millimolar range (2.0 and 1.7, respectively) [71, 76, 95]. Figure 8 shows the molecular structures of the α- and β-D-glucoses.

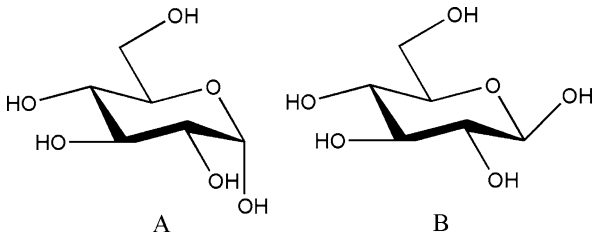

Fig. 8 Molecular structures of *A* α- and *B* β-D-glucose [71, 76, 95]

Glucose, on binding at the catalytic domain, upholds the less active T state by stabilization of the bunged pose of the 280s loop that obstructs the access for the substrate (e.g., glycogen) to the catalytic site [4].

5
Glucose Analog Inhibitors (GAIs) Against GPs

Figure 9 shows some of the early discovered GAIs against GPs, where experimental IC_{50} values calculated from A and B are 25.3 and 16.3 mM [71], respectively. For C, D, E, and F, the IC_{50} values were found to be 2.6, 0.032, 0.081, and 0.14 mM, respectively, [70]. For H, K, and L, the IC_{50} values were found to be 0.65, 0.44, and 0.37 mM, respectively, [72]. For compound I, the IC_{50} value was 0.014 mM [96], for compound J the IC_{50} value was 0.053 mM [66], and for compounds M and N the IC_{50} values were 0.0286 and 0.0031 mM, respectively [97].

5.1
D-Glucose Analogs

A number of β-D-glucose analogs were designed using the program GRID [98], which can predict energetically favorable substitutions and determine probable interaction sites between a functional group probe (e.g., hydroxyl, amino,

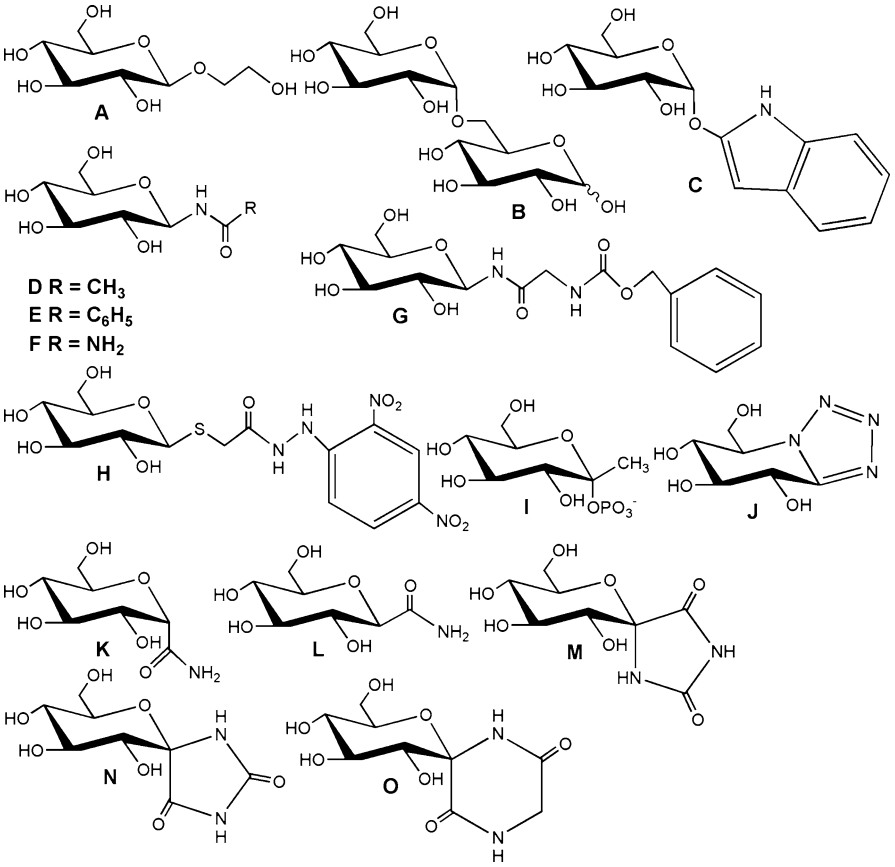

Fig. 9 Molecular structures of some of early discovered GAIs [7]

methyl, etc.) and the enzyme surface [4]. Figure 10 shows structures of some of the β-D-glucose analogs.

5.2 Glycosylidene Analogs

Several glycosilidene analogs with spiro-hydantoins and spioro-thiohydantoins exhibited potent inhibition against GPs. Figure 11 shows some of the examples.

For compounds A and B, the activities decreased due the replacement of S instead of O [97, 99]. Their K_i values were 3.1 and 5.1 μM, respectively. This means that O is more important for its binding with GP. But for C and D almost opposite results were observed, where their K_i values were > 11.5 and > 10 mM, respectively. The difference between A, B and C, D is the lack of the methoxy (= CH_2OH) group at the sugar ring, which resulted in almost com-

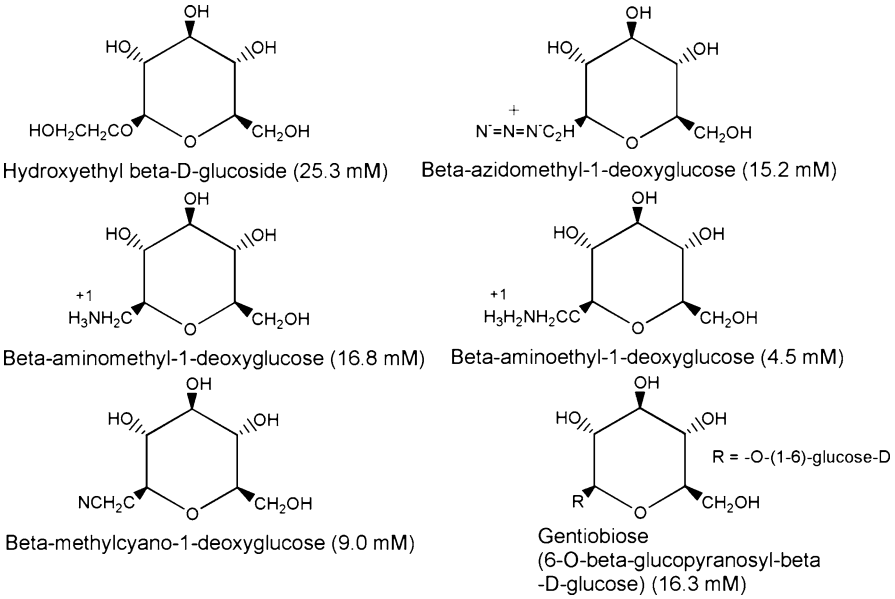

Fig. 10 Molecular structures of some of the β-D-glucose analogs (their IC_{50} values are shown in parenthesis) (modified from Oikonomakos et al. 2002) [4]

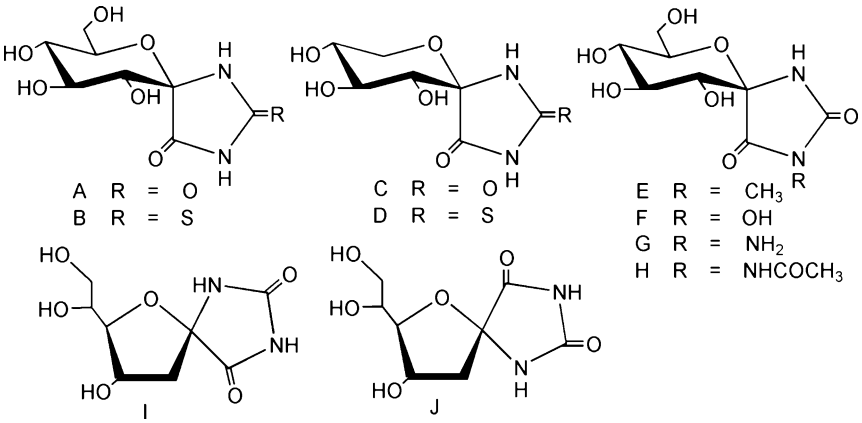

Fig. 11 Molecular structures of glycosylidene analogs experimentally proved as GP inhibitors [7]

plete loss of the inhibitory activity against GP. This shows that the $= CH_2OH$ side chain at the sugar ring is much more important than that of the O or S at the R position [97, 99, 100].

Substitutions of N-9 in the spirohydantoin as shown in compounds E to H (see Fig. 11) fetched about no enhancement of the inhibition. Their K_i values

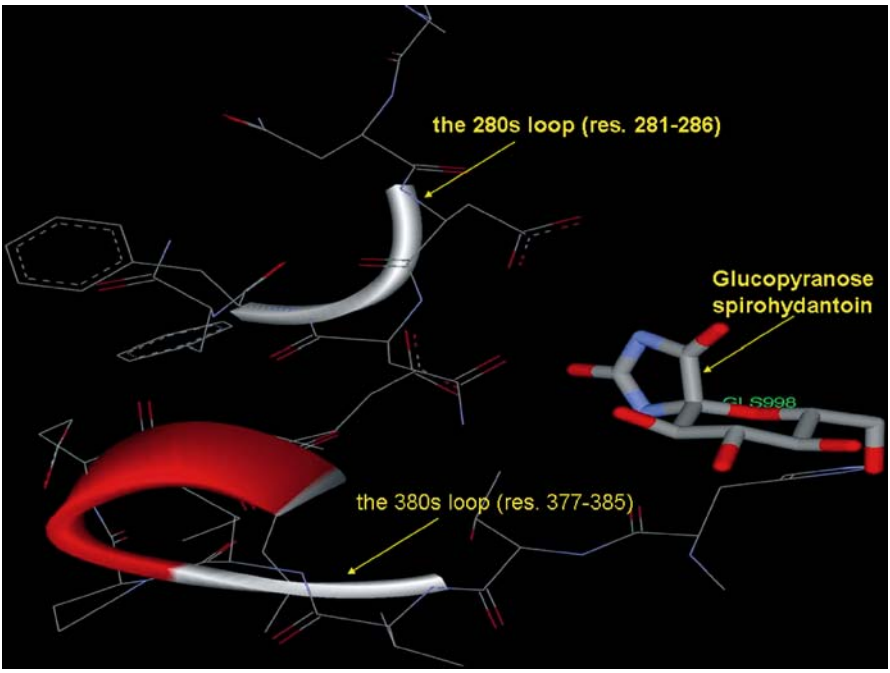

Fig. 12 Glucopyranose spirohydantoin (a pyranose analog of the potent herbicide, hydantocidin) has been identified as the most potent glucose analog inhibitor of GP*b* (PDB code 1A8I). Here, the molecule is bound in GP*b* at the small regions of the 280s and 380s loops [62]. The small molecule glucopyranose spirohydantoin is shown here as a *stick model*, the particular loops are shown in *solid ribbon*, and some other related parts of the protein GP*b* are shown in simple *line models*. The figure was created using the Accelrys DS Visualizer version 1.6

were 1.2, 0.039, 0.146 and 0.55 mM, respectively [101]. Alteration of the sugar ring to a furanoid structure as in I and J led to very weak binding, in agreement with previous interpretations with epimers and deoxy derivatives of D-glucose [7, 95, 102, 103]. The findings with C, D, I, and J, underline and confirm the high specificity of the GP active site towards a fully OH-substituted hexopyranoid sugar moiety of D-gluco configuration [7].

Figure 12 shows a crystal structure of a GP–glucopyranose spirohydantoin complex at 1.8 Å resolution (PDB code 1A8I) [62].

5.3 *N*-Acyl-β-D-glucopyranosylamides

Molecules having structural building blocks of the hydantoin moiety similar to A ($K_i = 0.032$ mM), in Fig. 13, can also bind with GPs [7]. Due to a novel synthetic approach [104], it has become possible to synthesize and

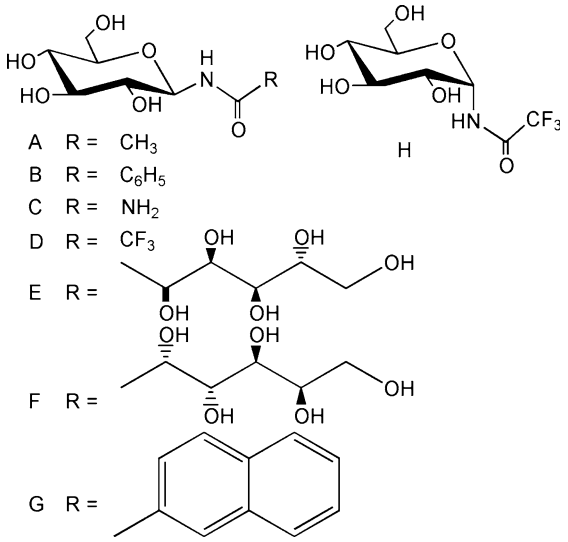

Fig. 13 Molecular structures of the *N*-acyl-β-D-glucopyranosylamide analogs [7]

explore anomeric pairs of *N*-trifluoroacetyl-D-glucopyranosylamine D (K_i = 0.71 mM) and H (*inactive*) (for structures see Fig. 13) [7]. Compared to A, the presence of the $-CF_3$ group in D significantly decreased the inhibitory activities against the enzyme GP, and the anomer H had totally lost the inhibition [7, 99]. These findings support the significance of structural building blocks of *N*-acyl-β-D-glucopyranosylamides. A hydrophobic amide side chain [104] in C (K_i = 0.341 mM) and F (K_i = 0.225 mM) exhibited weaker inhibitory profiles [7, 99]. Introduction of a naphthyl group, as in the case of G (K_i = 9.7 μM) resulted in a rather better inhibitor [7, 99]. These series of synthetic compounds and their in vitro experimental results recommend that analogous molecules with long aliphatic and/or hydrophobic side chains should be explored [7, 99].

5.4 *N*-Acyl-*N*′-β-D-glucopyranosyl Ureas

Analogs of *N*-acyl-*N*′-β-D-glucopyranosyl urea (compounds A–D in Fig. 14) [7, 53], can be regarded as "open" hydantoins [7]. Whereas the N-acetylated molecule A (K_i = 0.305 mM) [53] proved a weaker inhibitor than urea [7], increasing the hydrophobicity of the acyl side chain makes the binding stronger For example, the experimental K_i values of compounds B–D in Fig. 14were found to be 5.6, 13.0, and 0.4 μM, respectively [53]. The 2-naphthoyl urea (compound D, K_i = 0.4 μM) is to date the best glucose analog inhibitor against rabbit muscle GP*b* [7].

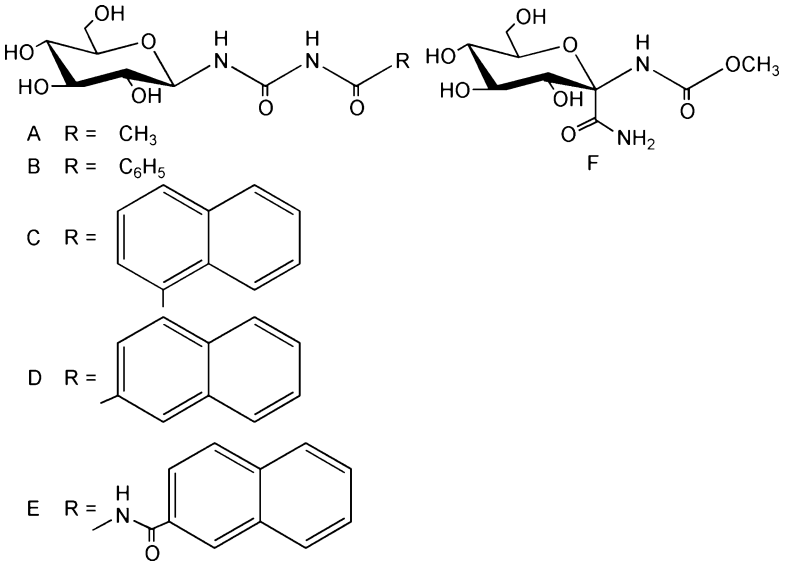

Fig. 14 Molecular structures of *N*-acyl-*N*′-β-D-glucopyranosyl ureas [7]

Placing the hydrophobic moiety farther from the sugar makes the binding stronger by more than one order of magnitude, although this distance must have an optimum, since compound E is a weak inhibitor. At 0.625 mM concentration, the observed inhibition was only ~ 45% [7].

These discoveries suggest that properly situated large non-polar groups are able to fit into the β-channel next to to the active site of the enzyme GPs [7].

A different means of "opening" the hydantoin ring is presented by the molecule F (K_i = 0.016 mM) [105] (for molecular structure see Fig. 14). Though there has not yet been a synthetic counterpart of F in the *N*-acyl-glycosylamie series, a comparison with molecule A (from Fig. 13 (K_i = 0.032 mM) shows that the presence of the carboxamido group (– $CONH_2$) in the α position may be beneficial for binding with GP [7].

5.5 D-Gluco-heptulosonamide Analogs

Hydroxyamide (compound A in Fig. 15, K_i = 3.1 mM) can be considered a fusion of α-D-glucose and 2,6-anhydro-heptonamide, though the bifuctional anomeric center brought about a significant decrease in the inhibition in comparison to both these two molecules [99]. The carboxamido-glucoside B (see Fig. 15, ~ 25% inhibition at 0.625 mM) of opposite anomeric configuration proved to have less inhibitory activity against rabbit muscle GP*b* [7].

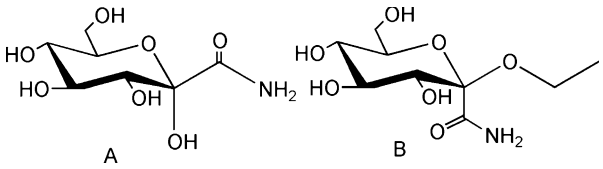

Fig. 15 Molecular structures of D-gluco-heptulosonamide analogs [7, 99]

6
Concluding Remarks, Future Challenges, and Recommendations

The inhibition of GP has established a potential approach in revising the exploitation of this enzyme, which is one of the most important key regulators of blood glucose levels. It suggests a new perception for combating NIDDM as an epidemically intensifying metabolic disorder. A motivating point about GP is that it has multiple binding pockets in its protein structure, which allows it to be targeted through diverse effectors [4, 7].

Theoretical (computational) calculations can also offer quantitative descriptors of physicochemical properties of the molecular structures, molecular interactions, and thermodynamics of interactions. Principally, extensive studies on the catalytic site of GP have been exploited in theoretical QSAR studies [4]. The techniques engaged correlate biochemical behaviors with the known crystallographic structures, and map regions around the inhibitor molecule and added water molecules to improve the in silico prediction [106–110].

Physicochemical explorations with glucose analogs have shown prospective blood glucose lowering in animal experimentations that are devoid of any noticeable signals of hypoglycemia. Since the inhibition of human liver GP also has metabolic significance, it is relatively certain that these compounds will lead to the development of novel inhibitors of GP and thus to a novel treatment for NIDDM or to an add-on to existing treatment [7]. Large numbers of molecules have been reported to possess moderate to potent inhibitory profiles against different types of GPs. Now it is time to prove their clinical efficacy and their specificity. Before going to clinical trials it is necessary to optimize the targeted molecule(s) for their safety profiles. So, there are questions of in vitro, and acute and chronic in vivo toxicity studies. As most of the potent inhibitors are synthetic in origin, the amounts of the particular molecules are not a big issue. There have been some reports published on the in vivo studies of some potential molecules on different mammalian models [22, 29, 111–120], but these data are not sufficient to lead to clinical trials. There is a need for long-term chronic toxicity studies on higher animals and also for genotoxic studies, which are also extremely important for long-term treatments.

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