Structure-based design of potent glycogen phosphorylase inhibitors

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INTRODUCTION

Diabetes is a disorder of metabolism and is widely recognized as one of the leading causes of death and disability. It is estimated that more than 180 million people worldwide have diabetes.[1] In the United States, more than 20 million people – about 7.0% of the population – have diabetes.[2] Diabetes is a lifelong condition that, if left untreated, can lead to serious complications such as nerve damage, kidney failure, blindness, and cardiovascular diseases.[3] Type 2 diabetes is a chronic metabolic disorder characterized by fed and fasting hyperglycemia. Glycogen phosphorylase (GP) is a key enzyme in the regulation of glycogen metabolism by catalyzing the breakdown of glycogen to glucose-1-phosphate. In muscle, glucose 1-phosphate is used to generate metabolic energy, whereas in liver it is also converted to glucose for export to peripheral tissues. There are three human isozymes of GP: liver, muscle, and brain, named to denote the tissues in which they are preferentially expressed. The muscle and brain isozymes serve the tissues in which they are found, whereas the liver isozyme meets the glycemic demands of the body as a whole. Previous reports have indicated that GP inhibition can lower blood glucose in diabetic models, thus validating it as a potential therapeutic target for treatment of type 2 diabetes.[4–6] The liver isozyme of human glycogen phosphorylase (HLGP) is considered to be the preferred target for therapeutic intervention with GP inhibitors because inhibition of muscle or brain GP could lead to undesirable side effects.

HLGP and human muscle glycogen phosphorylase (HMGP) are dimers composed of two identical monomers, with more than 800 amino acid residues in each. Glycogen phosphorylase exists in two interconvertible forms: a Ser14 phosphorylated high-activity form (GPa) and a dephosphorylated low-activity resting form (GPb). Both forms exist in equilibrium between two different conformational states: a more active R state and a less active T state.[7] The more active R state is induced by the substrate and by allosteric effectors such as adenosine monophosphate (AMP), whereas the less active T state is stabilized by inhibitor binding. X-ray crystallographic studies of inactive and active conformations of HLGPa demonstrated large conformational changes between the two states, including order/disorder transitions and changes in secondary structures.[8] The inactive conformation of GP was used as the target for inhibitor design and optimization.

Glycogen phosphorylase contains at least six potential regulatory sites (Figure 17.1): (1) the Ser14 phosphate recognition site (Ser14 phosphorylation induces conformational changes that alter GP activity); (2) the catalytic site that binds the substrates glycogen and glucose-1-P, as well as glucose and glucose analogs; and (3) the AMP allosteric site that binds AMP, IMP, ATP, and glucose-6-P. This site is about 35Å away from the catalytic site (Figure 17.1). The Bayer diacid compound W1807 [Figure 17.2(a)], a potent inhibitor of rabbit muscle glycogen phosphorylase (RMGP), binds at this site as determined by crystallographic analysis.[9,10] (4) The inhibitor site (also referred to as the purine nucleoside site) binds heterocyclic compounds such as caffeine [Figure 17.2(b)] and flavopiridol. This site is more than 10Å away from the catalytic site.[11] (5) The glycogen storage site. (6) The dimer interface site that binds indole derivative CP320626 [Figure 17.2(c)] and its analogs.[12] This site was identified as a new allosteric site by x-ray crystallographic analysis.[13–15] Four of these six regulatory sites are known to be inhibitor binding sites: the catalytic site, the AMP allosteric site, the inhibition site and the dimer interface site [Figure 17.1].

In this chapter, we describe the use of molecular modeling in the development of a series of potent GP inhibitors. We started from a lead series consisting of phenyl diacids with various substitutions on the pyridine ring (Table 17.1). Of these, the most potent compound is 4-(2-{[(4-nitropyridine-2-yl)carbonyl]amino}phenoxy)phthalic acid [compound **1a**, Figure 17.3(a)]. Due to the lack of competitive binding studies, modeling studies were undertaken to predict the most probable binding site for compound **1a**. These involved superposition[16] of compound **1a** onto inhibitors that are known to bind at different sites based on the x-ray crystal structures, as well as examination of the protein environment to determine the possibility of interaction with nearby residues. Ultimately, these analyses suggested that compound **1a** binds at the AMP allosteric site. The docking of compound **1a** inside the AMP allosteric site was further explored by Internal Coordinates Mechanics (ICM) calculations[17] with subsequent energy optimization.