Summary

Type II diabetes, one of the most common metabolic disorders, is characterized by hyperglycaemia in the context of insulin resistance. Today it represents more than 90% of all diabetics. Liver glycogen phosphorylase is a key enzyme in glycogen metabolism catalyzing the degradation of glycogen to glucose-1-phosphate. Since glycogen in the liver serves as a source of glucose to maintain blood glucose homeostasis glycogen phosphorylase is a validated pharmaceutical target for the discovery of novel antihyperglycaemic agents (inhibitors of the enzymatic action) to combat type II diabetes. The discovery of new inhibitors is based on glucose, the natural inhibitor of the enzyme, which binds at the active site. The goal of the present thesis was to discover glucose derivatives which would have stronger potency than glucose for glycogen phosphorylase. For this the method of structure-based-inhibitor design was used. The method is based on the structural analysis of the molecular details that govern inhibitor recognition and specificity by the active site, followed by the assessment of each new inhibitor, in spiral pathway between structural analysis and biochemical assessment which concludes to an inhibitor with strong potency.

In the framework of the present Thesis six different groups of glucose derivatives analogues have been studied initially with rabbit muscle glycogen phosphorylase and finally with human liver glycogen phosphorylase. Kinetic experiments used to determine the efficacy of these compounds and their K_i values while X-ray crystallographic studies of the protein-inhibitor complexes revealed the structural basis of their inhibitory potency.

The first group studied was the *C*5 halogen substituted glucopyranosyl nucleosides with halogens at the *C*5 position of uracil with **ClcClU** being the most potent (K_i =1.02 μM). The next group comprised by *C*5-alkynyl and alkylfurano[2,3-*d*] pyrimidine glucopyranonucleosides where alkynyl groups of variable length were introduced in the *C*5-position replacing the halogen with the aim to exploit interactions with residues of the hydrophobic β-pocket within the active site. The results from this group emphasized the importance of the linker group connecting the glucopyranose moiety with an R group. Therefore, in the next group of ligands linkers of different length were used with the aim to enable the R groups to form favourable interactions with residues in the β-pocket. *N*-acyl-β-D-glucopyranosylami-nes had the linker –NHCO–. A variety of R groups were used based on the virtual screening of 1888 compounds of the ZINC, a free database of commercially-available compounds. The most potent compounds of this group were used as a scaffold for

the design and study of a new group, the *N*-acyl-β-D-glucopyranosyl ureas where the –NHCO– linker was replaced by the –NHCONHCO– group. This proved to have the optimum length for inhibitory potency.

The last of inhibitor groups studied had heterocyclic groups as linkers and R substituents of variable size and length. The study of *C*-glucopynosyl- 1,2,4 triazoles and 4(5)-Aryl-2-*C*-glucopyranosyl-imida-zoles led to the discovery of very strong inhibitors with K_i values at the nanomolar range. The most potent (**BEva349**) bearing an imidazole ring linker with a K_i value of 26 nM is the most potent inhibitor of the catalytic site discovered thus far. The efficacy of these inhibitors was also tested in human liver glycogen phosphorylase. The results verified their strong inhibitory potencies against the pharmaceutical target while at the same time provided experimental evidence for the safe use of data with rabbit muscle glycogen phosphorylase for the design of potent inhibitors of human liver glycogen phosphorylase.