Synthesis and evaluation of a sugar-conjugated glibenclamide derivative

A. Korobeynikov[a], R. Schirrmacher[a], M. Schwanstecher[b], F. Rösch[a]
[a]Institut für Kernchemie, Johannes Gutenberg – Universität, Fritz-Straßmann-Weg 2, 55128 Mainz;
[b]Institut für Pharmakologie und Toxikologie, Universität, Beethovenstr. 55, 38106 Braunschweig

Diabetes mellitus, better known as "diabetes", is a chronic disease associated with abnormally high levels of the sugar glucose in the blood. There are two main types of the disease: insulin-dependent diabetes mellitus (IDDM, type 1), and non-insulin-dependent diabetes mellitus (NIDDM, type 2). IDDM is an autoimmune disease characterized by destruction of pancreatic β-cells being responsible for insulin secretion. It causes absolute insulin deficiency. NIDDM is associated with defects of insulin action (insulin resistance) and insulin secretion although pancreatic β-cells remain intact [1-3]. In this case, some pharmacological agents including sulfonylurea derivatives are commonly used to stimulate insulin secretion by binding to sulfonylurea receptors (SUR1) of β-cells.

Glibenclamide, one of the sulfonylurea derivatives, has a high binding affinity to human SUR1 [4]. This might allow using its ^{18}F-labelled analogues for visualizing and quantifying β-cells concentrations *in vivo* via positron emission tomography (PET).

Unfortunately glibenclamide has relatively high lipophily resulting in unspecific accumulation of a glibenclamide PET ligand in other organs. Thus, conjugation with a sugar moiety should decrease lipophilic properties of glibenclamide to an acceptable value not changing dramatically the binding affinity.

The synthesis of α-glucose-conjugated glibenclamide was carried out in eight reaction steps (Fig. 1) starting with the removing the acetyl group from the anomeric hydroxyl group of glucose pentaacetate (**1**) by means of benzylamine. Resulting tetraacetyl-D-glucose (**2**) was reacted with tert-butyl bromoacetate to yield the protected α-glucose-linker (**3**). Following deprotection with trifluoroacetic acid and chlorination in thionyl chloride gave the product (**5**), which was coupled with 4-amino-5-chloro-2-methoxybenzoic acid to yield the conjugate (**6**). This compound was then reacted with p-(2-aminoethyl)benzenesulfonamide through the formation of mixed anhydride by using ethylchloroformate and the product (**7**) was obtained. Reaction with cyclohexyl isocyanate in presence of copper chloride (I) gave the protected α-glucose-glibenclamide conjugate (**8**). The following deprotection with sodium methoxide yielded the final 4-[2-(α-D-glucopyranoside)-*O*-acetoyl]amino-*N*-{2-[4-({[(cyclohexylamino)carbonyl]amino}sulfonyl)-phenyl]ethyl}-5-chloro-2-methoxybenzamide (**9**).

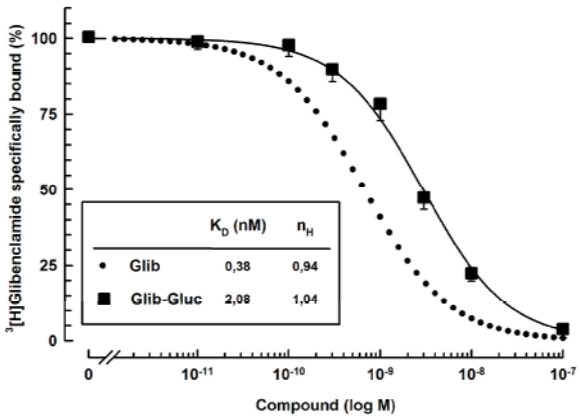

Fig.2

Competition binding experiments *in vitro* were performed as described [5] to assess the affinity of the glibenclamide-glucose conjugate for binding to human SUR1 (Fig. 2). The substance induced a complete monophasic inhibition curve (■) with a Hill coefficient (n_H) close to 1 (1.04) yielding a dissociation constant (K_D) of 2.08 nM. In parallel control displacement by unlabelled glibenclamide was assessed (K_D = 0.38 nM, n_H = 0.94, dotted line).

This result allows to go continue this study, the next step of which is the synthesis of glucose-conjugated glibenclamide precursor and its labeling with Fluor-18.

[1] Ronner P. et al. Diabetes, 42: 1760-1772 (1993).
[2] Schatz H. Diabetologie kompakt.- Berlin: Blackwell Wiss.- 2001.
[3] Balabolkin M. I. Diabetologia.- Moskau: Medizina.-2000.
[4] Ashcroft S., Ashcroft F. Biochimica et Biophisica Acta, 1175: 45-59 (1992).
[5] Schwanstecher M. et al. Br J Pharmacol, 106: 295-301 (1992).

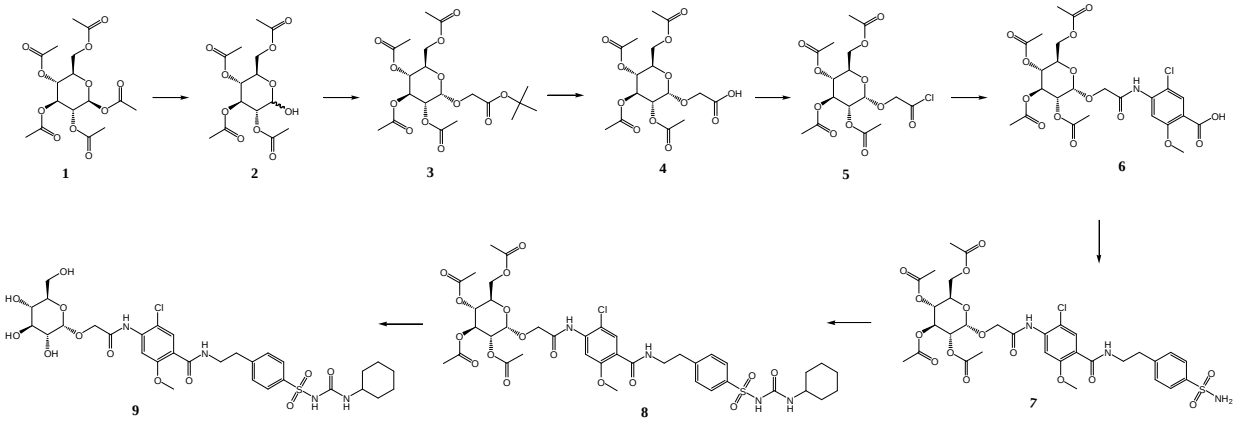

Fig. 1.