Investigation of α-glucosidase Inhibitory Effects of Some New Hydrazides and Molecular Modeling Studies

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Abstract

In this study, The ability to limit enzyme activity was explored for three substances, namely 4, 5, and 6 compounds. Inhibiting α-glucosidase activity is being examined as a possible way to manage type 2 diabetes, which is linked to several health hazards. According to the findings, the α-glucosidase enzyme activity was decreased by all three compounds—4, 5, and 6. Pictures show that the inhibition is concentration dependent . These results indicate that these substances might be used to treat disorders associated with α-glucosidase enzyme activity, such diabetes. The unique amino acid interactions between each chemical and the α-glucosidase enzyme are illuminated by the 2D interaction maps . Important amino acids involved in the inhibitory process, such as ARG 442, GLN 353, and GLU 411, were shown to have hydrogen bond interactions. Enzyme conformational changes and catalytic reaction halts are likely caused by the red separation contacts seen in the interaction maps, which indicate that water molecules have left the enzyme's active site. This study's findings provide credence to the idea that chemicals 4, 5, and might be used therapeutically to treat illnesses like type 2 diabetes, which are linked to high α-glucosidase activity. Validating these results and evaluating the safety and effectiveness of these molecules as possible anti-2 diabetes medicines will need more study, including as in vivo investigations and clinical trials.

Keywords: *α-glucosidase , Inhibition, Diabetes, Hydrazides*

1. Introduction

Type 2 diabetes (DM2) and associated cardiovascular diseases and cancer are an increasing problem around the globe, especially in the developed world [1]. Currently, in the Netherlands the prevalence of DM2 is approximately 3.5% and this number is expected to increase by at least 32% in the next decades. This is due to the changing demographic characteristics (more elderly people), increasing problem of overweight and the improved and early detection of patients with DM2 [2]. The diagnosis of DM2 is not clear-cut, but merely the result of an arbitrarily chosen point somewhere between the absence of insulin resistance and normal insulin secretion, and advanced peripheral insulin resistance and absence of insulin production. Therefore, the optimal moment to start treatment is not unequivocal. Specifi c criteria have been defi ned for those people who have raised post-prandial and/or fasting blood glucose, but who do not meet the criteria for DM2. This condition is referred to as ‘impaired glucose tolerance’ (IGT) when post-prandial blood glucose levels are elevated, and ‘impaired fasting blood glucose’ (IFBG) in case of elevated fasting blood glucose (criteria: Table 1). In this paper, the current evidence is reviewed for the use of AGIs as initial treatment for patients with DM2, or as treatment for patients with IGT and/or IFBG.

Diet and exercise is the fi rst step in the treatment of DM2. But if these measures alone fail to suffi ciently control blood glucose levels, starting oral drug therapy is recommended [3]. To date, 6 classes of oral antihyperglycemic drugs are available: biguanides (metformin), sulphonylurea (eg, tolbutamide), glinidines (eg, repaglinide), thiazolidinediones (eg, pioglitazone), dipeptidyl peptidase IV inhibitors (eg, sitagliptin) and alphaglucosidase inhibitors (AGIs; eg, acarbose) [4].

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Table 1 Current definitions for diabetes mellitus, impaired glucose tolerance and impaired fasting blood glucose

	WHO 2006	ADA 2007	Rutten et al 2006
Diabetes mellitus	FPG ≥ 7.0 *or* 2HPG ≥ 11.1	Symptoms of diabetes[a] plus CPG ≥ 11.1 *or* FPG ≥ 7.0 *or* 2HPG ≥ 11.1[b]	Symptoms of diabetes[a] plus CPG ≥ 11.1 *or* FPG ≥ 7.0 on two occasions
Impaired glucose tolerance	FPG < 7.0 *and* 2HPG 7.8–11.0	2HPG 7.8–11.0[c]	No definition
Impaired fasting blood glucose	FPG 6.1–6.9 *and (if measured)* 2HPG < 7.8	FPG 5.6–6.9[c]	FPG > 6.1 *and* <6.9[c]

AGIs reversibly inhibit a number of alpha-glucosidase enzymes (eg, maltase), consequently delaying the absorption of sugars from the gut [5]. In a recent study among healthy subjects it was suggested that the therapeutic effects of AGIs are not only based on a delayed digestion of complex carbohydrates, but also on metabolic effects of colonic starch fermentation [6]. Acarbose (Glucobay®) is the most widely prescribed AGI. The other AGIs are miglitol (Glyset®) and voglibose (Volix®, Basen®). AGIs might be a reasonable option as fi rst-line drug in the treatment of patients with DM2 as it specifi cally targets postprandial hyperglycemia, a possible independent risk factor for cardiovascular complications [7]. Although rare cases of hepatic injury were described, AGIs are expected to cause no hypoglycemic events or other life-threatening events, even at overdoses, and cause no weight gain [8].

2. Materials and Methods

2.1 Enzyme Activity Assay

One typical method for determining if a chemical or substance inhibits alpha-glucosidase activity in vitro is the alpha-glucosidase enzyme inhibition test. One enzyme that plays a role in digesting carbohydrates is alphaglucosidase. Its job is to hydrolyze complicated carbs into glucose. Treatment options for diseases like diabetes and obesity may benefit greatly by blocking alpha-glucosidase activity. To generate a stock solution, dissolve the molecule or substance in 1 milligramme of DMSO per millilitre of solvent. This will allow you to test it for alpha-glucosidase inhibition. Various concentrations for the test are achieved by diluting the stock solution. Recombinant enzymes may be used to produce alpha-glucosidase. The enzyme source is made ready by using appropriate procedures to isolate or purify alpha-glucosidase. In most cases, the enzyme is kept frozen and then thawed just before the test. In order to facilitate the alpha-glucosidase enzyme's optimum activity, a buffer solution is produced. With a pH of 6.8, the buffer includes phosphate. p-nitrophenyl-α-D-glucopyranoside (pNPG) is one of the particular substrates usually used to test alpha-glucosidase activity. In order to get the substrate to the right concentration, it is dissolved in the assay buffer. After the test sample, buffer, substrate, and D.W. have been combined, the reaction mixture may be created using the alpha-glucosidase enzyme. Finding the sweet spot for enzyme and substrate concentrations is essential. The negative control indicates maximal enzyme activity without any inhibitory impact, while the positive control reflects total suppression of alpha-glucosidase enzyme activity. The inclusion of these controls in the test ensures the validity of the data. To enable the enzyme-substrate reaction to take place, the reaction mixture is incubated at a certain temperature, usually 37°C, for a specified duration. Optimal incubation times could vary from experiment to experiment. Following the incubation time, the activity of the alpha-glucosidase enzyme is determined by using an appropriate detection technique. To produce a coloured product while using pNPG as the substrate, the reaction is usually halted by adding a stop solution, such sodium carbonate. A spectrophotometer is used to measure the product production by tracking the absorbance at a given wavelength, often around 405 nm. For each quantity of the tested drug or chemical, the percentage of alpha-glucosidase inhibition is determined using the absorbance values that were obtained. The inhibitory concentration (IC50),

which is the concentration needed to block 50% of the alpha-glucosidase enzyme activity, may be calculated by generating a doseresponse curve.

2.2 Molecular Docking

The Molegro Virtual Docker programme was used to conduct docking investigations in order to forecast the placement of molecules in enzyme active sites [9]. We used the 4EY7 PDB ID to access the AChE X-ray crystal structures available in the RCSB Protein Data Bank (RCSB PDB) [10]. After sketching out the synthetic compounds in 2D in Chemdraw, we imported them into MarvinSketch to double-check our work and generate the 3D SDF structures. Enzymes and novel compounds were optimised and regularised in docking investigations using Molegro Virtual Docker. At the location where the enzymes are active, a grid box was drawn with the crystal ligand coordinates in the middle. Each compound underwent ten trials at its target's active site. We used Discovery Studio Visualizer to examine and display the top-scoring positions.

3. Results and Discussion

Tabulated in Table 2 are the study's symbols and the IC_{50} for these samples.

Table 2 The signals employed and the sample's IC_{50}

Compounds	IC50 values µM
4	121.605
5	86.643
6	61.340

The fact that compound 4 may block the action of the α-glucosidase enzyme suggests it may be useful in treating degenerative diseases like 2 diabetes that involve α-glucosidase. The correlation between enzyme activity % and concentration 4 is graphically shown in Figure 1, which also offers a thorough description of the process.

The graph shows that as the value of 4 increases, the activity of the enzyme α-glucosidase decreases. Figure 2 shows a two-dimensional interaction map that shows the specific interactions between compound 4 and the enzyme's amino acids. The binding process was greatly facilitated by the contributions of amino acids GLU 411, ARG 442 and GLN 353 via hydrogen bond interactions. Through these interactions, the binding is stabilised and the inhibitory effects are enhanced. Another indicator that water molecules have departed the enzyme's active site is the presence of red separation contacts. Enzyme catalysis stops and the enzyme's structure changes as a consequence. A α-glucosidase inhibitor is compound 4, thus.

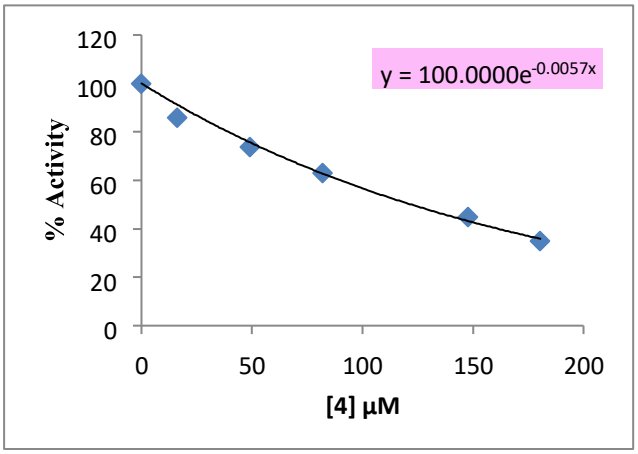

Figure 1 Reduce α-glucosidase enzyme activity from the compound 4

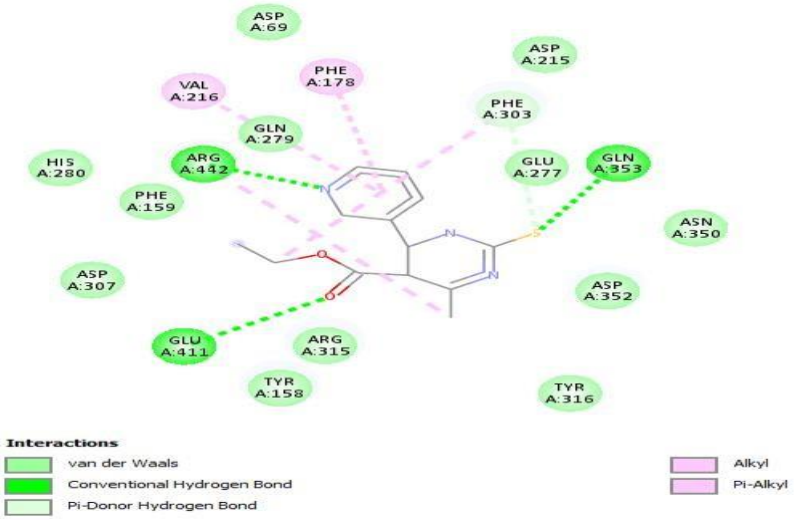

Figure 2 The 4 compound's two-dimensional structures with α-glucosidase enzyme

The fact that compound 5 may block the action of the α-glucosidase enzyme suggests it may be useful in treating degenerative diseases like 2 diabetes that involve α-glucosidase. The correlation between enzyme activity % and concentration 5 is graphically shown in Figure 3, which also offers a thorough description of the process.

The graph shows that as the value of 5 increases, the activity of the enzyme α-glucosidase decreases. Figure 4 shows a two-dimensional interaction map that shows the specific interactions between compound 5 and the enzyme's amino acids. The binding process was greatly facilitated by the contributions of amino acids GLU 411, TYR 158 and GLN 353 via hydrogen bond interactions. Through these interactions, the binding is stabilised and the inhibitory effects are enhanced. Another indicator that water molecules have departed the enzyme's active site is the presence of red separation contacts. Enzyme catalysis stops and the enzyme's structure changes as a consequence. A α-glucosidase inhibitor is compound 5, thus.

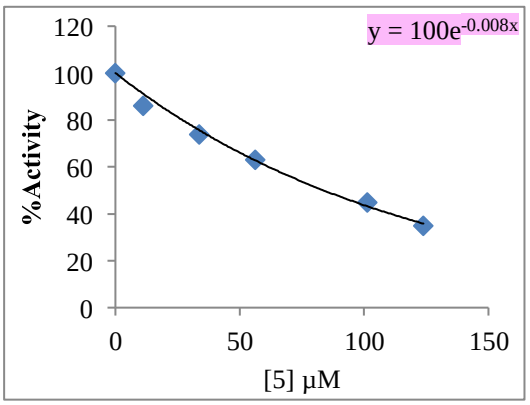

Figure 3 Reduce α-glucosidase activity from the compound 5

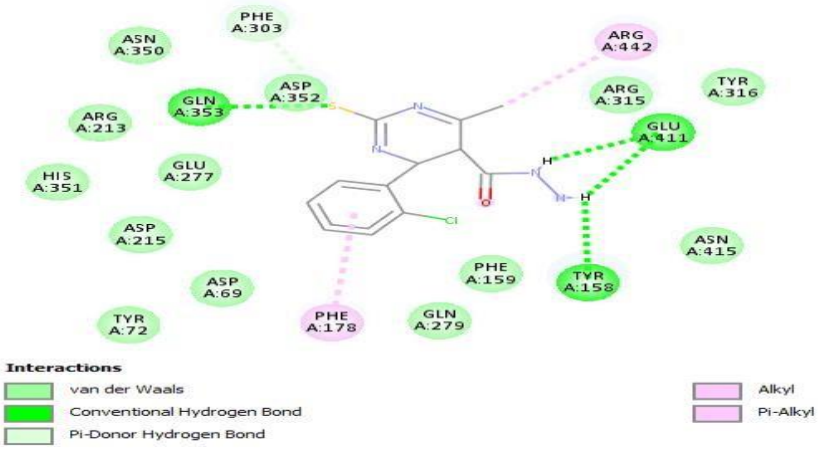

Figure 4 The 5 compound's two-dimensional structures with α-glucosidase enzyme

The fact that compound 6 may block the action of the α-glucosidase enzyme suggests it may be useful in treating degenerative diseases like 2 diabetes that involve α-glucosidase. The correlation between enzyme activity % and concentration compound 6 is graphically shown in Figure 5, which also offers a thorough description of the process.

The graph shows that as the value of 6 increases, the activity of the enzyme α-glucosidase decreases. Figure 6 shows a two-dimensional interaction map that shows the specific interactions between 6 compound and the enzyme's amino acids. The binding process was greatly facilitated by the contributions of amino acids GLU 277, ASP 352, TYR 158, ASP 215 and GLN 353 via hydrogen bond interactions. Through these interactions, the binding is stabilised and the inhibitory effects are enhanced. Another indicator that water molecules have departed the enzyme's active site is the presence of red separation contacts. Enzyme catalysis stops and the enzyme's structure changes as a consequence. A α-glucosidase inhibitor is compound 6, thus.

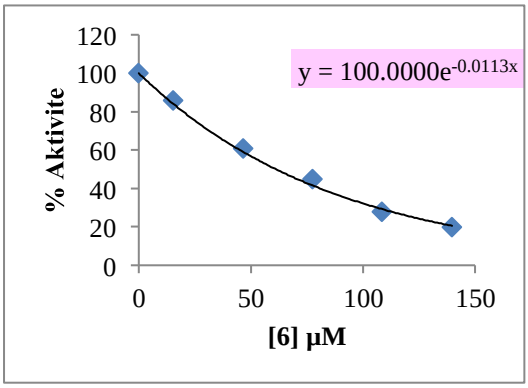

Figure 5 Reduce α-glucosidase enzyme activity from the compound 6

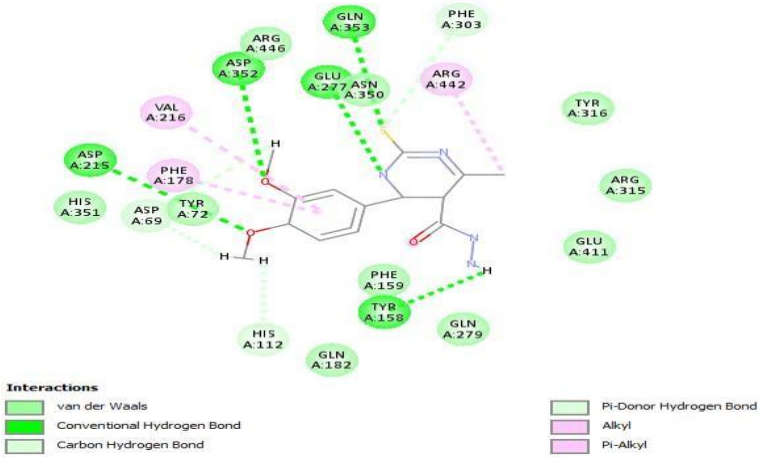

Figure 6 The compound 6 compound's two-dimensional structures with α-glucosidase enzyme.

4. Conclusion

In this study, the capacity of three compounds, namely 4, 5, and 6 compounds, to inhibit α-glucosidase activity was investigated. The treatment of type 2 diabetes and other conditions linked to increased α-glucosidase activity may be improved by considering this new opportunity. Concentration had a role in the inhibitory effect of the three medications on α-glucosidase activity. The 2D interaction maps highlighted the inhibitory mechanism by revealing interactions with specific amino acids via hydrogen bonds. As seen by the red separation contacts, the catalytic activity of the enzyme was hindered because water molecules were displaced from its active site. The findings indicate that compounds 4, 5, and 6 compounds might be effective in treating type 2 diabetes and other conditions associated with elevated α-glucosidase activity. Additional research, including clinical trials and in vivo studies, is necessary to validate these results and assess the efficacy and safety of these compounds as anti-type 2 diabetes medications. This finding is a huge step forward in the hunt for novel therapeutic techniques, and it might soon lead to improved health and a greater quality of life for those with α-glucosidase-related illnesses.

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