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Novel synthesis of dihydropyrimidines for α-glucosidase inhibition to treat type 2 diabetes: *In vitro* biological evaluation and *in silico* docking

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ABSTRACT

A convenient and efficient new method has been established for the synthesis of dihydropyrimidines by inexpensive and non-toxic N-acetyl glycine (NAG) catalysed reaction of aromatic aldehydes with ethyl acetoacetate and urea/thiourea. This method is applicable for various substituted aldehydes as well as urea and thiourea. It has also been used to synthesize bicyclic oxygen-bridged pyrimidine derivatives (**4d**, **4j**). The biological assay revealed that the majority of compounds synthesized displayed modest inhibitory activity against α-glucosidase at low micro-molar concentrations. Molecular docking studies were also performed on the most active compound, **4f** (with IC_{50} value 112.21 ± 0.97 μM), to show the enzyme – inhibitor interactions.

1. Introduction

α-Glucosidase (EC3.2.1.20) is an important enzyme found in the brush border surface of cells in the small intestine. During the digestion of food this enzyme hydrolyzes carbohydrates and produces α-D-glucose, which is absorbed in the blood stream, increases postprandial blood glucose levels and causes diabetes. Thus, for the control and prevention of diabetes, α-glucosidase inhibitors are of particular interest as they can help to reduce the carbohydrate digestion and subsequent monosaccharide absorption [1,2]. Blood glucose levels is very critical for diabetes mellitus management and levels should be maintained within an acceptable range (70–100 mg/dl) [3,4]. In addition to diabetes prevention, balanced levels of monosaccharides within the blood stream may also benefit to avoid hyperlipidemia, hyperlipoproteinemia and obesity [5]. α-Glucosidase also enables monosaccharide removal from viral glycoproteins, thus, its inhibitors could alter cell-to-cell signaling, virus recognition by the cell and could be used in the treatment of viral diseases, cancers and immune-regulations [6–10]. Acarbose, deoxynojirimycin, miglitol and voglibose are among the key known candidates which are used extensively for inhibiting the α-glucosidases (Fig. 1). Due to side effects and absorptivity problems associated with these inhibitors [11,12], new potent α-glucosidase inhibitors are highly desired.

Dihydropyrimidines (DHPMs) are considered pharmacologically very important molecules due to their number of biological activities, such as antiviral, antihypertensive, antibacterial, and antagonists [13–17]. Pyrimidines also occupy a unique position in the medicinal chemistry, as being part of nucleic acids [14]. Recently, pyrimidine derivatives have been reported as potent inhibitors of the enzymes responsible for diabetes [18], and particularly, pyrimidine fused heterocycles are identified as specific α-glucosidase inhibitors (Fig. 2) [19].

The Biginelli reaction is a multi-component one pot reaction which affords an efficient synthesis of DHPMs. Various synthetic methods have been reported for the preparation of DHPMs [20–22], however, to achieve better results in most of the reports metal catalysis has been extensively explored [23–27]. Metal catalysts are expensive, environmentally harmful and some of the

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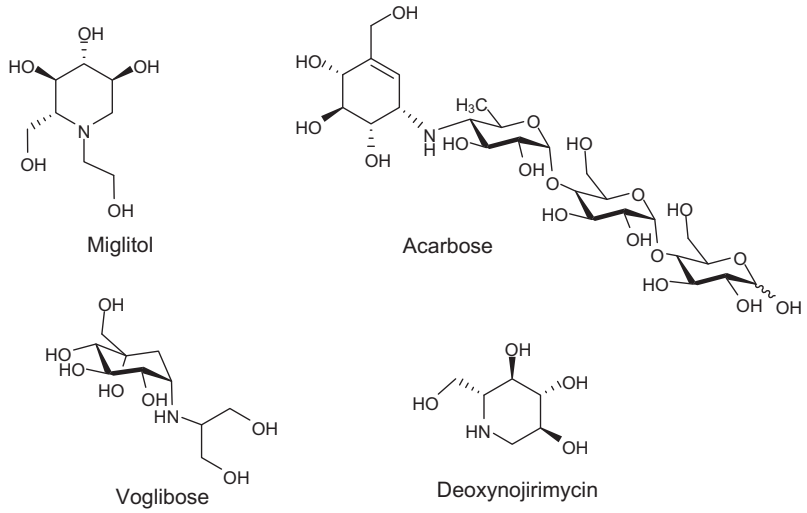


Fig. 1. Structures of acarbose, deoxynojirimycin, miglitol and voglibose.

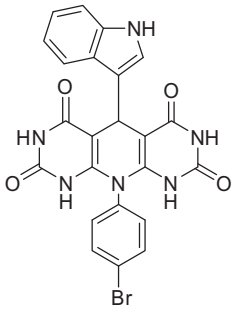


Fig. 2. The molecular structure of a pyrimidine based α-glucosidase inhibitor.

reported conditions require prolonged reaction time, and complete removal of metal traces from products is usually problematic.

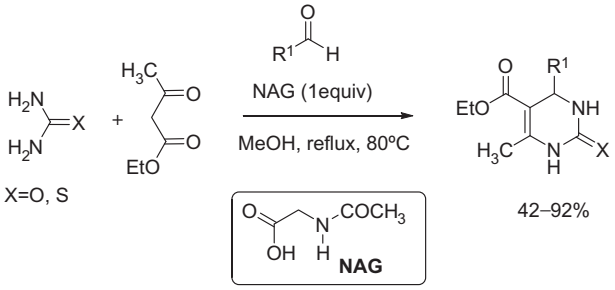
Metal free, simple and environmentally benign reaction conditions have attracted much attention [28–30]. Discrete organocatalysts can effectively accelerate chemical transformations with easy handling due to their moisture and oxygen stability. In addition, their syntheses are easily available with inexpensive costs and as well non-toxic nature. For this reason they are good candidates for synthesis of pharmaceutical products when compared to transition metal catalysts. We planned to search for a potential new organocatalyst which should give an efficient synthesis of dihydropyrimidines with the advantage of providing an easy separation workup. This led us to amino acids and especially *N*-protected amino acids; such as N-acetyl glycine (NAG). In this paper, we disclose the novel and a facile synthesis of dihydroprymidines under mild reaction conditions using NAG. *In vitro* α-glycosidase inhibitory activity for all the synthesized compounds and docking of the most active compound into the active site of the targeted protein to demonstrate the binding pattern are also described.

2. Results and discussion

2.1. Chemistry

We began our studies from the optimization of mole equivalents requirement of NAG. Different amounts (0.2–2 equivalents) of NAG were tested and one equivalent of NAG [31] gave high yield (92%) of the required pyrimidine **4a** from benzaldehyde **1**, urea **2** and ethyl acetoacetate **3**. Whereas, 0.2 and 0.5 equivalents of NAG afforded relatively poor yields and excess amount of catalyst (2 equiv) resulted almost equal yield (93%). Hence, one equivalent of the NAG was found to be the optimum amount to obtain the good yield. To demonstrate the scope of our new method, a number of pyrimidines bearing different substituents were accomplished in significant to excellent yields (40–92%) from urea or thiourea, selected aldehydes and ethyl acetoacetate, in the presence of NAG as a catalyst (Scheme 1). It was also found that urea could easily lead to cyclized product relatively in shorter period of time (Table 1, entries 1–5) while thiourea was less effective, producing dihydropyrimidine derivatives in moderate yields (Table 1, entries 6–10). The best results were obtained using 1 equiv. NAG, ethanol as solvent at 80 °C for 3 h. Notably, a variety of functional groups (OH, OCH_3, Cl) were tolerated under newly optimized reaction conditions.

According to previous reports, the use of salicylaldehyde in Biginelli reaction gave 4-(2-hydroxyphenyl) pyrimidines [32–38] but, in recent studies accomplished by various research groups have shown that oxygen-bridged pyrimidine derivatives are formed [39–43]. In order to explore the behavior of salicylaldehyde under our newly optimized reaction conditions and to compare with traditional reaction conditions (HCl in MeOH) [32–38]. We made two attempts (Scheme 2), and we observed the formation of 1,4-conjugate products **4d** and **4j** under our new optimized conditions instead of dihydropyrimidines **4m** which was obtained in traditional conditions. It is concluded that NAG can promote 1,4-conjugate addition by activating the α,β-unsaturated ester, while HCl could not support conjugate addition. The structures for compounds **4d**, **4j** and **4m** and relative stereochemistry in case of **4d** and **4j** were confirmed from the single crystal XRD (see Figs. 3–5).



Scheme 1. Synthetic protocol of dihydropyrimidines derivatives **4a–4l**.