The assessment of antidiabetic properties of novel synthetic curcumin analogues: α -amylase and α -glucosidase as the target enzymes

Fatemeh Hasaninezhad¹ · Zohreh Tavaf¹ · Farhad Panahi² · Maryam Nourisefat² · Ali Khalafi-Nezhad² · Reza Yousefi¹

Received: 3 April 2020 / Accepted: 2 November 2020 / Published online: 12 November 2020
© Springer Nature Switzerland AG 2020

Abstract

Diabetes mellitus is a metabolic disorder characterized by high blood glucose levels and instability in carbohydrate metabolism. For treating diabetes, one important therapeutic approach is reducing the postprandial hyperglycemia which can be managed by delaying the absorption of glucose through inhibition of the carbohydrate-hydrolyzing enzymes, α -amylase (α -Amy) and α -glucosidase (α -Gls) in the digestive tract. In this work, a new class of curcumin derivatives incorporating pyrano[2,3-d]pyrimidine heterocycles was synthesized using a multicomponent reaction between curcumin, aldehydes, and barbituric acid. Using UV-Vis spectroscopic method, the synthetic compounds were assessed for their inhibitory properties against α -Amy and α -Gls enzymes. Also, the antioxidant potential of these compounds was measured spectroscopically and compared with Trolox which is known as a gold standard to measure antioxidant capacity. The results of present study suggest that the curcumin derivatives were able to efficiently inhibit both yeast and mammalian α -Gls. In comparison with the antidiabetic medicine acarbose, the synthetic curcumin derivatives were also capable to inhibit more effectively the yeast α -Gls. The partial inhibitory effects of these compounds against pancreatic α -Amy were also important in the terms of avoiding development of the possible gastrointestinal side effects. Moreover, some of the curcumin derivatives indicated stronger antioxidant activity than Trolox. Overall, these synthetic curcumin analogues might be considered as novel molecular templates for development of efficient antidiabetic compounds with promising inhibitory activities against α -Amy and α -Gls enzymes.

Keywords Antidiabetic compounds · Enzyme inhibition · Antioxidant activity · Hyperglycemia · Curcumin

Introduction

Diabetes mellitus (DM) is currently a serious medical concern. This growing endocrine disturbance is connected with increased incidence and fatality, in addition to high cost of the health care. In 2000, about 171 million cases of DM have been reported, and it was expected that this number would become double (around 366 million) by 2030 [1]. It was demonstrated that DM and its associated problems are linked with the

enhancement of the oxidative stress, leading to disproportion between the generations of reactive oxygen species (ROS). The activity of the antioxidant defense system is also affected by DM [2]. Furthermore, the etiology of DM and its complications is controlled by ROS [3]. More importantly, DM causes important defects in insulin production or action, characterized by the hyperglycemia and alteration in the metabolism of carbohydrate, lipid and proteins [4]. As a well-known defect occurring in diabetes, postprandial hyperglycemia may result to a variety of secondary complications such as high venture for the cardiovascular illnesses [5], cataracts, atherosclerosis, retinopathy, nephropathy, neuropathy, and impaired wound healing [6].

Ingesting carbohydrate-rich diets could trigger the high blood glucose level because of the quick absorption of carbohydrates in small intestine. This phenomenon is assisted by the sugar hydrolysing enzymes such as α -glucosidase (α -Gls) which exist in the epithelial mucosa of the small intestine. Cleavage of the glycosidic bonds in the complex

✉ Farhad Panahi
panahi@shirazu.ac.ir

✉ Reza Yousefi
ryousefi@shirazu.ac.ir

¹ Protein Chemistry Laboratory (PCL), Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran

² Department of Chemistry, College of Sciences, Shiraz University, Shiraz, Iran

carbohydrates is the main task of these enzymes to release the absorbable monosaccharides [7]. In this regard, one of the important therapeutic approaches to treat diabetes is the control of postprandial hyperglycemia through inhibition of α -Gls. In fact, delays in the overall time of carbohydrate digestion in digestive tract are the aim of this treatment approach. On the other hand, sluggishness carbohydrate digestion can decrease the rate of glucose absorption to control the insulin levels and consequently postprandial blood glucose [8, 9].

Using α -Gls inhibitors, it is possible to have a therapeutic strategy to reduce the risks of diabetes. Also, other carbohydrate-mediated diseases, such as hyper lipoproteinemia and obesity can be controlled through α -Gls inhibition approach [10]. However, serious gastrointestinal side effects were observed due to non-selective and strong inhibition of pancreatic α -Amy which is one of the major drawbacks of the available α -Gls inhibitor drugs [11]. Consequently, development of strong α -Gls inhibitors with weak to moderate inhibitory action against α -Amy is an important object in this field of research. More importantly, it was illustrated that the compounds bearing more than one type of therapeutic actions (combination therapy) possesses significant role in the precautionary treatment of diabetic impediments [12]. In order to realize combination therapy in practice, one can use medicines with diverse methods of action. It was reported that compounds with both antioxidant and anti-glycation activities have shown remarkable reactivity in diabetes mellitus treatment [13]. Thus, by the use of known medicinal compounds such as curcumin which has previously shown different biological activities to synthesize new derivatives, it is possible to obtain new combinational therapeutic agents. Curcumin is a yellow color pigment in food industry which displays different biological properties such as antioxidant, anticancer, anti-inflammatory, and anti-HIV integrase [14]. This compound was also recommended by Chinese traditional medical prescriptions to be used against the diabetic complications [15]. Accordingly, in the current study, the effects of novel synthetic curcumin analogues on α -Glc/ α -Amy inhibition were evaluated. Also, antioxidant properties and mode of inhibitory action of these curcumin analogues against α -Glc were discussed.

Materials and methods

Materials

In this study, the yeast α -Gls (EC.3.2.1.20), *p*-nitrophenyl α -D-glucopyranoside (*p*NPG), porcine pancreatic α -Amy (EC 3.2.1.1), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ampicillin were purchased from Sigma-Aldrich Chemical Company. Dimethyl sulfoxide (DMSO) was used as solvent to prepare the stock solutions of curcumin and its synthetic derivatives. For the dilution of the stock

solutions, phosphate buffer was used. Chemicals were purchased from Fluka, Merck and Aldrich Chemical Companies and used as received without further purification. For recording ^1H NMR and ^{13}C NMR spectra we used a Bruker Avance DRX (250 MHz) and samples were dissolved in pure deuterated CDCl_3 with tetramethylsilane (TMS) as an internal standard. The reactions were monitored by TLC on silica gel PolyGram SILG/UV254 plates.

Methods

General procedure for the synthesis of compounds L₁–L₇

For the synthesis of curcumin-based pyrano[2,3-*d*] pyrimidines, we followed a procedure according to our previous reported synthetic methodology [16]. Accordingly, into a mechanically stirred solution of curcumin (1.0 mmol), aldehyde (1.0 mmol), and barbituric acid (1.0 mmol) in EtOH (3.0 mL) the magnetic nanoparticles functionalized sulfanilic acid (MNP-SAA) (8 mol%, 0.05 g) were added at 80 °C. The mixture was stirred within a specified period of time of 6–8 h. After completion of the reaction (controlled by TLC), the mixture was cooled to the room temperature. The MNP-SAA catalyst was separated from the reaction mixture using an external magnetic field. Then, some water was added for precipitation of the reaction mixture. If the precipitate was not observed immediately, it was kept in refrigerator overnight. The obtained precipitate was washed with water and dried in an oven. To obtain the pure product, recrystallization was carried out in *n*-hexane/ethylacetate solvents. The obtained products were characterized using different spectroscopic techniques and the results were compared with our previous reports in order to confirm the successful synthesis of the ligands and also their acceptable purity [16].

Rat intestinal acetone powder preparation

The acetone powder of rat intestine (25 rats with the bodyweights between 200 ± 20 g) was produced using a method reported previously in literature [17, 18]. The rat's sacrifice was accomplished under common anesthesia and their small intestines were cut from a point just under the entrance of the bile duct to around 6 cm on top of the cecum. The rat intestines were separated into the slighter parts, facilitating the washing process [19]. Subsequently, the intestinal segments were cleaned using 30 mL of cold NaCl (0.9%). All processes were carried out at 4 °C. The intestinal segments were homogenized using a Potter–Elvehjem type homogenizer in 2 vol of 5 mM phosphate buffer while pH was fixed at 7.0. Then, a 10 volume of the acetone solution (–20 °C) was added to the homogenates after 1 h. Afterward the mixture was shaken for 10 min, allowed to stand and next centrifuged for 30 min (at 1500×g). In this stage the precipitates were washed with cold acetone (12 vol) and the mixture

centrifuged for 30 min (at 1500×g). Finally, the precipitate was dried overnight (4 °C) and stored at low temperature (−20 °C).

The inhibition assay of yeast and rat α -Gls

The inhibition assay of yeast and rat enzymes was carried out based on our earlier reports with some modifications [17, 18]. The assay was done on micro plate reader (BioTekELx 808 instrument, USA) which is equipped with temperature control system. The activity of both yeast and mammalian α -Glc was measured with pNPG as a substrate reagent. When the pNPG is hydrolyzed by α -Glc, *p*-nitrophenol was released as a color agent, which can be monitored by its absorption at 405 nm. The inhibitory action of yeast enzyme (0.2 U/mL) was accomplished in phosphate buffer (100 mM; pH 7.0) at 25 °C for 10 min. The yeast enzyme assay was investigated with enhancement of pNPG concentration in the presence of different molar ratio of curcumin-derived inhibitors in the range of 0–100 μ M. To determine the activity of rat α -Gls, the intestinal acetone powder was dissolved in ice cold phosphate buffer (100 mM, pH 7.0) and sonicated for 25 s at 4 °C. Next, the suspension was centrifuged (10,500×g) at 4 °C, for 75 min. Then, the obtained supernatant was dialyzed using 10 mM potassium phosphate buffer (pH 7.0) for 24 h. The activity of rat α -Gls (0.15 U/mL) was obtained by increasing concentration of pNPG substrate (0.1–10 mM) at 37 °C. A mixture including rat α -Gls (0.15 U/mL) and different concentrations of each ligand was pre-incubated for 10 min at 37 °C, prior to adding pNPG. Subsequently, the increase of absorbance was pursued for 30 min (405 nm). Lineweaver–Burk analysis was used to obtain the mode of enzyme inhibition.

The enzyme activity at different concentrations of substrate was used to draw the Dixon plot. In this way, the activity of yeast and mouse enzymes was measured once in the absence of the inhibitors and several times in the presence of constant concentrations of each synthetic compound. To reach to Dixon plot, first the absorption against time was drawn and then the velocity was determined in different concentrations of the inhibitors and converted to $1/V$. We also used Dixon and Cortes transforms with the aim to obtain the IC_{50} values and inhibition constants (K_i). The slope of $1/IC_{50}$ vs v_0/v (relative velocity) in Cortes plot demonstrates the amount of inhibition constant [20]. In this study, Acarbose (pharmacological inhibitor) was used as a positive control. Also, all tests were repeated at least for three times.

Pancreatic α -Amy assay

We followed a method which has been previously published to assess the inhibition of α -Amy by the synthetic curcumin derivatives [34]. These synthetic compounds (250 mL, 100 μ M) were incubated with porcine α -Amy (250 mL, 0.4 mg/mL). The experiment was done in buffer C (20

mMNaPi, pH 6.9 bearing 6 mM sodium chloride) for 10 min at 25 °C. After pre-incubation, the starch solution (250 mL; 0.1%) which has been already prepared in buffer C was added to each tube. We then incubated the reaction mixture at 25 °C for 10 min. Finally, 1 mL of dinitrosalicylic acid (DNS) was used to stop the reaction progress. These tubes were then incubated for 5 min in a boiling water bath. After cooling down to the room temperature and subsequent to dilution by adding 10 mL distilled water, we measured the absorbance of each sample at 540 nm. The obtained absorption values were compared with that of the control buffer. The results were expressed as percentage of α -Amy inhibition, according to the following equation:

$$\%Inhibition = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}} \right] \times 100 \quad (1)$$

Antioxidant activity measurement of the curcumin-derived compounds

A T90⁺ UV-Vis spectrophotometer instrument (PG Instrument Ltd., UK) was used to measure the antioxidant activity of the curcumin-derived inhibitors. The antioxidant assay was done based on a decolorization system, recording reduction of ABTS radical cation (ABTS^{•+}) [21]. The final concentration of ABTS was fixed at 7 mM in double distilled water. As a result of reaction between ABTS and potassium persulfate (2.45 mM), ABTS radical cation (ABTS^{•+}) was produced. During formation of ABTS^{•+}, the reaction mixture was kept in the dark condition for 12–16 h before use. Then, the solution of ABTS radical was diluted in 5 mM phosphate buffer (pH 7.4) to obtain an absorbance value of 0.70 ± 0.02 at 734 nm. Also, the solution of the synthetic compound was diluted between 0 and 200 μ M in the same buffer. We added 10 μ L of each curcumin derivative to one milliliter of ABTS radical solution. At last, the absorbance was read at 734 nm in a kinetic fashion for 6 min. The disappearance of ABTS radical in the presence of each inhibitor was estimated as the percentage inhibition of absorbance at 734 nm.

$$ABTS^{\bullet+} \text{ inhibition } (\%) = \left[1 - \frac{A_{Sample}}{A_{Control}} \right] \times 100 \quad (2)$$

The Trolox equivalent antioxidant capacity (TEAC) of the synthetic inhibitors (μ M) was determined by comparing their percent inhibition with that of the standard curve for Trolox [22].

Assessment of the effect of curcumin based compounds on human lumen microflora

In this experiment, the sterilized Brain–Heart Infusion (BHI) agar medium was poured into sterile Petri dishes and permits it to solidify. The *Lactobacillus plantarum* (PTCC 1058) and

Escherichia coli (PTCC 1553) were provided by the Persian Microbial Type Culture Collection. After solidification, 100 μ L of each overnight culture of the bacteria in BHI broth with a density equivalent to 0.5 McFarland scale was swabbed into the individual plates, using sterile cotton swabs. Then, the wells of 6 mm diameter were made on BHI agar plates by a gel puncture and each compound with concentration of 100 μ M at the final volume of 20 μ L was poured onto each well of the plates. Ampicillin at the same concentration of compounds and 20 μ L BHI broth were used as positive and negative controls, respectively. After incubation at 37 °C for 24 h, the diameter of inhibition zone was measured.

Statistical analysis

The data were analyzed by Graphpad Prism 6.07 software. The differences between multiple groups were compared using parametric t-test with significant *p*-values (<0.05). The data were analyzed using three independent tests and expressed as mean $\pm$ SD.

Results and discussion

The chemistry of the synthetic curcumin-based derivatives

Diversity oriented synthesis (DOS) is one of the most widely used approaches in organic chemistry for preparing structurally different natural-like and biologically active compounds [23]. By applying this approach, it is possible to synthesize a large numbers of compound libraries using simple starting materials which are highly considered in the medicinal chemistry. Multicomponent reactions (MCRs), as a most widely used method of DOS, is very important in organic synthesis due to its high possibility for preparing of complex molecules using simple and available starting materials [24]. MCRs allow us to synthesize plenty of natural-based compounds, highly considered in the design of biologically active and biocompatible products [25]. When natural organic molecules to be used in MCR, it is possible to synthesize complex natural-like products which are very useful in drug design and synthesis. Along this line, we have disclosed some MCRs including small natural organic molecules such as carbohydrates and nucleosides for the synthesis of diverse biologically active compounds [26, 27]. We have also synthesized an interesting class of natural product-like compounds using curcumin as starting material. Curcumin has been indicated to be most active component of turmeric (*Curcuma longa*) plant [28]. Many published articles on this compound have described several biological properties ranging from antioxidant, anti-inflammatory to inhibition of angiogenesis [29]. Multiple cellular targets within the cells including different transcription

factors, kinases, receptors, inflammatory cytokines and growth factors have been suggested for curcumin [30]. This natural product indicates a limited cellular absorption, poor metabolism and rapid elimination which are major reasons for its poor bioavailability [31]. In order to overcome the mentioned is advantages, different strategies including synthesis of new curcumin analogues with improved bioavailability have been proposed [32]. Those from turmeric and synthetic analogues are two major groups of curcumin derivatives [33]. On the other hand, the curcumin analogues were designed by removing the β -ketone moiety to mono-ketone or changing to heteroaromatic ring and putting different substitutions on the benzene ring, coupling as co-polymer and metal complex [34]. Considering the chemical structure of curcumin, this valuable molecule has high potential to be used as starting material in MCRs for the synthesis of diverse complex molecules with important applications in the medicinal chemistry. Moreover, curcumin structure allows its versatile biological activities in both medicinal chemistry and food science [35]. However, new curcumin-based derivatives open up our hand in finding new medicinal applications of this valuable material. We have recently developed a multicomponent reaction which is applicable in the synthesis of a class of curcumin-based pyrano[2,3-d]pyrimidine analogues (Scheme 1).

Our initial assessment suggested this new class of curcumin derivatives as good antidiabetic compounds through inhibition of α -Amy and α -Gls with high antioxidant activity [15, 36]. These valuable results prompted us to synthesize other derivatives of these compounds in order to deep our insight in the inhibition activity of designed ligands with change of the molecular structures by selecting type of components. In this way seven new derivatives (**L₁–L₇**) were synthesized for the assessment of their inhibitory actions against two carbohydrate-hydrolyzing enzymes α -Amy and α -Gls (Scheme 1). Other biological properties including antioxidant activity of these compounds were also investigated.

The biological activities of synthetic curcumin-based derivatives

The assessment of biological activity of curcumin-based derivatives

Uncontrolled diabetes will finally culminate in development of many associated complications including heart disease, retinopathy, amputation, neuropathy and hypoglycemia. Due to many adverse effects associated with the long-term consumption of synthetic drugs, new trends are the medications provided by the natural based products which have indicated lesser complications [37]. During the previous decades many efforts have been done with the aim to introduce new drugs with pleiotropic and complementary functions which can be used in the treatment of diabetes mellitus. Curcumin possesses

Scheme 1 The chemical structure of synthesized curcumin derivatives

potent anti-inflammatory, antitumor, anti-HIV and antimicrobial properties [38].

The wound healing properties, antioxidant activity [39] and its effect in delaying diabetic cataract [40] suggest curcumin as a valuable precursor in the synthesis of novel medicinal compounds with application in the treatment of diabetes and its complications. The ability of curcumin to inhibit the intestinal carbohydrate hydrolyzing enzyme, α -Gls [41] was the reason behind the synthesis of novel derivatives of this compound with potential application to reduce the rise of postprandial blood glucose in the diabetic patients.

Inhibition parameters of curcumin derivatives against rat and yeast α -glucosidases

The poor clinical outcomes of current anti-diabetic medicine and increasing prevalence of type-II diabetes mellitus are two important reasons that led us to investigate on the new therapeutic approaches focusing to control this metabolic disorder in its initial stages [36]. In the small intestine, two carbohydrate-hydrolyzing enzymes α -Amy and α -Gls are responsible to digest the complex carbohydrates into the absorbable monosaccharides [42]. Therefore, among several types of available medicines, inhibitors of α -Gls which prevent the

absorption of monosaccharides in the small intestine are important to prevent diabetes and its complications in the initial stages. Moreover, α -Gls inhibitors have already indicated to suppress secretion of insulin after each meal and this event has less impact on decreasing capacity of insulin secretion by pancreatic β -cells [42]. In the current study, the inhibitory activities of novel curcumin derivatives (L₁–L₇) were examined against both yeast and rat α -Gls enzymes.

Our results showed that acarbose inhibits the yeast alpha-glucosidase enzyme with the IC₅₀ value of 206.57 mM, while the synthetic ligands have a more significant inhibitory effect on this enzyme. Also, acarbose inhibits mouse glucosidase with IC₅₀ value of 26.54, while L₁ and L₅ ligands inhibit this enzyme with the IC₅₀ values of 106.80 and 46.04 mM, respectively. Lineweaver–Burk and Cortes plots were used to determine the types of inhibition. Moreover, the inhibition constants were also identified by the aid of Dixon plot. As determined by the plots of Lineweaver–Burk and Cortes [20], L₁ and L₅ indicated a competitive mode of inhibition on mouse α -Gls (Fig. 1). Also, against the yeast enzyme, a similar mode of inhibition was observed for the synthetic ligands L₄ and L₆. Other curcumin derivative indicated a mixed-type of inhibition against the yeast enzyme (Fig. 2 and Table 1).

Fig. 1 The Lineweaver–Burk (a) and Dixon (b) plots of L_1 inhibitor against mouse α -glucosidase. The activity of mouse enzyme was measured with the substrate concentration ranging between 0.1 and 5 mM, in the presence of different concentrations of each inhibitor. The experiments performed in 10 mM phosphate buffer, pH 7.0 at 37 °C for 30 min. This figure represents the plots obtained for inhibitor L_1 . The Lineweaver–Burk and Dixon plots of other synthetic compounds are presented in the supporting information (Fig. S1)

The inhibition constants (K_{ic} , K_{iu}) are also shown in Table 1. However, the competitive inhibition mode of α -Gls is not very desirable because inhibition of this enzyme in the small intestine demands a higher dose of competitive inhibitor to overcome the competition with a carbohydrate rich diet. The Lineweaver–Burk and Dixon plots of other synthetic compounds are presented as the supporting information in Fig. S1 and Fig. S2.

Inhibition of α -Amy by curcumin-based derivatives

The large insoluble starch break into the smaller oligosaccharide molecules is made by the aid of α -Amy which prominently found in the pancreatic juice and saliva [43]. The inhibition of this enzyme also results in the reduction of glucose release but its complete inhibition can initiate undesirable effects such as intestinal disorder because of the gas production by gut microflora [44]. Because of this unwanted effect, the partial inhibition of α -Amy is desirable for modulating the rate of monosaccharide release from the complex polysaccharide such as starch [45]. In the current study the synthetic

Fig. 2 The Lineweaver–Burk (a) and Dixon (b) plots of L_5 inhibitor against yeast α -glucosidase. The enzyme activity was measured with the substrate concentration ranging between 0.1 and 4 mM in the presence of different concentrations of each inhibitor. The experiments were done in 100 mM phosphate buffer, pH 7.0 at 25 °C for 10 min. This figure represents the plots obtained for inhibitor L_5 . The Lineweaver–Burk and Dixon plots of other synthetic compounds are presented in the supporting information (Fig. S2)

curcumin derivatives were also used to study their inhibition against porcine pancreatic α -Amy.

The curcumin derivatives indicated a lower inhibition ability against alpha-amylase enzyme when compared to acarbose, which reduces activity of this enzyme to 42% of its original activity.

The activity of α -Amy was measured according to the procedure which indicated in Scheme 2.

The results have shown that α -Amy activity was reduced to the maximum level of 15% in the presence of L_4 and other curcumin derivatives demonstrated lower inhibitory action. The weak inhibitory action of the synthetic ligands compared to Acarbose (42% inhibition) against α -Amy is highly desirable in terms of their reduced susceptibility for possible development of the intestinal disorders (Fig. 3).

The assessment of antioxidant activity of synthetic curcumin derivatives

Chronic hyperglycemia activates glucose metabolism and associates with significant production of reactive oxygen species (ROS) and increasing oxidative stress in body. Under this situation the poor ability of the antioxidant defense system

Table 1 The IC_{50} ,^a Inhibition constants (K_i) values and inhibition mode of the synthetic curcumin derivatives

Entry	IC_{50} (μ M)		K_{ic} ^b (μ M)		K_{iu} ^b (μ M)		Type of inhibition	
	Yeast	Mouse	Yeast	Mouse	Yeast	Mouse	Yeast	Mouse
L ₁	12.41 ± 1.10	106.80 ± 2.10	10.23 ± 0.80	100.29 ± 2.40	37.62 ± 2.70	–	MC ^c	C ^c
L ₂	61.11 ± 3.50	–	55.62 ± 3.40	–	92.90 ± 3.60	–	MC	–
L ₃	36.64 ± 2.70	–	39.19 ± 2.00	–	93.10 ± 3.20	–	MC	–
L ₄	18.20 ± 1.50	–	16.44 ± 1.20	–	–	–	C	–
L ₅	27.60 ± 1.30	46.04 ± 2.10	19.14 ± 1.30	41.51 ± 0.90	71.68 ± 3.00	–	MC	C
L ₆	24.10 ± 0.90	–	21.60 ± 3.00	–	–	–	C	–
L ₇	63.73 ± 2.10	–	59.63 ± 3.20	–	107.00 ± 4.00	–	MC	–
AC ^d	206.57 ± 4.30	26.54 ± 2.57	166.70 ± 3.81	9.59 ± 2.80	–	–	C	C

^a The half maximal inhibitory concentration^b c and u respectively indicate competitive and un-competitive inhibitions^c MC and C respectively stand for mixed-competitive and competitive inhibitions^d Acarbose

to neutralize the mass production of oxidative agents may result in appearance of diabetes complications [46]. Oxidative stress associated with hyperglycemia is believed to have an important role in the etiology of diabetes and its complications [47]. Moreover, oxidative stress is highly

capable to trigger inflammatory cascade mediated many chronic disorders such as cancer, asthma, arthritis, and other diseases [48]. Therefore, those synthetic inhibitors displaying good antioxidant activity and improved anti-inflammatory properties are highly acceptable as antidiabetic medicines to

Scheme 2 The enzymatic process catalyzed by α -amylase enzyme. This scheme indicates the details of α -amylase activity measurement

Fig. 3 The inhibitory action of curcumin-based derivatives against porcine pancreatic α -amylase. α -amylase (0.5 mg/ml) was pre-incubated with each synthetic inhibitor for 10 min and then with starch solution for 30 min, at 25 °C. After that, the reaction mixture was incubated in a boiling water bath for 5 min in the presence of dinitrosalicylic acid. Subsequent to cooling down to room temperature, the mixture was

diluted by adding distilled water and then its absorbance was measured at 540 nm. The measured absorbance was compared with that of the control experiment and presented as percentage of α -amylase inhibition. This experiment was done in 20 mM sodium phosphate buffer, pH 6.9, containing 6 mM sodium chloride

be used by diabetic patients [49]. Curcumin-based heterocycle compounds contain electron-donating groups, therefore indicating antioxidant properties [50]. In the current study, the antioxidant activity of curcumin-based heterocycle compounds was determined using decolorization of the ABTS⁺ and the obtained results were shown in Figs. 4 and 5. The antioxidant capacity of the synthetic compounds was also calculated as TEAC which indicated in Table 2.

With the exception of L₂ and L₆, other curcumin derivatives indicated either comparable or higher antioxidant activity than the standard compound. The highest antioxidant activity was also observed for L₁ and L₅ which can be explained

with the feature of electron-donating substitutions in their chemical structures.

The effect of curcumin based compounds effect on human lumen microflora

The gut bacteria play important functions to regulate the development of intestine epithelium, maintaining mucosal homeostasis and repair, improving absorption of nutrients from food, and contribute to the innate immune system [51]. Today, over use of several antibiotics and other medicines cause important adverse effects on the intestinal normal flora resulting

Fig. 4 The effects of time on suppression of the absorbance of 3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺). The experiments were performed in different concentrations of each ligand (1.25–10 μ M). The curves represent the effects of the specific antioxidant on the suppression of the absorbance of the ABTS⁺ at 734 nm. The symbols used are as the following: Control ABTS⁺ radical cation (●), L₁(■), L₂(▲), L₃(▼), L₄(◆), L₅(○), L₆(□), L₇(Δ)

Fig. 5 The dose–response curve obtained by analysis a range of concentration of curcumin-derived compounds at the selected time points (1, 4 and 6 min). These plots represent the percentage inhibition of the absorbance of ABTS^{•+} solution as a function of concentration of the curcumin-derived compounds

important disturbance and complication of Gut. Therefore, the aim of this part was to evaluate the effect of these compounds on major bacteria existing in gut. To evaluate effect of these

compounds, the inhibition zone in well diffusion assay was performed against two important types of gut bacteria. The concentration of these curcumin derivatives and reference compound (ampicillin) was fixed at 100 μM (Fig. 6). As shown in Fig. 6, the inhibition zone was not evident for curcumin derivatives against *L. plantarum*.

In the case of *Escherichia coli*, only L₁ indicated an effect with the inhibition zone of 11 ± 0.1 mm, comparable with that of ampicillin (100 μM) as 15 ± 0.3 against *Lactobacillus plantarum* and 12 ± 0.5 against *Escherichia coli*. The little difference can be attributed to the cell wall composition of these bacterial target cells. Overall, our assessment confirmed that the curcumin derivatives except L₁ have no significant effect on the microflora existing in the intestinally. Therefore, the oral administration of these synthetic

Table 2 Comparison between antioxidant activity as trolox equivalent antioxidant capacity (TEAC) (μM) at specific time points

Ligand	TEAC±SD
L ₁	1.82 ± 0.07
L ₂	0.73 ± 0.02
L ₃	1.39 ± 0.09
L ₄	1.20 ± 0.10
L ₅	2.00 ± 0.09
L ₆	0.82 ± 0.06
L ₇	1.17 ± 0.10

Fig. 6 The effect of curcumin derivatives was assessed against *Lactobacillus plantarum* and *Escherichia coli* using well diffusion assay. The concentration of each compound was 100 μ M. The upper panel belongs to *Lactobacillus plantarum* while the lower panel stands *Escherichia coli*. Ampicillin (100 μ M) and Brain–Heart Infusion (BHI) broth were also used as positive and negative controls, respectively

compounds for treatment of diabetes may have little ecological disturbance in the normal intestinal microflora.

Conclusion

In brief, with the future rise in diabetic population worldwide, the search for active compounds with α -GIs inhibitory activity from medicinal plants has become a very meaningful task. In the current study, we have synthesized and assessed the novel curcumin-based heterocycle compounds for their inhibitory action against α -GIs and α -Amy. In fact, this study was done with the aim to search for the novel compounds with possible application in controlling the postprandial hyperglycemia in diabetic patients. Among seven curcumin derivatives which have been examined in the current study, only compounds **L₁** and **L₅** with the reasonable α -GIs inhibitory activity, poor α -Amy inhibition and important antioxidant properties were introduced as potentially important anti-diabetic agents. Therefore, these compounds can be potentially used for the more convenient management of postprandial hyperglycemia in diabetic patients. Moreover, these compounds can be evaluated for potential pharmacological activity against other metabolic diseases.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40200-020-00685-z>.

Acknowledgments The authors appreciatively acknowledge the financial support of Shiraz University and National Institute for Medical Research Development (NIMAD).

Author contributions RY, FP and AK conceived and designed research. FP and MN synthesized and characterized synthetic compounds. FH and

ZT conducted biological experiments. FP and RY analyzed data and wrote the manuscript. All authors read and approved the manuscript.

Funding This work was supported by NIMAD (grant number 964854).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest with the contents of this article. **Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s40200-020-00685-z>.

References

- Funke I, Melzig MF. Traditionally used plants in diabetes therapy: phytotherapeutics as inhibitors of alpha-amylase activity. *Rev Bras Farmacogn.* 2006;16:1–5.
- Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. *Med.Sci.* 2004;10:141–7.
- Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother.* 2005;59:365–73.
- Jaiswal N, Srivastava SP, Bhatia V, Mishra A, Sonkar AK. *J Diabetes Metab.* 2012;6:2.
- Carroll MF, Gutierrez A, Castro M, Tsewang D, Schade DS. Targeting postprandial hyperglycemia: a comparative study of insulinotropic agents in Type 2 diabetes. *J Clin Endocrinol Metab.* 2003;88:5248–54.
- Choudhary MI, Basha FZ, Abbas G, Khan SN, Shah SAA. *Pure Appl Chem.* 2007;79:2263–8.
- Chiba S. Molecular mechanism in α -glucosidase and glucoamylase. *Biosci Biotechnol Biochem.* 1997;61:1233–9.
- Kim KY, Nam KA, Kurihara H, Kim SM. Potent α -glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochem.* 2008;69:2820–5.

9. Chiasson JL, Rabasa-Lhoret R. *J Diabetes*. 2004;53:34–8.
10. Dwek RA, Butters TD, Platt FM, Zitzmann N. Targeting glycosylation as a therapeutic approach. *Drug Discov*. 2002;1:65–75.
11. Bachhawat JA, Shihabudeen MS, Thirumurugan K. *Int J Pharm Pharm Sci*. 2011;3:267–74.
12. Konya H, Miuchi M, Konishi K, Nagai E, Ueyama T, Kusunoki Y, et al. Pleiotropic Effects of Mitiglinide in Type 2 Diabetes Mellitus. *J Int Med Res*. 2009;37:1904–12.
13. Sharma OP. *Biochem Pharmacol*. 1976;15:1811–2.
14. Oglah MK, Mustafa YF. Curcumin analogs: synthesis and biological activities. *Med Chem Res*. 2020;29:479–86.
15. Du ZY, Liu RR, Shao WY, Mao XP, Ma L, Gu LQ. α -Glucosidase inhibition of natural curcuminoids and curcumin analogs. *Eur J Med Chem*. 2006;41:213–8.
16. Panahi F, Niknam E, Sarikhani S, Haghighi F, Khalafi-Nezhad A. Multicomponent synthesis of new curcumin-based pyrano[2,3-d]pyrimidine derivatives using a nano-magnetic solid acid catalyst. *New J Chem*. 2017;41:12293–302.
17. Yousefi R, Alavian-Mehr MM, Mokhtari F, Panahi F, Mehraban MH, Khalafi-Nezhad A, et al. *Med Chem*. 2013;28:1228–35.
18. B. B. Aggarwal, Y. J. Surh, S. Shishodia, Springer Science and Business Media. 595 (2007).
19. Panahi F, Yousefi R, Mehraban MH, Khalafi-Nezhad A. Synthesis of new pyrimidine-fused derivatives as potent and selective antidiabetic α -glucosidase inhibitors. *Carbohydr Res*. 2013;380:81–91.
20. Cortes A, Cascante M, Cardenas ML, Cornish-Bowden A. Relationships between inhibition constants, inhibitor concentrations for 50% inhibition and types of inhibition: new ways of analysing data. *Biochem J*. 2001;357:263–8.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231–7.
22. Wang H, Cao G, Prior RL, Agric J. Total antioxidant capacity of fruits. *Food Chem*. 1996;44:701–5.
23. O'Connor CJ, Beckmann HS, Spring DR. Diversity-oriented synthesis: producing chemical tools for dissecting biology. *Chem Soc Rev*. 2012;41:4444–56.
24. de Graaff C, Ruijter E, Orru RV. Recent developments in asymmetric multicomponent reactions. *Chem Soc Rev*. 2012;41:3969–4009.
25. Dömling A. Recent developments in isocyanide based multicomponent reactions in applied chemistry†. *Chem Rev*. 2006;106:17–89.
26. Panahi F, Yousefi R, Mehraban MH, Khalafi-Nezhad A. Synthesis of new pyrimidine-fused derivatives as potent and selective antidiabetic α -glucosidase inhibitors. *Carbohydr Res*. 2013;380:81–91.
27. Tavaf Z, Dangolani SK, Yousefi R, Panahi F, Shahsavani MB, Khalafi-Nezhad A. Synthesis of new curcumin derivatives as influential antidiabetic α -glucosidase and α -amylase inhibitors with anti-oxidant activity. *Carbohydr Res*. 2020;494:108069.
28. Gupta SC, Prasad S, Kim J, Patchva S, Webb LJ, Priyadarsini IK, et al. Multitargeting by curcumin as revealed by molecular interaction studies. *Nat Prod Rep*. 2011;28:1937–55.
29. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*. 2003;23:363–98.
30. Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB. Curcumin and cancer: An “old-age” disease with an “age-old” solution. *Cancer Lett*. 2008;267:133–64.
31. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm*. 2007;4:807–18.
32. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, et al. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem.Pharmacol*. 2008;76:1590–611.
33. Kiuchi F, Goto Y, Sugimoto N. Studies on crude drugs effective on visceral larva migrans. Part XVI. Nematocidal activity of turmeric: synergistic action of curcuminoids. *Chem Pharm Bull*. 1993;41:1640–3.
34. Adams BK, Ferstl EM, Davis MC, Herold M, Kurtkaya S, Camalier RF, et al. Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and anti-angiogenesis agents. *Bioorg Med Chem*. 2004;12:3871–83.
35. Qiu X, Du Y, Lou B, Zuo Y, Shao W, Huo Y, et al. Synthesis and identification of new 4-arylidene curcumin analogues as potential anticancer agents targeting nuclear factor- κ B signaling pathway. *J Med Chem*. 2010;53:8260–96.
36. Yousefi A, Panahi F, Sarikhani S, Zolghadr AR, Bahaoddini A, Khalafi-Nezhad A. Novel curcumin-based pyrano[2,3-d]pyrimidine anti-oxidant inhibitors for α -amylase and α -glucosidase: Implications for their pleiotropic effects against diabetes complications. *Int J Biol Macromol*. 2015;78:46–55.
37. Tabatabaei-Malazy O, Larijani B, Abdollahi M. *J Med Chem*. 2013;7:25–30.
38. Jordan WC, Drew CR. *J Natl Med Assoc*. 1996;88:333–8.
39. Mahmoud YA. Modulation of protein kinase C by curcumin; inhibition and activation switched by calcium ions. *Br J Pharmacol*. 2007;150:200–8.
40. Aldebasi YH, Aly SM, Rahmani AH. *Int J Physiol.Pathophysiol.Pharmacol*. 2013;5:194–9.
41. Kowluru RA, Kanwar M. *J Clin NutrMetab*. 2007;4:8–13.
42. Goda T, Suruga K, Komori A, Kuranuki S, Mochizuki K, Makita Y, et al. Effects of miglitol, an α -glucosidase inhibitor, on glycemic status and histopathological changes in islets in non-obese, non-insulin-dependent diabetic Goto-Kakizaki rats. *Br J Nutr*. 2007;98:702–10.
43. Afifi AF, Kamel EA, Khalil AA, Fawzi MFEI, Housery MM. *J Clin Nutr Metab*. 2008;3:14–21.
44. Cho M, Han JH, You S. *Br J Pharmacol*. 2011;44:1164–71.
45. Kim KT, Rioux LE, Turgeon SL. α -amylase and α -glucosidase inhibition is differentially modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*. *J.Phytochem*. 2014;98:27–33.
46. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. *Med Sci Monit*. 2004;10:141–7.
47. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed.Pharmacother*. 2005;59:365–73.
48. Paine A, Eiz-Vesper B, Blaszczak R, Immenschuh S. Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential. *Biochem.Pharmacol*. 2010;80:1895–903.
49. Abdollahi M, Tabatabaei-Malazy O, Larijani B. *Res Rev J Pharm Pharm Sci*. 2012;15:447–66.
50. Tavaf Z, Tabatabaei M, Khalafi-Nezhad A, Panahi F, Hosseini A. Green synthesis of silver nanoparticles by reduced glycyated casein adducts: Assessment of their antibacterial and antioxidant activity against *Streptococcus mutans*. *Eur J Integr Med*. 2015;7:294–302.
51. Kim KY, Nam KA, Kurihara H, Kim SM. Potent α -glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry*. 2008;69:2820–5.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.