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The α-glucosidase and α-amylase inhibitory activity from different chili pepper extracts

Watcharachaisoponsiri, T., Sornchan, P., Charoenkiatkul, S. and *Suttisansanee, U.

Institute of Nutrition, Mahidol University, Phutthamonthon 4 Rd.,Salaya, Phutthamonthon, NakhonPathom 73170

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Abstract

Diabetes is nowadays one of the leading causes of death among all health problems. Control of some key enzymes involving in starch degradation is considered a model pathway for new drug discovery for treatment/prevention of diabetes. Chili peppers have been demonstrated the ability to control diabetes through inactivation of α-glucosidase and α-amylase, the key enzymes that hydrolyze polysaccharide into glucose. The purpose of this experiment was to compare α-glucosidase and α-amylase inhibitory activities of different commercially available chili peppers in Thailand, including 'Yellow pepper', 'Bird chili', 'Green pepper', 'Cayenne Pepper', 'Red Chinda pepper', 'Green Chinda pepper', 'Young pepper', 'Chili Spur pepper' and 'Sweet pepper'. As results, 70% (v/v) aqueous ethanolic extracted chili peppers (5 mg/mL) exhibited anti-α-glucosidase activities within the range of 23-66% inhibition with Sweet pepper and Cayenne Pepper providing the highest and the lowest inhibitory activities, respectively. On the other hand, all chili peppers exhibited anti-α-amylase activities within the range of 27-58% inhibition with Green Chinda pepper and Sweet pepper providing the highest and the lowest inhibitory activities, respectively. Both enzyme inhibitions were dose-dependent, in which the inhibition was elevated as increasing concentration of chili pepper extract. Besides, these enzyme inhibitions were independent to fruit maturity but instead chili pepper species. Thus, chili peppers might be potential sources of natural products against diabetes and obesity through inhibition of α-glucosidase and α-amylase for future development of functional food, nutraceuticals or dietary supplement.

Introduction

Diabetes is a chronic disease, in which body cannot produce enough insulin or respond to the insulin. Diabetes is a public health problem, because it can cause acute or chronic complications that increased morbidity and mortality rates (Surya *et al.*, 2014). Diabetes can be divided into two groups, type 1 and type 2. The former involves immune system, which destroys islet cells that produce insulin in pancreas. As a result, insulin production is terminated. The later involves the body that continues to produce insulin, but it does not function properly due to insulin resistance.

Diabetes can be prevented by reducing sugar absorption through inhibition of α-amylase and α-glucosidase, the key enzymes that degrade carbohydrate into sugar. The α-amylase is a digestive enzyme that found in saliva and pancreas. It hydrolyzes α-1-4-glycosidic linkage on polysaccharide (starch), producing smaller sugar units such as disaccharide (maltose) and glucose. The digestion of starch is started in oral cavity by salivary α-amylase and continued to a small intestine by pancreatic α-amylase. The products of the reaction, glucose units, are absorbed into the wall of intestine. Glucoses are then delivered by blood to targeted cells throughout the body. Thus, α-amylase inhibition is important for delaying carbohydrate degradation and glucose adsorption.

Similarly, α-glucosidase can hydrolyze polysaccharide into glucose at terminal non-reducing α-1-4-glycosidic linkage in small intestine, where the enzyme is located. The product from α-glucosidase is monosaccharide such as glucose, which is absorbed into intestine wall. Thus, the inhibition of α-glucosidase can, as well, control diabetes by delaying carbohydrate degradation and glucose absorption.

Acarbose, a pseudo-oligosaccharide analogue, is used to treat diabetes through inhibition of α-amylase and α-glucosidase (Yamashita *et al.*, 1984). Acarbose is composed of acarviosin moiety, which is an unsaturated cyclitol unit, and 4,6-dideoxy 4-amino-D-glucose at the terminal reducing end. The drug possesses half maximal inhibitory concentrations

*Corresponding author.
Email: *uthaiwan.sut@mahidol.ac.th*
Tel: 0-2800-2380 ext. 422; Fax: 0-2441-9344

(IC_{50}) of 80 and 36 µg/mL for α-amylase and α-glucosidase inhibitions, respectively (Kwon *et al*., 2002; Kumar *et al.*, 2011). However, acarbose has side effects, including abdominal or stomach pain, bloated feeling or passing of gas, and diarrhea (He *et al*., 2014). Thus, natural plants or green medicines that can inhibit α-amylase and/or α-glucosidase without or less side effects are of current interest.

Chili pepper in the *Capsicum* genus, which can be found all over the world, is used as a food ingredient to add spicy flavor and as a food decoration due to their varieties of fruit colors (green, yellow, orange, red, purple and etc.). The taxonomic hierarchy of chili can be classified into the genus of *Capsicum* with five domesticated species that can grow around the world, including *C. annuum, C. frutescens, C. chinense, C. baccatum* and *C. pubescensa*. Since two species of chilies including *C. annuum* (Green pepper, Young pepper, Chili Spur pepper and Sweet pepper) and *C. fretescens* (Yellow pepper, Bird chili, Red Chinda pepper, Green Chinda pepper and Cayenne Pepper) are agriculturally and commercially available in Thailand. Chili has been widely studied regarding its health promotions on diabetes through inhibition of some key enzymes that control the disease (Oboh *et al*., 2011). Some bioactive compounds or polyphenols presenting in chili peppers have demonstrated the ability to control diabetes through inactivation of α-glucosidase and α-amylase by reduce disaccharides in the intestinal lumen (Hanhineva *et al.*, 2010). Nevertheless, only little information concerning anti-diabetic properties through enzymatic control of α-glucosidase and α-amylase activities on different varieties of chili pepper is available. Therefore, the purpose of this experiment was to compare α-glucosidase and α-amylase inhibitory activities of different commercially available chili peppers in Thailand, including 'Yellow pepper', 'Bird chili', 'Green pepper', 'Cayenne Pepper', 'Red Chinda pepper', 'Green Chinda pepper', 'Young pepper', 'Chili Spur pepper' and 'Sweet pepper'.

Materials and Methods

Preparation of chili pepper

Chili peppers including Yellow pepper, Bird chili, Green pepper, Cayenne Pepper, Red Chinda pepper, Green Chinda pepper, Young pepper, Chili Spur pepper and Sweet pepper were purchased from local market during June, 2014 from Phutthamonthon, Nakhon Pathom, Thailand (Table 1, Figure 1). The samples were clean with deionized water and cut into small pieces (approx. 0.5 x 0.5 cm). The samples were then freeze-dried (Heto PowerDry PL9000, Thermo Fisher Scientific, Waltham, MA, USA) before being ground into fine powder by a cyclotec sample mill (series 1903 with 200–240V and 50/60 Hz; FOSS, Höganäs, Sweden). The moisture content after freeze drying was determined using Association of Official Analytical Chemists (AOAC) (930.15, AOAC International, 2005). All samples were kept in vacuum bags and stored at –20°C

Table 1. The general information of chili peppers used in this study

Species	Common name	Fruit color	Maturity
Capsicum annuum	Green pepper	Green	Mature
	Young pepper	Green	Pre-mature
	Chili Spur pepper	Red	Mature
	Sweet pepper	Red	Mature
	Green Chinda pepper	Green	Mature
Capsicum frutescens	Yellow pepper	Yellow	Mature
	Bird chili	Orange	Mature
	Cayenne Pepper	Red	Mature
	Red Chinda pepper	Red	Mature

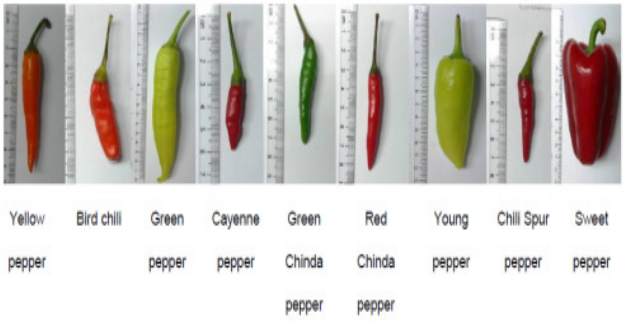

Figure 1. Various types of chili peppers including Yellow pepper, Bird chili, Green pepper, Cayenne pepper, Green Chinda pepper, Red Chinda pepper, Young pepper, Chili spur pepper and Sweet pepper

Extraction of chili pepper

Dry chili powder (0.2 g dry weight) was resuspended in 70% (v/v) aqueous ethanol (8 mL) and vortexed for 5 min. The mixture was sonicated for 10 min in a water bath sonicator (model B1510, 40 KHz; Bransonic Bransonic® Ultrasonic, Danbury, CT, USA) and continued shaking in a temperature-controlled water bath (Memmert GmbH, Wisconsin, USA) at 60°C for 1 hour. The sample was then centrifuged at 1190xg for another 10 min. The supernatant were collected and stored at -20°C for further analysis.

Determination of α-glucosidase inhibitory activities

The α-glucosidase inhibitory activity was determined by a colorimetric assay utilizing a well-established protocol (You *et al*., 2011) with some changes as follows. The enzyme reaction consisted of 0.05 U/mL *Saccharomyces cerevisiae* α-glucosidase (EC 3.2.1.20) (type I, ≥10 units/mg protein), 0.5 mM p-nitrophenyl-α–D-glucopyranoside (pNPG) and chili pepper extracted (5 mg/mL) in a 96–well plate. All chemicals were received from Sigma–Aldrich (St. Louis, MO, USA). The enzyme reaction was monitored at 405 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT) with a Gen5 data analysis software. The initial rate was fitted by the Michaelis–Menten equation with least squares fit parameter using a GraphPad Prism software version 5.00 (GraphPad Software, Inc., La Jolla, CA, USA). The enzymatic inhibitory activity was calculated as % inhibition using the following equation;

$$\% \text{ inhibition} = 100 \times (1 - ((B-b)/(A-a)))$$

where *A* was an initial velocity of the control reaction (without plant extract) with the enzyme, *a* was an initial velocity of the control reaction without enzyme, *B* was an initial velocity of the enzyme reaction with chili extract and b was an initial velocity of the reaction with chilli extract but without enzyme.

Determination of α-amylase inhibitory activities

The α-amylase inhibitory activity was determined by a colorimetric microplate assay utilizing a well-established protocol (Ingrid and Matthias, 2006) with some changes as follows. The enzyme reaction consisted of 4 mg porcine pancreatic α-amylase (EC 3.2.1.1) (type VI-B, ≥10 units/mg solid), 1.25 mM p-nitrophenyl-α-D-maltopentaoside (PNPG-5) and chili pepper extracted (5 mg/mL) in the 96–well plate. All chemicals were received from Sigma–Aldrich (St. Louis, MO, USA). The enzyme reaction was monitored at 405 nm using the microplate reader. The percentage of α-amylase inhibition was calculated using the equation as above.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) of triplicate assays. One way analysis of variance (ANOVA) and Turkey's multiple comparison tests were performed to determine the significant differences between values with $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corp, Armonk, NY).

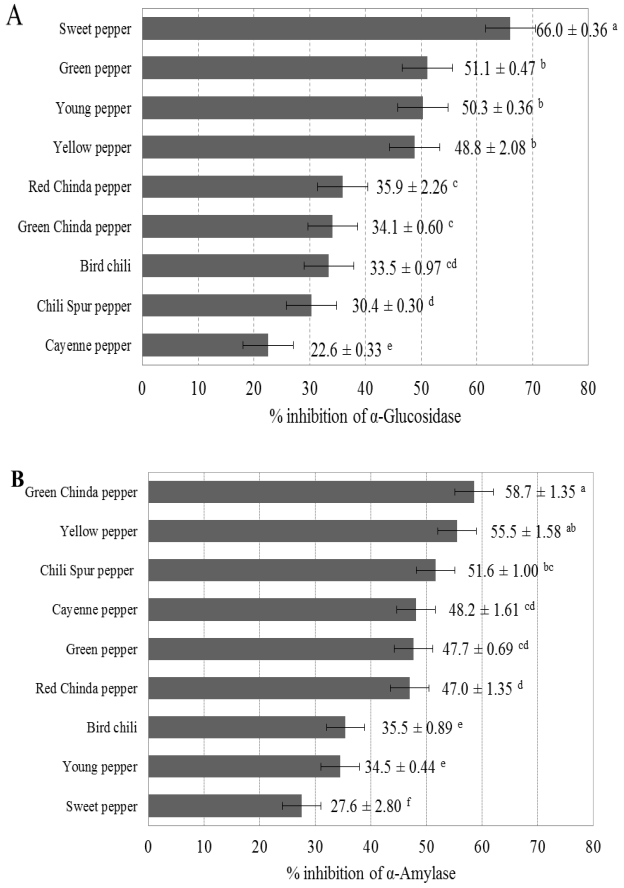

Figure 2. The inhibitory activities against (A) α-glucosidase and (B) α-amylase of different chili peppers (5 mg/mL) including 'Yellow pepper', 'Bird chili', 'Green pepper', 'Cayenne Pepper', 'Red Chinda pepper', 'Green Chinda pepper', 'Young pepper', 'Chili Spur pepper' and 'Sweet pepper'. The different letters showed statistically significant difference at p value < 0.05 using one-way ANOVA and Turkey's multiple comparison tests.

Results and Discussion

Chili is a plant in the *Capsicum* genus and Solanaceae family. This research was focused on biochemical properties against key enzymes that control diabetes from these two species of chili peppers. Starch hydrolysis is controlled by two crucial enzymes, α-amylase and α-glucosidase. These enzymes can degrade carbohydrate into glucose, which can be further absorbed into body. Overload of sugar consumption not only causes obesity, but also diabetes, the 6th cause of death among Thai public health problems in 2012 (Bureau of Non Communicable, 2012; Social and Quality of Life Data Base System, 2012). Thus, the inhibition of α-amylase and α-glucosidase is of current interest to prevent carbohydrate digestion, leading to controlling of obese and diabetic occurrences.

As a result, it was found that all investigated chili peppers could inactivate α-glucosidase with different degrees of inhibition (Figure 2A). Chili pepper

extracts (5 mg/mL) exhibited anti-α-glucosidase activities within the range of 23-66% inhibition. Sweet pepper exhibited the highest inhibitory activity (66% inhibition), followed by a group of middle inhibitory ranged peppers (48-51% inhibition including Green pepper, Young pepper and Yellow pepper), middle low inhibitory ranged peppers (30-36% inhibition including Red Chinda pepper, Green Chinda pepper, Bird chili and Chili Spur pepper) and low inhibitory ranged pepper (Cayenne Pepper with 23% inhibition), respectively. These results suggested that α-glucosidase inhibitory activities were independent of fruit maturity but instead particular chili pepper species.

The species of *C. annuum* was found to be medicinal plants with antidiabetic potential (Surya *et al.*, 2014). The sweet pepper *(Capsicum annuum)* that used in this experiment was in its red mature stage. The bioactive compounds in red sweet pepper that were previously analyzed included capsaicinoids (11.8 µg/100 g dry weight), quercetin (9.97 µg/g dry weight and 34 µg/g fresh weight), myricetin (244.3 µg/g dry weight) and luteolin (11 µg/g fresh weight) (Tundis *et al*., 2011; Bae *et al.*, 2012). Capsaicinoids are a group of bioactive compounds founded in chili peppers with pungent characteristic. Interestingly, peppers in *C. annuum* species were reported to contain capsiate (non-pungent capsaicinoid) with a greater anti-diabetic action than capsaicin (pungent capsaicinoid) (Kwon *et al.*, 2013). Capsiate could enhance hepatic insulin sensitivity during euglycemic hyperinsulinemia clamp, reduce hepatic glucose output, increase triglyceride accumulation and increase glycogen storage (Kwon *et al.*, 2013). Quercetin, myricetin and luteolin could, as well, act against α-glucosidase activity with the IC_{50} values of 1.59, 2.12 and 6 µg/mL, respectively (Ranilla *et al*., 2010). Comparing to acrabose, the anti-diabetic drug with the IC_{50} of 36 µg/mL (Kwon *et al.*, 2002), these compounds could be potentially used for diabetic treatment, and consumption of sweet pepper could possibly be used for diabetic prevention. Nevertheless, absorption and bioavailability of these bioactive compounds in each particular chili pepper are required to be further investigated. Additionally, detailed mechanism and interaction(s) within inhibitor-enzyme complex, which is a platform for enzyme structure based drug design, needed to be analyzed.

Interestingly, by using multispectroscopic methods and molecular docking technique, it was previously reported that the interactions between luteolin, a reversible inhibitor with inhibition constant (K_i) of 17.2 mM, and α-glucosidase are mainly hydrophobic interactions (Yan *et al*., 2014). Instead of binding directly into the active site of the enzyme (like a competitive inhibitor), luteolin, the non-competitive inhibitor, actually interacts near the catalytic pocket of α-glucosidase and indirectly blocks the entrance of the natural substrate, starch. This binding also allows enzyme conformational change and rearrangement such that the substrate could not enter the active site of the enzyme and is unable to be hydrolyzed. Thus, hydrolysis of starch and glucose production could not occur. This matter could lead to low absorption of glucose into blood system and, therefore, control of diabetes.

Likewise, the α-amylase inhibitory activities of investigated chili peppers indicated that all extracts (5 mg/mL) could inactivate α-amylase with different degrees of inhibition (Figure 2B). The inhibition was within the range of 27-58% with Green Chinda pepper exhibiting the highest inhibitory activity, followed by a group of middle inhibitory activities (47-55% inhibition including Yellow pepper, Chili Spur pepper, Cayenne Pepper, Green pepper and Red Chinda pepper) and a group of low inhibitory activities (27-35% inhibition including Bird chili, Young pepper and Sweet pepper), respectively. Interestingly, Sweet pepper with the highest α-glucosidase inhibitory activity exhibited the lowest α-amylase inhibitory activity (28% inhibition).

Green Chinda pepper *(C. frutescens)* is commonly used for flavoring in food due to its hot spicy taste. This species was found to contain various bioactive compounds that could act against α-amylase such as capsaicin, myricetin, quercetin, luteolin and linoleic acid (Tundis *et al.*, 2011; Wongsa *et al*., 2012). It was previously reported that capsaicin can decrease plasma glucose and maintain insulin levels in vivo (Chaiyasit *et al*., 2009). However, directed interaction between capsaicin and α-amylase regarding enzyme inhibition has not been reported. Nevertheless, high quantity of phenolics and capsaicins were found in red pepper stalk, the part with strong α-amylase inhibitory activity (Menichini *et al.*, 2009). Myricetin, quercetin and luteolin were reported to exhibited α-amylase inhibitory activity with IC_{50} of 151.12, 120.93 and 103.05 µg/mL (Tadera *et al.*, 2006). Besides, linoleic acid that was found in total lipophilic fraction exhibited the IC_{50} values of 8.7 and 29.0 µg/mL against α-amylase for big and medium pepper, respectively (Tundis *et al*., 2011). Comparing to acarbose with the IC_{50} of 80 µg/mL, these compounds could be potentially used for diabetic control through α-amylase inhibition. However, like α-glucosidase inhibition, further investigation in limited factors such as absorption, bioavailability,

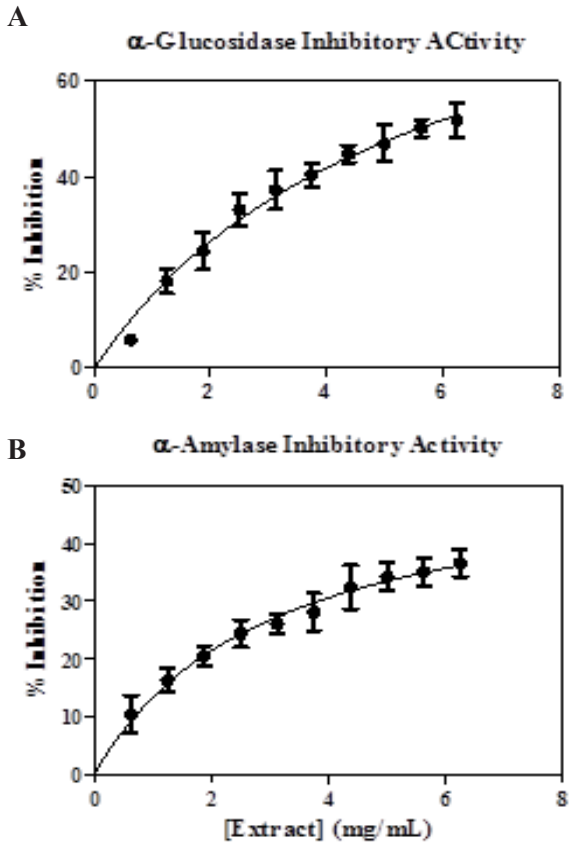

Figure 3. The non-linear, dose-dependent relationship of (A) α-glucosidase inhibition and (B) α-amylase inhibition of chili pepper extract (in this case, yellow pepper was used as an example consecutively)

and detailed inhibitor-enzyme mechanism regarding α-amylase inhibition are required.

Amylase inhibitor can be divided into two classes; non-proteinaceous and proteinaceous inhibitors. Non-proteinaceous inhibitors include several types of organic compounds such as acarbose, isoacarbose, acarviosine-glucose, hibiscus acid and the cyclodextrins. Some of these bioactive compounds can be isolated from plants such as hibiscus acid from Roselle tea *(Hibiscus sabdariffa)* (He *et al*., 2007). It was also found that quercetin, myricetin and luteolin could act as non-competitive inhibitors on α-amylase (Tadera *et al*., 2006; Yan *et al*., 2014). The α-amylase provides a binding site (not the active site) for the compounds with mainly hydrophobic interactions. The strength of inhibition is focused on cyclic moiety of the inhibitor, which resembles the structure of the enzyme's natural substrate.

Moreover, the α-glucosidase and α-amylase inhibitions were found to be dose-dependent, in which the inhibition was elevated as increasing concentration of chili pepper extract (Figure 3). The relationship between inhibitory efficacy and extract concentration was in a linear relation under low concentration of extract, indicating a one-to-one

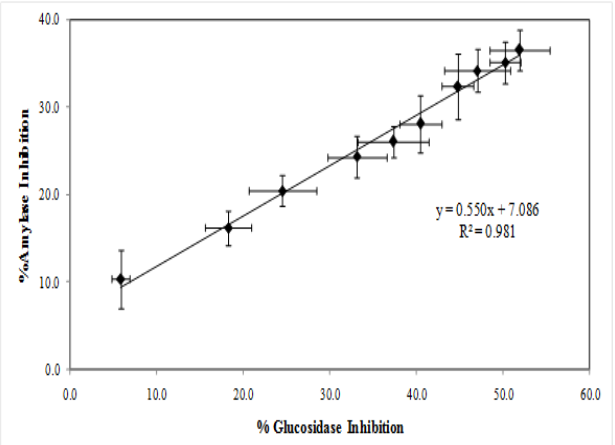

Figure 4. The Correlation between α-glucosidase and α-amylase inhibition of chili pepper extract (in this case, yellow pepper was used as an example consecutively)

interaction between inhibitor and enzyme. However, when the extract concentration was continuingly increased, slower rate of increased inhibition was observed. At this point, it might suggest that more enzyme molecules were occupied and have become limiting factor. After that, the inhibition became plateau regardless of increased extract concentration, suggesting that all enzyme molecules are occupied and inhibitor (chili pepper extracts) were in excess. Additionally, the correlation between α-glucosidase and α-amylase inhibitions of chili pepper extract was found to be a linear relation (Figure 4). It was possible that anti-α-glucosidase agents might as well possess anti-α-amylase activity (bifunctional properties). Nevertheless, more information on enzyme-inhibitor structural analyses and enzyme activity would be taken into account in order to confirm this hypothesis.

Conclusion

The investigation on α-glucosidase and α-amylase of chili peppers suggested that (1) all investigated chili peppers could inactivate α-glucosidase and α-amylase with different degrees of inhibition, (2) the inhibition was found to be dose-dependent and (3) type of the particular chili pepper was found to affect enzyme activities rather than ripening stages. From these results, it could be suggested that chili peppers could possibly be used to control diabetes through inhibition of two key enzymes, α-glucosidase and α-amylase, that degrade carbohydrate into sugar. The highest anti-α-glucosidase activities is sweet pepper, and the highest anti-α-amylase activities is green chinda pepper. Besides, these results also suggested that α-glucosidase and α-amylase inhibitory activities were independent to fruit maturity but instead chili pepper species. This information would support fundamental knowledge of functional food for health

benefit regarding diabetic prevention from chili peppers, potential development of food supplement and nutraceutical, and future drug design for diabetic treatment based on enzyme-drug interactions.

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