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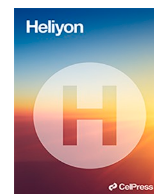

Review article

A review on the in vitro and in vivo screening of α-glucosidase inhibitors

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ABSTRACT

As a global metabolic disease, the control and treatment of diabetes have always been the focus of medical research. α-Glucosidase is a key enzyme in regulating blood glucose levels and has important applications in the treatment of diabetes. This review aims to explore the enzyme activity of α-glucosidase and its inhibition mechanism and evaluate the efficacy and limitations of existing inhibitor screening methods. First, the chemical structure, biological activity, and influencing factors of α-glucosidase on diabetes are discussed in detail. Then, the various methods that have been used to screen α-glucosidase inhibitors in recent years are reviewed, including in vivo animal experiments, in vitro experiments, and virtual molecular docking. The experimental principles, advantages, and limitations of each method and their application in discovering new inhibitors are also discussed. Finally, this review emphasizes the importance of developing efficient and safe α-glucosidase inhibitors, summarizes the advantages and disadvantages of various screening models, and proposes future research directions. This review comprehensively examines the enzyme activity of α-glucosidase and the screening methods for α-glucosidase inhibitors, provides an important perspective in the field of diabetes drug discovery and development, and provides a reference for future research.

1. Introduction

Diabetes is a chronic disease. Between 1990 and 2021, the global prevalence of age-standardized diabetes increased from 3.2 % to 6.1 %, with a growth rate of approximately 90 %. The global prevalence of diabetes is increasing, and this is not expected to decline in any country or region [1]. The International Diabetes Federation (IDF) estimated that 537 million adults aged between 20 and 79 years will have diabetes worldwide by 2021, and this number is expected to reach 783 million by 2045 [2]. According to the 2019 World Health Organization (WHO) classification, diabetes can be divided into the following six types [3]: type 1 diabetes (T1DM), type 2 diabetes (T2DM), mixed diabetes (slow development of immune-mediated diabetes in adults, ketosis-prone type 2 diabetes), other specific types (such as monogenic diabetes and pancreatic exocrine diseases), gestational hyperglycemia, and unclassified diabetes.

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Among them, T2DM is the most common type and accounts for more than 90 % of all diabetes cases in the world [1,2].

T2DM is caused by abnormal blood glucose levels in vivo due to insulin resistance (decreased sensitivity of insulin in target organs such as the liver, muscle, and adipose tissue, resulting in increased glucose in the liver) and islet β-cell dysfunction (insufficient insulin secretion) [2,4]. In 2021, a high body mass index (BMI) was estimated to be the major risk factor for T2DM globally, and other risk factors include diet, environment, occupation, tobacco use, and alcohol consumption [1]. Oral hypoglycemic drugs and lifestyle (exercise and diet management) can control hyperglycemia [5,6]. Currently, insulin, biguanides, second-generation sulfonylureas, thiazolidinediones, α-glucosidase inhibitors (AGIs), glucagon-like peptide 1 agonists, sodium and glucose cotransporter 2 inhibitors are some of the drugs used to treat diabetes [7].

α-Glucosidase (α-Glu) is a therapeutic target for the treatment of T2DM. Common AGIs include acarbose, misoglitol and voglibose [8,9]. Although these drugs can effectively control blood glucose levels, long-term use can cause adverse side effects, such as gastrointestinal disorders, obesity, hepatotoxicity, abdominal distension, diarrhea, and drug resistance [10,11]. Finding new, safe, and effective AGIs with few side effects has become a focus, and thus, the screening of AGIs from natural products has attracted increasing attention. Researchers have found that many bioactive compounds from the extracts of traditional medicinal plants and foods (such as fruits, vegetables, grains, and tea) can effectively inhibit the activity of α-Glu. Reported compounds include anthocyanins, flavonoids, alkaloids, and phenols [12–14].

Because the components of natural products are complex, it is important to use simple and efficient methods to screen for effective AGIs from natural products. In recent years, various new inhibitor screening methods have been developed. There has been literature that reviewed the in vitro screening methods for AGIs before 2019, and it focused on the screening methods for AGIs related to sensing strategies [15]. This review, however, mainly covers research literatures after 2019. The content encompasses both in vivo and in vitro screening methods for AGIs, and is updated and more comprehensive. In addition, this review also discusses the experimental principles, advantages, and limitations of the main methods, as well as their application in discovering new inhibitors. It provides a reference for the development and utilization of natural AGIs.

2. Chemical structure, biological activity, influencing factors of α-glu, and its effects on diabetes mellitus

α-Glu is a carbohydrate hydrolase found on the surface of intestinal cells and is produced in the small intestine [16]. This enzyme is widely distributed in microorganisms, plants, animals, and human tissues [17]. α-Glu is a common exosaccharide. Its molecular structure comprises a polypeptide chain and an active site that binds to its substrate. The amino acid sequence of the enzyme contains two main domains: N-terminal and C-terminal. The N-terminal domain contains a glycosidase active center that catalyzes the hydrolysis of the substrate, whereas the C-terminal domain is involved in substrate binding and stabilization [18]. The main biological activity of α-Glu is to catalyze the hydrolysis of glucose and other α-glucosides, which are hydrolyzed by the cleavage of the 1-glucosidic bond between the substrate hetero-carbon and the non-reducing end of the glucose residue, releasing D-glucose [19]. α-Glu is a key enzyme that hydrolyzes carbohydrates such as maltose and sucrose into monosaccharides, such as glucose and fructose. The resulting monosaccharides can directly enter cells and be absorbed, utilized, and supply energy to the body [17,20]. The activity of α-Glu is affected by many factors, including pH, temperature, substrate specificity, and enzyme concentration. Different influencing

Table 1
Summary of research reports on screening AGIs using an in vivo model.

Tested drugs	Modeling animal	Modeling method	Mode of administration	Administration dose	Ref.
The dry leaf extract of *M. multiflora*	Swiss male mice	STZ-NA -induced diabetic	Injection	150 mg/kg(STZ) and 50 mg/kg(NA)	[27]
Ageratina adenophora hydroalcoholic extract	Wistar albino rats	STZ-NA -induced diabetic	Injection	50 mg/kg(STZ) and 100 mg/kg(NA)	[28]
Q. coccifera leaves extracts	Swiss albino mice	Alloxan monohydrate-induced diabetic	Injection	180 ng/kg	[29]
The extract and phytoniosomes of *Tradescantia pallida* leaves	Swiss albino mice	Alloxan monohydrate -induced diabetic	Injection	150 mg/kg	[30]
The hydroalcohol fraction of *H. zeylanicum* leaves	Wistar albino rats	High-fat diet and STZ-induced diabetic	Injection	40 mg/kg	[31]
Triazole tethered thymol/carvacrol-coumarin hybrids	Female Wistar rats	STZ-induced diabetic	Injection	60 mg/kg	[32]
Protocatechuic acid	Male C57BL/6J mice	STZ-induced diabetic	Injection	40 mg/kg	[33]
Mangiferin-loaded solid lipid nanoparticles	Male Wistar rats	High-fat diet and STZ-induced diabetic	Injection	35 mg/kg	[34]
Ethyl acetate fractions of *Tectona grandis* crude extract	SD rats	Fructose-STZ-induced diabetic	Injection	10 % fructose and 40 mg/kg STZ	[35]
The n-butanol fraction of *F. tikoua*	Male C57BL/KsJ db/db mice	–	–	–	[36]
The eggplant extract	Fruit fly	Sucrose-induced diabetic	Oral	free access to sucrose	[37]
Flavonoids from *R. corchorifolius*	Male ICR mice	Sucrose, maltose or starch solution	Oral	2 g/kg	[38]

Note: indicates that no modeling is required.

factors may lead to changes in enzyme activity, thereby affecting carbohydrate digestion and metabolism. For example, an acidic environment is usually conducive to the activity of α-Glu because it neutralizes negatively charged groups, thereby increasing the affinity between the substrate and enzyme [21]. In addition, the change in enzyme concentration also affects the catalytic efficiency of the enzyme, and high concentrations may lead to excessive hydrolysis or inhibition.

α-Glu is a key therapeutic target for the treatment of diabetes. Inhibiting the activity of α-Glu can slow down the absorption rate of glucose in the intestine, reduce the peak value of postprandial blood glucose, and help control the fluctuation of blood glucose. This has a significant therapeutic effect on T2DM and impaired glucose tolerance in prediabetic patients, especially those with significantly elevated postprandial blood glucose levels [17,22]. In addition, AGIs have the advantage that they do not stimulate insulin secretion and, therefore, do not cause hypoglycemia, which is essential to avoiding blood glucose fluctuations and maintaining blood glucose stability throughout the day [23,24]. Studies [25,26] have shown that AGIs can reduce the risk of cardiovascular disease in patients with diabetes, providing a new strategy for the comprehensive treatment of diabetes.

3. In vivo screening

Screening in vivo generally involves creating models using compounds, such as streptozotocin (STZ), streptozotocin-nicotinamide (STZ-NA), and alloxan, that damage islet β-cells. These models can be created by intraperitoneal administration alone or by a high-sugar and high-fat diet combined with intraperitoneal administration of the above-mentioned drugs, resulting in partial necrosis of islet β-cells in experimental animals, resulting in T2DM [27–35]. Alternatively, a diabetic mouse model, such as an experiment with db/db mice, can be directly used [36]. In addition, a diabetic Drosophila model induced by sucrose has been used to screen AGIs [37]. Furthermore, without a T2DM animal model, normal mice can be given sucrose or maltose directly for glucose tolerance test. AGIs can be screened by measuring the blood glucose level at different time points after meals [38]. The screening of AGIs using various in vivo models, as reported in the literature, has been summarized in Table 1. Its common experimental model in vivo is shown in Fig. 1.

Oliveira et al. [27] found that the extract of dried leaves of *Myrcia multiflora* can inhibit the blood glucose of T2DM mice induced by STZ and has a protective effect on the kidney and liver. Jaber et al. [29] found that the methanol extract of *Quercus coccifera* leaves could control the alloxan monohydrate-induced increase in blood glucose in T2DM mice. Rout et al. [31] described a T2DM model induced by STZ after feeding Wistar albino rats a high-fat diet for 2 weeks and used this model to investigate the water-alcohol partial hypoglycemic effect of *Homalium zeylanicum* leaves. The results showed that the extract reduced the blood glucose levels in diabetic mice. Wang et al. [36] investigated the antidiabetic effect of the n-butanol fraction of *Ficus tikoua* using the male C57BL/KsJ db/db mice model and showed that the extract might ameliorate diabetes via reducing fasting and random blood glucose, improving oral glucose tolerance, and decreasing glycosylation of hemoglobin in vivo. Nwanna et al. [37] investigated the effect of an eggplant fruit diet on sucrose-induced metabolic dysfunction in diabetic *Drosophila melanogaster* and determined the homogenate extract of

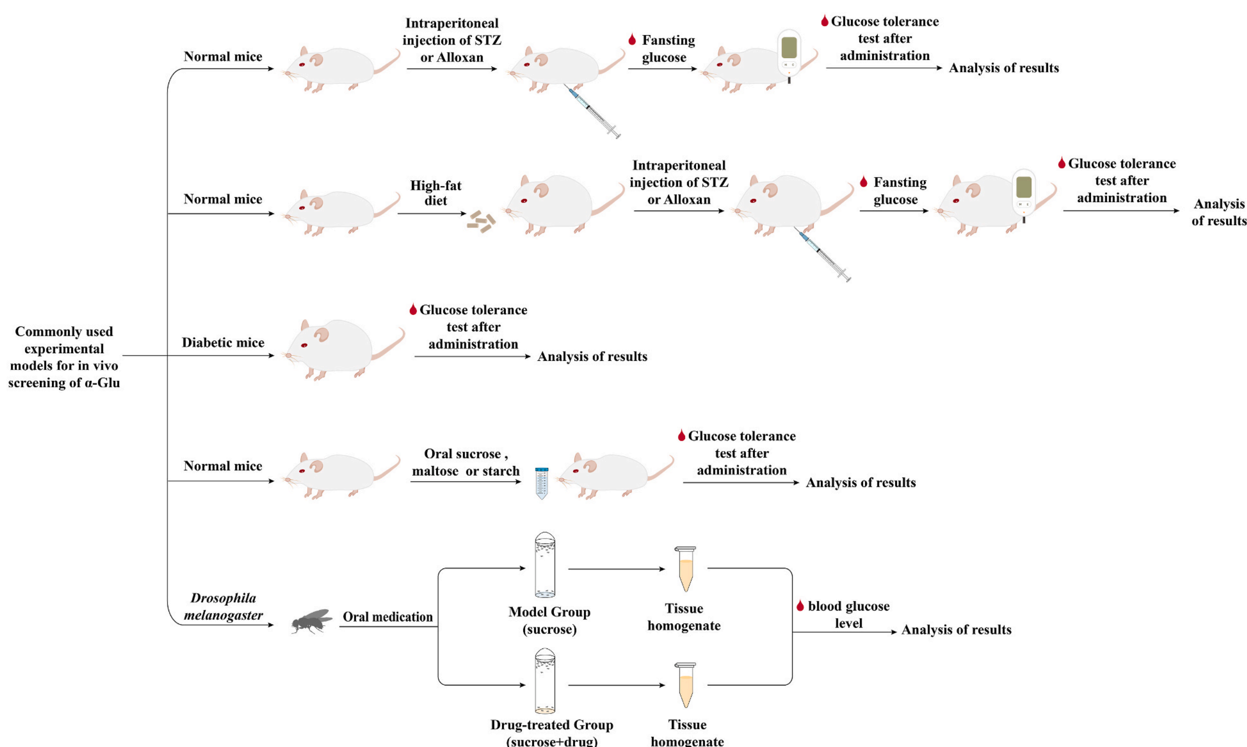

Fig. 1. Diagram of commonly used experimental models in vivo.

Drosophila tissue. The antidiabetic effect of the fruit was evaluated by measuring the body weight, α-amylase and α-Glu activity, and blood glucose levels. The fruit was found to improve hyperglycemia in diabetic *D. melanogaster*. This method is simple and inexpensive for studying *D. melanogaster*. Tian et al. [38] investigated the effects of flavonoids in *Rubus corchorifolius* fruit on postprandial hyperglycemia. A glucose tolerance test was performed after sucrose, maltose, or starch was directly given to normal mice. Blood glucose levels were measured at 0, 15, 30, 60, and 120 min. The results showed that quercetin-7-O-β-D-glucopyranoside and flavonoid enrichment could inhibit postprandial hyperglycemia by inhibiting intestinal α-Glu and α-amylase.

The advantages of screening AGIs using in vivo models are as follows: High physiological correlation: An in vivo model can better simulate the physiological environment of the human body, including the intestinal environment and flora, and thus will evaluate the efficacy and safety of AGIs more accurately.

Directly reflects the therapeutic effect: An in vivo model can directly observe the therapeutic effect of drugs on the disease, such as lowering the level of blood sugar. This is helpful for rapid screening of AGI drug candidates with significant efficacy.

Drug metabolism: An in vivo model can evaluate the metabolism of AGIs in the body, including metabolism in the liver, absorption, and excretion in the intestinal tract.

Multi-index evaluation: An in vivo model can evaluate the effects of AGIs on blood glucose, intestinal flora, intestinal hormones, and other indices simultaneously.

Safety assessment: An in vivo model can enable the detection of possible toxic and other adverse reactions, providing important information for subsequent clinical trials.

The disadvantages of screening AGIs using an in vivo model are as follows: High cost: In vivo experiments require additional resources, time, and money.

Ethical issues: Using animals for experiments involves animal welfare and ethical issues and requires strict review and approval.

Large variability: Owing to the differences among biological individuals, there may be greater variability in the results.

Screening speed: Compared to the in vitro experiment, the screening speed of the in vivo experiment is slower.

Generally, in vivo model screening of AGIs provides more comprehensive and accurate data but is also associated with higher costs and more effort. When choosing screening methods, researchers must weigh various factors to ensure that the research is both effective and economical.

4. In vitro screening

As an important means of screening for AGIs, in vitro screening methods can be categorized based on different experimental principles, such as screening of AGIs based on 4-nitrophenol-α-D-glucopyranoside (PNPG) as substrate, screening of AGIs based on Caco-2 cell model, screening of AGIs based on immobilized enzyme, screening of AGIs based on sensing strategy, screening of AGIs based on affinity ultrafiltration combined with mass spectrometry analysis, and screening of AGIs based on magnetic ligand fishing technology. Each screening method possesses its unique advantages and characteristics, and the following will elaborate on their principles, advantages, disadvantages, and other aspects.

4.1. Screening of AGIs based on PNPG as substrate

One of the classic methods for determining α-Glu activity and inhibitor screening is the PNPG method [39,40]. The principle behind the method is that PNPG can be hydrolyzed to the yellow product p-nitrophenol (PNP) under the influence of α-Glu with PNPG as substrate. After the addition of AGIs, PNP formation is inhibited. The colorimetric method or HPLC determination of the PNP content is used to calculate the inhibition rate and evaluate the activity of the inhibitors. When the ultraviolet detection method is used to determine the PNP content, there is a problem in that PNP may overlap with the visible absorption spectra of many AGIs, causing the method to have high interference and low sensitivity. When HPLC is used to determine the PNP content, it has higher accuracy than ultraviolet detection because it has the function of content analysis after separation.

Zhu et al. [39] used PNPG as a substrate to determine the activity of α-Glu by quantitative analysis of the PNP hydrolysate using HPLC. The IC_{50} of acarbose determined using this method was 2.07 mg/mL, which was close to the values reported in the literature, and the reliability of the method was verified. Teerapongpisan et al. [40] used the PNPG method to identify *Phaeanthus lucidus* Oliv. The α-Glu inhibitory activity of the compounds isolated from the ethyl acetate extract of the leaves was detected using a spectrophotometer. The results showed that Phaeanthuslucidine E had good α-Glu inhibitory activity, which was better than that of acarbose.

4.2. Screening of AGIs based on Caco-2 cell model

Caco-2 cells are cloned human colon adenocarcinoma cells, which resemble differentiated intestinal epithelial cells in structure and function, have microvilli and other structures, and contain enzymes related to the brush border epithelium of the small intestine. This Caco-2 cell model can be used to simulate intestinal transport in vivo because it can fuse and differentiate into intestinal epithelial cells and form a continuous monolayer under cell culture conditions. Therefore, Caco-2 cells are a good in vitro model for studying intestinal epithelial cell absorption and transport [41]. Two detection methods are available. (1) First, the Caco-2 monolayer is cultured, and the glucose-free medium of the sample and substrate is added to the top side with sucrose or maltose as the substrate, and the glucose-free medium is added to the base side. After incubation, the glucose content on the top side of the Caco-2 monolayer is determined using the glucose oxidase method to evaluate the inhibitory effect of the sample on α-Glu [42,43]. (2) Caco-2/TC7 cells (TC7 clones derived from the late passage of the parent Caco-2 cell line) are cultured to fully differentiate, and the intracellular enzymes are extracted using the

appropriate cell lysis method as a source of α-Glu. Specific enzyme activity determination methods, such as colorimetry and fluorescence, are used to determine the inhibitory effect of the inhibitor on the enzyme. The inhibitory effect of drugs on α-Glu can be evaluated by comparing the difference in enzyme activity between the drug-treated and untreated groups [44].

Studies by Noonong et al. [42] showed that compounds from *Lysiphyllum strychnifolium* (Craib) A.Schmitz, 3,5,7-trihydroxychromone-3-O-**α**-L-rhamnopyranoside and 3,5,7,3',5'-pentahydroxy-flavanonol-3-O-**α**-L-rhamnopyranoside, inhibited the activity of α-Glu in Caco-2 cells. Tu et al. [43] conducted Caco-2 cell experiments and found that the α-Glu inhibitory effect of *Lentinula edodes* polysaccharides resulted from a decrease in glucose transport of digested starch. Proença et al. [44] investigated the inhibitory effect of flavonoids on human sucrase-isomaltase (SI) in a Caco-2/TC7 cell model. Caco-2/TC7 cells were cultured, cell extracts were prepared to obtain SI as an enzyme source, and its inhibitory activity was determined. When sucrose and maltose were used as substrates, and a glucose oxidase/peroxidase assay was used to determine the inhibitory activity of SI, the tested flavonoids showed similar IC_{50} values. However, when PNPG was used as a substrate and determined using the classical PNPG method, flavonoids could not effectively inhibit SI.

4.3. Screening of AGIs based on immobilized enzyme

Immobilized enzymes are special enzymes that combine enzymes with specific carriers using chemical or physical methods and can catalyze repeatedly and continuously in a certain space. In recent years, great progress has been made in enzyme immobilization technology. Compared to the free enzyme, immobilized α-glucosidase showed better stability and reproducibility. Magnetic nanoparticles [45], metal-organic frameworks [46], polyvinylidene fluoride membranes [47], cellulose filter paper [48,49], capillary tubes [50], and other materials have been used as immobilization carriers for enzymes. The principle of this method is that the inhibitor sample is brought into contact with the immobilized α-Glu to allow them to interact under certain conditions. In this step, the inhibitor may bind to the active site of the enzyme and thus inhibit its catalysis of the substrate, usually sucrose or maltose. The activity of potential inhibitors can be evaluated indirectly by measuring the catalytic efficiency of the enzyme immobilized on the substrate, which can be achieved by measuring the conversion rate of the substrate to the product or the amount of product. Commonly used methods include colorimetry, fluorescence, capillary electrophoresis (CE), HPLC, and gas chromatography (GC). Because CE, HPLC, and MS have the advantages of high separation efficiency, short analysis time, high sensitivity, and low sample consumption, they are often used in combination with immobilized enzymes to screen enzyme inhibitors [45,46].

Cheng et al. [45] covalently bound α-Glu to magnetic Fe_3O_4 nanoparticles using (3-aminopropyl) triethoxysilane, and identified eight inhibitors from Polygonum cuspidatum extract by HPLC-MS/MS technique. Wan et al. [46] prepared a magnetic core-shell metal-organic framework composite (Fe_3O_4@CS@ZIF-8) and immobilized α-Glu as a carrier. Combined with CE detection, it was used for AGI screening 14 traditional Chinese medicine extracts. The results showed that rhubarb extract had good activity and verified the enzyme inhibitory activity of the three active components. Wen et al. [47] immobilized α-Glu on tannic acid/3-aminopropyltriethoxysilane co-precipitation polyvinylidene fluoride membrane (TA/APTES@PVDF) and screened potential AGIs from 13 traditional Chinese medicine extracts combined with CE detection, among which Sanguisorba Radix had the strongest

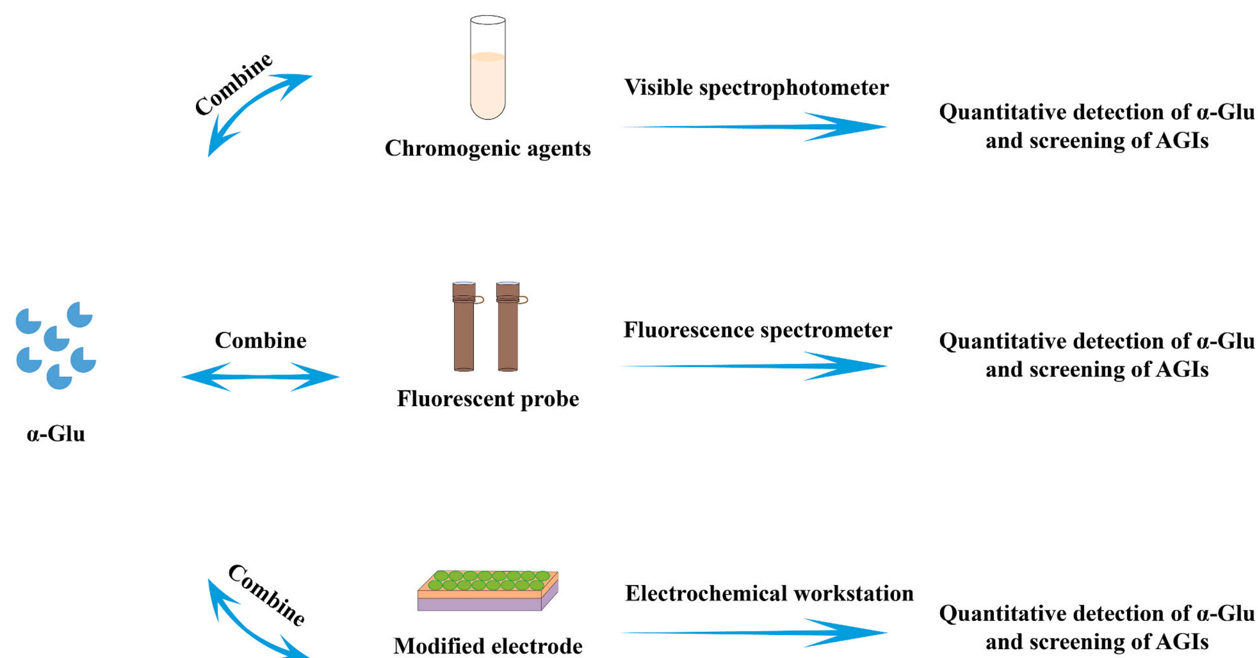

Fig. 2. Schematic diagram of sensing strategy.

inhibitory effect. The immobilized α-Glu was instantly separated from the culture medium and maintained an initial relative activity of 77.1 % after 11 cycles. In addition, it exhibited good reproducibility, stability, and stronger temperature and pH tolerance. Li et al. [48] prepared a new type of immobilized α-Glu through electrostatic interactions between a cellulose filter paper coated with dopamine-polyethyleneimine and α-Glu. Combined with the results of CE detection, it was successfully used for screening inhibitors in 10 aqueous extracts of traditional Chinese medicine. In addition, Li et al.immobilized α-Glu on the inner wall of the entrance of the capillary column functionalized by dopamine-polyethyleneimine co-deposition and established an online method for screening AGIs in an immobilized enzyme reactor based on CE. Using PNPG as a substrate, the change in the PNP yield was evaluated before and after the addition of inhibitors. This method screened sixteen drugs with inhibitory activity from 20 traditional Tibetan medicinal extracts [50].

4.4. Screening of AGIs based on sensing strategy

In recent years, various new sensing strategies, such as colorimetric sensing, fluorescence sensing, and electrochemical sensing, have been developed to improve the sensitivity, selectivity, reliability, and accuracy of α-Glu detection and AGI screening methods. A schematic of the sensing strategy is shown in Fig. 2.

4.4.1. Colorimetric sensing

The classical method for detecting α-Glu activity has the advantages of simple operation and low cost; however, its sensitivity is relatively low. Colorimetric sensing can not only be monitored by the naked eye but also has high sensitivity [51–53]. The principle of this method is the use of chromogenic agents that can react specifically with α-Glu. When these chromogenic agents are combined with α-Glu, a color change occurs, which can be observed and measured using the naked eye or a visible spectrophotometer. The change in color or wavelength is proportional to the activity of α-Glu, enabling the quantitative detection of α-Glu and the screening of AGIs. Zhang et al. [52] hydrothermally synthesized a nickel-based metal-organic framework (SDs/Fe/NiMOF) co-doped with sulfur and iron for the colorimetric detection of α-Glu. SDs/Fe/Ni-MOF exhibited high enzyme activity at the nanoscale. In the presence of H_2O_2, 3,3 ', 5,5'-tetrame-thylbenzidine(TMB) can be oxidized to oxTMB. α-Glu activity was detected using α-arbutin (α-Arb) as the substrate. α-Glu hydrolyzes α-Arb to release hydroquinone (HQ). HQ inhibits the activity of the nanoenzyme and reduces oxTMB to TMB. The activity of α-Glu was indirectly detected by the nanoenzyme. The linear range was 1.00–100 U/L, and the limit of detection (LOD) was about 0.525 U/L. This method has the advantages of high selectivity, wide detection range, and low detection limit and is suitable for detecting α-Glu activity in serum samples. Zhou et al. [53] synthesized a gold nanocluster-modified iron-cobalt oxide nanowires (His-AuNCs@FeCo-ONSs). A dual-mode (colorimetric/fluorescence) detection method for α-Glu was developed through the enzyme-enzyme cascade reaction of α-Glu, glucose oxidase, and His-AuNCs@FeCo-ONSs. In this dual mode, LOD of α-Glu are 2.2 U/L and 3.3 U/L, respectively, which can be used for determining α-Glu activity and AGIs screening.

4.4.2. Fluorescence sensing

The fluorescence sensing strategy has many advantages, such as simple operation, easy preparation, good selectivity, and high sensitivity [54–56]. The principle of this method is that the fluorescent group is connected to a molecule that can bind to α-Glu to form a fluorescent probe. When a fluorescent probe binds to α-Glu, it causes a change in fluorescence intensity or a shift in the fluorescence wavelength. This change can be measured using a fluorescence spectrometer to enable the quantitative detection of α-Glu and screening of AGIs. The fluorescence sensing method has the advantages of high sensitivity and high selectivity and is suitable for detecting low concentrations of α-Glu and screening AGIs. Geng et al. [56] developed a fluorescence method based on guar gum (GG), which enhances the emission of glutathione-terminated gold nanoclusters (GSH-AuNCs) for determining α-Glu activity and AGIs screening using GSH-AuNCs as fluorescent probes. In this method, GG can enhance the fluorescence emission of the GSH-AuNCs probe, and Fe^{3+} can dynamically quench the emission of AuNCs. After adding α-Glu and L-ascorbic acid-2-O-α-D-glucopyranosyl (AAG), the ascorbic acid produced by the hydrolysis of AAG in the presence of α-Glu can lead to the recovery of AuNCs emission, while the recovery of AuNCs emission can be inhibited in the presence of AGIs. The LOD of this method was 0.13 U/L, and the detection range was increased by five orders of magnitude from 0.2 to 4000 U/L, which is better than most current methods. This method has great potential for screening AGIs from natural plant extracts. Zhao et al. [57] proposed a fluorescence "on" biosensor based on water-soluble silicon-containing nanoparticles for detecting α-Glu activity and AGIs screening. In this platform, quercetin, kaempferol, and luteolin achieved similar or lower IC_{50} values, which verifies the ability of the platform to screen potential AGIs.

4.4.3. Electrochemical sensing

The electrochemical sensing method is characterized by a high sensitivity and low detection limit, and hence, it has attracted much attention in the detection of α-Glu activity [58–60]. The principle of the method is that an electrode is used that can specifically bind to α-Glu. When α-Glu binds to the modifier on the electrode surface, this changes the electrochemical properties of the electrode, such as changes in the current or potential. This change can be measured using an electrochemical workstation to enable the quantitative detection of α-Glu and screening of AGIs. The electrochemical sensing method has the advantages of fast response and simple operation and is suitable for real-time monitoring and high-throughput screening. Aschemacher et al. [58] developed an electrochemical sensor using carbon nanotubes and hydrophobic natural eutectic solvents to detect the activity of α-Glu in medicinal plant extracts. The detection range of α-Glu was 0.004–0.1 U/mL, and the LOD was 0.0013 U/mL. Zhang et al. [59] established a two-dimensional metal-organic skeleton (2D Fe-BTC) catalyzed luminol-H_2O_2 chemiluminescence (CL) reaction based on the cascade enzymatic reaction. The luminol-H_2O_2-Fe-BTC CL system can rapidly determine the activity of α-Glu and can be used for AGI screening. The linear range of α-Glu detection was 0.01–0.5 U/mL, and the LOD was 0.0074 U/mL. Shao et al. [60] synthesized Zr-based mixed connector

metal-organic frameworks (ML-MOFs) by a solvothermal method, and M6-MOFs exhibited the best electrochemiluminescence (ECL) performance. For the first time, Shao et al. used M6-MOFs to construct an ECL sensing platform for sensitive detection of α-Glu activity. Upon exposure to α-Glu, α-Arb was hydrolyzed to HQ, and HQ was oxidized to benzoquinone, which led to a decrease in the ECL intensity of the M6-MOFs. The degree of ECL quenching is proportional to the concentration of HQ produced. This method has the advantages of high specificity, stability, and reproducibility. α-Glu has a good linear correlation in the logarithm of 0.001–0.1 U/mL, and the LOD can be as low as 0.00088 U/mL.

4.5. Screening of AGIs based on affinity ultrafiltration combined with mass spectrometry analysis

Affinity ultrafiltration combined with mass spectrometry analysis is a typical affinity-selective mass spectrometry platform based on a solution [61–68]. The principle of screening AGIs is that the enzyme is incubated with an analyte solution, interacts with an affinity molecule to form an enzyme protein-ligand complex, and ultrafiltration is performed on a semi-permeable membrane with a certain cut-off molecular weight. The ultrafiltration membrane divides the mixed solution into bound and unbound parts. The enzyme protein-ligand complex is trapped on the membrane, and the unbound components can pass through the membrane. The trapped enzyme protein-ligand complex can release the ligand from the complex by adding organic solvents, such as methanol, or changing the pH, and then directly identifying the ligand by LC-MS [62], or indirectly determining the unbound substance [68]. Finally, by further structural analysis and activity verification of the selected potential inhibitors, mainly through computer-aided virtual screening and in vitro enzyme activity inhibition experiments, the binding mode, inhibitory activity, and mechanism of action of inhibitors with α-Glu can be determined to evaluate their potential as drugs. The Affinity ultrafiltration combined with mass spectrometry analysis screening flowchart is shown in Fig. 3. This method is characterized by high selectivity, high sensitivity, and rapidity and is suitable for screening potential AGIs from complex systems.

Lu et al. [61] screened AGIs from *Siraitia grosvenorii* roots by affinity ultrafiltration combined with the HPLC method, and identified 17 potential AGIs. Then the structures of the isolated active compounds were identified by nuclear magnetic resonance spectroscopy and HR-ESI-MS, and verified the α-Glu inhibitory activity of the active compounds by PNPG method and molecular docking. Among them, the compound siraitic acid II C showed the strongest inhibitory activity; the IC_{50} was 430.13 ± 13.33 μM, which was significantly better than the acarbose $IC_{50,}$ which was 1332.50 ± 58.53 μM. Li et al. [62] used bioaffinity ultrafiltration and HPLC-ESI-qTOFMS/MS to screen potential AGIs from *Cerasus humilis* (Bge.) Sok.leaf-tea. Ten potential AGIs were identified. Myricetin, guanosine, and quercetin were confirmed using the PNPG assay in vitro. Li et al. [63] screened and identified 36 potential AGIs in *Cyclocarya paliurus* leaves by affinity ultrafiltration combined with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS). Shi et al. [64] screened AGIs from the acetone extract of pomegranate peel by bioaffinity ultrafiltration and HPLC-ESI-MS/MS and screened 11 compounds, among which ellagic acid had the strongest α-Glu inhibitory activity. Finally, the α-Glu inhibitory effect of ellagic acid was confirmed using kinetic analysis, fluorescence spectrum analysis, molecular docking, and sucrose loading assays in vivo. Zhang et al. [65] screened potential α-Glu, pancreatic lipase, and COX-2 inhibitors from *Nelumbo nucifera* leaves by bioaffinity ultrafiltration combined with HPLC-MS/MS. Four potentially active compounds were screened and identified, and the α-Glu inhibitory activities of the candidate compounds were verified using PNPG and molecular docking. Han et al. [66] screened and identified 12 potential AGIs from a 30 % acetone extract of Tartary buckwheat shells by affinity ultrafiltration combined with HPLC-MS/MS. The enzyme inhibitory activities of these inhibitors were confirmed by PNPG experiments, kinetic analysis, circular dichroism spectrum analysis, and molecular docking. Feng et al. [67] used bioaffinity ultrafiltration combined with UHPLC Q-Exactive Plus Orbitrap HRMS from *Oxalis corniculate* L., 31 potential AGIs were screened. Among them, six compounds, such as quercetin and luteolin, had stronger inhibitory activity than acarbose, and the α-Glu inhibitory activity

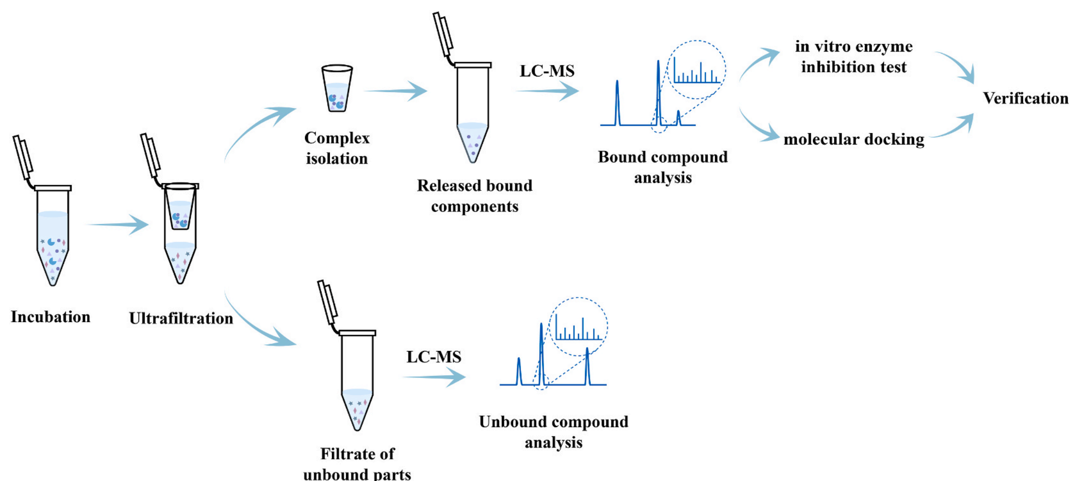

Fig. 3. Affinity ultrafiltration combined with mass spectrometry analysis screening flowchart.

of quercetin and luteolin was verified by fluorescence quenching assay and molecular docking.

4.6. Screening of AGIs based on magnetic ligand fishing technology

The magnetic ligand fishing technique was used to screen AGIs, mainly based on the specific binding of the ligand to α-Glu and the application of magnetic separation technology [69–72]. The specific principles are as follows. First, we should design and prepare magnetic ligands that can specifically bind α-Glu. This binding is based on interactions between ligands and enzymes, such as hydrogen bonding, van der Waals forces, or electrostatic interactions. When a magnetic ligand binds to α-Glu, a magnetic complex is formed. The sample containing potential AGIs is then mixed with the magnetic complex. In this process, the compounds that can inhibit the activity of α-Glu bind to the enzyme to form ternary complexes with magnetic ligands, and then the magnetic complexes with inhibitors can be easily separated from the mixture by an external magnetic field. This step is based on the magnetic responsiveness of magnetic ligands so that the complexes can be quickly and effectively collected under the action of a magnetic field. Then, the complexes are dissociated and eluted down using appropriate organic solvents or changing the pH, and the inhibitors are released from the magnetic ligand under the action of an external magnetic field and subsequently identified and analyzed by LC-MS technique. Finally, the enzyme inhibitory activity of the compound is verified using an in vitro enzyme inhibition test and molecular docking. The magnetic ligand fishing technology screening flowchart is shown in Fig. 4. This method combines the specificity of ligands and the high efficiency of magnetic separation and provides a rapid, simple, and reliable method for screening AGIs.

Jiang et al. [69] prepared AGIs-coated CuInS2/ZnS-Fe_3O_4@SiO_2 (AG-CIZSFS) nanocomposites by solvothermal and cross-linking methods, and established a bioanalysis platform combined with magnetic fluorescence ligand capture and in situ imaging techniques. Six potential compounds from the crude extract of *Agrimonia pilosa* Ledeb (APL), which showed stronger α-Glu inhibition than the crude extract of APL, were screened and identified. Their enzyme inhibitory activities were verified using the PNPG method and molecular docking. Shen et al. [70] prepared AGIs screened from *Epimedii Folium* extract using α-Glu magnetic beads and identified them by UPLC-MS/MS analysis. The results showed that the compounds identified from the extract were epimedin A, epimedin B, epimedin C, and other flavonoids. Finally, their enzyme inhibitory activities were verified using molecular docking and the PNPG method. Fan et al. [71] immobilized α-Glu on magnetic Fe_3O_4 nanoparticles and screened ligands from *Selaginella uncinata* using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Eleven biflavone ligands with α-Glu inhibitory activity were accurately and rapidly identified. In addition, these ligands were confirmed to be potential inhibitors of PNPG using molecular docking tests. Wubshet et al. [72] combined magnetic ligand capture and high-resolution inhibition analysis to evaluate the inhibitory activity of conjugates in ligand fishing experiments and identify α-Glu inhibitory ligands. This method identified seven bioflavonoids in the crude ethyl acetate extract of *Ginkgo biloba*.

4.7. Other in vitro screening methods

In addition to the in vitro screening methods mentioned above, methods for determining enzyme activity and inhibitor screening include high-performance thin layer chromatography (HPTLC) [73,74], near-infrared spectroscopy [75], capillary electrophoresis [76], online screening [77], and Raman spectroscopy [78]. Azadniya et al. [74] analyzed six kinds of baobab fruit powder using HPTLC

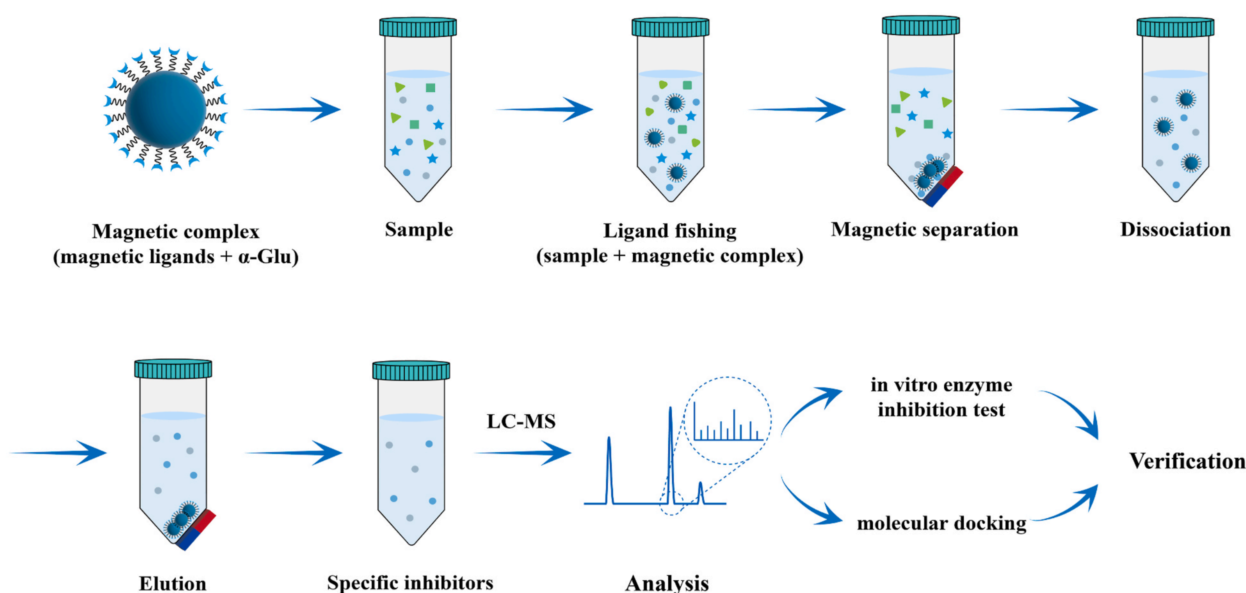

Fig. 4. Flow chart for screening of magnetic ligand fishing techniques.

combined with antibacterial and enzyme inhibition assays. HPTLC plates were immersed in an α-Glu solution, incubated at 37 °C for 30 min, and then incubated in a substrate solution at 37 °C for 30 min. The AGIs were detected as colorless (white) regions on a purple background. He et al. [75] proposed a new method for predicting the inhibitory activity of *Coreopsis tinctoria* extracts against α-Glu based on near-infrared spectroscopy. The model showed a good correlation between the near-infrared spectrum and the inhibitory activity of the *C. tinctoria* extract, and the inhibitory activity of the *C. tinctoria* extract on α-Glu was confirmed by UV spectroscopy. Zhang et al. [76] developed a new method for simultaneous screening of multiple bioactive compounds in one CE procedure, which was verified using the PNPG method and molecular docking. Finally, this method was used to select four compounds from 14 extracts of *Lycium barbarum*. Nan et al. [77] established an HPLC-DAD-MS^n-FLD system for the online screening of α-Glu and α-amylase inhibitors in *Prunus mume*. The sample solution was separated on an HPLC column, and the HPLC chromatogram was recorded using DAD. The eluent was then divided into two strands: one strand received MS data for structural identification, and the other reacted with the enzyme solution and was detected using a fluorescence detector. When the enzyme is inhibited by the active compounds, the fluorescence intensity decreases and forms a negative peak. Zhang et al. [78] developed photoaffinity microarray based on a high-resolution microconfocal Raman spectrometer. Using this method, more than ten components of Shenqi Jiangtang granules were converted into microarrays with small-molecule probes. Raman spectroscopy was used to investigate the interaction between active small molecules on the microarray and target proteins, and eight bioactive components specifically bound to α-Glu were screened. This method has the advantages of high efficiency, versatility, non-contact, label-free, and high spatial resolution. However, when the difference in binding ability between the target protein and a small molecule is small, it is difficult to evaluate its binding ability.

5. Molecular docking technology

The principle of screening AGIs by molecular docking is primarily based on computer simulation technology. Compounds with potential inhibitory activity are predicted and screened by simulating the interaction between small-molecule ligands and α-Glu [63, 69,79]. The specific operation methods are as follows: First, the three-dimensional structural model of α-Glu is constructed, and the location and characteristics of its active pocket are determined. The candidate small molecular ligands are then introduced into the docking software to match the active pocket of the enzyme by adjusting the spatial position and posture of the ligand. In the docking process, the software calculates the interaction energy between the ligand and the enzyme, including van der Waals forces and electrostatic and hydrophobic interactions. By constantly optimizing the position and posture of the ligand, we can determine the conformation with the lowest energy and the most stable binding, that is, the best binding mode. Finally, compounds with a strong binding ability to α-Glu are selected as potential AGIs based on the docking results. Their inhibitory activity and mechanism can be confirmed by further in vitro or in vivo experiments. Molecular docking technology has the advantages of high efficiency, high speed, and low cost and can screen potentially active compounds from a large-scale compound library, which provides strong support for the development of α-glucosidase inhibitors. At the same time, this technique can also be used to study the interaction mechanism between enzymes and inhibitors and provide a theoretical basis for drug design.

Li et al. [63] screened the potential AGIs from *C. paliurus* leaves using UPLC-QTOF-MS/MS and molecular docking techniques. The docking results showed that the six active components could be well embedded in the active pocket of α-Glu and had significant affinity interactions with key amino acid residues by forming hydrogen bonds, hydrophobic interactions, and van der Waals forces. The enzyme inhibitory effect of these components was confirmed using the PNPG method. Jiang et al. [69] used molecular docking to clarify the interaction between the compounds selected from the crude extract of APL and the binding pocket of α-Glu. These components bind tightly to key residues through interactions to produce α-Glu inhibitory activity. In addition, enzyme inhibitory activity was verified using the PNPG method.

6. Conclusions and prospects

6.1. Conclusions

α-Glu is considered a therapeutic target for treating T2DM, and AGIs are one of the main drugs for treating T2DM. Additionally, AGIs can be used in other situations. For example, they can be used to treat T1DM; however, they are usually combined with insulin to improve blood glucose control and reduce the occurrence of hypoglycemia. In addition, they can be used in people with impaired glucose tolerance to help prevent the development of diabetes. This shows that AGIs are of great importance in treating and preventing diabetes. Currently, the clinical application of the three types of AGIs commonly used in clinics is limited because of their side effects. Therefore, increasing attention has been paid to the screening of new safe and effective AGIs from natural products.

In vivo model screening of AGIs has many advantages, such as high physiological correlation, a direct reflection of therapeutic effect, understanding of drug metabolism, and multi-index evaluation, but it also has some disadvantages, such as high experimental cost, ethical problems, individual differences, and difficulty in controlling experimental conditions. Therefore, when selecting an in vivo model for AGI screening, we need to comprehensively consider the research purpose, resource conditions, ethical norms, and other factors.

There are many methods to screen AGIs in vitro, including the traditional PNPG method, Caco-2 cell method, immobilized enzyme method, sensing strategy, affinity ultrafiltration combined with mass spectrometry analysis, and magnetic ligand fishing technique, which have the advantages of simple operation, low cost, controllable conditions, and high-throughput screening; however, they also have some shortcomings such as limited physiological correlation, lack of information on drug metabolism, and differences in cell or enzyme sources. Therefore, when using in vitro models to screen AGIs, we need to fully understand their characteristics and limitations

and conduct a comprehensive evaluation combined with in vivo experiments and other research methods.

The screening of AGIs using the molecular docking technique is highly effective, and the results can be an important guide for further experimental design. Based on the docking score and binding mode, the most potentially effective compounds can be selected for further experimental verification to improve the success rate and efficiency of the experiment. However, the prediction results are still affected by many factors, such as algorithm accuracy, parameter setting, and compound structure complexity. The prediction accuracy can be further improved by optimizing the algorithm and parameter settings. Finally, it is necessary to combine in vitro and in vivo efficacy experiments to ensure the accuracy and reliability of screening results.

6.2. Prospects

Regarding the experimental methods for screening AGIs, the future development direction may be mainly expressed in the following aspects.

(1) Further optimization of high-throughput screening technology: Currently, high-throughput screening techniques, such as sensing strategy, affinity ultrafiltration combined with mass spectrometry analysis, and magnetic ligand fishing technology, have been applied to the screening of AGIs. In the future, these techniques should be further optimized to improve the accuracy and efficiency of screening. For example, faster and more accurate screening can be achieved by improving the screening model, optimizing reaction conditions, and improving the degree of automation.
(2) Screening of multi-target and multi-mechanism inhibitors: with an in-depth study of the mechanism of action of α-Glu, researchers may focus on compounds that can act on multiple targets simultaneously or through multiple mechanisms. These compounds may have stronger hypoglycemic effects and fewer side effects. Therefore, future screening methods should focus on discovering and verifying these compounds.
(3) Screening strategies based on artificial intelligence and machine learning: artificial intelligence and machine learning technologies have shown great potential in the field of drug discovery and development. In the future, these techniques could be widely used for filtering AGIs. For example, by training a machine learning model, we can intelligently screen many compound libraries and predict the biological activity of compounds to improve screening efficiency and success rate.

In summary, future experimental methods for screening AGIs will focus more on developing high-throughputand intelligence to promote continuous progress and innovation in this field.

Data availability statement

No data was used for the research described in the article.

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CRediT authorship contribution statement

Guangjuan Pan: Writing – original draft, Software, Resources, Investigation, Conceptualization. **Yantong Lu:** Resources, Investigation, Formal analysis, Conceptualization. **Zhiying Wei:** Supervision, Investigation, Formal analysis, Conceptualization. **Yaohua Li:** Supervision, Resources, Formal analysis, Conceptualization. **Li Li:** Supervision, Resources, Investigation, Formal analysis, Conceptualization. **Xiaojiao Pan:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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