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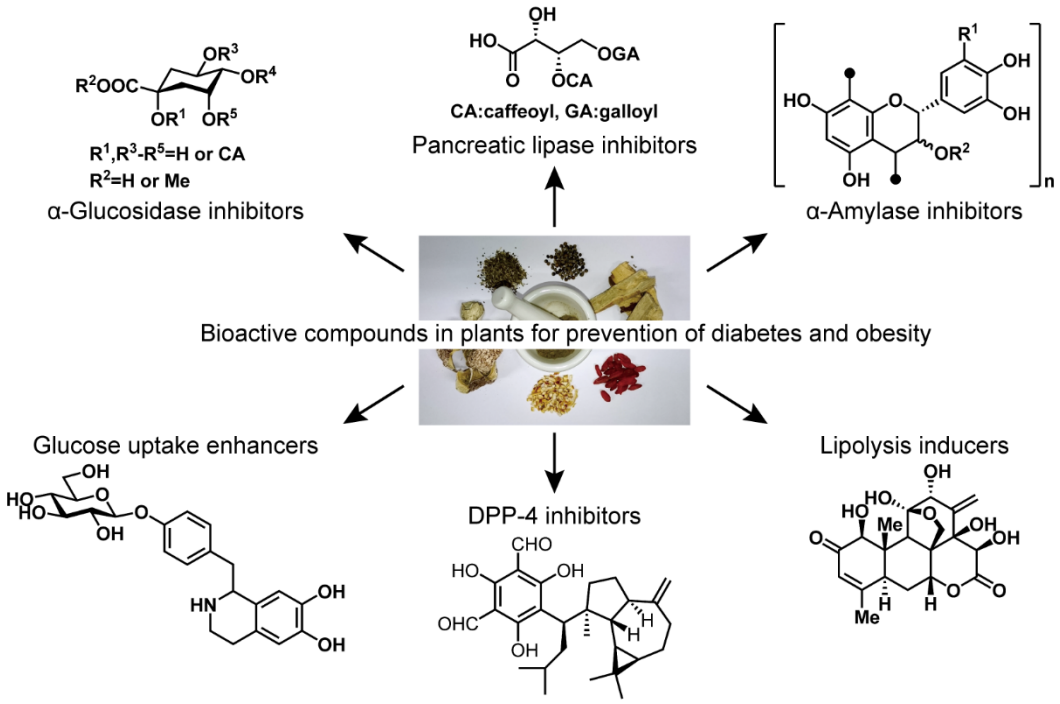
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Graphical abstract



Review

Bioactive compounds in plant materials for the prevention of diabetes and obesity

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Running Title: Anti-diabetes/obesity compounds in plant materials

Abstract

Plant materials have been widely studied for their preventive and therapeutic effects for type 2 diabetes mellitus (T2DM) and obesity. The effect of a plant material arises from its constituents, and the study of these bioactive compounds is important to achieve a deeper understanding of its effect at the molecular level. In particular, the study of the effects of such bioactive compounds on various biological processes, from digestion to cellular responses, is required to fully understand the overall effects of plant materials in these health contexts. In this review, I summarize the bioactive compounds we have recently studied in our research group that target digestive enzymes, dipeptidyl peptidase-4, myocyte glucose uptake, and lipid accumulation in adipocytes.

Keywords: diabetes, obesity, digestive enzyme, glucose uptake, lipolysis

Abbreviations:

AC: adenylyl cyclase, AMPK: AMP-activated protein kinase, βAR: β-adrenergic receptor, CA: catecholamine, cAMP: cyclic adenosine monophosphate, cGMP: cyclic guanosine monophosphate, DPP-4: dipeptidyl peptidase-4, ERK: extracellular signal-regulated kinase, GC: guanylyl cyclase, GH: growth hormone, GLP-1: glucagon-like peptide-1, GLUT: glucose transporter, HSL: hormone-sensitive lipase, IR: insulin receptor, IRS: insulin receptor substrate, MAPK: mitogen-activated protein kinase, MEK: MAPK/ERK kinase, MG: maltase-glucoamylase, NP: natriuretic peptide, NPR: natriuretic peptide receptor, mTORC2: mechanistic target of rapamycin complex-2, PC: proanthocyanidin, PI3K: phosphoinositide 3-kinase, PKA: cAMP-dependent protein kinase, PKB (AKT): protein kinase B, PKG: cGMP-dependent protein kinase, PPARγ: peroxisome proliferator-activated receptor-γ, SGLT1: sodium-dependent glucose transporter 1, SI: sucrase-isomaltase, T2DM: type 2 diabetes mellitus, TNFα: tumor necrosis factor-α.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a disease characterized by hyperglycemia that results from impaired secretion of insulin and/or sensitivity to insulin, which impairs glucose metabolism. High circulating concentrations of glucose cause damage to blood vessels, leading to the development of diabetic complications, including cardiovascular disease, renal failure, blindness, and neurological disorders [1]. Obesity is defined by a body mass index (BMI) above 30. The excess lipid accumulation in adipose tissue not only enlarges adipose depots, changing body appearance, but also influences the function of individual adipocytes, which increases the risk of various diseases, including T2DM, hyperlipidemia, and cardiovascular disease [2]. T2DM and obesity both represent significant current global health problems. In 2017, there were estimated to be 425 million adults with diabetes (IDF Diabetes Atlas), and 650 million obese individuals in the world (WHO Fact Sheet). Accordingly, numerous studies are conducted to aid understanding of these health problems [3,4].

The study of novel drug substances assists the accumulation of knowledge regarding T2DM and has provided a variety of choices for the treatment of the disease. Inadequate insulin secretion can be treated using insulin injection, sulfonylureas, glucagon-like peptide-1 analogs, or dipeptidyl peptidase-4 (DPP-4) inhibitors, while insulin resistance is treated with biguanides in the first instance, and potentially also with peroxisome proliferator-activated receptor-γ (PPARγ) agonists, to improve insulin sensitivity. Inhibitors of carbohydrate digestive enzymes and glucose transporter-2 also improve glucose homeostasis by reducing the absorption of carbohydrate from the intestine and permitting the loss of glucose in urine [5].

Although knowledge of the mechanisms of obesity has significantly expanded, anti-obesity medicines are less well developed. However, appetite suppressants such as lorcaserin (a serotonin 5-HT2C receptor agonist) and glucagon-like peptide-1 analogs are used, and

inhibitors of lipid digestive enzymes represent an alternative [6]. Although lipid metabolism is also being targeted for anti-obesity drug development, drugs with this mechanism are not yet available [7]. However, despite many effective medications having been developed, the numbers of people with T2DM and/or obesity continue to rise, indicating the requirement for a wider range of modalities, not only to treat, but also to prevent these health problems arising.

Plant materials are widely used to maintain human health. Traditional medications, food supplements, and functional foods all utilize the effects of plant materials to prevent disease and/or improve health, and these include numerous substances that are believed to target T2DM and obesity. The preventive effects of plant-derived substances against T2DM and obesity can generally be explained by specific bioactive compounds contained within them. A variety of biological processes can be targeted by plant compounds, including the digestion and absorption of nutrients in the gut, the transport of absorbed nutrients to tissues, and the accumulation and metabolism of nutrients in cells [8,9]. However, unlike conventional medicinal compounds, plant materials may target multiple processes due to the presence of numerous compounds within them, making their effects more complex and difficult to study.

Characterization of the bioactive compounds within plant materials is essential to improve our understanding of their anti-diabetic and anti-obesity potential. In this review, I summarize the bioactive compounds that we have recently identified in plant materials, which have potential for the prevention of T2DM and/or obesity. Pancreatic lipase inhibitors (compounds **1**–**5**) inhibit the digestion of triglycerides, which reduces the absorption of lipids from food. Intestinal α-glucosidase and pancreatic α-amylase inhibitors (**6**–**19**) inhibit the digestion of polysaccharides and delay their absorption, which can reduce post-prandial hyperglycemia. Dipeptidyl peptidase inhibitors (**20**–**24**) extend the half-life of incretin hormones, which increases insulin secretion from the pancreas, whereas glucose uptake enhancers (**25** and **26**) either have an additive effect to that of insulin, or stimulate additional

glucose disposal into muscle cells, which can ameliorate hyperglycemia. Finally, lipolytic compounds (**27** and **28**) stimulate lipolysis in adipocytes, reducing the size of the intracellular lipid droplets (Figure 1). Such mechanistic information aids our understanding of the effect of plant materials at the molecular level and may promote their appropriate use in the prevention of T2DM and obesity

Figure 1

2. Inhibitors of digestive enzymes

2-1. Pancreatic lipase inhibitors

Pancreatic lipase is a well-known target for prevention of obesity [10]. Food-derived lipids (triacylglycerol) form an emulsion with bile acids, and are then hydrolyzed by pancreatic lipase to produce free fatty acids and monoacylglycerol, which is efficiently absorbed from the intestine. The exact absorption mechanism of these hydrolysis products remain unclear, but the inhibition of pancreatic lipase reduces the absorption of lipids, which is an effective means of preventing obesity [11].

Our search for pancreatic lipase inhibitors, employing a model system using porcine pancreatic lipase as the enzyme and triolein emulsion as the substrate, resulted in the identification of hydroxychavicol (**1**), together with its dimers (**2**,**3**), from *Eugenia polyantha* [12], and phenolcarboxylic acid esters of L-threonic acid (**4**,**5**) from *Filipendula kamtschatica* [13].

E. polyantha (synonym *Syzygium polyanthum*) is a deciduous tropical tree, the leaves of which are aromatic, have a sour taste, and have been used as a spice in Indonesia. The inhibitory activity of **1** is moderate (half maximal inhibitory concentration [IC_{50}] 1.0 mM), but it is present at a high concentration in the plant (1.83% w/w in dried leaves), giving it potential for use in

the prevention of obesity.

F. kamtschatica is a plant that distributed from northern Japan, through the Kuril Islands, to the Kamchatka peninsula, and which is employed by the Ainu people as a traditional medicine for eczema and hives, or as an antidiarrheal agent. The yields from the isolation of **4** and **5** are 0.073% and 0.036%, and **5** shows potent inhibitory activity (IC_{50} 26 μM). In contrast, the inhibitory activity of **4** is relatively low (IC_{50} 246 μM), indicating the importance of the position of the caffeoyl moiety for the activity. The structures of **4** and **5** resemble that of diacylglycerol, which is an intermediate product of lipid digestion by pancreatic lipase. Therefore, substrate recognition by the lipase be at least partially responsible for the difference in inhibitory activity of the two compounds.

The inhibition of pancreatic lipase by plant components has been well studied and a variety of polyphenols, saponins, and triterpenes have been reported to have an inhibitory effect [10,14]. Further study of the lipase inhibitors present in plant materials will help us to understand the anti-obesity effects of such natural remedies.

2-2. Carbohydrate digestive enzyme inhibitors

When consumed, polysaccharides are initially hydrolyzed by salivary and pancreatic α-amylase to produce di- or trisaccharides, and then hydrolyzed to monosaccharides by intestinal α-glucosidase. Intestinal epithelial cells express several glucose transporters, including sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2), which efficiently transport the monosaccharides generated out of the gut lumen [15]. The inhibition of α-amylase and/or α-glucosidases delays the absorption of carbohydrates by reducing the production of monosaccharides, thereby attenuating the rapid post-prandial increase in blood glucose concentration [16,17]. Because high post-prandial blood glucose concentrations are known to predispose toward the development of T2DM, inhibitors of α-amylase and α-glucosidases may be effective in the prevention of T2DM.

2-2-1. Intestinal α-glucosidase inhibitors

We have searched for α-glucosidase inhibitors in plant materials using rat intestinal α-glucosidase as a model enzyme, and identified caffeoylquinic acids (**6**–**13**) from *Pluchea indica* and *Achillea millefolium* [18,19]. The inhibitory activity of these compounds ranges from as low as 2.0 μM for 3,4,5-tri-*O*-caffeoylquinic acid methyl ester (**11**) to >2,000 μM for chlorogenic acid (**6**), depending on the position and the number of caffeoyl groups.

α-glucosidase inhibitors have been quite widely identified in plant materials, with a number of plant-derived compounds (terpenes, alkaloids, quinones, flavonoids, phenylpropanoids, hydrolysable tannins, and azasugars) having been reported to inhibit intestinal α-glucosidase [17,20]. Some of these show quite high potency, for example deoxynojirimycin (IC_{50} = 0.36 μM [21]) from *Morus alba* (mulberry tree) and salacinol (IC_{50} = 6.0 μM [22]) from *Salacia* plants. However, to understand the potential utility of intestinal α-glucosidase inhibitors in greater depth, it should be appreciated that intestinal α-glucosidase is not a single enzyme but comprises two enzyme complexes, maltase-glucoamylase (MG) and sucrase-isomaltase (SI). Although their names indicate their major substrate, SI also recognizes maltose, which is the major disaccharide produced by the digestion of starch in the intestine. Thus, the hydrolysis of maltose is performed by both MG and SI, but the predominant enzyme differs, depending on the substrate concentration. MG has a higher affinity for maltose and is the predominant hydrolase at low substrate concentration, but is inhibited by the accumulation of products. Therefore, at high concentration, SI may be more significant for the digestion of maltose [23].

In most of the *in vitro* studies of the inhibitory activities of plant-derived compounds, a crude enzyme solution prepared from rodent intestine has been used. Therefore, The data obtained may reflect the inhibition of either MG, SI, or both. Thus, although the data give an indication of the effectiveness of the compound, to understand the properties of a candidate compound more clearly its inhibition of MG and SI should be separately assessed. One way to

accomplish this is to employ recombinant α-glucosidases [24], but for a more practical method we developed a single-step purification of MG from a crude enzyme solution by employing a feature of the non-competitive α-glucosidase inhibitor, 2-aminoresorcinol (**14**). 2-Aminoresorcinol (**14**) is a potent MG/SI inhibitor that was produced by the structural modification of baicalein [25]. Its mode of inhibition is non-competitive, which means that it targets the enzyme-substrate complex for inhibition, forming an enzyme-substrate-inhibitor complex. This also means that in the absence of the substrate, the inhibitor does not bind to the enzyme.

By employing the characteristic mode of inhibition of **14**, we designed and tested a novel method for the single-step affinity purification of the enzyme. A derivative of **14** is conjugated to sepharose gel and the crude enzyme, mixed with maltose, is passed through the gel. In the presence of maltose, the MG-maltose complex is trapped by **14** on the gel, while the other components of the solution are washed out. The trapped enzyme is then easily eluted using an elution buffer that does not contain maltose. Removal of the maltose decomposes the enzyme-substrate-inhibitor complex, such that the trapped enzyme is easily recovered, with no requirement for a change in salt concentration or pH, which can also elute non-selectively trapped proteins; or the use of an inhibitor in solution, which would have to be removed before using the purified enzyme.

The initial attempt at this method succeeded in purifying MG [26]. However, several problems, including the capacity, stability of the affinity gel, and the lack of purification of SI remained. By improving the method, we should have easier access to pure MG or SI for use in evaluating the specific activity of a candidate inhibitor against each enzyme complex, and therefore should be able to gain a better understanding of the inhibitory activity of plant-derived α-glucosidase inhibitors.

2-2-2. Pancreatic α-amylase inhibitors

As for the inhibition of α-glucosidase, many plant extracts have been shown to inhibit pancreatic α-amylase [17], including flavonoids, catechins, hydrolysable tannins, and proteins [27–30]. Our research group has also identified lupenone (**15**), a triterpene from *Abrus precatorius*, and corilagin (**16**) and macatannin B (**17**), elagitannins from *Phyllanthus urinaria*, as α-amylase inhibitors, by employing porcine pancreatic α-amylase as the model enzyme [31,32].

Knowledge regarding the ability of triterpenes to inhibit pancreatic α-amylase inhibition is limited. Oleanolic acid, ursolic acid, and lupeol have been reported to inhibit α-amylase [33], but compared with these compounds, **15** was more potent, achieving 84% inhibition at 50 μM, in comparison to 32% for ursolic acid, and nearly no inhibition by other related triterpenes at the same concentration. When the differences in the structures of the tested triterpenes were analyzed, the lupine skeleton and C-3 ketone group seemed to be most important for the inhibitory activity [31].

Several reports have suggested that α-amylase is inhibited by plant extracts containing ellagitannins, but knowledge regarding individual ellagitannins is limited to those isolated from *Rubus suavissimus* [34]. Ellagitannins from *R. suavissimus* caused 15–61% inhibition of salivary α-amylase when present at 10 μg/mL, but the inhibitory activities of **16** and **17** were much lower (21% and 32%, respectively, when present at 1.0 mM), which may be due to the differences between salivary and pancreatic α-amylase. Furthermore, although several compounds isolated from plant materials have been identified to be α-amylase inhibitors, we have frequently faced difficulties in the identification process, creating doubt regarding the bioactive principal responsible for the α-amylase inhibition.

α-Amylase is an enzyme that specifically hydrolyzes polysaccharides by recognizing multiple sugar units in the substrate-binding region using “subsites” [35,36]. Because a single subsite recognizes a single sugar unit, it is expected that small molecules that interact with a single subsite will bind with low affinity and show poor inhibitory activity. Therefore, we

speculated that the standard isolation methodology employed to identify small molecule inhibitors would not be suitable for the identification of α-amylase inhibitors in plant materials.

To confirm the importance of molecular size for the inhibition of α-amylase, an artificial α-amylase inhibitor comprising deoxynojirimycin and glucose, conjugated through an alkyl linker, was designed and synthesized. Deoxynojirimycin is a potent α-glucosidase inhibitor that does not inhibit α-amylase. However, α-amylase and α-glucosidase show similarity in their active centers [37], implying that deoxynojirimycin would occupy the subsite near the active center of α-amylase, and that the reason for the lack of inhibition of α-amylase demonstrated is low affinity due to interaction with a single subsite. Addition of a glucose moiety to deoxynojirimycin should increase this affinity by permitting occupation of an additional subsite, and the conjugate should function as an α-amylase inhibitor. The molecular size of the conjugate is controlled by modifying the length of the linker. Indeed, in practice the synthetic deoxynojirimycin-glucose conjugates (**18**) exhibited α-amylase inhibitory activity and there was a positive correlation between the length of the linker and their inhibitory activity (Figure 2). This finding implies that the size of an molecule is important for its α-amylase inhibitory efficacy [38].

Figure 2

In addition to the above, we suspected a contribution of large molecules in plant materials to α-amylase inhibition. Therefore, large molecules were isolated and a highly condensed proanthocyanidin (PC) in the root of *Astilbe thunbergii*, named AT-P (**19**), was identified to be a fairly potent α-amylase inhibitor (Table 1) [39]. However, the identification of PC as an α-amylase inhibitor was not novel. PCs from acacia bark [40], persimmon peel [41], sapodilla [42], and *Polygonum multiflorum* [43] have been previously reported to inhibit α-amylase (Table 1). Moreover, PCs in apple [44], grape skin [45], persimmon leaf [46], and almond seed

skin [47], have also been suggested to be the bioactive principal responsible for α-amylase inhibition, giving the strong impression that the study of PCs is important for our understanding of the α-amylase inhibitory properties of plant materials.

Table 1

PCs are often a mixture of condensed flavan-3-ol with different degrees of polymerization. It is difficult to determine the structure of PCs and therefore, assessment of their contribution to the α-amylase inhibition by plant materials might remain superficial. However, by separating PCs from the various plant materials and evaluating their individual α-amylase inhibitory activities, the α-amylase inhibitory properties of plant materials would be better understood.

3. Dipeptidyl peptidase-4 inhibitors

The secretion of insulin by pancreatic β-cells is a key step in the control of post-prandial blood glucose concentration. Pancreatic β-cells sense an elevation in blood glucose, which results in the secretion of insulin, and glucagon-like peptide-1 (GLP-1) enhances this response. GLP-1 is mainly secreted by intestinal L-cells upon stimulation by food components, but is rapidly degraded by DPP-4. Therefore, inhibitors of DPP-4 extend the half-life of GLP-1 in the circulation and are effective at ameliorating hyperglycemia and T2DM [48].

The search for DPP-4 inhibitors in plant materials using human recombinant DPP-4 has resulted in the identification of rugosin A (**20**) (IC_{50} 25.8 μM) and rugosin B (**21**) (IC_{50} 28.5 μM), along with related hydrolyzable tannins from rose (*Rosa gallica*) flower [49]. This research also identified macrocarpal A-C (**22**–**24**) from *Eucalyptus globulus* as a potent inhibitor of DPP-4 [50]. The activity of **24** was characteristic, showing a rapid increase in inhibition between 30 and 35 μM, which is likely due to an aggregation of the compound in solution. Furthermore, a variety of other plant-derived compounds have been reported to be

DPP-4 inhibitors, including peptides, flavonoids, resveratrol, cyanidins, and triterpenes [51–53].

4. Myocyte glucose uptake stimulators

Myocytes and adipocytes are insulin-sensitive cells that increase their uptake of glucose in response to insulin stimulation and thereby contribute to the insulin-stimulated reduction in blood glucose. Insulin resistance and insufficient insulin secretion are the two main defects in T2DM; therefore, enhancers of glucose uptake by myocytes and adipocytes can be effective for the prevention or treatment of T2DM [54]. Two types of plant-derived glucose uptake enhancer have been reported, one of which is an insulin-sensitizer. This type of compound typically has its effects by activating peroxisome proliferator-activated receptor-γ (PPARγ).

PPARγ is a nuclear receptor that is highly expressed in both white and brown adipose tissue. It is a master regulator of adipogenesis and modulates lipid metabolism by enhancing the transcription of genes that contain PPAR response elements (PPRE) [55]. It modulates insulin sensitivity by increasing the expression of insulin signaling pathway intermediates, including insulin receptor substrates (IRS), phosphoinositide 3-kinase (PI3K), and glucose transporter 4 (GLUT4) [55]. Greater expression of these proteins enhances the response of adipocytes to insulin, including insulin-stimulated glucose uptake.

Isoflavones and flavonoids are the major plant components with PPARγ agonist activity [56]. However, glycosylated isoflavones and flavonoids, which are common components of plant materials, are not reported to possess PPARγ agonist activity, with the exception of puerarin (**25**), the 8*C*-glucosylated daidzein that is found in in *Pueraria iobata* [57]. We have studied the difference between molecules containing *O*- and *C*-linkage and evaluated the role of *C*-glucoside using 3T3-L1 adipocytes. A structural study revealed that *O*-glucosides reduce the potency of isoflavones in the enhancement of insulin induced glucose uptake into 3T3-L1 cells, but *C*-glucoside does not, but instead supports this activity by increasing the solubility of

316 **25** in aqueous media [58].

317 Another type of glucose uptake enhancer is a compound that directly stimulates the
318 translocation of GLUT4 to the plasma membrane and enhances glucose uptake, independent
319 of insulin. The L6 muscle cell line expresses GLUT4 and is a suitable model in which to
320 evaluate this type of compound. Our search of plant materials for stimulators of glucose uptake
321 resulted in the identification of higenamine 4'-*O*-β-D-glucoside (**26**) from lotus (*Nelumbo*
322 *nucifera*) plumule, a plant component used to prepare tea in one region of Japan and employed
323 in Kampo medicine [59]. The aglycon version of higenamine (norcoclaurine) exists in the leaf
324 and embryo, but the glucoside was first form to be reported in the lotus plant. The configuration
325 of higenamine varies between parts of the lotus plant, with the *S*-enantiomer being found in the
326 leaves and the *R*-enantiomer in the embryo [60,61]. Our careful analysis of the configuration
327 of **26**, involving stereoselective synthesis of *R*/*S*-isomers, showed it to be present as 3/2 mixture
328 of diastereomers [62]. An additional study of **26** using a PI3K inhibitor (LY294002), AMPK
329 inhibitor (dorsomorphin), and β2-adrenergic receptor antagonist (ICI118,551), all of which are
330 specific inhibitors of proteins involved in GLUT4 translocation, revealed the β2-adrenergic
331 receptor to be the target protein (Figure 3) [59]. In addition, a study of the relationship between
332 structure and activity revealed higenamine (synonym: norcoclauline) to be the core structure
333 responsible for the activity, and showed that the *S*-enantiomer is more active than the *R*-
334 enantiomer [63].

335

336 Figure 3

337

338 The signaling mechanism connecting β2-adrenergic receptor and GLUT4 translocation has
339 been studied in detail and can provide an explanation for the effect of **26**. Activation of the β2-
340 adrenergic receptor activates adenylyl cyclase (AC), which generates cyclic adenosine
341 monophosphate (cAMP), activating cAMP-dependent protein kinase (PKA). PKA

phosphorylates the mechanistic target of rapamycin complex-2 (mTORC2), which stimulates the translocation of GLUT4 and enhances glucose uptake into muscle cells [64].

β2-adrenergic receptor agonists are widely used as bronchodilators to treat asthma but have also been studied for their potential to treat hyperglycemia [65]. In addition to **26**, *p*-synephrine from the orange flower (*Citrus aurantium*) has been identified as a glucose uptake enhancer by our group, as already published by Hong *et al* [66]. In this article, the involvement of β2-adrenergic receptor was not confirmed, but *p*-synephrine is known to be a β-adrenergic receptor agonist [67,68], and we have also confirmed the involvement of the receptor using specific inhibitors (unpublished data). Furthermore, several other plant compounds, including osthole, gramine, and hordenine, are also β2-adrenergic receptor agonists [69], suggesting these compounds are also worthy of further investigation, to develop understanding of the therapeutic effects of plant materials against diabetes.

5. Pro-lipolytic compounds that reduce adipocyte size

Enlargement of adipocytes through the accumulation of excess lipid is the cause of obesity, and is also associated with insulin resistance. Therefore, the control of lipid accumulation in adipocytes is likely to be beneficial in the prevention of T2DM, as well as obesity.

3T3-L1 cells are a widely accepted adipocyte cell line, and the accumulation of lipids in these cells can easily be evaluated by staining the lipid droplets with oil Red-O or Nile Red. Screening of medicinal plants for candidates that reduce lipid accumulation in 3T3-L1 adipocytes yielded *Eurycoma longifolia* Jack [70]. Two quassinoids from this plant, eurycomanone (**27**) (EC_{50} 14.6 μM) and 13β,21-epoxyeurycomanone (**28**) (EC_{50} 8.6 μM), were identified as bioactive principals that enhance lipolysis, thereby reducing cellular lipid content [71]. Many other plant extracts have also been reported to induce lipolysis, and several compounds have been identified as active compounds, including flavones and polymethoxyflavones [72–74], pterostilbene [75], arylbutanoid glycosides [76], aculeatin [77],

(3, 3-dimethylallyl)halfordinol [78], and arecoline [79]. However, although knowledge of plants containing pro-lipolytic compounds is gradually increasing, the exact mechanisms of action involved require clarification.

Adipocyte lipolysis is stimulated by a range of agents, including catecholamines, natriuretic peptides, TNF-α, and growth hormone [80]. Catecholamines stimulate lipolysis *via* the β3-adrenergic receptor, which signals through AC, cAMP, and PKA to phosphorylate hormone-sensitive lipase (HSL) and perilipin [80]. Natriuretic peptides act through natriuretic peptide receptors, which signal through guanylyl cyclase, cGMP, and cGMP-dependent protein kinase (PKG), which phosphorylates the same targets as PKA [80]. Finally, TNF-α and growth hormone bind to their specific receptors, which results in activation of mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK), to downregulate perilipin and enhance lipolysis (Figure 4) [81,82].

Figure 4

To identify the signaling pathway involved in the pro-lipolytic effect of eurycomanone (**27**), a PKA inhibitor (H-89) and an ERK inhibitor (PD98059) were co-incubated with this compound. PD98059 did not significantly affect the lipolytic activity of **27** and **28**, but H-89 diminished their activity. The contribution of PKA to the pro-lipolytic activity of **27** and **28** was also confirmed by the greater phosphorylation of the catalytic subunit of PKA. However, activation of the β-adrenergic receptor, upstream of PKA, was prevented by co-incubation with propranolol (a non-selective β-adrenergic receptor antagonist), meaning that the precise target protein of these compounds has yet to be identified (Figure 4) [71].

In summary, many of the pro-lipolytic compounds in plants are only known for their activity, while neither their direct targets, nor the mechanisms involved, have been studied. Therefore, a great deal of study remains to achieve understanding of the pro-lipolytic effects

of the plant materials and their bioactive constituents.

6. Conclusion and future perspectives

Plant materials contain numerous compounds with bioactivity against a variety of biological processes related to the development of T2DM and obesity. From the efforts of many researchers, knowledge of the anti-diabetic and anti-obesity activities of plant components is accumulating. However, it is still necessary to explore and study the bioactive compounds in plant materials in greater depth, to understand their effects more clearly.

In many cases, the effects of plant materials can be explained by study of the bioactive compounds they contain, which target well-studied biological processes, such as digestion. However, there are many processes that have not been extensively studied, but may be targets of unidentified bioactive compounds in plant materials. Therefore, analysis of a wide variety of biological processes as potential targets of the bioactive constituents of plant materials is important to fully understand the effects of those materials. In addition, the exact mechanisms and target proteins of these bioactive compounds should be explored more thoroughly to improve knowledge of how these bioactive compounds have their effects at a molecular level.

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641 Table 1. α-Amylase inhibitory activity of proanthocyanidins isolated from plants

Plant origin	IC_{50} (μg/mL)	mDP*	Active constituents**
A. thunbergii	1.7	11.8	C, EC, GC, EGC, (E)CG, (E)GCG
Persimmon peel	53.9% at 100 μg/mL	ND	ND
Acacia bark	38.0	4–8	5-deoxyflavan-3-ols
Sapodilla	4.2	9.0	C, EC, (E)CG, (E)GC, (E)GCG
P. multiflorum	2.9	32.6	C, EC, (E)CG

642 *mDP: mean degree of polymerization

643 **analyzed by thiol degradation. C: Catechin, EC: epicatechin, GC: gallocatechin, EGC:
644 epigallocatechin, (E)CG: (epi)catechin gallate, (E)GCG: (epi)gallocatechin gallate, ND: not
645 determined.

646

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Figures

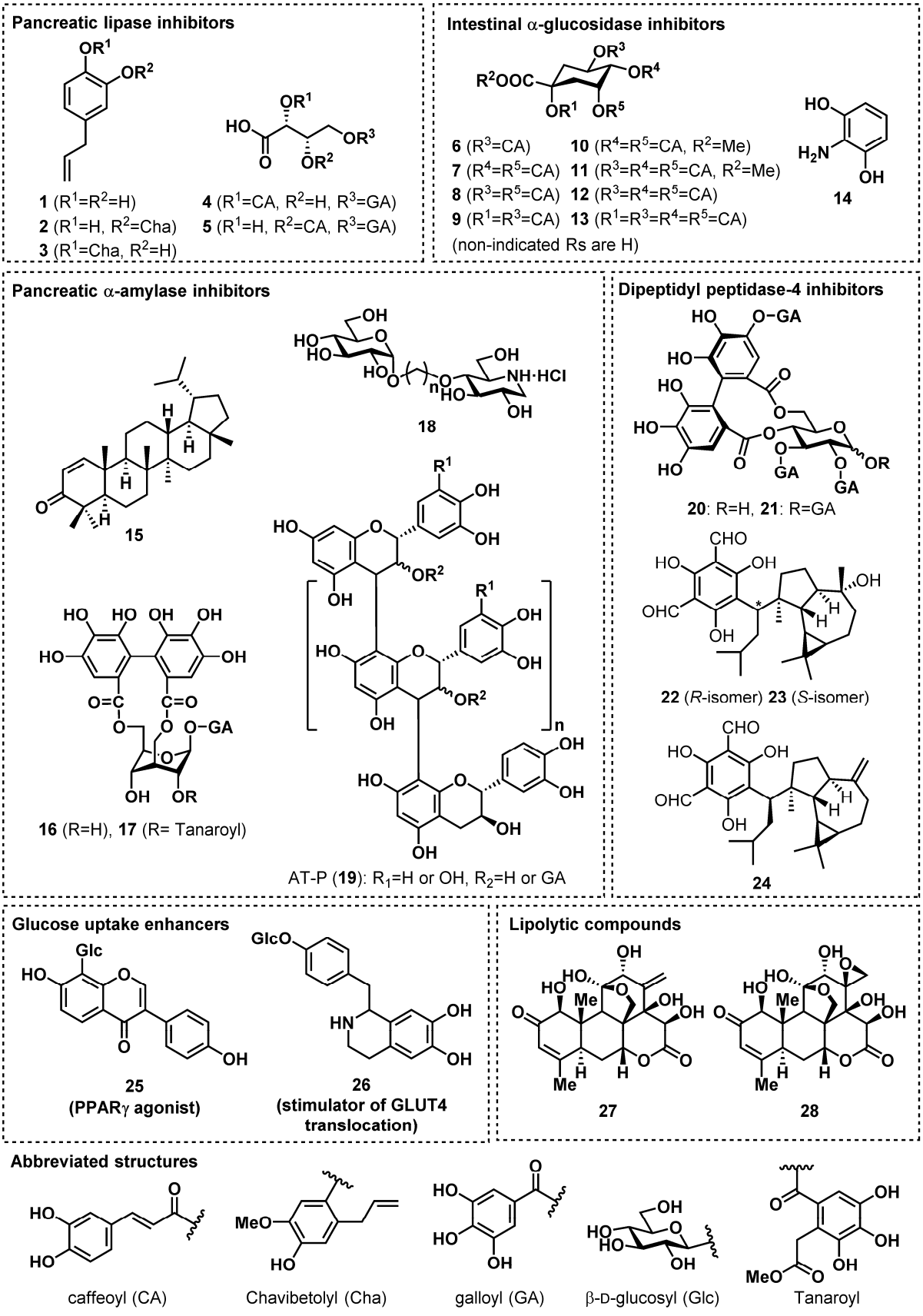


Figure 1. Structures of the studied compounds

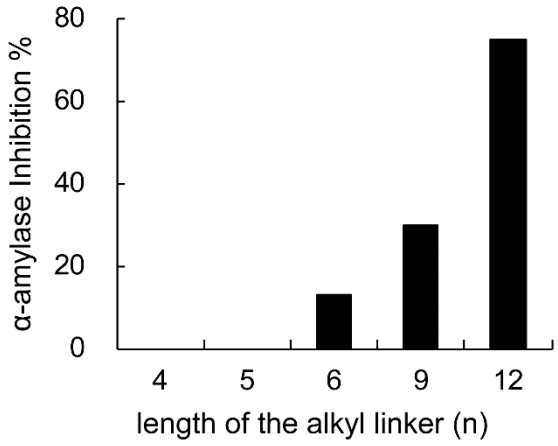
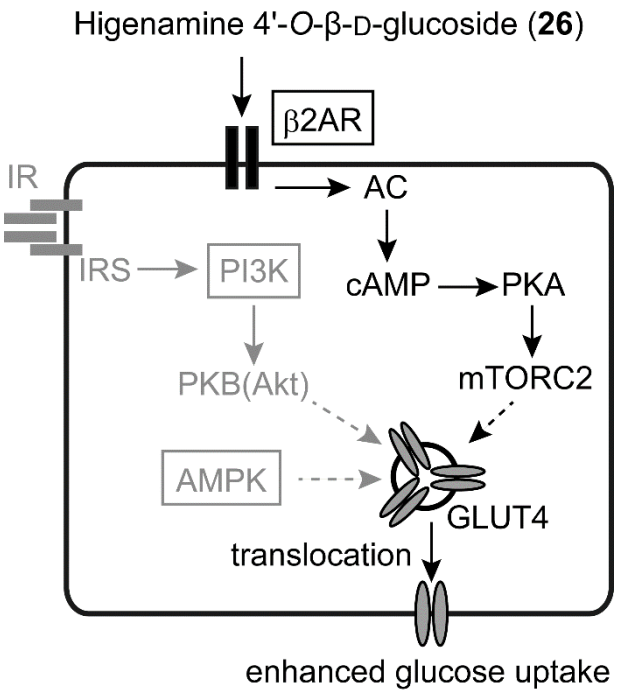


Figure 2. α-Amylase inhibitory activity of artificial inhibitors (18)

The numbers indicate the length of the alkyl linker between deoxynojirimycin and the glucose moiety indicated by 'n' in structure of compound **18** (Figure 1).



658

659 **Figure 3. The mechanism of the higher glucose uptake induced by higenamine 4'-*O*-β-D-**
660 **glucoside (26)**

661 Compound **26** activates the β2AR to increase glucose uptake. The proteins in boxes were
662 evaluated for their involvement and the other major pathways colored gray were not implicated.
663 β2AR: β2-adrenergic receptor, AC: adenylyl cyclase, cAMP: cyclic adenosine monophosphate,
664 PKA: cAMP-activated protein kinase A, mTORC2: mechanistic target of rapamycin complex-
665 2, GLUT4: glucose transporter 4, IR: insulin receptor, IRS: insulin receptor substrate, PI3K:
666 phosphoinositide 3-kinase, PKB (AKT): protein kinase B, AMPK: AMP-activated protein
667 kinase.

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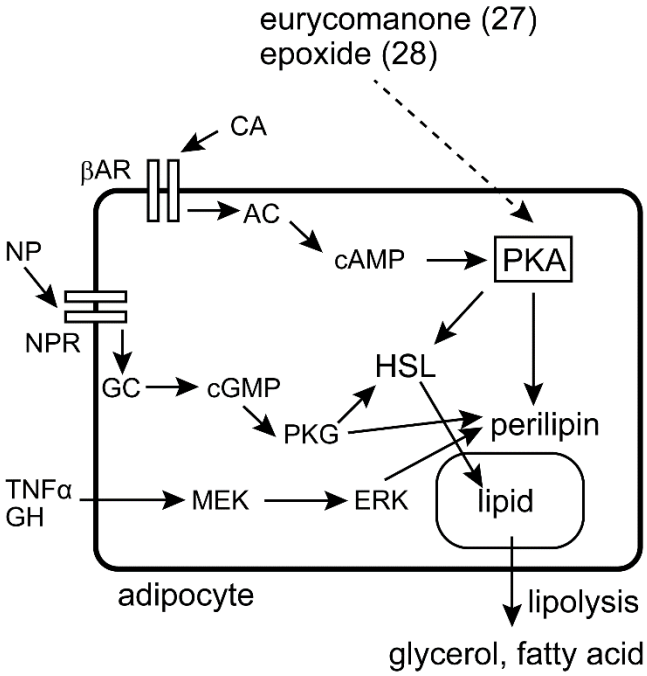


Figure 4. Lipolytic pathways and the target of eurycomanone (27) and its epoxide (28)

Compounds **27** and **28** have their effect through the activation of PKA. The involvement of ERK in this activity has been ruled out, but other proteins involved in lipolysis have not been studied in detail. AC: adenylyl cyclase, βAR: β-adrenergic receptor, CA: catecholamine, cAMP: cyclic adenosine monophosphate, cGMP: cyclic guanosine monophosphate, ERK: extracellular signal-regulated kinase, GC: guanylyl cyclase, GH: growth hormone, HSL: hormone-sensitive lipase, MEK: MAPK/ERK kinase, NP: natriuretic peptide, NPR: natriuretic peptide receptor, PKA: cAMP-activated protein kinase, PKG: cGMP-activated protein kinase, TNFα: tumor necrosis factor-α.