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Biflavones from *Ginkgo biloba* as novel pancreatic lipase inhibitors: Inhibition potentials and mechanism

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ABSTRACT

Reduction of lipid absorption has been recognized as an attractive approach for the discovery of new drugs to treat obesity and overweight. The leave extract of *Ginkgo biloba* has been widely used for the treatment of metabolic diseases (such as hyperlipidemia) in both eastern and western countries, but the bioactive compounds in *Ginkgo biloba* and the underlying mechanism have not been fully characterized. This study aimed to investigate the inhibition potentials and mechanism of major biflavones from *G. biloba* on pancreatic lipase (PL), a key target regulating lipid absorption. The results clearly demonstrated that all tested biflavones in *G. biloba* including isoginkgetin, bilobetin, ginkgetin and sciadopitysin, displayed strong to moderate inhibitory effects on PL with the IC_{50} values ranging from 2.90 μM to 12.78 μM. Further investigations on both inhibition kinetic analyses and docking simulations demonstrated that isoginkgetin, bilobetin and ginkgetin were potent PL inhibitors (K_i < 2.5 μM), which could create strong interactions with the catalytic triad of PL *via* hydrogen bonding. These findings provided a new powerful evidence for explaining the hypolipidemic effects of *G. biloba*, while these newly identified PL inhibitors from *G. biloba* could serve as lead compounds for the development of biflavonoid-type PL inhibitors.

1. Introduction

The prevalence of obesity and overweight has dramatically increased in both developed and developing countries, due to the modern lifestyle and an increase of consumption of high-fat and high-sugar diets [1–4]. Obesity and overweight are consistently associated with the elevated levels of blood glucose and lipid, which are important risk factors for the development of metabolic diseases including hypertension, arteriosclerosis, non-insulin-dependent diabetes mellitus, and coronary heart disease [2,5,6]. Over the past thirty years, many efforts have been implemented to find more promising targets or practical medications for the treatments of obesity. One of the practical strategies for the prevention of obesity is to reduce fat digestion and absorption in the digestive organs [7,8].

Pancreatic lipase (triacylglycerol acyl hydrolase, PL), which catalyzes the hydrolysis of triacylglycerides in the gastrointestinal tract, is the key enzyme for lipid absorption [7–9]. This enzyme is secreted from the pancreas and responsible for the hydrolysis of 50–70% of total dietary fats. More evidence has demonstrated that inhibition of PL and the associated reduction of lipid absorption is an attractive approach for the discovery of new drugs to treat obesity and overweight [10]. For example, orlistat, a well-known PL inhibitor, has been approved by the US Food and Drug Administration in 1999 to treat obesity [11]. Although orlistat displays potent anti-obesity effect, the drug can also cause non-negligible gastrointestinal side effects, such as faecal incontinence and stomach pain [12]. Thus, it is necessary to find more PL inhibitors with potent PL inhibition activity and improved safety profile for clinical use.

In recently years, there is a great deal of interest in discovering natural compounds from herbal medicines or edible plants as drug lead compounds, due to most of herbs displayed satisfying safety during long history of use for medical treatments [13–16]. *Ginkgo biloba*, a famous traditional Chinese herb medicine, has been used for medical purposes for a long history in many countries [17,18]. Now the extracts of *G. biloba* leaves are commonly used as dietary supplements or

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phytomedicines in both eastern and western countries [18,19]. Both basic researches and clinical trials have demonstrated that the extracts of *G. biloba* could bring beneficial effects on the treatment of metabolic diseases including hyperlipidemia, cardiovascular and arteriosclerosis, but the active compounds in *G. biloba* and the underlying mechanism have not been fully characterized. The extract of *G. biloba* leaves contains many bioactive compounds including terpene trilactones, biflavonoids and flavanol glycosides [20,21]. It has been reported that terpene trilactones including ginkgolides and bilobalide display moderate inhibitory effects against PL with the IC_{50} values from 22.9 μg/mL to 60.1 μg/mL [22]. However, the inhibitory effects of biflavonoids, another important class of bioactive compounds in *G. biloba* extract, have not been investigated yet.

In the present study, the inhibitory effects of four major biflavonoids distributed in *G. biloba* on PL were investigated and well characterized by a florescence-based biochemical assay. The preliminary screening demonstrated that all tested biflavones in *G. biloba* including isoginkgetin (1), bilobetin (2), ginkgetin (3) and sciadopitysin (4) (Fig. 1), displayed strong to moderate inhibitory effects towards PL, with the IC_{50} values ranging from 2.90 μM to 12.78 μM. To determine the inhibition mechanism of the tested biflavones on PL, the inhibition constant (K_i) and inhibition types of these compounds against PL were carefully characterized. Meanwhile, molecular docking simulations were also conducted to gain deep insights into the inhibitory behaviors of these biflavones against PL from the view of ligand-enzyme interactions. All these findings provided a new powerful evidence for explaining the hypolipidemic effects of *G. biloba*, while the newly identified natural PL inhibitors could also serve as lead compounds for the development of novel PL inhibitors.

2. Material and methods

2.1. Chemicals and reagents

The natural biflavones including isoginkgetin, bilobetin, ginkgetin, and sciadopitysin were isolated from the crude leaf extract of *Ginkgo biloba* by reversed-phase HPLC with an ODS column (4.6 × 150 mm, 5 μm) and UV detection at 270 nm. The chemical structures of these biflavones are fully characterized by NMR technique, and the ^{1}H NMR (500 MHz) and ^{13}C NMR(125 MHz)spectra of these four biflavones were presented in Figs. S1–S8 (Supplementary materials). The purities of these four biflavones were higher than 98% (determined by LC-UV). 4-Methylumbelliferone (4-MU), 4-methylumbelliferyl oleate (4-MUO) and porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (St. Louis, MO). Each compound (100 mM) were prepared in DMSO and stored at 4 °C until use. Stock solutions of 4-methylumbelliferyl oleate (100 mM) were prepared in DMSO and stored at −20 °C until use. 0.1 M citrate phosphate buffer (PH 7.4) was prepared using Millipore water and stored at 4 °C for further use. Millipore water (Millipore, Bedford, USA), LC grade DMSO (Tedia, USA) were used throughout.

2.2. Enzyme inhibition assays

The inhibitory effects against pancreatic lipase (Sigma type II) were investigated using 4-methylumbelliferyl oleate (4-MUO) as substrate [23]. Briefly, the incubation mixture with a total volume of 0.2 mL was consisted of lipase solution (0.01 mg/mL, final concentration), 0.1 M citrate phosphate buffer (0.1 M citrate-Na_2HPO_4, pH 7.4), and each

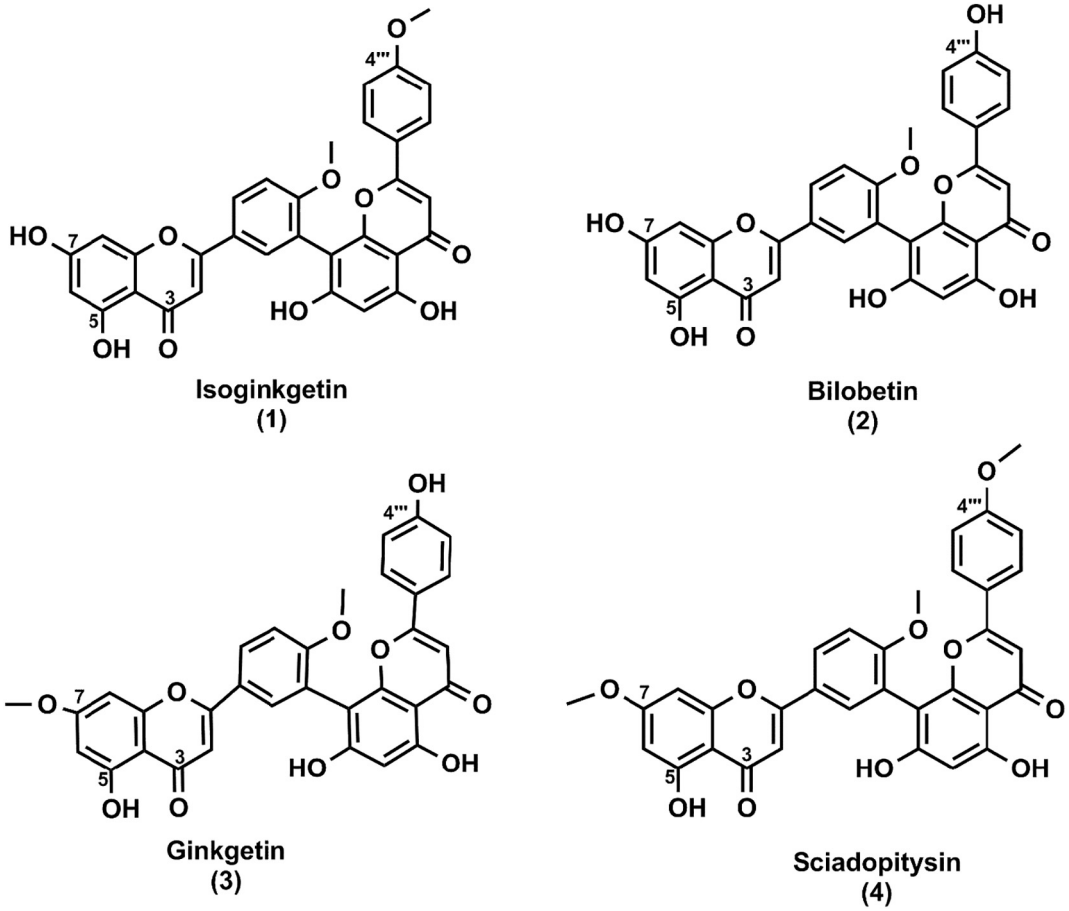

Fig. 1. Chemical structures of natural biflavones from *G. biloba*, including isoginkgetin (1), bilobetin (2), ginkgetin (3) and sciadopitysin (4).

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