Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2020, **18**(5): 369–378
doi: 10.1016/S1875-5364(20)30043-1
Chinese Journal of Natural Medicines

•Research article•

Pancreatic lipase inhibitory constituents from Fructus Psoraleae

HOU Xu-Dong[1Δ], SONG Li-Lin[2, 3Δ], CAO Yun-Feng[4Δ], WANG Yi-Nan[3], ZHOU Qi[1], FANG Sheng-Quan[3], WU Da-Chang[1], ZANG Shi-Zhu[1], CHEN Lu[1], BAI Yue[1], GE Guang-Bo[3*], HOU Jie[1*]

[1] *College of Basic Medical Sciences, Dalian Medical University, Dalian 116000, China;*
[2] *Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116000, China;*
[3] *Translational Medicine Center, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine & Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai 200000, China;*
[4] *Dalian Runsheng Kangtai Medical Laboratory Co. Ltd., Dalian 116000, China*

Available online 20 May, 2020

[ABSTRACT] Pancreatic lipase (PL), a crucial enzyme in the digestive system of mammals, has been proven as a therapeutic target to prevent and treat obesity. The purpose of this study is to evaluate and characterize the PL inhibition activities of the major constituents from Fructus Psoraleae (FP), one of the most frequently used Chinese herbs with lipid-lowering activity. To this end, a total of eleven major constituents isolated from Fructus Psoraleae have been obtained and their inhibition potentials against PL have been assayed by a fluorescence-based assay. Among all tested compounds, isobavachalcone, bavachalcone and corylifol A displayed strong inhibition on PL (IC_{50} < 10 μmol·L^{-1}). Inhibition kinetic analyses demonstrated that isobavachalcone, bavachalcone and corylifol A acted as mixed inhibitors against PL-mediated 4-methylumbelliferyl oleate (4-MUO) hydrolysis, with the K_i values of 1.61, 3.77 and 10.16 μmol·L^{-1}, respectively. Furthermore, docking simulations indicated that two chalcones (isobavachalcone and bavachalcone) could interact with the key residues located in the catalytic cavity of PL *via* hydrogen binding and hydrophobic interactions. Collectively, these finding provided solid evidence to support that Fructus Psoraleae contained bioactive compounds with lipid-lowering effects *via* targeting PL, and also suggested that the chalcones in Fructus Psoraleae could be used as ideal leading compounds to develop novel PL inhibitors.

[KEY WORDS] Pancreatic lipase; Fructus Psoraleae; Inhibition potential; Isobavachalcone; Bavachalcone
[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2020)05-0369-10

Introduction

Obesity, a chronic metabolic disorder, is associated with a variety of human diseases. According to the investigation of World Health Organization in 2016, the proportion of population with overweight and obesity around the world is nearly 50% [1]. Overweight (BMI higher than or equal to 25 kg·m^{-2}) and obesity (BMI higher than or equal to 30 kg·m^{-2}) will lead to excessive accumulation of fat and thus bring a range of disorders of the human body. Overweight or obesity is customarily provoked by a combination of inordinate food digestion, lack of lifestyle exercise, and genetic susceptibility [2, 3]. Accumulative evidences have indicated that overweight and obesity are closely linked with a variety of human diseases, such as cardiovascular diseases (hyperlipidemia, arteriosclerosis and hypertension), diabetes mellitus type 2, osteoarthritis, asthma and various types of cancer (liver, gallbladder, kidney and colon) [4-6]. Currently, a panel of many therapeutic strategies (such as dietotherapy, pharmacotherapy and surgical therapy) for the prevention and treatment of obesity and related diseases in the clinical seting. Among these therapeutic strategies, pancreatic lipase (PL) inhibition therapy has been proven to be one of the most effective options to prevent and

[Received on] 21-Oct.-2019
[Research funding] This work was supported by the National Key Research and Development Program of China (Nos. 2017YFC1700200, 2017YFC1702000); NSF of China (Nos. 81922070, 81973286, 81973393, 81773687); Natural Science Foundation of Liaoning Province (No. 20180530025); Program of Shanghai Academic/Technology Research Leader (No. 18XD1403600); Shuguang Program (No. 18SG40) supported by Shanghai Education Development Foundation and Shanghai Municipal Education Commission; Innovative Entrepreneurship Program of High-level Talents in Dalian (No. 2017RQ121); and Undergraduate Training Program for Innovation and Entrepreneurship from Shanghai University of Traditional Chinese Medicine (No. 201710141980301090 4).
[*]**Corresponding author]** E-mail: geguangbo@dicp.ac.cn (GE Guang-Bo); houjie@dlmedu.edu.cn (HOU Jie)
[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

treat obesity or obesity-associated metabolic diseases, such strategy may strongly blocks the absorption of dietary fat *via* inhibiting PL in the gastrointestinal system.

Pancreatic lipase (triacylglycerol acyl hydrolase, PL), a key hydrolase in the digestive system of mammals, is responsible for the hydrolysis of dietary triglycerides and thereby regulates the lipid absorption. Although there are several kinds of lipases in the digestive organs of human, including lingual lipase, gastric lipase and pancreatic lipase, PL plays a predominant role in the decomposition of triglycerides from dietary fats with the contribution of about 70% [7, 8]. Over the past few decades, many studies have been conducted to find efficacious PL inhibitors for the prevention and treatment of overweight and obesity, owing to the crucial role of PL in lipid absorption in humans [8]. Orlistat, a potent PL inhibitor that has been approved for the treatment of obesity in 1999, can covalently bind on the serine residual of the catalytic triad of PL, and thereby blocking the catalytic activity of PL [9, 10]. Although orlistat displays good performance in weight loss, some of the most frequent side effects of this agent (such as gastrointestinal toxicity, liver damage, pancreatic damage, kidney damage, metabolic system abnormalities and high cancer risk) strongly limits its clinical applications, especially for long-term treatment [11, 12]. Therefore, it is desirable to find more drug leads or candidates for the development of efficacious PL inhibitors with high efficacy and good safety profiles, which may provide alternative pharmacotherapy to prevent and treat obesity and obesity-associated disorders.

Over the past two decades, numerous phytochemicals and synthetic compounds have been found with PL inhibition activities [13]. Notably, natural products from herbs or medicinal plants are still one of the major sources for discovery of the lead compounds for drug development [14, 15]. Many previous studies have demonstrated that a wide range of natural compounds (such as flavonoids, bioflavones, triterpenes and other phenolic compounds) display strong PL inhibition activities [16-21]. *Fructus Psoraleae* (also named Bu-gu-zhi in Chinese, FP), a famous edible Chinese herb that have been frequently used in Asia countries for a long history, has been found with lipid-lowering activity and other beneficial effects for the prevention and treatment of metabolic disorders [22-27]. Recent investigations have found that the major constituents from *Fructus Psoraleae* display strong inhibition against a panel of serine hydrolases, such as carboxylesterase 1 and carboxylesterase 2 [28, 29]. Considering that the catalytic triad (Ser, His, and Glu) is highly conserved in all serine hydrolases, while the inhibitor spectra of mammalian serine hydrolases are highly overlapped [30], *Fructus Psoraleae* constituents may also inhibit the catalytic activities of PL, thereby regulate lipid absorption in mammals.

This study aimed to investigate the inhibitory effects and the inhibitory behaviors of the major constituents in *Fructus Psoraleae* on PL, a crucial target regulating lipid absorption in mammals. To this end, a total of eleven major constituents from *Fructus Psoraleae* were obtained and their ability to inhibit PL was carefully investigated by a routinely used biochemical assay. The results showed that three constituents from *Fructus Psoraleae* including isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) displayed strong PL inhibition activity, with IC_{50} values of less than 10 μmol·L^{-1}. Next, the inhibitory behaviours of two chalcones (isobavachalcone and bavachalcone, two potent PL inhibitor isolated from *Fructus Psoraleae*) was well-characterized by both inhibition kinetic assays and docking simulations. These studies provided a novel evidence to support the lipid-lowering effect of *Fructus Psoraleae*, while two chalcones with strong PL inhibition activity could also be used as lead compounds for the development of new PL inhibitors with improved potency and good safety profiles.

Material and Methods

Chemicals and reagents

The major constituents isolated from *Fructus Psoraleae*, including psoralen, isopsoralen, 8-methoxypsoralen, isobavachalcone, bavachalcone, corylifol A, corylin, bavachin, bavachinin A, bakuchiol and psoralidin (purities > 98%) were obtained from Chengdu preferred Biotech Co., Ltd., China. Porcine pancreatic lipase (PPL, type II, Lot No. SLBL2143V) and 4-methylumbelliferyl oleate (4-MUO, substrate) were obtained from Sigma-Aldrich, USA. The chemical structures of all tested constituents from *Fructus Psoraleae* (FP) were depicted in Fig. 1. The stock solution of all tested natural constituents were prepared using DMSO as the solvent and stored at 4 °C. Kaempferol (a known reversible inhibitor of PL) was provided by Chengdu preferred Biotech Co., Ltd., and used as a positive inhibitor in this study. The buffer of the entire experimental system was prepared according to a previous report [16]. Other reagents were of LC grade.

PL inhibition assay

To explore the inhibitory potential of each natural constituent from FP against porcine pancreatic lipase, the enzyme solution (10 μg·mL^{-1}, final concentration) was co-incubated with each inhibitor and 4-MUO (10 μmol·L^{-1}, final concentration). Explain in detail, 200 μL incubation solution contains 194 μL citrate-Na_2HPO_4 buffer, 2 μL pancreatic lipase solution and 2 μL each inhibitor (suitable concentration gradient). The mixture of enzyme solution, inhibitor solution and buffer were pre-incubate for 10 min before the enzyme was reacted with substrate. The substrate or each tested compound was dissolved in DMSO to a final concentration of DMSO at 1% in the incubation mixture (*V*/*V*, a concentration that had no effect on catalytic activity). The amount of 4-methylumbelliferone (the hydrolytic metabolite of 4-MUO in PL) released by the reaction of pancreatic lipase in all incubations was further assayed under excitation at 340 nm, while the fluorescence signals of the 4-methylumbelliferone were recorded at 460 nm by using microplate reader. The

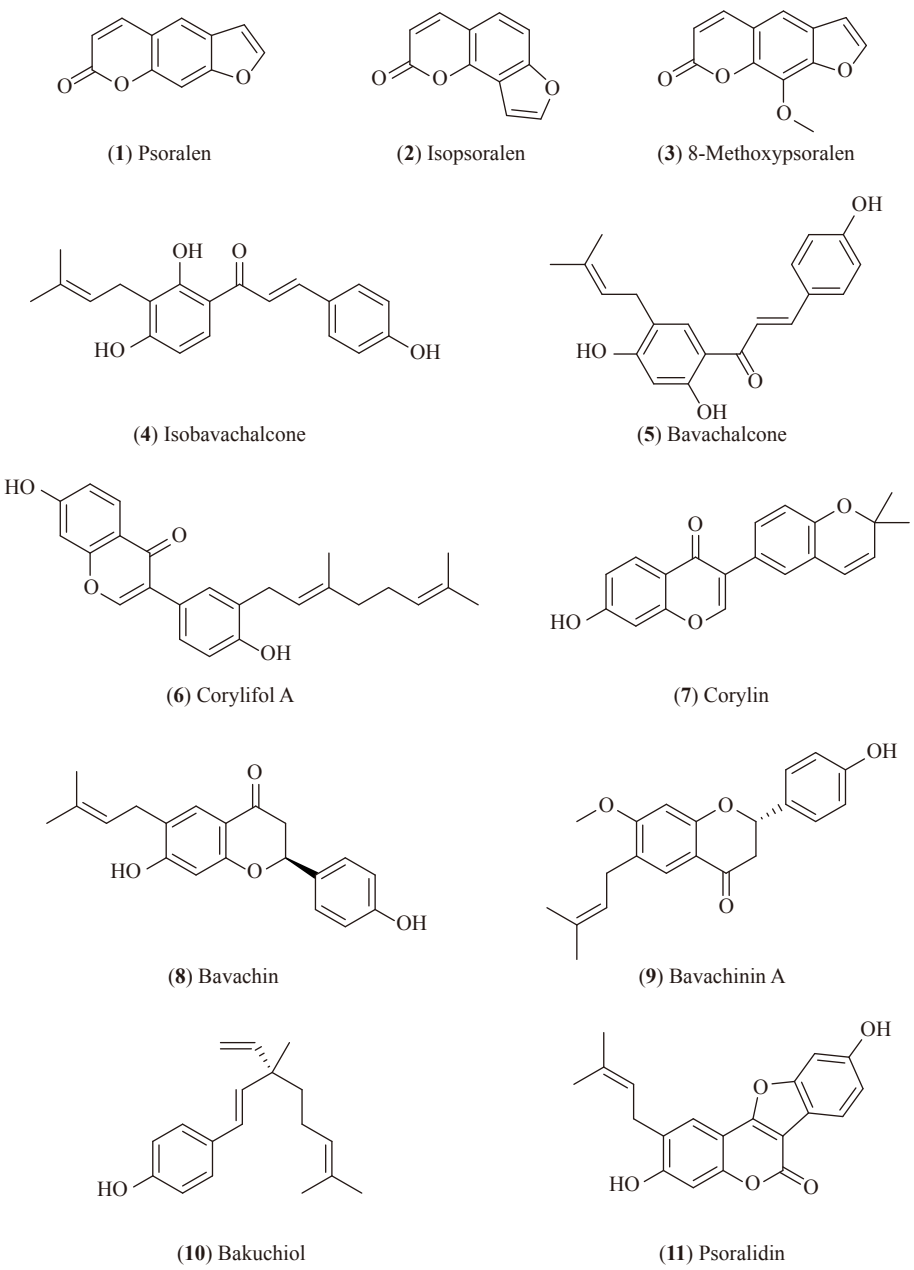

Fig. 1 The major constituents from Chinese herb Fructus Psoraleae (also named Bu-gu-zhi)

residual activity of PL in the presence of each inhibitor was determined using following formula, residual activity = (the hydrolytic rate in the presence of each inhibitor at a given concentration) / (the hydrolytic rate in the presence of DMSO) × 100%.

Inhibition kinetic analyses

The inhibition kinetic parameters (K_i values and the inhibition modes) of isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) against PL were determined by a set of assays using various substrate concentrations in the presence of different concentrations of each inhibitor. The related inhibition kinetic parameters were calculated by the inhibition kinetic plots and the Lineweaver-Burk plots. For reversible inhibition, the inhibition kinetic modes including competitive, noncompetitive and mixed inhibition. The following equations for competitive inhibition Eq. (1), noncompetitive inhibition Eq. (2), or mixed inhibition Eq. (3) were used to find the best suitable mode for fitting all kinetic data.

$$V = (V_{max}S)/[K_m(1 + I/K_i) + S] \quad (1)$$

$$V = (V_{max}S)/[(K_m + S)(1 + I/K_i)] \quad (2)$$

$$V = (V_{max}S)/[(K_m + S)(1 + I/\alpha K_i)] \quad (3)$$

where V is the velocity of the hydrolytic reaction; K_i is the inhibition constant describing the affinity of each inhibit-

or for the target enzyme; S and I is the substrate (4-MUO) and inhibitor concentration, respectively; V_{max} is the maximum velocity; K_m is the Michaelis-Menten constant (substrate concentration at 0.5 V_{max}) [31-33].

Molecular docking simulations

Molecular docking simulations were conducted by AutoDock Vina. The crystallographic structure data of porcine pancreatic lipase was taken from the Protein Data Bank (PDB:1ETH), and prepared by AutoDockTools 1.5.6 [34]. Interaction analysis and visualization were performed by Discovery Studio Visualizer (BIOVIA Discovery Studio 2019, Dassault Systèmes, SanDiego, USA) and PyMOL (The PyMOL Molecular Graphics System Version 2.3, Schrödinger, LLC., New York City, USA). The calculated affinity energy and X-Score were calculated by AutoDock Vina and X-Score v1.2 [35].

Statistical analysis

The IC_{50} and K_i values were estimated by the GraphPad Software and shown as mean ± SD.

Results

Inhibition potentials of the constituents in Fructus Psoraleae against PL

Firstly, the inhibition potentials of 11 major constituents in *Fructus Psoraleae* on pancreatic lipase (PL) were assayed using three different inhibitor concentrations (1, 10, 100 μmol·L^{-1}, final concentration). As shown in Fig. 2, the residual activities of bakuchiol (**10**), psoralen (**1**), bavachin (**8**), corylin (**7**), bavachinin A (**9**), corylifol A (**6**), isobavachalcone (**4**) and bavachalcone (**5**) at high dosage (100 μmol·L^{-1}, final concentration) were 53.13%, 53.08%, 15.06%, 9.03%, 8.9%, 6.56%, 6.53% and 3.61%, respectively. Meanwhile, isopsoralen (**2**), 8-methoxypsoralen (**3**) and psoralidin (**11**) displayed negligible effects on PL. To quantitatively evaluate the inhibition potency of these compounds on PL, the dose-inhibition curves of bakuchiol (**10**), psoralen (**1**), bavachin (**8**), corylin (**7**), bavachinin A (**9**), corylifol A (**6**), isobavachalcone (**4**) and bavachalcone (**5**) were plotted using a series of inhibitor concentrations. As shown in Fig. 3, these natural compounds inhibited PL activities in a dose-dependent manner. The IC_{50} values were determined as 108.3 ± 8.85, 84.18 ± 3.30, 29.55 ± 1.93, 27.15 ± 0.77, 17.15 ± 0.93, 7.81 ± 0.50, 5.73 ± 0.34 and 3.30 ± 0.24 μmol·L^{-1} for psoralen (**1**), bakuchiol (**10**), bavachin (**8**), corylin (**7**), bavachinin A (**9**), corylifol A (**6**), bavachalcone (**5**) and isobavachalcone (**4**), respectively (Table 1). Meanwhile, the IC_{50} value of the positive inhibitor (kaempferol) was 54.22 μmol·L^{-1}, which also determined under some conditions as mentioned above. These findings suggested that isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) displayed relatively strong inhibition on PL (IC_{50} values < 10 μmol·L^{-1}).

Inhibition kinetics of three PL inhibitors from Fructus Psoraleae

The above mentioned results encouraged us to further investigate the inhibitory behaviour of three constituents (**4**–**6**) from FP with strong PL inhibition activity. Before inhibition kinetic assays, time-dependent inhibition assays of three constituents on PL were performed to ascertain whether these enzymes are time-dependent inhibitors of PL. As shown in, isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) displayed similar inhibitory effects and closed IC_{50} values with different pre-incubation times, suggesting that these three natural compounds were reversible inhibitors of PL rather than irreversible inhibitors, which was much different from that of orlistat. Subsequently, a set of kinetic analyses were performed using varying concentrations of both the substrate and each PL inhibitor. As illustrated in Fig. 4, both inhibition kinetic plots and Lineweaver-Burk plots clearly demonstrated that isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) inhibited PL *via* a mixed manner. As listed in Table 1, the K_i values of isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) were evaluated as 1.61, 3.77 and 10.16 μmol·L^{-1}, respectively. These results demonstrated that isobavachalcone (**4**) and bavachalcone

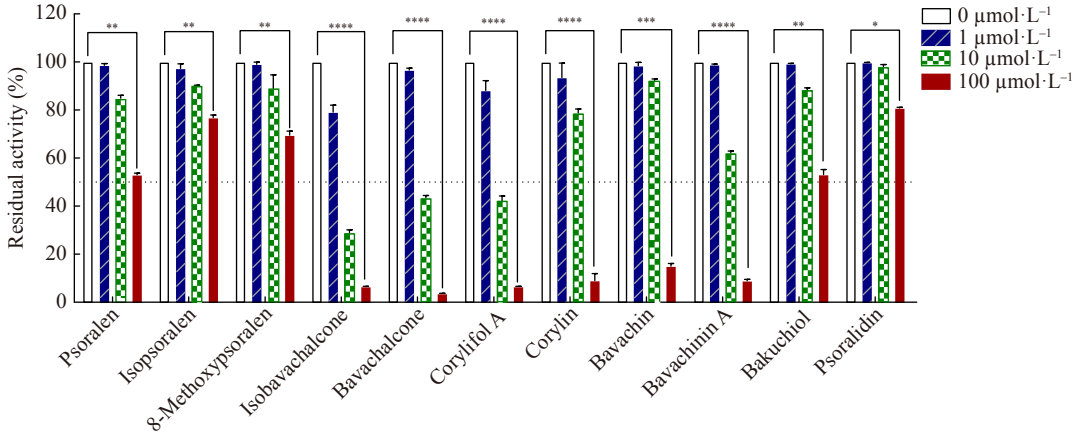

Fig. 2 The inhibitory effects of major constituents (0, 1, 10, 100 μmol·L^{-1}, final concentration) from *Fructus Psoraleae* on PL. All data were shown as mean ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ *vs* the control group (inhibitor concentration = 0 μmol·L^{-1}

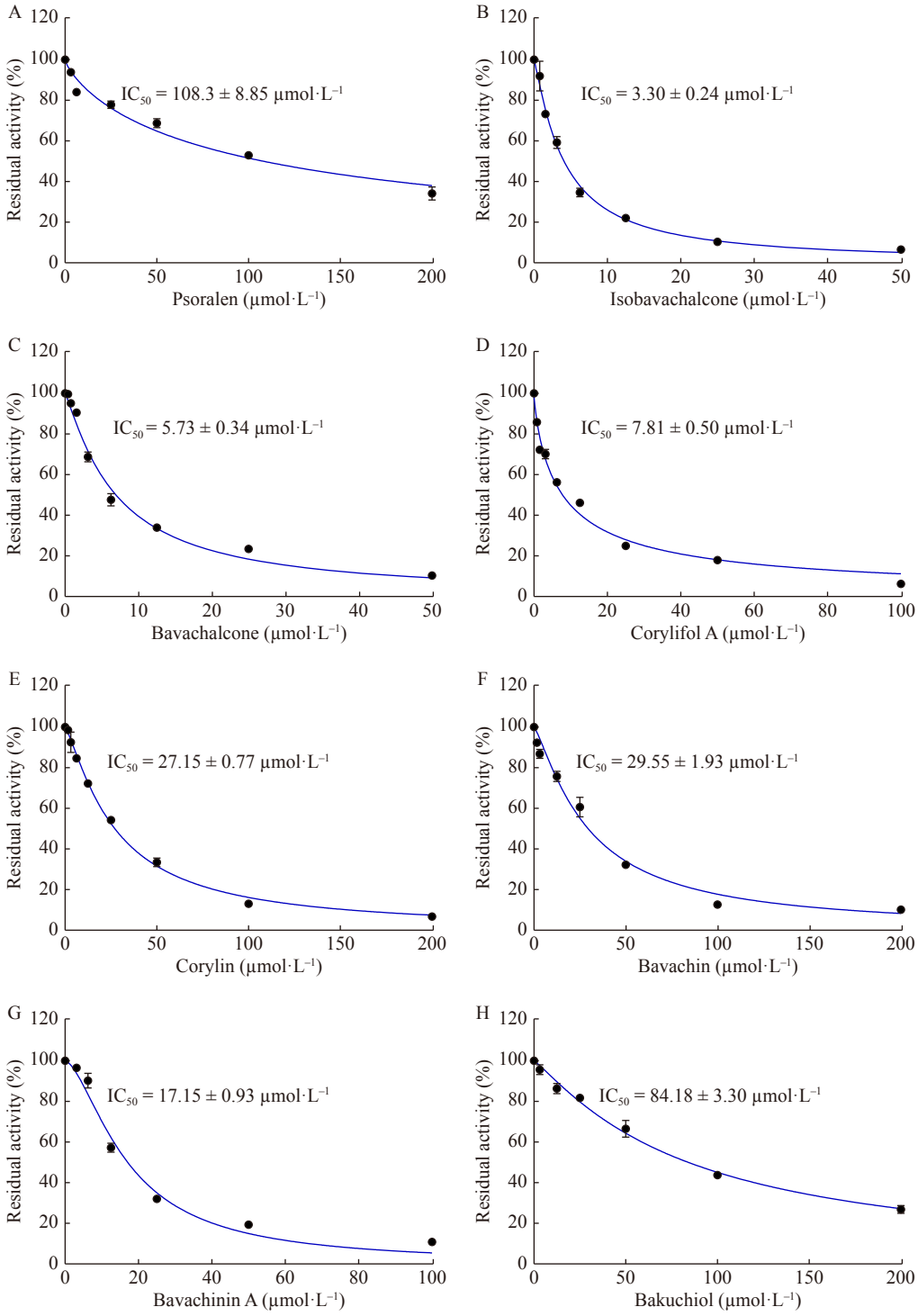

Fig. 3 The dose-inhibition curves of psoralen (A), isobavachalcone (B), bavachalconec (C), corylifol A (D), corylin (E), bavachin (F), bavachinin A (G) and bakuchiol (H). All data were shown as mean ± SD, $n = 3$

(**5**), two abundant natural chalcones isolated from *Fructus Psoraleae,* could potently inhibit the catalytic activities of PL *via* a mixed inhibition manner, with the K_i values of less than 3.8 μmol·L^{-1}.

Molecular docking simulations

For deeply understanding the interactions between PL and two newly identified chalcone-type PL inhibitors and PL, molecular docking simulations were conducted using a reported crystal structure of PL (PDB: 1ETH). As shown in Figs. 5A and 5B, both isobavachalcone (**4**) and bavachalcone (**5**) could be well-docked into the catalytic cavity of PL, within 4.3 angstrom to catalytic triad (Ser-153, Asp-177 and His-264). As shown in Figs. 5C and 5D, 2D representation analysis showed that bavachalcone (**5**) was able to form hydro-

Table 1 The IC_{50} values, K_i values and the inhibition modes of all tested natural constituents in *Fructus Psoraleae* on PL-mediated 4-MUO hydrolysis

Compounds	MW	IC_{50} (μmol·L^{-1})	K_i (μmol·L^{-1})	Inhibition mode	Goodness of fit (R^2)
Psoralen	186.16	108.3 ± 8.85	N.D.	–	–
Isopsoralen	186.16	> 100	N.D.	–	–
8-Methoxypsoralen	216.19	> 100	N.D.	–	–
Isobavachalcone	324.37	3.30 ± 0.24	1.61 ± 0.47	Mixed	0.97
Bavachalcone	324.37	5.73 ± 0.34	3.77 ± 0.89	Mixed	0.98
Corylifol A	390.47	7.81 ± 0.50	10.16 ± 1.91	Mixed	0.98
Corylin	320.35	27.15 ± 0.77	N.D.	–	–
Bavachin	324.37	29.55 ± 1.93	N.D.	–	–
Bavachinin A	338.40	17.15 ± 0.93	N.D.	–	–
Bakuchiol	256.38	84.18 ± 3.30	N.D.	–	–
Psoralidin	336.34	>100	N.D.	–	–
Kaempferol*	286.24	54.22	N.D.	–	–

* Kaempferol can used as a positive control

gen bonds with Asp-80, Ser-153 and His-264, while His-152, Phe-216 and Leu-265 could interact with bavachalcone *via* pi-cation, pi-pi and pi-sigma interactions, respectively. Similarly, isobavachalcone (**4**) could interact with Leu-265 *via* pi-sigma interactions and with Phe-216 *via* T-shaped pi-pi interactions, as well as with Phe-78 and Arg-257 *via* hydrogen bonding (Fig. 5). The binding affinity of isobavachalcone (4) and bavachalcone (5) on PL was listed in Table 2, and it was evident from Table 2 that both isobavachalcone (4) and bavachalcone (5) displayed similar binding affinity to PL.

Discussion

As one of the most popular herbal medicines, *Fructus Psoraleae* (the dried ripe fruits of *Psoralea corylifolia* L.) has been widely used in Asian countries for the prevention and treatment of a variety of disorders, such as asthma, diarrhea, and osteoporosis [24, 25]. Recent investigations have demonstrated that the crude extract of FP can strongly inhibit lipid accumulation and reduce serum lipid and hepatic triglyceride levels, suggesting that some natural constituents in this herb can regulate lipid metabolism [36]. Considering that *Fructus Psoraleae* are always oral administrated and the exposure of its constituents to the gastrointestinal system will be at a high level, the bioactive constituents in *Fructus Psoraleae* may interact with the key enzymes participating in lipid absorption and metabolism in the gastrointestinal system. In current study, the inhibition potentials of 11 major constituents isolated from *Fructus Psoraleae* towards PL, a key serine hydrolase in the gastrointestinal system that regulate lipid absorption in mammals, have been well-investigated for the first time. The major constituents in *Fructus Psoraleae* have been well-investigated, and several different classes of natural compounds (such as coumarins, chalcones, flavonoids and others) that bearing various scaffolds have been reported. In this study, our results clearly demonstrate that these constituents displays differential effects on PL. The chalcones (such as isobavachalcone and bavachalcone) exhibited strong inhibitory effects on PL (IC_{50} or K_i < 5 μmol·L^{-1}), the flavonoids (such as corylifol A, corylin, bavachin and bavachinin A) displayed moderate inhibition on PL (IC_{50} or K_i = 5–50 μmol·L^{-1}), while bakuchiol and the coumarins displayed weak inhibition on PL (IC_{50} or K_i > 50 μmol·L^{-1}). These finding suggest that the chalcones (including isobavachalcone and bavachalcone) are bioactive compounds with PL inhibitory effects.

The levels of the major constituents in *Fructus Psoraleae* have been accurately determined and reported by several groups [37-40]. Isobavachalcone (4), one of the most abundant constituents in *Fructus Psoraleae*, with the high content in this herb of 5.8 mg·g^{-1} dried material [41]. Notably, the recommended daily dose of *Fructus Psoraleae* in Chinese pharmacopoeia is 6–10 g for adult, which means that the daily dose of isobavachalcone (**4**) alone can up to 58 mg·d^{-1}. Considering that the total volume of the gastrointestinal system in human is about 4 L and the molecular weight of isobavachalcone (**4**) is 323.37 Da, the local exposure of this potent PL inhibitor to the human gastrointestinal tract could be up to 44.8 μmol·L^{-1}, which is much higher than the inhibition constant of isobavachalcone (**4**) towards PL (1.61 μmol·L^{-1}). Considering that other constituents in *Fructus Psoraleae* also display strong to moderate PL inhibition activity, it is easily conceivable that oral administration of *Fructus Psoraleae* or the herbal products containing *Fructus Psoraleae* are more likely to partially block the catalytic activities of PL in the gastrointestinal tract *in vivo*. It is worth noting that some constituents in FP (such as psoralen, isopsoralen and bakuchiol) have been reported with hepatotoxicity *via* targeting various off-targets [36, 42, 43], but the chalcones in FP (such as isobavachalcone and bavachalcone) displayed good safety profiles and beneficial effects for the treatment of a variety of disorders (such as osteoporosis and obesity) [44]. In future, it is

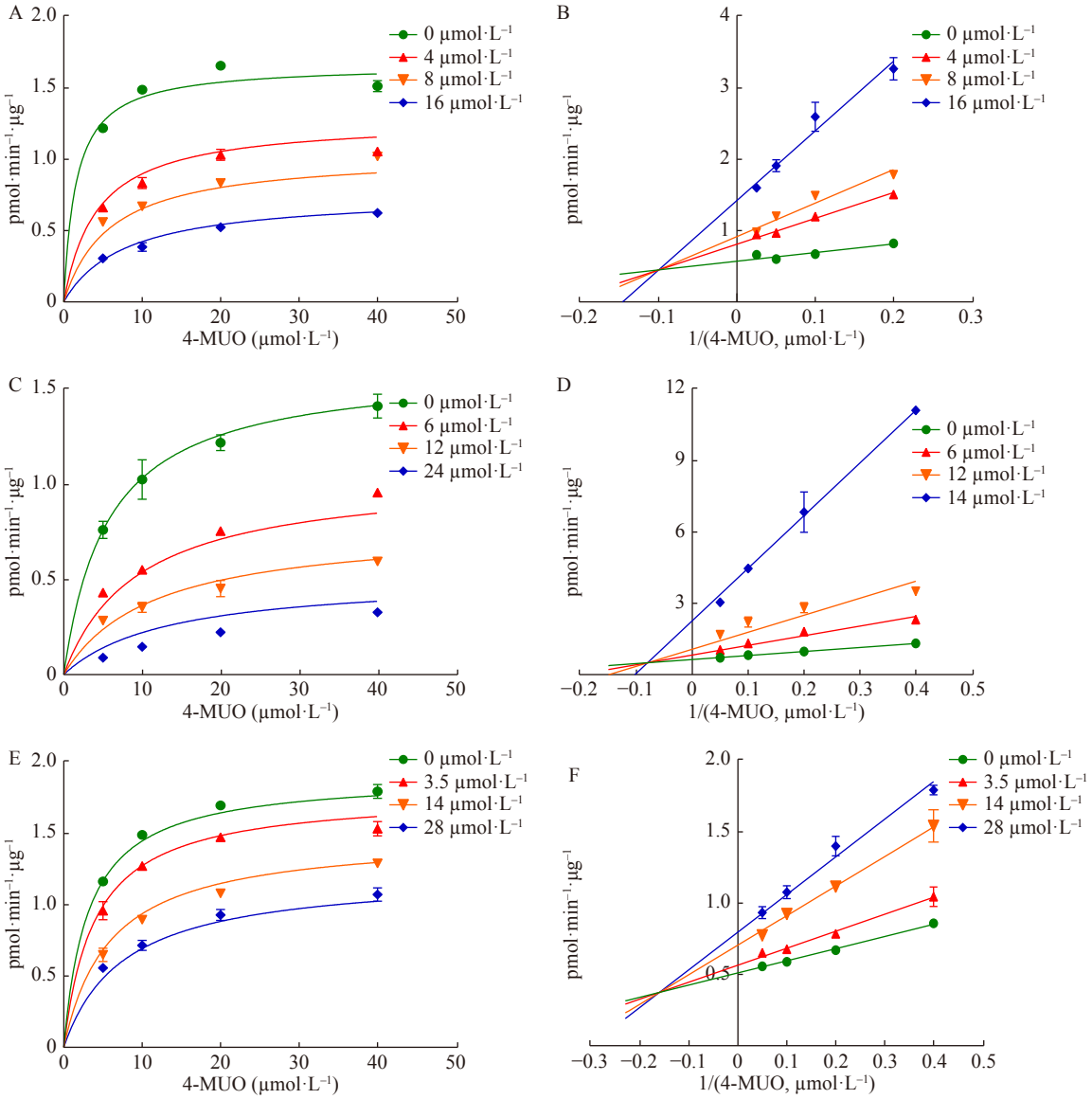

Fig. 4 The inhibition kinetics of three natural PL inhibitors from *Fructus Psoraleae*. Left: the inhibition kinetic plots of isobavachalcone (A), bavachalcone (C) and corylifol A (E) against PL-mediated 4-MUO hydrolysis. Right: the Lineweaver-Burk plots of isobavachalcone (B), bavachalcone (D) and corylifol A (F) against PL-mediated 4-MUO hydrolysis. All data were shown as mean ± SD

better to isolate the chalcones or get the fraction rich in these two chalcones from the crude extract of FP, which can be used to prepare a new drug or alternative medicines with good safety profiles and improved efficacy.

Although the results demonstrate that some constituents in *Fructus Psoraleae* (such as isobavachalcone and bavachalcone) can strongly inhibit PL, the PL inhibition activities of these natural chalcones are not good enough as orlistat, the marketed synthetic PL inhibitor [40]. Therefore, it is desirable to develop more efficacious PL inhibitors using these two newly identified natural chalcones as lead compounds. As mentioned above, the content of isobavachalcone (**4**) in *Fructus Psoraleae* is very high [37-40]. The medicinal chemists can easily get isobavachalcone from this herb and then to get a wide range of structurally diverse chalcone derivatives *via* chemical modifications. From the viewpoints of the chemical structure, isobavachalcone (**4**) contains several phenolic groups, which could be easily modified by the chemists. The chalcones could also be obtained *via* total synthesis [45], which strongly facilitates the medicinal chemists to design and synthesize structurally diverse derivatives for detailed structure-activity relationship studies. Notably, molecular docking simulations suggest that the C-4 phenolic group of both isobavachalcone (**4**) and bavachalcone (**5**) can strongly interact with the key residues of PL *via* hydrogen bonding, suggesting that the C-4 phenolic group is essential for PL inhibition. Hence, this key interaction can be conserved for the design and development of the next generation of PL inhibitors us-

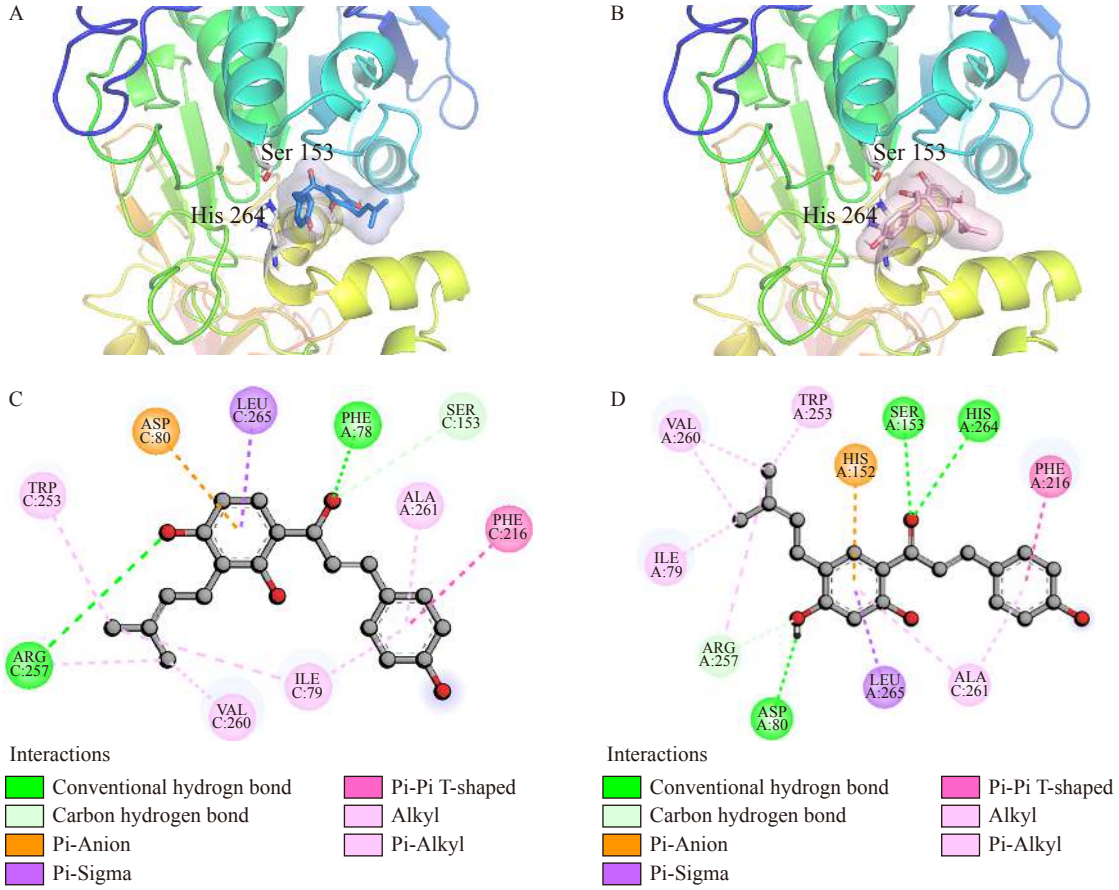

Fig. 5 A stereo view showed that isobavachalcone (A) and bavachalcone (B) could tightly bind on the catalytic cavity of PL. 2D representation of the interactions between isobavachalconec (C)/bavachalcone (D) and the residuals in the active site of pancreatic lipase

Table 2 The Affinity energy, X-Score and key interactions (H-bonds) of isobavachalcone (4) and bavachalcone (5) on the catalytic cavity of PL

Ligand	Affinity energy (kcal·mol^{-1})	X-Score	Key interactions (H-bonding)
Isobavachalcone	−9.4	6.89	Phe 78, Arg 257
Bavachalcone	−9.9	6.73	Ser 153, Asp 80, His 264

ing chalcone as the scaffold. Additionally, considering that most natural chalcones can be readily metabolized by hepatic drug metabolizing enzyme [46-48], some novel strategies should be used to improve the dissolution and/or the local exposure of these chalcones to the gastrointestinal system, which will be very helpful for enhancing the PL inhibition effects of these chalcones *in vivo*.

Conclusion

In summary, the inhibition potentials of major constituents in *Fructus Psoraleae* against PL, a crucial digestive enzyme controlling lipid metabolism in mammals, have been carefully evaluated and characterized. Among all tested compounds, isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) displayed strong PL inhibition activity (IC_{50} values < 10 μmol·L^{-1}). Inhibition kinetic analyses demonstrated that isobavachalcone (**4**), bavachalcone (**5**) and corylifol A (**6**) were mixed inhibitors against PL-mediated 4-MUO hydrolysis, with the K_i values of 1.61, 3.77 and 10.16 μmol·L^{-1}, respectively. Finally, docking simulations demonstrated that isobavachalcone (**4**) and bavachalcone (**5**) could interact with key residues located in the catalytic cavity of PL *via* hydrophobic interactions and hydrogen binding. Collectively, these results provided new evidence to support that *Fructus Psoraleae* contained bioactive compounds with lipid-lowering effects *via* targeting PL, and also suggested that the natural chalcones in *Fructus Psoraleae* could be used as ideal lead compounds to develop novel PL inhibitors.

References

[1] Qasim A, Turcotte M, De Souza RJ, *et al*. On the origin of obesity: identifying the biological, environmental and cultural drivers of genetic risk among human populations [J]. *Obes Rev*, 2018, **19**(2): 121–149.

[2] Jensen MD, Ryan DH, Apovian CM, *et al*. 2013

AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American college of cardiology/American heart association task force on practice guidelines and the obesity society [J]. *J Am Coll Cardiol*, 2014, **63**(25 Pt B): 2985–3025.

[3] Saunders KH, Umashanker D, Igel LI, *et al*. Obesity pharmacotherapy [J]. *Med Clin North Am*, 2018, **102**(1): 135–148.

[4] Haslam DW, James WP. Obesity [J]. *Lancet*, 2005, **366**(9492): 1197–1209.

[5] Poulain M, Doucet M, Major GC, *et al*. The effect of obesity on chronic respiratory diseases: pathophysiology and therapeutic strategies [J]. *Can Med Assoc J*, 2006, **174**(9): 1293–1299.

[6] Antonopoulos AS, Oikonomou EK, Antoniades C, *et al*. From the BMI paradox to the obesity paradox: the obesity-mortality association in coronary heart disease [J]. *Obes Rev*, 2016, **17**(10): 989–1000.

[7] Mukherjee M. Human digestive and metabolic lipases—a brief review [J]. *J Mol Catal B Enzym*, 2003, **22**(5-6): 369–376.

[8] Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential [J]. *Drug Discov Today*, 2007, **12**(19-20): 879–889.

[9] Weibel EK, Hadvary P, Hochuli E, *et al*. Lipstatin, an inhibitor of pancreatic lipase, produced by *Streptomyces toxytricini*. I. Producing organism, fermentation, isolation and biological activity [J]. *J Antibiot*, 1987, **40**(8): 1081–1085.

[10] De La Garza AL, Milagro FI, Boque N, *et al*. Natural inhibitors of pancreatic lipase as new players in obesity treatment [J]. *Planta Med*, 2011, **77**(8): 773–785.

[11] Chaput JP, St-Pierre S, Tremblay A. Currently available drugs for the treatment of obesity: Sibutramine and orlistat [J]. *Mini Rev Med Chem*, 2007, **7**(1): 3–10.

[12] Cheung BM, Cheung TT, Samaranayake NR. Safety of antiobesity drugs [J]. *Ther Adv Drug Saf*, 2013, **4**(4): 171–181.

[13] Lunagariya NA, Patel NK, Jagtap SC, *et al*. Inhibitors of pancreatic lipase: state of the art and clinical perspectives [J]. *EXCLI J*, 2014, **13**: 897–921.

[14] Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014 [J]. *J Nat Prod*, 2016, **79**(3): 629–661.

[15] Wang DD, Zou LW, Jin Q, *et al*. Recent progress in the discovery of natural inhibitors against human carboxylesterases [J]. *Fitoterapia*, 2017, **117**: 84–95.

[16] Hou XD, Ge GB, Weng ZM, *et al*. Natural constituents from Cortex Mori Radicis as new pancreatic lipase inhibitors [J]. *Bioorg Chem*, 2018, **80**: 577–584.

[17] Liu PK, Weng ZM, Ge GB, *et al*. Biflavones from *Ginkgo biloba* as novel pancreatic lipase inhibitors: Inhibition potentials and mechanism [J]. *Int J Biol Macromol*, 2018, **118**(Pt B): 2216–2223. DOI: 10.1016/j.ijbiomac.2018.07.085.

[18] Buchholz T, Melzig MF. Polyphenolic compounds as pancreatic lipase inhibitors [J]. *Planta Med*, 2015, **81**(10): 771–783.

[19] Rahim A, Takahashi Y, Yamaki K. Mode of pancreatic lipase inhibition activity *in vitro* by some flavonoids and non-flavonoid polyphenols [J]. *Food Res Int*, 2015, **75**: 289–294.

[20] Gutierrez-Grijalva EP, Antunes-Ricardo M, Acosta-Estrada BA, *et al*. Cellular antioxidant activity and *in vitro* inhibition of alpha-glucosidase, alpha-amylase and pancreatic lipase of oregano polyphenols under simulated gastrointestinal digestion [J]. *Food Res Int*, 2019, **116**: 676–686.

[21] Yuan Y, Zhang J, Fan J, *et al*. Microwave assisted extraction of phenolic compounds from four economic brown macroalgae species and evaluation of their antioxidant activities and inhibitory effects on alpha-amylase, alpha-glucosidase, pancreatic lipase and tyrosinase [J]. *Food Res Int*, 2018, **113**: 288–297.

[22] Chen CH, Hwang TL, Chen LC, *et al*. Isoflavones and anti-inflammatory constituents from the fruits of Psoralea corylifolia [J]. *Phytochemistry*, 2017, **143**: 186–193.

[23] Kim S, Lee S, Lee H, *et al*. Evaluation on antimicrobial activity of psoraleae semen extract controlling the growth of gram-positive bacteria [J]. *Korean J Food Sci Anim Resour*, 2017, **37**(4): 502–510.

[24] Jin H, Wang L, Xu C, *et al*. Effects of *Psoraleae fructus* and its major component psoralen on Th2 response in allergic asthma [J]. *Am J Chin Med*, 2014, **42**(3): 665–678.

[25] Li WD, Yan CP, Wu Y, *et al*. Osteoblasts proliferation and differentiation stimulating activities of the main components of *Fructus psoraleae* corylifoliae [J]. *Phytomedicine*, 2014, **21**(4): 400–405.

[26] Wu CR, Chang CL, Hsieh PY, *et al*. Psoralen and isopsoralen, two coumarins of *Psoraleae fructus*, can alleviate scopolamine-induced amnesia in rats [J]. *Planta Med*, 2007, **73**(3): 275–278.

[27] Chopra B, Dhingra AK, Dhar KL. Psoralea corylifolia L. (Buguchi)-folklore to modern evidence: review [J]. *Fitoterapia*, 2013, **90**: 44–56.

[28] Sun DX, Ge GB, Dong PP, *et al*. Inhibition behavior of fructus psoraleae 's ingredients towards human carboxylesterase 1 (hCES1) [J]. *Xenobiotica*, 2016, **46**(6): 503–510.

[29] Li YG, Hou J, Li SY, *et al*. Fructus Psoraleae contains natural compounds with potent inhibitory effects towards human carboxylesterase 2 [J]. *Fitoterapia*, 2015, **101**: 99–106.

[30] Wang D, Zou L, Jin Q, *et al*. Human carboxylesterases: a comprehensive review [J]. *Acta Pharm Sin B*, 2018, **8**(5): 699–712.

[31] Weng ZM, Ge GB, Dou TY, *et al*. Characterization and structure-activity relationship studies of flavonoids as inhibitors against human carboxylesterase 2 [J]. *Bioorg Chem*, 2018, **77**: 320–329.

[32] Zou LW, Li YG, Wang P, *et al*. Design, synthesis, and structure-activity relationship study of glycyrrhetinic acid derivatives as potent and selective inhibitors against human carboxylesterase 2 [J]. *Eur J Med Chem*, 2016, **112**: 280–288.

[33] Cheng X, Lv X, Qu H, *et al*. Comparison of the inhibition potentials of icotinib and erlotinib against human UDP-glucuronosyltransferase 1A1 [J]. *Acta Pharm Sin B*, 2017, **7**(6): 657–664.

[34] Trott O, Olson AJ. Software news and update AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading [J]. *J Comput Chem*, 2010, **31**(2): 455–461.

[35] Wang R, Lai L, Wang S. Further development and validation of empirical scoring functions for structure-based binding affinity prediction [J]. *J Comput Aided Mol Des*, 2002, **16**(1): 11–26.

[36] Li ZJ, Abulizi A, Zhao GL, *et al*. Bakuchiol contributes to the hepatotoxicity of *Psoralea corylifolia* in Rats [J]. *Phytother Res*, 2017, **31**(8): 1265–1272.

[37] Qiao CF, Han QB, Song JZ, *et al*. Chemical fingerprint and quantitative analysis of fructus psoraleae by high-performance liquid chromatography [J]. *J Sep Sci*, 2007, **30**(6): 813–818.

[38] Zhang Y, Chen Z, Xu X, *et al*. Rapid separation and simultaneous quantitative determination of 13 constituents in *Psoraleae fructus* by a single marker using high-performance liquid chromatography with diode array detection [J]. *J Sep Sci*, 2017, **40**(21): 4191–4202.

[39] Li Y, Wang F, Chen Z. Determination of bavachin and isobavachalcone in *Fructus psoraleae* by high-performance liquid chromatography with electrochemical detection [J]. *J Sep Sci*, 2011, **34**(5): 514–519.

[40] Luan L, Shen X, Liu X, *et al*. Qualitative analysis of Psoraleae Fructus by HPLC-DAD/TOF-MS fingerprint and quantitative analysis of multiple components by single marker [J]. *Biomed Chromatogr*, 2018, **32**(2).

[41] Bialecka-Florjanczyk E, Fabiszewska AU, Krzyczkowska J, *et*

al. Synthetic and natural lipase inhibitors [J]. *Mini Rev Med Chem*, 2018, **18**(8): 672–683.
[42] Wang Y, Zhang H, Jiang JM, *et al*. Hepatotoxicity induced by psoralen and isopsoralen from *Fructus psoraleae*: Wistar rats are more vulnerable than ICR mice [J]. *Food Chem Toxicol*, 2019, **125**: 133–140.
[43] Li A, Gao M, Zhao N, *et al*. Acute liver failure associated with *Fructus psoraleae*: a case report and literature review [J]. *BMC Complement Altern Med*, 2019, **19**(1): 84.
[44] Park CK, Lee Y, Chang EJ, *et al*. Bavachalcone inhibits osteoclast differentiation through suppression of NFATc1 induction by RANKL [J]. *Biochem Pharmacol*, 2008, **75**(11): 2175–2182.
[45] Mohamed MFA, Shaykoon MSA, Abdelrahman MH, *et al*. Design, synthesis, docking studies and biological evaluation of novel chalcone derivatives as potential histone deacetylase inhibitors [J]. *Bioorg Chem*, 2017, **72**: 32–41.
[46] Huang L, Nikolic D, Van Breemen RB. Hepatic metabolism of licochalcone A, a potential chemopreventive chalcone from licorice (*Glycyrrhiza inflata*), determined using liquid chromatography-tandem mass spectrometry [J]. *Anal Bioanal Chem*, 2017, **409**(30): 6937–6948.
[47] Zhou QH, Zhu YD, Zhang F, *et al*. Interactions of drug-metabolizing enzymes with the Chinese herb *Psoraleae fructus* [J]. *Chin J Nat Med*, 2019, **17**(11): 858–870. DOI: 10.1016/S1875-5364(19)30103-7 .
[48] Ge GB. Deciphering the metabolic fates of herbal constituents and the interactions of herbs with human metabolic system [J]. *Chin J Nat Med*, 2019, **17**(11): 801–802 DOI: 10.1016/S1875-5364(19)30098-6.

Cite this article as: HOU Xu-Dong, SONG Li-Lin, CAO Yun-Feng, WANG Yi-Nan, ZHOU Qi, FANG Sheng-Quan, WU Da-Chang, ZANG Shi-Zhu, CHEN Lu, BAI Yue, GE Guang-Bo, HOU Jie. Pancreatic lipase inhibitory constituents from Fructus Psoraleae [J]. *Chin J Nat Med*, 2020, **18**(5): 369-378.

GE Guang-Bo (Dr. & Prof.),Vice Dean of Institute of Interdisciplinary Medicine, Shanghai University of Traditional Chinese Medicine.Dr. Ge received his Ph.D degree in biochemical engineering in 2009 from Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS). After eight-year working at Dalian Institute of Chemical Physics, Dr. Ge joined in Shanghai University of Traditional Chinese Medicine as a full professor and vice dean of Institute of Interdisciplinary Medicine in 2017. His current studies are focused on drug metabolism and the interactions between drug metabolizing enzymes and small molecule ligands. Dr. Ge has developed more than ten novel optical substrates for enzymes, part of them have been used for drug discovery (such as enzyme inhibitors/modulators), drug/herb-drug interactions and other biomedical fields. Until now, he has found more than thirty potent or specific enzyme inhibitors from natural pharmaceutical resources. Now he is the author/coauthor of more than 180 publications in scientific journals, with the H-index of 33. His representative achievements have been published in the top journals such as J Am Chem Soc, Angew Chem Int Ed, Chem Sci, Biosensors & Bioelectronics, ACS Applied Materials & Interfaces, J Med Chem, Anal Chem, Chem Commun, Sens Actuator B-Chem, Anal Chim Acta, Chem Eur J, Eur J Med Chem.

HOU Jie (Dr. & Asso. Prof.),College of Basic Medical Sciences, Dalian Medical University. Dr. Hou received her Ph.D degree in applied chemistry in 2008 from Dalian University of Technology (DLUT). After graduation from DLUT, she worked as a lecturer in College of Pharmacy, Nankai University (NKU) for 4 years (2008-2012). In 2012, Dr Hou joined into Dalian Medical University as an associated professor. Her current researches are focused on the herbal regulation effects on activities of enzymes in gastrointestinal tract, and the relationship between the herbal medicine and enzymes related in glycolipid metabolisms. Until now, she has disclosed the active constituents in more than 10 kinds of herbal medicines had potential inhibition effects on key enzymes, such as pancreatic lipase, glucosidase, glucuronidase and et al. Now she is the author/coauthor of more than 70 publications in scientific journals. His representative achievements have been published in the top journals such as Int J Biol Macromol, Bioorg Chem, Biosensors & Bioelectronics, Food Chem Toxicol.