SHORT COMMUNICATION

Cryptotanshinone and tanshinone IIA from *Salvia miltiorrhiza* Bunge (Danshen) as a new class of potential pancreatic lipase inhibitors

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**ABSTRACT**

*Salvia miltiorrhiza* Bunge extract was investigated for the first time for its inhibitory activity against pancreatic lipase (PL), an important enzyme involved in the digestion of dietary fats. It showed a concentration-dependent activity with an IC\(_{50}\) value of 3.54 ± 0.22 mg/mL. Two compounds, cryptotanshinone and tanshinone IIA (the major lipophilic constituents of *S. miltiorrhiza*), have been selected as potential ligands of PL. Cryptotanshinone showed a higher lipase inhibitory activity (IC\(_{50}\) = 6.86 ± 0.43 μM) compared to the parent tanshinone IIA. Molecular docking studies were undertaken to establish whether a direct interaction of the principal constituents of the *S. miltiorrhiza* extract with the human pancreatic lipase could be evoked. All these findings provided new insights into the understanding of the interactions between natural constituents of *S. miltiorrhiza* extract and PL, also suggesting that cryptotanshinone could be used as lead compound for the development of efficacious PL inhibitors.

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1. Introduction

Obesity is characterized by an exceeding adipose biomass and adipose tissue expansion, so reducing the absorption of carbohydrates and lipids in the digestive system using anti-obesity drugs, has been considered as an effective therapeutic approach (Apovian 2016). An important enzyme involved in the digestion of dietary fats is pancreatic lipase (PL), a triacylglycerol acyl hydrolase, that is responsible for the hydrolysis of almost 60% triacylglycerides in the gastrointestinal tract (Wang et al. 2013). Natural products have been considered a source of safe and promising anti-obesity drugs (Marrelli et al. 2016). Salvia miltiorrhiza Bunge (Danshen) belongs to the Lamiaceae family. It has been widely used in Asian countries for treating different diseases and early pharmacological studies demonstrated its multiple bioactivities: cardiovascular and cerebrovascular effects as well as antioxidant, anti-inflammatory and anti-tumor properties (Jianping et al. 2018). These activities are due to the presence of lipophilic tanshinones (Li et al. 2018). In the present study S. miltiorrhiza extract and the major lipophilic compounds, cryptotanshinone and tanshinone IIA, were investigated for the first time as lipase inhibitors. Molecular docking studies on the crystallographic structures of pancreatic lipase have been carried out to investigate the binding mode of the compounds with the enzyme binding site.

2. Results and discussion

The lyophilized extract of S. miltiorrhiza (E.S. 1% tanshinones) showed a lipase inhibitory activity with an IC₅₀ value of 3.54 ± 0.22 mg/ml (Figure S1, Table S1). The potential contribution of tanshinone IIA (1) and cryptotanshinone (2) (Figure S2) was evaluated. Pancreatic lipase activity was significantly affected by 1 at the three highest concentration tested (*** p < 0.001, Dunnett’s multiple comparison test) (Figure 1). Compound 2 effectively inhibited pancreatic lipase activity at all the concentrations tested, with an IC₅₀ value of 6.86 ± 0.43 μM (Table S1). Even if there was a significant difference in the inhibition of lipase activity by the compounds, the effect was not as pronounced as with 1. The inhibition was observed at lower concentrations for 2, indicating a higher potency.

Figure 1. Pancreatic lipase inhibition induced by cryptotanshinone and tanshinone IIA. Data were expressed as means ± S. E. (n = 3). Mean values of samples showing significant difference from the control was denoted with * P < 0.05, ** P < 0.01, *** P < 0.001 in one-way ANOVA followed by Dunnett’s test.
between the biological activity of this compound and the positive control orlistat ($IC_{50}$ value = $0.036 \pm 0.002 \mu M$, *** $P < 0.001$, t-test), the biological activity of cryptotanshinone was significantly higher than those of rutin, chosen as natural reference compound ($IC_{50}$ value = $163.00 \pm 7.24 \mu M$, *** $P < 0.001$, t-test). Various pharmacological properties have been ascribed to cryptotanshinone in particular it is able to inhibit in a concentration dependent manner the rat platelet aggregation (Maione et al. 2015). Our data are in accordance with previous studies that showed that cryptotanshinone is able to reduce the concentration of body fat, serum cholesterol and triglyceride levels in mice (Kim et al. 2007). Subsequent studies also showed that cryptotanshinone can effectively inhibit adipogenesis (Rahman et al. 2016). In order to verify whether the inhibitory activity of $S. miltiorrhiza$ extract could be attributed to the direct interaction of 1 and 2 with the enzyme, molecular docking was performed by using a pancreatic lipase structure withdrawn from the Protein Data Bank (PDB). Different pancreatic lipase crystallographic structures have been deposited to the PDB, the most recent 1LPB entry was adopted. In this structure pancreatic lipase is complexed with two molecules of a C11 alkyl phosphonate inhibitor MUP. The crystallographic structure of pancreatic lipase includes three structural domains: a N-terminal domain containing the active site characterized by a Ser152-Asp176-Hys263 catalytic triad, a non-catalytic C-terminus surrounding a colipase binding site, and a lid-loop modulating the ligand entry into the active site. During docking experiments, the agliconic portion of Rutin (3) (Figure S2) was used as an anti-pancreatic lipase reference compound. Docking experiments showed that compounds 1–3 were able to interact with the enzyme accommodating in the same binding site of the crystallographic ligand, therefore they should be able to prevent the access of the natural ligand to the catalytic site. All the three molecules were find able to bind the protein active domain with comparable affinities ranging from $-9.9$ to $-11.1$ kcal/mol (Table S2). Furthermore, as easily hypothesized, compounds 1 and 2 interact with the catalytic core in a very similar way (Figure 2) since the structural differences present on the furan moiety do not significantly influence the overall interaction of ligands with the enzyme. In particular, the interaction with PL of both compounds 1 and 2 is made favorable by two H-bonds: O atoms at position 10 and 11, acting as H-acceptors, interact with the side chain of His263 and Ser152, respectively. Moreover, several

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Figure 2. Ligand-binding pocket of the active site of HPL; protein structural elements are represented as ribbons. (a) Superimposed binding modes of the agliconic portion of Rutin, 3 (yellow); Tanshinone IIA, 1 (cyan); Cryptotanshinone, 2 (magenta). The key residues are also indicated in the specific binding mode of (b) 1 and (c) 2.
hydrophobic interactions contribute to the complex stability. Here, for the first time, we have investigated the inhibitory effects of *S. miltiorrhiza* extract and its reference compounds, tanshinone IIA and cryptotanshinone, on pancreatic lipase. The results showed that the extract possesses moderate inhibitory effects on pancreatic lipase. Molecular docking simulations demonstrated that selected compounds were able to interact with the catalytic site of pancreatic lipase and can act as valuable enzyme ligands. All these findings provided new insights into the deep understanding of the interactions between natural constituents in *S. miltiorrhiza* extract and pancreatic lipase also suggesting that cryptotanshinone (2) could represent a suitable lead compound for the development of new pancreatic lipase inhibitors useful for the management of obesity and related disorders.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Apovian CM. 2016. Obesity: definition, comorbidities, causes, and burden. Am J Manag Care. 22(7 Suppl):s176–s185.
Jianping X, Kunhua W, Guojun Z, Lujing L, Dawei Y, Wenle W, Qiheng H, Yuan X, Yaqiong B, Min Y, Minhui L. 2018. Ethnopharmacology, phytochemistry, and pharmacology of Chinese *Salvia* species: A review. J Ethnopharmacol. 225:18–30.
Kim EJ, Jung SN, Son KH, Kim SR, Ha TY, Park MG, Jo IG, Park JG, Choe W, Kim SS, Ha J. 2007. Antidiabetes and antiobesity effect of cryptotanshinone via activation of AMP-activated protein kinase. Mol Pharmacol. 72(1):6272.
Li ZM, Xu SW, Liu PQ. 2018. *Salvia miltiorrhiza* Bunge (Danshen): A golden herbal medicine in cardiovascular therapeutics. Acta Pharmacol Sin. 39(5):802–824.
Maione F, Cantone V, Chini MG, De Feo V, Mascolo N, Bifulco G. 2015. Molecular mechanism of tanshinone IIA and cryptotanshinone in platelet anti-aggregating effects: an integrated study of pharmacology and computational analysis. Fitoterapia. 100:174–178.
Marrelli M, Conforti F, Araniti F, Statti GA. 2016. Effects of saponins on lipid metabolism: a review of potential health benefits in the treatment of obesity. Molecules. 21(10):1404.
Rahman N, Jeon M, Song HY, Kim YS. 2016. Cryptotanshinone, a compound of *Salvia miltiorrhiza* inhibits pre-adipocytes differentiation by regulation of adipogenesis-related genes expression via STAT3 signaling. Phytomedicine. 23(1):58–67.
Wang TY, Liu M, Portincasa P, Wang DQ. 2013. New insights into the molecular mechanism of intestinal fatty acid absorption. Eur J Clin Invest. 43(11):1203–1223.