Fitoterapia 101 (2015) 99–106

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

# *Fructus Psoraleae* contains natural compounds with potent inhibitory effects towards human carboxylesterase 2

Yao-Guang Li [a,b,1], Jie Hou [c,1], Shi-Yang Li [a], Xia Lv [a], Jing Ning [a,c], Ping Wang [a], Zhao-Ming Liu [a], Guang-Bo Ge [a,*], Jia-Yan Ren [b,*], Ling Yang [a,*]

[a] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[b] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu 030801, China
[c] Dalian Medical University, Dalian 116044, China

ARTICLE INFO

*Article history:*
Received 27 November 2014
Accepted in revised form 1 January 2015
Accepted 6 January 2015
Available online 13 January 2015

*Keywords:*
*Fructus Psoraleae*
Human carboxylesterase 2
Inhibitory effects
LC-DAD-ESI-MS/MS

ABSTRACT

*Fructus Psoraleae* (FP) is an edible Chinese herbal which is widely used in Asia for the treatment of various diseases including asthma, diarrhea, and osteoporosis. This study aimed to investigate the inhibitory effects of the crude ethanol extract from FP on human carboxylesterase 2 (hCE2), as well as to identity and characterize the naturally occurring inhibitors of hCE2 in FP. Our results demonstrated that the ethanol extract of FP displayed potent inhibitory effects towards hCE2, while five major bioactive constitutes in FP were efficiently identified by LC-DAD-ESI-MS/MS, with the aid of LC-based activity profiling. The identified bioactive compounds including neobavaisoflavone, isobavachalcone, bavachinin, corylifol A and bakuchiol were found to be naturally occurring potent inhibitors of hCE2, with low Ki values ranging from 0.62 μM to 3.89 μM. This is the first report of the chemical constitutes in FP as potent inhibitors of hCE2.



## 1. Introduction

Carboxylesterases (CEs, E.C.3.1.1.1), a well conserved multigene family of α/β-hydrolase fold enzymes, is widely distributed in numerous animal species and a variety of mammalian tissues. CEs catalyze the hydrolysis of a vast number of structurally diverse endogenous and exogenous substrates including fatty acid esters, environmental toxins and ester-containing drugs [1,2]. As an important class of phase I xenobiotic-metabolizing enzymes in mammals, CEs efficiently catalyze the cleavage of the ester bond in various carboxylic esters, releasing the corresponding alcohol and carboxylic acid fragments which are more polar and readily excreted than the parent ester [3]. Therefore, mammalian CEs are considered as important mediators for detoxification or metabolic activation of various ester drugs, environmental toxicants and carcinogens [4,5].

Human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2) are two major CEs distributed in human body, which are differentiated on the basis of tissue distribution, substrate specificity, immunological properties, and gene regulation [6]. hCE1 is primarily expressed in liver, with lesser amounts in intestine, kidney, lung, testes, heart, monocytes and macrophages [7], while hCE2 is primarily expressed in gastrointestinal tract but at relatively lower levels in liver [8,9]. The substrate specificities of hCE1 and hCE2 are also different. Generally, hCE1 preferentially hydrolyzes substrate with a small alcohol group and large acyl group [4]. In sharp contrast, hCE2 preferentially catalyzes substrates with a large alcohol group and small acyl group [3,10,11].

As the major carboxylesterase distributed in human intestine and tumor tissues, hCE2 is considered to play the most important role on the bioavailability of oral prodrugs, and the treatment outcomes of ester anti-cancer agents [7,8]. Some

* Corresponding authors. Tel.: +86 411 84379317; fax: +86 411 84676961.
*E-mail addresses:* geguangbo@dicp.ac.cn (G.-B. Ge), Renjiay@126.com (J.-Y. Ren), ylingdicp@gamil.com (L. Yang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fitote.2015.01.004
0367-326X/

intravenously administered prodrugs, for example, the anti-cancer agent CPT-11 (irinotecan), can be secreted into intestinal tracts through bile, but its toxic hydrolytic metabolite can be released rapidly after hydrolysis by hCE2 [12,13]. The accumulation of SN-38 in intestine has been recognized as the main reason which caused severe delayed-onset diarrhea, one of the most common side effects of CPT-11 [12]. Co-administration with potent hCE2 inhibitors can reduce the occurrence of CPT-11 induced life-threatening diarrhea in patients, and then improve the quality of life for the patient [14–16]. Therefore, it is urgently desirable to find potent inhibitors of hCE2 for alleviating the delayed-onset diarrhea induced by CPT-11.

In recently years, discovery of the selective and potent inhibitors of hCE2 from herbals for translational applications has attracted increasing attention, due to most of herbals display satisfying safety during long history of use for medical treatments [17–19]. These studies motivated us to find more potent hCE2 inhibitors from Chinese traditional herbals. In the past two years, a high-throughput screening campaign was carried out by us to find potent inhibitors of hCE2 from herbals, by using Fluorescein Diacetate (FD) as the fluorescent probe substrate for hCE2 [20]. More than one hundred edible herbals were extracted by 95% ethanol and then their inhibition activities towards hCE2 were screened. Among these herbal extracts, the ethanolic extract of *Fructus Psoraleae* (Bu-gu-zhi) exhibited the most potent inhibitory effect towards hCE2 (Table S1). *Fructus Psoraleae* (FP) is the dried ripe seeds of *Psoralea corylifolia L* (*Fabaceae*). As an edible Chinese herb, FP has been widely used in Asia, for alleviation of asthma and diarrhea, treatment of osteoporosis, osteomalacia, bone fracture and some skin diseases [21–25]. Many pharmacological studies around the world have clearly shown that FP and its major constitutes display multiple pharmacological activities including antioxidation, anticancer and anti-inflammatory [26–29]. However, the inhibitory effects of the crude extract from FP and its chemical constitutes on human carboxylesterase 2 (hCE2) have not been investigated. This study aimed to identify the major bioactive compounds in *Fructus Psoraleae* displaying potent inhibitory effects towards hCE2, as well as to characterize their potency for hCE2 inhibition. To this end, ultra-fast liquid chromatography (UFLC)-UV fingerprinting and the residual activity based screening were used to find naturally occurring hCE2 inhibitors in FP, and then the inhibitory effects of these bioactive compounds towards hCE2 were well-characterized by using FD assay.

## 2. Material and methods

### 2.1. Chemicals and reagents

Fluorescein diacetate (FD), fluorescein and Bis-p-nitrophenyl phosphate (BNPP) were purchased from TCI (Tokyo Japan). 2-(2-Benzoyl-3-methoxyphenyl) benzothiazole (BMBT) was synthesized from 2-(2-hydroxy-3-methoxyphenyl) benzothiazole (HMBT) as previously reported [30] its structure was fully characterized and was fully confirmed by $^{1}$H-NMR, $^{13}$C-NMR and high resolution mass spectrometry. Stock solutions of FD (1 mM) and BMBT (1 mM) were prepared in DMSO and stored at −20 °C until use. The authentic standards including neobavaisoflavone, isobavachalcone, bavachinin, corylifol A, and bakuchiol were purchased from Chengdu Pufei De Biotech Co., Ltd. (Chengdu, Sichuan, China). The purity of each standard compound was determined by HPLC-UV and their purities were over 98%. Phosphate buffer (pH 7.4) was prepared by using Millipore water and stored at 4 °C until use. Millipore water (Millipore, Bedford, USA), HPLC grade acetonitrile, methanol and formic acid (Tedia company, USA) were used throughout. The pooled human liver microsomes (HLM) from 50 donors were obtained from Celsis, Inc. (USA), and stored at −80 °C until use.

### 2.2. Plant material and extraction

The dried ripe seeds of *P. corylifolia L.* (Bu-gu-zhi) and other Chinese herbals were purchased from one pharmacy store in Dalian (Liaoning Province, China) in April, 2013. All plant samples were identified by Dr. Shi-Yang Li from Dalian Institute of Chemical Physics, based on morphological characters, according to the Pharmacopoeia of the People's Republic of China (Chemical Industry Press, Beijing 2005). The extraction and sample preparation were performed as follows. In brief, each sample (1.5 g of dried plant sample) was accurately weighted and ground to fine particles, and then sieved through a 40-mesh screen. Each powdered plant material was extracted with 95% ethanol (5 mL) by ultrasonication at room temperature for 30 min after 4 h immersion. The extraction was repeated three times, and the extracts were combined and concentrated *in vacuo*. The crude extract (59.8 mg) was then dissolved in ethanol, and then followed by centrifugation at 4000 rpm for 10 min at 4 °C by using Allegra 64R centrifuge (Beckman, USA). The supernatant was then transferred into a 5-mL volumetric flask as sample solution (~12 mg/mL) before UFLC analysis.

### 2.3. Chemical fingerprinting by UFLC-DAD & fraction collection

Chemical fingerprinting and fraction collection were performed on a Shimadzu (Kyoto, Japan) Prominence UFLC$^{TM}$ system equipped with a CBM-20A communications bus module, an SIL-20ACHT autosampler, two LC-20AD pumps, a DGU-20A3 vacuum degasser, a CTO-20AC column oven, an

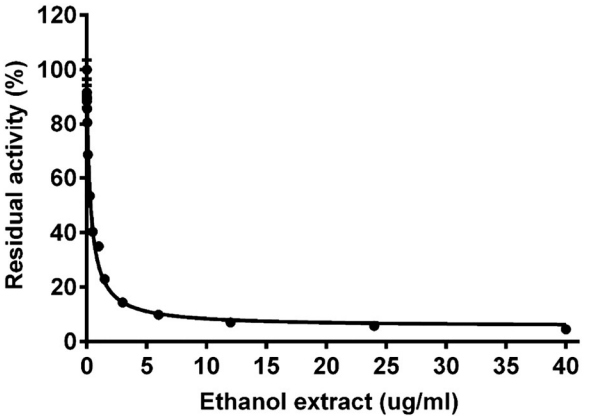


**Fig. 1.** The inhibition curve of the ethanolic extract from *Fructus Psoraleae* towards hCE2.