Journal of Pharmaceutical and Biomedical Analysis 161 (2018) 129–135

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Quantitative structure-activity relationship for estrogenic flavonoids from *Psoralea corylifolia*

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ARTICLE INFO

Article history:
Received 11 June 2018
Received in revised form 16 August 2018
Accepted 19 August 2018

Keywords:
Flavonoids from *Psoralea corylifolia*
Estrogenic activity
Fluorescence polarization
Computational modeling
QSAR

ABSTRACT

A combination of *in vitro* and *in silico* approaches was employed to investigate the estrogenic activities of flavonoid compounds from *Psoralea corylifolia*. In order to develop fluorescence polarization (FP) assay for flavonoids, a soluble recombinant protein human estrogen receptor α ligand binding domain (hERα-LBD) was produced in *Escherichia coli* strain. The competition binding experiment was performed by using coumestrol (CS) as a tracer. The result of FP assay suggested that the tested flavonoids can bind to hERα-LBD as affinity ligands, except for corylin. Then, molecular modeling was conducted to explore the binding modes between hERα-LBD and flavonoids. All the tested compounds fit into the hydrophobic binding pocket of hERα-LBD. The hydrophobic and hydrogen-bonding interactions are dominant forces to stabilize the flavonoids-hERα-LBD binding. It can be speculated from molecular docking study that the hydroxyl groups and prenyl group are essential for flavonoid compounds to possess estrogenic activities. Both methylation of hydroxyl group and cyclization of prenyl group significantly diminish the estrogenic potency of flavonoids. Furthermore, quantitative structure-activity relationship (QSAR) analysis was performed by the calculated binding energies of flavonoids coupled with their determined binding affinities. Comparison between the docking scores and the pIC_{50} values yields an R-squared value of 0.9722, indicating that the estrogenic potency of flavonoids is structure-dependent. In conclusion, molecular docking can potentially be applied for predicting the receptor-binding properties of undescribed compounds based on their molecular structure.

1. Introduction

As an erect annual herb, *Psoralea corylifolia* (Leguminosae) has been used in traditional practices of Ayurvedic and Chinese medicine [1–5]. It is widely distributed and considered as a natural alternative remedy due to its diverse beneficial effects, including hepatoprotective, estrogenic, antidepressant, antimicrobial, antioxidant, and antitumor activities [6,7]. The flavonoid compounds derived from the fruits of *Psoralea corylifolia* can be divided into isoflavones, flavanones and chalcones [8]. Multiple biological activities of these components have been confirmed, demonstrating their potential for treating diseases [6,9]. As phytoestrogens, flavonoids share similar structure with endogenous estrogens (such as 17β-estradiol). They can interfere with endocrine regulations in the human body through binding to estrogen receptors (ERs) [10–14].

Estrogen receptors are the transcriptional factors playing important roles by binding and activating estrogen response elements (EREs) on target genes, subsequently controlling cell proliferation and survival in normal mammary tissue [15]. They belong to the nuclear receptor (NR) superfamily [16] and participate in the regulation of reproduction, development, metabolism, and homeostasis [17,18]. Two isoforms of estrogen receptors (ERα and ERβ) have been identified and share the similar crystallographic structure [19–22]. Current available endocrine therapies for ER-positive breast cancers mainly focus on the selective estrogen receptor modulators (SERMs) [23]. They exert dual agonistic or antagonistic effect on ER transcription and have been applied for treating hormone responsive breast cancers for decades [24,25].

Estrogen mimetics including both natural and synthetic chemicals have been reported to selectively activate ERs [26–29]. Phytoestrogens, a group of plant-derived compounds with estrogenic properties [30], can structurally or functionally mimic

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https://doi.org/10.1016/j.jpba.2018.08.040
0731-7085/

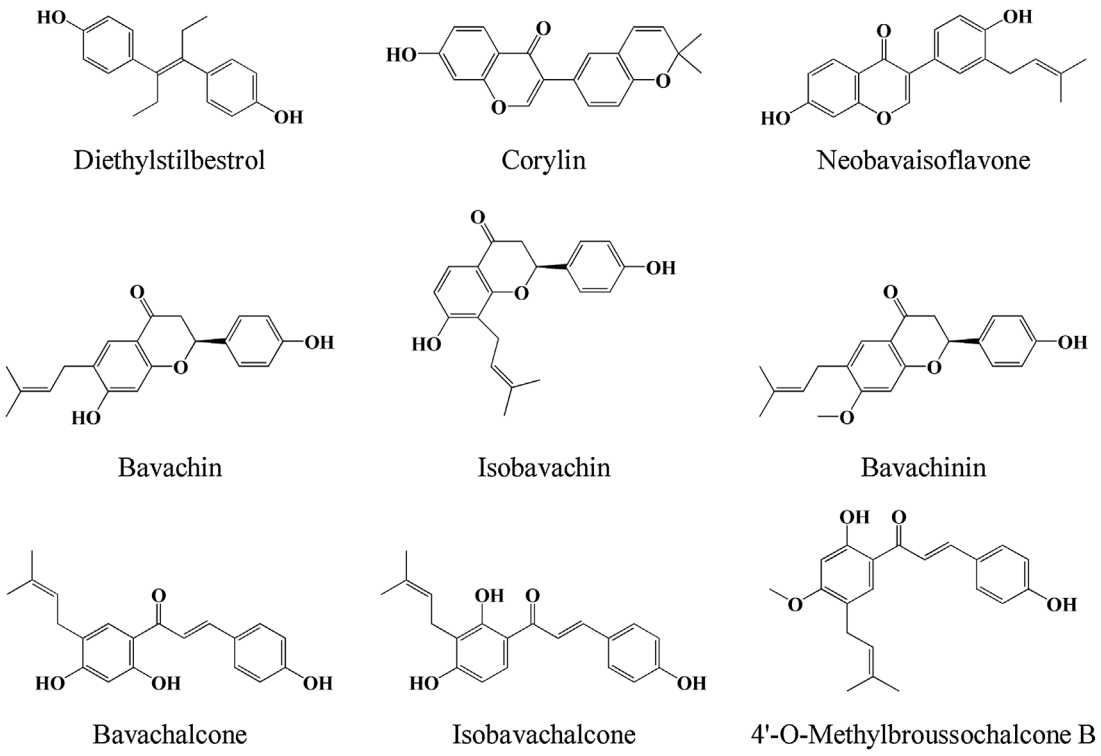

Fig. 1. Structures of the agonist diethylstilbestrol and the flavonoids from *Psoralea corylifolia*.

mammalian estrogens [31,32]. They are considered as a natural source of SERMs eliminating the side-effects of hormone replacement therapy. Furthermore, they are also known to exert widely benefits to human health, especially against cancer, osteoporosis, irregular menopause syndrome, cardiovascular disease, *etc.* [33,34]. Genistein is a typical phytoestrogen isolated from soybeans and belongs to isoflavonoids [35], a class of secondary metabolites that mainly occur in Leguminosae [36]. It has been confirmed to bind to the human estrogen receptors and disrupt normal estrogenic signaling. However, the estrogenic potential of many other naturally occurring flavonoid compounds and the underlying molecular mechanism of their pharmacological activities are still unclear. Hence, the present work focuses on the estrogenicity of flavonoids isolated from the fruits of *Psoralea corylifolia*.

A combination of *in vitro* and *in silico* approaches was employed to investigate the estrogenic activities of flavonoid compounds, including two isoflavones corylin and neobavaisoflavone, three flavanones bavachin, isobavachin and bavachinin, three chalcones bavachalcone, isobavachalcone, and 4′-O-methylbavachalcone. In order to develop fluorescence polarization assay for flavonoid compounds, a soluble recombinant protein human estrogen receptor α ligand binding domain (hERα-LBD) was produced first. The competition binding experiment was performed by using coumestrol (CS) as a tracer. Based on the determined binding affinities of hERα-LBD with flavonoids, molecular docking was conducted to explore their binding modes, in an attempt to establish a quantitative structure-activity relationship (QSAR) model for evaluating and predicting the estrogenic potential of flavonoid compounds.

2. Materials and methods

2.1. Materials and chemicals

Isopropyl β-D-1-thiogalactopyranoside (IPTG), dimethylsulfoxide (DMSO), and coumestrol (CS) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan). Corylin (≥98%), neobavaisoflavone (≥98%), bavachin (≥98%), isobavachin (≥98%), bavachinin (≥99%), bavachalcone (≥98%), isobavachalcone (≥98%), and 4′-O-methylbavachalcone (≥98%) were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). The structures of these flavonoid compounds are shown in Fig. 1. All other reagents used were of analytical grade.

2.2. Expression and purification of hERα-LBD

The coding sequences of human estrogen receptor α ligand binding domain (hERα-LBD) and glutathione S-transferase (GST) were inserted into the pGEX-4T-1 vector at restriction sites BamHI and XhoI. The expression plasmid pGEX-4T-1-hERα-LBD was introduced into *Escherichia coli* strain BL21(DE3)pLysS. Cells were treated with 0.5 mM IPTG overnight at 20 °C to induce the expression of hERα-LBD. A 0.22 μm membrane filter (Millipore, Bedford, MA, USA) was used to remove all the bacterial cells from suspension. Afterward, the supernatant was loaded onto an IDA-Ni^{2+} column (Novagen, Madison, WI, USA) to purify the target protein.

2.3. Fluorescence polarization assay

In this work, an autofluorescent exogenous estrogen coumestrol (CS) was employed as a probe. The protein hERα-LBD (250 nM) and the probe (10 nM) were mixed in a total volume of 290 μL and titrated with various concentrations of flavonoids (10 μL). The microplate was subjected to FlexStation 3 (Molecular Devices, Sunnyvale, CA, USA) after being incubated at room temperature for 2 h. The excitation and emission wavelengths were 355 and 405 nm, respectively. The IC_{50} value (concentration of flavonoid for 50% inhibition of binding between CS and hERα-LBD) was calculated according to a four parameter logistic equation $Y = (A - D) / [1 + (X / IC_{50})^B] + D$, where Y and X correspond to the polarization value and the tanshinone concentration, A and D are the polarization values at zero and an infinite concentration respectively, and B is the slope