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Psoralidin, a coumestan analogue, as a novel potent estrogen receptor signaling molecule isolated from *Psoralea corylifolia*

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ABSTRACT

A novel biological activity of psoralidin as an agonist for both estrogen receptor (ER)α and ERβ agonist has been demonstrated in our study. Psoralidin has been characterized as a full ER agonist, which activates the classical ER-signaling pathway in both ER-positive human breast and endometrial cell lines as well as non-human cultured cells transiently expressing either ERα or ERβ. The estrogenic activity was determined using the relative expression levels of either reporter or the endogenous genes dependent on the agonist-bound ER to the estrogen response element (ERE). Psoralidin at 10 μM was able to induce the maximum reporter gene expression corresponding to that of E_2-treated cells and such activation of the ERE-reporter gene by psoralidin was completely abolished by the cotreatment of a pure ER antagonist, implying that the biological activities of psoralidin are mediated by ER. Psoralidin was also able to induce the endogenous estrogen-responsive gene, *pS2*, in human breast cancer cells MCF-7. It was observed that activation of the classical ER-signaling pathway by psoralidin is mediated via induction of ER conformation by psoralidin and direct binding of the psoralidin–ER complex to the EREs present in the promoter region of estrogen-responsive genes, as shown by chromatin immunoprecipitation assay results. Finally, molecular docking of psoralidin to the ligand binding pocket of the ERα showed that psoralidin is able to mimic the binding interactions of E_2, and thus, it could act as an ER agonist in the cellular environment.

Psoralea corylifolia L. (PCL, Leguminosae) has been used in alternative medicine as an aphrodisiac and a tonic, and for the management of various diseases such as cardiovascular diseases, inflammatory diseases, hypertension, nephritis, and skin diseases.[1] It has been reported that PCL has antibacterial and antitumor activities as well as dilatory effects on coronary arteries and estrogen-like activities.[2] In particular, the seed extract of PCL is useful as a remedy for bone fractures, osteomalacia, and osteoporosis,[3,4] implying that it may contain a number of bioactive compounds with estrogenicity.

Studies have identified various phytoestrogens from the seeds of the PCL including bakuchiol, psoralen, isobavachalcone, isobavachromene, bavachinin, bavachin, corylifol A, and neobavaisoflavone.[5,6] These studies support the evidence that the use of PCL for osteoporosis or cardiovascular diseases is associated with the estrogenic properties of PCL through interaction with the estrogen receptor (ERs).

Psoralidin, 3,9-dihydroxy-2-(3-methylbut-2-enyl)-[1]benzofuro[3,2-*c*]chromen-6-one (Fig. 1), is a naturally occurring coumestan and it is isolated from the fractions of organic solvents such as ethylacetate, hexane, or *n*-butanol of the seed extract of PCL.[7] It has been demonstrated that psoralidin has a variety of biological activities such as anticancer, antioxidant, antibacterial, antidepressant, anti-inflammatory activities, and regulation of insulin signaling.[5,6] Our study identified a novel biological activity of psoralidin, showing that it is an ER signaling molecule likely responsible for the previously reported estrogenic activities of the seed extract of PCL. In this Letter, we describe the binding affinity of psoralidin toward both ERs α and β and its activation of the ER and estrogen response element (ERE)-mediated gene transcription in various

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cancer cell lines. Direct binding of psoralidin with ERα leading to activation of estrogen-responsive genes was confirmed by detection of EREs bound to a psoralidin–ERα complex by a chromatin immunoprecipitation (ChIP) assay. Finally, crucial interactions of psoralidin in the ligand binding pocket of the ERα was demonstrated using a molecular docking study to provide in silico evidence for the estrogenicity of psoralidin.

The relative binding affinity of psoralidin to both ERα and β was 0.56% and 0.042% compared to that of E_2 (set as 100%) and its IC_{50} values to replace the binding of E_2 to both receptors were 1.03 and 24.6 μM, respectively (Table 1). Our data imply that psoralidin is able to bind to both receptors within the micromolar range and has preferential affinity for ERα over ERβ. The receptor subtype selectivity obtained from the results of the binding affinity study was consistent with that of ERE-transcriptional activities, where the EC_{50} values of psoralidin in activating the ERE-mediated reporter gene transcription in either an ERα or β-dependent manner were 3.68 or 6.88 μM, respectively, resulting in its selectivity of ERα over ERβ of 1.86 (Table 2).

The effects of psoralidin on ERE-mediated gene transcription were evaluated in both human breast cancer cell lines, the MCF-7 cancer cell line and the Ishikawa endometrial cancer cell line, to differentiate its tissue selectivity. MCF-7 cells were 7.4-fold more sensitive to psoralidin than Ishikawa, implying that psoralidin might be less proliferative in the endometrium. Psoralidin treatment at 10 μM reached maximum ERE-mediated luciferase activity obtained at 1 nM of E_2. The transcriptional activation of the luciferase gene by psoralidin through interaction with the ERE was completely abolished by cotreatment of a pure antiestrogen, ICI 182,780 (ICI) (Fig. 2). These data imply that psoralidin is a full ER agonist in MCF-7 cells via the classical ER/ERE-mediated transcription pathway.

MCF-7 cells possess both ERα and β at different protein levels;[8] therefore, it was not clear which ER subtype mediates the induction of luciferase activity by psoralidin in this cell line. In order to investigate the ER subtype that affects the psoralidin-induced classical ER/ERE signaling pathway, ERE-luciferase activities were examined in ER-negative CV1 cells transfected with either ERα or ERβ. Psoralidin was able to induce ERE-luciferase activity via both receptors with EC_{50} of 3.68×10^{-6} and 6.88×10^{-6} M, respectively (Table 2). These data are consistent with the results from the receptor binding affinity assay in that psoralidin may display

Table 2
EC_{50} values of ERE-reporter gene transcription activities by psoralidin in various cell lines

Cell line	EC_{50} (μM)
MCF-7	1.85 ± 0.09
Ishikawa	27.4 ± 0.18
CV1 transfected with ERα	3.68 ± 0.4
CV1 transfected with ERβ	6.88 ± 0.9

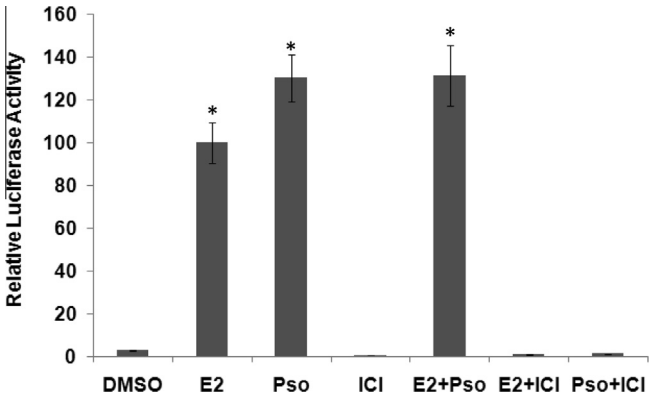

Figure 2. Relative luciferase activities in MCF-7 cells after treatment of psoralidin (Pso, 10 μM) and combination of psoralidin (10 μM) and ICI (1 μM). Relative luciferase activities were obtained when the luciferase activity of cells treated with E_2 (1 nM) was set as 100.

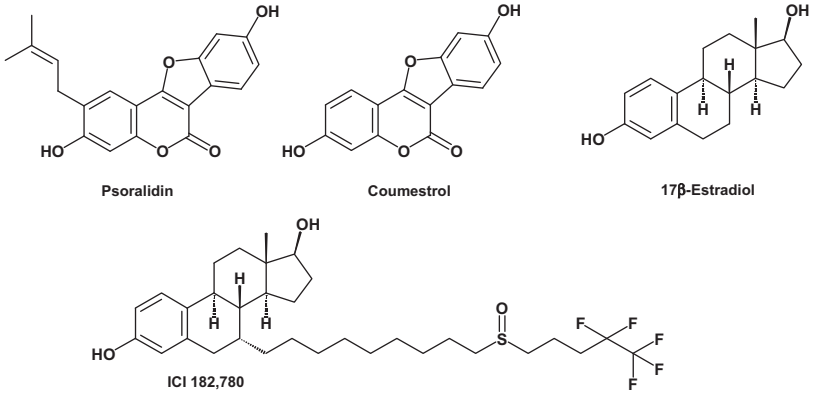

Figure 1. Structures of psoralidin, coumestrol, 17β-estradiol and ICI 182-780 used in this study.

Table 1
Binding affinities of psoralidin to both ERα and ERβ

Compound	ERα		ERβ		Selectivity[b]
	IC_{50} (μM)	RBA[a]	IC_{50} (μM)	RBA	
E_2	$5.76 \pm 0.4 \times 10^{-3}$	100	$7.06 \pm 0.4 \times 10^{-3}$	100	0.816
Psoralidin	1.03 ± 0.08	0.56	24.6 ± 0.9	0.03	0.0419

[a] RBA of psoralidin was calculated as the ratio of the concentrations of psoralidin required to reduce the specific radioligand binding by 50%, that is, the ratio of the IC_{50} values. The RBA value for E_2 was arbitrarily set at 100.
[b] Selectivity was calculated as the ratio of the IC_{50} value in binding to ERα and that in binding to ERβ. The greater the value is, the more selective it is toward ERα.