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8-Prenyl naringenin is a potent ERα selective phytoestrogen present in hops and beer☆

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1. Introduction

Phytoestrogens are compounds found in leafy plants and fungi that exhibit estrogenic activity both in vivo and in vitro. Chemically they refer to the classes of isoflavones, flavanones, coumestrans and resorcylic acid lactones. Circumstantial evidence suggests that phytoestrogens ingested with food have beneficial effects in the prevention of breast cancer, post-menopausal syndrome and atherosclerosis. However, the potency of most of these compounds is relatively low compared to 17β-estradiol. Recently, isolation of a potent phytoestrogen (8-prenyl naringenin (8-PN)) from the heart wood of an indigenous tree in Thailand (*Anaxagorea luzonensis* A. Gray) was reported [1]. 8-PN was also found in Compositae [2] and identified as the estrogenic component in hops (*Humulus lupulus* L.) and beer [3].

We studied in vitro receptor binding of both naturally found 8-PN enantiomers 2*S*(−)8-PN and 2*R*(+)8-PN compared to endogenous mammalian estrogens and other phytoestrogens, using recombinant human ERα/ERβ. As a striking result we found that both 8-PN enantiomers show high affinity and strong selectivity for ERα. 2*S*(−)8-PN exhibits an overall higher affinity for both receptors than 2*R*(+)8-PN.

Using a mammalian cell-based transient transactivation assay, we were able to demonstrate that 8-PN is the strongest plant-derived ERα agonist identified so far, being about 10 times more potent than coumestrol and approximately 100 times more potent than genistein. Surprisingly and in clear contrast to genistein, 8-PN is a much weaker agonist of ERβ than of ERα.

As determined by in vitro transactivation analysis, 8-PN displays only 70-fold weaker estrogenicity at ERα than 17β-estradiol, whereas its estrogenic potency is about 20,000-fold lower compared to 17β-estradiol when assessed in vivo by classic uterine growth assay and vagina growth assay in juvenile rats. These data suggest that 8-PN, the first ERα selective phytoestrogen, is a natural occurring selective estrogen receptor modulator (SERM) with remarkable potential for treatment of a variety of conditions associated with estrogen deficiency.

2. Materials and methods

2.1. Chemical synthesis of 8-PN

Synthesis of 8-prenyl naringenin was performed as described before [4] starting from commercially available naringenin. Pure enantiomeres were obtained from chiral HPLC.

2.2. In vitro receptor binding

Various compounds were tested for ER binding in a cell-free, 96-well competition assay using recombinant human ERα and ERβ from cytosolic SF9-cell extracts. Extracts were incubated with a total 5 nM of ^{3}H-labeled 17β-estradiol and the respective compound (in DMSO) for 1 h at 20 °C. Free ligand was subsequently removed by charcoal filtration and receptor bound ^{3}H-estradiol measured by scintillation counter.

2.3. In vitro transactivation/trans-suppression

In vitro estrogenity was assessed in U2-osteosarcoma cells (96-well plates) transient transfected with plasmids encoding for either ERα (pSG5-ERα/Heg0) or ERβ (pSG5-ERβ/ERβ0) and a luciferase reporter gene construct

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(pBL(ERE)$_2$*tk*Luc$^+$). Cells were kept in RPMI medium (glutamine, w/o phenol red) enriched with 10% fetal calf serum (FCS) at 37 °C and 5% CO_2. Twenty-four hours prior transfection, cells were split and grown in RPMI medium (glutamine, w/o phenol red) containing 10% charcoal-treated fetal calf serum (CCS). Cells were then treated with test compounds (in DMSO) 24 h after transfection and luciferase activity was determined 20 h later via luminometry.

2.4. In vivo uterine growth and vaginotrophic effect

To examine in vivo estrogenic activity, juvenile female rats were administrated a single daily dose of 8-PN (s.c., ethanol/arachis oil, three consecutive days of treatment) followed by autopsy at day 4. The relative increase of uterine and vagina wet weights was determined.

3. Results summary

I. The prenylflavanone 8-PN is the first known ERα selective phytoestrogen, exhibiting >2-fold higher affinity for ERα than ERβ measured by in vitro competitive binding assay.
II. As determined by transactivation studies, 8-PN is the strongest plant-derived ERα agonist identified so far, 10-fold more potent than coumestrol and 100-fold stronger than genistein but only 70 times weaker than 17β-estradiol.
III. Transactivational analysis reveals a >3.6-fold higher estrogenic activity of 8-PN at ERα than ERβ, a strong contrast to primarily ERβ activating coumestrol and genistein.
IV. 8-PN is a pure ER agonist in vitro, exhibiting an estrogenic activity profile comparable to estron.
V. In vivo estrogenic activity of 8-PN in reproductive tissue is about 20,000-fold weaker compared to 17β-estradiol.
VI. 2*S*(−)8-PN shows moderately higher ER affinity and estrogenic activity in vitro and in vivo than 2*R*(+) 8-PN.
VII. 8-PN is a natural SERM with promising potential for treatment of various estrogen deficiency-related conditions.

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