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Estrogenic effect of three substituted deoxybenzoins

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ABSTRACT

Deoxybenzoins (*1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone*) are possible precursors or metabolites of isoflavanones which may have xenoestrogenic potential on estrogen receptor (ER). In this study we evaluated three 2′-substituted deoxybenzoin derivatives for their estrogenic effect based upon their ability to affect the proliferation of ERα$^+$ MCF7 cells, ERβ$^+$ PC3 cells and Hep2 cells stably transfected and expressing either ERα or ERβ. These compounds designated as CMPD3, CMPD6 and CMPD9 had –COOH, $-(CH_2)_4-CH_3$ and $-CH_3$ substitutions, respectively on the 2′-position of the 2,4-dihydroxyphenyl ring of deoxybenzoin. We found that all three compounds increased the proliferation of ERα$^+$ MCF7 cells ($EC_{50} \sim 1–12$ μM) and ERα$^+$ Hep2 cells, while causing apoptosis in ERβ$^+$ PC3 cells ($IC_{50} \sim 1–5$ μM) and ERβ$^+$ Hep2 cells. The compounds also up-regulated the expression of estrogen sensitive genes, trefoil factor 1 (TFF1, previously known as pS2) and cathepsin-D (CTSD), in these cells. We performed *in vitro* ER transcription activation assays using Hep2 cells transiently co-transfected with estrogen response element driven luciferase and either ERα or ERβ vectors to ascertain the mechanism of action of these compounds through the 'classical' genomic pathway of estrogenic activity and to determine their ER subtype selectivity. Molecular docking of the compounds with the Ligand Binding Domain of ERα and ERβ showed similar docking scores (Glidescores of −6.5 to −8.5 kcal/mol) indicating that these compounds were ligands of both ERα and ERβ with similar affinity.

1. Introduction

Xenoestrogens are non-endogenous ligands for estrogenic receptors (ER) in eukaryotic cells. They may have little or no structural resemblance to the endogenous estrogens like 17-β estradiol, but they possess the essential pharmacophoric features to bind with the estrogen receptors. The extent and nature of this interaction determines the agonistic or the antagonistic outcome of these compounds on the estrogen responsive cells. There are two main subtypes of estrogen receptors in mammalian cells, estrogen receptor–alpha (ERα, NR3A1) and estrogen receptor–beta (ERβ, NR3A2), in addition to several other subtypes and splice variants reported [1,2]. These two subtypes, ERα and ERβ, show a high degree of structural homology in the Ligand Binding Domain (LBD) and recognize similar estrogen response elements (ERE, consensus sequence 5′-GGTCAnnnTGACC-3′) upstream of estrogen-modulated genes [3,4]. In addition to this 'classical' genomic pathway, ERs also perform non-genomic effects through membrane bound forms or soluble cytosolic forms [5,6]. Therefore the cellular responses to a xenoestrogen depends upon its receptor selectivity, binding affinity, agonistic/antagonistic effect and the relative expression levels of the ER subtypes and co-activators [2]. In cells with differential expression of the ER subtypes, ERα agonists produce concentration dependent mitogenic effect, while ERβ agonists cause apoptosis [2,7]. The ER antagonists cause conformational changes in the receptors such that the agonistic coactivators cannot bind, or allows corepressors to bind or reduces the stability of the receptors. Such growth regulating effect of these selective ER modulators (SERMs) on estrogen sensitive tissue types like mammary glands, ovaries and prostate gland allows their clinical use for conditions like cancers of these organs.

A large number of non-steroidal compounds from diverse sources are now being recognized as having xenoestrogenic effect. These include several plant metabolites, pesticides and industrial chemicals. Although health effects for some xenoestrogens like soy isoflavonoids are well established [8–10], unintended and unsuspected exposure to xenoestrogens can be potentially disruptive to the estrogen-dependent tissues. Substituted dehydroxybenzoins (*1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)* ethanone) are a class of non-steroidal compounds which are being evaluated for its anti-microbial activity [11], anti-oxidant effect [12], anti-inflammatory effect [13], vasodilator effect [14] and estrogenic effect [15,16]. It is also used industrially in manufactur-

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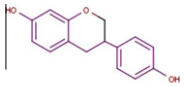
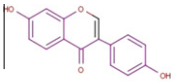
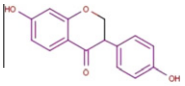
Table 1
The compounds described in this study.



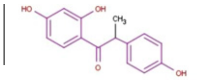
A. Equol
(Isoflavan)



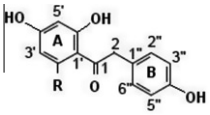
B. Genistein
(Isoflavone)



C. Dihydrodaidzein
(Isoflavanone)



D. *O*-desmethylangolensin
(αMethyldeoxybenzoin)



E. Deoxybenzoin (-*R* = -H)

Name	R	Reference
CMPD3	–COOH	*
CMPD6	–(CH2)4–CH3	*
CMPD9	–CH3	*
Compound **2**	–H	[14]
Compound **4**	–OH	[14]

* This study

ing of fire-retardant polymers [17]. Dehydroxybenzoins (DOBs) may also be bioavailable from the orally consumed isoflavones by microbial or endogenous metabolism. For example, *O*-desmethylangolensin may be produced (to varying degrees) from dietary daidzein by the intestinal flora [18].

In vitro cell proliferation assays are useful in identifying the agonist or antagonist properties of the growth modulating test compounds [19]. MCF7 is an immortal human cell line derived from adenocarcinoma of the breast. These cells predominantly express ERα subtype and are routinely used in *in vitro* screening for agonists and antagonists of ERα. In this cell type, ERα agonists decreases the doubling time and increases the expression of estrogen responsive genes, whereas *ERα* antagonists inhibits estrogen stimulated cell proliferation. PC3 is a testosterone independent human prostate cancer cell line with elevated ERβ expression. ERβ agonists like genistein reduces cell growth or induces apoptosis while ERβ antagonists blocks the inhibitory effect of agonists. In addition to the gross effects such as cell proliferation or inhibition, estrogenics also produce rapid upregulation of several genes, like trefoil factor 1 (TFF1, previously designated as pS2) and cathepsin-D (CTSD), at early stages of exposure [20–23].

Here we describe the estrogenic effect of three substituted dehydroxybenzoins (Table 1), determined by cell proliferation assay and expression of estrogen specific genes (TFF1 and CTSD), in MCF7 and PC3 cells. To ascertain that these effects were mediated through the ERs, the ability of these compounds to upregulate ERE-driven luciferase expression in Hep2 cells transiently transfected with ER (either ERα or ERβ) was determined. We also studied the ability of these compounds to produce cell proliferation or inhibition of Hep2 cells stably transfected and overexpressing either ERα or ERβ receptors.

2. Materials and methods

2.1. Synthesis of the compounds

The DOBs were synthesized by the approach described by [24]. Briefly, in boron trifluoride diethyl etherate ($BF_3O(C_2H_5)_2$), 4-hydroxy phenyl acetic acid was added with vigorous stirring and heating at 75 °C followed by the slow addition of corresponding resorcinol. The reaction mixture was cooled to room temperature, followed by the addition of *N*,*N*-dimethyl formamide (DMF), and $BF_3O(C_2H_5)_2$ at 0 °C. To this mixture, a solution of methane sulfonyl chloride in DMF was added at 50 °C and the temperature was raised to 110 °C for 3 h. After the completion of the reaction, reaction mixture was cooled and poured into ice-cold water. The oily phase was separated and extracted with ethyl acetate, the organic layer washed with water, dried and the solvent removed by evaporation. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane in the ratio of 2:1. All compounds were analysed by NMR (^{1}H NMR and ^{13}C NMR) on Bruker AVIII spectrometer and GC–MS (GCMate, JEOL Ltd.).

NMR spectra of the purified DOBs were recorded to ascertain the structure; the chemical shifts (δ) are expressed herein as ppm. GC–MS was used to determine the *m/z* of the $(M+H)^+$ species.

2.1.1. CMPD3 (3,5-dihydroxy-2-[2-(4-hydroxyphenyl)acetyl]benzoic acid)

^{1}H-NMR 4.29(2H, s, CH_2), 6.01(2H, s, H-3, 5), 6.61(2H, d, *J* = 8.0 Hz, H-3′, 5′), 7.18 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 8.09 (1H, br s,

Table 2
Primer sets used for the RT-PCR. All primer sets were designed to have at least one intron or intron–exon junction, on the genomic template, in order to minimize the chances of amplification from genomic DNA (if any). The primers for *GAPDH* and *CTSD* were designed using Primer-BLAST service from NCBI (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Target gene	Primer sequence	Amplicon size (bp) from cDNA	Reference
ER-α	→5′-TAC TGC ATC AGA TCC AAG GG-3′ ←5′-ATC AAT GGT GCA CTG GTT GG-3′	650	[19]
ER-β	→5′-TGA AAA GGA AGG TTA GTG GGA ACC-3′ ←5′-TGG TCA GGG ACA TCA TCA TGG-3′	528	[19]
TFF1 (pS2)	→5′-GGA GAA CAA GGT GAT CTG CG-3′ ←5′-CAC ACT CCT CTT CTG GAG GG-3′	236	[19]
GAPDH	→5′-TGC CTC CTG CAC CAC CAA CT-3′ ←5′-GCC TGC TTC ACC ACC TTC – 3′	349	*
CTSD	→5′-CCC GCA AGG CCT ACT GGC AG -3′ ←5′-CTG CAG CTC GCG CAC CTC AT-3′	146	*

* This study.