Dietary Phytoestrogens: Potential Selective Estrogen Enzyme Modulators?

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Abstract

Between one-third to one-half of all breast cancers are steroid sensitive. Steroid-pathway enzymes (sulfatase, 17β-hydroxysteroid dehydrogenases, aromatase and sulfotransferases) are thus prime candidates for therapeutic approaches based on the control of intact hormone activity. Some phytoestrogens, ubiquitous in our diet, are inhibitors of these enzymes. Such a therapeutic potential has stimulated research and progress has been achieved during the last years. Complementary to previous reviews on phytoestrogens, this contribution covers the estrogen pathway inhibition effects of these compounds and special attention will be given to isoflavonoids, flavonoids and lignans. Furthermore, the research on structurally-related compounds as therapeutic agents will be discussed briefly.

Key words
Phytoestrogens · aromatase · hydroxysteroid dehydrogenases · sulfatase · sulfotransferases · endocrine disrupters

Introduction

Over the past 15 years, there has been a tremendous increase in the number of papers published on the biological activities of phytoestrogens. The estrogen receptor binding is the best-documented biological action of phytoestrogens and, as estrogen agonists and antagonists, they can be classified as selective estrogen receptor modulators (SERMs) [1]. These compounds also have a diverse range of other biological effects including the potential to alter the biosynthesis of endogenous hormones through a number of pathways.

The high incidence of breast cancer in post-menopausal women and the lack of any correlation between the estrogen levels in plasma and the growth of breast cancer suggest that local estrogen synthesis plays an important role in the pathogenesis of estrogen-dependent breast cancer [2]. Two principal pathways are involved in the last steps of formation of 17β-estradiol: (i) the "aromatase pathway" which respectively transforms androstenedione (AD) to estrone (E₁) and testosterone (T) to estradiol (E₂) and (ii) the "sulfatase pathway" which converts estrone sulfate (E₁S) into estrone (E₁); estrone is then transformed into estradiol (E₂) by 17β-hydroxysteroid dehydrogenase type 1. By analogy with a selected estrogen receptor modulator (SERM), the concept of a selective estrogen enzyme modulator (SEEM) as a therapeutic agent has recently emerged [3], [4]. The SEEM can control the enzymatic mechanisms involved in the formation and transformation of estrogens (Fig. 1).

This contribution covers the inhibition of estrogen pathways by phytoestrogens. In this way, previous reviews will be complemented [5], [6], [7].

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Fig. 1 Enzymatic steps involved in the synthesis of estrogenic steroids: the concept of selective estrogen enzyme modulator (SEEM; from Refs. [3] and [4]). 17β-HSD-1 = 17β-hydroxysteroid dehydrogenase type 1. SEEM-I inhibits the estrone sulfotransferase; SEEM-II inhibits the 17β-HSD-1; SEEM-III inhibits the aromatase activities; and SEEM-IV stimulates the estrone sulfotransferase activity. T: testosterone; AND: androstenedione; E₁: estrone; E₂: estradiol; E₁S: estrone sulfate.

Phytoestrogens: General Aspects

Dietary phytoestrogens are defined as natural chemicals present in our diet that can mimic or modulate the action of endogenous estrogens, usually by binding to estrogen receptors [6], [7]. Based on their chemical structure, phytoestrogens can be classified into four main groups, i.e., isoflavonoids, flavonoids, stilbenes and lignans (Fig. 2). To be complete, as mentioned recently by Cos [7], some terpenoids and saponins have been reported to exert similar effects.

Isoflavones, found in Leguminosae (soybeans in particular) constitute the largest group of natural isoflavonoids. In this subclass, the most thoroughly investigated and interesting compounds are genistein (1), daidzein (2), biochanin A (3) and formononetin (4). Coumestans represent a fully oxidized version of pterocarpans and are included in the isoflavonoids classification. The most representative, coumestrol (10), has been isolated from a few fodder and pasture plants (e.g., clover, alfalfa) belonging to the Leguminosae. Estrogenic activities of isoflavonoids have been widely studied [8], [9], [10], [11] and coumestrol is the phytoestrogen that has the higher binding affinity for the estrogen receptors [9], [11].

Over 4000 flavonoids have been identified in plant sources. They are usually subdivided into flavonols (found throughout the plant foods), flavanones (catechins, epicatechins, gallocatechins, epigallocatechins), anthocyanidins, chalcones, flavonones and flavones. Chalcones, flavanones (citrus fruits) and flavones (spices and herbs) are described as minor flavonoids in spite of sometimes being present in food at a dietary significant concentration. Recent studies on the estrogenic activity of flavonoids in vitro revealed that the estrogenic potency of flavonoids is 10⁻² to 10⁻⁵-fold less than that of estradiol. However, 8-prenylnaringenin (20), a recently discovered flavanone from hops and beer, demonstrated a higher estrogenic activity than coumestrol or isoflavones [12], [13].

Stilbenes are 1,2-diarylethenes detected in relatively few plant families where they contribute to the resistance to microbial degradation and act as phytoalexins. The major dietary sources of stilbenes are grapes, grape juices, wine and peanuts. Among monomeric stilbenes, resveratrol (24) (3,5,4′-trihydroxystilbenene), produced by plants as a defence mechanism against Botrytis cinerea has been identified as the major active compound and most of the studies about estrogenic/antiestrogenic activity have focused on it [7], [14].

Current research is focusing on a number of lignans recently identified in humans and several animals. Lignans are widely distributed throughout the plant kingdom, where they are involved in plant defences, and the major sources are the outer layer of cereals, mainly rye and flaxseed. They are converted into active phytoestrogens called “mammalian enterolignans” by the proximal colon microflora [15]. Both trans-2,3-bis(3-hydroxybenzyl)-γ-butyrolactone and 2,3-bis(3-hydroxybenzyl)-butane-1,4-diol, known respectively as enterodiol (25) and enterolactone (26) have been described as the major lignans. Lignans are closely related to phenolic estrogens and may function as weak estrogens or estrogen antagonists [16].

SEEM 1: Sulfatase Inhibitors

Sulfatas are a group of hydrolytic enzymes that catalyse the conversion of various sulfated compounds to their corresponding unconjugated derivatives [17]. Desulfation of estrone sulfate by estrone sulfatase (SEEM 1) represents an important step in the transformation of inactive steroids to estrogenic hormones. Quantitative determinations in breast cancer tissues indicate that the “estrone sulfatase pathway” is about 130 – 200-fold more important than the aromatase pathway [3]. Inhibition by isoflavone metabolites has been recently reported [18]. The most potent inhibitor was daidzein 7,4′-bisulfate (7) which exhibited an IC₅₀ value of 0.8 µM. Daidzein 7-O-sulfate (5), daidzein 4′-O-sulfate (6) and genistein 7-O-sulfate (8) inhibited sulfatase activity with IC₅₀ values < 20 µM. These results are quite similar to values previously reported by Wong and Keung [19]. It seems that, since less than 0.1% of the conjugation products of isoflavones in humans are bisulfates [20], concentrations of daidzein 4′,7-bisulfate, even in those consuming high soy/isoflavone-supplemented diets, would not be sufficient to influence sulfatase activity in vivo [18].

None of the unconjugated flavonoids and isoflavonoids tested by Harris were found to have any effect on sulfatase (IC₅₀ > 25 µM) at concentrations likely to be achieved from the diet. This conclusion is the opposite to Huang’s paper where it was reported that kaempferol (11), quercetin (12) and naringenin (16) act significantly as estrogen sulfatase inhibitors [21].

SEEM 2: 17β-Hydroxysteroid Dehydrogenase

The 17β-hydroxysteroid dehydrogenases (SEEM 2), key enzymes acting at the last step of androgen and estrogen formation, are nicotinamide adenine dinucleotide [NAD(H)]- and/or its phosphate form [NADP(H)]-dependent enzymes that catalyse the oxidation and reduction of 17β-hydroxy and 17-ketosteroids in a postional and stereospecific manner [22]. 17β-HSD1 is expressed in steroidogenic tissues including estrogen target tissues such as normal and malignant breast tissues [23] and is a prognostic marker in breast cancer [24]. It catalyses predominantly the conversion of estrone to estradiol and can be considered to be pri-
Isoflavonoids

Isoflavones

<table>
<thead>
<tr>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4'</th>
</tr>
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<tr>
<td>HO</td>
<td>HO</td>
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Genistein (1)
Daidzein (2)
Biochanin A (3)
Formononetin (4)
Daidzein-7-O-glucoside (5)
Daidzein-4',7'-di-O-glucoside (6)
Genistein-7-O-glucoside (8)

OH OH OH OH
OH OH OH
OH OH OMe
OH OH OMe
H OMe OMe
H OMe OMe
OH OMe OMe
OH OMe OMe

3-Phenylchromans

Coumestans

HO OH

Equol (9)

Coumestrol (10)

Stilbenes

HO OH

Resveratrol (24)

Lignans

HO OH

Enterodiol (25)

HO OH

Enterolactone (26)

Flavonoids

Flavonols

5 7 8 4'

Kaempferol (11)
Quercetin (12)

Flavanols

Catechin (13)
Gallocatechin gallate (14)

Chalcones

Naringenin chalcone (15)

Flavanones

Naringenin (16)
Hesperetin (17)
Eriodictyol (18)
Naringin (19)
Naringenin (20)

5 7 8 3' 4'
OH OH H H OH
OH OH H OMe OH
OH hesp* CH₂CH₂-(C₆H₅)₂ H OH

*: naringenin flavonoid

Flavones

Chrysin (21)
Apigenin (22)
Luteolin (23)

5 7 3' 4'
OH OH H H
OH OH OMe OH
OH OH OH OH

3.1. Estrogen receptors

These receptors are intracellular and situated in the cytoplasm and nucleus of cells. Two major subtypes of ERs have been identified: ERα (ERα) and ERβ (ERβ). ERα is more abundant in breast tissue and is involved in breast cancer development, while ERβ is more abundant in the uterus and is involved in reproductive functions.

3.1.1. Estrogen receptor α (ERα)

ERα is the primary receptor for estrogen and is responsible for the majority of estrogenic effects. It acts as a transcription factor and regulates the expression of genes involved in cell growth, differentiation, and apoptosis.

3.1.2. Estrogen receptor β (ERβ)

ERβ is a less well-characterized receptor and has been implicated in a variety of functions, including cell proliferation, differentiation, and apoptosis.

3.2. Estrogen receptor antagonists

Estrogen receptor antagonists (ERAs) are substances that block the action of estrogen at the receptor level, thereby preventing estrogenic effects. These substances are used in the treatment of breast cancer and other estrogen-dependent conditions.

3.2.1. Selective estrogen receptor modulators (SERMs)

SERMs are a class of ERAs that have estrogenic effects in some tissues and anti-estrogenic effects in others. Examples include tamoxifen and raloxifene.

3.2.2. Pure anti-estrogens

Pure anti-estrogens are substances that have strong anti-estrogenic effects in all tissues. Examples include letrozole and anastrozole.

3.3. Estrogen receptor agonists

Estrogen receptor agonists (ERAs) are substances that activate the estrogen receptor and induce estrogenic effects. These substances are used in the treatment of menopausal symptoms and osteoporosis.

3.3.1. Estrogen replacement therapy (ERT)

ERT involves the use of estrogen to alleviate menopausal symptoms such as hot flashes and vaginal dryness. It is also used to treat osteoporosis.

3.3.2. Botanical estrogen supplements

Botanical estrogen supplements are derived from plants and are thought to have estrogenic properties. Examples include black cohosh and red clover.

3.3.3. Synthetic estrogen analogs

Synthetic estrogen analogs are substances that have been designed to mimic the effects of estrogen. Examples include estradiol and estrone.

SEEM 3: Aromatase

Aromatase converts the Δ4-3-one ring of C₁₉ androgenic steroids to the phenolic A ring of estrogen. The gene expressing cytochrome P-450arom, referred to as CYP 19, is part of the cytochrome P-450 superfamily. This enzyme is expressed in numerous tissues such as placenta, ovarian granulosa cells, testicular Leydig cells, adipose tissue, liver and brain [33] and the aromatase activity in breast cancer tissues has been demonstrated to be higher than in normal tissue [34]. The biochemical mechanism of aromatase has been studied extensively and consider-
able progress has been made in understanding the reactions catalysed by this enzyme. Hypotheses have been advanced for the mechanism of the third oxidation step in androstenedione conversion, the Akhtar peroxyn acid intermediate remaining the most accepted hypothesis [35]. Several main groups of phytoestrogens, e.g., flavones, flavanone and lignans modulate aromatase activity in vitro. An early study made by Kelli and Vickery [36] reported inhibition by chrysin (21) (IC\textsubscript{50} = 0.5 μM) and apigenin (22) (IC\textsubscript{50} = 1.2 μM). Numerous studies have confirmed the potential of flavones as aromatase inhibitors [26, 28, 37, 38, 39, 40, 41]. Potent inhibition of aromatase occurred with flavanones such as naringenin (16), hesperetin (17), eriodictyol (18) and naringin (19) or flavone precursors, i.e., naringenin chalcone (15), in the 1 – 10 μM range [26, 28, 39, 40, 42, 43]. However, natural flavones are consistently more potent inhibitors than flavanones [41] and all studies were made with racemic mixtures of flavanones. Nevertheless, a computer modelling [42] revealed that only the 2S-configuration isomer of naringenin could bind to the active site and the values for the natural S-configuration isomer may be lower than those reported. Other flavonoids such as flavanols [catechin (13), gallo catechin gallate (14)] [44, 45] or flavonols [kaempferol (11), quercetin (12)] are inactive or weak inhibitors [37, 38, 40].

The aromatase inhibition effect of isoflavonoids has been examined and these compounds were reported to be inactive in several studies [26, 27, 38, 39, 42, 46]. But, recently Almstrump [43] found that biochanin A (3) and formononetin (4) are aromatase inhibitors at low concentrations; moreover, except for genistein, all the studied isoflavonoids were both aromatase inhibitors at low concentrations (< 1 μM) and estrogenic at higher concentrations (> 1 μM) resulting in U-shaped dose-response curves. To be complete, Wang [38] and Adlercreutz [47] studied the inhibition of human aromatase by mammalian lignans. Lignans were shown to be weak inhibitors, e.g., enterolactone (26) (IC\textsubscript{50} = 14 μM [47], Ki = 14.4 μM [38]) or inactive, e.g., enterodiol (25) [38, 47].

Computer modelling and site-directed mutagenesis have revealed the structural features for flavonoids to inhibit aromatase [42]. Flavones bind to the active site in an orientation in which rings A and C of flavones mimic rings C and D of androstenedione. By doing so, the p-phenyl substituent (ring B) of flavones is oriented in a similar position to that of the substrate’s ring A. The C-4 keto group of flavones points towards the haeme prothetic group indicating that this group is essential for the inhibition [36, 48]. Isoflavones have the 4’-hydroxyphenyl group at position C-3, greatly reducing the ability to bind and inhibit aromatase. This computer modelling revealed that the 3-hydroxy group of flavonols significantly changes the orientation of ring B, resulting in a large decrease in the ability to inhibit aromatase.

Recently, Saarinen evaluated the in vivo effect on aromatase of selected flavonoids using uterotrophic tests in immature rats [49]. Phytoestrogens such as naringenin (16), apigenin (22) or luteolin (23) neither induced uterine growth nor reduced estrogen- or androgen-induced uterine growth. According to these authors, the inability of these flavonoids to inhibit aromatase may be due to their relative poor absorption and/or bioavailability.

**SEEM 4: Sulforaphenases**

The concept that steroid-modifying enzymes such as estrogen sulforaphenase (EST) have critical physiological roles in modifying steroid hormone action at target cells has been emerging based upon multiple studies in different systems [50]. Estrogen sulforaphenase (SULT 1E1) belongs to the family of cytosolic sulforaphenases [51] and catalyses the transfer of a sulfonate radical to the 3-hydroxy group of estrogens using 3’-phosphoadenosine 5’-phosphosulfate as a donor for the sulfonate group; it is well accepted that estrogen sulfonation diminishes the estrogen receptor binding activity **(SEEM 4)**. Okate reported that estrogen sulforaphenase (SULT 1E1), from both human recombinant sources and human mammary epithelial cells, was inhibited competitively by quercetin [12] and resveratrol [24], with IC\textsubscript{50} values of 0.61 μM and 0.36 μM, respectively, using recombinant estrogen sulforaphenase [52]. Phenol sulforaphenases (SULT 1A1/2, SULT 1A3, SULT 2A1) [53], [54] also sulfate estrogens at physiologically relevant concentrations and may represent a route for estrogen sulfation in some mammary tumour cells lines that lack estrogen sulforaphenase. SULT 1A1 was reported to sulfate phytoestrogens, e.g., isoflavonoids (1, 2, 4, 9) and flavonoids (11, 12, 13, 16, 17, 21, 22, 23) with IC\textsubscript{50} < 0.5 μM [18], [55], [56], [57] and genistein (1), daidzein (2), quercetin (8) and luteolin (23) inhibited SULT activity against a physiological concentration of estradiol with IC\textsubscript{50} values in the low micromolar range [57].

**Dietary Phytoestrogens: Potential Selective Estrogen Enzyme Modulators?**

Only partial information is available on the quantities of dietary phytoestrogens that are consumed daily throughout the world [58] and the bioavailability is clearly a crucial factor influencing the biological activity of these compounds [58], [59], [60], [61]. After absorption mainly as glycosides and hydrolysis of the sugar moiety, phytoestrogens are reconjugated predominantly to gluconic acid and to a lesser degree to sulfuric acid. Only a small portion of the free aglycone has been detected in blood, demonstrating that the rate of conjugation is high. Circulating levels of phytoestrogens (conjugated and unconjugated forms) in adults may reach 4 – 6 μM after ingestion large amounts of fruits, vegetables or soy derivatives [58], [60], a level which seems insufficient to inhibit in vivo aromatase and 17β-hydroxysteroid dehydrogenase. This hypothesis could be contradicted in at least two cases: the ingestion of high dose dietary supplements (>> 50 mg phytoestrogens/day) and the special case of infants. Concentrated isoflavones are available in pill form and are sold extensively in health food stores and on the internet. Recently, the ability of chrysin to inhibit aromatase has led to its marketing as a high-dose dietary supplement for body building and is subject to industrial development [62]. Soy milk is consumed in large quantities by babies or infants who are allergic to cow milk. Isoflavone intake can reach, when expressed relative to body weight, 5 – 10-fold the dose shown to exert a physiological effect on the hormononal regulation of women’s menstrual cycles [63].

Sulforaphenases are at least 10 times more sensitive to inhibition by flavonoids or isoflavonoids than aromatase or 17β-hydroxysteroid dehydrogenase and these enzymes may be inhib-
Phytoestrogens and, more generally, natural compounds are a mine for drug design and many of the molecules described can be viewed as templates for molecular modifications [15], [64], [65], [66]. Fig. 3 summarizes the recent developments in the research of structurally-related compounds as therapeutic agents. The development of dual inhibitors (sulfatase/aromatase, for example) could constitute a priority approach [68].

Another major question for the future is the biological evaluation of new emerging phytoestrogens such as prenylated flavonoids from hops [77], [78], terpenoids from black cohosh [79], [80] and liquorice [81], [82], saponins from Tribulus terrestris [83] and coumarins from Dong Quai [84] (Fig. 4). At this time, only few papers have appeared on steroid biosynthesis inhibition by such compounds [31], [85], [86], [87], [88], [89].

**Concluding Remarks**

Phytoestrogens are endocrine disruptors according to the definition of the European Commission. In view of the current data, phytoestrogens appear to have steroidal potency not only by acting on steroids receptors but also by modulating steroidogenesis enzymes; there are indications that potential risks may be present, but the magnitude of these risks cannot be determined exactly due to the limited or controversial data available. As concluded recently by Cos in a previous review [7], it is still premature to recommend specific amounts of dietary phytoestrogens.

**SEEM 1 : sulfatase inhibitors**

![Diagram of SEEM 1 sulfatase inhibitors](image)

- (27) OH OH OSO$_3$NH$_2$ inhibition of steroid sulfatase (at 10 μM) 93% [67]
- (28) OH OSO$_3$NH$_2$ OMe inhibition of steroid sulfatase (at 10 μM) 51% [67]
- (29) H OSO$_3$NH$_2$ H H inhibition of steroid sulfatase (at 10 μM) 90% [67]
- (30) OH H H OSO$_3$NH$_2$ 99% [68]

**SEEM 2 : 17β-HSD inhibitors**

(see also Refs. [69], [70], [71])

- (31) inhibition of 17β-HSD : 50,2% [72]
- (32) ctenoacetone precursor [73]

**SEEM 3 : Aromatase inhibitors**

- (33) IC$_{50}$ = 0.041 μM [74]
- (34) IC$_{50}$ = 0.62 μM [75]
- (35) R$_1$ = OMe ; R$_2$ = (4-pyridyldimethyl)
  IC$_{50}$ = 0.22 μM [76]
- (36) R1 = OH ; R$_2$ = (4-pyridyldimethyl)
  IC$_{50}$ = 0.28 μM [76]

Fig. 3 Structurally related compounds as potent selective estrogen enzyme modifiers (SEEMs).
References

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Fig. 4 Examples of emerging phytoestrogens.

(37) 27-deoxyacetin from black cohosh

(38) glycyrrhizic acid from liquorice

(39) protodioscin from Tribulus terrestris

(40) ligustilide from Dong Quai


89 Anderson ML. Inhibiting aromatase with specific dietary supplements. US Patent 2004156926; 2004