Jean-Philippe Basly[1]
Marie-Chantal Canivenc Lavier[2]

Dietary Phytoestrogens: Potential Selective Estrogen Enzyme Modulators?

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 intacrine 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 *s*elective *e*strogen *r*eceptor *m*odulators (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 (E_1) and testosterone (T) to estradiol (E_2) and (ii) the "sulfatase pathway" which converts estrone sulfate (E_1S) into estrone (E_1); estrone is then transformed into estradiol (E_2) by 17β-hydroxysteroid dehydrogenase type 1. By analogy with a selected estrogen receptor modulator (SERM), the concept of a *s*elective *e*strogen *e*nzyme *m*odulator (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].

Affiliation
[1] Faculté de Pharmacie, Université de Limoges, Limoges, France.
[2] UMR 1234, Toxicologie Alimentaire, INRA, Dijon, France

Correspondence
Dr. Jean-Philippe Basly · Faculté de Pharmacie · Université de Limoges · 2 rue du docteur Marcland · 87025 Limoges · France · Phone/Fax: +33-555-43-58-98 · E-mail: basly@pharma.unilim.fr

Received July 14, 2004 · **Accepted** January 7, 2005

Bibliography
Planta Med 2005; 71: 287–294 · © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2005-864092
ISSN 0032-0943

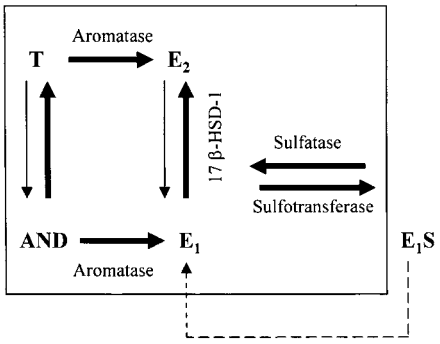


Fig. **1** Enzymatic steps involved in the synthesis of estrogenic steroids: the concept of selective estrogen enzyme modulator (SEEM; from Refs. [3] and [4]). 17β-HSD-1 = 17β-hydroxysteroid dehydrogenase type 1. **SEEM-I** inhibits the estrone sulfatase; **SEEM-II** inhibits the 17β-HSD-1; **SEEM-III** inhibits the aromatase activities; and **SEEM-IV** stimulates the estrone sulfotransferase activity. T: testosterone; AND: androstenedione; E_1: estrone; E_2: estradiol; E_1S: 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 formonetin (**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), flavanols (catechins, epicatechins, gallocatechins, epigallocatechins), anthocyanidins, chalcones, flavanones 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′-trihydroxystilbene), 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

Sulfatases 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 IC_{50} 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 IC_{50} 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 (IC_{50} > 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 estrone 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 positional 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-

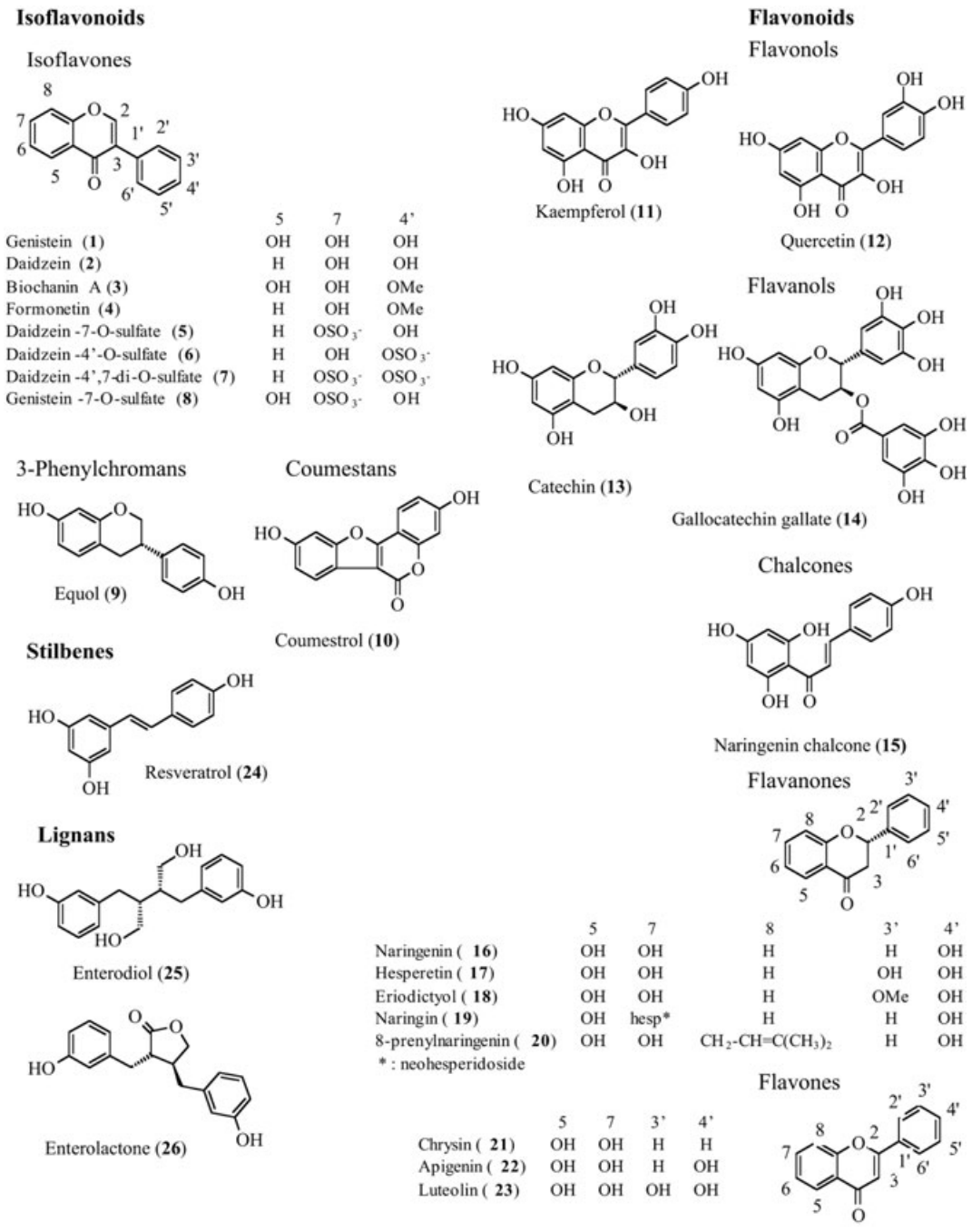


Fig. **2** Chemical structures of phytoestrogens discussed in this review.

marily responsible for estradiol biosynthesis in gonads and in peripheral tissues. Several phytoestrogens, i.e., genistein (**1**) (IC_{50} ~ 1.2 μM), daidzein (**2**) (IC_{50} ~ 1.2 μM), coumestrol (**10**) (IC_{50} ~ 0.12 μM), naringenin (**16**) (IC_{50} ~ 1.2 μM) and apigenin (**22**) (IC_{50} ~ 0.12 μM) were found to inhibit significantly estrone reduction catalysed by purified 17β-HSD1 or cultured T-47 D cells [25]. Le Bail [26], [27], [28] found almost similar results although these authors used human placental microsomes instead of purified enzyme for inhibition studies. On the contrary, Vinh [29] found no inhibitory activity for genistein even at 100 μM.

Recent works were focused on type 5 17β-HSD inhibition. This isoform is expressed in reproductive and hormone target tissues including breast tissues [24] and catalyses the conversion of androstenedione to testosterone [30]. Phytoestrogens, i.e., biochanin A (**3**), coumestrol (**10**) and quercetin (**12**), are potent inhibitors in the micromolar range [31], [32].

SEEM 3: Aromatase

Aromatase converts the Δ4 – 3-one ring of C_{19} 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 peroxy intermediate model remaining the most accepted hypothesis [35]. Several main groups of phytoestrogens, e.g., flavones, flavanones and lignans modulate aromatase activity *in vitro*. An early study made by Kellis and Vickery [36] reported inhibition by chrysin (**21**) (IC_{50} = 0.5 μM) and apigenin (**22**) (IC_{50} = 1.2 μM). Numerous studies had 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**), erodictyol (**18**) and naringin (**19**) or flavanone 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 2*S*-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**), gallocatechin gallate (**14**)] [44], [45] or flavonols [kaempferol (**11**), quercetin (**12**)] are inactive or weak inhibitors [37], [38], [40].

The aromatase inhibition effect of isoflavanoids has been examined and these compounds were reported to be inactive in several studies [26], [27], [38], [39], [42], [46]. But, recently Almstrup [43] found that biochanin A (3) and formonetin (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_{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 prosthetic 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: Sulfotransferases

The concept that steroid-modifying enzymes such as estrogen sulfotransferase (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 sulfotransferase (SULT 1E1) belongs to the family of cytosolic sulfotransferases [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**). Otake reported that estrogen sulfotransferase (SULT 1E), from both human recombinant sources and human mammary epithelial cells, was inhibited competitively by quercetin [12] and resveratrol [24], with IC_{50} values of 0.61 μM and 0.36 μM, respectively, using recombinant estrogen sulfotransferase [52]. Phenol sulfotransferases (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 sulfotransferase. 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_{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 IC50 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 glucuronic 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 soya 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 hormonal regulation of women's menstrual cycles [63].

Sulfotransferases 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-

ited by concentrations that occur *in vivo*. If the effects of phytoestrogens were exerted in breast tissues, phytoestrogen ingestion might contribute to an unfavourable ratio of estrogen/estrogen sulfate in tissues leading to the proliferation of hormone-dependent tumour cells [18]. Hence, as underlined by Harris [18], there is an urgent need for further work to clarify the influence of phytoestrogens on the sulfation/desulfation processes.

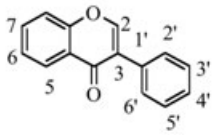
Phytoestrogens and, more generally, natural compounds are a mine for drug design and many of the molecules described can be viewed as templates for molecular modifications [15], [64], [65], [66]. Fig. **3** summarizes the recent developments in the research of structurally-related compounds as therapeutic 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].

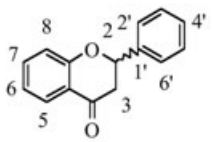
Concluding Remarks

Phytoestrogens are endocrine disrupters 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.

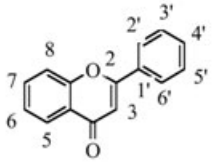
SEEM 1 : sulfatase inhibitors



	5	7	4'	inhibition of steroid sulfatase (at 10 µM)	reference
(27)	OH	OH	OSO_2NH_2	93 %	[67]

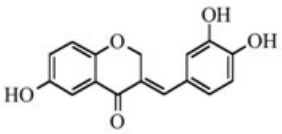


	5	7	4'	inhibition of steroid sulfatase (at 10 µM)	reference
(28)	OH	OSO_2NH_2	OMe	51 %	[67]



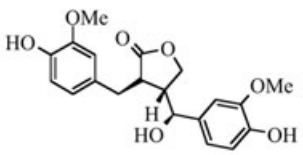
	5	6	7	4'	inhibition of steroid sulfatase (at 10 µM)	reference
(29)	H	OSO_2NH_2	H	H	90%	[67]
(30)	OH	H	H	OSO_2NH_2	99%	[68]

SEEM 2 : 17β-HSD inhibitors
(see also Refs. [69], [70], [71])

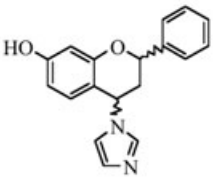


(**31**) inhibition of 17 β-HSD : 50,2% [72]

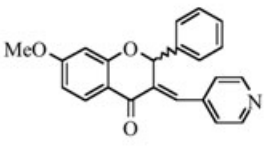
SEEM 3 : Aromatase inhibitors



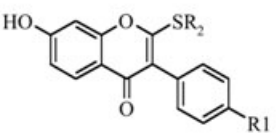
(**32**) enterolactone precursor [73]



(**33**) IC_{50} = 0.041 µM [74]

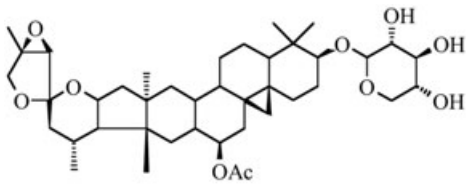


(**34**) IC_{50} = 0.62 µM [75]

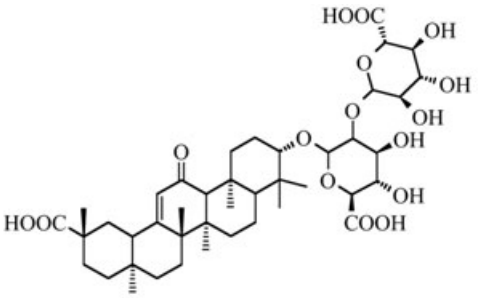


(**35**) R_1 = OMe ; R_2 = (4'-pyridyl)methyl
IC_{50} = 0.22 µM [76]
(**36**) R1 = OH ; R_2 = (4'-pyridyl)methyl
IC_{50} = 0.28 µM [76]

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

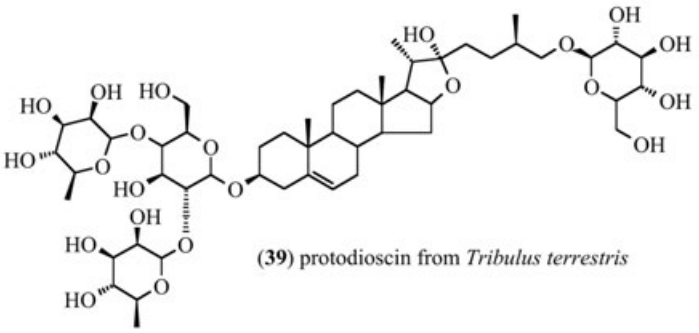


(37) 27-deoxyactein from black cohosh

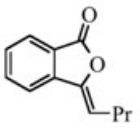


(38) glycyrrhizic acid from liquorice

Fig. **4** Examples of emerging phytoestrogens.



(39) protodioscin from *Tribulus terrestris*



(40) ligustilide from Dong Quai

References

[1] Brzezinski A, Debi A. Phytoestrogens: the "natural" selective estrogen receptor modulators? Eur J Obstet Gynecol Reprod Biol 1999; 85: 47 – 51

[2] Thijssen JHH. Local biosynthesis and metabolism of estrogens in the human breast. Maturitas 2004; 49: 25 – 33

[3] Chetrite GS, Pasqualini JR. The selective estrogen enzyme modulator (SEEM) in breast cancer. J Steroid Biochem Molec Biol 2001; 76: 95 – 104

[4] Pasqualini JR. The selective estrogen enzyme modulators in breast cancer: a review. Biochim Biophys Acta 2004; 1654: 123 – 43

[5] Diel P, Smolnikar K, Michna H. *In vitro* systems for the evaluation of the estrogenic activity of natural products. Planta Medica 1999; 65: 197 – 203

[6] Ososki AL, Kenelly EJ. Phytoestrogens: a review of the present state of research. Phytother Res 2003; 17: 845 – 69

[7] Cos P, De Bruyne T, Apers S, Vanden Bergue D, Pieters L, Vlietinck AJ. Phytoestrogens: Recent developments. Planta Medica 2003; 69: 589 – 99

[8] Miksicek RJ. Estrogenic flavonoids: structural requirements for biological activity. Proc Soc Exp Biol Med 1995; 208: 44 – 50

[9] Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van der Saag PT, Van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998; 10: 4252 – 63

[10] Rosenberg-Zand RJ, Jenkins DJA, Diamandis EP. Steroid hormone activity of flavonoids and related compounds. Breast Cancer Res Treat 2000; 62: 35 – 49

[11] Branham WS, Dial SL, Moland RG, Hass BS, Blair RM, Fang H, Shi L, Tong W, Perkins RG, Sheenan DM. Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. J. Nutr 2002; 132: 658 – 64

[12] Zierau O, Gester S, Schwab P, Metz P, Kolba S, Wulf M, Vollmer G. Estrogenic activity of the phytoestrogens naringenin, 6-(1,1-dimethylallyl)-naringenin and 8-prenylnaringenin. Planta Medica 2002; 68: 449 – 51

[13] Diel P, Thomae RB, Caldarelli A, Zierau O, Kolba S, Schmidt S, Schwab P, Metz P, Vollmer G. Regulation of gene expression by 8-prenylnaringenin in uterus and liver of Wistar rats. Planta Medica 2004; 70: 39 – 44

[14] Gehm BD, McAndrews JM, Chien PY, Jameson JL. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc Natl Acad Sci USA 1997; 94: 14138 – 43

[15] Dixon RA. Phytoestrogens. Annu Rev Plant Biol 2004; 55: 225 – 61

[16] Wang LQ. Mammalian phytoestrogens: enterodiol and enterolactone. J Chromatogr B 2002; 777: 289 – 309

[17] Poirier D, Ciobanu LC, Maltais R. Steroid Sulfatase inhibitors. Exp Opinion Ther Patents 1999; 9: 1083 – 99

[18] Harris RM, Wood DM, Bottomley L, Blagg S, Owen K, Hughes K, Waring RH, Kirk CJ. Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. J Clin Endocrinol Metab 2004; 89: 1779 – 87

[19] Wong CK, Keung WM. Daidzein sulfoconjugates are potent inhibitors of sterol sulfatase (EC 3.1.6.2). Biochem Biophys Res Commun 1997; 233: 579 – 83

[20] Clarke DB, Lloyd AS, Botting NP, Oldfield MF, Needs PW, Wiseman H. Measurement of intact sulfate and glucuronide phytoestrogen conjugates in human urine using isotope dilution liquid chromatography-tandem mass spectrometry with [$^{13}C_3$]isoflavone internal standards. Anal Biochem 2002; 309: 58 – 72

[21] Huang Z, Fasco MJ, Kaminsky LS. Inhibition of estrone sulfatase in human liver microsomes by quercetin and other flavonoids. J Steroid Biochem Molec Biol 1997; 63: 9 – 15

[22] Mindich R, Möller G, Adamski J. The role of 17-beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 2004; 218: 7 – 20

[23] Sasano H, Suzuki T, Takeyama J, Utsunomiya H, Ito K, Ariga N, Moriya T. 17-beta-hydroxysteroid dehydrogenase in human breast and endometrial carcinoma. A new development in intracrinology. Oncology 2000; 59: 5 – 12

[24] Oduwole OO, Li Y, Isomaa VV, Mäntyniemi A, Pulkka AE, Sioni Y, Vihko PT. 17ß-Hydroxysteroid dehydrogenase type 1 is an independent prognostic marker in breast cancer. Cancer Res 2004; 64: 7604 – 9

[25] Mäkelä S, Poutainen M, Kostian ML, Lehtimäki N, Strauss L, Santti R, Vihko R. Inhibition of 17β-hydroxysteroid oxidoreductase by flavonoids in breast and prostate cancer cells. Proc Soc Exp Biol Med 1998; 217: 310 – 6

[26] Le Bail JC, Laroche T, Marre-Fournier F, Habrioux G. Aromatase and 17β-hydroxysteroid dehydrogenase by flavonoids. Cancer Lett 1998; 133: 101 – 5

[27] Le Bail JC, Champavier Y, Chulia A, Habrioux G. Effects of phytoestrogens on aromatase, 3β-hydroxysteroid dehydrogenase Δ5/Δ4 isomerase, 17β-hydroxysteroid dehydrogenase activities and human breast cancer cells. Life Sci 2000; 66: 1281 – 91

[28] Le Bail JC, Pouget C, Fagnere C, Basly JP, Chulia AJ, Habrioux G. Chalcones are potent inhibitors of aromatase and 17β-hydroxysteroid dehydrogenase activities. Life Sci 2001; 68: 751 – 61

[29] Vinh TK, Nicholls PJ, Kirby AJ, Simons C. Evaluation of 7-hydroxy-flavones as inhibitors of oestrone and oestradiol biosynthesis. J Enz Inhib 2001; 16: 417 – 24
[30] Type 1 17β-HSD catalyses the conversion of androstenedione to testosterone but is mainly expressed in the testes and consequently is outside the scope of this review.
[31] Krazeisen A, Breitling R, Möller G, Adamski J. Phytoestrogens inhibit human 17beta-hydroxysteroid dehydrogenase type 5. Mol Cell Endocrinol 2001; 171: 151 – 62
[32] Krazeisen A, Breitling R, Möller G, Adamski J. Human 17beta-hydroxysteroid dehydrogenase type 5 is inhibited by dietary flavonoids. Adv Exp Med Biol 2002; 505: 151 – 61
[33] Harada N. Aromatase and intracrinology of estrogen in hormone-dependent tumors. Oncology 1999; 57: 7 – 16
[34] Chetrite GS, Corto-Prieto J, Philippe JC, Wright F. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Molec Biol 2000; 72: 23 – 7
[35] Akhtar M, Calder MR, Corina DL, Wright JN. Mechanistic studies on C-19 demethylation in oestrogen biosynthesis. Biochem J 1982; 201: 569 – 80
[36] Kellis JT, Vickery LE. Inhibition of human estrogen synthetase (aromatase) by flavones. Science 1984; 225: 1032 – 4
[37] Moochhala SM, Loke KH, Das NP. Spectral perturbation of human microsomal cytochrome P-450 by flavonoid binding. Biochem J 1988; 17: 755 – 62
[38] Wang C, Mäkelä T, Hase T, Adlercreutz H, Kurzer MS. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. J Steroid Biochem Molec Biol 1994; 50: 205 – 12
[39] Jeong HJ, Shin YG, Kim IH, Pezzuto JM. Inhibition of aromatase activity by flavonoids. Arch Pharm Res 1999; 22: 309 – 12
[40] Stresser DM, Turner SD, McNamara J, Stocker P, Miller VP, Crespi CL, Patten CJ. A high-throughput screen to identify inhibitors of aromatase (CYP19). Anal Biochem 2000; 284: 427 – 30
[41] Sanderson JT, Hordijk J, Denison MS, Springsteel MF, Nantz MH, van den Berg M. Induction and inhibition of aromatase (CYP19) activity by natural and synthetic flavonoid compounds in H295R human adrenocortical carcinoma cells. Toxicol Sci 2004; 82: 70 – 9
[42] Kao YC, Zhou CB, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site-directed mutagenesis study. Environ Health Persp 1998; 106: 85 – 92
[43] Almstrup K, Fernandez MF, Petersen JH, Olea N, Skakkeboæk NE, Leffers H. Dual effects of phytoestrogens result in U-shaped dose-response curves. Environ Health Persp 2002; 110: 743 – 8
[44] Campbell DR, Kurzer MS. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. J Steroid Biochem Molec Biol 1993; 46: 381 – 88
[45] Satoh K, Sakamoto Y, Ogata A, Nagai F, Mikuriya H, Numazawa M, Yamada K, Aoki N. Inhibition of aromatase activity by green tea extract catechins and their endocrinological effects of oral administration in rats. Food Chem Toxicol 2002; 40: 925 – 33
[46] White EL, Ross LJ, Steele VE, Kelloff GJ, Hill DL. Screening of potential cancer preventing chemicals as aromatase inhibitors in an *in vitro* assay. Anticancer Res 1999; 19: 1017 – 20
[47] Adlercreutz H, Bannwart C, Wähälä K, Mäkelä T, Brunow G, Hase T, Arosema PJ, Kellis JT, Vickery LE. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J Steroid Biochem Molec Biol 1993; 44: 147 – 53
[48] Ibrahim AR, Abul-Hajj YJ. Aromatase inhibition by flavonoids. J Steroid Biochem Molec Biol 1990; 34: 257 – 60
[49] Saarinen N, Joshi SC, Ahotupa M, Li X, Ammälä J, Mäkelä S, Santti R. No evidence for the in vivo activity of aromatase-inhibiting flavonoids. J Steroid Biochem Molecul Biol 2001; 78: 231 – 9
[50] Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MW, Bergman A, Safe SH, Kuiper GGJM, Schuur AG, Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 2000; 141: 1897 – 900
[51] Strott CA. Sulfonation and molecular action. Endocrin Rev 2002; 23: 703 – 732
[52] Otake Y, Nolan AL, Walle K, Walle T. Quercetin and resveratrol potently reduce estrogen sulfotransferase activity in normal human mammary epithelial cells. J Steroid Biochem Molec Biol 2000; 73: 265 – 70
[53] Falany JL, Falany CN. Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. Cancer Res 1996; 56: 1551 – 5
[54] Glatt H, Boeing H, Engelke CEH, Ma L, Kuhlow A, Pabel U, Pomplun D, Teubner W, Meinl W. Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. Mutation Res 2001; 482: 27 – 40
[55] Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T. Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. Drug Metab Dispos 1996; 24: 232 – 7
[56] Ghazali RA, Waring RH. The effects of flavonoids on human phenolsulphotransferases: potential in drug metabolism and chemoprevention. Life Sci 1999; 65: 1625 – 32
[57] Kirk CJ, Harris RM, Wood DM, Waring RH, Hughes PJ. Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous oestrogens? Biochem Soc Trans 2001; 29: 209 – 16
[58] Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Popyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79: 727 – 47
[59] Zhu M, Chen Y, Li RC. Oral absorption and bioavailability of tea catechins. Planta Medica 2000; 66: 444 – 7
[60] Rowlands I, Faughnan M, Hoey L, Wähälä K, Williamson G, Cassidy A. Bioavailability of phytoestrogens. Brit J Nutr 2003; 89: S45S58
[61] Spencer JPE, Abd El Mohsen M, Rice-Evans C. Cellular uptake and metabolism of flavonoids and their metabolites: implication for their bioavailability. Arch Biochem Biophys 2004; 423: 148 – 61
[62] Zeligs MA, Jacobs IC. Compositions and methods of adjusting steroid hormone metabolism through facilitated absorption of hydrophobic dietary compounds. US Patent 6,086,915, 2000,
[63] Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet 1997; 350: 23 – 7
[64] Bhat KPL, Pezzuto JM. Natural modulators of estrogen biosynthesis and function as chemopreventive agents. Arch Pharm Res 2001; 24: 73 – 84
[65] Kinghorn AD, Su BN, Jang DS, Chang LC, Lee D, Gu JQ, Carcache-Blanco EJ, Pawlus AD, Lee SK, Park EJ, Cuendet M, Gills JJ, Bhat K, Park HS, Mata-Greenwood E, Song LL, Jang M, Pezzuto JM. Natural inhibitors of carcinogenesis. Planta Medica 2004; 70: 691 – 705
[66] Nicolaou KC, Pfefferkorn JA, Roecker AJ, Cao GQ, Barluenga S, Mitchell HJ. Natural products-like combinatorial libraries based on privileged structures. 1. General principles and solid-phase synthesis of benzopyrans. J Am Chem Soc 2000; 122: 9939 – 53
[67] Reed JM, Potter BVL. Compounds with a sulfamate group. WO 9732872, 1997
[68] Reed JM, Potter BVL. Preparation of flavone, isoflavone and flavanone sulfamates as estrone sulfatase and/or aromatase inhibitors for treatment of breast and endometrial cancers. US Patent 6,187,766, 2003
[69] Yoshihama M, Nakakoshi M, Nakamura J, Nakayama S. Preparation of novel benzofuranone derivatives as remedies for hormone-dependent diseases. WO 9830556, 1998
[70] Hoffren AM, Murray CM, Hoffmann RD. Structure-based focusing using pharmacophores derived from the active site of 17β-hydroxysteroid dehydrogenase. Curr Pharm Des 2001; 7: 547 – 66
[71] Gobec S, Sova M, Kristan K, Rizner TL. Cinnamic acid esters as potent inhibitors of fungal 17β-hydroxysteroid dehydrogenase – a model enzyme of the short-chain dehydrogenase/reductase superfamily. Bioorg Med Chem Lett 2004; 14: 3933 – 6
[72] Yoshihama M, Nakakoshi M, Nakamura J, Nakayama S. Preparation of novel tetralone and benzopyranone derivatives as 17β-HSD inhibitors. WO 9832724, 1998
[73] Saarinen NM, Warri A, Makela SI, Eckerman C, Reunanen M, Ahotupa M, Salmi SM, Franke AA, Kangas L, Santti R. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from a coniferous tree (Picea abies). Nutr Cancer 2000; 36: 207 – 14
[74] Pouget C, Fagnere C, Basly JP, Habrioux G, Chulia AJ. Design, synthesis and evaluation of 4-imidazolylflavans as new leads for aromatase inhibition. Bioorg Med Chem Lett. 2002; 12: 2859 – 61
[75] Pouget C, Fagnere C, Basly JP, Habrioux G, Chulia AJ. New aromatase inhibitors. Synthesis and inhibitory activity of pyridinyl-substituted flavanone derivatives. Bioorg Med Chem Lett 2002; 12: 1059 – 61
[76] Kim YW, Hackett JC, Brueggemeier RW. Synthesis and aromatase inhibitory activity of novel pyridine-containing isoflavones. J Med Chem 2004; 47: 4032 – 40

[77] Kitaoka M, Kadokawa H, Sugano M, Ichikawa K, Taki M, Takaishi S, Iijima Y, Tsutsumi S, Boriboon M, Akiyama T. Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure-activity relationship. Planta Medica 1998; 64: 511 – 5
[78] Miyamoto M, Matsushita Y, Kiyokawa A, Fukuda C, Iijima Y, Sugano M, Akiyama T. Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 2). Estrogenic effects of 8-isopentenylnaringenin on bone metabolism. Planta Medica 1998; 64: 516 – 9
[79] Jarry H, Harnischfeger G, Duker E. The endocrine effects of constituents of *Cimicifuga racemosa*. 2. *In vitro* binding of constituents to estrogen receptors. Planta Medica 1985; 51: 316 – 9
[80] He K, Zheng B, Kim CH, Rogers L, Zheng Q. Direct analysis and identification of triterpene glycosides by LC/MS in black cohosh, *Cimicifuga racemosa*, and in several commercially available black cohosh products. Planta Medica 2000; 66: 635 – 40
[81] Takino Y, Koshioka M, Shiokawa M, Ishii Y, Maruyama S, Higashino M, Hayashi T. Quantitative determination of glycyrrhizic acid in liquorice roots and extracts by TLC-densitometry. Planta Medica 1979; 36: 74 – 8
[82] Liu J, Burdette JE, Xu H, Gu C, van Breemen RB, Bhat KP, Booth N, Constantinou AI, Pezzuto JM, Fong HH, Farnsworth NR, Bolton JL. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. J Agric Food Chem 2001; 49: 2472 – 9
[83] Hu K, Yao X. Protodioscin (NSC-698 796): its spectrum of cytotoxicity against sixty human cancer cell lines in an anticancer drug screen panel. Planta Medica 2002; 68: 297 – 301
[84] Lu GH, Chan K, Chan CL, Leung K, Jiang ZH, Zhao ZZ. Quantification of ligustilides in the roots of *Angelica sinensis* and related umbelliferous medicinal plants by high-performance liquid chromatography and liquid chromatography-mass spectrometry. J Chromatogr A 2004; 1046: 101 – 7
[85] Makela S, Poutanen M, Lehtimaeki J, Kostian ML, Santti R, Vihko R. Estrogen-specific 17 beta-hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. Proc Soc Exp Biol Med 1995; 208: 51 – 9
[86] Coldham NG, Sauer MJ. Identification, quantitation and biological activity of phytoestrogens in a dietary supplement for breast enhancement. Food Chem Toxicol 2001; 39: 1211 – 24
[87] Josephs RA, Guinn JS, Jennifer S, Harper ML, Askari F. Liquorice consumption and salivary testosterone concentrations. Lancet 2001; 358: 1613 – 4
[88] Armanini D, Bonanni G, Mattarello MJ, Fiore C, Sartorato P, Palermo M. Licorice consumption and serum testosterone in healthy man. Exp Clin Endocrinol Diabetes 2003; 111: 341 – 3
[89] Anderson ML. Inhibiting aromatase with specific dietary supplements. US Patent 2 004 156 926, 2004