

Natural Compounds with Aromatase Inhibitory Activity: An Update

Authors

Marcy J. Balunas^{1,2,3,4}, A. Douglas Kinghorn⁵

Affiliations

The affiliations are listed at the end of the article

Key words

- breast cancer
- aromatase
- natural compounds
- plants
- marine organisms
- fungi

Abstract

Several synthetic aromatase inhibitors are currently in clinical use for the treatment of postmenopausal women with hormone-receptor positive breast cancer. However, these treatments may lead to untoward side effects and so the search for new aromatase inhibitors continues, especially those for which the activity is pro-

moter-specific, targeting the breast-specific promoters I.3 and II. Recently, numerous natural compounds have been found to inhibit aromatase in noncellular, cellular, and *in vivo* studies. These investigations, covering the last two years, as well as additional studies that have focused on the evaluation of natural compounds as promoter-specific aromatase inhibitors or as aromatase inducers, are described in this review.

Introduction

Breast cancer is one of the leading causes of death in women in developed countries and is a growing public health concern in developing countries as well [1, 2]. Estrogens and the estrogen receptor are widely known to play an important role in breast cancer development and progression. Aromatase, a CYP19 enzyme, is the rate-limiting enzyme in the conversion of testosterone and androstenediol to the estrogens, estrone and estradiol. Aromatase inhibitors (AIs) have been shown to significantly reduce, and in most cases eliminate, the production of estrogens in postmenopausal women.

Aromatase inhibitors have shown a considerable clinical impact on the development and progression of breast cancer [3]. A recent study by the Cochrane Collaboration compared AIs with non-AI treatments of primary breast cancer, as well as comparing AIs to each other using data from 37 clinical trials [4]. It was found that AIs were advantageous over other types of endocrine therapies (e.g., tamoxifen), with the three clinically available AIs (anastrozole, exemestane, and letrozole) showing similar benefits on patient survival. The Cochrane Collaboration report also discussed the toxicity profiles of AIs in comparison with other types of endocrine therapies and found that AIs resulted in an increase of rash, diarrhea, and vomiting but a decrease in vaginal bleeding and

thromboembolism. Overall, the study found that AIs provided an increased survival benefit over other endocrine therapies, with acceptable toxicity profiles. Many physicians have begun prescribing AIs as a first-line treatment in postmenopausal breast cancer patients [3].

So if there are already clinically available AIs, why search for additional compounds that inhibit aromatase, inclusive of natural products? AIs do have several side effects, such as those listed above, but also lead to more severe side effects including effects on the bones, brain, and heart [5, 6]. Natural products with a long history of use, such as those from foods or from traditional medicines, that also exhibit aromatase inhibitory activity, may have less associated toxicity. Furthermore, natural compounds might help in the search for promoter-specific AIs, a new direction in AI research, and thus selectively target aromatase in the breast and reduce systemic toxicity.

This review summarizes the most recent advances in the search for natural product aromatase inhibitors. We focus primarily on natural compounds that have been reported in the two year period (February 2008 – January 2010) since the publication of our previous comprehensive review of natural product AIs that covered all of the relevant literature up to January 2008 [7]. Discussion of active compounds is organized based on aromatase activity in enzyme versus cellular and *in vivo* bioassays since the level of information provided by

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Correspondence

Dr. Marcy J. Balunas
Division of Medicinal Chemistry,
Department of Pharmaceutical
Sciences
School of Pharmacy
69 North Eagleville Road,
Unit 3092
Storrs, CT 06269
USA
Phone: + 1 86 0486 4066
Fax: + 1 86 0486 5792
marcy.balunas@uconn.edu

these types of assays can be drastically different. These more recent accounts will then be compared with the most potent active natural product AIs summarized in our previous review [7], followed by a discussion of the current research trends in AI research and future directions for natural product AIs.

Natural Products Active against Aromatase

Since January 2008, 31 natural product compounds have been reported with aromatase inhibitory activity (Tables 1, 2 and Figs. 1, 2). For the purpose of this review, activity will be defined as follows: in noncellular assays, strongly active compounds are those with IC_{50} values of $< 5 \mu M$, moderately active substances having IC_{50} values in the range of $> 5-10 \mu M$, and weakly active compounds with IC_{50} values of $> 11-25 \mu M$. In cellular assays, "strongly active" refers to compounds with an IC_{50} value of $< 10 \mu M$, "moderately active", $IC_{50} > 10-20 \mu M$, and "weakly active", $IC_{50} > 20-50 \mu M$. Not all reports have included an IC_{50} value and these will be treated on an individual basis. Compounds are numbered according to their position in the tables, where they are organized based on compound class. In the text, compounds are discussed based on their levels of activity with the most active compounds discussed first followed in decreasing order of activity.

Compounds active in noncellular AI assays

In noncellular testing, 24 active AI natural compounds were reported during the past two years. As in the previous review of natural product AIs, flavonoids were the most commonly reported class of active compounds found in noncellular assays (12 in total including six flavones, three flavanones, one biflavanone, one chalcone, one isoflavone), with active compounds also found among the xanthone (3), chromanone (2), fatty acid (2), terpenoid (2), alkaloid (1), coumarin (1), and depsidone (1) classes (Table 1, Fig. 1).

These compounds were isolated mainly from terrestrial plants, including several from botanical dietary supplements (phyto-medicines), with one report of AIs from marine fungi and the isolation of an active AI from a terrestrial endophytic fungus. Other compounds found to inhibit aromatase were commercially purchased but are commonly found from natural source organisms. Several types of noncellular assays were utilized for screening these natural product AIs. The most commonly reported assay system was the radiometric tritiated water release assay using microsomes from different sources, typically from human placentas, although one report utilized microsomes from HepG2 cell lysate [8] and two other reports used commercially available Supersomes® [9,10]. The other type of noncellular AI assay involved the use of a fluorometric substrate [either *O*-benzylfluorescein benzyl ester (DBF) or 7-methoxy-4-trifluoromethylcoumarin (MFC)] with purified aromatase enzyme. Because the radiometric and fluorometric assays are not readily comparable, the results from each type of assay will be discussed separately. In the radiometric assays, the most active natural product compounds were two terpenoids isolated from *Commiphora myrrha* (myrrh) (Table 1, Fig. 1) [11]. Thus, 2-methoxy-5-acetoxylfuranogermacr-1(10)-en-6-one (20) and dehydroabietic acid (21) were both found to have AI IC_{50} values in the high nanomolar range (210 nM and 320 nM, respectively). However, compound 21 was also found to inhibit the growth of HUVEC (human umbilical vein epithelial) cells with an IC_{50} of 69 nm, indicating a strong

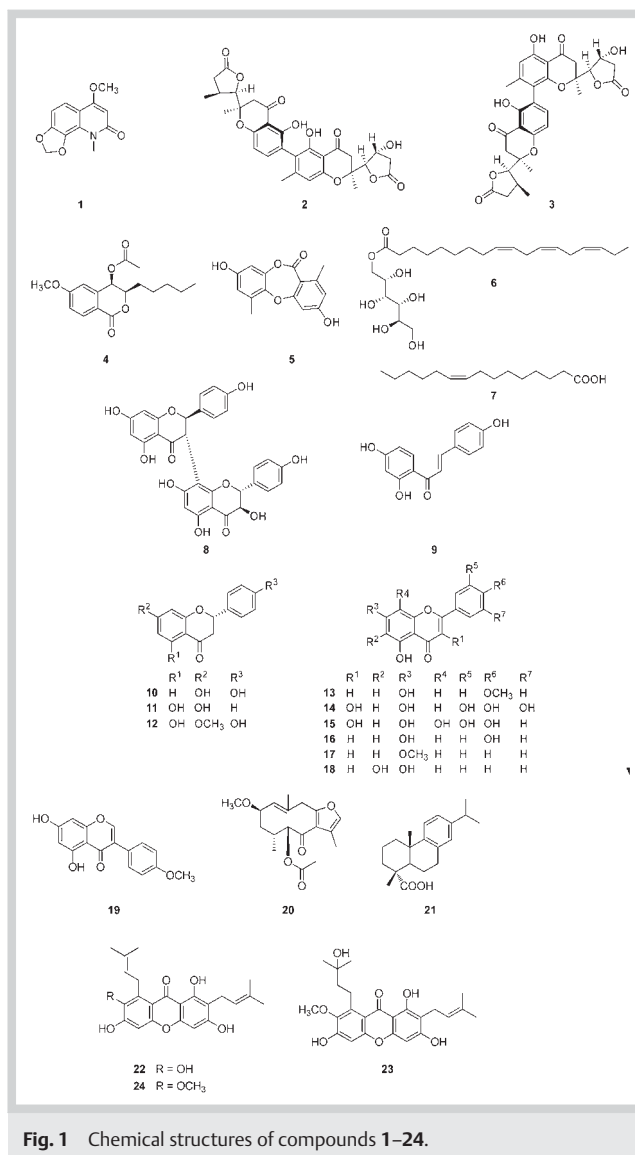


Fig. 1 Chemical structures of compounds 1–24.

cytotoxicity by this substance, which would most likely prohibit its further preclinical development as an AI. These compounds were further tested using an *in vitro* uterine contraction assay and found to be inactive.

Several other natural compounds were also found to be active in the noncellular radiometric assay (Table 1). Isoliquiritigenin (9), typically isolated from the botanical dietary supplement *Glycyrrhiza glabra* (licorice), was tested against a recombinant enzyme system involving human CYP19 Supersomes® and found to be strongly active with an AI IC_{50} of $3.8 \mu M$ [9]. In work carried out at The Ohio State University, three xanthones isolated from the widely-utilized botanical dietary supplement *Garcinia mangostana* (mangosteen), were found to be active in this assay, including the moderately active garcinone D (23, IC_{50} $5.2 \mu M$) and γ -mangostin (22, IC_{50} $6.9 \mu M$), and the weakly active α -mangostin (24, IC_{50} $20.7 \mu M$) [12]. These mangosteen xanthones were also tested in cellular AI assays and found to have varying levels of activity, as reported in the next section.

Two fatty acids, 9Z,12Z,15Z-octadecatrienoic acid sorbitol ester (6) [13], and linoleic acid (7) [14], were also found to be active in the noncellular radiometric AI assay. However, as previously re-

Table 1 Natural compounds active against aromatase in noncellular assays^a.

Compound Code	Source	Assay Type	Activity	Ref.
Alkaloid				
1	<i>Casimiroa edulis</i>	DBF enzyme ^b	3.92 μ M IC ₅₀	[19]
Chromanones				
2	<i>Monodictys putredinis</i>	DBF enzyme ^b	16.5 μ M IC ₅₀	[23]
3	<i>Monodictys putredinis</i>	DBF enzyme ^b	24.4 μ M IC ₅₀	[23]
Coumarin				
4	<i>Xyris pterygoblephara</i>	DBF enzyme ^b	1.6 μ M IC ₅₀	[17]
Depsidone				
5	<i>Corynespora cassicola</i> L36	MFC enzyme ^c	5.30 μ M IC ₅₀	[21]
Fatty Acids				
6	<i>Brassica campestris</i>	microsomes	7.80 μ g/mL IC ₅₀	[13]
7	<i>Brassaiopsis glomerata</i>	microsomes	7.4 PCA at 20 μ g/mL	[14]
Flavonoids				
Biflavanone				
8	<i>Garcinia kola</i>	DBF enzyme ^b	11.3 μ M IC ₅₀	[22]
Chalcone				
9	<i>Glycyrrhiza glabra</i> ^d	Supersomes [®]	3.8 μ M IC ₅₀	[9]
Flavanones				
10	commercial ^d	MFC enzyme ^c	0.34 μ M IC ₅₀	[16]
11	<i>Turnera diffusa</i>	HepG2 cell lysate	18.7 μ M IC ₅₀	[8]
12	<i>Prunus avium</i>	DBF enzyme ^b	1.2 μ M K _i	[20]
Flavones				
13	<i>Turnera diffusa</i>	HepG2 cell lysate	10.8 μ M IC ₅₀	[8]
14	commercial ^d	MFC enzyme ^c	10 μ M IC ₅₀	[16]
15	commercial ^d	MFC enzyme ^c	11 μ M IC ₅₀	[16]
16	commercial ^d	DBF enzyme ^b	1.0 μ M K _i	[20]
17	<i>Prunus avium</i>	DBF enzyme ^b	2.1 μ M K _i	[20]
18	commercial ^d	DBF enzyme ^b	5.1 μ M K _i	[20]
Isoflavone				
19	<i>Trifolium pratense</i> ^d	Supersomes [®]	12.5 μ M IC ₅₀	[10]
Terpenoids				
20	<i>Commiphora myrrha</i>	microsomes	0.21 μ M IC ₅₀	[11]
21	<i>Commiphora myrrha</i>	microsomes	0.32 μ M IC ₅₀	[11]
Xanthones				
22	<i>Garcinia mangostana</i>	microsomes	6.9 μ M IC ₅₀	[12]
23	<i>Garcinia mangostana</i>	microsomes	5.2 μ M IC ₅₀	[12]
24	<i>Garcinia mangostana</i>	microsomes	20.7 μ M IC ₅₀	[12]

^a The structures of compounds **1–24** are shown in **Fig. 1**; ^b DBF (O-benzylfluorescein benzyl ester) was used as substrate with purified aromatase enzyme; ^c MFC (7-methoxy-4-trifluoromethyl coumarin) was used as substrate with purified aromatase enzyme; ^d The source of the material in the literature reference was listed as commercial, but this compound is traditionally isolated from a natural product source

ported [15], unsaturated fatty acids have been shown to be interference compounds in non-cellular AI assays since they do not generally show activity in cellular AI screens. Another active AI natural product, the isoflavone biochanin A [**19**, purchased commercially but typically isolated from *Trifolium pratense* (red clover)], was tested using the same recombinant enzyme system using human CYP19 Supersomes[®] as mentioned above, resulting in weak activity with an AI IC₅₀ of 12.5 μ M [10]. Finally, using HepG2 cell lysate as the source of aromatase for their radiometric AI assay, Zhao and associates [8] isolated two compounds from *Turnera diffusa* (damiana), a plant which is purported to have aphrodisiac properties. They reported that the known flavonoids acacetin (**13**) and pinocembrin (**11**) exhibited AI IC₅₀ values of 10.8 and 18.7 μ M, respectively, causing them both to fall into the “weakly active” category, as delineated in this review.

In the noncellular fluorometric AI assays, the most active natural product reported in the last two years has been a commercially obtained sample of the licorice flavonoid, liquiritigenin (**10**, IC₅₀ 340 nM, [16]), using MFC as a substrate. This compound was initially found to be active using a molecular modeling program that

simulated the docking of the ligands in the active site of the aromatase enzyme. The investigators then evaluated their modeling hits using an *in vitro* fluorometric AI assay. Liquiritigenin (**10**) was the most active of the three hypothetical AI compounds tested, with myricetin (**14**, IC₅₀ 10 μ M) and gossypetin (**15**, IC₅₀ 11 μ M) being found to have more moderate or weak AI activity. Another strongly active compound in the fluorometric assay was the dihydroisocoumarin, (3*R*,4*R*)-(-)-6-methoxy-1-oxo-3-pentyl-3,4-dihydro-1*H*-isochromen-4-yl acetate (**4**), which exhibited an AI IC₅₀ of 1.6 μ M and was isolated from the Brazilian terrestrial plant *Xyris pterygoblephara* [17]. The quinolone alkaloid casimiroin (**1**) was originally isolated from *Casimiroa edulis* and found to inhibit both 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mutagenicity in *Salmonella typhimurium* and alveolar lesions in a mouse mammary gland organ culture *ex vivo* model (MMOC) [18]. In the course of follow-up synthetic and medicinal chemistry experiments, casimiroin was found to strongly inhibit aromatase (IC₅₀ 3.9 μ M) [19]. Furthermore, several analogues of casimiroin also exhibited potent AI activity using the DBF fluoromet-

Table 2 Natural product compounds active against aromatase in cellular and *in vivo* assays^a.

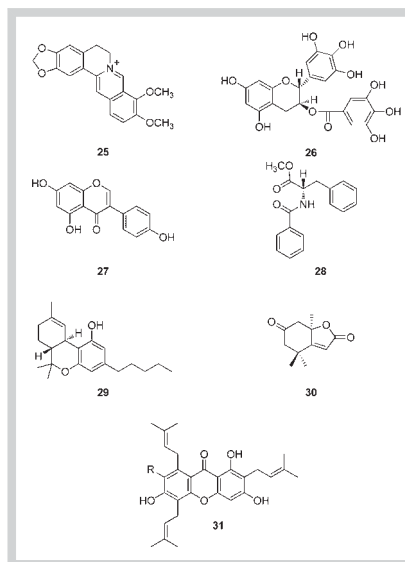
Compound Code	Source	Assay Type	Activity		Ref.
Alkaloid					
25	<i>Coptis japonica</i> ^b	MCF-7 cells	active	synergy w/fulvestrant	[24]
Flavonoids					
Chalcone					
9	<i>Glycyrrhiza glabra</i> ^b	MCF-7aro cells	3	$\mu\text{M } K_i$	[9]
9	<i>Glycyrrhiza glabra</i> ^b	xenograft mouse	deterred	growth	[9]
Catechin					
26	<i>Camellia sinensis</i> ^b	CaSki cells	76	% reduction in mRNA expression	[26]
26	<i>Camellia sinensis</i> ^b	HeLa cells	90	% reduction in mRNA expression	[26]
Isoflavones					
19	<i>Trifolium pratense</i> ^b	MCF-7aro cells	8	$\mu\text{M } \text{IC}_{50}$	[10]
19	<i>Trifolium pratense</i> ^b	MCF-7aro cells	10.8	$\mu\text{M } K_i$	[10]
27	<i>Glycine max</i> ^b	HepG2 cells	induced	expression at 1 μM	[27]
27	<i>Glycine max</i> ^c	zebrafish	induced	expression of <i>aro-B</i> gene	[28]
Peptide					
28	<i>Brassaiopsis glomerulata</i>	SK-BR-3 cells	33.3	PCA at 50 μM	[14]
Terpenoid					
29	<i>Cannabis sativa</i>	MCF-7 cells ^d	elevated	growth using AI	[29]
30	<i>Brassaiopsis glomerulata</i>	SK-BR-3 cells	21.8	PCA at 50 μM	[14]
Xanthones					
22	<i>Garcinia mangostana</i>	SK-BR-3 cells	4.97	$\mu\text{M } \text{IC}_{50}$	[12]
31	<i>Garcinia mangostana</i>	SK-BR-3 cells	32.3	PCA at 50 μM	[12]

^a The structures of compounds 25–31 are shown in **Fig. 2**; ^b The source of the material in the literature reference is listed as commercial but this compound is traditionally isolated from a natural product source; ^c No data were provided regarding the source material used in this study; ^d Δ^9 -THC induced

ric assay, with 5,6,8-trimethoxy-1,4-dimethylquinolin-2(1H)-one exhibiting an IC_{50} in the low nM range (100 nM) [19]. Other strongly active natural compounds in the fluorometric assays have included commercially obtained apigenin (**16**, K_i 1.0 μM) [20], and two other flavonoids isolated from the resin of *Prunus avium*, namely, sakuranetin (**12**, K_i 1.2 μM), and tectochrysin (**17**, K_i 2.1 μM) [20]. All three of these compounds were tested further for antifungal activity, for which they exhibited no activity, under the experimental conditions used. However, these compounds were also tested against cytochrome P450 enzymes and found to inhibit CYP1A1 and CYP3A4, potentially indicative of broad, nonspecific activity, thus making them less ideal candidates for further preclinical development.

Three compounds were found to have moderate activity in the fluorometric AI assays. Baicalein (**18**, K_i 5.1 μM) [20] was obtained commercially and, as was reported above for compounds **12**, **16**, and **17**, also inhibited the cytochrome P450 enzyme CYP3A4. Corynesidone A (**5**) was isolated from the terrestrial endophytic fungus *Corynespora cassicola* L36 and observed to moderately inhibit aromatase with an IC_{50} value of 5.3 μM [21]. All other isolates from this fungus were inactive against aromatase. Compound **5** was tested for cytotoxicity against a broad panel of cell types and demonstrated minimal cytotoxicity. Furthermore, corynesidone A exhibited strong antioxidant activity as measured using an oxygen radical absorbance capacity (ORAC) assay, but did not suppress the generation of TPA-induced superoxide anions in HL-60 cells [21]. Commercial myricetin (**14**), mentioned above during the discussion of liquiritigenin, also exhibited moderate AI activity with an IC_{50} of 10 μM [16].

Four natural compounds were reported with weak AI activity in the fluorometric assays during the period covered by the present review. Commercially obtained gossypetin (**15**), also mentioned above during the discussion of liquiritigenin, exhibited weak AI activity with an IC_{50} of 11 μM [16]. In the first reported instance of AI testing of a biflavonoid, the biflavanone, GB1 (5,7,4',3'',5'',7''-hep-

**Fig. 2** Chemical structures of compounds 25–31.

tahydroxy-3,8-biflavanone, **8**), from the terrestrial plant *Garcinia kola* (bitter kola, a component in many African herbal medicines) was reported to have an AI IC_{50} of 11.3 μM [22]. GB1 was also tested for α -glucosidase inhibition, antiplasmodial activity, and cytotoxicity and found to also have considerable antimalarial activity with no cytotoxicity. Although the authors reported the inhibition of α -glucosidase, the IC_{50} value determined in this study was as high as 900 μM , which would generally be considered inactive against this enzyme.

The final two compounds found to be weakly active in the fluorometric AI assay were two chromanones, monodictyochromes A (**3**, IC_{50} 24.4 μM) and B (**2**, IC_{50} 16.5 μM), isolated from the marine algal fungus *Monodictys putredinis* [23]. Monodictyochromes A and B were also tested against cytochrome P450 1A

(IC₅₀ values of 5.3 and 7.5 μM, respectively), and for induction of quinone reductase [concentrations to double activity (CD value) of 22.1 and 24.8 μM, respectively]. As in the case of previously mentioned compounds with several types of activity, these additional biological effects for monodictyochromes A and B may be indicative of broad, nonspecific action.

Compounds active in cellular and *in vivo* AI assays

Ten natural products were tested in cell-based *in vitro* and in *in vivo* testing during the reporting period of this review. Several of these compounds underwent substantial testing in numerous assay systems, presumably in an effort to pursue preclinical development. Flavonoids were again the most commonly tested class of compounds with four of the ten compounds tested falling into this class (two isoflavones, one chalcone, and one catechin). Two xanthenes underwent screening in cellular AI assays, as well as two terpenoids, one alkaloid, and one peptide (Table 2, Fig. 2). All of the active compounds tested in cellular and *in vivo* assays were either commercially purchased natural products or isolated from terrestrial plants. In most cases, each assay system was different and therefore findings from these reports will be discussed individually and grouped by compound.

The protoberberine alkaloid, berberine (25), was found to be the main active compound in the extract of *Coptis japonica* (part of the goldthread genus) and was subsequently purchased to afford sufficient material for further studies [24]. The research involved testing a combination of treatment with berberine and fulvestrant, a clinically utilized estrogen receptor antagonist that the authors indicated also acts as an aromatase antagonist. (However, this seems to be unsubstantiated from the reference used, since the most commonly accepted mechanism of action of fulvestrant is downregulation and degradation of the estrogen receptor [25]). Using the combination of berberine and fulvestrant, the authors found a synergistic inhibition of cell growth in MCF-7 cells, significantly different than both berberine and fulvestrant alone, which exhibited little to no inhibition.

Commercially purchased isoliquiritigenin (9) was found to be an active AI in three different test systems. In MCF-4aro cells (MCF-7 cells transfected with human *CYP19*), isoliquiritigenin was found to inhibit aromatase in a potent manner with a *K_i* of 3 μM [9]. They next tested isoliquiritigenin in a xenograft mouse model using athymic mice transplanted with MCF-7aro cells and found that the compound significantly suppressed xenograft growth (administered through feeding with doses of 0, 50, 150, or 500 ppm isoliquiritigenin supplementation). Finally, the effect of isoliquiritigenin was explored on specific aromatase promoters, namely, promoters I.3 and II, which are known to be breast-tissue specific, with the results indicating that the compound acts in a promoter-specific manner.

The most commonly reported catechin constituent of *Camellia sinensis* (green tea), (–)-epigallocatechin 3-gallate (EGCG, 26), was found to be active in cellular AI testing protocol, according to a recent report [26]. The regulation of aromatase by EGCG was studied in CaSki (HPV16 positive human cervical cancer) and HeLa (HPV18 positive human cervical cancer) cells, with this catechin derivative found to decrease mRNA expression in both cell types (after 12 h a 76% reduction was found in CaSki cells; after 24 h a 90% reduction was found in HeLa cells). The authors suggested that these effects on cervical cancer cells could be indicative of the potential use of EGCG in cervical cancer chemoprevention.

Two common natural isoflavones, biochanin A (19) and genistein (27), were reported as having AI activity. The first, biochanin A,

had previously been isolated from *Trifolium pretense* (red clover) and was obtained commercially for the studies in this report [10]. Compound 19 was reported to strongly inhibit aromatase in MCF-7aro cells (IC₅₀ 8 μM, *K_i* 10.8 μM). In addition, biochanin A was found to reduce mRNA expression in SK-BR-3 cells and was also found to act in a promoter-specific manner, upregulating breast-specific promoters I.3 and II, using luciferase reporter gene assays. Two separate studies have reported the aromatase activity of the *Glycine max* (soy) isoflavone, genistein (27). In the first study, genistein was obtained commercially and was found to induce aromatase activity in HepG2 cells using a concentration of 1 μM [27]. Genistein was also found to induce promoter-specific aromatase mRNA expression, with significant increases in promoters I.3 and II [27], although the authors used HepG2 cells for this analysis, in which aromatase expression is generally believed to be regulated primarily by promoter I.4. Further experiments were performed with this isoflavonoid and demonstrated the activation of protein kinases PKCα, P38, and ERK-1/2 and the activation of the transcriptional factor CREB. The inter-relationships between these protein kinases and the transcription factor with *CYP19* promoter II was hypothesized. The second study relating to the AI activity of genistein involved the use of zebrafish as an *in vivo* model to test AI activity [28]. After finding that genistein binds and activates estrogen receptors α, β, and γ, genistein was found also to induce expression of the *aromatase-B* gene (*aroB*) in the brain of zebrafish, using an estrogen-receptor dependent pathway.

In work carried out by the present authors, the peptide, *N*-benzoyl-L-phenylalanine methyl ester (28) was isolated from the Indonesian terrestrial plant *Brassaiopsis glomerulata*, and found to have weak AI activity in SK-BR-3 cells (33.3 PCA at 50 μM; no IC₅₀ reported) [14]. Another isolate from *B. glomerulata*, the terpenoid (–)-dehydroliolide (30), also showed AI activity in SK-BR-3 cells (21.8 PCA at 50 μM; no IC₅₀ reported) [14]. One other terpenoid derivative has been ascribed with indirect activity on aromatase during the period covered by this review, namely, Δ⁹-tetrahydrocannabinol (Δ⁹-THC, 29) isolated from *Cannabis sativa* (marijuana) [29]. Δ⁹-THC has been shown to cause MCF-7 cell proliferation and this growth was elevated by AIs, suggesting that aromatase plays a role in the Δ⁹-THC-induced proliferation of MCF-7 cells [29]. Finally, in our own work, two xanthenes from the botanical dietary supplement *Garcinia mangostana* (mangosteen) were found to be active when tested against SK-BR-3 cells, including the strongly active γ-mangostin (22, IC₅₀ 4.97 μM) and the weakly active garcinone E (31, 32.3 PCA at 50 μM) [12]. Garcinone D (23) and α-mangostin (24) were inactive in this cell-based assay. The potent enzymatic and cellular AI activities of γ-mangostin, one of the most abundant isolates from the pericarp of *Garcinia mangostana*, make this a promising lead for further *in vivo* and preclinical studies to determine the potential role of mangosteen botanical dietary supplements in cancer chemoprevention and/or chemotherapy for postmenopausal women with hormone-dependent breast cancer.

Comparison of the Most Recent and Previously Reported Natural Product AIs

Several of the compounds that have been reported to inhibit aromatase during the past two years have also previously undergone testing for AI activity as reported in our previous review [7]. Apigenin (16) was found to be strongly active in microsomes in re-

cent studies, which was also the case for much of the previous AI research on this compound, including noncellular and cellular tests. Myricetin (**14**) was also recently reported as a moderately active inhibitor of aromatase, consistent with previous literature reports on this compound. Biochanin A (**19**) was also recently found to be strongly active in cellular AI screening, whereas in previous studies the results for this compound were mixed, with some indicating inhibition of aromatase and others reporting inactivity. Isoliquiritigenin (**9**) and EGCG (**26**) were active in recent AI testing, and, as with biochanin A, these two compounds had previously been shown to have ambiguous aromatase activity. Two of the compounds reported herein as active, berberine (**25**) and genistein (**27**), have previously undergone AI testing and were found to be inactive in other assay systems. In the case of contradictory or ambiguous AI results for the same compounds, several possible variables should be considered, including the use of different assay systems (as mentioned above) and variations in the levels of compound purity, among others.

In comparing the levels of aromatase inhibition found in these more recent studies with those reported in the previous review, a decreased level of overall activity was noted. Several of most active natural product AIs found during the previous review are shown in **Fig. 3** [30–40]. In recent studies, three of the most active natural compounds in noncellular assays were found to have activity levels in the nanomolar range [liquiritigenin (**10**), 2-methoxy-5-acetoxymangostin (**20**), and dehydroabietic acid (**21**)]. However, in previous studies, eight compounds (**32–39**) were reported with nanomolar AI activity in noncellular assays. The biggest difference in the activity of the recently reported natural product AIs was found in the cellular and *in vivo* assays where much lower levels of activity were reported than in previous years. Several previously reported compounds exhibited nanomolar activity in cell-based AI screens (**16**, **32**, **33**, **37**, **40**) whereas all of the more recent reports are in the μM range [the three compounds considered strongly active in cell-based assay systems were γ -mangostin (**22**), isoliquiritigenin (**9**), and biochanin A (**19**)]. Interestingly, many of the more recent compounds have undergone substantially more molecular biology testing, resulting in reports of several active AIs that act on breast-tissue specific promoters.

Conclusions and Future Directions

Natural product aromatase research has continued to produce exciting, potent aromatase inhibitory lead compounds that provide interesting new avenues of investigation. Several natural compounds have recently been identified to exhibit nanomolar levels of inhibition of the aromatase enzyme, both in noncellular and cellular AI assays, as well as exhibiting activity using *in vivo* mouse and zebrafish models. Many of these potent natural product AIs need further investigation to help determine their potential for preclinical and clinical development. Several of the compounds in this review could be the subject of extensive medicinal chemistry to modify the natural product scaffold, in the search for more potent AIs.

In the broader aromatase research arena, the search for more potent AIs is no longer the only consideration. As shown in the case of several of the recent natural product isolates described above, aromatase researchers have begun to explore the potential of identifying inhibitors that function in a promoter-specific manner, thus targeting the breast-specific aromatase promoters I.3

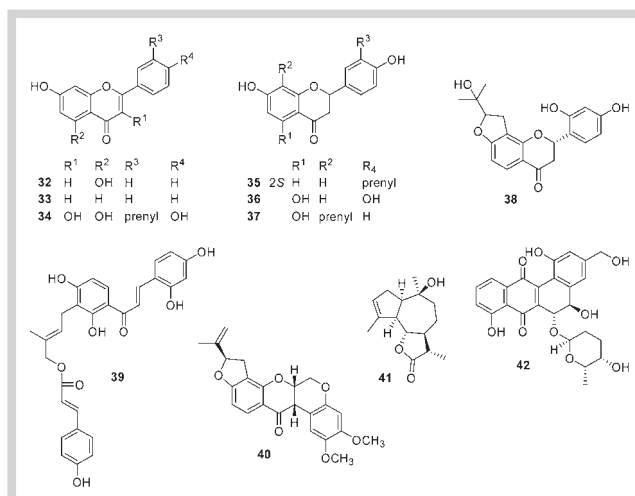


Fig. 3 Structures of strongly active aromatase inhibitors discussed in a previous review [7]. In cases above where absolute configuration is not indicated, no information was provided in the original literature.

and II [41,42]. Further studies are also utilizing new information about the aromatase enzyme X-ray structure to facilitate molecular modeling and docking studies [43]. Other research is exploring the use of combination treatments (e.g., aromatase inhibitors with endocrine disruptors) to circumvent breast cancer drug resistance [44]. Future investigations with natural product aromatase inhibitors will need to incorporate these types of multilateral studies to continue moving these exciting compounds from initial discovery to ultimate clinical utilization.

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Affiliations

- ¹ Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, School of Pharmacy, Storrs, CT, USA
- ² Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA
- ³ Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Clayton, Panamá
- ⁴ Smithsonian Tropical Research Institute, Ancón, Panamá
- ⁵ Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA

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