American Institute of Integrative Oncology
RESEARCH & EDUCATION

Foundations of Integrative Oncology
COURSE

GRAND ROUNDS CALL
With Dr. Nalini Chilkov
February 21st, 2018

5:30 PM Pacific / 6:30 PM Mountain / 7:30 PM Central / 8:30 PM Eastern

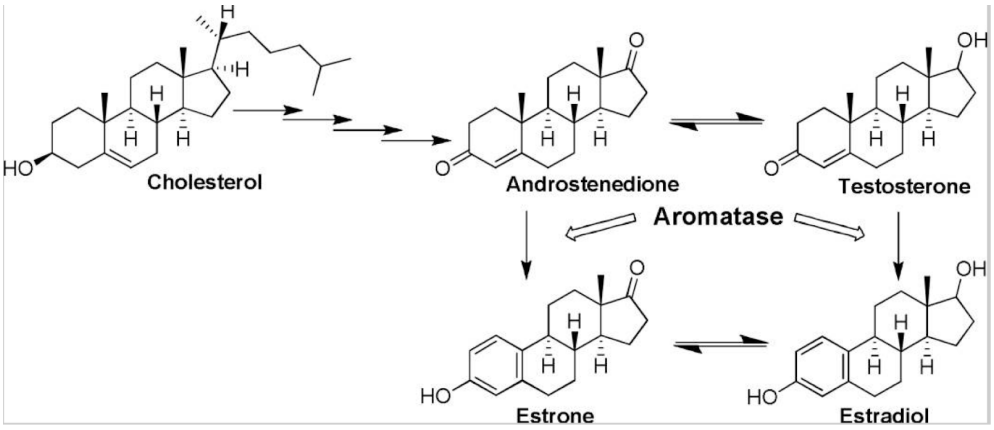
Clinical Pearl: Managing the Side Effects of Aromatase Inhibitors

Aromatase inhibitors are a class of drugs used in the treatment of breast cancer in postmenopausal women and gynecomastia in men.

- Preferred treatment for hormone receptor – positive breast cancer in postmenopausal women. Tamoxifen is used for premenopausal women.
- AIs are currently being tested as primary prevention therapy in large randomised trials involving tens of thousands of women at increased risk for breast cancer.

Mechanism:

- Reduce the amount of estrogen by interfering with its production
- Aromatase is a cytochrome P450 tissue enzyme and is responsible for catalyzing the biosynthesis of estrogens (estrone and estradiol) from androgens (androstenedione and testosterone)
- The concentration of estrogens has been shown to be as much as twenty-fold higher in breast cancer tissues than in the circulating plasma, suggesting locally increased aromatase expression for estrogen biosynthesis near or within the cancerous tissues



Both tamoxifen and AIs are effective for the adjuvant and neoadjuvant treatment of postmenopausal breast cancer.

The optimal choice of drug is dependent on the characteristics of the patient and tumor.

Adverse events associated with tamoxifen include increased risk of uterine cancers and thromboembolic events vs. an increased incidence of vaginal dryness, loss of libido, musculoskeletal pain and bone mineral density loss with AIs.

Rationale:

- 70% of Breast Cancers are ER+ - Estrogen Receptor Positive
- Estrogen promotes the growth and survival of normal and cancerous breast epithelial cells by binding and activating the estrogen receptor (ER)
- The activated receptor binds to gene promoters in the nucleus and activates many other genes responsible for cell division, inhibition of cell death, new blood vessel formation and protease activity

Selective Estrogen Receptor Modulator (SERM) Tamoxifen, Raloxifene
Interferes with the binding of estrogen to the ER and/or to the promoter elements of the genes it regulates. Selective ER modulators such as tamoxifen and raloxifene act in this manner.
AIs don't work as well at blocking estrogen produced in adipose tissue in obese women.

Ovarian Ablation

- Surgical: Ovarectomy
- Chemical: Gonadotrophin Luteinizing Releasing Hormone agonist: Lupron (Leuprolide) stops estrogen and testosterone production in ovary and testes
- Estrogen Receptor Down-Regulator: Fulvestrant (Faslodex)

Third Generation of Aromatase Inhibitors (100% effective):
Arimidex (Anastrozole) Aromasin (Exemestane) Femara (Letrozole)

Natural Aromatase Inhibitors (30-50% effective):

- **Scutellaria barbata** Ba Zhi Lian. chrysin and apigenin
- **Resveratrol** stilbenoid (1-2g daily)
- **Genestein** isoflavone
- **Chrysin** 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (500-1000 mg daily)
- **Urtica dioca** (stinging nettle root) Fatty acids octadecadienoic acid and docosapentaenoic acid and lignin secoisolariciresinol
- **Agaricus medicinal mushroom**: Mycelium of agaricus mushrooms used as an AI by Dr. Michael Traub. No human studies have been published.

Adverse Effects Result from Estrogen Depletion and Deprivation

- Musculoskeletal Pain: Arthralgias and Myalgias
- Increased Bone Turnover and Decreased Bone Density
- Vaginal dryness
- Loss of libido
- Painful intercourse
- Increase in Ischemic Heart Disease/Loss of Vasodilation of Coronary Arteries

Obesity (Subbaramaiah et al, 2011)

- Associated with inflammation and elevated aromatase expression

<u>Managing Adverse Effects:</u>

Acupuncture (Mao et al, 2009)

- Women with AI-induced arthralgias treated with Therapeutic Acupuncture (2x/week x 6 weeks) had significant improvement of joint pain and stiffness

Vitamin D (2K-10K iu/day - monitor serum levels)

- Significant inverse correlation between pain intensity and serum 25(OH)D levels (Waltman et al, 2009)
- Symptomatic patients were more likely to have had baseline levels below 40 ng/mL, compared with asymptomatic patients. Repletion to 25(OH)D levels >40 ng/mL is advisable. (Singer et al, 2014)
- Vitamin D has been shown to be effective in reducing the incidence and severity of arthralgia resulting from treatment with the aromatase inhibitor letrozole (Samuels, Schiff & Ben-Arye, 2014)

Exercise and Stretching

- **Yoga & Tai Chi** (Galantino, 2013) (Galantino et al, 2012) (Carson et al, 2009)

Maca

- Rich in sulpophanes and non-estrogenic plant sterols

Questions & Answers

Judy Pruzinsky: **You mentioned different protocols (such as immunotherapy etc) to avoid cutting. Do you like to avoid even biopsy cuts?**

Dr. Nalini:

- Biopsy is an essential part of diagnosis.
- Whenever it is possible to get a tissue sample, that is the only way to make a clear diagnosis and select appropriate tx.
- Similarly, if a tumor is operable surgical excision should be considered to reduce tumor burden. Immunotherapy and targeted therapies cannot be selected without tumor tissue analysis.

Todd Binkley: **Do you have a recommended list of (southern California) integrative (or at least open minded) oncologists to refer patients to?**

As most people on the call are not from SoCal and we have limited time together, let me make some general comments about what I look for in referring to an oncologist.

If you have a patient with a specific diagnosis, you can reach out for a referral and I would be happy to make an appropriate individualized suggestion.

There are no truly integrative oncologists in Southern California. There ARE collaborative oncologists, surgeons, radiologists. This is based on relationship.

Oncologists practice in a very controlled legal-medical setting and must adhere to **standard of care.** Only if the patient has failed standard of care can the oncologist get creative.

Instead I recommend that the patient have a top oncologist for their type of cancer who fully understands their disease and is apprised of all possible treatment options and studies. I am NOT anti-oncology.

Natural medicine alone typically is not sufficient for best outcomes and often leads to worse outcomes and premature death.

What to look for in an oncologist:
An oncologist who is patient centered, kind, communicative and respectful and a team player. The oncologist is the disease specialist. If the patient has a cancer that can be well treated by standard of care with good outcomes then that should usually be part of the plan. Then we step in and do the health side of the equation, help the patient to be educated, to get more individualized care and to have the tumor microenvironment and side effects well managed. I also partner with other physicians who do naturopathic IV therapies that I cannot do under my license.

- I refer patients to selected physicians whom I feel are a good fit for the patient.
- I refer to physicians with whom I have cultivated a relationship of mutual respect and collaboration.
- If I don't have a good referral, I will reach out to physicians and other health practitioners in my network for a recommendation.

Todd Binkley: **What are your specific product recommendations for your Top 10 Foundation Supplements?**

For example: *I've got a patient with a testicular mass (presumably seminoma), and liver enlargement. He hasn't submitted to a biopsy yet (I've encouraged him to do so immediately, but he's a chiropractor, hoping he can heal himself), so I'm thinking I'll recommend he start with your TOP TEN FOUNDATIONAL SUPPLEMENTS.*

Dr. Nalini:
The supplements that I use most are discussed in detail in the materia medica section of the course. There are also case studies which show supplements commonly used in my treatment plans. There are case studies from GRC that have good examples of supplements commonly used. Going through the course modules will give you a good

education. Invest the time so that you understand rationale, indications and contraindications.

1. **Foundation Five**
   - Cu Free Fe Free Multi (DFH Twice Daily Multi or DFH Metabolic Synergy or Thorne or Pure Encapsulations, Integrative Therapeutics Pro Thriver Wellness Multi)
   - EPA-DHA O3 FA (DFH Omegavail TG 1000 or Omegatropic)
   - Probiotic (Klaire Ther Biotic Complete or DFH Probiotic Supreme)
   - Vit D3 (DFH Vitamin D Supreme)
   - Minerals Magnesium glycinate plus Bone Mineral Formula copper-free (Osteoben DFH or Os Cap Thorne)
   - Vitamin C (DFH Stellar C)

2. Targeted Nutriceuticals and Botanicals to exert epigenetic impact on tumor microenvironment
- DFH Curcumevail or Thorne Meriva (Curcumin)
- DFH Broccoprotect or Thorne Crucera or NATURA Cell Guardian (Sulphoraphane)
- DFH Resveratrol Supreme or Thorne Polyresveratrol
- DFH EGCG or Thorne Green Tea Phytosome
- Clinical Synergy Mushroom Immune Max, Health Concerns Power Mushrooms, or NATURA Immucare I

Patient example: Testicular mass (presumably **seminoma**), and **liver enlargement.**
- LDH is 304 (was 197, 8 mo.s ago), AST 75 (was 20), CRP 8.1, CK 2237 (inexplicably—no exercise, BNP-11.7)

He hasn't submitted to a biopsy yet (I've encouraged him to do so immediately, but he's a chiropractor, hoping he can heal himself), ***so I'm thinking I'll recommend he start with the TOP TEN FOUNDATIONAL SUPPLEMENTS.***

There is no generic plan. The OutSmart Cancer System is not PROTOCOL based but SYSTEMS based. It is dynamic and customized for each patient. It is not formulaic.

Each patient must have an individualized care plan.
- Every single patient has a unique plan and that plan is adjusted as they move through different phases of the cancer journey, targeted to their tumor characteristics and tumor microenvironment, their developmental process of learning.
- The Outsmart Cancer System gives you guidelines and principles so that you can build a plan for each patient. This is how the best outcomes are realized. There is no one size fits all EVER.

You CAN certainly encourage any patient to get started with a HEALTH PLAN, however this type of cancer requires a medical oncologist on the team. This is not in any way cytotoxic and does not eradicate tumor cells.

To transform outcomes much more must be done.

Recommendation: Emphasize to the patient that there must also be a plan to eradicate the tumor cells. This rarely occurs in solid tumors that are aggressive. An integrative approach is required.
- With liver enlargement and elevated LFT, we need to know if he has metastatic disease or other hepatic disease.
- The BEST guidance you can give him is to insist he have a thorough oncology workup and a precise diagnosis and have an INTEGRATIVE PLAN best of both worlds.
- He may be in denial, so if you are the person he has turned to for guidance, deliver the hard truths. That is part of our role. Help the patient get real and face what is happening and take action.

Judy Pruzinsky: **You talk often about helping bone health. If somebody comes in, pre-cancer diagnosis, with osteoporosis and you have them on something like Osteoben, would you be doing therapeutic dosing that is much higher than recommended or would you go with the recommended dosing?**

Dr. Nalini:
- I work with the **recommended dosing** to make sufficient minerals available. Minerals won't deposit faster in the bone without drugs.
- Osteoporosis and bone metastases are metabolic diseases.
- Movement and weight-bearing exercise are encouraged depending on the patient. If a patient's skeleton is not

fragile and at risk for fractures, resistance training and exercise are recommended. Physical therapy is supportive for more fragile and elderly patients.
- Monitor patient's diet for excess protein.

Judy Pruzinsky: **I had a patient ask me about Resveratrol dosing. He made reference to the cancer protocol Rx of 3-5 grams/day of resveratrol compared to this statement from a tech report from DFH:**
Researchers at Harvard Medical School have found that resveratrol increases the production of a protein called SIRT1, and although it has not yet been confirmed in humans, in theory this action could increase human lifespan dramatically. One negative aspect of this research is that it would be impossible to consume, from normal food sources, the amount of resveratrol proven effective. The amounts used in one successful mouse study were approximately 22.4 mg/kg body weight per day. Scaling this amount to human body weights could imply an "equivalent human dose" of 1.5 to 2.0 grams/day, however if one compensates for the fact that humans have slower metabolic rates than mice, an equivalent human dose may be closer to a range of 200 mg/day. With this in mind Resveratrol Supreme was designed as the ultimate high potency, high quality 200mg trans-resveratrol formulation.
So should I be advising 3 of these per day? I usually dose 2 bid

Dr. Nalini:
1. Do not rely on mouse studies with regards to drug and nutraceutical and phytochemical metabolism in humans.
2. I usually give 200-500mg trans Resveratrol daily as a nutritional dose and 3000-5000mg per day as a therapeutic dose. I like Resveratrol Supreme (DFH) as it is resveratrol : Quercetin 1:1 Start with **2 bid**
3. Resveratrol is a multi tasker. There is a detailed discussion and good slides in the course on resveratrols many actions. Inflammation, Oxidative Stress, Mitochondrial Function via SIRT1, protects telomeres and DNA, crosses BBB, aromatase inhibitor, epigenetic effects on supporting expression of tumor suppressors and inhibiting expression of oncogenes
4. Always dose patients WITHIN your own clinical skill and knowledge. Do not copy my treatment plans. Think through the case yourself. Understand drug-herb drug nutrient interactions, the patient's digestive capacity and budget and do not overwhelm.

Do not give high pharmacologic doses unless you are clear that it is indicated, safe and there are no contraindications and you can manage any adverse reactions*

Judy Pruzinsky: **On the same topic of dosing, for the clinical studies would it be correct to assume that with something like curcumin it would be with standardized to 95% curcuminoids? If we wanted to give 3 grams of Curcumin daily, would we assume that was 3 grams at the 95% level of curcuminoids? If not, how would we adjust dosing?**

Dr. Nalini: **RE STANDARDIZATION of HERBAL PRODUCTS and EXTRACTS**
You definitely want to know the CONCENTRATION of your supplements and botanical extracts. You also want to know the concentration of a particular phytochemical….These are two different and somewhat confusing parameters

A powder that is 95% curcuminoids would not be the same as a powder that is 50% curcuminoids.

You will also see botanical extracts labeled 2:1, 5:1 herb-to-extract ratio. This means there are 2 parts (60g) of raw plant material to make 1 part (30g) of extract. This would be a STANDARDIZED extract so that the product is tested or assayed so that EQUAL amounts of active ingredients are the same from pill to pill or batch to batch.

An example of a 1:1 FLUID extract 1 gram of raw material makes 1 ml of liquid extract (the 1 ml contains the constituents from 1 gram of raw material). Like a tea which is a water extract.

A tincture is more dilute: 1:10 one part of dried weight of herb represent 10 parts by volume of tincture.

A 95% curcumin extract is a PURIFIED and concentrated product.
With a high concentration one particular constituent, in this case curcumin from Curcuma longa… This does NOT mean that 95 grams of dried curcuma rhizome was extracted down to 1 gram of extract. To get to high concentrations of one constituent an artificial process is used in manufacturing.

Resource: **AHPA's *Standardization of Botanical Products: White Paper***
http://www.ahpa.org/Education/TechnicalWorkshops/TabId/349/ArtMID/1109/ArticleID/225/White-Paper-Standardization.aspx

Judy Pruzinsky: **Still on the BCC path I was reading https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4744388 and came upon:**
Cutaneous basal cell carcinoma requires a specific stromal environment to maintain its morphological characteristics [49]. Key regulators of the biological behaviour of cutaneous basal cell carcinoma appear to be stromal fibroblasts and myofibroblasts [49]. Cutaneous basal cell carcinoma cells express bone morphogenetic protein (BMP) 2 and 4, while GREMLIN 1, a BMP antagonist is highly expressed in the stroma of the tumour but not in the dermis underlying normal keratinocytes. GREMLIN 1 counteracts the growth-inhibitory effect of BMPs and is therefore assumed to be an important agent supporting cutaneous basal cell carcinoma cell proliferation and survival. Matrix metalloproteinase expressed in the stroma of cutaneous basal cell carcinoma also plays an important role in regulating growth and other functions of cutaneous basal cell carcinoma cells [6]. It is probable that cutaneous basal cell carcinoma tumorigenesis depends substantially on specific factors produced by stroma damaged by UV, but on the rare occasions when cutaneous basal cell carcinomas occur at sites that are not exposed to sunlight, other biological factors drive its initiation and progression [5].

Would this lead you to believe that if a patient was taking Bone Morphogenic Proteins for osteoporosis, in the form of the TRF product from DFH, that this actually might be assisting as part of a BCC protocol??

Also you have spoken of certain lights to show BCC, was she referring to Raman spectroscopy? It seems that is still not in use diagnostically in the USA.

Dr. Nalini:
Bone morphogenic protein has been implicated in promoting cancer growth, therefore, I don't use this in my clinical practice.

Kamron Keep: **You've discussed intermittent fasting briefly in previous lectures. Could you provide a little more information on when and how you recommend this to clients on chemotherapy? I understand it may depend on their health going into and throughout chemotherapy, but I'm looking to gain more general guidance.**

Dr. Nalini: The rationale for IF is to put the body into mild ketosis. Healthy cells can function on ketones instead of glucose, but tumor cells are glucose dependent. By removing their main fuel the tumor cells are stressed.

- Only robust patients can do IF. A patient who is frail, underweight, sarcopenic or in cachexia cannot do IF.
- However, it is possible to eat a modified ketogenic diet and consume ketogenic shakes during the period before chemo infusions.
- Depending upon the patient that time interval may be 12 or 24 hours or even 48 hours in younger stronger patients.
- If the patient can fast on bone broth or a ketogenic shake during most of the infusion..that is best….but most pts do not want to do that.
- A modified ketogenic shake would have 20-30 grams protein, 30 grams fat and very little carbs, fiber (no fruit). Can add MCT oil. Can also drink Bone Broth for 10g protein per cup plus all of the minerals.

It is recommended as a lifestyle habit to fast from dinner to tomorrow's breakfast for 13+ hours.

This also triggers an immune response, triggers Sirtuins (Mitochondrial health) and reduces not only glucose but insulin levels and can help improve glycemic control and fat burning.

- Low CHO diets cause a drop in plasma insulin and lactate
- Low CHO diets can reduce insulin-mediated glucose uptake into tumor cells; hyperglycemia enhances proliferation in some tumors
- Insulin is the primary growth factor and driver of proliferation (insulin makes glucose available)
- Tumor size and growth is related to plasma insulin levels and plasma lactate levels

In a previous GRC I discussed Thomas Seyfried's published works on cancer as a metabolic and mitochondrial syndrome. His papers are posted in the resource library.

- *Cancer as a Mitochondrial Metabolic Disease*
- *Press Pulse A Novel Therapeutic Strategy for Metabolic Management of Cancer*

Research Highlight: Cancer as an Ecomolecular Disease and a Neoplastic Consortium

y Cajal, S. R., Capdevila, C., Hernandez-Losa, J., de Mattos, L., Ghosh, A., Lorent, J., ... & Topisirovic, I. (2017). **Cancer as an ecomolecular disease and a neoplastic consortium**. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer.

Abstract

Current anticancer paradigms largely target driver mutations considered integral for cancer cell survival and tumor progression. Although initially successful, many of these strategies are **unable to overcome the tremendous heterogeneity that characterizes advanced tumors, resulting in the emergence of resistant disease.**

Cancer is a rapidly evolving, multifactorial disease that accumulates numerous genetic and epigenetic alterations.

This results in wide phenotypic and molecular heterogeneity within the tumor, the complexity of which is further amplified through specific interactions between cancer cells and the tumor microenvironment. In this context, cancer may be perceived as an "ecomolecular" disease that involves cooperation between several neoplastic clones and their interactions with immune cells, stromal fibroblasts, and other cell types present in the microenvironment.

This collaboration is mediated by a variety of secreted factors. **Cancer is therefore analogous to complex ecosystems such as microbial consortia.** In the present article, we comment on the current paradigms and perspectives guiding the development of cancer diagnostics and therapeutics and **the potential application of systems biology to untangle the complexity of neoplasia.**

In our opinion, **conceptualization of neoplasia as an ecomolecular disease is warranted. Advances in knowledge pertinent to the complexity and dynamics of interactions within the cancer ecosystem are likely to improve understanding of tumor etiology, pathogenesis, and progression.** This knowledge is anticipated to facilitate the design of new and more effective therapeutic approaches that target the tumor ecosystem in its entirety.

Resources

Balunas, M. J., Su, B., Brueggemeier, R. W., & Kinghorn, A. D. (2008). Natural products as aromatase inhibitors. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 8(6), 646-682.

Carson, J. W., Carson, K. M., Porter, L. S., Keefe, F. J., & Seewaldt, V. L. (2009). Yoga of Awareness program for menopausal symptoms in breast cancer survivors: results from a randomized trial. Supportive care in cancer, 17(10), 1301-1309.

Fabian, C. J. (2007). The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. International journal of clinical practice, 61(12), 2051-2063.

Galantino, M. L. (2013). Tai chi for well-being of breast cancer survivors with aromatase inhibitor-associated arthralgias: a feasibility study. Alternative therapies in health and medicine, 19(6), 38.

Galantino, M. L., Greene, L., Archetto, B., Baumgartner, M., Hassall, P., Murphy, J. K., ... & Desai, K. (2012). A qualitative exploration of the impact of yoga on breast cancer survivors with aromatase inhibitor-associated arthralgias. Explore: The Journal of Science and Healing, 8(1), 40-47.

Mao, J. J., Bruner, D. W., Stricker, C., Farrar, J. T., Xie, S. X., Bowman, M. A., ... & DeMichele, A. (2009). Feasibility trial of electroacupuncture for aromatase inhibitor—related arthralgia in breast cancer survivors. Integrative cancer therapies, 8(2), 123-129.

Samuels, N., Schiff, E., & Ben-Arye, E. (2014). Non-herbal nutritional supplements for symptom relief in adjuvant breast cancer: creating a doctor–patient dialogue. BMJ supportive & palliative care, bmjspcare-2013.

Singer, O., Cigler, T., Moore, A. B., Levine, A. B., Do, H. T., & Mandl, L. A. (2014). Hypovitaminosis D is a predictor of aromatase inhibitor musculoskeletal symptoms. The breast journal, 20(2), 174-179.

Subbaramaiah, K., Howe, L. R., Bhardwaj, P., Du, B., Gravaghi, C., Yantiss, R. K., ... & Kopelovich, L. (2011). Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. Cancer prevention research, 4(3), 329-346.

Waltman, N. L., Ott, C. D., Twiss, J. J., Gross, G. J., & Lindsey, A. M. (2009). Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy. Cancer nursing, 32(2), 143.

y Cajal, S. R., Capdevila, C., Hernandez-Losa, J., de Mattos, L., Ghosh, A., Lorent, J., ... & Topisirovic, I. (2017). Cancer as an ecomolecular disease and a neoplastic consortium. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer.

Managing Adverse Effects of Aromatase Inhibitors

American Institute of Integrative Oncology RESEARCH & EDUCATION®

Aromatase Inhibitor: Definition

HORMONAL THERAPY

Aromatase inhibitors are a class of drugs used in the treatment of breast cancer in postmenopausal women and gynecomastia in men.

Preferred treatment for hormone receptor – positive breast cancer in postmenopausal women.

AIs are currently being tested as primary prevention therapy in large randomised trials involving tens of thousands of women at increased risk for breast cancer.

They may also be used off-label to reduce estrogen conversion when using external testosterone

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Aromatase Inhibitor: Mechanism

Inhibition of the effects of estrogen in Post Menopausal Women

Reduce the amount of estrogen by interfering with its production

Aromatase is a cytochrome P450 enzyme and is responsible for catalyzing the biosynthesis of estrogens (estrone and estradiol) from androgens (androstenedione and testosterone)

The concentration of estrogens has been shown to be as much as twenty-fold higher in breast cancer tissues than in the circulating plasma, suggesting locally increased aromatase expression for estrogen biosynthesis near or within the cancerous tissues

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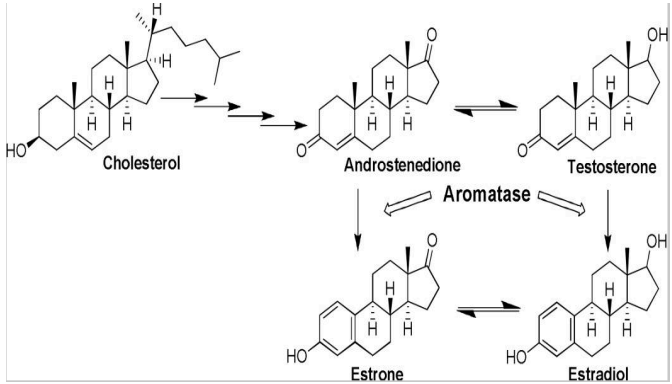

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HORMONAL THERAPY

Both tamoxifen and AIs are effective for the adjuvant and neoadjuvant treatment of postmenopausal breast cancer;

The optimal choice of drug is dependent on the characteristics of the patient and tumor.

Adverse events associated with tamoxifen include increased risk of uterine cancers and thromboembolic events vs. an increased incidence of vaginal dryness, loss of libido, musculoskeletal pain and bone mineral density loss with AIs.

Studies of AIs in the breast cancer prevention setting are ongoing.

Aromatase Inhibitor: Rationale For Use

70% of Breast Cancers are ER+ Estrogen Receptor Positive

Estrogen promotes the growth and survival of normal and cancerous breast epithelial cells by binding and activating the estrogen receptor (ER).

The activated receptor in turn binds to gene promoters in the nucleus and activates many other genes responsible for cell division, inhibition of cell death, new blood vessel formation and protease activity

HORMONAL THERAPY PREMENOPAUSAL WOMEN

Selective Estrogen Receptor Modulator (SERM) Tamoxifen, Raloxifene
Interfere with the binding of estrogen to the ER and/or to the promoter elements of the genes it regulates. Selective ER modulators such as tamoxifen and raloxifene act in this manner.

Ovarian Ablation
Surgical: Ovarectomy
Chemical: Gonadotrophin Luteinizing Releasing Hormone agonist: Lupron (Leuprolide) stops estrogen and testosterone production in ovary and testes
Estrogen Receptor Down-Regulator Fulvestrant (Faslodex)

Third Generation of Aromatase Inhibitors (100% effective)

Arimidex (Anastrozole) **Aromasin (Exemestane)** **Femara (Letrozole)**

Natural Aromatase Inhibitors. (30-50% effective)

Scutellaria barbata Ba Zhi Lian. chrysin and apigenin

Resveratrol stilbenoid

Genestein isoflavone

Chrysin 5,7,4'-trihydroxy-3',5'-dimethoxyflavone

Urtica dioca Fatty acids octadecadienoic acid and docosapentaenoic acid and lignin secoisolariciresinol

Obesity and Aromatase Expression

Cancer Prev Res (Phila) . 2011 March ; 4(3): 329–346. doi: 10.1158/1940-6207.CAPR-10-0381

Obesity is associated with inflammation and elevated aromatase

expression in the mouse mammary gland

Kotha Subbaramaiah1, Louise R. Howe2, Priya Bhardwaj1, Baoheng Du1, Claudia

Gravaghi1, Rhonda K. Yantiss3, Xi Kathy Zhou4, Victoria A. Blaho3, Timothy Hla3, Peiying

Yang5, Levy Kopelovich6, Clifford A. Hudis7, and Andrew J. Dannenberg1

Aromatase Inhibitor: Adverse Effects

Adverse Effects Result from Estrogen Depletion and Deprivation

- Musculoskeletal Pain: Arthralgias and Myalgias
- Increased Bone Turnover and Decreased Bone Density
- Vaginal dryness,
- Loss of libido
- Painful intercourse
- Increase in Ischemic Heart Disease/Loss of Vasodilation of Coronary Arteries

Aromatase Inhibitor SE: Electroacupuncture

From baseline to the end of intervention, **patients reported reduction in pain severity** (from 5.3 to 1.9), **stiffness** (from 6.9 to 2.4), **and joint symptom interference** (from 4.7 to 0.8), all P < .001; 11/12 **considered joint symptoms "very much better" Subjects also reported significant decrease in fatigue** (from 4.4 to 1.9, P = .005) and anxiety (from 7.1 to 4.8, P = .01). **No infection or development or worsening of lymphedema was observed.**

(10 treatments over 8 weeks)

Feasibility trial of electroacupuncture for aromatase inhibitor--related arthralgia in breast cancer survivors. Mao JJ, Bruner DW, Stricker C, Farrar JT, Xie SX, Bowman MA, Pucci D, Han X, DeMichele A.

Integr Cancer Ther. 2009 Jun;8(2):123-9

Aromatase Inhibitor SE: Acupuncture

Acupuncture 2x/week x 6 weeks Women with AI-induced arthralgias treated with Therapeutic Acupuncture had significant improvement of joint pain and stiffness,

which was not seen with Sham Acupuncture.… differences between TA and SA in pain severity (2.6 v 4.5; P = .003) and pain-related interference (2.5 v 4.5; P = . 002) at 6 weeks

Randomized, blinded, sham-controlled trial of **acupuncture** for the management of **aromatase inhibitor**-associated joint symptoms in women with early-stage breast cancer.

Crew KD, Capodice JL, Greenlee H, Brafman L, Fuentes D, Awad D, Yann Tsai W, Hershman DL. J Clin Oncol. 2010 Mar 1;28(7):1154-60.

Aromatase Inhibitor: Vitamin D

2K-10K iu/day -monitor serum level

There was a significant inverse correlation between pain intensity and serum 25(OH)D levels

Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy.Waltman NL, Ott CD, Twiss JJ, Gross GJ, Lindsey AM. Cancer Nurs. 2009 Mar-Apr;32(2):143-50

Symptomatic patients were more likely to have had baseline levels below 40 ng/mL, compared with asymptomatic patients. Repletion to 25(OH)D levels >40 ng/mL is advisable.

Hypovitaminosis D is a Predictor of Aromatase Inhibitor Musculoskeletal Symptoms. Breast J. 2014 Mar;20(2):174-9 Singer O et al

Aromatase Inhibitor SE: Arthralgias and Vitamin D

2K-10K iu/day monitor serum levels

Vitamin D has been shown to be effective in reducing the incidence and severity of arthralgia resulting from treatment with the aromatase inhibitor letrozole

BMJ Support Palliat Care. 2014 Sep;4(3):e1. doi: 10.1136/bmjspcare-2013-000463. Epub 2013 Feb 19.

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Natural Products as Aromatase Inhibitors

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Review

Cancer as an ecomolecular disease and a neoplastic consortium

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ABSTRACT

Current anticancer paradigms largely target driver mutations considered integral for cancer cell survival and tumor progression. Although initially successful, many of these strategies are unable to overcome the tremendous heterogeneity that characterizes advanced tumors, resulting in the emergence of resistant disease. Cancer is a rapidly evolving, multifactorial disease that accumulates numerous genetic and epigenetic alterations. This results in wide phenotypic and molecular heterogeneity within the tumor, the complexity of which is further amplified through specific interactions between cancer cells and the tumor microenvironment. In this context, cancer may be perceived as an "ecomolecular" disease that involves cooperation between several neoplastic clones and their interactions with immune cells, stromal fibroblasts, and other cell types present in the microenvironment. This collaboration is mediated by a variety of secreted factors. Cancer is therefore analogous to complex ecosystems such as microbial consortia.

In the present article, we comment on the current paradigms and perspectives guiding the development of cancer diagnostics and therapeutics and the potential application of systems biology to untangle the complexity of neoplasia. In our opinion, conceptualization of neoplasia as an ecomolecular disease is warranted. Advances in knowledge pertinent to the complexity and dynamics of interactions within the cancer ecosystem are likely to improve understanding of tumor etiology, pathogenesis, and progression. This knowledge is anticipated to facilitate the design of new and more effective therapeutic approaches that target the tumor ecosystem in its entirety.

1. Causes and consequences of cancer cell heterogeneity

Malignant tumors are enormously diverse. More than 250 clinicopathological types and thousands of varieties of neoplasia have so far been described. Moreover, cells within the same tumor are morphologically, phenotypically, and genetically heterogeneous, with further post-treatment diversification in metastases and recurrent lesions [1–3]. This inter- and intratumor heterogeneity manifests as a dramatic discrepancy in clinical features, prognoses, and therapeutic responses. Morphological patterns and other histological features that distinguish tumor types are already used to predict differences in prognosis (e.g. solid or macropapillary patterns are associated with worse survival in patients with lung adenocarcinoma) [4]. The same applies for a number of molecular alterations, some of which are used to guide clinical decisions [5,6]. Finally, intratumor heterogeneity, and the extent to which it occurs, can also be used as a prognostic indicator [7–10]. Hence, heterogeneity between and within tumors can affect clinical outcomes and guide therapeutic approaches.

2. Genomic heterogeneity

Recent studies using next-generation sequencing and single cell-based technologies have uncovered tremendous intratumor heterogeneity at the molecular level. For example, several studies have characterized the genomic landscape of primary tumors and metastatic lesions within the same patient [11,12,1,3–7,13]. These analyses

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revealed a constellation of genetic alterations in primary tumors and identified distinct clonal and subclonal architectures within both primary lesions and metastases, which indicated a number of seemingly different evolutionary routes that cancer cells can undertake within the same tumor [3,6–9]. Clear examples of genetic intratumor heterogeneity have been documented for neoplasia of the breast [14], lung [15,16], and kidney [17].

Indeed, in a manner analogous to the role of biodiversity in natural ecosystems, genetic diversity in cancer is thought to promote tumor fitness and is therefore a predictor of poor clinical outcomes [7–10]. Hence, it is important to understand how this intratumor heterogeneity occurs, as well as its significance in disease progression. The acquisition and maintenance of the "hallmarks of cancer" [18,19] are thought to occur stochastically with the accumulation of genetic alterations that are selected according to their contribution to cancer cell fitness, that is, whether they are driver or passenger events. This mirrors the Darwinian, step-wise, and reiterative process of clonal expansion, genetic diversification, and clonal selection of fitter populations [20]. One such example is cell competition, whereby fitter cells (winners) eliminate the surrounding cells (losers) by apoptosis [21]. There is however increasing evidence for a non-linear, branched evolution of neoplasia [7,16,22]. In this model, distinct, sometimes complementary, phenotypes emerge within a tumor, and each of these phenotypes is selected for simultaneously. The clones still originate from a common ancestor. However, in contrast to linear evolution, divergent clones evolve in parallel, resulting in multiple lineages that collectively contribute to the malignancy. This process of branched evolution appears to be especially applicable in the context of heterogeneous microenvironments because different selection forces may be operating concurrently in different areas of the tumor. In addition, it has recently been proposed that heterogeneity within certain tumor types may also occur via the "Big Bang" model, in which many mutations are acquired very early during tumor progression. In the absence of strong selective pressures at the initial stages of tumor progression, these mutations are likely to coexist. Theoretically, this state is maintained until a given stressor selects for the fittest clones. In this model, complete clonal sweeps are thought to be rare and the clones that survive stress may thus not have been the most dominant in the original tumor [23,24].

Regardless of its origin, genomic heterogeneity within tumors presents a challenge to both diagnosis and therapy. Genomic heterogeneity is associated with several important caveats when attempts are made to classify and prognosticate cancers. In recent decades, considerable effort has been directed toward generating a comprehensive catalog of the genes that initiate or cause cancer progression (drivers) and distinguishing them from genes that are a simple by-product of somatic evolution (passengers), for which a number of bioinformatic tools have been developed [25]. Even in those cases where potentially druggable alterations are found, the implementation of this framework is contradictory to the widely heterogeneous nature of most tumors because such studies are performed on restricted cell populations isolated from a small part of the tumor that are unlikely to reflect the full spectrum of the heterogeneity within a given neoplasia. As such, intratumor heterogeneity significantly confounds the interpretation of massive sequencing studies performed using single tumor samples, as minor clones may be masked and molecular studies may not be representative of the tumor as a whole. This makes it challenging to target single cancer-driving mutations because these mutations may be present in some but not all cancer cells in the same tumor. Furthermore, during cancer evolution, one driving genetic lesion may be replaced by another as it is becoming apparent that mutations essential for cancer development may not be required for disease progression [26].

Consistent with this tenet, even those tumors with a potent driver mutation show a temporal clinical response of months or years, followed by clinical relapse when that mutation is targeted. This eventual treatment failure is thought to be a consequence of the involvement of alternative genes or the activation of redundant pathways, as well as the inability of single drugs to target the entire subset of malignant cells [3,10,27]. For example, almost half of melanomas harbor BRAF-activating mutations (most commonly BRAFV600E), which lead to constitutive activation of the mitogen activated protein kinase (MAPK) pathway. BRAF inhibitors such as vemurafenib and dabrafenib are used to treat metastatic melanomas and initially cause tumor regression. However, resistance ensues, often due to downstream activation of MEK. To circumvent this, MEK inhibitors have been developed and used in combination with BRAF inhibitors. This extended the response to about a year, but alternative modes of resistance emerged, leading to recurrence [28]. Similar targeted therapies have been developed for a number of other oncogenes and cancers, but their clinical efficacy is usually lower than expected. Moreover, several important drivers have not yet been targeted. For instance, no effective therapies have been found for KRAS mutation-driven tumors, even though > 20% of all cancers harbor mutations in this gene and its aberrant activation is associated with resistance to anti-epidermal growth factor receptor (EGFR) therapies and other anticancer agents [29]. We posit that this is largely due to the tremendous genetic heterogeneity present in most tumors, which is further compounded by a variety of epigenetic mechanisms [30].

Finally, many genetic alterations vary widely from one patient to another (referred to as intertumor heterogeneity), making it difficult to form overarching conclusions regarding the importance of specific alterations. These caveats are further compounded in advanced disease, in which genetic alterations vary enormously between primary tumors and metastases and/or are affected by chemo- or radiotherapy.

3. Epigenetic heterogeneity

As proposed by Kolch et al. [30], genetic events are likely triggering elements of tumorigenesis, but much of the enormous plasticity of cancer cells to evolve different phenotypes, as well as their ability to adapt to challenging environments and withstand therapy, is encoded by constant perturbations in epigenetic programs and the rewiring of signaling networks, which display high flexibility and nonlinearity.

Indeed, overlaid onto genomic heterogeneity is epigenetic heterogeneity [31]. Unlike mutations, epigenetic changes do not affect the primary DNA sequence, but involve interactions among cells and their microenvironments, which lead to heritable changes in otherwise reversible phenomena such as chromatin modifications. In cancers, epigenetic heterogeneity can manifest as cellular hierarchies, similar to those observed in stem cell-associated systems, as well as the manifestation of cellular plasticity.

According to the hierarchical model of cancer, either a stem cell acquires a set of mutations that gives rise to a stem cell-like counterpart, referred to as a "cancer stem cell", or a cancer cell acquires stem cell-like properties [32]. Cancer stem cells can self-renew and give rise to the progeny of more differentiated cancer cells with a variety of different phenotypes. Consequently, they engender a hierarchy of cells that are all derivatives of the original mutated progenitor, contributing to the cellular heterogeneity of tumors [32]. The first study to describe the hierarchical model of cancer, led by Dick and colleagues [33], was based on a human acute myeloid leukemia model. This paradigm has since been extended to malignancies as diverse as breast cancer, glioma, and colon cancer [34–37]. Hence, hierarchical structures likely contribute to tumor heterogeneity in most cancer types. Interestingly, strategies for disrupting pathways that are thought to maintain stem-like and niche cell phenotypes such as inhibition of Wnt production have been proposed [38].

Epigenetic heterogeneity may also be acquired via "phenotypic plasticity". Phenotypically, plastic cancer cells can move back and forth through a continuum of cell fate specifications, from well-differentiated cell types to those with stem cell-like phenotypes [39]. For example, non-invasive epithelial-like $CD44^+/CD24^+$ breast cancer populations can give rise to highly invasive mesenchymal-like $CD44^+/CD24^-$ cells

Box 1
Big data in cancer.

All genomic interaction studies are currently performed by bioinformatics experts, whose role is becoming increasingly important. The methods used are diverse, and several computational data evaluation models have been described. For example, in some types of cancer, the main databases include the Catalogue of Somatic Mutations in Cancer (COSMIC), which encompasses more than 1.5 million individual mutations in 25,606 genes from almost 950,000 samples. Also important is the quantity of data held by the consortium formed by the Cancer Genome Project, the International Cancer Genome Consortium (ICGC), The Cancer Genome Atlas (TCGA), and the Encyclopedia of DNA Elements (ENCODE), which also investigates various structural and regulatory units of the human genome. Several platforms are used to analyze this huge quantity of data. These include Bionimbus, Bioconductor, CytoScape, and OncoDrive, which were designed to enable scientists to exchange databases and construct algorithms and mathematical models of cancer. With all of these databases, the main objective is to understand molecular alterations, mechanisms, and interactions between the different alterations and biochemical pathways in order to identify the real drivers of tumor progression.

Therefore, massive data are being incorporated from different types of tumors. These include histopathologic, immunohistochemical, molecular, and proteomic data, as well as data on microRNA. Data are also obtained from spectrophotometry, liquid chromatography, metabolomics, nuclear medicine and imaging, circulating tumor cells, and tumors implanted in murine models. Although these databases significantly contributed to the field of cancer biology, several issues such as misinterpretation of DNA damage during sample handling as bona fide somatic mutations in cancer specimens have been recognized (add PMID: 28209900).

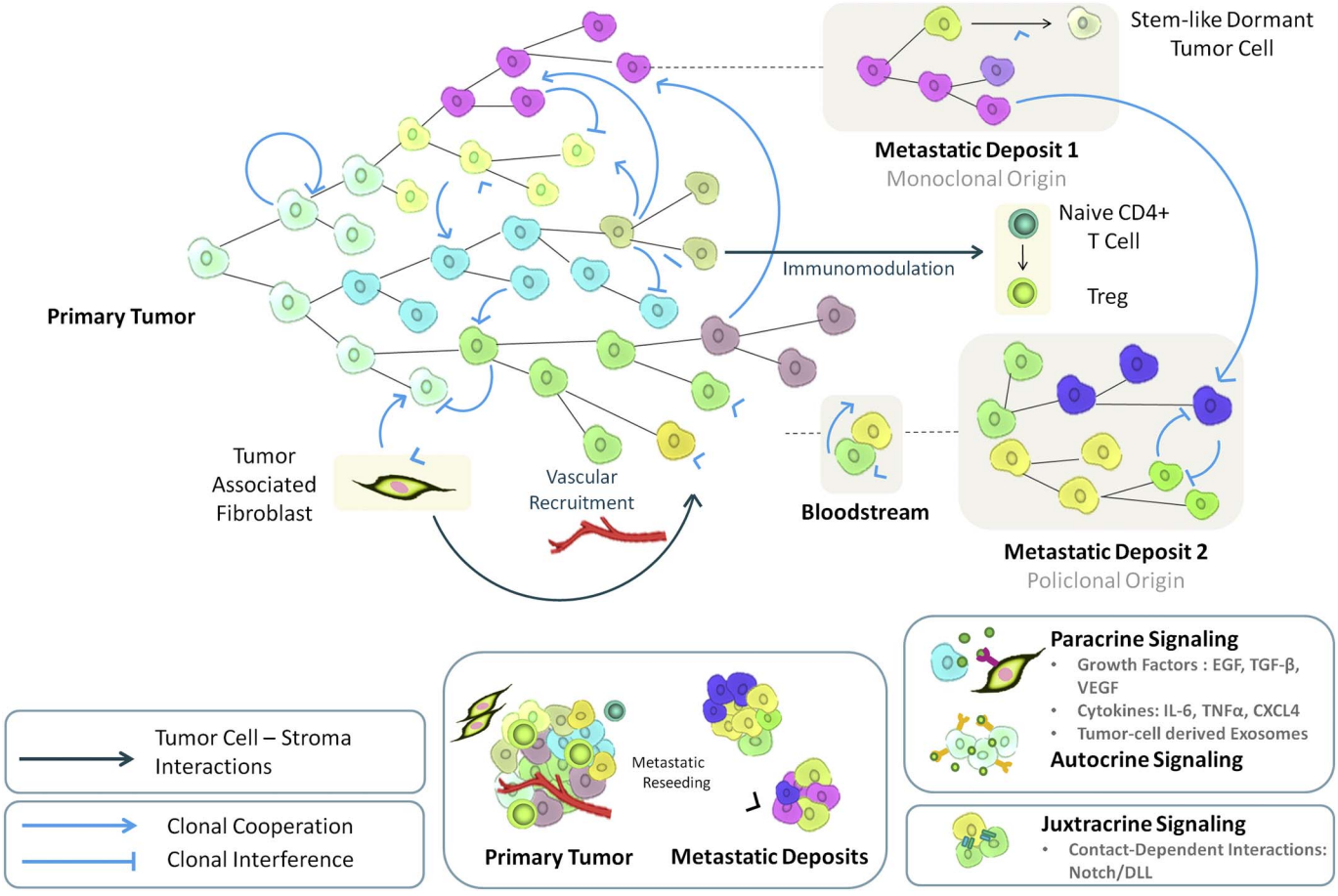

Fig. 1. Clonal interference and cooperation in tumor evolution. A regulatory architecture for intratumor heterogeneity.
This scheme illustrates how several clones are formed during tumor progression (each clone is indicated with a different color). Clones need to cooperate among themselves and with stromal and inflammatory cells. These interactions can be via paracrine, autocrine, or juxtacrine signaling. Metastases are formed by some clones that probably also need to cooperate among themselves to be able to grow in the metastatic niche.

both in vitro and in vivo [40]. While the emergence of cancer stem cells is a feature of plasticity, other phenomena such as epithelial-to-mesenchymal transition (EMT) also occur [41]. EMT is characterized by a loss of epithelial cell markers, such as epithelial (E)-cadherin, and the acquisition of mesenchymal markers, such as vimentin and neural (N)-cadherin [41]. In cancer, EMT is induced by a variety of transcription factors, signaling proteins, and aberrant regulation of various microRNAs (miRNAs) [42]. For example, upon exposure to tobacco, normal human bronchial epithelial cells undergo EMT, due to aberrant epigenetic silencing of miR-200 and miR-205 tumor suppressors [43,44]. EMT also correlates with an upregulation of pluripotency markers such as Nanog and Nodal [45,46], suggesting that cells that have undergone EMT may represent those with more stem cell-like phenotypes. Several studies have shown that plasticity can be induced

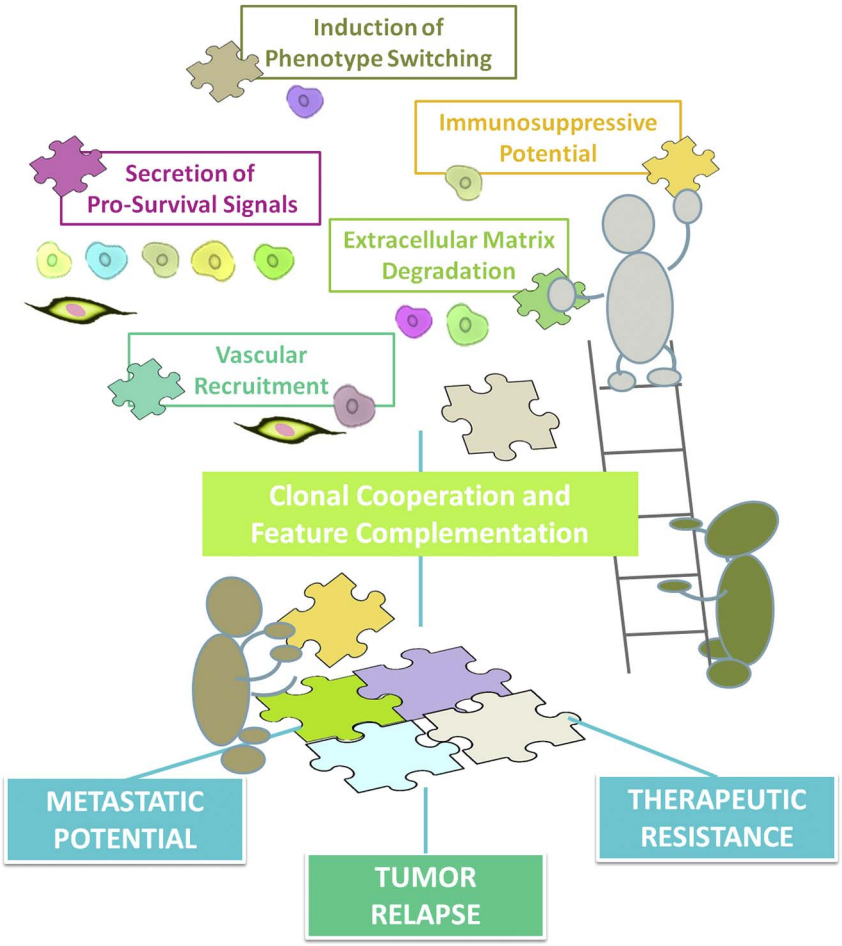

Fig. 2. Clonal cooperation in cancer. Emergent properties.
The clones needed to form the malignant tumor are selected based on oncogenic properties that have developed and may be shared with other clones not containing these properties, including shared use of proangiogenic factors or prosurvival signals secreted by some clones. We visualize this cooperation as pieces of a jigsaw puzzle, which, when completed, reveals the full picture, as a real emergent property.

by stresses such as hypoxia and chemotherapy, pointing to an adaptive mechanism that is driven by the microenvironment and can reset the equilibrium of a tumor to favor continued adaptation and progression [47–49]. Hence, differences in the tumor microenvironment that occur during progression or in response to therapies may also drive epigenetic heterogeneity concomitant with plasticity. This plasticity of cancer may generate and/or accelerate the selection of cellular clones with complementary features that lead to therapy resistance and favor cancer dissemination.

Similar to genomic heterogeneity, epigenetic heterogeneity can also limit the efficacy of targeted therapies. For example, chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL), which are associated with clone-specific BCR-ABL fusion [50], can be successfully treated by inhibiting BCR-ABL with imatinib. However, resistance and recurrence occur in some BCR-ABL$^+$ patients treated with imatinib. This is partly due to the inability of the drug to eradicate leukemic stem cells [50,51]. Leukemic stem cells appear to be epigenetically rewired to not always manifest oncogene addiction to BCR-ABL as compared to more differentiated progeny. This leads to residual disease persistence in patients treated with imatinib, highlighting the importance of understanding epigenetic heterogeneity for the success of the anticancer treatment [52,53].

4. Epigenetic changes and genomic instability go together

Genetic and epigenetic mechanisms also continuously engage and disengage a multitude of signaling pathways, resulting in dynamic restructuring of key cellular networks [54,30]. As one can imagine, mutations may activate specific pathways. However, the outcome of this activation depends on cellular context and epigenetic receptivity. Many such examples of the orchestration and modular activation of multiple signaling pathways have been described [30], including crosstalk between tyrosine kinase receptors. The function of EGFR is affected by gene amplifications and mutations, as well as the availability of other tyrosine kinase receptors for dimerization [30,55]. As such, the consequences of an EGFR mutation would depend on the epigenetically regulated expression of other receptors. EGFR activity is thought to be mostly mediated by the MAPK/ERK and PI3K signaling pathways [30]. However, the effects of EGFR on downstream signaling pathways appear to be modular. For instance, heterodimerization of EGFR with other receptors of the EGFR family such as HER2 and HER3 bolsters the activation of both MAPK/ERK and PI3K pathways [30]. EGFR has also been reported to engage AXL, which is transactivated by EGFR through heterodimerization. AXL, in turn, can be stimulated by platelet-derived growth factor and interact with additional receptors, including MET [30]. These examples illustrate the potential diversification of EGFR signaling in the context of neoplasia. They also highlight an important caveat that must be considered when deriving targeted therapies against this frequently mutated protein.

Cellular plasticity can also be influenced by the interplay between genomic and epigenetic mechanisms. For example, during EMT, signals from ligands, including TGF-β, lead to the orchestrated expression of transcription factors such as Snail-1, Slug, and ZEB-1, which repress the expression of specific epithelial genes (e.g. E-cadherin) while inducing the expression of mesenchyme-specific genes (e.g. vimentin) [56]. Several interconnecting positive and negative feedback loops comprising ERK, WNT, microRNAs, and other pathways have been proposed to govern complex perturbations in gene expression that underpin EMT [57–60]. Collectively, it is becoming obvious that the interplay between genomic, epigenetic, and signaling alterations in

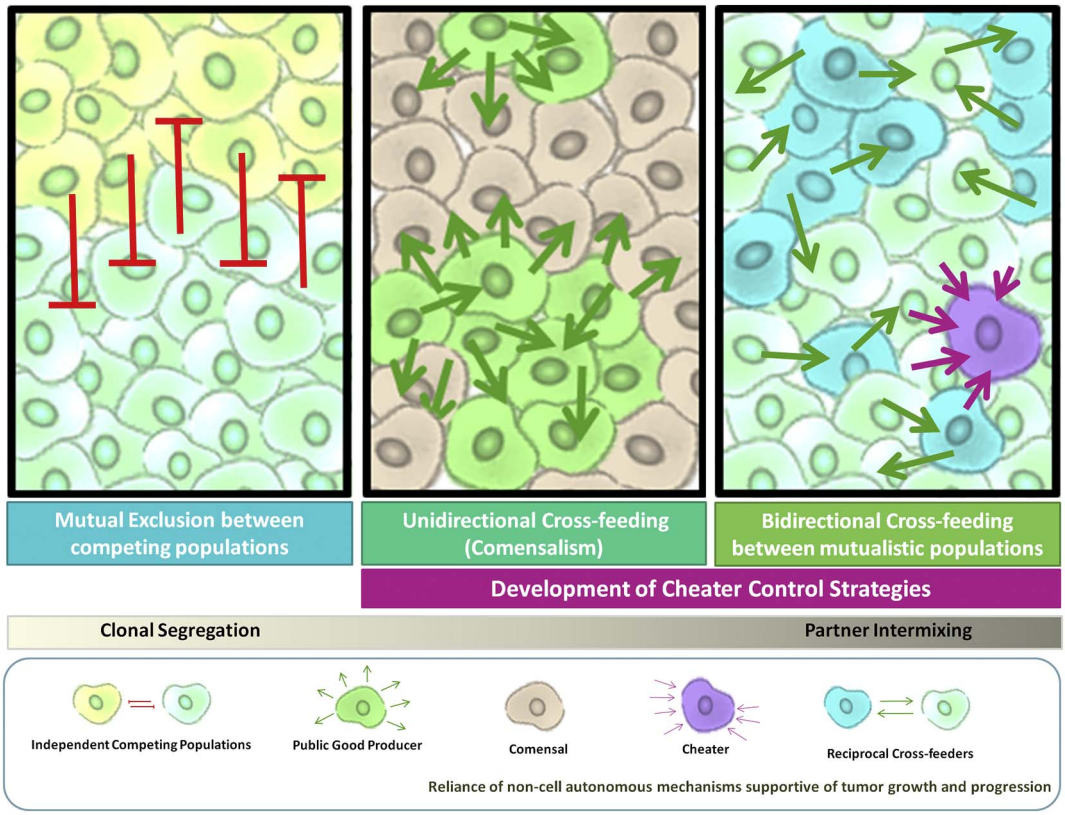

Fig. 3. Spatial and functional architecture in cancer. Clonal segregation and partner intermixing.
In malignant and invasive cells, clones may have bidirectional cross-feeding between mutualist populations. At the primary tumor, other clone strategies may be found such as competing populations and even unidirectional cross-feeding.

cancer may be infinitely more complex than initially anticipated, whereby genetic, epigenetic, and signaling perturbations diversify cells within the same tumor bed and lead to immense intratumor heterogeneity (see Box 1 big data and Box 1 ecological diversity).

5. Cancer as a consortium of cooperating malignant clones and microoenviromental cells

For several decades, authors such as Heppner [19,61–64] have been stressing that tumor progression requires the cooperation of several transformed cellular clones, as well as the active involvement of the microenvironment.

Thus, cancer could be considered a multicellular community. In ecology, the biological functions associated with interspecies interactions must be concomitantly more beneficial to the component species than their respective costs [65]. Complex multicellular systems, such as cancer, are thus likely to function in a similar fashion to microbial consortia (see Box 6 microbial consortium), wherein a spatial architecture and distribution of cellular clones ensures greater and mutual benefits. Positive and negative clonal cooperation (clonal interference), mediated both directly at the level of cellular contacts and indirectly via microenvironmental factors, cytokines, and/or exosomes, is therefore likely to play a major role in cancer evolution (see Figs. 1, 3). Tumor cells are thought to require a certain number of molecular alterations—just three according to Vogelstein [66,67]—to overcome senescence and acquire neoplastic properties. However, to generate metastases, a cancer cell must be able to overcome anoikis, invade and survive in the peripheral blood, and eventually grow in a remote organ. It is unlikely that these processes are achieved in isolation in a single clone. A more plausible explanation is that the metastatic potential of cancer cells is generated in cancer cell consortia, which facilitate tumor progression in an “ecomolecular” way in which several clones cooperate. These clones are synergistic and share the molecular and biochemical alterations required to generate an invasive tumor. Based on these observations, we propose that cancer should be perceived as an “ecomolecular” disease that involves cooperation between several neoplastic clones and their interactions with immune cells, stromal fibroblasts, and other cell types present in the microenvironment. Cancer is therefore analogous to complex ecosystems such as microbial consortia.

Clonal cooperation within cancer cell populations can explain phenomena such as the recently described cooperative invasion in melanoma [68] or circulating tumor cell (CTC) clusters [69] (see also Fig. 2). Interestingly, CTC clusters appear to display enhanced metastatic potential compared with single cells [70]. Polyclonal CTC clusters have been demonstrated in metastatic murine models [68,69,71,72], whereby seeding of different malignant cellular clones within CTC clusters can occur in parallel or at different moments (indirect clonal cooperation) [69–71]. These data support the concept of clonal cooperation and suggest that, in tumor growth, synergism between several complementary clones and local factors is needed for survival and invasion. Malignant cells can then stay quiescent for an extended period of time (“dormancy”). These cell populations evolve a self-induced latency state that allows them to evade immune response while promoting their long-term survival in micrometastatic deposits [73]. SOX transcription factors and the Wnt pathway have been proposed to “wake up” these clones [74]. However, accordingly to the idea of ecomolecular disease and tumor consortia, cooperation between several tumor clones may be required to trigger tumor growth in the metastatic niche. Furthermore, other studies have shown that this cooperation can also occur between malignant and non-malignant cell types [75,76]. For example, association with surrounding normal cells, including

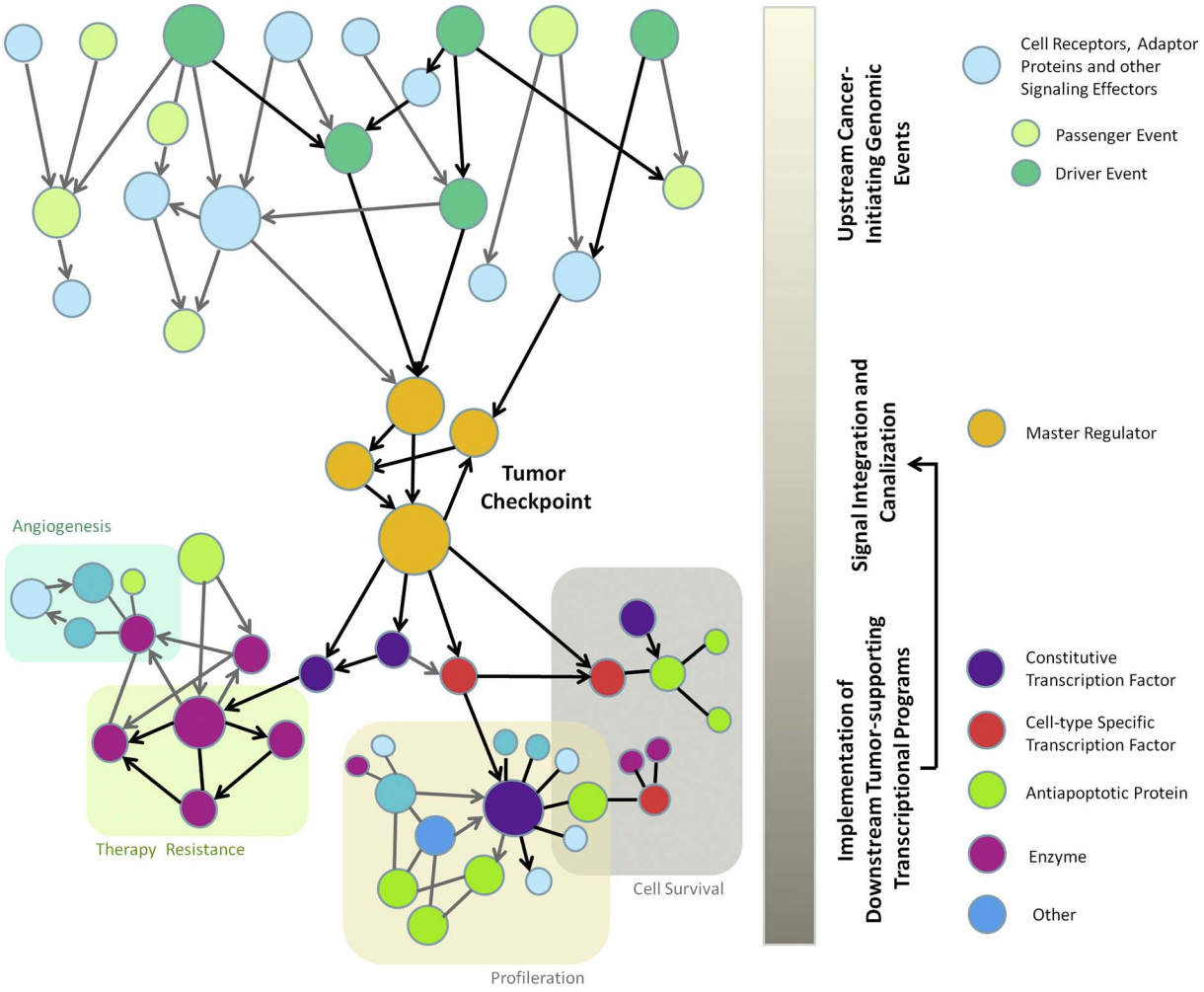

Fig. 4. Establishment and maintenance of the tumor cell state. Master regulators, tumor checkpoints, and tumor-supporting transcriptional signatures.
According to the hallmarks of Hanahan and Weinberg, tumor transformation and progression requires the disruption of several biochemical pathways. Large numbers of genetic alterations are observed in malignant tumors and numerous positive and negative feedback loops in and between those pathways. It therefore seems logical to look for central nodes, hubs, or funnel factors that control cell proliferation or the resistance of malignant cells to several cellular stresses. Systems biology is emerging as a powerful tool to identify the factors that commonly change within one tumor and in separate tumors among independent patients.

platelets, macrophages, and fibroblasts, increases the metastatic potential of cancer cells [75,76]. Notably, paraneoplastic phenomena are sometimes years ahead of the clinical detection of a malignant tumor. For example, paraneoplastic syndromes such as eosinophilia and thrombocytosis, as well as certain neurological disorders, can anticipate the early detection of tumors at an incipient clinical stage [77].

Clonal cooperation in cancer can be expanded to explain why a minimum number of cells is required for a clone to have sufficient biological fitness, a phenomenon known as the Allee effect [9,78]. The Allee effect explains why isolated cells often cannot grow in vitro or in human tumor explants and why rates of cancer initiation, invasion, and metastasis are relatively low when individual or a relatively low number of cells are used. This can be at least partly explained by an ability of clonal cooperation to bolster tumor growth, especially in situations of microenvironmental stress. For instance, autocrine production of growth-promoting and prosurvival factors may be insufficient to support neoplastic growth unless they are present in large quantities (i.e. from a greater number of cells). This is referred to as cooperative feeding and may be one of the major determinants of the Allee effect in cancer. The acquisition of driver events and the continuous shaping of the genomic landscape of a tumor could be understood either under the lens of the classical clonal theory or more recent evolutionary and developmental paradigms [23,24,79,80]. Herein, we propose that these theoretical frameworks should also include inter-clonal relationships other than competition (e.g. mutualism and commensalism) and consider sources of variability not necessarily contingent upon the genomic status of the cancer cell (i.e. epigenetic, post-transcriptional, and signaling pathway remodeling) [63].

Cancer cells interact with the microenvironment. The tumor microenvironment consists of various cell types, such as endothelial and immune cells, as well as inflammatory cells, fibroblasts, adipocytes, and mesenchymal stem cells. These cells are surrounded by heterogeneously deposited extracellular matrices and signaling proteins and are affected by changing biophysical properties such as pH and oxygenation [18,45,81,82]. A plethora of mechanisms ensure the adaptation of somatic cells to the multicellular development program of the organism, whereas deregulation of these mechanisms allows cancer cells to thrive and progress despite negative microenvironmental cues. Indeed, cancer cells actively enroll their healthy counterparts in tumor progression-supporting behaviors. Hence, the microenvironment is an active mediator of tumor progression and must be accounted for when cancer is conceptualized, prognosticated, and treated.

Environmental factors such as limited oxygen supply or lack of nutrients bolster the expression of multiple cytokines, pro-stromal, and inflammatory factors and thereby promote the recruitment of endothelial cells, macrophages, fibroblasts, and an array of inflammatory cells to hypoxic areas of the tumor [83,84]. Hypoxia is a potent activator of both metastasis and therapy resistance and, as described above,

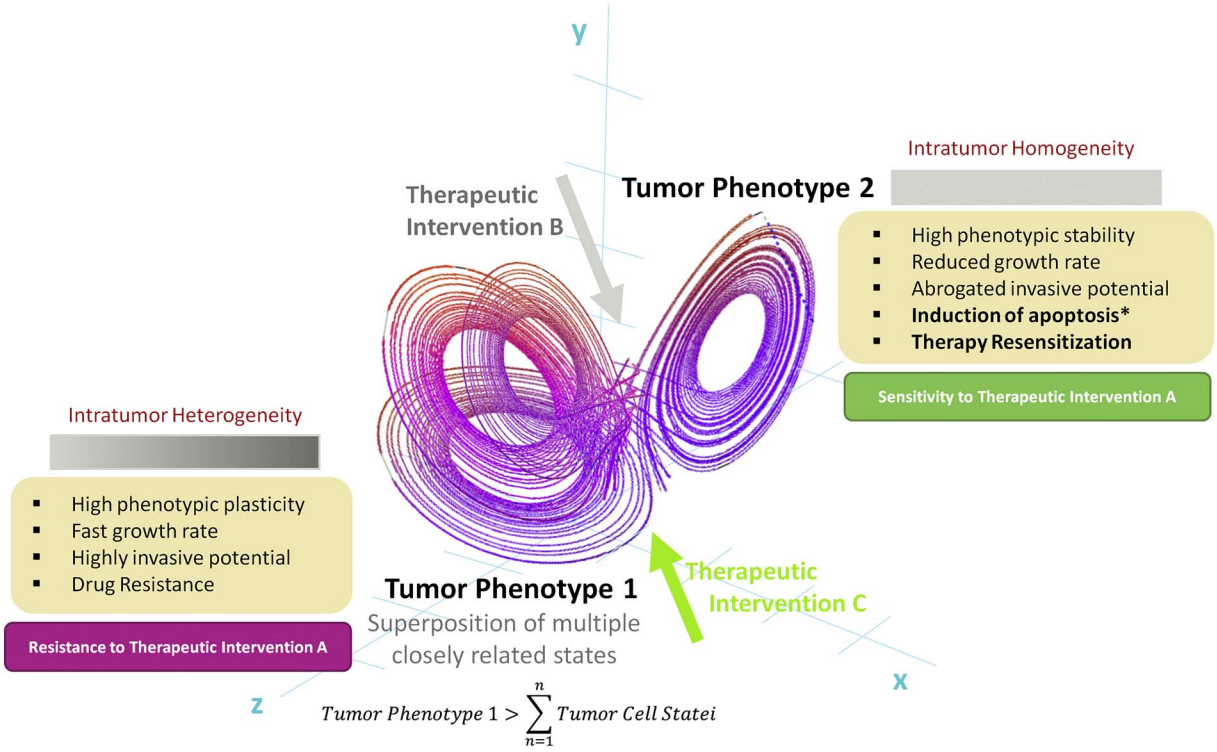

Fig. 5. Domesticating the chaos. A proposal for network rewiring in tumor cells.
In the complex interplay of the pathways activated in cancer, new therapeutic approaches have to be defined. According to chaos theory, it would be difficult to control each pathway, but there are options, such as reorientation of the signals to a pathway that is druggable. Thus, the networks responsible for the maintenance of a particular highly heterogeneous tumor phenotype would be shifted toward more manageable homogeneous states.

Box 2
Application of the theories of "ecological" diversity to the study of tumors.

Intratumor heterogeneity is increasingly studied by employing models used in ecology. Well-known indices have been applied to the study of breast cancer by various groups, including that of Polyak, in order to better understand the genetic and phenotypic diversity of breast cancer metastases. Such indices include the Shannon entropy index, which was described for the study of animal species and quantification of entropy, that is, to reflect information and uncertainty and to try to predict variations in the homogeneity. Also used has been the Simpson index, a diversity index that aims to validate the percentage of individuals who belong to a specific type of species by sub-classifying them into variants.

Use of mathematical approaches in conjunction with the Shannon entropy and the Simpson diversity index aim to better explain the molecular heterogeneity and diversity of tumors [63,64,125]. Other authors [126] have proposed the use of the quadratic entropy index (Rao) or index of ecological diversity, which has been studied in plant genealogy.

can induce stem cell phenotypes concomitant with the expression of cytokines such as IL-6. By changing the cytokine milieu, hypoxia promotes the acquisition of an immunosuppressive microenvironment, allowing cancer cells to evade destruction [85,86]. Alterations in cytokine secretion also promote metastasis by recruiting cells such as M2 macrophages that can facilitate invasion and cancer spread. Consistently, numerous studies have shown that hypoxia [87,88] and leucocytes [75] promote metastasis.

In addition to extracellular stimuli, an important determinant in tumor evolution involves interactions with the immune system (see Box 5). Indeed, the immune system can prevent, control, shape, and promote cancer through the process of immunoediting, during which tumor cells continually evolve in response to interactions with the immune compartment [85,89–91]. Immunoediting involves three phases: elimination, in which the immune system recognizes and eradicates cancer cells; equilibrium, in which the tumor is kept in check, or dormant, by co-existing with the immune system without growing; and escape, in which the tumor grows and can no longer be suppressed. Immune surveillance can be escaped via several mechanisms, including a reduction in tumor-associated antigens, resistance to apoptosis and immune suppression through the secretion of cytokines and metabolic factors, and suppressor cell recruitment and activation. Moreover, an altered transcriptional landscape in malignant cells increases immunogenic diversity by generating alternative protein isoforms [92]. The expression of alternative isoforms is associated with reduced signatures of T cell cytolytic activity and poor patient survival. Hence, epigenetic modifications, leading to altered isoform expression, could be how cancer cells adapt to and evade the immune system. These concepts highlight the importance of immune cells in tumor evolution and have been reviewed extensively elsewhere [85,89–91].

Based on these findings, we propose that cancer cells, through clonal interactions and crosstalk with their microenvironment, constitute a neoplastic consortium that functions analogously to that of their microbial counterparts. Mapping and dissection of the molecular underpinnings of neoplastic consortia will undoubtedly enhance understanding of cancer biology and provide the basis for more effective cancer treatments.

6. Cancer as an emergent property

The concept of emergent properties is commonly equated to that of a famous saying by Aristotle, “The whole is greater than the sum of its parts”, or more recently to the principle postulated by Kurt Koffka, “The whole is other than the sum of its parts” (see Box 6).

Emergent behavior is often unpredictable and unprecedented and may represent a new level in the evolution of the system. Emergent properties arise when a number of single components (e.g. pixels on a television screen, bees within a beehive, the subcellular machinery of the cell) interact in an environment and lead to complex collective behaviors that are difficult to grasp by simply monitoring the individual components of the system.

Cancer can be studied in a similar way to the pixels on a television screen: a single pixel reveals nothing: it is the sum of all of the pixels that conveys the meaning of an image. In other words, neoplasia is not the result of single genomic alterations or even multiple genomic alterations in a single cell, but the sum of all of the molecular changes undergone by a community of tumor cells, including those affecting signal transduction and gene regulatory networks, as well as the environment within which malignant cells reside. Similar concepts have been postulated in the neural networks theory [93], in which the synchronized activity of a set of neurons enables the perception of images and sounds to give rise to cerebral and cognitive functions. This is in opposition to concepts underlying current precision medicine strategies. Most precision-based approaches are based on the premise that an entire tumor can be eradicated by taking out a single driving factor. The rather disappointing outcomes of recent trials and single-cell studies demonstrating tremendous clonal heterogeneity suggest that Gestalt-like models should be considered to enhance the understanding of cancer complexity [11,12].

In a similar manner, we propose that cancer must be understood within the framework of a series of genetic alterations that appear to be coordinated with other molecular events, such as the epigenetic status of the cell, rewiring of signaling networks, and microenvironmental factors. This is analogous to the model in which individual pixels cooperate to form a complete picture—reuniting a minimum number of conditions to configure a circuit that provides cancer cells with required growth autonomy. But this paradigm must consider that the complexity of cancer is also likely to rely on interactions between tumor cell clones and associated normal cells. This complete set of properties, some of which differ between cell populations in the tumor, allows neoplasia to act as a cooperative and coordinated community, facilitating invasion and disease spread, and ultimately leading to the patient's demise. Thus, understanding of how these consortia of stromal and inflammatory cells interact with tumor cells is critical for developing more effective treatments [75,94–96].

7. Employing systems biology approaches to grasp the complexity of cancer ecosystems

Systems biology encompasses tools that hold great promise for deciphering the vulnerabilities of the tumor ecosystem as a whole. These studies are based on the premise that multiple oncogenic events converge on a relatively limited number of cellular networks (see Box 3 topologic analysis), which may contain essential or synthetically lethal clinically targetable hubs or factors [26,97] such as the eIF4F complex (see Box 4 central nodes). Targeting of these central nodes of cancer-specific networks (e.g. protein synthesis machinery) is thought to provide a sufficient therapeutic window to selectively target cancer ecosystems while causing minimal toxicity in normal tissues, which indeed is observed in preclinical studies. Nonetheless, many contemporary systems biology approaches do not consider intratumor heterogeneity. This is relevant as key functional nodes within the metabolic, signal transduction, and gene expression networks responsible for supporting the tumor phenotype are critically dependent on the heterogeneity of the tumor. Critical nodes of cancer-specific networks should thus be studied within specific cancer ecosystems. This aspect still represents a major challenge.

Given the large amount of data amassed on tumors in recent years at the clinical, morphological, and molecular levels, there is heightened interest in the development of powerful bioinformatics methods and well-curated databases to boost understanding of the complexity of tumor ecosystems. These data may also help to classify tumors according to histopathological, biochemical, and genomic features and thus facilitate tailoring of diagnosis and clinical management to the biological profile of a patient's tumor. Accordingly, multidimensional molecular and gene expression data, which are associated with the response to antitumor treatments and clinical progress, are thought to facilitate the selection of patients who are more likely to respond to targeted or “precision” therapies [98,99]. Several approaches that encompass deep-learning are being developed to harness information on intratumor heterogeneity and to identify and diagnose multiple tumor types [178,179] by integrating radiological, histological, gene expression, and in situ hybridization data.

There are ongoing large collaborative efforts such as the Cancer Cell Map Initiative [100] and others taken on by groups such as the Califano laboratory at Columbia University [26,101]. Consistent with the role of epigenetic and signaling programs in cancer development and progression, these efforts suggest that a functionally relevant characterization of all of the molecular alterations described in a patient's tumor will only be possible in the context of a topological study of all of the pathways and networks involved in tumorigenesis. According to such efforts, it appears that genomic and gene expression profiling—at both steady-state mRNA and proteome levels—must be appropriately integrated to identify the clinically targetable factors driving tumor progression in each individual patient. However, most current precision therapies target the mutated genes in a given tumor type, whereby it is thought that suppression of drivers will shut down downstream pathways that provide cancer cells with a selective growth advantage. However, the presence of evolutionary tolerable mutations in driver genes, in conjunction with the well-established ability of cancer cells to rewire their signaling pathways and intratumor heterogeneity, complicates such approaches.

Multi-institutional efforts are crucial to the development of more efficient treatment strategies. For example, the DARWIN trial (Deciphering Antitumor Response With Intratumor Heterogeneity; NCTO2183883) intended to define the relationship between driver clonality and the potential benefit of targeted therapy by assessing ctDNA and CTC and the TRACER trial (TRAcking Non-small Cell Lung Cancer Evolution Through Therapy [Rx]; NCTO1888601). Herein, several regions of tumor were sequenced before and after relapse in order to define the genomic landscape of tumors throughout evolution and to understand the impact of tumor heterogeneity on therapy responses [102,103]. This information is now being used to further refine clinical trials and to try to individualize treatments as much as possible in stratified patient groups. For example, in the emerging “N-of-1 trial” [104], the trial data are obtained from a single patient to determine the optimal intervention for that individual. However, these trials need increased attention in light of the era of personalized medicine.

VIPER analysis [97] and multiple concerted disruption [105] aim to integrate data on DNA alterations (mutations, amplifications, translocations, methylations, and deletions) with mRNA expression and protein levels. Although these approaches are likely to produce some meaningful data, given the complex relationships within the tumor microenvironment, it will also likely be pertinent to understand the dynamics of DNA alterations in relation to mRNA and protein levels occurring as a result of the interactions within the tumor ecosystem. Finally, these data should also be appropriately integrated with patients' clinical and family history. Collectively, − omics data generation, analysis, and interpretation, and their clinical applications, will require a joint effort from experts in diverse disciplines such as

Box 3
Topological analysis and study of biochemical and genetic alterations.

Several representative examples explore the interplay among biochemical pathways and clinicopathological data. The database Gene X Press includes gene modules that affect the activity of a tumor, such as those of the Gene Ontology project, which describes the potential pathways and abnormalities in tumors resulting from specific genetic alterations. In addition, Gene Microarray Pathway Profiler and Signaling Pathway Impact Analysis consider the position of a gene in a pathway. Similarly, some models associate genes in both cis and trans, making it possible to identify genes known as "masters". Study of cancer-related pathways has been proposed to consolidate understanding of biological mechanisms by means of algorithms. One example is the Pathway Recognition Algorithm, which uses integration data from oncogenomic models, allowing the number of copies of genes to be contrasted with mRNA expression, methylation, and microRNA expression.

medicine, biology, mathematics, statistics, bioinformatics, and systems biology [30,106,107] (see Box 1 big data and Box 2 ecological diversity).

The availability of system-wide data in a variety of cancers is facilitating the development of approaches that go beyond the classic, reductionist paradigms, which are limited to the association of single genes with cellular phenotypes and functions. Systems biology approaches consider the interplay between multiple molecular factors that underpin the development of a particular phenotype. Accordingly, rather than a genetic disease, cancer is now being perceived as a "disease of networks" [100]. Therefore, to fully grasp the complexity of the tumor ecosystem, the networks driving cancer need to be mapped and their dynamics and evolution over time need to be deciphered. Emerging data show that these cancer networks are constantly rewired in part by clonal interactions, changing microenvironments, and the acquisition of novel molecular alterations that are largely induced by anticancer treatments [106,108]. Hence, minor subpopulations that are not readily detectable in bulk tumors or that manifest the ability to adapt to hostile environments may emerge following treatments that specifically target cancer-driving mutations present in the predominant tumor subpopulations. These subpopulations are likely to result in refractory disease inasmuch as they do not harbor the vulnerabilities identified in the majority of the tumor.

In our opinion, these studies suggest that a shift in cancer treatment paradigms may be warranted. Interactions in the tumor ecosystem do not occur randomly: they appear to follow a series of principles. They contain highly connected core nodes known as "hubs". Hubs interconnect various pathways and are considered essential for the maintenance and integrity of the entire network and cellular ecosystems, whether healthy or pathogenic. These "network hubs" are usually encoded by well-conserved genes that play a role in key cellular activities [106–111]. Analogous to the "butterfly effect" in chaos theory, small alterations in these hubs can lead to major alterations in cellular functions (e.g. proliferation and invasion), whereby the differential reliance of cancer and normal cells on a given "network hub" is expected to provide a sufficient therapeutic window (see Box 7).

Therefore, systems biology approaches in cancer research hold a promise of identifying networks that are crucial for cancer cell survival and disease progression in the context of tumor ecosystems. These approaches are also thought to allow modeling and prediction of the response to drugs and the identification of key nodes or essential cancer networks [26,97]. Furthermore, recently developed methods exploit data-centered mathematical and computational methods, such as deep learning and evolutionary optimization algorithms, which are expected to facilitate mapping of the interactions between networks in systems of immense complexity such as the cancer ecosystem, as well as to detect similarities and discrepancies between different cancer ecosystems [30,108–111].

Thus, the application of current systems biology approaches and the development of novel approaches to study cancer may prove important in the following areas. **(1) Provision of detailed system level-aided and clinically oriented subclassifications of cancer types.** In this regard, the use of deep-learning approaches appears promising. **(2) Mapping of oncogenic networks and identification of their critical nodes to overcome the effects associated with intratumor heterogeneity.** A comprehensive understanding of the cellular networks that are altered in the tumorigenic state and in individual patients will be especially important to the study of the actions and interactions of cytotoxic drugs and other small molecule inhibitors with the cellular machinery. A large number of pharmacokinetic and pharmacodynamics factors, including drug half-life, potency, and efficiency of target inhibition or activation, as well as other parameters, will be required to accurately predict and guide therapeutic decisions (systems pharmacology). **(3) Informing future preclinical research and design of**

Box 4
Are central nodes of oncogenic networks targetable and could they overcome intratumor heterogeneity?

Compared with targeting of functionally redundant upstream regulators, targeting of central nodes of signaling networks that integrate multiple oncogenic signals may represent a valid strategy to overcome the capacity of neoplastic cells to rewire and become drug resistant [26]. Protein synthesis is frequently dysregulated in neoplasia. Differences in translational programs between normal and cancer cells are thought to provide a sufficient therapeutic window to selectively target cancer cells while causing minimal toxicity in normal tissues [127,128]. The eukaryotic translation initiation factor 4F (eIF4F) complex, which comprises a cap-binding subunit eIF4E, scaffolding protein eIF4G, and DEAD box RNA helicase eIF4A, recruits mRNA to the ribosome [129]. It is activated by the vast majority of oncogenes (e.g. c-MYC, HER2, PI3KCA) and inactivated by tumor suppressors (e.g. TSC1/2, PTEN) [128]. An increase in eIF4F levels is observed in the vast majority of cancers, where it results in a selective increase in the translation of mRNAs encoding pro-oncogenic factors such as cyclins, c-myc, and BCL-2 family members while not affecting the synthesis of housekeeping proteins such as actins and tubulins [130]. Elevated eIF4F levels are associated with chemoresistance and poor prognosis [131–133]. Moreover, activation of the eIF4F complex through multiple pathways diminishes the efficacy of a wide variety of oncogenic kinase inhibitors, including those targeting EGFR, HER2, PI3K, MAPK, and mTOR [134–146]. Given that the eIF4F complex plays a crucial role in cancer cell survival, irrespective of driver mutations or pathway rewiring [143], targeting of eIF4F may provide a means to address issues related to both intratumor heterogeneity and drug resistance [143,147,148,149,150]. Indeed, several preclinical studies have confirmed the validity of approaches that interfere with eIF4F assembly and/or function [145,149–154].

Box 5
Tumor microenvironment and therapeutic strategies.

Several therapeutic strategies have been proposed to target different cells in the tumor microenvironment. For example, cancer-associated fibroblasts (CAFs) take on hallmarks of transformation and are important mediators of cancer progression [155]. The tumor-supporting attributes of CAFs are acquired after exposure to tumor-derived factors such as TGF-β and become essential for tumor growth and metastasis. This is thought to at least in part be caused by the ability of CAFs to contribute essential growth factors within the cancer ecosystem and to chaperone cancer cells through the vasculature [156].

The interplay between cancer cells and the immune system is also highly important. Tumors achieve evasion via a number of mechanisms, including the expression of checkpoint proteins such as programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte-associated protein-4 (CTLA-4), as well as through the downregulation of immune-stimulating antigens [91]. Immuno-oncology therapies target such phenomena by stimulating the immune system via either passive or active approaches [89].

Active therapies include adoptive T cell transfer, vaccines, antigen-presenting cells such as dendritic cells, and oncolytic viruses [89]. Passive therapies are broadly aimed at fighting tumors by modifying signaling pathways that promote immunosuppression [89]. These approaches include checkpoint inhibitors such as ipilimumab (which targets CTLA4) and pembrolizumab and nivolumab (which target PD1R) as well as small molecules targeting immune modulators as diverse as cyclooxygenase-2 (COX-2) and chemokine receptor type-4 (CXCR-4) [89]. The most successful passive therapies thus far are the checkpoint inhibitor therapies. These therapies have been shown to eradicate some tumors by altering the tumor ecosystem in a manner that allows immune cells to regain control [89,155,157–159].

A promising approach to the treatment of malignant tumors is to target factors that confer them resistance to cellular stress. It is thought that acute stress (e.g. starvation, oxidative stress, chemotherapy, hypoxia) induces adaptation mechanisms in the translation machinery that are largely independent of the genetic and epigenetic makeup of cancer cells [160]. Although the best-explained mechanism of translational adaptation to stress comprises reduction in ternary complex recycling via eIF2αphosphorylation [161], recently emerging data show that various types of chemotherapeutics induce eIF4E phosphorylation via MAP kinase-interacting kinases (MNKs) [162]. MNKs are activated by ERK or stress-induced p38 kinase [163,164]. Phospho-eIF4E tends to selectively affect the translation of mRNAs encoding for secreted factors, cytokines, and matrix metalloproteinases, which play a major role in the interaction of cancer cells with their microenvironment [165]. Indeed, cancer cells whose eIF4E cannot be phosphorylated have severely impeded metastatic potential [166]. Therefore, eIF4E phosphorylation may be an essential mechanism of adaptation to stress downstream of p38 and ERK, and recently developed MNK inhibitors are showing early but promising results in combination with traditional therapeutic approaches. Moreover, levels of phospho-eIF4E appear to be consistently elevated in the vast majority of cells in the tumor [162]. Accordingly, it is expected that, during acute adaptation to chemotherapy-induced stress, phospho-eIF4E levels will be uniformly increased throughout the tumor and metastases, which suggests that MNK inhibitor and chemotherapeutic combinations may help to overcome issues associated with intratumor heterogeneity. Notably, the anticancer effects of MNK inhibitors have been shown in a number of preclinical models [167–171].

phase I clinical trials by anticipating therapy response in silico and predicting the best targets for each patient and tumor (personalized cancer therapy or precision medicine).

Altogether, the application of systems biology in cancer research may revolutionize the way we assess the molecular and biochemical changes in a single tumor and permit therapeutic approaches based on central targets. Nevertheless, some pitfalls or limitations can be envisioned. The great value of the available data and curated databases only materializes following detailed post hoc analyses. In-depth understanding of the properties of the system studied is required to use the data in such databases, and the heterogeneity in data quality, which is particularly observed during the developmental phase of -omics methods, is a major challenge that needs to be considered. For example, a recent method that has gathered substantial interest as providing a link between transcriptomes and proteomes is ribosome profiling [112]. Recently, there were concerns raised regarding biases in ribosome profiling data that could be associated with technical artifacts in cDNA library preparation and sequencing [113]. Therefore, although one of

Box 6
Microbial consortia as a paradigm for cancer understanding.

Clonal interrelationships have been extensively studied in microbial ecology, as exemplified by the microbial consortia. In microbiology, microbial populations inhabiting varying environments and/or responding to stress can cooperate with each other by forming well-structured communities in both space, time, and function [172–174,65]. Notably, the constitution of microbial consortia is an area of considerable interest in the biotechnological industry because such communities, while remarkably complex, show promise in overcoming the limitations imposed by approaches based on the use of a single strain [146]. In this regard, significant efforts have been made to engineer synthetic ecologic consortia, in which the interplay among members is expected to lead to a more sustainable, productive, predictable, and stable design [65].

The microbial interactions within consortia are mainly mediated by secreted factors, including metabolites. To this end, the stability of consortia depends on aspects as variable as cellular density, medium viscosity, and the localization and availability of resources and other metabolic products [65,174]. Spatial distribution of the involved populations (assortment), including cheaters (species that have access to group benefits but do not contribute to the other members of the group), is also thought to play a major role in the function of microbial consortia [65,174]. Cooperative interrelationships between microbes in consortia are commonly classified as non-reciprocal (commensalism) or reciprocal (mutualism) [65]. While competing populations with no metabolic interdependence tend to segregate (competitive exclusion), mutualism tends to drive partner intermixing [174]. Several studies have demonstrated that spatial self-organization, sometimes conditioned by phenomena as unpredictable as genetic drift, may provide a solution for the stability of intraspecific cooperation without the need for specific molecular mechanisms of partner recognition. This suggests that mapping of the spatial organization of a given consortium is likely to provide insights into its function.

Box 7
Emergent properties and the chaos theory in cancer research.

Emergent properties represent one of the most significant challenges for the engineering of complex systems. The system is different from the "sum" of its component parts, which in the context of cancer research hinders approaches centered on the study of cancer cells in isolation.

For example, a plethora of factors are involved in tumor progression and metastasis (e.g. genetic interactions, stromal cells, histiocytes, lymphocytes, and environmental conditions). Such complex interplay of large numbers of factors is challenging to predict, even with the most sophisticated software available today. Hence, this very feature of "chaos" should be accounted for in order to fully understand cancer.

The application of chaos theory may thus greatly help to explain the formation and progression of malignant tumors. In the three-body problem, Henri Poincaré [175] observed that the behavior of the heavenly bodies was extremely complicated and that this made it impossible to make long-term projections about their trajectories. He wrote, "A very small cause that escapes us determines a considerable effect which we cannot ignore, and then we say that the effect is due to chance….but it is not always so." He also observed that, "it may happen that small differences in the initial conditions produce very great ones in the final phenomenon. A small error in the former will produce an enormous error in the latter. Prediction becomes impossible and we have a fortuitous phenomenon." Chaos theory was postulated in the decades following the work of Poincaré. It is somewhat paradigmatic that in the 1960s, with the advent of the first computers, the meteorologist Edward Lorentz began to design calculations to predict the evolution of the weather. Based on a seemingly banal experiment, he realized that if he entered numbers into a new computer with three decimal places instead of six, the results were totally different. For years, Lorentz tried to find an explanation, and his efforts laid the foundation for what we now know as chaos theory. He also established the term "butterfly effect", in which small initial variations may produce enormous final variations, as pointed out by Poincaré. In the context of tumor biology, one can imagine how this might manifest: a small early event could be amplified and then even redirected by varietal competing factors.

Chaos theory brings an alternative approach to study of the complexity of tumor systems and is likely applicable to systems biology. For example, a recently published study [176] provides a very graphic summary of the discordance between massive sequencing studies, data analysis, and evaluation of data, depending on the platform used to interpret them. After comparing thousands of variants from several large numbers of tumors using various sets of transcripts with the platforms REFSEQ, ENSEMBLE, the annotation test, ANNOVAR, and other software packages, the authors came to an interesting conclusion: the results were highly variable and depended on the set of transcripts and software platform used. Moreover, the same sequences compared using different software applications showed discrepancies of $> 30\%$.

Given that, in principle, it was already impossible with the three-body problem to determine and predict the evolution of orbits, it is rather plausible that such a prediction may be even more "chaotic" in cellular models and biological systems with tens, if not thousands, of variables. However, chaos, while unpredictable, can be determined. In other words, chaos is not random, but has an underlying order. In this sense, especially at the physical level, but also at the biochemical level, modeling of enzyme behavior has permitted advances in attempts to predict what was previously perceived as the unpredictable. Chaos theory postulates the existence of clearly deterministic concepts that depend on the initial conditions and on the number of initial variables. In fact, recent research indicates that it might be possible to "train" chaos. For instance, Ott, Grebogy, and Yorke drew up a mathematical algorithm that could transform chaos into simple regular processes [177]. This mathematical approach has already been used in medicine. Ott, Grebogy, and Yorke also noted that, "it is not necessary to completely understand the chaotic process to regulate it". The proposed algorithm targets the direction of the process and tries to modify it with small adjustments to ensure that it "gets back on track". Therefore, chaotic systems are very flexible and can interrelate and modulate each other. This concept of chaos theory can be applied to one of the examples set out above, namely, control of cell stress and thus the promotion of factors mediating resistance to cell damage.

the advantages of -omics methods is commonly assumed to be their unbiased nature, this should always be questioned as hidden biases may be at play and distort interpretations. Moreover, application of -omics methods should be adapted to the underlying question so that the most complete understanding can be obtained. Thus, to accurately progress or understand cancer from a systems biology perspective, the methodology should be carefully and critically chosen. In addition, to avoid artifacts, rigorous validation of findings obtained using systems biology methods by orthogonal and well-established molecular biology and biochemistry techniques is also warranted.

8. Final considerations

It is clear that cancer cells vary from patient to patient as well as among themselves, even within the same tumor bed. Such heterogeneity can be a limiting factor in the identification of a single molecular marker associated with tumor aggressiveness, response to therapy, and prognosis. In this regard, CTCs or cell-free ctDNA in plasma and in cerebrospinal fluid and other biological fluids [114] may constitute a non-invasive source of genetic material that may allow identification of the genetic characteristics of tumors [115–119] and help to reveal their clonal relationships and hierarchical organization [120].

Efficiently applied systems biology studies, in conjunction with standard molecular biology and biochemical methods, may help to change many of the paradigms in cancer research by providing a way to assess multiple variables in a high-throughput and tumor ecosystem-wide manner. Furthermore, tumors constantly evolve new phenotypes, which enable cancer cells to withstand therapy, invade, and metastasize. In this sense, systems biology approaches can be used for modeling tumor evolution and experimentally testing these models, which will hopefully result in new tools to predict disease progression in the clinic [121–124].

We therefore propose the following considerations:

1. Tumor progression is characterized not only by the sequential accumulation of molecular aberrations, but also by the diversification and coexistence of various tumor cell clones with unique molecular profiles and distinctive behaviors. It is highly likely that these clones display synergistic properties that together contribute to the development of aggressive and invasive tumors. Factors released by the clones or environmental cells could then maintain the capabilities of

the consortium and could be new cancer treatment targets.
2. Interrelationships between molecular pathways and complex biological processes are not linear. Small adjustments can lead to disproportionate changes with major consequences.
3. Cancer is a dynamic system, in which the whole is different than the sum of the parts. Neoplastic ecosystems are quantitatively and qualitatively different to normal tissue systems and may exhibit less "ordered" hierarchy. Cancer networks are also modulated over time; consequently, prediction of their behavior is highly complex, if not impossible, within the framework of the current cancer biology paradigms.
4. Due to intratumor heterogeneity, most molecular data obtained in a small tumor sample are unlikely to be representative of the entire landscape of the tumor and/or metastases. Multisampling and longitudinal studies, as well as topologic and systems biology analyses, are needed to establish a more reliable correlation between real drivers and clinical response.
5. In light of recent findings on the complexity of tumor consortia, we envisage a slow accumulation of advances that will make cancer a chronic disease and reduce mortality by improving early diagnosis, identifying new therapeutic targets, and permitting immunotherapy advances. Significant improvements may be achieved in the long term if researchers can identify key target nodes supporting tumors (i.e. master regulators and funnel factors) that are largely independent of genetic makeup and microenvironment [26] (see also Fig. 4).
6. The biochemical alterations involved, while complex, are directly associated with spontaneous events that produce marked cellular biochemical and biological changes enabling cancer cell survival and tumor progression. This "chaotic advantage" of the biochemical regulation of the tumor may be exploitable by redirecting the action of the apparent "chaos", for example, by modulating factors involved in the cellular stress response. In such a framework, it will be vital to bring together research professionals (molecular biologists, bioinformatics, mathematicians, systems biology specialists) who can certify and validate the findings of systems biology studies and, perhaps even more importantly, establish standards for study design and database curation.

In summary, we propose that cancer is a complex consortium, characterized by collaboration among various cells (e.g. different cancer cell clones and inflammatory and stromal cells), which in concert result in the emergent properties of cancer. These emergent properties of cancer confer a malignant clinical phenotype of invasiveness and metastatic potential. Ecological, evolutionary, and molecular alterations of neoplasia are dynamic and change as the disease progresses. This highlights the importance of studying different areas of the tumor, during progression, recurrences, and metastases. Cancer biology, then, seems amenable to the application of ecological and evolutionary principles. It is thus expected that the effective treatments will be those that avoid or even exploit the clonal diversity of the tumor while still being selective [9]. The collaboration between tumor and non-tumor cells (e.g. via cell-cell interactions, metabolites, cytokines, and exosomes) in cancer ecosystems may open new lines of research that enable progress in the study of advanced cancers, whose survival expectations are still abysmal (see Fig. 5).

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Conflict of interest

None.

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REVIEW ARTICLE

The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer

C. J. Fabian

SUMMARY

The third-generation aromatase inhibitors (AIs) anastrozole, exemestane and letrozole have largely replaced tamoxifen as the preferred treatment for hormone receptor – positive breast cancer in postmenopausal women. Approximately 185,000 new cases of invasive breast cancer are diagnosed yearly, and at least half of these women are both postmenopausal and eligible for adjuvant therapy with AIs. In addition, AIs are currently being tested as primary prevention therapy in large randomised trials involving tens of thousands of women at increased risk for breast cancer. Given the volume of use, internists will increasingly see postmenopausal women who are taking or considering treatment with AIs. Physicians need to be able to: (i) briefly discuss the pros and cons of using a selective estrogen receptor modulator such as tamoxifen or raloxifene vs. an AI for risk reduction and (ii) recognise and manage AI-associated adverse events. The primary purpose of this review is to help internists with these two tasks.

Review Criteria

Expert opinion based on review of literature on relevant clinical trials.

Message for the Clinic

Both tamoxifen and AIs are effective for the adjuvant and neoadjuvant treatment of postmenopausal breast cancer; the optimal choice of drug is dependent on the characteristics of the patient and tumour. Adverse events with both drug classes are manageable. Adverse events associated with tamoxifen include increased risk of uterine cancers and thromboembolic events vs. an increased incidence of vaginal dryness, loss of libido, musculoskeletal pain and bone mineral density loss with AIs. Promising studies of AIs in the breast cancer prevention setting are ongoing.

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Introduction

Estrogen promotes the growth and survival of normal and cancerous breast epithelial cells by binding and activating the estrogen receptor (ER). The activated receptor in turn binds to gene promoters in the nucleus and activates many other genes responsible for cell division, inhibition of cell death, new blood vessel formation and protease activity. An increase in the proportion of cells that express ER is found at both the earliest stages of breast precancer and in approximately 70% of breast cancers (1). There are three ways in which estrogen-dependent processes important in the development and progression of the majority of breast cancers may be interrupted (Figure 1). The first is to interfere with the binding of estrogen to the ER and/or to the promoter elements of the genes it regulates. Selective ER modulators such as tamoxifen and raloxifene act in this manner. A second method is to reduce or eliminate ER expression. This is exemplified by fulvestrant, a selective ER down-regulator, which works by making less receptor available for binding to estrogen. The most direct means is to simply reduce the amount of estrogen by interfering with its production, via ovarian ablation in premenopausal women and use of aromatase inhibitors or inactivators (AIs) in postmenopausal women. Because of their effectiveness, AIs are quickly becoming the most frequently used antihormonal treatment for breast cancer in postmenopausal women. Further, AIs are now being tested in breast cancer prevention trials.

Aromatase inhibitors are not without adverse effects, which primarily stem from profound estrogen depletion. Many women will turn to their internists for advice about whether to take these drugs, as well as help in preventing and managing adverse events. The purpose of this article is to provide primary care physicians with a basic understanding of AIs to help facilitate these interactions.

What is an aromatase inhibitor and how does it work?

Aromatase inhibitors and inactivators interfere with the body's ability to produce estrogen from androgens by suppressing aromatase enzyme activity.

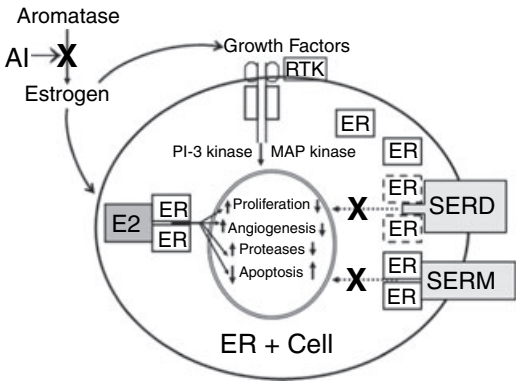

Figure 1 Schematic of metabolic pathways in an ER-positive cell that can be affected by AIs. The left side represents the active pathways and cellular responses under normal estrogen control. The right side depicts the blockade of pathways involving ERs and the resultant cellular responses. AI, aromatase inhibitor; E_2, estradiol; ER, estrogen receptor; MAP, mitogen-activated protein; PI-3, phosphoinositide-3; RTK, receptor tyrosine kinase; SERD, selective estrogen receptor down-regulator; SERM, selective estrogen receptor modulator

Before menopause, ovarian aromatase is responsible for the majority of circulating estrogen and is exquisitely sensitive to changes in luteinising hormone (LH). Following menopause, aromatase in fat and muscle may be responsible for much of the circulating estrogen. Aromatase in highly estrogen-sensitive tissues, such as the breast, uterus, vagina, bone, brain, heart and blood vessels, provides local estrogen in an autocrine fashion (Figure 2). The aromatase gene promoter in breast tissue is less sensitive

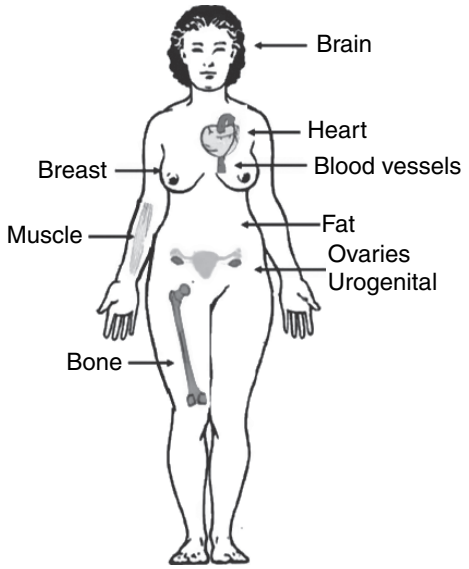

Figure 2 Schematic of organs with substantial aromatase activity

Table 1 Efficacy of aromatase suppression by three generations of AIs

Drug	Dose	% Inhibition
First generation		
Aminoglutethimide (1,3)	1 g	91
Second generation		
Fadrozole (100)	2 mg	82
Vorozole (5)	1 mg	93
Third generation		
Letrozole (100,101)	2.5 mg	99
Anastrozole (100,102)	1 mg	97
Exemestane (100,103,104)	25 mg	98

AIs, aromatase inhibitors.

than the gene promoter in the ovary to fluctuations in LH but much more sensitive to increases in inflammatory cytokines. Circulating inflammatory cytokines increase with age, and breast tissue inflammatory cytokines increase with proliferative breast disease and breast cancer. Thus, it comes as little surprise that breast aromatase activity is increased in proliferative breast disease and many cases of breast cancer (2).

Three generations of AIs have been developed (Table 1) (3–8). Each successive generation has been associated with higher specificity for the aromatase enzyme (Figure 3), fewer adverse events, and greater suppression of aromatase activity. The utility of first- and second-generation AIs was limited by adverse events, such as rash, fatigue, dizziness, ataxia, nausea and vomiting, as well as by a lack of enzyme selectivity. Third-generation AIs are superior to earlier versions because they are associated with fewer adverse events and greater suppression of aromatase activity. There are two classes of third-generation AIs. Non-steroidal AIs reversibly bind to the aromatase enzyme and include anastrozole and letrozole. The steroidal AI exemestane binds to aromatase irreversibly. All third-generation AIs are administered orally on a daily basis. Adverse events include hot flushes, vaginal dryness, loss of libido, fatigue, arthralgias, joint stiffness and loss of bone mineral density with subsequent increased risk of fracture (9). In premenopausal women, AIs have a limited ability to reduce circulating estrogen. Unlike postmenopausal women, premenopausal women have a large amount of aromatase substrate present in the ovary. The exquisite sensitivity of the ovarian aromatase promoter to gonadotrophins, which increase dramatically after AI administration, makes AIs less effective in inhibiting ovarian estrogen production. Thus, AIs are generally not given to premenopausal women for breast cancer

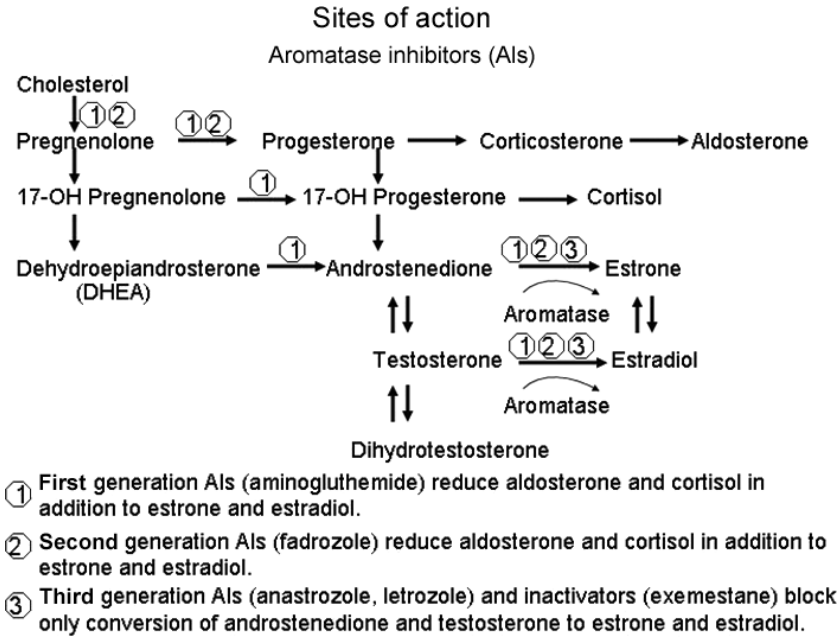

Figure 3 Metabolic pathways differentially targeted by aromatase inhibitors (AIs)

treatment without the addition of a medication to suppress the rise in gonadotrophins and subsequent increase in hormone levels (9).

Why do we need aromatase inhibitors?

For women with newly diagnosed hormone receptor positive ER+ cancers requiring systemic adjuvant therapy, 5 years of tamoxifen reduces the relative odds of recurrence by 40% and relative risk of death from breast cancer by 34% (10). At 15 years this equates to about a 12% absolute reduction in recurrence and a 9% absolute reduction in mortality, irrespective of nodal status. However, about a third of women diagnosed with ER-positive breast cancer will ultimately relapse despite adjuvant tamoxifen with or without chemotherapy (10). Women with hormone receptor-positive disease that has metastasised to organ sites distant from the breast almost always relapse following first-line antihormonal therapy with tamoxifen. More effective antihormonal treatment for tamoxifen-resistant tumours are needed.

There is some evidence suggesting a worse outcome with tamoxifen for women with ER-positive tumours that lack progesterone receptor (PgR), and/or exhibit overexpression of growth factor receptors such as human epidermal growth factor receptors 1and 2 (EGFR and HER-2/neu) (11,12). The observation that prolonged administration of tamoxifen may increase rather than decrease late recurrence rates (13) may be due to tamoxifen's ability to act as a partial estrogen agonist in breast tissue under conditions of growth factor receptor up-regulation, which commonly occurs after prolonged tamoxifen use (11,12). AIs appear to be more effective than tamoxifen in ER-positive tumours regardless of PgR or growth factor receptor status (14,15).

Treatment with AIs produce frequent and durable responses in postmenopausal women previously treated with tamoxifen or endocrine ablative surgery, and AIs are more effective than tamoxifen in producing responses and delaying progression in first-line treatment of metastatic disease (16). A recent meta-analysis concluded that in women with metastatic breast cancer, AIs show a survival benefit when compared with other endocrine therapy (17).

How are aromatase inhibitors currently used?

The third-generation AIs are currently the preferred first-line treatment for metastatic hormone receptor-positive tumours and have all been approved by the US Food and Drug Administration for adjuvant use in postmenopausal women before or after surgery for ER-positive and/or PgR-positive breast cancer (18). Although anecdotal responses have been observed in women with ER- and PgR-negative tumours, in current clinical practice, only postmenopausal women with ER-positive and/or PgR-positive tumours are selected for treatment with AIs (9,16). There are several clinical studies evaluating the use of AIs in premenopausal women combined with ovarian

suppression with a LH-releasing hormone (LHRH) analogue. AIs are generally not used off-label for premenopausal women except in special circumstances, such as prior tamoxifen failure or medical contraindications to tamoxifen. When AIs are used in premenopausal women they must be combined with surgical or medical ovarian ablation. Results with AIs in the adjuvant or neoadjuvant setting are detailed below.

Neoadjuvant therapy with aromatase inhibitors vs. tamoxifen

Systemic treatment administered before definitive surgery is termed neoadjuvant therapy and is often used in women who have clinically involved nodes or a tumour that is ≥ 3 cm. Under these circumstances the chance of occult metastatic disease is high, and the chance of breast conservation with a cosmetically acceptable outcome is low. Neoadjuvant treatment both increases the chance of breast conservation and promotes timely treatment of occult metastases. Pathological response to neoadjuvant chemotherapy is an important prognostic factor. Women with a pathological complete response in breast and lymph nodes to neoadjuvant chemotherapy have as much as a 95%, 5-year distant, disease-free survival (DFS) (19). Although pathological complete response rates after neoadjuvant chemotherapy are in the 20% or higher range for hormone receptor-negative tumours, they are rare with tumours that are hormone receptor-positive.

Neoadjuvant trials with antihormone therapy have generally shown that the chance of breast conservation is higher with AIs than tamoxifen and may be higher for AIs than for chemotherapy in women with hormone receptor positive tumours (19–22). In a trial comparing neoadjuvant letrozole with tamoxifen, the mammographic complete response rate with letrozole, although very low, was still higher than that observed for tamoxifen (20). In the Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen trial, women randomised to anastrozole alone were significantly more likely to have experienced sufficient tumour regression to be eligible for breast-conserving surgery than women randomised to tamoxifen or combined treatment (23). Neoadjuvant antihormonal therapy with an AI is a particularly attractive option for postmenopausal women who wish to attempt breast conservation and have strongly ER- and PgR-positive tumours that are ≥ 3 cm and have low proliferation rates.

Adjuvant therapy with aromatase inhibitors vs. tamoxifen

Clinical trials of AIs as adjuvant therapy have followed one of four approaches: (i) a head-to-head comparison of tamoxifen vs. an AI; (ii) extended adjuvant therapy following initial adjuvant therapy (5 years of an AI after 5 years of tamoxifen); (iii) switching to an AI for 2–3 years after 2–3 years of tamoxifen and (iv) combination therapy using both an AI and tamoxifen simultaneously. All AI approaches except the simultaneous combination of an AI and tamoxifen are associated with fewer breast cancer-related events than tamoxifen alone.

Head-to-head comparisons of an aromatase inhibitor and tamoxifen

The Anastrozole, Tamoxifen Alone or in Combination (ATAC) trial randomised more than 9000 women to 5 years of tamoxifen, anastrozole or both agents in combination. The combination treatment did not show a benefit and is not discussed further. Sixty-one per cent of women had no disease detected in their lymph nodes (referred to as node negative) at diagnosis. After 5 years of treatment, there was a significant improvement in DFS in the group of women treated with anastrozole alone regardless of tumour size, nodal status or use of adjuvant chemotherapy before the randomisation. There was a significant interaction with hormone receptor status: women who had ER-positive but PgR-negative tumours were likely to have a superior outcome with anastrozole, whereas women with tumours that were positive for both receptors did just as well with tamoxifen as with anastrozole. The absolute improvement in DFS with 5 years of anastrozole, compared with 5 years of tamoxifen, was 2.5% ($p = 0.005$). The incidence of contralateral breast cancer was reduced by 53% in women with hormone receptor-positive tumours. No overall survival benefit or significant reduction in deaths from breast cancer was demonstrated for anastrozole in this study. However, there appears to be an emerging survival benefit for women with ER-positive tumours who also had evidence of tumour cells in their draining lymph nodes (referred to as node positive) (24,25).

In the Breast International Group's Femara-Tamoxifen trial, also known as BIG 1–98, 5 years of adjuvant letrozole was compared with 5 years of tamoxifen in postmenopausal women with ER-positive and/or PgR-positive breast cancer. Eventually, this trial was modified with the addition of two treatment groups in which women were either switched from tamoxifen to letrozole or from letrozole to tamoxifen after the initial 2 years of treatment (26). Approximately 8000 patients were randomised to receive tamoxifen or letrozole as their initial therapy. Fifty-nine per cent of women were node negative, and the median age was 61. At a median follow-up of slightly more than 2 years, there was a

significant 3.4% absolute improvement in DFS with letrozole compared with tamoxifen. Women with PgR-positive and PgR-negative cancer appeared to benefit equally from letrozole compared with tamoxifen. An approximate 50% reduction in risk of contralateral breast cancer was observed. No significant overall survival benefit was reported, although there was a numeric reduction in deaths from breast cancer and an increase in deaths because of other causes in the group treated initially with letrozole (26). These results were recently updated analysing only those women randomised to 5 years of letrozole vs. placebo. At a median follow-up of 51 months there continues to be a 3% absolute improvement in DFS (18% relative reduction) following letrozole with no improvement in overall survival (27).

The ongoing Tamoxifen Exemestane Adjuvant Multi-institutional (TEAM) trial compares exemestane with tamoxifen as first-line adjuvant treatment. The TEAM trial is designed to compare DFS in patients treated with exemestane vs. tamoxifen at 2.75 years, and to compare DFS in patients treated with 5 years of up-front exemestane vs. tamoxifen for 2.5–3 years followed by 2–2.5 years of exemestane. Enrolment was completed in January 2006 ($n = 9786$). We are awaiting the efficacy results of this trial.

Aromatase inhibitors as extended endocrine adjuvant therapy

Given the appreciable late recurrence rates in women with ER-positive breast cancer following 5 years of adjuvant tamoxifen, the MA.17 trial was designed to determine whether 5 years of letrozole (after 5 years of adjuvant tamoxifen) would improve DFS compared with placebo. At a median follow-up of 2.4 years from the time of randomisation, letrozole improved DFS, compared with placebo, by a relative value of 43% and an absolute value of 6%. This was significant regardless of nodal status (28). The trial was unblinded, with women who received placebo given open-label treatment with letrozole on request (28). In an update of this study, a significant reduction in death from any cause was noted for node-positive women receiving letrozole (29). Incidence of menopause-related symptoms, new onset of osteoporosis, arthralgias and alopecia (generally minimal to mild) were all higher for women randomised to letrozole compared with placebo. There was no increase in the rate of bone fracture. There were some specific quality of life domains which were significantly worse with letrozole, including physical functioning, bodily pain, vitality, vasomotor symptoms and sexuality (30).

Switching therapy

The switching strategy was designed to: (i) combine the apparent superior efficacy of AIs with tamoxifen's favourable effects on bone and (ii) expose tumour cells to anti-hormonal therapies with two different mechanisms of action. Several adjuvant trials were designed in which, after 2–3 years of adjuvant tamoxifen, women were randomised to continue taking tamoxifen for another 2–3 years or switch to an AI. One such trial, the Intergroup Exemestane Study (IES), randomised 4742 postmenopausal women after 2–3 years of tamoxifen to exemestane 25 mg/day or to continued tamoxifen of sufficient duration to complete a 5-year course of adjuvant therapy (31). Fifty-one per cent of patients were node negative at baseline, and 81% were known to have ER-positive breast cancer. At a median follow-up of 30.6 months, exemestane was associated with a 32% reduction in risk of local or metastatic recurrence, contralateral breast cancer, or death, for an absolute benefit of 4.7% in terms of DFS compared with tamoxifen (31). A recent update at 58 months showed similar improvement in DFS in both the intent-to-treat (24%) and ER-positive/unknown population (26%). A 45% relative reduction in the incidence of contralateral breast cancer was observed. A 17% relative increase in overall survival ($p = 0.05$) was reported for women randomised to switch to exemestane compared with those remaining on tamoxifen if their tumours were ER-positive or ER unknown (32). Quality of life measured at 3- to 6-month intervals during the first 24 months was similar for women taking exemestane or tamoxifen (33).

In other switching trials, such as the Italian Tamoxifen Arimidex (ITA) trial and the Austrian Breast and Colorectal Study Group 8 (ABCSG 8)/Arimidex-Nolvadex (ARNO 95) combined analysis, switching to anastrozole after 2 years of tamoxifen was compared with continued tamoxifen treatment. A 39% relative improvement in DFS ($p = 0.049$) and 52% improvement in overall survival were seen at a median follow-up of 30 months in the ABCSG 8/ARNO 95. Improvement in DFS was observed for ITA (34,35).

In summary, all the adjuvant trials in postmenopausal women – whether they involved initial head-to-head comparison with tamoxifen (ATAC, BIG 1–98), switching to an AI after 2–3 years of tamoxifen (IES, ITA and ABCSG 8/ARNO 95), or administering 5 years of an AI after 5 years of tamoxifen – show improvement in DFS favouring the AI. An overall survival benefit is emerging in at least two of the switching trials in women randomised to 2–3 years of an AI following 2–3 years of tamoxifen vs. continuing on tamoxifen (32,35). No

significant overall survival benefit has been demonstrated to date for up-front AI administration with letrozole or anastrozole or extended adjuvant therapy with letrozole, although node-positive women appear to show a survival benefit. Follow-up in these trials is short, and an overall survival advantage is likely with up-front AI use. The lack of an early overall survival advantage with AIs in the up-front setting compared with the switch setting may be due to the fact that the switch trials, by excluding women who relapse on tamoxifen in the first 2–3 years, enroll women who are most likely to respond to antihormone therapy. At present, the American Society of Clinical Oncology Technical Assessment recommends that postmenopausal women with receptor-positive breast cancer receive an AI as part of their adjuvant therapy, either as initial therapy, as part of a switching strategy, or after 5 years of tamoxifen (18).

There is no clear advantage to one AI vs. another at the present time. Oncologists often select an AI depending on the type of adjuvant strategy they wish to employ. Several head-to-head trials comparing one AI to another in the adjuvant setting are ongoing. These include trials of anastrozole vs. exemestane and anastrozole vs. letrozole.

Use of aromatase inhibitors in premenopausal women

Responses have been observed in premenopausal women with concomitant goserelin and AI treatment following tamoxifen failure (36,37). This concept is also being tested in the adjuvant setting with the Suppression of Ovarian Function (SOFT) and Tamoxifen or Exemestane Plus Ovarian Ablation (TEXT) trials. In the SOFT trial, women who are premenopausal after any adjuvant chemotherapy and have ER-positive tumours are randomised to tamoxifen, tamoxifen plus an LHRH analogue or exemestane plus the LHRH analogue (other types of ovarian ablation are also allowed). In the TEXT trial, premenopausal women who may or may not have received chemotherapy are randomised to receive tamoxifen or exemestane, both with an LHRH analogue. The TEXT trial is nearing completion of accrual. It is not clear whether an AI with ovarian ablation will be as good as or better than tamoxifen with or without ovarian ablation at this time. If an AI is given to a premenopausal woman outside of these ongoing trials ovarian ablation with oophorectomy or ovarian suppression with an LHRH analogue must be given. If ovarian suppression with an LHRH analogue is chosen, serum estradiol levels must be monitored regularly to ensure that they remain in the postmenopausal range.

Adverse event profile of aromatase inhibitors compared with tamoxifen

The adverse event profile for AIs differs from that of tamoxifen. There is no increase in uterine cancers or thromboembolic events as is observed with tamoxifen, but with the exception of hot flushes. Women taking AIs are more likely to complain of symptoms related to estrogen deprivation. Women taking AIs are also more likely to report musculoskeletal adverse events than women taking tamoxifen. These are considered in detail below.

Gynaecological sequelae

Use of AIs is associated with a higher frequency of vaginal dryness, loss of libido and painful intercourse than is tamoxifen. There are fewer instances of vaginal bleeding and endometrial cancer with AIs than with tamoxifen (30,33,38). AIs are associated with hot flushes, but the proportion of women who exhibit vasomotor instability may be less than that seen with tamoxifen treatment (31,39). Younger age at initiation of treatment is associated with increased frequency of hot flushes (40).

Musculoskeletal effects

Studies of tamoxifen in postmenopausal women have shown reduction in bone turnover markers and an increase in bone density and the opposite effects with AIs (41–44). These differential effects are not surprising because tamoxifen exerts partial estrogen agonist effects on bone in postmenopausal women, and osteoporosis has been strongly associated with the low serum estrogen levels that occur following AI administration (45). Although a head-to-head comparison of the three third-generation AIs in the Letrozole, Exemestane, Anastrozole Pharmacodynamics study has shown a similar effect on markers of bone turnover for all three drugs (46), it has also been suggested that exemestane may be associated with less of a deleterious effect than is seen with the other third-generation AIs (47). Additional data are expected from a bone substudy in MA.27, an adjuvant trial comparing anastrozole with exemestane.

In adjuvant studies, all three third-generation AIs – anastrozole, letrozole and exemestane – have shown an increased risk of bone fracture compared with tamoxifen. The absolute differences, while statistically significant in the ATAC trial of anastrozole vs. tamoxifen and the BIG 1–98 trial of letrozole vs. tamoxifen, were only 1–4%. Most fractures were in the spine and not the hip (27,48). The difference in fracture rate approached, but did not reach, statistical significance in the IES trial (3.1% for women switching to exemestane vs. 2.3% in women contin-

uing on tamoxifen) (31). Letrozole given in MA.17 after 5 years of tamoxifen had a numerically higher fracture rate than placebo (5.3% vs. 4.3%), but like the IES trial, the absolute excess fracture rate was ≤ 1% and statistically insignificant (43). This would seem to indicate that tamoxifen taken before an AI provides some measure of bone mineral density protection in postmenopausal women.

Bisphosphonates can be used to prevent the bone mineral loss observed with AIs. This strategy was successfully used in the Zometa-Femara Adjuvant Synergy trials, and the Austrian Breast and Colorectal Cancer Study Group trial 12, in which an intravenous bisphosphonate, zoledronic acid, was administered every 6 months for the duration of AI therapy (49,50). Vitamin D supplementation is advisable in women with serum 25-OH vitamin D levels < 30 ng/ml because women with baseline vitamin D insufficiency are at an increased risk of bone loss when receiving AIs (51).

In randomised studies, arthralgias/myalgias have been reported significantly more frequently in women randomised to AIs than in those randomised to tamoxifen or placebo. The absolute frequency varies tremendously from trial to trial (5.4–37% for AIs vs. 3.6–26% for tamoxifen or placebo), which in turn probably reflects the method used to record the symptoms. The incidence of arthralgias and myalgias appear to be about two-thirds higher with an AI than with tamoxifen or placebo but usually improves with time (38). Two small studies have shown that women taking AIs for cancer therapy often have deficient or suboptimal 25-OH vitamin D levels in their serum (51,52). Improvements in myalgias and arthralgias were observed in a high proportion of women with deficient or suboptimal levels of vitamin D who were given prescription-strength vitamin D for 12 weeks (52). Serum 25-OH vitamin D is used to assess adequacy of total body vitamin D stores (53) and levels should be checked prior to starting AI treatment to make sure they are in the optimal range of 30–50 ng/ml (53–55). In general, each additional 1000 IU of vitamin D3 can be expected to increase 25-OH-D serum levels by 10 ng/ml. The addition of celecoxib 400 mg bid to exemestane reduced arthralgias and improved response rates in a placebo-controlled trial in women with metastatic disease (56). Prospective trials are under way to assess the prevalence of vitamin D deficiency in women undergoing adjuvant therapy with AIs, correlation with the development of myalgias/arthralgias and the relief of symptoms with vitamin D replacement.

Thromboembolic and cardiovascular effects

Aromatase inhibitors do not increase the risk of deep venous thrombosis; this differs from tamoxifen, for which the risk of deep venous thrombosis and pulmonary embolism is increased approximately twofold (57,58). Further, except for a higher frequency of occurrence in women over 50 and those with high body mass index, there does not appear to be an easily identified predisposing factor behind the majority of episodes of deep venous thrombosis associated with tamoxifen (59).

Aromatase inhibitors in adjuvant trials have been associated with an increase in ischaemic cardiovascular events and a numeric, but not statistically significant increase in cardiac deaths when compared with tamoxifen (25,30,31), but not when compared with placebo (29). AIs do not have a substantial effect on lipid metabolism (39,58). It is possible that, if there is an intrinsic adverse effect of AIs on ischaemic heart disease, it might be due to estrogen depletion in the coronary arteries leading to loss of the vasodilatory response of estrogen to stress (60). Alternatively, the observation might stem from a small cardio-protective benefit from tamoxifen rather than a deleterious effect of AIs. With the exception of triglycerides, tamoxifen has a favourable effect on the serum lipid profile (1) and tamoxifen has also been observed to improve endothelial function and reduce carotid intima–media thickness in postmenopausal women (61). Despite tamoxifen's favourable effects on some lipid and endothelial parameters, there is as yet no conclusive evidence that tamoxifen exhibits cardioprotective effects (62). The lack of significant cardiovascular benefit in most randomised trials for tamoxifen may be due to an increase in triglycerides and clot promoting proteins, which offset the beneficial cardiovascular effects of tamoxifen (1,59). An additional factor might be the widespread use of statins, which would obscure tamoxifen's favourable effects on cholesterol. In the ATAC trial, 4.1% of participants randomised to anastrozole vs. 3.4% of those randomised to tamoxifen died from ischaemic heart disease (25). In the IES trial, at 3-year follow-up, a higher number of cardiovascular deaths were reported for exemestane than for tamoxifen (1.1% vs. 0.8%) (31). In the BIG 1–98 trial, 2.5% of women randomised to letrozole had serious or fatal cardiac events compared with 1.1% taking tamoxifen; this was highly significant (27). There were also twice as many cardiac deaths with letrozole than with tamoxifen (13 vs. 6), but given the small number of events, the difference was not statistically significant.

Because the proportional differences in cardiac deaths observed in women randomised to AI vs. tamoxifen are < 1%, a potential increase in cardiovascular events is not likely to be a major concern for women undergoing cancer therapy with an AI. However, enthusiasm for AI use in the primary pre-

vention setting will be limited if AIs are found to be associated with a higher number of cardiac events compared with placebo or tamoxifen.

Management and prevention of adverse events

As AI use becomes more common, internists will undoubtedly be asked by their patients for help with management and prevention of adverse events, although the relative risks and benefits of AIs vs. other hormonal therapy will hopefully have been discussed by the patient's oncologist.

For vasomotor symptoms, non-hormonal methods such as selective serotonin reuptake inhibitors (SSRIs), gabapentin or clonidine should be tried first (63). In doses commonly needed for relief of hot flushes (75 mg venlafaxine, 20 mg fluoxetine and 300–900 mg gabapentin), side effects for these medications include drowsiness, dry mouth and dyspepsia. Use of SSRIs may also contribute to the loss of sexual interest.

Vaginal dryness that is not ameliorated with lubricants may be treated with poorly absorbed vaginal estrogens, such as oestradiol vaginal rings or tablets. However, a small study showed a significant increase in serum estrogen levels following use of these preparations (64). A weak preparation (1%) of testosterone with 2 mg of estriol (1 g administered 2–3 times weekly) is often effective for improving vaginal dryness, dyspareunia and libido. When women are taking AIs, testosterone cannot be readily converted to estradiol. Estriol is a very weak estrogen and likewise cannot be converted to estradiol (65). There is little information regarding the safety of this practice, particularly in women with prior breast cancer (66).

Aromatase inhibitors for breast cancer prevention

Tamoxifen fails to prevent ER-negative breast cancer, and one-third or more of ER-positive breast cancers (67–70). The incomplete efficacy, increased risk of serious adverse events, and the lack of survival benefit with tamoxifen given as primary prevention (66–70) fuels the effort to develop safer and more effective primary-prevention strategies. The superior DFS observed for AIs compared with tamoxifen in the adjuvant setting combined with the lack of increase in thromboembolic events or uterine cancer has led to the initiation of multiple primary-prevention trials in high-risk women without prior breast cancer. Currently, there are several major multi-institutional primary-prevention trials in postmenopausal women in which an AI is being compared with placebo (Table 2).

Of serious concern for prevention is the potential for increase in risk of bone fracture and cardiovascular disease related to long-term estrogen depletion with AIs. However, arthralgias, fatigue, dyspareunia, reduced libido and hot flushes may result in poor uptake and/or compliance. Ongoing phase III prevention trials will define the incidence of these adverse events relative to placebo in a healthy population, and potential solutions to avoid some of these problems in the prevention setting are already being explored.

One small study indicates that bone mineral loss after AIs is primarily limited to women with insufficient 25-OH vitamin D levels (71). Given the importance of adequate vitamin D in health, practitioners should strive to achieve 25-OH vitamin D levels of at least 30 ng/ml (55,72). Bisphosphonates have been found effective in preventing AI- and cancer-therapy-related bone mineral loss in the adjuvant setting (73,74). Along with exercise and appropriate supplementation of calcium and vitamin D, bisphosphonates could be used along with AIs to prevent bone loss. Very low-dose estradiol (0.015 mg estradiol patch replaced twice weekly) increased serum estradiol to a median of 12 pmol and may be effective in reducing the increased bone turnover associated with AI use (75).

Statins could be used along with AIs to improve both lipid profiles and endothelial function. There is

Table 2 Ongoing Multi-institutional Phase III Primary Prevention trials of AIs in postmenopausal women

Trial	Agents studied	Duration studied (years)
International Breast Cancer Intervention Study II	Anastrozole vs. placebo	5
Aromasin Prevention Study	Exemestane vs. placebo	3
National Cancer Institute of Canada Clinical Trials Group MAP.3 Breast Cancer Prevention Trial	Exemestane vs. placebo	5

AIs, aromatase inhibitors.

also a suggestion that long-term use of a lipophilic statin might reduce breast cancer risk (76), but results in case–control studies are mixed (77–79). However, because both statins and AIs are metabolised in the liver, pharmacological and pharmacodynamical studies need to be completed to better understand how concomitant administration might affect levels of both drugs.

Approximately one-quarter of perimenopausal and postmenopausal women take hormone replacement therapy for some period of time during menopause or menopause transition (80). Although other drugs give partial relief of symptoms associated with the climacteric, none is as effective as hormone replacement (81). The Women's Health Initiative (WHI) indicates a nonsignificant increase in the risk of breast cancer and coronary heart disease for women taking combined oral equine estrogen plus a progestin after 5 years. However, there was no increase in breast cancer risk in the WHI for women taking estrogen alone at a median follow-up time of ~7 years (82,83). In fact, updated results indicate that women aged 50–59 randomised to estrogen alone had a nonsignificant reduction in breast cancer and coronary heart disease. Further, for women aged 50–59 randomised to estrogen alone or combined estrogen plus progestin there was a significant 30% reduction in overall mortality compared with those randomised to placebo (84,85). The Million Women Study showed a modest increase in risk of breast cancer for hormone replacement therapy given by any route with the exception of vaginal hormones. Similar to the WHI, women taking estrogen and a progestin had a higher relative risk than those receiving estrogen alone (86). Few prevention options are available for those women who need hormone replacement for the management of menopausal symptoms and who are at increased risk for breast cancer because of family history or other factors. Tamoxifen and hormone replacement (usually transdermal) are commonly prescribed together in Europe, but this is generally not performed in the USA (87). Furthermore, updated analyses of the three major primary prevention trials of tamoxifen vs. placebo in which hormone replacement was allowed have yielded conflicting results. The Italian prevention trial conducted predominately in average risk hysterectomised women found a reduced risk of ER+ breast cancer with tamoxifen only in those women at increased risk because of hormone replacement or other factors (69). The Royal Marsden trial showed a reduced incidence of ER+ breast cancer whether women took hormone therapy or not. However, the International Breast Cancer Intervention Study 1 trial results indicated tamoxifen was not effective in women beginning hormone replacement therapy (HRT) during study (68–70).

Preclinical studies indicate that AIs might be effective in reducing the risk of breast cancer in hormonally intact animals under circumstances in which breast aromatase is up-regulated (88). In studies of postmenopausal women, breast estradiol levels have been found to be 10- to 50-fold higher than serum levels, and aromatase – which is up-regulated in proliferative breast disease – is responsible for much of this local synthesis (89,90). We have performed a 6-month pilot study of letrozole in high-risk women who continued to take their hormone replacement during the study period. An approximate two-thirds reduction in breast tissue proliferation (Ki-67) was observed after 6 months of letrozole. There was no increase in hot flushes or arthralgias for the majority of women in the trial (91). The concept of using an AI in women already receiving hormone replacement therapy will be explored further in a placebo-controlled, randomised, proof-of-principle trial in which change in Ki-67 in benign breast tissue is the primary end-point. In this ongoing study, the change in bone turnover markers and the cardiovascular risk biomarkers will also be explored.

Overcoming resistance to aromatase inhibitors

Even with an initial response to treatment, for women with metastatic disease, resistance eventually develops to AIs and clinical regrowth of tumour is observed. In most cases, the resistant cancer continues to be ER positive. There are several mechanisms of resistance demonstrated in animal models. These include: (i) development of hypersensitivity of the ER to very low levels of estrogen; (ii) up-regulation of growth factor receptors and/or associated signalling pathways (HER-2, EGFR and insulin growth factor receptor (IGFR)) (92,93). Reduction in the level of ER expression would theoretically reduce the sequelae of ER hypersensitivity and could be accomplished by increasing ER ubiquitisation with a drug such as fulvestrant, an ER down-regulator (94). Results from animal models suggest that the AI letrozole plus fulvestrant is more effective than either alone (93). Fulvestrant is often effective as antihormonal therapy following response and progression on an AI and is equally effective as anastrozole in women with metastatic disease who have previously been treated with tamoxifen (94,95). Fulvestrant plus anastrozole is currently being compared with anastrozole alone in metastatic disease in the co-operative group setting. The use of short courses of physiological or pharmacological doses of estradiol to induce apoptosis in breast

cancer cells with a hypersensitive ER in women whose tumours are resistant to multiple types of endocrine therapy including AIs has been suggested based upon preclinical models (96,97). Combination regimens of AIs and several types of growth factor receptor or activated pathway inhibitors are being explored (98–100).

Summary

The third-generation AIs are now preferred therapy for postmenopausal women with hormone receptor-positive tumours in both the early and metastatic settings. Switching from adjuvant tamoxifen to an AI (exemestane or anastrozole) after 2–3 years of tamoxifen has shown superior DFS and overall survival compared with continuing on tamoxifen. Using anastrozole or letrozole instead of adjuvant tamoxifen as initial therapy (with or without prior adjuvant chemotherapy) has also shown superior DFS. Finally, for women completing 5 years of tamoxifen, extended adjuvant antihormonal therapy with letrozole has shown a reduced recurrence rate, particularly for node-positive patients. American Society of Clinical Oncology guidelines recommend that an AI be included in a woman's adjuvant regimen if she has ER-positive and/or PgR-positive breast cancer. The decision to use AI as initial endocrine therapy, as opposed to switching to an AI after 2–3 years of tamoxifen therapy, is likely to be guided by the tumour characteristics. Patients who have ER-positive tumours with unfavourable characteristics, such as HER-2 positivity, PgR negativity or nodal positivity, are likely to be selected for immediate AI therapy. However, patients with ER-positive tumours without unfavourable characteristics are likely to be selected for tamoxifen treatment for 2–3 years before taking an AI for 2–3 years. Several ongoing clinical trials are examining the use of AIs in women at an elevated risk of developing breast cancer. Critical to the ultimate success of AIs in both the adjuvant and preventive settings will be management of adverse events, particularly bone mineral density loss, arthralgias and gynaecological sequelae.

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Natural Products as Aromatase Inhibitors

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Abstract

With the clinical success of several synthetic aromatase inhibitors (AIs) in the treatment of postmenopausal estrogen receptor-positive breast cancer, researchers have also been investigating also the potential of natural products as AIs. Natural products from terrestrial and marine organisms provide a chemically diverse array of compounds not always available through current synthetic chemistry techniques. Natural products that have been used traditionally for nutritional or medicinal purposes (e.g., botanical dietary supplements) may also afford AIs with reduced side effects. A thorough review of the literature regarding natural product extracts and secondary metabolites of plant, microbial, and marine origin that have been shown to exhibit aromatase inhibitory activity is presented herein.

Keywords

aromatase inhibitors; natural products; breast cancer; botanical dietary supplements

BREAST CANCER

Worldwide breast cancer estimates included over one million incident cases and almost 400,000 deaths in the year 2000 [1,2]. In the United States, over 178,000 women were expected to be diagnosed with breast cancer in 2007 with over 40,000 deaths occurring from the disease [3]. In developed countries, mortality from breast cancer has recently begun to decline, primarily due to earlier detection and improved treatments [4,5]. Breast cancer is thought to be a result of inherited genetic predisposition (e.g., mutations in genes such as *BRCA*-1, *BRCA*-2, *p53*, *PTEN/MMAC1*, and/or *ATM*) and/or environmental factors (e.g., radiation exposure, dietary factors, alcohol consumption, hormonal exposure) [2,6,7]. Numerous genetic mutations are necessary for breast cancer development and progression including the acquisition of the capabilities for self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis, known collectively as the "hallmarks of cancer" [8].

Numerous molecular targets have been identified as playing a significant role in breast cancer development and progression. Estrogens and the estrogen receptors (ERs) are widely

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recognized to play an important role in the development and progression of breast cancer, making estrogens and the ERs widely studied molecular targets [9–12]. Two of the endogenous estrogens found in humans include estradiol and estrone. In pre-menopausal women, estrogens are produced primarily through conversion of androgens in the ovaries while estrogen production in postmenopausal women occurs in only peripheral tissues [13,14]. Estrogens have various effects throughout the body, including positive effects on the brain, bone, heart, liver, and vagina, with negative effects such as increased risk of breast and uterine cancers with prolonged estrogen exposure [10,15,16]. Estrogens exhibit their effects through binding to one of two variants of ERs, ERα or ERβ [17,18]. Upon binding of estrogen, the ER dimerizes and binds to the estrogen-response element (ERE), causing transcription of estrogen dependent genes [19]. Estrogens influence breast cancer development and progression by various methods including stimulation of cell proliferation through the ERα pathway, direct increases in rates of genetic mutations, or effects on the DNA repair system [12,20–22].

Modulation of estrogen exposure as a treatment for breast cancer began as early as the late nineteenth century when complete ovariectomy was noticed to have favorable effects on cancerous progression [23]. While ovarian ablation (through surgery, irradiation, or medication) is still utilized clinically for some pre-menopausal breast cancer patients [19,24], extensive research has been performed to modify estrogen exposure pharmacologically. Modulation of estrogens and ERs can be accomplished by inhibiting ER binding, by downregulating ERs, or by decreasing estrogen production [24–26]. Tamoxifen (Nolvadex®), a selective estrogen receptor modulator (SERM) that works by blocking the binding of estrogen to the ER, has been considered the treatment of choice for estrogen abatement for the last twenty-five years [27,28]. However, tamoxifen acts as both an ER antagonist and agonist in various tissues and thus results in significant side-effects such as increased risk of endometrial cancer and thromboembolism [26]. This partial antagonist/agonist activity is also thought to lead to the development of drug resistance and eventual treatment failure for patients using tamoxifen [29,30]. Other SERMs, including raloxifene (Evista®, approved in United States for osteoporosis), and toremifene (Fareston®, approved in the United States to treat breast cancer) are in development to overcome these side effects and still maintain efficacy in breast cancer treatment [31–33]. Fulvestrant (Faslodex®) is a clinically approved estrogen receptor down-regulator currently used as second-line therapy in the treatment of postmenopausal metastatic breast cancer [34,35]. An important target to decrease estrogen production involves aromatase inhibition, which has found clinical utility in postmenopausal women with breast cancer.

AROMATASE INHIBITION AND BREAST CANCER

Aromatase is a cytochrome P450 enzyme and is responsible for catalyzing the biosynthesis of estrogens (estrone and estradiol) from androgens (androstenedione and testosterone) (Fig. 1) [36,37]. The aromatase enzyme is encoded by the aromatase gene *CYP*19 for which the expression is regulated by tissue-specific promoters, implying that aromatase expression is regulated differently in various tissues [38–41]. Aromatase has been found in numerous tissues throughout the body including breast, skin, brain, adipose, muscle, and bone [36,37,42]. The concentration of estrogens has been shown to be as much as twenty-fold higher in breast cancer tissues than in the circulating plasma, suggesting locally increased aromatase expression for estrogen biosynthesis near or within the cancerous tissues [13,43]. Inhibition of the aromatase enzyme has been shown to reduce estrogen production throughout the body to nearly undetectable levels and is proving to have significant affect on the development and progression of hormone-responsive breast cancers. As such, aromatase inhibitors (AIs) can be utilized as either anticancer agents or for cancer chemoprevention

[44–47]. However, the use of AIs for cancer chemotherapy or chemoprevention is limited to postmenopausal women or premenopausal women who have undergone ovarian ablation.

Aromatase inhibitors can be classified as either steroidal or nonsteroidal. Steroidal AIs (also known as Type I inhibitors) include competitive inhibitors and irreversible inhibitors, which covalently bind aromatase, producing enzyme inactivation. Nonsteroidal AIs (Type II inhibitors) reversibly bind the enzyme through interaction of a heteroatom on the inhibitor with the aromatase heme iron [42,48,49]. AIs have been clinically available since the introduction of aminoglutethimide (**1**, AG) in the late 1970's (Fig. 2) [42,50]. However, AG did not completely inhibit aromatase, resulting in decreased efficacy, nor did AG selectively inhibit aromatase, causing considerable side effects [50]. Second-generation AIs (Fig. 2) include formestane (**5**), which was administered through intramuscular injection [19], and vorozole, both having various limiting side-effects [51]. Three third-generation AIs are currently in clinical use, namely, anastrozole (**2**, Arimidex®), letrozole (**3**, Femara®), and exemestane (**6**, Aromasin®) (Fig. 2) [19,42,45,46,49,52]. These agents have shown nearly complete estrogen suppression and are highly selective for aromatase.

When compared with currently existing breast cancer therapies, aromatase inhibitors generally exhibit significantly improved efficacy with fewer side effects [53–55]. Current studies on synthetic AIs generally focus on combination treatment [56–58], resistance mechanisms [59–64], and/or improving their safety profile by reducing side effects [55,65–67].

Although synthetic AIs show a better side effect profile than tamoxifen, serious side effects still occur, generally related to estrogen deprivation [68–72]. Synthetic AIs may cause decreased bone mineral density, osteoporosis, and increases in musculoskeletal disorders [55,65,66,73–75]. Synthetic AIs also can result in increased cardiovascular events as well as altering the lipid profiles of patients [67,74,76]. Synthetic AIs can also affect cognition, decreasing the protective effects of estrogens on memory loss with aging [40,77]. Several quality of life side effects are also often seen with the use of synthetic AIs including diarrhea, vaginal dryness, diminished libido, and dyspareunia [54,78,79]. Some of the side effects of synthetic AIs can be partially alleviated using available therapies, including osteoporosis treatments and cholesterol-lowering medicines.

Even with the improved efficacy of AIs or other endocrine therapies, postmenopausal breast cancer patients eventually develop resistance to AIs causing relapse of the disease [59–64,80]. Generally, resistance involves tumor regrowth after 12–18 months of treatment and stable disease. Several mechanisms are thought to be involved in resistance to synthetic AIs including circumventing normal cellular pathways, enhancing sensitivity to existing estrogens, and/or redistributing estrogen receptors to extra-nuclear sites [59–64]. Several clinical trials are currently exploring the use of combination therapies with synthetic AIs and other compounds [e.g., epidermal growth factor receptor (EGFR) inhibitor gefitinib, HER-2/neu inhibitor trastuzumab, estrogen receptor degrader fulvestrant, and selective estrogen receptor modulators toremifene and raloxifene], hoping to extend the length of stable disease and reduce resistance mechanisms to synthetic AIs.

Two new aromatase inhibitors and one dietary supplement are currently undergoing clinical trials as single agent AIs (http://www.clinicaltrials.gov/). Atamestane (**7**, Fig. 2) is currently in two phase III clinical trials, including a recently completed study of atamestane with toremifene as compared with letrozole for advanced breast cancer and a study of toremifene with or without atamestane versus letrozole in women with metastatic breast cancer. In preclinical experiments, atamestane with or without toremifene was found to have fewer side-effects than letrozole, with favorable effects on bone, serum, and uterine markers [81].

Testolactone (**4**, Teslac®, Fig. 2) is considered a first generation AI and is currently approved for use in the United States for treatment of advanced breast cancer [82]. The AI activity of testolactone is thought to be competitive and irreversible, similar to other steroidal AIs. Testolactone is undergoing clinical trials for conditions other than breast cancer, including the recently completed study for the treatment of LHRH (lutenizing hormone-releasing hormone) resistant precocious puberty in girls (phase II), another recently completed study for the treatment of boys with precocious puberty (phase II), and as part of an ongoing study of a three drug combination therapy for children with congenital adrenal hyperplasia (phase I) [83,84]. Phase I clinical trials have begun on the botanical dietary supplement IH636 grape seed extract for the prevention of breast cancer in postmenopausal women who are at increased risk of developing breast cancer. The IH636 extract has a high concentration of proanthocyanidins and has been shown to inhibit aromatase using *in vitro* and *in vivo* models [85,86].

Even with the growing number of clinically used AIs including anastrozole, letrozole, exemestane, and other compounds in development there remains a need for improved AIs, due to the development of resistance to AIs and because of the side-effects associated with currently utilized compounds. New aromatase inhibitors could offer increased clinical efficacy and less severe side-effects. Although still theoretical, selective aromatase modulators (SAMs) may be found based on the evidence for tissue-specific promoters of aromatase expression [19,41,50]. Transcriptional regulation of aromatase is performed by several tissue-specific promoters, with normal breast adipose tissue utilizing PI.4 (major), PI.3 (minor), and PII (minor) promoters [46,87]. Promotors PI.3 and PII both direct aromatase expression in breast cancer tissues, while other tissues utilize various promoters to regulate aromatase expression (PI.1 – placenta; PI.4 – skin; PI.5 – fetal tissues; PI.6 – bone; PI.7 – vacular endothelial; PII – ovary and testis; PIf – brain) [46,87–89]. This tissue-specific regulation of aromatase expression by different promoters provides a possible mechanism for inhibiting aromatase expression in breast cancer tissues while continuing aromatase expression in peripheral tissues. For example, if PI.3 and PII could be downregulated in breast cancer tissues then there may be some minor side-effects in the ovary or testes, and the adipose tissue but the common side-effects of current AIs on the bone, brain, and cardiovascular system may be alleviated. Several researchers have been examining upstream targets that specifically influence promoters important in aromatase expression in breast cancer (e.g., COX-2 enzyme inhibitors that decrease aromatase expression involving PII and PI.4 [87] and liver receptor homologue (LRH)-1 modulators that decrease PII activity [90]).

NATURAL PRODUCTS AS AROMATASE INHIBITORS

With the clinical success of several synthetic aromatase inhibitors (AIs) for the treatment of postmenopausal breast cancer, researchers have been investigating the potential of natural products as AIs. Natural products have a long history of medicinal use in both traditional and modern societies, and have been utilized as herbal remedies, purified compounds, and as starting materials for combinatorial chemistry. Terrestrial flora and fauna, marine organisms, bacteria, fungi, and other microbes, provide a chemically diverse array of compounds not available through current synthetic chemistry techniques [e.g., 91–100]. Natural products that have been used traditionally for nutritional or medicinal purposes (for example, botanical dietary supplements and ethnobotanically utilized species) may also provide AIs with reduced side effects. Reduced side effects may be the result of compounds within the natural product matrix that inhibit aromatase while other compounds within the matrix alleviate some of the side effects of estrogen deprivation (e.g., phytoestrogens). As such, natural product AIs may be important for the translation of AIs from their current clinical uses as chemotherapeutic agents to future clinical uses in breast cancer chemoprevention.

New natural product AIs may be clinically useful for treating postmenopausal breast cancer and may also act as chemopreventive agents for preventing secondary recurrence of breast cancer.

Natural product AIs may also be important in the search for more potent AIs. Natural product compounds that significantly inhibit aromatase may be utilized to direct synthetic modification of natural product scaffolds to enhance aromatase inhibition. Furthermore, natural product AIs could also be used to explore regulation of aromatase through other pathways and receptors {e.g., modulation of liver receptor homologue-1 (LRH-1) an orphan receptor that regulates aromatase in adipose tissue, testis, and granulose cells as well as contribute to over-expression of aromatase in breast cancer patients [90,101]}. Natural product AIs could also be useful in the search for selective aromatase modulators (SAMs). Although still theoretical, selective aromatase modulators (SAMs) may be found based on the evidence for tissue-specific promoters of aromatase expression [19,41,50,102,103]. New natural product AIs could offer increased clinical efficacy and decreased side effects. Finally, screening for new natural product aromatase inhibitors may provide improved leads for future drug development.

The next sections of this article will detail natural product AIs that have been reported in the literature up to January 2008, beginning with a description of natural product extracts tested followed by a review of natural product compounds that have been tested.

NATURAL PRODUCT EXTRACTS TESTED FOR AROMATASE INHIBITION

Numerous natural product extracts have been tested for their ability to inhibit aromatase. Extracts evaluated have been produced mainly from edible plants and edible fungi, but have also included botanical dietary supplements, spices, teas, coffee, cycads, cigarettes and tobacco, traditional indigenous medicines, wine, and beer. Preparation of natural product extracts has rarely followed a standardized extract preparation method and in some cases this information has not been included in literature reports. Aromatase inhibition assays have varied widely, with the most common being a noncellular tritiated water release assay using microsomes from different sources, most commonly from human placentas. Although less frequent, cellular and *in vivo* aromatase inhibition assays have been utilized to test natural product extracts. In some cases other assays may be utilized to test for aromatase inhibition. Some studies did not report the assay utilized to determine aromatase inhibition activity. Assay results are presented in numerous forms [e.g., % inhibition, percent control activity (PCA), units/100 g], thus complicating the comparison of levels of aromatase inhibition activity from one sample to another. For the purposes of this review, the most active extracts in the microsomal assay will be discussed followed by discussion of the results of cellular and *in vivo* studies.

The most active natural product extracts from testing in the microsomal aromatase inhibition assay, reported as % inhibition, comprise the ethyl acetate partition of *Dioon spinulosum* Dyer ex Eichl. [104], the ethyl acetate partition of *Encephalartos ferox* Bertol. f. [104], a 75% methanol reflux extract of *Riedelia* Meisn. sp. [105], a 75% methanol reflux extract of *Viscum album* L. [105], the methanol partition of *Cycas rumphii* Miq. [104], the methanol and ethyl acetate partitions of *Cycas revoluta* Thunb. [104], a 75% methanol reflux extract of *Alpinia purpurata* K. Schum. [105], and a 75% methanol reflux extract of *Coccothrinax* Sarg. sp. [105]. The natural product extracts that were most active in the microsomal aromatase inhibition assay reported as PCA included five red wine varieties (*Vitis* L. sp.) from various wineries, with the most active being Cabernet Sauvignon from Tanglewood (France) [86,106,107]. The hexane partition of the leaves of *Brassaiopsis glomerulata* (Blume) Regel (Araliaceae) was found to be active in microsomes [108]. The methanol and

chloroform extracts of *Garcinia mangostana* L. (Clusiaceae) (mangosteen) were also strongly inhibitory against aromatase in microsomes [109].

When results were reported as μg/mL, the most active extracts in the microsomal assay included a water reflux extract of *Euonymus alatus* (Thunb.) Sielbold ("gui-jun woo" in Korean folk medicine), a dichloromethane partition of *Isodon excisus* Kudo var. *coreanus* [110], a water reflux extract of *Scutellaria barbata* D. Don [111], and a polyphenol-enhanced extract of green tea (*Camellia sinensis* Kuntze) [112]. Another study reported results in units/100 g wet weight (one unit was defined as the dose required for 50% inhibition) and found tea (*C. sinensis*), coffee (*Coffea* L. sp.), cocoa (*Theobroma cacao* L.), collards (*Brassica oleracea* L.), and tomato leaves (*Lycopersicon esculentum* Mill.) to strongly inhibit aromatase using a microsomal assay [113]. Interestingly, this study also reported that cigarette smoke (obtained using methylene chloride and aqueous traps) and tobacco leaves (70% ethanol extract; *Nicotiana tabacum* L.) also potently inhibited aromatase, as reported in cigarette equivalents [113].

The *Euonymus alatus* (Thunb.) Sielbold and *Scutellaria barabata* D. Don extracts mentioned above were subjected to further testing in both myometrial and leiomyonal cells with the extracts found to have stronger aromatase inhibition activity in leiomyonal cells [111]. Other active natural product extracts tested in cellular aromatase assays included xanthohumol-rich stout beer in choriocarcinoma-derived JAR cells [114], a water extract of grape seed extract (*Vitis* L. sp.) in MCF-7aro cells [85], a water reflux extract of white button mushrooms [*Agaricus bisporus* (J. Lange) Imbach] in MCF-7aro cells [115], red clover flowers (*Trifolium pratense* L.) in a MCF-7 cell dual assay for aromatase inhibition and estrogenicity [116], mangosteen (*Garcinia mangostana* L.) in SK-BR-3 cells [109], and *Brassaiopsis glomerulata* (Blume) Regel in SK-BR-3 cells [108]. The red clover flowers were found to inhibit aromatase at low concentrations and were also estrogenic at high concentrations.

One of the red wines [Pinot noir from Hacienda (Sonoma, CA); *Vitis* L. sp.] with demonstrated activity in the microsomal assay was further tested *in vivo* using an aromatase-transfected MCF-7 breast cancer xenograft mouse model and found to be active [86,106,107]. The grape seed extract (*Vitis* L. sp.) that exhibited aromatase inhibition in MCF-7aro cells was further tested using an *in vivo* MCF-7aro xenograft mouse model and found to reduce tumor weight [85]. This study also ascertained that grape seed extract suppressed exon I.3-, exon PII-, and exon I.6-containing aromatase mRNAs in MCF-7 and SK-BR-3 cells, which is interesting since promoters I.3 and II are important promoters for aromatase expression in breast cancer [87,102,117]. Furthermore, it was also found reported in this same study that grape seed extract down-regulated the transcription factors cyclic AMP-responsive element binding protein-1 (CREB-1) and glucocorticoid receptor (GR), which are up-regulators of aromatase gene expression [85]. Researchers at the City of Hope Comprehensive Cancer Center's Beckman Research Institute at Duarte, California, have begun recruiting patients for a Phase I clinical trial of IH636 grape seed proanthocyanidin extract in preventing breast cancer in postmenopausal women at risk of developing breast cancer (http://clinicaltrials.gov/ct/show/NCT00100893?order=59). The study lists aromatase inhibition as one of the possible mechanisms of action of grape seed extract.

Numerous other natural product extracts have been reported as "active" but actually, most of these exhibit only marginal to weak inhibition of aromatase (see Table 1).

NATURAL PRODUCT COMPOUNDS TESTED FOR AROMATASE INHIBITION

Quite a large number of small-molecule natural product secondary metabolites, of various compound classes, have been evaluated for their ability to inhibit the aromatase enzyme. As

with the natural product extracts reported in the literature, purified natural products have been tested in a variety of aromatase inhibition assays, with the most common being a noncellular tritiated water release assay using microsomes from different sources, typically from human placentas. Cellular and *in vivo* aromatase inhibition assays have been utilized to biologically evaluate some of the natural product compounds reported in the literature. Again, assay results have been presented in the literature in numerous forms, complicating the direct comparison of aromatase inhibition potency from compound to compound. For the purposes of this review, compounds are considered strongly active if their IC_{50} in microsomes was less than 5 μM and/or if their IC_{50} in cells was less than 10 μM, moderately active if their IC_{50} in microsomes was less than 10 μM and/or if their IC_{50} in cells was less than 20 μM, weakly active if their IC_{50} in microsomes was less than 25 μM and/or if their IC_{50} in cells was less than 50 μM, and inactive if their IC_{50} in microsomes was greater than 25 μM and/or if their IC_{50} in cells was greater than 50 μM. Natural product compounds are discussed according to compound class organized by the group most frequently tested for aromatase inhibition, beginning with flavonoids, followed by other classes listed alphabetically. Up to January 2008, 282 natural product compounds had been reported to be tested for aromatase inhibition in the literature, with 125 flavonoids, 36 terpenoids, 19 peptides, 18 lignans, 16 xanthones, 15 fatty acids, 10 alkaloids, and 43 miscellaneous compounds having been evaluated.

The various types of flavonoids previously tested for aromatase inhibition have comprised 37 flavones, 20 flavanones, 19 chalcones, 10 isoflavans, nine catechins, eight isoflavanones, six isoflavones, five pterocarpans, four rotenoids, two anthocyanins, two flavanols, two homoisoflavonoids, and one coumestan. Of the flavonoids tested, flavones have been tested most often and have been the most active (Table 2, Fig. 3). Chrysin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone, **11**) has shown strong aromatase inhibition in microsomes [118–124], JEG-3 cells [125], Arom+HEK 293 cells [125], human preadipocyte cells [126], H295R adrenocortical carcinoma cells [127], and in a MCF-7 dual assay for aromatase inhibition and estrogenicity (chrysin was also estrogenic at high concentrations) [116]. Chrysin (**11**) did not show activity using trout ovarian aromatase [128] or in endometrial cells [118].

Apigenin (5,7,4'-trihydroxyflavone, **8**) and quercetin (3,5,7,3',4'-pentahydroxyflavone, **37**) have been tested numerous times for aromatase inhibition. Apigenin (**8**) was found to be strongly active in microsomes [121–124], JEG-3 cells [125], Arom+HEK 293 cells [125], and granulose-luteal cells [129]. However, this flavone was found to be only moderately active in H295R adrenocortical carcinoma cells [127] and was not active using trout ovarian aromatase [128]. The pentahydroxylated flavone, quercetin (**37**), present in numerous plant species but reported in the aromatase literature as being isolated from *Epilobium capense* [130] and *Morinda citrifolia* L. (noni) [131], was found to be moderately active in two microsomal studies [120,122] but only weakly active in another microsomal study [130]. Quercetin (**37**) was not active in granulose-luteal cells [129], JEG-3 cells [125], H295R adrenocortical carcinoma cells [127], human preadipocyte cells [126], or using trout ovarian aromatase [128].

Reports of activity for unsubstituted flavone (**19**), a natural product derivative, have ranged from moderately active (8 μM IC_{50}) [122] to inactive (375.0 μM IC_{50}) [128] in microsomes. Flavone (**19**) was found to be weakly active in human preadipocyte cells [126] but inactive in JEG-3 cells [125], H295R adrenocortical carcinoma cells [127], and using trout ovarian aromatase [128].

7-Hydroxyflavone (**26**) has been tested several times and has shown strong aromatase inhibition in most microsomal assay testing [123,124,132]. 7-Hydroxyflavone (**26**) also exhibited strong activity in JEG-3 cells [125] and H295R adrenocortical carcinoma cells

[127] but was not active using trout ovarian aromatase [128]. Luteolin (5,7,3',4'-tetrahydroxyflavone, **31**) has shown strong activity in microsomal testing [120,121,133] and cellular testing with JEG-3 cells [125]. Luteolin (**31**) was only moderately active in preadipose cells [134]. 7,8-Dihydroxyflavone (**16**) was tested four times and has shown strong to moderate activity in microsomal testing [121,123].

Of the flavones tested three or less times, those with strong activity include 6-hydroxyflavone (**25**) in JEG-3 cells [125], 7,4'-dihydroxyflavone (**15**) in microsomes [132], 7-methoxyflavone (**32**) in microsomes [123,124] but not in H295R adrenocortical carcinoma cells [127], and isolicoflavonol (3,5,7-trihydroxy-3'-prenylflavone, **27**, isolated from *Broussonetia papyrifera*) in microsomes [135]. Moderately active flavones included broussoflavonol F (3,5,7-trihydroxy-8,3'-diprenylflavone, **10**, isolated from *B. papyrifera* Vent.) in microsomes [135], galangin (3,5,7-trihydroxyflavone, **20**) in JEG-3 cells [125], kaempferol (3,5,7,4'-tetrahydroxyflavone, **29**) in JEG-3 cells [125], 5,7,4'-trihydroxy-3'-methoxyflavone (**44**) in microsomes [136], and rutin (5,7,3',4'-tetrahydroxyflavone 3-diglucoside, **39**, isolated from *Vitis* L. sp.) [107].

When comparing aromatase inhibitory activity within the flavone compound class, several trends become apparent. Hydroxyl groups at positions 5, 7, and 4' generally increase aromatase inhibition activity [e.g., as in apigenin (**8**), luteolin (**31**), chrysin (**11**), and isolicoflavonol (**27**)], although hydroxylation at these positions is not always enough to provide strong aromatase inhibition [e.g., morin (**33**), quercetin (**37**)]. Methoxylation generally decreases aromatase inhibition activity [e.g., 7-hydroxyflavone (**26**) was more active than 7-methoxyflavone (**32**), apigenin (**8**) was more active than prunetin (**36**), and kaempferol (**29**) was more active than kaempferide (**28**)] except in the case of chrysin (**11**), which has two methoxyl groups and is one of the most active flavones tested thus far. Substitution at the C-3 position generally reduces activity [e.g., 3-hydroxyflavone (**21**), morin (**33**), quercetin (**37**), myricetin (**34**) and robinetin (**38**), which were all inactive or only weakly active], while prenylation seems to increase activity, as exemplified by isolicoflavonol (**27**) and broussoflavonol F (**10**).

Twenty flavanones have been tested for aromatase inhibition in the literature (Table 3, Fig. 4). Of these, naringenin (5,7,4'-flavanone, **59**) has been tested most often and has shown strong to moderate aromatase inhibition activity in microsomal testing [118,119,123,124]. This substance was found to be active in JEG-3 cells [125], Arom+HEK 293 cells [125], and inhibited aromatase at low concentrations in a MCF-7 dual assay for aromatase inhibition and estrogenicity [naringenin (**59**) was also estrogenic at high concentrations] [116]. Naringenin (**59**) was less active in H295R adenocortical carcinoma cells [127]. The (2*S*) stereoisomer of naringenin (**59**, isolated from *Broussonetia papyrifera* Vent.) [135] was less active than naringenin (**59**) when no stereochemistry was indicated.

Unsubstituted flavanone (**52**), a natural product derivative, was found to range from having moderate aromatase inhibition [121,122,132,133,137] to being inactive [128] in microsomal biological evaluations. Flavanone (**52**) was inactive using trout ovarian aromatase [128]. 7-Hydroxyflavanone (**56**) and 7-methoxyflavanone (**58**) were both found to be aromatase inhibitors in microsomes [133,137,138], with 7-hydroxyflavanone (**56**) exhibiting more potent activity than 7-methoxyflavanone (**58**). 7-Hydroxyflavanone (**56**) was also active in H295R cells but 7-methoxyflavanone was inactive [127]. Hesperetin (5,7,3'-trihydroxy-4'-methoxyflavanone, **53**) [121,133] and eriodictyol (5,7,3',4'-tetrahydroxyflavanone, **50**) [133] were each tested twice in microsomal aromatase assays and found to be strongly active. 8-Prenylnaringenin (**62**, isolated from *Humulus lupulus* L.) was one of the most active natural product compounds tested for aromatase inhibition in both microsomes and cell assays [114,139].

Of the flavanones tested only once, (2*S*)-2',4'-dihydroxy-2”-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**49**, isolated from *Broussonetia papyrifera* Vent.) [135], (2*S*)-abyssinone II (**45**, isolated from *B. papyrifera*), (2*S*)-5,7,2',4'-tetrahydroxyflavanone (**63**, isolated from *B. papyrifera*), (2*S*)-euchrenone a7 (**51**, isolated from *B. papyrifera*), 7,8-dihydroxyflavanone (**48**) [124], and naringin (**60**) [121] were found to be potent aromatase inhibitors using microsomal assays. Pinostrobin (5-hydroxy-7-methoxyflavanone, **61**) [125] was found to be active in JEG-3 cells [125].

When comparing the activity within the flavanone compound class, several trends are noticeable. Hydroxyl groups at positions 7 and 4' generally increases aromatase inhibition [e.g., eriodictyol (**50**), (2*S*)-abyssinone II (**45**), and (2*S*)-euchrenone a7 (**51**)]. Methoxylation, however, decreases activity [e.g., 7-hydroxyflavanone (**56**) was more active than 7-methoxyflavanone (**58**)]. Prenylation generally caused substantial increases in aromatase activity [e.g., 8-prenylnaringenin (**62**), (2*S*)-abyssinone II (**45**), and (2*S*)-euchrenone a7 (**51**)] except in the case of isoxanthohumol (**57**).

Nineteen chalcones have been tested for their ability to inhibit aromatase (Table 4, Fig. 5). 3'-[γ-Hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2',4'-tetrahydroxychalcone 11'-*O*-coumarate (**75**, isolated from *Broussonetia papyrifera* Vent.) [135], naringenin chalcone (4,2',4',6'-tetrahydroxychalcone, **78**) [133], eriodictyol chalcone (3,4,2',4',6'-pentahydroxychalcone, **68**) [133], and 2,4,2',4'-tetrahydroxy-3'-prenylchalcone (**82**, isolated from *B. papyrifera*) were the most active of the chalcones tested in microsomal assays. Butein (3,4,2',4'-tetrahydroxychalcone, **65**) was active in MCF-7aro cells [140], while xanthohumol (4,4',6'-trihydroxy-2'-methoxy-5'-prenylchalcone, **83**, isolated from *Humulus lupulus* L.) was active in SK-BR-3 cells [139]. Isoliquiritigenin (4,2',4'- trihydroxychalcone, **77**) isolated from licorice (*Glycyrrhiza glabra* L.) [141] and tonka bean (*Dipteryx odorata* Willd.) [142], was found to be inactive in microsomes [133,143] but strongly active in SK-BR-3 cells [143]. Isogemichalcone C (**76**) was also moderately active in a microsomal assay [135].

A couple of trends are discernible when comparing the aromatase inhibitory activity of structures within the chalcone compound class. Hydroxyl groups at positions 4, 2', and 4' have generally provided compounds with a greater degree of aromatase inhibition. The 1,2 double bond is necessary for activity [e.g., phloretin (**80**) was inactive while naringenin chalcone (**78**) was active]. In addition, methoxylation generally reduces activity [e.g., eriodictyol chalcone (**68**) was considerably more active than hesperetin chalcone (**69**); 3'-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2',4'-tetrahydroxychalcone 11'-*O*-coumarate (**75**) was more active than isogemichalcone C (**76**)].

Ten isoflavans were tested with four isoflavans found to be weakly active (Table 5, Fig. 6). 4'-*O*-Methylglabridin (**90**), isolated from licorice (*Glycyrrhiza glabra* L.), leiocin (**87**), isolated from *Berchemia discolor* Hemsl. [144], leiocinol (**88**), isolated from *B. discolor*, and methylequol (**89**) [145] were all weakly active in the microsomal assay.

Nine catechins were reported as being tested for their ability to inhibit aromatase (Table 6, Fig. 7). Epigallocatechin gallate [EGCG, **99**, isolated from *Camellia sinensis* Kuntze (green tea)], has been tested four times with results ranging from weakly active [146], when steroechemistry was not reported, to inactive for the (−) stereoisomer [112], in microsomal testing. However, an epidemiological study inferring aromatase inhibition through changes in estradiol levels demonstrated that estradiol levels were lower for people with higher EGCG (**99**) intake [147]. Furthermore, EGCG (**99**) has been tested using an *in vivo* Swiss-Webster mouse model measuring ovarian aromatase activity and was found to inhibit aromatase activity by 56% at 25 and 12.5 mg/kg [148]. Theaflavin (**101**) and theaflavin-3,3'-gallate (**102**), both isolated from *Camellia sinensis* Kuntze (black tea), were found to

strongly inhibit aromatase in microsomes [146]. (−)-Gallocatechin gallate (**100**), isolated from *C. sinensis* (green tea), was found to weakly inhibit aromatse in microsomes [112]. All other catechins tested were found to be inactive.

Aromatase inhibition testing has been reported for eight isoflavanones (**103–110**, Table 7, Fig. 8), with all isoflavanones found to be inactive in microsome testing [132,143].

From the literature, six isoflavones were tested for aromatase inhibition (Table 8, Fig. 9). The isoflavone biochanin A (5,7-dihydroxy-4'-methoxyisoflavone, **111**) was reported as either moderately active [121] or inactive [119,123,149] in microsomal assays but was strongly active in JEG-3 cells [125] and inactive in granulose-luteal cells [129], human preadipocyte cells [126], and against trout ovarian aromatase [128]. However, biochanin A (**111**) did inhibit aromatase at low concentrations using a MCF-7 dual assay for aromatase inhibition and estrogenicity and was estrogenic at high concentrations [116]. None of the other isoflavones inhibited aromatase.

Sixteen miscellaneous flavonoids were tested for their ability to inhibit aromatase (Table 9, Fig. 10). The coumestan, coumestrol (**119**), has been tested five times for aromatase activity and results have ranged from weakly active [123] in microsomal testing to moderately active in preadipose cells [134]. The only other miscellaneous flavonoid found to be active was a rotenoid, rotenone (**132**, a commercially available insecticide and a potent respiratory toxin), which was found to be strongly active in H295R adrenocortical carcinoma cells [127]. None of the flavanols, homoisoflavonoids, or pterocarpans were found to be active.

From the literature, ten alkaloids have been reported as being tested for aromatase inhibition (Table 10, Fig. 11). Five of these alkaloids were isolated from *Nicotiana tabacum* L. [113,150], with the others from *Hydrastis canadensis* L. (goldenseal), and *Piper* L. sp. [143]. None were found to inhibit aromatase.

Fifteen fatty acids have been tested for aromatase inhibition (Table 11, Fig. 12). Using the categories delineated above, one of the fatty acids, (10*E*,12*Z*)-9-oxo-10,12-octadecadienoic acid (**154**) isolated from *Urtica dioica* L. (stinging nettle) showed moderate aromatase inhibitory activity [151]. Two other fatty acids, (10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid (**149**) and docosapentaenoic acid (**146**) [152], showed weak aromatase inhibitory activity in microsomal testing [151]. However, though several unsaturated fatty acids exhibited strong aromatase inhibitiory activity during initial screening they were found to be inactive in cellular aromatase testing [152]. In bioassay-guided studies on natural product extracts for aromatase inhibition activity, fatty acids may be regarded as “interfering” substances, since they are active in noncellular, enzyme-based aromatase assays but do not inhibit aromatase in secondary cellular testing [152].

In previous literature reports, eighteen lignans were evaluated for aromatase inhibition (Table 12, Fig. 13). The mammalian lignans enterodiol (**166**) and enterolactone (**167**) were each tested three times, as was nordihydroguaiaretic acid (**172**). Enterolactone (**167**) was moderately active in microsomes and strongly active using Arom+HEK 293 cells [153]. Nordihydroguaiaretic acid (**172**) was weakly active in micromal testing [145], although this compound was also found to be inactive in microsomes by another group [154]. Of the other lignans tested, 4,4'-dihydroxyenterolactone (**164**) was moderately active and 4,4'-enterolactone (**165**) was weakly active in microsomal aromatase testing [145]. All other lignans tested were inactive, although nectandrin B (**171**), isolated from *Myristica argentea* Warb. [154], and secoisolariciresinol (**173**) isolated from *Urtica dioica* L. (stinging nettle) [155] were both previously reported as active compounds.

From the literature, nineteen natural product peptides were tested for aromatase inhibition (Table 13, Fig. 14). Sixteen peptides were isolated from an unidentified soil bacterium and were similar in structure, varying only in two side chains and two residues [156]. Most of these peptides from bacteria were inactive in microsomes, with SNA-60-367-6 (**186**) and -11 (**190**) being weakly active [156]. No cellular testing was done on these compounds. *N*-Benzoyl-L-phenylalanine methyl ester (**177**), isolated from *Brassaiopsis glomerulata* L., was found to be weakly active in SK-BR-3 cells [108].

A total of 36 terpenoids have been tested for aromatase inhibition, including ten diterpenoids, ten steroids, seven triterpenoids, five isoprenoids, two sesquiterpenoids, and two withanolides (Table 14, Fig. 15). Of the terpenoids tested, diterpenoids and steroids have been tested most often but were only found to be weakly inhibitory or inactive. The most active of the diterpenoids using recombinant yeast microsomes was the ring C-aromatized compound, standishinal (**203**), isolated from *Thuja standishii* Carrière [157]. Inflexin (**198**), an *ent*-kaurane diterpenoid, isolated from *Isodon excisus* Kudo var. *coreanus*, was also active in micromal aromatase testing [110]. These two diterpenes show little similarity, making structural comparisons within the diterpenoid class difficult. Ten steroids isolated from *Aglaia ponapensis* Kaneh. [158], *Albizia falcataria* (L.) Fosberg, and *Brassaiopsis glomerulata* (Blume) Regel [108] were found to be inactive in microsomal aromatase testing.

Of the seven triterpenoids ursolic acid (**227**), isolated from *Isodon excisus* Kudo var. *coreanus* [110] and *Urtica dioica* L. [155], was tested in microsomes and found to be moderately inhibitory once [110], but otherwise inactive. Another of the triterpenoids tested, aglaiaglabretol B (**223**) isolated from *Aglaia crassinervia* Kurz ex Hiern [159], was moderately active against SK-BR-3 cells [143]. However, aglaiaglabretol B (**223**) was also found to be cytotoxic during previous work [159], limiting the potential use of this compound as an aromatase inhibitor.

Of the five isoprenoids (−)-dehydrololiolide (**205**), isolated from *Brassaiopsis glomerulata* (Blume) Regel, moderately inhibited aromatase in SK-BR-3 cells [108]. The other four isoprenoids were inactive.

A sesquiterpene lactone, 11βH,13-dihydro-10-*epi*-8-deoxycumambrin (**211**), isolated from *Stevia yaconensis* Hieron. var. *subeglandulosa* [160], was found to be strongly active using microsomal aromatase testing [161]. Though the other sesquiterpene lactone 10-*epi*-8-deoxycumambrin B (**210**) was found to be moderately active in microsomes it was found to be cytotoxic in further testing [161]. The former was moderately active as an aromatase inhibitor in JEG-3 choriocarcinoma cells and was not cytotoxic [161].

The two withanolides, isolated from *Physalis philadelphica* Lam. (tomatillo, an edible fruit similar to tomato often used in salsa) [162], were found to be inactive against aromatase in microsome testing [143].

Sixteen xanthones were tested for aromatase inhibition in microsomes (Table 15, Fig. 16). Twelve xanthones were isolated from *Garcinia mangostana* L. (mangosteen) [163]. γ-Mangostin (**276**) and garcinone D (**270**), were found to be strongly active in microsomes and α-mangostin (**275**) and garcinone E (**271**) were found to be moderately active. The other xanthones from *G. mangostana* L. were inactive. Four xanthones were isolated from a marine fungus, *Monodictys putredinis* [164], and were found to be inactive in microsomal testing.

There have been 43 miscellaneous natural product compounds tested for aromatase inhibition in the literature (Table 16, Fig. 17). Fourteen benzenoids were tested, with

TAN-931 (**269**) isolated from the bacterium *Penicillium funiculosum* No. 8974 [165], being weakly active in microsomes. TAN-931 (**269**) was further tested *in vivo* using Sprague-Dawley rats and was found to reduce estradiol levels presumably, although not definitively, through aromatase inhibition [165]. All other benzenoids were inactive.

Seven anthraquinones have been tested, six of which were isolated from *Morinda citrifolia* L. (noni), a widely used botanical dietary supplement [166,167]. None of the anthraquinones isolated from *M. citrifolia* was found to be active in microsomal aromatase testing. Benzanthraquinone I (**249**), isolated from the bacteria *Streptomyces* S-11106, exhibited strong aromatase inhibitory activity in microsomes [168].

The stilbenoid, resveratrol (**286**), isolated from *Vitis* L. sp. [107], was reported to strongly inhibit aromatase in microsomes [107], to moderately inhibit aromatase in another microsomal test [136], and to be inactive when tested a third time [143]. One of the miscellaneous compounds, albanol A (**281**) isolated from *Broussonetia papyrifera* Vent. [135], was found to moderately inhibit aromatase in microsomes. All other miscellaneous compounds, including all alkanols, aromatic hydrocarbons, benzofurans, chlorophylls, diarylheptanoids, dioxadispiroketals, spiroketones, and tannins, were found to be inactive against aromatase.

CONCLUSIONS

Numerous natural product extracts, from plant, fungal, and microbial terrestrial and marine sources, have been evaluated for aromatase inhibition using various noncellular, cell-based, and *in vivo* assays. Some of the more active extracts included those of *Agaricus bisporus* (Lange) Imbach (white button mushrooms) [115] and *Vitis* L. sp. (grape and/or wine) [86,106,107], among others. Some aromatase activity-guided fractionation has been performed on *Vitis* sp. extracts, resulting in the isolation of various procyanidin dimers that have yet to be fully characterized [86]. Interestingly, several types of extracts and partitions of *A. bisporus* and a sample of *Vitis* sp. (grape) were subsequently tested for their ability to inhibit aromatase in microsomes and found to be inactive [143]. Several factors could be involved in the discrepancies between the literature results, including variations in the species collected, timing of collections, purity of materials extracted, preparation of extracts, and assay methodology.

Several other extracts were determined to inhibit aromatase in microsomes including from *Brassaiopsis glomerulata* (Blume) Regel [108] and *Garcinia mangostana* L. (mangosteen) [109], with both of these species having undergone activity-guided purification resulting in the isolation of compounds with AI activity. Extracts of several cycads were also found to be potent AIs [104] but, to date, their bioassay-guided fractionation has not been performed. Another active extract that has not undergone fractionation is *Euonymus alatus* [111]. Active compounds isolated from these extracts may provide potent AIs and possible leads for further development.

Nearly 300 natural product compounds have been evaluated for their ability to inhibit aromatase, in noncellular, cell-based, and *in vivo* aromatase inhibition assays. Flavonoids have been tested most frequently and generally found to be the most active class of natural product AI compounds. Some of the more active flavonoids included apigenin (**8**), chrysin (**11**), 7-hydroxyflavone (**26**), isolicoflavonol (**27**), (2*S*)-abyssinone II (**45**), (2*S*)-2',4'-dihydroxy-2”-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**49**), eriodictyol (**50**), 8-prenylnaringenin (**62**), 3'-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2',4'-tetrahydroxychalcone 11'-*O*-coumarate (**75**), isoliquiritigenin (**77**), and rotenone (**132**). Other very active AI compounds included the xanthone, γ-mangostin (**239**), the

sesquiterpene lactone, 11βH,13-dihydro-10-*epi*-8-deoxycumambrin (**211**), and the anthraquinone, benzanthraquinone I (**249**).

Since natural product drug discovery efforts frequently utilize non-cellular screening assays, several of the compounds reported to be active in non-cellular assays should be avoided by future AI drug discovery endeavors. This is exemplified by the unsaturated fatty acids, which are commonly found in natural product extracts and have been shown to interfere with non-cellular AI assays [152]. Several flavonoids have also been found to be active in non-cellular screening and inactive in cell-based assays. In natural product AI screening efforts it is recommended that extracts active in non-cellular bioassays be dereplicated for the presence of known aromatase inhibitors prior to expensive and time-consuming bioassay-guided fractionation.

All of the most active compounds were of the flavonoid or xanthone compound classes, with the exception of the active sesquiterpene lactone and the active anthraquinone. The ability of flavonoids to inhibit aromatase has been well established [169,170] and some flavonoids have continued into *in vivo* studies with various results [125,148]. Interestingly, Saarinen et al. [125] have shown that apigenin (**8**), chrysin (**11**), and naringenin (**59**) were all inactive using an *in vivo* AI mouse model. The AI activity of flavonoids needs further *in vivo* testing to substantiate the extensive and potent *in vitro* results. Various AI mouse models are currently available or in development, including a transgenic model that overexpresses aromatase [171], an aromatase-knockout mouse model [172], and a MCF-7 xenograft model [173].

Several natural product compounds have already undergone synthetic modifications to further enhance AI activity. Two separate syntheses have been performed on the strongly active flavone (2*S*)-abyssinone II (**45**) [174,175] and several analogues have been found to be more active than the natural compound. Synthesis of 7,8-benzoflavanones has provided several leads with potent AI activity [176]. Ursolic acid (**227**) derivatives were synthesized with resulting compounds having lower activity than the natural product [177]. The diterpenoid, standishinal (**203**), and several synthetic derivatives were subjected to AI testing with the most active compounds having a *cis*-configuration on the A/B ring [178]. Synthetic xanthones have only recently been tested for their ability to inhibit aromatase [48,179,180] with extremely potent AI activity in the nanomolar range. However, very few natural product or synthetic compounds have undergone extensive evaluation using additional *in vitro* or *in vivo* and preclinical models.

This review highlights several compound classes that may act as aromatase inhibitors (e.g., flavones, flavanones, chalcones, and xanthones) and other structural classes that are less active. These scaffolds may be utilized to direct synthetic modification of natural product scaffolds to enhance aromatase inhibition. New natural products or natural product analogues that inhibit aromatase may be clinically useful for treating postmenopausal breast cancer. Aromatase inhibitors may also act as chemopreventive agents for preventing secondary recurrence of breast cancer. Furthermore, AIs from edible plant materials may eventually be appropriate for primary prevention of breast cancer in postmenopausal women (e.g., lower toxicity due to history of human consumption). Botanical dietary supplements or foods that are ingested regularly and act as AIs may have a role in breast cancer chemoprevention or chemotherapy for postmenopausal women.

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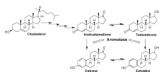


Fig. (1).
Conversion of cholesterol to androstenedione and testosterone, followed by aromatase catalyzed conversion to estrone and estradiol, respectively.

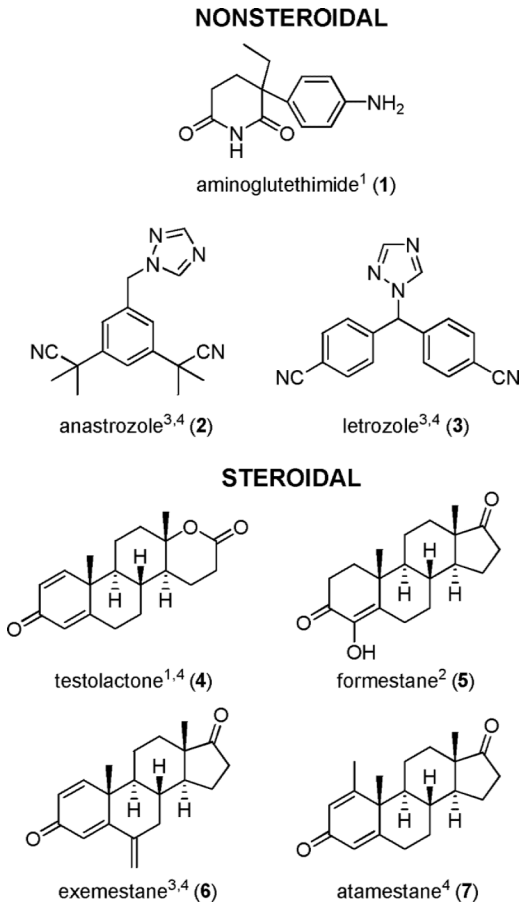
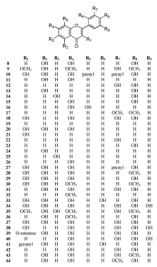


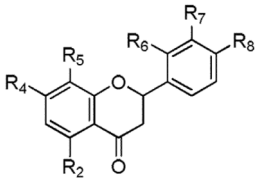
Fig. (2).

Examples of first[1], second[2], and third[3] generation AIs, including AIs currently in clinical trials[4]. All three third generation compounds are currently approved for clinical use.

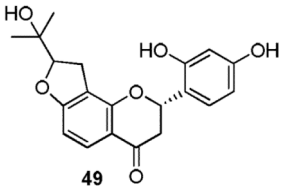


	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
6	H	OH	H	OH	H	H	OH	H
9	OCH_3	OH	H	OCH_3	H	H	OH	OCH_3	H
10	H	OH	H	OH	H	H	H	H	H
11	H	OH	H	OH	H	H	H	H	H
12	H	H	H	H	H	H	OH	OH	H
13	H	OH	H	H	H	H	H	OH	H
14	H	OH	H	H	H	H	H	OH	H
15	H	H	OH	H	H	H	H	OH	H
16	H	H	H	OH	H	H	H	H	H
17	H	H	H	H	H	H	OCH_3	OCH_3	H
18	H	H	H	OH	H	H	OH	OH	H
19	H	H	H	OH	H	H	H	H	H
20	OH	OH	H	OH	H	H	H	H	H
21	OH	H	H	H	H	H	H	H	H
22	H	H	H	H	H	H	OH	H	H
23	H	H	H	H	H	H	H	H	H
24	H	OH	H	H	H	H	H	H	H
25	H	H	OH	H	H	H	H	H	H
26	H	H	H	OH	H	H	H	H	H
27	OH	OH	H	OH	H	H	prenyl	OH	H
28	OH	OH	H	OH	H	H	H	OH	H
29	OH	OH	H	OH	H	H	H	OH	H
30	OH	OH	H	OCH_3	H	H	H	OCH_3	H
31	H	OH	H	OH	H	H	OH	OH	H
32	H	H	H	OCH_3	H	H	H	H	H
33	OH	OH	H	OH	H	H	OH	OH	H
34	OH	OH	H	OH	H	H	OH	OH	OH
35	OCH_3	OH	OH	OCH_3	H	H	OH	OCH_3	H
36	H	OH	H	OCH_3	H	H	H	OH	H
37	H	OH	H	OH	H	H	OH	OH	H
38	OH	OH	H	OH	H	H	OH	OH	OH
39	O-sulfonic	OH	H	OH	H	H	OH	OH	H
40	H	H	H	OH	H	H	OH	OH	OH
41	prenyl	OH	H	OH	H	H	OH	OH	H
42	H	OH	H	OH	H	H	OH	OH	H
43	H	OH	H	OH	H	H	OH	OCH_3	H
44	H	OH	H	OH	H	H	OCH_3	OH	H

Fig. (3).
Structures of natural product flavones tested for aromatase inhibition.

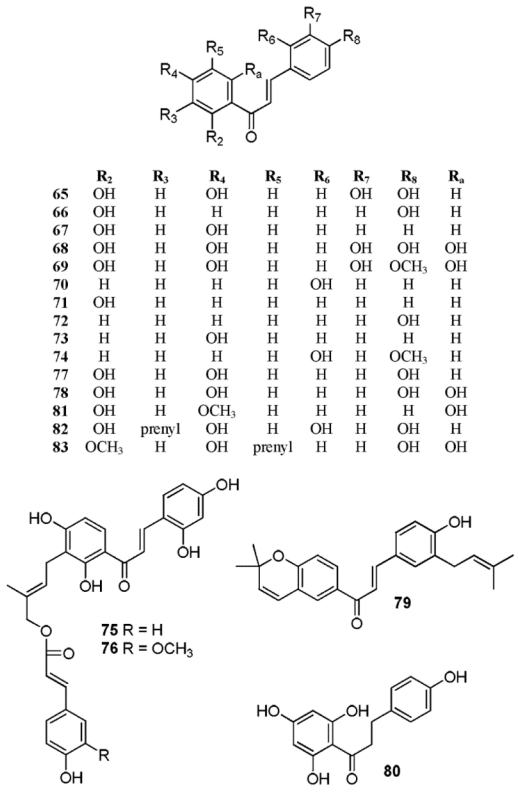


		R_2	R_4	R_5	R_6	R_7	R_8
45	2*S*	H	OH	H	H	prenyl	OH
46		H	H	H	H	OH	OH
47		OH	OH	H	H	H	H
48		H	OH	OH	H	H	H
50		OH	OH	H	H	OH	OH
51	2*S*	H	OH	prenyl	OH	H	OH
52		H	H	H	H	H	H
53		OH	OH	H	H	OH	OCH_3
54		OH	O-rutinoside	H	H	OH	OCH_3
55		H	H	H	H	H	OH
56		H	OH	H	H	H	H
57		OCH_3	OH	prenyl	H	H	OH
58		H	OCH_3	H	H	H	H
59	2*S*	OH	OH	H	H	H	OH
60	2*S*	OH	O-rutinose	H	H	H	OH
61		OH	OCH_3	H	H	H	H
62		OH	OH	prenyl	H	H	OH
63	2*S*	OH	OH	H	OH	H	OH
64		OH	OH	H	H	OCH_3	OH



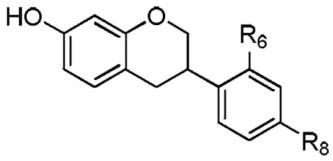
49

Fig. (4).
Structures of natural product flavanones tested for aromatase inhibition.

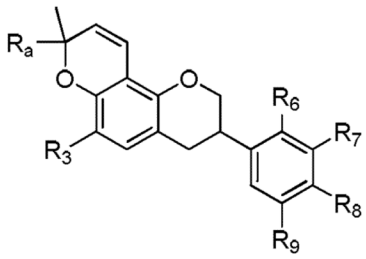


	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_a
65	OH	H	OH	H	H	OH	OH	H
66	OH	H	H	H	H	H	OH	H
67	OH	H	OH	H	H	H	H	H
68	OH	H	OH	H	H	OH	OH	OH
69	OH	H	OH	H	H	OH	OCH_3	OH
70	H	H	H	H	OH	H	H	H
71	OH	H	H	H	H	H	H	H
72	H	H	H	H	H	H	OH	H
73	H	H	OH	H	H	H	H	H
74	H	H	H	H	OH	H	OCH_3	H
77	OH	H	OH	H	H	H	OH	H
78	OH	H	OH	H	H	H	OH	OH
81	OH	H	OCH_3	H	H	H	H	OH
82	OH	prenyl	OH	H	OH	H	OH	H
83	OCH_3	H	OH	prenyl	H	H	OH	OH

Fig. (5).
Structures of natural product chalcones tested for aromatase inhibition.

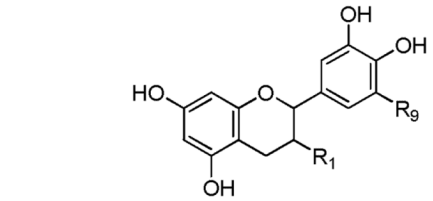


	R_6	R_8
84	H	OH
89	H	OCH_3
93	OCH_3	OCH_3



		R_3	R_6	R_7	R_8	R_9	R_a
85	3*S*	H	OH	H	OCH_3	H	phenyl
86	3*R*	H	OH	OH	OH	H	CH_3
87	3*S*	H	OH	H	-OCH_2O-		CH_3
88	3*S*	CH_3	OH	H	-OCH_2O-		CH_3
90	3*R*	H	OH	H	OCH_3		CH_3
91	3*S*	H	OH	H	-OCH_2O-		phenyl
92	3*S*	H	OH	OH	OCH_3		phenyl

Fig. (6).
Structures of natural product isoflavans tested for aromatase inhibition.



			R_1	R_9
94	2*R*	3*S*	OH	H
95	2*S*	3*R*	gallate	H
96	2*S*	3*R*	OH	H
97	2*R*	3*R*	gallate	H
98	2*R*	3*R*	OH	OH
99	2*R*	3*S*	gallate	OH
100	2*S*	3*R*	gallate	OH

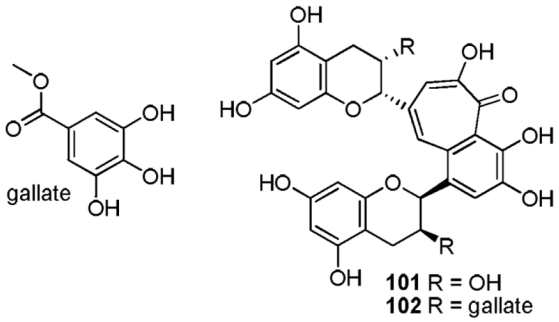


Fig. (7).
Structures of natural product catechins tested for aromatase inhibition.

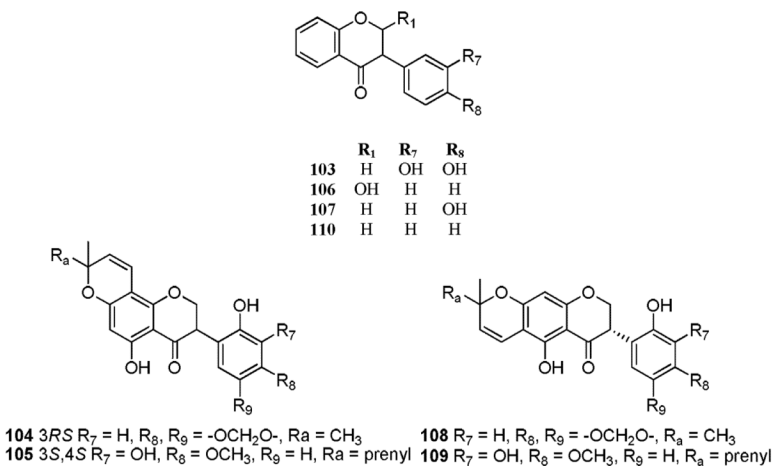
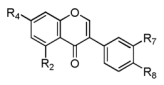


Fig. (8).
Structures of natural product isoflavanones tested for aromatase inhibition.



	R_2	R_4	R_7	R_8
111	OH	OH	H	OCH_3
112	H	OH	H	OH
113	H	OH	H	OCH_3
114	OH	OH	H	OH
115	H	H	H	H
116	H	OH	OH	OH

Fig. (9).
Structures of natural product isoflavones tested for aromatase inhibition.

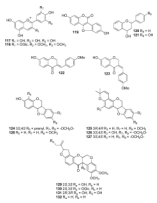


Fig. (10).
Structures of natural product flavonoids (not previously mentioned) tested for aromatase inhibition.

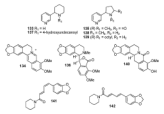


Fig. (11).
Structures of alkaloids tested for aromatase inhibition.

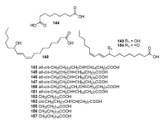


Fig. (12).
Structures of natural product fatty acids tested for aromatase inhibition.

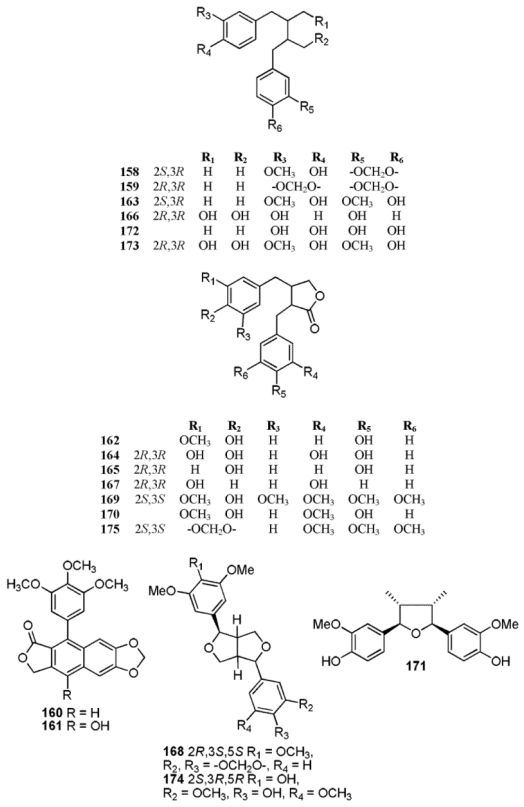
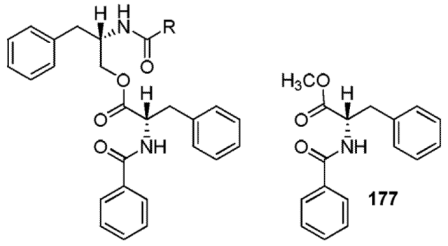
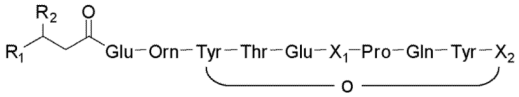


Fig. (13).
Structures of natural product lignans tested for aromatase inhibition.



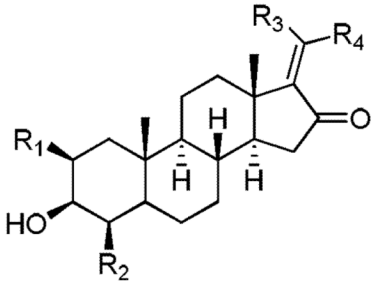
176 R = CH_3
178 R = phenyl

177

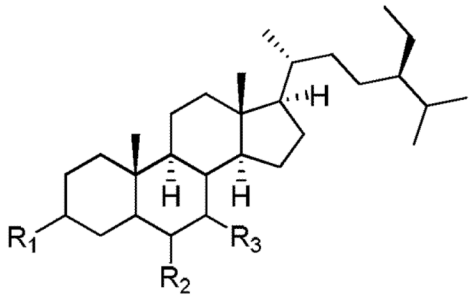


	X_1	X_2	R_1	R_2
179	Ala	Ile	$-CH_2(CH_2)_{10}CH(CH_3)_2$	OH
180	Aba	Ile	$-CH_2(CH_2)_{10}CH(CH_3)_2$	OH
181	Val	Ile	$-CH_2(CH_2)_{10}CH(CH_3)_2$	OH
182	Ala	Ile	$-CH_2(CH_2)_{10}CHCH_2CH_3$ CH_3	OH
183	Val	Val	$-CH_2(CH_2)_{13}CH_3$	OH
184	Val	Val	$-CH_2(CH_2)_{11}CH(CH_3)_2$	OH
185	Aba	Ile	$-CH_2(CH_2)_{10}CHCH_2CH_3$ CH_3	OH
186	Aba	Ile	$-CH_2(CH_2)_{11}CH(CH_3)_2$	OH
187	Val	Ile	$-CH_2(CH_2)_{10}CHCH_2CH_3$ CH_3	OH
188	Val	Ile	$-CH_2(CH_2)_{11}CH(CH_3)_2$	OH
189	Ala	Ile	$-CH_2(CH_2)_{12}CH_3$	H
190	Val	Ile	$-CH_2(CH_2)_{10}CH(CH_3)_2$	H
191	Val	Ile	$-CH_2(CH_2)_{12}CH_3$	H
192	Ala	Ile	$-CH_2(CH_2)_{11}CH(CH_3)_2$	H
193	Aba	Ile	$-CH_2(CH_2)_{10}CHCH_2CH_3$ CH_3	H
194	Val	Ile	$-CH_2(CH_2)_{11}CH(CH_3)_2$	H

Fig. (14).
Structures of natural product peptides tested for aromatase inhibition.



		R_1	R_2	R_3	R_4
212	H-5α	OH	H	CH_3	H
213	H-5α	OH	H	H	CH_3
220	$\Delta^{5(6)}$	H	OH	CH_3	H
221	$\Delta^{5(6)}$	H	OH	H	CH_3



			R_1	R_2	R_3
214	$\Delta^{4(5)}$	H-8β	=O	β-OH	H
215	$\Delta^{4(5)}$	H-8β	=O	H	β-OH
216	$\Delta^{7(8)}$	H-5α	β-OH	H	H
217	$\Delta^{7(8)}$	H-5α	β-OAc	H	H
218	$\Delta^{7(8)}$	H-5α	=O	H	H
219	$\Delta^{5(6)}$	H-8β	β-OH	H	H

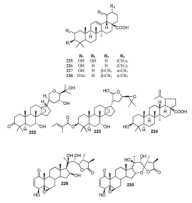
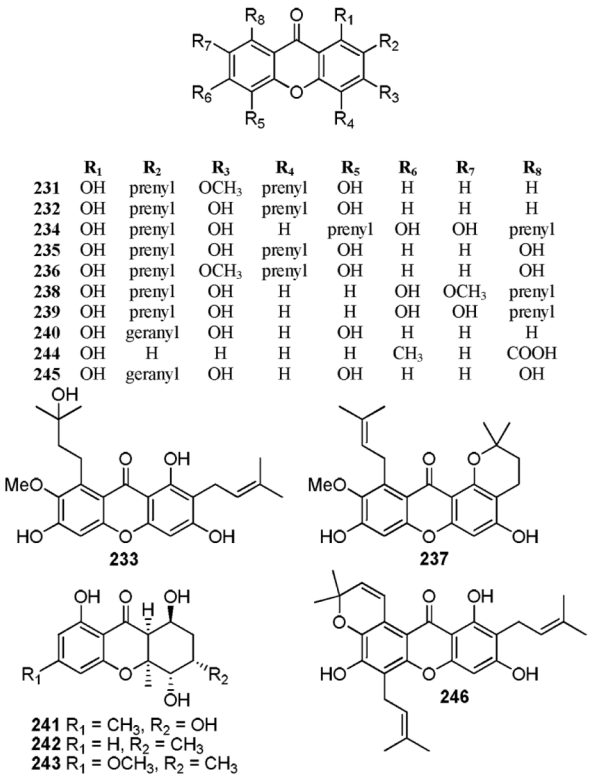


Fig. (15).
Structures of natural product terpenoids tested for aromatase inhibition.



	R1	R2	R3	R4	R5	R6	R7	R8
231	OH	prenyl	OCH_3	prenyl	OH	H	H	H
232	OH	prenyl	OH	prenyl	OH	H	H	H
234	OH	prenyl	OH	H	prenyl	OH	OH	prenyl
235	OH	prenyl	OH	prenyl	OH	H	H	OH
236	OH	prenyl	OCH_3	prenyl	OH	H	H	OH
238	OH	prenyl	OH	H	H	OH	OCH_3	prenyl
239	OH	prenyl	OH	H	H	OH	OH	prenyl
240	OH	geranyl	OH	H	OH	H	H	H
244	OH	H	H	H	H	CH_3	H	COOH
245	OH	geranyl	OH	H	OH	H	H	OH

Fig. (16).
Structures of natural product xanthones tested for aromatase inhibition.

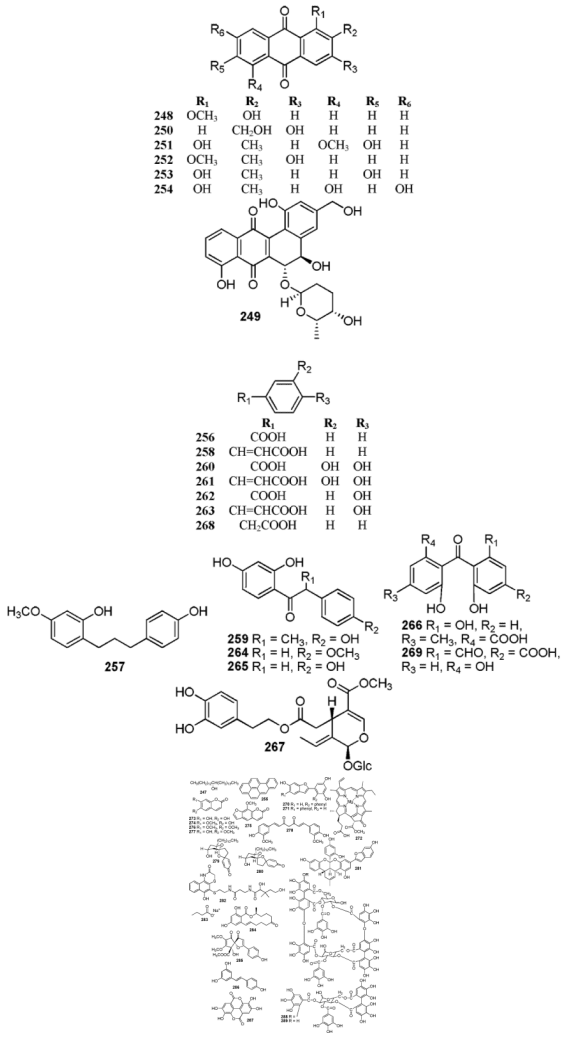


Fig. (17).
Structures of miscellaneous natural products (not previously mentioned) tested for aromatase inhibition.

Table 1

Previous literature reports of natural product extracts tested for aromatase inhibition

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Aesculus glabra	Ohio buckeye	Hippocastanaceae	plant	methanol ($CHCl_3$ partition)	microsomes	42.0	PCA at 20 μg/mL	[143]
Agaricus bisporus	baby button mushroom	Agaricaceae	fungus	water reflux	microsomes	~58	PCA at 100 μL	[115]
Agaricus bisporus	crimini mushroom	Agaricaceae	fungus	water reflux	microsomes	~46	PCA at 100 μL	[115]
Agaricus bisporus	portobello mushroom	Agaricaceae	fungus	water reflux	microsomes	~45	PCA at 100 μL	[115]
Agaricus bisporus	stuffing mushroom	Agaricaceae	fungus	water reflux	microsomes	~20	PCA at 100 μL	[115]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water reflux	microsomes	~35	PCA at 100 μL	[115]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water reflux	MCF-7aro cells	14	at 10 μL/mL	[115]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (air dried)	microsomes	83.1	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (air dried, hexane partition)	microsomes	71.1	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (air dried, $CHCl_3$ partition)	microsomes	51.7	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (air dried, water partition)	microsomes	63.1	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (air dried, butanol partition)	microsomes	82.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (dehydrated)	microsomes	94.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (dehydrated, hexane partition)	microsomes	55.3	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (dehydrated, $CHCl_3$ partition)	microsomes	54.7	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (dehydrated, water partition)	microsomes	73.5	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (dehydrated, butanol partition)	microsomes	55.0	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (fresh)	microsomes	66.4	PCA at 20 μg/mL	[143]

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Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (fresh, hexane partition)	microsomes	72.7	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (fresh, $CHCl_3$ partition)	microsomes	78.8	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (fresh, water partition)	microsomes	89.6	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (fresh, butanol partition)	microsomes	79.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	acetone (fresh)	microsomes	59.1	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	acetone (fresh, hexane partition)	microsomes	38.3	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	acetone (fresh, $CHCl_3$ partition)	microsomes	39.2	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	acetone (fresh, water partition)	microsomes	81.5	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	acetone (fresh, butanol partition)	microsomes	85.3	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water (reflux)	microsomes	96.2	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water (reflux, hexane partition)	microsomes	80.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water (reflux, $CHCl_3$ partition)	microsomes	56.1	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water (reflux, water partition)	microsomes	79.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	water (reflux, butanol partition)	microsomes	65.3	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (sautéed)	microsomes	85.8	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (sautéed, hexane partition)	microsomes	53.5	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (sautéed, $CHCl_3$ partition)	microsomes	68.2	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (sautéed, water partition)	microsomes	83.8	PCA at 20 μg/mL	[143]
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	methanol (sautéed, butanol partition)	microsomes	57.1	PCA at 20 μg/mL	[143]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Agaricus bisporus	white button mushroom	Agaricaceae	fungus	Dichloromethane	microsomes	54.4	PCA at 20 μg/mL	[143]
Agaricus bisporus	cremini mushroom	Agaricaceae	fungus	Dichloromethane	microsomes	65.7	PCA at 20 μg/mL	[143]
Agaricus bisporus	portobella mushroom	Agaricaceae	fungus	Dichloromethane	microsomes	59.1	PCA at 20 μg/mL	[143]
Agaricus blazei (1SY16)	almond mushroom	Agaricaceae	fungus	Unknown	microsomes	87.7	PCA at 20 μg/mL	[143]
Agaricus blazei	almond mushroom	Agaricaceae	fungus	Methanol	microsomes	75.2	PCA at 20 μg/mL	[143]
Agaricus blazei	almond mushroom	Agaricaceae	fungus	methanol (hexane partition)	microsomes	72.5	PCA at 20 μg/mL	[143]
Agaricus blazei	almond mushroom	Agaricaceae	fungus	methanol (dichloromethane partition)	microsomes	82.1	PCA at 20 μg/mL	[143]
Agaricus blazei	almond mushroom	Agaricaceae	fungus	methanol (water partition)	microsomes	88.4	PCA at 20 μg/mL	[143]
Agaricus blazei	almond mushroom	Agaricaceae	fungus	Dichloromethane	microsomes	74.5	PCA at 20 μg/mL	[143]
Allium sp.[a]	green onion	Liliaceae	plant	water reflux	microsomes	~75	PCA at 100 μL	[115]
Allium sp.[a]	green onions	Liliaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Allium sp.[a]	spanish onions	Liliaceae	plant	70% ethanol	microsomes	310	units/100 g	[113]
Allium sp.[a]	white onions	Liliaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Alpinia purpurata	red ginger	Zingerberaceae	plant	75% MeOH reflux	microsomes	~78	% inhib.	[105]
Althaea rosea var. *nigra*	hollyhock	Malvaceae	plant	Nd	immunocytochemistry in Leydig cells	weak		[181]
Apium graveolens [a]	celery	Apiaceae	plant	water reflux	microsomes	~80	PCA at 100 μL	[115]
Apium graveolens [a]	celery	Apiaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Asparagus officinalis [a]	asparagus	Liliaceae	plant	70% ethanol	microsomes	1300	units/100 g	[113]
Auricularia sp.	woodear mushroom	Auriculariaceae	fungus	water reflux	microsomes	~86	PCA at 100 μL	[115]
Brassaiopsis glomerulata (leaves)	none	Araliaceae	plant	methanol (hexane partition)	microsomes	6.9	PCA at 20 μg/mL	[143]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Brassaiopsis glomerulata (leaves)	none	Araliaceae	plant	methanol (ethyl acetate partition)	microsomes	59.3	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (leaves)	none	Araliaceae	plant	methanol (water partition)	microsomes	98.2	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (leaves)	none	Araliaceae	plant	methanol (hexane partition)	SK-BR-3 cells	7.2	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (leaves)	none	Araliaceae	plant	methanol (ethyl acetate partition)	SK-BR-3 cells	37.0	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (twigs)	none	Araliaceae	plant	methanol (hexane partition)	microsomes	35.6	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (twigs)	none	Araliaceae	plant	methanol (ethyl acetate partition)	microsomes	46.6	PCA at 20 μg/mL	[143]
Brassaiopsis glomerulata (twigs)	none	Araliaceae	plant	methanol (water partition)	microsomes	95.8	PCA at 20 μg/mL	[143]
Brassica juncea [a]	mustard (greens)	Brassicaceae	plant	70% ethanol	microsomes	2700	units/100 g	[113]
Brassica oleracea [a]	broccoli	Brassicaceae	plant	water reflux	microsomes	~85		[115]
Brassica oleracea [a]	broccoli	Brassicaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Brassica oleracea [a]	broccoli (leaves)	Brassicaceae	plant	70% ethanol	microsomes	3600	units/100 g	[113]
Brassica oleracea [a]	cabbage	Brassicaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Brassica oleracea [a]	cauliflower	Brassicaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Brassica oleracea [a]	collards	Brassicaceae	plant	70% ethanol	microsomes	8500	units/100 g	[113]
Brassica oleracea [a]	kale	Brassicaceae	plant	70% ethanol	microsomes	4700	units/100 g	[113]
Brassica rapa var. *rapa*[a]	turnips	Brassicaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Camellia sinensis [a]	black tea	Theaceae	plant	Nd	nd	>50	% inhib.	[182]
Camellia sinensis [a]	green tea	Theaceae	plant	Nd	nd	>50	% inhib.	[182]
Camellia sinensis [a]	green tea (polyphenone-60)	Theaceae	plant	Nd	microsomes	28	μg/mL IC_50	[112]
Camellia sinensis [a]	tea	Theaceae	plant	70% ethanol	microsomes	27000	units/100 g	[113]
Cantharellus sp.	chanterelle mushroom	Cantharellaceae	fungus	water reflux	microsomes	~80	PCA at 100 μL	[115]
Capsicum annuuma [a]	bell pepper	Solanaceae	plant	water reflux	microsomes	~89	PCA at 100 μL	[115]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Capsicum sp.[a]	pepper (leaves)	Solanaceae	plant	70% ethanol	microsomes	2800	units/100 g	[113]
Capsicum sp.[a]	peppers	Solanaceae	plant	70% ethanol	microsomes	330	units/100 g	[113]
Cestrum sp.	none	Solanaceae	plant	75% MeOH reflux	microsomes	~40	% inhib.	[105]
Chrysanthemum partheniuma [a]	feverfew	Asteraceae	plant	Nd		>50	% inhib.	[182]
Cichorium endivia [a]	endive	Asteraceae	plant	70% ethanol	microsomes	850	units/100 g	[113]
Cichorium endivia [a]	escarole	Asteraceae	plant	70% ethanol	microsomes	830	units/100 g	[113]
Citrus × *limona* [a]	lemons	Rutaceae	plant	70% ethanol	microsomes	660	units/100 g	[113]
Citrus paradisi [a]	grapefruit (juice)	Rutaceae	plant	Nd	microsomes	~68	PCA at 25 μL	[183]
Citrus sinensis [a]	orange	Rutaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Citrus sinensis [a]	orange (juice)	Rutaceae	plant	Nd	microsomes	~90	PCA at 25 μL	[183]
Coccothrinax sp.	none	Arecaceae	plant	75% MeOH reflux	microsomes	~70	% inhib.	[105]
Coffea sp.[a]	coffee	Rubiaceae	plant	70% ethanol	microsomes	13000	units/100 g	[113]
Coix lachrymal-jobi var. *ma-yuen*	adlay or Job's tears	Poaceae	plant	Methanol	rat granulose cells	inhibits	activity at 100 μg/mL	[184]
Cucumis melo [a]	cantaloupe	Cucurbitaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Cucumis sativus [a]	cucumber	Loasaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Curcuma longa [a]	turmeric	Zingiberaceae	plant	Nd	nd	>50	% inhib.	[182]
Cycas cairnsiana	none	Cycadaceae	plant	75% MeOH reflux (ethyl acetate partition)	microsomes	69	% inhib.	[104]
Cycas revoluta	sago palm	Cycadaceae	plant	75% MeOH reflux (methanol partition)	microsomes	79	% inhib.	[104]
Cycas revoluta	sago palm	Cycadaceae	plant	75% MeOH reflux (ethyl acetate partition)	microsomes	86	% inhib.	[104]
Cycas rumphii	none	Cycadaceae	plant	75% MeOH reflux (methanol partition)	microsomes	90	% inhib.	[104]
Cycas rumphii	none	Cycadaceae	plant	75% MeOH reflux (ethyl acetate partition)	microsomes	15	% inhib.	[104]
Daucus carota [a]	carrot	Apiaceae	plant	water reflux	microsomes	~74	PCA at 100 μL	[115]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Daucus carota [a]	carrot	Apiaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Dioon spinulosum	none	Zamiaceae	plant	75% MeOH reflux (methanol partition)	microsomes	40	% inhib.	[104]
Dioon spinulosum	none	Zamiaceae	plant	75% MeOH reflux (ethyl acetate partition)	microsomes	97	% inhib.	[104]
Encephalartos ferox	bread palm	Zamiaceae	plant	75% MeOH reflux (methanol partition)	microsomes	45	% inhib.	[104]
Encephalartos ferox	bread palm	Zamiaceae	plant	75% MeOH reflux (ethyl acetate partition)	microsomes	97	% inhib.	[104]
Epilobium capense	willowherb	Onagraceae	plant	aqueous methanol	microsomes	60	% inhib. at 200 μg	[130]
Epilobium capense	willowherb	Onagraceae	plant	Methanol	microsomes	54	% inhib. at 200 μg	[130]
Euonymus alatus	“gui-jun woo”	Celastraceae	plant	water reflux	microsomes	11	μg/mL IC_50	[111]
Euonymus alatus	“gui-jun woo”	Celastraceae	plant	water reflux	myometrial cells	0.80	μg/mL IC_50	[111]
Euonymus alatus	“gui-jun woo”	Celastraceae	plant	water reflux	leiomyonal cells	0.07	μg/mL IC_{50}	[111]
Flammulina velutipes	enoki mushroom		fungus	water reflux	microsomes	~78	PCA at 100 μL	[115]
Fragaria sp.[a]	strawberry (juice)	Rosaceae	plant	Nd	microsomes	~52	PCA at 25 μL	[183]
Fragaria sp.	strawberry	Rosaceae	plant	Methanol	microsomes	84.8	PCA at 20 μg/mL	[143]
Fragaria sp.	strawberry	Rosaceae	plant	Acetone	microsomes	65.8	PCA at 20 μg/mL	[143]
Fragaria sp.	strawberry	Rosaceae	plant	methanol/acetone	microsomes	84.3	PCA at 20 μg/mL	[143]
Garcinia mangostana	mangosteen	Clusiaceae	plant	Methanol	microsomes	18.9	PCA at 20 μg/mL	[109]
Garcinia mangostana	mangosteen	Clusiaceae	plant	methanol ($CHCl_3$ partition)	microsomes	29.8	PCA at 20 μg/mL	[109]
Garcinia mangostana	mangosteen	Clusiaceae	plant	Methanol	SK-BR-3 cells	24.1	PCA at 20 μg/mL	[109]
Garcinia mangostana	mangosteen	Clusiaceae	plant	methanol ($CHCl_3$ partition)	SK-BR-3 cells	16.5	PCA at 20 μg/mL	[109]
Glycyrrhiza glabra [a]	licorice	Fabaceae	plant	Nd	nd	>50	% inhib.	[182]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Glyxine max [a]	soy (infant formulas)	Fabaceae	plant	Nd	*in vivo* brain aromatase	none		[185]
Hericium erinaceus	lion's mane mushroom	Hericiaceae	fungus	Dichloromethane	microsomes	57.9	PCA at 20 μg/mL	[143]
Hordeum vulgare *Humulus lupulus*	alcohol free beer	Poaceae Cannabaceae	plant	Nd	choriocarcinoma-derived JAR cells	65.27	PCA	[114]
Hordeum vulgare [a] *Humulus lupulus* [a]	lager beer	Poaceae Cannabaceae	plant	Nd	choriocarcinoma-derived JAR cells	75.8	PCA	[114]
Hordeum vulgare [a] *Humulus lupulus* [a]	stout	Poaceae Cannabaceae	none	Nd	choriocarcinoma-derived JAR cells	33.9	PCA	[114]
Hordeum vulgare [a] *Humulus lupulus* [a]	xanthohumol-rich stout	Poaceae Cannabaceae	nd	Nd	choriocarcinoma-derived JAR cells	26.4	PCA	[114]
Isodon excisus var. *coreanus*	none	Lamiaceae	plant	methanol (diethyl ether partition)	microsomes	13.7	μg/mL IC_{50}	[110]
Lactuca sp.[a]	iceberg lettuce	Asteraceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Lactuca sp.[a]	romaine lettuce	Asteraceae	plant	70% ethanol	microsomes	560	units/100 g	[113]
Larrea tridentata [a]	chaparral	Zygophyllaceae	plant	Nd	nd	>50	% inhib.	[182]
Lentinula edodes	shiitake mushroom	Marasmiaceae	fungus	water reflux	microsomes	~62	PCA at 100 μL	[115]
Lentinus edodes	shiitake mushroom	Marasmiaceae	fungus	Dichloromethane	microsomes	76.5	PCA at 20 μg/mL	[143]
Lycopersicon esculentum [a]	tomato	Solanaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Lycopersicon esculentum [a]	tomato (leaves)	Solanaceae	plant	70% ethanol	microsomes	6000	units/100 g	[113]
Morinda citrifolia	noni	Rubiaceae	plant	Nd	nd	inhibits		[131]
Murraya paniculata	mock orange	Rutaceae	plant	75% MeOH reflux	microsomes	~68	% inhib.	[105]
Musa sp.[a]	banana	Musaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Nicotiana tabacum [a]	cigarette smoke	Solanaceae	plant	aqueous trap	microsomes	0.25	cigarette equivalents	[113]
Nicotiana tabacum [a]	cigarette smokea	Solanaceae	plant	methylene chloride trap	microsomes	0.07	cigarette equivalents	[113]
Nicotiana tabacum [a]	tobacco (leaves)	Solanaceae	plant	70% ethanol	microsomes	0.025	cigarette equivalents	[113]
Nicotiana tabacum [a]	tobacco (leaves)	Solanaceae	plant	70% ethanol	microsomes	590	units/100 g	[113]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Opuntia sp.[a]	cactus flower	Cactaceae	plant	water (autoclaved) (dichloromethane-methanol partition)	microsomes	~20	PCA at 100 μL	[186]
Opuntia sp.[a]	cactus flower	Cactaceae	plant	water (autoclaved) (diethyl ether subfraction)	microsomes	~17	PCA at 100 μL	[186]
Opuntia sp.[a]	cactus flower	Cactaceae	plant	water (autoclaved) (petroleum ether-diethyl ether subfraction)	microsomes	~10	PCA at 100 μL	[186]
Persea americana [a]	avocado (meat)	Lauraceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Persea americana [a]	beet (greens)	Amaranthaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Petroselinum crispum [a]	parsley	Apiaceae	plant	70% ethanol	microsomes	1200	units/100 g	[113]
Piper cubeba	none	Piperaceae	plant	96% ethanol	enzyme	<10	μg/mL IC_{50}	[187]
Pleurotus ostreatus	oyster mushroom	Tricholomataceae	fungus	water reflux	microsomes	~94	PCA at 100 μL	[115]
Pleurotus sp.	Italian brown mushroom	Tricholomataceae	fungus	water reflux	microsomes	~36	PCA at 100 μL	[115]
Plumbago capensis	leadwort	Plumbaginaceae	plant	75% MeOH reflux	microsomes	~8	% inhib.	[105]
Prunus persica [a]	peach (juice)	Rosaceae	plant	Nd	microsomes	~89	PCA at 25 μL	[183]
Prunus persica [a]	peach (juice)	Rosaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Prunus sp.[a]	plum (juice)	Rosaceae	plant	Nd	microsomes	~70	PCA at 25 μL	[183]
Prunus sp.[a]	plum	Rosaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Pternandra azurea	none	Melastomataceae	plant	methanol ($CHCl_3$ partition)	microsomes	70.1	PCA at 20 μg/mL	[143]
Punica granatum	pomegranate	Punicaceae	plant	fermented juice	microsomes	51	% inhib.	[188]
Punica granatum	pomegranate	Punicaceae	plant	pericarp polyphenols	microsomes	24	% inhib.	[188]
Pyrus malus [a]	apple (juice)	Rosaceae	plant	Nd	microsomes	~79	PCA at 25 μL	[183]
Pyrus malus [a]	apple	Rosaceae	plant	70% ethanol	microsomes	<80	units/100 g	[113]
Renealmia sp.	none	Bromeliaceae	plant	75% MeOH reflux	microsomes	~18	% inhib.	[105]
Riedelia sp.	none	Ericaceae	plant	75% MeOH reflux	microsomes	~97	% inhib.	[105]
Rubus occidentalis	black raspberry	Rosaceae	plant	none (dried fruit)	microsomes	80.8	PCA at 20 μg/mL	[143]

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Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Salix sp.[a]	willow bark	Salicaceae	plant	nd	nd	>50	% inhib.	[182]
Scutellaria barbata	skullcap	Lamiaceae	plant	water reflux	microsomes	23	μg/mL IC_{50}	[111]
Scutellaria barbata	skullcap	Lamiaceae	plant	water reflux	myometrial cells	15.00	μg/mL IC_{50}	[111]
Scutellaria barbata	skullcap	Lamiaceae	plant	water reflux	leiomyonal cells	1.01	μg/mL IC_{50}	[111]
Solanum melongena [a]	eggplant	Solanaceae	plant	70% ethanol	microsomes	190	units/100 g	[113]
Solanum melongena [a]	eggplant (leaves)	Solanaceae	plant	70% ethanol	microsomes	800	units/100 g	[113]
Solanum tuberosum [a]	potato	Solanaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Solanum tuberosum [a]	potato (leaves)	Solanaceae	plant	70% ethanol	microsomes	4500	units/100 g	[113]
Spinacia oleracea [a]	spinach	Amaranthaceae	plant	water reflux	microsomes	~83	PCA at 100 μL	[115]
Spinacia oleracea [a]	spinach	Amaranthaceae	plant	70% ethanol	microsomes	2400	units/100 g	[113]
Taraxacum officinale [a]	dandelion (greens)	Asteraceae	plant	70% ethanol	microsomes	2900	units/100 g	[113]
Theobroma cacao [a]	chocolate	Sterculiaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Theobroma cacao [a]	cocoa	Sterculiaceae	plant	70% ethanol	microsomes	9000	units/100 g	[113]
Trifolium pratense	red clover (flowers)	Fabaceae	plant	Nd	MCF-7 dual assay for AI and estrogenicity	inhibits	aromatase	[116]
Uncaria tomentosa [a]	cat's claw	Rubiaceae	plant	Nd	nd	>50	% inhib.	[182]
Vallaris sp.	none	Apocynaceae	plant	75% MeOH reflux	microsomes	~20	% inhib.	[105]
Viscum album	mistletoe	Viscaceae	plant	75% MeOH reflux	microsomes	~94	% inhib.	[105]
Vitis sp.[a]	black grape (juice)	Vitaceae	plant	Nd	microsomes	~23	PCA at 25 μL	[189]
Vitis sp.[a]	Cabernet Sauvignon, Glen Ellen Proprietor's Reserve (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	7.7	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Cabernet Sauvignon, San Andrés (Lontué Valley, Chile)	Vitaceae	plant	Nd	microsomes	0.36	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Cabernet Sauvignon, Tanglewood (France)	Vitaceae	plant	Nd	microsomes	0.29	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Champagne grape (juice)	Vitaceae	plant	Nd	microsomes	~90	PCA at 25 μL	[189]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Vitis sp.[a]	Chardonnay, Santa Rita Reserve (Casablanca Valley, Chile)	Vitaceae	plant	Nd	microsomes	80	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Chardonnay, Woodbridge (Woodbridge, CA)	Vitaceae	plant	Nd	microsomes	99.1	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Christmas rose grape (juice)	Vitaceae	plant	Nd	microsomes	~40	PCA at 25 μL	[189]
Vitis sp.[a]	Christmas rose grape (seed)	Vitaceae	plant	Nd	microsomes	~10	PCA at 25 μL	[189]
Vitis sp.[a]	Fumé Blanc, Domaine Napa (Napa Valley, CA)	Vitaceae	plant	Nd	microsomes	112.5	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	grape (seed)	Vitaceae	plant	Water	MCF-7aro cells	70.4	% inhib. at 40 μg/mL	[85]
Vitis sp.[a]	grape (seed)	Vitaceae	plant	Water	*in vivo* MCF-7aro xenograft	reduced	tumor weight	[85]
Vitis sp.[a]	grape (seed)	Vitaceae	plant	Water	MCF-7aro cells	80.5	% inhib. at 60 μg/mL	[85]
Vitis sp.[a]	grape (seed)	Vitaceae	plant	Water	*in vivo* MCF-7aro xenograft	reduced	tumor weight	[85]
Vitis sp.[a]	green seedless grape (juice)	Vitaceae	plant	Nd	microsomes	~38	PCA at 25 μL	[189]
Vitis sp.[a]	Merlot, Forest Ville (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	0.46	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Merlot, Hacienda, 1997 (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	3.29	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Merlot, Hacienda, 1998 (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	0.9	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Merlot, JW Morris Winery (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	0.42	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Pinot Noir, Cambiaso (Healdburg, CA)	Vitaceae	plant	Nd	microsomes	0.34	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Pinot Noir, Hacienda (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	2.16	PCA at 50 μL	[86, 106, 107]

Species Name	Common Name	Family	Type	Extraction Solvent	Assay Type	Activity		Ref.(s)
Vitis sp.[a]	Pinot Noir, Hacienda (Sonoma, CA)	Vitaceae	plant	Nd	microsomes	~8	PCA at 25 μL	[86, 106, 107]
Vitis sp.[a]	Pinot Noir, Hacienda (Sonoma, CA)	Vitaceae	plant	Nd	*in vivo* mouse	inhibits		[86, 106, 107]
Vitis sp.[a]	red globe grape (juice)	Vitaceae	plant	Nd	microsomes	~78	PCA at 25 μL	[189]
Vitis sp.[a]	red seedless grape (juice)	Vitaceae	plant	Nd	microsomes	~29	PCA at 25 μL	[183]
Vitis sp.[a]	red seedless grape (juice)	Vitaceae	plant	Nd	MCF-7aro cells	inhibits	aromatase	[183]
Vitis sp.[a]	red seedless grape (juice)	Vitaceae	plant	Nd	*in vivo* MCF-7aro xenograft	70	% reduced tumor size	[183]
Vitis sp.[a]	red seedless grape (juice)	Vitaceae	plant	Nd	microsomes	~30	PCA at 25 μL	[189]
Vitis sp.[a]	Sauvignon Blanc, Turning Leaf (Modesto, CA)	Vitaceae	plant	Nd	microsomes	106.5	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	seedless grape	Vitaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
Vitis sp.[a]	Zinfandel, Black Mountain (San Diego, CA)	Vitaceae	plant	Nd	microsomes	0.39	PCA at 50 μL	[86, 106, 107]
Vitis sp.[a]	Zinfandel, Sequoia Ridge (Graton, CA)	Vitaceae	plant	Nd	microsomes	0.39	PCA at 50 μL	[86, 106, 107]
Vitis sp.	grape	Vitaceae	plant	none (dried fruit)	microsomes	75.7	PCA at 20 μg/mL	[143]
Zingiber officinale [a]	ginger (root)	Zingerberaceae	plant	70% ethanol	microsomes	0	units/100 g	[113]
none	propolis	none	misc.	Nd	nd	>50	%inhib.	[182]

nd = no data

[a]Genus and species not provided by author.

Table 2

Previous literature reports of natural product flavones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
apigenin (**8**)	microsomes	1.2	μM IC_{50}	[122]
apigenin (**8**)	microsomes	2.9	μM IC_{50}	[123]
apigenin (**8**)	microsomes	4.2	μM IC_{50}	[190]
apigenin (**8**)	microsomes	10	μM IC_{50}	[177]
apigenin (**8**)	microsomes	15	μM IC_{50}	[136]
apigenin (**8**)	microsomes	0.9	μg/mL IC_{50}	[121]
apigenin (**8**)	microsomes (modified)	2.9	μM IC_{50}	[124]
apigenin (**8**)	spectrophotometric w/microsomes	0.9	K_s	[120]
apigenin (**8**)	trout ovarian aromatase	84.0	μM IC_{50}	[128]
apigenin (**8**)	JEG-3 cells	0.18	μM IC_{50}	[125]
apigenin (**8**)	Arom+HEK 293 cells	1.4	μM IC_{50}	[125]
apigenin (**8**)	H295R adrenocortical carcinoma cells	20	μM IC_{50}	[127]
apigenin (**8**)	granulose-luteal cells	inhibited	at 10 μmol/L for 24 h	[129]
ayanin (**9**)	microsomes	69.6	PCA at 20 μg/mL	[143]
broussoflavonol F (**10**)	microsomes	7.3	PCA at 20 μg/mL	[143]
broussoflavonol F (**10**)	microsomes	9.7	μM IC_{50}	[135]
broussoflavonol F (**10**)	SK-BR-3 cells	28.4	PCA at 50 μM	[143]
chrysin (**11**)	microsomes	0.5	μM IC_{50}	[122]
chrysin (**11**)	microsomes	0.7	μM IC_{50}	[123]
chrysin (**11**)	microsomes	1.1	μM IC_{50}	[191]
chrysin (**11**)	microsomes	8.9	μM IC_{50}	[136]
chrysin (**11**)	microsomes	1.1	μg/mL IC_{50}	[121]
chrysin (**11**)	microsomes	1	K_i	[118]
chrysin (**11**)	microsomes	2.6	K_i	[119]
chrysin (**11**)	microsomes (modified)	0.7	μM IC_{50}	[124]
chrysin (**11**)	spectrophotometric w/microsomes	0.5	K_s	[120]
chrysin (**11**)	trout ovarian aromatase	>1004	μM IC_{50}	[128]
chrysin (**11**)	JEG-3 cells	0.5	μM IC_{50}	[125]
chrysin (**11**)	Arom+HEK 293 cells	0.6	μM IC_{50}	[125]
chrysin (**11**)	human preadipocyte cells	4.6	μM IC_{50}	[126]
chrysin (**11**)	H295R adrenocortical carcinoma cells	7	μM IC_{50}	[127]
chrysin (**11**)	MCF-7 dual assay for AI and estrogenicity	inhibits		[116]
chrysin (**11**)	endometrial stromal cells	none		[118]
chrysin (**11**)	nd	11	μM IC_{50}	[192]
3',4'-dihydroxyflavone (**12**)	microsomes	90	μM IC_{50}	[132]

Compound Name	Assay Type	Activity		Ref.(s)
3',4'-dihydroxyflavone (**12**)	microsomes	100	μM IC_{50}	[136]
3',4'-dihydroxyflavone (**12**)	microsomes	>200	μM IC_{50}	[132]
5,4'-dihydroxyflavone (**13**)	microsomes	120	μM IC_{50}	[132]
6,4'-dihydroxyflavone (**14**)	microsomes	90	μM IC_{50}	[132]
7,4'-dihydroxyflavone (**15**)	microsomes	2	μM IC_{50}	[132]
7,4'-dihydroxyflavone (**15**)	trout ovarian aromatase	200.0	μM IC_{50}	[128]
7,8-dihydroxyflavone (**16**)	microsomes	8	μM IC_{50}	[123]
7,8-dihydroxyflavone (**16**)	microsomes	2.2	μg/mL IC_{50}	[121]
7,8-dihydroxyflavone (**16**)	microsomes	10	K_i	[119]
7,8-dihydroxyflavone (**16**)	nd	55	μM IC_{50}	[192]
3',4'-dimethoxyflavone (**17**)	microsomes	42	μM IC_{50}	[136]
fisetin (**18**)	microsomes	8.5	μg/mL IC_{50}	[121]
fisetin (**18**)	JEG-3 cells	55	μM IC_{50}	[125]
flavone (**19**)	microsomes	8	μM IC_{50}	[122]
flavone (**19**)	microsomes	10	μM IC_{50}	[132]
flavone (**19**)	microsomes	48	μM IC_{50}	[123]
flavone (**19**)	microsomes	67	μM IC_{50}	[136]
flavone (**19**)	microsomes	375.0	μM IC_{50}	[128]
flavone (**19**)	microsomes (modified)	48.0	μM IC_{50}	[124]
flavone (**19**)	trout ovarian aromatase	731.0	μM IC_{50}	[128]
flavone (**19**)	human preadipocyte cells	68	μM IC_{50}	[126]
flavone (**19**)	JEG-3 cells	>100	μM IC_{50}	[125]
flavone (**19**)	H295R adrenocortical carcinoma cells	none		[127]
galangin (**20**)	microsomes	95	K_i	[119]
galangin (**20**)	JEG-3 cells	12	μM IC_{50}	[125]
3-hydroxyflavone (**21**)	microsomes	140	μM IC_{50}	[132]
3'-hydroxyflavone (**22**)	microsomes	73	μM IC_{50}	[136]
4'-hydroxyflavone (**23**)	microsomes	180	μM IC_{50}	[132]
5-hydroxyflavone (**24**)	microsomes	100	μM IC_{50}	[132]
6-hydroxyflavone (**25**)	microsomes	80	μM IC_{50}	[132]
6-hydroxyflavone (**25**)	JEG-3 cells	5.5	μM IC_{50}	[125]
7-hydroxyflavone (**26**)	microsomes	0.2	μM IC_{50}	[123]
7-hydroxyflavone (**26**)	microsomes	0.5	μM IC_{50}	[132]
7-hydroxyflavone (**26**)	microsomes	8.2	μM IC_{50}	[136]
7-hydroxyflavone (**26**)	microsomes	30.5	μg/mL IC_{50}	[121]
7-hydroxyflavone (**26**)	microsomes (modified)	0.21	μM IC_{50}	[124]
7-hydroxyflavone (**26**)	trout ovarian aromatase	>1001	μM IC_{50}	[128]

Compound Name	Assay Type	Activity		Ref.(s)
7-hydroxyflavone (**26**)	JEG-3 cells	0.35	μM IC_{50}	[125]
7-hydroxyflavone (**26**)	H295R adrenocortical carcinoma cells	4	μM IC_{50}	[127]
isolicoflavonol (**27**)	microsomes	0.1	μM IC_{50}	[135]
kaempferide (**28**)	JEG-3 cells	80	μM IC_{50}	[125]
kaempferol (**29**)	microsomes	32	% inhib. at 50 μM	[130]
kaempferol (**29**)	JEG-3 cells	11	μM IC_{50}	[125]
kaempferol (**29**)	preadipose cells	61	μM IC_{50}	[134]
kaempferol 7,4'-dimethyl ether (**30**)	microsomes	45.6	PCA at 20 μg/mL	[143]
kaempferol 7,4'-dimethyl ether (**30**)	SK-BR-3 cells	99.2	PCA at 50 μM	[143]
luteolin (**31**)	microsomes	8.6	μM IC_{50}	[136]
luteolin (**31**)	microsomes	3.3	μg/mL IC_{50}	[121]
luteolin (**31**)	microsomes (modified)	1.2	μM IC_{50}	[133]
luteolin (**31**)	spectrophotometric w/microsomes	1.0	K_s	[120]
luteolin (**31**)	JEG-3 cells	2	μM IC_{50}	[125]
luteolin (**31**)	preadipose cells	25	μM IC_{50}	[134]
7-methoxyflavone (**32**)	microsomes	3.2	μM IC_{50}	[123]
7-methoxyflavone (**32**)	microsomes (modified)	3.2	μM IC_{50}	[124]
7-methoxyflavone (**32**)	H295R adrenocortical carcinoma cells	none		[127]
morin (**33**)	spectrophotometric w/microsomes	5.0	K_s	[120]
myricetin (**34**)	microsomes	5.6	μg/mL IC_{50}	[121]
myricetin (**34**)	microsomes	41	% inhib. at 50 μM	[130]
myricetin (**34**)	spectrophotometric w/microsomes	5.6	K_s	[120]
oxyayanin B (**35**)	microsomes	83.0	PCA at 20 μg/mL	[143]
prunetin (**36**)	microsomes	none	μM IC_{50}	[123]
prunetin (**36**)	microsomes	7.8	μg/mL IC_{50}	[121]
quercetin (**37**)	microsomes	12	μM IC_{50}	[122]
quercetin (**37**)	microsomes	35	% inhib. at 50 μM	[130]
quercetin (**37**)	spectrophotometric w/microsomes	4.7	K_s	[120]
quercetin (**37**)	trout ovarian aromatase	139.0	μM IC_{50}	[128]
quercetin (**37**)	JEG-3 cells	>100	μM IC_{50}	[125]
quercetin (**37**)	H295R adrenocortical carcinoma cells	none		[127]
quercetin (**37**)	human preadipocyte cells	none		[126]
quercetin (**37**)	granulose-luteal cells	none	at 10 μmol/L for 24h	[129]
quercetin (**37**)	nd	~85	% inhib. at 100 μM	[107]
quercetin (**37**)	nd	nd		[131]
robinetin (**38**)	microsomes	45.7	μg/mL IC_{50}	[121]
rutin (**39**)	human preadipocyte cells	none		[126]
rutin (**39**)	nd	~120	% inhib. at 100 μM	[107]

Compound Name	Assay Type	Activity		Ref.(s)
7,3',4',5'-tetrahydroxyflavone (**40**)	microsomes	45	μM IC_{50}	[136]
5,7,2',4'-tetrahydroxy-3-geranylflavone (**41**)	microsomes	24.0	μM IC_{50}	[135]
7,3',4'-trihydroxyflavone (**42**)	microsomes	38	μM IC_{50}	[136]
5,7,3'-trihydroxy-4'-methoxyflavone (**43**)	microsomes	27	μM IC_{50}	[136]
5,7,4'-trihydroxy-3'-methoxyflavone (**44**)	microsomes	7.2	μM IC_{50}	[136]

nd = no data

Table 3

Previous literature reports of natural product flavanones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
(2*S*)-abyssinone II (**45**)	microsomes	0.4	μM IC_{50}	[135]
3',4'-dihydroxyflavanone[a] (**46**)	microsomes	160	μM IC_{50}	[132]
5,7-dihydroxyflavanone[a] (**47**)	microsomes	10	μM IC_{50}	[136]
7,8-dihydroxyflavanone[a] (**48**)	microsomes (modified)	8.0	μM IC_{50}	[124]
(2*S*)-2',4'-dihydroxy-2”-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**49**)	microsomes	0.1	μM IC_{50}	[135]
eriodictyol[a] (**50**)	microsomes	5.3	μM IC_{50}	[136]
eriodictyol[a] (**50**)	microsomes (modified)	0.6	μM IC_{50}	[133]
(2*S*)-euchrenone a7 (**51**)	microsomes	3.4	μM IC_{50}	[135]
flavanone[a] (**52**)	microsomes	8	μM IC_{50}	[122]
flavanone[a] (**52**)	microsomes	8	μM IC_{50}	[132]
flavanone[a] (**52**)	microsomes	28.5	μM IC_{50}	[137]
flavanone[a] (**52**)	microsomes	32	μM IC_{50}	[136]
flavanone[a] (**52**)	microsomes	250.0	μM IC_{50}	[128]
flavanone[a] (**52**)	microsomes	8.7	μg/mL IC_{50}	[121]
flavanone[a] (**52**)	microsomes (modified)	13.8	μM IC_{50}	[133]
flavanone[a] (**52**)	trout ovarian aromatase	>1000	μM IC_{50}	[128]
hesperetin[a] (**53**)	microsomes	1.0	μg/mL IC_{50}	[121]
hesperetin[a] (**53**)	microsomes (modified)	3.3	μM IC_{50}	[133]
hesperidin[a] (**54**)	microsomes	40.9	μg/mL IC_{50}	[121]
4'-hydroxyflavanone[a] (**55**)	microsomes	10	μM IC_{50}	[132]
7-hydroxyflavanone[a] (**56**)	microsomes	3.8	μM IC_{50}	[138]
7-hydroxyflavanone[a] (**56**)	microsomes	10	μM IC_{50}	[136]
7-hydroxyflavanone[a] (**56**)	microsomes (modified)	2.4	μM IC_{50}	[133]
7-hydroxyflavanone[a] (**56**)	H295R adrenocortical carcinoma cells	65	μM IC_{50}	[127]
isoxanthohumol[a] (**57**)	choriocarcinoma-derived JAR cells	139.7	μM IC_{50}	[114]
isoxanthohumol[a] (**57**)	SK-BR-3 cells	25.4	μM IC_{50}	[139]
7-methoxyflavanone[a] (**58**)	microsomes	8.0	μM IC_{50}	[137]
7-methoxyflavanone[a] (**58**)	microsomes (modified)	2.6	μM IC_{50}	[124]
7-methoxyflavanone[a] (**58**)	H295R adrenocortical carcinoma cells	none		[127]
naringenin (**59**[a])	microsomes	2.9	μM IC_{50}	[191]
naringenin (**59**[a])	microsomes	9.2	μM IC_{50}	[123]

Compound Name	Assay Type	Activity		Ref.(s)
(2*S*)-naringenin (**59**)	microsomes	17.0	μM IC_{50}	[135]
naringenin (**59**[a])	microsomes	0.3	K_i	[118]
naringenin (**59**[a])	microsomes	5.1	K_i	[119]
naringenin (**59**[a])	microsomes (modified)	9.2	μM IC_{50}	[124]
naringenin (**59**[a])	JEG-3 cells	1.4	μM IC_{50}	[125]
naringenin (**59**[a])	Arom+HEK 293 cells	3.2	μM IC_{50}	[125]
naringenin (**59**[a])	H295R adrenocortical carcinoma cells	85	μM IC_{50}	[127]
naringenin (**59**[a])	MCF-7 dual assay for AI and estrogenicity	inhibits		[116]
naringenin (**59**[a])	rat granulose cells	inhibits		[184]
naringenin (**59**[a])	endometrial stromal cells	none		[118]
naringin (**60**)	microsomes	1.8	μg/mL IC_{50}	[121]
pinostrobin[a] (**61**)	JEG-3 cells	4	μM IC_{50}	[125]
8-prenylnaringenin[a] (**62**)	microsomes	0.2	μM IC_{50}	[191]
8-prenylnaringenin[a] (**62**)	choriocarcinoma-derived JAR cells	0.065	μM IC_{50}	[114]
8-prenylnaringenin[a] (**62**)	SK-BR-3 cells	0.08	μM IC_{50}	[139]
8-prenylnaringenin[a] (**62**)	breast adipose fibroblast cells	0.3	μM IC_{50}	[191]
(2*S*)-5,7,2',4'-tetrahydroxyflavanone (**63**)	microsomes	2.2	μM IC_{50}	[135]
5,7,4'-trihydroxy-3'-methoxyflavanone (**64**)	microsomes	25	μM IC_{50}	[136]

[a]Optical sign not provided by authors.

Table 4

Previous literature reports of natural product chalcones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
butein (**65**)	MCF-7aro cells	3.70	μM IC_{50}	[140]
4,2'-dihydroxychalcone (**66**)	microsomes (modified)	>50	μM IC_{50}	[133]
2',4'-dihydroxychalcone (**67**)	microsomes (modified)	>50	μM IC_{50}	[133]
eriodictyol chalcone (**68**)	microsomes (modified)	2.8	μM IC_{50}	[133]
hesperetin chalcone (**69**)	microsomes (modified)	24.2	μM IC_{50}	[133]
2-hydroxychalcone (**70**)	MCF-7aro cells	~45	PCA at 20 μM	[140]
2'-hydroxychalcone (**71**)	microsomes (modified)	>50	μM IC_{50}	[133]
2'-hydroxychalcone (**71**)	MCF-7aro cells	~30	PCA at 20 μM	[140]
4-hydroxychalcone (**72**)	microsomes (modified)	>50	μM IC_{50}	[133]
4-hydroxychalcone (**72**)	MCF-7aro cells	~60	PCA at 20 μM	[140]
4'-hydroxychalcone (**73**)	microsomes (modified)	30.6	μM IC_{50}	[133]
2-hydroxy-4-methoxychalcone (**74**)	microsomes (modified)	>50	μM IC_{50}	[133]
3'-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2',4'-tetrahydroxychalcone 11'-*O*-coumarate (**75**)	microsomes	0.5	μM IC_{50}	[135]
isogemichalcone C (**76**)	microsomes	7.1	μM IC_{50}	[135]
isoliquiritigenin (**77**)	microsomes	30.6	PCA at 20 μg/mL	[143]
isoliquiritigenin (**77**)	microsomes (modified)	34.6	μM IC_{50}	[133]
isoliquiritigenin (**77**)	SK-BR-3 cells	9.3	PCA at 50 μM	[143]
isoliquiritigenin (**77**)	MCF-7aro cells	~60	PCA at 20 μM	[140]
naringenin chalcone (**78**)	microsomes (modified)	2.6	μM IC_{50}	[133]
paratocarpin B (**79**)	microsomes	58.1	PCA at 20 μg/mL	[143]
phloretin (**80**)	microsomes (modified)	>50	μM IC_{50}	[133]
pinostrobin chalcone (**81**)	microsomes (modified)	14.3	μM IC_{50}	[133]
2,4,2',4'-tetrahydroxy-3'-prenylchalcone (**82**)	microsomes	3.3	PCA at 20 μg/mL	[143]
2,4,2',4'-tetrahydroxy-3'-prenylchalcone (**82**)	microsomes	4.6	μM IC_{50}	[135]
2,4,2',4'-tetrahydroxy-3'-prenylchalcone (**82**)	SK-BR-3 cells	10.6	PCA at 50 μM	[143]
xanthohumol (**83**)	SK-BR-3 cells	3.2	μM IC_{50}	[139]
xanthohumol (**83**)	choriocarcinoma-derived JAR cells	20.3	μM IC_{50}	[114]

Table 5

Previous literature reports of natural product isoflavans tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
equol (**84**)	microsomes	150	μM IC_{50}	[145]
equol (**84**)	microsomes	850.0	μM IC_{50}	[128]
equol (**84**)	trout ovarian aromatase	793.0	μM IC_{50}	[128]
equol (**84**)	human preadipocyte cells	none		[126]
heminitidulan (**85**)	microsomes	45.1	PCA at 20 μg/mL	[143]
3'-hydroxy-4'-*O*-methylglabridin (**86**)	microsomes	70.0	PCA at 20 μg/mL	[143]
leiocin (**87**)	microsomes	28.6	PCA at 20 μg/mL	[143]
leiocin (**87**)	SK-BR-3 cells	85.5	PCA at 50 μM	[143]
leiocinol (**88**)	microsomes	36.9	PCA at 20 μg/mL	[143]
leiocinol (**88**)	SK-BR-3 cells	101.8	PCA at 50 μM	[143]
methylequol (**89**)	microsomes	20	μM IC_{50}	[145]
4'-*O*-methylglabridin (**90**)	microsomes	25.2	PCA at 20 μg/mL	[143]
4'-*O*-methylglabridin (**90**)	SK-BR-3 cells	71.2	PCA at 50 μM	[143]
nitidulan (**91**)	microsomes	47.1	PCA at 20 μg/mL	[143]
nitidulan (**91**)	SK-BR-3 cells	59.1	PCA at 50 μM	[143]
nitidulin (**92**)	microsomes	71.2	PCA at 20 μg/mL	[143]
sativan (**93**)	microsomes	>50	μM IC_{50}	[123]

Table 6

Previous literature reports of natural product catechins tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
(+)-catechin (**94**)	microsomes	100.0	PCA at 20 μg/mL	[143]
(+)-catechin (**94**)	microsomes	none		[112]
(+)-catechin (**94**)	H295R adrenocortical carcinoma cells	none		[127]
catechin (**94**[a])	human preadipocyte cells	none		[126]
(−)-catechin gallate (**95**)	microsomes	55	μM IC_{50}	[112]
(−)-epicatechin (**96**)	microsomes	94.9	PCA at 20 μg/mL	[143]
(−)-epicatechin (**96**)	microsomes	none		[112]
(−)-epicatechin (**96**)	H295R adrenocortical carcinoma cells	none		[127]
(−)-epicatechin-3-*O*-gallate (**97**)	microsomes	67.1	PCA at 20 μg/mL	[143]
(−)-epicatechin gallate (**97**)	microsomes	20	% inhib. at 100 μM	[112]
epicatechin gallate (**97**[a])	*in vivo* Swiss-Webster mice ovarian aromatase activity	none		[148]
(−)-epigallocatechin (**98**)	microsomes	75.3	PCA at 20 μg/mL	[143]
(−)-epigallocatechin (**98**)	microsomes	100	μM IC_{50}	[112]
(−)-epigallocatechin-3-*O*-gallate (**99**)	microsomes	54.9	PCA at 20 μg/mL	[143]
epigallocatechin gallate (**99**[a])	microsomes	13.79	μM IC_{50}	[146]
(−)-epigallocatechin gallate (**99**)	microsomes	60	μM IC_{50}	[112]
epigallocatechin gallate (**99**[a])	*in vivo* Swiss-Webster mice ovarian aromatase activity	56	% inhib. at 25 μg/kg	[148]
epigallocatechin gallate (**99**[a])	epidemiological E_2 levels	lower	E_2 levels with higher EGCG intake	[147]
(−)-gallocatchin gallate (**100**)	microsomes	15	μM IC_{50}	[112]
theaflavin (**101**)	microsomes	4.17	μM IC_{50}	[146]
theaflavin-3,3'-digallate (**102**)	microsomes	3.45	μM IC_{50}	[146]

[a]Optical sign not provided by authors.

Table 7

Previous literature reports of natural product isoflavanones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
3',4'-dihydroxyisoflavanone (**103**)	microsomes	>200	μM IC_{50}	[132]
discoloranone A (**104**)	microsomes	85.8	PCA at 20 μg/mL	[143]
discoloranone B (**105**)	microsomes	53.5	PCA at 20 μg/mL	[143]
2-hydroxyisoflavanone (**106**)	microsomes	170	μM IC_{50}	[132]
4'-hydroxyisoflavanone (**107**)	microsomes	160	μM IC_{50}	[132]
isodiscoloranone A (**108**)	microsomes	91.5	PCA at 20 μg/mL	[143]
isodiscoloranone B (**109**)	microsomes	57.2	PCA at 20 μg/mL	[143]
isoflavanone (**110**)	microsomes	120	μM IC_{50}	[132]

Table 8

Previous literature reports of natural product isoflavones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
biochanin A (**111**)	microsomes	18.9	μg/mL IC_{50}	[121]
biochanin A (**111**)	microsomes	49	μM IC_{50}	[123]
biochanin A (**111**)	microsomes	94.50	μM IC_{50}	[149]
biochanin A (**111**)	microsomes	10.2	μg/mL IC_{50}	[121]
biochanin A (**111**)	microsomes	12	K_i	[119]
biochanin A (**111**)	trout ovarian aromatase	>1000	μM IC_{50}	[128]
biochanin A (**111**)	JEG-3 cells	4	μM IC_{50}	[125]
biochanin A (**111**)	human preadipocyte cells	113	μM IC_{50}	[126]
biochanin A (**111**)	granulosa-luteal cells	none	at 10 μmol/L for 24 h	[129]
biochanin A (**111**)	MCF-7 dual assay for AI and estrogenicity	inhibits		[116]
daidzein (**112**)	microsomes	none	μM IC_{50}	[123]
daidzein (**112**)	microsomes	>50	K_i	[118]
daidzein (**112**)	microsomes	none		[145]
daidzein (**112**)	trout ovarian aromatase	>1002	μM IC_{50}	[128]
daidzein (**112**)	endometrial stromal cells	none		[118]
daidzein (**112**)	human preadipocyte cells	none		[126]
formononetin (**113**)	microsomes	75.7	PCA at 20 μg/mL	[143]
formononetin (**113**)	microsomes	none	μM IC_{50}	[123]
formononetin (**113**)	MCF-7 dual assay for AI and estrogenicity	inhibits		[116]
genistein (**114**)	microsomes	none	μM IC_{50}	[123]
genistein (**114**)	microsomes	>50	K_i	[118]
genistein (**114**)	microsomes	123	K_i	[119]
genistein (**114**)	microsomes	none		[149]
genistein (**114**)	microsomes (modified)	none	μM IC_{50}	[124]
genistein (**114**)	trout ovarian aromatase	>1003	μM IC_{50}	[128]
genistein (**114**)	endometrial stromal cells	none		[118]
genistein (**114**)	MCF-7 dual assay for AI and estrogenicity	none		[116]
genistein (**114**)	H295R adrenocortical carcinoma cells	none		[127]
genistein (**114**)	human preadipocyte cells	none		[126]
isoflavone (**115**)	microsomes	>200	μM IC_{50}	[132]
7,3',4' -trihydroxyisoflavone (**116**)	microsomes	none	μM IC_{50}	[123]

Table 9

Previous literature reports of natural product flavonoids (not previously mentioned) tested for aromatase inhibition (listed alphabetically by compound class)

Compound Name	Compound Class	Assay Type	Activity		Ref.(s)
cyanidin (**117**)	anthocyanin	microsome	72	μM IC_{50}	[136]
malvidin-3-*O*-glucoside (**118**)	anthocyanin	microsome	299	μM IC_{50}	[136]
coumestrol (**119**)	coumestan	microsomes	25	μM IC_{50}	[123]
coumestrol (**119**)	coumestan	microsomes (modified)	50.6	% inhib. at 50 μM	[154]
coumestrol (**119**)	coumestan	microsomes (modified)	35.0	μM IC_{50}	[124]
coumestrol (**119**)	coumestan	trout ovarian aromatase	>1000	μM IC_{50}	[128]
coumestrol (**119**)	coumestan	preadipose cells	17	μM IC_{50}	[134]
flavan-4-ol (**120**)	flavanol	microsomes	120	μM IC_{50}	[132]
4'-hydroxyflavan-4-ol (**121**)	flavanol	microsomes	>200	μM IC_{50}	[132]
bonducellin (**122**)	homoisoflavonoid	microsomes	65.0	PCA at 20 μg/mL	[143]
isobonducellin (**123**)	homoisoflavonoid	microsomes	41.0	PCA at 20 μg/mL	[143]
isobonducellin (**123**)	homoisoflavonoid	SK-BR-3 cells	58.4	PCA at 50 μM	[143]
4'-dehydroxycabenegrin A (**124**)	pterocarpan	microsomes	50.9	PCA at 20 μg/mL	[143]
(−)-hemileiocarpin (**125**)	pterocarpan	microsomes	69.8	PCA at 20 μg/mL	[143]
2-hydroxyleiocarpin (**126**)	pterocarpan	microsomes	73.3	PCA at 20 μg/mL	[143]
leiocarpin (**127**)	pterocarpan	microsomes	83.9	PCA at 20 μg/mL	[143]
medicarpin (**128**)	pterocarpan	microsomes	>50	μM IC_{50}	[123]
amorphigenin (**129**)	rotenoid	microsomes	83.7	PCA at 20 μg/mL	[143]
amorphigenin glucoside (**130**)	rotenoid	microsomes	83.0	PCA at 20 μg/mL	[143]
dalbinol (**131**)	rotenoid	microsomes	86.5	PCA at 20 μg/mL	[143]
rotenone (**132**)	rotenoid	H295R adrenocortical carcinoma cells	0.30	μM IC_{50}	[127]

Table 10

Previous literature reports of alkaloids tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
anabasine (**133**)	microsomes	6600	μM IC_{50}	[113]
berberine (**134**)	microsomes	87.5	PCA at 20 μg/mL	[143]
cotinine (**135**)	microsomes	none		[113]
β-hydrastine (**136**)	microsomes	95.6	PCA at 20 μg/mL	[143]
N-(4-hydroxy-undecanoyl)anabasine (**137**)	microsomes	30	μM IC_{50}	[150]
nicotine (**138**)	microsomes	4	cigarette equiv.	[113]
nicotine (**138**)	microsomes	26000	μM IC_{50}	[113]
N-*n*-octanoylnornicotine (**139**)	microsomes	360	μM IC_{50}	[113]
N-*n*-octanoylnornicotine (**139**)	microsomes	360	μM IC_{50}	[150]
8-oxotetrahydrothalifendine (**140**)	microsomes	96.0	PCA at 20 μg/mL	[143]
1-[1-oxo-5(8,9-methylenedioxyphenyl)-2*E*,4*Z*-pentadienyl]-piperidine (**141**)	microsomes	97.7	PCA at 20 μg/mL	[143]
piperine (**142**)	microsomes	100.6	PCA at 20 μg/mL	[143]

Table 11

Previous literature reports of natural product fatty acids tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
arachidonic acid (**143**)	microsomes	11.5	PCA at 20 μg/mL	[152]
arachidonic acid (**143**)	microsomes	28.2	μM IC_{50}	[152]
arachidonic acid (**143**)	SK-BR-3 cells	147.2	PCA at 100 μM	[152]
azelaic acid (**144**)	microsomes	none		[113]
docosahexaenoic acid (**145**)	microsomes	12.4	PCA at 20 μg/mL	[152]
docosahexaenoic acid (**145**)	microsomes	33.2	μM IC_{50}	[152]
docosahexaenoic acid (**145**)	SK-BR-3 cells	98.2	PCA at 100 μM	[152]
docosapentaenoic acid (**146**)	microsomes	15.7	PCA at 20 μg/mL	[152]
docosapentaenoic acid (**146**)	microsomes	16.8	μM IC_{50}	[152]
docosapentaenoic acid (**146**)	SK-BR-3 cells	94.4	PCA at 100 μM	[152]
eicosapentaenoic acid (**147**)	microsomes	30.2	PCA at 20 μg/mL	[152]
eicosapentaenoic acid (**147**)	microsomes	53.2	μM IC_{50}	[152]
eicosapentaenoic acid (**147**)	SK-BR-3 cells	137.6	PCA at 100 μM	[152]
(9*Z*,11*E*)-12-hydroxy-9,11-octadecadienoic acid (**148**)	microsomes	15.9	%inhib. at 313.0 μM	[155]
(10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid (**149**)	microsomes	84	% inhib.	[151]
linoleic acid (**150**)	microsomes	22.5	PCA at 20 μg/mL	[152]
linoleic acid (**150**)	microsomes	7.4	PCA at 20 μg/mL	[108]
linoleic acid (**150**)	microsomes	48.0	μM IC_{50}	[152]
linoleic acid (**150**)	SK-BR-3 cells	147.6	PCA at 100 μM	[152]
α-linolenic acid (**151**)	microsomes	49.5	PCA at 20 μg/mL	[152]
α-linolenic acid (**151**)	microsomes	44.2	μM IC_{50}	[152]
α-linolenic acid (**151**)	SK-BR-3 cells	92.8	PCA at 100 μM	[152]
myristic acid (**152**)	microsomes	66.7	PCA at 20 μg/mL	[152]
oleic acid (**153**)	microsomes	19.5	PCA at 20 μg/mL	[152]
oleic acid (**153**)	microsomes	32.7	μM IC_{50}	[152]
oleic acid (**153**)	SK-BR-3 cells	99.3	PCA at 100 μM	[152]
(10*E*,12*Z*)-9-oxo-10,12-octadecadienoic acid (**154**)	microsomes	95	% inhib.	[151]
palmitic acid (**155**)	microsomes	83.2	PCA at 20 μg/mL	[152]
pentadecanoic acid (**156**)	microsomes	76.2	PCA at 20 μg/mL	[152]
stearic acid (**157**)	microsomes	89.4	PCA at 20 μg/mL	[152]

Table 12

Previous literature reports of natural product lignans tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
erythro-austrobailignan-6 (**158**)	microsomes (modified)	0	% inhib. at 50 μM	[154]
threo-austrobailignan-5 (**159**)	microsomes (modified)	0	% inhib. at 50 μM	[154]
dehydrodesoxypodophyllotoxin (**160**)	microsomes	96.0	PCA at 20 μg/mL	[143]
dehydropodophyllotoxin (**161**)	microsomes	88.1	PCA at 20 μg/mL	[143]
3'-demethoxymatairesinol[a] (**162**)	microsomes	37	μM IC_{50}	[145]
meso-dihydroguaiaretic acid (**163**)	microsomes (modified)	15.1	% inhib. at 50 μM	[154]
4,4'-dihydroxyenterolactone (**164**)	microsomes	6	μM IC_{50}	[145]
4,4'-enterolactone (**165**)	microsomes	15	μM IC_{50}	[145]
enterodiol (**166**)	microsomes	30	μM IC_{50}	[145]
enterodiol (**166**)	Arom+HEK 293 cells	>10	μM IC_{50}	[153]
enterodiol (**166**)	preadipose cells	>100	μM IC_{50}	[134]
enterolactone (**167**)	Arom+HEK 293 cells	8.90	μM IC_{50}	[153]
enterolactone (**167**)	microsomes	14	μM IC_{50}	[145]
enterolactone (**167**)	preadipose cells	74	μM IC_{50}	[134]
epiaschantin (**168**)	microsomes	76.7	PCA at 20 μg/mL	[143]
(−)-hernolactone (**169**)	microsomes	73.5	PCA at 20 μg/mL	[143]
matairesinol[a] (**170**)	Arom+HEK 293 cells	>10	μM IC_{50}	[153]
nectandrin B (**171**)	microsomes (modified)	30	% inhib. at 50 μM	[154]
nordihydroguaiaretic acid[a] (**172**)	microsomes	11	μM IC_{50}	[145]
nordihydroguaiaretic acid[a] (**172**)	microsomes (modified)	42	% inhib. at 50 μM	[154]
nordihydroguaiaretic acid[a] (**172**)	nd	68.70	μM IC_{50}	[149]
secoisolariciresinol (**173**)	microsomes	10.9	% inhib. at 409.0 μM	[155]
secoisolariciresinol (**173**)	Arom+HEK 293 cells	>10	μM IC_{50}	[153]
(−)-syringaresinol (**174**)	microsomes	60.2	PCA at 20 μg/mL	[143]
(−)-yatein (**175**)	microsomes	74.2	PCA at 20 μg/mL	[143]

nd = no data

[a] Optical information not provided by author.

Table 13

Previous literature reports of natural product peptides tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
N-acetyl-L-phenylalaninyl-*N*-benzoyl-L-phenylalaninate (**176**)	microsomes	83.0	PCA at 20 μg/mL	[108]
N-acetyl-L-phenylalaninyl-*N*-benzoyl-L-phenylalaninate (**176**)	SK-BR-3 cells	114.1	PCA at 50 μM	[108]
N-benzoyl-L-phenylalanine methyl ester (**177**)	microsomes	94.3	PCA at 20 μg/mL	[108]
N-benzoyl-L-phenylalanine methyl ester (**177**)	SK-BR-3 cells	33.3	PCA at 50 μM	[108]
N-benzoyl-L-phenylalaninyl-*N*-benzoyl-L-phenylalaninate (**178**)	microsomes	94.2	PCA at 20 μg/mL	[108]
N-benzoyl-L-phenylalaninyl-*N*-benzoyl-L-phenylalaninate (**178**)	SK-BR-3 cells	121.8	PCA at 50 μM	[108]
SNA-60-367-2 (**179**)	microsomes	60	% inhib. at 100 μg/mL	[156]
SNA-60-367-2 (**179**)	microsomes	63	μM IC_{50}	[156]
SNA-60-367-4 (**180**)	microsomes	65	% inhib. at 100 μg/mL	[156]
SNA-60-367-5 (**181**)	microsomes	63	% inhib. at 100 μg/mL	[156]
SNA-60-367-6 (**182**)	microsomes	74	% inhib. at 100 μg/mL	[156]
SNA-60-367-8 (**183**)	microsomes	61	% inhib. at 100 μg/mL	[156]
SNA-60-367-9 (**184**)	microsomes	55	% inhib. at 100 μg/mL	[156]
SNA-60-367-10 (**185**)	microsomes	68	% inhib. at 100 μg/mL	[156]
SNA-60-367-10 (**185**)	microsomes	42	μM IC_{50}	[156]
SNA-60-367-11 (**186**)	microsomes	72	% inhib. at 100 μg/mL	[156]
SNA-60-367-12 (**187**)	microsomes	60	% inhib. at 100 μg/mL	[156]
SNA-60-367-13 (**188**)	microsomes	50	% inhib. at 100 μg/mL	[156]
SNA-60-367-13 (**188**)	microsomes	66	μM IC_{50}	[156]
SNA-60-367-14 (**189**)	microsomes	31	% inhib. at 100 μg/mL	[156]
SNA-60-367-17 (**190**)	microsomes	48	% inhib. at 100 μg/mL	[156]
SNA-60-367-18 (**191**)	microsomes	49	% inhib. at 100 μg/mL	[156]
SNA-60-367-19 (**192**)	microsomes	49	% inhib. at 100 μg/mL	[156]
SNA-60-367-21 (**193**)	microsomes	36	% inhib. at 100 μg/mL	[156]
SNA-60-367-23 (**194**)	microsomes	32	% inhib. at 100 μg/mL	[156]

Table 14

Previous literature reports of natural product terpenoids tested for aromatase inhibition (listed alphabetically by compound class)

Compound Name	Compound Class	Assay Type	Activity		Ref.(s)
trans-communic acid (**195**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
12*S*-hydroxylabda-8(17),13(15), 14-trien-19-oic acid (**196**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
12-hydroxy-6,7-*seco*-abieta-8,11,13-triene-6,7-dial (**197**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
inflexin (**198**)	diterpenoid	microsomes	9.2	μg/mL IC_{50}	[110]
labda-8(17),13-dien-12*R*,15-olid-19-oic acid (**199**)	diterpenoid	recombinant yeast microsomes	7.2	% inhib. at 1 μM	[157]
12-methoxyabieta-8,11,13-trien-11-ol (**200**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
13-oxo-15,16-dinorlabda-8(17),11*E*-dien-19-oic acid (**201**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
14-oxo-15-norlabda-8(17),12*E*-dien-19-oic acid (**202**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
standishinal (**203**)	diterpenoid	recombinant yeast microsomes	50.2	% inhib. at 1 μM	[157]
totarol (**204**)	diterpenoid	recombinant yeast microsomes	0	% inhib. at 1 μM	[157]
(−)-dehydrololiolide (**205**)	isoprenoid	microsomes	91.5	PCA at 20 μg/mL	[108]
(−)-dehydrololiolide (**205**)	isoprenoid	SK-BR-3 cells	21.8	PCA at 50 μM	[108]
4-[(1*E*)-3-hydroxy-1-butenyl]-3,5,5-trimethyl-(4*R*)-2-cyclohexen-1-one (**206**)	isoprenoid	microsomes	93.5	PCA at 20 μg/mL	[143]
4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3 *E*-buten-2-one (**207**)	isoprenoid	microsomes	62.3	PCA at 20 μg/mL	[143]
loliolide (**208**)	isoprenoid	microsomes	84.6	PCA at 20 μg/mL	[143]
menthol (**209**)	isoprenoid	microsomes	none		[113]
10-*epi*-8-deoxycumambrin B (**210**)	sesquiterpenoid	microsomes	7.0	μM IC_{50}	[161]
11βH,13-dihydro-10-*epi*-8-deoxycumambrin (**211**)	sesquiterpenoid	microsomes	2.0	μM IC_{50}	[161]
11βH,13-dihydro-10-*epi*-8-deoxycumambrin (**211**)	sesquiterpenoid	JEG-3 choriocarcino ma cells	10	μM IC_{50}	[161]
2β,3β-dihydroxy-5-preg-17(20)-(*E*)-en-16-one (**212**)	steroid	microsomes	81.7	PCA at 20 μg/mL	[143]
2β,3β-dihydroxy-5-preg-17(20)-(*Z*)-en-16-one (**213**)	steroid	microsomes	77.4	PCA at 20 μg/mL	[143]
6β-hydroxystigmasta-4-en-3-one (**214**)	steroid	microsomes	94.2	PCA at 20 μg/mL	[108]
6β-hydroxystigmasta-4-en-3-one (**214**)	steroid	SK-BR-3 cells	46.3	PCA at 50 μM	[108]
7β-hydroxy-4,22-stigmastadien-3-one (**215**)	steroid	microsomes	79.8	PCA at 20 μg/mL	[108]
7β-hydroxy-4,22-stigmastadien-3-one (**215**)	steroid	SK-BR-3 cells	127.6	PCA at 50 μM	[108]
spinasterol (**216**)	steroid	microsomes	96.9	PCA at 20 μg/mL	[108]

Compound Name	Compound Class	Assay Type	Activity		Ref.(s)
spinasterol (**216**)	steroid	SK-BR-3 cells	103.5	PCA at 50 μM	[108]
spinasterol glucoside (**217**)	steroid	microsomes	93.1	PCA at 20 μg/mL	[143]
spinasterone (**218**)	steroid	microsomes	91.9	PCA at 20 μg/mL	[108]
spinasterone (**218**)	steroid	SK-BR-3 cells	98.6	PCA at 50 μM	[108]
stigmasterol (**219**)	steroid	microsomes	99.6	PCA at 20 μg/mL	[108]
stigmasterol (**219**)	steroid	SK-BR-3 cells	114.6	PCA at 50 μM	[108]
(*E*)-volkendousin (**220**)	steroid	microsomes	73.8	PCA at 20 μg/mL	[143]
(*Z*)-volkendousin (**221**)	steroid	microsomes	52.8	PCA at 20 μg/mL	[143]
aglaiaglabretol A (**222**)	triterpenoid	microsomes	97.4	PCA at 20 μg/mL	[143]
aglaiaglabretol B (**223**)	triterpenoid	microsomes	49.4	PCA at 20 μg/mL	[143]
aglaiaglabretol B (**223**)	triterpenoid	SK-BR-3 cells	16.5	PCA at 50 μM	[143]
betulinic acid (**224**)	triterpenoid	microsomes	89.5	PCA at 20 μg/mL	[143]
maslinic acid (**225**)	triterpenoid	microsomes	56.5	PCA at 20 μg/mL	[143]
oleanolic acid (**226**)	triterpenoid	microsomes	83.5	PCA at 20 μg/mL	[108]
oleanolic acid (**226**)	triterpenoid	microsomes	12.4	% inhib. at 40.7 μM	[155]
oleanolic acid (**226**)	triterpenoid	SK-BR-3 cells	93.5	PCA at 50 μM	[108]
ursolic acid (**227**)	triterpenoid	microsomes	103.1	PCA at 20 μg/mL	[143]
ursolic acid (**227**)	triterpenoid	microsomes	30.4	% inhib. at 81.5 μM	[155]
ursolic acid (**227**)	triterpenoid	microsomes	14.0	μg/mL IC_{50}	[110]
ursolic acid (**227**)	triterpenoids	microsomes	32	μM IC_{50}	[193]
ursolic acid 3-*O*-acetate (**228**)	triterpenoid	microsomes	42.7	μg/mL IC_{50}	[110]
ixocarpalactone A (**229**)	withanolide	microsomes	105.6	PCA at 20 μg/mL	[143]
ixocarpalactone B (**230**)	withanolide	microsomes	106.7	PCA at 20 μg/mL	[143]

Table 15

Previous literature reports of natural product xanthones tested for aromatase inhibition

Compound Name	Assay Type	Activity		Ref.(s)
cudraxanthone G (**231**)	microsomes	57.8	PCA at 20 μg/mL	[109]
8-deoxygartanin (**232**)	microsomes	82.6	PCA at 20 μg/mL	[109]
garcinone D (**233**)	microsomes	10.0	PCA at 20 μg/mL	[109]
garcinone D (**233**)	microsomes	5.16	μM IC_{50}	[109]
garcinone D (**233**)	SK-BR-3 cells	50.7	PCA at 50 μM	[109]
garcinone E (**234**)	microsomes	23.9	PCA at 20 μg/mL	[109]
garcinone E (**234**)	microsomes	25.14	μM IC_{50}	[109]
garcinone E (**234**)	SK-BR-3 cells	32.3	PCA at 50 μM	[109]
gartanin (**235**)	microsomes	75.9	PCA at 20 μg/mL	[109]
8-hydroxycudraxanthone G (**236**)	microsomes	55.1	PCA at 20 μg/mL	[109]
1-isomangostin (**237**)	microsomes	52.6	PCA at 20 μg/mL	[109]
α-mangostin (**238**)	microsomes	22.2	PCA at 20 μg/mL	[109]
α-mangostin (**238**)	microsomes	20.66	μM IC_{50}	[109]
α-mangostin (**238**)	SK-BR-3 cells	59.4	PCA at 50 μM	[109]
γ-mangostin (**239**)	microsomes	4.7	PCA at 20 μg/mL	[109]
γ-mangostin (**239**)	microsomes	6.88	μM IC_{50}	[109]
γ-mangostin (**239**)	SK-BR-3 cells	−0.5	PCA at 50 μM	[109]
γ-mangostin (**239**)	SK-BR-3 cells	4.97	μM IC_{50}	[109]
mangostinone (**240**)	microsomes	78.8	PCA at 20 μg/mL	[109]
monodictysin A (**241**)	DBF enzyme[1]	32	% inhib. at 50 μM	[164]
monodictysin B (**242**)	DBF enzyme[1]	9	% inhib. at 50 μM	[164]
monodictysin C (**243**)	DBF enzyme[1]	28.3	μM IC_{50}	[164]
monodictyxanthone (**244**)	DBF enzyme[1]	37	% inhib. at 50 μM	[164]
smeathxanthone A (**245**)	microsomes	80.8	PCA at 20 μg/mL	[109]
tovophylline A (**246**)	microsomes	74.7	PCA at 20 μg/mL	[109]

[1] DBF (*O*-benzylfluorescein benzyl ester) was used as substrate with purified aromatase enzyme

Table 16

Previous literature reports of miscellaneous natural products (not previously mentioned) tested for aromatase (listed alphabetically by compound class)

Compound Name	Compound Class	Assay Type	Activity		Ref.(s)
14-octacosanol (**247**)	alkanol	microsomes	24.3	% inhib. at 29.6 μM	[155]
alizarin-1-methyl ether (**248**)	anthraquinone	microsomes	82.5	PCA at 20 μg/mL	[143]
benzanthraquinone I (**249**)	anthraquinone		94	% inhib. at 25 μM	[168]
3-hydroxy-2-(hydroxymethyl)-anthraquinone (**250**)	anthraquinone	microsomes	82.1	PCA at 20 μg/mL	[143]
morindone-5-methyl ether (**251**)	anthraquinone	microsomes	92.5	PCA at 20 μg/mL	[143]
rubiadin-1-methyl ether (**252**)	anthraquinone	microsomes	99.0	PCA at 20 μg/mL	[143]
soranjidol (**253**)	anthraquinone	microsomes	96.2	PCA at 20 μg/mL	[143]
1,5,7-trihydroxy-2-methyl-anthraquinone (**254**)	anthraquinone	microsomes	50.5	PCA at 20 μg/mL	[143]
benzo[*a*]pyrene (**255**)	aromatic hydrocarbon	microsomes	none		[113]
benzoic acid (**256**)	benzenoid	microsomes	none		[113]
broussonin A (**257**)	benzenoid	microsomes	30.0	μM IC_{50}	[135]
trans-cinnamic acid (**258**)	benzenoid	microsomes	none		[113]
O-desmethylangolensin (**259**)	benzenoid	microsomes	160	μM IC_{50}	[145]
3,4-dihydroxybenzoic acid (**260**)	benzenoid	microsomes	none		[113]
3,4-dihydroxycinnamic acid (**261**)	benzenoid	microsomes	none		[113]
4-hydroxybenzoic acid (**262**)	benzenoid	microsomes	90.8	PCA at 20 μg/mL	[108]
4-hydroxybenzoic acid (**262**)	benzenoid	microsomes	none		[113]
4-hydroxybenzoic acid (**262**)	benzenoid	SK-BR-3 cells	84.3	PCA at 50 μM	[108]
4-hydroxycinnamic acid (**263**)	benzenoid	microsomes	none		[113]
MF-1 (**264**)	benzenoid	microsomes	30	μM IC_{50}	[145]
MF-2 (**265**)	benzenoid	microsomes	100	μM IC_{50}	[145]
monodictyphenone (**266**)	benzenoid	DBF enzyme[1]	25	% inhib. at 50 μM	[164]
oleuropein (**267**)	benzenoid	microsomes	27	μM IC_{50}	[136]
phenylacetic acid (**268**)	benzenoid	microsomes	none		[113]
TAN-931 (**269**)	benzenoid	microsomes	17.2	μM IC_{50}	[165]
TAN-931 (**269**)	benzenoid	*in vivo* Sprague-Dawley rats	reduced	estradiol levels	[165]

Compound Name	Compound Class	Assay Type	Activity		Ref.(s)
demethylmoracin I (**270**)	benzofuran	microsomes	31.1	μM IC_{50}	[135]
moracin N (**271**)	benzofuran	microsomes	31.1	μM IC_{50}	[135]
chlorophyllide *a* (**272**)	chlorophyll	microsomes	80.3	PCA at 20 μg/mL	[143]
esculetin (**273**)	coumarin	microsomes	>640	μM IC_{50}	[136]
isoscopoletin (**274**)	coumarin	microsomes	>640	μM IC_{50}	[136]
8-methoxypsoralen (**275**)	coumarin	*in vivo* female Wistar rats	decreased	aromatase protein	[194]
scoparon (**276**)	coumarin	microsomes	>640	μM IC_{50}	[136]
scopoletin (**277**)	coumarin	microsomes	>640	μM IC_{50}	[136]
curcumin (**278**)	diarylheptanoid	microsomes	none		[149]
aculeatin A (**279**)	dioxadispiroketal	microsomes	66.8	PCA at 20 μg/mL	[143]
aculeatin B (**280**)	dioxadispiroketal	microsomes	77.2	PCA at 20 μg/mL	[143]
albanol A (**281**)	miscellaneous	microsomes	7.5	μM IC_{50}	[135]
FR 901537 (**282**)	miscellaneous	nd	nd		[195]
sodium butyrate (**283**)	miscellaneous	breast adipose fibroblast cells	decreased	promoter specific aromatase mRNA	[196]
zearalenone (**284**)	miscellaneous	granulosa-luteal cells	inhibited	at 10 μmol/L for 24h	[129]
limnophilaspiroketone (**285**)	spiroketone	microsomes	106.2	PCA at 20 μg/mL	[143]
resveratrol (**286**)	stilbenoid	microsomes	51.9	PCA at 20 μg/mL	[143]
resveratrol (**286**)	stilbenoid	microsomes	12.8	μM IC_{50}	[136]
resveratrol (**286**)	stilbenoid	nd	~115	% inhib. at 100 μM	[107]
ellagic acid (**287**)	tannin	microsomes	99.5	PCA at 20 μg/mL	[143]
oenothein A (**288**)	tannin	microsomes	70	% inhib. at 50 μM	[130]
oenothein A (**288**)	tannin	nd	nd		[197]
oenothein B (**289**)	tannin	microsomes	33	% inhib. at 50 μM	[130]
oenothein B (**289**)	tannin	nd	nd		[197]

[1] DBF (*O*-benzylfluorescein benzyl ester) was used as substrate with purified aromatase enzyme nd = no data

ClinicalOncology News

Breast Cancer

JANUARY 24, 2018

Acupuncture Reduces Aromatase Inhibitor-Associated Joint Pain

Acupuncture is effective in reducing aromatase inhibitor (AI)-associated joint pain in women with breast cancer, according to SWOG S1200. The randomized blinded sham- and waitlist-controlled trial, presented at the 2017 annual San Antonio Breast Cancer Symposium (abstract GS4-04), demonstrated that the nonpharmacologic option may help to increase adherence to AIs, improving breast cancer outcomes.

“Identification of nonopioid options, such as acupuncture, for pain control is a public health priority. We feel there is now sufficient evidence to support insurance coverage of acupuncture for AI arthralgia,” said Dawn Hershman, MD, MS, the leader of the Breast Cancer Program at the Herbert Irving Comprehensive Cancer Center at Columbia University Medical Center, in New York City.

Dr. Hershman pointed out that AIs are extremely effective for the treatment of hormone receptor–positive breast cancer, useful in the adjuvant setting, in the metastatic setting, and in preventing breast cancer. “But they don’t work if women don’t take them, and the most common reason women stop taking the medication early is due to joint pain or arthralgia,” Dr. Hershman said.

Acupuncture is a popular, nonpharmacologic modality for the treatment of a variety of painful medical conditions. Several small studies have suggested that acupuncture may be beneficial for AI arthralgias; however, others have shown no benefit, Dr. Hershman said. The overall interpretation of these trials has been uncertain due to short duration, small sample sizes and differences in methodology.

In the new study, the researchers enrolled patients with stage I to III hormone-sensitive

breast cancer who were receiving a third-generation AI for at least 30 days prior to registration. Patients needed to have a score of at least 3 (range, 0-10) on the worst pain item of the Brief Pain Inventory (BPI), with symptoms starting or increased since AI initiation. Patients were not included if they were on opioids or corticosteroids, or were receiving alternative/physical therapy for the treatment of joint pain within 28 days before registration. No prior acupuncture treatment for joint symptoms at any time was allowed, but patients could enroll in the study if they had used acupuncture for other reasons more than 12 months beforehand. The median age of patients in the study was 60 years. Investigators randomly assigned 226 patients to receive true acupuncture given twice a week for six weeks (n=110), sham acupuncture (n=59) or waitlist control (n=57); true acupuncture was offered after 24 weeks.

The primary study end point was at six weeks, and the researchers tested maintenance of the intervention by evaluating true acupuncture once a week for an additional six weeks, sham acupuncture once a week for an additional six weeks, and a waitlist control. “We looked at the duration of the potential benefit by evaluating women without any intervention for a subsequent 12 weeks.” All women received acupuncture at the end of 24 weeks.

Patients in the true acupuncture group received standard traditional Chinese medicine point prescription to reduce pain and stress (30-45 minutes per session). Full-body, auricular and a joint-specific acupuncture protocol was tailored to the most painful joints. Patients in the sham acupuncture group received a shallow needle insertion utilizing thin and short needles at non-acupuncture points. “Prior studies have shown that sham acupuncture can result in physiologic effects,” Dr. Hershman said.

The primary outcome measure was worst pain score at six weeks on the BPI. “We found a significant difference in worst pain score comparing true acupuncture to sham acupuncture and comparing true acupuncture to waitlist control. We found no difference between the two groups of sham and waitlist control,” Dr. Hershman said.

The researchers also evaluated what they considered to be a significant meaningful drop: at least a 2-point change in pain from baseline at six months. “With true acupuncture, 58% of patients had a 2-point change compared to 31% in the sham and 30% in the waitlist control arm. Similar results were seen if we looked at a 50% change from baseline,” Dr. Hershman said. True acupuncture was better than either of the two

control groups for other six-week end points analyzed, including BPI average pain, BPI stiffness, the Western Ontario and McMaster Universities Osteoarthritis Index, and the modified Score for the Assessment and Quantification of Chronic Rheumatic Affections of the Hands. The toxicity of the intervention was limited to grade 1 bruising (47% in true acupuncture).

“We have shown consistently with multiple measures assessing pain and stiffness that true acupuncture generated better outcomes than either control group,” Dr. Hershman said. “Transitioning from twice-a-week to once-a-week acupuncture maintained the effect of the intervention. The intervention effects persisted 12 weeks following completion of the intervention.” The cost of the 12-week (18 sessions) intervention was approximately $1,250 ($65-$75 per session).

Commenting on the study after the presentation, Hope Rugo, MD, professor of medicine and director of the Breast Oncology Clinical Trials Program at the University of California, San Francisco, Helen Diller Family Comprehensive Cancer Center, congratulated the researchers. “This is an incredible study,” Dr. Rugo said. “It is something that really needed to be done and will be a big help for our patients.”

—Kate O’Rourke