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# Overview of The Physiology of Aromatase Enzyme Actions and Substances That Inhibit its Activities

I. C. Okoli[1*], U. C. Ezetoha[1], M. N. Opara[1], C. T. Ezeokeke[1] and F. C. Iheukwumere[2]
[1]Tropical Animal Health and Production Research Lab., Department of Animal Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State.
[2]Department of Animal Science/Fisheries, Abia State University, Uturu, Abia State, Nigeria
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## Abstract

Steroid hormones are synthesized through the mediations of a number of enzymes. Estrogen, the female steroid hormone is biosynthesized from androgen by aromatase enzyme. Aromatase is found mainly in estrogen producing cells of the body of mammals and in breast cancer tissues. It plays a role in follicular development where it produces estrogen that causes the growth and maturation of follicles. Its activity can be inhibited in the embryo of avian species to cause phenotypic sex reversal and in hormone dependent breast tumours to lower the growth stimulating effect. Numerous inhibitors of aromatase activity exist and are grouped into steroidal and non-steroidal inhibitors.

**Keywords;** Aromatase enzyme, Hormone, Cell, Inhibitors.

## 1.0 Introduction

An organism is a living chemical system in which substances are constantly changing. Underlying every change in the living world are chemical reactions. These include molecular reactions that may end in transformations into other kinds of molecules as well as materials break downs and reorganizations. We known that enzymes are critically involved in the starting and sustenance of these chemical processes; most chemical reactions would take place too slowly to sustain life if it were not for enzymes (Wessell and Hopson 1988). One of such is the synthesis of hormones.

The importance of hormones in the living organism cannot be over-emphasized. They produce and co-ordinate anatomical, physiological and behavioral changes in an animal (Purves *et al.* 2004). The male and female gonads as hormonal glands produce androgens and estrogens, which are steroids synthesized from cholesterol. Both males and females can synthesize androgen but females have higher level of the aromatase enzyme which is needed to convert androgen to estrogen. Without aromatase activity, female secondary sexual characteristics will not develop, accessory sex glands and mammary glands development will be affected, estrus cycle will not occur, uterine activity will be impeded and hence no reproduction (Purves *et al.* 2004).

In this paper, we discuss the pathway leading to the production of steroid hormones, the biochemistry and activity of aromatase enzyme and substances that inhibit its actions.

## 2.0 Overview of the structure and biosynthesis of steroid hormones

### 2.1 Structure of steroid hormones

Steroid hormones are derivatives of cholesterol that are synthesized by a variety of tissues, most prominently the adrenal glands and gonads. They are classified into five groups of molecules based primarily on the receptor to which they bind (Bowen 2001). These include glucocorticoids, mineralocorticoids, androgens, estrogens and progestogens or progestins. Steroid hormones have a basic nucleus or ring structure called cyclopentanoperhydrophenanthrene ring (see Hafez 1987; Okeudo 2000; Esonu 2000).

As shown in Figure 1, this nucleus consists of three, six-membered fully hydrogenated (perhydro) phenanthrene rings designated A, B and C; and onefive-membered cyclopentane ring designated D (Hafez 1987; Okeudo 2000). The nucleus has a flat

* Corresponding author's e-mail: dr_charleso@yahoo.com

structure with some substitute groups projecting above or below it. Groups above are denoted by solid lines and are called beta (β) while those below are indicated by dotted lines and are termed alpha (α). Carbon atoms are numbered as shown in Figure 1, and methyl side chains are linked to carbon atoms 10 and 13.

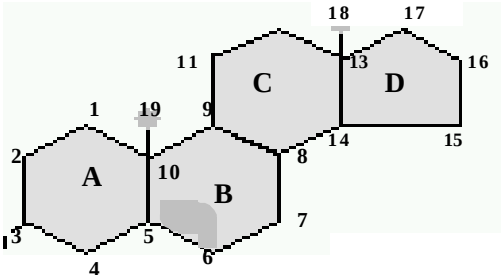


Figure 1: Cyclopentanoperhydrophenanthrene ring.
**(Source:** Okeudo 2000).

Differences in biological activities of the various steroid hormones are accounted for by the attachment of $O_2$, OH, $CH_2$ or $CH_3CH_2$ molecules at positions 3, 11 and 17 (Bath *et al.,* 1978), and by the number of carbon atoms present in them (Hafez, 1987). For example, an 18-carbon steroid will have estrogen activity, 19-carbon steroid will have androgen activity and 21 –carbon steroid will have progesterone property.

## 2.2 Biosynthesis of steroid hormones

The biosynthesis of steroid hormones requires a battery of oxidative enzymes located in both mitochondria and endoplasmic reticulum of the cell (see Table 1). Each enzyme is responsible for the conversion of one steroid to another. Cholesterol-containing low density lipoprotein is predominantly used in steroidogenesis. Within the cell, an enzyme, cholesterol ester hydrolase converts cholesterol ester to free cholesterol and this is mobilized to the mitochondria and internalized (CRRA 2000). This is the rate limiting step for the general steroidogenic pathway and is mediated by the steroidogenic acute regulatory protein (StAR).Once inside the mitochondria, cholesterol is converted to pregnenolone by the enzyme cytochrome P450 cholesterol side chain cleavage ($P450_{SCC}$) (Okeudo 2000).

Pregnenolone is not a hormone, but is the immediate precursor for the synthesis of all steroid hormones for all species and all tissues (CRRA 2000; Bowen 2001). Pregnenolone could be converted to progesterone by the enzyme 3 β-hydroxysteroid dehydrogenase (3β-HSD), or to 17 α - hyroxypregnenolone by the cytochrome P450 17α-hydroxylase ($P450_{17\text{-}OH}$).

In ruminant follicles, the expression of the enzyme,3β-HSD in the luteal and granulose cells leads to the production of progesterone from pregnenolone, while the activities of $P450_{17\text{-}OH}$, 3β-HSD and 17β-HSD lead to the synthesis of

Table 1: Enzymes involved in steriodogenesis.

| Common name | Old name | Current name |
|---|---|---|
| Side- chain cleavage enzyme; desmolase | $P450_{Scc}$ | CYPIIAI |
| 3beta-hydroxysteriod dehydrogenase | 3 beta-HSD | 3-beta HSD |
| 17alpha-hydroxylase/17,20 lyase | P450c17 | CYP17 |
| 21-hydroxylase | $P450_{C21}$ | CYP21A2 |
| 11 Beta-hydoxylase | $P450_{CII}$ | CYPIIBI |
| Aldosterone synthase | $P450_{CIIAS}$ | CYPIIB2 |
| *17beta- hydroxysteroid dehydrogenase | 17 beta-HSD | 17 beta-HSD |
| Aromatase | $P450_{arom}$ | CYP19 |

* Not included in the original table. Source: Bowen (2001).

androgens, androstenedione and testosterone. A good portion of these secreted androgens are absorbed by the neighboring granulosa cells and are converted to estrogens. The granulosa cells prefer to metabolize androstenedione to estrone by the enzyme, cytochrome P45O aromatase ($P450_{arom}$) before estrone is metabolized to estradiol by 17β-HSD. Alternatively,
testosterone could be metabolized to estradiol by $P450_{arom}$ (CRRA 2000).

The enzymes involved in steroidogenesis are regulated by the gonadotropins-follicle stimulating hormone (FSH) and lituenizing hormone (LH) and some growth factors (Armstrong and Webb 1997; CRRA 2000; Webb *et al.* 2004). The enzymes expressed in luteal and theca cells are in general regulated by LH, while FSH regulates the activities of $P450_{scc}$ and $P450_{arom}$. Hence, LH stimulates progesterone secretion from luteal cells and androgens from theca cells, whereas FSH stimulates progesterone and estradiol secretion from granulosa cells (see Frandson 1981).

## 3.0 The Estrogen Synthetase-Aromatase

Aromatase is the enzyme involved in the production of estrogen and acts by catalyzing the conversion of an androgen to an estrogen. It is predominantly found in estrogen producing cells of the adrenal glands, ovaries, placenta, adipose tissues and brain as well as human breast tissue (Bulun *et al.* 1993; Anonymous 1996; Miller *et al.* 1997; Brueggemier *et al.* 2005).

Aromatase is a cytochrome P4SO enzyme complex. Cytochrome P (CYP) enzyme complex is a suite of enzymes that use iron to oxidize things, often as part of the body's strategy to dispose of harmful substances by making them more water soluble (Anonymous 2000). They are usually found in the microsomal part of the cell and catalyses varieties of reactions. CYP component of the microsome of the cell strongly absorbs light at a wavelength of 450 nm when exposed to carbon monoxide, after extraction of the microsomal portion and adding haem-reducing agents (Simpson *et al.* 1994).

Aromatase is comprised of two major proteins (Simpson *et al.* 1993; Simpson *et al.* 1994). These include cytochrome $P4S0_{arom,}$ a hemoprotein that converts $C_{19}$ steroids to $C_{18}$ steroids containing a phenolic A ring (Kellis and Vickery 1987). A hemoprotein contains a haem group as the prosthetic component (Okeudo 2000). The heam group is a porphyrin molecule (large heterocyclic organic ring) complexed with one atom of iron. It functions in retaining oxygen and delivering it for enzymatic reactions (Wikipedia 2006).

The second protein in aromatase is NADPH-cytochrome P450 reductase, which transfers reducing equivalent to cytochrome $P45O_{arom}$. NADPH-cytochrome P450 reductase (CPR) is a flavoprotein containing both flavine adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and is the electron donor protein for several oxygenase enzymes found on the endoplasmic reticulum of most eukaryotic cells (Porter 2001).

### 3.1 Gene expression

The aromatase gene, designated CYP19, encodes the cytochrome $P450_{arom,}$ and this gene is located on chromosone 15q 21.1. The coding region is approximately 30 kb in size, and the regulatory region is approximately 93 kb (Simpson *et al.* 1993; Bulun *et al.* 2004). The aromatase gene consists of 10 exons and its full length cDNA of 3.4 kb encodes for a protein of 503 amino acids. The aromatase protein is a glycosylated protein with a molecular mass of 58,000 daltons (Gartner *et al.* 2001).

The regulation of aromatase is complex in various tissues, and several tissue-specific promoter regions have been identified upstream from the CYP19 gene (Dowsett 1993; Zhao *et al.* 1997). A promoter region is the site at which an RNA polymerase attaches to DNA and initiates transcription. These tissue-specific promoters include P1.1, P1.3, P1.4, P1.6, P1.7 and P11. Promoter P1.1 is the major promoter used in placental tissues and is the farthest upstream. Promoters P1.3, P1.4, P1.6 and P1.7 are the promoters used in extra-glandular sites. P1.4 is the primary promoter used in normal adipose tissue and is responsive to glucocorticoids and cytokines. Promoters P1.3 is also present in adipose tissues such as normal breast tissue and is elevated in breast cancer tissues. Promoter P11 is used in ovary and in breast cancer tissues and it contains a cAMP

responsive element (Zhao *et al.* 1996 and 1997).

## 4.0 Aromatase action in Estrogen biosynthesis

Estradiol, the most endogenous estrogen is biosynthesized from the androgens by the aromatase enzyme. The preferred substrate for the synthesis of estradiol is androstenedione, which it converts to estrone. Estrone is then acted upon by 17β-HSD to produce estradiol -17β. In the conversion of one mole of substrate into one mole of estrogen product, three moles of reduced form of NADPH and three moles of oxygen are used (Brueggemeier *et al.* 2005). Androstenedione is aromatized via three successive oxidation steps. The first two steps are the hydroxylations of the angular C-19 methyl group, while the final oxidation step proceeds with the aromatization of the A ring of the steroid and loss of the C-19 carbon atom as formic acid. This third step in the aromatase reaction oxidatively cleaves the $C_{10}$ –$C_{19}$ bond (Brueggemeier *et al.* 2005).

## 4.1 Aromatase expression in follicular development

During early follicular development, FSH binds to granulosa cells of primary follicles to stimulate production of estradiol by the enhancement of aromatase synthetase (Tonetta and Di Zerga 1989). Estradiol, in turn, induces the proliferation of granulosa cells and increases the sensitivity of the follicle to further gonadotropin stimulation. It can synergize with gonadotropins to increase ovarian weight, enhance proliferation of granulosa cells, and promote growth of preantral follicles and antrum formation (Frandson 1981; Tonetta and Di Zerga 1989).

However, as the synthesis of this steroid increases, it directly stimulates follicular growth. Estradiol not only enhances gonadotropin stimulations of LH and FSH receptors in granulosa cell but is also required for FSH induction of FSH receptors, increasing the number of its own receptor as well as increasing it own production by stimulating aromatase activity. Through two positive feedback loops (one at the pituitary and one at the ovary), it maintains the dominant follicle and ensure ovulation.

In the cattle (and other domestic animals), follicular growth occurs in waves and it is marked by changesin steriodogenic activities. Price *et al.* (1995) noted that small follicles contain relatively little estradiol, and that follicular fluid estradiol concentrations increases with follicle size in healthy growing follicles. Its concentration decreases in subordinate follicles while the dominant follicle is growing. But once the dominant follicle reaches maximum diameter, follicular fluid estradiol concentrations fall dramatically and decreases further once the follicles start regressing (Price *et al*. 1995). Examination of steroidogenic enzyme mRNA levels at different stages of development determines the point in the pathway that is responsible for increased or decreased secretion of estradiol by the follicle (Bao and Garverick 1998).

CRRA (2000) noted that during the pre-anatral and early antral stages, the granulosa cells express only FSH receptors and are thus steroidogenically inactive. However, Tonetta and di Zerga (1989) and Webb *et al.* (2004) noted that pre-antral follicles are capable of producing estradiol. Soon after the formation of theca cell layer, mRNA for LH receptors, $P450_{SCC}$, $P450_{17\text{-}0H}$ and 3â-HSD are first expressed (CRRA 2000; Webb *et al*. 2004). Thus, the theca cells are able to make progesterone and androgens.

## 5.0 Late Antral follicular stage

In the cattle, the later stage of antral development is characterized by two or three waves of follicular growth during each estrous cycle and each wave of growth is characterized by recruitment of follicles which continue to grow to about 6-8mm in diameter with an increase in FSH secretion (Webb *et al.* 2004).

When the follicles are recruited, there is induction of mRNA expression for $P450_{SCC}$ and $P450_{arom}$ in granulosa cells, and an increase in mRNA of the gonadotrophin receptors and steriodogenic enzymes with increasing follicular size. Thus, the granulosa cells can synthesize pregnenolone and convert androstenedione to estrone but cannot synthesize progesterone since they lack 3β–HSD. A study carried out on hypogonadotropic cattle (Garverick *et al.* 2002) showed that antral follicle growth at

this stage is under gonadotropic control.

### 5.1 Dominant follicular stage

In monovulatory species, one follicle is selected for continued growth and becomes dominant. As a growing follicle becomes a dominant follicle, the granulosa cells start to express mRNA for LH receptors and 3β–HSD (CRRA 2000). Thus, the cells are able to synthesize progesterone and respond to LH which is essential for dominant follicle maturation. As the dominant follicle grows, there are also increases in mRNA for $P450_{arom}$ in granulosa cells and StAR protein in theca cells. There is a decrease in all steriodogenic enzymes in granulosa cells of subordinate follicles which causes them to regress.

When the dominant follicle reaches the static phase of growth (maximum diameter: >8 mm), there is a reduction in mRNA for $P450_{SCC}$ in granulosa cells though there are no changes in $P450_{arom}$ mRNA. Thus, the follicle secretes less estradiol than growing follicles due to reduction in androgen precursor supply to granulosa cell. If the dominant follicle undergoes atresia, there is no further loss of mRNA that codes the steroidogenic enzymes in the theca cells but, granulosa cells suffer a loss of $P450_{SCC,}$ $P450_{arom}$, LH receptor and 3β –HSD mRNAs. Thus estradiol decreases further once the follicle starts regressing (CRRA 2000).

### 6.0 Aromatase expression in Avian Embryo

In mammals, the phenotype of the homogametic sex (female) develops in the (relative) absence of steroids while that of the heterogametic sex (male) is imposed by the early action of steroids($7^{th}$ week of development in human embryo). Until the time of the action of steroids, the embryo has the potential to develop into either sex. The presence of a Y chromosome normally causes the undifferentiated embryonic gonads to begin to produce androgens at the time. In response to the androgens, the reproductive system develops into that of a male.But if androgens are not produced at that time, female reproductive structures develop (Purves *et al. 2004*). The opposite situation exists in birds.

The heterogametic sex in avian species is the female and the presence of estrogens and their receptors plays a crucial role in female sexual differentiation (Bruggeman *et al.* 2002). Investigations on the time- and sex- dependent expression of the enzymes involved in steroidogenesis which determine the ratio of androgens to estrogens produced by the gonads show that lack of estrogen synthesis in the male appears to be due to the extremely low levels of 17β–HSD and P450 aromatase expression. In the females, there is extensive expression of the aromatase gene at about day 5-6 of incubation leading to estrogen synthesis, and specific expression of the estrogen receptor mRNA in the left gonad results in the development of a functional ovary (Bruggeman *et al.* 2002).

If the production or synthesis of estrogen is hindered at early period of incubation (especially before the fifth day), the sex of the developing embryo will be reversed phenotypically. This has been attempted by the use of aromatase inhibitors anti-estrogens, androgen and synthetic steroids.

### 6.1 Aromatase in Breast Cancer Tissues

Aromatase has been measured in the stromal cell component of normal breast and breast tumors; and has also been detected in the breast epithelial cells in vitro (Bulun *et al.* 1993; Reed *et al.* 1993; Miller *et al.* 1997; Quinn *et al.* 1999). Expression of aromatase is highest in or near breast tumor sites (Miller *et al*. 1997). Hormone dependent breast cancer contains estrogen receptors, and requires estrogen for tumor growth.

The increased expression of aromatase observed in breast cancer tissues is associated with a switch in the major promoter region used in gene expression with promoter PII (and P1.3) being the predominant promoter (Zhao *et al.* 1996). As a result of the use of alternate promoter, the regulation of estrogen biosynthesis switches from one controlled primarily by glucocorticoids and cytokines to a promoter regulated through cAMP-mediated pathways (Zhao *et al*. 1996). Prostaglandin $E_{2\,(}PGE_{2)}$ increases intracellular cAMP levels and stimulates estrogen biosynthesis.The growth stimulatory effect of estrogen in breast cancer could therefore be reduced by the use of anti-estrogens and aromatase inhibitors. Anti-estrogens compete for binding to the estrogen

receptors and hence reduce the number of receptors available for binding to the steroid (Jordan 1995; Carmicheal 1998). Example of such is the drug-tamoxifen. Aromatase inhibitors on the other hand help to decrease the circulating levels of estrogen. Effective aromatase inhibitors have been developed (Harvey *et al.* 1982; Banting *et al.* 1989; Cole and Robinson 1990; Brueggemeier 1994; Brodie *et al.* 1999; Santen and Harvey 1999; Simpson 2001).

## 6.2 Aromatase Inhibitors

Aromatase inhibitors work by preventing the synthesis of estrogens in the body. Investigations on the development of aromatase inhibitors began in 1970s and have expanded greatly in the past three decades (Brueggemeier *et al*. 2005). Effective aromatase inhibitors have been developed, and are grouped into steroidal and non-steroidal inhibitors.

### 6.2.1 Steriodal Inhibitors

Steroidal inhibitors build upon the basic androstenedione nucleus and incorporate chemical substitutions at varying positions on the steroid. Modifications at certain positions in the androstane steroid molecule will result in the inhibition of the enzyme. They are classified as competitive inhibitors and mechanism-based inhibitors.

### 6.2.2 Competitive Enzyme Inhibitors

The development of aromatase inhibitors began with the synthesis and biochemical evaluation of competitive inhibitors (Schwarzel 1973; Siiteri and Thompson 1975; Brueggemeier *et al*. 1978). Competitive inhibitors are molecules that compete with the substrate androstenedione for non covalent binding to the active site of the enzyme to decrease the amount of product formed. They bind to the enzyme in the same manner as the substrate androstenedione. 4-Hydroxyandrostenedione (4-OHA) was the prototype agent, but was later discovered to be a mechanism based enzyme inhibitor. Other examples include;

i 1-substituted aromatase inhibitors such as 1-methyl androstra -1, 4-diene-3, 17-dione (Henderson *et al*. 1986).

ii 7- substituted inhibitors which include 7α–(4-amino) phenylthio-4-androstene-3,17-dione (Brueggemeier *et al.* 1992); and

iii 19-substituted inhibitors such as thiiranes and oxiranes (Kellis *et al.,* 1987), and thiol and amino analogs (Bednarski 1985; Wright *et al*. 1985).

### 6.2.3 Mechanism based enzyme inhibitor or suicide inactivators:

A mechanism-based inhibitor mimics the substrate, is converted to a reactive intermediate and results in the inactivation of the enzyme. The inhibitor contains a chemical functionality that is acted upon by the enzyme during the normal catalytic process (Brueggemeier *et al.* 2005). They produce time dependent inactivation of the enzyme only in the presence of a cofactor, such as NADPH. The inactivation that occurs is due to irreversible, covalent binding of the inhibitors to the enzyme protein. Examples include 10-propargyl-4-estrene-3, 17-dione or MDL18, 962(see Metcalf *et al.* 1981; Marcotte and Robinson 1982), 4-Hydroxy-4-androstene-3, 17-dione or formestane (see Brodie *et al.,* 1981; Wiseman and Goa, 1996) and 6-methyleneandrost-1, 4-diene-3, 17-dione or exemestane (see Giudici *et al.* 1988; Zaccheo *et al.* 1989).

## 6.3 Nonsteroidal Inhibitors

These inhibitors possess a heteroatom (non carbon atom in a heterocyclic compound) as a common chemical feature and interfere with steroid hydroxylation by the binding of this heteroatom with the heme iron of the cytochrome P450 enzymes. Initial non-steroidal inhibitors were fewer enzymes specific and inhibited to varying degrees, other cytochrome P450-mediated hydroxylations of steroidogenesis. They include aminoglutethimide-like molecules, imidazole/triazole derivatives; and flavonoid analogs.

### 6.3.1 Aminoglutethimide and Imidazole/ Triazole Derivatives

Aminoglutethimide was the prototype for onesteroidal aromatase inhibitors (Cocconi 1994). It is referred to as first generation aromatase inhibitors. It is effective at decreasing aromatization but inhibits a number of other steroidogenic CYP450

enzymes which result in toxicity. It also has inhibiting effects on cortisol and aldosterone (Cocconi 1994).

Imidazole derivatives contain two nitrogen atoms in their five- membered aromatic ring. Example is Fadrazole (Trunet *et al.* 1997). It is referred to as the second generation inhibitor. It is more selective than aminoglutethimide, and its inhibitory activity is more potent but it still has some non selective inhibitory activity with respect to aldosterone, progesterone and corticosterone biosynthesis.

Several non-steroidal aromatase inhibitors containing a triazole ring (five-membered aromatic ring structure with three nitrogen atoms) have been developed. An example is Vorozole (Vanden *et al*. 1990; Wouters *et al*. 1994). Other triazole analogs are anastrozole or arimidex (Plourde *et al*. 1994) and letrozole (Bhatnagar *et al*. 1990). Invitro studies have shown that letrozole and anastrozole have no effect on the biosynthesis of other steroids and are more active inhibitors than aminoglutethimide.

### 6.3.2 Flavonoid derivatives

Flavonoids are found in plants. They are present in many food sources, such as fruits, vegetable, legumes and whole grains. The class of flavonoids encompasses flavones, isoflavones, flavonones and flavonols. They possess the benzopyranone ring system as the common chemical feature (Brueggemeier *et al.* 2005). Several flavonoids demonstrate inhibitory activities of the aromatase enzyme, thus lowering estrogen biosynthesis and circulating estrogen levels (Ibrahim and Abul-Haji 1990; Adlercreutz 1995; Kao *et al.* 1998; Le Bail e*t al.* 1998; Pouget *et al.* 2002).

## 7.0 Conclusion

Aromatase have been shown to be one of the enzymes involved in steriodogenesis. Its action on androstanedione or testosterone leads to the production of estrogen; estradiol being the most potent endogenous estrogen. The estrogen produced has several functions in female animals including the development of ovarian follicles and in bird, it induces sexual differentiation in the embryo. When the activity of the enzyme is inhibited, estrogen biosynthesis is hindered and its level is drastically reduced.

This has been used in effecting phenotypic sex reversal in birds and in treatment of hormone dependent tumors in humans. Inhibitors used recently include steroidal inhibitors such as formestane, 1-methyl-ADD and exemestane and non steroidal inhibitors such as aminoglutethimide, fadrazole, letrozole and flavonoids.

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