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Comprehensive Pharmacology and Clinical Efficacy of Aromatase Inhibitors

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Abstract

The goal of hormone therapy is to deprive breast tumours of estrogens, since estrogens have been implicated in the development or progression of tumours. This can be accomplished by the use of antiestrogens that block estrogen action or by inhibiting aromatase, the enzyme that catalyses the final and rate–limiting step in estrogen biosynthesis.

A number of steroidal and nonsteroidal compounds have been developed as aromatase inhibitors. This review highlights the valuable role that a few of these aromatase inhibitors have played, and continue to play, in the treatment of breast cancer. Following background information regarding the biochemistry of aromatase, the rationale for its inhibition, and an outline of the test systems for evalu-

ating and characterising aromatase inhibitors, the discussion focuses on the new generation of aromatase inhibitors that are in clinical trials or clinically available. Specifically, it discusses the pharmacology and clinical efficacy of formestane, exemestane, rogletimide, fadrozole, vorozole, anastrozole and letrozole.

The role of these agents as the optimal second-line agents (after tamoxifen) for the treatment of advanced breast cancer has been established; their prospects in other clinical settings and as potential breast cancer chemopreventives are warranted but are yet to be fully determined.

Aromatase (estrogen synthetase) is a membrane-bound enzyme complex consisting of a cytochrome P450 (CYP) homoprotein (aromatase, $P450_{arom}$) and a flavoprotein, reduced nicotinamide adenine dinucleotide diphosphate (NADPH)–cytochrome P450 reductase.[1] This enzyme complex catalyses the conversion of androgens into estrogens [exemplified by the conversion of androstenedione (1) into estrone (4); figure 1], a process believed to proceed through the participation of 3 sequential reactions at C-19, each requiring 1 molecule of NADPH and 1 molecule of molecular oxygen.[2] This overall process culminates in the extrusion of the C-19 atom as formic acid and the aromatisation of ring A.[3] The function of the reductase is to transfer, in a stepwise fashion, 2 electrons from NADPH to the haem iron of the cytochrome. The mechanisms of the reactions catalysed by aromatase have been studied over the years through the use of stereo- and regio-specifically labelled substrates, and the fate of each atom undergoing chemical change during the conversion is depicted in figure 1.[4] Excellent and recent reviews on the mechanism of aromatisation of androgens to estrogens are available.[4-6]

Research interest in aromatase and the aromatisation reaction (estrogen biosynthesis) continues to expand from basic endocrinological and reproductive biological studies to aromatase inhibition for the treatment of estrogen-dependent diseases and molecular biology studies. In this review, the pharmacology and clinical efficacy of those aromatase inhibitors that are in clinical trials or clinically available, and their place in the treatment of breast cancer, are comprehensively examined. The review has been mainly based on reviews, papers and abstracts published in the last 5 years up to June 1998. Most of the original articles cited were also consulted.

1. Aromatase and Breast Cancer

It is over 100 years since Sir Cecil Beatson demonstrated that ovariectomy resulted in tumour regression in patients with breast cancer.[7] Subsequently, estrogen was identified as the mediator of the ovarian dependency.[8] Nevertheless, the sensitivity of breast cancer to estrogens has been found to increase as patients age. Two-thirds of breast cancers in postmenopausal women (compared with less than half of those in premenopausal women) have tumours positive for estrogen receptors. Estrogen receptor–positive cancers are more responsive to hormonal therapy than cancers lacking these receptors. Thus, not only is breast cancer more prevalent in postmenopausal women, but it is also a more hormone-dependent disease.[9]

When ovarian steroid production declines because of the menopause, estrogens produced in peripheral tissue, such as adipose tissue, are the major sources of circulating estrogen. Therefore, in postmenopausal patients, total blockade of estrogens is more likely to be accomplished with systemic treatment rather than surgical removal of endocrine glands. Two pharmacological approaches are currently used to reduce estrogen effect: (i) inhibition of estrogen action by antiestrogens, which interact with estrogen receptors in the tumour; and (ii) inhibition of estrogen production by inhibitors of aromatase (estrogen synthetase).

The antiestrogen tamoxifen has become first-line treatment for postmenopausal patients with estrogen receptor–positive breast cancer, since it

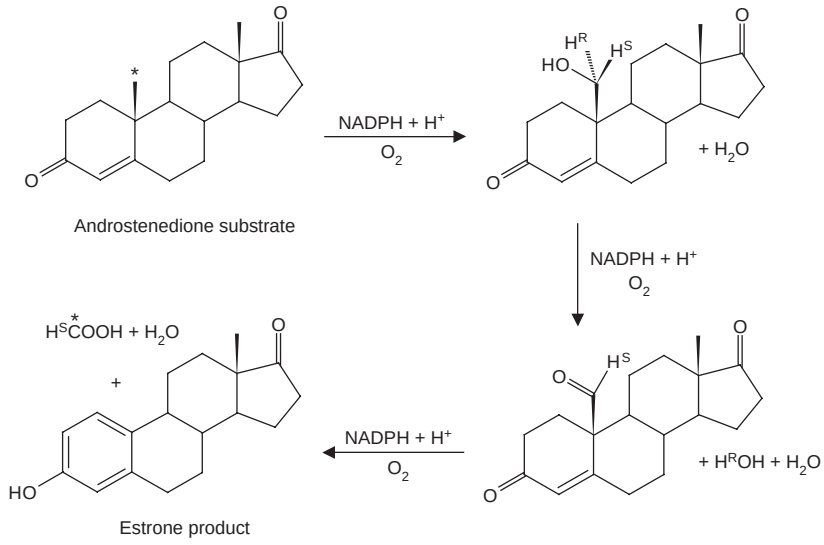


Fig. 1. Aromatisation of androgens to estrogens. The compounds on the right are intermediates in the reaction. **NADPH** = nicotinamide adenosine dinucleotide diphosphate.

was shown to produce significantly greater response rates in these patients than are achieved with cytotoxic agents and to be well tolerated.[10] However, resistance to tamoxifen inevitably develops, which results in disease progression. In 1973, we reported the first inhibitors of aromatase, the enzyme that controls the conversion of androstenedione and testosterone to estrone and estradiol, respectively.[11] We proposed these inhibitors for reducing estrogen production as a therapeutic strategy for breast cancer treatment. A number of compounds were identified that inhibit estrogen synthesis and cause mammary tumour regression in animal models.[12] One of these, formestane (4-hydroxyandrostenedione), is now proving to be effective in patients with tamoxifen-resistant breast cancer.[13,14] By reducing estrogen production, aromatase inhibitors can elicit further responses in some patients who have relapsed on antiestrogen therapy. Thus, aromatase inhibitors can extend the duration of response and quality of life for patients with breast cancer.

We also postulated that the more complete estrogen blockade via aromatase inhibition might result in greater tumour response than with tamoxifen, as tamoxifen is known to be a weak or partial estrogen agonist in addition to being an estrogen antagonist. Although some agonist effects may be beneficial (e.g. on bone and the cardiovascular system), the potentially adverse actions of tamoxifen on the endometrium are a cause of concern. Inhibitors of aromatase act by a different mechanism of action than tamoxifen and do not have estrogenic activity; therefore, they are not associated with endometrial effects.

2. Distribution and Regulation of Aromatase

Estrogen synthesis occurs in a variety of tissues in both males and females of most species. For example, aromatase has been identified in several locations of the brain, including the hypothalamus, amygdala and hippocampus.[15] In men, aromatase activity is associated with muscle, adipose tissue and the testis.[16-19] In women during pregnancy, estrogens are produced in high levels by the placenta where aromatase is expressed in the cytoplasm of syncytiotrophoblasts in the outer layer of the chorionic villi.[20,21] The granulosa cells are the major source of estrogen synthesis in the ovary,

although low levels of aromatase are expressed in the thecal compartment of the developing follicle.[22] Adipose tissue is considered to be the main site of extragonadal estrogen synthesis contributing to circulating estrogen levels in postmenopausal women.[23] However, levels of estradiol in breast tissues from postmenopausal women have been found to be 10 times higher than those in plasma.[24,25] A number of reports, including our own, indicate that aromatase activity as well as aromatase messenger RNA is present in normal breast tissue and breast tumours.[26-32]

Some clinical observations suggest that there may be a correlation between intratumoural aromatisation and tumour response to inhibition of estrogen synthesis in patients treated with aromatase inhibitors;[33,34] observations also suggest that local production of estrogens may have an important role in tumour proliferation. However, aromatase activity measured in human breast tumour homogenates has been reported to be relatively low and was thought to be insufficient to catalyse the formation of sufficient estrogen to activate the estrogen receptor.[35] Using both immunocytochemistry and *in situ* hybridisation, we recently determined the site of aromatisation to be mainly in the tumour epithelial cells in human breast cancers, although some stromal cells surrounding the tumour also expressed the enzyme.[32] Other, but not all, immunocytochemical studies have shown epithelial staining. These differences in results may be due to technique and/or antibodies.[36-38] In our studies, aromatase activity measured in cryosections of tumours correlated with a marker of proliferation (proliferating cell nuclear antigen score), suggesting that local concentrations of estrogens are sufficient to stimulate the growth of the tumour. We also found that among tumours that were stimulated by estrogens in histocultures, proliferation of some tumours was also enhanced by testosterone. This stimulation could be inhibited by the use of an aromatase inhibitor, suggesting that estrogens were produced by the tumours via aromatisation of testosterone. Thus, it appears that aromatase expressed in the tumour may play an important role by producing estrogens that act locally to stimulate tumour proliferation.

In humans, it is currently thought that there is only a single form of aromatase encoded by one gene, which has been mapped to chromosome 15.[39] The human cytochrome $P450_{arom}$ gene is composed of 10 exons, 1 of which (exon I) is a noncoding exon. The human aromatase cDNA is 2736 base pairs in length and encodes a 55kD protein of 503 amino acids. Regulation of the enzyme occurs in a tissue-specific manner facilitated by the use of alternative promoters.[40] The promoter utilised in the placenta is at least 40kb upstream from the translational start site. In the ovary, where the enzyme is regulated by follicle-stimulating hormone (FSH),[41] a promoter proximal to the translational start site, promoter II, is used. In adipose tissue, where glucocorticoids and cyclic AMP appear to be regulators of aromatase,[42,43] two promoters may be utilised – promoter II and another promoter as yet uncharacterised.

Steroidogenic P450 enzymes function as hydroxylases in the conversion of one steroid to another. The hydroxylations mediated by $P450_{arom}$ are characteristic of steroidogenic P450 enzymatic reactions. Nevertheless, there is only about 30% homology between $P450_{arom}$ and other cytochrome P450 enzymes.[44] The region of greatest homology among all steroidogenic P450 enzymes is the haem-binding region. Studies using the VGAP alignment program have shown that the human aromatase sequence has only 17.9 to 23.5% identical amino acids in common with the human adrenal side-chain cleavage enzyme, the 11β-hydroxylase, 17α-hydroxylase and other P450 enzymes. This low sequence identity indicates that $P450_{arom}$ belongs to a separate gene family, which has been designated CYP19.[45]

3. Rationale for Inhibition of Aromatase

Recognising the unique features of the reaction catalysed by $P450_{arom}$, we postulated that selective inhibition of $P450_{arom}$ might be achieved with substrate analogues.[11] In addition, since estrogen production is the last step (fig. 2, step 5) in the

biosynthetic sequence of steroid production, selective blockade of $P450_{arom}$ does not interfere with the production of other steroids, such as adrenal corticosteroids. For these reasons, $P450_{arom}$ is a particularly suitable enzyme target for selective inhibition. As indicated above in section 2, the low homology of $P450_{arom}$ with other steroidogenic enzymes is consistent with selective inhibition being a reasonable objective.

Following the publication of the first report of aromatase inhibitors from our group in 1973, development of aromatase inhibitors has progressed over the last 26 years.[11] These inhibitors include steroidal and nonsteroidal compounds and are classified as competitive inhibitors (type II), affinity labels and mechanism-based inhibitors (suicide inactivators or irreversible type I inhibitors). Summaries of research studies (basic and clinical aspects) on aromatase inhibitors have been published from 3 international conferences[46-49] and recent excellent reviews on aromatase inhibitors have also appeared.[5,50-52]

3.1 Systems for the Evaluation of Aromatase Inhibitors

Following the design and eventual synthesis of prospective aromatase inhibitors by the chemist, compounds are tested to evaluate their inhibitory potencies and other pharmacological properties towards aromatase enzyme. Consequently, appropriate *in vitro* as well as *in vivo* assays have been developed and are reviewed in this section.

3.1.1 In Vitro Assay

Microsomal preparations from human placenta provide the major source of aromatase,[53] although preparations from rat ovaries have also been utilised.[54] The assay is based on the method of Thompson and Siiteri[2] and depends on the findings by Brodie et al.[55] that the 1β hydrogen atom of androstenedione is eliminated during aromatisation. Tritiated androgens (usually [1β,2β-^{3}H]androstenedione or testosterone, and more recently [1β-^{3}H]androstenedione) are added to the assay buffer (0.1 mol/L potassium phosphate, pH 7.4) in

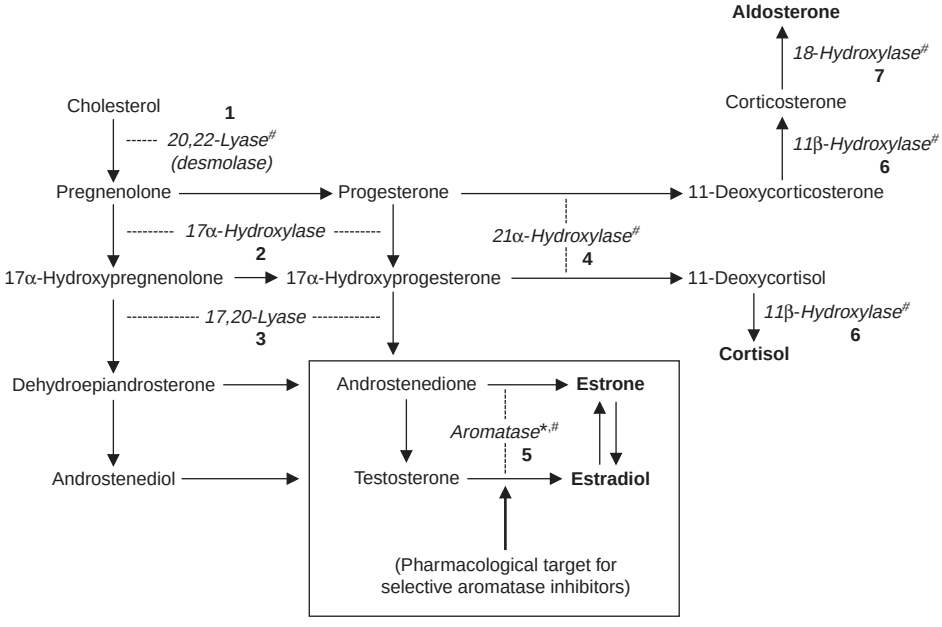


Fig. 2. Pathways of steroidogenesis in humans. Only the cytochrome P-450 enzyme systems are named and numbered; * = site of action of selective aromatase inhibitors; # = site of action of nonselective aromatase inhibitors.

the presence of an NADPH-generating system. After reaction initiation by addition of microsomal aromatase and incubation at 37°C in air, estrogen synthesis is measured indirectly by the amount of tritium released from the 1β position, which forms tritiated water. Utilisation of this assay system in the presence of a prospective inhibitor allows the potency of the inhibitor to be determined. Assays with [1β-^{3}H]androstenedione are now believed to be most appropriate, as significant loss of ^{3}H from the 2α position of the androgen substrates has been reported.[56,57] In addition, since androstenedione is 10-fold better substrate for aromatase than testosterone, assays that utilise testosterone may also not be completely accurate.

Classification of the inhibitor as reversible or irreversible can be made by preincubation of microsomes with inhibitor for various lengths of time, subsequent removal of inhibitor with dextran-coated charcoal, followed by reincubation with substrate and measuring residual enzyme inhibition.[58] Although this is a quick and easy assay for testing inhibitors, the product isolation method should be used to verify the presence of aromatase activity in tissue not previously known to express aromatase. The ^3H_2O assay has been found unreliable in some studies.[59]

Human choriocarcinoma cells, JEG-3 or JEG, have been used to study aromatase inhibitors in cell culture.[60] Although some investigators have used human estrogen-dependent breast cancer cells (MCF-7) for this purpose,[61,62] aromatase activity is generally very low. However, when transfected with the aromatase gene,[63] these cells (MCF-7_{CA}) have substantial aromatase activity. They have proved to be very useful for comparing aromatase inhibitors and determining their K_i values and other enzyme kinetic parameters in cell culture.[64,65] An advantage of using these MCF-7_{CA} cells is that they can be inoculated into athymic (nude) mice and grown as hormone-responsive tumours for studies of the antitumour effects of inhibitors (see later in this section).

The inhibitory potencies of aromatase inhibitors are usually expressed as concentrations giving 50% inhibition (IC_{50}) and/or K_i values. Because the aromatase inhibition assays are performed differently in various laboratories, and a wide range of K_m values for androstenedione have been obtained, inhibition data for the many aromatase inhibitors should be compared with caution. It should be noted that in comparing IC_{50} values, the concentration of the substrate used should be considered, whereas for K_i values the K_m values for the substrate used is important. In the latter case, the K_i/K_m ratio is thought to be a meaningful and appropriate indicator of activity. In addition, it may be useful to determine and compare the inhibitory potencies of prospective aromatase inhibitors with that of a known potent aromatase inhibitor under the same assay conditions.

3.1.2 In Vivo Assay

Compounds that show potent inhibition of aromatase in the *in vitro* assay are typically studied further in *in vivo* animal models. Their efficacy depends on: (i) aromatase enzyme turnover or ligand (aromatase inhibitor)/substrate concentrations; and (ii) the pharmacokinetics of the aromatase inhibitor.

Inhibition of Ovarian Ovulation

Determining inhibition of estrogen synthesis in the female rat is complicated by gonadotropin feedback regulation. For this reason, we used rats in proestrus to determine the effects of aromatase inhibitors in our early studies.[11] Animals with regular cycles were treated with aromatase inhibitors prior to the day of proestrus, then on the afternoon of the proestrus surge when estrogen levels were expected to be maximum, blood was collected from the ovarian vein by cannulation and serum assayed for estrogen concentrations by radioimmunoassay.[11,63]

We also developed a rat ovarian model using injections of gonadotropins to override cyclic fluctuations and to stimulate ovarian aromatase. The ovary luteinises and aromatase activity and estrogen secretion are maintained for several days at high levels. This model proved useful for studying direct effects of aromatase inhibitors on ovarian aromatase activity and estrogen secretion.[54,66]

Antitumour Effects

Inhibition of ovarian aromatase activity (estrogen biosynthesis), as well as the antitumour effects of aromatase inhibitors have been determined in female rats. The carcinogen 7,12-dimethylbenzanthracene (DMBA) is able to induce multiple mammary tumours 6 to 8 weeks following administration.[67] Since about 90% of these tumours are dependent on estrogens from the ovary, the ability of aromatase inhibitors to cause regression or arrest progression of tumour growth in these rats can be studied.[11,68] This model represents the premenopausal patient with breast cancer. However, the system is complicated by feedback processes which increase secretion of gonadotropins and ovarian aromatase activity. This can result in high estrogen levels that may override inhibition by some aromatase inhibitors.[69]

We recently developed another estrogen-dependent model for the evaluation of aromatase inhibitors which is more appropriate for the postmenopausal patient with breast cancer.[70] This model utilises human estrogen-dependent breast cancer cells transfected with the aromatase gene (MCF-7_{CA}). These cells are inoculated subcutaneously with Matrigel into ovariectomised nude mice. The cells produce sufficient estrogen by aromatisation to stimulate their proliferation and formation of tumours in the mice. In addition, the uterus is maintained in a condition similar to an intact animal. The model has proved useful in evaluating the antiestrogens.[71] Recently, the possibility that aromatase inhibitors used in combination with antiestrogens may be more effective than either treatment alone was investigated. However, the studies demonstrated no synergistic or additive effects with the combined treatment compared with aromatase inhibitors alone, although the combination was more effective than the antiestrogen alone. Although aromatase inhibitors did not show estrogenic effects on the uterus, when combined with tamoxifen the uterine weight was only slightly less than with tamoxifen alone.[72] The results suggest that antiestrogens and aromatase inhibitors are likely to be more beneficial when used in sequence, thus providing a longer duration of tumour response.

Peripheral Aromatisation

A direct and sensitive assessment of aromatase inhibitors on peripheral aromatisation *in vivo* is provided by procedures in which the impact of aromatase inhibitors on the level of aromatase enzyme and its activity are measured directly. The most appropriate animal model for this assay is the male rhesus monkey in which most of the circulating estrogen is extragonadal in origin.[73,74]

Following a continuous infusion of [7-^{3}H]androstenedione as labelled substrate and [4-^{14}C]estrone as an internal standard, both the rate and extent of conversion of androstenedione to estrone and estradiol in plasma can be measured.[75] This procedure has been used with some minor modifications to measure the extent of inhibition of aromatase activity in patients with breast cancer.[76-85] Differences in results between plasma levels and aromatisation indicated greater suppression when measured by peripheral aromatisation methods. This finding and the advent of very potent inhibitors led to the development of more sensitive methods for measuring serum/plasma concentration.

Serum Estrogen Levels

Circulating estrogen levels are low following menopause. Thus, accurate measurements following treatment with aromatase inhibitors requires highly sensitive assays. Studies with the new highly potent inhibitors necessitated development of improved methods.[86,87] Although serum levels are a useful indicator of circulating estrogens, they may not reflect local levels in the environment of the breast. Only a few studies have addressed this issue to date,[88] although levels of hormones in breast tissue have been studied in other contexts.[24,25]

3.1.3 Antitumour Assessment of Aromatase Inhibitors in Patients with Breast Cancer

Response to treatment with aromatase inhibitors is usually evaluated according to the International Union Against Cancer or the European Organization for Research and Treatment of Cancer

criteria.[89,90] It has been argued that stricter application of these response criteria leads to much lower response rates than those expected and reported in the past for the early aromatase inhibitors.

3.2 Classification of Aromatase Inhibitors

The chemical structures of prominent aromatase inhibitors in clinical trials and presently available are depicted in figure 3; these have been broadly classified as steroidal and nonsteroidal.

3.2.1 Steroidal Aromatase Inhibitors

Steroidal compounds that are structurally related to the natural substrate of aromatase make up about 80% of the aromatase inhibitors reported. A common and crucial feature of all 4 of the steroidal inhibitors evaluated clinically (formestane, exem-

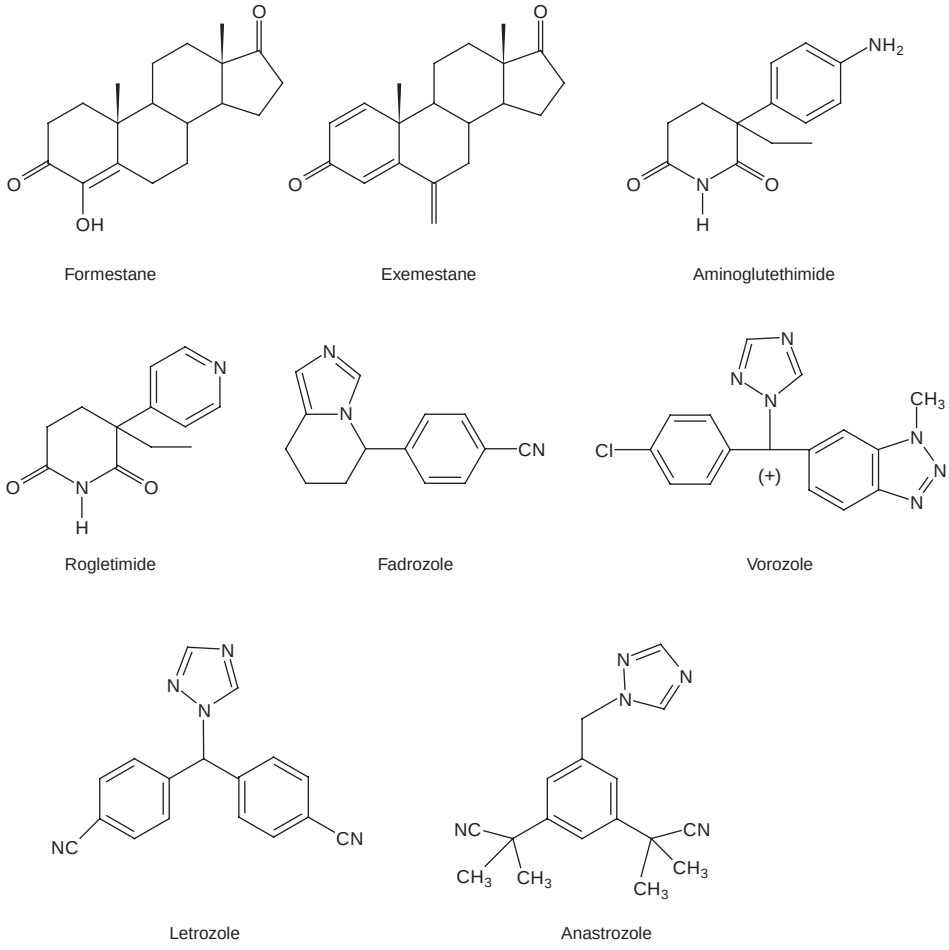


Fig. 3. Structures of aromatase inhibitors in breast cancer therapy. Formestane (4-androstenedione, 4-OHA); exemestane (6-methyleneandrosta-1,4-diene-3,17-dione, FCE-24304); aminoglutethimide; rogletimide [pyridoglutethimide, PyG, 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione], fadrozole [4-(5,6,7,8-tetrahydroimidazo[1,5I]pyridine-5-yl)benzonitrile monohydrochloride, CGS 16949A]; vorozole [6-[(4-chlorophenyl)-(1H-1,2,4-triazol-1-yl)methyl]-1-methyl-1H-benzotriazole, R-83842)]; letrozole [4-[1-(cyanophenyl)-1-(1,2,4-triazolyl)methyl]benzonitrile, CGS-20267]; and anastrozole [2,2′-[5-(1H-1,2,4-triazol-1-yl-methyl)-1,3-phenylene]bis(2-methylpropiononitrile), ZD-1033].

estane, atamestane and 10-propargylandrostenedione) is their ability to inactivate aromatase (they are usually referred to as 'suicide inactivators'). Suicide inactivators are thought to compete with the natural substrate and subsequently interact with the active site of the enzyme. They bind either very tightly or irreversibly to the enzyme, thus causing its inactivation.[91] Because they bind irreversibly to the enzyme due to covalent modification at the active site, these inhibitors are quite specific and have lasting effects *in vivo*. Thus, the continued presence of the drug to maintain inhibition is not necessary when using type I (suicide) inhibitors, and the chance of toxic adverse effects to the patient will therefore be reduced.

However, it should be noted that the vast majority of steroidal inhibitors are unlike the suicide inactivators, as they interact with the substrate-binding site of the enzyme in a competitive and reversible manner. Indeed, these types of inhibitors have not shown promise as candidates for clinical studies because of poor *in vivo* activity.[5] It is tempting to speculate that because aromatase has a relatively low K_m, high dose levels may be needed even for reversible inhibitors with apparently low K_i values. This assertion seems to apply only to the steroidal compounds when one considers the results of clinical studies with the nonsteroidal compounds which, although intrinsically reversible aromatase inhibitors, cause substantial suppression of estrogen synthesis (see section 4.2). The relatively short plasma half-lives of the reversible steroidal inhibitors may be the main reason for their mediocre efficacy *in vivo*.

Based on the current status of knowledge of studies (*in vitro* and *in vivo*) on steroidal aromatase inhibitors, their ability to inactivate aromatase seem to be crucial. This property is clearly illustrated by the results of preclinical studies with 2 steroidal compounds of similar structure, 6α-bromoandrostenedione, a competitive and reversible inhibitor of aromatase (K_i 3.4 nmol/L) and its 6β-epimer, a mechanism-based irreversible inactivator (K_i 0.8 μmol/L; K_{inact} 0.025 min^{-1}). Thus, Tochigi and colleagues[92] administered 6β-bromoandrostenedione to rats and found a 64% inactivation of ovarian aromatase activity compared with the control animals, whereas the 6α-bromoandrostenedione was ineffective at the same dose. It should be noted that the 6α-epimer is at least 235 times stronger than the 6β-epimer as an inhibitor of aromatase *in vitro*.[93] Clearly, therefore, the focus in the future design of prospective steroidal aromatase inhibitors should be directed towards compounds that may inhibit aromatase irreversibly.

3.2.2 Nonsteroidal Aromatase Inhibitors

The nonsteroidal inhibitors possess a heteroatom (usually of a nitrogen-containing heterocyclic moiety) as a common feature that interferes with steroidal hydroxylation by binding with the haem iron of cytochrome $P450_{arom}$. Like most steroidal inhibitors, these compounds are reversible inhibitors of aromatase. Although it is generally believed that most nonsteroidal type II inhibitors are less enzyme specific and will inhibit, to varying degrees, other cytochrome P450-mediated hydroxylations in steroidogenesis, the nonsteroidal inhibitors shown in figure 3, except for aminoglutethimide, are highly selective for aromatase. Since not all of the prospective nonsteroidal compounds that have been tested as inhibitors of aromatase are potent inhibitors of the enzyme, it seems reasonable to believe that the nature of non-heterocyclic moiety is also important. This portion of the molecule may interact with aromatase via hydrogen and/or van der Waals bonding. The degree of compatibility or synergism between binding to the haem iron and interaction with the protein residue may also be crucial.

4. Pharmacology and Clinical Efficacy of Aromatase Inhibitors

4.1 Early Inhibitors Used Clinically

Although a large number of aromatase inhibitors have been developed over the past 26 years, unfortunately only a relatively small number of these compounds are in clinical trials or clinically available. Prior to the advent of the aromatase inhibitor field in the early 1970s, 2 compounds, tes-

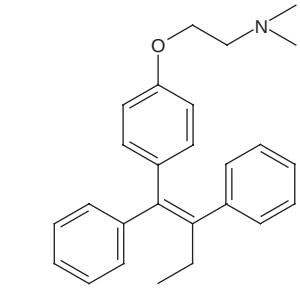
tolactone and aminoglutethimide, were being used for breast cancer treatment that later were found to be weak aromatase inhibitors. Testolactone has been used in the treatment of breast cancer since 1960,[94] but did not have a well established mechanism of action until the report of its inhibition of aromatase in 1979.[1,2,95] Aminoglutethimide became available for treatment of the same disease in 1967 because of its ability to inhibit adrenal steroid formation.[95,96] Subsequently, aminoglutethimide was found to reduce estrone concentrations while androstenedione levels were concomitantly increased.[97] Because of its availability and superior efficacy to testolactone in breast cancer therapy, aminoglutethimide has had an important place in demonstrating the utility of the concept of aromatase inhibition. Although aminoglutethimide exhibits similar efficacy in patients with breast cancer to the selective aromatase inhibitors (see section 4.2), it has significant adverse effects.[98] These properties of aminoglutethimide are in part due to its lack of specificity and its inhibition of multiple P450 enzymes, notably 11β-hydroxylase. Patients receiving aminoglutethimide therefore require concomitant use of cortisol replacement therapy.[97] Other adverse effects include somnolence and rashes. However, several additional effects were observed in patients. These included enhanced conversion of Δ^5 to Δ^4 steroids[99] and reduction of plasma levels of estrogen sulfate by increasing steroid metabolism in the liver.[100] These latter effects may occur as a result of induction of hepatic cytochrome P450 mixed function oxidases by aminoglutethimide.[101]

4.2 Selective Aromatase Inhibitors

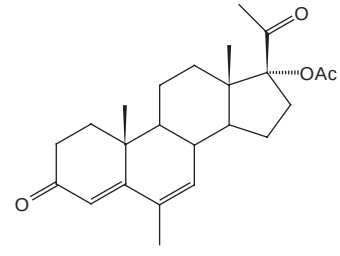
Significantly, the potential of specific and potent aromatase inhibitors discovered in the early 1970s (e.g. formestane)[11,54] for the treatment of advanced breast cancer in postmenopausal women provided the stimulus that led to the discovery of other aromatase inhibitors. In addition, following the recognition that aminoglutethimide was a nonspecific inhibitor of several steroidogenic P450 enzymes, including aromatase, the quest began for more selective and potent nonsteroidal aromatase inhibitors. In this section we only discuss in detail the pharmacology and clinical potential/efficacy of those compounds that have been studied in clinical trials or are approved for the treatment of breast cancer. These include formestane, rogletimide, fadrozole, vorozole, anastrozole and letrozole. These compounds have been given to sufficient numbers of patients with breast cancer to allow meaningful evaluation of the clinical data. The structures of these compounds are depicted in figure 3. The structures of the antiestrogen tamoxifen and of the progestin megestrol are shown in figure 4; their clinical efficacies in patients with breast cancer are usually compared with those of the aromatase inhibitors.

4.2.1 Formestane

During the testing of prospective aromatase inhibitors,[54] formestane was one of the first discovered as a potent and specific inhibitor of aromatase with good endocrine effects.[11,54] *In vitro*, it interacts with human placental aromatase with an



Tamoxifen



Megestrol

Fig. 4. Structures of tamoxifen and megestrol.

apparent K_i of 10.2 nmol/L, causing a rapid and irreversible inactivation with a K_{inact} of 0.41×10^{-3} s^{-1},[58,102] and it is referred to as a suicide inhibitor. Compared with aminoglutethimide, it is approximately 60 times more potent.[58,66] *In vivo*, formestane 50 mg/kg given subcutaneously twice daily almost completely inhibits ovarian and extragonadal estrogen synthesis and significantly reduces the growth of estrogen-dependent mammary tumours in rats.[66,103] These promising preclinical results[12,58,66,73] and initial clinical studies[13,14] provided the basis for subsequent clinical trials of formestane and its approval as treatment in postmenopausal women with breast cancer.

Clinical studies show that this compound reduces peripheral aromatisation and plasma estrogen levels.[77,104] Two different dosages of formestane administered by injection were evaluated in over 560 postmenopausal patients with breast cancer with advanced disease at different centres. Patients were either estrogen receptor–positive or of unknown receptor status and had relapsed from other hormonal treatments. Patients received formestane either as a 500mg intramuscular injection weekly, 500mg intramuscularly every 2 weeks or 250mg intramuscularly every 2 weeks. Comparison between the 250mg and 500mg intramuscular doses administered every 2 weeks showed similar degrees of estrogen suppression. The overall response rates observed in several studies were complete or partial tumour regression in 25 to 39% of patients, disease stabilisation in 22% of patients[105-107] and disease progression in the remaining women. When formestane 250 mg/day was administered orally to patients, the response rates were similar to those of patients receiving biweekly injections of 250 and 500mg.[108] Although there was no significant difference in response rates between the 2 routes of administration, recent studies have found that inhibition of peripheral aromatisation was less with oral formestane than with formestane administered by injection.[104] Biweekly injections of both dosages of formestane were very well tolerated, with mild adverse effects occurring in 17% of patients. Local reactions, usually sterile abscesses, occurred in less than 10% of the patients and were mainly a feature of the higher injected dose (500mg). Only 3 to 5% of patients discontinued treatment. The differences in estrogen reduction between the treatment groups were minor and there were no significant differences in clinical efficacy. Therefore, 250mg injected every 2 weeks, which results in fewer local reactions than the 500mg dose, is recommended for advanced breast cancer treatment in postmenopausal women.[109]

The lack of response of some patients to formestane treatment appears to be due to loss of hormone sensitivity of their tumours rather than to suboptimal dosages of formestane. Serum estradiol levels in patients were found to be suppressed by formestane without apparent differences between dosage regimens, and these suppressed levels were maintained for at least several months.[104] It therefore seems likely that tumours remained sensitive to estrogens in those patients who had relapsed from tamoxifen but subsequently responded to formestane treatment.[110] Several mechanisms by which tumours may become resistant to tamoxifen, yet retain hormone responsiveness, have been proposed.[111,112] Although most patients treated with aromatase inhibitors in studies to date have previously received tamoxifen as first-line therapy, a few studies of aromatase inhibitors in postmenopausal patients with breast cancer previously untreated with endocrine therapy have been conducted. No significant difference in response rates was found between formestane and tamoxifen when used as first-line therapy, although patients relapsed sooner from formestane.[113] Formestane is now approved in many countries for advanced cancer in postmenopausal women, making it the first selective aromatase inhibitor to become available and at the time was the first new treatment for breast cancer in 10 years.

4.2.2 Exemestane

Exemestane is another potent steroidal irreversible aromatase inhibitor.[114] *In vitro*, exemestane causes time-dependent irreversible inhibition of human placental aromatase with a half-life ($t_{½}$) of

13.8 minutes and a K_i of 26 nmol/L, and was found to be as potent as formestane (K_i 29 nmol/L under the same assay conditions).[115] It was also demonstrated that exemestane was highly specific for aromatase, as it did not inhibit desmolase or 5α-reductase.[115] *In vivo*, in rats with DMBA-induced tumours, exemestane 3 to 100 mg/kg subcutaneously daily caused potent inhibition of ovarian aromatase and significantly suppressed the growth of established tumours.[116,117] In addition, this study also showed a clear preventative effect on the appearance of new tumours during the treatment period. In another *in vivo* study,[118] both subcutaneous and oral exemestane were shown to be highly effective at inhibiting the growth of DMBA-induced mammary tumours in ovariectomised testosterone-treated rats (a model of postmenopausal mammary tumours). These encouraging preclinical studies have led to a number of clinical evaluations.

In a phase I clinical trial when single doses of exemestane 0.5 to 800mg were administered to healthy postmenopausal women, plasma estrogens were significantly (61 to 72%) suppressed; maximal estrogen suppression was attained with a dose of 25mg.[119] A similar observation of sustained suppression of plasma estrogens was made in postmenopausal patients with breast cancer during long-term treatment with exemestane 2.5 to 25 mg/day.[120] Following a suspicion that metabolites of exemestane may cross-react with antibodies in the radioimmunoassays and thus interfere with assays of plasma and urinary determinations, Johannessen et al.[121] developed a very specific and highly sensitive analytical method for measuring estrogen levels. Using this method, they found that long-term treatment with exemestane suppressed plasma and urinary estrogens by 85 to 94%.[121] These studies enabled them to recommend the drug dosage of 25 mg/day currently used in phase II and phase III trials.

Two large multicentre, multinational phase II studies have recently been conducted in patients failing to respond on tamoxifen treatment. Of a total of 265 patients enrolled, 202 were available for evaluation of response.[84] The overall objective response rate was 22% and disease stabilisation (≥24 weeks) was observed in an additional 24% of cases. Thus, 46% of patients benefited from the treatment. In another study,[84] the response to exemestane 200mg once daily was evaluated in 62 of 80 patients enrolled after progressing on treatment with a high dosage of aminoglutethimide (≥500mg once daily with glucocorticoids). A total of 24% of the patients obtained an objective response to exemestane therapy, whereas an additional 24% achieved stable disease for more than 24 weeks. These results are similar to those of other studies showing a lack of cross-resistance to aminoglutethimide and formestane.[122,123] Although exemestane is generally well tolerated, a number of mild to moderate adverse effects have been reported, including androgenic effects (hypertrichosis, hair loss, hoarseness and acne), hot flushes, increased sweating and nausea.

Studies are in progress to confirm the response to exemestane in patients relapsing on aminoglutethimide, some new nonsteroidal aromatase inhibitors or megestrol. In addition, a phase III trial comparing the response rate to exemestane 25mg once daily with treatment with megestrol is ongoing in Europe and the US.[84] Exemestane, an orally active, selective and long-lasting steroidal irreversible inhibitor of aromatase, is expected to become available soon for the treatment of patients with breast cancer.

4.2.3 Rogletimide

Since 1983, several groups have engaged in the synthesis of aromatase inhibitors that are structurally related to aminoglutethimide. Consequently, Jarman's group discovered rogletimide (pyridoglutethimide).[124] This compound is slightly less potent than aminoglutethimide, with K_i values of 1.1 and 0.6 μmol/L, respectively, but unlike aminoglutethimide does not inhibit desmolase.[124,125] This means that rogletimide can be used without cortisol replacement. Indeed, preclinical tests in mice demonstrated that rogletimide lacks the sedative and other CNS effects of aminoglutethimide.[126] *In vivo* in the rat, rabbit and in humans,

rogletimide is metabolised mainly to rogletimide N-oxide, which was shown to be inactive against aromatase. Thus, rogletimide does undergo metabolic inactivation *in vivo*, but to a lesser extent than observed with aminoglutethimide.[127]

In a clinical study that involved 4 postmenopausal women with breast cancer receiving rogletimide 500mg twice daily for 3 to 4 days, estradiol levels fell to about 31% of baseline values within 48 hours and remained suppressed during treatment.[128] Dowsett and colleagues[129] demonstrated in a study of 10 postmenopausal patients with breast cancer that significant suppression of estradiol occurred at a rogletimide dosage of 200mg twice daily with no significant additional suppression at higher dosages, i.e. 400, 800 or 1000mg. Although there were no significant endocrine effects at any dosage on the serum levels of cortisol, aldosterone, luteinising hormone, FSH, prolactin, sex hormone binding globulin or thyroid-stimulating hormone, there were dosage-related adverse effects, the most frequent being nausea and lethargy. Only 1 patient (10%) in this group showed an objective response to treatment.

A more recent study[130] with oral rogletimide 300 and 400mg twice daily showed serum and urine estrogen levels suppressed to less than 50% of baseline without effects on other serum steroid hormones. Adverse effects noted at both dosages were anorexia, nausea, heartburn/epigastric discomfort, fatigue, hot flashes, dizziness and lightheadedness.

Additional phase II trials have also been reported. In a study by Schulz et al.[131] that involved administration of rogletimide 300mg twice daily to 68 postmenopausal women with advanced/metastatic breast cancer, the highest objective response rate observed was only 14%, but 34% of the patients had their disease stabilised. The adverse effects were headaches and gastrointestinal symptoms. In another phase II study of rogletimide 200mg twice daily in 90 heavily pretreated patients with breast cancer, the results were disappointing; the objective response rate was only 4% and more adverse effects were reported.[132] These relatively low response values for rogletimide may preclude its further clinical evaluation as a prospective drug for treatment of breast cancer.

4.2.4 Fadrozole

Fadrozole is a second-generation nonsteroidal reversible inhibitor of aromatase. *In vitro*, it is a potent inhibitor of human placental aromatase with a K_i value of 1.6 nmol/L, making it approximately 400 times more potent than aminoglutethimide.[133] Selectivity towards aromatase was indicated by the significantly higher concentration of fadrozole required to inhibit other related steroidogenic P450 enzymes.[133] However, it is now known that fadrozole also inhibits 11β-hydroxylase and 18-hydroxylase, enzymes involved in the synthesis of cortisol and aldosterone.[134,135]

The results of *in vivo* studies with fadrozole in animals are impressive. Fadrozole 0.26 mg/kg orally almost completely reduced ovarian estrogen production in gonadotropin-primed rats.[133] Furthermore, at an oral dosage of 1 to 8 mg/day, fadrozole caused almost complete regression of hormone-dependent DMBA-induced mammary tumours in rats.[136]

A number of clinical trials have been conducted with fadrozole. Phase I studies reported excellent tolerability.[137-139] Fadrozole 2mg daily caused maximal estrogen suppression in postmenopausal patients.[138,139] In one study, Santen and colleagues[137] showed that aldosterone suppression occurred only at substantially higher dosages than those required for maximal estrogen suppression (8 and 16mg daily). Two phase II studies did not show a significant difference in toxicity or response between 1 and 4mg daily in one study or between 1, 2 and 4mg daily in the other study.[140,141] In contrast, clinical studies by Dowsett and colleagues[142,143] with fadrozole indicated a dosage-related suppression of estradiol levels between the dosages of 0.6 and 4mg daily and that aldosterone levels were suppressed by approximately 50% at the 4 mg/day dosage.

In order to clarify these discrepancies, Dowsett and colleagues have recently conducted 2 additional studies with fadrozole. A double-blind ran-

domised endocrine study of 3 doses of fadrozole (0.5, 1 and 2mg, all twice daily) was conducted in 80 (68 evaluable) postmenopausal patients with advanced breast cancer over a period of 3 months.[144] It was concluded that fadrozole achieved near-maximal suppression of estrogens at 1mg twice daily, and that its effects on aldosterone synthesis are unlikely to be of clinical significance. In the second study the objective was to evaluate response, time to treatment failure, duration of response and tolerability.[145] This randomised study involved the oral administration of fadrozole 0.5, 1 or 2mg twice daily to 80 postmenopausal patients with recurrent breast cancer after tamoxifen failure. The objective response rate was 17%. 15 patients (21%) had stabilised disease and 45 patients (63%) had progressive disease. There was no statistical difference between the dosage groups. The main adverse events were of mild to moderate severity: nausea in 11 patients (13.75%), hot flushes in 4 (5%) and somnolence in 3 (4%). These results revealed that fadrozole is a clinically active aromatase inhibitor with a low incidence of adverse effects.

Another randomised phase II study of fadrozole in postmenopausal patients with metastatic breast cancer has recently been reported by Miller and colleagues.[146] Three treatment groups received, respectively, oral fadrozole 0.6mg 3 times daily, 1mg twice daily and 2mg twice daily. The results were identical to those of Dowsett et al.[145] and confirm the modest activity of fadrozole in heterogeneous groups of patients with breast cancer at 3 different dosage levels.

A large randomised double-blind multi-institutional phase III trial[147] has been completed comparing fadrozole 1mg twice daily and megestrol 40mg 4 times daily in a total of 683 postmenopausal patients with metastatic breast cancer after progression from first-line hormonal therapy. It was concluded that fadrozole was as efficacious as megestrol in this group of patients. Adverse events were mild with both therapies, but megestrol was associated with higher frequency of bodyweight gain, fluid retention and dyspnoea, whereas fadrozole was associated with a higher frequency of nausea and vomiting.

Optically pure (–)-fadrozole is useful for the treatment of estrogen-dependent breast cancer and reduces the adverse effects associated with racemic fadrozole.[148]

4.2.5 Vorozole

Vorozole, a chiral nonsteroidal triazole derivative, is a potent and selective competitive inhibitor of aromatase. It is the (+)- (*d*-) enantiomer of the racemic mixture (R-76713). Although the aromatase inhibitory activity resides primarily in the (+)-enantiomer (R-83842), preclinical as well as clinical studies have also been conducted with racemic vorozole and to a lesser extent with the (–)-enantiomer (R-83839).[149-159] Racemic vorozole, (+)-vorozole and (–)-vorozole have K_i values for human placental aromatase of 1.3, 0.7 and 18 nmol/L, respectively. Our discussion will focus on (+)-vorozole and racemic vorozole.

Vorozole is approximately 1029 times more potent than aminoglutethimide in the human placental aromatase system,[149] is highly selective for aromatase[153] and does not suppress aldosterone production in rats.[154] In hormone-dependent DMBA-induced rat mammary tumours, vorozole 2.5 mg/kg twice daily reduced tumour growth to an extent equivalent to ovariectomy.[151]

In vivo peripheral aromatisation in healthy postmenopausal women was inhibited by 93, 93.2 and 94.4% by doses of 1.0, 2.5 and 5.0mg, respectively, of racemic vorozole.[155] These doses may be considered equivalent to 0.5, 1.25 and 2.5mg, respectively, of vorozole itself as the (–)-enantiomer has previously been shown to be inactive *in vivo* in rats.[151] In healthy male volunteers, a single oral dose (5 or 10mg) of racemic vorozole lowered plasma estradiol levels to the detection limit of the assay 4 and 8 hours following administration.[154,156] Dowsett and colleagues[157] have shown from their studies with patients with breast cancer a significant trend to increasing estradiol suppression with increasing doses of vorozole ranging from 1 to 5mg, but no significant difference between 2.5 and 5mg. These studies enabled clinicians to establish

a vorozole dosage of 2.5 mg/day for phase II clinical trials.

A phase II clinical study has been conducted of 29 patients with breast cancer with estrogen receptor–positive or unknown status who were treated with oral vorozole 2.5mg once daily.[158] Of the 27 patients evaluated, 3 patients (11%) had partial remission of their disease and disease stabilisation was observed in 50% of patients. The adverse effects were mild and included malaise, anorexia and nausea, hot flashes, fluid retention, vaginal infection, alopecia, lightheadedness and 1 allergic reaction which caused lip swelling. In a recent study,[159] 34 postmenopausal patients previously treated with tamoxifen in the adjuvant setting and/or for advanced disease were treated with vorozole 2.5mg once daily. The overall objective response rate was 21%. Tolerability was excellent to good in 97% of the patients, and the adverse effects were mild. Another phase II study involving 27 postmenopausal patients with advanced breast cancer treated with vorozole 2.5 mg/day has been completed with improved results.[160] All patients had been previously treated with tamoxifen as adjuvant (2 patients) or for advanced disease (24 patients), or both (1 patient). The overall objective response rate was 30%, and 33.3% of the patients had disease stabilisation. Treatment was very well tolerated, with mild adverse effects. From these results it was concluded that vorozole is an active second-line endocrine treatment deserving consideration for randomised comparison with other established agents (antiestrogens) used for estrogen therapy of breast cancer.

In 2 large phase III studies,[161,162] vorozole 2.5 mg/day demonstrated favourable clinical efficacy compared with aminoglutethimide and megestrol. Vorozole improved patient quality of life to a greater extent than aminoglutethimide, and appeared to be at least as well tolerated as megestrol but was better than aminoglutethimide. The most common adverse events with vorozole in this study were hot flushes and nausea, which were generally mild in severity. A comprehensive review of important studies (preclinical and clinical) on vorozole has appeared recently.[163] However, further development of vorozole has been halted.

4.2.6 Anastrozole

Anastrozole, an achiral benzyl triazole derivative, is a potent and selective aromatase inhibitor with an IC_{50} value of 15 nmol/L against human placental aromatase.[164] *In vivo* studies of ovarian aromatase activity in the rat show that an oral dose of anastrozole (0.1 mg/kg given on day 2 or 3 of the estrous cycle) completely blocked ovulation in the mature female and androstenedione-stimulated uterine development in prepubertal females. These effects have been attributed to inhibition of the normal preovulatory rise in ovarian follicular estrogen synthesis in mature females and inhibition of metabolism of the exogenous androstenedione by the immature ovary in prepubertal females. Twice-daily oral administration of ≥0.1 mg/kg of anastrozole to male pigtail monkeys inhibited peripheral aromatase, reducing circulating estradiol concentrations by 50 to 60%.[164] The selectivity of anastrozole for aromatase was also demonstrated as it did not interfere with the production of steroid hormones by other related cytochrome P450-dependent enzymes in a variety of pharmacological studies in the rat, dog and monkey.[164]

Anastrozole has been subjected to a broad range (at least 20 phase I studies) of rigorous studies in humans to determine its pharmacodynamics, pharmacokinetics and safety.[164-167] Because of its impressive pharmacological and clinical properties in breast tumours, it has now been extensively evaluated in the treatment of postmenopausal women with advanced breast cancer. In 2 recent studies of anastrozole, oral dosages of 1 and 10mg daily were compared with megestrol 40mg 4 times daily in postmenopausal patients with advanced breast cancer.[168,169] The 1mg dose of anastrozole was chosen because it was the lowest dose producing maximal suppression of serum estradiol concentrations. The 10mg dose was used because it had the potential for greater efficacy without additional toxicity. However, after a median follow-up of 6.1 months, there was no significant difference between the 2 dosages of anastrozole.[169] Com-

plete or partial responses lasting 3 to 8 months occurred in 10.3% of patients receiving anastrozole 1mg, 8.9% of patients receiving anastrozole 10mg and 7.9% of patients receiving megestrol. The disease was stabilised in 25.1, 22.6 and 26.1% of patients, respectively. Responses were observed in patients who progressed after receiving adjuvant tamoxifen as well as in patients who received tamoxifen for advanced disease. The main advantage of anastrozole was tolerability. Although all treatments were well tolerated, patients receiving megestrol experienced more bodyweight gain. Significant bodyweight gain in up to 64% of patients treated with megestrol has been reported.[170] Gastrointestinal disorders were more frequent with anastrozole but were usually in the form of mild transient effects. This resulted in a low (3%) incidence of withdrawal. In the entire clinical programme, there was no evidence that increasing patient age adversely affected the tolerability of anastrozole. As a consequence, no dosage modification is recommended for elderly patients.

Although anastrozole 10 mg/day was not shown to produce any additional clinical benefit over the 1 mg/day dosage, the tolerability profile was very similar to that of the lower dosage. Thus, the good tolerability of anastrozole 10 mg/day provides an excellent therapeutic margin for tolerability and selectivity over the recommended dosage of 1 mg/day. After 31 months, there was a significant improvement in overall survival of patients receiving anastrozole 1 mg/day in comparison with those receiving megestrol (median overall survival 26.7 months versus 22.5 months, respectively).[171] Anastrozole, a highly selective, once-daily, orally active nonsteroidal aromatase inhibitor, appears to be beneficial in postmenopausal women with advanced breast cancer. Anastrozole has recently been approved by the US Food and Drug Administration (FDA) and a number of other regulatory agencies around the world for this indication.[172] A review of its use in the management of postmenopausal women with advanced breast cancer has recently appeared.[173]

4.2.7 Letrozole

Letrozole is another potent nonsteroidal achiral triazolyl inhibitor of aromatase, with an IC_{50} value of 11.5 nmol/L against human placental aromatase.[174] It is highly selective for aromatase as it does not affect adrenal steroidogenesis *in vitro* or *in vivo* at concentrations and doses several orders of magnitude higher than those required to inhibit estrogen synthesis. In addition, it is approximately 200 and 10 000 times as potent as aminoglutethimide *in vitro* and *in vivo*, respectively.[174,175] In animal models, letrozole has been shown to cause almost complete regression of estrogen-dependent DMBA-induced mammary tumours.[175]

Phase I studies were conducted in healthy volunteers[176,177] and in postmenopausal patients with advanced breast cancer.[178,179] These have shown letrozole to be effective in suppressing estrogen and estradiol levels by more than 75 to 80% at 3 dosages of 0.1, 0.5 or 2.5 mg/day with no clinically relevant effects on other endocrine steroid hormones. Objective response rates of approximately 25% were recorded in these groups of patients after failure of previous therapy. Tolerability was excellent, with minimal adverse effects.[178-180]

A phase II study with letrozole in a larger group of 63 postmenopausal Japanese women with advanced or recurrent breast cancer has also been conducted.[181] Objective responses were observed in 28% of patients receiving 0.5 mg/day and in 39% of those receiving 1 mg/day. Stable disease was seen in 41% and 40% of patients in the 2 groups, respectively; few adverse events were reported. Letrozole, unlike anastrozole, seems to produce more responses at a higher dosage. A large, double-blind, multicentre trial (the AR/BC2) has been completed comparing letrozole 0.5mg once daily, letrozole 2.5mg once daily and megestrol 160mg once daily in a total of 551 postmenopausal women with advanced breast cancer previously treated with antiestrogens.[182,183] Letrozole 2.5mg once daily was statistically superior to megestrol in overall tumour response rate and time to treatment failure. Megestrol was associated with significantly more adverse experiences, which resulted in more pa-

tients withdrawing from treatment than with either dosage of letrozole. Megestrol also caused a ≥10% increase in bodyweight in more patients. In addition, there was a significantly greater benefit of the 2.5mg letrozole dosage compared with the 0.5mg dosage in terms of objective response rates, time to progression, time to treatment failure, and survival. These results suggest that letrozole is more effective and better tolerated than megestrol.

In a recent non-blind study,[184] 555 patients with advanced breast cancer previously treated with antiestrogens were randomly assigned to receive daily letrozole 2.5 or 0.5mg, or aminoglutethimide 250mg with hydrocortisone or cortisone acetate replacement. After 33 months, the overall objective response rates and median duration of response were 17.8% and 23.2 months for letrozole 2.5mg, 16.7% and 17.5 months for letrozole 0.5mg, and 11.2% and 12.3 months for aminoglutethimide. Letrozole was significantly superior to aminoglutethimide for time to progression. These results suggest that oral letrozole 2.5 mg/day can be recommended for the treatment of patients with advanced breast cancer. Letrozole received FDA approval in December 1996.

5. Discussion

The results of 2 types of randomised clinical trials with some aromatase inhibitors are summarised in table I. First, formestane[185] and fadrozole[148] are each compared with tamoxifen as first-line treatment for metastatic breast cancers. These data suggest that there is no significant difference between tamoxifen and either formestane or fadrozole as first-line endocrine therapeutic agent, and that there is no clear advantage to use of one drug over the other in terms of responses and duration of treatment, although duration of response appears to be longer with tamoxifen. Secondly, fadrozole, vorozole, anastrozole and letrozole are also each compared with megestrol and/or aminoglutethimide after tamoxifen failure in postmenopausal women with breast cancer, i.e. in second-line endocrine therapy.[161,162,168,182,183,186] Here,

Table I. Results of randomised phase III clinical studies of major aromatase inhibitors

Aromatase inhibitor	Eligibility	Dosage	Responders[a]/total	Response rate[a] (%)	Stable disease (%)	Time to progression (months)
Formestane[185]	First-line ER+/?	Formestane (intramuscular 250mg/2wk)	57/173	33	31	7.1
		Tamoxifen (30 mg/day)	65/175	37	34	9.8
Fadrozole[186]	First-line	Fadrozole (1mg bid)	21/103	20	54	5.2
		Tamoxifen (20 mg/day)	29/106	27	49	5.4
Fadrozole[147]	Second-line ER+/?	Fadrozole (1mg bid)	43/345	12	24	5.3
		Megestrol (40mg qid)	40/284	14	28	5.8
Vorozole[161]	Second-line	Vorozole (2.5 mg/day)	21/190	11	–	2.7
		Megestrol (40mg qid)	15/185	8	–	3.6
Vorozole[162]	Second-line	Vorozole (2.5 mg/day)	64/277	23	24	6.7
		Aminoglutethimide (250mg bid)	50/279	18	19	6.0
Anastrozole[168]	Second-line	Anastrozole (1 mg/day)	26/263	10	25	5.3
		Anastrozole (10 mg/day)	22/248	9	23	5.3
		Megestrol (40mg qid)	20/253	8	26	5.3
Letrozole[182,183]	Second-line ER+/?	Letrozole (0.5 mg/day)	24/188	13	13	5.1
		Letrozole (2.5 mg/day)	41/174	27	10	5.6
		Megestrol (40mg qid)	31/189	16	15	5.5

a Includes complete remission and partial remission.

bid = twice daily; **ER**+ = estrogen receptor–positive; **qid** = 4 times daily; **–** = indicates data not available; **?** = unknown estrogen receptor status.

most of the new aromatase inhibitors showed a similar antitumour activity to megestrol or aminoglutethimide in these heavily pretreated patients, although letrozole proved to be more effective than aminoglutethimide. In addition, whereas letrozole showed a dose-response relationship for efficacy, the efficacy of anastrozole was dose-independent but it may improve survival. The adverse effects were different, with more bodyweight increase and fluid retention with megestrol, and nausea, vomiting and hot flashes with the aromatase inhibitors. Overall, the new aromatase inhibitors were better tolerated with reduced toxicity than the standard second-line therapy with aminoglutethimide or megestrol. However, further studies in well characterised patients may distinguish the benefits of these agents.

The new aromatase inhibitors are very potent in blocking estrogen synthesis (for example, letrozole is approximately 10 000 times as potent as aminoglutethimide *in vivo*), have high selectivity and are well tolerated by patients. They are now likely to be the drugs of choice for second-line treatment for advanced breast cancer. Further clinical studies will determine their efficacy in first-line treatment in terms of long-term benefits and survival.

The potential utility of aromatase inhibitors in other settings is emerging and encouraging. Studies are now in progress[187,188] or are currently being launched to evaluate formestane, anastrozole, letrozole and other aromatase inhibitors both as first-line treatment or adjuvant treatment as an alternative or in combination with tamoxifen. Other trials to study the possible roles of aromatase inhibitors as chemopreventive agents for breast cancer are also in progress. The chemopreventive activity of vorozole in the methylnitrosourea-induced mammary tumour model in rats has been demonstrated.[189] A recent review article in this field by Kolloff and colleagues[190] is timely and desirable. In addition, a study by Takayama et al.,[191] who demonstrated the potential of anastrozole in the treatment of endometriosis, is notable. Other studies of these types would be of interest. A further stimulus to these studies is the recent and impressive finding from a long-term study that tamoxifen is an effective chemopreventive agent of breast cancer in high-risk women.[192,193]

6. Conclusion

The role of aromatase inhibitors as the optimal second-line agents for the treatment of advanced breast cancer has been established in large comparative clinical trials (table I). They are significantly superior to the progestins in terms of duration of overall clinical benefits, survival and tolerability. Thus, patients benefit from improved quality of life. The aromatase inhibitors that are clinically available in the treatment of postmenopausal women with breast cancer include formestane, anastrozole and letrozole. As drug resistance remains a major problem in cancer therapy, the variety of well-tolerated aromatase inhibitors with different chemical structures offers the possibility that several useful drugs now available to clinicians will improve the management of patients with breast cancer.

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