J. Enzyme Inhibition, 1990, Vol. 4, pp. 101–111
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BIOCHEMICAL AND MOLECULAR ASPECTS OF AROMATASE

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INTRODUCTION

The biosynthesis of estrogens from androgens is catalyzed by an enzyme complex termed aromatase. This cytochrome P-450 complex converts C_{19} steroids into C_{18} steroids containing an aromatic A ring. The isolation of aromatase activity in the microsomal membrane fraction from human term placenta was first reported by Ryan[1] in 1959. Early investigations on the biochemistry of aromatase focused on determination of substrate specificity[2–7] and cofactor requirements.[1,8] These studies demonstrated that three moles of NADPH and three moles of molecular oxygen are necessary for conversion of one mole of androstenedione, the preferred substrate for placental aromatase. Aromatase activity is found in numerous cells and tissues, including placenta, ovary,[9–15] testis,[16–20] adipose,[21–24] brain,[25,26] blastocyst,[27] and mammary carcinoma.[28–32] Research interests in the aromatization reaction continue to expand from basic endocrinology and reproductive biology studies to aromatase inhibition for treatment of estrogen-dependent cancers. The international conference held in March 1987 demonstrated the extent of research activities on aromatase.[33] This review will focus on recent advances in the biochemical and molecular aspects of the aromatase enzyme complex.

BIOCHEMISTRY

Mechanism of Aromatization

The elucidation of the mechanism of the aromatization reaction began in the early 1960's and continues to receive extensive study. Aromatization proceeds via three successive steps, with the first two steps being hydroxylations. The first step involves oxidation of the angular C-19 methyl group to provide 19-hydroxyandrostenedione. 19,19-Dihydroxyandrostenedione, isolated as 19-oxoandrostenedione, is formed by the second oxidation step. The exact mechanism of the last oxidation remains to be fully determined, with three postulated mechanisms under consideration (Figure 1).

Fishman and coworkers[34–36] proposed that the first two sequential hydroxylations at the C-19 position are followed by a third hydroxylation at the 2β-position, yielding 2β-hydroxy-19-oxoandrostenedione (Figure 1; Path a). Estrone results from the spontaneous collapse of this intermediate with liberation of formic acid. However, Caspi *et al.*[37] failed to show incorporation of the 2β-hydroxyl group into formic acid

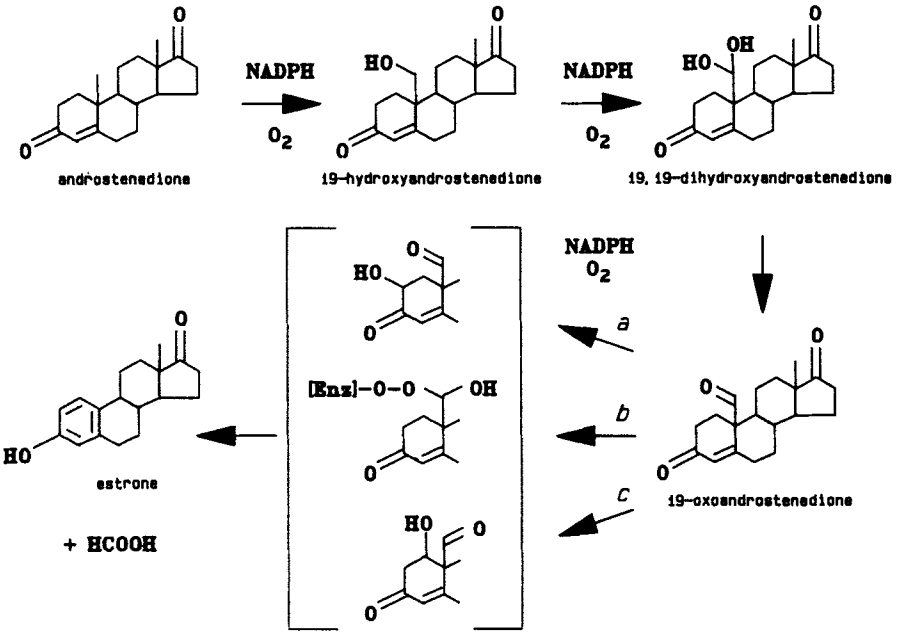


FIGURE 1 Mechanism of Aromatization.

under enzymatic or nonenzymatic condition with the synthesized [2β-^{18}O, 19-^{3}H]-2β-hydroxy-10β-formylandrost-4-ene-3,17-dione. Akhtar *et al.*[38-40] examined the loss of the C_{19}-methyl group during aromatization by use of $^{18}O_2$ and [^{3}H]-intermediates. The pro-R hydrogen of C_{19}-^{3}H intermediate is eliminated during aromatization and the pro-S hydrogen is incorporated into the extruded formic acid. Furthermore, oxygen atoms from the first and third oxidation steps also are incorporated into formic acid. These results have led to the proposal that the last oxidation step is a peroxidative attack at the C_{19} position[41] (Figure 1; Path b). A third possible mechanism involves the final hydroxylation occurring at the 1β-position and the subsequent stereoselective removal of the 2β-hydrogen[42] (Figure 1; Path c).

Purification of Aromatase

Aromatase is a membrane-bound cytochrome P-450 monooxygenase consisting of two proteins – aromatase cytochrome P-450 (P-450$_{arom}$) and NADPH-cytochrome P-450 reductase. Cytochrome P-450$_{arom}$ is a heme protein which binds the steroid substrate and molecular oxygen and catalyzes the oxidations. The reductase is a flavoprotein, is found ubiquitously in endoplasmic reticulum, and is responsible for transferring reducing equivalents from NADPH to cytochrome P-450$_{arom}$. Purification of cytochrome P-450$_{arom}$ proved to be very difficult because of its membrane-bound nature, instability and low tissue concentration. In 1981, Pasanen and Pelkonen[43] utilized phenyl Sepharose column chromatography for a rapid and efficient initial purification step for placental P-450's. In 1982, Osawa and his collaborators reported that two distinct active protein complexes (termed aromatase I and aroma-

tase II) were solubilized from human term placenta with deoxycholate and separated by DEAE cellulose chromatography.[44] Aromatase II converted androstenedione to estrone and was the major aromatase activity from placenta. In 1985, Mendelson *et al.*[45] isolated cytochrome P-450$_{arom}$ with a molecular weight of 55,000 and a specific content of 2 nmol/mg protein using immunoaffinity chromatography with monoclonal antibodies. Octylamino-agarose chromatography[46] and affinity chromatography[47] also yielded purified cytochrome P-450$_{arom}$ with low heme content. Chen *et al.*[48] reported the amino terminal sequence of the octylamino-agarose purified protein.

In 1987, Kellis and Vickery[49] developed a new isolation procedure for cytochrome P-450$_{arom}$ with high specific activity. The enzyme was extracted with sodium cholate, fractionated by ammonium sulfate precipitation, and subjected to column chromatographjy in the presence of substrate androstenedione and the nonionic detergent, Nonidet NP-40. This procedure yielded a highly purified and active cytochrome P-450$_{arom}$, with the highest specific content of 11.5 nmol of cytochrome P-450 per mg of protein reported. Reconstitution of this cytochrome P-450$_{arom}$ with NADPH-cytochrome P-450 reductase and phospholipid resulted in complete conversion of androstenedione to estrone, thus, demonstrating that one cytochrome P-450 protein catalyzes all three oxidation steps.

Molecular Biology of Aromatase

Knowledge of the molecular biology of aromatase has advanced greatly in the past three years, with the research group of Simpson, Mendelson and colleagues providing many insights. These researchers utilized monoclonal and polyclonal antibodies[45] to cytochrome P-450$_{arom}$ to screen a phage gt11 human placental cDNA library.[50,51] Initially, a complementary DNA clone coding for about 60% of the carboxy-terminal portion of aromatase was isolated. Restriction mapping and sequencing identified a full length cDNA complementary to mRNA encoding cytochrome P-450$_{arom}$.[52] The open reading frame encodes a protein of 503 amino acids; the report of Harada[53] agrees and confirms these results. The cDNA contains the sequences for the first 10 amino acids of the purified protein and the sequences of all four cysteine-containing tryptic peptides isolated by Chen *et al.*[48,54] from purified cytochrome P-450$_{arom}$. This cDNA sequence was inserted into COS1 monkey kidney cells, and aromatase mRNA and aromatase enzymatic activity were detected in transfected cells.[52] Finally, the cDNA clones have been utilized to examine the regulation of aromatase in ovarian and adipose tissues.[55–58]

AROMATASE INHIBITORS – MOLECULAR PROBES OF THE ACTIVE SITE

Inhibitors of aromatase have been developed over the past 15 years as potential therapeutic agents for controlling various physiological processes and/or disease states dependent upon estrogen biosynthesis. Currently, the most immediate use of aromatase inhibitors is the treatment of estrogen-dependent breast cancer. The aromatase inhibitors aminoglutethimide and 4-hydroxyandrostenedione have demonstrated therapeutic effectiveness in the treatment of hormone-dependent breast tumors in both animals[59–61] and humans.[62–64] Numerous steroidal and non-steroidal aromatase inhibitors have been prepared and evaluated since 1973. These inhibitors

have included competitive inhibitors, affinity labelling agents, and mechanism-based inhibitors ("suicide substrates"). Research summaries of aromatase inhibitors have been published from two international aromatase conferences,[33,65] and recent reviews on aromatase inhibitors have also appeared.[66-69]

Aromatase inhibitors have potential use not only as therapeutic agents, but also as biochemical tools. In particular, these agents can aid in studying the active site of the aromatase enzyme system. By designing steroidal analogs with substituents at various positions on the steroid nucleus, evaluation of the structural requirements needed for favorable interactions with the enzymatic site can be performed. Furthermore, selection of appropriate substituents may provide "molecular probes" for deducing the types of drug-macromolecular interactions, such as hydrogen bonding, van der Waal's forces, π-π interactions, or electrostatic forces.

Structure-Activity Relationships of Steroidal Inhibitors

In 1973, the first full report of inhibition of aromatase was published by Brodie and his colleagues,[70] who evaluated the relative inhibitory activity of 100 readily available steroids and nonsteroids in human placental microsomes. The initial structure- activity relationships for aromatase inhibition were established, and subsequently confirmed by other investigators.[71-73] In summary, effective inhibition is observed with C_{19} steroids possessing an A/B trans ring junction, a ketone functionality at C-3, and a 17-keto or 17β-hydroxyl substituent. Steroids with extended linear conjugation, e.g., 4,6-diene-3-one, 1,4,6-triene-3-one, and 4-ene-3,6-dione, are also effective inhibitors. Effective steroidal inhibitors that have been developed to date build upon this basic androstenedione nucleus and incorporate chemical substituents at varying positions on the steroid (Figure 2).

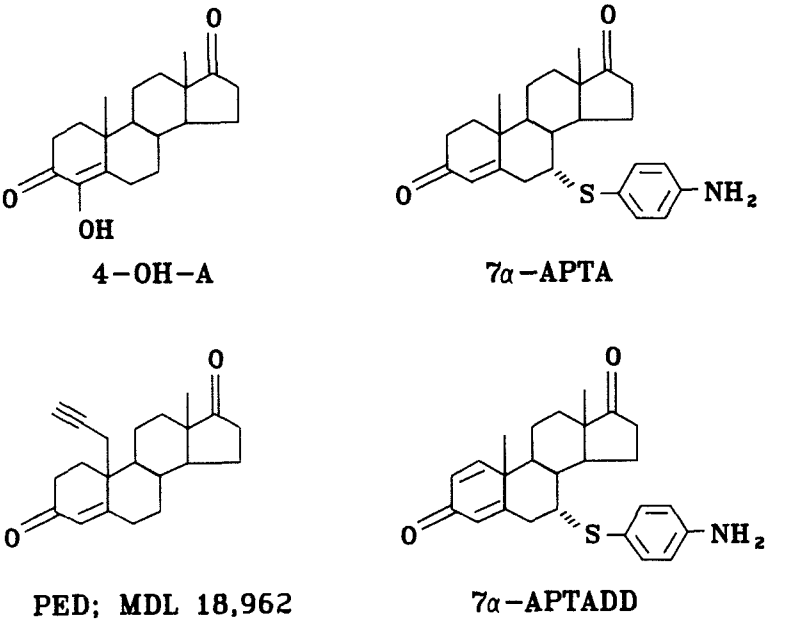


FIGURE 2 Steroidal Aromatase Inhibitors.

Modifications of the A-ring of the androstenedione molecule initially focused on substitution at C-4. 4-Hydroxy-4-androstene-3,17-dione (4-OHA) and 4-acetoxy-4-androstene-3,17-dione (4-AcOA) are effective inhibitors *in vitro*,[70,74] produce enzyme-mediated inactivation,[75] inhibit reproductive process *in vivo*,[76] and cause regression of hormone-dependent mammary rat tumors.[77,78] 4-OHA is now under clinical investigation for the treatment of advanced breast cancer in postmenopausal women.[79,80] Other C-4 substituents, such as thiols[81] and amines,[82] are not as effective as 4-OHA. A limited number of inhibitors with substituents at other positions of the A ring have been reported. 1-Methyl-1,4-androstadiene-3,17-dione is a potent inhibitor of aromatase *in vitro* and *in vivo* and suppresses gonadal and peripheral aromatization in juvenile female rats.[83] On the other hand, bulky substituents at the 1α-position are poor inhibitors.[73] At the C-3 position, replacement of the ketone with a methylene provided effective inhibitors.[84] Thus, the spacial requirements of the A-ring for binding of the steroidal inhibitor to aromatase are rather restrictive, permitting only small structural modifications to be made. Incorporation of the polar hydroxyl group at C-4 enhances inhibitory activity.

In contrast to the SAR of the A-ring, bulky substitutions at the C-7 position of the B-ring have provided several very potent aromatase inhibitors.[73] 7α-(4′-Amino)-phenylthio-4-androstene-3,17-dione (7α-APTA) is a very effective competitive inhibitor, with an apparent K_i of 18 nM. This inhibitor has also demonstrated effectiveness in inhibiting aromatase in cell cultures[32,85] and in treating hormone-dependent rat mammary tumors.[85,86] Evaluation of various substituted aromatic analogs of 7α-APTA provided no correlation between the electronic character of the substituents and inhibitory activity.[87] Affinity labeling derivatives of 7α-APTA produced inactivation of aromatase, with a [^{14}C]-analog demonstrating covalent binding to aromatase.[88,89] An effective mechanism-based inhibitor of aromatase, 7α-(4′-amino)-phenylthio-1,4-androstadiene-3,17-dione (7α-APTADD) with an apparent K_i of 9.9 nM, was also developed by this research group and has the most rapid rate of inactivation reported to date.[90] Recent results of various 7-substituted 4,6-androstadiene-3,17-dione derivatives[91,92] suggest that only those derivatives that can project the 7-aryl substituent into the 7α pocket are effective inhibitors. Modifications on other positions of the B-ring that have provided aromatase inhibitors include 6α-bromo and 6β-bromo analogs[93] and 6α- and 6β-hydroperoxyandrostenediones.[94] Overall, the most effective B-ring modified aromatase inhibitors are those with 7α-aryl derivatives, with several analogs having 2–10 times greater affinity for the enzyme than the substrate. These results suggest that additional interactions occur between the phenyl ring at the 7α-position and amino acids at or near the enzymatic site of aromatase, resulting in enhanced affinity.

The other position of androstenedione that has received considerable investigation is the C-19 methyl position, the site of enzymatic oxidation. 10-Propargyl-4-estrene-3,17-dione (PED; MDL 18962) was the first compound designed as a mechanism-based inhibitor of aromatase; it was synthesized and studied independently by three research groups.[95–97] This agent is an effective inhibitor *in vitro* and *in vivo*[98–103] and is now under clinical investigation. Other 19-substituted aromatase inhibitors evaluated include difluoro analogs,[104] thiiranes and oxiranes,[105–107] epoxysteroids,[108] and thiol and amino analogs.[109,110] The 19-R-isomers of the thiiranes and oxiranes were potent inhibitors and showed affinity 36 to 80-fold greater than the corresponding 19-S-isomers. Spectral titrations of microsomal preparation and purified P-450$_{arom}$ demonstrated that the binding of the 19R-isomers to the enzyme shifts the Soret maximum of

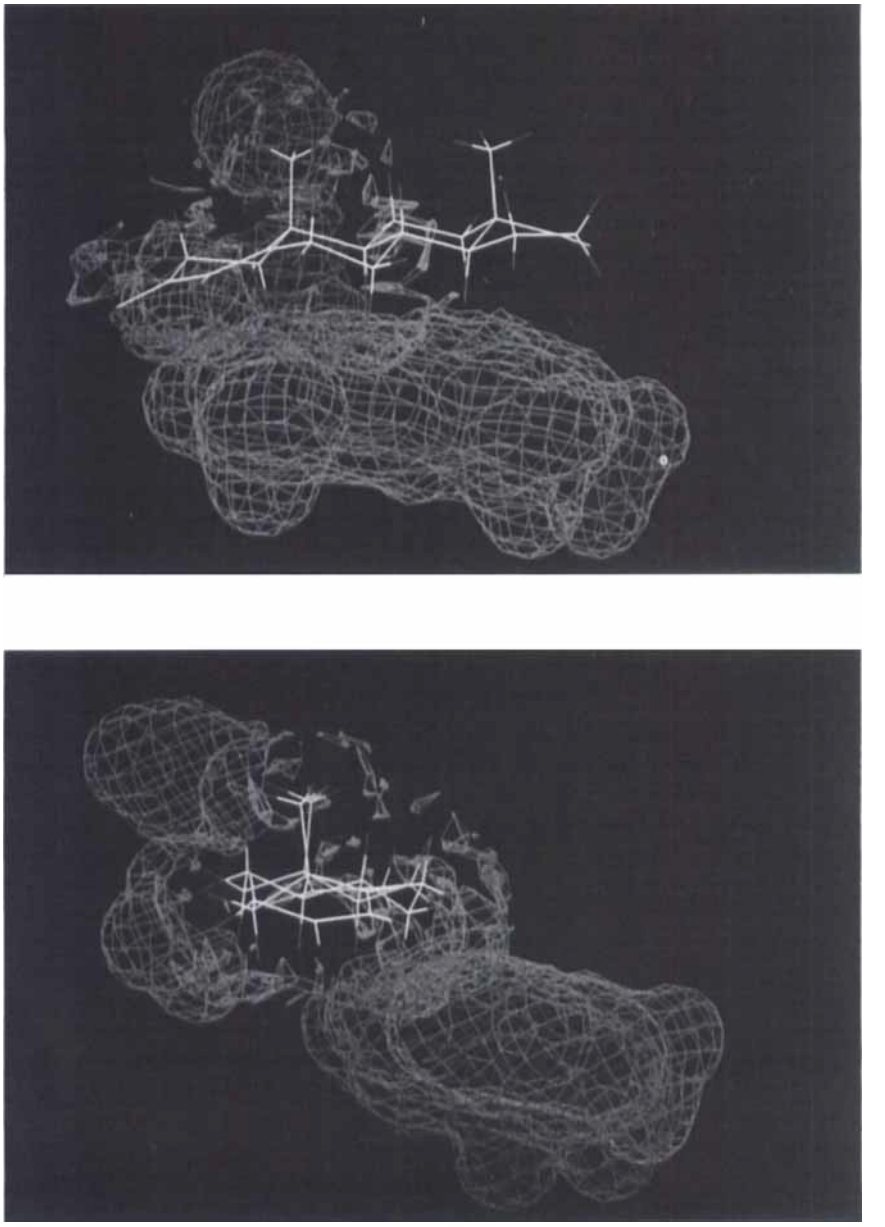


FIGURE 3 Spacial Map of the Active Site of Aromatase. Volume calculations were performed with SYBYL (Tripos Associates, St. Louis, MO) using energy-minimized structures of androstenedione and several potent aromatase inhibitors. Volume differences of the A and B ring between the inhibitors and androstenedione are superimposed around the stick structure of androstenedione. Figure 3A represents the view along the edge of the steroid nucleus, with the α-face below the steroid. Figure 3B represents another view rotated along the y axis, with the 3-keto functionality in the foreground and the D ring in the back. (See Rear for Colour Plate)

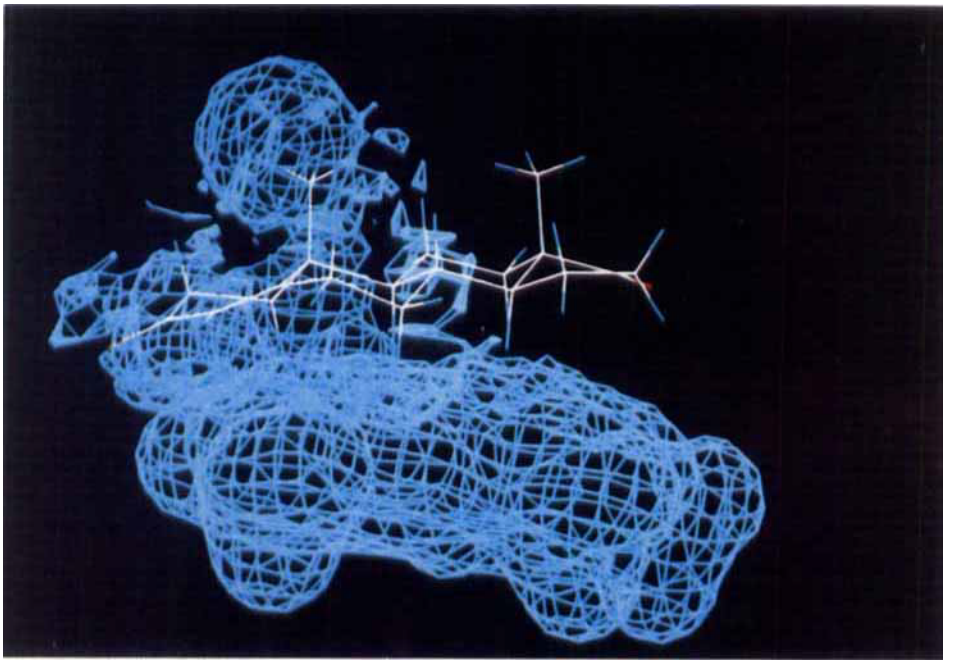


Figure 3A

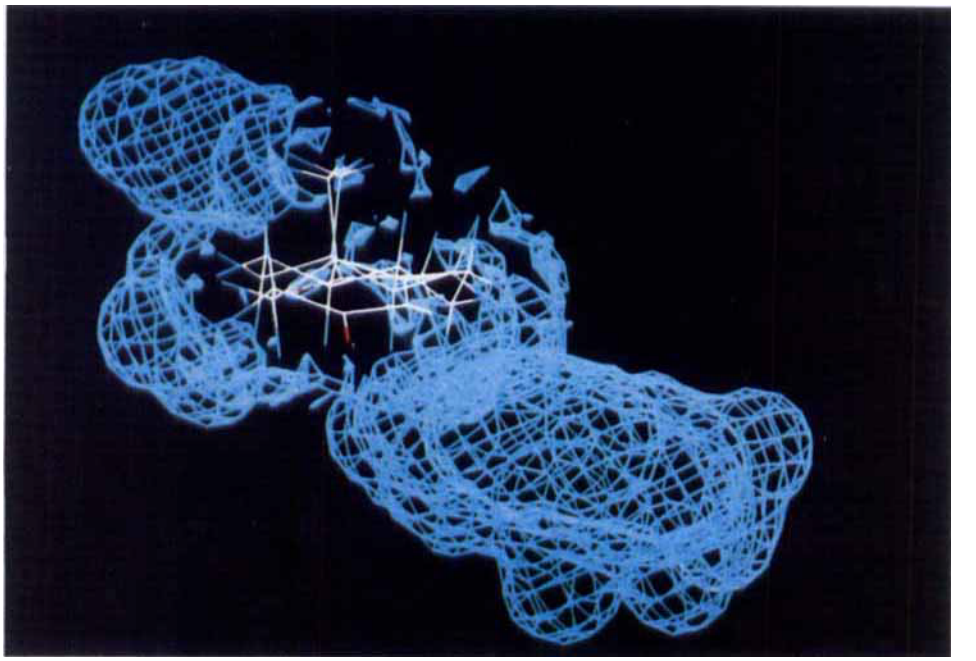


Figure 3B
COLOR PLATES for Brueggemeier Figure 3.
Special Map of the Active Site of Aromatase.

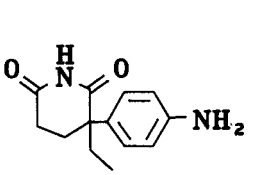
the ferric enzyme, producing the high spin form of the enzyme. Consequently, the oxygen atom of the oxirane and the sulfur atom of the thiirane are bound to the heme iron in the inhibitor complexes. Thus, effective inhibitors have been prepared with geometrically small functionalities at the C-19 position, suggesting that the active site of the enzyme can accommodate small changes in structure and will result in a catalytically active enzyme complex.

In summary, the structure-activity relationships of steroidal aromatase inhibitors suggest that the spacial requirements for interaction of agents with the active site of aromatase are very restrictive, permitting only small structural changes to be made on the A-ring and at C-19. Incorporation of aryl functionalities at the 7α-position of the steroid is the exception, and inhibitors with such modifications exhibit enhanced affinity for the enzyme. A spacial map of the active site of aromatase (Figure 3), derived from molecular modeling and computational chemistry of potent inhibitors,[92] provides a topological model which can serve to facilitate development of more effective aromatase inhibitors.

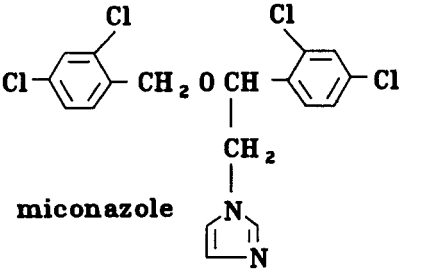
Structure-Activity Relationships of Nonsteroidal Inhibitors

Several nonsteroidal agents have demonstrated effective aromatase inhibition (Figure 4). These agents possess a heteroatom as a common chemical feature and interfere with steroid hydroxylations by the binding of this heteroatom with the heme iron of the cytochrome P-450's. In general, these inhibitors are less enzyme specific and will inhibit, to varying degrees, other cytochrome P-450-mediated hydroxylations in steroidogenesis.

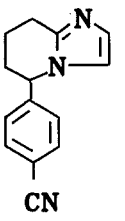
Aminoglutethimide (AG), an anticonvulsant, is an inhibitor of cholesterol side chain cleavage and of aromatase.[111-113] AG was withdrawn from use as an anti-



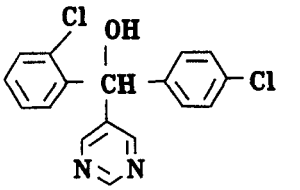
aminoglutethimide



miconazole



CSG 16949A



fenarimol

FIGURE 4 Nonsteroidal Aromatase Inhibitors.

convulsant in 1966 due to side effects of adrenal dysfunction. Nevertheless, AG was found to be an effective endocrine therapy for metastatic breast cancer in postmenopausal women,[114,115] and treatment with AG and hydrocortisone was equally effective as adrenalectomy in these patients.[116] Several closely related analogs of AG have been prepared[117-120] and exhibit similar aromatase inhibitory activity to AG with diminished effects on other cytochrome P-450 enzymes.

Several heteroaromatic compounds have also exhibited potent inhibition of aromatase. Miconazole, ketoconazole, clotrimazole and other imidazole derivatives are more effective than AG in inhibiting aromatase but are less effective than steroidal inhibitors.[121-123] Fenarimol, a pyrimidine carbinol agricultural fungicide, caused a dose-dependent decrease in aromatase activity *in vitro* and *in vivo*.[124,125] Recently, two selective aromatase inhibitors, CSG 16949A[126] and R 76713,[127] have been described and are now undergoing further drug development. Finally, flavonoid derivatives, such as 7,8-benzoflavone (α-naphthoflavone) and chrysin have shown aromatase inhibitory activity.[128,129] In summary, effective nonsteroidal aromatase inhibitors contain a heteroatom incorporated into or attached directly to a planar ring. This structural feature permits the molecule to potentially interact at the "A-ring" site and facilitate the binding of the heteroatom with the heme iron of the cytochrome P-450.

CONCLUSIONS

Significant progress on the biochemistry of aromatase has been made within the past few years, resulting in purified cytochrome P-450$_{arom}$, cDNA sequences, and information on the aromatase gene. Evaluations of various aromatase inhibitors have resulted in the development of structure-activity relationships of steroidal and nonsteroidal agents. Investigations with these agents and new inhibitors as "probes" of the active site of purified aromatase will provide valuable information on enzyme structure at the molecular level and can aid in elucidation of the final step of aromatization. Future research with purified protein and with the enzyme complex in its native, membrane-bound environment will enhance the development of more specific and effective inhibitors for the treatment of estrogen-dependent breast cancer.

References

1. Ryan, K.J. (1959) *J. Biol. Chem.*, **234**, 268.
2. Ryan, K.J. (1960) *Proc. Intern. Congr. Endocrinol.*, **350**, 697.
3. Ryan, K.J. (1961) *Proc. Intern. Congr. Biochem.*, **7**, 381.
4. Gual, C., Morato, T., Hayano, M., Gut, M. and Dorfman, R.I. (1962) *Endocrinol.*, **71**, 920.
5. Wilcox, R.B. and Engel, L.L. (1965) *Steroids*, **Suppl. 1**, 49.
6. Reed, K.C. and Ohno, S. (1976) *J. Biol. Chem.*, **251**, 1625.
7. Brodie, A.M.H., Schwarzel, W.C. and Brodie, H.J. (1976) *J. Steroid Biochem.*, **7**, 787.
8. Thompson, E.A. and Siiteri, P.K. (1974) *J. Biol. Chem.*, **249**, 5364.
9. Ryan, K.J. and Short, R.V. (1965) *Endocrinol.*, **76**, 108.
10. Makris, A. and Ryan, K.J. (1975) *Endocrinol.*, **96**, 694.
11. Makris, A. and Ryan, K.J. (1975) *Steroids*, **29**, 65.
12. Ryan, K.J. (1963) *Acta Endocrinol.*, **44**, 81.
13. Hammerstein, J., Rice, B.F. and Savard, K. (1964) *J. Clin. Endocrinol. Metab.*, **24**, 597.
14. Fahmy, D., Griffiths, K. and Turnbull, A.C. (1969) *J. Endocrinol.*, **44**, 133.
15. McKnatty, K.P., Baird, D.T., Bolton, A., Chambers, P., Corker, C.S. and McLean, H. (1976) *J. Endocrinol.*, **71**, 77.

16. Kelch, R.P., Jenner, M.R., Weinstein, R., Kaplan, S.L. and Grumback, M.M. (1972) *J. Clin. Invest.*, **51**, 824.
17. Payne, A.H., Kelch, R.P., Musich, S.S. and Halpern, M.E. (1976) *J. Clin. Endocrinol. Metab.*, **42**, 1081.
18. Fritz, I.B., Griswold, M.D., Louis, B.F. and Dorrington, J.H. (1976) *Mol. Cell. Endocrinol.*, **5**, 289.
19. Valladares, L.E. and Payne, A.H. (1979) *Endocrinol.*, **105**, 431.
20. Tsai-Morris, C.H., Aquilano, D.R. and Dufau, M.I. (1984) *Ann. NY Acad. Sci.*, **438**, 666.
21. Grodin, J.M., Siiteri, P.K. and MacDonald, P.C. (1973) *J. Clin. Endocrinol. Metab.*, **36**, 207.
22. Hemsell, D.L., Grodin, J.M., Breuner, P.F., Siiteri, P.K. and MacDonald, P.C. (1973) *J. Clin. Endocrinol. Metab.*, **38**, 476.
23. Edman, C.D., MacDonald, P.C. and Combes, B. (1975) *Gastroenterol.*, **69**, 819.
24. Edman, C.D. and MacDonald, P.C. (1978) *Am. J. Obstet. Gynecol.*, **130**, 456.
25. Naftolin, F., Ryan, K.J., Davis, I.J., Reddy, V.V., Flores, F., Petro, Z. and Kuhn, M. (1975) *Rec. Prog. Horm. Res.*, **31**, 295.
26. Roselli, C.E., Horton, L.E. and Resko, J.A. (1985) *Endocrinol.*, **117**, 2471.
27. Sengupta, J., Roy, S.K. and Manchada, S.K. (1982) *J. Reprod. Fertil.*, **66**, 63.
28. Dao, T.L., Varela, R. and Morreal, C. (1970) *Estrogen Target Tissues and Neoplasia*, (Dao, T.L., ed.) 163, Chicago, UNiversity of Chicago Press.
29. Li, K., Chandra, P., Foo, T., Adams, J.B. and McDonald, D., *Steroids*, **28**, 561.
30. Miller, W.R. and Forrest, A.P.M. (1976) *Brit. J. Cancer*, **33**, 116.
31. MacIndoe, J.H. (1979) *J. Clin. Endocrinol. Metab.*, **49**, 272.
32. Brueggemeier, R.W. and Katlic, N.E. (1987) *Cancer Res.*, **47**, 4548.
33. Santen, R.J., (ed.) (1987) *Steroids*, **50**, 1–665.
34. Hahn, E.F. and Fishman, J. (1984) *J. Biol. Chem.*, **259**, 4472.
35. Goto, J. and Fishman, J. (1977) *Science*, **195**, 80.
36. Fishman, J., Goto, J. and Raju, M.S. (1981) *J. Biol. Chem.*, **256**, 4466.
37. Caspi, E., Wicha, J., Arunachalam, T., Nelson, P. and Spiteller, G. (1984) *J. Amer. Chem. Soc.*, **106**, 7282.
38. Arigoni, D., Battaglia, R., Akhtar, M. and Smith, T. (1975) *J. Chem. Soc. Chem. Comm.*, 185.
39. Akhtar, M., Calder, M.R., Corina, D.L. and Wright, J.N. (1981) *J. Chem. Soc. Chem. Comm.*, **129**.
40. Akhtar, M., Calder, M.R., Corina, D.L. and Wright, J.N. (1982) *Biochem. J.*, **201**, 569.
41. Cole, P.A. and Robinson, C.H. (1988) *J. Am. Chem. Soc.*, **110**, 1284.
42. Townsley, J.D. and Brodie, H.J. (1968) *Biochemistry*, **7**, 33.
43. Pasanen, M. and Pelkonen, O. (1981) *Biochem. Biophys. Res. Comm.*, **103**, 1310.
44. Osawa, Y., Tochigi, B., Higasshiyama, T., Yarborough, C., Nakamura, T. and Yamamoto, T. (1982) *Cancer Res. (suppl.)*, **42**, 3299s.
45. Mendelson, C.R., Wright, E.E., Evans, C.T., Porter, J.C. and Simpson, E.R. (1985) *Arch. Biochem. Biophys.*, **243**, 480.
46. Nakajin, S., Shinoda, M. and Hall, P.F. (1986) *Biochem. Biophys. Res. Comm.*, **134**, 704.
47. Hagerman, D.D. (1987) *J. Biol. Chem.*, **262**, 2398.
48. Chen, S., Shively, J.E., Nakajin, S., Shinoda, M. and Hall, P.F. (1986) *Biochem. Biophys. Res. Comm.*, **135**, 713.
49. Kellis, J.K., Jr. and Vickery, L.E. (1987) *J. Biol. Chem.*, **262**, 4413.
50. Evans, C.T., Ledesma, D.B., Schulz, T.Z., Simpson, E.R. and Mendelson, C.R. (1986) *Proc. Natl. Acad. Sci.*, **83**, 6387.
51. Simpson, E.R., Evans, C.T., Powell, F.E., Ledesma, D.B. and Mendelson, C.R. (1987) *Mol. Cell. Endocrinol.*, **52**, 267.
52. Corbin, C.J., Grahan-Lorence, S., McPhaul, M., Mason, J.I., Mendelson, C.R. and Simpson, E.R. (1988) *Proc. Natl. Acad. Sci.*, **85**, 8948.
53. Harada, N. (1988) *Biochem. Biophys. Res. Comm.*, **156**, 725.
54. Chen, S., Besinan, M.J., Sparkes, R.S., Zollman, S., Klisak, I., Mohandes, T., Hall, P.F. and Shively, J.E. (1988) *DNA*, **7**, 27.
55. Steinkampf, M., Mendelson, C.R. and Simpson, E.R. (1987) *Mol. Endocrinol.*, **1**, 465.
56. Steinkampf, M., Mendelson, C.R. and Simpson, E.R. (1988) *Mol. Cell. Endocrinol.*, **59**, 93.
57. Evans, C.T., Merrill, J.C., Corbin, C.J., Saunders, C., Simpson, E.r. and Mendelson, C.R. (1987) *J. Biol. Chem.*, **269**, 6914.
58. Simpson, E.R., Merrill, J.C., Hollub, A.J., Grahan-Lorence, S. and Mendelson, C.R. (1989) *Endo. Rev.*, **10**, 136.
59. Brodie, A.M.H., Schwarzel, W.C., Shaikh, A.A. and Brodie, H.J. (1977) *Endocrinol.*, **100**, 1684.
60. Brodie, A.M.H., Marsh, D.A. and Brodie, H.J. (1979) *J. Steorid Biochem.*, **10**, 423.

61. Brodie, A.M.H., Brodie, H.J., Garrett, W.M., Hendrickson, J.R., Marsh, D.A. and Tsai-Morris, C.-H. (1982) *Biochem. Pharmacol.*, **31**, 2017.
62. Santen, R.J., Santner, S., Davis, B., Veldhuis, J., Samojlik, E. and Ruby, E. (1978) *J. Clin. Endocrinol. Metab.*, **47**, 1257.
63. Coombes, R.C., Goss, P., Dowsett, M., Gazet, J.-C. and Brodie, A.M.H. (1984) *Lancet*, **2**, 1237.
64. Sjoerdsma, A. (1981) *Clin. Pharmacol.*, **30**, 3.
65. Santen, R.J., Santner, S. and Lipton, (1982) *Cancer Res. (suppl.)*, **42**, 1s.
66. Johnston, J.O. and Metcalf, B.W. (1984) *Novel Approaches to Cancer Chemotherapy*, (Sunkara, P., Ed.) 307, New York, Academic Press.
67. Banting, L., Smith, H.J., James, M., Jones, G., Nazareth, W., Nichols, P.J., Hewlins, M.J.E. and Rowlands, M.G. (1988) *J. Enz. Inhibit.*, **2**, 215.
68. Covey, D.F. (1988) *Sterol. Biosynthesis Inhibitors*, (Berg, D. and Plempel, M., ed.), 534, Chichester, UK, Ellis Horwood.
69. Banting, L., Nichols, P.J., Shaw, M.A. and Smith, H.J. (1989) *Progress in Medicinal Chemistry*, **26**, 253.
70. Schwarzel, W.C., Kruggel, W.G. and Brodie, H.J. (1973) *Endicrinol.*, **92**, 866.
71. Schubert, K., Bvhme, K.H. and Hvrhold, C. (1974) *Endokrinol.*, **64**, 19.
72. Siiteri, P.K. and Thompson, E.A., Jr. (1975) *J. Steroid. Biochem.*, **6**, 317.
73. Brueggemeier, R.W., Floyd, E.E. and Counsell, R.E. (1978) *J. Med. Chem.*, **21**, 1007.
74. Marsh, D.A., Brodie, H.J., Garrett, W., Tsai-Morris, C.-H. and Brodie, A.M.H. (1985) *J. Med. Chem.*, **28**, 788.
75. Brodie, A.M.H., Garrett, W., Hendrickson, J.R., Tsai-Morris, C.-H., Marcotte, C.H. and Robinson, C.H. (1981) *Steroids*, **38**, 693.
76. Brodie, A.M.H., Schwarzel, W.C., Shaikh, A.A. and Brodie, H.J. (1977) *Endocrinol.*, **100**, 1684.
77. Wing, L.Y., Garrett, W. and Brodie, A.M.H. (1985) *Cancer Res.*, **45**, 2425.
78. Brodie, A.M.H., Garrett, W., Hendrickson, J.R. and Tsai-Morris, C.-H., (1982) *Cancer Res. (suppl.)*, **42**, 3360s.
79. Brodie, A.M.H., Wing, L.Y., Goss, P., Dowsett, M. and Coombs, R.C. (1986) *J. Steroid Biochem.*, **25**, 859.
80. Goss, P.E., Powles, T.J., Dowsett, M., Hutchison, G., Brodie, A.M.H., Gazet, J.-C. and Coombes, R.C. (1986) *Cancer Res.*, **46**, 4823.
81. Abul-Hajj, Y.J. (1986) *J. Med. Chem.*, **29**, 582.
82. di Salle, E., Ornati, G., Giuducu, D., Briatico, G. and Lombardi, P. (1986) *J. Steroid Biochem.*, **25**, 134S.
83. Henderson, D., Habenicht, U.-F., Nishino, Y., Kerb, U. and Etreby, M.F.El. (1986) *J. Steroid Biochem.*, **25**, 867.
84. Miyairi, S. and Fishman, J. (1986) *J. Biol. Chem.*, **261**, 6772.
85. Brueggemeier, R.W., Li, P.-K., Snider, C.E., Darby, M.V. and Katlic, N.E. (1987) *Steroids*, **50**, 163.
86. Brueggemeier, R.W. and Li, P.-K. (1988) *Cancer Res.*, **48**, 6808.
87. Darby, M.V., Lovett, J.A., Brueggemeier, R.W., Groziak, M.P. and Counsell, R.E. (1985) *J. Med. Chem.*, **28**, 803.
88. Snider, C.E. and Brueggemeier, R.W. (1985) *J. Steroid Biochem.*, **22**, 325.
89. Brueggemeier, R.W., Snider, C.E. and Counsell, R.E. (1982) *Cancer Res. (suppl.)*, **42**, 3334s.
90. Snider, C.E. and Brueggemeier, R.W. (1987) *J. Biol. Chem.*, **262**, 8685.
91. Li, P.-K. and Brueggemeier, R.W. (1990) *J. Med. Chem.*, **33**, 101.
92. Li, P.-K. and Brueggemeier, R.W. (1989) *J. Enz. Inhibit.*, this issue.
93. Osawa, Y., Osawa, Y. and Coon, M.J. (1987) *Endocrinol.*, **121**, 1010.
94. Tan, L., Hrycay, E.G. and Matsumoto, K. (1983) *J. Steroid Biochem.*, **19**, 1329.
95. Mann, J. and Pietrzak, B. (1987) *J. Chem. Soc. Perkin Trans. I*, 385.
96. Covey, D.F. and Hood, W.F. (1981) *J. Biol. Chem.*, **256**, 1076.
97. Marcotte, P.A. and Robinson, C.H. (1982) *Biochemistry*, **21**, 2773.
98. Flynn, G.S., Johnston, J.O., Wright, C.L. and Metcalf, B.W. (1981) *Biochem. Biophys. Res. Comm.*, **103**, 913.
99. Johnston, J.O., Wright, C.L. and Metcalf, B.W. (1984) *Endocrinol.*, **115**, 776.
100. Johnston, J.O., Wright, C.L. and Metcalf, B.W. (1984) *J. Steroid Biochem.*, **20**, 1221.
101. Longcope, C., Femino, A.M. and Johnston, J.O. (1988) *Endocrinol.*, **122**, 2007.
102. Johnston, J.O. (1987) *Steroids*, **50**, 106.
103. Ziminski, S.J., Brandt, M.E., Covey, D.F. and Puett, D. (1987) *Steroids*, **50**, 135.
104. Marcotte, P.A. and Robinson, C.H. (1982) *Steroids*, **39**, 325.
105. Kellis, J.T., Childers, W.E., Robinson, C.H. and Vickery, L.E. (1987) *J. Biol. Chem.*, **262**, 4421.

106. Childers, W.E. and Robinson, C.H. (1987) *J. Chem. Soc. Comm.*, 320.
107. Childers, W.E., Shih, M.J., Furth, P.S. and Robinson, C.H. (1987) *Steroids*, **50**, 121.
108. Shih, M.J., Carrell, M.H., Carrell, H.L., Wright, C.L., Johnston, J.O. and Robinson, C.H. (1987) *J. Chem. Soc. Chem. Comm.*, 213.
109. Bednarski, P.J., Porubek, D.J. and Nelson, S.D. (1985) *J. Med. Chem.*, **28**, 775.
110. Wright, J.N., Calder, M.R. and Akhtar, M. (1985) *J. Chem. Soc. Chem. Comm.*, 1733.
111. Dexter, R.N., Fishman, L.M., Ney, R.L. and Liddle, G.W. (1967) *J. Clin. Endocrinol. Metab.*, **27**, 473.
112. Salhanick, H.A. (1982) *Cancer Res. (suppl.)*, **42**, 3315s.
113. Graves, P.E. and Salhanick, H.A. (1979) *Endocrinol.*, **105**, 52.
114. Stuart-Harris, R.C. and Smith, I.E. (1984) *Cancer Treat. Rev.*, **11**, 189.
115. Santen, R.J., Badder, E., Leman, S., Harvey, H., Lipton, A., Boucher, A.E., Manni, A. and Rosen, H. (1982) *Breast Cancer Res. Treat.*, **2**, 375.
116. Santen, R.J., Worgul, T.J., Samojlik, E., Interrante, A., Boucher, A.E. and Well, S. A. (1981) *N. Eng. J. Med.*, **305**, 545.
117. Kellis, J.T., Jr and Vickery, L.E. (1984) *Endocrinol.*, **114**, 2128.
118. Pourgholami, M.H., Nicholls, P.J., Smith, H.J., Daly, M.J. and Dyas, J. (1987) *J. Steroid Biochem.*, **26**, 309.
119. Neidle, S. and Jarman, M. (1988) *Biochem. Pharmacol.*, **37**, 143.
120. Rowlands, M.G., Bunnett, M.A., Foster, A.B., Jarman, M., Stanek, J. and Schweizer, E. (1988) *J. Med. Chem.*, **31**, 971.
121. Hajek, K.K., Cook, N.I. and Novak, R.F. (1982) *J. Pharmac. Exp. Ther.*, **223**, 97.
122. Mason, J.I., Murry, B.A., Olcott, M. and Sheets, J.J. (1985) *Biochem. Pharmacol.*, **34**, 1087.
123. Ayub, M. and Levell, M. (1988) *J. Steroid Biochem.*, **31**, 65.
124. Hirsch, K.S., Weaver, D.E., Black, L.J., Falcone, J.F. and MacLusky, N.J. (1987) *Toxicol. Appl. Pharmacol.*, **91**, 235.
125. Hirsch, K.S., Jones, D., Lindstrom, T.D., Stamm, N.B., Sutton, G.P., Taylor, H.M. and Weaver, D.E. (1987) *Steroid*, **50**, 201.
126. Steele, R.E., Mellor, L.B., Sawyer, W.K., Wasvary, J.M. and Browne, L.J. (1987) *Steroids*, **50**, 147.
127. Wouters, W., DeCoster, R., Krekels, M., Van Dun, J., Berens, D., Haelterman, C., Raeymaekers, A., Freyne, E., Van Gelder, J., Venet, M. and Janssen, P.A. (1989) *J. Steroid Biochem.*, **32**, 781.
128. Kellis, J.T. and Vickery, L.E. (1984) *Science*, **225**, 1032.
129. Kellis, J.T., Nesnow, S. and Vickery, L.E. (1986) *Biochem. Pharmacol.*, **35**, 2887.