PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07H 15/12, C12N 5/02, 5/00
C12N 15/07, 15/12, A61K 37/50

A1

(11) International Publication Number: **WO 91/05794**

(43) International Publication Date: 2 May 1991 (02.05.91)

(21) International Application Number: PCT/US90/05873

(22) International Filing Date: 12 October 1990 (12.10.90)

(30) Priority data:
422,904 18 October 1989 (18.10.89) US

(60) Parent Application or Grant
(63) Related by Continuation
US 422,904 (CON)
Filed on 18 October 1989 (18.10.89)

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: STABLE MAMMALIAN CELL LINES THAT EXPRESS AROMATASE

(57) Abstract

Stable mammalian cell lines that express human aromatase and which are useful to screen anti-breast cancer drugs are disclosed.

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STABLE MAMMALIAN CELL LINES THAT EXPRESS AROMATASE

This application is a continuation of United States Serial No. 07/422,904 filed 18 October 1989.

FIELD OF THE INVENTION

This invention relates to mammalian cell lines which express aromatase. The cell lines are useful to screen aromatase inhibitors useful as anti-breast cancer drugs.

BACKGROUND OF THE INVENTION

Aromatase catalyzes the formation of C-18 estrogenic steroids from C-19 androgens. Aromatase inhibitors are useful in breast cancer therapy because of the central importance of estrogens to the development of such malignancies.

At the present time, in vitro methods are commonly used to screen aromatase inhibitors. The investigation can be carried out using a partially purified aromatase preparation. See Taniguchi, H., et al., Anal. Biochem. 181:167-171 (1989). Data from such methods may not represent the activity of the drugs in intact cells. There are also methods using animal tissues which express aromatase. See Schenkel, A.H., et al., J.Steroid Biochem. 33:125-131 (1989). These tissues express aromatase at such a low level that enzyme assay requires a long incubation. In addition, tissue specimens are usually heterogeneous. Therefore, it is difficult to compare data from experiment to experiment. Aromatase has been expressed in yeast. Pompon, D., et al., Molecular Endrocrinology 3:1477-1487 (1989). However, the yeast model is not preferred for screening drugs for humans.

The need for a reliable and a rapid method for the primary screening of aromatase inhibitors is apparent. Such a method requires stable mammalian cell lines which contain high levels of aromatase. Corbin, C.J., et al., Proc.Natl.Acad.Sci.USA 85:8948-8952 (1988) report the expression of human aromatase cDNA in mammalian COS cells through a transient expression method. Because the enzyme is expressed for only a short period of time, the Corbin cell lines are impractical for screening anti-breast cancer drugs.

SUMMARY OF THE INVENTION

This invention provides stable mammalian cell lines which contain high levels of aromatase useful for the primary screening of aromatase inhibitors. The uptake efficiency of such inhibitors can be evaluated because aromatase activity is measured using intact cells. The effect on cell growth is apparent from a comparison of growth rate in the presence and absence of inhibitors.

More particularly, this invention includes the construction of expression plasmids containing aromatase cDNA and mammalian cells transfected with such plasmids which express a functional aromatase protein. The aromatase expression product has enzymatic properties substantially identical to the enzyme in human placenta.

The expressed enzyme has the same Michales-Menken constant (Km) as the wild type enzyme and is coupled efficiently with the endogenous NADPH-cytochrome P-450 reductase. The activity of the expressed enzyme is inhibited by known aromatase inhibitors with Ki values similar to those reported in the literature. Accordingly, an important aspect of this

invention includes methods to screen aromatase inhibitors as drugs to treat, inter alia, estrogen dependent breast cancer.

DETAILED DESCRIPTION OF THE INVENTION

A full-length human placental aromatase cDNA clone "Aro 2" was isolated upon screening a human placental cDNA library with an aromatase cDNA probe and an oligonucleotide probe whose sequence was derived from a human aromatase genomic clone. Expression plasmids containing the aromatase cDNA clone were constructed. The enzyme was expressed at high levels in transfected mammalian cell lines. The expressed enzyme activity was inhibited by a known aromatase inhibitor, 4-hydroxyandrostenedione.

The transfected cell lines are useful in known assay procedures to screen aromatase inhibitors. Such assays may be performed directly on cultured cells without purifying the expressed enzyme.

DESCRIPTION OF THE FIGURES

Figure 1 depicts the nucleotide sequence of cDNA clone Aro 2 and the deduced amino acid sequence. The peptide sequence data confirms that the Aro 2 clone encodes for human placental aromatase. Regions corresponding to peptides determined by microsequencing methods are underlined.

Figure 2 shows the structure of an aromatase expression plasmid pH β-Aro useful to express aromatase in mammalian cell lines.

Figure 3 shows that aromatase expressed in CHO cells transfected with the plasmid of Figure 2 has activity following the normal Michales-Menken kinetics.

Figure 4 shows that the aromatase expressed in CHO cells transfected with the plasmid of Figure 2 is inhibited by 4-hydroxyandrostenedione.

Cloning and Analysis of Aro 2

Details of the cloning and analysis of aromatase cDNA, Aro 2 containing the full-length coding region are set forth in Pompon, D., et al., Expression of Human Placental Aromatase in Saccharomyces cerevisiae, Molecular Endocrinology 3:1477-1487 (1989). This paper is incorporated herein by express reference.

Design and Construction of Aromatase Expression Plasmid pAroX17

Constitutive or stable gene expression offers advantages over transient expression in the stable maintenance of transfected DNA in cells and in the possibility of isolating enough expressed protein for further biochemical and biophysical analyses. The yeast S. cerevisiae is a useful host for the expression of mammalian genes particularly cytochrome P-450s because the cells contain microsomal membranes, cytochrome P-450 reductase and cytochrome b_5 to allow membrane integration and catalytic stability. Gene expression in yeast can be accomplished by two ways. The first involves the stable integration of the foreign gene into the yeast nuclear DNA. This gives stably transformed cells expressing the heterologous protein at a low level. The alternative approach is to include the foreign gene in an autonomous multicopy replicate plasmid which is similar to, but more complex to that used to transform bacteria like Esherichia coli. Therefore, the transformed strain can express high levels of a heterologous protein. In addition, the presence of plasmids is stabilized by the maintenance of a constant selection pressure attributed by a marker being constructed into the plasmids. The second approach was used to express aromatase in yeast.

Figure 5 of Pompon, D., et al., supra, is the diagram for the construction of the aromatase expression plasmids. An adaptor with the following sequences

5′ GATCAGATCTATGGTTTTGGAAATGCTG 3′

3′ TCTAGATACCAAAACCTTTACGACCTAG 5′

was ligated to the BamHI restricted plasmid pYeDP1/8-2. Plasmids (i.e. PYeDP11) bearing the insert in the orientation in which the BamHI site just flanking the GAL10-CYC1 promoter was destroyed were selected. Plasmid pYeDP11, therefore, has a Bgl II site and a new unique BamHI site. The EcoRI fragment corresponding to the full-length aromatase cDNA was excised from the λgt11 vector by limited EcoRI digestion and cloned into the EcoRI site of pYeDP11 in the orientation that the 5′ end of the cDNA is next to the GAL10-CYC1 segment of the vector giving pExx1.

Deletion of the 5′-flanking sequences of aromatase cDNA was performed by full in vitro synthesis of a double stranded DNA encoding the amino-terminal part of the aromatase preceded by a synthetic adaptor containing Bgl II restriction site sequence from a single stranded M13 matrix. The 700 bp BamHI fragment of pExx1 was cloned in the BamHI site of phage M13MP18 in the orientation that bring the 3′-end of the cDNA fragment close to the EcoRI site of the vector. An adaptor-primer, with the sequence 5′-GATCAGATCTATGGTTTTGGAAATGCTG-3′, was hybridized with the single stranded phase DNA and was elongated using deoxynucleotide-triphosphates and the Klenow fragment of E. coli DNA polymerase I. This newly synthesized single stranded was purified, and the M13 reverse sequencing primer was then added to initiate the synthesis of the complementary strand.

By this approach, a double stranded DNA including a synthetic Bgl II site immediately flanking the transduction initiation codon of the aromatase cDNA was obtained. A double digestion of this DNA fragment by BamHI and Bgl II restriction endonucleases gave a 620 bp fragment encoding the amino-terminal part of aromatase. This fragment was cloned in the suitable orientation into the Bgl II-BamHI digested pExx1 plasmid to reconstitute a full-length aromatase coding sequence inserted into the yeast expression unit giving plasmid pAroX17.

Construction of Aromatase Expression
Plasmid, pH β-Aro (Figure 2)

pAroX17 was digested with restriction enzymes, Bgl II and Stu I, and the previously described 1.9 Kb fragment containing aromatase cDNA was purified. The end of the fragment created through restriction by Bgl II has a 3'-OH recessed end, and it was filled in to form blunt end by the addition of Klenow enzyme and the appropriate deoxynucleotides. This aromatase cDNA fragment was then ligated to a Sal I and Hind III restricted and bluntly ended expression vector, pH β Apr-1-neo (see Gunning, P., et al., Proc.Natl.Acad.Sci. USA 84:4831-4835 (1987)). Plasmids bearing the cDNA insert in the orientation in which the Bgl II site just flanking the β-actin promoter were selected and used for expression experiments.

Expression of Aromatase

Aromatase was expressed by human breast cancer cell lines MCF-7 and BT-20 and one non-cancerous human cell line HBL-100 transfected in known manner with the expression plasmid pH β-Aro. Aromatase was also expressed by the Chinese hamster ovary (CHO) cell line transfected with the pH β-Aro plasmid.

Aromatase Assay in Cells Transfected with pH β-Aro Plasmid

The transfected cell lines express high level of aromatase as indicated by activity measurement. The enzyme assay was performed directly on cultured cells without purifying the enzyme. Cells are grown to confluence on six-well cell culture plates. Cells are washed twice with serum free cell culture medium before assay. The substrate, androst-4-ene-3, 17-dione [1β,2β-^{3}H(N)] (specific activity, 43.1 Ci/mol), dissolved in serum free cell culture medium and filter-sterilized, is added into each well. After 30 min incubation at 37°C and followed by 5 min incubation on ice, 1 ml of culture medium is withdrawn from each well. The culture medium is initially mixed with equal volume of chloroform to extract unused substrate, and further mixed with dextran treated charcoal. Charcoal is removed by brief centrifugation, and the supernatant containing the product, tritiated water, is counted. The protein concentration is determined after dissolving cells with 0.5 N NaOH. Figure 3 serves as an example and shows that the aromatase expressed in CHO cells has activity following normal Michales-Menken kinetics. Table I shows that aromatase expressed in

these cell lines has Michales-Menken constant (K_m) and maximum velocity (V_{max}) similar to those calculated for aromatase in human placental microsomes.

TABLE I

Cell Lines	K_m (nM)	V_{max} (pmol $[^3H]$ H_2O Formed/hr/mg)
CHO	57.8	201.2
MCF-7	55.6	10
BT-20	64.5	62.5
HBL-100	39.9	26.7

Figure 4 shows that the expressed aromatase is inhibited by 4-hydroxyandrostenedione, a well-known aromatase inhibitor. A 50% inhibition of the activity would be achieved by the addition of 30 nM of the inhibitor, a concentration similar to that reported in the literature. This result indicates that this system will be useful to screen aromatase inhibitors as drugs to treat breast cancer.

WHAT IS CLAIMED IS:

1. Isolated cDNA comprising the nucleotide sequence depicted by Figure 1.

2. An isolated polypeptide comprising the amino acid sequence depicted by Figure 1.

3. The aromatase expression plasmid pH β-Aro.

4. A transfected mammalian cell which expresses aromatase.

5. A mammalian cell transfected with the plasmid pH β-Aro.

6. A human breast cancer cell transfected with plasmid pH β-Aro which expresses human aromatase.

7. A human breast cancer cell as defined by claim 6 which is an MCF-7 cell, a BT-20 cell, or an HBL-100 cell.

8. A process for screening human aromatase inhibitors which comprises determining the extent of inhibition of the activity of aromatase expressed by a cell as defined by claim 4, claim 5 or claim 6 caused by a known amount of said inhibitor.

FIG. 1-1

```
                                                    GGGAGGACGGAAGGTCCTGTG
CTCGGGATCTTCCAGACGTCGCGACTCTAAATTGCCCCCTCTGAGGTTAAGGAACACAAG      81
ATGGTTTTGGAAATGCTGAACCAGATACATTATAACATCACCAGCATCGTGCCTGAAGCC
 M  V  L  E  M  L  N  Q  I  H  Y  N  I  T  S  I  V  P  E  A
ATGCCTGCTGCCACCATGCCAGTCCTGCTCCTCACTGGCCTTTTTCTCTTGGTGTGGAAT     201
 M  P  A  A  T  M  P  V  L  L  L  T  G  L  F  L  L  V  W  N
TATGAGGGCACATCCTCAATACCAGGTCCTGGCTACTGCATGGGAATTGGACCCCTCATC
 Y  E  G  T  S  S  I  P  G  P  G  Y  C  M  G  I  G  P  L  I
TCCCACGGCAGATTCCTGTGGATGGGGATCGGCAGTGCCTGCAACTACTACAACCGGGTG     321
 S  H  G  R  F  L  W  M  G  I  G  S  A  C  N  Y  Y  N  R  V
TATGGAGAATTCATGCGAGTCTGGATCTCTGGAGAGGAAACACTCATTATCAGCAAGTCC
 Y  G  E  F  M  R  V  W  I  S  G  E  E  T  L  I  I  S  K  S
TCAAGTATGTTCCACATAATGAAGCACAATCATTACAGCTCTCGATTCGGCAGCAAACTT     441
 S  S  M  F  H  I  M  K  H  N  H  Y  S  S  R  F  G  S  K  L
GGGCTGCAGTGCATCGGTATGCATGAGAAAGGCATCATATTTAACAACAATCCAGAGCTC
 G  L  Q  C  I  G  M  H  E  K  G  I  I  F  N  N  N  P  E  L
TGGAAAACAACTCGACCCTTCTTTATGAAAGCTCTGTCAGGCCCCGGCCTTGTTCGTATG     561
 W  K  T  T  R  P  F  F  M  K  A  L  S  G  P  G  L  V  R  M
GTCACAGTCTGTGCTGAATCCCTCAAAACACATCTGGACAGGTTGGAGGAGGTGACCAAT
 V  T  V  C  A  E  S  L  K  T  H  L  D  R  L  E  E  V  T  N
GAATCGGGCTATGTGGACGTGTTGACCCTTCTGCGTCGTGTCATGCTGGACACCTCTAAC     681
 E  S  G  Y  V  D  V  L  T  L  L  R  R  V  M  L  D  T  S  N
ACGCTCTTCTTGAGGATCCCTTTGGACGAAAGTGCTATCGTGGTTAAAATCCAAGGTTAT
 T  L  F  L  R  I  P  L  D  E  S  A  I  V  V  K  I  Q  G  Y
TTTGATGCATGGCAAGCTCTCCTCATCAAACCAGACATCTTCTTTAAGATTTCTTGGCTA     801
 F  D  A  W  Q  A  L  L  I  K  P  D  I  F  F  K  I  S  W  L
TACAAAAAGTATGAGAAGTCTGTCAAGGATTTGAAAGATGCCATAGAAGTTCTGATAGCA
 Y  K  K  Y  E  K  S  V  K  D  L  K  D  A  I  E  V  L  I  A
GAAAAAAGACGCAGGATTTCCACAGAAGAGAAACTGGAAGAATGTATGGACTTTGCCACT     921
 E  K  R  R  R  I  S  T  E  E  K  L  E  E  C  M  D  F  A  T
GAGTTGATTTTAGCAGAGAAACGTGGTGACCTGACAAGAGAGAATGTGAACCAGTGCATA
 E  L  I  L  A  E  K  R  G  D  L  T  R  E  N  V  N  Q  C  I
TTGGAAATGCTGATCGCAGCTCCTGACACCATGTCTGTCTCTTTGTTCTTCATGCTATTT    1041
 L  E  M  L  I  A  A  P  D  T  M  S  V  S  L  F  F  M  L  F
CTCATTGCAAAGCACCCTAATGTTGAAGAGGCAATAATAAAGGAAATCCAGACTGTTATT
 L  I  A  K  H  P  N  V  E  E  A  I  I  K  E  I  Q  T  V  I
GGTGAGAGAGACATAAAGATTGATGATATACAAAAATTAAAAGTGATGGAAAACTTCATT    1161
 G  E  R  D  I  K  I  D  D  I  Q  K  L  K  V  M  E  N  F  I
```

```
TATGAGAGCATGCGGTACCAGCCTGTCGTGGACTTGGTCATGCGCAAAGCCTTAGAAGAT
 Y  E  S  M  R  Y  Q  P  V  V  D  L  V  M  R  K  A  L  E  D

GATGTAATCGATGGCTACCCAGTGAAAAAGGGGACAAACATTATCCTGAATATTGGAAGG  1281
 D  V  I  D  G  Y  P  V  K  K  G  T  N  I  I  L  N  I  G  R

ATGCACAGACTCGAGTTTTTCCCCAAACCCAATGAATTTACTCTTGAAAATTTTGCAAAG
 M  H  R  L  E  F  F  P  K  P  N  E  F  T  L  E  N  F  A  K

AATGTTCCTTATAGGTACTTTCAGCCATTTGGCTTTGGGCCCCGTGGCTGTGCAGGAAAG  1401
 N  V  P  Y  R  Y  F  Q  P  F  G  F  G  P  R  G  C  A  G  K

TACATCGCCATGGTGATGATGAAAGCCATCCTCGTTACACTTCTGAGACGATTCCACGTG
 V  I  A  M  V  M  M  K  A  I  L  V  T  L  L  R  R  F  H  V

AAGACATTGCAAGGACAGTGTGTTGAGAGCATACAGAAGATACACGACTTGTCCTTGCAC  1521
 K  T  L  Q  G  Q  C  V  E  S  I  Q  K  I  H  D  L  S  L  H

CCAGATGAGACTAAAAACATGCTGGAAATGATCTTTACCCCAAGAAACTCAGACAGGTGT
 P  D  E  T  K  N  M  L  E  M  I  F  T  P  R  N  S  D  R  C

CTGGAACACTAGAGAAGGCTGGTCAGTACCCACTCTGGAGCATTT-CTCATCAGTAGTTC  1641
 L  E  H                          t        -
                                  c        t
                                  t        -

ACATACAAATCATCCATCCTTGCCAATAGTGTCATCCTCACAGTGAACACTCAGTGGCCC
                                                          -
                                                          g
                                                          g

ATGGCATTTTATAGGCATACCTCCTATGGGTTGTCACCAAGCTAGGTGCTATTGGTCATC  1761
    c                        g    a
    a                        -    a
    c                        g    -

TGCTCCTGTTCACACCAGAGAACCAGGCTACAAGAGAAAAAGCAGAGGCCAAGAGTTTGA



GGG-AGAAATAGTCGGTGAAGAAACCGTATCCATAAAGACCCGATTCCACCAAATGTGCT  1881
   -                                          gg        t tg
   g                                          tt        g gc
   -                                          tt        t tg

TTGAGAAGGATAGGCCTTCATTAACAAAATGTATGTCTGGTTCCCCAGTAGAGCTCTACT



GCCTCAACCCAAGGGGATTTTTATGTCTGGGGCAGAAACACTCAAGTTGATTAGAAAGAC  2001
        c
        -
        c
```

FIG. 1-2

```
CAGGCCAATGTCAGGGTACCTGGGGCCAAACCCACCTGCTAGTGTGAATTAAAGTACTTT

AATTTTGTTTTCTGTGGAGGTGGAAAAGCAACATTCATAGTCTTTGGAGAAATGCTTAGA  2121
      g                                                     c
      c                                                     g
      g                                                     g

AATTCAGCATTTGACCCTTGCTGTGAATTAAGCCCAATTAATTCCTGTTTGTCTACATAT
            g
            a
            a

GATCTGTCTGTGGCAAAAGTTTAATCAGAGGAAATTCTTTCCCAGTCTGTCGATTTATGC  2241
                                        ggg
                                        tcc
                                        tcc

CTCAGCCACTTGCCTGTGCTACAATTCATTGTGTTACCTGTAGATTCAGGTAATACAAAC
                        g
                        a
                        a

TATATATAATCATCAAGTAATACAAACTAATTTAGTAATAGCCTGGGTTAAGTATTATTA  2361
t                                                     -
c                                                     t
t                                                     t

GGGCCCTGTGTCTGCTGTAGAAAAAAAAATTCACATGATGCACTTCAAATTCAAATAAAA


ATCCTTTTGGCATGTTCCCATTTTTGCTTAGCTCAATTAGTGTGGCTAACCAAGAGATAA  2481

CTGTAAATGTGACATTGATTTGCTCTTACTACAGCTTCAGTGATTGGGGGAGGAAAAGTC
                                      t
                                      a
                                      t

CCAACCCAATGGGCTCAAACTTCTAAGGGGTACTCCTCTCATCCCCTTATCCTTCTCCCT  2601


CGACATTTTCTCCCTCTTTCTTCCCATGACCCCAAAGC-AAGGGCAACAGATCAGTAAAG
                                      -
                                      c
                                      c

AACGTGGTCAGAGTAGAACCCCTGAAGTATTTTTTAATCCTACCTCAAAATTTAACAGTT  2721


ACCTGAGAGATTTAACATTATCTAGTTCATTGAATCATTGTATGTGGTCATGGATAAATT
                                t     t
                                t     t
                                -     -

GCACACCTTG                                                    2788
      c t
      t g
      c t
```

FIG. 1-3

FIG. 2

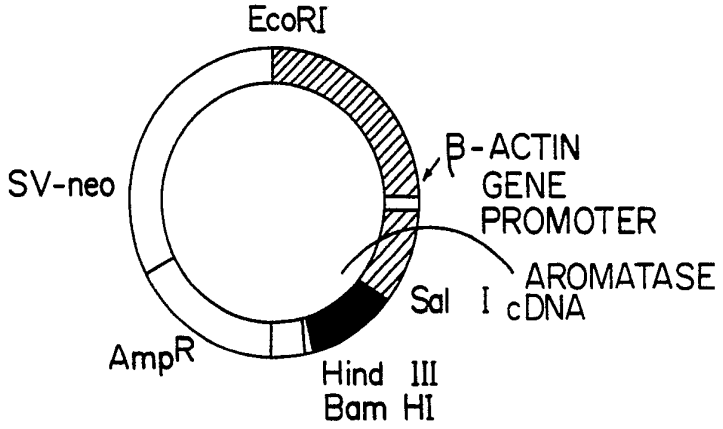


FIG. 3

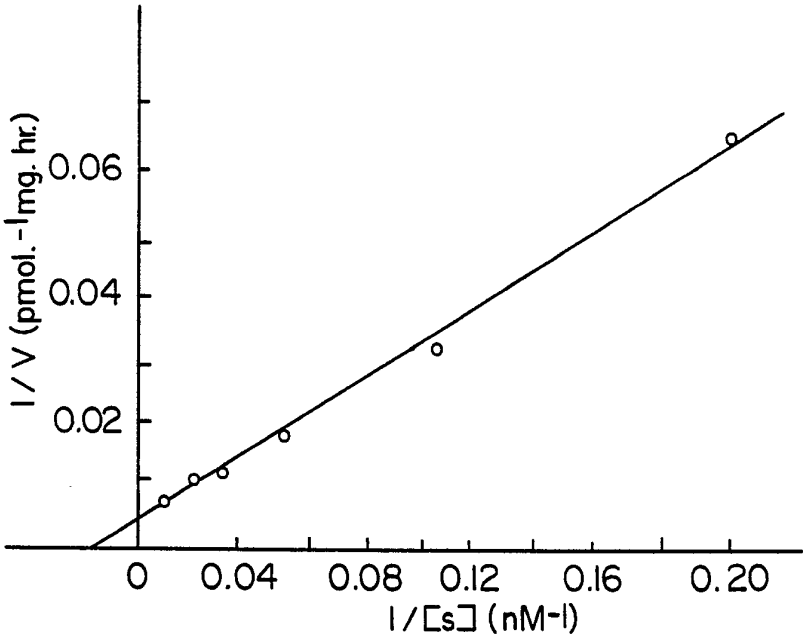
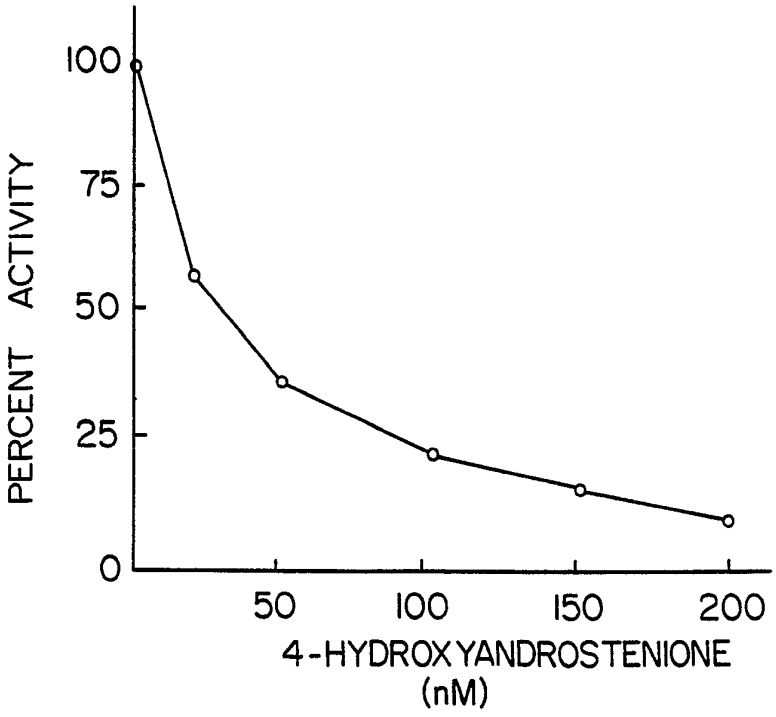


FIG. 4



INTERNATIONAL SEARCH REPORT

International Application PCT/US90/05873

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07H 15/12; C12N 5/02; C12N 5/00; C12N 15/07; C12N 15/12;A61K 37/50

US. CL.: 536/27; 435/189; 435/240.2; 435/320; 536/27; 424/94.4

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/27; 435/189; 435/240.2; 435/320; 424/94.4

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DATA BASES: DIALOG ONLINE (File CA, 1967-1990; FIle BIOSIS, 1969-1990; File MEDLINE, 1969-1990; File WPI, 1963-1990); AUTOMATED PATENT SYSTEM (FIle UPAT, 1975-1990). Keywords: Aromatase, MCF-7

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X/Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, Vol. 83, issued September 1986, EVANS et al., "Isolation and Characterization of a Complementary DNA Specific for Human Aromatase- System Cytochrome P-450 mRNA," pages 6387-6391, see e.g., Figure 2.	1 / 3-8
X/Y	MOLECULAR AND CELLULAR ENDOCRINOLOGY, Vol. 52, issued 1987, SIMPSON et al., "Sequencing of cDNA Inserts Encoding Aromatase Cytochrome P- 450 (p-450)," pages 267-272, see Figure 2.	1 / 3-8
X/Y	DNA, Vol. 7, No. 1, issued January 1988, CHEN et al., "Human Aromatase: cDNA Cloning, Southern Blot Analysis, and Assignment of the Gene to Chromosome 15," pages 27-38, see Figure 4.	1 / 3-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 January 1990	14 FEB 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Richard Lebovitz

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X/Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Vol. 156, No. 2, issued 31 October 1988, HARADA, "Cloning of a Complete cDNA Encoding Human Aromatase: Immunochemical Identification and Sequence Analysis," pages 725-732, see Figure 3.	1/3-8
X/Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, Vol. 85, issued December 1988, CORBIN et al., "Isolation of a Full-Length cDNA Insert Encoding Human Aromatase System Cytochrome P-450 and its Expression in Nonsteroidogenic Cells," pages 8948-8952, see Figure 1.	1/3-8
X/Y	MOLECULAR ENDOCRINOLOGY, Vol. 3, No. 9, issued 1989, POMPON et al., "Expression of Human Placental Aromatase in Saccharomyces cerevisiae," pages 1477-1486, see Figure 1.	1/3-8
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Vol. 134, no. 2, issued 29 January 1986, NAKAJIN et al., "Purification to Homogeneity of Aromatase From Human Placenta," pages 704-710, see page 707.	2
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, Vol. 84, issued July 1987, GUNNING et al., "A Human B-Actin Expression Vector System Directs High-Level Accumulation of Antisense Transcripts," pages 4831-4835, see entire document.	3-8
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 258, No. 20, issued 25 October 1983, LAI et al., "Regulated Expression of the Chicken Ovalbumin Gene in a Human Estrogen-Responsive Cell Line," pages 12693-12701, see e.g., ABSTRACT.	3-8

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	GENE, Vol. 69, issued 1988, SOMASEKHAR et al., "An Estrogen-Responsive Element From the 5'-Flanking Region of the Rat Prolactin Gene Functions in MCF-7 but not in Hela Cells, pages 23-28, see e.g., ABSTRACT.	3-8
Y,E	US, A, 4,985,352 (JULIUS et al.) 15 January 1991, see column 7, lines 27-36.	8