RAISBECK, LARA, RODRÍGUEZ & RUEDA
MIEMBROS DE LA FIRMA
BAKER & McKENZIE

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262 252

Señores
SUPERINTENDENCIA DE INDUSTRIA Y COMERCI
DIVISION DE NUEVAS CREACIONES
E. S.

SUPERINDUSTRIA Y COMERCIO
Radicacion : 01027558 00000004 Folios: 3
Fecha (AMD): 2003-07-23 12:51:07
Tramite : 002 PATENTES D 1 REGISTRO/ 536 PETICION
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Ref.: Expediente No. **01.027.558**
Solicitud de Patente de Invención Titulada:
"METABOLITOS AGONISTAS/ANTAGONISTAS DE ESTROGENO".
Solicitante: **PFIZER PRODUCTS INC.**

SOLICITUD DE EXAMEN DE FONDO SEGÚN EL ARTICULO 44 DE LA DECISION 486, GACETA No. 527

ALVARO CORREA ORDOÑEZ, mayor de edad y vecino de Bogotá, D.C., identificado según aparece al pie de mi firma, en mi condición de apoderado sustituto, de la sociedad Pfizer Products Inc., respetuosamente me permito solicitar, dando cumplimiento a lo establecido en el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina de Naciones, que se examine si la invención reúne los requisitos de patentabilidad previstos en la misma. Adjunto recibo por el monto de $ 335.000 pesos para cubrir la tasa correspondiente, según la Resolución 41687 del año 2002.

Atentamente,

ALVARO CORREA ORDOÑEZ
C.C. No. 79.143.366 de Usaquén
T.P. 20.281

Anexos: Sustitución de Poder
Recibo por $ 335.000 pesos

OLG/ P2001/0000822 230703.DOC

DILIGENCIA DE RECONOCIMIENTO
Y PRESENTACION PERSONAL

Ante el Notario Once del Circulo de Santafé de Bogotá, compareció [redacted]

Quién exhibió la C.C. No. [redacted]

expedida en Usaquen T.P. 20.28

y declaró que la firma y huella que aparecen en el presente memorial son suyas y que el contenido del mismo es cierto.

Memorial dirigido a interesado

Santafé de Bogotá D.C. 23 JUL 2005

Firma del Declarante

HUE[redacted]

Autorizó la anterior Diligencia

NOTARIO ONCE DEL CIRCULO DE SANTAFE DE BOGOTA D.C.

REPUBLICA DE COLOMBIA
NOTARIA DE CIRCULO DE
SANTAFE DE BOGOTA, D.C.

[redacted] NAVARRO DE NAUTIOTA
NOTARIA ONCE

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RAISBECK, LARA, RODRÍGUEZ & RUEDA
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BAKER & McKENZIE

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Señores
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

Ref.: Solicitud colombiana de patente No. **01.027.558**
Solicitante: **PFIZER PRODUCTS INC.**
"METABOLITOS AGONISTAS/ANTAGONISTAS DE ESTROGENO".

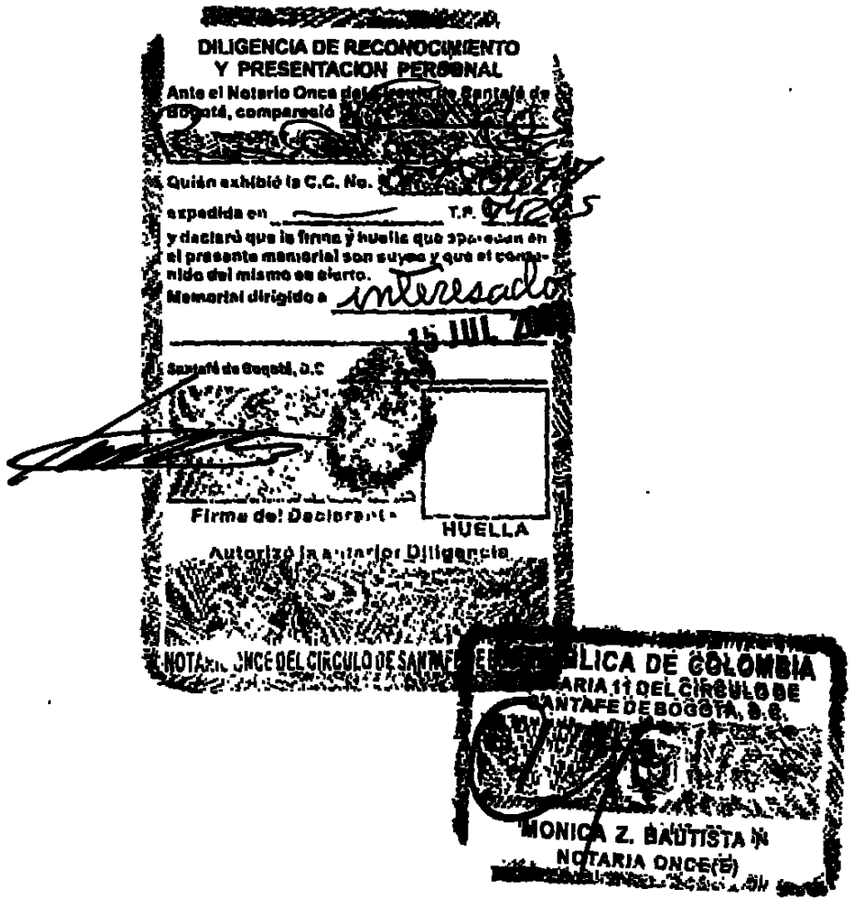
SUSTITUCIÓN DE PODER

CARLOS R. OLARTE, mayor y vecino de Bogotá. D.C., identificado como aparece al pie de mi firma, actuando en mi carácter de apoderado de la sociedad de la referencia, respetuosamente me permito manifestar que **SUSTITUYO** el poder a mi conferido por la mencionada sociedad en el doctor **ALVARO CORREA ORDOÑEZ,** identificado con la Cédula de Ciudadanía número 79.143.366 de Usaquén y con la Tarjeta Profesional número 20.281, para que sea él quien en adelante, asuma el trámite de la presente solicitud.

El apoderado sustituto queda con las mismas facultades del principal.

Atentamente,

CARLOS R OLARTE
C.C. No. 79.782.747 de Bogotá
T. P. No. 74.295 del Consejo Superior de la Judicatura



DILIGENCIA DE RECONOCIMIENTO
Y PRESENTACION PERSONAL

Ante el Notario Once del Círculo de Santafé de Bogotá, compareció

Quién exhibió la C.C. No.

expedida en T.P.

y declaró que la firma y huella que aparecen en el presente memorial son suyas y que el contenido del mismo es cierto.

Memorial dirigido a interesado

15 JUL

Santafé de Bogotá, D.C.

Firma del Declarante

HUELLA

Autorizó la anterior Diligencia

NOTARIA ONCE DEL CIRCULO DE SANTAFE

REPUBLICA DE COLOMBIA
NOTARIA 11 DEL CIRCULO DE
SANTAFE DE BOGOTA, D.C.

MONICA Z. BAUTISTA N
NOTARIA ONCE(E)

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264

SUPERINDUSTRIA Y COMERCIO
Radicacion : 01027358 00000004 Folios: 3
Fecha (AMD): 2003-07-23 12:51:07
Tramite : 002 PATENTES D 1 REGISTRO/ 536 PETICION
NIT : 800176089-2 Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 03 - 43,886
FECHA : JUNIO 24 DE 2003

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
JULIAN ANDRES VARGAS	CONSIGNACION	BANCO POPULAR	050-00110-6	13228244	24/06/2003	18,050,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	85 EXAMEN DE PATENTABILIDAD DE INVEN	335,000.00
		TOTAL : $	335,000.00

SON: TRESCIENTOS TREINTA Y CINCO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

255

Industria y Comercio
SUPERINTENDENCIA

265

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. **01-27558**

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **527**, DE

FECHA **30** DE **ABRIL** DE **2003**, EL TERMINO HABIL SEÑALADO POR LA LEY

EXPIRA EL **29** DE **JULIO** DE **2003**.

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI ____ _____ NO ____ X _____

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Santa Fe de Bogotá D.C. - Colombia

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SUPERINTENDENCIA DE INDUSTRIA Y C MERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020/
Bogotá, D.C.,

Doctor **Oficio Nº** 12923
Alvaro Correa Ordóñez
Apoderado
PFIZER PRODUCTS INC.

REFERENCIA	**Radicación**	**01 27558**
	Trámite	**002**
	Evento	**001**
	Actuación	**430**
	Folios	**002**

Como resultado del examen de patentabilidad de la solicitud de patente de invención Nº 01 27558, realizado según el artículo 46 de la decisión 486 de la comisión de la Comunidad Andina, se notifica:

SOLICITUD DE SUMINISTRO DE INFORMACIÓN

El art. 46 de la Decisión 486 establece que "*La Oficina Nacional competente podrá requerir el informe de expertos o de organismos científicos o tecnológicos que se consideren idóneos para que emitan opinión sobre la patentabilidad de la invención. Asimismo, cuando lo estime conveniente, podrá requerir informes de otras oficinas de propiedad industrial. De ser necesario, a efectos del examen de patentabilidad y a requerimiento de la oficina nacional competente, el solicitante proporcionará, en un plazo que no excederá de 3 meses, uno o más documentos relativos a una o más solicitudes extranjeras referidas total o parcialmente a la misma invención que se examina: ...b) copia de los resultados de exámenes de novedad o de patentabilidad efectuados respecto a esa solicitud extranjera...Si el solicitante no presentara los documentos requeridos dentro del plazo señalado en el presente artículo la oficina nacional competente denegará la patente*"

Como consecuencia de la búsqueda de anterioridades de materia relacionada con la de la presente solicitud, se determinó que existe en el estado de la técnica el siguiente documento que eventualmente afectaría los requisitos de patentabilidad de esta solicitud:

DATABASE CHEMABS
Chemical Abstracts service, Columbus, Ohio, US; STN, CAPLUS accession no. 2000:151487, XP002176764 abstract; RN 260357-98-0

Siendo que la publicación referenciada hace parte del resultado del examen de búsqueda realizado para la solicitud WO 0177093, equivalente a la solicitud colombiana aquí estudiada, y no siendo viable la consecución de dicho artículo por

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este despacho, se requiere que el solicitante, en virtud de lo establecido en el art. 46 de la Decisión 486 de la Comisión de la Comunidad Andina, para que proporcione la publicación completa, con lo que podrá realizarse un análisis absoluto de la patentabilidad de la invención correspondiente a la presente solicitud.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo 5 del título primero de la Circular Única Nº 10 de 2001.

Álix Carmenza Céspedes de Vergel
Jefe de la División de Nuevas Creaciones

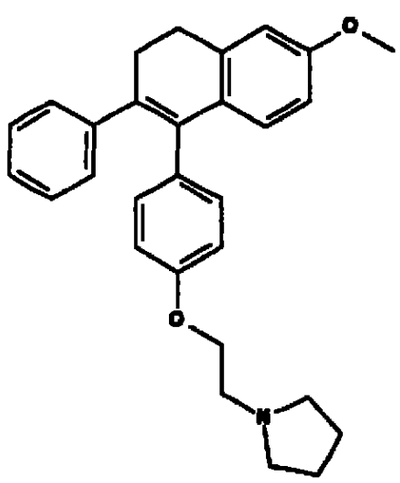
El anterior requerimiento fue notificado por fijación en lista, de fecha 09 NOV. 2006

CONCEPTO TÉCNICO Número 01713	**EXAMINADOR TÉCNICO** Código 202044

258 ~~268~~

Substance Summary:

Compound Displayed PubChem



SID: 159096
CID: 4416
NLM Toxicology: Link
Related Substances:
Same: 9 Links
Similar Substances: 74 Lin
Structure Search

Source: ChemIDplus (001845110)

Mesh | Synonyms | Properties | Descriptor

Medical Subject Annotations: (Total:1)

Nafoxidine
An estrogen antagonist that has been used in the treatment of breast cancer.

Show MeSH Tree Structure

Pharmacological Action:
Estrogen Antagonists
Antineoplastic Agents, Hormonal

PubMed via MeSH Choose by Subheadings:

administration and dosage | adverse effects | analogs and deriv

259 269

antagonists and inhibitors	chemical synthesis	chemistry
diagnostic use	metabolism	pharmacology
therapeutic use	toxicity	

Depositor-Supplied Synonyms: (Total: 11)

Display: Next 1 | All | Sort: Weigl

Nafoxidine
Nafoxidinum [INN-Latin]
Nafoxidina [INN-Spanish]
NSC 70735
BRN 1440873
5-20-01-00210 (Beilstein Handbook Reference)
1845-11-0
1847-63-8
Pyrrolidine, 1-(2-(p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy)ethyl)-
1-(2-(p-(3,4-Dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy)ethyl)pyrrolidine

Properties Computed from Structure:

Molecular Weight: 425.562 g/mol
Molecular Formula: $C_{29}H_{31}NO_2$

Hydrogen Bond Donor Count: 0
Hydrogen Bond Acceptor Count: 3
Rotatable Bond Count: 7

Descriptors Computed from Structure:

IUPAC Name: 1-[2-[4-(6-methoxy-2-phenyl-3,4-dihydronaphthalen-1-yl)phenoxy]ethyl]pyrrc
Canonical SMILES: COC1=CC2=C(C=C1)C(=C(CC2)C3=CC=CC=C3)C4=CC=C(C=C4)OCCN5
InChI: InChI=1/C29H31NO2/c1-31-26-14-16-28-24(21-26)11-15-27(22-7-3-2-4-8-22)29(28
23)32-20-19-30-17-5-6-18-30/h2-4,7-10,12-14,16,21H,5-6,11,15,17-20H2,1H3

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Biochem. J. (1977) 164, 659–667
Printed in Great Britain

Interaction of the Anti-Oestrogen, Nafoxidine Hydrochloride, with the Soluble Nuclear Oestradiol-Binding Protein in Chick Liver

By CATHERINE B. LAZIER and WILLIAM S. ALFORD
Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

(*Received* 3 *November* 1976)

Nafoxidine hydrochloride (Upjohn, 11100A) injected with oestradiol into immature chicks inhibits the hormone-induced increase in [^{3}H]oestradiol-binding activity in salt extracts of liver nuclei as well as the subsequent production by liver of egg-yolk phosphoprotein. Substantial inhibition of both oestradiol-induced responses is seen when nafoxidine is given in a dose approximately equimolar with that of oestradiol. *In vitro* nafoxidine competitively inhibits binding of [^{3}H]oestradiol in nuclear extracts. The K_i for the inhibition is 43 nM, which indicates an affinity of nafoxidine for the binding protein about 4% of that of oestradiol. The inhibitory action of nafoxidine *in vivo* thus is more potent than the relative binding affinity determined *in vitro* might indicate. One possible explanation is that the primary site of nafoxidine action is at a point proximal to nuclear receptor interaction. Nafoxidine injected alone into the chick does not induce phosphoprotein synthesis, but it does increase [^{3}H]oestradiol-binding activity in extracts of liver nuclei to a limited extent. No differences in the properties of the oestradiol-binding activity in extracts from nafoxidine-treated chicks or from oestradiol-treated chicks were detected. Chick liver cytosol does not contain detectable high-affinity oestradiol-binding activity. A low-affinity oestradiol-binding component with a sedimentation coefficient of 3.5S was found, but it was unaffected by treatment of chicks with either nafoxidine or oestradiol. The results suggest a difference in the mechanism of oestradiol action in the chick liver and in the widely studied rat uterus, on which the usual model for oestradiol action is largely based.

Non-steroidal anti-oestrogens have been useful probes in the investigation of the mechanism of action of oestradiol. Some of the earliest indications of the physiological relevance of oestradiol–cytosol receptor interaction came from experiments with anti-oestrogens (Jensen, 1966). These and other studies resulted in the current model for oestradiol action in the mammalian uterus, in which the hormone initially binds a specific high-affinity cytosol receptor, the complex undergoes a transformation from a 4S to a 5S species and migrates to the nucleus, where it interacts with specific acceptor sites in the chromatin and stimulates specific transcriptional events (Jensen *et al.*, 1974; O'Malley & Means, 1974).

The anti-oestrogen nafoxidine hydrochloride (1-{2-[*p*-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]ethyl}pyrrolidine hydrochloride; Upjohn 11100A) is a diphenyldihydronaphthalene derivative which has been widely used in mammalian systems (Jensen & DeSombre, 1973). It binds both the cytoplasmic and nuclear forms of oestradiol receptor in rat uterus (Rochefort *et al.*, 1972*a*). It exhibits both agonistic and antagonistic effects in rat uterus, and recent work suggests that a site of the antagonistic action is in the blockage or delay in replenishment of the cytoplasmic oestrogen receptor (Clark *et al.*, 1974; Katzenellenbogen & Ferguson, 1975; Capony & Rochefort, 1975).

In the chick liver, oestradiol induces the formation of the egg-yolk phosphoprotein precursor vitellogenin (Clemens, 1974; Deasy *et al.*, 1975). Particular interest in the mechanism of oestradiol action in this tissue derives from the fact that, with one exception (Arias & Warren, 1971), no groups have been able to demonstrate the presence of a typical high-affinity cytosol receptor for oestradiol (Mester & Baulieu, 1972; Onon & Bellé, 1973; Lazier, 1975). They were, however, able to show specific high-affinity binding of oestradiol in salt extracts of liver nuclei. Such activity is also present in an insoluble nuclear residue (Lebeau *et al.*, 1974) and in chromatin (Gschwendt & Kittstein, 1974). Treatment of immature chicks or of roosters with oestradiol results in a substantial increase in each of these binding activities preceding the production of vitellogenin. Rooster liver cytosol contains only a low-affinity binding protein for oestradiol (Gschwendt, 1975*a*).

Studies on anti-oestrogen action in chick liver were thus undertaken in an attempt to clarify the apparent difference in the interaction of oestradiol in this tissue and in the rat uterus. Indeed, two reports show that nafoxidine is anti-oestrogenic in a single injection in the chick and is not itself oestrogenic (Gschwendt, 1975*b*; Lazier, 1975). This contrasts with the situation in the rat (Clark *et al.*, 1974). Gschwendt (1975*b*) noted the inhibitory action of

nafoxidine on the rooster liver oestradiol-binding sites in chromatin. Here we examine the effect of nafoxidine in the presence or absence of oestradiol on the salt-soluble nuclear oestradiol-binding activity. Preliminary abstracts dealing with some of the findings have been presented elsewhere (Lazier & Alford, 1975, 1976b).

Materials and Methods

Chemicals

[2,4,6,7-^{3}H]Oestradiol-17β(114Ci/mmol) was obtained from New England Nuclear Corp., Montreal, Quebec. Radiochemical purity, monitored by t.l.c., was greater than 98%. [^{14}C]Formaldehyde was from New England Nuclear Corp. Unlabelled steroids, bovine serum albumin and puromycin dihydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Nafoxidine hydrochloride was a generous gift of the Upjohn Co., Kalamazoo, MI, U.S.A. The charcoal–dextran suspension was prepared from Norit A (Fisher Chemical Co., Fairlawn, NJ, U.S.A.) and Dextran T70 (Pharmacia, Montreal, Quebec, Canada).

Animals and injections

Male Cobb chicks (1–3 weeks old) were obtained from a local hatchery. Oestradiol, puromycin and nafoxidine were all dissolved in propylene glycol for injection (intraperitoneally) at concentrations of 25, 25 and 50mg/ml respectively. Doses were 2.5mg/100g body wt. for oestradiol, 2.5mg/100g for puromycin and 5.0mg/100g for nafoxidine, unless otherwise indicated.

Preparation of cell extracts

The crude nuclear pellet was prepared by centrifugation in sucrose-containing buffers (A and B) as described previously (Lazier, 1975). Nuclear extracts for sucrose-gradient centrifugation were prepared in the same buffers supplemented with thioglycerol (10mM) as recommended by Harrison & Toft (1975). This did not appear to affect the activity or stability of [^{3}H]oestradiol binding.

The washed crude nuclear pellets were homogenized in buffer C (0.5M-KCl/1.5mM-EDTA/10mM-Tris/HCl, pH7.4) (1g of tissue/ml). DNA in the nuclear homogenate was determined by the method of Burton (1970) and is expressed as μmol of nucleotide. The homogenate was frozen for at least 1h at −20°C, thawed and centrifuged at 37000*g* for 30min. The supernatant thus obtained is the nuclear extract. Washing the nuclear pellet with Triton X-100 solutions did not affect the yield of oestradiol-binding activity (per unit of DNA), indicating that the salt-soluble binder is actually located in the nuclei (Best-Belpomme *et al.*, 1975).

Cytosol was prepared by centrifugation of the initial 700*g* supernatant at 100000*g* for 1h.

Determination of [^{3}H]oestradiol binding

All cell extracts were preincubated with an equal volume of charcoal–dextran suspension (0.25% Norit A, 0.0025% dextran in 10mM-Tris/HCl/1.5mM-EDTA, pH7.4) for 30min at 37°C. Lazier (1975) showed that this treatment was necessary in extracts from oestradiol-treated chicks to expose specific sites for assay subsequently by incubation with [^{3}H]oestradiol at 2°C. Rochefort *et al.* (1972*a*) used the procedure to remove endogenous bound and unbound nafoxidine from uterine cell fractions. The nuclear oestradiol-binding protein of chick liver is stable throughout incubation at 37°C.

Incubation of the charcoal-treated extracts (0.6ml) with [^{3}H]oestradiol (0.25–2.5nM) was for 16h at 2°C. Unlabelled diethylstilboestrol (1000-fold excess) was included in parallel assay mixtures for determination of non-specific binding. After the incubation at 2°C, an equal volume of charcoal–dextran suspension (*concentration as above*) was added and after 30*min* the mixtures were centrifuged at 3000*g* for 10min. The supernatants were added to Aquasol (New England Nuclear Corp.) and the radioactivity corresponding to the bound [^{3}H]oestradiol was determined in a Nuclear–Chicago Isocap 300 liquid-scintillation spectrometer (efficiency approx. 35%). All data are given as specific [^{3}H]oestradiol binding, ascertained by subtracting the value for non-specific binding from that for total binding obtained on incubation with [^{3}H]oestradiol alone.

Preparation of serum and phosphoprotein P analysis

The procedures were exactly as described previously (Lazier, 1975).

Sucrose-gradient centrifugation

Sucrose (5–20%, w/v) gradients were prepared in 4.0ml polypropylene tubes (Martin & Ames, 1961). Stock solutions (0.5 and 28%, w/v) of sucrose in 10mM-Tris/HCl/1mM-EDTA/10mM-thioglycerol, pH7.5, were used to prepared low-salt gradients by using a Buchler gradient former. High-salt gradients were prepared by including 0.5M-KCl in both of the stock sucrose solutions. Cytosol (0.3ml), incubated for 30min at 2°C with 1nM-[^{3}H]oestradiol, was layered on the gradient, which was then centrifuged in a Spinco SW.56 rotor at 40000rev./min for 16h. Fractions (two drops each) were obtained by piercing the bottom of the tube. The fractions were added to Aquasol and radioactivity was determined as described above.

Sucrose gradients were also prepared from high- or low-salt stock sucrose solutions which contained [^{3}H]oestradiol (1 nM). Samples (0.2 ml) of nuclear extract were layered on the gradient, and centrifugation and collection of fractions were as described for conventional gradients. Bound [^{3}H]oestradiol in the fractions was determined by the charcoal-adsorption technique used by Harrison & Toft (1975). Charcoal–dextran suspension (0.4 ml) was added to each fraction, and after 1 min at 2°C the mixture was centrifuged at 3000*g* for 10 min. The charcoal–dextran suspension was more concentrated (0.5% charcoal, 0.05% dextran) than that used in other experiments reported here, and the exposure time to charcoal was briefer (1 min). However, preliminary experiments showed that the same amount of specific [^{3}H]oestradiol binding was measured by the two techniques.

Sedimentation values were determined by comparison with an internal ^{14}C-labelled bovine serum albumin marker (Martin & Ames, 1961) prepared by the method of Rice & Means (1971).

Results

Nafoxidine inhibition of oestradiol-induced [^{3}H]oestradiol binding and of phosphoprotein production

Table 1 shows the inhibitory effect of nafoxidine given intraperitoneally with oestradiol in several dose combinations on the hormone-induced increase in [^{3}H]oestradiol-binding activity in salt extracts of nuclei and on phosphoprotein production. The dose of oestradiol is similar to that found necessary for induction of phosphoprotein production by other investigators (Bauving & Gruber, 1971; Gschwendt & Kittstein, 1974; Denley *et al.*, 1975). Table 1 (*a*) shows that nafoxidine given in a dose twice that of oestradiol (w/w, or 1.2:1 on a molar basis) almost completely inhibited both responses at the times examined. With the maximum effective dose of oestradiol (10 mg/100 g; Lazier, 1975), 50% inhibition of phosphoprotein production at 64 h was seen with an equal dose of nafoxidine (w/w, or a molar ratio of nafoxidine to oestradiol of 0.6:1) (Table 1*b*). This contrasts with the usual doses required in the uterus, where anti-oestrogen/hormone molar ratios of 25–50 are generally used (Clark *et al.*, 1974; Katzenellenbogen & Ferguson, 1975). Table 1 also shows that nafoxidine itself did not induce phosphoprotein synthesis.

Effect of nafoxidine in vitro on the nuclear [^{3}H]oestradiol-binding protein

Nafoxidine added *in vitro* to nuclear extract prepared from livers of oestradiol-treated chicks inhibited binding of [^{3}H]oestradiol in a competitive manner. Fig. 1 shows a double-reciprocal plot for specific [^{3}H]oestradiol binding in the absence and presence of three concentrations of nafoxidine. A plot of the slopes versus inhibitor concentration (Fig. 1 inset) yields a straight line, suggesting simple linear competitive inhibition. The K_i for nafoxidine was

Table 1. *Anti-oestrogenic action of nafoxidine: effect of time and dose*

Specific binding of [^{3}H]oestradiol (1 nM) in nuclear extracts of liver, and serum phosphoprotein P contents were assayed as described in the Materials and Methods section. Each group represents the mean ± S.E.M. for duplicate determinations on three or four individual chicks. The numbers in parentheses refer to the dose of oestradiol or nafoxidine (mg/100 g body wt).

Treatment and dose	Time (h)	[^{3}H]Oestradiol bound (fmol/μmol of DNA)	Serum phosphoprotein P (μg/ml)
(*a*)			
Oestradiol (2.5)	15	48.1 ± 14.0	—
Oestradiol (2.5)+nafoxidine (5.0)	15	8.2 ± 4.2	—
Oestradiol (2.5)	48	47.6 ± 9.9	32.6 ± 8.6
Oestradiol (2.5)+nafoxidine (5.0)	48	16.9 ± 3.9	~1.0
Oestradiol (10)	48	92.0 ± 11.1	51.8 ± 14.0
Oestradiol (10)+nafoxidine (20)	48	16.9 ± 4.0	2.0 ± 0.1
(*b*)			
Oestradiol (10)	24	61.1 ± 14.0	—
Oestradiol (10)+nafoxidine (10)	24	48.8 ± 18.1	—
Oestradiol (10)	48	92.0 ± 11.1	51.8 ± 14.0
Oestradiol (10)+nafoxidine (10)	48	15.3 ± 4.4	13.8 ± 7.0
Oestradiol (10)	64	59.5 ± 4.6	118.3 ± 4.3
Oestradiol (10)+nafoxidine (10)	64	17.7 ± 4.0	60.5 ± 7.0
(*c*)			
Vehicle control	48	9.2 ± 1.2	~1.0
Nafoxidine (5.0)	48	17.4 ± 2.8	~1.0
Nafoxidine (10.0)	64	—	~1.0

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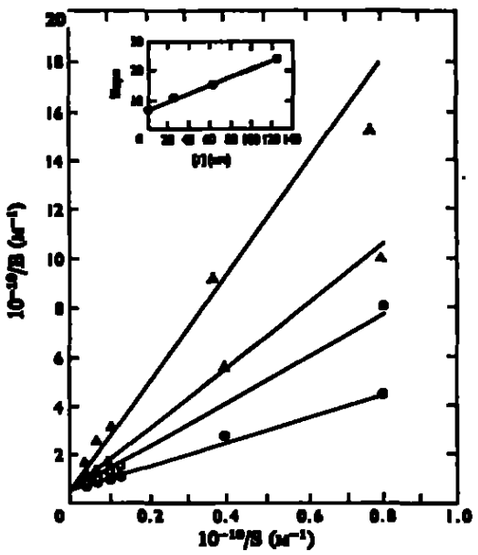

Fig. 1. *Double-reciprocal plot for nafoxidine inhibition of [^{3}H]oestradiol-binding activity in vitro*

Salt extract of liver nuclei was prepared 20 h after injection of oestradiol. Specific binding of various concentrations of [^{3}H]oestradiol in the absence and presence of three concentrations of nafoxidine was determined as described in the Materials and Methods section. ●, [^{3}H]Oestradiol alone; ○, +25 nM-nafoxidine; ▲, +62.5 nM-nafoxidine; △, +125 nM-nafoxidine. 1/B, Reciprocal of bound oestradiol; 1/S, reciprocal of [^{3}H]oestradiol added. Inset: a plot of the slopes from the double-reciprocal plot $\left[\frac{K_d}{B}\left(1+\frac{I}{K_i}\right)\right]$ versus inhibitor concentration (I).

43 nM, reflecting an affinity of nafoxidine for the receptor which is about 4% that of oestradiol. This contrasts sharply with the inhibitory potency of nafoxidine *in vivo* (see Table 1).

Effects of nafoxidine alone: time-course for the increase in [^{3}H]oestradiol-binding activity and the effect of puromycin

As was apparent in Table 1 and has been shown earlier (Lazier, 1975; Gschwendt, 1975*b*), nafoxidine itself does not induce phosphoprotein production. However, given *in vivo* it does result in a small but significant increase in [^{3}H]oestradiol binding in liver nuclear extracts. Fig. 2 shows the extent of the increase in extracts prepared at various times after nafoxidine injection. A comparable dose of oestradiol gave a much earlier and more extensive increase in nuclear-binding activity (Lazier, 1975). The early

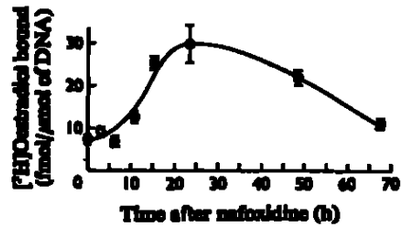

Fig. 2. *Time course of the increase in [^{3}H]oestradiol-binding activity in salt extracts of liver nuclei after nafoxidine injection*

Specific binding of 1 nM-[^{3}H]oestradiol was measured as described in the Materials and Methods section. Each point represents the mean ± S.E.M. for extracts from at least three individual chicks, assayed in duplicate.

(1½ h) increase in [^{3}H]oestradiol binding in nuclear extracts found after oestradiol injection was inhibited by cycloheximide and actinomycin D (Lazier, 1975). It was much more difficult to demonstrate an effect of translation or transcription inhibitors on the nafoxidine effect, because of the marked toxicity of these drugs in chicks (LeCount & Grey, 1972), and the relatively long exposure time required before a measurable nafoxidine response is seen. Cycloheximide given in the maximum non-lethal dose 1 h before nafoxidine had a marginal effect on the increase in [^{3}H]oestradiol binding measured 16 h after nafoxidine (results not shown). Puromycin was less toxic over the required 16 h time-period. Table 2 shows that a single dose of puromycin 1 h before nafoxidine partly inhibited the effect of nafoxidine. Two doses of puromycin, 1 h before and 10 h after nafoxidine, were inhibitory, but a single dose of puromycin 10 h after nafoxidine was ineffective. Thus protein synthesis may be important early in the response of chick liver to nafoxidine.

Properties of the nafoxidine-increased [^{3}H]oestradiol-binding activity

It was important to establish whether or not the [^{3}H]oestradiol-binding activity increased by nafoxidine in nuclear extracts was the same activity as that increased by oestradiol itself. Scatchard (1949) plots of the specific [^{3}H]oestradiol-binding activity in nuclear extracts from nafoxidine-treated and oestradiol-treated chicks gave K_d values of 1.8 nM and 1.7 nM respectively. Somewhat lower values have been reported for the oestradiol-induced activity by several groups (Mester & Baulieu, 1972; Onon & Bellé, 1973; Lazier, 1975). It is our experience, however, that liver preparations from different flocks of chicks may

Table 2. *Effect of puromycin on the nafoxidine-induced increase in nuclear [^{3}H]oestradiol-binding activity*
Puromycin was injected at the times shown relative to nafoxidine injection. All chicks were killed 15h after nafoxidine. Specific binding of [^{3}H]oestradiol (1nM) was determined in liver nuclear extracts as described in the Materials and Methods section. Data are expressed as mean values ±S.E.M. for duplicate determinations on three separate liver preparations.

Treatment	[^{3}H]Oestradiol-binding activity (fmol/μmol of DNA)
Nafoxidine	21.7±0.5
Nafoxidine+puromycin (−1h)	11.1±2.4
Nafoxidine+puromycin (−1h, +10h)	6.0±2.1
Nafoxidine+puromycin (+10h)	25.1±2.4
Control	4.2±2.0

Table 3. *Specificity of [^{3}H]oestradiol-binding activity in salt extracts of liver nuclei from oestradiol-treated and nafoxidine-treated chicks*
Binding of 1nM-[^{3}H]oestradiol was determined as outlined in the Materials and Methods section. The potential competitor was added to the assay mixture in the concentration indicated. The results are given as the mean±range for duplicate determinations.

Addition	Concentration	[^{3}H]Oestradiol-binding activity in liver nuclear extracts (c.p.m.)	
		Oestradiol-treated chicks	Nafoxidine-treated chicks
None	—	6185±129	2941±48
Diethylstilboestrol	1μM	1726±46	1628±24
Oestradiol-17β	1μM	1563±70	1672±18
Oestradiol-17β	10nM	2625±103	2047±10
Oestradiol-17α	1μM	1579±12	1585±88
Oestradiol-17α	10nM	3903±38	2377±57
Progesterone	1μM	6231±197	2855±50
Testosterone	1μM	6655±40	3112±37
Cortisol	1μM	6665±41	3114±48
Nafoxidine	100nM	2620±24	1890±11
Nafoxidine	10nM	3801±19	2325±32

yield values for the K_d for [^{3}H]oestradiol which vary from 0.5 to 2.0nM.

The specificity of the [^{3}H]oestradiol-binding activity in liver nuclear extracts from nafoxidine- and oestradiol-treated chicks is compared in Table 3. Progesterone, cortisol and testosterone had no effect in either case, but oestradiol-17β, oestradiol-17α, diethylstilboestrol and nafoxidine all suppress the binding of [^{3}H]oestradiol by both types of extract. Oestradiol-17α was less effective than oestradiol-17β at a concentration of 10nM. The partial competition by oestradiol-17α has been observed earlier (Master & Baulieu, 1972). In addition, Gschwendt & Kittstein (1974) showed that injection of oestradiol-17α into roosters resulted in an increase in [^{3}H]oestradiol-17β binding by liver chromatin and in phosphoprotein production. Chan & Common (1974) have suggested that oestradiol-17α is a major phenolic steroid in hen plasma.

Neither the [^{3}H]oestradiol-binding activity in liver nuclear extracts from oestradiol-treated chicks nor that in extracts from nafoxidine-treated chicks was reproducibly stable on centrifugation in conventional sucrose gradients. However, centrifugation in gradients containing an even distribution of [^{3}H]oestradiol did give consistent results. Such gradients have been reported by Harrison & Toft (1975) to give superior resolution of the oestradiol receptor from chick oviduct cytosol when compared with conventional sucrose gradients. Fig. 3 shows that both types of extract in high-salt gradients exhibit specific [^{3}H]oestradiol-binding activity with a sedimentation coefficient of 4.5S. In low-salt gradients a loss of about 30% of the activity in the 4.5S peak was seen for extracts from oestradiol-treated and nafoxidine-treated chicks (Lazier & Alford, 1976a). No other binding species appear, in contrast with the typical aggregation of the 5S nuclear uterine oestradiol receptor to an 8–10S species in low-salt conditions (Alberga *et al.*, 1970).

The temperature-sensitivity of the [^{3}H]oestradiol-binding activity was compared in both types of extract (Fig. 4). Similar inactivation curves were seen for both cases, with a half-life of 5min at about 55°C. The nuclear oestradiol-binding protein of chick liver is relatively temperature-stable compared with

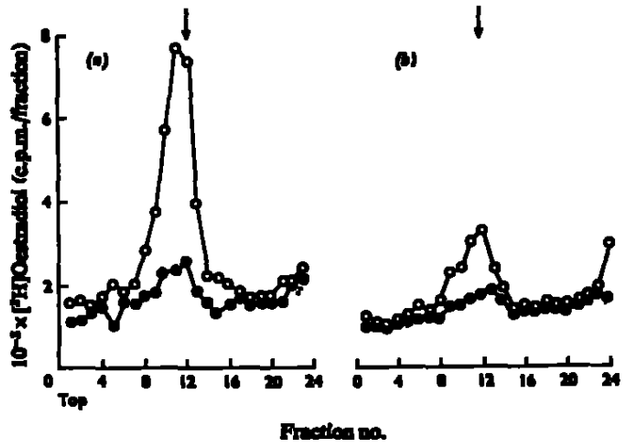

Fig. 3. *Sedimentation of the soluble nuclear [^{3}H]oestradiol-binding activity in sucrose gradients containing an even distribution of [^{3}H]oestradiol*

Nuclear extract (0.2ml) from oestradiol-treated (*a*) or nafoxidine-treated (*b*) chicks was applied to high-salt sucrose gradients containing an even distribution of 1nm-[^{3}H]oestradiol, as described in the Materials and Methods section. Chicks were injected 20h before death. ○, [^{3}H]Oestradiol; ●, [^{3}H]oestradiol in gradients run in the presence of 0.1μm-diethylstilboestrol; arrow represents the internal ^{14}C-labelled bovine serum albumin marker.

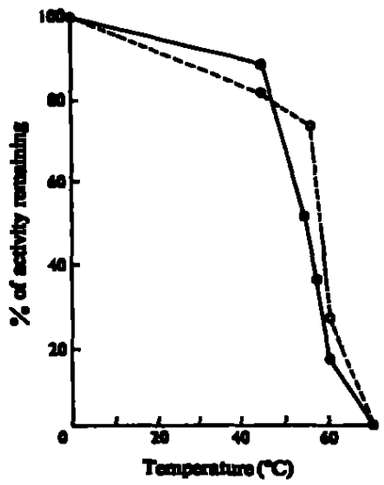

Fig. 4. *Temperature-sensitivity of [^{3}H]oestradiol-binding activity in nuclear extracts from oestradiol-treated or nafoxidine-treated chicks*

Extracts were preincubated for 5min at the indicated temperature, 0.6ml samples removed, cooled on ice and assayed in triplicate for specific binding of 2nm-[^{3}H]oestradiol as described in the Materials and Methods section. ●, Extract from oestradiol-treated chicks; ○, extract from nafoxidine-treated chicks. Chicks were injected 20h before death.

the nuclear receptor in uterus, where 50% of binding activity is lost after 5min at 45°C for receptor heated in the absence of oestradiol (Puca *et al.*, 1971).

Thus the high-affinity [^{3}H]oestradiol-binding activity in liver nuclear extracts from nafoxidine-treated chicks exhibits properties of [^{3}H]oestradiol affinity, sucrose-gradient sedimentation, hormone specificity and temperature sensitivity very similar to those for extracts from oestradiol-treated chicks.

Effect of nafoxidine on the low-affinity cytosol oestradiol-binding protein

Gschwendt (1975*a*) has characterized a low-affinity [^{3}H]oestradiol-binding protein in rooster liver cytosol which sedimented at about 4S in conventional sucrose gradients. We have confirmed his data for cytosol from immature chicks. On conventional sucrose gradients or gradients containing an even distribution of [^{3}H]oestradiol and an internal ^{14}C-labelled albumin marker, we find a single [^{3}H]oestradiol-binding species, sedimenting at 3.3–3.5S. Binding was not suppressed by inclusion of a 100-fold excess of diethylstilboestrol in the gradients. Fig. 5 shows conventional sucrose-gradient profiles for liver cytosol from control chicks and from chicks that had been given nafoxidine or oestradiol 20h previously. In rat uterus, exposure to nafoxidine for 23h results in marked depletion of the cytosol oestradiol receptor (Clark *et al.*, 1974). In the chick liver, however, injection of neither nafoxidine nor oestradiol had any

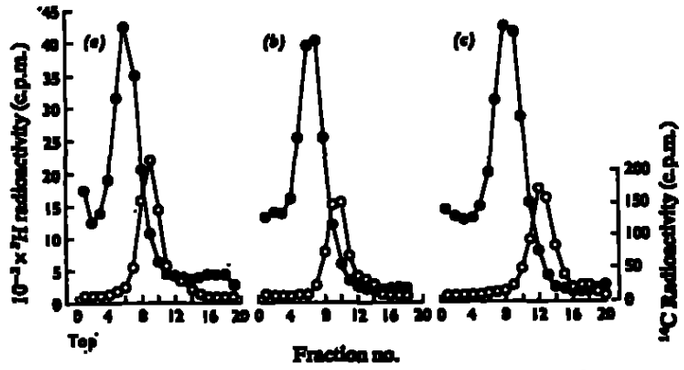

Fig. 5. *Sedimentation of cytosol on conventional sucrose gradients*

Cytosol was incubated for 30min at 2°C with 1nM-[^{3}H]oestradiol, and 0.3ml was applied to 5–20% sucrose gradients along with an internal ^{14}C-labelled albumin marker as described in the Materials and Methods section. (*a*) Liver cytosol from control chicks; (*b*) liver cytosol from oestradiol-treated chicks (20h); (*c*) liver cytosol from nafoxidine-treated chicks (20h). ●, [^{3}H]Oestradiol; ○, ^{14}C-labelled bovine serum albumin.

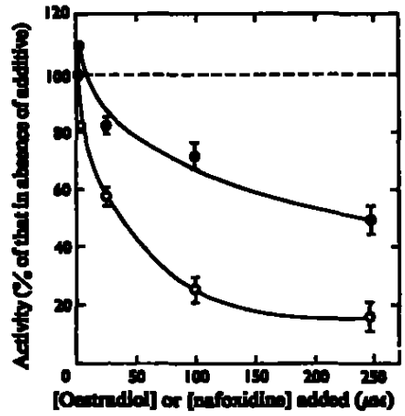

Fig. 6. *Competition of oestradiol and nafoxidine for [^{3}H]-oestradiol binding by cytosol*

Binding of 2nM-[^{3}H]oestradiol by cytosol (0.6ml) in the absence and presence of several concentrations of unlabelled oestradiol (○) or nafoxidine (●) was measured by the charcoal adsorption assay. Cytosol was prepared from 7-day-old chicks unexposed to exogenous oestradiol and was not preincubated with charcoal–dextran. Prior removal of endogenous steroids by charcoal–dextran incubation as described in the Materials and Methods section made no difference to the results. Each point represents the mean ±S.E.M. for triplicate determinations on three preparations.

apparent effect on the low-affinity oestradiol-binding protein of cytosol.

The effect of nafoxidine and of unlabelled oestradiol-17β on cytosol binding of [^{3}H]oestradiol was tested in a charcoal adsorption assay. Fig. 6 shows that very high concentrations of oestradiol-17β suppressed binding of 2nM-[^{3}H]oestradiol by cytosol. The concentration required to give 50% inhibition was 35μM. Nafoxidine was even less effective than oestradiol-17β; 250μM was required to give 50% inhibition.

Discussion

The inhibitory action of nafoxidine on oestradiol-induced vitellogenin synthesis by chick liver has been observed when nafoxidine is given with oestradiol (Lazier, 1975) or 30min before the hormone (Gschwendt, 1975*b*). We have taken serum phosphoprotein P content as a measure of vitellogenin synthesis, whereas Gschwendt (1975*b*) examined the vitellogenin protein by electrophoresis. Gschwendt (1975*b*) found that nafoxidine decreased the oestradiol stimulation of oestradiol-binding activity of isolated chromatin, and we show here that the anti-oestrogen also inhibits the oestradiol stimulation of the salt-soluble nuclear oestradiol-binding activity. Although the drug itself is not oestrogenic, its anti-oestrogenic potency is very marked, 50% inhibition of phosphoprotein synthesis being obtained by an anti-oestrogen/oestrogen molar ratio of about 0.6, by using the maximum effective dose of oestradiol. It is noteworthy that with this dose ratio, little inhibition of nuclear oestradiol-binding activity was seen at 24h, but at 48 and 64h the activity fell markedly. This suggests that nuclear receptor must be maintained in an adequate amount over a sustained period of time in order for a full phosphoprotein response to be obtained.

Gschwendt (1975*b*) reported that nafoxidine itself

stimulates the oestradiol-binding activity of rooster liver chromatin, paralleling our finding with the salt-soluble nuclear binding protein (Lazier, 1975). In an attempt to show whether or not nafoxidine influences the same binding protein as oestradiol, we examined several properties of the [^{3}H]oestradiol-binding activity in nuclear extracts from nafoxidine-treated and oestradiol-treated chicks. Both types of extract exhibit high-affinity binding of [^{3}H]oestradiol, similar hormone specificity, similar behaviour in high- and low-salt sucrose-gradient centrifugation and similar temperature-sensitivity. Although these techniques do not rule out subtle differences in the two oestradiol-binding activities, they show that the nafoxidine-induced activity is not grossly different from that induced by oestradiol. The explanation of the lack of oestrogenic activity of nafoxidine thus does not seem to lie in the production of markedly defective nuclear oestradiol receptor, but more likely in inadequate concentrations of the receptor being achieved over the required time period.

Lazier (1975) postulated that the increase in nuclear oestrogen receptor was an obligatory early action in the vitellogenic response in chick liver. The increase (at 1½h) was sensitive to actinomycin D and cycloheximide. Delaying the increase (with cycloheximide) resulted in a corresponding delay in phosphoprotein production. Both the extent of the phosphoprotein response and the increase in nuclear oestradiol-binding activity were proportional to the dose of oestradiol. Schneider & Gschwendt (1976) suggest that oestradiol treatment of roosters gives a rapid rise in soluble nuclear receptor (at 10min), followed by a decline and a second rise at 20min. The second rise was inhibited by cycloheximide. Joos *et al.* (1976) have demonstrated a very rapid rise in nuclear receptor, after injection of oestradiol via the portal vein, which was largely (but not completely) sensitive to cycloheximide. These experiments, and ours, agree in the important point that a stage dependent on protein synthesis is required to give the increase in nuclear oestrogen receptor, well before any vitellogenin production can be demonstrated by a very sensitive radio-isotopic technique (Bergink *et al.*, 1973). The mechanism for the increase in nuclear receptor could reflect synthesis of new receptor protein, or it could reflect an activation mechanism in which receptor was cleaved from a pre-receptor form by a rapidly turning-over peptidase. A small part of the increase may be accounted for by translocation of receptor from an unknown cell compartment.

Our data show that nafoxidine interferes with the oestradiol-induced increase in nuclear receptor content. The mechanism of nafoxidine inhibition appears to be more complex than solely direct blockade of the nuclear oestradiol-binding sites. This follows from our observation that the measured affinity of nafoxidine for the nuclear receptor *in vitro* is substantially less than that of oestradiol, whereas potent inhibition *in vivo* is obtained with equimolar doses of the drug and the hormone. The same discrepancy in the potency of nafoxidine *in vivo* and *in vitro* is apparent in the results of Gschwendt (1975*b*) for the effect of the drug on the oestradiol-binding sites in rooster liver chromatin. These observations can be explained in several ways. If the anti-oestrogenic action of the drug is in fact by competitive blockade of nuclear binding sites, it may be that much higher concentrations of nafoxidine relative to oestradiol are achieved in the liver cell through a difference in absorption or metabolism of the two compounds. It is also possible that nafoxidine is metabolized to a more potent inhibitor *in vivo*. An alternative explanation is that the primary site of nafoxidine action is not on the nuclear receptor itself, but at some point proximal to the nuclear receptor. Thus nafoxidine may interact with a pre-nuclear receptor present in some cell fraction other than the salt extract of nuclei, which has an affinity for nafoxidine equal to or greater than that of oestradiol. The nafoxidine–pre-nuclear receptor complex could not give the increase in nuclear receptor content as readily as can the oestradiol–pre-nuclear receptor complex. Nafoxidine itself therefore would give rise only to a limited amount of nuclear receptor but when given *in vivo* with oestradiol, the strong inhibitory effect of the drug would be obtained. A search for such a pre-nuclear receptor would be greatly facilitated by the use of radioactive nafoxidine, as would studies on the absorption, transport and metabolism of the drug. The competition studies reported here indicate that the putative pre-nuclear receptor is not present as a stable entity in cytosol. Another consideration is that nafoxidine could exert additional inhibitory mechanisms not related to the receptor system.

In rat uterus, the mediation of the oestradiol response by high-affinity cytosol receptor is widely recognized (Jensen *et al.*, 1974). The action of anti-oestrogens such as nafoxidine in depleting cytosol receptor has been shown by several groups (Rochefort *et al.*, 1972*a*, *b*; Clark *et al.*, 1974; Ruh & Ruh, 1974; Katzenellenbogen & Ferguson, 1975; Capony & Rochefort, 1975). Inhibition of replenishment may be the main focus of the anti-oestrogenic action. In studies in the rat on the inhibitory action of nafoxidine *in vivo* much higher doses of nafoxidine than of oestradiol are generally used (references cited above). Indeed Jensen *et al.* (1972) suggest that 100–1000-fold greater doses of nafoxidine than of oestradiol are required to see substantial inhibition *in vivo*. *In vitro* the affinity of nafoxidine for the uterine-cytosol oestrogen receptor is one-thirtieth that of oestradiol (Rochefort *et al.*, 1972*a*, *b*). Thus in the rat the anti-oestrogenic potency of nafoxidine *in vivo* more or less reflects the relative affinity of the drug for the cytosol receptor. In the chick, however, the drug is much more potent

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than the affinity for the nuclear receptor *in vitro* might indicate. This difference in nafoxidine action, along with its lack of oestrogenic activity, and the absence of a typical cytosol receptor in chick liver underline a possible difference in the mechanism of oestradiol action in chick liver and the rat uterus. Sheridan (1975) has reviewed several other cases of steroid-hormone action in which saturable nuclear binding of hormone is seen in the absence of classical cytosol receptor. It is possible, however, that cytosol receptor has escaped detection owing to instability. If such an activity is eventually found in the chick the nafoxidine results could imply that replenishment of cytosol receptor is required in the course of a primary response to oestradiol. This does not appear to be the case in the rat (Clark *et al.*, 1974).

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United States Patent [19]

Cameron et al.

[11] Patent Number: 5,552,412

[45] Date of Patent: Sep. 3, 1996

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[54] **5-SUBSTITUED-6-CYCLIC-5,6,7,8-TETRAHYDRONAPHTHALEN2-OL COMPOUNDS WHICH ARE USEFUL FOR TREATING OSTEOPOROSIS**

[75] Inventors: **Kimberly O. Cameron; Paul A. DaSilva Jardine; Robert L. Rossi, all of Groton, Conn.**

[73] Assignee: **Pfizer Inc, New York, N.Y.**

[21] Appl. No.: **369,954**

[22] Filed: **Jan. 9, 1995**

[51] Int. Cl.[6] **C07D 211/86; A61K 31/445; A61K 31/46; A61K 31/40**

[52] U.S. Cl. **514/317; 514/331; 514/428; 514/213; 540/582; 540/585; 546/192; 546/229; 546/233; 546/234; 546/236; 546/237; 548/566; 548/570; 548/571; 548/575**

[58] Field of Search **548/575, 566, 548/570, 571; 514/428, 317, 331, 213; 540/582, 585; 546/192, 229, 233, 234, 236, 237**

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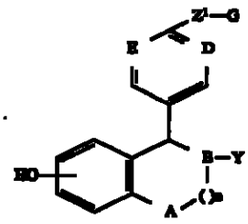
Jones, Charles D., et al., J. Med. Chem. (1984) 27, 1057–1066 "Antiestrogens. 2. Structure—Activity Studies in a Series of 3-Aroyl-2-arylbenzo[b]thiophene Derivatives Leading to [6-Hydroxy-2-(4-hydroxyphenl)benzo[b] thien-3-yl][4-[2-(1-piperidinyl)ethoxy]-phenyl]methanone Hydrochloride (LY156758), a Remarkably Effective Estrogen Antagonist with Only Minimal Intrinsic Estrogenicity".

Chemical Abstracts, vol. 65, No. 3, Abstract 5425b, Aug. 1, 1966.

Primary Examiner—Zinna Northington Davis
Attorney, Agent, or Firm—Peter C. Richardson; Gregg C. Benson; Mervin E. Brokke

[57] **ABSTRACT**

Compounds of this formula



are useful for treating or preventing, obesity, breast cancer, osteoporosis, endometriosis, cardiovascular disease and prostatic disease.

11 Claims, No Drawings

280

270

5-SUBSTITUED-6-CYCLIC-5,6,7,8-TETRAHYDRONAPHTHALEN2-OL COMPOUNDS WHICH ARE USEFUL FOR TREATING OSTEOPOROSIS

This invention relates to estrogen agonists and antagonists and their pharmaceutical uses.

BACKGROUND OF THE INVENTION

The value of naturally occurring estrogens and synthetic compositions demonstrating "estrogenic" activity has been in their medical and therapeutic uses. A traditional listing of the therapeutic applications for estrogens alone or in combination with other active agents includes: oral contraception; relief for the symptoms of menopause; prevention of threatened or habitual abortion; relief of dysmenorrhea; relief of dysfunctional uterine bleeding; an aid in ovarian development; treatment of acne; diminution of excessive growth of body hair in women (hirsutism); the prevention of cardiovascular disease; treatment of osteoporosis; treatment of prostatic carcinoma; and suppression of post-partum lactation [Goodman and Gilman, The Pharmacological Basis Of Therapeutics (Seventh Edition) Macmillan Publishing Company, 1985, pages 1421–1423]. Accordingly, there has been increasing interest in finding newly synthesized compositions and new uses for previously known compounds which are demonstrably estrogenic, this is, able to mimic the action of estrogen in estrogen responsive tissue.

From the viewpoint of pharmacologists interested in developing new drugs useful for the treatment of human diseases and specific pathological conditions, it is most important to procure compounds with some demonstrable estrogen-like function but which are devoid of proliferative side-effects. Exemplifying this latter view, osteoporosis, a disease in which bone becomes increasingly „more fragile, is greatly ameliorated by the use of fully active estrogens; however, due to the recognized increased risk of uterine cancer in patients chronically treated with active estrogens, it is not clinically advisable to treat osteoporosis in intact women with fully active estrogens for prolonged periods. Accordingly estrogen agonists are the primary interest and focus.

Osteoporosis is a systemic skeletal disease, characterized by low bone mass and deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. In the U.S., the condition affects more that 25 million people and causes more than 1.3 million fractures each year, including 500,000 spine, 250,000 hip and 240,000 wrist fractures annually. These cost the nation over $10 billion. Hip fractures are the most serious, with 5%–20% of patients dying within one year, and over 50% of survivors being incapacitated.

The elderly are at greatest risk of osteoporosis, and the problem is therefore predicted to increase significantly with the aging of the population. Worldwide fracture incidence is forecast to increase three-fold over the next 60 years, and one study estimates that there will be 4.5 million hip fractures worldwide in 2050.

Women are at greater risk of osteoporosis than men. Women experience a sharp acceleration of bone loss during the five years following menopause. Other factors that increase the risk include smoking, alcohol abuse, a sedentary lifestyle and low calcium intake.

Estrogen is the agent of choice in preventing osteoporosis or post menopausal bone loss in women; it is the only

treatment which unequivocally reduces fractures. However, estrogen stimulates the uterus and is associated with an increased risk of endometrial cancer. Although the risk of endometrial cancer is thought to be reduced by a concurrent use of a progestogen, there is still concern about possible increased risk of breast cancer with the use of estrogen.

Black, et al. in EP 0605193A1 report that estrogen, particularly when taken orally, lowers plasma levels of LDL and raises those of the beneficial high density lipoproteins (HDL's). Long-term estrogen therapy, however, has been implicated in a variety of disorders, including an increase in the risk of uterine cancer and possibly breast cancer, causing many women to avoid this treatment. Recently suggested therapeutic regimens, which seek to lessen the cancer risk, such as administering combinations of progestogen and estrogen, cause the patient to experience unacceptable bleeding. Furthermore, combining progesterone with estrogen seems to blunt the serum cholesterol lowering effects of estrogen. The significant undesirable effects associated with estrogen therapy support the need to develop alternative therapies for hypercholesterolemia that have the desirable effect on serum LDL but do not cause undesirable effects.

There is a need for improved estrogen agonists which exert selective effects on different tissues in the body. Tamoxifen, 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenyl-but-1-ene, is an antiestrogen which has a palliative effect on breast cancer, but is reported to have estrogenic activity in the uterus. Gill-Sharma, et al., *J. Reproduction and Fertility* (1993) 99, 395, disclose that tamoxifen at 200 and 400 mg/kg/day reduces the weights of the testes and secondary sex organs in male rats.

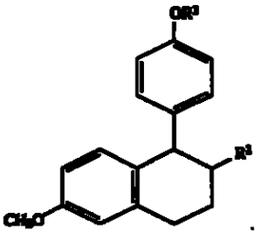
Recently it has been reported (Osteoporosis Conference Scrip No. 1812/13 Apr. 16/20, 1993, p. 29) that raloxifene, 6-hydroxy-2-(4-hydroxyphenyl)-3-[4 -(2-piperidinoethoxy) benzoyl] benzo[b] thiophene, mimics the favorable action of estrogen on bone and lipids but, unlike estrogen, has minimal uterine stimulatory effect. (Breast Cancer Res. Treat. 10(1). 1987 p 31–36 Jordan, V. C. et al.).

Neubener, et al., The Prostate 2:3:245 (1993) teach that raloxifene treatment of male rats produced regression of the ventral prostate.

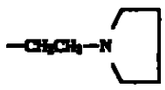
Raloxifene and related compounds are described as antiestrogen and antiandrogenic materials which are effective in the treatment of certain mammary and prostate cancers. See U.S. Pat. No. 4,418,068 and Charles D. Jones, et al., *J. Med. Chem.* 198.4, 27, 1057–1066.

Jones, et al in U.S. Pat. No. 4,133,814 describe derivatives of 2-phenyl-3-aroylbenzothiophene and 2-phenyl-3-aroylbenzothiophene-1-oxides which are useful as antifertility agents as well as suppressing the growth of mammary tumors.

Lednicer, et al., *J. Med. Chem.*, 12, 881 (1969) described estrogen antagonists of the structure

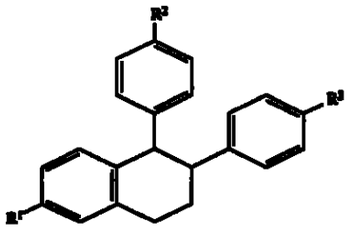


wherein R^2 is phenyl or cyclopentyl and R^3 is H,



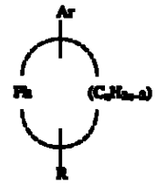
or $-CH_2CHOHCH_2OH$.

Bencze, et al., *J. Med. Chem.*, 10,138 (1967) prepared a series of tetrahydronaphthalenes intended to achieve separation of estrogenic, antifertility and hypocholesterolemic activities. These structures are the general formula



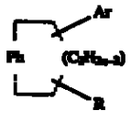
wherein R^1 is H or OCH_3; R^2 is H, OH, OCH_3, $OPO(OC_2H_5)_2$, $OCH_2CH_2N(C_2H_5)_2$, OCH_2COOH or $OCH(CH_3)COOH$.

U.S. Pat. No. 3,234,090 refers to compounds which have estrogenic and antifungal properties of the formula



in which Ph is a 1,2-phenylene radical, Ar is a monocyclic carbocyclic aryl group substituted by tertiary amino-lower alkyl-oxy, in which tertiary amino is separated from oxy by at least two carbon atoms, R is hydrogen, an aliphatic radical, a carbocyclic aryl radical, a carbocyclic aryl-aliphatic radical, a heterocyclic aryl radical or a heterocyclic aryl aliphatic radical, the group of the formula $-(C_nH_{2n-a})-$ stands for an unbranched alkylene radical having from three to five carbon atoms and carrying the groups Ar and R, salts, N-oxides, salts of N-oxides or quaternary ammonium compounds thereof, as well as procedure for the preparation of such compounds.

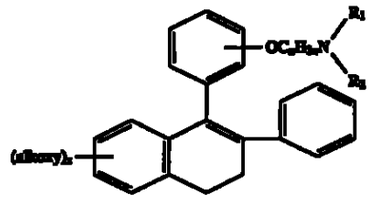
U.S. Pat. No. 3,277,106 refers to basic ethers with estrogenic, hypocholesterolemic and antifertility effects which are of the formula



in which Ph is a 1,2-phenylene radical, Ar is a monocyclic aryl radical substituted by at least one amino-lower alkyl-oxy group in which the nitrogen atom is separated from the oxygen atom by at least two carbon atoms, R is an aryl radical, and the portion $-(C_nH_{2n-a})-$ stands for lower alkylene forming with Ph a six- or seven-membered ring, two of the ring carbon atoms thereof carry the groups Ar and R,

salts, N-oxides, salts of N-oxides and quaternary ammonium compounds thereof.

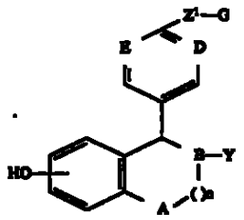
Lednicer, et al., in J. Med. Chem. 10, 78 (1967) and in U.S. Pat. No. 3,274,213 refer to compounds of the formula



wherein R_1 and R_2 are selected from the class consisting of lower alkyl and lower alkyl linked together to form a 5 to 7 ring member saturated heterocyclic radical.

SUMMARY OF THE INVENTION

This invention provides compounds of the formula



wherein:

A is selected from CH_2 and NR;

B, D and E are independently selected from CH and N;

Y is

(a) phenyl, optionally substituted with 1–3 substituents independently selected from R^4;

(b) naphthyl, optionally substituted with 1–3 substituents independently selected from R^4;

(c) C_3–C_8 cycloalkyl, optionally substituted with 1–2 substituents independently selected from R^4;

(d) C_3–C_8 cycloalkenyl, optionally substituted with 1–2 substituents independently selected from R^4;

(e) a five membered heterocycle containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2-$ and $-S(O)_n-$, optionally substituted with 1–3 substituents independently selected from R^4;

(f) a six membered heterocycle containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2-$ and $-S(O)_n-$ optionally substituted with 1–3 substituents independently selected from R^4; or

(g) a bicyclic ring system consisting of a five or six membered heterocyclic ring fused to a phenyl ring, said heterocyclic ring containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2-$ and $-S(O)_n-$, optionally substituted with 1–3 substituents independently selected from R^4;

Z^1 is

(a) $-(CH_2)_p W(CH_2)_q-$;

(b) $-O(CH_2)_p CR^3R^4-$;

(c) $-O(CH_2)_p W(CH_2)_q-$;

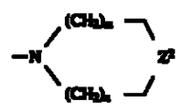
(d) $-OCHR^2CHR^3-$; or

(e) $-SCHR^2CHR^3-$;

G is

(a) $-NR^7R^8$;

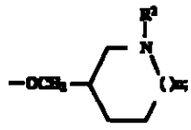
(b)



wherein n is 0, 1 or 2; m is 1, 2 or 3; Z^2 is -NH-, -O-, -S-, or $-CH_2-$; optionally fused on adjacent carbon atoms with one or two phenyl rings and, optionally independently substituted on carbon with one to three substituents and, optionally, independently on nitrogen with a chemically suitable substituent selected from R^4; or

(c) a bicyclic amine containing five to twelve carbon atoms, either bridged or fused and optionally substituted with 1–3 substituents independently selected from R^4; or

Z^1 and G in combination may be



W is

(a) $-CH_2-$;

(b) -CH=CH-;

(c) -O-;

(d) $-NR^2-$;

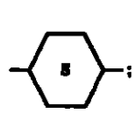
(e) $-s(O)_m-$;

(g) $-CR^2(OH)-$;

(h) $-CONR^2-$;

(i) $-NR^2CO-$;

(j)



(k) -C≡C-;

R is hydrogen or C_1–C_6 alkyl;

R^2 and R^3 are independently

(a) hydrogen; or

(b) C_1–C_4 alkyl;

R^4 is

(a) hydrogen;

(b) halogen;

(c) C_1–C_6 alkyl;

(d) C_1–C_4 alkoxy;

(e) C_1–C_4 acyloxy;

(f) C_1–C_4 alkylthio;

(g) C_1–C_4 alkylsulfinyl;

(h) C_1–C_4 alkylsulfonyl;

(i) hydroxy (C_1–C_4)alkyl;

(j) aryl (C_1–C_4)alkyl;

(k) $-CO_2H$;

(l) -CN;

(m) -CONHOR;

(n) $-SO_2NHR$;

(o) $-NH_2$;

(p) C_1–C_4 alkylamino;

(q) C_1–C_4 dialkylamino;

(r) $-NHSO_2R$;

(s) $-NO_2$;

(t) -aryl; or

(u) -OH.

R^5 and R^6 are independently C_1–C_6 alkyl or together form a C_3–C_{10} carbocyclic ring;

R^7 and R^8 are independently

(a) phenyl;

(b) a C_3–C_{10} carbocyclic ring, saturated or unsaturated;

(c) a C_3–C_{10} heterocyclic ring containing up to two heteroatoms, selected from -O-, -N- and -S-;

(d) H;

(e) C_1–C_6 alkyl; or

(f) form a 3 to 8 membered nitrogen containing ring with R^2 or R^6;

R^7 and R^8 in either linear or ring form may optionally be substituted with up to three substituents independently selected from C_1–C_6 alkyl, halogen, alkoxy, hydroxy and carboxy;

a ring formed by R^7 and R^8 may be optionally fused to a phenyl ring;

s is 0, 1 or 2;

m is 1, 2 or 3;

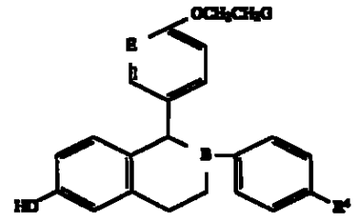
n is 0, 1 or 2;

p is 0, 1, 2 or 3;

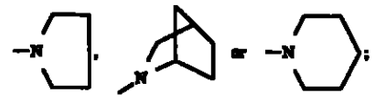
q is 0, 1, 2 or 3;

and optical and geometric isomers thereof; and atoxonic pharmacologically acceptable acid addition salts, N-oxides, and quaternary ammonium salts thereof.

Preferred compounds of the invention are of the formula:



wherein G is



R^4 is H, OH, F, or Cl; and B and E are independently selected from CH and N.

Especially preferred compounds are:

Cis-6-(4-fluoro-phenyl)-5-[4-(2-piperidin-1 -yl-ethoxy)-phenyl]-5,6,7,8 -tetrahydro-naphthalen-2-ol;

(-)-Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl] -5,6,7,8-tetrahydro-naphthalen-2-ol;

Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5, 6,7,8-tetrahydro-naphthalen 2-ol;

Cis-1-[6'-pyrrolodinoethoxy-3'-pyridyl]-2-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalen

1-(4'-Pyrrolidinoethoxyphenyl)-2-(4"-fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline;

Cis-6-(4-hydroxyphenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydro-naphthalen-2-ol; and

1-(4'-Pyrrolidinolethoxyphenyl)-2-phenyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline.

In another aspect this invention provides methods of treating or preventing a condition selected from breast cancer, osteoporosis, endometriosis and cardiovascular disease and hypercholesterolemia in male or female mammals and benign prostatic hypertrophy and prostatic carcinomas in male mammals which comprises administering to said mammal an amount of a compound of formula I and preferably a preferred compound of formula I compounds as described above which is effective in treating or preventing said condition.

In another aspect this invention provides a method of treating or preventing obesity in mammals which comprises administering to said mammal an amount of a compound of formula I and preferably a preferred compound of formula I as described above which is effective in treating or preventing obesity.

In yet another aspect this invention provides a pharmaceutical composition for treating or preventing breast cancer, osteoporosis, obesity, cardiovascular disease, hypercholesterolemia, endometriosis and prostatic disease comprising a compound of formula I and a pharmaceutically acceptable carrier.

In another aspect, this invention provides intermediate compounds useful in preparing compounds of Formula I. These are 1-[2-[4-(6-Methoxy-3,4-dihydronaphthalen-1-yl)phenoxy]ethyl]pyrrolidine and 1-[2-[4-(2-Bromo-6-methoxy-3,4-dihydronaphthalen-1-yl)phenoxy]ethyl]pyrrolidine.

DETAILED DESCRIPTION OF THE INVENTION

The terms C_1–C_3 chloroalkyl and C_1–C_3 fluoroalkyl include methyl, ethyl, propyl and isopropyl substituted to any desired degree with chlorine or fluorine atoms, from one atom to full substitution. The term C_5–C_7 cycloalkyl includes cyclopentyl, cyclohexyl and cycloheptyl.

Halo means chloro, bromo, iodo and fluoro. Aryl (Ar) includes phenyl and naphthyl optionally substituted with one to three substituents independently selected from R^4 as defined above. DTT means dithiothreitol. DMSO means dimethyl sulfoxide. EDTA means ethylene diamine tetra acetic acid.

Estrogen agonists are herein defined as chemical compounds capable of binding to the estrogen receptor sites in mammalian tissue, and mimicking the actions of estrogen in one or more tissues.

Estrogen antagonists are herein defined as chemical compounds capable of binding to the estrogen receptor sites in mammalian tissue, and blocking the actions of estrogen in one or more tissues.

One of ordinary skill will recognize that certain substituents listed in this invention will be chemically incompatible with one another or with the heteroatoms in the compounds, and will avoid these incompatibilities in selecting compounds of this invention. Likewise certain functional groups may require protecting groups during synthetic procedures which the chemist of ordinary skill will recognize.

The chemist of ordinary skill will recognize that certain compounds of this invention will contain atoms which may be in a particular optical or geometric configuration. All such isomers are included in this invention; exemplary levorotatory isomers in the cis configuration are preferred. Likewise, the chemist will recognize that various pharmaceutically acceptable esters and salts may be prepared from certain compounds of this invention. All of such esters and salts are included in this invention.

As used in this application, prostatic disease means benign prostatic hyperplasia or prostatic carcinoma.

The remedies for the prostatic diseases, breast cancer, obesity, cardiovascular disease, hypercholesterolemia and osteoporosis of this invention comprise, as active ingredient, a compound of formula I or a salt or ester thereof. The pharmaceutically acceptable salts of the compounds of formula I are salts of non-toxic type commonly used, such as salts with organic acids (e.g., formic, acetic, trifluoroacetic, citric, maleic, tartaric, methanesulfonic, benzenesulfonic or toluenesulfonic acids), inorganic acids (e.g. hydrochloric, hydrobromic, sulfuric or phosphoric acids), and amino acids (e.g., aspartic or glutamic acids). These salts may be prepared by the methods known to chemists of ordinary skill.

The remedies for prostatic diseases, breast cancer, obesity, cardiovascular disease, hypercholesterolemia and osteoporosis of this invention may be administered to animals including humans orally or parenterally in the conventional form of preparations, such as capsules, microcapsules, tablets, granules, powder, troches, pills, suppositories, injections, suspensions and syrups.

The remedies for prostatic diseases, breast cancer, obesity, cardiovascular disease, hypercholesterolemia and osteoporosis of this invention can be prepared by the methods commonly employed using conventional, organic or inorganic additives, such as an excipient (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate or calcium carbonate), a binder (e.g., cellulose, methylcellulose, hydroxymethylcellulose, polypropylpyrrolidone, polyvinylprolidone, gelatin, gum arabic, polyethyleneglycol, sucrose or starch), a disintegrator (e.g., starch, carboxymethylcellulose, hydroxypropylstarch, low substituted hydroxypropylcellulose, sodium bicarbonate, calcium phosphate or calcium citrate), a lubricant (e.g., magnesium stearate, light anhydrous silicic acid, talc or sodium lauryl sulfate), a flavoring agent (e.g., citric acid, menthol, glycine or orange powder), a preservative (e.g., sodium benzoate, sodium bisulfite, methylparaben or propylparaben), a stabilizer (e.g., citric acid, sodium citrate or acetic acid), a suspending agent (e.g., methylcellulose, polyvinylpyrrolidone or aluminum stearate), a dispersing agent (e.g., hydroxypropylmethylcellulose), a diluent (e.g., water), and base wax (e.g., cocoa butter, white petrolatum or polyethylene glycol). The amount of the active ingredient in the medical composition may be at a level that will exercise the desired therapeutical effect; for example, about 0.1 mg to 50 mg in unit dosage for both oral and parenteral administration.

The active ingredient may be usually administered once to four times a day with a unit dosage of 0.1 mg to 50 mg in human patients, but the above dosage may be properly varied depending on the age, body weight and medical condition of the patient and the type of administration. A preferred dose is 0.25 mg to 25 mg in human patients. One dose per day is preferred.

Compounds of this invention are readily prepared by the reactions illustrated in the schemes below.

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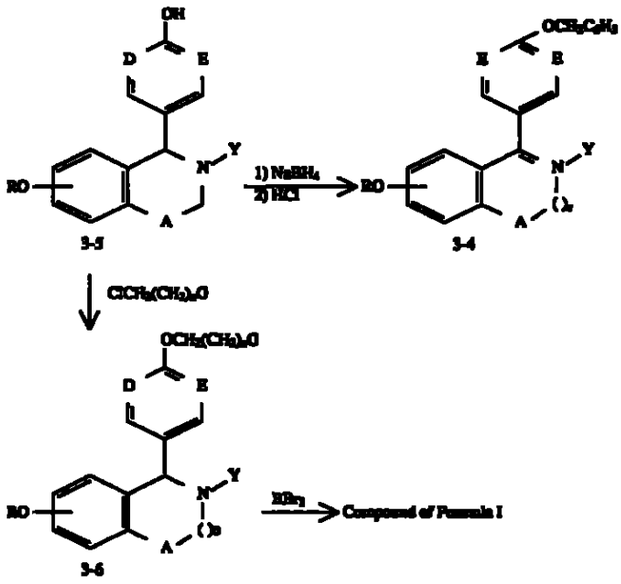


The compounds of this invention are valuable estrogen agonists and pharmaceutical agents or intermediates thereto. Those which are estrogen agonists are useful for oral contraception; relief for the symptoms of menopause; prevention of threatened or habitual abortion; relief of dysmenorrhea; relief of dysfunctional uterine bleeding; relief of endometriosis; an aid in ovarian development; treatment of acne; diminution of excessive growth of body hair in women (hirsutism); the prevention and treatment of cardiovascular disease; prevention and treatment of atherosclerosis; prevention and treatment of osteoporosis; treatment of benign prostatic hyperplasia and prostatic carcinoma obesity; and suppression of post-partum lactation. These agents also have a beneficial effect on plasma lipid levels and as such are useful in treating and preventing hypercholesterolemia.

While the compounds of this invention are estrogen agonists in bone, they are also antiestrogens in breast tissue and as such would be useful in the treatment and prevention of breast cancer.

Control and Prevention of Endometriosis

"The protocol for surgically inducing endometriosis is identical to that described by Jones, *Acta Endocrinol* (Copenh) 106,:282–8. Adult Charles River Sprague-Dawley CD® female rats (200–240 g) are used. An oblique ventral incision is made through the skin and musculature of the body wall. A segment of the right uterine horn is excised, the myometrium is separated from the endometrium, and the segment is cut longitudinally. A 5×5 mm section of the endometrium, with the epithelial lining apposed to the body wall, is sutured at its four corners to the muscle using polyester braid (Ethiflex, 7-0®). The criterion of a viable graft is the accumulation of fluid similar to that which occurs in the uterus as a result of oestrogen stimulation.

Three weeks after transplantation of the endometrial tissue (+3 weeks) the animals are laparotomized, the volume of the explant (lengthxwidthxheight) in mm was measured with calipers, and treatment is begun. The animals are injected sc for 3 weeks with 10 to 1000 μg/kg/day of a compound of Formula I. Animals bearing endometrial explants are injected sc with 0.1 ml/day of corn oil for 3 weeks served as controls. At the end of 3 week treatment period (+6 weeks), the animals are laparotomized and the volume of the explant determined. Eight weeks after cessation of treatment (+14 weeks) the animals are sacrificed; the explant are measured again. Statistical analysis of the explant volume is by an analysis of variance.

Effect on Prostate Weight

Male Sprague-Dawley rats, three months of age are administered by subcutaneous injection either vehicle (10% ethanol in water), estradiol (30 μg/kg), testosterone (1 mg/kg) or a compound of formula I daily for 14 days (n=6/group). After 14 days the animals are sacrificed, the prostate is removed and the wet prostate weight is determined. Mean weight is determined and statistical significance ($p<0.05$) is determined compared to the vehicle-treated group using Student's t-test.

The compounds of formula I significantly ($P<0.05$) decrease prostate weight compared to vehicle. Testosterone has no effect while estrogen at 30 μg/kg significantly reduces prostate weight.

Bone mineral density

Bone mineral density, a measure of bone mineral content, accounts for greater than 80% of a bone's strength. Loss of bone mineral density with age and/or disease reduces a bone's strength and renders it more prone to fracture. Bone mineral content is accurately measured in people and animals by dual x-ray absorptiometry (DEXA) such that changes as little as 1% can be quantified. We have utilized DEXA to evaluate changes in bone mineral density due to estrogen deficiency following ovariectomy (surgical removal of ovaries) and treatment with vehicle, estradiol (E2), keoxifen (raloxifen), or other estrogen agonists. The purpose of these studies is to evaluate the ability of the compounds of this invention to prevent estrogen deficiency bone loss as measured by DEXA.

Female (S-D) rats 4–6 months of age undergo bilateral ovariectomy or sham surgery and allowed to recover from

to a solution of 5-(6-methoxy-2-phenyl-3,4-dihydronaphthalen-1-yl)-2-(2 -pyrrolidin-1-ylethoxy)pyridine (286.4 mg, 0.6714 mmol) in acetic acid (50 mL). The mixture was hydrogenated on a Parr shaker at 50 psi and at 50° C. for 16 h. The catalyst was filtered off with the aid of celite and the acetic acid was removed in vacuo. ^{1}H NMR indicated that the reaction was incomplete and the residue was resubjected to the reaction conditions (50 psi and 60° C.) for an additional 6 h. The catalyst was removed via filtration through celite and the solvent was removed in vacuo. Radial chromatography (solvent gradient, CH_2Cl_2 to 10% MeOH in CH_2Cl_2) provided the desired material (207 mg, 72%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.19 (m, 4H), 6.84 (m, 3H), 6.75 (d, J=2.4 Hz, 1H), 6.68 (dd, J=2.4, 8.4 Hz, 1H), 6.59 (dd, J=2.4, 8.4 Hz, 1H), 6.40 (d, J=8.4 Hz, 1H), 4.35 (t, J=5.7 Hz, 2H), 4.21 (d, J=4.8 Hz, 1H), 3.82 (s, 3H), 3.38 (m, 1H), 3.06 (m, 2H), 2.90 (t, J=5.7 H 2.69 (m, 4H), 2.11 (m, 2H), 1.84 (m, 4H).

Step F

cis-6-Phenyl-5-[6-(2-pyrrolidin- 1-ylethoxy)pyridin-3-yl-5,6,7,8-tetrahydronaphthalen-2 -ol: To a solution of cis-5-(6-methoxy-2-phenyl-1,2,3,4-tetrahydronaphthalen-1-yl)-2-(2-pyrrolidin-1-ylethoxy)pyridine (69.6 mg, 0.162 mmol) in dry CH_2Cl_2 (3 mL) at 0° C. was added $AlCl_3$ (110 mg, 0.825 mmol) followed by excess EtSH (400 μL). After 0.5 h, the reaction was warmed to room temperature and additional $AlCl_3$ (130 mg) was added. After 0.5 h, aqueous $NaHCO_3$ (satd) was carefully added and the aqueous layer was extracted with CH_2Cl_2/MeOH (3x). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Radial chromatography (solvent gradient, CH_2Cl_2 to 15% MeOH in CH_2Cl_2) provided the deprotected material (64.6 mg, 96%) as an off-white solid. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.18 (m, 3H), 6.96 (d, J=2.4 Hz, 1H), 6.82 (m, 2H), 6.70 (d, J=2.4 Hz, 1H), 6.67 (d, J=8.4 Hz, 1H), 6.62 (dd, J =2.4, 8.5 Hz, 1H), 6.52 (dd, J=2.4, 8.4 Hz, 1H), 5.80 (d, J=8.5 Hz, 1H), 4.45 (m, 2H), 4.18 (d, J=4.8 Hz, 1H), 3.40 (m, 1H), 3.04 (m, 3H), 2.75 (m, 6H), 2.11 (m, 1H), 1.88 (m, 4H). The two enantiomers were isolated by chromatography on a 5 cm id×5 cm Chiracel OD column using 5% ethanol/95% heptane with 0.05% diethylamine. Enantiomer 1: R_t=17.96 min, $[\alpha]_D$=+242 (c=1, MeOH); Enantiomer 2: R_t=25.21 min, $[\alpha]_D$=−295 (c=1, MeOH).

EXAMPLE 7

Cis-6-[4-fluoro-phenyl)-5-[4-(2-piperidin- 1 -yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalen-2-ol

Step A

1 g of 1-[4'-piperidino ethoxy phenyl]-2-[4'-fluoro phenyl]- 6-methoxy-3,4-dihydronaphthalen (which can be made as in Example 1 but replacing phenylboronic acid in Step C with 4-fluorophenylboronic acid) in 35 ml acetic acid was added palladium hydroxide on carbon (20%, 1 g)(flame dried in vacuo). The mixture was hydrogenated on a Parr shaker at 50° C. and 50 psi for 4 hours. Filtration through Celite and concentration yielded 1.2 g of crude reaction product which was used without further purification in the next step.

^{1}H-NMR (250 MHZ, $CDCl_3$): δ 1.9 (m), 3.1 (m), 3.25 (m), 3.8 (s, 3H), 4.2 (d, 1H), 4.25 (bd), 6.35 (d, 2H), 6.5 (d, 2H), 6.65 (m), 6.75 (m), 6.8–6.88 (m) m/z 460 (M+1)

Step B

A solution of cis-1-[4'-piperidinoethoxy phenyl]-2-[4'-fluoro phenyl]-6-methoxy-1,2,3,4-tetrahydro naphthalene-1-yl] phenoxy}-ethyl)-piperidine (540 mg, 1.17 mmol) in anhydrous CH_2Cl_2 was cooled to 0° C. followed by the addition of BBr_3 [5.8 mL (1 M solution in CH_2Cl_2), 5.88 mmol] dropwise. The reaction was allowed to warm to room temperature and stirred for another hour. After the reaction was complete, the reaction was cooled back to 0° C. and aqueous $NaHCO_3$ was carefully added. The aqueous layer was extracted with CH_2Cl_2 (3x). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was radially chromatographed (solvent 4:1 ether/hexane, 1% triethylamine) to provide the deprotected product. The HCl salt product was form with 1 M HCl/Ether solution followed by trituration with EtOAc/THF to provide 126 mg of product. ^{1}H NMR (250 MHz, $CDCl_3$): δ 6.80 (m,4H), 6.63 (m, 4H), 6.50 (dd, 1H), 6.40 (d, 2H), 4.22 (dd, 3H), 3.72 (m, 2H), 3.48 (dd, 2H), 3.0 (bm, 2H), 1.83 (m, 9H), m/z 446 (M+1)

EXAMPLE 8

(−)Cis,6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy) phenyl]-5,6,7,8-tetrahydronaphthalen-2-ol

The racemic compound of Example 1 (3 g) was subjected to enantiomeric separation on a 5 cm×5 cm Chiralcell OD column employing 99.95% (5% EtOH/95% heptane)/0.05% diethylamine as the element to afford 1 g of a fast eluting (+) enantiomer and 1 g of a slow (−) eluting enantiomer, both of which possessed identical NMR, MS and TLC behavior as the racemate. Alternatively, a crystallization procedure using R-binap phosphoric acid can be employed to effect resolution. In 20 ml of methanol and 20 ml of methylene chloride was added 7.6g (0.0184 mol) of the product of Example 1 and 6.4g (0.0184 mol) of R-(−)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate. After solution was complete, evaporation of solvent followed by trituration with ether afforded 14.2 g of racemic salt. This solid was slurried in 500 ml of dioxane and 25 ml of methanol and the resulting mixture was heated until the initial solid dissolved. On standing for 1 hour, a white precipitate formed (6.8 g) which was collected and which HPLC (using the conditions above) indicated to be approximately 73% enantiomerically pure. This material was slurried in 250 ml of absolute ethanol and heated until solution was achieved, at which time the solution was allowed to stand at room temperature overnight. The collected crystals were washed with cold ethanol followed by ether to afford 3.1 g of 98% enantiomerically pure salt; a second crop of 588 mg was also obtained. Partitioning between 1:2 methanol/methylene chloride and 1% aqueous sodium hydroxide afforded the corresponding free base which was converted to the hydrochloride salt (HCl in dioxane). Recrystallization from acetonitrile/methylene chloride afforded the levorotatory preferred enantiomer

hydrochloride corresponding to Example 1. [α_D–330.6 (c=0.05,CH_2Cl_2]; mp 260°–263° C.

EXAMPLE 9

Cis-6-(4'-hydroxyphenyl)-5-[4-(2-piperidin-1-ylethoxy)phenyl]-5,6,7,8 -tetrahydronaphthalen-2-ol.

Following the procedures described for the preparation of Example 1, the title compound was obtained. ^{1}H NMR ($CDCl_3$): δ 3.12 (m, 1H); 3.90 (m, 2H); 4.15 (d, 1H); 6.15–6.72 (m, 11H); FAB MS (M+1) 430.

EXAMPLE 10

1 -(4'-Piperidinoethoxyphenyl)-2-(4"-hydroxyphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride

Using the procedure of Example 3 described in step G, substituting N-2-chloroethylpiperidine hydrochloride for N-2-chloroethylpyrrolidine hydrochloride, the title compound was obtained. ^{1}H NMR ($CDCl_3$): δ 2.65 (m, 2H); 2.75 (m, 2H); 5.45 (s, 1); 6.50–7.00 (m, 11H); FAB MS(M+1) 445.

EXAMPLE 11

1 -(4'-Pyrrolidinoethoxyphenyl)-2-(4"-fluorophenyl)-6-hydroxy- 1,2,3,4-tetrahydroisoquinoline hydrochloride

The title compound was obtained using the procedure of Example 3 described in step A, substituting 4-fluoroaniline for 4-methoxyaniline. ^{1}H NMR ($CDCl_3$): δ 2.12 (m, 2H); 3.65 (m, 2H); 4.45 (m, 2H); 6.10 (s, 1H); 7.5 (m, 2H); MS(M+1) 433.

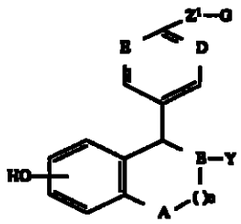
EXAMPLE 12

1–4-(Pyrrolidinoethoxyphenyl)-2-phenyl-6-hydroxy-1,2,3,4 -tetrahydroisoquinoline hydrochloride

The title compound was prepared using the procedure of Example 3 described in step A, substituting aniline for 4-methoxyaniline. ^{1}H NMR ($CDCl_3$): δ 1.70 (m, 4H); 2.70 (m, 2H); 4.00 (m, 2H); 5.70 (s, 1H); 6.60–7.25 (m, 12H); FAB MS(M+1) 415.

We claim:

1. A compound of the formula:



wherein:

A is CH_2;

B, D and E are CH;

Y is

(a) phenyl, optionally substituted with 1–3 substituents independently selected from R^4;

(b) naphthyl, optionally substituted with 1–3 substituents independently selected from R^4;

(c) C_3–C_8 cycloalkyl, optionally substituted with 1–2 substituents independently selected from R_4;

(d) C_3–C_8 cycloalkenyl, optionally substituted with 1–2 substituents independently selected from R^4;

(e) a five membered heterocycle containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2$- and $-S(O)_n$-, optionally substituted with 1–3 substituents independently selected from R^4;

(f) a six membered heterocycle containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2$- and $-S(O)_n$- optionally substituted with 1–3 substituents independently selected from R^4; or

(g) a bicyclic ring system consisting of a five or six membered heterocyclic ring fused to a phenyl ring, said heterocyclic ring containing up to two heteroatoms selected from the group consisting of -O-, $-NR^2$-, NR^2- and $-S(O)_n$-, optionally substituted with 1–3 substituents independently selected from R^4.

Z^1 is

(a) $-O(CH_2)_p$ CR^5R^6-;

(b) $-O(CH_2)_pW(CH_2)_q$-; or

(c) $-OCHR^5CHR^5$-;

G is $-NR^7R^8$;

W is

(a) $-CH_2$-;

(b) -CH═CH-;

(c) -O-;

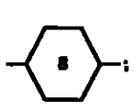
(d) $-NR^2$-;

(e) $-S(O)_n$-;

(g) $-CR^2(OH)$-;

(h) $-CONR^2$-;

(i) $-NR^2CO$-;



or

(k) -C≡C-;

R^2 and R^3 are independently

(a) hydrogen; or

(b) C_1–C_4 alkyl;

R^4 is

(a) hydrogen;

(b) halogen;

(c) C_1–C_8 alkyl;

(d) C_1–C_4 alkoxy;

(e) C_1–C_4 acyloxy;

(f) C_1–C_4 alkylthio;

(g) C_1–C_4 alkylsulfinyl;

(h) C_1–C_4 alkylsulfonyl;

(i) hydroxy (C_1–C_4)alkyl;

(j) aryl (C_1–C_4)alkyl;

(k) $-CO_2H$;

(l) -CN;

(m) -CONHOR;

(n) $-SO_2NHR$;

(o) $-NH_2$;

SUPERINTENDENCIA DE INDUSTRIA Y COMERCI
DIVISIÓN DE NUEVAS CREACIONES

2020/
Bogotá, D.C.,

Doctor
Alvaro Correa Ordoñez
Apoderado
PFIZER PRODUCTS INC.

Oficio Nº 12922

REFERENCIA	**Radicación**	**01 27558**
	Trámite	**002**
	Evento	**001**
	Actuación	**430**
	Folios	**011**

Como resultado del examen de patentabilidad de la solicitud de patente de invención Nº 01 27558, realizado según el artículo 45 de la decisión 486 de la comisión de la Comunidad Andina, se notifica:

1. DOCUMENTOS ESTUDIADOS:

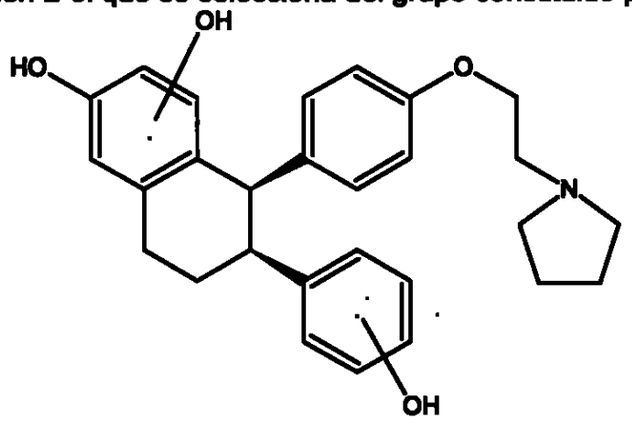
- El resumen y el capítulo descriptivo de la solicitud en los folios 105 y 15 a 62 respectivamente.
- Los ejemplos que ilustran la invención en los folios 63 a 84 y las figuras de los folios 107 a 124.
- Las cláusulas 1 a 11 relacionadas en el capítulo reivindicatorio de la solicitud que se encuentran en los folios 86 a 104.
- El primer examen de forma realizado a la solicitud de oficio No. 1490, que se encuentra en el folio 130.
- La respuesta al primer examen de forma, allegada mediante memorial de fecha 11/06/2001 que se encuentra en los folios 131 a 231.
- La copia de la solicitud original mediante la cual se reivindica prioridad de Estados Unidos US60/267.198 en los folios 157 a 231, junto con la traducción del capítulo reivindicatorio correspondiente en los folios 137 a 156.
- El memorial de fecha 09/04/2003 que contiene un nuevo capítulo reivindicatorio que reemplaza el presentado con anterioridad y se encuentra en los folios 234 a 261.
- La solicitud de examen de patentabilidad junto con el pago de la tasa correspondiente, allegada mediante memorial de fecha 23/07/2003, que se encuentra en los folios 262 a 264 y además contiene la sustitución de poder.

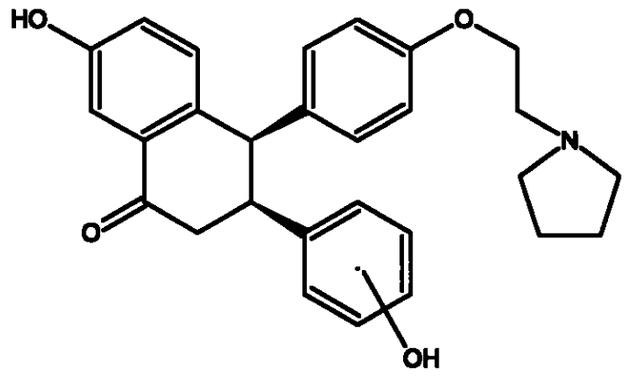
2. OBJETO DE LA INVENCIÓN:

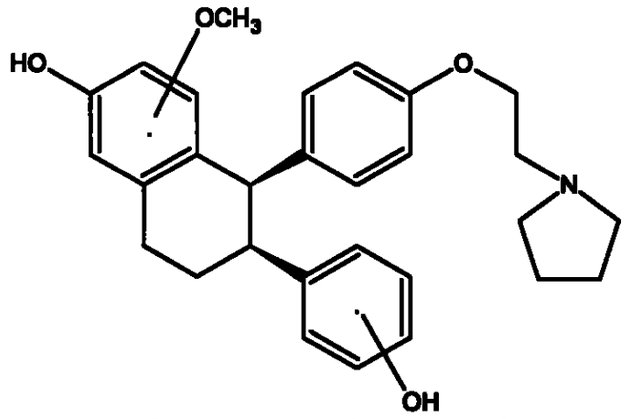
La solicitud hace referencia a un ***"Metabolitos agonistas/antagonistas de estrógeno"***

- **Reivindicación 1:**

Se reivindica un metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol que se selecciona del grupo constituido por:



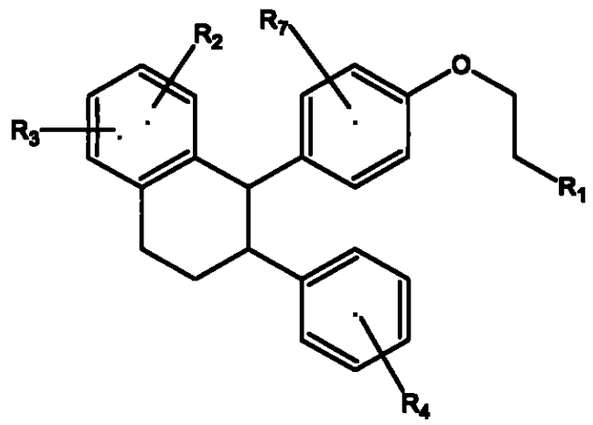




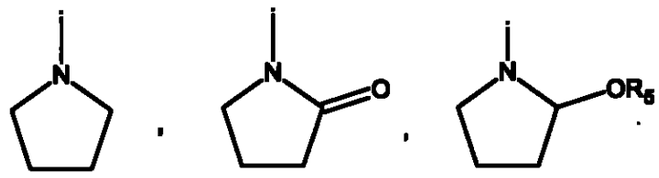
Y los estereoisómeros, tautómeros, regioisómeros e isómeros configuracionales de este y las sales farmacéuticamente aceptables de los mismos.

- **Reivindicación 2:**

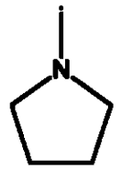
Se reivindica una cantidad efectiva de un metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol correspondiente a la fórmula (I):



En la que R1 se selecciona de:



$-NH(CH_2)_3COR_6$
R_5 se selecciona de H ó CH_3
R_2, R_3, R_4 y R_7 son iguales o diferentes y se seleccionan entre H y OR_5; y
R_6 se selecciona de –OH ó $NHCH_2COOH$, siempre que:

(a) si R_1 es  o $-NH(CH_2)_3COOH$ y
(b) R_2 es OH ó OCH_3 y R_3 y R_7 son H, o si R_1 es como se definió en (a) anteriormente, y
(c) R_2 y R_7 son H y R_3 es OH ó OCH_3,

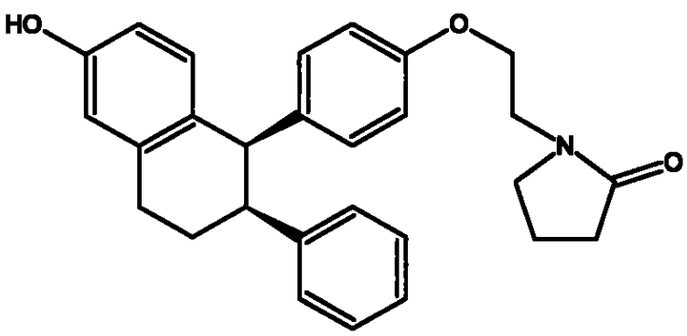
Entonces R_4 no es H.

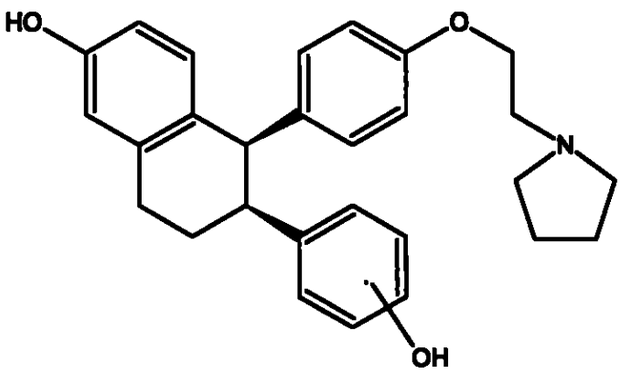
O un isómero óptico, estereoisómero, regioisómero o isómero configuracional o isómero geométrico de éste o un tautómero o una sal farmacéuticamente aceptable de este para ser administrada a un paciente para el tratamiento de osteoporosis, cáncer de mama, hiperlipidemia, aterosclerosis, enfermedad de Alzheimer, cataratas, pérdida de libido, disfunción sexual masculina, cáncer de colon, verrugas dérmicas, enfermedad autoinmune, acné, alopecia, enfermedad cardiovascular, cataratas, diabetes, endometriosis, disfunción sexual femenina, hiperglucemia, obesidad, trastorno obsesivo compulsivo, síndrome premenstrual, carcinoma prostático, hiperplasia prostática benigna, hipertensión pulmonar, daño por reperfusión, entre otras...

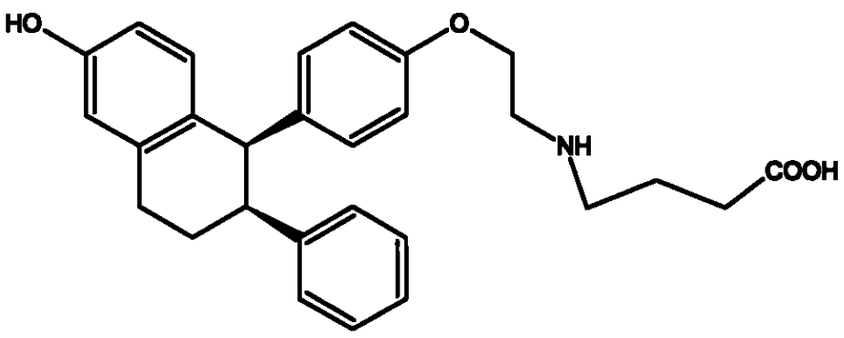
- **Reivindicación 3:**

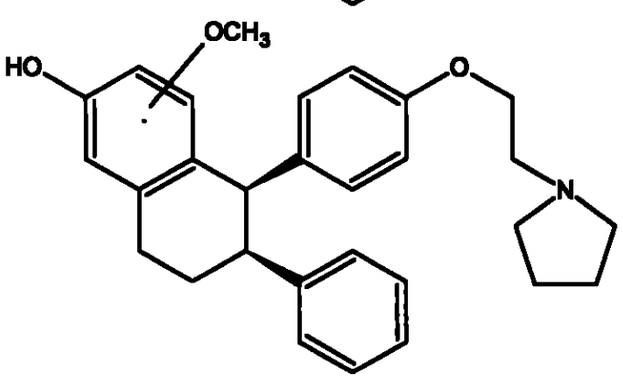
Se reivindica una cantidad efectiva de un metabolito según la cláusula 2, en donde dicho metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol se selecciona del grupo constituido por:

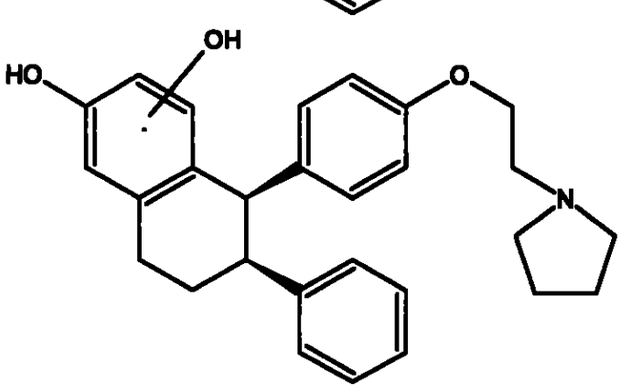
~~290~~ 280

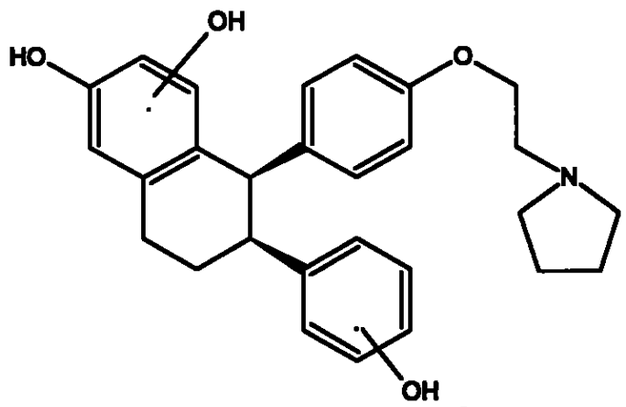








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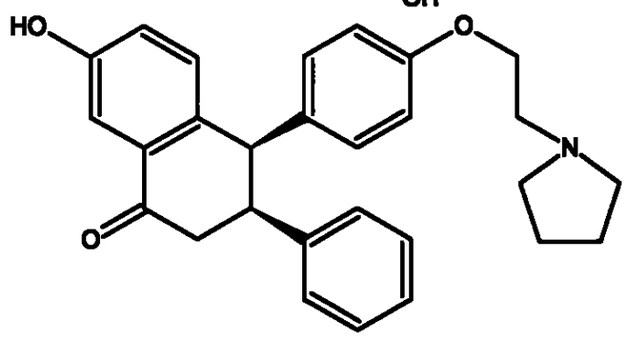
OH
O
N
OH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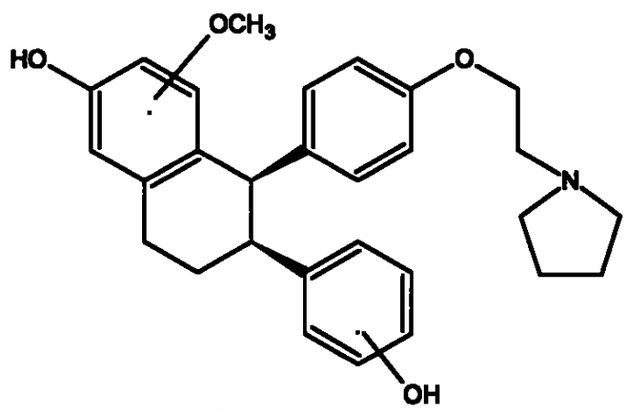
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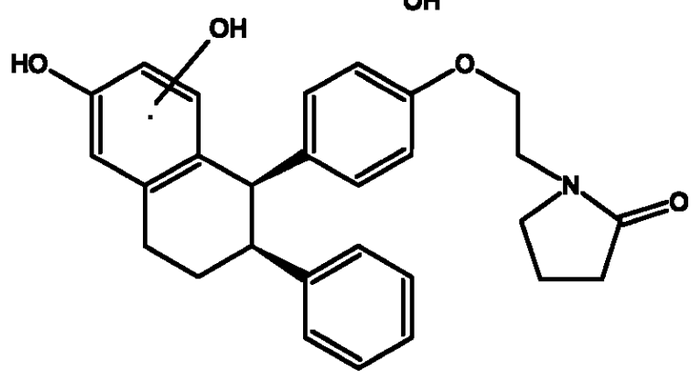
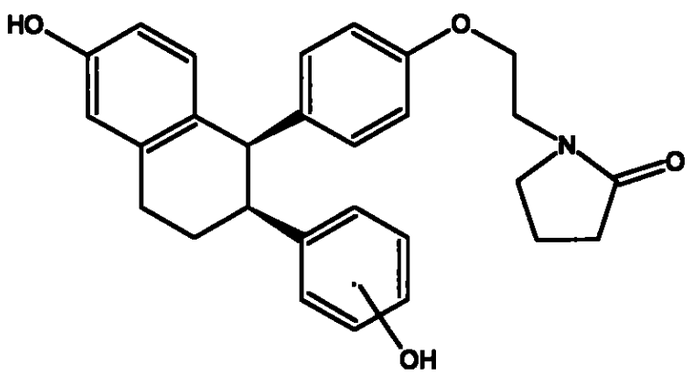
O
N
OH


HO
OH
O
N
O

282



Y los estereoisómeros, tautómeros, regioisómeros e isómeros configuracionales de éste, y las sales farmacéuticamente aceptables de éstos.

- **Reivindicaciones 4 y 5:**

Se reivindica una composición farmacéutica que comprende un metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol correspondiente a la fórmula (I).

Además se reivindica una composición farmacéutica según la cláusula 4, en donde dicho metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol se selecciona del grupo constituido por aquellos listados en la cláusula 3.

Una vez catalogada la invención ésta se clasificó en la sección: **A 61 K** $^{31}/_{085}$ // $^{31}/_{016}$ // $^{31}/_{40}$ de la **Clasificación Internacional de Patentes.**

3. CLARIDAD DE LA SOLICITUD:

El artículo 30 de la Decisión 486 contempla que ***"Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción".***

Teniendo en cuenta el alcance de la materia que se reivindica, esta Dependencia se permite señalar lo siguiente:

a) La expresión "metabolito" hace referencia al producto obtenido a través de la transformación biológica de las moléculas de un fármaco, una vez este se ha sometido a la dinámica de la absorción, distribución, metabolización y eliminación endógena, es decir el proceso – ADME - que tiene ocurrencia en el cuerpo humano.

b) Teniendo en cuenta que las características técnicas esenciales de la invención giran entorno a la generación espontánea de los metabolitos del fármaco: (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol, y que los mismos forman parte del estado de la técnica, porque de acuerdo con el documento US5552412, citado en el aparte descriptivo de la invención, del compuesto señalado se conoce desde 1996 fecha a partir de la cual se ha avanzado en el estudio de la farmacocinética que cursa el principio activo, y que además los metabolitos se producen gracias a un procedimiento netamente biológico, no es claro el verdadero alcance de la invención y en particular la materia que constituye en sí misma el producto de la actividad inventiva y creadora del hombre, más si se tiene en cuenta que los metabolitos

se generan de manera espontánea gracias a la biotransformación que sufren las moléculas una vez se administran a un paciente.

Así pues, si se tiene en cuenta que una reivindicación se construye en atención a las características determinantes que definen la materia a proteger y de aquello que la invención añade al estado de la técnica, resulta claro que de la lectura de la cláusula 1, este no es el panorama que se aprecia. El aparte "determinante", por el contrario, se deriva de resultados de estudios subsecuentes sobre la farmacocinética del compuesto – cláusulas 1 y 3 - , resultados que son producto de la biotransformación espontánea.

c) Tal como se presenta el capítulo reivindicatorio, es evidente que la invención se está definiendo en atención al producto obtenido una vez cursan las diferentes alternativas de transformación biológica del fármaco, el cual en su momento si constituyó una invención - **(-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol** - es decir *no se está caracterizando un producto por sus elementos sino por el resultado del proceso de biotransformación: esto es la generación de metabolitos una vez tiene curso la farmacocinética del compuesto.*

d) De otro lado, cuando se reivindica una cantidad efectiva de un compuesto farmacológicamente activo, esto es: en el preámbulo de la reivindicación se hace alusión a una dosificación específica, se debe continuar la cláusula en el aparte caracterizante por el rango de cantidades que al suministrarse sugieren un aporte novedoso e inventivo al estado de la técnica.

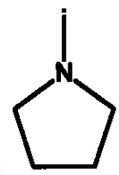
No es aceptable demandar en el preámbulo de la cláusula **"Una cantidad efectiva de un metabolito de (-)-cis-6-fenil-5-[4-(2-pirrolidin-1-iletoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol"** y definir la cantidad efectiva por los atributos específicos que definen un compuesto, es decir su estructura y elementos químicos, porque como bien se conoce el preámbulo de una reivindicación debe indicar el objeto real que constituye la invención, esto es la clase técnica que caso particular corresponde al compuesto, o si el interesado considera que corresponde a las cantidades terapéuticas, igualmente debe existir coherencia entre el objeto y reclamado y la definición de sus características esenciales que para el caso de las cantidades será el rango de dosificación.

Es importante aclarar en este punto que en el evento en que se pretenda protección para un rango de dosificación, es probable que la materia reclamada se vea afectada por el literal (d) del artículo 20 que exceptúa legalmente de patentabilidad aquellos objetos que se relacionan directamente con un método de tratamiento terapéutico o quirúrgico para humanos o animales, tal es el caso de la cláusula 2 que finaliza el aparte caracterizante señalando las bondades terapéuticas de los metabolitos así: .

"para ser administrada a un paciente para el tratamiento de osteoporosis, cáncer de mama, hiperlipidemia, aterosclerosis, enfermedad de Alzheimer, cataratas, pérdida de líbido, disfunción sexual masculina, cáncer de colon, verrugas dérmicas, enfermedad autoinmune, acné, alopecia, enfermedad cardiovascular, cataratas, diabetes, endometriosis, disfunción sexual femenina, hiperglucemia, obesidad, trastorno obsesivo compulsivo, síndrome premenstrual, carcinoma prostático, hiperplasia prostática benigna, hipertensión pulmonar, daño por reperfusión, artritis reumatoide, osteoartritis, seborrea, ginecomastia senil, deficiencia de testosterona y afecciones sensibles a la elevación de testosterona, síndrome de Turner, fibrosis uterina, vaginitis atrófica, incontinencia, cáncer de útero, hirsutismo, bulimia, anorexia, deseo sexual hipoactivo, trastorno de la excitación sexual, dispareunia, vaginismo, prolapso, infecciones del tracto urinario, apoplejía, infarto de miocardio, insuficiencia renal

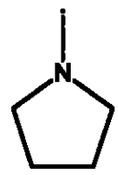
aguda o crónica, enfermedad oclusiva arterial periférica, fenómeno de Raynaud, cánceres de ovario, hígado y páncreas, así como de cáncer desmoide, glioma, carcinoma de células renales, curación de heridas, incremento de la frecuencia de orgasmos o disminución del pH vaginal".

e) Respecto de la definición y los atributos particulares de la estructura de fórmula (I) se objeta la forma poco clara como se señalan las connotaciones especiales que toma el radical R_1, el cuál cuando es $-NH(CH_2)_3COR_6$ deberá tener en cuenta las connotaciones del radical secundario R_6 el cual se define como:



–OH ó $NHCH_2COOH$, siempre que: si R_1 es o $-NH(CH_2)_3COOH$ y R_2 es OH ó OCH_3 y R_3 y R_7 son H, o si R_1 es como se definió en (a) anteriormente, y R_2 y R_7 son H y R_3 es OH ó OCH_3 entonces R_4 no es H.

Si se observa con detalle, se parte de la premisa que R_1 es $-NH(CH_2)_3COR_6$, entonces no es claro por qué dentro de las connotaciones de R_6 se toma como



condición especial que R_1 sea , si ya se había establecido que R_6 aplica sólo en el caso en que R_1 sea $-NH(CH_2)_3COR_6$.

4. DETERMINACIÓN DEL ESTADO DE LA TÉCNICA:

Consultadas las fuentes de información con que cuenta este despacho, se encontraron los siguientes documentos, anteriores a la fecha de presentación de la solicitud en estudio - 07.04.2000-, relacionados con la materia técnica reivindicada:

N°	Documento	Título	Fecha de Publicación
D1	US5552412	"5-substituted-6-ciclic-5,6,7,8-tetrahydronaphtalen-2-ol compounds which are useful for treating osteoporosis"	03/09/1996
D2	Artículo 1	"Interaction of the Anti-oestrogen, Nafoxidine hydrochloride, with the soluble nuclear oestradiol-binding protein in chick liver"	1977

Artículo 1: LAZIER Catherine B., ALFORD William S. *"Interaction of the Anti-oestrogen, Nafoxidine hydrochloride, with the soluble nuclear oestradiol-binding protein in chick liver"*. Biochem Journal (1997)164, 659 -667.

Anterioridades D1 y D2 en los folios 268 a 286.

5. EVALUACIÓN DE LOS REQUISITOS DE PATENTABILIDAD:

El artículo 14 de la decisión 486 de la comisión de la comunidad andina establece que: *"Los países miembros otorgarán patentes para las invenciones, sean de*

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producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial".

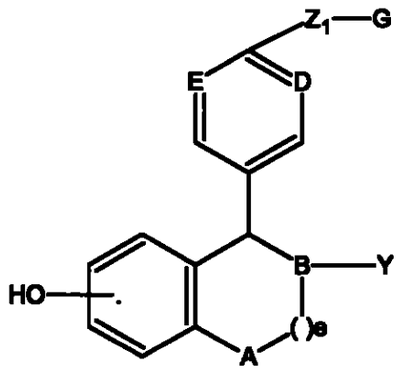
En cumplimiento del artículo 14 de la decisión 486 de la comisión de la comunidad andina, se procedió a evaluar los requisitos de novedad, nivel inventivo y aplicación industrial con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

5.1 Evaluación del NIVEL INVENTIVO:

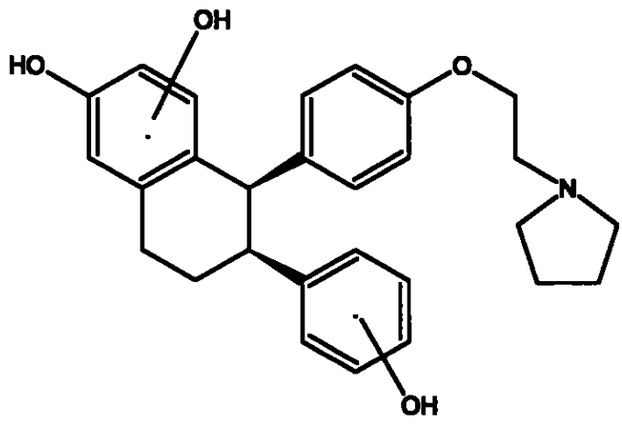
El artículo 18 de la decisión 486 dispone "*Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la Materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*"

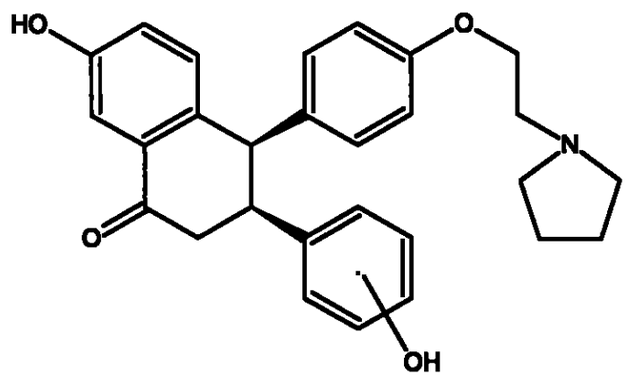
En atención al objeto reclamado existe en el estado de la técnica dos publicaciones químicas que hacen referencia a objetos similares a los reclamados por la solicitud en estudio y que afectan su altura inventiva.

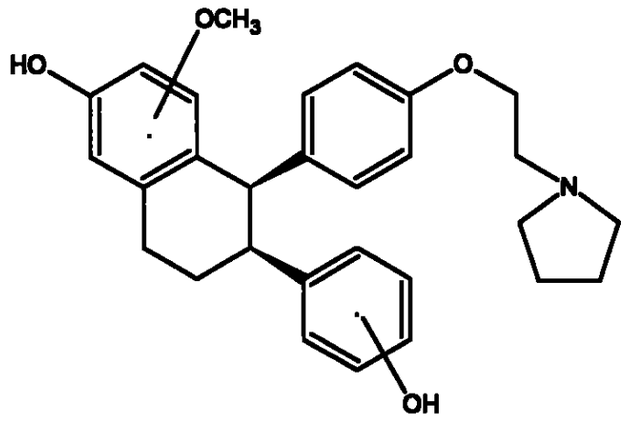
El documento **D1**, por ejemplo, comprende compuestos que presentan la estructura química:



En particular revela la molécula (cis),6-fenil-5-[4-(2-pirrolidin-1-il-etoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol, compuesto del cual se reivindican sus metabolitos y composiciones farmacéuticas que los contienen en las cláusulas 1 a 4, y que responden a las estructuras:

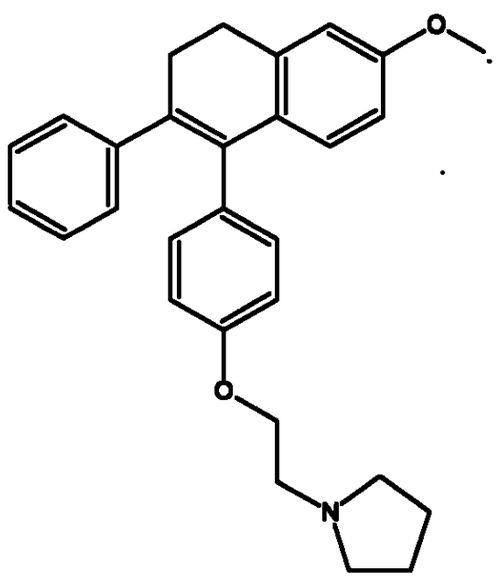






Que al compararse con la estructura contemplada en la anterioridad se hace palpable que la materia reclamada se deriva evidentemente del estado del arte.

De igual forma al comparar estructuralmente los compuestos de la invención con la estructura del antiestrogénico NAFOXIDINA, fármaco reconocido desde la década de los 70´s, y al cual se le han efectuado diversos estudios clínicos para determinar su mecanismo de acción en ensayos *in vitro* - documento D2 -, resulta claro que la materia reclamada no sólo constituye el producto esencial de la transformación biológica del compuesto *(cis),6-fenil-5-[4-(2-pirrolidin-1-il-etoxi)fenil]-5,6,7,8-tetrahidronaftalen-2-ol* si no que en realidad no existe verdadera actividad inventiva y creadora que haya dado lugar a su diseño estructural.



Es claro que para un experto en la materia técnica resulta evidente, a partir de las

enseñanzas reveladas por las anterioridades D1 y D2 y el conocimiento de conjunto sobre los procesos biológicos y la farmacocinética de las reacciones que sufren las sustancias activas en el cuerpo humano, llegar a obtener, aislar o diseñar los metabolitos generados en la transformación biológica – proceso endógeno – que sufren las moléculas activas.

Por lo anterior, los documentos citados pueden afectar el nivel inventivo de la presente solicitud, en el siguiente orden:

Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US5552412	1 a 4	Nivel Inventivo
D2	Artículo 1	1 a 4	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo 5 del título primero de la Circular Única Nº 10 de 2001.

Álix Carmenza Céspedes de Vergel
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 09 NOV. 2006

CONCEPTO TÉCNICO Número 01711	**EXAMINADOR TÉCNICO** Código 202044

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 1-27556 EDICTO No. 4492

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LA SECRETARÍA GENERAL AD-HOC
HACE SABER:

Que esta Superintendencia profirió Resolución No. 4827 de 26-FEB-2007. Que el contenido de la parte resolutiva es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

ARTICULO PRIMERO: Negar el privilegio de patente de invención para la solicitud correspondiente a "METABOLITOS AGONISTAS / ANTAGONISTAS DE ESTROGENO".

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor Álvaro Correa Ordoñez, o a quien haga sus veces, en su calidad de apoderado de Pfizer Products Inc., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los 26 FEB. 2007

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,

JAIME BUENO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:00 a.m. de hoy.

Secretaria General Ad-Hoc 13-MAR-2007 Este edicto se desfija hoy 27-MAR-2007 a las 4:30 p.m.
Secretaria General Ad-Hoc
EDICTO No. 4492