

A NOVEL CRYSTALLINE FORM OF 6-HYDROXY-
3-(4-[2-(PIPERIDIN-1-YL)ETHOXY]
PHENOXY)-2-(4-METHOXYPHENYL)BENZO[b]THIOPHENE HYDROCHLORIDE

5 **Background of the Invention**

6-Hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride (arxoxifene) was first described generically in U.S. Patent No. 5,510,357 and was specifically disclosed in U.S. Patent No. 5,723,474 ('474) and European Patent Application 0729956. Arxoxifene is a nonsteroidal mixed estrogen antagonist/agonist, useful for, inter alia, lowering serum cholesterol and for inhibiting hyperlipidemia, osteoporosis, estrogen dependent cancers including breast and uterine cancer, endometriosis, CNS disorders including Alzheimer's disease, aortal smooth muscle cell proliferation, and restenosis.

Specifically, arxoxifene is useful for, and is being clinically evaluated for the treatment of receptor positive metastatic breast cancer; the adjuvent treatment of receptor positive patients following appropriate systemic or local therapy; the reduction of recurrence of invasive and noninvasive breast cancer; and the reduction of the incidence of invasive breast cancer and ductal carcinoma in situ (DCIS). Arxoxifene is also useful in combination with radiotherapy, aromatase inhibitors, LHRH analogues, and acetyl choline esterase (AChE) inhibitors.

X-ray powder diffraction (XRD), thermogravimetric analysis (TGA), proton nuclear magnetic resonance (¹H NMR) and Karl Fischer (KF) analyses of bulk arxoxifene isolated by the procedures taught in '474 later indicated that said material was hydrated, poorly crystalline, and contained variable amounts of an organic volatile (ethyl acetate) in its lattice.

Poorly crystalline and/or amorphous materials are typically less desirable than highly crystalline materials for formulation processing. Amorphous compounds are chemically and physically less stable as they tend to adsorb significant amounts of water. The adsorption of water by an amorphous material in a gelatin capsule, for example, may cause the capsule to shrink or buckle as moisture is transferred from the capsule to the amorphous component. In addition, amorphous compounds have a tendency to precipitate out of solutions containing them. If an amorphous drug substance precipitates from a delivery solution, the dissolution and bioavailability properties of the drug may be negatively affected.

In addition, it is generally not desirable to formulate pharmaceuticals containing substantial amounts of organic solvent (e.g., ethyl acetate) due to potential solvent toxicity to the recipient thereof and changes in potency of the pharmaceutical as a function of the solvent. In addition, from a manufacturing perspective, it is also generally less desirable to prepare non-crystalline materials whenever said preparation involves a collection of the final product via filtration. Such filtrations are often more difficult to perform when the material collected is non-crystalline. Moreover, it is also generally less desirable, from a manufacturing perspective, to formulate pharmaceuticals containing substantial amounts of water (hydrates) because the level of hydration will typically be some function of the relative humidity at which the pharmaceutical is produced and stored. In other words, potency variability is typically more problematic with a hydrate relative to its anhydrous form.

Although the arzoxifene prepared by the procedures taught in '474 could be used as a pharmaceutical it would be highly desired and advantageous to find a more crystalline

form of arzoxifene that did not contain water nor an organic solvent within its crystal lattice which could be reproducibly and efficiently prepared on a commercial scale.

Summary of the Invention

The present invention is related to a non-solvated anhydrous crystalline form of 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride (F-V) having an X-ray diffraction pattern which comprises at least one of the following peaks: 7.3 ± 0.2 , 15.5 ± 0.2 , 15.9 ± 0.2 , and $17.6 \pm 0.2^\circ$ in 2θ when obtained from a copper radiation source.

In a first aspect of the present invention, there is provided non-solvated anhydrous crystalline 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride (F-V) having an X-ray diffraction pattern which comprises at least one of the following peaks: 7.3 ± 0.2 , 15.5 ± 0.2 , 15.9 ± 0.2 , and $17.6 \pm 0.2^\circ$ in 2θ when obtained from a copper radiation source ($\lambda=1.54056'$).

In addition, the present invention relates to a pharmaceutical formulation comprising F-V; one or more pharmaceutical carriers, diluents, or excipients; and optionally a stabilizing agent selected from methionine, acetylcysteine, cysteine or cysteine hydrochloride; and optionally estrogen, optionally progestin, optionally an aromatase inhibitor, optionally an LHRH analogue and optionally an acetyl choline esterase (AChE) inhibitor.

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In a second aspect of the present invention, there is provided a pharmaceutical formulation comprising the crystalline compound as defined above and one or more pharmaceutical carriers, diluents, or excipients.

In addition, the present invention is related to methods for using F-V to inhibit pathological conditions such as: uterine fibrosis, endometriosis, aortal smooth muscle cell proliferation, restenosis, breast cancer, uterine cancer, prostatic cancer, benign prostatic hyperplasia, bone loss, osteoporosis, cardiovascular disease, hyperlipidemia, CNS disorders, and Alzheimer's disease and for using F-V for the manufacture of a medicament for inhibiting same.

The present invention is further related to methods for using F-V to up-regulate choline acetyltransferase (ChAT) and for using F-V for the manufacture of a medicament for up-regulating same.

The present invention also relates to a process for preparing F-V which comprises crystallizing 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride from a crystallization solvent selected from the group consisting of: methanol or aqueous methanol, ethanol or isopropanol; and subsequently drying the resulting solid to a constant weight.

In a third aspect of the present invention, there is provided a process for preparing a compound as defined in the first aspect which comprises crystallizing 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride from a crystallization solvent selected from: methanol or aqueous methanol, ethanol or isopropanol; and subsequently drying the resulting solid to a constant weight.

In a fourth aspect of the present invention, there is provided use of a crystalline compound as defined above in the manufacture of a medicament for inhibiting a pathological condition selected from: uterine fibrosis, endometriosis, aortal smooth muscle cell proliferation, restenosis, breast cancer, uterine cancer, prostatic cancer, benign prostatic hyperplasia, bone loss, osteoporosis, cardiovascular disease, hyperlipidemia, CNS disorders, and Alzheimer's disease.

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Brief Description of the Figures

Figure 1 is a representative TGA trace of F-V.

Figure 2 is a representative DSC trace of F-V.

Figure 3 is a representative XRD Pattern for Form V.

Detailed Description of the Invention

F-V may be prepared by drying, either at ambient temperature or at slightly elevated temperature, the crystalline solid isolated at ambient temperature from crystallization of arzoxifene (or any polymorph/solvate thereof) from methanol, ethanol or isopropanol or aqueous mixtures of methanol. When using ethanol or isopropanol, the water content in said solvents is preferably less than 0.2% (A.C.S. spectrophotometric grade). Preferably the aqueous composition in methanol contains less than 30% by volume water. More preferably F-V is prepared by drying, either at ambient temperature or at slightly elevated temperature, the solid isolated from crystallization from aqueous methanol wherein the volume of water is between 20% and 5%. Most preferably F-V is prepared by drying at 50 to 70°C, under vacuum, the solid isolated at ambient temperature from crystallization of arzoxifene (or any polymorph/solvate thereof) from aqueous methanol wherein the water content by volume is 15%.

Typically, arzoxifene may be dissolved in methanol (about 1 g solute/20 ml of solvent) and optionally heated in order to effect dissolution of the arzoxifene starting material. Once dissolution is achieved, the solution may optionally be concentrated to about 1 g of solute/5 ml of solvent, e.g., by distillation, before allowing the solution to cool slowly to room temperature. Once at room temperature, the solution may optionally be cooled further (with the aid of an ice bath or refrigeration) to between 0 and 5°C. After a sufficient amount of time has elapsed for crystallization to occur, the F-V crystals may be collected by vacuum filtration and washed with cold (about 0°C) methanol before drying to a constant weight in vacuum. Slightly elevated drying temperatures (about 50°C for 12 to 48 hours) in the presence of a nitrogen purge are preferred. For commercial scale synthesis of F-V, it may be advantageous to seed the crystallization with F-V.

Suitable arzoxifene starting material for the above crystallization includes, but is not limited to, S-II, F-I, F-III (solvated and non-stoichiometric hydrated crystalline forms of arzoxifene described in PCT Patent Applications PCT/US00/16332 and PCT/US00/16333, the teachings of which are hereby incorporated by reference), arzoxifene prepared by the procedures taught in '474, or any mixture thereof. It is not important which form of arzoxifene one starts with because crystallization from anhydrous methanol, according to the procedures described herein, results in F-V crystals.

Characterization of F-V

Differential scanning calorimetry/thermogravimetric analysis (DSC/TGA), moisture sorption/desorption, and X-ray powder diffraction (XRD) methods were used to characterize F-V. TGA is a measure of the thermally induced weight loss of the material as a function of temperature. It is most

commonly used to study to study desolvation processes and
quantatively determine the total volatile content of a
solid. DSC is a technique that is often used to screen
compounds for polymorphism because the temperatures(s) at
5 which a physical change in a material occurs is usually
characteristic of that material. Moisture sorption
isotherms provide evaluation of the degree of hygroscopicity
associated with a given material and characterization of
non-hydrates and hydrates. Lastly, XRD is a technique that
10 detects long-range order in a crystalline material.

A representative TGA trace of F-V is shown in Figure 1.
Thermogravimetric analysis of F-V showed no weight loss
indicating the isolation of a non-solvated crystal form.
DSC analysis of F-V showed a sharp melting endotherm at 174-
15 175°C as shown in Figure 2, which is significantly higher
than that observed for F-III.

The moisture sorption/desorption isotherm obtained for
F-V showed a weight increase of 0.11% over the range of 0-
95% RH indicating a stable anhydrous crystal form with
20 little propensity to adsorb water or convert to a hydrated
form of arxoxifene.

The XRD pattern of F-V features sharp peaks and a flat
baseline, indicative of highly crystalline materials. The
angular peak positions in 2θ and corresponding I/I_0 data for
25 all peaks with intensities equal to or greater than 10% of
the largest peak for F-V is tabulated in Table 1. All data
in Table 1 are expressed with an accuracy of $\pm 0.2\%$.

Although many of the intense reflections are generally at
similar diffraction angles to those reported for S-II, F-I
and F-III, each of the forms gives a different powder
30 pattern, allowing for a clear distinction between S-II, F-I,
F-III and F-V.

Variable temperature x-ray powder diffraction analysis of F-V showed no significant change in the diffraction pattern up to 125°C which is consistent with the DSC profile indicating a stable crystal form.

5 It is well known in the crystallography art that, for any given crystal form, the relative intensities of the diffraction peaks may vary due to preferred orientation resulting from factors such as crystal morphology and habit. Where the effects of preferred orientation are present, peak
10 intensities are altered, but the characteristic peak positions of the polymorph are unchanged. See, e.g., The United States Pharmacopeia #23, National Formulary #18, pages 1843-1844, 1995. Furthermore, it is also well known in the crystallography art that, for any given crystal form,
15 the angular peak positions may vary slightly. For example, peak positions can shift due to a variation in the temperature at which a sample is analyzed, sample displacement, or the presence or absence of an internal standard. In the present case, a peak position variability
20 of ± 0.2 in 2θ will take into account these potential variations without hindering the unequivocal identification of F-V.

A well known and accepted method for searching crystal forms in the literature is the "Fink" method. The Fink
25 method uses the four most intense lines for the initial search followed by the next four most intense lines. In accord with the Fink method, based on peak intensities as well as peak position, F-V may be identified by the presence of peaks at 7.3 ± 0.2 , 15.5 ± 0.2 , 15.9 ± 0.2 , and $17.6 \pm$
30 0.2° in 2θ ; when the pattern is obtained from a copper radiation source. The presence of F-V may be further verified by peaks at 17.9 ± 0.2 , 18.2 ± 0.2 , 18.9 ± 0.2 , and

21.5 $\pm$ 0.2° in 2 θ ; when the pattern is obtained from a copper radiation source.

Table 1

Angle 2 θ	I/I ₀ (%)	Angle 2 θ	I/I ₀ (%)	Angle 2 θ	I/I ₀ (%)
7.3	45	17.6	72	22.6	19
9.0	22	17.9	83	23.3	20
10.0	10	18.2	56	24.4	46
12.8	39	18.9	82	25.8	38
14.6	15	19.8	27	27.4	32
15.5	50	21.5	100	28.2	18
15.9	64				

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F-V has several advantages over the prior art form of arzoxifene described in '474 and over F-I and F-III described in the previously incorporated by reference PCT applications. Relative to the arzoxifene produced by the procedures taught in '474, F-V is more stable at ambient temperature and is, therefore, more amenable to pharmaceutical development, i.e., development of a dosage formulation. In addition, unlike the form disclosed in '474, F-V is highly crystalline. Crystalline materials are generally less hygroscopic and more stable (e.g., less prone to chemical degradation, maintains consistent potency) than amorphous materials and are, therefore, more desirable for formulation processing. Furthermore, unlike the form of arzoxifene produced by the procedures taught in '474, which contained ethyl acetate and water in its lattice, F-V contains neither.

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Unlike S-II, F-I and F-III, F-V is truly an anhydrous form of arzoxifene which shows no propensity to adsorb water on changes in relative humidity. Furthermore, F-V's crystal lattice is stable up to its melting temperature. Moreover,

F-V has approximately a 10% higher aqueous solubility relative to F-III and is the thermodynamically most stable known form of arzoxifene.

5 Characterization Methods

DSC analysis was performed using a TA Instruments 2920 equipped with an auto-sampler and a refrigerated cooling device. The sample was enclosed in a crimped aluminum pan and analyzed vs. an empty reference pan. The heat flow was measured after equilibration at 30° C. The heating rate was 10 5°C per minute to 300°C. A graph of heat flow vs. temperature was integrated to identify any endothermic or exothermic events.

15 TGA analysis was performed using a TA Instruments 2950 equipped with an auto-sampler. The sample was loaded onto a tared aluminum pan and the temperature was ramped from ambient to 300°C at a rate of 10°C per minute. A graph of weight percent vs. temperature was integrated to determine the percent loss.

20 Moisture Sorption Isotherms were generated using a VTI SGA-100 flow instrument. The samples were analyzed at 25°C from 0-95% relative humidity (RH) for adsorption and from 95-5% RH for desorption in steps of 5% RH. The adsorption and desorption isotherms were generated as a graph of the % weight change vs. % RH.

25 X-ray powder diffraction patterns were obtained on a Siemens D5000 X-ray powder diffractometer which was equipped with a CuK α source (λ = 1.54056) operated at 50 kV and 40 mA with a Kevex solid state Si(Li) detector. The samples were scanned from 4 to 35° in 2 θ at 2.5 seconds per step size of 30 0.04°. The dry powders were packed into recessed top-loading sample holders and a smooth surface was obtained using a glass slide.

Variable temperature X-ray powder diffraction patterns were obtained on a Siemens D5000 X-ray powder diffractometer which was equipped with a $\text{CuK}\alpha$ source ($\lambda = 1.54056$) operated at 50kV and 40 mA with a scintillation detector and nickel filter. The powder was packed into a top-loading, recessed temperature controlled holder and a smooth surface was obtained for diffraction. The sample was scanned from 2 to 35° 2 θ at 2.5 seconds per step size of 0.04° beginning at 25°C after an equilibration time of 5 minutes. Subsequent scans were obtained at increasing temperature increments of 25°C to a maximum of 125°C.

The following examples further illustrate processes for preparing F-V. The examples are not intended to be limiting to the scope of these processes in any respect, and should not be so construed.

Examples

Example 1

Crystallization from Methanol Without Concentration

A 20.00 g sample of arzoxifene is combined with 500 ml of anhydrous methanol (HPLC grade) and heated to reflux. All of the solids dissolve to afford a homogeneous pale yellow solution. The solution is cooled to below reflux and 5.00 g of additional arzoxifene are added. The solution is re-heated to reflux to effect dissolution of all of the solids. The solution is slowly allowed to cool with agitation. At 50°C the solution is seeded with several milligrams of previously prepared F-V salt. The crystalline slurry is allowed to cool from 50°C to 30°C over a 1.25 hour period. At this point a large amount of white solids are present. The stirred slurry is immersed in an ice bath and

stirred for an additional 3 hours. The slurry is filtered using Whatman #1 filter paper and the white solid is washed with 50 ml of methanol pre-chilled to 0°C. The wet cake is dried for about 48 hours at 50°C under vacuum with a slight N₂ purge. Yield 15.94 g (63.8% yield). HPLC potency 89.4% (as free base), total related substances (TRS) 0.28%. Comparison of the product weight before and after drying showed the initial wet cake contained 65% solvent.

Example 2

Crystallization from Methanol With Concentration

A 25.00 g sample of arzoxifene is combined with 500 ml of anhydrous methanol (HPLC grade) and heated to reflux. All of the solids dissolved to afford a homogeneous pale yellow solution. The solution is concentrated by removal of 375 ml of distillate by atmospheric distillation. At this point, the reaction mixture is a clear homogeneous yellow solution. Reflux is broken and the solution is seeded with several milligrams of previously prepared F-V. After seeding, the mixture is allowed to cool to ambient temperature with slow agitation over a 1 hour period. During this time a large amount of white precipitate forms. The slurry is immersed in an ice bath and stirred for an additional 3 hours. The slurry is filtered using Whatman #1 filter paper and the white solid is washed with 50 ml of methanol pre-chilled to 0°C. The wet cake is dried for about 48 hours at 50°C under vacuum with a slight N₂ purge. Yield 22.44 g (89.8% yield). HPLC potency 91.3% (as free base), TRS 0.26%. Comparison of the product weight before and after drying showed the initial wet cake contained 31.5% solvent.

Example 3

30 Gallon Scale Recrystallization from Methanol

5 A 3.08 kg sample of arzoxifene is combined with 60 L of
anhydrous methanol (HPLC grade) and heated to reflux. All
of the solids dissolved to afford a pale yellow homogeneous
solution. The solution is concentrated by removal of 40 L of
distillate by atmospheric distillation. At this point, the
10 reaction mixture is a clear homogeneous yellow solution. The
reaction is cooled to break reflux and the manway is opened
at about 40°C to check for crystallization. Crystals are
observed and cooling is continued at a rate 12°C per hour to
a final temperature of 0°C. The crystallization slurry is
stirred overnight at 0°C and then filtered through a single
15 plate filter press. In order to remove all product from the
crystallization tank, the mother liquor is used as a tank
wash and then sent through the press. The wet cake is then
washed with 11.3 L of anhydrous methanol pre-chilled to 0°C.
The wet cake is dried by applying vacuum to the press and
20 running 50°C water through the jacket of the press. A
slight N₂ purge is applied after about 24 hours. Total
drying time is about 36 hours. The yield is 2.588 kg
(86.27%); HPLC potency 92.7 (as free base); TRS 0.39%.

Example 4

Crystallization from Ethanol

25 Punctilious ethanol (250 ml) and arzoxifene (10.0 g)
were combined and heated to reflux to effect dissolution.
The solution was allowed to cool to ambient temperature over
30 a 3 hour period during which time a white crystalline
precipitate formed. The solids were isolated by filtration
and vacuum dried overnight at 50°C with a slight N₂ purge.

Yield 5.50 g, m.p. 173°C (by DSC). An x-ray powder diffraction spectrum for this sample was obtained and was substantially identical to that of the F-V pattern disclosed in Figure 3.

Example 5

Crystallization from Isopropanol

Anhydrous isopropanol (250 ml) and azroxifene (10.0 g) were combined and heated to reflux to effect dissolution. Heat was removed and the solution seeded with several milligrams of F-V. The reaction mixture was allowed to cool to ambient temperature and stir overnight during which time a white precipitate formed. The solids were isolated by filtration to afford 12.11 g of wetcake. A 4.01 g sample of the wetcake was dried over night at 60°C under vacuum with a slight N₂ purge. Yield 2.72 g; m.p. 171.5°C (by DSC). An x-ray powder diffraction spectrum for this sample was obtained and was substantially identical to that of the F-V pattern disclosed in Figure 3.

Example 6

Preparation from Azroxifene Free Base

Azroxifene free base (5.07 g) was slurried in 65.0 ml of methanol. A solution of 1.41 ml of concentrated hydrochloric acid and 10.0 ml of water was added to the reaction mixture. The reaction mixture was heated to 55°C for 15 minutes to effect dissolution. The reaction mixture was cooled to 30°C and seeded with 50 mg of F-V. The reaction mixture was cooled to 10°C at a rate of 1°C/hr and stirred at that temperature for 8 hours. The solids were isolated by filtration, washed with methanol pre-chilled to 10°C and vacuum dried at 50°C over night with a light N₂

purge. Yield 4.42 g (87.7% yield); potency (HPLC) 99.7%; TRS 0.32%. An x-ray powder diffraction spectrum for this sample was obtained and was substantially identical to that of the F-V pattern disclosed in Figure 3.

Utilities

As used herein, the term "effective amount" means an amount of F-V that is capable of inhibiting conditions, or detrimental effects thereof, described herein. When F-V is co-administered with estrogen, progestin, an aromatase inhibitor, an LHRH analogue, or an AChE inhibitor, the term "effective amount" also means an amount of such an agent capable of producing its intended effect.

The terms "inhibiting" and "inhibit" include their generally accepted meaning, i.e., preventing, prohibiting, restraining, alleviating, ameliorating, slowing, stopping, or reversing the progression or severity of a pathological condition, or sequela thereof, described herein.

The terms "preventing", "prevention of", "prophylaxis", "prophylactic" and "prevent" are used herein interchangeably and refer to reducing the likelihood that the recipient of F-V will incur or develop any of the pathological conditions, or sequela thereof, described herein.

The terms "estrogen deprived" and "estrogen deprivation" refer to a condition, either naturally occurring or clinically induced, where a woman can not produce sufficient endogenous estrogenic hormones to maintain estrogen dependent functions, e.g., menses, homeostasis of bone mass, neuronal function, cardiovascular condition, etc. Such estrogen deprived situations arise from, but are not limited to, menopause and surgical or chemical ovariectomy, including its functional equivalent, e.g., medication with an aromatase inhibitor, GnRH agonists or antagonists, ICI 182780, and the like. Disease states

associated with an estrogen deprived state include, but are not limited to: bone loss, osteoporosis, cardiovascular disease and hyperlipidemia.

As used herein, the term "estrogen" includes steroidal compounds having estrogenic activity such as, for example, 17 β -estradiol, estrone, conjugated estrogen (Premarin[®]), equine estrogen 17 β -ethynyl estradiol, and the like. A preferred estrogen-based compound is Premarin[®], and norethynodrel.

As used herein, the term "progestin" includes compounds having progestational activity such as, for example, progesterone, norethynodrel, nongestrel, megestrol acetate, norethindrone, and the like. Norethindrone is a preferred progestin-based agent.

As used herein the term "aromatase inhibitor" includes compounds capable of inhibiting aromatase, for example commercially available inhibitors such as aminoglutemide (CYTANDREN[®]), Anastrozole (ARIMIDEX[®]), Letrozole (FEMARA[®]), Formestane (LENATRON[®]), Exemestane (AROMASIN[®]), and the like.

As used herein, the term "LHRH analogue" refers to an analogue of lutenizing hormone releasing hormone that inhibits estrogen production in a premenopausal women including for example, goserlin (ZOLADEX[®]), leuprolide (LUPRON[®]) and the like.

As used herein, the term "AChE inhibitor" includes compounds that inhibit acetyl choline esterase, for example, physostigmine salicylate, tacrine hydrochloride, donepezil hydrochloride and the like.

The term "up-regulate ChAT" refers to increasing the enzymatic activity of ChAT, i.e., promoting the conversion of choline to acetyl choline. This promotion would include an increase in the efficiency and/or rate of reaction of

ChAT and choline and/or an increase in the amount of ChAT present at the site of action. This increase in the amount of enzyme present may be due to gene regulation or other synthetic step of the enzyme's formation and/or a decrease in the enzyme's de-activation and metabolism.

Selected Testing Procedures

General Rat Preparation Procedure: Seventy-five day old (unless otherwise indicated) female Sprague Dawley rats (weight range of 200 to 225g) are obtained from Charles River Laboratories (Portage, MI). The animals are either bilaterally ovariectomized (OVX) or exposed to a Sham surgical procedure at Charles River Laboratories; and then shipped after one week. Upon arrival, they are housed in metal hanging cages in groups of 3 or 4 per cage and have *ad libitum* access to food (calcium content approximately 0.5%) and water for one week. Room temperature is maintained at $22.2^{\circ} \pm 1.7^{\circ}\text{C}$ with a minimum relative humidity of 40%. The photoperiod in the room was 12 hours light and 12 hours dark.

Dosing Regimen Tissue Collection: After a one week acclimation period (therefore, two weeks post-OVX) daily dosing with F-V is initiated. 17α -ethynyl estradiol or F-V is given orally, unless otherwise stated, as a suspension in 1% carboxymethylcellulose or dissolved in 20% cyclodextrin. Animals are dosed daily for 4 days. Following the dosing regimen, animals are weighed and anesthetized with a ketamine: Xylazine (2:1, v:v) mixture and a blood sample is collected by cardiac puncture. The animals are then sacrificed by asphyxiation with CO_2 , the uterus is removed through a midline incision, and a wet uterine weight is determined. 17α -ethynyl estradiol is obtained from Sigma Chemical Co., St. Louis, MO.

Cardiovascular Disease/Hyperlipidemia

The blood samples from above are allowed to clot at room temperature for 2 hours, and serum is obtained following centrifugation for 10 minutes at 3000 rpm. Serum cholesterol is determined using a Boehringer Mannheim Diagnostics high performance cholesterol assay. Briefly the cholesterol is oxidized to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide is then reacted with phenol and 4-aminophenazone in the presence of peroxidase to produce a p-quinone imine dye, which is read spectrophotometrically at 500 nm. Cholesterol concentration is then calculated against a standard curve. The entire assay is automated using a Biomek Automated Workstation.

Uterine Eosinophil Peroxidase (EPO) Assay

The uteri from above are kept at 4°C until time of enzymatic analysis. The uteri are then homogenized in 50 volumes of 50 mM Tris buffer (pH - 8.0) containing 0.005% Triton X-100. Upon addition of 0.01% hydrogen peroxide and 10 mM O-phenylenediamine (final concentrations) in Tris buffer, increase in absorbance is monitored for one minute at 450 nm. The presence of eosinophils in the uterus is an indication of estrogenic activity of a compound. The maximal velocity of a 15 second interval is determined over the initial, linear portion of the reaction curve.

Inhibition of Bone Loss (Osteoporosis) Test Procedure

Following the general preparation procedure described above, the rats are treated daily for thirty-five days (6 rats per treatment group) and sacrificed by carbon dioxide asphyxiation on the 36th day. The thirty-five day time period is sufficient to allow maximal reduction in bone density, measured as described herein. At the time of

sacrifice, the uteri are removed, dissected free of extraneous tissue, and the fluid contents are expelled before determination of wet weight in order to confirm estrogen deficiency associated with complete ovariectomy. Uterine weight is routinely reduced about 75% in response to ovariectomy. The uteri are then placed in 10% neutral buffered formalin to allow for subsequent histological analysis.

The right femurs are excised and digitized X-rays generated and analyzed by an image analysis program (NIH image) at the distal metaphysis. The proximal aspect of the tibiae from these animals are also scanned by quantitative computed tomography. In accordance with the above procedures, F-V or ethynyl estradiol (EE₂) in 20% hydroxypropyl β -cyclodextrin are orally administered to test animals. F-V is also useful in combination with estrogen or progestin.

MCF-7 Proliferation Assay

MCF-7 breast adenocarcinoma cells (ATCC HTB 22) are maintained in MEM (minimal essential medium, phenol red-free, Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (V/V), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES {(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])10 mM}, non-essential amino acids and bovine insulin (1 ug/mL) (maintenance medium). Ten days prior to assay, MCF-7 cells are switched to maintenance medium supplemented with 10% dextran coated charcoal stripped fetal bovine serum (DCC-FBS) assay medium) in place of 10% FBS to deplete internal stores of steroids. MCF-7 cells are removed from maintenance flasks using cell dissociation medium (Ca⁺⁺/Mg⁺⁺ free HBSS (phenol red-free) supplemented with 10 mM HEPES and 2 mM EDTA). Cells are washed twice with assay medium and adjusted to 80,000

cells/mL. Approximately 100 mL (8,000 cells) are added to flat-bottom microculture wells (Costar 3596) and incubated at 37°C in a 5% CO₂ humidified incubator for 48 hours to allow for cell adherence and equilibration after transfer. Serial dilutions of drugs or DMSO as a diluent control are prepared in assay medium and 50 mL transferred to triplicate microcultures followed by 50 mL assay medium for a final volume of 200 mL. After an additional 48 hours at 37°C in a 5% CO₂ humidified incubator, microcultures are pulsed with tritiated thymidine (1 uCi/well) for 4 hours. Cultures are terminated by freezing at -70°C for 24 hours followed by thawing and harvesting of microcultures using a Skatron Semiautomatic Cell Harvester. Samples are counted by liquid scintillation using a Wallac BetaPlace β counter.

DMBA-Induced Mammary Tumor Inhibition

Estrogen-dependent mammary tumors are produced in female Sprague-Dawley rats which are purchased from Harlan Industries, Indianapolis, Indiana. At about 55 days of age, the rats receive a single oral feeding of 20 mg of 7,12-dimethylbenz[a]anthracene (DMBA). About 6 weeks after DMBA administration, the mammary glands are palpated at weekly intervals for the appearance of tumors. Whenever one or more tumors appear, the longest and shortest diameters of each tumor are measured with a metric caliper, the measurements are recorded, and that animal is selected for experimentation. An attempt is made to uniformly distribute the various sizes of tumors in the treated and control groups such that average-sized tumors are equivalently distributed between test groups. Control groups and test groups for each experiment contain 5 to 9 animals.

F-V is administered either through intraperitoneal injections in 2% acacia, or orally. Orally administered

compounds are either dissolved or suspended in 0.2 mL corn oil. Each treatment, including acacia and corn oil control treatments, is administered once daily to each test animal. Following the initial tumor measurement and selection of test animals, tumors are measured each week by the above-mentioned method. The treatment and measurements of animals continue for 3 to 5 weeks at which time the final areas of the tumors are determined. For each compound and control treatment, the change in the mean tumor area is determined.

Uterine Fibrosis Test Procedures

Test 1: Between 3 and 20 women having uterine fibrosis are administered F-V. The amount of compound administered is from 0.1 to 1000 mg/day, and the period of administration is 3 months. The women are observed during the period of administration, and up to 3 months after discontinuance of administration, for effects on uterine fibrosis.

Test 2: The same procedure is used as in Test 1, except the period of administration is 6 months.

Test 3: The same procedure is used as in Test 1, except the period of administration is 1 year.

Test 4: Prolonged estrogen stimulation is used to induce leiomyomata in sexually mature female guinea pigs. Animals are dosed with estradiol 3-5 times per week by injection for 2-4 months or until tumors arise. Treatment consisting of F-V or vehicle is administered daily for 3-16 weeks and then animals are sacrificed and the uteri harvested and analyzed for tumor regression.

Test 5: Tissue from human leiomyomas are implanted into the peritoneal cavity and/or uterine myometrium of sexually mature, castrated, female, nude mice. Exogenous estrogen is supplied to induce growth of the explanted tissue. In some cases, the harvested tumor cells are cultured *in vitro* prior to implantation. Treatment consisting of F-V or vehicle is supplied by gastric lavage on a daily basis for 3-16 weeks and implants are removed and measured for growth or regression. At the time of sacrifice, the uteri are harvested to assess the status of the organ.

Test 6: Tissue from human uterine fibroid tumors is harvested and maintained, *in vitro*, as primary non-transformed cultures. Surgical specimens are pushed through a sterile mesh or sieve, or alternately teased apart from surrounding tissue to produce a single cell suspension. Cells are maintained in media containing 10% serum and antibiotic. Rates of growth in the presence and absence of estrogen are determined. Cells are assayed for their ability to produce complement component C3 and their response to growth factors and growth hormone. *In vitro* cultures are assessed for their proliferative response following treatment with progestins, GnRH, F-V, and vehicle. Levels of steroid hormone receptors are assessed weekly to determine whether important cell characteristics are maintained *in vitro*. Tissue from 5-25 patients is utilized.

Test 7: F-V's ability to inhibit estrogen-stimulated proliferation of leiomyoma-derived ELT cell lines is measured substantially as described in Fuchs-Young, et al., "Inhibition of Estrogen-Stimulated Growth of Uterine Leiomyomas by Selective Estrogen Receptor Modulators", *Mol. Car.*, 17(3):151-159 (1996), the teachings of which are herein incorporated by reference.

Endometriosis Test Procedures

5 Test 1: Twelve to thirty adult CD strain female rats are
used as test animals. They are divided into three groups of
equal numbers. The estrous cycle of all animals is
monitored. On the day of proestrus, surgery is performed on
each female. Females in each group have the left uterine
10 horn removed, sectioned into small squares, and the squares
are loosely sutured at various sites adjacent to the
mesenteric blood flow. In addition, females in Group 2 have
the ovaries removed. On the day following surgery, animals
in Groups 1 and 2 receive intraperitoneal injections of
water for 14 days whereas animals in Group 3 receive
15 intraperitoneal injections of 1.0 mg of F-V per kilogram of
body weight for the same duration. Following 14 days of
treatment, each female is sacrificed and the endometrial
explants, adrenals, remaining uterus, and ovaries, where
applicable, are removed and prepared for histological
20 examination. The ovaries and adrenals are weighed.

Test 2: Twelve to thirty adult CD strain female rats are
used as test animals. They are divided into two equal
groups. The estrous cycle of all animals is monitored. On
25 the day of proestrus, surgery is performed on each female.
Females in each group have the left uterine horn removed,
sectioned into small squares, and the squares are loosely
sutured at various sites adjacent to the mesenteric blood
flow. Approximately 50 days following surgery, animals
30 assigned to Group 1 receive intraperitoneal injections of
water for 21 days whereas animals in Group 2 receive
intraperitoneal injections of 1.0 mg of F-V per kilogram of
body weight for the same duration. Following 21 days of
treatment, each female is sacrificed and the endometrial

explants and adrenals are removed and weighed. The explants are measured as an indication of growth. Estrous cycles are monitored.

5 Test 3: Autographs of endometrial tissue are used to induce
endometriosis in rats and/or rabbits. Female animals at
reproductive maturity undergo bilateral oophorectomy, and
estrogen is supplied exogenously thus providing a specific
and constant level of hormone. Autologous endometrial
10 tissue is implanted in the peritoneum of 5-150 animals and
estrogen supplied to induce growth of the explanted tissue.
Treatment consisting of a compound of the present invention
is supplied by gastric lavage on a daily basis for 3-16
weeks, and implants are removed and measured for growth or
15 regression. At the time of sacrifice, the intact horn of
the uterus is harvested to assess status of endometrium.

Test 4: Tissue from human endometrial lesions is implanted
into the peritoneum of sexually mature, castrated, female,
20 nude mice. Exogenous estrogen is supplied to induce growth
of the explanted tissue. In some cases, the harvested
endometrial cells are cultured *in vitro* prior to
implantation. Treatment consisting of F-V supplied by
gastric lavage on a daily basis for 3-16 weeks, and implants
25 are removed and measured for growth or regression. At the
time of sacrifice, the uteri are harvested to assess the
status of the intact endometrium.

Test 5: Tissue from human endometrial lesions is harvested
and maintained *in vitro* as primary non-transformed cultures.
Surgical specimens are pushed through a sterile mesh or
sieve, or alternately teased apart from surrounding tissue
to produce a single cell suspension. Cells are maintained
30 in media containing 10% serum and antibiotic. Rates of

growth in the presence and absence of estrogen are determined. Cells are assayed for their ability to produce complement component C3 and their response to growth factors and growth hormone. *In vitro* cultures are assessed for their proliferative response following treatment with progestins, GnRH, F-V, and vehicle. Levels of steroid hormone receptors are assessed weekly to determine whether important cell characteristics are maintained *in vitro*. Tissue from 5-25 patients is utilized.

CNS Disorders Including Alzheimer's Disease

Estrogens, such as 17 β -estradiol, regulate gene transcription by binding to estrogen receptors (ER) which reside in the cytoplasm of certain cell populations. Ligand activation of the ER is a prerequisite for nuclear transport of the complex where binding to a 13 base-pair palindromic DNA consensus sequence (estrogen response element, or ERE) begins assembly of a transcriptional apparatus which culminates in the activation of appropriate target genes. A variety of genes have been identified which are regulated by estrogen. These include cytoskeletal proteins, neurotransmitter biosynthetic and metabolic enzymes and receptors, as well as other hormones and neuropeptides. ERE's have been identified in many estrogen-responsive genes including vitellogenin, c-fos, prolactin, and luteinizing hormone.

Of significance in the central nervous system, ERE-like sequences have been identified in p75^{NGF} and trkA, both of which serve as signaling molecules for the neurotrophins: nerve growth factor (NGF), brain derived nerve growth factor (BDNGF), and neurotrophin-3.

BDNF as well as NGF have been shown to promote the survival of cholinergic neurons in culture. It is postulated that if the interactions between neurotrophins

and estrogens are important for the development and survival of basal forebrain neurons (which degenerate in Alzheimer's disease) then clinical conditions in which an estrogen deficiency exists (as after menopause) may contribute to a loss of these neurons.

The following experiment is conducted in ovariectomized rats (prepared as described above) to determine the similarities and/or differences between F-V and estrogen at affecting gene expression in various brain regions. Six week old rats are dosed daily with subcutaneous injections of estradiol benzoate (0.03 mg/kg), F-V or vehicle (control). After five weeks of treatment, animals are sacrificed and their brains removed and hippocampi collected by microdissection. The hippocampi are fast frozen in liquid nitrogen and stored at -70°C . Total RNA is prepared from pooled tissue from the appropriate treatment and control groups and reverse transcribed using a 3' oligonucleotide primer which is selected for specific mRNA (poly-A+) populations. Polymerase chain reactions (PCR) are carried out in a cocktail consisting of: random 5' oligonucleotides (10 base-pairs in length; total of 150), reaction buffer, Taq polymerase, and a ^{32}p dTCP.

After 40 rounds of amplification, the reaction products are size fractionated on a 6% TBE-urea gel, dried and exposed to X-ray film. The resulting mRNA display patterns are compared between treatment groups.

Use of F-V in Conjunction with Estrogen

Peri- and post-menopausal women often undergo hormone replacement therapy (HRT) to combat negative consequences associated with the drop in circulating endogenous estrogen, e.g., to treat hot flashes. However, HRT has been associated with increased risks of certain cancers including

uterine and breast cancer. F-V may be employed in conjunction with HRT to inhibit these risks.

Use of F-V in Conjunction With an Aromatase Inhibitor

5 By definition, the ovaries of a postmenopausal woman are not functioning. Her only source of estrogen is through conversion of adrenal androgens to estrogens by the enzyme aromatase, which is found in peripheral tissues (including fat, muscle and the breast tumor itself). Thus, drugs that
10 inhibit aromatase (aromatase inhibitors) deplete the postmenopausal woman of circulating estrogen. Estrogen deprivation by means of aromatase inhibition is an important treatment option for patients with metastatic breast cancer. During therapy with an aromatase inhibitor, lack of
15 circulating estrogen may cause negative, unintended side-effects, for example on serum lipid levels. F-V may be employed to inhibit these negative effects.

Use of F-V in Conjunction with a LHRH Analogue

20 Continuous exposure to a LHRH (lutenizing hormone releasing hormone) analogue inhibits estrogen production in the premenopausal women by desensitizing the pituitary gland, which then no longer stimulates the ovaries to produce estrogen. The clinical effect is a "medical
25 oophrectomy" which is reversible upon cessation of the LHRH analogue. During therapy with a LHRH analogue, lack of circulating estrogen may cause negative, unintended side-effects, for example on serum lipid levels. F-V may be employed to inhibit these negative effects.

Increasing Levels of Acetyl Choline

30 It is known that patients suffering from Alzheimer's disease have a markedly smaller level of cholinergic neurons in the hippocampus than their non-Alzheimer peers. The

progressive loss of these cholinergic neurons appears to mirror the progressive loss in memory and cognitive function in these patients. It is thought that one reason for the decline of these neurons is the loss or decreased function of the neurotransmitter, acetyl choline.

The level of acetylcholine in a neuron is basically determined by where the equilibrium between its bio-synthesis and bio-degradation lies. The enzyme choline acetyltransferase (ChAT) is primarily responsible for its synthesis and acetylcholineesterase (AChE) for its degradation.

In the order to determine F-V's effect on levels of ChAT, the following experiment is performed: Following the general rat preparation procedure described above, 40 rats are dosed daily by subcutaneous injection or oral gavage with F-V at 3 mg/kg/day in a vehicle containing 10% cyclodextrin, estradiol benzoate at 0.03 or 0.3 mg/kg/day, or vehicle control. Animals are treated for 3 or 10 days. There are twenty animals per each dosing regimen. At the appropriate time intervals, the animals are sacrificed and their brains dissected. The particular portions of the brains are homogenized and assayed. Homogenates from the hippocampus and frontal cortex were processed and determination of ChAT activity is made by a radio-labelled assay of the bio-synthesis of acetyl choline. This procedure may be found in Schoepp et al., *J. Neural Transmiss.*, 78:183-193, 1989, the teachings of which are incorporated by reference.

As expected, in the OVX animals, ChAT levels are reduced >50% ($p < 0.001$) compared to the sham operated controls.

In another embodiment of the present invention, F-V is used in combination with an AChE inhibitor. Use of an AChE

inhibitor increases levels of acetylcholine by blocking its degradation via inhibition of AChE.

Benign Prostatic Hyperplasia (BPH)

5 For background on the link between estrogen action and treatment of BPH and prostate carcinoma, see PCT Application No. WO 98/07274, International Publication Date: October 15, 1998.

10 In the experiments described below, the ability of F-V to bind at estrogen receptors in several human prostatic cancer cell lines is evaluated.

15 Lysates of the LNCaP, DU-45 and PC-3 human prostatic cancer cell lines are prepared in a TEG medium comprising 50 mM Tris·HCl pH 7.4, 1.5 mM ethylenediamine tetraacetic acid (EDTA) 0.4 M KCl, 10% glycerol, 0.5 mM 2-ME, and 10 mM sodium molybdate further containing the protease inhibitors pepstatin (1 mg/mL), leupeptin (2 mg/mL), aprotinin (5 mg/mL) and phenylmethylsulfonyl fluoride (PMSF, 0.1 mM) (TEGP).

20 The cell lysates are centrifuged and the pellets resuspended in cold TEGP (1 mL TEGP/100 mg of pellet) and sonicated for 30 seconds (duty cycle 70%, output 1.8) on a Branson Model 450 Sonifier. Lysates are pelleted by centrifugation at 10,000 x G for 15 minutes at 4°C after
25 which the supernates are withdrawn and either used immediately or stored at -70°C.

30 Competitive Binding Assay: The binding buffer is TEG in which the 0.4 M KCl is replaced by 50 mM NaCl and to which 1 mg/mL of ovalbumin had been further added (TEGO). F-V is diluted to 20 nM in TEGO from which 3-fold serial dilutions are prepared. Assays are performed in round-bottom polypropylene microplates in triplicate microwells. Each well receives 35 µL of tritiated 17β-estradiol (0.5 nM, specific activity 60.1 Ci/mmol, DuPont-New England Nuclear,

Boston, MA) and 35 mL of cold competitor test compound (0.1 nM - 5 nM) or TEGO, and following incubation for 5 minutes at 4°C with shaking, 70 mL of MCF-7 cell line lysate.

Plates are incubated for 24 hours at 4°C after which time 70 mL of dextran-coated charcoal (DCC) is added to each well followed by vigorous shaking for 8 minutes at 4°C. The plates are then centrifuged at 1500 x G for 10 minutes at 4°C. Supernate is harvested from each well into a flexible polystyrene microplate for scintillation counting in a Wallac Micobeta Model 1450 counter. Radioactivity is expressed as disintegrations per minute (DPM) after correcting for counting efficiency (35-40%) and background. Additional controls are total counts and total counts + DCC to defined the lower limit of DCC extractable counts. The results of these competitive binding assays are expressed as mean percent bound (% Bound) +/- standard deviation using the formula:

$$\% \text{ Bound} = \frac{\text{DPM}_{\text{test compound}} - \text{DPM}_{\text{total count + DCC}}}{\text{DPM}_{\text{no test compound}} - \text{DPM}_{\text{total count + DCC}}} \times 100$$

Prevention of Breast Cancer

This invention also relates to the administration of F-V to a recipient who is at risk of developing *de novo* breast cancer. The term "*de novo*", as used herein, means the lack of transformation or metamorphosis of normal breast cells to cancerous or malignant cells in the first instance. Such a transformation may occur in stages in the same or daughter cells via an evolutionary process or may occur in a single, pivotal event. This *de novo* process is in contrast to the metastasis, colonization, or spreading of already transformed or malignant cells from the primary tumor site to new locations.

A person who is at no particular risk of developing breast cancer is one who may develop de novo breast cancer, has no evidence or suspicion of the potential of the disease above normal risk, and who has never had a diagnosis of having the disease. The greatest risk factor contributing to the development of breast carcinoma is a personal history of suffering from the disease, or an earlier occurrence of the disease, even if it is in remission with no evidence of its presence. Another risk factor is family history of the disease.

Induction of mammary tumors in rats by administration of the carcinogen N-nitroso-N-methylurea is a well-accepted animal model for the study of breast cancer and has been found suitable for analyzing the effect of chemopreventive agents.

In two separate studies, 55-day old female Sprague-Dawley rats are given an intravenous (Study 1) or intraperitoneal (Study 2) dose of 50 mg of N-nitroso-N-methylurea per kilogram of body weight one week prior to feeding *ad libitum* a diet into which varying amounts of F-V, (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine base (tamoxifen base), or control are blended.

In Study 1, the dietary doses of 60 mg/kg of diet and 20 mg/kg of diet translates into roughly comparable doses of 3 and 1 mg/kg of body weight for the test animals.

In Study 2, the dietary doses of 20, 6, 2, and 0.6 mg/kg of diet translates roughly into comparable doses of 1, 0.3, 0.1 and 0.03 mg/kg of body weight for the test animals.

Rats are observed for evidence of toxicity and are weighed and palpated for tumor formation once a week. The animals are sacrificed after thirteen weeks (Study 1) or eighteen weeks (Study 2) and tumors are confirmed and weighed at autopsy.

Formulations

The term "pharmaceutical" when used herein as an adjective means substantially non-deleterious to the recipient mammal. By "pharmaceutical formulation" it is meant the carrier, diluent, excipients and active ingredient(s) must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

F-V is preferably formulated prior to administration. The selection of the formulation should be decided by the attending physician taking into considerations the same factors involved with determining the effective amount.

The total active ingredients in such formulations comprises from 0.1% to 99.9% by weight of the formulation. Preferably, no more than two active ingredients are contained in said formulation. That is, it is preferred to formulate F-V with a second active ingredient selected from an estrogen, progestin, aromatase inhibitor, LHRH analogue and AChE inhibitor. Most preferred formulations are those where F-V is the sole active ingredient.

Pharmaceutical formulations of the present invention are prepared by procedures known in the art using well known and readily available ingredients. For example, F-V, either alone, or in combination with an estrogen, progestin, aromatase inhibitor, LHRH analogue, or an AChE inhibitor compound, are formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, solutions, injectables, aerosols, powders, and the like.

Pharmaceutical compositions of this invention for parenteral administration comprise sterile aqueous or non-aqueous solutions, dispersions, suspensions, or emulsions, as well as sterile powders which are reconstituted immediately prior to use into sterile solutions or

suspensions. Examples of suitable sterile aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, physiological saline solution, ethanol, polyols (such as glycerol, propylene glycol, poly(ethylene glycol), and the like), and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity is maintained, for example, by the use of coating materials such as lecithin, by the maintenance of proper particle size in the case of dispersions and suspensions, and by the use of surfactants.

Parenteral compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms is ensured by the inclusion of antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of injectable formulations may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug following subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline material of low water solubility or by dissolving or suspending the drug in an oil vehicle. In the case of the subcutaneous or intramuscular injection of a suspension containing a form of the drug with low water solubility, the rate of absorption of the drug depends upon its rate of dissolution.

Injectable "depot" formulations of F-V are made by forming microencapsulated matrices of the drug in biodegradable polymers such as poly(lactic acid),

poly(glycolic acid), copolymers of lactic and glycolic acid, poly (orthoesters), and poly (anhydrides) these materials which are described in the art. Depending upon the ratio of drug to polymer and the characteristics of the particular polymer employed, the rate of drug release can be controlled.

Injectable formulations are sterilized, for example, by filtration through bacterial-retaining filters, or by presterilization of the components of the mixture prior to their admixture, either at the time of manufacture or just prior to administration (as in the example of a dual chamber syringe package).

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, F-V is mixed with at least one inert, pharmaceutical carrier such as sodium citrate, or dicalcium phosphate, and/or (a) fillers or extenders such as starches, sugars including lactose and glucose, mannitol, and silicic acid, (b) binding agents such as carboxymethyl-cellulose and other cellulose derivatives, alginates, gelatin, poly(vinylpyrrolidone), sucrose and acacia, (c) humectants such as glycerol, (d) disintegrating agents such as agar-agar, calcium carbonate, sodium bicarbonate, potato or tapioca starch, alginic acid, silicates and sodium carbonate, (e) moisturizing agents such as glycerol; (f) solution retarding agents such as paraffin, (g) absorption accelerating agents such as quaternary ammonium compounds, (h) wetting agents such as cetyl alcohol and glycerin monostearate, (i) absorbents such as kaolin and bentonite clay, and (j) lubricants such as talc, calcium stearate, magnesium stearate, solid poly(ethylene glycols), sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also contain buffering agents.

Solid compositions of a similar type may also comprise the fill in soft or hard gelatin capsules using excipients such as lactose as well as high molecular weight poly(ethylene glycols) and the like.

5 Solid dosage forms such as tablets, dragees, capsules, pills and granules can also be prepared with coatings or shells such as enteric coatings or other coatings well known in the pharmaceutical formulating art. The coatings may contain opacifying agents or agents which release the active
10 ingredient(s) in a particular part of the digestive tract, as for example, acid soluble coatings for release of the active ingredient(s) in the stomach, or base soluble coatings for release of the active ingredient(s) in the intestinal tract.

15 The active ingredient(s) may also be microencapsulated in a sustained-release coating, with the microcapsules being made part of a pill or capsule formulation.

Liquid dosage forms for oral administration of F-V include solution, emulsions, suspensions, syrups and
20 elixirs. In addition to the active components, liquid formulations may include inert diluents commonly used in the art such as water or other pharmaceutical solvents, solubilizing agents and emulsifiers such as ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol,
25 benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, ground nut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, poly(ethylene glycols), fatty acid esters of sorbitol, and mixtures thereof.

30 Besides inert diluents, the liquid oral formulations may also include adjuvants such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Liquid suspension, in addition to the active ingredient(s) may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite clay, agar-agar, and tragacanth, and mixtures thereof.

Compositions for rectal or intravaginal administration are prepared by mixing F-V with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or any suppository wax which is a solid at room temperature, but liquid at body temperature and therefore melt in the rectum or vaginal cavity to release the active component(s). The compounds are dissolved in the melted wax, formed into the desired shape, and allowed to harden into the finished suppository formulation.

F-V may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposome formulations are formed by mono- or multilamellar hydrated liquid crystals which are dispersed in an aqueous medium. Any non-toxic, pharmaceutical, and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to F-V, stabilizers, excipients, preservatives, and the like. The preferred lipids are phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic.

Methods for forming liposomes are known in the art as described, for example, in Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N. Y. (1976), p. 33 et seq.

The following formulation examples are illustrative only and are not intended to limit the scope of the present invention.

Formulation 1: Tablets containing approximately 10 and 50 mgs, respectively, of F-V may be prepared as follows:

Ingredient	Quantity (mg/tablet)	Quantity (mg/tablet)
F-V	11.3	56.5
Lactose Anhydrous	176.8	128.2
Lactose Spray Dried Special	44.2	32.0
Povidone	11.0	13.0
Polysorbate 80	2.5	2.6
Crosspovidone (Inside)	6.25	6.24
Crosspovidone (Outside)	6.25	6.5
Magnesium Stearate	1.5	1.7
Microcrystalline Cellulose (Outside)	0.0	13.0

5 The components are blended and compressed to form tablets.

Alternatively, tablets each containing 2.5 - 1000 mg of F-V are made up as follows:

Formulation 2: Tablets

Ingredient	Quantity (mg/tablet)
F-V	25 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

F-V, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of

polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of medicament per 5 ml dose are made as follows:

Formulation 3: Suspensions

Ingredient	Quantity (mg/5 ml)
F-V	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 4: Intravenous Solution

Ingredient	Quantity
F-V	25 mg
Isotonic saline	1,000 mL

5 The solution of the above ingredients is intravenously administered to a patient at a rate of about 1 mL per minute.

Formulation 5: Tablets Containing Cysteine Hydrochloride

10 Core tablets weighing approximately 250 mg and containing approximately 10 mg or 20 mg of F-V are prepared generally as follows. The F-V, water soluble diluents (lactose monohydrate and anhydrous lactose), and a portion of the distintegrant (crospovidone) are blended in a high shear granulator. This blend is then wet massed in the high
15 shear granulator with an aqueous solution of povidone and polysorbate 80. The cysteine hydrochloride is also dissolved in the granulation solution and added during the wet mass step via the granulation solution. To maintain a constant tablet fill weight, the amount of lactose (lactose monohydrate and anhydrous lactose) is reduced corresponding
20 to the amount of cysteine hydrochloride added. Following a wet sizing step through a rotating impeller mill, the granules are dried using a fluid bed dryer. The dried granules are reduced to a suitable size with a rotating
25 impeller mill. The remaining ingredients (microcrystalline cellulose, magnesium stearate, and rest of the crospovidone) are added to the dried granules and blended. This mixture is then compressed into round shaped tablets using a conventional rotary tablet press. The unit formulae for
30 each of these lots are summarized in Table 2 which includes the amounts (mg/tablet) and type of excipient utilized in

each case. As seen from the table, the tablet cores for lots C and D included the application of a film coat which is applied via an aqueous dispersion in a side-vented coating pan fitted to a commercial air-handling unit.

Table 2

Tablet Unit Formulae (mg/tablet)

Ingredient	Lot A	Lot B	Lot C	Lot D
F-V (Base Equivalent)	11.31 (10)	11.31 (10)	22.73 (20)	22.73 (20)
L-Cysteine HCl Monohydrate	0.10	0.50	0.50	0.75
Povidone	12.50	12.50	12.50	12.50
Polysorbate 80	1.25	1.25	1.25	1.25
Anhydrous Lactose	148.67	148.35	139.22	139.02
Lactose Monohydrate	37.17	37.09	34.80	34.75
Crospovidone	12.50	12.50	12.50	12.50
Micro- crystalline Cellulose	25.00	25.00	25.00	25.00
Magnesium Stearate	1.50	1.50	1.50	1.50
Color Mixture Yellow	--	--	10.00	10.00

Formulation 6: Tablets Containing Methionine

Core tablets weighing approximately 250 mg and containing approximately 1 mg of F-V are prepared generally

as follows. F-V, water soluble diluents (lactose monohydrate and anhydrous lactose), and a portion of the disintegrant (crospovidone) are blended in a high shear granulator. This blend is then wet massed in the high shear granulator with an aqueous solution of the povidone and polysorbate 80. The methionine is also dissolved in the granulation solution and added during the wet mass step via the granulation solution. To maintain a constant tablet fill weight, the amount of lactose (lactose monohydrate and anhydrous lactose) is reduced corresponding to the amount of stabilizer added. Following a wet sizing step through a rotating impeller mill, the granules are dried using a fluid bed dryer. The dried granules are reduced to a suitable size with a rotating impeller mill. The remaining ingredients (microcrystalline cellulose, magnesium stearate, and the rest of crospovidone) are added to the dried granules and blended. This mixture is then compressed into round shaped tablets using a conventional rotary tablet press. The unit formulae for each of these lots are summarized in Table 3 which includes the amounts (mg/tablet) and type of excipient utilized in each case.

Table 3: Tablet Unit Formulae (mg/tablet)

Ingredient	Lot E	Lot F	Ingredient	Lot E	Lot F
Serm III HCl (Base Equiv.)	1.12 (1.0)	1.12 (1.0)	Lactose Monohydrate	38.88	38.78
Methionine	0.50	1.00	Crospovidone	12.50	12.50
Povidone	12.50	12.50	Micro- crystalline Cellulose	25.00	25.00
Polysorbate 80	2.50	2.50	Magnesium Stearate	1.50	1.50
Anhydrous Lactose	155.5	155.1			

Dosage

The specific dose of F-V administered according to this invention is determined by the particular circumstances surrounding each situation. These circumstances include, the route of administration, the prior medical history of the recipient, the pathological condition or symptom being treated, the severity of the condition/symptom being treated, and the age and sex of the recipient.

Generally, an effective minimum daily dose of F-V is about 1, 5, 10, 15, or 20 mg. Typically, an effective maximum dose is about 800, 100, 60, 50, or 40 mg. Most typically, the dose ranges between 15 mg and 60 mg. The exact dose may be determined, in accordance with the standard practice in the medical arts of "dose titrating" the recipient; that is, initially administering a low dose of the compound, and gradually increasing the dose until the desired therapeutic effect is observed.

Although it may be necessary to dose titrate the recipient with respect to the combination therapies discussed above, typical doses of active ingredients other than F-V are as follows: ethynyl estrogen (0.01 - 0.03 mg/day), mestranol (0.05 - 0.15 mg/day), conjugated estrogenic hormones (e.g., Premarin®, Wyeth-Ayerst; 0.3 - 2.5 mg/day), medroxyprogesterone (2.5 -10 mg/day), norethylnodrel (1.0 - 10.0 mg/day), nonethindrone (0.5 - 2.0 mg/day), aminoglutemide (250-1250 mg/day, preferably 250 mg four times per day), anastrozole (1-5 mg/day, preferably 1 mg once per day), letrozole (2.5-10 mg/day, preferably 2.5 mg once a day), formestane (250-1250 mg per week, preferably 250 mg twice weekly), exemestane (25-100 mg/day, preferably 25 mg once per day), goserlin (3-15 mg/three months, preferably 3.6-7.2 mg once every three months) and

leuprolide (3-15 mg/month, preferably 3.75-7.5 mg once every month).

Route of administration

5 F-V can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. The method of administration of each estrogen- and progestin-based agent is consistent with that which is known in the art. F-V,
10 alone or in combination with estrogen, progestin, or an AChE inhibitor generally will be administered in a convenient formulation.

The pharmaceutical compositions of this invention may be administered to humans and other mammals (e.g., dogs,
15 cats, horses, swine and the like) orally, rectally, intravaginally, parenterally, topically, buccally or sublingually, or nasally. The term "parenteral administration" refers herein to modes of administration which include intravenous, intramuscular, intraperitoneal,
20 instrasternal, subcutaneous, or intraarticular injection or infusion.

Mode/Length of Administration

For the majority of the methods of the present
25 invention, F-V is administered continuously, from 1 to 3 times daily or as often as needed to deliver an effective amount of F-V to the recipient. Cyclical therapy may especially be useful in the treatment of endometriosis or may be used acutely during painful attacks of the disease.
30 In the case of restenosis, therapy may be limited to short (1-6 months) intervals following medical procedures such as angioplasty.

CLAIMS

1. Non-solvated anhydrous crystalline 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride (F-V) having an X-ray diffraction pattern which comprises at least one of the following peaks: 7.3 ± 0.2 , 15.5 ± 0.2 , 15.9 ± 0.2 , and $17.6 \pm 0.2^\circ$ in 2θ when obtained from a copper radiation source ($\lambda = 1.54056 \text{ \AA}$).
2. The crystalline compound according to claim 1 wherein said X-ray diffraction pattern further comprises at least one of the following peaks: 17.9 ± 0.2 , 18.2 ± 0.2 , 18.9 ± 0.2 , and 21.5 ± 0.2 in 2θ .
3. A pharmaceutical formulation comprising the crystalline compound according to claim 1 or claim 2 and one or more pharmaceutical carriers, diluents, or excipients.
4. A process for preparing a compound according to claim 1 which comprises crystallizing 6-hydroxy-3-(4-[2-(piperidin-1-yl)ethoxy]phenoxy)-2-(4-methoxyphenyl)benzo[b]thiophene hydrochloride from a crystallization solvent selected from: methanol or aqueous methanol, ethanol or isopropanol; and subsequently drying the resulting solid to a constant weight.
5. The process according to claim 4 wherein said solvent is aqueous methanol and wherein the ratio of water to methanol (v:v) is between 20% and 5%.
6. The process according to claim 5 where the ratio is 15%.
7. The crystalline compound according to claim 1 or claim 2 for use in the inhibition of a pathological condition selected from: uterine fibrosis, endometriosis, aortal smooth muscle cell proliferation, restenosis, breast cancer, uterine cancer, prostatic cancer, benign prostatic hyperplasia, bone loss, osteoporosis, cardiovascular disease, hyperlipidemia, CNS disorders, and Alzheimer's disease.

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8. The compound according to claim 7 for use in the inhibition of osteoporosis.
9. Use of a crystalline compound according to claim 1 or claim 2 in the manufacture of a medicament for inhibiting a pathological condition selected from: uterine fibrosis, endometriosis, aortal smooth muscle cell proliferation, restenosis, breast cancer, uterine cancer, prostatic cancer, benign prostatic hyperplasia, bone loss, osteoporosis, cardiovascular disease, hyperlipidemia, CNS disorders, and Alzheimer's disease.
10. Use of a compound according to claim 9 in the manufacture of a medicament for inhibiting osteoporosis.