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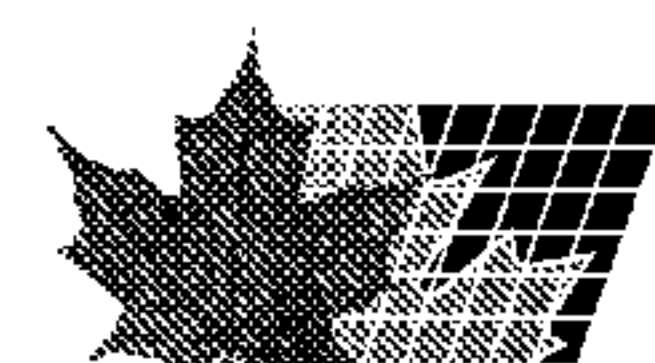
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(54) Titre : COMPOSITION ET METHODE POUR PREVENIR OU TRAITER LE CANCER DU SEIN

(54) Title: COMPOSITION FOR AND METHOD OF PREVENTING OR TREATING BREAST CANCER

(57) **Abrégé/Abstract:**

The present invention relates to compositions and methods for treating and preventing breast cancer. A method for treating breast cancer is provided in which tamoxifen and an isoflavone are coadministered to a woman having breast cancer. The tamoxifen is administered to prevent, minimize, or reverse the development or growth of the breast cancer. The isoflavone is administered to prevent or minimize tamoxifen induced uterotrophic effects, and preferably, endometrial cancer. Most preferably, administration of the isoflavone enhances the prevention, minimization, or reversal of the breast cancer. In another aspect, tamoxifen and an isoflavone are coadministered to woman predisposed to breast cancer to prevent breast cancer. A composition in accordance with the present invention contains tamoxifen in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer and an isoflavone in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects, and preferably in an amount sufficient to prevent or minimize endometrial cancer.



**COMPOSITION FOR AND METHOD OF PREVENTING OR TREATING
BREAST CANCER**

Abstract of the Invention

The present invention relates to compositions and methods for treating and preventing breast cancer. A method for treating breast cancer is provided in which tamoxifen and an isoflavone are coadministered to a woman having breast cancer. The tamoxifen is administered to prevent, minimize, or reverse the development or growth of the breast cancer. The isoflavone is administered to prevent or minimize tamoxifen induced uterotrophic effects, and preferably, endometrial cancer. Most preferably, administration of the isoflavone enhances the prevention, minimization, or reversal of the breast cancer. In another aspect, tamoxifen and an isoflavone are coadministered to woman predisposed to breast cancer to prevent breast cancer. A composition in accordance with the present invention contains tamoxifen in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer and an isoflavone in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects, and preferably in an amount sufficient to prevent or minimize endometrial cancer.

COMPOSITION FOR AND METHOD OF PREVENTING OR TREATING BREAST CANCER

Field of the Invention

The present invention relates to a composition containing tamoxifen and at least one isoflavone, and a method of treating breast cancer while inhibiting tamoxifen induced uterotrophic effects.

Background of the Invention

Breast cancer is one of the leading causes of cancer mortality among Western women, and is predicted to become a leading cause of cancer death in Oriental women in countries such as Japan in the near future. The American Cancer Society estimates that 1 in 9 women face a lifetime risk of this disease, which will prove fatal for about one-quarter of those afflicted with the disease.

Tamoxifen (Fig. 1), a synthetic nonsteroidal selective estrogen receptor modulator, has been used effectively in the treatment of breast cancer for over twenty years. Tamoxifen is one of the most widely prescribed antineoplastic agents in the United States and Great Britain, and is one of the initial hormonal treatments of choice in both premenopausal and postmenopausal women with estrogen receptor positive metastatic disease. Furthermore, adjuvant therapy studies show a substantial reduction of contralateral primary breast carcinoma in tamoxifen treated women, which indicates that tamoxifen may be of use in breast cancer prevention.

Tamoxifen has tissue-specific estrogenic and antiestrogenic effects. Estrogen, an ovarian hormone, increases the risk of breast and endometrial cancer by inducing an estrogen receptor mediated increase in the frequency of breast and endometrial cell division. Cell division is essential in the complex process of genesis of human cancer since it per se increases the risk of genetic error - particularly genetic errors such as inactivation of tumor suppressor genes.

Tamoxifen has antiestrogenic effects in breast tissue. Tamoxifen's antiestrogenic effect in breast tissue is a primary mechanism by which tamoxifen inhibits the

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proliferation of breast cancer cells. Tamoxifen competes with estrogen for binding to cytoplasmic estrogen receptors ("ER"), with subsequent inhibition by the tamoxifen/ER complex of many of the activities of endogenous estrogen within tumor cells. Endogenous estrogen binds with ERs to promote cellular activities such as estrogen/ER-mediated gene transcription, DNA synthesis, cancer cell growth, and increases in autocrine polypeptides such as transforming growth factor-alpha, epidermal growth factor, insulin-like growth factor-II, and other growth factors that may be involved in cell proliferation. Competitive inhibition of estrogen binding to ERs by tamoxifen reduces or prevents such cancer growth inducing cellular activities. As a result of tamoxifen's antiestrogenic activity in breast tissue, tamoxifen prevents the transition of breast cancer cells from the early G1 phase to the mid-G1 phase of the cell cycle and exhibits a cytostatic effect on breast cancer cells. Tamoxifen has been shown to reduce distant breast cancer metastasis as well as local-regional recurrence of such cancers in both node-negative and node-positive women.

Tamoxifen, however, has an estrogenic effect on uterine tissues when endogenous estrogen levels are low, which predominantly occurs in postmenopausal women and oophorectomized women. Uterine epithelial cell heights are significantly increased by the estrogenic effect of tamoxifen in these women, leading to uterine hypertrophy. Tamoxifen also causes marked uterine eosinophilia. These effects have been associated with endometrial carcinoma, and long term use of tamoxifen is linked to an increased risk of endometrial cancer, up to a fivefold excess of risk relative to women not treated with tamoxifen therapy. Therefore, application of tamoxifen for long term breast cancer prevention and long term treatment of breast cancer has significant associated risks. It is desirable to reduce or eliminate these risks.

Summary of the Invention

In one aspect, the present invention is a composition which is a combination of tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their respective naturally occurring glucosides and glucoside conjugates. Preferably, the tamoxifen is present in the composition in an amount

sufficient to prevent, minimize, or reverse the development or growth of breast cancer in a woman having or predisposed to breast cancer. Preferably, the isoflavone is present in the composition in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects and/or to prevent minimize, or reverse the development or growth of breast cancer in a woman having or predisposed to breast cancer. In a preferred embodiment, the isoflavone is present in the composition in an amount sufficient to prevent or minimize the risk of development of endometrial cancer.

In a another aspect, the present invention is a method of treating breast cancer in a woman. Tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates are co-administered to a woman having breast cancer. The tamoxifen is administered to prevent, minimize, or reverse the development or growth of breast cancer, and the isoflavone is administered to prevent or minimize tamoxifen induced uterotrophic effects and/or to prevent, minimize, or reverse the development or growth of breast cancer. In a preferred embodiment, the isoflavone is also administered to prevent or minimize the risk of development of endometrial cancer.

In yet another aspect, the present invention is a method of preventing breast cancer in a woman predisposed to breast cancer. Tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their respective naturally occurring glucosides and glucoside conjugates are co-administered to a woman predisposed to breast cancer. Tamoxifen is administered to prevent or minimize the risk of development of breast cancer. The isoflavone is administered to prevent or minimize tamoxifen induced uterotrophic effects. In a preferred embodiment, the isoflavone is also administered to prevent or minimize the risk of development of endometrial cancer.

Brief Description of the Drawings

Fig.1 is a depiction of the molecular structure of tamoxifen

Fig.2 is a depiction of the molecular structures of genistein, daidzein, glycitein, biochanin A, and formononetin.

Fig. 3 is a depiction of the molecular structures of specific naturally occurring glucosides and glucoside conjugates of genistein, daidzein, and glycitein.

Detailed Description of the Invention

As used herein, the term “ER” refers to “estrogen receptor”. The term “breast cancer” means any cancer having its origin in breast cells, and includes metastatic and local forms of breast cancer (node negative and node positive), as well as ER positive and ER negative forms of breast cancer. The term “uterotrophic effect” means the proliferation of uterine epithelial cells, which frequently is a side effect of administration of tamoxifen to women, and which appears to be directly related to development of endometrial cancer. As used herein “Mal” represents “malonyl” and “Ac” represents “acetyl”. The term “minimize”, or a derivative thereof, includes a complete or partial inhibition of a specified biological effect (which is apparent from the context in which the term minimize is used). The term “isoflavone” may mean a single isoflavone or plural isoflavones when the “isoflavone” is defined as at least one of a selected group of isoflavones. “Tamoxifen” means either tamoxifen or a pharmaceutically acceptable salt thereof. “Sequential” or “sequentially” as used herein to describe sequential administration of tamoxifen and an isoflavone means administration of desired amount of tamoxifen and isoflavone individually within a specified periodic period of time, for example daily, and is not intended to be limited to immediate consecutive administration of tamoxifen and the isoflavone.

The present invention resides in the discovery that the combination of tamoxifen with certain isoflavones can be used to treat or prevent breast cancer in a woman having or predisposed to breast cancer, and the isoflavones will prevent or minimize the development of uterotrophic effects and endometrial cancer, as well as enhance the prevention, minimization or reversal of the development or growth of the breast cancer. The isoflavones which are useful in the compositions and methods of present invention are genistein, daidzein, glycitein, biochanin A, formononetin and their naturally occurring glucosides and glucoside conjugates. An isoflavone glucoside refers herein to an isoflavone moiety having a carbohydrate monomer covalently bonded thereto, and an

isoflavone glucoside conjugate refers to an isoflavone glucoside having another molecular moiety, such as an ester, bonded to the carbohydrate portion of the isoflavone glucoside. These compounds are shown in Figs. 2 and 3.

Materials

Tamoxifen (2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine) (Fig. 1), as used in the methods and compositions of the present invention, can be prepared by established procedures. For example, tamoxifen may be prepared by the methods described in Belgian patent number 637,389 and British patent number 1,064,629, both of which may be referred to for further details. Tamoxifen is also commercially available, for example from the Aldrich Chemical Company, Inc., 940 West Saint Paul Avenue, Milwaukee, Wisconsin, 53233.

The isoflavone compounds used in the compositions and methods of the present invention are naturally occurring substances which may be found in plants such as legumes, clover and the root of the kudzu vine (*pueraria* root). Common legume sources of these isoflavone compounds include soy beans, chick peas and various other types of beans and peas. Clover sources of these isoflavone compounds include red clover and subterranean clover. Soy beans are a particularly preferred source of the isoflavone compounds (except biochanin A which is not present in soy).

The isoflavone compounds may be isolated from the plant sources in which they naturally occur, or may be synthetically prepared by processes known in the art. For example, daidzein may be isolated from red clover as disclosed by Wong (*J. Sci. Food Agr.*, Vol. 13, p. 304 (1962)) or may be isolated from the mold *Micromonospora halophytica* as provided by Ganguly and Sarre (*Chem. & Ind. (London)*, p. 201 (1970)), both references of which may be referred to for further details. Daidzein may be synthetically prepared by the methods provided by Baker et al (*J. Chem. Soc.*, p. 274 (1933)), Wesley et al (*Ber.* Vol. 66, p. 695 (1933)), Mahal et al (*J. Chem. Soc.*, p. 1769 (1934)), Baker et al (*J. Chem. Soc.*, p. 1852 (1953)), or Farkas (*Ber.* Vol. 90, p. 2940 (1957)), each reference of which may be referred to for further details. The isoflavone

glucoside daidzin may be synthetically prepared by the method of Farkas et al (*Ber.*, Vol. 92, p. 819 (1959)), which may be referred to for further details. The daidzein isoflavone glucoside conjugates 6'-O-Mal daidzin and 6'-O-Ac daidzin can be prepared by a conventional saponification of daidzin with a malonyl or an acetyl anhydride, respectively.

Genistein may be synthetically prepared by the methods provided by Baker et al (*J. Chem. Soc.*, p. 3115 (1928)); Narasimhachari et al (*J. Sci. Ind. Res.*, Vol. 12, p. 287 (1953)); Yoder et al, (*Proc. Iowa Acad. Sci.*, Vol. 61, p. 271 (1954) and Zemplen et al (*Acta. Chim. Acad. Sci. Hung.*, Vol. 19, p. 277 (1959)), each reference of which may be referred to for further details. The isoflavone glucoside genistin may be synthetically prepared by the method of Zemplen et al (*Ber.*, Vol. 76B, p. 1110 (1943)), which may be referred to for further details. The isoflavone glucoside conjugates of genistein, 6'-O- Mal genistin and 6'-O-Ac genistin, can be prepared by a conventional saponification of genistin with a malonyl or an acetyl anhydride, respectively.

Biochanin A can be synthetically prepared by the method provided by Baker et al (*Nature* 169:706 (1952)), which may be referred to for further details. Biochanin A can also be separated from red clover by the method provided by Pope et al (*Chem. & Ind. (London)* p. 1092 (1953)), which may be referred to for further details. Formononetin can be synthetically prepared by the methods disclosed by Wessely et al (*Ber.* 66:685 (1933) and Kagel et al (*Tetrahedron Letters*, p. 593 (1962)), both references which may be referred to for further details. Formononetin can be isolated from soybean meal by the method of Walz (*Ann.* 489:118 (1931)) or can be isolated from clover species by the method of Bradbury et al (*J. Chem. Soc.* p. 3447 (1951)), both references which may be referred to for further details.

It is preferred to extract the isoflavones useful in the compositions and methods of the present invention from the plant materials in which they naturally occur. A preferred method of isolating the isoflavone compounds is to extract the plant materials with an alcohol, preferably methanol or ethanol, or an aqueous solution, preferably an aqueous alkaline solution, to remove the isoflavones from the plant material. It is preferred to comminute the plant material before extracting the isoflavone compounds to maximize recovery of the isoflavone compounds from the plant material. The isoflavone

compounds can be isolated from the extract by conventional separation procedures such as reverse phase high performance liquid chromatography ("HPLC").

In a preferred embodiment, the isoflavone compounds genistein, genistin, 6'-O-Mal genistin, 6'-O-Ac genistin, daidzein, daidzin, 6'-O-Mal daidzin, 6'-O-Ac daidzin, glycitein, glycitin, and 6'-O-Mal glycitin are isolated from a soy material, preferably a commercially available soy material. Soy materials from which the isoflavone compounds can be isolated include: soy beans, dehulled soy beans, soy meal, soy flour, soy grits, soy flakes (full fat and defatted), soy cotyledons, soy molasses, soy protein concentrate, soy whey, soy whey protein, and soy protein isolate. In one embodiment, the isoflavones are extracted from soy beans, dehulled soy beans, soy meal, soy flour, soy grits, soy flakes, soy protein concentrate, soy whey protein, or soy protein isolate, preferably soy meal, soy flour, soy grits, or soy flakes, with a low molecular weight organic extractant, preferably an alcohol, ethyl acetate, acetone, or ether, and most preferably aqueous ethyl alcohol or methyl alcohol. Most preferably the extractant has a pH of about the isoelectric point of soy protein (about pH 4 to pH 5) to minimize the amount of soy protein extracted by the extractant.

The extractant containing the isoflavones is separated from the insoluble soy materials to form an isoflavone enriched extract. If desired, an isoflavone enriched material may be recovered by concentrating the extract to remove the solvent, thereby producing a solid isoflavone enriched material.

In a more preferred embodiment the isoflavone compounds are further purified from other soy materials soluble in the extract by contacting the extract with a material which adsorbs the isoflavones in the extract, and eluting the adsorbed isoflavones out of the adsorbent material with a solvent which causes the isoflavones to be differentially eluted from the adsorbent material.

In a preferred embodiment, the isoflavones are separated from impurities in the extract by a conventional reverse phase HPLC separation. After extraction of the isoflavones from the soy material and separation of the extract from the insoluble soy materials, the extract is filtered to remove insoluble materials that could plug an HPLC column. An HPLC column is prepared by packing a conventional commercially

available HPLC column with a particulate adsorbent material which will releasably bind the isoflavones and impurities in the extract in a compound specific manner. The adsorbent material may be any reverse phase HPLC packing material, however, a preferred packing material may be chosen by the criteria of load capacity, separation effectiveness, and cost. One such preferred packing material is Kromasil C18 16 μ m 100Å beads available from Eka Nobel, Nobel Industries, Sweden.

The filtered extract is passed through the packed HPLC column until all the binding sites of the column are fully saturated with isoflavones, which is detected by the appearance of isoflavones in the effluent from the column. The HPLC column may then be eluted with a solvent to effect the separation. In a preferred embodiment, the eluent is a polar solvent such as ethanol, methanol, ethyl acetate, or acetonitrile, and preferably is an aqueous alcohol having an alcohol content of between about 30% and about 90 %, most preferably about 50%, and most preferably the alcohol is ethanol.

The isoflavone compounds and impurities are separately collected from the column effluent. The isoflavone fractions of the eluent may be identified from other eluent fractions in accordance with conventional HPLC and analytical chemistry techniques. In a preferred embodiment the eluent fractions containing the aglucone isoflavones are collected separately since the aglucone isoflavones are believed to be particularly active tyrosine kinase inhibitors and anti-angiogenesis agents which inhibit the development or progression of breast cancer. Of the aglucone isoflavone materials, the fraction of effluent containing daidzein elutes from the column first, followed by a glycitein fraction, followed by the more polar genistein.

The isoflavone fractions of the eluent may be collected from the column, and the volatile content of the solvent (e.g. alcohol) can be removed by evaporation. The isoflavone compounds can be recovered directly if all of the solvent is removed by evaporation, or may be recovered by chilling the remaining solvent (e.g. water) to crystallize the isoflavones and centrifuging or filtering the remaining solvent away from the crystallized isoflavones.

In a particularly preferred embodiment the isoflavone glucosides and isoflavone glucoside conjugates -- 6'-O-Mal genistin, 6'-O-Ac genistin, 6'-O-Mal daidzin, 6'-O-Ac

daidzin, 6'-O-Mal glycitin, genistin, daidzin, and glycitin -- are converted to their respective aglucone isoflavone forms -- genistein, daidzein, and glycitein. The conversion of the isoflavone glucoside conjugates and the isoflavone glucosides to the aglucone isoflavones can be effected in the substrate from which the isoflavones are to be extracted prior to the extraction, or may be effected in the isoflavone enriched extract after separation of the extract from the insoluble materials. The aglucone isoflavone compounds are especially desirable in the compositions and methods of the present invention since, as noted above, they are believed to be particularly active in inhibiting angiogenesis and tyrosine kinase activity.

The isoflavone glucoside conjugates 6''-O-Mal genistin, 6''-O-Ac genistin, 6''-O-Mal daidzin, 6''-O-Ac daidzin, and 6''-O-Mal glycitin can be converted to their respective glucosides genistin, daidzin, and glycitin by forming an aqueous alkaline solution of the substrate containing the isoflavones having a pH of about 6 to about 13, preferably about pH 9 to about pH 11, and treating the aqueous alkaline solution at a temperature of about 2°C to about 121°C, preferably about 25°C to about 75°C, for a period of time sufficient to effect the conversion, preferably about 30 minutes to about 5 hours, more preferably about 30 minutes to about 1.5 hours. The isoflavone glucosides genistin, daidzin, and glycitin can be converted to their respective aglucone forms genistein, daidzein, and glycitein by contacting the isoflavone glucosides with an enzyme capable of cleaving a 1,4- β -glucoside bond -- preferably a commercially available beta-glucosidase enzyme, an alpha- or beta-galactosidase enzyme, a pectinase enzyme, a lactase enzyme, or a gluco-amylase enzyme -- at a pH at which the enzyme is active, typically from about pH 3 to about pH 9, and at a temperature of about 25°C to about 75°C, more preferably about 45°C to about 65°C, for a period of time sufficient to effect the conversion, typically about 1 hour to about 24 hours, preferably about 1 hour to about 3 hours.

The aglucone isoflavones can be separated from the substrate using conventional separation procedures. For example, the aglucone isoflavones may be extracted from the substrate with a low molecular weight alcohol. The aglucone isoflavones may be separated from the extract by conventional recrystallization processes, or by HPLC. In a particularly preferred embodiment, an isoflavone composition isolated from a soy

substrate for formulation into a composition of the present invention or for use in a method of the present invention includes at least 40% genistein, at least 15% daidzein, and at least 1% glycitein. In another particularly preferred embodiment of the invention, an isoflavone composition isolated from a soy substrate for formulation into a composition of the present invention or for use in a method of the present invention contains at least 85% genistein, at least 5% daidzein, and at least 0.5% glycitein. In yet another preferred embodiment, each isoflavone is recovered separately in pure form.

Several of the isoflavone compounds are commercially available, and may be purchased for formulation into compositions provided in the present invention, or used in the methods of the present invention. For example, genistein, daidzein, and glycitein are commercially available and may be purchased, for example, from Indofine Chemical Company Inc., P.O. Box 473, Somerville, New Jersey 08876, and biochanin A is available from Aldrich Chemical Company, Inc., 940 West Saint Paul Avenue, Milwaukee, Wisconsin 53233.

Method

In one aspect, the present invention is a method of treating or preventing breast cancer in a woman having or predisposed to having breast cancer by coadministering to the woman tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates. The tamoxifen prevents, minimizes, or reverses the development or growth of breast cancer. In one embodiment of the invention, the isoflavone prevents or minimizes tamoxifen induced uterotrophic effects and endometrial carcinoma. In another embodiment of the invention, the isoflavone enhances prevention, minimization, or reversal of the development or growth of breast cancer.

A. Method of treating breast cancer with tamoxifen and preventing or minimizing tamoxifen induced uterotrophic effects

One embodiment of the present invention is a method of treating breast cancer in which tamoxifen is administered to a woman having breast cancer, and at least one

isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates is co-administered to the woman to prevent or minimize any tamoxifen-induced uterotrophic effects. Tamoxifen is administered to the woman to prevent, minimize, or reverse the growth or development of breast cancer in the woman. An amount of tamoxifen sufficient to treat breast cancer in the method of the invention is at least 5 mg per day, preferably from 5 mg to 100 mg per day, more preferably from 10 mg to 50 mg per day, and most preferably from 15 mg to 45 mg per day. The tamoxifen may be administered in several doses per day to achieve the daily amount of tamoxifen sufficient to treat the breast cancer, however, it is preferred that the daily required amount of tamoxifen be administered in one or two doses.

The isoflavone is co-administered to the woman with the tamoxifen to prevent or minimize tamoxifen induced uterotrophic effects. The isoflavone can be co-administered concurrently or sequentially with the tamoxifen. Most preferably, tamoxifen and the isoflavone are co-administered concurrently in a composition of the present invention, described below, on a periodic basis, preferably daily. When administered sequentially the isoflavone and tamoxifen are administered as separate components on a periodic basis, preferably daily, although shorter and longer periods may be used. The isoflavone and tamoxifen can each be prepared as described above for sequential administration, or, if commercially available, may be purchased from a commercial vendor.

The amount of isoflavone sufficient to prevent or minimize tamoxifen induced uterotrophic effects when tamoxifen is administered to treat breast cancer depends on the amount of tamoxifen administered and the effectiveness of the selected isoflavone in competitively inhibiting tamoxifen induced uterotrophic effects. The isoflavones are weakly estrogenic substances that have estrogenic or antiestrogenic effects in certain tissues depending on the type of tissue and the concentration of endogenous estrogen or other compounds having estrogenic activity such as tamoxifen or tamoxifen metabolites. The isoflavones used in the present invention have an antiestrogenic effect in uterine tissues when concentrations of tamoxifen or estrogen are relatively high, such as induced by treatment of a woman with tamoxifen. One mechanism by which the isoflavones likely cause an antiestrogenic effect in uterine tissue in the presence of tamoxifen or

tamoxifen metabolites is by binding to uterine cell ERs and competitively inhibiting tamoxifen or tamoxifen metabolites from binding to the ERs. Unlike tamoxifen, the isoflavones do not cause an estrogenic response upon binding to the uterine cell ERs, therefore, the isoflavones prevent, inhibit, or minimize the uterotrophic effects caused by uterine endothelial cell tamoxifen/ER or tamoxifen-metabolite/ER complexes.

When tamoxifen is administered to treat breast cancer and the isoflavone is administered to inhibit tamoxifen induced uterotrophic effects, the isoflavone is administered in a weight/weight ratio of isoflavone:tamoxifen of at least 0.25:, more preferably from about 0.5:1 to about 100:1, and most preferably from about 1:1 to about 20:1. Typical daily doses of the isoflavone sufficient to minimize tamoxifen induced uterotrophic effects will be at least 1 mg per day, preferably from about 1 mg to about 1000 mg per day, more preferably from about 10 mg to about 500 mg per day, and most preferably from about 30 mg to about 300 mg per day. The isoflavone may be administered in several doses per day to achieve the daily amount of isoflavone sufficient to prevent or minimize tamoxifen induced uterotrophic effects, however, it is preferred that the daily required amount of isoflavone be administered in one or two doses.

In a particularly preferred embodiment of the method, co-administration of the isoflavone with tamoxifen in an amount sufficient to prevent or minimize uterotrophic effects is also effective to prevent or minimize the development of endometrial cancer. As noted above, tamoxifen causes an increased risk of development of endometrial cancer as a result of tamoxifen's estrogenic activity in uterine tissue and its uterotrophic effects. Co-administration of the isoflavone together with tamoxifen, therefore, preferably prevents or minimizes the development of endometrial cancer by preventing or minimizing tamoxifen induced uterotrophic effects.

B. Method of preventing or minimizing the risk of developing breast cancer while preventing or minimizing uterotrophic effects

Another embodiment of the present invention is a method of preventing or minimizing the risk of development of breast cancer in which tamoxifen is administered to a woman susceptible to developing breast cancer, and at least one isoflavone selected

from genistein, daidzein, glycitein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates is co-administered to the woman to prevent or minimize any tamoxifen-induced uterotrophic effects. Tamoxifen is administered to the woman in an amount sufficient to prevent or minimize the risk of development of breast cancer. The amount of tamoxifen sufficient to prevent or minimize the risk of development of breast cancer may be less than that required to treat breast cancer, and preferably is administered in a smaller amount than that utilized to treat breast cancer, particularly since preventative administration is likely to span a significant time period. An amount of tamoxifen sufficient to prevent or minimize the risk of development of breast cancer is at least 0.5 mg per day, preferably from about 0.5 mg to about 10 mg per day, more preferably from about 1 mg to about 5 mg per day, and most preferably from about 1.5 mg to about 4.5 mg per day. The tamoxifen may be administered in several doses per day to achieve the daily amount of tamoxifen sufficient to prevent or minimize the risk of development of breast cancer, however, it is preferred that the daily required amount of tamoxifen be administered in one dose.

The isoflavone is co-administered to the woman with the tamoxifen to prevent or minimize the tamoxifen induced uterotrophic effects. The isoflavone can be co-administered concurrently or sequentially with the tamoxifen. When administered sequentially the isoflavone and tamoxifen are administered as separate components on a periodic basis, preferably daily, although shorter and longer periods may be used. The isoflavone and tamoxifen can each be prepared as described above for sequential administration, or, if commercially available may be purchased from a commercial vendor. When administered concurrently, the isoflavone and tamoxifen may be combined into a composition of the present invention, as described below, which is effective to deliver tamoxifen and the isoflavone concurrently.

The amount of isoflavone sufficient to prevent or minimize tamoxifen induced uterotrophic effects when the tamoxifen is administered to prevent the development of breast cancer depends on the amount of tamoxifen administered and the effectiveness of the selected phytoestrogen in competitively inhibiting tamoxifen induced uterotrophic effects. The isoflavone is administered in a weight/weight ratio of isoflavone:tamoxifen

of at least 0.25:1, more preferably from about 0.5:1 to about 100:1, and most preferably from about 1:1 to about 20:1. Typical daily doses of the isoflavone sufficient to minimize tamoxifen induced uterotrophic effects, when the tamoxifen is administered in a dosage of between 0.5 mg per day to 10 mg per day, will be at least 0.125 mg per day, and more preferably from about 0.25 mg per day to about 1000 mg per day.

In a particularly preferred embodiment, co-administration of the isoflavone with tamoxifen to prevent or minimize the risk of development of breast cancer and to prevent or minimize uterotrophic effects is also effective to prevent or minimize the risk of development of endometrial cancer. The isoflavone-induced prevention or minimization of the risk of development of tamoxifen induced endometrial cancer removes the significant risk associated with long term administration of tamoxifen, and permits tamoxifen to be used in a long term breast cancer preventative regimen.

C Method of treating breast cancer with tamoxifen and isoflavones

Yet another embodiment of the present invention is a method of treating breast cancer in which tamoxifen is administered to a woman having breast cancer to prevent, minimize, or reverse the growth of the breast cancer, and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates is co-administered to the woman to enhance the prevention, minimization, or reversal of the growth of the breast cancer. Tamoxifen is administered to the woman to treat the breast cancer in the manner and in the dosages described above.

The isoflavone is administered to the woman in an amount sufficient to enhance the tamoxifen induced prevention, minimization, or reversal of the breast cancer. Preferably, the isoflavone is administered to the woman in an amount effective to prevent, minimize, or reverse the development or growth of breast cancer by itself.

The isoflavones utilized in the method of the invention prevent, minimize, or reverse the growth of breast cancer by several mechanisms. First, the isoflavones are anti-estrogenic in breast tissue, and serve to competitively inhibit estrogen induced cancerous breast cell division by binding to the ER of the cell, where the isoflavone/ER

complex inhibits cancer cell growth in much the same manner as tamoxifen (e.g. daidzein halts cell growth in the G1 phase of the cell cycle, genistein halts cell growth in the G2 phase of the cell cycle). Second, some of the isoflavones, particularly genistein and biochanin A, are tyrosine kinase inhibitors which inhibit enzymatic tyrosine kinase enzyme activity. Tyrosine kinase activity is necessary for cancerous cells to produce proteins required for cellular differentiation and growth. Third, the isoflavones inhibit angiogenesis, and thereby inhibit a cancerous cell mass from developing the network of blood vessels necessary to support the cell mass, limiting the sustainable growth of the cell mass. Fourth, the isoflavones decrease endogenous estrogen levels by interfering with pituitary and hypothalamus gland feedback mechanisms which regulate the release of gonadotropins such as estradiol. The effect of the combined mechanisms of action is to prevent, minimize, or reverse the growth of breast cancer in addition to the tamoxifen activity against breast cancer.

The amount of isoflavone sufficient to enhance the tamoxifen induced prevention, minimization, or reversal of breast cancer growth may be less than that required for prevention or minimization of tamoxifen induced uterotrophic effects since the isoflavone is being utilized in cooperation with tamoxifen in the breast tissue, rather than in opposition to tamoxifen as in the uterine tissues. The isoflavone, however, need not be administered in an amount lesser than that required to prevent or minimize tamoxifen induced uterotrophic effects, and is preferably administered in such amounts to achieve the dual goals of enhancing the prevention, minimization, or reversal of breast cancer and preventing or minimizing tamoxifen induced uterotrophic effects and/or endometrial cancer. Preferably the isoflavone is administered in a weight/weight ratio of isoflavone:tamoxifen of at least 0.1:1, and more preferably from about 0.5:1 to about 100:1, and most preferably from about 1:1 to about 20:1. Typical daily doses of the isoflavone sufficient to enhance tamoxifen's effects against breast cancer will be at least 0.5 mg per day, preferably from about 0.5 mg per day to about 1000 mg per day, and most preferably from about 3 mg per day to about 300 mg per day. The isoflavone may be administered in several doses per day to achieve the daily amount of isoflavones

sufficient to enhance tamoxifen's anti-cancer activity, however, it is preferred that the daily required amount of isoflavones be administered in one or two doses.

The isoflavone can be co-administered concurrently or sequentially with the tamoxifen to treat breast cancer and enhance the prevention, minimization, or reversal of the growth or development of the breast cancer with the isoflavone. When administered sequentially the isoflavone and tamoxifen are administered as separate components on a periodic basis, preferably daily, although shorter and longer periods may be used. The isoflavone and tamoxifen can each be prepared as described above for sequential administration, or, if commercially available may be purchased from a commercial vendor. When administered concurrently, the isoflavone and tamoxifen may be combined into a composition of the present invention, as described below, which is effective to deliver tamoxifen and the isoflavone concurrently.

Composition

The composition of the present invention includes tamoxifen and at least one of the isoflavones selected from genistein, daidzein, glycitein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates. The tamoxifen is present in the composition in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer in a woman. When the composition is to be used to treat breast cancer, at least 5 mg of tamoxifen are present in the composition, more preferably from about 5 mg to about 100 mg of tamoxifen, even more preferably from about 10 mg to about 50 mg of tamoxifen, and most preferably from about 15 mg to about 45 mg of tamoxifen. When the composition is to be used to prevent or minimize the risk of development of breast cancer in a woman predisposed to breast cancer, at least 0.5 mg of tamoxifen are present in the composition, more preferably from about 0.5 mg to about 10 mg of tamoxifen, even more preferably from about 1 mg to about 5 mg of tamoxifen, and most preferably from about 1.5 mg to about 4.5 mg of tamoxifen.

In one embodiment of the composition of the invention, the isoflavone is present in the composition in an amount sufficient to prevent or minimize uterotrophic effects, especially uterotrophic effects induced by tamoxifen. To prevent or minimize

uterotrophic effects of tamoxifen in the composition the isoflavone is present in the composition in a weight:weight ratio of isoflavone:tamoxifen of at least 0.25:1, and more preferably from about 0.5:1 to about 100:1, and most preferably from about 1:1 to about 20:1.

When the composition is to be used to treat breast cancer and to prevent or minimize uterotrophic effects, and the composition contains from about 5 mg to about 100 mg of tamoxifen, the composition contains at least 0.25 mg of the isoflavone, preferably from about 0.25 mg to about 1000 mg of the isoflavone, even more preferably from about 10 mg to about 500 mg of the isoflavone, and most preferably from about 30 mg to about 300 mg of the isoflavone. When the composition is to be used to prevent the development of breast cancer and to prevent or minimize uterotrophic effects, and the composition contains from about 0.5 mg to about 10 mg of tamoxifen, the composition contains at least 0.25 mg of the isoflavone, and more preferably from about 0.25 mg to about 50 mg of the isoflavone. Most preferably the isoflavone is present in the composition in an amount sufficient to prevent or minimize the risk of development of endometrial cancer, which is an amount equivalent to that for preventing or minimizing uterotrophic effects.

In another embodiment of the invention the isoflavone is present in the composition in an amount sufficient to enhance the tamoxifen induced prevention, minimization, or reversal of the growth of breast cancer. To enhance the tamoxifen induced prevention, minimization, or reversal of the growth of breast cancer the isoflavone is present in the composition in a weight:weight ratio of isoflavone:tamoxifen of at least 0.1:1, more preferably from about 0.5:1 to about 100:1, and most preferably from about 1:1 to about 20:1. When tamoxifen is present in the composition from about 5 mg to about 100 mg and the isoflavone is to be used to enhance the prevention, minimization, or reversal of the growth of breast cancer, the composition contains at least 0.5 mg of the isoflavone, more preferably from about 2.5 mg to about 1000 mg of the isoflavone, and most preferably from about 5 mg to about 500 mg of the isoflavone.

The above compositions preferably also include an excipient, most preferably a pharmaceutical excipient. Compositions containing an excipient and incorporating

tamoxifen and the isoflavone can be prepared by procedures known in the art. For example, tamoxifen and the isoflavone can be formulated into tablets, capsules, powders, suspensions, solutions for parenteral administration including intravenous, intramuscular, and subcutaneous administration, and into solutions for application onto patches for transdermal application with common and conventional carriers, binders, diluents, and excipients.

Inert pharmaceutically acceptable carriers useful to form pharmaceutical compositions in accordance with the present invention include starch, mannitol, calcium sulfate, dicalcium phosphate, magnesium stearate, silicic derivatives, and/or sugars such as sucrose, lactose, and glucose. Binding agents include carboxymethyl cellulose and other cellulose derivatives, gelatin, natural and synthetic gums including alginates such as sodium alginate, polyethylene glycol, waxes and the like. Diluents useful in the invention include a suitable oil, saline, sugar solutions such as aqueous dextrose or aqueous glucose, and glycols such as polyethylene or polypropylene glycol. Other excipients include lubricants such as sodium oleate, sodium acetate, sodium stearate, sodium chloride, sodium benzoate, talc, and magnesium stearate, and the like; disintegrating agents including agar, calcium carbonate, sodium bicarbonate, starch, xanthan gum, and the like; and adsorptive carriers such as bentonite and kaolin. Coloring and flavoring agents may also be added to the pharmaceutical compositions.

The following non-limiting formulations illustrate pharmaceutical compositions of the present invention.

FORMULATIONS

The following Formulations 1-4 illustrate pharmaceutical formulations including tamoxifen and an isoflavone.

Formulation 1

Gelatin capsules

Hard gelatin capsules are prepared using the following ingredients: Tamoxifen 0.5-100 mg/capsule; Isoflavone 0.1-1000 mg/capsule; Starch, NF 0 - 600 mg/capsule; Starch flowable powder 0 - 600 mg/ capsule; Silicone fluid 350 centistokes 0 -20 mg/capsule. The ingredients are mixed, passed through a sieve, and filled into capsules.

Formulation 2

Tablets

Tablets are prepared using the following ingredients: Tamoxifen 0.5-100 mg/tablet; Isoflavone 0.1-1000 mg/ tablet; Microcrystalline cellulose 20-300 mg/tablet; Starch 0-50 mg/tablet; Magnesium stearate or stearate acid 0-15 mg/tablet; Silicon dioxide, fumed 0-400 mg/tablet; silicon dioxide, colloidal 0-1 mg/tablet, and lactose 0-100 mg/tablet. The ingredients are blended and compressed to form tablets.

Formulation 3

Suspensions

Suspensions are prepared using the following ingredients: Tamoxifen 0.5-100 mg/5ml; Isoflavone 0.1-1000 mg/5ml; Sodium carboxymethyl cellulose 50-700 mg/5ml; Sodium benzoate 0-10 mg/5ml; Purified water 5 ml; and flavor and color agents as needed.

Formulation 4

Parenteral solutions

A parenteral composition is prepared by stirring 1.5% by weight of active ingredients (tamoxifen and isoflavone wt/wt ratio of from 10:1 to 1:10) in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilized.

The above description is intended to be illustrative of the present invention, and is not intended to be limiting. Other embodiments are within the claims.

What is claimed is:

1. A composition for preventing, minimizing, or reversing the development or growth of breast cancer comprising a combination of tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their respective naturally occurring glucosides and glucoside conjugates.
2. The composition of claim 1 wherein said tamoxifen is present in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer in a woman having breast cancer.
3. The composition of claim 2 wherein from about 5 mg to about 100 mg of tamoxifen are present in said composition.
4. The composition of claim 1 wherein said tamoxifen is present in said composition in an amount sufficient to prevent or minimize the development of breast cancer in a woman predisposed to breast cancer.
5. The composition of claim 4 wherein from about 0.5 mg to about 10 mg of tamoxifen are present in said composition.
6. The composition of claim 1 wherein said isoflavone is in a concentrated form derived from soy or clover.
7. The composition of claim 1 wherein said isoflavone is present in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer in a woman having breast cancer.

8. The composition of claim 7 wherein from about 0.5 mg to about 1000 mg of said isoflavone are present in said composition.
9. The composition of claim 1 wherein said isoflavone is present in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects in a woman having or predisposed to breast cancer.
10. The composition of claim 9 wherein from about 0.25 mg to about 1000 mg of said isoflavone are present in said composition.
11. The composition of claim 9 wherein said isoflavone is present in an amount sufficient to prevent or minimize the risk of endometrial cancer.
12. The composition of claim 1 wherein the weight:weight ratio of said isoflavone to said tamoxifen in said composition is at least 0.1:1.
13. The composition of claim 12 wherein the weight:weight ratio of said isoflavone to said tamoxifen in said composition is from about 0.5:1 to about 100:1.
14. The composition of claim 1 further comprising an excipient.
15. The use of a composition for treating breast cancer in a woman wherein said composition is a combination in administratable forms of tamoxifen and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin and their naturally occurring glucosides and glucoside conjugates.
16. The use according to claim 15 wherein said isoflavone is in a concentrated form derived from soy or clover.
17. The use according to claim 15 wherein the administratable form of said tamoxifen is in an amount sufficient to prevent, minimize, or reverse the development or growth of breast cancer.

18. The use according to claim 17 wherein the administratable form of said tamoxifen provides from about 5 mg to about 100 mg of said tamoxifen per day.

19. The use according to claim 15 wherein the administratable form of said isoflavone is in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects.

20. The use according to claim 19 wherein the administratable form of said isoflavone provides from about 0.25 mg to about 1000 mg of said isoflavone per day.

21. The use according to claim 19 wherein the administratable form of said isoflavone and said tamoxifen is in a weight:weight ratio of isoflavone:tamoxifen of from about 0.5:1 to about 100:1.

22. The use according to claim 19 wherein the administratable form of said isoflavone is in an amount to prevent or minimize the risk of endometrial cancer.

23. The use according to claim 15 wherein the administratable form of said isoflavone is in an amount sufficient to enhance prevention, minimization, or reversal of the development or growth of breast cancer.

24. The use according to claim 23 wherein the administratable form of said isoflavone provides from about 0.5 mg to about 1000 mg of isoflavone per day.

25. The use according to claim 15 wherein said administratable form of tamoxifen and said isoflavone is in a pill or capsule form for convenient use of tamoxifen and the isoflavone.

26. The use of a composition for preventing or minimizing the risk of developing breast cancer in a woman predisposed to breast cancer wherein the composition comprises the combination of tamoxifen and at least one phytoestrogenic isoflavone selected from genistein, daidzein, biochanin A, formononetin and their naturally occurring glucosides and

glucoside conjugates.

27. The use according to claim 26 wherein the administratable form of said tamoxifen is in an amount sufficient to prevent or minimize the risk of development of breast cancer.

28. The use according to claim 27 wherein said administratable form of said tamoxifen provides from about 0.5 mg to about 10 mg of said tamoxifen per day.

29. The use according to claim 26 wherein the administratable form of said isoflavone is in an amount sufficient to prevent or minimize tamoxifen induced uterotrophic effects.

30. The use according to claim 29 wherein the administratable form of said isoflavone and said tamoxifen is in a weight:weight ratio of isoflavone:tamoxifen of from about 0.5:1 to about 100:1.

31. The use according to claim 29 wherein the administratable form of said isoflavone provides from about 0.25 mg to about 50 mg of isoflavone per day.

32. The use according to claim 29 wherein the administratable form of said isoflavone is in an amount to prevent or minimize the risk of endometrial cancer.

33. The use according to claim 26 wherein the administratable form of said isoflavone is concentrated and derived from soy or clover.

34. The use according to claim 26 wherein the administratable form of said tamoxifen and said isoflavone is in a pill or capsule form for convenient use of the tamoxifen and the isoflavone.

FIG. 1

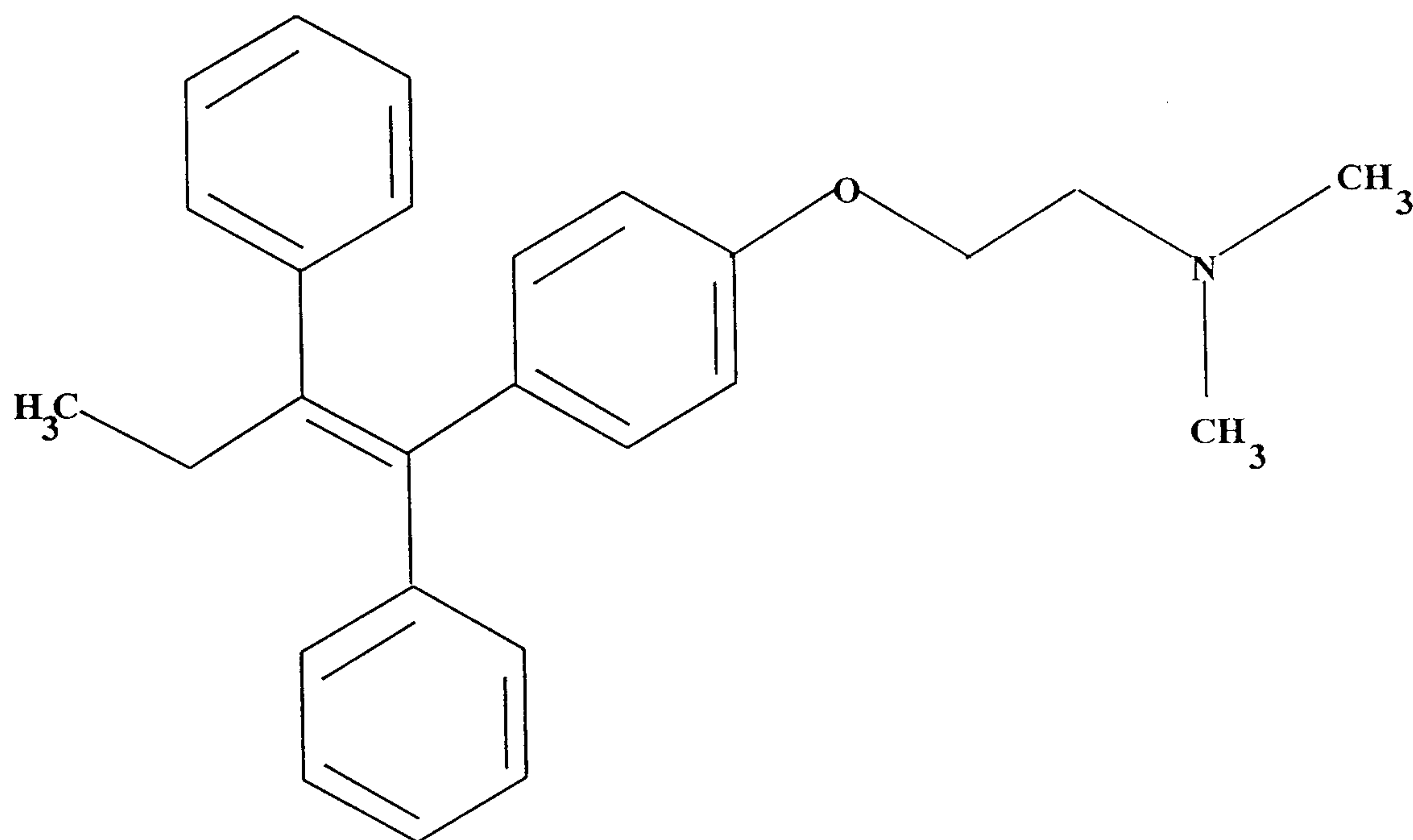
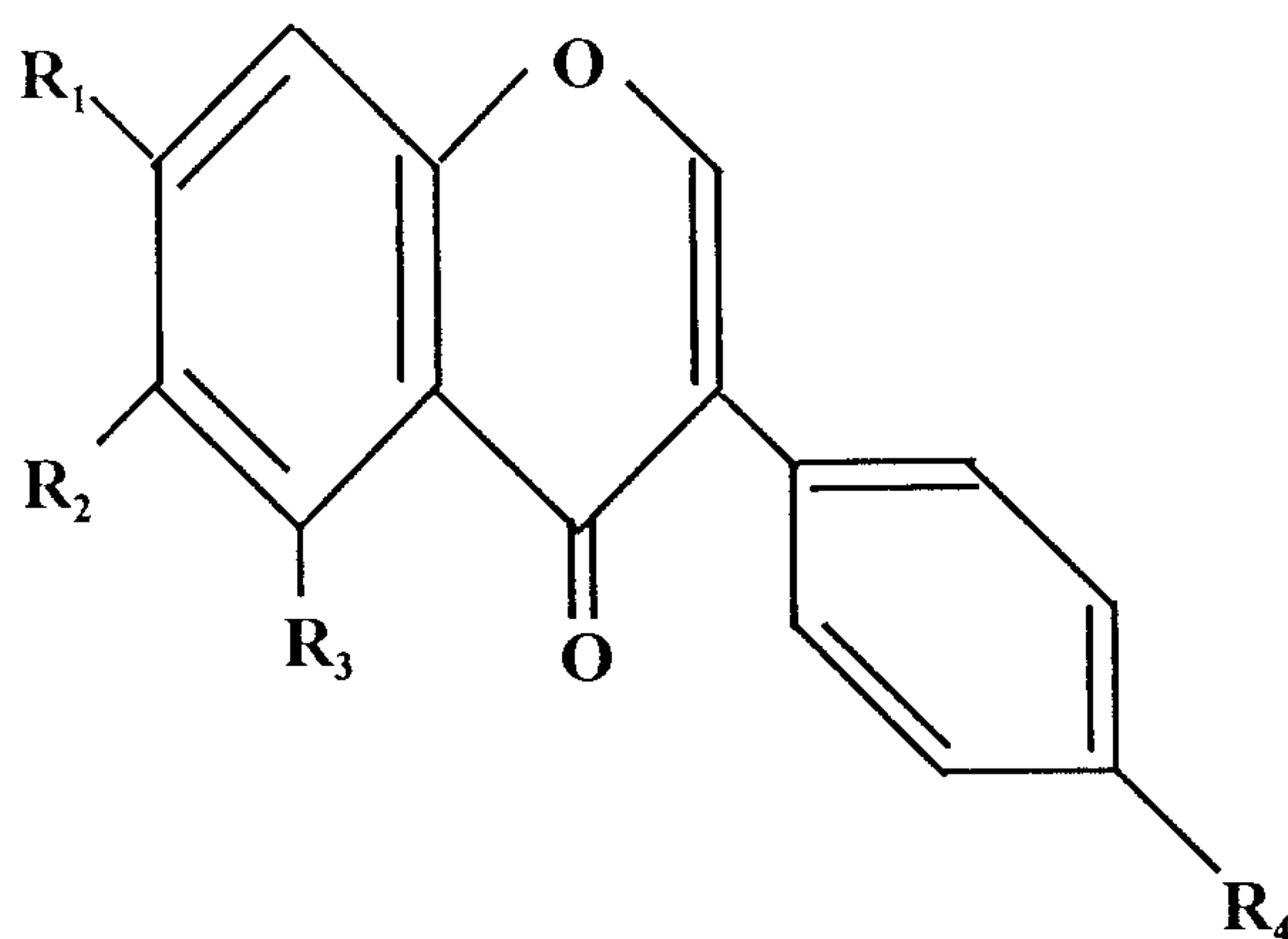
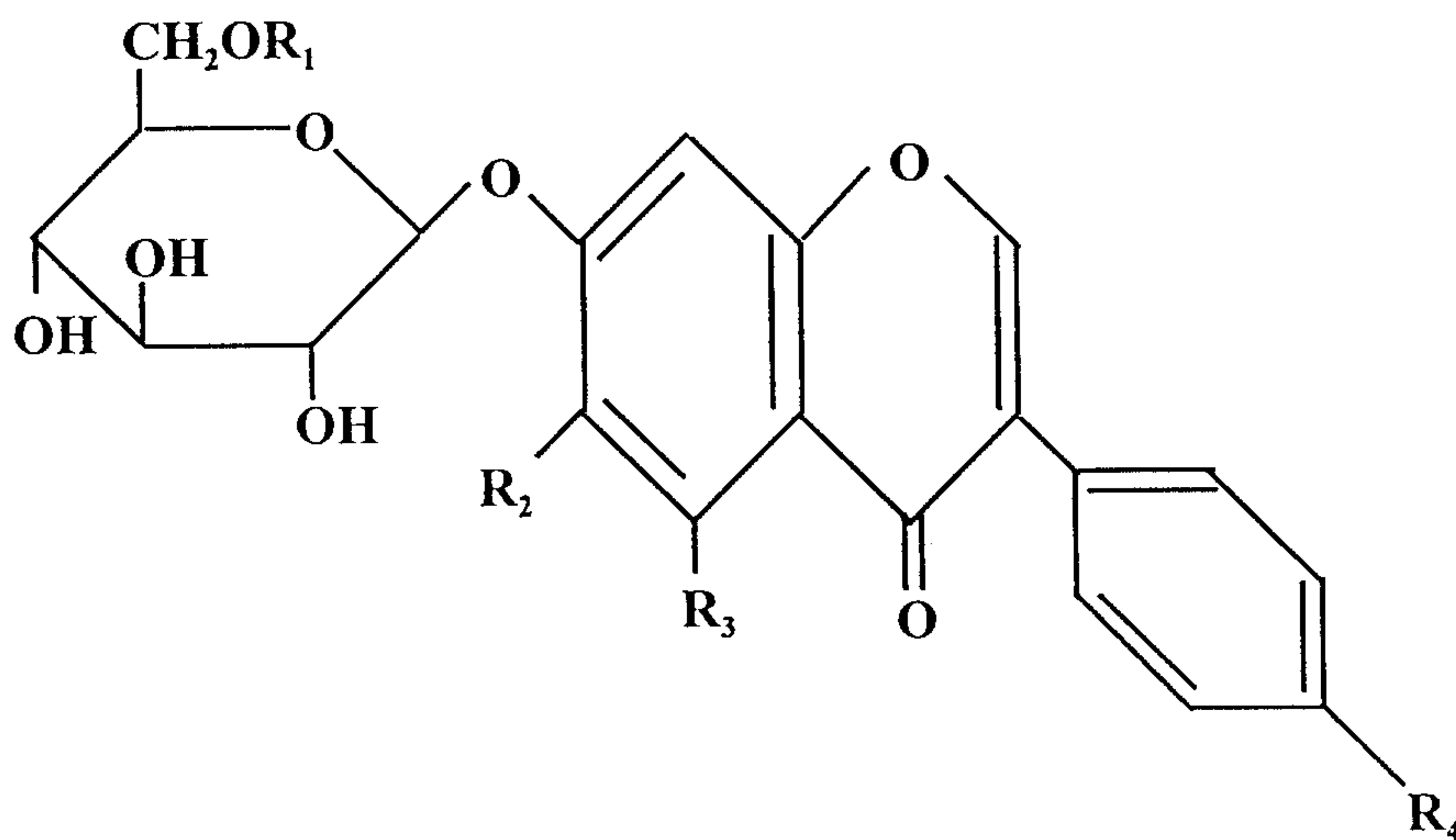


FIG. 2

Compound	R ₁	R ₂	R ₃	R ₄
Genistein	OH	H	OH	OH
Daidzein	OH	H	H	OH
Glycitein	OH	OCH ₃	H	OH
Biochanin A	OH	H	OH	OCH ₃
Formononetin	OH	H	H	OCH ₃

FIG. 3

Compound	R ₁	R ₂	R ₃	R ₄
Genistin	H	H	OH	OH
6'-OMal genistin	COCH ₂ CO ₂ H	H	OH	OH
6'-OAc genistin	COCH ₃	H	OH	OH
Daidzin	H	H	H	OH
6'-OMal daidzin	COCH ₂ CO ₂ H	H	H	OH
6'-OAc daidzin	COCH ₃	H	H	OH
Glycitin	H	OCH ₃	H	OH
6'-OMal glycitin	COCH ₃	OCH ₃	H	OH

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