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(54) Title: COMPOUNDS HAVING ANTIESTROGENIC AND TISSUE SELECTIVE ESTROGENIC PROPERTIES

(57) Abstract: Compounds for the treatment of estrogen-receptor related maladies are provided. In particular, the invention provides compounds that are tetrahydroquinoline phenylamide derivatives and are useful for the treatment of breast and prostate cancer, and osteoporosis.



WO 2005/016889 A1

## COMPOUNDS HAVING ANTIESTROGENIC AND TISSUE SELECTIVE ESTROGENIC PROPERTIES

### DESCRIPTION

#### BACKGROUND OF THE INVENTION

##### *Field of the Invention*

The invention generally relates to compounds that are useful for the treatment of estrogen-receptor related maladies. In particular, the invention provides tetrahydroquinoline phenylamide derivatives that are useful for the treatment of breast and prostate cancer, and osteoporosis.

##### *Background of the Invention*

It is commonly acknowledged that estrogen and the estrogen receptors (ERs) play essential roles in the development of breast tumors, although the precise mechanisms involved have not been determined. Several treatment regimens for breast cancer have been developed that utilize Selective Estrogen Receptors Modulators (SERMs). Tamoxifen, a “first generation” SERM, (Figure 7A) was developed more than 30 years ago and was approved by the Food and Drug Administration in 1985 for the treatment of breast cancers. Tamoxifen antagonizes or mimics the effect of estrogen in a variety of tissues. For example, tamoxifen acts as an antiestrogen in breast tissues and CNS system, and exerts estrogenic

effects in bone, cardiovascular and endometrium tissues. In bone system, it initially was suspected that tamoxifen's antiestrogenic effects might accelerate bone resorption and increase the risk of developing osteoporosis. However, in vitro and in vivo studies have demonstrated that tamoxifen performs as an estrogen in bone, promoting maintenance of bone density, and is thus useful in the treatment of osteoporosis.

Although tamoxifen shows some beneficial estrogenic effects, it also has been proposed to promote uterus and liver carcinogenesis. Some tumors become resistant to treatment with tamoxifen over time. Only a few tamoxifen alternatives have been developed, e.g. Toremifene, GW 5638 and Idoxifene, and second generation SERMs such as Raloxifene, which is currently in clinical trials. Unfortunately, it appears that raloxifene may display cross-resistance to tamoxifen resistant tumors.

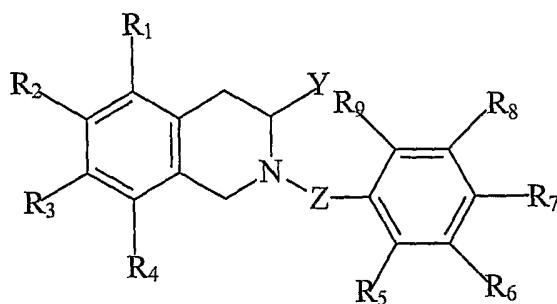
Estrogen receptors also play a role in prostate cancer, and in the development of osteoporosis. Thus, agents that modulate estrogen-receptors may also be useful for the treatment of those diseases.

There is thus an ongoing need to develop new compounds for the treatment of diseases related to estrogen receptor function, in particular for the treatment of breast and prostate cancer, and osteoporosis. This need is particularly acute in light of the tendency of tumors to become resistant to treatment with therapeutic agents after extended use, and in view of the desirability of discovering compounds with reduced deleterious side effects than those exhibited by currently known compounds.

### SUMMARY OF THE INVENTION

The invention provides a series of compounds possessing antiestrogenic and tissue-selective estrogenic properties. The compounds may be used in the treatment of estrogen receptor related diseases, including breast and prostate cancer, and osteoporosis.

It is an object of the present invention to provide a compound of generic formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R1, R2, R3, R4, R5, R6, R7, R8 and R9 are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R10 and -CH<sub>2</sub>-NH-R10; and R10 is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

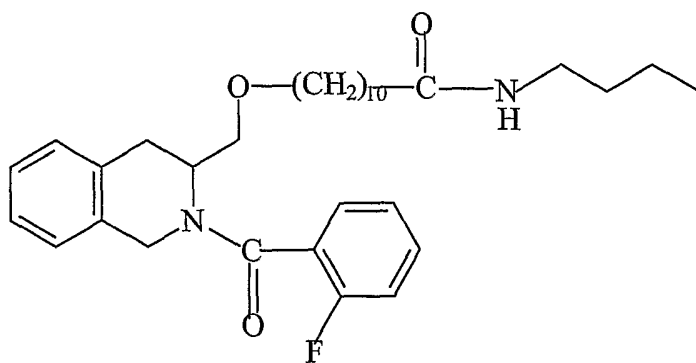
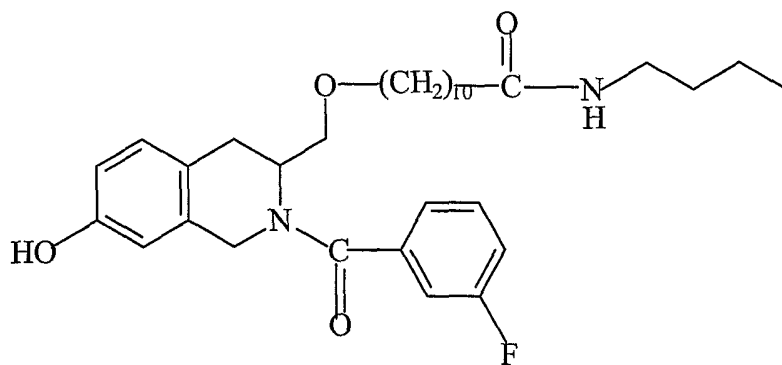
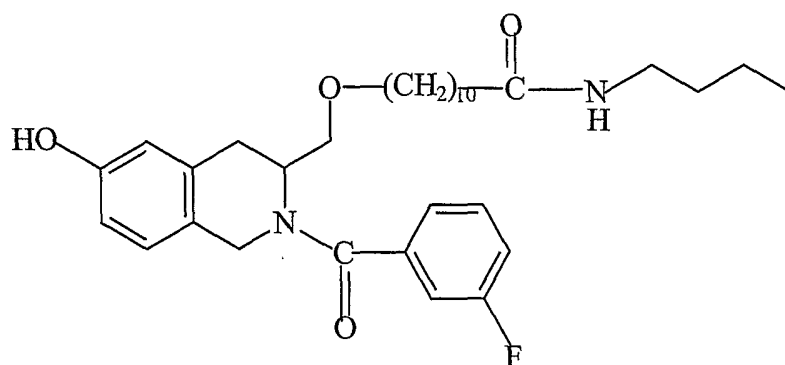
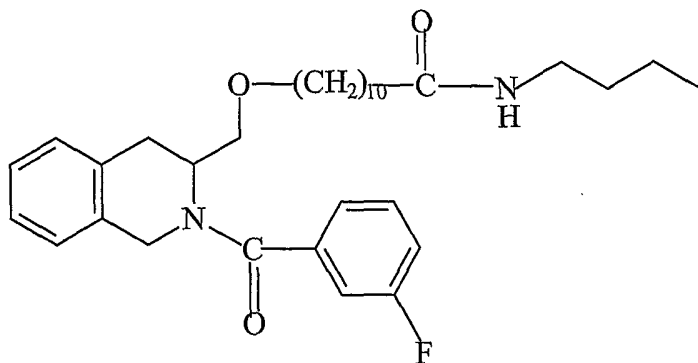
c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

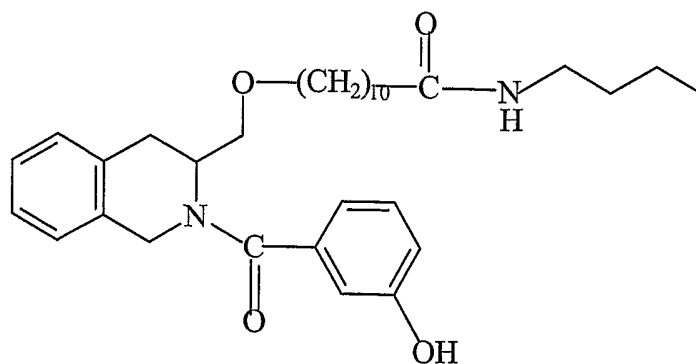
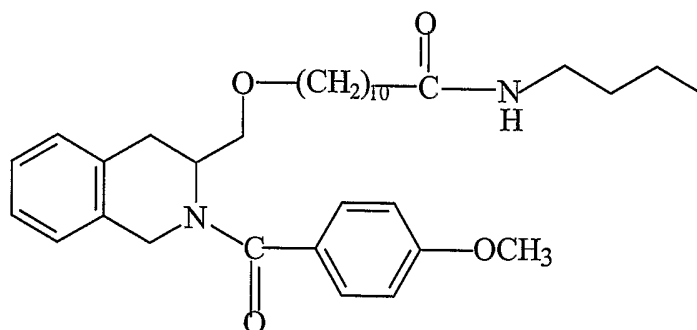
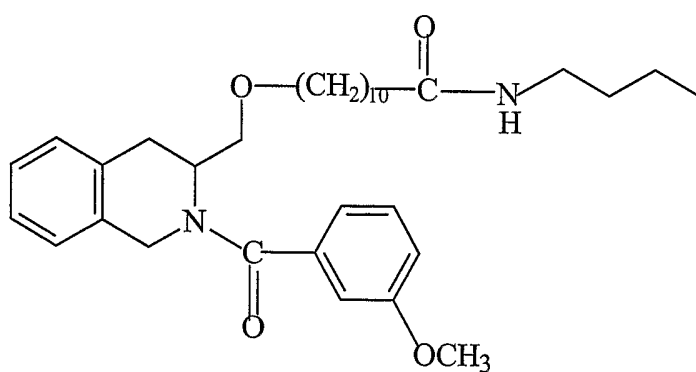
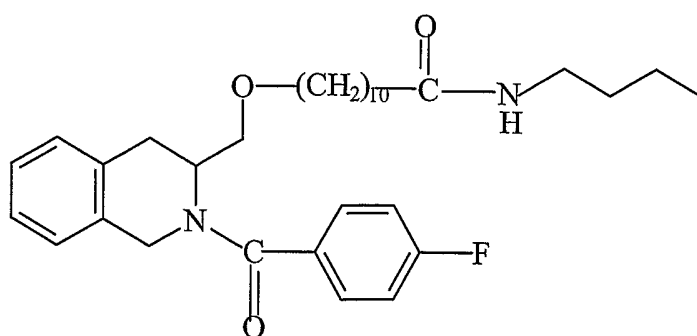
d) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-R11, where n = 1-10 and R11 is selected from: substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

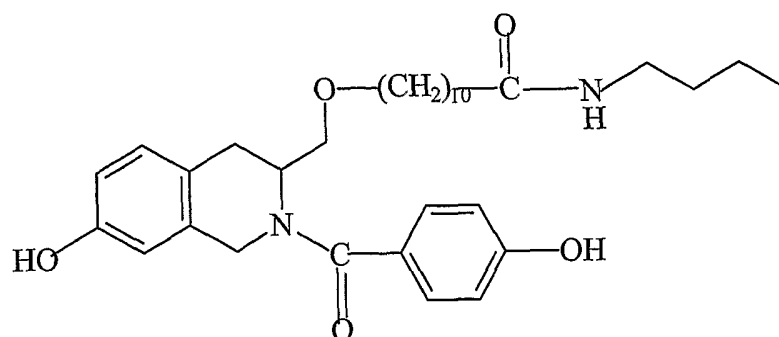
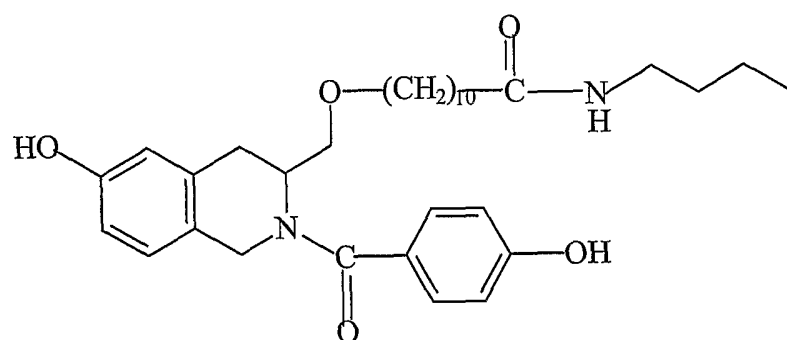
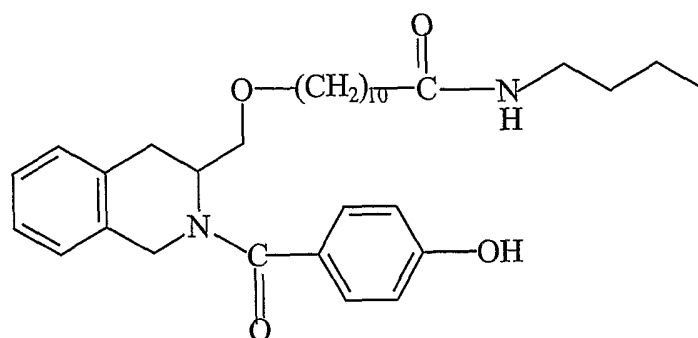
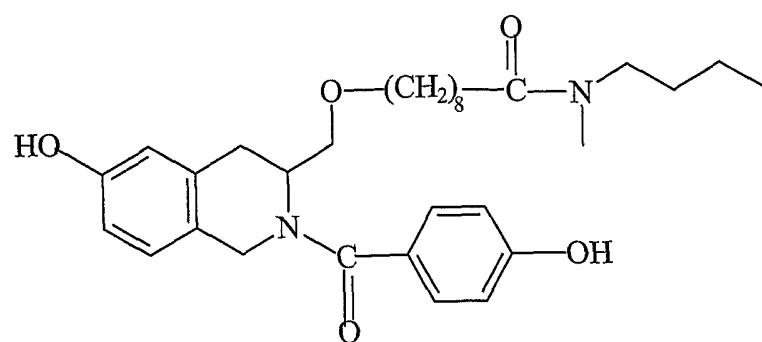
e) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-R11, where n = 1-10 and R11 is selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

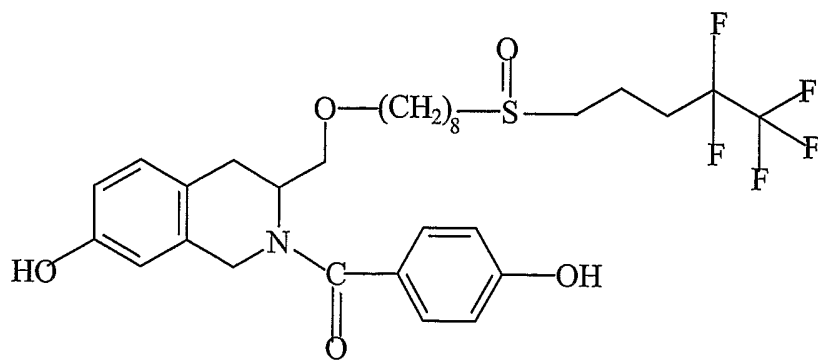
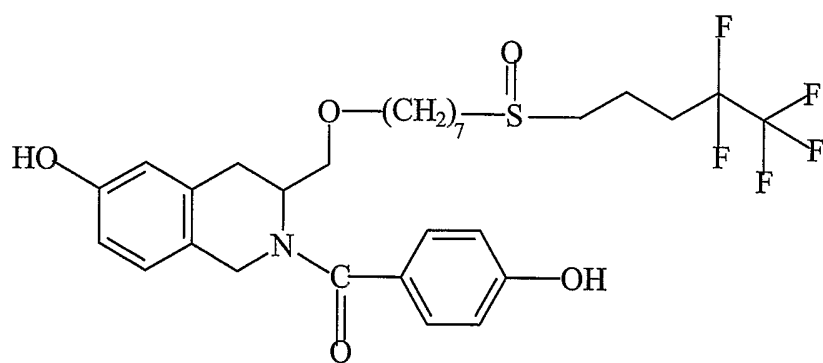
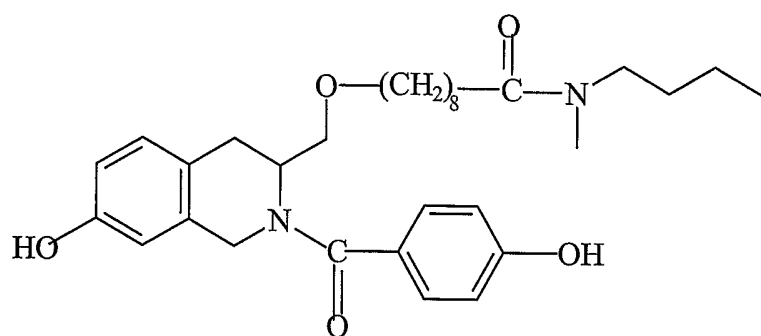
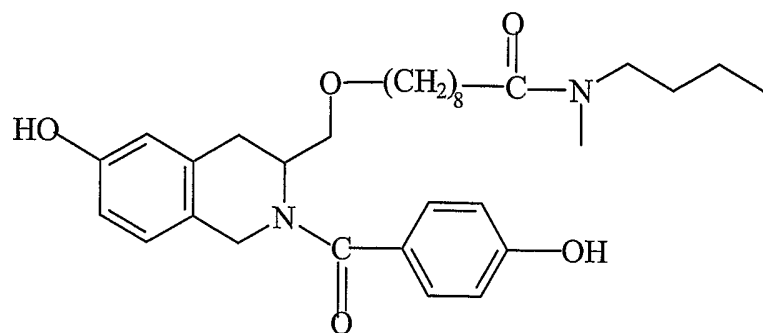
In a preferred embodiment of the invention, the substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl is -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CF<sub>2</sub>CF<sub>3</sub>. In other preferred embodiments, R1, R2, R3, R4, R5, R6, R7, R8 and R9 are F, OCH<sub>3</sub>, OH, CH<sub>3</sub> or Cl.

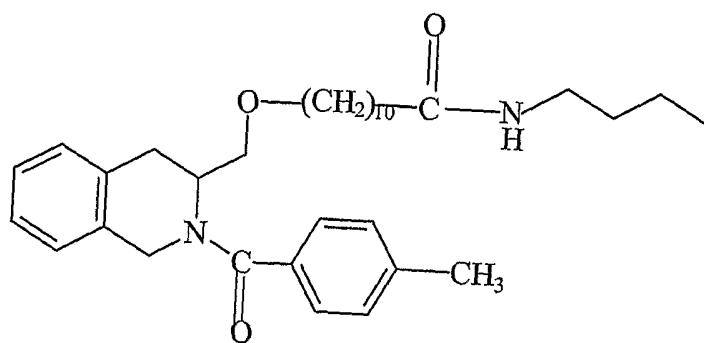
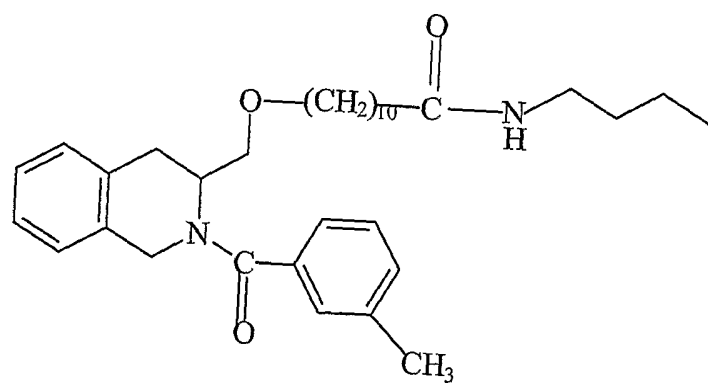
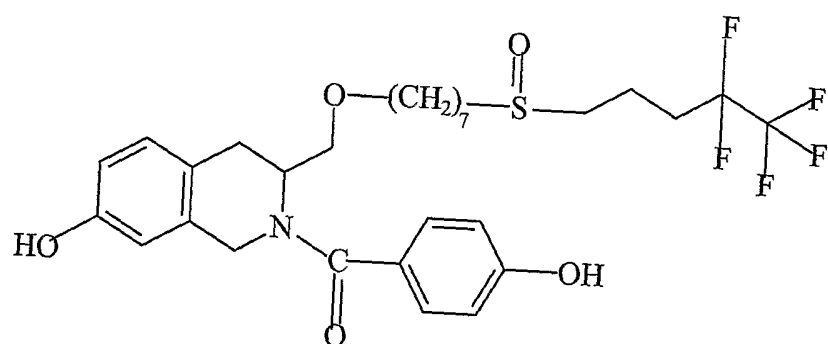
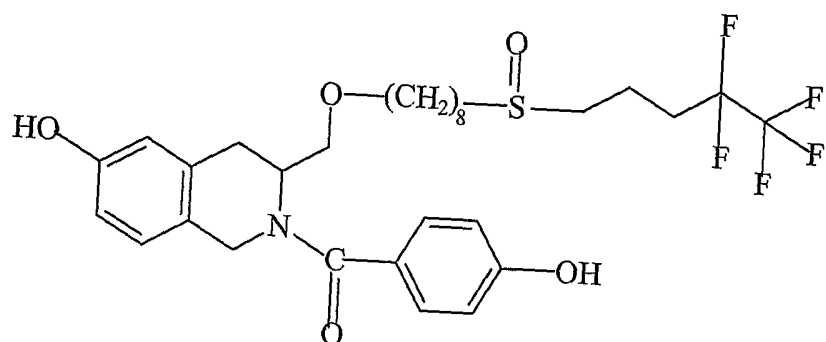
It is a further object of the invention to provide compounds with the following formulas:

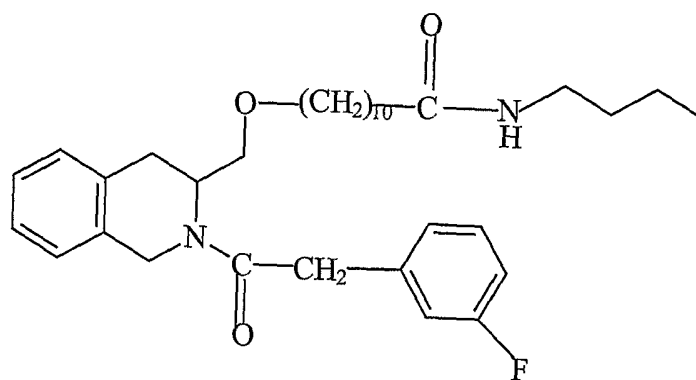
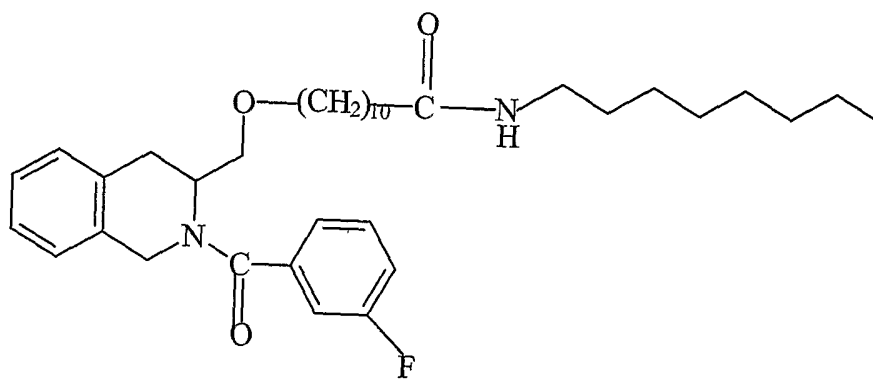
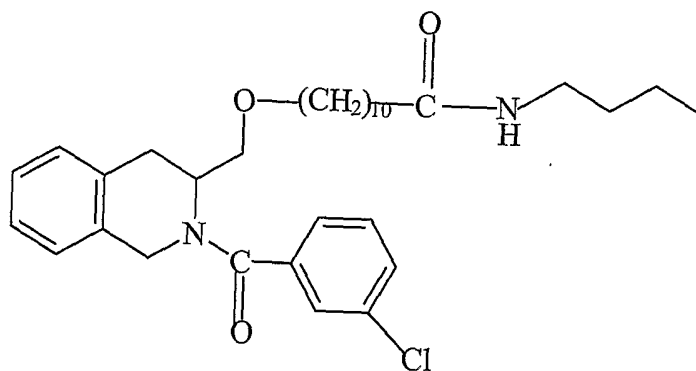




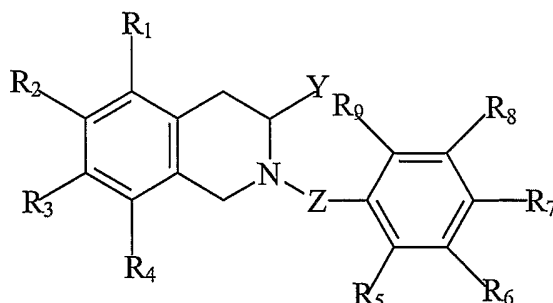








The invention further provides a method of inhibiting binding of estrogen *in vivo* or *in vitro* by providing a compound which binds to an estrogen binding site. The compound has the generic formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R<sub>10</sub> and -CH<sub>2</sub>-NH-R<sub>10</sub>; and R<sub>10</sub> is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

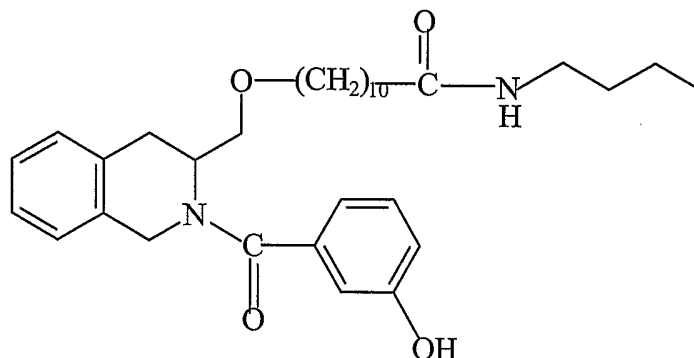
c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

d)  $-(\text{CH}_2)_n\text{-S(=O)-R11}$ , where  $n = 1-10$  and R11 is selected from: substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

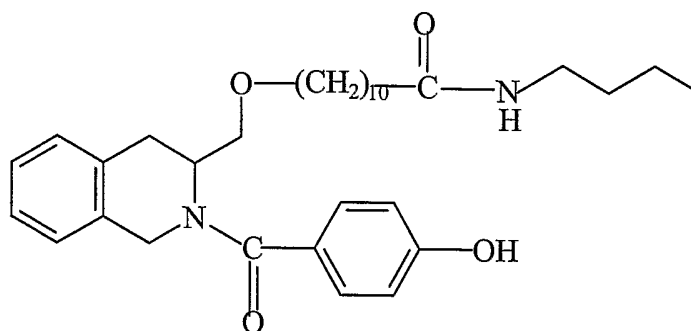
e)  $-(\text{CH}_2)_n\text{-SO}_2\text{-R11}$ , where  $n = 1-10$  and R11 is selected from the group consisting of substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

In a preferred embodiment of the invention, the substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CF}_2\text{CF}_3$ . In other preferred embodiments, R1, R2, R3, R4, R5, R6, R7, R8 and R9 are F,  $\text{OCH}_3$ , OH,  $\text{CH}_3$  or Cl.

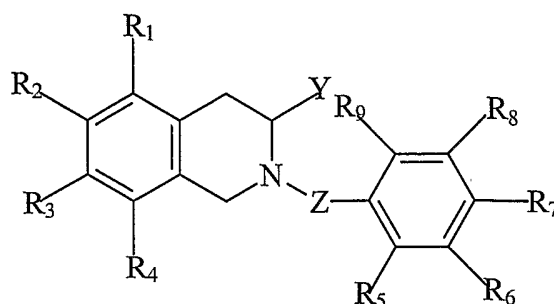
In preferred embodiments of the method, the compound is



or



The invention further provides a method for treating tamoxifen-resistant breast cancer tumors in a patient in need thereof. The method comprises the step of administering to the patient a compound of generic formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R<sub>10</sub> and -CH<sub>2</sub>-NH-R<sub>10</sub>; and

R<sub>10</sub> is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

d)  $-(\text{CH}_2)_n\text{-S(=O)-R11}$ , where  $n = 1-10$  and R11 is selected from: substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

e)  $-(\text{CH}_2)_n\text{-SO}_2\text{-R11}$ , where  $n = 1-10$  and R11 is selected from the group consisting of substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic synthesis scheme.

Figure 2. Schematic synthesis scheme.

Figure 3. Schematic synthesis scheme.

Figure 4. Antiestrogenic activity of compound 1 against various concentrations of estrogen in transient transfection reporter assay for hER $\alpha$  in MCF-7 cells.

Figure 5. Estrogenic activity comparison of compounds 4, 6 and 7 for wild type hER $\alpha$  and hER $\alpha$  D351Y in transient transfection receptor assay.

Figure 6. Proliferation effects of compounds 2, 4 and 6 against MDA-MB-231 cells.

### DETAILED DESCRIPTION OF THE PREFERRED

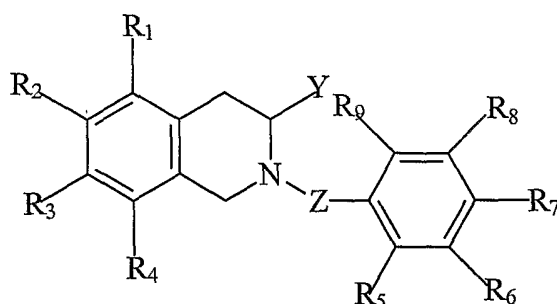
### EMBODIMENTS OF THE INVENTION

A series of compounds possessing antiestrogenic and tissue-selective estrogenic properties have been discovered. The compounds are tetrahydroquinoline phenylamide derivatives and may be used in the treatment of estrogen receptor related diseases, including breast and prostate cancer. In particular, the compounds will be of use in the treatment of advanced breast cancer, especially when tamoxifen (or other treatments modalities) have ceased to be effective. Currently, tamoxifen can be used to treat breast cancer for only about five years due to the development of tamoxifen resistance by the tumor cells. Unfortunately, it has been discovered that the related compound raloxifene may be cross-resistant to tamoxifen-resistant breast tumors, eliminating it as a potential alternative treatment. The compounds of the present invention, which are not cross-resistant to tamoxifen-resistant breast tumors, thus provide another much needed avenue of alternative treatment.

Further, apart from their usefulness as a cancer treatment, the compounds of the present invention will be also useful in the treatment of osteoporosis in a manner similar to tamoxifen.

The compounds of the present invention offer the advantage that they are easy to prepare compared to commercial products based on naturally occurring molecules (e.g. tamoxifen).

The compounds are based on the generic structure depicted in Formula 1:



Formula 1

in which

Z is selected from CO, CH<sub>2</sub>, and (COCH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R1, R2, R3, R4, R5, R6, R7, R8 and R9 are the same or different, and are selected from H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from -CH<sub>2</sub>-O-R10 and -CH<sub>2</sub>-NH-R10;

R10 is selected from:

- 1) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from: substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;
- 2) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from: substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

3)  $-(CH_2)_n-SO_2-N-R_{11}$ ,  $R_{12}$ , where  $n = 1-10$  and  $R_{11}$  and  $R_{12}$  are the same or different, and are selected from: substituted and unsubstituted  $C_1-C_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

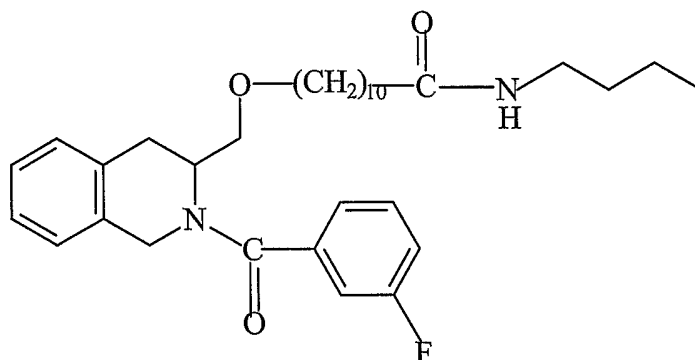
4)  $-(CH_2)_n-S(=O)-R_{11}$ , where  $n = 1-10$  and  $R_{11}$  is selected from: substituted and unsubstituted  $C_1-C_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

5)  $-(CH_2)_n-SO_2-R_{11}$ , where  $n = 1-10$  and  $R_{11}$  is selected from: substituted and unsubstituted  $C_1-C_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

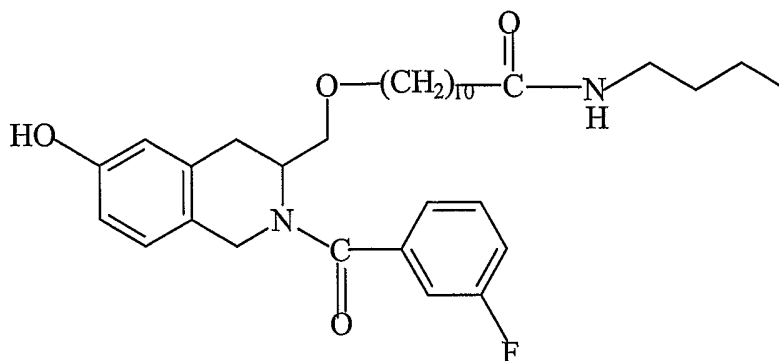
In a preferred embodiment of the invention, the substituted  $C_1-C_9$  alkyl is -  
 $CH_2CH_2CH_2CF_2CF_3$ ,

In other preferred embodiments,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are selected from F,  $OCH_3$ ,  $OOH$ ,  $CH_3$  and Cl.

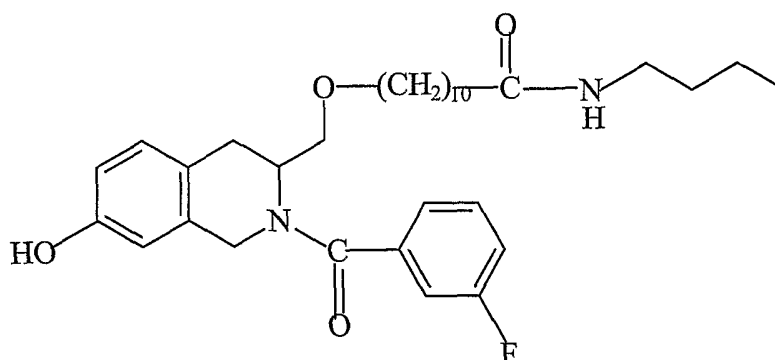
The following compounds illustrate preferred embodiments of the invention:



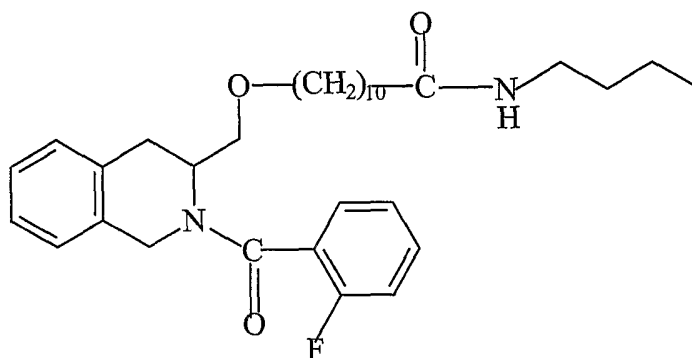
Formula 2. 11-[2-(3-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



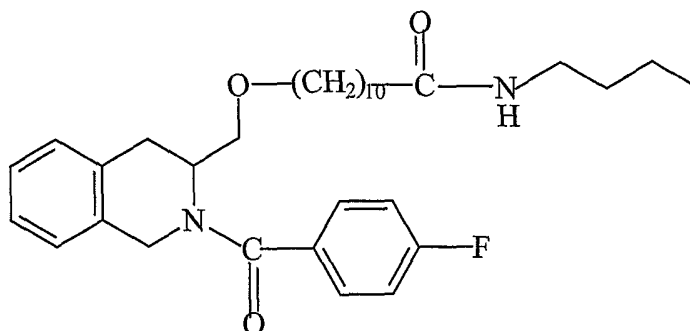
Formula 3. 11-[2-(3-Fluoro-benzoyl)-6-hydroxy-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-undecanoic acid butylamide.



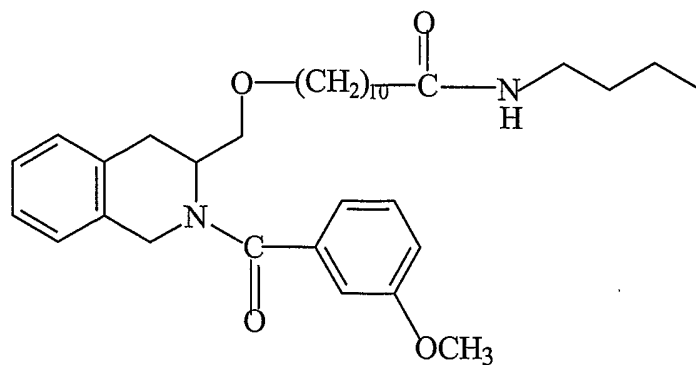
Formula 4. 11-[2-(3-Fluoro-benzoyl)-7-hydroxy-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-undecanoic acid butylamide.



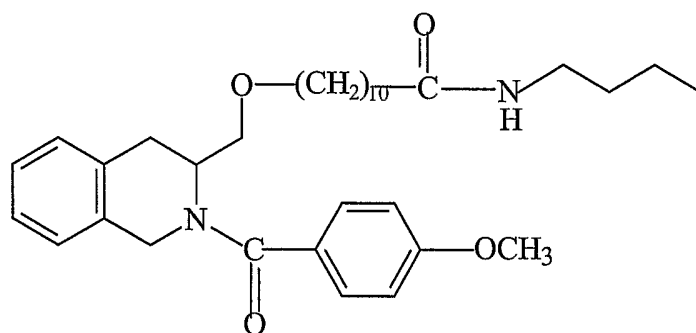
Formula 5. 11-[2-(2-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-undecanoic acid butylamide.



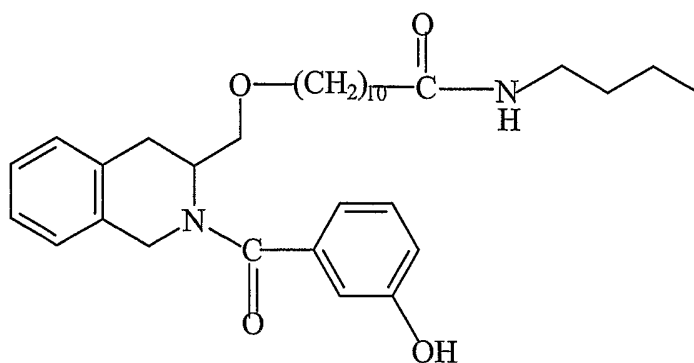
Formula 6. 11-[2-(4-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-undecanoic acid butylamide.



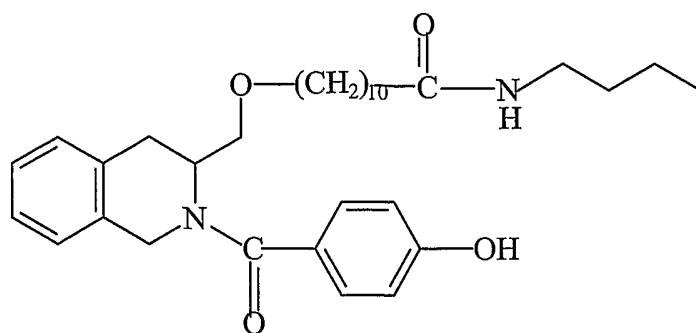
Formula 7. 11-[2-(3-Methoxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



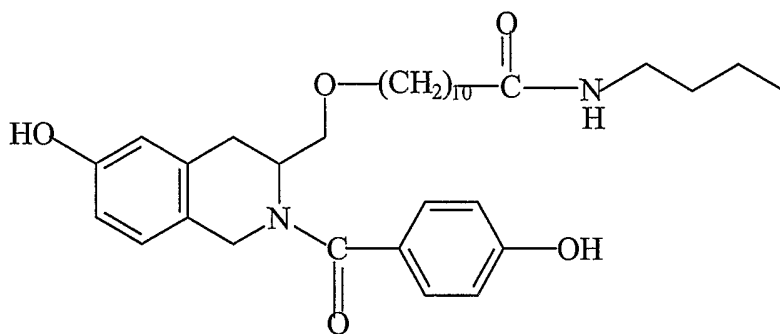
Formula 8. 11-[2-(4-Methoxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



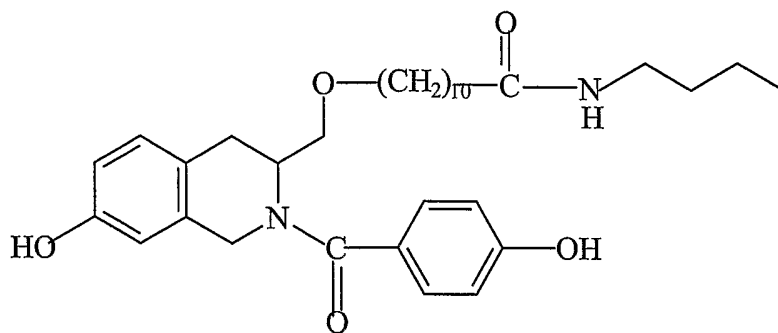
Formula 9. 11-[2-(3-Hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



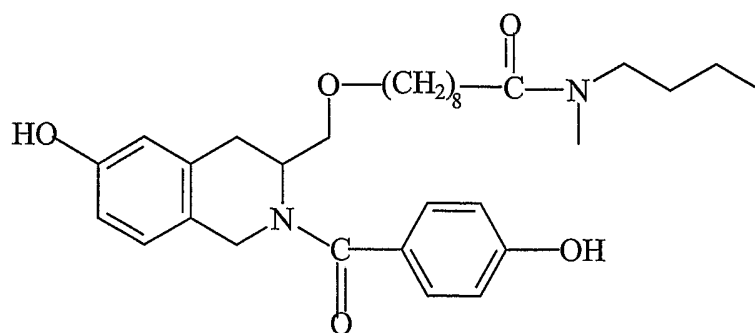
Formula 10. 11-[2-(4-Hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



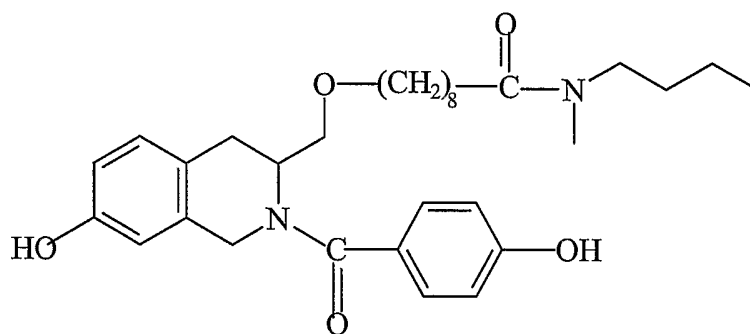
Formula 11. 11-[6-Hydroxy-2-(4-hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



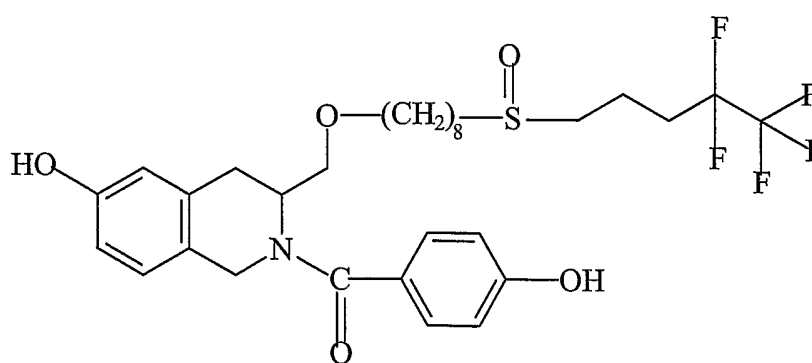
Formula 12. 11-[7-Hydroxy-2-(4-hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl]-undecanoic acid butylamide.



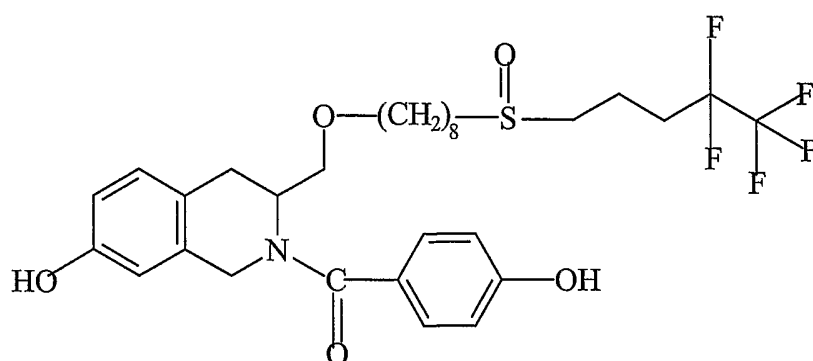
Formula 13. 9-[6-Hydroxy-2-(4-hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-nonanoic acid butylamide.



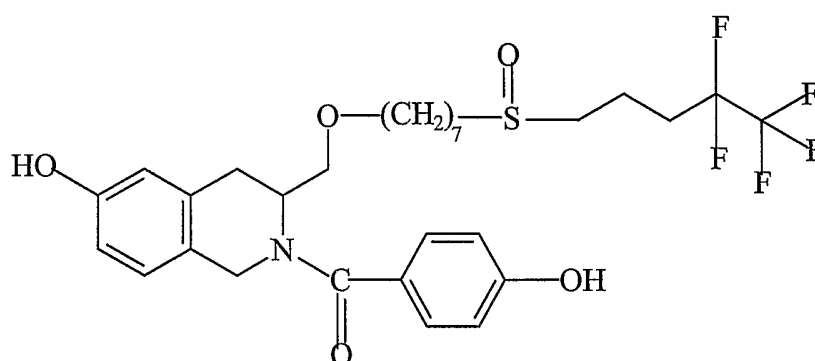
Formula 14. 9-[7-Hydroxy-2-(4-hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxyl-nonanoic acid butylamide.



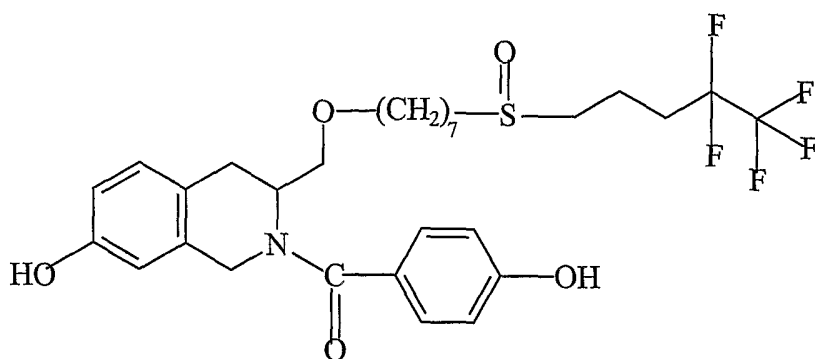
Formula 15. {6-Hydroxy-3-[8-(4, 4, 5, 5, 5-pentafluoro-pentane-1-sulfinyl)-octyloxymethyl]-3,4-dihydro-1H-isoquinolin-2-yl}-(4-hydroxy-phenyl)-methanone.



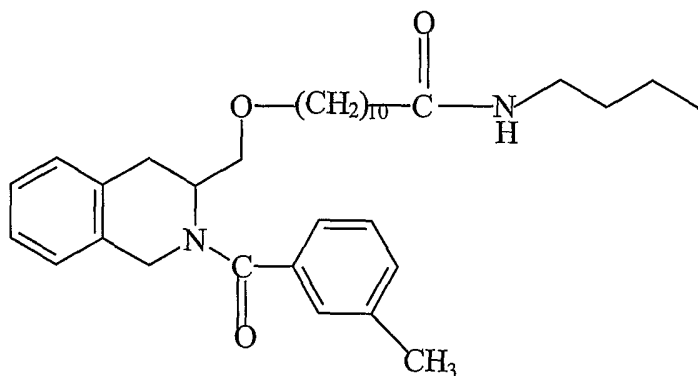
Formula 16. {7-Hydroxy-3-[8-(4, 4, 5, 5, 5-pentafluoro-pentane-1-sulfinyl)-octyloxymethyl]-3,4-dihydro-1*H*-isoquinolin-2-yl}-(4-hydroxy-phenyl)-methanone.



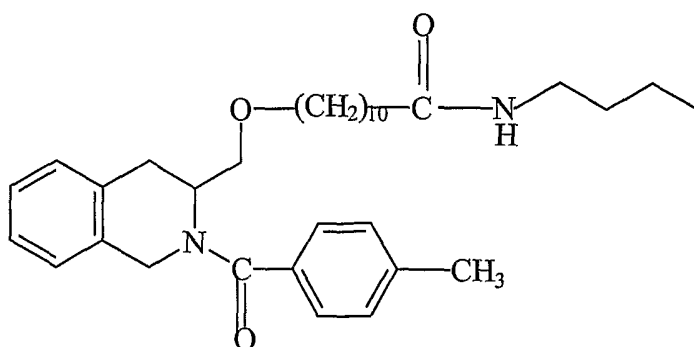
Formula 17. {6-Hydroxy-3-[7-(4, 4, 5, 5, 5-pentafluoro-pentane-1-sulfinyl)-heptyloxymethyl]-3,4-dihydro-1*H*-isoquinolin-2-yl}-(4-hydroxy-phenyl)-methanone.



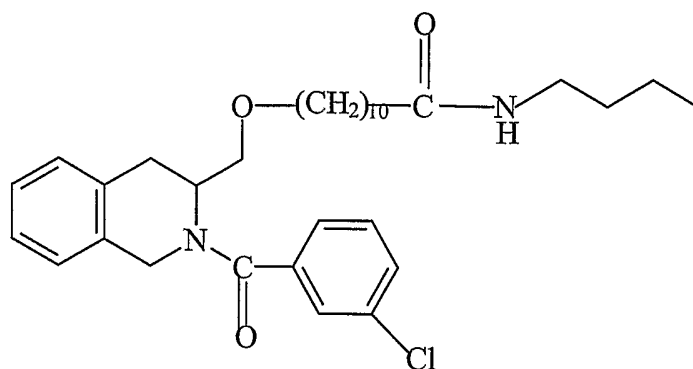
Formula 18. {-Hydroxy-3-[7-(4, 4, 5, 5, 5-pentafluoro-pentane-1-sulfinyl)-heptyloxymethyl]-3,4-dihydro-1*H*-isoquinolin-2-yl}-(4-hydroxy-phenyl)-methanone.



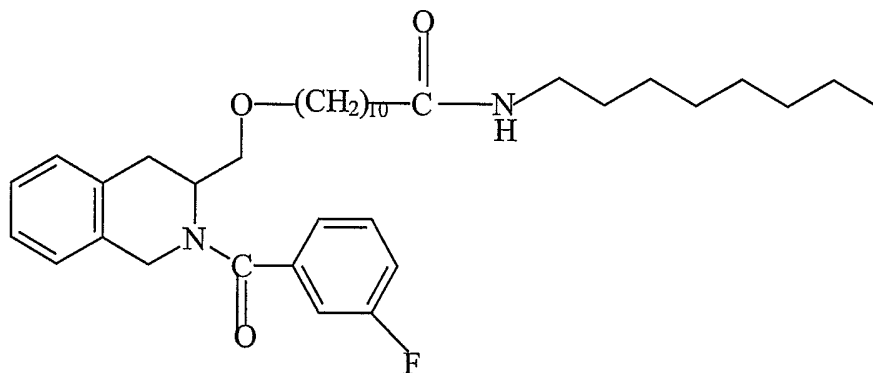
Formula 19. 11-[2-(3-Methyl-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide



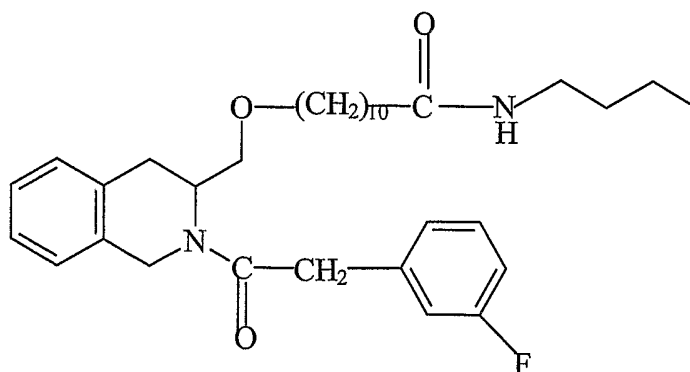
Formula 20. 11-[2-(4-Methyl-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide



Formula 21. 11-[2-(3-Chloro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide



Formula 22. 11-[2-(3-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide



Formula 23. 11-{2-[2-(3-Fluoro-phenyl)-acetyl]-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide

The present invention provides methods for the treatment of diseases involving estrogen receptors. In a preferred embodiment of the invention, the disease is breast cancer. In another embodiment of the invention, the disease is prostate cancer. By "breast cancer" and "prostate cancer" we mean both tumors that develop in the breast/prostate, and metastatic tumors that originated in breast/prostate tissue. By "treating" cancer we mean that a compound is administered in order to alleviate symptoms of the disease (e.g. to slow or stop growth of a tumor or to decrease tumor size, halt metastasis, etc.). Those of skill in the art will recognize that, in the treatment of cancer, administration of the compounds of the present invention may completely eradicate symptoms of the disease, or, alternatively, may attenuate or slow the progression of disease, which is also beneficial to the patient.

In particular, the compounds of the present invention may be useful for the treatment of breast cancer tumors that are resistant to tamoxifen.

In yet another embodiment of the invention, the disease that is treated is osteoporosis. By "treating" osteoporosis we mean that a compound is administered in order to alleviate symptoms of the disease (e.g. to increase bone density, or at least to prevent, stop or slow the loss of bone density). Those of skill in the art will recognize that, in the treatment of osteoporosis, administration of the compounds of the present invention may completely eradicate symptoms of the disease, or, alternatively, may attenuate or slow the progression of disease, which is also beneficial to the patient.

Use of the compounds will generally involve identifying patients suffering from estrogen-receptor related tumors (e.g. breast or prostate tumors), or alternatively, from osteoporosis, and administering the compounds in an acceptable form by an appropriate route. Administration may be oral or parenteral, including intravenously, intramuscularly, subcutaneously, etc., or by other routes (e.g. transdermal, sublingual, aerosol, suppository, etc.).

The compounds can be administered in the pure form or in a pharmaceutically acceptable formulation including suitable elixirs, binders, and the like or as pharmaceutically acceptable salts or other derivatives. It should be understood that the pharmaceutically

acceptable formulations and salts include liquid and solid materials conventionally utilized to prepare injectable dosage forms and solid dosage forms such as tablets and capsules. Water may be used for the preparation of injectable compositions which may also include conventional buffers and agents to render the injectable composition isotonic. Other potential additives include: colorants; surfactants (TWEEN, oleic acid, etc.); and binders or encapsulants (lactose, liposomes, etc). Solid diluents and excipients include lactose, starch, conventional disintegrating agents, coatings and the like. Preservatives such as methyl paraben or benzalkium chloride may also be used. Depending on the formulation, it is expected that the active composition will consist of 1-99% of the composition and the vehicular "carrier" will constitute 1-99% of the composition. The pharmaceutical compositions of the present invention may include any suitable pharmaceutically acceptable additives or adjuncts to the extent that they do not hinder or interfere with the therapeutic effect desired of the Pt complex.

The administration of pharmaceutical compositions of the present invention can be intermittent, or at a gradual or continuous, constant or controlled rate to a patient. In addition, the time of day and the number of times per day that the pharmaceutical formulation is administered can vary. Further, the preferred dosing schedule can vary depending upon factors such as the mode of delivery, gender, age, and other conditions of the patient, as well as tumor type, stage, grade and location.

The dosage to be administered may vary depending on the age, gender, weight and overall health status of the individual patient, as well as the nature of the cancer itself. The level of efficacy and optimal amount of dosage may vary somewhat from compound to compound.

## EXAMPLES

### Experimental Methods and Materials

#### A. Synthesis of N-Butyl-11-bromoundecanamide

11-Bromoundecanoic acid (45 mmol) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (200 ml) and tributylamine (54 mmol). After the mixture had cooled at  $-10^\circ\text{C}$ , isobutylchlorofomate (60 mmol) was added and allowed to react for 2hrs. At this moment, excess N-butylamine (225 mmol) was added and later the cooling bath was removed. After 3hrs,  $\text{CH}_2\text{Cl}_2$  was added and

the organic phase was washed with 1 N HCl, saturated NaHCO<sub>3</sub>, and water. After drying by MgSO<sub>4</sub>, the solvent was removed and the crude product was purified by column chromatography. The pure amide product was eluted with solvent, hexane (80)-EtOAc (20). The purified amide was further crystallized with hexane.

**General procedures for the synthesis of compounds of the invention, designated compounds 1-13**

Relative benzoic acid (phenyl acetic acid) and (s)-(-)-1, 2, 3, 4-tetrahydro-3-isoquinoline methanol were dissolved in DMF under nitrogen gas. The 1-hydroxybenzotriazole hydrate (HOBT) and 1-(3-(Dimethylamino) propyl)-3-ethyl-carbodiimide hydrochloride (DEC) were added into the solution and the reaction were stirred further. After 16 hrs, EtOAc was added into reaction solution and the solution was washed with 10% KHSO<sub>4</sub>, saturated NaHCO<sub>3</sub>, and brine. The resulted organic phase was dried by MgSO<sub>4</sub>. The crude product was purified by column chromatography. The purified compound was dissolved in DMF, NaH and N-butyl-11-bromoundecanamide was added into solution. The reaction solution was refluxed under nitrogen gas further. After 16 hrs, EtOAc was added into reaction solution and the solution was washed with H<sub>2</sub>O and brine. The resulted organic phase was dried by MgSO<sub>4</sub>. The crude product was further purified by column chromatograph to generate final pure product.

11-[2-(2-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 11): Yield: 71 %; <sup>1</sup>H NMR(CDCl<sub>3</sub>, 300 MHz): δ0.95 (t, 3H, CH<sub>3</sub>), 1.1-1.3 (m, 14H, CH<sub>2</sub>), 1.35-1.48 (m, 2H, CH<sub>2</sub>), 1.35-1.48 (m, 4H, CH<sub>2</sub>), 2.16-2.18(m, 2H, C(O)CH<sub>2</sub>), 2.75 (m, 2H, CH<sub>2</sub>), 3.1-3.26 (m, 4H, OCH<sub>2</sub> and NCH<sub>2</sub>), 3.41-3.52 (m, 2H, OCH<sub>2</sub>), 4.19 (s, broad, 1H, CH), 4.42 (s, 2H, NCH<sub>2</sub>), 6.92 (dd, 1H, ArH), 7.05-7.15 (m, 3H, ArH), 7.28-7.31 (m, 2H, ArH), 7.32-7.43 (m, 2H, ArH); Anal. Calcd. for C<sub>32</sub>H<sub>45</sub>FN<sub>2</sub>O<sub>3</sub>: C, 73.25 ; H, 8.64; N, 5.34. Found: C, 73.05; H, 8.41; N, 5.14.

11-[2-(3-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 12): Yield: 68 %; <sup>1</sup>H NMR(CDCl<sub>3</sub>, 300 MHz): δ0.93 (t, 3H, CH<sub>3</sub>), 1.2-1.3 (m, 14H, CH<sub>2</sub>), 1.36-1.51 (m, 2H, CH<sub>2</sub>), 1.58-1.61 (m, 4H, CH<sub>2</sub>), 2.14 (t, 2H, C(O)CH<sub>2</sub>), 2.6 (m, 2H, CH<sub>2</sub>), 3.13-3.20 (m, 2H, NCH<sub>2</sub>), 3.24 (t, 2H, OCH<sub>2</sub>), 3.70-3.75 (m, 2H, OCH<sub>2</sub>), 3.95-4.1 (m, 3H, CH and NCH<sub>2</sub>), 6.78 (m, 1H, ArH), 7.15 (m, 3H, ArH), 7.28

(m, 3H, ArH), 7.47 (s, 1H, ArH); Anal. Calcd. for  $C_{32}H_{45}FN_2O_3$ : C, 73.25 ; H, 8.64; N, 5.34. Found: C, 73.13; H, 8.38; N, 5.21.

11-[2-(4-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 13): Yield: 70 %;  $^1H$  NMR( $CDCl_3$ , 300 MHz):  $\delta$ 0.88 (t, 3H,  $CH_3$ ), 1.2-1.28 (m, 14H,  $CH_2$ ), 1.43-1.49 (m, 2H,  $CH_2$ ), 1.59-1.62 (m, 4H,  $CH_2$ ), 2.14 (m, 2H,  $C(O)CH_2$ ), 2.69 (m, 2H,  $CH_2$ ), 3.11-3.16 (m, 2H,  $NCH_2$ ), 3.23 (t, 2H,  $OCH_2$ ), 3.42 (m, 2H,  $OCH_2$ ), 4.36 (m, 3H, CH and  $NCH_2$ ), 7.06 (m, 1H, ArH), 7.10-7.14 (m, 3H, ArH), 7.19 (m, 2H, ArH), 7.45-7.49 (m, 2H, ArH); Anal. Calcd. for  $C_{32}H_{45}FN_2O_3$ : C, 73.25; H, 8.64; N, 5.34. Found: C, 73.08; H, 8.33; N, 5.18.

11-[2-(3-Methoxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 14): Yield: 71 %;  $^1H$  NMR( $CDCl_3$ , 300 MHz):  $\delta$ 0.91 (t, 3H,  $CH_3$ ), 1.27-1.47 (m, 16H,  $CH_2$ ), 1.53-1.58 (m, 4H,  $CH_2$ ), 2.12 (t, 2H,  $C(O)CH_2$ ), 2.65 (m, 2H,  $CH_2$ ), 3.2-3.3 (m, 4H,  $OCH_2$  and  $NCH_2$ ), 3.61 (d, 2H,  $OCH_2$ ,  $J=6$ Hz), 3.82, (s, 3H,  $OCH_3$ ), 4.27-4.37 (m, 2H, CH and  $NCH_2$ ), 6.95-7.01 (m, 4H, ArH), 7.12-7.19 (m, 2H, ArH), 7.28-7.32 (m, 2H, ArH); Anal. Calcd. for  $C_{33}H_{48}N_2O_4$ : C, 73.84; H, 9.01; N, 5.22. Found: C, 73.58; H, 8.77; N, 5.03.

11-[2-(4-Methoxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 15): Yield: 73 %;  $^1H$  NMR( $CDCl_3$ , 300 MHz):  $\delta$ 0.95 (t, 3H,  $CH_3$ ), 1.30 (m, 14H,  $CH_2$ ), 1.51 (m, 2H,  $CH_2$ ), 1.65 (m, 4H,  $CH_2$ ), 2.17 (t, 2H,  $C(O)CH_2$ ), 2.74 (m, 2H,  $CH_2$ ), 3.26 (m, 4H,  $OCH_2$  and  $NCH_2$ ), 3.45-3.47 (m, 2H,  $OCH_2$ ), 3.89, (s, 3H,  $OCH_3$ ), 4.35-4.50 (m, 2H, CH and  $NCH_2$ ), 6.95-6.98 (m, 4H, ArH), 7.26-7.30 (m, 2H, ArH), 7.46 (m, 2H, ArH); Anal. Calcd. for  $C_{33}H_{48}N_2O_4$ : C, 73.84; H, 9.01; N, 5.22. Found: C, 73.64; H, 8.89; N, 5.08.

11-[2-(3-Hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 16): Yield: 58 %;  $^1H$  NMR( $CDCl_3$ , 300 MHz):  $\delta$ 0.91 (t, 3H,  $CH_3$ ), 1.33-1.66 (m, 20H,  $CH_2$ ), 2.18 (t, 2H,  $C(O)CH_2$ ), 2.74 (m, 2H,  $CH_2$ ), 3.11 (m, 2H,  $NCH_2$ ), 3.4 (m, 2H,  $OCH_2$ ), 3.75 (m, 2H,  $OCH_2$ ), 4.36 (m, 3H, CH and  $NCH_2$ ), 6.85 (m, 1H, ArH), 6.88 (m, 1H, ArH), 7.08-7.19 (m, 5H, ArH), 7.4 (s, 1H, ArH); Anal. Calcd. for  $C_{32}H_{46}N_2O_4$ : C, 73.53; H, 8.87; N, 5.36. Found: C, 73.21; H, 8.72; N, 5.51.

11-[2-(4-Hydroxy-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 17): Yield: 55 %;  $^1H$  NMR( $CDCl_3$ , 300 MHz):  $\delta$ 0.92 (t, 3H,

$\text{CH}_3$ ), 1.27 (m, 14H,  $\text{CH}_2$ ), 1.29-1.59 (m, 6H,  $\text{CH}_2$ ), 2.19 (t, 2H,  $\text{C}(\text{O})\text{CH}_2$ ), 2.74 (m, 2H,  $\text{CH}_2$ ), 3.34-3.41 (m, 4H,  $\text{OCH}_2$  and  $\text{NCH}_2$ ), 3.65 (m, 2H,  $\text{OCH}_2$ ), 4.4 (m, 3H,  $\text{CH}$  and  $\text{NCH}_2$ ), 6.84 (m, 2H,  $\text{ArH}$ ), 7.18 (m, 3H,  $\text{ArH}$ ), 7.32 (m, 3H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{32}\text{H}_{46}\text{N}_2\text{O}_4$ : C, 73.53; H, 8.87; N, 5.36. Found: C, 73.58; H, 8.63; N, 5.41.

11-[2-(3-Methyl-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 18): Yield: 75 %;  $^1\text{H}$  NMR( $\text{CDCl}_3$ , 300 MHz):  $\delta$ 0.91 (t, 3H,  $\text{CH}_3$ ), 1.26 (m, 14H,  $\text{CH}_2$ ), 1.43-1.45 (m, 2H,  $\text{CH}_2$ ), 1.58 (m, 4H,  $\text{CH}_2$ ), 2.12 (t, 2H,  $\text{C}(\text{O})\text{CH}_2$ ), 2.38 (s, 2H,  $\text{CH}_3$ ), 2.68 (m, 2H,  $\text{CH}_2$ ), 3.18-3.29 (m, 4H,  $\text{OCH}_2$  and  $\text{NCH}_2$ ), 3.41-3.44 (m, 2H,  $\text{OCH}_2$ ), 4.2 (m, 3H,  $\text{CH}$  and  $\text{NCH}_2$ ), 6.88 (s, broad, 1H,  $\text{ArH}$ ), 7.05-7.27 (m, 7H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{33}\text{H}_{48}\text{N}_2\text{O}_3$ : C, 76.11; H, 9.29; N, 5.38. Found: C, 75.98; H, 9.37; N, 5.57.

11-[2-(4-Methyl-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 19): Yield: 71 %;  $^1\text{H}$  NMR( $\text{CDCl}_3$ , 300 MHz):  $\delta$ 0.91 (t, 3H,  $\text{CH}_3$ ), 1.26 (m, 14H,  $\text{CH}_2$ ), 1.40-1.45 (m, 2H,  $\text{CH}_2$ ), 1.59 (m, 4H,  $\text{CH}_2$ ), 2.11 (t, 2H,  $(\text{O})\text{CH}_2$ ), 2.67 (m, 2H,  $\text{CH}_2$ ), 3.10-3.23 (m, 4H,  $\text{OCH}_2$  and  $\text{NCH}_2$ ), 3.40-3.44 (m, 2H,  $\text{OCH}_2$ ), 4.28-4.48 (m, 3H,  $\text{CH}$  and  $\text{NCH}_2$ ), 6.89 (s, broad, 1H,  $\text{ArH}$ ), 7.10-7.21 (m, 5H,  $\text{ArH}$ ), 7.34 (m, 2H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{33}\text{H}_{48}\text{N}_2\text{O}_3$ : C, 76.11; H, 9.29; N, 5.38. Found: C, 75.87; H, 9.58; N, 5.43.

11-[2-(3-Chloro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid butylamide (compound 20): Yield: 77 %;  $^1\text{H}$  NMR( $\text{CDCl}_3$ , 300 MHz):  $\delta$ 0.92 (t, 3H,  $\text{CH}_3$ ), 1.27-1.35 (m, 14H,  $\text{CH}_2$ ), 1.43-1.49 (m, 2H,  $\text{CH}_2$ ), 1.62 (m, 4H,  $\text{CH}_2$ ), 2.15 (t, 2H,  $\text{C}(\text{O})\text{CH}_2$ ), 2.65 (m, 2H,  $\text{CH}_2$ ), 3.13-3.16 (m, 2H,  $\text{NCH}_2$ ), 3.25 (t, 2H,  $\text{OCH}_2$ ), 3.40 (d, 2H,  $\text{OCH}_2$ ,  $J=8.4\text{Hz}$ ), 4.28-4.34 (m, 1H,  $\text{CH}$ ), 4.45 (s, 2H,  $\text{NCH}_2$ ), 6.92 (m, 1H,  $\text{ArH}$ ), 7.12-7.20 (m, 3H,  $\text{ArH}$ ), 7.35-7.41 (m, 3H,  $\text{ArH}$ ), 7.49 (s, 1H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{32}\text{H}_{45}\text{ClN}_2\text{O}_3$ : C, 71.02; H, 8.38; N, 5.18. Found: C, 71.28; H, 8.53; N, 5.01.

11-[2-(3-Fluoro-benzoyl)-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy]-undecanoic acid octylamide (compound 21): Yield: 69 %;  $^1\text{H}$  NMR( $\text{CDCl}_3$ , 300 MHz):  $\delta$ 0.88 (t, 3H,  $\text{CH}_3$ ), 1.26 (m, 22H,  $\text{CH}_2$ ), 1.48 (m, 2H,  $\text{CH}_2$ ), 1.61 (m, 4H,  $\text{CH}_2$ ), 2.14 (t, 2H,  $\text{C}(\text{O})\text{CH}_2$ ), 2.64 (m, 2H,  $\text{CH}_2$ ), 3.21-3.25 (m, 4H,  $\text{OCH}_2$  and  $\text{NCH}_2$ ), 3.41 (m, 2H,  $\text{OCH}_2$ ), 4.29 (m, 1H,  $\text{CH}$ ), 4.34 (s, 2H,  $\text{NCH}_2$ ), 6.90 (m, 1H,  $\text{ArH}$ ), 7.12-7.2 (m, 6H,  $\text{ArH}$ ), 7.38 (s, 1H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{36}\text{H}_{53}\text{FN}_2\text{O}_3$ : C, 74.44; H, 9.20; N, 4.82. Found: C, 74.28; H, 9.36; N, 4.91.

11-{2-[2-(3-Fluoro-phenyl)-acetyl]-1,2,3,4-tetrahydro-isoquinolin-3-ylmethoxy}-undecanoic acid butylamide (compound 22): Yield: 53 %;  $^1\text{H}$  NMR( $\text{CDCl}_3$ , 300 MHz):  $\delta$ 0.92 (t, 3H,  $\text{CH}_3$ ), 1.25 (m, 14H,  $\text{CH}_2$ ), 1.45-1.47 (m, 2H,  $\text{CH}_2$ ), 1.61-1.66 (m, 4H,  $\text{CH}_2$ ), 2.13 (t, 2H,  $\text{C(O)CH}_2$ ), 2.85 (m, 2H,  $\text{CH}_2$ ), 3.02 (s, 2H,  $\text{C(O)CH}_2$ ), 3.23 (m, 4H,  $\text{OCH}_2$  and  $\text{NCH}_2$ ), 3.33 (m, 2H,  $\text{OCH}_2$ ), 4.31 (s, broad, 1H,  $\text{CH}$ ), 4.46 (s, 2H,  $\text{NCH}_2$ ), 6.89-6.99 (m, 4H,  $\text{ArH}$ ), 7.05-7.15 (m, 4H,  $\text{ArH}$ ); Anal. Calcd. for  $\text{C}_{33}\text{H}_{47}\text{FN}_2\text{O}_3$ : C, 73.57; H, 8.79; N, 5.20. Found: C, 73.67; H, 8.50; N, 4.91.

## B. Biological assays

### Plasmid preparation

The plasmids employed were the mammalian expression plasmid containing full length human ER cDNA, pCMV-hER, ER cDNA, pcDNA3.1-hER, human AIB1 cDNA, pcDNA3.1-AIB1 and the reporter gene expression plasmid containing three repeated estrogen receptor response elements, pGL-TATA-Luc. These four plasmids were used in the transient transfection reporter assays for estrogen receptor in human breast cancer cells. The yeast expression plasmid pCu424CUP1 and reporter gene plasmid pLG 178 for yeast-based human estrogen receptor reporter assays were purchased from ATCC (American Type Culture Collection Manassas, VA). The yeast expression plasmids, pGADT7 and pGBTT7, for yeast two-hybrid assay were purchased from Clontech (Palo Alto, CA). The vector pCu424CUP1 is constructed to express human estrogen receptor. pCUP-hER contains full length hER cDNA and can express hER in yeasts BJ3505. The expression of hER is regulated by CUP1 promoter. For the construction of pCUP-hER, the hER cDNA was generated by PCR and the primers, 5'-GGATCCATGACCATGACCCTCCACACC-3' (SEQ ID NO. 1) and 5'-GTCGACTCAGACTGTGGCAGGAAACCC-3' (SEQ ID NO. 2) were designed by inserting a BamHI site in front of the hER start codon and a SalI site after the hER stop codon. The pCMV-hER was used as the template of the PCR reaction. The resulting PCR product was further cloned into pCu424CUP1 between BamHI site and SalI site to generate pCUP-hER. pCUP-hER contains long form full length hER cDNA and can express hER in yeasts BJ3505. The expression of hER is also regulated by CUP1 promoter. For the construction of pCUP-hER, the hER cDNA was generated by PCR and the primers, 5'-GGATCCATGGATATAAAAACTCACCATC-3' (SEQ ID NO. 3) and 5'-GTCGACTCACTGAGACTGTGGGTCTTCTGG-3' (SEQ ID NO. 4) were designed by

inserting a BamHI site in front of the hER start codon and a SalI site after the hER stop codon. The pcDNA3.1-hER was used as the template of the PCR reaction. The PCR product was subsequently cloned into pCu424CUP1 between BamHI site and SalI site to generate pCUP-hER. The vectors, pGBKT7 and pGADT7 were used to perform yeast two-hybrid assays for hER. pGBKT7 contains the Gal-4 DNA binding domain which was fused with the bait protein. pGADT7 containing the Gal-4 activation domain which was fused further with the target protein. pGBT-hER contains full length hER cDNA and can express Gal4 DNA binding/hER in yeasts Y190. For the construction of pGBT-hER, the hER cDNA was generated by PCR and the primers, 5'-CATATGACCATGACCCTCCACACC-3' (SEQ ID NO. 5) and 5'-GGATCCTCAGACTGTGGCAGGGAAACCC-3' (SEQ ID NO. 6) were designed by inserting an NdeI site in front of the hER start codon and a BamHI site after the hER stop codon. The PCR product was cloned into pGBKT7 between NdeI and BamHI site. pGAD-hER contains full length hER cDNA and can express hER conjugated with Gal4 activation domain in yeasts Y190. For the construction of pGAD-hER, the hER was generated by PCR which used the same primers mentioned in the construction of pGBT-hER. The PCR product was cloned into pGADT7 between NdeI and BamHI site.

The vector, pLG 178 was used to construct reporter gene in yeast based estrogen receptor reporter transactivation assays. There are three repeated estrogen receptor response element GGTCACGCTGACC cloned in front of lacZ, reporter gene. The cDNA of three repeated ERE was generated by PCR in which the plasmid pERE3-TATA-CAT was used as the template. The primers, 5'-CTCGAGTGGTTTTTGACCCCGAACG-3' (SEQ ID NO. 7) and 5'-CTCGAGCCCGGGGTCTAGAAGATCC-3' (SEQ ID NO. 8), used in generating PCR product were designed by inserting XhoI site at 5' and 3' terminus. The PCR product was cloned into pLG 178 within XhoI sites to generate pLG 178ERE3. The sequence of all plasmids was confirmed by DNA sequence reactions performed by Iowa State University DNA sequencing facility (Ames, IA).

#### **Yeast strains**

The *S. cerevisiae* strain BJ3505 (MAT pep4: His3 prb1- 1.6R his3- 200 lys2-801 ura3-52 gal2 can1) obtained from ATCC was used in the estrogen receptor reporter transactivation assays. The yeast strain Y190 (MATa leu2-3 leu2-112 ura3-52 trp1-901 his3- 200 ade2-101 gal4 gal80 ura3 Gal-lacZ lys gal-his3 cyhR) obtained from ATCC was used in

the yeast two-hybrid assays. All yeast transformation was carried out following the lithium acetate transformation protocol.

#### **Site-directed mutagenesis**

The tyrosine mutation (pCMV-D351Y) at amino acid 351 was introduced by using the Quick Change Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and pCMV-ER was used as the template. The primers constructed were as follows: 5' primer (5'-GGCTTACTGACCAACCTGGCATACAGGGAGCTGGTTCAC-3') (SEQ ID NO. 9), the underlined nucleotide was changed to make the D351Y mutation) and a 3' primer (5'-GTGAACCAGCTCCCTGTATGCCAGGTTGGTCAGTAAGCC-3') (SEQ ID NO. 10).

#### **Reporter gene transactivation and $\beta$ -galactosidase assays for human estrogen receptor in yeast**

The transformed yeasts from early-mid-log phase growth (OD<sub>600</sub> nm approximately 1.0) were diluted to an OD<sub>600</sub> nm of 0.03 in selective medium plus 50  $\mu$ M CuSO<sub>4</sub> to induce estrogen receptor production. The diluted yeasts were aliquoted into 15-ml cap tubes containing culture medium 5 ml per tube and doses of either estradiol or test molecules, or both in methanol or DMSO were added. A solvent (methanol or DMSO) control was included in each experiment. The cultures were incubated overnight at 30 C with vigorous orbital shaking (300 rpm). After incubation, the yeast samples were diluted in appropriate selective medium to OD<sub>600</sub> of 0.3-0.4 and 100  $\mu$ l was added to each well of a 96-well microtiter plate. Each sample was assayed in triplicate. To each well, 100  $\mu$ l of assay buffer (60mM Na<sub>2</sub>HPO<sub>4</sub>, 40mM NaH<sub>2</sub>PO<sub>4</sub>, 10mM KCl, 1mM MgSO<sub>4</sub>, 2mg/ml 2-nitrophenyl-beta-D-galactosidase (ONPG), 0.1% SDS, 50 mM  $\beta$ -mercaptoethanol, and 200U/ l oxaliticase (Enzogenetics, Corvallis, OR) was added. The change of ortho nitrophenol, the yellow product that resulted from  $\beta$ -galactosidase cleavage of ONPG, was measured by using a kinetic microtiter plate reader (Molecular Device, Sunnyvale, CA).  $\beta$ -galactosidase activity is expressed as V<sub>max</sub> (mOD<sub>420</sub>/min) divided by cell density (OD<sub>590</sub>). The relative activity for test samples is the  $\beta$ -galactosidase activity of test sample over that of estradiol whose activity is 1.

#### **Yeast two-hybrid and filter lift assays**

The transformed yeasts with yeast two-hybrid vectors were cultured in synthetic medium lacking tryptophan, and leucine. The estradiol or/and test molecule was added to the

cultured medium after the transformed yeast cells were plated on nitrocellulose membrane. After stimulation with estradiol or test molecules for overnight, the nitrocellulose membranes plated with yeast cells were transferred to a new filter and the yeast cells were permeablized by three heat-freeze cycles. After that, the membranes were soaked in 0.4 ml Z-buffer/Xgal (60 mM  $\text{Na}_2\text{HPO}_4$ , 40mM  $\text{NaH}_2\text{PO}_4$ , 10mM  $\text{KCl}$ , 1mM  $\text{MgSO}_4$ , 0.1% SDS, 50mM  $\beta$ -mercaptoethanol, 20 mM Xgal). The pictures of the results were taken within 2 and 3 hours after the  $\beta$ -galactosidase assay was processed.

#### **Chemiluminescence-based competitive binding assay**

The HitHunter™ EFC estrogen receptor chemilinescence assay kit (Discoverx, Fremont, CA) was used to determine the ability of test molecules to displace ED-estrogen conjugate from hER $\alpha$  - ED-estrogen conjugate complex. The recombinant hER or hER (5 nM) was preincubated with ED-estrogen conjugate in screening buffer. After the preincubation, the test molecules and hER-ED-estrogen conjugate complex solution were added into the 96-well microplate to produce a final volume of 50  $\mu\text{l}$  per well. After the reaction was incubated at room temperature for 1.5 hrs, the EA solution and chemiluminescence substrate buffer were added into each well for 1 hr incubation, and the luminescence values were measured by using luminescence microplate reader, LumiCount (Packard, Boston, MA). The  $\text{IC}_{50}$  value of tested compounds was generated by graphfit software. The  $\text{IC}_{50}$  value was further converted to relative binding affinity (RBA) by using raloxifene's  $\text{IC}_{50}$  as the standard that was set to 1. The RBA value of each test molecule was calculated by using the equation; RBA equals ( $\text{IC}_{50}$  of raloxifene/ $\text{IC}_{50}$  of test molecule).

#### **Fluorescence-based competitive binding assays**

The estrogen receptor competitor assay kits (Panvera, Madison, WI) were used to determine the ability of test molecules to displace the fluormone, ES2, from hER -ES2 or hER -ES2 complex. Serial dilutions of each test molecule were prepared in methanol or DMSO. The recombinant hER or hER (7 nM) was preincubated with ES2 (1 nM) in screening buffer. After the preincubation, the test molecules and ER-ES2 complex solution were added into the 96-well microplate to produce a final volume of 100  $\mu\text{l}$  per well. The reaction was incubated at room temperature for 1 hr and the polarization values were measured by using fluorescence microplate reader, Polarion (Tecan, Research Triangle Park, NC) with excitation wavelength 495 nm and emission wavelength 535 nm. The polarization

value versus test molecule concentration curves was analyzed by graphfit software to generate  $IC_{50}$  values. The  $IC_{50}$  value was further converted to relative binding affinity (RBA) by using tamoxifen's  $IC_{50}$  as the standard that was set to 1. The RBA value of each test molecule was calculated by using the equation; RBA equals ( $IC_{50}$  of tamoxifen/ $IC_{50}$  of test molecule).

#### **Cell culture, transfection, luciferase assay and $\beta$ -galactosidase assay**

Human breast cancer cells, MCF-7 (ER positive) and MDA-MB-231 (ER negative), were purchased from ATCC. The cells routinely were cultured as monolayer in Dulbecco's modified minimal essential medium (GIBCO/BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), Penicillin (100 unit/ml)/Streptomycin (100 g/ml) and bovine insulin (0.005 mg/ml) (GIBCO/BRL, Grand Island, NY), and incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>/air.

For the transient transfection reporter assays, the cells were plated in triplicate in 12-well plates at a density of 300000 cells/well in the phenol red-free DMEM (GIBCO/BRL, Grand Island, NY) supplemented with 10% charcoal-stripped fetal bovine serum (Hyclone, Logan, UT), Penicillin (100 unit/ml)/Streptomycin (100 g/ml), 2mM-glutamine and 1mM sodium Pyruvate. 24 hrs later, the cells were transfected with three plasmids by using Superfect transfection kit (Qiagen, Valencia, CA). For the detection of hER activity, cells were transfected with 2 g hER expression plasmid (pCMV-ER), 6 g luciferase reporter plasmid containing estrogen receptor response element (PGL-TATA-Luc), and 600ng normalization control,  $\beta$ -galactosidase reporter plasmid (pCMV). For the detection of hER activity, cells were transfected with 3ug hER expression plasmid (pcDNA3.1-hER), 6 g luciferase reporter plasmid containing estrogen receptor response element (PGL-TATA-Luc), and 600ng pCMV. For determining the influence of ER's major coactivator in breast, AIB1, 2 g pcDNA3.1-AIB1 was added into the transfection plasmid solution mentioned previously.

The transfected cells were rinsed with PBS and treated with various concentrations of test molecules and one positive control (vehicle, DMSO or methanol) in phenol red-free culture medium. After incubation for further 24 hrs, the cells were washed with PBS and lysed with lysis buffer (Pierce, Rockford, IL). The lysate was used to determine the

luciferase activity for ER's activity and the  $\beta$ -galactosidase activity for the normalization of transfection efficiency.

For the luciferase activity assay, 20  $\mu$ l of lysate and 100  $\mu$ l luciferase assay buffer (Promega, Madison, WI) were added into a well of 96-well plate. The luminescence was detected by using luminescence microplate reader, LumiCount (Packard, Boston, MA). For the  $\beta$ -galactosidase activity assay, 20  $\mu$ l of lysate and 185  $\mu$ l  $\beta$ -galactosidase assay buffer (Clontech, Palo Alto, CA) were added into a well of 96-well plate. The  $\beta$ -galactosidase activity was measured as luminescence strength by using luminescence microplate reader, LumiCount (Packard, Boston, MA). The normalized reporter activity was calculated by the luciferase activity divided by that of  $\beta$ -galactosidase. For the estrogenic or antiestrogenic effects of test molecules, the normalized reporter activity value was further converted to relative normalized reporter activity by using the value of estradiol or DMSO as a standard that was set to 1.

#### **Cell proliferation assay**

MCF-7 or MDA-MB-231 cells were inoculated into 12-well culture plates at 10000 cells in 2ml maintained medium per well. Cells were allowed to attach to the bottom for 24 hrs incubation, then the seeding medium was removed and replaced by the experimental medium (phenol red-free DMEM supplemented with 5% charcoal-stripped fetal bovine serum and Penicillin (100 unit/ml)/Streptomycin (100  $\mu$ g/ml)). After 24 hr incubation, the test molecules dissolved in DMSO were added in the wells. The final concentration of DMSO in the culture medium did not exceed 0.1%. The culture was continued for 3 days and the medium and tested compounds were replaced every two days. The final cell numbers were estimated with CellTiter proliferation assay kit (Promega, Madison, WI) by measuring the absorbance at 570 nm, which is directly proportional to the number of living cells in the culture. Experiments were at least duplicated for each compound and the results are shown as average value of at least three individual testing with less than 15% error.

#### **Results and Discussion**

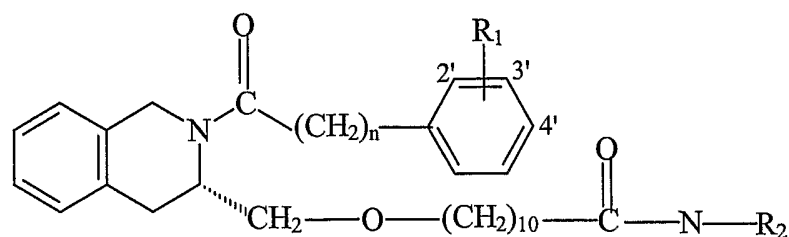
##### **Fluorescence-based binding affinity testing for compounds of the invention**

In this assay, recombinant hER and the commercial synthetic estrogen, ES2 containing fluorescence polarization property, are used. This assay is a homogenous assay. When the assay solution only contains ES2, the solution exerts weak fluorescence

polarization property due to the quick rotation of free ES2 molecule. On the contrary, when ES2 is mixed with hER, the rotation of ES2 is largely decreased due to its tight interaction with hER and the solution exerts strong fluorescence polarization property. Addition of the antiestrogen to the ES2/hER displaces ES2 from hER and results in the whole solution exerting less fluorescence polarization property than the solution without antiestrogen. Based on this principle, the higher the binding affinity of the tested compound, the lower fluorescence polarization value the tested solution would exert.

Table 1 shows the relative binding affinity of certain compounds of the present invention, designated compounds 1 to 12, where generic Tamoxifen was tested as reference and its binding affinity was set at 1. The result showed that among these compounds, compounds 6 and 7 have highest binding affinity and higher than tamoxifen. The preferable physical property of the substituted group on the second aromatic ring is hydrogen bond donor and hydrogen bond acceptor, and the favorable tendency of substituent's physical property for binding affinity is  $\text{OH} > \text{F} > \text{OCH}_3 > \text{CH}_3, \text{Cl}$ . For the substituted position on the second aromatic ring, substituents at 3'-position are slightly better than 4'-position and 2'-position is the least preferred for binding. Essentially, when the binding affinity of compounds 2, 11 and 12 are compared, it reveals that the length of the bridge and side chain seem not to influence the compound's binding affinity.

**Table 1.** Relative binding affinity of compound 1 to compound 12 for hER in fluorescence-based binding assay



| Compound      | n | R <sub>1</sub> | R <sub>2</sub> | Relative Binding Affinity |
|---------------|---|----------------|----------------|---------------------------|
| 1 (Formula 5) | 0 | 2'-F           | butyl          | 0.225                     |

|                 |   |                     |       |       |
|-----------------|---|---------------------|-------|-------|
| 2 (Formula 2)   | 0 | 3'-F                | butyl | 0.287 |
| 3 (Formula 6)   | 0 | 4'-F                | butyl | 0.264 |
| 4 (Formula 7)   | 0 | 3'-OCH <sub>3</sub> | butyl | 0.192 |
| 5 (Formula 8)   | 0 | 4'-OCH <sub>3</sub> | butyl | 0.14  |
| 6 (Formula 9)   | 0 | 3'-OH               | butyl | 5.0   |
| 7 (Formula 10)  | 0 | 4'-OH               | butyl | 1.79  |
| 8 (Formula 19)  | 0 | 3'-CH <sub>3</sub>  | butyl | 0.156 |
| 9 (Formula 20)  | 0 | 4'-CH <sub>3</sub>  | butyl | 0.132 |
| 10 (Formula 21) | 0 | 3'-Cl               | butyl | 0.156 |
| 11 (Formula 22) | 0 | 3'-F                | octyl | 0.177 |
| 12 (Formula 23) | 1 | 3'-F                | butyl | 0.267 |
| Tamoxifen       |   |                     |       | 1     |

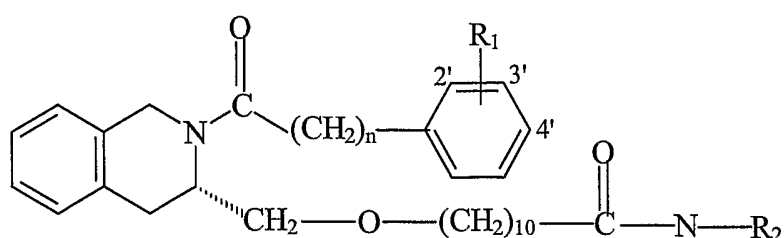
### Transient transfection reporter testing

The transient transfection reporter assay was used to determine the estrogenic or antiestrogenic activity of tested compounds in employed human breast cancer cells, MCF-7 cells that are cotransfected with hER $\alpha$  expression and reporter plasmids. For estrogenic activity, 5  $\mu$ M tested compound was added to transfected MCF-7 cells. For antiestrogenic activity, 5  $\mu$ M tested compound and 1 nM estradiol are added to transfected MCF-7 cells. The estrogenic (antiestrogenic) activity was determined by dividing the luciferase activity by the  $\beta$ -galactosidase activity. To determine the influence of estrogen receptor's major coactivator in breast cancer, AIB1, for the compounds' estrogenic activity, the expression vector of AIB1 was additionally cotransfected with the plasmids mentioned above. The results are shown in Figure 4. As can be seen, compound 1 can antagonize estrogen's action in a dose dependent manner.

Table 2 shows the estrogenic effects of compounds 1 to 12. In the same assay, DMSO was tested as reference and its estrogenic effect was set as 1. Tamoxifen and estrogen were also tested for comparison. Shown in the Table 2, tamoxifen's relative estrogenic effect is 1.385. Among the compounds 1 to 12, compound 7 exerts the highest estrogenic effect. Apart from compound 7, compounds 8, 9 and 12 have similar or higher estrogenic effects than tamoxifen. The other compounds 1, 2, 3, 4, 5, 6, 10 and 11 do not exert any estrogenic effects. Essentially, those compounds containing substituent on the 3' position of the second aromatic ring don't possess estrogenic effects, except compound 8 containing methyl group on the 3 position and compound 12 with longer bridge length. For compound containing

hydroxyl group and compound 9 containing methyl group on the 4' position of the second aromatic ring, both exert significant estrogenic effects.

**Table 2.** Relative estrogenic activity of compound 1 to 12 in transient transfection reporter assay for hER $\alpha$  in MCF-7 cells

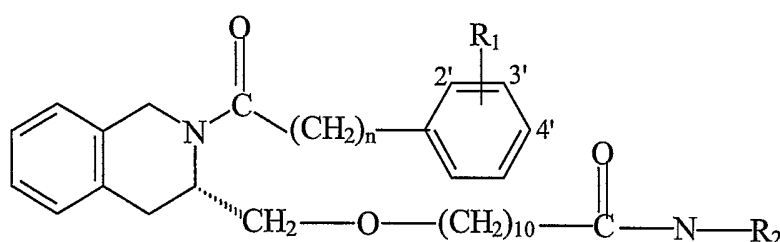


| Compound        | n | R <sub>1</sub>      | R <sub>2</sub> | Relative Estrogenic Activity at 2.5 mM |
|-----------------|---|---------------------|----------------|----------------------------------------|
| 1 (Formula 5)   | 0 | 2'-F                | butyl          | 1.02                                   |
| 2 (Formula 2)   | 0 | 3'-F                | butyl          | 0.9                                    |
| 3 (Formula 6)   | 0 | 4'-F                | butyl          | 1.09                                   |
| 4 (Formula 7)   | 0 | 3'-OCH <sub>3</sub> | butyl          | 0.78                                   |
| 5 (Formula 8)   | 0 | 4'-OCH <sub>3</sub> | butyl          | 1.06                                   |
| 6 (Formula 9)   | 0 | 3'-OH               | butyl          | 1.03                                   |
| 7 (Formula 10)  | 0 | 4'-OH               | butyl          | 2.05                                   |
| 8 (Formula 19)  | 0 | 3'-CH <sub>3</sub>  | butyl          | 1.32                                   |
| 9 (Formula 20)  | 0 | 4'-CH <sub>3</sub>  | butyl          | 1.72                                   |
| 10 (Formula 21) | 0 | 3'-Cl               | butyl          | 0.68                                   |
| 11 (Formula 22) | 0 | 3'-F                | octyl          | 0.83                                   |
| 12 (Formula 23) | 1 | 3'-F                | butyl          | 1.68                                   |
| DMSO            |   |                     |                | 1                                      |
| Tamoxifen       |   |                     |                | 1.385                                  |
| 1 nM E2         |   |                     |                | 5.92                                   |

Table 3 shows the antiestrogenic effects of several compounds of the present invention. In this assay, 1nM estrogen was used as reference and tamoxifen was tested for comparison. The compounds 2, 4, 6, 7, 8 and 10 showed 25% to 56% inhibition against estrogen, and tamoxifen showed 72% inhibition activity. Compound 6 containing hydroxyl

moiety on the 3' position of the second aromatic ring, had the strongest inhibition activity among the compounds and the rest of the compounds with the substituent on the 3' position of the second aromatic ring showed moderate inhibition activity, except compound 12. Compound 7 with hydroxyl group on the 4' position also exhibited moderate antiestrogenic activity. Based on the physical property of the substituents, hydrogen bond donor group, like hydroxyl, was optimal, and hydrogen bond acceptor group, like fluoro and methoxy, was secondly optimal. Unexpectedly, compound 8 with methyl group substituent on the 3 position exerts moderate antiestrogenic effect and it also possesses a moderate estrogenic effect.

**Table 3.** Relative antiestrogenic activity of compound 1 to 12 in transient transfection reporter assay for hER $\alpha$  in MCF-7 cells



| Compound        | n | R <sub>1</sub>      | R <sub>2</sub> | Relative Antiestrogenic Activity at 2.5 $\mu$ M |
|-----------------|---|---------------------|----------------|-------------------------------------------------|
| 1 (Formula 5)   | 0 | 2'-F                | butyl          | 1.17                                            |
| 2 (Formula 2)   | 0 | 3'-F                | butyl          | 0.75                                            |
| 3 (Formula 6)   | 0 | 4'-F                | butyl          | 0.93                                            |
| 4 (Formula 7)   | 0 | 3'-OCH <sub>3</sub> | butyl          | 0.79                                            |
| 5 (Formula 8)   | 0 | 4'-OCH <sub>3</sub> | butyl          | 1.64                                            |
| 6 (Formula 9)   | 0 | 3'-OH               | butyl          | 0.44                                            |
| 7 (Formula 10)  | 0 | 4'-OH               | butyl          | 0.76                                            |
| 8 (Formula 19)  | 0 | 3'-CH <sub>3</sub>  | butyl          | 0.75                                            |
| 9 (Formula 20)  | 0 | 4'-CH <sub>3</sub>  | butyl          | 1.34                                            |
| 10 (Formula 21) | 0 | 3'-Cl               | butyl          | 0.83                                            |
| 11 (Formula 22) | 0 | 3'-F                | octyl          | 1.11                                            |
| 12 (Formula 23) | 1 | 3'-F                | butyl          | 1.01                                            |

|           |  |  |  |      |
|-----------|--|--|--|------|
| Tamoxifen |  |  |  | 0.28 |
| 1 nM E2   |  |  |  | 1    |

Amino acid 351 (D351) of hER $\alpha$  plays an essential role in regulating estrogenic or antiestrogenic compound's binding to hER $\alpha$  and the recruitment of corepressors. Importantly, the point mutation of D351 to tyrosine (Y) was found to occur in tamoxifen treated breast tumors. The mutated D351Y can largely enhance 4-hydroxytamoxifen's estrogenic activity so the D351Y hER $\alpha$  mutant is implied to serve a key function for the formation of tamoxifen resistant breast tumors. Based on this observation the hER $\alpha$  D351Y mutant is generated by PCR-based site directed mutagenesis method for use in the transient transfection reporter assay. The main goal of this testing is to elucidate the existence of the pure antiestrogenic property of the compounds of the invention and to determine whether the compounds estrogenic activity will increase due to the presence of hER $\alpha$  D351Y or not.

Figure 5 shows the estrogenic activity of compounds 4, 6, and 7 against hER $\alpha$  D351Y. In this assay, DMSO was used as reference. Estrogen and tamoxifen were used as the comparison. The results shown in Figure 5 indicate that the estrogenic activity of estrogen does not increase and that of tamoxifen only moderately increases. However, the estrogenic activities of compounds 4, 6, and 7, do not increase when their estrogenic effects against hER $\alpha$  D351Y and hER $\alpha$  wild type are compared. Interestingly, the estrogenic effects of compounds 4, 6, and 7 against mutant hER $\alpha$  D351Y are even lower than that of DMSO.

This result demonstrates that these compounds can act as pure antiestrogens and they provide antiestrogenic effects against basal hER $\alpha$  effects.

#### **hER $\alpha$ positive breast cancer cell proliferation testing for compounds 1-13**

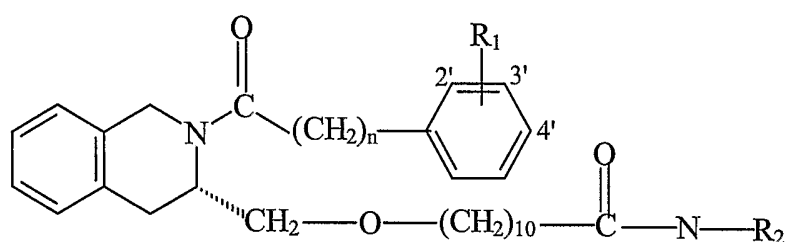
MCF-7 cell line was used to perform this assay and the description of this assay as mentioned above. Table 4 shows the inhibition activity of compounds 1 to 13 against MCF-7 cell proliferation. DMSO was used as reference. Estrogen and tamoxifen are also tested for comparison.

2nM estrogen stimulates the growth of DMSO-treated MCF-7 cells 150% compared to 58% by tamoxifen. For compounds 1 to 13, compounds 3, 8 and 10 only show slight inhibition effects. Compound 6 exerts the strongest inhibition effects with 38% inhibition. However, compounds 2 and 4 exert slightly lower inhibition effects than compound 6. The

results show that the compounds with substituent on the 3' position of the second aromatic ring exert much better anti-proliferation activity against hER $\alpha$  dependent breast cancer cells than those with substituent on the 4' position. The relationship between physical property of substituent and anti-proliferation activity is that hydrogen bond donor > hydrogen bond acceptor > hydrophobic group. This trend correlates with the trend from transient transfection reporter assay's antiestrogenic testing. In fact, those compounds that show good antiestrogenic effects in transient transfection reporter assay also possess good anti-proliferation activity against MCF-7 cells.

These results confirm that compounds of the present invention exhibit inhibition ability against hER $\alpha$ .

**Table 4.** Proliferation effects of compound 1 to 13 against MCF-7 cells



| Compound        | n | R <sub>1</sub>      | R <sub>2</sub> | Relative Cancer Cell Proliferation Effects at 5 $\mu$ M |
|-----------------|---|---------------------|----------------|---------------------------------------------------------|
| 1 (Formula 5)   | 0 | 2'-F                | butyl          | 1.06                                                    |
| 2 (Formula 2)   | 0 | 3'-F                | butyl          | 0.66                                                    |
| 3 (Formula 6)   | 0 | 4'-F                | butyl          | 0.91                                                    |
| 4 (Formula 7)   | 0 | 3'-OCH <sub>3</sub> | butyl          | 0.7                                                     |
| 5 (Formula 8)   | 0 | 4'-OCH <sub>3</sub> | butyl          | 0.98                                                    |
| 6 (Formula 9)   | 0 | 3'-OH               | butyl          | 0.62                                                    |
| 7 (Formula 10)  | 0 | 4'-OH               | butyl          | 0.95                                                    |
| 8 (Formula 19)  | 0 | 3'-CH <sub>3</sub>  | butyl          | 0.84                                                    |
| 9 (Formula 20)  | 0 | 4'-CH <sub>3</sub>  | butyl          | 1.01                                                    |
| 10 (Formula 21) | 0 | 3'-Cl               | butyl          | 0.86                                                    |
| 11 (Formula 22) | 0 | 3'-F                | octyl          | 1                                                       |
| 12 (Formula 23) | 1 | 3'-F                | butyl          | 1.2                                                     |

|                   |   |                    |       |      |
|-------------------|---|--------------------|-------|------|
| 13 (not depicted) | 0 | 4'-CF <sub>3</sub> | butyl | 1.01 |
| DMSO              |   |                    |       | 1    |
| Tamoxifen         |   |                    |       | 0.42 |
| 2 nM E2           |   |                    |       | 1.52 |

#### **hER negative breast cancer cell proliferation testing for compounds 2, 4 and 6**

In this assay, MDA-MB-231 breast cancer cell line, a hER negative cell line, was used. DMSO was used as reference. Estrogen and tamoxifen were also tested for comparison.

Figure 6 shows the proliferation activities of compounds 2, 4 and 6. As expected, estrogen does not promote the growth of MDA-MB-231 cells. Compounds 2, 4, and 6 also do not inhibit or stimulate the growth of MDA-MB-231 cells. However, tamoxifen still exerts moderate anti-proliferation effects against MDA-MB-231 cells. Tamoxifen's ER independent anti-proliferation activity has been proposed by several groups.

The results shown here further demonstrate that the inhibitory effects of compounds 2, 4, and 6 against hER $\alpha$  are specific.

#### **Yeast two-hybrid/filter lifting testing for compounds 2 and 6**

Dimerization of estrogen receptor plays an essential role in regulating estrogen receptor's functions including DNA binding, transactivation, and coactivator recruitment. In this study, yeast two hybrid and filter lift assays were used to determine the ability of compounds 2 and 6 to interrupt the dimerization of hER $\alpha$ . For setting up yeast two hybrid assay, the full length hER $\alpha$  was inserted into pGAD-GAL4 and pGBD-GAL4 vectors. In pGBD-GAL4, hER $\alpha$  was fused to the GAL4 DNA binding domain through its N terminus. In pGAD-GAL4, hER $\alpha$  was fused to the GAL4 activation domain through its N terminus. When the dimerization of hER $\alpha$  occurs mainly through its C terminus, the GAL4 DNA binding domain binds to the repeated GAL4 response element located in front of the  $\beta$ -gal reporter gene and the whole complex, GAL4-BD/hER $\alpha$ /hER $\alpha$ /GAL4-AD, turns on the synthesis of the product of  $\beta$ -gal reporter gene. The compound's inhibitory activity is inversely related to the activity of the reporter gene product,  $\beta$ -galactosidase. The higher the activity of  $\beta$ -galactosidase, the lower the compound's inhibitory activity against hER $\alpha$  dimerization. Filter lift assay in which X-gal was used, was employed to qualitatively

determine the activity of  $\beta$ -galactosidase. Increase in blue color formed from the tested yeast colony indicates that the tested compound does not possess significant inhibition activity.

Compound 2 and tamoxifen were tested either alone or in combination with estrogen. The results indicated that compound 2 and tamoxifen alone did not induce the dimerization of hER $\alpha$  based on the fact that their relative tested yeast colony did not change color. When combined with estrogen, both compounds exert only a weak inhibitory activity. Compound 6 and tamoxifen were also tested. The results showed that compound 6 and tamoxifen did not induce hER $\alpha$  dimerization. When estrogen's colony was used as a reference colony, the colony tested with compound 6 in combination with estrogen shows slight blue color change. However, the colony tested with tamoxifen in combination with estrogen shows moderate blue color change. Results observed for this test after one additional hour of color development further confirmed the result.

This result demonstrates that compound 6 can exert inhibitory activity against estrogen for the dimerization of hER $\alpha$  and its inhibitory activity is higher than tamoxifen's.

Since compound 6 has a hydrophobic side chain similar to ICI type of compounds, ICI182,780 (Figure 7B) was also tested in this assay for comparison. The results showed that Compound 6 and ICI182,780 alone do not promote the dimerization of hER $\alpha$ . However, when combined with estrogen, both compounds exert an obvious inhibitory activity against the dimerization of hER $\alpha$ . Unexpectedly, compound 6's inhibitory activity is similar to that of ICI182,780.

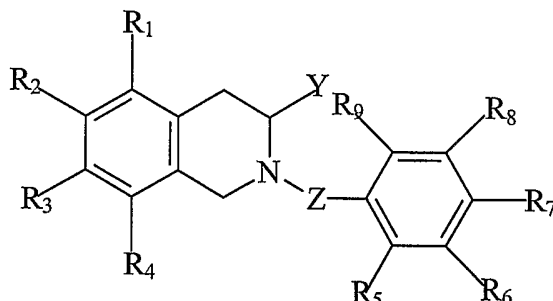
Taken together, these experimental results demonstrate that the compounds of the present invention function *in vivo* and *in vitro* as competitive inhibitors of estrogen binding to estrogen receptors.

While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claims. Accordingly, the present invention should not be limited to the embodiments as described above, but should further include all modifications and equivalents thereof within the spirit and scope of the description provided herein.

## CLAIMS

We claim:

1. A compound of formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R<sub>10</sub> and -CH<sub>2</sub>-NH-R<sub>10</sub>; and R<sub>10</sub> is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

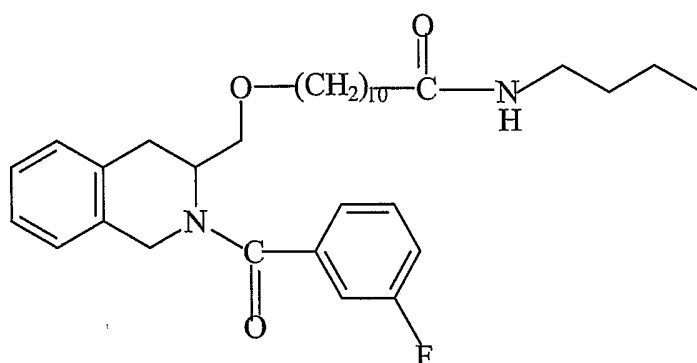
d)  $-(\text{CH}_2)_n\text{-S(=O)-R11}$ , where  $n = 1-10$  and R11 is selected from: substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

e)  $-(\text{CH}_2)_n\text{-SO}_2\text{-R11}$ , where  $n = 1-10$  and R11 is selected from the group consisting of substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

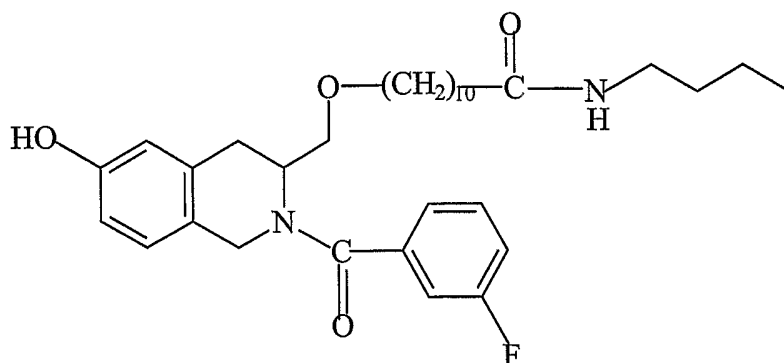
2. The compound of claim 1 wherein said substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl is  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CF}_2\text{CF}_3$ .

3. The compound of claim 1 wherein R1, R2, R3, R4, R5, R6, R7, R8 and R9 are selected from the group consisting of F,  $\text{OCH}_3$ , OH,  $\text{CH}_3$  and Cl.

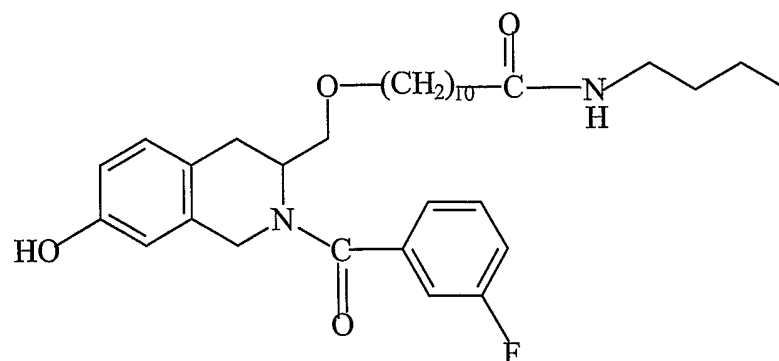
4. A compound of formula



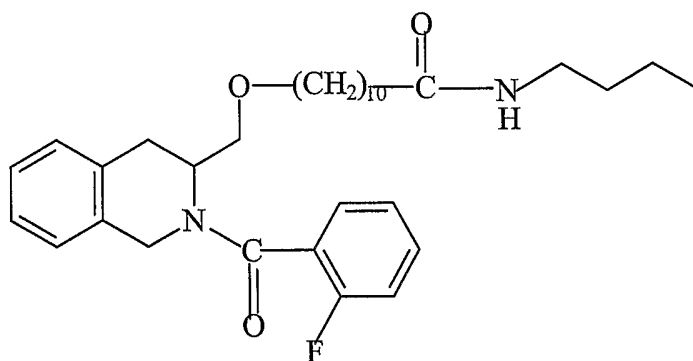
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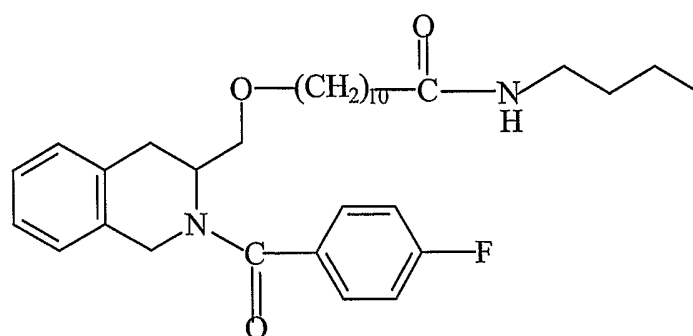
6. A compound of formula



7. A compound of formula

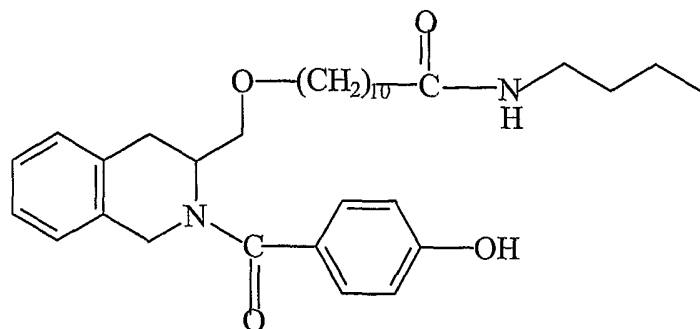


8. A compound of formula

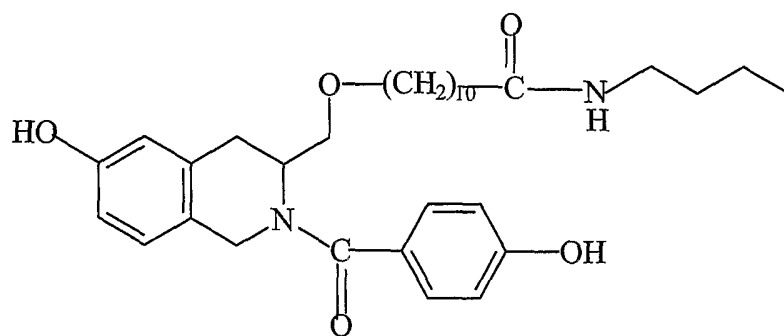




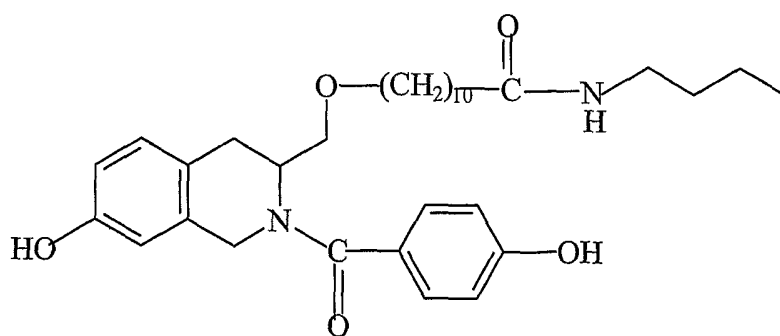
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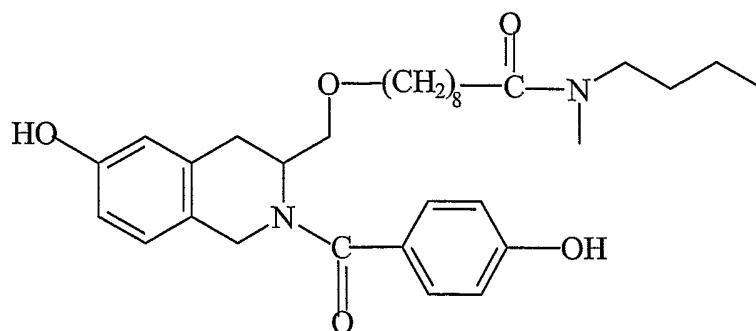
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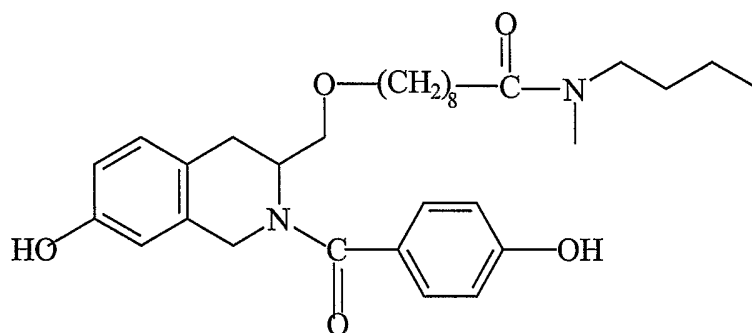
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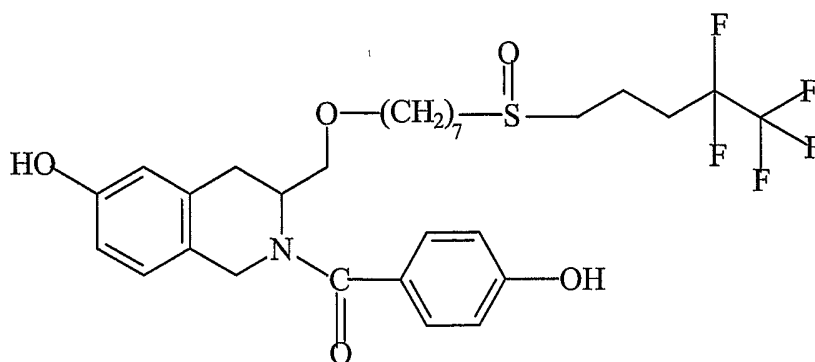
15. A compound of formula



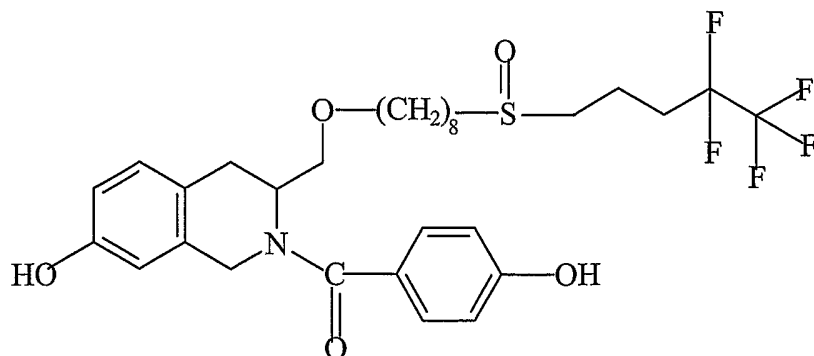
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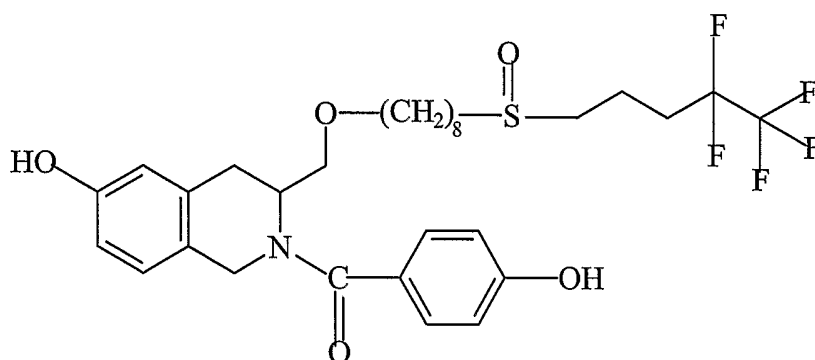
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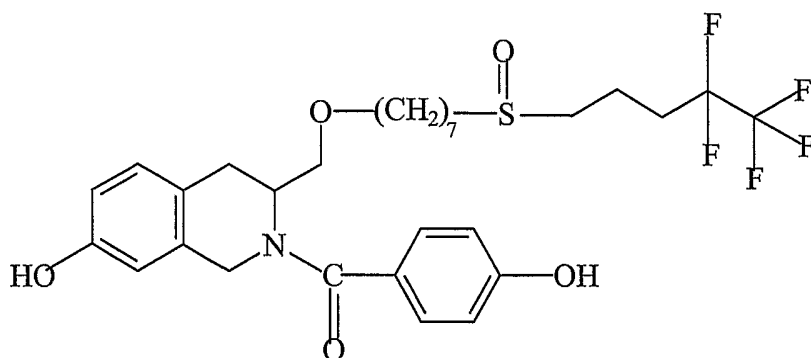
18. A compound of formula



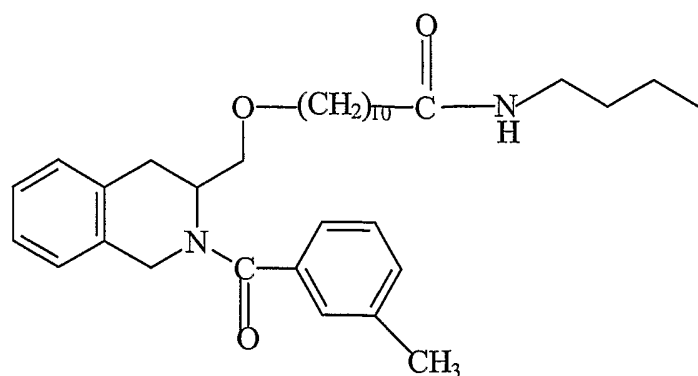
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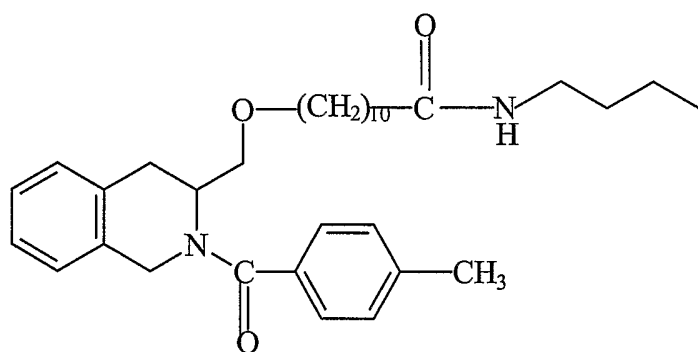
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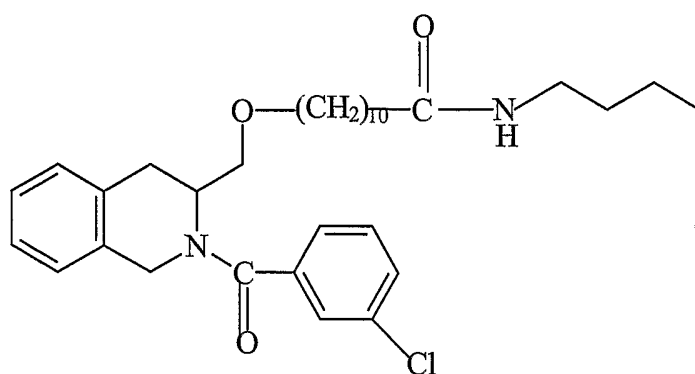
21. A compound of formula



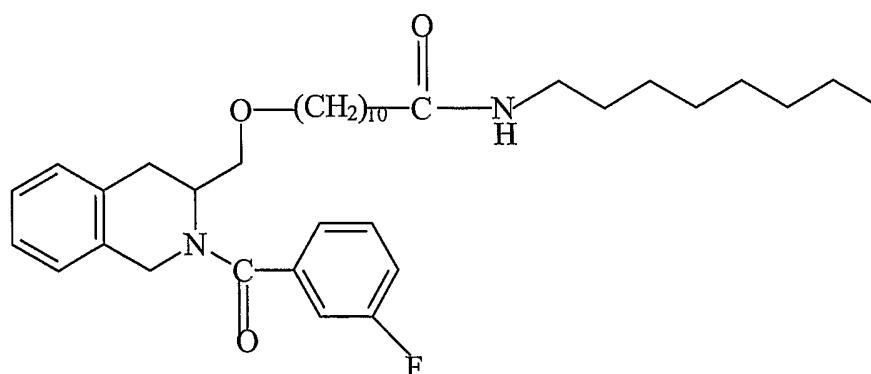
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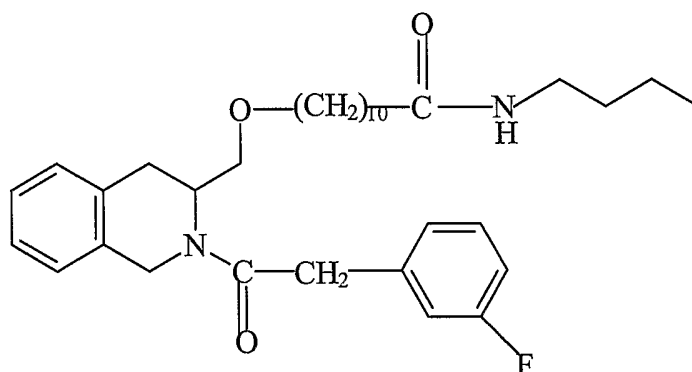
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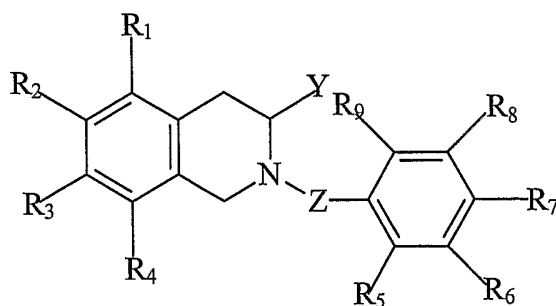
24. A compound of formula



25. A compound of formula



26. A method of inhibiting binding of estrogen *in vivo* or *in vitro* by providing a compound which binds to an estrogen binding site, said compound having the generic formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R1, R2, R3, R4, R5, R6, R7, R8 and R9 are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R10 and -CH<sub>2</sub>-NH-R10; and R10 is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R11, R12, where n = 1-10 and R11 and R12 are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

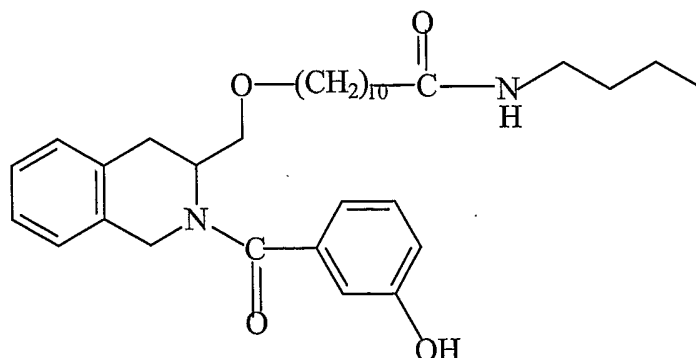
d) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-R11, where n = 1-10 and R11 is selected from: substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

e) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-R11, where n = 1-10 and R11 is selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

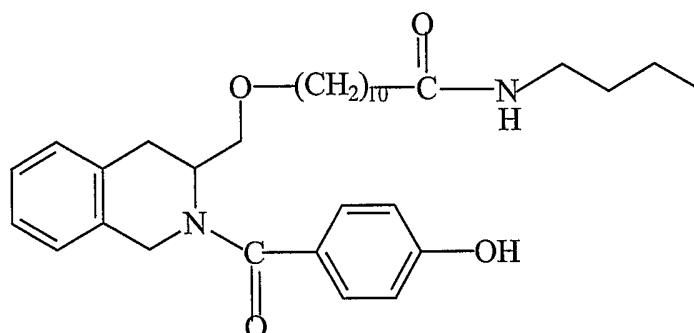
27. The method of claim 26, wherein said substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl is -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CF<sub>2</sub>CF<sub>3</sub>.

28. The compound of claim 26 wherein R1, R2, R3, R4, R5, R6, R7, R8 and R9 are selected from the group consisting of F, OCH<sub>3</sub>, OH, CH<sub>3</sub> and Cl.

29. The method of claim 26, wherein said compound is

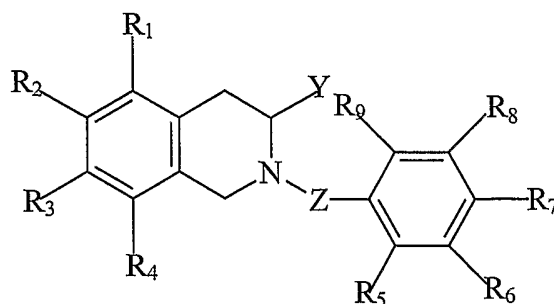


30. The method of claim 26, wherein said compound is



31. A method for treating tamoxifen-resistant breast cancer tumors in a patient in need thereof, comprising the step of

administering to said patient a compound of generic formula



wherein

Z is selected from the group consisting of CO, CH<sub>2</sub>, and CO(CH<sub>2</sub>)<sub>n</sub>, where n = 1 or 2;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are the same or different, and are selected from the group consisting of H, OH, halogens, R and OR, where R is a substituted or unsubstituted alkyl group having 1-4 carbons;

Y is selected from the group consisting of -CH<sub>2</sub>-O-R<sub>10</sub> and -CH<sub>2</sub>-NH-R<sub>10</sub>; and R<sub>10</sub> is selected from the group consisting of :

a) -(CH<sub>2</sub>)<sub>n</sub>-C(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

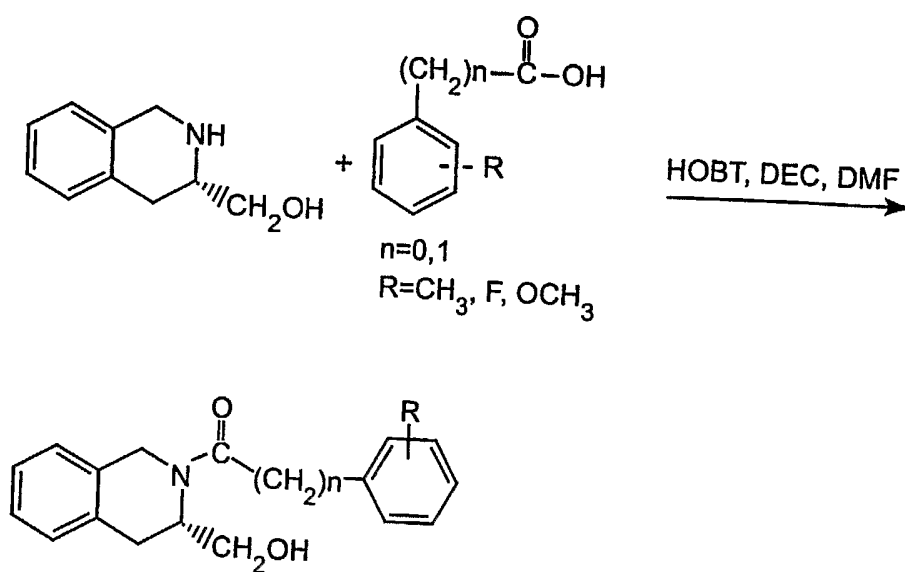
b) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

c) -(CH<sub>2</sub>)<sub>n</sub>-SO<sub>2</sub>-N-R<sub>11</sub>, R<sub>12</sub>, where n = 1-10 and R<sub>11</sub> and R<sub>12</sub> are the same or different, and are selected from the group consisting of substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl;

d) -(CH<sub>2</sub>)<sub>n</sub>-S(=O)-R<sub>11</sub>, where n = 1-10 and R<sub>11</sub> is selected from: substituted and unsubstituted C<sub>1</sub>-C<sub>9</sub> alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl; and

e)  $-(\text{CH}_2)_n\text{-SO}_2\text{-R11}$ , where  $n = 1\text{-}10$  and R11 is selected from the group consisting of substituted and unsubstituted  $\text{C}_1\text{-C}_9$  alkyl, substituted and unsubstituted cycloalkyl, and substituted and unsubstituted aryl.

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*Figure 1*

2/7

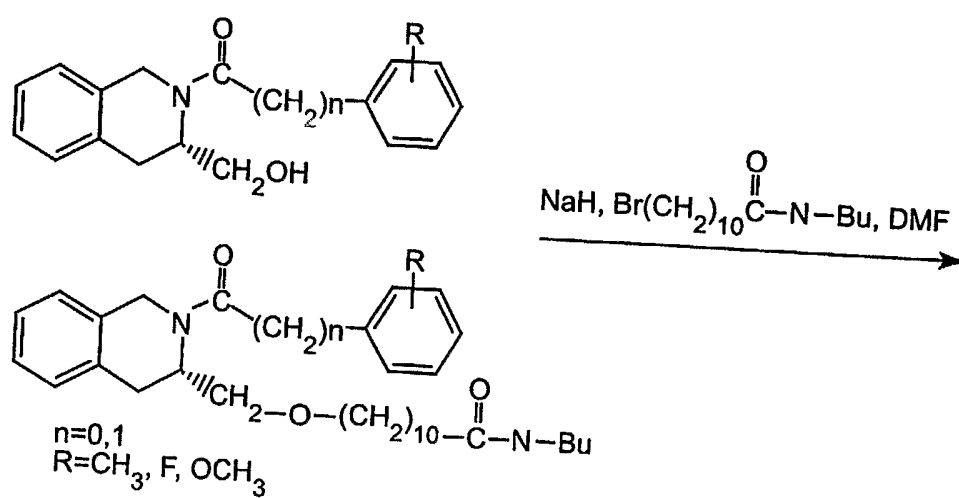
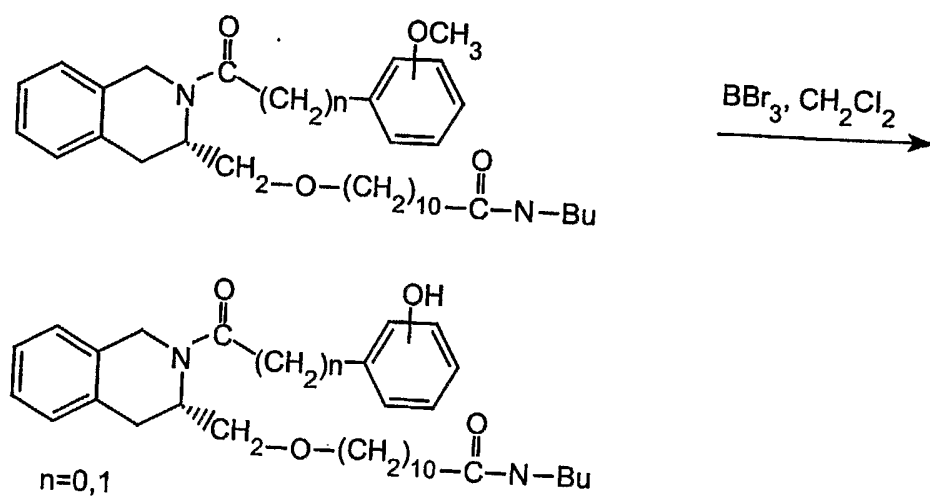


Figure 2

3/7

*Figure 3*

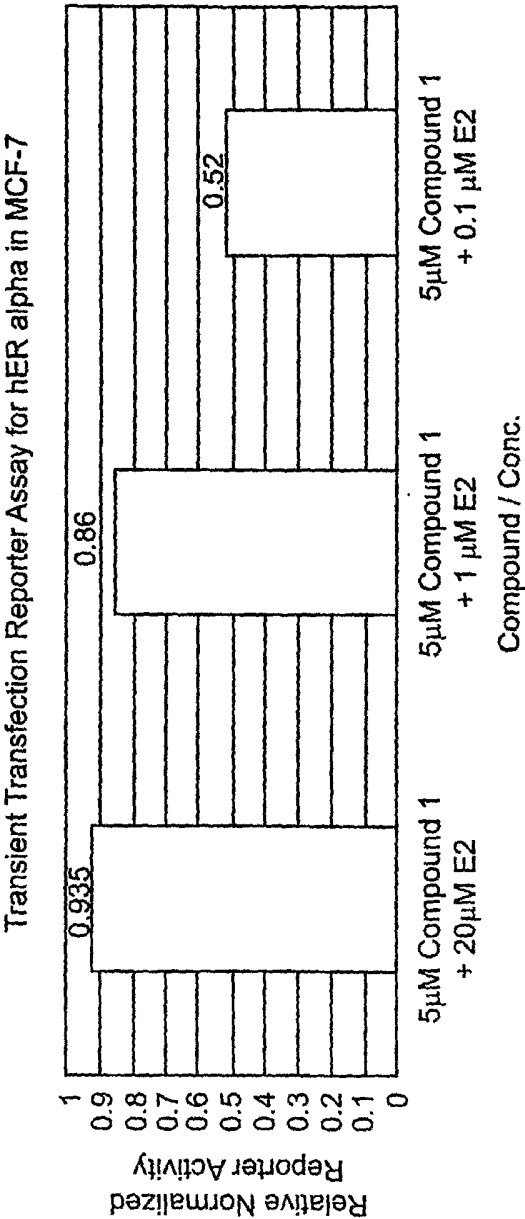


Figure 4

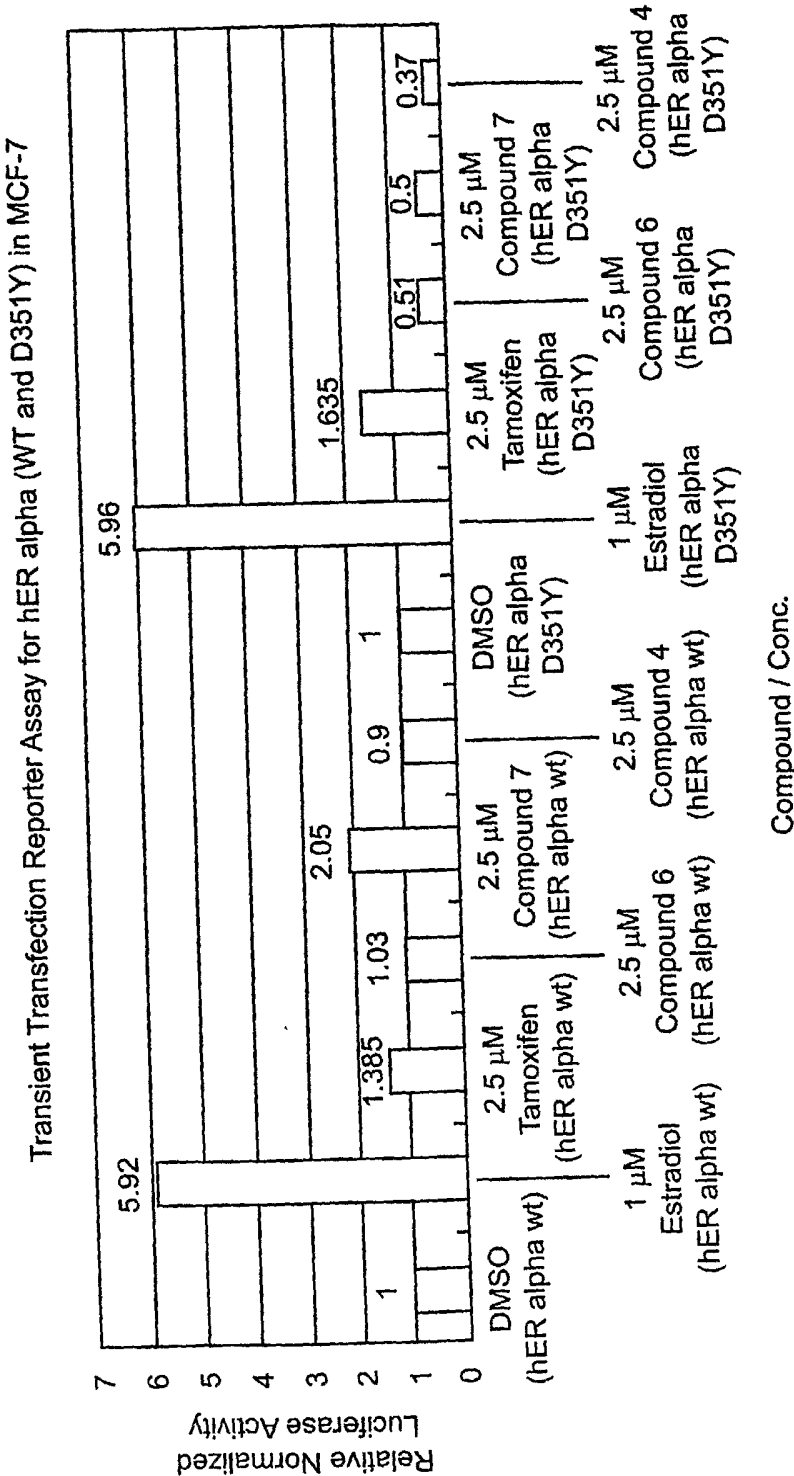


Figure 5

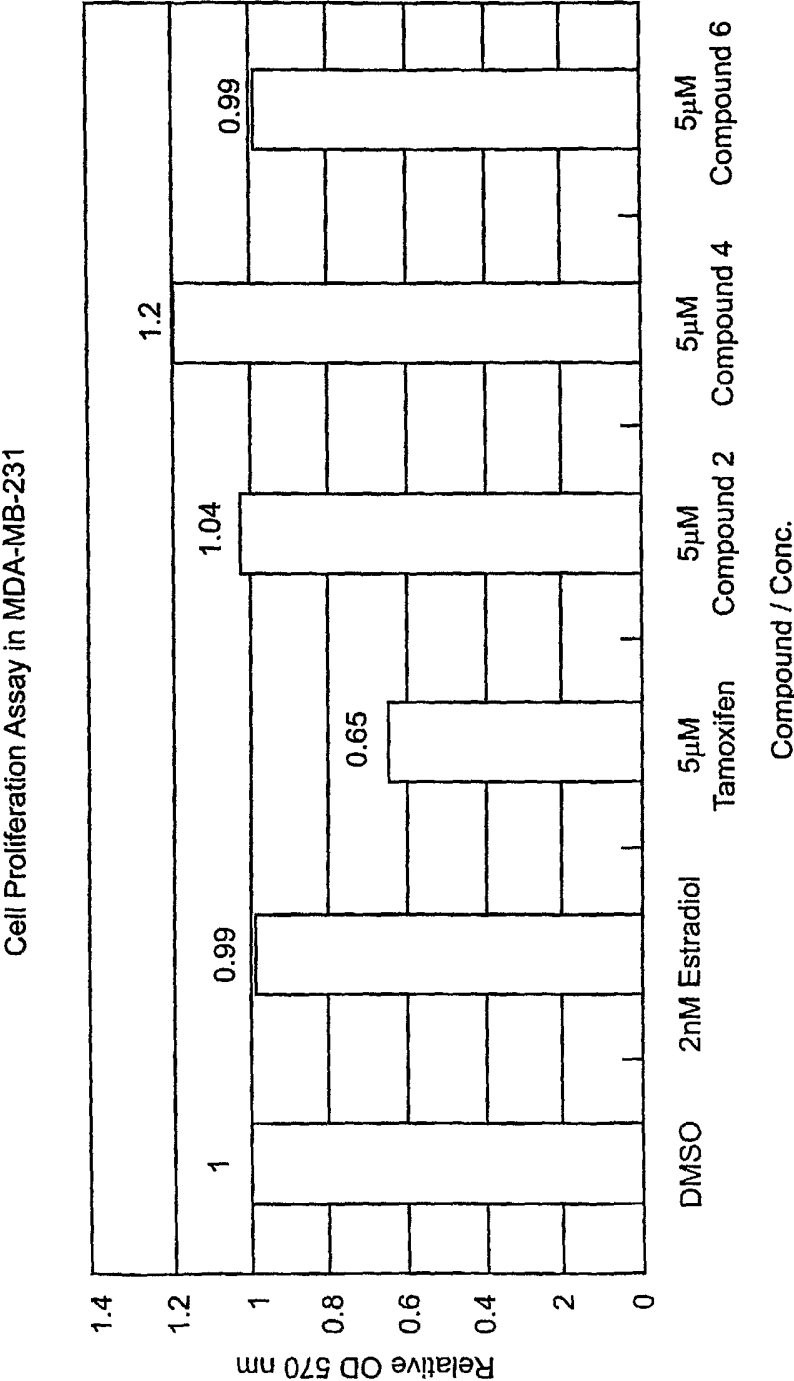
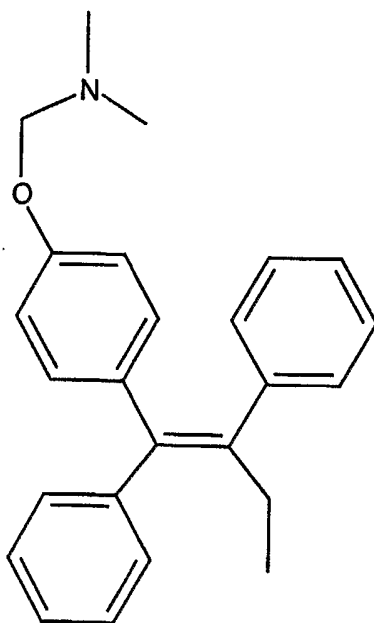
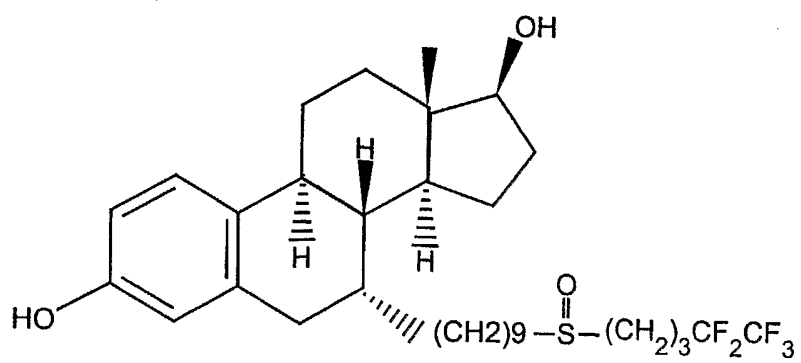


Figure 6



**Figure 7A**



*Figure 7B*

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/25186

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 217/00; A61K 31/47

US CL : 514/307; 546/146,149

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/307; 546/146,149

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
CAS ONLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages    | Relevant to claim No. |
|------------|---------------------------------------------------------------------------------------|-----------------------|
| A          | WO 95/35316 A1( ASTRA AKTIEBOLAG) 28 December 1995 (28.12.1995), see entire document. | 1-31                  |

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Further documents are listed in the continuation of Box C.

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See patent family annex.

|                                                                                                                                                                         |     |                                                                                                                                                                                                                                              |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents                                                                                                                                 | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
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