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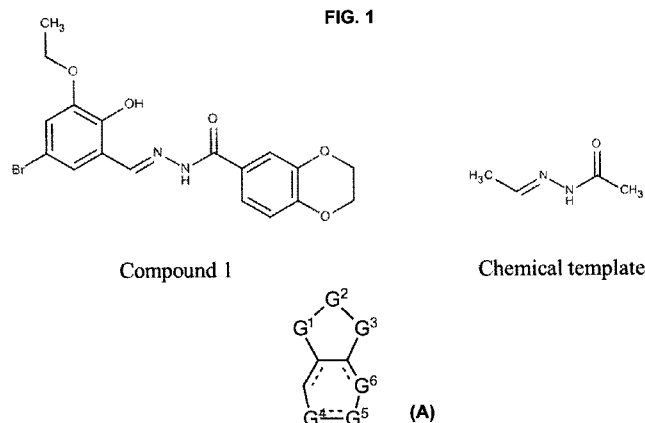
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(54) Title: INHIBITORS OF ESTROGEN RECEPTOR ALPHA AND THEIR USE AS THERAPEUTICS FOR CANCER



(57) Abstract: This invention provides of compounds having structures of Formula A, uses of these compounds for treatment of various indications, including breast cancer, as well as methods of treatment involving these compounds.

INHIBITORS OF ESTROGEN RECEPTOR ALPHA AND THEIR USE AS THERAPEUTICS FOR CANCER

TECHNICAL FIELD

This invention relates to therapeutics, their uses and methods for treatment. In particular the invention relates to therapies and methods of treatment for breast cancer.

BACKGROUND

Approximately 24,000 Canadian women will be diagnosed with breast cancer (BCa) and 5,000 will die from it this year alone (Canadian Cancer Society, 2013). Since more than 75% of BCa cases are estrogen receptor- α (ER α) positive, treatment of these cancers with anti-estrogens, such as Tamoxifen, has been the main therapeutic approach for more than 30 years. Tamoxifen is an ER α modulator, which demonstrates selective antagonist activity in the breast and agonist activity in the endometrium and bone. Unfortunately, one third of women treated with Tamoxifen for 5 years develop recurrent disease within 15 years. There are several molecular mechanisms involved in the development of such resistance, including mutations in ER α protein, loss of ER α expression and post-translational modifications of ER α . Experimental and clinical observations have suggested that ER α signaling continues to play an important role even after the development of resistance. Biopsies from BCa patients who relapsed on Tamoxifen, indicated that ER α expression was retained in more than 50% of the cases.

Commercial and clinical ER α inhibitors share a similar chemical scaffold and act by direct binding to the receptor's estrogen binding site (EBS). Thus, such closely related chemicals are similarly vulnerable to mutations arising around the EBS, which can confer resistance (Robinson et al., 2013; Toy et al., 2013). Moreover, cross-talk between ER α and activated growth factor receptor pathways and notch signaling pathways (Hao et al., 2010) have been shown to play a major role in enhanced receptor-coactivator interaction and thereby

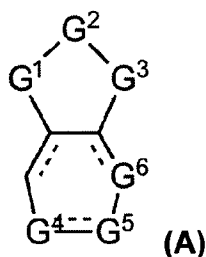
activating ER α , even in the absence of its native ligand, 17 β -estradiol (E2). Thus, drugs that target ER α EBS can become ineffective with time.

The AF2 pocket plays a pivotal role in activation and functioning of ER α . Upon binding of E2 ligand, ER α undergoes a series of conformational changes that allow the formation of AF2 on the surface of the receptor's ligand binding domain (ER α LBD). The AF2 is a deeply buried hydrophobic cavity, located adjacent to (but distinct from) the EBS. It recruits a variety of co-activators and mediates diverse functions of ER α . Recent findings suggest that ER α co-activators are differentially expressed in malignant tumours and that their functions may be altered, leading to tumour progression. For example, coactivators such as SRC-1 and CBP (which possess histone acetyl transferase activity) have been shown to be amplified in BCa. Hao et al suggested that AF2 can also directly recruit p300 coactivator, independently from E2 via the Notch-1 signaling pathway, which activates the ER α in BCa (Hao et al., 2010). In addition, Proline-glutamic acid-leucine-rich protein-1 (PELP1), a coactivator protein that is known to bind to the AF2 site plays a central role in ER α + metastasis (Habashy et al., 2010).

SUMMARY

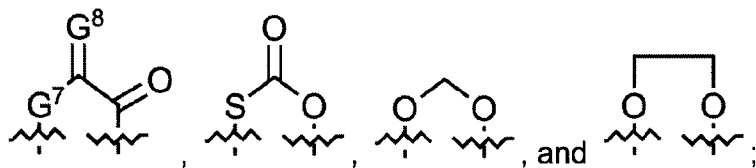
This invention relates to therapeutics, their uses and methods for treatment for breast cancer. In particular, therapeutics of the present invention may be effective by targeting the activation of function 2 site of estrogen receptor alpha and may be used a therapeutics for hormone-resistant and metatstatic breast cancer.

In illustrative embodiments of the present invention, there is provided compound of Formula A,

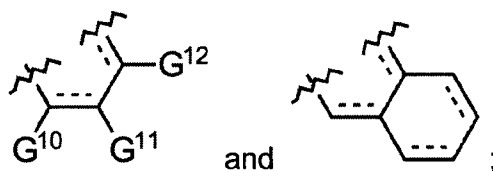


wherein

G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

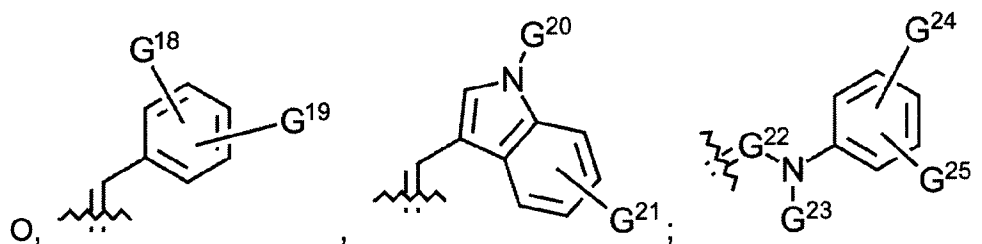


G^4 , G^5 , and G^6 , when taken together are selected from the group consisting of:

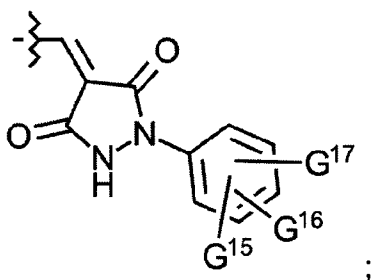


G^7 is selected from the group consisting of: S, CH_2 , and O;

G^8 is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , $\text{CH}_2\text{-CH}_3$, OCH_3 , NO_2 , and



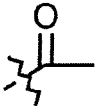
G^{15} , G^{16} , and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $\text{O-CH}_2\text{-CH}_3$, and NO_2 ;

G^{18} is selected from the group consisting of: OH, CH_3 , and $\text{CH}_2\text{-CH}_3$;

G^{19} is selected from the group consisting of: H, OH, CH₃, and CH₂-CH₃;

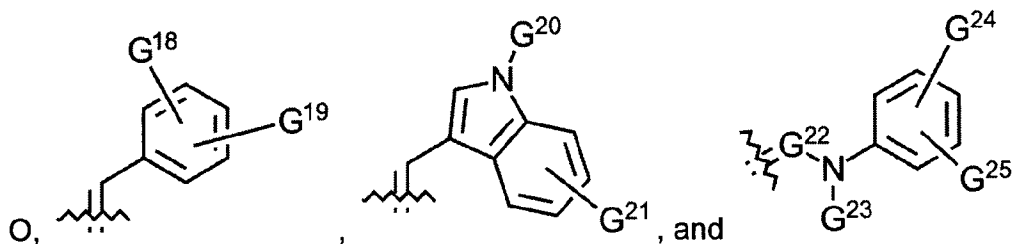
G^{20} and G^{21} are each independently selected from the group consisting of: H, CH₃, CH₂-CH₃, CH₂-CH₃-CH₃, and CH₃-CH₂-CH₃;

G^{22} is selected from the group consisting of: N and CH;

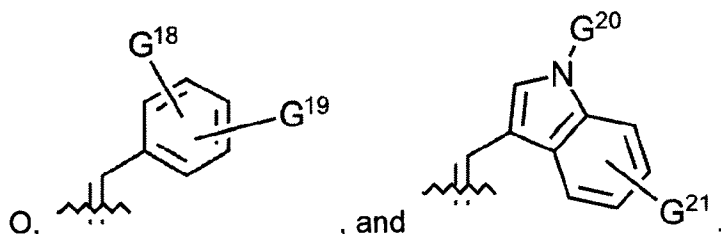
G^{23} is selected from the group consisting of: H and  ;

G^{24} and G^{25} are each independently selected from the group consisting of: H, Cl and CH₃, provided that when G^{25} is CH₃, then either i) G^{24} is Cl, or ii) G^7 is CH₂.

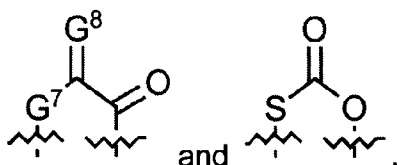
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^8 is selected from the group consisting of:



In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^8 is selected from the group consisting of:

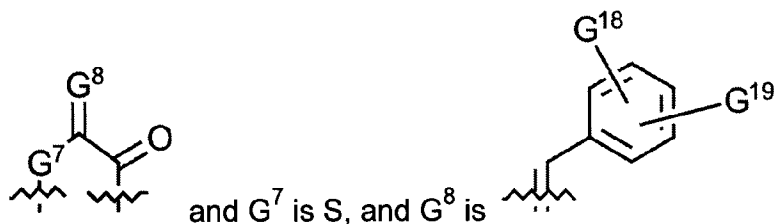


In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:



In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^7 is S.

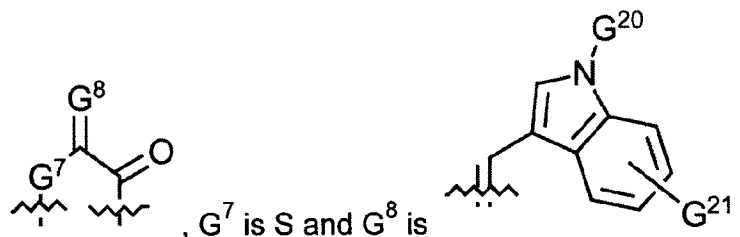
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^1 , G^2 , and G^3 , when taken together, are



In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{18} is OH.

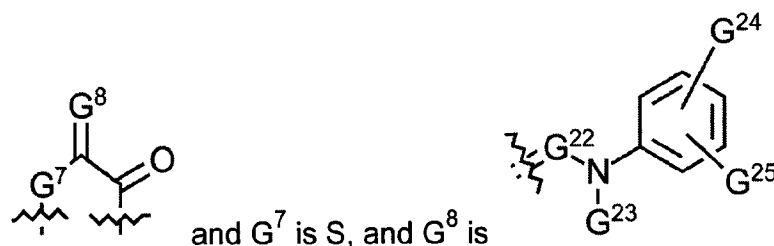
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{19} is OH.

In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^1 , G^2 , and G^3 , when taken together, are



In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{20} is H and G^{21} is CH_2-CH_3 .

In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^1 , G^2 , and G^3 , when taken together, are

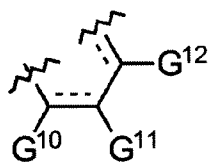


In illustrative embodiments of the present invention, there is provided a

compound described herein wherein G^{22} is CH_2 and G^{23} is .

In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{24} is Cl and G^{25} is H.

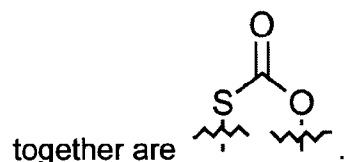
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^4 , G^5 , and G^6 , when taken together, are



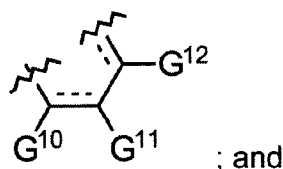
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{10} and G^{11} are each H.

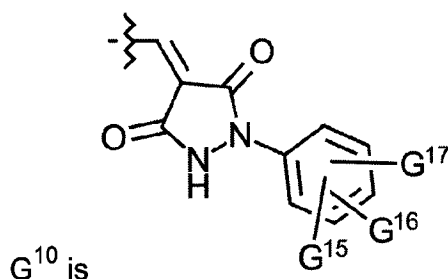
In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^{12} is CH_3 or F.

In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^8 is O and G^1 , G^2 , and G^3 , when taken



In illustrative embodiments of the present invention, there is provided a compound described herein wherein G^4 , G^5 , and G^6 , when taken together, are





In illustrative embodiments of the present invention, there is provided a compound described herein wherein G¹¹ is H; and G¹² is OCH₃.

In illustrative embodiments of the present invention, there is provided a pharmaceutical composition comprising a compound described herein.

In illustrative embodiments of the present invention, there is provided use of a compound described herein in the treatment of cancer.

In illustrative embodiments of the present invention, there is provided a method of medical treatment comprising administering to a subject having or suspected of having breast cancer a compound described herein.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention,

Figure 1 - Chemical template derived based on Compound 1 is used as a query to find analogues of compound 1 by 2D similarity search method.

Figures 2A, 2B, 2C, and 2D - Graphical representation of results from an experiment analyzing a Mammalian Two-Hybrid System.

Figures 3A, 3B, 3C and 3D - Graphical representation of results from an experiment analyzing Down-regulation of ER target genes.

Figure 4 - Graphical representation of results showing dose-response curves of VPC-16464 in an experiment analyzing cell viability as assessed by the Presto Blue viability assay.

Figure 5 - Graphical representation of results from an experiment analyzing Inhibition of constitutively active mutant form of ER.

Figures 6A and 6B – Graphic representation of ER α AF2 pocket and *in silico* screening pipeline. (A) Graphic representation of the AF2 site on the surface of the ER α -LBD [PDB: 3UUD]. (B) Virtual screening protocol used for the discovery of AF2 binders. The numbers indicate compounds obtained after each screening step.

Figures 7A, 7B, 7C and 7D – Graphical representation of results from an experiment showing that compounds inhibit ER α transcriptional activity and coactivator binding at the AF2 site.

Figure 8 – Graphical representation of results from an experiment showing dose response curves of compounds that are right-shifted in the presence of higher concentrations of coactivator peptide.

Figure 9 – Graphical representation of results from an experiment showing that compounds do not displace E2 from HBS of ER α .

Figure 10 – Graphical representation of results from an experiment showing dose response curves of compounds that do not shift in the presence of higher concentrations of E2.

Figures 11A, 11B, 11C and 11D – Graphical representation of results from an experiment showing that compounds show direct reversible binding to the ER α -LBD.

Figures 12A, 12B, and 12 C – Graphical representation of results from an experiment showing that compounds affect the viability of ER α -positive cells.

Figures 13A, 13B, 13C, and 13D – Graphical representation of results from an experiment showing that VPC-16230 and VPC-16225 inhibit ER α transcriptional activity in tamoxifen resistant cells.

Figures 14A, 14B and 14C – Graphical representation of results from an experiment showing that compounds inhibit mRNA and protein expression levels of ER α dependent genes.

Figures 15A, 15B, 15C, and 15D – Graphical representation of binding orientation of VPC-13002 and VPC-16230 inside the ER AF2 pocket. (A)

Overlay of the compound VPC-13002 binding pose over the α -helical LXXLL motif. Indole and aryl group of VPC-13002 overlaps with the Leucines at *i* and *i*+4 positions of the LXXLL motif of the co-activator, (B) Predicted binding orientation of VPC-13002 inside the ER AF2 site, (C) Overlay of VPC-16230 binding pose over the α -helical LXXLL motif, (D) Predicted binding orientation of VPC-16230 inside the ER AF2 site.

DETAILED DESCRIPTION

This invention is based in part on compounds described herein that modulate Estrogen Receptor alpha (ER α) activity. Specifically, compounds of the present invention show inhibition of identified ER α transcriptional activity by disrupting the interaction between the ER α and its co-activator proteins at the Activation Function-2 (AF2) regulatory interface of the receptor. This may make these compounds suitable for use in the treatment of cancer, in particular, breast cancer.

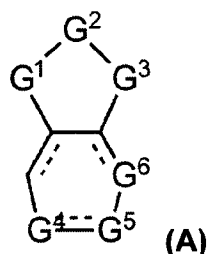
The present invention, at least in part, addresses the problem of rising drug resistance in BCa by using computer modeling, biological screening and medicinal chemistry to develop a class of drugs that directly target the co-activator binding pocket (AF2 site) on ER α (without any cross-reactivity toward the EBS) and therefore, may not suffer from EBS resistant mutations.

ER α belongs to the same nuclear receptor superfamily as the Androgen Receptor (AR), and although the nuclear receptors are involved in different physiological processes, all of them share the same domain organization. X-ray crystallography studies at the AF2 site have revealed that despite the high sequence homology between nuclear receptors' AF2, they possess different surface shapes and electrostatic characteristics, which may be exploited to achieve selectivity (Caboni et al., 2013).

A computational drug discovery pipeline was employed to identify more potent and selective inhibitors of ER α that specifically bind to the AF2 site and inhibit the interaction between ER α and its coactivator proteins. Compounds of the present invention significantly inhibit ER α transcriptional activity by blocking

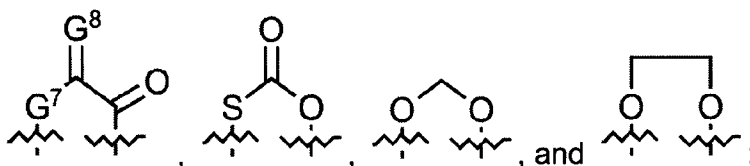
the association of coactivators at the AF2 site. They also demonstrate good anti-proliferative activity and reduced the expression of ER α -dependent genes. Compounds of the present invention also inhibit the growth of TamR cell lines in an ER α -specific manner.

In accordance with one embodiment, there is provided a compound or pharmaceutically acceptable salt having a structure of Formula A:

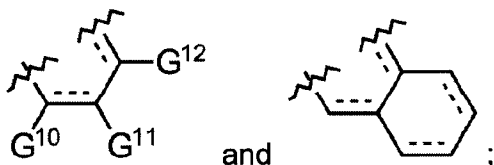


wherein

G¹, G², and G³, when taken together are selected from the group consisting of:

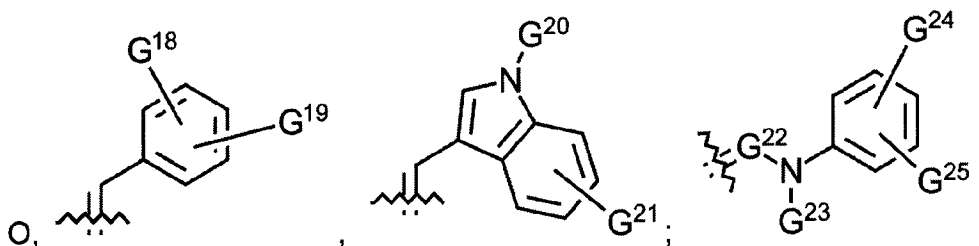


G⁴, G⁵, and G⁶, when taken together are selected from the group consisting of:

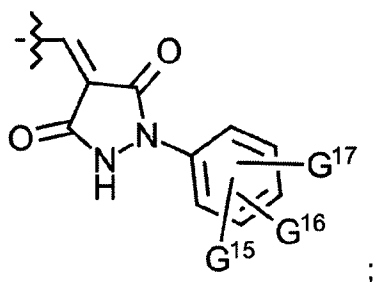


G⁷ is selected from the group consisting of: S, CH₂, and O;

G⁸ is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , CH_2-CH_3 , OCH_3 , NO_2 , and



G^{15} , G^{16} , and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $O-CH_2-CH_3$, and NO_2 ;

G^{18} is selected from the group consisting of: OH, CH_3 , and CH_2-CH_3 ;

G^{19} is selected from the group consisting of: H, OH, CH_3 , and CH_2-CH_3 ;

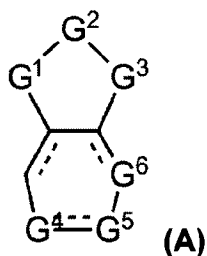
G^{20} and G^{21} are each independently selected from the group consisting of: H, CH_3 , CH_2-CH_3 , $CH_2-CH_3-CH_3$, and $CH_3-CH_2-CH_3$;

G^{22} is selected from the group consisting of: N and CH;

G^{23} is selected from the group consisting of: H and ;

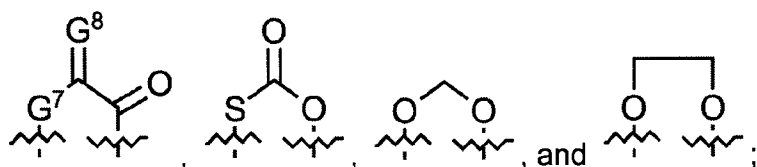
G^{24} and G^{25} are each independently selected from the group consisting of: H, Cl and CH_3 , provided that when G^{25} is CH_3 , then either i) G^{24} is Cl, or ii) G^7 is CH_2 .

In accordance with another embodiment, there is provided a use of a compound or pharmaceutically acceptable salt having a structure of Formula A:

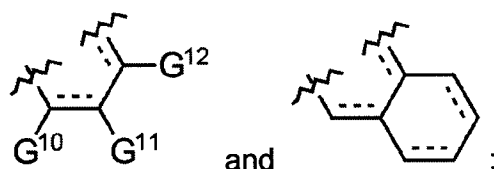


wherein

G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

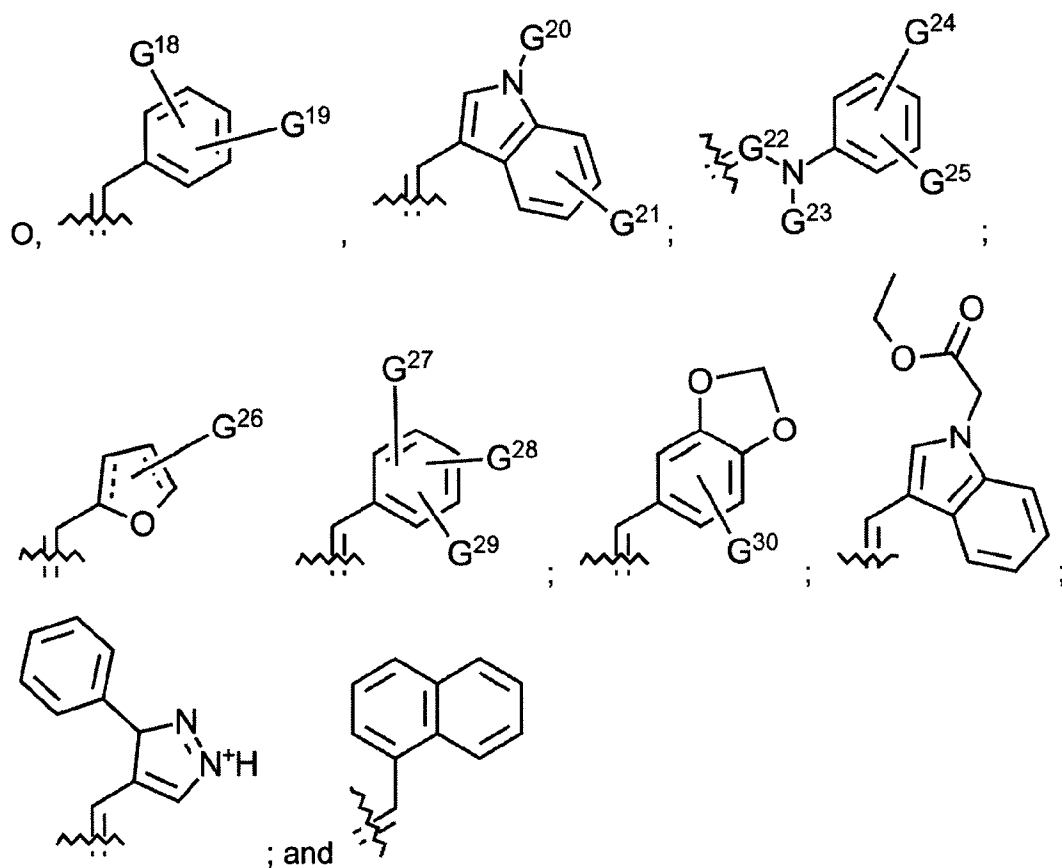


G^4 , G^5 , and G^6 , when taken together are selected from the group consisting of:

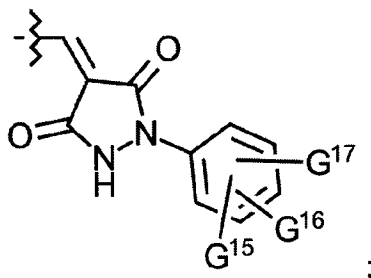


G^7 is selected from the group consisting of: S, CH_2 , and O;

G^8 is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , CH_2-CH_3 , OCH_3 , NO_2 , and



G^{15} , G^{16} , and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $O-CH_2-CH_3$ and NO_2 ;

G^{18} is selected from the group consisting of: OH and CH_3 , and CH_2-CH_3 ;

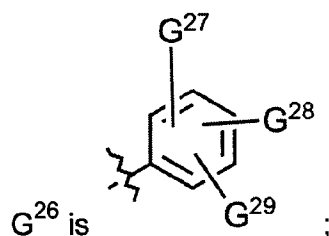
G^{19} is selected from the group consisting of: H, OH, CH_3 , and CH_2-CH_3 ;

G^{20} and G^{21} are each independently selected from the group consisting of: H, CH_3 , CH_2-CH_3 , $CH_2-CH_3-CH_3$, and $CH_3-CH_2-CH_3$;

G^{22} is selected from the group consisting of: N and CH;

G^{23} is selected from the group consisting of: H and  ;

G^{24} and G^{25} are each independently selected from the group consisting of: CH_3 , Cl, H, F and $O-CH_3$;



G^{27} , G^{28} , and G^{29} are each independently selected from the group consisting of: Cl, CH_3 , $O-CH_3$, $O-CH_2-CH_3$, and NO_2 ;

G^{30} is NO_2 or H.

In accordance with another embodiment, there is provided a use of a compound or pharmaceutically acceptable salt having a structure as outlined in Table 1, Table 2, Table 3, Table 4, Table 5, and/or Table 6.

In accordance with another embodiment, there is provided a use of a compound or pharmaceutically acceptable salt having a structure as outlined in Table 1, Table 2, Table 5, and/or Table 6.

In accordance with another embodiment, there is provided a commercial package, including a compound or pharmaceutically acceptable salt thereof as described herein and instructions for use in modulating Estrogen Receptor alpha (ER α) activity.

The compounds or pharmaceutically acceptable salt of the present invention may also be used in the manufacture of a medicament for modulating Estrogen Receptor alpha (ER α) activity. Alternatively, a pharmaceutical composition described herein may be used for modulating Estrogen Receptor alpha (ER α) activity. The modulating of Estrogen Receptor alpha (ER α) activity may be used for the treatment of cancer. The modulating of Estrogen Receptor alpha (ER α) activity may be used for the treatment of at least one indication selected from the group including: melanoma, colorectal cancer, pancreatic cancer, hepatocellular cancer, esophageal cancer, sarcoma, lung cancer, breast cancer, cervical cancer, ovarian cancer, or gastric cancer. The compounds or pharmaceutically acceptable salt thereof or pharmaceutical composition described herein may be used for the treatment of cancer wherein the cancer is primary or metastatic cancer.

The following tables provide the structures and measured activities of compounds described herein.

Table 1 – Structures and Measured Activities of Identified ER AF2 Inhibitors.			
VPC-ID	Structure	ER Inhibition IC ₅₀ (μM)	Peptide Displacement IC ₅₀ (μM)

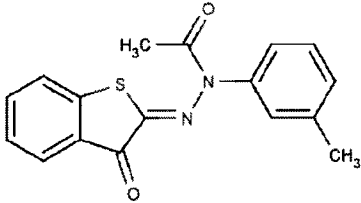
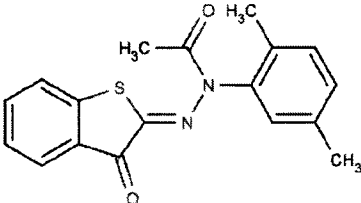
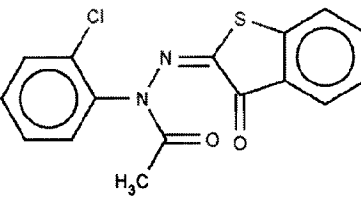
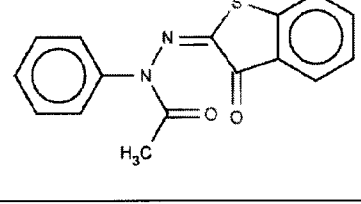
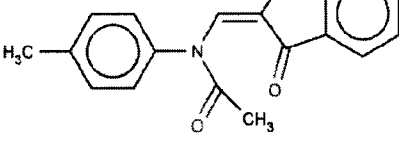
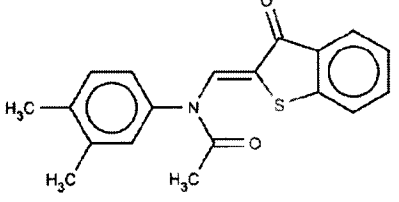
Table 1 – Structures and Measured Activities of Identified ER AF2 Inhibitors.			
16457		0.73	Active
16458		Not tested	Not tested
16464		2.33	Active
16465		5.33	Active
16475		12.25	Not tested
16484		9.833	Not tested

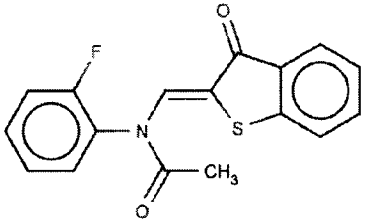
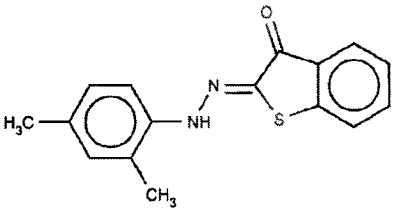
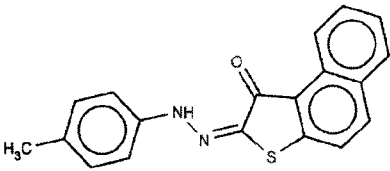
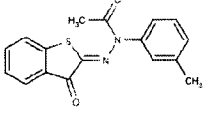
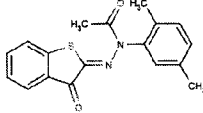
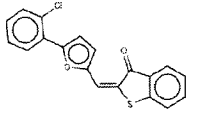
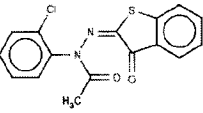
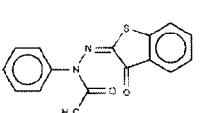
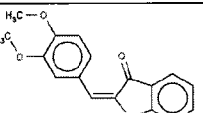
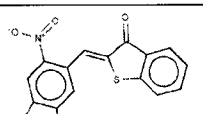
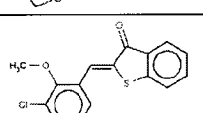
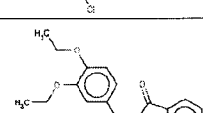
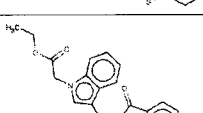
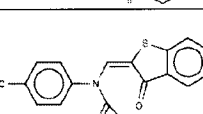
Table 1 – Structures and Measured Activities of Identified ER AF2 Inhibitors.			
16487		4.643	Not tested
16492		8.673	Not tested
16494		Not tested	Not tested

Table 2 – Structures and Measured Activities of Potential ER AF2 Inhibitors.
 Italicised ID numbers (in red) represent structures tested in TABLE 1.

Cell Inhibition (T47D cell line)						
compound #	ZINC ID	Structure	30uM	10uM	IC50 (uM)	Peptide displacement
16457	ZINC00227376		59.50	37.29	0.78	Not tested
16458	ZINC00227373		40.45	40.38	Not tested	Not tested
16459	ZINC00340283		98.52	95.60	6.54	Not tested
16464	ZINC00227362		102.57	100.63	2.33	Active
16465	ZINC00227356		100.23	99.57	5.33	Active
16466	ZINC00195408		91.45	88.16	1.55	Not tested
16467	ZINC00291665		92.26	64.18	12.5	Not tested
16468	ZINC00285355		83.90	79.55	1.163	Not tested
16469	ZINC00194123		81.34	71.77	3.214	Not tested
16473	ZINC00693656		85.51	79.52	3.08	Not tested
16475	ZINC00284077		83.99	4.87	12.25	Not tested

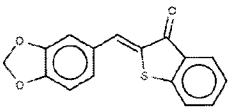
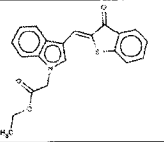
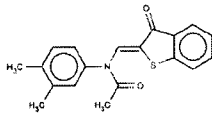
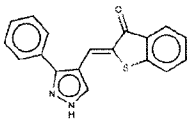
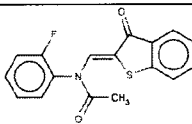
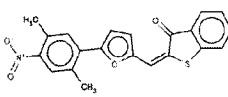
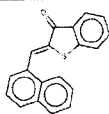
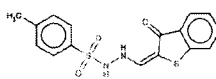
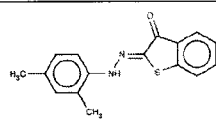
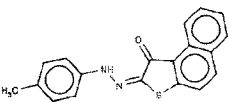
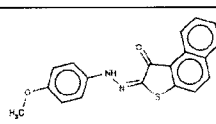
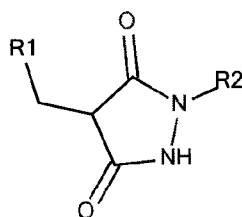
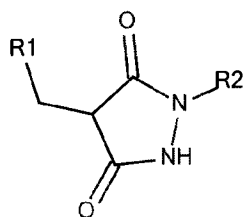
16476	ZINC00296948		55.43	59.37	1.082/1.2	Not tested
16480	ZINC13424355		79.46	63.35	0.35	Not tested
16484	ZINC00186136		90.16	23.41	9.833	Not tested
16485	ZINC00179559		93.59	73.66	0.6379	Not tested
16487	ZINC00521673		54.20	43.72	4.643	Not tested
16488	ZINC00809288		60.99	50.74	Not tested	Not tested
16490	ZINC01465938		81.71	79.30	0.7965	Not tested
16491	ZINC02287548		41.24	17.63	Not tested	Not tested
16492	ZINC02372749		98.46	74.99	8.673	Not tested
16494	ZINC04499527		42.77	37.38	Not tested	Not tested
16497	ZINC04499530		51.51	30.90	0.1827	Not tested

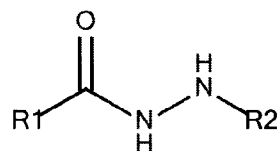
Table 3 – Structures and Measured Activities of Identified ER AF2 Inhibitors (pyrazolidine-3, 5 dione derivatives).

VPC-ID	R1	R2	Peptide Displacement IC ₅₀ (μM)	ER Inhibition IC ₅₀ (μM)
13002			2.46	7.60
16019			3.16	9.62
16041			4.33	8.24
16046			5.38	16.34
16038			7.98	9.75
16007			11.27	~25
16040			19.54	~35

Table 3 – Structures and Measured Activities of Identified ER AF2 Inhibitors
(pyrazolidine-3, 5 dione derivatives).

VPC-ID	R1	R2	Peptide Displacement IC ₅₀ (μM)	ER Inhibition IC ₅₀ (μM)
16003			29.10	> 50
16004			31.98	> 50
16021			>100	> 50

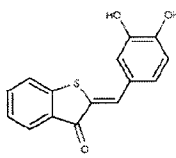
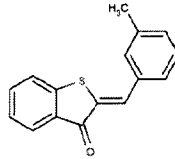
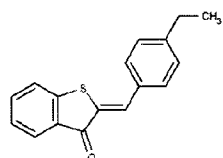
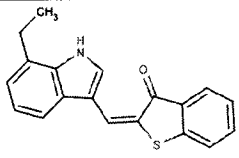
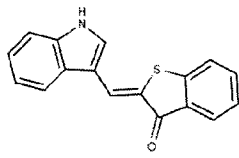
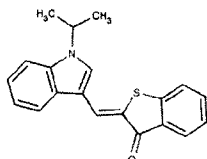
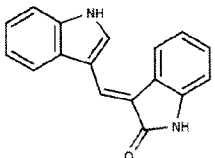
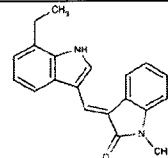
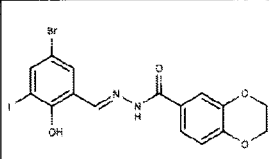
Table 4 – Structures and Measured Activities of Identified ER AF2 Inhibitors (acetohydrazide derivatives).

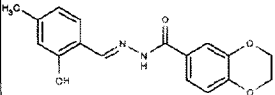
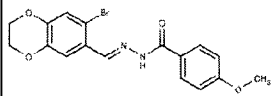
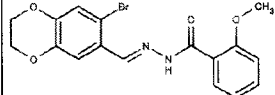
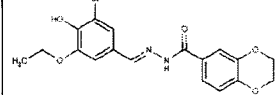
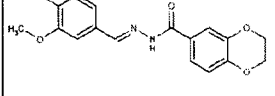
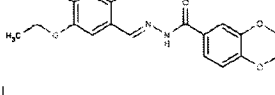
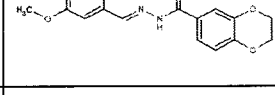
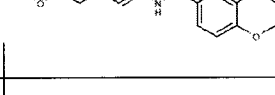
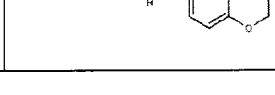


VPC-ID	R1	R2	Peptide Displacement IC ₅₀ (μM)	ER Inhibition IC ₅₀ (μM)
16230			2.98	5.81
16225			3.76	8.24
16222			8.64	9.32
16223			9.78	10.46
16236			16.27	22.01

Table 5 – Structures and Measured Activities of Potential ER AF2 Inhibitors.

Cell Inhibition (T47D cell line)					
Compound #	Structure	30uM	10uM	IC50 (uM)	Peptide displacement
16335		89.86	76.36	5.00	Active
16338		68.99	69.19	2.77	Active
16359		76.37	15.04	22.86	Active
16360		94.05	18.02	16.09	Active
16369		47.67	-85.21	0.33	Active
16390		72.38	10.33	22.16	Active
16394		42.24	12.20	25.45	Active
16395		89.57	-18.71	~ 13.65	Active

16408		88.72	73.65	0.83	Active
16411		84.32	31.13	15.78	Active
16415		76.28	72.64	2.60	Active
16417		70.45	61.06	0.20	Active
16434		89.65	71.70	Not tested	Active
16436		103.43	76.55	1.19	Active
16439		100.52	72.58	7.27	Inactive
16440		49.45	35.56	Not tested	Inactive
16441		98.03	74.85	0.80	Inactive

16442		82.21	36.75	2.32	Inactive
16443		89.68	20.06	Not tested	Inactive
16444		82.80	21.37	Not tested	Inactive
16445		16.60	6.52	Not tested	Active
16446		10.84	1.67	Not tested	Active
16447		63.83	46.54	Not tested	Inactive
16448		49.38	16.13	Not tested	Active
16449		-27.37	-0.64	Not tested	Inactive
16450		-18.95	-7.72	Not tested	Inactive

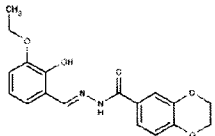
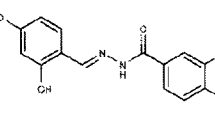
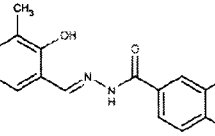
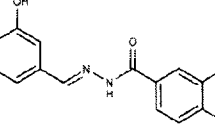
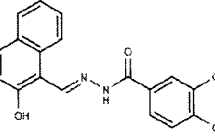
16451		71.22	71.53	Not tested	Inactive
16452		43.11	14.32	Not tested	Active
16453		101.79	72.35	Not tested	Inactive
16454		-111.21	-299.09	Not tested	Inactive
16455		95.16	82.22	Not tested	Inactive

Table 6 – Structures and Measured Activities of Potential ER AF2 Inhibitors.

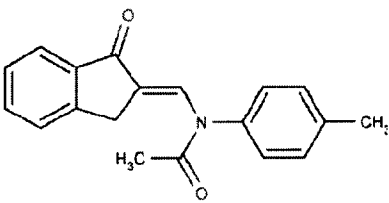
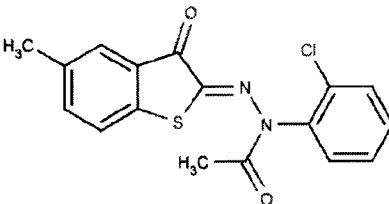
ID	Structure	ER IC50	peptide Disp
16600		bad curve	Not tested
16601		5.4	Active

Table 6 – Structures and Measured Activities of Potential ER AF2 Inhibitors.

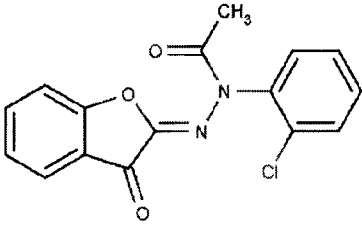
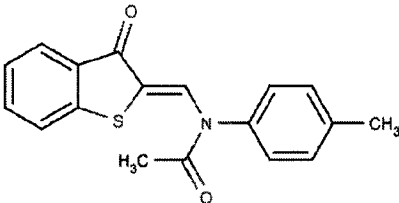
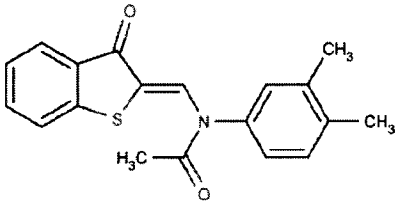
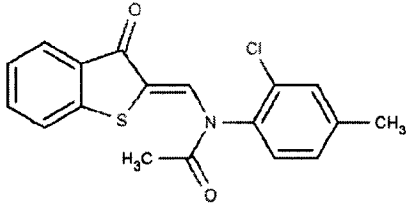
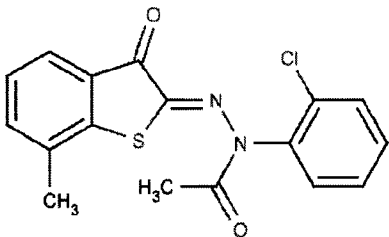
ID	Structure	ER IC50	peptide Disp
16602		bad curve	Not tested
16603		bad curve	Not tested
16604		bad curve	Not tested
16605		4.3	Active
16606		0.31	Active

Table 6 – Structures and Measured Activities of Potential ER AF2 Inhibitors.			
ID	Structure	ER IC50	peptide Disp
16607		2.7	Active

Those skilled in the art will appreciate that the point of covalent attachment of the moiety to the compounds as described herein may be, for example, and without limitation, cleaved under specified conditions. Specified conditions may include, for example, and without limitation, *in vivo* enzymatic or non-enzymatic means. Cleavage of the moiety may occur, for example, and without limitation, spontaneously, or it may be catalyzed, induced by another agent, or a change in a physical parameter or environmental parameter, for example, an enzyme, light, acid, temperature or pH. The moiety may be, for example, and without limitation, a protecting group that acts to mask a functional group, a group that acts as a substrate for one or more active or passive transport mechanisms, or a group that acts to impart or enhance a property of the compound, for example, solubility, bioavailability or localization.

In some embodiments, compounds of the present invention may be used for systemic treatment of cancer. In some embodiments, compounds of the present invention may be used for systemic treatment of at least one indication selected from the group including: melanoma, colorectal cancer, pancreatic cancer, hepatocellular cancer, esophageal cancer, sarcoma, lung cancer, breast cancer, cervical cancer, ovarian cancer, or gastric cancer. In some embodiments compounds may be used in the preparation of a medicament or a composition for systemic treatment of an indication described herein. In some embodiments,

methods of systemically treating any of the indications described herein are also provided.

Compounds as described herein may be in the free form or in the form of a salt thereof. In some embodiment, compounds as described herein may be in the form of a pharmaceutically acceptable salt, which are known in the art (Berge et al., 1977). Pharmaceutically acceptable salt as used herein includes, for example, salts that have the desired pharmacological activity of the parent compound (salts which retain the biological effectiveness and/or properties of the parent compound and which are not biologically and/or otherwise undesirable). Compounds as described herein having one or more functional groups capable of forming a salt may be, for example, formed as a pharmaceutically acceptable salt. Compounds containing one or more basic functional groups may be capable of forming a pharmaceutically acceptable salt with, for example, a pharmaceutically acceptable organic or inorganic acid. Pharmaceutically acceptable salts may be derived from, for example, and without limitation, acetic acid, adipic acid, alginic acid, aspartic acid, ascorbic acid, benzoic acid, benzenesulfonic acid, butyric acid, cinnamic acid, citric acid, camphoric acid, camphorsulfonic acid, cyclopentanepropionic acid, diethylacetic acid, digluconic acid, dodecylsulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, glucoheptanoic acid, gluconic acid, glycerophosphoric acid, glycolic acid, hemisulfonic acid, heptanoic acid, hexanoic acid, hydrochloric acid, hydrobromic acid, hydriodic acid, 2-hydroxyethanesulfonic acid, isonicotinic acid, lactic acid, malic acid, maleic acid, malonic acid, mandelic acid, methanesulfonic acid, 2-naphthalenesulfonic acid, naphthalenedisulphonic acid, p-toluenesulfonic acid, nicotinic acid, nitric acid, oxalic acid, pamoic acid, pectinic acid, 3-phenylpropionic acid, phosphoric acid, picric acid, pimelic acid, pivalic acid, propionic acid, pyruvic acid, salicylic acid, succinic acid, sulfuric acid, sulfamic acid, tartaric acid, thiocyanic acid or undecanoic acid. Compounds containing one or more acidic functional groups may be capable of forming pharmaceutically acceptable salts with a pharmaceutically acceptable base, for example, and without limitation, inorganic bases based on alkaline metals or alkaline earth

metals or organic bases such as primary amine compounds, secondary amine compounds, tertiary amine compounds, quaternary amine compounds, substituted amines, naturally occurring substituted amines, cyclic amines or basic ion-exchange resins. Pharmaceutically acceptable salts may be derived from, for example, and without limitation, a hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation such as ammonium, sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese or aluminum, ammonia, benzathine, meglumine, methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, glucamine, methylglucamine, theobromine, purines, piperazine, piperidine, procaine, N-ethylpiperidine, theobromine, tetramethylammonium compounds, tetraethylammonium compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, morpholine, N-methylmorpholine, N-ethylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine or polyamine resins. In some embodiments, compounds as described herein may contain both acidic and basic groups and may be in the form of inner salts or zwitterions, for example, and without limitation, betaines. Salts as described herein may be prepared by conventional processes known to a person skilled in the art, for example, and without limitation, by reacting the free form with an organic acid or inorganic acid or base, or by anion exchange or cation exchange from other salts. Those skilled in the art will appreciate that preparation of salts may occur *in situ* during isolation and purification of the compounds or preparation of salts may occur by separately reacting an isolated and purified compound.

In some embodiments, compounds and all different forms thereof (e.g. free forms, salts, polymorphs, isomeric forms) as described herein may be in the solvent addition form, for example, solvates. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent in physical association

the compound or salt thereof. The solvent may be, for example, and without limitation, a pharmaceutically acceptable solvent. For example, hydrates are formed when the solvent is water or alcoholates are formed when the solvent is an alcohol.

In some embodiments, compounds and all different forms thereof (e.g. free forms, salts, solvates, isomeric forms) as described herein may include crystalline and amorphous forms, for example, polymorphs, pseudopolymorphs, conformational polymorphs, amorphous forms, or a combination thereof. Polymorphs include different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability and/or solubility. Those skilled in the art will appreciate that various factors including recrystallization solvent, rate of crystallization and storage temperature may cause a single crystal form to dominate.

In some embodiments, compounds and all different forms thereof (e.g. free forms, salts, solvates, polymorphs) as described herein include isomers such as geometrical isomers, optical isomers based on asymmetric carbon, stereoisomers, tautomers, individual enantiomers, individual diastereomers, racemates, diastereomeric mixtures and combinations thereof, and are not limited by the description of the formula illustrated for the sake of convenience.

In some embodiments, pharmaceutical compositions as described herein may comprise a salt of such a compound, preferably a pharmaceutically or physiologically acceptable salt. Pharmaceutical preparations will typically comprise one or more carriers, excipients or diluents acceptable for the mode of administration of the preparation, be it by injection, inhalation, topical administration, lavage, or other modes suitable for the selected treatment. Suitable carriers, excipients or diluents (used interchangeably herein) are those known in the art for use in such modes of administration.

Suitable pharmaceutical compositions may be formulated by means known in the art and their mode of administration and dose determined by the

skilled practitioner. For parenteral administration, a compound may be dissolved in sterile water or saline or a pharmaceutically acceptable vehicle used for administration of non-water soluble compounds such as those used for vitamin K. For enteral administration, the compound may be administered in a tablet, capsule or dissolved in liquid form. The tablet or capsule may be enteric coated, or in a formulation for sustained release. Many suitable formulations are known, including, polymeric or protein microparticles encapsulating a compound to be released, ointments, pastes, gels, hydrogels, or solutions which can be used topically or locally to administer a compound. A sustained release patch or implant may be employed to provide release over a prolonged period of time. Many techniques known to one of skill in the art are described in *Remington: the Science & Practice of Pharmacy* by Alfonso Gennaro, 20th ed., Lippencott Williams & Wilkins, (2000). Formulations for parenteral administration may, for example, contain excipients, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

Compounds or pharmaceutical compositions as described herein or for use as described herein may be administered by means of a medical device or appliance such as an implant, graft, prosthesis, stent, etc. Also, implants may be devised which are intended to contain and release such compounds or compositions. An example would be an implant made of a polymeric material adapted to release the compound over a period of time.

An “effective amount” of a pharmaceutical composition as described herein includes a therapeutically effective amount or a prophylactically effective amount. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduced tumor size, increased life span or increased life expectancy. A therapeutically effective amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the subject, and the ability of the compound to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as smaller tumors, increased life span, or increased life expectancy. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount.

It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by medical practitioners. The amount of active compound(s) in the composition may vary according to factors such as the disease state, age, sex, and weight of the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

In some embodiments, compounds and all different forms thereof as described herein may be used, for example, and without limitation, in combination with other treatment methods for cancer. In other embodiments, compounds and all different forms thereof as described herein may be used, for example, and without limitation, in combination with other treatment methods for at least one indication selected from the group including: melanoma, colorectal cancer, pancreatic cancer, hepatocellular cancer, esophageal cancer, sarcoma, lung cancer, breast cancer, cervical cancer, ovarian cancer, or gastric cancer. The modulating of Estrogen Receptor alpha (ER α) activity may be used for the treatment of at least one indication wherein the cancer is primary or metastatic cancer. For example, compounds and all their different forms as described herein may be used as neoadjuvant (prior), adjunctive (during), and/or adjuvant (after) therapy with surgery, radiation (brachytherapy or external beam), or other therapies (eg. HIFU).

In general, compounds as described herein should be used without causing substantial toxicity. Toxicity of the compounds as described herein can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population). In some circumstances however, such as in severe disease conditions, it may be appropriate to administer substantial excesses of the compositions. Some compounds as described herein may be toxic at some concentrations. Titration studies may be used to determine toxic and non-toxic concentrations. Toxicity may be evaluated by examining a particular compound's or composition's specificity across cell lines using MDA-MB-453 and/or HeLa cells as a negative control that do not express ER. Animal studies may be used to provide an indication if the compound has any effects on other tissues. Systemic therapy that targets the ER will be unlikely to cause major problems to other tissues since antiandrogens and androgen insensitivity syndrome are not fatal.

Compounds as described herein may be administered to a subject. As used herein, a "subject" may be a human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc. The subject may be suspected of having or at risk for having cancer. The subject may be suspected of having or at risk for having one or more of melanoma, colorectal cancer, pancreatic cancer, hepatocellular cancer, esophageal cancer, sarcoma, lung cancer, breast cancer, cervical cancer, ovarian cancer, or gastric cancer. Diagnostic methods for cancer are known to those of ordinary skill in the art.

Some compounds and compositions as described herein may interfere with a mechanism specific to the interaction between ER α and its coactivator proteins. Various embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

Examples

The following examples are illustrative of some of the embodiments of the invention described herein. These examples do not limit the spirit or scope of the invention in any way.

Leads Generation:

The AF2 site represents a hydrophobic groove on the ER α surface flanked with charged amino acids that may be involved in the binding of coactivators (Figure 6A). Being a protein-protein interaction site, AF2 is a challenging target which nevertheless offers an attractive option for direct inhibition of ER α activation. *In silico* computational drug discovery methods were used to conduct a virtual screen of more than 4 million purchasable lead-like compounds from the ZINC database (Irwin, J. et al., 2012) to identify a list of potential AF2 binders. The *in silico* pipeline included large-scale docking, in-site rescoring, and consensus voting procedures (for details see Figure 6B). TABLE 3, TABLE 4, and TABLE 5 show tested compounds by structure and the associated identifiers.

All chemical structures were first collected and adjusted to proper protonation state, checked for errors and docked into the ER α AF2 pocket using the Glide SP program with default settings (Friesner et al., 2004). For this purpose a 3UUD crystal structure of the ER α with 1.6 Å resolution was used (Delfosse et al., 2012). As the result, a set of 2 million compounds that received a dock score ≤ 5.0 were re-docked into the 3UUD structure using another docking protocol – eHiTS (Zsoldos et al., 2007) with the corresponding docking score threshold set to 3.0. This step reduced the set of candidate AF2 binders to 500,000.

Next, to identify the most consistently predicted binding orientations of the compounds, r.m.s.d. was calculated between the docking poses generated by Glide and eHiTS protocols. Only molecules with docking poses having r.m.s.d. values below 2.0 Å were selected for further analysis. Furthermore, the selected ligands were subjected to additional on-site scoring using the LigX program and the pKi predicting module of the Molecular Operating Environment (MOE). With this information, a cumulative scoring vote of five different parameters (Glide score, eHiTS score, LigX score, and pKi predicted by the MOE) was generated with each molecule, receiving a binary 1.0 score for every “top 10% appearance”. The final cumulative vote was then used to select 5,000 compounds that consistently demonstrated high predicted binding affinity toward the targeted AF2 site. These compounds were then visually inspected, and a list of 100 candidates was identified for further testing.

Selected compounds were evaluated for their ability to inhibit ER α transcriptional activity using cellular screening assays in ER α -positive T47D-KBluc, a stable luciferase reporter BCa cell line. Twenty active compounds which inhibited the reporter gene expression by at least 50% at 12 and 30 μ M were selected for construction of dose response curves. Among these, 15 compounds demonstrated inhibition of ER α transcriptional activity in a dose dependent manner, with IC₅₀ values ranging from 5.8 to 100 μ M (Tables 3 & 4). Ten compounds summarized in Table 3 belong to the chemical class of pyrazolidine-3, 5 diones and five compounds from Table 4 are derivatives of carbonylhydrazide.

Among these, VPC-13002, VPC-16225 and VPC-16230 demonstrated significant inhibition of the reporter gene expression with IC_{50} 's of 7.6, 8.24 and 5.81 μ M respectively (Figure 7A - Dose response curves (0.1-50 μ M) of compounds VPC-13002, VPC-16225 and VPC-16230 (IC_{50} : 7.6, 8.24, 5.81 μ M respectively) showing inhibition of the ER α transcriptional activity as measured by luciferase reporter assay in T47D KBluc cells). The IC_{50} of OHT in this assay was established as 4.2 nM (Figure 7B - Dose response curve (0.000006-3 μ M) of OHT (IC_{50} : 4.2 nM) showing inhibition of the ER α transcriptional activity as measured by luciferase reporter assay in T47D KBluc cells. Data was fitted using log of concentration of the inhibitors Vs % activation with GraphPad Prism 5).

Binding of the identified small molecules to the AF2 site should inhibit E2 dependent co-activator peptide recruitment to this site. To test this hypothesis, the AF2 binders were analyzed by using Lanthascreen TR-FRET ER α co-activator assay kit from Life Technologies. Terbium labelled anti-GST antibody indirectly labels the ER α -LBD by binding to a GST tag on the protein. Binding of the agonist, E2 to the ER α causes conformational changes that result in an increase in the affinity of ER α for a fluorescently labeled co-activator peptide, Fluorescein-PGC-1a. PGC-1a has been shown to interact with the AF2 site of ER α in agonist-dependent manner (Tcherepanova et al., 2000). The close proximity of Fluorescein-PGC-1a to the terbium-labeled antibody causes an increase in the TR-FRET signal. When a compound binds to the AF2 site, the recruitment of the co-activator peptide is blocked, causing a decrease in the TR-FRET signal, which is measured as a ratio of emission at 520 nm to 495 nm.

Out of 15 chemicals tested, 9 demonstrated effective blocking of AF2/PGC-1a interaction in a concentration-dependent manner and their IC_{50} values were determined to range between 2 and 20 μ M (Tables 3 & 4). This suggests that the compounds bind to the AF2 site, thereby inhibiting coactivator recruitment. Small molecules VPC-13002, VPC-16225 and VPC-16230 that were potent in cellular assays demonstrated IC_{50} values of 2.46, 3.76 and 2.98 μ M respectively (Figure 7C - Dose response curves (0.1-50 μ M) of compounds VPC-13002, VPC-16225 and VPC-16230 (IC_{50} : 2.46, 3.76, 2.98 μ M respectively) for

displacement of the PGC-1a peptide from the AF2 site as measured by TR-FRET assay). The cold PGC1a peptide used as a control in this assay showed a dose-dependent decrease in the FRET signal with increase in concentration (Figure 7D - Dose dependent (1.8-50 μ M) behaviour of Cold PGC1a peptide for displacement of the Fluorescein-PGC-1a peptide from the AF2 site as measured by TR-FRET assay. Data was fitted using log of concentration of the inhibitors Vs emission ratio with GraphPad Prism 5. Data points represent average of two independent experiments performed in triplicates. Error bars indicate standard error of mean (SEM) for n=6 values).

The three compounds were tested for competition with increasing concentrations of Fluorescein-PGC1a (250, 500 and 1000 nM) to confirm their AF2-mediated mode of action. A right shift in the dose response curves of the compounds was observed in the presence of higher concentrations of Fluorescein-PGC1a (Figures 8A-C – Dose response curves (0.05-400 μ M) of VPC-13002 (A), VPC-16225 (B) and VPC-16230 (C) showing a right shift with increase in concentrations of Fluorescein-PGC1a generated by the TR-FRET assay. (Error bars indicate standard error of mean (SEM) for n=6 values. Data was fitted using log of concentration of the inhibitors Vs emission ratio with GraphPad Prism 5.). This suggests that the compounds bind to the AF2 site.

To confirm that the identified compounds bind to the ER α , the ER α LBD containing the avi-tag at the N terminus and a six residue histidine tag at the C terminus was cloned and purified. The ER α LBD is biotinylated on the avi-tag by the biotin ligase expressed by the bacterial cells co transformed with the biotin ligase plasmid. The bER- α LBD was purified by Ni-affinity chromatography and immobilized on streptavidin biosensor tip. The interaction between small molecule and the protein is measured in real time as a shift in the interference pattern of the incident light. A response profile is generated on the Octet system (Figure 8B). The binding of the identified six hits was confirmed in this assay. Figure 7C features the BLI data obtained for the most potent compound VPC-13002, demonstrating its direct and reversible interaction with ER α . It should be

noted that VPC-13002 could be fit with a simple 1:1 model even at higher concentration, suggesting single-site binding.

For the compounds to be AF2 specific, it is important to confirm that they do not bind at the HBS. To rule out interaction with HBS, active compounds were tested for E2 displacement using PolarScreen™ ER Alpha Competitor Assay kit from Life Technologies. Nine compounds which effectively inhibited coactivator recruitment were tested for E2 displacement at 20 μ M. Out of the nine compounds tested, six (including VPC-13002, VPC-16225 and VPC-16230) did not exhibit any detectible E2 displacement when tested at 20 μ M (see Figure 6). It should be noted that the K_d of FI-E2 with full length ER α in this assay is reported by the manufacturer as 18 ± 9 nM. FI-E2 was used at the recommended concentration of 4.5 nM in order to ensure that the lack of competition observed at 20 μ M was not due to the presence of an excess of FI-E2 ligand. To further confirm that the active compounds do not compete with E2 for binding at the HBS, an E2 ligand competition assay was performed. Results from this experiment demonstrate that these compounds did not displace FI-E2 even at the highest concentrations tested (3-150 μ M) (Figures 9 A-C - Dose response curve (3-150 μ M) of VPC-13002 (A), VPC-16225 (B), and VPC-16230 (C) for displacement of FI-E2 in fluorescence polarization assay. Data was fitted using log of concentration of the inhibitors Vs polarization with GraphPad Prism 5.), whereas the IC_{50} of unlabeled-E2 for displacement of FI-E2 from the HBS in this assay is 4.2 nM (Figure 9D - and E2 (0.01-1000 nM; IC_{50} : 4.2 nM) for displacement of FI-E2 in fluorescence polarization assay. Data was fitted using log of concentration of the inhibitors Vs polarization with GraphPad Prism 5.), which suggests that the estimated cellular IC_{50} 's do not reflect binding to the HBS.

This was also confirmed by measuring IC_{50} 's of the developed inhibitors in the presence of higher concentrations of E2 (luciferase assay on T47D-KBluc cell line). Since OHT (used as a positive control) competes with E2 for binding at the HBS, we observed a right-shift in the IC_{50} curve of OHT that was proportional to the fold increase in E2 (Figure 10A - Dose response curves (0.000095–50 μ M) of

OHT showing a right shift proportional to the fold increase in concentrations of E2 generated by the luciferase assay in T47D-KBluc cell line. Error bars indicate standard error of mean (SEM) for n=6 values. Data was fitted using log of concentration of the inhibitors Vs % activation with GraphPad Prism 5). On the contrary, IC₅₀ curves of VPC-16225 and VPC-16230 compounds did not show any significant shift (Figure 10B and 10C - (B) Dose response curves (0.1-50 μ M) of VPC-16225 and VPC-16230 (C) showing no significant right shift in the presence of higher concentrations of E2 as measured by luciferase assay in T47D-KBluc cell line. Error bars indicate standard error of mean (SEM) for n=6 values. Data was fitted using log of concentration of the inhibitors Vs % activation with GraphPad Prism 5). This confirmed that the compounds do not bind to the HBS.

To confirm that the identified inhibitors directly bind to the ER α , the ER α -LBD in fusion with an avi-tag at the N-terminus and a six residue histidine tag at the C-terminus was cloned and purified. The ER α -LBD was biotinylated on the avi-tag by a biotin ligase expressed by the bacterial cells co-transformed with the biotin ligase plasmid pBirACm. The bER α -LBD was purified by Ni-affinity chromatography and immobilized on streptavidin biosensor tip. The interaction between small molecule and protein is measured in real time as a shift in the interference pattern of the incident light. A response profile is generated on the Octet system.

The binding of the identified 6 compounds was confirmed using this assay. As an example, Figures 11A-D (BLI dose response curves (3-100 μ M) reflecting the binding of the compounds VPC-13002 (A), VPC-16225 (B) and VPC-16230 (C) to ER α -LBD in a dose dependent manner. PGC-1 α coactivator peptide is used as a positive control (D) features the BLI data obtained for the most potent compounds, VPC-13002, VPC-16225 and VPC-16230 along with the PGC-1 α peptide used as a control, demonstrating their direct and reversible interaction with ER α . Importantly, it should be noted that the binding curves of these compounds could fit with a simple 1:1 model even at higher concentration, suggesting their single-site ER binding.

To ascertain the growth inhibitory potential of VPC-13002, VPC-16225 and VPC-16230, their ability to inhibit the E2 stimulated growth of ER α -positive, MCF7, TamR3 and TamR6 BCa cells in MTS assays was evaluated. Cell viability was assessed after 96 h of incubation with each compound. General cell toxicity was assessed by measuring inhibition of growth in ER α -negative MDA-MB-453 and HeLa cell lines. The VPC-13002 molecule is a derivative of pyrazolidine-3, 5 dione that demonstrated certain toxicity in ER α -negative cells; hence, the growth inhibitory effect of this compound was not considered to be ER α -mediated (Figure 12A) (Figures 12A-D - Dose response curves (0.2-50 μ M) of the compounds showing decrease in cell viability as assessed by the MTS assay. The compounds, VPC-13002 (A), VPC-16225 (B) and VPC-16230 (C) inhibit the growth of ER α -positive MCF7 and tamoxifen resistant cells (TamR3 and TamR6) with very little effect on ER α -negative MDA-MB-453 and HeLa cells except VPC-13002, which is toxic in both the control cell lines. Data points represent average of two independent experiments performed in triplicates. Error bars indicate standard error of mean (SEM) for n=6 values. Data was fitted using log of concentration of the inhibitors Vs % growth with GraphPad Prism 5.) and the molecule was eliminated from further analysis. Figures 12B and 12C show that carbohydrazide derivatives, VPC-16225 and VPC-16230, exhibited growth inhibition of MCF7 cells in a concentration dependent manner in the range of 0.2 to 50 μ M (IC₅₀'s 6 and 7.8 μ M respectively) confirming their ER α -specific effect. Next, VPC-16225 and VPC-16230 in TamR3 and TamR6, was tested. These cell lines were derived from parental MCF7 cells upon prolonged treatment with tamoxifen and retained expression of ER α . Compared to controls, both VPC-16225 and VPC-16230 inhibited the proliferation of these cell lines in a dose dependent manner at the concentrations tested. The IC₅₀ values for VPC-16225 are 3.1 μ M and 4.1 μ M in TamR3 and TamR6 respectively. VPC-16230 had IC₅₀ values of 3.4 μ M and 6.3 μ M in TamR3 and TamR6 respectively. It may be noted that due to the development of resistance, the growth of the TamR3 and TamR6 cell lines is not affected by the presence of 1 μ M tamoxifen in the medium.

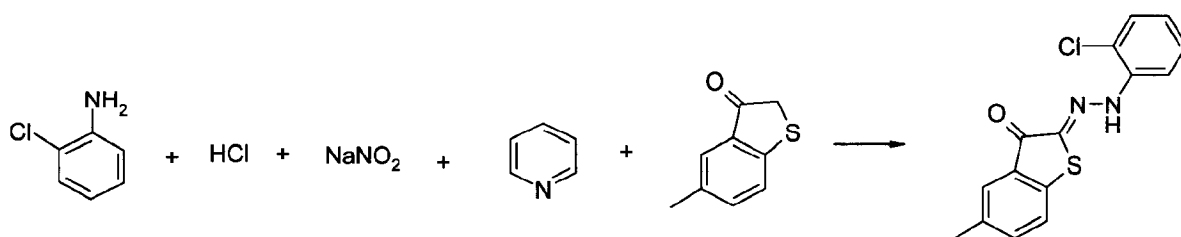
To confirm that the growth inhibition of tamoxifen-resistant cell lines, TamR3 and TamR6, was through inhibition of ER α activity, the ability of VPC-16225 and VPC-16230 to inhibit the expression of an estrogen responsive luciferase reporter gene was assessed. TamR3 and TamR6 cells were transiently transfected with the luciferase plasmid (3X ERE TATA luc) and then treated the following day with compounds at concentrations ranging from 0.1 to 50 μ M, all in the presence of 1 nM E2. Both compounds showed significant inhibition of E2-stimulated ER α transcriptional activity in the two cell lines as measured by the luminescence signal (Figure 13A-D - Dose response curves (0.1-50 μ M) of compound VPC-16225 for transcriptional inhibition of transiently transfected E2-responsive luciferase reporter in tamoxifen resistant cells TamR3, IC₅₀: 8.2 μ M (A) and TamR6, IC₅₀: 7.2 μ M (B). VPC-16230 inhibits ER α transcriptional activity in a dose dependent manner in TamR3, IC₅₀: 4.7 μ M (C) and TamR6, IC₅₀: 4.7 μ M (D). Dose response curves (0.000095–50 μ M) of Fulvestrant (IC₅₀ in TamR3: 0.09 μ M; IC₅₀ in TamR6: 0.04 μ M) and Tamoxifen (0.000095-6 μ M) have been shown for comparison. Data points represent average of two independent experiments performed in triplicates. Error bars indicate standard error of mean (SEM) for n=6 values. Data was fitted using log of concentration of the inhibitors Vs % activation with GraphPad Prism 5). Fulvestrant, which was used as a positive control in these cells, yielded IC₅₀ values of 0.09 and 0.04 μ M in TamR3 and TamR6 systems respectively. OHT was ineffective in inhibiting the transcriptional activity of ER α in these cells.

The ER α transcriptional inhibitory potential of VPC-16225 and VPC-16230 was evaluated by measuring the mRNA expression levels of the estrogen responsive genes, pS2, Cathepsin-D and CDC2 (Frasor et al., 2003; Carroll et al., 2006). MCF7 cells were treated with the test compounds for 24 h following which the mRNA was isolated and qRT-PCR analyses performed. While VPC-16225 did not show any significant effect, VPC-16230 considerably reduced mRNA levels of these genes in a dose dependent manner (Figure 14A - VPC-16230 significantly decreases mRNA levels of pS2, Cathepsin-D and CDC2 at 12 and 6 μ M in the presence of 1nM E2 in MCF7 cells. Data points represent

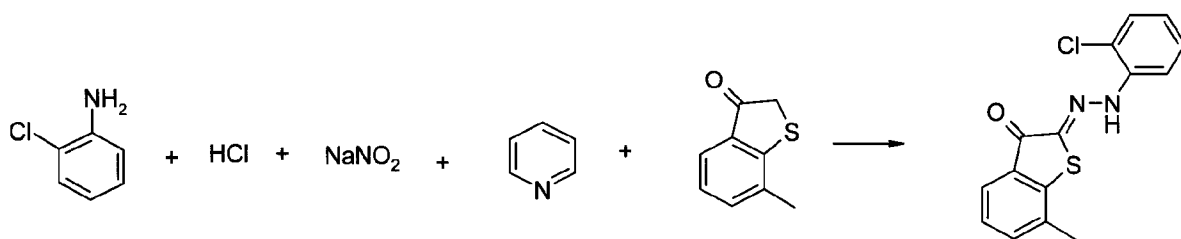
average of two independent experiments performed in triplicates. Error bars indicate standard error of mean (SEM) for n=6 values. A p value <0.05 was considered significant (*) compared to E2+DMSO control). However, when treated in the absence of E2, VPC-16230 did not significantly inhibit gene expression compared to the vehicle control (Figure 14B - VPC-16230 does not significantly decrease mRNA levels of pS2, Cathepsin-D and CDC2 at 12, 6 and 3 μ M in the absence of 1nM E2). The inhibition of gene expression in the presence of E2 was also observed at the protein level (Figure 14C - VPC-16230 decreases protein levels of pS2, Cathepsin-D and CDC2 at 12 and 6 μ M in MCF7 cells as observed by western blot). These results suggest that VPC-16230 is a strong inhibitor of ER α transcriptional activity in BCa cells and can be considered as an AF2-directed drug prototype in the study.

Procedure for preparation of diazocompounds - 1

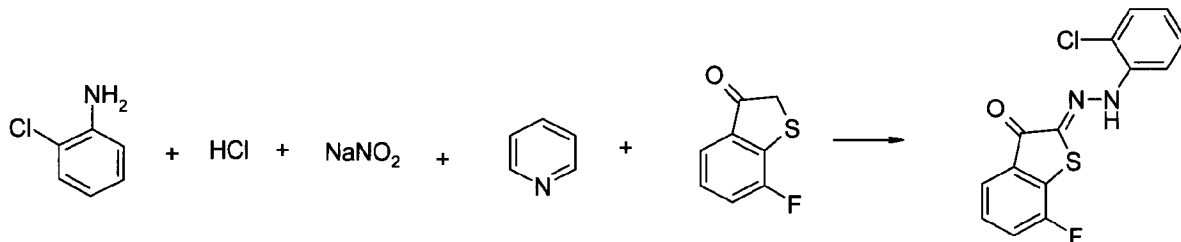
In first reactor amine (12mmol) was mixed with water (50ml) and treated with aq HCl (30mmol), then NaNO₂ (14.4mmol) in water (3ml) was added dropwise at 0-5C. Resulting mixture was stirred at 0-5C for 0.5h. Then mixture from first reactor was added portionally to solution of ketone (10mmol) and pyridine (40mmol) in EtOH (50ml) at 0-5C, resulting mixture was stirred for 0.5h at 0-5C, then 1h at rt. After product was filtered and dried on air.



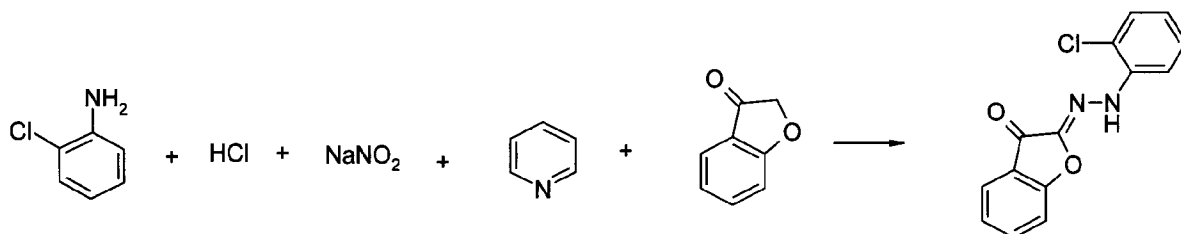
NMR SDA2760 LCMS SDA2759



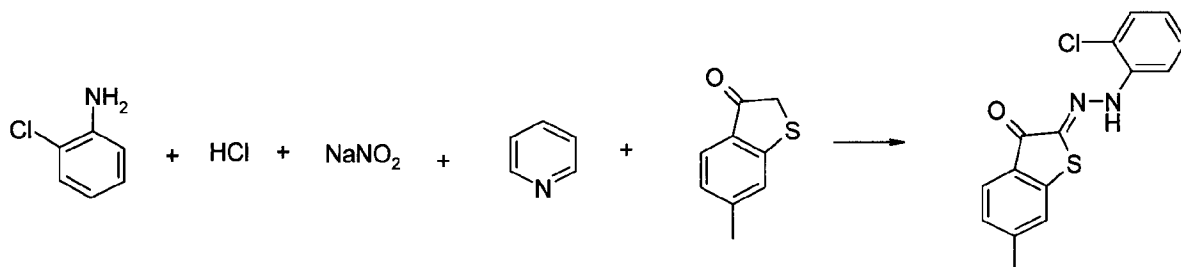
NMR SDA3586 LCMS SDA3589



NMR SDA3573 LCMS SDA3574



NMR SDA2876 LCMS SDA2877

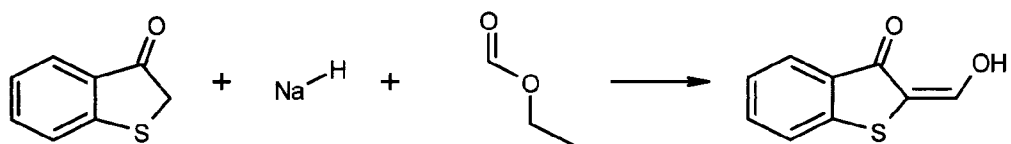


NMR SDA4157

Procedure for preparation of compounds - 2

To a mixture of 60% NaH (0.17mol) and HCOOEt (0.13mol) in toluene (200ml), ketone (0.1mol) was added dropwise at 0-5°C. Then mixture was stirred for 16h at rt. Then reaction mixture was evaporated to dryness, residue was dissolved in water and organic residues was extracted with MTBE from

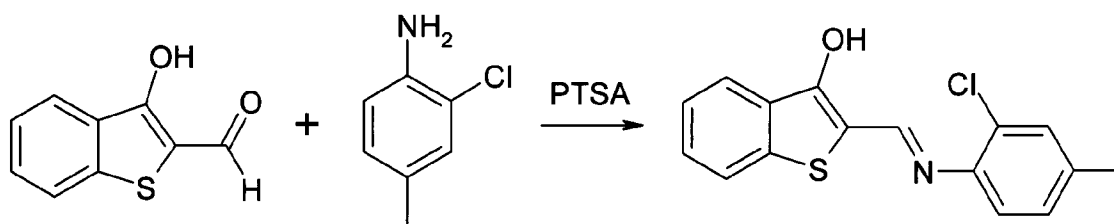
aqueous solution. Then water was acidified with AcOH to pH<7 and product was extracted with EtOAc:



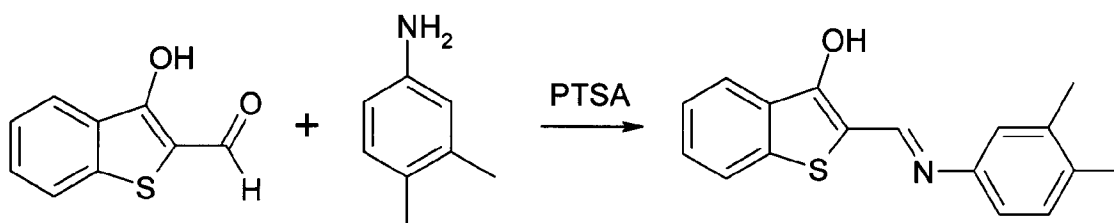
NMR SDA2880

Procedure for preparation of Shiff base - 3

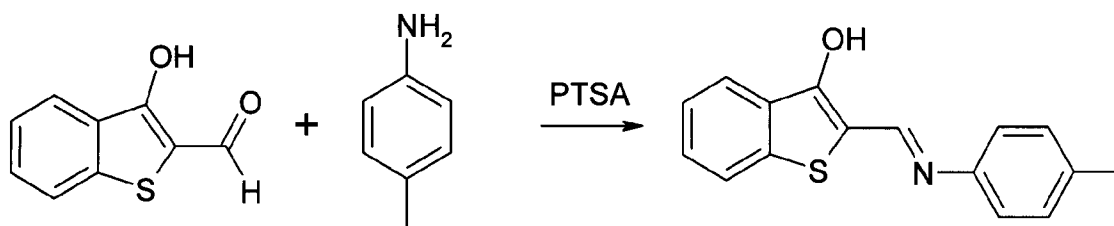
Mixture of formil compound (10mmol) and amine (25mmol) with catalytic amount of PTSA was refluxer for 4hrs, then reaction mixture was evaporated and residue was triturated with toluene (5ml) to obtain precipitate, wich was ffiltrated and washed with toluene (2x5ml):



NMR SDA3401 LCMS SDA3405



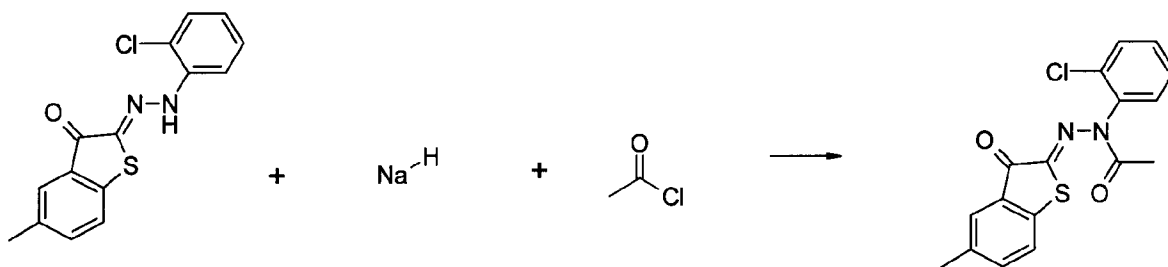
NMR SDA3404 LCMS SDA3476



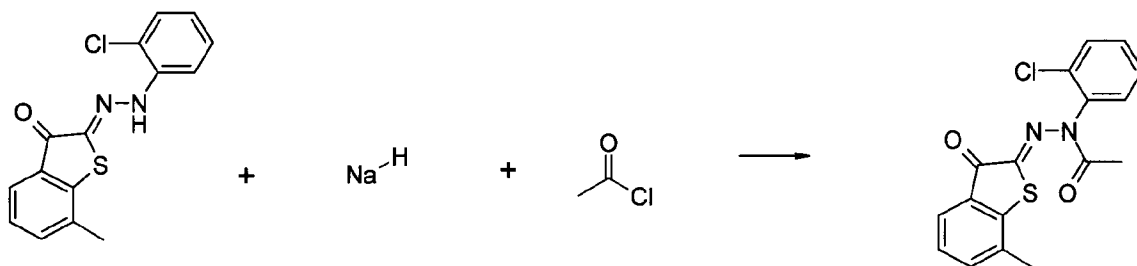
NMR 3399 LCMS 3403

Procedure for preparation of acylation - 4

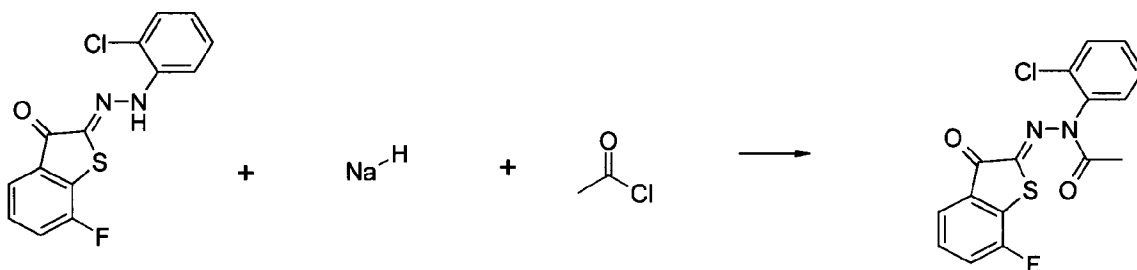
To a mixture of 60% NaH (20mmol) in DMF (50ml), aminocompound (10mol) was added at rt. Then mixture was stirred for 1h at rt. Then AcCl (30mmol) was added dropwise at rt and resulting mixture was stirred for 16hrs. Then reaction mixture was poured into water (250ml), precipitate was filtered dried, washed with water (2x50ml) then with Et₂O (2x5ml) and dried on air:



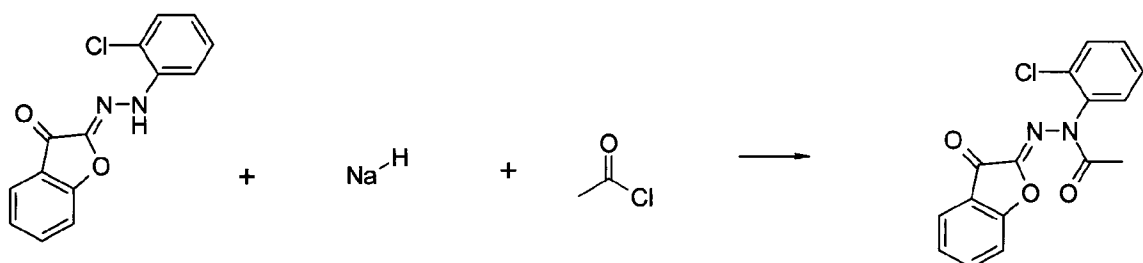
NMR 2855 LCMS 2856



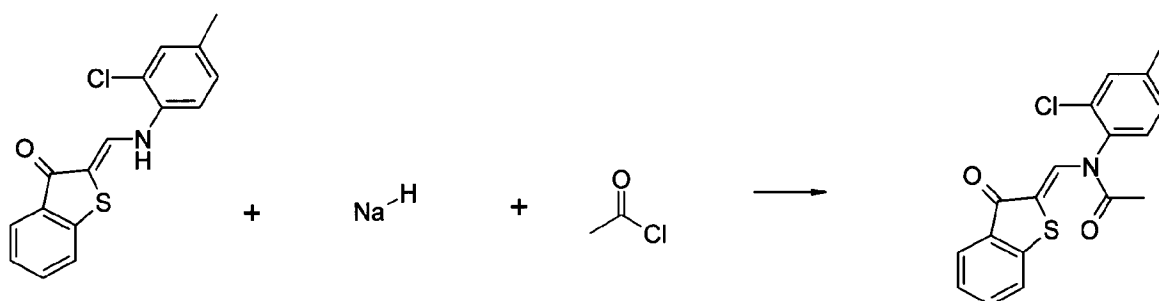
NMR 3697 LCMS 3676



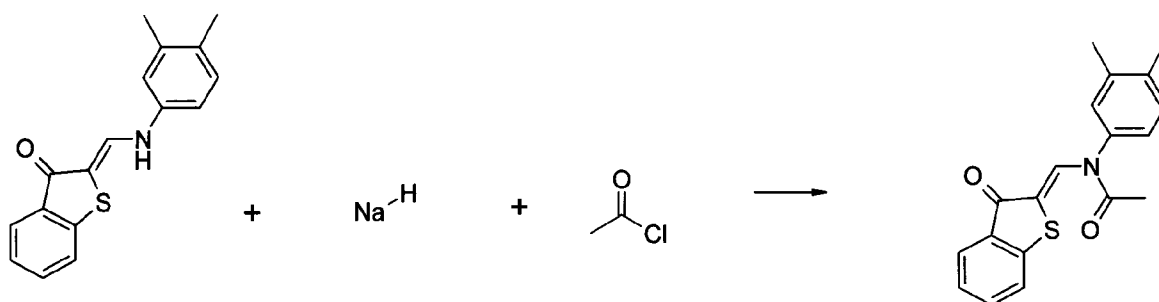
NMR 3646 LCMS 3580



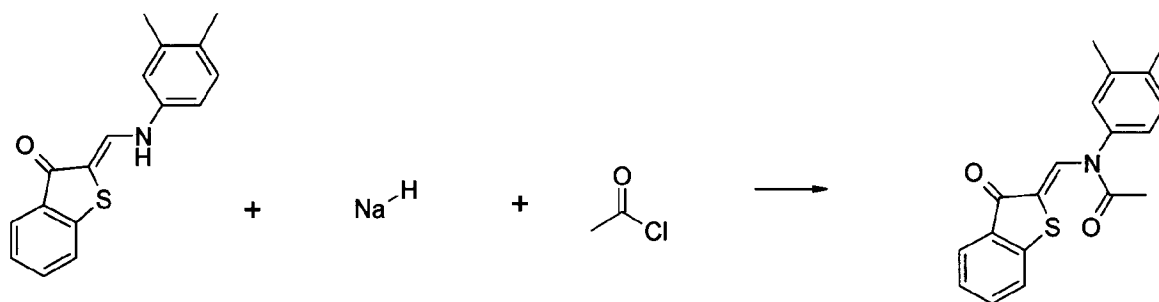
NMR 3151 LCMS 3086



NMR 3431 LCMS 3462



NMR 3432 LCMS 3463



NMR 3433 LCMS 3464

Identification of Analogues of Compound 1 by 2D Similarity Search Method:

Previous work identified several small molecules that specifically target the ER AF2 pocket (Singh et al., 2015). Among them, (2-((2-phenoxyethyl)thio)-1-(2-(p-tolyloxy) ethyl)-1H-benzo[d]imidazole) derivative (compound 1) was selected as a candidate. A chemical template was designed based on the structure of compound 1 (Figure 1; Singh et al., 2013), and a molecular similarity search was performed to identify the analogues compounds with different substitutions around template. Instant JChem, a 2D similarity searching tool from ChemAxon (Instant JChem 5.10) was employed to search through ZINC database 12.0.21 All software parameters were set to their default values. A total of 2000 ZINC compounds which generated Tanimoto coefficient above 0.6 with respect to the query structure were selected further analysis. These compounds were further evaluated using our established *in silico* pipeline as discussed below. Table 1 and Table 2 show tested compounds by structure and the associated identifiers.

Molecular Docking of Selected Compounds into the ER AF2 Pocket:

The AF2 site represents a hydrophobic groove located on the surface of the ER. Being a protein-protein interaction site, AF2 represents a challenging target. Nevertheless, it offers an attractive option for direct inhibition of the ER transactivation. Using an in-house computational drug discovery pipeline, the selected 2000 compounds were virtually tested. The *in silico* pipeline included molecular docking, on-site rescoring, and consensus voting procedures. Initially, all molecules were docked into the ER crystal structure (Protein Data Bank [PDB] ID code 3UUD, 2.4Å° resolution) using the Glide SP program (Friesner et al., 2004). Previous studies indicated that amino acids Val355, Ile358 and Leu379 are critical for hydrophobic contacts with AF2 binders and are critical for protein-ligand coordination. Therefore, the corresponding hydrophobic constraints were applied during the docking. Compounds that received a moderate to high score

by Glide SP were selected and re-docked into the 3UUD structure using the eHiTS docking protocol (Zsoldos et al., 2007). To improve the accuracy of the predicted binding orientation, the root-mean-square deviation (rmsd) was calculated for the docking poses generated by the Glide and eHiTS programs. Only molecules with docking poses with rmsd < 2.0 Å° were subjected to further analysis.

Next, the selected ligands underwent additional on-site rescoring using the Ligand Explorer (LigX) program and the pKi predicting module of the Molecular Operating Environment (MOE) (Chemical Computing Group). With this information, a cumulative scoring of four different predicted parameters (Glide score, eHiTS score, rmsd, LigX score, and pKi predicted by the MOE) was computed, with each molecule receiving a binary 1.0 score for every “top 20% appearance.” The final cumulative vote allowed for selecting 40 molecules associated with a higher probability of being BF3 binders. These compounds were then visually inspected and based on the commercial availability, 9 chemicals (Table 1) were purchased from chemical vendors.

Mammalian two-hybrid assay:

The direct effect of VPC-16464 on ER-coactivator interaction was assessed by mammalian two-hybrid system (Promega). The ER-LBD was cloned into the pACT vector to produce the ER-LBD protein fused to the VP16 activation domain. The SRC-3 coactivator peptide (aa 614-698) containing LXXLL motives 1 and 2 was cloned into the pBIND vector to produce the SRC-3 protein fused to the GAL4-DNA binding domain. MDA-MB-231 cells were transfected with pACT-ER-LBD, pBIND-SRC-3, luciferase reporter plasmid containing a GAL4 recognition sequence and a constitutively active renilla reporter plasmid. The cells were treated with VPC-16464 at 5 concentrations with a two-fold dilution starting from 50 µM. The compound inhibited the interaction between ER and SRC-3 in a dose dependent manner (Figure 2A). This provides direct evidence that the compound shows AF2 mediated activity.

TR-FRET assay:

To further confirm that VPC-16464 affects E2 mediated ER-coactivator interaction TR-FRET assay was used. When tested at 3-fold dilution range starting at 50 μ M the compound successfully displaced the Fluorescein-PGC-1 α and SRC-2-3 coactivator peptides in a dose dependent manner (Figure 2B).

Estrogen displacement:

To confirm that VPC-16464 does not bind to the conventional hormone binding site E2 displacement was assessed with the Polar Screen Estrogen Receptor- α Competitor Green Assay Kit (P2698, Life Technologies). The compound showed less than 40% inhibition at 50 μ M (Figure 2C).

Inhibition of transcriptional activity:

Blocking ER-coactivator interaction should affect the transcriptional activity of the receptor. Therefore, the effect of VPC-16464 on ER transcriptional activity was assessed in ER⁺ T47D-KBluc cells using a luciferase reporter assay. Serum starved cells were treated with the compound at concentrations ranging from 50-0.1 μ M in the presence of 1nM E2. Effect of the compound on transcription of estrogen responsive luciferase reporter gene was analysed. VPC-16464 showed dose dependent inhibition of ER driven luciferase gene expression with an IC₅₀ of 1.86 μ M (Figure 2D).

Down-regulation of ER target genes:

To further investigate the effect of inhibition of ER transcriptional activity by VPC-16464 we looked at ER regulated genes, Ps2, PR, CDC2 and Cathepsin-D in MCF7 and TamR3 cells. VPC-16464 down-regulated the expression of Ps2, PR and CDC-2 mRNA at all the concentrations tested in MCF7. However the effect on Cathepsin-D was not significant. In TamR3 cells similar effects were observed (Figures 3A-D). Cells were treated with the test compound for 24 hrs in the presence of 1nM estradiol (E2). 4-Hydroxytamoxifen (OHT) was used as control.

Inhibition of growth of ER⁺ and Tamoxifen-resistant BCa cells:

AF2 mediated inhibition of ER transcriptional activity should affect cell growth. Presto Blue cell viability assay was used to assess the growth inhibitory potential of VPC-16464. ER⁺ MCF7 and T47D and tamoxifen-resistant, TamR3 cells (Leung et al., 2010) were treated for 96h with 2-fold dilution range of the compound starting at 50 μ M. ER⁻ MDA-MB-453 cells were used as a negative control. The cells were treated in the presence of 1nM E2 for MCF7 and 1nME2 + 1 μ M OHT for TamR3 cells. The compound reduced the growth of the three cell lines without any effect on the ER⁻ cells (Figure 4). This rules out any off target effects.

Inhibition of constitutively active mutant form of ER:

The existence of constitutively active mutant forms of ER in clinical setting has been recently reported in patients who have developed resistance to tamoxifen. The Y537S mutant has been shown by different groups to be constitutively active (Robinson et al., 2013; Toy et al., 2013). The location of this residue outside the AF2 pocket is advantageous as the binding of VPC-16464 should not be affected by this mutation. This hypothesis was tested by creating site-directed mutagenesis on WT-ER to generate the Y537S mutant. MDA-MB-231 cells were transfected with plasmids encoding either wild type or mutant ER along with an estrogen responsive luciferase reporter plasmid and a constitutively active renilla reporter plasmid. The cells were treated with VPC-16464 at 4 concentrations starting from 50 μ M. The compound successfully inhibited both the wild type and mutant forms of the receptor (Figure 5). The expression levels of ER from corresponding treatments were analyzed by Western blot to confirm that the observed effect was not due to lower levels of the transfected receptor (Figure 5). This corroborates the idea that such clinically relevant mutant forms of the receptor which cause tamoxifen to act as agonist can be inhibited by targeting an alternative site on the receptor.

pSG5 plasmid encoding either the full length wild type (WT) or mutant forms of ER α (Y537S and Y537C) was transfected into MDA-MB-231 cells along with the 3X-ERE-TATA luc reporter plasmid. The cells were treated with 2-fold

dilution of VPC-16464 starting at 50 μ M in the presence of 1 nM E2. The compound successfully inhibited the constitutively active mutant forms of the receptor in a dose dependent manner compared to OHT which was ineffective even at 1 μ M.

Preparation of the protein structure for docking:

Virtual screening was carried out on the ER α crystal structure [PDB:3UUD] (1.60 Å resolution; Delfosse et al., 2012). To prepare the protein structure for docking, all solvent molecules were deleted, and bond orders for the ligand and the protein were adjusted. The missing hydrogen atoms were added, and side chains were then energy-minimized using the OPLS-2005 force field (as implemented by Maestro software; Maestro, 2008). The ligand binding region was defined by a 12 Å box centered on the crystallographic ligand of 3UUD. No van der Waals scaling factors were applied; the default settings were used for all other adjustable parameters.

Ligand preparation:

The ZINC database version 8.0 was used for virtual screening against the ER α AF2 site. The compounds were imported into a molecular database using the Molecular Operating Environment (MOE) version 2012 (MOE 2008). Hydrogen atoms were added after these structures were “washed” (a procedure including salt disconnection, removal of minor components, deprotonation of strong acids, and protonation of strong bases). The following energy minimization was performed with the MMFF94x force field, as implemented by the MOE, and optimized structures were exported into the Maestro suite in SD file format.

Virtual screening, consensus scoring and voting:

Initially, ~ 4 million compounds were docked into the AF2 site using Glide SP module. Next, approximately 2 million molecules were re-docked, which had a glide score $\leq$ 5.0, into the same binding cavity using the electronic high-throughput screening (eHiTS) docking module. A total of 5×10^5 structures, which

received eHiTS docking scores below the 3.0 threshold, were identified for further in silico refinement.

The determined docking poses of the 5×10^5 selected compounds were evaluated by (1) Glide docking score, (2) eHiTS docking score, (3) predicting pKi of protein-ligand binding using MOE SVL script scoring.svl to improve accuracy of the prediction of energies of hydrogen bonds and hydrophobic interactions, (4) calculating rigorous docking scores, defined by the Ligand Explorer (LigX) module of the MOE package, which accounts for receptor/ligand flexibility and (5) computing the root mean square deviation (r.m.s.d.) between docking poses generated by Glide and eHiTS programs to quantify their docking consistency. On the basis of these sorted output values from the above four procedures, each molecule would then receive a binary 1.0 vote for every "top 10% appearance". The final cumulative vote (with the maximum possible value of 5) was then used to rank the training set entries. On the basis of the cumulative count, the most highly voted (5×10^3) molecules were selected and their docking poses subjected to visual inspection.

After this final selection step, a list of 100 compounds was formed that were purchased and tested experimentally. From these 100 compounds, 14 were found to be active and demonstrated the ability to displace the coactivator peptide from the target AF2 site. Small molecules were purchased from established suppliers, including Asinex (Compound VPC-13002), ChemBridge (compounds VPC-16007, VPC-16003, VPC-16004, VPC-16222 and VPC-16223), ChemDiv (compound VPC-16019), TimTec (compounds VPC-16041, VPC-16046, VPC-16038, VPC-16040 and VPC-16021) and Vitas-M (compounds VPC-16230, VPC-16225, and VPC-16236).

Cell Culture - 1:

T47D-KBluc, MCF7, MDA-MB-453, Hela (ATCC, Manassas, VA, USA) and tamoxifen resistant cell lines, TamR3 and TamR6 (Leung et al., 2010) were cultured at 37°C in humidified incubator with 5% CO₂. The cell lines were maintained in the following culture media: MCF-7: phenol-red-free RPMI 1640

(Gibco, Life Technologies), supplemented with 10% fetal bovine serum (FBS) (Gibco, Life Technologies); T47D-KBluc: phenol red-free RPMI 1640 containing 4.5 g/liter glucose (Sigma-Aldrich), 10 mM Hepes (Sigma-Aldrich), pH 7.5, 1 mM sodium pyruvate (Life Technologies), 0.2 U/ml insulin (Sigma-Aldrich) and 10% FBS; MDA-MB-453 and Hela: dulbecco's modified eagles medium (DMEM) (Hyclone, Thermo Scientific) supplemented with 10% FBS (Gibco, Life Technologies); TamR3 and TamR6: phenol-red-free RPMI 1640, containing 10% charcoal stripped serum (CSS) (Gibco, Life Technologies) and 1 μ M tamoxifen (Sigma-Aldrich).

Cell Culture - 2:

T47D-KBluc, MDA-MB-231 and MDA-MB-453 cell lines were obtained from ATCC, Manassas, VA, USA. MCF7 cell line was a gift from Dr. Sandra Dunn (Division of Hematology and Oncology, Department of Pediatrics, University of British Columbia, Vancouver, Canada). Tamoxifen resistant cell line, TamR3 was kindly provided by Dr. Euphemia Leung (University of Auckland, New Zealand; Leung et al., 2010). Cells were cultured at 37°C in humidified incubator with 5% CO₂. The cell lines were maintained in the following culture media: MCF7: phenol-red-free RPMI 1640 (Gibco, Life Technologies), supplemented with 10% fetal bovine serum (FBS) (Gibco, Life Technologies); T47D-KBluc: phenol red-free RPMI 1640 containing 4.5 g/liter glucose (Sigma-Aldrich), 10 mM Hepes (Sigma-Aldrich), pH 7.5, 1 mM sodium pyruvate (Life Technologies), 0.2 U/ml insulin (Sigma-Aldrich) and 10% FBS; MDA-MB-231: phenol red free dulbecco's modified eagles medium (DMEM) (Hyclone, Thermo Scientific) supplemented with 10% FBS (Gibco, Life Technologies) MDA-MB-453: DMEM (Hyclone, Thermo Scientific) supplemented with 10% FBS (Gibco, Life Technologies); TamR3: phenol-red-free RPMI 1640, containing 10% charcoal stripped serum (CSS) (Gibco, Life Technologies) and 1 μ M tamoxifen (Sigma-Aldrich).

Chemicals and Antibodies:

17 β -Estradiol, 4-Hydroxytamoxifen (OHT) and tamoxifen were obtained from Sigma-Aldrich. E2 was dissolved in 100% ethanol. OHT, Tamoxifen and test compounds were dissolved in DMSO. Cold PGC1 α (Peroxisome proliferator-activated receptor gamma coactivator 1- α) peptide (EAEEPSLLKKLLAPANTQ) was synthesized from Elim Biopharmaceuticals, Inc. (CA, USA).

The rabbit monoclonal anti-pS2 antibody (EPR3972) was obtained from Abcam Inc. the mouse monoclonal anti-CDC2 antibody (JI-04-00640) was purchased from RayBiotech, Inc. The rabbit polyclonal antibody for α -Actin (A2066) was obtained from Sigma-Aldrich. Mouse monoclonal anti-Cathepsin-D antibody (C0715) were obtained from Sigma-Aldrich.

Luciferase ER- α transcriptional Assay:

ER α -positive T47D-KBluc human BCa cells were grown in phenol-red-free RPMI 1640 supplemented with 10% CSS for 5 days. The cells were seeded on a 96-well plate (2×10^4 cells/well). After 24 h, the cells were treated with either the test compounds or OHT in the presence of 1 nM E2. The test compounds were screened at two concentrations, 12 μ M and 30 μ M. OHT was added at a final concentration of 5 μ M. For generation of dose response curves, the compounds were added at a range of 0.1-50 μ M and OHT was added at a range of 0.000006–3 μ M. The medium contained 0.1% (v/v) ethanol and 0.1% (v/v) dimethyl sulfoxide (DMSO). 24 h after treatment, the medium was aspirated off and the cells were lysed by adding 50 μ L of 1 $\times$ passive lysis buffer (Promega). The plates were placed on a shaker at room temperature for 15 min and then subjected to two freeze–thaw cycles to help lyse the cells. Then 20 μ L of the lysate from each treatment was transferred onto a 96-well white flat-bottom plate (Corning) and the luminescence signal was measured after adding 50 μ L of the luciferase assay reagent (Promega) on TECAN M200pro plate reader. Differences in growth were normalized against total protein concentration measured by BCA assay.

To rule out binding at HBS, dose response curves (0.1-50 μM) of test compounds and (0.000095-50 μM) of OHT were generated in the presence of a set of higher concentrations (1,10,50,100 nM) of E2 following the same procedure as described above.

Transient Transfection:

For transient transfection pGL2.TATA.lnr.luc plasmid was used which contains three copies of vitellogenin estrogen response element (ERE) upstream of the TATA promoter (Addgene plasmid 11354). This is the same plasmid used to construct pGL2.TATA.lnr.luc.neo, used to create the stable cell line, T47D-KBluc.

Tamoxifen-resistant cells, TamR3 and TamR6, were grown in phenol-red-free RPMI 1640 supplemented with 10% CSS supplemented with 1 μM tamoxifen. The cells were seeded on a 96-well plate (2×10^4 cells/well). After 24 h, the cells were co-transfected with 50 ng each of the ER α responsive luciferase plasmid and a constitutive renilla reporter (to normalize for variations in transfection efficiency) using TransIT-2020 reagent (Mirus). Cells were treated next day with the test compounds in the presence of 1 nM E2. The compounds were added in a 2-fold dilution ranging from 0.1-50 μM . Tamoxifen was added at concentrations ranging from 0.000095-6 μM and fulvestrant was added in the range of 0.000095-50 μM . The medium contained 0.1% (v/v) ethanol and 0.1% (v/v) DMSO. 24 h after treatment, the medium was aspirated off and the cells were lysed by adding 50 μL of 1 $\times$ passive lysis buffer (Promega). Luciferase activities were assayed using the dual luciferase assay system (Promega).

TR-FRET ER- α coactivator assay:

Peptide displacement was assessed with the LanthaScreen TR-FRET ER- α Co-activator Assay Kit (PV4544, Life Technologies) as per instructions of the manufacturer. The compounds were tested in the range of 0.1-50 μM and cold PGC1 α was added at 3-fold dilution ranging from 1.8-50 μM .

For the peptide competition assay, the compounds were tested in the range of 0.05-400 μM in the presence of three different concentrations (250, 500

and 1000 nM) of Fluorescein-PGC1 α peptide and the recommended concentrations of GST tagged ER-LBD (7.25 nM), Terbium labelled anti-GST antibody (5 nM). Briefly, a 2-fold serial dilution of the test compounds was prepared at 100X final concentration in DMSO. The compounds were diluted 50-fold in complete assay buffer (assay buffer containing 5mM Dithiothreitol (DTT)) to get a 2X final concentration and 2% DMSO. The GST tagged ER-LBD was prepared at 4X final concentration in complete assay buffer, 4X Fluorescein-PGC1 α /4X Tb anti-GST antibody /4X EC₈₀ E2 mix was prepared separately in complete assay buffer. The EC₈₀ of E2 was determined to be 6.1 μ M in this assay. 10 μ l of the diluted test compounds was added to a black flat bottom 384-well plate followed by addition of 5 μ l of the 4X ER-LBD mix. 4X Fluorescein-PGC1 α /4X Tb anti-GST antibody/4X EC₈₀ E2 mix was added last. The plate was incubated at room temperature for 2 h and FRET was analysed on Synergy-4 multi-plate reader with the following setting: Excitation: 340 nm, Emission: 495 nm and 520 nm. The emission ratio (520:495) was analysed and plotted.

E2 displacement assay:

E2 displacement was assessed with the Polar Screen Estrogen Receptor- α Competitor Green Assay Kit (P2698, Life Technologies) as per the instructions of the manufacturer. For screening purposes, the compounds were tested at 20 μ M in the presence of 25nM full length ER α and 4.5nM fluorescein labelled-E2 (FI-E2). For E2 ligand competition assay, a 2-fold serial dilution of the test compounds was prepared at 100X final concentration in DMSO. The compounds were diluted 50-fold in assay buffer to get a 2X final concentration and 2% DMSO. 50 μ l of the diluted test compounds were added to a 50 μ l mixture containing 2X full length ER α and FI-E2 in each well to obtain final concentrations of 3-150 μ M of test compound in presence of 25nM full length ER α and 4.5nM FI-E2. Unlabelled -E2 was tested at concentrations ranging from (0.01-1000 nM). After 2h incubation, polarization was measured as per instructions of the manufacturer on TecanF500 plate reader.

Bio Layer Interferometry (BLI) assay:

The direct reversible interaction between small molecules and the ER- α was quantified by BLI using an OctetRED (ForteBio) apparatus. The LDB of the biotinylated ER α (bER α) was produced in situ with AviTag technology (Avidity). The AviTag sequence (GLNDIFEAQKIEWHE) was incorporated at the N terminus of the ER α -LBD (302-552). A six residue histidine tag was incorporated at the C-terminus of the ER α -LBD for purification of the protein. Escherichia coli strain BL21 containing both biotin ligase and ER α -LBD vectors was induced with 0.5 mM isopropyl- β -D-thiogalactopyranoside (IPTG) in the presence of 0.02 mM E2 and 0.15 mM biotin at 16°C overnight. The bacteria were then lysed by sonication, and the resulting lysate was purified by immobilized metal ion affinity chromatography (IMAC) with nickel-agarose beads (GE Healthcare) and cation-exchange chromatography (HiTrap SP). Purified and biotinylated protein (bER α -LBD at 0.05 mg/mL) was bound to the super-streptavidin sensors overnight at 4°C in assay buffer [20 mM Tris, pH 7.5, 500 mM NaCl, 0.2 mM tris (2-carboxyethyl) phosphine (TCEP), 0.02 mM E2, 5% glycerol and 5% DMSO]. The compounds were dissolved in the assay buffer in a 2-fold dilution series ranging from 3.1-100 μ M. In all experiments, a known AF2-interacting peptide, PGC-1a (Elim Biopharmaceuticals, CA, USA) was used as a control to confirm functionality of the bER α -LBD.

Cell Viability – 1:

Cell proliferation was determined using the MTS assay. Cells were seeded in 96-well plates at a density of 5×10^3 cells/well. MCF7, TamR3, TamR6, MDA-MB-453 and HeLa cells were seeded in their respective media. On the following day, the cells were treated with test compounds (0.2-50 μ M) in the presence of 1nM E2 and incubated at 37°C in 5% CO₂. After 96 h, 30 μ L of MTS reagent (CellTiter 961 Aqueous One Solution Reagent, Promega) was added and incubated for 90 mins at 37°C in 5% CO₂. The production of formazan was measured at 490 nm.

Cell Viability - 2:

Cell proliferation was determined using the presto blue cell viability assay. Cells were seeded in 96-well plates at a density of 5×10^3 cells/well. MCF7, TamR3, TamR6, MDA-MB-453 and HeLa cells were seeded in their respective media. On the following day, the cells were treated with test compounds (0.2-50 μ M) in the presence of 1nM E2 and incubated at 37°C in 5% CO₂. After 96 h, 30 μ L of MTS reagent (CellTiter 961 Aqueous One Solution Reagent, Promega) was added and incubated for 90 mins at 37°C in 5% CO₂. The production of formazan was measured at 490 nm.

qRT-PCR:

mRNA levels were analysed by quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). For this purpose, serum starved MCF7 cells were seeded onto 6 well plates at a density of 6×10^5 cells/well. After 24h, the cells were treated with the either VPC-16230 or OHT in the presence and absence of 1nM E2. RNA was isolated after 24h with TRIzol reagent and purified with the RNAeasy mini-kit (Qiagen). The purified mRNA was quantified using nanodrop. 0.5 μ g RNA was reverse transcribed using iScript kit (Biorad). 100 ng cDNA product was added to the primer mix. Final concentration of the primers was 5 pM. The sequences of the primers used in the qRT-PCR were as follows: pS2, forward 5'-TTGTGGTTTTCTGGTGTCA-3' and reverse 5'-GCAGATCCCTGCAGAAGTGT-3', Cathepsin-D, forward 5'-CAGAAGCTGGTGGACCAGAAC-3' and reverse 5'-TGCGGGTGACATTCAGGTAG-3', CDC2, forward 5'-ACTGGCTGATTTTGGCCTTG-3' and reverse 5'-TTGAGTAACGAGCTGACCCCA-3', GAPDH, forward 5'-TGCACCACCAACTGCTTAGC-3' and reverse 5'-GGCATGGACTGTGGTCATGAG-3'. The fold change in expression of the gene was calculated using the delta delta C_t method with GAPDH as the internal control.

Western Blotting:

MCF7 cells were serum starved in phenol red-free RPMI containing 10% CSS for five days. The cells were then seeded onto a 6-well plate at a density of 6×10^5 cells/well and treated the following day with the test compounds in the presence of 1nM E2. After 24h, the medium was aspirated off and the cells were washed with ice-cold phosphate-buffered saline (PBS). Cells were lysed in 1X radioimmunoprecipitation assay (RIPA) buffer containing 1 tablet of protease inhibitor cocktail (Roche). Cell debris was pelleted by centrifugation at 15000g for 10 min at 4°C. The supernatants were collected and quantified using the BCA assay. In each case, 25 µg of protein was loaded onto 15% (v/v) sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels, separated and transferred to PVDF membrane. Membranes were incubated with pS2, Cathepsin-D and CDC2 antibodies or control alpha-actin antibody. Bound antibodies were detected using horseradish peroxidase-conjugated secondary antibodies. Chemiluminescence was detected with ECL Detection Kit (GE Healthcare) and bands were visualised using the GBox imager (Syngene).

Statistical Analysis:

Data was analysed and dose response curves were generated using GraphPad Prism5. A p value <0.05 was considered significant (*).

List of abbreviations:

BCa: Breast cancer; ERα: Estrogen receptor-alpha; E2: Estradiol; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor type 2; IGF-1R: Insulin like growth factor-1; ERK: Extracellular-regulated kinase; Akt: Serine/threonine-specific protein kinase (PKB); AF2: Activation function-2; LBD: Ligand binding domain; HBS: hormone binding site; SRC-1: Steroid receptor coactivator 1; CBP: CREB-binding protein; MOE: Molecular Operating Environment; eHiTS: electronic high-throughput screening; ATCC: American type culture collection; FBS: Fetal bovine serum; CSS: Charcoal stripped serum; OHT: 4-Hydroxytamoxifen; DMSO: Dimethyl sulfoxide; TR-FRET: Time resolved-Fluorescence resonance energy transfer; BLI: Bio layer interferometry; bERα: biotinylated ERα; ERE: Estrogen response element; qRT-

PCR: Quantitative real-time reverse transcriptase-polymerase chain reaction; GAPDH: Glyceraldehyde phosphate dehydrogenase; PBS: Phosphate-buffered saline; RIPA: Radio immuno precipitation assay; SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis; BF3: Binding Function-3; AR: Androgen receptor.

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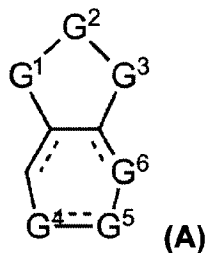
Zsoldos Z, Reid D, Simon A, Sadjad SB, Johnson AP: eHiTS: A new fast, exhaustive flexible ligand docking system. *J Mol Graph* 2007, 26(1):198-212.

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. Furthermore, numeric ranges are provided so that the range of values is recited in addition to the individual values within the recited range being specifically recited in the absence of the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Furthermore, material appearing in the background section of the specification is not an admission that such material is prior art to the invention. Any priority document(s) are incorporated herein by reference as if each individual priority document were specifically and individually indicated to be incorporated by reference herein and

as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

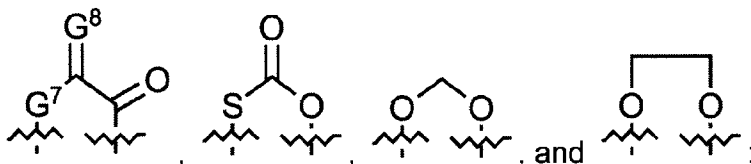
What is claimed is:

1. A compound of Formula A,

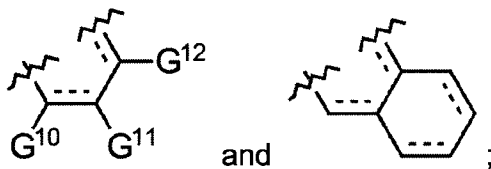


wherein

G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

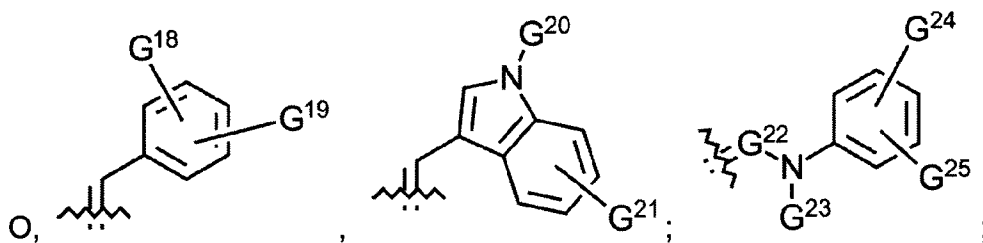


G^4 , G^5 , and G^6 , when taken together are selected from the group consisting of:

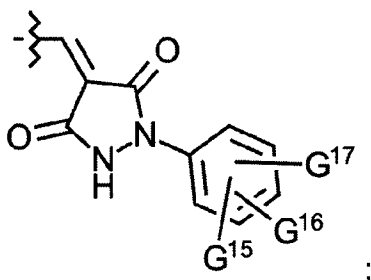


G^7 is selected from the group consisting of: S, CH_2 , and O;

G^8 is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , CH_2-CH_3 , OCH_3 , NO_2 , and



G^{15} , G^{16} , and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $O-CH_2-CH_3$, and NO_2 ;

G^{18} is selected from the group consisting of: OH, CH_3 , and CH_2-CH_3 ;

G^{19} is selected from the group consisting of: H, OH, CH_3 , and CH_2-CH_3 ;

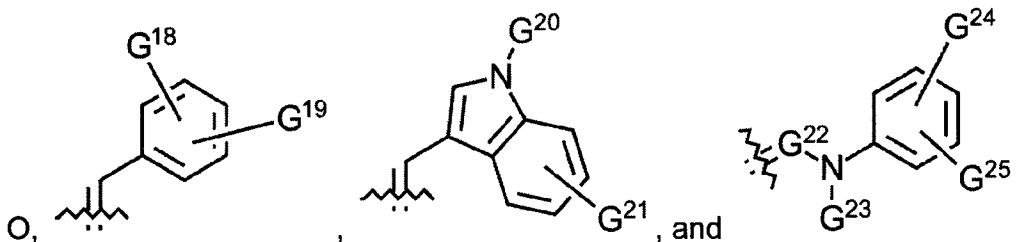
G^{20} and G^{21} are each independently selected from the group consisting of: H, CH_3 , CH_2-CH_3 , $CH_2-CH_3-CH_3$, and $CH_3-CH_2-CH_3$;

G^{22} is selected from the group consisting of: N and CH;

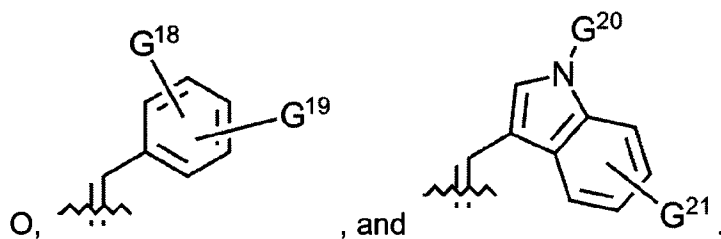
G^{23} is selected from the group consisting of: H and  ;

G^{24} and G^{25} are each independently selected from the group consisting of: H, Cl and CH_3 , provided that when G^{25} is CH_3 , then either i) G^{24} is Cl, or ii) G^7 is CH_2 .

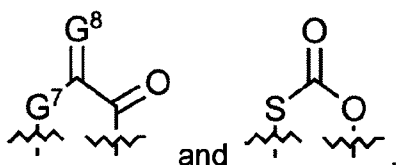
2. The compound of claim 1 wherein G^8 is selected from the group consisting of:



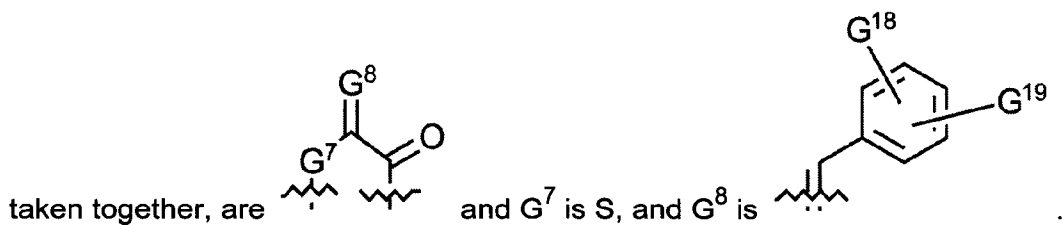
3. The compound of claim 1 wherein G^8 is selected from the group consisting of:



4. The compound of any one of claims 1 to 3 wherein G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

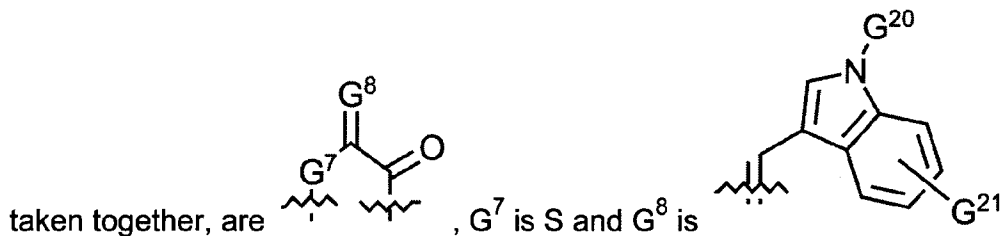


5. The compound of any one of claims 1 to 4 wherein G^7 is S.
6. The compound of any one of claims 1 to 4 wherein G^1 , G^2 , and G^3 , when



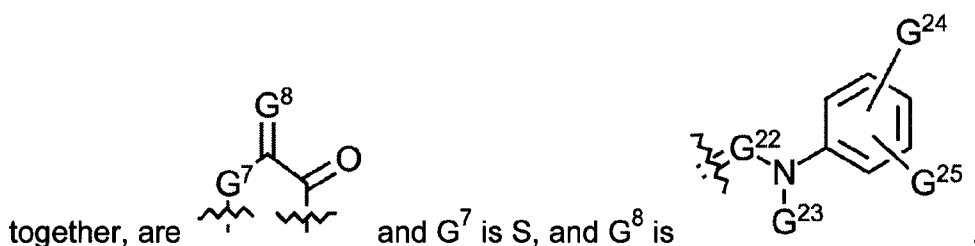
7. The compound of any one of claims 1 to 6 wherein G^{18} is OH.
8. The compound of any one of claims 1 to 7 wherein G^{19} is OH.

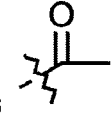
9. The compound of any one of claims 1 to 4 wherein G^1 , G^2 , and G^3 , when



10. The compound of claim 9 wherein G^{20} is H and G^{21} is $\text{CH}_2\text{-CH}_3$.

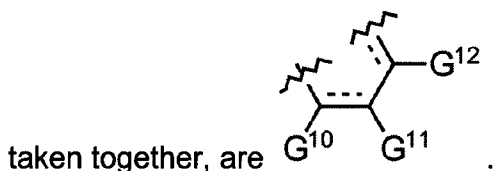
11. The compound of claim 1 or 2 wherein G^1 , G^2 , and G^3 , when taken



12. The compound of claim 11 wherein G^{22} is CH_2 and G^{23} is .

13. The compound of claim 11 or 12 wherein G^{24} is Cl and G^{25} is H.

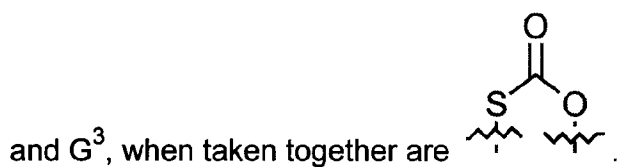
14. The compound of any one of claims 1 to 13 wherein G^4 , G^5 , and G^6 , when



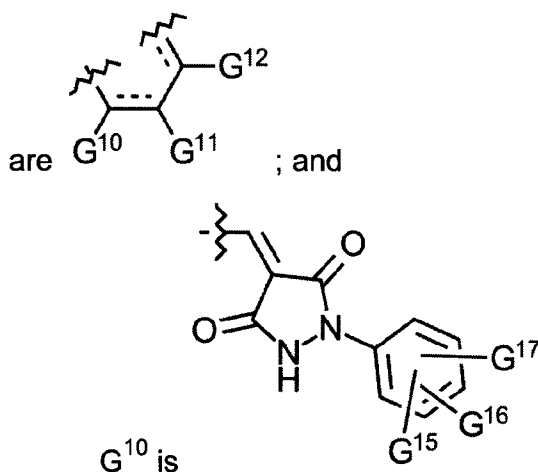
15. The compound of claim 14 wherein G^{10} and G^{11} are each H.

16. The compound of claim 15 wherein G^{12} is CH_3 or F.

17. The compound of any one of claims 1 to 3 wherein G^8 is O and G^1 , G^2 ,



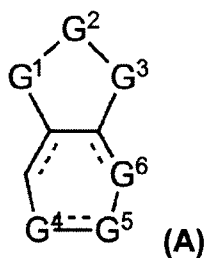
18. The compound of claim 17 wherein G^4 , G^5 , and G^6 , when taken together,



19. The compound of claim 17 or 18 wherein G^{11} is H; and G^{12} is OCH_3 .

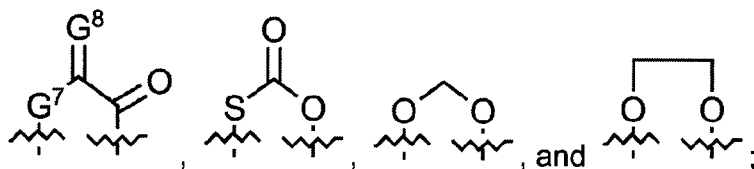
20. A pharmaceutical composition comprising a compound of any one of claims 1 to 19 and a pharmaceutically acceptable excipient.

21. Use of a compound of Formula A,

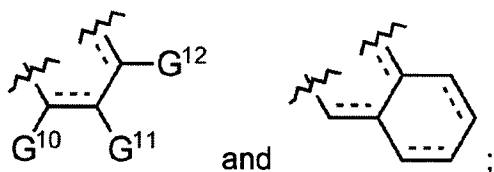


wherein

G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

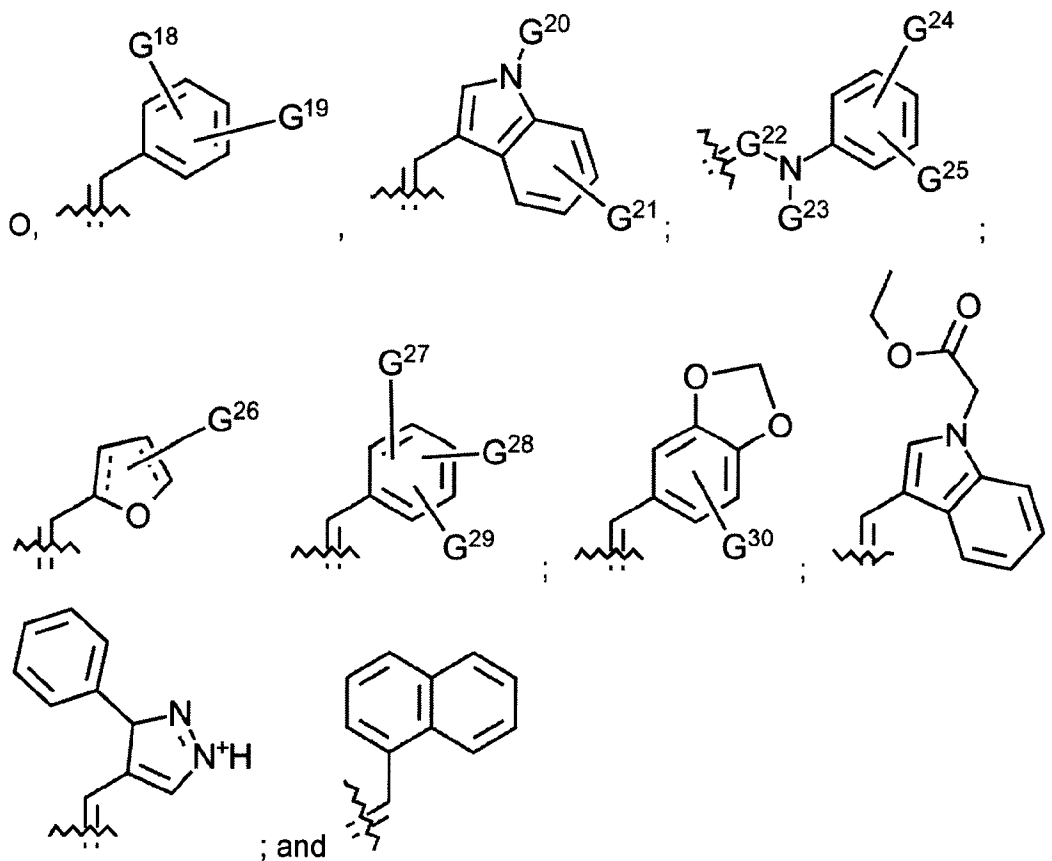


G^4 , G^5 , and G^6 , when taken together are selected from the group consisting of:

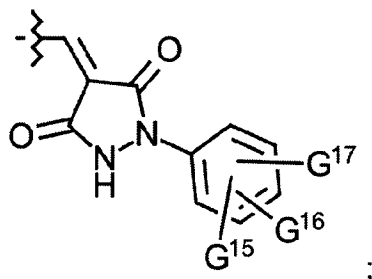


G^7 is selected from the group consisting of: S, CH_2 , and O;

G^8 is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , CH_2-CH_3 , OCH_3 , NO_2 , and



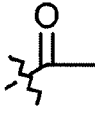
G^{15} , G^{16} , and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $O-CH_2-CH_3$ and NO_2 ;

G^{18} is selected from the group consisting of: OH and CH_3 , and CH_2-CH_3 ;

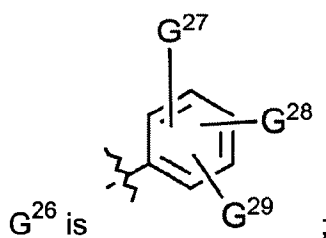
G^{19} is selected from the group consisting of: H, OH, CH_3 , and CH_2-CH_3 ;

G^{20} and G^{21} are each independently selected from the group consisting of: H, CH_3 , CH_2-CH_3 , $CH_2-CH_3-CH_3$, and $CH_3-CH_2-CH_3$;

G^{22} is selected from the group consisting of: N and CH;

G^{23} is selected from the group consisting of: H and  ;

G^{24} and G^{25} are each independently selected from the group consisting of: CH_3 , Cl, H, F and $O-CH_3$;

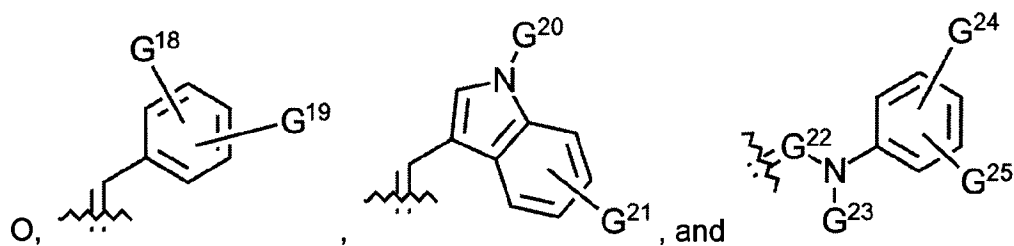


G^{27} , G^{28} , and G^{29} are each independently selected from the group consisting of: Cl, CH_3 , $O-CH_3$, $O-CH_2-CH_3$, and NO_2 ;

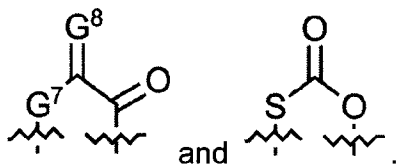
G^{30} is NO_2 or H;

in the treatment of cancer.

22. The use claim 21 wherein G^8 is selected from the group consisting of:

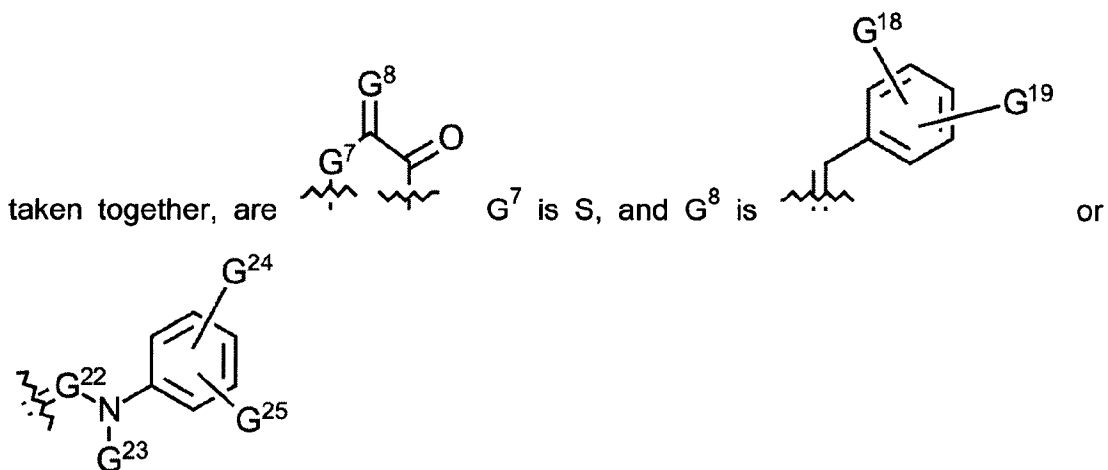


23. The use of claim 21 or 22 wherein G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:



24. The use of any one of claims 21 to 23 wherein G^7 is S.

25. The use of any one of claims 21 to 24 wherein G^1 , G^2 , and G^3 , when



26. The use of any one of claims 21 to 25 wherein G^{18} is OH.

27. The use of any one of claims 21 to 25 wherein G^{19} is OH.

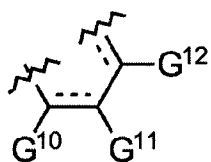
28. The use of any one of claims 21 to 25 wherein G^{22} is N and G^{23} is



29. The use of claim 28 wherein G^{24} is Cl.

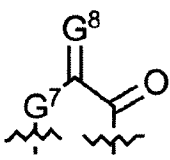
30. The use of claim 28 or 29 wherein G^{25} is CH_3 .

31. The use of any one of claims 28 to 30 wherein G^4 , G^5 , and G^6 , when

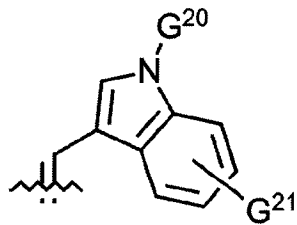


taken together are G^{10} G^{11} and G^{12} is F or CH_3 .

32. The use of any one of claims 21 to 23 wherein G^1 , G^2 , and G^3 , when

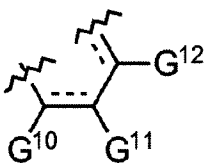


taken together, are G^7 is S and G^8 is



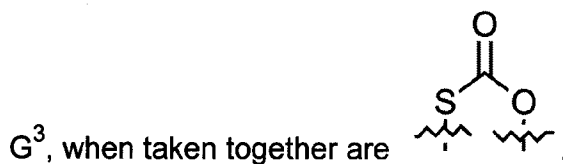
33. The use of claim 32 wherein G^{20} is H and G^{21} is CH_2-CH_3 .

34. The use of any one of claims 21 to 33 wherein G^4 , G^5 , and G^6 , when

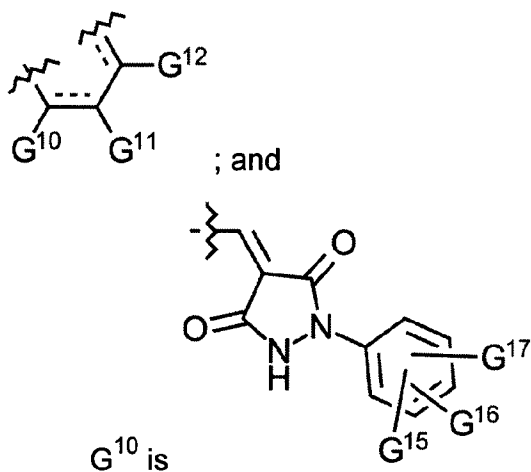


taken together, are G^{10} G^{11} ; and G^{10} , G^{11} , and G^{12} are each H.

35. The use of any one of claims 21 to 23 wherein G^8 is O and G^1 , G^2 , and

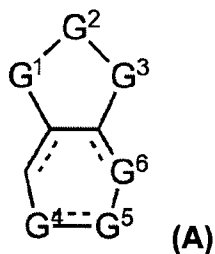


36. The use of claim 35 wherein G^4 , G^5 , and G^6 , when taken together, are



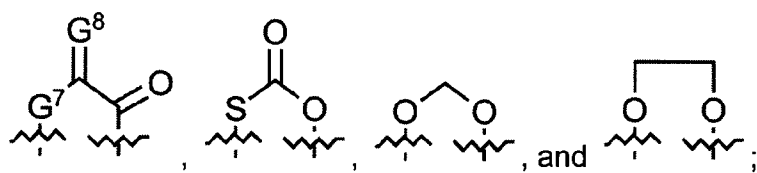
37. The use of claim 35 or 36 wherein G^{11} is H; and G^{12} is OCH_3 .

38. A method of medical treatment comprising administering to a subject having or suspected of having breast cancer a compound of Formula A,

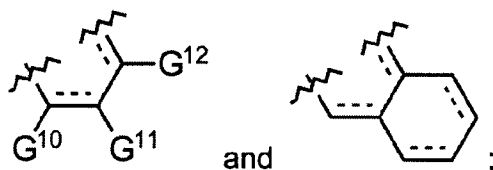


wherein

G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:

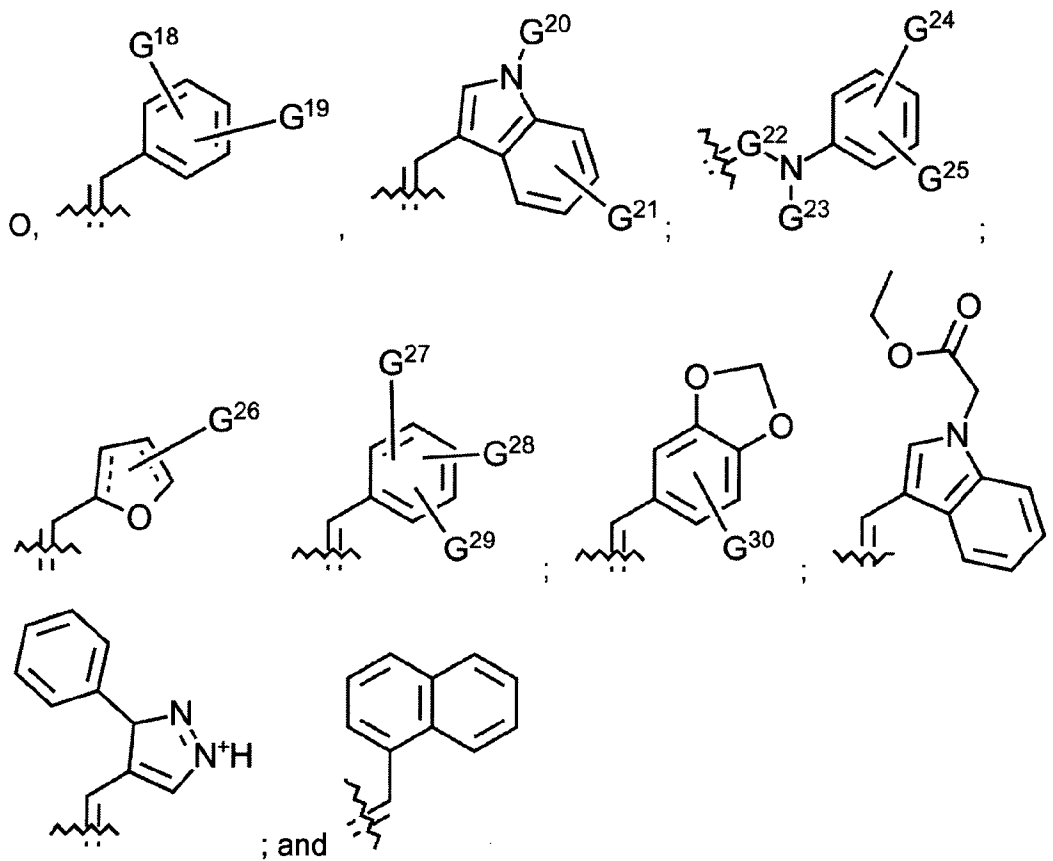


G^4 , G^5 , and G^6 , when taken together are selected from the group consisting of:

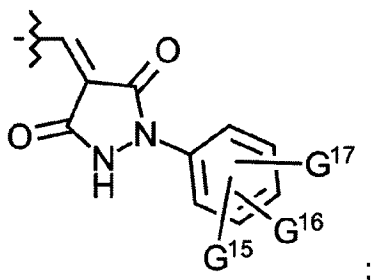


G^7 is selected from the group consisting of: S , CH_2 , and O ;

G^8 is selected from the group consisting of:



G^{10} , G^{11} , and G^{12} are each independently selected from the group consisting of: H, Cl, F, Br, CH_3 , CH_2-CH_3 , OCH_3 , NO_2 , and



G^{15} , G^{16} and G^{17} are each independently selected from the group consisting of: Cl, Br, F, CH_3 , OH, $O-CH_2-CH_3$ and NO_2 ;

G^{18} is selected from the group consisting of: OH, CH_3 and CH_2-CH_3 ;

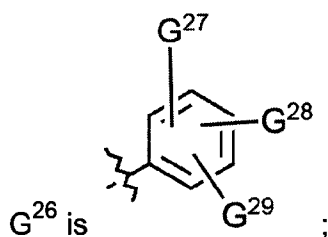
G^{19} is selected from the group consisting of: H, OH, CH_3 , and CH_2-CH_3 ;

G^{20} and G^{21} are each independently selected from the group consisting of: H and CH_3 , CH_2-CH_3 , $CH_2-CH_3-CH_3$, $CH_3-CH_2-CH_3$;

G^{22} is selected from the group consisting of: N and CH;

G^{23} is selected from the group consisting of: H and  ;

G^{24} and G^{25} are each independently selected from the group consisting of: CH_3 , Cl, H, F and OCH_3 ;

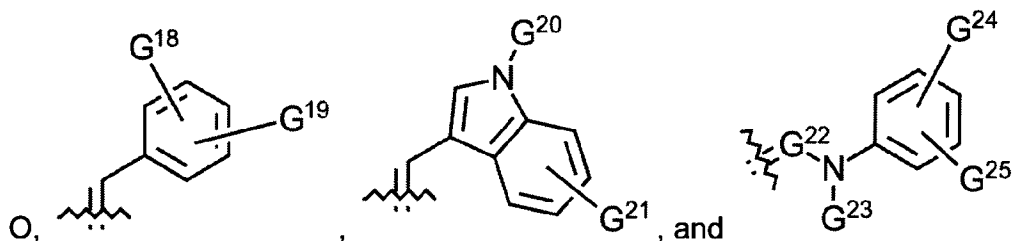


G^{27} , G^{28} , and G^{29} are each independently selected from the group consisting of: Cl, CH_3 , $O-CH_3$, $O-CH_2-CH_3$, and NO_2 ;

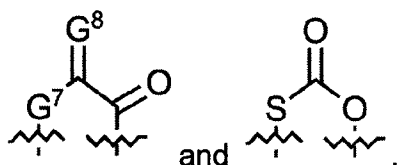
G^{30} is NO_2 or H;

in the treatment of cancer.

39. The method of medical treatment of claim 38 wherein G^8 is selected from the group consisting of:

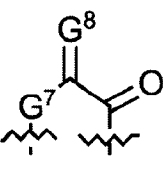


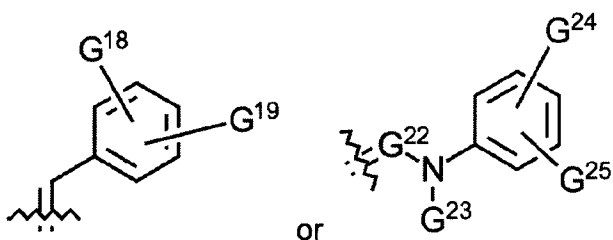
40. The method of medical treatment of claim 38 or 39 wherein G^1 , G^2 , and G^3 , when taken together are selected from the group consisting of:



41. The method of medical treatment of any one of claims 38 to 40 wherein G^7 is S.

42. The method of medical treatment of any one of claims 38 to 41 wherein

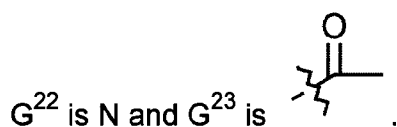
G^1 , G^2 , and G^3 , when taken together, are  G^7 is S, and G^8 is



43. The method of medical treatment of any one of claims 38 to 42 wherein G^{18} is OH.

44. The method of medical treatment of any one of claims 38 to 42 wherein G^{19} is OH.

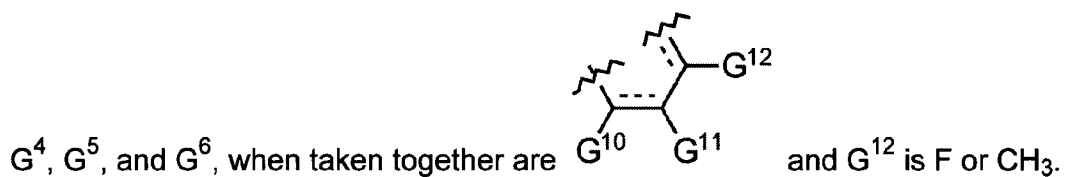
45. The method of medical treatment of any one of claims 38 to 42 wherein



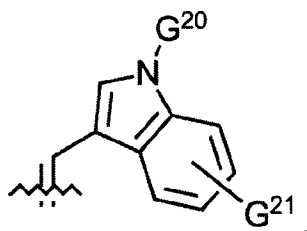
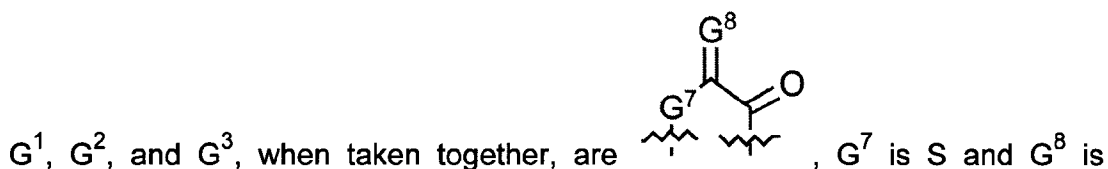
46. The method of medical treatment of claim 45 wherein G^{24} is Cl.

47. The method of medical treatment of claim 45 or 46 wherein G^{25} is CH_3 .

48. The method of medical treatment of any one of claims 45 to 47 wherein

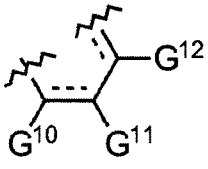


49. The method of medical treatment of any one of claims 38 to 40 wherein

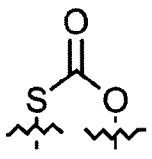


50. The method of medical treatment of claim 49 wherein G^{20} is H and G^{21} is CH_2-CH_3 .

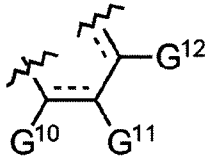
51. The method of medical treatment of any one of claims 38 to 50 wherein

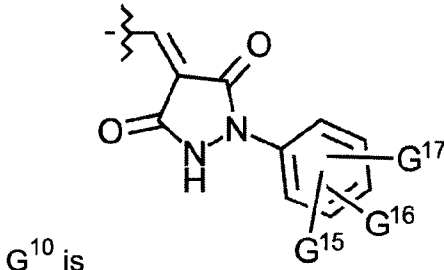
G^4 , G^5 , and G^6 , when taken together, are ; and G^{10} , G^{11} , and G^{12} are each H.

52. The method of medical treatment of any one of claims 38 to 40 wherein G^8

is O and G^1 , G^2 , and G^3 , when taken together are .

53. The method of medical treatment of claim 52 wherein G^4 , G^5 , and G^6 ,

when taken together, are ; and

G^{10} is .

54. The method of medical treatment of claim 52 or 53 wherein G^{11} is H; and G^{12} is OCH_3 .

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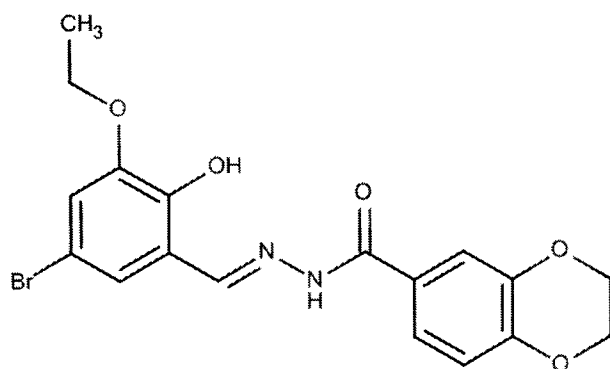
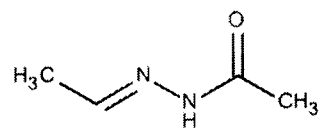
FIG. 1**Compound 1****Chemical template**

FIG. 2

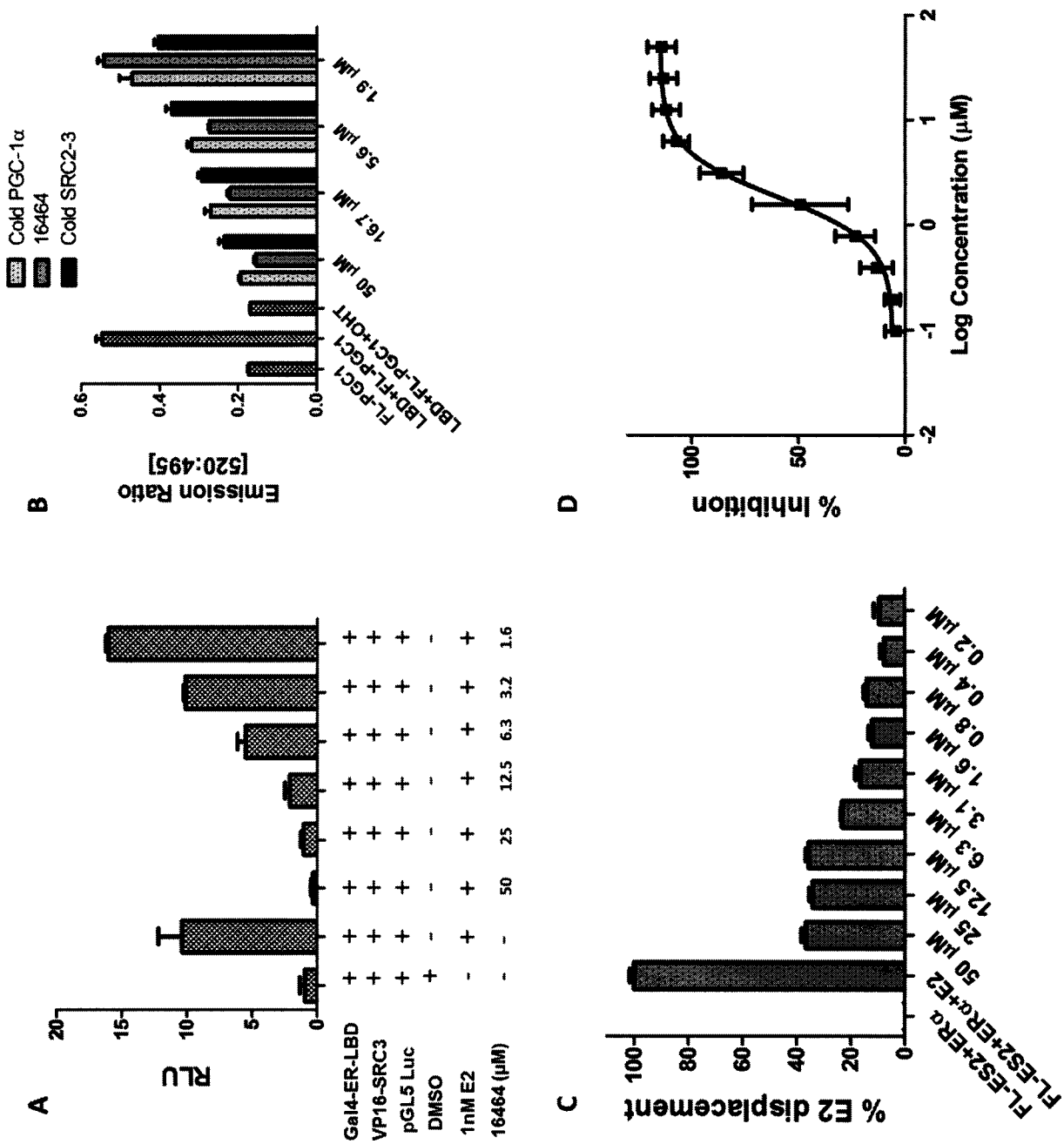


FIG. 3

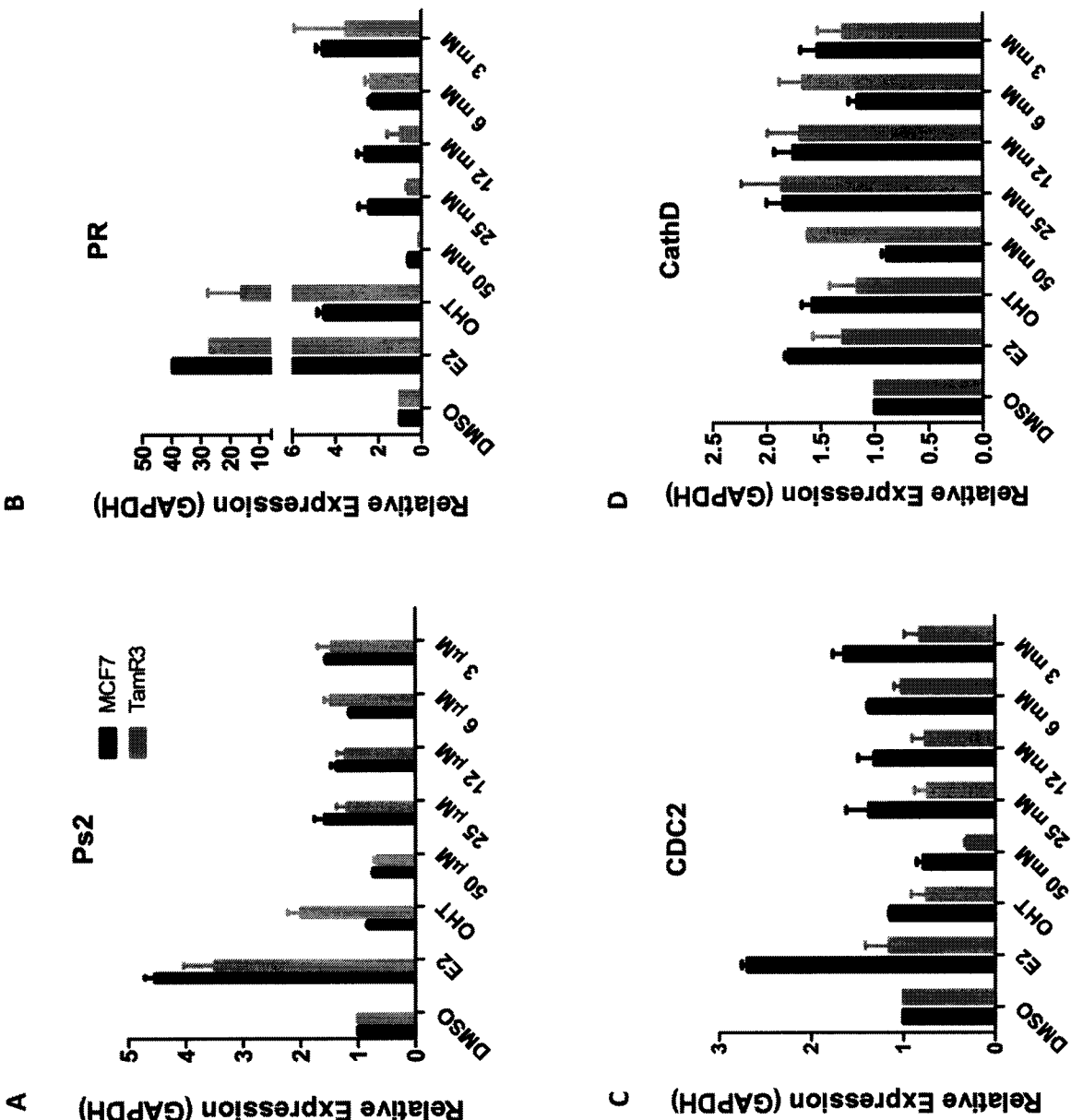
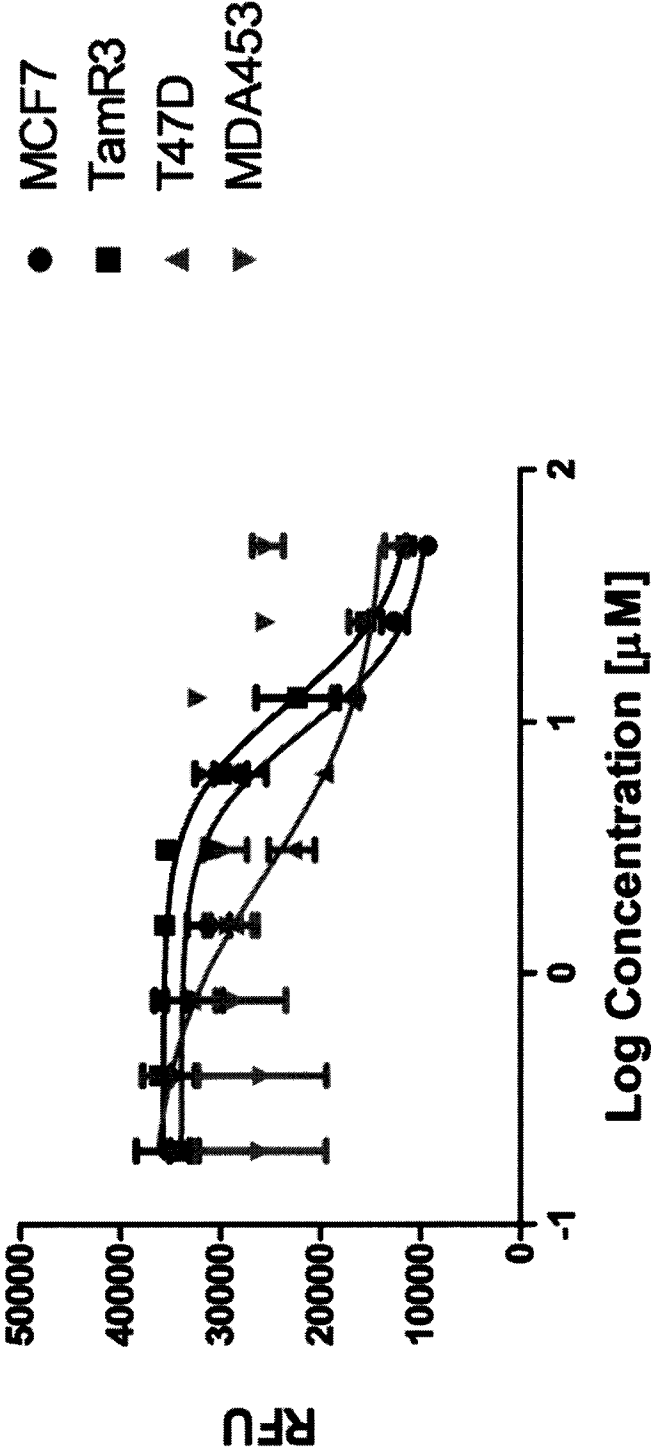


FIG. 4



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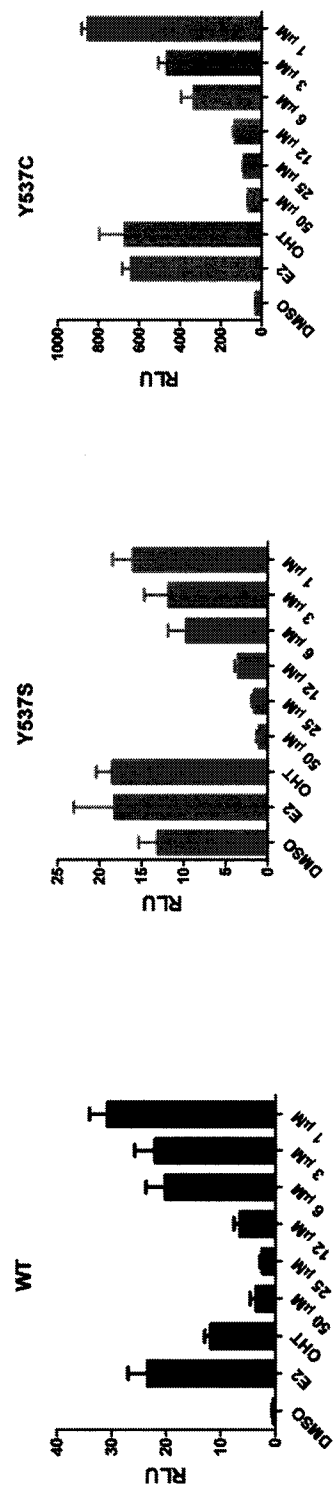


FIG. 5

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FIG. 6

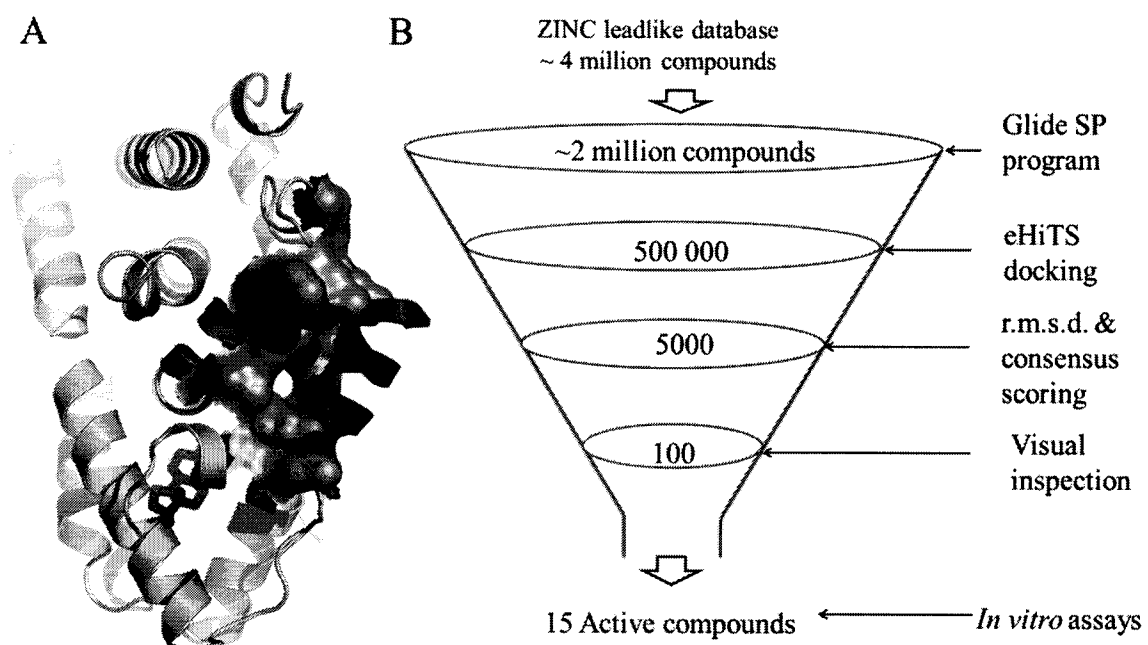
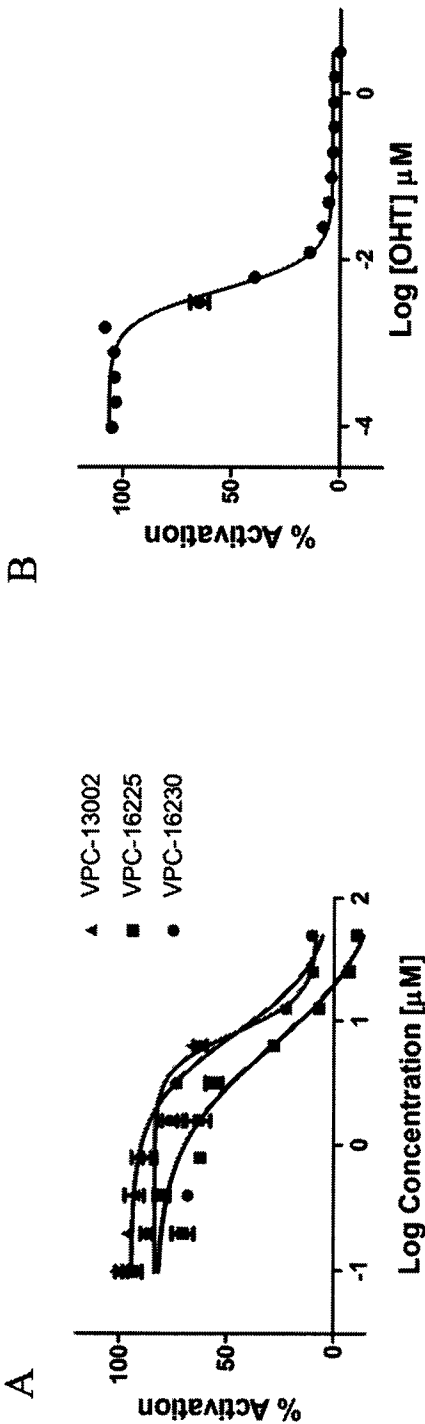


FIG. 7
Luciferase Assay



TR-FRET Assay

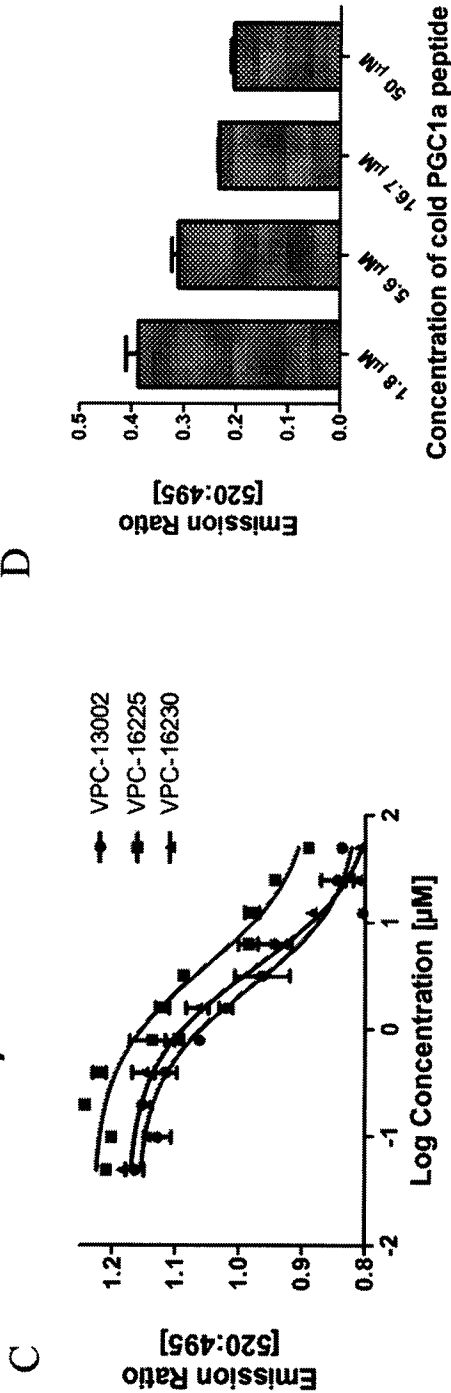


FIG. 8

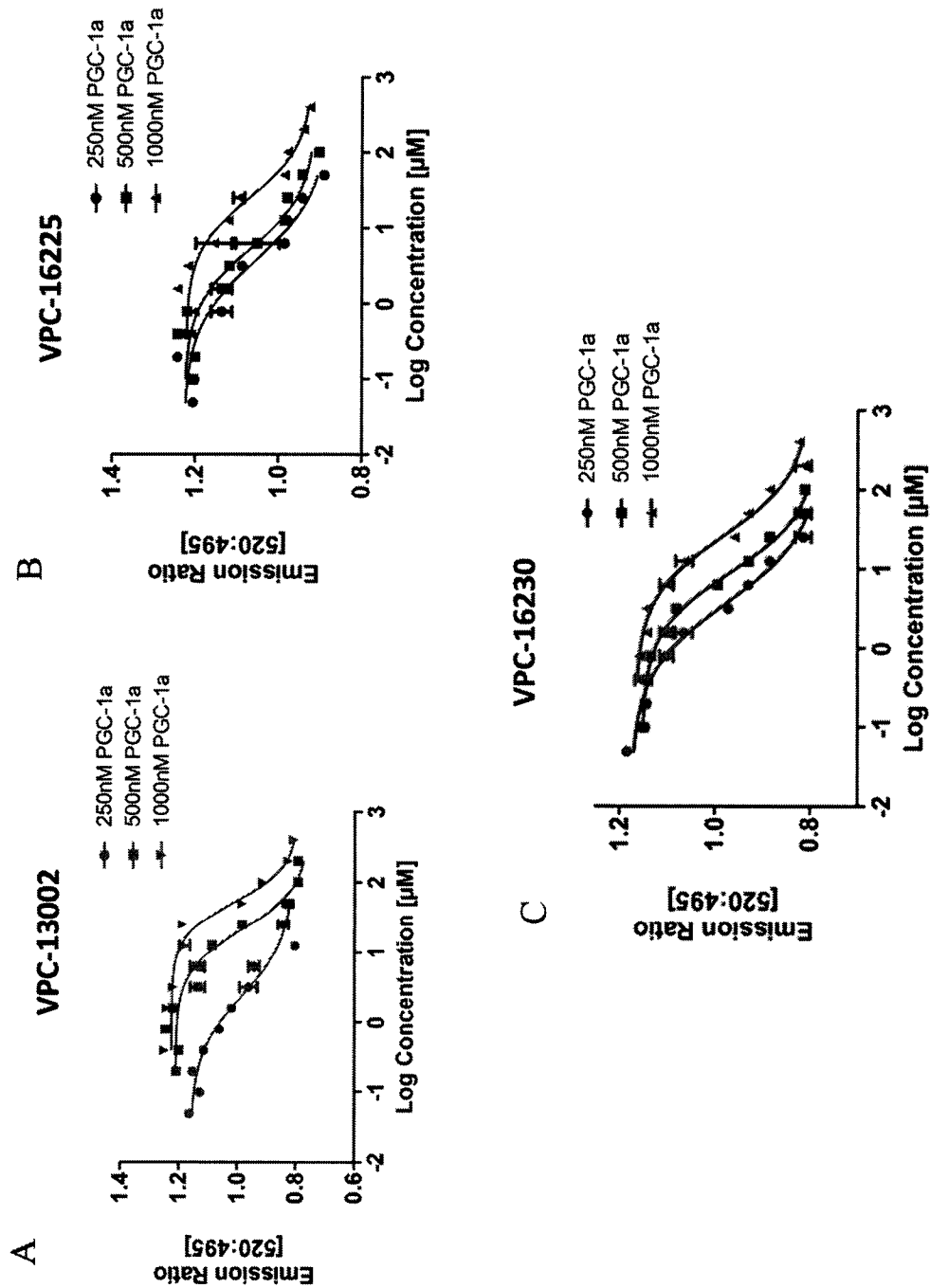


FIG. 9

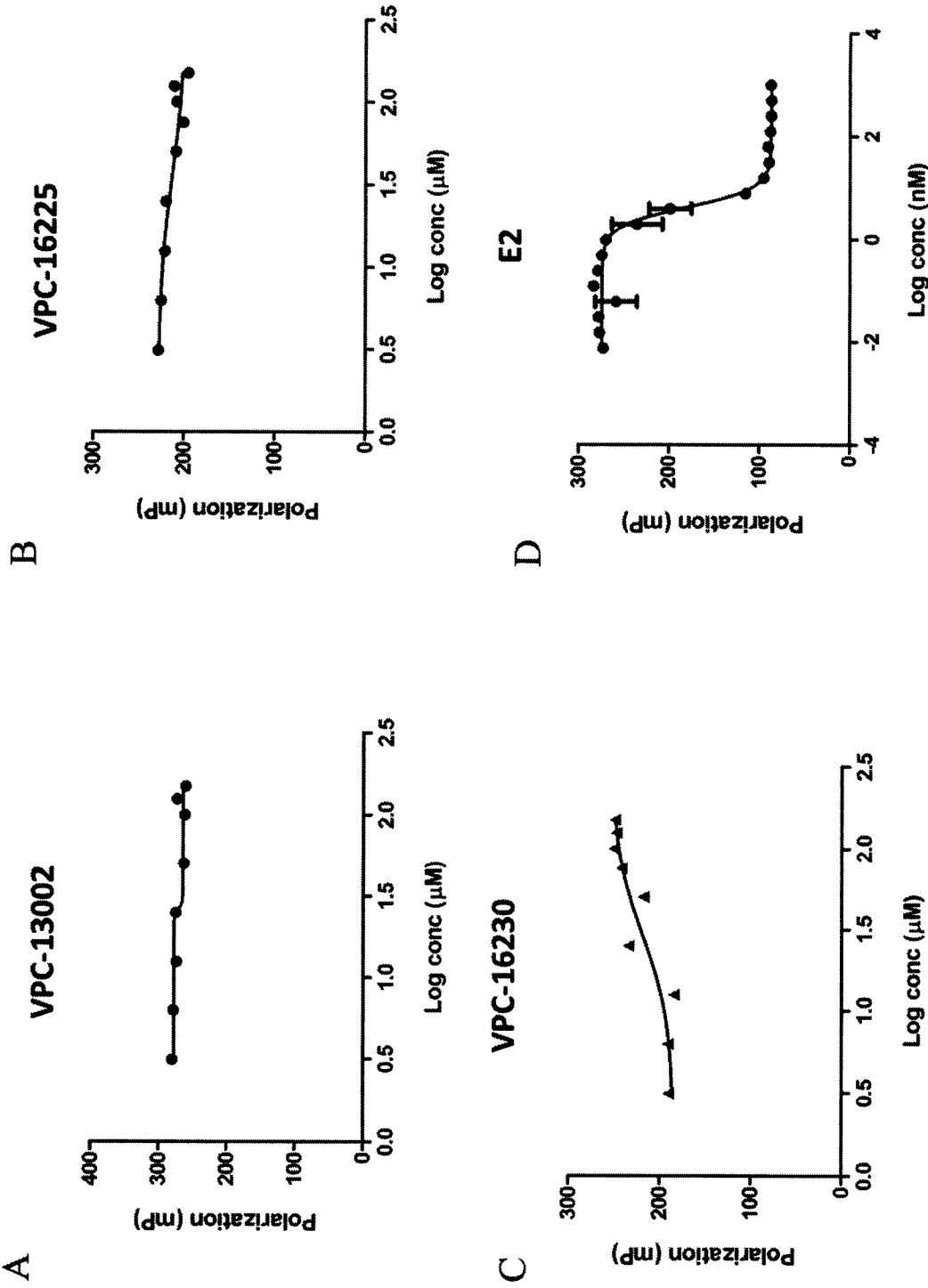


FIG. 10

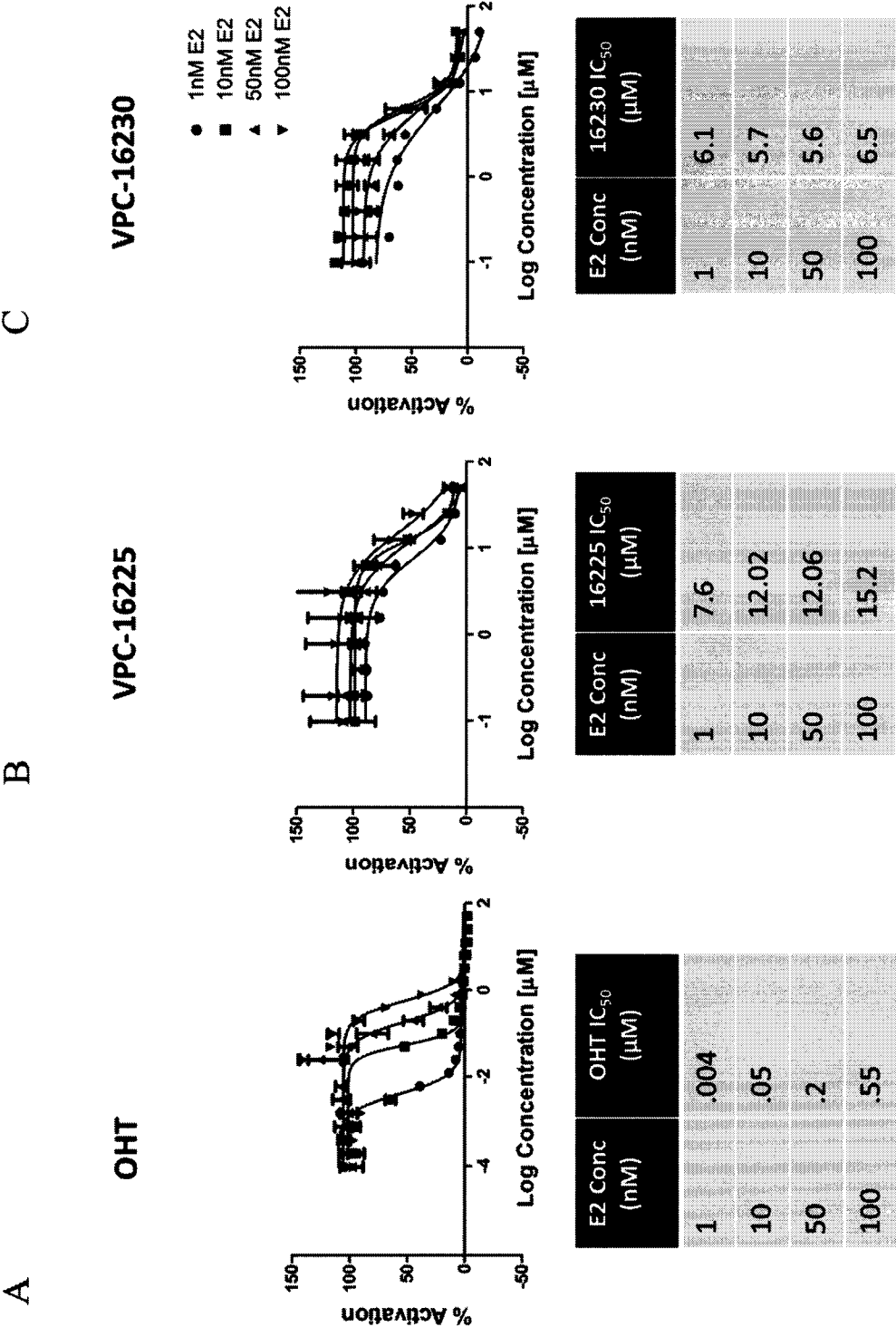
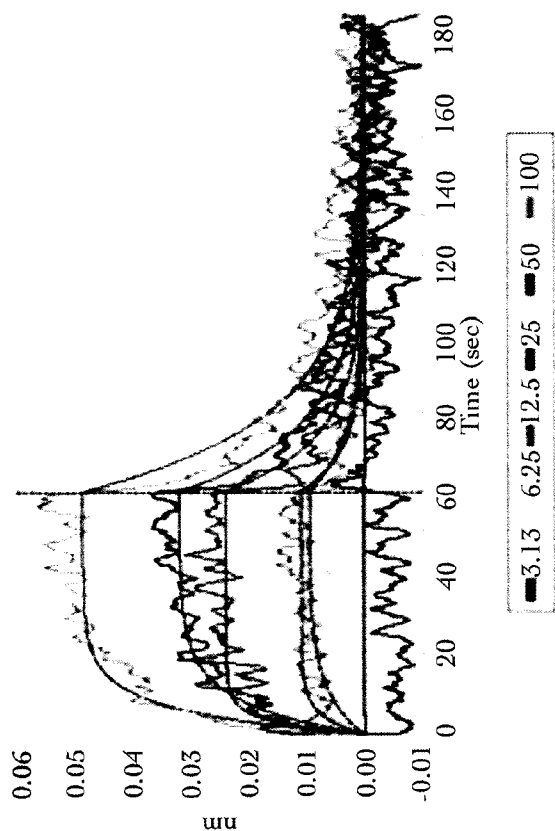
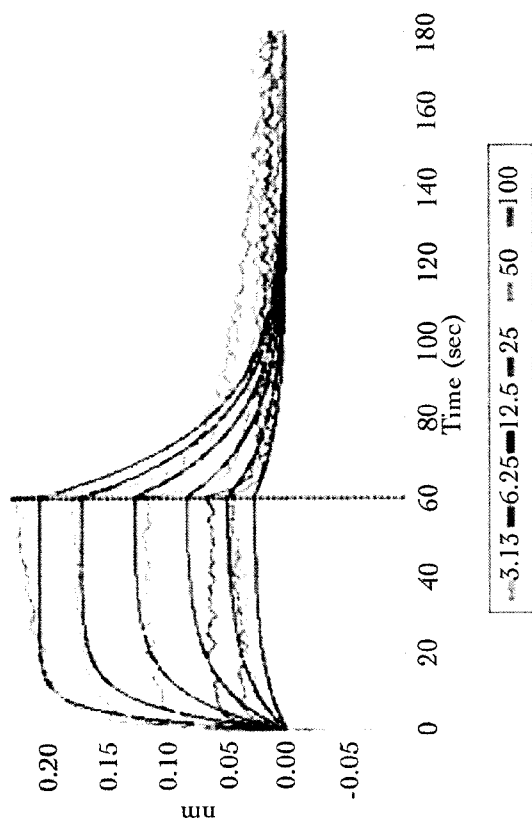


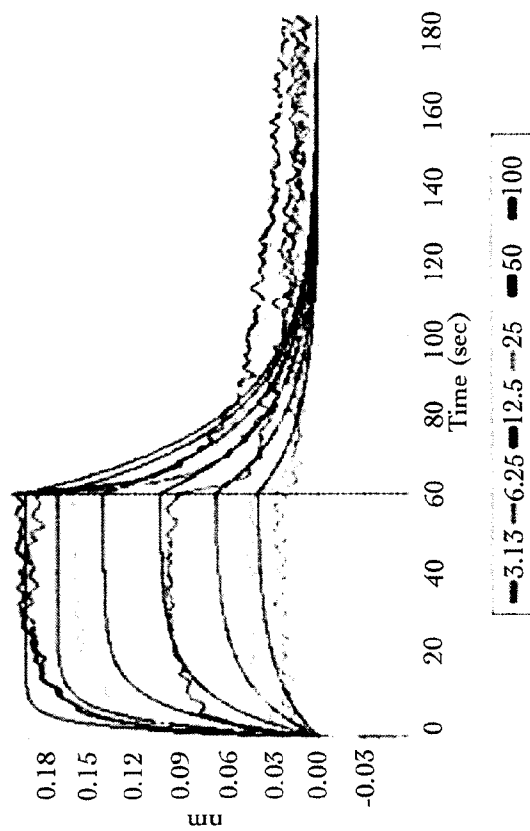
FIG. 11 A



B



C



D

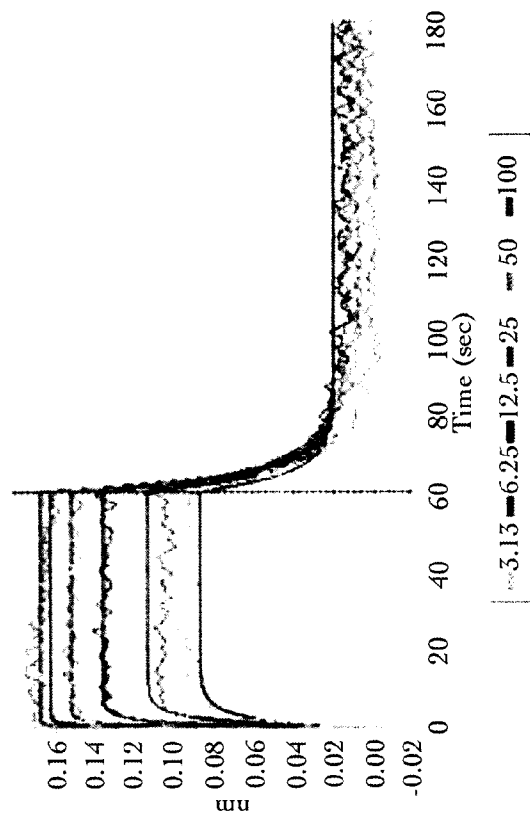


FIG. 12

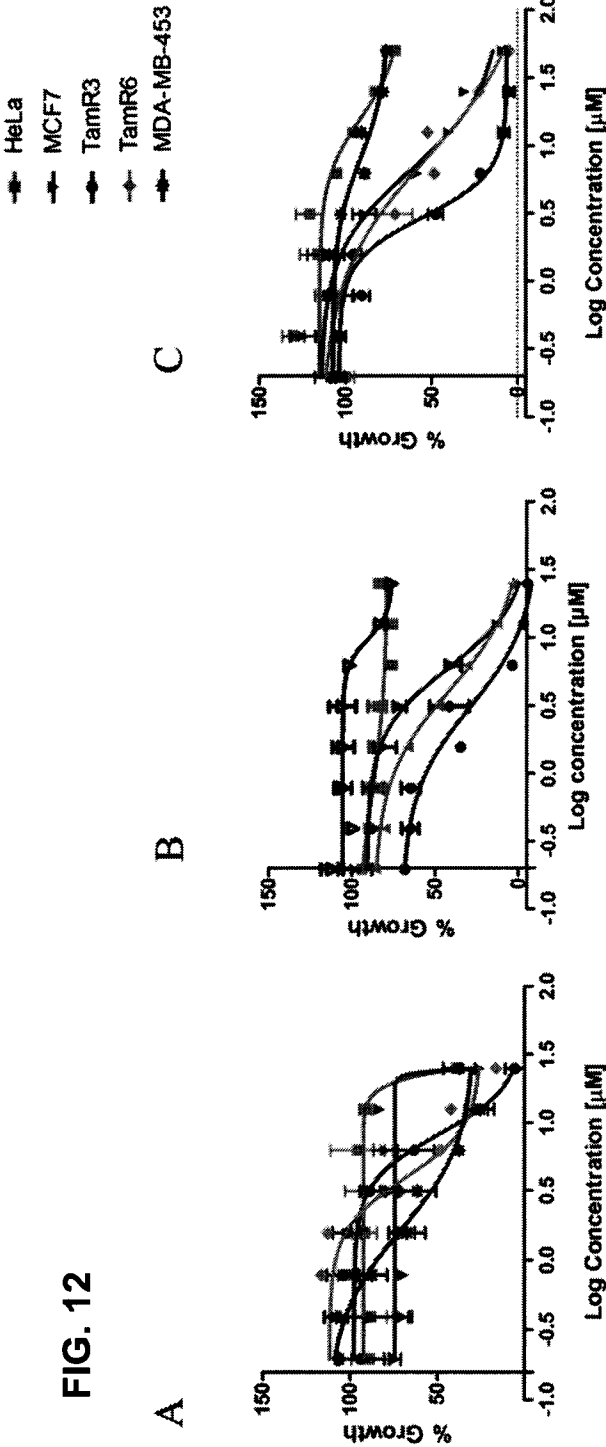


FIG. 13

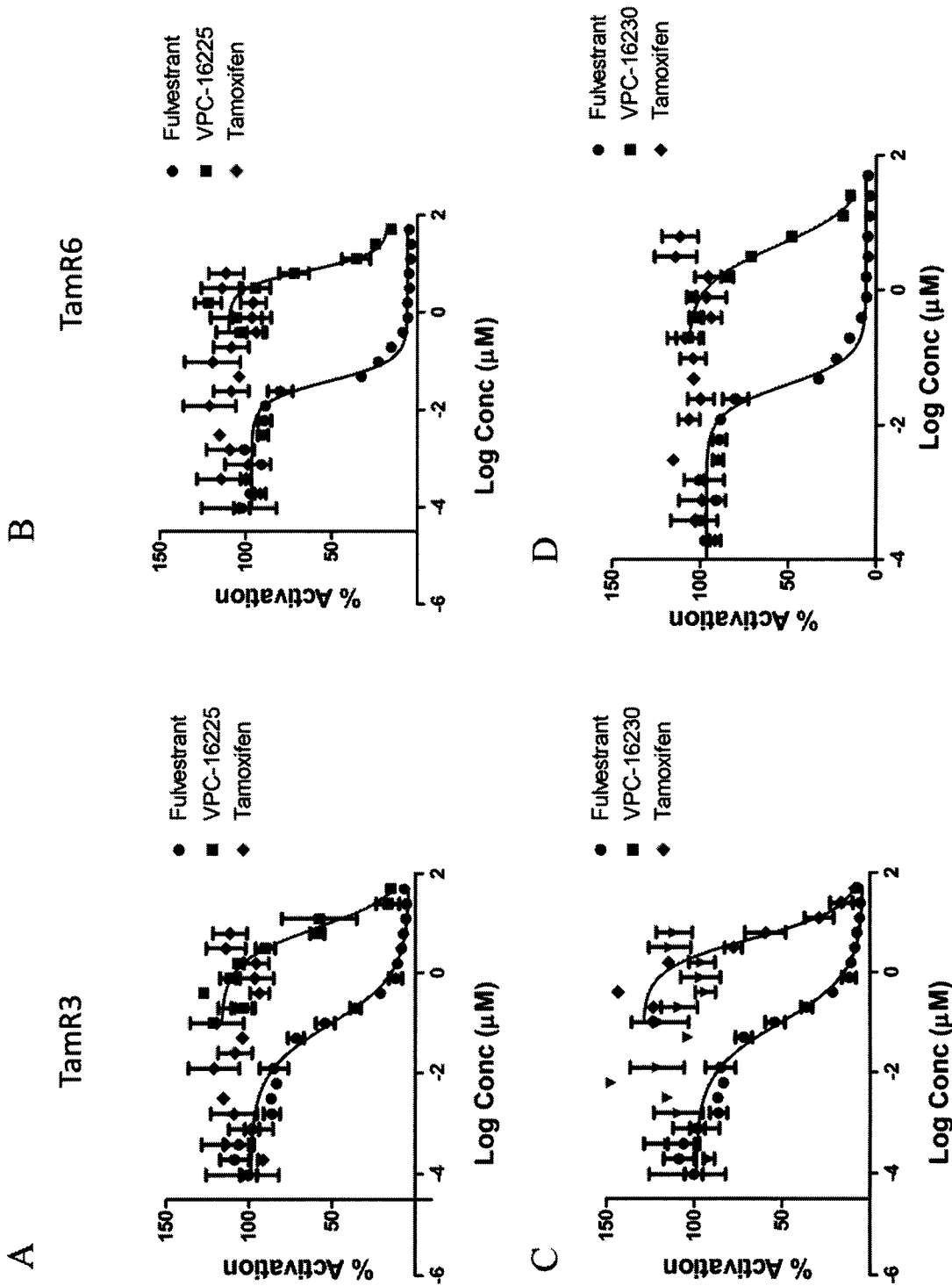
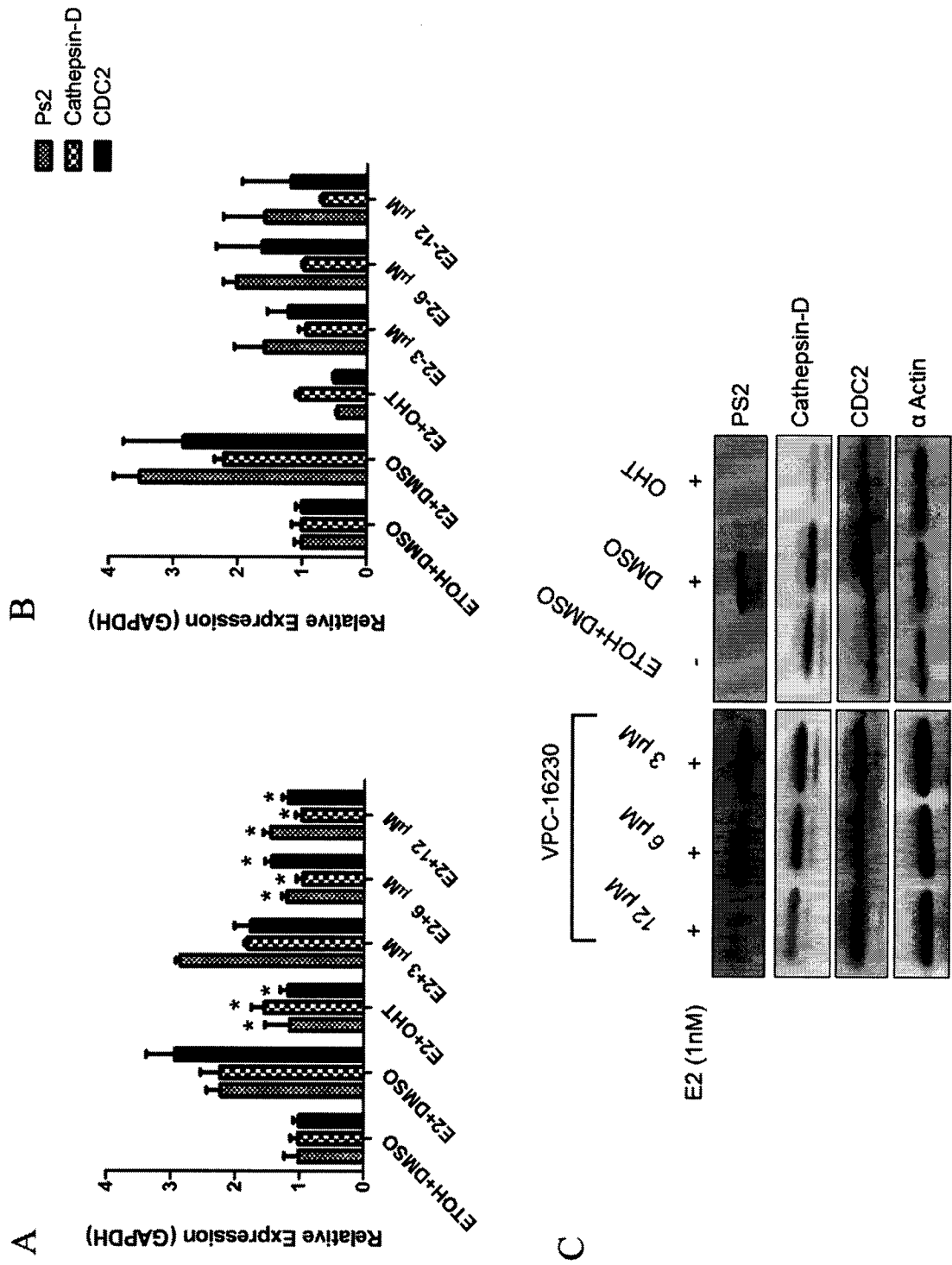
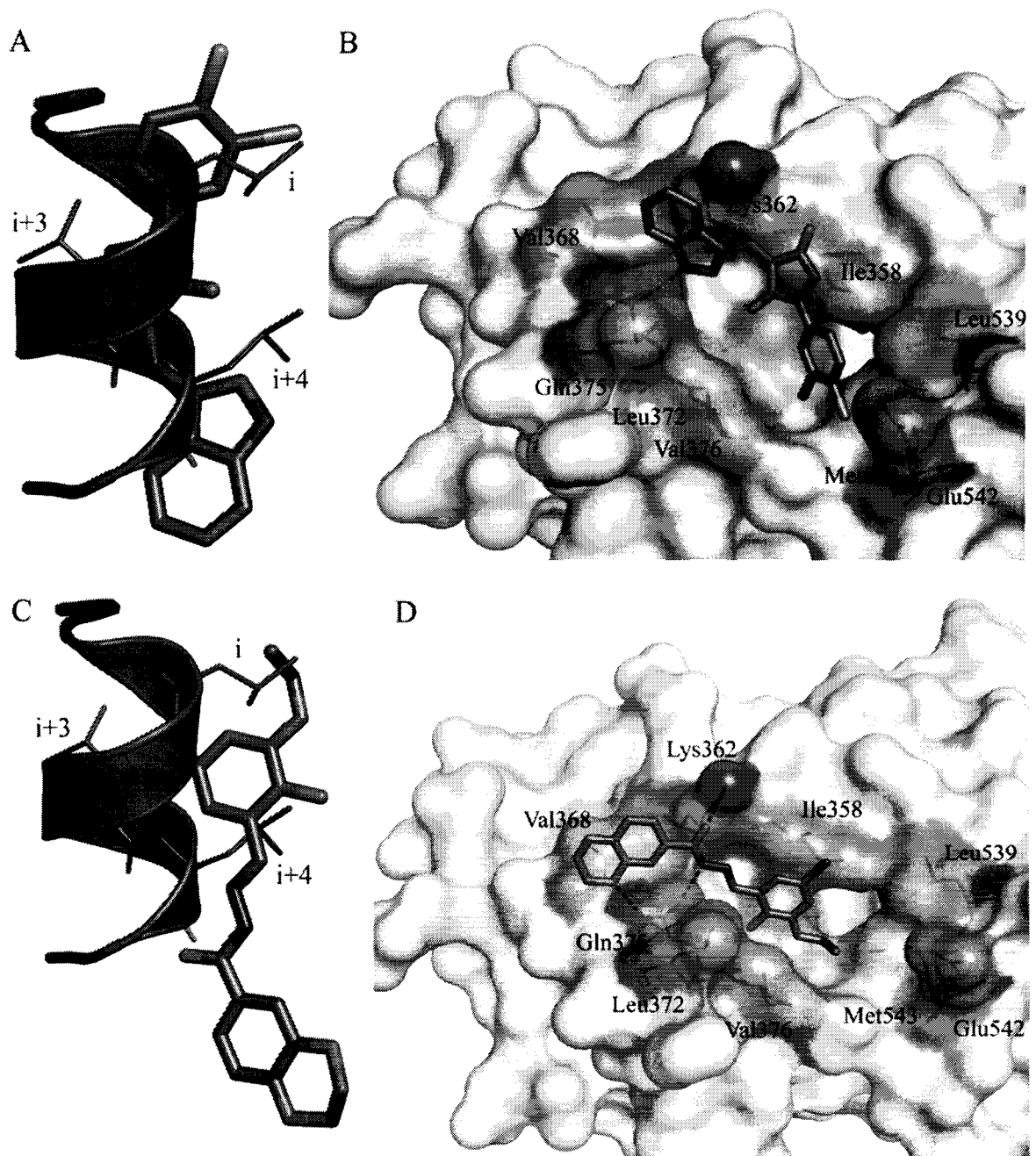


FIG. 14



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FIG. 15



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/000117

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07D 411/06** (2006.01), **A61K 31/166** (2006.01), **A61K 31/357** (2006.01), **A61K 31/36** (2006.01), **A61K 31/381** (2006.01), **A61K 31/404** (2006.01) (more IPCs on page 7)

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)

IPC (2006.01): **C07D 411/06**, **A61K 31/166**, **A61K 31/357**, **A61K 31/36**, **A61K 31/381**, **A61K 31/404**, **A61K 31/4152**, **A61K 31/4155**, **A61P 35/00**, **C07D 333/64**, **C07D 409/06**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
STN (Registry, CAPlus, CHEMCATS), Canadian Patent Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB568063A (SOCIETY OF CHEMICAL INDUSTRY IN BASLE), 16 March 1945 (16-03-1945) *see the compound of formula I *	1-5, 7, 8, 14-16
X	US2435018A (RUZICKA), 27 January 1948 (27-01-1948) *see column 1, line 35*	1-5, 7, 8, 14-16
X	US4133814A (JONES et al.), 09 January 1979 (09-01-1979) *see column 5, line 20*	1-5, 7, 8, 14-16
X	WO9820344A1 (JOUILLIE et al.), 14 May 1998 (14-05-1998) *see claim 1*	1-5, 7, 8, 14-16
X	WO9845272A1 (DEADY et al.), 15 October 1998 (15-10-1998) *see the examples*	1-5, 7, 8, 14-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
17 May 2016 (17-05-2016)

Date of mailing of the international search report
28 June 2016 (28-06-2016)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Guillaume Tessier (819) 639-8671

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/000117

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR2871375A1 (PLOS et al.), 16 December 2005 (16-12-2005) *see claim 1*	1-5, 7, 8, 14-16
X	EP1749822A1 (GUEDAT), 07 February 2007 (07-02-2007) *see page 25, compounds of formula 15a-j*	1-5, 7, 8, 14-16
X	PARK et al., "Identification of novel mPGES-1 inhibitors through screening of a chemical library", <i>Bioorganic & Medicinal Chemistry Letters</i> , 22 (2012), 7335-7339 *see figure 1, compound 9*	1-5, 7, 8, 14-16, 20-24, 26-31, 34, 38-41, 43-48, 51

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/000117

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 38-54
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 38-54 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 38-54.
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:
1-5, 7, 8, 14-16, 20-24, 26-31, 34, 38-41, 43-48, 51 (all in part)

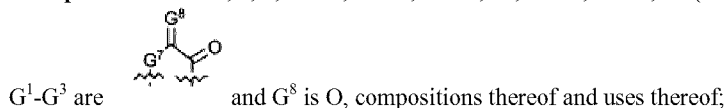
- Remark on Protest**
- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - ☐ No protest accompanied the payment of additional search fees.

A61K 31/4152 (2006.01), *A61K 31/4155* (2006.01), *A61P 35/00* (2006.01), *C07D 333/64* (2006.01),
C07D 409/06 (2006.01)

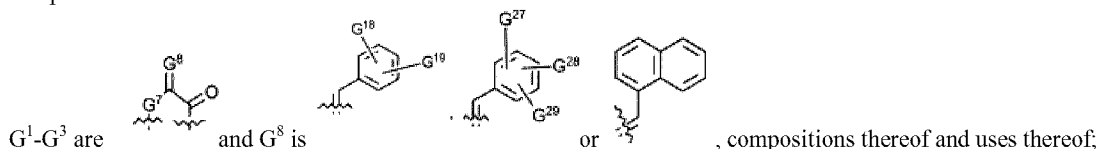
Continuation of Box III:

The claims are directed to a plurality of alleged inventions as follows:

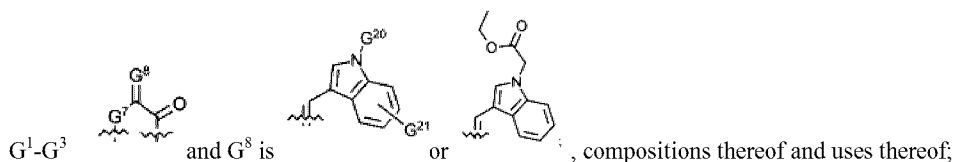
Group A - Claims 1-5, 7, 8, 14-16, 20-24, 26-31, 34, 38-41, 43-48, 51 (all in part) are directed to compounds of Formula A wherein



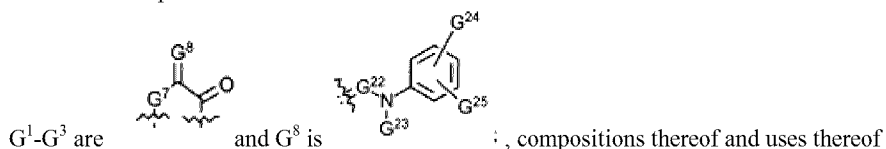
Group B - Claims 1-5 (in part), 6, 7-8 (in part), 14-16(in part), 20-24 (in part), 25, 26-31(in part), 34 (in part), 38-48 (in part), 51 are directed to compounds of Formula A wherein



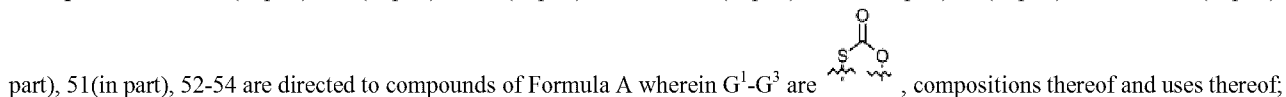
Group C - Claims 1-5 (in part), 7-8 (in part), 9, 10, 14-16 (in part), 20-24 (in part), 26-31 (in part), 32, 33, 34(in part), 38-41 (in part), 43-48 (in part), 49-50, 51(in part) are directed to compounds of Formula A wherein



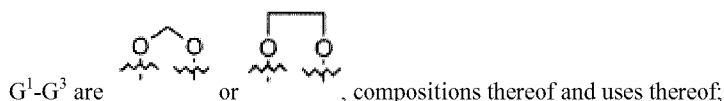
Group D - Claims 1-5 (in part), 7, 8 (in part), 11-14, 15-16 (in part), 20-24 (in part), 26-31(in part), 34(in part), 38-48(in part), 51(in part) are directed to compounds of Formula A wherein



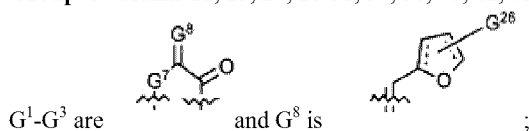
Group E - Claims 1-5 (in part), 7-8 (in part), 14-16(in part), 17-19, 20-24(in part), 26-31 (in part), 34(in part), 35-37, 38-41(in part), 43-48 (in



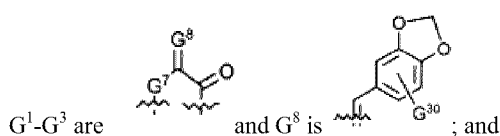
Group F - Claims 1-3, 5, 7, 8, 14-16, 20-22, 24, 26-31, 34, 38, 39, 41, 43-48, 51 (all in part) are directed to compounds of Formula A wherein



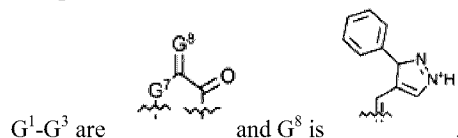
Group G - Claims 21, 23, 24, 26-31, 34, 38, 40, 41, 43-48, 51 (all in part) are directed to uses of compounds of Formula A wherein



Group H - Claims 21, 23, 24, 26-31, 34, 38, 40, 41, 43-48, 51(all in part) are directed to uses of compounds of Formula A wherein



Group I- Claims 21, 23, 24, 26-31, 34, 38, 40, 41, 43-48, 51(all in part) are directed to uses of compounds of Formula A wherein



Documents considered:

D1: WO2010129954A1 (SMITH et al.), 11 November 2010 (11-11-2010)

D2: WO2009155042A1 (BURSAVICH et al.) , 23 December 2009 (23-12-2009)

D3: CAS Registry Number® 328963-71-9, 21 October 2014 (21-11-2014)

An initial search revealed a large number of documents relevant to the issue of novelty and inventive step of the present claims. Each of D1 to D3 discloses compounds falling within the definition of general formula A, but in different groups of the alleged inventions as described above. As there is no novel structural feature common to all groups of the alleged inventions, the present claims lack unity *a posteriori* with respect to at least D1-D3 and are thus directed to different inventive concepts.

The claims must be limited to one inventive concept as set out in PCT Rule 13.

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