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(71) Applicant: **THE HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, INC.** [US/US]; 6720-A Rockledge Drive, Suite 100, Bethesda, Maryland 20817 (US).

(72) Inventors: **DOBI, Albert L.**; 14071 Pellita Terrace, Rockville, Maryland 20850 (US). **DALGARD, Clifton**; 3302 Winnett Road, Chevy Chase, Maryland 20815 (US). **SRIVASTAVA, Shiv**; 10520 Boswell Lane, Potomac, Maryland 20854 (US).

(74) Agents: **LOWEN, Gregory T.** et al.; 1111 Pennsylvania Ave NW, Washington, District of Columbia 20004 (US).

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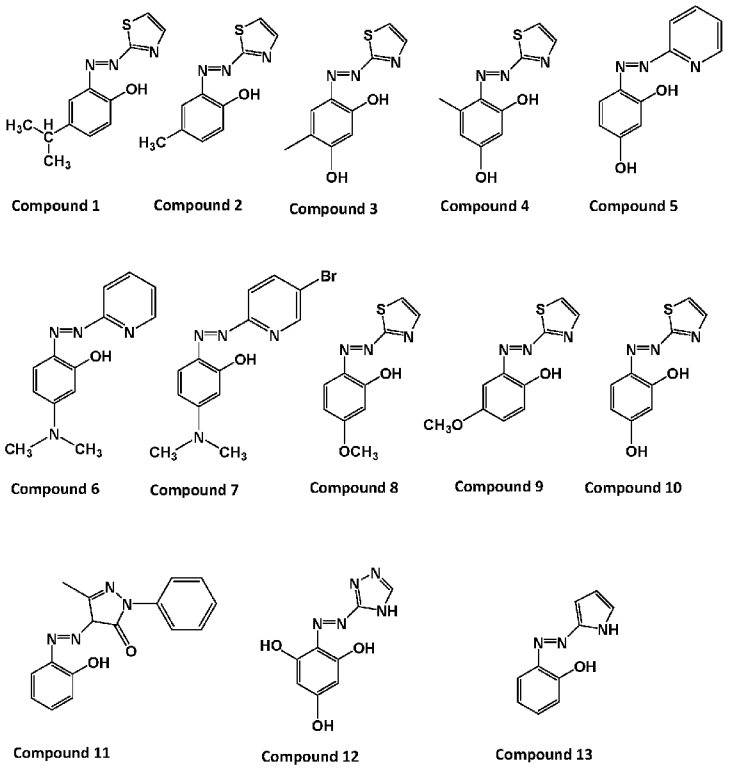
(54) Title: AZOPHENOLS AS ERG ONCOGENE INHIBITORS

Figure 1A



(57) Abstract: Selective azophenol inhibitors of a wild type or an altered ERG protein expression are described, where the inhibitors represent a compound of Formula (I) or Formula (II), wherein X, X1, X2, X3, X4, X5, R1 through R4 and R9 are as described. An aspect of the present invention relates to a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or Formula (II)

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Azophenols as ERG Oncogene Inhibitors

Background of the Invention

[0001] The *ETS* Related Gene (*ERG*) proto-oncogene was characterized more than twenty-five years ago (Rao *et al.*, 1987a; Rao *et al.*, 1987b; Reddy *et al.*, 1987) and belongs to a large family of *ETS* transcription factors that are both positive and negative regulators of gene expression (Watson *et al.*, 2010). These transcription factors are downstream effectors of the mitogenic signal transduction pathways involved in cell proliferation, cell differentiation, development, transformation, apoptosis, and immune regulation (Watson *et al.*, 2010; Sreenath *et al.*, 2011; Dobi *et al.*, 2013).

[0002] Prostate cancer (CaP) is the most frequently diagnosed non-skin malignancy and second leading cause of cancer related deaths among men in the western countries, with a projected 1.7 million newly diagnosed cases worldwide (International Agency for Research of Cancer, WHO, Press Release No 209, March 21, 2012). An estimated 2.9 million patients in the United States and 11 million world-wide are currently living with prostate cancer (http://globocan.iarc.fr/old/FactSheets/cancers/prostate-new.asp; http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-key-statistics). While early detected CaP due to PSA screening is managed effectively by surgery or radiation, a significant subset of CaP patients (20% to 40%) experience disease recurrence after definitive treatment and will require hormone ablation therapy (Eur. Urol. 2007 May; 51(5):1175-84, Epub 2007). Despite an initial response to therapy, metastatic CaP tumors eventually become refractory to hormone ablation therapy. For this subset of patients – *i.e.*, those having metastatic hormone refractory cancer, there is no effective cure.

[0003] The *ERG* gene is the most prevalent and validated genomic alteration in prostate cancer The *ERG* proto-oncogene is overexpressed in 60-70% of prostate tumors in patients of Caucasian ancestry as a result of recurrent gene fusions involving *TMPRSS2* and the *ETS* family of genes (Petrovics *et al.*, 2005; Tomlins *et al.*, 2005; reviewed in Kumar Sinha *et al.*, 2008; Rubin *et al.*, 2012). Emerging studies on human prostate cancer specimens and various experimental models underscore the causative oncogenic function of *ERG* in prostate cancer (Klezovitch *et al.*, 2008; Tomlins *et al.*, 2008; Sun

et al., 2008; Wang *et al.*, 2008). *ETS* factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to *PTEN* loss (Chen *et al.*, 2013; Nguyen *et al.*, 2015) Numerous reports have highlighted both diagnostic and prognostic features of the genomic activation of *ERG* revealing that about half the prostate tumors harbor the most common gene fusion that takes place between the androgen receptor-regulated *TMPRSS2* gene promoter and *ERG* protein coding sequence (reviewed in Kumar-Sinha *et al.*, 2008; Rubin *et al.*, 2012). Fusion between the *TMPRSS2* gene promoter and *ERG* results in the overexpression of N-terminally truncated or full-length forms of *ERG* (Klezovitch *et al.*, 2008; Sun *et al.*, 2008; Sreenath *et al.*, 2011). Fusion events between *ERG* and other androgen inducible promoter sequences, such as *SLC45A3* (Han *et al.*, 2008) and *NDRG1* have also been identified in prostate cancer (Pflueger *et al.*, 2009; Rubin *et al.*, 2012).

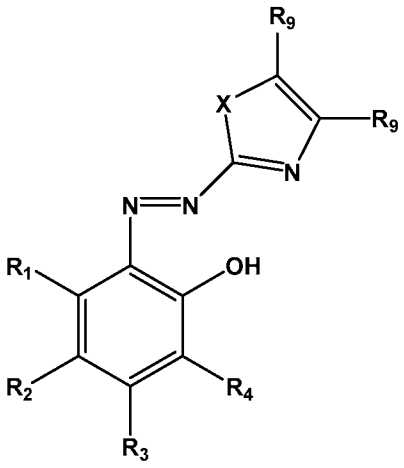
[0004] *ERG* expression in CaP is androgen receptor (AR) dependent. While AR signaling inhibitors are employed as therapeutics for treating CaP, compounds that selectively inhibit *ERG* expression are highly desirable. Up to 4 million of patients living with prostate cancer worldwide are expected to harbor *ERG* positive tumors (Farrell *et al.*, 2013). Compounds such as *ERGi*-USU are examples of such selective inhibitors that inhibit the ERG protein in *ERG* positive cancer cell lines with minimal effect on normal primary endothelial cells that endogenously express *ERG* - *i.e.*, *ERG* negative tumor or normal cells (PCT US2015/020172). The azophenols also selectively inhibit *ERG* expression and thus provide for the treatment of cancers or pathologic conditions associated with an *ERG* fusion event or *ERG* overexpression, including, for example, prostate cancer, Ewing's sarcoma, acute myeloid leukemia, megakaryoblastic leukemia, endothelial cancer and acute T-lymphoblastic leukemia.

[0005] A systematic screening of 456 known kinases in kinase ligand competition assays (Fabian *et al.*, 2005) indicated potential ligands for *RIO2* (Kd=200 nM). The *RIO* family of atypical serine/threonine kinases was first characterized in 1997 based on the studies of a right open reading frame (*RIO1*) gene, expressed constitutively at a low level in *Saccharomyces cerevisiae* (Angermayr *et al.*, 1997). Unexpectedly, *RIO* kinase 2 (*RIOK2*) protein levels were observed to decrease in *ERG* expressing VCaP cells in response to the azophenols of the invention with minimal effect on *RIOK2*

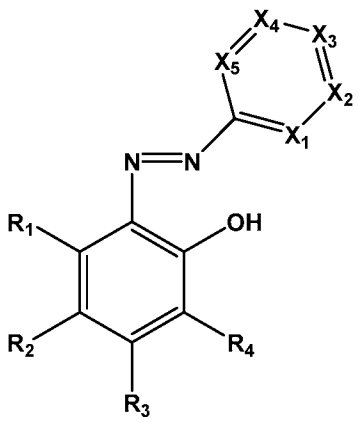
transcript levels as assessed by whole transcriptome analyses. In the present invention, *RIOK2* was investigated as a potential target of the *ERG* inhibitors described herein.

Summary of the Invention

[0006] An aspect of the present invention relates to a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or Formula (II)



Formula (I)



Formula (II)

or a pharmaceutically acceptable salt thereof, wherein:

X is NH, O or S;

X_1, X_2, X_3, X_4 and X_5 are independently N or CR_9, where only one of X_1, X_2, X_3, X_4 and X_5 is N;

R_1, R_2 and R_4 are independently selected from the group consisting of H, aryl, halogen, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl, wherein the aryl, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen,

hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_5R_6$, $-NR_5C(O)R_6$ and $-C(O)NR_5R_6$;

R_3 is selected from the group consisting of H, -OH, $-NR_5R_6$, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl, wherein the C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_5R_6$, $-NR_5C(O)R_6$ and $-C(O)NR_5R_6$;

R_5 and R_6 are independently selected from the group consisting of H, C_1-C_8 alkyl, aryl and C_3-C_7 cycloalkyl, or R_5 and R_6 taken together form a C_3-C_7 heterocycloalkyl wherein the C_1-C_8 alkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_7R_8$, $-NR_7C(O)R_8$ and $-C(O)NR_7R_8$;

R_7 and R_8 are independently selected from the group consisting of H and C_1-C_8 alkyl;

each R_9 is independently H, halogen, -CN, -OH, COOH, $-NR_{10}R_{11}$, C_1-C_{10} alkyl, C_3-C_7 cycloalkyl, C_1-C_{10} alkoxy and C_3-C_7 heterocycloalkyl wherein the C_1-C_{10} alkyl, C_3-C_7 cycloalkyl, C_1-C_{10} alkoxy and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_{10}R_{11}$, $-NR_{10}C(O)R_{11}$ and $-C(O)NR_{10}R_{11}$;

R_{10} and R_{11} are independently selected from the group consisting of H, C_1-C_8 alkyl and C_3-C_7 cycloalkyl, or R_{10} and R_{11} taken together form a C_3-C_7 heterocycloalkyl wherein the C_1-C_8 alkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halo, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_{12}R_{13}$, $-NR_{12}C(O)R_{13}$ and $-C(O)NR_{12}R_{13}$; and

R_{12} and R_{13} are independently selected from the group consisting of H and C_1-C_8 alkyl.

[0007] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; and R_1, R_2, R_3 and R_4 are independently selected from the group consisting of H and C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0008] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; and at least one of R_1, R_2, R_3 and R_4 is not H.

[0009] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_3 is H; and at least one of R_1, R_2 and R_4 is not H, such as C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0010] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_4 is H; and at least one of R_1, R_2 and R_3 is not H, such as C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0011] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_1 is H; and at least one of R_2, R_3 and R_4 is not H, such as C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0012] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_3 and R_4 are each H; and at least one of R_1 and R_2 is not H, such as C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0013] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_1, R_3 and R_4 are each H; and R_2 is not H, such as C_1-C_{10} alkyl, such as C_1-C_8 alkyl, such as C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl or pentyl.

[0014] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; R_1, R_3 and R_4 are each H; and R_2 is halogen. In a particular embodiment, R_2 is fluorine, bromine or chlorine.

[0015] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; each of R_2 and R_4 is H; R_1 is methyl; and R_3 is OH.

[0016] In an aspect of the invention directed to Formula (I), X is S; each R_9 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH.

[0017] In an aspect of the invention directed to Formula (I), X is NH; each R_9 is H; each of R_1, R_2 and R_4 is H; R_1 is methyl; and R_3 is OH.

[0018] In an aspect of the invention directed to Formula (I), X is NH; each R_9 is H; each of R_1, R_3 and R_4 is H; and R_2 is methyl or isopropyl.

[0019] In an aspect of the invention directed to Formula (I), X is O; each R_9 is H; each of R_1, R_3 and R_4 is H; and R_2 is methyl or isopropyl.

[0020] In an aspect of the invention directed to Formula (I), X is O; each R_9 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH.

[0021] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH.

[0022] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_3 and R_4 is H; and R_2 is halogen. In a particular embodiment, R_2 is fluorine, bromine or chlorine.

[0023] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_2 and R_4 is H; R_1 is methyl; and R_3 is OH.

[0024] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl.

[0025] In an aspect of the invention directed to Formula (II), X_2 is N; X_1, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH.

[0026] In an aspect of the invention directed to Formula (II), X_2 is N; X_1, X_3, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl.

[0027] In an aspect of the invention directed to Formula (II), X_3 is N; X_1, X_2, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH.

[0028] In an aspect of the invention directed to Formula (II), X_3 is N; X_1, X_2, X_4 and X_5 are CR_9; each R_9 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl.

[0029] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_3 is halogen and the R_9 at each of positions X_2, X_4 and X_5 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH. In a particular embodiment, the halogen at X_3 is F, Cl or Br, such as Br.

[0030] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_3 is halogen and the R_9 at each of positions X_2, X_4 and X_5 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl pentyl or hexyl. In a particular embodiment, the halogen at X_3 is F, Cl or Br, such as Br.

[0031] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_2 is halogen and the R_9 at each of positions X_3, X_4 and X_5 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH. In a particular embodiment, the halogen at X_2 is F, Cl or Br, such as Br.

[0032] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_2 is halogen and the R_9 at each of positions X_3, X_4 and X_5 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl. In a particular embodiment, the halogen at X_2 is F, Cl or Br, such as Br.

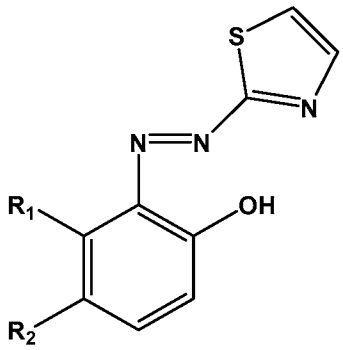
[0033] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_4 is halogen and the R_9 at each of positions X_2, X_3 and X_5 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH. In a particular embodiment, the halogen at X_4 is F, Cl or Br, such as Br.

[0034] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_4 is halogen and the R_9 at each of positions X_2, X_3 and X_5 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl. In a particular embodiment, the halogen at X_4 is F, Cl or Br, such as Br.

[0035] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_5 is halogen and the R_9 at each of positions X_2, X_3 and X_4 is H; each of R_1, R_2 and R_4 is H; and R_3 is OH. In a particular embodiment, the halogen at X_5 is F, Cl or Br, such as Br.

[0036] In an aspect of the invention directed to Formula (II), X_1 is N; X_2, X_3, X_4 and X_5 are CR_9, where the R_9 at position X_5 is halogen and the R_9 at each of positions X_2, X_3 and X_4 is H; each of R_1, R_2 and R_4 is H; R_3 is NR_5R_6; and R_5 and R_6 are each independently C_1-C_8 alkyl, such as methyl, ethyl, isopropyl, butyl, isobutyl, pentyl or hexyl. In a particular embodiment, the halogen at X_5 is F, Cl or Br, such as Br.

[0037] In an aspect of the invention, the compound of Formula (I) is a compound of Formula (III)



Formula (III)

or a pharmaceutically acceptable salt thereof,

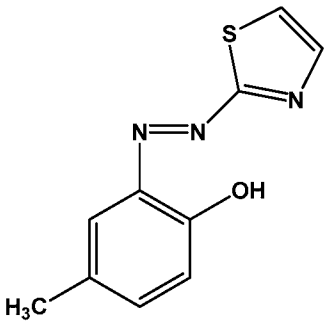
wherein R_1 and R_2 are as defined for Formula (I).

[0038] In an aspect of the invention, R_1 and R_2 of Formula (III) are independently selected from the group consisting of H, C_1-C_6 alkyl, such as C_1-C_4 alkyl, such as methyl, ethyl, isopropyl, butyl and isobutyl, where at least one of R_1 and R_2 is not H.

[0039] In an aspect of the invention, R_1 of Formula (III) is H and R_2 is C_1-C_6 alkyl, such as C_1-C_4 alkyl, such as methyl, ethyl, isopropyl, butyl or isobutyl.

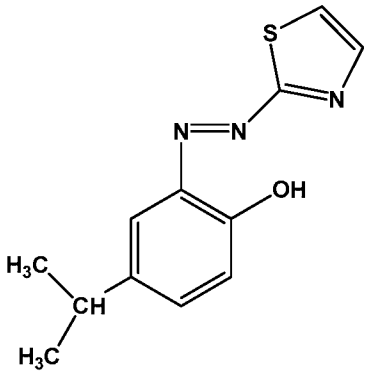
[0040] In an aspect of the invention, R_1 of Formula (III) is H and R_2 is C_1-C_6 alkyl.

[0041] In an aspect of the invention, the compound of Formula (I) is



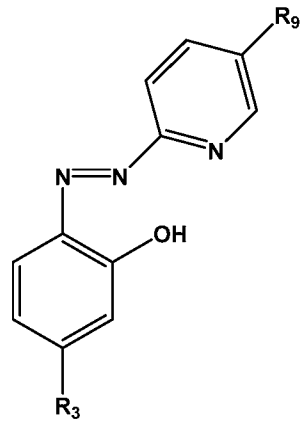
or a pharmaceutically acceptable salt thereof.

[0042] In an aspect of the invention, the compound of Formula (I) is



or a pharmaceutically acceptable salt thereof.

[0043] In an aspect of the invention, the compound of Formula (II) is a compound of Formula (IV)

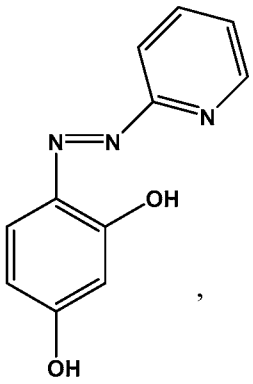


Formula (IV)

or a pharmaceutically acceptable salt thereof,

wherein R_3 and R_9 are as defined for Formula (II).

[0044] In an aspect of the invention, the compound of Formula (IV) is



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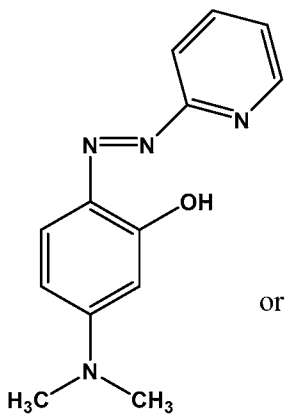
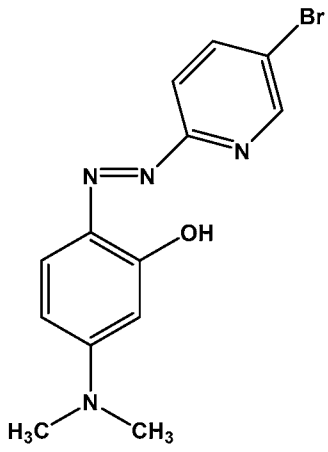


or

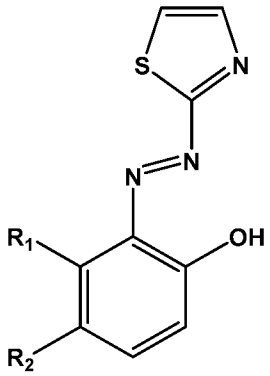


or a pharmaceutically acceptable salt thereof.

[0045] In an aspect of the invention, the compounds of Formulae (I), (II), (III), (IV) and (V) are effective in treating a disease selected from the group consisting of prostate cancer, Ewing's sarcoma, acute myeloid leukemia, acute T-lymphoblastic leukemia, endothelial cancer and colon cancer.

[0046] In an aspect of the invention, the disease is prostate cancer.

[0047] Another aspect of the invention is a compound of Formula (V)



Formula (V)

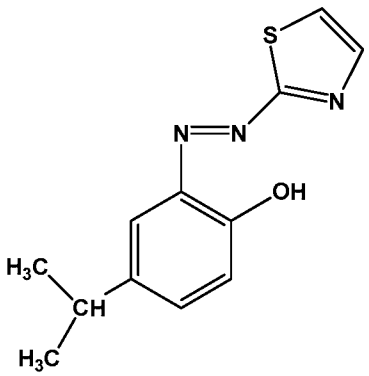
or a pharmaceutically acceptable salt thereof, wherein

R_1 is selected from the group consisting of H and optionally substituted C_1-C_{10} alkyl, such as C_1-C_9 alkyl, such as C_1-C_8 alkyl, such as C_1-C_7 alkyl, such as C_1-C_6 alkyl, such as C_1-C_5 alkyl, such as methyl, ethyl, isopropyl, butyl and isobutyl; and

R_2 is optionally substituted C_2-C_{10} alkyl, such as C_2-C_8 alkyl, such as C_2-C_7 alkyl, such as C_2-C_6 alkyl, such as C_2-C_5 alkyl, such as C_2-C_4 alkyl, such as isopropyl, butyl and isobutyl, with the proviso that when R_1 is H, R_2 is not ethyl, isobutyl or $-C(CH_3)_2-CH_2-C(CH_3)_3$.

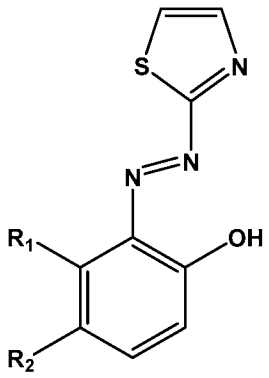
[0048] In an aspect of the invention, R_1 is H and R_2 is optionally substituted C_2-C_{10} alkyl, with the proviso that R_2 is not ethyl, isobutyl or $-C(CH_3)_2-CH_2-C(CH_3)_3$.

[0049] In an aspect of the invention, the compound of Formula (V) is



or a pharmaceutically acceptable salt thereof.

[0050] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (V)



Formula (V)

or a pharmaceutically acceptable salt thereof, wherein:

R_1 and R_2 are as defined for Formula (V).

[0051] In an aspect of the invention, the disease is prostate cancer.

[0052] Another aspect of the invention is a pharmaceutical composition comprising the compound of Formula (I) and an excipient.

[0053] Another aspect of the invention is a pharmaceutical composition comprising the compound of Formula (II) and an excipient.

[0054] Another aspect of the invention is a pharmaceutical composition comprising the compound of Formula (III) and an excipient.

[0055] Another aspect of the invention is a pharmaceutical composition comprising the compound of Formula (IV) and an excipient.

[0056] Another aspect of the invention is a pharmaceutical composition comprising the compound of Formula (V) and an excipient.

[0057] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (I) and an excipient.

[0058] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (II) and an excipient.

[0059] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (III) and an excipient.

[0060] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (IV) and an excipient.

[0061] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (V) and an excipient.

[0062] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a compound of

Formula (I) in combination with a therapeutically effective amount of a known *ERG* inhibitor.

[0063] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a compound of Formula (II) in combination with a therapeutically effective amount of a known *ERG* inhibitor.

[0064] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a compound of Formula (III) in combination with a therapeutically effective amount of a known *ERG* inhibitor.

[0065] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a compound of Formula (IV) in combination with a therapeutically effective amount of a known *ERG* inhibitor.

[0066] An aspect of the invention is a method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a compound of Formula (V) in combination with a therapeutically effective amount of a known *ERG* inhibitor.

Brief Description of the Drawings

[0067] The following figures are merely representative of selected embodiments of the present invention and are not intended to define or narrow the scope of the invention as

otherwise described herein. The chemical structures of Compounds 1 to 7 referred to in the Figures below are depicted in Figure 1A.

[0068] **Figure 1A** depicts the azophenol compounds 1-13 for which testing as ERG inhibitors is disclosed herein. **Figure 1B** represents exemplary substitution patterns for the compounds of Formula (I) as described herein. **Figures 1C** and **1D** depict the chemical structures of exemplary azophenol compounds of Formula (I) and Formula (II) as described herein.

[0069] **Figure 2** illustrates the inhibition of endogenous AR, PSA and ERG proteins in ERG harboring prostate cancer cells (VCaP) by Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0070] **Figure 3** illustrates the inhibition of the growth of ERG harboring prostate cancer cells (VCaP) by the Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0071] **Figure 4** illustrates the lack of inhibition of endogenous AR and PSA proteins in ERG negative prostate cancer cells (LNCaP) by Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0072] **Figure 5** illustrates that Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM do not affect the growth of ERG negative prostate cancer cells (LNCaP).

[0073] **Figure 6** illustrates that Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM do not inhibit endogenous AR protein in ERG negative prostate cancer cells (LAPC-4).

[0074] **Figure 7** illustrates that Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM do not affect the growth of ERG negative prostate cancer cells (LAPC-4).

[0075] **Figure 8** illustrates the inhibition of endogenous ERG and RIOK2 proteins in ERG harboring cancer cells (COLO320) by Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0076] **Figure 9** illustrates the inhibition of growth of ERG harboring colon cancer cells (COLO320) by Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0077] **Figure 10** illustrates the lack of inhibition of ERG and RIOK2 proteins in endogenous ERG expressing normal endothelial cells (HUVEC) by Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM.

[0078] **Figure 11** illustrates that Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM do not affect the growth of ERG harboring normal endothelial cells (HUVEC).

[0079] **Figure 12** illustrates that Compounds 1 and 2 at concentrations of 0.25 μM and 1 μM do not affect the growth of ERG negative prostate-derived immortalized cells (BPH-1 and RWPE-1).

[0080] **Figure 13** illustrates the inhibition of ERG protein in VCaP cells by Compound 1 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 1 on ERG protein in VCaP.

[0081] **Figure 14** illustrates the inhibition of RIOK2 protein in VCaP cells by Compound 1 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 1 on RIOK2 protein in VCaP.

[0082] **Figure 15** illustrates the inhibition of VCaP cell growth by Compound 1 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM.

[0083] **Figure 16** illustrates the inhibition of ERG protein in VCaP cells by Compound 2 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 2 on ERG protein in VCaP.

[0084] **Figure 17** illustrates the inhibition of RIOK2 protein in VCaP cells by Compound 2 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 2 on RIOK2 protein in VCaP.

[0085] **Figure 18** illustrates the inhibition of VCaP cell growth by Compound 2 at concentrations of 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM.

[0086] **Figure 19** illustrates the inhibition of VCaP cell growth by Compound 5 at concentrations of 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 5 on ERG protein in VCaP.

[0087] **Figure 20** illustrates the inhibition of VCaP cell growth by Compound 6 at concentrations of 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 6 on ERG protein in VCaP.

[0088] **Figure 21** illustrates the inhibition of VCaP cell growth by Compound 7 at concentrations of 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC_{50} of Compound 7 on ERG protein in VCaP.

[0089] **Figure 22** illustrates the inhibition of VCaP cell growth by Compound 5 at concentrations of 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC50 of Compound 5 on cell growth.

[0090] **Figure 23** illustrates the inhibition of VCaP cell growth by Compound 6 at concentrations of 0.02 μM, 0.05 μM, 0.10 μM, 0.20 μM and 0.40 μM and shows the IC50 of Compound 6 on cell growth.

[0091] **Figure 24** illustrates the inhibition of VCaP cell growth by Compound 7 at concentrations of 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM and 1 μM and shows the IC50 of Compound 7 on cell growth.

[0092] **Figure 25** illustrates the inhibition of VCaP cell growth by Compound 3 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 3 on ERG protein in VCaP.

[0093] **Figure 26** illustrates the inhibition of VCaP cell growth by Compound 4 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 4 on ERG protein in VCaP.

[0094] **Figure 27** illustrates the inhibition of VCaP cell growth by Compound 5 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 5 on ERG protein in VCaP.

[0095] **Figure 28** illustrates the inhibition of VCaP cell growth by Compound 3 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 3 on VCaP cell growth.

[0096] **Figure 29** illustrates the inhibition of VCaP cell growth by Compound 4 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 4 on VCaP cell growth.

[0097] **Figure 30** illustrates the inhibition of VCaP cell growth by Compound 5 at concentrations of 0.3 μM, 0.6 μM, 1.0 μM, 3.0 μM and 10.0 μM and shows the IC_{50} of Compound 5 on VCaP cell growth.

Detailed Description

Definitions

[0098] A "pharmaceutically acceptable salt" is a pharmaceutically acceptable, organic or inorganic acid or base salt of a compound of the invention. Representative pharmaceutically acceptable salts include, *e.g.*, alkaline metal salts, alkaline earth salts, ammonium salts, water-soluble and water-insoluble salts, such as the acetate, amsonate (4,4-diaminostilbene-2, 2 -disulfonate), benzenesulfonate, benzonate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium, calcium edetate, camsylate, carbonate, chloride, citrate, clavulariate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexafluorophosphate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, 3-hydroxy-2-naphthoate, oleate, oxalate, palmitate, pamoate (1,1-methene-bis-2-hydroxy-3-naphthoate, einbonate), pantothenate, phosphate/diphosphate, picrate, polygalacturonate, propionate, p-toluenesulfonate, salicylate, stearate, subacetate, succinate, sulfate, sulfosalicylate, suramate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate salts. A pharmaceutically acceptable salt can have more than one charged atom in its structure. In this instance the pharmaceutically acceptable salt can have multiple counterions. Thus, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterions.

[0099] The terms “treat”, “treating” and “treatment” refer to the amelioration or eradication of a disease or symptoms associated with a disease. In certain embodiments, such terms refer to minimizing the spread or worsening of the disease resulting from the administration of one or more prophylactic or therapeutic agents to a patient with such a disease.

[00100] The terms “prevent,” “preventing,” and “prevention” refer to the prevention of the onset, recurrence, or spread of the disease in a patient resulting from the administration of a prophylactic or therapeutic agent.

[00101] The term “effective amount” refers to an amount of a compound of the invention, or other active ingredient sufficient to provide a therapeutic or prophylactic benefit in the treatment or prevention of a disease or to delay or minimize symptoms associated with a disease. Further, a therapeutically effective amount with respect to a compound of the invention means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or prevention of a disease. Used in connection with a compound of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease, or enhances the therapeutic efficacy of or synergies with another therapeutic agent.

[00102] A “subject” includes an animal, such as a human, cow, horse, sheep, lamb, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit or guinea pig. The animal can be a mammal such as a non-primate and a primate (*e.g.*, monkey and human). In one embodiment, a subject is a human, such as a human infant, child, adolescent or adult.

[00103] The term "substituted", as used herein, refers to the replacement of at least one hydrogen atom of a molecular arrangement with a substituent. In the case of an oxo substituent ("=O"), two hydrogen atoms are replaced. When substituted, one or more of the groups below are "substituents." Substituents include, but are not limited to, halogen, hydroxy, oxo, cyano, nitro, amino, alkylamino, dialkylamino, alkyl, alkoxy, alkylthio, haloalkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocycloalkyl; -NRaRb, -NRaC(O)Rb, -NRaC(O)NRaNRb, -NRaC(=O)ORb, -NRaSO_2Rb, -C(=O)Ra, -C(=O)ORa, -C(=O)NRaRb, -OC(=O)NRaRb, -ORa, -SRa,

-SORa, -S(=O)$_2$Ra, -OS(=O)$_2$Ra and -S(=O)ORa. In addition, the above substituents may be further substituted with one or more of the above substituents, such that the substituent comprises a substituted alkyl, substituted aryl, substituted arylalkyl, substituted heterocycle, or substituted heterocycloalkyl. Ra and Rb in this context may be the same or different and, independently, hydrogen, alkyl, haloalkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heterocyclyl, substituted heterocyclyl, heterocycloalkyl or substituted heterocycloalkyl.

[00104] The term "unsubstituted", as used herein, refers to any compound that does not contain extra substituents attached to the compound. An unsubstituted compound refers to the chemical makeup of the compound without extra substituents, *e.g.*, the compound does not contain protecting group(s). For example, unsubstituted proline is a proline amino acid even though the amino group of proline may be considered disubstituted with alkyl groups.

[00105] The term "alkyl", as used herein, refers to any straight chain or branched, non-cyclic or cyclic, unsaturated or saturated aliphatic hydrocarbon containing from 1 to 10 carbon atoms, while the term "lower alkyl" has the same meaning as alkyl but contains from 1 to 6 carbon atoms. The term "higher alkyl" has the same meaning as alkyl but contains from 7 to 10 carbon atoms. Representative saturated straight chain alkyls include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and the like; while saturated branched alkyls include, but are not limited to, isopropyl, sec-butyl, isobutyl, tert-butyl, isopentyl, and the like. Cyclic alkyls may be obtained by joining two alkyl groups bound to the same atom or by joining two alkyl groups each bound to adjoining atoms. Representative saturated cyclic alkyls include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated cyclic alkyls include, but are not limited to, cyclopentenyl and cyclohexenyl, and the like. Cyclic alkyls are also referred to herein as a "homocycles" or "homocyclic rings." Unsaturated alkyls contain at least one double or triple bond between adjacent carbon atoms (referred to as an "alkenyl" or "alkynyl", respectively). Representative straight chain and branched alkenyls include, but are not limited to, ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like; while

representative straight chain and branched alkynyls include, but are not limited to, acetylenyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, 3-methyl-1-butynyl, and the like.

[00106] The term "aryl", as used herein, refers to any aromatic carbocyclic moiety such as, but not limited to, phenyl or naphthyl.

[00107] The term "arylalkyl", or "aralkyl" as used herein, refers to any alkyl having at least one alkyl hydrogen atom replaced with an aryl moiety, such as, but not limited to, benzyl, $-(CH_2)_2$-phenyl, $-(CH_2)_3$-phenyl, $-CH(phenyl)_2$, and the like.

[00108] The term "halogen", as used herein, refers to any fluoro, chloro, bromo, or iodo moiety.

[00109] The term "haloalkyl", as used herein, refers to any alkyl having at least one hydrogen atom replaced with halogen, such as trifluoromethyl, and the like.

[00110] The term "heteroaryl", as used herein, refers to any aromatic heterocycle ring of 5 to 10 members and having at least one heteroatom selected from nitrogen, oxygen and sulfur, and containing at least 1 carbon atom, including, but not limited to, both mono and bicyclic ring systems. Representative heteroaryls include, but are not limited to, furyl, benzofuranyl, thiophenyl, benzothiophenyl, pyrrolyl, indolyl, isoindolyl, azaindolyl, pyridyl, quinolinyl, isoquinolinyl, oxazolyl, isooxazolyl, benzoxazolyl, pyrazolyl, imidazolyl, benzimidazolyl, thiazolyl, benzothiazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, cinnolinyl, phthalazinyl, or quinazolinyl.

[00111] The term "heteroarylalkyl", as used herein, refers to any alkyl having at least one alkyl hydrogen atom replaced with a heteroaryl moiety, such as -CHpyridinyl, $-CH_2$pyrimidinyl, and the like.

[00112] The term "heterocycle" or "heterocyclic ring", as used herein, refers to any 3- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from 1 to 4 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle may be attached via any heteroatom or

carbon atom. Heterocycles may include heteroaryls exemplified by those defined above. Thus, in addition to the heteroaryls listed above, heterocycles may also include, but are not limited to, morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

[00113] The term "heterocycloalkyl", as used herein, refers to any alkyl having at least one alkyl hydrogen atom replaced with a heterocycle, such as -CH_2-morpholinyl, and the like.

[00114] The term "homocycle" or "cycloalkyl", as used herein, refers to any saturated or unsaturated (but not aromatic) carbocyclic ring containing from 3-7 carbon atoms, such as, but not limited to, cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclohexene, and the like.

[00115] The term "alkylamino", as used herein, refers to at least one alkyl moiety attached through a nitrogen bridge (*e.g.*, -N-alkyl or -N-(alkyl)-N-) including, but not limited to, methylamino, ethylamino, dimethylamino, diethylamino, and the like.

[00116] The term "alkyloxy" or "alkoxy", as used herein, refers to any alkyl moiety attached through an oxygen bridge (*e.g.*, -O-alkyl) such as, but not limited to, methoxy, ethoxy, and the like.

[00117] The term "alkylthio", as used herein, refers to any alkyl moiety attached through a sulfur bridge (*e.g.*, -S-alkyl) such as, but not limited to, methylthio, ethylthio, and the like.

[00118] The term "alkenyl" refers to an unbranched or branched hydrocarbon chain having one or more double bonds therein. The double bond of an alkenyl group can be unconjugated or conjugated to another unsaturated group. Suitable alkenyl groups include, but are not limited to vinyl, allyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl, 2-ethylhexenyl, 2-propyl-2-butenyl,4-(2-methyl-3-butene)-pentenyl. An alkenyl group can be unsubstituted or substituted with one or two suitable substituents.

[00119] The term "alkynyl" refers to unbranched or branched hydrocarbon chain having one or more triple bonds therein. The triple bond of an alkynyl group can be unconjugated or conjugated to another unsaturated group. Suitable alkynyl groups include, but are not limited to ethynyl, propynyl, butynyl, pentynyl, hexynyl, methylpropynyl, 4-methyl-1-butynyl, 4-propyl-2-pentynyl-, and 4-butyl-2-hexynyl. An alkynyl group can be unsubstituted or substituted with one or two suitable substituents.

Compounds and Methods

[00120] The present invention relates to selective azophenol *ERG* inhibitor compounds and to their use for treating or preventing a disease related to over-expression of an *ETS* Related Gene (*ERG*), a wild type *ERG* protein or an altered *ERG* protein in a subject. More specifically, the azophenol *ERG* inhibitors of the invention do not attenuate or inhibit androgen receptor (AR) signaling in *ERG* negative AR positive CaP cell lines tested and thus exhibit fewer toxic side effects when compared to conventional agents that inhibit AR signaling as the underlying mechanism for treating prostate cancer. Additionally, the azophenols of the invention inhibit *ERG* protein, and cell growth in the ERG positive tumor cell lines that do not express AR.

[00121] Approved strategies for the treatment of prostate cancer routinely entail therapeutic agents that attenuate or inhibit the activity of AR in prostate cancer cells. Because the expression of *ERG* in VCaP prostate cancer cells is regulated by AR, representative azophenol compounds of the invention were evaluated to determine if the observed ERG inhibitory activity was a result of AR inhibition. The azophenol compounds were observed to inhibit the expression of AR and prostate specific antigen (PSA) in VCaP prostate cancer cells.

[00122] To investigate whether the azophenol compounds of the invention are selective inhibitors of *ERG* protein expression, representative azophenol compounds were further tested for its ability to inhibit AR and PSA activity in the following AR positive/*ERG* negative prostate cancer cell lines: LNCaP (mutant AR positive and *ERG* negative), as well as in LAPC4 cells that are AR (wild type) positive and *ERG* negative.

[00123] Inhibition of AR by a representative azophenol compound of the invention is specific to VCaP cells. No AR inhibition was observed in other AR positive/*ERG* negative cell lines used in this screen.

[00124] In an exemplary embodiment, a method for treating or preventing a disease related to overexpression of wild type *ERG* protein or an altered *ERG* protein product of the E-Related Gene (*ERG*) in a subject by administering to the subject a therapeutically-effective amount of an azophenol compound of the invention that selectively inhibits *ERG* expression. While the exact mechanism by which *ERG* expression is lowered or inhibited is unknown, the azophenol compounds may, for example, influence *ERG* mRNA gene transcription, *ERG* mRNA translation, prevent *ERG* protein from attaining its functionally active tertiary structure or inhibit the growth of *ERG* positive tumors by altering the regulation of a gene that is essential for cell growth.

[00125] The azophenol compounds of the invention appear to selectively inhibit *ERG* expression in cancer cells without inhibiting the expression of *ERG* in normal endothelial cells. A representative azophenol compound of the invention inhibits expression of *ERG* in a dose dependent manner in *ERG* positive cancer cell lines. No measurable inhibition of *ERG* protein expression was observed in normal HUVEC cells, with basal expression of ERG, however.

[00126] *ERG* overexpression in cancer cells is believed to play a role in the development of oncogene addiction, a condition in which some *ERG* positive cancer cells depend on the activity of the *ERG* protein for their growth and survival. Inhibition or attenuation of *ERG* protein expression in *ERG* positive cancer cells, therefore, may arrest the growth and survival of cancer cells. As illustrated by the results of a cell growth inhibition study, inhibition of *ERG* expression prevents growth of *ERG* positive cancer cells. No cell growth inhibition effects were observed, however, for *ERG* negative prostate cancer cell lines, an *ERG* negative immortalized benign prostate cell line (BPH1), and for *ERG* positive normal cells. These results support the use of the azophenol compounds of the invention as candidate therapeutic agents for the selective treatment of *ERG* positive cancers, such as, for example, prostate cancer. In an exemplary embodiment, methods for treating *ERG* positive cancers using the azophenol

compounds of the invention therefore provide an unexpected approach for treating metastatic hormone refractory prostate cancer that is unresponsive to treatment with agents that attenuate or inhibit AR activity and/or ablate hormonal activity.

[00127] The azophenol inhibitors of *ERG* overexpression according to the invention may also be used in combination with one or more other therapeutic agents capable of treating cancers. In an exemplary embodiment, the azophenol compounds are used in combination with conventional inhibitors of AR activity for the treatment of prostate cancer, particularly for subject diagnosed with prostate cancer, in which the AR activity is amplified or super activated.

[00128] The azophenol compounds of the invention were further evaluated as a candidate therapeutic agent for treating a patient having an *ERG* positive cancer. Separate cultures of *ERG* positive VCaP prostate cancer cells and *ERG* negative LNCaP cells were used to test for selective inhibition of *ERG* expression and the cell growth inhibitory activity of several representative azophenol compounds. The azophenol compounds of the invention are selective inhibitors of *ERG* expression and growth of *ERG* positive cancer cells.

[00129] The above observations and the role of *ERG* in cancer cell growth support the use of *ERG* specific inhibitors, such the azophenol compounds of the invention, as viable therapeutic agents for treating cancers such as prostate cancer, colorectal cancer, Ewing sarcoma, a vascular tumor and leukemia. In an exemplary embodiment, the subject receiving treatment for cancer according to a method of the invention is a mammal. For instance, the methods and uses described herein are suitable for the treatment of cancers in humans. Alternatively, the methods and uses of the invention may be suitable in a veterinary context, wherein the subject includes, but is not limited to, a dog, cat, horse or cow.

[00130] In select embodiments of the invention, the azophenol *ERG* inhibitors are co-administered with at least one anti-cancer therapeutic agent. As used herein, "co-administer" indicates that each of the at least two components is administered during a time frame wherein the respective periods of biological activity or effects overlap. Thus, the term "co-administer" is intended to encompass sequential as well as

coextensive administration of the individual therapeutic components. Accordingly, "administering" the combination of components according to some of the methods of the present invention includes sequential as well as coextensive administration of the individual components of the present invention. Likewise, the phrase "combination of compounds" or "combination of components" and the like indicate that the individual components are coadministered, and these phrases do not necessarily mean that the compounds must be administered contemporaneously or coextensively. In addition, the routes of administration of the individual components need not be the same. In an exemplary embodiment, the azophenol compounds are administered in the same composition.

[00131] In an exemplary embodiment, at least one azophenol *ERG* inhibitor of the invention is co-administered with a prostate cancer therapy. In a more specific embodiment, the azophenol *ERG* inhibitors are co-administered with one or more of lutenizing hormone-releasing hormone (LHRH) analogs such as, but not limited to, leuprolide (Lupron®, Eligard®), goserelin (Zoladex®), triptorelin (Trelstar®), degarelix (Firmagon®), Abiraterone (Zytiga®) and histrelin (Vantas®). In other specific embodiments, the azophenol *ERG* inhibitors are co-administered with one or more of anti-androgen receptors such as, but not limited to, flutamide (Eulexin®), bicalutamide (Casodex®), Enzalutamide (Xtandi®) and nilutamide (Nilandron®). In other specific embodiments, the azophenol *ERG* inhibitors are co-administered with one or more chemotherapeutics such as, but not limited to, Docetaxel (Taxotere®), Cabazitaxel (Jevtana®), Mitoxantrone (Novantrone®), Estramustine (Emcyt®), Doxorubicin (Adriamycin®), Etoposide (VP-16), Vinblastine (Velban®), Paclitaxel (Taxol®), Carboplatin (Paraplatin®), Vinorelbine (Navelbine®) Abiraterone (Zytiga), ARN-509 (J@J), and Galeterone (Tokai).

[00132] In an exemplary embodiment, the azophenol *ERG* inhibitors are administered as a first line therapy. In other embodiments, the azophenol *ERG* inhibitors are administered as a second line therapy or a third line therapy. In still other embodiments, the azophenol *ERG* inhibitors are administered subsequent to a third line therapy. As used herein, a first line therapy is the therapeutic regimen that is first prescribed or followed upon diagnosis of a condition that warrants the use of an *ERG* inhibitor, such as but not

limited to prostate cancer. A second line therapy is the therapeutic regimen that is prescribed or followed upon diagnosis of a recurrence or metastasis of condition that warrants the use of an *ERG* inhibitor, such as but not limited to prostate cancer. Likewise, a third line therapy is the therapeutic regimen that is prescribed or followed upon diagnosis of a second recurrence or metastasis of condition that warrants the use of an *ERG* inhibitor, such as but not limited to prostate cancer. A therapy, for the purposes of determining which "line" of therapy as used herein, need not be a drug therapy. For example, a first line therapy, as used herein, may be surgical removal, or radiation therapy. Any therapy designed to remove, reduce or ablate the tumor or condition can be considered a "line" of therapy.

[00133] In other embodiments, the azophenol *ERG* inhibitors of the invention can be administered as a "maintenance" therapeutic. As used herein, a maintenance therapeutic is a therapeutic regimen that is prescribed or followed while the subject is free of any detectable condition requiring treatment, for example, after a tumor is surgically removed from the subject. In these embodiments, the *ERG* inhibitors can be taken, for example, after surgical resection, for a specified period of time such as, but not limited to, at least about six months, such as one year, two years, three years, four years or five years, after the removal or disappearance of the tumor or cancer.

Pharmaceutical Formulations, Routes of Administration and Dosing Regimen

[00134] Despite evidence generally associating *ERG* expression with cancer cell growth, the conventional art does not appear to consider a small molecule compound that selectively inhibits expression of *ERG* in cancer cells or the use of such selective *ERG* inhibitors as anti-neoplastic agents. The present invention provides azophenol compounds and their pharmaceutical compositions that are useful in treating a subject suffering from an *ERG* positive cancer, as more generally set forth above.

[00135] The azophenol compound or composition of the invention can be formulated as described herein and is suitable for administration in a therapeutically effective amount to the subject in any number of ways. A therapeutically effective amount of an azophenol compound as described herein depends upon the amounts and types of excipients used, the amounts and specific types of active ingredients in a dosage form,

and the route by which the compound is to be administered to patients. However, typical dosage forms of the invention comprise a compound or a pharmaceutically acceptable salt of the compound.

[00136] Typical dosage levels for the azophenol compounds generally range from about 0.001 to about 100 mg per kg of the subject's body weight per day which can be administered in single or multiple doses. An exemplary dosage is about 0.01 to about 25 mg/kg per day or about 0.05 to about 10 mg/kg per day. In other exemplary embodiments, the dosage level ranges from about 0.01 to about 25 mg/kg per day, such as about 0.05 to about 10 mg/kg per day, or about 0.1 to about 5 mg/kg per day.

[00137] A dose can typically range from about 0.1 mg to about 2000 mg per day and can be given as a single once-a-day dose or, alternatively, as divided doses throughout the day, optionally taken with food. In a particular embodiment, the daily dose is administered twice daily in equally divided doses. A daily dose range can range from about 5 mg to about 500 mg per day such as, for example, between about 10 mg and about 300 mg per day. In managing the patient, the therapy can be initiated at a lower dose, such as from about 1 mg to about 25 mg, and increased if necessary up to from about 200 mg to about 2000 mg per day as either a single dose or divided doses, depending on the subject's global response.

[00138] The azophenol *ERG* inhibitor compounds according to the invention may be delivered by oral, parenteral (*e.g.*, intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (*e.g.*, transdermal, local) routes of administration. The inhibitors can be formulated alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles that are appropriate for each route of administration.

[00139] For example, suitable oral compositions in accordance with the invention include, without limitation, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, syrups or elixirs. Inventive compositions suitable for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions. For example, liquid

formulations of the azophenol compounds can contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations of the azophenol *ERG* inhibitor.

[00140] For tablet compositions, typical non-toxic pharmaceutically acceptable excipients include, without limitation, inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents such as, for example, corn starch, or alginic acid; binding agents such as, for example, starch, gelatin or lubricating agents such as, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or, alternatively, they may be coated by known coating techniques to delay disintegration and absorption in the gastrointestinal tract and thereby to provide a sustained therapeutic action over a desired time period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

[00141] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent such as, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium such as, for example peanut oil, liquid paraffin or olive oil.

[00142] For aqueous suspensions the azophenol compound is admixed with excipients suitable for maintaining a stable suspension. Examples of such excipients include, without limitation, sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia.

[00143] Oral suspensions can also contain dispersing or wetting agents, such as naturally-occurring phosphatide such as, for example, lecithin, or condensation products of an alkylene oxide with fatty acids such as, for example, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols such as, for example, heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as, for example,

polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides such as, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives such as, for example, ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents such as sucrose or saccharin.

[00144] Sweetening agents such as those set forth above, and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

[00145] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water can provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as, for example, sweetening, flavoring and coloring agents, may also be present.

[00146] Syrups and elixirs may be formulated with sweetening agents such as, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and flavoring and coloring agents.

[00147] Compositions for parenteral administrations are formulated in a sterile medium suitable for intravenous, intramuscular or intrathecal delivery. A sterile injectable preparation of the azophenol compounds may be in the form of a sterile injectable solution or sterile injectable suspension. Non-toxic, parentally acceptable diluents or solvents such as, for example, 1,3-butanediol can be used to formulate the parenteral compositions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile oils also can be employed as a solvent or a suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic monoglycerides or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables.

[00148] Depending on the vehicle used and the concentration of the drug in the formulation, the parenteral formulation can contain other adjuvants such as local anesthetics, preservatives and buffering agents.

Examples

Cell Lines

[00149] Tumor cell lines VCaP, COLO320, KG-1, MOLT4, LNCaP, and MDA Pca2b were obtained from the American Tissue Culture Collection (ATCC; Manassas, VA). The cells were grown in ATCC- recommended cell culture media under cell growth promoting conditions as recommended by the supplier. Normal cells, such as HUVEC-primary cultures of human umbilical vein endothelial cells and the RWPE1 cell line established from normal adult prostate epithelial cells immortalized with human papilloma virus 18 were also obtained from ATCC. The BPH1 cell line derived from primary epithelial cell cultures immortalized with SV40 large T-antigen, were a gift from Dr. Simon Hayward (Vanderbilt University Medical Center). LAPC4, a metastatic prostate cancer cell line was a gift from Dr. Charles Sawyer (then at UCLA).

Reagents

[00150] *ERG* monoclonal antibody (CPDR *ERG*-MAb; 9FY, licensed to Biocare Medical, CA) was developed and characterized at the Center for Prostate Disease Research. Antibodies for the androgen receptor (AR; sc-816), glyceraldehyde phosphate dehydrogenase (GAPDH; sc-25778), and α-Tubulin (sc-5286) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody for prostate specific antigen (PSA; A0562012) was obtained from DakoCytomation (Carpinteria, CA). Antibodies for apoptosis (9915S) and cell cycle regulator (9932) sampler kits were purchased from Cell Signaling (Danvers, MA). Sheep anti-mouse IgG-HRP (NXA931) and donkey-anti rabbit IgG-HRP (NXA934V) were obtained from GE Health Care, Buckinghamshire, UK. A number of the azophenol compounds were obtained from the Stanford University School of Medicine. Many of the azophenol compounds disclosed herein are known in the prior art, typically for their use as dyes. The published methods for their syntheses represent routes by which the compounds of the invention were prepared.

General Protocol for Screening inhibitors of the *ERG* oncoprotein expression

[00151] The *TMPRSS2-ERG* fusion positive prostate cancer cell line, VCaP (ATCC), was used to identify the test compound inhibitors of *ERG* expression. Cells were grown in medium using conditions prescribed by the vendor. VCaP cells in logarithmic growth were seeded in a tissue culture dish at a cell density of 2×10^6 cells per plate. Following 48 hours incubation at 37°C, cells were exposed for a period of 48 hours with 0, 0.05, 0.25 and 0.5 μM concentrations of each test azophenol compound. The inhibition of *ERG* expression was evaluated by an In-cell Western blot assay (LI-COR Biosciences, Lincoln, NE) using the *ERG* specific CPDR *ERG*-MAb as further described below.

Selection of *ERG* siRNA as a Positive Control

[00152] Small interfering RNA (siRNA) oligo duplexes (5' CGA CAU CCU UCU CUC ACA UAU 3': si-1; and 5' UGA UGU UGA UAA AGC CUU A 3': si-2) against human *ERG* gene (Gene ID: 2078; Accession: NM_004449), were purchased from Dharmacon (Lafayette, CO) and were evaluated as positive controls for use in the *ERG* expression inhibition screens. Two siRNAs were chosen to primarily rule out off target or non-specific effects. Since both siRNAs showed identical results, si-1 was used in the *ERG* expression inhibitory studies described below. A non-targeting (NT) siRNA duplex was used as negative control (D-001206-13-20; Dharmacon, Lafayette, CO). Cells were cultured in their respective growth medium for 48 hours prior to transfection using a 50 nM concentration of the NT siRNA or *ERG* siRNA. Lipofectamine 2000® (Invitrogen, Carlsbad, CA) was used for transfection.

General Protocol for Evaluating the Inhibitory Effects of Test Compounds By Western Blot Analysis

[00153] Inhibition of ERG protein expression by the test compounds were determined by Western blot analysis. The ERG specific monoclonal antibody CPDR ERG-MAb was used as the primary antibody. In brief, Western blot was performed by running a fixed amount of total protein extracted from cell lysates of the treated cells using (4-12% Bis-Tris) gel by electrophoresis, followed by transfer to membrane and incubation with primary antibody and continued with HRP-conjugated secondary antibody. Cultured

cells were treated at specific dosages with each of the tested *ERG* inhibitors. Following incubation of the treated cells for an indicated time period, cells were lysed using Mammalian Protein Extraction Reagent (M-PER; Pierce, Rockford, IL) containing a protease inhibitor cocktail and phosphatase inhibitor cocktails I & III (Sigma, St Louis, MO). Cell lysates containing 50 μg of total protein were electrophoresed through 4-12% Bis-Tris Gel (Invitrogen, Carlsbad, CA) and the cellular proteins were transferred to PVDF membrane (Invitrogen, Carlsbad, CA). Membranes were incubated at 4°C for 12 hours with primary antibodies for AR, PSA, GAPDH, α-Tubulin, apoptosis markers and cell cycle regulators. Following exposure to primary antibodies, the membranes were washed with buffer (3X, 5 minutes each at room temperature) followed by incubation with relevant secondary antibodies for 1 hour at 24°C. Finally, the membranes were washed with buffer and developed using the ECL Western blot detection reagent (GE Health Care, Buckinghamshire, UK). The ERG protein expression of the test azophenol compounds were normalized with GAPDH.

Selective Inhibition of ERG Expression

[00154] In brief, ERG positive VCaP cells in logarithmic growth phase were plated in 10 cm tissue culture dish at a cell density of 2 x 10^6 cells per dish. The plated cells were treated with 0, 0.2, 0.4, 0.6, 0.8 and 1 μM concentrations of each azophenol compound for a period of 48 hours. Cells from each dish were then processed for Western blot analysis and alterations in the expression of ERG protein were monitored. Both ERG and GAPDH protein band were quantified from each concentration using Image J (NIH) and ERG band density was normalized with corresponding GAPDH protein control. Relative density of ERG in each concentration was calculated and with Graphpad Prism 6 software, the IC50 of each compound was calculated.

General Protocol for Cell Growth and Tumor Growth Inhibition Studies

[00155] The appropriate *ERG* positive cancer cells, control *ERG* negative cells or *ERG* positive normal cells were grown as adherent monolayers or suspensions in tissue culture dishes using the appropriate growth medium as suggested by the vendor. Approximately 48 hours following plating of cells, the appropriate test compound is added to each well of the tissue culture dish at a predetermined concentration. The medium was replenished

every 24 hours with fresh growth medium containing the same concentration of the same test compound for indicated period of the cell growth inhibition assay. Percent cell growth inhibition was calculated using a hemocytometer for estimating cell density in each of the test wells of the tissue culture dish and trypan blue dye exclusion microscopy and photography to estimate the fraction of viable cells in each test well.

[00156] To investigate whether the azophenol compounds selectively arrested the growth of ERG positive cancer cells (VCaP), VCaP cells were cultured to achieve cells in logarithmic growth and these cells were then plated in 6 well tissue culture dishes in duplicates at a cell density of 0.2×10^6 cells per well. The plated cells were exposed to 0, 0.2, 0.4, 0.6, 0.8 and 1 μM concentrations of each compound for a period of 8 days. At the end of time period, cells were recovered from the test plate, washed, trypsinized and the cell density of viable cells were determined with trypan blue dye staining method and automated cell counter Bio-Rad TC10. The IC50 of cell growth inhibition of each compound was determined by trypsinizing the cells at the end of the experiment and counting the cells by using the automated cell counter (Bio-Rad TC10 automated cell counter). The average cell numbers of each concentration were used to calculate the IC50 of each compound with the GraphPad Prism 6 software.

[00157] Male athymic nude mice 6-8 weeks old and weighing 27 to 30g were purchased from Charles River Laboratories. *ERG* harboring prostate cancer cells (VCaP) were trypsinized and washed twice with ice-cold PBS, and resuspended in ice-cold 50% matrigel in serum-free DMEM medium. A total of $4x10^6$ cells /0.1 mL /mouse were subcutaneously injected into lower right dorsal flank of the mice. Prior to injection, mice were anesthetized with inhalation anesthesia (isoflurane). Tumor growth was monitored weekly after injection. Three weeks later when tumors were palpable mice were randomly separated into 2 experimental groups and one control group of 7 mice in each group. In the treatment groups, mice were injected intraperitoneally (I.P) with 100 mg/kg of the test compound or 150 mg/kg of the test compound while the control group were injected with vehicle (1:1[v/v], DMSO/PEG300) only. Growth in tumor volume was recorded weekly by digital caliper measurements and tumor volumes calculated using the $1/2 (L \times W^2)$ formula, where L = length of tumor and W = width. Tumor volumes were compared between treated and control groups with repeated

measurements and statistical significance of the results between the groups computed using students t-test and p values calculated.

[00158] Table 1 below shows the results of testing of azophenol compounds.

Compound	Cell line	Tissue origin	*ERG* gene status	ERG protein inhibition (IC50)	Cell growth inhibition (IC50)
3	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	1.352	4.070
4	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	1.855	1.122
8	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D
9	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D
10	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D
5	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	0.5422	0.5572
6	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	0.2727	0.0739
7	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	0.5587	0.6260
11	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D
12	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D
13	VCaP	Metastatic prostate cancer	*TMPRSS2-ERG* fusion; ERG protein (N-33 aa-del)	N/D	N/D

N/D: Assay performed, inhibition not detected at the tested levels.

[00159] All publications cited herein are incorporated by reference in their entireties.

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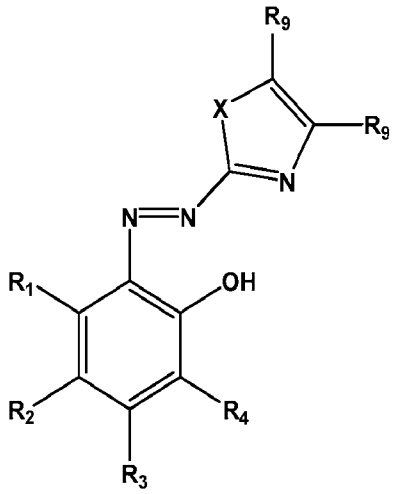
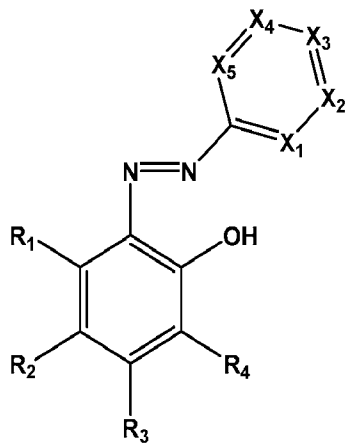
We Claim:

1. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or Formula (II)



Formula (I)



Formula (II)

or a pharmaceutically acceptable salt thereof,

wherein:

X is NH, O or S;

X_1, X_2, X_3, X_4 and X_5 are independently N or CR_9, where only one of X_1, X_2, X_3, X_4 and X_5 is N;

R_1, R_2 and R_4 are independently selected from the group consisting of H, aryl, halogen, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl, wherein the aryl, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_5R_6$, $-NR_5C(O)R_6$ and $-C(O)NR_5R_6$;

R_3 is selected from the group consisting of H, -OH, $-NR_5R_6$, C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl, wherein the C_1-C_{10} alkyl, C_1-C_{10} alkoxy, C_3-C_7 cycloalkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_5R_6$, $-NR_5C(O)R_6$ and $-C(O)NR_5R_6$;

R_5 and R_6 are independently selected from the group consisting of H, C_1-C_8 alkyl, aryl and C_3-C_7 cycloalkyl, or R_5 and R_6 taken together form a C_3-C_7 heterocycloalkyl wherein the C_1-C_8 alkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_7R_8$, $-NR_7C(O)R_8$ and $-C(O)NR_7R_8$;

R_7 and R_8 are independently selected from the group consisting of H and C_1-C_8 alkyl;

each R_9 is independently H, halogen, -CN, -OH, COOH, $-NR_{10}R_{11}$, C_1-C_{10} alkyl, C_3-C_7 cycloalkyl, C_1-C_{10} alkoxy and C_3-C_7 heterocycloalkyl wherein the C_1-C_{10} alkyl, C_3-C_7 cycloalkyl, C_1-C_{10} alkoxy and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting of C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halogen, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_{10}R_{11}$, $-NR_{10}C(O)R_{11}$ and $-C(O)NR_{10}R_{11}$;

R_{10} and R_{11} are independently selected from the group consisting of H, C_1-C_8 alkyl and C_3-C_7 cycloalkyl, or R_{10} and R_{11} taken together form a C_3-C_7 heterocycloalkyl wherein the C_1-C_8 alkyl and C_3-C_7 heterocycloalkyl are optionally substituted with one or more substituents selected from the group consisting C_1-C_8 alkyl, C_3-C_7 cycloalkyl, C_3-C_7 heterocycloalkyl, aryl, heteroaryl, halo, hydroxyl, -CN, -COOH, $-CF_3$, $-OCH_2F$, $-OCHF_2$, $-OC_1$-C_8 alkyl, -O-aryl, -O-heteroaryl, $-NR_{12}R_{13}$, $-NR_{12}C(O)R_{13}$ and $-C(O)NR_{12}R_{13}$; and

R_{12} and R_{13} are independently selected from the group consisting of H and C_1-C_8 alkyl.

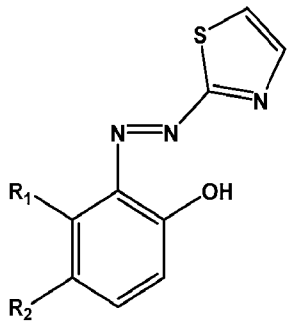
2. The method according to claim 1, wherein R_3 and R_4 in Formula (I) and Formula (II) are each H and at least one of R_1 and R_2 is not H.

3. The method according to claim 1, wherein R_1, R_3 and R_4 in Formula (I) and Formula (II) are each H and R_2 is not H.

4. The method according to claim 1, wherein R_3 in Formula (I) and Formula (II) is -OH.

5. The method according to claim 1, wherein R_3 in Formula (I) and Formula (II) is $-NR_5R_6$.

6. The method according to claim 1, wherein the compound of Formula (I) is a compound of Formula (III)



Formula (III)

or a pharmaceutically acceptable salt thereof,

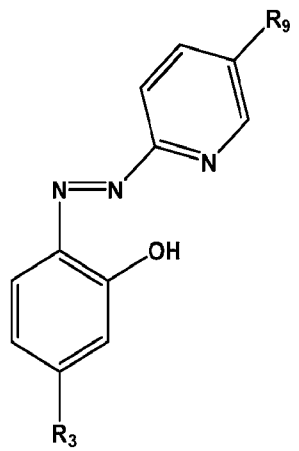
wherein:

R_1 and R_2 are as defined.

7. The method according to claim 6, wherein R_1 and R_2 are independently selected from the group consisting of H and C_1-C_{10} alkyl, where at least one of R_1 and R_2 is not H.

8. The method according to claim 7, wherein R_1 is H and R_2 is C_1-C_{10} alkyl.

9. The method according to claim 1, wherein the compound of Formula (II) is a compound of Formula (IV)



Formula (IV)

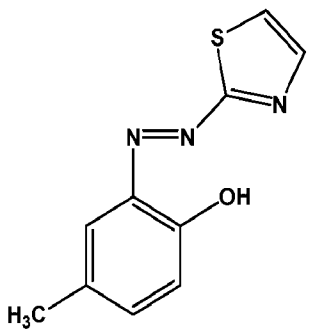
or a pharmaceutically acceptable salt thereof,

wherein:

R_3 and R_9 are as defined.

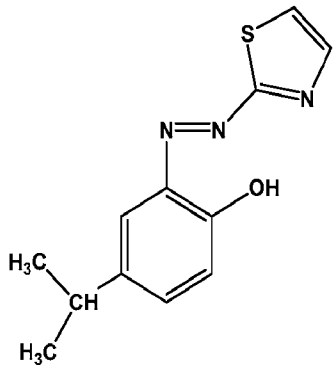
10. The method according to claim 9, wherein R_3 is OH or NR_5R_6; and R_9 is halogen.

11. The method according to claim 1, wherein the compound of Formula (I) is



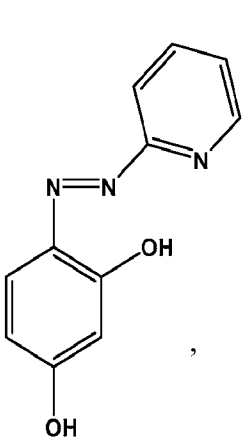
or a pharmaceutically acceptable salt thereof.

12. The method according to claim 1, wherein the compound of Formula (I) is

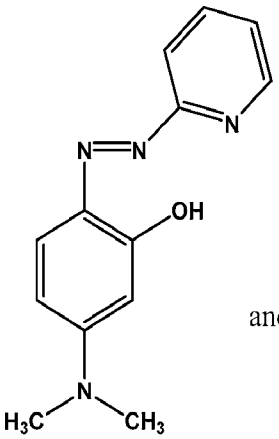


or a pharmaceutically acceptable salt thereof.

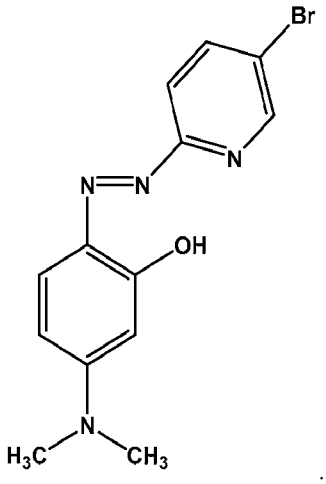
13. The method according to claim 9, wherein the compound of Formula (IV) is selected from the group consisting of:



,



and

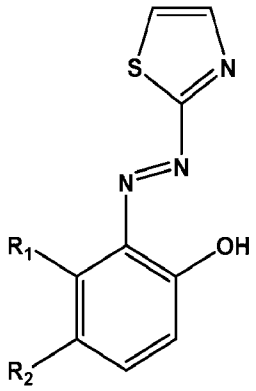


.

14. The method according to claim 1, wherein the disease is selected from the group consisting of prostate cancer, Ewing's sarcoma, acute myeloid leukemia, acute T-lymphoblastic leukemia, endothelial cancer and colon cancer.

15. The method according to claim 14, wherein the disease is prostate cancer.

16. A compound of Formula (V)



Formula (V)

or a pharmaceutically acceptable salt thereof,

wherein:

R_1 is selected from the group consisting of H and C_1-C_{10} alkyl; and

R_2 is C_2-C_{10} alkyl,

with the proviso that when R_1 is H, R_2 is not ethyl, isobutyl or $-C(CH_3)_2-CH_2-C(CH_3)_3$.

17. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (V) according to claim 16 or a pharmaceutically acceptable salt thereof.

18. The method according to claim 17, wherein the disease is prostate cancer.

19. A pharmaceutical composition comprising the compound of Formula (I) or Formula (II) according to claim 1 and an excipient.

20. A pharmaceutical composition comprising the compound of Formula (III) according to claim 6 and an excipient.

21. A pharmaceutical composition comprising the compound of Formula (IV) according to claim 9 and an excipient.

22. A pharmaceutical composition comprising the compound of Formula (V) according to claim 16 and an excipient.

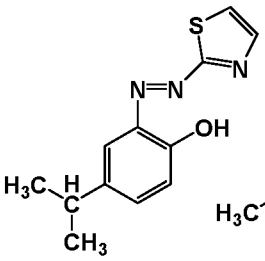
23. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to claim 19.

24. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to claim 20.

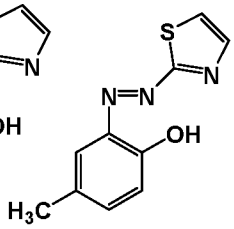
25. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising co-administering to the subject a therapeutically effective amount of a pharmaceutical composition according to claim 21.

26. A method for treating a disease associated with overexpression of wild type *ERG* protein, an altered *ERG* protein, *ERG* gene transcription or *ERG* mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to claim 22.

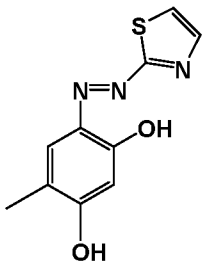
Figure 1A



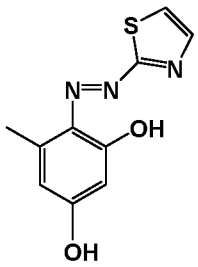
Compound 1



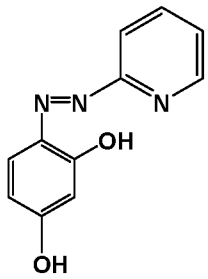
Compound 2



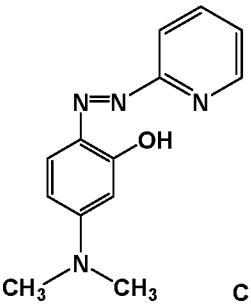
Compound 3



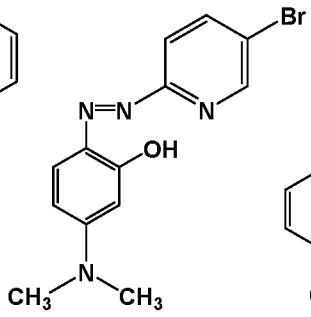
Compound 4



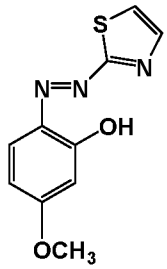
Compound 5



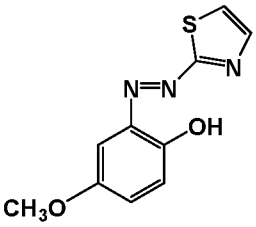
Compound 6



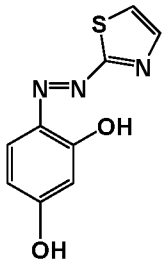
Compound 7



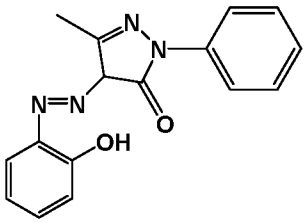
Compound 8



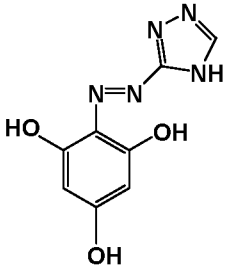
Compound 9



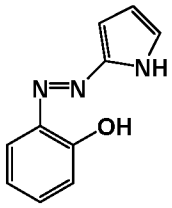
Compound 10



Compound 11



Compound 12



Compound 13

Figure 1B

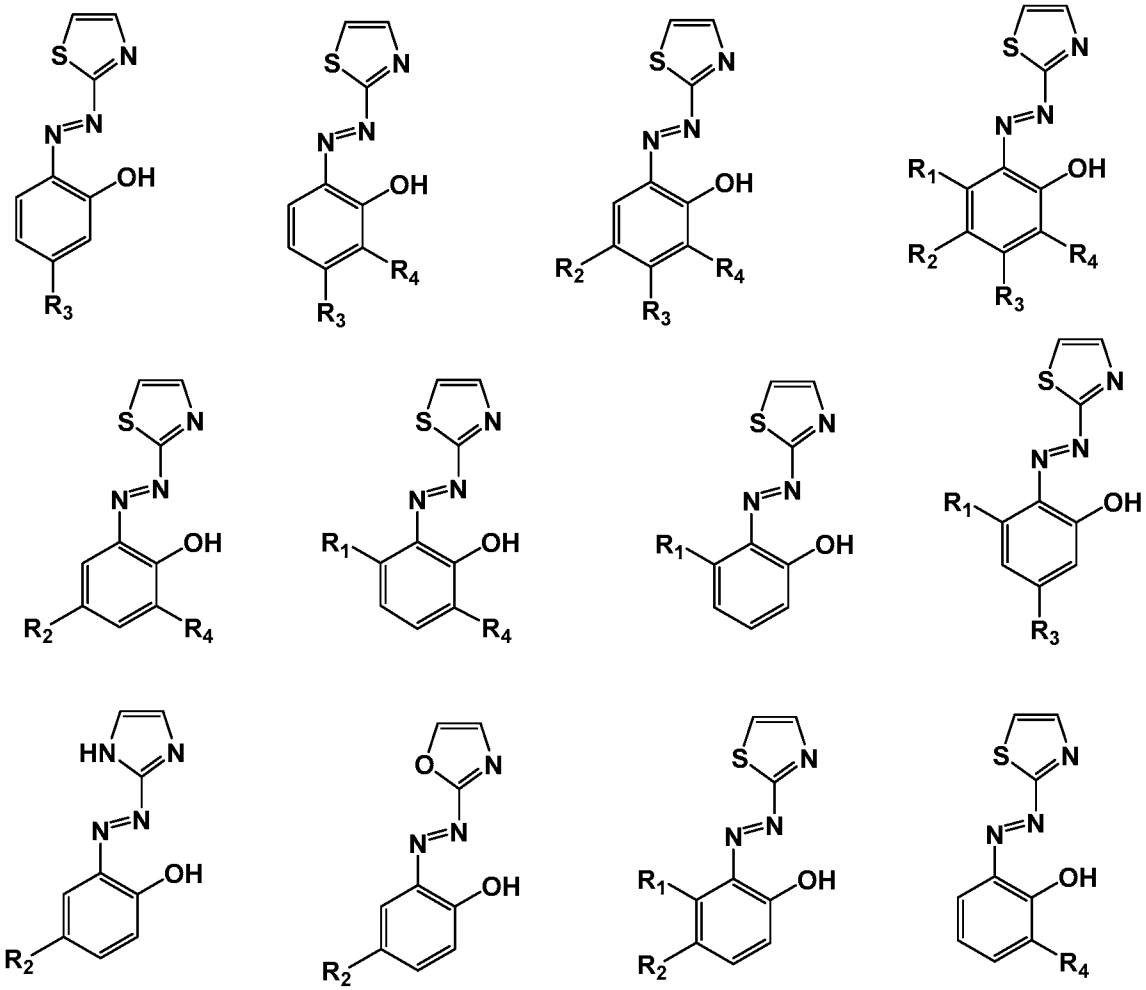


Figure 1C

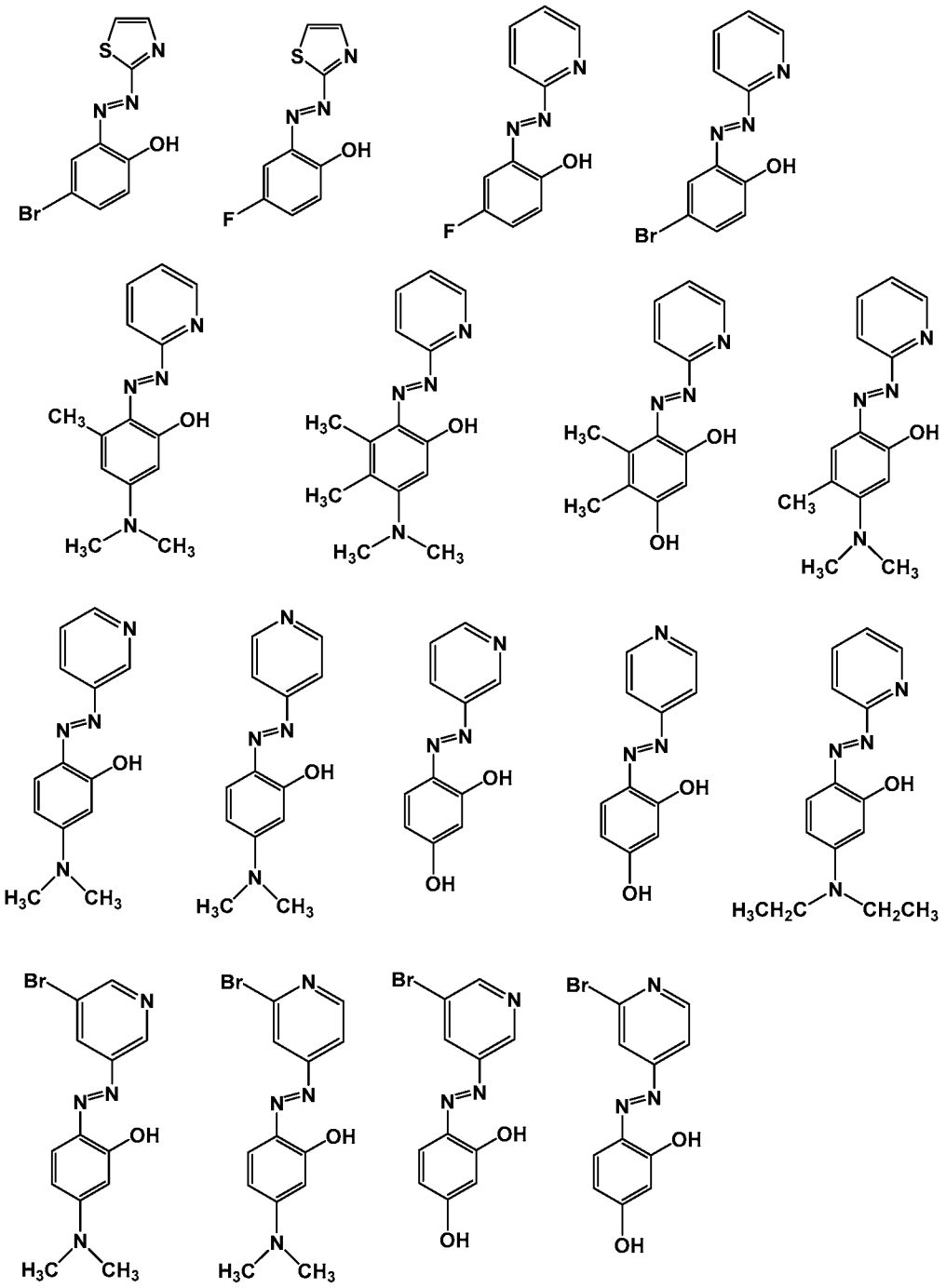


Figure 1D

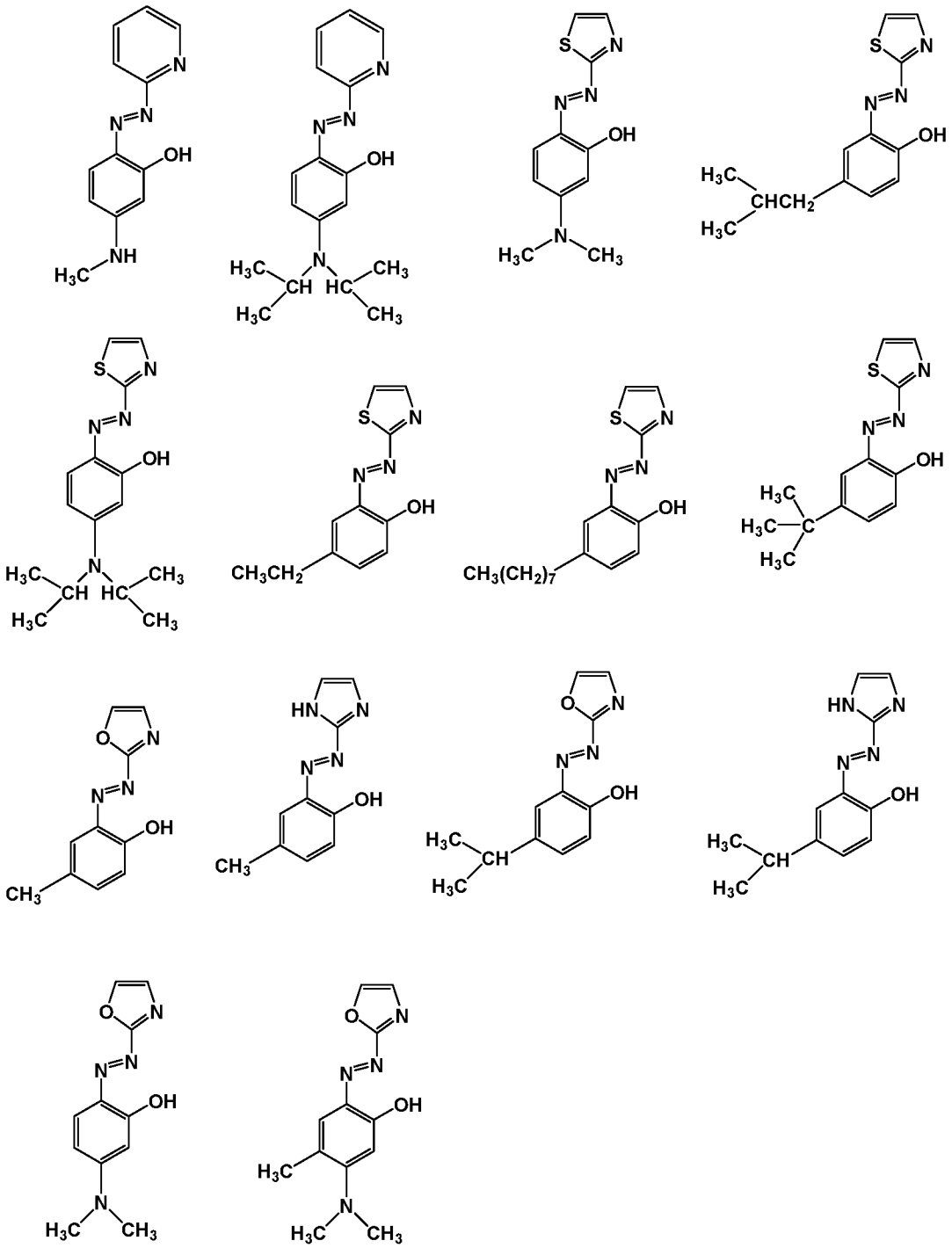
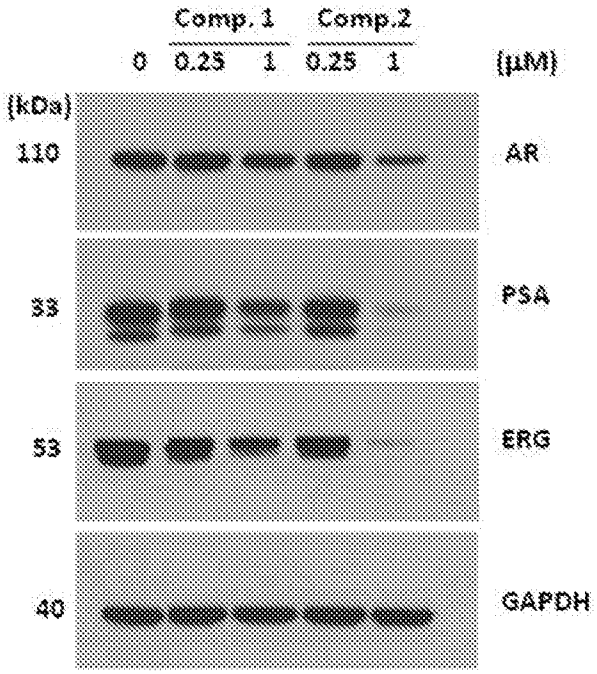


Figure 2


Comp. 1
Comp.2
0
0.25
1
0.25
1
(μM)
(kDa)
110
AR
33
PSA
53
ERG
40
GAPDH

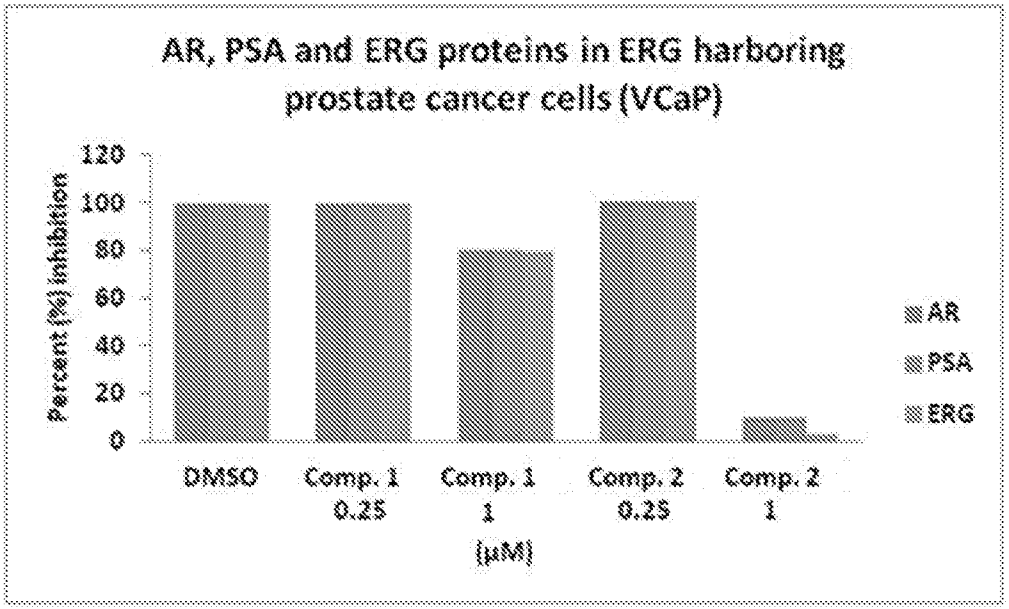
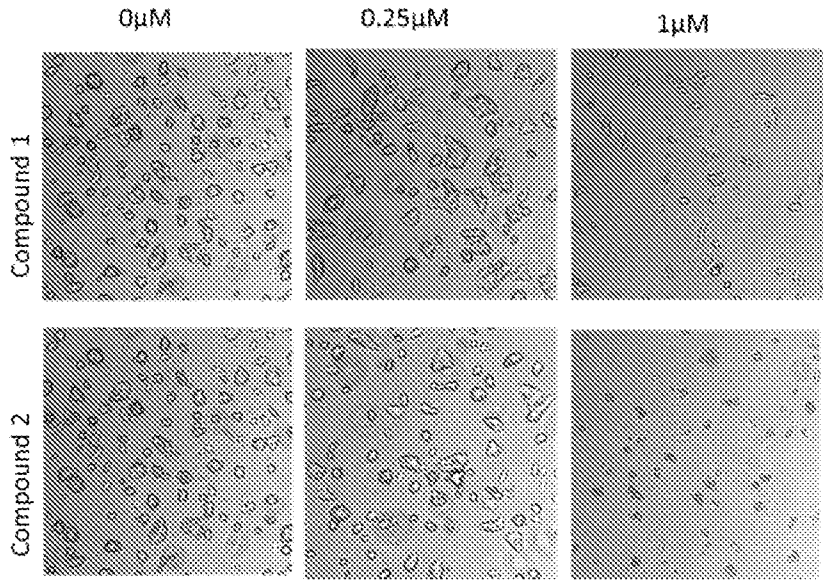

AR, PSA and ERG proteins in ERG harboring prostate cancer cells (VCaP)
Percent (%) inhibition
120
100
80
60
40
20
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)
AR
PSA
ERG

Figure 3


0μM
0.25μM
1μM
Compound 1
Compound 2

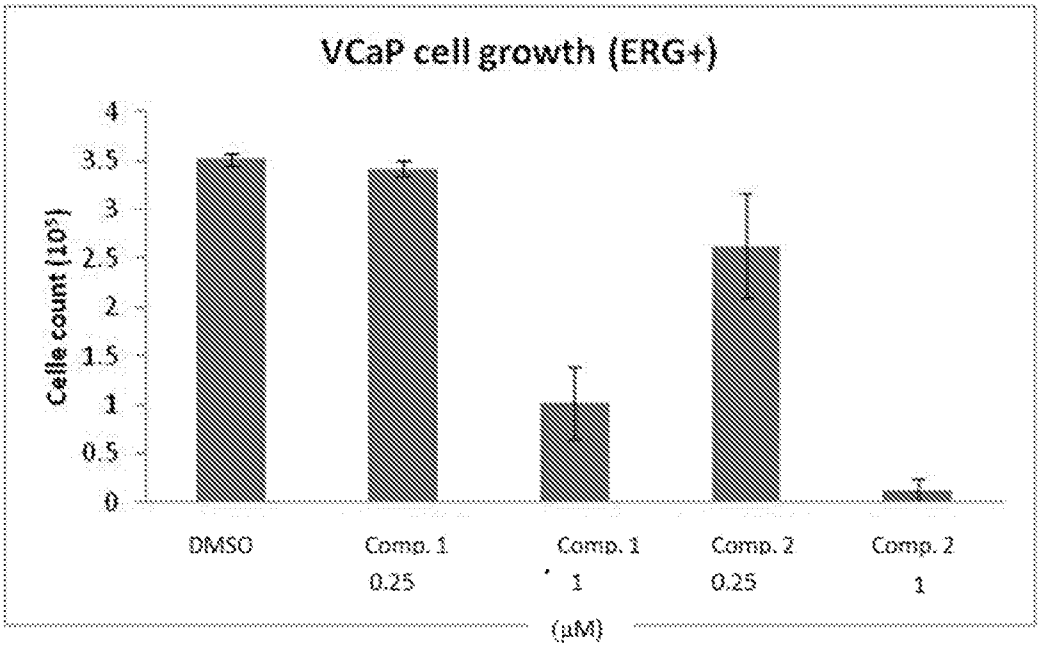
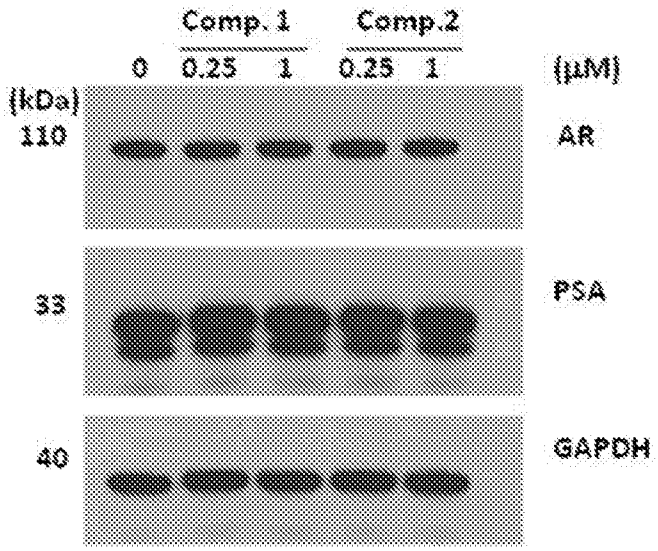

VCaP cell growth (ERG+)
Cells count (10^5)
4
3.5
3
2.5
2
1.5
1
0.5
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 4


Comp. 1
Comp.2
0
0.25
1
0.25
1
(μM)
(kDa)
110
AR
33
PSA
40
GAPDH

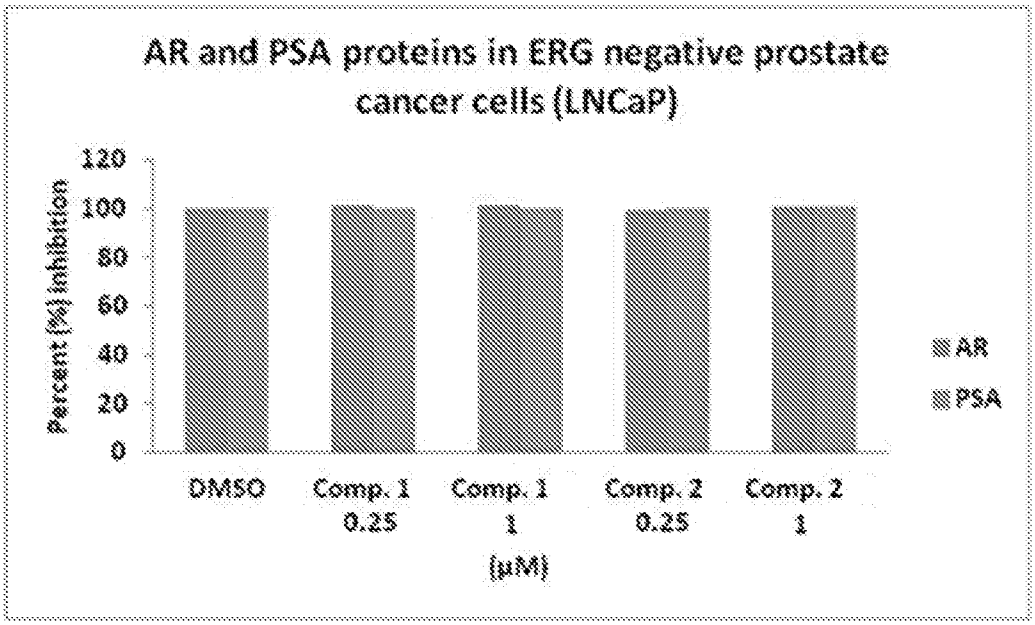
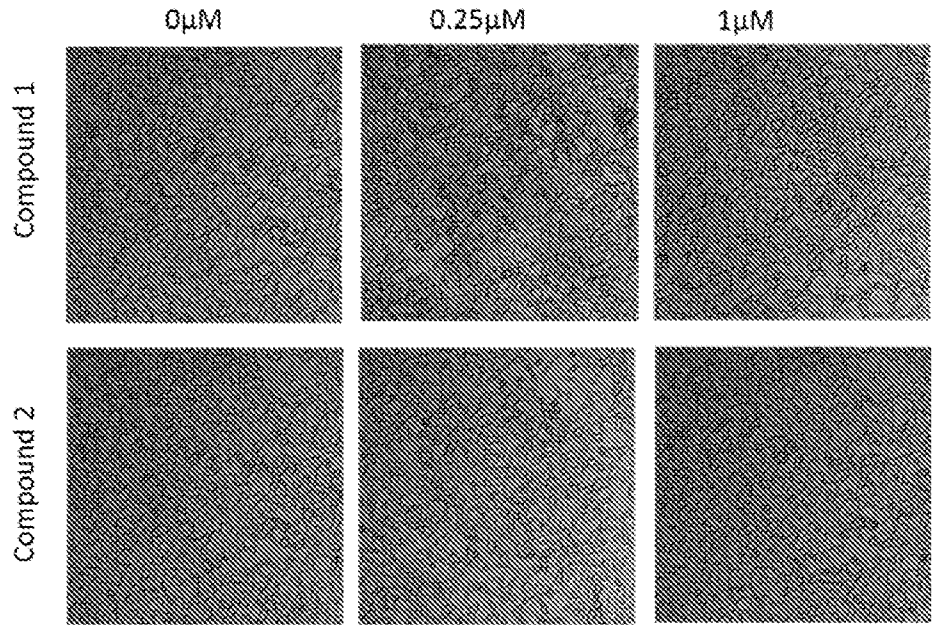

AR and PSA proteins in ERG negative prostate cancer cells (LNCaP)
Percent (%) inhibition
120
100
80
60
40
20
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)
AR
PSA

Figure 5


0μM
0.25μM
1μM
Compound 1
Compound 2

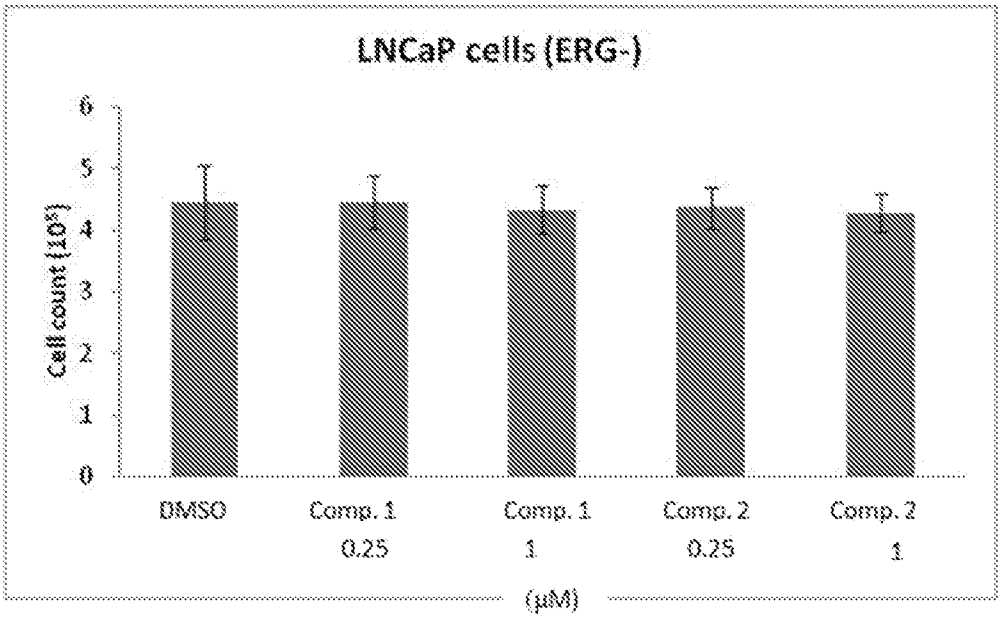
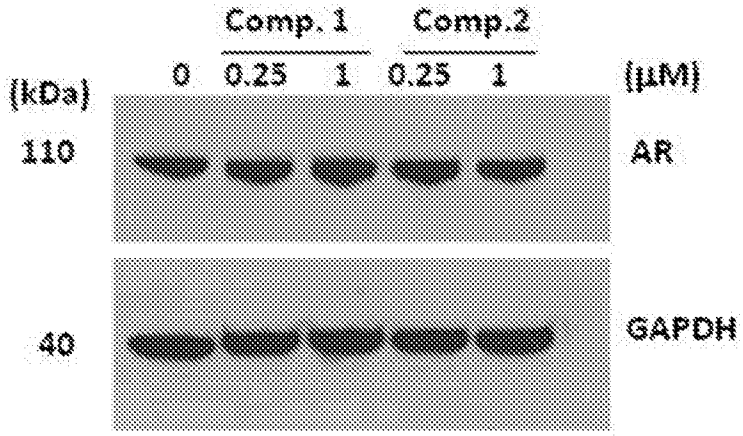

LNCaP cells (ERG-)
Cell count (10⁵)
0
1
2
3
4
5
6
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 6


Comp. 1
Comp.2
0
0.25
1
0.25
1
(μM)
(kDa)
110
AR
40
GAPDH

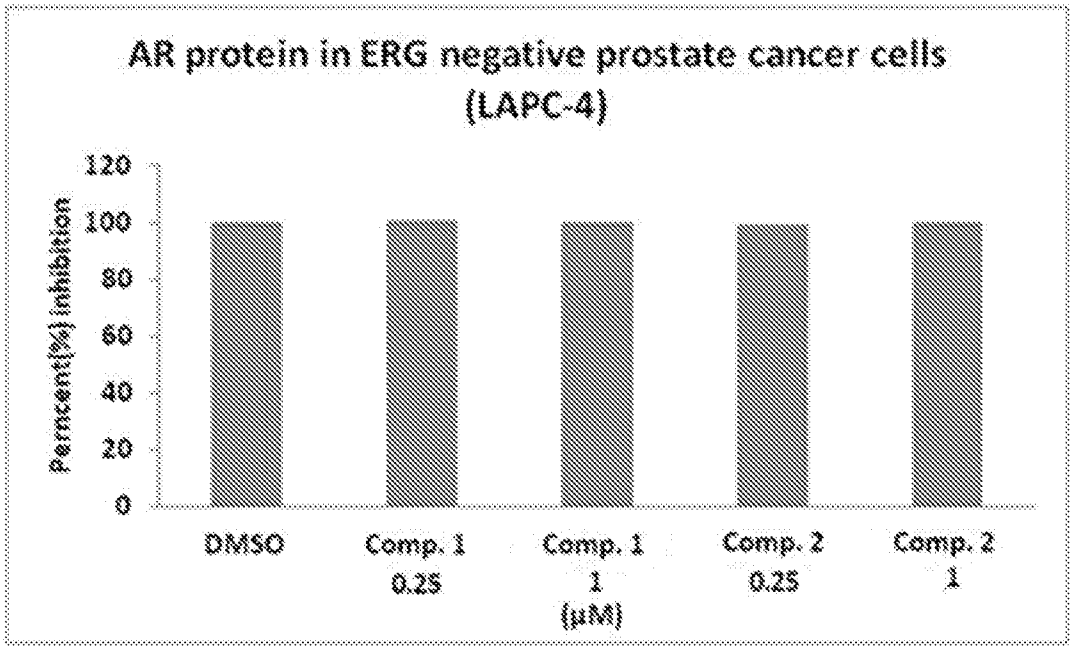
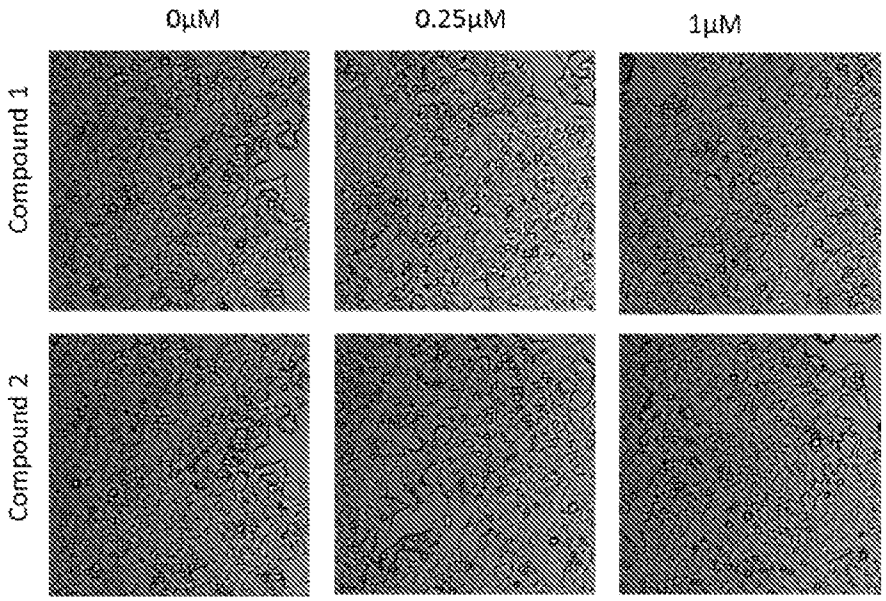

AR protein in ERG negative prostate cancer cells (LAPC-4)
Perncent(%) inhibition
120
100
80
60
40
20
0
DMSO
Comp. 1 0.25
Comp. 1 1 (μM)
Comp. 2 0.25
Comp. 2 1

Figure 7


0μM
0.25μM
1μM
Compound 1
Compound 2

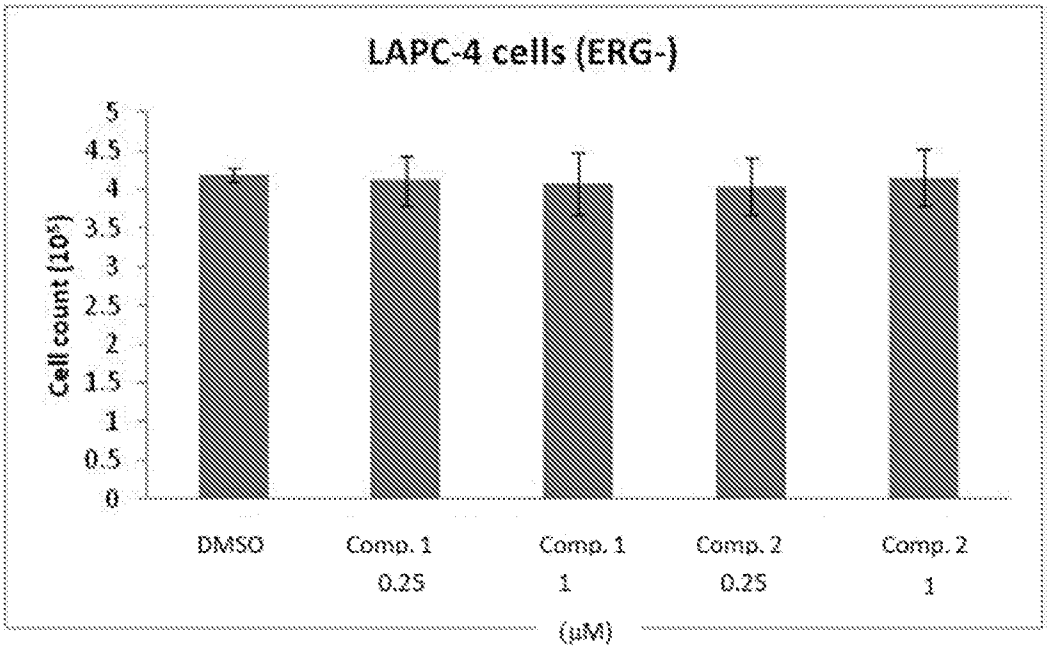
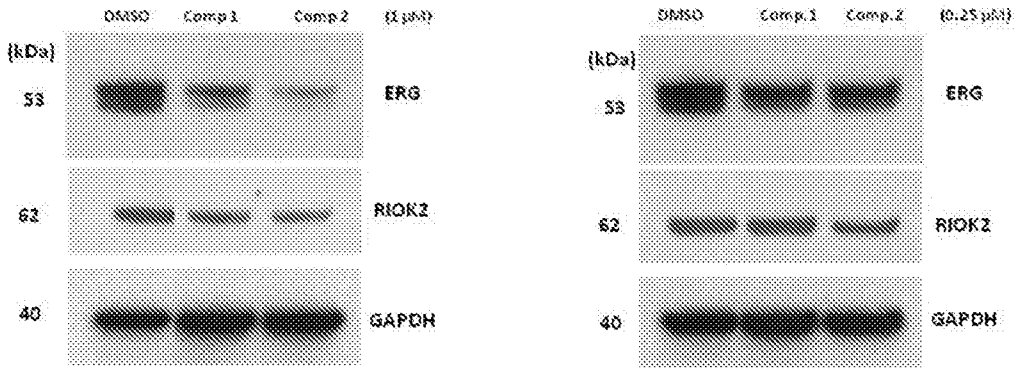
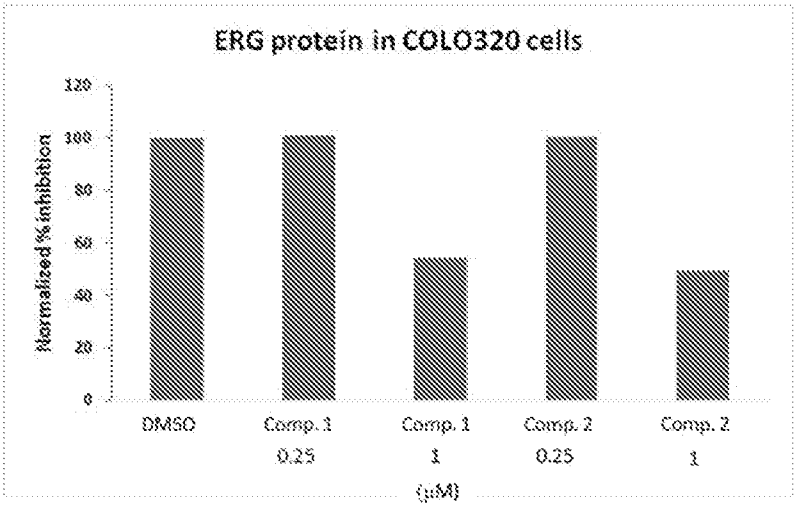

LAPC-4 cells (ERG-)
Cell count (10⁵)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 8


DMSO
Comp.1
Comp.2
(1 μM)
(kDa)
ERG
RIOK2
GAPDH
DMSO
Comp.1
Comp.2
(0.25 μM)
(kDa)
ERG
RIOK2
GAPDH


ERG protein in COLO320 cells
Normalized % inhibition
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

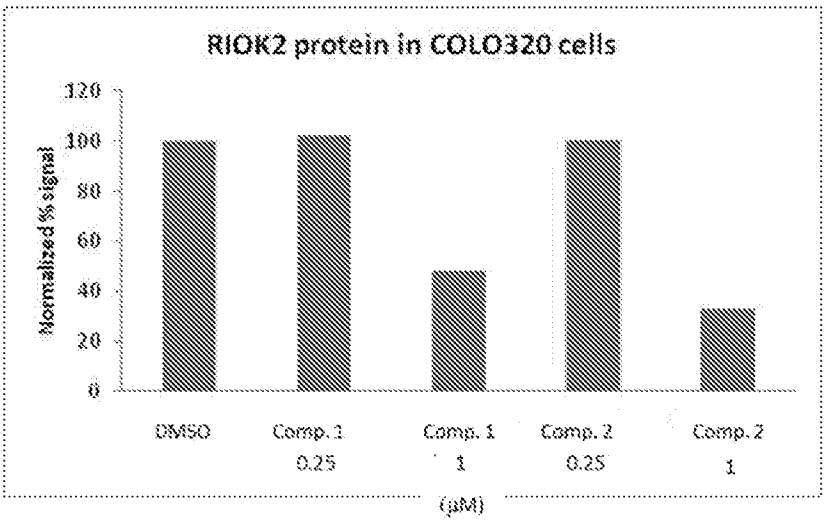
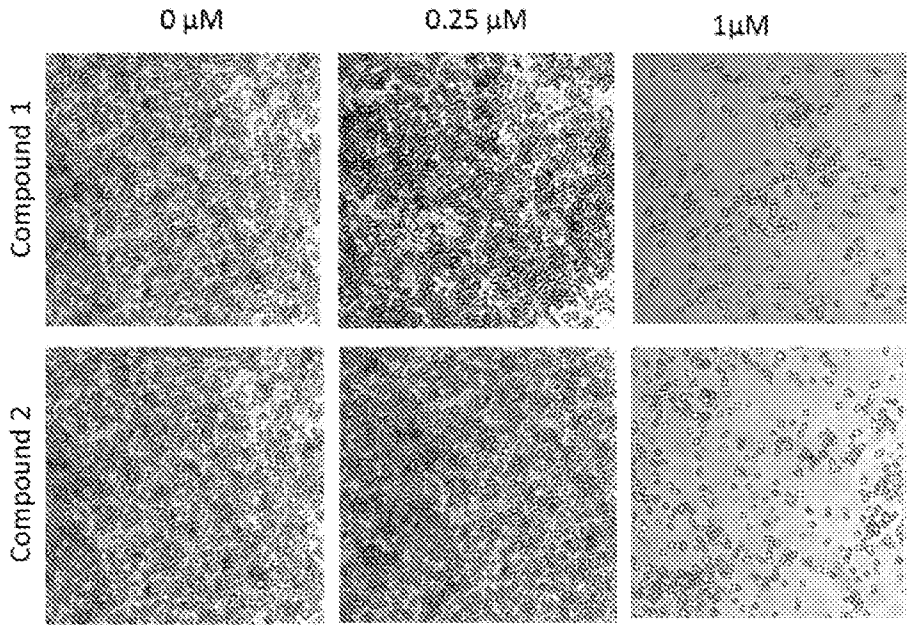

RIOK2 protein in COLO320 cells
Normalized % signal
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 9


0 µM
0.25 µM
1µM
Compound 1
Compound 2

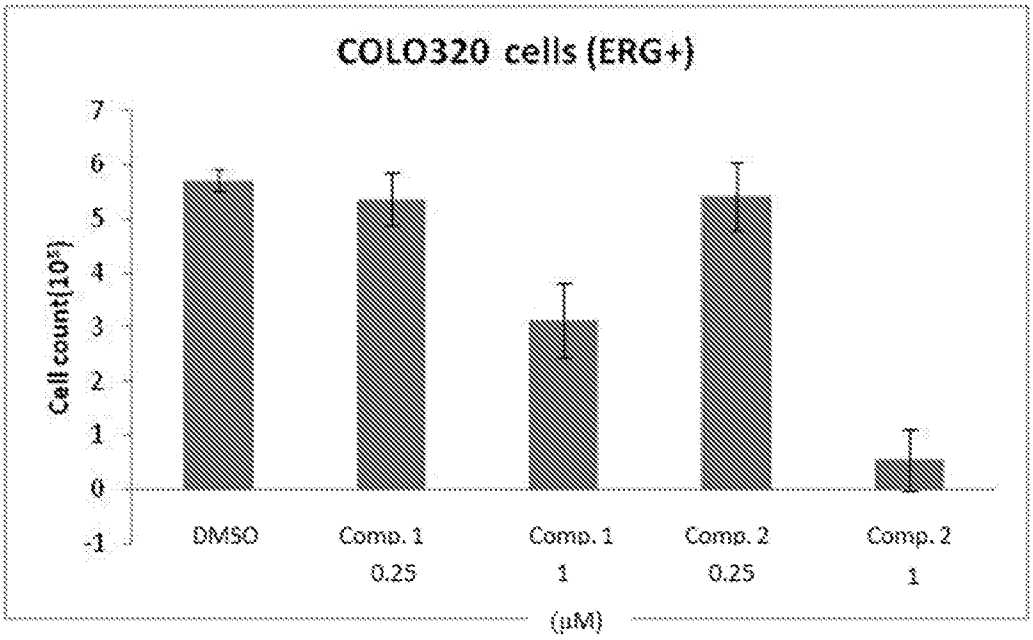
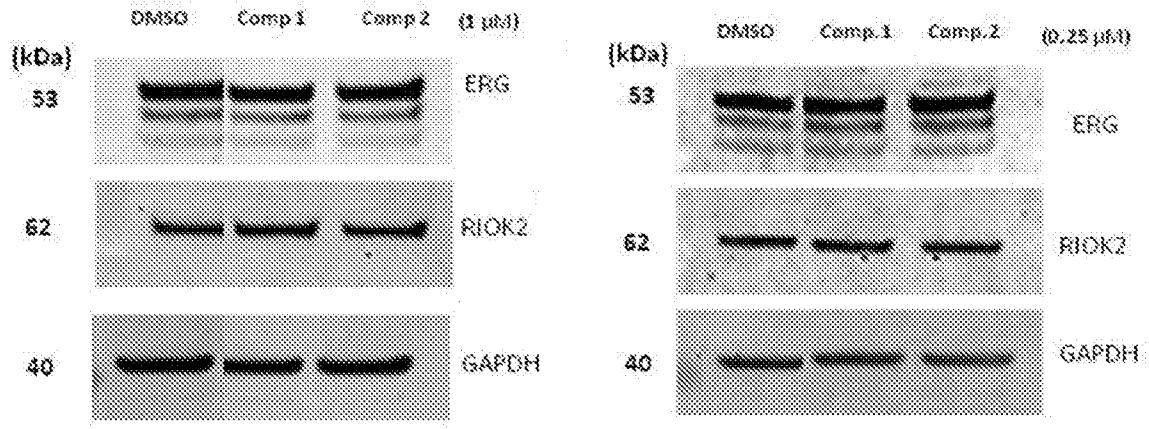
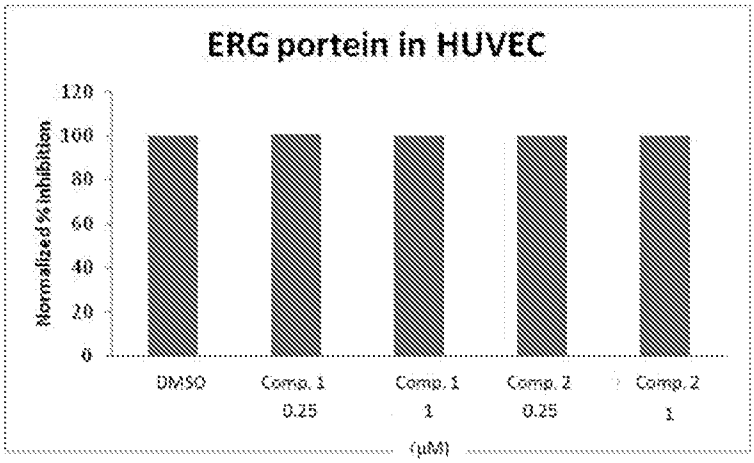

COLO320 cells (ERG+)
Cell count[10⁵]
7
6
5
4
3
2
1
0
-1
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(µM)

Figure 10


DMSO
Comp 1
Comp 2
(1 μM)
(kDa)
53
ERG
62
RIOK2
40
GAPDH
DMSO
Comp. 1
Comp. 2
(0.25 μM)
(kDa)
53
ERG
62
RIOK2
40
GAPDH


ERG portein in HUVEC
Normalized % inhibition
120
100
80
60
40
20
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

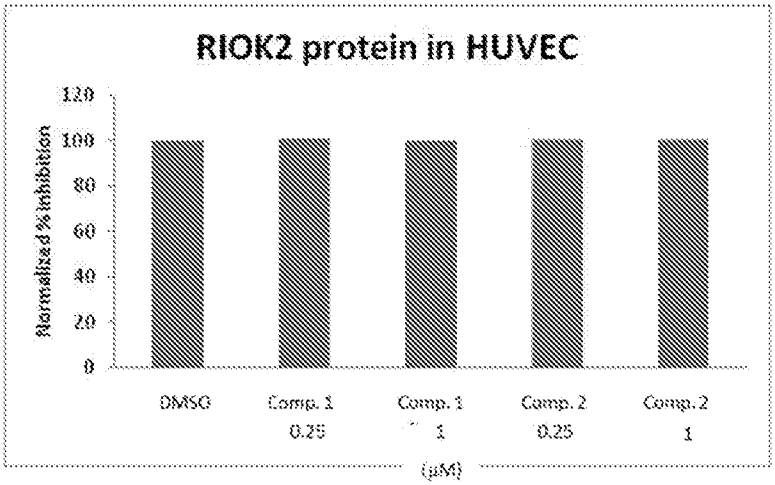
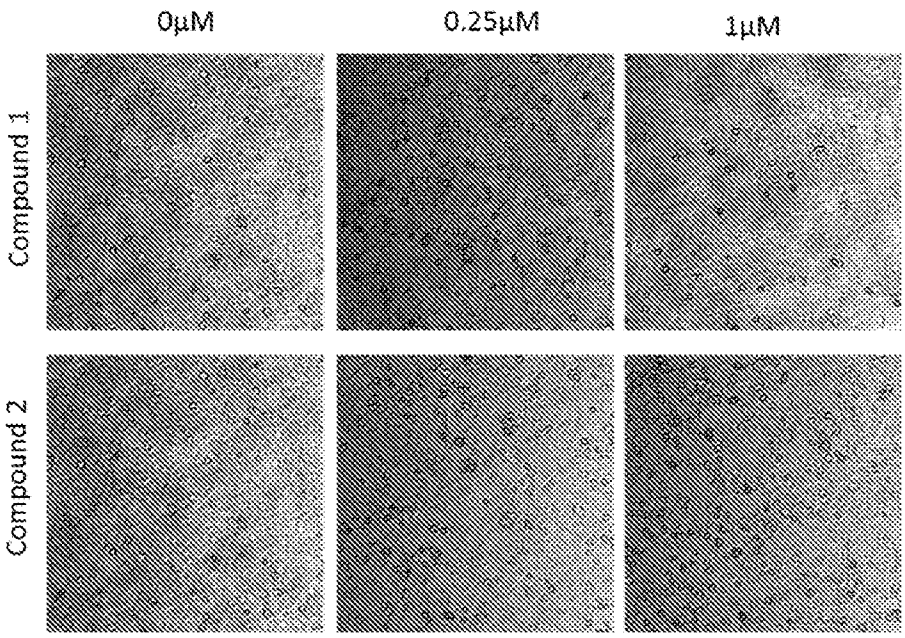

RIOK2 protein in HUVEC
Normalized % inhibition
120
100
80
60
40
20
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 11


0μM
0.25μM
1μM
Compound 1
Compound 2

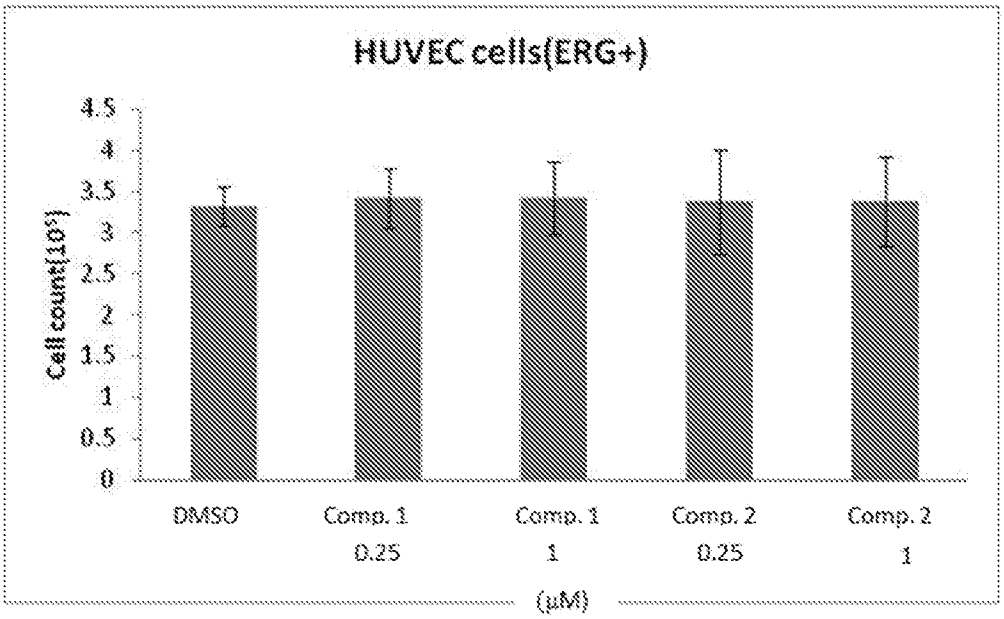
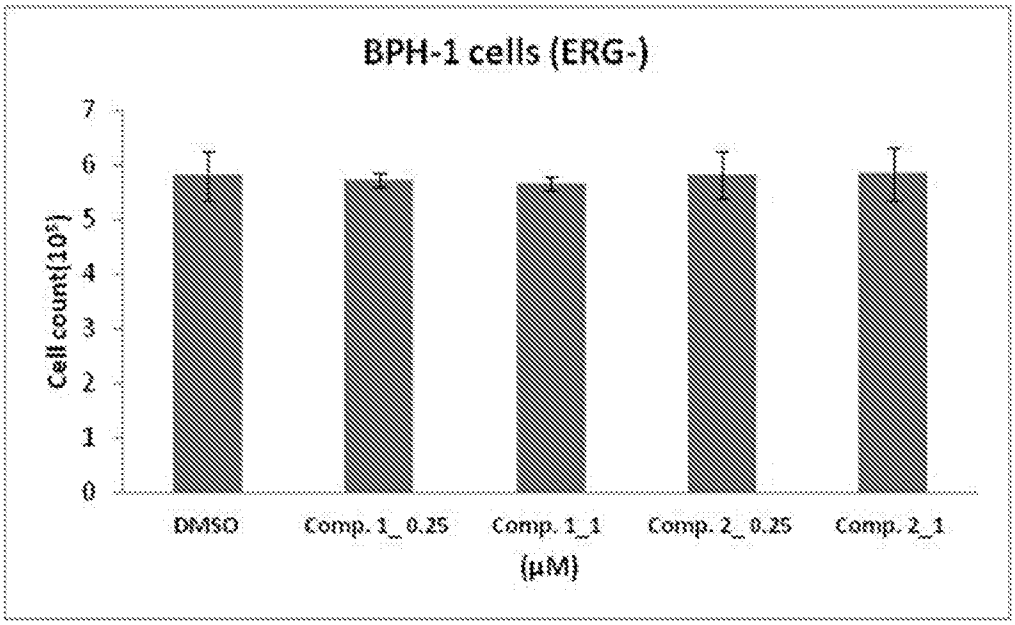

HUVEC cells(ERG+)
Cell count(10⁵)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
DMSO
Comp. 1 0.25
Comp. 1 1
Comp. 2 0.25
Comp. 2 1
(μM)

Figure 12


BPH-1 cells (ERG-)
Cell count(10^5)
0
1
2
3
4
5
6
7
DMSO
Comp. 1_ 0.25
Comp. 1_1
Comp. 2_ 0.25
Comp. 2_1
(µM)

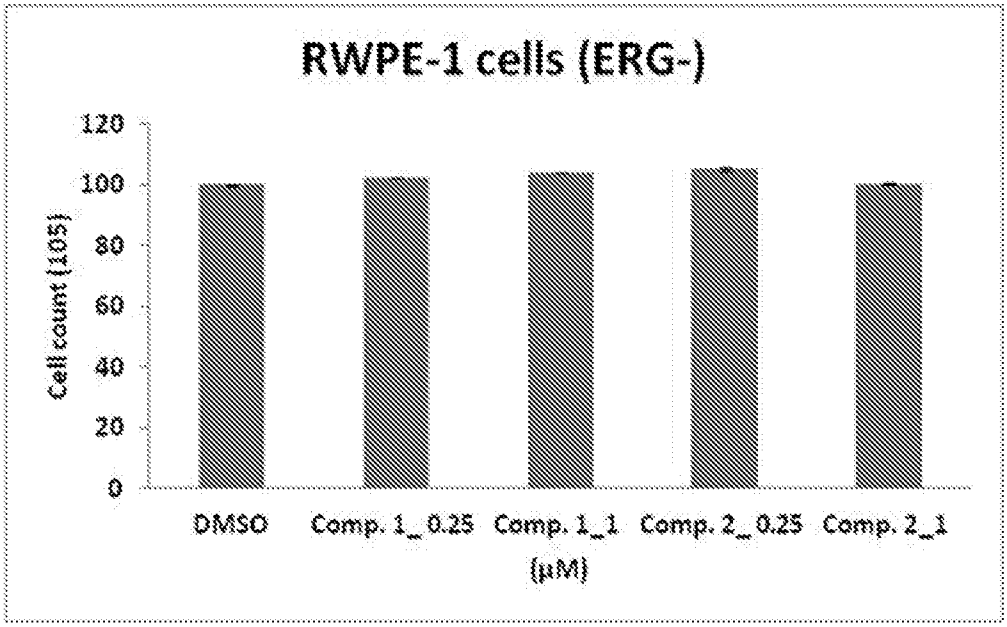
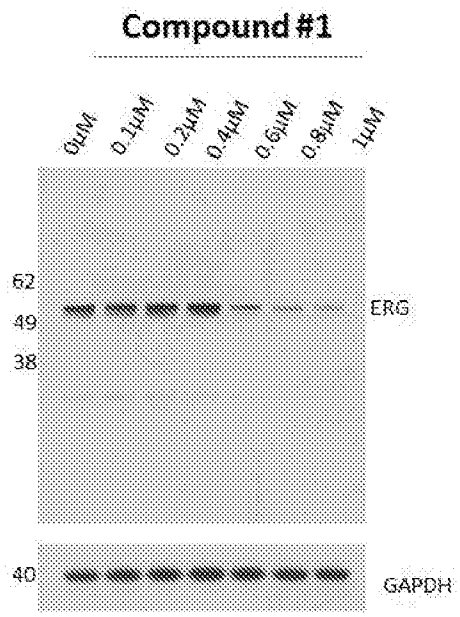
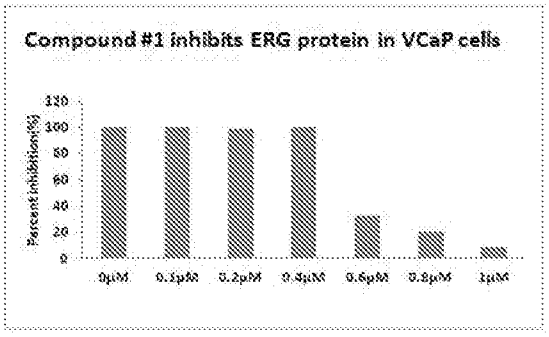

RWPE-1 cells (ERG-)
Cell count (105)
0
20
40
60
80
100
120
DMSO
Comp. 1_ 0.25
Comp. 1_1
Comp. 2_ 0.25
Comp. 2_1
(µM)

Figure 13


Compound #1
0uM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
62
49
38
ERG
40
GAPDH


Compound #1 inhibits ERG protein in VCaP cells
Percent inhibition(%)
120
100
80
60
40
20
0
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM

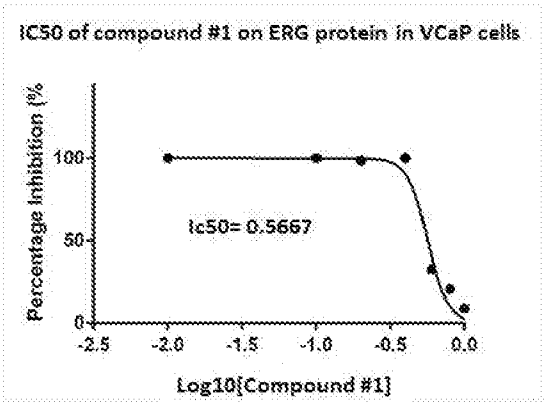
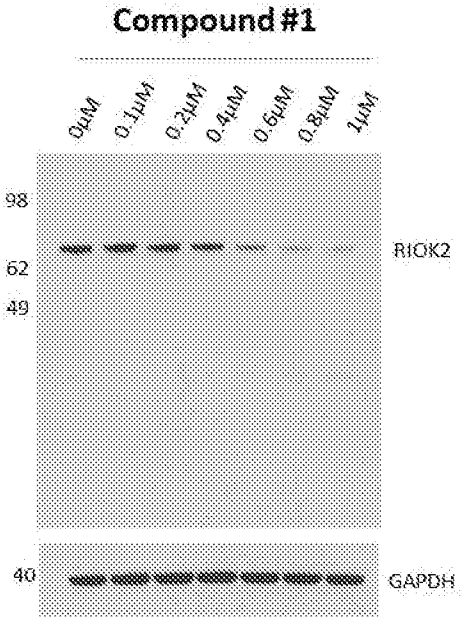
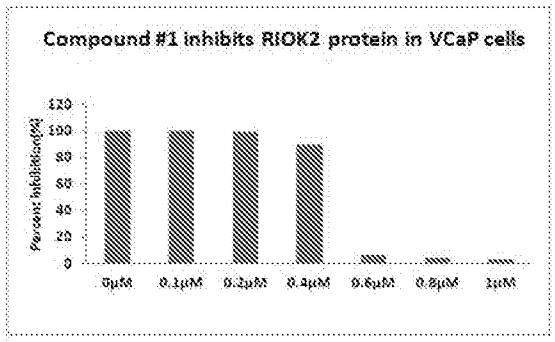

IC50 of compound #1 on ERG protein in VCaP cells
Percentage Inhibition (%
100
50
0
Ic50= 0.5667
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Log10[Compound #1]

Figure 14


Compound #1
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
98
62
49
40
RIOK2
GAPDH


Compound #1 inhibits RIOK2 protein in VCaP cells
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM

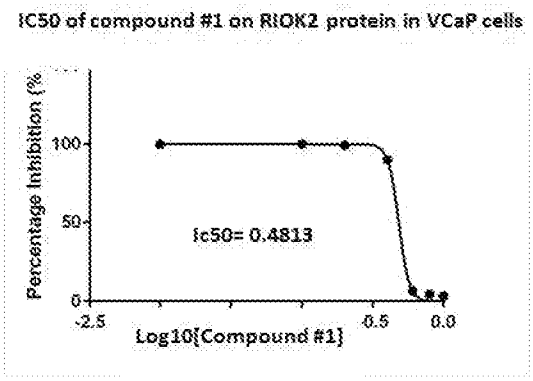

IC50 of compound #1 on RIOK2 protein in VCaP cells
Percentage Inhibition (%
100
50
0
-2.5
-0.5
0.0
Ic50= 0.4813
Log10[Compound #1]

Figure 15

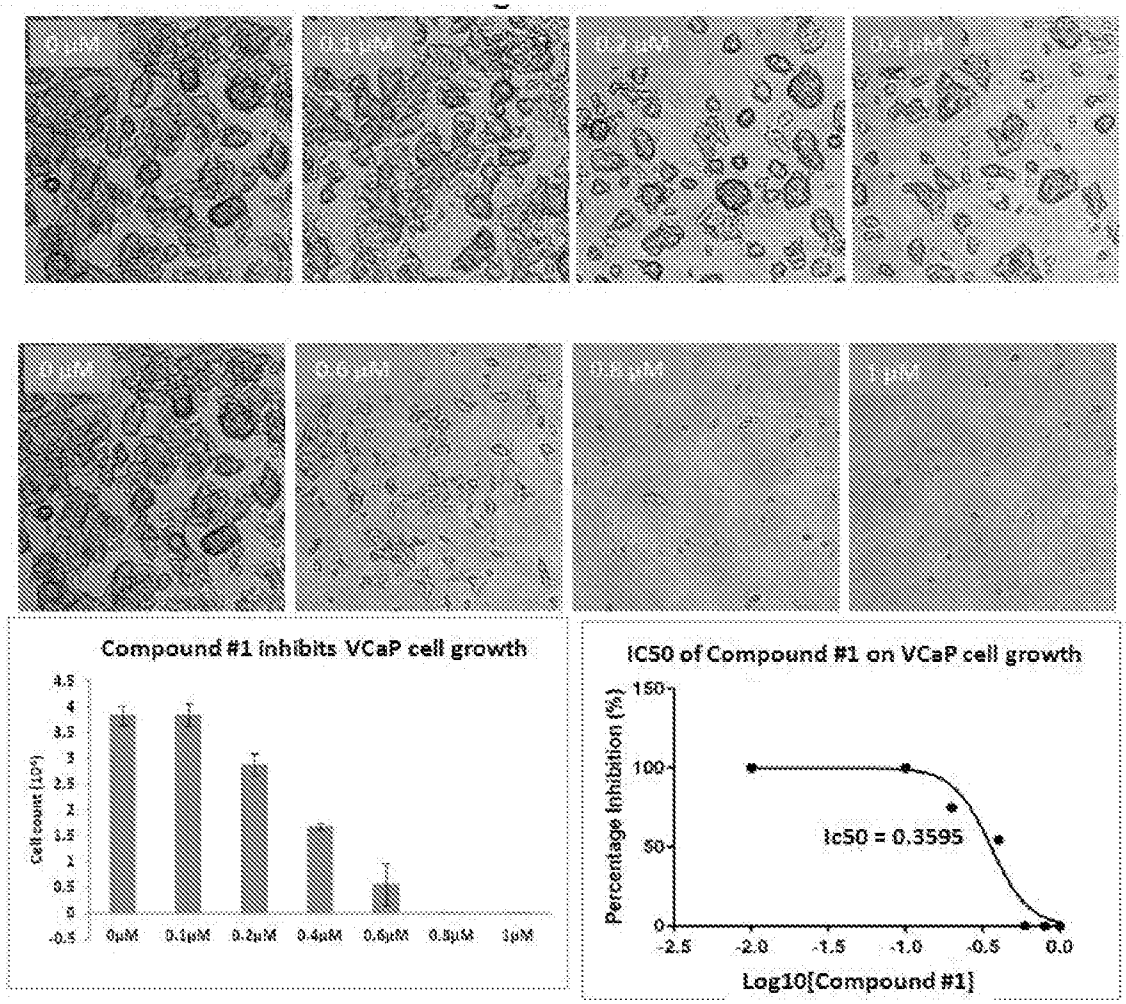

Compound #1 inhibits VCaP cell growth
Cell count (10^4)
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
IC50 of Compound #1 on VCaP cell growth
Percentage Inhibition (%)
IC50 = 0.3595
Log10[Compound #1]

Figure 16

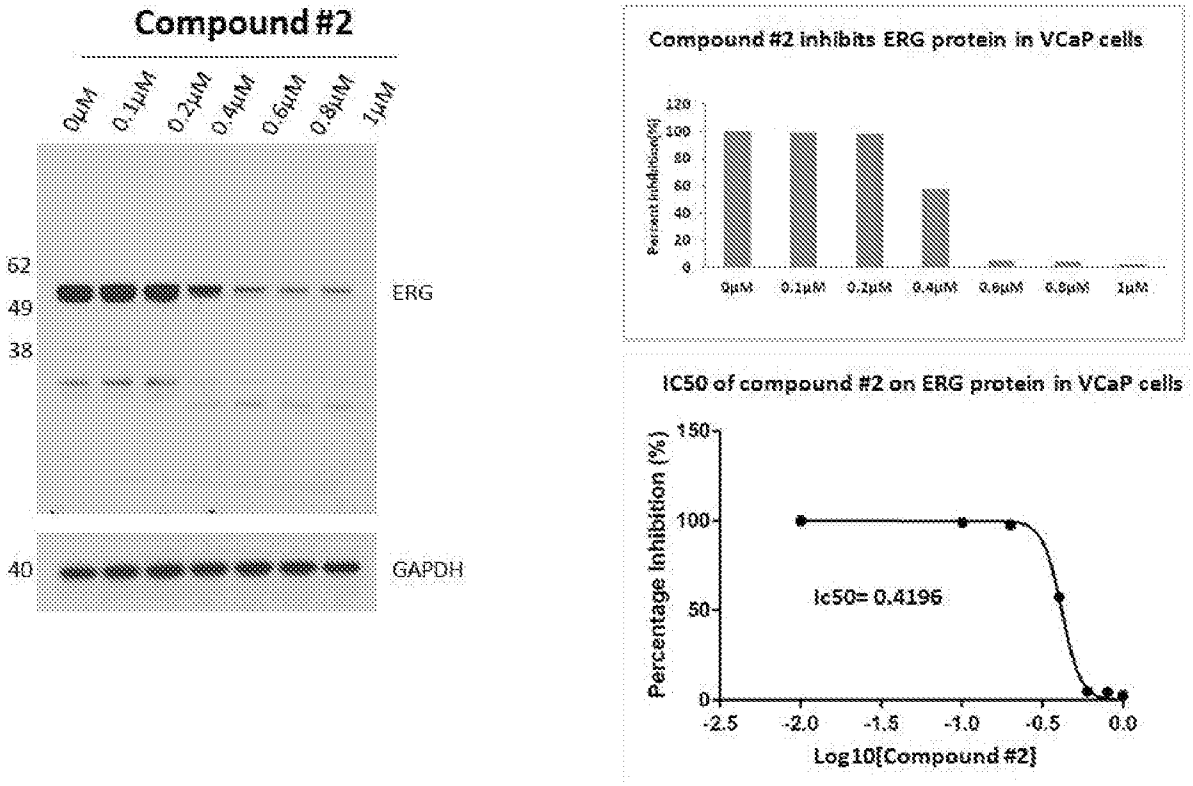

Compound #2
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
62
49
38
ERG
40
GAPDH
Compound #2 inhibits ERG protein in VCaP cells
Percent Inhibition(%)
120
100
80
60
40
20
0
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
IC50 of compound #2 on ERG protein in VCaP cells
Percentage Inhibition (%)
150
100
50
0
Ic50= 0.4196
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Log10[Compound #2]

Figure 17

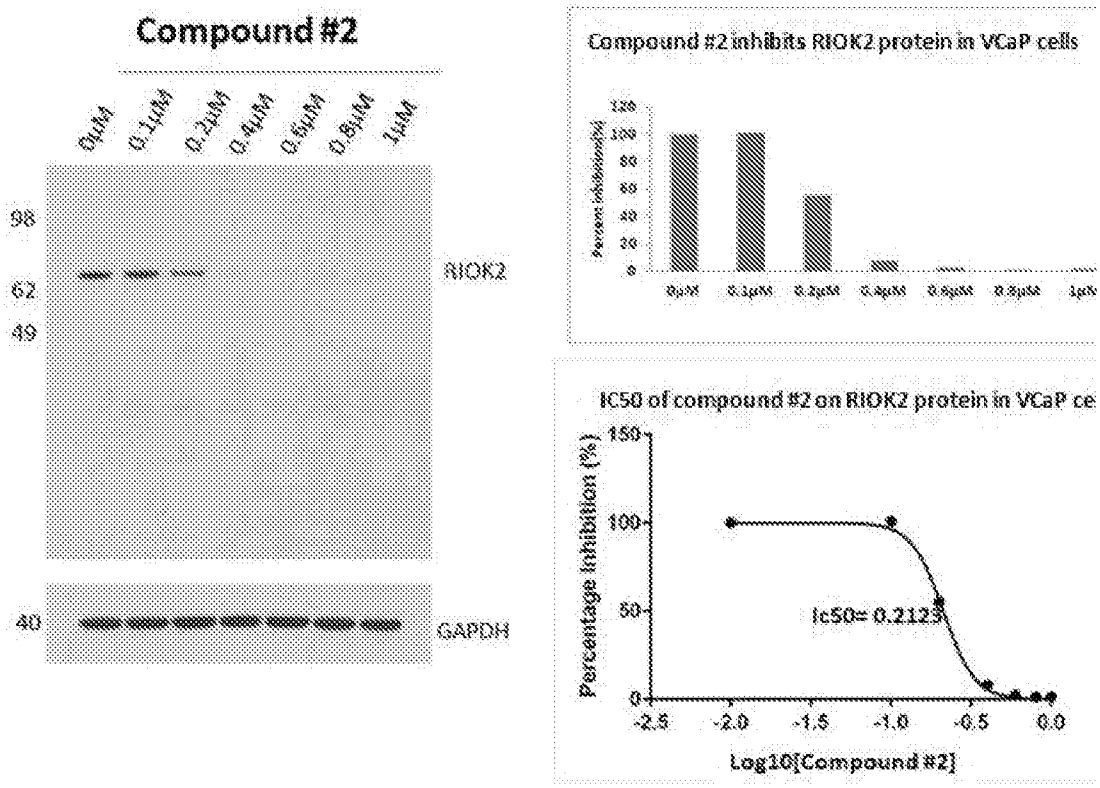
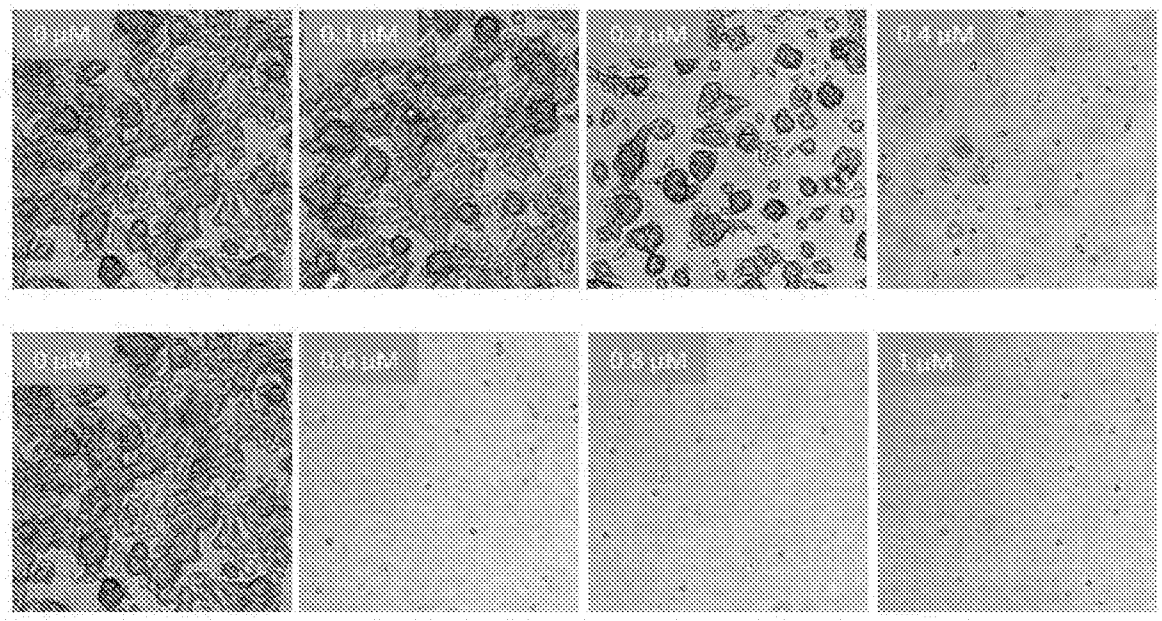
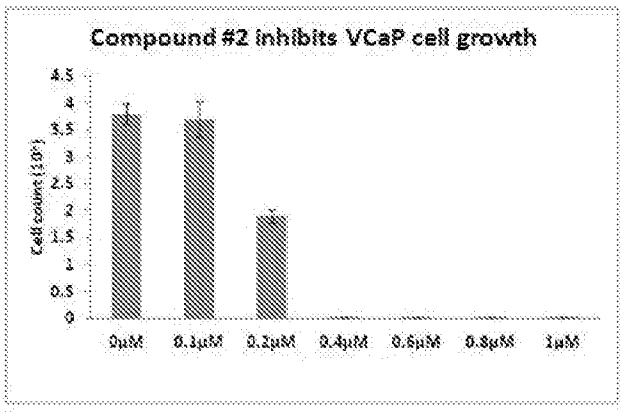

Compound #2
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM
98
62
49
RIOK2
40
GAPDH
Compound #2 inhibits RIOK2 protein in VCaP cells
IC50 of compound #2 on RIOK2 protein in VCaP ce
Percentage Inhibition (%)
150
100
50
0
Ic50= 0.2123
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Log10[Compound #2]

Figure 18




Compound #2 inhibits VCaP cell growth
Cell count (10⁵)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0μM
0.1μM
0.2μM
0.4μM
0.6μM
0.8μM
1μM

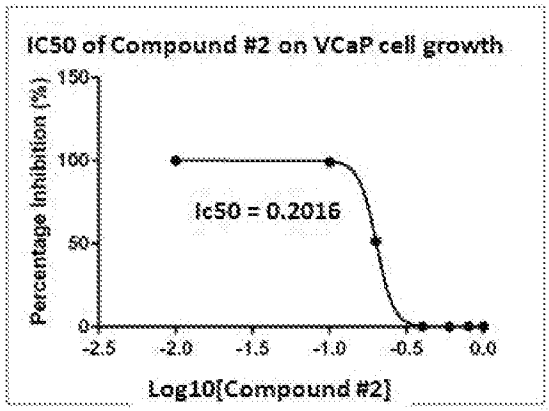
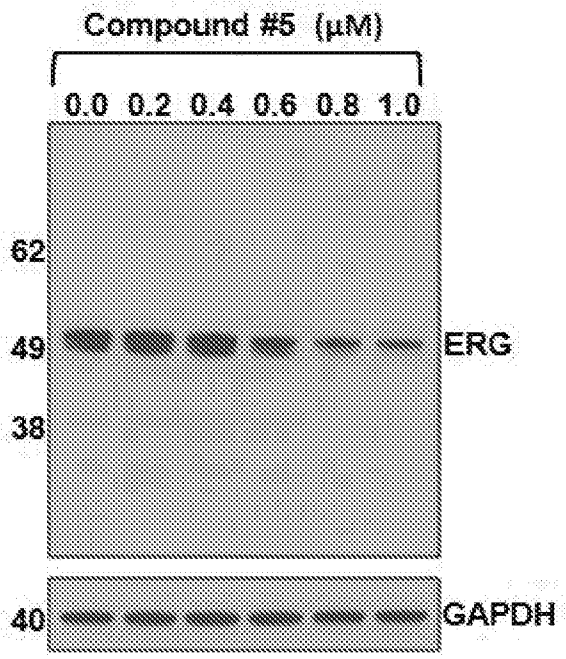

IC50 of Compound #2 on VCaP cell growth
Percentage Inhibition (%)
150
100
50
0
Ic50 = 0.2016
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Log10[Compound #2]

Figure 19


Compound #5 (μM)
0.0 0.2 0.4 0.6 0.8 1.0
62
49
38
ERG
40
GAPDH

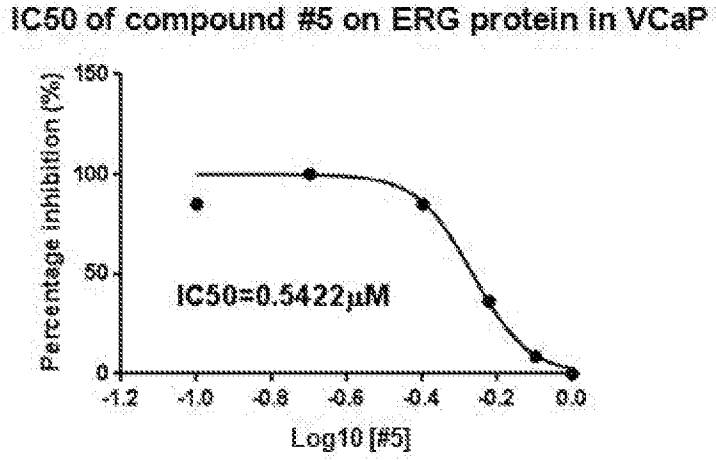
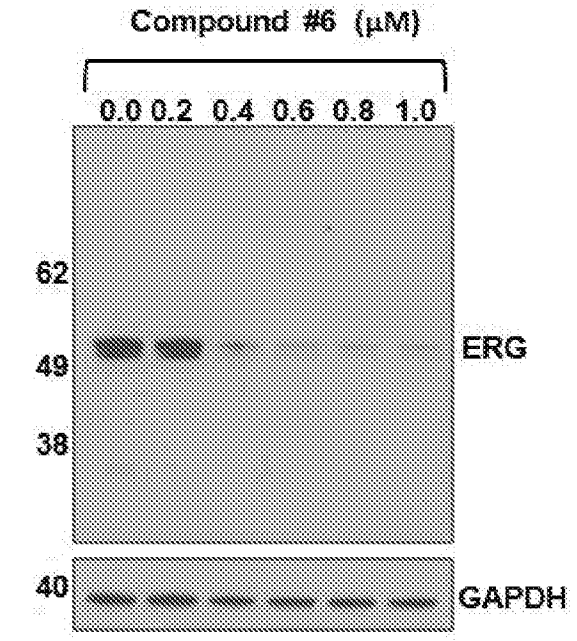

IC50 of compound #5 on ERG protein in VCaP
Percentage inhibition (%)
150
100
50
0
IC50=0.5422μM
-1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0
Log10 [#5]

Figure 20


Compound #6 (μM)
0.0 0.2 0.4 0.6 0.8 1.0
62
49
38
ERG
40
GAPDH

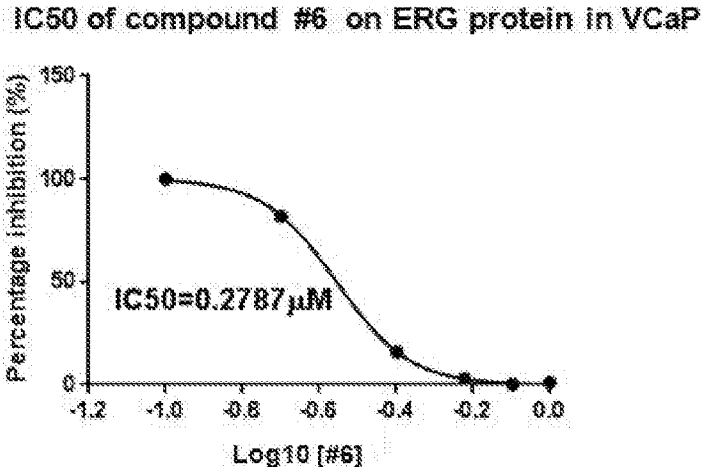
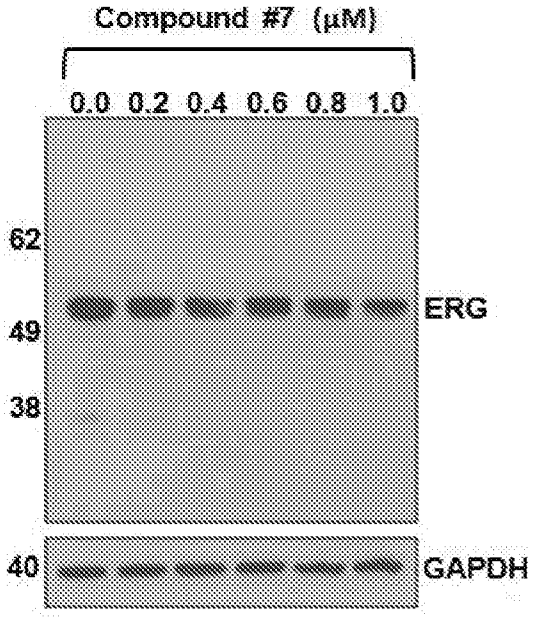

IC50 of compound #6 on ERG protein in VCaP
Percentage inhibition (%)
150
100
50
0
IC50=0.2787μM
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Log10 [#6]

Figure 21


Compound #7 (μM)
0.0 0.2 0.4 0.6 0.8 1.0
62
49
38
ERG
40
GAPDH

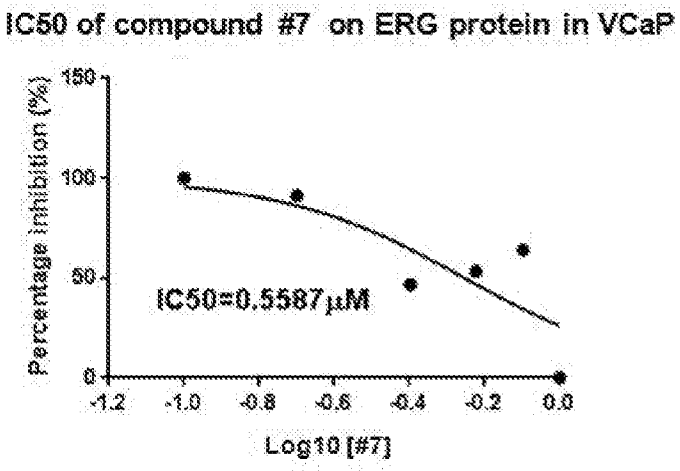
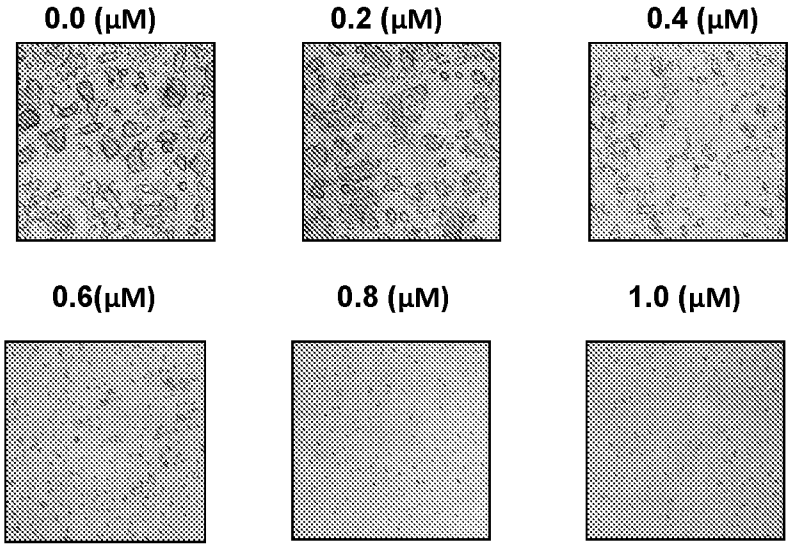

IC50 of compound #7 on ERG protein in VCaP
Percentage inhibition (%)
150
100
50
0
IC50=0.5587μM
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Log10 [#7]

Figure 22


0.0 (μM)
0.2 (μM)
0.4 (μM)
0.6(μM)
0.8 (μM)
1.0 (μM)

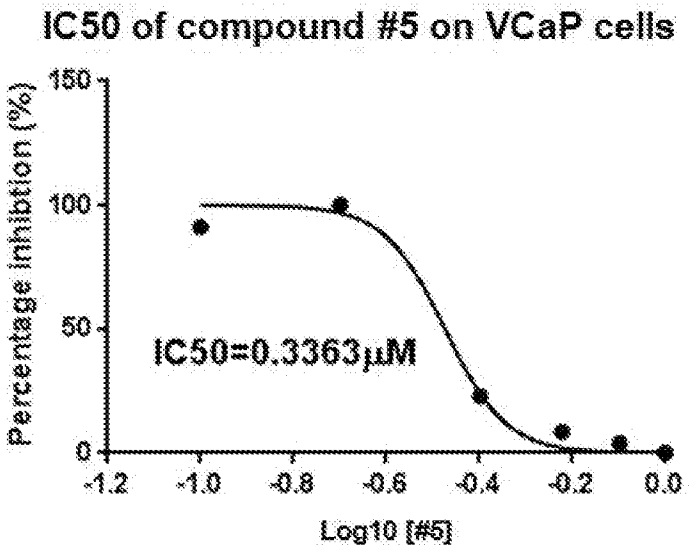
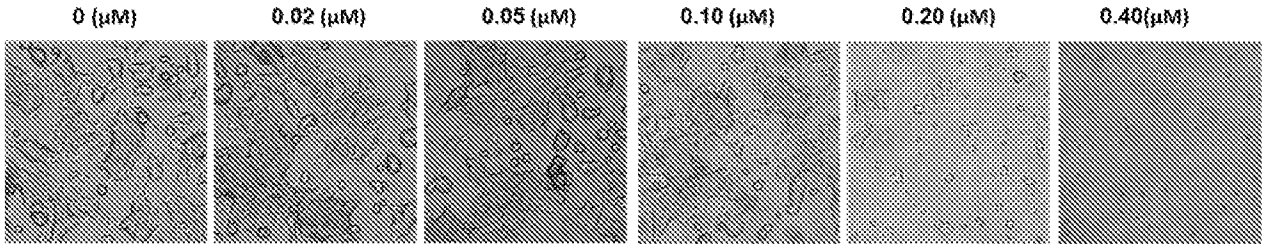

IC50 of compound #5 on VCaP cells
Percentage Inhibition (%)
150
100
50
0
IC50=0.3363μM
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Log10 [#5]

Figure 23


0 (μM)
0.02 (μM)
0.05 (μM)
0.10 (μM)
0.20 (μM)
0.40(μM)

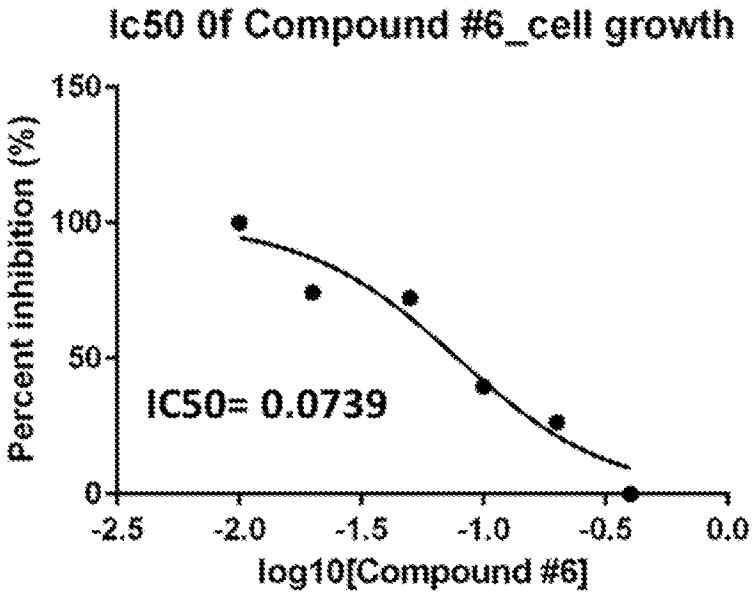
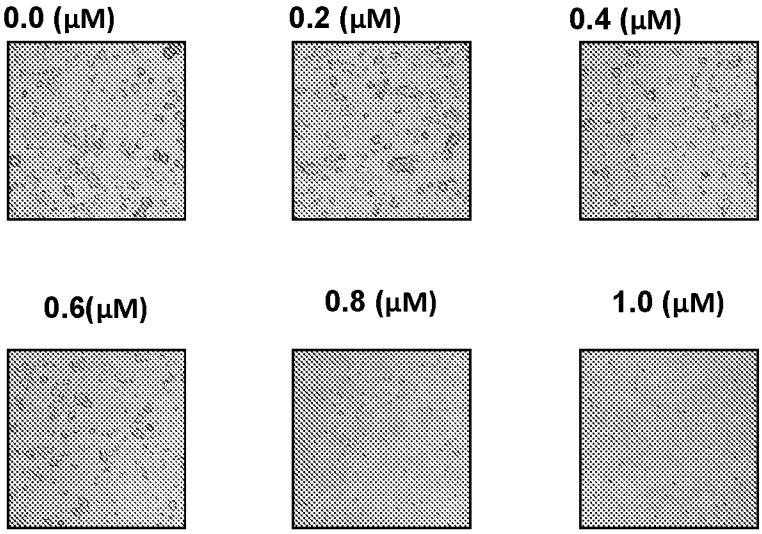

Ic50 0f Compound #6_cell growth
Percent inhibition (%)
150
100
50
0
IC50= 0.0739
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
log10[Compound #6]

Figure 24.


0.0 (μM)
0.2 (μM)
0.4 (μM)
0.6(μM)
0.8 (μM)
1.0 (μM)

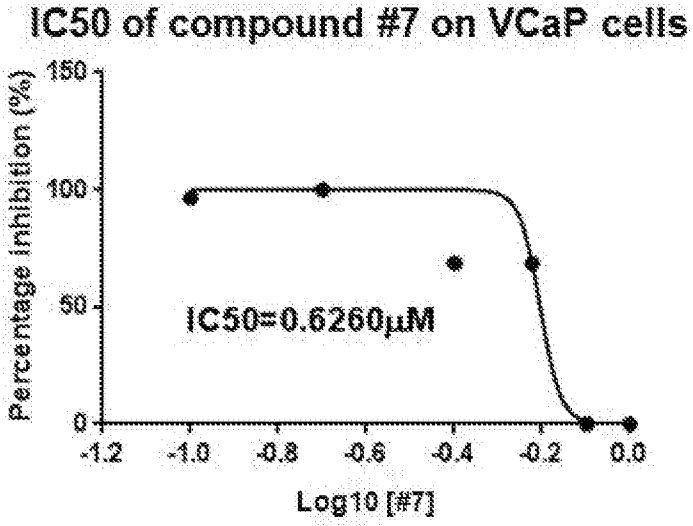
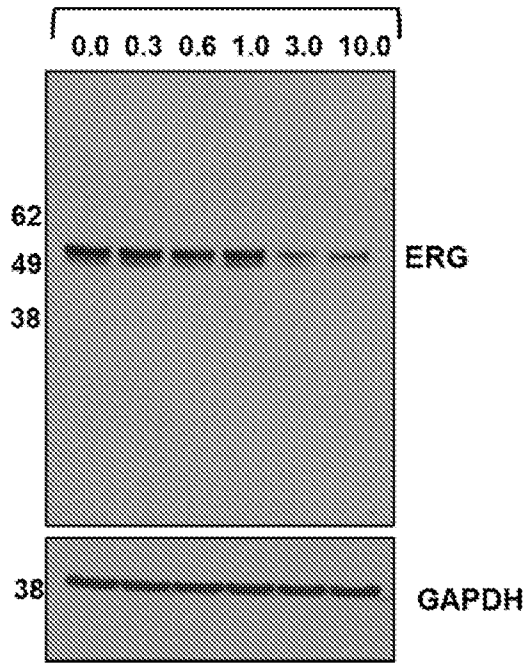

IC50 of compound #7 on VCaP cells
Percentage Inhibition (%)
150
100
50
0
IC50=0.6260μM
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Log10 [#7]

Figure 25


Compound #3 (μM)
0.0 0.3 0.6 1.0 3.0 10.0
62
49
38
ERG
38
GAPDH

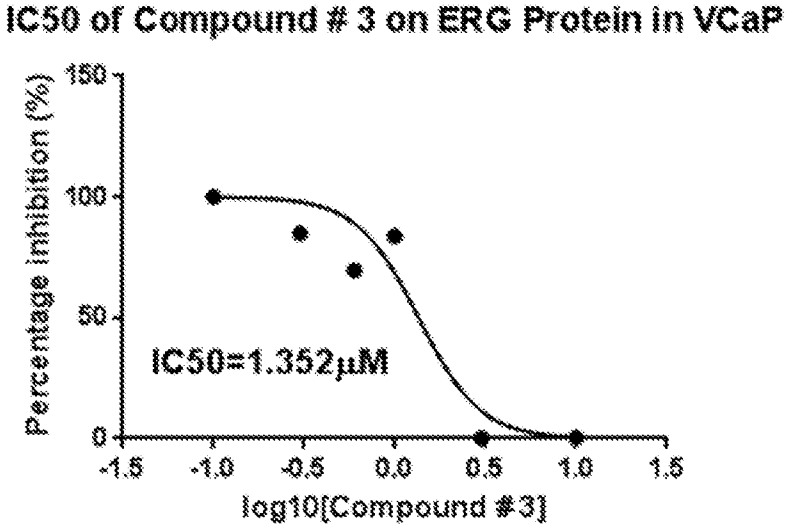
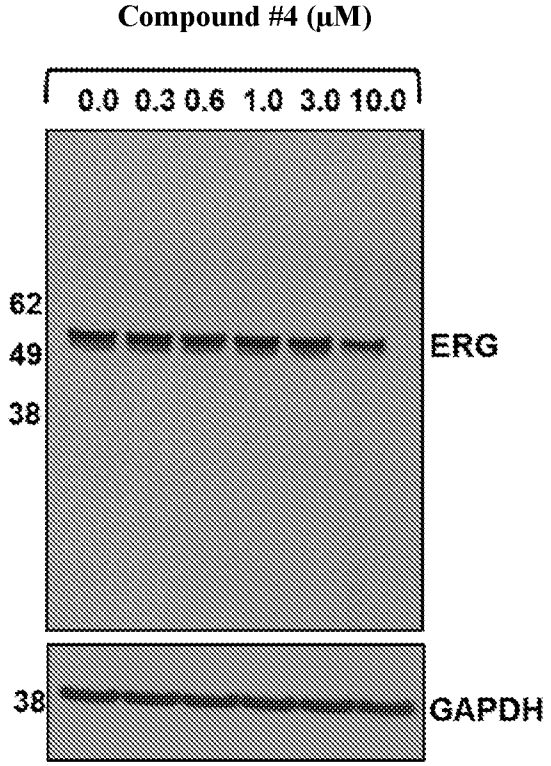

IC50 of Compound # 3 on ERG Protein in VCaP
Percentage inhibition (%)
150
100
50
0
IC50=1.352μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #3]

Figure 26


Compound #4 (μM)
0.0 0.3 0.6 1.0 3.0 10.0
62
49
38
ERG
38
GAPDH

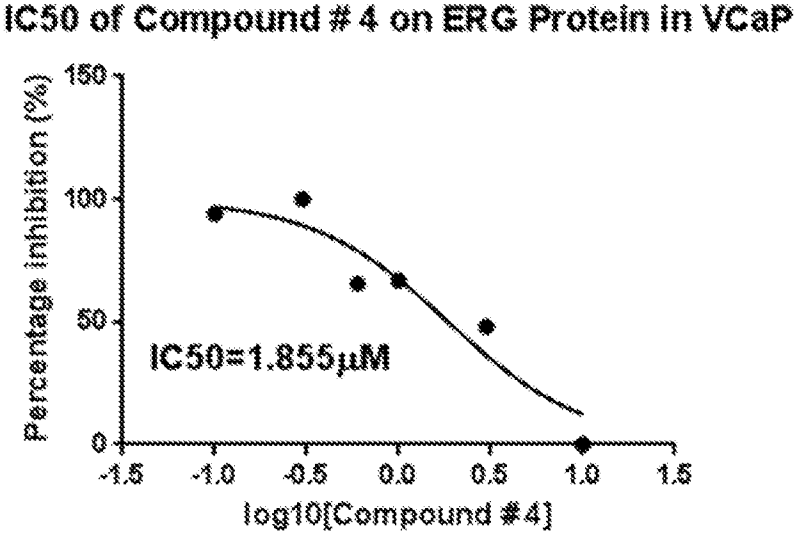
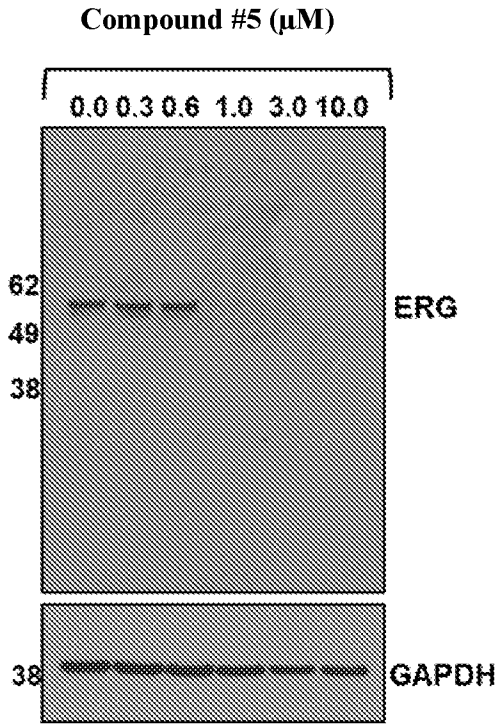

IC50 of Compound # 4 on ERG Protein in VCaP
Percentage inhibition (%)
150
100
50
0
IC50=1.855μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #4]

Figure 27


Compound #5 (μM)
0.0 0.3 0.6 1.0 3.0 10.0
62
49
38
ERG
38
GAPDH

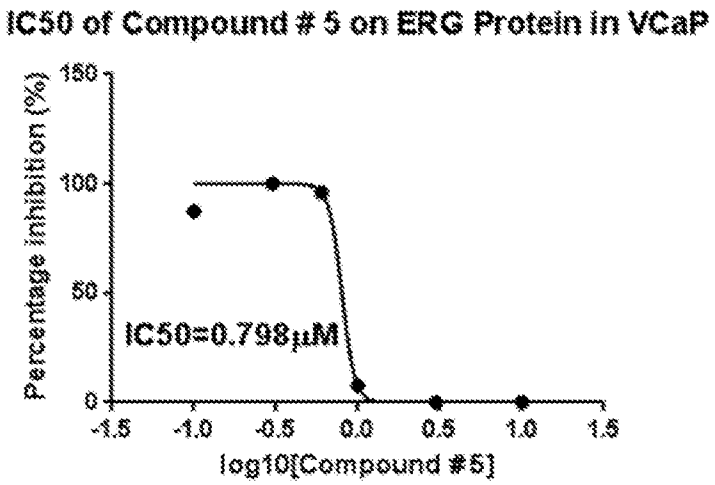
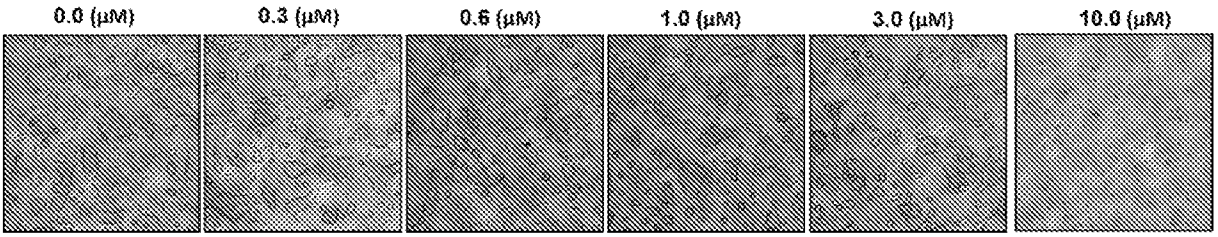

IC50 of Compound # 5 on ERG Protein in VCaP
Percentage Inhibition (%)
150
100
50
0
IC50=0.798μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #5]

Figure 28


0.0 (μM)
0.3 (μM)
0.6 (μM)
1.0 (μM)
3.0 (μM)
10.0 (μM)

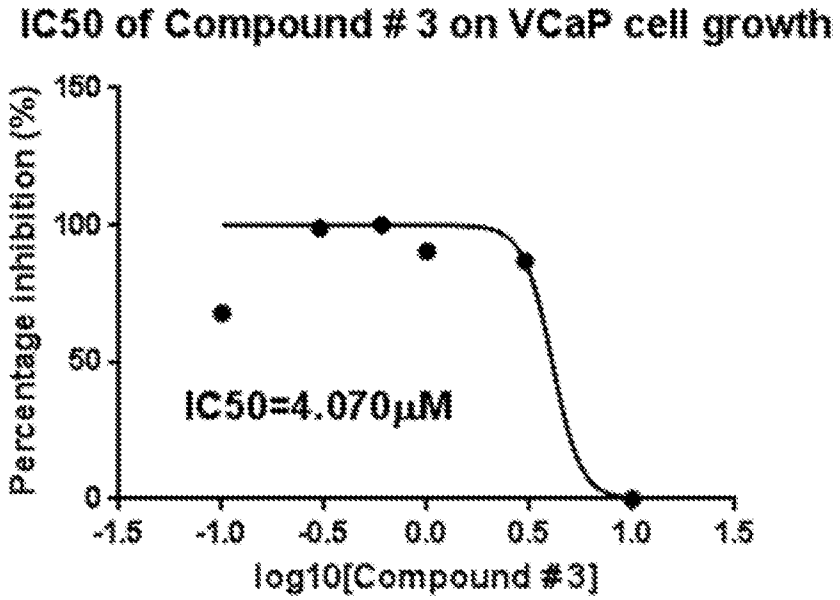
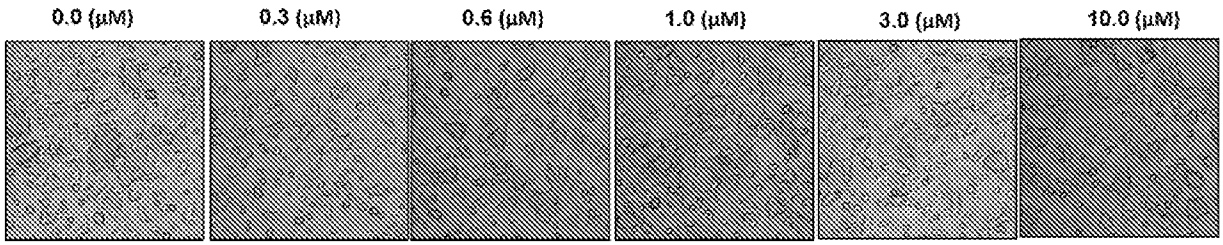

IC50 of Compound # 3 on VCaP cell growth
Percentage inhibition (%)
150
100
50
0
IC50=4.070μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #3]

Figure 29


0.0 (μM)
0.3 (μM)
0.6 (μM)
1.0 (μM)
3.0 (μM)
10.0 (μM)

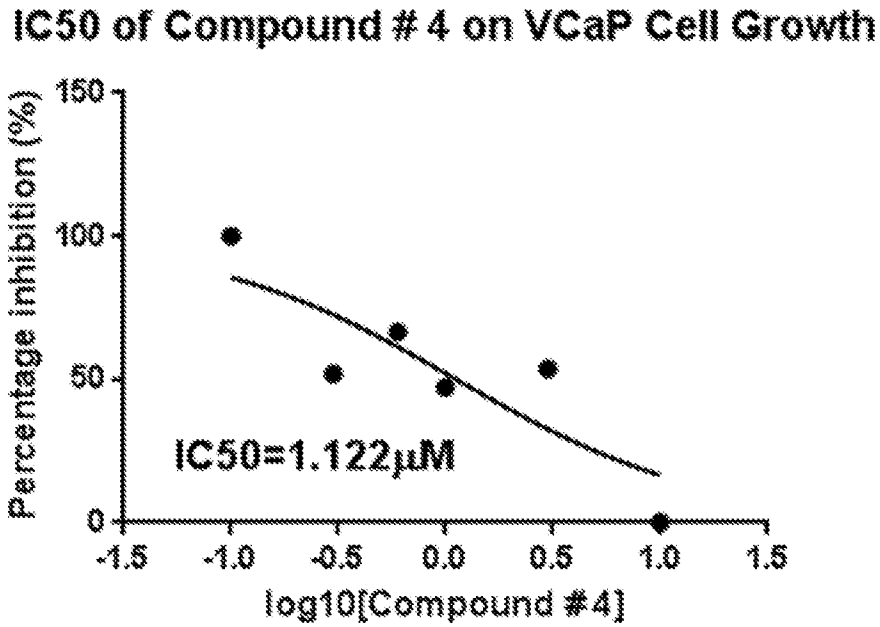
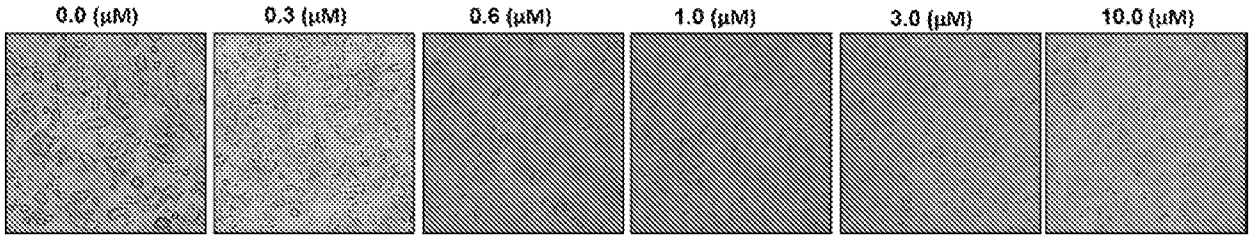
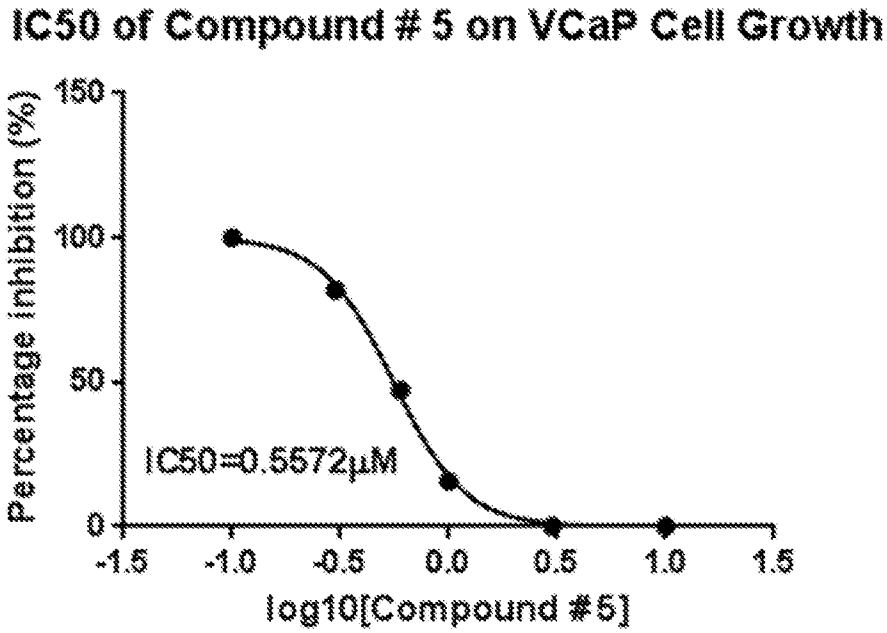

IC50 of Compound # 4 on VCaP Cell Growth
Percentage inhibition (%)
150
100
50
0
IC50=1.122μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #4]

Figure 30


0.0 (μM)
0.3 (μM)
0.6 (μM)
1.0 (μM)
3.0 (μM)
10.0 (μM)


IC50 of Compound # 5 on VCaP Cell Growth
Percentage inhibition (%)
150
100
50
0
IC50=0.5572μM
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
log10[Compound #5]

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/051098

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01)
CPC - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01)
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(8) - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01)
CPC - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/150; 514/183; 514/277; IPC(8) - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01); CPC - A61K 31/05; A61K 31/045; A61K 31/15; C07C 245/00; C07C 245/02; C07C 245/04 (2017.01) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, STN, PubChem, Google Patents, Google Scholar
Search terms used: Azophenol ERG cancer imidazole thiazole pyridine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/048718 A2 (INSTITUTE FOR CANCER RESEARCH D/B/A THE RESEARCH INSTITUTE OF FOX CHASE CANCER CENTER) 02 April 2015 (02.04.2015) entire document	1, 6-9, 12-26
A	PUBCHEM, Substance Record for SID 6506557 Create Date: 2005-09-14. [retrieved on 17 October 2016]. Retrieved from the Internet: <https://pubchem.ncbi.nlm.nih.gov/substance/6506557>. entire document	1, 6-9, 12-26
A	PUBCHEM, Substance Record for SID 162267588 Create Date: 2013-05-21. [retrieved on 11 January 2017]. Retrieved from the Internet: <https://pubchem.ncbi.nlm.nih.gov/substance/162267588>. entire document	1, 6-9, 12-26
A	PUBCHEM, Substance Record for SID 198947980 Create Date: 2014-08-25. [retrieved on 17 October 2016]. Retrieved from the Internet: <https://pubchem.ncbi.nlm.nih.gov/substance/198947980>. entire document	1, 6-9, 12-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" 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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" 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
"Y" 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

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2017	02 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/051098

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims 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. ☐ Claims 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 claims Nos.:

1, 6-9, 12-26

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 claims Nos.:

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.

Continued from Box No. III Observations where unity of invention is lacking

Claims 1, 14, 15, 19, and 23 have been analyzed subject to the restriction that the claims read on the Formula (I) as described in the Lack of Unity of Invention (See Box IV). The claims are restricted to a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein: X is NH; R1, R2, and R4 are independently H; R3 is H; each R9 is independently H.

In the first paid election, claims 1, 9, 13-15, 19, 21, 23, and 25 have been analyzed subject to the restriction that the claims read on the Formula (II) as described in the Response to the Invitation to Pay Additional Fees in the International Application dated 30 November 2016 as further restricted to the variable definitions listed below. The claims are restricted to a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (II) or a pharmaceutically acceptable salt thereof, wherein: X1 is N; X2, X3, X4 and X5 are CR9; R1, R2 and R4 are independently H; R3 is NR5R6; R5 an R6 are independently C1 alkyl; and each R9 is independently H; and pharmaceutical compositions thereof.

In the second paid election, claims 1, 6-8, 12, 14-20, 22-24, and 26 have been analyzed subject to the restriction that the claims read onthe Formula (II) as described in the Response to the Invitation to Pay Additional Fees in the International Application dated 30 November 2016 as further restricted to the variable definitions listed below. The claims are restricted to a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein: X1 is S; R1, R3 and R4 are independently H; R2 is C3 alkyl; and each R9 is independently H; and pharmaceutical compositions thereof.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-26 are drawn to methods for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, compounds of Formula (V) or pharmaceutically acceptable salts thereof, and pharmaceutical compositions thereof.

The first invention of Group I+ is restricted based on the proviso where only one of X1, X2, X3, X4, and X5 is N; and is restricted to a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein: X is NH; R1, R2, and R4 are independently H; R3 is H; each R9 is independently H; compounds of Formula (V) or pharmaceutically acceptable salts thereof; and pharmaceutical compositions thereof. It is believed that claims 1, 14, 15, 19, and 23 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (II) or a pharmaceutically acceptable salt thereof, wherein: X1, X2, X3, X4, and X5 are CR9; R1, R2, and R4 are independently C10 alkoxy; R3 is C10 alkyl; each R9 is independently -CN; compounds of Formula (V) or pharmaceutically acceptable salts thereof; and pharmaceutical compositions thereof. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables of X1, X2, X3, X4, X5, R1, R2, R3, R4, R9, and X.

The Groups I+ share the technical features of a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a compound of Formula (I) or the core structure Formula (II) or a pharmaceutically acceptable salt thereof; a compound having the core structure of Formula (V); a pharmaceutical composition comprising the compound of Formula (I) or Formula (II), a compound of Formula (III), a compound of Formula (IV), or a compound of Formula (V), and an excipient; and a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition. However, these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2015/048718 A2 to Institute for Cancer Research D/B/A The Research Institute of Fox Chase Cancer Center teaches a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom (Abstract, Cancer cells include...prostate...), comprising administering to the subject a therapeutically effective amount of a compound having the core structure of Formula (II) (Claims 1 and 6,...Evans Blue...; It is well known that Evans Blue has the core structure of Formula (II)); a pharmaceutical composition (Claim 101) comprising the compound having the core structure of Formula (II) (Claim 101,.Evans Blue...); a method for treating a disease associated with overexpression of wild type ERG protein, an altered ERG protein, ERG gene transcription or ERG mRNA translation in a subject suffering therefrom (Abstract, Cancer cells include...prostate...), comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition (Claims 1 and 6,...Evans Blue...; Pg. 10, Para. 4).

Additionally, Substance Record for SID 6506557 to PubChem teaches a compound having the core structure of Formula (V) (Pg. 3;... see shown structure...); a compound of Formula (I): wherein X is S; R1 and R2 are independently H, R4 is C1 alkyl; R3 is H; and each R9 is independently H (Pg. 3;...see shown structure...); and a compound of Formula (III), wherein R1 is H, and R2 is C1 alkyl (Pg. 3;... see shown structure...).

Additionally, Substance Record for SID 198947980 to PubChem teaches a compound of Formula (IV) or a pharmaceutically acceptable salt thereof, wherein R3 is hydrogen and R9 is hydrogen (Pg. 3;...see shown structure...).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.