(54) Title: ALDO-KETO REDUCTASE SUBFAMILY 1C3 (AKR1C3) INHIBITORS

(57) Abstract: The present invention relates to a novel class of AKR1C3 inhibitors, to compositions containing them, to methods for their preparation, and to methods of use thereof. The AKR1C3 inhibitors may be useful in the treatment of, for example, prostate cancer, benign prostate hyperplasia (BPH), lung cancer, acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, breast cancer, uterine cancer, uterine fibroids, endometriosis, myeloma and leiomyoma.

Figure 22A
ALDO-KETO REDUCTASE SUBFAMILY 1C3 (AKR1C3) INHIBITORS

FIELD OF THE INVENTION

[001] The present invention relates to a novel class of aldo-keto reductase inhibitors, aldo-keto reductase subfamily 1C3 (AKR1C3) inhibitors and to compositions containing AKR1C3 inhibitors, to methods for preparation of AKR1C3 inhibitors, and to methods of use thereof. The AKR1C3 inhibitors are useful in the treatment of, for example, prostate cancer, benign prostate hyperplasia (BPH), lung cancer, non-small cell lung cancer (NSCLC), acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, breast cancer, endometriosis, myeloma and leiomyoma. AKR1C3 inhibitors may also provide a function as inhibitors of AKR1C3-mediated androgen-dependent androgen receptor-transactivation, i.e., inhibitors of an AKR1C3 co-activation function, as described herein.

BACKGROUND OF THE INVENTION

[002] Androgens and estrogens, through their intracellular receptors, promote physiological and pathological developments. Both these classes of steroid hormones are critical for the growth of secondary sexual characteristics, bone, muscle, and for the function of neurological and other tissues. These hormones, their receptors and associated proteins have also been implicated in the transformation of normal reproductive tissues into cancerous tissues. A large proportion of androgens in men (40%), and the majority of estrogens in women (75% before menopause and close to 100% after menopause), are synthesized in peripheral target tissues from precursor steroids of adrenal origin. The genes encoding the enzymes responsible for the formation and metabolism of androgens and estrogens are expressed in a large series of peripheral tissues, thus providing the basis for a promising new area in hormone action, namely intracrinology. These steroidogenic and steroid metabolizing enzymes should become a major target of novel therapies for steroid-sensitive diseases, particularly breast and prostate cancer.

[003] Testosterone and dihydrotestosterone (DHT), the active circulating androgens, and estradiol, the potent circulating estrogen, are synthesized from the weak adrenally synthesized precursors androstenedione and estrone, respectively, by steroidogenic enzymes belonging to the class of hydroxysteroid dehydrogenases (HSDs). Enzymes belonging to the class of 17β-hydroxysteroid dehydrogenases (17P-HSDs) and aldo-keto reductases (AKR) play important roles in the synthesis of
potent androgens and estrogens. Potent endogenous androgens (testosterone and DHT) are synthesized by type 3 (testicular) and type 5 (peripheral tissues such as adrenal, prostate, etc.) 17P-HSDs.

[004] The human AKRIC isozymes are hydroxysteroid dehydrogenases, and are involved in the pre-receptor regulation of steroid hormone action. AKRIC isozymes regulate the concentration of active and inactive androgens, estrogens and progestins in target tissues by catalyzing the reduction of ketosteroids at positions C3, C17 or C20. AKRIC isozymes regulate the ligand occupancy and transactivation of steroid hormone receptors such as the androgen receptor, estrogen receptor, and progesterone receptor by altering the intracellular steroid hormone formation rates and thus the steroid hormone concentration in target tissues.

[005] Hormone dependent cancers, such as breast and prostate cancer, are dependent on the local milieu of estrogens or androgens, respectively. The AKRIC enzymes support the synthesis of these hormones locally. Microarray analysis of prostate cancer tissues has shown that the AKR1C3 isoform is over-expressed in advanced or metastatic prostate cancers compared to organ-confined prostate cancer. In addition, it has also been shown that androgen biosynthesis continues in peripheral tissues (i.e., not in testes) and supports the post-castration proliferation of prostate cancer (e.g. castration resistant prostate cancer (CRPC)) and that the AKR1C3 is the key enzyme that mediates this peripheral androgen synthesis.

[006] There are four isozymes in the aldo-keto reductase family 1 member C (AKRIC) family, which share more than 85% amino acid sequence identity: AKR1C1 (20a-HSD), AKR1C2 (type 3 3a-HSD), AKR1C3 (type 5 17β-HSD), and AKR1C4 (type 1 3a-HSD). Despite their high sequence identity, the four isozymes display different substrate preferences, inhibition profiles and tissue specific expression patterns. In addition to prostate cancer, alterations in the expression and function of AKRIC isoforms have been reported in other cancers, such as breast cancer, small cell lung cancer and myeloma, suggesting a role for these isozymes in the development of other cancers. Drugs that increase the function of AKR1C1 and AKR1C2 or drugs that decrease the function of AKR1C3 may be useful in treating various malignancies related to steroidal sex hormones.

[007] AKR1C3 is a 37 kDa cytosolic enzyme of the NAD dependent aldo-keto reductase family. AKR1C3 is also known as 17P-hydroxysteroid dehydrogenase type 5 (17βHSD5), 3otHSD2, and prostaglandin F synthase. Isoforms AKRIC 1-4 have overlapping function depending on substrate concentration and tissue distribution. AKRIC enzymes catalyze multiple enzymatic reactions to
include: conversion of the potent progestin progesterone to the weak progestin 20α-hydroxyprogesterone (AKR1C1, AKR1C3); conversion of the most potent endogenous androgen 5α-dihydrotestosterone (DHT) to the weak androgen 5α-androstane-3α,17β-diol (3α-diol) (AKR1C2); conversion of the weak adrenal androgen androstenedione (also known as androstene-3,17-dione, 4'dione, and A'dione) to the potent androgen testosterone (AKR1C3; also done by 17βHSD3 in Leydig cells); conversion of the weak androgen dehydroepiandrosterone (DHEA) to androstenediol (AKR1C3), a precursor to testosterone; conversion of the weak androgen 5α-androstanedione (5α-dione) to the more active androgen DHT (AKR1C3) (Figure 1); conversion of the weak estrogen estrone to 17β-estradiol (AKR1C3); and conversion of differentiative prostaglandin PGD2 to the proliferative prostaglandin PGF2α (AKR1C3). Therefore, inhibition of AKR1C3 activity may reduce the level of end products as described above. For example, inhibition of AKR1C3 activity may reduce the level of the potent androgens testosterone and 5α-dihydrotestosterone (DHT), and/or the potent estrogen 17β-estradiol and/or the proliferative prostaglandin PGF2α. Additionally, inhibition of AKR1C3 activity may increase the level of starting product as described above. For example, inhibition of AKR1C3 activity may increase the level of the potent progestin progesterone.

[008] AKR1C3 is expressed in numerous tissues including liver, prostate, testes, adrenals, uterus, breast, lung, kidney, bladder, ovary, adipose, and brain. AKR1C3 is considered an activating enzyme for the androgen receptor (AR) through biosynthesis of testosterone; and activating the estrogen receptor (ER) through biosynthesis of 17β-estradiol; and a deactivating enzyme of the peroxisome proliferator-activated receptor gamma (PPARγ) through the synthesis of PGF2α.

[009] AKR1C3 inhibitors may be advantageous in the treatment of prostate cancer. Numerous studies suggest that treatment of prostate cancer patients with gonadotropin releasing hormone antagonist-antiandrogen combinations often fails due to increased intratumoral androgen biosynthesis. CYP17A1 (17,20-lyase; 17α-hydroxylase) inhibitors such as ketoconazole and abiraterone inhibit conversion of pregnenolone and progesterone to DHEA and androstenedione, respectively, and exhibit moderate efficacy in CRPC. Unlike CYP17A1, AKR1C3 is upregulated in CRPC and represents a more specific target for preventing localized androgen biosynthesis (i.e. lyase inhibitors also prevent Cortisol synthesis leading to mineralocorticoid excess, especially abiraterone). In addition, other enzymatic roles of AKR1C3 promote prostate tumor growth. These include the effects on prostaglandin metabolism which increase prostate growth and tumor vascularity by decreasing PGJ2 resulting in reduction in PPARγ activity and up-regulation of COX-2. Conversion of DHT to 3α-diol is thought to
increase prostate cell proliferation through an AR-independent pathway involving epidermal growth factor-like pathway. Hence, inhibition of AKR1C3 may have direct anti-proliferative effects via decreases in estrogen and androgen synthesis intratumorally (as well as in adjacent tissues), which would limit the occupation of AR and ER. Further, indirect anti-proliferative effects of AKR1C3 inhibition may arise from increased levels of pro-differentiative ligands, e.g., PPARγ ligands and decreased levels of 3α-diol (EGF-like pathway).

[0010] AKR1C3 inhibitors may be advantageous for the treatment of breast cancer. Selective estrogen receptor modulators (SERMs) and aromatase inhibitors are widely used in treatment of ER positive breast cancer, which includes about 75% of the cases. Studies have shown that AKR1C3 is consistently over expressed in breast cancer ductal carcinoma in situ and invasive breast cancer ductal carcinoma, as well as being an indicator of poor prognosis for breast cancer. Multiple mechanisms of AKR1C3 promotion of breast tumor growth exist such as: a) conversion of androstenedione to testosterone provides a substrate for CYP19 aromatase to create 17P-estradiol; b) conversion of estrone (weak estrogen) to 17P-estradiol; c) reduction of the anti-proliferative effect of PGD2; and d) decrease in progesterone by inactivation to 20ot-progesterone which further increases ER:PR ratio. Thus inhibition of AKR1C3 should be therapeutic in breast cancer as inhibition of mechanisms a) and b) above should decrease intratumoral ER occupancy, and inhibition of mechanism c) should increase intratumoral occupancy of PPARγ. Further support for use of AKR1C3 inhibitors comes from a report of anti-proliferative effects in stably transfected MCF7 cells (Chemico-Biological Interactions 178 (2009), 221-227). AKR1C3 inhibitors may be advantageous for the treatment of AR-positive and ER-positive breast cancers. CRPC and refractory breast cancer are common cancers with low survival rates (less than 50% at 5 yrs). Currently there are no AKR1C3 inhibitors approved or in clinical trials.

[0011] AKR1C3 inhibitors may be useful for androgen-dependent conditions. Non limiting examples of such uses include: (a) treatment of adrenal adenomas, carcinoma, or hyperplasia; (b) treatment of Leydig cell tumors in men; (c) treatment of arhenoblastomas in women; (d) treatment of polycystic ovarian syndrome (PCOS) in women; e) treatment, prevention, decreasing the incidence of, halting and/or causing a regression of prostate cancer; f) other clinical, therapeutic, and/or diagnostic areas; or g) treatment and/or prevention of acne, seborrhea, hirsutism, baldness and alopecia.

[0012] AKR1C3 inhibitors may be useful for estrogen-dependent conditions. Non limiting examples of such uses include (a) treating, suppressing, inhibiting or reducing the amount of
precancerous precursors of prostate adenocarcinoma, for example, those having benign prostatic hyperplasia, prostatic intraepithelial neoplasia (PIN) or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of prostate cancer; (b) treating, preventing, suppressing, inhibiting, or reducing the incidence of osteoporosis, hot flashes, gynecomastia, and/or hair loss in male human subjects having prostate cancer; (c) treating, suppressing, inhibiting or reducing the risk of developing prostate cancer; or (d) treating, suppressing, inhibiting or reducing the risk of developing breast cancer in a subject.

[0013] AKR1C3 inhibitors may be useful for prostaglandin-dependent diseases. AKR1C3 is the only known PGF2 synthase in humans. Diseases related to increased PGF2 levels (or increased PGF2 synthase (AKR1C3) activity) include: endometriosis, inflammatory tachycardia, lung cancer, asthma and airway inflammation, type 2 diabetes, obesity, multiple inflammatory diseases, diseases related to oxidative stress, dysmenorrhea, and renal cell carcinoma. These diseases could result from direct increases in PGF2 levels or from decreases in PPAR-γ activity (as AKR1C3 sequesters the precursor of endogenous PPAR-γ ligand, which is PGJ2, to form PGF2). AKR1C3 also catalyzes the reduction of prostaglandin (PG) H(2) to PGF(2a) and PGD(2) to 9α-lip-PGF(2), which will limit the formation of anti-proliferative prostaglandins, including 15-deoxy-A(12,14)-PGJ(2), and contribute to proliferative signaling. AKR1C3 is overexpressed in a wide variety of cancers, including breast and prostate cancer. There is considerable interest in the development of an inhibitor of aldo-keto reductase (AKR) 1C3 (type 5 17P-hydroxysteroid dehydrogenase and prostaglandin F synthase) as a potential therapeutic for both hormone-dependent and hormone-independent cancers.

[0014] Consistent with the potential role for AKR1C3 in the initiation of parturition, indomethacin, which is a potent and isoform selective inhibitor of AKR1C3, has long been used for tocolysis (i.e., also called anti-contraction medications or labour repressants) or medications used to suppress premature labor (from the Greek tokos, childbirth, and lytic, capable of dissolving).

[0015] Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and flufenamic acid are known commercial inhibitors of AKR1C3. However, NSAIDs have significant cross-reactivity with cyclooxygenase enzymes (COX-1 and COX-2), which leads to side effects such as gastric irritation, ulcers, cardiovascular problems and others. Hence, there is a need in the art to develop new HSD inhibitors, for example, HSD inhibitors that are specific to AKR1C3 and that lack cross-reactivity with, for example, other AKR1C isoforms and HSD isoforms, and with other enzymes, such as COX
enzymes. The AKR1C3 inhibitors of this invention do not cross-react or have significantly reduced cross-reactivity with respect to CYP17A1, COX-1, COX-2, other AKR1C enzymes, 17βHSD3, and are not agonist or antagonists for steroid hormone receptors such as AR, ER, and PR. Yet, AKR1C3 inhibitors may inhibit the down-stream activities of steroid receptors, as AKR1C3 inhibitors may regulate the formation of ligands for these receptors. For example, an AKR1C3 inhibitor may decrease or inhibit the activity of the AR receptor by decreasing the amount of available testosterone and DHT. Additionally, an AKR1C3 inhibitor may decrease or inhibit the activity of an ER receptor (ER-alpha or ER-beta) by decreasing the amount of available estradiol. Further, an AKR1C3 inhibitor may increase or augment the activity of the PR receptor by decreasing the amount of available progesterone.

SUMMARY OF THE PRESENT INVENTION

[0016] In one aspect, this invention provides a compound of Formula I:

\[
\begin{align*}
\text{Formula I} \\
\end{align*}
\]

wherein,

\begin{align*}
A & = \text{O, N or C}; \\
B & = \text{N or C}; \\
R^1 & = \text{nothing, H, alkyl or -alkylene-CC>2R^3, in which } R^3 \text{ is H or alkyl; wherein, when } R^1 \text{ is nothing an oxo (C=O) group is formed;} \\
R^2 & = \text{H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkylene-CC>2R^3, in which } R^3 \text{ is H or alkyl;}
\end{align*}
R³ is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF₂OMe, CN, carboxyl, S0₂R² or S0₂NHR² in which R² is, in each case, independently, H or alkyl;

R⁴ is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, S0₂R² or S0₂NHR² in which R² is, in each case, independently, H or alkyl;

R⁵ is nothing, H, alkyl or -alkylene-C0₂R³, in which R³ is H or alkyl; wherein when R⁵ is nothing an oxo (C=O) group is formed;

R⁶ is nothing, H, alkyl or -alkylene-C0₂R³, in which R³ is H or alkyl; wherein when A is O, R⁶ is nothing;

n = 1, 2, 3, 4 or 5;

or its isomer, tautomer, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate, prodrug, metabolite or any combination thereof.

In some embodiments of Formula I, if A = C, R⁶ = H, B = N, and R⁵ is nothing, then R² is not H or substituted or unsubstituted cycloalkyl, or R¹ is not H or alkyl, or R³ is not OH, halogen, haloalkyl, or carboxy, or R⁴ is not H, alkyl, OH, halogen, haloalkyl, CN or carboxyl.

In one embodiment, this invention is directed to a compound of Formula II:

![Formula II](image_url)
wherein

\[ R^1 \text{ is } H, \text{ alkyl or -alkylene-C}_0^2 R^3, \text{ in which } R^3 \text{ is } H \text{ or alkyl}; \]

\[ R^2 \text{ is } H, \text{ substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkylene-C}_0^2 R^3, \text{ in which } R^3 \text{ is } H \text{ or alkyl}; \]

\[ R^3 \text{ is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF}_2\text{OMe}, \text{ CN, carboxyl, substituted or unsubstituted phenyl, } S0\text{ }R^2 \text{ or } S0\text{ }S0\text{ }NHR^2 \text{ in which } R^2 \text{ is, in each case, independently, } H \text{ or alkyl; } \]

\[ R^4 \text{ is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, } S0\text{ }R^2 \text{ or } S0\text{ }S0\text{ }NHR^2 \text{ in which } R^2 \text{ is, in each case, independently, } H \text{ or alkyl; } \]

\[ m = 1 \text{ or } 2; \]

and

\[ n = 1, 2, 3, 4 \text{ or } 5; \]

or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate, or any combination thereof.

[0019] In some embodiments of Formula II, if \( R^2 = H \) or substituted or unsubstituted cycloalkyl, then \( R^1 \) is not \( H \) or alkyl, or \( R^3 \) is not OH, halogen, haloalkyl, or carboxy, or \( R^4 \) is not \( H \), alkyl, OH, halogen, haloalkyl, CN or carboxyl.

[0020] In another aspect, the present invention relates to a pharmaceutical composition containing a compound of this invention, or its isomer, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate, prodrug, metabolite or any combination thereof, and a pharmaceutically acceptable carrier.

[0021] In a further aspect, the present invention relates to a method of treating, suppressing, inhibiting or reducing the incidence of, or delaying progression of a disorder or condition that responds to AKR1C3 inhibition comprising administering to a patient in need thereof a therapeutically effective amount of a compound of this invention or its isomer, pharmaceutically acceptable salt, polymorph,
metabolite, prodrug, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, such disorders and conditions include, but are not limited to prostate cancer, advanced prostate cancer, precancerous precursors of prostate adenocarcinoma, prostate intraepithelial neoplasia (PIN), castration resistant prostate cancer (CRPC), benign prostate hyperplasia (BPH), lung cancer, non-small cell lung cancer (NSCLC), acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, breast cancer, metastatic breast cancer, refractory breast cancer, AR-positive breast cancer, ER-alpha positive breast cancer, ER-beta positive breast cancer, uterine cancer including endometrial and cervical cancers, uterine fibroids including myomas, endometriosis, myeloma and leiomyoma.

[0022] In one embodiment, this invention provides a method of lowering total serum testosterone levels in a male subject comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of lowering serum testosterone in a male subject comprising administering a therapeutically effective amount of a compound of this invention. In yet another embodiment, this invention provides a method of lowering serum free testosterone in a male subject comprising administering a therapeutically effective amount of a compound of this invention. In still another embodiment, this invention provides a method of lowering serum levels of prostate-specific antigen (PSA) in a male subject comprising administering a therapeutically effective amount of a compound of this invention.

[0023] In one embodiment, this invention provides a method of lowering total serum testosterone levels in a male subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of total serum testosterone is independent of a reduction of serum luteinizing hormone levels. In another embodiment, this invention provides a method of lowering serum testosterone levels in a male subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In yet another embodiment, this invention provides a method of lowering serum free testosterone levels in a male subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In still another embodiment, this invention provides a method of lowering serum PSA levels in a male subject comprising administering a therapeutically effective amount of a compound of
this invention, wherein the lowering of serum PSA is independent of a reduction of serum luteinizing hormone levels.

[0024] In one embodiment, this invention provides a method of lowering total serum testosterone levels in a female subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of total serum testosterone is independent of a reduction of serum luteinizing hormone levels. In another embodiment, this invention provides a method of lowering serum testosterone levels in a female subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In yet another embodiment, this invention provides a method of lowering serum free testosterone levels in a female subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In still another embodiment, this invention provides a method of lowering serum PSA levels in a female subject comprising administering a therapeutically effective amount of a compound of this invention, wherein the lowering of serum PSA is independent of a reduction of serum luteinizing hormone levels.

[0025] In one embodiment, this invention provides a method of increasing survival of a subject with advanced prostate cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with castration-resistant prostate cancer (CRPC) comprising administering a therapeutically effective amount of a compound of this invention.

[0026] In one embodiment, this invention provides a method of prolonging progression-free survival of a subject with advanced prostate cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with castration-resistant prostate cancer (CRPC) comprising administering a therapeutically effective amount of a compound of this invention.

[0027] In one embodiment, this invention provides a method of lowering total serum estradiol levels in a subject comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of lowering serum free estradiol in
a subject comprising administering a therapeutically effective amount of a compound of this invention. In one embodiment, a subject is a male subject. In another embodiment, a subject is a female subject.

[0028] In one embodiment, this invention provides a method of increasing survival of a subject with advanced breast cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with refractory breast cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with AR-positive or ER-positive breast cancer comprising administering a therapeutically effective amount of a compound of this invention.

[0029] In one embodiment, this invention provides a method of prolonging progression-free survival of a subject with advanced breast cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with refractory breast cancer comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with AR-positive or ER-positive breast cancer comprising administering a therapeutically effective amount of a compound of this invention.

[0030] In one embodiment, this invention provides a method of treating, increasing survival and/or prolonging progression-free survival of a subject with uterine cancer comprising administering a therapeutically effective amount of a compound of this invention.

[0031] In one embodiment, this invention provides a method of treating a subject with uterine fibroids comprising administering a therapeutically effective amount of a compound of this invention.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0032] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:
As used herein, A'dione and 4'dione and androstenedione and androstene-3,17-dione are all interchangeable and refer to the same compound which is the endogeneous substrate of AKR1C3 in the synthesis of testosterone.

**Figure 1:** depicts steroidogenic pathways involved in androgen synthesis. Filled arrows indicate back door steroidogenic pathway.

**Figure 2:** depicts in vitro inhibition of AKR1C3 enzyme activity by compound 78 (Figure 2A, Figure 2B) and 67 (bottom image of Figure 2C). The amount of testosterone synthesized from androstenedione by AKR1C3 is represented in the line graph (Figure 2B). "T" represents testosterone. "4' dione" represents androstenedione.

**Figure 3:** depicts specificity of compound 78 for AKR1C3. 78 demonstrated no cross reaction with AKR1C1 (i.e. was not an AKR1C1 inhibitor). Figure 3A shows the TLC fractionation of progesterone (P) and 20a-hydroxy-progesterone (20a(OH)P). Figure 3B shows the quantification of radiolabeled 20a-hydroxy-progesterone. Figure 3C shows HEK-293-AKR1C3 enzyme activity. HEK-293 cells transfected with AKR1C3 were treated with [3H]-labeled androstenedione (10 uM). Medium was collected, evaporated to dryness, the pellet was resuspended in methanol, and the radiolabeled androstenedione and testosterone were fractionated using TLC.

**Figure 4:** depicts COX-1 inhibitory activity by compounds of this invention (67, 78, and 205) as compared to indomethacin.

**Figure 5:** depicts COX-2 inhibitory activity by compounds of this invention (67, 78, and 205) as compared to indomethacin.

**Figure 6:** depicts specificity of inhibition of AKR1C3 enzyme activity in vivo (cellular) by compound 78. A representative of n=3 experiments is shown here. Figures 6A and 6B demonstrated that 78, but not indomethacin, inhibited AKR1C3 enzyme activity in vivo in HEK-293 cells transfected with AKR1C3, inhibiting conversion of androstenedione to testosterone. Figure 6C depicts that 78, but not indomethacin, inhibited AKR1C3 enzyme activity in adrenal H295R cells. Figure 6D demonstrated that AKR1C3 is overexpressed in LNCaP cells transfected with AKR1C3 (LNCaP-AKR), and Figure 6E demonstrated that 78, but not indomethacin, inhibited AKR1C3 enzyme activity in prostate cancer LNCaP cells stably transfected with AKR1C3. Figure 6F depicts that 78 and 81, but not indomethacin, inhibited finasteride-dependent testosterone formation in prostate...
cancer LNCaP cells stably transfected with AKR1C3 (LNCaP-AKR). Key: A’dione-Androstenedione, T-Testosterone, Indo-Indomethacin.

[0040] **Figure 7**: depicts AKR1C3-dependent AR transactivation in cells transfected with AKR1C3.

[0041] **Figure 8**: depicts that 78 inhibited AKR1C3-dependent AR transactivation. Results shown in the figures are representative of three experiments. **Figure 8A** demonstrates that AKR1C3 augmented ligand-dependent AR transactivation. **Figure 8B** demonstrates that AKR1C3 inhibition by 78 dose-dependently blocked androstenedione-induced AR transactivation. **Figure 8C** demonstrates that neither 78 nor indomethacin reduced AKR1C3-dependent AR transactivation. 4’dione-androstenedione; RLU-relative light units; Ren RLU-renilla relative light units.

[0042] **Figure 9**: depicts proliferation inhibition by 78 of LNCaP cells (first two columns in **Figure 9A**) and LNCaP cells that are stably transfected with AKR1C3 (LNCaP-AKR) (columns 3-8 in **Figure 9A**). Open bars indicate vehicle addition; closed bars indicate 10 nM A’dione addition. **Figure 9B**: depicts the growth of a LNCaP-AKR tumor xenograft model and its inhibition using 78 (**Figure 9B**).

[0043] **Figure 10**: depicts that 78 selectively inhibited AKR1C3-dependent AR transactivation, but did not inhibit 17pHSD3-dependent AR transactivation.

[0044] **Figure 11** depicts that AKR1C3 transfected into HEK293 cells augmented A’dione-dependent AR transactivation. □ AKR1C3; ◻ Vector.

[0045] **Figure 12**: shows that AKR1C3 augmented AR in response to active androgens. **Figures 12A-12F** depicts that AKR1C3 increased active androgen-induced AR transactivation with a titration of the androgens: 4’dione; testosterone, DHT, R1881, a SARM ((5’)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide), and an inactive stereoisomer of the SARM ((R)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide), compared with vector, □ or ▲ indicates AKR1C3 transfected cells; ◻ indicates Vector (pCR3.1) transfected cells. **Figure 12G** depicts that AKR1C3 concentration-dependently increased R1881-induced AR transactivation. The amount of AKR1C3 containing vector transfected into cells is denoted as: ▼0.1 µg, □ 0.25 µg, ▲ 0.5 µg, ◻ 0.75 µg, and open square 1 µg. ◻ Vector (pCR3.1) denotes vector transfected cells. **Figure 12H** shows that AKR1C3 did not increase...
AR expression. **Figure 121** shows titration curves used to produce AR EC50 values, which demonstrated that 78 inhibited AKRIC3-induced androgen-dependent AR transactivation. o AKRIC3 (EC50 = 0.072 nM); T AKRIC3 + 78; »vector (EC50 = 0.1222 nM). **Figure 12J** shows R1881-induced AKRIC3-dependent AR transactivation is not observed with other AKRICs. o AKRIC3; ■ AKRIC4; T AKRIC1; Δ AKRIC2; · vector (pCR3.1). In line graphs of Figures 12A-12G and 12I-12J solid lines are vector transfected and broken lines are AKRIC (1, 2, 3 or 4) transfected cells. All figures are representative of n=3 experiments. **Figure 12K** depicts that AKRIC3 concentration-dependently increases R1881-induced AR transactivation. HEK-293 cells were transfected with indicated concentration of AKRIC3. Total amount of transfected plasmids were normalized to 1 μg with vector pCR3.1. Twenty-four hours after transfection the cells were treated with a titration of R1881 and luciferase assay performed. Bottom panel shows the AKRIC3 RNA levels.

[0046] **Figure 13** shows AKRIC3-dependent androgen-induced AR transactivation is not cell type dependent. **Figures 13A-E** depict AKRIC3 increased active androgen-induced AR transactivation in COS-1 cells with a titration of the androgens: A'dione (13A); testosterone (13B), DHT (13C), R1881 (13D), and a SARM ((5)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide) (13E), compared with vector; o AKRIC3; · Vector (pCR3.1). A'dione-androstenedione; RLU-relative light unites, SARM-selective androgen receptor modulator. Solid lines are vector pCR3.1 transfected cells and broken lines are AKRIC3 transfected cells.

[0047] **Figure 14** shows AKRIC3-dependent increase in transactivation was specific to AR. **Figures 14** illustrates specificity of AKRIC3-dependent transactivation with a titration of steroid receptors: A'dione-AR (14A), R1881-AR (14B), Dex-GR (14C), Prog-PR (14D), Estrogen-ERα (14E), Aldosterone-MR (14F), and Rosi-PPARy (14G), compared with vector; o AKRIC3; · Vector (pCR3.1). A'dione-androstenedione; Dex-dexamethasone; Prog-progesterone; estrogen-17P-estradiol; Rosi-rosiglitazone. Solid lines are pCR3.1 transfected cells and broken lines are AKRIC3 transfected cells.

[0048] **Figure 15**: shows that different domains mediated the enzymatic and activator functions of AKRIC3. **Figure 15A** depicts a map of various truncated constructs of AKRIC3 in pCR3.1 with their amino acid sequences. **Figures 15B-15F** and 15I-K show transactivation with AKRIC3 truncation constructs. **Figure 15G** depicts a TLC enzymatic assay with full length and truncated AKRIC3 constructs depicted in Figure 15A. Only full-length AKRIC3 (construct A) had
enzyme activity. Figure 15H shows that 78 requires full length AKR1C3 to inhibit its activator function (i.e., no inhibition was seen with construct D). In Figure 15B-15D, the solid line indicates cells were transfected with vector (pCR3.1), the dashed line indicates cells were transfected with 0.5 μg of AKR1C3 construct, and the dotted line indicates cells were transfected with 1.0 μg of AKR1C3 construct. In Figure 15E, the solid line indicates cells were transfected with vector (pCR3.1), the dashed line indicates cells were transfected with AKR1C3 construct A (full-length), and the dotted line indicates cells were transfected with AKR1C3 construct D. In Figure 15F, construct A is represented by the dashed lines.

[0049] Figure 16: depicts that AKR1C3 and AR physically interact in tissue culture and xenograft prostate cancer cells. Figure 16A: shows AKR1C3 physically interacted with AR in prostate cancer tissue culture cells, depicting co-immunoprecipitation of AR and AKR1C3. Analysis of the immunoprecipitant produced by immunoprecipitation (IP) of AKR1C3 demonstrated immunoreactivity with AR on immunoblot (IB). Figure 16B: shows colocalization of AR with AKR1C3 in prostate cancer tissue culture cells, by confocal microscopy. Figure 16C: shows endogenous AR and AKR1C3 interact in VCaP CRPC tumor xenografts, depicting co-immunoprecipitation of AR and AKR1C3. Analysis of the immunoprecipitant produced by immunoprecipitation (IP) of AR demonstrated immunoreactivity with AKR1C3 on immunoblot (IB).

[0050] Figure 17: depicts that AKR1C3 is recruited to the PSA promotor. Figure 17A: shows that AKR1C3 is recruited to PSA enhancer. Figure 17B: presents results of a ChIP assay. LNCaP-AKR1C3 cells were serum starved for 3 days and were treated for 2 hrs and recruitment of AKR1C3 on PSA enhancer was measured by ChIP assay.

[0051] Figure 18: depicts AR and AKR1C3 co-localized in LNCaP cells. Figure 18A shows AR-AKR1C3 colocalization demonstrated by laser confocal microscopy. Figure 18B shows that AKR1C3 and AR interacted by Duolink proximity ligation assay. Figure 18C shows that AR and AKR1C3 interacted in advanced prostate cancer.

[0052] Figure 19: shows AKR1C3 enhanced androgen signaling and prostate cancer xenograft growth. Figures 19A-19C show AKR1C3 siRNA inhibited AR function in LNCaP cells. siRNA treatments include cyclophilin siRNA (denoted as Cyclo.); AKR1C3 siRNA (denoted as AKR1C3); and no siRNA control (denoted as -). Figures 19D, 19E, Figure 19G and Figure 19H show AKR1C3 transfection increased androgen induced PSA gene expression. Figure 19D is the PSA gene
expression with a titration of A'dione and Figure 19E is with a titration of R1881. In both the figures, solid lines are vector pCR3.1 transfected and broken lines are AKR1C3 transfected. Figure 19F shows the 78 inhibited AKR1C3-dependent PSA gene expression in LNCaP cells. Figure 19G shows that AKR1C3 increased DHT-induced LNCaP tumor xenograft growth. Numbers within brackets indicate the number of animals with tumor uptake. Figures 19H shows AKR1C3 expression in cells transfected with AKR1C3 (closed bars) or vector (open bars; not visible in the figure) transfected cells. Figure 19I shows AKR1C3 enhanced androgen signaling in tumor xenografts. Open bars are LNCaP-Vector tumors (n=3) and filled bars are LNCaP-AKR1C3 tumors (n=6). Figure 19J shows FKBP51 protein, derived from an AR dependent gene, is increased in LNCaP-AKR1C3 xenografts. Figure 19K depicts over-expression of AKR1C3 increased LNCaP xenograft growth in intact mice. Figure 19L depicts AR target FKBP51 (right panel), but not AR (left panel), protein expression is increased in LNCaP-AKR1C3 xenograft tumors. Increased FKBP51 was observed despite little to no change in AR levels. Figure 19M depicts a Kaplan-Meier plot of tumor uptake in LNCaP-vector (solid line) or LNCaP-AKR1C3 (broken line) cells.

[0053] Figure 20: depicts crystals of purified AKR1C3.

[0054] Figure 21: depicts crystal structure of AKR1C3 in complex with compounds 45 and 2-(4-(bromomethyl)-3-hydroxyphenyl)-6-hydroxy-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2 H)-one in Figures 21A and 21B, respectively. The AKR1C3 crystal structures with 45 and 2-(4-(bromomethyl)-3-hydroxyphenyl)-6-hydroxy-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2 H)-one are superimposed in Figure 21C.

[0055] Figure 22: shows 78 inhibited AKR1C3 enzyme- and coactivator- activities in cells. Figure 22A shows 78, but not indomethacin, inhibited AKR1C3-mediated A'dione-induced ARE transactivation. Figure 22B shows 78 increased the EC50 of A'dione. Figure 22C shows 78 inhibition of AR transactivation was selective to AKR1C3. Figure 22D shows 78 inhibited AKR1C3-dependent R1881 induced AR transactivation. Figure 22E shows 78 required full length AKR1C3 to inhibit its coactivation effect. Figure 22F shows 78 did not cross react with AKR1C1. Figure 22G shows 78 inhibited AKR1C3-dependent A'dione-induced AR transactivation at all concentrations of AKR1C3.

[0056] Figure 23: shows 78 inhibited androgen signaling, and prostate cancer cell and tumor growth. Figure 23A shows 78 inhibited LNCaP-AKR1C3 cell growth. Figure 23B (top pane) shows 78 inhibited PSA gene expression in VCaP cells. Figure 23C shows 78 reduced the growth of LNCaP
prostate cancer xenograft growth. The bottom pane showed that finasteride (Fin) treatment enhanced PSA gene expression in VCaP cells. Figure 23D shows 78 reduced VCaP castration resistant prostate cancer xenograft growth. Figure 23E shows that serum PSA was suppressed in VCaP tumors treated with 78. At the end of the study, PSA was measured in the serum of CRPC VCaP tumor bearing animals treated with vehicle or 40 mg/kg compound 78. As demonstrated in the graph, 78 inhibited serum PSA by greater than 75% compared to vehicle treated animals. Numbers above bars are the mean of each group. N=5. Figure 23F shows the expression of steroidogenic enzymes in VCaP cells. RNA from VCaP cells was extracted and realtime PCR was performed for AKR1C1, AKR1C3, and 5α reductase-1. Values are expressed as gene normalized to GAPDH and expressed relative to AKR1C3 expression. Figure 23G shows 78 suppressed tumor PSA. The protein in Figure 26G was extracted from the tumors that are shown in Figure 23D, fractionated by SDS-PAGE and Western blotted for PSA and actin. Figure 23H depicts 78 as inversely correlated with tumor volume. 78 concentration in serum was measured and correlated with tumor volume shown in Figure 23D. *p<0.05. The in vitro figures are representative of n=3 and each experiment was performed with triplicate samples; Fin denotes finasteride treatment; 78 denotes treatment with 78; PSA- prostate specific antigen; SRD5otl denotes 5α-hydroxysteroid dehydrogenase subtype 1; LNCaP-AKR1C3 denotes LNCaP cells stably transfected with AKR1C3. In Figures 23A and 23B, * indicates significance at p<0.05 from vehicle treated samples. Figure 23I demonstrates a model describing AKR1C3’s dual function as an androstenedione activating enzyme and AR activating co-factor.

Figure 24: depicts concentration-dependent increase of AR activation, individually (Figure 24A, SRC-2; Figure 24B, AKR1C3) and together (Figure 24C, SRC-2 and AKR1C3), demonstrating cotransfection synergistically increased AR transactivation

Figure 25: depicts cyclophilin (Cyclo) (Figure 25A) and AKR1C3(Figure 25B) expression after siRNA transfection as shown in Figures 19A-19C.

Figure 26: depicts that AKR1C3 translocation to nucleus required AR. Figure 26A shows NIH3T3 cells stably transfected with AKR1C3, were infected with adenovirus LacZ, treated with 10 nM R1881, and the expression of AKR1C3 was detected by immunofluorescence using laser confocal microscopy demonstrating no nuclear translocation. Figure 26B shows NIH3T3 cells stably transfected with AKR1C3 were infected with adenovirus AR and were treated with 10 nM R1881.
Cells were fixed and AR (green) and AKR1C3 (red) were detected by immunofluorescence using laser confocal microscopy (Example 11), demonstrating AR and AKR1C3 in the nucleus.

[0060] **Figure 27:** depicts AKR1C3 migrated with AR. LNCaP-AKR1C3 cells were treated with 0.1 nM R1881 (top panels) or 0.1 nM R1881 and 10 μM SNARE-1. Cells were fixed and AR (green) and AKR1C3 (red) were detected by immunofluorescence using a laser confocal microscopy. (Key: SNARE-1-Selective Nuclear Androgen Receptor Exporter-1.) (Example 11).

[0061] **Figure 28:** depicts % conversion from 4-dione to Testosterone.

**DETAILED DESCRIPTION OF THE PRESENT INVENTION**

[0062] In one aspect, the present invention relates to compounds that act as hydroxysteroid dehydrogenase inhibitors (HDIs), such as compounds that act as inhibitors of 17P-hydroxysteroid dehydrogenases (17P-HSDs), for example, and/or compounds that act as selective inhibitors of AKR1C3 (type 5 17P-HSD or 17P-HSD5). In another aspect, the present invention relates to compounds that act as inhibitors of the AKR1C3 co-activation function, for example, inhibitors of AKR1C3 co-activation of androgen-dependent Androgen Receptor (AR)-transactivation.

[0063] In one embodiment, this invention is directed to a compound of Formula I:

\[
\begin{align*}
\text{A} & \text{ is } O, \text{N or C;} \\
\text{B} & \text{ is } N \text{ or C;} \\
\text{R}^1 & \text{ is an alkyl or alkenyl group,} \\
\text{R}^2 & \text{ is a hydroxy or amino group,} \\
\text{R}^3 & \text{ is a hydrophobic group,} \\
\text{R}^4 & \text{ is a hydrogen or an alkyl group,} \\
\text{R}^5 & \text{ is a hydroxy or amino group,} \\
\text{R}^6 & \text{ is a hydrogen or an alkyl group,} \\
\text{m} & \text{ is an integer from 1 to 5,} \\
\text{n} & \text{ is an integer from 1 to 5.}
\end{align*}
\]

**Formula I**

wherein,

A is O, N or C;

B is N or C;
R₁ is nothing, H, alkyl or -alkylene-C≥2R², in which R⁸ is H or alkyl; wherein, when R₁ is nothing an oxo (C=0) group is formed;

R² is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkyene-CC>2R₆, in which R₈ is H or alkyl;

R³ is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF₂OMe, CN, carboxyl, S0₂R₂, substituted or unsubstituted phenyl, S0₂NHR₂ or any combination thereof; in which R₂ is, in each case, independently, H or alkyl;

R⁴ is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, S0₂R₂, S0₂NHR₂ or any combination thereof; in which R₂ is, in each case, independently, H or alkyl;

R⁵ is nothing, H, alkyl or -alkylene-C0₂R₅, in which R₅ is H or alkyl; wherein when R₅ is nothing an oxo (C=0) group is formed.

R⁶ is nothing, H, alkyl or -alkylene-C0₂R₅, in which R₅ is H or alkyl; wherein, when A is O then R₆ is nothing;

m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;

or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0064] In some embodiments of Formula I, if A = C, R⁶ = H, B = N, and R⁵ is nothing, then R² is not H or substituted or unsubstituted cycloalkyl, or R₁ is not H or alkyl, or R³ is not OH, halogen, haloalkyl, or carboxyl, or R⁴ is not H, alkyl, OH, halogen, haloalkyl, CN or carboxyl.

[0065] In one embodiment, A of Formula I is C (carbon). In another embodiment, A of Formula I is N (nitrogen). In another embodiment A of Formula I is O (oxygen). In one embodiment, when A of Formula I is O, then R⁶ is nothing.
One of ordinary skill in the art will recognize that some of the compounds of Formula I may exist in different tautomeric forms.

In one embodiment, Formula I is represented by the structure of Formula IA:

\[
\text{Formula IA}
\]

wherein \( R^1, R^2, R^3, R^4, R^5, R^6, m \) and \( n \) are as described above for Formula I.

In another embodiment, Formula I is represented by the structure of Formula IB:

\[
\text{Formula IB}
\]

wherein \( R^1, R^2, R^3, R^4, R^5, m \) and \( n \) are as described above for Formula I.

In another embodiment, Formula I is represented by the structure of Formula IC:
Formula IC

wherein $R^1, R^2, R^3, R^4, R^5, R^6, m$ and $n$ are as described above for Formula I.

[0070] These two forms of Formula IC are used interchangeably herein, and both are encompassed within the present invention.

5 [0071] In certain embodiments, the compound of Formula I is selected from:
4-(4-trifluoromethyl)phenyl)naphthalene-1,6-diol (203),
methyl 2-((5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalen-2-yl)oxy)acetate (209),
dimethyl 2,2'-(4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy))diacetate (209A), and
methyl 2-((6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212).

10 [0072] In one embodiment, the compound of Formula I is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, $N$-oxide, hydrate or any combination thereof.

[0073] In one embodiment, the present invention relates to a compound of Formula II:

Formula II

wherein

$R^1$ is H, alkyl or -alkylene-C0$_2$R$^\psi$, in which R$^\psi$ is H or alkyl;

$R^2$ is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkylene-C0$_2$R$^\psi$, in which R$^\psi$ is H or alkyl;
R3 is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF2OMe, CN, carboxyl, SO2Rz, substituted or unsubstituted phenyl, SO2NHRz or any combination thereof in which Rz is, in each case, independently, H or alkyl;

R4 is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO2Rz, SO2NHRz or any combination thereof; in which Rz is, in each case, independently, H or alkyl;

m = 1 or 2; and

n = 1, 2, 3, 4 or 5;

or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0074] In some embodiments of Formula II, if R2 = H or substituted or unsubstituted cycloalkyl, then R1 is not H or alkyl, or R3 is not OH, halogen, haloalkyl, or carboxy, or R4 is not H, alkyl, OH, halogen, haloalkyl, CN or carboxyl.

[0075] One of ordinary skill in the art will recognize that some of the compounds of Formula II may exist in different tautomeric forms, for example, as shown below:

[0076] These two forms of compounds of Formula II are used interchangeably herein, and both are encompassed within the present invention.

[0077] In certain embodiments, the compound of Formula II is selected from:

4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2 H)-one (17),
6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2 H)-one (21),
6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2\textsubscript{H})-one (39),
4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),
2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2\textsubscript{H})-one (49),
4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2\textsubscript{H})-one (51),
4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2\textsubscript{H})-one (53),
4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-1(2\textsubscript{H})-one (55),
2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (59),
2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (61),
4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2\textsubscript{H})-one (63),
2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (70),
4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-1(2\textsubscript{H})-one (72),
6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (77),
4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-methoxyisoquinolin-1(2\textsubscript{H})-one (80),
methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1\textsubscript{H})-yl)acetate (82),
4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{H})-one (91)
4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{H})-one (106),
4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),
2-((2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109),
2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1\textsubscript{H})-yl)acetic acid (113),
4-(2-bromo-4,5-difluorophenyl)-6-methoxyisoquinolin-1(2H)-one (214),

4-(2-bromo-4,5-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-yl-one (215),

4\5'-difluoro-2'-(6-methoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-biphenyl]-4-carboxamide (216),

4\5'-difluoro-2'-(6-hydroxy-1-oxo-1,2-dihydropyridoisoquinolin-4-yl)-[1,1'-biphenyl]-4-carboxamide (217),

4-(3,4-difluorophenyl)-6-methoxyisoquinolin-1(2H)-one (219), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In one embodiment, the compound of Formula II is 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In another embodiment, the compound of Formula II is 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In another embodiment, the compound of Formula II is 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In yet another embodiment, the compound of Formula II is 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In still another embodiment, the compound of Formula II is 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
[0083] In a further embodiment, the compound of Formula II is 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0084] In a further embodiment, the compound of Formula II is 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0085] In a further embodiment, the compound of Formula II is 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0086] In a further embodiment, the compound of Formula II is 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[0087] In one embodiment, R^1 of Formula I and/or Formula II is H or alkyl. In one embodiment, R^1 is H or methyl. In one embodiment, R^1 is H. In one embodiment R^1 is methyl. In another embodiment, R^1 is -alkylene-CC>2R^2. In one embodiment R^2 is H. In another embodiment, R^2 is alkyl, e.g., methyl. In one embodiment, R^2 is -alkylene-CO_2CH_3, for example, R^2 is -CH_2CO_2CH_3. In one embodiment, R^1 is nothing, wherein if R^1 is nothing an oxo (=O) group is formed.

[0088] In one embodiment, R^2 of Formula I and/or Formula II is H, alkyl, alkenyl, or cycloalkyl. For example, R^2 is H, alkyl, or cycloalkyl. In one embodiment, R^2 is H. In another embodiment, R^2 is alkyl, for example, methyl, ethyl, propyl, butyl, etc. In one embodiment, R^2 is methyl or n-butyl. In one embodiment, R^2 is methyl. In one embodiment, R^2 is n-butyl. In one embodiment, R^2 is alkenyl. For example, in one embodiment, R^2 is halo-substituted n-propylene (e.g., dihalo-n-propyl-1-ene, such as -CH₂CH=CF₂). In another embodiment, R^2 is cycloalkyl, e.g., cyclopropyl or cyclohexyl. In one embodiment R^2 is cyclopropyl. In one embodiment R^2 is cyclohexyl. In another embodiment, R^2 is C₃-C₄ cycloalkyl, e.g., cyclopropyl. In another embodiment, the cycloalkyl is a heterocycloalkyl.

[0089] In another embodiment, R^2 of Formula I and/or Formula II is -alkylene-CC>2R^3. In one embodiment R^3 is H. In another embodiment, R^3 is alkyl, e.g., methyl. In one embodiment, R^3 is -alkylene-CO_2CH_3, for example, R^3 is -CH₂CO₂CH_3.
In certain embodiments, R<sub>2</sub> of Formula I and/or Formula II is H or alkyl (e.g., methyl, butyl). In one embodiment, R<sub>3</sub> of Formula I and/or Formula II is H or cycloalkyl. For example, in one embodiment, R<sub>2</sub> is H or cyclopropyl.

In certain embodiments, R<sub>3</sub> of Formula I and/or Formula II is, independently, hydroxyl, halogen, haloalkyl, CF<sub>2</sub>OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO<sub>2</sub>alkyl or SO<sub>2</sub>NHalkyl, or any combinations thereof. For example, R<sub>3</sub> is hydroxyl, halogen, haloalkyl, CF<sub>2</sub>OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO<sub>2</sub>CH<sub>3</sub> or SO<sub>2</sub>NHCH<sub>3</sub>, or any combinations thereof. For example, in certain embodiments, R<sub>3</sub> is hydroxyl, F, Cl, CF<sub>3</sub>, CN, carboxyl, SO<sub>2</sub>CH<sub>3</sub> or SO<sub>2</sub>NHCH<sub>3</sub>, or any combinations thereof. In another embodiment, the phenyl is substituted by one to four substituents selected from halogen, haloalkyl, CN, C(0)NH<sub>2</sub>, N0<sub>2</sub>, amine, amide, hydroxyl and alkyl.

In certain embodiments, R<sub>4</sub> of Formula I and/or Formula II is, independently, H, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO<sub>2</sub>alkyl or SO<sub>2</sub>NHalkyl, or any combinations thereof. In another embodiment, R<sub>4</sub> is hydrogen. For example, R<sub>4</sub> is hydroxyl, halogen, haloalkyl, CN, carboxyl, SO<sub>2</sub>CH<sub>3</sub> or SO<sub>2</sub>NHCH<sub>3</sub>, or any combinations thereof. For example, in certain embodiments, R<sub>4</sub> is hydroxyl, F, CF<sub>3</sub>, CN, carboxyl, SO<sub>2</sub>CH<sub>3</sub> or SO<sub>2</sub>NHCH<sub>3</sub>, or any combinations thereof.

In one embodiment, n of Formula I and/or Formula II is 1, 2 or 3. In one embodiment, n is 1. In another embodiment, n is 2. In a further embodiment, n is 3. In another embodiment, n is 2 or 3.

In one embodiment, m of Formula I and/or Formula II is 1 or 2. In one embodiment, m is 1. In another embodiment, m is 2.

In another embodiment, when n>1, R<sub>3</sub> is the same or different substituent. In another embodiment, when m>1, R<sub>4</sub> is the same or different substituent. In certain embodiments, R<sub>3</sub> of Formula I and/or Formula II is substituted up to five times by the same substituent. For example in one embodiment, R<sub>3</sub> is F and n is 3 such as in 3,4,5-trifluorophenyl, where R<sub>3</sub> is taken together with the phenyl ring to which it is attached. In certain embodiments, R<sub>3</sub> of Formula I and/or Formula II is substituted multiple times by up to five different substituents. For example in one embodiment, R<sub>3</sub> is CF<sub>3</sub> and F and n is 2, such as in 3-trifluoromethyl-4-fluorophenyl where R<sub>3</sub> is taken together with the phenyl ring to which it is attached; or any combinations thereof, such as 3,4-difluoro-6-bromophenyl where R<sub>3</sub> is taken together with the phenyl ring to which it is attached.

In another embodiment, where R<sub>3</sub> is unsubstituted phenyl, such as 3,4-difluoro-6-bromophenyl where R<sub>3</sub> is taken together with the phenyl ring to which it is attached.
In certain embodiments, the R³ substituent(s) of Formula I and/or Formula II, together with the phenyl ring to which it(they) is(are) attached forms a group selected from hydroxyphenyl (e.g., 4-hydroxyphenyl); (haloalkyl)phenyl (e.g., (trifluoromethyl)phenyl, such as 4- (trifluoromethyl)phenyl); (alkylsulfonyl)phenyl (e.g., (methylsulfonyl)phenyl such as 4- (methylsulfonyl)phenyl); carboxyphenyl (e.g., 4-carboxyphenyl); halophenyl (e.g., fluorophenyl, such as 3-fluorophenyl, 4-fluorophenyl); dihalophenyl (e.g., difluorophenyl, such as 3,5-difluorophenyl or 3-fluoro-4-chlorophenyl); trihalophenyl (e.g., trifluorophenyl, such as 3,4,5-trifluorophenyl); halo(hydroxy)phenyl (e.g., fluoro(hydroxy)phenyl, such as 3-fluoro-4-hydroxyphenyl); halo(haloalkyl)phenyl (e.g., fluoro(trifluoromethyl)phenyl, such as 3-(trifluoromethyl)-4-fluorophenyl, 3-fluoro-4-(trifluoromethyl)phenyl); halo(cyano)phenyl (e.g., fluoro(cyano)phenyl, such as 3-cyano-4-fluorophenyl and 4-cyano-3-fluorophenyl).

In further embodiments, R¹ of Formula I and/or Formula II is H, R² is H or cycloalkyl, R³ is halogen or haloalkyl and n is 2 or 3. For example, in one embodiment, R¹ is H, R² is H or cyclopropyl, R³ is F or CF₃ and n is 2 or 3.

In one embodiment, R⁵ of Formula I is H or alkyl. In one embodiment, R⁵ is H or methyl. In one embodiment, R⁵ is H. In one embodiment, R⁵ is methyl. In another embodiment, R⁵ is alkylene-CC>2R⁸. In one embodiment R⁸ is H. In another embodiment, R⁸ is alkyl, e.g., methyl. In one embodiment, R⁵ is alkylene-CO2CH₃, for example, R⁵ is -CH2CO2CH₃. In one embodiment, R⁵ is nothing wherein when R⁵ is nothing no oxo (C=O) group is formed.

In one embodiment, R⁶ of Formula I is nothing, H, alkyl or alkylene-CC>2R⁸, in which R⁸ is H or alkyl. In another embodiment, R⁶ is hydrogen. In another embodiment, R⁶ is alkyl such as methyl or ethyl. In one embodiment, when A is O, R⁶ is nothing. In one embodiment, when A is N, then R⁶ is H or alkyl. In another embodiment, when A is N, then R⁶ is alkylene-CC>2R⁸.

In one embodiment, this invention provides a compound of Formula I or II, or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, this invention provides a compound of Formula I or II, or an isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, N-oxide, hydrate or any combination thereof. In one embodiment, this invention provides a compound of Formula I or II, or an isomer, tautomer, pharmaceutically acceptable salt, polymorph, N-oxide, hydrate or any combination thereof. In one embodiment, this invention provides a compound of Formula I or II, or an isomer, tautomer, pharmaceutically acceptable salt, polymorph, N-oxide, hydrate or any combination thereof. In one embodiment, this invention provides a compound of Formula I or
II, or an isomer, tautomer, pharmaceutically acceptable salt, N-oxide, hydrate or any combination thereof. In one embodiment, this invention provides a compound of Formula I or II, or an isomer, pharmaceutically acceptable salt, or any combination thereof.

[00101] In another embodiment, this invention provides an isomer of a compound of Formula I or II. In another embodiment, this invention provides a metabolite of a compound of Formula I or II. In another embodiment, this invention provides a pharmaceutically acceptable salt of a compound of Formula I or II. In another embodiment, this invention provides a hydrate of a compound of Formula I or II. In another embodiment, this invention provides a tautomer of a compound of Formula I or II. In another embodiment, this invention provides an N-oxide of a compound of Formula I or II. In another embodiment, this invention provides a prodrug of a compound of Formula I or II. In another embodiment, this invention provides a polymorph of a compound of Formula I or II. In another embodiment, this invention provides a crystal of a compound of Formula I or II.

[00102] In one embodiment, compounds of this invention include:

4-(3-(bis(2,4-dimethylphenyl)methyl)-4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (72A),

6-(6-hydroxy-1-oxoisoquinolin-2(1H)-yl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (87),

6-methoxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (88),

4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (90),

6-hydroxy-4-(6-hydroxypyridin-3-yl)isoquinolin-1(2H)-one (93),

4-(6-hydroxypyridin-3-yl)-6-methoxyisoquinolin-1(2H)-one (93A),

6-hydroxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (104),

methyl 2-(bromomethyl)-4-(6-hydroxy-1-oxoisoquinolin-2(1H)-yl)benzoate (104A),

1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110),

2-((4-bromophenyl)sulfonyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl bromobenzenesulfonate (110A),
5J-dimethoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (207),
5,7-dihydroxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (208),
5-hydroxy-7-methoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (208A), and
1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl methanesulfonate (213).

or their prodrug, isomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00103] In one embodiment, a compound of this invention is a hydroxysteroid dehydrogenase inhibitor (HSDi). In one embodiment, an HSDi is an aldo-keto reductase inhibitor. In one embodiment, an HSDi, is an AKR1C3 inhibitor. In one embodiment, the HSDi of this invention is a compound of Formula I. In one embodiment, the HSDi is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00104] In one embodiment, a hydroxysteroid dehydrogenase inhibitor (HSDi) of this invention is a compound of Formula II. In one embodiment the HSDi is 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00105] In another embodiment, the HSDi is 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00106] In yet another embodiment, the HSDi is 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00107] In still another embodiment, the HSDi is 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
In a further embodiment, the HSDi is 2-fluoro-5-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, the HSDi is 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, the HSDi is 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, the HSDi is 4-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, the HSDi is 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In certain embodiments, the compounds of the present invention are active as selective hydroxysteroid dehydrogenase inhibitors (HSDi). In one embodiment, the compounds of the present invention are active as selective inhibitors of AKRIC. In another embodiment, the compounds of the present invention are active as selective inhibitors of AKR1C3.

In one embodiment, a selective inhibitor of AKR1C3 is 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In another embodiment, a selective inhibitor of AKR1C3 is 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
In yet another embodiment, a selective inhibitor of AKR1C3 is 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In still another embodiment, a selective inhibitor of AKR1C3 is 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, a selective inhibitor of AKR1C3 is 2-fluoro-5-(6-hydroxy-l-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, a selective inhibitor of AKR1C3 is 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, a selective inhibitor of AKR1C3 is 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In a further embodiment, a selective inhibitor of AKR1C3 is 4-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In one embodiment, a selective inhibitor of AKR1C3 is 4-(3,4,5-trifluorophenyl)naphthalene-l,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In one embodiment, a selective inhibitor of AKR1C3 is 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

As used herein the term "halogen" means F, Cl, Br, and I.
The term "alkyl" means a substituted or unsubstituted saturated hydrocarbon radical which may be straight-chain or branched-chain and contains about 1 to about 20 carbon atoms, for instance 1 to 12 carbon atoms, such as 1 to 8 carbon atoms, e.g., 1 to 4 carbon atoms. Suitable alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, and dodecyl. Other examples of suitable alkyl groups include, but are not limited to, 1-, 2- or 3-methylbutyl, 1,1-, 1,2- or 2,2-dimethylpropyl, 1-ethylpropyl, 1-, 2-, 3- or 4-methylpentyl, 1,1-, 1,2-, 1,3-, 2,2-, 2,3-, or 3,3-dimethylbutyl, 1- or 2-ethylbutyl, ethylmethylpropyl, trimethylpropyl, methylhexyl, dimethylpentyl, ethylpentyl, ethylmethylbutyl, dimethylbutyl, and the like.

Substituted alkyl groups are alkyl groups as described above which are substituted in one or more positions by, e.g., halogen, hydroxyl, amino, carboxy, and cyano, or any combinations thereof (e.g., CF₃, CHF₂). The use of the term "halogenated alkyl" does not mean that "alkyl" cannot be substituted by one or more halogen atoms. The use of the term "hydroxyalkyl" does not mean that "alkyl" cannot be substituted by one or more hydroxyl atoms.

The term "alkenyl" means a substituted or unsubstituted hydrocarbon radical which may be straight-chain or branched-chain, which contains one or more carbon-carbon double bonds, and which may comprise about 1 to about 20 carbon atoms, such as 1 to 12 carbon atoms, for instance 1 to 6 carbon atoms. Suitable alkenyl groups include ethenyl, propenyl, butenyl, etc.

Substituted alkenyl groups are alkenyl groups as described above which are substituted in one or more positions by, e.g., halogen, hydroxyl, amino, carboxy, cyano, or any combinations thereof.

The term "alkylene" means a linear saturated divalent hydrocarbon radical of one to eight carbon atoms or a branched saturated divalent hydrocarbon radical of three to six carbon atoms unless otherwise stated e.g., methylene, ethylene, propylene, 1-methylpropylene, 2-methylpropylene, butylene, pentylenes, and the like.

The term "alkynyl" means a substituted or unsubstituted aliphatic hydrocarbon radical which may be straight-chain or branched-chain and which contains one or more carbon-carbon triple bonds. Preferably the alkynyl group contains 2 to 15 carbon atoms, such as 2 to 12 carbon atoms, e.g., 2 to 8 carbon atoms. Suitable alkynyl groups include ethynyl, propynyl, butynyl, etc.
Substituted alkynyl groups are alkynyl groups as described above which are substituted in one or more positions by, e.g., halogen, hydroxyl, amino, carboxy, cyano, or any combinations thereof.

The term "alkylcycloalkyl" means a cycloalkyl-alkyl- group, where cycloalkyl and alkyl are as described above.

The term "amino" means -NH₂.

The term "alkylamino" means -NH(alkyl), wherein alkyl is as described above.

The term "dialkylamino" means -N(alkyl)₂, wherein alkyl is as described above.

The term "alkylsulfonyl" means a n-SO₂-alkyl group, wherein alkyl is as described above.

The term "alkylsulfinyl" means an-SO-alkyl group, wherein alkyl is as described above.

The term "carboxyl" means -C(0)OH.

The term "aryl" refers to an all-carbon monocyclic, heterocyclic or fused-ring polycyclic groups having a completely conjugated pi-electron system. Examples include phenyl, biphenyl, oligomeric phenyl groups, naphthalene, cummulenes, pyranal, pyrrolyl, pyrazinyl, pyrimidinyl, pyrazolyl, furanyl, thiophenyl, thiazolyl, imidazolyl, isoxazolyl, and the like. In another embodiment, the aryl refers to a 3-12 member ring. In another embodiment, the aryl refers to a 4-8 member ring. In another embodiment, the aryl refers to a 5 member ring. In another embodiment, the aryl refers to a 6 member ring. In another embodiment, the aryl may be substituted by one or more groups or unsubstituted ring, wherein said substituent is for example, but not limited to an halogen, haloalkyl, alkyl, cyano, nitro, carbonyl, amido, an amino or any combination thereof.

The term "arylsulfonyl" means an -SO₂-aryl group, wherein aryl is as described above.

The term "arylsulfinyl" means an-SO-aryl group, wherein aryl is as described above.
The term "cycloalkyl" means a monocyclic, bicyclic or tricyclic nonaromatic saturated hydrocarbon radical having 3 to 10 carbon atoms, such as 3 to 8 carbon atoms, for example, 3 to 6 carbon atoms. Suitable cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, norbornyl, 1-decalin, adamant-1-yl, and adamant-2-yl. Other suitable cycloalkyl groups include, but are not limited to, spiropentyl, bicyclo[2.1.0]pentyl, bicyclo[3.1.0]hexyl, spiro[2.4]heptyl, spiro[2.5]octyl, bicyclo[5.1.0]octyl, spiro[2.6]nonyl, bicyclo[2.2.0]hexyl, spiro[3.3]heptyl, bicyclo[4.2.0]octyl, and spiro[3.5]nonyl. Preferred cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The cycloalkyl group can be substituted, for example, by one or more halogens and/or alkyl groups.

The term "heterocycle" means a substituted or unsubstituted non-aromatic mono- or multicyclic ring system comprising 3 to 10 atoms, preferably 5 or 6 atoms, wherein at least one of the ring atoms is an N, O or S atom. Suitable heterocycle groups include, but are not limited to tetrahydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, isoxazolinyl, and the like.

Substituted heterocycle groups include the above-described heterocycle groups which are substituted one or more times by, for example, alkyl, alkenyl, alkynyl, cycloalkyl, alkycycloalkyl, aryl, alkylaryl, heteroaryl, alkyheteroaryl, heterocycle, alkylheterocycle, halogen, hydroxyl, cyano, alkoxyl, aryloxy, cycloalkyloxy, alkoxy carbonyl, carboxyl, amino, alkylamino, dialkylamino, -SH, thioalkyl, alkylsulfonyl, alkylsulfinyl, arylsulfonyl, arylsulfanyl, aminosulfonyl, aminosulfanyl, aroyl, acyl, or any combinations thereof.

The term "haloalkyl" means an alkyl group substituted by one or more halogens, for example, CF₃, CF₂CF₃, CH₂F, CHF₂, CH₂Br, and the like.

The term "hydroxyalkyl" means an alkyl group substituted by one or more hydroxyl groups, for example, CH₂OH, CH₃CH₂OH, and the like.

The term "acyl" means an HC(O)-, alkyl-C(O)-, or cycloalkyl-C(O)-, in which the alkyl and cycloalkyl groups are as previously described.

The term "alkoxy" means alkyl-O- groups and in which the alkyl portion is in accordance with the previous discussion. Suitable alkoxy groups include, but are not limited to, methoxy, difluoromethoxy, trifluoromethoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, i-butoxy,
pentoxy, hexoxy, heptoxyl, octoxy, and the like. For example, the alkoxy can be methoxy, difluoromethoxy, trifluoromethoxy or ethoxy.

[00149] The term "alkylheterocycle" refers to a heterocycle-alkyl-group wherein the heterocycle and alkyl portions are in accordance with the previous discussions.

[00150] The term "alkylthio" means an alkyl-S-group, in which the alkyl group is as previously described.

[00151] The term "arylthio" means an aryl-S-group, in which the aryl group is as previously described.

[00152] The term "alkoxycarbonyl" means an alkyl-O-CO-group, in which the alkyl group is as previously described.

[00153] The term "aminoalkyl" means a linear monovalent hydrocarbon radical of one to six carbon atoms, or a branched monovalent hydrocarbon radical of three to six carbons substituted with at least one, preferably one or two, -NRR' where R is hydrogen, alkyl, or -COR where R is alkyl, and R is selected from hydrogen, alkyl, hydroxyalkyl, alkoxylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or haloalkyl, e.g., aminomethyl, methyaminoethyl, 2-ethylamino-2-methylethyl, 1,3-diaminopropyl, dimethylaminoethyl, diethylaminoethyl, acetylaminoethyl, and the like.

[00154] The term "amidoalkyl" means a linear monovalent hydrocarbon radical of one to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbons substituted with at least one, preferably one or two, -(CO)NRR' where R is hydrogen, alkyl, or -COR where R is alkyl, and R' is selected from hydrogen, alkyl, hydroxyalkyl, alkoxylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or haloalkyl, e.g., CH₂CONH₂, CH₂CONHalkyl (e.g., CH₂CONHCH₃), CH₂CONH(alkyl)₂ (e.g., CH₂CON(CH₃)₂), and the like.

[00155] The term "aminosulfanyl" means a -SONRR' radical where R is independently hydrogen, alkyl, hydroxyalkyl, alkoxylalkyl, or aminoalkyl and R' is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocycl, heterocyclalkyl, hydroxyalkyl, alkoxylalkyl, or aminoalkyl as defined above, e.g., -SONH₂, methylaminosulfanyl, 2-dimethylaminosulfanyl, and the like.
The term "aminosulfonyl" means a -SO₂NRR' radical where R is independently hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, or aminoalkyl and R' is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocyclylalkyl, hydroxyalkyl, alkoxyalkyl, or aminoalkyl as defined above, e.g., -SO₂NH₂, methylaminosulfonyl, 2-dimethylaminosulfonyl, and the like.

One of ordinary skill in the art will recognize that some compounds of Formula I and Formula II can exist in different tautomeric and/or conformational and/or geometrical isomeric and/or optical isomeric forms. All of these forms, including cis isomers, trans isomers, diastereomeric mixtures, racemates, nonracemic mixtures of enantiomers, substantially pure, and pure enantiomers, are within the scope of the present invention. Substantially pure enantiomers contain no more than 5% w/w of the corresponding opposite enantiomer, e.g., no more than 2%, such as no more than 1%.

In one embodiment, the term "isomer" is meant to encompass optical isomers of the compound. In one embodiment, the term "isomer" is meant to encompass stereoisomers of the compound. In one embodiment, the term "isomer" is meant to encompass conformational isomers. In one embodiment, the term "isomer" is meant to encompass tautomers. It is to be understood that the present invention encompasses any optically-active, or stereoisomeric form, or mixtures thereof, and use of these for any application is to be considered within the scope of this invention.

In one embodiment, the compounds of Formulae I and II are substantially pure (E)-isomers. In another embodiment, the compounds of Formulae I and II are substantially pure (Z)-isomers. In another embodiment, the compounds of Formulae I and II are a mixture of (E) and the (Z) isomers. In one embodiment, the compounds of Formulae I and II are pure (E)-isomers. In another embodiment, the compounds of Formulae I and II are pure (Z)-isomers. In one embodiment, the compounds of Formulae I and II are substantially pure (R)-isomers. In another embodiment, the compounds of Formulae I and II are substantially pure (S)-isomers. In another embodiment, the compounds of Formulae I and II are a mixture of (R) and the (S) isomers. In one embodiment, the compounds of Formulae I and II are pure (R)-isomers. In another embodiment, the compounds of Formulae I and II are pure (S)-isomers.

As is known to an ordinary skilled artisan, optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example, by the formation of diastereoisomeric salts using an optically active acid or base or formation of covalent diastereomers.
Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid. Mixtures of diastereoisomers can be separated into their individual diastereomers on the basis of their physical and/or chemical differences by methods known to those skilled in the art, for example, by chromatography or fractional crystallization. The optically active bases or acids are then liberated from the separated diastereomeric salts. A different process for separation of optical isomers involves the use of chiral chromatography (e.g., chiral HPLC columns), with or without conventional derivation, optimally chosen to maximize the separation of the enantiomers. Suitable chiral HPLC columns are manufactured by Diacel, e.g., Chiracel OD and Chiracel OJ among many others, all routinely selectable. Enzymatic separations, with or without derivitization, are also useful. The optically active compounds of Formula I-II can likewise be obtained by utilizing optically active starting materials in chiral synthesis processes under reaction conditions which do not cause racemization.

[00161] In addition, one of ordinary skill in the art will recognize that the compounds can be used in different enriched isotopic forms, e.g., enriched in the content of $^2$H, $^3$H, $^{11}$C, $^{13}$C and/or $^{14}$C. In one particular embodiment, the compounds are deuterated. Such deuterated forms can be made via the procedure described in U.S. Patent Nos. 5,846,514 and 6,334,997. As described in U.S. Patent Nos. 5,846,514 and 6,334,997, deuteration can improve the efficacy and increase the duration of action of drugs.


[00163] Where applicable, the present invention also relates to useful forms of the compounds as disclosed herein, such as base free forms, and pharmaceutically acceptable salts or prodrugs of all the compounds of the present invention for which salts or prodrugs can be prepared. Pharmaceutically acceptable salts include those obtained by reacting the main compound, functioning as a base with an inorganic or organic acid to form a salt, for example, salts of hydrochloric acid, sulfuric acid,
phosphoric acid, methane sulfonic acid, camphor sulfonic acid, oxalic acid, maleic acid, succinic acid, citric acid, formic acid, hydrobromic acid, benzoic acid, tartaric acid, fumaric acid, salicylic acid, mandelic acid, and carbonic acid. Pharmaceutically acceptable salts also include those in which the main compound functions as an acid and is reacted with an appropriate base to form, e.g., sodium, potassium, calcium, magnesium, ammonium, and choline salts. Those skilled in the art will further recognize that acid addition salts of the claimed compounds may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods. Alternatively, alkali and alkaline earth metal salts can be prepared by reacting the compounds of the invention with the appropriate base via a variety of known methods.

[00164] The following are further examples of acid salts that can be obtained by reaction with inorganic or organic acids: acetates, adipates, alginates, citrates, aspartates, benzoates, benzenesulfonates, bisulfates, butyrates, camphorates, digluconates, cyclopentanepropionates, dodecylsulfates, ethanesulfonates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, fumarates, hydrobromides, hydroiodides, 2-hydroxy-ethanesulfonates, lactates, maleates, methanesulfonates, nicotinates, 2-naphthalenesulfonates, oxalates, palmoates, pectinates, persulfates, 3-phenylpropionates, picrates, pivalates, propionates, succinates, tartrates, thiocyanates, tosylates, mesylates and undecanoates.

[00165] For example, the pharmaceutically acceptable salt can be a hydrochloride, a hydrobromide, a hydroformate, a maleate or a sodium salt.

[00166] Preferably, the salts formed are pharmaceutically acceptable for administration to mammals. However, pharmaceutically unacceptable salts of the compounds are suitable as intermediates, for example, for isolating the compound as a salt and then converting the salt back to the free base compound by treatment with an alkaline reagent. The free base can then, if desired, be converted to a pharmaceutically acceptable acid addition salt.

[00167] One of ordinary skill in the art will also recognize that some of the compounds of Formula I and Formula II can exist in different polymorphic forms. As known in the art, polymorphism is an ability of a compound to crystallize as more than one distinct crystalline or "polymorphic" species. A polymorph is a solid crystalline phase of a compound with at least two different arrangements or polymorphic forms of that compound molecule in the solid state.
Polymorphic forms of any given compound are defined by the same chemical formula or composition and are as distinct in crystal structure as crystalline structures of two different chemical compounds.

In one embodiment, the compounds of Formula I and Formula II can exist in different solvate forms. Solvates of the compounds of the invention may form when solvent molecules are incorporated into the crystalline lattice structure of the compound molecule during the crystallization process. For example, a compound of Formula I or Formula II may exist in the form of a hydrate, such as, for example, a monohydrate, hemihydrate, sesquihydrate, dihydrate, trihydrate, or any combination thereof.

The term "prodrug" means a compound that is a drug precursor which upon administration to a subject undergoes chemical conversion by metabolic or chemical processes to yield a compound of the present invention. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.) the compounds of the present invention may be delivered in prodrug form. Thus, the present invention includes prodrugs of the disclosed compounds and methods of delivering the same. Prodrugs of a compound of the present invention may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound. Accordingly, prodrugs include, for example, compounds of the present invention wherein a hydroxy, amino, or carboxy group is bonded to any group that, when the prodrug is administered to a mammalian subject, cleaves to form a free hydroxyl, free amino, or carboxylic acid, respectively. Examples include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups; and alkyl, carbocyclic, aryl, and alkylaryl esters such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, cyclopropyl, phenyl, benzyl, and phenethyl esters, and the like. Such prodrugs are considered to be within the scope of this invention.

This invention provides, in other embodiments, metabolites of a compound of Formula I and Formula II. In one embodiment, the term "metabolite" refers to any substance produced from another substance by metabolism or a metabolic process.

In another aspect, the present invention relates to methods for preparing the compounds of Formula I and Formula II. The compounds of the present invention may be prepared by conventional methods, known to one or ordinary skill in the art. For example, some of the processes that can be used are given in the general reaction schemes outlined below. Modifications to these
exemplary reaction schemes will be readily apparent to those skilled in the art upon reading the present disclosure and examples which follow. All starting materials are commercially available or can be conventionally prepared from known starting materials, unless otherwise indicated.

General scheme for synthesis of HSDi compounds.

[00172] In one embodiment, the compounds of this invention are prepared according to the following scheme:

\[
\begin{align*}
\text{R}^2\text{Br} & \quad \text{Cul/L-proline} \\
\text{K}_2\text{CO}_3/\text{DMSO} & \quad \text{or} \\
\text{NaH}/\text{THF} & \\
\text{NBS} & \quad \text{THF} \\
\text{Pd}(\text{PPPh}_3)_4 & \quad \text{Cs}_2\text{CO}_3, \\
\text{DME}/\text{H}_2\text{O} & \\
\text{BBr}_3 & \quad \text{H}_2\text{O} \\
\end{align*}
\]

[00173] In one embodiment, the process for preparation of the compounds of this invention comprises an N-alkylation step of isoquinolines using alkyl bromide or aryl bromide (R²Br). In another embodiment, the N-alkylation can be achieved under dehydrogenation by NaH followed by addition of the corresponding alkylbromides. In another embodiment, for preparation of the compounds of this invention comprises bromination of the isoquinolinone at position 4 followed by substitution of the bromine by a substituted phenyl group [Ph(R³)_₅]. In another embodiment, the
compounds of this invention are prepared according to Example 1. In another embodiment, the compounds of this invention are prepared according to Example 2.

In one embodiment, the process for preparation of the compounds of this invention comprises a bromination of the naphthoid core template at position 4 followed by substitution of the bromine by a substituted phenyl group \([\text{Ph}(R^1)_n]\). In another embodiment, the compounds of this invention are prepared according to Example 1. In another embodiment, the compounds of this invention are prepared according to Example 2.

**Pharmaceutical Compositions**

The compounds of the invention can be administered alone or as an active ingredient of a formulation. Thus, the present invention also includes pharmaceutical compositions of compounds of Formula I and Formula II, containing, for example, one or more pharmaceutically acceptable carriers.

Numerous standard references are available that describe procedures for preparing various formulations suitable for administering the compounds according to the invention.

[00177] The mode of administration and dosage forms are closely related to the therapeutic amounts of the compounds or compositions which are desirable and efficacious for the given treatment application.

[00178] Suitable dosage forms include but are not limited to oral, rectal, sub-lingual, mucosal, nasal, ophthalmic, subcutaneous, intramuscular, intravenous, transdermal, spinal, intrathecal, intra-articular, intra-arterial, sub-arachinoid, bronchial, lymphatic, and intra-uterile administration, and other dosage forms for systemic delivery of active ingredients. Formulations suitable for oral administration are preferred.

[00179] To prepare such pharmaceutical dosage forms, the active ingredient may be mixed with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration.

[00180] In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed. Thus, for liquid oral preparations, such as, for example, suspensions, elixirs and solutions, suitable carriers and additives include water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like. For solid oral preparations such as, for example, powders, capsules and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. Due to their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form. If desired, tablets may be sugar coated or enteric coated by standard techniques.

[00181] For parenteral formulations, the carrier will usually comprise sterile water, though other ingredients, for example, ingredients that aid solubility or for preservation, may be included. Injectable solutions may also be prepared in which case appropriate stabilizing agents may be employed.
In some applications, it may be advantageous to utilize the active agent in a "vectorized" form, such as by encapsulation of the active agent in a liposome or other encapsulant medium, or by fixation of the active agent, e.g., by covalent bonding, chelation, or associative coordination, on a suitable biomolecule, such as those selected from proteins, lipoproteins, glycoproteins, and polysaccharides.

Treatment methods of the present invention using formulations suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets, or lozenges, each containing a predetermined amount of the active ingredient as, for example, a powder or granules. Optionally, a suspension in an aqueous liquor or a non-aqueous liquid may be employed, such as a syrup, an elixir, an emulsion, or a draught.

A tablet may be made by compression or molding, or wet granulation, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, with the active compound being in a free-flowing form such as a powder or granules which optionally is mixed with, for example, a binder, disintegrant, lubricant, inert diluent, surface active agent, or discharging agent. Molded tablets comprised of a mixture of the powdered active compound with a suitable carrier may be made by molding in a suitable machine.

A syrup may be made by adding the active compound to a concentrated aqueous solution of a sugar, for example sucrose, to which may also be added any accessory ingredient(s). Such accessory ingredient(s) may include flavorings, suitable preservative, agents to retard crystallization of the sugar, and agents to increase the solubility of any other ingredient, such as a polyhydroxy alcohol, for example glycerol or sorbitol.

Formulations suitable for parenteral administration may comprise a sterile aqueous preparation of the active compound, which preferably is isotonic with the blood of the recipient (e.g., physiological saline solution). Such formulations may include suspending agents and thickening agents and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose form.

Parenteral administration may comprise any suitable form of systemic delivery. Administration may for example be intravenous, intra-arterial, intrathecal, intramuscular, subcutaneous, intramuscular, intra-abdominal (e.g., intraperitoneal), etc., and may be effected by
infusion pumps (external or implantable) or any other suitable means appropriate to the desired administration modality.

[00188] Nasal and other mucosal spray formulations (e.g. inhalable forms) can comprise purified aqueous solutions of the active compounds with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal or other mucous membranes. Alternatively, they can be in the form of finely divided solid powders suspended in a gas carrier. Such formulations may be delivered by any suitable means or method, e.g., by nebulizer, atomizer, metered dose inhaler, or the like.

[00189] Formulations for rectal administration may be presented as a suppository with a suitable carrier such as cocoa butter, hydrogenated fats, or hydrogenated fatty carboxylic acids.

[00190] Transdermal formulations may be prepared by incorporating the active agent in a thixotropic or gelatinous carrier such as a cellulosic medium, e.g., methyl cellulose or hydroxyethyl cellulose, with the resulting formulation then being packed in a transdermal device adapted to be secured in dermal contact with the skin of a wearer.

[00191] In addition to the aforementioned ingredients, formulations of this invention may further include one or more accessory ingredient(s) selected from, for example, diluents, buffers, flavoring agents, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants), and the like.

[00192] The formulations of the present invention can have immediate release, sustained release, delayed-onset release or any other release profile known to one skilled in the art.

**Methods of Treatment**

[00193] According to other aspects of the present invention, methods for treating a condition that responds to a hydroxysteroid dehydrogenase inhibitor are provided. In certain embodiments, the compounds of the present invention may be useful as hydroxysteroid dehydrogenase inhibitors. For example, the compounds of the present invention may be useful as AKR1C inhibitors, for example AKR1C3 inhibitors. In one embodiment, the compounds of this invention may be useful as selective inhibitors of AKR1C3.
For example, some embodiments provide methods of treating a condition that responds to a hydroxysteroid dehydrogenase inhibitor comprising administering to a patient in need thereof an effective amount of a compound of the present invention.

In certain embodiments, the present invention provides methods of treatment of conditions related to cancer. For example, the present invention, in certain embodiments, provides methods of treatment of hormone dependent or hormone independent cancers. Non-limiting examples of hormone dependent cancers include prostate cancer, breast cancer, uterine fibroids including myomas and uterine cancer including cervical and endometrial cancers. Non-limiting examples of hormone independent cancers include lung cancer including non-small cell lung cancer, bladder cancer, colon cancer, leukemias including acute myelogenous leukemia, and/or lymphoma.

In one embodiment of this invention, a method of treating a condition is the method of treating prostate cancer. As used herein, the term "prostate cancer" refers to prostate cancer, primary prostate cancer, advanced prostate cancer, metastatic prostate cancer, hormone naïve prostate cancer, refractory prostate cancer and/or castration resistant prostate cancer (CRPC) or any combination thereof.

In another embodiment of this invention, a method of treating a condition is the method of treating breast cancer. As used herein, the term "breast cancer" refers to breast cancer; metastatic breast cancer; advanced breast cancer; refractory breast cancer; AR-positive breast cancer; ER-positive breast cancer, wherein ER-positive breast cancer may refer to ER-alpha-positive breast cancer and/or ER-beta-positive breast cancer; AR-positive refractory breast cancer; ER-positive refractory breast cancer; AR-positive metastatic breast cancer; ER-positive metastatic breast cancer; triple negative breast cancer; and/or breast cancer that has failed SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®), ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof.

As used herein, the term "metastasis" refers to the transfer of a disease from one organ or part thereof to another not directly connected with it. Metastasis can occur for example as a result of transfer of malignant cells from one organ (for example breast) to other organs.

In one embodiment, this invention provides a method of treating a subject suffering from prostate cancer, comprising the step of administering to said subject a compound of this invention,
or its isomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat prostate cancer in the subject.

[00200] As used herein, the term "treating" refers to treating, preventing, suppressing, inhibiting or delaying the progression of.

[00201] In one embodiment, the methods of this invention are directed to treating prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of prostate cancer. In another embodiment, this invention is directed to suppressing prostate cancer. In another embodiment, this invention is directed to reducing the incidence of prostate cancer. In another embodiment, this invention is directed to reducing the severity of prostate cancer. In another embodiment, this invention is directed to inhibiting prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with prostate cancer.

[00202] In one embodiment, the methods of this invention are directed to treating primary prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting primary prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of primary prostate cancer. In another embodiment, this invention is directed to suppressing primary prostate cancer. In another embodiment, this invention is directed to reducing the incidence of primary prostate cancer. In another embodiment, this invention is directed to reducing the severity of primary prostate cancer. In another embodiment, this invention is directed to inhibiting primary prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with primary prostate cancer.

[00203] In one embodiment, the methods of this invention are directed to treating hormone naive prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting hormone naive prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of hormone naive
prostate cancer. In another embodiment, this invention is directed to suppressing hormone naïve prostate cancer. In another embodiment, this invention is directed to reducing the incidence of hormone naïve prostate cancer. In another embodiment, this invention is directed to reducing the severity of hormone naïve prostate cancer. In another embodiment, this invention is directed to inhibiting hormone naïve prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with hormone naïve prostate cancer.

[00204] In one embodiment, the methods of this invention are directed to treating advanced prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting advanced prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of advanced prostate cancer. In another embodiment, this invention is directed to suppressing advanced prostate cancer. In another embodiment, this invention is directed to reducing the incidence of advanced prostate cancer. In another embodiment, this invention is directed to reducing the severity of advanced prostate cancer. In another embodiment, this invention is directed to inhibiting advanced prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with advanced prostate cancer.

[00205] In one embodiment, the methods of this invention are directed to treating refractory prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting refractory prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of refractory prostate cancer. In another embodiment, this invention is directed to suppressing refractory prostate cancer. In another embodiment, this invention is directed to reducing the incidence of refractory prostate cancer. In another embodiment, this invention is directed to reducing the severity of refractory prostate cancer. In another embodiment, this invention is directed to inhibiting refractory prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with refractory prostate cancer.

[00206] In one embodiment, the methods of this invention are directed to treating metastatic prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting metastatic prostate cancer. In one
embodiment, the methods of this invention are directed to palliative treatment of metastatic prostate cancer. In another embodiment, this invention is directed to suppressing metastatic prostate cancer. In another embodiment, this invention is directed to reducing the incidence of metastatic prostate cancer. In another embodiment, this invention is directed to reducing the severity of metastatic prostate cancer. In another embodiment, this invention is directed to inhibiting metastatic prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with metastatic prostate cancer.

[00207] In one embodiment, the methods of this invention are directed to treating castration resistant prostate cancer. In one embodiment, the methods of this invention are directed to suppressing, reducing the incidence, reducing the severity, or inhibiting castration resistant prostate cancer. In one embodiment, the methods of this invention are directed to palliative treatment of castration resistant prostate cancer. In another embodiment, this invention is directed to suppressing castration resistant prostate cancer. In another embodiment, this invention is directed to reducing the incidence of castration resistant prostate cancer. In another embodiment, this invention is directed to reducing the severity of castration resistant prostate cancer. In another embodiment, this invention is directed to inhibiting castration resistant prostate cancer. In another embodiment, this invention is directed to increasing the survival of a subject with castration resistant prostate cancer.

[00208] In one embodiment, this invention provides a method of delaying the progression of prostate cancer in a subject suffering from prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of prostate cancer in the subject. In another embodiment, this invention provides a method of delaying the progression of primary prostate cancer in a subject suffering from primary prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of primary prostate cancer in the subject. In still another embodiment, this invention provides a method of delaying the progression of hormone naïve prostate cancer in a subject suffering from prostate cancer, comprising the step of administering to
said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of prostate cancer in the subject. In another embodiment, this invention provides a method of delaying the progression of advanced prostate cancer in a subject suffering from advanced prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of advanced prostate cancer in the subject. In one embodiment, this invention provides a method of delaying the progression of metastatic prostate cancer in a subject suffering from metastatic prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of metastatic prostate cancer in the subject. In another embodiment, this invention provides a method of delaying the progression of refractory prostate cancer in a subject suffering from refractory prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of refractory prostate cancer in the subject. In one embodiment, this invention provides a method of delaying the progression of castration resistant prostate cancer in a subject suffering from castration resistant prostate cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of castration resistant prostate cancer in the subject.

[00209] In one embodiment, the methods of this invention make use of compounds of Formula I. In another embodiment, the methods of this invention make use of compounds of Formula II. In yet another embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with LH-RH agonist. In still another embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with an anti-
androgen. In a further embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00210] In one embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with leuprolide acetate (Lupron®).

[00211] In one embodiment, the methods of treating prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostate cancer make use of 6-methoxy-4-(3,4,5-
trifluorophenyl)isoquinolin-1(2 H\textsuperscript{+})-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostate cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H\textsuperscript{+})-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostate cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostate cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H\textsuperscript{+})-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00212] In one embodiment, the methods of treating primary prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating primary prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating primary prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating primary prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H\textsuperscript{+})-one (45), or its prodrug, tautomer, isomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating primary prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H\textsuperscript{+})-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating primary prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H\textsuperscript{+})-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating primary prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H\textsuperscript{+})-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating primary prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating primary prostate cancer make
use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating primary prostate cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating primary prostate cancer make use of 4-(6-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00213] In one embodiment, the methods of treating hormone naïve prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating hormone naïve prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating hormone naïve prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-l,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating hormone naïve prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating hormone naïve prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating hormone naïve prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating hormone naïve prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating hormone naïve prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
one embodiment, the methods of treating hormone naïve prostate cancer make use of 6-methoxy-4- (3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating hormone naïve prostate cancer make use of 4-(4-chloro-3- fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating hormone naïve prostate cancer make use of 4-(6-hydroxy-l- oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating hormone naïve prostate cancer make use of 4-(3,4- difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00214] In one embodiment, the methods of treating advanced prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating advanced prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating advanced prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-l,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating advanced prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating advanced prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating advanced prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced prostate cancer make use of 2-fluoro-5-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal,
N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced prostate cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced prostate cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced prostate cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced prostate cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00215] In one embodiment, the methods of treating metastatic prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating metastatic prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating metastatic prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating metastatic prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating metastatic prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating metastatic prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating metastatic prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile.
(84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, 
N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic prostate cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic prostate cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic prostate cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydrisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic prostate cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00216] In one embodiment, the methods of treating refractory prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating refractory prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating refractory prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating refractory prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating refractory prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating refractory prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating refractory
prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory prostate cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory prostate cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory prostate cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00217] In one embodiment, the methods of treating castration resistant prostate cancer make use of a compound of Formula I. In another embodiment, the methods of treating castration resistant prostate cancer make use of a compound of Formula II. In one embodiment, the methods of treating castration resistant prostate cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating castration resistant prostate cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating castration resistant prostate cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating castration resistant prostate cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating castration resistant prostate cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug,
isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating castration resistant prostate cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating castration resistant prostate cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 \(H\))-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating castration resistant prostate cancer make use of 24-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 \(H\))-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating castration resistant prostate cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating castration resistant prostate cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 \(H\))-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof.

[00218] In one embodiment, the methods of this invention make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel,
cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00219] In one embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In another embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another
embody, the methods of this invention make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00221] In one embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone). In still another embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer,
tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00222] In one embodiment, the methods of this invention make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00223] In one embodiment, the methods of this invention make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite,
pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00224] In one embodiment, the methods of this invention make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide),
chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, Adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00225] In one embodiment, the methods of this invention make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminoglutethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, Adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00226] In one embodiment, the methods of this invention make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its
prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 4-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminogluthethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00227] In one embodiment, the methods of this invention make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with LH-RH agonist. In another embodiment, the methods of this invention make use 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with leuprolide acetate (Lupron®). In still another embodiment, the methods of this invention make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with an anti-androgen. In a further embodiment, the methods of this invention make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in combination with gonadotropin releasing hormone agonists (e.g., leuprolide) or antagonists (e.g., degarelix), anti-androgens (e.g., bicalutamide, nilutamide, flutamide, enzalutamide (MDV3100), ketoconazole, aminogluthethamide), chemotherapeutic agents (e.g., docetaxel, paclitaxel, cabazitaxel, adriamycin, mitoxantrone, estramustine, cyclophosphamide), kinase inhibitors (imatinib (Gleevec®) or gefitinib (Iressa®)) or other prostate cancer therapies (e.g., vaccines (sipuleucel-T (Provenge®), GVAX, etc.), herbal (PC-SPES) or a lyase inhibitor (abiraterone).

[00228] As used herein, the term "primary prostate cancer" refers to prostate cancer that develops in the prostate.
As used herein, the term "hormone naïve prostate cancer" refers to prostate cancer that has not been treated with hormones such as estrogens or androgens, agents that block hormones such as LH-RH agonists or antagonists like leuprolide acetate (Lupron®) and degarelix, respectively.

As used herein, the term "refractory prostate cancer" refers to a prostate cancer that has not responded to treatment. In another embodiment, a "refractory prostate cancer" is a prostate cancer resistant to treatment. In one embodiment, refractory prostate cancer is refractory metastatic prostate cancer. In one embodiment, refractory prostate cancer has not responded to chemical/surgical castration, i.e., the reduction of available androgen/testosterone/DHT by chemical or surgical means. As used herein, "refractory prostate cancer", may in some embodiments be referred to as "androgen-independent prostate cancer". As used herein, "refractory prostate cancer", may in some embodiment be referred to as "castration resistant prostate cancer".

As used herein, the term "castration resistant prostate cancer" (CRPC) refers to advanced prostate cancer which was developed despite ongoing androgen deprivation therapy (ADT) and/or surgical castration. In another embodiment, ADT refers to treatment consisting of leuprolide acetate (Lupron®).

As used herein, the term "advanced prostate cancer" refers to metastatic cancer having originated in the prostate, and having widely metastasized to beyond the prostate such as the surrounding tissues to include the seminal vesicles the pelvic lymph nodes or bone, or to other parts of the body. Prostate cancer pathologies are graded with a Gleason grading from 1 to 5 in order of increasing malignancy. In another embodiment, patients with significant risk of progressive disease and/or death from prostate cancer should be included in the definition and that any patient with cancer outside the prostate capsule with disease stages as low as IIB clearly has "advanced" disease.

As used herein, the term "metastatic prostate cancer" refers to prostate cancer that has spread from the place where it first started to another place in the body. Other places in the body include but are not limited to, the lungs, liver and bone.
In further embodiments, the present invention provides methods of treatment of conditions related to the prostate. For example, the present invention provides methods of treatment of treatment of prostatic dysplasia, prostatic hyperplasia, benign prostate hyperplasia (BPH), and prostatitis. The present invention also provides methods of treatment of precancerous precursors of prostate adenocarcinoma. In one embodiment, the precancerous precursor of prostate adenocarcinoma is prostate intraepithelial neoplasia (PIN). In one embodiment, the PIN is high-grade PIN (HGPIN).

In some embodiments, the present invention provides methods of treatment conditions such as, but not limited to, prostate cancer, benign prostate hyperplasia (BPH), lung cancer, acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, breast cancer, endometriosis, myeloma and leiomyoma.

In one embodiment, this invention provides a method of treating a subject suffering from breast cancer, comprising the step of administering to said subject a compound of this invention, or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat breast cancer in the subject.

In one embodiment, the compounds of this invention are useful for a) treating a subject suffering from breast cancer; b) treating a subject suffering from metastatic breast cancer; c) treating a subject suffering from refractory breast cancer; d) treating a subject suffering from AR-positive breast cancer; e) treating a subject suffering from AR-positive refractory breast cancer; f) treating a subject suffering from AR-positive metastatic breast cancer; g) treating a subject suffering from triple negative breast cancer; h) treating a subject suffering from advanced breast cancer; i) treating a subject suffering breast cancer that has failed SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof; j) treating, preventing, suppressing or inhibiting metastasis in a subject suffering from breast cancer; k) prolonging survival of a subject with breast cancer, and/or l) prolonging the progression-free survival of a subject with breast cancer.

In one embodiment, a "refractory breast cancer" is a breast cancer that has not responded to treatment. In another embodiment, a "refractory breast cancer" is a breast cancer resistant to
treatment. In one embodiment, refractory breast cancer is refractory metastatic breast cancer. In one embodiment, refractory breast cancer has not responded to treatment with anthracyclines, taxanes, capecitabine, ixabepilone, SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof.

[00239] In one embodiment, a "triple negative breast cancer" is defined by lack of expression of estrogen, progesterone, and ErbB2 (also known as human epidermal growth factor receptor 2 (HER2)) receptors. This subgroup accounts for 15% of all types of breast cancer. This subtype of breast cancer is clinically characterized as more aggressive and less responsive to standard treatment and associated with poorer overall patient prognosis.

[00240] In one embodiment, the methods of this invention are directed to treating a subject suffering from AR-positive breast cancer, regardless of grade, stage or prior treatments.

[00241] In one embodiment, the methods of this invention are directed to treating a subject suffering from ER-positive breast cancer, regardless of grade, stage or prior treatments.

[00242] In one embodiment, the methods of this invention are first, second, third, or fourth line therapies for breast cancer. A first line therapy refers to a medical therapy recommended for the initial treatment of a disease, sign or symptom. A second line therapy therapy is given when initial treatment (first-line therapy) does not work, or stops working. Third line therapy is given when both initial treatment (first-line therapy) and subsequent treatment (second-line therapy) does not work, or stop working, etc.

[00243] In one embodiment, this invention provides a method of delaying the progression of breast cancer in a subject suffering from breast cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of breast cancer in the subject. In another embodiment, this invention provides a method of delaying the progression of advanced breast cancer in a subject suffering from advanced breast cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of advanced breast cancer in the subject. In one
embodiment, this invention provides a method of delaying the progression of metastatic breast cancer in a subject suffering from metastatic breast cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, $N$-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of metastatic breast cancer in the subject. In one embodiment, this invention provides a method of delaying the progression of hormone-resistant breast cancer in a subject suffering from hormone-resistant breast cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, $N$-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of hormone-resistant breast cancer in the subject.

[00244] In one embodiment, the methods of this invention make use of compounds of Formula I. In another embodiment, the methods of this invention make use of compounds of Formula II. In yet another embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with LH-RH agonist. In a further embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II in combination with leuprolide acetate (Lupron®).

[00245] In one embodiment, the methods of treating breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating breast cancer make use of 4-((3,4,5-trifluorophenyl)naphthalene-1,6-diyl) (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, $N$-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer make use of 6-hydroxy-2-methyl-4-((3,4,5-trifluorophenyl)isoquinolin-4(2 $H$)-one) (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, $N$-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating breast cancer make use of 2-cyclopropyl-6-hydroxy-4-((3,4,5-trifluorophenyl)isoquinolin-4(2 $H$)-one) (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, $N$-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating breast cancer make use of
6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2\textsubscript{\textit{H}})-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (77) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{\textit{H}})-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{\textit{H}})-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating advanced breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating advanced breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating advanced breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00246] In one embodiment, the methods of treating advanced breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating advanced breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating advanced breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating advanced breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{\textit{H}})-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
any combination thereof. In still another embodiment, the methods of treating advanced breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \text{H})-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating advanced breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 \text{H})-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating advanced breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \text{H})-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 \text{H})-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating advanced breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 \text{H})-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00247] In one embodiment, the methods of treating metastatic breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating metastatic breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating metastatic breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \text{H})-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating metastatic breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \text{H})-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or
any combination thereof. In still another embodiment, the methods of treating metastatic breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating metastatic breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2\textsubscript{H})-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating metastatic breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{H})-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating metastatic breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2\textsubscript{H})-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00248] In one embodiment, the methods of treating refractory breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating refractory breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating refractory breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating refractory breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\textsubscript{H})-one (67), or its prodrug,
isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating refractory breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating refractory breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating refractory breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00249] In one embodiment, the methods of treating AR-positive breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating AR-positive breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating AR-positive breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating AR-positive breast cancer
make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating AR-positive breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating AR-positive breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating AR-positive breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating refractory breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating AR-positive refractory breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt,
polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating AR-positive refractory breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating AR-positive refractory breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating AR-positive refractory breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating AR-positive refractory breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive refractory breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00251] In one embodiment, the methods of treating AR-positive metastatic breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating AR-positive metastatic breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating AR-positive metastatic breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating
AR-positive metastatic breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating AR-positive metastatic breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating AR-positive metastatic breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating AR-positive metastatic breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00252] In one embodiment, the methods of treating triple negative breast cancer make use of a compound of Formula I. In another embodiment, the methods of treating triple negative breast cancer make use of a compound of Formula II. In one embodiment, the methods of treating triple negative
breast cancer make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating triple negative breast cancer make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating triple negative breast cancer make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating triple negative breast cancer make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating triple negative breast cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating triple negative breast cancer make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating triple negative breast cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating triple negative breast cancer make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating triple negative breast cancer make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating triple negative breast cancer make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00253] In one embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab

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emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of a compound of Formula I. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of a compound of Formula II. In one embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant...
treatment, or any combination thereof make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isooquinolin-1(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®), ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating breast cancer that failed to respond to SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
In one embodiment, this invention provides a method of treating a subject suffering from uterine cancer, comprising the step of administering to said subject a compound of this invention, or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat uterine cancer in the subject. In one embodiment, uterine cancer refers to an endometrial cancer. In another embodiment, uterine cancer refers to a cervical cancer.

In one embodiment, the compounds of this invention are useful for a) treating a subject suffering from uterine cancer; b) treating a subject suffering from metastatic uterine cancer; c) treating a subject suffering from refractory uterine cancer; d) treating a subject suffering from advanced uterine cancer; e) treating, preventing, suppressing or inhibiting metastasis in a subject suffering from uterine cancer; f) prolonging survival of a subject with uterine cancer, and/or i) prolonging the progression-free survival of a subject with uterine cancer.

In one embodiment, a "refractory uterine cancer" is a uterine cancer that has not responded to treatment. In another embodiment, a "refractory uterine cancer" is a uterine cancer resistant to treatment. In one embodiment, refractory uterine cancer is refractory metastatic uterine cancer.

In one embodiment, this invention provides a method of delaying the progression of uterine cancer or uterine fibroids in a subject suffering from uterine cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of uterine cancer in the subject. In another embodiment, this invention provides a method of delaying the progression of advanced uterine cancer in a subject suffering from advanced uterine cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of advanced uterine cancer in the subject. In one embodiment, this invention provides a method of delaying the progression of metastatic uterine cancer in a subject suffering from metastatic uterine cancer, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination
thereof, or a composition comprising the same in an amount effective to delay the progression of metastatic uterine cancer in the subject.

[00258] In one embodiment, this invention provides a method of treating a subject suffering from uterine fibroids, comprising the step of administering to said subject a compound of this invention, or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat uterine fibroids in the subject. In one embodiment, uterine fibroids refers to myomas.

[00259] In one embodiment, the compounds of this invention are useful for treating a subject suffering from uterine fibroids.

[00260] In one embodiment, this invention provides a method of delaying the progression of uterine cancer or uterine fibroids in a subject suffering from uterine cancer or uterine fibroids, comprising the step of administering to said subject a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, or a composition comprising the same in an amount effective to delay the progression of uterine cancer or uterine fibroids in the subject.

[00261] In one embodiment, the methods of this invention make use of compounds of Formula I or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of this invention make use of compounds of Formula II or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In yet another embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II or their isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in combination with LH-RH agonist, in an amount effective to treat uterine cancer or uterine fibroids. In a further embodiment, the methods of this invention make use of compounds of Formula I and/or Formula II or their isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in combination with leuprolide acetate (Lupron®), in an amount effective to treat uterine cancer or uterine fibroids.
In one embodiment, the methods of treating uterine cancer or uterine fibroids and make use of a compound of Formula I or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer or uterine fibroids make use of a compound of Formula II or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, N-oxide, hydrate, prodrug or metabolite or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In one embodiment, the methods of treating uterine cancer or uterine fibroids make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer or uterine fibroids make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer or uterine fibroids make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In yet another embodiment, the methods of treating uterine cancer or uterine fibroids make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In still another embodiment, the methods of treating uterine cancer or uterine fibroids make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In a further embodiment, the methods of treating uterine cancer make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In one embodiment, the methods of treating uterine cancer or uterine fibroids make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (77), or its prodrug, isomer, tautomer, metabolite,
pharmacologically acceptable salt, polymorph, crystal, \(\text{N-oxide}\), hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer make use of \(4-(4\text{-chloro-3-fluorophenyl})-6\text{-hydroxyisoquinolin-1(2} H\text{-)}\text{one}\) (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(\text{N-oxide}\), hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer make use of \(4-(6\text{-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl})\text{benzonitrile}\) (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(\text{N-oxide}\), hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids. In another embodiment, the methods of treating uterine cancer make use of \(4-(3,4\text{-difluorophenyl})-6\text{-hydroxyisoquinolin-1(2} H\text{-)}\text{one}\) (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(\text{N-oxide}\), hydrate or any combination thereof, in an amount effective to treat uterine cancer or uterine fibroids.

[00263] In one embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, \(\text{N-oxide}\), metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to inhibit AKR1C3. In another embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, \(\text{N-oxide}\), metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to decrease androgen levels in said subject. In another embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, \(\text{N-oxide}\), metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to suppress the transcriptional activity of the androgen receptor in said subject. In a further embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, \(\text{N-oxide}\), metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to effect a change in an androgen-dependent condition.
In one embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to inhibit AKR1C3. In another embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to decrease estrogen levels in said subject. In a further embodiment, this invention provides a method of hormone therapy comprising the step of contacting an AKR1C3 enzyme of a subject with a compound of this invention or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, metabolite, prodrug or any combination thereof, or a composition comprising the same, in an amount effective to effect a change in an estrogen-dependent condition.

In another embodiment, this invention provides for the use of a compound as herein described, or its prodrug, analog, isomer, tautomer, metabolite, derivative, pharmaceutically acceptable salt, pharmaceutical product, polymorph, crystal, N-oxide, hydrate or any combination thereof, for treating, reducing the severity of, reducing the incidence of, or delaying the onset of lung cancer.

In another embodiment, this invention provides for the use of a compound as herein described, or its prodrug, analog, isomer, tautomer, metabolite, derivative, pharmaceutically acceptable salt, pharmaceutical product, polymorph, crystal, N-oxide, hydrate or any combination thereof, for treating, reducing the severity of, reducing the incidence of, and delaying the onset of non small cell lung cancer.

There are 3 PPAR genes described in xenopus, PPAR α, β, and γ. (Dreyer, 1992). Human PPAR-a has been recently described, although putatively described as a single gene, unlike the xenopus genes, with no closely related family members (Sher, 1993). In the Xenopus system, PPAR α, β and γ show very high amino acid identity in their DNA binding and ligand binding regions, and correspondingly have shown similar abilities to activate the same target DNA sequences, and to be activated by clofibrate and peroxisome proliferator agents (Dreyer, 1992). However, human PPAR α, and now γ, have different chromosomal locations. All species α, β, and γ show differences in A/B and
D regions, and all species demonstrate that α, β, and γ have different tissue expression patterns. Amino acid identity between the xenopus γ and human γ receptors, in critical regions, is high enough to expect similar function, and indeed, studies on the human PPAR α by Sher et al. (1993) show it has the same ability as xenopus and murine PPAR α to activate the same reporters. Thus, PPAR-γ, is likely to activate these reporters in a manner similar to xenopus PPAR-γ, based on the highly conserved identity in critical regions. However, differences in sequence are significant enough to expect differences in vivo.

[00268] In some embodiments, the present invention provides a method for treating, reducing the incidence, delaying the onset or progression, or reducing and/or abrogating the symptoms associated with a metabolic disease in a subject, comprising the step of administering to said subject a compound of this invention, or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat symptoms associated with a metabolic disease in the subject.

[00269] In one embodiment, this invention provides a method of treating a subject suffering from obesity-associated metabolic disorder, comprising the step of administering to said subject a compound of this invention, or its isomer, tautomer, pharmaceutically acceptable salt, pharmaceutical product, crystal, hydrate, N-oxide, prodrug, metabolite or any combination thereof, or a composition comprising the same in an amount effective to treat obesity-associated metabolic disorder in the subject.

[00270] In one embodiment, the compounds of this invention are useful for a) treating a subject suffering from obesity; b) preventing obesity in a subject; c) treating a subject suffering from obesity-associated metabolic disorder; d) preventing obesity-associated metabolic disorder in a subject; e) inducing anti-proliferative effects in a subject; f) treating a subject suffering from a prostaglandin-associated metabolic disorders; and g) preventing prostaglandin-associated metabolic disorders.

[00271] In one embodiment, the methods of treating obesity make use of a compound of Formula I. In another embodiment, the methods of treating obesity make use of a compound of Formula II. In one embodiment, the methods of treating obesity make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph,
crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \( \text{H} \))-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating obesity make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \( \text{H} \))-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating obesity make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \( \text{H} \))-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating obesity make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 \( \text{H} \))-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating obesity make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 \( \text{H} \))-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 \( \text{H} \))-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 \( \text{H} \))-one (220) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00272] In one embodiment, the methods of preventing obesity make use of a compound of Formula I. In another embodiment, the methods of preventing obesity make use of a compound of Formula II. In one embodiment, the methods of preventing obesity make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite,
pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of preventing obesity make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of preventing obesity make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of preventing obesity make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of preventing obesity make use of 2-fluoro-5-(6-hydroxy-l-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity make use of 4-(6-hydroxy-l-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

In one embodiment, the methods of treating obesity-associated metabolic disorder make use of a compound of Formula I. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of a compound of Formula II. In one embodiment, the methods of treating
obesity-associated metabolic disorder make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating obesity-associated metabolic disorder make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating obesity-associated metabolic disorder make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating obesity-associated metabolic disorder make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating obesity-associated metabolic disorder make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating obesity-associated metabolic disorder make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
In one embodiment, the methods of preventing obesity-associated metabolic disorder may use of a compound of Formula I. In another embodiment, the methods of preventing obesity-associated metabolic disorder may use of a compound of Formula II. In one embodiment, the methods of preventing obesity-associated metabolic disorder may use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity-associated metabolic disorder may use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of preventing obesity-associated metabolic disorder may use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of preventing obesity-associated metabolic disorder may use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of preventing obesity-associated metabolic disorder may use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of preventing obesity-associated metabolic disorder may use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity-associated metabolic disease may use of or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity-associated metabolic disease may use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity-associated metabolic disease may use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing obesity-associated metabolic disease may use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
metabolic disease make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2\(\text{H}\))-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof.

[00275] In one embodiment, the methods of inducing an anti-proliferative effect make use of a compound of Formula I. In another embodiment, the methods of inducing an anti-proliferative effect make use of a compound of Formula II. In one embodiment, the methods of inducing an anti-proliferative effect make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In another embodiment, the methods of inducing an anti-proliferative effect make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\(\text{H}\))-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of inducing an anti-proliferative effect make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\(\text{H}\))-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In still another embodiment, the methods of inducing an anti-proliferative effect make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\(\text{H}\))-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In a further embodiment, the methods of inducing an anti-proliferative effect make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2\(\text{H}\))-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In one embodiment, the methods of inducing an anti-proliferative effect make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In another embodiment, the methods of inducing an anti-proliferative effect make use of or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\(\text{H}\))-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In another embodiment, the methods of inducing an anti-proliferative effect make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\(\text{H}\))-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, \(N\)-oxide, hydrate or any combination thereof. In another embodiment, the methods of inducing an anti-proliferative effect make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-
yl)benzonitrile (108), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In another embodiment, the methods of inducing an anti-proliferative effect make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 *H*)-one (220), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof.

[00276] In one embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of a compound of Formula I. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of a compound of Formula II. In one embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 *H*)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 *H*)-one (67), or its prodrug, isomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In still another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 *H*)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In a further embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 *H*)-one (81), tautomer, or its prodrug, isomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In one embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 *H*)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, *N*-oxide, hydrate or any combination thereof. In
another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 2-fluoro-5-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (108) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(6-hydroxy-l-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00277] In one embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of a compound of Formula I. In another embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of a compound of Formula II. In one embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), or a prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In yet another embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In still another embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In a further embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of or 2-fluoro-5-(6-hydroxy-l-oxo-1,2-dihydroisoquinolin-4-
yl)benzonitrile (84), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In one embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of preventing prostaglandin-associated metabolic disorders make use of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (106), or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof. In another embodiment, the methods of treating prostaglandin-associated metabolic disorders make use of 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00278] As used herein, the term "obesity-associated metabolic disorder" refers, in one embodiment, to a disorder which results from, is a consequence of, is exacerbated by or is secondary to obesity. Non-limiting examples of such a disorder are osteoarthritis, Type II diabetes mellitus, increased blood pressure, stroke, and heart disease.

[00279] As used herein, the term "prostaglandin-associated metabolic disorder" refers, in one embodiment, to a disorder which results from, is a consequence of, or is exacerbated by changes in prostaglandin levels.

[00280] Cholesterol, triacylglycerol and other lipids are transported in body fluids by lipoproteins which may be classified according to their density, for example, the very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

[00281] It has been shown that high levels of LDL cholesterol in the blood correlate with atherosclerosis which is a progressive disease characterized in part by sedimentation of lipids in inner
walls of arteries, particularly of coronary arteries. It has also been shown that a high blood level of LDL cholesterol correlates with coronary heart disease. Also, a negative correlation exists between blood levels of HDL cholesterol and coronary heart disease.

[00282] The level of total cholesterol in blood, which is the sum of HDL cholesterol, LDL cholesterol, VLDL cholesterol and chylomicron cholesterol, is not necessarily predictive of the risk of coronary heart disease and atherosclerosis.

[00283] The correlation between atherosclerosis and LDL cholesterol levels, however, is much higher than a similar correlation between atherosclerosis and total serum cholesterol levels.

[00284] In one embodiment, this invention provides methods of use of the compounds as herein described for improving the lipid profile and/or reducing the circulating lipid levels in a subject. In some embodiments, according to this aspect of the invention, the subject suffers from one or more conditions selected from the group consisting of: atherosclerosis and its associated diseases, premature aging, Alzheimer's disease, stroke, toxic hepatitis, viral hepatitis, peripheral vascular insufficiency, renal disease, and hyperglycemia, and the invention provides for the administration of a compound or composition comprising the same, as herein described, which in some embodiments positively affects a lipid profile in the subject, which is one means by which the method is useful in treating the indicated diseases, disorders and conditions.

[00285] In one embodiment the invention provides for the treatment of atherosclerosis and its associated diseases, such as for example, cardiovascular disorders, cerebrovascular disorders, peripheral vascular disorders, intestinal vascular disorders, or combinations thereof.

[00286] In one embodiment cardiovascular disorders comprise of hypertension (HTN), coronary artery disease (CAD) or myocardial perfusion. In another embodiment this invention provides methods of use of the compounds as herein described for treating arteriosclerosis. In another embodiment this invention provides methods of use of the compounds as herein described for lowering blood pressure. In another embodiment this invention provides methods of use of the compounds as herein described for treating cardiac diseases and disorders comprising cardiomyopathy and cardiac dysfunctions such as myocardial infarction, cardiac hypertrophy and congestive heart failure. In another embodiment this
invention provides methods of use of the compounds as herein described for cardioprotection comprising cardioprotection in insulin resistance; treating diabetes type I ans II, metabolic syndrome, syndrome X and/or high blood pressure.

[00287] In one embodiment, the invention provides a method of treating, preventing, reducing the risk of mortality from cardiovascular and/or cerebrovascular disease in a subject, comprising administering a compound of this invention or its prodrug, analog, isomer, tautomer, metabolite, derivative, pharmaceutically acceptable salt, pharmaceutical product, polymorph, crystal, N-oxide, hydrate or any combination thereof, or a pharmaceutical composition comprising the same. In one embodiment, the method of treating cardiovascular and/or cerebrovascular disease makes use of a compound of Formula I. In another embodiment, the method of treating cardiovascular and/or cerebrovascular disease makes use of a compound of Formula II.

[00288] In one embodiment, compounds of this invention reduce LDL and total cholesterol levels. In another embodiment a compound of Formula I and/or a compound of Formula II reduces LDL and total cholesterol levels in a subject. In one embodiment, the compound is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin- 1(2 H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin- 1(2H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or their prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00289] In another embodiment, compounds of Formulae I and II are co-administered with HDL-elevating agents. In another embodiment, a compound of this invention is co-administered with an HDL-elevating agent. In another embodiment, HDL-elevating agents include niacin. In another embodiment the HDL-elevating agents include fibrates including gemfibrozil (Lopid), thiourea based gemfibrozil analogues, and fenofibrate (TriCor®). In another embodiment, HDL-elevating agents
include statins. In another embodiment, HDL-elevating agents include l-hydroxyalkyl-3-phenylthiourea, and analogs thereof.

[00290] In one embodiment, this invention provides a method of reducing circulating lipid levels in a subject, said method comprising administering a compound of this invention or its isomer, tautomer, prodrug, metabolite, pharmaceutically acceptable salt, hydrate, N-oxyde, or any combination thereof, or a composition comprising the same. In one embodiment, the subject suffers from atherosclerosis and its associated diseases, premature aging, Alzheimer’s disease, stroke, toxic hepatitis, viral hepatitis, peripheral vascular insufficiency, renal disease, hyperglycemia, or any combination thereof. In one embodiment, the compound is characterized by the structure of, 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or their prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxyde, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00291] In one embodiment, this invention provides a method of treating atherosclerosis and its associated diseases, such as, for example, cardiovascular disorders, cerebrovascular disorders, peripheral vascular disorders, or intestinal vascular disorders in a subject, the method comprising the step of administering to the subject compound of this invention or its pharmaceutically acceptable salt, isomer, tautomer, hydrate, N-oxyde, or any combination thereof, or a composition comprising the same. In one embodiment, the compound is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-
(108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H )-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00292] In certain embodiments, the present invention provides methods of treating a condition. In certain embodiments, the present invention provides methods of preventing a condition. In certain embodiments, the present invention provides methods of delaying the onset of a condition. In certain embodiments, the present invention provides methods of reducing the recurrence of a condition. In certain embodiments, the present invention provides methods of reducing the severity of a condition.

[00293] In one embodiment, the methods of the present invention are useful for treating prostate cancer. In one embodiment the methods of the present invention are useful for treating PIN. In one embodiment, the methods of the present invention are useful for treating breast cancer. In one embodiment, the methods of the present invention are useful for treating acne. In one embodiment, the methods of the present invention are useful for treating alopecia.

[00294] The inhibition of cyclooxygenase (COX) enzymes (e.g., COX-1 and/or COX-2) is undesirable for compounds that act as AKR1C3 inhibitors, to act as inhibition of cyclooxygenase enzymes, especially COX-1, typically leads to unwanted side effects in a patient, such as gastric irritation, ulcers and cardiovascular problems. In one embodiment, compounds of the present invention are much more potent inhibitors of AKR1C3 than they are inhibitors of cyclooxygenase.

[00295] In one embodiment, the compounds of the present invention lack cross-reactivity with cyclooxygenase (COX) enzymes. In one embodiment, the compounds of the present invention lack cross-reactivity with COX-1 enzyme. In one embodiment, the compounds of the present invention lack cross-reactivity with COX-2 enzyme. In one embodiment, the compounds of the present invention lack cross-reactivity with COX-1 and COX-2 enzymes. The term "cross-reactivity", as used herein, means that the ratio of inhibition of AKR1C3 to inhibition of cyclooxygenase for a compound of the present invention is greater than about 10-fold. In another embodiment, the ratio of inhibition is for example greater than about 100. In a further embodiment, the ratio of inhibition is greater than about 1000.
In one embodiment, the compounds of the present invention lack cross-reactivity with AKR1C1. In one embodiment, the compounds of the present invention lack cross-reactivity with AKR1C2. In one embodiment, the compounds of the present invention lack cross-reactivity with AKR1C4. In one embodiment, the compounds of the present invention lack cross-reactivity with 17PHSD3. In one embodiment, the compounds of the present invention lack cross-reactivity with 5α-reductase type 1. The term "cross-reactivity", as used herein, means that the ratio of inhibition of AKR1C3 to inhibition of AKR1C1, AKR1C2, AKR1C4, and 17βHSD3 respectively, for a compound of the present invention is greater than about 10-fold. In another embodiment, the ratio of inhibition is for example greater than about 100. In a further embodiment, the ratio of inhibition is greater than about 1000.

In certain embodiments, the methods described herein prevent or lessen typical side-effects associated with inhibition of AKR1C3 from occurring. In certain embodiments, the methods described herein prevent or lessen gastric irritation, ulcers, and/or cardiovascular problems.

In certain embodiments, a compound of this invention may be an estrogen receptor (ER) agonist. In other embodiment, a compound of this invention may be an ER antagonist. In still another embodiment, a compound of this invention may be an ER partial agonist/antagonist. In yet another embodiment, a compound of this invention is not an ER agonist. In a further embodiment, a compound of this invention is not an ER antagonist. In one embodiment, a compound of this invention is not an ER partial agonist/antagonist. In another embodiment, a compound of this invention does not bind to an ER.

In certain embodiments, an AKR1C3 inhibitor of this invention may modulate the activity of androgen receptor (AR) agonists. In other embodiments, an AKR1C3 inhibitor of this invention may modulate the activity of an AR antagonist. In still another embodiment, an AKR1C3 inhibitor of this invention may modulate the activity of a selective androgen receptor modulator (SARM). In yet another embodiment, a compound of this invention is not an AR agonist. In a further embodiment, a compound of this invention is not an AR antagonist. In one embodiment, a compound of this invention is not a SARM. In another embodiment, a compound of this invention does not bind to an AR. In certain embodiments, an AKR1C3 inhibitor of this invention may inhibit the action of a coactivator of the androgen receptor (AR). In certain embodiments, an AKR1C3 inhibitor of this invention may antagonize the action of a coactivator of the androgen receptor (AR). In certain embodiments, an AKR1C3 inhibitor of this invention may inhibit the action of a
coactivator of androgen-dependent androgen receptor (AR) activation. In certain embodiments, an AKR1C3 inhibitor of this invention may antagonize the action of a coactivator of androgen-dependent androgen receptor (AR) activation. In certain embodiments, an AKR1C3 inhibitor of this invention may inhibit the action of AKR1C3 as a coactivator of androgen-dependent androgen receptor (AR) activation. In certain embodiments, an AKR1C3 inhibitor of this invention may antagonize the action of AKR1C3 as a coactivator of androgen-dependent androgen receptor (AR) activation. In certain embodiments, an AKR1C3 inhibitor of this invention may antagonize the action of AKR1C3 as a coactivator of the androgen receptor (AR) activation independent of whether or not AR is bound to an androgen.

[00300] In one embodiment, the present invention provides a method of inhibiting androgen-dependent AR transactivation in a subject comprising administering a therapeutically effective amount of a compound of this invention. In another embodiment, the inhibition treats prostate cancer, primary prostate cancer, advanced prostate cancer, metastatic prostate cancer, hormone naïve prostate cancer, refractory prostate cancer or castration resistant prostate cancer (CRPC), or any combination thereof. In another embodiment the cancers overexpress AKR1C3. In another embodiment, the treatment is independent of inhibiting AKR1C3 enzyme activity. In another embodiment the treatment is independent of lowering endogenous androgen levels. In another embodiment the treatment is independent of lowering endogenous estrogen levels. In another embodiment the treatment is independent of lowering endogeneous proliferative prostaglandin levels. In another embodiment the method comprising the step of administering a compound of this invention or its pharmaceutically acceptable salt, isomer, tautomer, hydrate, N-oxide, or any combination thereof, or a composition comprising the same. In one embodiment, the compound is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), 4-(3-fluoro-4-( trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.
[00301] In one embodiment, this invention provides a method of treating AKR1C3 overexpressing tumors in a subject comprising administering a therapeutically effective amount of a compound of this invention. Non limiting examples of AKR1C3 overexpressing tumors include prostate cancer, primary prostate cancer, advanced prostate cancer, metastatic prostate cancer, hormone naïve prostate cancer, refractory prostate cancer or castration resistant prostate cancer (CRPC), or any combination thereof. In another embodiment the tumors are not androgen-dependent tumors. In another embodiment the tumors are not estrogen-dependent tumors. In another embodiment the method comprising the step of administering a compound of this invention or its pharmaceutically acceptable salt, isomer, tautomer, hydrate, N-oxide, or any combination thereof, or a composition comprising the same. In one embodiment, the compound is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

[00302] In one embodiment, this invention provides a method of lowering total serum testosterone levels in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of lowering serum testosterone in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In yet another embodiment, this invention provides a method of lowering serum free testosterone in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In still another embodiment, this invention provides a method of lowering serum levels of prostate-specific antigen (PSA) in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any
embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (77), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (84), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2*H*)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2*H*)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00303] In one embodiment, this invention provides a method of lowering total serum testosterone levels in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention, wherein the lowering of total serum testosterone is independent of a reduction of serum luteinizing hormone levels. In another embodiment, this invention provides a method of lowering serum testosterone levels in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In yet another embodiment, this invention provides a method of lowering serum free testosterone levels in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention, wherein the lowering of serum free testosterone is independent of a reduction of serum luteinizing hormone levels. In still another embodiment, this invention provides a method of lowering serum PSA levels in a male subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention, wherein the lowering of serum PSA is independent of a reduction of serum luteinizing hormone levels. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2*H*)-one
(78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (77), 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2H)-one (106), 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00304] In one embodiment, this invention provides a method of increasing survival of a subject with advanced prostate cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with castration-resistant prostate cancer (CRPC) comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00305] In one embodiment, this invention provides a method of prolonging progression-free survival of a subject with advanced prostate cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with castration-resistant prostate cancer (CRPC) comprising administering a therapeutically effective amount of an AKR1C3
inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00306] In one embodiment, this invention provides a method of lowering total serum estradiol levels in a subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of lowering serum free estradiol in a subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, a subject is a male subject. In another embodiment, a subject is a female subject. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.
[00307] In one embodiment, this invention provides a method of lowering intratumor levels of testosterone in a subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, a subject is a male subject. In another embodiment, a subject is a female subject. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00308] In one embodiment, this invention provides a method of lowering intratumor levels of DHT in a subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, a subject is a male subject. In another embodiment, a subject is a female subject. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or their prodrug, isomer, tautomer metabolite,
pharmaceutically acceptable salt, polymorph, crystal, \textit{N}-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00309] In one embodiment, this invention provides a method of lowering intratumor levels of estrogen in a subject comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, a subject is a male subject. In another embodiment, a subject is a female subject. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, \textit{N}-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-\textit{l}(2\textit{H})-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-\textit{l}(2\textit{H})-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-\textit{l}(2\textit{H})-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-\textit{l}(2\textit{H})-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-\textit{l}(2\textit{H})-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-\textit{l}(2\textit{H})-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-\textit{l}(2\textit{H})-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, \textit{N}-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00310] In one embodiment, this invention provides a method of increasing survival of a subject with advanced breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with refractory breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of increasing survival of a subject with AR-positive or ER-positive breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, \textit{N}-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-
l(2\(H\))-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2\(H\))-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2\(H\))-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2\(H\))-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00311] In one embodiment, this invention provides a method of prolonging progression-free survival of a subject with advanced breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with refractory breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In another embodiment, this invention provides a method of prolonging progression-free survival of a subject with AR-positive or ER-positive breast cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (67), 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2\(H\))-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\(H\))-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2\(H\))-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2\(H\))-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.
In one embodiment, this invention provides a method of increasing survival of a subject with advanced uterine cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

In one embodiment, this invention provides a method of prolonging progression-free survival of a subject with advanced uterine cancer comprising administering a therapeutically effective amount of an AKR1C3 inhibitor of this invention. In one embodiment, the AKR1C3 inhibitor is a compound of Formula I and/or Formula II or their prodrug, isomer, tautomer metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described. In one embodiment, the AKR1C3 inhibitor is characterized by the structure of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205), 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45), 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78), 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81), 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84), or 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77), or 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106), or 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108), 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (220) or their prodrug, isomer, tautomer metabolite,
pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof, or any embodiment thereof, as herein described.

[00314] In some embodiments, a compound of this invention is not a nuclear receptor binding agent.

[00315] In some embodiments, the compounds of the present invention are administered as a mono-therapy. In other embodiments, the compounds of the present invention are administered as part of a combination therapy. For example, compounds of this invention may be used in combination with other drugs or therapies that are used in the treatment of the diseases or conditions for which compounds of this invention are useful. In such combinations, each active ingredient can be administered either in accordance with their usual dosage range or a dose below their usual dosage range. For example, a compound of this invention may be adjunctively administered in combination with an antiandrogen, antiestrogen, anti-cancer drug, 5-alpha reductase inhibitor, aromatase inhibitor, GnRH agonist, GnRH antagonist, lyase inhibitor, progestin, prostate cancer vaccine, an agent that treats prostate cancer (docetaxel/prednisone, estramustine), an agent that treats breast cancer (Herceptin®, cyclophosphamide, methotrexate, fluorouracil, and doxorubicin (Adriamycin).

[00316] In one embodiment, the methods of the present invention comprise administering a compound of this invention as the sole active ingredient. However, also encompassed within the scope of the present invention are methods for treating a disorder that responds to an AKR1C3 inhibitor including treating and/or preventing prostate cancer, precancerous precursors of prostate adenocarcinoma, PIN, HGPIN, advanced prostate cancer, castration resistant prostate cancer, benign prostate hyperplasia (BPH), lung cancer, non-small cell lung cancer (NSCLC), acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, breast cancer, metastatic breast cancer, refractory breast cancer, AR-positive breast cancer, endometriosis, myeloma or leiomyoma which comprise administering a compound of this invention in combination with one or more therapeutic agents. These agents include, but are not limited to: selective estrogen receptor modulators (SERM), selective estrogen receptor degraders (fulvestrant), HER2 inhibitors (lapatinib, trastuzumab), bevacizumab, chemotherapeutic agents, taxanes, anthracyclines, epothilones, LHRH analogs, antiandrogens, antiestrogens, anticancer drugs, 5-alpha reductase inhibitors, aromatase inhibitors (exemestane, anastrozole, letrozole, vorozole,
formestane, fadrozole), progestins, agents acting through other nuclear hormone receptors, progesterone, estrogen, PDE5 inhibitors, apomorphine, bisphosphonate, growth factor inhibitors (such as those that inhibit VEGF, IGF and the like VEGF-A inhibitor (bevacizumab), selective androgen receptor modulators (SARMs), or one or more additional agents that treats diseases that respond to AKR1C3 inhibitors.

[00317] Thus, in one embodiment, the methods of the present invention comprise administering the compound of this invention, in combination with a chemotherapeutic agent. In one embodiment, the chemotherapeutic agent is a taxane. In another embodiment, the chemotherapeutic agent is an anthracycline. In one embodiment, the chemotherapeutic agent is an epothilone (ixabepilone). Thus, in one embodiment, the methods of the present invention comprise administering the compound of this invention in combination with an LHRH analog, taxanes, anthracyclines, or epothilones. In another embodiment, the methods of the present invention comprise administering the compound of this invention, in combination with an antiandrogen (bicalutamide, nilutamide, flutamide, or enzalutamide (MDV3100)). In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with an antiestrogen. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with an anticancer drug. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with a 5-alpha reductase inhibitor. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with an aromatase inhibitor. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with a progestin. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with an agent acting through other nuclear hormone receptors. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with a selective estrogen receptor modulator (SERM). In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with an estrogen. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with one or more additional agents that act through another hydroxysteroid dehydrogenase receptor. In another embodiment, the methods of the present invention comprise administering a compound of this invention, in combination with one or more additional agents that inhibit 17βHSD3. In another
embodiment, the methods of the present invention comprise administering a compound of this
invention, in combination with one or more additional agents that act through AKR1C1. In another
embodiment, the methods of the present invention comprise administering a compound of this
invention, in combination with one or more additional agents that act through AKR1C2. In another
embodiment, the methods of the present invention comprise administering a compound of this
invention, in combination with one or more additional agents that act through AKR1C4.

[00318] In one embodiment, the methods of this invention make use of compounds of
Formula I and/or Formula II in combination with PPARα ligands such as bezafibrate, fenofibrate,
gemfibrozil. In another embodiment, the methods of this invention make use of compounds of
Formula I and/or Formula II in combination with PPARγ ligands such as darglitazone, pioglitazone,
rosiglitazone, isaglitazone, rivoglitazone, netoglitazone. In yet another embodiment, the methods of
this invention make use of compounds of Formula I and/or Formula II in combination with Dual
acting PPAR ligands, such as naveglitazar, farglitzar, tesaglitazar, ragaglitazar, oxeglitazar, PN-
2034, PPAR δ. In yet another embodiment, the methods of this invention make use of compounds
of Formula I and/or Formula II in combination with 17-ketoreductase inhibitors, 3β-ΔHΔ4,6-
isomerase inhibitors, 3P-AHA4,5-isomerase inhibitors, 17,20 desmolase inhibitors, p450c17
inhibitors, p450ssc inhibitors, 17,20-lyase inhibitors, or combinations thereof.

[00319] In one embodiment, the agent treating the endocrine system is a peroxisome
proliferator-activated receptor ligand. In some embodiments, peroxisome proliferator-activated
receptor ligands include but are not limited to bezafibrate, fenofibrate, gemfibrozil, darglitazone,
pioglitazone, rosiglitazone, isaglitazone, rivoglitazone, netoglitazone, naveglitazar, farglitzar,
tesaglitazar, ragaglitazar, oxeglitazar, or PN-2034. In one embodiment, the methods of this
invention make use of compounds of Formula I and/or Formula II in combination with a
peroxisome proliferators-activated receptor ligand. In one embodiment, the methods of this
invention make use of compounds of Formula I and/or Formula II in combination with bezafibrate,
fenofibrate, gemfibrozil, darglitazone, pioglitazone, rosiglitazone, isaglitazone, rivoglitazone,
netoglitazone, naveglitazar, farglitzar, tesaglitazar, ragaglitazar, oxeglitazar, or PN-2034.

[00320] Such other drug(s) may be administered, by a route and in an amount commonly
used therefor, contemporaneously or sequentially with a compound of this invention. When a
compound of this invention is used contemporaneously with one or more other drugs, a
pharmaceutical unit dosage form containing such other drugs in addition to the compound of this
invention may be employed. Accordingly, the pharmaceutical compositions of the present invention
include those that also contain one or more other active ingredients, in addition to a compound of this invention.

[00321] By adjunctive administration is meant simultaneous administration of the compounds in the same-dosage form, simultaneous administration in separate dosage forms, and separate administration of the compounds.

[00322] In one embodiment, the methods of this invention may comprise administration of a compound of this invention at various dosages. In one embodiment, the compound of this invention is administered at a dosage of about 0.1 to about 200 mg per day. In one embodiment, the compound of this invention is administered at a dose of about 0.1 to about 10 mg, or in another embodiment, 0.1 to about 25 mg, or in another embodiment, about 0.1 to about 60 mg, or in another embodiment, about 0.5 to about 15 mg, or in another embodiment, about 0.5 to about 30 mg, or in another embodiment, about 0.5 to about 25 mg, or in another embodiment, about 0.5 to about 60 mg, or in another embodiment, about 0.75 to about 15 mg, or in another embodiment, 0.75 to about 60 mg, or in another embodiment, about 1 to about 5 mg, or in another embodiment, about 1 to about 15 mg, or in another embodiment, about 1 to about 30 mg, or in another embodiment, about 1 to about 60 mg, or in another embodiment, about 30 to about 75 mg, or in another embodiment, about 75 mg to about 300 mg, or in another embodiment, about 250 to about 750 mg, or in another embodiment, about 100 to about 2000 mg.

[00323] In one embodiment, the methods of this invention may comprise administration of a compound of this invention at various dosages. In one embodiment, a compound of this invention is administered at a dosage of about 0.1 mg. In another embodiment a compound of this invention is administered at a dosage of about 0.5 mg, about 1 mg, about 2.5 mg, about 5 mg, about 7.5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 500 mg, or about 1000 mg.

[00324] The term "treating" means to relieve, alleviate, delay, reduce, reverse, improve or prevent at least one symptom of a condition in a subject. The term "treating" may also mean to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a condition. The compounds of the present invention may be
administered as a mono-therapy or administered as part of a combination therapy. For example, one or more of the compounds of the present invention may be co-administered or used in combination with one or more additional therapies known in the art.

[00325] An "effective amount" means the amount of a compound of this invention that, when administered to a patient (e.g., a mammal) for treating a disease, is sufficient to effect such treatment for the disease, or an amount of a compound of this invention that is sufficient for inhibiting AKR1C3 to achieve an objective of the invention. The "effective amount" will vary depending on the compound, the disease and its severity and the age, weight, etc., of the patient to be treated. In certain embodiments, the "effective amount" selectively inhibits AKR1C3.

[00326] As used herein, the term "administering" refers to bringing a subject in contact with a compound of the present invention. As used herein, administration can be accomplished in vitro, i.e. in a test tube, or in vivo, i.e. in cells or in tissues of living organisms, for example humans. In one embodiment, the present invention encompasses administering the compounds of the present invention to a subject.

[00327] A subject or patient in whom administration of the therapeutic compound is an effective therapeutic regimen for a disease or disorder is preferably a human, but can be any animal, including a laboratory animal in the context of a preclinical trial or screening or activity experiment. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods, compounds and compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, humans, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., avian species, such as chickens, turkeys, songbirds, etc., i.e., for veterinary medical use.

[00328] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviations, per practice in the art. Alternatively, "about" with respect to the compositions can mean plus or minus a range of up to 20%, preferably up to 10%, more preferably up to 5%.
It is to be understood that any use of any of the compounds as herein described may be used in the treatment of any disease, disorder or condition as described herein, and represents an embodiment of this invention.

In some embodiments, the term "comprise" or grammatical forms thereof, refers to the inclusion of the indicated active agent, such as the compound of this invention, as well as inclusion of other active agents, and pharmaceutically acceptable carriers, excipients, emollients, stabilizers, etc., as are known in the pharmaceutical industry. In some embodiments, the term "consisting essentially of" refers to a composition, whose only active ingredient is the indicated active ingredient, however, other compounds may be included which are for stabilizing, preserving, etc. the formulation, but are not involved directly in the therapeutic effect of the indicated active ingredient. In some embodiments, the term "consisting essentially of" may refer to components, which exert a therapeutic effect via a mechanism distinct from that of the indicated active ingredient. In some embodiments, the term "consisting essentially of" may refer to components, which exert a therapeutic effect and belong to a class of compounds distinct from that of the indicated active ingredient. In some embodiments, the term "consisting essentially of" may refer to components which facilitate the release of the active ingredient. In some embodiments, the term "consisting" refers to a composition, which contains the active ingredient and a pharmaceutically acceptable carrier or excipient.

In one embodiment, the present invention provides combined preparations. In one embodiment, the term "a combined preparation" defines especially a "kit of parts" in the sense that the combination partners as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners i.e., simultaneously, concurrently, separately or sequentially. In some embodiments, the parts of the kit of parts can then, e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the combination partners, in some embodiments, can be administered in the combined preparation. In one embodiment, the combined preparation can be varied, e.g., in order to cope with
the needs of a patient subpopulation to be treated or the needs of the single patient which different needs can be due to a particular disease, severity of a disease, age, sex, or body weight as can be readily made by a person skilled in the art.

[00332] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. While the invention has been depicted and described by reference to exemplary embodiments of the invention, such a reference does not imply a limitation on the invention, and no such limitation is to be inferred. The invention is capable of considerable modification, alteration, and equivalents in form and function, as will occur to those ordinarily skilled in the pertinent arts having the benefit of this disclosure. The depicted and described embodiments of the invention are exemplary only, and are not exhaustive of the scope of the invention. Consequently, the invention is intended to be limited only by the spirit and scope of the appended claims, giving full cognizance to equivalence in all respects.

[00333] All references cited herein are hereby incorporated by reference in their entirety.

EXAMPLES

EXAMPLE 1: Synthesis of isoquinolin-1(2H)-one AKR1C3 inhibitors.

[00334] Example 1A: Synthesis of 4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2H)-one (17).
Synthesis of 4-bromo-6-methoxyisoquinolin-1(2H)-one (15): 6-Methoxyisoquinoline-1-ol (1) (2.00 g, 11.42 mmol) was dissolved in 30 mL of anhydrous THF at room temperature. N-Bromosuccinimide (2.03 g, 11.42 mmol) in 30 mL of anhydrous THF was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature overnight. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$/acetone = 19/1 v/v) to give a pale-yellow solid product, 2.80 g, 96.6% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 11.43 (s, 1H), 8.15 (d, 1H, $J$ = 9.0 Hz), 7.54 (d, 1H, $J$ = 3.9 Hz), 7.17 (dd, 1H, $J_1$ = 9.0 Hz, $J_2$ = 2.4 Hz), 7.10 (d, 1H, $J$ = 2.4 Hz), 3.92 (s, 3H).

Synthesis of 6-methoxy-4-(4-methoxyphenyl)isoquinolin-1(2H)-one (16): 4-Bromo-6-methoxyisoquinolin-1(2H)-one (15) (0.81 g, 3.23 mmol), tetrakis (triphenylphosphine) palladium (0.18 g, 0.16 mmol), potassium carbonate (0.88 g, 6.38 mmol) and 4-methoxyphenylboronic acid (0.58 g, 3.83 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and a reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction mixture was diluted with 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO$_4$ followed by filtration with metal scavenger and concentrated to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 9/1 v/v) to give a white solid product, 0.11 g, 12.3% yield. MS: m/z 282.0 [M+H]$^+$. $^1$H NMR (DMSO-$d_4$, 300 MHz) $\delta$ 11.23 (d, 1H, $J$ = 5.7 Hz), 8.21 (d,
1H, J = 8.7 Hz), 7.36 (d, 2H, J = 8.7 Hz), 7.13 (dd, 1H, J1 = 8.7 Hz, J2 = 2.4 Hz), 7.04 (d, 2H, J = 8.7 Hz), 7.00 (d, 1H, J = 5.7 Hz), 6.87 (d, 1H, J = 2.4 Hz), 3.81 (s, 3H), 3.75 (s, 3H).

[00337] Synthesis of 4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2 H)-one (17): 6-Methoxy-4-(4-methoxyphenyl)isoquinolin-1(2 H)-one (16) (0.10 g, 0.355 mmol) was dissolved in anhydrous methylene chloride (30 mL) in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. BBr3 (1.78 mL of 1 M CH2Cl2 solution, 1.78 mmol) was added via a syringe dropwise with stirring at room temperature. The resulted solution was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of water and 5 mL of methanol at 0 °C. The solution was then stirred at room temperature for two hours. CH2Cl2 layer was separated and the aqueous layer was extracted with CH2Cl2/MeOH (9/1 v/v, 3x50 mL). The organic layers were combined and dried over anhydrous MgSO4. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH2Cl2/MeOH = 9/1 v/v) to give a white solid product, 65 mg, 68.4% yield. MS: m/e 265.8 [M-H]-. 1H NMR (DMSO-4, 300 MHz) δ 11.17 (d, 1H, J = 5.7 Hz), 9.53 (s, 1H), 8.20 (d, 1H, J = 9.0 Hz), 7.22 (d, 2H, J = 8.4 Hz), 7.12 (dd, 1H, J1 = 9.0 Hz, J2 = 2.4 Hz), 6.95 (d, 1H, J = 6.0 Hz), 6.87 (s, 1H), 6.86 (m, 3H), 6.50 (s, 1H), 3.76 (s, 3H).

Synthesis of 6-methoxy-2-methylisoquinolin-1(2H)-one (18): 6-Methoxyisoquinoline-l-ol (1) (1.00 g, 5.71 mmol), copper(I)iodide (0.22 g, 1.14 mmol), L-proline (0.26 g, 2.28 mmol) and anhydrous potassium carbonate (1.58 g, 11.42 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 40 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 120 °C under argon atmosphere. Then, methyl iodide (3.24 g, 22.84 mmol) was added via a syringe. The reaction mixture was stirred at 100 °C for 5 hours. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were separated, washed with brine (2 x 20 mL) and concentrated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, 34 (34/Acetone = 4/1, v/v) to give a white solid product, 0.85 g, 78.7% yield. MS: 212.0 [M+Na]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.10 (d, 1H, J = 8.7 Hz), 7.42 (d, 1H, J = 7.1 Hz), 7.10 (d, 1H, J = 2.7 Hz), 7.05 (dd, 1H, J1 = 8.7 Hz, J2 = 2.7 Hz), 6.52 (d, 1H, J = 7.1 Hz), 3.86 (s, 3H), 3.46 (s, 3H).

Synthesis of 4-bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19): 6-Methoxy-2-methylisoquinolin-1(2H)-one (18) (0.80 g, 4.23 mmol) and N-bromosuccinimide (0.90 g, 5.07 mmol)
were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (30 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 2 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CILCVAcetone = 17/3 v/v) to give a white solid product, 1.08 g, 95.6% yield. MS: 290.0 [M+H]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.16 (d, 1H, J = 8.7 Hz), 7.93 (s, 1H), 7.16 (dd, 1H, J1 = 9.0 Hz, J2 = 2.4 Hz), 7.06 (d, 1H, J = 2.4 Hz), 3.92 (s, 3H), 3.47 (s, 3H).

Synthesis of 6-methoxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2H)-one (20): 4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.35 g, 1.31 mmol), tetrakis(triphenylphosphine)palladium (76 mg, 0.066 mmol), potassium carbonate (0.36 g, 2.62 mmol) and 4-(trifluoromethyl)phenylboronic acid (0.30 g, 1.57 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 3 hours. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO4 followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH2Cl2/Acetone = 4/1 v/v) to give a white solid product, 0.40 g, 90.9% yield. MS: m/z 356.1 [M+Na]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.26 (d, 1H, J = 9.0 Hz), 7.86 (d, 2H, J = 8.1 Hz), 7.72 (d, 2H, J = 8.1 Hz), 7.58 (s, 1H), 7.17 (dd, 1H, J = 9.0 Hz, J = 2.4Hz), 6.87 (d, 1H, J = 2.4 Hz), 3.78 (s, 3H), 3.54 (s, 3H).

Synthesis of 6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2H)-one (21): 6-Methoxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2H)-one (20) (0.38 g, 1.14 mmol) was dissolved in 30 mL of anhydrous CH2Cl2 in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr3 (3.42 mL of 1.0M CH2Cl2 solution, 3.42 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature for 20 hours, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH2Cl2 layer was separated. The aqueous layer was extracted with CH2Cl2 (3 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO4. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH2Cl2/MeOH = 9/1 v/v) to give a white solid product,
0.33 g, 90.7% yield. MS: m/e 317.9 [M-H]−. 1H NMR (DMSO-d6, 300 MHz) δ 10.23 (s, 1H), 8.17 (d, 1H, J = 9.0 Hz), 7.86 (d, 2H, J = 8.4 Hz), 7.67 (d, 2H, J = 8.4 Hz), 7.50 (s, 1H), 6.97 (dd, 1H, J = 8.7 Hz, J2 = 2.1 Hz), 6.78 (d, 1H, J = 2.1 Hz), 3.51 (s, 3H).

Example 1C: Synthesis of 6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2H)-one (39).

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{N} \quad \text{CH}_3 \\
\text{Br} & \quad \text{Pd(PPh)_3} \\
\rightarrow & \quad \text{K}_2\text{CO}_3 \\
\text{DME/H}_2\text{O} \\
\text{H}_3\text{CO}_2\text{S} & \quad \text{B} \quad \text{OH} \\
\text{HO} & \quad \text{BBr}_3 \\
\text{HO} & \quad \text{N} \quad \text{CH}_3 \\
\text{O} & \quad \text{OH} \\
\text{O} & \quad \text{OSO}_2 \\
\end{align*}
\]

[00343] Synthesis of 6-methoxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2H)-one (38): 4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.56 g, 2.09 mmol), tetrakis(triphenylphosphine)palladium (0.12 mg, 0.105 mmol), potassium carbonate (0.58 g, 4.18 mmol) and 4-(methylsulfonyl)phenylboronic acid (0.50 g, 2.51 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 3 hours. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3×50 mL). The extracts were combined, washed with brine (2×10 mL) and dried over anhydrous MgSO4 followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH2Cl2/Acetone = 4/1 v/v) to give a white solid product, 0.61 g, 84.9% yield. MS: m/z 344.0 [M+H]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.27 (d, 1H, J = 9.0 Hz), 8.04 (d, 2H, J = 8.4 Hz), 7.77 (d, 2H, J = 8.4Hz), 7.60 (s, 1H), 7.18 (dd, 1H, J1 = 9.0 Hz, J2 = 2.4Hz), 6.90 (d, 1H, J = 2.4 Hz), 3.79 (s, 3H), 3.54 (s, 3H), 3.29 (s, 3H).
[00344] Synthesis of 6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-(2\(^H\))-one (39): 6-Methoxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-(2\(^H\))-one (38) (0.50 g, 1.46 mmol) was dissolved in 30 mL of anhydrous CH\(_2\)Cl\(_2\) in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr\(_3\) (8.0 mL of 1.0M CH\(_2\)Cl\(_2\) solution, 8.0 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature for two days, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH\(_2\)Cl\(_2\) layer was separated. The aqueous layer was extracted with EtOAc (5 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO\(_4\). The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH\(_2\)Cl\(_2\)/MeOH = 19/1 v/v) to give a white solid product, 0.45 g, 93.9% yield. MS: m/e 327.9 [M-H].\(^{1}\)H NMR (DMSO-d6, 300 MHz) \(\delta\) 10.27 (s, 1H), 8.18 (d, 1H, \(J = 8.7\) Hz), 8.05 (d, 2H, \(J = 8.4\) Hz), 7.02 (d, 2H, \(J = 8.4\) Hz), 7.52 (s, 1H), 6.98 (dd, 1H, \(J_1 = 8.7\) Hz, \(J_2 = 2.4\) Hz), 6.80 (d, 1H, \(J = 2.4\) Hz), 3.52 (s, 3H), 3.30 (s, 3H).

[00345] Example ID: Synthesis of 4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41).
Synthesis of methyl 4-(6-methoxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoate (40): 4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.52 g, 1.94 mmol), tetrakis(triphenylphosphine)palladium (0.11 mg, 0.097 mmol), potassium carbonate (0.54 g, 3.88 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.42 g, 2.33 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 6 hours. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with CH₂Cl₂ (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 17/3 v/v) to give a white solid product, 0.52 g, 82.9% yield. MS: m/z 346.0 [M+Na]⁺. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.26 (d, 1H, J = 8.7 Hz), 8.08 (d, 2H, J = 8.4 Hz), 7.65 (d, 2H, J = 8.4Hz), 7.58 (s, 1H), 7.17 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4Hz), 6.90 (d, 1H, J = 2.4 Hz), 3.90 (s, 3H), 3.77 (s, 3H), 3.54 (s, 3H).

Synthesis of 4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41): Methyl 4-(6-methoxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoate (40) (0.46 g, 1.42 mmol) was suspended in 20 mL of anhydrous m-xylene in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (1.78 g, 7.11 mmol) was added via a syringe with stirring at room temperature. After stirred and heated to 100 °C overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (5 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/acetone/MeOH = 8/1/1 v/v/v) to give a pale-yellow solid product, 0.35 g, 83.5% yield. MS: m/e 293.9 [M-H]⁻. ¹H NMR (DMSO-4, 300 MHz) δ 13.08 (s, 1H), 10.23 (s, 1H), 8.17 (d, 1H, J = 8.7 Hz), 8.05 (d, 2H, J = 8.4 Hz), 7.57 (d, 2H, J = 8.4 Hz), 7.49 (s, 1H), 6.97 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.1 Hz), 6.82 (d, 1H, J₁ = 2.1 Hz), 3.51 (s, 3H).

Example IE: Synthesis of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45).
Synthesis of 6-methoxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (44):

4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.53 g, 1.98 mmol), tetrakis(triphenylphosphine)palladium (0.11 g, 0.099 mmol), potassium carbonate (0.55 g, 3.96 mmol) and 3,4,5-trifluorophenylboronic acid (0.42 g, 2.37 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. Toluene (50 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with EtOAc (2x30 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO$_4$ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CILCVAcetone = 17/3 v/v) to give a white solid product, 0.53 g, 84.1% yield.

MS: m/z 342.0 [M+Na]$^+$. $^1$H NMR (DMSO-$d_6$, 500 MHz) δ 8.26 (d, 1H, $J = 9.0$ Hz), 7.59 (s, 1H), 7.51 (d, 2H, $J = 8.0$ Hz), 7.18 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz), 6.89 (d, 1H, $J = 2.0$ Hz), 3.82 (s, 3H), 3.54 (s, 3H).

Synthesis of 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45):

6-Methoxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (44) (0.45 g, 1.41 mmol) was dissolved in 30 mL of anhydrous methylene chloride in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr$_3$ (7.05 mL of 1M CH$_2$Cl$_2$ solution, 7.05 mmol) was added via a syringe with stirring at room temperature.
stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (2 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CILC^/MeOH = 19/1 v/v) to give a pale-yellow solid product, 0.32 g, 74.4% yield. MS: m/e 303.8 [M-H]: ¹H NMR (DMSO-d₆, 300 MHz) δ 10.29 (s, 1H), 8.16 (d, 1H, J = 8.7 Hz), 7.50 (s, 1H), 7.48-7.43 (m, 2H), 7.08 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.1 Hz), 6.80 (d, 1H, J = 2.1 Hz), 3.50 (s, 3H).

Example IF: Synthesis of 2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2H)-one (49).

[00351]

Synthesis of 2-cyclohexyl-6-methoxyisoquinolin-1(2H)-one (46): 6-Methoxy-1 H-isochromen-1-one (1.00 g, 5.68 mmol) and cyclohexylamine (10.14 g, 0.102 mol) were placed in a dry 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The
reaction mixture was stirred and heated to reflux for 5 hours under argon atmosphere. Then, the reaction solution was quenched by adding 100 mL of 2N HCl solution at room temperature. The mixture was extracted with ethyl acetate (3x30 mL). The extracts were separated, washed with brine (2 x 20 mL) and concentrated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$/Acetone = 97/3, v/v) to give a white solid product, 0.55 g, 89.8% yield. MS: m/z 412.0 [M+H]$^+$.

$^1$H NMR (DMSO-d$_6$, 500 MHz) δ 8.12 (d, 1H, $J = 8.7$ Hz), 7.48 (d, 1H, $J = 7.5$ Hz), 7.09 (d, 1H, $J = 2.4$ Hz), 7.05 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 6.56 (d, 1H, $J = 7.8$ Hz), 4.82-4.72 (m, 1H), 3.86 (s, 3H), 1.86-1.58 (m, 7H), 1.48-1.35 (m, 2H), 1.27-1.14 (m, 1H).

[00353] Synthesis of 4-bromo-2-cyclohexyl-6-methoxyisoquinolin-1(2$H$)-one (47): 2-Cyclohexyl-6-methoxyisoquinolin-1(2$H$)-one (46) (0.96 g, 3.73 mmol) and N-bromosuccinimide (0.80 g, 4.48 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (30 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 6 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$/Acetone = 97/3 v/v) to give a white solid product, 1.20 g, 95.7% yield. MS: 336.0 [M+H]$^+$. $^1$H NMR (DMSO-d$_6$, 300 MHz) δ 8.20 (d, 1H, $J = 9.0$ Hz), 7.87 (s, 1H), 7.18 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 7.08 (d, 1H, $J = 2.4$ Hz), 4.79-4.69 (m, 1H), 3.92 (s, 3H), 1.85-1.63 (m, 7H), 1.46-1.21 (m, 3H).

[00354] Synthesis of 2-cyclohexyl-6-methoxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2$H$)-one (48): 4-Bromo-2-cyclohexyl-6-methoxyisoquinolin-1(2$H$)-one (47) (0.50 g, 1.49 mmol), tetrakis(triphenylphosphine)palladium (86 mg, 0.074 mmol), potassium carbonate (0.41 g, 2.97 mmol) and 4-(methylsulfonyl)phenylboronic acid (0.36 g, 1.79 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO$_4$ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$/Acetone = 19/1 v/v) to give a white solid product, 0.55 g, 89.8% yield. MS: m/z 412.0 [M+H]$^+$. $^1$H NMR (DMSO-d$_6$, 500 MHz) δ...
MHz) \( \delta \) 8.29 (d, 1H, \( J = 9.0 \) Hz), 8.04 (d, 2H, \( J = 8.5 \) Hz), 7.78 (d, 2H, \( J = 8.5 \) Hz), 7.54 (s, 1H), 7.19 (dd, 1H, \( J_1 = 9.0 \) Hz, \( J_2 = 2.5 \) Hz), 6.89 (d, 1H, \( J = 2.5 \) Hz), 4.86-4.80 (m, 1H), 3.79 (s, 3H), 3.29 (s, 3H), 1.85-1.76 (m, 6H), 1.67-1.64 (m, 1H), 1.43-1.38 (m, 2H), 1.27-1.19 (m, 1H).

[00355] **Synthesis of 2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2H)-one (49):** 2-Cyclohexyl-6-methoxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2H)-one (48) (0.58 g, 1.41 mmol) was dissolved in 30 mL of anhydrous CH\(_2\)Cl\(_2\) in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr\(_3\) (5.60 mL of 1.0M CH\(_2\)Cl\(_2\) solution, 5.60 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH\(_2\)Cl\(_2\) layer was separated. The aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO\(_4\). The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH\(_2\)Cl\(_2\)/MeOH = 19/1 v/v) to give a white solid product, 0.46 g, 82.1% yield. MS: m/e 396.1 [M-H]\(^-\) \( ^1\)H NMR (DMSO-\(d_6\), 300 MHz) \( \delta \) 10.27 (s, 1H), 8.20 (d, 1H, \( J = 8.7 \) Hz), 8.04 (d, 2H, \( J = 8.1 \) Hz), 7.73 (d, 2H, \( J = 8.1 \) Hz), 7.47 (s, 1H), 6.99 (dd, 1H, \( J_1 = 8.7 \) Hz, \( J_2 = 2.1 \) Hz), 6.79 (d, 1H, \( J = 2.1 \) Hz), 4.82 (m, 1H), 3.30 (s, 3H), 1.85-1.63 (m, 7H), 1.41-1.39 (m, 2H), 1.23-1.15 (m, 1H).

[00356] **Example 1G: Synthesis of 4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (51).**
[00357] **Synthesis of 4-(4-fluorophenyl)-6-methoxy-2-methylisoquinolin-1(2 H)-one (50):**

4-Bromo-6-methoxy-2-methylisoquinolin-1(2 H)-one (19) (0.51 g, 1.90 mmol), tetrakis(triphenylphosphine)palladium (0.11 g, 0.095 mmol), potassium carbonate (0.53 g, 3.80 mmol) and 4-fluorophenylboronic acid (0.32 g, 2.28 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. Then, the reaction mixture was diluted by adding 100 mL of water at room temperature. The mixture was extracted with EtOAc (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO$_4$ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$/Acetone = 97/3 v/v) to give a white solid product, 0.47 g, 87.2% yield. MS: m/z 284.0 [M+H]$^+$. $^1$H NMR (DMSO-$_4$, 300 MHz) $\delta$ 8.25 (d, 1H, $J$ = 8.7 Hz), 7.53-7.47 (m, 3H), 7.37-7.30 (m, 2H), 7.15 (dd, 1H, $J_1$ = 8.7 Hz, $J_2$ = 2.4 Hz), 6.81 (d, 1H, $J$ = 2.4 Hz), 3.76 (s, 3H), 3.52 (s, 3H).

[00358] **Synthesis of 4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (51):**

4-(4-Fluorophenyl)-6-methoxy-2-methylisoquinolin-1(2 H)-one (50) (0.42 g, 1.48 mmol) was dissolved in 30 mL of anhydrous methylene chloride in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr$_3$ (6.0 mL of 1M CH$_2$Cl$_2$ solution, 6.0 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO$_4$. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 19/1 v/v) to give a white solid product, 0.35 g, 87.7% yield. MS: m/e 267.8 [M-H]$^-$. $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ 10.21 (s, 1H), 8.16 (d, 1H, $J$ = 8.7 Hz), 7.48-7.43 (m, 2H), 7.37-7.30 (m, 2H), 6.96 (dd, 1H, $J_1$ = 9.0 Hz, $J_2$ = 2.4 Hz), 6.74 (d, 1H, $J$ = 2.4 Hz), 3.50 (s, 3H).

[00359] **Example 1H: Synthesis of 4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (53).**
Synthesis of 4-(3-fluorophenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (52): 4-Bromo-6-methoxy-2-methylisoquinolin-l(2H)-one (19) (0.50 g, 1.87 mmol), tetrakis(triphenylphosphine)palladium (0.11 g, 0.095 mmol), potassium carbonate (0.53 g, 3.80 mmol) and 3-fluorophenylboronic acid (0.31 g, 2.24 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. Then, the reaction mixture was diluted by adding 100 mL of water at room temperature. The mixture was extracted with EtOAc (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 19/1 v/v) to give a white solid product, 0.47 g, 89.5% yield. MS: m/z 284.2 [M+H]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.25 (d, 1H, J = 9.0 Hz), 7.59-7.51 (m, 2H), 7.35-7.24 (m, 2H), 7.16 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 6.88 (d, 1H, J = 2.4 Hz), 3.77 (s, 3H), 3.53 (s, 3H).

Synthesis of 4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (53): 4-(3-Fluorophenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (52) (0.44 g, 1.55 mmol) was dissolved in 30 mL of anhydrous methylene chloride in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (6.50 mL of 1M CH₂Cl₂ solution, 6.50 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5
mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 23/1 v/v) to give a white solid product, 0.40 g, 95.7% yield. MS: m/e 267.8 [M-H]-. ¹H NMR (DMSO-4, 300 MHz) δ 10.24 (s, 1H), 8.16 (d, 1H, J = 9.0 Hz), 7.56-7.52 (m, 1H), 7.46 (s, 1H), 7.29-7.24 (m, 3H), 6.97 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 6.82 (d, 1H, J = 2.0 Hz), 3.50 (s, 3H).

Example II: Synthesis of 4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (55).

[00362]

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{Br} \\
\text{N} & \quad \text{CH}_3 \\
\text{H}_3\text{CO} & \quad \text{F} \\
\text{O} & \quad \text{CH}_3 \\
\text{H}_3\text{CO} & \quad \text{F} \\
\text{O} & \quad \text{CH}_3
\end{align*}
\]

19

Pd(PPh₃)₄/K₂CO₃

DME/H₂O

[00363]

Synthesis of 4-(3-fluoro-4-methoxyphenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (54): 4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.48 g, 1.79 mmol), tetrakis(triphenylphosphine)palladium (0.10 g, 0.090 mmol), potassium carbonate (0.50 g, 3.58 mmol) and 3-fluoro-4-methoxyphenylboronic acid (0.37 g, 2.15 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane(30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. Then, the reaction mixture was diluted by adding 100 mL of water at room temperature. The mixture was extracted with EtOAc (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow
residue was purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$/Acetone = 96/4 v/v) to give a white solid product, 0.52 g, 92.9% yield. MS: m/z 336.2 [M+Na]$^+$. $^1$H NMR (DMSO-$d_6$, 300 MHz) δ 8.23 (d, 1H, $J = 9.0$ Hz), 7.39-7.22 (m, 3H), 7.14 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz), 6.87 (d, 1H, $J = 2.4$ Hz), 3.90 (s, 3H), 3.77 (s, 3H), 3.51 (s, 3H).

[00364] **Synthesis of 4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (55):** 4-(3-Fluoro-4-methoxyphenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (54) (0.54 g, 1.72 mmol) was dissolved in 30 mL of anhydrous methylene chloride in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr$_3$ (9.0 mL of 1M CH$_2$Cl$_2$ solution, 9.0 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO$_4$. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 19/1 v/v) to give a white solid product, 0.45 g, 91.6% yield. MS: m/e 283.9 [M-H]$:^1$H NMR (DMSO-$d_6$, 300 MHz) δ 10.18 (s, 1H), 9.99 (s, 1H), 8.14 (d, 1H, $J = 9.0$ Hz), 7.35 (s, 1H), 7.19 (d, 1H, $J = 11.5$ Hz), 7.08-7.02 (m, 2H), 6.94 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 2.0$ Hz), 6.81 (d, 1H, $J = 2.0$ Hz), 3.48 (s, 3H).

[00365] **Example lj: Synthesis of 2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (59).**
Synthesis of 2-butyl-6-methoxyisoquinolin-1(2H)-one (56): 6-Methoxy-1H-isochromen-1-one (1.00 g, 5.68 mmol) and butylamine (4.15 g, 56.76 mmol) were placed in a dry 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction mixture was stirred and heated to reflux overnight under argon atmosphere. Then, the reaction solution was quenched by adding 100 mL of 2N HCl solution at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were separated, washed with brine (2x20 mL) and concentrated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, CH2Cl2/Acetone = 97/3, v/v) to give a pale-yellow oil product, 0.30 g, 22.9% yield. MS: 231.9 [M+H]+. 1H NMR (DMSO-<¾, 500 MHz) δ 8.10 (d, 1H, J = 9.0 Hz), 7.41 (d, 1H, J = 7.5 Hz), 7.09 (d, 1H, J = 2.5 Hz), 7.05 (dd, 1H, J = 9.0 Hz, J = 2.5 Hz), 6.53 (d, 1H, J = 7.0 Hz), 3.91 (t, 2H, J = 7.5 Hz), 3.86 (s, 3H), 1.63 (m, 2H), 1.28 (m, 2H), 0.90 (t, 3H, J = 7.5 Hz).

Synthesis of 4-bromo-2-butyl-6-methoxyisoquinolin-1(2H)-one (57): 2-Butyl-6-methoxyisoquinolin-1(2H)-one (56) (0.20 g, 0.87 mmol) and N-bromosuccinimide (0.18 g, 1.04 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar
and septa. Anhydrous tetrahydrofuran (30 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 6 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$-acetone = 96/4 v/v) to give a white solid product, 0.24 g, 89.6% yield. MS: m/z 332.0 [M+H]+. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 8.18 (d, 1H, $J = 8.7$ Hz), 7.94 (s, 1H), 7.18 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz), 7.08 (d, 1H, $J = 2.4$ Hz), 3.93 (t, 2H, $J = 7.5$ Hz), 3.92 (s, 3H), 1.64 (m, 2H), 1.28 (m, 2H), 0.90 (t, 3H, $J = 7.5$ Hz).

[00368] **Synthesis of 2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (58):** 4-Bromo-2-butyl-6-methoxyisoquinolin-1(2H)-one (57) (0.20 g, 0.65 mmol), tetrakis(triphenylphosphine)palladium (37 mg, 0.032 mmol), cesium carbonate (0.42 g, 1.29 mmol) and 3,4,5-trifluorophenylboronic acid (0.17 g, 0.97 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 6 hours. The reaction solution was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO$_4$ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$-acetone = 96/4 v/v) to give a white solid product, 0.23 g, 98.7% yield. MS: m/z 362.3 [M+H]+. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 8.24 (d, 1H, $J = 9.0$ Hz), 7.54 (s, 1H), 7.51-7.48 (m, 2H), 7.16 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 2.5$ Hz), 6.87 (d, 1H, $J = 2.5$ Hz), 3.96 (t, 2H, $J = 7.5$ Hz), 3.80 (s, 3H), 1.68 (m, 2H), 1.32 (m, 2H), 0.91 (t, 3H, $J = 7.5$ Hz).

[00369] **Synthesis of 2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (59):** 2-Butyl-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (58) (0.23 g, 0.64 mmol) was dissolved in 30 mL of anhydrous CH$_2$Cl$_2$ in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr$_3$ (3.18 mL of 1.0M CH$_2$Cl$_2$ solution, 3.18 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH$_2$Cl$_2$ layer was separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO$_4$. The solvent was removed under reduced
The residue was purified by column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 19/1 v/v) to give a white solid product, 0.19 g, 86.0% yield. MS: m/e 345.9 [M-H]. $^1$H NMR (DMSO-$d_6$, 300 MHz) δ 10.29 (s, 1H), 8.16 (d, 1H, J = 8.7 Hz), 7.52-7.43 (m, 3H), 6.98 (dd, 1H, $J_1$ = 8.7 Hz, $J_2$ = 2.1 Hz), 6.81 (d, 1H, $J$ = 2.1 Hz), 3.94 (t, 2H, $J$ = 7.5 Hz), 1.66 (m, 2H), 1.31 (m, 2H), 0.91 (t, 3H, $J$ = 7.5 Hz).

[00370] Example IK: Synthesis of 2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (61).

![Chemical Structure 47](image1)

![Chemical Structure 60](image2)

![Chemical Structure 61](image3)

[00371] Synthesis of 2-cyclohexyl-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (60): 4-Bromo-2-cyclohexyl-6-methoxyisoquinolin-1(2H)-one (47) (0.37 g, 1.10 mmol), tetrakis(triphenylphosphine)palladium (64 mg, 0.055 mmol), cesium carbonate (0.72 g, 2.20 mmol) and 3,4,5-trifluorophenylboronic acid (0.23 g, 1.32 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 6 hours. The reaction solution was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate
The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 49/1 v/v) to give a white solid product, 0.40 g, 93.9% yield. MS: m/z 388.1 [M+H]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.26 (d, 1H, J = 9.0 Hz), 7.53-7.45 (m, 3H), 7.17 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 6.86 (d, 1H, J = 2.4 Hz), 4.86-4.76 (m, 1H), 3.79 (s, 3H), 1.86-1.64 (m, 7H), 1.43-1.39 (m, 2H), 1.29-1.21 (m, 1H).

Example 1L: Synthesis of 4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (63).

[00372] Synthesis of 2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (61): 2-Cyclohexyl-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1 (2H)-one (60) (0.40 g, 1.03 mmol) was dissolved in 30 mL of anhydrous CH₂Cl₂ in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (5.16 mL of 1.0M CH₂Cl₂ solution, 5.16 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 97/3 v/v) to give a white solid product, 0.38 g, 98.6% yield. MS: m/e 372.0 [M-H]- ¹H NMR (DMSO-d₆, 300 MHz) δ 10.29 (s, 1H), 8.17 (d, 1H, J = 8.7 Hz), 7.49-7.44 (m, 3H), 6.98 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.4 Hz), 6.79 (d, 1H, J = 2.4 Hz), 4.80 (m, 1H), 1.85-1.64 (m, 7H), 1.43-1.39 (m, 2H), 1.28-1.16 (m, 1H).

Example 1L: Synthesis of 4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (63).
Synthesis of 4-(3,5-difluorophenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (62):

4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.30 g, 1.12 mmol), tetrakis(triphenylphosphine)palladium (65 mg, 0.056 mmol), cesium carbonate (0.73 g, 2.24 mmol) and 3,5-difluorophenylboronic acid (0.21 g, 1.34 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. Then, the reaction mixture was diluted by adding 100 mL of water at room temperature. The mixture was extracted with EtOAc (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 49/1 v/v) to give a white solid product, 0.28 g, 83.1% yield. MS: m/z 302.0 [M+H]+. ¹H NMR (DMSO-4, 300 MHz) δ 8.25 (d, 1H, J = 8.7 Hz), 7.59 (s, 1H), 7.34-7.24 (m, 3H), 7.17 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.1 Hz), 6.89 (d, 1H, J = 2.1 Hz), 3.79 (s, 3H), 3.52 (s, 3H).

Synthesis of 4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (63):

4-(3,5-Difluorophenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (62) (0.23 g, 0.76 mmol)
was dissolved in 30 mL of anhydrous methylene chloride in a dry and argon flushed 150 mL single-
necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr$_3$ (3.82 mL of 1M CH$_2$Cl$_2$ solution, 3.82 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The mixture was extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO$_4$. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CTLC$^V$/MeOH = 97/3 v/v) to give a white solid product, 0.20 g, 91.3% yield. MS: m/e 285.8 [M-H]. $^1$H NMR (DMSO-$_d6$, 300 MHz) δ 10.28 (s, 1H), 8.16 (d, 1H, $J = 9.0$ Hz), 7.53 (s, 1H), 7.35-7.27 (m, 1H), 7.24-7.17 (m, 2H), 6.97 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz), 6.84 (d, 1H, $J = 2.4$ Hz), 3.50 (s, 3H).

Example 1M: Synthesis of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67).
Synthesis of 2-cyclopropyl-6-methoxyisoquinolin-1(2H)-one (64): 6-Methoxyisoquinoline-1-ol (1) (2.00 g, 11.42 mmol), copper(I) iodide (0.44 g, 2.28 mmol), L-proline (0.53 g, 4.57 mmol) and anhydrous potassium carbonate (3.16 g, 22.84 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 40 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 90 °C under argon atmosphere. Then, bromocyclopropane (2.76 g, 22.84 mmol) was added via a syringe. The reaction mixture was stirred at 90 °C overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (5x30 mL). The extracts were separated, washed with brine (2 x 20 mL) and concentrated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, O4 (O4/Acetone = 19/1, v/v) to give a white solid product, 0.45 g, 18.3% yield. MS: 237.9 [M+Na]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.12 (d, 1H, J = 8.7 Hz), 7.35 (d, 1H, J = 7.2 Hz), 7.12 (d, 1H, J = 2.4 Hz), 7.07 (dd, 1H, J1 = 8.7 Hz, J2 = 2.4 Hz), 6.57 (d, 1H, J = 7.5 Hz), 6.03-5.90 (m, 1H), 5.19-5.05 (m, 2H), 4.58-4.55 (m, 2H), 3.87 (s, 3H).

Synthesis of 4-bromo-2-cyclopropyl-6-methoxyisoquinolin-1(2H)-one (65): 2-Cyclopropyl-6-methoxyisoquinolin-1(2H)-one (64) (0.44 g, 2.04 mmol) and N-bromosuccinimide (0.44 g, 2.45 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (30 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 6 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH2Cl2/Acetone = 49/1 v/v) to give a white solid product, 0.28 g, 46.7% yield. MS: 316.0 [M+Na]+. 1H NMR (DMSO-d6, 300 MHz) δ 8.20 (d, 1H, J = 9.0 Hz), 7.87 (s, 1H), 7.20 (dd, 1H, J1 = 9.0 Hz, J2 = 2.4 Hz), 7.10 (d, 1H, J = 2.4 Hz), 6.03-5.90 (m, 1H), 5.21-5.10 (m, 2H), 4.57 (d, 2H, J = 5.4 Hz), 3.93 (s, 3H).

Synthesis of 2-cyclopropyl-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (66): 4-Bromo-2-cyclopropyl-6-methoxyisoquinolin-1(2H)-one (65) (0.20 g, 0.68 mmol), tetrakis(triphenylphosphine)palladium (39 mg, 0.034 mmol), cesium carbonate (0.44 g, 1.36 mmol) and 3,4,5-trifluorophenylboronic acid (0.18 g, 1.02 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere.
The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 97/3 v/v) to give a white solid product, 0.11 g, 46.8% yield. MS: m/z 368.1 [M+Na]⁺. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.25 (d, 1H, J = 9.0 Hz), 7.54-7.44 (m, 3H), 7.18 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.4Hz), 6.87 (d, 1H, J = 2.4 Hz), 6.06-5.93 (m, 1H), 5.19 (d, 1H, J = 2.4 Hz), 5.15 (dd, 1H, J₁ = 10.2 Hz, J₂ = 1.2 Hz), 4.60 (d, 2H, J = 5.7 Hz), 3.80 (s, 3H).

[00380] Synthesis of 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (67): 2-Cyclopropyl-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1 (2H)-one (66) (0.11 g, 0.32 mmol) was dissolved in 30 mL of anhydrous CH₂Cl₂ in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (1.27 mL of 1.0M CH₂Cl₂ solution, 1.27 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give a white solid product, 80 mg, 75.5% yield. MS: m/e 329.9 [M-H]⁻. ¹H NMR (DMSO-d₆, 500 MHz) δ 10.34 (s, 1H), 8.17 (d, 1H, J = 8.5 Hz), 7.47-7.44 (m, 2H), 7.42 (s, 1H), 7.00 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 6.81 (s, 1H), 6.02-5.94 (m, 1H), 5.19-5.14 (m, 2H), 4.58 (d, 2H, J = 5.5 Hz).

[00381] Example IN: Synthesis of 2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (70).
[00382] Synthesis of 2-(3,3-difluoroallyl)-6-methoxyisoquinolin-1(2 \textit{H})-one (68): 6-Methoxyisoquinoline-1-ol (1) (1.00 g, 5.71 mmol), copper(I) iodide (0.22 g, 1.14 mmol), L-proline (0.26 g, 2.28 mmol) and anhydrous potassium carbonate (1.58 g, 11.42 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 30 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 90 °C under argon atmosphere. Then, 3-iodo-1,1,1-trifluoropropane (2.02 g, 11.42 mmol) was added via a syringe. The reaction mixture was stirred at 90 °C overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (5x30 mL). The extracts were separated, washed with brine (2 x 20 mL) and concentrated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, CH$_2$Cl$_2$/Acetone = 49/1, v/v) to give a white solid product, 0.38 g, 26.6% yield. MS: 274.0
[M+Na]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.11 (d, 1H, J = 8.7 Hz), 7.42 (d, 1H, J = 2.4 Hz), 7.07 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.4 Hz), 6.57 (d, 1H, J = 7.2 Hz), 4.87 (td, J₁ = 7.8 Hz, J₂ = 2.4 Hz), 4.54 (d, 2H, J = 7.8 Hz), 3.87 (s, 3H).

[00383] Synthesis of 4-bromo-2-(3,3-difluoroallyl)-6-methoxyisoquinolin-1(2 H)-one (69):
2-(3,3-Difluoroallyl)-6-methoxyisoquinolin-1(2 H)-one (68) (0.36 g, 1.44 mmol) and N-bromosuccinimide (0.31 g, 1.72 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (30 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 6 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/Acetone = 97/3 v/v) to give a white solid product, 0.45 g, 95.1% yield. MS: 354.0 [M+Na]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.18 (d, 1H, J = 9.0 Hz), 7.93 (s, 1H), 7.19 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 7.08 (d, 1H, J = 2.4 Hz), 4.97-4.82 (m, 1H), 4.56 (d, 2H, J = 7.5 Hz), 3.92 (s, 3H).

[00384] Synthesis of 2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (70):
4-Bromo-2-(3,3-difluoroallyl)-6-methoxyisoquinolin-1(2 H)-one (69) (0.50 g, 1.51 mmol), tetrakis(triphenylphosphine)palladium (87 mg, 0.076 mmol), cesium carbonate (0.984 g, 3.02 mmol) and 3,4,5-trifluorophenylboronic acid (0.40 g, 2.27 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 6 hours. The reaction solution was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 97/3 v/v) to give a white solid product, 0.35 g, 60.8% yield. MS: m/z 382.1 [M+H]+. ¹H NMR (DMSO-d₆, 500 MHz) δ 8.25 (d, 1H, J = 9.0 Hz), 7.55 (s, 1H), 7.53-7.49 (m, 2H), 7.19 (d, 1H, J = 9.0 Hz), 6.88 (s, 1H), 4.96-4.88 (m, 1H), 4.60 (d, 2H, J = 7.5 Hz), 3.81 (s, 3H).

[00385] Example IN: Synthesis of 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (72).

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Synthesis of 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-methoxy-2-methylisoquinolin-1(2H)-one (71): 4-Bromo-6-methoxy-2-methylisoquinolin-1(2H)-one (19) (0.21 g, 0.78 mmol), tetrakis(triphenylphosphine)palladium (68 mg, 0.059 mmol), cesium carbonate (0.51 g, 1.57 mmol) and 2-(4-fluoro-3-(trifluoromethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.34 g, 1.18 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. Then, the reaction mixture was diluted by adding 50 mL of water at room temperature. The mixture was extracted with EtOAc (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/Acetone = 19/1 v/v) to give a white solid product, 0.25 g, 98.2% yield. MS: m/z 374.1 [M+Na]⁺. ¹H NMR (DMSO-4, 500 MHz) δ 8.25 (d, 1H, J = 8.5 Hz), 7.89-7.86 (m, 1H), 7.84 (d, 1H, J = 7.0 Hz), 7.67-7.63 (m, 1H), 7.60 (s, 1H), 7.17 (dd, 1H, J₁ = 9.5 Hz, J₂ = 2.0 Hz), 6.78 (d, 1H, J = 2.0 Hz), 3.77 (s, 3H), 3.53 (s, 3H).

Synthesis of 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (72) and 4-(3-(bis(2,4-dimethylphenyl)methyl)-4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (72A): 4-(4-Fluoro-3-(trifluoromethyl)phenyl)-6-
methoxy-2-methylisoquinolin-l(2 H)-one (71) (0.20 g, 0.57 mmol) was dissolved in 30 mL of anhydrous m-xylene in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (0.71g, 2.85 mmol) was added via a syringe with stirring at room temperature. After stirred and heated to 90 °C for 3 hours, the reaction was quenched by adding 50 mL of water and 5 mL of methanol at 0 °C. The mixture was stirred at room temperature for two hours and extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CILCVMeOH = 19/1 v/v) to give 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one as a white solid, 0.10 g, 52.1% yield and 4-(3-(bis(2,4-dimethylphenyl)methyl)-4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one as a white solid, 30 mg, 10.7% yield.

4-(4-Fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (72): MS: m/e 336.0 [M-H]⁻. ¹H NMR (DMSO-d₆, 500 MHz) δ 10.31 (s, 1H), 8.17 (d, 1H, J = 9.0 Hz), 7.89 (d, 1H, J = 8.0 Hz), 7.64-7.62 (m, 1H), 7.57-7.53 (m, 2H), 6.97 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.5 Hz), 6.68 (d, 1H, J = 2.5 Hz), 3.51 (s, 3H).

4-(3-(bis(2,4-dimethylphenyl)methyl)-4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (72A): MS: m/e 490.1 [M-H]⁻. ¹H NMR (DMSO-d₆, 500 MHz) δ 10.20 (s, 1H), 8.10 (d, 1H, J = 9.0 Hz), 7.34-7.33 (m, 3H), 7.02 (s, 2H), 6.94 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 6.91 (d, 2H, J = 7.5 Hz), 6.88 (d, 1H, J = 7.0 Hz), 6.63 (d, 2H, J = 8.0 Hz), 6.53 (d, 1H, J = 1.5 Hz), 5.84 (s, 1H), 3.45 (s, 3H), 2.22 (s, 6H), 2.11 (s, 6H).

Example 10: Synthesis of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (77) and 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78).
Synthesis of (4-bromo-6-methoxy-l-oxoisoquinolin-2(lH)-yl)methyl acetate (76): 4-Bromo-6-methoxyisoquinolin-l(2H)-one (15) (5.00 g, 19.68 mmol) was dissolved in 80 mL of anhydrous THF at room temperature under argon. NaH (0.95 g of 60% wt in mineral oil, 23.62 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for 3 hours. Then, bromomethyl acetate (4.52 g, 29.52 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature for 5 hours. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution at 0 °C. The solution was stirred at room temperature for one hour. THF solvent was removed. The aqueous residue was extracted with ethyl acetate (3x50 mL). The extracts were dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 19/1 v/v) to give a white solid product, 5.65 g, 88.0 % yield. MS: 350.0 [M+Na]⁺. ¹H NMR (DMSO-4, 500 MHz) δ 8.19 (d, 1H, J = 9.0 Hz), 7.97 (s, 1H), 7.21 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 7.08 (s, 1H), 5.84 (s, 2H), 3.93 (s, 3H), 2.06 (s, 3H).

Synthesis of 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (77): (4-Bromo-6-methoxy-l-oxoisoquinolin-2(lH)-yl)methyl acetate (76) (2.00 g, 6.13 mmol), tetrakis(triphenylphosphine)palladium (0.71 g, 0.61 mmol), cesium carbonate (3.99 g, 12.26 mmol) and 3,4,5-trifluorophenylboronic acid (1.62 g, 9.20 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (60 mL) and water (20 mL) were added via a syringe under argon atmosphere.
The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2x20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CH₂Cl₂:MeOH = 19/1 v/v) to give a white solid product, 1.45 g, 77.5% yield. MS: m/z 304.0 [M-H]⁻. ¹H NMR (DMSO-d₆, 300 MHz) δ 11.42 (s, 1H), 8.21 (d, 1H, 7 = 9.0 Hz), 7.51-7.43 (m, 2H), 7.17-7.14 (m, 2H), 6.86 (d, 1H, 7 = 2.4 Hz), 3.79 (s, 3H).

Synthesis of 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78): 6-Methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77) (0.98 g, 3.21 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (50 mL) was added via a syringe at room temperature. BBr₃ (4.02 g, 16.05 mmol) was added dropwise with stirring at room temperature. The resulted solution was heated to 80 °C for 8 hours. 100 mL of water and 10 mL of methanol were added to quench the reaction at 0 °C. The solution was stirred at room temperature for two hours and extracted with EtOAc/MeOH (9/1 v/v, 3x50 mL). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂:MeOH = 9/1 v/v) to give a white solid product, 0.68 g, 73.1% yield. MS: m/e 289.9 [M-H]⁻. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.29 (s, 1H), 10.28 (s, 1H), 8.13 (d, 1H, 7 = 9.0 Hz), 7.45 (d, 1H, 7 = 8.0 Hz), 7.43 (d, 1H, 7 = 7.5 Hz), 7.10 (d, 1H, 7 = 5.5 Hz), 6.96 (dd, 1H, 7i = 9.0 Hz, 7₂ = 1.5 Hz), 6.79 (d, 1H, 7 = 1.5 Hz).

Example IP: Synthesis of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81).
Synthesis of (4-bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)acetic acid (76): 4-Bromo-6-methoxyisoquinolin-1(2H)-one (15) (5.00 g, 19.68 mmol) was dissolved in 80 mL of anhydrous THF at room temperature under argon. NaH (0.95 g of 60%wt in mineral oil, 23.62 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for 3 hours. Then, bromomethyl acetate (4.52 g, 29.52 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature for 5 hours. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution at 0 °C. The solution was stirred at room temperature for one hour. THF solvent was removed. The aqueous residue was extracted with ethyl acetate 3x50 mL. The extracts were dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CILCVAcetone = 19/1 v/v) to give a white solid product, 5.65 g, 88.0% yield. MS: 350.0 [M+Na]+. ¹H NMR (DMSO-d⁶, 500 MHz) δ 8.19 (d, 1H, J = 9.0 Hz), 7.97 (s, 1H), 7.21 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 7.08 (s, 1H), 5.84 (s, 2H), 3.93 (s, 3H), 2.06 (s, 3H).

Synthesis of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-methoxyisoquinolin-1(2H)-one (80): (4-Bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)methyl acetate (76) (2.00 g, 6.13 mmol), tetrakis(triphenylphosphine)palladium (0.71 g, 0.61 mmol), cesium carbonate (3.99 g, 12.26 mmol) and 3-fluoro-4-trifluoromethylphenylboronic acid (2.66 g, 9.20 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (60 mL) and water (20 mL) were added via a syringe under argon...
atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2x20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give a white solid product, 1.35 g, 65.2% yield. MS: m/z 338.2 [M+H]⁺. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.50 (d, 1H, J = 3.5 Hz), 8.23 (d, 1H, J = 8.5 Hz), 7.86 (t, 1H, J = 8.0 Hz), 7.66 (d, 1H, J = 11.5 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.28 (d, 1H, J = 5.0 Hz), 7.16 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 6.90 (d, 1H, J = 2.0 Hz), 3.79 (s, 3H).

[00396] Synthesis of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxyisoquinolin-1(2H)-one (81): 4-(3-Fluoro-4-(trifluoromethyl)phenyl)-6-methoxyisoquinolin-1(2H)-one (80) (0.90 g, 2.67 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (50 mL) was added via a syringe at room temperature. BBr₃ (3.34 g, 13.34 mmol) was added dropwise with stirring at room temperature. The resulting solution was heated to 70 °C for 8 hours. 100 mL of water and 10 mL of methanol were added to quench the reaction at 0 °C. The solution was stirred at room temperature for two hours and extracted with EtOAc/MeOH (9/1 v/v, 3x50 mL). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.66 g, 76.8% yield. MS: m/z 321.9 [M-H]⁻. ¹H NMR (DMSO-d₆, 300 MHz) δ 11.33 (d, 1H, J = 6.0 Hz), 10.26 (s, 1H), 8.14 (d, 1H, J = 8.7 Hz), 7.89 (t, 1H, J = 8.1 Hz), 7.62 (d, 1H, J = 11.7 Hz), 7.48 (d, 1H, J = 8.1 Hz), 7.18 (d, 1H, J = 6.3 Hz), 6.97 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.4 Hz), 6.81 (d, 1H, J = 2.4 Hz).

[00397] Example 1Q: Synthesis of methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82).
[00398] **Synthesis of methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate** (82): 6-Hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78) (0.28 g, 0.96 mmol) was dissolved in 80 mL of anhydrous DMF at room temperature under argon. NaH (0.12 g of 60%wt in mineral oil, 2.88 mmol) was added in 4 portions at 0 °C. The solution was stirred at room temperature for two hours. The white precipitate was filtered, washed overnight, dried anhydrous MgSO₄, and then concentrated at room temperature. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 24/1 v/v) to give a white solid product, 0.40 g, 95.7 % yield. MS: 458.1 [M+Na]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.22 (d, 1H, J = 8.7 Hz), 7.60 (s, 1H), 7.43 (d, 1H, J = 9.0 Hz), 7.42 (d, 1H, J = 9.0 Hz), 7.22 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 6.86 (d, 1H, J = 2.4 Hz), 4.91 (s, 2H), 4.79 (s, 2H), 3.69 (s, 6H).

[00399] **Example 1R: Synthesis of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile** (84).

![Synthesis diagram](image)

[00400] **Synthesis of 4-bromo-6-hydroxyisoquinolin-1(2H)-one** (83): 4-Bromo-6-methoxyisoquinolin-1(2H)-one (15) (1.44 g, 5.67 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous m-xylene (50 mL) was added via a syringe at room temperature. BBr₃ (7.10 g, 28.34 mmol) was added dropwise with stirring at room temperature. The resulting solution was heated to reflux overnight. 100 mL of water and 10 mL of methanol were added to quench the reaction at 0 °C. The solution was stirred at room temperature for two hours. The white precipitate was filtered, washed...
with water (2×20 mL) and dried in air. The solution was extracted with EtOAc/MeOH (9/1 v/v, 3×50 mL). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue and solid were combined and purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 1.25 g, 91.9% yield.

[00401] Synthesis of 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84): 4-Bromo-6-hydroxyisoquinolin-1(2 H)-one (83) (0.45 g, 1.87 mmol), tetrakis(triphenylphosphine)palladium (0.22 g, 0.19 mmol), cesium carbonate (2.44 g, 7.48 mmol) and 2-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (0.70 g, 2.81 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3×50 mL). The extracts were combined, washed with brine (2×10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.36 g, 69.2% yield. MS: m/e 278.8 [M-H]⁻ NMR (DMSO-d₆, 500 MHz) δ 11.29 (d, 1H, J = 4.5 Hz), 10.28 (s, 1H), 8.13 (d, 1H, J = 8.5 Hz), 8.00 (dd, 1H, J₁ = 6.5 Hz, J₂ = 2.0 Hz), 7.85-7.82 (m, 1H), 7.66-7.63 (m, 1H), 7.11 (d, 1H, J = 6.0 Hz), 6.96 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 6.68 (d, 1H, J = 2.0 Hz).

[00402] Example IS: Synthesis of 6-(6-hydroxy-1-oxoisoquinolin-2(1 H)-yl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (87).
Synthesis of 6-(6-methoxy-1-oxoisoquinolin-2(1H)-yl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (86): 6-Methoxyisoquinoline-1-ol (1) (1.00 g, 5.71 mmol), 6-bromo-4-methyl-2-oxo-2H-chromene-3-carbonitrile (1.81 g, 6.85 mmol), copper(I) iodide (0.22 g, 1.14 mmol), L-proline (0.26 g, 2.28 mmol) and anhydrous potassium carbonate (1.58 g, 11.42 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 50 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 90 °C overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was stirred at room temperature for one hour. The mixture was extracted with EtOAc (5x50 mL). The organic layers were combined, washed with brine (2x30 mL), dried over anhydrous MgSO4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH2Cl2/acetone = 9/1 v/v) to give a yellow solid product, 0.38 g, 18.6 % yield. MS: 359.2 [M+H]+. 1H NMR (DMSO-4, 500 MHz) δ 8.17 (d, 1H, J = 9.0 Hz), 8.12 (s, 1H), 7.94 (d, 1H, J = 9.0 Hz), 7.64 (d, 1H, J = 8.5 Hz), 7.61 (d, 1H, J = 7.0 Hz), 7.24 (s, 1H), 7.14 (d, 1H, J = 7.5 Hz), 6.71 (d, 1H, J = 7.0 Hz), 5.76 (s, 3H), 3.91 (s, 3H).

Synthesis of 6-(6-hydroxy-1-oxoisoquinolin-2(1H)-yl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (87): 6-(6-Methoxy-1-oxoisoquinolin-2(1H)-yl)-4-methyl-2-oxo-2H-chromene-3-carbonitrile (86) (0.19 g, 0.53 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (30 mL) was added via a syringe at room temperature. BBr3 (0.66 g, 2.65 mmol) was added dropwise with stirring at room temperature. The resulted solution was heated to 90 °C for 3 hours. 50 mL of water and 10 mL of methanol were added to quench the reaction at 0 °C.
The solution was stirred at room temperature for two hours and extracted with EtOAc (3x50 mL). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 47/3 v/v) to give a yellow solid product, 0.10 g, 55.6% yield. MS: m/e 342.9 [M-H]⁻. ¹H NMR (DMSO-d₆, 500 MHz)  δ 10.45 (s, 1H), 8.11-8.10 (m, 2H), 7.91 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.44 (d, 1H, J = 7.5 Hz), 7.01-6.97 (m, 2H), 6.62 (d, 1H, J = 7.0 Hz), 2.75 (s, 3H).

Example IT: Synthesis of 6-methoxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (88).

![Chemical structure of 6-methoxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (88).

Synthesis of 6-methoxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (88): 6-Methoxyisoquinoline-1-ol (1) (1.00 g, 5.71 mmol), 5-bromoisobenzofuran-1(3H)-one (1.46 g, 6.85 mmol), copper(I) iodide (0.22 g, 1.14 mmol), L-proline (0.26 g, 2.28 mmol) and anhydrous potassium carbonate (1.58 g, 11.42 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 50 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 90 °C overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was stirred at room temperature for one hour. The mixture was extracted with EtOAc (5x50 mL). The organic layers were combined, washed with brine (2x30 mL), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 97/3 v/v) to give a white solid product, 0.78 g, 44.6% yield. MS: 330.0 [M+Na]⁺. ¹H NMR (DMSO-d₆, 500 MHz)  δ 8.17 (d, 1H, J = 9.0 Hz), 7.89 (d, 1H, J = 8.0 Hz), 7.83 (s, 1H), 7.69 (d, 1H, J = 8.0 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 2.0 Hz), 7.14 (dd, 1H, J₁ = 8.0 Hz, J₂ = 2.0 Hz), 6.71 (d, 1H, J = 8.0 Hz), 5.48 (s, 2H), 3.91 (s, 3H).
Example 1U: Synthesis of 4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (90).

**Synthesis of 4-bromoisoquinolin-1(2H)-one (89):** Isoquinolin-1-ol (1.00 g, 6.89 mmol) and N-bromosuccinimide (1.47 g, 8.27 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (50 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature overnight. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CILC\(^{+}\)/MeOH = 99/1 v/v) to give a white solid product, 1.08 g, 70.1% yield. MS: 223.6 [M-H]. \(^1\)H NMR (DMSO-4, 300 MHz) \(\delta\) 11.59 (s, 1H), 8.23 (dd, 1H, \(J_1 = 7.8\) Hz, \(J_2 = 0.9\) Hz), 7.89-7.83 (m, 1H), 7.78-7.75 (m, 1H), 7.63-7.65 (m, 2H).

**Synthesis of 4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (90):** 4-Bromoisoquinolin-1(2H)-one (89) (0.50 g, 2.23 mmol), tetrakis(triphenylphosphine)palladium (0.26 g, 0.22 mmol), cesium carbonate (2.91 g, 3.35 mmol) and 3,4,5-trifluorophenylboronic acid (0.59 g, 3.35 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (5x20 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO\(_4\) followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column
chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give a white solid product, 0.45 g, 73.7% yield. MS: m/z 279.0 [M+Na]+. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.60 (s, 1H), 8.29 (d, 1H, J = 7.5 Hz), 7.73 (t, 1H, J = 7.0 Hz), 7.58-7.52 (m, 2H), 7.48-7.45 (m, 2H), 7.20 (s, 1H).

Example IV: Synthesis of 4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinol-1(2H)-one (91).

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[00409] Synthesis of 4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinol-1(2H)-one (91).

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[00410] Synthesis of (4-bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)methyl acetate (76): 4-Bromo-6-methoxyisoquinolin-1(2H)-one (15) (5.00 g, 19.68 mmol) was dissolved in 80 mL of anhydrous THF at room temperature under argon. NaH (0.95 g of 60%wt in mineral oil, 23.62 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for 3 hours. Then, bromomethyl acetate (4.52 g, 29.52 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature for 5 hours. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution at 0 °C. The solution was stirred at room temperature for one hour. THF solvent was removed. The aqueous residue was extracted with ethyl acetate (3x50 mL). The extracts were dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 19/1 v/v) to give a white solid product, 5.65 g, 88.0 % yield. MS: 350.0 [M+Na]+. ¹H NMR (DMSO-d₄, 500 MHz) δ 8.19 (d, 1H, J = 9.0 Hz), 7.97 (s, 1H), 7.21 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 7.08 (s, 1H), 5.84 (s, 2H), 3.93 (s, 3H), 2.06 (s, 3H).
Synthesis of 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-methoxyisoquinolin-1(2H)-one (80): (4-Bromo-6-methoxy-1-oxoisooquinolin-2(1H)-yl)methyl acetate (76) (2.00 g, 6.13 mmol), tetrakis(triphenylphosphine)palladium (0.71 g, 0.61 mmol), cesium carbonate (3.99 g, 12.26 mmol) and 2-(3-fluoro-4-(trifluoromethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.66 g, 9.20 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (60 mL) and water (20 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2x20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CILC\textregistered/MeOH = 19/1 v/v) to give a white solid product, 1.35 g, 65.2% yield. MS: m/z 338.2 [M+H]+. 1H NMR (DMSO-d$_6$, 500 MHz) δ 11.50 (d, 1H, J = 3.5 Hz), 8.23 (d, 1H, J = 8.5 Hz), 7.86 (t, 1H, J = 8.0 Hz), 7.66 (d, 1H, J = 11.5 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.28 (d, 1H, J = 5.0 Hz), 7.16 (dd, 1H, J$_1$ = 9.0 Hz, J$_2$ = 2.0 Hz), 6.90 (d, 1H, J = 2.0 Hz), 3.79 (s, 3H).

Synthesis of 4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-methoxyisoquinolin-1(2H)-one (91): 4-(3-Fluoro-4-(trifluoromethyl)phenyl)-6-methoxyisoquinolin-1(2H)-one (80) (8.68 g, 25.74 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (100 mL) was added via a syringe at room temperature. BB$_r_3$ (25.80 g, 0.103 mol) was added dropwise with stirring at room temperature. The resulted solution was heated to 70 °C for 8 hours. The reaction was quenched by pouring the reaction mixture to 200 mL of ice water and 20 mL of methanol. The resulted mixture was stirred at room temperature for two hours. The white precipitate was isolated, washed with water (2x50 mL) and dried under vacuum. The solid was subjected to column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 93/7 v/v) to give a white solid product, 6.52 g, 75.8% yield. MS: m/e 334.1 [M-H]−. 1H NMR (DMSO-d$_6$, 500 MHz) δ 11.33 (d, 1H, J = 5.0 Hz), 10.28 (s, 1H), 8.14 (d, 1H, J = 9.0 Hz), 7.44 (dd, 1H, J$_1$ = 12.0 Hz, J$_2$ = 1.5 Hz), 7.40 (dd, 1H, J$_1$ = 8.0 Hz, J$_2$ = 1.5 Hz), 7.16 (d, 1H, J = 5.5 Hz), 6.98 (dd, 1H, J$_1$ = 9.0 Hz, J$_2$ = 2.0 Hz), 6.84 (d, 1H, J = 2.0 Hz), 3.89 (s, 3H).

Example 1W: Synthesis of 6-hydroxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93) and 6-methoxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93A).
Synthesis of 6-methoxy-4-(6-methoxypyridin-3-yl)isoquinolin-1(2H)-one (92):

(4-Bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)methyl acetate (76) (0.74 g, 2.27 mmol), tetrakis(triphenylphosphine)palladium (0.131 g, 0.114 mmol), cesium carbonate (1.48 g, 4.54 mmol) and 6-methoxypyridin-3-ylboronic acid (0.42 g, 2.72 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2x20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give a white solid product, 0.48 g, 75.0% yield. MS: m/z 283.2 [M+H]+. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.34 (d, 1H, J = 5.0 Hz), 8.22-8.21 (m, 2H), 7.81 (dd, 1H, J₁ = 8.5 Hz, J₂ = 2.5 Hz), 7.14 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.0 Hz), 7.11 (d, 1H, J = 5.0 Hz), 6.93 (d, 1H, J = 8.5 Hz), 6.77 (d, 1H, J = 2.5 Hz), 3.91 (s, 3H), 3.77 (s, 3H).

[00415] Synthesis of 6-hydroxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93) and 6-methoxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93A):

6-Methoxy-4-(6-methoxypyridin-3-yl)isoquinolin-1(2H)-one (92) (0.30 g, 1.06 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (30 mL) was added via a syringe at room temperature. BBr₃ (1.31 g, 5.30 mmol) was added dropwise with stirring at room temperature. The resulted solution was heated to 80 °C for 8 hours. The reaction was quenched by adding 50 mL of ice water and 10 mL of methanol at 0 °C. The resulted mixture was stirred at room temperature for two hours and...
neutralized by adding saturated NaHCO₃ solution. The volatile was removed under reduced pressure. The residue was subjected to column chromatography (silica-gel, CH2Cl2/MeOH = 17/3 v/v) to give solid compounds.

[00416] 6-Hydroxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93): as a white solid product, 0.16 g, 59.5% yield. MS: m/z 277.0 [M+Na]⁺. ¹H NMR (DMSO-4, 500 MHz) δ 11.0 (s, 1H), 8.06 (d, 1H, 7 = 9.0 Hz), 7.46 (dd, 1H, 7i = 9.3 Hz, 7₂ = 2.4 Hz), 7.38 (d, 1H, 7 = 2.4 Hz), 7.02 (s, 1H), 6.97 (dd, 1H, 7i = 8.7 Hz, 7₂ = 2.4 Hz), 6.71 (d, 1H, 7 = 2.1 Hz), 6.41 (d, 1H, J = 9.3 Hz); and

[00417] 6-Methoxy-4-(6-oxo-1,6-dihydropyridin-3-yl)isoquinolin-1(2H)-one (93A): as a pale-yellow solid, 10 mg, 3.5% yield. MS: m/z 291.0 [M+Na]⁺. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.8 (d, 1H, J = 5.4 Hz), 10.21 (s, 1H), 8.18 (dd, 1H, 7i = 2.4 Hz, 7₂ = 0.3Hz), 8.12 (d, 1H, 7 = 8.7 Hz), 7.76 (dd, 1H, 7i = 8.7 Hz, 7₂ = 2.4 Hz), 7.03 (d, 1H, 7 = 5.7 Hz), 6.97-6.92 (m, 2H), 6.70 (d, 1H, 7 = 2.1 Hz), 3.91 (s, 3H).

[00418] Example IX: Synthesis of 6-hydroxy-2-(l-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (104) and methyl 2-(bromomethyl)-4-(6-hydroxy-1-oxoisouquinolin-2(1H)-yl)benzoate (104A).
and anhydrous potassium carbonate (1.58 g, 11.42 mmol) were placed in a dry 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. The reaction flask was vacuumed and refilled with dry argon. 50 mL of anhydrous methyl sulfoxide was added via a syringe. The reaction mixture was stirred and heated to 90 °C overnight. The reaction was quenched by adding 100 mL of water at room temperature. The mixture was stirred at room temperature for one hour. The mixture was extracted with EtOAc (5x50 mL). The organic layers were combined, washed with brine (2x30 ml), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 97/3 v/v) to give a white solid product, 0.78 g, 44.6% yield. MS: 

\[ 330.0 \text{ [M+Na}^+] \]

1H NMR (DMSO-d₆, 500 MHz) δ 8.17 (d, 1H, J = 9.0 Hz), 7.89 (d, 1H, J = 8.0 Hz), 7.83 (s, 1H), 7.69 (d, 1H, J = 8.0 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 2.0 Hz), 7.14 (dd, 1H, J₁ = 8.0 Hz, J₂ = 2.0 Hz), 6.71 (d, 1H, J = 8.0 Hz), 5.48 (s, 2H), 3.91 (s, 3H).

[00420] Synthesis of 6-hydroxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (104) and methyl 2-(bromomethyl)-4-(6-hydroxy-1-oxoisooquinolin-2(1H)-yl)benzoate (104A). 6-Methoxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (103) (0.12 g, 0.39 mmol) was placed in a dry and argon flushed 100 mL single-necked round-bottomed flask fitted with a stirring bar, reflux condenser and an argon inlet. Anhydrous 1,2-dichloroethane (30 mL) was added via a syringe at room temperature. BBr₃ (0.49, 1.95 mmol) was added via a syringe with stirring at room temperature. The resulted solution was heated to 80 °C for 5 hours. The reaction mixture was poured into 100 mL of ice water and 10 mL methanol. After stirred at room temperature for two hours, the solution was extracted with EtOAc (3 x 50 mL). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give white solid products.

[00421] 6-Hydroxy-2-(1-oxo-1,3-dihydroisobenzofuran-5-yl)isoquinolin-1(2H)-one (104): 32 mg, 28.1% yield. MS: m/e 292.1 [M-H]⁻. 1H NMR (DMSO-d₄, 500 MHz) δ 10.47 (s, 1H), 8.11 (d, 1H, J = 9.0 Hz), 7.97 (d, 1H, J = 8.0 Hz), 7.82 (s, 1H), 7.67 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 7.5 Hz), 7.00 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.5 Hz), 6.97 (d, 1H, J = 2.5 Hz), 6.62 (d, 1H, J = 2.5 Hz), 5.47 (s, 2H); and

[00422] Methyl 2-(bromomethyl)-4-(6-hydroxy-1-oxoisooquinolin-2(1H)-yl)benzoate (104A): 45 mg, 29.8% yield. MS: m/e 387.8 [M-H]⁻. 1H NMR (DMSO-d₆, 500 MHz) δ 10.48 (s, 1H), 8.10 (d, 1H, J = 8.5 Hz), 8.00 (d, 1H, J = 8.5 Hz), 7.75 (d, 1H, J = 2.0 Hz), 7.58 (dd, 1H, J₁ =
Example 1Y: Synthesis of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106).

**Synthesis of 4-(4-chloro-3-fluorophenyl)-6-methoxyisoquinolin-1(2H)-one (105):**

(4-Bromo-6-methoxy-1-oxoisooquinolin-2(1H)-yl)methyl acetate (76) (0.65 g, 1.99 mmol), tetrakis(triphenylphosphine)palladium (0.115 g, 0.10 mmol), cesium carbonate (1.30 g, 3.99 mmol) and 4-chloro-3-fluorophenylboronic acid (0.42 g, 2.39 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2x20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CH$_2$Cl$_2$/MeOH = 19/1 v/v) to give a white solid product, 0.48 g, 79.3% yield. MS: m/e 301.8 [M-H]$^-$. $^1$H NMR (DMSO-$_4$, 500 MHz) $\delta$ 11.41 (d, 1H, $J = 4.0$ Hz), 8.21 (d, 1H, $J = 9.0$ Hz), 7.67 (t, 1H, $J = 8.0$ Hz), 7.54 (dd, 1H, $J_1 = 10.5$ Hz, $J_2 = 2.0$ Hz), 7.33 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz), 7.17-7.13 (m, 2H), 6.86 (d, 1H, $J = 2.5$ Hz), 3.87 (s, 3H).
[00425] **Synthesis of 4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106):**

4-(4-Chloro-3-fluorophenyl)-6-methoxyisoquinolin-1(2H)-one (105) (0.35 g, 1.15 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (30 mL) was added via a syringe at room temperature. BBr₃ (1.44 g, 5.76 mmol) was added dropwise with stirring at room temperature. The resulted solution was heated to 80 °C for 6 hours. The reaction was quenched by adding 50 mL of ice water and 10 mL of methanol at 0 °C. The resulted mixture was extracted with EtOAc (3x50 mL). The organic layer was separated, dried over anhydrous MgSO₄ and concentrated to dryness under reduced pressure. The residue was subjected to column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.28 g, 84.1% yield. MS: m/e 287.8 [M-H]⁻.

[00426] **Example 1Z: Synthesis of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108).**

![Chemical structure](image)

[00427] **Synthesis of 4-(6-methoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (107):**

(4-Bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)methyl acetate (76) (0.65 g, 1.99 mmol), tetrakis(triphenylphosphine)palladium (0.115 g, 0.10 mmol), cesium carbonate (1.30 g, 3.99 mmol) and 4-cyanophenylboronic acid (0.35 g, 2.39 mmol) were placed in a dry and argon flushed 250 mL
three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 100 mL of water at room temperature. The white precipitate was filtered out, washed with water (2×20 mL), dried under vacuum and purified by flash column chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give a white solid product, 0.46 g, 83.6% yield. MS: m/e 274.8 [M-H]-. ¹H NMR (DMSO-d₆, 500 MHz) δ 11.46 (d, 1H, J = 4.5 Hz), 8.22 (d, 1H, J = 9.0 Hz), 7.94 (d, 2H, J = 8.0 Hz), 7.68 (d, 2H, J = 8.5 Hz), 7.19 (d, 1H, J = 5.5 Hz), 7.16 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.5 Hz), 6.85 (d, 1H, J = 2.0 Hz), 3.77 (s, 3H).

[00429] **Synthesis of 4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108):**

4-(6-Methoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (107) (0.42 g, 1.52 mmol) was placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous chlorobenzene (30 mL) was added via a syringe at room temperature. BBr₃ (1.90 g, 7.60 mmol) was added dropwise with stirring at room temperature. The resulted solution was heated to 80 °C for 6 hours. The reaction was quenched by adding 50 mL of ice water and 10 mL of methanol at 0 °C. The resulted mixture was extracted with EtOAc (3x50 mL). The organic layer was separated, dried over anhydrous MgSO₄ and concentrated to dryness under reduced pressure. The residue was subjected to column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.35 g, 87.7% yield. MS: m/e 260.8 [M-H].

¹H NMR (DMSO-d₆, 500 MHz) δ 11.31 (d, 1H, J = 5.0 Hz), 10.28 (s, 1H), 8.14 (d, 1H, J = 9.0 Hz), 7.95 (d, 2H, J = 8.5 Hz), 7.63 (d, 2H, J = 8.0 Hz), 7.12 (d, 1H, J = 6.0 Hz), 6.97 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.5 Hz), 6.77 (d, 1H, J = 2.5 Hz).

[00429] **Example 1AA: Synthesis of 2-((2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109).**
Synthesis of methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82): 6-Hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78) (0.28 g, 0.96 mmol) was dissolved in 80 mL of anhydrous DMF at room temperature under argon. NaH (0.12 g of 60% wt in mineral oil, 2.88 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for 3 hours. Then, bromomethyl acetate (0.44 g, 2.88 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution at 0 °C. The solution was stirred at room temperature for one hour. THF solvent was removed. The aqueous residue was extracted with ethyl acetate (3x50 mL). The extracts were dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 24/1 v/v) to give a white solid product, 0.40 g, 95.7% yield. MS: 458.1 [M+Na]⁺. ¹H NMR (DMSO-d₄, 500 MHz) δ 8.22 (d, 1H, J = 8.7 Hz), 7.60 (s, 1H), 7.43 (d, 1H, J = 9.0 Hz), 7.42 (d, 1H, J = 9.0 Hz), 7.22 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 6.86 (d, 1H, J = 2.4 Hz), 4.91 (s, 2H), 4.79 (s, 2H), 3.69 (s, 6H).

Synthesis of 2-((2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109): Methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82) (0.17 g, 0.39 mmol) was placed in a dry...
250 mL single-necked round-bottomed flask fitted with a stirring bar and sealed with a rubber stopper. Anhydrous dichloromethane (30 mL) was added via syringe at room temperature. BBr₃ (1.95 mL of 1M CH₂Cl₂ solution, 1.95 mmol) was added dropwise with stirring at room temperature. The resulted solution was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of ice water and 10 mL of methanol at 0 °C. The resulted mixture was extracted with EtOAc (3x50 mL). The organic layer was separated, dried over anhydrous MgSO₄ and concentrated to dryness under reduced pressure. The residue was subjected to column chromatography (silica-gel, CHCl₃/MeOH = 9/1 v/v) to give a white solid product, 0.13 g, 79.0% yield. MS: m/e 419.9 [M-H]⁻. 

[00432] Example 1AB: Synthesis of 1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110) and 2-(4-bromophenylsulfonyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110A).

[00433] Synthesis of 1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110) and 2-(4-bromophenylsulfonyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110A): 6-Hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78) (0.50 g, 1.72 mmol) was dissolved in 30 mL of dry DMF under argon. NaH (0.21 g, 60%wt in mineral oil, 5.16 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. Then, bromobenzenesulfonyl chloride (0.66 g, 2.58 mmol) was added. The resulted mixture was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution. The mixture was extracted with EtOAc. The organic layer was separated, dried over anhydrous MgSO₄ and
concentrated to dryness. The residue was purified by column (silica-gel, CH2Cl2) to give white solid products.

[00434] l-oxo-4-(3,4,5-trifluorophenyl)-l,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110): 0.19 g, 21.6% yield. MS: m/z 533.8 [M+Na]+. 1H NMR (DMSO-d6, 500 MHz) δ 11.77 (d, 1H, J = 5.5 Hz), 8.33 (d, 1H, J = 8.5 Hz), 7.82 (d, 2H, J = 8.5 Hz), 7.75 (d, 2H, J = 8.5 Hz), 7.34 (dd, 1H, J1 = 8.5 Hz, J2 = 2.0 Hz), 7.27-7.24 (m, 3H), 6.83 (d, 1H, J = 2.0 Hz) and;

[00435] 2-(4-bromophenylsulfonf)-l-oxo-4-(3,4,5-trifluorophenyl)-l,2-dihydroisoquinolin-6-yl 4-bromobenzenesulfonate (110A): 0.48 g, 38.4% yield. MS: m/z 729.9 [M+H]+. 1H NMR (DMSO-d6, 500 MHz) δ 8.26 (d, 1H, J = 8.5 Hz), 8.10 (d, 2H, J = 8.5 Hz), 7.92 (d, 2H, J = 8.5 Hz), 7.86 (s, 1H), 7.80 (d, 2H, J = 8.5 Hz), 7.73 (d, 2H, J = 8.5 Hz), 7.41-7.38 (m, 3H), 6.84 (s, 1H).

[00436] Example 1AC: Synthesis of 2-(6-hydroxy-l-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(l H)-yl)acetic acid (113).

[00437] Synthesis of l-oxo-4-(3,4,5-trifluorophenyl)-l,2-dihydroisoquinolin-6-yl benzoate (111): 6-Hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78) (0.70 g, 2.40 mmol) was dissolved in 40 mL of dry DMF under argon. NaH (0.12 g, 60% wt in mineral oil, 2.88
mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. Then, benzoyl chloride (0.51 g, 3.60 mmol) was added via a syringe. The resulted mixture was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of water. The solution was extracted with EtOAc. The organic layer was separated, dried over anhydrous MgSO₄ and concentrated to dryness. The residue was purified by column (silica-gel, EtOAc) to give a white solid product, 0.30 g, 32.0% yield. MS: m/e 393.9 [M-H]⁻. ¹H NMR (DMSO-δ₆, 500 MHz) δ 11.67 (d, 1H, J = 5.5 Hz), 8.38 (d, 1H, J = 8.5 Hz), 8.13 (d, 2H, J = 8.0 Hz), 7.77-7.74 (m, 1H), 7.62-7.59 (m, 2H), 7.50 (d, 1H, J = 9.0 Hz), 7.46-7.43 (m, 2H), 7.41 (s, 1H), 7.25 (d, 1H, J = 5.5 Hz).

[00438] **Synthesis of 2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl benzoate (112):**

1-Oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl benzoate (111) (0.18 g, 0.46 mmol) was dissolved in 30 mL of anhydrous THF at room temperature under argon. NaH (36 mg of 60%wt in mineral oil, 0.91 mmol) was added at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for one hour. Then, methyl 2-bromoacetate (0.10 g, 1.50 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature for 6 hours. The reaction was quenched by adding 50 mL of saturated NH₄Cl solution at 0 °C. The solution was stirred at room temperature for one hour. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were washed with brine (2x20 mL), dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH₂Cl₂/acetone = 9/1 v/v) to give a white solid product, 0.20 g, 94.0% yield. MS: 490.1 [M+Na]⁺. ¹H NMR (DMSO-δ₆, 500 MHz) δ 8.40 (d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 8.0 Hz), 7.77-7.74 (m, 1H), 7.68 (s, 1H), 7.63-7.59 (m, 2H), 7.55 (d, 1H, J = 8.5 Hz), 7.48-7.45 (m, 3H), 4.85 (s, 2H), 3.72 (s, 3H).

[00439] **Synthesis of 2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetic acid (113):**

2-(2-Methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl benzoate (112) (0.20 g, 0.43 mmol) was dissolved in 30 mL of anhydrous dichloromethane at room temperature. BBr₃ (4.30 mL of 1M CH₂Cl₂ solution, 4.30 mmol) was added at room temperature. The reaction solution was stirred at room temperature overnight. The reaction was quenched by adding 100 mL of water and 10 mL of methanol. The organic phase was separated, dried over anhydrous MgSO₄ and concentrated to dryness. The residue was purified by column (silica-gel, CH₂Cl₂/methanol = 7/3 v/v) to give a white solid product, 0.10 g, 67.1% yield. MS: 347.9 [M-H]⁻. ¹H NMR (DMSO-δ₆, 500 MHz) δ 8.08 (d, 1H, J = 8.7 Hz), 7.39-7.35 (m, 3H), 6.95 (dd, 1H, J₁ = 8.7 Hz, J₂ = 1.5 Hz), 6.81 (s, 1H), 4.44 (s, 2H).
Example IAD: Synthesis of l-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl methanesulfonate (213).

6-Hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (0.22 g, 0.76 mmol) was dissolved in 30 mL of anhydrous DMF at room temperature under argon. NaH (30 mg, 60% wt in mineral oil, 0.76 mmol) was added in portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for 2 hours. Then, methanesulfonyl chloride (0.104 g, 0.91 mmol) was added via a syringe at room temperature. The resulted mixture was stirred at room temperature for 3 hours. The reaction was quenched by adding 100 mL of saturated NH₄Cl solution. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were dried over anhydrous MgSO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CPLC^/MeOH = 9/1 v/v) to give a white solid product, 0.20 g, 71.7 % yield. MS: 367.8 [M-H]. ¹H NMR (DMSO-d⁶, 400 MHz): δ 11.78 (s, 1H), 8.39 (d, 1H, J = 8.0 Hz), 7.52-7.47 (m, 3H), 7.42 (s, 1H), 7.30 (d, 1H, J = 4.0 Hz), 3.44 (s, 3H).

Example 1AE: Synthesis of 4-(2-bromo-4,5-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-yl-one (215).
Synthesis of 4-(2-bromo-4,5-difluorophenyl)-6-methoxyisoquinolin-1(2H)-one (214): (4-
Bromo-6-methoxy-l-oxoisoquinolin-2(1H)-yl)methyl acetate (0.83 g, 2.54 mmol),
tetrakis(triphenylphosphine)palladium (0.147 g, 0.127 mmol), cesium carbonate (1.66 g,
5.08 mmol), (2-biphenyl)di-ieri-butylphosphine (0.151 g, 0.508 mmol) and (2-bromo-4,5-
difluorophenyl)boronic acid (0.72 g, 3.05 mmol) were placed in a dry and argon flushed 250 mL
three-necked round-bottomed flask fitted with a stirring bar and a reflux condenser. 1,2-
Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere.
The reaction solution was stirred and heated to reflux for 6 hours. The reaction mixture was diluted
with 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50
mL). The organic layers were combined, dried over anhydrous MgSO₄, filtered and concentrated to
dryness. The residue was purified by flash column chromatography (silica gel, CILCVmethanol =
9/1 v/v) to give a white solid product, 0.58g, 62.4% yield. MS: 365.8[M-H]⁺. ¹H NMR (DMSO-d₆,
400 MHz) δ 11.36 (d, 1H, J = 4.0 Hz), 8.20 (d, 1H, J = 8.0 Hz), 8.04-8.00 (m, 1H), 7.67-7.55 (m,
1H), 7.15-7.11 (m, 2H), 6.36 (d, 1H, J = 4.0 Hz), 3.73 (s, 3H).
[00441] Synthesis of 4-(2-bromo-4,5-difluorophenyl)-6-hydroxyisoquinolin-l(2 H)-yl)-
one (215): 4-(2-Bromo-4,5-difluorophenyl)-6-methoxyisoquinolin-l(2 H)-one (214) (0.414 g, 1.13 mmol) was dissolved in 30 mL of anhydrous CH₂Cl₂ in a dry and argon flushed 150 mL single-
necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (5.65 mL of 1.0M CH₂Cl₂ solution, 5.65 mmol) was added via a syringe with stirring at room temperature. After refluxed for 6 hours, the reaction was quenched by adding 100 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure.

The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.28 g, 70.4% yield. MS: 351.7 [M-H]⁻. ¹H NMR (DMSO-4, 400 MHz) δ
11.22 (d, 1H, J = 4.0 Hz), 8.10 (d, 1H, J = 8.0 Hz), 8.05-8.01 (m, 1H), 7.68-7.63 (m, 1H), 7.65 (d, 1H, J = 8.0 Hz), 6.94 (dd, 1H, J₁ = 8.0 Hz, J₂ = 4.0 Hz), 6.29 (d, 1H, J = 4.0 Hz).

[00442] Example 1AF: Synthesis of 4',5'-difluoro-2'-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-[00443]
4-yl)-[l,l'-biphenyl]-4-carboxamide (218).
Synthesis of 4',5'-difluoro-2'-(6-methoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-biphenyl]-4-carbonitrile (216): 4-(2-Bromo-4,5-difluorophenyl)-6-methoxyisoquinolin-1(2H)-one (214) (0.20 g, 0.546 mmol), tetrakis(triphenylphosphine)palladium (32 mg, 0.0273 mmol), cesium carbonate (0.355 g, 1.09 mmol) and 4-cyanophenylboronic acid (0.12 g, 0.819 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and a reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction mixture was diluted with 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The organic layers were combined, dried over anhydrous MgSO4, filtered and concentrated to dryness. The residue was purified by flash column chromatography (silica gel, CH2Cl2/methanol = 9/1 v/v) to give a white solid product, 0.19 g, 89.6% yield. MS: 387.0[M-H]-. 1H NMR (DMSO-d6, 400 MHz) δ 11.27 (d, 1H, J = 4.0 Hz), 8.06 (d, 1H, J = 8.0 Hz), 7.68 (d, 2H, J = 8.0 Hz), 7.42 (d, 2H, J = 8.0 Hz), 7.03 (d, 1H, J = 8.0 Hz), 6.99 (dd, 1H, J1= 8.0 Hz, J2 = 4.0 Hz), 6.38 (s, 1H), 3.68 (s, 3H).

Synthesis of 4',5'-difluoro-2'-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-biphenyl]-4-carbonitrile (217): 4',5'-difluoro-2'-(6-methoxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-
biphenyl]-4-carbonitrile (216) (0.17 g, 0.438 mmol) was dissolved in 30 mL of anhydrous CH2Cl2 in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (2.19 mL of 1.0M CH2Cl2 solution, 2.19 mmol) was added via a syringe with stirring at room temperature. After refluxed overnight, the reaction was quenched by adding 100 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH2Cl2 layer was separated. The aqueous layer was extracted with CH2Cl2 (3 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.12 g, 73.3% yield. MS: 372.8 [M-H]⁻. 

**HNMR (DMSO-d6, 400 MHz)** δ 11.06 (d, 1H, J = 4.0 Hz), 10.12 (s, 1H), 7.99 (d, 1H, J = 8.0 Hz), 7.70 (d, 2H, J = 8.0 Hz), 7.69-7.66 (m, 1H), 7.60-7.57 (m, 1H), 7.37 (d, 2H, J = 8.0 Hz), 6.89 (d, 1H, J = 4.0 Hz), 6.84 (d, 1H, J = 8.0 Hz), 6.39 (s, 1H).

**Synthesis of 4%5'-difluoro-2'-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-biphenyl]-4-carboxamide (218):** 4',5'-Difluoro-2'-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)-[1,1'-biphenyl]-4-carbonitrile (217) (70 mg, 0.187 mmol), acetaldehyde oxime (55.2 g, 0.935 mmol) and tris(triphenylphosphine)rhodium(I) chloride (3.46 mg, 0.0037 mmol) were mixed together in 20 mL of toluene under argon. The reaction mixture was stirred and heated to reflux for 8 hours. Then, toluene was removed. The residue was subjected to column chromatography (silica-gel, CH2Cl2/MeOH = 9/1 v/v) to give a white solid product, 58 mg, 79.4% yield, MS: 390.9 [M-H]⁻. 

**HNMR (DMSO-d6, 400 MHz):** δ 11.05 (d, 1H, J = 4.0 Hz), 10.16 (s, 1H), 8.00 (d, 1H, J = 8.0 Hz), 7.90 (s, 1H), 7.69 (d, 2H, J = 8.0 Hz), 7.65-7.54 (m, 2H), 7.33 (s, 1H), 7.26 (d, 2H, J = 8.0 Hz), 6.88-6.84 (m, 2H), 6.44 (d, 1H, J = 4.0 Hz).

**Example 1AG: Synthesis of 4-(3,4-difluorophenyl)-6-methoxyisoquinolin-1(2 H)-one[00444] (219) and. 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2 H)-one (220):**
Synthesis of 4-(3,4-difluorophenyl)-6-methoxyisoquinolin-1(2H)-one (219) and 4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220): (4-Bromo-6-methoxy-1-oxoisoquinolin-2(1H)-yl)methyl acetate (0.83 g, 2.54 mmol), tetrakis(triphenylphosphine)palladium (0.147 g, 0.127 mmol), cesium carbonate (1.66 g, 5.08 mmol), (2-biphenyl)di-ieri-butylphosphine (0.151 g, 0.508 mmol) and (2-bromo-4,5-difluorophenyl)boronic acid (0.72 g, 3.05 mmol) were placed in a dry and argon flushed 250 mL three-necked round-bottomed flask fitted with a stirring bar and a reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux for 6 hours. The reaction mixture was diluted with 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The organic layers were combined, dried over anhydrous MgSO₄, filtered and concentrated to dryness. The residue was purified by flash column chromatography (silica gel, CILCVmethanol = 9/1 v/v) to give a white solid product, 0.18 g, 24.7% yield. This product was dissolved in 30 mL of anhydrous CH₂Cl₂ in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (3.13 mL of 1.0M CH₂Cl₂ solution, 3.13mmol) was added via a syringe with stirring at room temperature. After refluxed overnight, the reaction was quenched by adding 100 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure.
The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 9/1 v/v) to give a white solid product, 0.11 g, 64.7% yield. MS: 217.8 [M-H]. ¹H NMR (DMSO-d₆, 400 MHz) δ 11.21 (s, 1H), 10.24 (s, 1H), 8.12 (d, 1H, J = 8.0 Hz), 7.58-7.53 (m, 2H), 7.28-7.24 (m, 1H), 7.05 (d, 1H, J = 8.0 Hz), 6.95 (dd, 1H, J₁ = 8.0 Hz, J₂ = 4.0 Hz), 6.76 (d, 1H, J = 4.0 Hz).

**EXAMPLE 2:**

Synthesis of naphthalene (and other) HSDi templates.

Example 2A: Synthesis of 4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203).

![Chemical structure of compounds](image)

[00446] **Synthesis of 4-bromo-1,6-dimethoxynaphthalene (201):** 1,6-Dimethoxynaphthalene (200) (3.03 g, 16.10 mmol) and N-bromosuccinimide (2.87 g, 16.10 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (40 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature overnight. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexanes) to give a pale-yellow solid product, 3.58 g, 83.3% yield. MS: 269.0 [M+H]. ¹H NMR (DMSO-d₆, 500 MHz) δ 8.17 (d, 1H, J = 9.0 Hz), 7.62 (d, 1H, J = 8.0 Hz).
Hz), 7.44 (d, 1H, J = 2.5 Hz), 7.14 (dd, 1H, J = 9.0 Hz, J = 2.5 Hz), 6.55 (d, 1H, J = 8.5 Hz), 3.96 (s, 3H), 3.95 (s, 3H).

[00447] Synthesis of 1,6-dimethoxy-4-(4-(trifluoromethyl)phenyl)naphthalene (202): 4-Bromo-1,6-dimethoxynaphthalene (201) (0.57 g, 2.13 mmol), tetrakis(triphenylphosphine)palladium (0.12 g, 0.11 mmol), potassium carbonate (0.59 g, 4.27 mmol) and 4-(trifluoromethyl)phenylboronic acid (0.49 g, 2.56 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction was quenched by adding 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, hexanes) to give a white solid product, 0.65 g, 91.7% yield. MS: m/z 333.2 [M+H]+. ¹H NMR (DMSO-d₆, 500 MHz) δ 8.18 (d, 1H, J = 9.5 Hz), 7.86 (d, 2H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.0 Hz), 7.37 (d, 1H, J = 9.5 Hz), 7.22 (dd, 1H, J = 9.0 Hz, J = 2.5 Hz), 7.11 (d, 1H, J = 2.0 Hz), 6.93 (d, 1H, J = 8.0 Hz), 3.99 (s, 3H), 3.73 (s, 3H).

[00448] Synthesis of 4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203): 1,6-Dimethoxy-4-(4-(trifluoromethyl)phenyl)naphthalene (202) (0.53 g, 1.59 mmol) was dissolved in 30 mL of anhydrous CH₂Cl₂ in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (7.95 mL of 1.0 M CH₂Cl₂ solution, 7.95 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature for 3 hours, the reaction was quenched by adding 100 mL of water and 10 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂ /acetone = 97/3 v/v) to give a white solid product, 0.40 g, 82.6% yield. MS: m/z 302.9 [M-H]-. ¹H NMR (DMSO-d₆, 300 MHz) δ 10.19 (s, 1H), 9.63 (s, 1H), 8.08 (d, 1H, J = 9.0 Hz), 7.83 (d, 2H, J = 8.4 Hz), 7.63 (d, 2H, J = 8.5 Hz), 7.16 (d, 1H, J = 7.8 Hz), 7.02 (dd, 1H, J = 8.7 Hz, J = 2.4 Hz), 7.01 (s, 1H), 6.73 (d, 1H, J = 7.8 Hz).
Example 2B: Synthesis of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205).

\[
\begin{align*}
\text{Br} & \quad \text{OH} \\
\text{HCO} & \quad \text{HCO} \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{201} & \quad \text{204}
\end{align*}
\]

Synthesis of 1,6-dimethoxy-4-(3,4,5-trifluorophenyl)naphthalene (204): 4-Bromo-1,6-dimethoxynaphthalene (201) (0.60 g, 2.25 mmol), tetrakis(triphenylphosphine)palladium (0.13 mg, 0.11 mmol), cesium carbonate (1.46 g, 4.49 mmol) and 4-(3,4,5-trifluorophenyl)boronic acid (0.47 g, 2.70 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction was diluted by adding 100 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO\(_4\) followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, hexanes/acetone = 98/2 v/v) to give a white solid product, 0.70 g, 97.9% yield. MS: m/z 319.0 [M+H]+. \(^1\)H NMR (DMSO-\(d_6\), 300 MHz) \(\delta\) 8.16 (d, 1H, \(J = 9.3\) Hz), 7.45 (d, 1H, \(J = 9.0\) Hz), 7.42 (d, 1H, \(J = 9.0\) Hz), 7.34 (d, 2H, \(J = 8.1\) Hz), 7.20 (dd, 1H, \(J_1 = 9.3\) Hz, \(J_2 = 2.4\) Hz), 7.09 (d, 1H, \(J = 2.4\) Hz), 6.90 (d, 1H, \(J = 8.1\) Hz), 3.99 (s, 3H), 3.76 (s, 3H).

Synthesis of 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205): 1,6-Dimethoxy-4-(3,4,5-trifluorophenyl)naphthalene (204) (0.65 g, 2.04 mmol) was dissolved in 30 mL of anhydrous CH\(_2\)Cl\(_2\) in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted
with a stirring bar and an argon inlet. BBr$_3$ (8.50 mL of 1.0M CH$_2$Cl$_2$ solution, 8.50 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature for 3 hours, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for 2 hours. The CH$_2$Cl$_2$ layer was separated. The aqueous layer was extracted with EtOAc (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO$_4$. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH$_2$Cl$_2$/acetone = 98/2 v/v) to give a pale-yellow solid product, 0.45 g, 76.3% yield. MS: m/e 288.9 [M-H]. $^1$H NMR (DMSO-$_4$, 300 MHz) δ 10.22 (s, 1H), 9.69 (s, 1H), 8.07 (d, 1H, $J$ = 7.5 Hz), 7.38 (d, 1H, $J$ = 9.0 Hz), 7.35 (d, 1H, $J$ = 9.0 Hz), 7.15 (d, 1H, $J$ = 7.8 Hz), 7.02 (dd, 1H, $J_1$ = 7.8 Hz, $J_2$ = 2.4 Hz), 7.01 (s, 1H), 6.70 (d, 1H, $J$ = 7.8 Hz).

Example 2C: Synthesis of 5,7-dimethoxy-8-(3,4,5-trifluorophenyl)-2 $H$-chromen-2-one (207), 5,7-dihydroxy-8-(3,4,5-trifluorophenyl)-2 $H$-chromen-2-one (208), and 5-hydroxy-7-methoxy-8-(3,4,5-trifluorophenyl)-2 $H$-chromen-2-one (208A).
Synthesis of 8-bromo-5,7-dimethoxy-2H-chromen-2-one (206): 5,7-Dimethoxy-2H-chromen-2-one (1.00 g, 4.85 mmol) and N-bromosuccinimide (1.04 g, 5.82 mmol) were placed in a dry 250 mL single-necked round-bottomed flask fitted with a stirring bar and septa. Anhydrous tetrahydrofuran (50 mL) was added via a syringe under argon atmosphere at room temperature. The reaction mixture was allowed to stir at room temperature for 4 hours. Then, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH$_2$C$_2$) to give a white solid product, 1.25 g, 90.6% yield. MS: 309.0 [M+Na]$^+$. $^1$H NMR (DMSO-4, 300 MHz) $\delta$ 7.98 (d, 1H, $J = 9.6$ Hz), 6.72 (s, 1H), 6.21 (d, 1H, $J = 9.6$ Hz), 3.99 (s, 3H), 3.97 (s, 3H).
Synthesis of 5,7-dimethoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (207): 8-Bromo-5,7-dimethoxy-2H-chromen-2-one (206) (0.50 g, 1.75 mmol), tetrakis(triphenylphosphine)palladium (0.10 g, 0.088 mmol), cesium carbonate (1.14 g, 3.51 mmol) and 3,4,5-trifluorophenylboronic acid (0.46 g, 2.63 mmol) were placed in a dry and argon flushed 150 mL three-necked round-bottomed flask fitted with a stirring bar and reflux condenser. 1,2-Dimethoxyethane (30 mL) and water (10 mL) were added via a syringe under argon atmosphere. The reaction solution was stirred and heated to reflux overnight. The reaction solution was diluted by adding 50 mL of water at room temperature. The mixture was extracted with ethyl acetate (3x50 mL). The extracts were combined, washed with brine (2x10 mL) and dried over anhydrous MgSO₄ followed by filtration and concentration to give a yellow residue. The yellow residue was purified by flash column chromatography (silica-gel, ¾ (¼) to give a white solid product, 0.18 g, 30.5% yield. MS: m/z 359.1 [M+Na]+. ¹H NMR (DMSO-d₆, 300 MHz) δ 8.07 (d, 1H, J = 9.4 Hz), 7.37 (d, 1H, J = 6.8 Hz), 7.34 (d, 1H, J = 6.8 Hz), 6.77 (s, 1H), 6.20 (d, 1H, J = 9.6 Hz), 4.02 (s, 3H), 3.89 (s, 3H).

Synthesis of 5,7-dihydroxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (208) and 5-hydroxy-7-methoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (208A): 5,7-Dimethoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (207) (0.10 g, 0.30 mmol) was suspended in 30 mL of anhydrous m-xylene in a dry and argon flushed 150 mL single-necked round-bottomed flask fitted with a stirring bar and an argon inlet. BBr₃ (0.37 g, 1.49 mmol) was added via a syringe with stirring at room temperature. After stirred at room temperature overnight, the reaction was quenched by adding 50 mL of water and 5 mL of methanol. The solution was stirred at room temperature for one hour. The CH₂Cl₂ layer was separated. The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The organic layers were separated, combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica-gel, CH₂Cl₂/MeOH = 19/1 v/v) to give 5,7-dihydroxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one as a white solid, 20 mg, 21.9% yield and 5-hydroxy-7-methoxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one as a white solid, 25 mg, 26.1% yield.

5,7-Dihydroxy-8-(3,4,5-trifluorophenyl)-2H-chromen-2-one (208): MS: m/z 306.8 [M-H]. ¹H NMR (DMSO-d₆, 500 MHz) δ 10.87 (s, 1H), 10.63 (s, 1H), 8.01 (d, 1H, J = 9.5 Hz), 7.34 (d, 1H, J = 7.0 Hz), 7.32 (d, 1H, J = 7.0 Hz), 6.49 (s, 1H), 6.07 (d, 1H, J = 10.0 Hz).
5-Hydroxy-7-methoxy-8-(3,4,5-trifluorophenyl)-2 H-chromen-2-one (208A): MS: m/e 320.9 [M-H]+. 1H NMR (DMSO- d6, 500 MHz) δ 10.88 (s, 1H), 8.03 (d, 1H, J = 10.0 Hz), 7.37 (d, 1H, J = 7.0 Hz), 7.35 (d, 1H, J = 7.0 Hz), 6.55 (s, 1H), 6.15 (d, 1H, J = 9.5 Hz), 3.91 (s, 3H).

Example 2D: Synthesis of methyl 2-(5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yloxy)acetate (209) and dimethyl 2,2’-(4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy)diacetate (209A):

![Chemical structures](image)

Synthesis of methyl 2-(5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yloxy)acetate (209) and dimethyl 2,2’-(4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy)diacetate (209A): 4-(3,4,5-Trifluorophenyl)naphthalene-1,6-diol (205) (0.23 g, 0.79 mmol) was dissolved in 20 mL of anhydrous DMF at room temperature under argon. NaH (64 mg of 60% wt in mineral oil, 1.58 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for one hour. Then, methyl 2-bromoacetate (0.12 g, 0.79 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of saturated NH4Cl solution at 0 °C. The solution was stirred at room temperature for one hour. The mixture was extracted with ethyl acetate 3x50 mL. The extracts were washed with brine (2x20 mL), dried over anhydrous MgSO4, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH2Cl2/acetone = 99/1 v/v) to give compound methyl 2-(5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yloxy)acetate (209) as a white solid product, 80 mg, 26.4 % yield.

Methyl 2-(5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yloxy)acetate (209): MS: 361.0 [M-H]. 1H NMR (DMSO- d6, 300 MHz) δ 9.81 (s, 1H), 8.16 (d, 1H, J = 9.0 Hz),
7.44-7.38 (m, 2H), 7.25 (d, 1H, J = 7.8 Hz), 7.12 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.4 Hz), 7.03 (s, 1H), 6.74 (d, 1H, J = 8.1 Hz), 5.01 (s, 2H), 3.74 (s, 6H).

[00461] Dimethyl 2,2’-(4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy)diacetate (209A): MS: 457.1 [M+Na]+. 1H NMR (DMSO-d₆, 300 MHz) δ 8.25 (d, 1H, J = 9.0 Hz), 7.43-7.28 (m, 4H), 7.01 (d, 1H, J = 2.4 Hz), 6.78 (d, 1H, J = 8.1 Hz), 5.05 (s, 2H), 3.74 (s, 3H), 3.68 (s, 3H).

[00462] Example 2E: Synthesis of methyl 2-(6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212).

[00463] Synthesis of 5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yl acetate (210): 4-(3,4,5-Trifluorophenyl)naphthalene-1,6-diol (205) (1.22 g, 4.20 mmol) was dissolved in 30 mL of dry DMF under argon. NaH (0.17 g, 60%wt in mineral oil, 4.20 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. Then, acetyl chloride (0.33 g, 4.20 mmol) was added via a syringe. The resulted mixture was stirred for additional 2 hours at room temperature. The reaction was quenched by adding 50 mL of water. The solution was extracted with EtOAc. The organic layer was separated, dried over anhydrous MgSO₄ and concentrated to dryness. The residue was purified by column (silica-gel, Hexanes/EtOAc) to give a white solid product, 0.45 g, 32.3% yield. MS: m/e 330.9 [M-H]-. 1H NMR (DMSO-d₆, 500 MHz) δ 9.97 (s,
1H), 7.86 (d, 1H, J = 9.0 Hz), 7.53 (s, 1H), 7.51-7.48 (m, 2H), 7.37 (d, 1H, J = 8.0 Hz), 7.16 (dd, 1H, J1 = 9.0 Hz, J2 = 2.5 Hz), 7.12 (d, 1H, J = 8.0 Hz), 7.06 (d, 1H, J = 2.0 Hz), 2.46 (s, 3H).

[00464] Synthesis of methyl 2-(6-acetoxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yloxy)acetate (211): 5-Hydroxy-8-(3,4,5-trifluorophenyl)naphthalene-2-yl acetate (210) (0.33 g, 0.99 mmol) was dissolved in 30 mL of anhydrous THF at room temperature under argon. NaH (0.12 g of 60%wt in mineral oil, 2.98 mmol) was added in 4 portions at 0 °C in an ice bath. The reaction mixture was stirred at room temperature for one hour. Then, methyl 2-bromoacetate (0.46 g, 2.98 mmol) was added via a syringe at 0 °C. The resulted mixture was stirred at room temperature overnight. The reaction was quenched by adding 50 mL of saturated NH4Cl solution at 0 °C. The solution was stirred at room temperature for one hour. The mixture was extracted with ethyl acetate (3×50 mL). The extracts were washed with brine (2×20 mL), dried over anhydrous MgSO4, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH2Cl2/acetone = 99/1 v/v) to give compound methyl 2-(6-acetoxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yloxy)acetate (211) as a white solid product, 0.12 g, 30.0 % yield. MS: 427.1 [M+Na]+. 1H NMR (DMSO-d6, 500 MHz) δ 7.96 (d, 1H, J = 9.0 Hz), 7.49-7.44 (m, 3H), 7.33 (dd, 1H, J1 = 9.0 Hz, J2 = 2.5 Hz), 7.25 (d, 1H, J = 7.5 Hz), 7.04 (d, 1H, J = 2.0 Hz), 4.86 (s, 2H), 2.47 (s, 3H).

[00465] Synthesis of methyl 2-(6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yloxy)acetate (212): Methyl 2-(6-acetoxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yloxy)acetate (211) (50 mg, 0.124 mmol) was dissolved in 30 mL of MeOH/H2O (1:1) at room temperature. 20 mL of 6N HCl solution was added at room temperature. The reaction solution was stirred and heated to reflux overnight and extracted with EtOAc. The organic phase was separated, dried over anhydrous MgSO4 and concentrated to dryness. The residue was purified by column (silica-gel, CH2Cl2/Acetone = 4/1 v/v) to give a white solid product, 40 mg, 89.3% yield. MS: 360.9 [M-H]-.

1H NMR (DMSO-d6, 500 MHz) δ 10.38 (s, 1H), 8.16 (d, 1H, J = 9.0 Hz), 7.35 (t, 2H, J = 8.0 Hz), 7.24 (d, 1H, J = 8.0 Hz), 7.19 (dd, 1H, Ji = 9.0 Hz, J2 = 2.5 Hz), 6.99 (d, 1H, J = 2.5 Hz), 6.81 (d, 1H, J = 8.0 Hz), 4.81 (s, 2H), 3.68 (s, 3H).

**EXAMPLES 3-6**

**Material and Methods for Examples 3-16**

[00466] The materials and methods presented here were used throughout Examples 3-16 unless stated otherwise in the Example.
[00467] **Reagents:** AR antibody, PG-21, were obtained from Millipore (Billerica, MA), AR antibody, AR N-20, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), AKR1C3 mouse monoclonal antibody was obtained from Sigma (St. Louis, MO) and AKR1C3 rabbit polyclonal antibody was obtained from Life Technologies (Carlsbad, CA). Actin antibody was procured from Chemicon International (Temecula, CA). Accell siRNAs were purchased from Dharmaco (Lafayette, CO). Human PSA ELISA was procured from R&D Systems (Minneapolis, MN). All other reagents used were analytical grade from Fisher.

[00468] **Cloning and protein purification:** AKR1C1-C4 cDNAs were cloned into the pCR3.1 vector backbone. AKR1C1 and AKR1C3 construct for protein purification were cloned in pGEX 4T-1. All cloned plasmids were sequenced to ensure absence of any mutations. For AKR1C3 protein production, 10 ml bacterial culture was inoculated in 1 liter of LB and protein synthesis was induced by 1 mM IPTG (Isopropyl β-D-1-Galactopyranoside) for 5 hrs at 37°C with shaking at 250 rpm. The bacterial cells were lysed by freeze thaw cycles, and the protein was purified using glutathione sepharose beads (Amersham, Piscataway, NJ). Point mutations were made by Quickchange site directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). All the other plasmids were described earlier (see Narayananan R. *et al.* Cancer Res 70(2):842-851, 2010 which is incorporated by reference). GRE-LUC and SRC-2 coactivator plasmids were kindly provided by Dr. Nancy L. Weigel and Dr. Bert W O'Malley (Baylor College of Medicine, Houston, TX).

[00469] **AKR1C3 enzyme activity and thin layer chromatography (TLC):** Reaction mixture containing 50 μl phosphate buffer, 0.2 μl NADPH (100 mM), 0.5 μl purified AKR1C3, 0.25 μl \[^{14}\text{C}]-\text{androstene-3,17-dione}\ (\[^{14}\text{C}]-\text{A’dione}\) or \[^{14}\text{C}]-\text{4’dione}\ or \[^{14}\text{C}]-\text{androstenedione}\) (48 mCi/mmol; Perkin Elmer) was incubated at 37 °C for 60 min. For the progesterone to 20 αt(OH) progesterone conversion, \[^{14}\text{C}]-\text{A’dione}\) was replaced with \[^{14}\text{C}]-\text{progesterone}\ (53 mCi/mmol; American Radiolabeled Chemicals, St. Louis, MO). The reaction was stopped by the addition of 400 μl ethyl acetate, vortexed, and spun for 5 min at 2000 rpm. The upper layer was transferred to a new tube and air dried. The pellet was resuspended in 25 μl of methanol, spotted on TLC plates and fractionated in TLC chamber with 3:1 chloroform:ethyl acetate. Once the mobile phase reached the top, the plates were dried and exposed to a phoshoimager screen overnight.
[00470]  **Cell culture:** All cells were obtained from ATCC (Manassas, VA) and were grown according to the instructions provided. For the ChIP and co-immunoprecipitation assays, cells were plated in 10 cm dishes at 5 million cells per dish in medium supplemented with 1% charcoal stripped FBS (csFBS). The cells were maintained in 1% csFBS for 3 days to reduce basal occupancy of promoters with medium changed on days 1 and before treatment on day 3.

[00471]  **Transfection and transactivation assay:** For transfection, cells were plated at 100,000 cells per well of a 24-well plate in DME+5% csFBS. The cells were transfected using Lipofectamine (Invitrogen, Carlsbad, CA) with indicated plasmids. The cells were treated 24 hrs after transfection as indicated in the figures and the luciferase assay (Dual Luciferase assay kit, Promega) performed 48 hrs after transfection. Transfections of siRNAs (Accell siRNAs, Dharmacon Inc.) in LNCaP cells were performed in Accell siRNA delivery medium once on day 0 and then on day 3 with medium change on day 3.

[00472]  Transfections of LNCaP cells with Amaxa electroporator (Amaxa Inc., Gaithersburg, MD) were performed using solution R and program T-009 according to the manufacturer’s protocol. Twenty-four hours after transfection, medium was changed and the cells were treated as indicated in the figures.

[00473]  Stable LNCaP and NIH3T3 cell lines were generated by lentiviral infection of AKR1C3 cloned into pLenti U6 Pgk-puro vector as described earlier (see Yang C.H., *et al.* *J Biol Chem* 286(45):39172-39178, 2011 which is incorporated by reference; and Yang C.H., *et al.* *Cancer Res* 70(20):8108-8116, 2010 which is incorporated by reference). LacZ and AR adenovirus were made at Seven Hills Bioreagents (Cincinnati, OH).

[00474]  **Co-immunoprecipitation and Western blotting:** Co-immunoprecipitation and western blotting were described earlier (see Narayanan R, *et al.* *Mol Cell Biol* 25(1):264-277, 2005 which is incorporated by reference). Co-immunoprecipitation was performed with rabbit polyclonal AKR1C3 antibody in cells treated for 6 hrs.

[00475]  **Confocal microscopy:** Cells were plated at 150,000 cells/well of a 24-well plate on collagen coated coverslips. Medium was replaced to RPMI+1% csFBS without phenol red and
maintained in this medium for 3 days. Cells were treated for the indicated periods and were fixed and imaged using confocal microscopy (Carl Zeiss) as described earlier (see Narayanan R, et al. Mol Cell Biol 25(8):2885-2898, 2005 which is incorporated by reference). In some instances where indicated, cells were serum starved for three days prior to treatment.

[00476] Chromatin immunoprecipitation assay: ChIP assays were performed with AR N-20 and AKR1C3 rabbit polyclonal antibodies as described earlier (see Narayanan R, et al. Mol Endocrinol 22(I):2448-2465, 2008).

[00477] Duolink proximity ligation assay: Duolink kit (O'link, Uppsala, Sweden) was purchased and was used to demonstrate the interaction between AR and AKR1C3 (see Saint-Lu N, et al. Arterioscler Thromb Vase Biol 32(4):894-901, 2012). Images were obtained using deconvolution fluorescent microscopy.

[00478] RNA isolation and gene expression: RNA was isolated using cell to ct kit (Applied Biosystems, Carlsbad, CA) and realtime PCR was performed using TaqMan primers and probes from Applied Biosystems on ABI 7900 (Applied Biosystems).

[00479] Growth Assay: LNCaP cells were plated at 10,000 cells per well of a 96 well plate in RPMI supplemented with 1% csFBS. The cells were treated as indicated in the figures. Cell viability was measured using sulforhodamine blue (SRB) reagent.

[00480] Tumor xenograft experiments: All animal protocols were approved by The University of Tennessee Animal Care and Use Research Committee. Nude mice obtained from Harlan (Indianapolis, IN) were housed with five animals per cage and were allowed free access to water and commercial rodent chow (Harlan Teklad 22/5 rodent diet - 8640). During the course of the study, the animals were maintained on a 12 hr light:dark cycle. Xenograft experiments were performed as previously published. Briefly, a mixture of 2x10^6 LNCaP or VCaP cells was suspended in 0.0375 ml RPMI+10% FBS and 0.0625 ml Matrigel/animal and was injected s.c. Once the tumor size reached 100 mm^3, the animals were castrated or sham operated, randomized, DHT pellets were implanted subcutaneously or not supplemented and treated as indicated in the
figures. Tumor volume and body weight were measured. Tumor volume was calculated using the formula length*width*width*0.5236.

[00481] **Prostate cancer specimen:** Section of a prostate cancer specimen with gleason score 7 (4+3) was obtained under The University of Tennessee, Institutional Review Board (IRB) approval. The specimen was subjected to Duolink's PLA with AR and AKR1C3 antibodies. For negative control, the sample was probed only with AKR1C3 antibody.

**EXAMPLE 3:**

**Novel small molecules inhibit AKR1C3 activity**

A novel series of AKR1C3 inhibitors was designed and synthesized. The activity of the most potent inhibitor, compound 78, was compared to known AKR1C3 inhibitor, indomethacin, in *in vitro* purified- and cell based- enzyme assays.

**EXAMPLE 3A: Purified enzyme assays.**

[00482] **Purified AKR1C assays:** Purified AKR1C3 enzyme was incubated with ATP and [¹⁴C]-androstenedione (A'dione) (12 μM) in the presence of a titration of putative AKR1C3 inhibitors (e.g., 78, indomethacin, 67), for 60 min. At the end of incubation period, the reaction mixture was evaporated to dryness and the pellet was resuspended in methanol. Radiolabeled A'dione and testosterone were fractionated using TLC and quantified using phosphoimager.

[00483] Representative results for compound 78 are shown in Figure 2A. The amount of testosterone synthesized from androstenedione (labeled 4'dione) by AKR1C3 is represented in the line graph (Figure 2B). The results demonstrated that 78 inhibited *in vitro* AKR1C3 enzyme activity comparably to indomethacin (Indomet). Figure 2C shows that 67 also completely inhibited purified AKR1C3 activity at 10 μM. Table 1 reports the percent of AKR1C3 inhibition at 10 μM concentration of AKR1C3 inhibitors in this purified AKR1C3 TLC based enzyme assay. Table 2 reports the data from the same assay for other compounds of this invention.

[00484] Representative results for IC₅₀ values for compounds of this invention, generated in competition with 100 nM 4'dione in HEK-293 cells stably transfected with AKR1C3 and using Mass Spec are reported in Table 3. In addition, Table 3 presents % HSD inhibition compared with control.
IC_{50} determination method: HEK-293 cells were stably transfected with AKR1C3 (HEK-293-
AKR1C3) using lentivirus and the cells were selected using puromycin. AKR1C3 expression was checked by Western blot to ensure that the cells over-express AKR1C3. HEK-293-AKR1C3 cells were plated in 24 well plates at 125,000 cells/well in DME+5% csFBS without phenol red. Medium was changed to RPMI+1%csFBS without phenol red and maintained in this medium for 3 days to reduce basal hormone levels. Medium was changed again on day 4 and were treated with 100 nM androstenedione alone or in combination with a dose response of individual inhibitor. Twenty four hours after treatment, medium was collected and injected in LC-MS/MS to detect the amount of testosterone formed by AKR1C3. The data was plotted in SigmaPlot (Systat Software Inc.) and the IC_{50} values for inhibitors were calculated.

[00485] % AKR1C3 inhibition method: Reaction mixture containing 50 µι phosphate buffer, 0.2 µι NADPH (100 mM), 0.5 µι purified AKR1C3, 0.25 µι [\textsuperscript{14}C]-A'dione (48 mCi/mmol; Perkin Elmer) was incubated at 37°C for 60 min. The reaction was stopped by the addition of 400 µι ethyl acetate, vortexed, and spun for 5 min at 2000 rpm. The upper layer was transferred to a new tube and air dried. The pellet was resuspended in 25 µι of methanol, spotted on TLC plates and fractionated in TLC chamber with 3:1 chloroform:ethyl acetate. Once the mobile phase reached the top, the plates were dried and exposed to a phosphoimager screen overnight.

% Liver microsome inhibition: Stock solutions of perspective AKR1C3 inhibitors were prepared in DMSO at 5 mM and were incubated with liver microsomes (0.1 mg/mL). Incubations were conducted at 37 °C in a shaking water bath for 10 minutes (microsomes). Incubation durations were selected based on time course study (microsomes) or published literature (recombinant enzymes). Prepared samples were analyzed using LC-MS/MS.

Table 1: AKR1C3 inhibition by some compounds of this invention

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<td>67</td>
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Table 2: AKR1C3 inhibition by other compounds of this invention.

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**NAME**

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DKB-476-065  
Rutin
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<td>HSD-5 (10uM)</td>
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<td>3,4, dimethoxy cinnamic acid</td>
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EXAMPLE 3B: Lack of cross-reactivity.

Specificity assay: Purified AKR1C1 or AKR1C3 enzyme was incubated with ATP, and [14C]-progesterone (10 µM) in the presence or absence of 10 µM 78 (a representative AKR1C3 inhibitor) for 60 min. At the end of incubation period, the reaction mixture was evaporated to dryness, the pellet was resuspended in methanol, and the [14C] progesterone and [14C] 20a-hydroxy-progesterone (20a(OH)P) were fractionated using TLC (Figure 3A). [14C] progesterone and [14C] 20a(OH) were quantified using phosphoimager and the amount of 20a-hydroxy-progesterone synthesized from progesterone (P) by AKRs is represented in the bar graph (Figure 3B). These data demonstrated that 78 did not cross react with AKR1C1 (i.e. 78 inhibited AKR1C3 but not AKR1C1 with regard to the substrate progesterone) in these assays.

Purified COX-1 and COX-2 assays:

COX Inhibition Assay

COX inhibitory activity was determined using a commercially available reagent kit from Cayman Chemical Company (#700100). The assay was performed according to the manufacturer's guidelines. Briefly, either ovine COX-1 or human recombinant COX-2 was incubated in the presence of increasing concentrations of investigational compound dissolved in DMSO (5% final reaction volume). Arachidonic Acid (final concentration 10 µM) along with 10-acetyl-3,7- dihydroxyphenoxazine (ADHP), a fluorometric substrate, was added to the reaction mix and the formation of Resorufin (Ex5 505 nm Em 585,595) was measured using a Biotek Synergy 4 plate reader. All values were background subtracted and represented as a percentage of total activity (vehicle alone).

Results

The commercially available COX inhibitor indomethacin potently inhibited the activity of COX-1 (IC50 = 16.2 nM) whereas 205 required more than 500 nM to inhibit fifty percent of Resorufin formation (Figure 4). Both 78 and 67 required even more drug to inhibit COX-1 activity with IC50 values determined to be greater than 5,000 nM and 50,000 nM, respectively. Indomethacin was much less potent against COX-2 with an IC50 of 2.33 µM (Figure 5). 205 demonstrated similar activity with an IC50 of 1.80 µM whereas 67 and 78 required in excess of 500 µM to inhibit fifty percent of Resorufin formation in these assay conditions.
EXAMPLE 3C: Transfected cell assays.

AKR1C3 inhibition in AKR1C3 transfected HEK-293 cells:

HEK-293 cells were transiently transfected with 1 µg AKR1C3 and treated with a titration of 78 or indomethacin in the presence of [14C]-A’dione (1 µM). After over-night incubation, A’dione and testosterone in the medium were fractionated using TLC and were quantified using phosphorimager. (Figure 6A). Percent testosterone synthesized from A’dione is represented in the line graph (Figure 6B). Compound 78, but not indomethacin, inhibited cellular conversion of androstenedione to testosterone.

Conversion of A’dione to T

AKR1C3 stably transfected HEK-293 cells were again transfected transiently with AKR1C3 (to increase levels) in serum starved conditions for 3 days. Cells were then treated with AKR1C3 inhibitor. 30 minutes later, cells were treated with 1 µM androstenedione for 24 hours, medium collected, and the amount of synthesized testosterone detected by TLC were exposed to phosphor screen and quantitated by phosphor imager (Figure 28 and Table 4).

Table 4

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<th>Compound Number</th>
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<td>213</td>
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<tr>
<td>215</td>
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</table>
AKR1C3 inhibition in H295R adrenal cells:

H295R adrenal cells were treated with 78 or indomethacin followed by $^{14}$C androstenedione. Medium was collected and the amount of androstenedione and testosterone secreted in the medium was measured by TLC. Percent testosterone synthesized from androstenedione is indicated as a bar graph (Figure 6C). Compound 78 but not indomethacin inhibited AKR1C3 enzyme activity in adrenal H295R cells.

AKR1C3 inhibition in LNCaP or LNCaP-AKR1C3 cells:

Representative of n=3 experiments is depicted here (Figures 6D-6F). Western blot showing AKR1C3 expression in LNCaP prostate cancer cells or LNCaP cells stably transfected with AKR1C3 (LNCaP-AKR) is presented in Figure 6D. LNCaP or LNCaP-AKR1C3 transfected cells (LNCaP-AKR) were treated with $[^{14}]$C-A’dione (12 μM) in the presence or absence of 10 μM 78 or indomethacin. Medium was collected and the amount of testosterone synthesized was detected by TLC and quantified using phosphorimagery. Percent testosterone synthesized from androstenedione is indicated as a bar graph (Figure 6E). Compound 78, but not indomethacin, inhibited AKR1C3 enzyme activity in LNCaP cells stably transfected with AKR1C3. This suggests that 78 could be used to inhibit AKR1C3 in the prostate cancer setting.

Since 5α-reductase converts testosterone to DHT, experiments were performed to test if finasteride, a 5α-reductase inhibitor, would increase testosterone levels in LNCaP-AKR1C3 cells. LNCaP or LNCaP-AKR1C3 cells were treated with $[^{14}]$C-A’dione (1μM) in the presence or absence of 10 μM finasteride, 78, 81 or indomethacin (indo).
Results presented in Figure 6F demonstrated that finasteride blocked the conversion of testosterone to DHT in LNCaP-AKR1C3 cells, thereby significantly increasing the testosterone levels. Percent testosterone (%T) formed was calculated as indicated above and represented as numbers below the TLC image. By acting upstream of 5α-reductase in the steroidogenic pathway, 78 completely blocked the formation of testosterone from A'dione, indicating the ability of AKR1C3 inhibitors, unlike 5α-reductase inhibitors, to reduce testosterone levels. Representative of n=3 experiments is depicted here. A'dione-Androstenedione; T-Testosterone; TLC-Thin Layer Chromatography; LNCaP-mock cells were transfected with vector only; LNCaP-AKR were transfected with AKR1C3; vehicle; Indo denotes treatment with indomethacin.

Cumulatively these experiments establish that 78 is cell penetrant and achieves nM potency in AKR1C3 inhibition in these cellular models, suggesting much higher potency in cell-based systems than was predicted by the purified AKR1C3 enzyme assays of Example 3A. Surprisingly, indomethacin did not perform comparably in these cellular assays, perhaps due to low cellular penetration associated with the carboxylate group. An advantage of the cellular assays is that they screen for cell penetration and AKR1C3 activity in a single assay.

EXAMPLE 4

Inhibition of AKR1C3-dependent AR transactivation

Objective: Androstenedione (A'dione) is a partial agonist in vitro (activates AR by itself with EC50 of ~100 nM and partially antagonizes DHT-dependent AR agonism). However, it gets converted to testosterone in testes, prostate and adrenals by various hydroxysteroid dehydrogenases (HSDs) and activates AR robustly. The objective of this study is to establish a transient transfection assay to screen our ligands of 17P-HSD5 (AKR1C3) for ability to inhibit AR function in the cellular context.

Results: In vector (pCR3.1) transfected HEK-293 cells, A'dione did not activate AR until 100 nM (shown as 10⁻⁷ M). However, in AKR1C3 transfected cells, A'dione activated AR starting at 1 nM. The ability of 10 nM A'dione to activate AR was comparable to 1 nM testosterone (Figure 7).

Compound 78 inhibited AKR1C3-dependent AR transactivation: HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 vector...
backbone or pCR3.1 AKR1C3. Twenty-four hours after transfection, the cells were treated with a titration of androstenedione (4’dione) or testosterone. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Transfection of AKR1C3 into HEK-293 cells augmented androgen-dependent AR transactivation (Figure 8A).

**Compound 78 inhibits AKR1C3-mediated androstenedione-induced AR transactivation:**

[00498] HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 AKR1C3. Twenty-four hours after transfection, the cells were treated with 10 nM androstenedione alone or in combination with the indicated concentrations of 78 or indomethacin. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Compound 78, but not indomethacin, inhibited AKR1C3-mediated androstenedione-induced AR transactivation (Figure 8B).

[00499] **Compound 78 did not inhibit AKR1C1-dependent AR transactivation** (Figure 8C): HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 vector backbone or pCR3.1 AKR1C1. Twenty-four hours after transfection, the cells were treated with 10 nM androstenedione alone or in combination with the indicated concentrations of 78 or indomethacin. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase.

These assays established that compounds of this invention are cell penetrant, potent AKR1C3 inhibitors and that this activity is able to potently inhibit AR transactivation in the cellular context. These compounds should be able to prevent intracrine synthesis of testosterone and DHT. This intracrine synthesis of androgens by AKR1C3 is significantly up-regulated in CRPC. Correspondingly, the AKR1C3 inhibitors of this invention should be able to effectively inhibit the AR signaling axis in prostate cancer at any stage of development in the *in vivo* setting (i.e. in xenografts or in patients).

**EXAMPLE 5**

**Compound 78 inhibits the proliferation of LNCaP-AKRIC3 cells and LNCaP-AKRIC3 tumor xenograft**
[00500] LNCaP cells stably transfected with AKR1C3 (LNCaP-AKR) were treated with 10 nM androstenedione (4’dione) alone or in combination with 10 µM 78 or indomethacin. Cell proliferation was measured by sulforhodamine B assay. Androstenedione very weakly induced the proliferation of LNCaP cells transfected with vector. On the other hand, LNCaP cells stably over-expressing AKR1C3 (LNCaP-AKR) were very responsive to 4’dione-induced proliferation, which was blocked by compound 78, but not by indomethacin (Figure 9A; open bars - vehicle treated; closed bars - 4’dione treated).

[00501] LNCaP or LNCaP cells stably transfected with AKR1C3 (LNCaP-AKR) were injected subcutaneously in nude mice (n=5). Once the tumor reaches 100 mm³, the mice were castrated and vehicle or androstenedione pellets were implanted subcutaneously. The animals were simultaneously treated with vehicle or 30 mg/kg/day of 78 subcutaneously for 2 weeks. Tumor volume was measured and represented as percent change in tumor volume from the day of castration. Compound 78 inhibited the LNCaP-AKR tumor xenograft (Figure 9B) to some degree. The efficacy in this model may have been limited by the bioavailability of 78 in the tumor.

EXAMPLE 6

Selective Inhibition of AKR1C3-Dependent, but not 17pHSD3-Dependent, AR Transactivation

[00502] HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 AKR1C3 or 17βHSD3. Twenty-four hours after transfection, the cells were treated with 10 nM androstenedione alone or in combination with the indicated µM concentrations of 78. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase.

[00503] Results shown in Figure 10 are representative of three experiments. 4’dione-androstenedione; RLU-relative light units; Ren RLU-renilla relative light units. In cells transfected with vector (pCR3.1), 4’dione (10 nM) did not increase AR transactivation. However, in AKR1C3 or 17βHSD3 transfected cells, 4’dione substantially increased AR transactivation, presumably by conversion of 4’dione to T. This demonstrates that the increased AR transactivation was AKR1C3- or 17βHSD3- dependent, respectively. Compound 78 dose-dependently decreased AKRIC3-dependent AR transactivation, but did not inhibit 17PHSD3-dependent AR transactivation at 78 levels of <10 µM. This suggests that AKR1C3 inhibitors of this invention can selectively block peripheral (i.e.,
AKR1C3-dependent) androgen synthesis. Thus, the anti-androgenic effect is targeted to only cells expressing or over-expressing AKR1C3, unlike anti-androgens or LH-RH agonists and antagonists. This will suppress intratumoral androgen synthesis in tumors expressing or overexpressing AKR1C3.

**Example 7**

**AKR1C3 Androgen-Dependent AR transactivation**

**AR transactivation as AKR1C3 functional assay**

**AKR1C3 augments androgen-dependent AR transactivation.**

[00504] HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and 1 μg vector or AKR1C3 using lipofectamine tranfections reagent. Twenty-four hours after transfection, the cells were treated with a titration of A’dione. Cells were harvested forty-eight hours after transfection and firefly luciferase levels were measured and normalized to renilla luciferase.

[00505] AKR1C3 converts A’dione to testosterone resulting in a ligand with stronger AR activity. Figure 11 shows that AR activity in response to A’dione in HEK-293 cells transfected with AKR1C3 was higher than the AR activity in vector transfected cells. Over-expression of AKR1C3 significantly reduced the EC₅₀ of A’dione to transactivate AR from 415 nM to 175 nM, as shown in Table 5 below.

[00506] Table 5: AKR1C3 overexpression in prostate cancer may amplify or hypersensitize the AR signaling pathway.
These results suggest AKR1C3 overexpression in prostate cancer may amplify or hypersensitize the AR signaling pathway.

**AKR1C3 augments androgen-dependent AR transactivation.**

AR transactivation studies were carried out in AKR1C3 transfected HEK-293 cells using A'dione, three 17-hydroxy AR agonists (testosterone, DHT and R1881) and a nonsteroidal selective androgen receptor modulator (SARM)((5)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide) to confirm that the ability of AKR1C3 to amplify AR transactivation was selective for 17-keto steroids.

HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 μg pCR3.1 vector backbone or pCR3.1 AKR1C3. Twenty-four hours after transfection, the cells were treated with a titration of androstenedione (A'dione), testosterone, DHT, R1881, selective androgen receptor modulator (SARM)((5)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide) or an "inactive SARM" that is devoid of AR binding activity ((a)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide). Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase.
The results show that cells transfected with AKR1C3 have augmented androgen-dependent AR transactivation (Figures 12A-12F). Results shown in Figures 12A-12F are representative of three experiments. (Key: 4'dione-androstenedione; RLU-relative light units; Ren RLU-renilla relative light units; open circles (or closed triangles in Figure 12F) represent cells transfected with AKR1C3; closed circles represent cells transfected with backbone vector (pCR3.1)).

Unexpectedly, AKR1C3 increased AR transactivation in response to all ligands that bind to the AR (Figures 12A-12E), but not the the 4'-isomer of the SARM that is devoid of AR binding, which is not an AR ligand (Figure 12F closed triangles). AKR1C3 not only reduced the EC50 of these ligands (Table 5 above), but also increased the maximum level of AR transactivation (E max).

As only A'dione is a substrate for AKR1C3, the increased AR transactivation is AR ligand, i.e. androgen-dependent, not AKR1C3 ligand-dependent. The absence of the increased AR transactivation with the non-androgen ("inactive SARM") supports this explanation. This suggests that AKR1C3 has a stimulatory effect on androgen-dependent AR transactivation which is not related to the enzyme activity of AKR1C3, which is consistent with a previously unreported co-activator activity of AKR1C3 with respect to AR transactivation, as reported herein for the first time.

The AKR1C3 inhibitor, acting as androgen-dependent coactivator, reduced the EC50 of steroidal and nonsteroidal (SARM) agonists, suggesting that AKR1C3 increased the androgen signaling pathway through a mechanism independent of its enzymatic function (these androgens, except for A'dione, are not substrates). This suggests that an AKR1C3 inhibitor may be able to suppress the androgen axis in prostate cancers in which the AR is activated by a broadened spectrum of compounds, such as endogenous glucocorticoids. Broadened androgen agonism is a commonly observed feature of CRPC, and is poorly treated by current AR ligands.

**Increase in AR transactivation by AKR1C3 is not due to increased AR expression**

HEK-293 cells were transfected with the indicated concentration of AKR1C3. The total amount of transfected plasmids were normalized to 1 μg with vector pCR3.1. Twenty-four hours after transfection, the cells were treated with a titration of R1881. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Figure 12K shows the AKR1C3 RNA levels.
[00514] Cells transfected with vector or AKR1C3 and treated with 10 nM R1881 were fractionated by SDS-PAGE and Western blots were probed for AR.

[00515] Varying the level of AKR1C3 expression showed that 0.5 µg of AKR1C3 plasmid DNA increased AR transactivation in a concentration-dependent manner (Figure 12G) without altering the expression of AR (Figure 12H). Results shown in Figures 12G and 12H are representative of three experiments. (Key: SARM-selective androgen receptor modulator; AR-Androgen Receptor; 4’dione-androstenedione; RLU-relative light units; Ren RLU-renilla relative light units.). The SARM compound is (5’)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide.

Enhanced AR transactivation of diverse AR ligands is not an artifact

[00516] In order to ensure that the ability of AKR1C3 to enhance AR transactivation of diverse AR ligands was not an artifact of cell type or transfection system, control experiments using different cell types (COS-1 and NIH 3T3), various transfection conditions (lipofectamine, fugene, and Amaza electroporator), and cells stably expressing AKR1C3 using a lentivirus system were performed.

[00517] COS-1 cells were transfected with 0.25 µg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 or pCR3.1-AKRIC3. Twenty-four hours after transfection the cells were treated with a titration of the indicated androgens. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Results shown in the figures are representative of three experiments.

[00518] The results in COS-1 cells (Figure 13A-E; and data not shown) demonstrated that the AR activation effect of AKR1C3 was not unique to a cell type or transfection condition.

Specificity of transactivation for the AR

[00519] Steroid receptors share sequence homology in many of their functional domains, facilitating their interaction with the same coactivator. Transactivation experiments were performed with glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), estrogen receptor (ERa), and peroxisome proliferator-activated receptor γ (PPARγ) to determine AKR1C3’s selectivity for AR.

[00520] HEK-293 cells were transfected with 0.25 µg GRE-LUC (AR, GR, PR, and MR),
ERE-LUC (ERα) or PPRE-LUC (PPARγ), 50 ng respective receptors, 10 ng CMV-renilla luciferase and 1 μg pCR3.1 or pCR3.1-AKRIC3. Twenty-four hours after transfection the cells were treated with a titration of the indicated ligands. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Results shown in Figures 14A-14G are representative of three experiments.

[00521] AKR1C3 only increased AR function, and did not affect the function of other receptors in response to their respective ligands (Figure 14A-G). (Open - AKR1C3 transfected; closed circles - vector (pCR3.1) transfected.) The specificity of the AKR1C3 coactivator function for AR (and not other NHR) supports its role as a therapeutic target for any androgen-dependent condition in which AKR1C3 is expressed or overexpressed.

**Compound 78 inhibits AKRIC3-induced, androgen-dependent AR transactivation (i.e., inhibits the co-activator function of AKRIC3 on AR transactivation).**

[00522] HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase and 1 μg pCR3.1 or AKR1C3. Twenty-four hours after transfection, the cells were treated with a titration of R1881 alone or in combination with 10 μM of 78. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. This demonstrates that an AKR1C3 ligand can inhibit or attenuate androgen-dependent AR transactivation. In other words, the compounds of this invention can reverse the co-activator function of AKR1C3, suggesting utility in any AR-dependent disease, including advanced stages of prostate cancer.

[00523] The results show that compound 78 inhibits AKRIC3-induced androgen-dependent AR transactivation (Figure 121). Results shown in Figure 121 are representative of three experiments. 4'dione-androstenedione; RLU-relative light units; Ren RLU-renilla relative light units; closed circles represent pCR3.1 transfected cells; open circles represent AKR1C3 transfected cells; closed triangles represent AKRI C3 transfected cells treated with 78.

**AR augmentation (i.e., coactivator) effect is selective to AKR1C3.**

[00524] Since the members of AKRIC family share high sequence homology, the effect of AKRIC isoforms on AR transactivation in response to R1881 was tested in HEK-293 cells.
[00525] HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 vector or various AKR1Cs. Twenty-four hours after transfection, the cells were treated with a titration of R1881. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase.

[00526] The results show that R1881-induced AR transactivation was augmented only by AKR1C3 (open circles), but not by AKR1C1, 1C2, or 1C4, indicating the selective ability of AKR1C3 to function as an AR activator. (Figure 12J). Results shown in Figure 12J are representative of three experiments.

Example 8

**Different regions of AKR1C3 mediate the enzymatic and activation functions**

[00527] In order to determine the regions responsible for the enzymatic and activation functions of AKR1C3, a variety of truncated and mutated AKR1C3 constructs were generated and incorporated into a pCR3.1 vector (Figure 15A). AR transactivation studies in response to 4'dione and R1881 were performed. The constructs identified as A-H were used throughout this example.

**R1881 and 4'dione mediate AKR1C3-induced AR transactivation through different domains.**

[00528] HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and vector or the indicated AKR1C3 constructs. Twenty-four hours after transfection, the cells were treated with a titration of androstenedione (4'dione) or R1881, as indicated in the panels. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase. Figure 15B, left (4'dione treatment) and right (R1881 treatment) panels: 1 µg pCR3.1 (solid line) or 0.5 µg (broken line) and 1 µg (dotted line) of full length AKR1C3; Figure 15C, left (4'dione treatment) and right (R1881 treatment) panels: 0.5 µg (broken line) and 1 µg (dotted line) of construct B; Figure 15D, left (4'dione treatment) and right (R1881 treatment) panels: 0.5 µg (broken line) and 1 µg (dotted line) of construct C; Figure 15E left panel: 1 µg construct A and D and treated with 4'dione (solid line pCR3.1 backbone, broken line construct A, and dotted line construct D); Figure15E right panel: 1 ug construct A and D and treated with R1881 (solid line pCR3.1 backbone, broken line construct A, and dotted line construct D); Figure 15F: transfected with 1 µg of all constructs; Figure 15I: 1 µg (dotted lines) constructs A, D, F, or G, or
pCR3.1 vector (solid line); and Figure 15J: (R1881 treatment) and Figure 15K: (A’dione treatment), pCR3.1 (solid line), or constructs A or H (broken lines).

[00529] Results showed amino acids 1-182 failed to mediate the effects of A’dione or R1881. While full length AKR1C3 was required for the effects of A’dione, amino acids 1-282 were sufficient to mediate the effects of R1881 (Figures 15B-15F and 15H). The region of AKR1C3 spanning amino acids 171-237 (construct G) was sufficient to mediate the effects of R1881. Point mutation F306A that eliminates the binding of A’dione to AKR1C3 also eliminated its effect on AR transactivation in response to A’dione, but not to R1881. The enzymatic functions of these constructs were confirmed by TLC (Figure 15G). These results suggest that the full length protein was required to mediate AKR1C3’s enzymatic functions, but that amino acids 171-237 were sufficient to mediate the AR activation.

[00530] Figure 15F indicates that full-length AKR1C3 (construct A) is required to demonstrate the observed AKR1C3-induced androgen-dependent AR transactivation (i.e., co-activator function of AKR1C3). Thus, inhibition of AKR1C3 by 78 required full-length AKR1C3.

**TLC enzymatic assay with full length and truncated AKR1C3 constructs.**

[00531] To corroborate that only the full length possesses the enzymatic function, HEK-293 cells were transfected with 1μg vector or the indicated AKR1C3 constructs and treated with 1 μM rC]Adione. Medium was collected and the synthesized testosterone was detected by TLC.

[00532] The results in Figure 15G, show that only full length AKR1C3 (construct A) mediates the enzymatic conversion.

**Compound 78 requires full length AKR1C3 to inhibit its co-activator function.**

[00533] HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng pCR3.1 hAR, 10 ng CMV-renilla luciferase, 1 μg of constructs A-G. Twenty-four hours after transfection, the cells were treated with a titration of androstenedione (4’dione). Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase.

[00534] Since AKR1C3 inhibitors like 78 bind to the substrate binding pocket and the pocket includes amino acids in C terminal domain, 78 was unable to inhibit AKR1C3-dependent R1881-
induced AR transactivation for constructs truncated to only include amino acids 1-282 (construct D which is C-terminally truncated) (Figure 15H).

**Example 9**

**AKR1C3 Interacts with the Androgen Receptor (AR)**

[00535] The ability of AKR1C3 to selectively potentiate AR function suggested that it might possibly function as an AR selective coactivator. In order to classify a protein as a coactivator, it must 1) interact with the receptor, 2) increase the activity of the receptor and 3) get recruited to the responsive element of a target gene. All of these criteria are satisfied in the Examples set forth herein. Criteria 1) is reported in Examples 9 and 11; criteria 2) is reported in Example 7; and criteria 3) is reported in Example 10; suggesting that AKR1C3 acts as a coactivator of the androgen receptor.

[00536] To determine if AR and AKR1C3 interact in tissue culture cells, co-immunoprecipitation studies were conducted in LNCaP-AKR1C3 (AKR1C3) or LNCaP-vector (vector) cells. Cells were treated with R1881 and the protein extracts were immunoprecipitated with AKR1C3 antibody and immunoblotted for AR.

[00537] LNCaP cells stably transfected with vector or AKR1C3 were serum starved for 2 days and treated with vehicle or 10 nM R1881 for 6 hours. Cells were harvested, protein extracted, immunoprecipitated (IP) with AKR1C3 antibody and Western-blotted for AR. Western blot for AR with 10% of the total protein extract was simultaneously performed as input control.

[00538] In an alternative method, confocal microscopy was used to determine if AR and AKR1C3 co-localize in prostate cancer VCaP tissue culture cells. VCaP prostate cancer cells were plated on coverslips and were serum starved for three days. The cells were then treated with R1881 or A’dione for 6 hours and were formalin fixed and immunostained with AR and AKR1C3 antibodies. The localization of AR and AKR1C3 were imaged using confocal microscopy.

[00539] To determine if endogenous AR and AKR1C3 interact in VCaP CRPC tumor xenografts, protein was extracted from VCaP xenograft (n=3) and was subjected to immunoprecipitation (IP) with AR antibody (AR N20 from SantaCruz Biotechnology) or IgG. The immunoprecipitated samples and 20% input samples were fractionated on a SDS PAGE and Western blotted with AKR1C3 antibody (Sigma) and AR antibody.
Results:

The results shown in Figure 16A demonstrated that AR and AKR1C3 interacted in a ligand-dependent manner in LNCaP-AKR1C3 cells, but not in LNCaP-vector cells. In support, the results shown in Figure 16B demonstrated interaction between AR and AKR1C3 in VCaP cells. Figure 16C presents data showing robust interaction between endogenous AR and AKR1C3 in VCaP CRPC tumor xenografts, as demonstrated by the IP with AR antibody as compared to the IP with IgG.

Example 10

AKR1C3 is recruited to PSA enhancer.

If AKR1C3 interacts with AR, it could also be recruited to the response element (ARE) of an AR target gene. LNCaP-AKR1C3 or LNCaP-vector cells were treated with R1881 or A’dione and the recruitment of AKR1C3 to the ARE on the PSA enhancer was examined using chromatin immunoprecipitation (ChIP) assay.

LNCaP- AKR1C3 (LN-AKR) or LNCaP-Vector (LN-Vector) cells were maintained in serum free conditions for three days. Cells were then treated with 100 nM A’dione or 10 nM R1881 for 2 hours and a chromatin immunoprecipitation (ChIP) assay was performed with AR (Figure 17A, left panels) or AKR1C3 (Figure 17A, right panels) antibodies. DNA was purified and realtime PCR was performed for PSA enhancer and normalized to input.

Results showed that AKR1C3 was recruited to the PSA enhancer both in response to its substrate, A’dione, as well as in response to R1881 (Figure 17A, right panel). Although AR was recruited to the PSA enhancer in LNCaP-vector cells (Figure 17A, left panel), the recruitment of AKR1C3 to the PSA enhancer could not be detected in LNCaP-vector cells due to its limited expression.

Example 11

AR and AKR1C3 co-localize in LNCaP cells and in Advanced Prostate Cancer.

The results presented in Examples 9 and 10 demonstrated that a steroidogenic enzyme is capable of acting as an interacting partner of a steroid receptor. Several confirmatory studies were undertaken to further establish that this interaction occurs.
LNCaP cells stably transfected with lentivirus AKR1C3 were plated on coverslips and serum starved for 2 days. Cells were then treated with vehicle, 100 nM A'dione, or 10 nM R1881 for 6 hrs. Cells were fixed and immunostained with primary antibodies specific to AR and AKR1C3 and fluorescent tagged secondary antibodies. The immunofluorescent signals were captured by laser confocal microscopy.

Immunofluorescence studies in LNCaP-AKR1C3 cells performed using laser confocal microscopy (Figure 18A) showed that both AR and AKR1C3 were cytoplasmic in the absence of AR ligands, but translocated into the nucleus upon binding of a non-AKR1C3-substrate AR ligand (R1881) or A'dione. The migratory patterns for AR and AKR1C3 overlapped substantially, supporting the idea that the two proteins interact with each other.

In order to ensure that AKR1C3’s translocation is dependent on AR, and is not cell type dependent, NIH3T3 cells stably transfected with AKR1C3 were infected with adenovirus expressing Lac Z (Figure 26A) or AR (Figure 26B) and were treated with 10 nM R1881. Although cells were treated with R1881, AKR1C3 was cytoplasmic in the absence of AR and translocated into the nucleus only in the presence of AR, indicating the requirement for the AR presence for AKR1C3 to translocate into nucleus.

AR antagonist, SNARE-1, was known to inhibit ligand-dependent AR nuclear translocation. Therefore, the translocation of AR and AKR1C3 in response to R1881 in the presence or absence of SNARE-1 was also tested. R1881 efficiently translocated AR into the nucleus and AKR1C3 co-translocated with AR. However, when cells were treated with 10 µM SNARE-1, AR only partially translocated into the nucleus and predominantly remained in the cytoplasm. AKR1C3 followed the same pattern (Figure 27).

Figure 16 shows that AR and AKR1C3 co-localize in LNCaP-AKR1 C3 cells.

The Duolink proximity ligation assay (PLA) detects protein-protein interaction by fluorescent visualization (Soderberg et al., 2006). DNA attached to the secondary antibodies is ligated and amplified and the amplified DNA fluoresces red only if the two proteins are in proximity.

LNCaP-AKR1C3 or LNCaP-Vector cells were plated on coverslips, serum starved for 2 days and were treated overnight with 100 nM A’dione. Cells were fixed and incubated with AR and/or AKR1C3 antibodies. The fluorescent DNA attached to secondary antibodies was amplified and
the fluorescent DNA signal detected by deconvolution fluorescent microscopy. Nucleus was stained with DAPI.

The results of treating LNCaP-AKR1C3 (LNCaP-AKR) cells with R1881 and subjecting them to PLA showed that while AR and AKR1C3 interaction (represented by red fluorescence) was detected in LNCaP-AKR1C3 cells (Figure 18B, lower panel), interaction was undetected in LNCaP-Vector cells or in the absence of one of the antibodies (Figure 18B, middle panel).

**AKR1C3 and AR interacts in advanced prostate cancer**

To determine if the interaction between AR and AKR1C3 observed in prostate cancer cells was also observed in human prostate cancer, a prostate cancer specimen (Gleason sum 4+3=7) was subjected to PLA with AR and/or AKR1C3 antibody.

A tissue section from Gleason score 7 prostate cancer was subjected to Duolink proximity ligation assay with AR and AKR1C3 antibodies (Figure 18C, positive top panels) or in the absence of AR antibody (Figure 18C, negative control bottom panels). Nucleus was counterstained with DAPI and images captured using deconvolution microscopy. Images are representative of n = 3; A’dione-androstenedione; IP-immunoprecipitation; IB-Immunoblotting; ChIP-chromatin immunoprecipitation.

Results showed that while the interaction between the two proteins (as visualized by red fluorescent spots) was clearly observed in the tissue (Figure 18C top panel), the interaction was not detected in the absence of one of the antibodies (Figure 18C bottom panel). These results suggest that the AR and AKR1C3 not only interact in cells, but also in advanced prostate cancer.

**Example 12**

**AR Function in LNCaP cells is affected by AKR1C3 Expression**

**Endogenous AKR1C3 expression is important for AR function in LNCaP cells.**

Since the LNCaP cells express modest amounts of AKR1C3, siRNA was used to understand AKR1C3’s role in AR function.
LNCaP cells were transfected with siRNA constructs for cyclophilin (Cycle), or AKR1C3 or with no siRNA [i.e., not transfected with siRNA which is labeled as (-)]. Six days after transfection (with medium change and a second transfection after 3 days), the cells were treated with vehicle (open bars) or 0.1 nM R1881 (closed bars) for 24 hrs., RNA was extracted and the expression of AR target androgen responsive genes (PSA and FKB51) was measured by real time PCR and normalized to GAPDH (Figures 19A-19B). Secreted PSA in R1881-treated cell culture medium was also measured in the medium of siRNA transfected cells treated with R1881 and measured by ELISA (Figure 19C). Figures 19A and 19B demonstrates the reduction in target gene expression by siRNAs.

Results shows that siRNA to AKR1C3 significantly reduced the ligand-induced expression of AR-dependent genes such as PSA and FKB51, and PSA protein (Figures 19A-19C), compared to cyclophilin or no siRNA conditions. Figure 25 demonstrates the reduction in target gene expression by siRNAs.

**AKR1C3 over-expression increases the androgen-dependent PSA gene expression in LNCaP cells.**

Although AKR1C3 amplified the function of AR in a transactivation system, its effects on endogenous gene expression in prostate cancer cells were not known. PSA gene expression was measured in LNCaP cells transfected with AKR1C3 using Amaxa electroporator and treated with increasing concentrations of A’dione (Figure 19D) or R1881 (Figure 19E).

With reference to Figures 19D, 19E and 19H, LNCaP cells were transfected with 10 μg vector (solid line) or AKR1C3 (broken line) using Amaxa electroporator. Cells were maintained in serum free condition for 3 days and treated for 12 hrs with the indicated concentrations of A’dione (Figure 19D) or R1881 (Figure 19E). EC5₀ of R1881 induced PSA gene expression is given at the top of Figure 19E. RNA was extracted and realtime PCR for PSA gene expression was performed and normalized to GAPDH. Figure 19H demonstrates that AKR1C3 was overexpressed in cells transfected with AKR1C3 (closed bar) or vector (open bar; not visible in figure) transfected cells.

Results showed that AKR1C3 over-expression increased PSA gene expression in response to both A’dione and R1881 (Figures 19D and 19E).

**Compound 78 inhibits the AKRIC3-induced PSA gene expression and cell growth**
[00562] Since AKR1C3 induced PSA gene expression, the effect of 78 on this amplified response was tested. LNCaP cells were transfected with vector (pCR3.1) or AKR1C3 using the Amaxa electroporator. LNCaP-AKR1C3 cells were treated with vehicle or 10 µM 78 in the presence of R1881 or A’dione. PSA gene expression was measured and normalized to GAPDH.

[00563] Results demonstrated that 78 at 10 µM concentration, was very effective in inhibiting the AKR1C3-dependent, A’dione- and R1881- induced PSA gene expression (Figure 19F), with results similar to those observed with siRNA in LNCaP cells. In accordance with the PSA results, LNCaP-AKR1C3 cells demonstrated increased growth in response to A’dione, which was completely inhibited by 78 (Figure 23A).

AKR1C3 over-expression promotes prostate cancer xenograft implantation and growth.

[00564] LNCaP cells were transfected with vector (pCR3.1) or AKR1C3 using Amaxa electroporator. LNCaP-vector (solid line, diamond) or LNCaP-AKR1C3 (broken line, square) cells were mixed with matrigel and implanted (2 million cells/mouse) subcutaneously in nude mice (n=1/l/group). On the day of implantation, animals were castrated and a 90 day sustained release DHT pellet (12.5 mg) was implanted under the skin (Figure 19G). Figure 19K presents data wherein LNCaP-AKR1C3 or LNCaP-vector cells were subcutaneously implanted in intact nude mice (N=5). Numbers in the brackets are tumor bearing animals relative to the total number of animals in the group. Tumor volume was measured biweekly. Figure 19M is a Kaplan-Meier plot of tumor uptake in LNCap-vector (solid line) or LNCap-AKR1C3 (broken line) tumors. Western blots from Figure 19J were quantified and expressed as target protein normalized to actin and the cells were injected subcutaneously in nude mice. Tumor growth was monitored and volume was measured during the course of the study (n=5).

[00565] Results showed that AKR1C3 transfected tumors had a greater incidence of tumor implantation and a higher rate of tumor growth than LNCaP-vector tumors (Figures 19G and 19K). Consistently, the tumor incidence was two times higher in LNCaP-AKR1C3 bearing animals than LNCaP-vector bearing animals. Expression of AR-dependent genes such as FKBP51 and TMPRSS2 increased significantly (Figure 19I) and their protein levels were higher in LNCaP- AKR1C3 tumors (Figure 19J), indicating the role for AKR1C3 to enhance AR signaling in androgen-dependent prostate cancer and CRPC progression. A significant increase in the androgen-dependent FKBP51 protein
expression (Figure 19L, right panel) was observed in LNCaP-AKRIC3 tumors without concomitant increase in AR protein levels (Figure 19L, left panel), suggesting an AKR1C3 coactivation of AR.

[00566] Figure 19G and 19K show that over-expression of AKR1C3 increased tumor volume.

AKR1C3 enhances androgen signaling in tumor xenografts

[00567] After sacrificing the animals described above, tumors were excised and expression of the indicated genes was measured using realtime PCR and normalized to GADPH. Figure 19I shows that over-expression of AKR1C3 enhanced expression of FKBP51 and TMPRSS2 (open bars are LNCaP-vector tumors (n=3) and filled bars are LNCaP-AKRIC3 tumors (n=6).

[00568] Protein was extracted from the above indicated tumors and was fractionated on SDS-PAGE and Western blotted with FKBP51, AKR1C3 and actin antibodies. Figure 19J shows the resultant Western blot, demonstrating that FKBP51 protein is increased in LNCaP-AKRIC3 xenografts.

Example 13

AKR1C3 Crystallography

[00569] Methods. AKR1C3 was cloned into pGEX4T-1 and transformed into E. coli BL21-DE3. GST-AKR1C3 was expressed in one liter of LB media by induction with 0.1 mM IPTG at 37 °C for 4-6 hours. The cell pellet was resuspended in 10 mL lysis buffer (10% glycerol, 50 mM Tris pH 7.5, 0.5 mM EDTA, 0.8% w-octyl-P-glucoside, 0.1 mM PMSF, 1 mM NADP, 10 mM DTT, 1 mg/mL lysozyme, 10 U/mL DNase, 10 mM MgCl₂), subjected to three freeze/thaw cycles and centrifuged at 40,000 x g for 20 min. The clarified lysate was incubated with 3 mL glutathione sepharose resin for 2 hrs at 4 °C and the resin was washed with PBS containing 1 mM DTT and 0.2% w-octyl-P-glucoside. The fusion protein was cleaved overnight with 500 units thrombin at 4 °C and supernatant was placed on a fast blue column in Buffer A (20 mM Tris pH 7.5, 10% glycerol, 1 mM DTT) and eluted with Buffer B (Buffer A + 1M NaCl). Buffer exchange and protein concentration were performed with a Millipore lOcD cut-off concentrator to 500 µL in a buffer containing 10 mM KH₂PO₄ pH 7.0, 1 mM EDTA, 0.5% decyl-maltoside, and 1.2 mM NADP. Drug of interest was added before crystallization trials to a final concentration of 100 µM. Best crystals were grown with reservoir solutions containing 25-33% PEG3350 and 0.1 M Hepes pH 7.5 using hanging drops (Figure 20). Crystals were
cryoprotected in 35% PEG3350 and 0.1 M Hepes pH 7.5. Diffraction data were collected using a Rigaku RU300 rotating anode generator and an R-axis IV++ image plate (Rigaku), and processed with Crystal Clear software (Molecular Structure Corporation). Structures were solved using molecular replacement with the androstenedione-AKR3 complex (PDB code 1FX0).

Results. Electron density maps of the AKR1C3-(cmpd 45) complex at 1.9 Å resolution (Figure 21A, 21B) demonstrated a bifurcated hydrogen bond between the hydroxyl group of the ligand to H17 and Y55. The carbonyl group of 45 interacts with a network of water molecules in a hydrophilic region. One of the meto-fluoro groups is in hydrogen bond range with the side chain of Y216. Analogs containing an N-substituted phenyl ring such as 2-(4-(bromomethyl)-3-hydroxyphenyl)-6-hydroxy-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2 H)-one result in displacement of W277 and rearrangement of F316 to be accommodated within the binding pocket (Figure 21C) as determined by comparing the crystal structure of the AKR1C3-(2-(4-(bromomethyl)-3-hydroxyphenyl)-6-hydroxy-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2 H)-one) complex at 1.9 Å. Such bulky N-substitutions also result in an altered binding conformation as shown.

Example 14

Compound 78 inhibits AKR1C3 enzyme- and coactivator-activities in cells

Compound 78 selectively inhibits AKR1C3-dependent AR transactivation

HEK-293 cells were transfected with 0.25 μg GRE-LUC, 10 ng CMV-LUC, 50 ng CMV hAR, and 1 μg vector or AKR1C3. The cells were treated with 10 nM A’dione alone or in combination with a titration of 78 or indomethacin, and luciferase assay performed as indicated above. Figure 22A shows 78 but not indomethacin, inhibits AKR1C3-mediated A’dione induced AR transactivation.

HEK-293 cells were transfected as indicated above for Figure 22A. Twenty-four hours after transfection the cells were treated with a titration of A’dione alone or in combination with 10 μM 78 and luciferase assay performed as described above. Figure 22B shows 78 increases the EC50 of A’dione. EC50 values are given at the top of the figure.

HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and 1 μg pCR3.1, pCR3.1-AKR1C3 or pCR3.1 17βHSD3. Cells were treated
with 10 nM A'dione in the presence or absence of a titration of 78 (µM) and transactivation assay performed as indicated in above. Figure 22C shows 78 inhibition of AR transactivation is selective to AKR1C3.

[00574] To understand if a competitive inhibitor of AKR1C3 such as 78 has the potential to inhibit R1881-induced AKR1C3-dependent AR transactivation, HEK-293 cells transfected with vector or AKR1C3 were treated with increasing concentrations of R1881 alone or in combination with 78. HEK-293 cells were transfected as indicated above for Figure 22A. Twenty-four hours after transfection the cells were treated with a titration of R1881 alone or in combination with 10 µM 78 and luciferase assay was performed.

[00575] Results showed that 78 completely inhibited the AKR1C3-dependent transactivation induced by R1881 (Figure 22D, solid line is vector transfected cells, broken line is AKR1C3 transfected cells, and dotted line is AKR1C3 transfected cells treated with 78). EC50 of R1881 under various conditions is also presented. This suggests that even in the absence of peripheral testosterone synthesis by AKR1C3 (i.e., no A'dione substrate present), an AKR1C3 inhibitor can still suppress AR signaling.

[00576] HEK-293 cells were transfected as indicated above with 1 µg vector or construct D (AA's 1-282; Figure 15A) and were treated with a titration of R1881 alone or in combination with 10 µM 78. Luciferase assay was performed 24 hrs after treatment. Though R1881 required amino acids 1-282 of AKR1C3 to elicit its coactivation, 78 required full length AKR1C3 to inhibit its coactivation effect (Figure 22E).

[00577] To examine cross reactivity of 78 with AKRICI, HEK-293 cells were transfected with 0.25 µg ERE-LUC, 50 ng pCR3.1 hERβ, 10 ng CMV-renilla luciferase and 1 µg pCR3.1 vector backbone or pCR3.1 AKRICI. Twenty-four hours after transfection the cells were treated with 100 nM DHT alone or in combination with the indicated concentrations of 78 or indomethacin. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase (Figure 22F). Results shown in Figure 22F are representative of three experiments. A'dione-androstenedione; RLU-relative light units.

[00578] To examine concentration dependence of 78 inhibition, HEK-293 cells were transfected with 0.25 µg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and various
concentrations of pCR3.1 or AKR1C3. Twenty-four hours after transfection the cells were treated with 10 nM androstenedione alone or in combination with the indicated concentrations of 78. Cells were harvested forty-eight hours after transfection and firefly luciferase assay was performed and normalized to renilla luciferase (Figure 22G). Androstenedione-androstenedione; RLU-relative light units.

Therefore, results showed that in agreement with the enzyme inhibition results, 78, not indomethacin, effectively inhibited the AKR1C3-dependent A’dione-induced AR transactivation (Figure 22A and 22B), but not the 17P-HSD3 (Figure 22C) and AKR1C1-dependent transactivation (Figure 22F). This inhibition was observed at all AKR1C3 levels, indicating the potency of 78 to block AKR1C3 enzyme activity (Figure 22G). Results shown are representative of three experiments.

**Example 15**

**Compound 78 inhibits androgen signaling and prostate cancer cell and tumor growth**

**Testosterone levels are reduced by AKR1C3 inhibition and not by 5α-reductase inhibition**

As intratumoral levels of testosterone can drive prostate cancer progression, the activities of 78 and finasteride were tested to reduce AR signaling and testosterone production in VCaP, which are CRPC cells that express endogenously high levels of AKR1C1, AKR1C3 and 5α-reductase type-1 (Figure 23). LNCaP-vector (open bars) or LNCaP-AKR1C3 (filled bars) cells were treated with vehicle or 10 μM 78 for 3 days. Cells were fixed and stained with sulforhodamine Blue (SRB) and the optical density (OD) was measured at 535 nm. Figure 23A shows 78 inhibited LNCaP-AKR1C3 cell growth.

VCaP prostate cancer cells were maintained in serum free medium for 3 days and treated with vehicle or 10 μM 78 (Figure 23B-upper panel) or 10 μM finasteride (Fin; Figure 23B-lower panel). RNA was extracted and PSA gene expression was measured and normalized to GAPDH. Figure 23B shows 78 inhibited PSA gene expression in VCaP cells.

The results demonstrated that while finasteride increased PSA gene expression under serum-starved conditions, 78 significantly inhibited PSA gene expression (Figure 23B). The ability of finasteride to increase PSA gene expression could be due to the higher expression of AKR1C1 (Figure 23F) as finasteride significantly increased testosterone levels (Table 6).
Table 6  **Finasteride Blocked DHT Synthesis, Increasing the Levels of Testosterone in these cells.**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Finasteride (10 µM)</th>
<th>Testosterone (nM)</th>
<th>DHT (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNCaP-Vector</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LNCaP-1C3</td>
<td>-</td>
<td>0.77</td>
<td>0</td>
</tr>
<tr>
<td>VCaP</td>
<td>-</td>
<td>3.42</td>
<td>4.34</td>
</tr>
<tr>
<td>HEK-293</td>
<td>-</td>
<td>780.11</td>
<td>32.4</td>
</tr>
<tr>
<td>LNCaP-Mock</td>
<td>+</td>
<td>3.67</td>
<td>0</td>
</tr>
<tr>
<td>LNCaP-1C3</td>
<td>+</td>
<td>17.16</td>
<td>0</td>
</tr>
<tr>
<td>VCaP</td>
<td>+</td>
<td>44.38</td>
<td>0</td>
</tr>
</tbody>
</table>

Indicated cells were treated with 1 µM androstenedione in the presence or absence of 10 µM finasteride and the amount of testosterone and DHT formed was measured by Mass Spectrometry. LNCaP-1C3 denotes AKR1C3 transfected LNCaP cells. Mock is the same as vector.

Finasteride increased testosterone concentrations in LNCaP-1C3 and VCaP cells by ~20- and ~10-fold, respectively. This suggests that an advantage of AKR1C3 inhibition, over 5α-reduction inhibition, was the suppression of AR signaling without increased testosterone.

**78 inhibits prostate cancer xenograft growth**

LNCaP-AKR1C3 cells (2 million cells/mouse) were subcutaneously implanted in nude mice (n=7). Animals bearing LNCaP-AKR1C3 tumors were castrated when tumors reached 100 mm³ and were allowed to progress. Once the tumors reached 200 mm³, the animals were treated with vehicle or 78 at 30 mg/kg/day s.c. for 2 weeks. Alternatively, VCaP cells (2 million cells/mouse) were subcutaneously implanted in nude mice (n=5). Once tumors reached 300 mm³, the animals were castrated and the tumors were allowed to develop as castration resistant prostate cancer. Once the tumors re-emerged, the animals were randomized and treated with vehicle or 40 mg/kg 78 subcutaneously. Tumor volumes were measured biweekly. * p<0.05.
The growth of AKR1C3 dependent-tumors were slowed by treatment with 78 (Figure 23C). Comparable inhibition was also observed in another CRPC xenograft model utilizing VCaP cells, where 78 effectively reduced the tumor growth by 50% (Figure 23D).

At the end of the study, PSA was measured in the serum of CRPC VCaP tumor bearing animals treated with vehicle or 40 mg/kg compound 78. As demonstrated in the graph (Figure 23E), 78 inhibited serum PSA by greater than 75% compared to vehicle treated animals. Numbers above bars is the mean of each group. N=5.

Protein was extracted from tumors shown in Figure 23D, fractionated by SDS-PAGE and Western blotted for PSA and actin. Figure 23G shows 78 reduced tumor PSA to below detectable levels.

To ensure that the tumor volume reduction is due to the exposure to 78, drug concentration was measured in the serum of animals bearing VCaP xenograft and correlated with the final tumor volume. As shown in Figure 23H, the concentration of 78 in serum inversely correlated with tumor volume, indicating that increased exposure to an AKR1C3 inhibitor such as 78 will impede the growth of CRPC tumors.

For Figures 23A-D *in vitro* figures are representative of n=3 and each experiment was performed with triplicate samples; fin-Finasteride; cyclo-cyclophilin; A'dione-androstenedione; LNCaP-AKRlC3-LNCaP cells stably transfected with AKR1C3.

**Example 16**

**AKR1C3 and SRC-2 synergize to increase AR transactivation**

To determine if AKR1C3 and a bonafide coactivator (SRC-2) that is over-expressed in prostate cancer can synergistically increase AR transactivation, plasmids encoding both proteins were transfected in HEK-293 cells and AR transactivation studies were performed.

HEK-293 cells were transfected with 0.25 μg GRE-LUC, 50 ng CMV hAR, 10 ng CMV-renilla luciferase and indicated concentrations of SRC-2 (Figure 24A), AKR1C3 (1C3; Figure 24B) or combination of both (Figure 24C). Twenty-four hours after transfection the cells were treated with a titration of R1881. Cells were harvested forty-eight hours after transfection and firefly luciferase was measured and normalized to renilla luciferase.
Results showed that while SRC-2 (Figure 24A) and AKR1C3 (Figure 24B) concentration-dependently increased AR transactivation, co-transfection of sub-optimal concentrations of the two plasmids synergistically increased AR transactivation (Figure 24C), suggesting different interaction sites with AR. The increase in AR transactivation facilitated by AKR1C3 was comparable to that observed with an established coactivator, SRC-2 (Figures 24A-24C), indicative of the robustness in the ability of AKR1C3 to augment AR function. AKR1C3 over-expression was confirmed by realtime rtPCR (Figure 12K). Results shown in the figures are representative of three experiments. IC3-AKR1C3; RLU-relative light units.

Example 17

Weak ERα and ERβ binding and transactivation activities of representative compounds

Binding Method: Recombinant ER-ot or ER-β ligand binding domain (LBD) was combined with [3H]Estradiol (PerkinElmer, Waltham, MA) in buffer A (10 mM Tris, pH 7.4, 1.5 mM disodium EDTA, 0.25 M sucrose, 10 mM sodium molybdate, 1 mM PMSF) to determine the equilibrium dissociation constant (K_d) of [3H]E2. Protein was incubated with increasing concentrations of [3H]E2 with and without a high concentration of unlabeled E2 at 4 °C for 18 h in order to determine total and non-specific binding. Non-specific binding was then subtracted from total binding to determine specific binding. Ligand binding curves were analyzed by nonlinear regression with one site saturation to determine the K_d of E2 (ER-ot: 0.65 nM; ER-β: 1.83 nM). In addition, the concentration of [3H]E2 required to saturate ER-ot and ER-β LBD was determined to be 1-3 nM. Increasing concentrations of ER ligands (range: 10^-11 to 10^-6 M) were incubated with [3H]E2 (1-3 nM) and ER LBD (α or β) using the conditions described above.

Transactivation method: HEK-293 cells were plated in 24 well plates at 125,000 cells/well in DME + 5% csFBS without phenol red and transfected with 25 ng of mammalian expression plasmids for ER-alpha or ER-beta in combination with 0.25 ugERE-LUC and 5 ng CMV-renilla luc as transfection control. Twenty four hours after transfection, the cells were treated with the indicated drugs alone or in combination with estradiol (antagonist mode) and luciferase assay performed 48 hrs after transfection. Firefly luciferase was normalized to renilla luciferase.

The compounds described in Table 7 below do not have significant binding to ER-alpha or ER-beta.
<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Structure</th>
<th>$K_i$ (nM) ERα</th>
<th>$K_i$(nM) ERβ</th>
<th>Agonist</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2H)-one</td>
<td><img src="image" alt="Structure 17" /></td>
<td>&gt;1506</td>
<td>&gt;2201</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>6-hydroxy-2-methyl-4-(4-trifluoromethyl)phenylisoquinolin-1(2H)-one</td>
<td><img src="image" alt="Structure 21" /></td>
<td>&gt;1506</td>
<td>&gt;2201</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenylisoquinolin-1(2H)-one</td>
<td><img src="image" alt="Structure 39" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid</td>
<td><img src="image" alt="Structure 41" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one</td>
<td><img src="image" alt="Structure 45" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>&gt;3000</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>51</td>
<td>4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one</td>
<td><img src="image" alt="Structure 51" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>40.6% of E2 @10k nM</td>
<td>44.2% of E2 @10k nM</td>
</tr>
<tr>
<td>Number</td>
<td>Name</td>
<td>Structure</td>
<td>(K_i) (nM)</td>
<td>(K_i) (nM)</td>
<td>(\text{Agonist ER}\alpha)</td>
<td>(\text{Agonist ER}\beta)</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>-----------</td>
<td>---------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>203</td>
<td>4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol</td>
<td><img src="image1" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>&gt;3000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>205</td>
<td>4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol</td>
<td><img src="image2" alt="Structure" /></td>
<td>16.7% of E2 @10k nM</td>
<td>7.48% of E2 @10k nM</td>
<td>72.9% of E2 @10k nM</td>
<td>69.7% of E2 @10k nM</td>
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<td>53</td>
<td>4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinol in-1(2H)-one</td>
<td><img src="image3" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>28.5% of E2 @10k nM</td>
<td>16.0% of E2 @10k nM</td>
</tr>
<tr>
<td>55</td>
<td>4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinol in-1(2H)-one</td>
<td><img src="image4" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>7.52% of E2 @10k nM</td>
<td>44.4% of E2 @1000 nM</td>
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<tr>
<td>59</td>
<td>2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one</td>
<td><img src="image5" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>72.1% of E2 @10k nM</td>
<td>56.6% of E2 @1000 nM</td>
</tr>
<tr>
<td>61</td>
<td>2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one</td>
<td><img src="image6" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>67.3% of E2 @10k nM</td>
<td>66.0% of E2 @10k nM</td>
</tr>
<tr>
<td>63</td>
<td>4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinol in-I(2T)-one</td>
<td><img src="image7" alt="Structure" /></td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>40.9% of E2 @10k nM</td>
<td>42.5% of E2 @10k nM</td>
</tr>
</tbody>
</table>
Specifically, the compounds of Table 7 and the compounds of this invention had negligible or no ER binding and transactivation. It would not be expected, that compounds structurally similar to ER-beta agonists would have AKR1C3 inhibitor activity. Much less, would it be expected that the AKR1C3 inhibitors would suppress androgen-dependent AR activation.

While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the
art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.
What is claimed is:

1. A compound of Formula I:

\[
\text{Formula I}
\]

wherein,

A is 0, N or C;

B is N or C;

\(R^1\) is nothing, H, alkyl or \(-\text{alkylene-CC}>_2\text{R}\), in which \text{R}\ is H or alkyl; wherein, when \(R^1\) is nothing an oxo (C=0) group is formed;

\(R^2\) is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkylene-C0 \(\text{C}_2\text{R}\), in which \text{R}\ is H or alkyl;

\(R^3\) is, in each case, independently selected from hydroxyl, halogen, haloalkyl, \(\text{CF}_2\text{OMe}, \text{CN, carboxyl, substituted or unsubstituted phenyl, S}^0\text{R}^2, \text{S}^0\text{NHR}^2\) or any combination thereof in which \(\text{R}\) is, in each case, independently, H or alkyl;

\(R^4\) is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, \(\text{CN, carboxyl, S}^0\text{R}^2, \text{S}^0\text{NHR}^2\) or any combination thereof; in which \(\text{R}\) is, in each case, independently, H or alkyl;

\(R^5\) is nothing, H, alkyl or \(-\text{alkylene-C0}_2\text{R}\), in which \text{R}\ is H or alkyl; wherein when \(R^5\) is nothing an oxo (C=0) group is formed.

\(R^6\) is nothing, H, alkyl or \(-\text{alkylene-C0}_2\text{R}\), in which \text{R}\ is H or alkyl; wherein, when A is 0 then \(R^6\) is nothing;
m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;

or its isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N'-oxide, hydrate or any combination thereof.

2. The compound according to claim 1, wherein A and B are C (carbon), forming a naphthalene compound.

3. The compound according to claim 1, wherein R^1 is H or alkyl.

4. The compound according to claim 1, wherein R^2 is H, alkyl or cycloalkyl.

5. The compound according to claim 1, wherein n is 2 or 3.

6. The compound according to claim 1, wherein A and B are C (carbon), R^1 is H, R^2 is H, R^3 is halogen or haloalkyl, R^4 is H, R^5 is H, R^6 is H and n is 2 or 3.

7. The compound according to claim 6, wherein said compound is 4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205) or 4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203) or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N'-oxide, hydrate or any combination thereof.

8. A compound of Formula II:

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{R}^2 \\
\text{R}^1 \\
\text{R}^3_n \\
\text{O}_m(R^4) \\
\end{array}
\]

wherein

R^1 is H, alkyl or -alkylene-C_0^2R^5, in which R^5 is H or alkyl;
R\textsuperscript{2} is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkylenec-C\textsubscript{0}\textsuperscript{2}R\textsuperscript{3}, in which R\textsuperscript{3} is H or alkyl;

R\textsuperscript{3} is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF\textsubscript{2}OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO\textsubscript{2}R\textsuperscript{2} or S0\textsubscript{2}NHR\textsuperscript{2} in which R\textsuperscript{2} is, in each case, independently, H or alkyl;

R\textsuperscript{4} is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO\textsubscript{2}R\textsuperscript{2} or S0\textsubscript{2}NHR\textsuperscript{2} in which R\textsuperscript{2} is, in each case, independently, H or alkyl;

m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;

or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate, or any combination thereof.

9. The compound according to claim 8, wherein R\textsuperscript{1} is H or alkyl.

10. The compound according to claim 8, wherein R\textsuperscript{2} is H, alkyl or cycloalkyl.

11. The compound according to claim 8, wherein n is 2 or 3.

12. The compound according to claim 8, wherein R\textsuperscript{4} is H.

13. The compound according to claim 8, wherein R\textsuperscript{1} is H, R\textsuperscript{2} is H or cycloalkyl, R\textsuperscript{3} is halogen or haloalkyl, R\textsuperscript{4} is H and n is 2 or 3.

14. The compound according to claim 8, wherein said compound is selected from:

6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45),

2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl) isoquinolin-l(2 H)-one (67),

6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin- l(2 H)-one (78),

4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81),
2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84),
4-(4-hydroxyphenyl)-6-methoxyisoquinolin-l(2 H)-one (17),
6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2 H)-one (21),
6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2 H)-one (39),
4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),
2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2 H)-one (49),
4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (51),
4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (53),
4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (55),
2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (59),
2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (61),
4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (63),
2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (70),
4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (72),
6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77),
2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (80),
methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1 H)-yl)acetate (82),
4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (91),
4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-l(2 H)-one (106),
4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),
2-((2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109),
2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetic acid (113), and

4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220),

or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

15. A pharmaceutical composition comprising a compound according to any one of claims 1-14 and a pharmaceutically acceptable carrier.

16. A method of inhibiting a hydroxysteroid dehydrogenase in a patient in need thereof comprising administering to said patient a compound according to any one of claims 1-14.

17. The method according to claim 16, wherein said administration selectively inhibits an AKR1C3 enzyme activity.

18. A method of treating a disorder that responds to a hydroxysteroid dehydrogenase inhibitor comprising administering to a patient in need thereof, a therapeutically effective amount of compound according to any one of claims 1-14.

19. The method according to claim 18, wherein said administration selectively inhibits an AKR1C3 enzyme.

20. The method of claim 19, wherein said disorder is prostate cancer.

21. The method according to claim 20, wherein said disorder is selected from prostate cancer, primary prostate cancer, advanced prostate cancer, metastatic prostate cancer, hormone naïve prostate cancer, refractory prostate cancer or castration resistant prostate cancer (CRPC), or any combination thereof.

22. The method according to claim 19, wherein the patient has precancerous precursors of prostate adenocarcinoma.

23. The method of claim 22, wherein the precancerous precursor of prostate adenocarcinoma is prostate intraepithelial neoplasia (PIN).

24. The method according to claim 23, wherein the PIN is high-grade PIN (HGPIN).
25. The method of claim 21, wherein said administration prolongs progression-free survival or overall survival in a man with castration-resistant prostate cancer or advanced metastatic prostate cancer.

26. The method according to claim 19, wherein said disorder is selected from breast cancer; metastatic breast cancer; advanced breast cancer; refractory breast cancer; AR-positive breast cancer; ER-positive breast cancer; AR-positive refractory breast cancer; ER-positive refractory breast cancer; AR-positive metastatic breast cancer; ER-positive metastatic breast cancer; triple negative breast cancer; and/or breast cancer that has failed SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof.

27. The method of claim 26, wherein said patient has breast cancer that has failed SERM (tamoxifen, toremifene), aromatase inhibitor (AI), trastuzumab (Herceptin®, ado-trastuzumab emtansine), pertuzumab (Perjeta), lapatinib, exemestane (Aromasin®), bevacizumab (Avastin®), fulvestrant treatment, or any combination thereof.

28. The method of claim 26, wherein said administration prolongs progression-free survival or overall survival in a subject with refractory breast cancer or advanced metastatic breast cancer.

29. The method according to claim 19, wherein the disorder is selected from benign prostate hyperplasia (BPH), lung cancer, non-small cell lung cancer (NSCLC), acne, seborrhea, hirsutism, baldness, alopecia, precocious puberty, adrenal hypertrophy, polycystic ovary syndrome, endometriosis, myeloma or leiomyoma.

30. A method of lowering serum testosterone levels in a male subject consisting essentially of administering a therapeutically effective amount of a compound of Formula I, or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof;
wherein,

A is 0, N or C;

B is N or C;

R₁ is nothing, H, alkyl or -alkylene-CC>2R₂, in which R₂ is H or alkyl; wherein, when R₁ is nothing an oxo (C=0) group is formed;

R₂ is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkyene-C0_2R₃, in which R₃ is H or alkyl;

R₃ is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF₂OMe, CN, carboxyl, substituted or unsubstituted phenyl, S0₂R₂, S0₂NHR₂ or any combination thereof in which R₂ is, in each case, independently, H or alkyl;

R₄ is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, S0₂R₂, S0₂NHR₂ or any combination thereof; in which R₂ is, in each case, independently, H or alkyl;

R₅ is nothing, H, alkyl or -alkylene-C0_2R₆, in which R₆ is H or alkyl; wherein when R₅ is nothing an oxo (C=0) group is formed.

R₆ is nothing, H, alkyl or -alkylene-C0_2R₆, in which R₆ is H or alkyl; wherein, when A is 0 then R₆ is nothing;

m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;
in an amount effective to lower serum testosterone.

31. The method of claim 30, wherein said serum testosterone levels are total serum testosterone levels.

32. The method of claim 30, wherein said serum testosterone levels are free serum testosterone levels.

33. The method of claim 30, wherein said compound of Formula I is selected from:

6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (45),
2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl) isoquinolin-1(2H)-one (67),
6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (78),
4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2H)-one (81),
2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84),
4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2H)-one (17),
6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2H)-one (21),
6-hydroxy-2-methyl-4-(4-(methylsulfanyl)phenyl) isoquinolin-1(2H)-one (39),
4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),
2-cyclohexyl-6-hydroxy-4-(4-(methylsulfanyl)phenyl)isoquinolin-1(2H)-one (49),
4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (51),
4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (53),
4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (55),
2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (59),
2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (61),
4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2H)-one (63),
2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl) isoquinolin-1(2\,H)-one (70),
4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin4(2\,H)-one (72),
6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2\,H)-one (77),
2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (80),
methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1\,H)-yl)acetate (82),
4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\,H)-one (91)
4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2\,H)-one (106),
4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),
2-((2-(2-methoxy-2-oxoethyl)4-oxo4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109), and
2-(6-hydroxy-4-oxo-4-(3,4,5trifluorophenyl)isoquinolin-2(1\,H)-yl)acetic acid (113),
4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205);
4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203);
methyl 2-((5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalen-2-yl)oxy)acetate (209);
dimethyl 2,2’-((4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy))diacetate (209A);
methyl 2-((6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212); and
4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2\,H)-one (220), or
its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

34. The method of claim 30, wherein said lowering of serum testosterone is independent of a reduction of serum luteinizing hormone levels.

35. The method of claim 30, wherein said lowering of serum testosterone lowers prostate-specific antigen (PSA).
A method of lowering serum testosterone levels in a male subject consisting essentially of administering a therapeutically effective amount of a compound of Formula II, or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof;

![Chemical Structure](image)

(II)

wherein

R\(^1\) is H, alkyl or -alkylene-C\(\text{O}_2\)R\(^3\), in which R\(^3\) is H or alkyl;

R\(^2\) is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkylene-C\(\text{O}_2\)R\(^3\), in which R\(^3\) is H or alkyl;

R\(^3\) is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF\(_2\)OMe, CN, carboxyl, substituted or unsubstituted phenyl, S\(\text{O}_2\)R\(^z\) or S\(\text{O}_2\)NHR\(^z\) in which R\(^z\) is, in each case, independently, H or alkyl;

R\(^4\) is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, S\(\text{O}_2\)R\(^z\) or S\(\text{O}_2\)NHR\(^z\) in which R\(^z\) is, in each case, independently, H or alkyl;

m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;

in an amount effective to lower serum testosterone.
37. The method of claim 36, wherein said serum testosterone levels are total serum testosterone levels.

38. The method of claim 36, wherein said serum testosterone levels are free serum testosterone levels.

39. The method of claim 36, wherein said compound of Formula II is selected from:

6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45),
2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl) isoquinolin-l(2 H)-one (67),
6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (78),
4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81),
2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84),
4-(4-hydroxyphenyl)-6-methoxyisoquinolin-l(2 H)-one (17),
6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2 H)-one (21),
6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl) isquinolin-l(2 H)-one (39),
4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),
2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2 H)-one (49),
4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (51),
4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (53),
4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (55),
2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (59),
2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (61),
4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (63),
2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl) isoquinolin-l(2 H)-one (70),
4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (72),
6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2\text{H})-one (77),

2-fluoro-5-(6-hydroxy-l-oxo,l,2-dihydroisoquinolin-4-yl)benzonitrile (80),

methyl 2-(6-(2-methoxy-2-oxoethoxy)-l-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(l\text{H})-yl)acetate (82),

4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-64iydroxyisoquinolin-l(2\text{H})-one (91)

4-(4-chloro-3-fluorophenyl)-64iydroxyisoquinolin-l(2\text{H})-one (106),

4-(64iydroxy- l-oxo- 1,2-dihydroisoquinolin-4-yl)benzonitrile (108),

2-((2-(2-methoxy-2-xoemyl)4-oxo4-(3,4,54rifluorophenyl)-l,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109),

2-(64iydroxy-l-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(l\text{H})-yl)acetic acid (113), and

4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-l(2\text{H})-one (220), or

its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

40. The method of claim 36, wherein said lowering of serum testosterone is independent of a reduction of serum luteinizing hormone levels.

41. The method of claim 36, wherein said lowering of serum testosterone lower prostate-specific antigen (PSA).

42. A method of lowering serum estradiol levels in a subject consisting essentially of administering a therapeutically effective amount of a compound of Formula I, or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof:
wherein,

A is 0, N or C;

B is N or C;

$R^1$ is nothing, H, alkyl or -alkylene-CC>2$R^5$, in which $R^5$ is H or alkyl; wherein, when $R^1$ is nothing an oxo (C=0) group is formed;

$R^2$ is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkylene-C0 $\equiv R^7$, in which $R^7$ is H or alkyl;

$R^3$ is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF$_2$OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO$_2$R$^Z$, SO$_2$NHR$^Z$ or any combination thereof in which $R^Z$ is, in each case, independently, H or alkyl;

$R^4$ is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO$_2$R$^Z$, SO$_2$NHR$^Z$ or any combination thereof; in which $R^Z$ is, in each case, independently, H or alkyl;

$R^5$ is nothing, H, alkyl or -alkylene-C0 $\equiv R^8$, in which $R^8$ is H or alkyl; wherein when $R^5$ is nothing an oxo (C=0) group is formed.

$R^6$ is nothing, H, alkyl or -alkylene-C0 $\equiv R^8$, in which $R^8$ is H or alkyl; wherein, when A is 0 then $R^6$ is nothing;

$m = 1$ or $2$;

and

$n = 1, 2, 3, 4$ or $5$;
43. The method of claim 42, wherein said serum estradiol levels are total serum estradiol levels.

44. The method of claim 42, wherein said serum estradiol levels are free serum estradiol levels.

45. The method of claim 42, wherein said compound of Formula I is selected from:

6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (45),

2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (67),

6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (78),

4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-1(2 H)-one (81),

2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84),

4-(4-hydroxyphenyl)-6-methoxyisoquinolin-1(2 H)-one (17),

6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-1(2 H)-one (21),

6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2 H)-one (39),

4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),

2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-1(2 H)-one (49),

4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (51),

4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (53),

4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (55),

2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (59),

2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2 H)-one (61),

4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-1(2 H)-one (63),

in an amount effective to lower serum estradiol.
2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl) isoquinolin-1(2H)-one (70),

4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin4(2H)-one (72),

6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77),

2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (80),

methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82),

4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (91)

4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106),

4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),

2-((2-(2-methoxy-2-oxoethyl)-1-oxo-4-(3,4,5-Mfluorophenyl)4,2-dmydroisoquinolin-6-yl)oxy)acetic acid (109), and

2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetic acid (113),

4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205);

4-(4-(trifluoromethyl)phenyl)naphthalene- 1,6-diol (203);

methyl 2-((5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalen-2-yl)oxy)acetate (209);

dimethyl 2,2′-((4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy))diacetate (209A);

methyl 2-((6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212); and

4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or

its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N'-oxide, hydrate or any combination thereof.

46. The method of claim 42, wherein said lowering of serum estradiol is independent of a reduction of serum luteinizing hormone levels.

47. A method of inhibiting androgen-dependent AR transactivation in a subject comprising administering a therapeutically effective amount of a compound of Formula I, or its prodrug,
isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof:

![Chemical Structure](image)

wherein,

A is 0, N or C;

B is N or C;

\( R^1 \) is nothing, H, alkyl or -alkylene-C0 \(_2\)R\(^x\), in which R\(^x\) is H or alkyl; wherein, when \( R^1 \) is nothing an oxo (C=O) group is formed;

\( R^2 \) is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkyne-CC>\(_2\)R\(^y\), in which R\(^y\) is H or alkyl;

\( R^3 \) is, in each case independently selected from hydroxyl, halogen, haloalkyl, CF\(_2\)OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO \(_2\)R\(^z\), SO \(_2\)NHR\(^z\) or any combination thereof in which R\(^z\) is, in each case, independently, H or alkyl;

\( R^4 \) is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO \(_2\)R\(^z\), SO \(_2\)NHR\(^z\) or any combination thereof; in which R\(^z\) is, in each case, independently, H or alkyl;

\( R^5 \) is nothing, H, alkyl or -alkylene-CC>\(_2\)R\(^x\), in which R\(^x\) is H or alkyl; wherein when \( R^5 \) is nothing an oxo (C=O) group is formed.

\( R^6 \) is nothing, H, alkyl or -alkylene-C0 \(_2\)R\(^x\), in which R\(^x\) is H or alkyl; wherein, when A is 0 then \( R^6 \) is nothing;
in an amount effective to inhibit androgen-dependent AR transactivation.

48. The method of claim 47, wherein said compound is represented by a compound of Formula II, or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof;

![Chemical Structure](image)

wherein

R^1 is H, alkyl or alkylene-C\(_2\)R^5, in which R^5 is H or alkyl;

R^2 is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkylene-C\(_2\)R^5, in which R^5 is H or alkyl;

R^3 is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF\(_2\)OMe, CN, carboxyl, substituted or unsubstituted phenyl, SO\(_2\)R^z or SO\(_2\)NHR^z in which R^z is, in each case, independently, H or alkyl;

R^4 is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, SO\(_2\)R^z or SO\(_2\)NHR^z in which R^z is, in each case, independently, H or alkyl;

m = 1 or 2;

and
n = 1, 2, 3, 4 or 5.

49. The method of claim 47, wherein said compound of Formula I is selected from:

- 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (45),
- 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl) isoquinolin-l(2 H)-one (67),
- 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin- l(2 H)-one (78),
- 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2 H)-one (81),
- 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (84),
- 4-(4-hydroxyphenyl)-6-methoxyisoquinolin-l(2 H)-one (17),
- 6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2 H)-one (21),
- 6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl) isoquinolin-l(2 H)-one (39),
- 4-(6-hydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-4-yl)benzoic acid (41),
- 2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2 H)-one (49),
- 4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (51),
- 4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin- l(2 H)-one (53),
- 4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (55),
- 2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (59),
- 2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (61),
- 4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (63),
- 2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl) isoquinolin-l(2 H)-one (70),
- 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2 H)-one (72),
- 6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2 H)-one (77),
- 2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (80),
methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82),

4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (91)

4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106),

4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),

2-((2-(2-methoxy-2-oxoethyl)-4-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109), and

2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetic acid (113),

4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205);

4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203);

methyl 2-((5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalen-2-yl)oxy)acetate (209);

dimethyl 2,2’-((4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy))diacetate (209A);

methyl 2-((6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212); and

4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or

its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

50. The method of claim 47, wherein said inhibition treats prostate cancer, primary prostate cancer, advanced prostate cancer, metastatic prostate cancer, hormone naive prostate cancer, refractory prostate cancer or castration resistant prostate cancer (CRPC), or any combination thereof;

51. The method of claim 50, in which said cancers overexpress AKR1C3.

52. The method of claim 50, wherein said treatment is independent of inhibiting AKR1C3 enzyme activity.

53. The method of claim 50, wherein said treatment is independent of lowering endogenous androgen levels.

54. The method of claim 50, wherein said treatment is independent of lowering endogenous estrogen levels.
55. A method of treating AKR1C3 overexpressing tumors in a subject comprising
administering a therapeutically effective amount of a compound of Formula I, or its prodrug,
isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide,
hydrate or any combination thereof:

\[
\text{\begin{align*}
\begin{array}{c}
\text{(I)} \\
\text{A} \\
\text{B} \\
\text{R}^1 \\
\text{R}^2 \\
\text{R}^3 \\
\text{R}^4 \\
\text{R}^5 \\
\end{array}
\end{align*}
\]

wherein,

\(A\) is 0, N or C;

\(B\) is N or C;

\(R^1\) is nothing, H, alkyl or -alkylene-CC\(\longrightarrow\)R\(^8\), in which \(R^8\) is H or alkyl; wherein, when
\(R^1\) is nothing an oxo (C=0) group is formed;

\(R^2\) is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl,
substituted or unsubstituted cycloalkyl, or substituted or unsubstituted alkyne-C0\(\longrightarrow\)R\(^7\), in
which \(R^7\) is H or alkyl;

\(R^3\) is, in each case independently selected from hydroxyl, halogen, haloalkyl,
CF\(_2\)OMe, CN, carboxyl, substituted or unsubstituted phenyl, S0\(\longrightarrow\)R\(^z\), S0\(\longrightarrow\)NHR\(^z\) or any
combination thereof in which \(R^z\) is, in each case, independently, H or alkyl;

\(R^4\) is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen,
haloalkyl, CN, carboxyl, SC\(\longrightarrow\)R\(^z\), SC\(\longrightarrow\)NHR\(^z\) or any combination thereof; in which \(R^z\) is, in
each case, independently, H or alkyl;

\(R^5\) is nothing, H, alkyl or -alkylene-C0\(\longrightarrow\)R\(^8\), in which \(R^8\) is H or alkyl; wherein when
\(R^5\) is nothing an oxo (C=0) group is formed.
R^6 is nothing, H, alkyl or -alkylene-CC>2R^x, in which R^x is H or alkyl; wherein, when A is 0 then R^6 is nothing;

m = 1 or 2;

and

n = 1, 2, 3, 4 or 5;

in an amount effective to treat AKR1C3 overexpressing tumors.

56. The method of claim 55, wherein said compound is represented by a compound of Formula II, or its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof;

![Chemical Structure](image)

wherein

R^1 is H, alkyl or -alkylene-C0_2R^5, in which R^5 is H or alkyl;

R^2 is H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkyne-C0_2R^5, in which R^5 is H or alkyl;

R^3 is, in each case, independently selected from hydroxyl, halogen, haloalkyl, CF_2OMe, CN, carboxyl, substituted or unsubstituted phenyl, S0_2R^z or S0_2NHR^z in which R^z is, in each case, independently, H or alkyl;

R^4 is, in each case, independently selected from hydrogen, alkyl, hydroxyl, halogen, haloalkyl, CN, carboxyl, S0_2R^z or S0_2NHR^z in which R^z is, in each case, independently, H or alkyl;
m = 1 or 2;

and

n = 1, 2, 3, 4 or 5.

57. The method of claim 55, wherein said compound of Formula I is selected from:

- 6-hydroxy-2-methyl-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (45),
- 2-cyclopropyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (67),
- 6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (78),
- 4-(3-fluoro-4-(trifluoromethyl)phenyl)-6-hydroxy-isoquinolin-l(2H)-one (81),
- 2-fluoro-5-(6-hydroxy-l-oxo-l,2-dihydroisoquinolin-4-yl)benzonitrile (84),
- 4-(4-hydroxyphenyl)-6-methoxyisoquinolin-l(2H)-one (17),
- 6-hydroxy-2-methyl-4-(4-(trifluoromethyl)phenyl)isoquinolin-l(2H)-one (21),
- 6-hydroxy-2-methyl-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2H)-one (39),
- 4-(6-hydroxy-2-methyl-l-oxo-l,2-dihydroisoquinolin-4-yl)benzoic acid (41),
- 2-cyclohexyl-6-hydroxy-4-(4-(methylsulfonyl)phenyl)isoquinolin-l(2H)-one (49),
- 4-(4-fluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2H)-one (51),
- 4-(3-fluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2H)-one (53),
- 4-(3-fluoro-4-hydroxyphenyl)-6-hydroxy-2-methylisoquinolin-l(2H)-one (55),
- 2-butyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (59),
- 2-cyclohexyl-6-hydroxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (61),
- 4-(3,5-difluorophenyl)-6-hydroxy-2-methylisoquinolin-l(2H)-one (63),
- 2-(3,3-difluoroallyl)-6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-l(2H)-one (70),
- 4-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-2-methylisoquinolin-l(2H)-one (72),
6-methoxy-4-(3,4,5-trifluorophenyl)isoquinolin-1(2H)-one (77),

2-fluoro-5-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (80),
methyl 2-(6-(2-methoxy-2-oxoethoxy)-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetate (82),

4-(4-(difluoro(methoxy)methyl)-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (91)
4-(4-chloro-3-fluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (106),
4-(6-hydroxy-1-oxo-1,2-dihydroisoquinolin-4-yl)benzonitrile (108),
2-((2-(2-methoxy-2-oxoethyl)4-oxo-4-(3,4,5-trifluorophenyl)-1,2-dihydroisoquinolin-6-yl)oxy)acetic acid (109), and

2-(6-hydroxy-1-oxo-4-(3,4,5-trifluorophenyl)isoquinolin-2(1H)-yl)acetic acid (113),
4-(3,4,5-trifluorophenyl)naphthalene-1,6-diol (205);
4-(4-(trifluoromethyl)phenyl)naphthalene-1,6-diol (203);
methyl 2-((5-hydroxy-8-(3,4,5-trifluorophenyl)naphthalen-2-yl)oxy)acetate (209);
dimethyl 2,2′-((4-(3,4,5-trifluorophenyl)naphthalene-1,6-diyl)bis(oxy))diacetate (209A); methyl 2-((6-hydroxy-4-(3,4,5-trifluorophenyl)naphthalen-1-yl)oxy)acetate (212); and
4-(3,4-difluorophenyl)-6-hydroxyisoquinolin-1(2H)-one (220) or
its prodrug, isomer, tautomer, metabolite, pharmaceutically acceptable salt, polymorph, crystal, N-oxide, hydrate or any combination thereof.

58. The method of claim 55, wherein said tumors are not androgen-dependent tumors.

59. The method of claim 55, wherein said tumors are not estrogen-dependent tumors.
Figure 1

Cholesterol → Androstenediol

Pregnenolone → Progesterone → 20α-OH-Progesterone

DHEA → Androstenedione → Estrone

Androstenedione

20α-OH-Progesterone

Estrone

Testosterone → Estradiol → DHT

17β-Hydroxysteroid dehydrogenase (AKR1C3)

Androsterone
Figure 3

<table>
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<tr>
<th></th>
<th>AKR1C3</th>
<th>AKR1C1</th>
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<td>- 78</td>
<td>- 78</td>
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</table>

P

20α(OH)P

% 20α(OH)P

- 78

AKR1C3

- 78

AKR1C1

Vector AKR1C3

A'dione

T

3A

3B

3C
Figure 4

COX-1 Inhibition vs. log [Drug] (M)

- Indomethacin IC_{50} = 16.2 nM
- Compd 205 IC_{50} = > 500 nM
- Compd 67 IC_{50} = > 50 μM
- Compd 78 IC_{50} = > 500 μM

Graph showing the percentage of total activity vs. log concentration of drug.
Figure 8

AKR1C3 + testosterone
AKR1C3 + 4’-dione
pCR3.1 + testosterone
pCR3.1 + 4’-dione

8A

8B

8C
### Figure 16

<table>
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<tr>
<th>Vector</th>
<th>AKR1C3</th>
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<tr>
<td>R1881</td>
<td>-</td>
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</tbody>
</table>

**IP:** AKR1C3  
**IB:** AR  
**AR:** 10% Input  

### Figure 16A

### Figure 16B

- **AR**  
- **AKR1C3**  
- **Merge**

- 100 nM A'stione  
- 10 nM R1881

### Figure 16C

<table>
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<th>AR</th>
<th>IgG</th>
<th>20% input</th>
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<td>3</td>
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</table>

**AKR1C**  
**AR**
INTERNATIONAL SEARCH REPORT

International application No. PCT/US1 3/32674

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): C07D 21/22, 21/00 (2013.01)
USPC - 546/141, 146, 139, 112, 26, 1

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): C07D 21/722, 217/00; A01N 43/42; A61K 3/14/7 (2013.01)
USPC: 546/141, 146, 139, 112, 26, 1; 514/307, 309, 299, 279, 277, 183, 1

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

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


C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>US 2010/0256698 A1 (TROTTER, BW et al.) October 7, 2010; paragraphs [0012], [0062]-[0064], [0092], [01 15]-[01 17], [0150], [0239]</td>
<td>1. 3-5, 8-14, 15/1, 15/3-5, 15/8-14</td>
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<td>Y</td>
<td>WO 2010/096801 A1 (DALTON, JT et al.) August 26, 2010; paragraphs [0041], [0043], [0059]</td>
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<tr>
<td>Y</td>
<td>US 2006/0270591 A1 (CHANG, C) November 30, 2006; paragraphs [0370], [0488]</td>
<td>47-54, 57(B), 58</td>
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</table>

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
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  - "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)
  - "G" 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 novel or 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

K document member of the same patent family

Date of the actual completion of the international search: 23 May 2013 (23.05.2013)

Date of mailing of the international search report: 05 JUN 2013

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Authorized officer: Shane Thomas

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PCT OSP: 571-272-7774

Form PCT/ISA/2.10 (second sheet) (July 2009)
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<td>Y</td>
<td>US 2004/0171006 A1 (XIAO, Y) September 2, 2004; paragraphs (0226), (0236)</td>
<td>55, 56, 57(A), 57(B), 58</td>
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<td>Y</td>
<td>US 5112869 A (WATANABE, KA et al.) May 12, 1992; abstract</td>
<td>2, 6, 7, 15/2, 15/6-7, 16/2, 16/6-7, 17/16/2, 17/16/6-7, 18/2, 18/6-7, 19/18/2 19/18/6-7, 20/19/18/2, 20/19/18/6-7, 21/20/19/18/2, 21/20/19/18/6-7, 26/19/18/2, 26/19/18/6-7, 29/19/18/2, 29/19/18/6-7, 22/19/18/2, 22/19/18/6-7, 23/22/19/18/2, 23/22/19/18/6-7, 24/23/22/19/18/2, 24/23/22/19/18/6-7, 25/21/20/19/18/2, 25/21/20/19/18/6-7, 27/26/19/18/2, 27/26/19/18/6-7, 28/26/19/18/2, 28/26/19/18/6-7</td>
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<td>US 2011/0071146 A1 (NIIMI, T et al.) March 24, 2011; abstract; paragraphs [0038], [0055]</td>
<td>16/1-14, 17/15/1-14, 18/1-14, 18/15/1-14, 19/18/1-14, 20/19/18/1-14, 21/20/19/18/1-14, 22/19/18/1-14, 23/22/19/18/1-14, 24/23/22/19/18/1-14, 25/21/20/19/18/1-14, 26/19/18/1-14, 27/26/19/18/1-14, 28/26/19/18/1-14, 29/19/18/1-14</td>
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<td>US 2010/0286204 A1 (VICKER, N et al.) November 11, 2010; paragraphs [0170]-[0172]</td>
<td>22/19/18/1-14, 23/22/19/18/1-14, 24/23/22/19/18/1-14</td>
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<td>Y</td>
<td>WO 2012/008634 A2 (PERAMBAKAM, SM et al.) January 12, 2012; paragraphs [0104], [0108], [0113]</td>
<td>25/21/20/19/18/1-14</td>
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<td>Y</td>
<td>US 2010/0029734 A1 (WHITE, D et al.) February 4, 2010; abstract</td>
<td>27/26/19/18/1-14</td>
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<td>Y</td>
<td>BYRNS, MC et al. An Indomethacin Analogue, N-(4-Chlorobenzoyl)-melatonin, Is A Selective Inhibitor Of Aldo-keto Reductase 1C3 (Type 2 3Alpha-HSD, Type 5 17Beta-HSD, And Prostaglandin F Synthase), A Potential Target For The Treatment Of Hormone Dependent And Hormone Independent Malignancies. Biochem Pharmacol. 15 January 2008; Vol. 75; No. 2: 484-493.</td>
<td>57(B), 58</td>
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