ANDROGEN RECEPTOR DOWN-REGULATING AGENTS AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/782,383, filed March 14, 2013, which application is incorporated herein by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with the support of the United States government under Contract number CA117991and CA129379 by the National Institutes of Health.

BACKGROUND OF THE INVENTION

[0003] The present disclosure provides the design and synthesis of novel steroidal compounds that cause down-regulation of the androgen receptor (AR), both full length and splice variant. The compounds are potential agents for the treatment of all forms of prostate cancer and other diseases that depend on functional AR.

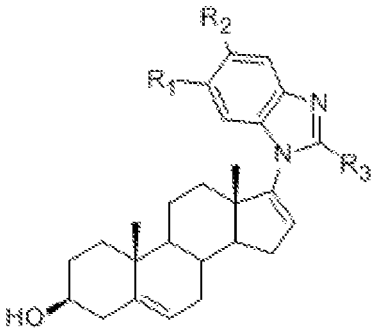
[0004] Compelling laboratory and clinical evidences strongly indicates that incurable castration-resistant prostate cancer (CRPC) remains dependent on functional androgen receptor (AR), AR-mediated processes, and the availability of intra-prostatic intracellular androgens. Unlike early stage prostate cancer (ESPC), CRCP is not responsive to classical AR antagonist, [hydroxyflutamide (**1**) or bicalutamide (**2**); **Figure 1**] or androgen deprivation therapy (luteinizing hormone-releasing hormone agonists/antagonists). Therefore, recent strategies have focused on the development of *more potent* androgen synthesis inhibitorsor AR antagonists. These research efforts have led to ongoing clinical evaluations/approvals of three potent CYP17 inhibitors, abiraterone acetate (Zytiga, **3a**), TAK-700 (Orteronel, **4**) and VN/124-1 (TOK-001 or galeterone, **5**), and two potent AR antagonists, MDV3100 (enzalutamide, **6**) and ARN-509 (**7**). The chemical structures of these clinical compounds are presented in **Figure 1**.

[0005] Despite the substantial clinical efficacy with Zytiga in patients with post-docetaxel CRPC, resistance to this therapy has already been reported. Resistance to MDV3100 treatment has also been reported. Reactivation of AR signaling following Zytiga or MDV3100 treatment might occur by several mechanisms, prominent of which is a switching of transcription program under the control of AR signaling. Indeed, it may not be possible to inhibit the new AR-regulated transcription program by currently available therapies and some of the promising agents in clinical development. If so, substantial down-regulation of AR (full length and truncated forms) expression would be a promising strategy for future studies.

[0006] Herein, we report several novel compounds which exhibit the abilities to induce AR (*full length and truncated*) ablation at low micromolar concentrations and with improved anti-proliferative (AP) activities. This study expands the current understanding of the optimal pharmacophore requirements for AR degradation/down-regulator (ARD) activity and their capabilities in regulating the activity of the AR (i.e., AR inactivation).

SUMMARY OF THE INVENTION

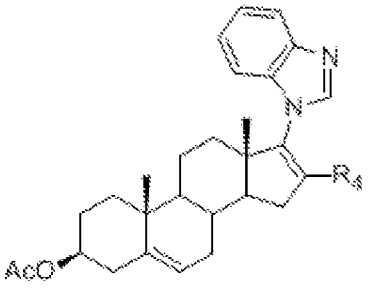
[0007] In one aspect, the present disclosure provides a compound of Formula I:



or pharmaceutically acceptable salt thereof, wherein: each of R_1 and R_2 is independently hydrogen, alkoxy, or CN; R_3 is hydrogen or halo; and wherein at least one of R_1, R_2, R_3 is not hydrogen.

[0008] In some cases, R_1 or R_2 can be CN. In other cases, R_1 can be alkoxy. For example, R_1 can be methoxy. In further cases, R_3 can be halo. For example, R_3 can be chloro.

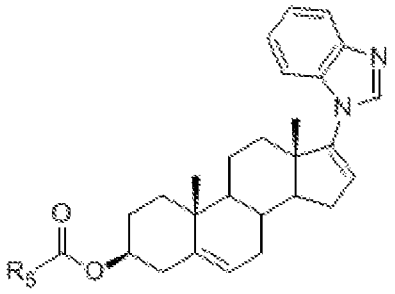
[0009] In another aspect, the present disclosure provides a compound of Formula II:



or pharmaceutically acceptable salt thereof, wherein: R_4 is $-CNHR_{10}$ or $-C=NR_{10}$; R_{10} is alkyl or aryl, optionally substituted by one or more R_{11} substituents; and R_{11} is halogen, alkoxy, or CN.

[0010] In some cases, R_4 can be $-CNHR_{10}$. In other cases, R_4 can be $-C=NR_{10}$. In some examples, R_{10} can alkyl. In other examples, R_{10} can be aryl. In further examples, R_{10} can be aryl substituted with one or more alkoxy groups.

[0011] In yet another aspect, the present disclosure provides a compound of Formula II:



or pharmaceutically acceptable salt thereof, wherein: R_5 is heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents; R_{12} is $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; and with the proviso that R_5 is not imidazole.

[0012] In some cases, R_5 can be heteroaryl. In some examples, R_5 can be pyridyl. For example, R_5 can be 3-pyridyl. In other examples, R_5 can be triazole. In other cases, R_5 can be arylalkyl. In yet other cases, R_5 can be cycloalkenyl. In further cases, R_5 can be alkoxyalkyl. In some examples, R_{12} can be $-CO_2H$ or $-CH_2CO_2H$.

[0013] In a further aspect, the present disclosure provides a pharmaceutical composition comprising one or more compounds or pharmaceutically acceptable salts of the present disclosure and a pharmaceutically acceptable excipient, carrier or diluent.

[0014] In one aspect, the present disclosure provides a method of treating cancer, a disease or a condition in a subject in need thereof, comprising: administering to the subject an effective amount of a compound, pharmaceutically acceptable salt or composition of the present disclosure.

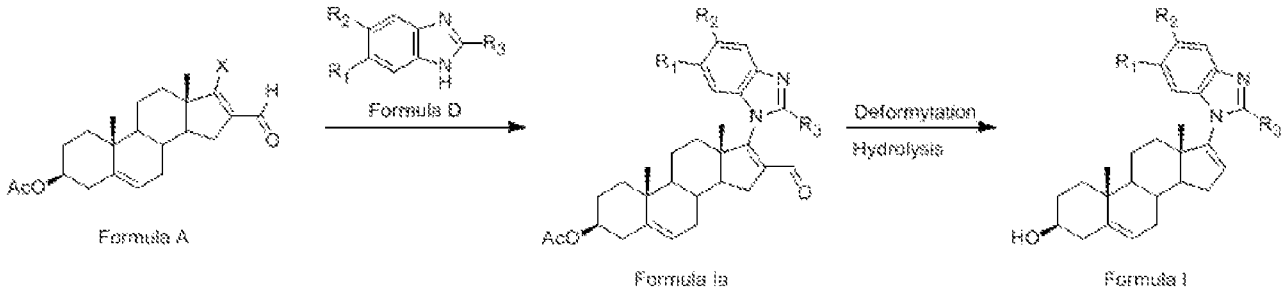
[0015] In some cases, the method can further comprise administering to the subject an effective amount of an anti-androgen, a CYP 17 inhibitor, a luteinizing hormone-releasing hormone agonist, a drug for preventing androgen production, an estrogen, or a chemotherapy drug. In some cases, the compound, pharmaceutically acceptable salt or composition is administered in combination with a hormone therapy, a chemotherapy, a radiation therapy, an immunotherapy, or surgery. In further cases, the cancer, the disease or the condition can be selected from prostate cancer, breast cancer, ovarian cancer, urogenital cancer, or prostate hyperplasia.

[0016] In another aspect, the present disclosure provides a method for inhibiting androgen receptor activity in a subject in need thereof, comprising administering to the subject an effective amount of a compound, pharmaceutically acceptable salt or composition of the present disclosure

[0017] In yet another aspect, the present disclosure provides a method for inhibiting androgen receptor activity in a cell, comprising contacting the cell with an effective amount of a

compound, pharmaceutically acceptable salt or composition of the disclosure, and thereby inhibiting androgen receptor activity in the cell.

[0018] In one aspect, the present invention provides a method for synthesizing a compound or pharmaceutically acceptable salt of Formula I, comprising the steps of:

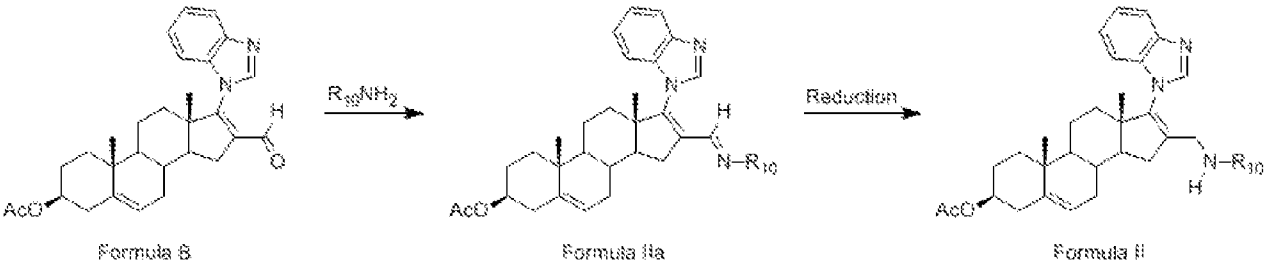


a. allowing a compound of Formula A to react with a benzimidazole of Formula D under conditions that are effective for synthesizing a compound of Formula Ia; and
b. deformylating and hydrolyzing the compound of Formula Ia;

wherein X can be halo; each of R_1 and R_2 can be independently hydrogen, alkoxy, or CN; R_3 can be hydrogen or halo; and wherein at least one of R_1, R_2, R_3 can be not hydrogen.

[0019] In some cases, the compound of Formula Ia is deformylated with a Pd catalyst.

In another aspect, the present disclosure provides a method for synthesizing a compound or pharmaceutically acceptable salt of Formula II, comprising the steps of:

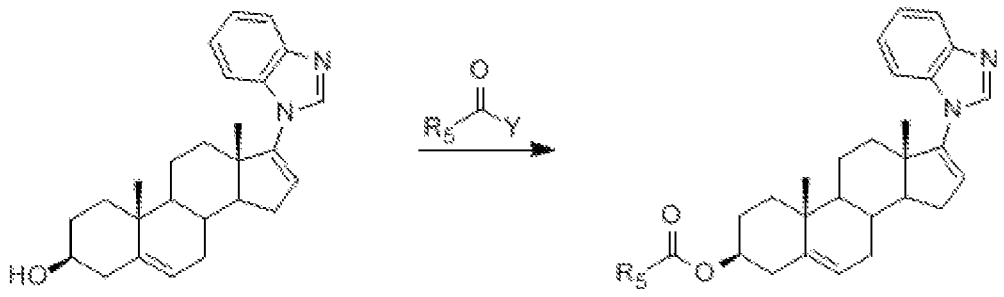


a. allowing a compound of Formula B to react with a substituted amine $R_{10}NH_2$ under conditions that are effective for synthesizing a compound of Formula IIa; and
b. reducing the compound of Formula IIa;

wherein R_{10} can be alkyl or aryl, optionally substituted by one or more R_{11} substituents; and R_{11} can be halogen, alkoxy, or CN.

[0020] In some cases, the compound of Formula IIa can be reduced by $NaBH_4$.

[0021] In yet another aspect, the present disclosure provides a method for synthesizing a compound or pharmaceutically acceptable salt of Formula III, comprising:



Formula C Formula III

allowing a compound of Formula C to react with an acylating agent $R_5C(O)Y$ under conditions that are effective for synthesizing a compound of Formula III; wherein R_5 can be heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents; and R_{12} can be $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; with the proviso that R_5 is not imidazole.

[0022] In some cases, the acylating agent $R_5C(O)Y$ can be an activated ester. In other cases, Y can be $-OC(O)R_5$. In further cases, Y can be R_5.

INCORPORATION BY REFERENCE

[0023] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0025] **Figure 1** illustrates the chemical structures of flutamide (**1**), bicalutamide (**2**), abiraterone acetate (Zytiga, **3a**), abiraterone alcohol (**3b**), TAK-700 (Orteronel, **4**), VN/124-1 (TOK-001 or Galeterone, **5**), MDV3100 (Enzalutamide, **6**) and ARN-509 (**7**).

[0026] **Figure 2** illustrates the chemical structures of dihydrotestosterone (DHT, **8**), metribolone (R1881, **9**), fulvestrant (**10**) and GW5638 (**11**).

[0027] **Figure 3** provides a stereo view of the binding mode of **5** (cap, green) in the active site hAR.

[0028] **Figure 4** summarizes the effects of the compounds on dihydrotestosterone (DHT)-stimulated transcription of AR.

[0029] **Figure 5A** illustrates the competitive inhibition of [3H]R1881 binding of compounds **2**, **3b**, **5**, **6** and **36** to AR in LNCaP cells; **Figure 5B** illustrates the competitive inhibition of [3H]R1881 binding of compounds **5**, **16**, **36**, **43** and **47** to AR in LNCaP cells.

[0030] **Figure 6A-E** illustrates the differential effect of compounds on suppressing AR expression in LNCaP and CWR22rv1 prostate cancer cells.

[0031] **Figure 7** summarizes the effects of compounds **5**, **32**, **36**, **47** and **48** on: i) cell viability (blue); ii) DHT-induced AR transactivation (green); and iii) AR protein expression following treatment with 15 μM of each compound for 24 h.

[0032] **Figure 8A** provides a stereoview of the binding mode of **47** (cap, brick red) in the active site of AR; **Figure 8B** provides a stereoview of the binding mode of **36** (cap, element color) in the active site of AR.

[0033] **Figure 9** is a synthetic scheme for the synthesis of C-17 benzimidazole compounds.

[0034] **Figure 10** is a synthetic scheme for the synthesis of C-16 substituted compounds.

[0035] **Figure 11** a synthetic scheme for the synthesis of C-3 modified compounds.

DETAILED DESCRIPTION

[0036] Design Strategy

[0037] Using structure activity analysis, a series of novel C-3, C-16 and C-17 galeterone analogs were prepared and evaluated for their effects on the modulation of the androgen receptor (AR).

[0038] On the basis that compound **5** binds to the AR ligand binding domain (LBD) to induce AR degradation, the published data of crystal structures of steroid ligand dihydrotestosterone (DHT, **8**) and of metribolone (R1881, **9**) (**Figure 2**) bound to AR LBD were examined. The hydrogen bonding interaction with Arg752 and Gln711 on one end (polar function at position C-3 of steroidal nucleus) and hydrogen bond to Asn705 and Thr877 on the other end (polar function at C-17 position of steroid scaffold) of the LBD may constitute the most important recognition elements for ligand affinity. Furthermore, previous studies have described the critical role of helix-12 in conformational changes that can induce antagonism in various nuclear receptors. It has been hypothesized that pushing helix 12 into an open conformation is the mechanism leading to antagonism for estrogen receptor alpha (ERα) and other nuclear receptors. Indeed, the distortion of helix-12 in the ERα structure complexed with known ERα down-regulators such as fulvestrant (**10**) and GW5638 (**11**) (**Figure 2**) can be critical for their receptor degradation activity. Following the binding of compound 5 to the LBD of AR, the bulky C-17 benzimidazole (BzIm) group of **5** may cause distortion of helix-12 to induce AR degradation. Modifications that allow for additional interactions between a small molecule and receptor may appear to play key determinants for designing new AR down-regulators with potential clinical

use. Furthermore, synthetic modifications of **5** were considered because the resulting fundamental chemical and physical changes may affect molecular shapes, bond angles, and partition coefficients. Different substituents can have different hydrophobic interactions, size, and electrostatic effects that can influence interaction of a ligand with its target receptor. These rational considerations provided the impetus for the systematic modifications of moieties tethered to C-17, C-16 and C-3 as described below.

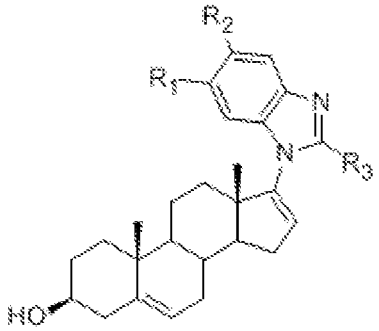
[0039] *C-17 modifications:* To explore the structure activity relationship (SAR) of the C-17 benzimidazole moiety of **5**, analogs with varied ring nitrogen atoms, increased aliphatic/aromatic hydrophobicity, and aromatic substituents to generate compounds **16-22** were designed and synthesized, as outlined in **Figure 9**.

[0040] *C-16 modifications:* Several C-16 substituted analogs (compounds **25**, **28** and **31**) of **5**, tethered with bulky aliphatic and aromatic groups (**Figure 10**) were designed and synthesized.

[0041] *C-3 modifications:* Molecular docking of **5** with human androgen receptor (hAR) ligand binding domain shows that C-3 hydroxyl group forms multiple hydrogen bonding with Arg-752 and Phe764 (**Figure 3**). Arginine is a polar hydrophilic amino acid which contains a positively charged guanidine group. On the basis of the hypothesis that any substitution at C3 which increases interaction with Arg752 may increase AR down-regulating activity, various C-3 modified compounds were designed and synthesized (**33-49**, **Figure 11**).

[0042] Compounds and Pharmaceutically Acceptable Salts

[0043] In one aspect, the present disclosure provides a compound of Formula I:

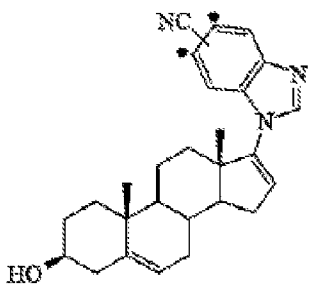


or pharmaceutically acceptable salt thereof,

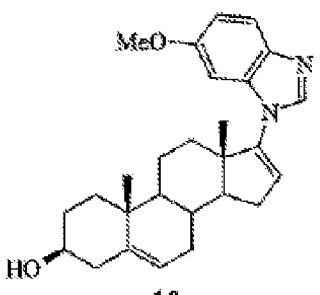
wherein each of R_1 and R_2 is independently hydrogen, alkoxy, or CN; R_3 is hydrogen or halo; and wherein at least one of R_1, R_2, R_3 is not hydrogen.

[0044] In some cases, R_1 can be CN. In other cases, R_2 can be CN. In yet other cases, R_1 can be alkoxy (e.g. methoxy). In yet other cases, R_3 can be halo (e.g. chloro).

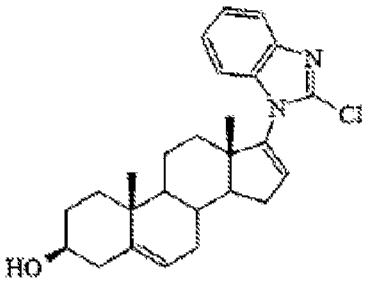
[0045] Exemplary compounds of Formula I include but are not limited to:



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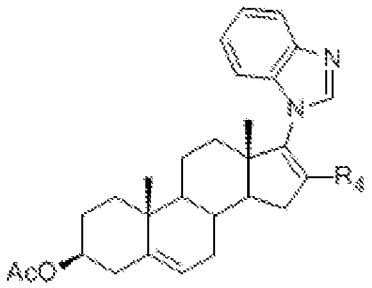


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[0046] In another aspect, the present disclosure provides a compound of Formula II:

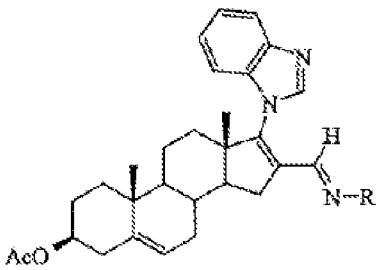


or pharmaceutically acceptable salt thereof,

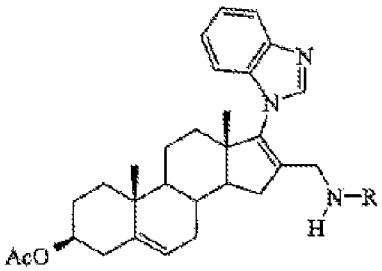
wherein R_4 is -CNHR$_{10}$ or -C=NR$_{10}$; R$_{10}$ is alkyl or aryl, optionally substituted by one or more R$_{11}$ substituents; and R$_{11}$ is halogen, alkoxy, or CN.

[0047] In some cases, R_4 can be -CNHR$_{10}$. In other cases, R_4 can be –C=NR$_{10}$. In some examples, R$_{10}$ can be alkyl (e.g. isopentyl). In other examples, R$_{10}$ can be aryl (e.g. phenyl). In further examples, R$_{10}$ can be further substituted with one or more alkoxy groups (e.g. dimethoxy).

[0048] Exemplary compounds of Formula II include but are not limited to:

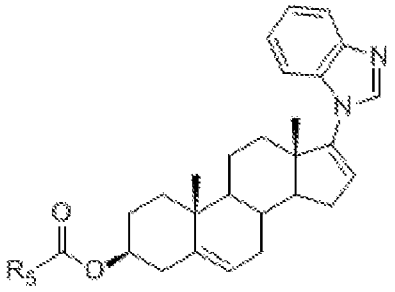


23, R = isopentyl
26, R = phenyl
29, R = 3,4-dimethoxy benzene



24, R = isopentyl
27, R = phenyl
30, R = 3,4-dimethoxy benzene

[0049] In yet another aspect, the present disclosure provides a compound of Formula III:

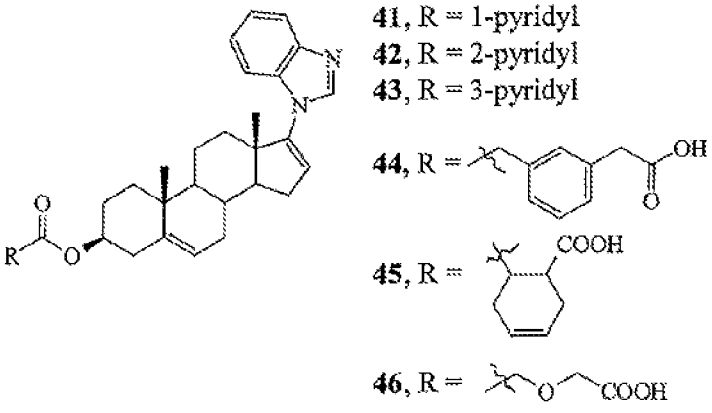


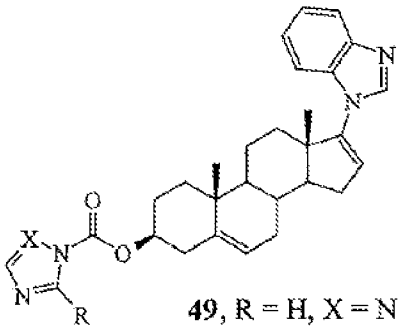
or pharmaceutically acceptable salt thereof,

wherein R_5 is heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents; R_{12} is $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; with the proviso that R_5 is not imidazole.

[0050] In some cases, R_5 can be heteroaryl. In some examples, R_5 can be pyridyl (e.g. 1-pyridyl, 2-pyridyl, 3-pyridyl). In other examples, R_5 can be triazole. In other cases, R_5 can be arylalkyl (e.g. benzyl). In yet other cases, R_5 can be cycloalkenyl (e.g. cyclohexenyl). In further cases, R_5 can be alkoxyalkyl (e.g. methoxymethyl). In various examples, R_{12} can be $-CO_2H$ or $-CH_2CO_2H$.

[0051] Exemplary compounds of Formula III include but are not limited to:

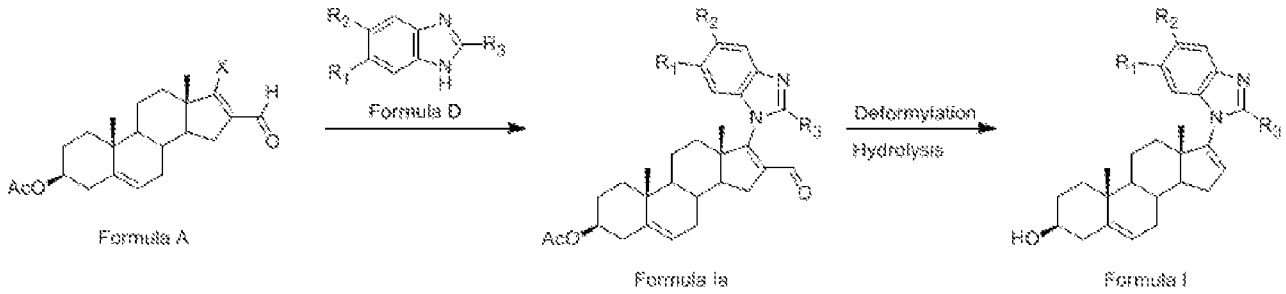




[0052] Reaction Schemes

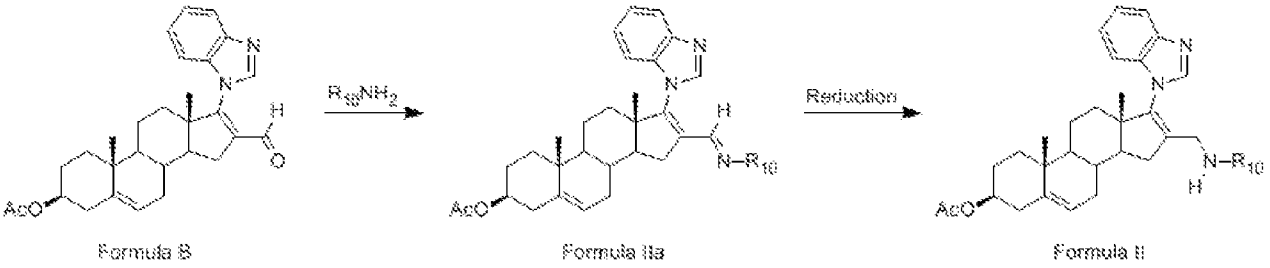
[0053] The compounds and pharmaceutically acceptable salts disclosed herein may be prepared by the routes described below. Materials used herein are either commercially available or prepared by synthetic methods generally known in the art. These schemes are not limited to the compounds listed or by any particular substituents employed for illustrative purposes. Numbering does not necessarily correspond to that of claims or other tables.

[0054] In one aspect, the present disclosure provides a method for synthesizing a compound of Formula I by allowing a compound of Formula A to react with a benzimidazole of Formula D under conditions that are effective for synthesizing a compound of Formula Ia:



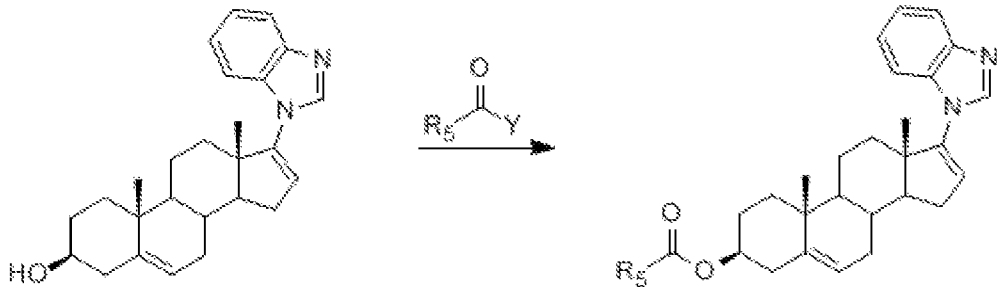
wherein X can be halo; each of R_1 and R_2 can be independently hydrogen, alkoxy, or CN; R_3 can be hydrogen or halo; and wherein at least one of R_1, R_2, R_3 is not hydrogen. The compound of Formula A can react with the benzimidazole under basic conditions. The compound of Formula Ia can then be deformylated and hydrolyzed to afford the compound of Formula I. In some examples, the deformylation can be in the presence of a catalyst. For example, the catalyst can be a Pd catalyst (e.g. 10% Pd on carbon). In some examples, the hydrolysis can be performed in the presence of an aqueous base.

[0055] In another aspect, the present disclosure provides a method for synthesizing a compound of Formula II by allowing a compound of Formula B to react with a substituted amine $R_{10}NH_2$ under conditions that are effective for synthesizing a compound of Formula IIa:



wherein R_{10} can be alkyl or aryl, optionally substituted by one or more R_{11} substituents; and R_{11} can be halogen, alkoxy, or CN. The compound of Formula IIa can then be reduced to afford the compound of Formula II. In some cases, the compound of Formula IIa can then be reduced by a reducing agent (e.g. $NaBH_4$).

[0056] In yet another aspect, the present disclosure provides a method for synthesizing a compound of Formula III allowing a compound of Formula C to react with an acylating agent $R_5C(O)Y$ under conditions that are effective for synthesizing a compound of Formula III:



Formula C Formula III

wherein R_5 can be heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents; and R_{12} can be $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; with the proviso that R_5 is not imidazole. In some cases, the acylating agent $R_5C(O)Y$ can be an activated ester (e.g. Y = DMAP). In other cases, the acylating agent $R_5C(O)Y$ can be an acid anhydride (e.g. Y = $-OC(O)R_5$). In yet other cases, Y can be R_5 (e.g. R_5 = triazole).

[0057] Compositions and Methods

[0058] The present disclosure also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and one or more of the compounds or salts discussed above. Suitable pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. The choice of carrier will be determined, in part, by the particular composition and by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of the pharmaceutical compositions of the present invention.

[0059] The present disclosure also relates to method of treating diseases or conditions, such as cancer or other urogenital diseases and conditions, including, without limitation, breast cancer, prostate cancer, other urogenital cancers, prostate hyperplasia, or other androgen-related diseases or conditions, by administering to a subject in need thereof an effective amount of a compound or salt in accordance with the present disclosure. The term "treating" is used conventionally, e.g., the management or care of a subject for the purpose of combating, alleviating, reducing, relieving, improving, etc., one or more of the symptoms associated with the prostate disease. Examples of prostate diseases that can be treated include, e.g., prostatic hyperplasia (BPH), and prostate cancer (e.g., prostatic adenocarcinoma). The treatment can be prophylactic or therapeutic. "Prophylactic" refers to any degree in inhibition of the onset of a cellular disorder, including complete inhibition, such as in a patient expected to soon exhibit the cellular disorder. "Therapeutic" refers to any degree in inhibition or any degree of beneficial effects on the disorder in the mammal (e.g., human), e.g., inhibition of the growth or metastasis of a tumor.

[0060] One skilled in the art will appreciate that suitable methods of administering a compound or salt of the present disclosure to an animal, e.g., a mammal such as a human, are known. Although more than one route can be used to administer a particular composition, a particular route can provide a more immediate and more effective result than another route.

[0061] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of one or more compound or salt of this disclosure dissolved in a diluent, such as water or saline, (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules, (c) suspensions in an appropriate liquid, and (d) suitable emulsions.

[0062] Tablet forms can include one or more of lactose, mannitol, cornstarch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically acceptable and compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

[0063] The compound or salt of the disclosure, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, hydrofluorocarbon (such as HFC 134a and/or 227), nitrogen, and the like.

[0064] Formulations suitable for parenteral administration include aqueous and non-aqueous solutions, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0065] The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to affect a therapeutic response in the animal over a reasonable time frame. The specific dose level and frequency of dosage may vary, depending upon a

variety of factors, including the activity of the specific active compound, its metabolic stability and length of action, rate of excretion, mode and time of administration, the age, body weight, health condition, gender, diet, etc., of the subject, and the severity of, for example, the prostate cancer or hyperplasia. Any effective amount of the compound can be administered, e.g., from about 1 mg to about 500 mg per day, about 50 mg to about 150 mg per day, etc. In one embodiment of this invention, a suitable dosage for internal administration is 0.01 to 100 mg/kg of body weight per day, such as 0.01 to 35 mg/kg of body weight per day or 0.05 to 5 mg/kg of body weight per day. A suitable concentration of the compound in pharmaceutical compositions for topical administration is 0.05 to 15% (by weight), preferably 0.02 to 5%, and more preferably 0.1 to 3%. The compound or salt of this disclosure can be administered in such dosages in any form by any effective route, including, e.g., oral, parenteral, enteral, intraperitoneal, topical, transdermal (e.g., using any standard patch), ophthalmic, nasally, local, non-oral, such as aerosal, spray, inhalation, subcutaneous, intravenous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal, etc.

[0066] As discussed above, the compound or salt of the present disclosure can be administered alone, or in combination with any ingredient(s), active or inactive, such as with a pharmaceutically acceptable excipient, carrier or diluent. The compound or salt of the present disclosure can also be used in combination with other cancer treatments and drugs. For example, the compound or salt of this disclosure can be used as a part of or in combination with known cancer treatments such as hormone therapy, chemotherapy, radiation therapy, immunotherapy, and/or surgery. In one embodiment of this invention, one or more of the compounds or salts described above is/are used in combination with one or more known and available drugs or other compounds. Exemplary drugs and/or hormones for use in combination with the compounds or salts of this invention for treating cancer or other conditions or diseases discussed above include, without limitation, anti-androgonens such as flutamide and nilutamide; a CYP17 inhibitor such as abiraterone; luteinizing hormone-releasing hormone agonists such as leuprolide, goserelin and buserelin; drugs that prevent the adrenal glands from making androgens such as ketoconazole and aminoglutethimide; and estrogens. Other suitable and exemplary cancer drugs, common for use in chemotherapy, include, without limitation, cyclophosphamide, methotrexate, 5-Fluorouracil (5-FU), doxorubicin, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mechlorethamin, melphalan, procarbazine, bleomycin, doxorubicin, idarubicin mitoxantrone, chlorodeoxyadenosine, cytarabine, fludarabine, 6-mercaptopurine, methotrexate, 6-thioguanine, pentostatin, etoposide, gemcitabine, steroid creams, coritcosteroids, prednisone, and dexamethasone.

[0067] The compounds or salts of this disclosure can be administered to a patient at any time as determined by the treating physician. For example, the compounds or salts of this disclosure can be administered for treating a patient during one or more of Stages II-IV of the cancer.

[0068] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[0069] Example 1. Overall Synthetic Approach of Androgen Receptor Down-Regulating Agents

[0070] In this study, twenty six novel compounds were synthesized as outlined in **Figure 9** (for C-17 modified series), **Figure 10** (C-16 modified series), and **Figure 11** (C-3 modified series).

[0071] The preparation of new 17-hetereoaryl substituted compounds (**16-22**) from the key intermediate, 3β-acetoxy-17-chloro-16-formylandtrosta-5,16-diene (**13**) followed the sequence: 17-heteroaryl-16-formyl intermediate → 16-deformylated intermediate → 3-deacetylated final product (not shown in **Figure 9**), similar to the synthetic route to compound **5** outlined in **Figure 9**. The key intermediate in the synthesis of all the compounds, **13**, was prepared following a Vilsmeier-Haack reaction of the commercially available 3β-acetoxyandrost-5-en-17-one (**12**) with phosphoryl chloride ($POCl_3$) and dimethylformamide (DMF).

[0072] For the synthesis of 3β-acetoxy-16-formyl-17-1*H*-heteroaryls (**14, 17a**, **18a**, **19a**, **20a**, and **22a**), the corresponding heteroaryls were each treated with **13** in the presence of K_2CO_3 in DMF at approximately 80 °C to give the desired intermediates (structures of intermediates not shown except **14**) in near quantitative yields. However, because of weak basicity of indole, we used indole-3-carbaldehyde instead for the synthesis of 17-indole-3-carbaldehyde (**16a**) intermediate following the same procedure with excellent yield. Attempts to condense 6-chloropurine with **13** in the presence of K_2CO_3 in DMF resulted in inseparable N^9/N^7 isomers (~ 6/4 ratio as indicated by TLC) in very low yield. Therefore, a N^9-purine alkylation procedure was used, in which **13** was reacted with 6-chloropurine in presence of tetrabutylammonium fluoride (TBAF) in THF at 50 °C to give the desired intermediate (**21a**) in excellent yield. TLC analysis indicated that N^7-purine alkylation was almost negligible and the N^9-purine was easily

purified following recrystallization in ethanol. The positional isomers of the 16-formyl derivatives (6-methoxy-BzIm **19a1** and 5-methoxy-BzIm **19a2**) were separated at this stage and their structures were confirmed on the basis of reported aromatic proton resonances for related 5- and 6-methoxy benzyl compounds.

[0073] Various attempts to separate positional isomers of 5(6) nitrile-benzimidazole intermediates of compound **18** at all stages were unsuccessful. The 5(6)-nitrile-benzimidazole and 2,3-diaminonaphthalene required for synthesis of **18a** and **20a** were synthesized starting from 3,4-diaminobenzonitrile and benzo[f]benzimidazole respectively by refluxing with formic acid. The 16-formyl intermediates (**14**, **17a** – **21a**; only structure of **14** shown) were each smoothly deformylated with 10% palladium on activated charcoal (Pd/C) in refluxing benzonitrile to give the corresponding deformylated compounds **15**, **17b**, **18b**, **19b**, **20b** and **21b,** respectively (structures not shown except **15**) in high yields. Similarly, the two formyl groups of 17-indole-3-carbaldehyde intermediate (**16a**) were deformylated with 10% Pd/C as described above with good yield to give **16b**. Deformylation of **22a** was achieved by refluxing with readily available chlorotris(triphenylphosphine) rhodium(I) in toluene to give **22b** in low yield.Unexpectedly, the 5-methoxy-16-formyl derivative **19a2** did not undergo deformylation using both methods. Hydrolysis of **15**, **16b-22b** with 10% methanolic-KOH gave target compounds **5**, **16**, **17**, **18**, **19**, **20**, **21** and **22**, respectively in high yields.

[0074] The C-16 substituted compounds were synthesized starting from **14** as illustrated in **Figure 10**. The intermediate imines **23, 26** and **29** were synthesized by refluxing *i*-pentylamine, aniline and 3,4-dimethoxyaniline, respectively with **14** in ethanol in presence of molecular sieves. Subsequent reduction of these imines with sodium borohydride ($NaBH_4$) in ice-cold methanol gave 3-acetoxy-16-alkylamine intermediates **24, 27** and **30**, respectively. Following hydrolysis of the 3β-acetoxy groups in compounds **24, 27** and **30,** the desired 16-substituted compounds, **25, 28**, and **31**, respectively, were obtained in excellent yields.

[0075] The C-3 modified compounds were synthesized as depicted in **Figure 13**. Δ^4-3-Oxo compound (**32**) was synthesized via modified Oppenauer oxidation of **5** by using *N*-methylpiperidone and aluminum isopropoxide. Oxidation of **5** with Dess-Martin periodinane in dichloromethane (DCM) afforded the Δ^5-3-oxo compound **33** in 70% yield. The mesyl (**34**) and tosyl (**35**) derivatives of **5** were readily synthesized by reacting with methanesulfonyl and toluenesulfonyl chloride, respectively. The C-3 oxime derivatives (hydroxime: **36**, phenyloxime: **37**, methyloxime: **38** and benzyloxime: **39**) were obtained by refluxing ketone (**32**) with the respective substituted hydroxylamine hydrochloride, using ethanol/methanol solvent mixture in presence of sodium acetate. Of all oximes, only biologically active oxime (**36**) was further

purified to separate *E*- and *Z*- geometrical isomers by combined purification methods (column chromatography, preparative TLC, and recrystallization). Addition of MeLi to the C-3-keto group of **32** afforded two distereomeric (3α- and 3β) alcohols (**40**) which we did not separate due to modest biological activity.

[0076] The ester derivatives (**41** - **46**) of **5** were synthesized from **5** by two different methods as described below. The pyridinecarboxylates (**41**, **42** and **43**) and carboxylate of 1,3-phenyldiacetic acid (**44**) of **5** were prepared using the mixed anhydride method via condensations with the respective anhydrides (pyridinecarboxylic acid/1,3-phenyldiacetic acid and 2-methyl-6-nitrobenzoic) in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine (TEA) with varying yields (39-90%). The ester **45** (72% yield) and **46** (28% yield) were synthesized by refluxing 1,2,3,6-tetrahydropthalic and diglycolic anhydrides respectively with **5** in the presence of DMAP in pyridine. Finally the carbamates (imidazole: **47**, 2-mehtylimidazole: **48** and 1,2,4-triazole: **49**) were synthesized in modest to high yield (67-80%) by reacting **5** with 1,1-carbonylbis(2-methylimidazole) (CDI) and carbonylditriazole (CDT), respectively in acetonitrile and DMC solvent mixture. The compounds described were rigorously characterized by physical and spectroscopic (IR, ^{1}H and ^{13}C NMR, and HRMS) analysis (see Example 8 for details). Most of the novel compounds were then subjected to *in vitro* biological activity studies as described in detail in the following sections.

[0077] Example 2: Biological Effects of Compounds on Transcriptional Activation of Androgen Receptor

[0078] After synthesizing the compounds, a luciferase reporter assay was used to determine whether the novel compounds also affect AR transcriptional activation (screening assay). Specifically, a luciferase experiment utilizing LNCaP cells dual transfected with the probasin luciferase reporter construct ARR2-luc and the Renilla luciferase reporting vector pRL-null was used.

[0079] The LNCaP cells were purchased from American Type Culture Collection- ATCC (Rockville, MD, USA). Cells were maintained in ATCC recommended culture media with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA, USA) and 1% penicillin/streptomycin (invitrogen). Cells were grown as a monolayer in T75 or T150 tissue culture flasks in a humidified incubator (5% CO_2, 95% air) at 37 ^{o}C. CWR22rv1 cells are a gift from Dr. Marja Nevalainen of Thomas Jefferson University, Philadelphia.

[0080] For the transcriptional activation luciferase assay, LNCaP cells were transferred to steroid-free medium 3 days before the start of the experiment and plated at 1 x 10^5 per well in

steroid-free medium. The cells were dual transfected with ARR2-Luc and the Renilla luciferase reporter vector pRL-null. After a 24-h incubation period at 37 °C, the cells were incubated in fresh phenol red–free RPMI 1640 containing 5% charcoal-stripped fetal bovine serum and treated with 10 nmol/L dihydrotestosterone, ethanol vehicle, and/or the selected compounds in triplicate. After an 18-h treatment period, the cells were washed twice with ice-cold Dulbecco's PBS and assayed using the Dual Luciferase kit (Promega) according to the manufacturer's protocol.Cells were lysed with 100 μL of luciferase lysing buffer, collected in a microcentrifuge tube, and pelleted by centrifugation. Supernatants (20 μL aliquots) were transferred to corresponding wells of opaque 96-well multiwall plates. Luciferase Assay Reagent was added to each well, and the light produced during the luciferase reaction was measured in a Victor 1420 scanning multiwell spectrophotometer (Wallac, Inc.). After measurement, Stop and Glo reagent (Promega) was added to quench the firefly luciferase signal and initiate the Renilla luciferase luminescence. Renilla luciferase luminescence was also measured in the Victor 1420.The results are presented as the fold induction (i.e., the relative luciferase activity of the treated cells divided by that of the control) normalized to that of the Renilla.

[0081] Luciferase expression was increased by approximately 100-fold after 10 nM DHT treatment for 24 hours. The ability of the novel compounds (10 μM) to affect DHT mediated AR transcription was assessed. **Figure 4** shows the effects of our most potent compounds. These compounds were able to substantially inhibit DHT mediated transcription, with inhibition ranging from ~65-100%.

[0082] Example 3: Androgen Receptor Binding Assays

[0083] In addition to AR down-regulation, compound **5** reduces androgen action through inhibition of androgen binding and subsequently reduces AR mediated transcriptional activity. Whole cell competitive binding assays with the synthetic ligand methyltrienolone (R1881) were used to assess the AR binding affinities of the novel compounds in comparison to **5**, and the FDA approved anti-androgens bicalutamide (**2**) and enzalutamide (**6**), and abiraterone alcohol (**3b**) as shown in **Figure 5A**.

[0084] The androgen receptor competitive binding assays were performed with the synthetic androgen methyltrienolone [^{3}H]R1881. Wells in 24-well multiwell dishes were coated with poly-l-lysine (0.05 mg/ml) for 30 minutes, rinsed with sterilized, distilled water, and dried for 2 hours. To determine the kinetics of [^{3}H]R1881 binding to the LNCaP AR cells were plated (2-3 x 10^5 cells/well) in 24 well multiwell dishes in steroid-free medium and allowed to attach. The following day the medium was replaced with serum-free, steroid free RPMI supplemented with

0.1 % BSA and containing [^{3}H]R1881 (0.01-10 nM) in the presence or absence of a 200 fold excess of cold DHT, to determine nonspecific binding, and 1μM triamcinolone acetonide to saturate progesterone and glucocorticoid receptors. Following a 2 hour incubation period at 37˚C, cells were washed twice with ice-cold DPBS and solubilized in DPBS containing 0.5 % SDS and 20 % glycerol. Extracts were removed and cell associated radioactivity counted in a scintillation counter. The data was analyzed, including K_d and B_{max} determination, by nonlinear regression using Graphpad Prism software (GraphPad Software, Inc, San Diego, CA). When the concentration of [^{3}H]R1881 required to almost saturate AR in both cell lines was established (5.0 nM), the ability of the test compounds (1 nM-10 μM) to displace [^{3}H]R1881 (5.0 nM) from the receptors was determined as described above. The IC_{50} of each compound was determined by nonlinear regression with Graphpad Prism software (GraphPad Software, Inc, San Diego, CA).

[0085] The compounds with the greatest ability to displace [^{3}H]R1881 were **5** and **6**, with IC_{50} values of 670 nM and 915 nM, respectively. Compound **2** was slightly weaker with an IC_{50} of 1.4 μM. We did not calculate the IC_{50} value of **3b** because of the *shallow steepness* of the AR binding curve, a phenomenon which indicates interaction of **3b** with *more than one receptor population*. A recent study also noted unusual AR binding characteristics with **3b**.[49] Interestingly, AR-binding assays using LNCaP cells shows that **6** was not as potent as previously reported for assays using LNCaP cells transfected with wild type AR[10] and was not significantly different from the binding affinity of bicalutamide (**2**). The new compounds were not as potent as **5** at inhibiting androgen binding at the concentrations tested (**Figure 5B**). For example, compound **36** showed the strongest inhibition of [^{3}H]R1881 binding of all the new compounds tested (~40%) at 10 μM. At 30 μM, **36** inhibited [^{3}H]R1881 binding to by ~80%, while **43** inhibited by ~53%. The most effective AR antagonist, **47**, did not strongly compete for the AR binding site, exhibiting only 20% displacement at a 30 μM concentration. It is relevant to state here that other investigators have recently reported the discovery of small-molecule androgen receptor down-regulators and anti-androgens that bind weakly to the AR.

[0086] Example 4: Effects on AR Down-Regulation, Transactivation and Anti-Proliferative Activity

[0087] To explore the AR down-regulation effects, LNCaP cells were treated with each of the compounds (**5**, **6**, **16-20**, **25**, **28**, **32**, **34**, **36**, **38**, **39**, **42**, **43**, **47-49**) of interest for 24 h followed by western blot analysis. As shown in **Figures 6A-C** most of the new compounds significantly caused AR down-regulation in LNCaP cells, with compound **47** being the most potent and proved to be greater than 8-fold more active than compound **5** at 15 μM. The ability of

compounds **5** and **47** to suppress AR expression was further demonstrated by immunocytochemical analysis (**Figure 6D**).

[0088] For the immunocytochemical analysis: LNCaP cells were plated in 8 chamber vessel tissue culture treated glass slide (0.025 X 10^6 cells/well), for 12h and then treated with 5uM of VN/124-1 or VNPT55 for 48h. Cells were washed twice with PBS and fixed in 3.7% formaldehyde for 10mins and permeabilized with 0.25% triton in PBS for another 5mins after several washes. Cells blocked with 5% BSA with 0.5% NP40 in PBS and incubated with anti-AR (1:600 dilution; cell signaling) in 2.5% BSA in PBS overnight. Cells were incubated for 1h with secondary antibody Alexa Fluor 488-conjugate anti-rabbit IgG(H+L) at 1:1000(Cell Signaling) and nuclear counterstain for 5mins (DAPI at 1:5000). All images were taken using the Nikon TE2000 microscope.

[0089] As shown in **Figure 6D**, exposure of LNCaP cells to 5 μM of compounds **5** and **47** for 48 h led to significant decrease in AR levels in the nucleus, in a fashion that mimics the western blot analysis data (*vide supra*). These data are similar to those reported for analogs of ciglitazone, a novel class of AR-ablative agents.

[0090] Due to the potential implication of AR splice variants lacking the ligand-binding domain (truncated AR) in driving the progression of CRPC, the effects of the compounds on the down-regulation of AR-3 (also called AR-V7) were determined. As shown in **Figure 6E**, galeterone (**5**) and some of our new compounds, **31**, **32**, **36** and **47** caused significant down-regulation of both full-length and truncated AR in CWR22rv1 prostate cancer cell line. Interestingly, AR-3 was more susceptible to the compounds than the full-length AR in this cell line. In contrast, MDV3100 did not affect the expression levels of either full-length or splice variant forms of AR. A number of natural products and related analogs have been shown to degrade both full-length and truncated AR in several human prostate cancer cell lines. However, except for the curcumin analog, ASC-J9 that possesses excellent drug-like properties, most of these compounds are poor drug candidates because of modest potencies and/or toxic nature. If adequately developed, the unique AR depleting agents provided in the present disclosure may be more effective against CRPC than agents that obligatorily bind to specific region(s) of AR to elicit inactivation of AR.

[0091] To determine whether AR down-regulation or AR transcriptional deactivation (AR inactivation) was contributing to the anti-proliferative activity, LNCaP cells were treated with 15μM of selected active compounds (**5**, **36**, **32, 47** and **48**,) for 24 hours and cell viability, AR transcriptional (luciferase) assay and AR western blot analysis were performed. As shown in **Figure 7,** the down-regulation of AR and inhibition of AR mediated transcription occurs before cell growth inhibition, which suggest that compound-induced AR inactivation contributes to

their anti-proliferative activities. These compounds also induced significant PARP cleavage in LNCaP and CWR22rv1 cells which suggest their abilities to induce apoptosis.

[0092] Example 5. CYP17 Inhibition Studies

[0093] Some compounds were evaluated for their ability to inhibit CYP17 enzyme. A truncated version of human CYP17A1 (CYP171dH) was expressed in *E. coli* and then purified to homogeneity. IC_{50} values of the compounds were determined from dose-response curves and are listed in **Table 1**.

Table 1: IC_{50} values of select compounds for inhibition of CYP17

Compounds	IC_{50} (μM)[a]
16	130
36	258
47	122
48	93.7
For comparison	
Abiraterone alcohol (**3b**)	0.206
Galeterone (**5**)	0.752
VN/85-1	0.125

[a] IC_{50} value is the concentration of inhibitor to inhibit the CYP17 enzyme activity by 50%, each in duplicate. IC_{50} values were each determined from dose-response curves.

[0094] The IC_{50} values of abiraterone alcohol (3b, a CYP17 inhibitor recently approved for prostate cancer therapy), galaterone and 3β-hydroxy-17-(1*H*-imidazole-1-yl)androsta-5,16-diene (VN/85-1, structure not shown, believed to be the most potent CYP17 inhibitor) were also determined in the same assay system for comparison (used as positive controls). As expected, these new compounds (**16**, **36**, **47** and **48**) with IC_{50} values in the high micromolar range (93.7 – 258 μM) were weak inhibitors of CYP17, reinforcing the previously established structural requirements for potent steroidal CYP17 inhibitors, including, no tolerance of bulky moieties at C-3 and appropriately positioned C-17 heterocyclic heteroatom. As expected, the well-established CYP17 inhibitors exhibited exquisite inhibition of the enzyme with IC_{50} values in the nanomolar range (**Table 1**).

[0095] Example 6. Anti-proliferative (anti-cancer) and androgen receptor down-regulating activities: Structure activity relationships (SAR)

[0096] In view of the hypothesis that the extent of AR degradation induced by compound **5** and the new analogs may correlate with their ability to inhibit proliferation of prostate cancer cells (LNCaP), these two activities were assessed using Western blot analyses and MTT assays.

[0097] For the Western blot analyses, LNCaP or CWR22v1 prostate cancer cells were cultured. The cells were then treated with the indicated compounds and whole cell lysates were prepared using RIPA lysis buffer (Sigma Aldrich) and protease and phosphatase inhibitors (Sigma Aldrich). All of the antibodies were ordered from cell signaling technology. Protein content was determined using the Bradford Assay (Bio-Rad, Hercules, CA, USA). Protein was subjected to SDS–PAGE and transferred onto nitrocellulosemembrane. Membranes were then incubated with secondary antibody (cell signaling technology) at room temperature for 1 hour. Bands were visualized by chemiluminescence (Millipore). Protein expression was normalized to β-actin and densitometry was carried out using Image J or ImageQuant 5.0 (Molecular Dynamics, Sunnyvale, CA, USA). CWR22Rv1 cells were used for endogenous levels of splice variant AR-3. Protein levels were analyzed with respective antibodies; full length AR and β-actin antibodies were purchased from cell signaling, antibody specific for splice variant AR-3 was obtained from Dr. Yun Qiu, University of Maryland, School of Medicine, Baltimore.

[0098] For the MTT colorimetric assay, cells were seeded in 96-well plates (Corning Costar) at a density of 5 x 10^3 cells per well. The cells were then allowed to adhere to the plate for 24 hours and treated with various concentrations of compounds dissolved in 95% EtOH. The cells were treated for 7 days with renewal of test compound and media on day 4. On the 7th day, medium was renewed and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (Sigma, St Louis, MO, USA) solution (0.5 mg MTT per ml of media) was added to the medium such that the ratio of MTT: medium was 1:10. The cells were incubated with MTT for 2 hours. The medium was then aspirated and DMSO was added to solubilize the violet MTT-formazan product. The absorbance at 562 nm was measured by spectrophotometry (Biotek Inc.).

[0099] *C-17 modification:*Indole **16** was synthesized and tested to assess the effect of decreased polarity at C17 position, due to absence of N-3 of BzIm ring. Unexpectedly, the compound caused up-regulation of AR (**Figure 6A**) and completely lost anticancer activity (GI_{50} >100 μM, **Table 2**) in comparison to lead compound **5** (GI_{50} = 3.35 μM).

Table 2: GI_{50} values of C-17 modified compounds

Compounds	GI_{50} (μM)[a]
Abiraterone (**3b**)	1.97
Galeterone (**5**)	3.35
MDV3100 (**6**)	5.12
16	>100
17	47.72
18	2.81
19	4.26
20	37.10
21	13.48
22	10.13

[a] The GI_{50} were determined from dose-response curves (by nonlinear regression analysis using GraphPad Prism) compiled from at least three independent experiments, SEM < 10%, and represents the compound concentration required to inhibit cell growth by 50%.

[00100] Increasing the number of nitrogen C-17 heterocycle by substituting with 6-chloropurine (**21**), caused a 4-fold reduction in anti-proliferative activity (GI_{50} = 13.48 μM). Introducing cyano group (**18**) displayed potent anti-proliferative activity (GI_{50} = 2.81 μM), but with diminished AR down-regulation (ARD) activity. Introduction of aliphatic hydrophobicity on BzIm ring by substituting methyl group on 5, 6 position (**17**) resulted into substantial loss of anti-proliferative (GI_{50} = 42.72 μM) and ARD activities, whereas substituting mono methoxy group (**19**) at 6th position of BzIm ring displayed no modulation of ARDA or anticancer activity (GI_{50} = 4.26 μM). Increasing aromatic hydrophobicity by replacing BzIm with naphtho[*2,3-d*]imidazole ring (**20**) caused significant loss of ARDA and anticancer activity (GI_{50} = 19.10 μM). Substituting 2-chloro BzIm (**22**) caused a 3-fold loss in anti-proliferative activity. None of the C17 modified molecules were superior to our lead compound **5**, and this clearly indicates that the BzIm ring at C17 position of lead **5** is essential and optimal for ARDA and anti-proliferative activity.

[00101] *C-16 modification:* Tethering aliphatic hydrophobic groups (isopentyl: **25**); aromatic (benzyl: **28**; dimetoxybenzyl: **31**) to increase bulk at C16 position resulted in significant loss of ARD and anticancer activities (GI_{50s} = 18.31, 22.13 and >100 μM, respectively; **Table 3**).

Table 3: GI_{50} values of C-16 modified compounds

Compounds	GI_{50} (μM)[a]
25	18.31
28	22.13
31	>100

[a] The GI_{50} were determined from dose-response curves (by nonlinear regression analysis using GraphPad Prism) compiled from at least three independent experiments using LNCaP cells, SEM < 10%, and represents the compound concentration required to inhibit cell growth by 50%.

[00102] *C-3 modification:* In an attempt to better understand the role played by OH and O in the ARD/anti-proliferative activities of compounds **5** and **32**, and to possibly achieve enhanced interaction with Arg in the AR ligand biding domain, a number of C-3 modified analogs were designed, synthesized and tested. First, oxidation of **5** or reductive alkylation of **32** to give 3-oxo-Δ^5 compound, **33** and 3-hydroxy-3-methyl compound, **40**, respectively, lead to significant loss (~5-fold) in anti-proliferative activity (**Table 4**).

Table 4: GI_{50} values of C-3 modified compounds

Compounds	GI_{50} (μM)[a]	Compounds	GI_{50} (μM)[a]
32	2.64	**40**	13.34
33	15.96	**41**	NT[b]
34	42.13	**42**	NT[b]
35	47.18	**43**	2.57
36	1.91	**44**	7.78
36E	2.03	**45**	8.22
36Z	1.95	**46**	9.13
37	NT[b]	**47**	0.87
38	3.38	**48**	5.34
39	5. 57	**49**	6.67

[a] The GI_{50} were determined from dose-response curves (by nonlinear regression analysis using GraphPad Prism) compiled from at least three independent experiments, SEM < 10%, and represents the compound concentration required to inhibit cell growth by 50%.
[b] Not tested due to insolubility in ethanol.

[00103] Conversion of compound **5** to the mesyl (**34**) and tosyl (**35**) derivatives also gave compounds with mediocre anti-proliferative activities, with GI_{50} values of 42.13 and 47.18 μM, respectively. On the contrary, introduction of oxime moieties at C-3 yielded compounds (*E/Z* oxime mixtures) with similar or better activities compared to compounds **5** and **32**. Thus, the simple oxime (**36**), and the related methyl- (**38**) and benzyl- (**39**) analogs exhibited GI_{50} values of 1.91, 3.38 and 5.57 μM, respectively. The biological activities of the phenyl oxime (**37**) were not assessed because of its limited solubility in ethanol or DMSO. Considering the promising and superior activity of *E/Z* mixture of oximes **36**, and the possibility that the pure *E* and *Z* had different anti-proliferative activities, it was surprising that **36*E*** and **36*Z*** isomers exhibited similar potencies, with GI_{50} values of 2.03 and 1.95 μM, respectively.

[00104] On the basis of known ester based anticancer drugs, such as docetaxel, cabazitaxel and esters in clinical development such as bevirimat and related analogs, three pyridinecarboxylate derivatives of compound **5**, including **41-43**, were synthesized. Of these compounds, the isonicotinoyl derivative **43** exhibited similar anti-proliferative activity (GI_{50} = 2.57 μM) as **5**. Here again, the biological activities of compounds **41** and **42** were not assessed because of their limited solubilities in ethanol or DMSO. The related analogs tethered to lipophilic ester side chain with a carboxylic acid terminus (**44-46**) exhibited potencies ~2.5-fold worse than compound **5**. Finally, evaluation of C-3 carbamates was performed because of: 1) precedence of drugs with carbamate moieties such as the widely use anthielmintics albendazole, fenbendazole and mebendazole; 2) the added feature of lowering the lipohilicity of compound **5**, which should also increase solubilities and perhaps physiological relevance. Of the three heteroaryl carbamates tested, the imidazoly carbamate **47** with a GI_{50} value of 0.87 μM was shown to be the most active, being ~4-fold superior to compound **5**. Introduction of 2^1-methyl as in carbamate **48** caused a 6-fold decrease in activity relative to **47**, similar to ~8-fold decrease in activity following replacement of the imidazole moiety with 1,2,4-triazole as exhibited by compound **49**.

[00105] Example 7. Docking studies

[00106] As stated in the design strategy section above, the docking studies were based on the well-established molecular determinants responsible for affinity of ligands to the AR. Three compounds (**5**, **36** and **47**) were each docked into active site of AR. R1881 (**9**) was included as a positive control. We found that docked **9** showed similar orientation and interactions as in the published crystal structure of **9** bound to the AR (data not shown). As clearly depicted in **Figure 3**, when compound **5** was modeled in the AR-LBD binding site, **5** made the crucial H-bond

interaction to Arg752 and Phe764 and the steroid scaffold showed hydrophobic interactions with surrounding amino acids similar to interactions of **9** with the AR-LBD. The two nitrogen atoms of the BzIm group of 5 showed no clear interactions with Asn705 and Thr877, unlike the interaction of **9** to Asn705 and Thr877 which occurs through the 17β hydroxyl group. The observed moderate binding affinity of **5** to AR (IC_{50} = 680 nM *versus* IC_{50} = 4 nM for DHT) may be due to subtle albeit favorable hydrophobic/hydrophilic interactions between the BzIm group of **5** and other surrounding amino acid residues in the active site.

[00107] Replacing the C-3-hydroxy group of **5**, with carbonylimidazole to give **47** appears to show better interactions with the key amino acid residues of the hAR LBD (**Figure 8A**). The carbonyl of the carbamate group interacts directly with Gln711 and indirectly with other amino acids (Met745 and Arg752) via one H_2O molecule. The electron cloud on imidazole ring also interacts with Arg752. Similar to the interactions seen with **5**, the two nitrogen atoms of the BzIm group of **47** showed no clear interactions with Asn705 and Thr877. Docking of the 3-oxime compound **36** to the LBD of hAR appear to show different types of interactions in which the oxygen and hydrogen atoms of the oxime show hydrogen bonds with Arg752 and Phe764, respectively (**Figure 8B**). In contrast to the interactions seen with compounds **5** and **47**, the N-3 of the C-17 BzIm moiety exhibited hydrogen bonding with Thr877. It would appear that the spatial arrangement among the hydrogen bonding functional groups and the steroid core of these compounds is the most appropriate for forming the necessary hydrogen bonds and hydrophobic interaction with hAR LBD. Unexpectedly, in spite of the significant hydrogen bonding networks exhibited by these compounds in these docking analyses, no significant experimental binding to AR were observed, except that our lead compound **5** exhibited moderate binding relative to the endogenous AR ligands.

[00108] Example 8. General Synthetic Procedures and Chemical Characterization of Androgen Receptor Down-Regulating Agents

[00109] ***Materials and Methods:***

[00110] Melting points (mp) were determined with a Fischer-Johns melting point apparatus and are uncorrected. Proton magnetic resonance spectra (^{1}H NMR) spectra were recorded in $CDCl_3$ or DMSO-d_6 at 500 or 400 MHz with Me_4Si as an internal standard using a Varian Inova 500 or Bruker 400 MHz spectrometers. ^{13}C NMR spectra were recorded in $CDCl_3$ using Bruker 400 or 500 MHz spectrometers. High-resolution mass spectra (HRMS) were determined on a Bruker 12Tesla APEX-Qe FTICR-MS by positive ion ESI mode by Ms. Susan A. Hatcher, Facility Director, College of Sciences Major Instrumentation Cluster, Old Dominion University, Norfolk, VA. Epiandrosterone acetate, and all other chemicals, reagents were

purchased from Sigma–Aldrich. Dihydrotestosterone (DHT) used in the biological experiments was synthesized. All compounds were stored in the cold (0–8 °C). Silica gel plates (Merck F254) were used for thin-layer chromatography, while flash column chromatography (FCC) was performed on silica gel (230-400 mesh, 60 Å). The preparative TLC performed on Silica gel GF (Analtec 500 microns) plates. Pet ether refers to light petroleum, b.p. 40-60 °C.

[00111] **3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (13):** This compound prepared from 3β-acetoxyandrost-5-en-17-one (Epinadrosterone acetate, **12**) as previously described, provided spectral and analytical data as reported.

[00112] **General method A: Synthesis of 3β-Acetoxy-17-(1*H*-heteroaryl-1-yl)-16-formylandrosta-5,16-diene (14, 16a-18a, 19a1, 19a2, 20a, and 22a):** A 25 mL RB flask equipped with a magnetic stir bar and condenser was charged with 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**13**, 0.38 g, 1 mmol), corresponding heteroaryl (3 mmol) and K_2CO_3 (0.41 g, 3 mmol) in dry DMF (~7.5 mL) was stirred at 80 °C under Ar and monitored by TLC. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (50 mL) and the resulting precipitate was filtered, washed with water and dried to give crude product. Purification by the FCC [petroleum ether/EtOAc/TEA (6:4:0.3)] gave the desired pure compounds. Above listed intermediate compounds were synthesized (using reactants, reagent and solvent ratio), isolated and purified by using this method unless otherwise stated.

[00113] **3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (14)**: Compound **14** prepared by following general Method A, reacting **13** (2.5 g, 6.65 mmol) with benzimidazole (2.35 g, 19.9 mmol) in presence of K_2CO_3 (2.76 g, 19.9 mmol) in dry DMF at 80 °C for 1.5 h. Followed by FCC purification provided pure **14** with identical spectral and analytical data as we previously reported.

[00114] **3β-Acetoxy-17-(3-formyl-1*H*-indol-1-yl)-16-formylandrosta-5,16-diene (16a):** Compound **16a** prepared by following general method A, reacting **13** (1 g, 2.66 mmol) with indole-3-carbaldehyde (0.5 g, 3.44 mmol) in presence of K_2CO_3 (0.5 g, 3.62 mmol) in dry DMF (15 mL) at 80 °C for 8 h. Purification by FCC [petroleum ether/EtOAc (7:3)] gave 1.1 g (85%) of pure **16a**: mp 206-208 °C; IR (Neat) 2935, 2852, 1729, 1665, 1635, 1453, 1374, 1239, 1032, 783 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.01 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 2.06 (s, 3 H, 3β-$OCOCH_3$), 4.65 (dt, J = 12.2, 6.5 Hz, 1 H, 3 α -H), 5.46 (br, 1 H, 6-H), 7.29 (s, 1 H, 2'-H), 7.39 (m, 2 H, aromatic-Hs), 7.80 (d, J = 14.9 Hz, 1 H, aromatic-H), 8.36 (m, 1 H, aromatic-H), 9.58 (br, 1 H, 16-CHO) and 10.15 (s, 1 H, indole-CHO).

[00115] **3β-Acetoxy-17-(5, 6-dimethyl-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (17a)**: Compound **17a** prepared by following general method A, reacting **13** (0.5 g,

1.33 mmol) with 5,6-dimethylbenzimidazole (0.54 g, 4.0 mmol) in presence of K_2CO_3 (0.55 g, 4.0 mmol) in dry DMF (10 mL) at 80 °C for 5 h. Purification by FCC gave 0.46 g (70.7%) of pure **17a**: mp 174-175 °C; IR (Neat) 2941, 2852, 1727, 1672, 1622, 1463, 1487, 1365, 1236, 1029, 897, 843, 717, 657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 3 H, 18-CH_3), 1.16 (br. s, 3 H, 19-CH_3), 2.03 (s, 3 H, 3β-$OCOCH_3$), 2.35 (s, 3 H, aromatic-CH_3) 2.38 (s, 3 H, aromatic-CH_3), 4.64 (m, 1 H, 3 α -H), 5.44 (br, 1 H, 6-H), 7.02 (br. s, 1 H, aromatic-Hs), 7.59 (s, 1 H, aromatic-H), 7.87 (s, 1 H, 2'-H) and 9.60 (s, 1 H, 16-CHO).

[00116] 3*β*-Acetoxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (18a): Compound **18a** prepared by following general method A, reacting **13** (0.5 g, 1.33 mmol) with 5(6)-nitrilebenzimidazole (0.38 g, 2.65 mmol) in presence of K_2CO_3 (0.55 g, 4.0 mmol) in dry DMF (10 mL) at 80 °C for 5 h. Purification by short column [petroleum ether/EtOAc/TEA (6:4:0.1)] gave 0.28 g (43.5%) of pure **18a**: mp 146-147 °C; IR (Neat) 2935, 2226, 1726, 1673, 1470, 1238 1032, 906, 728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 3 H, 18-CH_3), 1.19 (br. s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 4.62 (dt, J = 10.1, 5.3 Hz, 1 H, 3 α -H), 5.44 (br, 1 H, 6-H), 7.61 - 7.96 (m, 3 H, aromatic-H), 8.21 (s, 1 H, 2'-H) and 9.52 (s, 1 H, 16-CHO).

[00117] 3*β*-Acetoxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (19a1) and 3*β*-Acetoxy-17-(5-methoxy-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (19a2): Compound **19a1** and **19a2** prepared by following general method A, reacting **13** (0.5 g, 1.33 mmol) with 5(6)-methoxybenzimidazole (0.59 g, 4.0 mmol) in presence of K_2CO_3 (0.55 g, 4.0 mmol) in dry DMF (10 mL) at 80 °C for 3 h. Purification by FCC [petroleum ether/EtOAc/TEA (7.5:2:0.5)] gave first less polar 6-methoxy derivative (**19a1**) 0.15 g (24%): mp 242-245 °C; IR (Neat) 2935, 1721, 1673, 1502, 1440, 1249, 1220, 1032, 805, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 3 H, 18-CH_3), 1.18 (br. s, 3 H, 19-CH_3), 2.03 (s, 3 H, 3β-$OCOCH_3$), 3.82 (s, 3 H, -OCH_3), 4.62 (dt, J = 11.2, 6.6 Hz, 1 H, 3 α -H), 5.44 (t, 1 H, J = 1.84 Hz, 6-H), 6.70 (m, 1 H, aromatic-H) 6.95 (m, 1 H, aromatic-H), 7.70 (m, 1 H, aromatic-H), 7.87 (s, 1 H, 2'-H) and 9.61 (s, 1 H, 16-CHO). Subsequently more polar 5-methoxy derivative (**19a2**) 0.13 g (20%): mp 228-231 °C; IR (Neat) 2936, 2852, 1722, 1673, 1481, 1341, 1245, 1031, 897, 800, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 3 H, 18-CH_3), 1.16 (br. s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 3.88 (s, 3 H, -OCH_3), 4.63 (m, 1 H, 3 α -H), 5.44 (d, J = 5.6 Hz, 1 H, 6-H), 6.98 (m, 1 H, aromatic-H) 7.29 (m, 1 H, aromatic-H), 7.30 (m, 1 H, aromatic-H), 7.92 (s, 1 H, 2'-H) and 9.61 (s, 1 H, 16-CHO). About 0.11 g of mixture of **19a1** and **19a2** also collected (overall yield is 61%)

[00118] **3*β*-Acetoxy-17-(1*H*-benzo[f]benzimidazol-1-yl)-16-formylandrosta-5,16-diene (20a)**: Compound **20a** prepared by following general method A, reacting **13** (0.38 g, 1 mmol) with 1H-benzo[f]benzimidazole (0.2 g, 1.2 mmol) in presence of K_2CO_3 (0.207g, 1.5 mmol) in dry DMF (3 mL) at 80 °C for 2 h. Purification by FCC [petroleum ether/EtOAc/TEA (6:4:0.3)] gave 0.37 g (72%) of pure compound **20a**: mp 158-160 °C; IR ($CHCl_3$) 3691, 3024, 2951, 2359, 1725, 1670, 1604, 1491, 1452, 1375, 1253, 1032, 897, 852, 818, 700, 657, 618, 576, 565, 550, 529, 511, 476 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.05 (s, 6H, 18 and 19-CH_3), 2.04 (s, 3 H, 3α-OCH_3), 4.62 (m, 1 H, 3β-H), 5.44 (br, s, 6-H) 7.46 (br. s, 2 H, aromatic-H), 7.94 (s, 2 H, aromatic-H), 8.04 (m, 1 H, aromatic-H), 8.15 (s, 1 H, aromatic-H) 8.33 (s, 1 H, 2'-H) and 9.71 (s, 1 H, 16-CHO).

[00119] **3*β*-Acetoxy-17-(6-Chloro-9*H*-purin-9-yl)-16-formylandrosta-5,16-diene (21a)**: A mixture of **13** (2.43 g, 6.46 mmol), 6-chloropurine (0.5 g, 3.23 mmol) and TBAF (1.69 g, 6.46) in dry THF (40 mL) was stirred at 50 °C under Ar for 48 h. After cooling to room temperature, the reaction mixture concentrated and poured onto ice-cold water (250 mL) and the resulting precipitate was filtered, washed with water and dried to give a crude product. Purification by FCC [DCM/Methanol (9.7:0.3)] and then recrystallized with hot ethanol to give 0.82 g (51.3%) of pure **21a**: mp 140-142 °C; IR (Neat) 2943, 2853, 1729, 1672, 1584, 1556, 1435, 1236, 1032, 939, cm-1; ^1H NMR (500 MHz, $CDCl_3$) δ 1.07 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 4.61 (m, 1 H, 3 α -H), 5.43 (br, 1 H, 6-H), 8.20 (s, 1 H, 2'-H), 8.79 (s, 1 H, aromatic-H), and 9.53 (s, 1 H, 16-CHO).

[00120] **3*β*-Acetoxy-17-(2-chloro-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (22a)**: Compound **22a** prepared by following general method A, reacting **13** (0.5 g, 1.33 mmol) with 2-chlorobenzimidazole (0.6 g, 4.0 mmol) in presence of K_2CO_3 (0.55 g, 4.0 mmol) in dry DMF (10 mL) at 80° C for 50 h. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (250 mL) and the resulting emulsion was extracted with DCM, organic layer dried and evaporated. Purification by FCC [petroleum ether/EtOAc (8:2)] gave 0.27 g (41.1%) of pure **22a**: mp 203 °C; IR (Neat) 2936, 1731, 1679, 1448, 1244, 1033, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.06 (s, 3 H, 18-CH_3), 1.16 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 4.62 (m, 1 H, 3 α -H), 5.43 (br, 1 H, 6-H), 7.17 (d, 1 H, J = 7.9 Hz, aromatic-H), 7.34 (m, 2 H, aromatic-Hs), 7.74 (d, 1 H, J = 7.4 Hz, aromatic-H) and 9.37 (s, 1 H, 16-CHO).

[00121] **General method B: Synthesis of 3*β*-Acetoxy-17-(1*H*-heteroaryl-1-yl)-androsta-5,16-diene (15, 16b-21b):** A solution of 3*β*-Acetoxy-17-(1H-heteroaryl-1-yl)-16-formylandrosta-5,16-diene (**14**, **17a-21a**) in dry benzonitrile (10 mL) was refluxed in the presence of 10% Pd/C (50% weight of reactant) under Ar and monitored by TLC. After cooling

to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated, and the residue was purified by FCC on silica gel, using petroleum ether/EtOAc/TEA (7.5:3:0.5) solvent system. Above listed intermediate compounds were synthesized (using reactants, reagent and solvent ratio), isolated and purified by using this method unless otherwise stated.

[00122] 3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (15): Compound **15** prepared by refluxing **14** (2.04 g, 4.45 mmol), with 10% Pd/C (1.0 g) in dry benzonitrile (10 mL) for 5 h. Followed by FCC purification provided pure **15** with identical spectral and analytical data as we previously reported.

[00123] 3β-Acetoxy-17-(1*H*-indol-1-yl)-androsta-5,16-diene (16b): Compound **16b** prepared by following general method B, refluxing **16a** (0.17 g, 0.36 mmol), with 10% Pd/C (0.085 g) in dry benzonitrile (3 mL) for 24 h, then about 0.030 g of Pd/C and solvent (1 mL) added and further refluxed for 12 h. Purification by FCC gave 0.12 g (77.5%) of pure **16b**: mp 182-185 °C; IR (Neat) 2936, 2854, 1727, 1631, 1455, 1368, 1249, 1030, 721, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.95 (s, 3 H, 18-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.99 (s, 3 H, 3β-$OCOCH_3$), 4.47 (m, 1 H, 3α-H), 5.42 (br, 1 H, 6-H), 5.88 (s, 1 H, 16-H), 6.57 (m, 1 H, 3'-H), 7.05 (m, 1 H, 2'-H), 7.15 (m, 1 H, aromatic-H), 7.37 (d, J = 3.2 Hz, 1 H, aromatic-H), 7.50 (d, J = 8.0 Hz, 1 H, aromatic-H), and 7.57 (d, J = 7.7 Hz, 1 H, aromatic-H).

[00124] 3β-Acetoxy-17-(5,6-dimethyl-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (17b): Compound **17b** prepared by following general method B, refluxing **17a** (0.15 g, 0.308 mmol), with 10% Pd/C (0.075 g) in dry benzonitrile (2 mL) for 7 h. Purification by FCC gave 0.12 g (84.8%) of pure **17b**: mp 159-162 °C; IR (Neat) 2926, 2852, 1729, 1626, 1491, 1462, 1369, 1236, 1030, 846, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ1.03 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 2.06 (s, 3 H, 3β-$OCOCH_3$), 2.40 (s, 6H, 2 X aromatic-CH_3), 4.64 (m, 1 H, 3α-H), 5.45 (br, 1 H, 6-H), 5.96 (s, 1 H, 16-H), 7.26 (s, 1 H, aromatic-H), 7.58 (s, 1 H, aromatic-H), and 7.87 (s, 1 H, 2'-H).

[00125] 3β-Acetoxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (18b): Compound **18b** prepared by following general method B, refluxing **18a** (0.15 g, 0.31 mmol) 10% Pd/C (0.075 g) in dry benzonitrile (2 mL) for 24 h. Purification by FCC gave 0.09 g (63.5%) of pure **18b**: mp 204-206 °C; IR (Neat) 2939, 2222, 1731, 1487, 1247, 1030, 822, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ1.07 (s, 3 H, 18-CH_3), 1.18 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 4.62 (m, 1 H, 3α-H), 5.44 (m, 1 H, 6-H), 6.03 (m, 1 H, 16-H), 7.54 -8.15 (m, 4 H, aromatic-H).

[00126] **3*β*-Acetoxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (19b):** Compound **19b** prepared by following general method B, refluxing **19a1** (0.15 g, 0.307 mmol), with 10% Pd/C (0.075 g) in dry benzonitrile (2 mL) for 72 h, then about 0.030 g of Pd/C added and further refluxed for 12 h. Purification by FCC gave 0.05 g (35%) of pure sticky compound **19b**: IR (Neat) 2940, 1713, 1496, 1363, 1237, 1216, 1030, 816, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ1.01 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 3.88 (s, 3 H, -OCH_3), 4.63 (m, 1 H, 3α-H), 5.44 (s, 1 H, 6-H), 5.96 (br, 1 H, 16-H), 6.92 (m, 2 H, aromatic-Hs), 7.69 (d, 1 H, J = 8.7 Hz, aromatic-H), and 7.85 (s, 1 H, 2'-H).

[00127] **3*β*-Acetoxy-17-(1*H*-benzo[f]benzimidazol-1-yl)-androsta-5,16-diene (20b):** Compound **20b** prepared by following general method B, refluxing **20a** (0.2 g, 4.45 mmol), with 10% Pd/C (0.1 g) in dry benzonitrile (4 mL) for 5 h. Purification by FCC gave 0.14 g (73.8%) of pure **20b**: mp 144-146 °C; IR ($CHCl_3$) 3687, 2947, 2854, 2358, 2340, 1725, 1633, 1609, 1557, 1489, 1454, 1373, 1291,1253, 1195, 1136, 1031, 985, 910, 839, 735, 665, 590, 544, 533,513, 502, 488 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) δ 1.08 (s, 3 H,18-CH_3), 1.10 (s, 3 H, 19-CH_3), 2.01 (s, 3 H, 3*β*-OCH_3), 4.62 (m,1H, 3α-H), 5.45 (br,s,6-H), 6.11 (s, 1 H, 16-H), 7.42 (m, 2 H, aromatic-Hs), 7.92 (m, 2 H, aromatic-H), 8.04 (m, 1 H, aromatic-H), 8.15 (s, 1 H, aromatic-H) and 8.29 (s, 1 H, 2'-H).

[00128] **3*β*-Acetoxy-17-(6-Chloro-9*H*-purin-9-yl)-androsta-5,16-diene (21b):** Compound **21b** prepared by following general method B, refluxing **21a** (0.4 g, 0.81 mmol), with 10% Pd/C (0.4 g, i.e., equal weight of **21a**) in dry benzonitrile (7.5 mL) for 4 h. Cooled to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated, and carried to next step without purification.

[00129] **3*β*-Acetoxy-17-(2-chloro-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (22b)**. A solution of 3*β*-Acetoxy-17-(2-chlorobenzimidazol-1-yl)-16-formylandrosta-5,16-diene (**22a**), (0.15 g, 0.304 mmol) in dry toluene (3 mL) was refluxed in the presence of chlorotris (triphenylphosphine) rhodium (I) (0.29 g, 0.311 mmol) for 60 h. After cooling to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated, and the residue was purified by FCC [petroleum ether/EtOAc (8:2)] to give 0.04 g (28%) of pure **22b**: mp 161-165 °C; IR (Neat) 2926, 2853, 1629, 1403, 1462, 1369, 1233, 1035, 847 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ1.05 (d, 6H, 18 and 19-CH_3), 2.04 (s, 3 H, 3β-$OCOCH_3$), 4.62 (m, 1 H, 3α-H), 5.44 (m, 1 H, 6-H), 6.06 (s, 1 H, 16-H), 7.33 (m, 1 H, aromatic-H), 7.52 (m, 1 H, aromatic-H), and 7.68 (m, 2 H, aromatic-H).

[00130] General method C: Synthesis of 3β-Hydroxy-17-(1*H*-heteroaryl-1-yl)-androsta-5,16-diene (5, 16-22) and 3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylamino)methyl)-androsta-5,16-diene (25, 28 and 31): The acetate (**15, 16b-22b, 24, 27, 30**) (1 g) was dissolved in methanol (15 mL) under an inert Ar atmosphere, and the resulting solution was treated with 10% methanolic KOH (5 mL). The mixture was stirred at room temperature, monitored by TLC. Reaction mixture concentrated under vacuum, ice water (100 mL) added, and the resulting white precipitate was filtered, washed with water and dried. FCC on a short silica gel column, eluting with petroleum ether/EtOAc (6:4) to obtain pure target compounds. Above listed final compounds were synthesized (using reactants, reagent and solvent ratio), isolated and purified by using this method unless otherwise stated.

[00131] 3β-Hydroxy-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (5)[24] Compound **5** prepared by following general method C, treating acetate solution of **15** (1 g 3.02 mmol) in methanol (15 mL) with 10% methanolic KOH (5 mL) for 1.5 h. Purification by FCC over short column provided pure **5** with identical spectral and analytical data as we previously reported.

[00132] 3β-Hydroxy-17-(1*H*-indol-1-yl)-androsta-5,16-diene (16): Compound **16** prepared by slightly modifying general method C. The acetate solution of **16b** (0.09 g 0.2 mmol) in methanol (1.5 mL) was refluxed with 10% methanolic KOH (1 mL) for 3 h. Purification by FCC over short column short column gave pure **16** (0.076 g, 98.7%), mp 142-145 °C; IR (Neat) 3305, 2931, 2836, 1625, 1455, 1327, 1225, 10598, 1042, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.00 (s, 3 H, 18-CH_3), 1.06 (s, 3 H, 19-CH_3), 3.54 (m, 1 H, 3α-H), 5.41 (br, 1 H, 6-H), 5.85 (s, 1 H, 16-H), 6.55 (m, 1 H, 3'-H), 7.11 (m, 1 H, 2'-H), 7.19 (dd, J = 8.4, 5.7 Hz, 2 H, aromatic-Hs), 7.51 (d, 1 H, J = 8.3 Hz, aromatic-H), and 7.60 (d, 1 H, J = 7.8 Hz, aromatic-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 149.6, 141.2, 137.2, 128.4, 126.9, 122.0, 121.7, 120.6, 119.6, 111.3, 102.4, 71.7, 55.9, 50.6, 47.3, 42.0, 37.2, 36.8, 35.1, 31.6, 30.2, 20.8, 19.4, 16.0; HRMS calcd 410.2454 ($C_{27}H_{33}ON.Na^+$), found 410.2460.

[00133] 3β-Hydroxy-17-(5, 6-dimethyl-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (17): Compound **17** prepared by following general method C by treating acetate solution of **17b** (0.1 g 0.22 mmol) in methanol (2 mL) with 10% methanolic KOH (1 mL) for 3 h. Purification by FCC over short column provided pure **17** (0.05 g, 55%), mp 194-196 °C; IR (Neat) 3262, 2925, 2896, 2848, 1628, 1493, 1481, 1371, 1058, 834, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.06 (s, 3 H, 19-CH_3), 2.38 (s, 6H, 2 x aromatic-CH_3), 3.55 (m, 1 H, 3α-H), 5.41 (m, 1 H, 6-H), 5.95 (t, J = 2.6 Hz, 16-H), 7.25 (s, 1 H, aromatic-H), 7.57 (s, 1 H, aromatic-H), and 7.87 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 147.3, 141.3, 132.7, 131.6, 123.4, 121.1,

119.9, 111.3, 71.6, 55.9, 50.5, 47.2, 42.3, 37.2, 34.9, 31.6, 30.37, 20.6, 19.3, 16.0; HRMS calcd 439.2719 ($C_{28}H_{36}ON_2.Na^+$), found 439.2726.

[00134] 3β-Hydroxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (18): Compound **18** prepared according to general method C by treating acetate solution of **18b** (0.075 g 0.165 mmol) in methanol (1.5 mL) with 10% methanolic KOH (1 mL) for 2 h. Purification by FCC over short column provided pure **18** (0.055 g, 80.8%), mp 192-193 °C; IR (Neat) 3409, 3285, 2928, 2226, 1654, 1614, 1469, 1229, 1059, 801, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.01 (d, 3 H, 18-CH_3), 1.06 (d, 3 H, 19-CH_3), 3.55 (tdq, J = 9.0, 4.7, 2.6 Hz, 1 H, 3α-H), 5.40 (dp, J = 4.8, 1.7 Hz, 6-H), 6.02 (m, 1 H, 16-H), 7.52–8.15 (m, 4 H, aromatic-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 146.7, 144.8, 141.5, 127.0, 126.4, 125.5, 121.5, 119.8, 116.4, 112.4, 106.8, 106.1, 71.7, 56.1, 50.6, 47.5, 42.4, 37.3, 36.9, 34.9, 31.7, 30.6, 20.8, 19.5, 16.2, 15.0; HRMS calcd 414.2539 ($C_{27}H_{31}ON_3.H^+$), found 414.2532.

[00135] 3β-Hydroxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (19): Compound **19** prepared according to general method C by treating acetate solution of **19b** (0.05 g 0.11 mmol) in methanol (1 mL) with 10% methanolic KOH (1 mL) for 3 h. Purification by FCC over short column provided pure **19** (0.03 g, 55%), mp 169-179 °C; IR (Neat) 3339, 2933, 1614, 1501, 1450, 1283, 1068, 906, 813, 728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 3 H, 18-CH_3), 1.06 (s, 3 H, 19-CH_3), 3.58 (m, 1 H, 3α-H), 3.86 (s, 3 H, -OCH_3), 5.41 (t, 1 H, J = 2.42 Hz, 6-H), 5.95 (t, 1 H, J = 1.48 Hz,16-H), 6.92 (m, 2 H, aromatic-H), 7.67 (m, 1 H, aromatic-H), and 7.58 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 157.32, 147.6, 141.5, 137.9, 135.4, 124.0, 121.2, 120.7, 111.6, 95.2, 71.7, 56.2, 50.7, 47.5, 42.5, 37.4, 35.1, 31.8, 30.6, 20.9, 19.5, 16.2; HRMS calcd 441.2512 ($C_{27}H_{34}O_2N_2.Na^+$), found 441.2507.

[00136] 3β-Hydroxy-17-(1*H*-benzo[f]benzimidazol-1-yl)-androsta-5,16-diene (20): Compound **20** prepared according to general method C by treating acetate solution of **20b** (0.1 g ,0.32 mmol) in methanol (5 mL) with 10% methanolic KOH (1 mL) for 1.5 h. Purification by crystallization from EtOAc/Methanol gave **20** (0.075 g, 74%), mp 150-152 °C; IR ($CHCl_3$) 2934, 2339, 1609, 1490, 1453, 1291, 1040, 837, 808, 705, 663, 608, 578, 550, 517 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.09 (s, 6H, 18 and 19-CH_3), 3.57 (m, 1 H, 3α-H), 5.44 (br, s, 6-H), 6.13 (s,1 H, 16-H), 7.44 (m, 2 H, aromatic-Hs), 7.94 (m, 2 H, aromatic-H), 8.03 (m, 1 H, aromatic-H), 8.18 (s, 1 H, aromatic-H) and 8.31 (s, 1 H, 2'-H). HRMS calcd 461.2563 ($C_{30}H_{34}N_2O.Na+$), found 461.2570.

[00137] 3β-Hydroxy-17-(6-Chloro-9*H*-purin-9-yl)-androsta-5,16-diene (21): Compound **21** prepared according to general method C by treating acetate solution of **21b** (0.04 g 0.085 mmol) in methanol (1 mL) with 10% methanolic KOH (1 mL) for 3 h. Purification by

FCC over short column [DCM/methanol/TEA (9.7:0.3:0.05)] to obtain pure **21** (0.03 g, 82.6%), mp 272-274 °C; IR (Neat) 3385, 2928, 2604, 2498, 1664, 1516, 1433, 1346, 1040, 805, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.12 (s, 3 H, 18-CH_3), 1.23 (s, 3 H, 19-CH_3), 3.50 (m, 1 H, 3α-H), 5.41 (br, 1 H, 6-H), 5.59 (s, 1 H, 16-H), 8.11 (s, 1 H, 2'-H), 8.40 (s, 1 H, aromatic-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.1, 153.4, 141.6, 139.4, 121.9, 120.8, 71.2, 56.3, 53.1, 50.1, 47.0, 46.0, 36.9, 31.2, 19.5, 15.0, 11.7, 8.9, 8.8; HRMS calcd 871.3952 $(C_{24}H_{29}ClON_4)_2.Na^+$, found 871.3972

[00138] 3*β*-Hydroxy-17-(2-chloro-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (22): Compound **22** prepared according to general method C by treating acetate solution of **22b** (0.03 g 0.064 mmol) in methanol (0.75 mL) with 10% methanolic KOH (1 mL) for 3 h. Purification by FCC over short column [petroleum ether/EtOAc (7:3)] to obtain pure **22** (0.025 g, 91.6%), mp 83-86 °C; IR (Neat) 3346, 2929, 1449, 1267, 1121, 1071, 1040, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.05 (br, 6H, 18 and 19-CH_3), 3.54 (m, 1 H, 3α-H), 5.41 (br, 1 H, 6-H), 6.04 (m, 1 H, 16-H), 7.25 (m, 1 H, aromatic-H), 7.31 (m, 1 H, aromatic-H), and 7.68 (m, 2 H, aromatic-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 141.5, 133.2, 129.9, 123.3, 121.2, 111.5, 71.9, 55.9, 50.8, 42.5, 38.9, 37.3, 37.0, 34.0, 31.8, 30.6, 24.0, 23.2, 20.73, 19.5, 17.3, 16.4; HRMS calcd 445.2017 $(C_{28}H_{36}ON_2.Na^+)$, found 445.2020.

[00139] General method D: Synthesis of 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylimino)methyl)-androsta-5,16-diene (23, 26 and 29): The title compounds were prepared by refluxing a solution of 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (**14**) (1 equivalent), corresponding primary amine (2 equivalent), molecular sieves (~25% weight of **14**) and ethanol under Ar for 3-12 h. Reaction mixture was filtered, concentrated under vacuum, residue stirred with water and resulting crude product filtered. Purification by the FCC on silica gel column [petroleum ether/EtOAc (1:1)] gave the desired pure compounds. Above listed compounds were synthesized (using reactants, reagent and solvent ratio), isolated and purified by using this method unless otherwise stated.

[00140] 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-(isopentylimino)methyl)-androsta-5,16-diene (23): Compound **23** prepared by following general method D, refluxing **14** (0.4 g, 0.87 mmol), isopentylamine (0.15 g, 1.7 mmol), molecular sieves (0.2 g) in ethanol (5 mL) for 3 hours. Followed purification by FCC gave 0.41 g (89%) **23**: mp sinters at 135 °C, melts at 145°C; IR (Neat) 2934, 2851, 1726, 1676, 1640, 1490, 1453, 1247, 1219, 1032, 744 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 0.87 (d, 6H, aliphatic-CH_3), 1.07 (s, 3 H, 18-CH_3), 1.16 (s, 3 H,19-CH_3), 2.06 (s, 3 H, 3β-$OCOCH_3$), 4.64 (m, 1 H, 3 α -H), 5.46 (br. s, 1 H, 6-H), 7.30 (s, 1 H,

imine-CH), 7.34 (m, 2 H, aromatic-Hs), 7.72 (s, 1 H, aromatic-H), 7.87 (s, 1 H, aromatic-H), and 7.94 (s, 1 H, 2'-H).

[00141] General method E: Synthesis of 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylamino)methyl)-androsta-5,16-diene (24, 27 and 30): To ice cold solution of 16-enamines (**23**/ 2**6**/**30**) (1 mole equivalent) in methanol added $NaBH_4$ (0.5 mole equivalent) in three portions over 30 minutes. Reaction continued for 1.5-5 h then neutralized with acetic acid, evaporated, residue treated with water and filtered. Crude product carried to next step without purification.

[00142] 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((isopentylamino)methyl)-androsta-5,16-diene (24): Compound **24** prepared by following general method E, reacting **23** (0.1 g, 0.2 mmol) in methanol (1.5 mL) with $NaBH_4$ (0.0035 g, 0.09 mmol) at °C for 1.5 h. The crude product **24** (0.09 g, 89%) was carried to next step without purification.

[00143] 3*β*-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((isopentylamino)methyl)-androsta-5,16-diene (25): Compound **25** prepared by following general method C, treating methanolic solution (1 mL) of crude acetate **24** (0.08 g 0.15 mmol) with 10% methanolic KOH (0.75 mL) for 3h. Followed purification by passing through short silica bed [DCM/ethanol (9.5:0.5)] to give **25** (0.065 g, 88%), mp 111-113 °C; IR (Neat) 3281, 2927, 2850, 1487, 1454, 1374, 1224, 1061, 1007, 765, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.81 (d, 6H, alphatic-CH_3), 1.04 (s, 6H, 18, 19-CH_3), 3.55 (m, 1 H, 3α-H), 5.41 (br, 1 H, 6-H), 7.19-7.43 (m, 3 H, aromatic-Hs), 7.75-7.82 (m, 1 H, aromatic-H), and 8.1 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 142.8, 140.0, 134.8, 123.4, 122.4, 120.2, 110.8, 71.5, 55.9, 50.7, 48.9, 42.3, 38.9, 36.8, 34.6, , 32.4, 31.6, 30.3, 26.0, 22.6, 20.5, 19.3, 16.0, 15.8; HRMS calcd 510.3454 ($C_{32}H_{45}ON_3.Na^+$), found 510.34509

[00144] 3*β*-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-(phenylimino)methyl)-androsta-5,16-diene (26): Compound **26** prepared by following synthetic method D, refluxing **14** (0.15 g, 0.33 mmol), aniline (0.06 g, 0.65 mmol), molecular sieves (0.04 g) in ethanol (2 mL) for 3 h. Purification by passing through a silica bed gave 0.15 g (85.9%) **26**: mp sinters at 85-90 °C, melts at 125°C; IR (Neat) 2973, 2932, 2822, 1727, 1635, 1589, 1486, 1453, 1239, 1219, 1029, 764 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.10 (s, 3 H, 18-CH_3) 1.23 (s, 3 H, 19-CH_3), 2.06 (s, 3 H, 3β-$OCOCH_3$), 4.65 (m, 1 H, 3α-H), 5.49 (br, 1 H, 6-H), 6.96 (m, 2 H, aromatic-Hs) 7.17 (m, 1 H, aromatic-H) 7.26 (s, 1 H, imine-CH), 7.35 (m, 4 H, aromatic-Hs), 7.87 (m, 1 H, aromatic-H), 7.94 (m, 1 H, aromatic-H) and 7.99 (s, 1 H, 2'-H).

[00145] **3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((phenylamino)methyl)-androsta-5,16-diene** (**27**): Compound **27** prepared by following General synthetic method E, reacting **26** (0.1 g, 0.19 mmol) in methanol (1.5 mL) with $NaBH_4$ (0.0035 g, 0.09 mmol) at °C for 1.5 h. The crude **27** carried to next step without purification.

[00146] **3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((phenylamino)methyl)-androsta-5,16-diene** (**28**): Compound **28** prepared by following General method C, treating methanolic solution (1mL) of crude acetate **27** with 10% methanolic KOH (0.75 mL) for 3 h. Followed purification by passing through short silica bed [DCM/ethanol (9.5:0.5)] gave **28** (0.08 g, 86%), mp 130-132 °C; IR (Neat) 3329, 2928, 2852, 1602, 1418, 1375, 1217, 1058, 1007, 833, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.04 (s, 3 H, 19-CH_3), 3.54 (m, 1 H, 3α-H), 3.65 (br. s, 2 H, -CH_2), 5.38 (t, 1 H, J = 2.62 Hz, 6-H), 6.40 (t, 2 H, J = 8.8 Hz, aromatic-Hs), 6.69 (d, 1 H, J = 7.3 Hz, aromatic-H), 7.08 (m, 2 H, aromatic-Hs), 7.20-7.33 (m, 3 H, aromatic-Hs), 7.74-7.84 (m, 1 H, aromatic-H), and 7.79 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 147.6, 141.3, 138.7, 123.7, 122.5, 129.9, 120.4, 118.0, 113.0, 110.8, 71.6, 54.7, 50.6, 48.0, 42.2, 36.8, 34.4, 32.4, 31.1, 30.3, 20.5, 19.3, 15.8. HRMS calcd 516.2985 ($C_{33}H_{39}ON_3.Na^+$), found 516.2981

[00147] **3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-((3,4-dimethoxyphenyl)imino) methyl)-androsta-5,16-diene** (**29**)**:** Compound **29** prepared by following general method D, refluxing **14** (0.3 g, 0.65 mmol), 3,4-dimethoxy aniline (0.2 g, 1.3 mmol), molecular sieves (0.075 g) in ethanol (2 mL) for overnight. Purification by FCC [petroleum ether/EtOAc (1:1)] gave 0.29 g (74.5%) **29**: mp sinters at 115 °C, melts at 130°C; IR (Neat) 2937, 2904, 2852, 1729, 1586, 1509, 1451, 1372, 1233, 1125, 1026, 765 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.10 (s, 3 H, 18-CH_3) 1.23 (s, 3 H, 19-CH_3), 2.06 (s, 3 H, 3β-$OCOCH_3$), 3.84 (m, 6H, 2 X OCH_3), 4.64 (m, 1 H, 3α-H), 5.48 (br. s, 1 H, 6-H), 6.56 (m, 2 H, aromatic-Hs) 6.73 (m, 1 H, aromatic-H) 7.36 (m, 3 H, aromatic-2Hs and imine-CH), 7.88 (m, 1 H, aromatic-H), 7.95 (m, 1 H, aromatic-H), and 8.00 (s, 1 H, 2^1-H).

[00148] **3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-(((3,4-dimethoxyphenyl)amino) methyl)-androsta-5,16-diene** (**30**)**:** Compound **30** prepared by following General synthetic method E, reacting **29** (0.15 g, 0.25 mmol) in methanol (2.5 mL) with $NaBH_4$ (0.05 g, 0.126 mmol) at °C for 5 h. The crude **30** carried to next step without purification.

[00149] **3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-(((3,4-dimethoxyphenyl)amino) methyl)-androsta-5,16-diene** (**31**)**:** Compound **31** prepared by following method C, treating methanolic solution of (2 mL) of crude acetate **30** with 10% methanolic KOH (0.75 mL). Subsequent purification by FCC [DCM/ethanol (9.7: 0.3)] to give **31** (0.11 g, 79.6%), mp sinters

at 120 °C melts 135 °C; IR (Neat) 3351, 2929, 2852, 1612, 1514, 1454, 1229, 1136, 1025, 765, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 3.53 (m, 1 H, 3α-H), 3.61 (br, 2 H, N-CH_2), 3.74-3.77 (s, 6H, 2 X OCH_3), 5.37 (br, 1 H, 6-H), 5.95 (br, 1 H, aromatic-1"-H), 6.04 (d, J = 2.6 Hz, 1 H, aromatic-5"-H), 6.64 (br, 1 H, aromatic-6"-H), 7.21-7.31 (m, 3 H, aromatic-Hs), 7.74-7.83 (m, 1 H, aromatic-H), and 7.79 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 149.9, 142.2, 138.8, 123.7, 122.5, 120.9, 112.9, 110.3, 103.8, 99.4, 71.5, 56.6, 55.7, 50.6. 48.3, 42.8, 4.1, 34.7, 32.2, 31.1, 30.0, 20.5, 19.3, 15.8. HRMS calcd 576.3196 ($C_{35}H_{43}O_3N_3.Na^+$), found 576.3188.

[00150] **17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-dien-3-one (32):** This compound prepared from **5** as previously described, provided spectral and analytical data as reported.[24] ^{13}C NMR (500 MHz, $CDCl_3$) δ 199.4, 170.5, 147.2, 143.5, 141.1, 134.7, 124.3, 124.3, 123.5, 122.6, 122.5, 111.3, 54.3, 54.2, 47.4, 38.9, 35.9, 35.8, 34.1, 33.8, 32.8, 31.4, 30.4, 17.5, 17.3, 16.3.

[00151] **17-(1*H*-Benzimidazol-1-yl)-androsta-5,16-dien-3-one (33):** To a ice cold solution of **5** (0.05 g, 0.13 mmol) in dry DCM (3 mL) was added Dess-Martin periodinane (0.11 g, 0.26 mmol) and the mixture was stirred at ice cold temperature for 5 h. Then it was diluted with ether and was quenched with a mixture of saturated aqueous $NaHCO_3/Na_2S_2O_3$ (1:3). The organic layer was washed with brine and dried over Na_2SO_4, then solvent was evaporated under vacuum and the crude product was purified by FCC [DCM/ethanol/TEA (30:1:0.05)] to give the title compound **33** (0.035 g, 70%): mp 170-172 °C; IR (Neat) 2941, 1711, 1491, 1451, 1226, 751 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.05 (s, 3 H, 18-CH_3), 1.24 (s, 3 H, 19-CH_3), 5.41 (t, 1 H, J = 2.5 Hz, 6-H), 5.99 (br, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.49 (d, J = 6.9 Hz, 1 H, aromatic-H), 7.81 (m, 1 H, aromatic-H), and 7.96 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 209.9, 147.3, 143.5, 139.2, 134.8, 124.3, 123.5, 122.8, 122.6, 122.0, 120.5, 111.3, 56.0, 49.9, 49.7, 47.5, 37.74, 37.4, 37.0, 31.3, 31.1, 30.4, 19.3, 19.2, 16.8, 16.2. HRMS calcd 409.2250 ($C_{26}H_{30}ON_2.Na^+$), found 409.2258.

[00152] **3β-Mesyloxy-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-dien (34)**: To ice cold solution of **5** (0.4 g, 1.03 mmol) in pyridine (5 ml), was added methanesulfonyl chloride (0.68 g, 6 mmol). Reaction mixture stirred at 0° C for 5 h, then room temperature for 8 h and quenched to 75 ml ice-water mixture. The resulting yellow solid was, filtered, washed, dried and the crude product was purified by FCC [DCM/ethanol (1.5%)] to give the title compound **34** (0.4 g, 83%), mp 177-179 °C (lit.[15] 149-150 °C); IR (Neat) 2944, 1486, 1452, 1326, 1170, 938, 765 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.09 (s, 3 H, 19-CH_3), 3.03 (s, 3 H, mesyl-Hs), 4.56 (m, 1 H, 3α-H), 5.49 (br, 1 H, 6-H), 6.0 (m, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.49 (m, 1 H, aromatic-H), 7.82 (m, 1 H, aromatic-H), and 7.97 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz,

$CDCl_3$) δ 147.1, 143.3, 141.6, 139.1, 134.6, 123.4, 120.2, 81.6, 55.7, 50.3, 47.2, 39.2, 36.8, 34.8, 31.1, 28.9, 20.6, 19.1, 16.0. HRMS calcd 955.4472 $(C_{26}H_{30}ON_2)_2Na^+$, found 955.4468.

[00153] **3β-Tosyloxy-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-dien (35)**: To a cold (0° C) solution of **5** (0.1 g, 0.26 mmol) in pyridine (3 ml), was added tosyl chloride (0.06 g, 0.31 mmol). Reaction mixture stirred at 0° C for 5 h, then room temperature for 3 h and quenched to 30 ml ice-water mixture. The resulting yellow solid was filtered, washed, dried and the crude product was purified by FCC [DCM/Ethanol (1.0%)]. Resulting sticky solid was dissolved in 1.5 ml of EtOAc and about 10 ml of petroleum ether added slowly with stirring, the resulting turbid solution stirred at room temperature for 30 min, to give free flowing solid of title compound **35** (0.115 g, 84.5%), mp 139-141 °C; IR (Neat) 2948, 2850, 1490, 1451, 1329, 1171, 917, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.99 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 2.44 (s, 3 H, 4"-CH_3), 4.35 (m, 1 H, 3α-H), 5.37 (m, 1 H, 6-H), 5.97 (m, 1 H, 16-H), 7.25-7.34 (m, 3 H, aromatic-Hs), 7.35-7.37 (m, 2 H, 2", 6"-Hs), 7.48 (m, 1 H, aromatic-H), 7.79 (m, 3 H, aromatic-H and 3", 5"-H), and 7.95 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 147.0, 144.5, 141.6, 139.3, 134.6, 129.8, 127.6, 123.5, 122.5, 120.6, 111.1, 82.1, 55.7, 50.3, 47.2, 38.9, 36.8, 34.8, 30.3, 28.5, 21.7, 20.57, 19.1. HRMS calcd 565.2495 ($C_{33}H_{38}O3N_2S.Na^+$), found 565.2506.

[00154] **General method F: Synthesis of 3-(Substituted-oximino)-17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-diene (36-39)**: To a refluxing solution of ketone **32** (1 mole equivalent) in ethanol-methanol (2:1) solvent mixture, add a solution of sodium acetate (9.4 mole equivalent), corresponding substituted-oxamine hydrochloride (10.5 mole equivalent) in distilled water (10 mole equivalent). Reflux continued for 2-3 h, then concentrated, residue treated with water and crude product filtered. Purification FCC over silica using 5% ethanolic DCM gave pure oximes.

[00155] **3-((*EZ*)-Hydroximino)-17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-diene (36)**: Compound **36** prepared by following general method F. To a refluxing solution of **32** (0.08 g, 0.194 mmol) in ethanol-methanol (2 mL) added a solution of sodium acetate (0.15 g, 1.83 mmol), hydroxylamine .HCl (0.07 g, 2.04 mmol) in 0.75 ml distilled water. The reflux continued for 2 h and subsequent purification by FCC gave compound (mixture of *EZ* isomers) **36** (0.06 g, 77%): mp sinters at 145 °C, melts 155-160 °C; IR (Neat) 3181, 2929, 2853, 1609, 1453, 1226, 847 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.02 (s, 3 H, 18-CH_3), 1.11-1.15 (s, 3 H, 19-CH_3), 5.81 and 6.52 (~57% and 33% for *E* and *Z* isomers respectively) of (s, 1 H, 4-H), 5.95 (br, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.47 (m, 1 H, aromatic-H), 7.81 (m, 1 H, aromatic-H), and 7.95 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.64, 156.6, 154.5, 147.0, 142.9, 134.5, 124.3, 122.6,

117.8, 111.2, 55.3, 54.2, 47.3, 38.1, 34.6, 32.8, 30.3, 24.6, 20.9, 18.7, 17.9, 16.1. HRMS calcd 424.2359 ($C_{26}H_{31}ON_3.Na^+$), found 424.2363.

[00156] **Separation of *E* and *Z* isomers of 36:** Initially *EZ* mixture was purified by FCC using petroleum ether and EtOAc (1:1) mixture. This provided better purity of individual isomers with slight contamination of each in one another. The major product **36*E*** was further purified by crystallization with hot EtOAc which resulted into pure single isomer **36*E***: mp 218-221 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.02 (s, 3 H, 18-CH_3), 1.11 (s, 3 H, 19-CH_3), 5.85 (s, 1 H, 4-H), 5.98 (s, 1 H, 16-H), 7.28 - 7.36 (m, 2 H, aromatic-Hs), 7.44 - 7.55 (m, 1 H, aromatic-H), 7.79 - 7.88 (m, 1 H, aromatic-H), 7.97 (s, 1 H, 2'-H), 9.04 (br. s., 1 H, -OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 156.7, 154.4, 147.1, 143.1, 141.6, 134.5, 124.1, 123.5, 122.5, 120.2, 117.9, 111.1, 55.3, 54.0, 47.3, 38.1, 34.8, 34.6, 34.2, 32.2 , 31.5, 30.2, 21.1, 18.7, 17.6, 16.1. Where, **36*Z*** was further purified by preparative TLC using petroleum-ether, EtOAc (1:1) as solvent system: mp 158-162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.02 (s, 3 H, 18-CH_3), 1.15 (s, 3 H, 19-CH_3), 5.97 (br s., 1 H, 16-H), 6.53 (s, 1 H, 4-H), 7.27 - 7.34 (m, 2 H, aromatic-Hs), 7.44 - 7.52 (m, 1 H, aromatic-H), 7.76 - 7.87 (m, 1 H, aromatic-H), 7.7 (s, 1 H, 2'-H), 8.87 (br. s., 1 H, -OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 158.5, 147.0, 143.1, 141.6, 134.5, 124.2, 123.5, 122.6, 120.2, 117.7, 111.1, 55.2, 54.2, 47.3, 39.0, 38.1, 36.1, 34.8, 34.2, 32.8, 31.8, 30.2, 24.7, 20.9, 17.9, 16.1.

[00157] **3-((*EZ*)-*O*-Phenyloxime)-17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-diene (37):** Compound **37** prepared by following general method F. To a refluxing solution of **32** (0.05g, 0.13 mmol) in ethanol-methanol (2ml) added a solution of sodium acetate (0.1 g, 1.22 mmol), phenoxamine .HCl (0.2 g, 1.35 mmol) in 0.5 ml distilled water. The reflux continued for 2 h and subsequent purification by FCC gave compound (mixture of *EZ* isomers) **37** (0.04 g, 64%): mp 96-98 °C; IR (Neat) 2935, 2854, 1627, 1590, 1487, 1216, 897 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 1.05 (s, 3 H, 18-CH_3), 1.16-1.20 (s, 3 H, 19-CH_3), 6.00 (s, 1 H, 4-H and 16-H), 6.00 and 6.67 (~55% and 45% for *E* and *Z* isomers respectively) (s, 1 H, 4-H), 7.01 (m, 1 H, aromatic-H), 7.22 (m, 2 H, aromatic-Hs), 7.32 (m, 4 H, aromatic-Hs), 7.52 (m, 1 H, Aromatic-H), 7.83 (m, 1 H, aromatic-Hs) and 7.97 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 160.6, 159.5, 158.0, 156.0, 147.1, 129.2, 124.2, 123.5, 121.7, 120.2, 117.4, 114.7, 111.2, 55.3, 55.0, 47.3, 38.2, 36.0, 34.1, 32.4, 30.2, 24.6, 21.0, 20.0, 17.6, 16.1. HRMS calcd 500.2672 ($C_{32}H_{35}ON_3.Na^+$), found 500.2677.

[00158] **3-((*EZ*)-*O*-Methyloxime)-17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-diene (38)**: Compound **38** prepared by following general method F. To a refluxing solution of **32** (0.075g, 0.194 mmol) in ethanol-methanol (2 ml) added a solution of sodium acetate (0.15 g,

1.83 mmol), methoxyamine.HCl (0.17 g, 2.04 mmol) in 0.75 ml distilled water. The reflux continued for 3 h and subsequent purification by FCC gave compound (mixture of *EZ* isomers) **38** (0.072 g, 89%): mp 94-96 °C; IR (Neat) 2935, 2854, 1628, 1489, 1452, 1226, 1050, 743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.04 (s, 3 H, 18-CH_3), 1.11 (s, 3 H, 19-CH_3), 3.89 (s, 3 H, OCH_3), 5.83 and 6.44 (~69% and 31% for *E* and *Z* isomers respectively) (s, 1 H, 4-H), 6.03 (m, 1 H, 16-H), 7.35 (m, 2 H, aromatic-Hs), 7.53 (m, 1 H, aromatic-H), 7.87 (m, 1 H, aromatic-H), and 8.06 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.7, 156.0, 154.5, 153.1, 146.7, 125.2, 124.0, 123.3, 119.6, 117.7, 111.2, 61.6, 55.3, 54.2, 47.3, 38.0, 34.2, 32.2, 31.5, 30.3, 24.7, 21.0, 19.2, 17.6, 16.1. HRMS calcd 438.2515 ($C_{27}H_{33}ON_3.Na^+$), found 438.2520.

[00159] 3-((*EZ*)-(*O*-Phenylmethyl)oxime)-17-(1*H*-Benzimidazol-1-yl)-androsta-4,16-diene (39): Compound **39** prepared by following general method F. To a refluxing solution of **32** (0.075g, 0.194 mmol) in ethanol-methanol (2 ml) added a solution of sodium acetate (0.15 g, 1.83 mmol), benzyloxyamine.HCl (0.33 g, 2.04 mmol) in 0.75 ml distilled water. The reflux continued for 3 h and subsequent purification by FCC gave compound (mixture of *EZ* isomers) **39** (0.092 g, 96%) which solidifies on storage: mp sinters 66-68 °C, melts 77-79 °C; IR (Neat) 2935, 2854, 1627, 1609, 1489, 1452, 1225, 1015, 864 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.10 (s, 3 H, 19-CH_3), 5.10 (s, 2 H, OCH_2), 5.83 and 6.52 (~69% and 31% for *E* and *Z* isomers respectively) (s, 1 H, 4-H), 5.97 (s, 1 H, 16-H), 7.25 (br, 3 H, aromatic-Hs), 7.37 (m, 4 H, aromatic-Hs), 7.48 (m, 1 H, aromatic-H), 7.82 (m, 1 H, aromatic-H)and 7.95 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 156.4, 154.6, 153.5, 147.0, 138.1, 127.9, 122.8, 120.0, 117.8, 111.3, 55.4, 54.0, 47.3, 38.0, 34.6, 32.2, 30.3, 24.7, 21.0, 19.6, 17.9, 16.1. HRMS calcd 514.2828 ($C_{33}H_{37}ON_3.Na^+$), found 514.2834.

[00160] 3-Methyl-3-hydroxy-17-(1*H*-benzimidazol-1-yl)-androsta-4,16-diene (40): To a solution of ketone (**32**) (0.1 g, 0.26 mmol) in dry THF (3 mL) was added MeLi (1.6 M solution in ether, 0.41 mL, 0.60 mmol) at - 60° C, and the resulting mixture was stirred at 0° C for 1 h then room temperature for 3 h. The reaction was quenched with saturated aqueous NH_4Cl and was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4, and the solvent was removed under vacuum. The residue was purified by short FCC [petroleum ether, EtOAc, TEA (60:40: 0.5)] to afford product **40** (0.05 g, 48%); mp 95-97 °C; IR (Neat) 3329, 2827, 2853, 1489, 1453, 1376, 1292, 1226, 1133, 918, 741 cm^{-1};^1H NMR (500 MHz, $CDCl_3$) δ 1.00 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 1.27 (s, 3 H, C3-CH_3), 5.25 (t, J = 1.6 Hz, 1 H, 6-H), 5.96 (t, 1 H, J = 1.52 Hz, 16-H), 7.29 (m, 2 H, aromatic-Hs), 7.49 (m, 1 H, aromatic-H), 7.82 (dd, J = 7.0, 2.6 Hz, 1 H, aromatic- H), and 7.95 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz,

$CDCl_3$) δ 145.3, 127.6, 124.4, 123.6, 122.7, 120.4, 111.4, 70.1, 55.7, 54.8, 37.8, 35.6, 35.3, 34.7, 32.5, 30.4, 28.5, 21.1, 18.8, 16.3. HRMS calcd 425.2563 ($C_{27}H_{34}ON_2.Na^+$), found 425.2570.

[00161] **General method G**: Mixed anhydride method for the synthesis of aromatic/heteroaromatic esters (**41-44**): 2-Methyl-6-nitrobenzoic anhydride (0.39 mmol) was added to a solution of pyridinecaboxylic acid (0.386 mmol) and DMAP (0.29 mmol) in THF (1 ml), and the resulting mixture was allowed to stand at room temperature for 5min. A solution of **5** (0.193 mmol) in THF (1 ml) was mixed with the above reagent mixture and then with TEA (0.1 ml). This reaction mixture was allowed to stand at room temperature for 2 h. Reaction mixture absorbed on silica and purified by FCC using 2% ethanol in DCM in presence of traces of TEA (0.06%). The picolinoyl, nicotinoyl, isonoctinoyl and 1,3-phenyldiacetic acid esters derivatives were synthesized in a manner similar to the above. TLC and ^1H NMR and HRMS analyses revealed that the presence of other esters derived from 2-methyl-6-nitrobenzoic anhydride is absent.

[00162] **3*β*-(Pyridine-2-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (41)**: Compound **41** prepared by following general method G, using 2-Methyl-6-nitrobenzoic anhydride (0.13 g, 0.39 mmol), picolinic acid (0.05 g, 0.39 mmol), 4-DMAP (0.04 g, 0.29 mmol), THF (1 ml), **5** (0.075 g, 0.19 mmol), THF (1 ml) and TEA (0.1 ml). FCC gave pure **41** (0.09 g, 90%): mp 243-44 °C; IR (Neat) 2942, 2852, 1729, 1496, 1286, 1227, 1139, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.12 (s, 3 H, 19-CH_3), 4.99 (m, 1 H, 3α-H), 5.49 (t, 1 H, J = 1.98 Hz, 6-H), 5.99 (t, 1 H, J = 1.42 Hz, 16-H), 7.32 (m, 2 H, aromatic-Hs), 7.46-7.50 (m, 2 H, picolinoyl-5-H and aromatic-H), 7.80-7.84 (m, 1 H, aromatic- H), and (1H, picolinoyl-4-H), 7.96 (s, 1 H, 2'-H), 8.15 (br, 1 H, picolinoyl-3-H), 8.79 (m, 1 H, picolinoyl-6-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.9, 150.1, 148.7, 143.4, 141.8, 140.2, 137.2, 134.7, 127.0, 125.4, 124.4, 123.6, 122.7, 120.3, 111.4, 75.6, 56.0, 50.6, 47.4, 38.2, 37.2, 35.0, 31.3, 30.5, 27.8, 20.82, 19.5, 17.0. HRMS calcd 516.2621 ($C_{32}H_{35}O_2N_3.Na^+$), found 516.2614.

[00163] **3*β*-(Pyridine-3-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (42)**: Compound **42** prepared by following general method G, using 2-Methyl-6-nitrobenzoic anhydride (0.13 g, 0.39 mmol), nicotinic acid (0.05 g, 0.39 mmol), 4-DMAP (0.035 g, 0.29 mmol), THF (1 ml), **5** (0.075 g, 0.19 mmol), THF (1 ml) and TEA (0.1 ml). FCC gave pure **42** (0.85 g, 89%): mp 206-207 °C; IR (Neat) 3435, 2942, 2851, 1710, 1496, 1285, 1120, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.13 (s, 3 H, 19-CH_3), 4.93 (m, 1 H, 3α-H), 5.49 (br, 1 H, 6-H), 5.99 (t, 1 H, J = 1.46 Hz, 16-H), 7.32 (m, 2 H, aromatic-Hs), 7.41 (m,1H, nicotinoyl-5-H), 7.50 (m, 1 H, aromatic-H), 7.83 (m, 1 H, aromatic- H), 7.98 (s, 1 H, 2'-H), 8.33 (m, 1 H, nicotinoyl-4-H), 8.79 (m, 1 H, nicotinoyl-6-H), 9.23 (br. s, 1 H, nicotinoyl-2-H); ^{13}C

NMR (500 MHz, $CDCl_3$) δ 164.9, 153.5, 151.1, 147.3, 141.8, 140.0, 137.3, 126.8, 124.4, 123.6, 122.7, 120.4, 111.4, 75.2, 55.0, 50.6, 47.4, 38.3, 37.1, 35.0, 31.3, 30.5, 20.8, 19.5, 16.2. HRMS calcd 516.2621 ($C_{32}H_{35}O_2N_3.Na^+$), found 516.2617.

[00164] **3β-(Pyridine-4-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (43)**: Compound **43** prepared by following general method G, using 2-Methyl-6-nitrobenzoic anhydride (0.13 g, 0.39 mmol), isonicotinic acid (0.05 g, 0.39 mmol), 4-DMAP (0.035 g, 0.29 mmol), THF (1 ml), **5** (0.075 g, 0.19 mmol) , THF (1 ml) and TEA (0.1 ml). FCC gave pure **43** (0.064 g, 67%): mp 184-85 °C; IR (Neat) 2944, 2953, 1719, 1489, 1282, 1124, 745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.13 (s, 3 H, 19-CH_3), 4.90 (m, 1 H, 3α-H), 5.49 (br, 1 H, 6-H), 5.99 (s, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.49 (m, 1 H, aromatic-H), 7.81 (m, 1 H, aromatic- H), 7.85 (m, 2 H, isonicotinoyl-3, 5-Hs), 7.96 (s, 1 H, 2'-H), and 8.78 (m, 2 H, isonicotinoyl-2, 6-Hs); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.7, 150.8, 147.4, 143.5, 141.8, 139.9, 138.1, 134.8, 124.3, 123.6, 122.7, 120.4, 111.3, 75.6, 56.0, 50.6, 47.4, 38.2, 37.0, 35.0, 31.3, 30.5, 27.9, 19.5, 16.2. HRMS calcd 516.2621 ($C_{32}H_{35}O_2N_3.Na^+$), found 516.2615.

[00165] **3β-(3-(Oxycarbonyl)phenylacetic acid)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (44)**: Compound **41** prepared by following general method G, using 2-Methyl-6-nitrobenzoic anhydride (0.18 g, 0.51 mmol) was added to a solution of 1, 3-phenyldiacetic acid (0.1 g, 0.51 mmol) and DMAP (0.05 g, 0.39 mmol) in THF (2 ml), **5** (0.1 g, 0.26 mmol), THF (1 ml) and TEA (0.15 ml). FCC gave pure **44** (0.055 g, 39.81%): mp 222-23 °C; IR (Neat) 2944, 1734, 1610, 1454, 1337, 1204, 1165, 1003 749 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.99 (s, 3 H, 18-CH_3), 1.05 (s, 3 H, 19-CH_3), 3.59 (s, 2 H, CH_2-Hs), 3.64 (s, 2 H, CH_2-Hs), 4.63 (m, 1 H, 3α-H), 5.40 (br, 1 H, 6-H), 5.98 (m, 1 H, 16-H), 7.18-7.23 (m, 3 H, aromatic-Hs), 7.27-7.31 (m, 3 H, aromatic-H), 7.47 (m, 1 H, aromatic-H), 7.81 (m, 1 H, aromatic-H) 8.01 (s, 1 H, 2'-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.2, 147.1, 141.8, 140.3, 135.0, 134.6, 130.5, 128.9, 128.0, 125.0, 123.9, 122.16, 120.0, 111.5, 74.4, 56.0, 50.5, 47.4, 45.6, 41.8, 38.2, 37.0, 37.0, 31.3, 30.5, 27.82, 20.8, 19.4, 16.1, 8.7. HRMS calcd 587.2880 ($C_{36}H_{40}O_4N_2.Na^+$), found 587.2876

[00166] **3β-(6-(Cyclohex-3-enecaboxylic acid)carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (45)**: A mixture of **5** (0.1 g, 0.26 mmol), DMAP (0.035 g, 0.28 mmol), 1,2,3,6-tetrahydrophthalic anhydride (0.13 g, 0.85 mmol) and pyridine (3 mL) was refluxed for 3 hrs. Cooled to room temperature and quenched to water. Precipitate was extracted with EtOAc, dried with Na_2SO_4, evaporated and the residue was purified by FCC [petroleum ether/EtOAc/TEA (9.5:0.3:0.2)] to give 0.1 g (71.9%) of pure compound **45**: mp 178-179 °C; IR (Neat) 2931, 1724, 1453, 1225, 1195 and 743 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 -1.04 (m, 6H, 18-CH_3 and19-CH_3), 4.64 (m, 1 H, 3α-H), 5.40 (br, 1 H, 6-H), 5.69 (m, 2 H, *c*-hexyl-4, c-

hexyl-5, Hs), 5.96 (s, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.50 (d, 1 H, aromatic-H), 7.84 (1 H, m, aromatic-H) 8.05 (s, 1 H, 2'-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 177.3, 173.5, 1401.0, 126.0, 125.3, 124.8, 123.8, 123.0, 121.9, 120.0, 111.4, 73.8, 55.9, 50.5, 47.4, 45.4, 40.7, 38.2, 37.1, 34.9, 31.3, 30.5, 27.7, 26.4, 19.4, 16.2, 8.8. HRMS calcd 563.2880 ($C_{34}H_{40}N_2O_4.Na^+$), found 563.2879.

[00167] 3*β*-(Oxycarbonyl-(methoxy) acetic acid)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (46): A mixture of **5** (0.1 g, 0.26 mmol), DMAP (0.035 g, 0.28 mmol), diglycolic anhydride (0.1 g, 0.85 mmol) and pyridine (3 mL) was refluxed for 3 hrs. Cooled to room temperature and quenched to water. Precipitate was extracted with EtOAc, dried with Na_2SO_4, evaporated and the residue was purified by FCC [petroleum ether/EtOAc/TEA (9.5:0.3:0.2)] to give 0.05 g (28.6%) of pure compound **46**: mp 214-215 °C; IR (Neat) 2934, 1722, 1456, 1225, 1147 and 745 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (s, 3 H, 18-CH_3), 1.07 (s, 3 H, 19-CH_3), 4.25 (s, 2 H, CH_2), 4.26 (s, 2 H, CH_2), 4.74 (m, 1 H, 3α-H), 5.45 (br, 1 H, 6-H), 6.00 (m, 1 H, 16-H), 7.32 (m, 2 H, aromatic-Hs), 7.49 (m,1H, aromatic-H), 7.82 (m, 1 H, aromatic-H), 8.06 (s, 1 H, 2' aromatic- H); ^{13}C NMR (500 MHz, $CDCl_3$); δ 172.9, 169.9, 147.0, 141.7, 140.0, 134.4, 125.4, 124.2, 123.4, 119.7, 111.6, 75.0, 69.1, 68.8, 56.0, 50.5, 47.4, 38.2, 37.0, 34.9, 31.3, 31.1, 30.5, 27.8, 20.8, 19.4, 16.2. HRMS calcd 527.2516 ($C_{30}H_{36}N_2O_5.Na^+$), found 527.2516.

[00168] 3*β*-(1*H*-Imidazole-1-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (47): A solution of **5** (0.15 g, 0.38 mmol), CDI (0.125 g, 0.77 mmol) in anhydrous acetonitrile (2 mL) and DCM (1 mL) stirred at room temperature for 2 h. Then solvent evaporated, residue treated with water, and extracted with DCM. The crude white product obtained on evaporation of solvent was purified by FCC using 1.7% methanol in DCM in presence of traces of TEA (0.06%) to give **47 (**0.135 g, 72%): mp 194-96 °C; IR (Neat) 2965, 2923, 2839, 1754, 1488, 1452, 1392, 1292, 834, 773 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.12 (s, 3 H, 19-CH_3), 4.85 (m, 1 H, 3α-H), 5.51 (br, 1 H, 6-H), 5.99 (s, 1 H, 16-H), 7.07 (s, 1 H, 4"-H), 7.30 (m, 2 H, aromatic-Hs), 7.43 (s, 1 H, aromatic-H), 7.49 (m, 1 H, 5"-H) 7.81 (m, 1 H, aromatic- H), 7.96 (s, 1 H, 2'-H) and 8.13 (s, 1 H, 2"-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 148.1, 147.1, 143.3, 141.3, 139.1, 137.1, 134.6, 130.6, 124.1, 123.1, 120.2, 117.1, 111.1, 78.4, 55.7, 50.6, 47.2, 37.9, 36.8, 34.8, 31.1, 30.3, 27.6, 20.6, 19.3, 16.0. HRMS calcd 505.2573 ($C_{30}H_{34}O_2N_4.Na^+$), found 505.2577.

[00169] 3*β*-(2-Methyl-1*H*-imidazole-1-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (48): A solution of **5** (0.075 g, 0.193 mmol), 1,1-carbonylbis(2-methylimidazole) (0.05 g, 0.214 mmol) in anhydrous acetonitrile (1.5 mL) and DCM (0.75 mL) was refluxed over-night. The solvent evaporated, residue treated with water, and extracted with

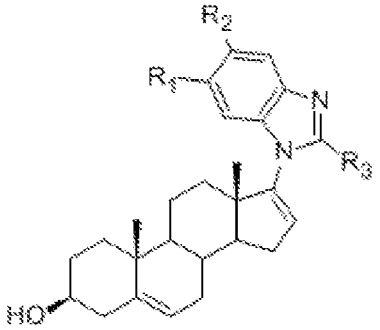
DCM. The crude white product obtained on evaporation of solvent was purified by FCC using 4 % ethanol in DCM in presence of traces of TEA (0.06%). The product was triturated with petroleum ether to give **48 (**0.065 g, 67%): mp 186-187 °C; IR (Neat) 2935, 2855, 1749, 1452, 1394, 1291, 1146, 983 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.12 (s, 3 H, 19-CH_3), 2.64 (s, 3 H, 2"-CH_3), 4.80 (m, 1 H, 3α-H), 5.51 (m, 1 H, 6-H), 5.99 (m, 1 H, 16-H), 6.84 (s, 1 H, 5"-H), 7.29 (m, 2 H, aromatic-Hs), 7.35 (s, 1 H, aromatic-H), 7.48 (m, H, aromatic-H) 7.81 (m, 1 H, 4"-H), and 7.96 (s, 1 H, 2'- H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 149.0, 147.9, 147.1, 143. 3, 141.6, 139.2, 134.6, 127.8, 123.4, 122.5, 120.2, 118.1, 111.1, 78.0, 55.7, 50.3, 47.2, 38.0, 36.8, 34.8, 31.1, 30.3, 27.7, 20.6, 19.3, 16.9, 16.0. HRMS calcd 519.2730 ($C_{31}H_{36}O_2N_4.Na^+$), found 519.2730.

[00170] 3*β*-(1*H*-1,2,4-Triazole-1-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (49): A solution of **5** (0.15 g, 0.386 mmol), CDT (0.19 g, 1.16 mmol) in anhydrous acetonitrile (3 mL) and DCM (1.5 mL) was refluxed for 3h. The solvent evaporated, residue treated with water, and extracted with DCM. The crude white product obtained on evaporation of solvent was purified by FCC using 4 % Ethanol in DCM in presence of traces of TEA (0.06%). The product was triturated with petroleum ether to give **49** (0.15 g, 80%): mp 205-206 °C; IR (Neat) 2950, 2855, 1776, 1489, 1375, 1289, 978, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.12 (s, 3 H, 19-CH_3), 4.96 (m, 1 H, 3α-H), 5.52 (m, 1 H, 6-H), 5.99 (s, 1 H, 16-H), 7.30 (m, 2 H, aromatic-Hs), 7.50 (t, 1 H, J = 3.8 Hz, aromatic -H), 7.81 (m, H, aromatic-H), 7.96 (s, 1 H, 2'-H), 8.07 (s, 1 H, 5"-H), and 8.83 (s, 1 H, 3"-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 153.8, 147.3, 145.8, 143,5, 141.8, 139.2, 134.7, 124.3, 123.6, 122.7, 120.4, 111.3, 80.0, 55.9, 50.5, 47.4, 37.9, 37.0, 35.0, 31.3, 30.5, 27.6, 20.8, 19.4, 16.2. HRMS calcd 506.2526 ($C_{29}H_{33}O_2N_5.Na^+$), found 506.2525.

CLAIMS

WHAT IS CLAIMED IS:

1. A compound of Formula I:



or pharmaceutically acceptable salt thereof, wherein:

each of R_1 and R_2 is independently hydrogen, alkoxy, or CN;

R_3 is hydrogen or halo; and

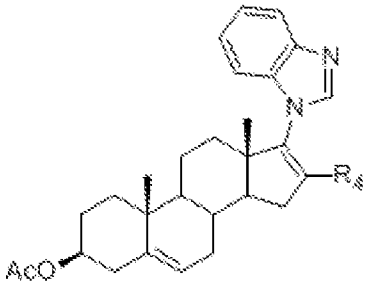
wherein at least one of R_1, R_2, R_3 is not hydrogen.

2. The compound or pharmaceutically acceptable salt of claim 1, wherein R_1 or R_2 is CN.

3. The compound or pharmaceutically acceptable salt of claim 1, wherein R_1 is alkoxy.

4. The compound or pharmaceutically acceptable salt of claim 1, wherein R_3 is halo.

5. A compound of Formula II:



or pharmaceutically acceptable salt thereof, wherein:

R_4 is $-CNHR_{10}$ or $-C=NR_{10}$;

R_{10} is alkyl or aryl, optionally substituted by one or more R_{11} substituents; and

R_{11} is halogen, alkoxy, or CN.

6. The compound or pharmaceutically acceptable salt of claim 5, wherein R_4 is $-CNHR_{10}$.

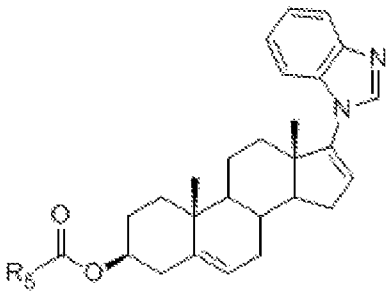
7. The compound or pharmaceutically acceptable salt of claim 5, wherein R_4 is $-C=NR_{10}$.

8. The compound or pharmaceutically acceptable salt of claim 5, wherein R_{10} is alkyl.

9. The compound or pharmaceutically acceptable salt of claim 5, wherein R_{10} is aryl.

10. The compound or pharmaceutically acceptable salt of claim 9, wherein R_{10} is aryl substituted with one or more alkoxy groups.

11. A compound of Formula III:



or pharmaceutically acceptable salt thereof, wherein:

R_5 is heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents;

R_{12} is $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; and

with the proviso that R_5 is not imidazole.

12. The compound or pharmaceutically acceptable salt of claim 11, wherein R_5 is heteroaryl.

13. The compound or pharmaceutically acceptable salt of claim 12, wherein R_5 is pyridyl.

14. The compound or pharmaceutically acceptable salt of claim 13, wherein R_5 is 3-pyridyl.

15. The compound or pharmaceutically acceptable salt of claim 12, wherein R_5 is triazole.

16. The compound or pharmaceutically acceptable salt of claim 11, wherein R_5 is arylalkyl.

17. The compound or pharmaceutically acceptable salt of claim 11, wherein R_5 is cycloalkenyl.

18. The compound or pharmaceutically acceptable salt of claim 11, wherein R_5 is alkoxyalkyl

19. The compound or pharmaceutically acceptable salt of claim 11, wherein R_{12} is $-CO_2H$ or $-CH_2CO_2H$.

20. A pharmaceutical composition comprising one or more compounds or pharmaceutically acceptable salts of any of claims 1-19 and a pharmaceutically acceptable excipient, carrier or diluent.

21. A method of treating cancer, a disease or a condition in a subject in need thereof, comprising: administering to said subject an effective amount of a compound, pharmaceutically acceptable salt or composition of any one of Claims 1-20.

22. The method of claim 21, further comprising administering to the subject an effective amount of an anti-androgen, a CYP 17 inhibitor, a luteinizing hormone-releasing hormone agonist, a drug for preventing androgen production, an estrogen, or a chemotherapy drug.

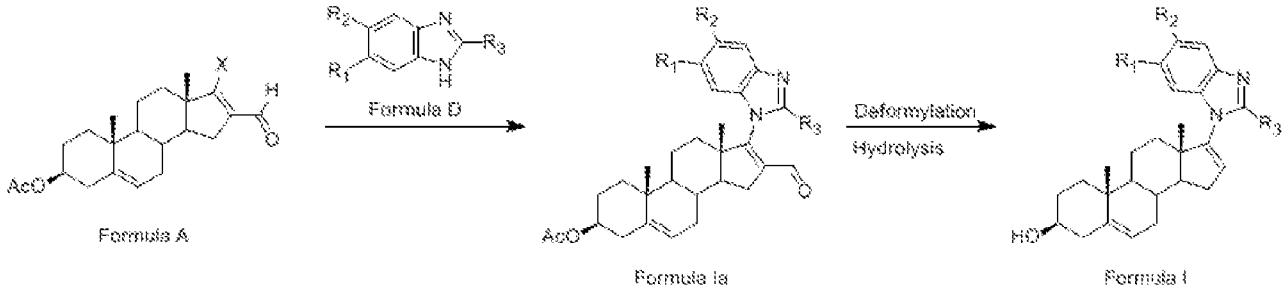
23. The method of claim 21, wherein the compound, pharmaceutically acceptable salt or composition is administered in combination with a hormone therapy, a chemotherapy, a radiation therapy, an immunotherapy, or surgery.

24. The method of claim 21, wherein the cancer, the disease or the condition is selected from prostate cancer, breast cancer, ovarian cancer, urogenital cancer, or prostate hyperplasia.

25. A method for inhibiting androgen receptor activity in a subject in need thereof, comprising administering to said subject an effective amount of a compound, pharmaceutically acceptable salt or composition of any one of Claims 1-20.

26. A method for inhibiting androgen receptor activity in a cell, comprising contacting said cell with an effective amount of a compound, pharmaceutically acceptable salt or composition of any one of Claims 1-20, thereby inhibiting androgen receptor activity in said cell.

27. A method for synthesizing a compound or pharmaceutically acceptable salt of Formula I, comprising the steps of:



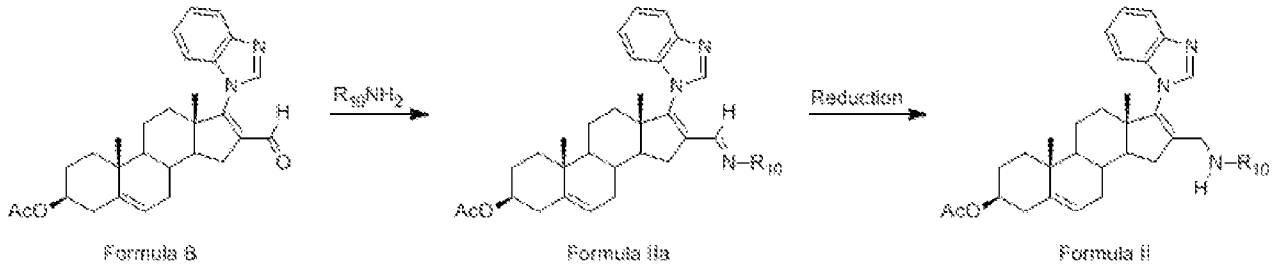
a. allowing a compound of Formula A to react with a benzimidazole of Formula D under conditions that are effective for synthesizing a compound of Formula Ia; and

b. deformylating and hydrolyzing the compound of Formula Ia;

wherein X is halo; each of R_1 and R_2 is independently hydrogen, alkoxy, or CN; R_3 can be hydrogen or halo; and wherein at least one of R_1, R_2, R_3 is not hydrogen.

28. The method of claim 27, wherein the compound of Formula Ia is deformylated with a Pd catalyst.

29. A method for synthesizing a compound or pharmaceutically acceptable salt of Formula II, comprising the steps of:



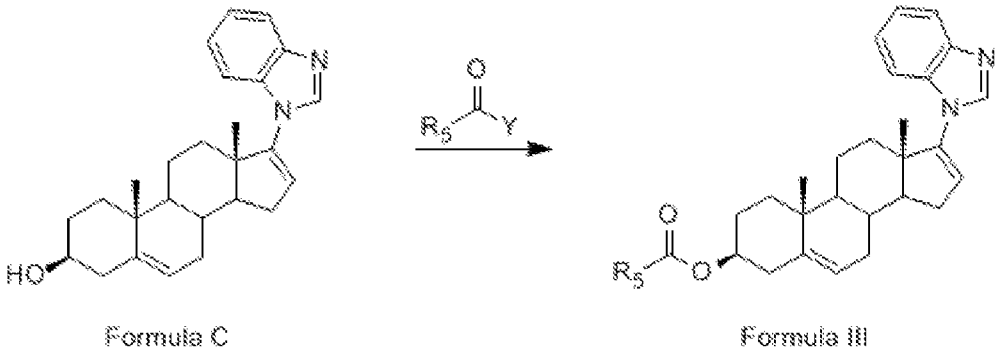
a. allowing a compound of Formula B to react with a substituted amine $R_{10}NH_2$ under conditions that are effective for synthesizing a compound of Formula IIa; and

b. reducing the compound of Formula IIa;

wherein R_{10} is alkyl or aryl, optionally substituted by one or more R_{11} substituents; and R_{11} is halogen, alkoxy, or CN.

30. The method of claim 29, wherein the compound of Formula IIa is reduced by $NaBH_4$.

31. A method for synthesizing a compound or pharmaceutically acceptable salt of Formula III, comprising:



allowing a compound of Formula C to react with an acylating agent $R_5C(O)Y$ under conditions that are effective for synthesizing a compound of Formula III; wherein R_5 is heteroaryl, arylalkyl, cycloalkenyl, alkoxyalkyl, optionally substituted with one or more R_{12} substituents; and R_{12} is $-(CH_2)_n-CO_2H$, wherein n is 0, 1, 2, or 3; with the proviso that R_5 is not imidazole.

32. The method of claim 31, wherein the acylating agent $R_5C(O)Y$ is an activated ester.

33. The method of claim 31, wherein Y is $-OC(O)R_5$.

34. The method of claim 31, wherein Y is R_5.