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Systematic Structure Modifications of Multitarget Prostate Cancer Drug Candidate Galeterone To Produce Novel Androgen Receptor Down-Regulating Agents as an Approach to Treatment of Advanced Prostate Cancer

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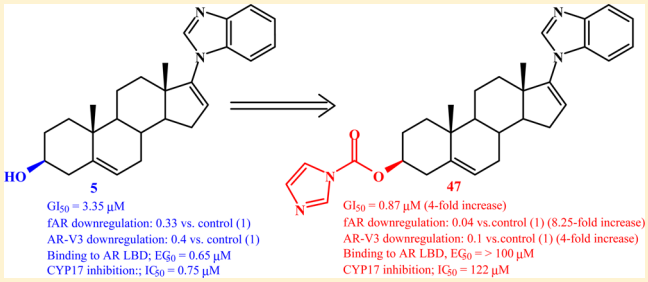
Supporting Information

ABSTRACT: As part of our program to explore the influence of small structural modifications of our drug candidate 3β-(hydroxy)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (galeterone, **5**) on the modulation of the androgen receptor (AR), we have prepared and evaluated a series of novel C-3, C-16, and C-17 analogues. Using structure activity analysis, we established that the benzimidazole moiety at C-17 is essential and optimal and also that hydrophilic and heteroaromatic groups at C-3 enhance both antiproliferative (AP) and AR degrading (ARD) activities. The most potent antiproliferative compounds were 3β-(1*H*-imidazole-1-carboxylate)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (**47**), 3-((*EZ*)-hydroximino)-17-(1*H*-benzimidazol-1-yl)androsta-4,16-diene (**36**), and 3β-(pyridine-4-carboxylate)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (**43**), with GI_{50} values of 0.87, 1.91, and 2.57 μM, respectively. Compared to **5**, compound **47** was 4- and 8-fold more potent with respect to AP and ARD activities, respectively. Importantly, we also discovered that our compounds, including **5**, **36**, **43**, and **47**, could degrade both full-length and truncated ARs in CWR22rv1 human prostate cancer cells. With these activities, they have potential for development as new drugs for the treatment of all forms of prostate cancer.



INTRODUCTION

Compelling laboratory and clinical evidence strongly indicates that incurable castration-resistant prostate cancer (CRPC) remains dependent on functional androgen receptor (AR), AR-mediated processes,[1] and the availability of intraprostatic intracellular androgens.[2] 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 inhibitors[2d] or AR antagonists.[3] These research efforts have led to ongoing clinical evaluations/approvals of three potent CYP17 inhibitors, abiraterone acetate (Zytiga, **3a**),[4] TAK-700 (orteronel, **4**),[5] and VN/124-1 (TOK-001 or galeterone, **5**),[1e,2d,6] and two potent AR antagonists, MDV3100 (enzalutamide, **6**)[7] and ARN-509 (**7**).[3a] The chemical structures of these clinical compounds are presented in Figure 1.

Despite the substantial clinical efficacy with **3a** in patients with post-docetaxel CRPC,[8] resistance to this therapy has already been reported.[9] Resistance to **6** treatment has also been reported.[10] Reactivation of AR signaling following **3a** or **6** treatments might occur by several mechanisms, prominent of which is a switching of transcription program under the control of AR signaling.[11] Indeed, it may not be possible to inhibit the new AR-regulated transcription program by currently available

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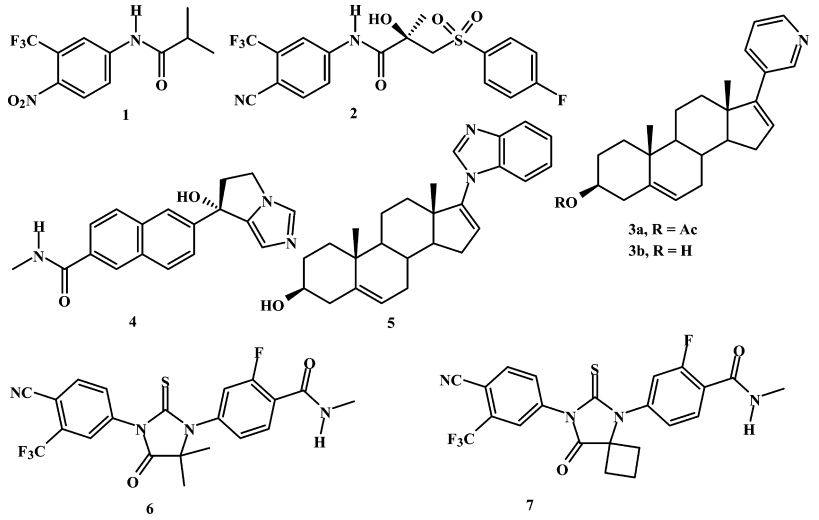

Figure 1. Chemical structures of compounds **1**–**7**.

therapies and some of the promising agents in clinical development. If so, substantial degradation of AR (full length and truncated forms) expression would be a promising strategy for future studies.

Although our clinical agent **5** was originally designed as a CYP17 inhibitor, we have unequivocally established through several in vitro and in vivo experiments that it also has other important desirable antiprostate cancer activities, acting as a potent anti-androgen and an AR degrading agent.[6,12] Because of our desire to invent more efficacious antiprostate cancer agents, we were eager to exploit compound **5**'s scaffold as a strategy to novel potent/efficacious AR degrading agents (ARDAs) with improved druglike properties. We also took into serious consideration published data of crystal structures of steroid ligand dihydrotestosterone (DHT, **8**) and of metribolone (R1881, **9**) (Figure 2) bound to AR LBD in our

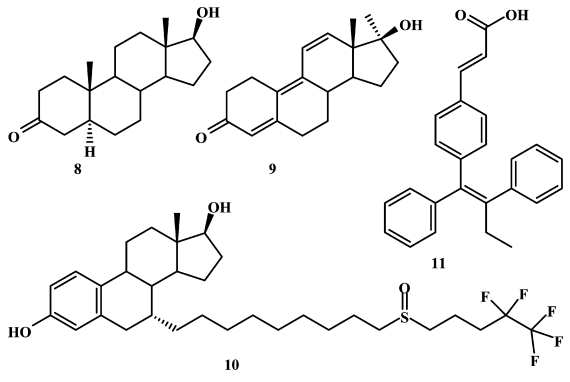

Figure 2. Chemical structures compounds **8**–**11**.

medicinal chemistry design strategy.[13] In addition we considered knowledge of related ERα structure complexed with known ERα down-regulators such as fulvestrant (**10**) and GW5638 (**11**) (Figure 2).[14,15] Specifically, we conducted a systematic structure modification of compound **5** to see if we could obtain more potent ARDAs. Herein, we report that lead optimization of **5** gave rise to several novel compounds that exhibit the abilities to induce AR (full length and truncated) ablation at low micromolar concentrations and with improved antiproliferative (AP) activities. This study expands our 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). A preliminary account of part of this work has recently been reported.[16]

RESULTS AND DISCUSSION

Design Strategy. Modifications that allow for additional interactions between a small molecule and receptor appear to be key determinants for designing new AR down-regulators with potential clinical use.[17] 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.[18] These rational considerations provided the impetus for the systematic modifications of moieties tethered to C-17, C-16, and C-3 as described below.

C-17 Modifications. To explore the structure–activity relationship (SAR) of the C-17 benzimidazole moiety of **5**, we synthesized analogues with varied ring nitrogen atoms, with increased aliphatic/aromatic hydrophobicity and with aromatic substituents to generate compounds **16**–**22** as outlined in Scheme 1.

C-16 Modifications. On the basis of previous studies by Roy et al.[19] of novel C-16 steroids that exhibit strong AR binding affinity and antiandrogenic activity, we designed and synthesized several C-16 substituted analogues (compounds **25**, **28**, and **31**) of **5**, tethered with bulky aliphatic and aromatic groups (Scheme 2).

C-3 Modifications. On the basis of studies of DHT/testosterone interactions with AR, it is well established that the interaction with Arg752 occurs via C-3 ketone.[20] Arginine is a polar hydrophilic amino acid that contains a positively charged guanidine group. On the basis of the hypothesis that any substitution at C-3 that increases interaction with Arg752 may increase AR down-regulating activity, we designed and synthesized various C-3 modified compounds (**33**–**49**, Scheme 3).

Chemistry. In this study, 26 novel compounds are described and are based on the structures of our clinical candidate, compound **5**, as outlined in Scheme 1 (for C-17 modified

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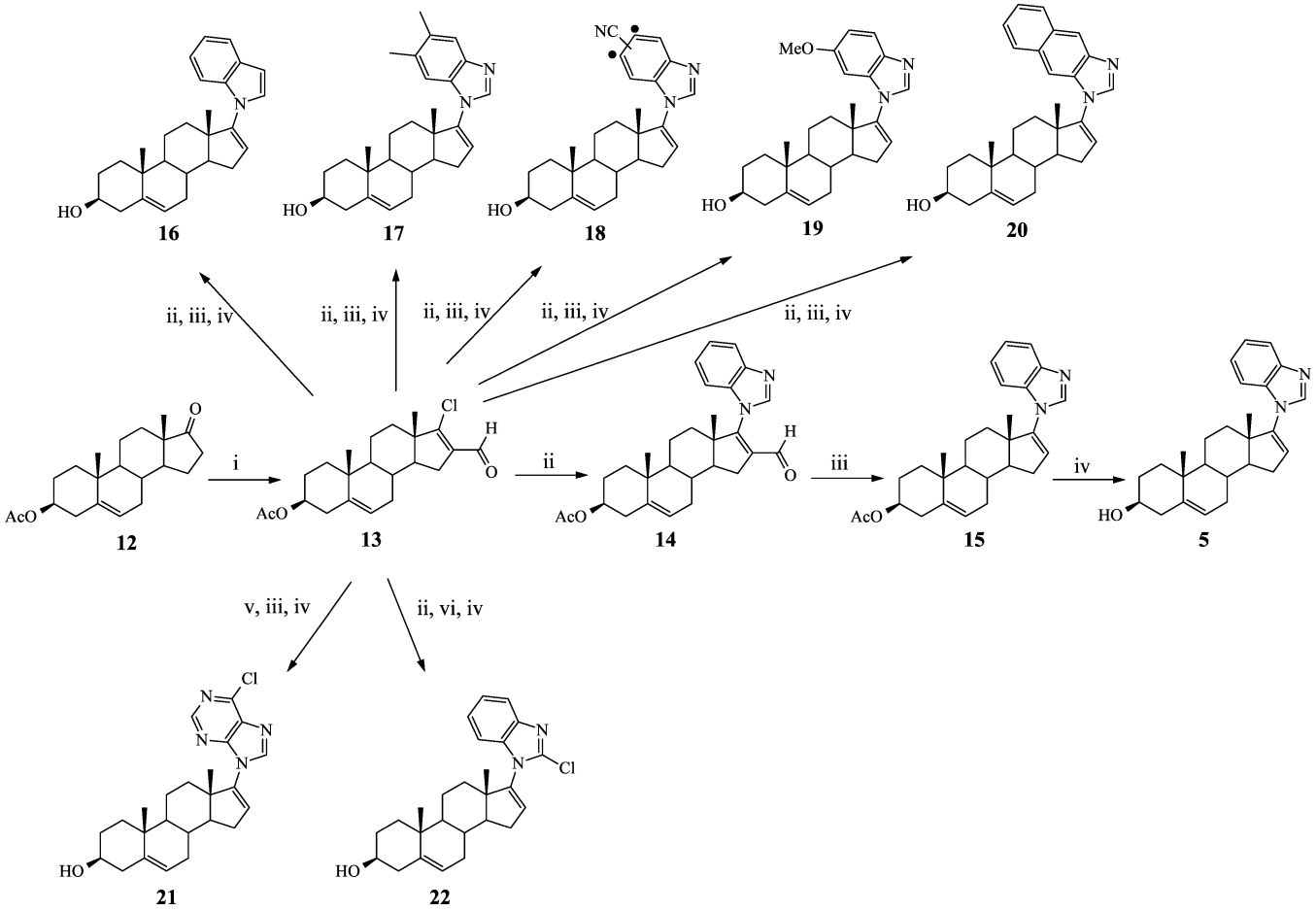
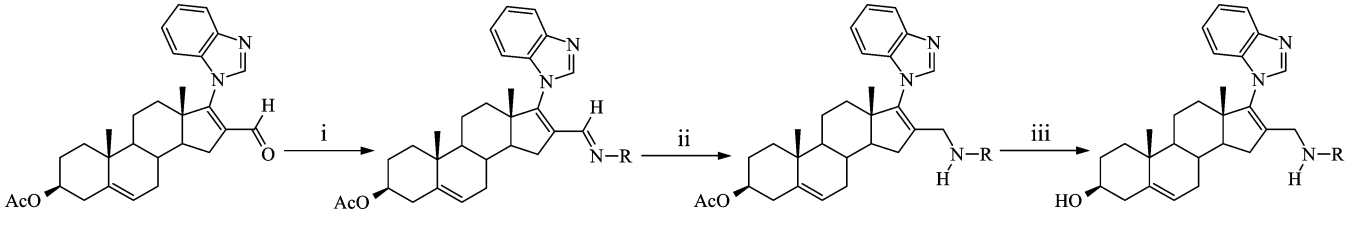
Scheme 1. Synthesis of C-17 Benzimidazole Compounds (5, 16−22)[a]



[a]Reagents and conditions: (i) $POCl_3$-DMF, $CHCl_3$, Ar, reflux; (ii) benzimidazole or indole-3-carbaldehyde or 5,6-dimethylbenzimidazole or 5(6)-cyanobenzimidazole or 5(6)-methoxybenzimidazole or naphtho[46]imidazole or 2-chlorobenzimidazole, K_2CO_3, DMF, Ar, 80 °C; (iii) 10% Pd on activated charcoal, PhCN, reflux; (iv) 10% methanolic KOH, Ar, rt (2−3 h); (v) 6-chloropurine, TBAF, THF, Ar, 50 °C; (vi) chlorotri(triphenylphosphine)rhodium[I], $PhCH_3$, Ar, reflux.

Scheme 2. Synthesis of C-16 Substituted Compounds (25, 28, and 31)[a]



14

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

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

25, R = isopentyl
28, R = phenyl
31, R = 3,4-dimethoxy benzene

[a]Reagents and conditions: (i) substituted amines, molecular sieves, EtOH, Ar, reflux (3−7 h); (ii) MeOH, $NaBH_4$, ice cold (2 h), rt (3 h); (iii) MeOH, 10% methanolic−KOH, Ar, rt (2−3 h).

series), Scheme 2 (C-16 modified series), and Scheme 3 (C-3 modified series). The preparation of new 17-hetereoaryl substituted compounds (**16**−**22**) from the key intermediate 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**13**) followed the sequence 17-heteroaryl-16-formyl intermediate → 16-deformylated intermediate → 3-deacetylated final product (not shown in Scheme 1), similar to the synthetic route to compound **5** outlined in Scheme 1. The key intermediate in our synthesis of all the compounds, **13**, was prepared following our established procedure for Vilsmeier−Haack reaction of the commercially available 3β-acetoxyandrost-5-en-17-one (**12**) with phosphoryl chloride ($POCl_3$) and dimethylformamide (DMF) as previously reported.[12a,21] 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

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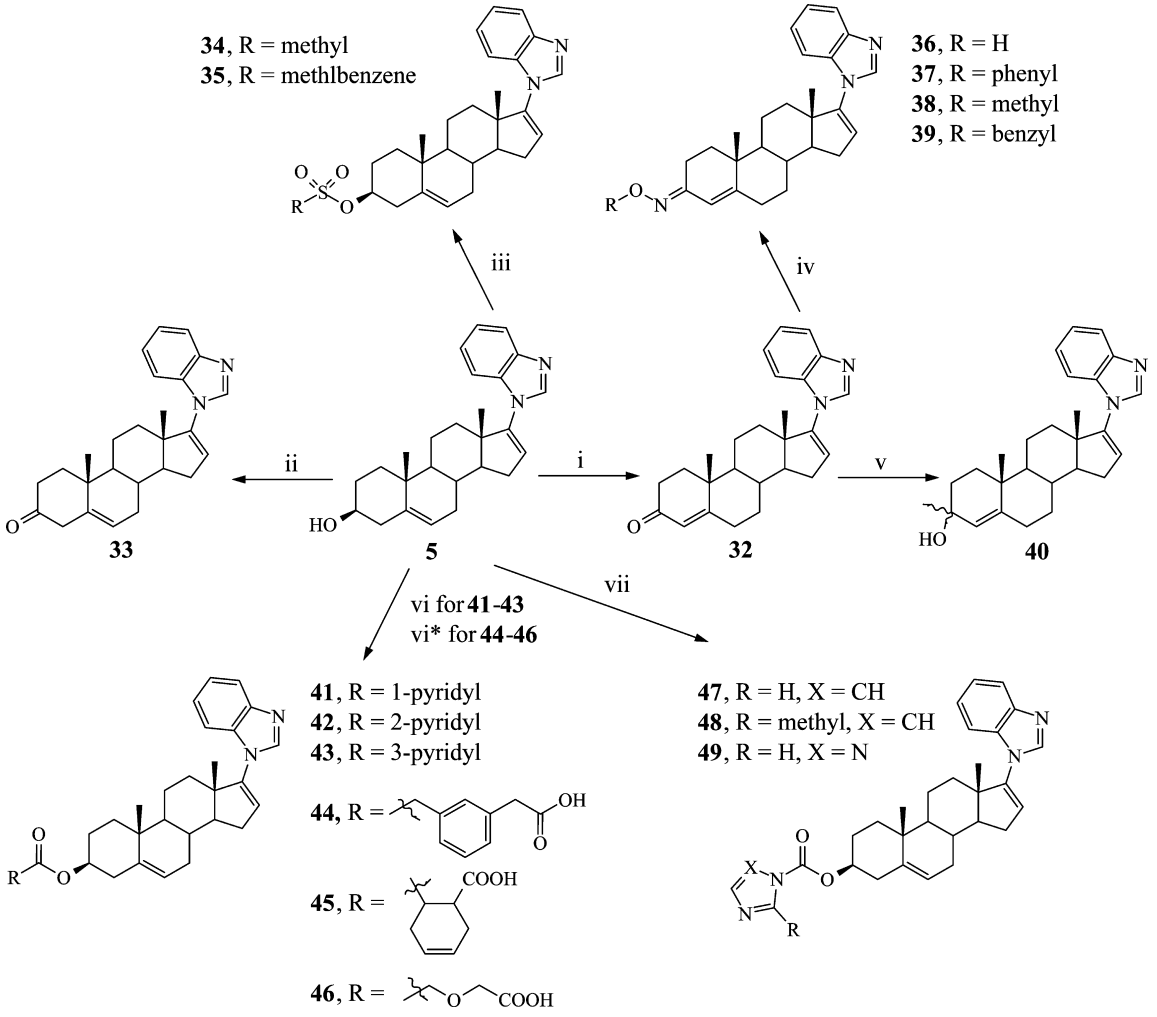
Scheme 3. Synthesis of C-3 Modified Compound (33−49)[a]



[a]Reagents and conditions: (i) Al(*i*-PrO)$_3$, 1-methyl-4-piperidone, toluene, reflux; (ii) Dess−Martin periodinane, MDC, 0 °C, rt (5 h); (iii) mesyl chloride or tosyl chloride, pyridine, ice cold (5 h), rt (5 h); (iv) substituted hydroxylamine·HCl, sodium acetate, MeOH, EtOH, Ar, reflux (2−3 h); (v) MeLi, THF, Ar, −60 °C (1 h), rt (2 h); (vi) pyridinecarboxylic acid, 2-methyl-6-nitobenzoic anhydride, DMAP, TEA, THF, rt (1 h); (vi*) acid anhydride, DMAP, pyridine, reflux; (vii) 1,1′-carbonylbisimidazole or 1,1′-carbonylbis(2-methylimidazole) or 1,1′-carbonyl-di-(1,2,4-triazole), CH_3CN, Ar, rt/reflux.

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, we adopted a reported N^9-purine alkylation procedure,[22] in which **13** was reacted with 6-chloropurine in the 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-methoxybenzyl compounds. Various attempts to separate positional isomers of 5(6)-nitrilebenzimidazole intermediates of compound **18** at all stages were unsuccessful. The 5(6)-nitrilebenzimidazole and 2,3-diaminonaphthalene required for synthesis of **18a** and **20a** were synthesized by following a reported procedure starting from 3,4-diaminobenzonitrile and benzo[*f*]benzimidazole, respectively, by refluxing with formic acid.[23] 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.[12a] 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.[12a] Unexpectedly, the 5-methoxy-16-formyl derivative **19a2** did not undergo deformylation using both methods. Hydrolysis of **15** and **16b−22b** with 10% methanolic KOH gave target compounds **5**, **16**, **17**, **18**, **19**, **20**, **21**, and **22**, respectively, in high yields.

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The C-16 substituted compounds were synthesized starting from **14** as illustrated in Scheme 2. The intermediate imines **23**, **26**, and **29** were synthesized by refluxing isopentylamine, aniline, and 3,4-dimethoxyaniline, respectively, with **14** in ethanol in the presence of molecular sieves. Subsequent reduction of these imines with sodium borohydride ($NaBH_4$) in ice-cold methanol[24] gave 3-acetoxy-16-alkylamine intermediates **24**, **27**, and **30**, respectively. Following hydrolysis of the 3β-acetoxy groups in compounds **24**, **27**, and **30**, we obtained the desired 16-substituted compounds **25, 28**, and **31**, respectively, in excellent yields.

The C-3 modified compounds were synthesized as depicted in Scheme 3. Δ^4-3-Oxo compound (**32**) was synthesized as we previously described via modified Oppenauer oxidation of **5** by using *N*-methylpiperidone and aluminum isopropoxide.[12a] Oxidation of **5** with Dess–Martin periodinane in dichloromethane (DCM)[25] 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 chloride and toluenesulfonyl chloride, respectively. The C-3 oxime derivatives (hydroxime, **36**; phenyloxime, **37**; methyloxime, **38**; benzyloxime, **39**) were obtained by refluxing ketone (**32**) with the respective substituted hydroxylamine hydrochloride, using ethanol/methanol solvent mixture in the presence of sodium acetate.[26] Of all the 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**) that we did not separate because of modest biological activity.

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)[27] with varying yields (39–90%). The ester **45** (72% yield) and **46** (28% yield) were synthesized by refluxing 1,2,3,6-tetrahydrophthalic and diglycolic anhydrides, respectively, with **5** in the presence of DMAP in pyridine.[28] Finally the carbamates (imidazole, **47**; 2-methylimidazole, **48**; 1,2,4-triazole, **49**) were synthesized in modest to high yields (67–80%) by reacting **5** with CDI, 1,1-carbonylbis(2-methylimidazole) and carbonylditriazole (CDT), respectively, in acetonitrile and DMC solvent mixture.[29] Except for the 3-mesyl (**34**), **5**, and **32**, all the compounds described in this study are novel and were rigorously characterized by physical and spectroscopic (IR, ^1H and ^{13}C NMR, and HRMS) analyses. Most of our novel compounds were then subjected to in vitro biological activity studies as described in detail in the following sections.

Biological Studies: Effects of Compounds on Transcriptional Activation of Androgen Receptor in LNCaP Cells. After synthesizing the compounds, we used a luciferase reporter assay to determine whether the novel compounds also affect AR transcriptional activation (screening assay). Specifically, we performed a luciferase experiment utilizing LNCaP cells dual-transfected with the probasin luciferase reporter construct ARR2-luc and the *Renilla* luciferase reporting vector pRL-null as we previously described and reported in the methods section.[6,12a,d] Luciferase expression was increased by approximately 100-fold after 10 nM DHT treatment for 24 h. The ability of the novel compounds (10 μM) to affect DHT mediated AR transcription was assessed. Figure 3 shows the

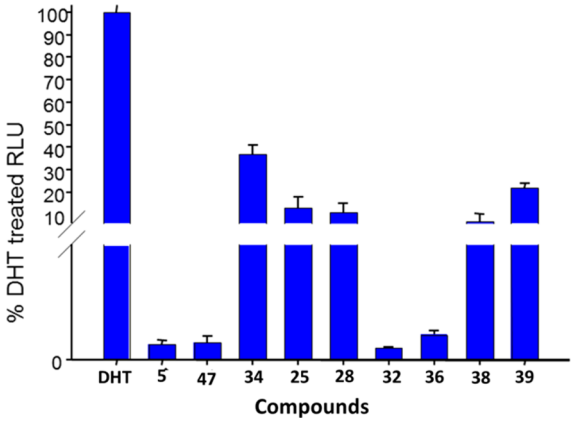

Figure 3. Effects of compounds at 10 μM on dihydrotestosterone (DHT) stimulated transcription of AR. LNCaP cells were transfected with the ARR2 reporter construct + the *Renilla* luciferase reporting vector pRL-null and treated with novel compounds for 24 h in the presence of 10 nM dihydrotestosterone (DHT). Control was baseline activity without androgen stimulation. Androgen-stimulated luciferase activity (luminescence) was measured in a Victor 1420 plate reader. 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*.

effects of our most potent compounds. These compounds were able to substantially inhibit DHT mediated transcription, with inhibition ranging from ~65% to 100%, and the order of potency was **32**, **5**, **47** > **36** > **38** > **28** > **25** > **39** > **34**.

Androgen Receptor Binding Assays. In addition to AR down-regulation, we have previously shown that compound **5** reduces androgen action through inhibition of androgen binding and subsequently reduces AR mediated transcriptional activity. We used whole cell competitive binding assays with the synthetic ligand methyltrienolone (R1881, **9**) to assess the AR binding affinities of our novel compounds in comparison to **5**, the FDA approved anti-androgens **2** and **6**, and CYP17 inhibitor **3b** as shown in Figure 4A. The compounds with the greatest ability to displace [^3H]**9** were **5** and **6**, with EC_{50} values of 670 and 915 nM, respectively. Compound **2** was slightly weaker with an EC_{50} of 1.4 μM. We did not calculate the EC_{50} value of **3b** because of the shallow steepness of the AR binding curve, a phenomenon that indicates interaction of **3b** with more than one receptor population.[30] A recent study also noted unusual (shallow steepness of the AR binding curve) AR binding characteristics with **3b**.[31] Interestingly, AR-binding assays using the MDA-MB-453 cell showed that **6** was not as potent as previously reported for assays using LNCaP cells transfected with wild type AR[3a] and was not significantly different from the binding affinity of **2**. Specifically, the binding affinity data were as follows: **6**, EC_{50} = 49 nM; **2**, EC_{50} = 31 nM.[3a] Our new compounds were not as potent as **5** at inhibiting androgen binding at the concentrations tested (Figure 4B). For example, compound **36** showed the strongest inhibition of [^3H]**9** binding of all the new compounds tested (~40%) at 10 μM. At 30 μM, **36** inhibited [^3H]**9** binding to by ~80% while **43** inhibited by ~53%. Unexpectedly, our most effective AR antagonist, **47**, did not strongly compete for the AR binding site, exhibiting only 20% displacement at a 30 μM.

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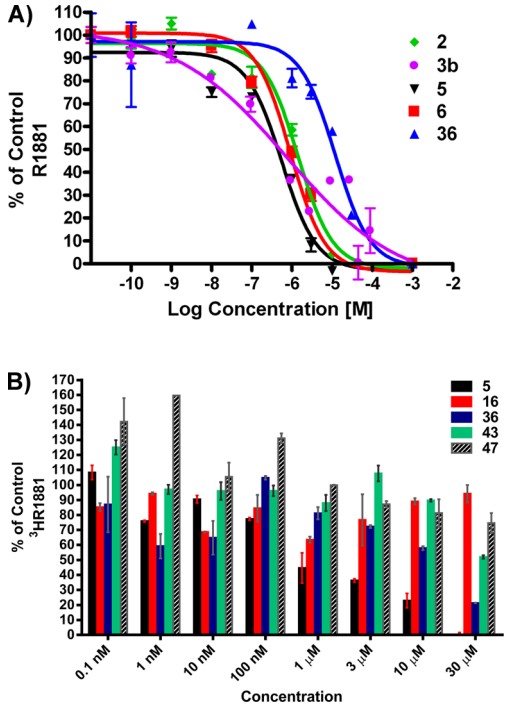

Figure 4. (A) Competitive inhibition of [^{3}H]R1881 binding of compounds **2**, **3b**, **5**, **6**, and **36** to AR in LNCaP cells. Error bars are SD; n = 3. (B) Competitive inhibition of [^{3}H]R1881 binding of compounds **5**, **16**, **36**, **43**, and **47** to AR in LNCaP cells. Error bars are SD; n = 3.

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.[32]

Effects of Compounds on AR Degradation, Transactivation, and Antiproliferative Activity. To explore the effects of our compounds on AR degradation, 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 Figure 5A−C, most of the new compounds significantly caused AR degradation in LNCaP cells, with compound **47** being the most potent and greater than 8-fold more active than compound **5** at 15 μM. In contrast, we note that a few compounds (**16**, **17**, **20**, and **49**) caused significant up-regulation of AR, a phenomenon that will be investigated in future studies. The ability of compounds **5** and **47** to suppress protein AR expression was further demonstrated by immunocytochemical analysis (Figure 5D). As shown, exposure of LNCaP cells to 5 μM **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 analogues of ciglitazone, a novel class of AR-ablative agents.[18c]

Because of the reported implication of AR splice variants lacking the ligand-binding domain (truncated AR) in driving the progression of CRPC,[33] we next determined the effects of our compounds on the down-regulation of AR-3 (also called AR-V7).[33c,d] As shown in Figure 5E, we observed that compound **5** and some of our new compounds, **31**, **32**, **36**, and **47**, caused significant down-regulation of both full-length and truncated AR in the CWR22rv1 prostate cancer cell line. Interestingly, we found that AR-3 was more susceptible to our compounds than the full-length AR in this cell line. In contrast, compound **6** did not affect the expression levels of either full-length or splice variant forms of AR. It is important to state here that a number of natural products[34] and related analogues[35] have been shown to degrade both full-length and truncated AR in several human prostate cancer cell lines. However, except for the curcumin analogue ASC-J9 that possesses excellent druglike properties,[35] most of these compounds are poor drug candidates because of modest potencies and/or toxic nature. Irrespective of how our compounds and others cause degradation of both forms of AR, such unique AR depleting agents if adequately developed may be more effective against CRPC than agents that obligatorily bind to specific region(s) of AR to elicit inactivation of AR.[36]

To determine whether AR degradation or AR transcriptional deactivation (AR inactivation) was contributing to the antiproliferative activity, we treated the LNCaP cells with 15 μM selected active compounds (**5**, **36**, **32**, **47**, and **48**) for 24 h and performed cell viability, AR transcriptional (luciferase) assay, and AR Western blot analysis. As shown in Figure 6, the degradation of AR and inhibition of AR mediated transcription occur before cell growth inhibition, which suggests that compound-induced AR inactivation contributes to their antiproliferative activities. These compounds also induced significant PARP cleavage in LNCaP and CWR22rv1 cells, which suggests their abilities to induce apoptosis (data not shown).

CYP17 (17α-Hydroxylase Activity) Inhibition Studies. A few compounds were evaluated for their ability to inhibit CYP17 enzyme. The assay was kindly performed by Dr. Emily Scott and colleagues according to their recently reported procedure in which a truncated version of human CYP17A1 (CYP171dH) was expressed in *E. coli* and then purified to homogeneity.[37] IC_{50} values of the compounds were determined from dose−response curves and are listed in Table 1. 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[12a,21]) were also determined in the same assay system for comparison (used as positive controls). As expected, these new compounds (**16**, **36**, **43**, **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.[21,38] As expected, the well-established CYP17 inhibitors exhibited exquisite inhibition of the enzyme with IC_{50} values in the nanomolar range (Table 1).[21,38b]

Antiproliferative (Anticancer) and Androgen Receptor Down-Regulating Activities: Structure−Activity Relationships (SARs). Because of our hypothesis that the extent of AR degradation induced by compound **5** and possibly the new analogues would correlate with their ability to inhibit proliferation of prostate cancer cells (LNCaP), we assessed these two activities using Western blot analyses and MTT assays.

C-17 Modification. Initially we synthesized and tested indole **16** to assess the effect of decreased polarity at C-17 position due to absence of N-3 of BzIm ring. Unexpectedly, the compound caused up-regulation of AR (Figure 5A) and completely lost anticancer activity (GI_{50} > 100 μM, Table 2)

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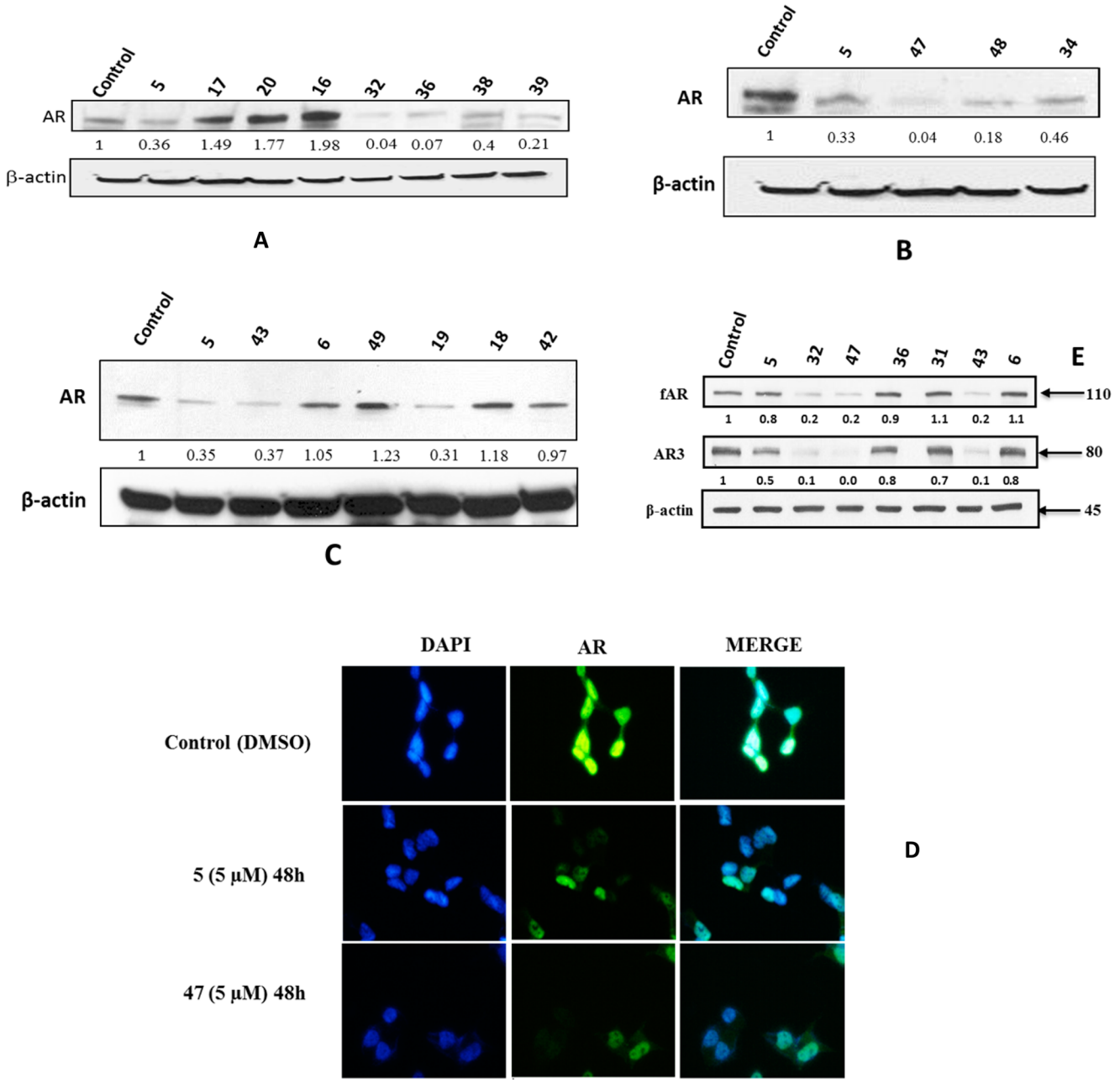

Figure 5. (A–E) Differential effect of compounds on suppressing AR expression in LNCaP and CWR22Rv1 prostate cancer cells. (A–C) Western blot analysis of AR expression in LNCaP cells treated with various compounds. Cells were exposed to individual compounds (15 μM) for 24 h, and the protein lysates were subjected to Western blot analysis. (D) Immunocytochemical analysis of the effect of 5 μM each of compounds **5** and **47** on suppressing AR expression in LNCaP cells after 48 h of exposure. Cells were stained with an-AR x-terminal antibody (green). The nuclear counterstaining was achieved using 4′,6-diamino-2-phenylindole (DAPI). (E) Effects of compounds on the expression of fAR and tAR-3 in CWR22Rv1 cells. Cells were exposed to individual compounds (15 μM) for 24 h, and the protein lysates were subjected to Western blot analysis. Western blots were done in duplicate or triplicate. The numbers below the blots represent respective densitometry intensities.

in comparison to lead compound **5** (GI_{50} = 3.35 μM). Increasing the number of nitrogen C-17 heterocycle by substituting with 6-chloropurine (**21**), caused a 4-fold reduction in antiproliferative activity (GI_{50} = 13.48 μM). Introducing a cyano group (**18**) displayed potent antiproliferative activity (GI_{50} = 2.81 μM) but with diminished AR down-regulation (ARD) activity. Introduction of aliphatic hydrophobicity on the BzIm ring by substituting methyl groups at the 5, 6 positions (**17**) resulted in substantial loss of antiproliferative (GI_{50} = 42.72 μM) and ARD activities, whereas substituting a monomethoxy group (**19**) at the sixth position of the 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 antiproliferative activity. None of the C-17 modified molecules were superior to our lead compound **5**, and this clearly indicates that the BzIm ring at the C-17 position of lead **5** is essential and optimal for ARDA and antiproliferative activity.

C-16 Modification. Our strategy to increase bulk at the C-16 position by tethering aliphatic hydrophobic groups (isopentyl,

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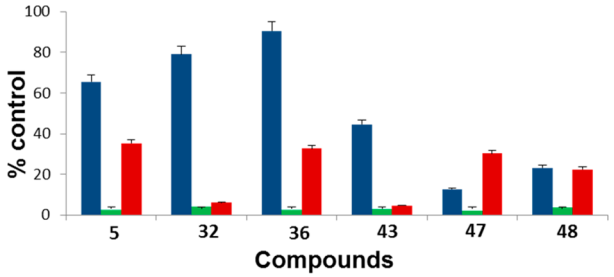

Figure 6. Effects of compounds **5**, **32**, **36**, **43**, **47**, and **48** on (i) cell viability (blue), (i) DHT-induced AR transactivation (green), and (iii) AR protein expression following treatment with 15 μM each compound for 24 h using LNCaP cells. Error bars are SD; n = 3.

Table 1. IC_{50} of Select Compounds for Inhibition of CYP17 (17α-Hydroxylase Activity)

compd	IC_{50} (μM)[a]
16	130
36	258
43	132
47	122
48	93.7
For comparison	
3b	0.206
5	0.752
VN/85-1	0.125

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

Table 2. GI_{50} of C-17 Modified Compounds

compd	GI_{50} (μM)[a]
3b	1.97
5	3.35
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} values 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%.

25) and aromatic groups (benzyl, **28**; dimethoxybenzyl, **31**) resulted in significant loss of ARD and anticancer activities (GI_{50s} of 18.31, 22.13, and >100 μM, respectively; Table 3).

C-3 Modification. In an attempt to better understand the role played by OH and O in the ARD/antiproliferative activities of compounds **5** and **32** and to possibly achieve enhanced interaction with Arg in the AR ligand biding domain, we designed, synthesized, and tested a number of C-3 modified analogues. First, oxidation of **5** or reductive alkylation of **32** to give 3-oxo-Δ^5 compound **33** or 3-hydroxy-3-methyl compound **40**, respectively, led to significant loss (~5-fold) of antiproliferative activity (Table 4). Conversion of compound **5** to the mesyl (**34**) and tosyl (**35**) derivatives also gave compounds with mediocre antiproliferative activities, with GI_{50} values of

Table 3. GI_{50} of C-16 Modified Compounds

compd	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%.

Table 4. GI_{50} of C-3 Modified Compounds

compd	GI_{50} (μM)[a]	compd	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
36*E*	2.03	**45**	8.22
36*Z*	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} values 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 because of insolubility in ethanol.

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**) analogues exhibited GI_{50} values of 1.91, 3.38, and 5.57 μM, respectively. We could not assess the biological activities of the phenyloxime (**37**) because of its limited solubility in ethanol or DMSO. Considering the promising and superior activity of an E/Z mixture of oxime **36** and the possibility that the pure E and Z had different antiproliferative activities, we were surprised that **36*E*** and **36*Z*** isomers exhibited similar potencies, with GI_{50} values of 2.03 and 1.95 μM, respectively.

On the basis of known ester based anticancer drugs, such as docetaxel and cabazitaxel,[39] and esters in clinical development such as bevirimat and related analogues,[28] we first synthesized three pyridinecarboxylate derivatives of compound **5**, including **41**–**43**. Of these compounds, the isonicotinoyl derivative **43** exhibited similar antiproliferative activity (GI_{50} = 2.57 μM) as **5**. Here again, we could not assess the biological activities of compounds **41** and **42** because of their limited solubilities in ethanol or DMSO. The related analogues tethered to the lipophilic ester side chain with a carboxylic acid terminus (**44**–**46**) exhibited potencies ~2.5-fold worse than compound **5**. Finally, we considered evaluation of C-3 carbamates because of (1) the precedence of drugs with carbamate moieties such as the widely used anthelmintics albendazole, fenbendazole, and mebendazole;[40] (2) the added feature of lowering the lipohilicity of compound **5**, which should also increase solubilities and perhaps physiological relevance.[41] Of the three heteroaryl carbamates tested, the imidazolyl 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

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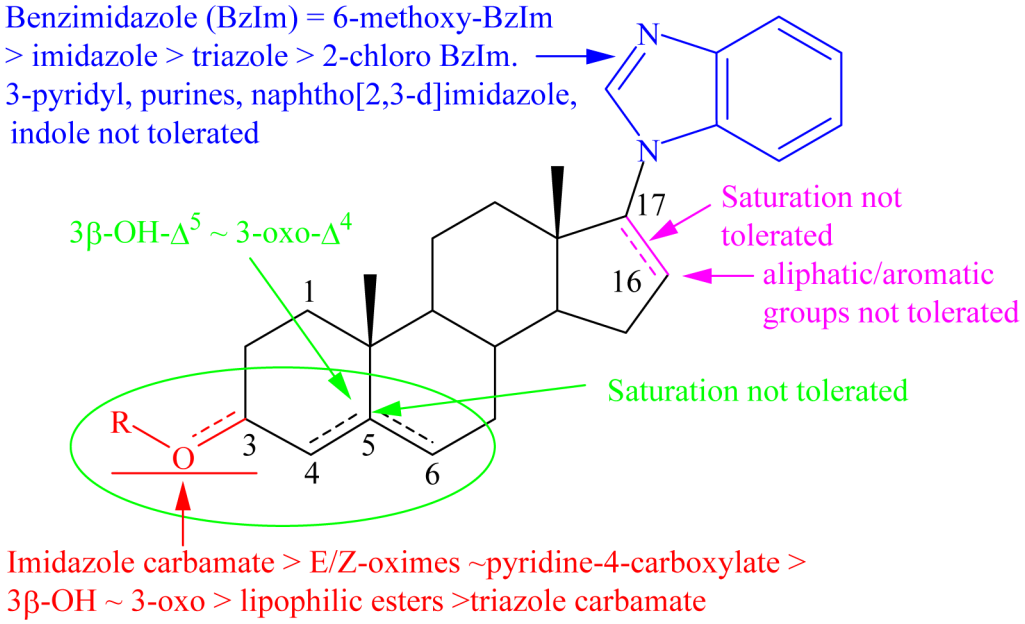

Figure 7. In vitro SAR of 3,16/17-substituted steroid derivatives as androgen receptor degrading agents..

2^1-methyl as in carbamate **48** caused a 6-fold decrease in activity relative to **47**, similar to ~8-fold decrease in activity.

CONCLUDING REMARKS

Our study has shown that potent antiproliferative (AP) and AR down-regulating (ARD) activities can be retained in compound **5** by modification of the 3β-OH to appropriate carbamate (**47**). We also establish that the C-17 benzimidazole group is essential and optimal for both AP and ARD activities and substituents tethered to C-16 are not tolerated. A summary of the in vitro structure−activity relationship (SAR) of these steroidal compounds as androgen receptor degrading agents (ARDAs) is presented in Figure 7. Importantly, we show that binding affinity to the LBD of AR is not essential for potent AP/ARD activities and that some of our compounds also exhibit exquisite depletion of both full-length and splice variant ARs. The significance of these novel findings has important implications because our novel compounds, including galeterone currently in phase 2 clinical trials in prostate cancer patients, can prevent androgen receptor activation by any known means. Although we have yet to conduct in vivo anti-prostate tumor efficacy studies with our carbamate compound **47**, on the basis of the strong promising data presented in this study and also that the carbamate moiety plays an important role in medicinal chemistry, being found in many drugs and prodrugs,[28,39,42] we strongly believe that **47** is a strong candidate for further development as a potential drug for the treatment of all forms of prostate cancer in humans. Consequently, in vivo antitumor efficacy evaluations of compound **47** and other promising compounds in castration resistant models of prostate cancer are in progress.

EXPERIMENTAL SECTION

Chemistry. 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 spectrometer. ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker 400 or 500 MHz spectrometer. High-resolution mass spectra were obtained on a Bruker 12 T APEX-Qe FTICR-MS instrument by positive ion ESI mode by Susan A. Hatcher, Facility Director, College of Sciences Major Instrumentation Cluster, Old Dominion University, Norfolk, VA. Epiandrosterone acetate and all other chemicals and reagents were purchased from Sigma-Aldrich. Dihydrotestosterone (DHT) used in the biological experiments was synthesized following our recently reported procedure.[43] Tritiated [^{3}H]R1881 was purchased from Perkin-Elmer LAS, while MDV3100 was purchased from Sequoiq Resrach Products Ltd., Pangbourne, U.K. Compounds **3a** and **3b** were synthesized in our lab. 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). Preparative TLC was performed using silica gel GF (Analtec 500 μm) plates. Petroleum ether refers to light petroleum, bp 40−60 °C. The purity of all final compounds was determined to be at least 95% pure by a combination of HPLC, NMR, and HRMS.

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.[21]

General Method A: Synthesis of 3β-Acetoxy-17-(1*H*-heteroaryl-1-yl)-16-formylandrosta-5,16-dienes (14, 16a−18a, 19a1, 19a2, 20a, and 22a).[12a] A 25 mL round-bottom 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), the corresponding heteroaryl (3 mmol), and K_2CO_3 (0.41 g, 3 mmol) in dry DMF (~7.5 mL), and the mixture 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 FCC [petroleum ether/EtOAc/TEA (6:4:0.3)] gave the desired pure compounds. The above listed intermediate compounds were synthesized (using reactants, reagent, and solvent ratio), isolated, and purified by using this method unless otherwise stated.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (14). Compound **14** was prepared by following general method A, reacting **13** (2.5 g, 6.65 mmol) with benzimidazole (2.35 g, 19.9 mmol) in the 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.[12a]

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3β-Acetoxy-17-(3-formyl-1*H*-indol-1-yl)-16-formylandrosta-5,16-diene (16a). Compound **16a** was prepared by following general method A, reacting **13** (1 g, 2.66 mmol) with indole-3-carbaldehyde (0.5 g, 3.44 mmol) in the 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).

3β-Acetoxy-17-(5, 6-dimethyl-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (17a). Compound **17a** was 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 the 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).

3β-Acetoxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (18a). Compound **18a** was prepared by following general method A, reacting **13** (0.5 g, 1.33 mmol) with 5(6)-nitrilebenzimidazole[23] (0.38 g, 2.65 mmol) in the 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).

3β-Acetoxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (19a1) and 3β-Acetoxy-17-(5-methoxy-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (19a2). Compounds **19a1** and **19a2** were 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 the 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 the less polar 6-methoxy derivative (**19a1**) and subsequently the more polar 5-methoxy derivative (**19a2**).

19a1. Yield, 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).

19a2. Yield, 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** was also collected (overall yield is 61%).

3β-Acetoxy-17-(1*H*-benzo[*f*]benzimidazol-1-yl)-16-formylandrosta-5,16-diene (20a). Compound **20a** was prepared by following general method A, reacting **13** (0.38 g, 1 mmol) with 1*H*-benzo[*f*]benzimidazole (0.2 g, 1.2 mmol) in the 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).

3β-Acetoxy-17-(6-Chloro-9*H*-purin-9-yl)-16-formylandrosta-5,16-diene (21a).[22] 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 was 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 recrystallization with hot ethanol gave 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).

3β-Acetoxy-17-(2-chloro-1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (22a). Compound **22a** was prepared by following general method A, reacting **13** (0.5 g, 1.33 mmol) with 2-chlorobenzimidazole (0.6 g, 4.0 mmol) in the 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. The organic layer was 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).

General Method B: Synthesis of 3β-Acetoxy-17-(1*H*-heteroaryl-1-yl)androsta-5,16-dienes (15, 16b−21b). A solution of 3β-acetoxy-17-(1*H*-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 the mixture was cooled 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. The above listed intermediate compounds were synthesized (using reactants, reagent, and solvent ratio), isolated, and purified by using this method unless otherwise stated.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (15). Compound **15** was 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, which provided pure **15** with identical spectral and analytical data as we previously reported.[12a]

3β-Acetoxy-17-(1*H*-indol-1-yl)androsta-5,16-diene (16b). Compound **16b** was 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) were added, and the mixture was 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).

3β-Acetoxy-17-(5,6-dimethyl-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (17b). Compound **17b** was 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.

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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 × 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).

3β-Acetoxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)androsta-5,16-diene (18b). Compound **18b** was prepared by following general method B, refluxing **18a** (0.15 g, 0.31 mmol) with 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).

3β-Acetoxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)androsta-5,16-diene (19b). Compound **19b** was 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 was added, and the mixture was 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).

3β-Acetoxy-17-(1*H*-benzo[*f*]benzimidazol-1-yl)androsta-5,16-diene (20b). Compound **20b** was 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, 3β-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).

3β-Acetoxy-17-(6-Chloro-9*H*-purin-9-yl)androsta-5,16-diene (21b). Compound **21b** was 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. After the mixture was cooled to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated and carried to the next step without purification.

3β-Acetoxy-17-(2-chloro-1*H*-benzimidazol-1-yl)androsta-5,16-diene (22b). A solution of 3β-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 the mixture was cooled 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, $CDCl_3$) δ 1.05 (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).

General Method C: Synthesis of 3β-Hydroxy-17-(1*H*-heteroaryl-1-yl)androsta-5,16-dienes (5, 16−22) and 3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylamino)methyl)androsta-5,16-dienes (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 and monitored by TLC. Reaction mixture was concentrated under vacuum. Ice−water (100 mL) was added, and the resulting white precipitate was filtered, washed with water, and dried. FCC was done on a short silica gel column, eluting with petroleum ether/EtOAc (6:4) to obtain pure target compounds. The above listed final compounds were synthesized (using reactants, reagent, and solvent ratio), isolated, and purified by using this method unless otherwise stated.

3β-Hydroxy-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (5).[12a] Compound **5** was 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 a short column provided pure **5** with identical spectral and analytical data as we previously reported. Previously unreported ^{13}C NMR data **5**: ^{13}C NMR (400 MHz, $CDCl_3$) δ147.1, 143.1, 141.6, 141.3, 134.5, 124.1, 123.4, 122.5, 120.9, 120.1, 111.1, 71.4, 55.8, 50.5, 47.2, 42.2, 37.1, 36.7, 34.8, 31.6, 31.1, 30.3, 30.3, 20.6, 19.3, 16.0.

3β-Hydroxy-17-(1*H*-indol-1-yl)-androsta-5,16-diene (16). Compound **16** was 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 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 ($C_{27}H_{33}ON{\cdot}Na^+$), found 410.2460.

3β-Hydroxy-17-(5, 6-dimethyl-1*H*-benzimidazol-1-yl)-androsta-5,16-diene (17). Compound **17** was 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 a 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 × 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 ($C_{28}H_{36}ON_2{\cdot}Na^+$), found 439.2726.

3β-Hydroxy-17-(5(6)-nitrile-1*H*-benzimidazol-1-yl)androsta-5,16-diene (18). Compound **18** was 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 a 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 ($C_{27}H_{31}ON_3H^+$), found 414.2532.

3β-Hydroxy-17-(6-methoxy-1*H*-benzimidazol-1-yl)androsta-5,16-diene (19). Compound **19** was 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 a 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 ($C_{27}H_{34}O_2N_2{\cdot}Na^+$), found 441.2507.

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3β-Hydroxy-17-(1*H*-benzo[*f*]benzimidazol-1-yl)androsta-5,16-diene (20). Compound **20** was 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.

3β-Hydroxy-17-(6-chloro-9*H*-purin-9-yl)androsta-5,16-diene (21). Compound **21** was 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 a short column [DCM/methanol/TEA (9.7:0.3:0.05)] gave 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

3β-Hydroxy-17-(2-chloro-1*H*-benzimidazol-1-yl)androsta-5,16-diene (22). Compound **22** was 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)] gave 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.

General Method D: Synthesis of 3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylimino)methyl)androsta-5,16-dienes (23, 26, and 29). The title compounds were prepared by refluxing a solution of 3β-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 and concentrated under vacuum. The residue was stirred with water and the resulting crude product filtered. Purification by the FCC on silica gel column [petroleum ether/EtOAc (1:1)] gave the desired pure compounds. The above listed compounds were synthesized (using reactants, reagent, and solvent ratio), isolated, and purified by using this method unless otherwise stated.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-(isopentylimino)methyl)androsta-5,16-diene (23). Compound **23** was 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 h. Purification followed by FCC gave 0.41 g (89%) of **23**: 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 (400 MHz, $CDCl_3$) 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).

General Method E: Synthesis of 3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((alkyl/arylamino)methyl)-androsta-5,16-dienes (24, 27, and 30). To an ice cold solution of 16-enamines (**23**/**26**/**30**) (1 mol equiv) in methanol was added $NaBH_4$ (0.5 mol equiv) in three portions over 30 min. Reaction continued for 1.5−5 h and then neutralized with acetic acid. The mixture was evaporated and the residue treated with water and filtered. Crude product was carried to the next step without purification.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((isopentylamino)methyl)androsta-5,16-diene (24). Compound **24** was 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 the next step without purification.

3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((isopentylamino)-methyl)androsta-5,16-diene (25). Compound **25** was 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 3 h. Purification followed by passage through a short silica bed [DCM/ethanol (9.5:0.5)] gave **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 510.345 09.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-(phenylimino)methyl)androsta-5,16-diene (26). Compound **26** was prepared by following synthetic method D, refluxing **14** (0.15 g, 0.33 mmol), aniline (0.06 g, 0.65 mmol), and 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%) of **26**: 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).

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((phenylamino)-methyl)androsta-5,16-diene (27). Compound **27** was 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** was carried to the next step without purification.

3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-((phenylamino)-methyl)androsta-5,16-diene (28). Compound **28** was prepared by following general method C, treating methanolic solution (1 mL) of crude acetate **27** with 10% methanolic KOH (0.75 mL) for 3 h. Purification followed by passage through a 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

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-((*EZ*)-((3,4-dimethoxyphenyl)imino)methyl)androsta-5,16-diene (29). Compound **29** was prepared by following general method D, refluxing **14** (0.3 g, 0.65 mmol), 3,4-dimethoxyaniline (0.2 g, 1.3 mmol), and molecular sieves (0.075 g) in ethanol (2 mL) overnight. Purification by FCC [petroleum ether/EtOAc (1:1)] gave 0.29 g (74.5%) of **29**: 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 × 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).

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-(((3,4-dimethoxyphenyl)amino)methyl)androsta-5,16-diene (30). Compound **30** was prepared by following general synthetic method

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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** was carried to the next step without purification.

3β-Hydoxy-17-(1*H*-benzimidazol-1-yl)-16-(((3,4-dimethoxyphenyl)amino)methyl)androsta-5,16-diene (31). Compound **31** was 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)] gave **31** (0.11 g, 79.6%): 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 × 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 ($C_{35}H_{43}O_3N_3{\cdot}Na^+$), found 576.3188.

17-(1*H*-Benzimidazol-1-yl)androsta-4,16-dien-3-one (32). This compound was prepared from **5** as previously described, providing spectral and analytical data as reported.[12a] ^{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.

17-(1*H*-Benzimidazol-1-yl)androsta-5,16-dien-3-one (33). To an 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 ($C_{26}H_{30}ON_2{\cdot}Na^+$), found 409.2258.

3β-Mesyloxy-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (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 and then at room temperature for 8 h and quenched with 75 mL of ice−water. 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.[6] 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.

3β-Tosyloxy-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (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 was stirred at 0 °C for 5 h and then at room temperature for 3 h and quenched with 30 mL of ice−water. The resulting yellow solid was filtered, washed, and 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 was 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 ($C_{33}H_{38}O3N_2S{\cdot}Na^+$), found 565.2506.

General Method F: Synthesis of 3-(Substituted-oximino)-17-(1*H*-benzimidazol-1-yl)androsta-4,16-dienes (36−39). To a refluxing solution of ketone **32** (1 mol equivalent) in ethanol−methanol (2:1) solvent mixture was added a solution of sodium acetate (9.4 mol equiv) and the corresponding substituted oxamine hydrochloride (10.5 mol equiv) in distilled water (10 mol equiv). Reflux continued for 2−3 h. Then the mixture was concentrated. The residue was treated with water and the crude product filtered. Purification FCC over silica using 5% ethanolic DCM gave pure oximes.

3-((*EZ*)-Hydroximino)-17-(1*H*-benzimidazol-1-yl)androsta-4,16-diene (36). Compound **36** was prepared by following general method F. To a refluxing solution of **32** (0.08 g, 0.194 mmol) in ethanol−methanol (2 mL) was added a solution of sodium acetate (0.15 g, 1.83 mmol) and hydroxylamine·HCl (0.07 g, 2.04 mmol) in 0.75 mL of distilled water. The reflux continued for 2 h, and subsequent purification by FCC gave compound **36** (mixture of *EZ* isomers) (0.06 g, 77%): 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) (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 ($C_{26}H_{31}ON_3{\cdot}Na^+$), found 424.2363.

Separation of *E* and *Z* Isomers of 36. Initially the *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 in pure single isomer **36*E***: mp 218−221 °C; ^1H 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. **36*Z*** was further purified by preparative TLC using petroleum ether/EtOAc (1:1) as solvent system: mp 158−162 °C; ^1H 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.

3-((*EZ*)-*O*-Phenyloxime)-17-(1*H*-benzimidazol-1-yl)androsta-4,16-diene (37). Compound **37** was prepared by following general method F. To a refluxing solution of **32** (0.05g, 0.13 mmol) in ethanol−methanol (2 mL) was added a solution of sodium acetate (0.1 g, 1.22 mmol) and phenoxamine·HCl (0.2 g, 1.35 mmol) in 0.5 mL of distilled water. The reflux continued for 2 h, and subsequent purification by FCC gave compound **37** (mixture of *EZ* isomers) (0.04 g, 64%): mp 96−98 °C; IR (neat) 2935, 2854, 1627, 1590, 1487, 1216, 897 cm^{-1}; ^1H 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,

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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 ($C_{32}H_{35}ON_3{\cdot}Na^+$), found 500.2677.

3-((*EZ*)-*O*-Methyloxime)-17-(1*H*-benzimidazol-1-yl)androsta-4,16-diene (38). Compound **38** was prepared by following general method F. To a refluxing solution of **32** (0.075g, 0.194 mmol) in ethanol−methanol (2 mL) was added a solution of sodium acetate (0.15 g, 1.83 mmol) and methoxyamine·HCl (0.17 g, 2.04 mmol) in 0.75 mL of distilled water. The reflux continued for 3 h, and subsequent purification by FCC gave compound **38** (mixture of *EZ* isomers) (0.072 g, 89%): mp 94−96 °C; IR (neat) 2935, 2854, 1628, 1489, 1452, 1226, 1050, 743 cm^{-1}; ^{1}H 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 ($C_{27}H_{33}ON_3{\cdot}Na^+$), found 438.2520.

3-((*EZ*)-(*O*-Phenylmethyl)oxime)-17-(1*H*-benzimidazol-1-yl)-androsta-4,16-diene (39). Compound **39** was prepared by following general method F. To a refluxing solution of **32** (0.075g, 0.194 mmol) in ethanol−methanol (2 mL) was added a solution of sodium acetate (0.15 g, 1.83 mmol) and benzyloxyamine·HCl (0.33 g, 2.04 mmol) in 0.75 mL of distilled water. The reflux continued for 3 h, and subsequent purification by FCC gave compound **39** (mixture of *EZ* isomers) (0.092 g, 96%) which solidifies on storage: sinters 66−68 °C, melts 77−79 °C; IR (neat) 2935, 2854, 1627, 1609, 1489, 1452, 1225, 1015, 864 cm^{-1}; ^{1}H 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 ($C_{33}H_{37}ON_3{\cdot}Na^+$), found 514.2834.

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 and then at 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}; ^{1}H 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 ($C_{27}H_{34}ON_2{\cdot}Na^+$), found 425.2570.

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 pyridinecarboxylic 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 5 min. 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 was absorbed on silica and purified by FCC using 2% ethanol in DCM in the 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 ^{1}H NMR and HRMS analyses revealed that the presence of other esters derived from 2-methyl-6-nitrobenzoic anhydride is absent.

3β-(Pyridine-2-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (41). Compound **41** was 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}; ^{1}H 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; 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 ($C_{32}H_{35}O_2N_3{\cdot}Na^+$), found 516.2614.

3β-(Pyridine-3-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (42). Compound **42** was 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}; ^{1}H 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 ($C_{32}H_{35}O_2N_3{\cdot}Na^+$), found 516.2617.

3β-(Pyridine-4-carboxylate)-17-(1*H*-benzimidazol-1-yl)-androsta-5,16-diene (43). Compound **43** was 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}; ^{1}H 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 ($C_{32}H_{35}O_2N_3{\cdot}Na^+$), found 516.2615.

3β-(3-(Oxycarbonyl)phenylacetic acid)-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (44). Compound **41** was prepared by following general method G. 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}; ^{1}H 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 ($C_{36}H_{40}O_4N_2{\cdot}Na^+$), found 587.2876

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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 h. The mixture was cooled to room temperature, and the reaction was quenched with 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 ($C_{34}H_{40}N_2O_4{\cdot}Na^+$), found 563.2879.

3β-(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 h. The mixture was cooled to room temperature, and the reaction was quenched with 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}; ^1H 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 ($C_{30}H_{36}N_2O_5{\cdot}Na^+$), found 527.2516.

3β-(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) was stirred at room temperature for 2 h. Then the solvent was evaporated and the 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 the 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}; ^1H 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 ($C_{30}H_{34}O_2N_4{\cdot}Na^+$), found 505.2577.

3β-(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) and 1,1-carbonylbis(2-methylimidazole) (0.05 g, 0.214 mmol) in anhydrous acetonitrile (1.5 mL) and DCM (0.75 mL) was refluxed overnight. The solvent was evaporated. The residue was 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 the 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 ($C_{31}H_{36}O_2N_4{\cdot}Na^+$), found 519.2730.

3β-(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 3 h. The solvent was evaporated and the 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 the 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 ($C_{29}H_{33}O_2N_5{\cdot}Na^+$), found 506.2525.

Biology Experiments. Cell Culture. LNCaP cells were purchased from American Type Culture Collection (ATCC; Rockville, MD, U.S.). Cells were maintained in ATCC recommended culture medium with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA, U.S.) 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 °C. CWR22rv1 cells are a gift from Dr. Marja Nevalainen of Thomas Jefferson University, Philadelphia, PA.

Cell Growth Inhibition (MTT Colorimetric Assay). The cells were seeded in 96-well plates (Corning Costar) at a density of 5×10^3 cells per well. Cells were allowed to adhere to the plate for 24 h and then treated with various concentrations of compounds dissolved in 95% EtOH. Cells were treated for 7 days with renewal of test compound and medium on day 4. On the seventh day, medium was renewed and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) (Sigma, St. Louis, MO, U.S.) solution (0.5 mg of MTT per mL of medium) was added to the medium such that the ratio of MTT/medium was 1:10. The cells were incubated with MTT for 2 h. 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.).

Transcriptional Activation Luciferase Assay. LNCaP cells were transferred to steroid-free medium 3 days before the start of the experiment and plated at 1×10^5 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*.

Western Blot Analysis. For immunoblot detection of various proteins, LNCaP or CWR22v1 prostate cancer cells were cultured. Cells were treated with indicated compounds, and whole cell lysates were prepared as described in ref 44 using RIPA lysis buffer (Sigma Aldrich) and protease and phosphatase inhibitors (Sigma Aldrich). All

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of the antibodies were ordered from Cell Signaling Technology. Protein content was determined using the Bradford assay (Bio-Rad, Hercules, CA, U.S.). Protein was subjected to SDS−PAGE and transferred onto nitrocellulose membrane. Membranes were then incubated with secondary antibody (Cell Signaling Technology) at room temperature for 1 h. 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, U.S.). 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, MD.[33c]

Androgen Receptor Competitive Binding Assay. Competitive binding assays were performed with the synthetic androgen methyltrienolone [^{3}H]R1881 essentially as described by Wong et al. and Yarbrough et al.[45] Wells in 24-well multiwell dishes were coated with poly L-lysine (0.05 mg/mL) for 30 min, rinsed with sterilized, distilled water, and dried for 2 h. To determine the kinetics of [^{3}H]R1881 binding to the LNCaP, AR cells were plated ($(2-3) \times 10^5$ 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 1 μM triamcinolone acetonide to saturate progesterone and glucocorticoid receptors. Following a 2 h 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 were analyzed, and K_d and B_{max} determined, 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 to 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).

Immunocytochemical Analysis. LNCaP cells were plated in eight-chamber vessel tissue culture treated glass slide (0.025×10^6 cells/well) for 12 h and then treated with 5 μM VN/124-1 or VNPT55 for 48 h. Cells were washed twice with PBS and fixed in 3.7% formaldehyde for 10 min and permeabilized with 0.25% Triton in PBS for another 5 min after several washes. Cells were 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 1 h with secondary antibody Alexa Fluor 488 conjugate anti-rabbit IgG(H+L) at 1:1000 (Cell Signaling) and nuclear counterstain for 5 min (DAPI at 1:5000). All images were taken using the Nikon TE2000 microscope.

CYP17 Inhibition Assay. The assay was kindly performed by Dr. Emily Scott and colleagues according to their recently reported procedure in which a truncated version of human CYP17A1 (CYP171dH) was expressed in *E. coli* and then purified to homogeneity.[37] IC_{50} values of the compounds were determined from dose−response curves. 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[12a,21]) were also determined in the same assay system for comparison (used as positive controls).

■ ASSOCIATED CONTENT

Supporting Information

HPLC chromatograms and high resolution mass spectral data for compounds **16**−**22**, **25**, **28**, **31**, and **33**−**49**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): Vincent C. O. Njar is the lead inventor of compound **5** patents and technologies thereof owned by the University of Maryland, Baltimore, and licensed to Tokai Pharmaceuticals, Inc. The other authors declare no potential conflict of interest. A patent application to protect the novel compounds has been filed.

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■ ABBREVIATIONS USED

AR, androgen receptor; ARD, androgen receptor down-regulation; AP, antiproliferative; BzIm, benzimidazole; BSA, bovine serum albumin; CRPC, castration resistant prostate cancer; CYP, cytochrome P450; CYP17A, 17α-hydroxylase/17,20-lyase; DAPI, 6-diamino-2-phenylindole; DHT, dihydrotestosterone; DBPS, Dulbecco's phosphate buffered saline; ESPC, early stage prostate cancer; FBS, fetal bovine serum; FCC, flash column chromatography; GI_{50}, compound concentration required to inhibit cell growth by 50%; HRMS, high-resolution mass spectrometry; hAR, human androgen receptor; IC_{50}, compound concentration required to inhibit cell growth by 50%; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; PDB, Protein Data Bank; SDS, sodium dodecyl sulfate

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