Journal of Medicinal Chemistry

Drug Annotation

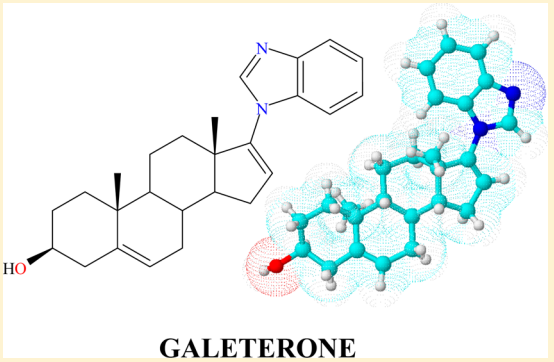
pubs.acs.org/jmc

Discovery and Development of Galeterone (TOK-001 or VN/124-1) for the Treatment of All Stages of Prostate Cancer

Vincent C. O. Njar*[,†,‡,§] and Angela M. H. Brodie[†,§]

[†]Department of Pharmacology, [‡]Center for Biomolecular Therapeutics, and [§]Marlene Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, Maryland 21201-1559, United States

ABSTRACT: In our effort to discover potent and specific inhibitors of 17α-hydroxylase/17,20-lyase (CYP17), the key enzyme which catalyzes the biosynthesis of androgens from progestins, 3β-(hydroxy)-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (Galeterone or TOK-001, formerly called VN/124-1) was identified as a selective development candidate which modulates multiple targets in the androgen receptor (AR) signaling pathway. This drug annotation summarizes the mechanisms of action, scientific rationale, medicinal chemistry, pharmacokinetic properties, and human efficacy data for galeterone, which has successfully completed phase II clinical development in men with castration resistant (advanced) prostate cancer (CRPC). Phase III clinical studies in CRPC patients are scheduled to begin in early 2015.



GALETERONE

INTRODUCTION

On the basis of the success of aromatase inhibitors (AIs, inhibitors of CYP19, the enzyme that catalyzes the conversion of androgens to estrogens) in the treatment of postmenopausal women with breast cancer,[1] we decided that the same approach might be used to target the inhibition of androgen synthesis involved in prostate cancer development and proliferation. We were also inspired by the realization that the development of new aromatase inhibitors might no longer be an attractive research endeavor because, at the time, four novel aromatase inhibitors, including 4-hydroxyandrostenedione (formestane), letrozole, arimidex, and exemestane were already approved for clinical use.[2] This early work resulted in our early publications in this field of research.[3]

In this drug annotation, we tell the drug discovery story and the interesting challenges that were overcome by outlining a medicinal chemistry case study of galeterone, describing the disease target(s), mechanism(s) of action, and the scientific rationale for advancing galeterone to clinical trials in men with prostate cancer (Figure 1).

STRUCTURE

Galeterone, 3β-hydroxy-17-(1*H*-benzimidazole-1-yl)androsta-5,16-diene (Figure 2), is a 17-heteroazole steroidal analogue scheduled to enter phase 3 clinical trial for castration resistant prostate cancer (CRPC).

DISEASE TARGET

Prostate cancer (PC) is the most commonly diagnosed cancer and the second leading cause of cancer-related death in American men. It is estimated that approximately 240,000 new cases of prostate cancer will be diagnosed and that nearly 30,000 prostate cancer-related deaths will occur in the United States in 2014.[5] PC is often diagnosed at a stage when it is restrained to the prostate gland and its immediate surroundings. At this stage, PC is treated either with prostatectomy (surgical removal of the prostate gland) or with radiotherapy. For most men, these procedures are successful in curing the disease. However, for some men, these procedures are not curative, and their prostate cancer continues to spread to other organs of the body. Men whose disease progresses following surgery or radiation are considered to have advanced prostate cancer.[6]

PC growth and progression are stimulated by androgens acting through the androgen receptor (AR). Androgen levels are mainly regulated through the hypothalamic−pituitary−adrenal/gonadal axis (Figure 3). Because androgens, predominantly testosterone (T) and dihydrotestosterone (DHT), are the primary fuels of advanced prostate cancer growth, the first-line therapy for advanced prostate cancer typically entails treatment with a class of drugs known as luteinizing hormone releasing hormone (LHRH) agonists, which reduce testosterone to castrate levels. Another class of marketed hormonal drugs, known as antiandrogens, block the binding of T and DHT to the AR.[7]

Nevertheless, resistance to these therapies usually occurs, rendering them ineffective over time. Approximately 60% of PC patients are expected to have tumor recurrence after five years despite the use of androgen deprivation therapy. This stage of PC is referred to as castration resistant prostate cancer (CRPC).[7] In CRPC patients, tumor growth occurs even while circulating androgens remain at castrate levels because tumor cells develop the capacity for alternative androgen

Received: August 12, 2014

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

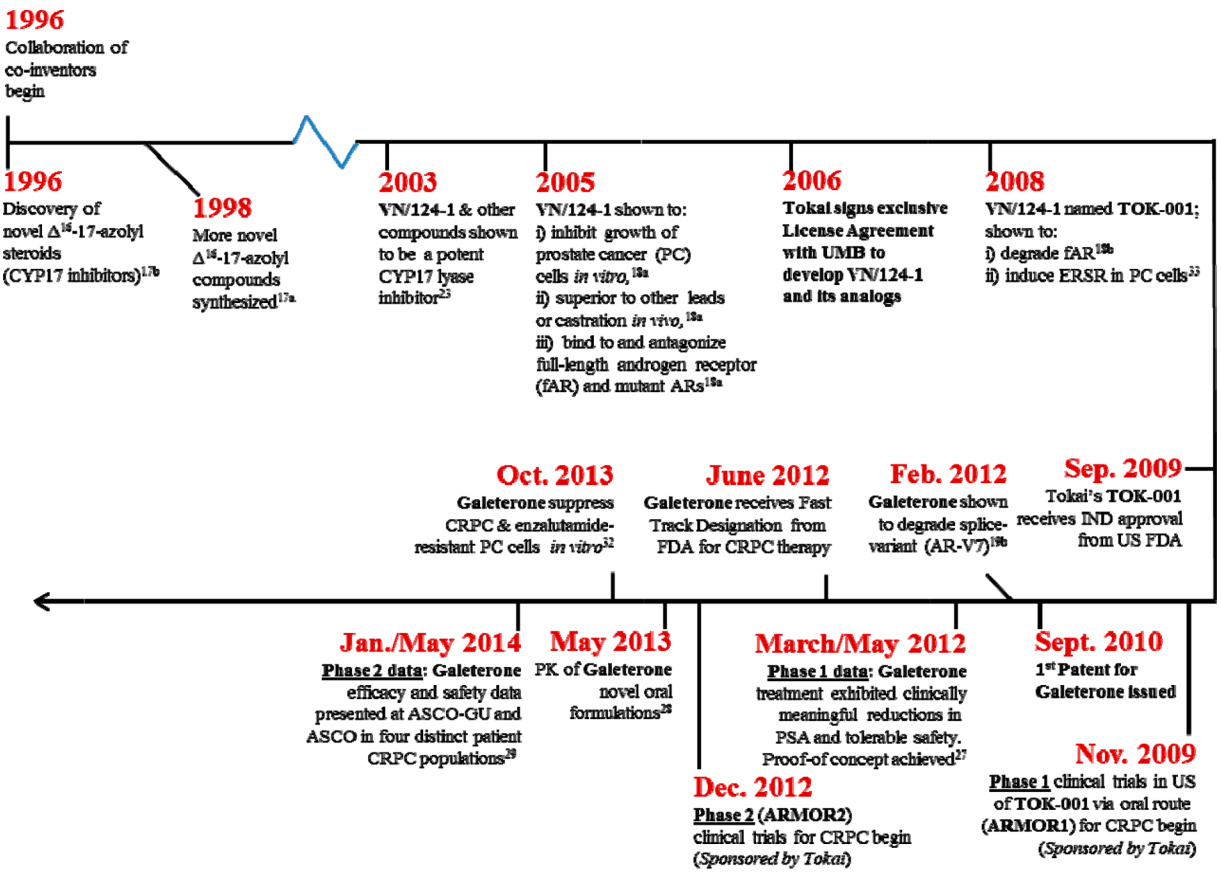


Figure 1. Important research and commercial/clinical milestones in the discovery and development of galeterone: (UMB = University of Maryland, Baltimore).

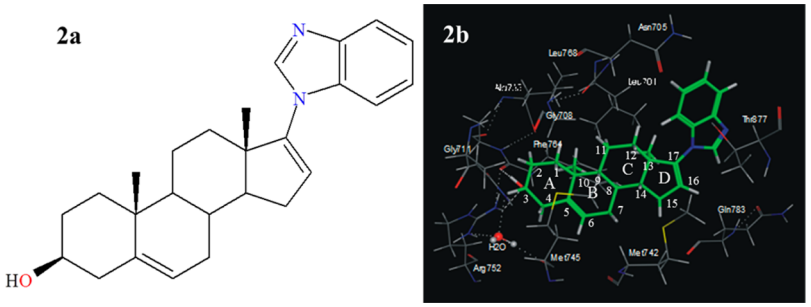


Figure 2. (a) 2-D structure of galeterone. (b) Stereo view of the binding mode of galeterone docked in the active site of human androgen receptor (hAR).[4] Stereo view of the binding mode of galeterone (cap, green) in the active site of hAR. The active site residues in the binding pocket are shown in stick model with element colors and active site bound water molecule in ball−stick model with element color. Hydrogen bonding interaction between the hydroxyl group of ligand-Gly711 and Phe764 is shown as white dots, and within the amino acids, the water-amino acid is also shown as white dashed lines.

biosynthesis. In addition, the cells learn how to thrive in the presence of very low androgen levels, due in part to AR overexpression. Since 2010, the US Food and Drug Administration (FDA) has approved two new antihormonal agents for the treatment of metastatic CRPC: abiraterone acetate (**2**, Zytiga) which is administered with prednisone, and enzalutamide (**3**, Xtandi) (Figure 4).[8] Each drug disrupts a part of the AR signaling pathway, the key driver of CRPC. It should be noted that following oral administration of prodrug, **2**, the release of abiraterone (**1**) is very rapid.[9] Compound **1** inhibits CYP17 to block androgen production, while **3** competitively blocks androgen binding to the AR. Compound **3** is a second generation antiandrogen shown to be more efficacious than bicalutamide (**5**, Casodex) (Figure 4), a widely used first generation antiandrogen.[7,10] However, there is a strong need for drugs with improved efficacy and safety profiles to treat early stage, hormone sensitive prostate cancer and CRPC. In this respect, it is worth noting that an analogue of compound **3**, i.e., ARN-509 (**6**) (Figure 4),[11] a potent AR antagonist is currently undergoing Phase 2 trials in patients with biochemically relapsed, hormone-sensitive prostate cancer (NCT01790126). In addition, another potent nonsteroidal inhibitor of CYP17, TAK-700 (Orteronel) (**7**) (Figure 4),[12] advanced rapidly to Phase 3 trials in patients with metastatic, castration-resistant prostate cancer (mCRPC).[13] Disappointingly, analysis of the Phase 3 data indicated that **7** plus

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

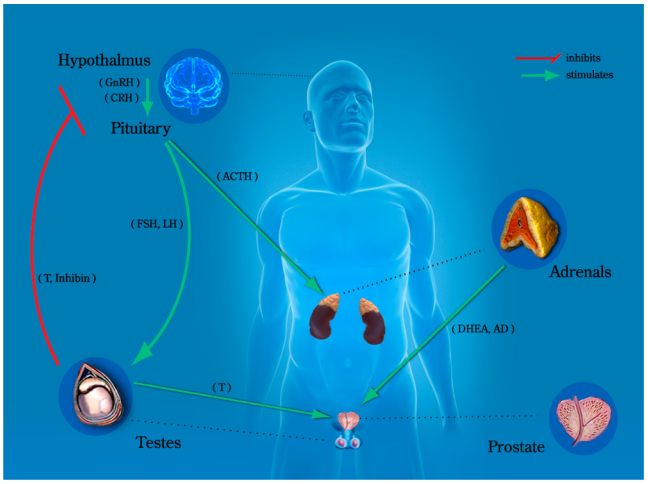


Figure 3. Endocrine control of prostatic growth.[6] The growth and development of the normal prostate requires a functioning androgen signaling pathway, which is regulated by the hypothalamic–pituitary–adrenal/gonadal axis. Androgens [testosterone (T), androstenedione (AD), and dehydroepiandrosterone (DHEA)] and other steroids are synthesized in the testes or adrenal glands and released into the circulation in response to specific hormonal signals [follicle stimulating hormone (FSH), gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), and luteinizing hormone releasing hormone (LHRH)]. Testosterone is transported by steroid hormone binding globulin (SHBG) to the prostate, where it is predominantly converted by 5α-reductase to its more active metabolite, 5α-dihydrotestosterone (DHT). The adrenals are stimulated to produce AD and DHEA by adrenocorticotropic hormone, released by the pituitary. Note: Circulating AD and DHEA are precursors of T which is mostly converted to DHT. Reprinted with permission from ref 6. Copyright 2011 Elsevier.

prednisone did not meet the primary end point of improved overall survival (OS) when compared to that of the control arm.[13] Thus, further development of compound **7** has been terminated.[11b,14]

SCIENTIFIC RATIONALE FOR BRINGING GALETERONE TO THE CLINIC

Because of the modest clinical success of ketoconazole (**4**) (Figure 4), an FDA approved antifungal drug and a modest CYP17 inhibitor),[15] we became interested in generating potent and specific inhibitors of CYP17 with the hope of eliminating the side effects (attributed to the promiscuous inhibition of several cytochrome P450 enzymes) of ketoconazole. The design of our steroidal CYP17 inhibitors was based on the structures of the natural substrates (progesterone and pregnenolone) of CYP17 with modifications mainly at the 17,20-side chain (azole heterocycles and other nitrogen containing moieties) and D-ring; sites at or close to the positions which may interact with the enzyme's active site (i.e., α-orientations at C-16, C-17, and C-20).[3b,16] We envisioned that these substrate analogues will interact with the steroid binding site of CYP17 in addition to coordination with the enzyme's heme iron resulting in highly specific and tight binding inhibitors (Figure 5).[3b,16,17]

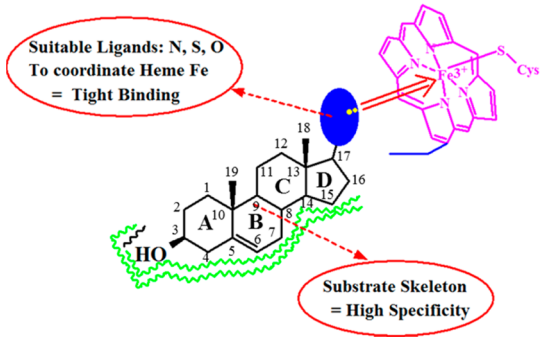


Figure 5. Proposed model for the CYP17/inhibitor complex.

Over the course of our studies, several lead CYP17 inhibitors have been discovered. Critical to this work was the invention of a novel nucleophilic vinylic "addition–elimination" substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene

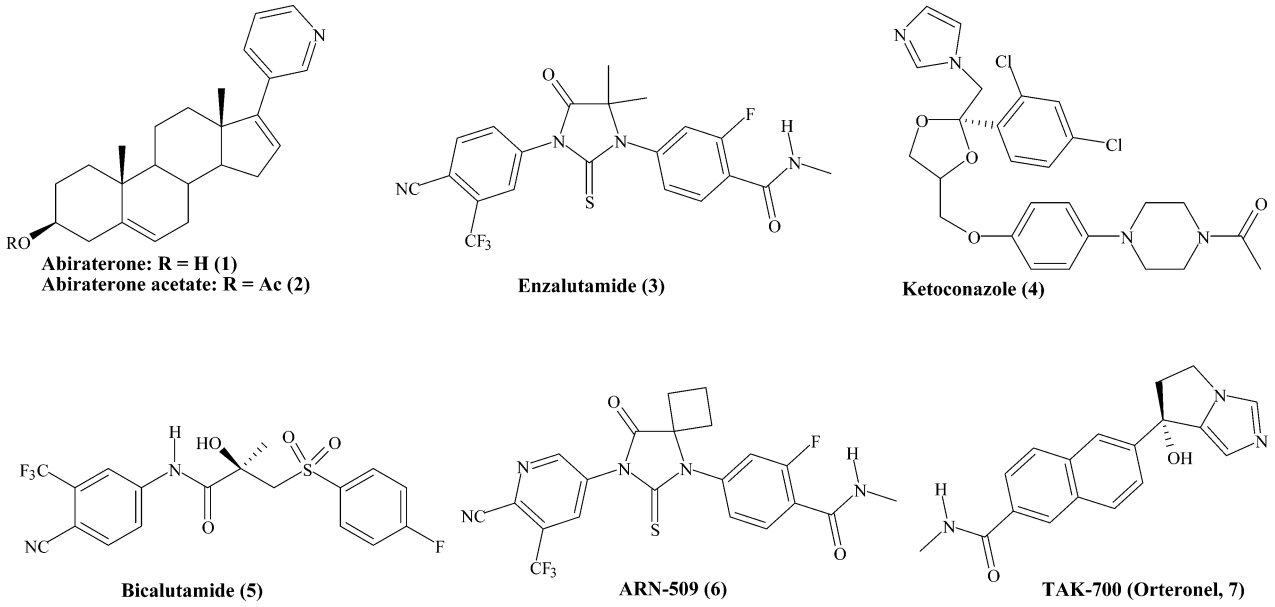


Figure 4. Structure of FDA approved antihormonal drugs.

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX–XXX

(**8**), to facilitate the synthesis of unique steroidal Δ^{16}-17 azoles (Figure 6).[17b] The uniqueness stems from the fact that the

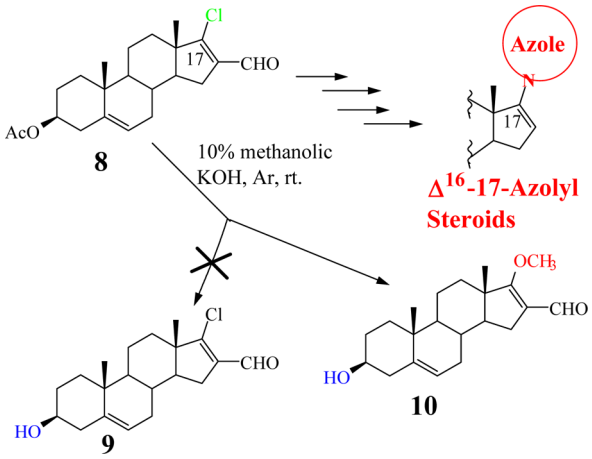


Figure 6. Key nucleophilic vinylic "addition–elimination" substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**8**).

azole moiety is tethered to the steroid scaffold through N1 of the azole, hitherto unknown C-17 steroidal azoles. As with most important discoveries, this high-yield and expeditious reaction was discovered serendipitously,[17b] where intended hydrolysis of compound **8** to give the corresponding 3β-hydroxy-17-chloro-16-formylandrosta-5,16-diene (**9**), instead provided 3β-hydroxy-17-methoxy-16-formylandrosta-5,16-diene (**10**) in quantitative yield, pointing to the possibility that the chlorine in compound **8** could be readily displaced by various nitrogen nucleophiles under appropriate reaction conditions (Figure 6).

Two other important findings were (i) the discovery that our lead CYP17 inhibitors were potent antagonists of the AR with activities that were superior to those of compound **5**[18] and (ii) that our CYP17 inhibitors were also AR degrading agents (ARDAs).[18b,19] Following many *in vivo* antitumor efficacy studies, galeterone was identified as the *first example of an antihormonal agent* [an inhibitor of androgen synthesis (CYP17 inhibitor)/antiandrogen/AR degrading agent (ARDA)] that is significantly more efficacious than castration in the suppression of androgen-dependent prostate tumor growth.[18,19] Because of its impressive anticancer properties, galeterone was selected as a clinical candidate.

STRUCTURE–ACTIVITY RELATIONSHIP LEADING TO GALETERONE: KEY CHALLENGES

On the basis of our design strategy described above and summarized in Figure 5, we set out to synthesize several steroidal compounds tethered with the 1,2,4-triazole moiety or azide at steroidal C-20 and in some cases with the introduction of 17β-hydroxyl or the 16/17 double bond (**11**–**16**) (Figure 7). However, the realization that these initial set of compounds were *mediocre CYP17 inhibitors* suggested to us the importance of regio-architecture to the production of potent CYP17 inhibitors. This finding was also unexpected because we had previously shown that 20-amino and 20,21-aziridinyl pregnene steroids were potent inhibitors of CYP17.[3b]

Following our discovery of a general method (*vide supra*) for introducing the Δ^{16}-17 azole functionality into ring D (Figure 6),[17b] we readily synthesized a variety of novel Δ^{16}-17 azole

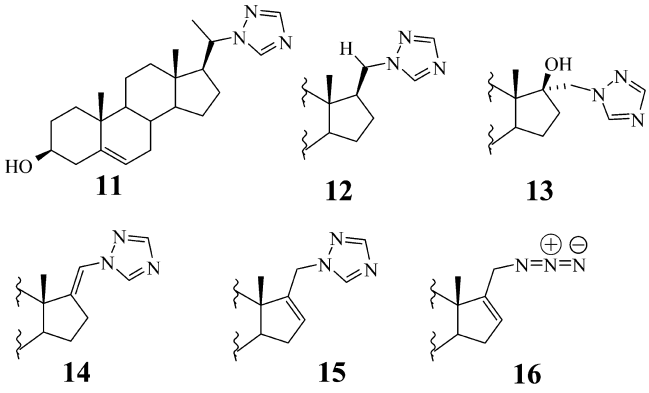


Figure 7. C-20 1,2,4-triazole and azide steroids.

steroidal compounds (**17**–**26**) and related analogues containing a saturated D-ring (**27** and **28**) (Figure 8).[17a]

Evaluation of these compounds for the inhibition of human CYP17 enzyme (Table 1) revealed that whereas the imidazole and triazole compounds (**17**, **18** **20**, and **24**–**26**) were potent inhibitors of CYP17, the 1*H*-pyrazole (**23**) was a modest inhibitor and that unexpectedly, the 2*H*-1,2,3-triazole (**19**) and the two tetrazole regioisomers (**21** and **22**) were noninhibitory. Compounds with the corresponding Δ^4-3-one moieties (**24**–**26**) were also potent CYP17 inhibitors. Because these Δ^{16}-17-azole compounds of Figure 8 are structurally similar, the striking difference in their CYP17 inhibitory activities may be due to the differences in their basicity profiles, properties imposed by the inherent different electronic character of each of the azole heterocycles. These data also strongly suggested that the presence of a nitrogen atom at either the 3′ or 4′ position seems important for potent inhibition of the enzyme. The requirement for a 16,17-double bond also descended from the observation that compounds **27** and **28** (without Δ^{16}) exhibited diminished potency compared to that of the corresponding parent Δ^{16} compounds, **17** and **18**, respectively. We also determined that bulky groups were not tolerated at the 3β position.[17a]

In other studies not presented here, we also showed that compound **18** was a slow-binding inhibitor of CYP17.[17a] Using UV–vis difference spectroscopy, we demonstrated that most of these inhibitors induced a type II difference spectrum, indicating the coordination of steroidal nitrogen to the heme iron of the CYP17 enzyme.[17]

On the basis of CYP17 inhibitory activities, compounds **17**, **18**, and **20** were selected as *lead compounds* and were each subjected to rigorous pharmacological studies in models of human prostate cancer *in vitro* and *in vivo*. In these preclinical biological studies, we clearly established that the lead compounds, especially, **18** and **20**, possessed several anticancer properties that target the AR. These include (i) CYP17 inhibition to block androgen (T and DHT) synthesis *in vivo*,[20] (ii) direct competitive binding to the ligand binding domain (LBD) of AR and concomitant AR antagonism,[17a,21] and (iii) significant growth inhibition of LNCaP human prostate tumor xenografts.[20a,21c] In addition, the pharmacokinetics in mice of these lead compounds was also determined.[22]

A critical review of our data highlighted the realization that our best leads were only as potent as castration in suppression of androgen-dependent prostate tumor growth. This realization led to the design, synthesis, and evaluations of a new class of potent CYP17 inhibitors/antiandrogens, with the emergence of

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX–XXX

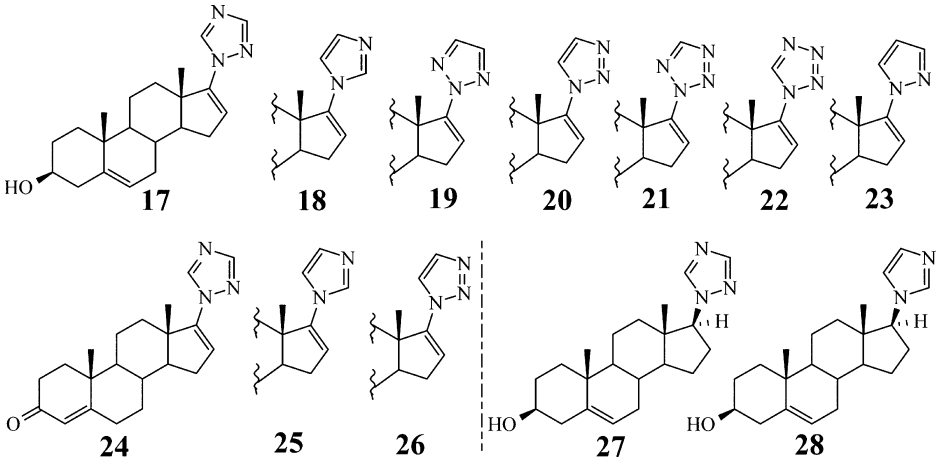


Figure 8. Variety of Δ^{16}-17 azolyl steroids and related compounds (compounds **18**–**23** have the same steroid scaffold as **17**, while compounds **25** and **26** have the same scaffold as **24**).

Table 1. Inhibition of Human CYP17 by Azolyl Compounds[a]

compds[b]	% inhibition[c]	IC_{50} (nM)[d]	K_i (nM)[e,f]
17	60	90	23.0
18	97	8	1.2
19	NI	-	-
20	94	13	1.4
21	NI	-	-
22	NI	-	-
23	40	-	-
24	-	55	41
25	-	7	1.9
26	-	19	8
27	-	219	-
28	-	62	-
for comparison **4**	67	78	38

[a]- = not determined. NI = no inhibition up to 10 μM. [b]Each inhibitor concentration was 150 nM for the initial screening assays. [c]Concentration of substrate, 17α-hydroxypregnenolone = 10 μM. [d]Mean ± SDM of at least two experiments. [e]K_i values were determined as described previously.[17a] [f]K_m for substrate, 17α-hydroxypregnenolone = 560 nM.

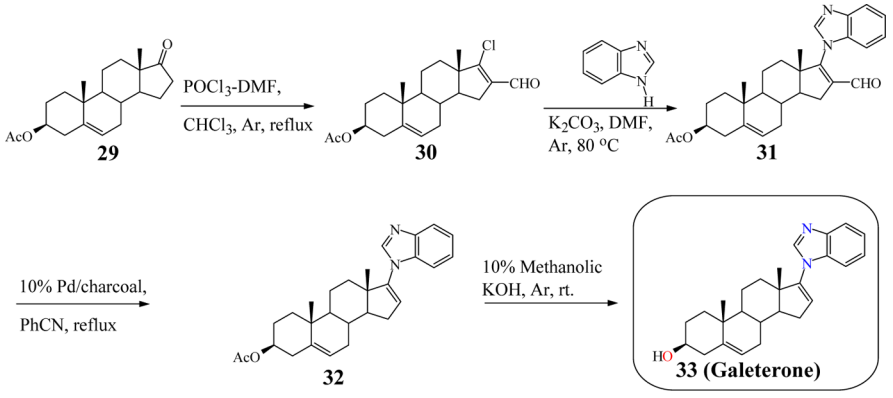
the clinical candidate galeterone (**33**).[18a,23] The stimulus for preparing this class of C-17 heteroaryl steroids was based on the desire to incorporate benzimidazole, benzotriazole, pyrimidine, and pyrazine moieties, the so-called "privileged sub-structures,"[24] in the new molecules. These scaffolds, especially the benzimidazole scaffold, continue to receive extensive attention in medicinal chemistry because of their diverse portfolio of biological activities and because they are entities of a variety of useful drugs.[24]

CHEMISTRY

The synthesis of galeterone is presented in Scheme 1. The key intermediate in our synthesis of galeterone, 3β-acetoxy-17-chloro-16-formylandtrosta-5,16-dine (**30**), was obtained by our routine procedure as previously described starting from the commercially available 3β-acetoxyandrost-5-en-17-one (**29**) (Scheme 1).[17,18] Treatment of **30** with benzimidazole in the presence of K_2CO_3 in DMF at approximately 80 °C gave the desired 3β-acetoxy-17-1*H*-benzimidazole (**31**) in near quantitative yield. Compound **31** was smoothly deformylated with 10% palladium on activated charcoal in refluxing benzonitrile to give compound **32** in 93% yield, from which hydrolysis gave the required 3β-hydroxy 17-benzimidazole (**33**) (VN/124-1; galeterone).

It is important to state here that a facile and large scale good manufacturing practice (GMP) synthesis/production of galeterone was developed in 2009 by Tokai Pharmaceuticals Inc. but is yet to appear in the literature. We are also aware that the commercial synthetic procedure retains the basic tenets of the original procedure.[17,18] The major changes of the GMP

Scheme 1. Synthesis of Galeterone



DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

process involve the purification and isolation of the intermediates and the final product.[18a]

IN VITRO PHARMACOLOGY

Utilizing intact-cells CYP17 expressing *E. coli*,[21a] galeterone was shown to be a potent inhibitor of the enzyme with an IC_{50} value of 300 nM, being 6-fold less potent than our previously reported compound **18** (IC_{50} = 50 nM).[23] Under the same assay conditions, abiraterone had an IC_{50} value of 800 nM. However, unlike abiraterone, galeterone was subsequently found to disrupt androgen signaling through multiple targets.[18] The increased efficacy of galeterone in several prostate cancer models both *in vitro* and *in vivo* is believed to arise from its ability to downregulate the AR as well as competitively block androgen binding. In competitive binding studies against the synthetic androgen [^{3}H]R1881, galeterone (EC_{50} = 845 nM) was equipotent to bicalutamide (EC_{50} = 971 nM) in LNCaP cells but had a slightly higher affinity for the wild-type receptor in PC3-AR cells (galeterone, EC_{50} = 405 nM versus casodex EC_{50} = 4,300 nM). Transcriptional activation assays utilizing a luciferase reporter showed galeterone to be a pure AR antagonist of the wild-type AR and the T877A mutation found in LNCaP cells.[18] In prostate cancer cell lines, galeterone inhibited the growth of CRPCs (IC_{50} = 2.9 μM), which had increased AR expression and were no longer sensitive to bicalutamide (IC_{50} = 18 μM).[25] In addition, galeterone demonstrated superior synergy for growth inhibition in combination with everolimus or gefitinib compared with bicalutamide.[25]

IN VIVO PHARMACOLOGY [PHARMACOKINETICS (PK) ANTI-TUMOR EFFICACY] IN PRECLINICAL SPECIES

The pharmacokinetic properties in male SCID mouse for galeterone were studied following our previously described procedure for other CYP17 inhibitors.[20a,22] The results are summarized in Table 2.

Table 2. Pharmacokinetic Parameters for Galeterone (50 and 100 mg/kg) in Mouse after s.c.[a] Administration

	galeterone	
parameter[b]	50 mg/kg	100 mg/kg
$t_{1/2}$ (min)	44.17 ± 1.15	36.6 ± 1.6
K_{el} (min^{-1})	56.5 ± 0.94	68.49 ± 1.26
AUC (min·μg/mL)	1440.00 ± 60.23	1813.94 ± 10.94
T_{max} (min)	30.00 ± 0.0	30.00 ± 0.0
C_{max} (μg/mL)	16.82 ± 0.37	32.23 ± 0.34
MRT (min)	65.40 ± 0.60	60.46 ± 1.54
V_d (mL/kg)	2098.99 ± 4.11	3276.39 ± 26.71

[a]Galeterone was formulated in 100 μL of vehicle (40% β-cyclodextrin in water). [b]Values are expressed as the mean ± SE, n = 5.

On reverse phase HPLC, galeterone [retention time (rt) = 21.6 min] was well resolved from the internal standard (**18**, rt =11.5 min), a metabolite (rt =17.3 min), and other endogenous compounds in mouse plasma. The calibration curves derived for galeterone were linear and reproducible (data not shown), the inter- and intra-assay variability was less than 10%, and its limit of detection was 100 ng/mL. The HPLC assay was validated and used to monitor galeterone in mice plasma.

Following subcutaneous administration (s.c.), the plasma concentration of galeterone declined exponentially with a mean half-life of about 44.17 min and an elimination rate constant of 56.5 min^{-1}. Galeterone was cleared at a rate of 1986.14 mL/h/kg from the systemic circulation and was not detected 6 h after administration. The calculated noncompartmental pharmacokinetic parameters based on the plasma concentration profile following subcutaneous administration of galeterone are shown in Table 2. After s.c. administration of galeterone, the observed plasma concentration in mice reached peak levels 30.0 min post-dose. Galeterone is well absorbed from the subcutaneous site, and the area under the curve for the plasma concentration versus time profiles after s.c. administration increased proportionately to dose as the administration dose was changed from 50 to 100 mg/kg. Furthermore, the elimination half-life, and mean residence time were relatively constant as the dose of galeterone was increased from 50 to 100 mg/kg (Table 2). These results indicate that the pharmacokinetic profile of galeterone is dose independent.

Galeterone (0.15 mmol/kg, s.c. twice daily) caused a 93.8% reduction (P = 0.00065) in the mean final LAPC-4 xenograft volume compared with that of the controls (Figure 9), and this

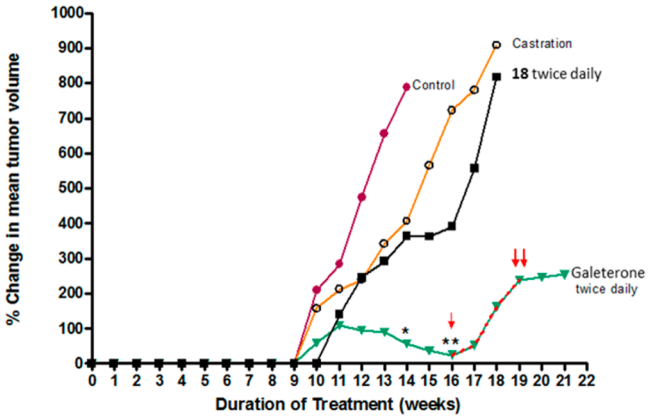


Figure 9. Effects of galeterone and compound **18**, and castration on the formation and growth of LAPC4 prostate tumors in male SCID mice. Three × 10^7 LAPC-4 cells were injected s.c. into the dorsal flank of SCID mice. One group of mice was castrated. The other groups of mice received the vehicle, galeterone (0.15 mmol/kg twice-daily), or **18** (0.15 mmol/kg twice-daily). Daily treatment with galeterone or **18** was initiated 1 day after cell inoculation. Tumors volumes were measured weekly, and the percentage of change in tumor volume was determined after 16 weeks of treatment. * indicates significant difference of galeterone versus the control, castration, and **18** at week 14 (P = 0.00065, 0.05, and 0.0097, respectively). ** indicates significant difference of galeterone versus castration and **18** at week 16 (P = 0.047 and 0.0047, respectively). ↓−↓↓: period of reduced administration dose of galeterone. At ↓, the dose was reduced by 78.6%, and after ↓↓, the usual dose was resumed.

efficacy was significantly more effective than castration or treatment with compound **18**, one of our early lead compounds with a 6-fold higher CYP17 inhibitory potency than galeterone.[18a] At the time of this study, we speculated that the superior antitumor efficacy of galeterone could in part be due to its better pharmacokinetics and or pharmacodynamics properties. Another notable aspect of this study was the observation, for the first time, of an antihormonal agent (an inhibitor of androgen synthesis (CYP17 inhibitor)/antiandrogen) that was significantly more effective than castration.[18a] On

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

the basis of these impressive preclinical data, galeterone and related compounds were licensed to Tokai Pharmaceuticals Inc., Cambridge, MA who subsequently conducted advanced preclinical studies to enable the ongoing clinical development (*vide infra*).

We note that since this initial impressive antitumor study reported in 2005, three other independent antitumor efficacy studies of galeterone have been reported by our groups. In the report by Vasaitis et al.,[18b] we showed that galeterone (0.13 mmol/kg, s.c., twice daily) was very effective in preventing the formation of LAPC4 tumors (6.94 versus 2410.28 mm^3 in the control group). Galeterone (0.15 mmol/kg, s.c., twice daily) plus castration induced *regression* of LAPC-4 tumor xenografts by 26.55% and 60.67%, respectively. In the latter study, we measure AR protein levels within the tumors from control and treated animals and found that treatment with galeterone caused marked depletion of AR protein expression. Interestingly, the xenograft data also showed that galeterone was more efficacious than either casodex or castration. In this report, by using cyclohexamide to inhibit new protein synthesis and measuring the rate of AR degradation, we also determined that galenterone's down-regulation of the AR level was, in part, due to increased AR degradation. In another study,[26] we also demonstrated that galeterone plus everolimus acted in concert to reduce castration-resistant prostate cancer (HP-LNCaP) tumor growth rates. Finally, in 2011 using an *in vivo* androgen-dependent LAPC-4 human prostate cancer xenograft model,[19a] we demonstrated for the first time that galeterone was more efficacious than abiraterone acetate (**2**) or castration. As observed previously,[18b] treatment with galeterone caused marked depletion of AR protein expression.

These preclinical studies enabled us to ascertain the mechanisms of action of galeterone. Specifically, galeterone inhibits CYP17, antagonizes AR, and induces degradation of AR (Figure 10). Indeed, this suggests that this multitarget approach would reduce the risk of patients developing resistance to therapy and may translate to an advantage in progression-free survival and overall survival. The above studies clearly demonstrate that, galeterone is a first-in-class, multitarget, oral small molecule for the treatment of prostate cancer that disrupts AR signaling via three distinct mechanisms of action.

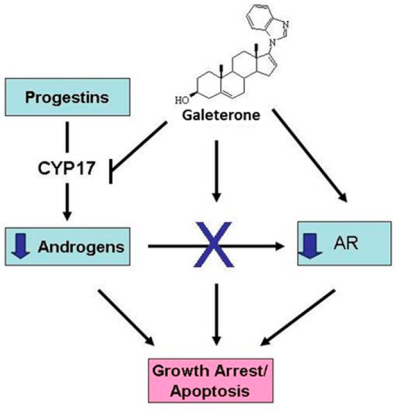


Figure 10. Multiple mechanisms of galeterone inhibition along the androgen axis. Galeterone can inhibit androgen signaling at three separate points. (1) Inhibition of CYP17 systemically blocks production of androgens from all tissues. (2) The molecule prevents binding of androgens to the AR and (3) induces degradation of the receptor.

SAFETY PHARMACOLOGY AND PHARMACEUTICAL PROPERTIES

Rigorous studies directed by Tokai Pharmaceuticals Inc. were conducted to establish the pharmaceutical properties and safety of galeterone. These studies although not available in the literature were deemed adequate [*IND 104,183 for* **33** *(TOK-001 or galeterone): Safe to Proceed*] and enabled the granting of Investigation New Drug (IND) status to galeterone by the US Food and Drug Administration on September 4, 2009.

CLINICAL TRIALS WITH GALETERONE: FIRST IN HUMAN STUDIES

On the basis of the preclinical studies reviewed above and favorable toxicology and pharmacokinetics, an IND application for galeterone was approved by the FDA for Phase 1 trials with an orally administered galeterone, as a powder in capsule (PIC).

Tokai's clinical development program for the evaluation of galeterone in patients with prostate cancer is designated ARMOR (androgen receptor modulation optimized for response). The ARMOR1 clinical trial was a 49 patient, multicenter, open-label, dose-escalation study of galeterone that utilized PIC formulations for the treatment of chemotherapy naïve nonmetastatic and metastatic CRPC (http://clinicaltrials.gov/show/NCT00959959). Co-administration of prednisone was not required, and there were no events of adrenal mineralocorticoid excess (AME), a clinical syndrome that is commonly associated with other CYP17 inhibitors, were observed in the study. Overall, galeterone was well tolerated. Approximately 90% of treatment emergent adverse events were of grade 1 or grade 2 in severity and were generally manageable and reversible. Prostate specific antigen (PSA) reductions were seen in a majority of patients: 24 (49%) patients had ≥30% maximum PSA reductions, including 11 patients (22%) with ≥50% maximal PSA reductions. Furthermore, tumor reductions, another sign of antitumor activity were observed radiographically. In summary, galeterone was well tolerated, with all cohorts showing an acceptable safety profile. Indeed, this was the first proof-of-concept study for galeterone in patients with CRPC.[27] On the basis of these promising Phase 1 data, galeterone received the Fast Track designation from the FDA for the potential treatment of CRPC.

A significant food effect, similar to abiraterone, was found when galeterone, as a PIC, was dosed in a phase I CRPC trial and confirmed in a PK study in human volunteers. Following reformulation of galeterone into a spray dried dispersion formulation,[28] the ARMOR2 clinical trial (~136 patients, multicenter study) was initiated as a two-part Phase 2 study to confirm the recommended Phase 2 dose of reformulated galeterone (Part 1) and to demonstrate safety and efficacy at the selected 2550 mg dose for Part 1 in four distinct CRPC patient cohorts (Part 2) (http://clinicaltrials.gov/show/NCT01709734). Following the conclusion of Part 1 of the study, a once-daily galeterone dose of 2550 mg was selected for Part 2, based on safety, efficacy, and pharmacokinetic data. Data from this ongoing study, as presented during ASCO 2014 showed that for 51 patients followed for at least 12 weeks across all doses, 82% achieved a PSA decline of at least 30% (PSA30), and 75% achieved a PSA decline of at least 50%

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

(PSA50). In metastatic treatment naïve CRCP, data from 21 patients showed that 85% achieved a PSA30 and that 77% achieved a PSA50. Furthermore, initial data in abiraterone refractory patients showed both biochemical PSA activity and stable disease after 12 weeks of dosing. Galeterone, at the recommended dose was safe and well tolerated in 87 patients with CRPC.[29] Approximately 90% of all treatment emergent adverse events were of grade 1 or 2 in maximum severity and were generally manageable and reversible. Results from the ongoing Phase 2 trial will be used to guide the strategy for pivotal Phase 3 trials planned for initiation in early 2015. In summary, a total of 107 CRPC patients were dosed at the target dose of 2550 mg of galeterone. Recruitment for Phase 2 trial has been closed, and treatment with galeterone therapy is ongoing for patients still on the study.

FOLLOW-ON PRE-CLINICAL STUDIES WITH GALETERONE

Follow-on preclinical studies can provide important data that can have profound impact on ongoing clinical drug development programs. This strategy represents a *"laboratory-to-clinic-to-laboratory-to-clinic"* drug discovery and development program. Indeed, this has been the case with some highly significant follow-on studies with galeterone. These include the following: (i) On the basis of the findings that patients in the Phase 1 study with galeterone did not experience events of AME, a clinical syndrome that is commonly associated with other CYP17 inhibitors, a comprehensive study was conducted by Tokai researchers to assess the impact of galeterone and other clinically used CYP inhibitors on CYP17 (17α-hydroxylase and 17,20-lyase activities) and global steroidogenesis.[30] Galeterone, unlike the other three inhibitors tested, was shown to be a selective and potent CYP17 lyase inhibitor and exhibited minimal evidence of deleterious steroid changes associated with mineralocorticoid excess (ME).[30] These preclinical data recapitulated Phase 1 clinical experience where no ME was observed. (ii) To improve drug exposure and eliminate the significant food effect seen when galeterone was administered as a PIC, an oral formulation was developed that had improved oral bioavailability and favorable pharmacokinetics.[28] (iii) The unexpected finding that galeterone also degrades splice variant androgen receptors (AR-3/AR-V7 and AR^{v568s})[19b] which are up-regulated in CRPC and in patients resistant to abiraterone and enzalutamide[31] has impacted the design of parts of pending Phase 3 clinical trials. (iv) Finally, in a collaboration between Tokai and researchers at Vancouver Prostate Centre, Canada, it was recently reported that galeterone suppressed castration-resistant and enzalutamide-resistant prostate cancer growth *in vitro*. Galeterone also blocked nuclear translocation and decreased AR dependent genes (PSA, TMPRSS2, and Nkx3.1) expressed in CRPC and enzalutamide-resistant cell lines.[32] Together, these studies strongly suggest that galeterone may represent the next generation of antihormonal therapy for patients with multiple stages of prostate cancer, including CRPC, drug-resistant prostate cancer, and tumors expressing AR splice variants.

CONCLUSIONS

Galeterone is a first-in-class, multitarget, oral small molecule in development for the treatment of prostate cancer. Galeterone acts by disrupting AR signaling via three independent points in the pathway. This unique small molecule has the potential to change the therapeutic management of CRPC. Galeterone selectively inhibits CYP17 lyase activity to prevent androgen synthesis, antagonizes androgen binding to the AR, and causes degradation of both full-length and splice variant ARs. Galeterone was discovered in a collaboration between a medicinal chemist and a pharmacologist/cancer biologist, who set out some 18 years ago to discover and develop potent inhibitors of CYP17 inspired by the clinical success of the aromatase (CYP19) inhibitors in breast cancer patients.[1a] Through this collaboration, one of us has over the years established a competitive and comprehensive laboratory with expertise not only in medicinal chemistry but also in oncopharmacology and cancer biology.

The mechanisms of the anticancer effects of galeterone are not completely understood, but it has been shown to impact multiple targets that disrupt AR signaling. Its actions selectively alter the transcription of genes and the function of proteins that are regulators of proliferation, migration, and the death of malignant cells. Galeterone was invented by hypothesis-driven research based on the knowledge that androgens are the key drivers of prostate cancer cell growth. Its design was also based on the knowledge of the mechanism of action of CYPs and in particular CYP17 and with benefit from intuitive medicinal chemistry knowledge and strategies.[3b,16,17,19b] Although the initial approach was to identify small molecules (potential drugs) with high specificity for inhibition of CYP17, we followed the scientific data acquired during the course of our research that enabled the identification of other desirable antiprostate cancer targets, namely, AR antagonism and AR degradation. Other potentially clinically relevant targets of galeterone continue to emerge.[33]

Galeterone also teaches us another important lesson. The goal in drug discovery and development is to create a new drug with an acceptable therapeutic index and favorable medicinal and drug-like properties. Whereas galeterone has a 6-fold lower CYP17 inhibitory potency, compared to our most potent CYP17 inhibitor, **18**, the latter compound exhibited only a modest *in vivo* antiprostate tumor efficacy in a head-to-head study with galeterone.[18a] Thus, although galeterone has a lower potency for CYP17 inhibition used in the early screens, we believe that galeterone's two other mechanisms of action, i.e., AR antagonism and AR degradation, led to an overall more efficacious agent.

Since we first reported that AR degradation contributes to antitumor efficacy of galeterone,[18b] several academic and corporate laboratories have attempted to synthesize small-molecule androgen receptor degrading agents (ARDAs). However, it remains to be determined whether a molecule with specific ARDA activity will be therapeutically more useful than a multitarget agent, such as galeterone that disrupts AR signaling, for the treatment of advanced prostate cancer.

EXPERIMENTAL SECTION

Chemistry. General procedures and techniques were identical with those previously reported.[17,18a] Infrared spectra were recorded on a PerkinElmer 1600 FTIR spectrometer using solutions in $CHCl_3$. High-resolution mass spectra (HRMS) were determined on a 3-T Finnigan FTMS-2000 FT mass spectrometer, in ESI mode (The Ohio State University, Department of Chemistry). As a criterion of purity for key target compounds, we provided high resolution mass spectral data with HPLC chromatographic data indicating compound homogeneity. Low-resolution mass spectra (LRMS) were determined on a Finnegan LCR-MS. Melting points (mp) were determined with a Fischer Johns melting point apparatus and are uncorrected. Dehydroepiandrosterone

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

and dehydroepiandrosterone acetate were purchased from Aldrich, Milwaukee, WI. 5-Tributylstannylpyrimidine and 2-tributylstannylpyrazine were purchased from Frontier Scientific, Inc., Logan, UT. The purity of all compounds is as previously reported in our original articles, determined to be at least 95% pure by a combination of HPLC, NMR, and HRMS.

3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (30). This compound, prepared from 3β-acetoxyandrost-5-en-17-one (**29**) as previously described, provided spectral and analytical data as described.[17]

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (31). A mixture of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (**30**, 2.5 g, 6.65 mmol), benzimidazole (2.35 g, 19.9 mmol), and K_2CO_3 (2.76 g, 23.9 mmol) in dry DMF (20 mL) was stirred at ca. 80 °C under Ar for 1.5 h. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (250 mL), and the resulting precipitate was filtered, washed with water, and dried to give a crude dirty white solid (ca. 2.9 g). Purification by FCC [petroleum ether/EtOAc/Et_3N (6:4:0.3)] gave 2.7 g (88.7%) of pure compound **31**: mp 227−230 °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 (300 MHz, $CDCl_3$) δ 1.07 (s, 6H, 18- and 19-CH_3), 2.04 (s, 3H, 3β-OCH_3), 4.60 (m, 1H, 3α-H), 5.43 (br s, 1H, 6-H), 7.35 (br. s, 2H, aromatic-Hs), 7.85 (s, 1H, aromatic-H), 7.98 (s, 1H, aromatic-H), 7.98 (s, 1H, 2^1-H) and 9.59 (s, 1H, 16-CHO). HRMS calcd 481.2462 ($C_{29}H_{34}O_3N_2.Na^+$), found 481.2454.

3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (32). A solution of 3β-acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (**31**, 2.04 g, 4.45 mmol) in dry benzonitrile (10 mL) was refluxed in the presence of 10% palladium on activated charcoal (1.02 g, i.e., 50% weight of **29**) for 5 h. After cooling to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated, and the residue was purified by FCC [petroleum ether/EtOAc/Et_3N (7.5:3:0.5)] to give 1.41 g (73.8%) of pure compound **32**: mp 159−160 °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 (300 MHz, $CDCl_3$) δ 1.02 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 2.04 (s, 3H, 3β-OCH_3), 4.62 (m, 1H, 3α-H), 5.43 (br s, 1H, 6-H), 5.98 (s, 1H, 16-H), 7.30 (m, 2H, aromatic-Hs), 7.49 (s, 1H, aromatic-H), 7.81 (s, 1H, aromatic-H), and 7.95 (s, 1H, 2^1-H). HRMS calcd 453.2512 ($C_{28}H_{34}O_2N_2.Na^+$), found 453.2511.

3β-Hydroxy-17-(1*H*-benzimidazol-1-yl)androsta-5,16-diene (33, Galeterone). The acetate **30** (1.3 g 3.02 mmol) was dissolved in methanol (20 mL) under an inert Ar atmosphere and the resulting solution treated with 10% methanolic KOH (8 mL). The mixture was stirred at room temperature for 1.5 h and then concentrated under reduced pressure at approximately 40 °C to a volume of 10 mL. This solution was poured into ice water (300 mL), and the resulting white precipitate was filtered, washed with water, and dried. Crystallization from EtOAc/MeOH gave **33** (1.10 g, 94%), mp 189−190 °C; IR ($CHCl_3$) 2934, 2339, 1609, 1490, 1453, 1291, 1040, 837, 808, 705, 663, 608, 578, 550, 517 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.02 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 3.55 (m, 1H, 3α-H), 5.41 (br s, 1H, 6-H), 5.99 (s, 1H, 16-H), 7.30 (m, 2H, aromatic-Hs), 7.54 (s, 1H, aromatic-H), 7.80 (s, 1H, aromatic-H), and 7.96 (s, 1H, 2^1-H). HRMS calcd 411.2407 ($C_{26}H_{32}ON_2.Na^+$), found 411.2396.

AUTHOR INFORMATION

Corresponding Author

*Department of Pharmacology, University of Maryland School of Medicine, 685 West Baltimore Street, HSF1, Suite 580E, Baltimore, MD 21201, USA. Tel: (410) 706 6364. Fax: (410) 706 0032. E-mail: vnjar@som.umaryland.edu.

Author Contributions

V.C.O.N. designed and synthesized the novel compounds and wrote the draft manuscript; V.C.N.O. and A.M.H.B. designed and supervised the biological/pharmacological experiments, reviewed, revised, and finalized the manuscript.

Notes

The authors declare the following competing financial interest(s): The authors, Vincent C. O. Njar and Angela M. H. Brodie declare competing financial interest because both of them are co-inventors of galeterone. Galeterone patents and technologies thereof owned by the University of Maryland, Baltimore, have been licensed to Tokai Pharmaceuticals, Inc. Cambridge, Mass, USA. V.C.O.N. (Lead Inventor) and A.M.H.B. also consult for Tokai.

ACKNOWLEDGMENTS

The preclinical studies reviewed in this drug annotation were supported over the years by grants from NIH and NCI (R01 CA027440 (Brodie AMH, PI, Njar VCO co-I); R21 CA117991 (Njar VCO, PI)) and also from Tokai Pharmaceuticals Inc. Follow-on preclinical studies were supported in part by NIH grant R01 CA 129379 (Njar VCO, PI) and grants from Tokai Pharmaceuticals Inc. The clinical studies were sponsored by Tokai Pharmaceuticals Inc. The studies have also been possible by efforts of dedicated graduate students and postdoctoral fellows who are coauthors on our original research and review articles cited in this report. We also thank UMB's Office of Research and Development (ORD) for the intellectual property protection for galeterone and related compounds and also for securing license agreements with Tokai Pharmaceuticals Inc. We sincerely thank the agencies and all the persons that made the invention of galeterone possible. Galeterone could not have advanced to this stage of clinical development without the foresight of Tokai Pharmaceuticals Inc. that dedicated all their resources and efforts to the development of galeterone.

ABBREVIATIONS USED

AD, androstenedione; AI, aromatase inhibitors; ME, mineralocorticoid excess; AME, adrenal mineralocorticoid excess; ARDAs, androgen receptor degrading agents; ARMOR, androgen receptor modulation optimized for response; AR, androgen receptor; CRPC, castration resistant prostate cancer; CYP17, cytochrome P450 17α-hydroxylase, C17,20-lyase; CYP19, cytochrome P45 aromatase; Gal, galeterone; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; FDA, Food and Drug Administration; FCC, flash column chromatography; FSH, follicle stimulating hormone; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; NCI, National Cancer Institute; NIH, National Institutes of Health; PC, prostate cancer; mCRPC, metastatic castration resistant prostate cancer; PIC, powder in capsule; PK, pharmacokinetics; SHBG, steroid hormone binding globulin; T, testosterone

REFERENCES

(1) (a) Brodie, A. M.; Dowsett, M.; Coombes, R. C. Aromatase inhibitors as new endocrine therapy for breast cancer. *Cancer Treat. Res.* **1988**, *39*, 51−65. (b) Chumsri, S.; Howes, T.; Bao, T.; Sabnis, G.; Brodie, A. Aromatase, aromatase inhibitors, and breast cancer. *J. Steroid Biochem. Mol. Biol.* **2011**, *125* (1−2), 13−22. (c) Schneider, R.; Barakat, A.; Pippen, J.; Osborne, C. Aromatase inhibitors in the treatment of breast cancer in post-menopausal female patients: an update. *Breast Cancer* **2011**, *3*, 113−125.

(2) Brodie, A. M. Aromatase, its inhibitors and their use in breast cancer treatment. *Pharmacol. Ther.* **1993**, *60* (3), 501−515.

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

(3) (a) Li, J.; Li, Y.; Son, C.; Banks, P.; Brodie, A. 4-pregnene-3-one-20 beta-carboxaldehyde: a potent inhibitor of 17 alpha-hydroxylase/C17,20-lyase and of 5 alpha-reductase. *J. Steroid Biochem. Mol. Biol.* **1992**, *42* (3−4), 313−320. (b) Njar, V. C.; Hector, M.; Hartmann, R. W. 20-amino and 20,21-aziridinyl pregnene steroids: development of potent inhibitors of 17 alpha-hydroxylase/C17,20-lyase (P450 17). *Bioorg. Med. Chem.* **1996**, *4* (9), 1447−1453.

(4) Pereira de Jesus-Tran, K.; Cote, P. L.; Cantin, L.; Blanchet, J.; Labrie, F.; Breton, R. Comparison of crystal structures of human androgen receptor ligand-binding domain complexed with various agonists reveals molecular determinants responsible for binding affinity. *Protein Sci.* **2006**, *15* (5), 987−999.

(5) Siegel, R.; Ma, J.; Zou, Z.; Jemal, A. Cancer Statistics, 2014. *CA: Cancer J. Clin.* **2014**, *64* (1), 9−29.

(6) Vasaitis, T.; Bruno, R. D.; Njar, V. C. O. CYP17 inhibitors for prostate cancer therapy. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 23−31.

(7) Vasaitis, T. S.; Njar, V. C. Novel, potent anti-androgens of therapeutic potential: recent advances and promising developments. *Future Med. Chem.* **2010**, *2* (4), 667−680.

(8) (a) Logothetis, C. J.; Efstathiou, E.; Manuguid, F.; Kirkpatrick, P. Abiraterone acetate. *Nature Rev. Drug Discovery* **2011**, *10* (8), 573−574. (b) Rehman, Y.; Rosenberg, J. E. Abiraterone acetate: oral androgen biosynthesis inhibitor for treatment of castration-resistant prostate cancer. *Drug Des., Dev. Ther.* **2012**, *6*, 13−18. (c) Semenas, J.; Dizeyi, N.; Persson, J. L. Enzalutamide as a second generation antiandrogen for treatment of advanced prostate cancer. *Drug Des. Dev. Thera.* **2013**, *7*, 875−881. (d) Yin, L.; Hu, Q.; Hartmann, R. W. Recent progress in pharmaceutical therapies for castration-resistant prostate cancer. *Int. J. Mol. Sci.* **2013**, *14* (7), 13958−13978.

(9) Ryan, C. J.; Smith, M. R.; Fong, L.; Rosenberg, J. E.; Kantoff, P.; Raynaud, F.; Martins, V.; Lee, G.; Kheoh, T.; Kim, J.; Molina, A.; Small, E. J. Phase I clinical trial of the CYP17 inhibitor abiraterone acetate demonstrating clinical activity in patients with castration-resistant prostate cancer who received prior ketoconazole therapy. *J. Clin. Oncol.* **2010**, *28* (9), 1481−1488.

(10) Anderson, J. The role of antiandrogen monotherapy in the treatment of prostate cancer. *BJU Int.* **2003**, *91* (5), 455−461.

(11) (a) Clegg, N. J.; Wongvipat, J.; Joseph, J. D.; Tran, C.; Ouk, S.; Dilhas, A.; Chen, Y.; Grillot, K.; Bischoff, E. D.; Cai, L.; Aparicio, A.; Dorow, S.; Arora, V.; Shao, G.; Qian, J.; Zhao, H.; Yang, G.; Cao, C.; Sensintaffar, J.; Wasielewska, T.; Herbert, M. R.; Bonnefous, C.; Darimont, B.; Scher, H. I.; Smith-Jones, P.; Klang, M.; Smith, N. D.; De Stanchina, E.; Wu, N.; Ouerfelli, O.; Rix, P. J.; Heyman, R. A.; Jung, M. E.; Sawyers, C. L.; Hager, J. H. ARN-509: a novel antiandrogen for prostate cancer treatment. *Cancer Res.* **2012**, *72* (6), 1494−1503. (b) Kim, W.; Ryan, C. J.; et al.121 Quo vadis: Advanced prostate cancer-clinical care and clinical research in the era of multiple androgen receptor-directed therapies. *Cancer* **2015**, 361−371.

(12) Kaku, T.; Hitaka, T.; Ojida, A.; Matsunaga, N.; Adachi, M.; Tanaka, T.; Hara, T.; Yamaoka, M.; Kusaka, M.; Okuda, T.; Asahi, S.; Furuya, S.; Tasaka, A. Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer. *Bioorg. Med. Chem.* **2011**, *19* (21), 6383−6399.

(13) Dreicer, J. R.; Qudar S.; et al. Results from a phase 3, randomized, double-blind, multicenter, placebo-controlled trial of orteronel (TAK-700) plus prednisone in patients with metastatic castratio-resistant prostate cancer (mCRPC) that has progressed during or following decetaxel-based therapy (ELM-PC 5 trial). *J. Clin. Oncol.* **2014**, *32* (Suppl 4), Abstract 7.

(14) Takeda announces unblinding of phase 3 study of orteronel in patients with metastatic castration-resistant prostate cancer that progressed post-chemotherapy based on interim analysis [press release]; 2013; http://www.takeda.com/news/2013/20130726_5894.html.

(15) Mahler, C.; Verhelst, J.; Denis, L. Ketoconazole and liarozole in the treatment of advanced prostatic cancer. *Cancer* **1993**, *71* (3 Suppl), 1068−1073.

(16) Njar, V. C.; Brodie, A. M. Inhibitors of 17alpha-hydroxylase/17,20-lyase (CYP17): potential agents for the treatment of prostate cancer. *Curr. Pharm. Des.* **1999**, *5* (3), 163−180.

(17) (a) Njar, V. C.; Kato, K.; Nnane, I. P.; Grigoryev, D. N.; Long, B. J.; Brodie, A. M. Novel 17-azolyl steroids, potent inhibitors of human cytochrome 17 alpha-hydroxylase-C17,20-lyase (P450(17) alpha): potential agents for the treatment of prostate cancer. *J. Med. Chem.* **1998**, *41* (6), 902−912. (b) Njar, V. C.; Klus, G. T.; Brodie, A. Nucleophilic vinylic "addition-elimination" substitution reaction of 3 beta-acetoaxy-17-chloro-16-formylandrosta-5,16-diene: A novel and general route to 17-substituted-delta-steroids. Part 1. Synthesis of novel 17-azolyl steroids; inhibitors of 17alpha-hydroxylase/17,20-lyase (P45017alpha). *Bioorg. Med. Chem. Lett.* **1996**, 2777−2782.

(18) (a) Handratta, V. D.; Vasaitis, T. S.; Njar, V. C.; Gediya, L. K.; Kataria, R.; Chopra, P.; Newman, D., Jr.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. Novel C-17-heteroaryl steroidal CYP17 inhibitors/antiandrogens: synthesis, in vitro biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model. *J. Med. Chem.* **2005**, *48* (8), 2972−2984. (b) Vasaitis, T.; Belosay, A.; Schayowitz, A.; Khandelwal, A.; Chopra, P.; Gediya, L. K.; Guo, Z.; Fang, H. B.; Njar, V. C.; Brodie, A. M. Androgen receptor inactivation contributes to antitumor efficacy of 17{alpha}-hydroxylase/17,20-lyase inhibitor 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene in prostate cancer. *Mol. Cancer Ther.* **2008**, *7* (8), 2348−2357.

(19) (a) Bruno, R. D.; Vasaitis, T. S.; Gediya, L. K.; Purushottamachar, P.; Godbole, A. M.; Ates-Alagoz, Z.; Brodie, A. M.; Njar, V. C. Synthesis and biological evaluations of putative metabolically stable analogs of VN/124−1 (TOK-001): head to head anti-tumor efficacy evaluation of VN/124−1 (TOK-001) and abiraterone in LAPC-4 human prostate cancer xenograft model. *Steroids* **2011**, *76* (12), 1268−1279. (b) Purushottamachar, P.; Godbole, A. M.; Gediya, L. K.; Martin, M. S.; Vasaitis, T. S.; Kwegyir-Afful, A. K.; Ramalingam, S.; Ates-Alagoz, Z.; Njar, V. C. 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. *J. Med. Chem.* **2013**, *56* (12), 4880−4898.

(20) (a) Handratta, V. D.; Jelovac, D.; Long, B. J.; Kataria, R.; Nnane, I. P.; Njar, V. C.; Brodie, A. M. Potent CYP17 inhibitors: improved syntheses, pharmacokinetics and anti-tumor activity in the LNCaP human prostate cancer model. *J. Steroid Biochem. Mol. Biol.* **2004**, *92* (3), 155−165. (b) Nnane, I. P.; Njar, V. C.; Liu, Y.; Lu, Q.; Brodie, A. M. Effects of novel 17-azolyl compounds on androgen synthesis in vitro and in vivo. *J. Steroid Biochem. Mol. Biol.* **1999**, *71* (3−4), 145−152.

(21) (a) Grigoryev, D. N.; Kato, K.; Njar, V. C.; Long, B. J.; Ling, Y. Z.; Wang, X.; Mohler, J.; Brodie, A. M. Cytochrome P450c17-expressing Escherichia coli as a first-step screening system for 17alpha-hydroxylase-C17,20-lyase inhibitors. *Anal. Biochem.* **1999**, *267* (2), 319−330. (b) Grigoryev, D. N.; Long, B. J.; Njar, V. C.; Brodie, A. H. Pregnenolone stimulates LNCaP prostate cancer cell growth via the mutated androgen receptor. *J. Steroid Biochem. Mol. Biol.* **2000**, *75* (1), 1−10. (c) Grigoryev, D. N.; Long, B. J.; Nnane, I. P.; Njar, V. C.; Liu, Y.; Brodie, A. M. Effects of new 17alpha-hydroxylase/C(17,20)-lyase inhibitors on LNCaP prostate cancer cell growth in vitro and in vivo. *Br. J. Cancer* **1999**, *81* (4), 622−630.

(22) (a) Nnane, I. P.; Njar, V. C.; Brodie, A. A. Pharmacokinetic profile of 3beta-hydroxy-17-(1H-1,2,3-triazol-1-yl)androsta-5,16-diene (VN/87−1), a potent androgen synthesis inhibitor, in mice. *J. Steroid Biochem. Mol. Biol.* **2001**, *78* (3), 241−246. (b) Nnane, I. P.; Njar, V. C.; Brodie, A. M. Pharmacokinetics of novel inhibitors of androgen synthesis after intravenous administration in mice. *Cancer Chemother. Pharmacol.* **2003**, *51* (6), 519−524.

(23) Clement, O. O.; Freeman, C. M.; Hartmann, R. W.; Handratta, V. D.; Vasaitis, T. S.; Brodie, A. M.; Njar, V. C. Three dimensional pharmacophore modeling of human CYP17 inhibitors. Potential agents for prostate cancer therapy. *J. Med. Chem.* **2003**, *46* (12), 2345−2351.

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX

(24) Nicolaou, K.; Pfefferkorn, J.; Roecker, A.; Cao, G.; Barluenga, S.; Mitchell, H. Natural product-like combinatoriallLibrarires based on privileged structures. 1. General principles and solid phase synthesis of benzopyrans. *J. Am. Chem. Soc.* **2000**, *122*, 9939−9953.
(25) Schayowitz, A.; Sabnis, G.; Njar, V. C.; Brodie, A. M. Synergistic effect of a novel antiandrogen, VN/124−1, and signal transduction inhibitors in prostate cancer progression to hormone independence in vitro. *Mol. Cancer Ther.* **2008**, *7* (1), 121−132.
(26) Schayowitz, A.; Sabnis, G.; Goloubeva, O.; Njar, V. C.; Brodie, A. M. Prolonging hormone sensitivity in prostate cancer xenografts through dual inhibition of AR and mTOR. *Br. J. Cancer* **2010**, *103* (7), 1001−1007.
(27) (a) Montgomery, G.; Eisenberger, M.; Rettig, M.; Chu, F.; Pili, R.; Stephenson, J.; Vogelzang, N. J.; Corey, E.; Morrison, J.; Talpin, M.-E. In *Phase 1 Clinical Trial of Galeterone (TOK-001) a Multifunctional Antiandrogen and CYP17 Inhibitor in Castration Resistant Prostate Cancer (CRPC)*; 2012 Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, Jun 1−5; American Society of Clinical Oncology: Alexandria, VA, 2012; p 4665. (b) Taplin M.-E., Chu F., Morrison J. P., Pili R., Rettig M. B., Stephenson J., Vogelzang N. J., Montgomery R. B. In *ARMOR1: Safety of galeterone (TOK-001) in a Phase 1 clinical trial in chemotherapy naive patients with castration-resistant prostate cancer (CRCP)*; 2012 American Association for Cancer Research Meeting, Chicago, IL, Mar 31−Apr 4; American Association for Cancer Research: Philadelphia, PA, 2012; pp CT-07.
(28) Kramer, W. G.; Vince, B.; McGarry, C. In *Comparison of the Pharmacokinetics (PK) of Galeterone Novel Oral Formulations*. 2013 Meeting of the American Society of Clinical Oncology (ASCO), Chicago, IL, May 31−Jun 4; American Society of Clinical Oncology: Alexandria, VA, 2013; p e16075.
(29) (a) Montgomery, G.; Eisenberger, M. A.; Heath, E. I.; Sartor, A. O.; Chu, F.; Shore, N. D.; Edenfield, W. J.; Koletsky, A. J.; Lipsitz, D. U.; Cochran, J. S.; Nordquist, L. T.; Roberts, J.; Talpin, M.-E. *Galeterone in Men with CRPC: Results in Four Distinct Patient Populations from ARMOR2 Study*; 2014 Meeting of the American Society of Clinical Oncology (ASCO), May 3−Jun 3, 2014; American Society of Clinical Oncology (ASCO): Alexandria, VA, 2014. (b) Taplin, M.-E.; Montgomery, R. B. *ARMOR2: Galeterone in progressive CRPC patients who have failed primary therapy*; 2014 American Society of Clinical Genitourinary Cancers Symposium (ASCO GU), San Francisco, CA, Jan 30−Feb 2, 2014; American Society of Clinical Oncology (ASCO): Alexandria, VA, 2014; p 71.
(30) Jacoby, D. B.; Williams, M. D. In *Galeterone, Abiraterone, Orteronel and Ketoconazole Exhibit Differential Inhibitory Effects on CYP17 and Steoidogenesis*; 2013 American Society of Clinical Oncology Genitourinary Cancers Symposium (ASCO GU), Feb 14−16, Orlando, FL, 2013; American Society of Clinical Oncology (ASCO): Alexandria, VA, p 184.
(31) Yuan, X.; Cai, C.; Chen, S.; Chen, S.; Yu, Z.; Balk, S. P. Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis. *Oncogene* **2014**, 33 (22), 2815−2825.
(32) Nakouzi N. A.; Wang, C.; Bishop J. L.; Jacoby, J.; Gleave, M.; Zoubeidi, A. In *Galeterone Suppresses Castration-Resistant and Enzalutamide-Resistant Prostate Cancer Growth in Vitro*; AACR-NCI-EORTC 2013 International Conference on Molecular Targets and Cancer Therapeutics, Oct 19−23, Boston, MA; American Association for Cancer Research: Philadelphia, PA, 2013; p C89.
(33) Bruno, R. D.; Gover, T. D.; Burger, A. M.; Brodie, A. M.; Njar, V. C. 17alpha-Hydroxylase/17,20 lyase inhibitor VN/124−1 inhibits growth of androgen-independent prostate cancer cells via induction of the endoplasmic reticulum stress response. *Mol. Cancer Ther.* **2008**, *7* (9), 2828−2836.

■ NOTE ADDED IN PROOF

During the review of this manuscript, Tokai Pharmaceuticals announced that the company remains on track to initiate the pivotal Phase 3 ARMOR3-SV trial, the first biomarker-based registration clinical trial of its kind in prostate cancer, in the first half of 2015 with top line results targeted for the end of 2016. In ARMOR3-SV, patients will be screen and those testing positive for AR-V7 will then be randomized to receive either galeterone or enzalutamide.

DOI: 10.1021/jm501239f
J. Med. Chem. XXXX, XXX, XXX−XXX