ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Discovery of BMS-641988, a Novel Androgen Receptor Antagonist for the Treatment of Prostate Cancer

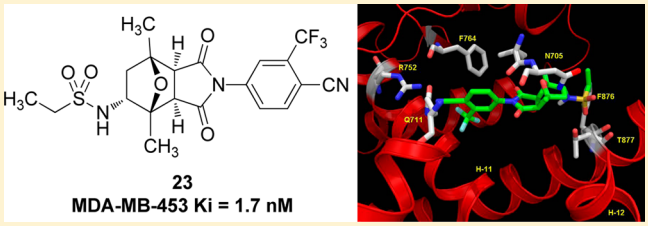
Aaron Balog,*[,†] Richard Rampulla,[†] Gregory S. Martin,[‡] Stanley R. Krystek,[†] Ricardo Attar,[†] Janet Dell-John,[†] John D. DiMarco,[†] David Fairfax,[‡] Jack Gougoutas,[†] Christian L. Holst,[‡] Andrew Nation,[†] Cheryl Rizzo,[†] Lana M. Rossiter,[‡] Liang Schweizer,[†] Weifang Shan,[†] Steven Spergel,[†] Thomas Spires,[†] Georgia Cornelius,[†] Marco Gottardis,[†] George Trainor,[†] Gregory D. Vite,[†] and Mark E. Salvati[†]

[†]Bristol Myers-Squibb Research and Development, Princeton, New Jersey 08543, United States

[‡]Albany Molecular Research Inc., 21 Corporate Circle, Albany, New York 12203, United States

Supporting Information

ABSTRACT: BMS-641988 (**23**) is a novel, nonsteroidal androgen receptor antagonist designed for the treatment of prostate cancer. The compound has high binding affinity for the AR and acts as a functional antagonist *in vitro*. BMS-641988 is efficacious in multiple human prostate cancer xenograft models, including CWR22-BMSLD1 where it displays superior efficacy relative to bicalutamide. Based on its promising preclinical profile, BMS-641988 was selected for clinical development.



KEYWORDS: *Prostate cancer, androgen receptor, CRPC, BMS-641988*

Carcinoma of the prostate (CaP) is the most common malignancy among men in the US and the second most common cause of cancer-related death worldwide after lung cancer.[1] The androgen receptor (AR) is a member of the nuclear hormone superfamily of ligand-induced transcription factors and is a key signaling pathway leading to the emergence of CaP. Androgen ablation, by surgical or chemical castration in combination with an antiandrogen such as hydroxyflutamide (**1**) or bicalutamide (**2**), has been the standard of care for advanced CaP for many years.[2] This therapy is initially effective in 80−90% of patients; however, >50% of the patients will ultimately develop castration resistant prostate cancer (CRPC) after ∼18 months.[3] The treatment of CRPC is challenging due to the sustained AR signaling, which is the result of AR overexpression/activation and the presence of activating AR mutations.[4] Furthermore, it has been reported that CRPC tumors express the necessary cytochrome P450 enzymes for intratumoral androgen production, thus bypassing the effects of chemical castration, which targets only gonadal androgen production.[5] These findings suggest that CRPC remains AR dependent, and effective therapies must target AR signaling directly with improved next-generation AR antagonists.

MDV3100 (enzalutamide, **3**) is a potent AR antagonist that was recently approved by the US Food and Drug Administration for the treatment of metastatic CRPC patients that have progressed post-treatment with docetaxel.[6] Although enzalutamide has shown promise in treating these patients, nearly all patients go on to develop resistance to enzalutamide via AR mutations.[4] Thus, there is a need for novel antiandrogens with distinct interactions in the AR ligand binding domain that could be dosed together or sequentially in the clinic to combat the potential pathways leading to CRPC progression. Our laboratory has been focused on the rational structural-based design of structurally novel, nonsteroidal small molecule AR antagonists for the potential treatment of CRPC.

Previously, we have reported a series of [2.2.1] carbobicyclic[7] and oxabicyclic[8] succinimide based AR antagonists (**4** and **5**, Figure 1). These compounds demonstrated potent binding affinity (K_i) and functional antagonist activity (IC_{50}) against the wild-type AR as found in the MDA-MB-453 cell-line (Table 1). These compounds compared favorably in terms of potency to the clinically used antiandrogens hydroxyflutamide (**1**) and bicalutamide (**2**). We designed a series of oxabicyclic-based AR

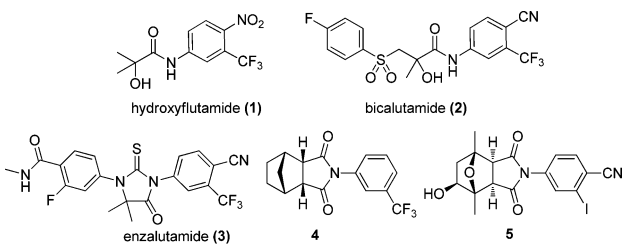


Figure 1. Known androgen receptor antagonists.

Received: April 24, 2015
Accepted: June 19, 2015
Published: June 19, 2015

DOI: 10.1021/acsmedchemlett.5b00173
ACS Med. Chem. Lett. 2015, 6, 908−912

Table 1. *In Vitro* Biological Activity

#	MDA-MB-453 K_i (nM)[a]	MDA-MB-453 IC_{50} (nM)[b]
2	64	173
5	8	10
11	1600	>5000
12	23	22
13	50	34
14	31	60
15	3.0	23
16	50	34
17	31	60
18	7.0	6
19	12	20
20	10	1.0
21	1.0	10
22	2.0	7.0
23	1.7	16

[a]Binding (K_i) determined through direct displacement with [^{3}H]-DHT in the MDA-MB-453 cell-line [b]Functional antagonist activity (IC_{50}) in the MDA-MB-453 cell-line determined through a transiently transfected reporter system utilizing the secreted alkaline phosphatase reporter gene driven by the AR-dependent PSA promoter.

antagonists, such as **5**, that demonstrated a superior pharmacokinetic (PK) profile compared to the carbocycles such as **4**. Based on our understanding of the need for sustained AR suppression in an effective AR antagonist, we expected that robust PK would be essential for an efficacious AR antagonist.[8] Accordingly, compound **5** was shown to be efficacious in the CWR22-BMSLD1 human prostate cancer xenograft model, where bicalutamide shows only limited efficacy.[8] We looked to incorporate PK properties critical for an effective AR antagonist, such as log $T_{1/2}$, with a broader activity profile than is seen with first generation agents such as bicalutamide. Accordingly, we screened out agents against the human CaP model CWR22, which has been shown to be refractory to both bicalutamide and hydroxyflutamide. Based on this result, we wanted to expand the scope of this series by using a structure-based approach to identify even more potent AR antagonists.

Utilizing available X-ray cocrystal structures of the AR generated at BMS, we developed a molecular model of the WT AR LBD (Figure 2) to aid in the identification of new AR antagonists.[9,10] In this model, interactions of N-705 with both the C-5 hydroxyl and the bridging oxygen of compound **5** are evident. Additional interactions between R752 and Q711 and the aryl nitrile functionality of compound **5** are also present. We postulated that endosubstitution at C-5 or C-6 of the bicycle would result in a direct interaction with helix-12 (H-12), possibly creating a more classical AR antagonist conformation, similar to that predicted for bicalutamide. The key hydrogen bonding interaction with N-705 would be maintained by the bridging oxygen as seen with bicalutamide (**2**), which is shown in orange. Thus, we set out to investigate the effect of various substitutions on the endoface of the [2.2.1]-oxabicyclic core of compound **5**.

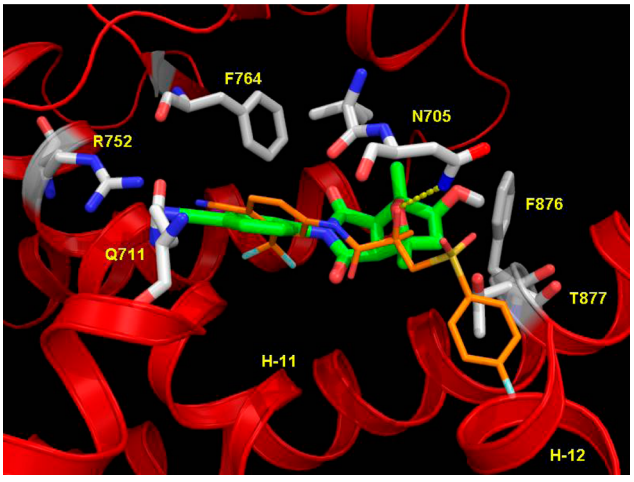


Figure 2. Compound **5** docked into a model of the wild-type AR ligand binding domain.

Our efforts started with the synthesis of compounds **11**−**13** by the synthetic route shown in Scheme 1. Maleimide **8** was prepared from the aniline **6** and maleic anhydride (**7**) under standard conditions.[11] Diels−Alder cycloaddition between **8** and the MEM ester of 2,5-dimethyl-3-furoic acid occurred at 120 °C to give only the desired exoisomer **9** after precipitation. Catalytic hydrogenation led to formation of the endoester by selective reduction from the beta-face of the olefin. Normal-phase chiral HPLC separation of the racemic endoester gave the desired enantiomer **10** in 45% yield and >99% ee. The optical isomer depicted by compound **10** was determined to be optimal for potent AR antagonist activity, and the absolute and relative stereochemistry was eventually confirmed by X-ray crystallographic analysis of compound **23**.[12] Treatment of **10** with 3 N HCl gave the key intermediate acid **11** in good yield. Compounds **12** and **13** were prepared by standard ester and amide formation conditions.

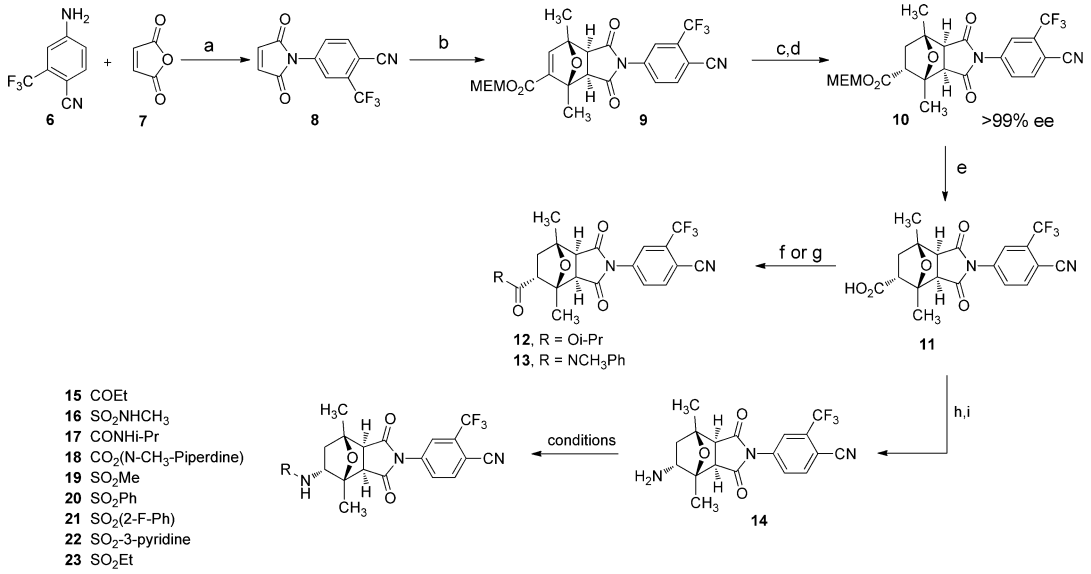
The acid **11** was found to have poor potency in our cellular *in vitro* assays (Table 1), but the ester **12** and amide **13** had promising binding and functional antagonist activity in the MDA-MB-453 cell-line. Unfortunately, neither the amide nor the ester had potency that was superior to the exohydroxy analogue **5**, so we investigated additional functionalities on the oxabicycle in an effort to find highly potent AR antagonists. The endoamine **14** could be prepared from the acid **11** by sequential Curtius rearrangement[13] and subsequent TFA-promoted cleavage of the resulting Teoc-carbamate in 77% yield. Compound **14** had only modest affinity to the AR but offered a good handle with which to further functionalize the oxa-bicyclic core.

A series of amides, sulfamides, carbamates, ureas, and sulfonamides was prepared from the amine **14** in library format. Standard coupling techniques were utilized to prepare analogues **15**−**23** (see Supporting Information); the corresponding *in vitro* biological data is shown in Table 1. In general, these analogues were potent binders to, and functional antagonists of, the wild-type AR present in the MDA-MB-453 cell line. The amide **15** and the carbamate **18** had excellent potency *in vitro*, while the sulfamide **16** and urea **17** were weakly active. The sulfonamides **19**−**23** had the best overall *in vitro* profile, with robust affinity and potent antagonist activity, superior to that for bicalutamide (**2**).

We next wanted to investigate the pharmacodynamic effects of these novel AR antagonists *in vivo*. Compounds **13**−**23** were progressed into the immature rat prostate weight (IRPW) PK/PD model, where the compound effect on AR-dependent growth of the prostate and seminal vesicles was measured (Table 2).[14] In this model, compounds were dosed orally once a day at 1 or 10 mg/kg for 4 days with plasma concentrations of drug measured 2 h postdose on day 4. Agents that effectively

DOI: 10.1021/acsmedchemlett.5b00173
ACS Med. Chem. Lett. 2015, 6, 908−912

Scheme 1. Synthetic Route to Endo-Substituted Bicycles[a]



[a]Reagents and conditions: (a)HOAc, 110 °C, 88%; (b) MEM 2,5-dimethyl-3-furoate, 120 °C, 33%, exo-isomer only; (c) H_2, Pd/C, EtOAc, 1 atm, 50%; (d) chiral HPLC separation, 45%, >99% ee; (e) 3 N HCl, THF, 22 °C, 98%; (f) $(COCl)_2$, DCM then i-PrOH, TEA, 97%; (g) EDC, HOBt, DIEA, *N*-methylaniline, DMF, 88%; (h) 2-trimethylsilylethanol, DPPA, TEA, 4 Å MS, 1,4-dioxane, 75 °C, 78%; (i) TFA, CH_2Cl_2, 22 °C, 99%.

Table 2. Immature Rat Prostate Weight Assay Results

#	IRPW 1 mg/kg SV/FB[a]	IRPW 10 mg/kg SV/FB[a]	exposure 1 mg/kg (μM)[b]	exposure 10 mg/kg (μM)[b]
2	69 ± 24	41 ± 4	2.3 ± 0.4	9.5 ± 1.4
13	88 ± 19	43 ± 8.0	1.8 ± 0.68	12.0 ± 0.82
14	89 ± 22	89 ± 8.1	0.08 ± 0.02	0.48 ± 0.32
15	87 ± 39	34 ± 6.0	24 ± 1.7	190 ± 73
16	89 ± 5.9	36 ± 4.2	0.01 ± 0.001	0.23 ± 0.08
17	115 ± 3.9	56 ± 8.9	8.3 ± 0.4	60 ± 6.1
18	105 ± 24	87 ± 16	0.012 ± 0.004	0.076 ± 0.017
19	64 ± 13	26 ± 4.2	0.29 ± 0.07	4.0 ± 1.2
20	63 ± 14	31 ± 5.2	0.23 ± 0.03	2.4 ± 0.52
21	56 ± 6.5	24 ± 1.8	0.16 ± 0.02	1.9 ± 0.06
22	44 ± 8.6	23 ± 11	0.05 ± 0.01	0.52 ± 0.09
23	58 ± 13	26 ± 3.0	0.79 ± 0.17	4.0 ± 0.42

[a]SV/FB is the percentage of weight of the seminal vesicles over the full body weight of the rat (n = 3) where testosterone treated control = 100% and sham = 10%. [b]Plasma exposure measured 2 h postdose on day 4.

block the proliferative effect of the AR in these tissues would result in a decrease in the total weight of organs relative to a control group. As expected, the amide **13** had poor pharmacodynamic effect in this model, most likely due to modest functional antagonist potency. The amide **15** and the urea **17** gave only modest PD effects even with very high exposure after 1 and 10 mg/kg doses. This result correlated with the very high serum protein binding measured for these two compounds (>99% in mouse serum). The sulfonamide **16** and the carbamate **18** also had only modest PD effects, but this was most likely due to poor exposure relative to compound **2**. The sulfonamide series stood out in the IRPW model by having excellent PD with modest exposure, suggesting superior *in vivo* potency compared to the ureas, amides, carbamates, and sulfamides. Compounds **19**–**23** all demonstrated robust PD at a 10 mg/kg dose with exposures significantly less than observed for bicalutamide. Of these promising analogues, the ethyl sulfonamide **23** was chosen for further studies due to robust potency *in vivo* and a promising PK profile in rats.

Compound **23** was further profiled to determine *in vitro* safety and ADME properties (Table 3). Inhibition of human cytochrome P450 (CYP) isoforms is very weak (>40 μM), and there is very low potential for CYP induction based on the human PXR transactivation assay. Plasma protein-binding of **23** was measured by equilibrium dialysis, and very low levels of plasma protein binding were observed in all species (>10% free). Compound **23** demonstrated excellent metabolic stability in hepatocyte incubations, and the predicted clearance in all species is low, especially for human. Finally, the PK properties of compound **23** were assessed in mouse, rat, and dog following both oral and IV doses. Consistent with the predicted hepatic clearance, compound **23** demonstrated moderate to long half-lives and very low clearance across species.

Compound **23** was then tested in the human prostate cancer xenograft model CWR22-BMSLD1 (Figure 3).[15] Treatment with bicalutamide (**2**) (150 mg/kg, po, qd × 35 days) resulted in good tumor growth inhibition for the initial 10 days, followed by regrowth of the tumor at a rate that was similar to control. When compound **23** was dosed (90 mg/kg, po, qd × 45 days) excellent tumor growth inhibition was observed over the entire dosing period, demonstrating superior efficacy to bicalutamide (**2**). Additionally, we investigated the possibility of treating bicalutamide resistant tumors in this model by allowing the bicalutamide-treated tumors to triple in size followed by a switch to treatment with compound **23**. As shown in Figure 3, tumor growth continued for ∼10 days after switching to compound **23**, followed by nearly complete tumor growth inhibition for the remainder of dosing period. We were encouraged by this result as it gave strong evidence that compound **23** has the potential to treat forms of prostate cancer resistant to bicalutamide (**2**).

Docking compound **23** into the wild-type AR ligand binding domain (Figure 4)[10] revealed the H-bond from N-705 to the

DOI: 10.1021/acsmedchemlett.5b00173
ACS Med. Chem. Lett. 2015, 6, 908–912

Table 3. Summary of Androgen Receptor Biological Data and ADME Properties for Compound 23[a]

assay	results
WT AR binding (K_i)	1.7 ± 0.56 nM
MDA 453 (IC_{50})	16 ± 3 nM
LNCaP (IC_{50})	153 ± 77 nM
human CYP (1A2, 2B6, 2C8, 2C9, 2D6, 3A4) IC_{50}	>40 μM
PXR-TA EC_{50}	>50 μM
hERG inhibition @ 30 μM	0%
protein binding (% free): mouse, rat, dog, human	14.8, 10.6, 16.5, 10.4
rate of metabolism in hepatocytes: mouse, rat, dog, monkey, human (pmol/min/10^6 cells)	0, 26, 3.0, 1.0, 2.0
predicted clearance: mouse, rat, dog, monkey, human (mL/min/kg)	0, 16, 1.8, 0.6, 0.8
IV/PO PK: $T_{1/2}$ (h), CL (mL/min/kg), $AUC_{0-\infty}$ (μM·h), %F	
mouse (5/10 mg/kg)	2.4, 4.5, 39.1/74.1, 94.7
rat (5/10 mg/kg)	3.7, 6.5, 31.7/40.5, 63.9
dog (1/2 mg/kg)	21.8, 0.35, 102.1/160.2,79

[a]IC_{50} values are an average of three experiments.

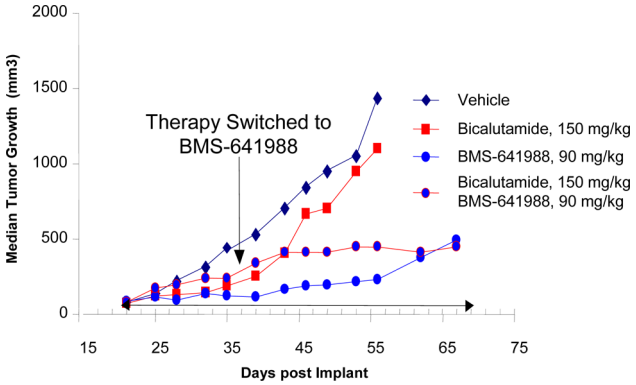


Figure 3. In vivo efficacy of compound **23** in the CWR22-BMSLD1 prostate cancer xenograft model.

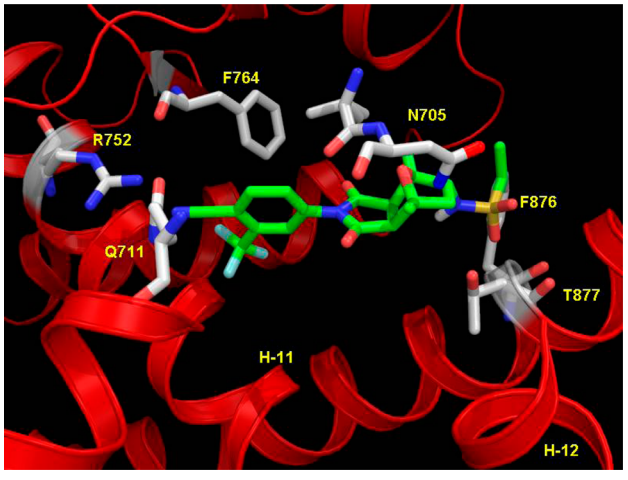


Figure 4. Compound **23** docked into a model of the WT AR ligand binding domain.

bridging oxygen is likely intact as observed for compound **5**. To accommodate the endosulfonamide at C-5, F876 must reorient resulting in a positional shift of Helix-11. This significant shift of Helix-11 results in a change in the overall architecture of the LBD, potentially giving compound **23** the promising antagonist profile presented here.

Further profiling of compound **23** demonstrated an acceptable preclinical safety profile both *in vitro* and *in vivo*. This compound was selected for clinical development and advanced into phase I clinical trials.[15,16]

In summary, we have utilized structure-based design to identify a new series of amino [2.2.1]-oxabicyclosuccinimide AR antagonists. Lead molecules demonstrated potent antagonist activity in cellular binding and transactivation assays *in vitro* and had robust PK/PD profiles in the IRPW model. Compound **23** was shown to be superior to bicalutamide in the CWR22-BMSLD1 human CaP tumor xenograft model and has the potential to address acquired bicalutamide resistance based on the results from these studies.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and characterization data for compounds **9**–**23** and biological methods. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00173.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: aaron.balog@bms.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Linda Fleming and the Department of Discovery Synthesis at Bristol-Myers Squibb for preparation of key intermediates. We also acknowledge the Departments of Lead Discovery & Optimization and Discovery Analytical Sciences for ADME and compound characterization support.

■ DEDICATION

We would like to dedicate this paper to the memory our colleague John D. DiMarco.

■ REFERENCES

(1) Ghafoor, A.; Jemal, A.; Thomas, H.; Murray, T.; Thun, M. Cancer statistics for African Americans. *Cancer J. Clin.* **2002**, *52*, 322–325.

(2) Crawford, E. D.; DeAntonio, E. P. Current status of combined androgen blockade: optimal therapy for advanced prostate cancer. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 1062.

DOI: 10.1021/acsmedchemlett.5b00173
ACS Med. Chem. Lett. 2015, 6, 908–912

(3) Dalesio, O. Maximum androgen blockade in advanced prostate cancer: an overview of the randomized trials. *Lancet* **2000**, *355*, 1491−1498.
(4) Scher, H.; Sawyers, C. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen receptor signaling axis. *J. Clin. Oncol.* **2005**, *23*, 8253.
(5) Locke, J. A.; Guns, E. S.; Lubik, A. A.; Adomat, H. A.; Hendy, S. C.; Wood, C. A.; Ettinger, S. L.; Gleave, M. E.; Nelson, C. C. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res.* **2008**, *68* (15), 6407−6415.
(6) Ning, Y. M.; Pierce, W.; Maher, V. E.; Karuri, S.; Tang, S. H.; Chiu, H.-J.; Palmby, T.; Zirkelback, J. F.; Marathe, D.; Mehrotra, N.; Liu, Q.; Ghosh, D.; Cottrell, C. L.; Leighton, J.; Sridhara, R.; Ibrahim, A.; Justice, R.; Pazdur, R. Enzalutamide for treatment of patients with metastatic castration-resistant prostate cancer who have previously received docetaxel; United States Food and Drug Administration approval summary. *Clin. Cancer Res.* **2013**, *19* (22), 6067−6073.
(7) Salvati, M.; Balog, A.; Wei, D.; Pickering, D.; Attar, R.; Geng, J.; Rizzo, C.; Hunt, J.; Weinmann, R.; Martinez, R. Identification of a novel class of androgen receptor antagonists based on the bicyclic-1H-isoindole-1,3(2H)-dione nucleus. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 389.
(8) Salvati, M.; Balog, A.; Shan, W.; Rampulla, R.; Giese, S.; Mitt, T.; Furch, J.; Vite, G.; Attar, R.; Maria, J.-K.; Geng, J.; Rizzo, C.; Gottardis, M.; Krystek, S.; Gougoutas, J.; Galella, M.; Obermeier, M.; Fura, A.; Chandrasens, G. Identification and optimization of a novel series of [2.2.1]-oxabicyclo imide-based androgen receptor antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1910−1915.
(9) Sack, J. S.; Kish, K.; Wang, C.; Attar, R.; Kiefer, S. E.; Yongmi, W.; Ginger, Y.; Scheffler, J. E.; Salvati, M. E.; Krystek, S. R.; Weinmann, R.; Einspahr, H. R. Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural ligand dihydrotestosterone. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 4904.
(10) Molecular modeling was performed using ICM software (Molsoft LLC, San Diego, CA). The docking of the ligands into WT AR LBD was carried out using the ICM docking procedure, which is a two step process. Initial docking of ligands was carried out using grid potential representation of the receptor and flexible ligand. Five grid potentials describe the shape, hydrophobicity, electrostatics, and hydrogen bonding potential of the receptor. The conformations from the grid were then optimized with a full atom representation of the receptor and flexible ligand, by an ICM stochastic global optimization algorithm as implemented in version 2.7 of the Molsoft ICM program. (a) Molsoft. *ICM 2.7 Program Manual*; Molsoft: San Diego, CA, 1998. (b) Totrov, M.; Abagyan, R. *Proteins Suppl. 1* **1997**, 215.
(11) Salvati, M. E.; Balog, A.; Pickering, D. A.; Giese, S.; Fura, A.; Li, W.; Patel, R.; Hanson, R. L.; Mitt, T.; Roberge, J.; Corte, J. R.; Spergel, S. H.; Rampulla, R. A.; Misra, r.; Xiao, H.-Y. Preparation of fused succinimides as modulators of nuclear hormone function. WO 20040077605.
(12) The absolute and relative stereochemistry of compound **23** was confirmed by single-crystal X-ray crystallography. The coordinates have been deposited in the Cambridge Crystallographic Data Center, #779603.
(13) Smith, P. A. S. Curtius reaction. *Org. React.* **1946**, *3*, 337.
(14) Hershberger, L. G.; Shipley, E. G.; Meyer, R. K. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. *Proc. Soc. Exp. Biol. Med.* **1953**, *83*, 175.
(15) Attar, R. M.; Kunkel, M. J.; Balog, A.; Cvijic, M. E.; Dell-John, J.; Rizzo, C. A.; Schweizer, L.; Spires, T. E.; Platero, J. S.; Obermeier, M.; Shan, W.; Salvati, M. E.; Foster, W. R.; Dinchuk, J.; Chen, S.-J.; Vite, G.; Kramer, R.; Gottardis, M. Discovery of BMS-641988, a novel and potent inhibitor of androgen receptor signaling for the treatment of prostate cancer. *Cancer Res.* **2009**, *69*, 6522−6529.
(16) Rathkopf, D.; Liu, G.; Carducci, M. A.; Eisenberger, M. A.; Anand, A.; Morris, M. J.; Slovin, S. F.; Sasaki, Y.; Takahashi, S.; Ozono, S.; Fung, N. K. E.; Cheng, S.; Gan, J.; Gottardis, M.; Obermeier, M. T.; Reddy, J.; Zhang, S.; Vakkalagadda, B. J.; Alland, L.; Wliding, G.; Scher, H. I. Phase I dose-escalation study of the novel antiandrogen BMS-641988 in patients with castration-resistant prostate cancer. *Clinc. Cancer Res.* **2010**, *17* (4), 880−887.

DOI: 10.1021/acsmedchemlett.5b00173
ACS Med. Chem. Lett. 2015, 6, 908−912