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Identification of a novel class of androgen receptor antagonists based on the bicyclic-1H-isoindole-1,3(2H)-dione nucleus

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Abstract—A novel series of isoindoledione based compounds were identified as potent antagonists of the androgen receptor (AR). SAR around this series revealed dramatic differences in binding and function in mutant variants (MT) of the AR as compared to the wild type (WT) receptor. Optimization of the aniline portion revealed substitution patterns, which yielded potent antagonist activity against the WT AR as well as the MT AR found in the LNCaP and PCa2b human prostate tumor cell lines.

Carcinoma of the prostate (CaP) is the second leading cause of cancer related death in men with an estimated 182,000 new cases diagnosed and 40,000 deaths each year in the United States.[1] The androgen receptor (AR) is a ligand binding transcription factor in the nuclear hormone receptor super family and is a key molecular target in the etiology and progression of prostate cancer. Binding of androgens, such as testosterone (T) and dihydrotestosterone (DHT), to the AR provides the mitogenic signal for the growth of CaP. For the past 50 years, androgen ablation via castration has been the most effective therapy for the treatment of advanced CaP in the clinic. A more recent treatment alternative, complete androgen blockade (CAB) is accomplished by treatment with an antiandrogen in addition to chemical castration with aluteinizing hormone releasing hormone agonist.[2] Although this approach initially shows a 80–90% response rate,[3] when treatment is continued for 1–2 years, approximately 50% of patients progress to fatal androgen independent disease.[4] Clearly, there is an unmet medical need for the treatment of advanced CaP. For this reason, we are interested in identifying novel small-molecule antagonists of the AR that are more effective than the current therapies at increasing the response period of advanced androgen dependent CaP to androgen ablation therapy.

Our search for a new series of small molecule AR antagonists involved a focused screening approach based upon the current clinically used antiandrogens Bicalutamide[3] (**1**) and hydroxyflutamide[3] (**2**) (Table 1). From a series of 350 available compounds, we identified a unique series of bicyclic-1H-isoindole-1,3(2H)-dione analogs, which demonstrate potent binding (K_i) to, and functional antagonism (IC_{50}) of the wild type (WT) AR as found in the MDA-453 cell line (Table 1).[5] These compounds also demonstrate potent binding (K_i) to and functional antagonism (IC_{50}) of the mutant (MT) T877A AR as found in the human prostate cancer cell line LNCaP.[6] In addition to binding and functional effects, the lead compounds inhibit the androgen dependent growth of the human prostate cancer cell line MDA-MB-PCa2b, which contains an AR with mutations at

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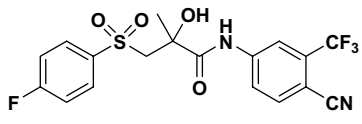
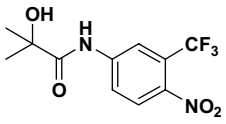
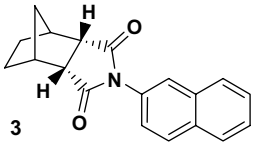
Table 1. Initial AR antagonist leads



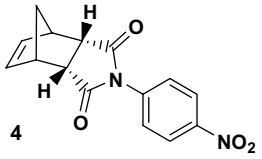
Bicalutamide (1)



Hydroxyflutamide (2)



3



4

#	MDA-453 K_i nM[a]	MDA-453 IC_{50} nM[b]	LNCaP K_i nM[a]	LNCaP IC_{50} nM[b]	PCa2b IC_{50} nM[c]
1	64	173	35	400	725
2	43	26	2	Ag[d]	Ag[d]
3	284	1200	11	84	265
4	192	664	12	30	179

#: Compound number.

[a] Binding (K_i) determined through direct displacement with [^{3}H]-DHT in the MDA-453 cell line or in the LNCaP cell line.[5]

[b] Functional antagonist activity (IC_{50}) determined through a transiently transfected reporter assay system utilizing a secreted alkaline phosphatase (SEAP) reporter construct and a PSA AR promoter domain.[5]

[c] Inhibition of cell proliferation determined by [^{3}H]-thymidine incorporation over a 72 h growth period.[5]

[d] Ag: Compound shows ability to activate AR in absence of DHT.

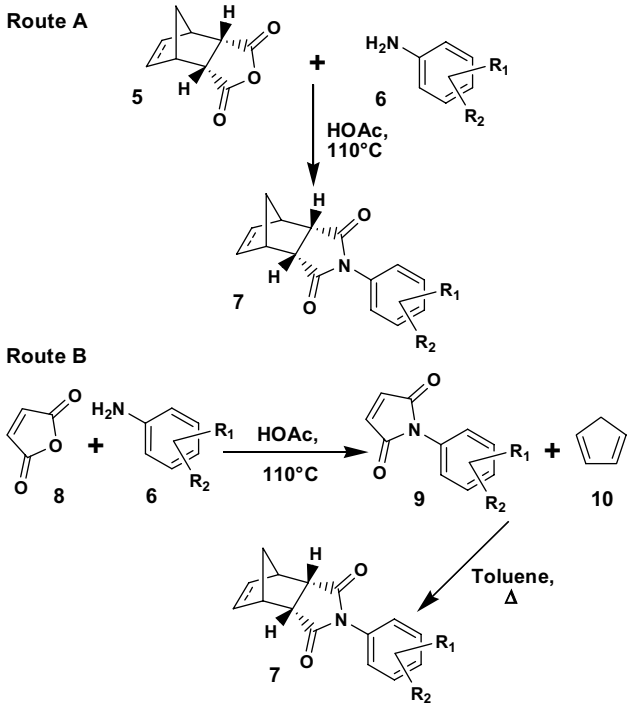


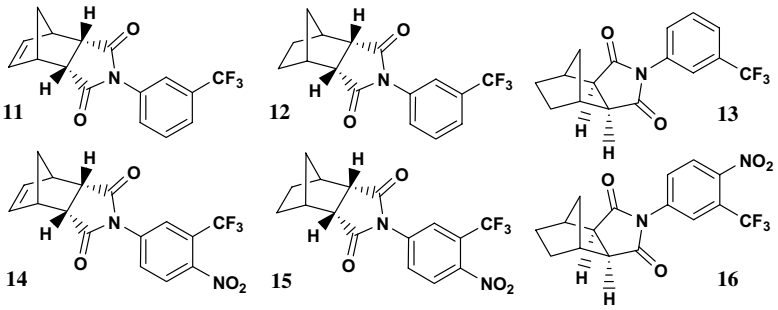
Figure 1. (Route A) Solution phase parallel synthesis approach through bicyclic anhydride intermediates. (Route B) Linear parallel synthesis approach through Diels–Alder reaction.

both L701H and T877A.[4,7] Table 1 demonstrates the activity profile of our initial leads, **3** and **4**, as compared to other clinically used antiandrogens. Both **3** and **4** demonstrates a profile similar to bicalutamide in their ability to block multiple isoforms of the AR. This is in contrast to hydroxyflutamide, which demonstrate an antagonist profile to the WT AR, but converts to an agonist with the MT AR isoforms.

To rapidly expand upon our initial leads, we applied two parallel synthesis approaches. As shown in Figure 1, Route A utilized a series of commercially available bicyclic anhydride intermediates, generally described as compound **5**, as well as an array of commercially available substituted anilines, generally described as compound **6**. Heating of the anhydrides with the aniline, as previously described,[5] yielded the desired final products **7** in acceptable yields. This process was optimized to be run in a 48-well reaction block with no additional purification required.[5] For those anilines that were unreactive, as well as to address other bicyclic ring systems not attainable through the anhydride intermediate, a linear approach (Route B) was applied. For Route B, maleic anhydride was treated with an array of substituted anilines to yield the N-aryl maleimide intermediate **9**. Cycloaddition of **9** with a diene, such as cyclopentadiene **10**, under a variety of Diels–Alder compatible conditions, yielded the final product **7**. For Route B, the unsaturated final product is obtained and can be reduced by treatment with Pd/C (10%) under a balloon atmosphere of H_2. In addition, Route B may yield a mixture of *endo* and *exo*-isomers depending on the conditions employed and the aniline or diene used. Through Routes A and B we were rapidly able to probe the SAR of the aniline as well as the bicyclic portion of the molecule. In this article, we will focus on the SAR results for a series of [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs.

Table 2 shows the SAR trends about the imide and bicyclic portion of a series of [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs. In general, reduction of the 5,6-olefin led to a significant increase in binding to both the WT and T877A MT AR. For the WT AR, the increased binding was accompanied by increased functional antagonist activity (compare compounds **11**–**12** and **14**–**15** in the MDA-453 cell line). For the MT AR found in the LNCaP and PCa2b cell lines, reduction of the 5,6-olefin did not significantly change the functional activity across the series of analogs examined. Throughout the series, the *endo*-isomer showed significantly better binding and functional antagonist activity toward the WT AR as compared to the *exo*-isomer. Remarkably, for the MT AR found in the LNCaP and PCa2b cell lines, little preference was seen for the *exo* versus the *endo*-isomer in terms of binding or functional activity (see examples **12**–**13**). It is interesting to note that the lack of specificity seen with the MT AR isoforms is consistent with the theory that such mutations serve as gain of function changes, allowing the receptor to utilize an array of different ligands in the absence of DHT.[8] Since most literature in the field points to the WT AR as being the predominant isoform found in advanced CaP,[9] we chose to pursue the *endo* isomers in further SAR studies.

Table 2. SAR around the bicycle-core of a series of [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs



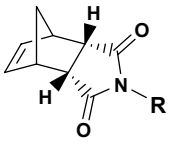
#	MDA-453 K_i nM[a]	MDA-453 IC_{50} nM[b]	LNCaP K_i nM[a]	LNCaP IC_{50} nM[b]	PCa2b IC_{50} nM[c]
11	632	744	7	35	410
12	319	127	3.4	7	NT[d]
13	1615	317	2	12	205
14	55	119	5	Ag	Ag
15	5	12	2	Ag	Ag
16	117	114	2	Ag	NT

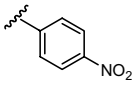
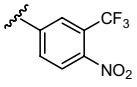
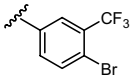
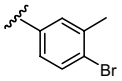
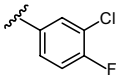
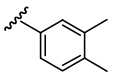
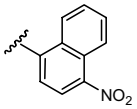
[a–c,#] See footnotes for Table 1.

[d] Ag: Compound were run through the assays described in footnotes 'b' and 'c' in the presence or absence of the AR agonist ligand DHT. In the absence of DHT, these compounds were able to activate the AR in the transaction assays or promote the growth of the PCa2b cell line, thus behaving as agonist (Ag) ligands for the AR. In the presence of DHT these compounds showed minimal to no measurable antagonist activity.

Table 3 shows the SAR trends about the aniline portion of a series of the [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs. In general, for binding (K_i) to the WT AR, substitution was well tolerated at the 2, 3, and

Table 3. SAR around the aniline system for a series of [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs



#	R	MDA-453 K_i nM[a]	MDA-453 IC_{50} nM[b]	LNCaP K_i nM[a]	LNCaP IC_{50} nM[b]	PCa2b IC_{50} nM[c]
4		192	664	12	30	179
14		55	119	5	Ag[d]	Ag
17		46	146	0.6	Ag	Ag
18		125	220	3.6	Ag	Ag
19		177	517	2	33	132
20		400	2014	3.4	10	96
21		1	>5	0.5	Ag	Ag

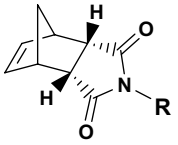
[a–c,#] See footnotes for Table 1.

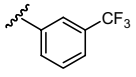
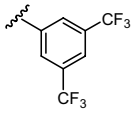
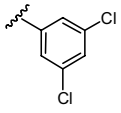
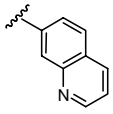
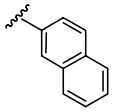
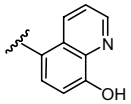
[d] Ag: Compound were run through the assays described in footnotes 'b' and 'c' in the presence or absence of the AR agonist ligand DHT. In the absence of DHT, these compounds were able to activate the AR in the transaction assays or promote the growth of the PCa2b cell line, thus behaving as agonist (Ag) ligands for the AR. In the presence of DHT these compounds showed minimal to no measurable antagonist activity.

4-positions of the aniline ring, with electron withdrawing groups preferred at positions 3 and 4. Substitution at the 5-position of the aniline was also tolerated and will be discussed later in this article. Electron withdrawing groups at the 4-position had the greatest impact on binding to the WT AR, with an additive effect seen when an additional electron withdrawing group was placed at the 3-position (compare compounds **4**–**14**), consistent with SAR reported with other antiandrogens described in the literature.[10] Small substituents, such as halogens or a methyl group, were well tolerated at the 2-position and they also gave an additive effect when electron withdrawing groups were at the 3- and 4-position of the aniline system (data not shown). In general, potent binding to the WT AR correlated well with potent antagonist activity for most analogs examined. Despite the fact that the AR found in the LNCaP cell line differs from the WT AR in the MDA-453 cell line only by a change from a threonine to an alanine at residue 877 in the ligand binding domain, SAR for the T877A MT AR was dramatically different from that seen for the WT AR. In general, binding to the T877A MT AR demonstrated a broad tolerance for substitution around the aniline ring. The dramatic preference for electron withdrawing groups seen with the WT AR was not as prevalent for the T877A MT AR (compare compounds **14**–**20**) and even large substitutions, which resulted in almost complete loss of binding to the WT AR, demonstrated potent binding to the T877A MT AR (data not shown). The functional SAR (IC_{50}) for the T877A MT AR (LNCaP) as well as the PCa2b cell line showed little correlation with the WT AR (MDA-453). For the T877A MT AR, little correlation was seen between binding and function, with compounds of similar binding affinities displaying either an agonist or an antagonist profile, as exemplified by compounds **18** and **20**. For aniline systems substituted at the 4-position or the 3- and 4-positions, no clear trends could be identified relative to the functional activity against the MT AR isoforms. Despite the fact that the PCa2b cell line contains a second mutation in the AR LBD (L701H) in addition to the T877A mutation found in the AR of the LNCaP cell line, functional activity between the two cell lines matched extremely well. This is an important observation as it correlates the ability of a compound to antagonize the AR in a reporter assay system (LNCaP cell line assay) with the ability to block AR dependent cellular proliferation (PCa2b cell line assay) in the background of a human prostate cancer cell line.

The goal of this initial series of analogs was to probe the influence of the aniline portion of the molecule and iden-

Table 4. SAR for a series of [2.2.1]-bicyclo-1H-isoindole-1,3(2H)-dione analogs



#	R	MD-453 K_i nM[a]	MD-A453 IC_{50} nM[b]	LNCaP K_i nM[a]	LNCaP IC_{50} nM[a]	PCa2b IC_{50} nM[c]
11		632	661	7	35	410
22		401	1746	24	72	198
23		158	307	6	41	180
24		530	1038	13	52	234
25		284	1210	11	84	265
26		162	418	32	1790	3639

[a–c,#] See footnotes for Table 1.

tify those features, which maximized binding while consistently yielding an antagonist profile against both the WT and MT isoforms of the AR. With the optimal substitution pattern for the aniline portion of the molecule better defined, we could next turn our attention toward optimization of potency and selectivity by modifications about the bicyclic and imide portion of the molecule. Although most of the aniline systems, which gave high affinity binding to the WT AR showed an agonist profile with the MT AR isoforms, two SAR patterns were identified, which provided potent binding and an antagonist profile across all the AR isoforms. As shown in Table 4, anilines substituted with electron withdrawing groups at the 3- or 3- and 5-positions (compounds **11**, **22**, and **23**) consistently demonstrated binding and an antagonist profile against all AR isoforms. Surprisingly, in the case of 3,5-disubstituted anilines, further substitution at the 4-position, even with electron withdrawing groups anticipated to increase binding, resulted in a loss of binding and functional activity across all AR isoforms (data not shown). It was also noted that incorporation of a 2-naphthyl group (compare compounds **24**–**25**) consistently gave a full antagonist profile against all AR isoforms across a range of substitutions on the aryl ring. This is in contrast to 1-naphthyl analogs, which gave variable data depending upon the substitution pattern about the aryl ring (compare compounds **21**–**26**).

In summary, we have developed and characterized a novel series of bicyclic-1H-isoindole-1,3(2H)-dione based AR antagonists. These compounds demonstrate potent binding to and functional antagonist activity against the WT AR. SAR revealed that for this series of AR antagonists, functional activity was governed by the aniline portion of the molecule. Optimization of the aniline portion revealed substitution patterns, which yielded potent binding and functional antagonist activity toward the WT AR and T877A MT AR found in the LNCaP human prostate cancer cell line, as well as potent growth inhibitory activity against the MDA-MB-PCa2b human prostate cancer cell line, which contains a doubly mutated L701H and T877A AR. With the optimal substitution pattern for the aniline portion of the molecule better defined, we will next turn our attention toward optimization of potency and selectivity by modifications about the bicyclic and imide portion of the molecule. These approaches will be discussed in future publications.

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