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BRIEF ARTICLE

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Unexpected Binding Orientation of Bulky-B-Ring Anti-Androgens and Implications for Future Drug Targets†

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Supporting Information

ABSTRACT: Several new androgen receptor antagonists were synthesized and found to have varying activities across typically anti-androgen resistant mutants (Thr877 → Ala and Trp741 → Leu) and markedly improved potency over previously reported pan-antagonists. X-ray crystallography of a new anti-androgen in an androgen receptor mutant (Thr877 → Ala) shows that the receptor can accommodate the added bulk presented by phenyl to naphthyl substitution, casting doubt on previous reports of predicted binding orientation and the causes of antagonism in bulky-B-ring antagonists.

INTRODUCTION

Prostate cancer, the second most frequent cause of cancer death in men, is treated with nonsteroidal anti-androgens such as bicalutamide (**1**, Figure 1) and hydroxyflutamide that antagonize the androgen receptor (AR) pathway, preventing androgen-dependent cell growth. In AR wild type (AR(wt)), **1** and hydroxyflutamide act as antagonists. Long-term treatment with anti-androgens results in anti-androgen resistance in prostate cancer cells or androgen withdrawal syndrome in patients. This phenomenon is thought to arise from, among other mechanisms, mutations to several key residues in the ligand-binding domain. Commonly studied mutations in AR include Thr877 → Ala (AR(T877A)), Trp741 → Leu (AR(W741L)), and Trp741 → Cys (AR(W741C)), in which varying agonism and antagonism are observed with use of those drugs. Specifically, **1** is an agonist in AR(W741L) and AR(W741C)[1] while hydroxyflutamide is an agonist in AR(T877A).[2]

In contrast, AR(wt) agonists such as S-22 (**2**)[3] are probably agonists in all of these mutants, since they will geometrically fit no worse with the bulk-reducing mutations than in AR(wt). The difference in AR(wt) activity between agonists such as **2** and antagonists such as **1** seems to arise from the linker oxygen's smaller size relative to the sulfonyl group of **1**. This bulk in antagonist drugs, though small, is thought to push the AR helix 12 (H12) away from the binding pocket, disturbing the agonist conformation of the receptor, the current theoretical key to antagonism.[4]

In a recent communication to *Journal of the American Chemical Society* on the use of molecular design to circumvent anti-androgen resistance in prostate cancer, McGinley and Koh predicted the orientation of bulky nonpolar substituents in analogues of the nonsteroidal anti-androgen **1** in molecular modeling and the apparent contact with H12.[5] This was the case in AR(wt) and all mutants for three reported compounds **3**, **4**, and **5** (Figure 1), all of which had K_i in the 2−5 μM range and IC_{50} in the 3−20 μM range. The study utilized our X-ray structure of **1** bound in agonist mode to AR(W741L) with H12 deleted to predict binding of their antagonists in the antagonist mode.[6]

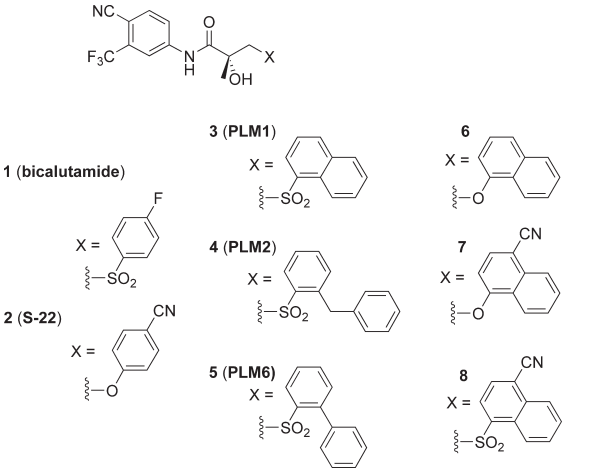

Figure 1. Structures of **1** and **2**, McGinley and Koh's analogues **3**−**5**,[5] and new analogues **6**−**8**.

Here we report findings of X-ray crystallographic structures and biological evaluation of several new compounds (**6**, **7**, and **8**, Figure 1) with markedly increased binding affinity but in some cases similar pan-antagonism as those reported by McGinley and Koh.[5] The X-ray structure of **7** in AR(T877A) has an orientation of the bulky naphthyl substituent that disagrees strikingly with the previous researchers' predictions.

Noting the value of the findings with **3**, we sought to improve upon these structures by varying the linker group of **3** from SO_2 to O (**6**) and improving binding by adding a CN group (**7**). We then attempted an improved activity SO_2-linked compound (**8**). Ether and amine linkages have been used in our labs in the past to

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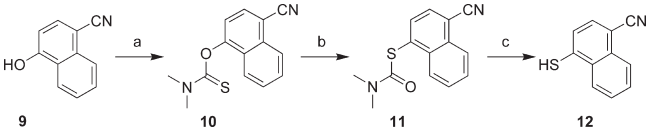
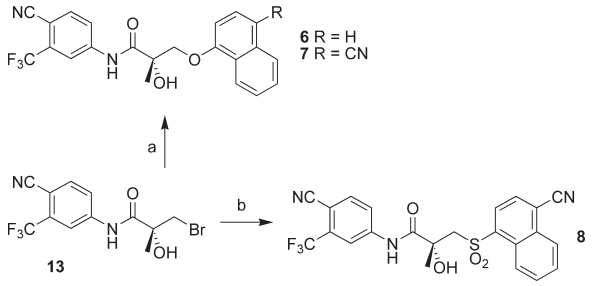
Scheme 1. Synthesis of 4-Cyano-1-naphthothionitrile (12)[a]



[a] Conditions: (a) dabco, *N*,*N*-dimethylthiocarbamoyl chloride, DMF, 70 °C; (b) 210 °C, argon atmosphere, 2 h; (c) THF, KH, MeOH, rt, 3 h.

Scheme 2. Synthesis of 6−8[a]



[a] Conditions: (a) (i) K_2CO_3, acetone, reflux 3 h; (ii) 1-naphthol, 2-propanol, K_2CO_3 reflux 18 h, **6** or 4-hydroxynaphtho-1-nitrile (**7**); (b) (i) sodium 4-cyanonaphthylthiolate, THF, 25 °C; (ii) MCPBA, dichloromethane.

Table 1. AR(wt) Relative Binding Affinities[a]

compd	RBA (%)
DHT	100 ± 11.6
1	0.69 ± 0.07
2	5.5 ± 1.8
6	0.97 ± 0.03
7	1.84 ± 0.7
8	0.37 ± 0.02

[a] Binding affinities were determined in a radiolabeled competitive assay and expressed relative to DHT.[11]

produce several potent AR modulators including **2** (agonist, 1 nM).[3,7]

CHEMISTRY

A previously reported method was used for preparation of 4-hydroxy-1-naphthonitrile (**9**).[8] A reported synthesis of 4-cyanothiophenol was adapted to obtain 4-mercapto-1-naphthonitrile (**12**) from **9** via thermal isomerization of a *N*,*N*-dimethylthiocarbamoyl fragment.[9] (Scheme 1)

Compounds **6**−**8** were all synthesized from previously known **13**[10] using varying coupling methods (Scheme 2). Epoxide formation from **13** using K_2CO_3 followed by treatment with 1-naphthol or 4-hydroxy-1-naphthonitrile in the presence of K_2CO_3 afforded **6** and **7**, respectively. Treatment of **13** with sodium 4-cyano-1-thionaphthylate in THF at room temperature followed by oxidation with *m*CPBA afforded **8**.

IN VITRO RESULTS

Compounds **6**−**8** were evaluated in vitro for agonist and antagonist effects on AR(wt), AR(W741L), and AR(T877A) as well as relative binding affinities (RBA) compared to dihydrotestosterone (DHT).[11] **1** bound the AR(wt) with an RBA of 0.69

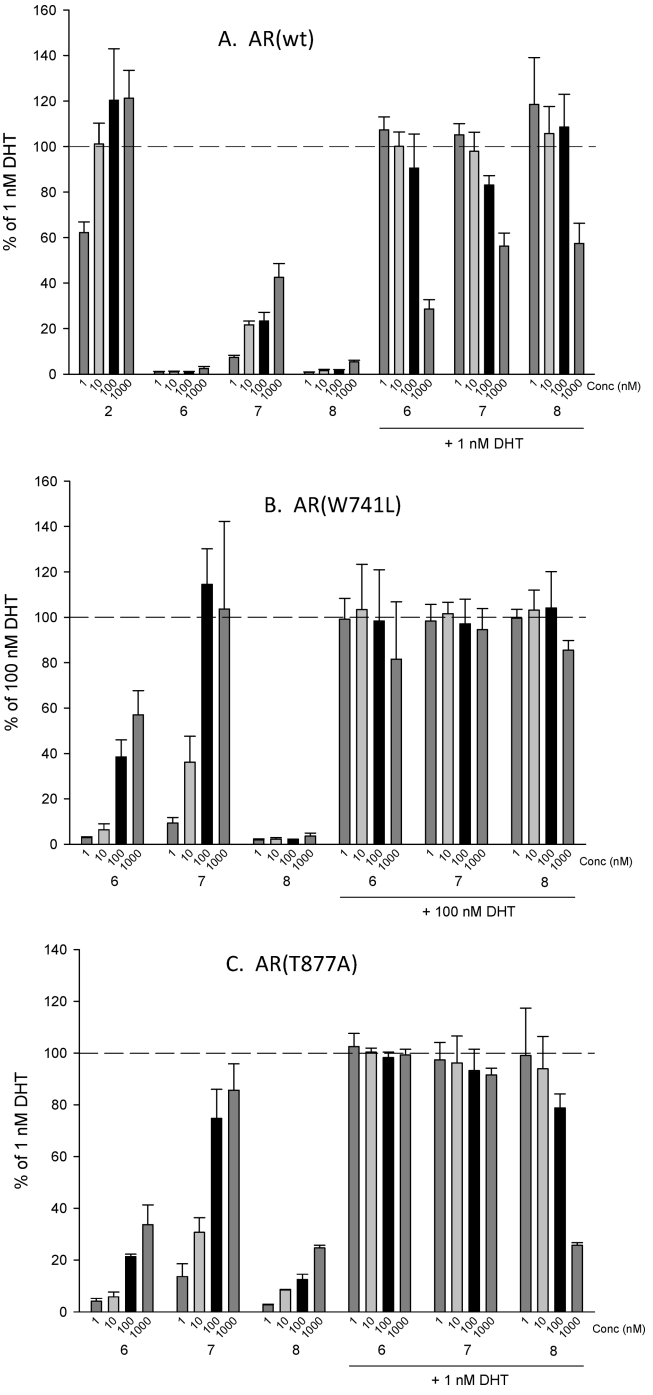


Figure 2. In vitro activity of anti-androgens. The functional activity of each compound was determined by the ability of each ligand to induce or suppress AR(wt), AR(W741L), or AR(T877A)-mediated transcriptional activation in a cotransfection system.[11] The activity of each compound (final concentrations of 1−1000 nM) was determined by incubating cells in the absence (agonist assays) or presence (antagonist assays) of DHT and expressed as the percentage of that induced by DHT. (A) Agonist and antagonist activity with or without the presence of 1 nM DHT of each compound in AR(wt). (B) Agonist and antagonist activity with or without the presence of 100 nM DHT in AR(W741L). (C) Agonist and antagonist activity with or without the presence of 1 nM DHT of each compound in AR(T877A).

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(Table 1). Addition of the naphthyl ring and 4-CN (**8**) decreased the binding affinity. However, replacing the sulfonyl group with an ether linkage and adding the naphthyl ring (**6**) increased affinity. Binding affinity was further enhanced by addition of a 4-CN group (**7**).

Where **2** is a full agonist, **6**−**8** were antagonists in the AR(wt) with varying activity (Figure 2A). **7** is also a partial agonist in AR(wt), while **8** is not. **8** displays weak antagonist activity at the highest concentration tested (1 μM), by inhibiting DHT-induced transactivation by 15% (Figure 2B). In the AR(T877A) mutant, **8** inhibited the activity of DHT by 61% (Figure 2C). **1** inhibited the activity of DHT by 84% in the AR(T877A) (not shown). In the AR(W741L) mutant, **1** (not shown) and **7** (Figure 2B) are full agonists.

Of the studied compounds, **7** displayed the highest affinity but did not exhibit pan-antagonism. As such, a crystal structure was obtained for the **7**/AR(T877A) complex. The scaffold of **7** overlays closely with that of **2** in AR(wt), reported here for the first time (Figure 3). To our surprise, **7**/AR(T877A) did not show the orientation of the distal naphthyl ring predicted by McGinley and Koh.[5] Rather, the distal ring of the naphthyl group experiences an edge-to-face $\pi-\pi$ interaction with the A-ring and is situated between W741 and M742 opposite H12. In addition, M745 shifts relative to the structure of **2**/AR(WT) to accommodate the bulk (Figure 4). Interestingly, there is a cascade of effects that are set off by the bulk accommodation; the distal naphthyl ring causes M742 to reorient (not shown), which pushes M745 toward the A-ring of **7**, causing a steric interaction. This steric interaction explains the lowered binding affinity of **7** compared to **2**.

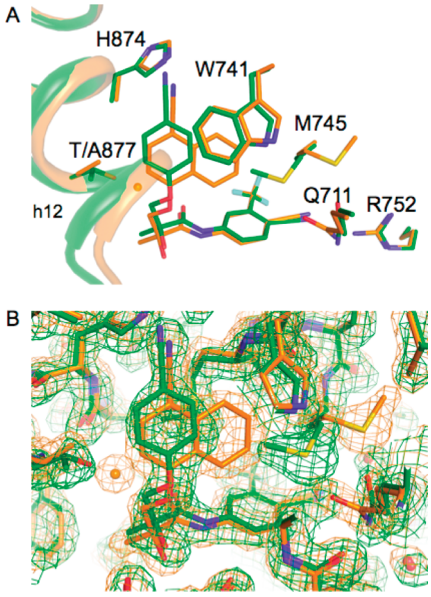

Figure 3. Overlaid X-ray structures of **2** (green carbons) and **7** (orange carbons) cocrystallized with AR(T877A) with (A) and without (B) electron density maps. The orientation of **7** is closely overlaid with **2**, and the distal naphthyl ring extends directly away from H12 toward M745.

Considering the similarities of the structures, the orientation of the naphthyl ring is expected to be highly conserved across **3** and **6**−**8**. The **7**/AR(T877A) complex is in transcriptionally active form, which does not explain how the naphthyl moiety causes antagonism in AR(WT), since the ring does not appear to appreciably interact with the T877/T877A space. This does, however, suggest that the binding of **7** in AR(WT) in antagonist mode would not be different with respect to the naphthyl group's orientation and that the naphthyl group orientation should be conserved across the ligands considered here. Therefore, the linker must be responsible for the observed differences in agonism and antagonism. Further supporting this is the fact that **7** is a partial agonist in AR(wt) while **8** shows no agonist activity.

The differences observed across the range of linkers are highlighted by the differences in the O-linked complexes **7** and **2** from **1** and its SO_2 linker. The molecules are separated with respect to binding not because of their B-ring size or shape but because of their linker size and orientation. In the structure of **1**/AR(W741L) the sulfonyl group is directed away from the rest of the ligand and forced to impinge on the space occupied by H12, causing antagonism.[6] In contrast are the intramolecular H-bond to the amide experienced by the **2** and **7** ether linkers.

Results of **3**, **4**, and **5** were predicted by the deletion of H12 in silico, which provided ample space for the distal naphthyl ring of **3**, but in the presence of H12, the ring's true preferred position is opposite the modeled results. This approach of H12 deletion is predicated on the assumption that AR in its inactive form is somewhat similar to ER, but it is well established in other receptors of the same family that the movement of H12 away from the active conformation can be much more subtle as in the case of PR.[4] This unfortunately suggests that deletion of H12 in silico is not as effective a predictive tool as it once appeared.

In sum, it is apparent that antagonism can be controlled by bulk extending from not only the B-ring but also an appropriate linker. Work is currently underway in our hands to explore the role of bulkier, branched linker groups.

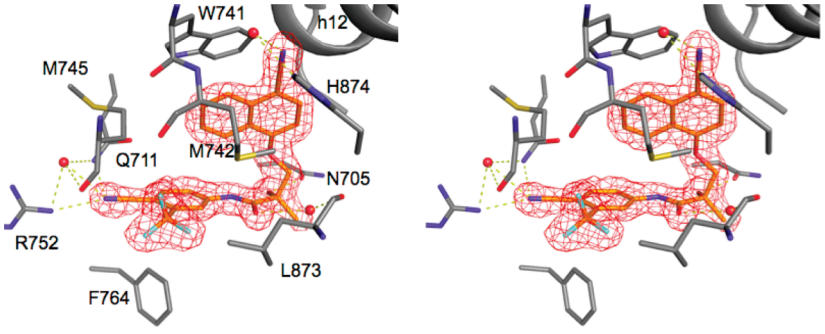

Figure 4. Stereoview of the X-ray structure of **7** (orange) cocrystallized with AR(T877A). There is an intermolecular $\pi-\pi$ interaction between the naphthyl ring of **7** and the W741 indolyl ring, which is 3.1 Å from the naphthyl ring.

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■ ASSOCIATED CONTENT

Supporting Information. Synthesis procedures and characterization data, including NMR, MS, and CHN results; X-ray crystallographic data for complexes **2**/AR(WT) and **7**/AR(T877A). This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

[†]PDB codes: WT-S-22, 3RLJ; T877A-7, 3RLL.

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■ ABBREVIATIONS USED

AR, androgen receptor; AR(T877A), Thr877 → Ala; AR(W741C), Trp741 → Cys; AR(W741L), Trp741 → Leu; AR(wt), wild-type androgen receptor; Dabco, 1,4-diazabicyclo[2.2.2]octane; DHT, dihydrotestosterone; DMF, dimethylformamide; H12, androgen receptor helix 12; MCPBA, *m*-chloroperoxybenzoic acid; RBA, relative binding affinity; rt, room temperature; THF, tetrahydrofuran

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