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NOTE

# Arabilin overcomes resistance to AR-targeted therapy

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The androgen receptor (AR) has a major role in the development of the prostate and in regulating genes encoding proteins for normal prostate function. In prostate cancer, increased level of AR in tumor cells promote AR signaling.[1] Therefore, androgen deprivation by medical or surgical castration is the mainstay treatment for men with advanced prostate cancer, and an increased understanding of the mechanisms of resistance to castration over the past decade has led to the discovery of novel agents.[2]

AR antagonists compete with dihydrotestosterone (DHT) for binding to the AR, thereby inducing changes in AR structure that impair transcriptional activity and inhibit the growth of prostate cancer cells. AR was characterized in the late 1960s, leading to the development of synthetic AR antagonists; this led to flutamide being approved in 1989, followed by bicalutamide (Figure 1a). Furthermore, a second-generation AR antagonist, enzalutamide, has been developed out of the need for more effective and long-term AR inhibition, and has recently been approved for patients with castration-resistant prostate cancer (CRPC) (Figure 1b).[3] However, long-term treatment with these first- and second-generation AR antagonists can lead to AR point mutations that are linked to the development of resistance.

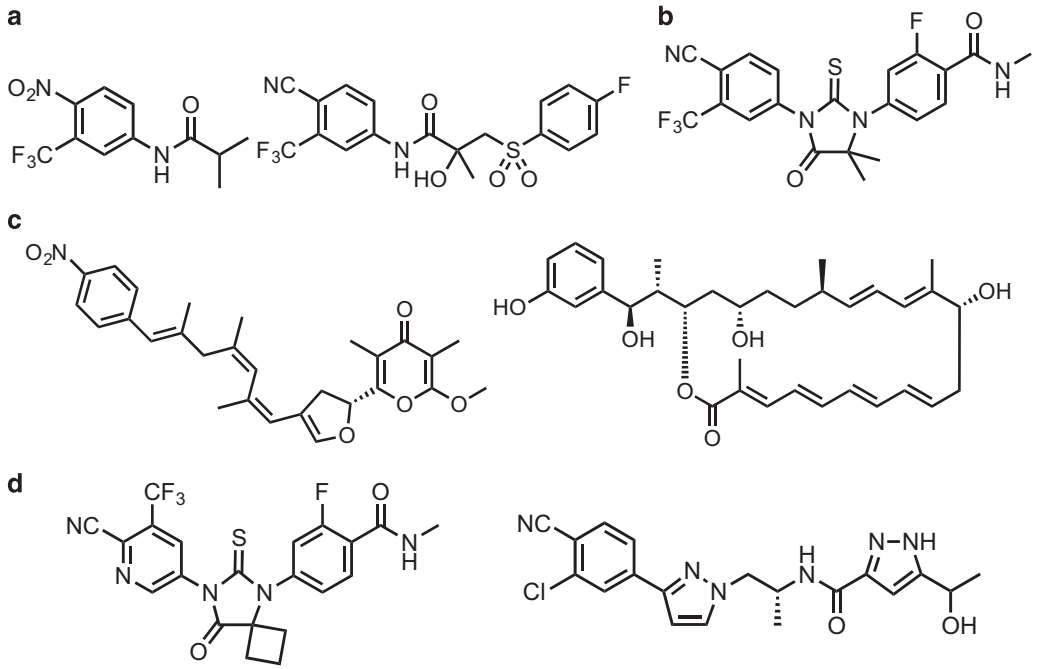


**Figure 1** (**a**) The structure of first-generation AR antagonists (flutamide (left) and bicalutamide (right)). (**b**) The structure of a second-generation AR antagonist (enzalutamide). (**c**) The structure of the AR antagonists we discovered (arabilin (left) and antarlide A (right)). (**d**) The structure of new-generation AR antagonists (apalutamide (left) and darolutamide (right)). AR, androgen receptor.

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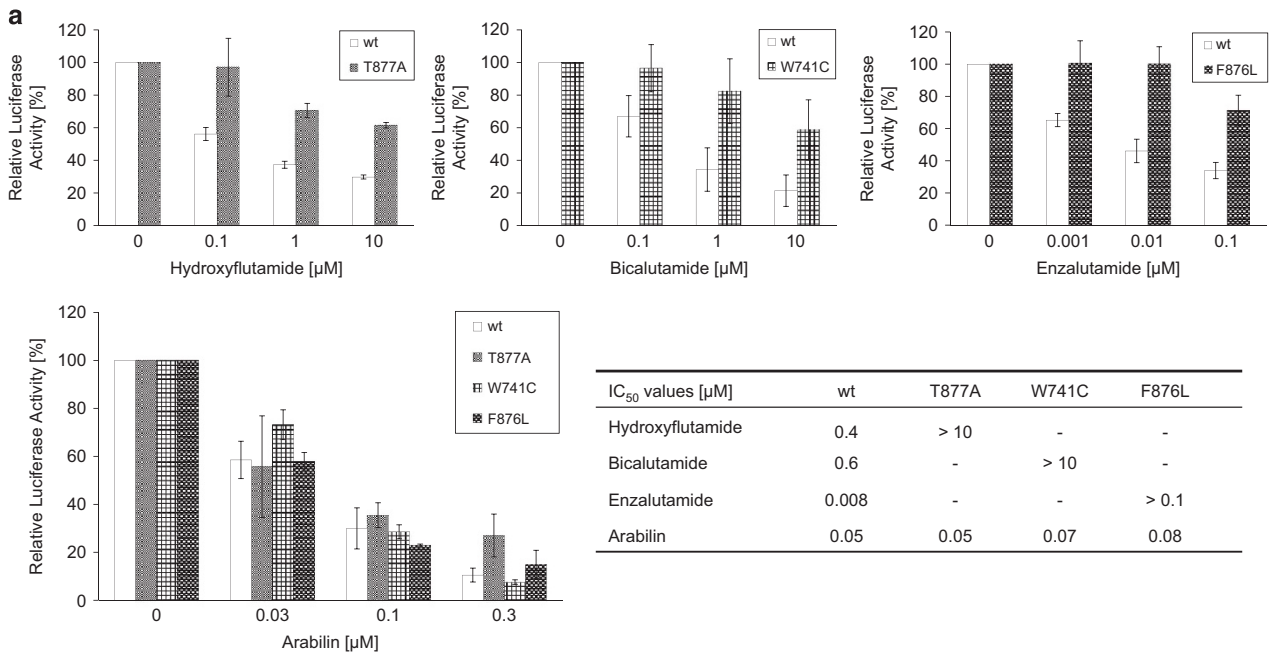
Thus, the development of a new type of AR antagonist is an attractive strategy to overcome prostate cancers that are resistant to known antagonists.

Previously, we reported the isolation and structural determination of arabilin as a potent AR antagonist from *Streptomyces* sp. MK756-CF1 (Figure 1c).[4] Arabilin is a novel polypropionate-derived metabolite with a *p*-nitrophenyl group and a substituted γ-pyrone ring. Total synthesis of arabilin was completed by Lim and Parker.[5]

Arabilin inhibits the binding of DHT to AR in a dose-dependent manner at an $IC_{50}$ of 11 μM.[4] However, it does not affect the binding of estradiol to the estrogen receptor. Furthermore, arabilin inhibits not only the DHT-induced expression of prostate-specific antigen (PSA) mRNA in prostate cancer LNCaP cells, but also inhibits the DHT-induced growth of LNCaP cells. In this study, we evaluated whether arabilin is capable of inhibitory activity against mutant ARs, which are linked to the development of resistance to first- and second-generation AR antagonists (T877A for flutamide resistance,[6] W741C for bicalutamide resistance[7] and F876L for enzalutamide resistance[8]).

Previously, we constructed these mutant AR plasmids by inverse PCR and established a luciferase reporter assay system using them.[9] The effects of anti-androgens on mutants AR (T877A), AR (W741C) and AR (F876L) were studied in transactivation assays in HEK293T cells transiently transfected with expression vectors encoding the corresponding mutant ARs and an androgen-responsive




| $IC_{50}$ values [μM] | wt | T877A | W741C | F876L |
|---|---|---|---|---|
| Hydroxyflutamide | 0.4 | > 10 | - | - |
| Bicalutamide | 0.6 | - | > 10 | - |
| Enzalutamide | 0.008 | - | - | > 0.1 |
| Arabilin | 0.05 | 0.05 | 0.07 | 0.08 |

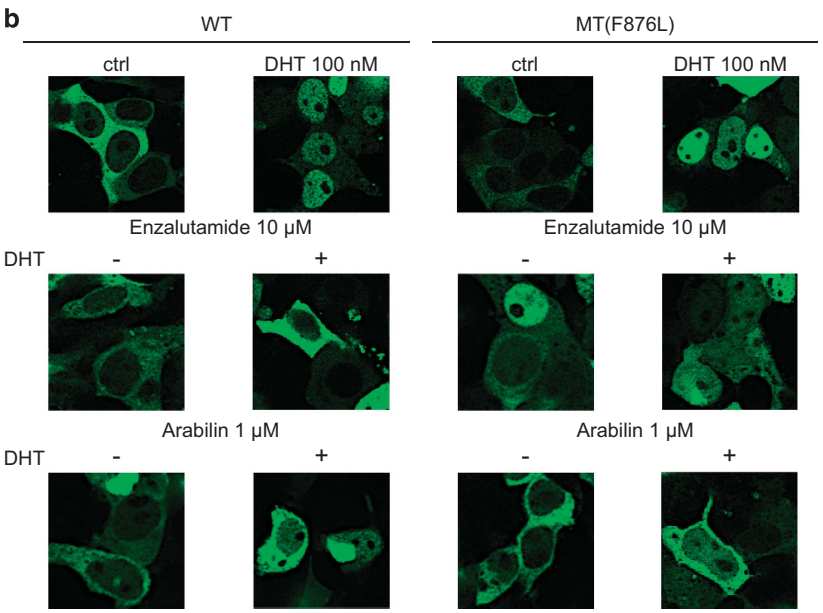



**Figure 2** (**a**) Effects of arabilin on transcriptional activity of wild-type or mutant ARs. Bars represent relative transcriptional activity as percentage of control (DHT set as 100%). $IC_{50}$ values are presented in the table. (**b**) Effects of arabilin on nuclear translocation of wild-type or mutant AR (F876L). AR, androgen receptor; DHT, dihydrotestosterone.

luciferase reporter gene construct. The cells were then treated with DHT (100 nM) and AR antagonists (arabilin, hydroxyflutamide, bicalutamide or enzalutamide). After 24 h, cells were harvested and assayed for luciferase activity. As shown in Figure 2a, hydroxyflutamide inhibited DHT-induced transcriptional activity of wild-type AR in a dose-dependent manner, but not that of flutamide-resistant AR (T877A). Similarly, bicalutamide and enzalutamide inhibited DHT-induced transcriptional activity of wild-type AR, but not that of the corresponding mutant ARs. Though these AR antagonists inhibited DHT-induced transcriptional activities of the corresponding mutant ARs weakly, this is because these AR antagonists act as partial antagonists.[10] On the other hand, arabilin inhibited the transcriptional activities not only of wild-type AR, but also of all tested mutant ARs at similar concentrations (Figure 2a), indicating that arabilin can overcome resistance against both first- and second-generation AR antagonists. Next, we examined the effect of arabilin on the nuclear translocation of wild-type and mutant AR. For this, wild-type GFP-AR or mutant GFP-AR (F876L) was overexpressed in HEK293T cells, and their subcellular localizations were observed with confocal microscopy.[9] As shown in Figure 2b, in wild-type AR-expressing cells, AR was predominantly cytoplasmic. This localization was not affected by the addition of either enzalutamide or arabilin. Treatment of cells with DHT (100 nM) for 1 h induced nuclear translocation of AR, and this translocation of AR was inhibited by the addition of enzalutamide or arabilin. In mutant AR (F876L)-expressing cells, mutant AR was also predominantly cytoplasmic in the absence of DHT, whereas treatment of cells with DHT (100 nM) for 1 h induced nuclear translocation of AR. As it has been reported that the F876L substitution in AR switches enzalutamide from an antagonist to an agonist,[8] treatment of cells with enzalutamide alone for 1 h induced nuclear translocation of GFP-AR (F876L). In this condition, arabilin did not induce nuclear translocation of mutant AR (F876L). These results indicated that arabilin does not act as an agonist in enzalutamide-resistant cells. Moreover, when the cells were treated with DHT (100 nM), arabilin inhibited DHT-induced nuclear translocation of not only wild-type AR but also mutant AR (F876L). These results also indicated that arabilin inhibits DHT-induced nuclear translocation of AR in enzalutamide-resistant cells.

Despite significant recent advances in the treatment of CRPC, many patients demonstrate limited clinical response. As a result, a number of new-generation AR antagonists are in clinical development.[11] Apalutamide (ARN-509) is currently in phase III clinical trials for CRPC; however, it does not show antagonist activity against mutant AR (F876L), possibly due to structural similarity with enzalutamide (Figure 1d).[12] As the diversity of chemical scaffolds of clinically used AR antagonists has thus far been narrow, new structures are of interest as they might have properties that differ from current AR antagonists. Darolutamide (ODM-201) is a synthetic AR antagonist presently in a phase III study, and it is both novel and structurally distinct from any known AR antagonist including the enzalutamide (Figure 1d).[13] Darolutamide has been shown to be a full antagonist of mutant AR (F876L), AR (W741L) and AR (T877A). We have recently isolated novel compounds, antarlides A–E, from *Streptomyces* sp. BB47, as AR antagonists.[9] Antarlides have a novel macrocyclic structure with a 22-membered ring. Antarlides inhibited the transcriptional activity of not only wild-type AR but also of mutant ARs. Therefore, the unique structure may be important for developing AR antagonists that can overcome resistance to AR-targeted therapy. Arabilin is also structurally distinct from any known AR antagonist, including second-generation AR antagonists, as judged from the chemical space based on E-Dragon.[14] In conclusion, our data showing that arabilin can overcome resistance to clinically used AR antagonists indicate that it has potential to be developed as a new-generation AR antagonist.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

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1 Heinlein, C. A. & Chang, C. Androgen receptor in prostate cancer. *Endocr. Rev.* **25**, 276–308 (2004).
2 Chen, C. D. *et al.* Molecular determinants of resistance to antiandrogen therapy. *Nat. Med.* **10**, 33–39 (2004).
3 Tran, C. *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* **324**, 787–790 (2009).
4 Kawamura, T. *et al.* Isolation and structure elucidation of a novel androgen antagonist, arabilin, produced by *Streptomyces* sp. MK756-CF1. *J. Antibiot.* **63**, 601–605 (2010).
5 Lim, H. N. & Parker, K. A. Total synthesis of the potent androgen receptor antagonist (-)-arabilin: a strategic, biomimetic [1,7]-hydrogen shift. *J. Am. Chem. Soc.* **133**, 20149–20151 (2011).
6 Ozers, M. S. *et al.* The androgen receptor T877A mutant recruits LXXLL and FXXLF peptides differently than wild-type androgen receptor in a time-resolved fluorescence resonance energy transfer assay. *Biochemistry* **46**, 683–695 (2007).
7 Yoshida, T. *et al.* Antiandrogen bicalutamide promotes tumor growth in a novel androgen-dependent prostate cancer xenograft model derived from a bicalutamide-treated patient. *Cancer Res.* **65**, 9611–9616 (2005).
8 Korpal, M. *et al.* An F876L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). *Cancer Discov.* **3**, 1030–1043 (2013).
9 Saito, S., Fujimaki, T., Panbangred, P., Igarashi, Y. & Imoto, M. Antarlides: a new type of androgen receptor (AR) antagonist that overcomes resistance to AR-targeted therapy. *Angew. Chem. Int. Ed.* **55**, 2728–2732 (2016).
10 Lallous, N. *et al.* Functional analysis of androgen receptor mutations that confer anti-androgen resistance identified in circulating cell-free DNA from prostate cancer patients. *Genome Biol.* **17**, 10 (2016).
11 Antonarakis, E. S. *et al.* Targeting the N-terminal domain of the androgen receptor: a new approach for the treatment of advanced prostate cancer. *Oncologist* **21**, 1–9 (2016).
12 Balbas, M. D. *et al.* Overcoming mutation-based resistance to antiandrogens with rational drug design. *Elife* **2**, e00499 (2013).
13 Moilanen, A. M. *et al.* Discovery of ODM-201, a new-generation androgen receptor inhibitor targeting resistance mechanisms to androgen signaling-directed prostate cancer therapies. *Sci. Rep.* **5**, 12007 (2015).
14 Tashiro, E. & Imoto, M. Chemical biology of compounds obtained from screening using disease models. *Arch. Pharm. Res.* **38**, 1651–1660 (2015).