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Targeting prostate cancer with compounds possessing dual activity as androgen receptor antagonists and HDAC6 inhibitors

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ABSTRACT

While enzalutamide and abiraterone are approved for treatment of metastatic castration-resistant prostate cancer (mCRPC), approximately 20–40% of patients have no response to these agents. It has been stipulated that the lack of response and the development of secondary resistance to these drugs may be due to the presence of AR splice variants. HDAC6 has a role in regulating the androgen receptor (AR) by modulating heat shock protein 90 (Hsp90) acetylation, which controls the nuclear localization and activation of the AR in androgen-dependent and independent scenarios. With dual-acting AR–HDAC6 inhibitors it should be possible to target patients who don't respond to enzalutamide. Herein, we describe the design, synthesis and biological evaluation of dual-acting compounds which target AR and are also specific towards HDAC6. Our efforts led to compound **10** which was found to have potent dual activity (HDAC6 IC_{50} = 0.0356 μM and AR binding IC_{50} = <0.03 μM). Compound **10** was further evaluated for antagonist and other cell-based activities, in vitro stability and pharmacokinetics.

It is widely recognized that chemotherapy drugs are most effective when given in combination. The rationale for combination therapy is to harness disparate mechanisms, thereby reducing the likelihood of resistance. Deriving from the same principles, it is possible to have a single molecule with dual activity. Cabozantinib is a small molecule tyrosine kinase inhibitor of cMet and VEGFR2; this dual activity has culminated in US FDA approvals for medullary thyroid cancer and advanced renal cell carcinoma and it is currently being tested in the clinic for various other cancers, including prostate cancer (PC).[1] At the research level, a recent Letter describes the synthesis and evaluation of dual-acting estrogen receptor (ER) and histone deacetylase (HDAC) inhibitors (HDACi).[2,3] These ER–HDAC inhibitors combined ERα antagonist activity with potent HDAC inhibitor activity, resulting in better anti-tumor efficacy in ERα positive breast cancer cells in vitro when compared to the approved drug Tamoxifen.

HDACs modulate histone acetylation, which controls gene expression. HDAC inhibitors have been studied and tested in cancer treatment with numerous agents approved and others undergoing clinical trials.[4,5] HDAC6 has been implicated in the pathogenesis and treatment of cancer[6] and its role in regulating the androgen receptor (AR) by modulating heat shock protein 90 (Hsp90) acetylation has also been studied.[7,8] Hsp90 acetylation controls the stability, nuclear localization and activation of the AR in androgen-dependent and independent scenarios. Inhibition of HDAC6 therefore provides an opportunity to target castration resistant prostate cancer.[6–9]

Enzalutamide has proven to be clinically beneficial in metastatic castration-resistant prostate cancer (mCRPC).[10–12] While enzalutamide and abiraterone[13] are approved for treatment of mCRPC, approximately 20–40% of patients have no response to these agents. Moreover, it has been stipulated that the lack of response and the development of secondary resistance to these drugs may be due to the presence of AR splice variants.[14] Meanwhile, the clinical evaluation of HDAC inhibitors as monotherapy for prostate cancer has not been promising. However using a

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combination of an HDAC inhibitor with antiandrogens, a synergistic increase in cytotoxicity has been demonstrated in a number of hormone-sensitive and -resistant preclinical models.[15] In such scenarios, dual-acting AR–HDAC6 inhibitors may be of value. The idea of using AR binding as a means of directing HDACi to prostate tumors has been explored, where AR binding is suggested as a homing device.[3] These authors reported using cyanonilutamide as the AR binding element in their designs of dual AR-HDACi.[3] Herein, we describe our efforts to make dual-acting compounds which target AR and are also specific towards HDAC6, using enzalutamide as the AR binding element. During the preparation of our manuscript, a similar hybrid molecule generated from enzalutamide and HDAC inhibitor such as Vorinostat was reported to inhibit viability of enzalutamide resistant PC cells by downregulating HSP90 and AR.[16]

In the design of our dual AR–HDAC6 inhibitors, the intent was to maintain AR antagonist activity, while also inhibiting HDAC6. We first examined the binding mode of enzalutamide, which when compared to conventional agents like Bicalutamide, binds to the AR with higher affinity and demonstrates pure antagonist activity in preclinical models.[10] After preparing the 3D structure of enzalutamide using LigPrep,[17] we performed IFD to the ligand binding domain (LBD) of human AR (pdb code: 1T63)[18] to understand its binding mode. The computational methods used are described in Supporting information (SI) section. Figure 1a illustrates the key interactions in 2D, highlighting the hydrophobic nature of the LBD and active site hydrogen bonds. The poses obtained from induced fit docking (IFD) shows that the trifluoromethyl group makes favorable van Der Waals (vDW) contacts with hydrophobic residues Val746, Met749, Phe764 and Leu873. Also the A ring of enzalutamide (Fig. 1b) forms a T-shaped pi-pi stacking interaction with Phe764. The cyano group forms H-bonds with two key active site residues—Arg752 and amide of Gln711. This group occupies the same position as the keto group in the endogenous substrate dihydrotestosterone (DHT). The amide oxygen is involved in an H-bond with Asn705. We also observed that the methyl amide part of enzalutamide points towards Helix12 (Fig. 1b) of the LBD, which was crucial for our design strategy moving forward.

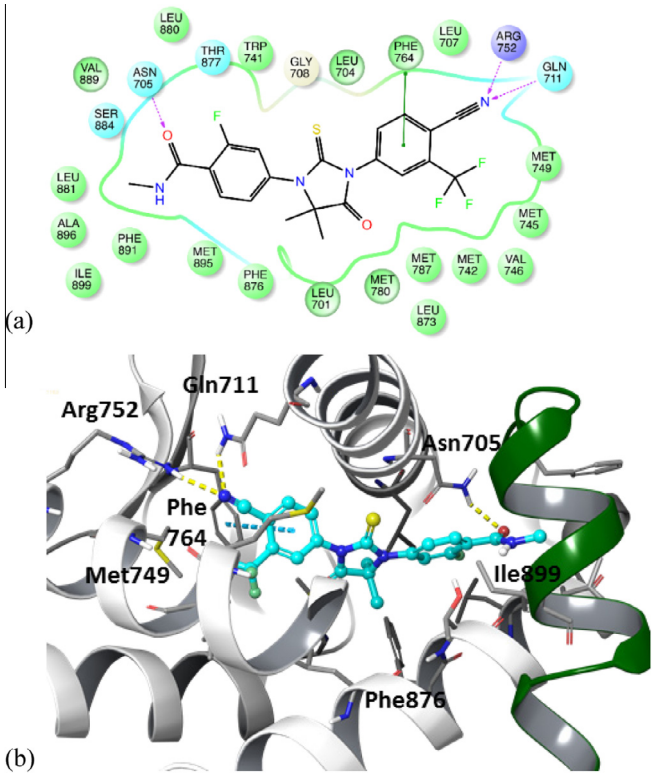

Figure 1. IFD pose of enzalutamide bound to AR. (a) Interaction of enzalutamide with AR represented in 2D. (b) The methyl amide points towards Helix12 shown in green. Enzalutamide is shown in blue ball and stick model. The key residues that interact with enzalutamide are labeled. The residues Arg752 and amide of Gln711 form H-bonds with the cyano group whereas Asn705 engages the methyl amide tail of enzalutamide. Phe764 forms a pi-pi stacking interaction with the cyanotrifluoromethyl phenyl-ring.

HDAC inhibitors typically have a zinc binding group (ZBG) which is attached to a cap group through a hydrophobic linker. Many of the reported HDAC inhibitors have an aromatic cap group such as indole (Panobinostat[19]) or phenyl (suberoylanilide hydroxamic acid, SAHA/Vorinostat[19]) groups. Until recently, no structure for HDAC6 or any other class IIb HDACs was available to guide inhibitor design. Two recent Letters detailing the structure of human and zebrafish HDAC6[20,21] have added valuable insights to our design approach. Crystal structures of Vorinostat and Panobinostat bound to HDAC6[21] show that the hydroxamic acid in these pan–HDAC inhibitors interacted with the active-site Zn^{2+} in a bidentate mode that was distinctly different from the HDAC6-selective inhibitor *N*-hydroxy-4-(2-((2-hydroxyethyl)(phenyl)amino)-2-oxoethyl)benzamide (HPOB), which had a monodentate interaction with Zn^{2+}. Moreover, the cap group of these pan–HDAC inhibitors appear to make interactions with the L1 loop (D460–P484) that may favor binding to HDAC1–3. Despite having almost the same first zinc coordination shell, different hydroxamate-zinc coordination modes are observed in HDACs. For example, the zinc binding mode is bidentate in HDAC8 (PDB code 1T69) and monodentate in HDAC7 (PDB code C0Z20). The nature of this coordination appears to be strongly driven by the local environment around zinc and water in this binding channel.[22] Without further detailed investigation and comparing binding energies of the ligands being investigated here (such as using high level quantum mechanical methods), it is not possible to determine if the zinc coordination mode is indeed a key player for selectivity.

With the knowledge that the methyl amide attached to the distal phenyl group of enzalutamide points towards Helix12, we proposed to use this phenyl group as the point of attachment of the HDAC inhibitor, through a suitable linker, as illustrated in Figure 2. The cyanotrifluoromethyl phenyl group (A-ring) and the thiohydantoin (B-ring) would be maintained to participate in key interactions with the AR ligand binding domain. In this design, the HDAC inhibitor is positioned such that it putatively points towards Helix12, thus maintaining AR antagonist activity. Enzalutamide would replace the aromatic cap of typical HDAC inhibitors. We planned to explore a variety of linkers as well as zinc-binding groups to accomplish our objective of dual AR–HDAC6 inhibition.

Our compound designs included a number of published Zn chelating groups (Fig. 3). These incorporated a thiophene trifluoromethyl ketone (**1**), 2-aminobenzamides (**2**, **11** and **12**), hydroxypyridones (**4**, **5**), hydroxylpyridine-thione (**3**), sulfamides (**6**, **7**) and hydroxamic acids (**8**, **9**, **10**, **13** and **14**).

In order to synthesize the proposed compounds, we prepared two key intermediates **18** and **19**. The synthesis of compound **18** has been reported;[23] a similar process was used to synthesize the bromophenyl compound **19**. The routes for the synthesis of the target molecules are shown in Scheme 1. The details of the syntheses can be found in SI.

Our evaluation of the synthesized compounds began with binding assays for AR and HDAC family proteins (Table 1). To identify activity against HDAC family proteins we screened for inhibition of several Class I HDACs (HDAC1, 2, and 3) and our target, the Class IIb HDAC6 using a fluorogenic readout of de-acetylation of target peptides to quantitate de-acetylation activity.[24] While pan–HDAC inhibitors have shown therapeutic promise, we focused on the described synergy of HDAC6 specific inhibition with an AR