EPI-001 is a selective peroxisome proliferator-activated receptor-gamma modulator with inhibitory effects on androgen receptor expression and activity in prostate cancer

Lucas J. Brand1,2, Margaret E. Olson3, Preethi Ravindranathan4, Hong Guo5, Aaron M. Kempema3, Timothy E. Andrews3, Xiaoli Chen5, Ganesh V. Raj4, Daniel A. Harki3, Scott M. Dehm2,6

1Graduate Program in Microbiology, Immunology and Cancer Biology, University of Minnesota, Minneapolis, MN, USA
2Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA
3Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA
4Department of Urology, University of Texas Southwestern Medical Center, Dallas, Texas, USA
5Department of Food Science and Nutrition, University of Minnesota, Minneapolis, MN, USA
6Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

Correspondence to:
Scott M. Dehm, e-mail: dehm@umn.edu

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ABSTRACT

The androgen receptor (AR) is a driver of prostate cancer (PCa) cell growth and disease progression. Therapies for advanced PCa exploit AR dependence by blocking the production or action of androgens, but these interventions inevitably fail via multiple mechanisms including mutation or deletion of the AR ligand binding domain (LBD). Thus, the development of new inhibitors which act through non-LBD interfaces is an unmet clinical need. EPI-001 is a bisphenol A-derived compound shown to bind covalently and inhibit the AR NH2-terminal domain (NTD). Here, we demonstrate that EPI-001 has general thiol alkylating activity, resulting in multilevel inhibitory effects on AR in PCa cell lines and tissues. At least one secondary mechanism of action associated with AR inhibition was found to be selective modulation of peroxisome proliferator activated receptor-gamma (PPARγ). These multi-level effects of EPI-001 resulted in inhibition of transcriptional activation units (TAUs) 1 and 5 of the AR NTD, and reduced AR expression. EPI-001 inhibited growth of AR-positive and AR-negative PCa cell lines, with the highest sensitivity observed in LNCaP cells. Overall, this study provides new mechanistic insights to the chemical biology of EPI-001, and raises key issues regarding the use of covalent inhibitors of the intrinsically unstructured AR NTD.

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed male cancer in the US with approximately 233,000 new cases and 30,000 deaths predicted in 2014 [1]. Normal and cancerous prostate tissues are dependent on activation of the androgen receptor (AR) to support cell proliferation and survival [2, 3]. Thus, inhibiting AR activation serves as the basis for treating metastatic disease [4]. However, these therapies ultimately fail via a variety of molecular mechanisms [5]. Importantly, castration-resistant PCa (CRPC) tumors remain AR-dependent, as evidenced by the increased overall survival of patients treated with the second-generation androgen deprivation therapies enzalutamide [6–8] and abiraterone [9]. Despite these advances, resistance to enzalutamide and abiraterone is frequent and several AR re-activation mechanisms have been reported as likely drivers [10–14]. Therefore, development of novel AR-targeted therapeutics that are active in CRPC remains an important area of investigation [15].
The AR is a modular steroid hormone receptor transcription factor with the primary transcriptional activation function mapping to Transcriptional Activation Units (TAU1 and TAU5) in the intrinsically unstructured AR NH2-terminal domain (NTD) [16, 17]. The functional importance of these domains is evidenced by the expression of AR splice variant proteins in CRPC, which are constitutively active AR species composed of the AR NTD and central DNA binding domain (DBD), but lacking the regulatory LBD [18, 19]. This highlights the clinical need for new therapeutics that exert action through non-LBD interfaces on the AR protein [18, 19]. EPI-001, a Bisphenol A diglycidyl ether (BADGE) derivative, was identified as a specific inhibitor of the AR that bind covalently to an undetermined structural motif in the AR NTD and inhibited the growth of androgen sensitive PCa and CRPC cells in vitro and in vivo [20, 21]. Here, we interrogated the mechanism by which EPI-001 inhibits the AR NTD. We show that EPI-001 is a general thiol modifier with myriad effects on AR expression and activity, and selectively modulates peroxisome proliferator-activated receptor-gamma (PPARγ) activity. Overall, this study provides novel insights to EPI-001 chemical biology that will be critical for ongoing development of AR NTD inhibitors.

RESULTS

EPI-001 inhibits transcriptional activity of both AR TAU1 and TAU5

LNCaP cells were treated with a range of EPI-001 concentrations to identify doses that effectively inhibited AR-responsive luciferase reporters. Contrary to previous reports showing that 10 μM EPI-001 achieved robust AR inhibition [20], we observed that a 50 μM dose was required (Supplementary Figure S4). To identify the specific AR TAU through which 50 μM EPI-001 inhibited AR activity, we performed promoter tethering assays with an ARNTD hybrid wherein the AR DBD had been replaced with the yeast Gal4 DBD (Figure 1A, construct 2). As a negative control, we used bisphenol A bis(2,3-dihydroxypropyl) ether (BABDHE), as it is structurally similar to EPI-001 but contains a diol instead of a reactive chlorohydrin (Figure 1B) [21]. EPI-001 inhibited ligand-dependent ARNTD transcriptional activity in LNCaP cells (Figures 1C and 1D), as well as aberrant, ligand-independent ARNTD transcriptional activity in the CRPC C4-2 cell line (Figure 1D). Deletion of TAU5 from ARNTD increased androgen-dependent ARNTD activity and decreased androgen-independent ARNTD activity, consistent with previous reports [22], but this deletion did not affect responsiveness to EPI-001 (Figure 1D). Conversely, deletion of TAU1 decreased androgen-dependent and independent modes of ARNTD transcriptional activity in LNCaP and C4-2 cells (Figure 1D). This precluded evaluation of EPI-001 effects on TAU1 in LNCaP, but residual androgen-independent ARNTD transcriptional activity in C4-2 cells remained responsive to EPI-001 (Figure 1D). To test the responsiveness of discrete AR TAU1 to EPI-001 directly, we tethered the entire AR NTD, or TAU1 or TAU5 fragments to the Gal4 DBD (Figure 1B, constructs 5–7). In all cell lines tested, EPI-001 inhibited transcriptional activity of the NTD-Gal4 hybrid (Figures 1E, 1F, and Supplementary Figure S5). The Gal4-TAU1 and Gal4-TAU5 fusion proteins displayed cell line-specific transcriptional activity, likely due to inefficient expression in PCa cell lines (Figures 1E, 1F, and Supplementary Figure S5).

In 293T fibroblasts, transcriptional activity of the Gal4-TAU1 and –TAU5 constructs was potently inhibited by EPI-001 (Figures 1E and 1F). These data agree with previous reports of direct AR inhibition by EPI-001, but extend this knowledge by demonstrating the effects could not be mapped to a discrete AR TAU. This indicates two possible scenarios: 1) EPI-001 binds specifically to both TAU1 and TAU5, or 2) EPI-001 has a more general effect on transcriptional activities of TAU1 and TAU5.

EPI-001 inhibits endogenous AR mRNA and protein expression

Interestingly, we observed that endogenous AR protein levels were consistently repressed in PCa cell lines treated with EPI-001 (Figure 1C). To explore this phenomenon, we tested the effect of EPI-001 on AR protein levels in a panel of androgen sensitive PCa (Figure 2A) and CRPC (Figure 2B) cell lines. In these cell lines, EPI-001 treatment decreased expression of full-length AR protein to varying degrees (Figures 2A and 2B). AR protein loss occurred between 8 and 16 hours of treatment and was independent of the proteasome (Supplementary Figure S6). In line with this, AR mRNA expression in LNCaP and C4-2 cells was reduced in response to EPI-001 at time points preceding the observed decreases in AR protein expression (Figure 2C). EPI-001 also inhibited the mRNA expression of AR and the AR target gene PSA in LAPC4 cells (Supplementary Figure S7A). EPI-001 treatment also decreased expression of truncated AR variant (AR-v) proteins expressed in the CRPC 22Rv1 cell line (Figure 2B). Interestingly, AR mRNA (Supplementary Figure S7B) and protein expression (Figure 2B) in CWR-R1 cells did not respond to EPI-001, nor did EPI-001 inhibit the expression of the AR target gene FKBP5 (Supplementary Figure S7B).

To test if the effects of EPI-001 on AR expression were due to decreased AR mRNA stability, we treated LNCaP with Actinomycin D alone or in combination with EPI-001. Treatment with EPI-001 did not accelerate AR mRNA decay following transcriptional blockade with Actinomycin D (Supplementary Figure S8). Consistent with this, we found that 50 μM EPI-001 reduced the rate of nascent AR mRNA synthesis in LNCaP cells (Figure 2D). Collectively, these data demonstrate that EPI-001 inhibits transcription of the AR gene.
Inhibition of AR expression correlates with reduced cell growth in PCa and CRPC cell lines

Based on these findings, we reasoned that inhibition of AR synthesis could be an important component of the EPI-001 anti-AR mechanism. EPI-001 inhibited growth of LNCaP cells at low concentrations, but in all other PCa cell lines, the concentrations at which EPI-001 inhibited growth (Figure 3A, Supplementary Figure S9) were the same concentrations that inhibited expression of AR or AR-V protein levels (Figure 3B). BABDHE also inhibited PCa and CRPC growth and AR expression, although higher doses were required than for EPI-001 (Supplementary Figure S10A & 10B), indicating the EPI-001 chlorohydrin moiety is important for inhibition of AR expression. Surprisingly, EPI-001 also inhibited growth of AR-negative PC-3 and DU 145 cell lines (Figure 3C), as well as the T47D

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breast carcinoma cell line (Supplementary Figures S11A and S11B). In T47D, biphasic modulation of AR as well as estrogen and progesterone receptors (ER and PR) was observed (Supplementary Figure S11C). These data signify AR-independent effects of EPI-001 in multiple cell types.

Figure 2: EPI-001 inhibits endogenous AR expression at the mRNA level. (A) Androgen sensitive PCa and (B) CRPC cell lines were treated overnight in serum-free medium with 1 nM DHT and/or 50 μM EPI-001 as indicated, and analyzed by western blot. Densitometry data for both full length (AR-fl) and truncated variant (AR-v) isoforms are provided. (C) AR mRNA expression was analyzed by qRT-PCR at indicated time-points in LNCaP (left) and C4-2 (right) cells treated with 50 μM EPI-001. (D) LNCaP cells were treated with 50 μM EPI-001 or vehicle control for 8 h in serum free medium. Nascent transcripts were isolated and subjected to qRT-PCR using primers for AR pre-mRNA (Exon 1 FW & Intron 1 RV) or spliced mRNA (Exon 1 FW & Exon 2 RV). Bars depict mean +/- standard deviation (C: n = 3 from a triplicate experiment representative of two biological replicates; D: n = 6 from two biological replicates performed in triplicate). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
EPI-001 action in PCa cells is similar to the PPARγ agonist, troglitazone

Bisphenol A Diglycidyl Ether (BADGE), which is related structurally to EPI-001 but contains a bis-epoxide, has been shown to act as a selective PPARγ modulator (SPPARM) with diverse effects in different cell types [23–25]. Given that PPARγ has also been shown to play a role in prostate development and maintenance [26], and PPARγ agonists such as troglitazone have been demonstrated to inhibit AR expression and PCa cell growth in vitro and in vivo [27–29], we reasoned that PPARγ modulation may be an unanticipated activity of EPI-001 in PCa cells. Indeed, troglitazone or EPI-001 caused inhibition of AR transcriptional activity in promoter reporter assays in LNCaP cells (Figure 4A) at doses that correlated with inhibition of AR protein expression (Figure 4B). Furthermore, treatment of LNCaP cells with troglitazone or EPI-001 resulted in dose-dependent reduction of AR protein levels as well as induction of p21 and p27 (Figure 4B). Troglitazone inhibited AR expression at lower doses than observed in prior studies [28, 30], which may be due to the absence of serum in the cell culture medium during drug treatment in our study. Finally, troglitazone treatment also inhibited the activity of AR Gal4, as well as the Gal4-tethered AR NTD, TAU1, and TAU5 (Figure 4C), analogous to the effect of EPI-001 in these models (Figures 1D and 1F).

Figure 3: Dose-dependent inhibition of AR expression and PCa/CRPC cell growth mediated by EPI-001. (A) LNCaP, C4-2, and 22Rv1 cells were treated for 7 days in steroid-depleted medium containing 1 nM DHT and/or EPI-001 as indicated. Growth was monitored by crystal violet staining. Bars depict mean +/− standard deviation (n = 3 from a triplicate experiment representative of two biological replicates). (B) LNCaP, C4-2, and 22Rv1 cells were treated for 24 hours in serum free medium as in (A) and subjected to western blot. Densitometry data are provided. (C) AR-negative PC-3 and DU 145 cells were treated with EPI-001 and analyzed for growth exactly as in A. Bars depict mean +/− standard deviation (n = 3 from a triplicate experiment representative of two biological replicates). *P < 0.05; **P < 0.01; ***P < 0.001, ****P < 0.0001.
To expand these observations to clinical disease, we treated fresh human PCa tissue maintained as explants [31–33] with troglitazone and EPI-001 (Figure 5A). The doses of EPI-001 and troglitazone used in this model were increased 2- to 4-fold relative to \textit{in vitro} experiments to reflect the higher doses of drug that have been used for \textit{in vivo} [21] or \textit{ex vivo} [34] experimentation. Importantly, both EPI-001 and troglitazone effected decreases in AR protein, AR mRNA, and AR target gene expression in PCa explants (Figure 5B and 5C).

\textbf{EPI-001 is a selective modulator of PPARγ in PCa cells}

We next tested for SPPARM activity of EPI-001 in PCa cells. Similar to troglitazone, EPI-001 activated a PPARγ-response element (PPRE)-regulated luciferase reporter in LNCaP cells (Figure 6A). This SPPARM activity was AR-independent, as troglitazone and EPI-001 both induced mRNA expression of the PPARγ targets CIDEC [35], TXNIP [30], and PDK4 [26] in the AR-null PC-3 cell line [27] (Figure 6B). However, in 3T3-L1 cells that had been differentiated to PPARγ-positive adipocytes, EPI-001 repressed expression of classical PPARγ target genes aP2 and LPL (Supplementary Figure S12A) and inhibited lipid droplet formation (Supplementary Figure S12B). These effects are consistent with previous reports of BADGE-mediated repression of PPARγ activity in 3T3-L1 adipocytes at micromolar concentrations [23]. Taken together, these cell type-specific PPARγ agonist/antagonist effects support a SPPARM function for EPI-001, with thiazolidinedione-like effects on PPARγ activity in PCa cells.
To investigate the relationship between EPI-001-mediated PPARγ activation and AR inhibition, we knocked down PPARγ with siRNA in LNCaP cells. Despite effective silencing of PPARγ expression, both troglitazone and EPI-001 maintained robust inhibition of AR protein expression (Supplementary Figure S13A). This finding is consistent with a previous study showing that troglitazone-mediated inhibition of AR expression is due to PPARγ-independent degradation of the transcription factor Sp1 [28]. However, EPI-001 had no effect on Sp1 levels (Supplementary Figure 13B). Conversely, siRNA-mediated knock down of PPARγ partially rescued the inhibition of AR transcriptional activity effected by EPI-001, but not troglitazone (Figure 6C), demonstrating that PPARγ participates in EPI-001-mediated inhibition of AR transcriptional activity, but not inhibition of AR expression.

EPI-001 forms covalent adducts with thiols in vitro

Because SPPARM activity did not fully account for the multi-level anti-AR effects of EPI-001, we considered the fact that Bisphenol A (BPA) and BADGE are endocrine disruptors used in the production of polycarbonate plastics and epoxy resins [36, 37]. The epoxide rings in BADGE and related compounds readily undergo hydrolysis and hydrochlorination reactions with substrates in aqueous solution [38], resulting in hydroxylated and halogenated derivatives, of which EPI-001 (BADGE.HCl.H2O) is one example [39]. Chlorohydrin moieties also have the potential to spontaneously interconvert to epoxides in aqueous solution [40]. Therefore, we used HPLC to interrogate whether EPI-001 can convert to a BADGE-like mono-epoxide in solution (Compound 2, Supplementary Figure S2). Indeed, the epoxide was observed after 12h incubation at neutral and basic pH (Figure 7A and 7B), but not under acidic conditions (Supplementary Figure S14A & S14B). The identity of compound 2 was confirmed by co-injection with an authentic standard and LC-MS analysis (Supplementary Figure S15). BADGE has been shown to react with nucleophilic side chains of food proteins in plastic-lined cans [41], which is the same reaction proposed for the specific AR-binding mechanism of EPI-001 [21]. In a previous study, non-specific reactivity of EPI-001 with nucleophilic thiols was not observed [21]. However, given our observation that EPI-001 spontaneously converts to the epoxide at neutral and basic pH, and that BADGE is reactive to nucleophiles, we queried reactivity of EPI-001 with the nucleophilic thiols glutathione, 2-mercaptoethanol, and cysteamine at various pH conditions (Figure 7C). No EPI-001:thiol adducts were formed under acidic conditions (Supplementary Figure S16A & S16B, and Supplementary Figure S17). However, reaction of EPI-001 with glutathione resulted in a trace amount of thiol adduct formation at pH
7.4, and nearly complete conversion to the glutathione adduct at pH 9.4 after 12 hours (Figure 7D). Similarly, 2-mercaptoethanol displayed limited adduct formation at neutral pH, but underwent complete conversion to the EPI-001:thiol adduct at basic pH (Figure 7D). Finally, EPI-001 did not react with cysteamine at pH 7.4, but displayed nearly complete adduct formation at pH 9.4 (Figure 7D). All EPI-001-thiol adducts were confirmed by mass spectrometry (Supplementary Figure S18A–S18C). Additionally, the monoepoxide, compound 2, formed adducts with all thiols examined at pH 7.4, but displayed nearly complete adduct formation at pH 9.4 (Figure 7D). These data indicate that EPI-001 spontaneously converts to the more reactive epoxide in solution at neutral and basic pH. Furthermore, EPI-001 extensively alkylates thiols under basic conditions with appreciable amounts of EPI-001:thiol adducts observed at neutral (7.4) pH. Our results suggest that EPI-001 is a reactive electrophile which may display some selectivity in modulation of proteins by virtue of local pH influence.

**DISCUSSION**

In this study, we describe unanticipated multi-level effects of EPI-001 on the AR and PPARγ pathways, leading to inhibition of cell growth. In previous reports, EPI-001 was shown to bind specifically to the AR NTD through a nucleophilic substitution reaction with the EPI-001 chlorohydrin group [21], thereby inhibiting AR activity via occlusion of an unidentified CBP binding domain [20]. We were unable to nominate a discrete AR NTD motif that could account for a specific mechanism of EPI-001-mediated AR transcriptional repression in this study. Conversely, we found that EPI-001 inhibited synthesis of AR in PCa cell lines and clinical tissues at doses that corresponded with the inhibition of AR target genes and PCa cell growth. The LNCaP cell line was an exception to this general dose relationship between AR expression inhibition and cell growth inhibition, displaying the highest sensitivity to EPI-001- and BABDHE-mediated growth inhibition. This is important,
Figure 7: EPI-001 converts in solution to a reactive epoxide and forms covalent adducts with thiols. (A) Scheme for conversion of EPI-001 to compound 2. EPI-001 was shaken at 37°C in PBS/DMSO at pH 2.4, 7.4, and 9.4. (B) HPLC chromatograms for conversion of EPI-001 to compound 2. Reaction mixtures were analyzed by LC-MS to confirm the presence of epoxide; m/z [M+H]+ 359.2 (calc’d); 359.0 (found). (C) Scheme for covalent modification of EPI-001 by reactive thiols. Solutions of EPI-001 and thiols in PBS/DMSO at pH 2.4, 7.4, and 9.4, respectively, were shaken at 37°C. (D) HPLC chromatograms for covalent adduct formation between EPI-001 and thiols (t = 12 h). New peaks that arose during the course of the reaction and are distinct from background signals (Supplementary Figure 20A) are denoted with an asterisk. Percent remaining was calculated by dividing the amount of measured EPI-001 remaining at t = 12 h by the amount remaining at t ~ 30 min and multiplying by 100%. Experiments were performed in triplicate and values shown are mean ± standard deviation. EPI-001-thiol adducts were characterized by LC-MS.
as the majority of pre-clinical data supporting the efficacy and specificity of EPI-001 for AR has been generated using the LNCaP model [20, 21]. Moreover, we found that EPI-001 inhibited the growth of AR-negative PC-3 and DU 145 cells. These data conflict with a previous report [20], but we propose that this discrepancy is due to two key differences in experimental design. First, our study incorporated longer-term (i.e. 7 day) growth assays as opposed to early timepoint (i.e. 3 day) BrdU incorporation readouts. Secondly, previous reports used 10 μM EPI-001 to treat PC-3 and DU 145, a dose which was not inhibitory to the growth of PC-3 and DU 145 in our study, but inhibitory LNCaP cells. These data highlight the cell line-specific responses to EPI-001, which supported earlier conclusions of AR specificity.

Our data indicate that PPARγ activation represents at least one AR-independent activity of EPI-001 in PCa cells. However, EPI-001 displayed PPARγ inhibitory activity in a classical 3T3-L1 adipocyte model, indicating SPPARM activity as opposed to pure agonist activity. SPPARM activity for EPI-001 is consistent with studies demonstrating that the chemically-related compound, BADGE, is a SPPARM that binds to the PPARγ LBD [23] and exhibits distinct molecular effects in PCa and 3T3-L1 cells when compared with synthetic thiazolidinedione PPARγ agonists [23–25] including troglitazone [36–38]. Furthermore, our data from thiol reactivity assays demonstrate that small molecule thiolates (e.g., glutathione, 2-mercaptoethanol, cysteamine) are readily alkylated by EPI-001 and this reactivity is attenuated at acid and neutral pH. Consequently, our data suggest that any protein bearing an accessible nucleophilic residue within a suitably basic binding pocket may be a target for covalent modification by EPI-001. This is further supported by the established reactivity of BADGE in vitro [41], and our data that EPI-001 is converted to an analogous epoxide (compound 2) in solution at physiological pH. Collectively, these data suggest EPI-001 and BADGE bear substantial proteome reactivity features in addition to their reported interactions with AR and PPARγ.

These new data indicate that structural changes to the core bisphenol of EPI-001 as well as the covalent warhead may be required to mitigate the AR-independent effects reported in this study and in the toxicology literature [36–38, 41, 42]. However, this task is complicated because no 3-dimensional structure has been reported for the intrinsically disordered AR NTD [43, 44], which impedes the rational design of improved inhibitors. Nevertheless, EPI-002, the (2R, 20S) isomer of racemic EPI-001, has been shown to display stronger AR interactions and reduced toxicity in mice [21], indicating this direction may be feasible. Our findings that EPI-001-mediated inhibition of AR activity is associated with inhibition of AR expression and activation of PPARγ in PCa, coupled with the finding that EPI-001 can capture nucleophilic thiols, will be important for ongoing pre-clinical development of EPI-001 and other anti-AR compounds that target functional domains independent of the AR LBD.

MATERIALS AND METHODS

Cell culture and growth assays

LNCaP, C4-2, DU 145, VCaP, 22Rv1, 293T, and PC-3 cell lines were obtained from ATCC. The ATCC validates the authenticity of these cell lines via short tandem repeat (STR) analysis. CWR-R1 prostate cancer cells were the generous gift of Dr. Elizabeth Wilson (University of North Carolina at Chapel Hill, Chapel Hill, NC). CWR-R1 cells were authenticated by sequence-based validation of two characteristic AR mutations: a H874Y mutation in the LBD, and a 50 kb deletion in AR intron 1 [45]. VCaP and 293T were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% FBS. All other cell lines were maintained in RPMI 1640 medium with 10% FBS, and all cell lines were maintained in 100 Units/mL Penicillin + 0.1 mg/mL Streptomycin. Cells were cultured in a 37°C incubator with 5% CO2 for no longer than 15 passages after resuscitation of frozen stocks. Cell growth was assessed by crystal violet staining as previously described [46].

Reagents

Dihydrotestosterone (DHT), mibolerone (MIB), and BABDHE (Bisphenol A bis [2,3-dihydroxypropyl] ether) were purchased from Sigma. Enzalutamide (ENZ) was purchased from Selleck Chemicals. Troglitazone was purchased from Cayman Chemical. EPI-001 (Bisphenol A [3-chloro-2-hydroxypropyl] [2,3-dihydroxypropyl] ether) was synthesized (See Supplementary Methods) or purchased from commercial sources (Santa Cruz Biotechnology or Sigma-Aldrich). EPI-001 was analyzed for purity via HPLC and NMR (Supplementary Methods, Supplementary Figures S1 and S2). EPI-001 dissolved for culture medium was used for all experiments (Supplementary Methods, Supplementary Figure S3). All other drugs were suspended in DMSO with the exception of DHT, MIB, and ENZ, which were prepared in absolute ethanol. Final DMSO or ethanol concentrations did not exceed 0.1% (v/v) in culture medium.

Plasmids

Plasmids encoding human AR (p5HBhAR-A), ARGal4, NTD-Gal4, ARGal4ΔTAU1, ARGal4ΔTAU5, sPSA-Luciferase (also referred to as PSAenh(ARE)-LUC), and sPSAGal4-Luciferase (also referred to as PSAenh(GAL4)-LUC) have been described [22]. SV40-Renilla, CMV-Renilla, and pG5-Luciferase were purchased from...
Promega. PPREx3-TK-Luciferase has been described [47], and was obtained from Addgene. The Gal4 DBD expression plasmid (pM) was purchased from Clontech. Gal4-TAU1 (AR a.a. 101–360) and Gal4-TAU5 (AR a.a. 361–490) were constructed as described in the Supplementary Methods and Supplementary Table 1.

**Cell transfection**

LNCaP cells were transfected via single-pulse electroporation as previously described [48]. C4-2 cells were transfected with Superfect reagent (Qiagen) according to manufacturer specifications. 293T cells were transfected with Lipofectamine 2000 (Life Technologies) according to manufacturer specifications. Treatment of transfected cells with androgen and/or drug was performed for 8 hours or overnight in serum-free medium as indicated.

**Dual luciferase assays**

Transfected cells were lysed in 1X Passive Lysis Buffer (Promega) and subjected to dual luciferase assays using a Dual Luciferase Assay Kit (Promega) as previously described [48].

**Western blot**

Western blotting with antibodies listed in Supplementary Table 2 was performed as previously described [49].

**Nascent RNA labeling and isolation**

Nascent transcripts were isolated using the Click-iT Nascent RNA Capture Kit (Life Technologies) according to manufacturer specifications. First-strand cDNA synthesis was performed using the SuperScript VILO cDNA synthesis kit (Life Technologies) according to manufacturer specifications and quantified via qRT-PCR. Detailed information regarding reagent concentrations and time of treatment can be found in the Supplementary Methods.

**Quantitative RT-PCR**

RNA isolation and quantitative RT-PCR analysis were performed as described [49] using primers listed in Supplementary Table 3.

**Prostate cancer explants**

Patient tissues were obtained from the University of Texas Southwestern Medical Center tissue core under UT Southwestern Medical Center and explant studies were performed as previously described [31, 32]. Detailed information on tissue treatment, dosing, timing, and processing can be found in the Supplementary Methods.

**pH stability and thiol reactivity studies**

Solutions of reduced l-glutathione, 2-mercaptoethanol, and cysteamine in phosphate buffered saline were adjusted to the desired pH (2.4, 7.4, or 9.4). EPI-001 or monoepoxide control (Compound 2, Supplementary Figure S2) were added to thiol solutions and aliquots of reactions were analyzed by reverse phase HPLC and LC-MS at the indicated time points. To quantify the amount of parent compound remaining, the area under the curve (AUC) of the parent compound was divided by the AUC of an internal standard. Further information regarding thiol reactivity, HPLC, and LC-MS conditions are included in the Supplementary Methods.

**Data analysis and statistics**

All statistical comparisons were made using the two-tailed Student’s t-Test with a P value of 0.05 or less considered significant.

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**Conflicts of interest**

The authors declare no conflicts of interest.

**Editorial note**

This paper has been accepted based in part on peer-review conducted by another journal and the authors’ response and revisions as well as expedited peer-review in Oncotarget.

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