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PRECLINICAL STUDIES

Synthesis and in vitro characterization of ionone-based compounds as dual inhibitors of the androgen receptor and NF-κB

Weiguo Liu · Jinming Zhou · Guoyan Geng · Rongtuan Lin · Jian Hui Wu

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Summary Current therapeutic strategy for advanced prostate cancer is to suppress the androgen receptor (AR) signaling. However, lethal castration-resistant prostate cancer (CRPC) arises due to AR reactivation via multiple mechanisms, including mutations in the AR and cross-talk with other pathways such as NF-κB. We have previously identified two ionone-based antiandrogens (SC97 and SC245), which are full antagonists of the wild type and the clinically-relevant T877A, W741C and H874Y mutated ARs. Here, we discovered SC97 and SC245 also inhibit NF-κB. By synthesizing a series of derivatives of these two compounds, we have discovered a novel compound **3b** that potently inhibits both AR and NF-κB signalling, including the AR F876L mutant. Compound **3b** showed low micromolar antiproliferative activites in C4-2B and 22Rv1 cells, which express mutated ARs and are androgen-independent, as well as DU-145 and PC-3 cells, which exhibit constitutively activated NF-κB signalling. Our studies indicate **3b** is effective against the CRPC cells.

Keywords Prostate cancer · Androgen receptor · Antiandrogen · NF-κB · IKKβ

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W. Liu · J. Zhou · G. Geng · R. Lin · J. H. Wu
Segal Cancer Center, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, 3755 Cote-Ste-Catherine, Rd., Montreal, QC H3T 1E2, Canada

R. Lin · J. H. Wu (✉)
Departments of Oncology and Medicine, McGill University, 546 Pine Ave. W, Montreal, QC H2W 1S6, Canada
e-mail: jian.h.wu@mcgill.ca

Abbreviations

AR	Androgen receptor
Bic	Bicalutamide
CRPC	Castration-resistant prostate cancer
DHT	Di-hydrotestosterone
WT	Wild type

Introduction

Proliferation and survival of prostate cancer cells are critically dependent on the androgen receptor (AR) signaling. This provides the rationale for androgen ablation therapy, which aims to suppress the AR signaling by reducing level of androgens (via castration) and antagonizing the AR by antiandrogens, such as flutamide, nilutamide and bicalutamide [1]. However, lethal castration-resistant prostate cancer (CRPC) arises due to AR reactivation. Current treatment modalities for patients with established CRPC are limited to docetaxel-based chemotherapy, with the median survival time <2 years [2, 3]. Enzalutamide (MDV3100) and abiraterone acetate were recently approved by FDA for treating CRPC patients. Enzalutamide is a second-generation antiandrogen that acts as a full antagonist even under elevated level of the AR. Abiraterone acetate is an oral 17α-hydroxylase inhibitor that blocks steroid biosynthesis in the adrenal gland and possibly within the tumor [4]. Clinical data from phase I/II and III clinical trials of both Abiraterone and enzalutamide have reported a high level of antitumor activity in CRPC patients, revealing that AR signaling is a driver of the CRPC cells [5–7]. It has been established that, in CRPC cells, AR is activated by multiple mechanisms that can no longer be effectively suppressed by castration and conventional antiandrogen agents, such as bicalutamide [6, 8]. Mutations

in the AR ligand-binding domain and crosstalk of the AR with other deregulated pathways (such as NF-κB) are two important mechanisms that may account for persistent AR activation in the CRPC cells [9–11].

The incidence of AR mutation in advanced prostate cancer is estimated to be in the range of 10−40% [9, 12]. The T877A and H874Y mutations are two most commonly identified variants in tissue specimens of patients with advanced prostate cancer. The T877A and H874Y mutated ARs were reported to be activated by antiandrogen hydroxyflutamide, androgens and a series of other sex steroids [11]. The T877A mutant is activated by nilutamide, another clinically used antiandrogen [13]. The W741C mutation was derived from liver metastatic tissue of a patient treated with bicalutamide and died of CRPC. Yoshida et al. have demonstrated that bicalutamide is paradoxically an agonist of the W741C mutant [10]. Thus, it appears the AR mutations could contribute to aberrant AR activation in the CRPC cells. Importantly, the AR F876L mutant was recently found to confer resistance to enzalutamide [14, 15].

The NF-κB plays a key role in inflammation, immune response, cell proliferation and protection against apoptosis [16]. Constitutive NF-κB activity is common in solid tumors [17], including metastatic prostate cancer [18]. Androgen-independent prostate cancer cell lines PC-3 and DU145 exhibit a high constitutive activation of IκB kinase (IKKβ) and NF-κB signaling [19]. IKKβ is critical for the NF-κB activation and a series of IKKβ inhibitors are currently in preclinical development for various cancers [20]. Accumulating evidence indicate there are important crosstalk between AR signaling and NF-κB pathway: i) NF-κB/p65 induces AR expression. Forced over-expression of NK-κB increased sensitivity of AR-positive prostate cancer cells to low concentrations of androgen, enhancing their growth and survival [21]; ii) The NF-κB-regulated IL-6 stimulates growth of prostate cancer in vitro and in vivo through activation of the AR [22]. Further, IL-6 regulates androgen synthesis in prostate cancer cells [23], and increases prostate cancer cells' resistance to bicalutamide [24]. Increased serum levels of IL-6 have been reported in patients with CRPC [25]. It was demonstrated that the NF-κB pathway controls the progression of prostate cancer to androgen-independent growth in vitro and in vivo [26]. Therefore, IKKβ/NF-κB/IL-6 axis is likely contributing to aberrant activation of the AR in CRPC cells.

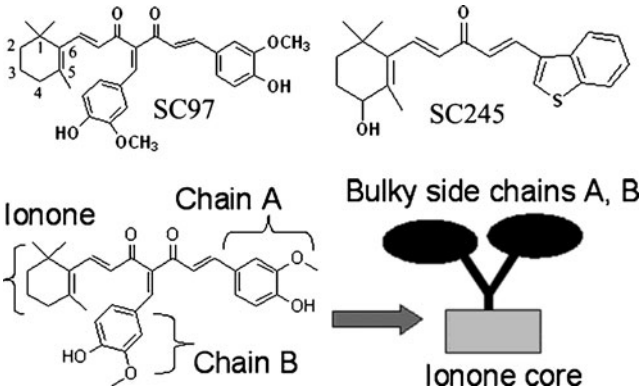
Our previous work has identified two novel ionone-based antiandrogens (referred to as SC97 and SC245), which are full antagonists of the wild type and the T877A, W741C and H874Y mutated ARs (Scheme 1) [27, 28]. Using PolarScreen AR competitor assay kit (P3018, Invitrogen), we showed that SC97 binds with the AR ligand-binding domain. Our molecular modeling studies indicated that the ionone portion of SC97 is anchoring inside the hormone binding pocket, whereas chains A and B are protruding toward the helix-12 of the AR ligand-binding domain (Scheme 1) [27]. We have synthesized a series of ionone-based compounds with chemical modifications at side chains [27–29]. In the present work, we discovered SC97 and SC245 also inhibit NF-κB activation. To optimize SC97 and SC245, we have synthesized a series of their derivatives with modifications at ionone cores. Our work has led to discovery of a novel compound **3b** that potently inhibits both AR and NF-κB signaling.

Materials and methods

General All reagents for chemical syntheses were purchased from Sigma-Aldrich (Oakville, ON, Canada). Bicalutamide and enzalutamide were purchased from Toronto Research Chemicals (North York, ON, Canada). All of the ^{1}H NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 500 MHz on proton. Mass measurements were performed on a LC-MSD-TOF instrument from Agilent technologies in positive electrospray mode. Purity was determined by HPLC (Waters Alliance 2695–2996) and the purities of compounds **3a-3d** and **4a-4f** were ≥ 95 %.

Luciferase assays for AR activation Point mutation of human AR (F876L) was introduced in pCMV-AR plasmid using Quickchange mutagenesis according to the manufacturer's instructions (Stratagene) (referred to as pCMV-AR-F876L). Plasmid pCMV-AR-W741C was kindly provided by Dr Osmu Ogawa (Kyoto University, Kyoto, Japan). PC-3 cells was seeded at a density of 1×10^{5} cells per well in 24-well microtiter plates and 24 h later, the cells were cotransfected with PSA-luc and pCMV-AR-W741C or pCMV-AR-F876L expressing plasmid and Renilla null luciferase using LipofectamineTM 2000 reagent (Invitrogen) following manufacturer's protocol. Five hours after transfection, the medium was changed to phenol red-free RPMI 1640 supplemented with 10 % charcoal-stripped FBS and 16 h later, the cells were exposed to DMSO vehicle, 10 nM DHT or compounds in the presence 10 nM DHT. After further 24 h, the reporter gene activities were measured by dual-luciferase reporter assay, according to the manufacturer's instructions (Promega). All experiments were performed in triplicate and repeated at least twice.

Luciferase assays for NF-κB activation Transfections for luciferase assay were carried out in HEK293 cells. Subconfluent HEK293 cells seeded on 24-well plates were transiently co-transfected with 30 ng of pRLTK reporter (*Renilla* luciferase for internal control), 100 ng of NF-κB-Luc reporter (firefly luciferase, experimental reporter) and



Scheme 1 Chemical structures of ionone-based antiandrogens SC97 and SC245 and the diagram showing the two bulky sidechains of SC97

100 ng of plasmid encoding IKKβ by calcium phosphate co-precipitation method. The total amounts of DNA were kept constant by supplementation with an empty vector (pcDNA3.1). At 24 h after transfection, the reporter gene activities were measured by dual-luciferase reporter assay, according to the manufacturer's instructions (Promega).

Quantification of IL-6 protein concentration DU145 cells were exposed to DMSO vehicle and compounds at designated doses for 24 h. The IL-6 level of cell culture supernates was determined using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacture's protocol (R&D Systems, Inc., Minneapolis, MN, USA). Each sample was tested in duplicate. After development of the colorimetric reaction, the absorbance at 450 nm was quantitated by means of a microplate reader. The absorbance readings were then converted to picograms per milliliter (pg/mL) based on standard curves obtained with recombinant cytokines. The lower limit of sensitivity of this kit was 3.1 pg/mL.

Western blot analysis HEK293 cells were pretreated with compounds for 12 h, then 10 ng/mL TNFα for 30 min. LNCaP cells in RPMI 1640 medium supplemented with 10 % FBS were exposed to DMSO vehicle or test compounds at the designated concentrations for 24 h. Additional details were provided in Supporting Materials.

MTT assays The growth inhibition assay was performed as described [27]. Briefly, LNCaP, C4-2B, 22Rv1, PC-3 and Du145 are maintained in RPMI 1640 supplemented with 10 % FBS. Cells were seeded at a density of $6–7\times10^3$ cells per well in 96-well plates. After overnight incubation, cells in fresh RPMI 1640 supplemented with 10 % FBS were exposed to DMSO vehicle control (0.5 %) and test compounds at designated concentrations for 72 h. Viable cells were evaluated by MTT assays. Experiments were performed in triplicate and repeated at least twice.

General procedure for the synthesis of 3a−3d After the mixture of di-ketone (**2a−2d)** (6.4 mmol) and $^{n}Bu_3BO_3$ (3.08 mL, 11.4 mmol) in 50 mL of anhydrous ethanol was stirred at 40°C for 0.5 h, 4-hydroxy-3-methoxycyclohexanecarbaldehyde (2.4 g, 15.4 mmol) was added into the mixture and stirred for 10 min. Next, $^{n}BuNH_2$ (278 μL, 2.82 μmol) was added into the mixture. The mixture reacted at room temperature for 4 days. The reaction was monitored by TLC. The solvent was distilled off under reduced pressure when the reaction completed. Ethyl acetate (30 mL) and 10 % HCl (aq) (8 mL) were added and stirred for 0.5 h at 60 °C. Saturated brine (30 ml) was added to the above mixture. The aqueous phase was extracted with ethyl acetate (20 mL×3). The combined organic phase was dried over Na_2SO_4. The organic solvent was distilled off under reduced pressure after filtering out the solid. The residue was purified through chromatography on silica gel to give **3a−3d** (elutant: n-hexane and ethyl acetate).

3a (Yellow solid, 19 % in yield), 1H NMR (500 MHz, CD_3COCD_3): δ 8.00 (1H, s), 7.79 (1H, d, J=15.5 Hz), 7.66 (1H, d, J=16.1 Hz), 7.54 (1H, m), 7.53 (1H, m), 7.46 (1H, d, J=2.0 Hz), 7.42 (1H, d, J=1.5 Hz), 7.05 (1H, d, J=9.3 Hz), 6.84 (1H, d, J=8.3 Hz), 6.75 (1H, td, J=10.0, 15.5 Hz), 6.41 (1H, d, J=16.0 Hz), 5.61 (1H, s), 4.09 (3H, s), 3.99 (3H, s), 2.57 (1H, d, J=10.0Hz), 1.43–1.85 (4H, m), 1.14 (3H, s), 1.06 (3H, s), 1.01 (3H, s). ESI-TOF-MS, m/z: 503.24 $[M + H]^+$.

3b (Yellow solid, 17 %), 1H NMR (500 MHz, CD_3COCD_3): δ 7.91 (1H, s), 7.69 (1H, d, J=15.5 Hz), 7.41 (1H, d, J=16.0 Hz), 7.38 (1H, m), 7.26 (1H, m), 7.22 (1H, d, J=2.0 Hz), 7.14 (1H, d, J=1.5 Hz), 7.06 (1H, d, J=9.0 Hz), 6.94 (1H, d, J=8.5 Hz), 6.55 (1H, d, J=15.5 Hz), 6.23 (1H, d, J=16.0 Hz),3.99 (1H, s), 3.96 (3H, s), 3.87 (3H, s), 1.44–1.85 (4H, m), 1.75 (3H, s), 1.07 (3H, s), 0.96 (3H, s). ESI-TOF-MS, m/z: 519.24 $[M + H]^+$.

3c (Yellow solid, 23 % in yield), 1H NMR (500 MHz, CD_3COCD_3): δ 7.85 (1H, s), 7.82 (1H, d, J=15.5 Hz), 7.71 (1H, d, J=16.0 Hz), 7.61 (1H, m), 7.59 (1H, m), 7.52 (1H, d, J=16.0 Hz), 7.44 (1H, d, J=1.5 Hz), 7.35 (1H, d, J=9.5 Hz), 7.21 (1H, d, J=2.0 Hz), 6.89 (1H, d, J=8.5 Hz), 6.75 (1H, d, J=15.5 Hz), 5.21 (1H, s), 3.99 (3H, s), 3.91 (3H, s), 1.61 (3H, s), 1.43–1.84 (4H, m), 1.11 (3H, s), 0.98 (3H, s). ESI-TOF-MS, m/z: 519.24 $[M + H]^+$.

3d (Yellow solid, 18.5 % in yield), 1H NMR (500 MHz, CD_3COCD_3): δ 7.96 (1H, s), 7.67 (1H, d, J=15.5 Hz), 7.56 (1H, d, J=16.1 Hz), 7.41 (1H, d, J=2.0 Hz), 7.32 (1H, d, J=1.5 Hz), 7.23 (1H, m), 7.16 (1H, m), 7.06 (1H, d, J=8.5 Hz), 6.85 (1H, d, J=8.5 Hz), 6.75 (1H, d, J=15.5 Hz), 6.38 (1H, d, J=16.0 Hz), 3.96 (3H, s), 3.87 (3H, s), 1.43–1.85 (6H, m), 1.16 (3H, s), 1.02 (3H, s), 0.97 (3H, s). ESI-TOF-MS, m/z: 519.24 $[M + H]^+$.

Starting material α-ionone (**1a**) and β-ionone (**1c**) are commercially available (Sigma-Aldrich). Procedures for preparation of the intermediates **1b**, **1d** and **2a**−**2d** as well as preparation of compounds **4a**−**4f** were reported in the Supplementary Materials.

Results

1) SC97 and SC245 inhibit NF-κB activation and suppress IL-6 secretion

To evaluate effect of SC97 and SC245 on the NF-κB signaling, HEk293 cells were transiently transfected with NF-κB-luc and IKKβ-expressing plasmid or pcDNA3.1 empty vector. Transfected cells were exposed to SC97 and SC245 at designated doses for 24 h. The study indicated that SC97 and SC245 inhibit NF-κB activation in a dose-dependent manner (Fig. 1a). By Western blot analysis in HEK293 cells, we further showed that SC97, like PS-1145 (a known IKK_β inhibitor), suppresses TNFα-induced degradation of the IκBα protein, a key substrate for IKK_β (Fig. 1b), indicating SC97 is an inhibitor of IKK_β. In particular, SC97 at 5 μM has substantially reduced IKKβ protein expression (Fig. 1b). By ELISA assays, we showed that SC97 and SC2454 dose-dependently suppress IL-6 secretion of DU145 prostate cancer cells (Fig. 1c).

2) Synthesis of novel derivatives of SC97 and SC245

To explore ionone core, we have synthesized four SC97 derivatives **3a**−**3d** and six SC245 derivatives **4a**−**4f** (Schemes 2, 3 and 4). Compounds **3a**−**3d** were synthesized as outlined in Scheme 2. Condensation of (E)-6-(2,6,6-trimethylcyclohex-2-enyl)hex-5-ene-2,4-dione (**2a**) with 4-hydroxy-3-methoxybenz aldehyde furnished compounds **3a**. Compounds **4a**−**4c** were synthesized according to Scheme 3, and **4d**−**4f** were synthesized according to Scheme 4.

3) Compound **3b** potently inhibits NF-kB and AR signaling

To evaluate effect of compounds **3a**−**3d** and **4a**−**4f** on the NF-κB signaling, HEK293 cells were transiently transfected with NF-κB-luc and IKKβ-expressing plasmid. Transfected cells were exposed to the compounds at designated concentrations for 24 h. As shown in Fig. 2, Compounds **3c** and **3d** at 5 μM are more potent than SC97 in suppressing NF-κB activation, while **3b** is comparable to that of SC97. Among SC245 analogues **4a**−**4f**, compound **4e** is more potent than SC245 (Fig. 2).

To study effect of compounds **3a**−**3d** and **4a**−**4f** on the AR signaling, LNCaP cells were transiently transfected with PSA-luc reporter and were exposed to DMSO vehicle control, 1 nM DHT or compounds at designated concentrations in the presence of 1 nM DHT. Antiandrogen bicalutamide (Bic) was included as a positive control (Fig. 3). As indicated by the fold of suppression, compound **3b** at 2.5 μM is much more potent than SC97 in suppressing DHT-induced AR activation, whereas compounds **4d** and **4e** are more potent than SC245 (Fig. 3). These studies indicated that introduction of a hydroxyl group to position 4 of the ionone ring of SC97 (Scheme 1) has substantially increased its anti-androgenic activity, while such modification only has minor impact on the inhibitory activity against the NF-κB signaling (compare **3b** and SC97 in Figs. 2 and 3). By AR-dependent reporter assay in PC3 cells, we showed that **3b** significantly suppresses DHT-induced activation of the W741C and F876L mutated ARs (Fig. 4).

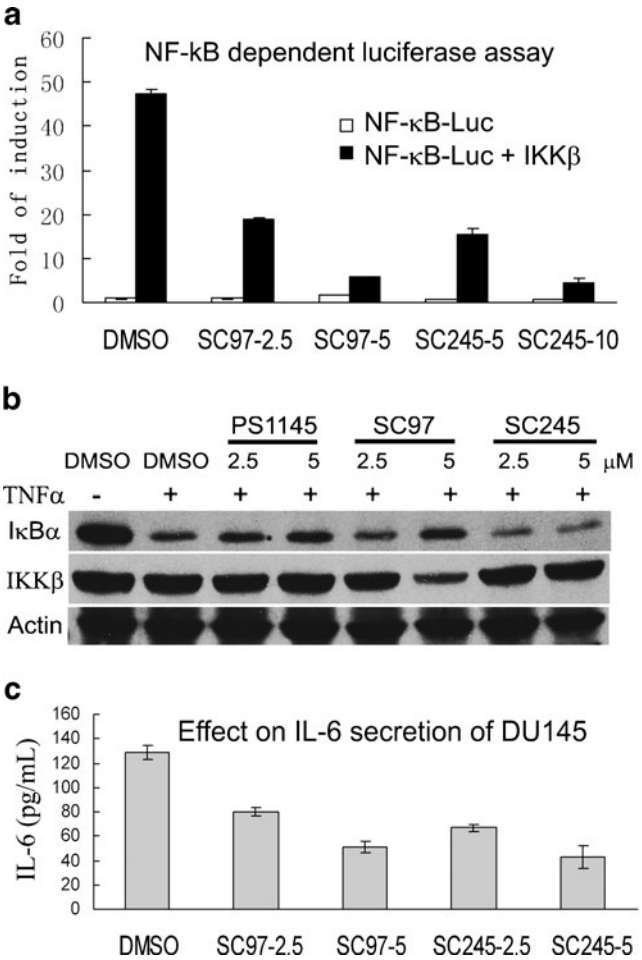
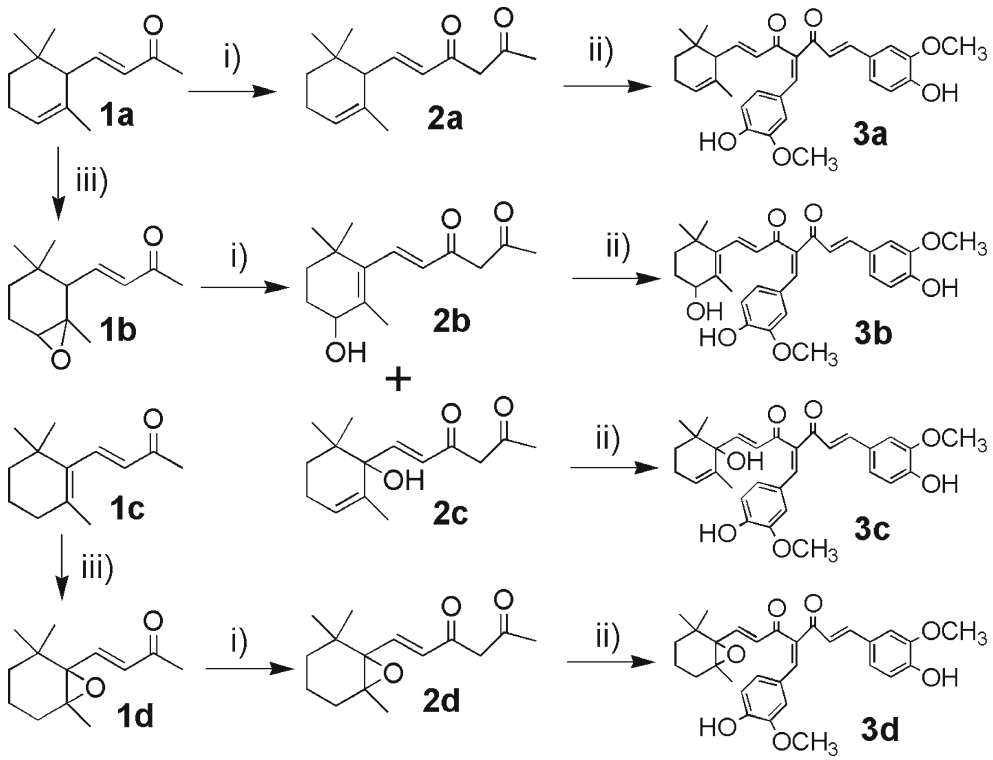


Fig. 1 **a** Compounds SC97 and SC245 dose-dependently suppress NF-κB activation. HEK293 cells were transiently transfected with NF-κB-luc, IKKβ-expressing plasmids (*black block*) or pcDNA3.1 vector (*white block*) and pRL-TK. Fold of induction related to the cells that were transfected with pcDNA3.1 vector and exposed to DMSO vehicle was shown; **b** Western blot analysis indicates that SC97 and PS1145 suppress TNFα-induced degradation of the IκBα protein in HEK293 cells. HEK293 cells were pretreated with compounds for 12 h, then 10 ng/mL TNFα for 30 min; **c** SC97 and SC245 suppress IL-6 secretion of DU-145 prostate cancer cells. The IL-6 level of cell culture supernates was determined using ELISA kit (R&D Systems, Inc., MN, USA)



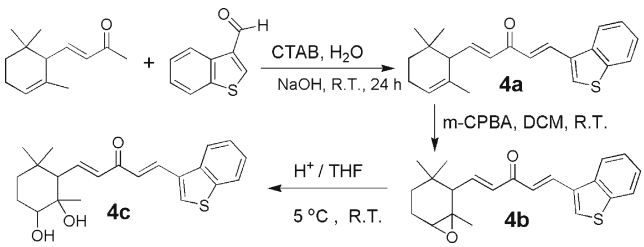
Scheme 2 Synthesis of compounds **3a–3d** and their intermediates: i) CH_3ONa, ethyl acetate, 38^0C; ii) n-Bu_3BO_3, n-$BuNH_2$, room temperature (R.T.), 4 days; and iii) MCPBA, CH_2Cl_2, R.T.

4) SC97 and Compound **3b** suppressed PSA expression and induced apoptosis in LNCaP cells

To determine whether SC97 and compound **3b** suppress PSA expression and induce apoptosis in LNCaP cells, LNCaP cells in complete medium were exposed to DMSO vehicle control, SC97 and compound **3b** at 2.5, and 5 μM for 24 h. Bic at 2.5 μM was included as a positive control. Western blot analysis indicated SC97 and **3b** dose-dependently suppressed PSA protein expression and induced caspase-3 activation and specific proteolytic cleavage of poly(ADP-ribose) polymerase (PARP) in LNCaP cells (Fig. 5). The study indicated that **3b** is more potent than SC97 in suppressing PSA expression and inducing apoptosis in LNCaP cells.

5) Antiproliferative activities in a panel of prostate cancer cell lines

LNCaP and C4-2B prostate cancer cells express endogenous T877A mutated AR. LNCaP cells are androgen-dependent and C4-2B cells are androgen-independent. The 22Rv1 cells are AR-positive and androgen-independent, whereas PC-3 and DU-145 cells are AR-negative and androgen-independent. The cytotoxicity effect of compounds **3a−3d** and **4a−4f** in a panel of prostate cancer cell lines, including LNCaP, C4-2B, 22Rv1, PC-3 and DU-145, were evaluated by MTT assay (Table 1). Among the SC97 analogues **3a−3d**, compound **3b** showed potent cytotoxicity in LNCaP, C4-2B and 22Rv1 cells, with IC_{50} values in the range of 1.2–3.8 μM (Table 1). Among the SC245 analogues **4a−4f**, compound **4e** showed low micromolar activities in LNCaP, C4-2B and 22Rv1 cells (Table 1).

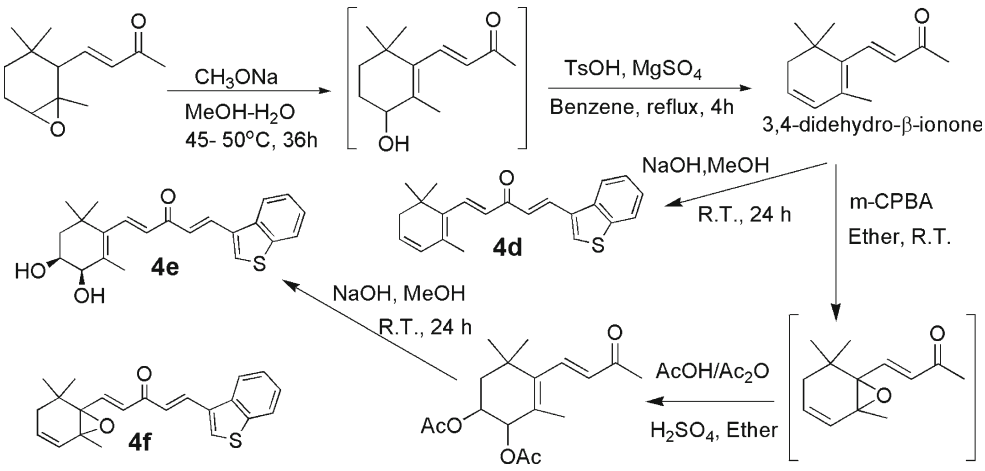


Scheme 3 Synthesis of compounds **4a–4c**. CTAB, cetyltrimethyl ammonium bromide

Discussion

Current therapeutic strategy for advanced prostate cancer is to suppress the AR signalling. Despite of initial response to castration and conventional antiandrogens, such as bicalutamide, lethal CRPC arises and remains addicted to the AR signalling [6, 30]. In particular, abiraterone and enzalutamide were recently approved by FDA for the treatment of CRPC. However, reports to date suggest that resistance to these agents invariably develops within 1−3 years in CRPC patients and is characterized with a rising

Scheme 4 Synthesis of compounds **4d–4f**



serum prostate-specific antigen (PSA), suggesting aberrant re-activation of the AR [8, 30, 31]. The key challenge in the field is how to suppress the continued AR signalling in CRPC cells [32]. To date, intensive research in CRPC has revealed that the AR in CRPC cells could be activated by multiple mechanisms, including mutations in AR, rendering the AR promiscuous so that it can be activated by a broad range of non-androgen ligands, even antiandrogens [33] and androgen-independent activation of the AR via cross-talk with other factors [34, 35]. In particular, it was demonstrated that IL-6 (a NF-κB regulated cytokine) stimulates prostate cancer growth in vitro and in vivo through activation of the AR and increase prostate cancer cell resistance to bicalutamide [22, 24]. Further, Yemelyanov et al. found that IKK$_\beta$ kinase, a master regulator of the NF-κB activation, is constitutively active in tissue specimens from advanced prostate cancer [36]. Thus, co-inhibition of the AR and NF-κB appears to be an attractive strategy for battling advanced prostate cancer.

Our previous work has identified two novel ionone-based antiandrogens SC97 and SC245, which are full antagonists of

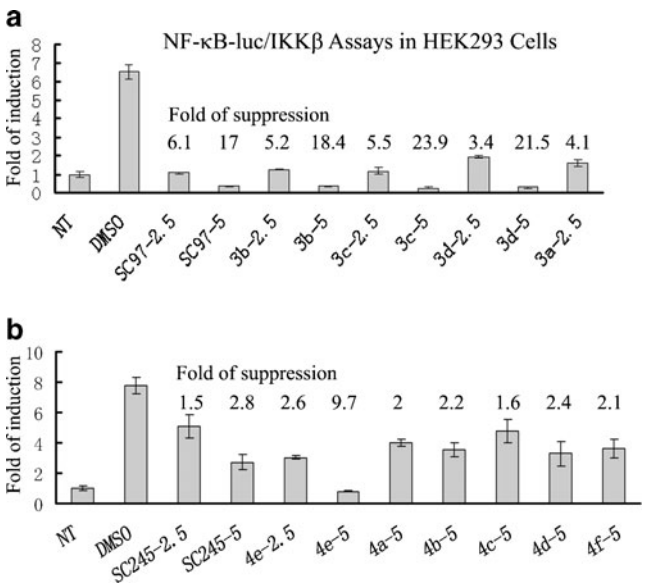


Fig. 2 Effect of compounds SC97, SC245, **3a–3d** and **4a–4f** on the NF-κB-dependent luciferase activity induced by expression of IKKβ. HEK293 cells were transiently cotransfected with NF-κB-Luc, IKKβ-expressing plasmids and pRL-TK (internal control) and were exposed to DMSO vehicle or compounds at designated doses (μM) for 24 h. NT, HEK293 cells were cotransfected with NF-κB-Luc, pcDNA3.1 empty vector and pRL-TK. Fold of induction related to the NT was shown. Fold of suppression related to the DMSO vehicle control was also indicated

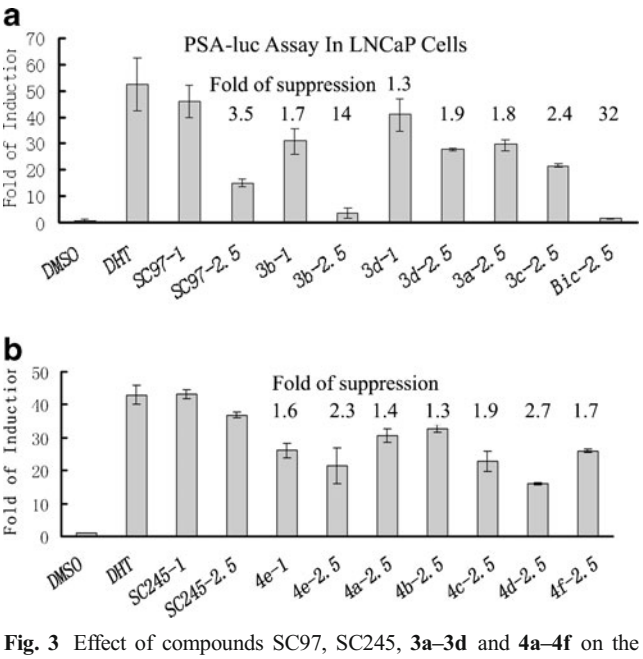


Fig. 3 Effect of compounds SC97, SC245, **3a–3d** and **4a–4f** on the DHT-induced AR activation in LNCaP cells. LNCaP cells were transiently transfected with PSA-luc (reporter) and pRL-TK and were exposed to DMSO vehicle, 1 nM DHT or compounds at the designated concentrations (μM) in the presence of 1 nM DHT for 24 h. Fold of induction related to the DMSO vehicle control was shown. Fold of suppression related to the cells that were exposed to 1 nM DHT alone was also indicated. Bars, standard deviations. Bic (bicalutamide) was included as a positive control

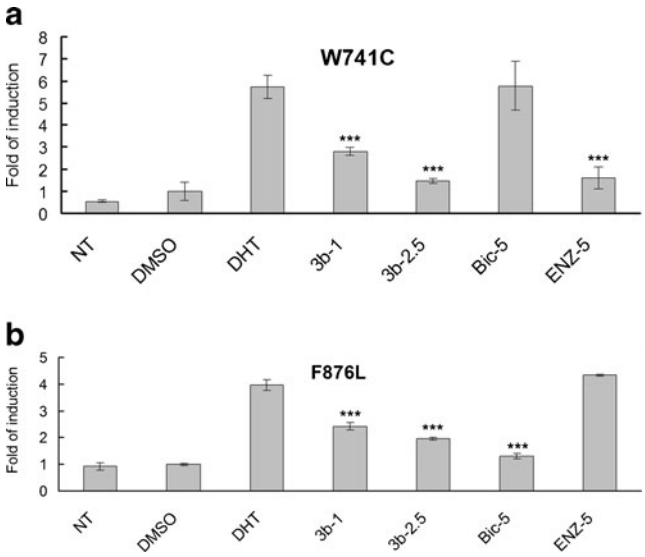


Fig. 4 Compound **3b** dose-dependently suppressed DHT-induced activation of the AR W741C (**a**) and F876L (**b**) mutants. ENZ, enzalutamide. For NT, only the PSA-luc, pCMV empty vector and pRL-TK were co-transfected into PC3 cells. For all other wells, the PSA-luc, AR mutant W741C or F876L expressing plasmid and pRL-TK were co-transfected into PC3 cells. The cells were exposed to DMSO vehicle, 10 nM DHT or compounds in the presence of 10 nM DHT for 24 h. Fold of induction were calculated relative to the DMSO wells. ***p <0.0001 when compared with DHT alone

the wild type and the T877A, W741C and H874Y mutated ARs [27, 28]. Using PolarScreen AR competitor assay kit (P3018, Invitrogen), we showed that SC97 binds with the AR ligand-binding domain [27]. In this work, we discovered that SC97 and SC245 also inhibits NF-κB and dose-dependently suppress IL-6 secretion in DU-145 prostate cancer cells. Our study indicated that SC97 suppresses IKK_{β} expression (Fig. 1b). Further work is needed to characterize molecular mechanism that account for this activity.

In an effort to optimize SC97 and SC245, we have focused on chemical modifications at the ionone ring and discovered compound **3b** that is more potent than SC97 in suppressing AR signalling in LNCaP cells (Figs. 3 and 5) and as potent as SC97 in inhibiting NF-κB activation (Fig. 2). Importantly, **3b** dose-dependently suppressed DHT-inducted activation of the AR W741C and F876L mutants. In contrast, bicalutamide is

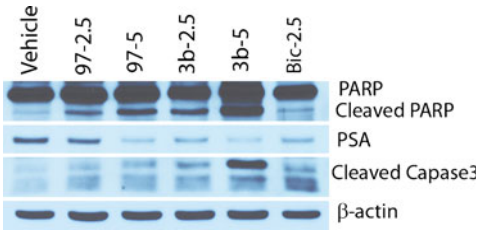


Fig. 5 SC97 and compound **3b** dose-dependently suppressed PSA expression and induced apoptosis in LNCaP cells. LNCaP cells were exposed to DMSO vehicle or compounds for 24 h. Bic was included as a positive control

Table 1 The cytotoxicity of compounds 3a–3d and 4a–4f in five prostate cancer cell lines (IC_{50}, μM)

	LNCaP	C4-2B	22Rv1	PC-3	DU145
SC97	1.3	1.6	2.2	4.2	2.9
SC245	0.6	0.7	1.1	2.0	N.D.
3a	4	5.7	9.2	4.5	8.5
3b	1.2	2.8	3.8	3.1	5.6
3c	1.7	3.3	5.1	3.9	8.3
3d	2.1	4.1	8.1	4.2	7.8
4a	8.1	>20	15.3	>20	>20
4b	2.9	9.0	6.8	N.D.	6.9
4c	1	5.6	4.2	N.D.	3.7
4d	9.2	13.1	15.6	N.D.	17.2
4e	1.8	4.1	4.1	N.D.	6.3
4f	5.8	8.7	8.2	6.6	12.6

N.D. not determined. Values for SC97 and SC245 were taken from references [27, 28]

inactive against the W741C mutant, whereas the second-generation antiandrogen enzalutamide is inactive against the F876L mutant (Fig. 4). This could have clinically significant as the W741C mutant was characterized from CRPC patients who were treated with bicluamide [10]. The F876L mutant was recently found in CRPC patients who progress on treatment of an enzalutamide analogue [14, 15]. Overall, our studies indicated that **3b** is a potent inhibitor of both AR and NF-κB signaling. The C4-2B and 22Rv1 cells are AR-positive and are androgen-independent. C4-2B cells express T877A mutated AR, whereas 22Rv1 cells express H874Y mutated ARs. Androgen-independent PC-3 and DU145 cells are AR-negative and exhibit a high constitutive activation of IKKβ and NF-κB signaling [19]. Compound **3b** showed low micromolar antiproliferative activities in C4-2B, 22Rv1, PC-3 and DU-145 cells (Table 1), indicating compound **3b** is an effective agent against the CRPC cells.

Conclusion

In present study, we discovered that ionone-based antiandrogen SC97 and SC245 also inhibit NF-κB activation and dose-dependently suppress IL-6 secretion in DU-145 prostate cancer cells. By introducing a hydroxyl group into position-4 of the ionone ring at SC97, we have obtained compound **3b** that is substantially more potent than SC97 in suppressing DHT-induced AR activation and as potent as SC97 in inhibiting NF-κB activation. Importantly, compound **3b** significantly inhibits F876L mutant which confers resistance to enzalutamide. Further, compound **3b** showed potent cytotoxicity in both AR-positive and AR-negative androgen-independent prostate cancer cells.

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Conflict of interest The authors declare that they have no conflict of interest.

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