Drug Development Research

WILEY

DRUG DEVELOPMENT RESEARCH

RESEARCH ARTICLE OPEN ACCESS

Inhibition of Steroidogenesis in Prostate Cancer Cells by Both a Natural and Another Synthetic Steroid

Marisa Cabeza[1] | Karla Mejía[1] | Fernando García[1] | Ivonne Heuze[1] | Miguel Morales[2] | Mauricio Rodríguez-Dorantes[2]

[1]Departamento de Sistemas Biológicos y de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico | [2]Laboratorio de Oncogenómica, Instituto Nacional de Medicina Genómica, Mexico City, Mexico

Correspondence: Marisa Cabeza (marisa@correo.xoc.uam.mx)

Received: 18 December 2024 | **Revised:** 12 February 2025 | **Accepted:** 8 March 2025

Funding: This study was conducted with funds approved by the Universidad Autónoma Metropolitana-Xochimilco.

Keywords: β-sitosterol | 17β-hydroxysteroid dehydrogenase activity | androstane derivative | LNCaP cells proliferation | type 1 5α-reductase activity

ABSTRACT

Studies suggest that vegetarians and Asians have lower mortality rates from prostate cancer compared to men who follow a Western diet. β-sitosterol, a key compound of plant-based diets, has been found to induce significant changes in the ultrasonic structure of the prostatic adenomas, making it a promising candidate for further prostate cancer research. Consequently, we investigated the potential of β-sitosterol and the synthetic derivative **2** as potent inhibitors of androgen synthesis, a critical process for the growth and survival of prostate tumor LNCaP cells. Solubilized LNCaP microsomes were used as a source of SRD5A1 and AKR1C3 to monitor androgen synthesis from labeled androstenedione, both in the presence and absence of β-sitosterol or **2**. Furthermore, the effect of these steroids on LNCaP viability was determined using the MTT method. Our findings revealed significant insights into the androgen synthesis pathways in LNCaP cells. The most efficient metabolic route for dihydrotestosterone formation was the conversion of androstenedione to 5α-androstanedione rather than from testosterone in LNCaP. This conclusion is supported by the Vmax values for 5α-androstanedione formation (271.05 ± 5.0 ng/mg protein/min) and the Vmax of testosterone formation (80.1 ± 8.0 ng/mg protein/min). Both β-sitosterol and **2** demonstrated substantial inhibitory effects of these enzymes for dihydrotestosterone formation and significantly reduced cell viability, highlighting their therapeutic potential. These findings enhance our understanding of the inhibitory effects of β-sitosterol and **2** on LNCaP cells and suggest their promising application in the treatment of prostate cancer.

1 | Introduction

Prostate cancer is one of the most common types of cancer worldwide and represents a leading cause of cancer-related death in men (Ferlay et al. 2021). Its treatment depends on the tumor grade, stage, and the patient's age. Therapeutic options range from active surveillance to a combination of surgery, chemotherapy, radiotherapy, and/or androgen deprivation therapy (ADT). Active surveillance is the preferred approach for patients with localized and low-risk diseases. This fact involves monitoring disease progression through periodic prostate-specific antigen (PSA) tests, physical exams, prostate biopsies, or a combination of these to intervene only when significant disease progression is observed (Litwin and Tan 2017). Active surveillance management can reduce the frequency of adverse effects associated with immediate treatment in men with low-risk prostate cancer, many of whom may never experience cancer-related symptoms (Garisto and Klotz 2017; Tosoian et al. 2011). In patients with more advanced or residual disease after a prostatectomy, ADT is the therapy of choice, as it has been shown to reduce tumor volume and PSA levels significantly (Rebello et al. 2021). However, after 18–24 months of ADT, many patients experience a rise in PSA levels and progress to castration-resistant prostate cancer (CRPC) (Huang et al. 2018).

The progression of prostate cancer to CRPC is primarily associated with the tumor's capacity to synthesize androgens within the tumor microenvironment, alongside support from adrenal androgens. Additionally, alterations in androgen receptor (AR) signaling play a significant role in this process. These alterations may involve increased AR expression, mutations in the AR gene, or the emergence of AR variants, which can activate the androgen signaling pathway without ligands (Dong et al. 2019).

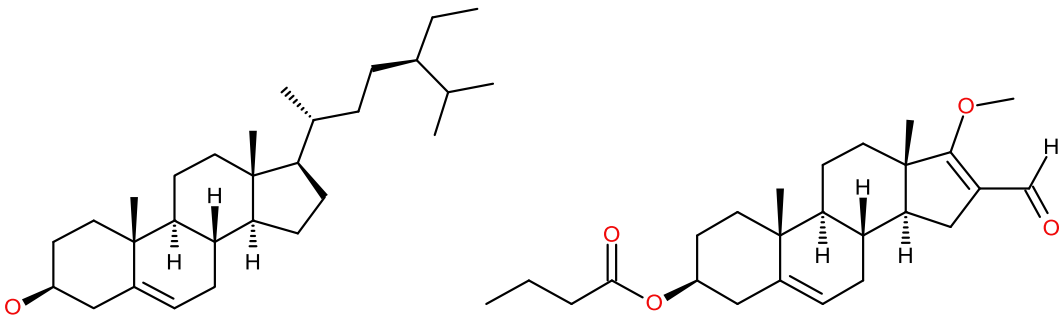
In this context, it has been proposed that the enzyme 5α-reductase type 1 (SRD5A1), which is overexpressed in tumors, may contribute to androgen synthesis in CRPC. Likewise, the enzyme 17β-hydroxysteroid dehydrogenase (AKR1C3), also overexpressed in CRPC, favors the conversion of 5α-dione to dihydrotestosterone (DHT) instead of the conversion of 4-dione to testosterone, which facilitates the production of androgens by the tumor (Figure 2) (Afzal et al. 2022). Therefore, inhibiting these enzymes could represent a viable therapeutic strategy for this disease.

Recent research has highlighted the potential therapeutic effects of β-sitosterol (SIT), a plant compound, on various cancers, including breast, colon, gastric, renal, liver, and pancreatic cancers. SIT has demonstrated the ability to induce apoptosis (Pradhan et al. 2019; Salamatullah et al. 2021; Baskar et al. 2010) and inhibit cell proliferation (Sharmila and Sindhu 2017; Cao 2018), migration, and invasion (Jang et al. 2023; Matsushita et al. 2020). However, the precise mechanisms of its action remain unclear, particularly concerning prostate cancer. Data in the literature indicates that the prevalence of latent infiltrative prostate tumors varies significantly among different racial groups, mirroring the incidence of clinical prostate carcinoma (Shimizu et al. 1991). For example, the prevalence among Black Americans is 36.9%, among whites 34.6%, among Colombians 31.5%, and among Japanese immigrants in Hawaii 25.6%. Interestingly, Japanese individuals living in Japan have a prevalence rate of 20.5%, comparable to their counterparts in Hawaii (Tsugane et al. 1990; Awad et al. 2001).

Such difference in the incidence of prostate carcinoma between the Japanese and other groups could be attributed to the abundant consumption of plant-based foods in their diet. Studies show that both vegetarians and Asians exhibit lower mortality rates from prostate cancer compared to men who follow a Western diet (Shimizu et al. 1991).

β-Sitosterol (SIT), shown in Figure 1, is an abundant compound found in plant-based diets and has various medical uses. Its effects on prostate cancer have been particularly noteworthy—a 2-month treatment with SIT induced significant changes in the ultrasound structure of prostatic adenoma (Awad et al. 2001). In vitro, SIT inhibited the growth and metastasis of the human prostate cancer cell line PC-3 (von Holtz et al. 1998) and increased the apoptosis of LNCaP cells (Cabeza et al. 2003).

Previous research has demonstrated the effect of SIT as an in vitro inhibitor of the activity of 5α-reductase type 2 (SRD5A 2) in the prostate of humans and hamsters (Cabeza et al. 2023). In humans, SRD5A2 is found in the genital skin, epididymis, seminal vesicles, and liver (Russell and Wilson 1994). Moreover, the prostatic isozyme SRD5A2, a type 1 isozyme of steroid 5α-reductase (SRD5A1), has also been found in the human prostate (Levy et al. 1990; Normington and Russell 1992; Thomas et al. 2005). SRD5A1 is also expressed in genital and nongenital skin, the liver, certain brain regions, the epididymis, seminal vesicles, testes, the adrenal glands, and the kidneys (Normington and Russell 1992). Two different genes encode for these isoenzymes. The gene encoding SRD5A1 is overexpressed in castration-resistant prostate cancer (CRPC) (Thomas et al. 2005). This could explain the ability of tumor cells to survive in a low-androgen environment.



(1) Stigmast-5-en-3β-ol
or
β-Sitosterol (SIT)
or
5-cholestene-24β-ethyl-3β-ol

(2) 2,16-Formyl-metoxyandrost-5,16-dien-3β-il butyrate (**5c**)

SRD5A2 IC_{50} = 0.050±0.0095 nM
D
oes not bind to the androgen receptor
S
ource:Sánchez-Márquez et al.,2016

FIGURE 1 | Structures of β-sitosterol (1) and steroid (2). The structure of β-sitosterol is similar to that of cholesterol, but it shows an ethyl group at C-24. β-sitosterol is a bioactive phytosterol biosynthesized from squalene in plants (Hang and Dussault 2010). Steroid 2 was previously synthesized by our group (2,16-formyl-17-methoxyandrost-5,16-dien-3β-yl butyrate (**5c**)) (Sánchez-Márquez et al. 2016). This steroid was previously identified as an inhibitor of the activity of 5α-reductase type 2.

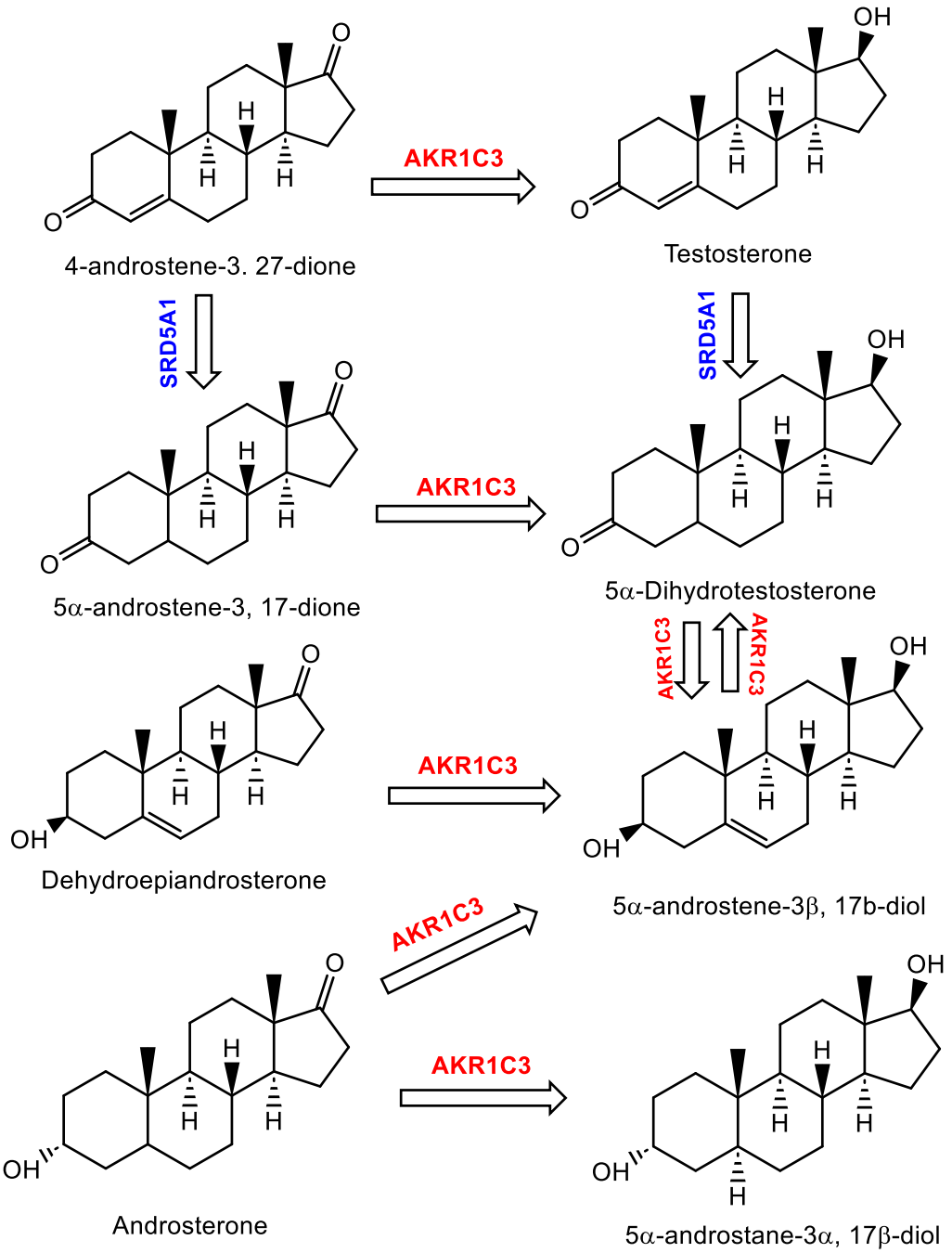


FIGURE 2 | Reduction of testosterone to dihydrotestosterone by the enzyme 3-oxo-5-α-steroid 4-dehydrogenase type 1 (5α-reductase, SRD5A1). Reduction of androgenic 17-ketosteroids by AKR1C3 (Pradhan et al. 2019).

Moreover, the increase in AKR1C3 expression, which reduces 17-keto to 17-hydroxysteroids (Figure 2) (Fung et al. 2006), favors the transformation of 5α-dione to DHT rather than the 4-dione to T synthesis pathway (Figure 2). These findings suggest that AKR1C3 is a therapeutic target. AKR1C3 is normally expressed in the liver, lungs, small intestine, prostate, mammary glands (Penning et al. 2000), adrenal glands (Nakamura et al. 2009), brain, kidneys, placenta, colon, spleen, and testes. It is one of the most expressed genes in CRPC due to the low serum androgen levels.

With the overexpression of AKR1C3, the tumor can produce its androgens, allowing it to grow without these hormones (Montgomery 2008). This phenomenon is observed in prostate cancer cells cultured in androgen-deprived media and in prostate cancer cell xenografts grown in castrated mice (Hang and Dussault 2010). The AKR1C3 gene can be repressed by the AR and its agonists (Stanbrough 2006; Yepuru 2013), but it can also act as a coactivator of such an AR (Yepuru 2013).

The LNCaP prostate cancer cell line is known for overexpressing the SRD5A1 and AKR1C3 genes (Nakamura et al. 2009; Wu et al. 2013; Biancolella et al. 2007; Mostaghel 2019) while exhibiting the presence of the AR. These characteristics make it an ideal model to study the

antiandrogenic and antiproliferative effects of various molecules. So far, the effects of SIT and steroid **2** on the inhibition of SRD5A1 and AKR1C3 activity have not been reported. Hence, this study aims to evaluate their potential, specifically determining whether these steroids can block the activity of SRD5A1 and AKR1C3 in the LNCaP cell line and demonstrate their antiproliferative effects on these cells.

2 | Experimental Procedures

2.1 | Chemicals and Radioactive Materials

Perkin Elmer (Metrix, Mexico City) supplied androstenedione (1,2,6,7-^{3}H) with a specific activity of 3519 GBq/mmol. Steraloids (Wilton, NH, USA) provided the unlabeled steroids: T, DHT, androstenedione (4-dione), androstenedione (5α-dione), and 5-cholesten-24β-ethyl-3β-ol (SIT). Sigma (Merck, Mexico) delivered reduced nicotinamide adenine dinucleotide phosphate (NADPH). The SIT was recrystallized until the melting point reached 136°C–137°C (Sen et al. 2012).

The finasteride (N-(1,1-di-methoxymethyl)-3-oxo-4-aza-5α-androst-1-eno-17β-carboxamide, FIN), an aza-steroid sold in Mexico as Proscar (treatment for benign prostate hypertrophy) or as Propecia (therapy for male pattern hair loss) (Merck, Sharp, and Dohme), was used as a positive control in these experiments. The FIN was obtained from Proscar by a meticulous extraction process. The tablets were crushed and extracted with chloroform, the solvent was removed in vacuo, and the residue was filtered by silica gel column chromatography. The melting point of the isolated FIN (252°C–254°C) was identical to that reported in the literature (Trapani 2002). We also used a compound synthesized by our group, 16-formyl-17-methoxyandrost-5,16-dien-3β-yl-butyrate (steroid 2), Figure 1. This steroid was identified as an inhibitor of the enzyme 5α-reductase type 2, showing similar activity to FIN and SIT on the human prostate (Russell and Wilson 1994).

2.2 | Biological Material

2.2.1 | LNCaP Cell Cultures

Cell passages 25–50 of the androgen-dependent LNCaP cell line provided by American Type Culture Collection (ATCC) (San Diego, CA, USA) were meticulously seeded in a flask and maintained in a carefully prepared Roswell Park Memorial Institute (RPMI) growth medium containing 10% of fetal bovine serum (FBS, Thermo Scientific, Mexico City) at pH 7.4 and under precise temperature conditions of 37° C and 5% CO_2 until they grew to confluence.

Later, we then added 1:1 trypsin–EDTA (0.25%) in phosphate-buffered saline (PBS) and waited 3 min. Then, we supplemented phenol-free filtered RPMI growth medium with 10% charcoal-stripped fetal bovine serum and centrifuged. The cell pellet was dissolved in phenol-free filtered RPMI growth 10% charcoal-stripped fetal bovine serum, and the cells were counted.

2.2.2 | Preparation of the Solubilized Microsomes From LNCaP Cells

To prepare solubilized microsomes from the confluent cultures of LNCaP cells, we thoroughly adopted the method described by Levy et al. We withdrew the medium, and the cells were chased with PBS (1×). Then, phenol-free filtered RPMI growth medium with 10% charcoal-stripped fetal bovine serum was added and incubated for 24 h in the same conditions reported before. The medium was then removed, and LNCaP cells were treated with 1 mL of buffer A (100 mM sodium citrate, 100 mM potassium chloride, 1 mM dithiothreitol (DTT), 0.4% Lubrol PX, (Garner 2008) 20% glycerol, and 10 mM NADPH). Cells were incubated for 60 min on a bed of ice, scraped and transferred to a Potter-Elvehjem homogenizer, and homogenized on ice for 10 min. The lysate was passed through a 21 G needle 5 times, then incubated on ice for 30 min, and finally centrifuged at 100,000 g for 1 h at 0°C. The proteins of the solubilized microsomes (supernatant) were quantified using the Bradford, 1976 method and aliquoted in microcentrifuge tubes labeled and frozen at −80°C for future use.

2.2.3 | Determination of the Conversion of 4-Dione to T and 5α-Dione to DHT in LNCaP Cells

Studies have reported the presence of the SRD5A1 and AKR1C3K isoenzymes in the wild LNCaP cell line (Wu et al. 1993). These isoenzymes play a crucial role in androgens' metabolism, particularly in converting testosterone (T) to its more potent form, DHT. Therefore, we followed the conversion of radiolabeled 4-dione to T, 4-dione to 5α-dione, and 5α-dione to DHT (Figure 2) using the solubilized microsome as a source of these enzymes (Normington and Russell 1992; Wu et al. 1993) in the presence or absence of SIT or steroid **2**. We decided to use wild LNCaP because this model is closer to reality due to the interaction of enzymes and their metabolites.

To obtain saturation plots, we performed two independent experiments in duplicate ($n = 2$), where we prepared several tubes containing a mixture of 2 nM [^{3}H]-4-dione and increasing concentrations of unlabeled 4-dione (ranging from 0.002 to 20.0 μmol/L). The mixture included 200 μM NADPH and 2 mM DTT in a final 200 μL of 20 mM phosphate buffer at pH 7.5. The reaction commenced once 30 μg/30 μL of solubilized microsomes were added. The tubes were then incubated in a water bath at 37.5°C for 15 min (Cabeza et al. 2023). We also prepared a control assay without the enzymatic fraction. After extraction with ethyl acetate and evaporation to dryness, SRD5A1 activity was identified using thin-layer chromatography (TLC). Steroid extracts were spotted onto high-performance thin-layer chromatography (HPTLC) Keiselgel 60 F254 (Merck, CDMX) plates with an automatic autospotter. Standards for 4-dione, 5α-dione, DHT, and T were applied to the corresponding lanes on both sides of the plate. The plates were developed in a 9:1 chloroform/acetone mixture and dried at room temperature. Radioactivity was scanned using a Bioscanner AR-2000 (Bioscan, Washington, DC, USA). T and 4-dione standards were identified by fluorescence under a ultraviolet lamp ($\lambda = 254$ nm, UVP, Upland, CA, USA), while DHT and 5α-dione were detected using a phosphomolybdic acid reagent.

2.2.4 | Determination of SRD5A and AKR1C3K Activity in the Presence or Absence of SIT or the Steroid 2

Two independent assays determined the SRD5A and AKR1C3K activity in the presence or absence of SIT or steroid **2** (Figure 1). This determination was crucial as it enabled the assessment of the inhibitory effects of SIT and steroid **2** on these enzymes.

We prepared test tubes containing the same mixture described in Section 2.1, adding 0.7 or 1.4 μM of SIT and 1.4 μM of compound 2, dissolved in ethanol/dimethyl sulfoxide (DMSO), to the assays. The solvent was then evaporated. The test tubes, each containing 30 μg/30 μL of protein, were incubated under the same conditions described in Section 2.1.

The residue obtained after extraction with ethyl acetate (2.1) was dissolved in a 1:1 chloroform/methanol mixture and applied to an HPTLC Keiselgel 60 F254 plate (Merck, CDMX) using an autospotter. Standards for 4-dione, 5α-dione, DHT, and T were dotted on each plate side in 4 lanes. The plates were then left to dry at room temperature. The plates were developed, and the standards were identified following the procedures detailed in Section 2.1.

The radioactive zones were identified based on their identical chromatographic behavior (Rf value) compared to the standards used. The total conversion rate to T, 5α-dione, and DHT was calculated by considering the percentage conversion of the radiolabeled precursor, its specific activity, the unlabeled 4-dione added to the assay, and the protein quantified by the Bradford Method (P). The final data were plotted in ng/mg P/min. Michaelis–Menten kinetics for the Km, Vmax, apparent (app) Kmapp, Vmaxapp, and Ki values were calculated using the Sigma Plot Software (https://systatsoftware.com/).

2.2.5 | Determination of the Effect of SIT on LNCaP Cell Proliferation

To determine the effect of SIT on the proliferation of metastatic prostate cancer cells, LNCaP cells were used at a density of 3×10^4 cells/well in 96-well container plates. Cells were allowed to adhere to the plate for 24 h in a Roswell Park Memorial Institute medium (RPMI-1640, phenol-free, and 10% charcoal-stripped fetal bovine serum).

LNCaP cell monolayers were washed with PBS. Subsequently, we separately added different concentrations (10^{-9} to 10^{-4}) of SIT or **2** (dissolved in 0.5% DMSO) plus 1 nM unlabeled T to each of the wells. Additionally, as viability controls, we added T (1 nM) or T + FIN (25 μM) (Baskar et al. 2010) to other wells. Separately, 100 pM of unlabeled 4-dione, 100 pM of 4-dione plus different concentrations of SIT (10^{-9} to 10^{-4}), or 1 μM of steroid **2** dissolved in 0.5% DMSO, were added to other wells. The plates were incubated at 37°C in a humidified atmosphere, with 5% CO_2–95% air in a VWR incubator (Symphony 1, 4 A, VWR, CDMX).

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyl tetrazolium bromide method) (Denizot 1986; Liu 1997) was employed to measure LNCaP cell proliferation. This method (chosen for its reliability and accuracy) involves the dissolution of formazan crystals formed from the reaction with 12 mM MTT in 100 μL of DMSO. The resulting absorbance was measured by spectrophotometry at 570 nm using a Biotek Epoch Microplate Spectrophotometer, Thermo Fisher Scientific, USA. Finasteride was used as an inhibitor of SRD5A2 activity in the trial. All assays were quadrupled in three independent experiments.

2.3 | Statistical Methods

The results of the viability experiments were subjected to one-way ANOVA using Dunnettt's post hoc test, according to the JMP 18 software package. Dunnettt's test allowed for multiple pairwise comparisons, comparing the measures of the treatments to a single predetermined control measure. In the experiment, treatment with T or 4-dione was considered the independent variable, and the combined treatment of T or 4-dione, plus the enzyme inhibitors FIN, **2**, and SIT, as dependent variables. The analysis of variance allowed us to identify interactions between the evaluated agents, administered concomitantly, with the hormonal substrates. Significance was determined by a p-value equal to or less than 0.02.

3 | Results and Discussion

3.1 | Conversion of 4-Dione to Testosterone

The thin-layer chromatography (TLC) analysis revealed the presence of multiple radioactive metabolites derived from radiolabeled 4-dione, representing a notable advancement in our research. Table 1 illustrates the conversion process of 4-dione into various metabolites. These results were calculated

TABLE 1 | Final rates of 4-dione conversion to T, DHT, and 5α-dione in the absence of inhibitors.

[4-Dione] (μM)	Rate of formation 5α-dione (ng/mg of protein/min)	Rate of formation DHT (ng/mg of protein/min)	Rate of formation T (ng/mg of protein/min)
2	8	11	4
5	11.4	20	9.5
7	44.4	48	12
11.0	72.8	118.4	17.5
16.0	112.4	140	80
20.0	271	141.7	43.7

considering the total radioactivity obtained from the Bioscanner readings across the chromatographic plate, the unlabeled 4-dione introduced during the experiment, and the data quantified from control samples lacking solubilized microsomes. The resulting data were plotted using SigmaPlot software version 15, applying the appropriate mathematical equation for analysis.

Equation: Standard Curves; Five Parameter Logistic Curve

$$xb = EC50*10\hat{}((1/Hillslope)*\log(2\hat{}(1/s - 1))$$

$$f1 = \min + (\max - \min)/(1 + (xb/x)\ \hat{}Hillslope)\ \hat{}s$$

$$f = \text{if}\ (x <= 0;\ \text{if}\ (Hillslope > 0;\ minimax);\ f1)$$

The radiolabeled 4-dione was identified in the plate by its chromatographic behavior, which matched that of the 4-dione standard. The average conversion of radiolabeled 4-dione to various metabolites in two independent experiments and their standard deviations were established as the control for these transformations (Table 1).

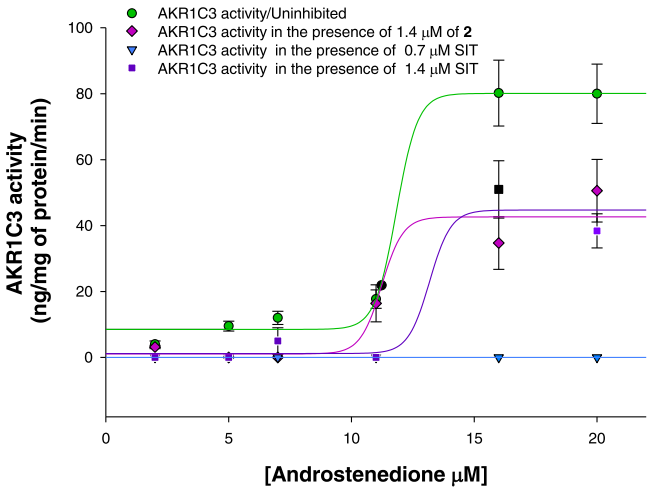


FIGURE 3 | Two separate experiments determined the conversion of labeled and unlabeled androstenedione (4-dione) by incubating it with solubilized microsomes from LNCaP cells. As described in the methods section, thin-layer chromatography separated and identified the formed metabolites. The conversion of 4-dione to testosterone (T) confirmed the activity of AKR1C3 in the solubilized microsomes from LNCaP cells and served as a control. Compared to this control, the results showed that 1.4 μM of steroid 2 and 1.4 μM of SIT inhibited this conversion. Additionally, 0.7 μM of SIT eliminated this transformation compared to the control.

The conversion of 4-dione to testosterone (T) suggests the involvement of the enzyme AKR1C3 in the solubilized microsome fraction derived from LNCaP cells (refer to Figure 2) (Mostaghel 2019) (Figure 3). Furthermore, compounds **2** and SIT were observed to inhibit the conversion of 4-dione to T at a concentration of 1.4 μM compared to the control (uninhibited 4-dione). Notably, a concentration of 0.7 μM SIT completely obstructed this conversion, as depicted in Figure 3, in contrast to the control (uninhibited 4-dione).

The Km and Kmapp values were comparable, whereas the Vmax and Vmaxapp values exhibited significant differences (see Table 2). These findings indicate that AKR1C3 binds to steroid **2**, forming the AKR1C3-4-dione complex and AKR1C3-4-dione-**2** with high affinity, as evidenced by the low Ki values in Table 2. These results suggest a type of noncompetitive inhibition. Furthermore, similar to steroid **2**, the SIT inhibited the activity of AKR1C3 at a concentration of 1.4 μM (refer to Table 2). Conversely, the observed reduction in T formation at 0.7 μM of SIT indicates a mixed type of inhibition (see Table 2).

3.2 | Formation of DHT in LNCaP Cells

The TLC results showed that radiolabelled DHT was produced from 5α-dione, Table 1 (refer to Figure 2) under inhibitor-free conditions due to the presence of AKR1C3 in the incubation medium (refer to Figure 2). Steroid **2** or 0.7 μM SIT effectively inhibited this conversion, as shown in Figure 4. Furthermore, a specific concentration of 1.4 μM SIT completely blocked the activity of AKR1C3, as depicted in Figure 4, highlighting the significance of particular concentrations in SIT inhibitory effects on DHT formation.

The kinetic parameters Km, Vmax, and Kmapp, $Vmax^{app}$ for converting 5α-dione to DHT in the absence and presence of inhibitors are shown in Table 3. The results ($n = 2$) showed that steroid **2** favored binding with the AKR1C3-5α-dione complex to form AKR1C3-5α-dione-**2**. The low Ki value indicated a high-affinity binding with this inhibitor, Table 3. Moreover, the Km and Kmapp values are very similar ($p > 0.05$). Still, the Vmax-app turned out to be very different from the Vmax ($p < 0.05$),

TABLE 2 | Kinetic properties of the AKR1C3 (testosterone formation from 4-dione) present in the soluble fraction of microsomes from LNCaP cells in the absence or presence of inhibitors.

Inhibitor	Km (μM)	Vmax (ng/mgP/min)	Kmapp (μM)	$Vmax^{app}$ (ng/mgP/min)	Ki (μM)	*R* value
Uninhibited Testosterone conversion	11.83 ± 0.6	80.1 ± 8.0				1
Steroid **2**			11.2 ± 0.4	42.7 ± 1.0	0.13	1
SIT 0.7 μM			2.0 ± 0.5	0	0.7	
SIT 1.4 μM			13.2 ± 1.9	44.7 ± 0.6	0.11	1

Note: Km, Km app, Vmax, and Vmaxapp are indicated as the mean ± SD ($n = 2$).

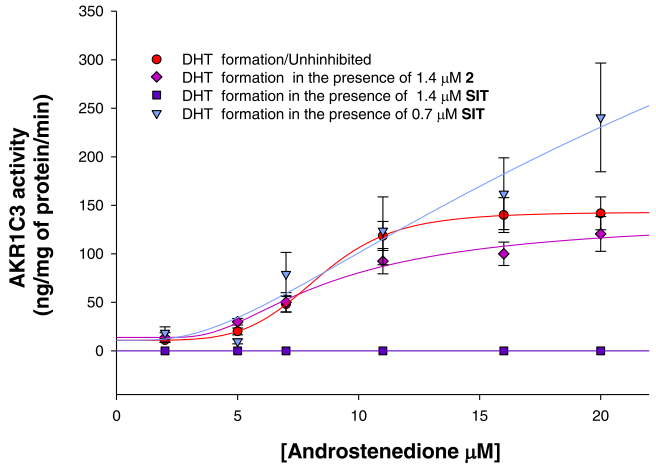


FIGURE 4 | The conversion of labeled and unlabeled androstenedione (4-dione) to 5α-androstanedione (5α-dione) and later to dihydrotestosterone (DHT) was tracked using solubilized microsomes from LNCaP cells as the enzyme sources. Radiolabeled 5α-dione and DHT were identified by TLC due to their identical chromatographic behavior to the standards of these steroids, as described in the methods. The conversion of 4-dione to 5α-dione and DHT was considered a control. The plot indicated that 1.4 μM of steroid **2** inhibited AKR1C3 activity compared to the control. Additionally, 0.7 μM of SIT slightly blocked this synthesis, while 1.4 μM of SIT eliminated it.

indicating a type of noncompetitive inhibition produced by steroid **2**.

However, data demonstrated that Kmapp differed from Km in the presence of 0.7 μM SIT (Table 3). Importantly, a Vmaxapp value was similar to the Vmax Table 3, thus indicating the formation of an AKR1C3-SIT complex. These data suggest a competitive inhibition type of SIT with the enzyme, a key aspect of our experiment. However, the lack of DHT formation in the presence of 1.4 μM SIT (Table 3) suggests a mixed kind of inhibition produced by this concentration.

3.3 | Conversion of 4-Dione to 5α-Dione

The findings from the TLC analysis revealed the conversion of androstenedione (4-dione) to 5α-androstanedione (5α-dione) in the solubilized microsomes from LNCaP cells, as depicted in Figure 5A. This transformation was attributed to SRD5A1 activity (Stanbrough 2006) and was the experimental control. The results indicated that **2** or SIT effectively inhibited this pathway compared to the control, as illustrated in Figure 5B. Notably, 1.4 μM SIT effectively blocked this conversion. These experiments were independently conducted in two trials, providing valuable insights into androgen biosynthesis in LNCaP cells.

TABLE 3 | Kinetic properties of the AKR1C3 (dihydrotestosterone formation from 4-dione) present in the soluble fraction of microsomes from LNCaP cells in the absence or presence of inhibitors.

Inhibitor	Km (μM)	Vmax (ng/mgP/min)	Kmapp (μM)	$Vmax^{app}$ (ng/mgP/min)	Ki (μM)	R value
Uninhibited DHT-synthesis	8.42 ± 1.3	133.59 ± 29				0.98
Steroid **2**			7.276 ± 2.2	104.29 ± 8	0.19	0.99
SIT 0.7 μM			11.7 ± 0.9	197.7 ± 35	1.79	0.96
SIT 1.4 μM			0	0	0	

Note: Km, Km app, Vmax, and Vmax app are indicated as the mean ± SD ($n = 2$).

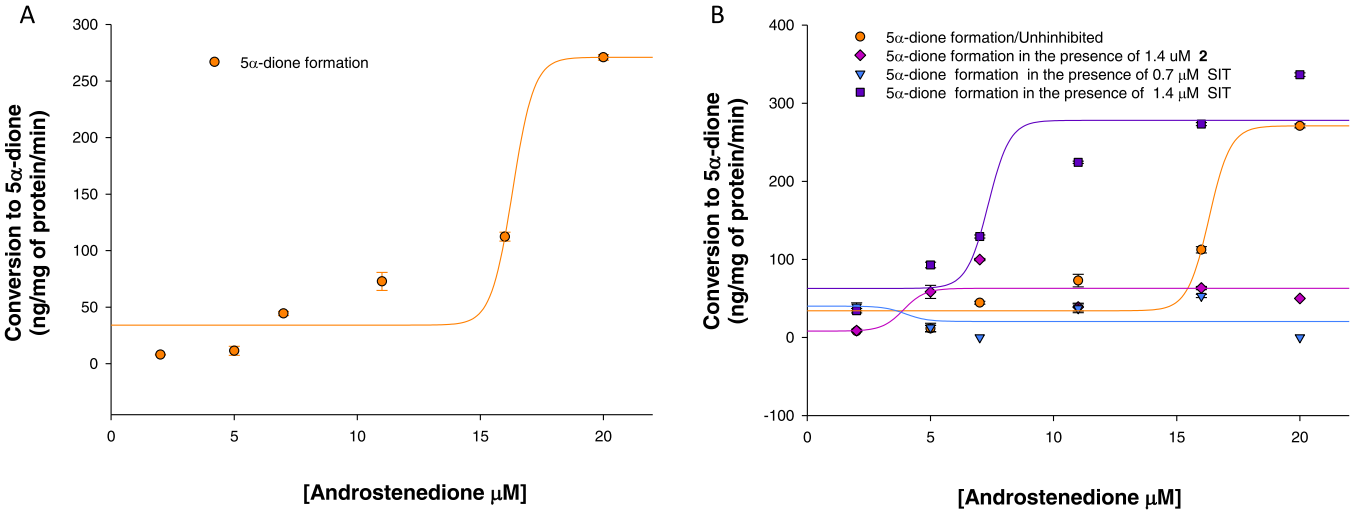


FIGURE 5 | The synthesis of labeled 5α-androstanedione (5α-dione) from radiolabeled androstenedione (4-dione) was tracked using solubilized microsomes from LNCaP cells as the enzyme source; TLC identified radiolabeled 5α-dione because of the identical chromatographic behavior to the 5α-dione standard, as we detailed in the methods. This conversion suggested the activity of type 1 5α-reductase enzyme (SRD5A1). A. The conversion of 4-dione to 5α-dione was considered the control. B. The plot showed that 1.4 μM of steroid 2 inhibited SRD5A1 activity compared to the control. In addition, 0.7 μM of SIT blocked this synthesis slightly, while 1.4 μM of SIT eliminated it.

The analysis of the kinetic parameters of SRD5A1 shows that the presence of steroid **2** or 0.7 μM SIT leads to significant differences in Km, Km app, Vmax, and Vmax app (see Table 4). These findings suggest that 1.4 μM of steroid **2** or 0.7 μM SIT inhibited the activity of SRD5A1 in a mixed manner with high affinity, as indicated by the low Ki value. Furthermore, in the presence of 1.4 μM SIT, the Vmax app aligns with Vmax, while the Km app deviates from Km. The data indicate that as the

TABLE 4 | A detailed analysis of the kinetic properties of SRD5A1 (5α-dione formation from 4-dione) found in the soluble fraction of microsomes from LNCaP cells.

Inhibitor	Km (μM)	Vmax (ng/mgP/min)	Kmapp (μM)	$Vmax^{app}$ (ng/mgP/min)	Ki (μM)	*R* value
Uninhibited 5α-dione synthesis	16.3 ± 0.7	271.05 ± 5.0				0.97
Steroid **2**	—		3.9	62.93 ± 8.0	0.48	1.0
SIT 0.7 μM	—		3.9	20 ± 2.0	0.22	0.97
SIT 1.4 μM	—		7.3	278 ± 6.5	1.1	0.94

Note: These properties are detailed in the absence or presence of inhibitors, providing a thorough understanding of the process. The values of Km, Km app, Vmax, and Vmax app are indicated as the mean ± SD ($n = 2$), ensuring the reliability and robustness of the results.

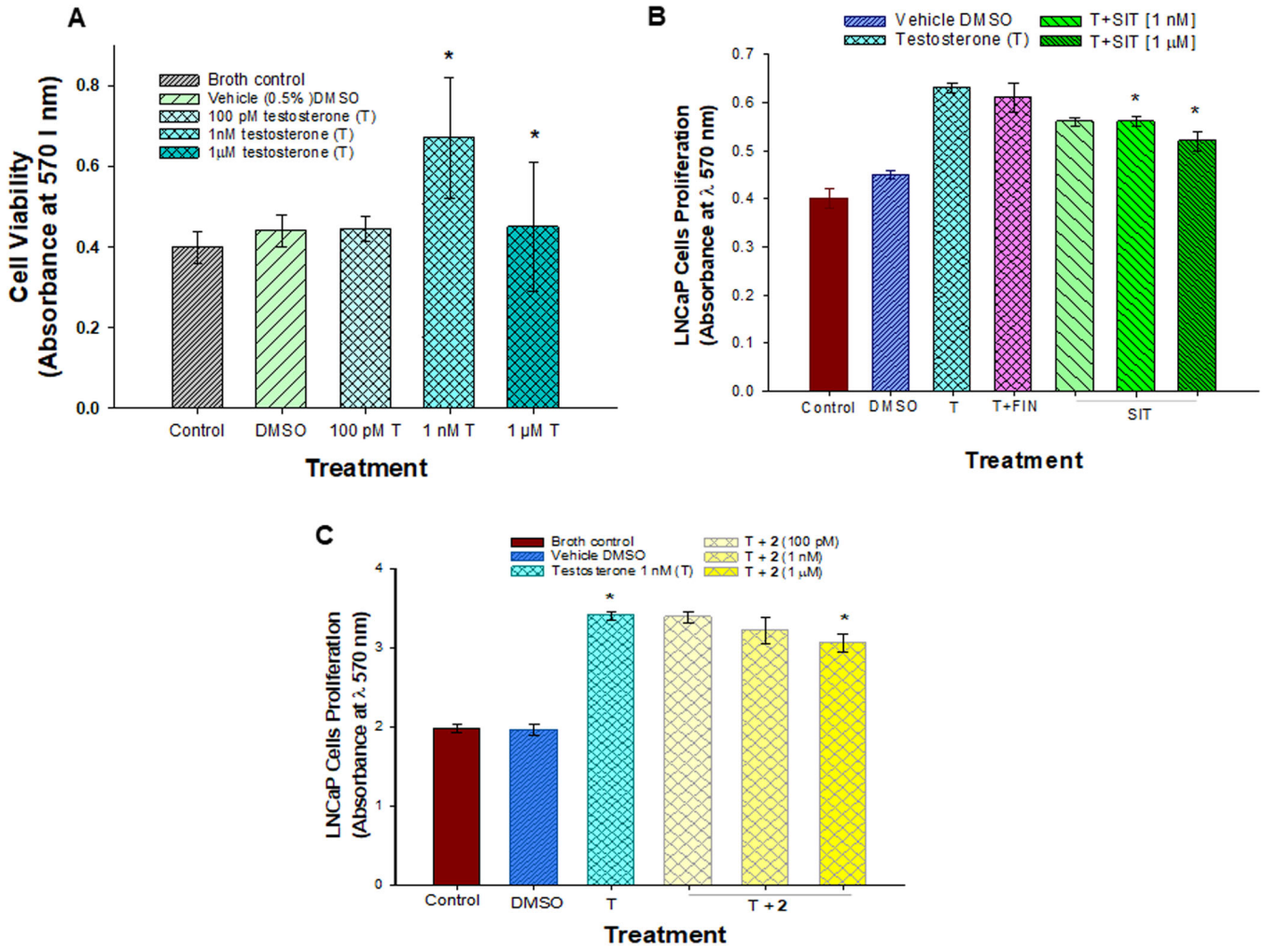


FIGURE 6 | LNCaP cells were seeded at a density of 3×10^4 cells per well in 96-well plates and allowed to adhere for 24 h. Different concentrations of SIT or **2**, along with unlabeled T, were added to separate wells. Viability controls, including T or T + FIN, were added to other wells. The plates were then incubated at 37°C, and cell proliferation was measured using the MTT method. A Impact of different testosterone (T) concentrations on LNCaP proliferation: 1 nM significantly increased LNCaP proliferation in four independent experiments (*). B In four independent experiments, FIN did not inhibit cell proliferation. However, SIT inhibited T-stimulated LNCaP proliferation: * Indicates a statistically significant difference ($p < 0.02$) with T-stimulated LNCaP cells. C The activity of 2 in T-stimulated LNCaP proliferation in four separate experiments. * Indicates a significant difference ($p < 0.02$) with T-stimulated LNCaP cells.

concentration of SIT increases, SRD5A1 exhibits competitive inhibition.

The data set also demonstrated that the Vmax value for forming T from 4-dione is lower than that for forming 5α-dione from the same precursor (4-dione). Table 2 and 4 (refer to Figure 2). These results are consistent with the Chang et al. 2011 findings, where the silencing of SRD5A1 expression blocked the conversion of 4-dione into 5α-dione and its eventual conversion to DHT in CRPC.

3.4 | Effect of SIT and Steroid 2 on LNCaP Cells

The impact of varying concentrations of T on LNCaP cells is depicted in Figure 6A. The analysis of variance and Dunnettt test, comparing the mean values of the samples with that of the vehicle-treated control (DMSO), revealed a significant increase in cell viability with 1 nM of T ($p < 0.02$) compared to vehicle-treated cells (Thomas et al. 2009). The effect of SIT on cell viability compared to T-treated cells is illustrated in Figure 6B. ANOVA and Dunnettt's test indicated a significant decrease ($p < 0.02$) in the growth of LNCaP cells with SIT treatment (Figure 5). In contrast, FIN, a selective inhibitor of SRD5A2, did not impact these cells, suggesting the absence of SRD5A2 activity. On the other hand, ANOVA analyses and the Dunnett test indicated that steroid **2** significantly ($p < 0.02$) reduced cell viability at a concentration of 1 μM, Figure 6C.

The impact of various concentrations of androstenedione (4-dione) on the proliferation of LNCaP cells is depicted in Figure 7A. The concentration of 100 pM, which significantly increased LNCaP proliferation ($p < 0.02$), suggests a potential role for androgens in the progression of prostate cancer. The activity of different concentrations of SIT and 1 μM of steroid **2** on 4-dione LNCaP stimulation is illustrated in Figure 7B. SIT did not reduce LNCaP cell proliferation at the three tested concentrations, whereas 1 μM of steroid **2** significantly decreased this proliferation ($p < 0.02$). SIT did not decrease LNCaP cell proliferation in the three tested concentrations, whereas 1 μM of steroid **2** reduced proliferation considerably ($p < 0.02$).

4 | Conclusion

The most efficient metabolic pathway for the formation of DHT in wild LNCaP cells was from the conversion of 4-dione to 5α-dione rather than from T. This conclusion is supported by the Vmax values for the formation of 5α-dione (271.05 ± 5.0 ng/mg protein/min) and the Vmax for the formation of T (80.1 ± 8.0 ng/mg protein/min) from 4-dione as a common precursor. These data suggest that the overexpression of SRD5A1 increases the conversion of 4-dione to 5α-dione more efficiently than the pathway from 4-dione to T (Figure 2) despite the overexpression of AKR1C3 in these cells. In these metabolic pathways, SIT at both concentrations inhibited the activity of both enzymes, as did 1.4 μM of steroid **2**.

Despite the overexpression of these enzymes in wild LNCaP cells, SIT acted more effectively in reducing proliferation when the precursor present in the assay was T, which was converted to DHT. Meanwhile, compound **2** in the assay proved more effective in reducing proliferation when the precursor present was 4-dione. The 4-dione, in the presence of **2**, reduced its

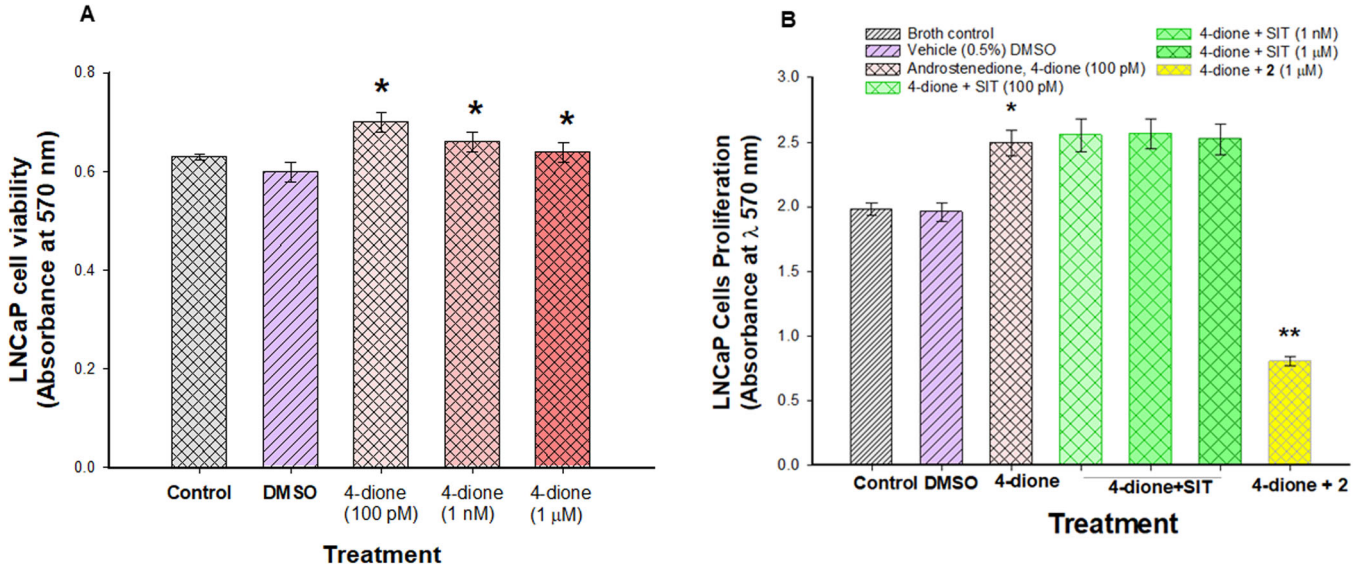


FIGURE 7 | LNCaP cells were seeded at a density of 3×10^4 cells per well in 96-well plates and allowed to adhere for 24 h. Different concentrations of SIT and **2**, along with unlabeled androstenedione (4-dione), were added to separate wells. Viability controls were made, adding 4-dione or vehicle (DMSO) to other wells. The plates were then incubated at 37°C, and cell proliferation was measured using the MTT Method. A Different 4-dione concentrations increased LNCaP proliferation compared with vehicle-treated cells in four independent experiments, suggesting a potential role for androgens in prostate cancer progression. B ANOVA and Dunnett test indicated that 1 μM Steroid 2 significantly decreased LNCaP cell proliferation in four separate experiments ($p < 0.02$). However, SIT did not differ from the 4-dione control in four independent experiments ($p > 0.02$). * Indicates a statistically significant difference ($p < 0.02$) with the vehicle-treated cells. ** Indicates a significant difference with the 4-dione-stimulated LNCaP proliferation ($p < 0.02$).

conversion to 5α-dione and DHT in these tumor cells. Consequently, both compounds could have therapeutic potential for CRPC.

Author Contributions

All the authors contributed equally to the development of this study and consented to.

Acknowledgments

This study was conducted with funds approved by the Universidad Autónoma Metropolitana-Xochimilco for the project "Mechanism of Action of Steroid Hormones in Different Tissues," which was approved by the publication Divisional Council on July 24, 2024, during its 14/24 session with agreement 14/24.3.8.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data for this experiment is available in the Supporting material file and the repositories of the Hormone Laboratory of Universidad Autónoma Metropolitana-Xochimilco.

References

Afzal, O., M. H. Akhter, I. Ahmad, et al. 2022. "A β-Sitosterol Encapsulated Biocompatible Alginate/Chitosan Polymer Nanocomposite for the Treatment of Breast Cancer." *Pharmaceutics* 14, no. 8: 1711. https://doi.org/10.3390/pharmaceutics14081711.

Awad, A. B., C. S. Fink, H. Williams, and U. Kim. 2001. "In Vitro and In Vivo (SCID Mice) Effects of Phytosterols on the Growth and Dissemination of Human Prostate Cancer PC-3 Cells." *European Journal of Cancer Prevention* 10, no. 6: 507–513.

Baskar, A. A., S. Ignacimuthu, G. M. Paulraj, and K. S. Al Numair. 2010. "Chemopreventive Potential of β-Sitosterol in Experimental Colon Cancer Model—An In Vitro and In Vivo Study." *BMC Complementary and Alternative Medicine* 10: 24. https://doi.org/10.1186/1472-6882-10-24.

Biancolella, M., A. Valentini, D. Minella, et al. 2007. "Effects of Dutasteride on the Expression of Genes Related to Androgen Metabolism and Related Pathway in Human Prostate Cancer Cell Lines." *Investigational New Drugs* 25: 491–497. https://doi.org/10.1007/s10637-007-9070-7.

Cabeza, M., E. Bratoeff, I. Heuze, E. Ramírez, M. Sánchez, and E. Flores. 2003. "Effect of Beta-Sitosterol as Inhibitor of 5 Alpha-Reductase in Hamster Prostate." *Proceedings of the Western Pharmacology Society* 46: 153–155.

Cabeza, V. J. S., F. Martínez Zimmerer, J. Soriano, and Y. Heuze. 2023. "β-Sitosterol as a Competitive Inhibitor of the Human Type 2 5α-Reductase." *EC Pharmacology and Toxicology* 11: 17–27.

Cao, Z., X. Wang, and L. Lu, et al. 2019. "β-Sitosterol and Gemcitabine Exhibit Synergistic Anti-Pancreatic Cancer Activity by Modulating Apoptosis and Inhibiting Epithelial-Mesenchymal Transition by Deactivating Akt/GSK-3β Signaling." *Frontiers in Pharmacology* 9: 1525. https://doi.org/10.3389/fphar.2018.01525.

Chang, K. H., R. Li, M. Papari-Zareei, et al. 2011. "Dihydrotestosterone Synthesis Bypasses Testosterone to Drive Castration-Resistant Prostate Cancer." *Proceedings of the National Academy of Sciences of the USA* 108, no. 33: 13728–13733. https://doi.org/10.1073/pnas.1107898108.

Denizot, F., and R. Lang. 1986. "Rapid Colorimetric Assay for Cell Growth and Survival Identification to the Tetrazoliurn Dye Procedure Giving Improved Sensitivity and Reliability." *Journal of Immunological Methods* 89, no. 2: 271–274.

Dong, L., R. C. Zieren, W. Xue, T. M. de Reijke, and K. J. Pienta. 2019. "Metastatic Prostate Cancer Remains Incurable; Why?" *Asian Journal of Urology* 6, no. 1: 26–41. https://doi.org/10.1016/j.ajur.2018.11.005.

Ferlay, J., M. Colombet, and I. Soerjomataram, et al. 2021. "Cancer Statistics for the Year 2020: An Overview." *International Journal of Cancer* 149, no. 4: 778–789. https://doi.org/10.1002/ijc.33588.

Fung, K. M., E. N. S. Samara, and C. Wong, et al. 2006. "Increased Expression of Type 2 3α-Hydroxysteroid Dehydrogenase/Type 5 17β-Hydroxysteroid Dehydrogenase (AKR1C3) and Its Relationship With Androgen Receptor in Prostate Carcinoma." *Endocrine-Related Cancer* 13, no. 1: 169–180. https://doi.org/10.1677/erc.1.01048.

Garisto, J. D., and L. Klotz. 2017. "Active Surveillance for Prostate Cancer: How to Do It Right." *Oncology* 31, no. 5: 333–340. 345.

Hang, J., and P. Dussault. 2010. "A Concise Synthesis of β-Sitosterol and Other Phytosterols." *Steroids* 75, no. 12: 879–883. https://doi.org/10.1016/j.steroids.2010.05.016.

von Holtz, R. L., C. S. Fink, and A. B. Awad. 1998. "β-Sitosterol Activates the Sphingomyelin Cycle and Induces Apoptosis in LNCaP Human Prostate Cancer Cells." *Nutrition and Cancer* 32, no. 1: 8–12. https://doi.org/10.1080/01635589809514709.

Huang, Y., X. Jiang, X. Liang, and G. Jiang. 2018. "Molecular and Cellular Mechanisms of Castration-Resistant Prostate Cancer." *Oncology Letters* 15, no. 5: 6063–6076. https://doi.org/10.3892/ol.2018.8123.

Garner, A. E., D. A. Smith, and N. M. Hooper. 2008. "Visualization of Detergent Solubilization of Membranes: Implications for the Isolation of Rafts." *Biophysical Journal* 94, no. 4: 1326–1340. https://doi.org/10.1529/biophysj.107.114108.

Jang, H. J., S. Lee, E. Hong, et al. 2023. "Mychonastes sp. 246 Suppresses Human Pancreatic Cancer Cell Growth via IGFBP3-PI3K-mTOR Signaling." *Journal of Microbiology and Biotechnology* 33, no. 4: 449–462. https://doi.org/10.4014/jmb.2211.11010.

Levy, M. A., M. Brandt, and A. T. Greway. 1990. "Mechanistic Studies With Solubilized Rat Liver Steroid 5α-Reductase: Elucidation of the Kinetic Mechanism." *Biochemistry* 29, no. 11: 2808–2815. https://doi.org/10.1021/bi00463a025.

Litwin, M. S., and H. J. Tan. 2017. "The Diagnosis and Treatment of Prostate Cancer: A Review." *Journal of the American Medical Association* 317, no. 24: 2532–2542. https://doi.org/10.1001/jama.2017.7248.

Liu, Y., D. A. Peterson, H. Kimura, and D. Schubert. 1997. "Mechanism of Cellular 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Reduction." *Journal of Neurochemistry* 69, no. 2: 581–593. https://doi.org/10.1046/j.1471-4159.1997.69020581.x.

Matsushita, M., K. Fujita, and N. Nonomura. 2020. "Influence of Diet and Nutrition on Prostate Cancer." *International Journal of Molecular Sciences* 21, no. 4: 1447. https://doi.org/10.3390/ijms21041447.

Montgomery, R. B., E. A. Mostaghel, R. Vessella, D. L. Hess, R. F. Kalhorn, and C. S. Higano. 2008. "Maintenance of Intratumoral Androgens in Metastatic Prostate Cancer: A Mechanism for Castration-Resistant Tumor Growth." *Cancer Research* 68: 4447–4454.

Mostaghel, E. A., A. Zhang, S. Hernandez, et al. 2019. "Contribution of Adrenal Glands to Intratumor Androgens and Growth of Castration-Resistant Prostate Cancer." *Clinical Cancer Research* 25, no. 1: 426–439. https://doi.org/10.1158/1078-0432.CCR-18-1431.

Nakamura, Y., P. J. Hornsby, and P. Casson, et al. 2009. "Type 5 17beta-Hydroxysteroid Dehydrogenase (AKR1C3) Contributes to Testosterone Production in the Adrenal Reticularis." *Journal of Clinical Endocrinology & Metabolism* 94, no. 6: 2192–2198. https://doi.org/10.1210/jc.2008-2374.

Normington, K., and D. W. Russell. 1992. "Tissue Distribution and Kinetic Characteristics of Rat Steroid 5 α-Reductase Isozymes. Evidence

for Distinct Physiological Functions." *Journal of Biological Chemistry* 267, no. 27: 19548–19554.

Penning, T. M., M. E. Burczynski, and J. M. Jez, et al. 2000. "Human 3α-Hydroxysteroid Dehydrogenase Isoforms (AKR1C1-AKR1C4) of the Aldo-Keto Reductase Superfamily: Functional Plasticity and Tissue Distribution Reveals Roles in the Inactivation and Formation of Male and Female Sex Hormones." *Biochemical Journal* 351, no. 1: 67–77.

Pradhan, N., S. Parbin, and C. Kausar, et al. 2019. "Paederia foetida Induces Anticancer Activity by Modulating Chromatin Modification Enzymes and Altering Pro-Inflammatory Cytokine Gene Expression in Human Prostate Cancer Cells." *Food and Chemical Toxicology* 130: 161–173. https://doi.org/10.1016/j.fct.2019.05.016.

Rebello, R. J., C. Oing, K. E. Knudsen, et al. 2021. "Prostate Cancer." *Nature Reviews Disease Primers* 7, no. 1: 9. https://doi.org/10.1038/s41572-020-00243-0.

Russell, D. W., and J. D. Wilson. 1994. "Steroid 5α-Reductase: Two Genes/Two Enzymes." *Annual Review of Biochemistry* 63, no. 1: 25–61. https://doi.org/10.1146/annurev.bi.63.070194.000325.

Salamatullah, A. M., P. Subash-Babu, A. Nassrallah, A. A. Alshatwi, and M. S. Alkaltham. 2021. "Cyclotrisiloxan and β-Sitosterol Rich *Cassia alata* (L.) Flower Inhibit HT-115 Human Colon Cancer Cell Growth via Mitochondrial-Dependent Apoptotic Stimulation." *Saudi Journal of Biological Sciences* 28, no. 10: 6009–6016. https://doi.org/10.1016/j.sjbs.2021.06.065.

Sánchez-Márquez, A., Y. Arellano, and E. Bratoeff, et al. 2016. "Synthesis and Biological Evaluation of Esters of 16-Formyl-17-Methoxy-Dehydroepiandrosterone Derivatives as Inhibitors of 5α-Reductase Type 2." *Journal of Enzyme Inhibition and Medicinal Chemistry* 31, no. 6: 1170–1176. https://doi.org/10.3109/14756366.2015.1103235.

Sen, A., P. Dhavan, K. K. Shukla, S. Singh, and G. Tejovathi. 2012. "Analysis of IR, NMR and Antimicrobial Activity of β-Sitosterol Isolated From Momordica charantia." *Science Secure Journal of Biotechnology* 1: 9–13. http://www.researchgate.net/publication/235995077_Analysis_of_IR_NMR_and_Antimicrobial_Activity_of_Sitosterol_Isolated_from_Momordica_charantia/file/3deec515591cbc2a35.pdf.

Sharmila, R., and G. Sindhu. 2017. "Modulation of Angiogenesis, Proliferative Response, and Apoptosis by β-Sitosterol in Rat Model of Renal Carcinogenesis." *Indian Journal of Clinical Biochemistry* 32, no. 2: 142–152. https://doi.org/10.1007/s12291-016-0583-8.

Shimizu, H., R. Ross, L. Bernstein, R. Yatani, B. Henderson, and T. Mack. 1991. "Cancers of the Prostate and Breast Among Japanese and White Immigrants in Los Angeles County." *British Journal of Cancer* 63, no. 6: 963–966. https://doi.org/10.1038/bjc.1991.210.

Stanbrough, M., G. J. Bubley, K. Ross, et al. 2006. "Increased Expression of Genes Converting Adrenal Androgens to Testosterone in Androgen-Independent Prostate Cancer." *Cancer Research* 66, no. 5: 2815–2825. https://doi.org/10.1158/0008-5472.CAN-05-4000.

Thomas, L. N., C. B. Lazier, and R. Gupta, et al. 2005. "Differential Alterations in 5α-Reductase Type 1 and Type 2 Levels During Development and Progression of Prostate Cancer." *Prostate* 63, no. 3: 231–239. https://doi.org/10.1002/pros.20188.

Thomas, L. N., R. C. Douglas, R. S. Rittmaster, and C. K. Too. 2009. "Overexpression of 5 Alpha-Reductase Type 1 Increases Sensitivity of Prostate Cancer Cells to Low Concentrations of Testosterone." *Prostate* 69, no. 6: 595–602. https://doi.org/10.1002/pros.20911.

Tosoian, J. J., B. J. Trock, and P. Landis, et al. 2011. "Active Surveillance Program for Prostate Cancer: An Update of the Johns Hopkins Experience." *Journal of Clinical Oncology* 29, no. 16: 2185–2190. https://doi.org/10.1200/jco.2010.32.8112.

Trapani, G., L. Dazzi, M. G. Pisu, A. Reho, E. Seu, and G. Biggio. 2002. "A Rapid Method for Obtaining Finasteride, a 5alpha-Reductase Inhibitor, From Commercial Tablets." *Brain Research Protocols* 9, no. 2: 130–134. https://doi.org/10.1016/s1385-299x(02)00146-0.

Tsugane, S., J. M. P. de Souza, and M. L. Costa, et al. 1990. "Cancer Incidence Rates Among Japanese Immigrants in the City of São Paulo, Brazil, 1969–78." *Cancer Causes & Control* 1, no. 2: 189–193. https://doi.org/10.1007/bf00053172.

Wu, L., M. Einstein, W. M. Geissler, H. K. Chan, K. O. Elliston, and S. Andersson. 1993. "Expression Cloning and Characterization of Human 17 Beta-Hydroxysteroid Dehydrogenase Type 2, a Microsomal Enzyme Possessing 20 Alpha-Hydroxysteroid Dehydrogenase Activity." *Journal of Biological Chemistry* 268, no. 17: 12964–12969.

Wu, Y., A. Godoy, F. Azzouni, J. H. Wilton, C. Ip, and J. L. Mohler. 2013. "Prostate Cancer Cells Differ in Testosterone Accumulation, Dihydrotestosterone Conversion, and Androgen Receptor Signaling Response to Steroid 5α-Reductase Inhibitors." *Prostate* 73, no. 13: 1470–1482.

Yepuru, M., Z. Wu, A. Kulkarni, et al. 2013. "The Steroidogenic Enzyme AKR1C3 Is a Novel Androgen Receptor-Selective Coactivator That Promotes Prostate Cancer Growth." *Clinical Cancer Research* 19, no. 20: 5613–5625.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.